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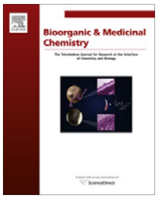

Synthetic biotinylated tetra β(1→5) galactofuranoside for in vitro aspergillosis diagnosis

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Dedicated to Jacques H. van Boom (1937–2004) in memoriam

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ABSTRACT

The synthesis of a tetra β(1→5) galactofuranoside was achieved using a thioglycoside donor with a methyl *tert*-butyl phenyl thio leaving group. This tetrasaccharide was conjugated to biotin and validated as antigen with the monoclonal antibody used for clinical detection of *Aspergillus fumigatus* galactomannan on streptavidin-coated microplates. Then we have shown its ability to detect antibodies associated with *A. fumigatus* induced disease by using sera from patients with Allergic broncho-pulmonary aspergillosis (ABPA) and correlated the results of antibody detection with those gained with a commercially available diagnostic test.

1. Introduction

Aspergillus fumigatus is a very common air-borne mould with some pathogenic potential leading to different types of disease depending on individual susceptibility and/or intensity of exposure.[1] These comprise ‘Farmer’s lung’ where repeated intense exposure to *A. fumigatus* spores (conidia) generates high levels of anti-*A. fumigatus* immunoglobulins (Ig) of IgG isotype leading to semi-delayed type hypersensitivity inducing pathology based on immune complexes. Allergic broncho-pulmonary aspergillosis (ABPA) occurring in patients with defect in mucobronchial clearance is characterized by limited *A. fumigatus* growth inducing local damage with an host reaction comprising IgG but also anti-*A. fumigatus* IgE; an ‘hypersensitivity type‘ also found in patients with so called ‘aspergillus asthma’.[2] In local pulmonary settings characterized by lungs deterged cavities created by tuberculosis or cancer surgery after effects, *A. fumigatus* develops easily under its mycelial form, creating real fungus balls presenting a threat for vascular system. Such a development is associated with very high levels of anti-*A. fumigatus* IgG.[3] Finally, invasive aspergillosis infection (IAI) affects severely immunosuppressed hospital patients, mainly in onco-hematology units. In such conditions where patients are devoid of pulmonary defenses as a result of aplasia induced chemotherapy *A. fumigatus* conidia germinate and extensive spreading mycelial growth quickly invade the lungs threatening patients life. For all these forms of diseases caused by a single organism, the biological diagnosis is essential, however the isolation/ identification of *A. fumigatus* by conventional mycology procedures is not always feasible or representative of the pathogenicity of this opportunistic pathogen.[4] All diagnostic strategies refer to serology using more or less refined antigens.[5] Among which *A. fumigatus* antigen galactomannan is quantitatively and qualitatively major.[6] All clinical forms of aspergillosis occurring in immunocompetent patients are characterized by an Ig response against this antigen, whereas for IAI monoclonal antibody based detection of galacto-mannanemia has gained recognition by physicians as a reliable criteria for diagnosis.[7] Sensitivity of this test may be due to massive expression of the detected galactofuranoside epitopes

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on *A. fumigatus*, which, beside the galactomannan, is also covalently attached to cell wall polysaccharides, associated to secreted glycoproteins and different glycolipids.[8] With regard to the importance of this epitope, we decided to include it in our program on the preparation of fungal antigenic oligosaccharides, in particular of their biotinylated conjugates. We described here the synthesis of a biotinylated tetragalactofuranoside (**A**) based on the identification of the *A. fumigatus* epitope circulating in IAI patients' sera.[9] We used this approach previously for creating a bank of oligomannosides mimicking parts of the yeast oligomannose repertoire for the diagnosis and pathophysiological analysis of *Candida* infections[10] and Crohn disease.[11]

2. Results and discussion

β-Galactofuranoside is an important component of parasite and bacteria oligosaccharides[12] and increasing synthetic work have been done[13] in particular in the fields of *Trypanosoma cruzi* and *mycobacteria* glycans.

Jacques van Boom's group had developed efficient synthesis of β Galf 1,5 oligosaccharides (from 4[14] to 7[15] units) in 1987–89 using Helferich activation ($Hg(CN)_2$, $HgBr_2$) of a glycosyl chloride (Fig. 1).

However, as far as we know, these oligosaccharides were not evaluated as antigens in aspergillosis. We decided to synthesize a biotin-conjugated tetragalactofuranoside (**A**) to evaluate this antigen as a medical tool. The retrosynthesis scheme is depicted in Scheme 1. The synthesis relies on a key-elongating thioglycoside **8** (Schemes 1 and 2). The thiol used in this compound is 2-methyl 5-*tert*-butyl thiophenol; it is a commercially available, less toxic and odorless alternative[16] to thiophenol. Position to be glycosylated (position 5) is temporary protected by a levulinate. A benzoate group in position 2 is used as a participating group. The preparation of compound **8** is described in Scheme 2.

D-Galactose was benzoylated in hot pyridine by benzoyl chloride according to Gallo-Rodriguez et al.[17] We faced first difficulties to reproduce the original results and to obtain pure *furano* perbenzoate (a detailed protocol is described in experimental part, in particular for the selective crystallization of *furano* forms). The anomeric benzoate was exchanged to a thioaryl group in the presence of $BF_3{\cdot}Et_2O$. After de-O-benzoylation, an isopropylidene acetal was introduced to selectively protect the positions 5 and 6. Benzoates were re-introduced on positions 2 and 3 and the isopropylidene group removed. A sequential bis acylation (benzoate, then levulinate) afforded compound **8** in a 23% overall yield from **1**. The position of the benzoate in position 6 in compound **7** was ascertained by ^1H NMR H-6 signals moved from 3.85 in **6** to 4.50 ppm in **7**, meanwhile H-5 signal moved slightly from 4.30 to 4.50 ppm,

With compound **8** in hand, the construction of the oligosaccharidic chain is straightforward and depicted on Scheme 3. First, the ethyl 6-hydroxy hexanoate was glycosylated with **8** to give **9**. The β configuration of **9** was checked in NMR: ^1H NMR (H-1 singlet, H-2 $J_{2,3}$ = 1.5 Hz) and ^{13}C NMR C-1 105.6 ppm) in agreement with van Boom spectral data.[14,15] Compound **9** was then de-*O*-levulinated[18] and glycosylated with **8**. The sequence was repeated until the tetrasaccharide **15** was reached. The overall yields were good (60–75%) and not size dependant. N-Bromo succinimide (NBS) was preferred to N-iodo succinimide (NIS); it usually gives better result with poorly reactive benzoylated thioglycosidic donors and it is also more stable and less expensive.

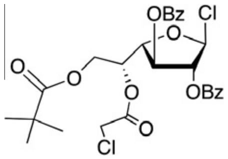


Figure 1. Repeating unit precursor used in van Boom's group.

The compound **15** was then converted in to the per-acetylated acid **18** by a three steps sequence: trans-esterification of levulinate and benzoate ester functions with basic methanol to give **16**, then saponification (aq NaOH) of the methyl ester (→**17**) and acetylation to give **18** (Scheme 4).

The two steps procedure avoided the contamination of **17** by levulinic and benzoic acids—they were easily removed as methyl esters. The acid **18** was then coupled with a biotin containing amine **19**[10] and the acetates were removed to give **A**.

2.1. Reactivity of A with monoclonal antibodies

The reactivity of **A** was tested with two rat monoclonal antibodies:

EBA2, a rat monoclonal immunoglobulin M (IgM) antibody, directed against *Aspergillus* β-galactofuranoside glycoconjugates. It is included in the commercial Platelia *Aspergillus* kit (Bio-Rad).

EBCA-1, a rat IgM monoclonal antibody, directed against *Candida* α-1,2 linked mannopentaose was used here as negative control. The later antibody was also used in the commercial Platelia *Candida* antigen kit for the detection of circulating *Candida* mannan.[19] Both monoclonal antibodies (MAbs) were HRP-labeled.

Two monoclonal antibodies were tested with **A** (0.1 μg/ml) coated on the streptavidin plates.

