Carbohydrate Research 360 (2012) 56–68

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Synthesis of pentasaccharides corresponding to the glycoform II of the outer core region of the *Pseudomonas aeruginosa* lipopolysaccharide

Bozhena S. Komarova [a], Yury E. Tsvetkov [a], Gerald B. Pier [b], Nikolay E. Nifantiev [a,*]

[a] Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow, Russia
[b] Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 29 June 2012
Accepted 30 July 2012
Available online 7 August 2012

Keywords:
Cystic fibrosis
Pseudomonas aeruginosa
Lipopolysaccharide
Outer core region
Pentasaccharide
Synthesis

ABSTRACT

Cystic fibrosis (CF) is a congenital disease caused by a mutation in a gene responsible for the synthesis of a membrane protein called the cystic fibrosis transmembrane conductance regulator (CFTR). Resistance to *Pseudomonas aeruginosa* infection is closely related to the biological properties of CFTR; however, these properties have not been clearly linked to the known role of CFTR as a chloride and bicarbonate ion channel. Indeed, data indicate that CFTR is an epithelial cell receptor for *P. aeruginosa*, with CFTR binding to the oligosaccharide of the outer core region of the bacterial lipopolysaccharide (LPS), of which two distinct glycoforms have been identified. Binding leads to effective innate immunity to clear this pathogen in individuals with wild-type CFTR. To reveal the molecular basis of elimination of the bacterium through this interaction, the synthesis of pentasaccharides corresponding to both glycoforms of the outer core region of *P. aeruginosa* LPS was undertaken. Here we report the synthesis of the glycoform II. Like glycoform I, it was prepared as three pentasaccharides bearing naturally occurring *N*-alanyl and *N*-acetyl substituents in the galactosamine moiety as well as unnatural *N*-acetylalanine to reveal the role of the amino group in the alanyl substituent. Key features of the synthesis were two α-glucosylations with glucosyl donors bearing α-stereodirecting acyl groups at O-6 and/or O-3 and high-yielding reduction of the azido group followed by N-acylation and final O-debenzylation.

1. Introduction

Cystic fibrosis (CF) is a congenital disease[1,2] in which numerous primary and secondary defects, such as impairment of mucociliary clearance of organisms in the lung, occur in mucosal tissues due to lack of functional cystic fibrosis transmembrane conductance regulator (CFTR) protein.[3] The hallmark of CF is chronic infection of the affected lung tissues by *Pseudomonas aeruginosa* leading to chronic and pathologic inflammation with eventual development of chronic obstructive lung disease phenotype and eventually to early death.[4] In healthy individuals, the process of efficient *P. aeruginosa* elimination from the lungs goes through a prerequisite step of the bacterium interacting with CFTR,[3,5,6] embedded in the plasma membrane of lung cells, after which the ingestion of a portion of the bacterial organisms by lung cells follows and leads to efficient clearance of this organism from the lung. In CF patients, the gene that codes CFTR is defective,[7] leading to low levels of mutant CFTR protein expression on lung cells and, hence, to low level of *P. aeruginosa* bound to and ingested by lung cells.

An epitope on the bacterial surface that is recognized by wild type CFTR is the outer core region of the bacterial lipopolysaccharide that is produced as two glycoforms (Fig. 1).[8,9] Both glycoforms can be simultaneously expressed on the surface of a given strain of *P. aeruginosa*.

Three pentasaccharides corresponding to the glycoform I were recently synthesized in our laboratory.[10] Here we report the synthesis of three pentasaccharides **1a–c** (Fig. 2) that have a common backbone corresponding to glycoform II. The compounds bear different *N*-acyl substituents on the galactosamine residue, which are necessary to define the role of the free amino group on the alanine substituent in the interaction of *P. aeruginosa* with wild type CFTR. These pentasaccharides, together with the previously obtained pentasaccharides corresponding to the glycoform I,[10] will be used for investigating the molecular basis leading to the failure to eliminate *P. aeruginosa* from the lungs of patients with CF.

2. Results and discussion

Like glycoform I,[10] the structure of glycoform II comprises two α-linked glucose residues and a vicinal 3,4-branching in the galactosamine moiety. Accordingly, similar approaches were used for the assembly of the oligosaccharide backbone of the glycoform II. Highly stereoselective α-glucosylation, which is not usually a simple task,[11,12] was achieved by application of glucosyl donors[10] with a non-participating benzyl group at O-2 and acyl groups at

* Corresponding author. Tel./fax: +7 499 135 8784.
E-mail address: nen@ioc.ac.ru (N.E. Nifantiev).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.07.019

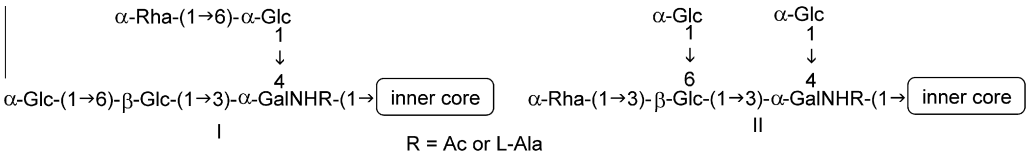


Figure 1. Glycoforms of the outer core region of the *P. aeruginosa*[6,7] lipopolysaccharide.

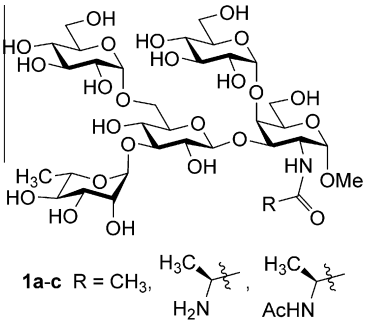
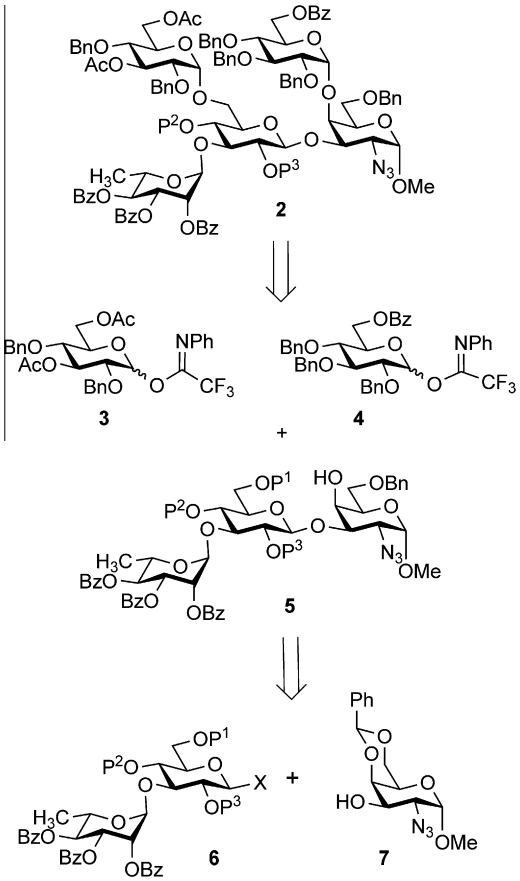


Figure 2. Structure of the target pentasaccharides.



Scheme 1. Retrosynthetic analysis of the pentasaccharide precursor.

O-6 and/or O-3 capable of anchimeric participation. The effect of remote acyl groups on the stereoselectivity of glycosylation in *gluco*-,[13–17] *fuco*-,[18] *xylo*-,[19] and *altro*-series[20] was directly or indirectly confirmed by multiple data obtained by different research groups. While the nature of this effect is still controversial,[21–23] it was successfully applied by us for the synthesis of α-fuco-,[18,23–25] α-xylo-,[19] α-glucurono-,[25] and α-glucooligosaccharides.[10,26] The most efficient leaving group upon glycosylation with partially O-acylated donors[26] was shown to be *N*-phenyltrifluoroacetimidoyl group.[27] Such donors are more stable and less prone to decomposition under glycosylation conditions than common trichloroacetimidates, while retaining all the advantages of Schmidt's donors.

The only applicable sequence of glycosylations to build the vicinal 3,4-branching in the galactosamine moiety proved to be initial β-(1→3)-glucosylation followed by α-(1→4)-glucosylation.[26] But the glycoform II also comprises an additional 3,6-branching that includes an α-L-Rha-(1→3)-β-D-Glc fragment. In the case of the glycoform I, the α-rhamnose unit was introduced at the last step of the assembly of the pentasaccharide backbone.[10] For glycoform II it seemed to be more expedient to start the synthesis from an α-L-Rha-(1→3)-β-D-Glc disaccharide to minimize manipulations of the protecting group.

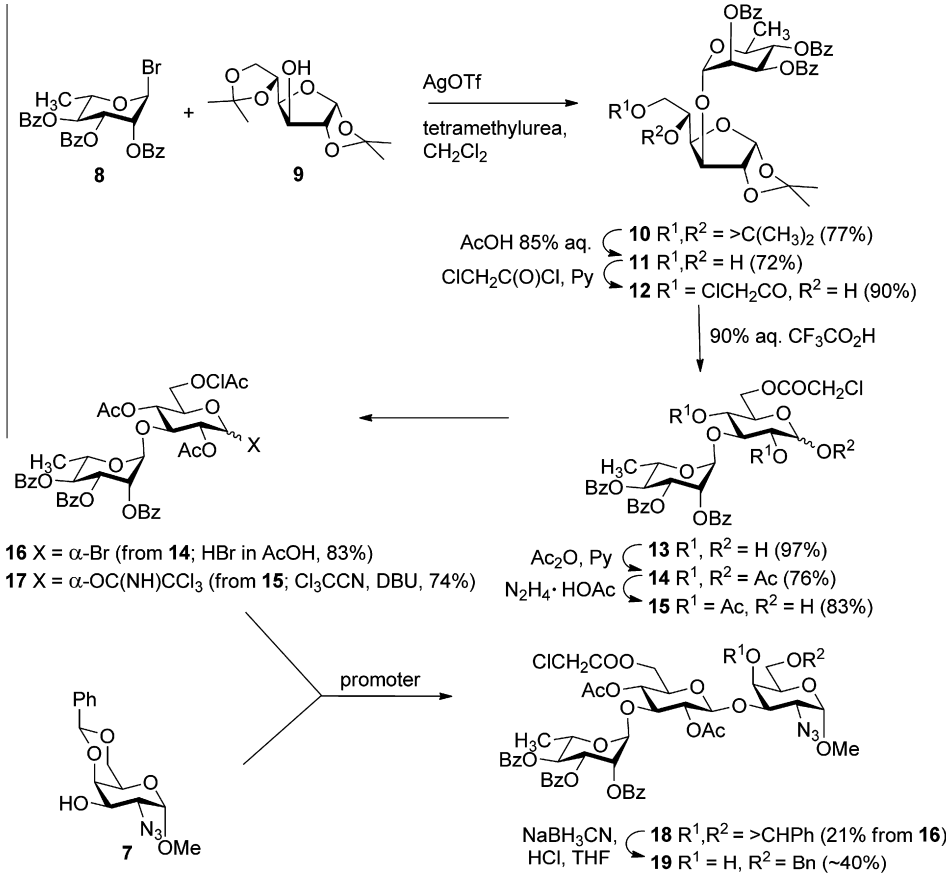
Taking the above considerations into account, the retrosynthetic scheme for the assembly of protected pentasaccharide **2** was proposed (Scheme 1). Pentasaccharide **2** could be cleaved on the α-glucoside linkages into α-glucosyl donors **3** and **4** and trisaccharide **5** containing free 4-OH group in the GalN residue and a selectively removable protecting group P^1 at O-6 of β-glucose. Trisaccharide **5** could be divided, in turn, into GalN acceptor **7** and disaccharide donor **6**.

