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# STUDIES ON C-5 EPIMERISATION OF 2-AMINO-2-DEOXY AND 2-AZIDO-2-DEOXY DERIVATIVES OF ALLYL β-D-GALACTOPYRANOSIDURONIC ACID ESTERS

PER J. GAREGG AND MARTIN HARALDSSON

*Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)*

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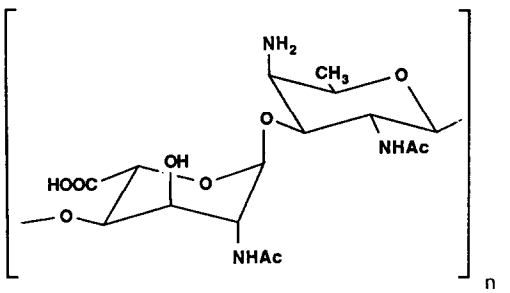
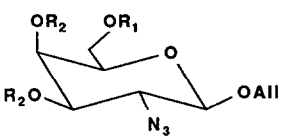
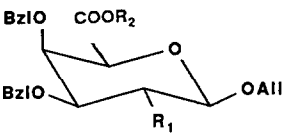
The *Shigella sonnei* phase 1 O-antigen is a linear polysaccharide containing a β-(1-4')-linked repeating unit of 2-acetamido-3-*O*-[(2-acetamido-2-deoxy-α-L-altropyranosyl)uronic acid]-4-amino-2,4,6-trideoxy-D-galactopyranose[1]. In studies directed towards the synthesis of this unit, glycosidically bound to a linking arm for making the appropriate artificial antigen, we had to devise a reasonable route to a suitable (2-acetamido-2-deoxy-α-L-altropyranosyl)uronic acid unit. Kovac and coworkers have reported that epimerisation of the 2,3,4 tri-*O*-benzyl or methyl ethers of methyl (methyl β-D-galacto-pyranosid)uronate upon treatment under mild basic conditions gives about 20-25% epimerisation[2]. Obviously, for our purposes it would be possible to recover the D-sugar by chromatography and recycle several times. However, we found that some of the 2-amino derivatives in the present work gave equilibria which are much more in favour of the L-*altro* configuration. Furthermore, the equilibrium appears to be strongly influenced by the nature of the C-6 ester group and we now report these results.

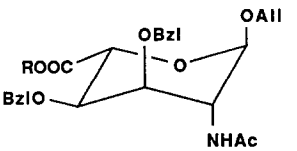



1 $R_1$=$R_2$= H
2 $R_1$=Tr $R_2$= H
3 $R_1$=Tr $R_2$=Bzl
4 $R_1$= H $R_2$=Bzl




5 $R_1$=$N_3$, $R_2$=$Bu^t$
6 $R_1$=$NH_2$ $R_2$=$Bu^t$
7 $R_1$=NHAc $R_2$=$Bu^t$
9 $R_1$=NHAc $R_2$=Bzl
11 $R_1$=NHAc $R_2$=Me




8 R=Bzl
10 R=Me

Azidonitration of 3,4,6-tri-O-acetyl-D-galactal and treatment of the crude azido-nitrates with sodium allyloxide yielded allyl 2-azido-2-deoxy-β-D-galactopyranoside (**1**) in 57% yield. Compound **1** was tritylated in the 6-position and the product (**2**) was benzylated in the 3,4-positions. The product **3** was then detritylated to give compound **4**, oxidation of which with pyridinium dichromate[3] in acetic acid and *tert.*-butanol gave the uronic acid *tert.*-butyl ester **5**. This in turn was converted by treatment with hydrogen sulfide to the amino compound **6**, and, further by acetylation to the acetamido compound **7**.

The approximate product ratios obtained upon C-5 epimerisation by treatment with sodium hydroxide was determined for the *tert.*-butyl esters **5-7**. Surprisingly, the ratios were in favour of the L-*altro* sugar, the 2-azidodeoxy, 2-aminodeoxy and the 2-acetamidodeoxy derivatives all giving a ratio of 2:3 for the D and L-sugars. The corresponding ratios for the benzyl ester **9** was 1:1 and those for the two methyl esters **10** and **11** were 3:2 and 9:1, respectively. It would appear that for these less sterically hindered esters, saponification competes with C-5 epimerisation, preventing true equilibration at C-5 of the pyranose rings, since the uronate anions would be expected to be equilibrated at a much slower rate than the esters.