Figure 2A shows a dose-dependent signal for EBA2 and an absence of signal for EBCA-1. These results show the specificity of EBA2 for A.

Figure 2B shows the inhibition of exogenous A antigen (soluble) on the interaction of EBA2 antibody with **A** coated plate. EBA2 reactivity is inhibited according to the increased concentration of **A** in solution.

2.2. Detection of anti-A antibodies in patients with allergic broncho-pulmonary aspergillosis (ABPA)

The series of experiments were conducted with 29 sera from 14 patients: 5 patients with ABPA (20 sera) and 9 healthy blood donors as control group (9 sera). For each patient, whole venous blood was collected and separated by centrifugation for serological analyses. Two tests were compared:

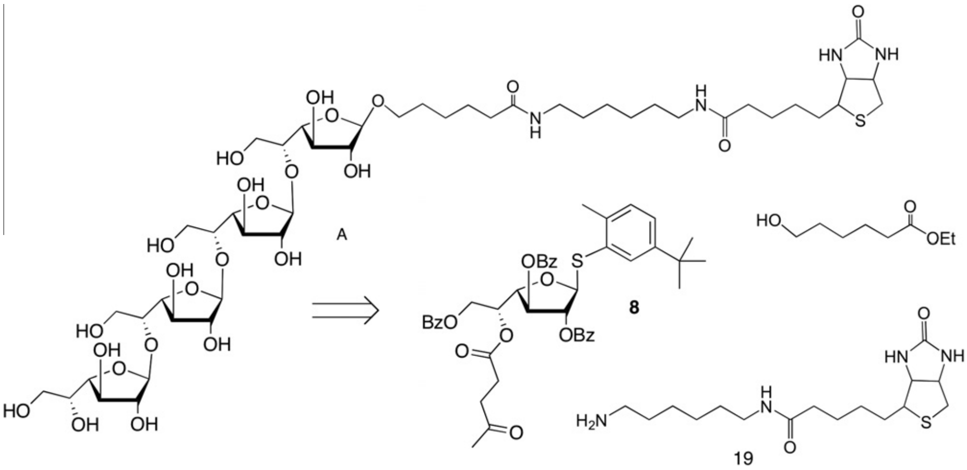
The first is based on the tetrasaccharide **A** on streptavidine coated microplate in ELISA protocol: streptavidin-plates were first coated overnight at room temperature with **A** at a concentration of 0.1 μg/mL. The day after, the plates were washed and saturated (BSA, 1%). Patients' sera were diluted (1:200) in a phosphate buffer.

The second was the serion commercial kit: IgG *Aspergillus fumigatus* Serion Elisa Classic kit. It was designed for the detection of anti-*Aspergillus fumigatus* antibodies (Institut Virion/Serion GmbH, Würzburg, Germany) and uses a whole cell wall extract from *Aspergillus fumigatus*. Serion test (http://www.virion-serion.de/322.0.html) was used according to manufacturer instructions

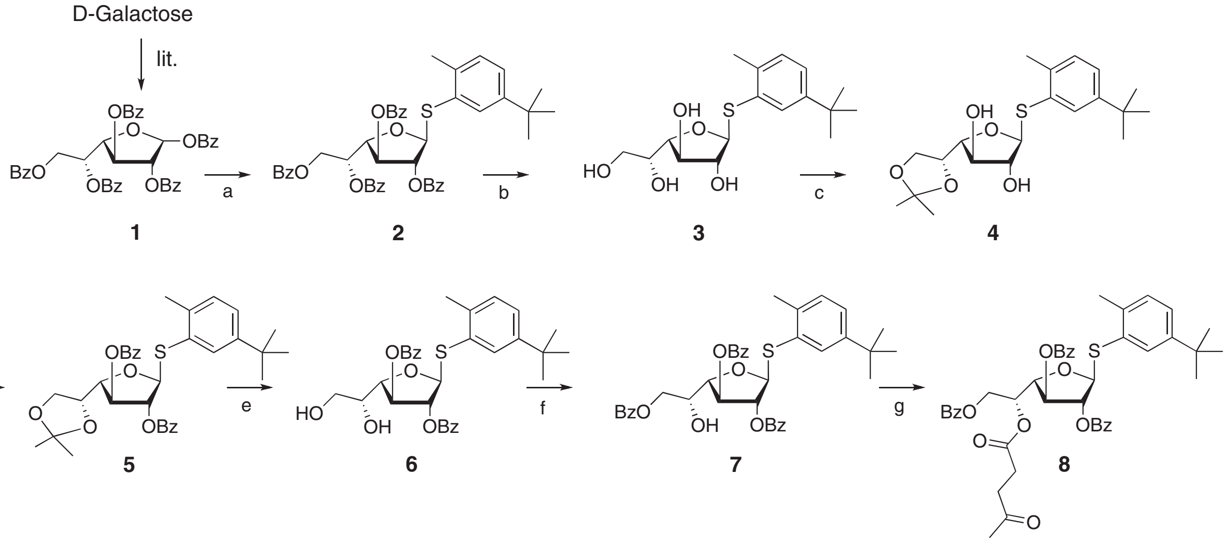
Figure 3 shows individual reactivity of ABPA patient sera and healthy controls in both tests. This figure represents the correlation between antibody titers observed for both Serion and **A**-based tests. An excellent correlation between both tests was observed for ABPA patients and control sera reactivity (r = 0. 96).

2.3. Conclusion, perspectives

We described the synthesis of a biotinylated tetrasaccharide composed of β (1,5) gal*f* units, analogous of galf oligosaccharides



Scheme 1. Retrosynthetic scheme of the target biotinylated β(1→5) tetragalactofuranoside (A).



Scheme 2. Reagents and conditions: (a) 2-methyl-5-*tert*-butyl-thiophenol, $BF_3 \cdot Et_2O$, CH_2Cl_2, 97%; (b) NaOMe, MeOH, 94%; (c) 2,2-dimethoxypropane, CSA, acetone, 90%; (d) BzCl, DMAP, NEt_3, CH_2Cl_2, 99%; (e) aq AcOH 100%; (f) BzCl, DMAP, NEt_3, CH_2Cl_2, −20 °C, 51%; (g) levulinic acid (LevOH), DCC, DMAP, CH_2Cl_2, 78%.

of *Aspergillus fumigatus* using odorless thioglycoside chemistry. The conjugate was successfully coated on streptavidine plates and recognized by a monoclonal antibody used for clinical purposes and described for reacting primarily with this epitope. The analysis of ABPA patients' antibody reactivity against this epitope compared favourably with commercial kit using *A. fumigatus* whole cell extract. This strongly suggests the diagnostic potential of this construct and open perspectives to be explored for various diseases induced by *A. fumigatus*. This could include extension to IgE isotype detection for ABPA or *A. fumigatus* asthma. For IAI the constructed probe makes accessible the assessment of a possible balance between circulation of this epitope in patient's sera and homologous antibody response during the time course of infection. Such has been demonstrated during the time course of *Candida albicans* infection depending on patients' immunodepression period, with a complementation of antibody and antigen detection tests increasing diagnostic sensitivity. Finally as this new *A. fumigatus* neo-antigen complements those existing for *C. albicans*, inclusion of both types of biotin tagged oligosaccharide (BTO) in a chip or on microspheres could cover analysis of patients antibody response towards the two major fungal pathogens causing medical and economic problems in developed countries.