Two synthetic approaches toward a trisaccharide of general formula **5** were explored. The first approach was based on the obvious choice of di-isopropylideneglucose **9** as a precursor of the 3-*O*-rhamnosylated β-glucose moiety (Scheme 2). Glycosylation of **9** with rhamnosyl bromide **8** gave disaccharide **10**,[28] which provided 5,6-diol **11**[28] upon mild acidic hydrolysis. The primary hydroxyl group in **11** was selectively protected with a chloroacetyl group (→ **12**), then the 1,2-*O*-isopropylidene group was removed to produce pyranose triol **13**. Acetylation of **13** gave triacetate **14**, and subsequent anomeric deacetylation afforded hemiacetal **15**. Both latter steps needed strict control to prevent the reactive chloroacetyl group from substitution with pyridine or hydrazine.

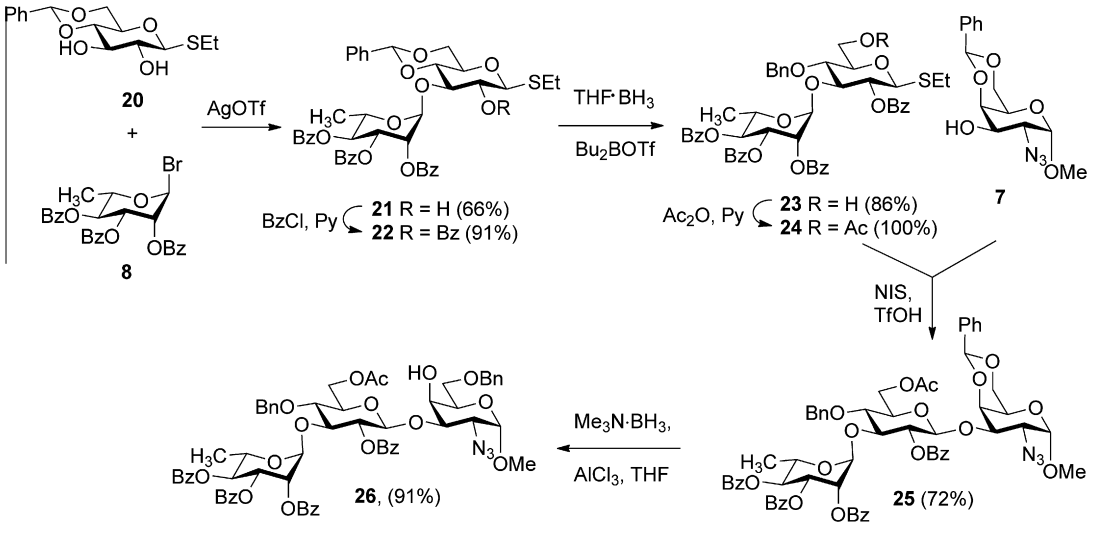
Triacetate **14** and hemiacetal **15** were converted by standard procedures into bromide **16** and trichloroacetimidate **17** respectively. Coupling of these glycosyl donors with 2-azido-2-deoxygalactoside **7** proceeded with low effectiveness; the best yield of trisaccharide **18** (21%) was achieved with trichloroacetimidate **17** in the presence of Bu_2BOTf. Furthermore, subsequent reductive opening of the benzylidene acetal ring in **18** with $NaBH_3CN$ in the presence of HCl was also not clear and afforded 6-*O*-benzyl ether **19** in moderate yield and as a hardly separable mixture with some unidentified contaminants. Multiple stages and modest yields on the final steps made the above approach impractical for the preparative synthesis of key trisaccharide structure **5**.

Therefore, another approach toward **5** based on regioselective glycosylation[29–31] of 2,3-diol **20** was developed (Scheme 3). AgOTf-promoted coupling of rhamnosyl bromide **8** with **20** proceeded with good regioselectivity and afforded disaccharide **21** in 66% yield along with a byproduct, which was likely the product of bis-glycosylation. After conventional benzoylation of the only hydroxyl group in **21**, tetrabenzoate **22** was obtained.

The location of the benzoyl group at O-2 and, hence, the rhamnosyl residue at O-3 was inferred from the low-field chemical shift of the signal for H-2 (δ 5.46) in the ^{1}H NMR spectrum of **22**.



Scheme 2. First approach toward trisaccharide structure **5**.



Scheme 3. Second approach toward trisaccharide structure **5**.

Then the benzylidene acetal was subjected to the reductive opening under the conditions (BH_3·THF, Bu_2BOTf)[32] providing the formation of a benzyl group at O-4 and a free hydroxyl group at C-6. Conventional acetylation of resulting **23** afforded acetate **24**. The positions of signals for H-6 (δ 4.54 and 4.31) and C-6 (δ 62.9) in the ^1H and ^{13}C NMR spectra of **24** confirmed the location of the acetyl group at O-6. Thioglycoside **24** corresponds to the necessary disaccharide donor of general formula **6** (Scheme 1) having selectively removable 6-*O*-acetyl group. Moreover, according to the published data,[33] the presence of 4-*O*-benzyl group was thought to enhance the glycosylating activity of **24** as compared to totally acetylated counterparts **16** and **17**. Indeed, NIS–TfOH-promoted glycosylation of acceptor **7** with thioglycoside **24** afforded trisaccharide **25** in 72% yield, that is, three times as high as the best yield achieved with donors **16** or **17**. Selective reductive opening of the benzylidene ring in **25**, this time under the

conditions directing the benzyl group to O-6 ($Me_3N{\cdot}BH_3$, $AlCl_3$, water),[34] resulted in the formation of key trisaccharide **26** corresponding to structure **5** (Scheme 1).

In principal, the product of 6-O-deacetylation of compound **26** could be subjected to simultaneous bis-α-glucosylation that would directly lead to the target pentasaccharide structure. However, possible formation of a difficult to separate mixture of four stereoisomers prompted us to prefer a scheme with two consecutive α-glucosylations (Scheme 4). First glucosylation of 4-OH in the GalN residue was carried out with *N*-phenyltrifluoroacetimidate **4**. 6-*O*-Benzoyl group in **4** is able to control the anomeric stereoselectivity; on the other hand, it allows selective liberation of 6-OH in the β-glucose unit due to stability under the conditions of acidic O-deacetylation.[35] TMSOTf-promoted glycosylation of acceptor **26** with imidate **4** resulted in the formation of an anomeric mixture **27** that was subjected, without separation, to acidic O-deacetylation. Pure α-anomer **28** was isolated from the resulting mixture by preparative HPLC in 76% overall yield. *N*-Phenyltrifluoroacetimidate **3**, which was previously shown to be the most stereoselective α-glucosyl donor,[26] was used for final glycosylation of **28**. As a result, target pentasaccharide **29** was obtained in 80% yield.

After completion of the assembly of the pentasaccharide backbone, it was necessary to reduce the azido group, introduce various *N*-acyl substituents, and remove *O*-acyl and benzyl protecting groups. To this aim, protected pentasaccharide **29** was firstly deacylated with methanolic MeONa to produce compound **30** (Scheme 5). It was shown during the synthesis of the glycoform I[10] that dithiothreitol (DTT) in the presence of di-isopropylamine in MeCN efficiently reduced the pentasaccharide azide to the corresponding amine, while some other widely used methods, including catalytic hydrogenation,[36–38] did not provide good results. Here we found that the application of aqueous MeCN as the reaction solvent considerably accelerated the reduction. Reduction of azide **30** under these conditions resulted in the formation of amine **31** in high yield. Amine **31** could be isolated and characterized; however, direct N-acylation of crude amine proved to be more practical. Thus, treatment of **31**, obtained by simple concentration of the reaction mixture, with Ac_2O in MeOH gave acetamide **32** in 84% overall yield.

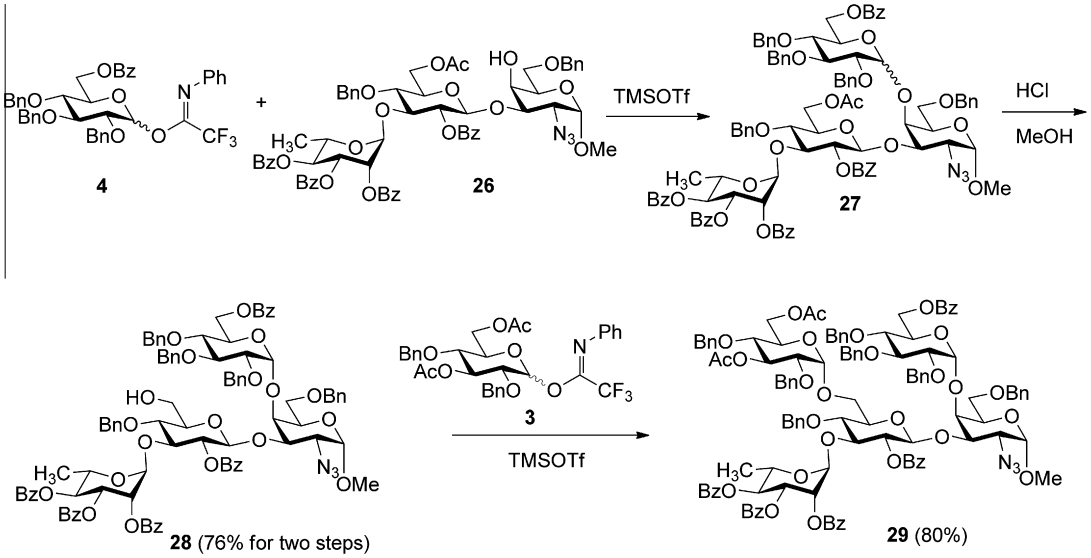
Similarly, acylation of crude **31** with alanine active ester **33** provided *N*-alanyl derivative **34** with a yield of 91%. The presence of signals for the acetyl (s at δ 1.95) and alanyl (d at δ 1.30; q at δ 4.10) groups, as well as downfield shifts of the signals for H-2 of the GalN residue as compared to that of parent amine **31** in the ^{1}H NMR spectra of **32** (δ 3.33 → 4.53) and **34** (δ 3.33 → 4.49) proved their structure. Benzyl protecting groups in both **32** and **34** were efficiently removed by conventional catalytic hydrogenolysis to produce N-acetylated **1a**, the first of the three target pentasaccharides, and **35** respectively. The *N*-Boc protection in the alanyl moiety of **35** was quantitatively removed by treatment with neat TFA to give the second pentasaccharide **1b**. Finally, conventional N-acetylation of **1b** provided the third target compound **1c**. The homogeneity of **1a–c** was ensured by HPLC purification, and their structure was assessed by ^{1}H and ^{13}C NMR (see Tables 1 and 2) and HRMS data.

To summarize, three pentasaccharide α-methylglycosides **1a–c** bearing *N*-acetyl, *N*-(L-alanyl) and *N*-(*N*-acetyl-L-alanyl) substituents in the galactosamine residue were prepared for biological investigations of the molecular basis of the CFTR-mediated binding and elimination of *Pseudomonas aeruginosa* from the lung. A combination of anomeric stereocontrol using remote acyl groups and the *N*-phenyltrifluoroacetimidoyl leaving group provided glucosyl donors, which proved to be an effective tool for α-glucosylation, including that of sterically and electronically demanding glycosyl acceptors. DTT in aqueous acetonitrile in the presence of di-isopropylamine provided the high-yielding transformation of the pentasaccharide azide to the corresponding amine, a key intermediate for final N-modification leading to three target pentasaccharides.

