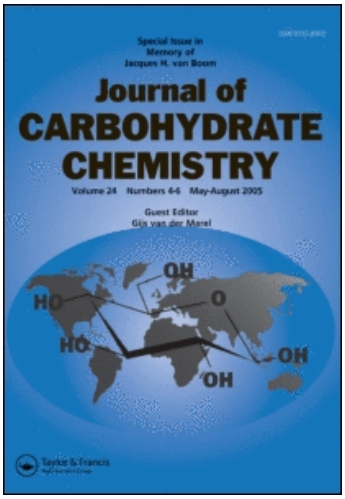

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Battistelli, Chiara Laura , De Castro, Cristina , Iadonisi, Alfonso , Lanzetta, Rosa , Mangoni, Lorenzo and Parrilli, Michelangelo(1999) 'Synthesis and NMR Characterisation of Methyl Mono- and Di-*O*-α-L-Rhamnopyranosyl-α-D-Glucopyranosiduronic Acids', Journal of Carbohydrate Chemistry, 18: 1, 69 — 86
To link to this Article: DOI: 10.1080/07328309908543980
URL: http://dx.doi.org/10.1080/07328309908543980

J. CARBOHYDRATE CHEMISTRY, 18(1), 69-86 (1999)

SYNTHESIS AND NMR CHARACTERISATION OF METHYL MONO- AND DI-*O*-α-L-RHAMNOPYRANOSYL-α-D-GLUCOPYRANOSIDURONIC ACIDS

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Received April 1, 1998 - Final Form September 22, 1998

ABSTRACT

The synthesis and NMR characterisation of methyl mono- and di-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acids **1-6** are described. Two commercial starting products were used: methyl α-D-glucopyranoside **7** for the preparation of **1** and **2**, and methyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside **8** for **3-6**. Oxidation reaction of the hydroxymethyl group of glucose to a carboxylic acid group was performed by sodium hypochlorite 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-mediated procedure after the coupling reaction. Glycosylation was carried out using the trichloroacetimidate approach with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter, resulting in a completely stereoselective formation of the α glycosyl linkage.

INTRODUCTION

The degradation with lithium-ethylenediamine is widely used for structural analysis of acid polysaccharides, that are selectively cleaved at the acidic unit.[1] Albersheim[2] put in evidence that the course of the reaction was affected by the glycosylation pattern of the

uronic unit. No further investigation on this topic was reported. In order to gain more insight into the mechanism of this reaction we needed model oligosaccharides containing one unit of α-D-glucopyranosiduronic acids since they are representative of common structural features of many natural polysaccharides[3] and generally difficult to obtain by acid hydrolysis of polysaccharides. In addition the complete NMR characterisation of the prepared compounds might be useful for analytical purposes.

To our knowledge, none of the methyl oligosaccharides **1-6**, described in this communication, was prepared before. However, two rather laborious syntheses of **1** in hemiacetalic form[4] and α-rhamnosylation of position 3 and 4 of methyl glucuronate 1,2-cyano ethylidene have been already described.[5,6] As will be shown, the latter procedure is not practical for the synthesis of unprotected oligosaccharides.

RESULTS AND DISCUSSION

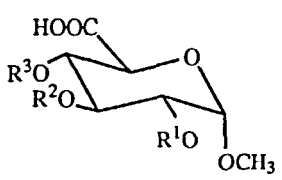
Two commercial D-glucose derivatives were chosen as convenient precursors for D-glucuronic acid units: methyl α-D-glucopyranoside **7** for the preparation of methyl 4-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acid **1** and methyl 3,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **2**, whereas methyl 3-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acid **3**, methyl 2-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acid **4**, methyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **5** and methyl 2,3-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **6** were prepared from methyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside **8**.

The choice of the above precursors implied that the oxidation of the hydroxymethyl group of glucose to a carboxylic acid group had to be performed after the glycosylation step. In fact the inverse sequence would have required the protection of the carboxyl group as an ester whose deprotection, in alkaline medium, would have led to a rather extensive β-elimination reaction,[7] as preliminary experiments we performed had shown, even under very mild conditions.

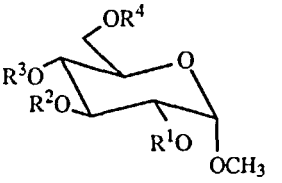
All the glycosylation reactions were carried out with the trichloroacetimidate approach[8] using 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate **9**[9] as the glycosyl donor. As expected, because of the acyl protective groups of **9**, only α glycosides were isolated as shown by $^1J_{C\text{-}H}$ values (169-172 Hz) for the anomeric carbons of all rhamnose residues of **1-6**. In our glycosylation reactions TMSOTf turned out to be a more efficient promoter than boron trifluoride etherate ($BF_3{\cdot}OEt_2$).

In all cases the hydroxymethyl oxidations were performed by the sodium hypochlorite TEMPO-mediated procedure,[10] which shows a high selectivity towards

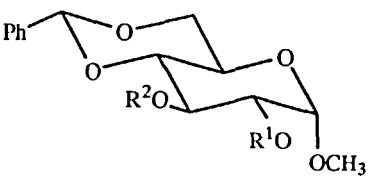
primary hydroxyl functions in the presence of secondary ones. Oxidations were performed either under aqueous conditions[11] or under two-phase water-dichloromethane conditions.[12,13]



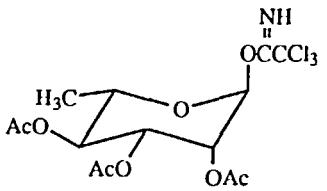
	R1	R2	R3
1	H	H	W
2	H	W	W
3	H	W	H
4	W	H	H
5	W	H	W
6	W	W	H
20	Y	Y	H
24	Bz	Y	H
29	Y	H	H
34	Y	H	Y



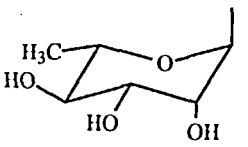
	R1	R2	R3	R4
7	H	H	H	H
10	Bz	H	Bz	Bz
11	Bz	Bz	H	Bz
12	Bz	H	H	Bz
13	Bz	Y	Bz	Bz
14	Bz	Bz	Y	Bz
15	Bz	Y	Y	Bz
16	H	H	W	H
17	H	W	W	H
19	Y	Y	H	H
23	Bz	Y	H	H
28	Y	H	H	H
30	Bz	Bn	H	Bn
31	H	Bn	H	Bn
32	Y	Bn	Y	Bn
33	Y	H	Y	H

