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Original article

Monovalent mannose-based DC-SIGN antagonists: Targeting the hydrophobic groove of the receptor

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ABSTRACT

Dendritic cell-specific, intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is a C-type lectin expressed specifically on dendritic cells. It is a primary site for recognition and binding of various pathogens and thus a promising therapeutic target for inhibition of pathogen entry and subsequent prevention of immune defense cell infection. We report the design and synthesis of D-mannose-based DC-SIGN antagonists bearing diaryl substituted 1,3-diaminopropanol or glycerol moieties incorporated to target the hydrophobic groove of the receptor. The designed glycomimetics were evaluated by *in vitro* assay of the isolated DC-SIGN extracellular domain for their ability to compete with HIV-1 gp120 for binding to the DC-SIGN carbohydrate recognition domain. Compounds **14d** and **14e**, that display IC_{50} values of 40 μM and 50 μM, are among the most potent monovalent DC-SIGN antagonists reported. The antagonistic effect of all the synthesized compounds was further evaluated by a one-point *in vitro* assay that measures DC adhesion. Compounds **14d**, **14e**, **18d** and **18e** were shown to act as functional antagonists of DC-SIGN-mediated DC adhesion. The binding mode of **14d** was also studied by molecular docking and molecular dynamics simulation, which revealed flexibility of **14d** in the binding site and provides a basis for further optimization.

1. Introduction

In 2012, the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported, between 2005 and 2011, a significant reduction in the rate of new HIV infections and a 25% decline in AIDS-related deaths – the result of increased access of patients to antiretroviral therapy [1]. Despite this fact, HIV is still one of the leading causes of morbidity and mortality worldwide. Since HIV-1 infections occur mainly *via* sexual transmission through mucosal surfaces, microbicides for topical vaginal or rectal administration constitute a promising approach for preventing sexually transmitted HIV-1 infections [2].

DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) is a C-type lectin expressed specifically on dendritic cells (DC). Its functions span from acting as an adhesion molecule that enables DC migration, pathogen/antigen recognition, internalization and processing, to binding of DC to $CD4^+$ T cells *via* ICAM-3 [3]. DC-SIGN recognizes and binds distinct molecular patterns (PAMPs – Pathogen-Associated Molecular Patterns) of a number of pathogens, including HIV-1 [4]. On binding, DC-SIGN–pathogen complexes are internalized, degraded into smaller fragments in lysosomes and conjugated with MHC class-II proteins to initiate a humoral immune response from T cells. DC-SIGN also activates signal transduction pathways that modulate DC-maturation status, and alters the DC-cytokine profile by tailoring toll-like receptor signaling [5].

Besides being a gatekeeper of DCs, DC-SIGN is involved in the initial step of HIV-1 infection, since primary contact of HIV-1 with immature DCs proceeds through interaction of HIV-1 envelope

Abbreviations: CRD, carbohydrate recognition domain; DBU, 1,8-diazabicyclo [5.4.0]undec-7-ene; DC, dendritic cell; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DMF, *N*,*N*-dimethylformamide; ECD, extracellular domain; MD, molecular dynamics; PAMPs, pathogen-associated molecular patterns; PI, propidium iodide; RMSD, root mean square distance; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

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glycoprotein gp120 and DC-SIGN. The DC-SIGN–HIV-1 complex is quickly endocytosed and most of the virus degraded. However, a portion of the HIV-1 avoids lytic degradation and escapes the host immune system [6,7]. DC-SIGN-bound HIV-1 is then transmitted effectively to recipient $CD4^+$ T cells, which leads to their productive infection [8]. Thus, inhibition of HIV-1 interaction with DC-SIGN represents a promising therapeutic approach for inhibition of viral entry and subsequent infection of immune defense cells [9].

DC-SIGN specifically recognizes highly mannosylated viral and bacterial PAMPs (HIV-1 gp120, GP Ebola virus, ManLAM *Mycobacterium tuberculosis*) [7,10] and mannose and fucose-based oligosaccharides such as blood group antigen $Lewis^x$ (Galβ4[Fucα3]GlcNAc). It has a higher specificity for fucose-containing than for mannose-containing oligosaccharides [11]. It exhibits very weak affinities for D-mannose (K_i = 13.1 mM) and L-fucose (K_i = 6.7 mM) [12,13], but mannose- and fucose-based glycomimetics designed to inhibit DC-SIGN are reported to have higher affinity [14]. The challenging task of the design of therapeutically useful DC-SIGN antagonists can be divided into three approaches – (*i*) design of monovalent glycomimetics [15–19], (*ii*) multimeric presentation of monosaccharides/oligosaccharides and glycomimetics [9,20–25] and (*iii*) discovery of non-carbohydrate ligands [26–29].

Recently, we reported a focused library of pseudomannobioside-based DC-SIGN antagonists bearing additional hydrophobic groups designed to target the lipophilic pocket of the receptor. Compounds inhibited DC-SIGN-mediated adhesion of immature DC to mannan-coated plates, with IC_{50} values in the lower micromolar range [18]. In the present study, we describe the design, synthesis and biological evaluation of more flexible D-mannose-based monovalent glycomimetics bearing either a 1,3-diaminopropan-2-ol or a glycerol linker between α-D-mannose and different aryl moieties. These aromatic groups were incorporated in the structure to yield possible hydrophobic and/or π–π interactions in the hydrophobic groove of the DC-SIGN carbohydrate recognition domain (CRD) and consequently to increase the binding affinity of the compounds. Selection of candidate molecules for the synthesis was supported by molecular modeling. The designed glycomimetics were evaluated, in an *in vitro* assay on the isolated DC-SIGN extracellular domain, for their ability to compete with HIV-1 gp120 for binding to DC-SIGN extracellular domain (ECD) and to prevent DC adhesion to mannan-coated plates, using our recently described assay [30].

2. Results and discussion

2.1. Design

DC-SIGN is a type II transmembrane protein that consists of an extracellular domain composed of a neck repeat domain, a CRD, and a transmembrane domain with a short cytosolic tail attached. The neck region is involved in the tetramerization of the receptor, which enables multivalent interaction with glycan ligands [13], while the CRD, which defines the ligand specificity, is involved in ligand binding. The primary carbohydrate-binding site of DC-SIGN CRD contains octacoordinated Ca^{2+} that forms six coordination bonds with the protein and two with the sugar moiety of the binding oligosaccharide. In the case of D-mannose, the equatorial 3- and 4-hydroxyl groups not only form coordination bonds with Ca^{2+}, but also form hydrogen bonds with the side chains of Glu347 and Glu354, residues that also interact with a Ca^{2+} ion [12]. The natural disaccharide Manα1-2Man displays two different binding modes of the core mannose residue in the Ca^{2+} binding site [31]. In the major binding mode, 3- and 4-hydroxyl groups of the reducing end mannose ring interact with Ca^{2+} while, in the minor mode, coordination of the Ca^{2+} occurs through the same groups of the non-reducing terminal mannose residue [32]. In contrast to the multiple binding modes of natural ligands in the Ca^{2+} binding site, it has recently been shown that pseudo-1,2-mannobioside glycomimetic possesses a unique binding mode [19].

In the present study, we have designed ligands, based on the D-mannose sugar that serves as an anchor for binding to the Ca^{2+} binding site of the DC-SIGN CRD. To improve the binding affinity of D-mannose we have incorporated variously substituted diaryl moieties that target the hydrophobic groove of the receptor defined by the side chain of Phe313 (Fig. 1). This amino acid residue presumably contributes steric hindrance to the binding of linear oligosaccharides, but enables binding and increases affinity of the

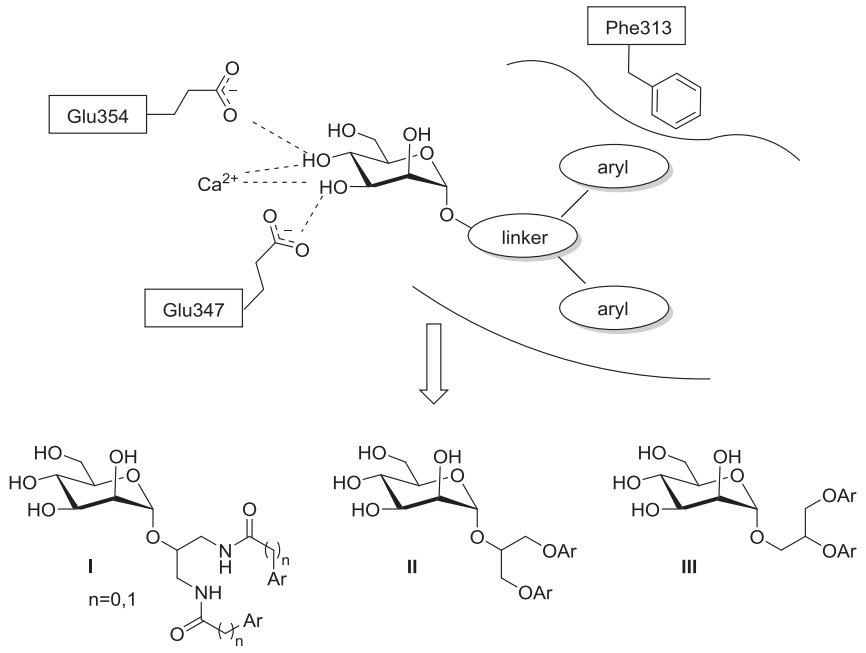
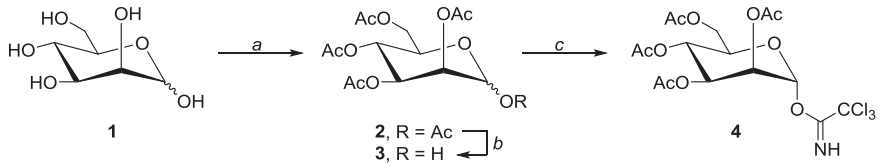


Fig. 1. Design of potential D-mannose-based DC-SIGN antagonists bearing aromatic moieties designed to target the hydrophobic groove of DC-SIGN CRD.



Scheme 1. *Reagents and conditions*. a) Ac_2O, pyridine, r.t., 24 h; b) hydrazine hydrate, AcOH, *N*,*N*-dimethylformamide, 50 °C, 24 h; *c*) Cl_3CCN, DBU, CH_2Cl_2, r.t., 3 h.

branched oligosaccharides. In the crystal structure of DC-SIGN CRD in complex with tetramannoside Man_4 (PDB entry: 1SL4) Phe313 forms weak hydrophobic interactions with the Man2 residue of Man_4 [12]. Since there are two hydrophobic pockets formed by the Phe313 phenyl ring, one facing the Ca^{2+} binding site and the other behind the Phe313 side chain, our ligands were designed to target both of them with the two aryl moieties attached through the linker to the D-mannose core. Aromatic rings have often been used to improve the binding affinity of glycomimetics targeting DC-SIGN [14,33] and other lectins [34–36]. Preliminary docking studies using FlexX [37,38], as available in LeadIT [39], identified 1,3-diaminopropanol and glycerol as linkers of suitable length between D-mannose and aryl moieties, enabling the latter to interact with the two hydrophobic pockets defined by Phe313 (Fig. 1). However, the results of docking a small set of the designed ligands showed comparable binding poses and scoring function scores. Hence, it was not possible to predict the binding affinity of compounds bearing variously substituted aryl rings. A small library of diaryl-based 1,3-diaminopropan-2-ol (**I**, Fig. 1) and glycerol (**II** and **III**, Fig. 1) derivatives was therefore prepared to evaluate our hypothesis and to provide some insight into the preliminary structure–activity relationship.

2.2. Chemistry

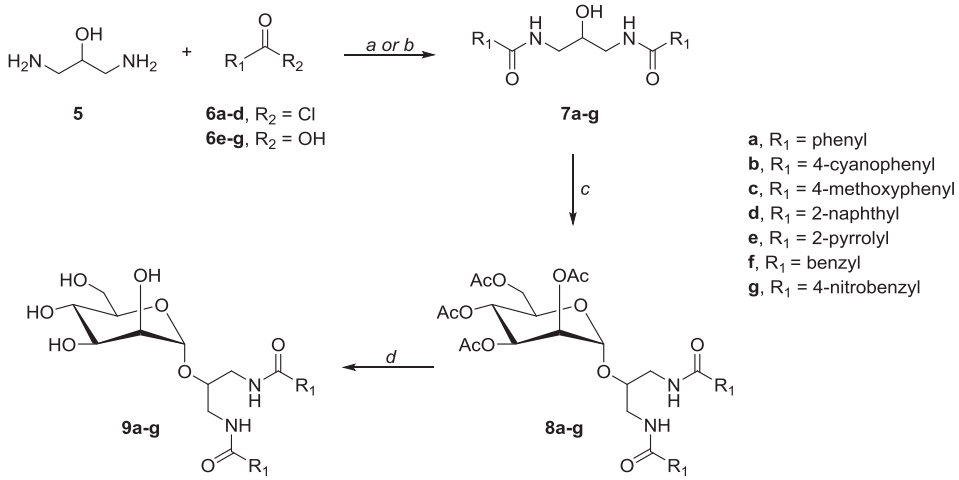
Synthesis of the designed DC-SIGN antagonists **9a**–**g** (Schemes 1 and 2) started from D-mannose (**1**), which was first per-*O*-acetylated using acetic anhydride in dry pyridine to give compound **2**. The anomeric hydroxyl group of **2** was removed selectively by hydrazine hydrate in *N*,*N*-dimethylformamide (DMF) and the compound **3** thus obtained was activated with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base to yield trichloroacetimidate **4** in the α conformation only. 2-Hydroxypropan-1,3-bisamides **7a**–**d** (Scheme 2) were synthesized from 1,3-diaminopropan-2-ol (**5**) and the corresponding acyl chlorides **6a**–**d**, while propanols **7e**–**g** were obtained by EDC/HOBt-promoted coupling of **5** with carboxylic acids **6e**–**g**. Mannosylation of propanols **7a**–**g** with trichloroacetimidate **4** and with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter yielded α-D-mannosides **8a**–**g** stereospecifically. Deacetylation using Zemplén conditions gave the final α-D-mannosides **9a**–**g**.

1,3-Diarylglycerols **12a**–**f** were obtained from epichlorohydrin (**10**) and corresponding phenols **11a**–**c** or naphthols **11d**–**f** using sodium ethoxide in absolute ethanol as a base (Scheme 3). Surprisingly, when 2-naphthol (**11f**) was used as an alcohol, only compound **12f** was isolated as a product, with no expected 1,3-bis(naphthalene-2-yloxy)propan-2-ol, while in all other cases 1,3-diaryl-substituted glycerols **12a**–**e** were obtained as the main products.

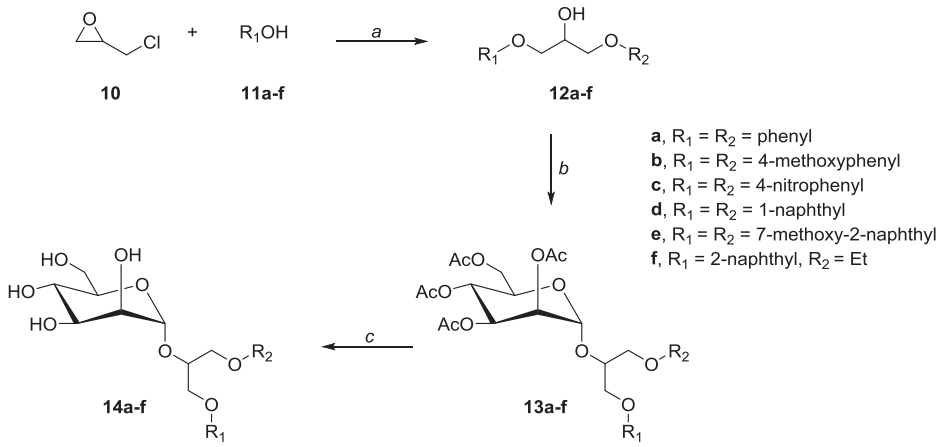
1,2-Diarylglycerols **16a**–**f** (Scheme 4) were synthesized from 2,3-dibromopropan-1-ol (**15**) and phenols **11a**–**f** using the same reaction conditions as for **12a**–**f**. Further, glycerols **12a**–**f** and **16a**–**f** were mannosylated with **4** and TMSOTf, and the obtained α-D-mannosides **13a**–**f** and **17a**–**f** deprotected using the Zemplén method to give the final compounds **14a**–**f** and **18a**–**f**. In contrast to symmetric 1,3-diarylglycerols **12a**–**e**, alcohols **12f** and **16a**–**f** were obtained as racemic mixtures, which also gave protected mannosides **13f** and **17a**–**f**, as well as final compounds **14f** and **18a**–**f** as mixtures of two diastereomers that could not be separated using the methodology we used for isolating and purifying of the parent symmetric compounds.

2.3. Biological activity

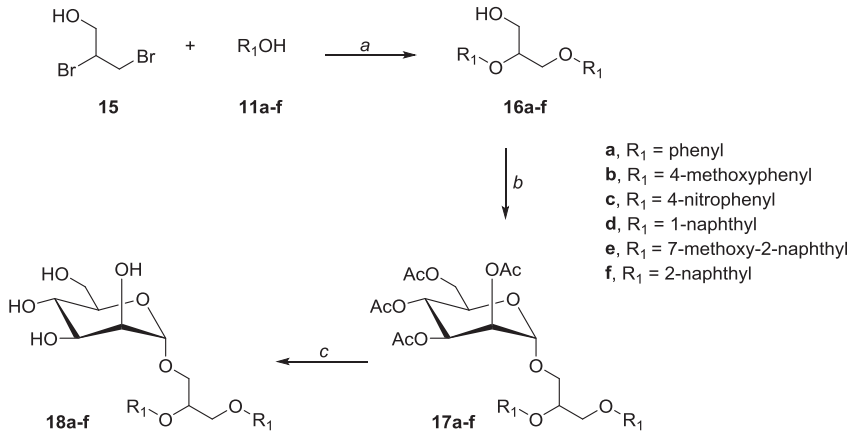
The designed mannose-based glycomimetics **9a**–**g**, **14a**–**f** and **18a**–**f** were evaluated *in vitro* on isolated DC-SIGN extracellular domain for their ability to compete with biotin-labeled HIV-1 gp120 for binding to DC-SIGN CRD (Tables 1 and 2). All final



Scheme 2. *Reagents and conditions*. a) for **7a**–**d**: Et_3N, CH_2Cl_2, 0 °C, 1 h; b) for **7e**–**g**: EDC/HOBt, *N*,*N*-dimethylformamide, *N*-methylmorpholine, r.t., 24 h; c) **4**, TMSOTf, CH_2Cl_2, 0 °C, then r.t., 24 h; d) NaOMe, MeOH, 30 min, then Amberlite® IR120 H, 15 min.



