Bioorganic & Medicinal Chemistry 20 (2012) 1817–1831

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Biotin sulfone tagged oligomannosides as immunogens for eliciting antibodies against specific mannan epitopes

Guillaume Despras [a], Raymond Robert [c], Boualem Sendid [b], Emeline Machez [b], Daniel Poulain [b], Jean-Maurice Mallet [a,*]

[a] Université Paris 6, Ecole Normale Supérieure, Département de Chimie, UMR CNRS 7203, 24 rue Lhomond, 75005 Paris, France
[b] Université Lille Nord de France, Inserm U995, Groupe Candida et Candidoses, Faculté de Médecine Henri Warembourg, Pôle Recherche, Centre Hospitalier Régional Universitaire de Lille, Rue Emile Laine, 59045 Lille Cedex, France
[c] Université Angers, Groupe d'Etude des Interactions Hôte-Pathogène (GEIHP), UPRES EA 3142, Institut de Biologie Santé – CHU, Allée du Pont, Centre Hospitalier Universitaire d'Angers, 4 Rue Larrey 49 933 Angers Cedex 9, France

ARTICLE INFO

Article history:
Received 4 October 2011
Revised 19 December 2011
Accepted 21 December 2011
Available online 11 January 2012

Dedicated to Professor Marc Julia (1922–2010) in memoriam

Keywords:
Glycosylation
Odorless thiol
Biotinylation
Biotin sulfone
Thioglycoside
Crohn disease
ASCA
Antibodies
Steptavidin
Biotin tagged oligosaccharides

ABSTRACT

Biotinylated tri and tetrasaccharide: α Man (1→3) α Man (1→2) α Man; α Man (1→3) α Man (1→2) α Man (1→2) α Man were prepared using methyl tertbutyl phenyl thioglycosides glycosyl donors (MBP) and biotin sulfone strategy. Three key mannosyl thioglycosidic donors have been prepared: one for 1→2 linkage and two for the 1→3 linkage (protected with a 4,6-*O*-benzylidene or a 4,6-di-*O*-benzyl). The benzyliden protected one was not found reactive enough, and the benzylated donor was preferred. These biotinylated oligomanosides were evaluated as antigen in Crohn disease diagnosis and used coupled to streptavidin as hapten for eliciting polyclonal antibodies in mice.

1. Introduction

Anti-yeast mannan antibodies are used for diagnosing human diseases. The most widely used tests consist in the detection of anti-*Candida albicans* mannan antibodies for the diagnosis of systemic nosocomial infections determined by this species[1] and Crohn's disease. Crohn's disease is a chronic inflammatory bowel disease, of unknown etiology, where anti-*Saccharomyces cerevisae* mannan antibodies (named ASCA) are commonly associated with severe ileal forms and young age at onset.[2] The reason why patients produce ASCA is unknown but the observation of ASCA in first degree healthy relatives of Crohn's disease patients suggest that their generation may be associated with a genetic basis.[3] Concerning the immunogen(s) of ASCA, clinical and experimental evidence has been gained that *C. albicans*, by altering its oligomannose repertoire in vivo, may be at the origin of ASCA.[4] Identified ASCA epitopes are the tetramannoside α Man (1→3) α Man (1→2) α Man (1→2) α Man (1→2) and the trimannoside: α Man (1→3) α Man (1→2) α Man (1→2).[5–7] For both diseases immunochemical studies have highlighted the very finely tuned process by which mammal immune systems recognize oligomannose epitopes, depending on the mannose linkage types and chain lengths.[8–10] In this respect the use of synthetic oligomannosides of defined structures to dissect the specificity of the human response associated with diseases, protection or inflammation contributed to a better understanding of pathophysiological mechanisms.[5,11]

Our last developments in this approach consisted in the use of biotin tagged oligosaccharides (BTO) and biotin sulfone tagged oligosaccharides (BSTO) as versatile tools through their coupling to streptavidin.[12–14] Among the advantages conferred by this coupling is that it could transform the haptenic B[S]TO in an immunogen. We therefore investigate how injection of the BSTO-streptavidin complex could generate antibodies. For establishing this proof of concept we focused on the tetrasaccharide αMan (1→3) αMan (1→2)

* Corresponding author.
E-mail address: Jean-Maurice.Mallet@ens.fr (J.-M. Mallet).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.12.048

αMan (1→2) α Man which represents the major epitope supporting the human antibody response during Crohn's disease. The reason for this choice lies in the absence of monoclonal antibody displaying this specificity among dozens of anti-mannan antibodies so far described,[8] a quite surprising observation regarding the prominent expression of this structure in *S. cerevisiae* mannan or in *C. albicans* mannan grown at pH 2.[6] Thus, overcoming the lack of production of anti-Crohn's disease major epitope through conventional immunization procedure was a secondary aim in order to provide experimental antibodies that can be useful for analyzing Crohn's disease pathophysiological mechanisms.

2. Results and discussion

Biotinylated synthetic oligosaccharides are an attractive tool, as streptavidin coated plates and beads are commercially available and widely use in serological assays. As biotin is chemically reactive, and not compatible with carbohydrate protecting group manipulation, we described recently the use of biotin sulfone tagged oligomannosides (BSTO) for antigen immobilization in candidiasis[12] or allergy[13] diagnosis. Biotin sulfone is as efficient as biotin in streptavidin binding. We want here to extend this strategy in 'Crohn' antigens, the target molecules are depicted in Figure 1.

2.1. Chemical synthesis

Thioglycosides **8**, **12**, and **20** (Fig. 2) were selected as key intermediates for the synthesis of the protected precursors of BSTO. The 1,2-trans stereoselectivity relies on a 2-*O*-benzoyl participating group.

As depicted in the retrosynthetic analysis (Scheme 1), the hexanoic spacer moiety used for the biotin ligation is introduced first; then, the protected oligosaccharides are obtained through iterative glycosylation/deprotection sequences. Mannosyl donors were the thioglycosidic block **8** for (→2 αMan 1→) linkage and the blocks **12** or **20** for (→3 αMan 1→) linkage.

Monosaccharides **8, 12** and **20** have been prepared from a commercially available odorless thiol (5-methyl 2-*tert*-butylthiophenol, Mbp-SH).[14,15] Compound **8** (Scheme 2) was prepared from the known orthoester **4**,[16] which was opened in acetic acid. The resulting anomeric acetate **5** was converted into the anomeric thioglycoside **6** (α/β mixture: 7/3) in presence of Mbp-SH and boron trifluoride etherate. The 2-*O*-acetate group of **6** was removed in Zemplèn conditions and the resulting free alcohol was benzoylated to afford donor **8**. Benzoates are generally better participating group than acetates in glycosylations (Scheme 2).

Donor **12** was prepared from D-mannose as depicted on Scheme 3:

The tetraol **9** was selectively reacted with PhCHO in dichloromethane in the presence of a catalytic amount of D(+)-camphorsulfonic acid (CSA). The compound **10** was next selectively benzoylated on the position 2 in a two steps sequence through the formation of a 2,3-orthobenzoate followed by its selective opening in acidic conditions. The donor **12** was finally obtained by the protection of the

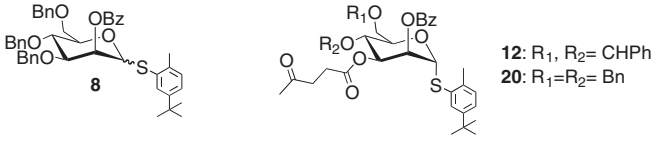


Figure 2. Key building blocks.

free alcohol function of **11** with a levulinate group. These two blocks in hand, the preparation of the protected trisaccharide was started first. The α(1,2) mono and disaccharidic core (**14**, **15** and **16**) have been already prepared using 2-naphtyl thioglycosides.[12] Here, they were synthesized in slightly better yields using Mbp thioglycoside **8** (Scheme 4) activated with *N*-iodosuccinimide and triflic acid.

The disaccharide **17** was obtained by coupling (NIS, TfOH) the donor **12** with the acceptor **14** (Scheme 5). Surprisingly, the yield was low (34%) and TLC analysis revealed a very slow and incomplete conversion of the donor. The use of the more reactive NBS (1.5 equiv) in the presence of 0.1 equiv of TfOH, at room temperature did not improve the yield (31%). This result was probably due to the strained conformation—induced by the benzylidene cycle—which could slow down the formation of an oxycarbenium like intermediate.

To circumvent this problem, another donor **20**, which is benzylated on positions 4 and 6, was synthesized (Scheme 6) from **9**. The tetraol was selectively silylated with *tert*-butyldiphenylsilyl chloride at room temperature. Compound **18** was converted into **19** after four consecutive steps without intermediate purifications. Positions 2 and 3 were first protected by a benzoate orthoester with trimethyl orthobenzoate. The resulting compound was desilylated by using *n*-tetrabutylammonium fluoride then positions 4 and 6 were benzylated and, finally, the orthoester was hydrolyzed to give a free hydroxyl group in position 3. The final step was the formation of the levulinoyl ester with the system levulinic acid/DCC/DMAP.

Hopefully, the glycosylation of **14** with the donor **20** gave a better yield of disaccharide **21** (78%), which confirmed the previous hypothesis (Scheme 7). The 3-*O*-levulinate group was cleaved with hydrazinium acetate in methanol and dichloromethane then the trisaccharide **23** was obtained after a second glycosylation using the donor **8**.

The tetrasaccharide **26** was constructed in a similar way from the disaccharidic acceptor **16** (Scheme 8).

As depicted in Scheme 9, the synthesis of the disaccharide **29** started from the donor **20** and ethyl 6-hydroxy-hexanoate to give the compound **27** in a moderate yield (59%). The deprotection of the position 3 and a glycosylation involving the donor **8** afforded the disaccharide **29**.

2.2. Final steps: biotinylation and deprotection

The final steps are depicted in Scheme 10. The biotin sulfone strategy was used:[12] the protected carbohydrate moiety and the biotin derivative was coupled first in organic solvent, the sulfur atom of biotin was oxidized into sulfone and the glycoconjugate was deprotected in the last step using classical benzyl hydrogenolysis. After the saponification of all ester groups (step a, Scheme 10), the

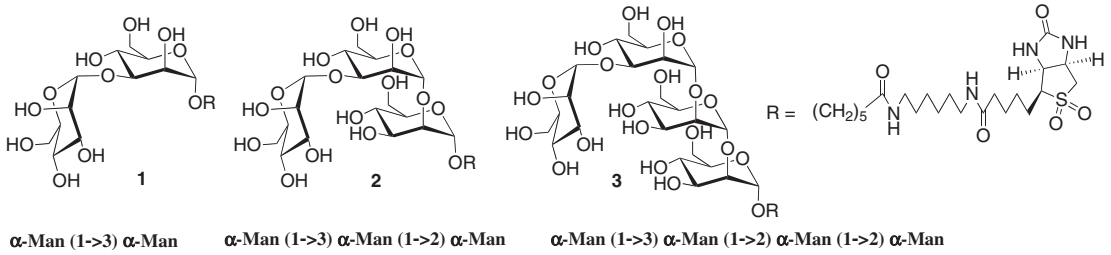
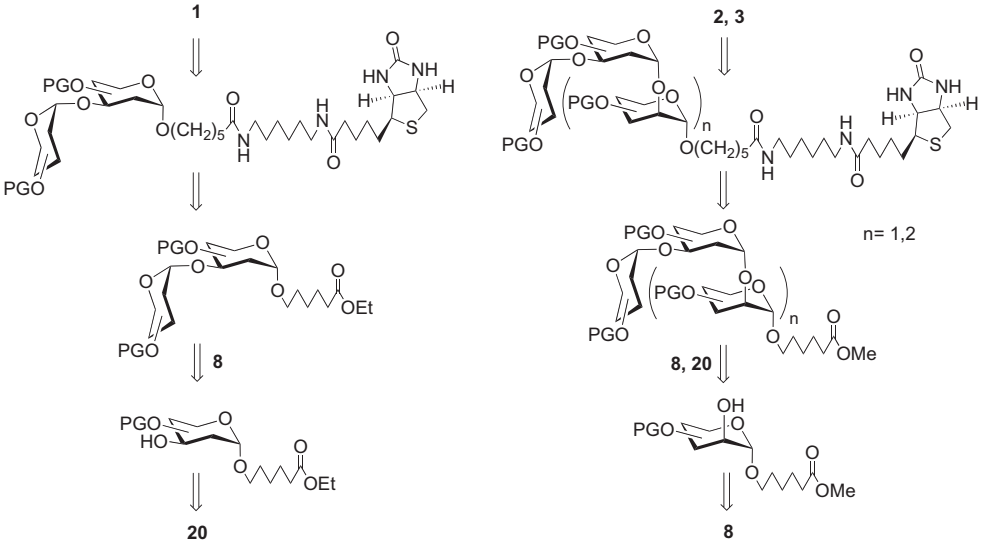
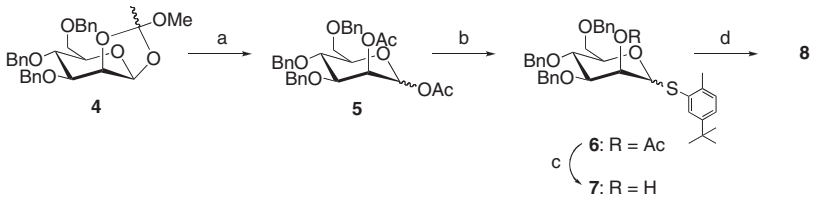


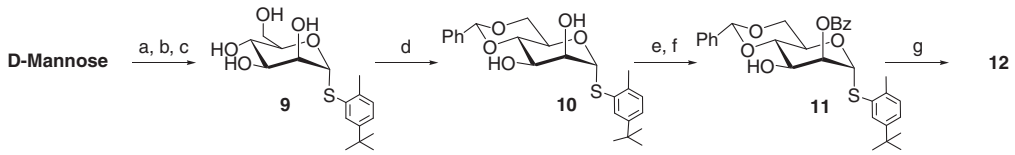
Figure 1. Synthesized BSTO.



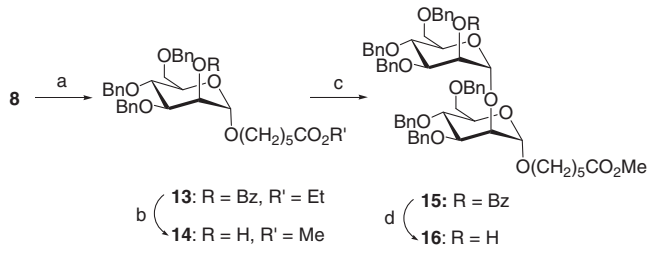
Scheme 1. Retrosynthetic analysis.



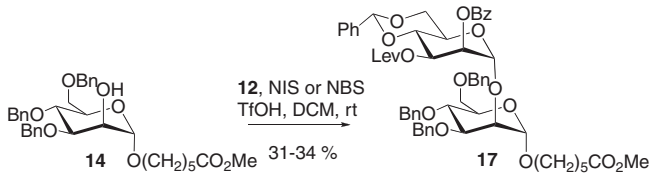
Scheme 2. Reagents and conditions: (a) AcOH, rt; (b) Mbp-SH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 75% two steps; (c) NaOMe, MeOH, rt, 93%; (d) BzCl, NEt_3, DMAP, CH_2Cl_2, rt, 83%.



Scheme 3. Reagents and conditions: (a) Ac_2O, Pyr, rt; (b) Mbp-SH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt 75%; (c) Na, MeOH, rt; (d) PhCHO, CSA, CH_2Cl_2, rt, 62%; (e) $PhC(OMe)_3$, CSA, rt; (f) AcOH 80%, 0 °C, 1 h, 69% two steps; (g) LevOH, DCC, DMAP, CH_2Cl_2, rt, 98%.



Scheme 4. Reagents and conditions: (a) Ethyl 6-hydroxy-hexanoate, NIS, TfOH, CH_2Cl_2, rt, 77%; (b) Na, MeOH, rt, 91%; (c) **8**, NIS, TfOH, CH_2Cl_2, rt, 80%; (d) NaOMe, MeOH, rt, 85%.



Scheme 5. Synthesis of the 1,2-disaccharide using the donor **12**.

biotinylated amine **30**[12] (Fig. 3) was coupled by using 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (EDC), *N*,*N*-dimethyl-amino-pyridine (DMAP) in *N*,*N*-dimethylformamide (step b).