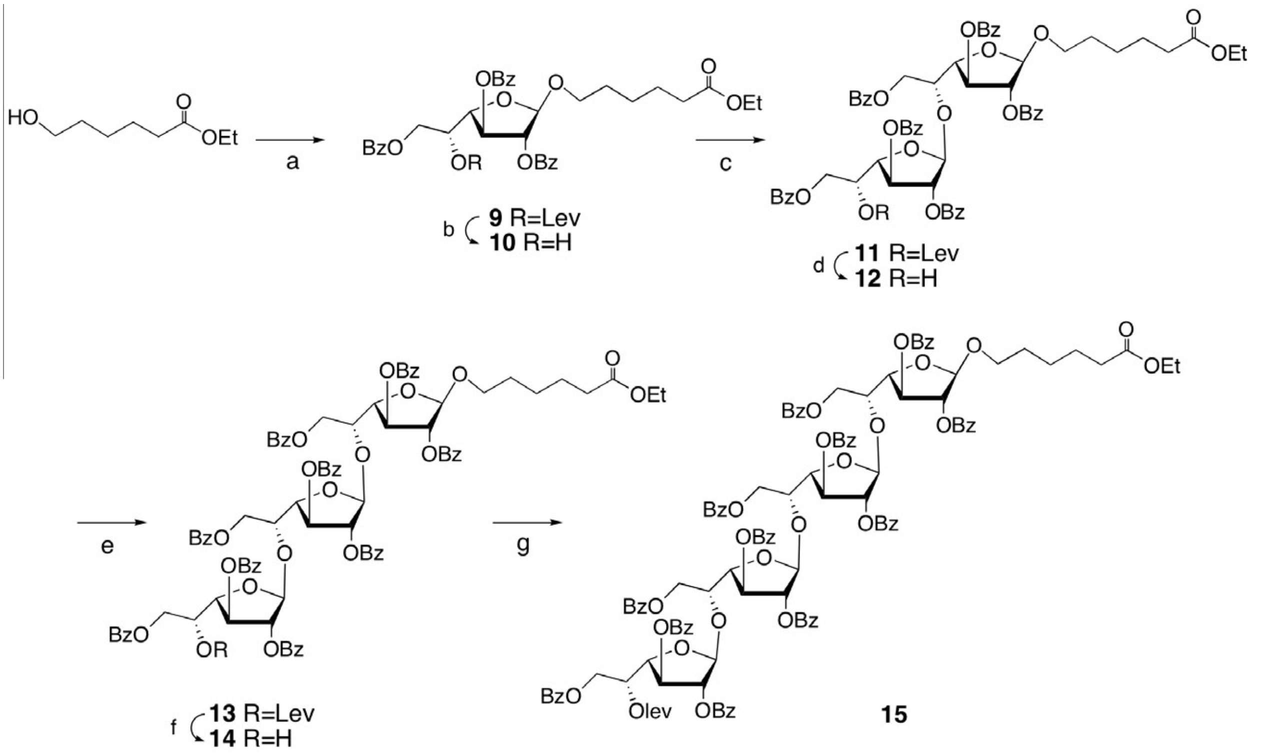
3. Experimental part

3.1. Monoclonal antibodies

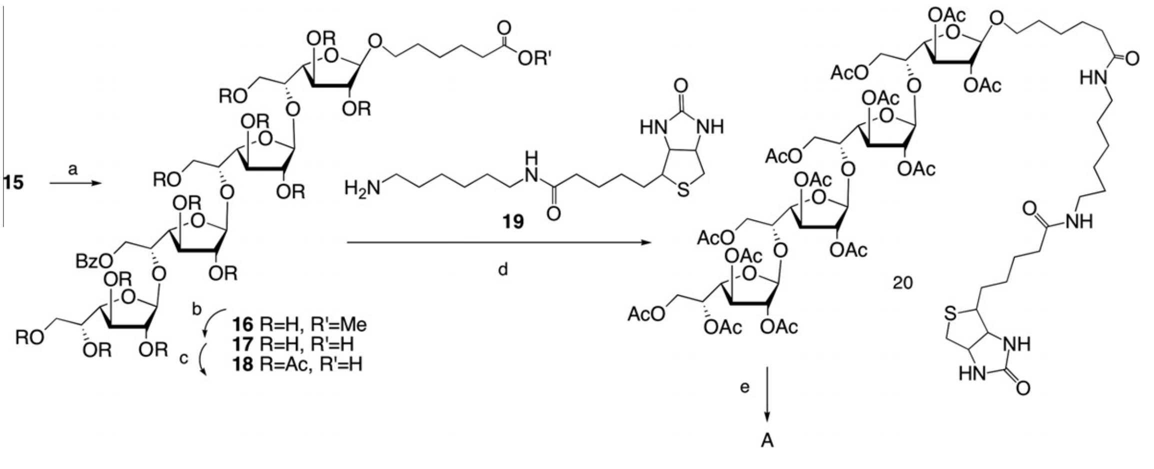
Both monoclonal antibodies (MAbs) were HRP-labeled. These peroxydase-labeled MAbs were diluted from 1:10 to 1:800 in a wash buffer (Platelia *Candida*® Ag Kit, Bio-Rad) and 100 μL were added to the plates for 1 h at 37 °C. After four washes with R2 1X buffer, 200 μL of chromogen tetramethylbenzidine (Platelia *Candida*® Ag Kit, Bio-Rad) was used for 30 min at 37 °C, in darkness. The plates were read at 450 nm on a PR3100 microplate reader (Bio-Rad). Different concentrations of soluble A were also used in an inhibition ELISA test. Each concentration of A was incubated with EBA2 (with a final volume of 100 μL) for 1 h at 37 °C before ELISA test as described above. Exogenous A was used at a concentration of 0, 2, 4 or 8 μg/mL.

3.2. Detection of anti-A antibodies in patients with allergic broncho-pulmonary aspergillosis (ABPA)

Serion test was used according to manufacturer instructions. Briefly, patients sera were diluted 1:500 in a dilution buffer (Phos-



Scheme 3. Reagents and conditions: (a) NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 68%; (b) N_2H_4 (0.5 M) in pyridine/AcOH (4:1), 67%; (c) NBS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 60%; (d) N_2H_4 (0.5 M) in pyridine/AcOH (4:1), 96%; (e) NBS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 0 °C, 68%; (f) N_2H_4 (0.5 M) in pyridine/AcOH (4:1), 70%; (g) NBS, TfOH, molecular sieves 4 Å, CH_2Cl_2, 0 °C, 75%.



Scheme 4. Reagents and conditions: (a) MeONa, MeOH, 2 h, 20 °C; (b) NaOH, H_2O, 2 h, 50 °C; (c) Ac_2O, DMAP, Pyr, 15 h, 20 °C; (d) **19**, EDC, DMAP, pyr., 15 h, 60 °C (overall yield 10%); (e) MeONa, MeOH, 15 h, 20 °C, 81%.

phate, Tween20, sodium azide) and added (100 µL per well) to the plates for 1 h at 37 °C. After washing with a solution of (sodium chloride, Tween 20, TRIS 30 mM, sodium azide), a goat anti-human polyclonal antibody phosphatase alcaline labeled was added (100 µL per well) for 30 min at 37 °C. After washes, a color reaction was detected by incubation with 100 µL of para-nitrophenyl phosphate solution for 30 min at 37 °C. After addition of stop solution (1.2 M NaOH), absorbance was read at 405 nm.

For A-ELISA, streptavidin-plates were first coated overnight at room temperature with A at a concentration of 0.1 µg/mL. The day after, the plates were washed and saturated (BSA, 1%). Patients' sera were diluted 1:200 in Phosphate buffer. 100 µL were added to the wells and incubated for 1 h at 37 °C. After four washes, a peroxydase-labeled anti-human IgG was used as conjugate. A color reaction was detected by incubation with tetramethyl-benzidine solution for 30 min. Absorbance was read at 450 nm.