Scheme 3. *Reagents and conditions.* a) NaOEt, EtOH, reflux, 24 h; b) **4**, TMSOTf, CH_2Cl_2, 0 °C, then r.t., 24 h; c) NaOMe, MeOH, 30 min, then Amberlite® IR120 H, 15 min.

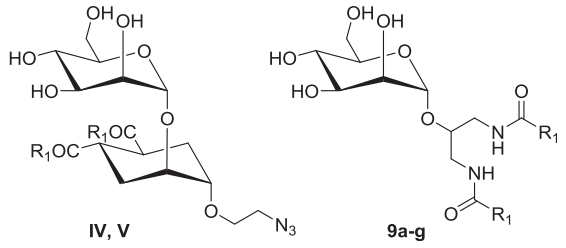


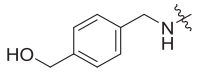
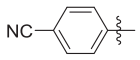
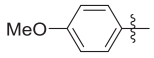
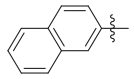
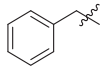
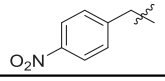
Scheme 4. *Reagents and conditions.* a) NaOEt, EtOH, reflux, 24 h; b) **4**, TMSOTf, CH_2Cl_2, 0 °C, then r.t., 24 h; c) NaOMe, MeOH, 30 min, then Amberlite® IR120 H, 15 min.

compounds were tested in the presence of detergent Tween®-20 to avoid nonspecific inhibition due to aggregate formation [40]. L-Fucose was used as a positive control in the assay and found to inhibit the binding of gp120 to DC-SIGN CRD with an IC_{50} value of 2.95 mM (Table 1), in good agreement with its K_i value of 6.7 mM [13]. We have also evaluated the activity of the two known pseudomannobioside-based DC-SIGN antagonists, **IV** [17,19] and **V** [41] (Table 1). Compounds **V** and **IV** have been reported to inhibit DC-SIGN binding to mannosylated BSA in the SPR assay, with IC_{50} values of 325 μM and 1020 μM [17]. In our assay, these two compounds showed comparable potencies of inhibition of gp120 binding to DC-SIGN ECD with IC_{50} values of 420 μM and 2760 μM, which shows the assay to be suitable for determining the potency of the newly designed DC-SIGN ligands.

First, a series of amides bearing the 1,3-diaminopropan-2-ol linker, **9a**–**g**, were synthesized and their activity evaluated on the isolated DC-SIGN extracellular domain of the receptor (Table 1). Phenyl-based compound **9a** showed a very weak affinity for DC-SIGN ECD, with an IC_{50} value of 8.79 mM. Incorporation of the electron withdrawing cyano group (**9b**) and of the electron donor methoxy group (**9c**) on the *para* position of the phenyl ring increased binding affinity by approximately 2-fold (IC_{50} values of 4.37 mM and 3.89 mM). The potency was further improved by replacing the phenyl ring by a 2-naphthyl ring to give compound **9d**, found to be the most potent DC-SIGN antagonist of this series with an IC_{50} value of 1.34 mM. Compound **9e**, carrying a pyrrole moiety, had negligible potency (IC_{50} = 13.25 mM). Similarly, extending the molecule by an additional methylene group between the linker and the phenyl ring, as in the benzyl-based compound **9f**, had a negative effect on affinity (IC_{50} = 14.94 mM), although an additional nitro group on the *para* position of the benzyl moiety (**9g**) increased potency slightly (IC_{50} = 6.49 mM). Furthermore, DC-SIGN ligands were evaluated by a one-point *in vitro* assay that measures DC-SIGN-mediated immature dendritic cell adhesion to mannan-coated plates [18,30]. The results demonstrate that the synthesized compounds **9a**–**g** inhibit DC adhesion (results represent DC adhesion percentage, Table 1) only weakly at a concentration of 500 μM. The amide bond between the aromatic moiety and the linker in compounds **9a**–**g** is rigid and may not allow optimal orientation of the aromatic moieties in the hydrophobic pocket formed by Phe313. We therefore replaced the 1,3-diaminopropan-2-ol by a glycerol linker to give more flexible ethers **14a**–**f** and **18a**–**f** (Table 2). Compounds **14a**–**f** possess a symmetrical 1,3-diaryloxypropan-2-ol group, in contrast to compounds **18a**–**f** with an asymmetric 2,3-diaryloxypropan-1-ol group that makes them mixtures of two diastereomers. Phenyl-based compound **14a** showed weak activity, with an IC_{50} value of 2.41 mM, while compound **18a**, like compound **9a**, was devoid of affinity (IC_{50} = 8.64 mM). The effect of the electron donor methoxy group on the *para* position of the phenyl ring depends on the symmetry of the diaryl group. In the case of symmetrical compound **14b** (IC_{50} = 5.15 mM) there is a negative effect on the affinity, while compound **18b** (IC_{50} = 1.01 mM) showed a more than 8-fold greater potency than its parent compound **18a**. The 4-nitrophenyl-

Table 1
IC_{50} values of L-fucose, compounds **IV**, **V** and **9a**–**g** determined in the isolated DC-SIGN extracellular domain assay and DC adhesion assay.



Compd	R_1	DC-SIGN IC_{50} [mM][a]	DC adh. [%][b]
L-fucose	/	2.95 ± 0.24	/
[c]**IV**	OMe	2.76 ± 0.34	/
[d]**V**		0.42 ± 0.07	/
9a		8.79 ± 0.81	100 ± 0.13
9b		4.37 ± 0.24	89.6 ± 0.11
9c		3.89 ± 0.18	95.5 ± 0.24
9d		1.34 ± 0.08	95.2 ± 0.10
9e		13.25 ± 0.28	95.7 ± 0.22
9f		14.94 ± 2.26	73.0 ± 0.21
9g		6.49 ± 0.10	81.2 ± 0.16

[a] IC_{50} of compounds in the DC-SIGN extracellular domain assay.
[b] % DC adhesion at 0.5 mM of the tested compound.
[c] DC-SIGN antagonist with an IC_{50} value of 1020 μM in the SPR competition test with immobilized Man-BSA [17,19].
[d] DC-SIGN antagonist with IC_{50} value of 325 μM in the SPR competition test with immobilized Man-BSA [41].

based compound **18c** (IC_{50} = 5.73 mM) showed weaker activity than **18b**; the activity of **14c** could not be determined because of its low solubility. The highest concentration of the stock solution that could be prepared for **14c** and for the poorly soluble 1-naphthyl-based **18d** and 7-methoxy-2-naphthyl-based **18e** was 0.5 mM and these compounds showed no activity at this concentration. In contrast, their analogs **14d** and **14e** were found to be the most potent DC-SIGN antagonists designed and synthesized in our library. Both compounds inhibited gp120 binding to DC-SIGN ECD with IC_{50} values in the low micromolar range (IC_{50}s for **14d** and **14e** were 40 μM and 50 μM). 2-Naphthyl-based compound **18f** exhibited modest potency with IC_{50} value of 395 μM, while the mono-2-naphthyl derivative **14f** was inactive. Thus, the dinaphthyl-based compounds **14d**, **14e** and **18f** are the most potent compounds of our focused library of mannosides, showing promising affinity for DC-SIGN CRD, and are among the most potent monovalent carbohydrate-based DC-SIGN antagonists so far [14]. Compounds **14d**, **14e**, **18d**, and **18e** also inhibited DC adhesion at concentrations of 500 μM (presented as DC adhesion percentage, Table 2). These results correlate quite well with the results of the competitive gp120-displacement assay, and offer additional evidence that compounds with a 1,3-diaryloxypropan-2-ol group and large hydrophobic moieties attached act as functional antagonists of the DC-SIGN primary function of adhesion molecule.

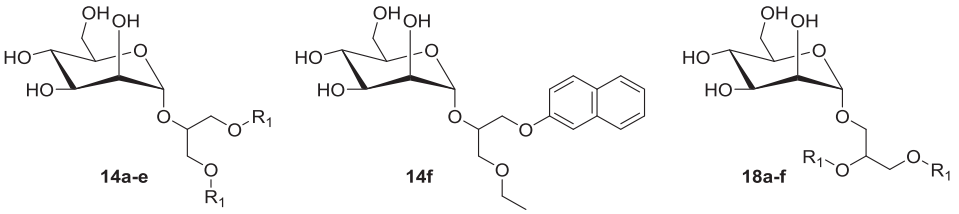
The potential cytotoxicity of representative synthetic DC-SIGN antagonists on target cells was evaluated with a propidium iodide (PI) uptake assay (see Experimental section) of DC cultures treated with compounds **9b**, **9d**, **14d**, **14e**, **18b** or **18f** for 24 h. All the compounds are shown to be non-cytotoxic to DC at 50 μM (Fig. 2). Moderate cytotoxicity on DC was observed for compounds **14d** and **14e** at 100 μM, while compounds **9d**, **14d**, **14e** and **18f** show cytotoxic effects on DC at the highest tested concentration of 250 μM. These results imply that their antagonistic effect on DC-SIGN DC adhesion could be the consequence of the cytotoxic effect. However, the cytotoxicity is measured after 24 h while the dendritic cell adhesion measurement takes no more than 90 min and, during this period, only negligible cytotoxicity was observed, even at the highest assayed concentration. We therefore conclude that the compounds assayed, although cytotoxic at the highest assayed concentration and during prolonged period, also serve as inhibitors of DC adhesion.

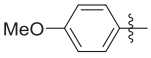
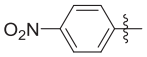
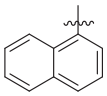
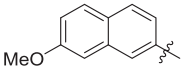
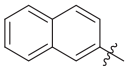
2.4. Molecular modeling

Plausible binding modes of the most potent DC-SIGN antagonists to DC-SIGN CRD, **14d**, **14e** and **18f**, were explored, initially using the molecular docking tool FlexX [37,38] as available in LeadIT [39]. In the crystal structure of DC-SIGN CRD in complex with Man_4 (PDB entry: 1SL4), the binding site was defined as the area within 7 Å from the ligand. In order to predict the binding orientation of the discovered antagonists correctly, we have validated the docking protocol by redocking Man_4 in the defined binding site under pharmacophore type constraints. To place the core mannose residue correctly, the side chain carboxylate groups of Glu347 and Glu354 were defined as hydrogen bond acceptors that form hydrogen bonds with 3- and 4-hydroxyl groups of the core mannose residue in the crystal structure. Further, to account correctly for complex interactions between Ca^{2+} and mannose, a pharmacophore with spherical coordination around Ca^{2+} was additionally defined. The described docking protocol reproduced the binding of Man_4 with an all heavy atom root mean square distance (RMSD) of 3.9 Å, which is usually not sufficient for further docking studies. However, the binding mode of the Man1 and Man2 residues of Man_4 was reproduced reasonably well with an RMSD value of 1.6 Å. The Man1 and Man2 residues of Man_4 form several interactions with the protein while, in contrast, Man3 and Man4 residues point towards the solvent and only interact weakly with DC-SIGN CRD. This is why the program was not able to reproduce the binding of the latter two residues, which resulted in a high RMSD for Man_4. Since the designed ligands are composed of only one mannose residue, reproduction of the core mannose binding mode is the important one. Given that the RMSD value for Man1 was only 0.8 Å, we concluded that the docking protocol is suitable for predicting the binding poses of our novel DC-SIGN antagonists. Indeed, in all our docking experiments this docking protocol correctly placed the mannose moiety of the designed ligands in the defined Ca^{2+} binding site.

The calculated binding modes of DC-SIGN antagonists **14d** (Fig. 2), **14e** and **18f** are as expected. The D-mannose residues of all three antagonists bind in a manner consistent with the defined pharmacophore type constraints, forming hydrogen bonds with the side chain carboxylates of Glu347 and Glu354 and coordination bonds with Ca^{2+}. In the case of antagonists **14d** and (*S*)-enantiomer of **18f**, the D-mannose residue is predicted to bind in the same

Table 2
IC_{50} values of compounds **14a**–**f** and **18a**–**f** determined in the isolated DC-SIGN extracellular domain assay and the DC adhesion assay.



R_1	Compd	[a]DC-SIGN IC_{50} [mM]	[b]DC adh. [%]	Compd	[a]DC-SIGN IC_{50} [mM]	[b]DC adh. [%]
	**14a**	2.41 ± 0.15	102 ± 0.07	**18a**	8.64 ± 5.77	104 ± 0.06
	**14b**	5.15 ± 1.72	83.3 ± 0.15	**18b**	1.01 ± 0.19	80.7 ± 0.18
	**14c**	>0.5	59.0 ± 0.14	**18c**	5.73 ± 1.32	76.2 ± 0.03
	**14d**	0.04 ± 0.03	0.904 ± 0.23	**18d**	>0.5	3.73 ± 0.54
	**14e**	0.05 ± 0.01	7.55 ± 0.11	**18e**	>0.5	5.29 ± 0.17
				**18f**	0.395 ± 0.08	87.6 ± 0.12
–	**14f**	7.16 ± 0.73	80.4 ± 0.09			

[a] IC_{50} of compounds in the DC-SIGN extracellular domain assay.
[b] % DC adhesion at 0.5 mM of the tested compound.

manner as Man1 of Man_4, while the D-mannose moiety of **14e** is rotated by 180°, as observed in the case of dimannoside Manα1-2Man in complex with DC-SIGN CRD (PDB entry: 2IT6) [31]. Both aromatic moieties of **14d**, **14e** and (*S*)-**18f** form hydrophobic and/or π-π interactions with the Phe313 side chain, while (*R*)-**18f** cannot interact with the protein in the expected binding mode.

Superposition of crystal structures of DC-SIGN CRD in complex with mannose-based oligosaccharides (PDB entries: 1SL4, 1SL6, 2IT5, 2IT6) [12,31] or glycomimetics (PDB entry: 2XR5) [19] shows that Phe313 is rather flexible, which has to be considered in the design of ligands and in molecular modeling studies. We have therefore studied the binding mode of compound **14d** in DC-SIGN CRD by molecular dynamics simulation. The FlexX-calculated pose of **14d** in complex with DC-SIGN CRD (Fig. 3) was taken as the starting structure. This was first solvated using the TIP3P water model and then subjected to 8.5 ns molecular dynamics simulation. There are several parameters that have to be monitored to confirm the physical stability of a MD simulation, including volume, temperature and total energy. All these parameters of the system were stable during the production run (Fig. 4a–c). The next important parameter is the RMSD of the backbone atoms that can provide information about the structural stability of the system during the MD simulation. It increased gradually during equilibration of the system and then remained stable during the production run (Fig. 4d). On the contrary, monitoring the RMSD of the ligand showed significant fluctuations during the equilibration and in the beginning of the production run, but then remained fairly stable for the last 3 ns of the simulation at around 2.3 Å (Fig. 4d). Detailed analysis of the trajectory revealed that the conformation, as well as the binding mode, of **14d** changed significantly from the starting FlexX-calculated binding pose. While the position and conformation of the mannose moiety of **14d** remained nearly constant during the simulation, the glycerol moiety with the two naphthalene rings attached showed much more flexibility and adopted a stable binding mode towards the end of the simulation. One of the naphthalene rings of **14d** remained in contact with Phe313 most of the simulation time, while the orientation of the other one changes and extends towards the groove formed by the Arg345 and Asn362 side chains (Fig. 5). Thus, analysis of the MD trajectory shows that the D-mannose residue indeed serves as an anchor for the binding of **14d** to DC-SIGN CRD, while the remaining part of the ligand, designed to interact with Phe313, shows much more flexibility. This behavior of the ligand in DC-SIGN CRD is probably one of the

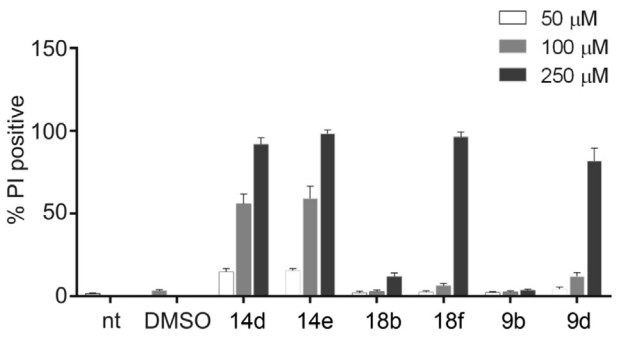


Fig. 2. Cytotoxicity of mannose-based DC-SIGN inhibitors **9b**, **9d**, **14d**, **14e**, **18b** and **18e**.

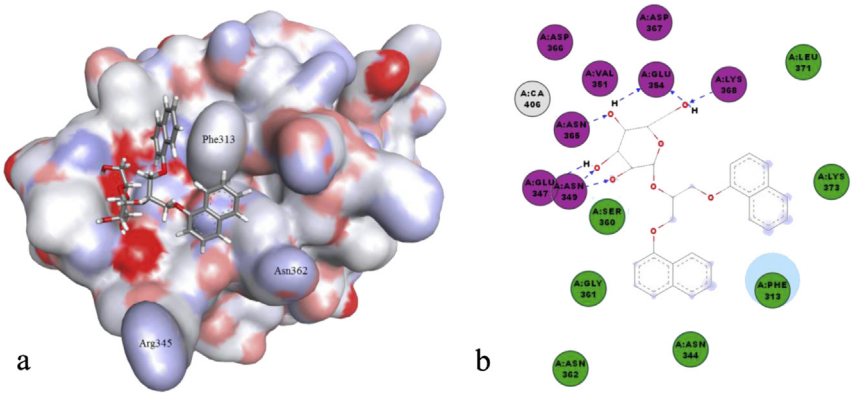


Fig. 3. FlexX-calculated binding mode of **14d** in DC-SIGN CRD. a) Surface representation of DC-SIGN CRD in complex with **14d**; b) 2D diagram showing the interactions of **14d** in complex with DC-SIGN CRD residues.

reasons for the affinity being limited to the micromolar level, which could be improved with the design of conformationally more constricted analogs of **14d** or by strengthening interaction forces by forming the interactions other than hydrophobic/van der Waals interactions.