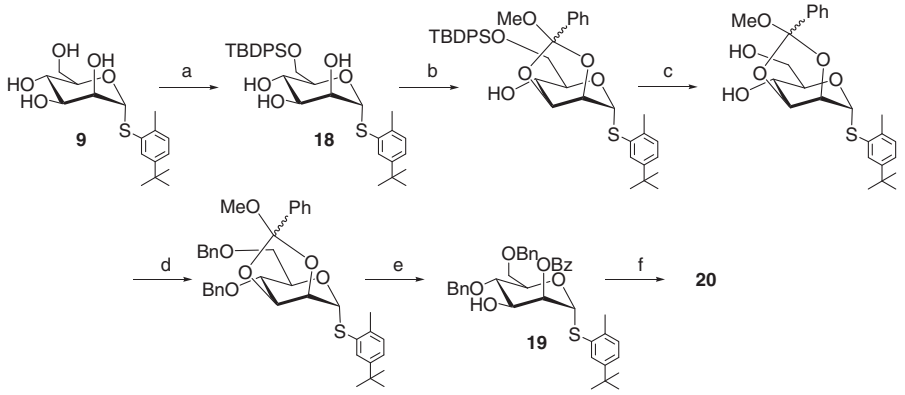
The three conjugates **32**,**35**,**38** were obtained in good yields (Scheme 10, Table 1) and could be easily purified. The presence of some free—but poorly reactive—hydroxyl group on the oligosaccharide did not interfere with the coupling step, but we observed that the presence of too many free hydroxyl groups dramatically reduced the yield of the coupling reaction (probably because of side reactions or reduced solubility).

The sulfur of the biotin was oxidized in sulfone with *meta*-chloroperbenzoic acid (*m*-CPBA in dichloromethane) (step d). The benzyl ethers cleavage was done in presence of hydrogen and of a catalytic amount of activated palladium to give deprotected glycoconjugates **1**, **2** and **3**, in good yields (step e).

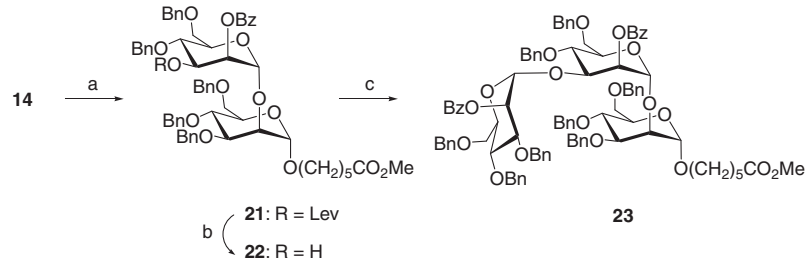
3. Preliminary immunological evaluations

3.1. Validation of 3 as diagnostic tools

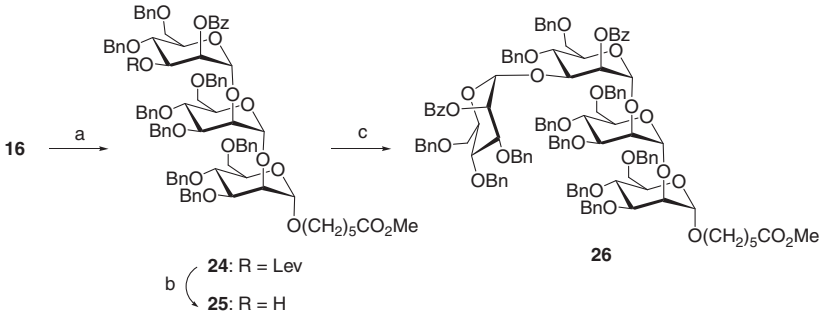
Biotinylated oligomannosides were coated on avidin coated, fluorescent and magnetic beads for use in bio-plex system. The



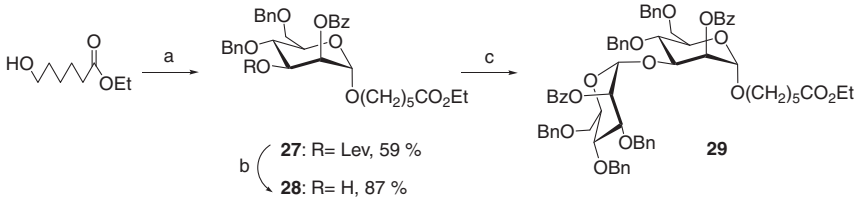
Scheme 6. Reagents and conditions: (a) TBDPSCl, Pyr, rt, 73%; (b) $PhC(OMe)_3$, CSA, DMF, rt; (c) TBAF, THF, rt; (d) BnBr, NaH, DMF, 0 °C to rt; (e) AcOH/water (8:2), THF, rt, 53% after four steps; (f) LevOH, DCC, DMAP, CH_2Cl_2, rt, 83%.



Scheme 7. Reagents and conditions: (a) **20**, NIS, TfOH, CH_2Cl_2, 0 °C, 78%; (b) N_2H_4, AcOH, MeOH, CH_2Cl_2, rt, 80%; (c) **8**, NIS, TfOH, CH_2Cl_2, 0 °C, 80%.



Scheme 8. Reagents and conditions: (a) **20**, NIS, TfOH, CH_2Cl_2, rt, 79%; (b) N_2H_4, AcOH, MeOH, CH_2Cl_2, rt, 91%; (c) **8**, NIS, TfOH, CH_2Cl_2, rt, 86%.



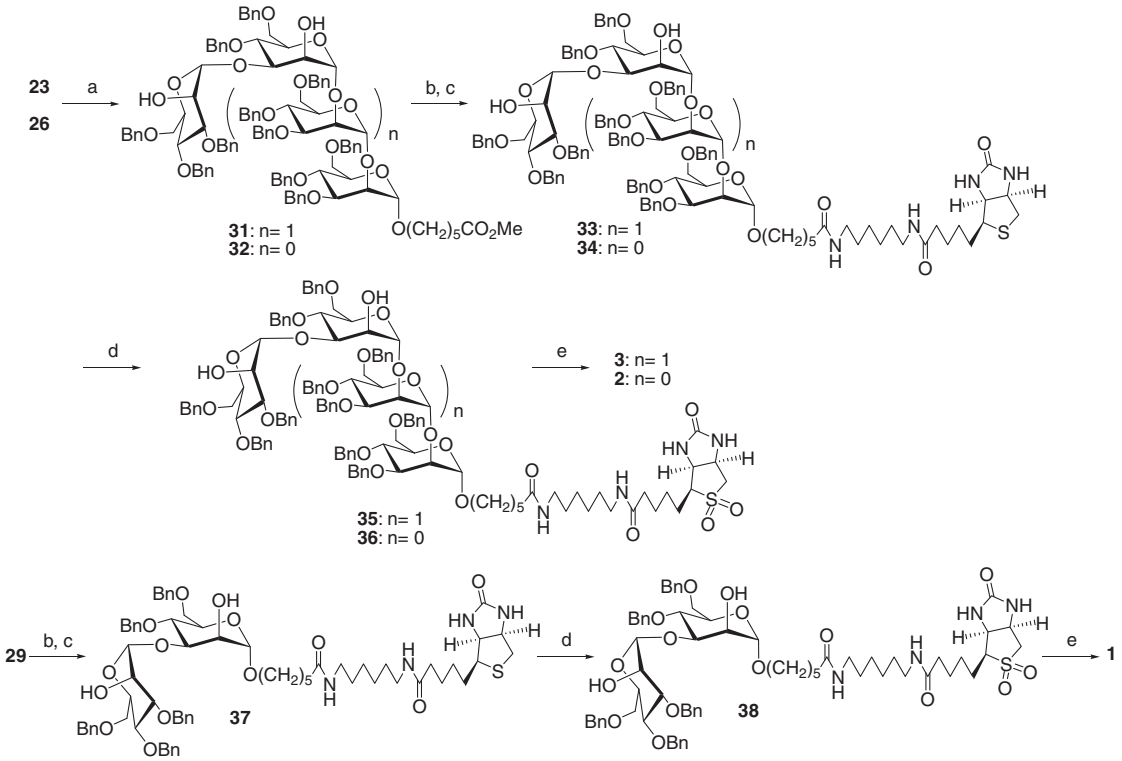
Scheme 9. Reagents and conditions: (a) **20**, NIS, TfOH, CH_2Cl_2, rt, 59%; (b) N_2H_4, AcOH, MeOH, CH_2Cl_2, rt, 87%; (c) **8**, NIS, TfOH, CH_2Cl_2, 0 °C, 78%.

tetrasaccharide **3** showed the ability to discriminate IgG responses associated with Crohn Disease (Fig. 4).

3.2. Immunization of mice with 3/streptavidin complex

To evaluate the immunogenicity of this epitope, the tetramannoside **3** was coupled to a protein carrier and injected to mice to induced antibody response. The high stability of biotin/streptavidin complex was used to prepare the glycoconjugate. Two mice were immunized with a preformed complex between **3** and streptavidin. Four other mice were used as controls (two with adjuvant alone, two with streptavidin).

Figure 5 shows the reactivity of sera IgM of these six mice against the conjugates **1**, **2**, **3** and also against related oligomannosides (**2a**, **2b**, **3b**, **4b** (Table 2) previously prepared). Mouse anti-oligomannoside antibodies were detected using xMAP technology in bio-plex system. The data are expressed as MFI. (Median fluorescence intensity). For each BSTO, a negative control (beads + PBS + conjugate-PE) was also tested. Mice immunization with **3** coupled to streptavidin generated IgM that preferentially reacted



Scheme 10. Reagents and conditions: (a) NaOMe, MeOH, rt; (b) aq NaOH, THF, 60 °C; (c) **30**, EDC, DMAP, DMF, 60 °C; (d) *m*-CPBA, CH_2Cl_2, rt; (e) H_2, Pd/C, MeOH, rt. (see yields in Table 1).

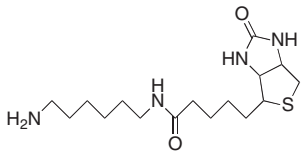


Figure 3. Compound **30**.

with the homologous **3** antigen and the BSTO series with terminal α-1,3 mannose while displaying low reactivity with α-1,2 mannose and β-1,2 mannose series. Low or no reactivity was observed for IgA and IgG, whatever the oligomannosides used.

3.3. Antibodies bind to *S. cerevisiae* mannan but also to *C. albicans* mannan

Mice immunization with **3** coupled to streptavidin generated antibodies that reacted preferentially with yeast mannans with higher expression of the corresponding oligomannose epitope. As shown on Figure 6 the mouse immunoglobulins (IgM) reacted more on *S. cerevisiae* mannan than on *C. albican*s mannan extracted from cultures at pH 7. However when the mannan was extracted from *C. albicans* grown at pH 2 as conditions known to induce a higher expression of ASCA epitopes[6] a higher reactivity of the mouse serum was observed.

4. Conclusion

Biotin sulfone tagged oligomannosides (BSTO) were prepared in an efficient way using odorless thioglycosidic donors and biotin sulfone strategies. This easy access to biotinylated oligosaccharide allows us to explore an innovative way to prepare antibodies by direct immunization of animals using streptavidin as a carrier. We have shown here that using BSTO coupled to streptavidin, we were able to generate a murine specific polyclonal antibody response that was revealed by using the same BSTO epitope. This proof of concept open the way for generating murine monoclonal antibodies of perfectly defined specificity. Regarding this Crohn's disease major epitope that we used here as a model, such a monoclonal antibody would represent a useful tool to analyse its expression on various microbes possibly involved in Crohn's disease (*C. albicans, Mycobacterium paratuberculosis, E. coli* AIEC)[17] or even as a (neo) autoantigen in inflamed tissues.[18]

Table 1
Biotinylation deprotection steps

Reactions and overall yields	Step a	Steps b,c	Step d	Step e
29 → **1** (33%)	—	47% (two steps)	76%	93%
23 → **2** (44%)	75%	72%	—	82% (two steps)
26 → **3** (43%)	81%	75%	76%	94%

5. Experimental

5.1. Chemical synthesis

5.1.1. General procedures

All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting points were determined in capillary tubes in a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 digital polarimeter at 22 ± 3 °C. Compound purity was checked by TLC on Silica gel 60 F_{254} (E.

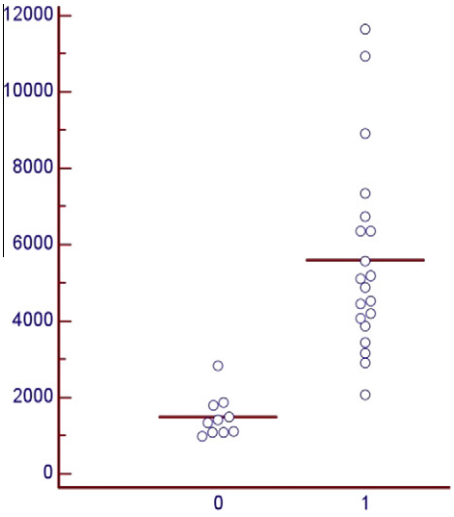
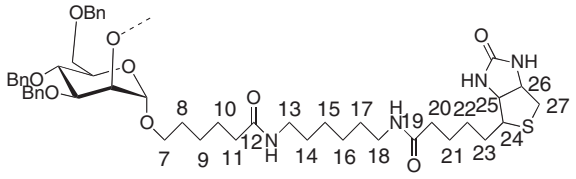


Figure 4. IgG against **3** in CD patient (right) compare to healthy patient (left), data are expressed as Median fluorescence intensity MFI.

Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica gel 60 (E. Merck). ^{1}H NMR spectra were recorded with Brüker AM 250, AM 400 instruments. Chemical ionization and FAB mass spectrometry were recorded with Jeol MS700: CI (gas: ammonia); FAB (matrix: NBA, NaI). Atom numbers in NMR assignments are as follow:



5.2. General procedure for glycosylation reactions

5.2.1. Procedure A

To a solution of donor (1 mmol) in dry CH_2Cl_2 (5–10 ml) was added under argon ethyl 6-hydroxy-hexanoate (1.5 mmol) and 4 Å molecular sieves (mass = donor + acceptor weight) then the mixture was stirred for 15 min. *N*-Iodosuccinimide (3 mmol) and trifluoromethanesulfonic acid (0.3 mmol) were successively added and after stirring 30 min at room temperature, the mixture was diluted with CH_2Cl_2, filtered on a celite pad and washed with satd aq $NaHCO_3$ and satd aq $Na_2S_2O_3$. The aqueous layer was extracted with CH_2Cl_2 then the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography.

Table 2
Additional BSTO used in bioplex detection of IgM (see figure 5).

α 1→3,1→2 series	α 1→2 series	β 1→2 series
1: Man- α-1,3 Man	**2a**: di- α (1,2) mannoside	**2b**: di- β (1,2) mannoside
2: tri- α (1,3) mannoside		**3b**: tri- β (1,2) mannoside
3: tetra- α (1,3) mannoside		**4b**: tetra- β (1,2) mannoside

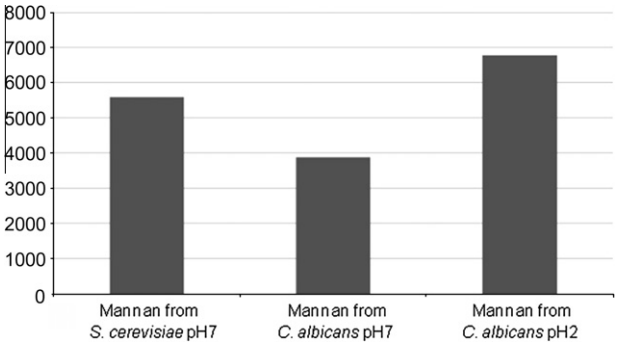


Figure 6. Elisa on coated mannan with immunized mouse 1 serum.

