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A divergent chemoenzymatic route to an intermediate for nucleoside analogues

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Abstract—Two regioisomeric isoxazoline monoacetates **1** and **2** were synthesized from the corresponding diacetate **3** via PPL or PLE catalyzed hydrolysis. With both the enzymes, the initial regioselectivity (~3–4:1) was offset by an intramolecular acyl transfer. In addition to a non-enzymatic catalysis for the acyl transfer, preliminary experiments do suggest a definite but minor role of enzyme for this intramolecular acyl transfer. Compounds **1** and **2** may serve as intermediates for nucleoside analogues.

Nucleoside analogues continue to draw attention of scientists working in areas related to chemistry or biology due to the widespread application of these molecules as therapeutic agents especially as antiviral compounds.[1] The majority of such analogues concentrated upon modification of the natural substrates in the sugar or the base moiety. The most notable structural variations are found in the furanose ring with its replacement by acyclic chain[2] or carbocyclic[3] or even heterocyclic rings[4] to give many biologically interesting compounds. We became interested to synthesize a modified sugar unit, which is conceptually a hybrid of the natural furanose and an azasugar as represented by **A** and **B**. Recently, Chiacchio et al.[5] synthesized a similar molecule namely a 3′-aza-2′-oxy system (**C**) via a nitrone cycloaddition approach; however, the lack of functionality at 3′-position prohibited its use for incorporation into DNA or RNA chains. The modified sugar unit proposed in our strategy can serve as open-ended nucleoside analogues. In this communication, we report a chemoenzymatic route to differentially protected isoxazolidine monoacetates **1** and **2**. These compounds, in principle, can be converted to various nucleoside analogues represented by **A** or **B**. The other interesting feature of the synthesis is the observation of an intramolecular acyl transfer.

The retrosynthesis (shown in Scheme 1) for the intermediates involves two important reactions: formation of the five-membered heterocycle by a 1,3-dipolar cycloaddition[6] and the differentiation of the hydroxyl groups both of which are primary. The latter can be, in principle, executable through enzymatic intervention, either by hydrolysis of the diacetate **6a**–**c** or by *trans*-acylation of the diol **3a**–**c**.[7] In line with that approach, as a first step, we carried out 1,3-dipolar cycloaddition of N-benzylmethylene nitrone[8] with *cis*-2-butene-1,4-diol in refluxing benzene for 72 h (6 h if carried out in refluxing toluene). The resulting isoxazolidine diol, isolated pure by Si-gel column chromatography in 80% yield, was smoothly acetylated to the diacetates **6a**–**c**, which were subjected to enzymatic hydrolysis using three well known hydrolytic enzymes, namely Porcine Liver Esterase (PLE), Porcine Pancreatic Lipase (PPL) and *Candida cylindracea* (CCL) (Scheme 2).[7] The results are quite interesting and are shown in Table 1.

All the substrates underwent hydrolysis in presence of PPL or PLE to generate the two monoacetates in moderate yields. CCL was unsuitable as the hydrolysis was very slow. The ratio of the two isomers was close to 1:1 for both the PPL and PLE after ~60–80% conversion. One striking feature of the hydrolysis was that during the monitoring of the reaction by TLC, initially the regioisomer **1a**–**c** could be seen as major isomer in the TLC; as the conversion became high, the other regioisomer **2a**–**c** started to appear and the ratio became almost 1:1 at the end of the reaction indicating the dependence of regioselectivity upon the extent of hydrolysis. It also varies, although to a lesser extent, on the

