Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2018, **16**, 1188

Synthesis of oligosaccharides related to galactomannans from *Aspergillus fumigatus* and their NMR spectral data†

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Received 7th November 2017,
Accepted 3rd January 2018

DOI: 10.1039/c7ob02734f

rsc.li/obc

The synthesis of model oligosaccharides related to antigenic galactomannans of the dangerous fungal pathogen *Aspergillus fumigatus* has been performed employing pyranoside-*into*-furanoside (PIF) rearrangement and controlled O(5) → O(6) benzoyl migration as key synthetic methods. The prepared compounds along with some previously synthesized oligosaccharides were studied by NMR spectroscopy with the full assignment of ^{1}H and ^{13}C signals and the determination of ^{13}C NMR glycosylation effects. The obtained NMR database on ^{13}C NMR chemical shifts for oligosaccharides representing galactomannan fragments forms the basis for further structural analysis of galactomannan related polysaccharides by a non-destructive approach based on the calculation of the ^{13}C NMR spectra of polysaccharides by additive schemes.

Introduction

Aspergillus fumigatus is an opportunistic fungal pathogen that can cause allergic bronchopulmonary aspergillosis, chronic aspergillosis and especially invasive aspergillosis (IA) – a life threatening disease with high morbidity and mortality rates among immunocompromised patients.[1–3] A major carbohydrate antigen produced by *A. fumigatus* is the polysaccharide galactomannan.[4] Its identification in a patient's serum employing a commercial sandwich enzyme immunoassay is used for the diagnosis of IA.[5]

Galactomannan is a complex heteropolysaccharide whose samples can be highly structurally diverse depending on the conditions of *A. fumigatus* cultivation. Despite numerous structural studies, some inconsistencies can be seen among the published data. Latgé *et al.* proposed a structure for two forms[4,6] of galactomannan representing in both cases an α-(1→2)- and α-(1→6)-linked poly-D-mannoside backbone bearing β-(1→5)-linked oligogalactofuranoside side chains attached to some of the mannose units *via* either β-(1→3)- or β-(1→6)-bonds (Fig. 1A). Recently, Shibata *et al.*[7] also revealed the presence of the β-(1→6)-linkage within the galactofuranoside chain[8] and the β-(1→2)- but not the β-(1→3)-attachment of the galactofuranoside side chain to the mannan backbone (Fig. 1B). Significant variations in the polysaccharide structure have been reported under different culture growth conditions.[7]

Polysaccharides structurally related to galactomannans from *A. fumigatus* have been reported in other fungal species in particular *Aspergillus ochraceus*,[9] *Malassezia furfur*,[10] *Malassezia pachydermatis*,[10] *Trichophyton rubrum*,[11] *Trichophyton mentagrophytes*,[11] *Paracoccidioides brasiliensis*[12] and others.[13] This stimulates the development of sensitive methods for the structural analysis of galactomannans. NMR spectroscopy is the most potent non-destructive method used to accomplish these tasks.

The chemical syntheses of galactofuranoside-containing oligosaccharides, related to mycobacterial arabinogalactan[14–16] or *Aspergillus* galactomannan[17–19] and others,[20] were previously reported. Surprisingly, there are well visible contradictions among the published ^{13}C NMR data for very similar Gal*f*-residues present in these oligosaccharides. For example, the ^{13}C NMR chemical shifts of Gal*f*-residues at the non-reducing end linked *via* (1→5)-bonds to the next Gal*f*-ring reported by Lowary *et al.*,[14] Reynolds *et al.*[15] and Gallo-Rodriguez *et al.*[17] (see compounds **A–C** in Table 1) are quite different. Similar deviations are found in data published for the terminal unit in Gal*f*-(1→6)-Gal*f*-fragments in oligosaccharides **D** and **E**. These examples of spectral discrepancy cannot be attributed to the differences of the used spectra recording conditions (temperature and concentration) or the remote influence of structural fragments along the oligosaccharide chains and probably are the results of the tentative assignment of NMR signals. Thus, a

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† Electronic supplementary information (ESI) available: NMR data and copies of ^{1}H and ^{13}C spectra. See DOI: 10.1039/c7ob02734f

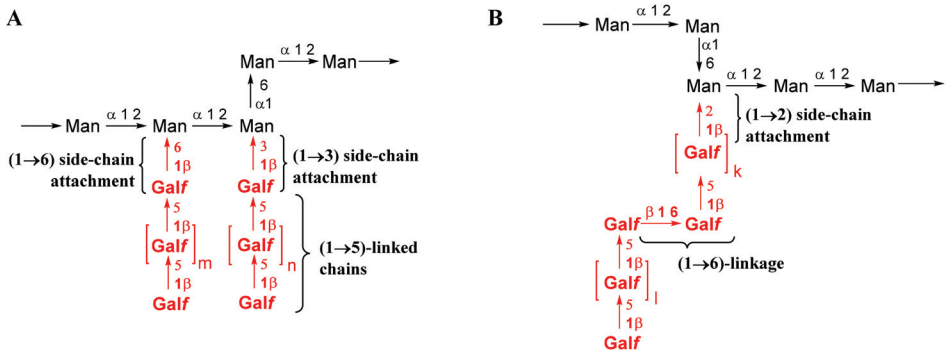


Fig. 1 Proposed structure of galactomannan (A) reported by Latgé *et al.* and[4] (B) additional structural elements reported by Shibata *et al.*[7]

Table 1 Assigned signals in the ^{13}C NMR spectra for the previously described oligosaccharides **A–E**. (δ, ppm; D_2O)

#	δ(C1)	δ(C2)	δ(C3)	δ(C4)	δ(C5)	δ(C6)
A[15]	107.71	81.58	76.74	82.81	70.77	62.07
B[14]	108.7	82.7	77.6	85.5	76.7	61.9
C[17]	109.0	83.6	78.7	84.6	n.d.[a]	64.2
D[15]	108.25	81.24	77.08	83.31	71.09	63.10
E[14]	107.9	81.2	77.6	83.9	70.4	61.9

[a] Not described. **A**[15] β-D-Gal*f*-(1→5)-β-D-Gal*f*-OC_8H_{17}. **B**[14] β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-OC_8H_{17}. **C**[17] β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-STol. **D**[15] β-D-Gal*f*-(1→6)-β-D-Gal*f*-OC_8H_{17}. **E**[14] β-D-Gal*f*-(1→6)-β-D-Gal*f*-(1→5)-β-D-Gal*f*-OC_8H_{17}.

careful revision of the previous data is required for their further use in the NMR analysis of fungal galactomannans.

Herein, we report an NMR spectral study of a series of synthetic galactomannan related oligosaccharides **1–13** (Fig. 2). This work is aimed towards the development of NMR database suitable for further structural analysis of *Aspergillus* galactomannan and structurally related oligo- and polysaccharides. The synthesis of compounds **1–6**, **8** and **13** is also reported, while the preparation of oligosaccharides **7** and **9–12** was previously published.[21,22]

Results and discussion

Synthesis of oligosaccharides

The investigation of fine correlation between a galactomannan structure and NMR chemical shift values requires a representative series of model oligosaccharides with strictly defined composition containing different fragments of studied polysaccharide. Previously,[21] we reported the synthesis of two galactomannan related pentasaccharides **7** and **10** and oligosaccharides **9**, **11** and **12**.[22] In the present work, additionally required models such as homo-galactofuranosides **1–4** containing both β-(1→5)- and β-(1→6)-linked galactofuranosyl units and hetero-

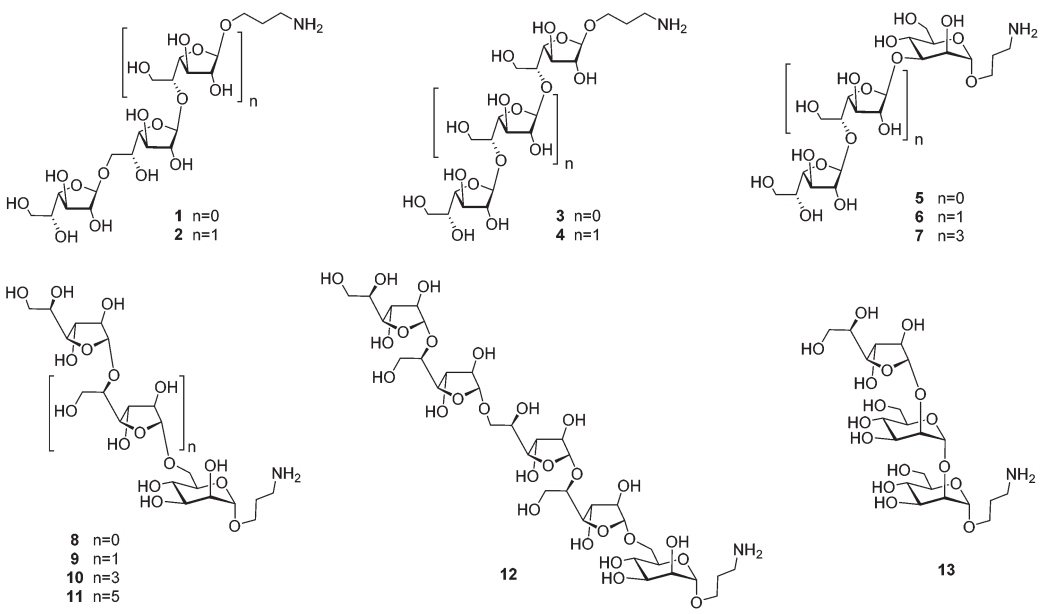


Fig. 2 Studied library of oligosaccharides related to galactomannans of *A. fumigatus*.

saccharides **5**, **6**, **8** and **13** have been obtained (Fig. 2). For their synthesis, glycosyl donor **16** [22] was chosen as a key galactofuranosyl building block. Its main feature was the presence of Fmoc-protection at O(6) which could be selectively removed by deblocking either a 6-OH or 5-OH group depending on the reaction conditions.[22] This made building block **16** suitable for assembling both types of linkages in the oligogalactofuranosyl chains.

