Synthesis of Trisaccharides Related to the Branching Region of the O-Antigen Polysaccharides from *Escherichia coli* O35 and *Salmonella arizona* O62

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The synthesis of three trisaccharides related to the branching region in the O-antigen polysaccharides from *E. coli* O35 and *S. arizona* O62 is reported. A regioselective β-glycosylation was performed at O-3 of a methyl α-L-rhamnopyranoside derivative. *cis*-Glycosylation of an α-D-galactosamine derivative at O-2 of the methyl α-L-rhamnopyranoside took place in excellent yield (90%) to produce, after deprotection, compound **1**. Functionalization at C-6 of the α-D-galactosamine residue by regioselective TEMPO oxidation resulted in the corresponding uronic acid, which subsequently gave **2**. Treatment of an activated ester, derived from the uronic acid, with ammonia provided the desired carboxamide, and subsequently **3**.

Introduction

Carbohydrates are commonly found as glycoconjugates in biological systems.[1] These include glycolipids on cell surfaces and glycoproteins in serum. Oligosaccharides of different structure form the basis for biological determinants such as blood groups on erythrocytes. Bacteria, which are usually surrounded by polysaccharides, can be divided into Gram-positive and Gram-negative. In the latter a lipopolysaccharide (LPS) is present.[2] This is a complex molecule consisting of lipids that anchors the saccharide part, which can be of different degrees of polymerization, in the cell membrane. The LPS often contains repeating units of three to six sugar residues with a degree of polymerization usually between 10 and 30. This part is known as the O-antigen and is responsible for the antigenic behavior of the bacteria and can be used for serotyping.

We recently determined the structure of the O-antigen polysaccharide from *Escherichia coli* O35, which has six sugars in the repeating unit.[3] The structure is branched with a β-linked 2-acetamido-2-deoxy-D-glucose residue (β-D-Glc*p*NAc) at O-3, and an α-linked 2-acetamido-2-deoxy-D-galacturonamide residue (α-D-Gal*p*NAcAN) at O-2, of an α-linked L-rhamnose residue (α-L-Rha*p*). This part of the structure is the basis for the target trisaccharide **3** (see Figure 1).

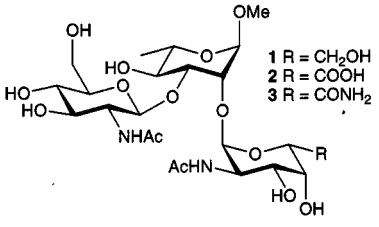

Figure 1. Synthesis target trisaccharides **1**−**3**

A detailed analysis of the ^{1}H NMR spectra also indicated that the polymer consisted of eight repeating units and that the biological repeating unit was terminated by a β-D-Glc*p*NAc residue. Thus, the branched trisaccharide element is at the non-reducing end of the polymer. The structure of the O-antigen is quite similar to that of *Salmonella arizona* O62,[4] the only difference being a terminal α-linked 2-acetamido-2-deoxy-α-D-galacturonic acid residue (α-D-Gal*p*NAcA) instead of the α-D-Gal*p*NAcAN residue. Subsequently, the target trisaccharide is given by **2**. It could also be shown that a rabbit antiserum specific for *Salmonella arizonae* O62 in an enzyme immunoassay reacts equally well with both LPSs thus confirming the similarity between the O-antigens, the only difference being a carboxyl function vs. a primary amide group.

Negative charges and the spacing between the carboxyl groups in polysaccharides have been shown to play an important role in the antigenicity of the polymer.[5] Thus, in order to probe such effects we set out to synthesize oligosaccharides of the branching region of the O-antigenic polysaccharides, including the uncharged target trisaccharide **1**.

Results and Discussion

In oligosaccharide synthesis, the oxidation to a carboxylic acid group followed by functionalization if necessary, can be performed prior to[6] glycosylation steps or afterwards.[7] In the latter case, additional complications due to a modified reactivity of either glycosyl donors or acceptors, or both, are not present. Also, the introduction of the carboxyl function at a late stage in the synthetic sequence facilitates further functionalization of a certain intermediate as will be shown below. 2,2,6,6-Tetramethyl-1-piperidinyloxy radical (TEMPO)[8] mediated oxidation has become a powerful tool for the regioselective oxidation of primary over secondary hydroxyl groups. We have previously de-

