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THE SYNTHESIS OF (1→4)-LINKED α-L-RHAMNANS

NELLY N. MALYSHEVA AND NIKOLAY K. KOCHETKOV
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)
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ABSTRACT

(1→4)-Linked α-L-rhamnans with d.p. 22 and 40 have been obtained by triphenylmethylium perchlorate-catalysed polycondensation of 3-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-4-*O*-trityl-L-rhamnopyranose and 3-*O*-benzoyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-4-*O*-trityl-L-rhamnopyranose, respectively.

INTRODUCTION

Glycosylation, involving the interaction of trityl ethers with 1,2-*O*-cyanoethylidene derivatives of sugars in the presence of triphenylmethylium perchlorate[1], generates 1,2-*trans*-glycosidic linkages stereospecifically, and offers the possibility for the directed synthesis of polysaccharides of various types. A general method of synthesis of 1,2-*O*-cyanoethylidene derivatives of sugars[2] has made accessible the appropriate trityl ethers of cyanoethylidene derivatives of mono- and oligo-saccharides. The polycondensation of such monomers has been used to synthesise homo- and hetero-polysaccharides with a regular structure[6,7], including the O-antigenic polysaccharide of *Salmonella newington*[8,9]. In almost every reaction, a 1,2-*trans*-glycosidic linkage was formed; the only exception so far was the synthesis of a (1→3)-linked α-L-rhamnan[5], when a secondary, equatorial hydroxyl group was involved in glycosylation.

In extending the scope of this reaction, we now report on the synthesis of (1→4)-linked α-L-rhamnans.

RESULTS AND DISCUSSION

The monomer required for this synthesis was a derivative of 1,2-*O*-cyanoethylidene-4-*O*-trityl-β-L-rhamnose with a temporary protecting-group at O-3. In previous polycondensations of cyanoethylidene monomers[3–9], the acetate group was used for protection. Taking into account the influence of the nature of the protecting groups on the extent of polymerisation of sugar monomers[10], we performed the synthesis of (1→4)-linked α-L-rhamnans using monomers protected with acetyl and benzoyl groups.

Compounds **1** and **2** were obtained by selective substitution[5] of HO-3 in 1,2-*O*-cyanoethylidene derivatives of rhamnose. Treatment of 1,2-*O*-[1-(*exo*-cyano)ethyli-

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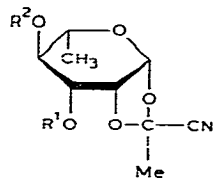

1 R^1 = Ac, R^2 = Tr
2 R^1 = Bz, R^2 = Tr
3 R^1 = R^2 = H
4 R^1 = Ac, R^2 = H
5 R^1 = Bz, R^2 = H

dene]-β-L-rhamnopyranose (**3**) with 1 equiv. of acetyl or benzoyl chloride in pyridine gave the acetate (**4**) and benzoate (**5**), respectively, in high yields as crystalline substances.

Tritylation of **4** and **5** with triphenylmethylium perchlorate in the presence of 2,4,6-collidine[11] proceeded with difficulty and produced **1** and **2** in yields of only 20–25%. Attempts to improve the yields by increasing the reaction time or the amount of tritylating agent failed. After column chromatography, **1** and **2** were obtained crystalline, and their structures were proved by p.m.r. spectroscopy. Location of the acetyl group in **1** and the benzoyl group in **2** at O-3 was indicated by the chemical shifts (δ 5.1 and 5.17, respectively) of the signals for H-3. The resonances for H-4 were at δ 3.32 and 3.65, respectively. The assignments of signals to H-3,4 were confirmed by double-resonance experiments. Tritylation of **4** and **5** causes marked upfield shifts of the resonances for Me groups (acetate, cyanoethylidene, and rhamnose). Additional proof of structure of **1** and **2** was effected by methylation analysis. Thus, application, in sequence, of deacylation, methylation, formolysis, hydrolysis, borohydride reduction, and acetylation afforded 3-*O*-methylrhamnitol tetra-acetate from **1** and **2** as the only product; this compound was identified by comparison (g.l.c.–m.s.) with an authentic sample.