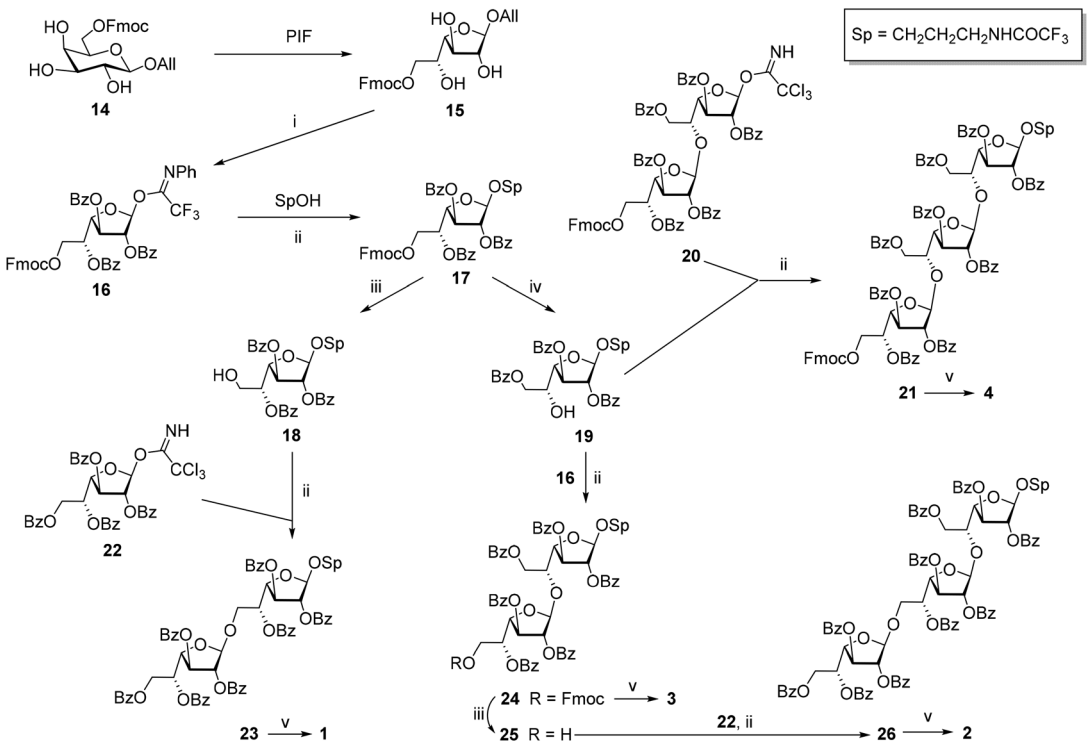
The synthesis of glycosyl donor **16** was performed according to the procedure using pyranoside-*into*-furanoside (PIF) rearrangement,[23,24] first described in 2014. Starting pyranoside **14** was initially transformed into the corresponding furanoside **15** and then subjected to subsequent per-*O*-benzoylation, deallylation and imidation (**15** → **16**). The glycosylation of 3-trifluoroacetamidopropanol with donor **16** in the presence of TMSOTf gave **17**, a precursor to disaccharides **1** and **3** with β-(1→6)- and β-(1→5)-bonds, respectively (Scheme 1).

The removal of Fmoc-protection in monosaccharide **17** by treatment with morpholine in DMF gave product **18** with the free OH-group at C-6, which was further glycosylated with imidate **22** [25] yielding β-linked disaccharide **23**. The configuration of the newly formed bond was confirmed by the singlet shape of the H(1)′-signal ($J_{H1',H2'}$ < 1.0 Hz) and the characteristic low-field chemical shift of C(1)′ (105.86 ppm) in the NMR spectra. One-step deblocking of the obtained disaccharide **23** gave target compound **1**.

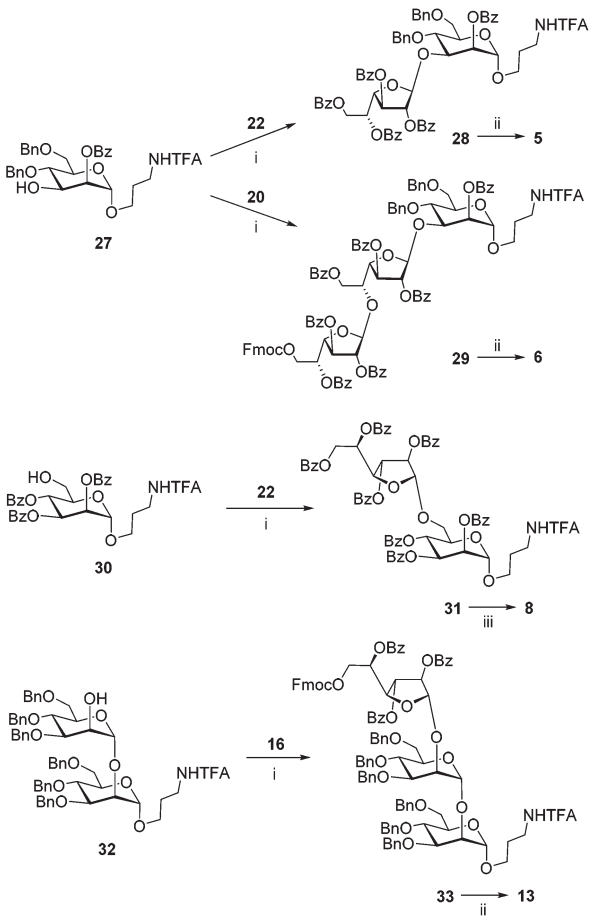
Alternatively, the removal of Fmoc-protection in **17** under conditions activating O(5) → O(6) benzoyl migration[22] (pyrrolidine in CH_2Cl_2) afforded 5-OH derivative **19** in 92% yield. The structure of product **19** was confirmed by ^{1}H NMR chemical shifts (4.43 ppm for H(5), and 4.51 and 4.62 ppm for H(6) in **19** *vs.* 5.61 ppm for H(5) and 4.05 ppm for both H(6) in **18**). The glycosylation of acceptor **19** with donors **16** and **20** [22] in the presence of TMSOTf gave β-linked di- **24** and trisaccharide **21**, respectively. Following the deblocking of the obtained oligosaccharides resulted in the formation of target compounds **3** and **4**.

Disaccharide **24** was also used for the preparation of trisaccharide **2** with alternating (1→6)- and (1→5)-linkages. For this purpose, Fmoc-protection in **24** was removed under conditions preventing benzoate migration (morpholine in DMF) to give acceptor **25** with the free hydroxyl group at O′(6) which was further glycosylated with imidate **22**. The deblocking of the obtained protected derivative **26** afforded target trisaccharide **2**.

Hetero-oligosaccharides **5**, **6**, **8** and **13** were prepared using mannoside acceptors **27**, **30** and **32** (Scheme 2). For the preparation of the oligosaccharides with the (1→3)-linkage between



Scheme 1 Synthesis of the model oligosaccharides **1–4**. Reagents and conditions: PIF: (1) Py·SO_3, HSO_3Cl, DMF, 20 °C, 16 h, then an excess of NH_4HCO_3 aq.; (2) IR-120(H^+), dioxane–DMF, 60 °C, 45 min, 66% on 2 steps; (i) (1) BzCl, Py, CH_2Cl_2, 20 °C, 3 h; (2) $PdCl_2$, MeOH : CH_2Cl_2, 20 °C, 2.5 h; (3) $CF_3C(NPh)Cl$, Cs_2CO_3, CH_2Cl_2, 20 °C, 1 h, 60% on 3 steps; (ii) TMSOTf, MS300 AW, CH_2Cl_2, –80 → –10 °C, 50 min, 87% for **17**, 96% for **21**, 71% for **23**, 94% for **24**, 76% for **26**; (iii) morpholine, DMF, rt, 35 min, 86% for **18**, 78% for **25**; (iv) pyrrolidine, CH_2Cl_2, rt, 40 min, 92%; (v): MeONa, MeOH–H_2O, rt, overnight, 82% for **1**, 89% for **2**, 83% for **3**, 89% for **4**.



Scheme 2 Synthesis of the model oligosaccharides **5**, **6**, **8** and **13**. Reagents and conditions: (i) TMSOTf, MS300 AW, CH_2Cl_2, −80 → −10 °C, 50 min, 95% for **28**, 67% for **29**, 90% for **31**, 77% for **33**; (ii) (1) H_2, Pd/C, EtOAc, MeOH, rt, 1 h; (2) NaOH, MeOH–H_2O, rt, 1 h, 62% for **5** 80% for **6**, 80% for **13**; (iii) NaOMe, MeOH–H_2O, rt, overnight, 77%.

galactofuranose and mannose, the glycosylations of acceptor **27** [21] with either monosaccharide **22** [25] or disaccharide **20** [22] donors were performed. The disaccharide with the (1→6)-linkage was prepared by the glycosylation of acceptor **30** [21] with donor **22**.[22] The trisaccharide with the (1→2)-linkage was prepared by the glycosylation of acceptor **32** [26] with donor **16**. All the couplings proceeded with good yields and excellent β-stereoselectivity. The deblocking of the resulting protected precursors **28**, **29**, **31** and **33** gave target model oligosaccharides **5**, **6**, **8** and **13**, respectively.

NMR analysis of oligosaccharides

A complete signal assignment in the ^{1}H and ^{13}C NMR spectra of oligosaccharides **1–13** was successfully performed employing 2D NMR experiments: COSY, TOCSY, ROESY, HSQC, and HMBC. The chemical shifts were referenced to CH_3CN (δ ^{1}H – 2.06; ^{13}C – 1.47 ppm) used as an internal standard. The ^{13}C chemical shifts of the studied compounds are summarized in Table 2 (for ^{1}H chemical shifts see Table S1 in the ESI†). The ^{13}C chemical shifts were measured using ^{1}H-decoupled 1D ^{13}C NMR spectra, and the ^{1}H chemical shifts of overlapping signals were established from 2D ^{1}H–^{13}C HSQC spectra using the centres of the corresponding correlation signals.

It can be noted that the assignment of signals summarized in Table 2 has certain, often quite substantial deviations from the previously reported chemical shifts (see Introduction), in particular for the signals of C(1), C(6) and some other carbons. Typically, in the previous papers dedicated to the synthesis of Gal*f*-containing oligosaccharides, NMR signal assignment procedures were described only briefly and the resulting attribution was probably tentative or based on possibly erroneous data from older studies.

The studied series of oligosaccharides (**1–13**) contained 10 types of galactofuranosyl residues which differed in (1) the type of the glycoside linkage in them and (2) the type of the carbohydrate unit as the aglycon (Fig. 3). The variation in chemical shifts among different compounds for the same type of residue did not exceed ±0.1 ppm. The analysis of chemical shifts did not reveal any chain length influence or influence from distant residues which allowed the use of these data for the spectral investigation of different types of oligo- and polysaccharides.

An important feature for the analysis of the NMR spectra of large carbohydrate chains is the determination of ^{13}C NMR glycosylation effects.[27] They are the differences in chemical shifts for the corresponding carbon atoms within a residue taken as a part of an oligo- or polysaccharide and for the same residue in the parent mono- or oligosaccharide, non-glycosylated at the position under consideration. The differences in chemical shifts for atoms involved directly in the glycosidic linkage are called the α-effects of glycosylation.

The differences in chemical shifts for atoms bonded to the substituted carbon are the β-effects of glycosylation. To calculate the glycosylation effects, the ^{13}C NMR chemical shifts of β-galactofuranose (detected as minor signals in the spectrum of D-galactose solution in D_2O), α-D-Man-O$(CH_2)_3NH_2$ [28] and α-D-Man-(1→2)-α-D-Man-O$(CH_2)_3NH_2$ [26] were used. For example, Fig. 4 shows two monosaccharides used for the calculation of the glycosylation effects in the spectrum of disaccharide **5**.