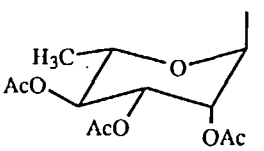


	R1	R2
8	H	H
18	Y	Y
21	Bz	H
22	Bz	Y
25	Bz	Bn
26	H	Bn
27	Y	Bn

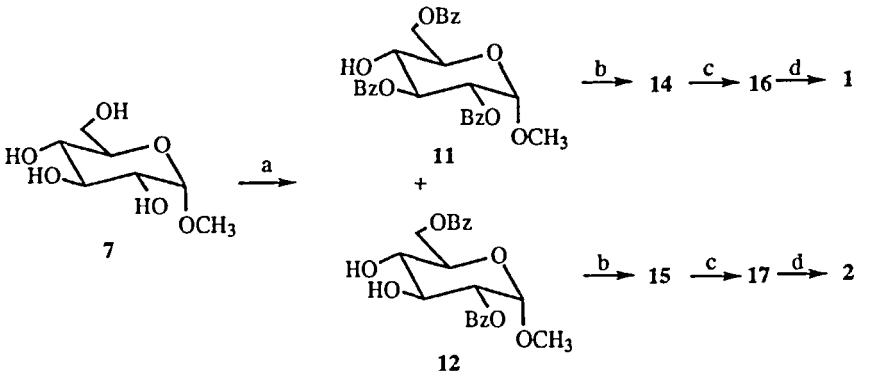


9

W= 

Y= 

As far as the preparation of compounds **1** and **2** is concerned (Scheme 1), methyl α-D-glucopyranoside **7** was selectively benzoylated, exploiting the reactivity order of secondary hydroxyl groups of **7**, which is 2-OH>3-OH>4-OH.[14] The benzoylation of **7** was performed utilising both 3 and 2 equivalents of benzoyl chloride in order to increase the yield of methyl tri-*O*-benzoyl-substituted and di-*O*-benzoyl-substituted glucopyranosides, respectively. Under the first condition the crude reaction product showed 1:2:2.4 molar ratios of **10**:**11**:**12** by integration of proton NMR signals of H-4 (at δ 5.41) of **10**, H-3 (at δ 5.81) of **11** and H-4 (at δ 3.61) of **12**, besides a small amount of



Scheme 1. a) BzCl/pyr; b) **9**/TMSOTf/CH_2Cl_2; c) MeONa/MeOH; d) TEMPO/NaBr/NaClO/H_2O/pH 10-11/0 °C.

methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside. Under the other condition 1:2.9:12.4 molar ratios of **10**:**11**:**12** were evaluated. From a preparative point of view only compound **12** was isolated in pure form in a convenient amount by silica gel column chromatography (see Experimental), while compounds **10** and **11** were obtained mainly as a mixture. Both **12** and this mixture were separately coupled with **9** to give **15** and a mixture of **13** and **14**, respectively. From this latter mixture only the predominant compound **14** was isolated pure by silica gel chromatography. The deprotection of **14** and **15** with sodium methoxide gave **16** and **17**, whose hydroxymethyl functions were finally selectively oxidised with the sodium hypochlorite TEMPO-mediated procedure to give compounds **1** and **2**, as shown by the presence of carbonyl signals in their ^{13}C NMR spectra (Table 1 and 2).

Different from the reported syntheses of hemiacetalic form of **1**,[4] our strategy exploits a glycosyl acceptor **11** which can be readily prepared in a single step from **7**. Moreover, it's noteworthy that the use of acyl protecting groups in the donor and in the acceptor didn't hamper the achievement of a good coupling yield (87%).

The preparation of **3**-**6** starting from **8** is shown in Scheme 2.

Compound **6** was obtained by direct coupling of **8** with **9**, followed by acid hydrolysis to remove the benzylidene group of **18**, by oxidation of the hydroxymethyl function of **19**, and by final total deprotection of **20**.

To prepare **3**, compound **8** was regioselectively benzoylated at the 2 position to form **21** using 1-(benzoyloxy)benzotriazole (BtOBz) under very mild reaction conditions.[15] Compound **21** was then submitted to the described sequence of coupling,

Table 1. ^{1}H and ^{13}C NMR spectral data[a,b] for compounds **1**, **3**, **4**.

	1		**3**		**4**	
Rha	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	4.62 d ($J_{1,2}$=1.8)	103.5	4.96 d ($J_{1,2}$=1.6)	103.6	4.95 d ($J_{1,2}$=2.0)	103.1
2	3.81 dd ($J_{2,3}$=3.5)	72.5	3.98 dd ($J_{2,3}$=3.6)	72.6	4.04 dd ($J_{2,3}$=3.4)	70.4
3	3.69 dd ($J_{3,4}$=9.8)	72.6	3.68 dd ($J_{3,4}$=9.5)	72.2	3.76 dd ($J_{3,4}$=9.8)	72.1
4	3.31 t ($J_{4,5}$=9.8)	74.2	3.36 t ($J_{4,5}$=9.5)	74.3	3.44 t ($J_{4,5}$=9.8)	72.3
5	3.88 dq ($J_{5,6}$=6.5)	71.5	3.92 dq ($J_{5,6}$=6.6)	71.2	3.69 dq ($J_{5,6}$=6.3)	69.7
6	1.11 d	18.8	1.15 d	18.8	1.30 d	17.2
GlcA						
1	4.75 d ($J_{1,2}$=3.8)	101.8	4.75 d ($J_{1,2}$=3.8)	102.1	4.94 d ($J_{1,2}$=3.8)	99.4
2	3.50 dd ($J_{2,3}$=9.7)	73.8	3.62 dd ($J_{2,3}$=9.3)	73.6	3.63 dd ($J_{2,3}$=9.8)	79.6
3	3.63 t ($J_{3,4}$=9.7)	73.5	3.67 t ($J_{3,4}$=9.3)	81.8	3.75 t ($J_{3,4}$=9.8)	70.5
4	3.55 t ($J_{4,5}$=9.7)	81.3	3.56 t ($J_{4,5}$=9.3)	72.6	3.61 t ($J_{4,5}$=9.8)	71.7
5	4.11 d	71.9	4.09 d	73.1	4.14 d	70.7
6	-	175.3	-	175.3	-	173.8
OCH_3	3.31 s	57.9	3.36 s	58.0	3.44 s	55.9

a. Recorded in D_2O at 30 °C and pH 1-2.
b. Chemical shifts are expressed in ppm; coupling costants in parentheses are reported in Hz.

hydrolysis, oxidation and deprotection as above to give compounds **22**, **23**, **24** and **3**, respectively.