The polycondensation of **1** and **2** was performed[9] in dichloromethane at room temperature with 10 mol% of triphenylmethylium perchlorate as catalyst for 50, 70, and 90 h, and 7 days. Similar results were obtained (yield, d.p. of polymer), and a reaction time of 70 h was chosen for preparative experiments.

Polycondensation of **1** and **2** followed by deacylation gave polymers (**7** and **8**, respectively) with similar properties, including low solubility in water although not as low as that of the synthetic, (1→3)-linked α-L-rhamnan[5]. Hydrolysis of **7** and **8** with formic acid and then with hydrochloric acid gave rhamnose as the only product.

Methylation analysis (formolysis, hydrolysis, borohydride reduction, and acetylation) of **7** and **8** gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol (**9**) and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol (**10**), identified by comparison (g.l.c., g.l.c.–m.s.) with authentic samples and in the ratios 1:22 and 1:40, respectively. The absence of other partially methylated rhamnitols proved the absence of branching, the regular (1→4)-linked structure of the polymers, and the regiospecificity of the polycondensation. The **9**/**10** ratios indicated the molecular weights of **7** and **8** to be ~3300 and ~6000, respectively, and reflects the, as yet unexplained, influence of the nature of the protecting groups (*i.e.*, acetyl and benzoyl) on d.p.

TABLE I

^{13}C-N.M.R. DATA FOR THE METHYLATED RHAMNANS

Rhamnan	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*MeO-2*	*MeO-3*
7	98.7	76.7	81.65	79.2	67.9	18.1	58.5	56.8
8	98.6	76.7	81.6	79.2	67.8	18.1	58.5	56.8

This finding suggests that the replacement of acetate by benzoate for protection could help to increase the molecular weight of polysaccharides obtainable by polycondensation of cyanoethylidene derivatives of sugars. The reason for the substantial increase of molecular weight in the rhamnan synthesis remains unclear and further study is required.

The oligosaccharide fraction obtained in the above polycondensation reactions contained hepta-decasaccharides with only (1→4)-glycosidic linkages (methylation analysis).

Determination of the configuration of the linkages in the synthetic rhamnans was accomplished by ^{13}C-n.m.r. spectroscopy. Since the polysaccharides **7** and **8** had low solubilities in water, their methylated derivatives (prepared by the Hakomori method[12]) were studied together with appropriate methylated methyl β- and α-L-rhamnosides (see ref. 5) as model compounds. The results are presented in Table I. The spectra of **7** and **8** were almost identical. The chemical shifts of the signals for C-1 (98.7 and 98.6 p.p.m.) are diagnostic for rhamnose derivatives, and those for C-5 (67.9 and 67.8 p.p.m.) are similar to those (98.3 and 67.8 p.p.m.) for α-rhamnosides (*cf.* 102 and 72 p.p.m. for β-rhamnosides). Thus, β-rhamnosidic linkages were absent from each polysaccharide. Consequently, the synthetic rhamnan is completely stereoregular and the polycondensation reaction proceeds stereospecifically.

The results reported here further illustrate the scope for polysaccharide synthesis by polycondensation of sugar cyanoethylidene derivatives.