3. Conclusion

We have designed and synthesized a series of D-mannose-based DC-SIGN antagonists bearing two hydrophobic residues on 1,3-diaminopropan-2-ol or a glycerol linker attached to D-mannose in the α configuration. Final compounds **9a**–**g**, **14a**–**f** and **18a**–**f** were tested in an *in vitro* assay on the isolated DC-SIGN extracellular domain for their ability to compete with HIV-1 gp120 for binding to DC-SIGN CRD. In the series of bisarylamides **9a**–**g**, the 2-naphthyl-based compound **9d** showed the most potent activity, with an IC_{50} of 1.34 mM. Replacement of the amide bond in 1,3-diaminopropan-2-ols **9a**–**g** by an ether bond in the more flexible 1,3-diarylglycerols (**14a**–**f**) or 2,3-diarylglycerols (**18a**–**f**) resulted in the most potent DC-SIGN antagonists **14d** and **14e**. The 1-naphthyl-based **14d** and the 7-methoxy-2-naphthyl-based **14e** inhibited the binding of HIV-1 gp120 with IC_{50}s of 40 and 50 μM, respectively, and are thus the most potent D-mannose-based monovalent DC-SIGN antagonists reported to date. Of the 2,3-diarylglycerols **18a**–**f**, compound **18e**,

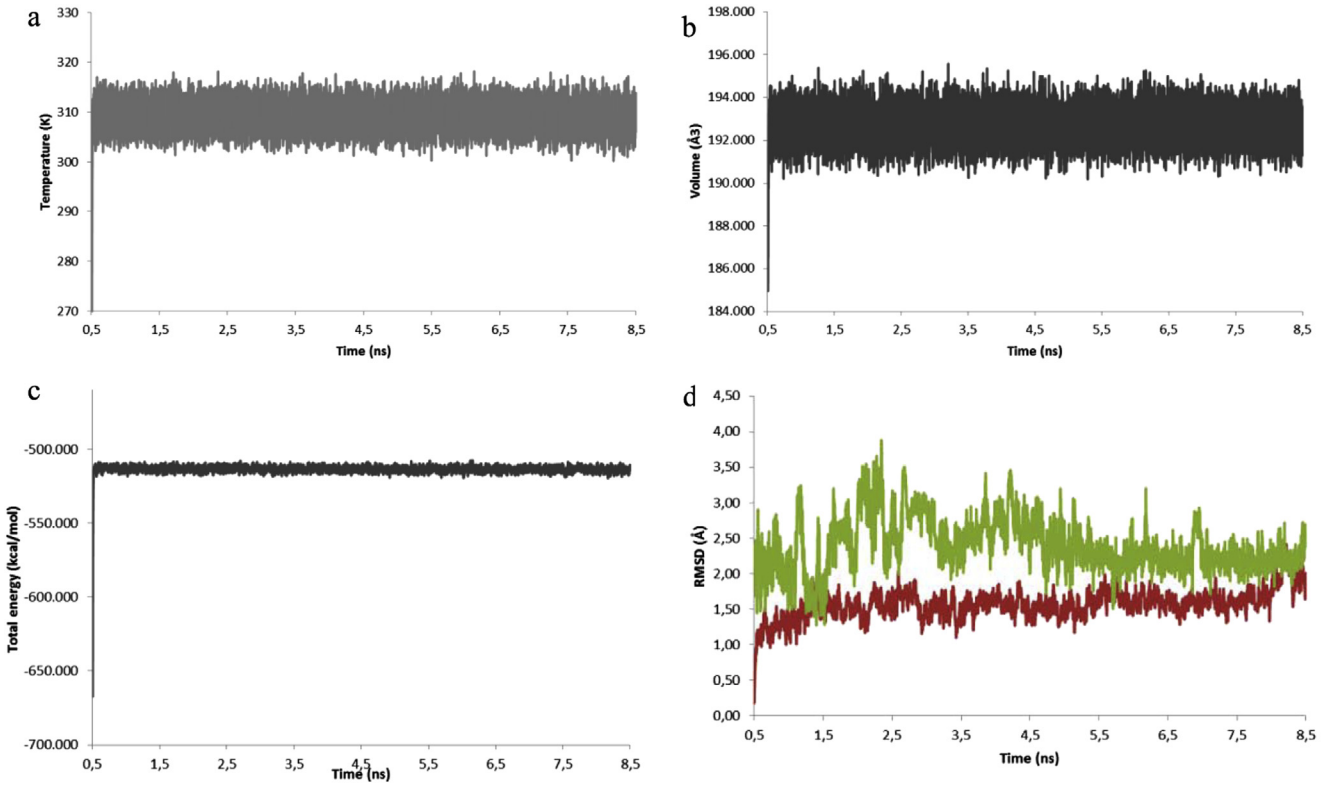


Fig. 4. a) Temperature, b) volume and c) total energy *vs* time plots during the MD simulation of DC-SIGN CRD in complex with **14d**; d) Backbone RMSD (in red) and ligand (**14d**) RMSD (in green) *vs* time plots during the MD simulation of DC-SIGN CRD in complex with **14d**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

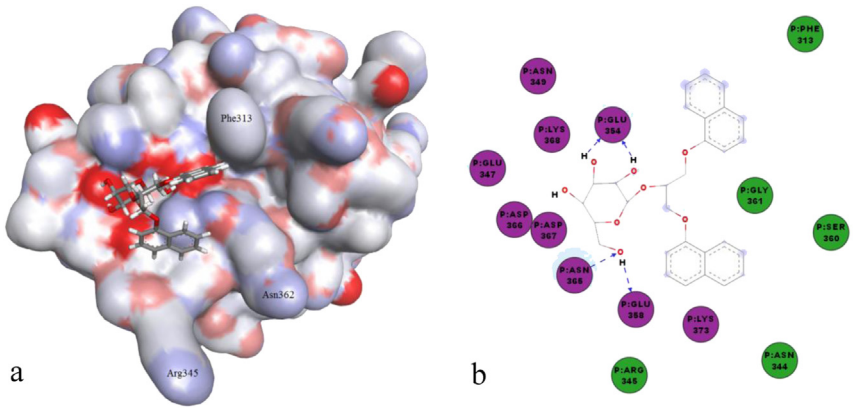


Fig. 5. Binding mode of the molecular dynamics average structure of **14d** to DC-SIGN CRD. a) Surface representation of DC-SIGN CRD in complex with **14d**; b) 2D diagram showing the interactions of **14d** in complex with DC-SIGN CRD residues.

bearing a 2-naphthyl moiety, displayed the highest affinity, with an IC_{50} of 395 μM.

The antagonistic effect of all the synthesized compounds was further evaluated by a one-point *in vitro* assay that measures DC adhesion. Compounds **14d**, **14e**, **18d** and **18e** were shown to act as functional antagonists of DC-SIGN-mediated DC adhesion. The binding modes of antagonists **14d**, **14e** and **18f**, studied by molecular docking, predicted hydrophobic interactions of both aryl substituents with the side chain of Phe313 and their embedment in the groove occupied by 3 out of the 4 mannose residues in Man_4 tetrasaccharide. However, the molecular dynamics simulation of **14d** in complex with DC-SIGN CRD revealed the flexibility of the ligand in the binding site as well as a final binding mode different from that predicted by docking. The more reliable binding mode of **14d** to DC-SIGN CRD determined by MD simulation provides a starting point for further optimization towards monovalent glycomimetics with improved affinity for DC-SIGN.

4. Experimental protocols

4.1. Chemistry

Dichloromethane was dried over calcium hydride and *N*,*N*-dimethylformamide over activated molecular sieves. All reagents were used as received from commercial sources without further purification unless otherwise indicated. Analytical TLC was performed on Merck silica gel ($60F_{254}$) plates (0.25 mm) and components visualized with staining reagents or ultraviolet light. Flash column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker AVANCE III spectrometer in DMSO-d_6, $CDCl_3$ or CD_3OD solution, with TMS as internal standard at 25 °C. Spectra were assigned using gradient COSY, HSQC and DEPT experiments. IR spectra were recorded on a Perkin–Elmer Spectrum BX FT-IR spectrometer. Mass spectra were obtained using a VGAnalytical Autospec Q mass spectrometer. All reported yields refer to purified products. Optical rotation was measured on a Perkin–Elmer 241 MC polarimeter. The reported values for specific rotation are the average of 5 successive measurements using an integration time of 5 s.

4.1.1. 1,2,3,4,6-Penta-O-acetyl-D-mannopyranose (**2**)

Acetic anhydride (20 mL, 0.21 mol) was added drop-wise to a stirred solution of D-mannose (4.981 g, 41.5 mmol) in anhydrous pyridine (25 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred overnight. Ethyl acetate (100 mL) was added and the organic phase successively washed with saturated aqueous $NaHCO_3$ solution (2 × 80 mL), 1 M HCl (2 × 80 mL) and brine (80 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The compound was obtained (mixture of α and β anomer: α/β = 33:67) as a white solid. Yield: 12.699 g (79.0%); colorless oil; $[\alpha]_D$ +24.9 (*c* 0.23, MeOH); IR (NaCl) ν 2991, 1740, 1434, 1368, 1207, 1147, 1086, 1048, 971, 786, 685, 600 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), signals of α-anomer, δ 1.98 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 3.79 (ddd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{5,6'}$ = 5.3 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 4.11 (dd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 4.28 (dd, 1H, $J_{5,6'}$ = 5.3 Hz, $J_{6,6'}$ = 12.4 Hz, H-6′), 5.11 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.27 (t, 1H, $J_{3,4;\ 4,5}$ = 10.0 Hz, H-4), 5.46 (dd, 1H, $J_{1,2}$ = 1.2 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.84 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1); signals of β-anomer, δ 1.98 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 3.99–4.05 (m, 1H, H-5), 4.07 (dd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 4.26 (dd, 1H, $J_{5,6'}$ = 4.9 Hz, $J_{6,6'}$ = 12.4 Hz, H-6′), 5.23 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 5.31–5.34 (m, 2H, H-3, H-4), 6.06 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1) ppm.

4.1.2. 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranose (**3**)

To a solution of hydrazine hydrate (5.03 mL, 56.2 mmol) in *N*,*N*-dimethylformamide (20 mL) acetic acid was added (3.75 mL, 65.5 mmol). A solution of **2** (18.277 g, 46.9 mmol) in *N*,*N*-dimethylformamide (10 mL) was added and the reaction mixture stirred at 50 °C overnight. The mixture was concentrated *in vacuo*, then water (70 mL) was added and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed successively with 1 M HCl (3 × 100 mL), saturated aqueous $NaHCO_3$ solution (2 × 100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. Yield: 8.801 g (54.0%); colorless oil; $[\alpha]_D$ +13.1 (*c* 0.25, MeOH); IR (NaCl) ν 3446, 2961, 1739, 1434, 1369, 1214, 1163, 1125, 1043, 977, 907, 792, 600 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 2.02 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 3.35 (d, 1H, J = 4.2 Hz, OH), 4.16 (dt, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,6'}$ = 4.4 Hz, H-6), 4.24–4.30 (m, 2H, H-5, H-6′), 5.27–5.35 (m, 3H, H-1, H-2, H-4), 5.45 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 10.0 Hz, H-3) ppm.

4.1.3. 2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**4**)

To a stirred solution of **3** (3.600 g, 12.8 mmol) in dry dichloromethane (50 mL) were added trichloroacetonitrile (15.4 mL,

0.15 mol) and DBU (0.534 mL, 3.58 mmol). The reaction mixture was stirred for 3 h at room temperature. The crude product was then concentrated *in vacuo* and purified by flash column chromatography using ethyl acetate/hexane (1:3) as eluent. Yield: 3.518 g (65.0%); yellow oil; $[\alpha]_D$ +9.3 (*c* 0.26, MeOH); IR (NaCl) ν 3321, 2941, 1744, 1678, 1637, 1534, 1434, 1368, 1211, 1155, 1087, 1043, 973, 938, 835, 795, 643, 599, 578 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.01 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 4.15–4.22 (m, 2H, H-5, H-6), 4.28 (dd, 1H, $J_{5,6'}$ = 4.7 Hz, $J_{6,6'}$ = 12.0 Hz, H-6′), 5.40–5.42 (m, 2H, H-3, H-4), 5.47–5.48 (m, 1H, H-2), 6.29 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 8.79 (s, 1H, NH) ppm.

4.1.4. General procedure A. Synthesis of bisarylamides

A solution of 1,3-diaminopropan-2-ol (5.55 mmol) and triethylamine (15.8 mmol) in dry dichloromethane (10 mL) was cooled to 0 °C and a solution of acyl chloride (10.56 mmol) added dropwise. The reaction mixture was stirred for 1 h and the precipitate was filtered off.

*4.1.4.1. N,N′-(2-Hydroxypropane-1,3-diyl)dibenzamide (**7a**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and benzoyl chloride (1.29 mL, 11.1 mmol) according to the general procedure A. Yield: 0.576 g (34.8%); white powder; mp 160 °C; IR (KBr) ν 3284, 2932, 2874, 1750, 1648, 1622, 1602, 1541, 1489, 1423, 1315, 1219, 1182, 1091, 1029, 984, 933, 845, 802, 696, 672 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.60–3.73 (m, 4H, 2× CH_2), 4.02–4.08 (m, 1H, CH), 4.38 (d, 1H, J = 4.2 Hz, OH), 7.33 (t, 2H, J = 6.2 Hz, 2× CONH), 7.44–7.57 (m, 6H, Ar–H), 7.86–7.89 (m, 4H, Ar–H) ppm; HRMS (ESI+): *m*/*z* for $C_{17}H_{19}N_2O_3$ ($[M + H]^+$): calcd 299.1396; found 299.1391.

*4.1.4.2. N,N′-(2-Hydroxypropane-1,3-diyl)bis(4-cyanobenzamide) (**7b**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 4-cyanobenzoyl chloride (1.785 g, 10.56 mmol) according to the general procedure A. Yield: 0.677 g (36.8%); white amorphous powder; IR (KBr) ν 3378, 2232, 1636, 1546, 1499, 1288, 1220, 1096, 1069, 1018, 984, 853, 761, 685, 636, 565, 545 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.25–3.42 (m, 4H, 2× CH_2), 3.82–3.90 (m, 1H, CH), 5.18 (d, 1H, J = 5.2 Hz, OH), 7.95–8.03 (m, 8H, Ar–H), 8.77 (t, 2H, J = 5.8 Hz, CONH) ppm; MS (ESI+): *m*/*z* (%) = 349 ($[M + H]^+$, 100).

*4.1.4.3. N,N′-(2-Hydroxypropane-1,3-diyl)bis(4-methoxybenzamide) (**7c**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 4-methoxybenzoyl chloride (1.802 g, 10.56 mmol) according to the general procedure A. Crude product was recrystallized from ethanol. Yield: 1.715 g (90.0%); white amorphous powder; IR (KBr) ν 3475, 3304, 2962, 1636, 1551, 1508, 1329, 1305, 1253, 1185, 1114, 1089, 1036, 848, 766, 687 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.25–3.36 (m, 4H, 2× CH_2), 3.78–3.81 (m, 7H, CH, 2× OCH_3), 5.11 (d, 1H, J = 5.0 Hz, OH), 7.00 (d, 4H, J = 8.9 Hz, Ar–H), 7.85 (d, 4H, J = 8.9 Hz, Ar–H), 8.36 (t, 2H, J = 5.7 Hz, CONH) ppm; MS (ESI+): *m*/*z* (%) = 359 ($[M + H]^+$, 100).

*4.1.4.4. N,N′-(2-Hydroxypropane-1,3-diyl)bis(2-naphthamide) (**7d**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 2-naphthoyl chloride (2.013 g, 10.56 mmol) according to the general procedure A. Yield: 1.715 g (90.0%); off-white amorphous powder; IR (KBr) ν 3448, 3277, 3056, 1619, 1558, 1503, 1458, 1432, 1338, 1251, 1205, 1147, 1125, 1015, 955, 915, 871, 839, 809, 780, 764, 734, 670 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.37–3.51 (m, 4H, 2× CH_2), 3.91–3.98 (m, 1H, CH), 5.21 (d, 1H, J = 5.1 Hz, OH), 7.57–7.64 (m, 4H, Ar–H), 7.96–8.03 (m, 8H, Ar–H), 8.50 (s, 2H, Ar–H), 8.70 (t, 2H, J = 5.8 Hz, CONH) ppm; MS (ESI+): *m*/*z* (%) = 399 ($[M + H]^+$, 100).

4.1.5. General procedure B. EDC/HOBt-promoted coupling

A solution of 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol), carboxylic acid (10.56 mmol) and HOBt (1.80 g, 13.34 mmol) in DMF (8 mL) was prepared and the pH adjusted to 8 with *N*-methylmorpholine. The solution was cooled to 0 °C, then EDC·HCl (2.60 g, 13.34 mmol) and dichloromethane (2 mL) were added and the reaction mixture stirred overnight at room temperature. The solvent was evaporated *in vacuo* and the oily residue dissolved in ethyl acetate (50 mL) and washed successively with saturated aqueous NaHCO3 solution (3 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure.

*4.1.5.1. N,N′-(2-Hydroxypropane-1,3-diyl)bis(1H-pyrrole-2-carboxamide) (**7e**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 1*H*-pyrrole-2-carboxylic acid (1.173 g, 10.56 mmol) according to the general procedure B. Yield: 1.234 g (81.2%); yellowish amorphous powder; IR (KBr) ν 3300, 1619, 1570, 1527, 1407, 1327, 1208, 1130, 1103, 1085, 1040, 996, 837, 772, 743, 604 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.26 (t, 4H, J = 5.9 Hz, 2× CH_2), 3.69–3.75 (m, 1H, CH), 5.15 (br s, 1H, OH), 6.09 (dd, 2H, J_1 = 2.5 Hz, J_2 = 3.6 Hz, Ar–H), 6.80 (dd, 2H, J_1 = 1.4 Hz, J_2 = 3.6 Hz, Ar–H), 6.86 (dd, 2H, J_1 = 1.4 Hz, J_2 = 2.5 Hz, Ar–H), 8.05 (t, 2H, J = 5.9 Hz, CONH), 11.47 (s, 2H, NH) ppm; MS (ESI+): *m*/*z* (%) = 277 ($[M + H]^+$, 100).

*4.1.5.2. N,N′-(2-Hydroxypropane-1,3-diyl)bis(2-phenylacetamide) (**7f**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 2-phenylacetic acid (1.513 g, 10.56 mmol) according to the general procedure B. Yield: 1.454 g (81.2%); white amorphous powder; IR (KBr) ν 3391, 3253, 3083, 1621, 1561, 1493, 1454, 1428, 1356, 1268, 1160, 1124, 1028, 866, 768, 715, 694, 621, 548 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.96–3.02 (m, 2H, $CHCH_2$), 3.07–3.13 (m, 2H, $CHCH_2$), 3.43 (s, 4H, 2× $COCH_2$), 3.51–3.56 (m, 1H, CH), 5.07 (br s, 1H, OH), 7.19–7.31 (m, 10H, Ar–H), 8.06 (t, 2H, J = 5.7 Hz, CONH) ppm; MS (ESI+): *m*/*z* (%) = 327 ($[M + H]^+$, 100).