Compound **21** was also benzylated at the 3 position to give methyl 2-*O*-benzoyl-3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside **25** employing benzyl trichloroacetimidate. This reagent allowed us to perform benzylations in the presence of both ester and acetal protecting groups owing to the very mild acidic conditions required.[16] To prepare **4**, the 2 position of **25** was deprotected by treatment with sodium methoxide, affording **26**, which was then glycosylated with compound **9** to give **27**. The removal of both benzyl and benzylidene protecting groups was achieved by Pd-catalyzed transfer hydrogenolysis using formic acid[17] to give **28**. The latter compound was oxidised at the hydroxymethyl position to yield **29**, which gave **4** by total deprotection.

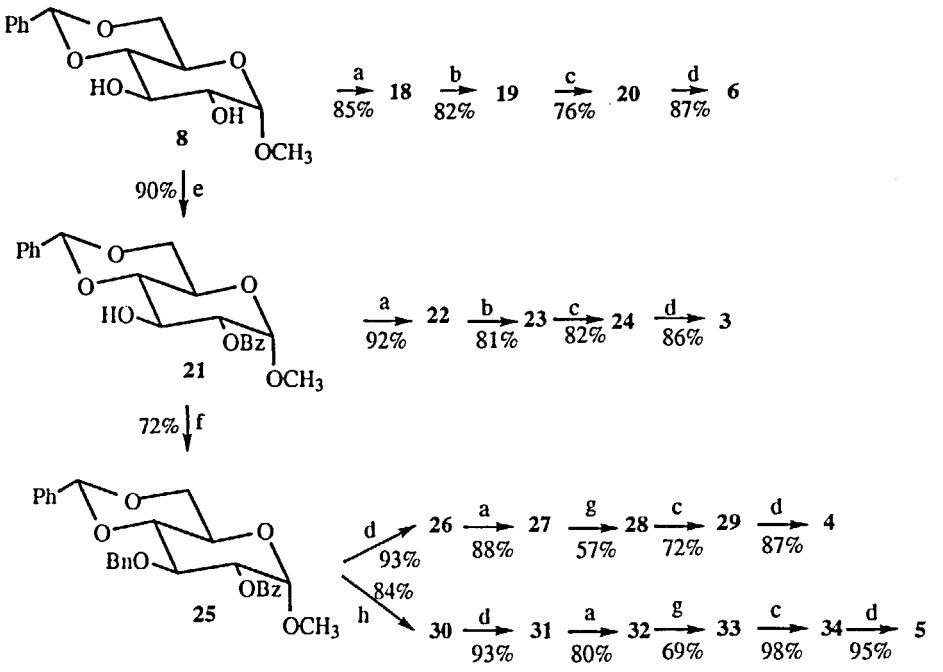
Compound **5** was prepared from **25** by regioselective reductive opening of the benzylidene acetal using sodium cyanoborohydride/hydrogen chloride[18] which afforded

Table 2. ^{1}H and ^{13}C NMR spectral data[a,b] for compounds **2**, **5**, **6**.

	2		**5**		**6**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
unit	Rha (1→3)-		Rha (1→4)-		Rha (1→3)-	
1	5.33 d ($J_{1,2}$=1.5)	101.3	4.96 d ($J_{1,2}$=1.5)	103.6	4.97 d ($J_{1,2}$=2.1)	102.1
2	4.08 dd ($J_{2,3}$=3.7)	70.6	4.03 dd ($J_{2,3}$=2.9)	70.9[c]	3.94 dd ($J_{2,3}$=3.3)	71.2
3	3.79 dd ($J_{3,4}$=9.8)	70.6	3.77 dd ($J_{3,4}$=9.8)	71.0[c]	3.76 dd ($J_{3,4}$=9.5)	70.8
4	3.45 t ($J_{4,5}$=9.5)	72.8	3.48 t ($J_{4,5}$=9.8)	72.8	3.44 t ($J_{4,5}$=9.5)	72.7
5	3.92 dq ($J_{5,6}$=6.7)	70.3	3.70 dq ($J_{5,6}$=6.4)	70.1	4.02 dq ($J_{5,6}$= 6.7)	69.6
6	1.31 d	17.5	1.31 d	17.6	1.24 d	17.2
unit	Rha (1→4)-		Rha (1→2)-		Rha (1→2)-	
1	4.92 d ($J_{1,2}$=1.5)	100.4	4.73 d ($J_{1,2}$=1.5)	101.8	4.96 d ($J_{1,2}$=2.1)	103.4
2	3.93 dd ($J_{2,3}$=3.7)	70.3	3.93 dd ($J_{2,3}$=3.0)	71.1[c]	3.93 dd ($J_{2,3}$=3.3)	70.1
3	3.72 dd ($J_{3,4}$=9.5)	70.6	3.73 dd ($J_{3,4}$=9.8)	71.6	3.77 dd ($J_{3,4}$=9.8)	70.8
4	3.43 t ($J_{4,5}$=9.5)	72.6	3.45 t ($J_{4,5}$=9.8)	72.8	3.46 t ($J_{4,5}$=9.8)	72.6
5	3.92 dq ($J_{5,6}$= 6.7)	70.1	4.01 dq ($J_{5,6}$= 6.7)	69.9	3.72 dq ($J_{5,6}$= 6.7)	71.1
6	1.30 d	17.5	1.23 d	17.3	1.32 d	17.5
unit	GlcA		GlcA		GlcA	
1	4.83 d ($J_{1,2}$=3.7)	100.4	4.92 d ($J_{1,2}$=3.9)	99.6	4.94 d ($J_{1,2}$=3.2)	99.5
2	3.81 dd ($J_{2,3}$=9.3)	72.0	3.63 dd ($J_{2,3}$=9.8)	80.4[d]	3.76 dd ($J_{2,3}$=9.8)	81.1
3	3.99 t ($J_{3,4}$=9.0)	77.0	3.77 t ($J_{3,4}$=9.8)	71.1[c]	3.81 t ($J_{3,4}$=9.8)	79.1
4	3.86 t ($J_{4,5}$=9.0)	75.4	3.65 t ($J_{4,5}$=9.8)	79.9[d]	3.69 t ($J_{4,5}$=9.8)	71.1
5	4.19 d	71.1	4.07 d	71.2[c]	4.08 d	71.8
6	-	173.8	-	174.9	-	174.4
OCH_3	3.44 s	56.2	3.44 s	56.1	3.47 s	56.1

a. Recorded in D_2O at 30 °C and at pH 1-2.
b. Chemical shifts are expressed in ppm; coupling costants in parentheses are reported in Hz.
c,d. Interchangeable values.

the 6-*O*-benzyl ether **30** as the major product (84%). The structure of **30** was inferred from the presence in its ^{1}H NMR spectrum of a D_2O exchangeable signal at δ 2.53 which appeared as a doublet indicating a hydroxyl group linked to a methine carbon. Removal of the 2-*O*-benzoyl group gave **31** which was submitted to the same reaction sequence from **26** to give **32**, **33**, **34** and finally **5**.