## EXPERIMENTAL

*General methods.* -- Melting points are corrected. Concentrations were performed under reduced pressure at bath temperatures < 40°. Optical rotations were recorded for 1% solutions in chloroform unless otherwise stated, at ambient temperatures, using a Perkin Elmer 241 polarimeter. $^1H$ and $^{13}C$ n.m.r. spectra were recorded for $D_2O$ or $CDCl_3$ solutions with JEOL GX-270 or FX-100 instruments. TMS (for $CDCl_3$ solutions) and TSP (for $D_2O$ solutions) were used as internal standards. Column chromatography was performed on silica gel (Matrex Silica 60A, 35-70, Amicon).

*Allyl 2-azido-2-deoxy-β-D-galactopyranoside* (**1**). -- The product of azidonitration of D-galactal[4] was treated with sodium allyloxide (50 mL 1M) at room temperature for 15 min. The solution was then neutralised with acetic acid and concentrated. Column chromatography of the residue (ethyl acetate) gave **1** (4.4 g, 57%), $[\alpha]_D$ +54° (*c* 1, water).

*Allyl 2-azido-2-deoxy-6-O-triphenylmethyl-β-D-galactopyranoside* (**2**). -- Compound **1** (5.6 g) was stirred with trityl chloride (7.6 g) and silver nitrate (4.6 g) in pyridine (100 mL) at room temperature overnight. The reaction mixture was partitioned between dichloromethane and water and the organic layer was concentrated. Column chromatography of the residue (toluene - ethyl acetate 9:1) gave **2** (10.7 g, 96%), $[\alpha]_D$ -3°.

*Allyl 2-azido-3,4-di-O-benzyl-2-deoxy-6-O-triphenylmethyl-β-D-galactopyranoside* (**3**). -- Compound **2** (5.1 g) in *N,N*-dimethyl formamide (100 mL) was treated with benzyl bromide (7.8 mL) and sodium hydride (1 g) at room temperature for 1.5 h. Methanol (10 mL ) was added to remove excess benzyl bromide. The product was partitioned between toluene and water and the organic layer was concentrated. Column chromatography of the residue (toluene) gave **3**, (6.3 g 90%), m.p. 124-126° (ethyl acetate - light petroleum) $[\alpha]_D$ -16°.

*Anal.* Calcd. for $C_{42}H_{41}N_3O_5$: C, 75.5; H, 6.1; N, 6.3. Found: C, 75.7; H, 6.2; N, 6.4.

*Allyl 2-azido-3,4-di-O-benzyl-2-deoxy-β-D-galactopyranoside* (**4**). Compound **3** (7.2 g) was treated with trifluoroacetic acid (200 mL 1% in dichloromethane) at room temperature for 20 h. The solution was neutralised by shaking with aqueous sodium hydrogencarbonate and the organic layer was concentrated. Column chromatography of the residue (toluene - ethyl acetate 6:1) gave **4**, (3.8 g, 83%), m.p. 66-69° (ethyl acetate - light petroleum), $[\alpha]_D$ -40°.

*Anal.* Calcd. for $C_{23}H_{27}N_3O_5$: C, 65.0; H, 6.3; N, 9.9. Found: C, 64.6; H, 6.5; N, 9.8.

tert.-*Butyl (allyl 2-azido-3,4-di*-O-*benzyl-2-deoxy*-β-D-*galactopyranosid)uronate* (**5**). -- A solution of **4** (3.6 g) in dichloromethane (40 mL) was treated with acetic anhydride (10 mL), *tert.*-butanol (10 mL) and pyridinium dichromate (7 g)[3] with stirring at room temperature for 2 h. Ethyl acetate (40 mL) was added and stirring was continued overnight. The mixture was filtered through a short column of silica gel which was washed with ethyl acetate. Concentration of the filtrate and column chromatography of the residue (toluene - ethyl acetate 20:1 gave **5** (2.4 g, 57%), m.p. 84-85° (diethyl ether - light petroleum) $[\alpha]_D$ +16°.

