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Facile access to pseudo-thio-1,2-dimannoside, a new glycomimetic DC-SIGN antagonist

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ABSTRACT

The synthesis and conformational analysis of pseudo-thio-1,2-dimannoside are described. This molecule mimics mannobioside (Manα(1,2)Man) and is an analog of pseudo-1,2-dimannoside, with expected increased stability to enzymatic hydrolysis. A short and efficient synthesis was developed based on an epoxide ring-opening reaction by a mannosyl thiolate, generated *in situ* from the corresponding thioacetate. NMR-NOESY studies supported by MM3* calculations showed that the pseudo-thio-1,2-dimannoside shares the conformational behavior of the pseudo-1,2-dimannoside and is a structural mimic of the natural disaccharide. Its affinity for DC-SIGN was measured by SPR and found to be comparable to the corresponding *O*-linked analog, offering good opportunities for further developments.

1. Introduction

Over the past decades, an increased understanding of glycans ability to encode biochemical information relevant to both physiological and pathological conditions has stimulated a fast growth of glycochemical research.[1–4] One of the important objectives in this field is the development of glycomimetic structures, targeted against sugar binding proteins (called lectins) and able to alter the read-out of sugar-encoded information. In this context, glycomimetics have been proposed both as probes of biological processes and for medicinal applications.[5–9]

Many modifications have been introduced in the structure of carbohydrates to generate glycomimetics with improved drug-like characteristics and stability to enzymatic degradation.[10] In particular, thioglycosides have raised a great deal of interest, because the *S*-glycosidic linkage is typically more resistant toward both acid-catalyzed and enzymatic hydrolysis than *O*-glycosides,[11,12] and yet oxygen and sulfur share similar bonding and geometries, allowing structural similarity. From a structural point of view, the C—S bond is longer than the corresponding C—O bond by about 0.4 Å and the *exo*-anomeric effect in thiodisaccharides is less pronounced than in regular sugars.[13] As a result, an increased conformational flexibility of the glycosidic torsions is often observed.[14]

We previously reported that the pseudo-1,2-dimannoside **1** is a structural mimic of 1,2-mannobioside (Manα(1,2)Man) and a slow-reacting substrate of jack-bean mannosidase.[15] Like the natural disaccharide, this molecule is an antagonist[16] of the dendritic cell receptor DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3 (ICAM-3)-Grabbing Non-integrin), a tetrameric C-type lectin involved in the recognition of viruses and pathogens at the mucosal level.[17] Based on this framework, we have developed a family of mannose-based DC-SIGN monovalent and polyvalent ligands that were shown to work as potent inhibitors of DC-SIGN mediated viral infections.[18–21] In an effort to improve the hydrolytic stability of **1** and to possibly simplify its synthesis, we have examined the corresponding pseudo-thio-1,2-dimannoside **2** (Fig. 1).

We herein report the synthesis of **2**, an analysis of its conformation based on NMR-NOESY studies (in D_2O) supported

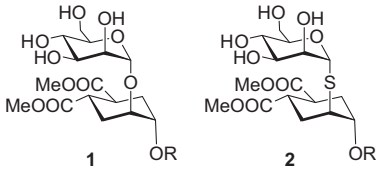

Fig. 1. Pseudo-1,2-dimannoside **1** and its thio analog **2**.

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by computational modeling, and the evaluation of its interaction with DC-SIGN by Surface Plasmon Resonance (SPR).

2. Results

2.1. Synthesis of the pseudo-thio-1,2-dimannoside

The synthesis of *S*-linked glycosides can be readily achieved by reaction of thiolates with glycosyl donors.[22] For the case at hand, however, a more expeditious approach was employed, exploiting nucleophilic opening of epoxide **3**[16] (Scheme 1) by a glycosyl thiol (1-thio sugar). Epoxide **3** is indeed a known intermediate in the synthesis of **1**, while the required glycosyl thiol can be generated *in situ* from 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-α-D-mannopyranose **4** by selective *S*-deacetylation (Scheme 1).[23,24] This allows, in principle, a one-pot synthesis of the pseudo-thio-1,2-dimannoside framework **5**.

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-α-D-mannopyranose **4** is a known compound,[23] which is most conveniently prepared from penta-*O*-acetylmannopyranose **6** by acid catalyzed reaction with thioacetic acid **7** (Scheme 2). The reaction affords **4** as a 9:1 α:β mixture (61% yield), from which pure **α-4** can be chromatographically isolated.

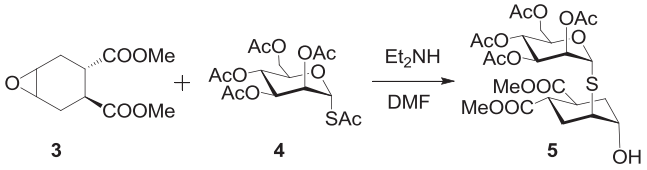
Selective thio-deacetylation of purified **α-4** with excess Et_2NH affords the anomeric thiol **8**, in equilibrium with the more reactive thiolate **9** (Scheme 3). Alkylation of **9** with primary iodides has recently been reported for the synthesis of *S*-linked 1,6-oligomannosides.[24] Alkylation with **3** (DMF, room temperature) gave almost full conversion of the epoxide in 4 h (as evaluated by TLC and ^{1}H NMR monitoring), affording product **5** in 68% yield after chromatography. Addition of MeOH (5 mol equiv) as a supplementary proton source did not modify the outcome of the reaction.