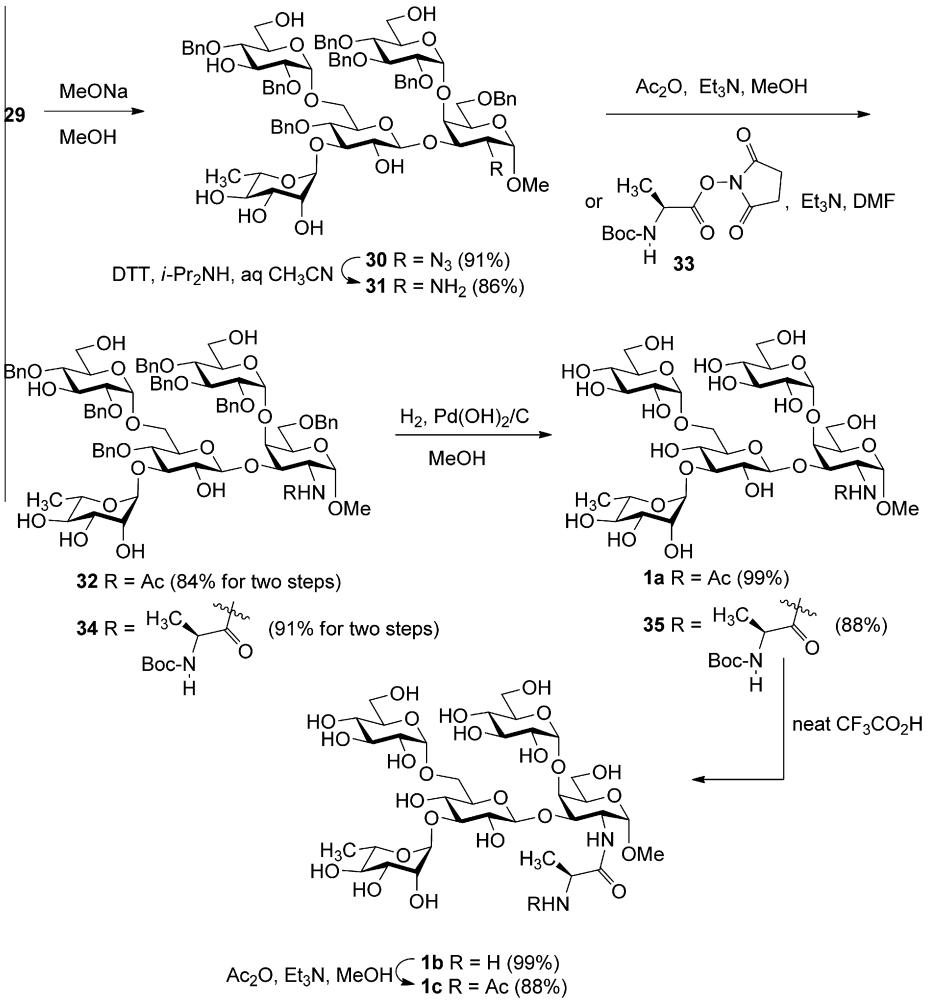
3. Experimental section

3.1. General experimental methods

All glycosylation reactions were carried out under dry Ar. Molecular sieves for glycosylation reactions were crushed and activated prior to application at 180 °C in vacuum of an oil pump during 2 h. Acetonitrile was distilled from P_2O_5 and then from CaH_2 under Ar. Dichloromethane was successively distilled from diethanolamine, P_2O_5, and CaH_2 under Ar. For glycosylation reactions, dichloromethane was freshly redistilled from CaH_2. THF was distilled from sodium benzophenone ketyl. DMF was distilled under reduced pressure (17 mm Hg) from phthalic anhydride and then from CaH_2. Pyridine was dried by distillation from P_2O_5. Analytical thin-layer chromatography (TLC) was performed on Silica



Scheme 4. Synthesis of protected pentasaccharide **29**.



Scheme 5. Synthesis of pentasaccharides **1a–c**.

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, Mostain reagent (ceric sulfate (1% w/v) and ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous H_2SO_4) or a solution of ninhydrin (300 mg) in a mixture of n-butanol (100 mL) and AcOH (3 mL). Column chromatography was performed on Silica Gel 60, 40–63 μm (Merck). Optical rotation values were measured using a JASCO DIP-360 polarimeter at the ambient temperature in solvents specified. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-300, Bruker AMX-400, Bruker DRX-500, and Bruker Avance spectrometers. ^{1}H NMR chemical shifts were referenced to residual signal of $CHCl_3$ (δ_H 7.27) or CH_3OH (δ_H 3.33). ^{13}C chemical shifts were referenced to the central resonance of $CDCl_3$ (δ_C 77.0) and CD_3OD (δ_C 49.0). Signal assignment in ^{1}H and ^{13}C NMR spectra was made using COSY, TOCSY, and ^{1}H–^{13}C HSQC techniques. High-resolution mass spectra were acquired by electrospray ionization on a MicrOTOF II (Bruker Daltonics) instrument.[39]

3.2. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-α-D-glucofuranose (10)

A solution of diacetoneglucose **9** (0.99 g, 3.79 mmol) and bromide **8** (3.07 g, 5.70 mmol) in a mixture of toluene (22 mL) and CH_2Cl_2 (49 mL) was stirred at rt with powdered mol. sieve 4 Å (500 mg) for 1 h. A solution of AgOTf (0.26 M, 22 mL) in toluene was added to the mixture, and stirring was continued until the full consumption of **9** (~2 h). After adding pyridine (5 mL), the reaction mixture was stirred for 20 min, filtered through a Celite layer, the filtrate was diluted with dichloromethane, washed with a mixture of satd aq $NaHCO_3$ and 1 M $Na_2S_2O_3$ (1:1), and then with water. The organic layer was separated, concentrated, and the residue was subjected to column chromatography (toluene–EtOAc, 17:1) to provide disaccharide **10** (2.10 g, 77%) as an amorphous solid: R_f 0.6 (toluene–EtOAc, 7:2). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.15–7.15 (m, 15H, Ar), 1.55 (s, 3H, C–C*H*$_3$), 1.45 (s, 6H, 2 C–C*H*$_3$), 1.37 (s, 3H, C–C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 165.8, 165.5 (C=O), 133.6–125.3 (Ar), 112.0, 109.2 (*C*(CH$_3$)$_2$), 26.8, 26.1, 25.3 (C(*C*H$_3$)$_2$). ESI HRMS: $[M+Na]^+$ calcd for $C_{39}H_{42}O_{13}$ + Na: 741.2518, found 741.2525.

3.3. 1,2-*O*-Isopropylidene-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-α-D-glucofuranose (11)

A solution of disaccharide **10** (1.00 g, 1.39 mmol) in 85% aq AcOH (20 mL) was kept at 35 °C until disappearance of the starting material (~24 h). The reaction mixture was diluted with toluene (40 mL) and concentrated. The residue was purified by column chromatography (toluene–acetone, 20:1 → 5:1) to give diol **11** (0.68 g, 72%) as an amorphous solid: R_f 0.21 (toluene–acetone, 5:1). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.05–7.05 (m, 15H, Ar), 3.03 (br s, 1H, OH), 2.81 (br s, 1H, OH), 1.45 (c, 3H, C–C*H*$_3$), 1.26 (c, 3H, C–C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 165.7,

Table 1
^{1}H NMR chemical shifts (δ, ppm) and coupling constants (J, Hz) for compounds **10–35**, **1a–c**

Compound	Monosaccharide unit	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
10	α-L-Rha-(1→3)	5,23	5,65	5,81 (10,2)	5,74 (9,9)	4,66	1,37	
	α-D-Glc-*f*	6,02 (3,6)	4,65	4,50 (3,1)	4,21 (9,1)	4,55 (6,2)	4,31 (5,9)	3,26 (8,3)
11	α-L-Rha-(1→3)	5,24	5,64 (3,5)	5,79 (10,2)	5,70 (10,0)	4,52	1,38 (6,2)	
	α-D-Glc-*f*	6,01 (3,8)	4,66 (3,7)	4,51 (2,9)	4,26 (9,1)	4,15 (3,1)	4,01 (5,2)	3,87 (11,3)
12	α-L-Rha-(1→3)	5,22	5,62 (3,4)	5,78 (10,2)	5,64 (9,8)	4,48	1,34 (6,2)	
	α-D-D-Glc-*f*	5,98 (3,6)	4,65	4,50 (2,9)	4,26 (9,2)	4,31	4,66	4,41
13	α-L-Rha-(1→3)	5,41	5,82	5,83	5,70	4,53	1,37	
α-Isomer	α-D-GlcOH	5,30 (3,0)	3,78 (9,0)	3,92 (9,0)	3,54 (9,0)	4,11	4,49	
13	α-L-Rha-(1→3)	5,43	5,82	5,83	5,70	4,51	1,37	
β-Isomer	β-D-GlcOH	4,69 (8,0)	3,58	3,69	3,60	3,61	4,54	4,46
14	α-L-Rha-(1→3)	5.19	5.57	5.7–5.58		4.16	1.30	
α-Isomer	α-D-GlcOAc	6.38 (3.6)	5.19	4.19	5.25	4.06	4.33	4.21
14	α-L-Rha-(1→3)	5.13	5.49	5.7–5.58		4.16	1.30	
β-Isomer	β—D-GlcOAc	5.68	5.30	4.01	5.24	3.77	4.36	4.24
15	α-L-Rha-(1→3)	5.19	5.63	5.70–5.62		4.19	1.31	
α-Isomer	α-D-GlcOH	5.56 (3.5)	4.99 (10.0)	4.31	5.11	4.23	4.36	4.26
15	α-L-Rha-(1→3)	5.17	5.56	5.70–5.62		4.19	1.31	
β-Isomer	β-D-GlcOH	4.70 (9.0)	5.02	4.01	5.11	3.71	4.36	4.26
16	α-D-Glc-Br	6,71 (4,0)	4,93 (9,7)	4,33 (9,4)	5,31 (9,7)	4,26 (4,6)	4,44	4,27 (13,1)
	α-L-Rha-(1→3)	5,24	5,62	5,65	5,70	4,16 (6,2)	1,34	
17	α-D-Glc-OC(NH)CCl$_3$	6,64 (3,6)	5,24	4,36	5,33	4,18	4,38	4,26
	α-L-Rha-(1→3)	5,24	5,62	5,68	5,70	4,21 (6,2)	1,35	
18	α-D-GalN-OMe	4,92 (3,4)	3,91	4,10	4,37	3,66	4,26	4,11
	β-D-Glc-(1→3)	4,79 (8,0)	5,26	3,94	5,17	3,62	4,38	4,26
	α-L-Rha-(1→3)	5,09	5,46	5,57	5,62 (9,5)	4,15	1,26	
21	α-L-Rha-(1→3)	5.54	5.74 (3.4)	5.80 (10.1)	5.57 (10.1)	4.53 (6.2)	1.01	
	β-D-Glc-SEt	4.43 (9.8)	3.70 (9.1)	4.02 (9.5)	3.73 (4.9)	3.56 (0)	4.38 (10.5)	3.81
22	α-L-Rha-(1→3)	5.09	5.39 (3.6)	5.76 (10.1)	5.46	4.45	1.36	
	β-D-Glc-SEt	4.67 (10.1)	5.48 (9.5)	4.26 (9.2)	3.87 (9.0)	3.65	4.45	3.86
23	α-L-Rha-(1→3)	5.35	5.52 (3.5)	5.82 (10.2)	5.54 (10.0)	4.39 (6.1)	1.07	
	β-D-Glc-SEt	4.66 (10.0)	5.46 (9.6)	4.29 (9.1)	3.88	3.61	4.04	3.88
24	α-L-Rha-(1→3)	5.34	5.50 (3.3)	5.81 (10.2)	5.52 (10.1)	4.33 (6.1)	1.05	
	β-D-Glc-SEt	4.60 (10.1)	5.46 (9.5)	4.27 (9.2)	3.83 (9.6)	3.74	4.54	4.31 (11.8)
25	α-L-Rha-(1→3)	5.33	5.57	5.83 (10.1)	5.54	4.35 (6.1)	1.08	
	β-D-Glc-(1→3)	4.96 (7.6)	5.54	4.29	3.91 (9.5)	3.75	4.77	4.24
	α-D-GalN-OMe	4.85 (3.3)	3.81 (10.7)	4.09 (2.9)	4.48	3.68	4.27	4.12
26	α-D-GalN-OMe	4.78	3.61	3.96	4.17	3.97	3.79	3.72
	β-D-Glc-(1→3)	4.86 (7.9)	5.48 (8.7)	4.29 (9.3)	3.81	3.76	4.58 (4.5)	4.25 (11.9)
	α-L-Rha-(1→3)	5.36	5.53 (3.2)	5.82 (10.1)	5.54	4.34 (6.1)	1.09	
28	α-D-GalN-OMe	4.82 (3.5)	3.61	3.96	4.34	4.28	3.91	3.53
α-Isomer	β-D-Glc-(1→3)	4.86	5.57	4.29	4.21 (9,5)	3.61	4.00	3.95
	α-L-Rha-(1→3)	5.39	5.56 (3,3)	5.85 (10,1)	5.53 (10,1)	4.43 (5,1)	1.09	
	α-D-Glc-(1→4)	5.01	3.55	4.11 (9,4)	3.82 (9,6)	4.53 (10,1)	5.34 (11,7)	4.73
29	α-D-GalN-OMe	5.06	3.60	4.05	4.26	3.39	3.72	3.67
	β-D-Glc-(1→3)	4.85	5.43	4.23 (9.4)	3.88	3.82	4.04	
	α-L-Rha-(1→3)	5.32	5.48 (3.1)	5.74 (10.0)	5.47	4.30 (6.1)	1.07	
	α-D-Glc-(1→6)	5.20 (2.9)	3.43	5.58 (9.6)	3.41	4.02	4.21	4.13
	α-D-Glc-(1→4)	4.81	3.59	4.10	3.83	4.50	5.11	4.63
30	α-D-GalN-OMe	4.88 (3.6)	3.78	4.14 (2.7)	4.27	4.04	3.84	3.62
	β-D-Glc-(1→3)	4.48 (8.0)	3.38	3.80	3.43	3.59	3.76	3.61
	α-L-Rha-(1→3)	5.44	4.03	3.76	3.40	3.86 (6.2)	1.09	
	α-D-Glc-(1→6)	4.76	3.34	4.00 (9.3)	3.32	3.68	3.72	3.53
	α-D-Glc-(1→4)	5.01 (3.3)	3.38	3.92 (9.4)	3.58	4.19	3.95	3.70
31	α-D-GalN-OMe	4.71	3.33	3.87	4.24	4.02	3.86	3.64
	β-D-Glc-(1→3)	4.45 (7.9)	3.41	3.80			3.79	3.57
	α-L-Rha-(1→3)	5.43	4.00	3.76		3.85 (6.2)	1.09	
	α-D-Glc-(1→6)	4.81	3.32	3.97	3.31	3.72	3.73	3.49
	α-D-Glc-(1→4)	5.00 (3.2)		3.92	3.57	4.21	3.96	3.71
32	α-D-GalN-OMe	4.73	4.53	4.02	4.19	4.03	3.82	3.65
	β-D-Glc-(1→3)	4.41	3.35	3.76	3.40	3.59	3.76	3.64
	α-L-Rha-(1→3)	5.42	3.97	3.75	3.38	3.84	1.07	
	α-D-Glc-(1→6)	4.80	3.34	3.96	3.29	3.70	3.73	3.51
	α-D-Glc-(1→4)	4.99	3.37	3.97	3.53	4.21	3.95	3.67
34	α-D-GalN-OMe	4.84	4.52	4.06	4.16	4.04	3.82	3.63
	β-D-Glc-(1→3)	4.43 (8.0)	3.35	3.77	3.43	3.58	3.75	3.69
	α-L-Rha-(1→3)	5.41	3.96	3.73	3.38	3.84 (6.2)	1.07	
	α-D-Glc-(1→6)	4.74	3.34	3.99	3.30	3.68	3.75	3.53
	α-D-Glc-(1→4)	5.00	3.39	3.98	3.52	4.19	3.93	3.67
35	α-D-GalN-OMe	4.85 (3.6)	4.47 (11.2)	4.13	4.28	4.04	3.91	
	β-D-Glc-(1→3)	4.51 (7.8)	3.32	3.63	3.53	3.61	3.92	4.51
	α-L-Rha-(1→3)	5.18	4.05	3.81	3.47	4.01 (6,3)	1.26	
	α-D-Glc-(1→6)	5.02 (3.5)	3.59	3.71	3.43	3.73	3.89	5.02
	α-D-Glc-(1→4)	4.99	3.52	3.87	3.52	4.21	3.85	4.99