Scheme 2. a) 9/TMSOTf/CH_2Cl_2; b) TFA/$CHCl_3$; c) TEMPO/KBr/NaClO/TBAC/$NaHCO_3$/CH_2Cl_2/H_2O/0 °C; d) MeONa/MeOH; e) BtOBz/Et_3N/CH_2Cl_2; f) TfOH/CH_2Cl_2/benzyltrichloroacetimidate; g) Pd/C/MeOH/HCOOH; h) $NaCNBH_3$/HCl/EtO_2/THF.

As outlined for the synthesis of **1**, the general strategy here illustrated for compound **1-6**, based on the glycosylation/regioselective oxidation sequence, reduces the number of steps required in the preparation of the glycosyl acceptors and in the deprotection of the target oligosaccharides. Furthermore, this approach bypasses the problems connected with the expected lower reactivity in glycosylations of the glucuronic acid alcoholic functions,[4] prevents the occurrence of degrading β-elimination reactions and allows use of a peracylated glycosyl donor which guarantees excellent stereochemical control in the coupling step. The overall yields for compounds **1**, **2** and **3-6**, from **7** and **8**, respectively, ranged between 20% and 50% and are higher than those reported for analogous derivatives.[4-6]

The complete assignment of ^1H and ^{13}C signals of compound **1-6** (Tables 1 and 2) was performed by one- and two-dimensional NMR experiments. Starting from anomeric signals the proton connectivity was established for all compounds by COSY and one-dimensional HOHAHA experiments. The heterocorrelated experiments *via* one-bond

allowed us to assign the related ^{13}C signals. Identification of the signals of each rhamnose unit for trisaccharide compounds **2**, **5**, **6** was established on the basis of long-range heteronuclear correlation experiments. In particular for methyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **5**, the anomeric carbons at δ 101.8 of Rha(1→2) unit and at δ 103.6 of Rha(1→4) unit were correlated with the proton signals at δ 3.63 dd and δ 3.65 t, which had been assigned to H-2 and H-4 of the glucuronic acid residue, respectively, on the basis of COSY experiment and multiplicity pattern. As for 2,3-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **6**, the proton signals at δ 3.81 t and δ 3.76 dd assigned to H-3 and H-2 of the glucuronic acid residue were correlated to carbon signals at δ 102.1 of Rha(1→3) and at 103.4 of Rha(1→2), respectively. Accordingly, the anomeric proton signals at δ 4.97 d and δ 4.96 d of Rha(1→3) and Rha(1→2) units were correlated to carbon signals at δ 79.1 and δ 81.1 of C-3 and C-2 of glucuronic acid unit, respectively. As for methyl 3,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid **2**, the proton signals at δ 3.86 t and δ 3.99 dd assigned to H-4 and H-3 of the glucuronic acid residue were correlated to carbon signals at δ 100.4 of Rha(1→4) and at 101.3 of Rha(1→3), respectively. Accordingly, the anomeric proton signals at δ 4.92 d and δ 5.33 d of Rha(1→4) and Rha(1→3) units were correlated to carbon signals at δ 75.4 and δ 77.0 of C-4 and C-3 of glucuronic acid unit, respectively.

By comparison of ^{1}H NMR chemical shift data of **1**-**6**, it is possible to note some interesting differences. The anomeric signal of the rhamnose residue (δ 5.33) linked at the 3 position of the uronic unit in compound **2** occurs at very low-field with respect to anomeric signals of the other compounds (δ 4.92-4.97). The lack of this shift in methyl 3-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acid **3**, suggests that it is due to the presence of a rhamnopyranosyl unit linked at the 4-position of the uronic residue. Other peculiar shifts at high-field can be found for the rhamnose unit linked at the 2-position of the uronic unit in compound **5** (δ 4.73) and for the rhamnose unit in **1** (δ 4.62). These shifts might be useful for analytical purposes.

EXPERIMENTAL

General methods. Optical rotations have been determined with a Perkin Elmer 141 polarimeter (589 nm), at 20 °C, with a concentration expressed in g/100 mL. ^{1}H (250 and 400 MHz) and ^{13}C (62.89 and 100 MHz) NMR spectra were recorded using Bruker AM 250 or DRX 400 Avance spectrometers equipped with a dual probe and a multinuclear inverse Z-grad probe, respectively. ^{1}H chemical shifts were measured relative to TMS (in

$CDCl_3$ and CD_3OD) or sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (in D_2O) and ^{13}C chemical shifts in D_2O relative to 1,4-dioxane (δ 67.4), all as internal standards. The spectra were recorded with standard Bruker pulse sequences. 2D homuclear shift correlation (COSY)[19] data were collected in the phase sensitive mode using the TPPI method and using gradient pulses for selection with multiple quantum filter. Typically, data sets of 2048 (t_2) x 512 (t_1) complex points were collected with 16 scans per FID, and a sweep width in both dimensions of 6 ppm. COSY spectra were processed with shifted sine-bell in both dimensions. 1D homonuclear Hartman-Hann correlation HOHAHA[20] were recorded using MLEV17 sequence for mixing selective excitation with a shaped pulse z-filter. Mixing times of 30, 50 and 100 ms were used. A gradient heteronuclear single quantum coherence (HSQC)[21] data set was collected in the phase sensitive mode using echo-antiecho gradient selection with decoupling during acquisition. Typically, a data set of 1024 x 256 complex points was acquired with 64 scans. The spectral width was 6 ppm for proton and 180 ppm for carbon. Data were processed with a Lorentzian-to-Gaussian weighting function applied to t_2 and a shifted squared sine-bell function and zero-filling applied to t_1. The heteronuclear multiple bond correlation (HMBC)[22] spectrum was recorded with low-pass J-filter to suppress one-bond correlation, no decoupling during acquisition and using gradient pulses for selection. A delay of 60 ms was used for the evolution of long-range correlations. The spectrum was processed with a shifted sine-bell in both dimensions. TLC chromatography was performed using silica gel plates F_{254} (Merck). All compounds were revealed by spraying plates with a saturated solution of CrO_3 in concd H_2SO_4, followed by heating at 120 °C. Silica gel 60 (Merck) was used for column chromatography (CC). Whenever anhydrous reaction conditions were required, dry solvents stored over molecular sieves were used, under dry argon atmosphere. Gel filtration chromatography was performed on a Bio-Gel P-2 (Bio-Rad) column. Total carbohydrates were determined by the phenol-H_2SO_4 test.[23]

Methyl 2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (10), Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (11) and Methyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside (12). To a stirred solution of methyl α-D-glucopyranoside **7** (585 mg, 3.0 mmol) in dry pyridine (8 mL) at 0 °C was added dropwise benzoyl chloride (1.0 mL, 9.0 mmol). After stirring at 0 °C for 1 h and at 4 °C for 12 h, water (5 mL) and CH_2Cl_2 were added to the reaction mixture. The organic phase was washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel CC (4:6 diethyl ether/hexane) to give methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (122 mg, 7%), **11** (91 mg, 6%), a mixture of **10** and **11** (959 mg, 63%) and **12** (242 mg, 20%). The same reaction was performed using a 1:2 molar ratio between **7** and benzoyl chloride to give by chromatography methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-

glucopyranoside (12 mg, 1%), a mixture of **10** and **11** (200 mg, 20%), compound **12** (391 mg, 50%) and a mixture of monobenzoylated derivatives.