5.2.2. Procedure B

To a solution of acceptor (1 mmol) and donor (1.2 mmol) in dry CH_2Cl_2 (5–10 ml) was added under argon 4 Å molecular sieves

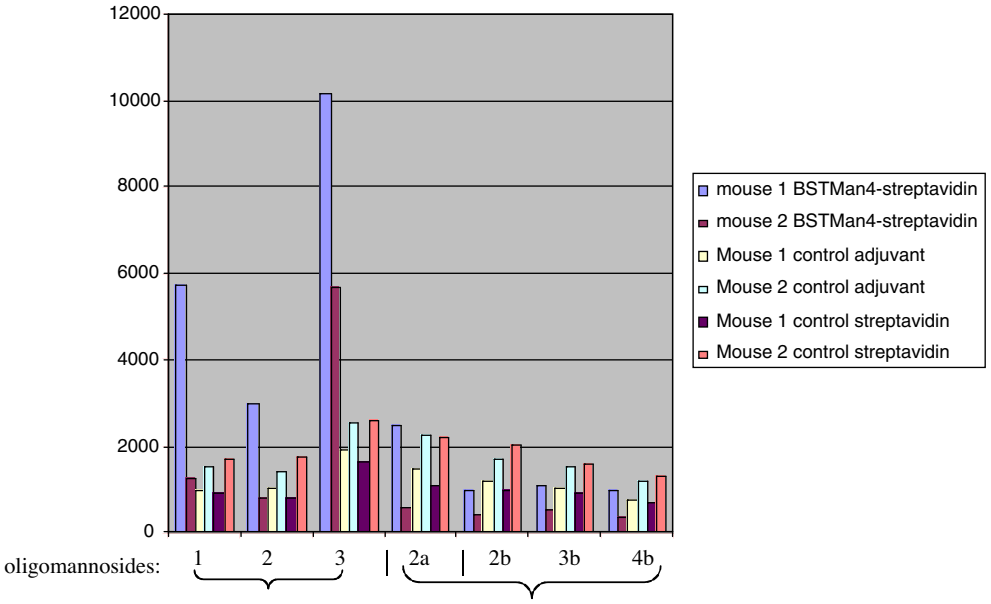


Figure 5. Reactivity of various BSTOs with mouse's serum in bioplex detection of IgM: BSTO used from left to right (**1**,**2**,**3** from this work and **2a**, **2b**, **3b**, **4b** from[12] (Table 2)).

(mass = donor + acceptor weight) and the mixture was stirred for 15 min at room temperature then cooled to 0 °C. *N*-Iodosuccinimide (2.5 mmol) and trifluoromethanesulfonic acid (0.3 mmol) were successively added and after stirring 30 min at 0 °C, the mixture was diluted with CH_2Cl_2, filtered on a celite pad and washed with satd aq $NaHCO_3$ and satd aq $Na_2S_2O_3$. The aqueous layer was extracted with CH_2Cl_2 then the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography.

5.2.3. Procedure C

Same procedure than B with *N*-bromosuccinimide (1.5 mmol) and trifluoromethanesulfonic acid (0.1 mmol).

5.3. General procedure for Zemplen ester cleavage

To a solution of acetylated/benzoylated compound (1 mmol) in dry methanol (2 ml) was added under argon sodium (0.3 mmol). The reaction mixture was stirred at room temperature for 4 h then neutralized with IR-120-H^+ amberlite resin, filtered and concentrated. Debenzoylated compounds were purified by column chromatography.

5.4. General procedure for levulinate cleavage

To a solution of protected carbohydrate (1 mmol) in dry CH_2Cl_2 (20 ml) was added under argon hydrazine acetate (2.2 mmol) in dry methanol (4 ml). After stirring overnight at room temperature, the mixture was washed with satd aq $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered and concentrated and the residue was purified by flash chromatography.

5.5. General procedure for saponification–biotinylation sequence

Oligosaccharide (1 mmol) was dissolved in THF (18 ml) and 10 M NaOH (6 ml) then the solution was stirred overnight at 60 °C. IR-120-H^+ amberlite resin was added and the mixture was filtrated then concentrated. The residue was dissolved in dry DMF (10 ml) with **30** (2 mmol) then EDC (2 mmol) and DMAP (0.045 g, 1.5 mmol) were added. The mixture was stirred at 60 °C for 2 days then concentrated. The residue was taken up in CH_2Cl_2 and washed with 1 M HCl then with brine. The aqueous layer was extracted with CH_2Cl_2 then the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography.

5.6. General procedure for biotin oxidation

To a solution of biotin conjugate (1 mmol) in dry CH_2Cl_2 (30 ml) was added under argon 3-chloroperbenzoic acid (3 mmol). The mixture was stirred at room temperature overnight then diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 then the combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash chromatography afforded pure biotin–sulfone conjugate.

5.7. General procedure for hydrogenolysis

To a solution of benzylated biotin–sulfone conjugate (0.100 g) in methanol (1 ml) was added Pd/C (10%) (0.5 g/g of substrate). Vacuum and H_2 were alternated then the mixture was stirred at room temperature under H_2 overnight (1 atm). The suspension was filtered through a celite pad then concentrated. The residue was dissolved in water and washed with CH_2Cl_2. The aqueous layer was filtered over PTFE 0.45 μm syringe filter and lyophilized to give final compound.

5.7.1. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (6)

Orthoester **4** (15 g, 30.0 mmol) was dissolved under argon in acetic acid (35 ml). The mixture was stirred at room temperature for 30 min then concentrated and dried under vacuum. The resulting syrup (**5**) was dissolved under argon in anhydrous toluene (30 ml) then 2-methyl 5-*tert*-butylthiophenol (6.5 ml, 35.0 mmol) and $BF_3{\cdot}Et_2O$ (7.5 ml, 59.0 mmol) were added. The solution was stirred at room temperature for 2 h then poured into satd aq $NaHCO_3$ (250 ml). The aqueous layer was extracted with CH_2Cl_2 then the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 9/1→8/2) to yield the mixture of anomers (α/β: 7/3) **6** (14.5 g, 75%) as a syrup. R_f: 0.21 (cyclohexane/ethyl acetate: 9/1) HRMS: [M+NH_4^+] calculated for $C_{40}H_{46}O_6S$ 672.335, found 672.336. α anomer ^{1}H NMR (400 MHz, $CDCl_3$): 7.64–7.18 (m, 18H, H Ar), 5.74 (s, 1H, H-2), 5.57 (s, 1H, H-1), 4.98 (d, 1H, J_{gem} = 10.0 Hz, C*H*Ph), 4.84 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.75 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.68 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.61 (d, 1H, J_{gem} = 10.0 Hz, C*H*Ph), 4.55 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.42 (m, 1H, H-5), 4.21 (m, 2H, H-3, H-4), 3.98 (dd, 1H, J_{gem} = 10.0 Hz, $J_{6,5}$ = 4.0 Hz, H-6a), 3.77 (dd, 1H, $J_{6,5}$ = 2.0 Hz, H-6b), 2.47 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 1.38 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 170.3 (CO), 149–127 (24C, C Ar), 78.6 (C-1), 75.2 (*C*H_2Ph), 74.3 (C-4), 73.3 (*C*H_2Ph), 72.6 (C-5), 71.8 (*C*H_2Ph), 70.6 (C-2), 68.6 (C-6), 31.2 (*t*-Bu), 26.8 (qC *t*-Bu), 21.0 (CH_3), 20.2 (CH_3).

5.7.2. (2-Methyl-5-*tert*-butylphenyl) 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (7)

Compound **6** (14.5 g, 22.0 mmol) was submitted to the general procedure for Zemplen deacetylation to give **7** (13.5 g, quantitative) as a syrup. R_f: 0.58 (cyclohexane/ethyl acetate: 7/3) HRMS: [M+NH_4^+] calculated for $C_{38}H_{44}O_5S$ 630.325, found 630.325. α anomer ^{1}H NMR (400 MHz, $CDCl_3$): 7.80–7.20 (m, 18H, H Ar), 5.73 (d, 1H, J = 1.0 Hz, H-1), 4.98 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.87 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.83 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.75 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.67 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.58 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.45 (m, 1H, H-2), 4.42 (m, 1H, H-5), 4.15 (dd, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.0 Hz, H-4), 4.08 (dd, 1H, $J_{3,2}$ = 3.0 Hz, H-3), 3.96 (dd, 1H, J_{gem} = 11.0 Hz, $J_{6,5}$ = 4.0 Hz, H-6a), 3.76 (dd, 1H, $J_{6,5}$ = 2.0 Hz, H-6b), 2.88 (s, 1H, OH), 2.49 (s, 3H, CH_3), 1.42 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 149.5–124.5 (24C, C Ar), 87.0 (C-1), 80.3 (C-3), 75.1 (*C*H_2Ph), 74.2 (C-4), 73.3 (*C*H_2Ph), 72.3 (C-5), 71.9 (*C*H_2Ph), 70.0 (C-2), 68.5 (C-6), 34.3 (qC *t*-Bu), 31.2 (*t*-Bu), 20.1 (CH_3).

5.7.3. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α,β-D-mannopyranoside (8)

To a solution of **7** (12.6 g, 20.0 mmol) in dry CH_2Cl_2 were added under argon triethylamine (20 ml, 144 mmol), benzoyl chloride (4.8 ml, 41.0 mmol) and 4-dimethylaminopyridine (0.500 g, 4.10 mmol). The reaction mixture was stirred at room temperature for 24 h then diluted in CH_2Cl_2 and washed with 1 M HCl. The aqueous layer was extracted with CH_2Cl_2 then the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 9/1 → 8/2) to yield **8** (12.26 g, 83%) as a syrup. R_f: 0.73 (cyclohexane/ethyl acetate: 7/3) HRMS: [M+Na+] calculated for $C_{45}H_{48}O_6S$ 739.306, found 739.310. α anomer: ^{1}H NMR (400 MHz, $CDCl_3$). 8.27–7.28 (m, 23H, H Ar), 6.11 (dd, 1H, $J_{2,1}$ = 2.0 Hz, $J_{2,3}$ = 2.8 Hz, H-2), 5.75 (d, 1H, H-1), 5.10 (d, 1H, J_{gem} = 10.8 Hz, C*H*Ph), 5.01 (d, 1H, J_{gem} = 11.3 Hz, C*H*Ph), 4.89 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.80

(d, 1H, J_{gem} = 11.3 Hz, C*H*Ph), 4.78 (d, 1H, J_{gem} = 10.8 Hz, C*H*Ph), 4.67 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.60 (ddd, 1H, $J_{5,4}$ = 10.0 Hz, $J_{5,6}$ = 3.6 Hz, $J_{5,6}$ = 1.6 Hz, H-5), 4.41 (dd, 1H, $J_{4,3}$ = 10.0 Hz, H-4), 4.32 (dd, 1H, H-3), 4.17 (dd, 1H, J_{gem} = 11.0 Hz, $J_{6,5}$ = 3.6 Hz, H-6a), 3.92 (dd, 1H, $J_{6,5}$ = 1.6 Hz, H-6b), 2.60 (s, 3H, CH_3), 1.46 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 165.5 (CO Bz), 149.6–127.5 (30C, C Ar), 86.2 (C-1), 78.5 (C-3), 75.2 (CH_2Ph), 74.3 (C-4), 73.3 (CH_2Ph), 72.8 (C-5), 71.5 (CH_2Ph), 70.8 (C-2), 68.8 (C-6), 34.3 (qC *t*-Bu), 31.2 (*t*-Bu), 20.3 (CH_3).

5.7.4. (2-Methyl-5-*tert*-butylthiophenyl) 1-thio-α-D-mannopyranoside (9)

To a solution of D-mannose (30 g, 167 mmol) in anhydrous pyridine (140 ml) was added under argon acetic anhydride (110 ml, 1.17 mol). The mixture was stirred overnight at room temperature then methanol (150 ml) was added and solvents were removed under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with 1 M HCl and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in dry CH_2Cl_2 (160 ml) under argon then 2-methyl 5-*tert*-butylthiophenol (46 ml, 250 mmol) and $BF_3{\cdot}Et_2O$ (64 ml, 505 mmol) were added. The solution was stirred overnight at room temperature then poured into satd aq $NaHCO_3$ (250 ml). The aqueous layer was extracted with CH_2Cl_2 then the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 1/1 then acetone) to yield **9** (43 g, 75%) as a white solid. R_f: 0.05 (cyclohexane/ethyl acetate: 1/1). $[\alpha]_D^{20}$ −18 (c = 8, $CHCl_3$) HRMS: [M+Na$^+$] calculated for $C_{17}H_{26}O_5S$ 365.1399, found 365.1395. ^{1}H NMR (400 MHz, $CDCl_3$): 7.51 (d, 1H, $J_{ortho\text{-}para}$ = 2.0 Hz, H_{ortho}), 7.19 (dd, 1H, $J_{para\text{-}meta}$ = 8.0 Hz, $J_{para\text{-}ortho}$ = 2.0 Hz, H_{para}), 7.09 (d, 1H, $J_{meta\text{-}para}$ = 8.0 Hz, H_{meta}), 5.44 (s, 1H, H-1), 4.82 (br s, 4H, OH), 4.27 (s, 1H, H-2), 4.11–4.03 (m, 3H, H-4, H-5, H-6a), 3.95 (d, 1H $J_{3,4}$ = 5.7 Hz, H-3), 3.73 (d, 1H, J_{gem} = 11.9 Hz, H-6b), 2.33 (s, 3H, CH_3), 1.27 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 150.1 (qC Ar), 137.3 (qC Ar), 132.7 (qC Ar), 130.6 (C_{ortho}), 130.4 (C_{meta}), 125.5 (C_{para}), 88.6 (C-1), 73.8 (C-4), 73.3 (C-2), 72.6 (C-3), 66.7 (C-5), 61.3 (C-6), 34.8 (qC *t*-Bu), 31.7 (3C, *t*-Bu), 20.7 (CH_3).

5.7.5. (2-Methyl-5-*tert*-butylthiophenyl) 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (10)

To a mixture of **9** (2.65 g, 7.74 mmol), D(+)-camphorsulfonic acid (0.180 g, 0.77 mmol) and 4 Å molecular sieves (2 g) in anhydrous CH_2Cl_2 was added under argon benzaldehyde (0.870 ml, 8.61 mmol). The mixture was stirred at room temperature for 5 h then D(+)-camphorsulfonic acid (0.90 g, 0.39 mmol) and benzaldehyde (0.160 ml, 1.55 mmol) were added. After stirring overnight, the solution was filtered through a celite pad then concentrated. The residue was chromatographied on silica gel (cyclohexane/ethyl acetate: 7/3) to yield **10** (2.070 g, 62%) as a white foam. R_f: 0.34 (cyclohexane/ethyl acetate: 7/3). $[\alpha]_D^{20}$ −82 (c = 6, $CHCl_3$) HRMS: [M+Na$^+$] calculated for $C_{24}H_{30}O_5S$ 453.1712, found 4535.1716. ^{1}H NMR (400 MHz, $CDCl_3$): 7.63–7.38 (8H, H Ar), 5.60 (s, 1H, C*H*Ph benzylidene), 5.52 (s, 1H, H-1), 4.95 (s, 2H, OH), 4.39 (ddd, 1H, $J_{5,4}$ = $J_{5,6}$ = 9.9 Hz, $J_{5,6}$ = 4.9 Hz, H-5), 4.32 (d, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 4.23 (dd, 1H, J_{gem} = 10.3 Hz, $J_{6,5}$ = 4.9 Hz, H-6a), 4.18 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 3.88 (dd, 1H, H-6b), 2.44 (s, 3H, CH_3), 1.34 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 150.2, 125.5 (12C, C Ar), 102.8 (C*H*Ph benzylidene), 88.3 (C-1), 79.5 (C-4), 73.2 (C-2), 69.5 (C-3), 69.0 (C-6), 64.9 (C-5), 34.9 (qC *t*-Bu), 31.7 (*t*-Bu), 20.7 (CH_3).

