Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 1312–1321
Carbohydrate RESEARCH

Preparation of the pentasaccharide hapten of the GPL of *Mycobacterium avium* serovar 19 by achieving the glycosylation of a tertiary hydroxyl group

Anikó Fekete,[a] Katalin Gyergyói,[a] Katalin E. Kövér,[b] István Bajza[a] and András Lipták[a,c,*]

[a]*Research Group for Carbohydrates of the Hungarian Academy of Sciences, PO Box 94, H-4010 Debrecen, Hungary*
[b]*Institute of Inorganic and Analytical Chemistry, University of Debrecen, PO Box 21, H-4010 Debrecen, Hungary*
[c]*Institute of Biochemistry, University of Debrecen, PO Box 55, H-4010 Debrecen, Hungary*

Received 24 January 2006; received in revised form 13 April 2006; accepted 20 April 2006
Available online 18 May 2006

Dedicated to Professor Dr. Richard R. Schmidt on the occasion of his 70th birthday

Abstract—The chemical synthesis of the glycopeptidolipid-type pentasaccharide hapten of *Mycobacterium avium* serovar 19 with a trifluoroacetamido spacer at the reducing end is described. The spacer-armed pentasaccharide **31**, when conjugated to an immunogenic protein, can be applied to the serodiagnosis of mycobacterial infections. The questionable structure of the penultimate monosaccharide unit was clarified as 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-L-mannopyranose. The occurrence of the 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-L-talopyranose could be excluded by the presence of the large H-1′–H-2′ coupling constant, which proves the $^4C_1$ (L) conformation as the favoured one.

Keywords: Mycobacteria; Hapten; Oligosaccharides

1. Introduction

The cell-surface antigen of *Mycobacterium avium* serovar 19 consists of a pentasaccharide chain and a peptidolipid aglycone moiety, which is anchored in the cell membrane.[1] Some mycobacteria are the so-called opportunistic microorganisms and because they cause serious infections among the immunocompromised persons, mainly among the victims of AIDS, fast and reliable serodiagnosis of the infections is an important clinical task. These immunological events are determined by the carbohydrate part of the cell-surface antigens and thus the synthesis of these outer chains, or nonreducing oligosaccharide fragments of them, are also of interest.

The structural elucidation[2] of the pentasaccharide (**1**, Fig. 1) left one question open: the stereochemistry at C-4 of the fourth monosaccharide unit. It could have either the L-*rhamno*- or 6-deoxy-L-*talo*-configuration. The other delicate structural characteristic of the pentasaccharide is that the tertiary hydroxyl group is glycosylated. To the best of our knowledge, this is the first example of the existence of such a structural unit among naturally occurring oligosaccharides and no synthetic methodology has been developed for its preparation.

2. Results and discussion

Our intention was to prepare the pentasaccharide using a 3+2 block strategy (Scheme 1). Earlier we have reported the synthesis of the two possible penultimate units, namely methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-mannopyranoside (**2**) and its C-4 epimer methyl 6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside

* Corresponding author. Tel.: +36 52 512900/22256; fax: +36 52 512913; e-mail: liptaka@puma.unideb.hu

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.04.038

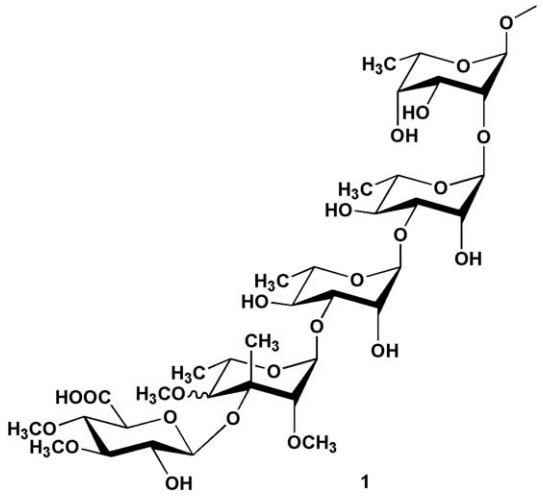


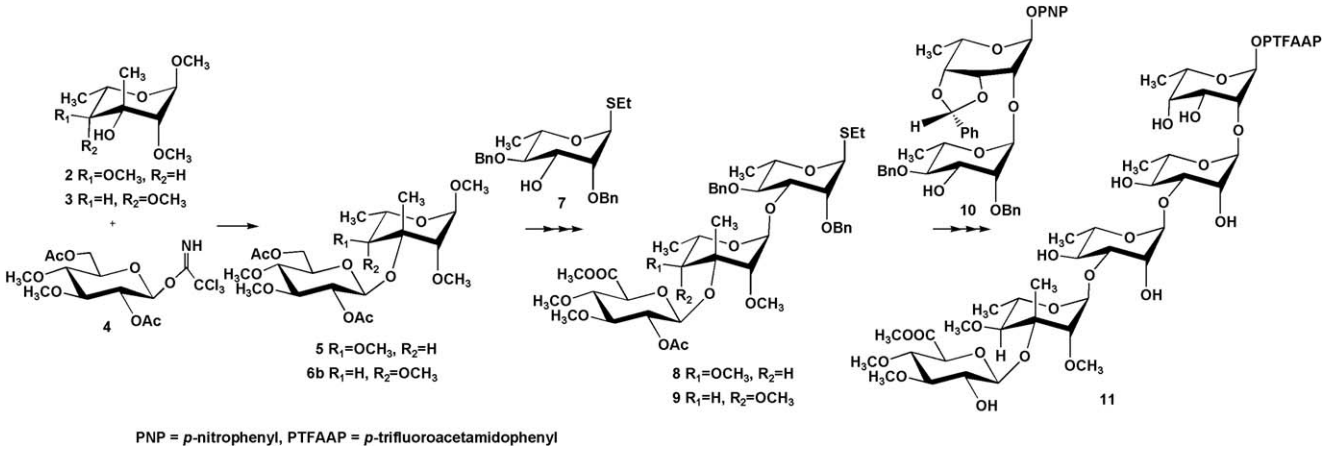
Figure 1. Structure of the pentasaccharide hapten of the serovariant 19 of the *Mycobacterium avium* complex.

(**3**),[3] the thioglycoside acceptor **7**, and the Rha*p*→6-deoxy-Tal*p* (**10**) disaccharide glycosyl acceptor.[4]

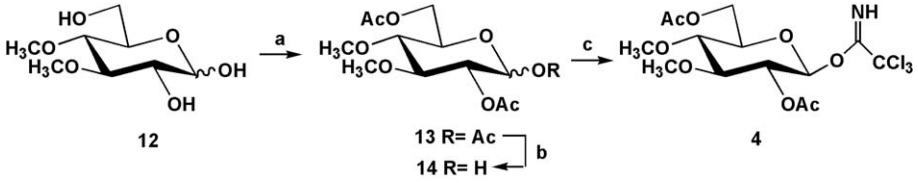
For the synthesis of the disaccharides at the nonreducing end, we used 2,6-di-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl trichloroacetimidate (**4**) as the glycosyl donor, which was prepared as follows (Scheme 2). Treatment of the 3,4-di-*O*-methyl-D-glucopyranose (**12**)[5] with acetic anhydride in pyridine gave the triacetate **13**. The anomeric acetyl group was selectively deprotected with hydrazine acetate in DMF to yield hemiacetal **14**. The trichloroacetimidate **4** was obtained from **14** using standard procedures.[7] We used TMSOTf[7] for the activation of the trichloroacetimidate **4** (Scheme 3). Thus, reaction of the donor **4** with acceptor **2** resulted in the disaccharide **5**, containing a β-interglycosidic linkage, in a yield of 42%.

