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Synthetic yeast oligomannosides as biological probes: α-D-Man*p* (1→3) α-D-Man*p* (1→2) α-D-Man*p* and α-D-Man*p* (1→3) α-D-Man*p* (1→2) α-D-Man*p* (1→2) α-D-Man*p* as Crohn's disease markers

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Abstract—The anti-*Saccharomyces cerevisiae* antibodies (ASCA) are markers for Crohn's disease used for diagnostic, phenotypic characterization and sero-epidemiological studies. Antibody detection is made by different immunoenzymatic tests using *S. cerevisiae* mannans which are both complex and poorly standardized antigens. Here we construct the major discriminating epitopes comprised within this antigen. When coupled to linker arm and a peptidic carrier to functionalize microtiter plates, they were able to discriminate serological responses between Crohn's disease and ulcerative colitis, another form of inflammatory bowel disease.

1. Introduction

Chronic inflammatory bowel diseases (IBD) including Crohn's disease (CD) and ulcerative colitis (UC), are common in developed countries. The diagnostic accuracy of conventional clinical, radiological, endoscopic and histological assessment in their diagnosis is generally good. Nevertheless diagnostic dilemnas sometimes persist and non-invasive accurate serological assays are still desirable. CD and not UC was found to be associated, in a large number of cases (60%),[1] to the presence of antibodies directed against *S. cerevisae* mannan.[2] This observation has led to the development of a useful diagnostic test (the so-called ASCA test, anti-*Saccharomyces cerevisae* antibodies).[3] However, ASCA test is not fully satisfactory, it uses as an antigen a not well defined mixture of oligosaccharides (various lengths and structures). The global antibody response assessed on such poorly standardized biological material has been shown to be not reproducible in several studies.[4,5] Two different studies have shown that among the large number of carbohydrate epitopes expressed in *S. cerevisae* mannan, the major epitopes were a trimannoside (α-D-Man*p* (**1→3**) α-D-Man*p* (**1→2**) α-D-Man*p*)[6] and a tetramannoside (α-D-Man*p* (**1→3**) α-D-Man*p* (**1→2**) α-D-Man*p* (**1→2**) α-D-Man*p*).[7] These oligomannosidic structures have been found only in yeast cell walls in *S. cerevisiae*,[8,9] *Candida albicans* and *C. stellatoidea*[10–12] mannans. They have been also found in glucosylated form in *Cryptococcus laurentii*[13] and in phosphorylated form in *Pichia holstii*.[14] In order to improve the selectivity of the ASCA test, we decided to design a more precise evaluation of antibodies directed against these two epitopes using synthetic oligomannosides.[15] Oligosaccharides are not able to bind directly to polystyrene plate, they are too hydrophilic. The binding is made possible through either a direct covalent coupling to chemically reactive microplate[16] or through formation of a neoglycopeptide. In this approach the oligomannosides were coupled first through a linker arm to a peptidic carrier.

We describe here the chemical synthesis[17] of two oligomannosides **1** and **2**, their conjugates to polylysine (Fig. 1), and their use for microplate functionalization.

Synthetic poly-L-lysine was selected here as chemically

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1 α–D-Man*p* (1->3) α-D-Man*p* (1–>2) α–D-Man*p* (1->2) α-D-Man*p* O $(CH_2)_8$-COOH
2 α–D-Man*p* (1->3) α-D-Man*p* (1–>2) α–D-Man*p* O $(CH_2)_8$-COOH
1-lys [α–D-Man*p* (1->3) α-D-Man*p* (1–>2) α–D-Man*p* (1->2) α-D-Man*p* O $(CH_2)_8$-CO]$_x$-poly-L-lysine
2-lys [α–D-Man*p* (1->3) α-D-Man*p* (1–>2) α–D-Man*p* O $(CH_2)_8$-CO]$_x$- poly-L-lysine

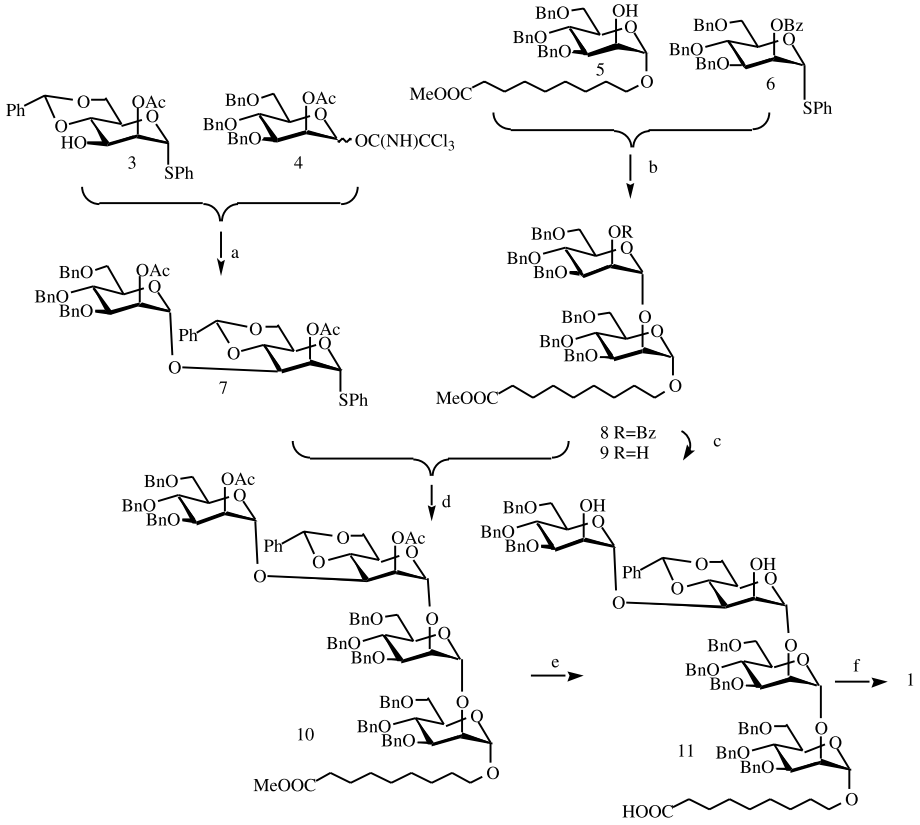
Figure 1.

well defined carrier.[18] We report the diagnostic performance of a new immunoassay using this neo-antigen to functionalize microtiter plates in its ability to discriminate serological responses from patients with CD and UC.[3]

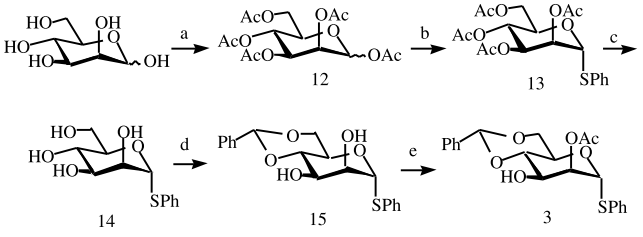
2. Chemical synthesis of 1 and 2

The blockwise synthesis of the tetrasaccharide **1**, depicted Scheme 1, is based on the condensation of two disaccharidic blocks **7** and **9**.