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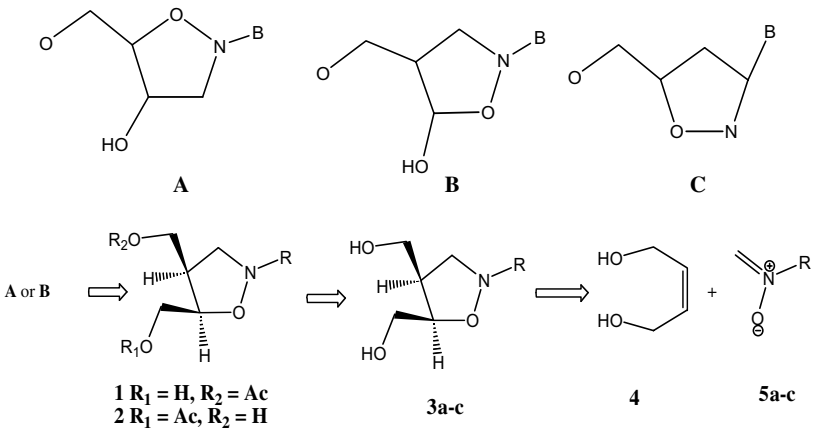
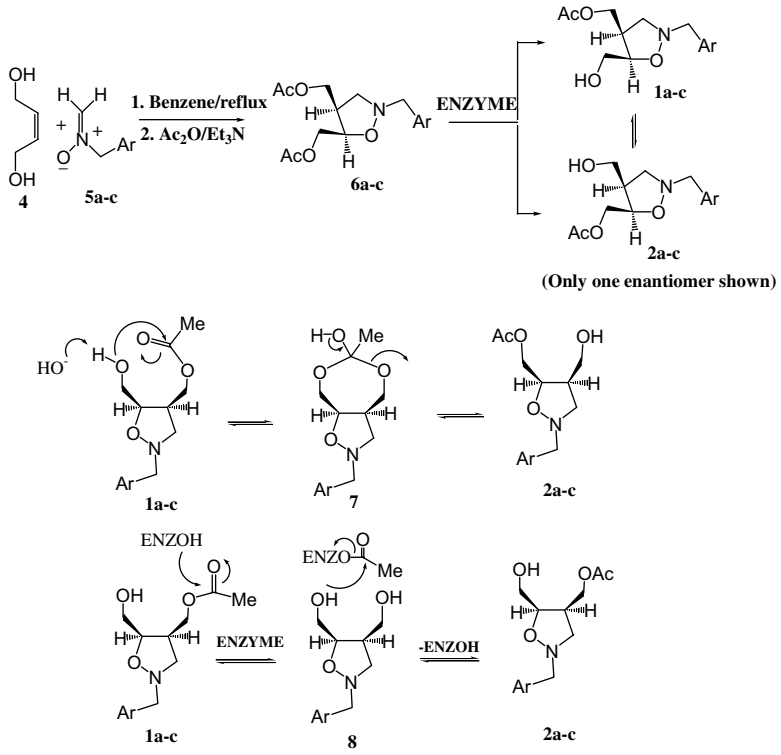


Scheme 1.



Scheme 2.

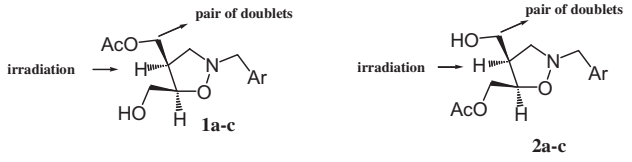
Table 1. Results of enzymatic hydrolysis of various diacetates (**6a–c**)

Substrate/enzyme	% Conversion	Reaction time (h)	Product	Ratio
6a PLE	5	5	**1a + 2a**	~2.5:1 Conversion very low
	62	48		1:1
6b PLE	4	5	**1b + 2b**	~2.5:1 Conversion very low
	60	48		1:1
6c PLE	5	5	**1c + 2c**	~2.5:1 Conversion very low
	60	48		1:1
6a PPL	20	5	**1a + 2a**	3:1
	80	24		1:1
6b PPL	18	5	**1b + 2b**	4:1
	72	24		1:1
6c PPL	15	5	**1c + 2c**	3.5:1
	77	24		1:1

type of enzyme used. For example, with PLE for which the reaction was much slower, the initial regioselectivity (~2.5:1) was obtained at about 5% conversion after 5 h. By the time the conversion reaches ~10%, which took 10 h, the ratio had already become 1:1. In case of PPL, the selectivity was highest (4:1) for ~20% conversion. The electronic or steric nature of the aryl group attached to the N does not play a significant role on the selectivity. There was no diol formation, showing that both the monoacetates **1** and **2** are poor substrates for both the enzymes. Gratifyingly, **1** and **2** could be separated by radial chromatography using hexane–ethyl acetate (1:1). Regarding characterization of the structures, simple ^{1}H and ^{13}C NMR could not distinguish between the two structures. The matter was finally resolved by decoupling experiments. Thus, for the monoacetate **1a–c**, multiplet centering at δ 3.45 for the hydroxymethylene collapsed to a pair of doublets upon irradiation of the signal at δ 3.05 for H-4 with the acetoxymethylene remaining unaffected. Similarly for **2a–c**, irradiation of the one proton multiplet at δ 3.0 at C-4 led to the collapse of the signals for acetoxymethylene into pair of doublets (Scheme 3).