However, when this reaction was attempted in the case of acceptor **3**, we isolated two products (Scheme 4). After NMR spectroscopic investigations, it was evident that both were disaccharides, one with the α (**6a**, 25%) and the other with the desired β (**6b**, 34%) interglycosidic linkage, despite the presence of the participating substituent at C-2. In addition to the well-known factors (the type of the leaving group at the anomeric centre, the promoter, the substituents on the carbohydrates, the solvent and the temperature) unfavourable steric interactions between the glycosyl donor and the acceptor may influence the stereochemical outcome (α/β ratio) of glycosylations.[9] In our case, acceptor **3** is an extremely overcrowded compound in which four rather bulky substituents are present in a 1,3-*cis*-diaxial arrangement.

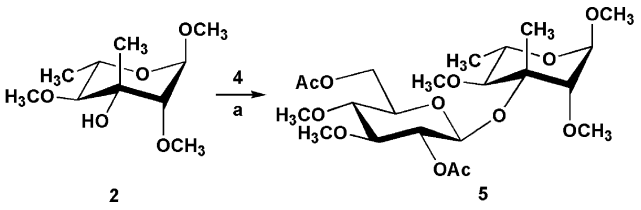
We observed another structural feature of compounds **6a** and **6b**: the change in conformation of the 6-deoxy-Tal*p* unit. Having measured the ^{1}H–^{1}H coupling constants, it was clear that the ring possessed the $^{4}C_{1}$ (L), not $^{1}C_{4}$ (L), conformation. For the explanation of this phenomenon, we synthesized each of the methyl ethers of the two sugars and compared their



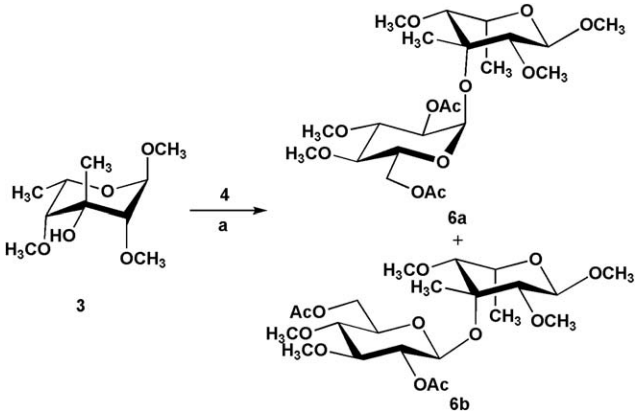
Scheme 1. Synthetic plan for target pentasaccharide **11**.



Scheme 2. Reagents and conditions: (a) Ac_2O, pyridine, rt, 86%; (b) hydrazine acetate, DMF, rt, 92%; (c) trichloroacetonitrile, K_2CO_3, CH_2Cl_2, rt, 61%.



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −50 °C, 42%.



Scheme 4. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −50 °C, 25% for **6a** and 34% for **6b**.

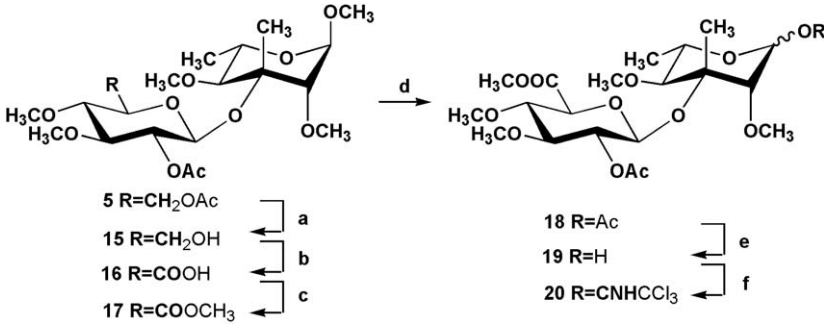
conformational properties. All of the methyl 6-deoxy-3-*C*-methyl-α-L-mannopyranoside derivatives adopt the $^1C_4$ (L) conformation. The situation in the case of the methyl 6-deoxy-3-*C*-methyl-α-L-talopyranoside derivatives is different: for the fully substituted glycosides the $^4C_1$ (L) conformation is predominant. However, it is to be noted that all of the mono- and disubstituted talopyranoside derivatives exist exclusively in the $^1C_4$ (L) conformation.[10]

The next step in our strategy was oxidation at the position 6′ in disaccharides **15** and **21** (Scheme 5). Thus compound **5** was treated with catalytic sodium methoxide in methanol to obtain the selectively 6-O-deprotected **15**,[11] which was oxidized using Jones' conditions[12] to yield the glucuronic acid **16** that was converted to methyl ester **17**. For the further transformation of **6b**, we had to choose another sequence: we used TEMPO-mediated selective oxidation[13] of the diol **21** (Scheme 6). Treatment of **6b** with catalytic sodium methoxide showed no selectivity in spite of the fact that the 2′-OAc was isolated and a bulky aglycone was also present.[11] Thus, treatment of **21** with TEMPO/NaOCl gave **22**, which was transformed to its methyl ester **23** with methyl iodide in DMF.

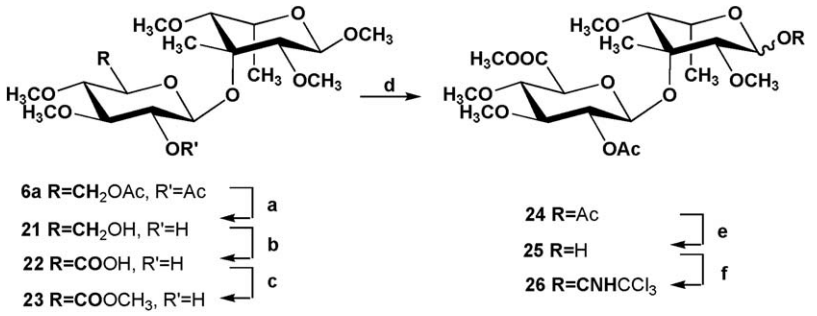
Because there were substantial differences in the NMR data of compounds **5**, **6b** and **17**, **23**, the comparison of the spectra of our two synthetic disaccharides with the spectrum of the native saccharide could eliminate the uncertainty in the structure. However, one more question had to be answered: does the conformation of the 6-deoxy-Tal*p* unit change when the methyl aglycone is replaced by a sugar ring?

Disaccharides **17** and **23** were thus transformed into glycosyl donors **20** and **26**, respectively, through acetolysis of the methyl aglycone (→**18**, →**24**). This reaction had to be carried out under mild conditions to prevent the cleavage of the interglycosidic bond. For the selective anomeric deprotection of **19**, hydrazine acetate[14] was used; however, applying the same conditions for acetate **24** gave the hemiacetal **25** in low yield. Of several other methods for the selective anomeric deacetylation, the use of dibutyltin oxide[15] proved to be the best. In the next steps, glycosyl trichloroacetamidates **20** and **26** were prepared under standard conditions[7] from hemiacetals **20** and **25**. The TMSOTf-activated glycosylation of the thioglycoside acceptor **7**[4] with **20** and **26** resulted in trisaccharides **8** (35%) and **9** (26%), respectively (Scheme 7).

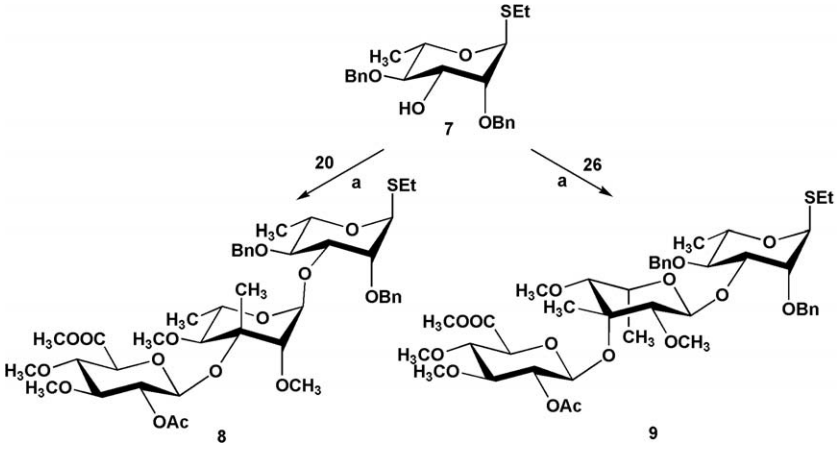
The NMR data of these two trisaccharides are listed in Table 1. It should be noted that compounds having the 6-deoxy-Tal*p* unit as a building block were more sensitive than their Rha*p*-analogues and decomposed on standing. Because it was evident from the NMR data of **9** that the replacement of the methyl aglycone by a sugar residue has no effect on the conformation of the 6-deoxy-Tal*p* unit, and because the NMR data for



Scheme 5. Reagents and conditions: (a) $NaOCH_3$, CH_3OH, rt, 86%; (b) CrO_3, 3.5 M H_2SO_4, acetone; (c) CH_2N_2 in Et_2O, CH_2Cl_2, rt, 64% for two steps; (d) Ac_2O, AcOH, TFA, 60 °C, 67%; (e) hydrazine acetate, DMF, rt, 82%; (c) trichloroacetonitrile, K_2CO_3, CH_2Cl_2, rt, 63%.