The α-selectivity of this glycosylation was controlled by the participation of the acetyl group at position 2 of **7**. The disaccharidic donor unit **7** was prepared of an imidate **4** and a thiophenyl acceptor **3**. The acceptor unit **9** was prepared by debenzoylation of **8** obtained though condensation of thiophenyl mannoside **6** with the acceptor **5**. In these cases, the complete α selectivity also relies on the neighboring group participation. The complete α-selectivity of each glycosylation was confirmed by NMR: the coupling constants between anomeric carbon (C-1) and anomeric hydrogen (H-1) were around 175 Hz, as expected for α anomers:[19] **7** *J*(C1, H-1)=175 Hz; **8** *J*(C1, H-1)=172 Hz and **10** *J*(C1, H-1)=172 Hz. It must be around 156 Hz for a β linkage. The tetrasaccharide **10** was deprotected in two



Scheme 1. Preparation of **1**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C, 89%; (b) NIS, TfOH cat, CH_2Cl_2, −20 °C, 80%; (c) MeONa, MeOH, rt, 90%. (d) NIS, TfOH cat, CH_2Cl_2, −20 °C, 72%; (e) aq NaOH, THF, 60 °C, 82%; (f) H_2, 10% Pd/C, MeOH, 83%.



Scheme 2. Preparation of **3**: Reagents and conditions: (a) Ac_2O, pyr; (b) PhSH, $BF_3.Et_2O$, CH_2Cl_2, 76% two steps; (c) MeONa, MeOH; (d) $PhCH(OMe)_2$, HBF_4/Et_2O, CH_2Cl_2; (e) CSA cat, $CH_3CH(OEt)_3$, then 80% aq AcOH, 75% two steps.

steps: saponification of ester functions by sodium hydroxide in hot aq THF, then hydrogenolysis of the benzyl groups to give **1**.

The synthesis started from four monosaccharidic blocks: **4**[20] and **6**[21] were prepared according literature procedure. Compound **3** was prepared by Lemieux's selective acetylation[22] of known[23,24] **15** (see Scheme 2). The preparation of **15** was optimized on a large scale (100 g) in particular, the monobenzylidenation of **14**[25] was improved using dichloromethane as a solvent instead of DMF (see Section 6).

Compound **5**[26] was prepared by NBS/triflic acid glycosylation of 2-*O*-benzoylated thioglycoside **6** of 8-methoxy carbonyloctanol **16** to give **17**. In this case, the use of NIS/triflic acid was ineffective. Compound **16**[27] was prepared[28] from methyl oleate (ozonolysis in dichloromethane then $NaBH_4$ reduction in methanol). It occurred that the initial preparation from azelaic acid was found much less convenient. The glycoside **17** was then debenzoylated to give the glycosyl acceptor **5** (Scheme 3).

The protected trisaccharide **18** (Scheme 4) was obtained by NIS/triflic acid glycosylation of **5** and **7** (75%). Treatment by sodium hydroxide in aq THF gave **19** and final hydrogenolysis of the benzyl groups gave **2** (Scheme 4).

3. Preparation of the glycoconjugates

Compounds **1** and **2** were then coupled with poly L-lysine hydrobromide (30–70 kδ) using 1-(3-dimethylamino-propyl)-3-ethyl carbodiimide (EDC) at pH 4.6 (0.1 M aq morpholino ethyl sulfonate (MES) buffer) at rt to give **1-lys** and **2-lys**. A 6/1 weight ratio was used in order to have a 15% maximum sugar content in the glycosylated peptides. The yields were 50% after chromatography on sephadex G 25. The sugar content (ca. 10%) was estimated by 400 MHz ^1H NMR in D_2O.

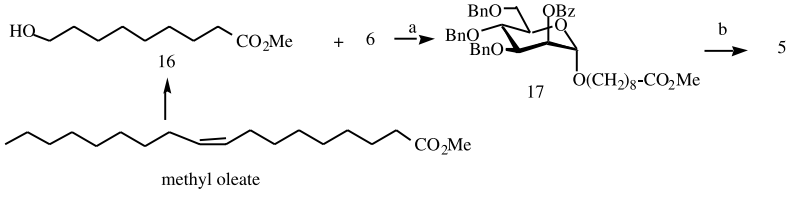
4. Evaluation of the polylysine conjugates: antibodies response in ELISA against synthetic oligomannosides

The series of experiments were conducted on sera from 90 patients with inflammatory bowel disease. Diagnosis of CD and UC was established by endoscopic, histologic and clinical criteria. There were 49 patients with Crohn's disease (19 males, 30 females, mean age 32 years), 41 patients with ulcerative colitis (22 males, 19 females, mean age: 33 years) and 46 healthy blood donors, as control group. For each patient, whole venous blood was collected and serum was separated by centrifugation for serological analyses (Figs. 2 and 3).

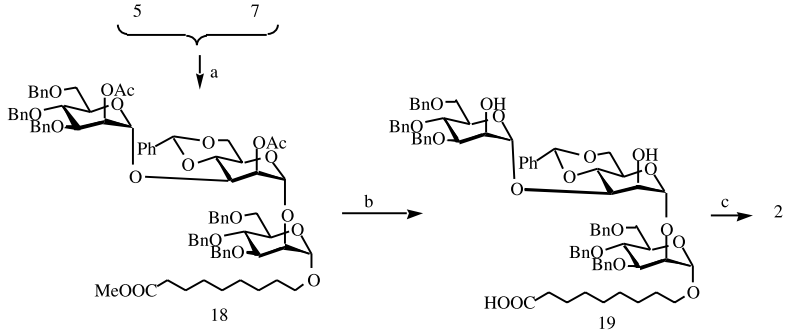
Figure 2 shows the results observed when immunoglobulins of patients with CD and UC or healthy controls reacting with synthetic trimannoside were quantified by ELISA. The mean value of trimannoside titers is 19.73 arbitrary units (a.u.) for CD, 3.70 for UC and 5.23 for control group. $p=0.0006$ for CD versus UC and $p=0.044$ for controls versus CD.

In the same way, on tetramannoside antigen (Fig. 3), the mean value of the antibodies titers for CD patients is twice higher than for UC patients (24.97 vs 12.67 respectively, $p=0.0002$). For healthy subjects, the mean value is 9.38 a.u. and $p<0.0001$ comparing to CD.

Both synthetic αMan1-3αMan1-2Man-Lys and αMan1-3αMan1-2αMan1-2 Man-lys displayed a significantly stronger reactivity with sera of CD patient than with sera of UC patients. These results demonstrate that synthetic oligomannosides have the ability to discriminate between the two types of IBD pathologies.



Scheme 3. Preparation of **5**: Reagents and conditions: (a) NBS, TfOH, CH_2Cl_2, −20 °C, 47%; (b) MeONa, MeOH, rt, 100%.



Scheme 4. Preparation of **2**: Reagents and conditions: (a) NIS, TfOH cat, CH_2Cl_2, −20 °C, 75%; (b) aq NaOH, THF, 60 °C, 73%; (c) H_2, 10% Pd/C, MeOH, 95%.

