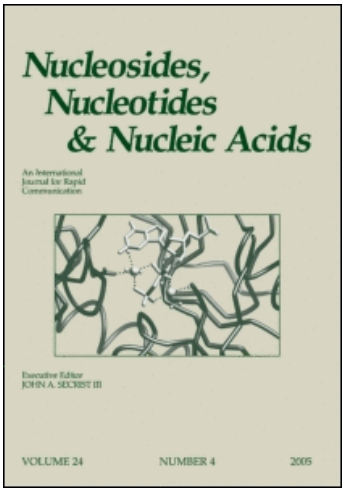

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Enzyme-Mediated Protecting Group Chemistry on the Hydroxyl Groups of Nucleosides

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ENZYME-MEDIATED PROTECTING GROUP CHEMISTRY ON THE HYDROXYL GROUPS OF NUCLEOSIDES

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Abstract: Enzymes are well known catalysts for carrying out regio- and stereoselective reactions in organic synthesis. Results from enzyme-mediated protections and deprotections of the hydroxyl groups of pentofuranose nucleosides are discussed in this review.

INTRODUCTION

Nucleosides are by virtue of their crucial roles in storage and transfer of metabolic energy and genetic information among the most important biomolecules, which has stimulated much interest in nucleoside analogues as potential chemotherapeutic agents. During the last ten years, the following two points have been key factors responsible for the sharply rising focus on nucleoside chemistry: a) the discovery of 2',3'-dideoxynucleosides like AZT, ddC and d4T as anti-HIV agents,[1-3] and b) the emergence of antisense and antigene oligonucleotides as potential and selective inhibitors of gene expression.[4-6] In these contexts, the intrinsic problems in nucleoside chemistry, *e.g.* the presence of multiple functionalities which are difficult to protect selectively and the absence of stereocontrol when condensing a 2-deoxy carbohydrate with a nucleobase,[7] have been emphasised. Whereas enzymes have been widely used in the organic chemistry of carbohydrates,[8-13] their implication in the chemistry of nucleosides[14] has been rather slow, except for the use of glycosyl transferring enzymes.[15] The use of enzymes for protecting group transformations in nucleoside chemistry[16] is presented in this review.

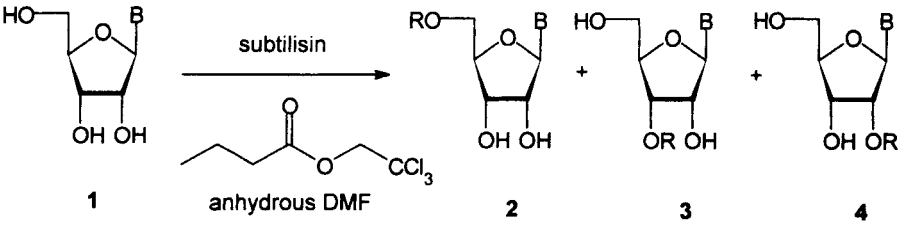
ENZYMATIC ACYLATIONS

The selective protection and deprotection of the hydroxyl groups of nucleosides is important for synthesis of nucleotides and nucleosides and for preparation of pro-drugs or analogues of antiviral agents. Although several chemical methods are available for the regioselective acylation of the carbohydrate moiety of nucleosides, recently developed enzymatic methods of acylation and deacylation offer significant advantages with respect to yield, regioselectivity and the number of synthetic steps which have to be carried out.

The enzymatic acylation of the carbohydrate moieties of nucleosides in dry organic solvents has shown substantial success. While direct enzymatic esterification of nucleosides with acids is often not practical, good to excellent yields of selectively protected acyl nucleosides have been obtained using transesterification techniques. Thus, transfer of a butyryl moiety from trichloroethyl butyrate to adenosine and uridine has been carried out in the presence of the proteolytic enzyme subtilisin in anhydrous DMF.[17] Although the yields and the regioselectivities of the enzymatic acylations generally were poor, the hydroxyl group at the 5'-position of adenosine and uridine were preferentially acylated (Scheme 1). The reactivity and selectivity of subtilisin was later increased by changing the solvent and the acylating agent trichloroethyl butyrate in DMF to trifluoroethyl butyrate in anhydrous pyridine, thus affording 5'-*O*-acylated derivatives of uridine, *N*-4-anisoylcytidine, adenosine and *N*-6-benzoyladenosine in 67-82% yield.[18]