Scheme 6. Reagents and conditions: (a) $NaOCH_3$, CH_3OH, rt, 95%; (b) TEMPO, NaOCl, KBr, $NaHCO_3$; (c) CH_3I, DMF, 0 °C to rt, 71% for two steps; (d) Ac_2O, AcOH, TFA, 60 °C, 64%; (e) *n*-Bu_2SnO, CH_3OH, 50 °C, 80%; (c) trichloroacetonitrile, K_2CO_3, CH_2Cl_2, rt, 82%.



Scheme 7. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −50 °C, 35% for **8** and 26% for **9**.

8 showed good agreement with the literature data,[2] we concluded that the natural saccharide had the penultimate sugar unit with Rha*p* configuration.

Therefore, we prepared just one pentasaccharide, **27**, from **8** as the trisaccharide donor and **10**[4] as acceptor using the NIS/TfOH promoter system[6,8] (Scheme 8). The product was obtained in 30% yield. The NMR data of this pentasaccharide is provided in Table 2. The sequence of the deprotection of **27** was the following: the OPNP-aglycone was converted to *p*-trifluoroacetamidophenyl group one through mild reduction under hydrogen in the presence of PtO_2 and amide-formation, then the acetyl group was cleaved and finally catalytic hydrogenation over $Pd(OH)_2$ resulted in the deprotected pentasaccharide **11** (for the NMR data, see Table 2) (Scheme 9).

In conclusion, we have developed a chemical synthesis of a spacer-armed pentasaccharide hapten of the *M. avium* serovar 19. Brennan and co-workers[2] have established the structure of the tetraglycosyl alditol released from GPL antigen of *M. avium* serovariant 19. The ^1H NMR data of the synthetic pentasaccharide and that of the native one showed good agreement. In the anomeric proton signals in the ^1H NMR spectra of the tetraglycosyl alditol obtained from natural sources, there were three ~1 Hz and one 7.75 Hz coupling constants. The last of these coupling constants (7.75 Hz) was assigned to the 3,4-di-*O*-methyl-β-D-glucuronic acid moiety. These anomeric proton signals may prove that the penultimate building block possesses 6-deoxy-L-*manno* (and not 6-deoxy-L-*talo*) configuration.

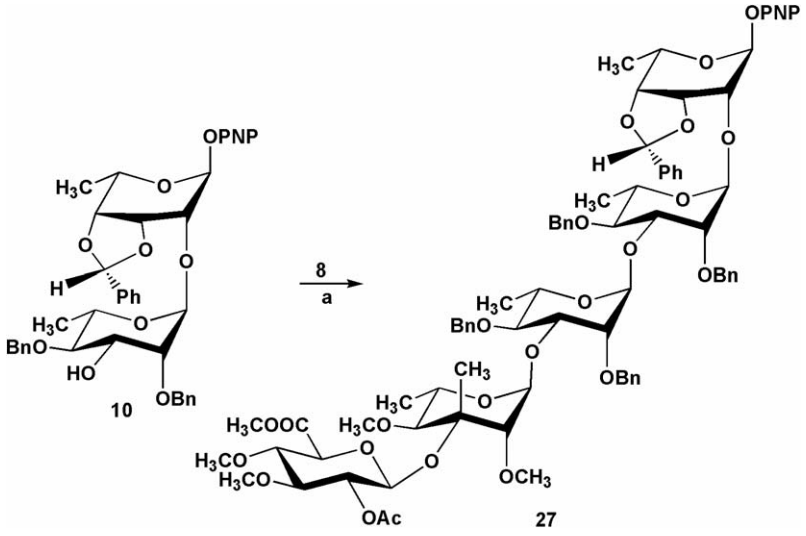
3. Experimental

3.1. General methods

Optical rotations were measured at rt with a Perkin–Elmer 241 automatic polarimeter in $CHCl_3$. Melting points were determined on a Kofler apparatus and are uncorrected. TLC was performed on Kieselgel 60 F254 (Merck) with detection by charring with 50% aqueous sulfuric acid. Column chromatography was performed on Silica Gel 60 (Merck 63–200 mesh). ^1H (200, 360 and 500 MHz) and ^{13}C NMR (50.3, 90.54, 125.76 MHz) spectra were recorded with Bruker WP-200 SY, Bruker AM-360 and Bruker DRX-500 spectrometers. Internal references: TMS (0.000 ppm for ^1H), $CDCl_3$ (77.00 ppm for

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) data of compounds **8** and **9**: δ_H, δ_C (ppm), J (Hz)

	8			**9**	
	δ ^{1}H	δ ^{13}C		δ ^{1}H	δ ^{13}C
Rha*p*			Rha*p*		
1	5.31 ($J_{1,2} = 1.5$)	81.1	1	5.34 ($J_{1,2} = 5$)	81.3
2	3.86 ($J_{2,3} = 3$)	80.9	2	3.86 ($J_{2,3} = 4$)	80.5
3	3.97 ($J_{3,4} = 9.5$)	79.6	3	3.97 ($J_{3,4} = 11.5$)	80.7
4	3.59 ($J_{4,5} = 9.5$)	80.7	4	3.54 ($J_{4,5} = 11.5$)	80.8
5	4.06	68.7	5	4.06	68.8
CH_3	1.27 ($J_{5,6} = 6$)	18.3	CH_3	1.25 ($J_{5,6} = 7$)	17.5
SCH_2CH_3	1.21, 2.60	18.5, 25.5	SCH_2CH_3	1.27, 2.60	17.1, 25.7
3-*C*-Me-Rha*p*			Tal*p*		
1	5.00 ($J_{1,2} = 1.5$)	99.8	1	5.07 ($J_{1,2} = 3$)	100.2
2	3.18	83.8	2	2.96	83.3
3	—		3	—	
4	3.22 ($J_{4,5} = 7$)	84.0	4	2.8 ($J_{4,5} = 3.5$)	84.4
5	3.65	67.7	5	3.91	67.4
CH_3(3)	1.37	16.1	CH_3(3)	1.42	18.1
CH_3(6)	1.21 ($J_{5,6} = 6$)	15.3	CH_3(6)	1.29 ($J_{5,6} = 7$)	15.2
Glc*p*A			Glc*p*A		
1	4.76 ($J_{1,2} = 8$)	95.8	1	4.76 ($J_{1,2} = 9$)	96.0
2	5.02 ($J_{2,3} = 9.5$)	72.6	2	4.99 ($J_{2,3} = 11$)	73.1
3	3.32 ($J_{3,4} = 9.5$)	84.5	3	3.32 ($J_{3,4} = 11$)	84.6
4	3.58 ($J_{4,5} = 9.5$)	80.7	4	3.61 ($J_{4,5} = 12.5$)	80.8
5	3.76	74.6	5	3.77	74.4
$COOCH_3$	3.66	52.4	$COOCH_3$	3.68	52.8



Scheme 8. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, −30 °C, 30%.