Compound **5** was obtained as a single isomer from a completely selective opening of **3**, affording *trans*-diaxial cyclohexane disubstitution[#] (Scheme 1). Analysis of the coupling constants in the ^{1}H NMR spectrum (CD_3OD, Fig. 2) clearly shows that the conformation of the cyclohexane ring depicted in Scheme 1 and Fig. 2, carrying both carbomethoxy groups in the equatorial position is populated by ≥90%. This feature is characteristic of vicinal *trans*-cyclohexanedicarboxylic acid derivatives,[25] including the pseudo-1,2-dimannoside **1**,[15] and it allows the distal substituents in **2** to occupy the axial position, thus mimicking the 3D features of an α-mannopyranose residue.

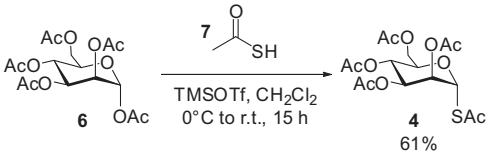
The tetra-*O*-acetyl-pseudo-thio-1,2-dimannoside **5** was deprotected (MeONa in MeOH), giving **2** (R = H) in quantitative yield (Scheme 4). To allow the synthesis of multivalent derivatives of **2**, we also pursued selective functionalization of the free alcohol group in the 1′ position of **5**. As a model reaction, we initially showed that **5** could be transformed in the pivaloate **10** (pivaloyl chloride, CH_2Cl_2, pyridine) and selectively deacetylated under Zemplén conditions (0.02 M MeONa in MeOH, room temperature, 5 min) to afford **11** (Scheme 4) in quantitative yield.

This set the stage for the synthesis of **14** (Scheme 5), a pseudo-thio-1,2-dimannoside functionalized with an azide tether, which allows for further manipulation and transformation into multivalent constructs. Compound **14** was obtained by reaction of **5** with 2,2-dimethyl-3-azido-propanoyl chloride **12**, synthesized according to a method described by Sewald et al.[26] (with modifications), to afford the ester **13** in 79% yield. Carefully controlled Zemplén deacetylation of **13**, as shown above, afforded **14** in quantitative yield.

[#] A second stereoisomer (≤3%) was observed in the ^{1}H NMR spectrum ($CDCl_3$) of the crude reaction product, deriving from opening of the minor enantiomer of **3** that has 94% e.e. This stereoisomer could be chromatographically removed from **5** (4:6 Hex:EtOAc).



Scheme 1. One-pot synthesis of **5**.



Scheme 2. Synthesis of **4** from penta-*O*-acetylmannopyranose **6**.

2.2. Conformational analysis of the pseudo-thio-1,2-dimannoside

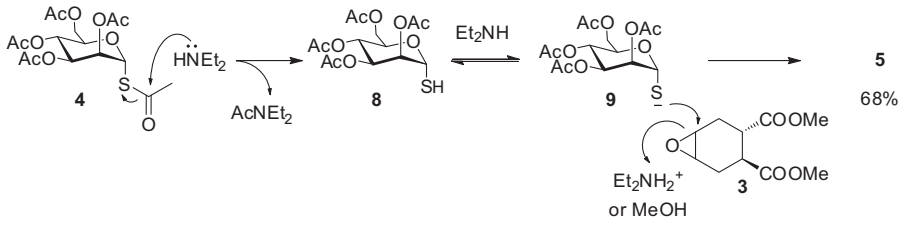
The conformation of **2** (R = H) was investigated by NOESY experiments; the data collected were correlated to computational models and compared to similar information on the pseudo-1,2-dimannoside **1**.

A previous NMR study on **1**[15] had revealed that this mimic has a conformational distribution similar to the natural disaccharide Manα(1,2)Man. Two conformations, dubbed *E* (for Extended) and *S* (for Stacked), are in fast equilibrium around the (pseudo)glycosidic bonds and are characterized by two mutually exclusive NOE contacts: the H1-H1′ contact for the *S* conformer, and the H1-H3′$_{eq}$ contact for the *E* conformer (Fig. 3a, the unconventional numbering of the cyclohexane ring was adopted for easier comparison with natural sugars). A qualitative analysis of the NOE data allowed deducing that the *E* conformer of **1** is the preferred conformation in solution (D_2O). Interestingly, the *E* conformation was also the one observed in the X-ray of the DC-SIGN:**1** complex.[27]