5.7.6. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (11)

Compound **10** (2.67 g, 6.20 mmol) and D(+)-camphorsulfonic acid (0.290 g, 1.25 mmol) were dissolved under argon in triethylorthobenzoate (16 ml). The reaction mixture was stirred at room temperature for 1 h then cooled to 0 °C and 20% aq acetic acid was added. The solution was stirred for 1 h then poured into satd aq $NaHCO_3$ (100 ml). The aqueous layer was extracted with CH_2Cl_2 then the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 9/1) to yield **11** (2.30 g, 70%). R_f: 0.38 (cyclohexane/ethyl acetate: 8/2). $[\alpha]_D^{20}$ +2 (c = 3, $CHCl_3$) HRMS: [M+Na$^+$] calculated for $C_{31}H_{34}O_6S$ 557.1974, found 557.1979. ^{1}H NMR (400 MHz, $CDCl_3$): 8.19–7.24 (13H, H Ar), 5.77 (d, 1H, $J_{2,3}$ = 2.8 Hz, H-2), 5.75 (s, 1H, C*H*Ph benzylidene), 5.61 (s, 1H, H-1), 4.58 (m, 1H, H-5), 4.41 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 4.35 (dd, 1H, J_{gem} = 10.3 Hz, $J_{6,5}$ = 4.7 Hz, H-6a), 4.23 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.98 (dd, 1H, $J_{6,5}$ = 5.3 Hz, H-6b), 2.56 (s, 3H, CH_3), 1.39 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 166.5 (CO), 150.3–126.0 (18C, C Ar), 102.8 (C*H*Ph benzylidene), 87.3 (C-1), 80.1 (C-4), 75.2 (C-2), 69.0 (C-6), 68.3 (C-3), 65.3 (C-5), 34.9 (qC *t*-Bu), 31.8 (*t*-Bu), 20.9 (CH_3).

5.7.7. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (12)

To a solution of **11** (2.09 g, 3.91 mmol) in dry CH_2Cl_2 (8 ml) were added under argon 4-dimethylaminopyridine (0.10 g, 0.08 mmol), levulinic acid (0.900 g, 7.75 mmol) and dicyclohexylcarboxydiimide (1.6 g, 7.75 mmol). The mixture was stirred at room temperature for 1 h then concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 8/2) to yield **12** (2.46 g, quantitative) as a white foam. R_f: 0.38 (cyclohexane/ethyl acetate: 8/2). $[\alpha]_D^{20}$ +41 (c = 7, $CHCl_3$) HRMS: [M+Na$^+$] calculated for $C_{36}H_{40}O_8S$ 655.2342, found 655.2337. ^{1}H NMR (400 MHz, $CDCl_3$): 8.14–7.19 (13H, H Ar), 5.91 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.7 Hz, H-2), 5.70 (s, 1H, C*H*Ph benzylidene), 5.62 (dd, 1H, $J_{3,4}$ = 10.3 Hz, H-3), 5.54 (s, 1H, H-1), 4.63 (m, 1H, H-5), 4.32 (m, 2H, H-4, H-6a), 3.98 (dd, 1H, J_{gem} = $J_{6,5}$ = 10.3 Hz, H-6b), 2.80–2.52 (m, 4H, CH_2 Lev), 2.50 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 1.34 (s, 9H, *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 206.5 (CO), 172.2 (CO), 165.8 (CO), 150.3–126.1 (18C, C Ar), 102.4 (C*H*Ph benzylidene), 87.10 (C-1), 77.1 (C-4), 72.9 (C-2), 69.5 (C-3), 69.0 (C-6), 65.7 (C-5), 38.3 (CH_2 Lev), 34.5 (qC *t*-Bu), 31.7 (*t*-Bu), 28.3 (CH_2 Lev), 20.8 (CH_3).

5.7.8. 5-Ethoxycarbonylpentyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (13)

Donor **8** (0.408 g, 0.570 mmol) was submitted to the general procedure A for glycosylation. The crude product was purified by flash chromatography (cyclohexane/EtOAc: 9/1) to yield **13** (0.306 g, 77%) as a syrup. R_f: 0.45 (cyclohexane/EtOAc: 8/2); $[\alpha]_D^{20}$ −7 (c = 0.7, $CHCl_3$); HRMS: [M+NH$_4$]$^+$ calcd for $C_{42}H_{52}O_9N$ 730.3642, found 714.3646. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15–7.18 (m, 20H, H Ar), 5.64 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 2.5 Hz, H-2), 4.97 (d, 1H, H-1), 4.91 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.84 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.78 (d, 1H, J_{gem} = 11.9 Hz, C*H*Ph), 4.62 (d, 1H, J_{gem} = 11.9 Hz, C*H*Ph), 4.59 (d, 1H, J_{gem} = 11.5 Hz, C*H*Ph), 4.57 (d, 1H, J_{gem} = 11.5 Hz, C*H*Ph), 4.20–4.09 (m, 4H, H-12, H-3, H-4), 3.96–3.91 (dd, 1H, J_{gem} = 10.4 Hz, $J_{6,5}$ = 3.9 Hz, H-6a), 3.91–3.86 (m, 1H, H-5), 3.84–3.79 (dd, 1H, $J_{6,5}$ = 1.5 Hz, H-6b), 3.78–3.72 et 3.52–3.43 (m, 2H, H-7a, H-7b), 2.35 (t, 2H, J = 7.5 Hz, 2H-11), 1.73–1.60 (m, 4H, 2H-8, 2H-10), 1.46–1.38 (m, 2H, 2H-9), 1.32–1.24 (t, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.8 (CO), 165.8 (CO), 138.4–127.5 (24C, C Ar), 97.8 (C-1), 78.3 (C-3 or C-4), 74.3 (C-3 or C-4), 70.9 (C-5), 68.2 (C-2), 74.9, 73.2, 71.7 (3C, 3 CH_2Ph), 67.7 (C-6), 34.2 (C-11), 29.1 (C-10), 25.7 (C-9), 24.7 (C-8), 14.2 (CH_3).

5.7.9. 5-Methoxycarbonylpentyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14)

Compound **13** (1.288 g, 1.848 mmol) was submitted to the general procedure for Zemplen debenzoylation. The crude product was purified by column chromatography (cyclohexane/EtOAc: 7/3 to 6/4) to give **14** (0.951 g, 89%) as a syrup. R_f: 0.60 (cyclohexane/EtOAc: 1/1). $[\alpha]_D^{25}$ +36 (c = 0.8, $CHCl_3$); HRMS: $[M+NH_4]^+$ calcd for $C_{34}H_{46}O_8N$ 596.3223, found 596.3231. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.48–7.20 (m, 15H, H Ar), 4.96 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 4.90 (d, 1H, J_{gem} = 11.1 Hz, C*H*Ph), 4.79 (d, 1H, J_{gem} = 11.1 Hz, C*H*Ph), 4.74 (d, 1H, J_{gem} = 11.8 Hz, CHPh), 4.72 (d, 1H, J_{gem} = 11.8 Hz, C*H*Ph), 4.61 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.57 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.09 (m, 1H, H-2), 3.98–3.90 (m, 2H, H-3, H-4), 3.87–3.81 (m, 2H, H-5, H-7a), 3.80–3.74 (m, 2H, H-7b, H-6a), 3.74–3.69 (m, 3H, CH_3), 3.53–3.44 (m, 1H, H-6b), 2.83 (br s, 1H, OH), 2.37 (t, 2H, J = 7.5 Hz, H-11), 1.75–1.60 (m, 4H, 2H-8, 2H-10), 1.48–1.38 (m, 2H, 2H-9).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.8 (CO), 138.0–127.3 (18C, C Ar), 99.1 (C-1), 80.1 (C-3 or C-4), 74.1 (C-3 or C-4), 70.9 (C-5), 68.2 (C-2), 74.9 (*C*H_2Ph), 73.2 (*C*H_2Ph), 71.7 (*C*H_2Ph), 67.2 (C-6), 33.7 (C-11), 28.9 (C-10), 25.5 (C-9), 24.5 (C-8).

5.7.10. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (15)

Acceptor **14** (0.919 g, 1.588 mmol) and donor **8** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/EtOAc: 8/2) to yield **15** (1.433 g, 80%) as a syrup. R_f: 0.35 (cyclohexane/EtOAc: 8/2); $[\alpha]_D^{20}$ +1 (c = 1.0, $CHCl_3$); HRMS: $[M+NH_4]^+$ calcd for $C_{68}H_{78}O_{14}N$ 1132.5422, found 1132.5433. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.14–7.26 (m, 35H, H Ar), 5.83 (dd, 1H, $J_{1',2'}$ = 2.1 Hz, $J_{2',3'}$ = 2.8 Hz, H-2′), 5.25 (d, 1H, H-1′), 4.95 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.93–4.49 (m, 12H, C*H*Ph), 4.18–4.13 (m, 1H, H-3′), 4.05 (dd, 1H, $J_{2,3}$ = 2.6 Hz, H-2), 3.97 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 3.94–3.87 (m, 3H), 3.85–3.74 (m, 5H), 3.70 (s, 3H, CH_3), 3.69–3.63 (m, 1H, H-7a), 3.36–3.30 (m, 1H, H-7b), 2.34 (t, 2H, J = 7.5 Hz, 2H-11), 1.68–1.53 (m, 4H, 2H-10, 2H-8), 1.39–1.31 (m, 2H, 2H-9).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.0 (CO), 138.0–127.3 (42C, CAr), 99.6 (C-1′), 98.7 (C-1), 79.7 (C-3 or C-4), 78.1 (C-3 or C-4), 75.3 (C-2), 74.7 (C-3), 74.3, 75.2, 75.1, 73.3, 73.2, 72.1, 71.6 (6C, 6 *C*H_2Ph), 69.3 (C-6), 69.0 (C-2′), 67.7 (C-7), 51.4 (CH_3), 33.9 (C-11), 29.1 (C-8), 25.7 (C-9), 24.7 (C-10).

5.7.11. 5-Methoxycarbonylpentyl 2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16)

Compound **15** was submitted to the general procedure for Zemplen debenzoylation. The crude product was purified by column chromatography (cyclohexane/EtOAc: 7/3) to yield **16** as a syrup. Yield: 85%.; R_f: 0.65 (cyclohexane/EtOAc: 1/1); $[\alpha]_D^{20}$ +34 (c = 0.5, $CHCl_3$). HRMS: $[M+Na]^+$ calcd for $C_{61}H_{70}O_{13}N$ 1033.4716, found 1033.4727.; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.39–7.30 (m, 30H, H Ar), 5.19 (br s, 1H, H-1′), 5.25 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.90–4.53 (m, 12H, C*H*Ph), 4.20–4.14 (m, 1H, H-2′), 4.06 (m, 1H, H-2), 4.03–3.99 (m, 1H, H-5 or H-5′), 3.96 (dd, 1H, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.92 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.2 Hz, H-3′), 3.88–3.74 (m, 5H, H-4, H-4′, H-5 or H-5′, 2H-6), 3.70 (s, 3H, CH_3), 3.65–3.59 (m, 1H, H-7a), 3.32–3.26 (m, 1H, H-7b), 2.33 (t, 2H, J = 7.5 Hz, 2H-11), 2.09 (s, 2H, OH), 1.68–1.48 (m, 4H, 2H-10, 2H-8), 1.37–1.29 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.0 (CO), 138.6–127.3 (36C, C Ar), 101.0 (C-1′), 98.7 (C-1), 79.9 (C-3′), 79.7 (C-3), 75.0 (C-2), 74.8 (C-4 or C-4′), 74.4 (C-4 or C-4′), 75.1, 74.9, 73.3, 73.2, 72.1, 72.0 (6C, 6 *C*H_2Ph), 71.8 (C-5 or C-5′), 71.5 (C-5 or C-5′), 69.3 (C-6 or C-6′), 69.2 (C-6 or C-6′), 68.5 (C-2′), 67.4 (C-7), 53.4, 51.4 (CH_3), 33.9 (C-11), 29.1 (C-8), 25.7 (C-9), 24.7 (C-10).

5.7.12. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-3-*O*-levulinoyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (17)

Acceptor **14** and donor **12** were submitted to the general procedures B and C for glycosylation. The crude product was purified by flash chromatography (cyclohexane/EtOAc: 7/3) Syrup. Yield: 34% via procedure B, 31% via procedure C. R_f: 0.50 (cyclohexane/EtOAc: 6/4). $[\alpha]_D^{20}$ −2 (c = 4, $CHCl_3$).HRMS: $[M+Na^+]$ calcd for $C_{59}H_{66}O_{16}Na$ 1053.4249, found 1053.4254. ^1H NMR (400 MHz, $CDCl_3$) δ 8.14–7.20 (m, 25H, H Ar), 5.83 (dd, 1H, $J_{2',3'}$ = 3.5 Hz, $J_{2',1'}$ = 1.6 Hz, H-2′), 5.64 (s, 1H, C*H*Ph benzyliden), 5.62 (dd, 1H, $J_{3',4'}$ = 9.9 Hz, $J_{3',2'}$ = 3.6 Hz, H-3′), 5.19 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1′), 4.91 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.88 (d, 1H, J = 10.8 Hz, *C*H_2Ph), 4.77 (d, 1H, J = 11.8 Hz, C*H*Ph), 4.72–4.67 (m, 3H, C*H*Ph), 4.58 (d, 1H, J = 10.8 Hz, C*H*Ph), 4.35 (dd, 1H, $J_{6b',6a'}$ = 10.3 Hz, $J_{6b',5'}$ = 4.3 Hz, H-6b′), 4.18 (m, 1H, H-4′), 4.03 (s, 1H, H-2), 3.98–3.90 (m, 3H, H-5′, H-6a′, H-3), 3.84 (m, 5H, H-4, H-5, H-6a, H-6b, H_7), 3.68 (s, 3H, H-12), 3.45–3.41 (m, 1H, H_7), 2.81, 2.47 (m, 4H, CH_2 Lev), 2.34 (t, 2H, 2H-11), 2.11 (s, 3H, $COCH_3$ Lev), 1.71–1.58 (m, 4H, 2H-8, 2H-10), 1.44–1.37 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.8 ($COCH_3$ Lev), 171.9 (OCO–CH_2 Lev), 165.6 (OCO-Ph), 138.7–126.7 (30C, arom), 102.4 (C*H*Ph benzyliden), 100.5 (C-1′), 99.1 (C-1), 80.4 (C-3), 76.9 (C-4′), 75.9 (C-2), 75.7 (CH_2Ph), 75.4 (C-5′), 73.7 (*C*H_2Ph), 73.0 (*C*H_2Ph), 72.4 (C-4 ou C-5), 70.8 (C-2′), 69.8 (C-6), 69.2 (C-6′), 67.9 (C-7), 64.6 (C-4 ou C-5), 51.9 (C-12), 38.4 (CH_2 Lev), 34.4 (C-11), 30.2 ($COCH_3$ Lev), 29.5 (C-8), 28.4 (CH_2 Lev), 26.2 (C-9), 25.1 (C-10).

5.7.13. (2-Methyl-5-*tert*-butylthiophenyl) 6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (18)

To a solution of **9** (3.720 g, 10.86 mmol) in dry pyridine (15 ml) was added under argon at 0 °C *tert*-butyldiphenylsilyl chloride (6.20 ml, 23.89 mmol). The mixture was stirred overnight at 0 °C then methanol was added and the solvent was removed. The residue was dissolved in CH_2Cl_2, washed with 1 M HCl and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/methanol: 96/4) to yield **18** (4.614 g, 73%) as a syrup. R_f: 0.39 (CH_2Cl_2/methanol: 95/5). $[\alpha]_D^{20}$ +124 (c = 1, $CHCl_3$). HRMS: $[M+Na^+]$ calculated for $C_{33}H_{44}O_5$ SSi 603.25709, found 603.29784. ^1H NMR (400 MHz, $CDCl_3$): 7.70–7.66 (m, 4H, H Ar), 7.50 (d, 1H, J = 3.0 Hz, H Ar), 7.47–7.36 (m, 7H, H Ar), 7.20 (dd, 1H, J = 3.0 Hz, J = 9.0 Hz, H Ar), 7.13 (d, 1H, J = 9.0 Hz, H Ar), 5.47 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 4.27 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 4.25–4.19 (m, 1H, H-3), 4.07 (dd, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.0 Hz, H-4), 4.00–3.88 (m, 3H, H-5, 2H-6), 2.86 (br s, 4H, OH), 2.37 (s, 3H, CH_3), 1.23 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 149.7–124.8 (13C, C Ar), 87.7 (C-1), 72.2 (C-2), 72.1 (C-5), 71.2 (C-3), 71.1 (C-4), 65.4 (C-6), 54.3 (Cq *t*-Bu), 34.4 (Cq *t*-Bu), 31.3 (*t*-Bu), 26.9 (*t*-Bu), 20.3 (CH_3).