^{13}C for organic solutions). Generally, the ^{1}H and ^{13}C NMR assignments have been established from one-dimensional NMR spectra. In a few cases, the proton-signal assignments were supported by analysis of two-dimensional ^{1}H–^{1}H correlation spectra (COSY) and selective TOCSY experiments, as well as the carbon-signal assignments by two-dimensional ^{13}C–^{1}H correlation maps (HETCOR). Elemental analyses were performed at the analytical laboratories in Debrecen. Abbreviations: Ac = acetyl, Bn = benzyl, PNP = *p*-nitrophenyl, PTFAAP = *p*-trifluoroacetamidophenyl.

3.2. 1,2,6-Tri-*O*-acetyl-3,4-di-*O*-methyl-D-glucopyranose (13)

To a solution of 3,4-di-*O*-methyl-D-glucopyranose **12**[5] (3.94 g, 18.94 mmol) in pyridine (50 mL) was added Ac_2O (20 mL). After stirring for 2 h at rt the mixture was concentrated. The residue was diluted with CH_2Cl_2, extracted with aq 1 M HCl and satd aq $NaHCO_3$ solution, dried and concentrated. The crude product was purified by silica column chromatography (7:3, hexane/EtOAc) to yield **13** (5.48 g, 86%) as a colourless syrup: $[\alpha]_D$

Table 2. ^{1}H and ^{13}C NMR data of compounds **27** and **11**: δ_H, δ_C (ppm), J (Hz)

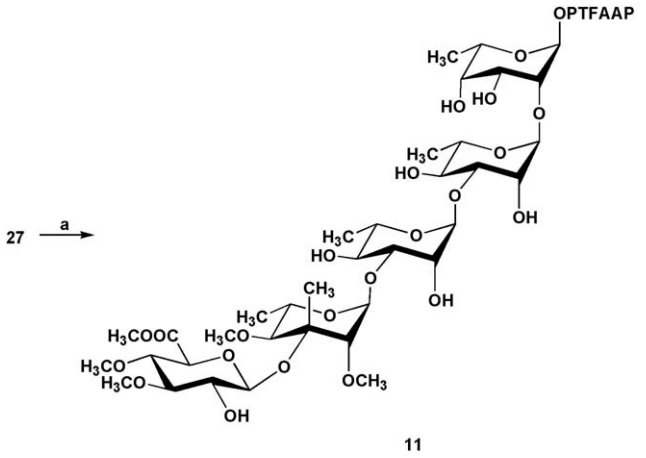
	27[a]		**11**[b]		
	δ ^{1}H	δ ^{13}C		δ ^{1}H	δ ^{13}C
Tal*p*			Tal*p*		
1	5.66 ($J_{1,2}=6$) ($J_{C1,H1}=175.5$)	98.6	1	5.59 ($J_{1,2}=1$)	98.2
2	4.24 ($J_{2,3}=3$)	71.3	2	4.09 ($J_{2,3}=3.5$)	77.0
3	4.60 ($J_{3,4}=8$)	74.9	3	4.20 ($J_{3,4}=3.5$)	65.8
4	4.25 ($J_{4,5}=2$)	76.5	4	3.73 ($J_{4,5}=1$)	71.6
5	4.03	67.8	5	4.17	68.4
CH_3	1.30 ($J_{5,6}=6.5$)	15.8	CH_3	1.16 ($J_{5,6}=6.5$)	15.6
PhCH	5.81				
Rha*p*			Rha*p*		
1	5.05 ($J_{1,2}=1.8$) ($J_{C1,H1}=169.5$)	98.9	1	4.98 ($J_{1,2}=1$)	102.6
2	3.73 ($J_{2,3}=3$)	79.6	2	4.09 ($J_{2,3}=3$)	70.1
3	4.05 ($J_{3,4}=9$)	79.3	3	3.82 ($J_{3,4}=9.5$)	78.2
4	3.50 ($J_{4,5}=9$)	80.5	4	3.48 ($J_{4,5}=9.5$)	71.5
5	3.79	68.9	5	3.81	67.1
CH_3(3)	1.18 ($J_{5,6}=6$)	18.5	CH_3(3)	1.26 ($J_{5,6}=6.5$)	17.1
Rha*p*			Rha*p*		
1	5.03 ($J_{1,2}=2$) ($J_{C1,H1}=169.5$)	95.5	1	5.00 ($J_{1,2}=1$)	102.8
2	3.82 ($J_{2,3}=3$)	78.4	2	4.13 ($J_{2,3}=3$)	70.0
3	3.96 ($J_{3,4}=9$)	78.2	3	3.82 ($J_{3,4}=9.5$)	78.2
4	3.51 ($J_{4,5}=9.5$)	80.6	4	3.53 ($J_{4,5}=9.5$)	71.3
5	3.90	68.8		3.81	69.6
CH_3	1.36 ($J_{5,6}=6$)	18.3	CH_3	1.23 ($J_{5,6}=6.5$)	17.1
3-*C*-Me-Rha*p*			3-*C*-Me-Rha*p*		
1	4.97 ($J_{1,2}=1.5$) ($J_{C1,H1}=169.5$)	99.9	1	5.05 ($J_{1,2}=1$)	98.9
2	3.12	83.8	2	3.53	83.2
3	—		3	—	
4	3.17 ($J_{4,5}=9.5$)	83.9	4	3.78 ($J_{4,5}=9.5$)	69.8
5	3.60	67.6	5	3.14	84.6
CH_3(3)	1.36	16.0	CH_3(3)	1.41	
CH_3(6)	1.17 ($J_{5,6}=6$)	18.5	CH_3(6)	1.23 ($J_{5,6}=6.5$)	17.1
Glc*p*A			Glc*p*A		
1	4.72 ($J_{1,2}=8$) ($J_{C1,H1}=159.5$)	95.7	1	4.78 ($J_{1,2}=7.5$)	96.7
2	5.00 ($J_{2,3}=10$)	72.6	2	3.42 ($J_{2,3}=9.5$)	72.6
3	3.30 ($J_{3,4}=10$)	84.5	3	3.35 ($J_{3,4}=9.5$)	85.2
4	3.56 ($J_{4,5}=10$)	80.6	4	3.43 ($J_{4,5}=9.5$)	80.8
5	3.74	74.5	5	4.05	73.2
COO*CH*$_3$	3.73	52.5	COO*CH*$_3$	3.78	53.4

[a] In $CDCl_3$.
[b] In CD_3OD.

+55.0 (*c* 1.1, $CHCl_3$); **13** (α): ^{1}H NMR ($CDCl_3$): δ 6.21 (d, 1H, $J_{1,2}=3.5$ Hz, H-1), 4.90 (dd, 1H, $J_{2,3}=9.5$ Hz, H-2), 4.31 (dd, 1H, $J_{6,6'}=11.5$ Hz, H-6′), 4.24 (dd, 1H, H-6), 3.48 (ddd, 1H, $J_{5,6'}=2.5$ Hz, $J_{5,6}=4$ Hz, H-5), 3.56, 3.59 (2s, 3-3H, OC*H*$_3$), 3.50 (s, 1H, OH), 3.25, 3.61 (2 dd, 1-1H, $J_{3,4}=8.5$ Hz, $J_{4.5}=9.5$ Hz, H-3, H-4), 2.07, 2.10, 2.15 (3s, 9H, 3CO*CH*$_3$); ^{13}C NMR ($CDCl_3$): δ 168.9, 169.8, 170.6 (3*C*OCH$_3$) 89.5 (C-1), 81.1, 71.2, 71.5, 79.0 (C-2, C-3, C-4, C-5), 62.5 (C-6), 60.8 (×2) (2O*C*H$_3$), 20.7, 20.8 (3CO*C*H$_3$). Anal. Calcd for $C_{14}H_{22}O_9$: C, 50.30; H, 6.63. Found: C, 50.42; H, 6.49.

