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Synthesis of *O*-β-D-Galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-L-serine

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The title compound (*2*) has been synthesized, using silver triflate-promoted 1,2-*trans*-glycoside formation in the construction of the glycosidic linkages. The fully protected galactosylgalactosyl bromide *3* and the xylosylserine derivative *4*, with a free hydroxyl group in the 4-position of the xylose residue, were synthesized and then condensed to afford the protected galactosylgalactosylxylosylserine *5*. This was deprotected to give *2*.

The synthesis of *O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-L-serine (*1*), which is a fragment of the carbohydrate-protein linkage region in various proteoglycans, has previously been communicated.[1] We now report the synthesis of the larger fragment *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-L-serine (*2*) which was needed for biosynthetic and NMR studies.

β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→3)-L-Ser

1

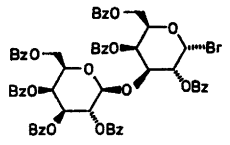
β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→3)-L-Ser

2

The synthesis strategy adopted was to make two dimers, the galactosylgalactosyl bromide *3* and the xylosylserine derivative *4* and then condense these to yield protected *2* (*5*).

Silver triflate-promoted reaction of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide with 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose,[2] followed by removal of protecting groups, yielded 3-*O*-β-D-galactopyranosyl-D-galactose, which was transferred into the fully benzoylated α-pyranosyl bromide (*3*).

Benzyl 2,3-anhydro-β-D-ribopyranoside was treated with allyl bromide and silver oxide in dimethyl formamide. The resulting 4-*O*-allyl derivative on treatment with aqueous sodium hydroxide yielded benzyl 4-*O*-allyl-β-D-xylopyranoside, in analogy with the reaction of the corresponding 4-*O*-methyl ether.[3] Acidic hydrolysis, followed by benzoylation, yielded 4-*O*-allyl-1,2,3-tri-*O*-benzoyl-β-D-xylopyranose, which was transferred into the α-xylopyranosyl bromide. Silver triflate-promoted condensation of this bromide with *N*-carbobenzyloxy-L-serine benzyl ester yielded a xylosylserine derivative, the allyl group of which was selectively removed by isomerization to a 1-propenyl group, catalyzed by tris(triphenylphosphine)rhodium(I) chloride,[4] followed by mild acidic hydrolysis. The resulting xylosylserine derivative *4*, with



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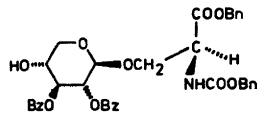

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a free hydroxyl at C-4, was condensed with *3*, again in a silver triflate-promoted reaction. A mixture of the β-galactoside *5* (44 %) and the corresponding α-galactoside (10 %) was obtained, and the products were separated by chromatography. Formation of α-galactopyranosides under conventional Koenigs-Knorr conditions has been observed earlier.[5] Removal of protecting groups from *5*, first by catalytic hy-

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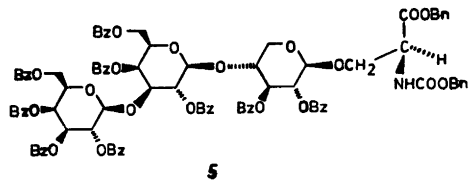

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drogenation over palladium on charcoal and then by treatment with sodium methoxide in methanol-chloroform, yielded *2*. The ^{13}C NMR spectrum of *2* was in agreement with the postulated structure and also showed that no racemization of the L-serine residue had occurred.[1]

In the previous synthesis of *1*,[1] benzoyl groups were removed by treatment with methanolic ammonia. Under these conditions considerable racemization of the L-serine residues occurred, as demonstrated by ^{13}C NMR spectroscopy. The synthesis of *1* was therefore repeated, starting from 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide and *4*, and the debenzoylation performed as above. The ^{13}C NMR spectrum showed that the substance was pure and that no racemization had occurred.

In the silver triflate-promoted [6] glycosylations, the yields of β-glycosides obtained varied between 44 and 74 %.