A similar analysis performed on **2** (R = H, D_2O, 500 MHz) showed an intense H1-H3′$_{eq}$ cross-peak (Fig. 4), while the H1-H1′ contact was revealed as a weak cross-peak (Fig. 4, inset) using 800 ms mixing time. A conformational search of **2** performed with the MM3* force field supported the existence of both *S* and *E* conformers of similar strain energy (Fig. 3b. Both R = Me and R = H were calculated, obtaining the same trends. Data are shown for R = Me). However, due to the increased length of the C—S bond relative to the C—O bond, the calculated H1-H1′ distance in the *S* conformation of **2** (***S*** in Fig. 3b) is 3.1 Å, as opposed to 2.6 Å in **1** (Fig. 3a), in qualitative agreement with a reduced intensity of the H1-H1′ cross-peak. Overall, the data are consistent with both the pseudo-1,2-dimannoside **1** and the pseudo-thio-1,2-dimannoside **2** populating the same conformational space and mimicking the conformational features of the native Manα(1,2)Man sugar.

2.3. Surface plasmon resonance inhibition studies with DC-SIGN

In order to investigate the biological activity of the pseudo-thio-1,2-dimannoside and compare it to the *O*-analog, compounds **2** (R = H) and **11** were tested by a well-established Surface Plasmon Resonance (SPR) inhibition assays.[27] In this test, increasing concentrations of the glycomimetics are used to inhibit the binding of DC-SIGN ECD (extra cellular domain) to mannosylated BSA immobilized on an SPR sensor chip. The calculated IC_{50} values for **1** (R = $CH_2CH_2N_3$), **2** and **11** in the same campaign (720 μM, 776 μM and 776 μM, respectively) are remarkably similar to one another, showing that the replacement of the glycosidic oxygen atom with a sulfur atom is not detrimental to the binding affinity



Scheme 3. Proposed mechanism for the one-pot epoxide opening reaction.

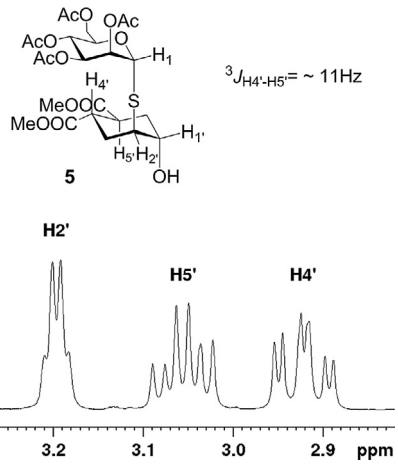


Fig. 2. Characterization of **5**. Coupling constant analysis (CD_3OD) shows that the conformation of the cyclohexane chair depicted here corresponds to ≥90% of the conformer population. The unconventional numbering of the cyclohexane ring was adopted for easier comparison with the natural Manα(1,2)Man disaccharide.

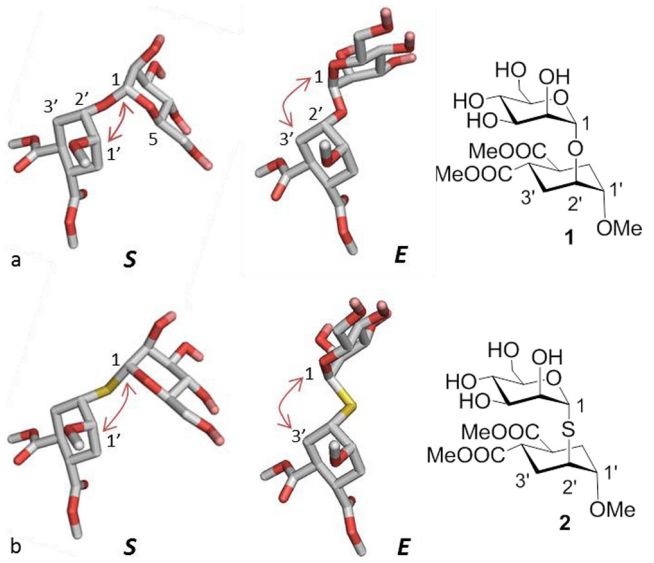
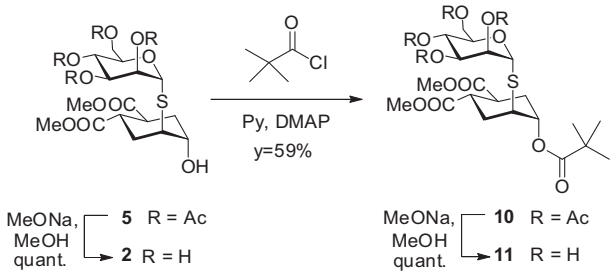
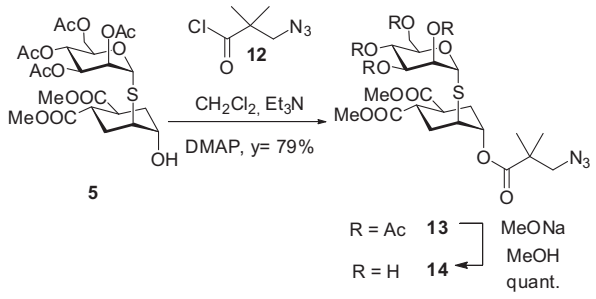


Fig. 3. (a) Stacked (*S*) and Extended (*E*) conformations of (a) the pseudo-1,2-dimannoside **1**. (b) the pseudo-thio-1,2-dimannoside **2**. The mutually exclusive NOE contacts defining the conformation are indicated by arrows.