3.3. 2,6-Di-*O*-acetyl-3,4-di-*O*-methyl-D-glucopyranose (14)

To a solution of compound **13** (600 mg, 1.79 mmol) in dry DMF (20 mL) was added hydrazine acetate (300 mg, 3.24 mmol). After stirring for 4 h at rt the mixture was diluted with CH_2Cl_2, extracted with H_2O, dried and concentrated. The crude product was purified by silica column chromatography (3:2, hexane/EtOAc) to give **14** (480 mg, 92%) as a colourless syrup: **14** (α): ^{1}H NMR ($CDCl_3$): δ 5.35 (d, 1H, $J_{1,2}=3.5$ Hz, H-1), 4.73 (dd, 1H, $J_{2,3}=10$ Hz, H-2), 4.21 (dd, 1H, $J_{6,6'}=12$ Hz, H-6′), 4.36 (dd, 1H, H-6), 4.00 (ddd, 1H, $J_{5,6'}=2$ Hz, $J_{5,6}=4.5$ Hz, H-5), 3.59, 3.53 (2s, 3-3H, OC*H*$_3$), 3.68 (dd, 1H, $J_{3,4}=9$ Hz, H-3), 3.61 (dd, 1H, $J_{4.5}=10$ Hz, H-4), 2.15, 2.11 (2s, 3-3H, 2CO*CH*$_3$); ^{13}C NMR ($CDCl_3$): δ 170.9, 170.4 (2*C*OCH$_3$) 90.3 (C-1), 79.7, 79.5, 73.3, 68.8 (C-2, C-3, C-4, C-5), 63.0 (C-6), 60.9, 60.7 (2O*C*H$_3$), 21.0, 20.9 (2CO*C*H$_3$). Anal. Calcd for $C_{12}H_{20}O_8$: C, 49.31; H, 6.90. Found: C, 49.45; H, 6.82.



Scheme 9. Reagents and conditions: (a) PtO_2, H_2, EtOAc; trifluoroacetic anhydride, pyridine; $NaOCH_3$, CH_3OH; $Pd(OH)_2$, H_2, CH_3OH, 13% for four steps.

3.4. 2,6-Di-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl trichloroacetimidate (4)

To a solution of compound **14** (50 mg, 0.17 mmol) in dry CH_2Cl_2 (5 mL) were added 0.5 mL trichloroacetonitrile (0.5 mL, 5 mmol) and K_2CO_3 (400 mg, 5.05 mmol). After stirring for 1 day at rt, the mixture was diluted with CH_2Cl_2, filtered and concentrated. The residue was purified by silica column chromatography (9:1, hexane/EtOAc containing 1% Et_3N) to give **4** (45 mg, 61%) as a syrup: ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1H, NH), 5.74 (d, 1H, $J_{1,2} = 8$ Hz, H-1), 5.17 (dd, 1H, $J_{2,3} = 8$ Hz, H-2), 4.31 (dd, 1H, $J_{6,6'} = 11.5$ Hz, H-6′), 4.37 (dd, 1H, H-6), 3.64 (ddd, 1H, $J_{5,6'} = 2$ Hz, $J_{5,6} = 4.5$ Hz, H-5), 3.56, 3.54 (2s, 3-3H, OCH_3), 3.36, 3.42 (2 dd, 1-1H, $J_{3,4} = 8$ Hz, $J_{4,5} = 8$ Hz, H-3, H-4), 2.08, 2.10 (2s, 3-3H, 2COCH_3); ^{13}C NMR ($CDCl_3$): δ 95.8 (C-1), 84.3, 78.8, 73.6, 71.5 (C-2, C-3, C-4, C-5), 62.6 (C-6), 60.6, 60.4 (2OCH_3), 20.8 (2COCH_3).

3.5. Methyl 2,6-di-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranoside (5)

The solution of the donor **4** (560 mg, 1.38 mmol) and acceptor **2**[3] (180 mg, 0.8 mmol) in dry CH_2Cl_2 (4 mL) was cooled to −50 °C, then TMSOTf (78 μL, 0.41 mmol) was added dropwise. After stirring for 30 min, Et_3N (100 μL) was added. The mixture was diluted with CH_2Cl_2, extracted with H_2O, dried and concentrated. The crude product was purified by column chromatography (95:5, CH_2Cl_2/acetone) to yield **5** (165 mg, 42%) as a colourless syrup: $[\alpha]_D$ −25.45 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.98 (dd, 1H, $J_{2',3'} = 9.6$ Hz, H-2′), 4.75 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 4.6 (d, 1H, $J_{1,2} = 1.8$ Hz, H-1), 4.37 (dd, 1H, $J_{6'a,6'b} = 11.8$ Hz, H-6′a), 4.22 (dd, 1H, H-6′b), 3.54 (br m, 1H, H-5), 3.42 (ddd, 1H, $J_{5',6'a} = 2.1$ Hz, $J_{5',6'b} = 6.1$ Hz, H-5′), 3.31 (dd, 1H, $J_{3',4'} = 8.9$ Hz, H-3′), 3.21 (d, 1H, H-2), 3.20 (dd, 1H, $J_{4',5'} = 9.8$ Hz, H-4′), 3.17 (d, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.32, 3.40, 3.41, 3.50 (4s, 12H, 4OCH_3), 2.08, 2.06 (2s, 3-3H, 2COCH_3), 1.36 (s, 3H, CH_3(3)), 1.28 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 170.6, 169.2 (2*C*OCH$_3$), 99.0 (C-1), 95.5 (C-1′), 85.4 (C-3′), 84.2 (C-4), 83.7 (C-2), 80.6 (C-3), 79.8 (C-4′), 73.3 (C-5′), 73.1 (C-2′), 67.2 (C-5), 63.4 (C-6′), 61.4, 60.7, 59.3, 55.2 (4O*C*H$_3$), 21.2, 21.0 (CO*C*H$_3$), 18.5 (C-6), 15.8 (CH$_3$(3)). Anal. Calcd for $C_{22}H_{38}O_{12}$: C, 53.43; H, 7.74. Found: C, 53.58; H, 7.53.

3.6. Methyl 2,6-di-*O*-acetyl-3,4-di-*O*-methyl-α-D-glucopyranosyl-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside (6a) and methyl 2,6-di-*O*-acetyl-3,4-di-*O*-methyl-α-D-glucopyranosyl-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside (6b)