*Anal.* Calcd. for $C_{27}H_{33}N_3O_6$: C, 65.4; H, 6.7; N, 8.5. Found: C, 65.1; H, 6.6; N, 8.5.

tert.-*Butyl (allyl 2-amino-3,4-di*-O-*benzyl-2-deoxy*-β-D-*galactopyranosid)uronate* (**6**). -- A slow stream of hydrogen sulfide was passed through a solution of **5** (265 mg) in pyridine (2 mL) and triethylamine (1 mL) at room temperature for 4 h. The product was concentrated and the residue was concentrated several times from water. Column chromatography of the residue (toluene - ethyl acetate 1:2) gave **6** (130 mg, 52%), m.p. 112-113° (dichloromethane - diethyl ether), $[\alpha]_D$ +22°.

*Anal.* Calcd. for $C_{27}H_{35}NO_6$: C, 69.1; H, 7.5; N, 3.0. Found: C, 68.8; H, 7.6; N, 2.9.

tert.-*Butyl (allyl 2-acetamido-3,4-di*-O-*benzyl-2-deoxy*-β-D-*galactopyranosid)uronate* (**7**). -- A slow stream of hydrogen sulfide was passed through a solution of **5** (834 mg) in pyridine (6 mL) and triethylamine (3 mL) at room temperature for 4 h. Acetic anhydride (3mL) was added slowly. The product was concentrated and the residue was concentrated once from water. Column chromatography of the residue (toluene - ethyl acetate 1:1) gave **7** (745 mg, 86%), $[\alpha]_D$ +34°.

*Benzyl (allyl 2-acetamido-3,4-di*-O-*benzyl-2-deoxy*-α-L-*altropyranosid)uronate* (**8**) and *benzyl (allyl 2-acetamido-3,4-di*-O-*benzyl-2-deoxy*-β-D-*galactopyranosid)uronate* (**9**). -- *Method A*: Hot aqueous sodium hydroxide (5 mL 2 M) was added to a solution of **7** (410 mg in methanol (5 mL) boiling under reflux. After 30 min at this temperature, the solution was cooled, diluted with water (50 mL) and washed with dichloromethane (2 x 10 mL). Aqueous hydrogen chloride (2 M) was added to the aqueous phase to pH 1 and the products were extracted with dichloromethane. Concentration of the organic phase gave a residue which was dissolved in tetrabutylammonium hydroxide (10 mL, 0.6M) and extracted with dichloromethane (10 mL). After drying ($MgSO_4$), benzyl bromide (0.1 mL) was added, and the solution was boiled under reflux for 2 h. The solution was washed with water (20 ml), and concentrated. Column chromatography of the residue (isooctane - acetone 3:2) gave **8** (203 mg, 51%), m.p. 113-114° (from diethyl ether - light petroleum, $[\alpha]_D$ -109° and **9** (138 mg, 34%), $[\alpha]_D$ +10°.

*Anal.* Calcd. for compound **8**, $C_{32}H_{35}O_7N$: C, 70.4; H, 6.5; N, 2.6. Found: C, 70.2; H, 6.4; N, 2.5.

*Method B.* Compound **5** was treated as for **7** with base for 5 h. It was not possible to keep the resulting acid salt in water solution, and therefore direct acidification, transformation into the tetrabutylammonium salts and esterification were performed. The ratio of the two C-5 epimers was estimated from $^{13}C$ n.m.r.. Treatment of the mixture with hydrogen sulfide in pyridine and triethylamine as described for **7** gave compounds **8** and **9** in a ratio of 3:2. *Method C.* Compound **6** was treated with base as described above for 30 min. After acidification, the product was treated with acetic anhydride and subsequently esterified as described above to give **8** and **9** in a ratio of 3:2. *Method D.* Compound **9** was treated as described in method A to yield **8** and **9** in a ratio of 1:1.