Compound **10**: Rf 0.68 (diethyl ether/hexane 8:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-7.35 (15H, H-Ph), 5.41 (t, 1H, $J_{4,3} = J_{4,5} = 10.2$ Hz, H-4), 5.15 (2H, H-1, H-2), 4.65 (dd, 1H, $J_{6,6'} = 12.2$ Hz, $J_{6,5} = 4.5$ Hz, H-6), 4.48 (2H, H-3, H-6'), 4.35 (m, 1H, H-5), 3.49 (s, 3H, OCH_3), 2.81 (d, 1H, OH-3).

Compound **11**: Rf 0.68 (diethyl ether/hexane 8:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-7.35 (15H, H-Ph), 5.81 (t, 1H, $J_{3,2} = J_{3,4} = 10.2$ Hz, H-3), 5.29 (dd, 1H, $J_{2,1} = 3.8$ Hz, H-2), 5.16 (d, 1H, H-1), 4.82 (dd, 1H, $J_{6,6'} = 12.2$ Hz, $J_{6,5} = 4.5$ Hz, H-6), 4.65 (dd, 1H, $J_{6',5} = 2.1$ Hz, H-6'), 4.13 (m, 1H, H-5), 3.89 (dt, 1H, $J_{4,5} = 9.8$ Hz, $J_{4,OH} = 3.2$ Hz, H-4), 3.48 (s, 3H, OCH_3), 3.36 (d, 1H, $J_{3,OH} = 2.7$ Hz, OH-4).

Compound **12**: Rf 0.26 (diethyl ether/hexane 8:2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21-7.46 (10H, H-Ph), 5.07 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.96 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 4.82 (dd, 1H, $J_{6,6'} = 12.4$ Hz, $J_{6,5} = 4.1$ Hz, H-6), 4.54 (dd, 1H, $J_{6',5} = 1.7$ Hz, H-6'), 4.19 (t, 1H, $J_{3,4} = 9.8$ Hz, H-3), 3.97 (m, 1H, H-5), 3.61 (t, 1H, $J_{3,2} = J_{3,4} = 9.8$ Hz, H-4), 3.43 (s, 3H, OCH_3).

The ^{1}H NMR spectra in pyridine of compouds **10-12** were identical with those reported.[14]

Methyl 4-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (14). To the solution of **11** and **10** in a 2:1 ratio (342 mg, 0.7 mmol) and **9** (577 mg, 1.4 mmol) in dry CH_2Cl_2 (7 mL) under argon at rt was added a 0.02 M solution of TMSOTf (350 μL) in dry CH_2Cl_2. After 2 h the mixture was neutralised with $NaHCO_3$, filtered and concentrated *in vacuo*. The residue was purified by silica gel CC (diethyl ether/hexane 4:6) to yield **14** (318 mg, 87%); Rf 0.45 (diethyl ether/hexane 8:2). This product was used in the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21-7.36 (15H, H-Ph), 6.03 (t, 1H, $J_{3,2} = J_{3,4}$ 9.8 Hz, H-3 Glc), 5.31-4.92 (6H, protons on oxygen-bearing carbons), 4.83 (dd, 1H, $J_{6',6} = 12.2$ Hz, H-6 Glc), 4.59 (dd, 1H, $J_{6',6} = 12.2$ Hz, $J_{6',5} = 4.1$ Hz, H-6' Glc), 4.23 (m, 1H, H-5 Glc), 4.12 (t, 1H, $J_{4,5} = J_{4,3} = 9.8$ Hz, H-4 Glc), 3.79 (m, 1H, H-5 Rha), 3.45 (s, 3H, OCH_3), 2.03 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$), 0.72 (d, 3H, $J_{6,5} = 6.1$ Hz H-6 Rha).

Methyl 4-*O*-α-L-rhamnopyranosyl-α-D-glucopyranoside (16). A solution of **14** (32 mg, 0.04 mmol) in dry MeOH (1 mL) was treated with 1 M NaOMe/MeOH (0.2 mL). After 1 h the solution was neutralised with Amberlite IR-120H$^+$, filtered through cotton and concentrated *in vacuo*. The residue was **16** (13 mg, 93%); Rf 0.63 (isopropyl alcohol/water 9:1). This product was used directly for preparetion of **1**. ^{1}H NMR (400 MHz, D_2O) δ 4.84 (brs, 1H, H-1 Rha), 4.79 (d, $J_{1,2} = 3.9$ Hz, H-1 Glc),

4.12-3.40 (10H, protons on oxygen-bearing carbons), 3.39 (s, 3H, OCH_3), 1.24 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 4-*O*-α-L-rhamnopyranosyl-α-D-glucopyranosiduronic acid (1). A solution of **16** (80 mg, 0.20 mmol), TEMPO (0.20 mg, 1.50 μmol), KBr (11 mg, 0.10 mmol) in deionised water (10 mL) was cooled to 0 °C in an ice-water bath. Sodium hypochlorite (4% water solution, 400 μL) was added dropwise to the mixture. The pH value was kept at 10-11 by dropwise addition of a 0.5 N NaOH solution. After a reaction time of 1 h MeOH (30 mL) was added and the solution was neutralised with 4 M HCl. The mixture was concentrated to give a solid, which was purified by a gel filtration chromatography to yield **1** as an amorphous solid (80 mg, 95%); Rf 0.17 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$+38.3° (*c* 1.3, water); ^1H and ^{13}C NMR see Table 1.

Anal. Calcd for $C_{13}H_{22}O_{11}$ (354.3): C, 44.1; H, 6.3. Found: C, 44.2; H, 6.3.