The α-effects for anomeric carbon atoms are most informative for the elucidation of monosaccharide sequences in oligo- or polysaccharide chains. They are dependent on the direction of the linkage and the stereochemical configuration of the anomeric center involved, and are not influenced by the type of substitution in the glycosylating residue under consideration. The exceptions to this rule are 2-*O*-substituted residues due to the interference of the β-effects of the substitution with the α-effects of glycosylation.

The C(1) glycosylation effects for differently linked β-Gal*f* residues are shown in Table 3. It can be seen that their values are different for 2- (4.8 ppm, entry 1), 3- (3.4 ppm, entry 2), 5- (6.1 ppm, entry 4) and 6-linked (6.8 ppm, entries 3 and 5) glycosylating furanoses, but the two latter disacchar-

Table 2 ^{13}C NMR chemical shifts (δ, ppm; D_2O) of oligosaccharides **1–13**[a]

#	Unit	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
1	β-D-Gal*f*-OH	101.57	81.92	76.35	82.58	71.27	63.33
	β-D-Gal*f*-(1→	108.40	81.58	77.40	83.62	71.45	63.30
	→6)-β-D-Gal*f*-O$(CH_2)_3NH_2$	107.68	81.30	77.17	84.04	70.23	69.58
2	β-D-Gal*f*-(1→	108.49	81.59	77.37	83.56	71.45	63.34
	→6)-β-D-Gal*f*-(1→	107.51	81.83	77.28	83.67	70.16	69.89
	→5)-β-D-Gal*f*-O$(CH_2)_3NH_2$	107.69	81.44	77.08	82.83	76.48	61.35
3	β-D-Gal*f*-(1→	107.58	81.84	77.17	83.38	71.20	63.38
	→5)-β-D-Gal*f*-O$(CH_2)_3NH_2$	107.65	81.43	76.99	82.69	76.53	61.41
4	β-D-Gal*f*-(1→	107.69	81.96[d]	77.15	83.33	71.19	63.43
	→5)-β-D-Gal*f*-(1→	107.50	81.89[e]	77.08[d]	82.29	76.23	61.70
	→5)-β-D-Gal*f*-O$(CH_2)_3NH_2$	107.69	81.47	77.06[e]	82.75	76.66	61.39
5	β-D-Gal*f*-(1→	105.02	81.97	77.70	83.66	71.45	63.44
	→3)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.20	67.28	76.10	65.66	73.43	61.45
6	β-D-Gal*f*-(1→	107.81	82.00	77.20	83.36	71.25	63.48
	→5)-β-D-Gal*f*-(1→	105.07	81.94	77.62	82.77	76.60	61.86
	→3)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.25	67.34	76.26	65.68	73.45	61.69
7	β-D-Gal*f*-(1→	107.74	82.02[d]	77.06[d]	83.37	71.24	63.48
	→5)-β-D-Gal*f*-(1→[b]	107.74	82.05[e]	77.12[e]	82.19	76.22	61.69
				77.19	82.26	76.36	
	→5)-β-D-Gal*f*-(1→	105.12	81.94	77.65	82.73	76.63	61.79
	→3)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.24	67.36	76.29	65.70	73.45	61.84
8	β-D-Gal*f*-(1→	108.41	81.50	77.31	83.56	71.44	63.28
	→6)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.39	70.45	71.03	67.25	72.30	67.41
9	β-D-Gal*f*-(1→	107.63	81.87	77.17	83.33	71.18	63.43[d]
	→5)-β-D-Gal*f*-(1→	108.37	81.65	77.23	82.49	76.50	61.55[e]
	→6)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.47	70.52	71.13	67.27	72.39	67.45
10	β-D-Gal*f*-(1→	107.67	81.86[d]	77.10	83.26	71.11	63.40
	→5)-β-D-Gal*f*-(1→[b]	107.53	81.95[e]	76.98	82.14	76.15	61.73
		107.57	81.97	77.10		76.27	
	→5)-β-D-Gal*f*-(1→	108.37	81.66	77.29	82.46	76.60	61.51
	→6)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.46	70.51	71.15	67.26	72.37	67.43
11	β-D-Gal*f*-(1→	107.68	81.87[d]	77.10	83.27	71.16	63.41
	→5)-β-D-Gal*f*-(1→[c]	107.54	81.96[e]	76.98	82.15	76.25	61.70
		107.58	81.98	77.04		76.22	61.73
				77.11			
	→5)-β-D-Gal*f*-(1→	108.38	81.67	77.29	82.46	76.59	61.50
	→6)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.47	70.51	71.13	67.25	72.38	67.44
12	β-D-Gal*f*-(1→	107.68	81.85	77.13	83.26	71.15	63.42
	→5)-β-D-Gal*f*-(1→	108.43	81.69[d]	77.32	82.54[d]	76.47	61.63
	→6)-β-D-Gal*f*-(1→	107.51	81.85	77.23	83.67	70.20	69.91
	→5)-β-D-Gal*f*-(1→	108.43	81.63[e]	77.32	82.49[e]	76.36	61.50
	→6)-α-D-Man*p*-O$(CH_2)_3NH_2$	100.47	70.51	71.13	67.26	72.37	67.44
13	β-D-Gal*f*-(1→	106.40	81.64	77.17	83.49	71.29	63.36
	→2)-α-D-Man*p*-(1→	100.25	75.42	70.13	67.80	74.03	61.64
	→2)-α-D-Man*p*-O$(CH_2)_3NH_2$	98.81	79.43	70.79	67.59	73.51	61.60

[a] Aglycon signals δ (ppm): Man*p*-O$\underline{C}H_2$-CH_2-CH_2-NH_2 – 65.68, Man*p*-OCH_2-$\underline{C}H_2$-CH_2-NH_2 – 27.21, Man*p*-OCH_2-CH_2-$\underline{C}H_2$-NH_2 – 38.13; Gal*f*-O$\underline{C}H_2$-CH_2-CH_2-NH_2 – 66.31, Gal*f*-OCH_2-$\underline{C}H_2$-CH_2-NH_2 – 27.19, Gal*f*-OCH_2-CH_2-$\underline{C}H_2$-NH_2 – 38.44, (internal standard $\underline{C}H_3CN$ – 1.47); [b] Two internal residues. [c] Four internal residues.[d,e] The assignment in this pair of chemical shifts may be reversed.

ides are not distinguishable on the basis of the glycosylation effects for C(1). Unfortunately, H(1) chemical shifts for the glycosylating Gal*f* residues in these two disaccharide fragments are also very close (5.02–5.04 ppm, Table S1†). In this case, more information can be given by the α-effects of substitution at C(6) of the glycosylated sugar residues, α-D-Man*p* (5.8 ppm) and β-D-Gal*f* (6.5 ppm). Additionally, the positions and integral intensities of H(6a,b)/C(6) correlation peaks in the HSQC spectra can also be used for the quantitative assessment of the relative content of the two disaccharide fragments in the polymer. However, this two-dimensional approach requires full analysis and certain assignment of NMR spectra.

NMR analysis of natural polysaccharides

All types of differently substituted Gal*f* residues were characterized by specific ^{13}C chemical shift patterns and ^{13}C NMR glycosylation effects which permitted their unambiguous identification in the ^{13}C-NMR spectra of natural polysaccharides. These signals could be regarded as "structure reporting" signals[29,30] suitable for the identification of the characteristic fragments of galactomannans and related polysaccharides which are usually available in very limited amounts and thus produce spectra with low signal intensities.

For example, the C(1) resonances of β-galactofuranoside residues represented the highest interest as structure report-

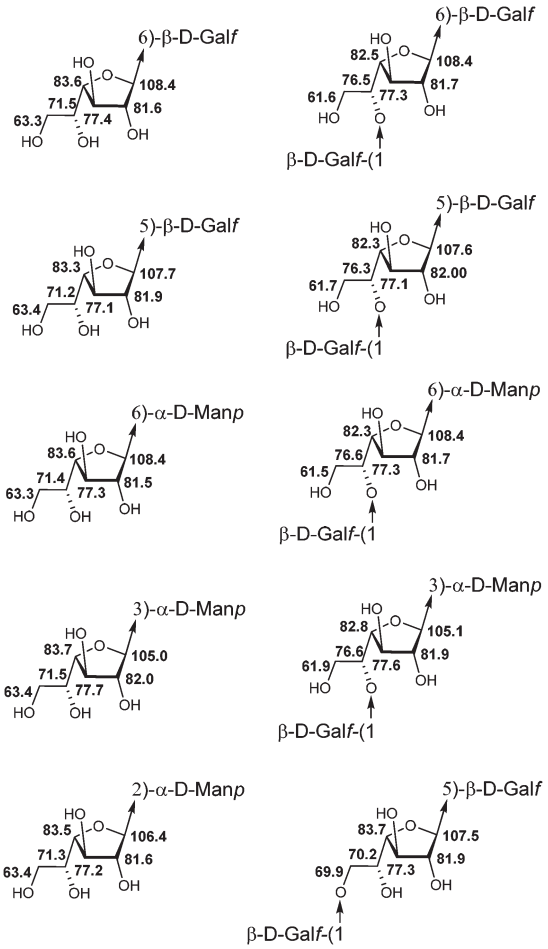


Fig. 3 Averaged ^{13}C NMR chemical shifts (δ, ppm; D_2O) of galactofuranosyl residues of different fragments of galactomannan chains.

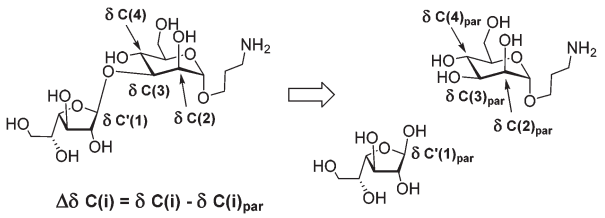


Fig. 4 Illustration of the calculation of ^{13}C NMR glycosylation effects ($\Delta\delta$) in disaccharide **5**.