5.7.14. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (19)

Compound **18** (1.03 g, 1.77 mmol) was dissolved in dry *N*,*N*-dimethylformamide (2 ml) and trimethylorthobenzoate (1.2 ml, 7.08 mmol). The solution was stirred under vacuum (ca 10 mbar) for 10 min then D(+)-camphorsulfonic acid (0.020 g, 0.088 mmol) was added. The mixture was stirred under vacuum for 3 h then triethylamine (0.5 ml) was added and the reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2, washed with water then the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated.

The crude residue was dissolved in dry THF (5 ml) containing *n*-tetrabutylammonium fluoride (1.67 g, 3.09 mmol). The mixture was stirred at room temperature for 3 h then concentrated. The residue was taken up in CH_2Cl_2, washed with brine then the

aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated.

To a solution of the previous residue in dry *N*,*N*-dimethylformamide (10 ml) was added benzyl bromide (0.65 ml, 3.09 mmol) under argon. The solution was cooled to 0 °C then sodium hydride (0.250 g, 3.60 mmol) was added portionwise. The mixture was stirred at room temperature overnight then methanol was added and concentrated. The residue was dissolved in CH_2Cl_2, washed with brine then the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and concentrated.

The residue was dissolved in a mixture of THF (5 ml), acetic acid (4 ml) and water (1 ml) and stirred at room temperature for 2 h. The solution was diluted with CH_2Cl_2 then washed with satd aq $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 then the combined organic layers were dried ($MgSO_4$), filtered and concentrated. Flash chromatography (cyclohexane/ethyl acetate: 95/5 → 90/10) gave compound **19** (0.582 g, 53%) as a syrup. $[\alpha]_D^{20}$ +77 (c = 1,$CHCl_3$). HRMS: [M+Na^+] calculated for $C_{38}H_{42}O_6S$ 649.25943, found 649.26066. ^{1}H NMR (400 MHz, $CDCl_3$): 8.03 (d, 2H, J = 9.6 Hz, H ortho Bz), 7.57–7.56 (m, 2H, H Ar), 7.39–7.15 (m, 14H, H Ar), 5.65 (dd, 1H, $J_{2,1}$ = 1.6 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.56 (d, 1H, H-1), 5.33 (d, 1H, J_{gem} = 11.2 Hz, C*H*Ph), 4.75–4.70 (m, 2H, 2 C*H*Ph), 4.53 (d, 1H, J_{gem} = 12.0 Hz, C*H*Ph), 4.43–4.39 (m, 1H, H-5), 4.29 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 4.14 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.00(dd, 1H, J_{gem} = 12.0 Hz, $J_{6,5}$ = 4.0 Hz, H-6a), 3.76 (dd, 1H, $J_{6,5}$ = 1.6 Hz, H-6b), 2.43 (s, 3H, CH_3), 1.28 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1 (CO), 149.8–125.4 (24C, C Ar), 86.2 (C-1), 75.9 (C-4), 75.0 (*C*H_2Ph), 74.8 (C-2), 73.6 (*C*H_2Ph), 72.8 (C-5), 71.3 (C-3), 70.0 (C-6), 34.5 (qC *t*-Bu), 31.4 (*t*-Bu), 20.5 (CH_3).

5.7.15. (2-Methyl-5-*tert*-butylthiophenyl) 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (20)

To a solution of **19** (2.14 g, 3.41 mmol) in dry CH_2Cl_2 (5 ml) were added under argon 4-dimethylaminopyridine (0.085 g, 0.68 mmol), levulinic acid (0.800 g, 6.82 mmol) and dicyclohexylcarboxydiimide (1.4 g, 6.82 mmol). The mixture was stirred at room temperature overnight then concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate: 8/2) to yield **20** (2.06 g, 83%) as a syrup. $[\alpha]_D^{20}$ +61 (c = 1, $CHCl_3$): *HRMS*: [M+Na^+] calculated for $C_{43}H_{48}O_8S$ 747.29621, found 747.29711. ^{1}H NMR (400 MHz, CDCl3) δ 8.07 (dd, J = 1.2 Hz, J = 8.4 Hz, 2H, H ortho Bz), 7.63–7.57 (m, 2H, H Ar), 7.44–7.27 (m, 12H, H Ar), 7.23 (dd, J = 2.0 Hz, J = 8.0 Hz, 1H, H Ar), 7.15 (d, J = 8.0 Hz, 1H, H Ar), 5.81 (dd, $J_{2,1}$ = 1.8 Hz, $J_{2,3}$ = 3.1 Hz, 1H, H-2), 5.55–5.51 (m, 2H, H-1, H-3), 4.80–4.74 (m, 2H, C*H*Ph), 4.63 (d, J_{gem} = 11.1 Hz, 1H, C*H*Ph), 4.54 (d, J_{gem} = 12.0 Hz, 1H, C*H*Ph), 4.49 (m, 1H, H-5), 4.34 (dd, $J_{4,3}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.03 (dd, $J_{6,5}$ = 3.2 Hz, J_{gem} = 11.1 Hz, H-6a), 3.76 (dd, $J_{6,5}$ = 1.7 Hz, H-6b), 2.82–2.74 (m, 1H, CH_2 Lev), 2.69–2.46 (m, 3H, CH_2 Lev), 2.45 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CDCl3) δ 206.62 (CO), 172.26 (CO), 165.9 (CO), 150.2–125.8 (21 C, C Ar), 86.3 (C-1), 75.4 (*C*H_2Ph), 74.0 (*C*H_2Ph), 73.4 (2C, C-4, C-5), 73.3 (C-3), 72.7 (C-2), 69.1 (C-6), 38.3 (CH_2 Lev), 31.8 (*t*Bu), 30.2 (CH_3), 28.4 (CH_2 Lev), 20.9 (CH_3).

5.7.16. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-3-*O*-levulinoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (21)

Acceptor **14** and donor **20** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 7/3). Syrup. Yield: 78%. $[\alpha]_D^{20}$ +5 (c = 1,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{66}H_{74}O_{16}$ 1145.48691, found 1145.48687. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, J = 1.1 Hz, J = 8.2 Hz, 2H, H ortho Bz), 7.65–7.59 (m, 1H, H Ar), 7.47–7.17 (m, 26H, H Ar), 7.16–7.10 (m, 1H, H Ar), 5.72 (dd, $J_{2',1'}$ = 2.0 Hz, $J_{2',3'}$ = 3.1 Hz, 1H, H-2′), 5.56 (dd, $J_{3',4'}$ = 9.2 Hz, 1H, H-3′), 5.13 (d, 1H, H-1′), 4.90 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.85 (d, J_{gem} = 10.9 Hz, 1H, C*H*Ph), 4.77–4.62 (m, 6H, C*H*Ph), 4.58–4.51 (m, 3H, C*H*Ph), 4.14 (m, 2H, H-4, H-4′), 4.00–3.97 (m, 1H, H-2), 3.93–3.84 (m, 3H, H-3, 1H-5, H-6a), 3.81–3.72 (m, 4H, 1H-5, 3H-6), 3.69–3.62 (m, 4H, H-7a, CH_3), 3.34–3.29 (m, 1H, H-7b), 2.80–2.72 (m, 1H, CH_2 Lev), 2.69–2.60 (m, 1H, CH_2 Lev), 2.57–2.41 (m, 2H, CH_2 Lev), 2.31 (t, J = 7.5 Hz, 2H, 2H-11), 2.11 (s, 3H, CH_3), 1.67–1.51 (m, 4H, 2H-8, 2H-10), 1.37–1.27 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7 (CO), 174.55 (CO), 171.9 (CO), 165.6 (CO), 139.0–127.7 (30 C, C Ar), 99.9 (C-1′), 99.0 (C-1), 80.2 (C-3), 76.4 (C-2), 75.7 (*C*H_2Ph), 75.4 (1C-5), 75.1 (*C*H_2Ph), 73.8 (*C*H_2Ph), 73.6 (*C*H_2Ph), 73.5 (1C-4), 72.8 (2C, C-3′, *C*H_2Ph), 72.4 (1C-5), 72.2 (1C-4), 70.8 (C-2′), 69.8 (1C-6), 69.4 (1C-6), 67.8 (C-7), 51.9 (CH_3), 38.3 (CH_2 Lev), 34.4 (C-11), 30.2 (CH_3), 29.5 (C-8), 28.4 (CH_2 Lev), 26.1 (C-9), 25.1 (C-10).

5.7.17. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (22)

Compound **21** was submitted to the general procedure for levulinate cleavage. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 7/3). Syrup. Yield: 80%. $[\alpha]_D^{20}$ +20 (c = 1,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{61}H_{68}O_{14}$ 1047.45013, found 1047.45063. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.3 Hz, 2H, H ortho Bz), 7.61 (t, J = 7.4 Hz, 1H, H Ar), 7.47–7.16 (m, 27H), 5.56 (dd, $J_{2',3'}$ = 2.8 Hz, 1H, H-2′), 5.19 (d, $J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 4.92 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.87 (d, J_{gem} = 10.7 Hz, 1H, C*H*Ph), 4.81 (d, J_{gem} = 11.2 Hz, 1H, C*H*Ph), 4.75 (d, J_{gem} = 11.9 Hz, 1H, C*H*Ph), 4.70–4.54 (m, 7H, C*H*Ph), 4.34 (dd, $J_{3',4'}$ = 9.6 Hz, 1H, H-3′), 4.09–4.03 (m, 1H, H-3), 4.00–3.87 (m, 5H, H-2, H-4, H-4′, H-5′, H-6a), 3.83–3.70 (m, 4H, H-5, 3H-6), 3.67 (s, 3H, CH_3), 3.66–3.60 (m, 1H, H-7a), 3.33–3.27 (m, 1H, H-7b), 2.31 (t, J = 7.5 Hz, 2H, 2H-11), 1.69–1.59 (m, 2H, 2H-10), 1.57–1.50 (m, 2H, 2H-8), 1.36–1.28 (m, 2H, 2H-9). ^{13}C NMR (63 MHz, $CDCl_3$) δ 174.1 (CO), 166.1 (CO), 138.6–127.4 (30C, C Ar), 99.5 (C-1′), 98.7 (C-1), 79.7, 75.8 (C-4 or C-4′), 75.8 (C-4 or C-4′), 75.3 (*C*H_2Ph), 74.8 (*C*H_2Ph), 74.8, 73.5 (*C*H_2Ph), 73.3 (*C*H_2Ph), 72.9 (C-2′), 72.2 (*C*H_2Ph), 71.9 (C-3 or C-5), 71.8 (C-3 or C-5), 70.6 (C-3′), 69.3 (1C-6), 69.2 (1C-6), 67.5 (C-7), 51.5 (CH_3), 34.0 (C-11), 29.1 (C-8), 25.7 (C-9), 24.7 (C-10).

5.7.18. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (23)

Acceptor **22** and donor **8** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 75/25). Syrup. Yield: 80%. $[\alpha]_D^{20}$ −9 (c = 1,$CHCl_3$). HRMS: [M+2Na^+]/2 calculated for $C_{95}H_{100}O_{20}$ 803.83132, found 803.83203. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 1.2 Hz, J = 8.3 Hz, 2H, H ortho Bz), 8.03 (dd, J = 1.2 Hz, J = 8.3 Hz, 2H, H ortho Bz), 7.63–7.56 (m, 1H, H Ar), 7.56–7.49 (m, 1H, H Ar), 7.44–7.07 (m, 54H, H Ar), 5.67–5.64 (m, 2H, H-2′, H-2″), 5.32 (d, 1H, $J_{1'',2''}$ = 1.4 Hz, H-1″), 5.22 (d, $J_{1',2'}$ = 1.9 Hz, 1H, H-1′), 4.90 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.87–4.76 (m, 3H, C*H*Ph), 4.73–4.46 (m, 11H, C*H*Ph), 4.41 (dd, $J_{3',2'}$ = 3.2 Hz, $J_{3',4'}$ = 9.1 Hz, 1H, H-3′), 4.37 (d, J_{gem} = 11.3 Hz, 1H, C*H*Ph), 4.31 (d, J_{gem} = 11.9 Hz, 1H, C*H*Ph), 4.17 (m, 2H, H-4, H-4′), 4.09–4.04 (m, 1H, 1H-5), 4.01 (dd, $J_{3''-2''}$ = 3.0 Hz, $J_{3''-4''}$ = 9.5 Hz, 1H, H-3″), 3.93–3.69 (m, 8H, H-2, 2H-5, 4H-6, H-4), 3.66–3.56 (m, 6H, 2H-6, H-7a, CH_3), 3.29–3.23 (m, 1H, H-7b), 2.28 (t, J = 7.5 Hz, 2H, 2H-11), 1.64–1.56 (m, 2H, 2H-10), 1.54–

1.46 (m, 2H, 2H-8), 1.33–1.25 (m, 2H, 2H-9). ^{13}C NMR (63 MHz, $CDCl_3$) δ 174.1 (CO), 165.5 (CO), 165.4 (CO), 138.8–127.2 (60C, C Ar), 99.6 (C-1″), 99.4 (C-1′), 98.6 (C-1), 79.3, 78.0 (C-3′), 75.3 (*C*H_2Ph), 75.3 (*C*H_2Ph), 74.9 (C-4), 74.8 (C-4′ or C-4″), 74.7 (*C*H_2Ph), 73.9 (C-4′ or C-4″), 73.4 (*C*H_2Ph), 73.3 (*C*H_2Ph), 73.2 (*C*H_2Ph), 72.6, 72.3 (C-2′), 72.1 (*C*H_2Ph), 71.2 (1C-5), 71.8, 71.6 (*C*H_2Ph), 69.4 (1C-6), 69.2 (C-2″), 69.1 (1C-6), 68.5 (1C-6), 67.5 (C-7), 51.5 (CH_3), 34.0 (C-11), 29.1 (C-8), 25.7 (C-9), 24.7 (C-10).

5.7.19. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-benzoyl-3-*O*-levulinoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (24)

Acceptor **16** and donor **20** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 7/3). Syrup. Yield: 79%. $[\alpha]_D^{20}$ −97 (c = 1.5,$CHCl_3$): HRMS**:** [M+Na$^+$] calculated for $C_{93}H_{102}O_{21}$ 1578.68398, found 1578.68472. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 1.2 Hz, J = 8.3 Hz, 2H, H ortho Bz), 7.63 (t, J = 7.5 Hz, 1H, H Ar), 7.46–7.05 (m, 42H, H Ar), 5.71 (dd, $J_{2''\text{-}1''}$ = 2.0 Hz, $J_{2''\text{-}3''}$ = 3.1 Hz, 1H, H-2″), 5.56 (dd, $J_{3''\text{-}4''}$ = 9.7 Hz, 1H, H-3″), 5.23 (d, $J_{1',2'}$ = 1.6 Hz, 1H, H-1′), 5.08 (d, 1H, H-1″), 4.93 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.86 (m, 2H, C*H*Ph), 4.75–4.51 (m, 13H, C*H*Ph), 4.38 (d, J_{gem} = 11.9 Hz, 1H, C*H*Ph), 4.20 (dd, $J_{4''\text{-}5''}$ = 9.7 Hz, 1H, H-4″), 4.13–4.09 (m, 1H, H-2′), 4.05–4.02 (m, 2H, H-2, H-5″), 4.02–3.70 (m, 11H, 2H-3, 2H-4, 2H-5, 5H-6), 3.67 (s, 3H, CH_3), 3.60–3.54 (m, 2H, H-7a, 1H-6), 3.23 (m, 1H, H-7b), 2.81–2.73 (m, 1H, CH_2 Lev), 2.67–2.61 (m, 1H, CH_2 Lev), 2.55–2.41 (m, 2H, CH_2 Lev), 2.30 (t, J = 7.6 Hz, 2H, 2H-11), 2.12 (s, 3H, Me), 1.65–1.57 (m, 2H, 2H-10), 1.54–1.46 (m, 2H, 2H-8), 1.29 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.7–127.7 (60 C, C Ar), 100.8 (C-1′), 99.7 (C-1″), 99.2 (C-1), 80.2 (C-3 or C-3′), 79.9 (C-3or C-3′), 76.4 (C-2′), 75.6 (*C*H_2Ph), 75.6 (*C*H_2Ph), 75.5, 75.3, 75.2 (*C*H_2Ph), 75.1 (C-2), 73.8 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.6 (*C*H_2Ph), 73.3 (C-4″), 72.8, 72.7 (C-3″), 72.5 (*C*H_2Ph), 72.3, 72.3, 70.7 (C-2″), 70.1 (1C-6), 69.7 (1C-6), 69.1 (1C-6), 67.8 (C-7), 51.9 (CH_3), 38.4 (CH_2 Lev), 34.4 (C-11), 30.2 (CH_3), 29.6 (C-8), 28.4 (CH_2 Lev), 26.1 (C-9), 25.1 (C-10).