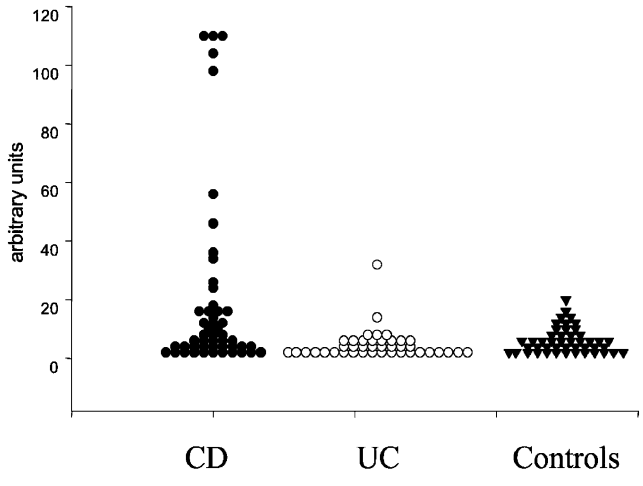


Figure 2. Reactivity of patients against synthetic trimannoside **2-lys**: CD patients (black circle), UC patients (white circle) or controls (black triangle).

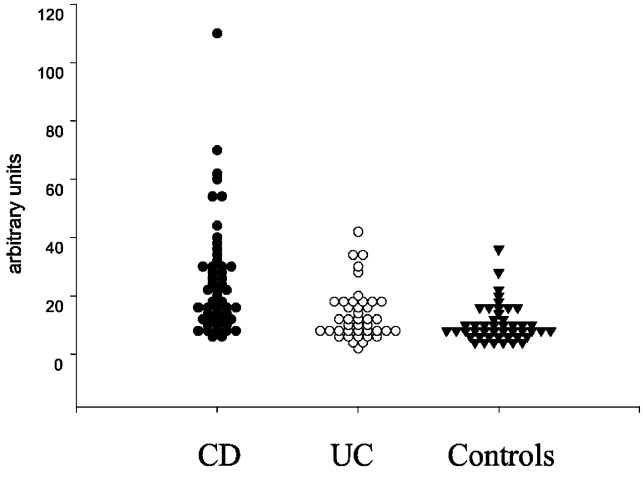


Figure 3. Reactivity of patients against synthetic tetramannoside **1-lys**: CD patients (black circle), UC patients (white circle) or controls (black triangle).

5. Conclusion

Following the development of the original ASCA test detection has been widely used for differential diagnosis among IBD patients. Other indications have been proposed such as disease monitoring and genetic counselling. More recently ASCA response and titers have been associated with CD phenotypes.[29,30] However, ASCA detection is not standardized and none of the numerous antigens currently used is chemically defined. This impairs both inter-laboratory reproducibility and basic analysis of the significance of the corresponding antibodies. The *S. cerevisiae* mannan is a complex repertoire of oligomannose epitopes varying among strains[31] and growth conditions.[16,29,32,33] The major epitope supporting the specific response in CD patients versus UC patients has been identified previously by two independent research groups as corresponding to the structure α-D-Man (**1**→**3**) [α-D-Man(**1**→**2**)]$_n$ α-D-Man (n=1 or 2).[7,6] The aim of the study was to assess the potential of a test involving synthetic analogues of these structures as an antigen. The preliminary data gained in this study show that a signal specific for a pathology was indeed detected. It therefore open perspectives about development of highly standardized tests as well as providing tools to dissect the genetic basis of oligomannoside repertoire humoral recognition.[34]

6. Experimental

6.1. 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. Merck) with detection by charring with sulfuric acid. Column chromatography were performed on Silica gel 60 (E. Merck). ^{1}H NMR spectra were recorded with Bruker AM 250, AM 400 instruments. Mass spectroscopy analyses were performed on a Nermag R 10-10. Elemental analyses were performed by Service d'Analyse de Université Pierre et Marie Curie.

6.1.1. Phenyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (7). To a stirred solution of **4**α/β (731 mg, 1.15 mmol), **3** (420 mg, 1.04 mmol) and 4 Å molecular sieves (1.1 g) in anhydrous dichloromethane (12 mL) was added, at −20 °C and under argon, TMSOTf (22 μL, 0.115 mmol). After stirring for 1 h at −20 °C, the mixture was neutralized with saturated sodium hydrogen carbonate and filtered through Celite. The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (20% EtOAc in cyclohexane) to afford **7** (626 mg, 68%) as a white powder. $[\alpha]_D$ +119 (c 0.55 in chloroform); mp: 58–59 °C (cyclohexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15–7.25 (m, 25H, arom.), 5.69 (s, 1H, benzylidene), 5.55 (dd, 1H, J_{2-1}=1.8 Hz, J_{2-3}=2.7 Hz, H-2D), 5.52 (dd, 1H, J_{2-1}=1.3 Hz, J_{2-3}=3.4 Hz, H-2C), 5.49 (d, 1H, H-1C), 5.34 (d, 1H, H-1D), 4.89 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.77 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.74 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.56 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.56 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.54 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.42 (ddd, 1H, J_{5-4}=9.8 Hz, J_{5-6b}=4.9 Hz, J_{5-6a}=10.3 Hz, H-5C), 4.40 (dd, 1H, J_{3-4}=9.8 Hz, H-3C), 4.30 (dd, 1H, J_{6b-6a}=10.3 Hz, H-6Cb), 4.20 (t, 1H, H-4C), 3.97–3.87 (m, 4H, H-5D, H-4D, H-3D and H-6Ca), 3.86 (dd, 1H, J_{6b-6a}=12.0 Hz, J_{6b-5}=3.9 Hz, H-6Db), 3.78 (dd, 1H, J_{6a-5}=3.3 Hz, H-6Da), 2.21 and 2.16 (2 s, 6H, 2 O–C=O–CH_3); ^{13}C NMR (100 MHz): δ 170.1 and 169.7 (2 –O–*C*=O–CH_3), 138.4, 138.2, 137.9, 136.9 and 133.0 (5 C arom.), 132.0–125.9 (25 CH arom.), 101.3 (benzylidene), 98.8 (C-1D), 86.8 (C-1C), 78.9 (C-4C), 75.6, 74.1 and 72.1 (C-3D, C-4D and C-5D), 74.9 (CH_2Ph), 73.4 (CH_2Ph), 73.1 (C-2C), 71.7 (CH_2Ph), 70.8 (C-3C), 68.6 (C-6D), 68.4 (C-2D), 68.2 (C-6C), 64.6 (C-5C), 21.0 and 20.8 (2 –O–C=O–*C*H_3); MS m/z (CI, NH_3): 894.3 (M+NH_4)$^+$; Anal. Calcd for $C_{50}H_{52}O_{12}S$ (877.02): C 68.47, H 5.98. Found: C 68.36, H 6.15.