Methyl 3,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,6-di-*O*-benzoyl-α-D-glucopyranoside (15). A solution of **12** (206 mg, 0.5 mmol) and **9** (670 mg, 1.5 mmol) in dry CH_2Cl_2 (2 mL) was treated with 0.02 M TMSOTf/ CH_2Cl_2 (510 μL) as described for the synthesis of **14**. Usual work-up and silica gel CC (diethyl ether/hexane 4:6) afforded **15** (383 mg, 81%) Rf 0.45 (diethyl ether/hexane 8:2). This product was used in the next step without further purification. ^1H NMR (250 MHz, $CDCl_3$) δ 8.1-7.4 (10H, aromatic protons), 5.4-3.9 (17H, protons on oxygen-bearing carbons), 3.32 (s, 3H, OCH_3), 2.10 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.92 (s, 3H, $COCH_3$), 1.86 (s, 3H, $COCH_3$), 1.77 (s, 3H, $COCH_3$), 1.24 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha), 1.11 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 3,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranoside (17). Compound **15** (353 mg, 0.36 mmol) in dry MeOH (1 mL) was treated with 1 M NaOMe/MeOH (0.2 mL), as described for the synthesis of **16**. Usual work-up gave **17** (170 mg, 98%), Rf 0.64 (isopropyl alcohol/water 85:15). This product was used in the next step without further purification. ^1H NMR (250 MHz, D_2O) δ 5.31 (s, 1H, H-1 Rha), 5.09 (s, 1H, H-1 Rha), 4.77 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1 Glc), 4.14-3.70 (12H, protons on oxygen-bearing carbons), 3.44 (m, 1H, H-4 Rha), 3.45 (m, 1H, H-4 Rha), 3.43 (s, 3H, OCH_3), 1.31 (d, 6H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 3,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (2). A solution of **17** (180 mg, 0.37 mmol), TEMPO (0.39 mg, 2.7 μmol), KBr (17.4 mg, 0.15 mmol), deionised water (1 mL) and sodium hypochlorite (4% water solution, 2.4 mL) was treated as described for the synthesis of **1**. Usual work-up and gel filtration chromatography afforded **2** (152 mg, 82%) as an amorphous solid; Rf 0.46 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$ -43.6 ° (*c* 4.3, water); ^1H and ^{13}C NMR see Table 2.

Anal. Calcd for $C_{19}H_{32}O_{15}$ (500.4): C, 45.6; H, 6.4. Found: C, 45.5; H, 6.3.

Methyl 2,3-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (18). A solution of **8** (127 mg, 0.45 mmol) and **9** (501 mg, 1.15 mmol) in dry CH_2Cl_2 (2 mL) was treated with 0.1 M TMSOTf/CH_2Cl_2 (90 μL) as described for the synthesis of **14**. Usual work-up and silica gel CC (diethyl ether/hexane 4:6) gave **18** (306 mg, 85%), Rf 0.23 (diethyl ether/hexane 8:2). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.53–7.28 (5H, H-Ph), 5.57 (s, 1H, C*H*Ph), 5.50-3.40 (17H, protons on oxygen-bearing carbons), 3.42 (s, 3H, OCH_3), 2.15 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.98 (s, 6H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.23 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha), 0.76 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,3-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranoside (19). A solution of **18** (255 mg, 0.31 mmol) in trifluoroacetic acid/$CHCl_3$/water 1:9:0.5 (5 mL) was stirred at 0 °C for 4 h. The solution was diluted with ethyl acetate, washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The residue was purified by silica gel CC ($CHCl_3$/MeOH 95:5) and yielded **19** (179 mg, 82%), Rf 0.12 (diethyl ether). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 6.81 (brs, 1H, OH), 6.52 (brs, 1H, OH), 5.40-3.50 (17H, protons on oxygen-bearing carbons), 3.39 (s, 3H, OCH_3), 2.13 (s, 6H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.22 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha), 1.21 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,3-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (20). To a solution of **19** (163 mg, 0.22 mmol) in dichloromethane (2 mL) was added a 0.39 mM CH_2Cl_2 solution of TEMPO (570 μL), and a saturated aqueous solution of $NaHCO_3$ (440 μL) containing KBr and tetrabutylammonium chloride (TBAC) in 0.05 M and 0.029 M, respectively. To the mixture stirred at 0 °C was added dropwise in 30 min a 0.305 M solution of NaOCl (2.05 mL) in saturated aqueous solution of $NaHCO_3$. After stirring at 0 °C for 1 h, the mixture was acidified to pH 3 with 1 M HCl, diluted with ethyl acetate, washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The residue was purified by silica gel CC ($CHCl_3$/MeOH 9:1) and yielded **20** (121 mg, 76%); Rf 0.3 (CH_2Cl_2/MeOH/AcOH 9:1:0.1). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 5.35–3.66 (15H, protons on oxygen-bearing carbons), 3.47 (s, 3H, OCH_3), 2.15 (s, 6H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.23 (d, 6H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,3-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (6). Compound **20** (110 mg, 0.15 mmol) in dry MeOH (2 mL) was treated with 1 M NaOMe/MeOH (0.5 mL), as described for the synthesis of **16**. Usual work-up of the residue gave **6** as an amorphous solid (127 mg, 87%); R_f 0.14 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$ -11.6° (*c* 2.0, water); ^1H and ^{13}C NMR see Table 2.

Anal. Calcd for $C_{19}H_{32}O_{15}$ (500.4): C, 45.6; H, 6.4. Found: C, 45.7; H, 6.2.

Methyl 2-*O*-benzoyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (21). Compound **8** (55 mg, 0.2 mmol) dissolved in dry CH_2Cl_2 (1 mL) was treated with 1-(benzoyloxy)benzotriazole (BtOBz, 47 mg, 0.2 mmol) and triethylamine (30 mL, 0.2 μmol) as reported.[15] The crude product was subjected to silica gel CC (diethyl ether/hexane 3:7) to afford **21** (68 mg, 90%); R_f 0.53 (diethyl ether/hexane 1:1); amorphous solid $[\alpha]_D$ + 103.0 (*c* 2.3, $CHCl_3$) [lit.[15] $[\alpha]_D$ + 107.0]; ^1H NMR (250 MHz, $CDCl_3$) δ 8.15–7.35 (10H, H-Ph), 5.61 (s, 1H, C*H*Ph), 5.09 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 5.06 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 4.41-4.32 (2H, H-3 and H-6'), 3.94 (m, 1H, H-5), 3.81 (t, 1H, $J_{4,5}$ =$J_{4,3}$ = 9.8 Hz, H-4), 3.65 (t, 1H, $J_{6,5}$ = $J_{6,6'}$= 9.8 Hz, H-6), 3.41 (s, 3H, OCH_3).