5.7.20. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (25)

Compound **24** was submitted to the general procedure for levulinate cleavage. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 75/25) to yield **25** (1.07 g, 91%) as a syrup. $[\alpha]_D^{20}$ +22 (c = 1,$CHCl_3$): HRMS**:** [M+Na$^+$] calculated for $C_{88}H_{96}O_{19}$ 1479.64380, found 1479.64329. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, J = 1.1 Hz, J = 8.2 Hz, 2H, H ortho Bz), 7.66–7.59 (m, 1H, H Ar), 7.49–7.11 (m, 41H, H Ar), 7.08 (t, J = 7.4 Hz, 1H, H Ar), 5.55 (dd, $J_{2''\text{-}1''}$ = 1.6 Hz, $J_{2''\text{-}3''}$ = 3.1 Hz, 1H, H-2″), 5.28 (d, $J_{1',2'}$ = 1.6 Hz, 1H, H-1′), 5.12 (d, 1H, H-1″), 4.92 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.91–4.78 (m, 3H, C*H*Ph), 4.75–4.52 (m, 12H, C*H*Ph), 4.41 (d, J_{gem} = 11.9 Hz, 1H, C*H*Ph), 4.34 (dd, $J_{3''\text{-}4''}$ = 8.8 Hz, 1H, H-3″), 4.13–4.09 (m, 1H, H-2′), 4.08–3.94 (m, 5H, H-2, H-3′, H-4″), 3.92–3.70 (m, 9H, 5H-6), 3.68 (s, 3H, CH_3), 3.64–3.57 (m, 2H, H-6a, H-7a), 3.30–3.24 (m, 1H, H-7b), 2.31 (t, J = 7.5 Hz, 2H, 2H-11), 1.92 (s, 1H, OH), 1.66–1.59 (m, 2H, 2H-10), 1.56–1.49 (m, 2H, 2H-8), 1.36–1.26 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.6–127.8 (45 C, C Ar), 101.0 (C-1′), 99.7 (C-1″), 99.2 (C-1), 80.1, 79.6 (C-3′), 76.4 (C-2′), 76.0, 75.6, 75.5 (*C*H_2Ph), 75.3, 75.2, 73.8 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.6 (*C*H_2Ph), 73.2 (C-2″), 72.7 (*C*H_2Ph), 72.6, 72.5 (*C*H_2Ph), 72.3, 72.2, 70.9 (C-3″), 69.9 (1C-6), 69.7 (1C-6), 69.4 (1C-6), 67.8 (C-7), 51.9 (CH_3), 34.4 (C-11), 29.6 (C-8), 26.2 (C-9), 25.2 (C-10).

5.7.21. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (26)

Acceptor **25** and donor **8** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 8/2). Syrup. Yield: 86%. $[\alpha]_D^{20}$ +5 (c = 1,$CHCl_3$): HRMS**:** [M+Na$^+$] calculated for $C_{122}H_{128}NaO_{25}^+$ 2015.86369, found 2015.86336.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (dd, J = 1.1 Hz, J = 8.2 Hz, 2H, H ortho Bz), 8.07 (dd, J = 1.2 Hz, J = 8.3 Hz, 2H, H ortho Bz), 7.67–7.62 (m, 1H, H Ar), 7.59–7.54 (m, 1H, H Ar), 7.46–7.10 (m, 58H, H Ar), 7.01 (t, J = 7.4 Hz, 1H, H Ar), 5.74–5.70 (m, 1H, H-2‴), 5.68–5.65 (m, 1H, H-2″), 5.36 (d, $J_{1'''\text{-}2'''}$ = 1.5 Hz, 1H, H-1‴), 5.29 (d, $J_{1',2'}$ = 1.4 Hz, 1H, H-1′), 5.17 (d, $J_{1''\text{-}2''}$ = 1.6 Hz 1H, H-1″), 4.93 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.92–4.80 (m, 4H, C*H*Ph), 4.75–4.50 (m, 15H, C*H*Ph), 4.45 (dd, $J_{3''\text{-}2''}$ = 3.1 Hz, $J_{3''\text{-}4''}$ = 9.4 Hz, 1H, H-3″), 4.43–4.31 (m, 3H, C*H*Ph), 4.27–4.20 (m, 2H, H-4‴, H-4″), 4.08 (m, 1H, H-2′), 4.05–3.93 (m, 7H, H-2, H-3‴, 2H-5), 3.88 (dd, $J_{3,2}$ = 2.8 Hz, $J_{3,4}$ = 9.1 Hz, 1H, H-3 or H-3′), 3.85–3.70 (m, 9H, 5H-6), 3.69–3.64 (m, 4H, 1H-6, CH_3), 3.62–3.56 (m, 3H, 2H-6, H-7a), 3.27–3.22 (m, 1H, H-7b), 2.30 (t, J = 7.5 Hz, 2H, 2H-11), 1.66–1.58 (m, 2H, 2H-10), 1.55–1.47 (m, 2H, 2H-8), 1.35–1.25 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.6–127.7 (65C, C Ar), 101.0 (C-1′), 100.0 (C-1‴), 99.6 (C-1″), 99.2 (C-1), 80.1 (C-3 or C-3′), 79.4, 78.4, 78.0 (C-3″), 77.0 (C-2′), 75.8 (*C*H_2Ph), 75.6 (*C*H_2Ph), 75.6, 75.3, 75.2 (*C*H_2Ph), 75.0 (C-4‴ or C-4″), 74.3 (C-4‴ or C-4″), 73.7 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.6 (*C*H_2Ph), 73.0, 72.7 (C-2″), 72.5, 72.5 (*C*H_2Ph), 72.4, 72.2, 72.0 (*C*H_2Ph), 70.1, 69.7 (2C, 2C-6), 69.6 (C-2‴), 69.3 (1C-6), 68.8 (1C-6), 67.8 (C-7), 51.9 (CH_3), 34.4 (C-11), 29.6 (C-8), 26.1 (C-9), 25.2 (C-10).

5.7.22. 5-Ethoxycarbonylpentyl 2-*O*-benzoyl-3-*O*-levulinoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (27)

Donor **20** (0.296 g, 0.408 mmol) was submitted to the general procedure A for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 7/3) to yield **27** (0.170 g, 59%) as a syrup. $[\alpha]_D^{20}$ −8 (c = 0.3, $CHCl_3$): HRMS**:** [M+Na$^+$] calculated for $C_{40}H_{48}O_{11}$ 727.30888, found 727.30989. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, J = 1.2 Hz, J = 8.3 Hz, 2H, H ortho Bz), 7.62–7.55 (m, 1H, H Ar), 7.44–7.19 (m, 13H, H Ar), 5.46 (m, 2H, H-2, H-3), 4.91 (s, 1H, H-1), 4.76 (d, J_{gem} = 11.9 Hz, 1H, C*H*Ph), 4.67 (d, J_{gem} = 11.1 Hz, 1H, C*H*Ph), 4.56–4.51 (m, 2H, C*H*Ph) 4.17 (dd, $J_{4,3}$ = $J_{4,5}$ = 9.3 Hz, 1H, H-4), 4.12 (q, J = 7.1 Hz, 2H, CH_2 Et), 3.94–3.88 (m, 2H, H-5, 1H-6), 3.80–3.68 (m, 2H, 1H-6, H-7a), 3.48–3.42 (m, 1H, H-7b), 2.76–2.68 (m, 1H, CH_2 Lev), 2.63–2.56 (m, 1H, CH_2 Lev), 2.53–2.36 (m, 2H, CH_2 Lev), 2.30 (t, J = 7.5 Hz, 2H, 2H-11), 2.09 (s, 3H, CH_3), 1.68–1.57 (m, 4H, 2H-8, 2H-10), 1.43–1.34 (m, 2H, 2H-9), 1.25 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7 (CO), 174.1 (CO), 172.2 (CO), 165.9 (CO), 138.7–128.0 (18C, C Ar), 98.0 (C-1), 75.3 (*C*H_2Ph), 73.9 (*C*H_2Ph), 73.3 (C-4), 73.0 (C-3), 71.9 (C-5), 70.9 (C-2), 69.2 (C-6), 68.3 (C-7), 60.6 (CH_2 Et), 38.3 (CH_2 Lev), 34.6 (C-11), 31.4 (CH_3 Lev), 29.58 (C-8 or C-10), 28.4 (CH_2 Lev), 26.1 (C-9), 25.1 (C-8 or C-10), 14.7 (CH_3 Et).

5.7.23. 5-Ethoxycarbonylpentyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (28)

Compound **27** was submitted to the general procedure for levulinate cleavage. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 8/2). Syrup. Yield: 87%. $[\alpha]_D^{20}$ −3 (c = 1, $CHCl_3$). HRMS: [M+Na]$^+$ calcd for $C_{35}H_{42}O_9Na$ 629.27210, found 629.27213.^{1}H NMR (250 MHz, CDCl3) δ 8.04–7.23 (m, 15H, H Ar), 5.34 (dd, $J_{2,1}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, 1H, H-2), 4.95 (d, 1H, H-1), 4.84–4.71 (m, 2H, C*H*Ph), 4.66 4.54 (m, 2H, C*H*Ph), 4.31–4.22 (m, 1H, H-3), 4.12 (q, J = 7.1 Hz, 2H, CH2 Et), 4.00 (dd,

$J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 3.92–3.65 (m, 4H, H-5, 2H-6, H-7a), 3.49–3.40 (m, 1H, H-7b), 2.30 (t, J = 7.5 Hz, 2H, 2H-11), 2.11 (s, 1H, OH), 1.72–1.53 (m, 4H, 2H-8, 2H-10), 1.40–1.34 (m, 2H, 2H-9), 1.25 (t, J = 7.1 Hz, 3H, CH3 Et). ^{13}C NMR (63 MHz, $CDCl_3$) δ 133.28–127.57 (18C, C Ar), 97.52 (C-1), 75.82 (C-4), 74.97 (*C*H_2Ph), 73.53 (*C*H_2Ph), 73.04 (C-2), 71.40 (C-5), 70.65 (C-3), 69.02 (C-6), 67.83 (C-7), 60.26 (CH_2 Et), 34.23 (C-11), 29.13 (C-8 or C-10), 25.74 (C-9), 24.73 (C-8 or C-10), 14.27 (CH_3 Et).

5.7.24. 5-Ethoxycarbonylpentyl 2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranoside (29)

Acceptor **28** and donor **8** were submitted to the general procedure B for glycosylation. The crude product was purified by flash chromatography (cyclohexane/ethyl acetate: 8/2). Syrup. Yield: 78%. $[\alpha]_D^{20}$ −23 (c = 1,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{69}H_{74}O_{15}$ 1165.49199, found 1165.49272. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10–8.02 (m, 4H, H ortho Bz), 7.62–7.51 (m, 2H, H Ar), 7.43–7.21 (m, 24H, H Ar), 7.20–7.13 (m, 5H, H Ar), 7.09 (dd, J = 3.0 Hz, 6.5, 2H, H Ar), 5.65 (dd, $J_{2',3'}$ = 3.0 Hz, 1H, H-2′), 5.48 (dd, $J_{2,3}$ = 3.0 Hz, 1H, H-2), 5.33 (d, $J_{1',2'}$ = 1.7 Hz, 1H, H-1′), 4.97 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.83–4.72 (m, 4H, C*H*Ph), 4.64–4.44 (m, 5H, C*H*Ph), 4.41–4.35 (m, 2H, H-3, C*H*Ph), 4.22–4.08 (m, 4H, H-4, H-4′, CH_2 Et), 3.99 (dd, $J_{3',4'}$ = 9.4 Hz, 1H, H-3′), 3.90 (m, 2H, H-5′, 1H-6), 3.86–3.81 (m, 1H, H-5), 3.77–3.64 (m, 4H, 3H-6, H-7a), 3.45–3.40 (m, 1H, H-7b), 2.30 (t, J = 7.6 Hz, 2H, 2H-11), 1.68–1.54 (m, 4H, 2H-8, 2H-10), 1.42–1.33 (m, 2H, 2H-9), 1.24 (t, J = 7.1 Hz, 3H, CH_3 Et). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.04 (CO), 166.24 (CO), 165.88 (CO), 139.03–127.72 (42 C, C Ar), 100.28 (C-1′), 97.59 (C-1), 78.72 (C-3), 78.24 (C-3′), 75.91 (*C*H_2Ph), 75.05 (*C*H_2Ph), 74.99 (C-4), 74.32 (C-4′), 73.88 (*C*H_2Ph), 73.77 (*C*H_2Ph), 72.91 (C-2), 72.87 (C-5′), 71.99 (C-5), 69.70 (C-2′), 69.25 (1C-6), 68.95 (1C-6), 68.29 (1C-7), 60.61 (CH_2 Et), 34.61 (C-11), 29.52 (C-8 or C-10), 26.10 (C-9), 25.12 (C-8 or C-10), 14.66 (CH_3 Et).

5.7.25. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopy ranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (31)

To a solution of **26** (1.14 g, 0.57 mmol) in dry methanol (10 ml) and dry THF (2 ml) was added under argon sodium (0.007 g, 0.28 mmol). The reaction mixture was stirred at 40 °C overnight then neutralized with IR-120-H^+ amberlite resin, filtered and concentrated. The crude product was purified by column chromatography (cyclohexane/ethyl acetate: 75/25) to give **31** (0.83 g, 81%) as a syrup. $[\alpha]_D^{20}$ +32 (c = 1,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{108}H_{120}O_{23}$ 1808.81462, found 1808.81244. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.12 (m, 55H, H Ar), 5.21 (d, $J_{1'''-2'''}$ = 1.6 Hz, 1H, H-1‴), 5.08 (d, $J_{1',2'}$ = 1.3 Hz, 1H, H-1′), 5.00 (d, $J_{1''-2''}$ = 1.6 Hz, 1H, H-1″), 4.92 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.86 (d, J_{gem} = 10.9 Hz, 1H, C*H*Ph), 4.81 (d, J_{gem} = 10.8 Hz, 2H, C*H*Ph), 4.67 (m, 3H, C*H*Ph), 4.61–4.46 (m, 13H, C*H*Ph), 4.46–4.38 (m, 2H, C*H*Ph), 4.37–4.34 (m, 1H, H-2″), 4.34–4.26 (m, 2H, 1 C*H*Ph), 4.12–4.08 (m, 1H, H-2‴), 4.07–4.03 (m, 1H, H-3″), 4.01–3.94 (m, 3H, H-2, H-2′), 3.93–3.67 (m, 14H), 3.64 (s, 3H, CH_3), 3.63–3.45 (m, 5H), 3.24–3.18 (m, 1H, H-7a), 2.27 (t, J = 7.6 Hz, 2H, 2H-11), 1.62–1.54 (m, 2H, 2H-10), 1.51– 1.44 (m, 2H, 2H-8), 1.31–1.22 (m, 2H, 2H-9). ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.9–127.7 (66C, C Ar), 102.4 (C-1″), 101.3 (C-1‴), 100.3 (C-1′), 99.2 (C-1), 82.9 (C-3″), 80.5, 79.8, 75.7 (C-2), 75.6 (*C*H_2Ph), 75.5 (*C*H_2Ph), 75.4 (*C*H_2Ph), 75.4 (C-2‴), 75.3, 75.1, 74.2, 73.8 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.6 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.6, 72.3 (*C*H_2Ph), 72.2, 72.1, 72.1 (*C*H_2Ph), 71.9, 70.1 (1C-6), 69.8 (1C-6), 69.5 (1C-6), 69.3 (2C, C-2′, C-2″), 67.8 (C-7), 51.9 (CH_3), 34.4 (C-11), 29.6 (C-8), 26.1 (C-9), 25.1 (C-10).