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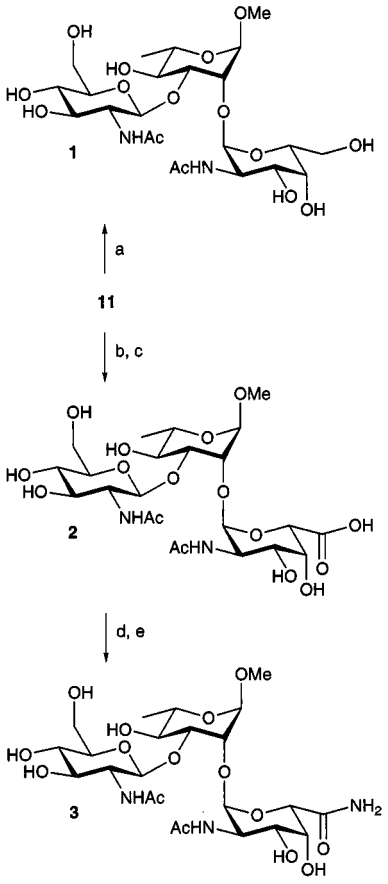
veloped a method for the determination of the absolute configuration of chiral glycerol residues in natural products based on TEMPO oxidation and analysis of the glyceric acids formed.[9] In our approach to synthesize the trisaccharides **1**−**3** (Scheme 1), the partially protected trisaccharide **11** becomes the key intermediate in the synthesis, since all three target trisaccharides are provided from the same precursor.



Scheme 1. Reagents and conditions: (a) 2 M HCl, 2 h, room temp., 88%; (b) TEMPO, NaOCl, NaOH aqueous, 1 h, room temp.; (c) 4 M HCl, room temp., 80% over two steps; (d) TSTU, DMF, 30 min., room temp.; (e) NH_3 (g), DMF, 45 min., 0 °C, 90% over two steps

This approach depends upon the efficiency of the regioselective TEMPO oxidation of a hydroxymethyl group into the desired carboxylic acid functionality, and subsequent transformation into a primary amide.

The key monosaccharide elements employed in the synthesis are shown in Figure 2.

The rhamnoside acceptor **4** was obtained from α-L-Rha*p*-OMe in 80% yield over three steps, by adapting a procedure previously described by Rainer et al.[10] The regioselective glycosylation of diol **4** with the glycosyl donor **5**[11] (Scheme 2) provided the disaccharide **7** (confirmed by 1D ^1H,^1H-NOESY NMR experiments) in 59% yield (H-1 of the β-linked residue showed $J_{1,2}$ = 8.3 Hz), together with the corresponding β-(1→2)-coupled disaccharide (19%) and the 2,3-disubstituted trisaccharide (12%).

By accepting a lower yield of **7** in this step, we avoided an inconvenient protecting group manipulation at both the

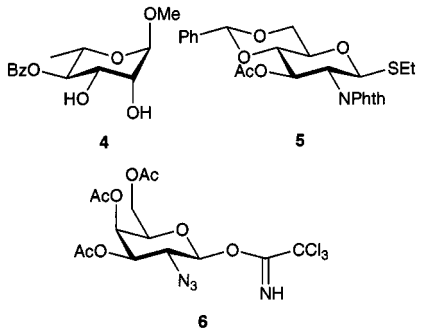
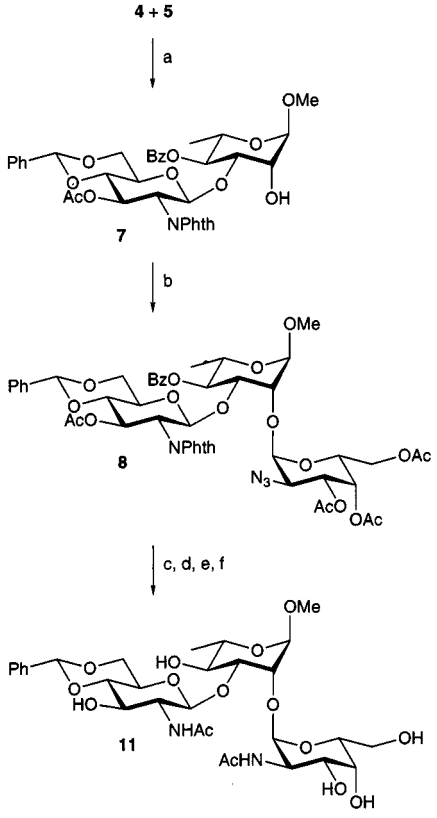


