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Synthesis of rhamnogalacturonan I oligosaccharides: synthesis of a tetrasaccharide intermediate

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Abstract

A hydroxyl protected tetrasaccharide intermediate, corresponding to a segment of the rhamnogalacturonan I polysaccharide, has been synthesized using the glycosyl imidate technique. This tetrasaccharide is designed to allow for further elongation and branching.

The study of the molecular mechanisms of plant glycobiology is a new and expanding field. It is becoming clear that the role of complex carbohydrates in plants goes beyond energy storage molecules or structural elements.[1–4] Rhamnogalacturonan I (RG-I) is a complex polysaccharide found in plant tissues. The backbone of RG-I is composed of an [–α-D-galactopyranosyluronic acid-(1-2)-α-L-rhamnopyranosyl-(1-4)–] repeating unit. The 4-position of the rhamnosyl residues are substituted with side chains containing L-arabinose, D-galactose, L-fucose and D-glucuronic acid residues in linear and branched configurations.[5] Monoclonal antibodies are being used as molecular probes to investigate the function of this and other plant polysaccharides in plant growth and development.[6,7] The results of these types of studies are meaningful only if the binding specificities of the antibodies in use are fully understood. Oligosaccharides of known structure and high purity are invaluable, when used in competitive binding assays, in characterizing the specificity of an antibody binding site. It is difficult to obtain pure samples of oligosaccharides in sufficient quantities for such studies, hence the need to prepare such structures by chemical synthesis. This paper describes the overall strategy for the synthesis of branched RG-I oligosaccharides, and the synthesis of a tetrasaccharide intermediate corresponding to a segment of the RG-I polysaccharide backbone (Fig. 1).

Tetrasaccharide **1** is a key intermediate in our overall synthetic strategy to synthesize RG-I oligosaccharides. The protecting group pattern of compound **1** is designed so that the 2-*O*-acetyl group of the terminal rhamnosyl unit may be selectively removed. This allows for chain extension by addition of disaccharide **21**. Alternatively, removal of the 4-*O*-allyl groups of the rhamnosyl residues allows for addition of further glycosyl units to give branched oligosaccharides. Finally, removal of the blocking

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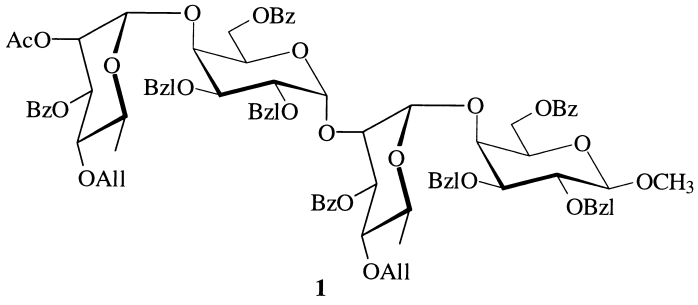

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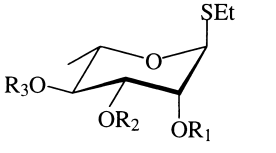
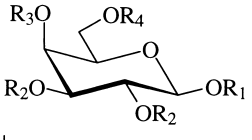
Figure 1.

groups, and oxidation[8] of the primary hydroxyls of the galactosyl residues introduces the carboxylic acid groups found in the native RG-I polysaccharide. This strategy allows the oligosaccharide to be built up using the more stable galactose as a temporary substitute for galacturonic acid.

The rhamnosyl donor **6** was prepared from the ethyl thioglycoside **2**[9] in a series of reactions that was performed without purification between steps (Scheme 1). Compound **2** was treated with trimethyl orthoacetate in dimethylformamide and a catalytic amount of *p*-toluenesulfonic acid. The resulting orthoester **3** was then treated with allyl bromide in dimethylformamide to give **4**. The orthoester of **4** was then opened under acidic conditions to give the 2-*O*-acetyl derivative **5**. The hydroxyl group of **5** was then benzoylated to give the fully blocked rhamnosyl donor **6**.