EXPERIMENTAL

General methods were the same as those described before.[1]

3-O-(2,3,4,6-Tetra-O-benzoyl-β-D-galactopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-galactofuranose. Silver triflate (1.79 g) and *s*-collidine (0.72 ml) in 1:1 nitromethane-toluene (10 ml) were added with stirring and cooling at −25 °C, to 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (4.48 g) and 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose [2] (1.67 g) in 1:1 nitromethane-toluene (20 ml). After 5 min at −25 °C a little *s*-collidine was added to neutralize the mixture. The solution was diluted with diethyl ether (100 ml) filtered and washed successively with aqueous sodium thiosulfate, water, 2 M aqueous sulfuric acid and aqueous sodium hydrogen carbonate. Drying ($MgSO_4$), filtration and concentration afforded a syrup which was purified by chromatography on a column of silica gel (700 g) (toluene−ethyl acetate 8:2). The title compound thus obtained (3.63 g, 67 %) had $[\alpha]_D$ +64° (*c* 0.5, $CHCl_3$).

3-O-β-D-Galactopyranosyl-D-galactose. Sodium methoxide (1 mmol) was added to 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (3.4 g) in methanol (100 ml). After 24 h at room temperature, the solution was neutralized (Dowex-50, H^+) and concentrated. The residue was partitioned between water and 1:1 diethyl ether−light petroleum. The aqueous phase was concentrated and the residue treated with 80 % aqueous acetic acid at 70 °C for 3.5 h. A TLC examination [1] (ethyl acetate−acetic acid−methanol−water, 12:3:3:2) revealed the presence of the title disaccharide, contaminated with small amounts of D-galactose. Concentration afforded the title compound (1.43 g). Crystallization from ethanol−water afforded the monohydrate, m.p. 161−164 °C, $[\alpha]_D$ +83° (5 min)→+61° (2 h, const., *c* 0.5, H_2O) (lit. [7] m.p. 163−170 °C, $[\alpha]_D$ +60° (2 h, const.).

2,4,6-Tri-O-benzoyl-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-α-D-galactopyranosyl bromide (3). Benzoyl chloride (6.4 ml) was added dropwise with stirring to crude 3-*O*-β-D-galactopyranosyl-D-galactose (1.18 g) in pyridine (50 ml) cooled in ice-water. The solution was left at room temperature overnight, worked up as usual and purified by column chromatography on silica gel (toluene−ethyl acetate 8:2) to afford each of the two anomers of the octabenzoate (altogether 2.70 g, 67 % from 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose). Acetic acid saturated with hydrogen bromide (5 ml) was added to the anomeric mixture of 3-*O*-β-D-galactopyranosyl-D-galactose octabenzoate (2.47 g) in dichloromethane (10 ml) at room temperature. After 30 min, TLC showed complete conversion into a single, faster-moving product. Dilution with dichloromethane, washing with ice-water and then with aqueous sodium bicarbonate, drying ($MgSO_4$), filtering and concentration afforded syrupy *3*, which was used directly in the next step without further purification.

Benzyl 4-O-allyl-2,3-anhydro-β-D-ribopyranoside. Allyl bromide (9.5 ml) and silver oxide (10 g) were added to a solution of benzyl 2,3-anhydro-β-D-ribopyranoside [3] (5.0 g) in dimethyl formamide (50 ml). After stirring in the dark at room temperature overnight, methanol (5 ml) and silver oxide (4.0 g) were added and the stirring continued for 1 h. The mixture was filtered through Celite, diluted with diethyl ether, washed with aqueous sodium thiosulfate and concentrated. Recrystallization from ethanol afforded the title compound (5.1 g, 86 %), m.p. 35−36 °C $[\alpha]_D$ −17° (*c* 0.5, $CHCl_3$). Anal. $C_{15}H_{18}O_4$: C, H.

Benzyl-4-O-allyl-β-D-xylopyranoside. Benzyl 4-*O*-allyl-2,3-anhydro-β-D-ribopyranoside (5.0 g) in 5 % aqueous sodium hydroxide (500 ml) was kept at 100 °C for 20 h, cooled and neutralized with aqueous sulfuric acid. Extraction with chloroform, drying ($MgSO_4$) filtration and concentration afforded the title compound (4.4 g 83 %) as an oily semisolid which was used directly in the next step.