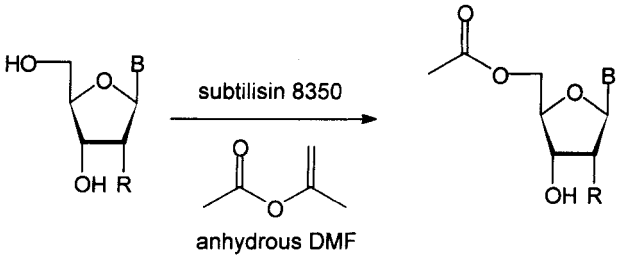
Highly regioselective acetyl transfer reactions to the carbohydrate parts of nucleosides has been carried out by employing a subtilisin mutant (subtilisin 8350, obtained *via* site-specific mutations) in anhydrous DMF.[19,20] This mutant enzyme transfers the acetyl group from isopropenyl acetate to the primary hydroxyl groups of various ribonucleosides and 2'-deoxyribonucleosides in very high yields (Scheme 2). The high regioselectivity of the mutant enzyme was attributed to the fact that it binds the reaction transition state more strongly than does the parent enzyme.

Selective introduction of protecting groups at the different hydroxyl functions of nucleosides have also been achieved by means of lipases. The regioselectivity of the acyl group transfer can be varied by changing the lipase. Unprotected thymidine and 2'-deoxyadenosine were converted to 3'-*O*-acylated/alkoxycarbonylated derivatives by use of Amano PS lipase[21,22] as catalyst. Thus, oxime esters in pyridine and alkoxycarbonyl



R	B	Isolated yield (%)	Product ratio (%)		
			2	3	4
n-C_3H_7CO	adenin-9-yl	35	85	15	-
n-C_3H_7CO	uracil-1-yl	43	55	35	10

SCHEME 1



B	thymin-1-yl	uridin-1-yl	cytosin-1-yl		adenin-1-yl	
R	H	OH	H	OH	H	OH
Yield (%)	100	80	90	80	80	65

SCHEME 2

oxime esters in THF were used as acyl and alkoxycarbonyl transferring agents affording 3'-*O*-monoprotected derivatives in yields of 54-83% and 64-82%, respectively. If a lipase from *Candida antartica* (SP 435) was used, 5'-*O*-acylated nucleosides were obtained.[23-25] These reactions were performed in THF or in dioxane on a variety of ribonucleosides and 2'-deoxyribonucleosides. Generally, the yields and regioselectivities were excellent, but small quantities of 3'-*O*-mono- and 3',5'-di-*O*-acylated products were observed in the case of 2'-deoxyribonucleosides (Scheme 3).[23] Nucleosides bearing a cytosine were found to be unreactive with oxime esters under these conditions.

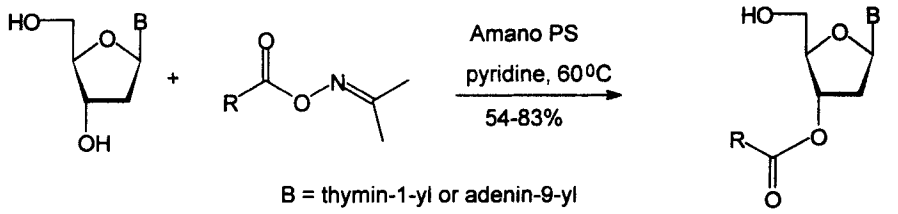
Acylation of cytidine and 2'-deoxycytidine with acid anhydrides and SP 435 resulted in formation of *N*,5'-*O*-diacylated compound.[23] Lipase SP 435 in THF has been shown to be the best combination, if compared to a wide variety of enzymes (*i.e.* PSL, PPL, CCL), for 5'-*O*-acylation and 5'-*O*-alkoxycarbonylation of 2,3'-anhydrothymidine, 2,2'-anhydrouridine, *ara*-uridine, *N*-butyryl-*ara*-cytidine and the α- and *xylo*-epimers of thymidine using butyric anhydride and benzyloxycarbonyl-*O*-acetoxime as acyldonors, respectively (Scheme 4).[25a]

Acylation of 2'-deoxy-5-substituted uridine nucleosides with acid anhydrides in the presence of a lipase from *Pseudomonas fluorescence* (PFL) in organic solvents showed only moderate selectivity towards the 5'-hydroxyl group.[26] When Amano PS was used instead, the 3'-hydroxyl function was acylated almost exclusively (Scheme 5).[27]