To a solution of the donor **4** (690 mg, 1.58 mmol) and acceptor **3**[3] (520 mg, 2.37 mmol) in dry CH_2Cl_2 (5 mL), TMSOTf (86 μL, 0.45 mmol) was added at −50 °C. After 20 min. stirring at −50 °C, Et_3N (100 μL) was added. The mixture was diluted with CH_2Cl_2 and after extractive work-up the crude syrup was purified by silica column chromatography (7:3 → 1:1, hexane/EtOAc) to give pure **6a** (195 mg, 25%) and **6b** (265 mg, 34%) both as colourless syrups: **6a**: $[\alpha]_D$ +53.9 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.48 (d, 1H, $J_{1',2'} = 3.8$ Hz, H-1′), 4.77 (d, 1H, $J_{1,2} = 7.1$ Hz, H-1), 4.63 (dd, 1H, $J_{2',3'} = 10$ Hz, H-2′), 4.28 (m, 1-1H, H-5, H-6′a), 4.19 (m, 1-1H, H-5′, H-6′b), 3.57 (t, 1H, $J_{3',4'} = 8.9$ Hz, H-3′), 3.57, 3.53, 3.50, 3.47, 3.39 (5s, 15H, OCH_3), 3.10 (t, 1H, $J_{4',5'} = 8.9$ Hz, H-4′), 3.02 (d, 1H, $J_{4,5} = 6$ Hz, H-4), 2.69 (d, 1H, H-2), 2.10, 2.09 (2s, 3-3H, COCH_3), 1.47 (s, 3H, CH$_3$(3)), 1.44 (d, 3H, $J_{5,6} = 7$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 170.7, 170.0 (*C*OCH$_3$), 97.7 (C-1), 91.6 (C-1′), 84.3 (C-2), 83.0 (C-4), 82.0 (C-3′), 80.2 (C-3), 80.0 (C-4′), 73.5 (C-2′), 69.2 (C-5), 68.2 (C-5′), 63.7 (C-6′), 61.1, 60.8, 60.6, 59.5, 56.3 (5O*C*H$_3$), 21.2 (CO*C*H$_3$), 19.0 (CH$_3$(3)), 14.2 (C-6). Compound **6b**: $[\alpha]_D$ −24.2 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.91 (dd, 1H, $J_{2',3'} = 9.2$ Hz, H-2′), 4.75 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.69 (d, 1H, $J_{1,2} = 4.1$ Hz, H-1), 4.33 (dd, 1H, $J_{6'a,6'b} = 11.7$ Hz, H-6′a), 4.18 (dd, 1H, H-6′b), 4.04 (br m, 1H, H-5), 3.40 (ddd, 1H, $J_{5',6'a} = 2.3$ Hz, $J_{5',6'b} = 6.5$ Hz, H-5′), 3.29 (t, 1H, $J_{3',4'} = 8.8$ Hz, H-3′), 3.19 (dd, 1H, $J_{4',5'} = 9.8$ Hz, H-4′), 2.90 (d, 1H, $J_{4,5} = 3.5$ Hz, H-4), 2.83 (d, 1H, H-2), 3.49, 3.41, 3.40, 3.35 (4s, 15H, OCH_3), 2.06, 2.04 (2s, 3-3H, COCH_3), 1.38 (s, 3H, CH$_3$(3)), 1.26 (d, 3H, $J_{5,6} = 6.8$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 170.6, 169.4 (*C*OCH$_3$), 99.3 (C-1), 95.8 (C-1′), 85.4 (C-3′), 83.9 (C-4), 83.5 (C-2), 80.1 (C-4′), 79.2 (C-3), 73.3 (C-2′), 73.1 (C-5′), 67.6 (C-5), 63.7 (C-6′), 60.4, 61.0, 59.9, 55.8 (O*C*H$_3$), 21.2, 20.8 (CO*C*H$_3$), 20.4 (CH$_3$(3)), 16.0 (C-6). Anal. Calcd for $C_{22}H_{38}O_{12}$: C, 53.43; H, 7.74. Found: C, 53.40; H, 7.83.

3.7. Methyl 2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranoside (15)

To a solution of compound **5** (160 mg, 0.32 mmol) in CH_3OH (10 mL), 1 M $NaOCH_3$ in CH_3OH (60 μL, 0.06 mmol) was added. After stirring at rt for 8 h, the mixture was neutralized with Amberlite IR 120 (H^+) resin, filtered and the filtrate was concentrated. The crude product was purified by column chromatography (9:1, CH_2Cl_2/acetone) to afford **15** (125 mg, 86%) as a colourless syrup: $[\alpha]_D$ −32.4 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.96 (t, 1H, $J_{2',3'} = 8$ Hz, H-2′), 4.82 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 4.67 (d, 1H, $J_{1,2} = 2$ Hz, H-1), 3.87 (dd, 1H, $J_{5',6'a} = 2$ Hz, $J_{6'a,6'b} = 12$ Hz, H-6′a), 3.76 (dd, 1H, $J_{5',6'b} = 3$ Hz, H-6′b), 3.57, 3.53, 3.44, 3.35 (4s, 15H, 5OCH_3), 3.19 (d, 1H, $J_{1,2} = 2$ Hz, H-2), 3.16 (d, 1H, $J_{4,5} = 9.5$ Hz, H-4), 2.11 (s, 3H, COCH_3), 1.37 (s, 3H, CH_3(3)), 1.29 (d, 3H, $J_{5,6} = 6$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.3 (*C*OCH$_3$), 98.3 (C-1), 95.0 (C-1′), 87.8, 84.2, 83.0, 78.8, 75.6, 72.9, 67.1 (C-3′, C-4, C-2, C-4, C-5′, C-2′, C-5), 80.9 (C-3), 61.9 (C-6′), 61.2, 60.4, 60.0, 58.8, 55.03 (5O*C*H$_3$), 21.0 (CO*C*H$_3$), 18.2 (C-6), 16.4 (CH$_3$(3)). Anal. Calcd for $C_{20}H_{36}O_{11}$: C, 53.09; H, 8.02. Found: C, 53.17; H, 8.13.

3.8. Methyl [methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranoside (17)

To a solution of **15** (70 mg, 0.15 mmol) in acetone (3 mL), CrO_3 (170 mg, 1.7 mmol dissolved in 500 μL of 3.5 M H_2SO_4) was added. After stirring for 1 h at rt, the mixture was poured onto ice-cold H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O and concentrated. The crude product (**16**) was dissolved in CH_2Cl_2 (5 mL) and a solution of CH_2N_2 in Et_2O was added dropwise until TLC analysis showed complete conversion. The mixture was concentrated and the residue was purified by column chromatography (7:3, hexane/EtOAc) to give **17** (46 mg, 64%) as a syrup: $[\alpha]_D$ −50.7 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.03 (dd, 1H, $J_{1',2'} = 8$ Hz, $J_{2',3'} = 9.5$ Hz, H-2′), 4.8 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 4.62 (d, 1H, $J_{1,2} = 2$ Hz, H-1), 3.78 (s, 3H, COOCH_3), 3.54, 3.51, 3.42, 3.36, 3.34 (5s, 15H, OCH_3), 3.19 (d, 1H, $J_{4,5} =$ 9.5 Hz, H-4), 3.18 (d, 1H, H-2), 2.10 (s, 3H, COCH_3), 1.36 (s, 3H, CH$_3$(3)), 1.28 (d, 1H, $J_{5,6} = 6$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.1 (*C*OCH$_3$), 168.4 (*C*OOCH$_3$), 98.4 (C-1), 95.6 (C-1′), 84.3, 83.8, 83.1, 80.3, 74.3, 72.4, 67.0 (C-3′, C-4, C-2, C-4, C-5′, C-2′, C-5), 81.0 (C-3), 61.2, 60.41, 60.2, 58.6, 55.0 (O*C*H$_3$), 52.4 (COO*C*H$_3$), 20.9 (CO*C*H$_3$), 18.1 (C-6), 15.9 (CH$_3$(3)). Anal. Calcd for $C_{21}H_{38}O_{12}$: C, 52.27; H, 7.94. Found: C, 52.34; H, 7.85.

3.9. [Methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranuronyl)uronate]-(1→3)-1-*O*-acetyl-3-*C*-methyl-2,4-di-*O*-methyl-L-rhamnopyranose (18)

To a solution of compound **17** (70 mg, 0.14 mmol) in Ac_2O (1 mL) and AcOH (1 mL), TFA (50 μL, 0.65 mmol) was added. After stirring for 1 day at 60 °C the mixture was concentrated, the crude product was purified by column chromatography (85:15, CH_2Cl_2/EtOAc) to yield **17** (48 mg, 67%) as a syrup: $[\alpha]_D$ −53.8 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.05 (d, 1H, $J_{1,2} = 2$ Hz, H-1), 5.04 (dd, 1H, $J_{2',3'} = 9.5$ Hz, H-2′), 4.8 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1′), 3.78 (s, 3H, COOCH_3), 3.54, 3.53, 3.4, 3.36 (4s, 12H, 4OCH_3), 3.33 (d, 1H, $J_{4,5} = 9.5$ Hz, H-4), 3.22 (d, 1H, H-2), 2.10, 2.09 (2s, 3-3H, 2COCH_3), 1.39 (s, 3H, CH$_3$(3)), 1.29 (d, 1H, $J_{5,6} = 6$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.1, 169.1 (2*C*OCH$_3$), 168.3 (*C*OOCH$_3$), 90.9 (C-1), 95.6 (C-1′), 84.3, 83.2, 82.1, 80.3, 74.4, 72.4, 69.5 (C-3′, C-4, C-2, C-4, C-5′, C-2′, C-5), 80.2 (C-3), 61.3, 60.5, 60.3, 58.7 (4O*C*H$_3$), 52.4 (COO*C*H$_3$), 21.3 (2CO*C*H$_3$), 18.2 (C-6), 15.9 (CH$_3$(3)). Anal. Calcd for $C_{22}H_{36}O_{13}$: C, 51.96; H, 7.14. Found: C, 51.86; H, 7.19.