(*continued on next page*)

Table 1 (*continued*)

Compound	Monosaccharide unit	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)
1a	α-D-GalN-OMe	4.83 (3.6)	4.45 (11.2)	4.08 (2.5)	4.27	4.03	3.90	
	β-D-Glc-(1→3)	4.49 (8.1)	3.32 (8.6)	3.59	3.51	3.62	3.90	4.49
	α-L-Rha-(1→3)	5.17	4.04	3.80 (9.7)	3.46	4.00 (6.3)	1,3	
	α-D-Glc-(1→6)	5.01 (3.7)	3.59	3.70 (9.3)	3.42	3.74	3.87	5,01
	α-D-Glc-(1→4)	4.98 (3.5)	3.51	3.85	3.52	4,21	3.83	
1b	α-D-GalN-OMe	4.84 (3.7)	4.47 (11.3)	4.16 (2.8)	4.29	4.03	3.89	
	β-D-Glc-(1→3)	4.50 (8.1)	3.30 (8.6)	3.56	3.51	3.61	3.90	4.50
	α-L-Rha-(1→3)	5.16	4.02	3.79	3.45	3.96 (6.3)	1.25	
	α-D-Glc-(1→6)	5.09 (3.7)	3.57	3.70 (9.6)	3.41	3.71	3.87	5.09
	α-D-Glc-(1→4)	4.98 (3.6)	3.52	3.83	3.52	4.20	3.82	
1c	α-D-GalN-OMe	4.80 (3,6)	4.45 (11.3)	4.14 (2.7)	4.27	4.03	3.88	
	β-D-Glc-(1→3)	4.51 (8.0)	3.31 (8.5)	3.60	3.51	3.62	3.91	4.51
	α-L-Rha-(1→3)	5.17	4.04	3.79	3.46	4.00 (6.3)	1.27	
	α-D-Glc-(1→6)	5.01 (3.7)	3.57	3.71	3.42	3.73	3.87	5.01
	α-D-Glc-(1→4)	4.99 (3.5)	3.52	3.84	3.52	4.20	3.82	

165.6 (CO), 133.6–128.3 (Ar), 112.0 ($C(CH_3)_2$), 26.7, 26.1 ($C(CH_3)_2$). Anal. Calcd for $C_{36}H_{38}O_{13}$: C, 63.71; H, 5.64. Found: C, 63.93; H, 5.68.

3.4. 6-*O*-Chloroacetyl-1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-α-D-glucofuranose (12)

To a solution of diol **11** (1.1 g, 1.6 mmol) in dry CH_2Cl_2 (10.8 mL) were added chloroacetyl chloride (144 μL, 1.6 mmol) and pyridine (130 μL, 1.6 mmol) at −15 °C. After 30 min, more pyridine (30 μL, 0.4 mmol) was added and the mixture was stirred for 1 h. To bring the reaction to completion, more chloroacetyl chloride (30 μL, 0.38 mmol) and pyridine (30 μL, 0.4 mmol) were added and stirring was continued for next 30 min. After quenching with water (4 mL), the reaction mixture was poured into 1 M HCl (80 mL) and extracted with CH_2Cl_2. Combined organic extracts were washed with water and concentrated. Column chromatography of the residue (toluene–EtOAc, 10:1) afforded disaccharide **12** (1.10 g, 90%) as an amorphous solid: R_f 0.54 (toluene–acetone, 4:1). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.11–7.10 (15H, m, Ar), 4,35 (2H, s, $ClCH_2$), 1,50 (3H, s, C–CH_3), 1,33 (c, 3H, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 167.9 ($ClCH_2CO$), 165.8, 165.7, 165.6 (PhCO), 133.6, 133.3, 133.2 (*ipso*-C, Bz), 129.9, 129.7, 129.6, 129.2, 129.1, 129.0, 128.4, 128.3, 128.2 (Ph), 112.2 ($C(CH_3)_2$), 40.8 ($ClCH_2$), 26.7, 26.2 ($C(CH_3)_2$). Anal. Calcd for $C_{38}H_{39}ClO_{14}$: C, 60.44; H, 5.21. Found: C, 60.51; H, 5.39.

3.5. 6-*O*-Chloroacetyl-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-D-glucopyranose (13)

Aq CF_3CO_2H (90%, 18 mL) was added to a chilled (+4 °C) solution of **12** (1.0 g, 1.4 mmol) in CH_2Cl_2 (2 mL). The mixture was kept at rt for 1 h, diluted with toluene (50 mL) and concentrated in vacuum. Residual CF_3CO_2H was removed by coevaporation with toluene. Triol **13** (933 mg, 97%) was purified by column chromatography (toluene–acetone, 4:1): R_f 0.23 (toluene–acetone, 4:1). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.05–7.12 (m, Ar), 4.12 (s, $ClCH_2$). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 166.1 ($ClCH_2CO$), 165.8, 165.7 (PhCO), 133.6, 133.3 (*ipso*-C, Bz), 129.9, 129.7, 129.0, 128.8, 128.6, 128.4, 128.3 (Ph), 40.8 ($ClCH_2CO$). ESI HRMS: $[M+Na]^+$ calcd for $C_{35}H_{35}ClO_{14}$ + Na: 737.1608, found 737.1614.

3.6. 1,2,4-Tri-*O*-acetyl-6-*O*-chloroacetyl-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-D-glucopyranose (14)

A solution of triol **13** (856 mg, 1.20 mmol) in Ac_2O (17 mL) and pyridine (17 mL) was kept for 6 h, then the reaction mixture was poured into a mixture of crushed ice and satd aq $NaHCO_3$. The aqueous solution was extracted four times with EtOAc and combined organic extracts were washed successively with water, 1 M HCl, and water. The organic layer was concentrated, and the residue was subjected to column chromatography (toluene–methyl *tert*-butyl ether, 11:1) to give triacetate **14** (768 mg, 76%) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.10–7.10 (m, Ar), 4.16–4.14 (m, $ClCH_2$), 2.34–2.10 (cluster of s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 169.6, 169.5, 169.2, 168.7 (CH_3CO), 167.2 ($ClCH_2CO$), 165.8, 165.3, 165.2 (PhCO), 40.7 (ClH_2CCO), 21.1, 21.0, 21.0, 20.9, 20.7, 20.5 (CH_3CO). ESI HRMS: $[M+Na]^+$ calcd for $C_{41}H_{41}ClO_{17}$ + Na: 863.1924, found 863.1927.

3.7. 2,4-Di-*O*-acetyl-6-*O*-chloroacetyl-3-*O*-(2,3,4-tri-*O*-benzoyl-β-L-rhamnopyranosyl)-D-glucopyranose (15)

To a solution of compound **14** (251 mg, 0.30 mmol) in DMF (4.3 mL) was added N_2H_4·AcOH (41 mg, 0.45 mmol). After 2.5 h, the reaction mixture was poured into satd aq $NaHCO_3$ and the aqueous solution was extracted four times with EtOAc. Combined organic extracts were washed with water, dried with Na_2SO_4, and concentrated. The product **15** (198 mg, 83%) was isolated by column chromatography (toluene–acetone, 5:1): ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.11–7.21 (m, Ar), 4.19 (s, $ClCH_2$), 2.39, 2.32, 2.31, 2.19 (4 s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.8, 169.7 (CH_3CO), 167.3 ($ClCH_2CO$), 165.8, 165.6, 165.3 (PhCO), 133.6, 133.5, 133.1 (*ipso*-C, Ph), 129.8, 129.7, 129.7, 129.2, 129.1, 129.0, 128.6, 128.4, 128.2 (Ph), 40.7 ($ClCH_2CO$), 21.1, 20.8, 20.7 (CH_3CO). Anal. Calcd for $C_{39}H_{39}ClO_{16}$: C, 58.61; H, 4.92. Found: C, 59.01; H, 4.69.