In order to obtain the corresponding product distribution of **8** and **9**, starting from the methyl ester analogues of **7** and its C-5 epimer, compound **7** was treated as described above, except that benzyl bromide was replaced with iodomethane.

From the methyl esters **10** and **11**, **8** and **9** were then prepared as follows: *Method E.* Compound **10** was treated as described in method A to yield **8** and **9** in a ratio of 1:9. *Method F.* Compound **11** was treated as described in method A to yield **8** and **9** in a ratio of 2:3.

*Methyl (allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-*L*-altropyranosid)uronate* (**10**) and *methyl (allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-*D*-galactopyranosid)uronate* (**11**). -- Compound **10** had $[\alpha]_D$ -97°, and compound **11** had $[\alpha]_D$ +31°.

$^{13}C$ N.m.r. data

| Compound | C-1 | Allyl, benzyl, trityl, ring-carbons and C-6. (δ) |
|---|---|---|
| 1 | 101.5 | 61.7 64.2 68.7 71.5 72.4 76.0 |
| 2 | 101.2 | 63.0 64.2 68.8 70.2 72.5 73.7 87.2 |
| 3 | 101.1 | 60.4 60.5 62.8 64.1 68.6 70.0 72.3 73.6 86.9 |
| 4 | 101.3 | 61.7 63.3 70.2 71.6 72.8 74.3 74.8 80.8 |
| 5 | 100.9 | 62.7 70.1 72.7 73.9 74.4 74.8 80.2 82.6 |
| 6 | 103.1 | 52.1 70.0 71.9 73.3 74.2 74.3 82.2 82.6 |
| 7 | 98.3 | 55.0 70.0 72.5 73.8 74.7 75.0 76.9 82.3 |
| 8 | 98.6 (167) | 49.6 67.0 68.8 69.0 70.9 71.2 72.7 73.3 |
| 9 | 98.3 (166) | 55.0 66.9 70.2 72.6 73.7 74.3 74.6 76.8 |
| 10 | 98.5 (167) | 49.5 68.5 68.8 71.0 71.2 72.5 73.3 |
| 11 | 98.2 (164) | 55.2 70.2 72.6 73.9 74.4 74.5 76.7 |

Figures in brackets are C-H coupling constants in Hz.

$^1H$ N.m.r. data (δ)

| | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | $J_{1,2}$ (Hz) | $J_{4,5}$ (Hz) |
|---|---|---|---|---|---|---|---|---|
| 1 | 4.51 | 3.51 | 3.65 | 3.91 | 3.65 | 3.74 3.78 | 8.1 | |
| 2 | 4.22 | 3.47 | nd | nd | nd | nd | 7.9 | |
| 3 | 4.21 | 3.79 | 3.27 | 3.83 | 3.33 | 3.20 3.49 | 8.1 | |
| 4 | 4.26 | 3.87 | 3.33 | 3.76 | 3.50 | nd | 8.1 | |
| 5 | 4.25 | 3.89 | 3.36 | 4.23 | 3.85 | | 8.1 | 1.3 |
| 6 | 3.39 | 3.38 | 4.22 | 4.29 | 3.95 | | 6.4 | 1.5 |
| 7 | 5.09 | 3.47 | 4.52 | 4.29 | 4.02 | | 8.2 | 1.1 |
| 8 | 4.87 | 4.38 | 3.92 | 3.83 | 4.78 | | 2.6 | 8.1 |
| 9 | 5.12 | 3.49 | 4.53 | 4.27 | 4.16 | | 7.7 | 0.9 |
| 10 | 4.86 | 4.40 | 3.93 | 3.82 | 4.74 | | 2.6 | 8.2 |
| 11 | 5.13 | 3.49 | 4.54 | 4.30 | 4.15 | | 8.2 | 1.3 |

nd = not determined

The $J_{1,2}$ and $J_{4,5}$ coupling constants for compounds **8** and **10** indicate that for these, the $^1C_4$ conformation predominates.

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