Bioorganic & Medicinal Chemistry Letters, Vol.2, No.5, pp. 423-426, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

Glycosidases catalyzed synthesis of 2-deoxy-β-glycosides

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(*Received* 24 *February* 1992)

Abstract: β-glycosidases are used for the stereoselective glycosylation of glycals. The one pot preparation of 2-deoxy-β-glycosides containing potentially sensitive glycal units is very advantageous since no protection-deprotection steps are required.

The 2-deoxyglycoside unit is the constituent of a variety of important active natural products such as antibiotics[1,2]. Numerous reports have appeared for the preparation of 2-deoxysugars in a multistep sequence. Of these, the acid catalyzed addition of water or alcohol to acetylated glycals appears to be the most direct method[3-6]. However the protected glycals often give rearranged products in acidic medium. A general procedure was reported recently for the preparation of 2-deoxysugars and their α-glycosides in mild conditions using either a sulfonic acid resin[7] or the triphenylphosphine-hydrobromide catalyzed addition of alcohols to glucal triacetate[8].

A different approach has been developed based on oxidative coupling of glycal units via onium intermediates[9-12]. Recently the controlled linking to monosaccharide derivatives has been devised by reacting two differently substituted glucals with a collidine complex of iodonium perchlorate yielding an [α(1→4)] disaccharide[13]. The iodine offers the possibility of removal by hydride reduction. A second method, developed by the same group is based on the addition of glucal derivatives on an epoxyglucose. In this case the β-disaccharides are obtained [14]. Deoxygenation of the C-2 hydroxyl group has been described[15].

Yet, to date, the methods remain unattractive as many different protection-deprotection strategies are required and rearranged products are often observed under acidic conditions.

The enzyme catalyzed synthesis of carbohydrates appears to hold considerable promise[16] and the use of glycosidases has been applied to the preparation of different products in recent years[17].

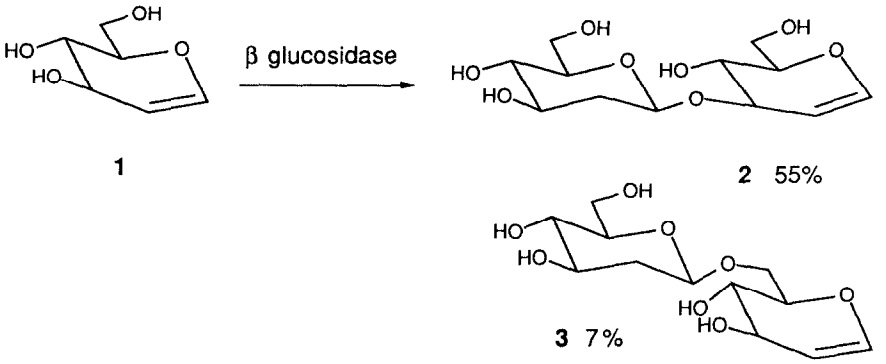
So an alternative for the synthesis of deoxysugar derivatives would be the enzyme-catalyzed addition of alcohols to glycals. In this case no protection of the glycal moiety is necessary. In fact it is known that the addition of water to glucal or galactal is catalyzed by glucosidases or galactosidases respectively and gives the expected 2-deoxysugar[18]. The condensation of glycerol has also been achieved albeit in low yields (7-10%) [19].

The recent report of the use of glycosidases for the synthesis of β-deoxyglycosides derivatives[20] prompted us to present some of our own results.

We have shown in a previous paper that glycosidases catalyze the stereospecific synthesis of glycosyl-aminoacids conjugates [21] and we were interested to see if the same enzymes could be used in the preparation of the 2-deoxy-O-glycosylaminoacids analogues.