The dependence of regioselectivity on the extent of hydrolysis can be explained if it is assumed that isomerization of **1** to **2** via intramolecular acetyl transfer occurs during hydrolysis. This transfer can be catalyzed by base (the pH of hydrolysis was 7.8) or by the enzyme. To distinguish between the two processes, one of monoacetate **1a–c** was kept at pH 7.8 with and without the enzyme. In both the cases, equilibration between **1** and **2** occurred at almost equal rates. The pH was sufficiently alkaline to cause the isomerization and thus no conclusion could be drawn about the role of the enzyme. When the pH was maintained at 7.0, intramolecular acyl transfer still took place in absence of enzyme but at a slower rate (~4 times). This rate was then compared with that in the presence of enzyme. The rate was found to be slightly higher as indicated by HPLC analysis.[9] However, since the two isomers could not be fully resolved by HPLC, the experiment was repeated using ^{1}H NMR and again slight but definite rate enhancement of isomerization was observed (see Fig. 1). Increasing the amount of enzyme caused a slight rise in the isomerization rate (Table 2). Our conclusion from these experiments are the following: the intramolecular acyl transfer is mostly chemically catalyzed and enzyme only plays a minor role; since the monoacetates are both poor substrates for the enzyme, the marginal increase in the rate of isomerization in the presence of enzyme is mostly masked by the comparatively higher rate of isomerization catalyzed by water. Intramolecular acyl transfer has been reported by Schneider and co-workers[8] in PPL-catalyzed hydrolysis of *cis*-1,2-diacetoxycycloalkanols. The authors indicated that the transfer might be taking place during work up. In our case, direct HPLC analysis indicated that the isomerization occurred in the reaction medium.

Definitive comments could not be made at this point regarding the enantioselectivity as methods (chiral shift NMR experiments or chiral HPLC) to determine enantiomeric excesses failed. However, these appeared to be small as indicated by the low optical rotation values. The fact that we could prepare both the monoacetates in pure form is significant as these can be transformed, in principle, into a variety of nucleoside analogues as shown in Scheme 4. All these transformations have extensive literature precedences.[10] Future work will concentrate on this aspect as well as improvement of stereo- and regioselectivity by transacetylation of diol to circumvent the problem of acetyl transfer. [11]



Scheme 3.

Table 2. Ratio of **1b** and **2b** after 72 h starting with pure **1a**

Without enzyme (Fig. 1a)	With enzyme (10 mg) (Fig. 1b)	With enzyme (100 mg) (Fig. 1c)
1.65	1.48	1.18

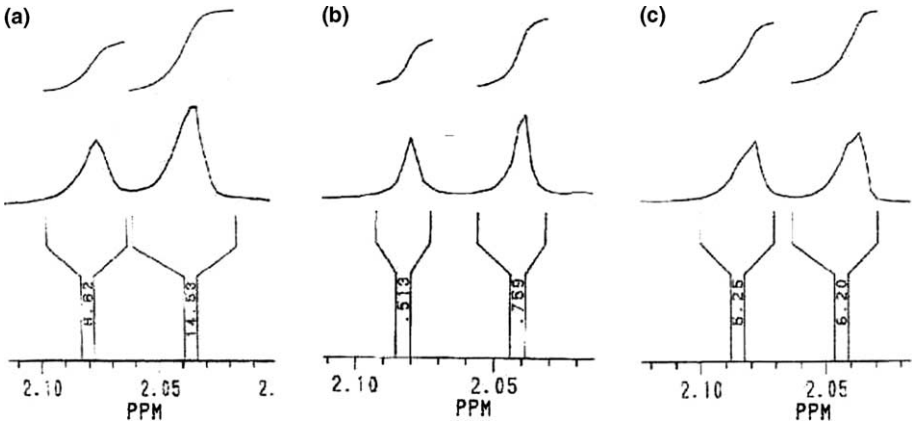
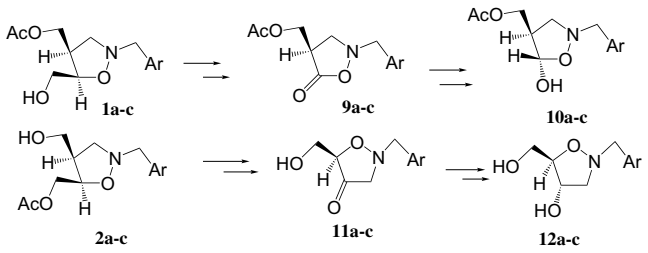


Figure 1.



