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Enzymatic synthesis of alkyl arabinofuranosides using a thermostable α-L-arabinofuranosidase

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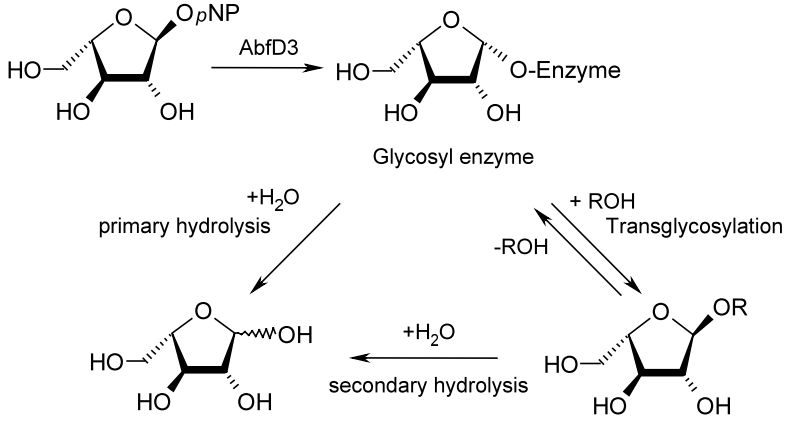
Abstract—A thermostable α-L-arabinofuranosidase was tested for its ability to perform transglycosylation with different alcohol acceptors. Reactions were characterized by high rates with optimal synthesis being obtained within 10 min. Both primary and secondary alcohols could act as acceptors in transarabinosylation but yields of alkyl arabinosides decreased with increasing alkyl chain length.

Enzymatic methods for the organic syntheses of sugar-based compounds have been increasingly developed and adopted over the last decade. This is because enzymes present several advantages when compared to traditional catalysts: high anomeric specificity, mild reaction conditions, no protection/deprotection steps of the reactive hydroxyl groups.[1]

The formation of glycosidic bonds is either catalysed by glycosyl transferases (EC 2.4) or glycosyl hydrolases (EC 3.2.1). Until recently, the use of the former has been restricted due to the limited availability of these enzymes and the need for costly substrates.[1] In contrast, glycosyl hydrolases are abundant and extensively used. These enzymes, which normally cleave glycosidic bonds, can be employed as catalysts under appropriate conditions to synthesize oligosaccharides[2–6] and a variety of glycoconjugates, including alkylated glycosides.[1]

Although a large number of glycosyl hydrolases with different specificities are known to perform transglycosylation,[1,7–9] the majority of reports have described the



Scheme 1.

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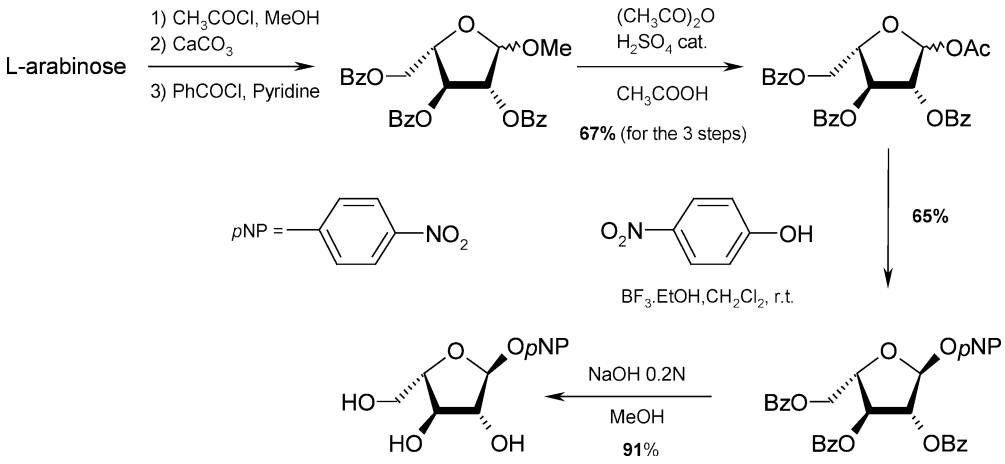

Scheme 2.

use of gluco- and galactosidases for transglucosylation or transgalactosylation, respectively, in the presence of various nucleophile acceptors. This tendency reflects the high availability and the wide-ranging applications of glucosides and galactosides. In contrast, fewer reports have described enzymatic catalysts for C-5 sugar transformations, despite the recent development of medically-relevant L-arabinose-based compounds.[10] In this letter, we report the synthesis of alkyl L-arabinofuranosides using a purified thermostable transglycosylating α-L-arabinofuranosidase (EC 3.2.1.55).

The reactions between *p*-nitrophenyl-α-L-arabinofuranoside (*p*NP-ara) and various primary and secondary alcohols are illustrated in Scheme 1.

In order to perform enzyme-catalyzed reactions, recombinant arabinofuranosidase (AbfD3) was first expressed in *Escherichia coli* and purified as previously described.[11,12] Although the synthesis of *p*NP-ara was previously described,[13,14] we carried out a multi-gram scale synthesis according to methods reported for other monosaccharides. Methyl L-arabinofuranoside[15] was first benzoylated and then transformed in 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-L-arabinofuranose.[16] Glycosylation with *p*-nitrophenol was achieved[17] and the benzoyl groups were removed with dilute sodium hydroxide to yield *p*NP-ara in a 40% overall yield (Scheme 2).