Selective acylations of each of the three hydroxyl groups of the sugar moiety of 5-fluorouridine and uridine have been achieved with different lipases and acid anhydrides in dioxane (Scheme 6).[28-29] Using *n*-octanoic anhydride as acyl donor, Amano PS and KWI-56 (a lipase from *Pseudomonas sp.*) induced highly efficient and selective acylation of the 3'-hydroxyl group of 5-fluorouridine and uridine, whereas SP 435 catalysed the preferential acylation of 5'-hydroxyl group of 5-fluorouridine, uridine and *ara*-uridine. Although, the conversion was poor, lipase M (a lipase from *Mucor javanicus*) selectively acylated the hydroxyl group at the C-2' position of 5-fluorouridine.[28]

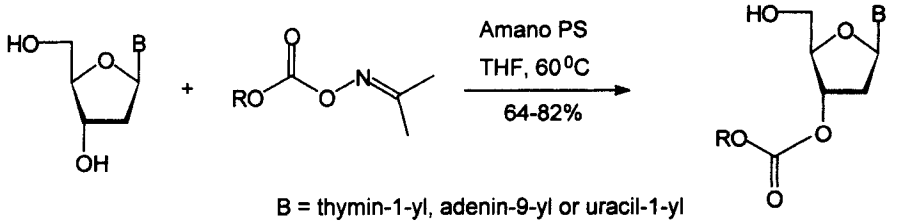
ENZYMATIC DEACYLATIONS

Enzyme-catalysed deacylations have been applied as a method to selectively remove one or more acyl groups from polyacylated nucleosides. Early studies focused on the use of the dihydrocinnamoyl group as an enzyme-labile protecting group removable by α-