Table 3 ^{13}C NMR glycosylation effects ($\Delta\delta$, ppm)

#	Linkage	α′-Effect, C(1)	α-Effect	β-Effects
1	β-D-Gal*f*-(1→2)-α-D-Man	4.8	(C2): 4.9	(C1): −2.7 (C3): −0.8
2	β-D-Gal*f*-(1→3)-α-D-Man	3.4	(C3): 4.9	(C2): −3.3 (C4): −1.7
3	β-D-Gal*f*-(1→6)-α-D-Man	6.8	(C6): 5.8	(C5): −1.1
4	β-D-Gal*f*-(1→5)-β-D-Gal*f*	6.1	(C5): 5.0	(C4): −1.0 (C6): −1.7
5	β-D-Gal*f*-(1→6)-β-D-Gal*f*	6.8	(C6): 6.5	(C5): −1.0

Table 4 ^{13}C NMR C(1)-shifts (δ, ppm; D_2O) of β-galactofuranoside residues in oligo- and polysaccharides with different aglycons

#	Terminal β-D-Gal*f*-units with different aglycon counterparts	C(1) in model oligo-saccharide	C(1) in polysaccharide (lit. data)
1	β-D-Gal*f*-(1→2)-D-Man	106.4	106.5[7 a]
2	β-D-Gal*f*-(1→3)-D-Man	105.0	105.4[11 b] 105.9[11 c]
3	β-D-Gal*f*-(1→5)-D-Gal*f*	107.6	107.7[7 a]
4	β-D-Gal*f*-(1→6)-D-Gal*f*/Man	108.4	108.5[7 a]

[a] Each chemical shift taken from ref. 7 has been decremented by 0.20 ppm for consistency with our chemical shift reference. [b] (1→6)-Mannosylated D-Man unit. [c] (1→2)-Mannosylated D-Man unit.

ing signals because they are located in a specific range (105–109 ppm) different from the anomeric signals of mannopyranoside units. The chemical shifts of β-galactofuranoside residues were strongly influenced by the nature of monosaccharide substituents at the anomeric site connected *via* β-(1→2)-, β-(1→3)-, β-(1→5)-, or β-(1→6)-linkages as illustrated by ^{13}C NMR data for model oligosaccharides (Table 4). They coincided well with the corresponding chemical shifts in the spectrum of galactomannan.[7,11] A small deviation (of 0.9 ppm) was observed only in the case of anomeric carbon in the β-galactofuranoside unit connected *via* the (1→3)-linkage to the (1→2)-mannosylated D-Man residue of the mannan backbone (see entry 2 in Table 4) and could be attributed to 2,3-vicinal branching.

To illustrate the applicability of the above mentioned structure reporting signals for the investigation of fungal galactomannans, we analysed the spectrum of galactomannan reported by Latgé *et al.*[4,6] Despite the fact that the presence of the β-D-Gal*f*-(1→3)-D-Man fragment was claimed by the authors, the signal of the anomeric carbon of the corresponding β-D-Gal*f*-unit was not observed in the spectrum (Fig. 1 in ref. 4). Hence, in the present study, the ^{13}C NMR spectrum was recorded for the galactomannan obtained under the reported conditions[4] and it contained the discussed signal. Due to its low intensity in the 1D ^{13}C NMR spectrum, the presence of the β-(1→3)-linked galactofuranoside unit was detected by a 2D HSQC spectral protocol (Fig. 5A).

The full assignment of the signals in the HSQC spectrum (Fig. 5A) revealed the presence of all the types of carbohydrate residues reported by Latgé *et al.*[4,6] However, the careful analysis of low intensity signals (Fig. 5B) suggested the presence of a very minor portion of structural elements reported by Shibata *et al.*:[7] the (1→2)-linkage between β-D-Gal*f* and α-D-Man residues and O(6)-glycosylated β-D-Gal*f* residues (see Fig. 1B). Their presence was confirmed by characteristic ^{1}H and ^{13}C chemical shifts that perfectly matched the data obtained for the corresponding model compounds **13** and **12**.

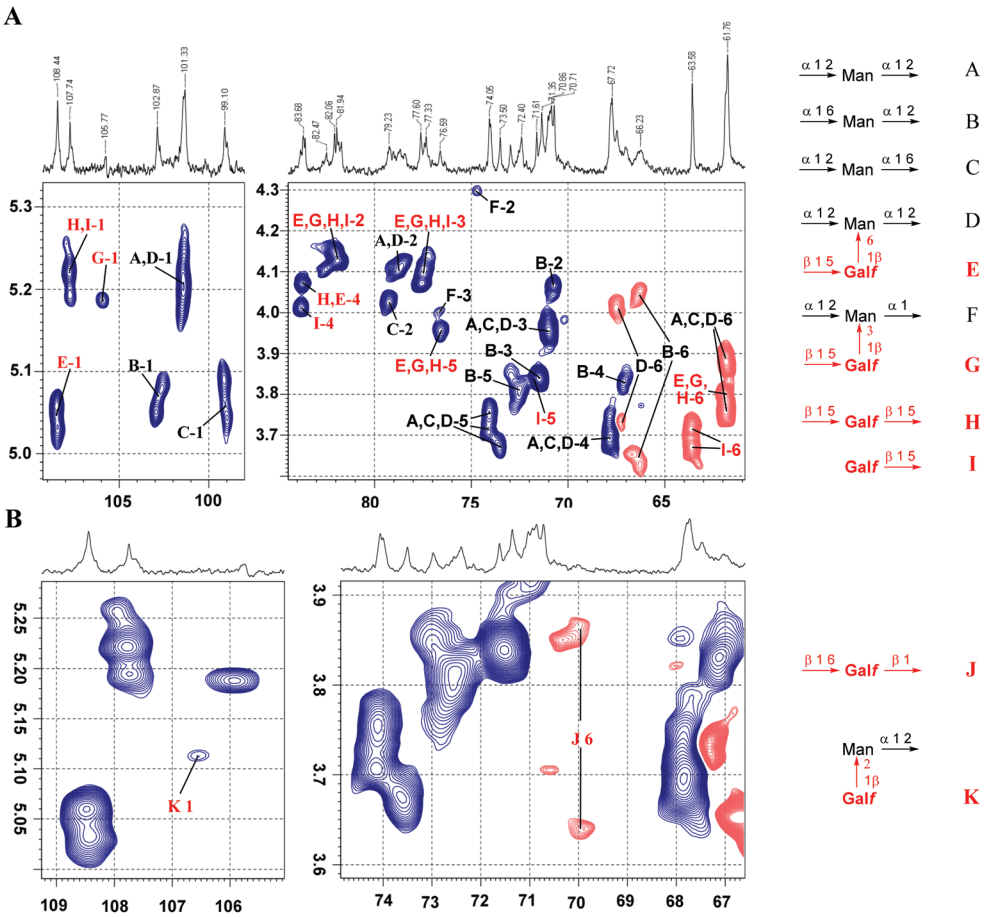


Fig. 5 Application of structure reporting signals for the analysis of natural galactomannan from *A. fumigatus*. (A) Overview of the HSQC spectrum with the assignment of major signals: anomeric (left) and polyol (right) region. (B) Analysis of low intensity signals reporting the structural elements as shown in Fig. 1B. The blue color indicates the positive phase signals referred to as CH or CH_3, whereas the red color indicates the negative phase signals referred to as CH_2 groups.

Conclusions

A series of model oligosaccharides representing key structural fragments of *Aspergillus* galactomannans was synthesised and studied by NMR spectroscopy. The obtained NMR database on ^{13}C NMR chemical shifts for different galactomannan fragments is shown to be useful for the verification of the previously established structures and further development of a non-destructive approach towards the structural analysis of galactomannan related polysaccharides based on the calculation of their ^{13}C NMR spectra by additive schemes.[31–35]

Experimental

General methods

All solvents were distilled and dried if necessary according to standard procedures (CH_2Cl_2, MeOH, toluene, and EtOAc) or purchased as dry (DMF and CH_3CN, Sigma-Aldrich). Commercial chemicals were used without purification unless noted. All glycosylation reactions were carried out using dry solvents under an Ar atmosphere. Molecular sieves for glycosylation reactions were activated prior to application at 180 °C under vacuum from an oil pump for 2 h. Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 aluminium sheets (Merck), and visualization was accomplished using UV light or by charring at ~150 °C with 10% (v/v) H_3PO_4 in ethanol. Column chromatography was performed on Silica Gel 60, 40–63 μm (Merck). Gel-filtration was performed on a TSK-40 HW(S) column (400 × 17 mm) by elution with 0.1 M AcOH in water at a flow rate of 0.5 mL min^{-1}. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-400, Bruker DRX-500, and Bruker AV-600 spectrometers. The spectra of compounds **1–13** were recorded in a Shigemi NMR microtube in D_2O (0.25 mL). Chemical shifts were referenced to residual solvent signals. For samples in D_2O, acetonitrile was used as an internal standard. The average sample concentration was 30 μmol mL^{-1}. The signal assignment in the ^1H and ^{13}C NMR spectra was made using COSY, TOCSY, and ^1H–^{13}C HSQC and HMBC techniques. High-resolution mass spectra (HR MS) were recorded on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measure-

ments were performed in a positive ion mode (interface capillary voltage −4500 V); mass range from *m*/*z* 50 to *m*/*z* 3000 Da; external or internal calibration was made with Electrospray Calibrant Solution (Fluka). A syringe injection was used for solutions in a mixture of acetonitrile and water (50 : 50 v/v, flow rate 3 μL min^{-1}). Nitrogen was applied as a dry gas; the interface temperature was set at 180 °C.

Preparation of the galactomannan sample. *Aspergillus* galactomannan was obtained as described.[4,6] The NMR spectra of galactomannan (solution of 4 mg samples in 0.3 mL of D_2O) were recorded at 333 K.

Glycosylation with imidate donors (general procedure). A carefully dried mixture of imidate donor and glycosyl acceptor was dissolved in CH_2Cl_2 and molecular sieves MS300 AW (100 mg per 1 mL of the reaction mixture) were added. After 10 min of stirring, the temperature was decreased to −80 °C and TMSOTf (0.40 eq. to imidate donor) was added. The mixture was stirred for 50 min and the temperature was gradually raised to −10 °C and then the mixture was quenched with one drop of Et_3N. The reaction mixture was purified by column chromatography on silica gel (toluene–EtOAc, gradient 8 : 1 → 3 : 1) to give the glycosylation product.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-galactofuranoside 17. The glycosylation of 3-trifluoroacetamidopropanol (22 mg, 0.13 mmol) with donor **16** (57 mg, 0.064 mmol) in 3 mL of CH_2Cl_2 as described in the general procedure gave monosaccharide **17** (49 mg, 87%) as a colourless syrup. R_f = 0.26 (toluene : EtOAc 10 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10–7.13 (m, 23H, Fmoc, Bz), 5.98–5.92 (m, 1H, H-5), 5.65 (d, *J* = 5.5 Hz, 1H, H-3), 5.39 (s, 1H, H-2), 5.29 (s, 1H, H-1), 4.71–4.62 (m, 3H, H-4, 2 × H-6), 4.39 (dd, *J* = 10.3, 7.6 Hz, 1H, Fmoc-CH_2), 4.31 (dd, *J* = 10.3, 7.5 Hz, 1H, Fmoc-CH_2), 4.21 (t, *J* = 7.5 Hz, 1H, Fmoc-CH), 3.98–3.94 (m, 1H, OC*H*H′CH_2), 3.67–3.57 (m, 2H, OCH*H*′CH_2, CH_2C*H*H′NH), 3.54–3.48 (m, 1H, CH_2CH*H*′NH), 2.01–1.90 (m, 2H, $CH_2C\mathit{H}_2CH_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.82 (Ar), 133.75 (Ar), 133.54 (Ar), 130.18 (Ar), 130.01 (Ar), 128.64 (Ar), 128.02 (Ar), 127.31 (Ar), 125.35 (Ar), 125.30 (Ar), 120.15 (Ar), 106.53 (C-1), 82.90 (C-2), 81.22 (C-4), 77.29 (C-3), 70.42 (Fmoc-CH_2), 70.10 (C-5), 66.61 (OCH_2CH_2), 66.23 (C-6), 46.75 (Fmoc-CH), 38.21 (CH_2CH_2NH), 28.29 ($CH_2CH_2CH_2$). HRMS (ESI): M = $C_{47}H_{40}F_3NO_{12}$. Calcd *m*/*z* for $[M + Na]^+$ 890.2395, found 890.2386.