		R_1	R_2	R_3
a	**2**	H	H	H
b	**3**	$CCH_3(OCH_3)$		H
b	**4**	$CCH_3(OCH_3)$		All
c	**5**	Ac	H	All
d	**6**	Ac	Bz	All



		R_2	R_3	R_4
e	**7, 12**	H	H	H
e	**8, 13**	H	CHPh	
f	**9, 14**	Bzl	CHPh	
g	**10, 15**	Bzl	H	H
h	**11, 16**	Bzl	H	Bz

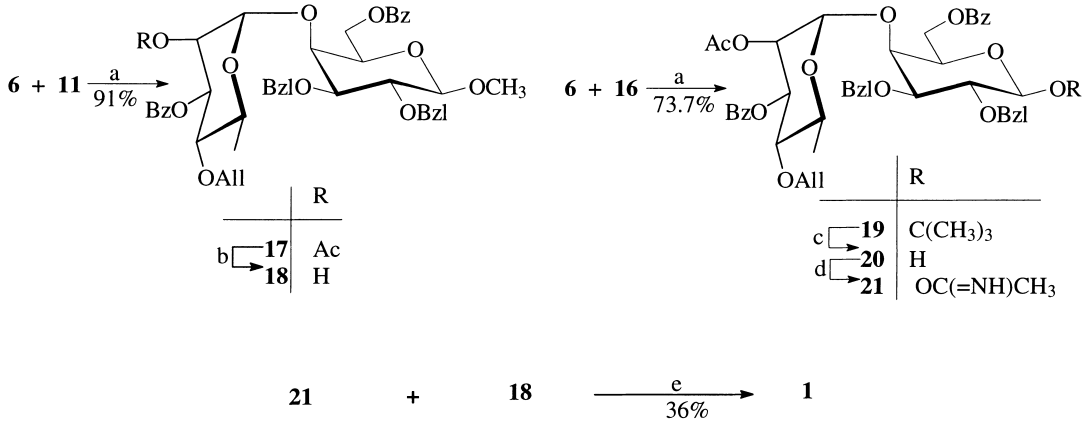
For compounds **7-11** R_1=CH_3, and for compounds **12-16** R_1=$C(CH_3)_3$

Scheme 1. *Reagents and conditions*: (a) trimethyl orthoacetate, PTSA, in DMF; (b) allyl bromide, NaH, in DMF; (c) 80% aqueous acetic acid; (d) benzoyl chloride in pyridine, **6** from **2** 69.8% yield; (e) α,α-dimethoxytoluene, PTSA, in DMF, 80°C, **8** from **7** 79.3% yield, **13** from **12** 45% yield; (f) benzyl bromide, NaH, in DMF: (g) HCl in MeOH (0.5 mL of a 1.0 M soln), in CH_2Cl_2; (h) benzoyl chloride, NaOH (1.25 M)/CH_2Cl_2, $[CH_3(CH_2)_3]_4N(HSO_4)$, **11** from **8** 70.1% yield, **16** from **13** 60.3% yield

Galactosyl acceptor **11** was prepared starting with methyl β-D-galactopyranoside **7**. The 2- and 3-positions were protected as benzyl ethers by treatment of the 4,6-benzylidene derivative **8** with benzyl bromide and sodium hydride in dimethylformamide to give **9**. The benzylidene group was removed and the resulting diol **10** was selectively benzoylated under phase transfer conditions[10] to give the 6-*O*-benzoyl derivative **11**. The galactosyl donor **16** was prepared in a similar series of reactions starting from *tert*-butyl β-D-galactopyranoside **12**[11] (prepared from tetra-*O*-acetyl-D-galactopyranosyl bromide and *tert*-butanol).