6.1.2. 8-Methoxycarbonyloctyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (8). To a stirred solution of **6** (430 mg, 665 μmol), **5** (412 mg, 665 μmol) and 4 Å molecular sieves (1 g) in anhydrous dichloromethane (9 mL) were successively added, at −20 °C and under argon, NIS (306 mg, 1.33 mmol) and TfOH (11.7 μL, 133 μmol). After stirring for 30 min at −20 °C, the reaction mixture was diluted with dichloromethane, neutralized with saturated sodium hydrogen carbonate and filtered through Celite. The organic layer was washed with sodium thiosulfate, brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (20% EtOAc in cyclohexane) to afford **8** (615 mg, 80%) as a colorless oil. $[\alpha]_D$ +5 (*c* 0.6, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15–7.25 (m, 35H, arom.), 5.85 (dd, 1H, J_{2-1}=1.8 Hz, J_{2-3}=3.3 Hz, H-2B), 5.27 (d, 1H, H-1B), 4.96 (d, 1H, J_{1-2}=1.6 Hz, H-1A), 4.93 (d, 1H, J_{gem}=10.7 Hz, CHPh), 4.92 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.83 (d, 1H, J_{gem}=11.1 Hz, CHPh), 4.78 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.76 (s, 2H, CH_2Ph), 4.74 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.63 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.62 (d, 1H, J_{gem}=10.7 Hz, CHPh), 4.58 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.57 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.51 (d, 1H, J_{gem}=11.1 Hz, CHPh), 4.19 (dd, 1H, J_{3-4}=9.0 Hz, H-3B), 4.12–4.09 (m, 2H, H-5B and H-4B), 4.07 (dd, 1H, J_{2-3}=2.9 Hz, H-2A), 4.00 (dd, 1H, J_{3-4}=9.2 Hz, H-3A), 3.94 (dd, 1H, J_{6b-6a}=10.5 Hz, J_{6b-5}=3.0 Hz, H-6Bb), 3.92 (t, 1H, J_{4-5}=9.2 Hz, H-4A), 3.85–3.77 (m, 4H, H-6A, H-6Ba and H-5A), 3.71 (s, 3H, $-CO_2CH_3$), 3.67 (dt, 1H, J_{gem}=9.5 Hz, J_{CH-CH2}=6.7 Hz, –O–C*H*–CH_2–), 3.35 (dt, 1H, J_{CH-CH2}=6.7 Hz, –O–C*H*–CH_2–), 2.36 (t, 2H, *J*=7.5 Hz, –C*H*$_2$–CO_2CH_3), 1.69–1.65, 1.57–1.54 and 1.35–1.33 (m, 12H, $-(CH_2)_6-$); ^{13}C NMR (100 MHz): δ 174.2 ($-CO_2CH_3$), 165.4 (–O–C=O), 138.5, 138.4, 2×138.3, 138.2, 137.9 and 129.9 (7 C arom.), 133.0–127.3 (35 CH arom), 99.5 (C-1B), 98.6 (C-1A), 79.7 (C-3A), 78.0 (C-3B), 75.2 (C-2A), 75.1 (CH_2Ph), 75.0 (CH_2Ph), 74.6 (C-4B), 74.3 (C-4A), 73.3 (CH_2Ph), 73.2 (CH_2Ph), 72.1 (CH_2Ph), 71.9 (C-5B), 71.7 (C-5A), 71.6 (CH_2Ph), 69.2 (C-6A), 69.1 (C-6B), 68.9 (C-2B), 67.6 (–O–CH_2–), 51.4 ($-CO_2CH_3$), 34.0 (–C*H*$_2$–CO_2CH_3), 29.3, 2×29.1, 29.0, 26.0 and 24.9 ($-(CH_2)_6-$); MS *m*/*z* (CI, NH_3): 1174.5 $(M+NH_4)^+$; Anal. Calcd for $C_{71}H_{80}O_{14}$ (1157.40): C 73.68, H 6.96. Found: C 73.61, H 7.11.

6.1.3. 8-Methoxycarbonyloctyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (9). Sodium (11 mg) was added to a stirred solution of **8** (540 mg, 467 μmol) in a mixture of methanol/dichloromethane (1:1, 5 mL) After stirring for 1 h, the reaction mixture was neutralized with Amberlite IR 120 (H^+), filtered and concentrated. The residue was purified by flash chromatography (27% EtOAc in cyclohexane) to afford **9** (442 mg, 90%) as a colorless oil. $[\alpha]_D$ +34 (*c* 0.7, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.30 (m, 30H, arom.), 5.21 (d, 1H, J_{1-2}=1.4 Hz, H-1B), 4.95 (d, 1H, J_{1-2}=1.7 Hz, H-1A), 4.89 (d, 1H, J_{gem}=10.6 Hz, CHPh), 4.87 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.75 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.75 (d, 1H, J_{gem}=11.6 Hz, CHPh), 4.71 (d, 1H, J_{gem}=11.6 Hz, CHPh), 4.69 (d, 1H, J_{gem}=12.1 Hz, CHPh), 4.64 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.59 (d, 1H, J_{gem}=10.6 Hz, CHPh), 4.59 (d, 1H, J_{gem}=12.2 Hz, CHPh), 4.58 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.56 (d, 1H, J_{gem}=12.1 Hz, CHPh), 4.54 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.20–4.17 (m, 1H, H-2B), 4.08 (dd, 1H, J_{2-3}=2.9 Hz, H-2A), 4.03–3.99 (m, 1H, H-5B), 3.99 (dd, 1H, J_{3-4}=9.3 Hz, H-3A), 3.93 (dd, 1H, J_{3-4}=9.1 Hz, H-3B), 3.89 (t, 1H, J_{4-5}=9.3 Hz, H-4A), 3.86 (t, 1H, J_{4-5}=9.1 Hz, H-4B), 3.86 (dd, 1H, J_{6b-6a}=11.1 Hz, J_{6b-5}=4.9 Hz, H-6Ab), 3.82–3.75 (m, 4H, H-6Aa, H-6B and H-5A), 3.71 (s, 3H, $-CO_2CH_3$), 3.65 (dt, 1H, J_{gem}=9.5 Hz, J_{CH-CH2}=6.8 Hz, –O–C*H*–CH_2–), 3.30 (dt, 1H, J_{CH-CH2}=6.8 Hz, –O–C*H*–CH_2–), 2.51 (d, 1H, J_{OH-2}=1.9 Hz, OH), 2.34 (t, 2H, *J*=7.6 Hz, –C*H*$_2$–CO_2CH_3), 1.70–1.63, 1.56–1.49 and 1.37–1.27 (m, 12H, $-(CH_2)_6-$); ^{13}C NMR (100 MHz): δ 174.3 ($-CO_2CH_3$), 138.5, 2×138.3, 138.2, 138.1 and 137.9 (6 C arom.), 128.4–127.3 (30 CH arom), 101.0 (C-1B), 98.7 (C-1A), 79.9 (C-3B), 79.8 (C-3A), 75.1 (CH_2Ph), 74.9 (CH_2Ph), 74.9 (C-2A), 74.7 (C-4A), 74.3 (C-4B), 73.3 (CH_2Ph), 73.2 (CH_2Ph), 72.8 (CH_2Ph), 72.0 (CH_2Ph), 71.7 (C-5B), 71.6 (C-5A), 69.2 (C-6A), 69.0 (C-6B), 68.4 (C-2B), 67.6 (–O–CH_2–), 51.4 ($-CO_2CH_3$), 34.0 (–CH_2–CO_2CH_3), 29.4, 29.2, 29.1, 29.0, 26.0 and 24.9 ($-(CH_2)_6-$); MS *m*/*z* (CI, NH_3): 1070.5 $(M+NH_4)^+$; Anal. Calcd for $C_{64}H_{76}O_{13}$ (1053.30): C 72.98, H 7.27. Found: C 72.89, H 7.43.