Figure 2. Key monosaccharides used in the synthesis



Scheme 2. Reagents and conditions: (a) NIS/AgOTf, CH_2Cl_2, −30 °C, 59%; (b) **6**, TMSOTf, Et_2O, −20 °C, 90%; (c) ethylenediamine, *n*-butyl alcohol, 4 h, 90 °C; (d) Ac_2O/pyridine, 1 h, room temp., 91% over two steps; (e) AcSH/pyridine, 36 h, room temp., 90%; (f) NaOMe/MeOH, 1.5 h, room temp., 89%

mono- and disaccharide levels. The subsequent installation of the α-galactosamine group was performed using the known trichloroacetimidate donor **6**.[12] The coupling in diethyl ether, mediated by a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf), afforded the trisaccharide **8** in 90% yield (H-1 of the α-linked residue showed $J_{1,2}$ = 3.1 Hz). The phthaloyl group was removed efficiently from **8** by treatment with ethylenediamine in *n*-butyl alcohol followed by acetylation with acetic anhydride/pyridine to furnish the intermediate trisaccharide **9** in 91% yield over two steps.

There are a wide variety of methods available for the transformation of an azide group to an *N*-acetyl function.

The galactosamine unit prompted us to look carefully for an efficient method. Reduction of the azide by 1,4-dithiothreitol (DTT) in the presence of diisopropylethylamine (DIPEA), and subsequent acetylation, would give the desired compound.[13] However, the yields were mediocre and large amounts of the reducing agent were needed. Instead, treating trisaccharide **9** with a mixture of thioacetic acid/pyridine (1:1)[14] gave the trisaccharide **10** in 90% yield. Removal of the *O*-acetyl groups under Zemplen conditions (NaOMe/methanol) gave the key trisaccharide **11** in 89% yield.

To provide the first target compound **1**, acidic hydrolysis of the 4,6-benzylidene-protected trisaccharide **11** with 2 M HCl gave, after gel filtration, **1** in 88% yield. In order to maintain high regioselectivity and a short reaction time the TEMPO oxidation should be conducted in a pH range of 9−11.[15] The TEMPO oxidation of trisaccharide **11** was conducted at pH 9.5−10.5, and subsequent hydrolysis of the 4,6-benzylidene group gave, after lyophilization and purification by gel filtration, **2** in 80% yield. In order to facilitate the amide formation, the carboxylic acid **2** was activated using *O*-(*N*-succinimidyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TSTU)[16] in DMF as solvent. Treatment of the activated ester with ammonia provided the desired carboxamide **3** in 90% yield.

In summary, three functionalized trisaccharides have been synthesized where the key transformations were performed just prior to deprotection. A slightly lower yield (59%) was accepted at the early stage for the regioselective glycosidic coupling, which also alleviated some protecting group transformations. In all other cases, yields were ≥ 80%, representing an efficient and highly *divergent* synthesis.

Experimental Section

General: Concentrations were performed under reduced pressure at temperatures < 40 °C (bath). NMR spectra were recorded at 25 °C for solutions in $CDCl_3$ or [D_4]MeOH or at 27 °C in D_2O using 300−600 MHz (^{1}H frequency) spectrometers. Chemical shifts are reported in ppm relative to internal $SiMe_4$ (δ_H = 0.00, δ_C = 0.0), internal sodium 4,4-dimethyl-4-sila(2,2,3,3-D_4)pentanoate (δ_H = 0.00) or internal acetone (δ_C = 31.0). High Resolution Fast Atom Bombardment Mass Spectrometry (HR-FABMS) was performed in the positive mode at a resolution of 10 000 using triethylene glycol or 3-nitrobenzyl alcohol as a matrix.

Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-α-L-rhamnopyranoside (7): Compounds **4** (1 g, 3.54 mmol) and **5** (1.63 g, 3.34 mmol) were mixed together with 4 Å molecular sieves (2 g) in CH_2Cl_2 (20 mL), and cooled to −30 °C. NIS (910 mg, 4.04 mmol) and AgOTf (3.46 mg, 1.35 mmol) were added whilst stirring and the temperature was allowed to reach −10 °C. The reaction was complete after 30 min. and triethylamine (1 mL) was added. The reaction mixture was filtered through celite and concentrated. The crude residue was purified by flash column chromatography (silica gel, toluene/EtOAc 2:1) to give **7** (1.4 g, 59%). − ^{13}C NMR ($CDCl_3$): δ = 17.3, 20.4, 54.9, 55.1, 65.8, 66.2, 68.4, 69.4, 70.2, 71.9, 78.7, 79.4, 99.2, 99.8, 101.5, 122.9, 126.0−129.2, 132.5, 133.6, 136.5, 164.6, 169.7. − HR-FABMS [M + H]$^+$: calcd. for $C_{37}H_{38}NO_{13}$ 704.2343; found 704.2332.

Methyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→2)-[3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)]-4-*O*-benzoyl-α-L-rhamnopyranoside (8): Compounds **6** (642 mg, 1.386 mmol) and **7** (752 mg, 1.07 mmol) were mixed together with 4 Å molecular sieves (2 g) in Et_2O (30 mL) and cooled to −20 °C. A catalytic amount of TMSOTf (20 μL, 0.01 equiv.) was then added. After 20 min. TLC indicated complete conversion. The reaction was then quenched with triethylamine (1 mL), diluted with CH_2Cl_2 (20 mL), filtered through celite and concentrated. Column chromatography (silica gel, toluene/EtOAc 1:1) gave **8** (979 mg, 90%). − ^{13}C NMR ($CDCl_3$): δ = 17.1, 20.4, 20.7 (2C), 20.9, 54.8, 55.0, 57.2, 60.5, 66.1, 66.3, 66.7, 67.1, 67.7, 68.6 (2C), 72.9, 74.3, 74.4, 79.2, 96.0, 96.9, 99.6, 101.5, 122.9, 123.9, 125.1, 126.0, 128.0−129.6, 130.8, 131.0, 132.9, 133.4, 133.6, 136.6, 164.6, 166.9, 167.2, 168.7, 169.2, 169.7, 170.4. − FABMS: [M + H]$^+$ *m*/*z* = 1017.1.

Methyl 2-Deoxy-2-*N*-acetyl-α-D-galactopyranosyl-(1→2)-[4,6-*O*-benzylidene-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranoside (11): Ethylenediamine (1 mL) was added to a suspension of trisaccharide **8** (413 mg, 0.406 mmol) in *n*-butyl alcohol (10 mL). The reaction mixture was then stirred for 4 h at 90 °C. Evaporation to dryness was followed by azeotropic evaporation with toluene and then with ethanol. The resulting syrup was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) was added and stirred at room temperature. After 1 h the acetylation was finished. Evaporation and purification by flash column chromatography (silica gel, toluene/acetone 2:1) gave the intermediate trisaccharide **9** (397 mg, 91%).

A 1:1 v/v mixture (4 mL) of AcSH and pyridine was added to **9**. The reaction mixture was then stirred for 36 h. The mixture was concentrated with an N_2 flow and the residue purified by flash column chromatography (silica gel, toluene/EtOAc 1:1 followed by toluene/acetone 1:1) to give **10** (363 mg, 90%). − FABMS: [M + H]$^+$ *m*/*z* = 945.2.

The trisaccharide **10** was dissolved in methanol (10 mL) and treated with NaOMe (0.1 M, 5 mL). The reaction mixture was stirred for 1.5 h at room temperature. Column chromatography (silica gel, CH_2Cl_2/MeOH 3:1) gave **11** (230 mg, 89%). − ^{13}C NMR ([D_4]MeOH): δ = 18.1, 22.8, 23.1, 51.6, 55.2, 58.1, 62.5, 67.6, 69.5 (2C), 70.0, 70.1, 71.4, 72.6, 73.5, 76.3, 79.2, 82.6, 96.6, 99.3, 102.7, 104.8, 127.3, 128.8−130.4, 133.3, 138.9, 173.6, 173.7. − FABMS: [M + H]$^+$ *m*/*z* = 673.2.

Methyl 2-Deoxy-2-*N*-acetyl-α-D-galactopyranosyl-(1→2)-[2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranoside (1): The trisaccharide **11** (100 mg, 9.53 mmol) was treated with 2 M HCl for 2 h. When TLC (EtOAc/AcOH/MeOH/H_2O 12:3:3:1) indicated complete reaction the mixture was lyophilized, and the product was purified by gel filtration chromatography (Bio-Gel P-2, pyridinium acetate buffer, pH 5.4) to give **1** (50 mg, 88%). − ^{1}H NMR (D_2O): δ = 1.32 (d, *J* = 6 Hz, 3 H), 2.04 (s, 3 H), 2.06 (s, 3 H), 3.40 (s, 3 H), 3.42−3.46 (2 H), 3.54 (dd, 1 H), 3.59 (dd, *J* = 9.0 Hz, 1 H), 3.68−3.81 (5 H), 3.89−3.95 (3 H), 4.08 (2 H), 4.16 (dd, *J* = 3, 10 Hz), 4.39 (dd, *J* = 3 Hz, 1 H), 4.69 (d, *J* = 1.8 Hz, 1 H), 4.71 (d, *J* = 8 Hz, 1 H), 5.02 (d, *J* = 3 Hz, 1 H). − ^{13}C NMR (D_2O): δ = 17.4, 22.7, 23.0, 50.1, 55.5, 56.4, 62.1, 68.0, 69.0, 69.8, 70.7, 71.1, 72.4, 74.6, 75.4, 76.5, 78.2, 95.8, 98.5, 103.5, 174.9. − HR-FABMS [M + H]$^+$: calcd. for $C_{23}H_{41}N_2O_{15}$ 585.2507; found 585.2507.