Transglycosylations were performed by incubating *p*NP-ara (5 mM in water) and alcohol acceptors (23%, v/v) in the presence of AbfD3 (59 IU) at 60°C. The progress of reactions was monitored using TLC in AcOEt/AcOH/H_2O (7/2/2). In the absence of alcohol acceptors, *p*NP-arabinobiose was formed indicating that *p*NP-ara can act both as the donor and the acceptor (data not shown). In contrast, in the presence of alcohols, only alkylated products were formed, suggesting that, in the role of nucleophile acceptor, alcohols outcompete both water and *p*NP-ara. In order to maximise product formation it was necessary to identify the optimal reaction time. By varying incubation time it was possible to demonstrate that, regardless of the alcohol used, the maximum yield was obtained after 10 min of incubation at 60°C. Longer incubation times led to the gradual AbfD3-catalyzed hydrolysis of the reaction products. This reaction rate is very fast compared to many of the other glycosyl hydrolase-catalyzed reactions described in the literature, although other examples of fast reactions have been reported.[18,19] Once maximum product yield was achieved, the enzyme was inactivated to avoid hydrolysis (100°C, 10 min) and preparative TLC (AcOEt/AcOH/H_2O: 7/2/2) was performed to facilitate reaction product purification. Accurate quantification of product yield was achieved using reverse phase HPLC (equipped with a dynamic light scattering detector and a C-18 column from which products were eluted using a gradually increasing gradient of acetonitrile in water: 2–60% v/v during 10 min, flow rate 1 mL/min). Table 1 shows the maximum yields obtained with several different alcohols. Propan-2-ol was the least reactive acceptor (24%), while methanol and ethanol gave the highest yields (54%). Up to a chain length of four carbons (with the exception of propan-2-ol), a very gradual decrease in yield was observed. However, in the presence of *n*-pentanol no product was detected, even when the reaction was performed in the presence of a variety of co-solvents.

Table 1. Alkyl arabinosides obtained with AbfD3 in the presence of *p*NP-ara and alcohols

Acceptors	Products[a]	Yields[b] (%)
Methanol	Methyl-α-L-arabinofuranoside	54
Ethanol	Ethyl-α-L-arabinofuranoside	54
n-Propanol	*n*-Propyl-α-L-arabinofuranoside	45
Propan-2-ol	*i*-Propyl-α-L-arabinofuranoside	24
n-Butanol	*n*-Butyl-α-L-arabinofuranoside	42
Butan-2-ol	*i*-Butyl-α-L-arabinofuranoside	41
Allyl alcohol[c]	Allyl-α-L-arabinofuranoside	33
n-Pentanol	None detected	Not applicable

[a] Structures resolved by ^{1}H NMR analysis.
[b] Yields determined by HPLC and based on *p*NP-ara initially added.
[c] CH_3CN (24%, v/v) was used as cosolvent.

While the influence of increasing carbon number on transglycosylation has already been reported,[20,21] the sudden loss of activity in the presence of *n*-pentanol is rather surprising and suggests that either we had not succeeded in identifying the appropriate co-solvent or that AbfD3 is rapidly inactivated in the presence of higher alcohols.

With the exception of ethane-1,2-diol which gave a 20% mixed product yield, all the alkylated reactions products were successfully purified and analysed using ^{1}H NMR spectroscopy. In all cases, only the corresponding α-L-arabinofuranosides were detected, witnessed by H-1 and H-2 coupling constants typical of a *trans*-relationship ($J_{1,2}$ <2 Hz). This is coherent with the notion that the use of glycosidases leads to very tight anomeric selectivity. Moreover, these results confirm the findings of previous work on the hydrolytic mechanism of F-51 arabinofuranosidases,[12,22] which suggested that hydrolysis proceeds via a retention mechanism in which the anomeric configuration is conserved. Additionally, AbfD3 was able to efficiently transfer the arabinofuranose moiety onto secondary alcohols. Indeed, in the case of the butyl alcohols, reaction yields were very similar (42 and 41% for butan-1 and 2-ol, respectively). In the case of a chiral secondary alcohol (butan-2-ol), formation of both diastereomers was obtained in almost identical proportions according to ^{1}H NMR. This latter observation indicates that the action of AbfD3 was not enantiospecific. Although not all the glycosidases tested have been found to be enantiospecific, various reports have indicated that glycosidase enantioselectivity is highly variable and can depend on the exact source of the enzyme. Likewise, in the case of some β-galactosidases from *E. coli* preferential transfer of galactosyl moieties to the (*R*)-enantiomers of chiral alcohols was observed,[23,24] whereas the β-galactosidase from *Sulfolobus solfataricus* was highly selective for the (*S*)-enantiomer in the case of the secondary hydroxyl group of propane-1,2-diol.[25]

In conclusion, this preliminary survey of AbfD3 transglycosylation activity has revealed that this enzyme may be a useful tool for chemoenzymatic syntheses involving L-arabinose. Further experiments are now underway to investigate the ability of AbfD3 to perform transglycosylation in the presence of other nucleophile acceptors, especially sugars other than L-arabinose.

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