Initial experiments were conducted using glucal and protected serine, with β-glucosidase as catalyst and buffer at pH 4 as reaction medium. In this case 2-deoxyglucose is obtained as the main product. The possibility of using organic co-colvents was examined since many enzymatic synthesis have been achieved in these media using proteases, lipases[22] and sometimes glycosidases[23]. Of the different solvents examined we found that acetone was the best for β-glucosidase as observed by Beau *et al.*[20]. In fact if the reaction is performed in acetone containing as little as 5% water two new products are formed and complete disappearance of glucal is observed after 18 hours. No condensation of glucal with serine is seen but instead two glucal units have reacted leading to the disaccharides **2** and **3.**

Optimisation of the reaction conditions has been performed without serine to give a 62% overall yield of the deoxysugar-glucal derivatives **2** and **3** after purification. The major product is the disaccharide **2** with a (1→3)-β-glycosidic linkage (55%). The assignment was based upon ^{1}H and ^{13}C n.m.r. data. The same disaccharide **2** has been obtained as a by-product by Lehmann[24] in a 9% yield. The better yield observed in our condensations is due to the high proportion of organic cosolvent. The disaccharide **2** can be separated easily from the isomer **3** with the (1→6)-β-glycosidic bond. The anomeric α-linked disaccharides were not observed (at least by n.m.r.).

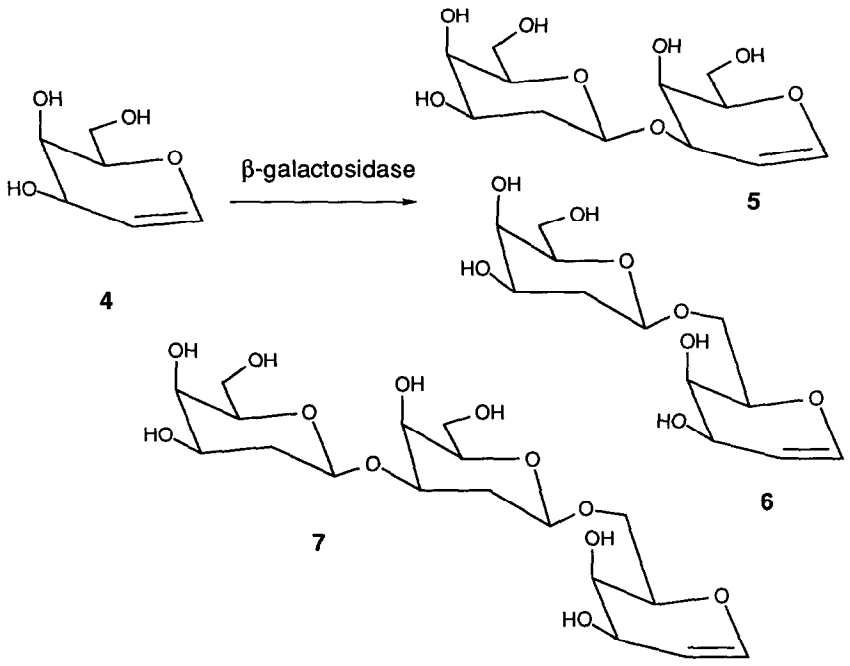


In a representative procedure, glucal (146 mg, 1 mmole) was incubated at 37°C with β-glucosidase from almonds (150 mg, 650 units), 60 ml of 0.1M acetate buffer pH 4 and 2 ml of acetone. The mixture was left for 36 hours, at which time all of the glucal had been consumed. The solvent was removed and the products purified on a RP-18 column (LiChroprep, Merck) with water as eluent. Product **2** (80 mg) is obtained in a 55% yield and product **3** (10 mg) in a 7% yield. A small amount of 2-deoxyglucose as well as traces of unidentified material were also observed. ^{1}H and ^{13}C n.m.r. spectra show that only one type of glycosidic linkage is present. A downfield shift is observed for the C-3 carbon in the disaccharide **2** and for the C-6 carbon in product **3**[25].

This method is very attractive as compared to the one developed by Danishefsky[15] to prepare the same O-(2-deoxy-β-D-glucopyranosyl)-(1→3)-1,5-anhydro-2-deoxy-D-arabino-hex-1-enopyranose **2**. No protection, deprotection of the different hydroxy groups are necessary. It is a one step synthesis, performed under mild

conditions and in good yields. As expected only the β anomer of the deoxyglucose unit is obtained. This disaccharide contains a potentially sensitive glucal that might function for an other deoxygenation sequence.