*4.1.5.3. N,N′-(2-Hydroxypropane-1,3-diyl)bis(2-(4-nitrophenyl)acetamide) (**7g**).* Prepared from 1,3-diaminopropan-2-ol (0.500 g, 5.55 mmol) and 2-(4-nitrophenyl)acetic acid (1.913 g, 10.56 mmol) according to the general procedure B. Yield: 2.260 g (98.0%); yellowish amorphous powder; IR (KBr) ν 3291, 1630, 1539, 1352, 1255, 1204, 1121, 856, 816, 710, 578 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.96–3.02 (m, 2H, $CHCH_2$), 3.10–3.16 (m, 2H, $CHCH_2$), 3.51–3.58 (m, 1H, CH), 3.62 (s, 4H, 2× $COCH_2$), 7.54 (d, 4H, J = 8.9 Hz, Ar–H), 8.16 (d, 4H, J = 8.9 Hz, Ar–H), 8.26 (t, 2H, J = 5.6 Hz, CONH) ppm; MS (ESI+): *m*/*z* (%) = 417 ($[M + H]^+$, 100).

4.1.6. General procedure C. Synthesis of diarylglycerols

To a freshly prepared solution of sodium ethoxide (20 mmol) in absolute ethanol (50 mL) phenol or naphthol (20 mmol) was added. After stirring at room temperature for 15 min epichlorohydrin (10 mmol) was added and the reaction mixture heated under reflux overnight. The solvent was evaporated under reduced pressure, the crude residue suspended in 2 M NaOH (50 mL) and extracted with ethyl acetate (3 × 50 mL). Combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed *in vacuo*.

*4.1.6.1. 1,3-Diphenoxypropan-2-ol (**12a**).* Prepared from phenol (0.941 g, 10 mmol) and epichlorohydrin (0.392 mL, 5 mmol) according to the general procedure C. The crude product was purified by flash column chromatography using dichloromethane/methanol (60:1) as eluent. Yield: 0.420 g (34.4%); white crystalline solid; mp 79–80 °C; IR (KBr) ν 3515, 3058, 2944, 1599, 1587, 1497, 1457, 1443,

1323, 1294, 1244, 1176, 1117, 1084, 1058, 1035, 1013, 992, 904, 829, 815, 755, 691, 637, 575, 509 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.68 (d, 1H, $J = 5.2$ Hz, OH), 4.18 (dd, 2H, $J_1 = 5.0$ Hz, $J_2 = 9.2$ Hz, 2× CH_2–H_A), 4.21 (dd, 2H, $J_1 = 5.0$ Hz, $J_2 = 9.2$ Hz, 2× CH_2–H_B), 4.40–4.47 (m, 1H, CH), 6.96–7.03 (m, 6H, Ar–H), 7.31–7.36 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.6 (2× CH_2), 68.8 (CH), 114.5, 121.2, 129.5, 158.3 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{15}H_{17}O_3$ ($[M + H]^+$): calcd 245.1178; found 245.1174.

*4.1.6.2. 1,3-Bis(4-methoxyphenoxy)propan-2-ol (**12b**).* Prepared from 4-methoxyphenol (2.480 g, 20 mmol) and epichlorohydrin (0.784 mL, 10 mmol) according to the general procedure C. The crude product was recrystallized from ethyl acetate/hexane. Yield: 0.900 g (14.8%); white crystalline solid; mp 80–82 °C; IR (KBr) ν 3576, 3004, 2947, 1628, 1609, 1516, 1466, 1445, 1389, 1253, 1211, 1166, 1119, 1030, 998, 860, 839, 630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 6H, 2× CH_3), 3.94 (dd, 2H, $J_1 = 5.3$ Hz, $J_2 = 9.8$ Hz, 2× CH_2-H_A), 4.00 (dd, 2H, $J_1 = 5.3$ Hz, $J_2 = 9.2$ Hz, 2× CH_2-H_B), 4.07–4.13 (m, 1H, CH), 6.84–6.91 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8 (2× OCH_3), 69.0 (2× CH_2), 69.5 (CH), 114.7, 115.6, 152.6, 154.2 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{17}H_{21}O_5$ ($[M + H]^+$): calcd 305.1389; found 305.1391.

*4.1.6.3. 1,3-Bis(4-nitrophenoxy)propan-2-ol (**12c**).* Prepared from 4-nitroxyphenol (2.783 g, 20 mmol) and epichlorohydrin (0.784 mL, 10 mmol) according to the general procedure C. The crude product was recrystallized from ethyl acetate/hexane. Yield: 0.233 g (7.0%); white crystalline solid; mp 122–123 °C; IR (KBr) ν 3572, 3115, 1610, 1592, 1509, 1466, 1337, 1301, 1258, 1174, 1027, 942, 848, 751, 690, 668, 652, 629 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.18–4.72 (m, 5H, CH, 2× CH_2), 5.63 (d, 1H, $J = 4.6$ Hz, OH), 7.18–7.22 (m, 4H, Ar–H), 8.20–8.24 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.3 (2× CH_2), 69.2 (CH), 114.6, 126.0, 142.1, 163.1 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{15}H_{15}N_2O_7$ ($[M + H]^+$): calcd 335.0879; found 335.0880.

*4.1.6.4. 1,3-Bis(naphthalen-1-yloxy)propan-2-ol (**12d**).* Prepared from 1-naphthol (2.516 g, 17.5 mmol) and epichlorohydrin (0.684 mL, 8.73 mmol) according to the general procedure C. The crude product was purified by flash column chromatography using dichloromethane as eluent. Yield: 1.918 g (64.0%); white crystalline solid; mp 85–86 °C; IR (KBr) ν 3432, 2932, 2880, 1595, 1518, 1456, 1430, 1268, 1239, 1178, 1112, 1070, 947, 899, 826, 770 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.47 (dd, 2H, $J_1 = 4.5$ Hz, $J_2 = 8.8$ Hz, 2× CH_2–H_A), 4.51 (dd, 2H, $J_1 = 4.5$ Hz, $J_2 = 8.8$ Hz, 2× CH_2–H_B), 4.73–4.80 (m, 1H, CH), 6.93 (dd, 2H, $J_1 = 0.8$ Hz, $J_2 = 7.6$ Hz, Ar–H), 7.42 (t, 2H, $J = 7.6$ Hz, Ar–H), 7.48–7.56 (m, 6H, Ar–H), 7.83–7.86 (m, 2H, Ar–H), 8.29–8.32 (m, 2H, Ar–H) ppm; HRMS (ESI+): *m/z* for $C_{23}H_{21}O_3$ ($[M + H]^+$): calcd 345.1491; found 345.1495.

*4.1.6.5. 1,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-2-ol (**12e**).* Prepared from 7-methoxy-2-naphthol (2.521 g, 14.5 mmol) and epichlorohydrin (0.567 mL, 7.24 mmol) according to the general procedure C. The crude product was recrystallized from ethyl acetate. Yield: 0.816 g (27.9%); white crystalline solid; mp 143–144 °C; IR (KBr) ν 3493, 2939, 2882, 2837, 1513, 1467, 1456, 1442, 1388, 1294, 1240, 1179, 1122, 1050, 984, 828, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 6H, 2× OCH_3), 4.33 (dd, 2H, $J_1 = 4.7$ Hz, $J_2 = 9.0$ Hz, 2× CH_2–H_A), 4.36 (dd, 2H, $J_1 = 4.7$ Hz, $J_2 = 9.0$ Hz, 2× CH_2–H_B), 4.52–4.59 (m, 1H, CH), 7.02–7.08 (m, 6H, Ar–H), 7.15 (d, 2H, $J = 2.5$ Hz, Ar–H), 7.69 (d, 2H, $J = 5.8$ Hz, Ar–H), 7.71 (d, 2H, $J = 5.8$ Hz, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3 (2× OCH_3), 68.8 (2× CH_2), 68.9 (CH), 105.3, 106.4, 116.0, 116.4, 124.5, 129.2, 129.3, 135.8, 157.0, 158.3 (20× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{25}H_{25}O_5$ ($[M + H]^+$): calcd 405.1702; found 405.1705.

*4.1.6.6. 1-Ethoxy-3-(naphthalen-2-yloxy)propan-2-ol (**12f**).* Prepared from 2-naphthol (2.880 g, 20.0 mmol) and epichlorohydrin (0.783 mL, 10.0 mmol) according to the general procedure C. The crude product was purified by flash column chromatography using dichloromethane as eluent. Yield: 0.150 g (6.2%); brown oil; IR (NaCl) ν 3340, 2975, 2885, 1925, 1631, 1602, 1381, 1272, 1218, 1183, 1090, 1050, 881, 838, 805, 748 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.31 (t, 3H, $J = 7.0$ Hz, $CH_2\underline{CH_3}$), 3.44–3.53 (m, 4H, 2× CH_2), 3.97–4.04 (m, 2H, $\underline{CH_2}CH_3$), 4.07–4.11 (m, 1H, CH), 5.16 (d, 1H, $J = 5.0$ Hz, OH), 7.18 (dd, 1H, $J_1 = 2.6$ Hz, $J_2 = 9.0$ Hz, Ar–H), 7.33 (d, 1H, $J = 1.5$ Hz, Ar–H), 7.34–7.37 (m, 1H, Ar–H), 7.46 (ddd, 1H, $J_1 = 1.3$ Hz, $J_2 = 6.9$ Hz, $J_3 = 8.2$ Hz, Ar–H), 7.80–7.84 (m, 3H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2 ($CH_2\underline{CH_3}$), 67.1 ($\underline{CH_2}CH_3$), 69.1, 69.2 (2× CH_2), 71.4 (CH), 106.9, 118.8, 123.8, 126.5, 126.8, 127.7, 129.1, 129.5, 134.5, 156.6 (10× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{15}H_{18}O_3$ ($[M + H]^+$): calcd 247.1334; found 247.1339.

4.1.7. General procedure D. Synthesis of diarylglycerols

To a freshly prepared solution of sodium ethoxide (20 mmol) in absolute ethanol (50 mL) phenol or naphthol (20 mmol) was added. After stirring at room temperature for 15 min 2,3-dibromopropan-1-ol (10 mmol) was added and the reaction mixture heated under reflux overnight. The solvent was evaporated under reduced pressure, the crude residue suspended in 2 M NaOH (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed *in vacuo*.

*4.1.7.1. 2,3-Diphenoxypropan-1-ol (**16a**).* Prepared from phenol (1.881 g, 20.0 mmol) and 2,3-dibromopropan-1-ol (1.03 mL, 10.0 mmol) according to the general procedure D. The crude product was purified by flash column chromatography using dichloromethane/methanol (70:1) as eluent. Yield: 1.187 g (48.6%); white crystalline solid; mp 80–82 °C; IR (KBr) ν 3515, 3058, 2944, 1598, 1587, 1497, 1456, 1443, 1322, 1294, 1244, 1175, 1117, 1084, 1058, 1035, 1013, 992, 904, 828, 815, 755, 691, 637, 575, 541, 509 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.66 (d, 1H, $J = 5.2$ Hz, OH), 4.15–4.23 (m, 4H, 2× CH_2), 4.40–4.47 (m, 1H, CH), 6.96–7.03 (m, 6H, Ar–H), 7.29–7.35 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.7 (2× CH_2), 68.8 (CH), 114.6, 121.3, 129.6, 158.4 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{15}H_{17}O_3$ ($[M + H]^+$): calcd 245.1178; found 245.1173.

*4.1.7.2. 2,3-Bis(4-methoxyphenoxy)propan-1-ol (**16b**).* Prepared from 4-methoxyphenol (2.483 g, 20.0 mmol) and 2,3-dibromopropan-1-ol (1.03 mL, 10.0 mmol) according to the general procedure D. The crude product was recrystallized from ethyl acetate/hexane. Yield: 2.756 g (90.7%); white crystalline solid; mp 92–93 °C; IR (KBr) ν 3493, 3104, 3098, 2880, 1879, 1512, 1456, 1387, 1294, 1229, 1178, 1116, 1049, 983, 951, 863, 827, 781, 745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.64 (d, 1H, $J = 5.0$ Hz, OH), 3.80 (s, 6H, 2× CH_3), 4.08–4.16 (m, 4H, 2× CH_2), 4.34–4.40 (m, 1H, CH), 6.84–6.92 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.7 (2× OCH_3), 68.9 (2× CH_2), 69.5 (CH), 114.7, 115.6, 152.6, 154.2 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{17}H_{21}O_5$ ($[M + H]^+$): calcd 305.1389; found 305.1383.

*4.1.7.3. 2,3-Bis(4-nitrophenoxy)propan-1-ol (**16c**).* Prepared from 4-nitrophenol (2.780 g, 20.0 mmol) and 2,3-dibromopropan-1-ol (1.03 mL, 10.0 mmol) according to the general procedure D. The crude product was recrystallized from ethyl acetate/hexane. Yield: 1.543 g (44.8%); off-white crystalline solid; mp 135–136 °C; IR (KBr) ν 3513, 3111, 2936, 1607, 1590, 1506, 1449, 1340, 1299, 1259, 1173, 1112, 1048, 1032, 948, 900, 865, 838, 753, 698, 690, 656, 632, 592 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.69 (s, 1H, OH), 4.29 (dd,

2H, $J_1 = 5.0$ Hz, $J_2 = 8.9$ Hz, 2× CH_2–H_A), 4.32 (dd, 2H, $J_1 = 4.0$ Hz, $J_2 = 8.9$ Hz, 2× CH_2–H_B), 4.50–4.54 (m, 1H, CH), 7.02–7.06 (m, 4H, Ar–H), 8.23–8.27 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.2 (2× CH_2), 68.5 (CH), 113.6, 124.7, 140.3, 162.6 (12× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{15}H_{15}N_2O_7$ ($[M + H]^+$): calcd 335.0879; found 335.0874.

*4.1.7.4. 2,3-Bis(naphthalen-1-yloxy)propan-1-ol (**16d**).* Prepared from 1-naphthol (2.883 g, 20.0 mmol) and 2,3-dibromopropan-1-ol (1.03 mL, 10.0 mmol) according to the general procedure D. The crude product was purified by flash column chromatography using dichloromethane/methanol (100:1) as eluent. Yield: 1.215 g (35.3%); brown solidified oil; IR (KBr) ν 3422, 3053, 2931, 1595, 1578, 1508, 1456, 1400, 1267, 1239, 1178, 1102, 1072, 945, 897, 796, 770, 731, 572 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (d, 1H, $J = 5.3$ Hz, OH), 4.47 (dd, 2H, $J_1 = 5.0$ Hz, $J_2 = 8.9$ Hz, 2× CH_2–H_A), 4.51 (dd, 2H, $J_1 = 4.3$ Hz, $J_2 = 8.9$ Hz, 2× CH_2–H_B), 4.72–4.79 (m, 1H, CH), 6.93 (dd, 2H, $J_1 = 0.8$ Hz, $J_2 = 7.6$ Hz, Ar–H), 7.42 (t, 2H, $J = 7.6$ Hz, Ar–H), 7.50–7.56 (m, 6H, Ar–H), 7.84–7.86 (m, 2H, Ar–H), 8.30–8.33 (m, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.1 (CH), 69.2 (2× CH_2), 105.1, 121.0, 121.7, 125.4, 125.5, 125.9, 126.6, 127.7, 134.6, 154.1 (20× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{23}H_{21}O_3$ ($[M + H]^+$): calcd 345.1491; found 345.1498.

*4.1.7.5. 2,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-1-ol (**16e**).* Prepared from 7-methoxy-2-naphthol (3.480 g, 20.0 mmol) and 2,3-dibromopropan-1-ol (1.03 mL, 10.0 mmol) according to the general procedure D. The crude product was recrystallized from ethyl acetate/hexane. Yield: 1.844 g (45.6%); white crystalline solid; mp 147–149 °C; IR (KBr) ν 3576, 3004, 2947, 1628, 1609, 1516, 1466, 1445, 1389, 1254, 1226, 1187, 1166, 1120, 1039, 998, 860, 839, 816, 629 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 6H, 2× OCH_3), 4.19 (dd, 2H, $J_1 = 5.8$ Hz, $J_2 = 9.9$ Hz, 2× CH_2–H_A), 4.24 (dd, 2H, $J_1 = 4.7$ Hz, $J_2 = 9.9$ Hz, 2× CH_2–H_B), 4.29–4.34 (m, 1H, CH), 5.54 (d, 1H, $J = 4.5$ Hz, OH), 6.99 (dd, 2H, $J_1 = 2.6$ Hz, $J_2 = 8.9$ Hz, Ar–H), 7.04 (dd, 2H, $J_1 = 2.6$ Hz, $J_2 = 8.9$ Hz, Ar–H), 7.23 (d, 2H, $J = 2.5$ Hz, Ar–H), 7.29 (d, 2H, $J = 2.5$ Hz, Ar–H), 7.72 (d, 2H, $J = 5.0$ Hz, Ar–H), 7.75 (d, 2H, $J = 5.0$ Hz, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3 (2× OCH_3), 68.8 (CH, 2× CH_2), 105.3, 106.5, 116.0, 116.4, 124.5, 129.2, 129.3, 135.8, 157.0, 158.3 (20× Ar–C) ppm. HRMS (ESI+): *m/z* for $C_{25}H_{25}O_5$ ($[M + H]^+$): calcd 405.1702; found 405.1699.

*4.1.7.6. 2,3-Bis(naphthalen-2-yloxy)propan-1-ol (**16f**).* Prepared from 2-naphthol (0.971 g, 6.73 mmol) and 2,3-dibromopropan-1-ol (0.364 mL, 3.37 mmol) according to the general procedure D. The crude product was purified by flash column chromatography using dichloromethane as eluent. Yield: 0.381 g (32.9%); brown solidified oil; IR (KBr) ν 3421, 3056, 2930, 1630, 1600, 1510, 1456, 1390, 1258, 1217, 1182, 1118, 1036, 992, 960, 910, 837, 815, 743, 622 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.69 (br s, 1H, OH), 4.29–4.40 (m, 4H, 2× CH_2), 4.55–4.61 (m, 1H, CH), 7.18–7.25 (m, 4H, Ar–H), 7.37–7.41 (m, 2H, Ar–H), 7.46–7.50 (m, 2H, Ar–H), 7.76–7.82 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.0 (CH, 2× CH_2), 107.0, 118.7, 124.0, 126.6, 126.9, 127.7, 129.2, 129.7, 134.5, 156.4 (20× Ar–C) ppm. HRMS for $C_{23}H_{21}O_3$ ($[M + H]^+$): calcd 345.1491; found 345.1496.

4.1.8. General procedure E. Glycosylation

To a solution of diarylalcohol (1.2 mmol) and **4** (1 mmol) in dry dichloromethane (20 mL) under argon trimethylsilyl trifluoromethanesulfonate (TMSOTf) (1.3 mmol) was added at 0 °C. After stirring at 0 °C for 30 min the reaction mixture was allowed to warm to room temperature and stirred overnight. Et_3N (2.6 mmol) was then added, the solvent removed under reduced pressure and crude product purified by flash column chromatography.