EXPERIMENTAL

Dichloromethane was washed with conc. H_2SO_4 and water, dried ($CaCl_2$), and distilled from P_2O_5 and then from CaH_2. Chloroform was distilled from $CaCO_3$, and benzene was distilled from Na and then from CaH_2. Nitromethane was distilled from urea at 100 mmHg and then from CaH_2. T.l.c. was performed on silica gel LS/5–40 μm (CSSR) with benzene–ether mixtures *A*, 1:1; and *B*, 98:2. Column chromatography was performed on silica gel L (100–160 μm, CSSR) with a gradient of benzene–ether. P.m.r. spectra (internal Me_4Si) were recorded with a Tesla BS-497 (100 MHz, CSSR) spectrometer, and ^{13}C-n.m.r. spectra with a Bruker-Physic WP-60 instrument (15.08 MHz). I.r. spectra (KBr discs) were recorded with a UR-20 spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polari-

meter, and melting points with a Kofler apparatus. G.l.c. was performed with a LChM-8-MD instrument and a steel column (3 m) of 5% SE-30 on Chromaton N-AW with nitrogen as the carrier gas. G.l.c.–m.s. was performed on a Varian MAT-111 (Gnom) instrument equipped with a steel column (1 m) packed with 5% of SE-30 on Chromaton N-AW-DMCS, using helium as the carrier gas. Solutions were concentrated *in vacuo* at 40°.

3-O-Acetyl-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnopyranose (**4**). — To a solution of 1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-L-rhamnopyranose[5] (1.18 g) in pyridine (20 ml) was added a solution of acetyl chloride (0.4 ml) in benzene during 30 min with stirring and cooling (~20°). The mixture was kept for 30 min at −20°, stored for 12 h at 0°, and concentrated, and the residue was subjected to column chromatography, to yield **4** (1.2 g, 86%), m.p. 106–107° (from ether–light petroleum), $[\alpha]_D$ +40° (*c* 2, chloroform), R_F 0.4 (solvent *A*). P.m.r. data ($CDCl_3$): δ 5.38 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.05 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 9 Hz, H-3), 4.53 (dd, 1 H, H-2), 3.2–3.7 (m, 2 H, H-4,5), 2.2 (s, 3 H, OAc), 1.89 (s, 3 H, cyanoethylidene Me), and 1.32 (d, 3 H, $J_{5,6}$ 6 Hz, Me of rhamnose).

Anal. Calc. for $C_{11}H_{15}NO_6$: C, 51.36; H, 5.88; N, 5.44. Found: C, 51.25; H, 5.80; N, 5.31.

3-O-Acetyl-1,2-O-[1-(exo-cyano)ethylidene]-4-O-trityl-β-L-rhamnopyranose (**1**). — To a solution of **4** (1.1 g) in dry dichloromethane (30 ml) was added 2,4,6-collidine (0.67 ml) and triphenylmethylium perchlorate[13] (1.7 g) during 10 min with stirring at room temperature. The mixture was stored for 2 h, diluted with chloroform (50 ml), washed with water (5 × 50 ml), and concentrated. The syrupy residue was extracted with ether (5 × 20 ml), the combined extracts were concentrated, and the residue was subjected to column chromatography, to yield the chromatographically homogeneous product (0.75 g). Crystallisation from methanol gave **1** (0.5 g, 24%), m.p. 184–185°, $[\alpha]_D$ −51.5° (*c* 2, chloroform), R_F 0.5 (solvent *B*). P.m.r. data (CCl_4): δ 7.2–7.6 (m, 15 H, 3 Ph), 5.35 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.1 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 9 Hz, H-3), 4.2 (dd, 1 H, H-2), 3.7 (dq, 1 H, $J_{5,6}$ 6, $J_{4,5}$ 8 Hz, H-5), 3.22 (dd, 1 H, H-4), 1.55 (s, 3 H, cyanoethylidene Me), 1.35 (s, 3 H, OAc), and 1.01 (d, 3 H, $J_{5,6}$ 6 Hz, Me of rhamnose).

Anal. Calc. for $C_{30}H_{29}NO_6$: C, 72.13; H, 5.85; N, 2.80. Found: C, 72.28; H, 5.75; N, 2.67.