Scheme 4.

Selected NMR ($CDCl_3$, 200 MHz) data:

For **6a:** δ 7.35–7.27 (5H, m, Ar-*H*), 4.35–4.33 (2H, m, *H*-5, ArCH_2), 4.22–4.12 (4H, m, $2\times$CH_2OAc), 3.90 (1H, br m, ArCH_2), 3.08 (3H, m, *H*-3, *H*-4), 2.08 (3H, s, OAc), 2.05 (3H, s, OAc).

For **1a**: δ 7.43–7.25 (5H, m, Ar-*H*), 4.28 (2H, m, *H*-5, ArCH_2), 4.02 (2H, m, CH_2OAc), 3.80 (3H, m, CH_2OH, ArCH_2), 3.07 (2H, m, *H*-3), 2.35 (1H, m, *H*-4), 2.05 (3H, s, OAc).

For **2a:** δ 7.35–7.29 (5H, m, Ar-*H*), 4.71 (1H, m, *H*-5), 4.41 (2H, m, ArCH_2), 4.19 (2H, m, CH_2OAc), 3.0 (4H, m, CH_2OH, H-3), 2.40 (1H, m, *H*-4), 2.08 (3H, s, OAc).

For **6b:** δ 7.24 (2H, d, J = 8.8 Hz, Ar-*H*), 6.85 (2H, d, J = 8.8 Hz, Ar-*H*), 4.33 (2H, m, H-5, ArCH_2), 4.14 (4H, m, $2\times$CH_2OAc), 3.88 (1H, m, ArCH_2), 3.79 (3H, s, OMe), 3.05 (3H, m, *H*-3, *H*-4), 2.07 (3H, s, OAc), 2.04 (3H, s, OAc).

For **1b**: δ 7.24 (2H, d, J = 8.6 Hz, Ar-*H*), 6.85 (2H, d, J = 8.6 Hz, Ar-*H*), 4.40 (2H, m, *H*-5, ArCH_2), 4.20 (2H, m, CH_2OAc), 3.87–3.65 (3H, m, CH_2OH, ArCH_2), 3.75 (3H, s, OMe), 2.96–2.86 (3H, m, *H*-3, *H*-4), 2.07 (3H, s, OAc).

For **2b**: δ 7.25 (2H, d, J = 8.8 Hz, Ar-*H*), 6.86 (2H, d, J = 8.8 Hz, Ar-*H*), 4.27 (3H, m, H-5, CH_2OAc), 3.94–3.64 (3H, m, ArCH_2, CH_2OH), 3.79 (3H, s, OMe), 3.06 (3H, m, CH_2OH, *H*-3), 2.70 (1H, m, *H*-4), 2.4 (3H, br s, OAc).

For **6c:** δ 6.93 (1H, s, Ar-*H*), 6.78 (2H, m, Ar-*H*), 4.36 (2H, m, *H*-5, ArCH_2), 4.18 (4H, m, $2\times$CH_2OAc), 4.01–3.94 (1H, m, ArCH_2), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 3.06 (3H, m, *H*-3, *H*-4), 2.08 (3H, s, OAc), 2.05 (3H, s, OAc).

For **1c**: δ 6.93 (1H, m, Ar-*H*), 6.85 (2H, m, Ar-*H*), 4.44–4.39 (2H, m, *H*-5, ArCH_2), 4.32–4.20 (2H, m, CH_2OAc), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 3.76 (3H, m, CH_2OH, ArCH_2), 2.95 (3H, m, *H*-3, *H*-4), 2.08 (3H, s, OAc).

For **2c**: δ 6.84 (1H, s, Ar-*H*), 6.79 (2H, m, Ar-*H*), 4.31–4.37 (4H, m, *H*-5, CH_2OAc, ArCH_2), 3.96–3.67 (3H, m, CH_2OH, ArCH_2), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 3.07 (3H, m, *H*-3, *H*-4), 2.04 (3H, s, OAc).

Acknowledgements

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