Scheme 4. Deprotection and selective functionalization of **5**.



Scheme 5. Synthesis of the azido-tethered pseudo-thio-1,2-dimannoside **14**.

(see Figs. 5 and S1–S3). Previous results on pseudo-1,2-dimannosides variously substituted at position 1′ had shown a minor influence on DC-SIGN affinity, as expected on the basis of the X-ray structure of the complex, which shows the 1′ ether chain pointing away from the protein binding site. This was confirmed for the thio-analog by comparison of the IC_{50} measured for **2** and **11**. The low, submillimolar activity of these monovalent ligands is characteristic of oligosaccharide-protein interactions and is overcome, both in Nature and in the development of active antagonists, by exploiting multivalent interactions.[8]

3. Discussion and conclusions

Antagonists of the C-type lectin DC-SIGN are promising therapeutic agents against viral infections.[9] Mimics of the 1,2-mannobioside disaccharide unit, Manα(1,2)Man, presented as appropriate multivalent constructs have shown an ability to interact with the protein and to antagonize DC-SIGN mediated HIV *trans* infection of $CD4^+$ T lymphocytes. Mimicry of the Manα(1,2)Man unit has been obtained with pseudo-1,2-dimannoside **1**, where the reducing end mannose is replaced by a conformationally locked cyclohexanediol.[9] More recently, we and others have shown that similar results can be obtained using a real D-carbamannose unit.[28] Mimic **1**, as well as the carbamannose analog, are slower substrates than the natural disaccharide for hydrolytic enzymes and thus have a better outlook as drug candidates. The group of Borbás has recently reported the synthesis of a thio-linked Manα(1,2)Man disaccharide by photoinduced hydrothiolation of a 2,3-unsaturated glucoside[29] and its use for the synthesis of self-assembling glycoconjugates.[30]

We have presented here the pseudo-thiodisaccharide analog of **1**, **2**, which is expected to be further stabilized against enzymatic and chemical hydrolysis. The compound was synthesized in a straightforward one-pot process from tetra-*O*-acetylmannose-1-thioacetate **4** and epoxide **3**, both easily accessible substrates.

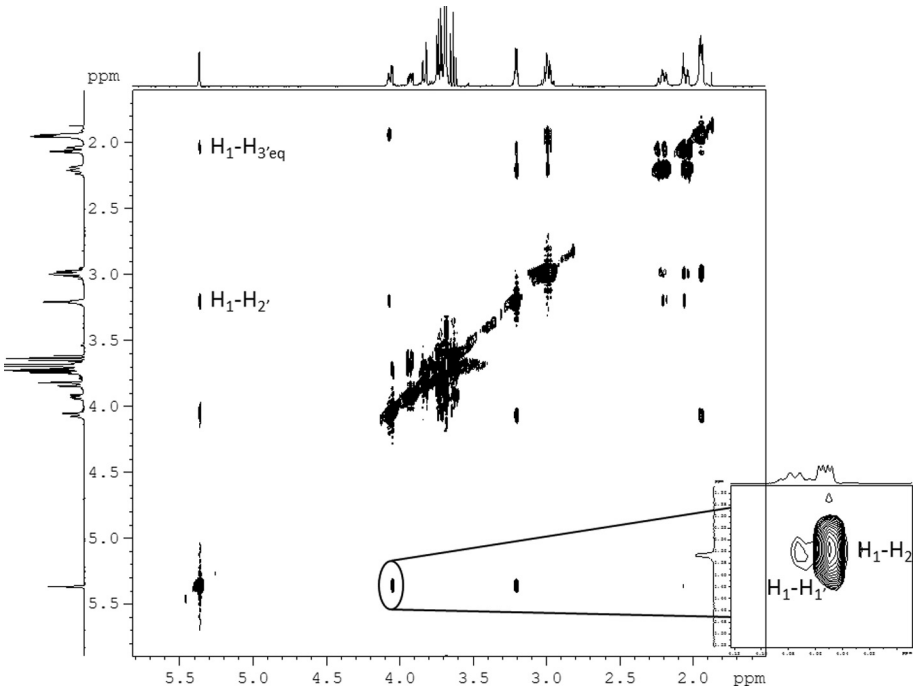


Fig. 4. NOESY spectrum (500 MHz, D_2O, 300 K, 800 ms of mixing time) of compound **2** (R = H) performed with solvent suppression.