3.10. [Methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranuronyl)uronate]-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-L-rhamnopyranose (19)

To a solution of compound **18** (60 mg, 0.12 mmol) in dry DMF (2 mL), hydrazine acetate (32 mg, 0.35 mmol) was added. After stirring for 1 day at rt the mixture was diluted with CH_2Cl_2, extracted with H_2O, dried and concentrated. The residue was purified by column chromatography (1:1, hexane/EtOAc) to give **19** (46 mg, 82%) as a syrup: $[\alpha]_D$ −19.9 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.18 (d, 1H, $J_{1,2} = 2$ Hz, H-1), 4.99 (dd, 1H, $J_{2',3'} = 9$ Hz, H-2′), 4.82 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 4.75 (s, 1H, OH) 3.81 (s, 3H, COOCH_3), 3.65, 3.54, 3.5, 3.45 (4s, 12H, OCH_3), 2.1 (s, 3H, COCH_3), 1.32 (s, 3H, CH$_3$(3)), 1.3 (d, 1H, $J_{5,6} = 6$ Hz, H-6); ^{13}C NMR: δ 169.0 (*C*OCH$_3$), 168.5 (*C*OOCH$_3$), 92.2 (C-1), 96,2 (C-1′), 85.7, 84.5, 84.3, 80.7, 73.9, 72.5, 70.4 (C-3′, C-4, C-2, C-4, C-5′, C-2′, C-5), 81.9 (C-3), 62.7, 60.8, 60.4, 58.8, (4O*C*H$_3$), 52.6 (COO*C*H$_3$), 20.9 (CO*C*H$_3$), 18.5 (C-6), 14.1 (CH$_3$(3)). Anal. Calcd for $C_{20}H_{34}O_{12}$: C, 51.50; H, 7.35. Found: C, 51.56; H, 7.29.

3.11. Methyl 3,4-di-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside (21)

To a solution of compound **6b** (48 mg, 0.1 mmol) in CH_3OH (3 mL), 2 M $NaOCH_3$ in CH_3OH (10 μL) was added. After stirring for 3 h at rt the mixture was neutralized with Amberlite IR 120 (H^+), filtered, concentrated and the residue was purified by silica column

chromatography (6:4, CH_2Cl_2/acetone) to yield pure **21** (39 mg, 95%) as a colourless syrup. $[\alpha]_D$ −43.0 (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.84 (br s, 1H, OH-2′), 4.78 (d, 1H, $J_{1,2} = 6$ Hz, H-1), 4.54 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1′), 4.25 (br m, 1H, $J_{4,5} = 4.5$ Hz, H-5), 3.67, 3.54, 3.52, 3.49, 3.45 (5s, 15H, OCH_3), 3.20 (br s, 1H, OH-6′), 2.81 (d, 1H, H-2), 1.49 (s, 3H, CH_3(3)), 1.40 (d, 3H, $J_{5,6} = 7$ Hz, H-6). Anal. Calcd for $C_{18}H_{34}O_{10}$: C, 52.67; H, 8.35. Found: C, 52.49; H, 8.52.

3.12. Methyl [methyl (3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranoside (23)

Compound **21** (48 mg, 0.11 mmol) was suspended in satd aq $NaHCO_3$ (2 mL), KBr (1 mg, 0.008 mmol) and 2,2,6,6-tetramethylpiperidine-1-oxyl (2 mg, 0.013 mmol) were added and the mixture was cooled to 0 °C, then NaOCl (1 mL) was added dropwise. The resulting mixture was stirred for 1 day at rt and then concentrated. To the solid residue **22** were added anhydrous DMF (4 mL) and CH_3I (10 μL) at 0 °C. After stirring for 1 day at rt, the mixture was concentrated, diluted with CH_2CH_2, extracted with H_2O, dried and concentrated. The resulting syrup was purified by silica column chromatography (8:2, CH_2Cl_2/acetone) to afford **23** (30 mg, 71%) as a colourless syrup: $[\alpha]_D$ −64.5 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.74 (br s, 1H, OH-2′), 4.48 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 4.69 (d, 1H, $J_{1,2} = 4.5$ Hz, H-1), 4.06 (br m, 1H, H-5), 3.73, 3.59, 3.44, 3.44, 3.43, 3.33 (6s, 18H, OCH_3), 3.07 (d, 1H, $J_{4,5} = 4$ Hz, H-4), 2.80 (d, 1H, H-2), 1.41 (s, 3H, CH_3(3)), 1.32 (d, 3H, $J_{5,6} = 6.5$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 168.8 (*C*OOCH$_3$), 99.2 (C-1), 97.8 (C-1′), 86.1, 83.0, 80.4, 74.4, 74.3, 67.2 (C-3′, C-4, C-2, C-4′, C-2′, C-5′ and C-5), 79.9 (C-3), 61.0, 60.5, 60.4, 55.6 (5O*C*H$_3$), 52.4 (COO*C*H$_3$), 21.0 (CH_3(3)), 15.9 (C-6). Anal. Calcd for $C_{19}H_{34}O_{11}$: C, 52.05; H, 7.82. Found: C, 52.17; H, 7.52.

3.13. [Methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-1-*O*-acetyl-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranose (24)

To a solution of the compound **23** (50 mg, 0.11 mmol) in Ac_2O (1 mL) and AcOH (1 mL), TFA (85 μL, 1.1 mmol) was added and the mixture was stirred for 1 day at 60 °C. The mixture was concentrated, the residue was purified by silica column chromatography (9:1, CH_2Cl_2/acetone) to yield **24** (36 mg, 64%) as a colourless syrup: $[\alpha]_D$ +40.0 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.97 (dd, 1H, $J_{2',3'} = 9$ Hz, H-2′), 4.89 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1′), 6.14 (d, 1H, $J_{1,2} = 2$ Hz, H-1), 4.18 (br m, 1H, H-5), 3.79 (s, 3H, COOCH_3), 3.52, 3.51, 3.45, 3.44 (4s, 12H, 3OCH_3), 2.99 (d, 1H, $J_{4,5} = 3.5$ Hz, H-4), 2.96 (d, 1H, H-2), 2.10, 2.08 (2s, 3-3H, 2COCH_3), 1.44 (s, 3H, CH_3(3)), 1.34 (d, 1H, $J_{5,6} = 6.5$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.2, 168.8 (2*C*OCH$_3$), 168.6 (*C*OOCH$_3$), 96.0 (C-1), 91.2 (C-1′), 84.4, 83.2, 82.3, 80.3, 74.1, 72.6, 69.7 (C-3′, C-4, C-2, C-4′, C-2′, C-5′, C-5), 78.9 (C-3), 61.0, 60.5, 60.3, 59.8, (4O*C*H$_3$), 52.4 (COO*C*H$_3$), 21.2, 20.96 (2CO*C*H$_3$), 20.6 (C-6), 15.5 (CH_3(3)). Anal. Calcd for $C_{22}H_{36}O_{13}$: C, 51.96; H, 7.14. Found: C, 51.78; H, 7.23.

3.14. [Methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranose (25)

To a solution of the compound **24** (22 mg, 0.043 mmol) in CH_3OH (0.5 mL), *n*-Bu_2SnO (5.4 mg, 0.021 mmol) was added. After stirring for 4 h at 50 °C the mixture was concentrated. The residue was purified by silica column chromatography (8:2, CH_2Cl_2/acetone, 1% Et_3N) to give **25** (16 mg, 80%) as a syrup. ^1H NMR ($CDCl_3$): δ 5.02 (dd, 1H, $J_{2',3'} = 9$ Hz, H-2′), 4.90 (d, 1H, $J_{1',2'} = 8$ Hz, H-1′), 5.15 (d, 1H, $J_{1,2} = 3$ Hz, H-1), 3.80 (s, 3H, COOCH_3), 3.58, 3.55, 3.47, 3.41 (4s, 12H, 4OCH_3), 2.12 (s, 3H, COCH_3), 1.26 (s, 3H, CH_3(3)), 1.40 (d, 1H, $J_{5,6}$ 6.5 Hz, H-6).