3.3. Chemical synthesis

3.3.1. General procedures

All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. 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 was performed on Silica Gel 60

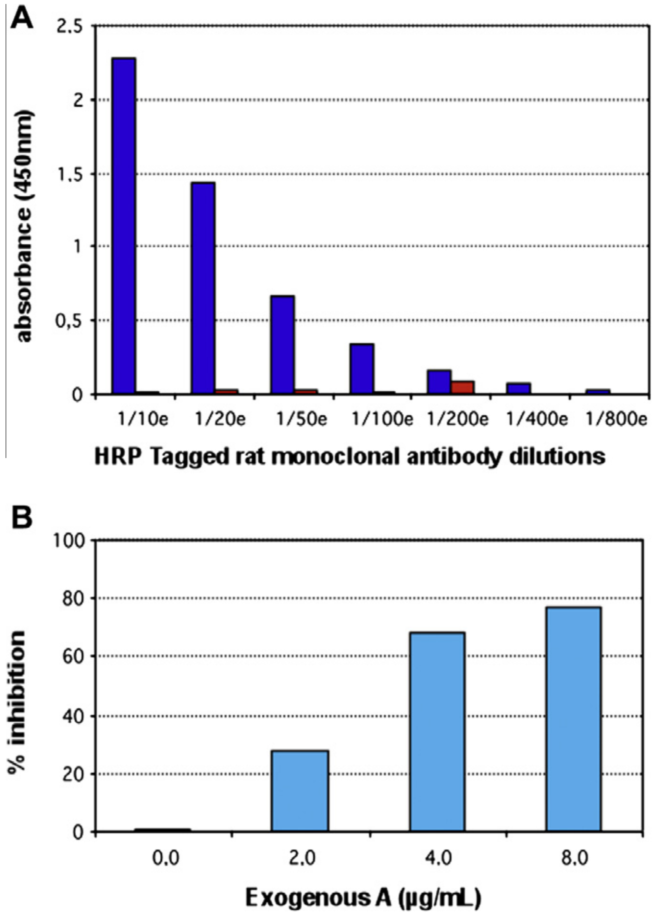


Figure 2. (A) Reactivity of **A** with monoclonal antibodies. Blue bars correspond to EBA2 reactivity and red bars to control (EBCA-1). (B) Inhibition of the binding of MAb EBA2 to **A**. Results were expressed as percentage of inhibition compared to sample incubated without inhibitor (exogenous A 0 µg/mL).

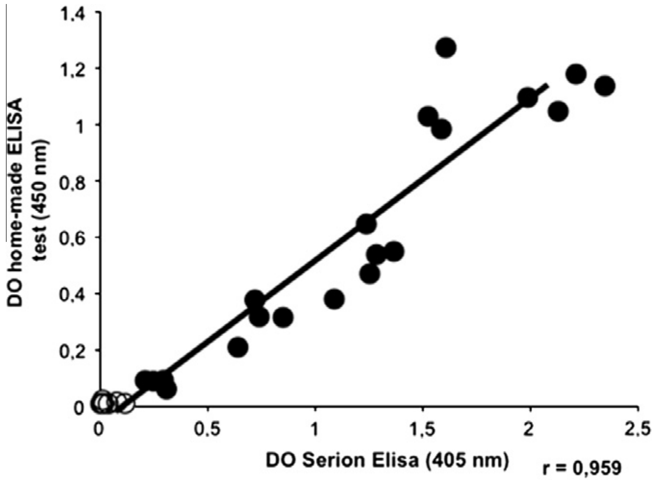


Figure 3. Correlation between the IgG seroreactivity of A and the whole cell wall extract of *A. fumigatus* (Serion Elisa Classic kit) using sera from patients with ABPA (Black circle) and healthy subjects (white circle). *r*: is the correlation coefficient.

(E. Merck) using cyclohexane (Cyhex) and Ethyl acetate. ^{1}H NMR spectra were recorded with Brüker AM 250, AM 400 instruments. Carbons of the hexyl chain are numbered from 7 to 12 in NMR spectra assignments. Chemical ionization and FAB mass spectrometry were recorded with Jeol MS700: CI (gas: ammonia); FAB (matrix: NBA, NaI).

3.3.2. 1,2,3,5,6-Penta-*O*-benzoyl-α,β-D-galactofuranoside (1)

The solution of anhydrous D-galactose (10.00 g, 55.51 mmol) in anhydrous pyridine (150 mL) was refluxed for 2 h then benzoyl chloride (40 mL, 344 mmol, 6.2 equiv) was added in one portion through the condenser. The mixture was stirred at 60 °C for 1 h30, then cool down at room temperature. Pyridine was evaporated. The solution of the residue in CH_2Cl_2 was washed with aq HCl (1 M), aq $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated to give a red solid which was dissolved in boiling EtOH (1500 mL). The mixture was cooled down to room temperature for 3 days and the solids were removed by filtration to give **1** (29.52 g, 75%) as a white powder (mixture of the two anomers $\alpha = 55\%$, $\beta = 45\%$). R_f: 0.50 (α and β) (Cyhex/EtOAc 7:3). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 4.60–4.80 (m, 4H, $H_4\alpha$, $H_{4\beta}$, $H_{6\alpha}$, $H_{6\beta}$), 5.15–5.25 (m, 2H, $H_{2\alpha}$, $H_{5\alpha}$), 5.75–5.85 (m, 2H, $H_{2\beta}$, $H_{5\beta}$), 6.00–6.10 (m, 1H, $H_{3\alpha}$), 6.20 (t, 1H, $J_{H3\beta H2\beta} = J_{H3\beta H4\beta} = 7.1$ Hz, $H_{3\beta}$), 6.20 (s, 1H, $H_{1\alpha}$), 6.25 (d, 1H, $J_{H1\beta H2\beta} = 4.7$ Hz, $H_{1\beta}$), 7.00–8.10 (m, 25H, H Ar).

3.3.3. (2-Methyl-5-*tert*-butylphenyl) 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (2)

To a solution of **1** (29.52 g, 42.17 mmol, 1 equiv) in anhydrous CH_2Cl_2 (100 mL), were added under argon at 0 °C: 2-methyl-5-*tert*-butylthiophenol (10.00 mL, 54.82 mmol, 1.3 equiv) and boron trifluoride etherate (10.70 mL, 84.34 mmol, 2 equiv). The mixture was stirred for 1 h30 at room temperature and was neutralised with a saturated aq $NaHCO_3$ solution at 0 °C. The product was extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (Cyhex then Cyhex/EtOAc 9:1) to give 31.09 g of **2** (97%) as a white foam. $[\alpha]_D^{25}$ −29 (*c* 2, $CHCl_3$). MS DCI+-HRMS *m*/*z* $[M+NH_4]^+$ calcd for $C_{45}H_{46}NO_9S$ 776.2893, found 776.2897. R_f: 0.53 (Cyhex/EtOAc 8:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.31 (s, 9H, *t*Bu), 2.50 (s, 3H, CH_3), 4.75–4.85 (m, 2H, H_6), 5.03 (t, 1H, $J_{H4H3} = J_{H4H5} = 4.3$ Hz, H_4), 5.77–5.80 (m, 2H, H_2, H_3), 5.85 (s, 1H, H_1), 6.17 (dt, 1H, $J_{H5H6a} = 6.8$ Hz, $J_{H5H6b} = J_{H5H4} = 4.3$ Hz, H_5), 7.10–8.10 (m, 23H, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.4 (CH_3), 31.1 (*t*Bu), 34.3 (Cq *t*Bu), 63.5 (C_6), 70.4 (C_5), 77.8 (C_2 or C_3), 81.6 (C_4), 82.5 (C_2 or C_3), 91.4 (C_1), 124.9–133.5 (C Ar), 138.2 (Cq), 149.7 (Cq), 165.2–165.9 (4 CO Bz).

3.3.4. (2-Methyl-5-*tert*-butylphenyl) 1-thio-β-D-galactofuranoside (3)

To a solution of **2** (31.09 g, 40.97 mmol, 1 equiv) in anhydrous methanol (300 mL) was added sodium (200 mg, 8.2 mmol, 0.2 equiv). The mixture was stirred overnight and then neutralised (IR-120 H^+ resin), filtered and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 5:5 then EtOAc 100%) to give 14.04 g of **3** (94%) as a syrup, used as such in the next step. R_f: 0.45 (EtOAc).