Methyl 2-*O*-benzoyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (22). A solution of **21** (347 mg, 0.89 mmol) and **9** (778 mg, 1.8 mmol) in dry CH_2Cl_2 (3 mL) was treated as described for the synthesis of **14** adding 0.02 M TMSOTf/CH_2Cl_2 (450 μL). After usual work-up, the residue was purified by silica gel CC (ethyl acetate/hexane 1:9→3:7) affording **22** (543 mg, 92%); R_f 0.44 (hexane/ethyl acetate 6:4). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 8.10–7.29 (10H, H-Ph), 5.61 (s, 1H, C*H*Ph), 5.29-3.69 (12H, protons on oxygen-bearing carbons), 3.40 (s, 3H, OCH_3), 1.95 (s, 3H, $COCH_3$), 1.93 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$), 0.78 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2-*O*-benzoyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranoside (23). A solution of **22** (480 mg, 0.73 mmol) in trifluoroacetic acid/$CHCl_3$/water 1:9:0.5 (15 mL) was treated as described for the synthesis of **19**. The residue was purified by silica gel CC (ethyl acetate/hexane 4:6) and yielded **23** (326 mg, 81%); Rf 0.77 ($CHCl_3$/MeOH 9:1). This product was used in the next step without further purification. ^1H NMR (250 MHz, $CDCl_3$) δ 8.08–7.39 (5H, H-Ph), 5.31-3.73 (12H, protons on oxygen-bearing carbons), 3.40 (s, 3H, OCH_3), 2.04 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$), 1.27 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2-*O*-benzoyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (24). Compound **23** (303 mg, 0.53 mmol) was oxidised as described for the synthesis of **20**, using TEMPO/CH_2Cl_2 (1.4 mL), $NaHCO_3$ sat./KBr/TBAC (520 μL) and $NaHCO_3$ sat./NaOCl (6.5 mL). Usual work-up and silica gel

CC (CH_2Cl_2/MeOH 99:1→95:5) yielded **24** (285 mg, 82%); Rf 0.30 ($CHCl_3$/MeOH/AcOH 9:1:0.1). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01–7.44 (5H, H-Ph), 5.22-4.85 (7H, protons on oxygen-bearing carbons), 4.45 (m, 1H, 5-Rha), 4.03 (d, 1H, $J_{5,4}$ = 10.2 Hz, 5-GlcA), 3.79 (t, 1H, $J_{4,3} = J_{4,5}$ 9.8 Hz, H-4 GlcA), 3.44 (s, 3H, OCH_3), 2.01 (s, 3H, $COCH_3$), 1.88 (s, 3H, $COCH_3$), 1.85 (s, 3H, $COCH_3$), 1.16 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 3-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (3). Compound **24** (255 mg, 0.44 mmol) in dry MeOH (2 mL) was deprotected using 1M NaOMe/MeOH (0.5 mL) as described for the synthesis of **16.** Usual work-up yielded **3** as an amorphous solid (133 mg, 86%); Rf 0.18 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$ +31.3° (*c* 1.7, water); ^1H and ^{13}C NMR see Table 1.

Anal. Calcd for $C_{13}H_{22}O_{11}$ (354.3): C, 44.1; H, 6.3. Found: C, 44.1; H, 6.2.

Methyl 2-*O*-benzoyl-3-*O*-benzyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (25). Trifluoromethanesulfonic acid (TfOH, 161 μL) was added to a stirred solution of **21** (1.817 g, 4.7 mmol) and benzyl trichloroacetimidate (2.22 mL, 11.8 mmol) in dry CH_2Cl_2 (10 mL). After 7 h the solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The residue was purified by silica gel CC (diethyl ether/hexane 2:8) and yielded **25** (1.608 g, 72%); Rf 0.63 (diethyl ether/hexane 1:1). This product was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 8.15–7.35 (15H, H-Ph), 5.61 (s, 1H, C*H*Ph), 5.20-5.05 (2H, H-1, H-2), 4.85 (ABq, 2H, J_{gem}= 11.5 Hz, O*CH*$_2$Ph), 4.25 (dd, 1H, $J_{6',5}$ = 4.3 Hz, $J_{6,6'}$= 12.5 Hz, H-6'), 4.20-3.74 (4H, H-3, H-4, H-5, H-6), 3.41 (s, 3H, OCH_3).

Methyl 3-*O*-benzyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (26). Compound **25** (758 mg, 1.59 mmol) in dry MeOH (2 mL) was treated with 1 M NaOMe/MeOH (0.5 mL) as described for the synthesis of **16.** Usual work-up and silica gel CC (diethyl ether/hexane 3:7→1:1) afforded **26** (550 mg, 93%); Rf 0.23 (diethyl ether/hexane 7:3); ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.28 (10H, H-Ph), 5.59 (s, 1H, C*H*Ph), 4.92 (ABq, 2H, J_{gem}=11.5 Hz, O*CH*$_2$Ph), 4.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.31 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.89-3.65 (5H, protons on oxygen-bearing carbons), 3.47 (s, 3H, OCH_3). The ^1H NMR spectrum was identical with the one reported.[24]

Methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3-*O*-benzyl (*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (27). Compound **26** (36 mg, 0.1 mmol) and **9** (90 mg, 0.21 mmol) in dry CH_2Cl_2 (2 mL) was treated as described for the synthesis of **14**, using 0.01 M TMSOTf/CH_2Cl_2 (97 μL). Usual work-up and silica gel CC gave **27** (55 mg, 88%); Rf 0.52 (diethyl ether/hexane 7:3). This product was used in

the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.51–7.20 (10H, H-Ph), 5.58 (s, 1H, C*H*Ph), 5.50-3.40 (12H, protons on oxygen-bearing carbons), 3.43 (s, 3H, OCH_3), 2.14 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.24 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranoside (28). A solution of **27** (247 mg, 0.38 mmol) in MeOH (2 mL) was added to a stirred suspension of Pd-C (10%, 3 g) in MeOH (16 mL) under argon containing 10% of formic acid. The suspension was placed in a sonic bath, the temperature of which rose from 20 °C to 50 °C in 2 h. The suspension was decanted, filtered through Celite and the filtrates were concentrated *in vacuo*. The residue was purified by silica gel CC ($CHCl_3$-MeOH 98:2→9:1) and yielded **28** (88 mg, 57%); Rf 0.41 ($CHCl_3$/MeOH 9:1). This product was used in the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.38–5.28 (2H, H-2 Rha and H-3 Rha), 5.08 (t, 1H, $J_{4,3} = J_{4,5}$ = 9.4 Hz, H-4 Rha), 4.93 (d, 1H, $J_{1,2}$ = 0.9 Hz, H-1 Rha), 4.84 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1 Glc), 4.07 (m, 1H, H-5 Rha), 3.92 (t, 1H, $J_{3,4} = J_{3,2}$ = 9.7 Hz, H-3 Glc), 3.68-3.54 (4H, H-4 Glc, H-5 Glc, H-6 and H-6' Glc), 3.45 (dd, 1H, $J_{2,3}$ = 9.8 Hz H-2 Glc), 3.40 (s, 3H, OCH_3), 2.15 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.22 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (29). Compound **28** (150 mg, 0.30 mmol) was oxidised as described for the synthesis of **20**, using TEMPO/CH_2Cl_2 (820 μL), $NaHCO_3$ sat./KBr/TBAC (640 μL) and $NaHCO_3$ sat./NaOCl (3.0 mL). Usual work up gave **29** (110 mg, 72%), which was used in the next step without further purification. Rf 0.26 ($CHCl_3$/MeOH/water 8:2:0.1). ^{1}H NMR (250 MHz, CD_3OD) δ 5.50–3.40 (10H, protons on oxygen-bearing carbons), 3.44 (s, 3H, OCH_3), 2.14 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.22 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (4). A solution of **29** (110 mg, 0.20 mmol) in dry MeOH (5 mL) was treated with 1 M NaOMe/MeOH (2.5 mL) as described for the synthesis of **16**. Usual work up yielded **4** as an amorphous solid (70 mg, 87%); Rf 0.16 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$ +26.7° (*c* 0.43, water); ^{1}H and ^{13}C NMR see Table 1.