Removal of Fmoc-protection without O(5) → O(6) Bz-migration (general procedure). To a solution of 6-*O*-Fmoc-protected saccharide (0.078 mmol) in dry DMF (0.4 mL), morpholine (20 μL) was added. After 35 min, the reaction mixture was poured into 1 M HCl (aq., 50 mL) and extracted with CH_2Cl_2 (50 mL × 2), and the combined organic phase was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (toluene–EtOAc, gradient 6 : 1 → 3 : 1) to afford the 6-OH product.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 18. The removal of Fmoc-protection without Bz-migration in monosaccharide **17** (68 mg, 0.078 mmol) as described in the general procedure gave the product **18** (43 mg, 86%) as a colorless syrup. R_f = 0.44 (toluene : EtOAc 5 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.3 Hz, 2H, *o*-Ph), 8.03 (d, *J* = 7.3 Hz, 2H, *o*-Ph), 7.95 (d, *J* = 7.2 Hz, 2H, *o*-Ph), 7.61–7.28 (m, 9H, Ph), 5.66–5.58 (m, 2H, H-3, H-5), 5.41 (d, *J* = 1.5 Hz, 1H, H-2), 5.30 (s, 1H, H-1), 4.72 (dd, *J* = 5.5, 3.7 Hz, 1H, H-4), 4.10–3.99 (m, 2H, H-6_a, H-6_b), 3.95 (dt, *J* = 10.5, 5.2 Hz, 1H, OCH_2), 3.70–3.47 (m, 3H, OCH_2, CH_2N), 2.69 (br. s, 1H, OH), 2.02–1.86 (m, 2H, $OCH_2C\mathit{H}_2CH_2$N). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.23 (Ph*C*(O)), 165.88 (Ph*C*(O)), 133.69, 133.64, 133.34, 129.91, 129.88, 129.85, 129.48, 128.82, 128.74, 128.53, 128.43 (Ar), 106.24 (C-1), 82.78 (C-2), 81.41 (C-4), 77.26 (C-3), 73.06 (C-5), 66.19 (OCH_2), 61.87 (C-6), 37.90 (CH_2N), 28.30 ($OCH_2C\mathit{H}_2CH_2$N). HRMS (ESI): M = $C_{32}H_{30}F_3NO_{10}$. Calcd *m*/*z* for $[M + Na]^+$ 668.1714, found 668.1707.

Removal of Fmoc-protection with O(5) → O(6) Bz-migration: preparation of 3-trifluoroacetamidopropyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside 19. To a solution of **17** (49 mg, 0.057 mmol) in dry CH_2Cl_2 (0.5 mL), pyrrolidine (24 μL) was added. After 40 min, the reaction mixture was diluted with CH_2Cl_2 and washed with 1 M HCl (aq.). The organic phase was concentrated *in vacuo*. The residue was purified by column chromatography (toluene : EtOAc 5 : 1) to afford the compound **19** (33 mg, 92%). R_f = 0.17 (toluene : EtOAc 5 : 1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12–8.00 (m, 6H, arom.), 7.63–7.39 (m, 9H, arom.), 7.30–7.20 (m, 2H, arom.), 5.72 (dd, *J* = 5.6, 2.2 Hz, 1H, H-3), 5.43 (d, *J* = 2.3 Hz, 1H, H-2), 5.22 (s, 1H, H-1), 4.62 (dd, *J* = 11.6, 6.3 Hz, 1H, H-6), 4.51 (dd, *J* = 11.6, 4.7 Hz, 1H, H-6′), 4.44–4.42 (m, 1H, H-5), 4.40 (dd, *J* = 5.6, 2.4 Hz, 1H, H-4), 3.89 (m, 1H, OC*H*H′CH_2), 3.63–3.53 (m, 2H, OC*H*H′CH_2, CH_2C*H*H′NH), 3.47–3.45 (m, 1H, CH_2CH*H*′NH), 2.82 (d, *J* = 8.4 Hz, 1H, 5-O*H*), 1.95–1.84 (m, 2H, $CH_2C\mathit{H}_2CH_2$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.79 (arom.), 133.74 (arom.), 133.23 (arom.), 129.90 (arom.), 129.83 (arom.), 129.64 (arom.), 128.61 (arom.), 128.56 (arom.), 128.42 (arom.), 106.27 (C-1), 82.71 (C-4), 82.25 (C-2), 77.49 (C-3), 68.81 (C-5), 66.33 (OCH_2CH_2), 65.97 (C-6), 38.04 (CH_2CH_2NH), 28.19 ($CH_2CH_2CH_2$). HRMS (ESI): M = $C_{32}H_{30}F_3NO_{10}$. Calcd *m*/*z* for $[M + Na]^+$ 668.1714, found 668.1714.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-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 (21). The glycosylation of acceptor **19** (8 mg, 0.0125 mmol) with disaccharide donor **20** (19 mg, 0.014 mmol) in 1 mL of CH_2Cl_2 as described in the general procedure gave trisaccharide **21** (22 mg, 96%) as a colorless syrup. R_f = 0.55 (toluene : EtOAc 5 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08–7.09 (m, 53H, Ph), 6.00–5.95 (m, 1H, H-5^{III}), 5.87 (dd, *J* = 5.5, 1.8 Hz, 1H, H-3^{I}), 5.84 (d, *J* = 5.0 Hz, 1H, H-3^{II}), 5.79 (s, 1H, H-1^{III}), 5.70 (s, 1H, H-1^{II}), 5.67–5.64 (m, 2H, H-2^{II}, H-2^{III}), 5.56 (d, *J* = 4.1 Hz, 1H, H-3^{III}), 5.35 (d, *J* = 1.7 Hz, 1H, H-2^{I}), 5.17 (s, 1H, H-1^{I}), 4.95 (dd, *J* = 5.1, 3.4 Hz, 1H, H-4^{III}), 4.82 (dd, *J* = 4.9, 3.2 Hz, 1H, H-4^{II}), 4.80–4.58 (m, 7H, H-6_a^{II}, H-6_a^{I}, H-6_a^{III}, H-6_b^{II}, H-6_b^{I}, H-5^{II}, H-5^{I}), 4.55–4.47 (m, 2H, H-4^{I}, H-6_b^{III}), 4.23 (dd, *J* = 10.0, 7.3 Hz, 1H, Fmoc-CH_2), 4.17–4.05 (m, 2H, Fmoc-CH_2, Fmoc-C*H*), 3.86 (dt, *J* = 10.3, 5.3 Hz, 1H, OCH_2), 3.61–3.49 (m, 2H, CH_2N, OCH_2), 3.47–3.38 (m, 1H,

CH_2N), 1.90–1.79 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.19, 165.84, 165.68, 165.54, 165.29, 165.20, 154.76, 143.41, 143.25, 141.15 (quat. Ph), 133.63, 133.52, 133.44, 133.30, 133.25, 133.17, 133.11, 133.05, 132.99, 130.03, 129.81, 129.76, 129.70, 129.66, 129.61, 129.38, 128.85, 128.63, 128.54, 128.44, 128.30, 128.10, 127.74, 127.10, 126.35, 125.26, 125.20, 120.45, 119.88 (Ph), 106.00 (C-1^{I}), 105.53 (C-1^{II}), 105.47 (C-1^{III}), 83.42 (C-4^{II}), 82.48 (C-2^{I}), 82.26 (C-4^{I}), 82.02 (C-2^{III}), 81.90 (C-2^{II}), 81.74 (C-4^{III}), 77.66 (C-3^{III}), 77.22 (C-3^{II}), 76.57 (C-3^{I}), 73.69 (C-5^{I}), 73.17 (C-5^{II}), 70.17 (C-5^{III}), 70.10 (Fmoc-CH_2), 66.71 (C-6^{III}), 66.30 (OCH_2), 65.35 (C-6^{II}), 63.98 (C-6^{I}), 46.54 (Fmoc-CH), 38.15 (CH_2N), 28.14 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{101}H_{88}F_3NO_{26}$. Calcd *m*/*z* for $[M + Na]^+$ 1810.5439, found 1810.5402.

Deblocking (general procedure). A solution of MeONa (0.1 M) in MeOH (1 mL) was added to the protected oligosaccharide (0.014 mmol) and the mixture was stirred for 1 h. Then, 1 drop of water was added and the reaction mixture was left overnight. Base was neutralized with AcOH (10 μL), and the reaction mixture was diluted with water and concentrated *in vacuo*. Gel chromatography on TSK-40 HW(S) followed by lyophilization afforded the unprotected compound as a white foam.

3-Aminopropyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside 4. The deblocking of compound **21** (22 mg, 0.012 mmol) as described in the general procedure afforded trisaccharide **4** (6 mg, 89%) as a white foam. HRMS (ESI): M = $C_{21}H_{39}NO_{16}$. Calcd *m*/*z* for $[M + H]^+$ 562.2342, found 562.2350.

3-Trifluoroacetamidopropyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 23. The glycosylation of acceptor **18** (13 mg, 0.020 mmol) with donor **22** (19 mg, 0.025 mmol) in 1 mL of CH_2Cl_2 as described in the general procedure gave disaccharide **23** (17 mg, 71%) as a colorless syrup. R_f = 0.45 (toluene : EtOAc 5 : 1). ^1H NMR (600 MHz, $CDCl_3$) δ 8.07–7.75 (m, 14H, *o*-Ph), 7.56–7.21 (m, 21H, Ph), 6.09 (dt, *J* = 7.4, 3.8 Hz, 1H, H-5), 5.87 (td, *J* = 6.3, 3.7 Hz, 1H, H-5), 5.66 (dd, *J* = 5.4, 1.5 Hz, 1H, H-3), 5.60 (d, *J* = 5.0 Hz, 1H, H-3), 5.44 (d, *J* = 0.9 Hz, 1H, H-2), 5.40 (s, 1H, H-1), 5.34 (d, *J* = 1.6 Hz, 1H, H-2), 5.24 (s, 1H, H-1), 4.81–4.70 (m, 4H, 2 × H-4, H-6_a^{II}, H-6_b^{II}), 4.18 (dd, *J* = 10.3, 6.7 Hz, 1H, H-6_a^{I}), 4.01 (dd, *J* = 10.3, 6.1 Hz, 1H, H-6_b^{I}), 3.90 (dt, *J* = 10.4, 5.2 Hz, 1H, OCH_2), 3.62–3.49 (m, 2H, OCH_2, CH_2N), 3.45–3.38 (m, 1H, CH_2N), 1.86–1.73 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.22, 165.97, 165.72, 165.70, 165.67, 165.63, 165.34 (PhC(O)), 133.58, 133.54, 133.48, 133.34, 133.24, 133.07, 129.96, 129.92, 129.85, 129.80, 129.73, 129.56, 129.51, 129.42, 128.87, 128.75, 128.70, 128.51, 128.46, 128.44, 128.41, 128.36, 128.32 (Ph), 106.56 (C-1), 105.86 (C-1), 83.06 (C-4), 81.96 (C-2), 81.78 (C-2), 81.34 (C-4), 77.51 (C-3), 77.12 (C-3), 70.78 (C-5), 70.18 (C-5), 66.64 (C-6), 65.28 (OCH_2), 63.67 (C-6×), 37.97 (CH_2N), 27.99 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{66}H_{56}F_3NO_{19}$. Calcd *m*/*z* for $[M + Na]^+$ 1246.3291, found 1246.3265.

3-Aminopropyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside 1. The deblocking of compound **23** (17 mg, 0.014 mmol) as described in the general procedure afforded disaccharide **1** (4.5 mg, 82%) as a white foam. HRMS (ESI): M = $C_{15}H_{29}NO_{11}$. Calcd *m*/*z* for $[M + H]^+$ 400.1813, found 400.1813.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside 24. The glycosylation of acceptor **19** (27 mg, 0.042 mmol) with donor **16** (45 mg, 0.051 mmol) in 1 mL of CH_2Cl_2 as described in the general procedure gave disaccharide **24** (52 mg, 94%) as a colorless syrup. R_f = 0.54 (toluene : EtOAc 5 : 1). ^1H NMR (500 MHz, $CDCl_3$) δ 8.07–7.68 (m, 12H, arom.), 7.56–7.14 (m, 26H, arom.), 6.07–6.00 (m, 1H, H-5^{II}), 5.88 (d, *J* = 5.7 Hz, 1H, H-3), 5.76 (s, 1H, H-1), 5.64 (s, 1H, H-2), 5.58 (d, *J* = 4.7 Hz, 1H, H-3), 5.37 (s, 1H, H-2), 5.20 (s, 1H. H-1), 4.98 (t, *J* = 3.9 Hz, 1H, H-4), 4.76 (dd, *J* = 11.7, 4.1 Hz, 1H, H-6_a), 4.71–4.52 (m, 5H, 3 × H-6, H-5^{I}, H-4), 4.28 (dd, *J* = 10.1, 7.9 Hz, 1H, Fmoc-CH_2), 4.23–4.17 (m, 1H, Fmoc-CH_2), 4.11 (t, *J* = 7.5 Hz, 1H, Fmoc-CH), 3.88 (dt, *J* = 10.0, 5.1 Hz, 1H, OCH_2), 3.60–3.42 (m, 3H, OCH_2, CH_2N), 1.91–1.77 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 166.18, 165.96, 165.72, 165.59, 165.52, 165.25 (PhC(O)), 154.83, 143.34, 143.17, 141.17, 133.63, 133.34, 133.22, 133.13, 130.02, 129.82, 129.78, 129.66, 129.57, 129.39, 128.74, 128.61, 128.52, 128.39, 128.35, 128.22, 127.79, 127.77, 127.12, 127.06, 125.22, 125.16, 119.92 (Ph), 106.14 (C-1), 105.54 (C-1), 82.81 (C-4), 82.19 (C-2), 81.97 (C-2), 81.77 (C-4), 77.72 (C-3), 77.23 (C-3), 76.53 (C-5), 73.39 (C-5), 70.17 (Fmoc-CH_2), 66.58 (C-6), 66.47 (OCH_2), 64.23 (C-6), 46.53 (Fmoc-CH), 38.08 (CH_2N), 28.04 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{74}H_{62}F_3NO_{20}$. Calcd *m*/*z* for $[M + Na]^+$ 1364.3709, found 1364.3705.

3-Aminopropyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside 3. The deblocking of compound **24** (19 mg, 0.014 mmol) as described in the general procedure afforded disaccharide **3** (4.7 mg, 83%) as a white foam. HRMS (ESI): M = $C_{15}H_{29}NO_{11}$. Calcd *m*/*z* for $[M + H]^+$ 400.1813, found 400.1817.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside 25. The removal of Fmoc-protection without Bz-migration in disaccharide **24** (38 mg, 0.028 mmol) as described in the general procedure gave the product **25** (25 mg, 78%) as a colorless syrup. R_f = 0.58 (toluene : EtOAc 3 : 1). ^1H NMR (600 MHz, $CDCl_3$) δ 8.06–7.81 (m, 12H, *o*-Ph), 7.60–7.19 (m, 18H, Ph), 5.89 (dd, *J* = 5.7, 2.3 Hz, 1H, H-3), 5.76 (s, 1H, H-1), 5.66 (d, *J* = 1.4 Hz, 1H, H-2), 5.64–5.60 (m, 1H, H-5^{II}), 5.60–5.57 (m, 1H, H-3), 5.37 (d, *J* = 2.3 Hz, 1H, H-2), 5.21 (s, 1H, H-1), 5.05 (dd, *J* = 5.3, 2.8 Hz, 1H, H-4), 4.78 (dd, *J* = 11.8, 4.3 Hz, 1H, H-6_a^{I}), 4.71 (dd, *J* = 11.8, 6.8 Hz, 1H, H-6_b^{I}), 4.62–4.57 (m, 2H, H-4, H-5^{I}), 4.05–3.88 (m, 3H, H-6_a^{II}, H-6_b^{II}, OCH_2), 3.62–3.45 (m, 3H, OCH_2, CH_2N), 3.23 (br. s, 1H, OH), 1.97–1.81 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.29, 166.23, 166.03, 165.63, 165.31 (PhC(O)), 133.88, 133.67, 133.35, 133.17, 129.91, 129.89, 129.84, 129.79, 129.65, 128.85, 128.76, 128.61, 128.41, 128.38, 128.35, 128.22 (Ph), 106.24 (C-1), 105.86 (C-1), 82.74 (C-4), 82.35 (C-2), 82.02 (C-2), 81.63 (C-4), 77.69 (C-3), 73.66 (C-3), 72.97 (C-5), 66.42 (OCH_2), 64.43 (C-6),

61.34 (C-6), 38.08 (CH_2N), 28.11 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{59}H_{52}F_3NO_{18}$. Calcd *m*/*z* for $[M + Na]^+$ 1142.3029, found 1142.3030.

3-Trifluoroacetamidopropyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside 26. The glycosylation of acceptor **25** (25 mg, 0.022 mmol) with donor **22** (21 mg, 0.028 mmol) in 1 mL of CH_2Cl_2 as described in the general procedure gave trisaccharide **26** (28 mg, 76%) as a colorless syrup. R_f = 0.55 (toluene : EtOAc 5 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–7.72 (m, 20H, *o*-Ph), 7.53–7.13 (m, 30H, Ph), 6.10–6.02 (m, 1H, H-5), 5.99 (dt, *J* = 7.7, 4.0 Hz, 1H, H-5), 5.87 (dd, *J* = 5.8, 2.3 Hz, 1H, H-3), 5.75 (s, 1H, H-2), 5.64–5.61 (m, 2H, H-2, H-3), 5.54 (d, *J* = 5.0 Hz, 1H, H-3), 5.35–5.34 (m, 2H, 2 × H-2), 5.24 (s, 1H, H-1), 5.18 (s, 1H, H-1), 4.94 (t, *J* = 4.1 Hz, 1H, H-4), 4.79–4.56 (m, 6H, H-4, 4 × H-6, H-5^I), 4.53 (dd, *J* = 5.7, 3.9 Hz, 1H, H-4), 4.19–4.10 (m, 1H, H-6$_a^{II}$), 4.01 (dd, *J* = 11.2, 7.4 Hz, 1H, H-6$_b^{II}$), 3.87 (dt, *J* = 10.1, 5.0 Hz, 1H, OCH_2), 3.61–3.36 (m, 4H, OCH_2, CH_2N), 1.89–1.66 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 166.17, 166.09, 165.88, 165.75, 165.62, 165.54, 165.25, 165.13 (Ph*C*(O)), 133.61, 133.54, 133.27, 133.22, 133.11, 132.96, 129.89, 129.78, 129.74, 129.69, 129.66, 129.60, 129.50, 128.88, 128.86, 128.84, 128.79, 128.77, 128.66, 128.57, 128.48, 128.37, 128.32, 128.28, 128.16 (Ph), 106.61 (C-1), 106.09 (C-1), 105.46 (C-1), 82.84 (C-4), 82.74 (C-2), 81.95 (2 × C-4), 81.88 (2 × C-2), 77.77 (C-3), 77.48 (C-3), 76.48 (C-3), 73.17 (C-4), 71.75 (C-5), 70.42 (C-5), 67.43 (C-6^{II}), 66.41 (OCH_2), 64.25 (C-6), 63.82 (C-6), 38.08 (CH_2N), 28.05 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{93}H_{78}F_3NO_{27}$. Calcd *m*/*z* for $[M + Na]^+$ 1720.4606, found 1720.4585.

3-Aminopropyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside 2. The deblocking of compound **26** (28 mg, 0.016 mmol) as described in the general procedure afforded disaccharide **2** (8 mg, 89%) as a white foam. HRMS (ESI): M = $C_{15}H_{29}NO_{11}$. Calcd *m*/*z* for $[M + Na]^+$ 422.1633, found 422.1639.

3-Trifluoroacetamidopropyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside 28. The glycosylation of acceptor **27** (19 mg, 0.031 mmol) with donor **22** (23 mg, 0.031 mmol) in 1 mL of CH_2Cl_2 as described in the general procedure gave disaccharide **28** (36 mg, 95%) as a colorless syrup. R_f = 0.38 (toluene : EtOAc 10 : 1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11–7.07 (m, 35H), 6.05 (ddd, 1H, H-5^{II}), 5.72 (s, 1H, H-1^{II}), 5.67 (dd, $J_{1,2}$ < 2 Hz, $J_{2,3}$ = 3.0 Hz, 1H, H-2^I), 5.58 (dd, $J_{2,3}$ = 1.4 Hz, $J_{3,4}$ = 5.8 Hz, 1H, H-3^{II}), 5.55 (d, 1H, H-2^{II}), 5.01 (d, 1H, H-1^I), 4.93 (d, *J* = 11.3 Hz, 1H, PhC*H*H′), 4.73 (dd, $J_{4,5}$ = 2.8 Hz, 1H, H-4^{II}), 4.72(dd, $J_{5,6}$ = 7.65 Hz, $J_{6,6'}$ = 11.5 Hz, 1H, H-6a^{II}), 4.69 (d, *J* = 11.9 Hz, 1H, PhCH*H*′), 4.66 (dd, $J_{5,6'}$ = 5.15 Hz, 1H, H-6b^{II}), 4.57 (d, 1H, PhC*H*H′), 4.53 (d, 1H, PhCH*H*′), 4.50 (dd, $J_{3,4}$ = 9.5 Hz, 1H, H-3^I), 4.11 (t, *J* = 9.3 Hz, 1H, H-4^I), 3.85 (m, 1H, OC*H*H′ CH_2), 3.78–3.77 (m, 2H, H-5^I, H-6a^I), 3.72 (d, *J* = 8.5 Hz, 1H, H-6b^I), 3.56 (m, 2H, OCH*H*′CH_2, C*H*H′N), 3.44 (m, 1H, CH*H*′N), 1.90 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 133.37, 133.28, 133.23, 133.16, 130.03, 129.93, 129.86, 129.80, 128.44, 128.37, 128.33, 128.27, 127.61, 127.57, 127.51, 127.47 (Ph), 102.28 (C-1^{II}), 97.76 (C-1^I), 82.36 (C-2^{II}), 81.52 (C-4^{II}), 78.04 (C-3^{II}), 75.22 (PhCH_2), 73.60 (C-3^I), 73.45 (PhCH_2), 73.36 (C-4^I), 72.08 (C-5^I), 69.69 (C-5^{II}), 68.96 (C-6^I), 67.99 (C-2^I), 65.51 (OCH_2), 63.55 (C-6^{II}), 37.85 (CH_2N), 28.45 ($CH_2CH_2CH_2$). HRMS (ESI): M = $C_{66}H_{60}F_3NO_{17}$. Calcd *m*/*z* for $[M + Na]^+$ 1218.3706, found 1218.370313.

3-Aminopropyl β-D-galactofuranosyl-(1→3)-α-D-mannopyranoside 5. To a solution of compound **28** (35 mg, 0.029 mmol) in EtOAc–MeOH 1 : 1 (1 mL), $Pd(OH)_2$ was added and the mixture was vigorously stirred overnight under a H_2 atmosphere. Then it was filtered through a Celite layer and concentrated *in vacuo*. To the residue, a solution of MeONa (0.1 M) in MeOH (0.6 mL) was added and the mixture was stirred for 1 h. Then, 1 drop of water was added and the reaction mixture was left overnight. Base was neutralized with AcOH (6 μL), and the reaction mixture was diluted with water and concentrated *in vacuo*. Gel chromatography on TSK-40 HW(S) followed by lyophilization afforded **5** (7.2 mg, 62%) as a white foam. HRMS (ESI): M = $C_{15}H_{29}NO_{11}$. Calcd *m*/*z* for $[M + H]^+$ 400.1813, found 400.1816.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside 29. The glycosylation of acceptor **27** (6 mg, 0.01 mmol) with donor **20** (14 mg, 0.01 mmol) in 0.5 mL of CH_2Cl_2 as described in the general procedure gave trisaccharide **29** (12 mg, 67%) as a colorless syrup. R_f = 0.54 (toluene : EtOAc 5 : 1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07–7.12 (m, 53H, arom.), 5.99–5.95 (m, 1H, H-5^{III}), 5.74 (s, 1H, H-1^{III}), 5.72 (dd, $J_{3,4}$ = 6.3 Hz, 1.9 Hz, 1H, H-3^{II}), 5.65 (d, *J* = 1.5 Hz, 1H, H-2^{III}), 5.64–5.61 (m, 2H, H-2^I, H-1^{II}), 5.57 (dd, *J* = 5.5, 1.2 Hz, 1H, H-3^{III}), 5.53 (d, *J* = 2.0 Hz, 1H, H-2^{II}), 4.97 (dd, *J* = 5.4, 3.4 Hz, 1H, H-4^{III}), 4.91–4.87 (m, 2H, H-1^I, PhCH_2), 4.67 (dd, *J* = 12.4, 4.1 Hz, 2H, H-6$_a^{III}$), 4.65–4.58 (m, 3H, PhCH_2, H-5^{II}, H-6$_a^{II}$), 4.56 (dd, *J* = 6.2, 3.9 Hz, 1H, H-4^{II}), 4.55–4.49 (m, 3H, H-6$_b^{II}$, H-6$_b^{III}$, PhCH_2), 4.45 (d, *J* = 11.9 Hz, 1H, PhCH_2), 4.37 (dd, *J* = 9.5, 3.1 Hz, 1H, H-3^I), 4.29 (dd, *J* = 10.5, 7.4 Hz, 1H, Fmoc-CH_2), 4.24 (dd, *J* = 10.5, 7.6 Hz, 1H, Fmoc-CH_2), 4.11 (t, *J* = 7.4 Hz, 1H, Fmoc-C*H*), 4.04 (t, *J* = 9.5 Hz, 1H, H-4^I), 3.73–3.68 (m, 1H, OCH_2), 3.59 (dd, *J* = 10.7, 3.9 Hz, 1H, H-6$_a^I$), 3.56–3.50 (m, 2H, H-6$_b^I$, H-5^I), 3.48–3.41 (m, 2H, OCH_2, NCH_2), 3.37–3.30 (m, 1H, CH_2N), 1.92–1.81 (m, 2H, $OCH_2CH_2CH_2N$). ^{13}C NMR (150 MHz, $CDCl_3$) δ = 166.40, 165.85, 165.67, 165.54, 165.53, 165.20, 165.04 (quat. Ph), 133.30, 133.14, 133.05, 130.03, 129.86, 129.82, 129.65, 128.43, 128.35, 128.33, 128.29, 128.25, 127.72, 127.52, 127.44, 127.08, 125.18, 119.89 (Ph), 105.36 (C-1^{III}), 102.11 (C-1^{II}), 97.78 (C-1^I), 82.43 (C-2^{II}), 82.27 (C-2^{III}), 82.10 (C-4^{II}), 81.75 (C-4^{III}), 77.73 (C-3^{II}), 77.56 (C-3^{III}), 75.11 (PhCH_2), 73.74 (C-3^I), 73.58 (C-5^{II}), 73.44 (PhCH_2), 73.18 (C-4^I), 71.84 (C-5^I), 70.25 (C-5^{III}), 70.05 (Fmoc-CH_2), 68.83 (C-6^I), 67.79 (C-2^I), 66.87 (C-6^{III}), 65.45 (OCH_2), 64.83 (C-6^{II}), 46.61 (Fmoc-*C*H), 37.75 (CH_2N), 28.45 ($OCH_2CH_2CH_2N$). HRMS (ESI): M = $C_{101}H_{88}NF_3NO_{26}$. Calcd *m*/*z* for $[M + Na]^+$ 1810.5439, found 1810.5402.

3-Aminopropyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→3)-α-D-mannopyranoside 6. The removal of all protec-

tive groups in compound **29** (12 mg, 0.0067 mmol) as described for the preparation of disaccharide **5** gave **6** (3 mg, 80%) as a white foam. HRMS (ESI): M = $C_{21}H_{39}NO_{16}$. Calcd *m*/*z* for $[M + Na]^+$ 584.2161, found 584.2158.

3-Trifluoroacetamidopropyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 31. The glycosylation of acceptor **30** (25 mg, 0.039 mmol) with donor **22** (32 mg, 0.043 mmol) in 1.5 mL of CH_2Cl_2 as described in the general procedure gave disaccharide **31** (43 mg, 90%) as a colorless syrup. R_f = 0.38 (toluene : EtOAc 10 : 1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14–7.15 (m, 35H), 6.06 (ddd, $J_{4,5}$ = 3.4 Hz, $J_{5,6}$ = 4.5 Hz, $J_{5,6'}$ = 7.8 Hz, 1H, H-5^{II}), 5.88 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.9 Hz, 1H, H-3^{I}), 5.84 (m, 1H, H-4^{I}), 5.68 (dd, $J_{1,2}$ = 1.7 Hz, 1H, H-2^{I}), 5.63 (dd, $J_{2,3}$ = 1.0 Hz, 1H, H-3^{II}), 5.56 (s, 1H, H-1^{II}), 5.53 (d, 1H, H-2^{II}), 5.08 (d, 1H, H-1^{I}), 4.77–4.74 (m, 2H, H-6^{II}, H-6′^{II}), 4.69 (dd, 1H, H-4^{II}), 4.35 (m, 1H, H-5^{I}), 4.12–3.91 (m, 3H, H-6a^{I}, H-6b′^{I}, OC*H*H′), 3.74–3.49 (m, 3H, OCH*H*′, CH_2N), 2.04 (m, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 133.65, 133.58, 133.54, 133.52, 133.31, 133.24, 133.13, 130.00, 129.91, 129.89, 129.79, 129.74, 128.65, 128.49, 128.44, 128.38, 128.32 (*Ph*C(O)), 106.49 (C-1^{II}), 97.52 (C-1^{I}), 82.06 (C-2^{II}), 81.26 (C-4^{II}), 77.63 (C-3^{II}), 71.34 (C-5^{I}), 70.39 (C-2^{I}), 70.19 (C-5^{II}), 69.83 (C-3^{I}), 67.15 (C-4^{I}), 66.27 (O*C*H$_2$), 66.07 (C-6^{I}), 63.66 (C-6^{II}), 37.61 (*C*H$_2$N), 28.52 (CH$_2$*C*H$_2$CH$_2$). HRMS (ESI): M = $C_{66}H_{56}F_3NO_{19}$. Calcd *m*/*z* for $[M + Na]^+$ 1246.3291, found 1246.3255.

3-Aminopropyl β-D-galactofuranosyl-(1→6)-α-D-mannopyranoside 8. The deblocking of compound **31** (35 mg, 0.029 mmol) as described in the general procedure afforded disaccharide **8** (8.8 mg, 77%) as a white foam. HRMS (ESI): M = $C_{15}H_{29}NO_{11}$. Calcd *m*/*z* for $[M + H]^+$ 400.1813, found 400.1816.