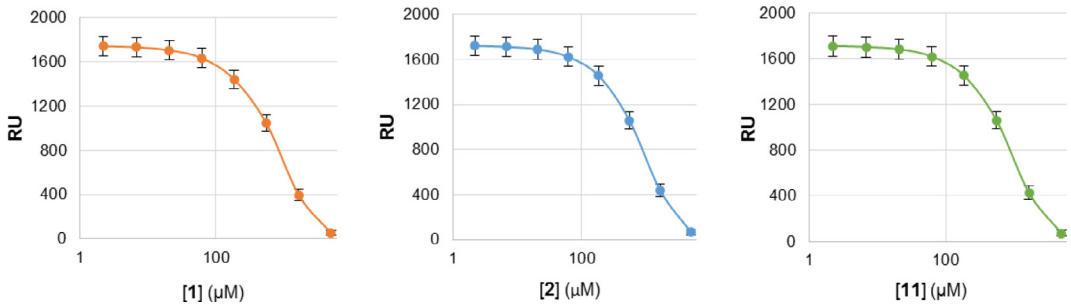


Fig. 5. Inhibition of DC-SIGN ECD binding to immobilized ManBSA by **1**, **2** (R = H), and **11** was measured by SPR.

The synthesis of thiodisaccharides by ring-opening of sugar epoxides with glycosyl thiols has been reported before,[31] but we have coupled this reaction with an *in situ* generation of the reactive thiolate from the anomeric thioacetate. This procedure implies several advantages compared to the classical glycosylation conditions. First of all, the one-pot synthesis allows improving the efficiency of the process whereby the reactants are subjected to successive transformations in the same reaction vessel, minimizing the purification steps. Moreover, the *S*-linkage can be formed avoiding the strict requirements for the standard glycosylation reaction (low reaction temperature, anhydrous conditions, etc.) and minimizing formation of disulfides by oxidation of the glycosidic thiol.

Conformational analysis of the pseudo-thio-1,2-dimannoside **2** by NMR-NOESY studies, supported by molecular modeling, strongly suggest that the molecule is a *bona fide* structural mimic of the natural Manα(1,2)Man sugar and shares the conformational features of the *O*-linked analog. Interaction studies with DC-SIGN, performed by Surface Plasmon Resonance confirm that the molecule acts as a DC-SIGN antagonist and has the same potency of analog **1**.

Last, but not least, we were able to equip this molecule with a stable, azido-terminated ester tether which survived Zemplén deprotection conditions and will be instrumental for the synthesis of multivalent constructs. Multivalent presentation of glycomimetic ligands is a well-established approach to circumvent the intrinsically low affinity of sugar-protein interactions and obtain highly active antagonists. Given its easy synthesis and expected metabolic stability, ligand **2** has the potential to replace **1** and its derivatives in the construction of multivalent DC-SIGN antagonists with antiviral action.

4. Experimental

4.1. General information

Chemicals were purchased from commercial sources and used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed under nitrogen atmosphere. Anhydrous solvents were purchased from Sigma-Aldrich®. Reactions were monitored by analytical thin-layer chromatography (TLC) performed on Silica Gel 60 F254 plates (Merck) with UV detection (254 nm) and/or staining with ceric ammonium molybdate acid solution, potassium permanganate alkaline solution. Silica gel 60 (40–63 μm) (Merck) was used for flash column chromatography. NMR experiments were recorded on a Bruker AVANCE-400 MHz instrument at 298 K or a Bruker AVANCE-500 MHz at 300 K. Chemical shifts (δ) are reported

in ppm. The ^{1}H and ^{13}C NMR resonances of compounds were assigned with the assistance of COSY and HSQC experiments. Multiplicities are assigned as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet). Mass spectra were recorded on Apex II ICR FTMS (ESI ionization-HRMS), Waters Micromass Q-TOF (ESI ionization-HRMS) or ThermoFischer LCQ apparatus (ESI ionization). Specific optical rotation values were measured using a Perkin-Elmer 241, at 589 nm in a 1 dm cell.

4.2. NMR NOESY experiments

NMR spectra of **2** (R = H) were obtained using 5 mg of **2** in 500 μL D_2O (24 mM). NOESY spectra were acquired using a Bruker 500 MHz instrument at 300 K. Spectra (8 scans and 256 increments) were collected using three mixing times (300, 600 and 800 ms). Water suppression was achieved by excitation–sculpting pulse sequence.

4.3. Synthesis

Compound **3** was prepared as previously described.[16] Compound **4** was prepared from commercially available penta-*O*-acetylmannopyranose **6** following a reported procedure[23] and 2,2-dimethyl-3-azido-propanoylchloride **12** was synthesized from commercially available 2,2-dimethyl-3-chloro-propanoic acid according to Sewald,[26] with modifications.