3-O-Benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-β-L-rhamnopyranose (**5**). — To a solution of 1,2-*O*-[1-(*exo*-cyano)ethylidene]-β-L-rhamnopyranose (1.42 g) in dry pyridine (20 ml) was added a solution of benzoyl chloride (0.8 ml) in dry pyridine (3 ml) during 1 h with stirring and cooling (~−20°). The mixture was stored for 72 h at room temperature and then concentrated to dryness, and the residue was subjected to column chromatography, to yield **5** (1.7 g, 85%), m.p. 142–143° (from ether–light petroleum), $[\alpha]_D$ +9° (*c* 2, chloroform), R_F 0.6 (solvent *A*). P.m.r. data ($CDCl_3$): δ 7.2–8.2 (m, 5 H, Ph), 5.38 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.25 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 9 Hz, H-3), 4.58 (dd, 1 H, H-2), 3.36 (t, 1 H, $J_{4,5}$ 9 Hz, H-4), 3.47 (dq,

1 H, $J_{5,6}$ 6 Hz, H-5), 1.82 (s, 3 H, cyanoethylidene Me), and 1.25 (d, 3 H, Me of rhamnose).

Anal. Calc. for $C_{16}H_{17}NO_6$: C, 60.18; H, 5.37; N, 4.39. Found: C, 59.97; H, 5.30; N, 4.21.

3-O-Benzoyl-1,2-O-[1-(exo-cyano)ethylidene]-4-O-trityl-β-L-rhamnopyranose (**2**). — To a solution of **5** (4 g) in dry dichloromethane (50 ml) was added 2,4,6-collidine (3 ml) and triphenylmethylium perchlorate (5 g) during 20 min with stirring at room temperature. The mixture was stored for 2 h and then worked-up as described above to give, after column chromatography, a chromatographically homogeneous product (1.57 g). Recrystallisation from benzene–light petroleum gave **2** (1.33 g, 20%), m.p. 182–183°, $[\alpha]_D$ −71.5° (*c* 2, chloroform), R_F 0.6 (solvent *B*). P.m.r. data ($CDCl_3$): δ 7.1–7.8 (m, 20 H, 4 Ph), 5.48 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.17 (dd, 1 H, $J_{2,3}$ 5, $J_{3,4}$ 6 Hz, H-3), 4.56 (dd, 1 H, H-2), 3.85 (quin, 1 H, $J_{4,5}$ 6 Hz, H-5), 3.65 (t, 1 H, H-4), 1.55 (s, 3 H, cyanoethylidene Me), and 1.02 (d, 3 H, $J_{5,6}$ 6 Hz, Me of rhamnose).

Anal. Calc. for $C_{35}H_{31}NO_6$: C, 74.85; H, 5.56; N, 2.49. Found: C, 74.35; H, 5.50, N, 2.32.

Polycondensations. — (*a*) **1**. The vacuum technique[9] was used. The reaction was performed in five ⅄-shaped tubes. In one limb of each tube was placed a solution of **1** (0.1 g, 0.2 mmol) in benzene (1 ml), and in the other limb, a solution of triphenylmethylium perchlorate (7 mg, 0.02 mmol) in nitromethane (0.3 ml). The solutions were freeze-dried (10^{-3} mmHg). Dichloromethane (2 ml, twice distilled from CaH_2 at 10^{-3} mmHg) was distilled into each tube. The solutions were mixed and left at room temperature for 70 h in the dark. Each tube was then connected with the atmosphere, and the yellow mixtures therein were treated with 90% aqueous trifluoroacetic acid (1 ml) for 30 min at room temperature. Pyridine (2 ml) was then added to each tube. The five mixtures were combined, diluted with chloroform (50 ml), washed with water (5 × 50 ml), and concentrated. The residue was washed with hexane (5 × 50 ml), to remove triphenylmethyl cyanide, to give the product **6** (226 mg) of the polycondensation.