4-O-Allyl-1,2,3-tri-O-benzoyl-β-D-xylopyranose. Benzyl 4-*O*-allyl-β-D-xylopyranoside (3.9 g)

was hydrolyzed with 0.25 M aqueous sulfuric acid at 100 °C overnight. Neutralization with barium carbonate, filtration and concentration afforded a product which was treated with benzoyl chloride (5 ml) in pyridine (50 ml) and worked up in the usual way. Purification on a column of silica gel (550 g, toluene–ethyl acetate 95:5) afforded the title compound (4.50 g, 64 %) which, following crystallization from methanol had m.p. 98–100 °C $[\alpha]_D$ +32° (*c* 0.5, $CHCl_3$). Anal. $C_{29}H_{26}O_8$: C, H. ^1H NMR: δ 6.15 ppm (d, *J* 6 Hz, H-1).

3-O-(*4*-O-*Allyl-2,3,di*-O-*benzoyl-β*-D-*xylopyranosyl*)-N-*carbobenzyloxy*-L-*serine benzyl ester*. Acetic acid, saturated with hydrogen bromide (10 ml) was added to 4-*O*-allyl-1,2,3-tri-*O*-benzoyl-*β*-D-xylopyranose (3.0 g) in dichloromethane (20 ml) at room temperature. After 10 min, when TLC indicated the presence of a single product, the solution was diluted with dichloromethane, washed with ice-water and then with aqueous sodium bicarbonate, dried ($MgSO_4$), filtered and concentrated. The glycosyl bromide was dried by repeated co-concentrations with dichloromethane, and then dissolved in 1:1 nitromethane–toluene (20 ml). *N*-Carbobenzyloxy-L-serine benzyl ester (2.04 g) was added. Silver triflate (1.67 g) and *s*-collidine (0.66 ml) in 1:1 nitromethane–toluene (10 ml) were added at −25 °C. After 3 min more *s*-collidine (0.5 ml) was added and the mixture worked up as described in the glycosylation above. Chromatography on a silica gel column (500 g, toluene–ethyl acetate 8:2) afforded the title compound (3.2 g, 74 %), $[\alpha]_D$ +35° (*c* 0.5, $CHCl_3$).

3-O-*(2,3-di*-O-*Benzoyl-β*-D-*xylopyranosyl)*-N-*carbobenzyloxy*-L-*serine benzyl ester (4)*. 1,4-Diazabicyclo[2.2.2]octane (50 mg) and tris-(triphenylphosphine)rhodium(I) chloride (250 mg) were added to 3-*O*-(4-*O*-allyl-2,3-di-*O*-benzoyl-*β*-D-xylopyranosyl)-*N*-carbobenzyloxy-L-serine benzyl ester (2.53 g) in ethanol–benzene–water 40:16:15 (122 ml). After boiling under reflux for 4 h, the mixture was worked up as described before.[4] The product, in acetone–water 10:1 (44 ml), was stirred at room temperature with mercury(II) oxide (1.0 g). Mercury(II) chloride (1.0 g) in acetone–water 10:1 (15 ml) was added dropwise and the mixture was stirred for 1 h. Filtration, dilution with diethyl ether, washing with aqueous potassium iodide, drying ($MgSO_4$), filtration and concentration afforded a product which was separated into its components by silica gel (500 g) column chromatography. Toluene–ethyl acetate (65:35) eluted first unchanged starting material (0.32 g) and then the title compound (1.58 g, 66 %) $[\alpha]_D$ +32° (*c* 0.5, $CHCl_3$). ^1H NMR: δ 3.00 ppm (d, OH) 4.68 (d, $J \sim 6$ Hz, H-1). ^{13}C NMR: δ 100.88 ppm (C-1, xylose) 54.35 (C-2, serine).

O-*(2,3,4,6-Tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzoyl-β*-D-*galactyopyranosyl)-(1→4)*-O-*(2,3-di*-O-*benzoyl-β*-D-*xylopyranosyl)-(1→3)*-N-*carbobenzyloxy*-L-*serine benzyl ester (5)*. Silver triflate (0.45 g) and *s*-collidine (0.15 ml) in 1:1 nitromethane–toluene (4.5 ml) were added to the bromide *3* (prepared from 1.65 g 3-*O*-*β*-D-galactopyranosyl-D-galactose octabenzoate) and compound *4* (1.16 g) in 1:1 nitromethane–toluene (7 ml) at −25 °C. After 5 min, the mixture was neutralized with excess *s*-collidine and worked up as described in the above glycosidations, except that 1:1 diethyl ether–ethyl acetate, instead of diethyl ether was used in the initial dilution. Chromatography on a silica gel column (200 g, toluene–ethyl acetate 75:25) first eluted the α-linked product (230 mg, 10 %), $[\alpha]_D$ +82° (*c* 0.5, $CHCl_3$) and then the title compound (920 mg, 44 %), $[\alpha]_D$ +37° (*c* 0.5, $CHCl_3$). ^{13}C NMR: δ 54.0 ppm (C-2, serine residue), 99.1, 101.5 and 102.1 (anomeric carbons, two galactose and one xylose residue).

