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Syntheses of ß-2-Deoxy-D-Glycosides Assisted by Glycosidases

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Key Words: enzyme-catalyzed glycosylation; ß-D-2-deoxy-glycoside synthesis; glycals; glycosidases

Abstract: Enzyme-catalyzed preparation of ß-2-deoxy-D-glucosides and galactosides including disaccharides has been achieved using the corresponding glycals as substrates.

Major advances in the chemical synthesis of oligosaccharides have been made during the last decade in terms of efficiency and selectivity[1]. To circumvent the unavoidable lengthy synthetic routes to these polyfunctional substances, enzyme-catalyzed syntheses have emerged as a valuable strategic choice. Two solutions can be set up: using either glycosyltransferases[2] which are generally highly specific enzymes towards *both* the glycosyl donor and acceptor, or glycosylhydrolases which are capable of catalyzing glycosyl transfer reactions by inverse hydrolysis or transglycosylation[3,4]. Good yields and highly predictable results are possible with glycosyltransferases but multienzymatic systems involving cofactors are necessary. From a synthetic viewpoint, the more readily available glycosylhydrolases offer the advantage of a simple catalytic system and accept a broader structural range of alcohol acceptors while retaining the capacity to create stereospecifically glycosidic bonds. However, technical "tricks" have to be set up to compensate for the thermodynamic disadvantage. One is to operate in organic solvents[5] to reduce the amount of water. Another would be to select the catalysis of a reaction in which no water appears in the chemical equilibrium. We now report the results of preliminary experiments which show that pyranoid glycals (1,5-anhydro-2-deoxy-hex-1-enitols) can be used as ß-2-deoxyglycosyl donors in transfer reactions on a preparative scale, catalyzed by glycosidases. The stereoselective chemical synthesis of ß-2-deoxy-glycosides, a problem of current interest, is only possible through a stereodirecting auxiliary group equatorially disposed at C-2, which is removed in a later step.[6] A one-pot enzymatic approach offers an attractive alternative.

Glycals, well known inhibitors of most glycosidases, slowly undergo an enzyme-catalyzed addition of water or alcohols[7] as shown in experiments designed mostly for mechanistic data, e.g., for defining the orientation of the functional groups involved in the catalytic process. The almond ß-glucosidase [E.C.3.2.1.21] and the *Aspergillus oryzae* ß-galactosidase [E.C.3.2.1.23], two commercially available (e → e)[7] glycosidases, were selected for this work.

The enzyme-catalyzed reactions were first examined in a variety of organic solvents (dichloromethane, acetone, acetonitrile) having a minimal water content.[5] The two enzymes[8] were, however, completely inactive under such conditions. After multiple trials, the best results were obtained as follows: The glycals (initial concentration of 0.04-0.06 mM) in a mixture of a citrate-phosphate buffer (pH 5) and an organic co-solvent (70% acetone for the ß-glucosidase and 75% acetonitrile for the ß-galactosidase) were treated by the corresponding enzymes (2000 U/mmol of the glycal) in the presence of alcohol acceptors. For the most significant results of the enzymic 2-deoxy-glycosylations, see the Table.

The reactions were monitored by t.l.c. and stopped when the glycal was almost consumed. In the ß-glucosidase-catalyzed reaction, conversion was high but hydration and/or cleavage of the glycoside formed was the major competing pathway even with a great excess of alcohol (60 equiv., entries 1, 2, glycosides **5** and **6**)[9]. Interestingly, the 6-deoxy analogue **3** was still a substrate and no noticeable 2,6-dideoxy-D-

Entry	Glycal	Alcohol Acceptor (equiv.)	Reaction Time (days)	Product	Yield[a] (%)
ß-D-glucosidase					
1	**1**	MeOH (60)	3	**5** (+4)	30 (-60)
2	**1**	allyl alcohol (60)	3	**6** (+4)	35 (-64)
3	**3**	MeOH (60)	14	**7**	60
4	**3**	allyl alcohol (60)	14	**8**	60
ß-D-galactosidase					
5	**2**	MeOH (50)	5	**9**	50
6	**2**	allyl alcohol (50)	5	**10**	36
7	**2**	**11** (3)	4	**16**	50
8	**2**	**12** (3)	5	**17**	33
9	**2**	**13** (3)	4	**18**	14
10	**2**	**14** (3)	2	**19**	30
11	**2**	**15** (3)	4	**20**	24

[a]Isolated yield based on the glycal.

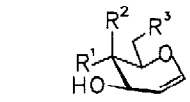
Table: Enzymic 2-deoxy-glycosylation

The reaction mixture containing the glycal and the acceptor (3 to 60 equiv.) in ~0.08-0.02M citrate-phosphate buffer, pH 5 and acetone (70%, ß-glucosidase) or acetonitrile (75%, ß-galactosidase) was stirred at room temperature in the presence of the enzyme (2000 U/mmol of glycal) until the glycal was almost consumed as indicated by t.l.c. The mixture was then filtered, the filtrate was concentrated and the products were purified by column chromatography on silica gel (ß-glucosidase reaction). For the ß-galactosidase reaction the filtrate was concentrated and the dried residue was acetylated (pyridine-acetic anhydride). The acetylated products were purified by column chromatography on silica gel.