*4.1.8.1. 1,3-Dibenzamidopropan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8a**).* Prepared from **7a** (0.177 g, 0.59 mmol) and **4** (0.449 g, 0.86 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using dichloromethane/methanol (20:1) as eluent. Yield: 0.228 g (61.1%); white crystalline solid; mp 68–70 °C; $[\alpha]_D$ +35.6 (*c* 0.15, MeOH); IR (KBr) ν 3323, 1743, 1643, 1579, 1533, 1489, 1432, 1377, 1217, 1163, 1132, 1043, 977, 694, 598 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 2.04 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 3.27–3.35 (m, 2H, CH_2), 4.04–4.21 (m, 5H, H-5, H-6, CH, CH_2), 4.32 (dd, 1H, $J_{5,6'} = 6.7$ Hz, $J_{6,6'} = 12.8$ Hz, H-6′), 5.12 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.27–5.32 (m, 2H, H-2, H-4), 5.39 (dd, 1H, $J_{2,3} = 3.3$ Hz, $J_{3,4} = 10.1$ Hz, H-3), 7.19–7.22 (m, 1H, CONH), 7.40–7.43 (m, 1H, CONH), 7.49–7.59 (m, 6H, Ar–H), 7.92–7.94 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.6, 20.7, 20.9 (4× $COCH_3$), 38.9, 40.24 (2× CH_2), 62.7 (C-6), 66.2 (C-4), 68.9 (C-3), 69.2 (C-2), 69.65 (C-5), 77.2 (CH), 96.7 (C-1), 127.1, 127.2, 128.79, 131.8, 131.9, 133.6, 133.8 (12× Ar–C), 168.1, 168.4 (2× CONH), 169.9, 170.1, 170.2, 170.7 (4× CO) ppm; HRMS (ESI−): *m/z* for $C_{31}H_{35}N_2O_{12}$ ($[M - H]^-$): calcd 627.2190; found 627.2198.

*4.1.8.2. 1,3-Bis(4-cyanobenzamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8b**).* Prepared from **7b** (0.423 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.510 g (78.5%); white crystalline solid; mp 110 °C; $[\alpha]_D$ +55.0 (*c* 0.17, MeOH); IR (KBr) ν 3422, 2233, 1752, 1638, 1543, 1374, 1227, 1135, 1046, 858, 766, 600 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.99 (s, 3H, $COCH_3$), 2.08 (s, 6H, 2× $COCH_3$), 2.18 (s, 3H, $COCH_3$), 3.27–3.37 (m, 2H, CH_2), 3.96–4.08 (m, 3H, H-5, CH_2), 4.12–4.19 (m, 2H, CH, H-6), 4.29 (dd, 1H, $J_{5,6'} = 5.8$ Hz, $J_{6,6'} = 12.1$ Hz, H-6′), 5.07 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.25 (dd, 1H, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.29 (d, 1H, $J_{3,4;\ 4,5} = 9.8$ Hz, H-4), 5.34 (dd, 1H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 9.8$ Hz, H-3), 7.32–7.36 (m, 1H, CONH), 7.55–7.58 (m, 1H, CONH), 7.79 (d, 4H, $J = 7.9$ Hz, Ar–H), 8.02 (dd, 4H, $J_1 = 1.5$ Hz, $J_2 = 8.6$ Hz, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.6, 20.7, 20.8, 20.9 (4× $COCH_3$), 39.2, 40.4 (2× CH_2), 62.8 (C-6), 66.2 (C-4), 68.8 (C-3), 69.3 (C-2), 69.5 (C-5), 75.3 (CH), 96.8 (C-1), 115.4, 115.5, 118.0, 127.9, 128.0, 132.5, 137.3, 137.5 (12× Ar–C), 166.3, 166.8 (2× CONH), 169.8, 170.3, 170.4, 170.8 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{33}H_{35}N_4O_{12}$ ($[M + H]^+$): calcd 679.2251; found 679.2268.

*4.1.8.3. 1,3-Bis(4-methoxybenzamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8c**).* Prepared from **7c** (0.456 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.250 g (35.6%); white amorphous powder; mp 103–104 °C; $[\alpha]_D$ +12.0 (*c* 0.19, MeOH); IR (KBr) ν 3448, 2965, 1752, 1627, 1551, 1508, 1375, 1256, 1178, 1117, 1053, 978, 837, 745, 687, 604 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 2.04 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 3.22–3.31 (m, 2H, CH_2), 3.89 (2× s, 6H, 2× OCH_3), 4.02–4.19 (m, 5H, H-5, H-6, CH, CH_2), 4.31 (dd, 1H, $J_{5,6'} = 6.5$ Hz, $J_{6,6'} = 12.6$ Hz, H-6′), 5.11 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1), 5.27–5.32 (m, 2H, H-2, H-4), 5.38 (dd, 1H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 6.97–7.00 (m, 4H, Ar–H), 7.17–7.20 (m, 1H, CONH), 7.36–7.40 (m, 1H, CONH), 7.88–7.91 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.6, 20.7, 20.8, 20.9 (4× $COCH_3$), 41.9 (2× CH_2), 55.4 (2× OCH_3), 62.5 (C-6), 66.1 (C-4), 68.4 (C-3), 68.7 (C-2), 69.0 (C-5), 69.9 (CH), 92.2 (C-1), 113.8, 126.2, 128.8, 128.9 (12× Ar–C), 162.4, 167.8 (2× CONH), 169.7, 169.9, 170.1, 170.7 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{33}H_{41}N_2O_{14}$ ($[M + H]^+$): calcd 689.2558; found 689.2545.

*4.1.8.4. 1,3-Bis(2-naphthamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8d**).* Prepared from **7d** (0.485 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.278 g (38.0%); white powder; mp 119 °C; $[\alpha]_D$ +46.4 (*c* 0.13, MeOH); IR (KBr) *ν* 3443, 1752, 1624, 1543, 1370, 1302, 1223, 1131, 1047, 972, 867, 825, 780 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *δ* 2.05 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.21 (s, 3H, $COCH_3$), 3.39–3.47 (m, 2H, CH_2), 4.17–4.26 (m, 5H, H-5, H-6, CH, CH_2), 4.34 (dd, 1H, $J_{5,6'}$ = 5.9 Hz, $J_{6,6'}$ = 11.9 Hz, H-6′), 5.18 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.29–5.36 (m, 2H, H-2, H-4), 5.44 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 7.38–7.42 (m, 1H, CONH), 7.56–7.64 (m, 5H, Ar–H, CONH), 7.91–7.93 (m, 2H, Ar–H), 7.96–8.04 (m, 6H, Ar–H), 8.49 (s, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 20.6, 20.7, 20.8, 20.9 (4× $COCH_3$), 39.3, 40.5 (2× CH_2), 62.8 (C-6), 66.2 (C-4), 69.0 (C-3), 69.3 (C-2), 69.7 (C-5), 75.7 (CH), 96.8 (C-1), 123.6, 123.7, 126.8, 127.7, 127.8, 128.0, 128.6, 129.1, 129.2, 130.9, 131.0, 132.7, 134.9, 135.0 (20× Ar–C), 168.2, 168.68 (2× CONH), 169.7, 170.3, 170.8 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{39}H_{41}N_2O_{12}$ ($[M + H]^+$): calcd 729.2660; found 729.2662.

*4.1.8.5. 1,3-Bis(1H-pyrrole-2-carboxamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8e**).* Prepared from **7e** (0.337 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.155 g (25.6%); yellowish amorphous powder; IR (KBr) *ν* 3399, 2957, 1751, 1636, 1560, 1526, 1371, 1325, 1230, 1133, 1046, 980, 747, 602 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *δ* 2.04 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 3.30–3.39 (m, 2H, CH_2), 3.89–3.99 (m, 3H, H-5, CH_2), 4.12–4.20 (m, 3H, H-6, CH), 4.30 (dd, 1H, $J_{5,6'}$ = 5.8 Hz, $J_{6,6'}$ = 11.8 Hz, H-6′), 5.08 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.26–5.30 (m, 2H, H-2, H-4), 5.38 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 6.27–6.30 (m, 2H, Ar–H), 6.78–6.83 (m, 2H, Ar–H), 6.95–6.98 (m, 3H, 2× Ar–H, CONH), 7.08–7.11 (m, 1H, CONH) ppm; HRMS (ESI+): *m/z* for $C_{27}H_{35}N_4O_{12}$ ($[M + H]^+$): calcd 607.2251; found 607.2237.

*4.1.8.6. 1,3-Bis(2-phenylacetamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8f**).* Prepared from **7f** (0.397 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.400 g (60.0%); white powder; mp 112–113 °C; $[\alpha]_D$ +22.7 (*c* 0.15, MeOH); IR (KBr) *ν* 3422, 1752, 1662, 1261, 1172, 1037, 849, 763, 732, 642, 518 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *δ* 2.04 (s, 6H, 2× $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 3.03–3.13 (m, 2H, CH_2), 3.43–3.53 (m, 2H, CH_2), 3.60 (2× s, 4H, 2× CH_2Ph), 3.74 (td, 1H, $J_{5,6'}$ = 4.9 Hz, $J_{4,5}$ = 10.0 Hz, H-5), 3.86–3.91 (m, 1H, CH), 4.04 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,6'}$ = 12.1 Hz, H-6), 4.15 (dd, 1H, $J_{5,6'}$ = 4.9 Hz, $J_{6,6'}$ = 12.1 Hz, H-6′), 4.87 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.10 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 5.17–5.23 (m, 2H, H-3, H-4), 6.17–6.23 (m, 1H, CONH), 6.31–6.35 (m, 1H, CONH), 7.29–7.40 (m, 10H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 20.6, 20.7, 20.9, 21.1 (4× $COCH_3$), 39.0, 40.1 (2× CH_2), 43.6, 43.7 (2× CH_2), 62.7 (C-6), 66.2 (C-4), 68.8 (C-3), 69.1 (C-2), 69.5 (C-5), 76.2 (CH), 96.9 (C-1), 127.3, 127.4, 129.0, 129.1, 129.3, 134.7 (12× Ar–C), 169.7, 170.1, 170.6, 171.2, 172.0, 172.2 (2× CONH, 4× CO) ppm; HRMS (ESI+): *m/z* for $C_{33}H_{41}N_2O_{12}$ ($[M + H]^+$): calcd 657.2660; found 657.2678.

*4.1.8.7. 1,3-Bis(2-(4-nitrophenyl)acetamido)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**8g**).* Prepared from **7g** (0.507 g, 1.22 mmol) and **4** (0.500 g, 1.02 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (3:1) as eluent. Yield: 0.190 g (25.0%); yellowish powder; mp 140–143 °C; $[\alpha]_D$ +17.2 (*c* 0.03, MeOH); IR (KBr) *ν* 3428, 1750, 1655, 1508, 1262, 1172, 1039, 859, 763, 645 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), *δ* 2.05 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 3.02–3.16 (m, 2H, CH_2), 3.47–3.62 (m, 2H, CH_2), 3.69 (2× s, 4H, 2× CH_2Ph), 3.76–3.80 (m, 1H, H-5), 3.98–4.02 (m, 1H, CH), 4.14 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,6'}$ = 12.3 Hz, H-6), 4.24 (dd, 1H, $J_{5,6'}$ = 5.6 Hz, $J_{6,6'}$ = 12.3 Hz, H-6′), 4.90 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.11 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.20 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.1 Hz, H-3), 5.26 (t, 1H, $J_{3,4;\ 4,5}$ = 10.1 Hz, H-4), 6.39–6.45 (m, 1H, CONH), 6.57–6.63 (m, 1H, CONH), 7.50 (dd, 4H, J_1 = 2.0 Hz, J_2 = 8.8 Hz, Ar–H), 8.23 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 20.7, 20.8, 20.9 (4× $COCH_3$), 39.0, 40.2 (2× CH_2), 43.1 (2× CH_2), 62.8 (C-6), 66.2 (C-4), 68.7 (C-3), 69.2 (C-2), 69.5 (C-5), 75.9 (CH), 97.0 (C-1), 124.0, 124.1, 130.1, 130.2, 142.0, 142.1 (12× Ar–C), 169.7, 170.1, 170.2, 170.4, 170.5, 170.8 (2× CONH, 4× CO) ppm; HRMS (ESI+): *m/z* for $C_{33}H_{39}N_4O_{16}$ ($[M + H]^+$): calcd 747.2361; found 747.2350.

*4.1.8.8. 1,3-Diphenoxypropan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13a**).* Prepared from **12a** (0.222 g, 0.91 mmol) and **4** (0.395 g, 0.76 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.196 g (42.0%); yellow oil; $[\alpha]_D$ +36.3 (*c* 0.20, MeOH); ^1H NMR (400 MHz, $CDCl_3$), *δ* 2.01 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 4.06 (dt, 1H, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 3.9 Hz, H-6), 4.16–4.25 (m, 4H, CH_2CHCH_2), 4.31–4.37 (m, 2H, H-5, H-6′), 4.45–4.51 (m, 1H, CH_2CHCH_2), 5.23 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.32 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.35 (d, 1H, $J_{3,4}$ = 10.0 Hz, H-4), 5.39 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 6.92–6.95 (m, 4H, Ar–H), 6.98–7.03 (m, 2H, Ar–H), 7.30–7.35 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 20.7, 21.0 (4× $COCH_3$), 62.3 (C-6), 66.0 (C-4), 67.1, 67.5 (2× CH_2), 68.7 (C-3), 69.0 (C-2), 69.7 (C-5), 75.0 (CH), 97.3 (C-1), 114.4, 114.6, 121.3, 121.4, 129.6, 129.7, 158.2, 158.3 (12× Ar–C), 169.8, 169.9, 170.0, 170.7 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{29}H_{34}O_{12}Na$ ($[M + Na^+]^+$): calcd 597.1948; found 597.1946.

*4.1.8.9. 1,3-Bis(4-methoxyphenoxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13b**).* Prepared from **12b** (0.350 g, 1.15 mmol) and **4** (0.500 g, 0.96 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.244 g (33.4%); yellow oil; $[\alpha]_D$ +35.9 (*c* 0.20, MeOH); IR (NaCl) *ν* 3349, 2974, 1756, 1508, 1379, 1231, 1089, 1049, 881 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), *δ* 1.94 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 3.70 (s, 6H, 2× OCH_3), 3.98 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,6'}$ = 12.2 Hz, H-6), 4.09–4.24 (m, 6H, CH_2CHCH_2, H-5, H-6′), 4.29–4.34 (m, 1H, CH_2CHCH_2), 5.09–5.17 (m, 3H, H-2, H-3, H-4), 5.24 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 6.85–6.94 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 20.7, 21.0 (4× $COCH_3$), 55.7 (2× OCH_3), 62.3 (C-6), 66.0 (C-4), 68.1, 68.3 (2× CH_2), 68.7 (C-2), 69.0 (C-3), 69.7 (C-5), 75.2 (CH), 97.3 (C-1), 114.7, 114.8, 115.4, 115.7, 152.4, 152.5, 154.2, 154.3 (12× Ar–C), 169.8, 169.9, 170.0, 170.7 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{31}H_{39}O_{14}$ ($[M + H]^+$): calcd 635.2340; found 635.2352.

*4.1.8.10. 1,3-Bis(4-nitrophenoxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13c**).* Prepared from **12c** (0.200 g, 0.60 mmol) and **4** (0.284 g, 0.54 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.260 g (72.0%); %); white solid; mp 110–111 °C; $[\alpha]_D$ +28.0 (*c* 0.23, MeOH); IR (KBr) *ν* 3375, 2938, 1756, 1594, 1508, 1457, 1369, 1340,

1256, 1175, 1113, 1041, 1011, 979, 862, 848, 754, 692, 676, 650 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), δ 1.92 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 3.99 (dd, 1H, $J_{5,6} = 2.6$ Hz, $J_{6,6'} = 12.2$ Hz, H-6), 4.13–4.17 (m, 2H, H-5, H-6′), 4.41–4.49 (m, 5H, CH_2CHCH_2, CH_2CHCH_2), 5.05 (dd, 1H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 10.2$ Hz, H-3), 5.09–5.12 (m, 2H, H-2, H-4), 5.29 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 7.22–7.24 (m, 4H, Ar–H), 8.21–8.26 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.7, 20.8, 20.9 (4× $COCH_3$), 62.4 (C-6), 66.0 (C-4), 67.5, 67.9 (2× CH_2), 68.6 (C-3), 69.1 (C-2), 69.6 (C-5), 74.5 (CH), 97.4 (C-1), 114.5, 114.7, 126.0, 126.1, 142.2, 162.9 (12× Ar–C), 169.7, 170.0, 170.1, 170.6 (4× CO) ppm; HRMS (ESI+): m/z for $C_{29}H_{32}N_2O_{16}Na$ ($[M + Na^+]^+$): calcd 687.1650; found 687.1652.

*4.1.8.11. 1,3-Bis(naphthalen-1-yloxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13d**).* Prepared from **12d** (0.643 g, 1.87 mmol) and **4** (0.815 g, 1.56 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.377 g (34.0%); yellow solid; mp 78 °C; $[\alpha]_D$ +26.6 (*c* 0.23, MeOH); IR (KBr) ν 3463, 3054, 2937, 1751, 1596, 1581, 1508, 1458, 1395, 1369, 1226, 1137, 1046, 980, 793, 772, 599, 571 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 1.99 (s, 6H, 2× $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 3.89 (dd, 1H, $J_{5,6} = 2.4$ Hz, $J_{6,6'} = 12.4$ Hz, H-6), 4.10 (dd, 1H, $J_{5,6'} = 5.0$ Hz, $J_{6,6'} = 12.4$ Hz, H-6′), 4.28 (ddd, 1H, $J_{5,6} = 2.3$ Hz, $J_{5,6'} = 5.0$ Hz, $J_{4,5} = 10.1$ Hz, H-5), 4.46–4.60 (m, 4H, CH_2CHCH_2), 4.77–4.83 (m, 1H, CH_2CHCH_2), 5.31 (t, 1H, $J_{3,4;\ 4,5} = 10.0$ Hz, H-4), 5.35 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.40 (dd, 1H, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 5.44 (dd, 1H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 6.90–6.96 (m, 2H, Ar–H), 7.39–7.44 (m, 2H, Ar–H), 7.48–7.56 (m, 6H, Ar–H), 7.81–7.87 (m, 2H, Ar–H), 8.23–8.31 (m, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.6, 20.7, 20.8, 20.9 (4× $COCH_3$), 62.2 (C-6), 65.9 (C-4), 67.5, 67.9 (2× CH_2), 68.9 (C-3), 69.0 (C-2), 69.7 (C-5), 75.2 (CH), 97.5 (C-1), 105.0, 105.1, 121.1, 121.2, 121.7, 121.8, 125.4, 125.5, 125.7, 125.8, 126.5, 126.6, 127.6, 127.7, 134.5, 134.6, 153.9 (20× Ar–C), 169,7, 169.9, 170.0, 170.6 (4× CO) ppm; HRMS (ESI+): m/z for $C_{37}H_{39}O_{12}$ ($[M + H]^+$): calcd 675.2442; found 675.2426.