Anal. Calcd for $C_{13}H_{22}O_{11}$ (354.3): C, 44.1; H, 6.3. Found: C, 44.3; H, 6.1.

Methyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-glucopyranoside (30). A saturated ethereal HCl solution was added to a 0 °C stirred mixture of **25** (77 mg, 0.16 mmol) and $NaCNBH_3$ (102 mg, 1.62 mmol) in dry tetrahydrofuran (5 mL) until the solution became acid. After 1 h at 0 °C the mixture was poured into ice-water, and the product was extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$

solution, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The residue was purified by silica gel CC (diethyl ether/hexane 2:8→4:6) and yielded **30** (63 mg, 84%); Rf 0.30 (diethyl ether/hexane 1:1). This product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.22 (15 H, H-Ph), 5.11 (2H, H-1 and H-2), 4.81 (AB_q, 2H, J_{gem} = 11.5 Hz, OC*H*$_2$Ph), 4.64 (AB_q, 2H, J_{gem} = 11.5 Hz, OC*H*$_2$Ph), 4.04 (t, 1H, $J_{3,2} = J_{3,4}$ = 9.8 Hz, H-3), 3.88-3.76 (4H, H-4, H-5, H-6', H-6), 3.40 (s, 3H, OCH_3), 2.53 (d, 1H, $J_{4,OH}$ =1.96 Hz, OH-4).

Methyl 3,6-di-*O*-benzyl-α-D-glucopyranoside (31). A solution of **30** (310 mg, 0.65 mmol) in dry MeOH (3 mL) was treated with 1M NaOMe/MeOH (0.5 mL) as described for the synthesis of **16**. Usual work-up and silica gel CC (diethyl ether/hexane 4:6) afforded **31** (220 mg, 93%); Rf 0.44 (diethyl ether/hexane 9:1); amorphous solid $[\alpha]_D$ +72.5° (*c* 2.9, $CHCl_3$) [lit.[25] $[\alpha]_D$ +70.1°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.27 (10H, H-Ph), 4.90 (AB_q, 2H, J_{gem} = 11.6 Hz, OC*H*$_2$Ph), 4.80 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1), 4.62 (AB_q, 2H, J_{gem} = 11.5 Hz, OC*H*$_2$Ph), 3.82-3.61 (6H, protons on oxygen-bearing carbons), 3.47 (s, 3H, OCH_3), 2.63 (brs, 1H, OH), 2.44 (brs, 1H, OH).

Methyl 2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,6-di-*O*-benzyl-α-D-glucopyranoside (32). A solution of **31** (67 mg, 0.18 mmol) and **9** (234 mg, 0.54 mmol) in dry CH_2Cl_2 (2 mL) was treated as described for the synthesis of **14**, with 0.02 M TMSOTf/CH_2Cl_2 (180 μL). Usual work-up and silica gel CC (diethyl ether/hexane 3:7) gave **32** (128 mg, 80%); Rf 0.84 ($CHCl_3$/MeOH 98:2). This product was used in the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.42–7.22 (10H, H-Ph), 5.41-3.62 (21H, protons on oxygen-bearing carbons), 3.41 (s, 3H, OCH_3), 2.08 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.01 (s, 6H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.22 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha), 0.85 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranoside (33). A solution of **32** (70 mg, 0.08 mmol) in MeOH (2 mL) and the suspension of Pd-C (10%, 350 mg) in MeOH (10 mL) containing 10% of formic acid were treated as described for the synthesis of **28**. The residue was purified by silica gel CC ($CHCl_3$/MeOH 98:2→95:5) to yield **33** (36 mg, 69%); Rf 0.48 ($CHCl_3$/MeOH 9:1). This product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44–3.37 (17H, protons on oxygen-bearing carbons), 3.42 (s, 3H, OCH_3), 2.18 (s, 6H, $COCH_3$), 2.08 (s, 6H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.25 (d, 6H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (34). Compound **33** (33 mg, 0.04 mmol) was oxidised as described for the synthesis of **20**, using TEMPO/CH_2Cl_2 (115 μL), $NaHCO_3$

sat./KBr/TBAC (90 μL) and $NaHCO_3$ sat./NaOCl (420 μL). Usual work-up gave **34** (33 mg, 98%), used in the next step without further purification; Rf 0.5 (CH_2Cl_2/MeOH/AcOH 9:1:0.1); ^1H NMR (250 MHz, $CDCl_3$) δ 5.37–3.55 (15H, protons on oxygen-bearing carbons), 3.46 (s, 3H, OCH_3), 2.15 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.25 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha), 1.22 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6 Rha).

Methyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranosiduronic acid (5). A solution of **34** (33 mg, 0.04 mmol) in dry MeOH (2 mL) was treated with 1 M NaOMe/MeOH (0.5 mL) as described for the synthesis of **16**. Usual work up yielded **5** as an amorphous solid (21 mg, 95%); Rf 0.18 (isopropyl alcohol/water/AcOH 9:1:0.1); $[\alpha]_D$ -12.8° (*c* 2.1, water); ^1H and ^{13}C NMR see Table 2.

Anal. Calcd for $C_{19}H_{32}O_{15}$ (500.4): C, 45.6; H, 6.4. Found: C, 45.5; H, 6.5.

ACKNOWLEDGEMENTS

NMR spectra were recorded at the Centro di Metodologie Chimico-Fisiche of the University Federico II of Naples. This research was supported by Ministero dell' Università e della Ricerca Scientifica e Tecnologica and by Consiglio Nazionale delle Ricerche.

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