3.3.5. (2-Methyl-5-*tert*-butylphenyl) 5,6-*O*-isopropylidene-1-thio-β-D-galactofuranoside (4)

To a solution of **3** (13.19 g, 38.52 mmol, 1 equiv) in anhydrous acetone (200 mL) were added 2,2-dimethoxypropane (7.16 mL, 57.8 mmol, 1.5 equiv) and camphorsulphonic acid (2.68 g, 11.5 mmol, 0.3 equiv). The mixture was stirred at room temperature for 2 h. NEt_3 (2.140 mL, 15.41 mmol, 0.4 equiv) was added and the solution was concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 6:4) to give

13.26 g of **4** (90%) as a syrup. R_f: 0.40 (Cyhex/EtOAc 5:5). ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 1.25 (s, 9H, *t*Bu), 1.30–1.40 (2s, 6H, 2 CH_3 isopropylidene), 2.30 (s, 3H, CH_3 thio), 3.80–4.60 (m, 6H, H_2, H_3, H_4, H_5, $2H_6$), 5.40 (s, 1H, H_1), 7.00–7.50 (m, 3H, H Ar).

3.3.6. (2-Methyl-5-*tert*-butylphenyl) 2,3-di-*O*-benzoyl-5,6-*O*-isopropylidene-1-thio-β-D-galactofuranoside (5)

To a solution of **4** (13.26 g, 34.67 mmol, 1 equiv) in anhydrous CH_2Cl_2 (150 mL) were added NEt_3 (15.5 mL, 111 mmol, 3.2 equiv), DMAP (0.847 mg, 6.9 mmol, 0.2 equiv) and benzoyl chloride (12.08 mL, 104 mmol, 3 equiv). The mixture was stirred at room temperature for 45 min. The crude is washed with water and a saturated aq $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (Cyhex 100% then Cyhex/EtOAc 8:2) to give 19.68 g (96%) of **5** as a syrup. R_f: 0.23 (Cyhex/EtOAc 9:1). $[\alpha]_D^{25}$ −44 (*c* 3, $CHCl_3$). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{34}H_{42}O_7NS$ 608.2676, found 608.2690. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 9H, *t*Bu), 1.45–1.47 (2s, 6H, 2 CH_3 isopropylidene), 2.53 (s, 3H, CH_3 thio), 4.07 (dd, 1H, J_{H6aH6b} = 8.7 Hz, J_{H6aH5} = 6.0 Hz, H_{6a}), 4.2 (dd, 1H, J_{H6bH6a} = 8.7 Hz, J_{H6bH5} = 6.6 Hz, H_{6b}), 4.60–4.65 (m, 2H, H_4, H_5), 5.65 (dt, *J* = 1.0 Hz, *J* = 2.2 Hz, H_3), 5.75 (m, 2H, H_1, H_2), 7.20–8.30 (m, 13H, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.4 (CH_3 thio), 25.3–26.2 (2 CH_3 isopropylidene), 31.1 (*t*Bu), 34.3 (Cq *t*Bu), 65.6 (C_6), 75.1 (C_4 or C_5), 77.3 (C_3), 82.2 (C_2), 82.8 (C_4 or C_5), 91.3 (H_1), 109.9 (Cq isopropylidene), 125.4–134.4 (C Ar),137.6 (Cq thio), 149.6 (Cq thio), 165.1–165.5 (2 CO Bz).

3.3.7. (2-Methyl-5-*tert*-butylphenyl) 2,3-di-*O*-benzoyl-1-thio-β-D-galactofuranoside (6)

Compound **5** (19.67 g, 33.33 mmol) was dissolved in glacial AcOH (160 mL) and the solution was heated at 80 °C before water (40 mL) was added through the condenser. The mixture was stirred at 80 °C for 1 h30 and concentrated. The residue was purified by column chromatography on silica gel (Cyhex 100% then Cyhex/EtOAc 6:4) to give 18.33 g (100%) of **6** as a syrup. R_f: 0.55 (Cyhex/EtOAc 5:5). $[\alpha]_D^{25}$ −54 (*c* 2, $CHCl_3$). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{31}H_{38}NO_7S$ 568.2363, found 568.2363. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 9H, *t*Bu), 2.51 (s, 3H, CH_3 thio), 3.70–3.90 (2 dd, 2H, H_6), 4.25 (m, 1H, H_5), 4.63 (dd, 1H, H_4), 5.72–5.75 (m, 2H, H_1, H_3), 5.78 (t, 1H, $J_{H2H1} = J_{H2H3}$ = 1.5 Hz, H_2), 7.00–8.10 (m, 13H, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 20.4 (CH_3 thio), 31.1 (*t*Bu), 34.4 (Cq *t*Bu), 64.3 (C_6), 70.4 (C_5), 78.1 (C_3), 81.9 (C_2), 84.1 (C_4), 91.3 (C_1), 128.4–133.7 (Cq Ar), 137.6 (Cq thio), 149.6 (Cq thio), 165.2–165.9 (2 CO Bz).

3.3.8. (2-Methyl-5-*tert*-butylphenyl) 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (7)

To a cooled (−20 °C) solution of **6** (18.33 g, 33.33 mmol, 1 equiv) in anhydrous CH_2Cl_2 (150 mL) were added: DMAP (828 mg, 10.0 mmol, 0.3 equiv), NEt_3 (5.7 mL, 40.7 mmol, 1.2 equiv). Benzoyl chloride (4.33 mL, 36.7 mmol, 1.1 equiv) was then added dropwise with an addition funnel. The mixture was warmed at 0 °C and was stirred for 1 h, and washed with water. The organic layer was dried over $MgSO_4$, filtered concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 8:2) to give 11.34 g (51%) of **7** as a syrup used as such in the next step.

3.3.9. (2-Methyl-5-*tert*-butylphenyl) 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-1-thio-β-D-galactofuranoside (8)

To a solution of **7** (11.40 g, 17.32 mmol, 1 equiv) in anhydrous CH_2Cl_2 (100 mL) were added levulinic acid (4.02 g, 34.6 mmol, 2 equiv), DMAP (211 mg, 1.73 mmol, 0.1 equiv) and DCC (7.13 g, 34.64 mmol, 2equiv). The mixture was stirred at room temperature for 1 h30, filtrated and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 10.22 g (78%) of **8** as a syrup. R_f: 0.37 (Cyhex/EtOAc 7:3). $[\alpha]_D^{25}$ −26 (*c* 2, $CHCl_3$). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{43}H_{48}NO_{10}S$ 770.2993, found 770.2986. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (s, 9H, *t*Bu), 2.10 (s, 3H, CH_3 Lev), 2.55–2.78 (m, 4H, 2 CH_2 Lev), 4.57 (dd, 1H, J_{H6aH6b} = 11.8 Hz, J_{H6aH5} = 7.2 Hz, H_{6a},), 4.67 (dd, 1H, J_{H6bH6a} = 11.8 Hz, J_{H6bH5} = 4.2 Hz, H_{6b}), 4.87 (t, 1H, $J_{H4H3} = J_{H4H5}$ = 4.6 Hz, H_4), 5.65 (m, 1H, H_3), 5.78–5.80 (m, 2H, H_1, H_2), 5.84 (dt, 1H, $J_{H5H4} = J_{H5H6a}$ = 4.2 Hz, J_{H5H6b} = 7.2 Hz, H_5), 7.20–8.20 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.4 (CH_3 thio), 27.9 (CH_2 Lev), 29.6 (CH_3 Lev), 31.2 (*t*Bu), 34.2 (Cq *t*Bu), 37.8 (CH_2 Lev), 63.1 (C_6), 69.9 (C_5), 77.3 (C_3), 81.1 (C_4), 82.1 (C_2), 91.1 (C_1), 125.3–149.7 (C Ar), 165.2–165.9 (3 CO Bz), 171.9 (CO ester Lev), 205.8 (CO ketone Lev).