3.15. Ethyl [methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (8)

To a solution of compound **19** (50 mg, 0.11 mmol) in dry CH_2Cl_2 (5 mL), trichloroacetonitrile (314 μL, 1.5 mmol) and K_2CO_3 (500 mg, 5.05 mmol) were added. After stirring for 1 day at rt the mixture was diluted with CH_2Cl_2, filtered and concentrated to afford **20** (42 mg, 63%) as a syrup, which was used in the next step without purification. The solution of the crude product **20** (42 mg, 0.07 mmol) and acceptor **7**[4] (46.5 mg, 0.12 mmol) in dry CH_2Cl_2 (500 μL) was cooled to −50 °C, then TMSOTf (3.5 μL, 0.018 mmol) was added dropwise and the mixture was stirred for 30 min. Et_3N (50 μL) was added, the mixture was diluted with CH_2Cl_2, extracted with H_2O, dried and concentrated. The resulting syrup was purified by silica column chromatography (7:3, hexane/EtOAc) to yield **8** (20 mg, 35%) as a syrup: $[\alpha]_D$ −67.4 (*c* 1.1, $CHCl_3$); ^1H and ^{13}C NMR data are collected in Table 1. MALDIMS *m*/*z* calcd for $C_{42}H_{60}O_{15}S_1$: 836.36 [M]. Found: 859.41 $[M+Na]^+$.

3.16. Ethyl [methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-6-deoxy-3-*C*-methyl-2,4-di-*O*-methyl-α-L-talopyranosyl-(1→3)-2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (9)

To a solution of the compound **25** (30 mg, 0.06 mmol) in dry CH_2Cl_2 (5 mL), trichloroacetonitrile (1 mL,

4.8 mmol) and K_2CO_3 (500 mg, 5.05 mmol) were added and the mixture was stirred for 1 day at rt. The mixture was diluted with CH_2Cl_2, filtered and concentrated to afford **26** (32 mg, 82%) as a syrup. The solution of the crude product **26** (40 mg, 0.07 mmol) and acceptor **7**[4] (60 mg, 0.15 mmol) in dry CH_2Cl_2 (200 μL) was cooled to −50 °C, then TMSOTf (3.5 μL, 0.018 mmol) was added dropwise and the mixture was stirred for 30 min. Et_3N (50 μL) was added. The mixture was diluted with CH_2Cl_2, extracted with H_2O, dried and concentrated. The residue was purified by silica column chromatography (6:4, hexane/EtOAc) to yield **9** (15 mg, 26%) as a colourless syrup: $[\alpha]_D$ −47.3 (*c* 0.3). ^1H and ^{13}C NMR data are collected in Table 1. MALDIMS calcd for $C_{42}H_{60}O_{15}S_1$: 836.36 [M]. Found: 860.47 $[M+Na]^+$.

3.17. *p*-Nitrophenyl [methyl (2-*O*-acetyl-3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-6-deoxy-3,4-*O*-benzylidene-α-L-talopyranoside (27)

The solution of donor **8** (25 mg, 0.03 mmol) and acceptor **10**[4] (42 mg, 0.06 mmol) in dry CH_2Cl_2 (400 μL) was cooled to −30 °C, then solution of NIS (8.2 mg, 0.036 mmol) and TfOH (1 μL, 0.004 mmol) in CH_2Cl_2 (200 μL) was added dropwise and the mixture was stirred for 30 min. Et_3N (50 μL) was added, the mixture was diluted with CH_2Cl_2, extracted with aqueous 10% $Na_2S_2O_3$ and H_2O, then dried and concentrated. The resulting syrup was purified using HPLC to give **27** (13 mg, 30%) as a colourless syrup: $[\alpha]_D$ −70.7 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR data are collected in Table 2. MALDIMS *m*/*z* calcd for $C_{79}H_{95}N_1O_{26}$: 1473.61 [M]. Found: 1496.92 $[M+Na]^+$.

3.18. *p*-Trifluoroacetamidophenyl [methyl (3,4-di-*O*-methyl-β-D-glucopyranosyl)uronate]-(1→3)-3-*C*-methyl-2,4-di-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-6-deoxy-α-L-talopyranoside (11)

To a solution of compound **27** (13 mg, 0.009 mmol) in EtOAc (2 mL), PtO_2 (10 mg) was added. After stirring for 2 h under H_2 at rt, the mixture was cooled to 0 °C, then pyridine (100 μL) and trifluoroacetic anhydride (50 μL, 0.36 mmol) were added. The mixture was stirred for 1 h then filtered and concentrated. The residue was purified by silica column chromatography (8:2, CH_2Cl_2/EtOAc) to yield the corresponding trifluoroacetamidophenyl derivative. The colourless syrup (7 mg) was dissolved in dry CH_3OH (2 mL) and stirred with 0.1 M $NaOCH_3$ in CH_3OH (10 μL) for 1 day at rt. The mixture was then neutralized with Amberlite IR 120(H^+), filtered and concentrated. The crude syrup was purified by silica column chromatography (6:4, CH_2Cl_2/EtOAc) to give 5 mg of the deacetylated congener. The colourless syrup was dissolved in dry CH_3OH, $Pd(OH)_2$ was added and the mixture was stirred for 1 day at rt. The mixture was filtered and concentrated. The crude syrup was purified on HPLC (SUPELCOSIL SPLC-Si, 254 nm, 85:15, EtOAc/hexane) to yield **11** (1.2 mg, 13%) as a colourless syrup: $[\alpha]_D$ −50.2 (*c* 0.1, H_2O); ^1H and ^{13}C NMR data are collected in Table 2. MALDIMS *m*/*z* calcd for $C_{44}H_{66}F_3N_1O_{24}$: 1049.39 [M]. Found: 1072.93 $[M+Na]^+$.

Acknowledgements

The Hungarian Science Fund supported the work of I. Bajza (OTKA T 035128), A. Lipták (OTKA T 048798), K. E. Kövér (OTKA T 048713) and the Ministry of Education (PÁL 48/2005) supported that of A. Lipták.

References

1. (a) Lipták, A.; Borbás, A.; Bajza, I. *Med. Res. Rev.* **1994**, *14*, 307–352; (b) Aspinall, G. O.; Chatterjee, D.; Brennan, P. J. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 169–242.
2. Chatterjee, D.; Bozic, C.; Aspinall, G. O.; Brennan, P. J. *J. Biol. Chem.* **1988**, *263*, 4092–4097.
3. Gyergyói, K.; Tóth, A.; Bajza, I.; Lipták, A. *Synlett* **1998**, 127–128.
4. Kerékgyártó, J.; Szurmai, Z.; Lipták, A. *Carbohydr. Res.* **1993**, *245*, 65–80.
5. Bell, D. J.; Greville, G. D. *J. Chem. Soc.* **1950**, 1902–1904.
6. Fügedi, P.; Garegg, P. J.; Norberg, T. *Glycoconjugate J.* **1987**, *4*, 97–108.
7. Schmidt, R. R. *Pure Appl. Chem.* **1987**, *61*, 1257–1270.
8. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
9. Spijker, N. M.; van Boeckel, C. A. A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 180–183.
10. Tóth, A.; Remenyik, J.; Bajza, I.; Lipták, A. *Arkivoc* **2003**, *V*, 28–45.
11. (a) Szurmai, Z.; Lipták, A. *Carbohydr. Res.* **1982**, *107*, 33–41; (b) Szurmai, Z.; Lipták, A.; Snatzke, G. *Carbohydr. Res.* **1990**, *200*, 201–208.
12. Butterworth, R. F.; Hanessian, S. *Synthesis* **1971**, 70–89.
13. de Noy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Synthesis* **1996**, 1153–1175.
14. Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39*, 368–373.
15. Herzig, J.; Nudelman, A.; Gottlieb, H. E. *Carbohydr. Res.* **1988**, *177*, 21–28.