6.1.4. 8-Methoxycarbonyloctyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (10). To a stirred solution of **7** (158 mg, 180 μmol), **9** (186 mg, 180 μmol) and 4 Å molecular sieves (500 mg) in anhydrous dichloromethane (5 mL) were successively added NIS (81 mg, 360 μmol) and TfOH (4 μL, 54 μmol), at −20 °C and under argon. After stirring for 30 min at −20 °C, the reaction mixture was diluted with dichloromethane, neutralized with saturated sodium hydrogen carbonate and filtered through Celite. The organic layer was washed with sodium thiosulfate, brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (17% EtOAc in cyclohexane) to afford **10** (236 mg, 72%) as a colorless oil. $[\alpha]_D$ +29 (*c* 0.85 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.25 (m, 50H, arom.), 5.66 (s, 1H, benzylidene), 5.56 (dd, 1H, J_{2-1}=1.5 Hz, J_{2-3}=3.0 Hz, H-2D), 5.44 (dd, 1H, J_{2-1}=1.3 Hz, J_{2-3}=3.5 Hz, H-2C), 5.32 (se, 1H, H-1D), 5.20 (se, 1H, H-1B), 5.04 (se, 1H, H-1C), 4.94 (se, 1H, H-1A), 4.88 (d, 1H, J_{gem}=10.6 Hz, CHPh), 4.88 (d, 1H, J_{gem}=10.7 Hz, CHPh), 4.87 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.75 (d, 1H, J_{gem}=12.4 Hz, CHPh), 4.75 (d, 1H, J_{gem}=11.2 Hz, CHPh), 4.72 (s, 2H, CH_2Ph), 4.67 (d, 1H, J_{gem}=12.3 Hz, CHPh), 4.67 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.62 (d, 1H, J_{gem}=12.4 Hz, CHPh), 4.61 (d, 1H, J_{gem}=10.6 Hz, CHPh), 4.60 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.58 (s, 2H, CH_2Ph), 4.57 (d, 1H, J_{gem}=11.2 Hz, CHPh), 4.53 (d, 1H, J_{gem}=10.9 Hz, CHPh), 4.52 (d, 1H, J_{gem}=10.7 Hz, CHPh), 4.33 (d, 1H, J_{gem}=12.3 Hz, CHPh), 4.43 (dd, 1H, J_{3-4}=9.3 Hz, H-3C), 4.21 (dd, 1H, J_{6b-6a}=10.4 Hz, J_{6b-5}=4.5 Hz, H-6Cb), 4.14–4.12 (m, 1H, H-2B), 4.09 (t, 1H, J_{4-5}=9.3 Hz, H-4C), 4.07–4.04 (m, 1H, H-5C), 4.03 (t, 1H, J_{4-3}=J_{4-5}=9.9 Hz, H-4D), 4.01–4.00 (m, 1H, H-2A), 3.98–3.96 (m, 1H, H-3B), 3.93 (dd, 1H, J_{3-4}=9.9 Hz, H-3D), 3.88–3.85 (m, 1H, H-5D), 3.71 (s, 3H, $-CO_2CH_3$), 3.62–3.59 (m, 1H, H-6Da), 3.60–3.56 (m, 1H, –O–C*H*–CH_2–), 3.25 (dt, 1H, J_{gem}=9.4 Hz, J_{CH-CH2}=6.6 Hz, –O–C*H*–CH_2–), 2.34

(t, 2H, $J_{CH2-CH2}$=7.5 Hz, –CH_2–CO_2CH_3), 2.14 and 2.13 (2 s, 6H, 2 O–C=O–CH_3), 1.70–1.62, 1.54–1.47 and 1.35–1.25 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 174.3 (–CO_2CH_3), 170.1 and 169.5 (2 –O–C=O–CH_3), 138.6, 138.5, 2×138.3, 2×138.2, 2×138.1, 137.9 and 137.0 (10 C arom.), 128.7–125.9 (50 CH arom.), 101.2 (benzylidene), 100.6 (C-1B, $^1J_{C-H}$=171.3 Hz), 99.8 (C-1C, $^1J_{C-H}$=172.2 Hz), 98.8 (C-1D, $^1J_{C-H}$=174.0 Hz), 98.6 (C-1A, $^1J_{C-H}$=170.5 Hz), 79.3 (C-3B), 78.9 (C-4C), 77.7 (C-3D), 75.5 (C-2A), 75.4 (C-2B), 75.1 (CH_2Ph), 75.0 (CH_2Ph), 74.8 (CH_2Ph), 73.9 (C-4D), 3×73.2 (3 CH_2Ph), 72.1 (CH_2Ph), 72.0 (CH_2Ph), 72.0 (C-5D), 71.7 (CH_2Ph), 71.4 (C-2C), 70.8 (C-3C), 68.1 (C-2D), 69.3, 69.2, 68.1 et 68.1 (4 C-6C), 67.6 (–O–CH_2–), 51.4 (–CO_2CH$_3$), 34.0 (–CH$_2$–CO_2CH_3), 29.4, 29.2, 29.1, 29.0, 26.0 and 24.9 (–$(CH_2)_6$–), 21.0 and 20.8 (2 –O–C=O–CH_3); MS *m/z* (CI, NH_3): 1836.5 $(M+NH_4)^+$; Anal. Calcd for $C_{108}H_{122}O_{25}$ (1820.026): C 71.27, H 6.75. Found: C 71.08, H 6.94.

6.1.5. 8-Carboxyloctyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-(4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (11). A mixture of **10** (200 mg, 110 μmol), 0.1 N aq NaOH (5 mL) and THF (5 mL) was heated at reflux for 15 h, cooled to room temperature, acidified with of 1 N aq HCl and extracted with dichloromethane. The organic layer was dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (40% EtOAc in cyclohexane) to afford **11** (155 mg, 82%) as a colorless oil. $[\alpha]_D$ +33 (*c* 0.5 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.25 (m, 50H, arom.), 5.60 (s, 1H, benzylidene), 5.21 (d, 1H, J_{1-2}=1.3 Hz, H-1B), 5.16 (d, 1H, J_{1-2}=1.4 Hz, H-1D), 5.12 (d, 1H, J_{1-2}=1.1 Hz, H-1C), 4.97 (d, 1H, J_{1-2}=1.2 Hz, H-1A), 4.42–4.41 (m, 1H, H-2C), 4.18–4.17 (m, 1H, H-2B), 4.08 (m, 1H, H-2D), 4.00 (dd, 1H, J_{2-3}=3.1 Hz, H-2A), 3.60 (dt, 1H, J_{gem}=9.5 Hz, J_{CH-CH2}=6.5 Hz, –O–C*H*–CH_2–), 3.27 (dt, 1H, J_{CH-CH2}=6.5 Hz, –O–C*H*–CH_2–), 2.35 (t, 2H, J=7.5 Hz, –CH_2–CO_2H), 1.70–1.60, 1.54–1.47 and 1.35–1.25 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 178.5 (–CO_2H), 102.3 (C-1C), 101.7 (benzylidene), 100.9 (C-1B), 99.8 (C-1D), 98.5 (C-1A), 75.7 (C-2A), 74.8 (C-2B), 69.6 (C-2C), 68.5 (C-2D), 67.6 (–O–CH_2–), 33.8 (–CH$_2$–CO_2H), 29.3, 29.0, 28.9, 28.7, 25.9 and 24.5 (–$(CH_2)_6$–); MS *m/z* (CI, NH_3): 1738.9 $(M+NH_4)^+$; Anal. Calcd for $C_{103}H_{116}O_{23}$ (1722.059): C 71.84, H 6.79. Found: C 71.73, H 6.91.