3.3.10. 5-Carboxyethylpentyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranoside (9)

To a solution of **8** (3.060 g, 4.069 mmol, 1 equiv) in anhydrous CH_2Cl_2 (20 mL) was added under argon and at 0 °C: molecular sieves 4 Å (1.50 g), ethyl 6-hydroxy hexanoate (800 μL, 4.88 mmol, 1.2 equiv), NIS (2.28 g, 10.15 mmol, 2.5 equiv) and triflic acid (180 μL, 2.03 mmol, 0.5 equiv). The mixture was stirred at 0 °C for 30 min, filtered, and successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 2.020 g of **9** (68%) as a syrup. R_f: 0.34 (Cyhex/EtOAc 7:3). $[\alpha]_D^{25}$ −14 (*c* 1, $CHCl_3$). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{40}H_{48}NO_{13}$ 750.3120, found 750.3115. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.40–1.45 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.11 (s, 3H, CH_3 Lev), 2.30 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.55–2.75 (m, 4H, $2CH_2$ Lev), 3.64 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.81 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.57 (dd, 1H, J_{H4H3} = 4.3 Hz, J_{H4H5} = 4.2 Hz, H_4), 4.63 (dd, 1H, J_{H6aH6b} = 12.0 Hz, J_{H6aH5} = 7.2 Hz, H_{6a}), 4.72 (dd, 1H, J_{H6bH6a} = 12.0 Hz, J_{H6bH5} = 4.2 Hz, H_{6b}), 5.30 (s, 1H, H_1), 5.51 (d, 1H, J_{H2H3} = 1.5 Hz, H_2), 5.65 (dd, 1H, J_{H3H2} = 1.5 Hz, J_{H3H4} = 4.3 Hz, H_3), 5.78 (dt, 1H, $J_{H5H4} = J_{H5H6b}$ = 4.2 Hz, J_{H5H6a} = 7.2 Hz, H_5), 7.30–8.13 (m, 15H, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3 Ethyl), 24.6 (C_8 or C_{10}), 25.6 (C_9), 27.9 (CH_2 Lev), 29.1 (C_8 or C_{10}), 29.6 (CH_3 Lev), 34.2 (C_{11}), 37.8 (CH_2 Lev), 60.1 (CH_2 Ethyl), 63.2 (C_6), 67.3 (C_7), 70.0 (C_5), 77.3 (C_3), 80.9 (C_4), 81.8 (C_2), 105.6 (C_1), 128.3–133.0 (C Ar), 165.3–166.0 (3 CO Bz), 172.0 (CO ester Lev), 173.5 (COOEt), 205.9 (CO ketone Lev).

3.3.11. 5-Carboxyethylpentyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (10)

Compound **9** (2.02 g, 2.76 mmol) was dissolved in a solution (0.5 M) of hydrazine monohydrate (28 mL) in pyridine/AcOH (4:1). The mixture was stirred at room temperature for 30 min and concentrated. The residue was dissolved in CH_2Cl_2, washed with aq (1 M) HCl and aq $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 1.158 g of **10** (67%) as a syrup. R_f: 0.37 (Cyhex/EtOAc 7:3). $[\alpha]_D^{25}$ −10 (*c* 1, $CHCl_3$). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{35}H_{42}NO_{11}$ 652.2752, found 652.2728. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.45 (m, 2H, H_9), 1.52–1.73 (m, 4H, H_8, H_{10}), 2.21 (t, 2H, *J* = 7.5 Hz, H_{11}), 3.50 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 6.2 Hz, H_{7a}), 3.70 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.57 (dd, 1H, *J* = 4.8 Hz, *J* = 2.1 Hz, H_4), 4.40–4.52 (m, 2H, H_5, H_6), 4.57 (m, 1H, H_{6a}), 5.25 (s, 1H, H_1), 5.50 (s, 1H, H_2), 5.64 (dt, 1H,

$J_{H3H2} = J_{H3H4}$ = 4.8 Hz, J_{H3OH} = 0.7 Hz, H_3), 7.22–8.00 (m, 15H, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.3 (CH_3 Ethyl), 24.7 (C_8 or C_{10}), 25.7 (C_9), 29.1 (C_8 or C_{10}), 34.2 (C_{11}), 60.2 (CH_2 Ethyl), 66.2 (C_6), 67.3 (C_7), 69.1 (C_5), 78.1 (C_3), 81.5 (C_2), 83.1 (C_4), 105.7 (C_1), 128.4–133.6 (C Ar), 165.3–166.5 (3 CO Bz), 173.6 (COOEt).

3.3.12. 5-Carboxyethylpentyl 5-*O*-(2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (11)

To a mixture of **10** (1.158 g, 1.83 mmol, 1 equiv) and **8** (1.511 g, 2.00 mmol, 1.1 equiv) and molecular sieves 4Å (1.00 g) in anhydrous CH_2Cl_2 (30 mL), were added at 0 °C under argon: NBS (814 mg, 4.57 mmol, 2.5 equiv) and TfOH (80 µL, 0.91 mmol, 0.5 equiv). After 30 min, the mixture was filtered, and successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 1.31 g of **11** (60%) as a white foam. R_f: 0.24 (Cyhex/EtOAc 7:3). MS DCI+-HRMS *m*/*z* $[M+NH_4]^+$ calcd for $C_{67}H_{70}NO_{21}$ 1224.4435, found 1224.4430. $[\alpha]_D^{25}$ +2 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.43 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.11 (s, 3H, CH_3 Lev), 2.30 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.55–2.75 (m, 4H, $2CH_2$ Lev), 3.61 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.80 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.50–4.58 (m, 2H, H_4, H_{6a}), 4.68–4.83 (m, 4H, H_5, H_{6a}, $2H_6$), 4.92 (t, 1H, $J_{H4aH3a} = J_{H4aH5a}$ = 4.7 Hz, H_{4a}), 5.25 (s, 1H, H_1), 5.49 (d, 1H, J_{H2H3} = 1.4 Hz, H_2), 5.42 (dd, 1H, J_{H3aH4a} = 5.2 Hz, J_{H3aH2a} = 1.6 Hz, H_{3a}), 5.73–5.75 (d, 1H, J_{H2aH3a} = 1.6 Hz, H_{2a}), 5.75–5.85 (m, 3H, H_{1a}, H_3, H_{4a}), 7.20–8.20 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3 Ethyl), 24.6 (C_8 or C_{10}), 25.6 (C_9), 27.9 (CH_2 Lev), 29.1 (C_8 or C_{10}), 39.5 (CH_3 Lev), 34.1 (H_{11}), 37.8 (CH_2 Lev), 60.1 (CH_2 Ethyl), 63.2 (C_6), 64.5 (C_{6a}), 67.2 (C_7), 70.0 (C_{5a}), 73.1 (C_5), 76.7–77.0 (C_3, C_{3a}), 81.6–81.7 (C_2, C_{2a}, C_{4a}), 82.2 (C_4), 105.2 (C_{1a}), 105.4 (C_1), 128.1–133.4 (C Ar), 165.1–166.0 (6 CO Bz), 171.9 (CO ester Lev), 173.5 (COOEt), 205.8 (CO ketone Lev).

3.3.13. 5-Carboxyethylpentyl 5-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (12)

Compound **11** (1.00 g, 0.83 mmol) was dissolved in a 0.5 M solution of hydrazine monohydrate (8 mL) in pyridine/AcOH (4:1). The mixture was stirred at room temperature for 30 min and concentrated. The residue was dissolved in CH_2Cl_2, washed with aq HCl (1 M) and $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 883 mg of **12** (96%) as a syrup. R_f: 0.35 (Cyhex/EtOAc 7:3). $[\alpha]_D^{25}$ −4 (*c* 3, $CHCl_3$). MS DCI+-HRMS *m*/*z* $[M+Na]^+$ calcd for $C_{62}H_{60}O_{19}Na$ 1131.3629, found 1131.3624. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.43 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.30 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.86 (d, 1H, J_{OHH5a} = 8.0 Hz, OH), 3.47 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.80 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.45 (br, 1H, H_5), 4.53–4.58 (m, 3H, H_4, H_{6a}), 4.68–4.83 (m, 3H, H_4, H_6), 5.20 (s, 1H, H_1), 5.49 (d, 1H, J_{H2H3} = 1.5 Hz, H_2), 5.70 (dd, 1H, J_{H3aH2a} = 1.7 Hz, J_{H3aH4a} = 5.1 Hz, H_{3a}), 5.78 (d, 1H, J_{H2aH3a} = 1.7 Hz, H_{2a}), 5.80 (s, 1H, H_{1a}), 5.84 (dd, 1H, J_{H3H2} = 1.5 Hz, J_{H3H4} = 5.2 Hz, H_3), 7.30–8.20 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3 Ethyl), 24.6 (C_8 or C_{10}), 25.6 (C_9), 29.1 (C_8 or C_{10}), 34.3 (H_{11}), 60.2 (CH_2 Ethyl), 64.6 (C_6), 66.1 (C_{6a}), 67.2 (C_7), 69.4 (C_{5a}), 73.8 (C_5), 77.0 (C_3), 78.0 (C_{3a}), 81.6 (C_{2a}), 81.8 (C_2), 82.1 (C_4), 83.5 (C_5), 105.5 (C_1, C_{1a}), 128.1–133.4 (C Ar), 165.1–166.4 (6 CO Bz), 173.6 (COOEt).