*4.1.8.12. 1,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13e**).* Prepared from **12e** (0.776 g, 1.92 mmol) and **4** (0.835 g, 1.60 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:3) as eluent. Yield: 0.410 g (33.0%); yellow solid; mp 88–89 °C; $[\alpha]_D$ +33.8 (*c* 0.21, MeOH); IR (KBr) ν 3448, 2940, 1751, 1633, 1516, 1466, 1388, 1222, 1162, 1137, 1042, 980, 860, 833, 599 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.01 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.21 (s, 3H, $COCH_3$), 3.93 (s, 6H, 2× OCH_3), 4.09 (dd, 1H, $J_{5,6} = 2.0$ Hz, $J_{6,6'} = 12.0$ Hz, H-6), 4.29–4.42 (m, 6H, H-5, H-6′, CH_2CHCH_2), 4.58–4.63 (m, 1H, CH_2CHCH_2), 5.28 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.32–5.38 (m, 2H, H-2, H-4), 5.42 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 7.00–7.08 (m, 6H, Ar–H), 7.14 (dd, 2H, $J_1 = 2.5$ Hz, $J_2 = 7.1$ Hz, Ar–H), 7.67–7.72 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.7, 21.0 (4× $COCH_3$), 55.3 (2× OCH_3), 62.3 (C-6), 66.0 (C-4), 67.2, 67.7 (2× CH_2), 68.8 (C-3), 69.0 (C-2), 69.7 (C-5), 74.9 (CH), 97.3 (C-1), 105.3, 106.3, 106.4, 115.8, 116.0, 116.4, 116.5, 124.5, 124.6, 129.2, 129.4, 129.5, 135.8, 135.9, 156.8, 156.9, 158.2, 158.3 (20× Ar–C), 169.8, 169.9, 170.1, 170.7 (4× CO) ppm; HRMS (ESI+): m/z for $C_{39}H_{43}O_{14}$ ($[M + H]^+$): calcd 735.2653; found 735.2661.

*4.1.8.13. 1-Ethoxy-3-(naphthalen-2-yloxy)propan-2-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**13f**).* Prepared from **12f** (0.282 g, 1.14 mmol) and **4** (0.543 g, 1.04 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:3) as eluent. Compound was obtained as a mixture of two diastereomers in 1:1 ration. Yield: 0.250 g (41.7%); yellow solid; mp 74–75 °C; $[\alpha]_D$ +55.7 (*c* 0.30, MeOH); IR (KBr) ν 3460, 2934, 1751, 1628, 1601, 1507, 1375, 1219, 1184, 1054, 983, 851, 750, 694, 624, 597 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21–1.26 (m, 6H, 2× CH_2CH_3), 2.01–2.02 (2× s, 9H, 3× CH_3), 2.04 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.20 (s, 6H, 2× CH_3), 3.54–3.60 (m, 4H, 2× CH_2CH_3), 3.62–3.69 (m, 4H), 4.06–4.24 (m, 6H), 4.29–4.43 (m, 6H, 2× CH), 5.20 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.25 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1′), 5.32–5.45 (m, 6H), 7.12–7.19 (m, 4H, Ar–H), 7.34–7.39 (m, 2H, Ar–H), 7.44–7.49 (m, 2H, Ar–H), 7.74–7.80 (m, 6H, Ar–H) ppm; HRMS (ESI+): m/z for $C_{29}H_{37}O_{12}$ ($[M + H]^+$): calcd 577.2285; found 577.2275.

*4.1.8.14. 2,3-Diphenoxypropan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17a**).* Prepared from **16a** (0.332 g, 1.36 mmol) and **4** (0.592 g, 1.13 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.154 g (20.0%); yellow oil; $[\alpha]_D$ +7.3 (*c* 0.30, MeOH); IR (NaCl) ν 3446, 2936, 1742, 1599, 1587, 1496, 1456, 1369, 1218, 1174, 1218, 1174, 1134, 1042, 980, 910, 825, 754, 691, 598 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), δ 1.93 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 3.98 (dd, 1H, $J_{5,6} = 2.6$ Hz, $J_{6,6'} = 12.2$ Hz, H-6), 4.12–4.31 (m, 6H, H-5, H-6′, CH_2CHCH_2), 4.36–4.41 (m, 1H, CH_2CHCH_2), 5.13–5.15 (m, 3H, H-2, H-3, H-4), 5.27 (d, 1H, $J_{1,2} = 0.9$ Hz, H-1), 6.93–7.00 (m, 6H, Ar–H), 7.28–7.34 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.7, 21.0 (4× $COCH_3$), 62.3 (C-6), 66.0 (C-4), 67.2, 67.5 (2× CH_2), 68.7 (C-3), 69.0 (C-2), 69.7 (C-5), 75.0 (CH), 97.3 (C-1), 114.4, 114.6, 121.3, 121.4, 129.6, 129.7, 1582, 158.3 (12× Ar–C), 169.8, 169.9, 170.0, 170.8 (4× CO) ppm; HRMS (ESI+): m/z for $C_{29}H_{34}O_{12}Na$ ($[M + Na^+]^+$): calcd 597.1948; found 597.1946.

*4.1.8.15. 2,3-Bis(4-methoxyphenoxy)propan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17b**).* Prepared from **16b** (0.405 g, 1.33 mmol) and **4** (0.633 g, 1.21 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.405 g (52.7%); yellow oil; $[\alpha]_D$ +36.9 (*c* 0.20, MeOH); IR (NaCl) ν 3350, 2975, 2887, 1752, 1381, 1089, 1050, 881, 462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.01 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.05 (dd, 1H, $J_{5,6} = 3.2$ Hz, $J_{6,6'} = 10.8$ Hz, H-6), 4.07–4.16 (m, 6H, H-5, H-6′, CH_2CHCH_2), 4.39–4.44 (m, 1H, CH_2CHCH_2), 5.20 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.31 (dd, 1H, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.2$ Hz, H-2), 5.34 (d, 1H, $J_{3,4} = 9.5$ Hz, H-4), 5.38 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 6.83–6.89 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 20.7, 21.0 (4× $COCH_3$), 55.7 (2× OCH_3), 62.3 (C-6), 66.0 (C-4), 68.1, 68.3 (2× CH_2), 68.7 (C-3), 69.0 (C-2), 69.7 (C-5), 75.2 (CH), 97.3 (C-1), 114.7, 114.8, 115.4, 115.7, 152.5, 152.6, 154.2, 154.3 (12× Ar–C), 169.8, 169.9, 170.0, 170.7 (4× CO) ppm; HRMS (ESI+): m/z for $C_{31}H_{39}O_{14}$ ($[M + H]^+$): calcd 635.2340; found 635.2330.

*4.1.8.16. 2,3-Bis(4-nitrophenoxy)propan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17c**).* Prepared from **16c** (0.425 g, 1.27 mmol) and **4** (0.554 g, 1.06 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.389 g (46.0%); white solid; mp 128–129 °C; $[\alpha]_D$ +30.7 (*c* 0.19, MeOH); IR (KBr) ν 3511, 3086, 2940, 1756, 1594, 1509, 1498, 1458, 1370, 1341, 1256, 1175, 1113, 1041, 1010, 979, 862, 848, 753, 691, 675, 597 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), δ 1.92 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 3.98 (dd, 1H, $J_{5,6} = 2.6$ Hz, $J_{6,6'} = 12.0$ Hz, H-6), 4.13–4.17 (m, 2H, H-5, H-6′), 4.38–

4.52 (m, 5H, C$\underline{H}_2$CHC$\underline{H}_2$, CH$_2$C$\underline{H}$CH$_2$), 5.05 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 10.2 Hz, H-3), 5.09–5.15 (m, 2H, H-2, H-4), 5.29 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.21–7.26 (m, 4H, Ar–H), 8.22–8.26 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CDCl$_3$), δ 20.6, 20.7, 20.9 (4× CO$\underline{C}$H$_3$), 62.4 (C-6), 66.0 (C-4), 67.5, 67.9 (2× CH$_2$), 68.6 (C-3), 69.1 (C-2), 69.6 (C-5), 74.5 (CH), 97.4 (C-1), 114.5, 114.6, 126.0, 126.1, 142.1, 163.0 (12× Ar–C), 169.7, 170.0, 170.1, 170.6 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{29}H_{32}N_2O_{16}Na$ ([M + Na$^+$]$^+$): calcd 687.1650; found 687.1652.

*4.1.8.17. 2,3-Bis(naphthalen-1-yloxy)propan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17d**).* Prepared from **16d** (0.280 g, 0.81 mmol) and **4** (0.386 g, 0.74 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.403 g (80.1%); brown oil; [α]$_D$ +4.5 (*c* 0.23, MeOH); IR (KBr) ν 3375, 3246, 1697, 1617, 1508, 1387, 1240, 1109, 930, 836, 771, 649, 619 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$), δ 1.90 (s, 3H, COC$\underline{H}_3$), 1.92 (s, 3H, COC$\underline{H}_3$), 1.96 (s, 3H, COC$\underline{H}_3$), 2.12 (s, 3H, COC$\underline{H}_3$), 3.79 (dd, 1H, $J_{5,6}$ = 2.5 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 3.94 (dd, 1H, $J_{5,6'}$ = 4.9 Hz, $J_{6,6'}$ = 12.3 Hz, H-6′), 4.28 (ddd, 1H, $J_{5,6}$ = 2.5 Hz, $J_{5,6'}$ = 4.9 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 4.52–4.64 (m, 4H, C$\underline{H}_2$CHC$\underline{H}_2$), 4.71–4.75 (m, 1H, CH$_2$C$\underline{H}$CH$_2$), 5.12 (t, 1H, $J_{3,4}$ = 9.9 Hz, H-4), 5.20 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.9 Hz, H-3), 5.25 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 5.45 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.09–7.14 (m, 2H, Ar–H), 7.42–7.47 (m, 2H, Ar–H), 7.48–7.57 (m, 6H, Ar–H), 7.88–7.91 (m, 2H, Ar–H), 8.17–8.23 (m, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, CDCl$_3$), δ 20.7, 20.8, 20.9 (4× CO$\underline{C}$H$_3$), 62.2 (C-6), 65.9 (C-4), 67.5, 67.9 (2× CH$_2$), 68.9 (C-3), 69.0 (C-2), 69.6 (C-5), 75.2 (CH), 97.5 (C-1), 105.0, 105.1, 121.1, 121.2, 121.7, 121.8, 125.4, 125.5, 125.7, 125.8, 126.6, 126.7, 127.6, 127.7, 134.5, 134.6, 153.9, 163.8 (20× Ar–C), 169.8, 170.0, 170.1, 170.6 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{37}H_{39}O_{12}$ ([M + H]$^+$): calcd 675.2442; found 675.2448.

*4.1.8.18. 2,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17e**).* Prepared from **16e** (0.382 g, 0.94 mmol) and **4** (0.449 g, 0.86 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.410 g (65.0%); brown oil; [α]$_D$ +28.4 (*c* 0.17, MeOH); IR (KBr) ν 3421, 2946, 1627, 1516, 1465, 1388, 1253, 1226, 1210, 1187, 1166, 1119, 1030, 860, 840, 801 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$), δ 1.93 (s, 3H, COC$\underline{H}_3$), 1.94 (s, 3H, COC$\underline{H}_3$), 2.01 (s, 3H, COC$\underline{H}_3$), 2.14 (s, 3H, COC$\underline{H}_3$), 3.85 (s, 3H, OCH$_3$), 3.86 (s, 3H, OCH$_3$), 4.00 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,6'}$ = 12.2 Hz, H-6), 4.17 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$ = 12.2 Hz, H-6′), 4.31 (ddd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{5,6'}$ = 4.8 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 4.34–4.47 (m, 4H, C$\underline{H}_2$CHC$\underline{H}_2$), 4.51–4.56 (m, 1H, CH$_2$C$\underline{H}$CH$_2$), 5.15–5.17 (m, 2H, H-3, H-4), 5.21 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.35 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.00 (dd, 2H, J_1 = 2.6 Hz, J_2 = 8.9 Hz, Ar–H), 7.06 (ddd, 2H, J_1 = 2.6 Hz, J_2 = 8.9 Hz, J_3 = 14.1 Hz, Ar–H), 7.22 (t, 2H, *J* = 2.8 Hz, Ar–H), 7.34 (t, 2H, *J* = 2.8 Hz, Ar–H), 7.74–7.79 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CDCl$_3$), δ 20.7, 21.0 (4× CO$\underline{C}$H$_3$), 55.3 (2× OCH$_3$), 62.3 (C-6), 66.0 (C-4), 67.2, 67.7 (2× CH$_2$), 68.8 (C-3), 69.0 (C-2), 69.7 (C-5), 74.9 (CH), 97.3 (C-1), 105.3, 106.3, 106.4, 115.8, 116.0, 116.4, 116.5, 124.5, 124.6, 129.2, 129.4, 129.5, 135.8, 135.9, 156.8, 156.9, 158.3, 158.4 (20× Ar–C), 169.8, 170.0, 170.1, 170.8 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{39}H_{43}O_{14}$ ([M + H]$^+$): calcd 735.2653; found 735.2632.

*4.1.8.19. 2,3-Bis(naphthalen-2-yloxy)propan-1-yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**17f**).* Prepared from **16f** (0.281 g, 0.82 mmol) and **4** (0.387 g, 0.74 mmol) according to the general procedure E. The crude product was purified by flash column chromatography using ethyl acetate/hexane (1:2) as eluent. Yield: 0.378 g (75.6%); yellow oil; [α]$_D$ +43.4 (*c* 0.22, MeOH); IR (KBr) ν 3463, 3057, 1751, 1629, 1601, 1512, 1465, 1369, 1217, 1181, 1136, 1045, 979, 838, 749, 623 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$), δ 2.02 (s, 3H, COC$\underline{H}_3$), 2.03 (s, 6H, 2× COC$\underline{H}_3$), 2.21 (s, 3H, COC$\underline{H}_3$), 4.01 (dd, 1H, $J_{5,6}$ = 2.1 Hz, $J_{6,6'}$ = 12.0 Hz, H-6), 4.31–4.42 (m, 6H, H-5, H-6′, C$\underline{H}_2$CHC$\underline{H}_2$), 4.60–4.65 (m, 1H, CH$_2$C$\underline{H}$CH$_2$), 5.30 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.33–5.38 (m, 2H, H-2, H-4), 5.44 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 7.16–7.24 (m, 4H, Ar–H), 7.36–7.41 (m, 2H, Ar–H), 7.46–7.50 (m, 2H, Ar–H), 7.76–7.82 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, CDCl$_3$), δ 20.7, 21.0 (4× CO$\underline{C}$H$_3$), 62.4 (C-6), 66.0 (C-4), 67.2, 67.7 (2× CH$_2$), 68.8 (C-3), 69.0 (C-2), 69.7 (C-5), 74.9 (CH), 97.3 (C-1), 106.8, 106.9, 118.5, 118.7, 123.9, 124.0, 126.5, 126.6, 126.8, 127.7, 129.2, 129.3, 129.7, 129.8, 134.4, 134.5, 156.2, 156.3 (20× Ar–C), 169.8, 170.0, 170.1, 170.7 (4× CO) ppm; HRMS (ESI+): *m/z* for $C_{37}H_{39}O_{12}$ ([M + H]$^+$): calcd 675.2442; found 675.2440.

4.1.9. General procedure F. Zemplén deacetylation

The protected mannopyranoside (1 mmol) was dissolved in dry methanol and sodium methanolate solution (30 wt. % in methanol, 0.01 mmol) was added. The reaction mixture was stirred at room temperature for 30 min and then the acidic ion exchange resin Amberlite® IR120 H was added for neutralization. After stirring the mixture for 10 min, the resin was filtered off, washed with methanol and the solvent removed *in vacuo*. Final compounds were obtained in quantitative yield and no further purification was carried out.

*4.1.9.1. 1,3-Dibenzamidopropan-2-yl α-D-mannopyranoside (**9a**).* Prepared from **8a** (0.164 g, 0.27 mmol) according to the general procedure F. Yield: 0.124 g (100%); white crystalline solid; mp 46–48 °C; [α]$_D$ +33.0 (*c* 0.17, MeOH); IR (KBr) ν 3398, 3069, 2933, 1635, 1576, 1534, 1489, 1435, 1292, 1125, 1096, 1023, 978, 929, 804, 692 cm^{-1}; ^{1}H NMR (400 MHz, CD$_3$OD), δ 3.53–3.80 (m, 9H, 2× CH$_2$, H-3, H-4, H-5, H-6, H-6′), 3.86 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.07–4.13 (m, 1H, CH), 5.05 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 7.47–7.52 (m, 4H, Ar–H), 7.54–7.59 (m, 2H, Ar–H), 7.87–7.91 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD$_3$OD), δ 41.4, 43.0 (2× CH$_2$), 62.8 (C-6), 68.6 (C-4), 72.4 (C-3), 72.5 (C-2), 75.3 (C-5), 76.9 (CH), 101.3 (C-1), 128.4, 128.5, 129.6, 129.7, 135.4, 135.6 (12× Ar–C), 170.5, 170.6 (2× CO) ppm; HRMS (ESI+): *m/z* for $C_{23}H_{27}N_2O_8$ ([M + H]$^+$): calcd 459.1767; found 459.1773.

*4.1.9.2. 1,3-Bis(4-cyanobenzamido)propan-2-yl α-D-mannopyranoside (**9b**).* Prepared from **8b** (0.350 g, 0.52 mmol) according to the general procedure F. Yield: 0.215 g (81.2%); white crystalline solid; mp 122–124 °C; [α]$_D$ +37.0 (*c* 0.15, MeOH); IR (KBr) ν 3392, 2933, 2232, 1648, 1543, 1498, 1438, 1287, 1127, 1099, 1054, 977, 858, 765, 679 cm^{-1}; ^{1}H NMR (400 MHz, CD$_3$OD), δ 3.54–3.75 (m, 8H, 2× CH$_2$, H-3, H-4, H-5, H-6), 3.78 (dd, 1H, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 11.3 Hz, H-6′), 3.84 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.08–4.14 (m, 1H, CH), 5.02 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.85–7.90 (m, 4H, Ar–H), 8.01–8.06 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD$_3$OD), δ 41.9, 43.3 (2× CH$_2$), 62.9 (C-6), 68.6 (C-4), 72.3 (C-3), 72.4 (C-2), 75.3 (C-5), 77.0 (CH), 101.3 (C-1), 116.1, 116.2, 119.0, 119.1, 129.3, 129.4, 133.5, 133.6, 139.6, 139.8 (12× Ar–C), 168.7, 168.8 (2× CO) ppm; HRMS (ESI+): *m/z* for $C_{25}H_{25}N_4O_8$ ([M − H]$^-$): calcd 509.1672; found 509.1680.