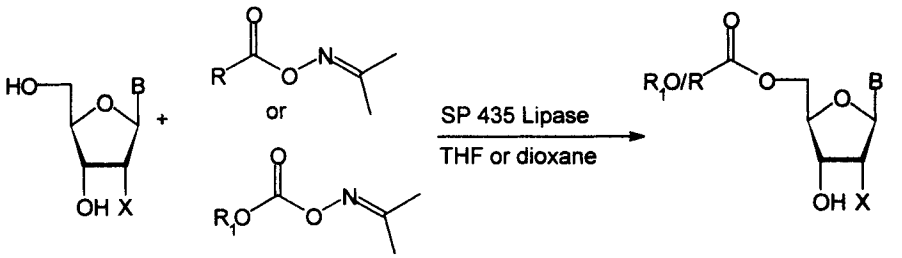
B = thymin-1-yl or adenin-9-yl

R = alkyl, 1-propenyl or phenyl



B = thymin-1-yl, adenin-9-yl or uracil-1-yl

R = alkyl, benzyl, vinyl or allyl



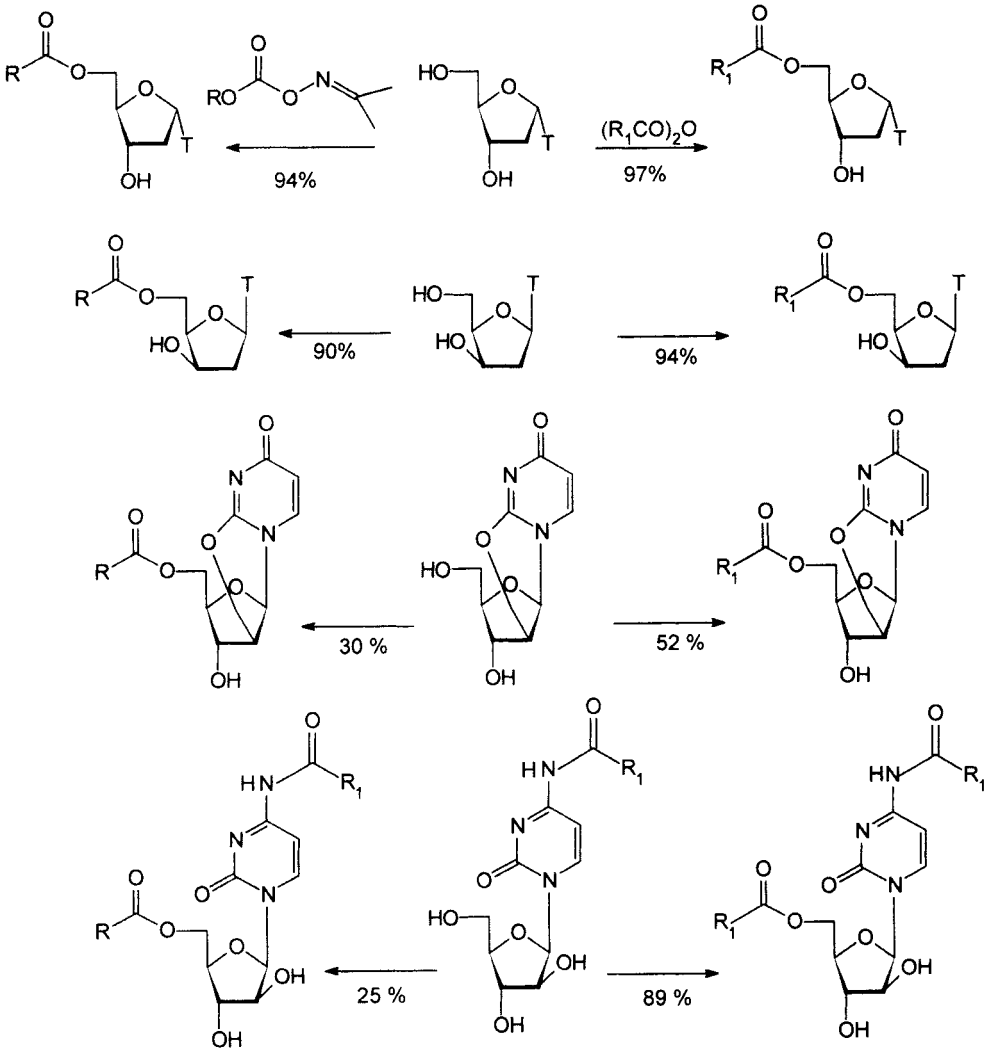
B = thymin-1-yl, hypoxanthin-9-yl, adenin-9-yl or uracil-1-yl

X = H or OH

R = alkyl, vinyl, allyl, phenyl or chloroalkyl

R_1 = alkyl or vinyl

SCHEME 3

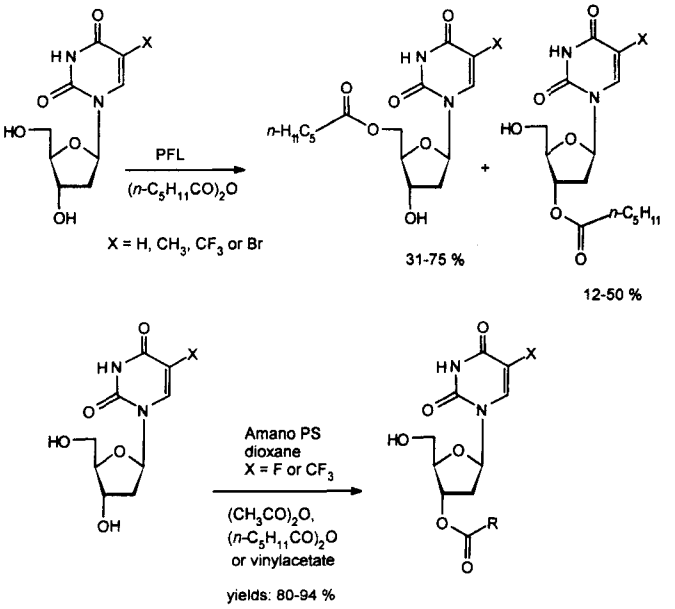


R = $PhCH_2$; R_1 = $CH_3CH_2CH_2$

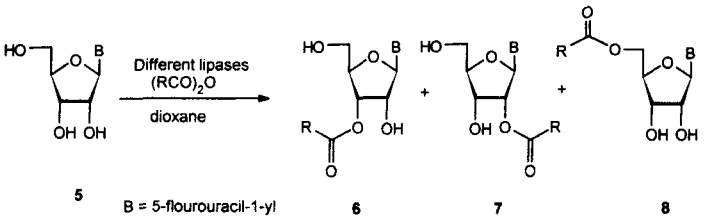
T = thymin-1-yl; U = uracil-1-yl

Enzyme used: SP 435 lipase

SCHEME 4



SCHEME 5



R	lipase	conversion (%)	Yield (%)		
			6	7	8
n-C_7H_{15}	Amano PS	100	92	1	trace
n-C_7H_{15}	KWI-56	98	86	2	4
n-C_7H_{15}	SP 435	100	trace	1	90
n-C_7H_{15}	Lipase M	50	3	42	-