O-*β*-D-*Galactopyranosyl-(1→3)*-O-*β*-D-*galactopyranosyl-(1→4)*-O-*β*-D-*xylopyranosyl-(1→3)*-L-*serine(2)*. The above galactosylgalactosylxylosylserine derivative (1.0 g) in 50:50:8 ethyl acetate–acetic acid–water (108 ml) was hydrogenated at 0.5 MPa with 10 % palladium on charcoal. Filtration and concentration left a residue which was dissolved in 1:2 chloroform-methanol (39 ml) and treated with methanolic sodium methoxide (5.5 ml, from 100 mg sodium in 10 ml methanol) at room temperature for 4 h, when TLC (ethyl acetate–acetic acid–methanol–water, 12:3:3:2) showed the absence of UV absorbing carbohydrate components. The solution was carefully neutralized to pH 7 with 1 M aqueous hydrochloric acid, diluted with water (100 ml) and washed with diethyl ether. The aqueous phase was concentrated to a small volume and then lyophilized after adjusting the pH to 5–6. The residue was fractionated on a Sephadex G-15 column to remove salts and minor impurities to yield the title compound *2* (0.23 g, 70 %), $[\alpha]_D$ −12° (*c* 0.3, H_2O). Sugar analysis [8] showed the presence of galactose and xylose in the ratio 2:1. ^{13}C NMR: δ 55.7 ppm (serine C-2), 62.2, 62.1 (two galactose C-6), 64.1 (xylose C-5), 68.9 (serine C-3), 69.6–77.5 (10 signals corresponding to terminal galactose C-2, C-3, C-4 and C-5, non-terminal galactose C-2, C-4, C-5 and xylose C-2, C-3 and C-4), 83.2 (non-terminal galactose C-3), 102.5, 103.7 and 105.4 (two galactose, one xylose C-1), 172.7 (serine C-1).

O-*(2,3,4,6-Tetra*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2,3-di*-O-*benzoyl-β*-D-*xylopyranosyl)*-(1→3)-N-*carbobenzyloxy*-L-*serine benzyl ester*. This compound was prepared using the same experimental conditions as those described in the condensation of *3* and *4*. Thus the condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactosyl bromide (725 mg) with *4* (535 mg) afforded, after chromatography, the title compound (539 mg, 54 %). The ^{13}C NMR spectrum and optical rotation were indistinguishable from those obtained for the same compound in previous work.[1]

O-β-D-*Galactopyranosyl*-(1→4)-O-β-D-*xylopyranosyl*-(*1→3*)-L-*serine* (*1*). The above galactosylxylosylserine derivative (630 mg) was hydrogenated and debenzoylated as described above in the preparation of *2* to yield the title compound *1* (138 mg, 68 %), $[\alpha]_D$ −28° (*c* 0.5, H_2O). The ^{13}C NMR spectrum was identical to the one previously published [1] except that no splitting of serine and xylose C-1 carbons was observed, showing that no racemization had occurred in the deprotection.

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REFERENCES

1. Erbing, B., Lindberg, B. and Norberg, T. *Acta Chem. Scand. B 32* (1978) 308.
2. Morgenlie, S. *Acta Chem. Scand. 27* (1973) 3609.
3. Garegg, P. J. *Acta Chem. Scand. 14* (1960) 957.
4. Gent, P. A. and Gigg, R. *J. Chem. Soc. Perkin Trans. 1* (1974) 1835.
5. Wickberg, B. *Acta Chem. Scand. 12* (1958) 1187.
6. Garegg, P. J. and Norberg, T. *Acta Chem. Scand. B 33* (1979) 116.
7. Ball, D. H. and Jones, J. K. N. *J. Chem. Soc.* (1958) 905.
8. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem. 37* (1965) 1602.

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