3.3.14. 5-Carboxyethylpentyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (13)

To a mixture of **12** (883 mg, 0.797 mmol, 1 equiv) and **8** (720 mg, 0.956 mmol, 1.2 equiv) and molecular sieves 4Å (1.00 g) in anhydrous CH_2Cl_2 (10 mL), were added at 0 °C under argon: NBS (355 mg, 2.00 mmol, 2.5 equiv) and TfOH (35 µL, 0.40 mmol, 0.5 equiv). After 30 min, the mixture was filtered and successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 911 mg of **13** (68%) as a white foam. R_f: 0.11 (Cyhex/EtOAc 7:3). $[\alpha]_D^{25}$-1 (*c* 1, $CHCl_3$). MS FAB+-HRMS *m*/*z* $[M+Na]^+$ calcd for $C_{94}H_{88}NaO_{29}$ 1703.5309, found 1703.5341. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.42 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.11 (s, 3H, CH_3 Lev), 2.29 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.40–2.70 (m, 4H, $2CH_2$ Lev), 3.47 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.81 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.30–4.52 (m, 2H, H_4, H_{6b}), 4.65–4.82 (m, 7H, H_5, H_{5a}, $2H_6$, $2H_{6a}$, H_{6b}), 4.88–4.93 (m, 2H, H_{4a}, H_{4b}), 5.40 (s, 1H, H_1), 5.49 (d, 1H, J_{H2H3} = 0.9 Hz, H_2), 5.55 (dd, 1H, J_{H3bH4b} = 5.2 Hz, J_{H3bH2b} = 1.8 Hz, H_{3b}), 5.71 (d, 1H, J_{H2aH3a} = 1.6 Hz, H_{2a}), 5.74 (d, 1H, J_{H2bH3b} = 1.8 Hz, H_{2b}), 5.75–5.80 (m, 3H, H_{1a}, H_{1b}, H_{5b}), 5.83–5.88 (m, 2H, H_3, H_{3a}), 7.30–8.30 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3 Ethyl), 24.6 (C_8 or C_{10}), 25.6 (C_9), 27.8 (CH_2 Lev), 29.1 (C_8 or C_{10}), 29.5 (CH_3 Lev), 34.1 (H_{11}), 37.8 (CH_2 Lev), 60.2 (CH_2 Ethyl), 63.6 (C_{6b}), 64.3 (C_6), 65.0 (C_{6a}), 67.1 (C_7), 70.0 (C_{5b}), 73.0–73.1(C_5, C_{5a}), 77.0–77.1 (C_3, C_{3a}, C_{3b}), 81.6–81.8 (C_2, C_{2a}, C_{2b}, C_{4a}), 82.5 (C_{4b}), 105.1–105.3 (C_1, C_{1a}, C_{1b}), 128.0–132.8 (C Ar), 165.0–166.1 (CO Bz), 171.9 (CO ester Lev), 173.5 (COOEt), 205.8 (CO ketone Lev).

3.3.15. 5-Carboxyethylpentyl (2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (14)

Compound **13** (911 mg, 0.542 mmol, 1 equiv) was dissolved in a 0.5 M solution of hydrazine monohydrate (6 mL) in pyridine/AcOH (4:1). The mixture was stirred at room temperature for 30 min and concentrated. The residue was dissolved in CH_2Cl_2, washed with (1 M) HCl and $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (Cyhex/EtOAc 7:3) to give 598 mg of **14** (70%) as a syrup. R_f: 0.50 (Cyhex/EtOAc 6:4). $[\alpha]_D^{25}$ −3.0 (*c* 1, $CHCl_3$). MS FAB+-HRMS *m*/*z* [M+Na] calcd for $C_{89}H_{82}NaO_{27}$ 1605.4941 found 1605.4941. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.42 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.30 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.86 (d, 1H, J_{OHH5b} = 7.3 Hz, OH), 3.47 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.80 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.40 (broad s, 1H, H_{5b}), 4.48–4.50 (m, 2H, $2H_{6b}$), 4.51–4.55 (dd, 1H, J_{H4H3} = 5.3 Hz, J_{H4H5} = 3.5 Hz, H_4), 4.65–4.83 (m, 7H, H_{4b}, H_5, H_{5a}, $2H_6$, $2H_{6a}$), 4.93 (dd, 1H, *J* = 5.1 Hz, *J* = 2.8 Hz, H_{4a}), 5.32 (s, 1H, H_1), 5.50 (d, 1H, J_{H2H3} = 1.2 Hz, H_2), 5.69 (dd, 1H, J_{H3bH4b} = 5.2 Hz, J_{H3bH2b} = 1.9 Hz, H_{3b}), 5.72 (d, 1H, J_{H2aH3a} = 1.5 Hz, H_{2a}), 5.76 (d, 1H, J_{H2bH3b} = 1.9 Hz, H_{2b}), 5.79 (1s, 1H, H_{1a}), 5.80 (s, 1H, H_{1b}), 5.83–5.90 (m, 2H, H_3, H_{3a}), 7.30–8.30 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2 (CH_3 Ethyl), 24.7 (C_8 or C_{10}), 25.6 (C_9), 29.0 (C_8 or C_{10}), 34.1 (H_{11}), 60.1 (CH_2 Ethyl), 64.4–65.0 (C_6, C_{6a}), 66.2 (C_{6b}), 67.1 (C_7), 69.4 (C_{5b}), 73.0–73.1 (C_5, C_{5a}), 77.0–77.1 (C_3, C_{3a}), 77.8 (C_{3b}), 81.6 (C_2, C_{2b}), 81.8 (C_{2a}), 82.5 (C_4), 82.8 (C_{4a}), 83.5 (C_{4b}), 105.1 (C_{1a} or C_{1b}), 105.4 (C_1), 105.6 (C_{1a} or C_{1b}), 128.1–133.4 (C ar), 165.0–166.3 (9 CO Bz), 173.5 (COOEt).

3.3.16. 5-Carboxyethylpentyl (2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (15)