Reaction of **11** with **6** in dichloromethane with *N*-iodosuccinimide and a catalytic amount of triflic acid[12,13] gave the disaccharide **17** (Scheme 2). Similarly, reaction of **16** with **6** gave disaccharide **19**. Selective removal of the 2-*O*-acetyl group of **17** was achieved by treatment with methanolic HCl[14] to

give disaccharide **18**. The ^{1}H-NMR spectrum of **18** showed the loss of the signal due to the acetyl group, and the H-2 of the rhamnosyl residue had shifted to 4.34 ppm for compound **18**, from 5.61 ppm for compound **17**, consistent with deacetylation of the 2′-position. Disaccharide **19** was transformed into a glycosyl donor by treatment of a dichloromethane solution of **19** with trichloroacetic acid to give a mixture of hemiacetals **20**,[15,16] which where then reacted with trichloroacetonitrile and DBU[17] to give the trichloroacetimidate derivative **21**. Disaccharides **18** and **21** were then reacted together with catalytic amounts of trimethylsilyl triflate[18] to give the tetrasaccharide **1**.[19] The newly formed glycosidic linkage between the galactosyl and the rhamnosyl residues of **1** was formed in the desired α-configuration. This determination was made based on the ^{13}C–^{1}H coupling constant for C-1 of the internal galactosyl unit. This coupling constant was measured from a ^{1}H-detected ^{13}C–^{1}H correlation spectrum of **1** and found to be 159 Hz; a value characteristic of α-glycosidic linkages. One-dimensional and two-dimensional NMR spectra[20] for all products were consistent with the proposed structures.



Scheme 2. *Reagents and conditions*: (a) *N*-iodosuccinimide, triflic acid (cat.), in CH_2Cl_2; (b) methanolic HCl in CH_2Cl_2, 58.5% yield; (c) trichloroacetic acid, in CH_2Cl_2, 62%; (d) trichloroacetonitrile, DBU, in CH_2Cl_2; (e) trimethylsilyl triflate in CH_2Cl_2

In a test reaction, using disaccharide **17** as a model compound for tetrasaccharide **1**, the 4-*O*-allyl group of **17** was successfully removed. This result indicates that selective deprotection of the 4-positions of tetrasaccharide **1** should be possible, and hence generation of branched oligosaccharides. Tetrasaccharide **1** represents a key synthetic intermediate in the synthesis of branched chain oligosaccharides corresponding to sequences found in the RG-I polysaccharide.