5.7.26. 5-Methoxycarbonylpentyl 2-*O*-(3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (32)

Compound **23** (0.109 g, 0.070 mmol) was submitted to the general procedure for Zemplen debenzoylation. The crude product was purified by column chromatography (cyclohexane/ethyl acetate: 7/3) to give **32** (0.071 g, 75%) as a syrup. $[\alpha]_D^{20}$ +27 (c = 0.4, $CHCl_3$): HRMS**:** [M+2Na^+]/2 calculated for $C_{81}H_{92}O_{18}$ 699.30340, found 699.30437. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.15 (m, 50H), 5.06 (d, J = 1.4, 1H), 5.00 (d, J = 1.5, 1H), 4.89 (dd, J = 6.2, 8.3, 2H), 4.83 (d, J = 11.0, 1H), 4.71 (s, 1H), 4.70–4.62 (m, 5H), 4.60–4.41 (m, 11H), 4.38 (dd, J = 2.6, 6.0, 1H), 4.30 (t, J = 9.1, 1H), 4.05 (dd, J = 3.0, 9.3, 1H), 4.01–3.89 (m, 5H), 3.88–3.78 (m, 5H), 3.78–3.68 (m, 7H), 3.66 (s, 3H), 3.59 (ddd, J = 8.3, 11.1, 13.3, 3H), 3.52–3.46 (m, 1H), 3.23 (dt, J = 6.5, 9.4, 1H), 2.42 (d, J = 1.8, 1H), 2.29 (t, J = 7.5, 2H), 1.83 (s, 1H), 1.65–1.56 (m, 3H), 1.54–1.45 (m, 2H), 1.34–1.24 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.59, 128.47, 128.37, 128.33, 128.30, 128.10, 128.04, 127.98, 127.93, 127.87, 127.81, 127.69, 127.62, 127.58, 127.52, 127.49, 127.37, 102.21, 99.87, 98.82, 82.61, 80.12, 79.74, 75.24, 75.08, 74.94, 74.68, 73.88, 73.48, 73.38, 73.29, 72.16, 71.96, 71.86, 71.66, 71.49, 69.45, 69.35, 69.15, 69.00, 68.88, 67.41, 51.51, 33.99, 29.16, 25.75, 24.76.

5.7.27. 5-Carboxypentyl 2-*O*-(2-*O*-(3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside biotin conjugate (33)

Compound **31** (0.44 g, 0.247 mmol) was submitted to the general procedure for saponification–biotinylation sequence. The crude product was purified by column chromatography (CH_2Cl_2/methanol: 95/5) to give **32** (0.393 g, 75%) as a syrup. $[\alpha]_D^{20}$ +39 (c = 1,$CHCl_3$). HRMS**:** [M+2Na^+]/2 calculated for $C_{123}H_{146}O_{24}N_4S$ 1070.99328, found 1070.99258. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.03 (m, 55H, H Ar), 6.39 (s, 1H, NH), 6.25 (br s, 1H, NH_{urea}), 5.94 (s, 1H, NH), 5.11 (s, 1H, H-1‴), 4.99 (s, 1H, H-1′), 4.92 (s, 1H, H-1″), 4.83 (s, 1H, H-1), 4.79–4.71 (m, 3H, C*H*Ph), 4.60–4.54 (m, 3H, C*H*Ph), 4.52–4.37 (m, 12H, C*H*Ph), 4.32 (m, 3H, H-26, 2 C*H*Ph), 4.28–4.13 (m, 4H, H-2″, 1H-5, H-25, 1 C*H*Ph), 4.00 (s, 1H, H-2‴), 3.98–3.36 (m, 23H), 3.20–2.96 (m, 5H, H-7b, 2H-13, 2H-18), 2.74 (dd, $J_{27\text{-}26}$ = 4.4 Hz, J_{gem} = 12.7 Hz, 1H, 1H-27), 2.60 (m, 1H, 1H-27), 2.14 (t, J = 6.8 Hz, 2H, 2H-11 or 2H-20), 2.03 (t, J = 7.3 Hz, 2H, 2H-11 or 2H-20), 1.67–1.14 (m, 20H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22, 2H-23). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.6–127.5 (66C, C Ar), 102.0 (C-1″), 101.0 (C-1‴), 100.1 (C-1′), 98.8 (C-1), 82.5, 80.2, 79.5, 77.3, 75.4 (C-2), 75.3 (*C*H_2Ph), 75.1 (*C*H_2Ph), 75.0, 74.9 (C-2‴), 74.7, 73.8, 73.5 (*C*H_2Ph), 73.4 (*C*H_2Ph), 73.3 (*C*H_2Ph), 72.3 (*C*H_2Ph), 72.2, 72.0 (*C*H_2Ph), 71.9, 71.8, 71.7 (*C*H_2Ph), 71.6, 69.7 (1C-6), 69.5 (1C-6), 69.2 (1C-6), 69.0 (C-2″), 68.9 (C-2′), 67.5 (C-7), 62.06 (C-25), 60.3 (C-26), 55.5 (C-24), 40.5 (C-27), 39.2 (2C, C-13, C-18), 36.6 (C-11 or C-20), 35.7 (C-11 or C-20), 29.4– 25.7 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.28. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-benzoyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-ben zyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyran oside biotin conjugate (34)

Compound **32** (0.44 g, 0.247 mmol) was submitted to the general procedure for saponification–biotinylation sequence. The crude product was purified by column chromatography (CH_2Cl_2 / methanol: 95/5). Syrup. Yield: 72%. $[\alpha]_D^{20}$ +36 (c = 1,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{96}H_{118}O_{21}N_4S$ 1718.79343, found 1718.79428. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.12 (m, 40H, H

Ar), 6.87 (s, 1H, NH_{urea}), 6.49 (s, 1H, NH), 6.23 (s, 1H, NH_{urea}), 5.95 (t, J = 5.2 Hz, 1H, NH), 5.06 (d, $J_{1',2'}$ = 1.0 Hz, 1H, H-1′), 4.98 (s, 1H, H-1″), 4.88 (d, $J_{1,2}$ = 1.3 Hz, 1H, H-1), 4.85 (d, J_{gem} = 10.7 Hz, 1H, C*H*Ph), 4.80 (d, J_{gem} = 10.9 Hz, 1H, C*H*Ph), 4.69–4.39(m, 16H, H-25, H-26, 14 C*H*Ph), 4.36–4.31 (m, 1H, H-2″), 4.34–4.31 (m, 1H, 1H-5), 4.05–3.97 (m, 3H, H-2, H-2′, H-3″), 3.95–3.82 (m, 3H, H-3, H-3′, 1H-5), 3.80 (t, J = 9.5 Hz, 2H, H-4″, 1H-4), 3.77–3.66 (m, 6H, 1H-5, 5H-6), 3.62 (dd, J = 9.1 Hz, J = 9.8 Hz, 1H, 1H-4), 3.58–3.52 (m, 1H, H-7a), 3.48 (dd, $J_{6,5}$ = 8.1 Hz, J_{gem} = 9.5 Hz, 1H, 1H-6), 3.27–3.21 (m, 1H, H-7b), 3.20–3.12 (m, 4H, 2H-13, 2H-18), 3.05 (s, 1H, H-24), 2.90–2.84 (m, 1H, 1H-27), 2.58 (br s, 1H, 1H-27), 2.24–2.15 (m, 2H, 2H-11 or 2H-20), 2.09 (t, J = 7.6 Hz, 2H, 2H-11 or 2H-20), 1.91–1.22 (m, 20H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22, 2H-23). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C-12 or C-19), 173.3 (C-12 or C-19), 162.9 (C_{urea}), 138.6–127.5 (48C, C Ar), 102.2 (C-1′), 100.1 (C-1″), 98.8 (C-1), 82.3 (C-3″), 80.1 (C-3 or C-3′), 79.7 (C-3 or C-3′), 75.3 (*C*H_2Ph), 75.1 (*C*H_2Ph), 75.1 (*C*H_2Ph), 75.0 (C-2 or C-2′), 74.9 (1C-4), 74.7 (1C-4), 73.9 (1C-4), 73.5 (*C*H_2Ph), 73.4 (*C*H_2Ph), 73.3 (*C*H_2Ph), 72.1 (*C*H_2Ph), 71.9 (*C*H_2Ph), 71.8 (1C-5), 71.7 (1C-5), 71.5 (1C-5), 69.5 (1C-6), 69.4 (1C-6), 69.1 (C-2″), 68.9 (C-2 or C-2′), 67.5 (C-7), 61.3 (C-27), 54.1 (C-25 or C-26), 49.7 (C-25 or C-26), 39.1 (2C, C-13, C-18), 36.6 (C-11 or C-20), 35.6 (C-11 or C-20), 29.7–14.2.(C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.29. 5-Carboxypentyl 2-*O*-(2-*O*-(3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside oxidized biotin conjugate (35)

Compound **33** (0.345 g, 0.163 mmol) was submitted to the general procedure for biotin oxidation. The crude product was purified by column chromatography (CH_2Cl_2/methanol: 93/7 → 92/8) to give compound **35** (0.265 g, 76%) as a syrup. $[\alpha]_D^{20}$ +31 (c = 1,$CHCl_3$). HRMS**:** [M+Na^+]/2 calculated for $C_{123}H_{146}O_{26}N_4S$ 1086.98820, found 1086.98804. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.12 (m, 55H, H Ar), 6.85 (s, 1H, NH_{urea}), 6.52 (br s, 1H, NH), 6.20 (br s, 1H, NH_{urea}), 5.88 (s, 1H, NH), 5.20 (s, 1H, H-1‴), 5.08 (s, 1H, H-1′), 5.03 (s, 1H, H-1″), 4.92 (s, 1H, H-1), 4.89–4.78 (m, 3H, C*H*Ph), 4.70–4.63 (m, 3H, C*H*Ph), 4.61–4.46 (m, 13H, C*H*Ph), 4.45–4.37 (m, 4H, H-25, H-26, 2 C*H*Ph), 4.37–4.25 (m, 3H, H-2″, 1H-5, 1 C*H*Ph), 4.11 (s, 1H, H-2‴), 4.07–3.99 (m, 2H, H-2′, H-3‴), 3.99–3.46 (m, 20H, H-2, 3H-3, 4H-4, 3H-5, 8H-6, H-7a), 3.28–3.00 (m, 7H, H-7b, 2H-13, 2H-18, 2H-27), 2.86 (s, 1H, H-24), 2.20 (m, 2H, 2H-11 or 2H-20), 2.09 (t, J = 7.5 Hz, 2H, 2H-11 or 2H-20), 1.91–1.28 (m, 20H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22, 2H-23). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0 (C-12 or C-19), 173.7 (C-12 or C-19), 163.3 (C_{urea}), 139.1–127.8 (66C, C Ar), 102.3 (C-1″), 101.3 (C-1‴), 100.4 (C-1′), 99.1 (C-1), 82.9 (C-3″), 80.5, 79.9, 75.7 (C-2), 75.6 (*C*H_2Ph), 75.5 (*C*H_2Ph), 75.3, 75.3, 75.1 (C-2‴), 74.2, 73.8 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.6 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.6, 72.3 (*C*H_2Ph), 72.2, 72.2, 72.1 (*C*H_2Ph), 71.9, 70.0 (1C-6), 69.8 (1C-6), 69.5 (C-2″), 69.3 (C-2′), 67.9 (C-7), 61.5 (C-24), 54.5 (C-25 or C-26), 54.5 (C-27), 50.1 (C-25 or C-26), 39.6 (C-13 or C-18), 39.5 (C-13 or C-18), 37.0 (C-11 or C-20), 35.9 (C-11 or C-20), 31.4–21.6 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.30. 5-Carboxypentyl 3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranoside biotin conjugate (37)

Compound **29** was submitted to the general procedure for saponification–biotinylation sequence. The crude product was purified by column chromatography (CH_2Cl_2 /methanol: 9/1). Syrup. Yield: 47%. $[\alpha]_D^{20}$ +33 (c = 0.4,$CHCl_3$): HRMS: [M+Na^+] calculated for $C_{108}H_{120}O_{23}$ 1253.60665, found 1253.60778. ^{1}H NMR (400 MHz, $CDCl_3$): 7.33–7.32 (m, 21H, H Ar), 7.18–7.14 (m, 4H, H Ar), 6.45 (t, 1H, J = 5.7 Hz, NH), 6.41 (br s, 1H, NH_{urea}), 6.01 (t, 1H, J = 5.7 Hz, NH), 5.08 (s, 1H, H-1′), 4.82 (d, 1H, J_{gem} = 10.9 Hz, C*H*Ph), 4.66–4.49 (m, 9H, H-1, 8 C*H*Ph), 4.46 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.39 (d, 1H, J_{gem} = 11.0 Hz, C*H*Ph), 4.37–4.34 (m, 1H, H-26), 4.29–4.24 (m, 1H, H-5′), 4.20–4.17 (m, 1H, H-25), 4.13 (s, 1H, H-2), 4.04 (s, 1H, H-2′), 3.95 (dd, 1H, $J_{3,2}$ = 3.0 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 3.88 (dd, 1H, $J_{3',2'}$ = 3.1 Hz, $J_{3',4'}$ = 8.8 Hz, H-3′), 3.82–3.77 (m, 2H, H-4, 1H-6), 3.71–3.60 (m, 5H, H-4′, H-5, 2H-6, H-7a), 3.54–3.50 (m, 1H, 1H-6), 3.36–3.30 (m, 1H, H-7b), 3.20–3.15 (m, 4H, 2H-13, 2H-18), 3.09–3.04 (m, 1H, H-24), 2.78 (dd, 1H, J_{gem} = 12.9 Hz, $J_{27\text{-}26}$ = 4.9 Hz, 1H-27), 2.62 (d, 1H, J_{gem} = 12.9 Hz, 1H-27), 2.18 (t, 2H, J = 7.3 Hz, 2H-11 or 2H-20), 2.13 (t, 2H, J = 7.6 Hz, 2H-11 or 2H-20), 1.72–1.24 (m, 20H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22, 2H-23). ^{13}C NMR (100 MHz, $CDCl_3$): 173.4 (C-12 or C-19), 173.3 (C-12 or C-19), 164.0 (C_{urea}), 138.4–137.4 (6qC Ar), 128.5–127.6 (C ar), 100.6 (C-1′), 100.3 (C-1), 82.5 (C-3), 80.1 (C-3′), 75.2 (*C*H_2Ph), 75.1 (*C*H_2Ph), 74.8 (C-4′), 74.0 (C-4), 73.6 (*C*H_2Ph), 73.4 (*C*H_2Ph), 71.9 (*C*H_2Ph), 71.5 (C-5′), 71.3 (C-5), 69.5 (1C-6), 69.3 (C-2), 68.9 (C-2′), 67.5 (C-7), 61.3 (C-25), 60.2 (C-26), 55.7 (C-24), 40.5 (C-27), 39.0 (C-13, C-18), 36.6 (C-11 or C-20), 35.9 (C-11 or C-20), 29.4–25.6 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.31. 5-Carboxypentyl 3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-4,6-di-*O*-benzyl-α-D-mannopyranoside oxidize biotin conjugate (38)

Compound **37** was submitted to the general procedure for biotin oxidation. The crude product was purified by column chromatography (CH_2Cl_2/methanol: 9/1). Syrup. Yield: 76%. $[\alpha]_D^{20}$ +36 (c = 1, $CHCl_3$). HRMS: [M+Na]$^+$ calcd for $C_{69}H_{90}O_{16}N_4Na$ 1285.59647, found 1285.59746. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.15 (m, 25H, H Ar), 6.81 (s, 1H, NH_{urea}), 6.64 (s, 1H, NH), 6.26 (s, 1H, NH_{urea}), 6.14 (s, 1H, NH), 5.11 (s, 1H, H-1′), 4.85 (d, J = 10.8 Hz, 1H, C*H*Ph), 4.69–4.41 (m, 12H, H-1, H-25, H-26, 9 C*H*Ph), 4.33–4.24 (m, 1H, H-5′), 4.15 (s, 1H, H-2), 4.06 (s, 1H, H-2′), 3.98 (dd, $J_{3,2}$ = 2.0 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 3.92 (dd, $J_{3',2'}$ = 2.4 Hz, $J_{3',4'}$ = 8.8 Hz, 1H, H-3′), 3.84–3.55 (m, 8H, H-4, H-4′, H-5, 4H-6, H-7a), 3.39–3.32 (m, 1H, H-7b), 3.29–3.04 (m, 6H, 2H-13, 2H-18, 2H-27), 2.93 (br s, 1H, H-24), 2.23–2.13 (m, 4H, 2H-11, 2H-20), 1.75–1.35 (m, 20H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22, 2H-23). ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.5 (C-12 or C-19), 173.3 (C-12 or C-19), 163.1 (C_{urea}), 138.4–127.5 (30 C, C Ar), 100.6 (C-1′), 100.2 (C-1), 82.4 (C-3), 80.0 (C-3′), 75.2 (*C*H_2Ph), 75.1 (*C*H_2Ph), 74.8 (C-4 or C-4′), 74.0 (C-4 or C-4′), 73.6 (*C*H_2Ph), 73.4 (*C*H_2Ph), 71.9 (*C*H_2Ph), 71.5 (C-5′), 71.3 (C-5), 69.5 (C-6′), 69.4 (C-2), 69.3 (C-6), 68.9(C-2″), 67.5 (C-7), 39.1 (2C, C-13, C-18), 36.6 (C-11 or C-20), 35.6 (C-11 or C-20), 29.7–25.6 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.32. Compound (1)