Methyl 2-Deoxy-2-*N*-acetyl-α-D-galactopyranosyluronic acid-(1→2)-[2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranoside (2): Compound **11** (130 mg, 0.152 mmol) was dissolved in water. NaBr (2 mg, 0.02 mmol) and TEMPO (2 mg) were then added. A solution of 12%NaOCl, with pH set to 10 with 1 M HCl, was added to the reaction mixture. The pH in the reaction was carefully monitored between 9.5 and 10.5. After 1 h, TLC indicated that all the starting material was consumed. Acidification with 4 M HCl furnished the removal of the 4,6-benzylidene group. Lyophilization and purification by gel filtration chromatography (Bio-Gel P-2, water, 1% *n*-butyl alcohol) gave the target compound **2** (100 mg, 80%). − ^{1}H NMR (D_2O): δ = 1.28 (d, *J* = 6 Hz, 3 H), 2.01 (s, 3 H), 2.04 (s, 3 H), 3.21 (s, 3 H), 3.25 (dd, *J* = 10 Hz, 1 H), 3.47−3.56 (3 H), 3.60−3.69 (2 H), 3.71−3.76 (2 H), 3.98 (dd, *J* = 2, 10 Hz), 4.07 (dd, *J* = 3, 10 Hz), 4.15 (2 H), 4.37 (dd, *J* = 1, 3 Hz), 4.52 (d, *J* = 8 Hz, 1 H), 4.57 (d, *J* = 2 Hz, 1 H), 5.00 (1 H), 5.06 (d, *J* = 3 Hz, 1 H). − ^{13}C NMR (D_2O): δ = 17.4, 22.7, 23.0, 50.6, 55.6, 56.4, 61.5, 67.0, 69.0, 69.8, 70.7, 71.1, 72.4, 74.6, 75.4, 76.5, 78.2, 95.8, 98.5, 103.5, 175.0. − HR-FABMS $[M + H]^+$: calcd. for $C_{23}H_{39}N_2O_{16}$ 599.2300; found 599.2264.

Methyl 2-Deoxy-2-*N*-acetyl-α-D-galactopyranosyluronamide-(1→2)-[2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranoside (3): Compound **2** (50 mg, 0.0835 mmol) was dissolved in DMF (2 mL), and then *N*,*N*-diisopropylethylamine (22 μL, 0.125 mmol) and TSTU (38 mg, 0.125 mmol) were added. After 30 min. TLC (EtOAc/AcOH/MeOH/H_2O 12:3:3:1) indicated complete formation of the active ester. After cooling to 0 °C, ammonia was bubbled through the solution for 15 min., and the mixture was then left at 0 °C for 30 min. Residual ammonia was removed with air flow and the reaction mixture was applied to a Bio-Gel P-2 column from which the target compound **3** was isolated (46 mg, 90%). − ^{1}H NMR (D_2O): δ = 1.28 (d, *J* = 6 Hz, 3 H), 2.01 (s, 3 H), 2.04 (s, 3 H), 3.26 (dd, *J* = 10 Hz, 1 H), 3.38 (dd, *J* = 3, 10 Hz), 3.40 (s, 3 H), 3.53 (dd, *J* = 3, 10 Hz), 3.56 (dd, *J* = 10 Hz, 1 H), 3.36−3.73 (3 H), 3.80 (dd, *J* = 3, 10 Hz), 3.91 (dd, *J* = 3, 10 Hz), 4.05 (dd, 1 H, *J* = 3, 10 Hz), 4.18 (dd, *J* = 3, 10 Hz), 4.20 (dd, *J* = 1, 3 Hz), 4.37 (1 H), 4.60 (d, *J* = 8 Hz, 1 H), 4.69 (d, *J* = 2 Hz, 1 H), 4.93 (d, *J* = 1 Hz, 1 H), 5.12 (d, *J* = 3 Hz, 1 H). − ^{13}C NMR (D_2O): δ = 17.4, 22.7, 23.0, 50.2, 55.5, 56.6, 62.2, 67.7, 69.7 (2C), 71.1, 71.8, 71.9, 74.4, 75.1, 76.7, 79.3, 95.5, 98.3, 104.1, 174.3, 174.9, 175.0. − HR-FABMS $[M + Na]^+$: calcd. for $C_{23}H_{39}N_3NaO_{15}$ 620.2279; found 620.2276.

Acknowledgments

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