*4.1.9.3. 1,3-Bis(4-methoxybenzamido)propan-2-yl α-D-mannopyranoside (**9c**).* Prepared from **8c** (0.205 g, 0.30 mmol) according to the general procedure F. Yield: 0.133 g (85.7%); white crystalline solid; mp 110–111 °C; [α]$_D$ +34.6 (*c* 0.10, MeOH); IR (KBr) ν 3403, 2934, 1607, 1542, 1458, 1300, 1257, 1182, 1027, 845, 767, 608 cm^{-1}; ^{1}H NMR (400 MHz, CD$_3$OD), δ 3.50–3.79 (m, 9H, 2× CH$_2$, H-3, H-4, H-5, H-6, H-6′), 3.85 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.87 (2× s, 6H, 2× OCH$_3$), 4.04–4.09 (m, 1H, CH), 5.04 (d, 1H, $J_{1,2}$ = 1.7 Hz,

H-1), 7.00–7.03 (m, 4H, Ar–H), 7.85–7.89 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 41.3, 42.8 (2× CH_2), 55.9 (2× OCH_3), 62.8 (C-6), 68.5 (C-4), 72.3 (C-3), 72.4 (C-2), 75.3 (C-5), 76.8 (CH), 101.2 (C-1), 114.7, 114.8, 127.4, 127.6, 130.2, 130.3 (12× Ar–C), 164.1, 170.2 (2× CO) ppm; HRMS (ESI+): *m*/*z* for $C_{25}H_{33}N_2O_{10}$ ($[M + H]^+$): calcd 521.2135; found 521.2142.

*4.1.9.4. 1,3-Bis(2-naphthamido)propan-2-yl α-D-mannopyranoside (**9d**).* Prepared from **8d** (0.250 g, 0.343 mmol) according to the general procedure F. Yield: 0.144 g (75.0%); white crystalline solid; mp 118–119 °C; $[\alpha]_D$ +21.0 (*c* 0.18, MeOH); IR (KBr) ν 3369, 2930, 1638, 1542, 1432, 1300, 1237, 1053, 907, 864, 824, 777, 760 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.63–3.83 (m, 9H, 2× CH_2, H-3, H-4, H-5, H-6, H-6′), 3.91 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.18–4.23 (m, 1H, CH), 5.12 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.56–7.63 (m, 4H, Ar–H), 7.93–8.02 (m, 8H, Ar–H), 8.46 (d, 2H, *J* = 11.3 Hz, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 41.6, 43.2 (2× CH_2), 62.8 (C-6), 68.6 (C-4), 72.4 (C-3), 72.5 (C-2), 75.3 (C-5), 76.8 (CH), 101.3 (C-1), 124.9, 125.0, 127.8, 127.9, 128.7, 128.8, 128.9, 129.0, 129.1, 129.4, 129.5, 130.1, 130.2, 132.6, 132.7, 134.0, 134.1, 136.4 (20× Ar–C), 170.6, 170.7 (2× CO) ppm; HRMS (ESI+): *m*/*z* for $C_{31}H_{33}N_2O_8$ ($[M + H]^+$): calcd 561.2237; found 561.2245.

*4.1.9.5. 1,3-Bis(1H-pyrrole-2-carboxamido)propan-2-yl α-D-mannopyranoside (**9e**).* Prepared from **8e** (0.097 g, 0.16 mmol) according to the general procedure F. Yield: 0.065 g (92.0%); white crystalline solid; mp 135–137 °C; $[\alpha]_D$ +39.5 (*c* 0.07, MeOH); IR (KBr) ν 3400, 2930, 1629, 1560, 1523, 1408, 1328, 1205, 1128, 1044, 884, 745, 605 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.45–3.67 (m, 6H, 2× CH_2, H-4, H-6), 3.71–3.81 (m, 3H, H-3, H-5, H-6′), 3.85 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.97–4.02 (m, 1H, CH), 5.01 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.19 (ddd, 2H, J_1 = 2.0 Hz, J_2 = 2.5 Hz, J_3 = 3.8 Hz, Ar–H), 6.86 (ddd, 2H, J_1 = 1.4 Hz, J_2 = 3.8 Hz, J_3 = 4.6 Hz, Ar–H), 6.93–6.94 (dd, 2H, J_1 = 1.4 Hz, J_2 = 2.5 Hz, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 40.5, 42.0 (2× CH_2), 62.8 (C-6), 68.56 (C-4), 72.3 (C-3), 72.4 (C-2), 75.2 (C-5), 77.2 (CH), 101.2 (C-1), 110.2, 110.3, 111.8, 112.2, 123.0, 123.1, 126.7, 126.8 (8× Ar–C), 164.0, 164.1 (2× CO) ppm; HRMS (ESI+): *m*/*z* for $C_{19}H_{27}N_4O_8$ ($[M + H]^+$): calcd 439.1829; found 439.1822.

*4.1.9.6. 1,3-Bis(2-phenylacetamido)propan-2-yl α-D-mannopyranoside (**9f**).* Prepared from **8f** (0.310 g, 0.47 mmol) according to the general procedure F. Yield: 0.231 g (100.0%); colorless oil; $[\alpha]_D$ +27.3 (*c* 0.12, MeOH); IR (KBr) ν cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.23 (dd, 1H, J_1 = 5.6 Hz, J_2 = 14.3 Hz, CH_2–H_A), 3.34–3.39 (m, 3H, CH_2, CH_2–H_B), 3.55 (s, 2H, $COCH_2$), 3.56 (s, 2H, $COCH_2$), 3.59–3.72 (m, 4H, H-3, H-5, H-6, H-6′), 3.77 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.78–3.82 (m, 1H, CH), 3.88 (d, 1H, *J* = 10.0 Hz, H-4), 4.89 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 7.24–7.35 (m, 10H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 40.9, 42.3 (2× CH_2), 43.8, 43.9 (2× CH_2CO), 63.0 (C-6), 68.8 (C-4), 72.2 (C-3), 72.4 (C-2), 75.3 (C-5), 76.7 (CH), 101.0 (C-1), 127.9, 128.0, 129.6, 129.7, 130.1, 130.2, 136.8, 136.9 (12× Ar–C), 174.5, 174.6 (2× CO) ppm; HRMS (ESI+): *m*/*z* for $C_{25}H_{33}N_2O_8$ ($[M + H]^+$): calcd 489.2237; found 489.2227.

*4.1.9.7. 1,3-Bis(2-(4-nitrophenyl)acetamido)propan-2-yl α-D-mannopyranoside (**9g**).* Prepared from **8g** (0.143 g, 0.19 mmol) according to the general procedure F. Yield: 0.111 g (100%); brown oil; $[\alpha]_D$ +25.3 (*c* 0.08, MeOH); IR (KBr) ν cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.27 (dd, 1H, J_1 = 5.6 Hz, J_2 = 14.2 Hz, CH_2–H_A), 3.35–3.42 (m, 3H, CH_2, CH_2–H_B), 3.56–3.68 (m, 4H, H-3, H-5, H-6, H-6′), 3.69 (s, 2H, $COCH_2$), 3.71 (s, 2H, $COCH_2$), 3.76 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.81–3.84 (m, 1H, CH), 3.89 (d, 1H, *J* = 9.3 Hz, H-4), 4.88 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 7.54–7.58 (m, 4H, Ar–H), 8.19–8.23 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 41.2, 42.5 (2× CH_2), 43.2, 43.4 (2× $COCH_2$), 63.1 (C-6), 68.8 (C-4), 72.2 (C-3), 72.4 (C-2), 75.4 (C-5), 76.9 (CH), 101.0 (C-1), 124.6, 124.7, 131.4, 131.5, 144.6, 144.7, 148.4, 148.5 (12× Ar–C), 172.9, 173.0 (2× CO) ppm; HRMS (ESI+): *m*/*z* for $C_{25}H_{31}N_4O_{12}$ ($[M + H]^+$): calcd 579.1938; found 579.1939.

*4.1.9.8. 1,3-Diphenoxypropan-2-yl α-D-mannopyranoside (**14a**).* Prepared from **13a** (0.141 g, 0.23 mmol) according to the general procedure F. Yield: 0.134 g (100%); white crystalline solid; mp 128 °C; $[\alpha]_D$ +32.8 (*c* 0.23, MeOH); IR (KBr) ν 3421, 2928, 1599, 1457, 1241, 1173, 1134, 1068, 977, 865, 753, 690 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.66–3.78 (m, 4H, H-3, H-4, H-6, H-6′), 3.82 (ddd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{5,6'}$ = 4.8 Hz, $J_{4,5}$ = 10.6 Hz, H-5), 3.86 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 4.19–4.32 (m, 4H, CH_2CHCH_2), 4.44–4.49 (m, 1H, CH_2CHCH_2), 5.16 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.92–7.00 (m, 6H, Ar–H), 7.27–7.31 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 62.7 (C-6), 68.4 (C-4), 68.8, 68.9 (2× CH_2), 72.3 (C-3), 72.4 (C-2), 74.9 (C-5), 75.6 (CH), 101.7 (C-1), 115.6, 115.7, 122.0, 122.2, 130.5, 130.6, 160.1, 160.2 (12× Ar–C) ppm; HRMS (ESI–): *m*/*z* for $C_{21}H_{25}O_8$ ($[M - H]^-$): calcd 405.1549; found 405.1542.

*4.1.9.9. 1,3-Bis(4-methoxyphenoxy)propan-2-yl α-D-mannopyranoside (**14b**).* Prepared from **13b** (0.190 g, 0.30 mmol) according to the general procedure F. Yield: 0.140 g (100%); orange solid; mp 69–71 °C; $[\alpha]_D$ +35.0 (*c* 0.20, MeOH); IR (KBr) ν 3378, 2925, 2832, 1507, 1458, 1289, 1232, 1133, 1107, 1064, 1044, 978, 824, 754, 670, 520 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.65–3.74 (m, 3H, H-3, H-4, H-6), 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.77–3.84 (m, 2H, H-5, H-6′), 3.86 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 4.11–4.25 (m, 4H, CH_2CHCH_2), 4.37–4.42 (m, 1H, CH_2CHCH_2), 5.14 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.84–6.87 (m, 4H, Ar–H), 6.89–6.93 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 56.1 (2× OCH_3), 62.8 (C-6), 68.4 (C-4), 69.5, 69.7 (2× CH_2), 72.3 (C-3), 72.5 (C-2), 74.8 (C-5), 75.7 (CH), 101.7 (C-1), 115.6, 115.8, 116.6, 116.7, 154.2, 154.4, 155.6, 155.7 (12× Ar–C) ppm; HRMS (ESI–): *m*/*z* for $C_{23}H_{29}O_{10}$ ($[M - H]^-$): calcd 465.1761; found 465.1757.

*4.1.9.10. 1,3-Bis(4-nitrophenoxy)propan-2-yl α-D-mannopyranoside (**14c**).* Prepared from **13c** (0.107 g, 0.16 mmol) according to the general procedure F. Yield: 0.080 g (100%); yellow solid; mp 98–99 °C; $[\alpha]_D$ +18.7 (*c* 0.25, MeOH); IR (KBr) ν 3420, 2928, 1592, 1507, 1340, 1299, 1256, 1175, 1111, 1028, 863, 844, 751, 689 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.64–3.74 (m, 3H, H-3, H-4, H-6), 3.78 (dd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{4,5}$ = 8.8 Hz, H-5), 3.81–3.85 (m, 2H, H-2, H-6′), 4.38–4.48 (m, 4H, CH_2CHCH_2), 4.52–4.56 (m, 1H, CH_2CHCH_2), 5.15 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.17–7.19 (m, 4H, Ar–H), 8.23–8.26 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 63.0 (C-6), 68.5 (C-4), 69.3, 69.4 (2× CH_2), 72.2 (C-2), 72.4 (C-3), 75.3 (C-5, CH), 101.9 (C-1), 115.9, 116.0, 126.8, 126.9, 143.1, 143.2, 165.1, 165.2 (12× Ar–C) ppm; HRMS (ESI–): *m*/*z* for $C_{21}H_{23}N_2O_{12}$ ($[M - H]^-$): calcd 495.1251; found 495.1264.

*4.1.9.11. 1,3-Bis(naphthalen-1-yloxy)propan-2-yl α-D-mannopyranoside (**14d**).* Prepared from **13d** (0.327 g, 0.45 mmol) according to the general procedure F. Yield: 0.251 g (100%); white crystalline solid; mp 192–194 °C; $[\alpha]_D$ +8.5 (*c* 0.23, MeOH); IR (KBr) ν 3421, 2927, 1580, 1457, 1267, 1238, 1102, 1069, 865, 792, 771, 572 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), δ 3.65–3.72 (m, 2H, H-4, H-6), 3.75 (dd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{6,6'}$ = 11.3 Hz, H-6′), 3.81 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.88 (ddd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{5,6'}$ = 5.4 Hz, $J_{4,5}$ = 8.2 Hz, H-5), 3.94 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.53–4.65 (m, 4H, CH_2CHCH_2), 4.79–4.82 (m, 1H, CH_2CHCH_2), 5.34 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.01–7.04 (m, 2H, Ar–H), 7.38–7.42 (m, 2H, Ar–H), 7.45–7.52 (m, 6H, Ar–H), 7.80 (m, 2H, Ar–H), 8.26–8.30 (m, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), δ 62.7 (C-6), 68.3

(C-4), 69.3, 69.4 (2× CH_2), 72.4 (C-2), 72.5 (C-3), 75.1 (C-5), 75.9 (CH), 101.9 (C-1), 106.2, 106.3, 121.2, 121.7, 126.3, 126.4, 126.9, 127.0, 127.1, 127.4, 128.5, 128.6, 136.0, 136.1, 155.6, 155.7 (20× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{29}H_{29}O_8$ ([M − H]$^-$): calcd 505.1862; found 505.1869.

*4.1.9.12. 1,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-2-yl α-D-mannopyranoside (**14e**).* Prepared from **13e** (0.359 g, 0.46 mmol) according to the general procedure F. Yield: 0.281 g (100%); white crystalline solid; mp 178–179 °C; $[\alpha]_D$ +19.0 (*c* 0.25, MeOH); IR (KBr) *ν* 3398, 2933, 1630, 1516, 1465, 1387, 1256, 1210, 1185, 1160, 1137, 1023, 975, 839, 806 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.70 (t, 1H, $J_{3,4;\ 4,5}$ = 9.6 Hz, H-4), 3.73–3.85 (m, 3H, H-3, H-6, H-6′), 3.88–3.91 (m, 7H, H-5, 2× OCH_3), 3.92 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.36–4.49 (m, 4H, CH_2CHCH_2), 4.58–4.64 (m, 1H, CH_2CHCH_2), 5.24 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.97 (ddd, 2H, J_1 = 0.95 Hz, J_2 = 2.5 Hz, J_3 = 8.8 Hz, Ar–H), 7.05 (ddd, 2H, J_1 = 1.7 Hz, J_2 = 2.5 Hz, J_3 = 8.8 Hz, Ar–H), 7.15 (dd, 2H, J_1 = 2.3 Hz, J_2 = 8.8 Hz, Ar–H), 7.25 (d, 2H, *J* = 2.6 Hz, Ar–H), 7.65–7.70 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), *δ* 55.9 (2× OCH_3), 62.8 (C-6), 68.5 (C-4), 68.9, 69.0 (2× CH_2), 72.4 (C-2), 72.6 (C-3), 75.2 (C-5), 75.3 (CH), 101.8 (C-1), 106.5, 106.6, 107.7, 107.8, 117.2, 117.3, 117.4, 117.5, 125.9, 126.0, 130.2, 130.4, 130.5, 137.6, 158.6, 158.7, 159.8, 159.9 (20× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{31}H_{33}O_{10}$ ([M − H]$^-$): calcd 565.2074; found 565.2073.

*4.1.9.13. 1-Ethoxy-3-(naphthalen-2-yloxy)propan-2-yl α-D-mannopyranoside (**14f**).* Prepared from **13f** (0.200 g, 0.35 mmol) according to the general procedure F. Compound was obtained as a mixture of two diastereomers in 1:1 ratio. Yield: 0.142 g (100%); yellow solid; mp 188–190 °C; $[\alpha]_D$ +33.9 (*c* 0.27, MeOH); IR (KBr) *ν* 3404, 2929, 1602, 1458, 1258, 1218, 1183, 1119, 1046, 978, 837, 747 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 121–1.25 (2× t, 6H, 2× CH_2CH_3), 3.56–3.64 (m, 4H, 2× CH_2CH_3), 3.67–3.81 (m, 12H, 2× CH_2CHCH_2), 3.85–3.89 (m, 4H), 4.18–4.34 (m, 6H, 2× CH_2CHCH_2), 5.11 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.16 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1′), 7.15–7.19 (m, 2H, Ar–H), 7.26–7.28 (m, 2H, Ar–H), 7.31–7.36 (m, 2H, Ar–H), 7.41–7.46 (m, 2H, Ar–H), 7.75–7.79 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), *δ* 15.5 (2× CH_2CH_3), 62.6, 62.7 (2× C-6), 68.0, 68.1 (2× CH_2CH_3), 68.4, 68.5 (2× C-4), 69.1, 69.4, 71.2, 71.5 (4× CH_2), 72.3, 72.4 (2× C-2), 72.5, 72.6 (2× C-3), 74.7, 74.9 (2× C-5), 75.8, 75.9 (2× CH), 101.4, 101.7 (2× C-1), 107.7, 107.9, 119.7, 119.8, 124.7, 124.8, 127.3, 127.4, 127.9, 128.6, 130.4, 130.5, 130.6, 130.7, 136.1, 158.0, 158.1 (20× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{21}H_{27}O_8$ ([M − H]$^-$): calcd 407.1706; found 407.1702.

*4.1.9.14. 2,3-Diphenoxypropan-1-yl α-D-mannopyranoside (**18a**).* Prepared from **17a** (0.154 g, 1.36 mmol) according to the general procedure F. Yield: 0.109 g (100.0%); orange oil; $[\alpha]_D$ +45.2 (*c* 0.20, MeOH); IR (KBr) *ν* 3421, 2929, 1599, 1496, 1457, 1301, 1243, 1174, 1134, 1058, 976, 882, 816, 753, 690, 508 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.67–3.78 (m, 4H, H-3, H-4, H-6, H-6′), 3.83 (ddd, 1H, $J_{5,6}$ = 2.5 Hz, $J_{5,6'}$ = 5.1 Hz, $J_{4,5}$ = 8.2 Hz, H-5), 3.87 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 4.18–4.30 (m, 4H, CH_2CHCH_2), 4.43–4.48 (m, 1H, CH_2CHCH_2), 5.16 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.93–6.99 (m, 6H, Ar–H), 7.26–7.31 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), *δ* 62.8 (C-6), 68.4 (C-4), 68.7, 68.9 (2× CH_2), 72.3 (C-2), 72.5 (C-3), 74.9 (C-5), 75.6 (CH), 101.7 (C-1), 115.6, 115.7, 122.0, 122.1, 130.5, 130.6, 160.1, 160.2 (12× Ar–C) ppm; HRMS (ESI+): *m/z* for $C_{21}H_{26}O_8Na$ ([M + Na$^+$]$^+$): calcd 429.1525; found 429.1528.