Compound **39** was submitted to the general procedure for hydrogenolysis to give **1** (93%) as a white solid. $[\alpha]_D^{20}$ +59 (c = 0.8, MeOH). HRMS: [M+Na^+] calculated for $C_{34}H_{60}O_{16}N_4S$ 835.36172, found 835.36161. ^{1}H NMR (400 MHz, D_2O) δ 5.05 (d, $J_{1',2'}$ = 1.2 Hz, 1H, H-1′), 4.76 (d, $J_{1,2}$ = 1.0 Hz, 1H, H-1), 4.72–4.63 (m, 2H, H-25, H-26), 4.00 (s, 2H, H-2, H-2′), 3.81 (m, 4H, H-3, H-3′, 2H-6), 3.74–3.54 (m, 7H, H-4, H-4′, H-5, H-5′, 2H-6, H-7a), 3.51–3.39 (m, 2H, H-7b, 1H-27), 3.35 (m, 1H, H-24), 3.28 (d, J_{gem} = 14.9 Hz, 1H, 1H-27), 3.10 (m, 4H, 2H-13, 2H-18), 2.19 (m, 4H, 2H-11, 2H-20), 1.88–1.80 (m, 1H, 1H-23), 1.70–1.65 (m, 1H, 1H-23), 1.64–1.23 (m, 18H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22). ^{13}C NMR (100 MHz, D_2O) δ 177.1 (C-12 or C-19), 176.7 (C-12 or C-19), 102.7 (C-1′), 100.0 (C-1), 78.6 (C-3 or C-3′), 73.7 (C-4 or C-4′), 73.3 (C-4 or C-4′), 70.7 (C-3 or C-3′), 70.4 (C-2 or C-2′), 70.1 (C-2 or C-2′), 68.0 (C-7), 67.2 (C-5 or C-5′), 66.5 (C-5 or C-5′),

61.4 (C-6 or C-6′), 61.2 (C-6 or C-6′), 60.7 (C-24), 54.6 (C-25 or C-26), 54.1 (C-27), 50.1 (C-25 or C-26), 39.6 (C-13 or C-18), 39.5 (C-13 or C-18), 36.1 (C-11 or C-20), 35.8 (C-11 or C-20), 28.6–21.3 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.33. Compound (2)

Compound **34** submitted to the general procedure for biotin oxidation. The crude product was submitted to the general procedure for hydrogenolysis to give **2** (82%, two steps) as a white solid. $[\alpha]_D^{20}$ +44 (c = 0.5, MeOH). HRMS: [M+Na$^+$] calculated for $C_{40}H_{70}O_{21}N_4S$ 997.41455, found 997.41443. ^{1}H NMR (400 MHz, D_2O) δ 5.15 (d, $J_{1,2}$ = 1.3 Hz, 1H, 1H-1), 5.09 (d, $J_{1,2}$ = 0.7 Hz, 1H, 1H-1), 5.01 (d, $J_{1,2}$ = 1.3 Hz, 1H, 1H-1), 4.24 (dd, $J_{2,3}$ = 3.0 Hz, 1H, 1H-2), 4.08 (dd, $J_{2,3\ =\ 3.3}$ Hz, 1H, 1H-2), 3.98–3.90 (m, 7H, 1H-2, 3H-3, 3H-6), 3.85–3.58 (m, 10H, 3H-4, 3H-5, 3H-6, H-7a), 3.57–3.47 (m, 2H, H-7b, 1H-27), 3.43 (m, 1H, H-24), 3.36 (d, J_{gem} = 15.0 Hz, 1H, 1H-27), 3.21–3.14 (m, 4H, 2H-13, 2H-18), 2.31–2.21 (m, 4H, 2H-11, 2H-20), 1.99–1.88 (m, 1H, 1H-23), 1.83–1.74 (m, 1H, 1H-23), 1.73–1.25 (m, 18H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22). ^{13}C NMR (100 MHz, D_2O) δ 176.8 (C-12 or C-19), 176.4 (C-12 or C-19), 164.2 (C_{urea}), 102.3 (1C-1), 102.2 (1C-1), 98.1 (1C-1), 78.8 (1C-2), 77.9 (1C-3), 73.5 (1C-4), 73.4 (1C-4), 72.8 (1C-4), 70.4 (2C, 2C-3), 70.1 (1C-2), 69.6 (1C-2), 67.74 (C-7), 67.0 (1C-5), 66.9 (1C-5), 66.4 (1C-5), 61.1 (2C, 2C-6), 61.0 (1C-6), 60.4 (C-24), 54.2 (C-25 or C-26), 53.8 (C-27), 49.8 (C-25 or C-26), 39.2 (2C, C-13, C-18), 35.8 (C-11 or C-20), 35.4 (C-11 or C-20), 28.2–21.0 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.7.34. Compound (3)

Compound **33** (0.118 g, 0.055 mmol) was submitted to the general procedure for hydrogenolysis to give **3** (0.059 g, 94%) as a white solid). $[\alpha]_D^{20}$ +54 (c = 0.5, MeOH). HRMS: [M+Na$^+$] calculated for $C_{46}H_{80}O_{26}N_4S$ 1159.46737, found 1159.46708. [M+Na$^+$]/2 calculated for $C_{46}H_{80}O_{26}N_4S$ 591.22830, found 591.22799. ^{1}H NMR (400 MHz, D_2O) δ 5.28 (d, $J_{1''',2'''}$ = 1.3 Hz, 1H, H-1‴), 5.13 (d, $J_{1',2'}$ = 1.4 Hz, 1H, H-1′), 5.07 (s, 1H, H-1″), 5.02 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.78–4.69 (m, 2H, H-25, H-26), 4.21 (dd, $J_{2,3}$ = 3.0 Hz, 1H, H-2), 4.10 (dd, $J_{2''',3'''}$ = 2.9 Hz, 1H, H-2‴), 4.06 (dd, $J_{2',3'}$ = 3.3 Hz, 1H, H-2′), 3.96–3.85 (m, 9H, H-2″, H-3, H-3′, H-3‴), 3.83–3.57 (m, 12H), 3.55–3.46 (m, 2H, H-7a, 1H-27), 3.42 (m, 1H, H-24), 3.35 (d, J_{gem} = 14.9 Hz, 1H, 1H-27), 3.19–3.13 (m, 4H, 2H-13, 2H-18), 2.24 (m, 4H, 2H-11, 2H-20), 1.97–1.87 (m, 1H, 1H-23), 1.81–1.71 (m, 1H, 1H-23), 1.70–1.28 (m, 18H, 2H-8, 2H-9, 2H-10, 2H-14, 2H-15, 2H-16, 2H-17, 2H-21, 2H-22). ^{13}C NMR (100 MHz, D_2O) δ 177.1 (C-12 or C-19), 176.7 (C-12 or C-19), 164.4 (C_{urea}), 102.5 (C-1or C-1′), 102.5 (C-1 or C-1′), 101.0 (C-1‴), 98.4 (C-1″), 79.3, 78.9 (C-2‴), 78.2 (C-2″), 73.7, 73.6, 73.1, 70.7, 70.4, 70.3 (C-2′), 69.9 (C-2), 68.1 (C-7), 67.4, 67.3, 67.2, 66.6, 61.5 (1C-6), 61.4 (1C-6), 61.3 (1C-6), 61.2 (1C-6), 60.7 (C-24), 54.5 (C-25 or C-26), 54.1 (C-27), 50.0 (C-25 or C-26), 39.6 (C-13 or C-18), 39.5 (C-13 or C-18), 36.1 (C-11 or C-20), 35.7 (C-11 or C-20), 28.5–21.3 (C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-21, C-22, C-23).

5.8. Biological evaluation

5.8.1. Immunogen preparation

The streptavidin molecule consists of four subunits and binds four D-biotin molecules. To saturate streptavidin biotine sites, an excess of **3** was used. Compound **3** and streptavidin were dissolved in PBS to make 1 mg/mL and 10 mg/mL stock solutions, respectively. The **3**-streptavidin complex was prepared the day of injection by mixing a solution of **3 (60 μL)** with a solution of streptavidin (74 μL) in PBS (166 μL). After incubation at 37 °C for 1 h, the **3**-streptavidin complex was emulsified with 300 μL of complete Freund's adjuvant (Sigma, St. Louis, Mo.) for the first injection and in incomplete Freund's adjuvant (Sigma) for the subsequent injections.

5.8.2. Immunization and antisera

Two 7 week-old female BALB/c mice (Iffa-Credo, L'Arbresle, France) received five subcutaneous injections (200 μL each) of immunogen (**3**-streptavidin) emulsified with adjuvant at 1-week intervals. Controls included two mice immunized with streptavidin emulsified with adjuvant and two mice treated with adjuvant only. On day 35, blood samples were obtained via the retroorbital venous. After incubation at room temperature for 1 h they were centrifuged and the serums were stored frozen. Appropriately qualified personnel performed all animal protocols.

5.8.3. Elisa

Antibodies to *Saccharomyces cerevisiae* or to *C. albicans* Phosphopeptidomannan–PPM—were detected by enzyme linked immunosorbent assay (ELISA) by using plates coated with PPM from *S. cerevisiae* SU1 [7] or from *C. albicans* grown at pH 6 [1] or pH 2 [6].

5.9. Luminex

5.9.1. Coupling reaction

We used the Luminex's xMAP technology, a microsphere based multiplexing system. The BSTOs were coated on magnetic beads; for this, we coated the avidin on the carboxylated surface of beads according to a protocol in 3 steps: activation of beads with *N*-hydroxysulfosuccinimide (S-NHS, 94.33 mg/mL) and 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (EDC, 50 mg/mL) for 30 min, at room temperature, with slow agitation. After magnetization and three washes with PBS (pH 7,4), a solution of avidin (4 mg/mL in PBS pH 7.4) was incubated for 3 h, at room temperature, with slow agitation. The beads were saturated with a solution of hydroxylamine (250 mM in PBS, pH 7.4), then magnetized and washed three times with diluent particle (Bio-Rad, France). The beads coated with avidin, were then placed in contact with the BSTOs, for 1 h, at room temperature. After magnetization and three washes, the beads were saturated with PBS, BSA 10%, overnight, at 4 °C. The beads were stored in diluent particle, in darkness, at 4 °C.

5.9.2. Multiplex quantification of BSTOs reactivity

The beads coated with different oligomannosides were incubated with serum of mouse immunized with 3-streptavidin for 30 min, in darkness, at 37 °C. The beads and serum were used at 1:1000 and 1:100 in PBS (pH 7.4), respectively. After magnetization and three washes (PBS, Tween 20 1%), a phycoerythrin labelled anti-mouse IgG, M or A was used as conjugate at 5 μg/mL (Southern Biotech, USA). After an incubation of 30 min, magnetization and three washes, the beads were resuspended in sheath fluid in a tube test and the reaction was monitored on a Luminex Laboratory MAP system 100 (Luminex, USA) at 532 nm. The results are expressed as median fluorescence intensity (MFI) determined for 100 microspheres of each BSTO identified by its microsphere number.

Acknowledgment

Financial support from the French National Research Agency (ANR CaBMT) is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.048.

References and notes

1. Sendid, B.; Tabouret, M.; Poirot, J. L.; Mathieu, D.; Fruit, J.; Poulain, D. *J. Clin. Microbiol.* **1999**, *37*, 1510.
2. Quinton, J. F.; Sendid, B.; Reumaux, D.; Duthilleul, P.; Cortot, A.; Grandbastien, B.; Charrier, G.; Targan, S. R.; Colombel, J. F.; Poulain, D. *Gut* **1998**, *42*, 788–791.
3. Sendid, B.; Quinton, J. F.; Charrier, G.; Goulet, O.; Cortot, A.; Grandbastien, B.; Poulain, D.; Colombel, J.-F. *Am. J. Gastroenterol.* **1998**, *93*, 1306–1310.
4. Standaert-Vitse, A.; Sendid, B.; Joossens, M.; François, N.; Vandewalle-El Khoury, P.; Branche, J.; Van Kruiningen, H.; Jouault, T.; Rutgeerts, P.; Gower-Rousseau, C.; Libersa, C.; Neut, C.; Broly, F.; Chamaillard, M.; Vermeire, S.; Poulain, D.; Colombel, J. F. *Am. J. Gastroenterol.* **2009**, *104*, 1745–1753.
5. Vandewalle, P.; Colombel, J-F.; Joossens, S.; Standaert-Vitse, A.; Collot, M.; Halfvarson, J.; Hadrich, I.; Landers, C. J.; Vermeire, S.; Rutgeerts, P.; Targan, S. R.; Mallet, J-M.; Chamaillard, M.; Sendid, B.; Poulain, D. *Am. J. Gastroenterol.* **2008**, *103*, 949–957.
6. Standaert-Vitse, A.; Jouault, T.; Vandewalle, P.; Mille, C.; Seddik, M.; Sendid, B.; Mallet, J.-M.; Colombel, J.-F.; Poulain, D. *Gastroenterology* **2006**, *130*, 1764–1775.
7. Sendid, B.; Colombel, J. F.; Jacquinot, P. M.; Faille, C.; Fruit, J.; Cortot, A.; Lucidarme, D.; Camus, D.; Poulain, D. *Clin. Diagn. Lab. Immunol.* **1996**, *3*, 219–226.
8. Sendid, B.; Jouault, T.; Vitse, A.; Fradin, C.; Colombel, J.-F.; Poulain, D. *Med. Sci.* **2009**, *25*, 473–481.
9. Poulain, D.; Sendid, B.; Standaert-Vitse, A.; Fradin, C.; Jouault, T.; Jawhara, S.; Colombel, J.-F. *Dig. Dis.* **2009**, *27*, 104–110.
10. Poulain, D.; Jouault, T. *Curr. Opin. Microbiol.* **2004**, *7*, 342–349.
11. Chevalier, R.; Esnault, J.; Vandewalle, P.; Sendid, B.; Colombel, J.-F.; Poulain, D.; Mallet, J.-M. *Tetrahedron* **2005**, *61*, 7669–7677.
12. Collot, M.; Sendid, B.; Fievez, A.; Savaux, C.; Standaert-Vitse, A.; Tabouret, M.; Drucbert, A.-S.; Danzé, P.-M.; Poulain, D.; Mallet, J.-M. *J. Med. Chem.* **2008**, *51*, 6201–6210.
13. Collot, M.; Wilson, I. B. H.; Bublin, K.; Hoffmann-Sommergruber, K.; Mallet, J.-M. *Bioorg. Med. Chem.* **2011**, *19*, 1306–1320.
14. Cattiaux, L.; Sendid, B.; Collot, M.; Machez, E.; Poulain, D.; Mallet, J.-M. *Bioorg. Med. Chem.* **2011**, *19*, 547–555.
15. Collot, M.; Savreux, J.; Mallet, J.-M. *Tetrahedron* **2008**, *64*, 1523–1535.
16. Franks, N. E.; Montgomery, R. *Carbohydr. Res.* **1968**, *6*, 286–298.
17. Chassaing, B.; Darfeuille-Michaud, A. *Gastroenterology* **2011**, *140*, 1720–1728.
18. Tsianos, E. V.; Katsanos, K. *World J. Gastroenterol.* **2009**, *15*, 521–525.