4.3.1. 2,3,4,6-Tetra-O-acetyl-1-S-acetyl-α-D-mannopyranose 4

To a solution of penta-*O*-acetylmannopyranose **6** (1.021 g, 2.62 mmol) in dry CH_2Cl_2 (1.75 mL), thioacetic acid **7** (558 μL, 7.85 mmol) was added under nitrogen atmosphere. TMSOTf (473 μL, 2.62 mmol) was added dropwise at 0 °C and the resulting mixture was stirred at room temperature overnight. After 15 h, TLC showed complete conversion of the starting material. The reaction mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ and H_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (2:8 CH_2Cl_2:iPr_2O) to afford **4** (654 mg, 1.61 mmol) in 61% yield. TLC: R_f = 0.35 (2:8 CH_2Cl_2:iPr_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93 (d, J = 1.6 Hz, 1H), 5.33 (dd, J = 10 Hz, 1H), 5.31 (dd, J = 3.2 Hz, J = 1.6 Hz, 1H), 5.08 (dd, J = 10 Hz, J = 3.2 Hz, 1H), 4.26 (dd, J = 12.4 Hz, 4.8 Hz, 1H), 4.05 (dd, J = 12.4 Hz, 2.4 Hz, 1H), 3.91 (ddd, J = 10 Hz, 4.8 Hz, 2.4 Hz, 1H), 2.41 (s, 3H), 2.16, 2.06, 2.02, 1.97 (s, 3H each); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.3, 170.5, 169.8, 169.7, 169.4, 80.1, 72.4, 70.9, 69.8, 65.6, 62.1, 31.2, 20.8, 20.6, 20.6, 20.5.

4.3.2. Compound 5

Diethylamine (75 μL, 0.72 mmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-α-D-mannopyranose **4** (200 mg, 0.49 mmol) and epoxide **3** (81 mg, 0.38 mmol) in dry DMF (630 μL). The reaction mixture was stirred for 4 h, then diluted with EtOAc, washed with 1 M HCl and H_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (4:6 Hex:EtOAc) to afford **5** (149 mg, 0.26 mmol) in 68% yield. TLC: R_f = 0.3 (4:6 Hex:EtOAc); $[\alpha]_D^{14}$ ($CHCl_3$, c 1.03): +68; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.37 (dd, J = 3.2 Hz, 1.5 Hz, 1H), 5.33 (d, J = 1.3 Hz, 1H), 5.26 (t, J = 9.9 Hz, 1H), 5.19 (dd, J = 9.9 Hz, 3.2 Hz, 1H), 4.40–4.34 (m, 1H), 4.24 (dd, J = 12 Hz, 6.4 Hz, 1H), 4.11 (dd, J = 12.3 Hz, 2.6 Hz, 1H), 3.86–3.78 (m, 1H), 3.71 (s, 3H), 3.69 (s, 3H), 3.22–3.14 (m, 1H), 3.10–2.98 (m, 2H), 2.2–2.11 (m, 4H), 2.08 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.93–1.76 (m, 2H); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.45 (bs, 1H); 5.37 (dd, J = 3.2 Hz, 1.5 Hz, 1H), 5.24 (t, J = 9.9 Hz, 1H), 5.19 (dd, J = 9.9 Hz, 3.2 Hz, 1H), 4.38–4.32 (m, 1H), 4.25 (dd, J = 12 Hz, 6.4 Hz, 1H), 4.12 (dd, J = 12 Hz, 2.6 Hz, 1H), 4.0 (q, J = 3.3 Hz, 1H), 3.68 (s, 6H), 3.20 (q, J = 3.6 Hz, 1H), 3.05 (dt, J = 11 Hz, 5.5 Hz, 1H), 2.92 (dt, J = 11 Hz, 3.8 Hz, 1H), 2.22–2.32 (m, 1H), 2.15 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.06–2.00 (m, 1H) 1.97 (s, 3H), 1.97–1.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.1, 173.8, 170.6, 169.9, 169.7, 169.6, 82.6, 71.1, 69.5, 69.3, 69.1, 66.3, 62.5, 52.2, 52.1, 48.7, 40.3, 39.6, 31.3, 28.6, 20.8, 20.6, 20.6, 20.5; MS (ESI) calcd for $C_{24}H_{34}O_{14}S$ [M+Na]$^+$ m/z: 601.16, found 601.42; HR-MS (ESI) calcd for $C_{24}H_{34}O_{14}S$ [M+Na]$^+$ m/z: 601.15615, found 601.15571.