A solution of **6** (226 mg) in chloroform (4 ml) and methanol (3 ml) was stirred with M methanolic sodium methoxide (2 ml) at room temperature for 48 h and then concentrated. The product did not possess i.r. carbonyl absorption. The residue was washed with water (5 × 10 ml) by decantation and then acetone (3 × 10 ml), and then dried *in vacuo* to yield the water-insoluble rhamnan (**7**) as a white solid (104 mg, 71%). The combined aqueous solution was neutralised with KU-2 (H^+) resin, filtered, treated with IRA-410 (HCO_3^-) resin, and concentrated to dryness, and the residue was dried *in vacuo* to yield an oligosaccharide fraction **11** (39 mg, 27%), $[\alpha]_D$ −27° (*c* 2, water).

(*b*) **2**. The reaction was performed as in (*a*). In one limb of each tube was placed a solution of **2** (170 mg, 0.3 mmol) in benzene (1 ml), and in the other, a solution of triphenylmethylium perchlorate (10.5 mg, 0.03 mmol) in nitromethane (0.3 ml), and the solutions were freeze-dried. Dichloromethane (2 ml) was distilled

into each tube, and the solutions of monomer and catalyst were mixed, left at room temperature for 96 h, and then worked-up as described above, to give **12** (435 mg).

A solution of **12** (435 mg) in chloroform (6 ml) and methanol (4 ml) was stirred with M methanolic sodium methoxide (4 ml) at room temperature for 48 h and then concentrated to dryness, and the residue was washed with ether (5 × 10 ml). The product, which had no i.r. absorption for carbonyl, was washed with water (5 × 10 ml) and acetone (3 × 10 ml), and then dried *in vacuo*, to yield the water-insoluble rhamnan **8** (180 mg, 82%) as a white solid. The combined aqueous solution was treated with KU-2 (H^+) resin and then IRA-410 (HCO_3^-) resin, and concentrated to dryness, and the residue was dried *in vacuo*, to yield the oligosaccharide fraction **13** (30 mg, 15%), $[\alpha]_D$ −23° (*c* 1, water).

Polycondensation products. — (*a*) *Hydrolysis.* The polysaccharide (5 mg) or the oligosaccharide fraction (5 mg) was treated with 85% formic acid (2 ml) at 100° for 2 h, and the solution then concentrated. A solution of the residue in M HCl (2 ml) was heated at 100° for 12 h and then concentrated, and water (3 × 5 ml) was distilled from the residue which was treated with sodium borohydride (20 mg) in water (2 ml) at room temperature for 12 h. The solution was deionised with KU-2 (H^+) resin, which was then collected and washed with water (2 × 3 ml). The combined filtrate and washings were concentrated to dryness, and methanol (3 × 5 ml) was distilled from the residue which was then treated with 1:1 acetic anhydride–pyridine (2 ml) at room temperature for 12 h. Rhamnitol penta-acetate was identified as the single product by g.l.c.

(*b*) *Methylation analysis.* The polysaccharide (5 mg) or the oligosaccharide fraction (5 mg) was methylated twice by the Hakomori method[12] (NaH, 20 mg; Me_2SO, 3 ml; MeI, 1 ml). The reaction mixture was diluted with chloroform (10 ml), washed with water (5 × 5 ml), and concentrated. The residue was subjected, in sequence, to formolysis, hydrolysis, borohydride reduction, and acetylation, as described above, to give only the diacetate of 2,3,4-tri-*O*-methylrhamnitol (**9**) and the triacetate of 2,3-di-*O*-methylrhamnitol (**10**) (in the ratio 1:22 for **7**, 1:10 for **11**, 1:40 for **8**, 1:7 for **13**), which were identified by g.l.c. in comparison with authentic samples and by g.l.c.–m.s. The mass spectra of **9** and **10** contained the following, characteristic peaks: *m/z* 89, 101, 115, 117, 131, 161, and 175 (for **9**), and 87, 101, 113, 117, 129, 143, 161, and 203 (for **10**).

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