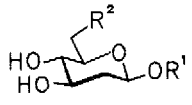
arabino-hexose formation was noted which explains the higher yields in glycosides (60%, entries 3, 4). The enzyme was however unable, under these conditions, to catalyze the glycosides synthesis using carbohydrates as acceptors (sucrose, mannitol). These results agree with two previous observations: the inability of the almond ß-glucosidase to catalyze the polymerization of ß-D-cellobiosyl fluoride[11], and inhibition studies that suggest a hydrophobic binding subsite for the aglycon moiety of the glycosidic substrates[12].

A. orizae ß-galactosidase behaved similarly with simple alcohols for which large excesses were needed (50 equiv.) to obtain an acceptable conversion (entries 5, 6). In contrast to the ß-glucosidase, this enzyme[13] was able to catalyze the addition of a variety of sugar alcohol acceptors onto the galactal and some selected examples are given in the Table (entries 7-11). This disaccharide synthesis deserves several comments: *a)* The reaction worked with 3 equiv. of the acceptor, an important attribute for any synthetic use. *b)* With simple α-galactosides as acceptors, only ß-(1→6) linkages are formed and the conversion efficiency is modulated by the glycosidic substituent's nature (entries 7-9). An increase in the size (or in the hydrophobicity) reduces the yield of the glycoside formation [from 50% (methyl glycoside) to 14% (benzyl glycoside)]. *c)* The regioselectivity of the reaction is under the influence of the glycosyl acceptor structure. An unexpected ß-(1→5) linkage (compound **19**) is selectively[14] formed with mannitol **14** while

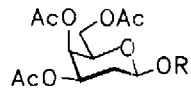
the enzyme catalyzes the formation of a ß-(1→3) linkage with ß-thioxyloside **15**, an acceptor molecule in which no primary hydroxyl group is available. *d)* The limiting yields obtained in the experiments (14-50%, entries 7-11) are **not** a consequence of competing hydration (and/or cleavage of the glycoside formed) because the amount of 2-deoxy-D-galactose isolated in these experiments was low (0-10%) and multiple transfers may be involved.[15]



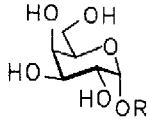
1 $R^1 = R^3 = OH$; $R^2 = H$
2 $R^1 = H$; $R^2 = R^3 = OH$
3 $R^1 = OH$; $R^2 = R^3 = H$



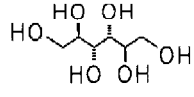
4 $R^1 = H$; $R^2 = OH$
5 $R^1 = Me$; $R^2 = OH$
6 $R^1 = All$; $R^2 = OH$
7 $R^1 = Me$; $R^2 = H$
8 $R^1 = All$; $R^2 = H$



9 R = Me
10 R = All



11 R = Me
12 R = All
13 R = Bn



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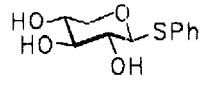

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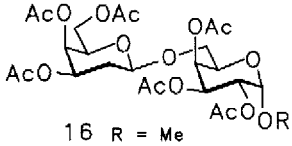
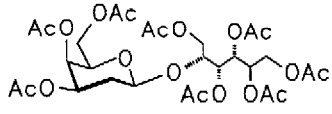


16 R = Me
17 R = All
18 R = Bn



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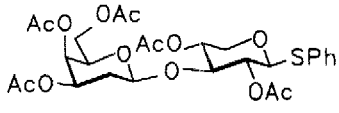

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The one-pot enzymic preparation of ß-D-2-deoxy glycosides, although unpredictable at the present time, merits further investigations, particularly with other sources of enzymes and work along these lines is in progress in our group.

References and Notes

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8. In these experiments, the enzyme preparations were obtained by lyophilizing the commercial preparations (Sigma) previously dissolved in a citrate-phosphate buffer adjusted at pH 5.
9. All new compounds gave satisfactory spectral and/or analytical data.
10. Selected ^{1}H-n.m.r. data ($CDCl_3$, 300 MHz, TMS as internal standard):
    5-8: δ 4.54-4.60 (dd, $J_{1,2ax}$ 9.6-9.8, $J_{1,2eq}$ 1.5-2.0 Hz, H-1)
    9, 10: δ 4.49-4.61 (dd, $J_{1,2ax}$ ~ 9, $J_{1,2eq}$ ~ 3 Hz, H-1)
    16-18, 20: δ 4.55-4.71 (dd, $J_{1',2'ax}$ 8.1-8.9, $J_{1',2'eq}$ 3.0-2.2 Hz, H-1')
    19: δ 4.75 (dd, $J_{1',2'a}$ 7.0, $J_{1',2'b}$ 5.0 Hz, H-1')
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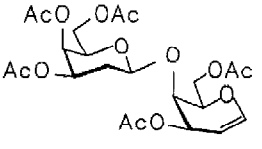
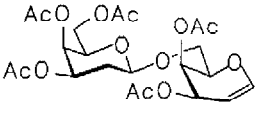
13. Commercial *A. orizae* ß-galactosidase can be used as is with simple alcohols. The preparation contains, however, starch and an active α-glucosidase which perturbs the experiments when using carbohydrate acceptors. Prior to use, the crude enzyme was hence incubated for 2 days at room temperature in a 0.08M citrate-phosphate pH 5 buffer and ultrafiltrated. This treatment eliminates most of the oligo(poly)saccharides present (81% of the original ß-galactosidase activity was recovered).
14. Another unidentified 2-deoxy-galactoside is also formed in minor amounts (<2%).
15. In a separate experiment where no carbohydrate acceptor was added to the reaction mixture, a small amount (11.5% after 5 days of reaction; 55% of galactal recovered) of an unseparable mixture of ***i*** and ***ii*** (ratio 5:1) was isolated after acetylation.



i



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