4.3.3. Compound 10

To a solution of compound **5** (40 mg, 0.069 mmol) in dry pyridine (150 μL), DMAP (8 mg, 0.069 mmol) and pivaloyl chloride (43 μL, 0.35 mmol) were added. The mixture was stirred at room temperature under nitrogen atmosphere. After 5 h, a second aliquot of pivaloyl chloride (25 μL, 0.21 mmol) was added. After reaction completion (24 h, monitored by TLC, 6:4 Hex:EtOAc) the mixture was diluted with EtOAc, washed with 1 M HCl, H_2O and satd aq $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (6:4 Hex:EtOAc) yielded product **10** (27 mg) in 59% yield. TLC: R_f 0.3 (6:4 Hex:EtOAc); $[\alpha]_D^{22}$ ($CHCl_3$, c 1.61): +75; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.38 (d, J = 1.5 Hz, 1H), 5.35 (dd, J = 3.2 Hz, 1.6 Hz, 1H), 5.29 (t, J = 9.7 Hz, 1H), 5.18 (dd, J = 10.0 Hz, 3.3 Hz, 1H), 5.08–5.04 (m, 1H), 4.36–4.27 (m, 2H), 4.16–4.07 (m, 1H), 3.7 (s, 3H), 3.69 (s, 3H), 3.34–3.29 (m, 1H), 3.01–2.87 (m, 2H), 2.15 (s, 3H), 2.13–2.00 (m, 10*H*), 1.99 (s, 3H) 1.2 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.0, 174.2, 174.1, 170.5, 169.8, 169.7, 169.5, 82.9, 70.8, 69.7, 69.6, 69.2, 65.9, 62.3, 52.0, 52.0, 43.6, 39.6, 39.5, 38.8, 28.7, 27.9, 27.0, 20.8, 20.6, 20.5, 20.5; MS (ESI) calcd for $C_{29}H_{42}O_{15}S$ [M+Na]$^+$ m/z: 685.21, found 685.29.

4.3.4. 3-Azido-2,2-dimethylpropanoyl chloride 12

2,2-Dimethyl-3-chloro-propanoic acid (400 mg, 2.97 mmol) was dissolved in dry DMF (14.6 mL) and NaN_3 (990 mg, 15.2 mmol) was added. The mixture was stirred at 50 °C under nitrogen atmosphere. After 3 days, the reaction was allowed to reach room temperature. The mixture was diluted with CH_2Cl_2 and filtered over a celite pad to remove unreacted NaN_3. The solvent was evaporated at reduced pressure giving 3-azido-2,2-dimethylpropanoic acid in quantitative yield (425 mg). The analytical data were fully consistent with those reported in the literature[32] (^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.42 (s, 2H), 1.25 (s, 6H)) and the compound was used without further purification.

The crude azide (143 mg, 1.00 mmol) was dissolved in dry CH_2Cl_2 (3.3 mL) and oxalyl chloride (254 μL, 2.00 mmol) was added drop wise to the solution. After the addition of one drop of dry DMF, the mixture was refluxed for 3 h. The solution was cooled to room temperature and the solvent was carefully removed in vacuo to yield 3-azido-2,2-dimethylpropanoyl chloride **12**. The complete conversion was confirmed by NMR analysis and the product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.53 (s, 2H), 1.35 (s, 6H).

4.3.5. Compound 13

To a solution of compound **5** (30 mg, 0.052 mmol) in dry CH_2Cl_2 (400 μL) and Et3 N (16 μL, 0.117 mmol), DMAP (2 mg, 0.015 mmol) was added. The solution was cooled to 0 °C and compound **12** (19 mg, 0.116 mmol) was added. The mixture was stirred at room temperature under nitrogen atmosphere. After reaction completion (3 h, monitored by TLC, 1:1 Hex:EtOAc) the mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ and 1 M HCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (6:4 Hex:EtOAc) yielded product **13** (29 mg) in 79% yield. TLC: R_f 0.5 (1:1 Hex:EtOAc); $[\alpha]_D^{16}$ ($CHCl_3$, c 1.08): +67; ^{1}H NMR (400 MHz, $CDCl_3$):

δ = 5.39 (m, 1H), 5.37–5.34 (m, 1H), 5.30 (t, J = 9.5 Hz, 1H), 5.19 (dd, J = 9.7 Hz, 3.6 Hz, 1H), 5.14–5.11 (m, 1H), 4.37–4.30 (m, 2H), 4.15–4.07 (m, 1H), 3.71 (s, 3H), 3.71 (s, 3H), 3.42 (s, 2H), 3.36–3.33 (m, 1H), 3.03–2.89 (m, 2H), 2.17 (s, 3H), 2.14–2.06 (m, 4H), 2.18 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.23 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.6, 174.5, 174.3, 170.8, 170.2, 170.0, 169.9, 83.3, 71.1, 70.9, 70.0, 69.6, 66.3, 62.6, 59.9, 52.4, 44.2, 43.8, 40.0, 39.9, 29.1, 28.2, 23.3, 23.3, 21.1, 20.9, 20.9, 20.9; MS (ESI) calcd for $C_{29}H_{41}N_3O_{15}S$ $[M+Na]^+$ m/z: 726.2; found 726.8.

4.3.6. General procedure for the Zemplén deacetylation

A MeONa/MeOH solution was added to the substrate dissolved in dry MeOH under nitrogen atmosphere (substrate and MeONa final concentration 0.02 M) and the reaction mixture was stirred at room temperature. After completion, (approx. 10 min, monitored by TLC) the reaction was quenched with AMBERLITE IR-120 H^+ till neutral pH, the resin was filtered off and the solvent was evaporated at reduced pressure, obtaining the pure product.