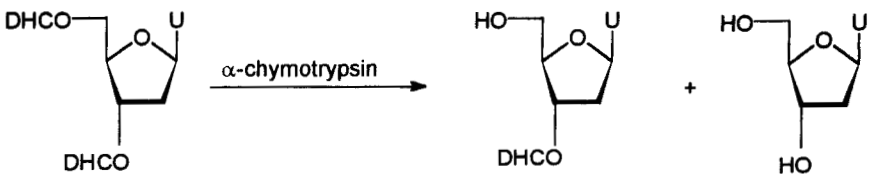
SCHEME 6

chymotrypsin.[30,31] Although the enzyme showed an interesting tendency to attack preferably the 5'-position (Scheme 7), this approach has not been further exploited.

The lipase PFL and the protease subtilisin has been used to effect regioselective deacylation of several 2'-deoxy-5-substituted uracil nucleosides in phosphate buffer with DMF as co-solvent.[32] Thus, PFL preferably removes the acyl group on the secondary hydroxyl function leading to the 5'-esters in reasonably good yields. Contrary, subtilisin shows preference for 5'-acyl group cleavage rendering the 3'-esters available (Scheme 8). With both enzymes, also the completely deprotected nucleosides were obtained in yields of 6-71%. Promising results were obtained when a lipase from porcine pancreas (PPL) in neat phosphate buffer was used for deacylation of 3',5'-di-*O*-acetylthymidine.[33] PPL attacks the 5'-*O*-acetyl group selectively leading to 3'-*O*-acetylthymidine in 98% yield. A contrasting result was obtained with the lipase from *Candida cylindracea* which catalysed hydrolysis of the 3'-ester three times faster than the 5'-ester.[33]

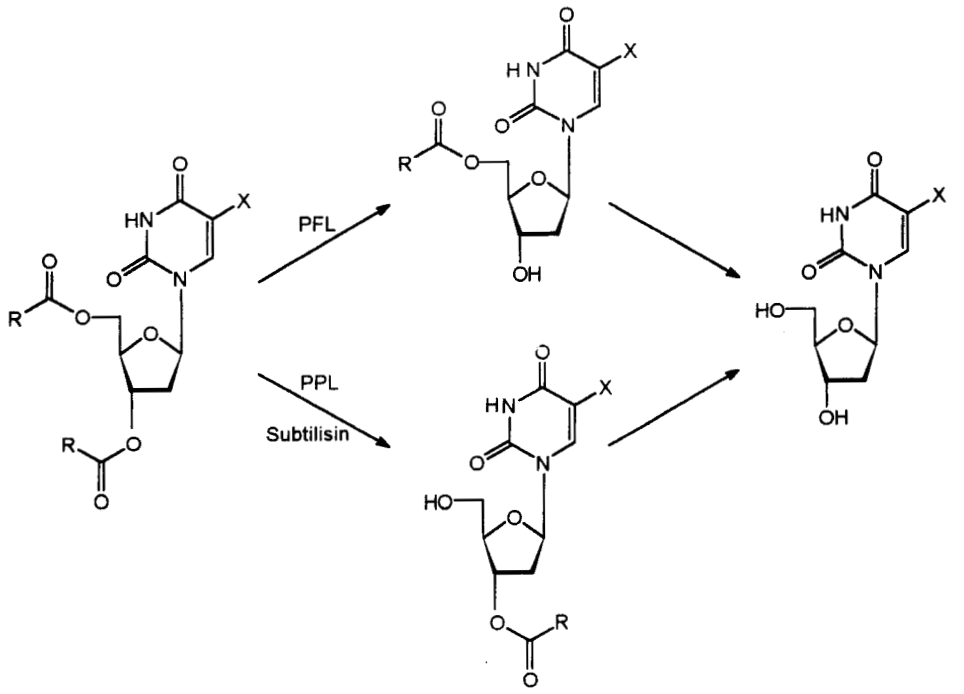
Regioselective hydrolysis of 2',3',5'-tri-*O*-acylated arabinofuranosyl nucleosides has been carried out with pig liver esterase (PLE) in phosphate buffer with ethanol as co-solvent leading to 2'-*O*-monoacylated nucleosides in high yield (Scheme 9).[34] Similar results were obtained on 9-(2,3,5-tri-*O*-acyl-β-D-arabinofuranosyl)adenine with a cell paste of *Bacillus subtilis*.[35] Subtilisin in phosphate buffer with or without organic co-solvents (DMF or dioxane) selectively hydrolysed the 5'-position of 2',3',5'-tri-*O*-acylated pyrimidine and purine ribonucleosides to give 2',3'-di-*O*-acylribonucleosides in 40-92% yield (Scheme 9).[36]