3.8. 2,4-Di-*O*-acetyl-6-*O*-chloroacetyl-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-α-D-glucopyranosyl bromide (16)

A solution of HBr in AcOH (33% wt, 0.45 mL) was added to a solution of **14** (68 mg, 0.081 mmol) in CH_2Cl_2 (0.50 mL). The reaction mixture was kept for 2 h at rt, diluted with CH_2Cl_2 (50 mL) and poured into ice water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), combined organic extracts were washed with satd aq $NaHCO_3$, and concentrated. Column chromatography (gradient elution, toluene–methyl *tert*-butyl ether, 25:1 → 50:3) of the residue afforded bromide **16** (58 mg, 83%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.12–7.20 (15H, m, Ar), 4.18 (2H, s, $ClCH_2$), 2.31, 2.19 (6H, 2 s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$ for the signals of sugar ring carbons see

Table 2
^{13}C NMR chemical shifts (δ, ppm) for compounds **10–35**, **1a–c**

Compound	Monosaccharide residue	C-1	C-2	C-3	C-4	C-5	C-6
10	α-L-Rha-(1→3)	94,9	71,1	70,0	71,6	67,1	17,2
	α-D-Glc-*f*	105,4	81,9	77,0	81,0	72,0	68,3
11	α-L-Rha-(1→3)	96,0	71,1	69,9	71,5	67,7	17,5
	α-D-Glc-*f*	105,2	82,0	79,1	79,6	68,5	64,4
12	α-L-Rha-(1→3)	95,9	71,0	69,8	71,5	67,2	17,5
	α-D-Glc-*f*	105,2	81,9	78,7	78,9	67,7	68,4
13	α-L-Rha-(1→3)	99,0	70,7	70,1	71,3	67,6	17,5
α-Isomer	α-D-GlcOH	92,4	71,4	83,6	69,0	69,3	65,0
13	α-L-Rha-(1→3)	98,8	70,6	70,2	71,3	67,6	17,5
β-Isomer	β-D-GlcOH	96,6	74,3	85,0	73,5	69,0	65,0
14	α-L-Rha-(1→3)	99.7	70.7	71.1 or 69.7[a]		67.9	17.5
α-Isomer	α-D-GlcOAc	89.2	70.6	78.2	68.7	70.0	63.1
14	α-L-Rha-(1→3)	99.8	70.7	71.1 or 69.7[a]		68.0	17.4
β-Isomer	β-D-GlcOAc	91.9	70.6	81.6	68.9	72.7	63.1
15	α-L-Rha-(1→3)	99.9	70.7	71.3 or 69.8[a]		67.8	17.6
α-Isomer	α-D-GlcOAc	90.2	72.7	78.2	69.3	67.5	63.6
15	α-L-Rha-(1→3)	99.9		71.1 or 69.7[a]			17.6
β-Isomer	β-D-GlcOAc	95.9	74.6	81.1	69.3	72.0	63.5
16	α-D-Glc-Br	87,1	72,4	78,3	67,8	72,3	62,6
	α-L-Rha-(1→3)	99,8	70,6	69,7 or 71,2[a]		67,9	17,6
17	α-D-Glc-OC(NH)CCl$_3$	93,1	71,4	77,9	68,5	70,2	63,1
	α-L-Rha-(1→3)	99,6	70,6	71,2 or 69,7[a]		67,9	17,6
18	α-D-GalN-OMe	99,6	59,2	75,1	75,7	63,0	69,0
	β-D-Glc-(1→3)	101,7	71,5	81,9	69,6	72,0	63,3
	α-L-Rha-(1→3)	99,6	70,9	69,6	71,2	67,9	17,4
21	α-L-Rha-(1→3)	97.5	70.7	70.2	71.7	66.3	17.0
	β-D-Glc-SEt	87.3	74.2	77.5	7.9	71.1	68.7
22	α-L-Rha-(1→3)	97.8	70.5	69.5	71.6	66.6	16.8
	β-D-Glc-SEt	84.4	72.9	77.1	79.0	71.2	68.7
23	α-L-Rha-(1→3)	97.9	70.7	69.4	71.7	67.3	17.3
	β-D-Glc-SEt	83.8	73,0	79.4	76.4	80.1	61.7
24	α-L-Rha-(1→3)	97.9	70.7	69.3	71.7	67.5	17.3
	β-D-Glc-SEt	83.7	72.8	79.4	77.0	77.2	62.9
25	α-L-Rha-(1→3)	97.7	70,7	69,5	74,0	67,4	17,3
	β-D-Glc-(1→3)	101.9	71.8	77.9	76.7	73.5	62.0
	α-D-GalN-OMe	99.7	58.9	75.4	75.6	63.0	69.1
26	α-D-GalN-OMe	98.9	59.0	77.8	68.5	68.8	69.4
	β-D-Glc-(1→3)	101.4	73.8	77.5	76.7	73.6	62.4
	α-L-Rha-(1→3)	97.8	70.6	69.3	71.6	67.5	17.3
28	α-D-GalN-OMe	99.2	59.0	78.8	77.2	73.0	67.7
α-Isomer	β-D-Glc-(1→3)	104.0	73.5	77.8	75.6	76.2	61.4
	α-L-Rha-(1→3)	98.1	70.6	69.4	71.9	67.2	17.3
	α-D-Glc-(1→4)	99.0	80.8	81.9	78.0	69.5	62.9
29	α-D-GalN-OMe	98.4	59.3	77.9	77.7	70.5	69.3
	β-D-Glc-(1→3)	103.4	73.7	77.9	77.1	76.2	67.2
	α-L-Rha-(1→3)	98.1	70.5	69.3	71.9	67.2	17.3
	α-D-Glc-(1→6)	96.6	77.4	73.2	76.0	68.9	63.3
	α-D-Glc-(1→4)	98.9	81.1	81.9	78.2	69.3	63.5
30	α-D-GalN-OMe	100.4	61.4	78.2	78.3	72.1	71.2
	β-D-Glc-(1→3)	106.3	76.4	82.0	78.8	75.7	67.9
	α-L-Rha-(1→3)	102.6	72.4	72.4	74.2	70.3	18.1
	α-D-Glc-(1→6)	98.2	81.6	74.8	80.1	72.5	62.6
	α-D-Glc-(1→4)	98.8	82.0	82.9	79.3	72.8	62.1
31	α-D-GalN-OMe	98.2	52.3	82.2	77.7	72.5	71.2
	β-D-Glc-(1→3)	107.2	76.6	82.0			68.1
	α-L-Rha-(1→3)	102.5	72.4	72.4		70.3	18.1
	α-D-Glc-(1→6)	101.0	81.8	74.8	80.3	72.4	62.8
	α-D-Glc-(1→4)	98.7	81.9	83.0	79.3	72.6	62.0
32	α-D-GalN-OMe	100.1	50.7	78.1	78.6	72.2	71.4
	β-D-Glc-(1→3)	106.4	76.2	81.6	78.8	75.8	68.0
	α-L-Rha-(1→3)	102.5	72.4	72.3	74.0	70.2	18.1
	α-D-Glc-(1→6)	98.2	81.6	74.8	80.1	72.6	62.8
	α-D-Glc-(1→4)	99.1	82.1	83.0	79.4	72.8	62.3
34	α-D-GalN-OMe	98.1	50.8	77.2	78.9	72.1	71.3
	β-D-Glc-(1→3)	105.7	76.5	81.5	78.7	76.1	67.8
	α-L-Rha-(1→3)	102.4	72.3	72.4	74.1	70.2	18.1
	α-D-Glc-(1→6)	100.0	81.6	74.8	80.0	72.7	62.7
	α-D-Glc-(1→4)	99.3	82.1	83.0	79.4	72.9	62.3
	α-D-GalN-OMe	99.8	50.7	77.4	73.5	73.3	62.0
	β-D-Glc-(1→3)	105.6	75.2	83.3	77.4	75.9	67.7
35	α-L-Rha-(1→3)	102.3	71.8	71.7	73.5	70.3	18.0
	α-D-Glc-(1→6)	99.5	72.9	74.7	71.1	73.4	62.2
	α-D-Glc-(1→4)	100.6	73.4	74.1	70.7	72.9	61.6
	α-D-GalN-OMe	99.9	50.5	78.3	77.4	73.4	62.0

(*continued on next page*)

Table 2 (*continued*)

Compound	Monosaccharide residue	C-1	C-2	C-3	C-4	C-5	C-6
	β-D-Glc-(1→3)	106.2	75.0	83.6	69.9	75.7	67.8
1a	α-L-Rha-(1→3)	102.3	71.8	71.6	73.4	70.3	18.0
	α-D-Glc-(1→6)	99.4	72.9	74.7	71.1	73.4	62.3
	α-D-Glc-(1→4)	100.5	73.4	74.1	70.7	72.7	61.6
	α-D-GalN-OMe	99.5	51.0	77.2	77.2	73.2	62.0
	α-D-Glc-(1→3)	105.7	7.0	83.3	69.8	75.9	67.8
1b	α-L-Rha-(1→3)	102.2	71.7	71.8	73.4	70.3	18.0
	α-D-Glc-(1→6)	99.5	72.9	74.7	71.1	73.3	62.3
	α-D-Glc-(1→4)	100.4	73.4	74.1	70.7	72.8	61.7
	α-D-GalN-OMe	99.8	50.8	77.2	77.4	73.3	62.0
	β-D-Glc-(1→3)	105.6	75.1	83.5	69.9	75.9	67.8
1c	α-L-Rha-(1→3)	102.3	71.8	71.7	73.5	70.3	18.0
	α-D-Glc-(1→6)	99.5	72.9	74.8	71.1	73.4	62.3
	α-D-Glc-(1→4)	100.5	73.4	74.2	70.7	72.8	61.7

[a] Assignment of these signals may be interchanged.

Table 2): δ 170.3, 169.4, (CH_3CO), 167.1 ($ClCH_2CO$), 165.7, 165.6, 165.4 (PhCO), 133.6, 133.4, 133.1 (*ipso*-C, Ph), 40.6 ($ClCH_2CO$), 21.0, 20.6 (CH_3CO). ESI HRMS: $[M+Na]^+$ calcd for $C_{39}H_{38}BrClO_{15}$ + Na: 883.0975, found 883.0983.

3.9. 2,4-Di-*O*-acetyl-6-*O*-chloroacetyl-3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-α-D-glucopyranosyl trichloroacetimidate (17)

Cl_3CCN (76 μL, 0.754 mmol) and DBU (6 μL, 0.038 mmol) were added to a solution of hemiacetal **15** (100 mg, 0.126 mmol) in dry CH_2Cl_2 (1.5 mL) at −18 °C. The reaction mixture was allowed to attain rt within 1.5 h and applied onto a silica gel column. Elution with toluene–methyl *tert*-butyl ether (25:2) provided trichloroacetimidate **17** (88 mg, 74%): R_f 0.2 (toluene–methyl *tert*-butyl ether, 35:2). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.78 (1H, s, $Cl_3CC(NH)O$), 8.11–7.15 (15H, m, Ar), 4.17 (m, $ClCH_2$), 2.23, 2.18 (6H, 2 s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.4, 169.5 (CH_3CO), 167.1 ($ClCH_2CO$), 165.8, 165.6, 165.3 (PhCO), 160.7 ($Cl_3CC(NH)$), 133.6, 133.4, 133.1 (*ipso*-C, Ph), 129.8, 129.7, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.3 (Ar), 40.1 ($ClCH_2CO$), 21.0, 20.5 (CH_3CO). ESI HRMS: $[M+Na]^+$ calcd for $C_{41}H_{39}Cl_4NO_{16}$ + Na: 964.0915, found 964.0936.