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19. The yield for this reaction is not optimised. The main product of the reaction on TLC corresponded to the isolated tetrasaccharide; however, the reaction was carried out on relatively small scale (33 mg of **18**) and losses were incurred during work-up and chromatography.
20. ^{1}H (300.13 MHz) NMR data for selected compounds. **1**: Chemical shifts for ^{1}H and ^{13}C were measured from a COSY spectrum and a ^{1}H-detected ^{13}C–^{1}H correlation spectrum, respectively. 8.15–7.10 (40H, aromatic), 5.77 (2H, $OCH_2CH{=}CH_2$ for the two allyl groups), 5.66 (H-2), 5.60 (H-3), 5.54 (H-3), 5.29 (H-1), 5.06 (H-1), 4.81 and 4.50 (ABq, OCH_2Ph), 4.79 and 4.61 (ABq, OCH_2Ph), 4.78 and 4. 62 (ABq, OCH_2Ph), 4.63 (H-6$_a$), 4.56 (H-6$_b$), 4.53 (H-1), 4.51 and 4.38 (ABq, OCH_2Ph), 4.42 (H-2), 4.32 (H-1), 4.13 (H-4), 4.03 (H-5), 4.00 (H-5), 3.96 (H-4), 3.95 (H-6$_a$), 3.89 (H-5), 3.88 (H-3 and H-6$_b$), 3.83 (H-2), 3.80 (H-5), 3.75 (H-2), 3.60 (3H, OCH_3), 3.61 (H-4), 3.52 (H-3), 3.46 (H-4), 1.98 (3H, $OCOCH_3$), 1.37 (3H, H$_3$-6), 1.19 (3H, H$_3$-6); ^{13}C (75.47 MHz) 105.23 ($J_{^{13}C-\,^{1}H}$ 159 Hz, C-1), 99.81 ($J_{^{13}C-\,^{1}H}$ 173 Hz, C-1), 98.39 ($J_{^{13}C-\,^{1}H}$ 168 Hz, C-1), 94.78 ($J_{^{13}C-\,^{1}H}$ 169 Hz, C-1). **18**: 8.07 (m, 4H, aromatic), 7.63–7.20 (m, 16H, aromatic), 5.80 (m, 1H, $OCH_2CH{=}CH_2$), 5.46 (dd, 1H, $J_{3',2'}$ 3.0 Hz, $J_{3',4'}$ 7.0 Hz, H-3′), 5.19 and 5.08 (m, 2H, $OCH_2CH{=}CH_2$), 5.17 (1H, H-1′), 4.93 and 4.74 (ABq, 2H, $J_{A,B}$ 11.0 Hz, OCH_2Ph), 4.79 and 4.61 (ABq, 2H, $J_{A,B}$ 11.5 Hz, OCH_2Ph), 4.59 (dd, 1H, $J_{6b,5}$ 6.5 Hz, $J_{6a,6b}$ 11.0 Hz, H-6$_a$), 4.51 (dd, 1H, $J_{6b,5}$ 5.9 Hz, H-6$_b$), 4.34 (m, 1H, H-2′), 4.30 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.23 and 4.11 (m, 2H, $OCH_2CH{=}CH_2$), 4.05 (m, 1H, H-4), 3.97 (dq, 1H, $J_{5',4'}$ 9.0 Hz, H-5′), 3.77 (m, 2H, H-5 and H-2), 3.58 (s, 3H, OCH_3), 3.54 (dd, 1H, $J_{3,4}$ 3.0 Hz, $J_{3,2}$ 8.5 Hz, H-3), 3.50 (dd, 1H, H-4′), 2.29 (m, 1H, $OH_{2'}$), 1.31 (d, 3H, $J_{6'5'}$ 6.0 Hz, H$_3$-6′). **19**: 8.01 (m, 4H, aromatic), 7.61–7.17 (m, 16H, aromatic), 5.79 (m, 1H, $OCH_2CH{=}CH_2$), 5.64 (t, 1H, $J_{2',1'+2',3'}$ 5.5 Hz, H-2′), 5.56 (dd, 1H, $J_{3',2'}$ 3.2 Hz, $J_{3',4'}$ 8.5 Hz, H-3′), 5.20 (d, 1H, $J_{1',2'}$ 2.5 Hz, H-1′), 5.18 and 5.07 (m, 2H, $OCH_2CH{=}CH_2$), 4.97 and 4.77 (ABq, 2H, $J_{A,B}$ 10.5 Hz, OCH_2Ph), 4.77 and 4.63 (ABq, 2H, $J_{A,B}$ 11.3 Hz, OCH_2Ph), 4.53 (m, 2H, H-6$_a$ and H-6$_b$), 4.51 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.18 and 4.09 (m, 2H, $OCH_2CH{=}CH_2$), 4.06 (m, 1H, H-4), 4.02 (dq, 1H, $J_{5',4'}$ 9.2 Hz, H-5′), 3,80 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.75 (m, 1H, H-5), 3.52 (dd, 1H, $J_{3,4}$ 3.0 Hz, H-3), 3.48 (t, 1H, $J_{4',3'+4'5'}$ 17.5 Hz, H-4′), 1.95 (s, 3H, $OCOCH_3$), 1.35 (d, 3H, $J_{6'5'}$ 6.0 Hz, H$_3$-6′), 1.30 (s, 9H, $OC(CH_3)_3$).