Experiments have also been performed with E. Coli β-galactosidase and galactal. In this case the reaction is much slower, as was already observed in the enzymatic catalyzed hydration of this substrate[18]. If acetone is used no condensation occurs. No other solvent was found suitable. If water alone is used as reaction medium three new products are obtained with an overall yield of 49%. Only traces of 2-deoxygalactal are observed and galactal is not completely consumed (in our reaction conditions) even after four days of incubation. The β-(1→3) and β-(1→6) disaccharides **5** and **6** are formed but could not be separated by reverse phase chromatography on RP-18 (yield 21%; **5**:**6**, 3:2). They have been identified and separated as their tritylated derivative (6,6' ditrylated derivative for **5**: major isomer; 6' monotritylated derivative for **6**: minor isomer). More interesting is the formation of the trisaccharide **7** (29%) containing two 2-deoxy-β-galactosyl units and galactal as the third sugar, leaving the double bond free for further condensation. N.m.r. COSY spectra (proton-proton and carbon-proton shift correlations) as well as DEPT experiments showed cross-peaks which enabled the assignment for the struture of the trisaccharide[25].



These syntheses demonstrate that glycosidases can be used as catalyst for the stereoselective glycosylation of glycals. The one pot preparation of 2-deoxy-β-saccharide glycals is very advantageous since no protection-deprotection steps are required. Work is in progress to explore the catalytic properties of the glycosidases for the assembly of 2-deoxysaccharides units. The galactosidase catalyzed condensations merit further investigation. In principle, it should be possible to orientate the reaction towards the formation of the di- or the tri-saccharide glycals.

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25 **2**: ^{1}H n.m.r. (D_2O, 300 MHz) δ 6.42 (d, $J_{1,2}$ 6.2 Hz, H-1 glucal), 4.9-4.85 (H-2 glucal, H-1 deoxyglucose), 4.38 (br d, $J_{3,4}$ 6.32 Hz, H-3 glucal), 3.95-3.88 (H-5 glucal, H-6 deoxyglucose), 3.85-3.8 (H-4, H-6,6' glucal), 3.77-3.67 (H-3, H-6' deoxyglucose), 3.42-3.36 (H-5 deoxyglucose), 3.25 (H-4 deoxyglucose), 2.24-1.53 (H-2 deoxyglucose); ^{13}C n.m.r. δ for deoxyglucose 98.82 (C-1), 76.87 (C-5), 71.69 (C-4), 71.18 (C-3), 61.70 (C-6), 39.30 (C-2); for glucal 145.64 (C-1), 100.37 (C-2), 78.82 (C-5), 76.87 (C-3), 67.59 (C-4), 60.71 (C-6). F.a.b. m.s. $(M+H)^+$ calc. 292.28, found 292.2; m.p. 184°C.

3: ^{1}H NMR (D_2O) δ 6.42 (d, $J_{1,2}$ 6.1 Hz, $J_{1,3}$ 1.5 Hz, H-1 glucal), 4.85 (H-2 glucal), 4.73 (H-1 deoxyglucose, dd, J= 9.8, 1.7 Hz), 4.18 (H-3, H-6 glucal), 4.0 (H-5 glucal), 3.90 (H-4 glucal, H-6 deoxyglucose), 3.67 (H-3, H-6' deoxyglucose, H-6' glucal), 3.36 (H-5 deoxyglucose), 3.24 (H-4 deoxyglucose), 2.26-1.5 (H-2 deoxyglucose; ^{13}C n.m.r. δ for deoxyglucose 101.14 (C-1), 76.92 (C-5), 71.81 (C-4), 71.25 (C-3), 61.80 (C-6), 38.90 (C-2); for glucal 144.30 (C-1), 103.97 (C-2), 77.81 (C-5), 69.70 (C-3), 69.04 (C-4), 68.74 (C-6). F.a.b. m.s. $(M+H)^+$ found 292.1.