3.10. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-6-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (18)

One molar solution of Bu_2BOTf in CH_2Cl_2 (4 μL) was added to a stirred mixture of donor **17** (33 mg, 0.035 mmol), acceptor **7** (9 mg, 0.029 mmol), and powdered mol. sieve AW-300 (55 mg) in freshly distilled CH_2Cl_2 (750 μL) at −40 °C. The resulting mixture was stirred at −30 °C for 1 h, diluted with CH_2Cl_2 (20 mL), and filtered through a Celite pad. The filtrate was washed with satd aq $NaHCO_3$, water, and concentrated. Purification of the residue by column chromatography ($CHCl_3$–acetone, 55:1 → 42:1) gave trisaccharide **18** (7 mg, 21%) as an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.08–7.20 (20H, m, Ar), 5.58 (1H, s, PhC*H*), 4.08 (2H, m, $ClCH_2$), 3.44 (OCH_3), 2.25 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 169.7 (CH_3CO), 167.1 ($ClCH_2CO$), 165.8, 165.7, 165.1 (PhCO), 137.7 (*ipso*-C, PhCH), 133.6, 133.4, 133.0 (*ipso*-C, Bz), 129.8, 129.7, 129.2, 128.8, 128.6, 128.4, 128.2, 128.1 (Ar), 100.5 (PhCH), 55.6 (OCH_3), 40.7 ($ClCH_2CO$), 21.2, 20.8 (CH_3CO). ESI HRMS: $[M+Na]^+$ calcd for $C_{53}H_{54}ClN_3O_{20}$ + Na: 1110.2881, found 1110.2880.

3.11. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-6-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (19)

$NaBH_3CN$ (4 mg, 0.064 mmol) and 4 M solution of HCl in dioxane (16 μL, 0.061 mmol) were consecutively added to a mixture of trisaccharide **18** (10 mg, 0.009 mmol) and mol. sieve 4 Å (28 mg) in dry THF (0.5 mL) at +4 °C. The resulting mixture was stirred for 1.5 h at rt, then made neutral by adding satd aq $NaHCO_3$. The solution was extracted with EtOAc, the combined extracts were washed with water and concentrated to give a chromatographically homogeneous product with R_f 0.27 (toluene–acetone, 11:2). NMR examination revealed this product to be a mixture of **19** and, presumably, its positional isomer with a 4-*O*-benzyl group in a ratio of about 1:1. ^{1}H and ^{13}C NMR spectra of this mixture showed the absence of signals corresponding to CH of the benzylidene group (δ_H 5.59, δ_C 100.5 ppm). The ^{13}C NMR spectrum of the mixture contained signals of double intensities for C1 of the α-$GalN_3$ and α-Rha units (δ 99.1 and 99.8) and two signals for C1 of the β-Glc residue (δ 101.8 and 101.6).

3.12. Ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (21)

A solution of donor **8** (1.15 g, 2.13 mmol, 1.14 equiv) and acceptor **20** (0.58 g, 1.86 mmol) in dry CH_2Cl_2 (12 mL) was added to a suspension of AgOTf (0.55 g, 2.14 mmol) and 2,6-di-*tret*-butyl-4-methylpyridine (0.31 g, 1.47 mmol, 0.98 equiv) in hexane (12.5 mL) at −12 °C under argon. The mixture was stirred at this temperature for 2 h, then the reaction was quenched with Et_3N (0.5 mL). The resulting mixture was diluted with CH_2Cl_2 (75 mL), washed consecutively with a mixture of 1 M $Na_2S_2O_3$ and satd aq $NaHCO_3$ (1:1), water, 1 M HCl, and water. The organic phase was concentrated and the residue was subjected to silica gel column chromatography (toluene–acetone, 50:1→20:1) to yield disaccharide **21** (0.93 g, 66%) as a colorless foam, R_f 0.37 (toluene–acetone, 15:1), $[\alpha]_D$ 12.0 (*c* 0.94, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.01–7.12 (20H, m, Ar); 5.64 (1H, s, PhC*H*); 2.75 (2H, m, SCH_2CH_3); 1.33 (3H, t, SCH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 165.7; 165.6 (PhCO); 137.2 (*ipso*-C, PhCH); 133.3; 133.1; 133.0 (*ipso*-C, Bz); 129.9; 129.7; 129.6; 129.5; 129.4; 129.3; 129.0; 128.5; 128.3; 128.2; 128.1; 126.2 (Ph); 101.7 (PhCH); 24.7 (SCH_2CH_3); 15.4 (SCH_2CH_3). Anal. Calcd for $C_{42}H_{42}O_{12}S$: C, 65.45; H, 5.49. Found: C, 65.44; H, 5.49.

3.13. Ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (22)

Benzoyl chloride (0.82 mL, 7.03 mmol) and DMAP (40 mg, 0.33 mmol) were added to a solution of alcohol **21** (0.77 g, 1.00 mmol) in a mixture of CH_2Cl_2 (5 mL) and pyridine (10 mL) at +4 °C. The reaction mixture was kept at rt for 72 h, diluted with EtOAc (250 mL) and washed successively with satd aq $NaHCO_3$, water, 1 M HCl, and finally with water. The organic phase was concentrated and the residue was purified by column chromatography (toluene–methyl *tert*-butyl ether, 50:1) to afford product **22** (0.80 g, 91%) as a colorless foam, R_f 0.22 (toluene–methyl *tert*-butyl ether, 40:1), $[\alpha]_D$ 80.6 (*c* 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.01–7.12 (25H, m, Ar); 5.66 (1H, s, PhC*H*); 2.75 (2H, m, SCH_2CH$_3$); 1.33 (3H, t, SCH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 165.6; 165.3; 165.0; 164.5 (PhCO); 137.0 (*ipso*-C, PhCH); 133.2; 133.11; 133.07; 133.0 (*ipso*-C, Bz); 130.0; 129.7; 129.4; 129.3; 129.2; 129.0; 128.4; 128.3; 128.2; 128.1 (Ph); 102.0 (PhCH); 24.1 (SCH$_2$CH$_3$); 14.8 (SCH$_2$CH$_3$). Anal. Calcd for $C_{49}H_{46}O_{13}S$: C, 67.26; H, 5.30. Found: C, 67.49; H, 5.42.

3.14. Ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside (23)

A solution of Bu_2BOTf (0.85 M, 0.65 mL, 0.55 mmol) in CH_2Cl_2 was added dropwise to a solution of **22** (0.42 g, 0.50 mmol) and $H_3B{\cdot}THF$ (8.4 mmol) in THF (4.5 mL) at +4 °C. The mixture stirred was for 2 h at the same temperature, then the excess of BH_3 and Bu_2BOTf was destroyed by adding MeOH (1.5 mL) and Et_3N (4.0 mL). If the used solution of $H_3B{\cdot}THF$ did not contain any stabilizer, the reaction mixture could be simply concentrated in a vacuum. If a commercial solution of $H_3B{\cdot}THF$ containing a stabilizer was used, the reaction mixtures should be washed with aq. $NaHCO_3$ before concentration. MeOH (3 × 5 mL) was evaporated from the residue obtained after concentration of the solution, and pure **23** (0.38 g, 86%) was isolated by silica gel column chromatography (toluene–methyl *tert*-butyl ether, 11:1 → 10:1), R_f 0.50 (toluene–acetone, 10:1) as a syrup, $[\alpha]_D$ 53.9 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.10–7.20 (25H, m, Ar); 5.07 (1H, d, J_{gem} = 11.3 Hz, PhCH_2); 4.91 (1H, d, J_{gem} = 11.3 Hz, PhCH_2); 2.79 (2H, m, SCH_2CH$_3$); 1.29 (3H, t, SCH$_2$CH_3). ^{13}C NMR (150 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 165.7; 165.2; 165.0; 164.7 (PhCO); 137.6 (*ipso*-C, PhCH$_2$); 133.2; 133.1; 133.0 (*ipso*-C, Bz); 130.0; 129.7; 129.6; 129.5; 129.3; 129.2; 128.4; 128.2; 127.8; 127.4 (Ph); 75.3 (PhCH$_2$), 24.2 (SCH$_2$CH$_3$); 14.8 (SCH$_2$CH$_3$). Anal. Calcd for $C_{49}H_{48}O_{13}S$: C, 67.11; H, 5.52. Found: C, 67.21; H, 5.54.

3.15. Ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-6-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside (24)

Disaccharide **23** (1.39 g, 1.59 mmol) was acetylated with Ac_2O (15 mL) in pyridine (15 mL). When the reaction ended, the mixture was diluted with $CHCl_3$ (150 mL) and poured into cold water, the organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 30 mL). The combined organic extracts were successively washed with satd aq $NaHCO_3$ (three times), water, 1 M HCl, and water (200 mL each time). The solvent was evaporated, and the residue was dried in vacuum to give disaccharide **24** (1.44 g, 97%), R_f 0.66 (toluene–aceton, 20:2.8) as a syrup, $[\alpha]_D$ 73,8 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.02–7.15 (25H, m, Ar); 5.05 (1H, d, J_{gem} = 11.1 Hz, PhCH_2); 4.74 (1H, d, J_{gem} = 11.1 Hz, PhCH_2); 2.70 (2H, m, SCH_2CH$_3$); 2.12 (3H, s, CH$_3$CO); 1.22 (3H, t, SCH$_2$CH_3). ^{13}C NMR (150 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.6 (CH$_3$CO); 165.7; 165.3; 165.0; 164.7 (PhCO); 137.2 (*ipso*-C, PhCH$_2$); 133.2; 133.1; 133.0 (*ipso*-C, Bz); 130.2; 129.6; 129.4; 129.2; 129.0; 128.9; 128.7; 128.4; 128.2; 127.9; 127.4 (Ph); 75.4 (PhCH$_2$), 24.1 (SCH$_2$CH$_3$); 20.9 (CH$_3$CO); 14.8 (SCH$_2$CH$_3$). Anal. Calcd for $C_{51}H_{50}O_{14}S$: C, 66.65; H, 5.48. Found: C, 66.69; H, 5.52.

3.16. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-6-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-β-D-D-glucopyranosyl-(1→3)-2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (25)

A mixture of donor **24** (250 mg, 0.27 mmol), acceptor **7** (74 mg, 0.24 mmol), and powdered mol. sieve AW-300 (900 mg) in dry CH_2Cl_2 (8 mL) was stirred for 1 h under argon. Then NIS (65 mg, 0.27 mmol) and TfOH (3 μL, 0.011 mmol) were added at −20 °C, and stirring was continued for 1 h. The reaction was quenched by adding di-isopropylamine (100 μL), the mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a pad of Celite. The filtrate was washed with a mixture of satd aq $NaHCO_3$ and 1 M $Na_2S_2O_3$ (1:1), and the solvent was evaporated. Silica gel column chromatography of the residue (toluene–methyl *tert*-butyl ether, 6:1 → 5.5:1) gave trisaccharide **25** (201 mg, 72%) as a syrup, R_f 0.40 (toluene–acetone, 20:2.8), $[\alpha]_D$ 89.0 (*c* 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.10–7.20 (30H, m, Ar); 5.64 (1H, s, PhC*H*); 5.07 (1H, d, J_{gem} = 11.2 Hz, PhCH_2); 4.77 (1H, d, J_{gem} = 11.2 Hz, PhCH_2); 3.41 (3H, s, CH$_3$O), 2.09 (3H, s, CH$_3$CO). ^{13}C NMR (75 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.5 (CH$_3$CO); 165.7; 165.3; 164.9; 165.7 (PhCO); 137.9; 137.3 (*ipso*-C, PhCH, PhCH$_2$); 133.2; 133.1; 133.0 (*ipso*-C, Bz); 129.9; 129.7; 129.6; 129.5; 129.3; 129.2; 128.7; 128.5; 128.4; 128.3; 128.2; 128.1; 128.0; 127.4; 126.1 (Ph); 75.4 (PhCH$_2$), 20.9 (CH$_3$CO). Anal. Calcd for $C_{63}H_{61}N_3O_{19}$: C, 65.00; H, 5.28; N, 3.61. Found: C, 64.73; H, 5.25; N, 3.83.