6.1.6. 8-Carboxyloctyl (α-D-mannopyranosyl)-(1→3)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (1). A solution of **11** (100 mg, 58 μmol) in MeOH (5 mL) was stirred under H_2 atmosphere in the presence of 10% Pd/C (20 mg) for 1 h at room temperature, filtered through Celite and concentrated. The residue was partitioned between distilled water and dichloromethane. The aqueous layer was filtered (membrane 0.22 μm) and lyophilized to afford **1** (40 mg, 83%) as a white amorphous powder. ^{1}H NMR (400 MHz, D_2O): δ 5.25 (d, 1H, J_{1-2}=1.2 Hz, H-1B), 5.10 (d, 1H, J_{1-2}=1.3 Hz, H-1D), 5.05 (d, 1H, J_{1-2}=0.8 Hz, H-1A), 4.99 (d, 1H, J_{1-2}=1.4 Hz, H-1C), 4.19 (dd, 1H, J_{2-3}=3.0 Hz, H-2C), 4.07 (dd, 1H, H-2B), 4.03 (dd, 1H, H-2D), 3.90–3.88 (m, 1H, H-2A), 3.92 (dd, 1H, J_{3-4}=9.6 Hz, H-3B), 3.91 (dd, 1H, J_{3-4}=9.4 Hz, H-3C), 3.72–3.65 (m, 1H, –O–C*H*–CH_2–), 3.49 (dt, 1H, J_{gem}=10.0 Hz, J_{CH-CH2}=6.2 Hz, –O–C*H*–CH_2–), 2.32 (t, 2H, J=7.4 Hz, –CH_2–CO_2H), 1.60–1.50 and 1.33–1.26 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 178.3 (–CO_2H), 102.5 (C-1D), 102.4 (C-1C), 101.0 (C-1B), 98.3 (C-1A), 79.3 (C-2A), 78.9 (C-2B), 78.2 (C-3C), 3×73.6 and 73.0 (4 C-5), 70.6 (C-3D), 70.5 (C-3A), 70.3 (C-2D), 70.2 (C-3B), 69.9 (C-2C), 68.3 (–O–CH_2–), 67.4, 2×67.2 and 66.5 (4 C-4), 2×61.4, 61.3 and 61.2 (4 C-6), 34.6 (–CH$_2$–CO_2H), 28.7, 2×28.5, 28.4, 25.6 and 24.6 (–$(CH_2)_6$–); MS *m/z* (HRMS): $(M+Na)^+$ calcd: 845.3267; found: 845.3295.

6.1.7. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (13). Acetic anhydride (345 mL) was added, at 0 °C to a solution of D-mannose (100 g, 556 mmol) in pyridine (500 mL). The solution was stirred overnight and concentrated. A solution of the residue in dichloromethane, was washed with 1 M aq HCl, aq sodium hydrogen carbonate, and water, dried ($MgSO_4$) and concentrated to give **12**.

Thiophenol (85.5 mL, 1.5 equiv) and BF_3Et_2O (350 mL, 5 equiv) were added to a solution of compound **12** in anhydrous dichloromethane (500 mL). The solution was stirred overnight at rt, carefully neutralized by aq sodium hydrogen carbonate, dried ($MgSO_4$) and concentrated. The residue was crystallized in diethyl ether/cyclohexane to give **13** (192.6 g, 76%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.33–7.13 (m, 5H, arom.), 5.35–5.33 (m, 2H, H-1 et H-2), 5.17–5.15 (m, 2H, H-3 et H-4), 4.39 (ddd, 1H, J_{5-6b}=5.2 Hz, J_{5-6a}=2.3 Hz et J_{5-4}=9.2 Hz, H-5), 4.15 (dd, 1H, J_{6b-6a}=12.2 Hz, H-6b), 3.94 (dd, 1H, H-6a), 2.00, 1.92, 1.89 et 1.86 (4 s, 12H, 4 –O–C=O–CH_3).

6.1.8. Phenyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (15). Sodium (1.15 g, 0.1 M) was added to a solution of **13** (92.55 g, 203 mmol) in methanol (470 mL). The solution was stirred at rt overnight, neutralized with Amberlite IR 120 (H+) and concentrated and dried under vacuum on P_2O_5 to give **14**.

Benzaldehyde dimethyl acetal (31.2 mL, 1.05 equiv) was added to suspension of **14** in anhydrous dichloromethane (1 L). HBF_4 (25.8 mL, 1.7 equiv, 50% in ethyl ether) was then added to the cooled solution (0 °C). The reaction mixture was stirred at rt overnight, neutralized (Et_3N) and concentrated. Th residue was crystallized in ethanol to give **15** (57.7 g, 79%) ^{1}H NMR (250 MHz, CD_3OD): δ 7.57–7.27 (m, 10H, arom.), 5.62 (s, 1H, benzylidene), 5.49 (d, 1H, J_{1-2}=1.5 Hz, H-1), 4.24 (m, 1H, H-5), 4.18 (d, 1H, J_{2-3}=3.5 Hz, H-2), 4.13 (dd, 1H, J_{6b-6a}=10.0 Hz et J_{6b-5}=5.0 Hz, H-6b), 4.03 (t, 1H, J_{4-3}=J_{4-5}=10.0 Hz, H-4), 3.95 (dd, 1H, H-3), 3.84 (t, 1H, H-6a).

6.1.9. Phenyl 2-*O*-acetyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (3). Camphor sulfonic acid (292 mg, 1.2 mmol) was added to a stirred solution of **15** (2 g, 5.5 mmol) in triethyl orthoacetate (10 mL) at room temperature, After stirring for 30 min, aq acetic acid 80% (14.4 mL) was added to the cooled (0 °C) reaction mixture and then stirred 1 h at room temperature. Solvents were removed in vacuum and the residue was purified by flash

chromatography (33% EtOAc in cyclohexane) to afford **3** (1.67 g, 75%) as a white powder. $[\alpha]_D$ +169 (*c* 1.0, chloroform); mp: 157–158 °C (cyclohexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56–7.30 (m, 10H, arom.), 5.66 (s, 1H, benzylidene), 5.52 (s, 1H, H-1), 5.51 (dd, 1H, $J_{2-1}=1.3$ Hz, $J_{2-3}=3.3$ Hz, H-2), 4.41 (ddd, 1H, $J_{5-4}=9.7$ Hz, $J_{5-6a}=10.3$ Hz, $J_{5-6b}=4.9$ Hz, H-5), 4.29 (dd, 1H, $J_{6b-6a}=10.3$ Hz, H-6b), 4.28-4.25 (m, 1H, H-3), 4.04 (t, 1H, $J_{4-3}=9.7$ Hz, H-4), 3.89 (t, 1H, H-6a), 2.65 (d, 1H, $J_{OH-3}=3.5$ Hz, OH), 2.21 (s, 3H, O–C=O–CH_3); ^{13}C NMR (100 MHz): δ 170.3 (–O–*C*=O–CH_3), 136.9, 133.0 (2 C arom.), 132.0–126.2 (10 CH arom.), 102.2 (benzylidene), 86.8 (C-1), 79.0 (C-4), 73.5 (C-2), 68.3 (C-6), 67.7 (C-3), 64.5 (C-5A), 20.9 (–O–C=O–*C*H_3); MS *m*/*z* (CI): 403.2 $(M+H)^+$; Anal. Calcd for $C_{21}H_{22}O_6S$ (402.46): C 62.67, H 5.51. Found: C 62.66, H 5.54.