3-Trifluoroacetamidopropyl 2,3,5-tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-β-D-galactofuranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside 33. The glycosylation of acceptor **32** (28 mg, 0.027 mmol) with donor **16** (20 mg, 0.023 mmol) in 2.0 mL of CH_2Cl_2 as described in the general procedure gave trisaccharide **33** (30 mg, 77%) as a colorless syrup. R_f = 0.38 (toluene : EtOAc 5 : 1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (d, *J* = 7.2 Hz, 2H, *o*-Ph), 8.02 (d, *J* = 7.3 Hz, 2H, *o*-Ph), 7.81 (d, *J* = 7.3 Hz, 2H, *o*-Ph), 7.71 (dd, *J* = 7.6, 3.0 Hz, 2H, Ph), 7.52–7.06 (m, 44H, arom.), 6.97 (t, *J* = 7.4 Hz, 1H, Ph), 6.89 (br s, 1H, NH), 5.97–5.93 (m, 1H, H-5^{III}), 5.50 (d, *J* = 4.1 Hz, 1H, H-3^{III}), 5.48 (s, 1H, H-2^{III}), 5.20 (s, 1H, H-1^{III}), 5.10 (d, *J* = 1.5 Hz, 1H, H-1^{II}), 4.88 (d, *J* = 1.6 Hz, 1H, H-1^{I}), 4.82 (d, *J* = 10.8 Hz, 1H, PhC*H*H′), 4.74–4.44 (m, 11H, 10 × PhC*H*H′, H-6a^{III}), 4.34 (d, *J* = 10.9 Hz, 1H, PhC*H*H′), 4.29–4.15 (m, 4H, H-2^{II}, Fmoc-CH_2, H-6b^{III}), 4.09 (t, *J* = 7.6 Hz, 1H, Fmoc-C*H*), 4.03–4.00 (m, 1H, H-2^{I}), 3.95–3.61 (m, 11H, H-3^{I}, H-4^{I}, H-5^{I}, H-6a^{I}, H-6b^{I}, H-3^{II}, H-4^{II}, H-5^{II}, H-6a^{II}, H-6b^{II}, OC*H*H′CH$_2$), 3.43–3.17 (m, 3H, OCH*H*′CH$_2$C*H*$_2$NH), 1.77–1.66 (m, 2H, CH$_2$C*H*$_2$CH$_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.69, 165.29, 154.93, 143.43, 141.33, 138.44, 138.38, 133.54, 133.42, 133.26, 130.15, 129.98, 128.61–128.36, 128.09, 127.90, 127.71, 127.39, 127.27, 125.40, 125.36, 120.05 (arom.), 103.75 (C-1^{III}), 99.40 (C-1^{II}), 99.24 (C-1^{I}), 82.50 (C-4^{III}), 81.43 (C-2^{III}), 79.90 (C-3^{I}), 78.18 (C-3^{II}), 77.91 (C-3^{III}), 75.74, 75.34, 75.14, 75.06, 74.89, 73.70, 73.55, 72.69, 72.37 (6 × PhC*H*$_2$, C-2^{I}, C-4^{I}, C-5^{I}, C-2^{II}, C-4^{II}, C-5^{II}), 70.57 (C-5^{III}), 70.25 (Fmoc-C*H*$_2$), 69.99 (C-6^{II}), 69.59 (C-6^{I}), 66.88 (C-6^{III}), 66.02 (OC*H*$_2$CH$_2$), 46.72 (Fmoc-*C*H), 38.15 (*C*H$_2$NH), 28.26 (OCH$_2$*C*H$_2$CH$_2$NH). HRMS (ESI): M = $C_{101}H_{96}F_3NO_{22}$. Calcd *m*/*z* for $[M + Na]^+$ 1754.6268, found 1754.6258.

3-Aminopropyl β-D-galactofuranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside 13. The removal of all protective groups in compound **33** (25 mg, 0.014 mmol) as described for the preparation of disaccharide **5** gave **13** (6.5 mg, 80%) as a white foam. HRMS (ESI): M = $C_{21}H_{39}NO_{16}$. Calcd *m*/*z* for $[M + H]^+$ 562.2342, found 562.2342.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by RSF grant 14-23-00199 (NEN). We thank Dr Thierry Fontaine for providing the galactomannan sample. We thank Dr A. O. Chizhov for recording high resolution mass spectra in the Department of Structural Studies of the Zelinsky Institute of Organic Chemistry, Moscow.

References

1 J. R. Köhler, B. Hube, R. Puccia, A. Casadevall and J. R. Perfect, *Microbiol. Spectrum*, 2017, **5**, DOI: 10.1128/microbiolspec.FUNK-0014-2016.
2 R. R. Herbrecht, D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J.-W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke and B. de Pauw, *N. Engl. J. Med.*, 2002, **347**, 408–415.
3 H. Kume, T. Yamazaki, T. Togano, M. Abe, H. Tanuma, S. Kawana and M. Okudaira, *Med. Mycol. J.*, 2011, **52**, 117–127.
4 J.-P. Latgé, H. Kobayashi, J.-P. Debeaupuis, M. Diaquin, J. Sarfati, J.-M. Wieruszeski, E. Parra, J.-P. Bouchara and B. Fournet, *Infect. Immun.*, 1994, **62**, 5424–5433.
5 D. Stynen, A. Goris, J. Sarfati and J. P. Latgé, *J. Clin. Microbiol.*, 1995, **33**, 497–500.
6 C. Costachel, B. Coddeville, J.-P. Latgé and T. Fontaine, *J. Biol. Chem.*, 2005, **280**, 39835–39842.
7 A. Kudoh, Y. Okawa and N. Shibata, *Glycobiology*, 2015, **25**, 74–87.
8 A. W. Van Bruggen-Van Der Lugt, H. J. Kamphuis, G. A. De Ruiter, P. Mischnick, J. H. Van Boom and F. M. Rombouts, *J. Bacteriol.*, 1992, **174**, 6096–6102.

9 S. Guo, W. Mao, M. Yan, C. Zhao, N. Li, J. Shan, C. Lin, X. Liu, T. Guo, T. Guo and S. Wang, *Carbohydr. Polym.*, 2014, **105**, 325–333.
10 N. Shibata, T. Saitoh, Y. Tadokoro and Y. Okawa, *Microbiology*, 2009, **155**, 3420–3429.
11 K. Ikuta, N. Shibata, J. S. Blake, M. V. Dahl, R. D. Nelson, K. Hisamichi, H. Kobayashi, S. Suzuki and Y. Okawa, *Biochem. J.*, 1997, **323**, 297–305.
12 R. P. Da Silva, C. Heiss, I. Black, P. Azadi, J. Q. Gerlach, L. R. Travassos, L. Joshi, M. Kilcoyne and R. Puccia, *Sci. Rep.*, 2015, **5**, 14213.
13 E. A. Leitão, V. C. Bittencourt, R. M. Haido, A. P. Valente, J. Peter-Katalinic, M. Letzel, L. M. de Souza and E. Barreto-Bergter, *Glycobiology*, 2003, **13**, 681–692.
14 G. C. Completo and T. L. Lowary, *J. Org. Chem.*, 2008, **73**, 4513–4525.
15 A. K. Pathak, V. Pathak, L. Seitz, J. A. Maddry, S. S. Gurcha, G. S. Besra, W. J. Suling and R. C. Reynolds, *Bioorg. Med. Chem.*, 2001, **9**, 3129–3143.
16 L. Gandolfi-Donadío, C. Gallo-Rodriguez and R. M. de Lederkremer, *J. Org. Chem.*, 2003, **68**, 6928–6934.
17 M. J. Tilve, C. R. Cori and C. Gallo-Rodriguez, *J. Org. Chem.*, 2016, **81**, 9585–9594.
18 L. Cattiaux, B. Sendid, M. Collot, E. Machez, D. Poulain and J.-M. Mallet, *Bioorg. Med. Chem.*, 2011, **19**, 547–555.
19 M. Fu, G. Zhang and J. Ning, *Carbohydr. Res.*, 2005, **340**, 25–30.
20 R. Euzen, V. Ferrières and D. Plusquellec, *Carbohydr. Res.*, 2006, **341**, 2759–2768.
21 D. A. Argunov, V. B. Krylov and N. E. Nifantiev, *Org. Biomol. Chem.*, 2015, **13**, 3255–3267.
22 D. A. Argunov, V. B. Krylov and N. E. Nifantiev, *Org. Lett.*, 2016, **18**, 5504–5507.
23 V. B. Krylov, D. A. Argunov, D. Z. Vinnitskiy, S. A. Verkhnyatskaya, A. G. Gerbst, N. E. Ustyuzhanina, A. S. Dmitrenok, J. Huebner, O. Holst, H.-C. Siebert and N. E. Nifantiev, *Chem. – Eur. J.*, 2014, **20**, 16516–16522.
24 V. B. Krylov, D. A. Argunov, D. Z. Vinnitskiy, A. G. Gerbst, N. E. Ustyuzhanina, A. S. Dmitrenok and N. E. Nifantiev, *Synlett*, 2016, 1659–1664.
25 C. Gallo-Rodriguez, L. Gandolfi and R. M. de Lederkremer, *Org. Lett.*, 1999, **1**, 245–248.
26 A. A. Karelin, Y. E. Tsvetkov, G. Kogan, S. Bystricky and N. E. Nifantiev, *Russ. J. Bioorg. Chem.*, 2007, **33**, 110–121.
27 A. S. Shashkov, G. M. Lipkind, Y. A. Knirel and N. K. Kochetkov, *Magn. Reson. Chem.*, 1988, **26**, 735–747.
28 B. Schumann, S. G. Parameswarappa, M. P. Lisboa, N. Kottari, F. Guidetti, C. L. Pereira and P. H. Seeberger, *Angew. Chem., Int. Ed.*, 2016, **55**, 14431–14434.
29 H. Van Halbeek, *Biochem. Soc. Trans.*, 1984, **12**, 601–605.
30 J. F. Vliegenthart, L. Dorland and H. van Halbeek, *Adv. Carbohydr. Chem. Biochem.*, 1983, **41**, 209–374.
31 G. M. Lipkind, A. S. Shashkov, Y. A. Knirel, E. V. Vinogradov and N. K. Kochetkov, *Carbohydr. Res.*, 1988, **175**, 59–75.
32 G. M. Lipkind, A. S. Shashkov, N. E. Nifant'ev and N. K. Kochetkov, *Carbohydr. Res.*, 1992, **237**, 11–22.
33 F. Lisacek, J. Mariethoz, D. Alocci, P. M. Rudd, J. L. Abrahams, M. P. Campbell, N. H. Packer, J. Ståhle, G. Widmalm, E. Mullen, B. Adamczyk, M. A. Rojas-Macias, C. Jin and N. G. Karlsson, *Methods Mol. Biol.*, 2017, **1503**, 235–264.
34 M. Lundborg and G. Widmalm, *Anal. Chem.*, 2011, **83**, 1514–1517.
35 A. G. Gerbst, V. B. Krylov, D. Z. Vinnitskiy, A. S. Dmitrenok, A. S. Shashkov and N. E. Nifantiev, *Carbohydr. Res.*, 2015, **417**, 1–10.