3.17. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-6-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (26)

To a solution of benzylidene acetal **25** (1.38 g, 1.2 mmol) in dry THF (75 mL) were added $Me_3N{\cdot}BH_3$ (0.35 g, 4.7 mmol) and $AlCl_3$ (0.94 g, 7.0 mmol), then water (46 μL, 2.6 mmol) was added to the resulting mixture at +4 °C. The reaction mixture was stirred at rt for 20 h, then 1 M HCl (500 mL) and water (500 mL) were added. The mixture was extracted with EtOAc (3 × 170 mL), and the combined extracts were washed with satd aq $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated. Pure **26** (1.26 g, 91%) was isolated as a syrup by silica gel column chromatography (toluene–EtOAc, 80:12 → 80:16), R_f 0.22 (petroleum ether–EtOAc, 7:3), $[\alpha]_D$ 100 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.08–7.20 (30H, m, Ar); 5.10 (1H, d, J_{gem} = 11.2 Hz, PhCH_2); 4.79 (1H, d, J_{gem} = 11.1 Hz, PhCH_2); 4.65 (1H, d, J_{gem} = 11.9 Hz, PhCH_2); 4.58 (1H, d, J_{gem} = 12.0 Hz, PhCH_2); 3.41 (3H, s, CH$_3$O), 2.11 (3H, s, CH$_3$CO). ^{13}C NMR (150 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.6 (CH$_3$CO); 165.7; 165.3; 165.0; 164.7 (PhCO); 138.1; 137.0 (*ipso*-C, PhCH, PhCH$_2$); 133.2; 133.1; 133.0; 132.9 (*ipso*-C, Bz); 129.9; 12.7; 129.6; 129.3; 129.2; 129.1; 128.5; 128.4; 128.2; 128.1; 128.0; 127.7 (Ph); 75.5, 73.6 (PhCH$_2$), 20.8 (COCH$_3$). Anal. Calcd for $C_{63}H_{63}N_3O_{19}$: C, 64.88; H, 5.45; N, 3.60. Found: C, 64.65; H, 5.59; N, 3.56.

3.18. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-[6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (28)

Dry toluene (2 × 5 mL) was evaporated from a mixture of donor **3** (0.68 g, 0.93 mmol) and acceptor **26** (0.98 g, 0.84 mmol), the residue was dissolved in dry CH_2Cl_2 (30 mL) and mol. sieve AW-300 (3.5 g) was added. The mixture was stirred for 1 h at rt, cooled to −30 °C, and TMSOTf (10 μL, 0.055 mmol) was added. Stirring was continued for 1.5 hours, and then the reaction was quenched by adding di-isopropylamine (0.2 mL). The mixture was diluted with CH_2Cl_2 (30 mL), filtered through a pad of Celite, and the filtrate was washed with satd aq $NaHCO_3$ and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). Combined organic solutions were concentrated and the residue was subjected to chromatographic purification (toluene–CH_3CN, 19:1 → 17:1) to yield tetrasaccharide **27** (1.22 g, 90%) as an anomeric mixture, R_f 0.42 (toluene–CH_3CN, 10:1). To a solution of **27** in a mixture of CH_2Cl_2 (35 mL) and MeOH (35 mL) was added methanolic hydrogen chloride (35 mL) prepared by addition of AcCl (8 mL) to MeOH (35 mL). After being kept for 3 h at rt, the mixture was diluted with CH_2Cl_2 (150 mL) and washed with satd aq $NaHCO_3$ solution. Aqueous layer was extracted back with CH_2Cl_2 (3 × 70 mL), and the combined organic solutions were concentrated. The residue was firstly purified by conventional silica gel column chromatography (toluene–CH_3CN, 30:4), and then the anomers were separated by preparative HPLC on a 5 μ silica gel column (250 × 21.2 mm) in toluene–CH_3CN (60:4) to provide pure α-tetrasaccharide **28** (0.96 g, 76% over two steps) as a syrup, R_f 0.23 (toluene–CH_3CN, 15:1), $[\alpha]_D$ 36.2 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.12–7.18 (55H, m, Ar); 5.11 (1H, m, PhC*H*$_2$); 5.05–5.01; 4.97–4.91; 4.87–4.84; 4.71 (m, PhC*H*$_2$); 3.39 (3H, s, CH_3O); 3.13 (1H, br d, OH). ^{13}C NMR (150 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 166.3; 165.7; 165.1; 164.5 (Ph*C*O); 138.6; 138.4; 138.3; 138.1; 138.0 (*ipso*-C, $PhCH_2$); 133.1; 133.0; 132.8; 132.6 (*ipso*-C, Bz); 130.4; 129.9; 129.8; 129.6; 129.3; 128.5; 128.4; 128.3; 128.2; 128.1; 128.0; 127.6; 127.4 (Ph), 75.7, 75.3, 74.9, 74.1 ($PhCH_2$), 55.3 (*C*H_3O). Anal. Calcd for $C_{95}H_{93}N_3O_{24}$: C, 68.70; H, 5.64; N, 2.53. Found: C, 68.52; H, 5.80; N, 2.71.

3.19. Methyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-[3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-[6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (29)

A solution of donor **2** (404.0 mg, 0.66 mmol) and acceptor **28** (794 mg, 0.48 mmol) in CH_2Cl_2 (17 mL) was added to powdered mol. sieve AW-300 (2.3 g), the resulting mixture was stirred at rt for 1 h, and then cooled to −20 °C. A solution of TMSOTf (25 μL, 0.07 mmol), prepared by mixing of 25 μL TMSOTf and 0.5 mL of CH_2Cl_2, was added. The same solution was added three times (each time 10 μL) within next 3 h. When TLC revealed full conversion of acceptor **28**, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and filtered through a pad of Celite. The filtrate was washed with satd aq $NaHCO_3$ and concentrated. The residue was purified by silica gel column chromatography (toluene–CH_3CN, 16:1). The isolated mixture (0.83 g) of pentasaccharide anomers was separated by preparative HPLC on a 5 μ silica gel column (250 × 21.2 mm) in toluene–CH_3CN (735:40) to yield pure α-isomer **29** (0.80 g, 80%) as a syrup, R_f 0.16 (toluene–CH_3CN, 3:0.2), $[\alpha]_D$ 82.1 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, for the signals of sugar ring protons see Table 1): δ 8.09–7.16 (50H, m, Ar); 5.07–4.96 (3H, m, H-1^I, PhC*H*$_2$), 4.92–4.80 (7H, m, PhC*H*$_2$, H-1^{II}, H-1^V); 4.67, 4.54–4.44 (6H, m, H-5^V, PhC*H*$_2$), 3.48 (OCH_3), 2.05–2.00 (6H, 2 s, 2 CH_3CO). ^{13}C NMR (125 MHz, $CDCl_3$, for the signals of sugar ring carbons see Table 2): δ 170.5, 169.5 (CH_3CO); 166.1, 165.7, 165.0, 164.5, 164.3 (Ph*C*O); 138.6, 138.4, 138.3, 137.7, 137.4 (*ipso*-C, Bz); 133.1, 133.0, 132.8, 132.7, 132.6 (*ipso*-C, $PhCH_2$); 130.3, 129.7, 129.6, 129.3, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.3 (Ph); 75.7, 75.4, 74.8, 74.0, 73.8, 72.8, 72.1 ($PhCH_2$); 55.2 (OCH_3); 21.0, 20.7 (*C*H_3CO). Anal. Calcd for $C_{119}H_{119}N_3O_{31}$: C, 68.48; H, 5.75; N, 2.01. Found: C, 68.40; H, 5.83; N, 2.10.

3.20. Methyl α-L-rhamnopyranosyl-(1→3)-[2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→6)]-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-amino-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (31)

To a solution of **29** (495 mg, 0.24 mmol) in a mixture of CH_2Cl_2 (10 mL) and MeOH (10 mL) was added 1 M methanolic MeONa (3 mL). After being kept for 24 h, the mixture was neutralized with Amberlyte IRA-120 (H^+). The resin was filtered off, washed with MeOH, and the combined filtrates were concentrated. Silica gel column chromatography (toluene–EtOH, 17:1) of the residue provided **30** (109.5 mg, 91%), R_f 0.2 (toluene–EtOH, 10:1). Deacylated pentasaccharide **30** was dissolved in a mixture of CH_3CN (2.0 mL) and water (0.4 mL), and then di-isopropylamine (100 μL) and DTT (30.3 mg, 0.196 mmol) were added. The mixture was kept at rt until complete transformation of the starting azide, the solvents were evaporated, and pure amine **31** (92.7 mg, 86%) was isolated as a syrup by silica gel column chromatography ($CHCl_3$–MeOH, 50:1 → 10:1), R_f 0.29 ($CHCl_3$–MeOH, 7:1), $[\alpha]_D$ 77.0 (*c* 1, MeOH). ^1H NMR (600 MHz, CD_3OD, for the signals of sugar ring protons see Table 1): δ 7.42–7.09 (30H, m, Ar), 4.93–4.89, 4.84–4.63, 4.59–4.53, 4.39–3.99 (PhC*H*$_2$), 3.38 (OCH_3). ^{13}C NMR (150 MHz, CD_3OD, for the signals of sugar ring carbons see Table 2): δ 139.9, 139.7, 139.5 (*ipso*-C, Ph), 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6 (Ph), 78.8, 76.3, 76.1, 75.8, 75.6, 74.7, 74.6, 74.4, 74.0 (PhCH_2 and C4, C5 of β-D-Glc, C4 of α-L-Rha), 55.8 (OCH_3). ESI HRMS: $[M+H]^+$ calcd for $C_{80}H_{97}NO_{24}$ + H: 1456.6479, found 1456.6473.

3.21. Methyl α-L-rhamnopyranosyl-(1→3)-[2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→6)]-4-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-galactopyranoside (32)

Azide **30** (74.0 mg, 0.05 mmol) was reduced with DTT as described in the previous experiment, the reaction mixture was concentrated, and toluene (2 × 2.0 mL) was evaporated from the residue. A solution of the residue in MeOH (1.0 mL) was treated with Ac_2O (9.4 μL, 0.10 mmol) and Et_3N (7.0 μL, 0.05 mmol) for 1 h, then the resulting mixture was taken to dryness. Column chromatography (toluene–EtOH, 60:1 → 10:1) of the residue gave acetamide **32** (63.1 mg, 84% over two steps from azide **30**) as a syrup, R_f 0.18 (toluene–EtOH, 10:1). $[\alpha]_D$ 71.6 (*c* 1, MeOH), ^1H NMR (600 MHz, CD_3OD, for the signals of sugar ring protons see Table 1): δ 7.42–7.18 (30H, m, Ar), 4.91, 4.89, 4.83 (3H, 3 d, PhC*H*$_2$), 4.76–4.54 (6H, m, H-1^I, PhC*H*$_2$, H-2^1), 4,40 (1H, s, PhC*H*$_2$), 4,28 (1H, d, J_{gem} = 11,1 Hz, PhC*H*$_2$), 4,23 (1H, d, J_{gem} = 11,1 Hz, PhC*H*$_2$); 3.34 (OCH_3); 1.95 (CH_3CON). ^{13}C NMR (150 MHz, CD_3OD, for the signals of sugar ring carbons see Table 2): δ 174.4 (CO); 140.2, 120.3, 139.9, 139.8, 139.6, 139.5 (*ipso*-C, $PhCH_2$); 129.6, 129.5, 129.4, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5 (Ph); 76.4, 76.2, 76.0, 75.9, 74.6, 74.4, 74.2 (PhCH_2); 55.7 (OCH_3); 23.0 (CH_3CON). ESI HRMS: $[M+Na]^+$ calcd for $C_{82}H_{99}NO_{25}$ + Na 1520.6404, found 1520.6415.