6.1.10. 8-Methoxycarbonyloctyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (18). Glycosylation of **7** (236 mg, 269 μmol) and **5** (165 mg, 269 μmol), as described for **10**, yielded **18** (275 mg, 75%) as a colorless oil. $[\alpha]_D$ +26 (*c* 0.4 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54–7.21 (m, 35H, arom.), 5.67 (s, 1H, benzylidene), 5.54 (dd, 1H, $J_{2-1}=1.8$ Hz, $J_{2-3}=3.3$ Hz, H-2C), 5.45 (dd, 1H, $J_{2-1}=1.5$ Hz, $J_{2-3}=3.5$ Hz, H-2B), 5.31 (d, 1H, $J_{1-2}=1.8$ Hz, H-1C), 5.11 (d, 1H, H-1B), 4.88 (d, 1H, $J_{1-2}=1.9$ Hz, H-1A), 4.87 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.85 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.73 (d, 1H, $J_{gem}=11.2$ Hz, CHPh), 4.71 (s, 2H, CH_2Ph), 4.70 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.67 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.63 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.54 (d, 1H, $J_{gem}=11.2$ Hz, CHPh), 4.52 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.50 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.42 (dd, 1H, $J_{3-4}=9.5$ Hz, H-3B), 4.34 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.32 (dd, 1H, $J_{6b-6a}=10.3$ Hz, $J_{6b-5}=4.3$ Hz, H-6Bb), 4.09 (t, 1H, $J_{4-5}=9.4$ Hz, H-4B), 4.07–3.99 (m, 3H, H-5B, H-2A and H-4C), 3.96 (dd, 1H, $J_{3-2}=3.0$ Hz, $J_{3-4}=8.5$ Hz, H-3A), 3.91 (dd, 1H, $J_{3-4}=9.5$ Hz, H-3C), 3.89–3.75 (m, 7H, H-5C, H-6Ba, H-4A, H-5A, H-6Cb and H-6A), 3.70 (s, 3H, –CO_2CH_3), 3.69 (dt, 1H, $J_{gem}=9.6$ Hz, $J_{CH-CH2}=6.9$ Hz, –O–C*H*–CH_2–), 3.58 (dd, 1H, $J_{6a-6b}=10.8$ Hz, $J_{6a-5}=1.6$ Hz, H-6Ca), 3.41 (dt, 1H, $J_{CH-CH2}=6.6$ Hz, –O–C*H*–CH_2–), 2.34 (t, 2H, $J=7.5$ Hz, C*H*$_2$–CO_2CH_3), 2.12 and 2.09 (2 s, 6H, 2 O–C=O–CH_3), 1.67–1.64, 1.59–1.56 and 1.36–1.30 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 174.3 (–*C*O_2CH_3), 170.1 and 169.5 (2 –O–*C*=O–CH_3), 138.6, 138.4, 138.3, 138.2, 138.1, 138.0 and 137.0 (7 C arom.), 128.8–125.9 (35 CH arom.), 101.4 (benzylidene), 99.9 (C-1B, $^1J_{C-H}=173.1$ Hz), 98.8 (C-1C, $^1J_{C-H}=174.0$ Hz), 98.6 (C-1A, $^1J_{C-H}=169.0$ Hz), 79.7 (C-3A), 78.9 (C-4B), 77.7 (C-3C), 75.2 (CH_2Ph), 2×74.9 (C-2A and C-4A), 74.9 (CH_2Ph), 73.9 (C-4C), 2×73.2 (2 CH_2Ph), 72.1 (CH_2Ph), 72.0 (C-5C), 71.7 (C-5A), 71.6 (CH_2Ph), 71.4 (C-2B), 70.9 (C-3B), 69.1 (C-6A), 68.5 (C-2C), 68.4 (C-6B), 68.2 (C-6C), 67.6 (–O–CH_2–), 63.9 (C-5B), 51.4 (–CO_2*C*H_3), 34.0 (–*C*H_2–CO_2CH_3), 29.4, 29.2, 29.1, 29.0, 26.0 and 24.9 (–$(CH_2)_6$–), 21.0 and 20.8 (2 –O–C=O–*C*H_3); MS *m*/*z* (CI, NH_3): 1404 $(M+NH_4)^+$; Anal. Calcd for $C_{81}H_{94}O_{20}$ (1387.636): C 70.11, H 6.82. Found: C 70.03, H 6.88.