4.3.6.1. Pseudo-thio-1,2-dimannoside 2 (R = H). TLC: R_f 0.4 (8:2 CH_2Cl_2:MeOH); $[\alpha]_D^{19}$ (MeOH, c 0.51): +155; ^{1}H NMR (400 MHz, D_2O): 5.38 (d, J = 1.5 Hz, 1H), 4.12–4.07 (m, 1H), 4.06 (dd, J = 1.5 Hz, 3.2 Hz, 1H), 3.95 (ddd, J = 1.9 Hz, 6.2 Hz, 9.0 Hz, 1H), 3.85 (dd, J = 2.2 Hz, 12.4 Hz, 1H), 3.77–3.71 (m, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 3.64 (dd, J = 9.8 Hz, 9.5 Hz, 1H), 3.25–3.19 (m, 1H), 3.06–2.95 (m, 2H), 2.27–2.16 (m, 1H), 2.11–2.03 (m, 1H), 2.00–1.92 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ = 177.3, 177.0, 84.9, 73.4, 71.7, 71.0, 67.7, 67.0, 60.8, 52.6, 45.4, 40.0, 39.0, 29.9, 27.5; HR-MS (ESI) calcd for $C_{16}H_{26}O_{10}S$ $[M+Na]^+$ m/z: 433.11389; found 433.11346.

*4.3.6.2. Compound **11**.* TLC: R_f 0.24 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D^{20}$ (MeOH, c 1.01): +128; ^{1}H NMR (400 MHz, D_2O): 5.41 (d, J = 1.1 Hz, 1H), 5.11–5.08 (m, 1H), 4.08 (dd, J = 1.5 Hz, 3.3 Hz, 1H), 3.95 (ddd, J = 2.4 Hz, 5.8 Hz, 9.4 Hz, 1H), 3.85 (dd, J = 2.4 Hz, 12.5 Hz, 1H), 3.79–3.72 (m, 2H), 3.71 (s, 3H), 3.70 (s, 3H), 3.69–3.63 (m, 1H), 3.42–3.38 (m, 1H), 3.05–2.92 (m, 2H), 2.23–2.01 (m, 4H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, D_2O): δ = 180.3, 176.8, 176.8, 85.3, 73.6, 71.7, 71.0, 70.6, 66.8, 60.6, 52.7, 52.7, 42.8, 39.9, 39.6, 38.7, 28.4, 27.3, 26.2; HR-MS (ESI) calcd for $C_{21}H_{34}O_{11}S$ $[M+Na]^+$ m/z: 517.17140; found 517.17166.

*4.3.6.3. Compound **14**.* TLC: R_f 0.37 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D^{20}$ (MeOH, c 0.81): +112; ^{1}H NMR (400 MHz, MeOD): δ = 5.40–5.36 (m, 1H), 5.17–5.12 (m, 1H), 3.95 (dd, J = 1.6 Hz, 3.2 Hz, 1H), 3.88–3.81 (m, 2H), 3.75 (dd, J = 6.0 Hz, 12.2 Hz, 1H), 3.72–3.68 (m, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.62 (dd, J = 3.2 Hz, 9.2 Hz, 1H), 3.50 (s, 2H), 3.43–3.38 (m, 1H), 3.00–2.89 (m, 2H), 2.19–2.10 (m, 2H), 2.08–2.02 (m, 2H), 1.24 (s, 6H). ^{13}C NMR (100 MHz, MeOD): δ = 176.1, 176.0, 176.0, 87.1, 75.7, 73.6, 73.1, 72.4, 68.5, 62.5, 60.8, 52.6, 52.6, 45.0, 44.1, 41.1, 40.9, 30.0, 29.0, 23.3, 23.3; HR-MS (ESI) calcd for $C_{21}H_{33}N_3O_{11}S$ $[M+Na]^+$ m/z: 558.17280; found 558.17371.

4.4. Surface plasmon resonance (SPR) analysis

The extracellular domain (ECD) of DC-SIGN (residues 66-404) was overexpressed and purified as previously described.[33] The SPR experiments were performed on a BIAcore T200 using a CM3 sensor chip. Flow cells were activated as described.[34] Flow cell one was functionalised with BSA and blocked with ethanolamine and subsequently used as a control surface. Flow cells 2 and 3 were treated with BSA-Manα1-3[Manα1-6]Man (Dextra) (60 μg/ml) in 10 mM NaOAc pH 4 to reach different binding densities and blocked with ethanolamine. The final densities on flow cells 2 and 3 were 2579 and 2923 RU, respectively. The affinity of the various compounds for DC-SIGN ECD were evaluated *via* an established inhibition assay[35] in which DC-SIGN ECD was injected at 20 μM alone or in the presence of increasing concentration of inhibitors (ranging from 0 to 5 mM). Injections were performed at 5 μL/min using 25 mM Tris-HCl pH 8, 150 mM NaCl, 4 mM $CaCl_2$, 0,05% P20 surfactant as running buffer. The surface was regenerated by the injection of 50 mM EDTA.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.03.046.

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