*4.1.9.15. 2,3-Bis(4-methoxyphenoxy)propan-1-yl α-D-mannopyranoside (**18b**).* Prepared from **17b** (0.310 g, 0.49 mmol) according to the general procedure F. Yield: 0.228 g (100%); white solid; mp 90–91 °C; $[\alpha]_D$ +39.3 (*c* 0.22, MeOH); IR (KBr) *ν* 3366, 2917, 2065, 1594, 1508, 1458, 1289, 1231, 1181, 1133, 1107, 1064, 1044, 978, 932, 883, 824, 753, 676, 520 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.68 (t, 1H, $J_{3,4;\ 4,5}$ = 9.5 Hz, H-4), 3.71–3.76 (m, 8H, H-3, H-6, 2× OCH_3), 3.78–3.84 (m, 2H, H-5, H-6′), 3.86 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 4.11–4.25 (m, 4H, CH_2CHCH_2), 4.37–4.42 (m, 1H, CH_2CHCH_2), 5.14 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.84–6.88 (m, 4H, Ar–H), 6.89–6.93 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), *δ* 56.1 (2× OCH_3), 62.8 (C-6), 68.4 (C-4), 69.5, 69.7 (2× CH_2), 72.3 (C-2), 72.5 (C-3), 74.9 (C-5), 75.7 (CH), 101.7 (C-1), 115.7, 116.6, 116.7, 154.2, 154.3, 155.6, 155.7 (12× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{23}H_{29}O_{10}$ ([M − H]$^-$): calcd 465.1761; found 465.1751.

*4.1.9.16. 2,3-Bis(4-nitrophenoxy)propan-1-yl α-D-mannopyranoside (**18c**).* Prepared from **17c** (0.324 g, 0.49 mmol) according to the general procedure F. Yield: 0.242 g (100%); brown solid; mp 176–179 °C; $[\alpha]_D$ +22.3 (*c* 0.22, MeOH); IR (KBr) *ν* 3421, 1593, 1507, 1340, 1254, 1174, 1111, 1024, 863, 844, 751, 690 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.64 (t, 1H, $J_{3,4;\ 4,5}$ = 9.3 Hz, H-4), 3.66–3.74 (m, 2H, H-3, H-6), 3.77–3.85 (m, 3H, H-2, H-5, H-6′), 4.38–4.48 (m, 4H, CH_2CHCH_2), 4.53–4.58 (m, 1H, CH_2CHCH_2), 5.15 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.16–7.20 (m, 4H, Ar–H), 8.23–8.26 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, CD_3OD), *δ* 62.9 (C-6), 68.5 (C-4), 69.3, 69.4 (2× CH_2), 72.2 (C-2), 72.4 (C-3), 75.3 (C-5, CH), 101.9 (C-1), 116.0, 126.9, 143.1, 143.2, 165.1, 165.2 (12× Ar–C) ppm; HRMS (ESI+): *m/z* for $C_{21}H_{24}N_2O_{12}Na$ ([M + Na$^+$]$^+$): calcd 519.1227; found 519.1218.

*4.1.9.17. 2,3-Bis(naphthalen-1-yloxy)propan-1-yl α-D-mannopyranoside (**18d**).* Prepared from **17d** (0.183 g, 0.27 mmol) according to the general procedure F. Yield: 0.137 g (100%); light brown solid; mp 151–153 °C; $[\alpha]_D$ +23.4 (*c* 0.24, MeOH); IR (KBr) *ν* 3380, 2929, 1670, 1595, 1579, 1507, 1460, 1395, 1340, 1267, 1238, 1178, 1136, 1102, 1069, 977, 833, 793, 769, 680 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.65–3.72 (m, 2H, H-4, H-6), 3.76 (dd, 1H, $J_{5,6'}$ = 2.3 Hz, $J_{6,6'}$ = 11.8 Hz, H-6′), 3.81 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.88 (ddd, 1H, $J_{5,6}$ = 2.3 Hz, $J_{5,6'}$ = 5.5 Hz, $J_{4,5}$ = 9.9 Hz, H-5), 3.94 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.53–4.64 (m, 4H, CH_2CHCH_2), 4.78–4.83 (m, 1H, CH_2CHCH_2), 5.34 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.02 (ddd, 2H, J_1 = 0.9 Hz, J_2 = 2.8 Hz, J_3 = 7.6 Hz, Ar–H), 7.38–7.51 (m, 8H, Ar–H), 7.80–7.83 (m, 2H, Ar–H), 8.26–8.30 (m, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 62.7 (C-6), 68.3 (C-4), 69.3, 69.4 (2× CH_2), 72.4 (C-3), 72.5 (C-2), 75.2 (C-5), 75.8 (CH), 101.9 (C-1), 106.2, 106.3, 121.6, 121.7, 122.8, 123.0, 126.3, 126.4, 126.9, 127.0, 127.1, 127.4, 128.5, 128.6, 136.0, 136.1, 155.6, 155.7 (20× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{29}H_{29}O_8$ ([M − H]$^-$): calcd 505.1862; found 505.1872.

*4.1.9.18. 2,3-Bis((7-methoxynaphthalen-2-yl)oxy)propan-1-yl α-D-mannopyranoside (**18e**).* Prepared from **17e** (0.200 g, 0.27 mmol) according to the general procedure F. Yield: 0.154 g (100%); white solid; mp 135 °C; $[\alpha]_D$ +31.1 (*c* 0.22, MeOH); IR (KBr) *ν* 3445, 2932, 1630, 1609, 1516, 1448, 1387, 1257, 1209, 1185, 1165, 1137, 1023, 974, 842, 831, 808, 631 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), *δ* 3.70 (t, 1H, $J_{3,4;\ 4,5}$ = 9.7 Hz, H-4), 3.73–3.84 (m, 3H, H-3, H-6, H-6′), 3.89–3.90 (m, 7H, H-5, 2× OCH_3), 3.92 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.36–4.49 (m, 4H, CH_2CHCH_2), 4.59–4.64 (m, 1H, CH_2CHCH_2), 5.24 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 6.97 (ddd, 2H, J_1 = 0.97 Hz, J_2 = 2.5 Hz, J_3 = 8.8 Hz, Ar–H), 7.05 (m, 2H, Ar–H), 7.15 (dd, 2H, J_1 = 2.4 Hz, J_2 = 8.9 Hz, Ar–H), 7.25 (d, 2H, *J* = 2.6 Hz, Ar–H), 7.65–7.70 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), *δ* 55.9 (2× OCH_3), 62.9 (C-6), 68.5 (C-4), 68.9, 69.0 (2× CH_2), 72.4 (C-3), 72.5 (C-2), 75.3 (C-5), 75.8 (CH), 101.8 (C-1), 106.5, 106.6, 107.7, 107.8, 117.2, 117.3, 117.4, 117.5, 125.9, 126.0, 130.0, 130.4, 130.5, 137.6, 158.6. 158.7, 159.8 (20× Ar–C) ppm; HRMS (ESI−): *m/z* for $C_{31}H_{33}O_{10}$ ([M − H]$^-$): calcd 565.2074; found 565.2073.

*4.1.9.19. 2,3-Bis(naphthalen-2-yloxy)propan-1-yl α-D-mannopyranoside (**18f**).* Prepared from **17f** (0.237 g, 0.35 mmol) according to the general procedure F. Yield: 0.178 g (100%); yellow solid; mp 188–

189 °C; $[\alpha]_D$ +31.9 (*c* 0.25, MeOH); IR (KBr) ν 3430, 1629, 1600, 1511, 1456, 1256, 1216, 1181, 1120, 1059, 975, 835, 745, 623 cm^{-1}; ^1H NMR (400 MHz, CD_3OD), δ 3.71 (t, 1H, $J_{3,4;\ 4,5} = 9.6$ Hz, H-4), 3.73–3.84 (m, 4H, H-3, H-5, H-6, H-6′), 3.92 (dd, 1H, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.3$ Hz, H-2), 4.38–4.51 (m, 4H, $C\underline{H}_2CHC\underline{H}_2$), 4.61–4.65 (m, 1H, $CH_2C\underline{H}CH_2$), 5.25 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 7.17–7.23 (m, 2H, Ar–H), 7.32–7.36 (m, 4H, Ar–H), 7.41–7.45 (m, 2H, Ar–H), 7.76–7.80 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 62.6 (C-6), 68.4 (C-4), 68.9, 69.0 (2× CH_2), 72.4 (C-3), 72.5 (C-2), 74.9 (C-5), 75.5 (CH), 101.8 (C-1), 107.9, 108.0, 119.7, 119.8, 124.7, 124.8, 127.4, 127.5, 128.0, 128.6, 130.4, 130.5, 130.6, 130.7, 136.1, 158.0, 158.1 (20× Ar–C) ppm; HRMS (ESI−): *m*/*z* for $C_{29}H_{30}O_8$ ($[M - H]^-$): calcd 505.1862; found 505.1854.

4.2. Molecular modeling

4.2.1. Computer hardware and software

All the computational work was performed on two workstations. Accelrys Discovery Studio 3.0 (DS) [42] was running on a workstation with Intel core i7 860 CPU processor, 8 GB RAM, two 750 GB hard drives and a Nvidia GT220 GPU graphic card, running Centos 5.5. LeadIT (version 2.1.2.) [39] and NAMD (version 2.9) [43] were running on four octal core AMD Opteron CPU processors, 16 GB RAM, two 750 GB hard drives, running 64-bit Scientific Linux 6.0.

4.2.2. Ligand and protein preparation

The tridimensional models of target compounds (Tables 1 and 2) were built from a standard fragment library in Accelrys Discovery Studio 3.0 (DS) [42]. The geometries of the molecules were optimized using CHARMM force field [44] with MMFF94 [45] partial atomic charges. The energy was minimized using the Smart Minimizer algorithm in DS until the gradient value was smaller than 0.001 kcal/(mol Å). The optimized structure was further refined with GAMESS interface in ChemBio3D Ultra 13.0 using semi-empirical PM3 method, QA optimization algorithm and Gasteiger Hückel charges for all atoms for 100 steps [46].

The crystal structure of DC-SIGN CRD in complex with tetramannoside Man_4 (PDB entry: 1SL4) was taken as a receptor for docking calculations using FlexX [37,38] as available in LeadIT [39]. Receptor was prepared in LeadIT graphical user interface using the Receptor wizard. Amino acid residues within a radius of 7 Å around Man_4 were defined as the binding site. Hydrogen atoms were added to the binding site residues and correct tautomers and protonation states were assigned. Water molecules and the ligand were deleted from the crystal structure, while Ca^{2+} ion in the Ca^{2+} binding site was defined as part of the receptor. Pharmacophore type constraints in FlexX-Pharm were defined in order to correctly place the core mannose residue during the docking validation procedure (see below). First, PharmMetal pharmacophore with spherical coordination around Ca^{2+} was defined to account correctly for complex interactions between Ca^{2+} (406) and Man1 residue of Man_4 (in the validation procedure) and mannose residue of the designed ligands (in the docking procedure). Secondly, side chain carboxylate groups of Glu347 and Glu354 were defined as hydrogen bond acceptors, which form hydrogen bonds with 3- and 4-hydroxyl groups of mannose residue in the crystal structure. All three selected pharmacophore type constraints were specified as essential. Additionally, we have defined Phe313 side chain as a phenyl ring center in FlexX-Pharm, but the docking poses were comparable to those without this pharmacophore constraint. Hence, this constraint was not used in docking validation and ligand docking.

4.2.3. Validation of the docking protocol and ligand docking

The FlexX molecular docking program, as available in LeadIT, was used for ligand docking. Hybrid algorithm (enthalpy and entropy driven ligand binding) was used to place the 'base fragment'. The Maximum number of solutions per iteration and the Maximum number of solutions per fragmentation parameter values were increased to 1000, while other parameters were set at their default values.

In order to validate our docking protocol, crystal structure ligand Man_4 was docked into the defined Ca^{2+} binding site of the receptor using the above described docking parameters and pharmacophore constraints. The protocol was able to reproduce the binding of the Man1 residue interacting with the Ca^{2+} and both Glu residues very well (RMSD value of 0.8 Å). The RMSD value for Man1 and Man2 residues of Man_4 was 1.6 Å, while RMSD value for the complete Man_4 was 3.9 Å. Such a result is expected, since Man2, Man3 and Man4 residues point towards the solvent and form only weak interactions with the protein. Nevertheless, we concluded that our docking protocol is suitable for binding mode studies of the designed DC-SIGN ligands, which were docked using the same settings as were used for docking protocol validation. Proposed binding modes and scoring function scores of the top five highest scored docking poses per ligand were evaluated and the highest ranked binding pose was used for graphical representation in DS.

4.2.4. Molecular dynamics

The molecular dynamics package NAMD (version 2.9) [43] and CHARMM22 force field [44] were used for molecular dynamics simulations of DC-SIGN–**14d** complex. The best scored docking pose of compound **14d** in complex with DC-SIGN CRD was used as the initial structure for MD simulation. Molecular mechanics parameters for compound **14d** were estimated using ParamChem tool [47–49]. Steepest descent (10 000 steps) and adopted basis Newton–Raphson (10 000 steps) energy minimizations were first performed to remove atomic clashes and to optimize the atomic coordinates of the protein–ligand complex. The structure of the energy minimized complex for MD simulation was prepared using *psfgen* in VMD (version 1.9.1.) [50]. The complex was then embedded in a box of water, which was modeled explicitly by a TIP3P model [51]. The system was neutralized by addition of KCl at 0.4 M concentration. The MD simulation was carried out in the NPT ensemble employing periodic boundary conditions. Langevin dynamics and Langevin piston methods were used for temperature (300 K) and pressure (1 atm) control, respectively. Short- and long-range forces were calculated every 1 and 2 timesteps, respectively, with a time step of 2.0 ps. The smooth particle mesh Ewald method [52] was used to calculate electrostatic interactions. The short-range interactions were cut off at 12 Å. All chemical bonds between hydrogen and heavy atoms were held fixed using SHAKE algorithm [53]. The simulation consisted of three consecutive steps: (*i*) solvent equilibration for 0.5 ns with ligand and protein constrained harmonically around the initial structure; (*ii*) equilibration of the complete system for 1 ns with ligand and protein released; (*iii*) an unconstrained 7 ns production run to allow the protein and the ligand to position themselves according to physical forces between them. The trajectory of the equilibration and production run was used for analysis in VMD. The average structure of the last ns of the production run was calculated and used for analysis of binding mode of **14d**.

4.3. Biological assays

4.3.1. Production of soluble DC-SIGN ECD

DC-SIGN ECD protein (residues 66-404) were overexpressed and purified according to the already published procedure [15,22,54].

4.3.2. Competitive gp120-displacement assay

The binding affinities of the synthesized compounds to DC-SIGN ECD were characterized by a solid-phase competitive displacement

assay. gp120 (Human Immunodeficiency Virus type 1/HIV-1 gp120 Protein, 100 mcg, 11233-V08H, Sino Biological Inc., China) was dissolved in aqueous solution of NaCl (0.05 M, 0.300 mL) at 30 °C, and then further diluted with 0.600 mL of water, and 0.100 mL of 1 M $NaHCO_{3(aq)}$. Biotin *N*-hydroxysuccinimide ester (1 mg) was dissolved in *N*,*N*-dimethylformamide (1 mL) and 0.100 mL of the solution was added to the solution of gp120. The reaction mixture was incubated for 90 min at 30 °C and dialyzed with a cut-off value of 12.000–14.000 for 3 h at RT against buffer 1 (3 L, 20 mM Tris, 150 mM NaCl, pH = 7.4). After dialysis, the solution was centrifuged for 5 min at 5400 rpm and diluted with buffer 2 (20 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, pH = 7.4) to a volume of 5 mL to yield the final stock solution of biotin-labeled gp120. 4 μL of soluble DC-SIGN ECD (stock solution 100 mcg/0.100 mL in 25 mM Tris pH 8, 150 mM NaCl, 4 mM $CaCl_2$) was diluted in 10.2 mL of buffer 2 and adsorbed to 96-well (100 μL/well) high-binding microtiter plates (Greiner, Lumitrac 600, white) overnight at 4 °C. The remaining DC-SIGN ECD solution was thrown away and non-specific receptor-binding sites were blocked with 1% BSA in buffer 2 (200 μL/well). Following incubation for 1 h at RT, the plates were washed twice with buffer 3 (buffer 2 containing 0.1% of Tween 20). The potential DC-SIGN antagonists were serially diluted with buffer 3 and solutions added (50 μL/well) at the same time as biotinylated gp120 (50 μL/well) to each well. The plates were incubated for 2 h at RT and then washed twice with buffer 3. In each well, peroxidase-conjugated antibiotin goat antibody (100 μL/well, 1:1000 dilution of purchased solution in buffer 3 + 0.1% of BSA, Anti-Biotin Goat pAb Peroxidase Conjugate, 0.5 mg/mL, Calbiochem, Merck Millipore, Germany) was added and incubated for another hour. The microtiter plates were washed with buffer 3 three times. Finally, chemiluminescence substrate (50 μL/well, BM chemiluminiscence ELISA substrate (POD), Cat. No. 1582950, Roche Diagnostics GmbH, Germany) was added and the luminescence detected with a Synergy H4 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Inc.). Positive controls received no antagonists while negative controls received no ligands. L-Fucose was used as the internal standard. The assays were performed at least in triplicate. The mean experimental data were fitted to the sigmoid model and IC_{50} values were calculated from the dose–response curve (OriginPro, OriginLab®, Versions 7.5 and 8.0).

4.3.3. *Measurement of dendritic cell adhesion with CFSE fluorescence assay*

The dendritic cell adhesion assay was performed as described in our previous publications [18,30].

4.3.4. *Cytotoxicity of mannose-based DC-SIGN antagonists*

To evaluate the potential cytotoxicity of the synthesized DC-SIGN antagonists on target cells we performed a propidium iodide (PI) up-take assay of DC cultures treated with various compounds for 24 h.

Dendritic cells were differentiated as described previously [18,30,55]. After 5 days, immature DCs were seeded at 0.5×10^6 cells/ml and treated with various concentrations (50, 100 and 250 μM) of compounds. After 24 h, the cells were harvested and incubated with PI for 15 min. Afterward, the cells were analyzed on a flow cytometer to determine the percentage of PI positive cells, which equals the percentage of dead cells in the culture.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.047.

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