To a mixture of **14** (400 mg, 0.25 mmol, 1 equiv) and **8** (285 mg, 0.38 mmol, 1.2 equiv) and molecular sieves 4 Å (500 mg) in anhydrous CH_2Cl_2 (10 mL), were added at 0 °C under argon: NBS (112 mg, 0.63 mmol, 2.5 equiv) and TfOH (10 μL, 0.126 mmol, 0.5 equiv). After 30 min, the mixture was filtered, and successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (cyhex/EtOAc 7:3) to give 408 mg of **15** (75%) as a white foam. R_f: 0.41 (Cyhex/EtOAc 6:4). $[\alpha]_D^{25}$ −18 (*c* 2, $CHCl_3$). MS FAB+-HRMS *m*/*z* $[M+Na]^+$ calcd for $C_{121}H_{110}NaO_{37}$ 2177.6624, found 2177.6628. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, *J* = 7.1 Hz, CH_3 Ethyl), 1.42 (m, 2H, H_9), 1.63–1.73 (m, 4H, H_8, H_{10}), 2.11 (s, 3H, CH_3 Lev), 2.30 (t, 2H, *J* = 7.5 Hz, H_{11}), 2.40–2.70 (m, 4H, $2CH_2$ Lev), 3.47 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.81 (dt, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.09 (q, 2H, *J* = 7.1 Hz, CH_2 Ethyl), 4.45–4.53 (m, 2H, H_4, $1H_{6b}$), 4.60–4.80 (m, 10H, H_5, H_{5a}, H_{5c}, $1H_{6b}$, $2H_6$, $2H_{6a}$, $2H_{6c}$), 4.80–4.90 (m, 3H, H_{4a}, H_{4b}, H_{4c}), 5.22 (s, 1H, H_1), 5.46 (s, 1H, H_2), 5.53 (dd, 1H, J_{H3bH2b} = 1.8 Hz, J_{H3bH4b} = 5.2 Hz, H_{3b}), 5.68–5.75 (m, 4H, H_{2a}, H_{2b}, H_{2c}, H_{5b}), 5.75–5.80 (m, 3H, H_{1a}, H_{1b}, H_{1c}), 5.80–5.88 (m, 3H, H_3, H_{3a}, H_{3c}), 7.30–8.30 (m, H Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (CH_3 Ethyl), 24.6 (C_8 or C_{10}), 25.6 (C_9), 27.8 (CH_2 Lev), 29.1 (C_8 or C_{10}), 29.5 (CH_3 Lev), 34.1 (H_{11}), 37.8 (CH_2 Lev), 61.8 (CH_2 Ethyl), 63.5 (C_{6b}), 64.9–66.0 (C_6, C_{6a}, C_{6c}), 67.2 (C_7), 71.1 (C_{5b}), 74.3 (C_5, C_{5a}, C_{5c}), 82.0–82.5 (C_2, C_{2a}, C_{2b}, C_{2c}, C_{4a}), 83.0 (C_4), 83.5 (C_{4c}), 84.1 (C_{4b}), 105.4–106 (C_1, C_{1a}, C_{1b}, C_{1c}), 128.0–132.8 (C Ar), 165.0–166.1 (12 CO Bz), 171.9 (CO ester Lev), 173.5 (COOEt), 205.8 (CO ketone Lev).

3.3.17. 5-Carboxypentyl (2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl)-(1→5)-(2,3,6-tri-*O*-acetyl-β-D-galactofuranoside (18)

To an heterogeneous solution of **15** (219 mg, 0.10 mmol, 1 equiv) in anhydrous methanol (25 mL) was added under argon sodium (3 mg, 0.1 mmol, 1equiv). The mixture was stirred at room temperature for 2 h, neutralised with IR 120 H^+ and concentrated. The residue **16** was washed with cyclohexane in order to extract methyl esters. The crude product **16** was dissolved in water (2 mL) and 2 mL of aq 10 M NaOH were added. The mixture was stirred at 50 °C for 2 h, cooled down to room temperature, neutralised (IR 120 H+), and concentrated. The residue **17** was dissolved in pyridine (5 mL) then DMAP (4 mg, 0.003 mol, 0.3 equiv) and acetic anhydride (190 μL, 2.0 mmol, 20 equiv) were added. The mixture was stirred at room temperature overnight. The solvents were coevaporated with toluene to obtain compound **18**.

3.3.18. Compound 20

To a mixture of **18** (0.1 mmol), DMAP (13 mg, 0.1 mmol, 1 equiv) and EDC (39 mg, 0.2 mmol, 2 equiv) in anhydrous pyridine (3 mL) was added a solution of **19** (140 mg, 0.31 mmol, 3 equiv) in anhydrous pyridine (2 mL). The mixture was stirred overnight at 60 °C. The solution was concentrated and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to give 17 mg of **20** (10%) as a syrup. R_f: 0.28 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{25}$ −56 (*c* 2, $CHCl_3$). MS FAB+-HRMS *m*/*z* $[M+Na]^+$ calcd for $C_{172}H_{106}N_4NaO_{37}S$ 1673.5154, found 1673.5194. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.25–1.75 (m, 20H, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}), 2.00–2.13 (m, 39H, OAc), 2.13–2.25 (m, 4H, H_{11}, H_{20}), 2.75 (d, 1H, $J_{H27aH27b}$ = 12.8 Hz, H_{27a}), 2.96 (dd, 1H, $J_{H27bH27a}$ = 12.8 Hz, $J_{H27bH25}$ = 4.5 Hz, H_{27b}), 3.16 (m, 1H, H_{24}), 3.17 (q, 4H, H_{18}, H_{13}), 3.46 (m, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.66 (m, 1H, J_{H7bH7a} = 9.6 Hz, J_{H7bH8} = 6.6 Hz, H_{7b}), 4.15–4.40 (m, 16H, H_{25} and 15H saccharides), 4.53 (m, 1H, H_{26}), 5.00–5.45 (m, 13H, H saccharides), 6.0 (t, 1H, NH), 6.25 (t, 2H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.0–22.1 (13 CH_3 OAc), 25.9–30.1 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}, C_{23}), 36.3–37.0 (C_{11}, C_{20}), 39.4 (C_{18}, C_3), 40.9 (C_{27}), 55.9 (C_{24}), 60.5 (C_{25}), 60.6 (C_{26}), 63.2–64.6 (C_6, C_{6a}, C_{6b}, C_{6c}), 68.1 (C_7), 69.6–72.5 (C saccharides),77 (C saccharide), 81.0–82.4 (C saccharides), 104.9–105.8 (C_1, C_{1a}, C_{1b}, C_{1c}), 164.1 (CO urea), 169–171 (13 CO OAc), 173.5 (CO amide), 173.6 (CO amid).

3.3.19. Compound A

To a solution of **17** (17 mg, 0.010 mmol) in methanol (5 mL) was added sodium (0.1 mg). The mixture was stirred at room temperature overnight. The solution was neutralised (IR 120 H+), filtered off and concentrated. The residue was dissolved in water (HPLC grade) and washed with CH_2Cl_2. The aqueous layer was frozen with liq. N_2 and lyophilised to give 9.2 mg of **18** (81%) as a white powder. MS ESI *m*/*z* $[M+Na]^+$ calcd for $C_{46}H_{80}N_4NaO_{24}S$ 1127.4775, found 1127.4781. $[\alpha]_D^{25}$ −58 (*c* 1 (H_2O/MeOH 1:1). ^1H NMR (400 MHz, D_2O) δ (ppm): 1.25–1.75 (m, 20H, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}), 2.10–2.25 (m, 4H, H_{11}, H_{20}), 2.66 (d, 1H, $J_{H27aH27b}$ = 13.0 Hz, H_{27a}), 2.87 (dd, 1H, $J_{H27bH27a}$ = 13.0 Hz, $J_{H27bH26}$ = 5.0 Hz, H_{27b}), 3.00–3.10 (m, 4H, H_{13}, H_{18}), 3.20 (m, 1H, H_{24}), 3.44 (dt, 1H, J_{H7aH7b} = 9.6 Hz, J_{H7aH8} = 5.3 Hz, H_{7a}), 3.50–4.25 (m, 24H, H saccharide except H_1), 4.27 (dd, 1H, J_{H25H26} = 4.5 Hz, J_{H25H24} = 8.0 Hz, H_{25}), 4.46 (dd, 1H, J_{H26H25} = 4.5 Hz, $J_{H26H27a}$ = 8.5 Hz, H_{26}), 4.82 (d, 1H, $1H_1$, *J* = 2.3 Hz), 5.10–5.12 (m, 2H, $2H_1$), 5.13–5.14 (d, 1H, $1H_1$, *J* = 2.0 Hz). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 25.0–28.7 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}, C_{23}), 35.9–36.1 (C_{11}, C_{20}), 39.5 (C_{18}, C_3), 40.1 (C_{27}), 55.8 (C_{24}), 60.6 (C_{26}), 61.4 (C saccharide), 62.4 (C_{25}), 63.1 (C saccharide), 68.6 (C_7), 70.8–82.9 (22 C saccharides), 107.3–107.5 (4 C1 saccharides), 165.6 (CO urea), 176.9–177.0 (CO amide).

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