6.1.11. 8-Carboxyloctyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-(4,6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (19). Saponification of **18** (236 mg, 172 μmol) as described for **10**, yielded **19** (160 mg, 73%) as a colorless oil. $[\alpha]_D$ +41 (*c* 0.3 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76–7.28 (m, 35H, arom.), 5.62 (s, 1H, benzylidene), 5.13 (d, 1H, $J_{1-2}=1.3$ Hz, H-1C), 5.09 (se, 1H, H-1B), 4.90 (d, 1H, $J_{gem}=10.3$ Hz, CHPh), 4.89 (se, 1H, H-1A), 4.85 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.74 (d, 1H, $J_{gem}=12.3$ Hz, CHPh), 4.73 (s, 2H, CH_2Ph), 4.72 (s, 2H, CH_2Ph), 4.64 (d, 1H, $J_{gem}=12.3$ Hz, CHPh), 4.58 (d, 1H, $J_{gem}=10.3$ Hz, CHPh), 4.55 (d, 1H, $J_{gem}=11.2$ Hz, CHPh), 4.49 (d, 1H, $J_{gem}=11.2$ Hz, CHPh), 4.48 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.43–4.42 (m, 1H, H-2B), 4.29 (dd, 1H, $J_{6b-6a}=10.2$ Hz, $J_{6b-5}=4.6$ Hz, H-6Bb), 4.21–4.16 (m, 2H, H-3B and H-5C), 4.12 (t, 1H, $J_{4-3}=J_{4-5}=9.6$ Hz, H-4B), 4.07–4.00 (m, 3H, H-2A, H-2C and H-5B), 3.98 (dd, 1H, $J_{3-2}=2.9$ Hz, $J_{3-4}=9.2$ Hz, H-3A), 3.97 (dd, 1H, $J_{3-2}=3.2$ Hz, $J_{3-4}=9.0$ Hz, H-3C), 3.91 (dd, 1H, $J_{6a-5}=10.0$ Hz, H-6Ba), 3.89 (t, 1H, $J_{4-3}=J_{4-5}=9.3$ Hz, H-4A), 3.84–3.74 (m, 3H, H-5A, H-6A and H-6Cb), 3.74–3.68 (m, 1H, –O–C*H*–CH_2–), 3.68 (t, 1H, $J_{4-5}=9.0$ Hz, H-4C), 3.55 (dd, 1H, $J_{6a-6b}=9.8$ Hz, $J_{6a-5}=7.6$ Hz, H-6Ca), 3.43 (dt, 1H, $J_{gem}=9.6$ Hz, $J_{CH-CH2}=6.5$ Hz, –O–C*H*–CH_2–), 2.37 (t, 2H, $J=7.4$ Hz, –C*H*$_2$–CO_2H), 1.70–1.64, 1.61–1.56 and 1.38–1.32 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 178.8 (–CO_2H), 138.4, 138.2, 138.1, 137.8, 137.6, 137.5 and 137.2 (7 C arom.), 128.9–127.3 (35 CH arom.), 102.5 (C-1B), 101.8 (benzylidene), 99.8 (C-1C), 98.8 (C-1A), 79.7 (C-3A), 79.6 (C-3C), 77.3 (C-4B), 76.8 (C-3B), 75.2 (CH_2Ph), 74.9 (CH_2Ph), 74.8 (C-2A and C-4A), 74.5 (C-4C), 73.3 (CH_2Ph), 73.2 (CH_2Ph), 72.3 (CH_2Ph), 72.0 (CH_2Ph), 71.7 (C-5A), 71.5 (C-5C), 69.5 (C-2B), 69.1 (C-6A), 68.9 (C-6C), 68.7 (C-6B), 68.5 (C-2C), 67.6 (–O–CH_2–), 64.1 (C-5B), 33.8 (–*C*H_2–CO_2H), 29.3, 29.0, 28.9, 28.8, 25.9 and 24.6 (–$(CH_2)_6$–); MS *m*/*z* (CI, NH_3): 1306 $(M+NH_4)^+$; Anal. Calcd for $C_{76}H_{88}O_{18}$ (1289.534): C 70.78, H 6.87. Found: C 70.69, H 7.03.

6.1.12. 8-Carboxyloctyl (α-D-mannopyranosyl)-(1→3)-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (2). Hydrogenolysis of **19** (164 mg, 127.5 μmol), as described for **1**, yielded **2** (80 mg, 95%) as white amorphous powder. ^{1}H NMR (400 MHz, D_2O): δ 5.10 (s, 1H, H-1C), 5.04 (s, 1H, H-1A), 4.96 (s, 1H, H-1B), 4.19 (dd, 1H, $J_{2-1}=1.3$ Hz, $J_{2-3}=1.4$ Hz, H-2B), 4.03 (dd, 1H, $J_{2-1}=1.5$ Hz, $J_{2-3}=1.6$ Hz, H-2C), 3.93–3.88 (m, 2H, H-2A and H-3B), 3.86–3.82 (m, 2H, H-3A and H-3C), 3.72–3.65 (m, 1H, –O–C*H*–CH_2–), 3.49 (dt, 1H, $J_{gem}=10.4$ Hz, $J_{CH-CH2}=6.1$ Hz, –O–C*H*–CH_2–), 2.32 (t, 2H, $J=7.35$ Hz, C*H*$_2$–CO_2CH_3), 1.60–1.52 and 1.34–1.25 (m, 12H, –$(CH_2)_6$–); ^{13}C NMR (100 MHz): δ 180.2 (–CO_2H), 102.6 (C-1B), 102.5 (C-1C), 98.3 (C-1A), 79.0 (C-2A), 78.1 (C-3B), 73.6, 73.6 and 73.0 (3 C-5), 70.6 (C-3C), 70.6 (C-3A), 70.3 (C-2C), 69.8 (C-2B), 68.3 (–O–CH_2–), 2×67.2 and 66.6 (3 C-4), 61.4, 61.3 and 61.2 (3 C-6), 34.6 (–*C*H_2–CO_2H), 28.7, 28.6, 28.5, 28.4, 25.6 and 24.8 (–$(CH_2)_6$–); MS *m*/*z* (HRMS): $(M+Na)^+$ calcd: 683.2738; found: 683.2748.

6.2. Conjugation to poly L-lysine

The coupling buffer (0.1 M MES buffer, pH 4.6) was prepared as follow: 4-Morpholino ethane sulfonic acid

hydrate (Acros 17259, 1 g) was dissolved in water (50 mL), the pH was adjusted to 4.6 with conc aq NaOH.

Poly L-Lysine.hydrobromide (Sigma 2636, mol wt 30,000–70,000, 100 mg) was dissolved in MES buffer (4 mL). Compound **1** or **2** (20 mg) was dissolved in MES buffer (10 mL). The two solutions were mixed and EDC (Acros 17144, 50 mg) was added at rt. Another EDC portion (50 mg) was added after 5 h and the reaction mixture was stirred for 16 h at rt, and concentrated. The residue was purified on Sephadex G25 (column length 40 cm, diameter 2.6 cm) using water as eluent. The yields were 50%, the sugar content was 10% as evaluated by 400 MHz ^{1}H NMR.

6.3. Detection of antibodies against synthetic oligomannosides

Tetramannoside-poly-lysine **1-lys** and trimannoside-poly-lysine **2-lys** were used as antigens in an enzyme-linked immuno-sorbent assay. Plates were first coated overnight at room temperature with 200 μL of tetramannoside (**1-lys**) or trimannoside (**2-lys**) at a concentration of 10 μg/mL in phosphate buffered saline (PBS) 0.15 M. The day after, the plates were washed in PBS-Tween 0.2% and saturated with Glucose (5%) Bovine Serum Albumin (0.6%) in PBS 0.15 M. Patients sera were diluted 1:400 in a sample diluent (kit Platelia Candida Ab Biorad)) and added to the plates for one hour at 37 °C. After three washing with TNT (Tris 0.05 M, NaCl 0.15 M, Tween 20 0.1%), a peroxydase-labeled goat antihuman immunoglobulin (G, A, M) (H and L chains) (Biorad, Marnes la Coquette, France) was used as conjugate. A color reaction was detected by incubation with 200 μL of tetramethylbenzidine solution for 30 min. Plates were read at 450 nm on a MRX2 (Dynex, France) automatic reader. A pool of sera from CD patients strongly reacting with both oligomannosides was selected for standardizing the tests and diluted from 1:100 to 1:6400. Each sample was tested in duplicate, the mean of the optical density in the two wells was calculated and reactivity of individual sera was expressed through the use of a program from Menarini laboratories as a percentage of the highest reactivity observed with the standard arbitrarily defined as 100%.

6.4. Statistical analysis

The statistical program used was Statview. The mean values were calculated on both antigen for CD and UC patients, and comparison between quantitative variables on the different groups was performed by the nonparametric Mann-Whitney test. Results were considered statistically significant when the two-side probability was less than 0.05.

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