3.22. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)] -β-D-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-α-D-galactopyranoside (1a)

Partially benzylated derivative **32** (61.2 mg, 0.041 mmol) was subjected to catalytic hydrogenolysis in MeOH (3 mL) in the presence of $Pd(OH)_2$/C (33 mg). When the reaction was complete, the mixture was filtered through a pad of Celite, the catalyst was washed with MeOH, and the resulting filtrate was concentrated. Product **1a** (35.0 mg, 99%) was isolated by reversed phase C18 HPLC (250 × 10 mm column) in H_2O–CH_3CN (99:1), R_f 0.23 (*n*-butanol–EtOH–H_2O–NH_4OH, 5:5:4:1)as an amorphous powder, $[\alpha]_D$ 87.3 (*c* 1, H_2O). ^1H NMR (600 MHz, D_2O, for the signals of sugar ring protons see Table 1): δ 3.42 (OCH_3); 2.02 (CH_3CON). ^{13}C NMR (150 MHz, D_2O, for the signals of sugar ring carbons see Table 2): δ 176.1 (CO), 50.5 (OCH_3), 23.6 (CH_3CO). ESI HRMS: $[M+Na]^+$ calcd for $C_{33}H_{57}NO_{25}$ + Na 890.3117, found 890.3112.

3.23. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)] -β -D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-[*N*-(*tert*-butoxycarbonyl)-L-alanylamino]-2-deoxy-α-D-galactopyranoside (35)

Azide **30** (22.6 mg, 0.015 mmol) was reduced with DTT as described for **31**, the reaction mixture was concentrated and coevaporated twice with toluene (1.0 mL). To a solution of the residue in dry DMF (0.5 mL) alanine active ester **33** (6.5 mg, 0.023 mmol) and Et_3N (3 μL, 0.022 mmol) were added. After 2.5 h and 24 h, more active ester **33** (4 mg each time) was added. When the reaction was complete, DMF was evaporated in vacuum of an oil pump and its traces were removed by coevaporation with toluene. Product **34** (22.2 mg, 91% for two steps) was isolated by silica gel column chromatography (toluene–EtOH, 50:1), R_f 0.29 (toluene–EtOH, 8.5:1). ^1H NMR (600 MHz, CD_3OD, for the signals of sugar ring protons see Table 1): 4.09 (1H, q, CH of Ala), 3.35 (3H, s, OCH_3), 1.44 (9H, s, $(CH_3)_3CO$), 1.30 (3H, d, CH_3 of Ala). ^{13}C NMR (150 MHz, CD_3OD, for the signals of sugar ring carbons see Table 2): δ 55.9 (OCH_3), 51.6 (CHNH of Ala), 18.1 (CH_3 of Ala). Compound **34** (22.2 mg, 0.0137 mmol) was subjected to catalytic hydrogenolysis as described for **1a**. Purification by reversed phase C18 HPLC (250 × 10 mm column) in H_2O–CH_3CN (93:7) afforded **35** (12.0 mg, 88%) as an amorphous powder, R_f 0.20 (*n*-butanol–EtOH–H_2O–NH_4OH, 5:5:4:1), $[\alpha]_D$ 73.6 (*c* 1, H_2O). ^1H NMR (600 MHz, D_2O, for the signals of sugar ring protons see Table 1): δ 4.13 (1H, m, CH of Ala), 3.44 (3H, s, OCH_3), 1.47 (9H, s, $(CH_3)_3CO$), 1.36 (3H, d, $J_{CH3,CH}$ = 7.2 Hz, CH_3 of Ala). ^{13}C NMR (150 MHz, D_2O, for the signals of sugar ring carbons see Table 2): δ 55.9 (OCH_3), 52.1 (CHNH of Ala), 29.2 ($(CH_3)_3CO$), 18.4 (CH_3 of Ala). ESI HRMS: $[M+H]^+$ calcd for $C_{39}H_{68}N_2O_{27}$ + H 997.4088, found 997.4085.

3.24. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)] -β-D-glucopyranosyl-(1→3)-[α-D-D-glucopyranosyl-(1→4)]-2-(L-alanylamino)-2-deoxy-α-D-galactopyranoside (1b)

Anhydrous TFA (2.0 mL) was added to lyophilized and dried over P_2O_5 **35** (44.1 mg, 0.044 mmol). The reaction mixture was kept at 22 °C for 4.5 min, diluted with toluene (2.0 mL) and concentrated in vacuum at the bath temperature <35 °C. The residue was coevaporated with toluene (2 × 2 mL), dried under vacuum using an oil pump, and lyophilized from water to provide amine **1b** (44.0 mg, 99%) as an amorphous powder, R_f 0.26 (*n*-butanol–EtOH–H_2O–NH_4OH, 5:5:4:1), $[\alpha]_D$ 62.6 (*c* 1, H_2O). ^1H NMR (600 MHz, D_2O, for the signals of sugar ring protons see Table 1): δ 4.08 (1H, m, CH of Ala), 1.57 (3H, d, $J_{CH3,CH}$ = 6,2 Hz, CH_3 of Ala). ^{13}C NMR (150 MHz, D_2O, for the signals of sugar ring carbons see Table 2): 172.5 (CO of Ala), 50.9 (CH of Ala), 18.0 (CH_3 of Ala). ESI HRMS: $[M+Na]^+$ calcd for $C_{34}H_{60}N_2O_{25}$ + Na 919.3383, found 919.3374.

3.25. Methyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-2-(*N*-acetyl-L-alanylamino)-2-deoxy-α-D-galactopyranoside (1c)

Derivative **1c** (26.0 mg, 80%) was prepared by N-acetylation of **1b** (34.9 mg, 0.035 mmol) as described for **1a**. Purification of the product was accomplished by gel-permeation chromatography on a TSK HW-40(S) column in 0.1 M AcOH. For **1c** R_f 0.31 (*n*-butanol–EtOH–H_2O–NH_4OH, 5:5:4:1), $[\alpha]_D$ 62.0 (*c* 1, H_2O). ^1H NMR (600 MHz, D_2O, for the signals of sugar ring protons see Table 1): δ 4.32 (1H, m, $J_{CH,CH3}$ = 7,2 Hz, CH of Ala), 4.71 (OCH_3), 2.04 (3H, s, CH_3CON), 1.39 (3H, d, $J_{CH3,CH}$ = 7,1 Hz, CH_3 of Ala). ^{13}C NMR (150 MHz, D_2O, for the signals of sugar ring carbons see Table 2): δ 177.0 (CH_3CO); 175.5 (CO of Ala); 51.4 (CH of Ala); 48.2 (OCH_3); 23.2 (CH_3CON); 18.0 (CH_3 of Ala). ESI HRMS: $[M+Na]^+$ calcd for $C_{36}H_{62}N_2O_{26}$ + Na 961.3494, found 961.3489.

Acknowledgments

The authors are grateful to Dr. A. A. Grachev and Dr. A. O. Chizhov for recording of NMR and mass spectra respectively.

References

1. Campodonico, V. L.; Gadjeva, M.; Paradis-Bleau, C.; Uluer, A.; Pier, G. B. *Trends Mol. Med.* **2008**, *14*, 120–133.
2. Ratjen, F.; Döring, G. *Lancet* **2003**, *361*, 681–689.
3. Pier, G. B. *Curr. Opin. Microbiol.* **2002**, *5*, 81–86.
4. Li, Z.; Kosorok, M. R.; Farrell, P. M.; Laxova, A.; West, S. E.; Green, C. G.; Collins, J.; Rock, M. J.; Splaingard, M. L. *JAMA* **2005**, *293*, 581–588.
5. Pier, G. B.; Grout, M.; Zaidi, T. S.; Olsen, J. C.; Johnson, L. G.; Yankaskas, J. R.; Goldberg, J. B. *Science* **1996**, *271*, 64–67.
6. Pier, G. B.; Grout, M.; Zaidi, T. S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12088–12093.
7. Collins, F. S. *Science* **1992**, *256*, 774–779.
8. Bystrova, O. V.; Lindner, B.; Moll, H.; Kocharova, N. A.; Knirel, Y. A.; Zähringer, U.; Pier, G. B. *Carbohydr. Res.* **2003**, *338*, 1895–1905.
9. Zaidi, T. S.; Fleiszig, S. M. J.; Preston, M. J.; Goldberg, J. B.; Pier, G. B. *Invest. Ophthalmol. Vis. Sci.* **1996**, *37*, 976–986.
10. Komarova, B. S.; Tsvetkov, Y. E.; Pier, G. B.; Nifantiev, N. E. *J. Org. Chem.* **2008**, *73*, 8411–8421.
11. Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35–79.
12. Demchenko, A. V. *Synlett* **2003**, 1225–1240.
13. Koto, S.; Morishima, N.; Kihara, Y.; Suzuki, H.; Kosugi, S.; Zen, S. *Bull. Chem. Soc. Jpn.* **1982**, *56*, 188–191.
14. Kronzer, F. J.; Schuerch, C. *Carbohydr. Res.* **1973**, *27*, 379–390.
15. Flowers, H. M. *Carbohydr. Res.* **1971**, *18*, 211–218.
16. van den Bos, L. J.; Codée, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165–2168.
17. Eby, R.; Schuerch, C. *Carbohydr. Res.* **1974**, *34*, 79–90.
18. Gerbst, A. G.; Ustuzhanina, N. E.; Grachev, A. A.; Khatuntseva, E. A.; Tsvetkov, D. E.; Whitfield, D. M.; Berces, A.; Nifantiev, N. E. J. *Carbohydr. Chem.* **2001**, *20*, 821–831.
19. Krylov, V.; Ustyuzhanina, N.; Grachev, A.; Bakker, H.; Nifantiev, N. *Synthesis* **2007**, *20*, 3147–3154.
20. Chiba, S.; Kitamura, M.; Narasaka, K. *J. Am. Chem. Soc.* **2006**, *128*, 6931–6937.
21. De Meo, C.; Kamat, M. N.; Demchenko, A. V. *Eur. J. Org. Chem.* **2005**, 706–711.
22. Crich, D.; Hu, T.; Cai, F. *J. Org. Chem.* **2008**, *73*, 8942–8953.
23. Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 17705–17713.
24. Gerbst, A. G.; Ustuzhanina, N. E.; Grachev, A. A.; Tsvetkov, D. E.; Khatuntseva, E. A.; Nifant'ev, N. E. *Mendeleev Commun.* **1999**, 114–116.
25. Zlotina, N. S.; Ustuzhanina, N. E.; Grachev, A. A.; Gerbst, A. G.; Nifantiev, N. E. J. *Carbohydr. Chem.* **2008**, *27*, 429–445.
26. Komarova, B. S.; Tsvetkov, Y. E.; Knirel, Y. A.; Zähringer, U.; Pier, G. B.; Nifantiev, N. E. *Tetrahedron Lett.* **2006**, *47*, 3583–3587.
27. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.
28. Duynstee, H. I.; de Koning, M. C.; Ovaa, H.; van der Marel, G. A.; van Boom, J. H. *Eur. J. Org. Chem.* **1999**, 2623–2632.
29. Verduyn, R.; Douwes, M.; van der Klein, P. A. M.; Moesinger, E. M.; Marel, G. A.; van Boom, J. H. *Tetrahedron* **1993**, *49*, 7301–7316.
30. Hiroshi, T.; Tetsuya, K.; Takashi, T.; Yoshiyuki, A.; Naohito, O. *Chem. Commun.* **2010**, *46*, 8249–8251.

31. Rising, T. W. D. F.; Claridge, T. D. W.; Davies, N.; Gamblin, D. P.; Moir, J. W. B.; Fairbanks, A. J. *Carbohydr. Res.* **2006**, *341*, 1574–1596.
32. Johnsson, R.; Olsson, D.; Ellervik, U. *J. Org. Chem.* **2008**, *73*, 5226–5237.
33. Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51–65.
34. Sherman, A. A.; Mironov, Y. V.; Yudina, O. N.; Nifantiev, N. E. *Carbohydr. Res.* **2003**, *338*, 697–703.
35. Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8–11.
36. Boer, J.; Blount, K. F.; Luedtke, N. W.; Elson-Schwab, L.; Tor, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 927–932.
37. Kleinert, M.; Röckendorf, N.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2004**, 3931–3940.
38. Lindhorst, T. K.; Kötter, S.; Krallmann-Wenzel, U.; Ehlers, S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 823–831.
39. Belyakov, P. A.; Kadentsev, V. I.; Chizhov, A. O.; Kolotyrkina, N. G.; Shashkov, A. S.; Ananikov, V. P. *Mendeleev Commun.* **2010**, *20*, 125–131.