Free or immobilised penicillin amidase has been reported to catalyse complete hydrolysis of 6-*N*-phenacetyl and 2-*N*-phenylacetyl protecting groups on the exocyclic amino groups in 2'-deoxyadenosine and 2'-deoxyguanosine, respectively.[37] The diastereoselectivity of deacetylations mediated by wheat germ lipase (WGL), porcine liver esterase (PLE), porcine pancreas lipase (PPL) and α-chymotrypsin have been examined on a 1:1 mixture of α- and β-3',5'-di-*O*-acetylthymidine and a 2:1 mixture of α- and β-1-(2-deoxy-3,4-di-*O*-acetyl-D-ribopyranosyl)thymine in a pure aqueous medium as well as in a partly organic one.[38] WGL and PLE in pure phosphate buffer afforded the β-anomer thymidine as the only completely deacetylated compound in 29% and 31% yield, respectively, whereas no clear cut diastereoselectivity was observed for the pyranose derivatives (Scheme 10).



DHC = $C_6H_5(CH_2)_2C(=O)$ U = uracil-1-yl

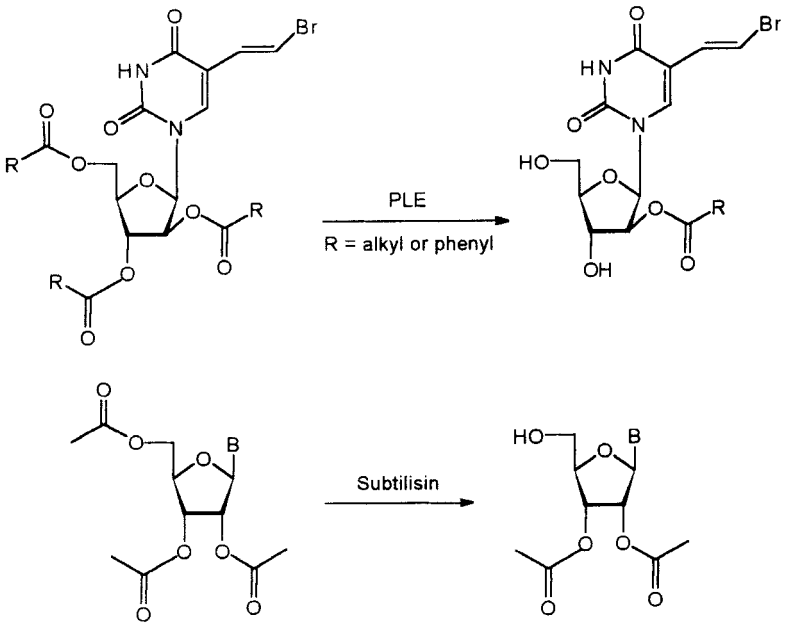
SCHEME 7



X = H, Br, F, CH_3 or CF_3

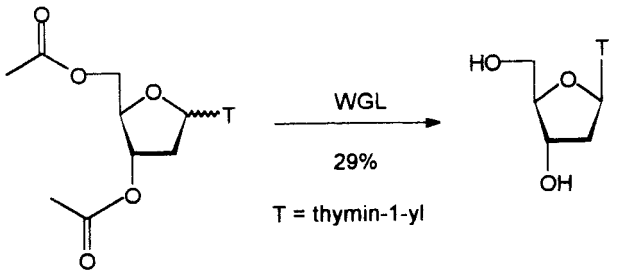
R = *n*-C_5H_{11} or CH_3

SCHEME 8



B = uracil-1-yl, cytosin-1-yl, adenin-9-yl, hypoxanthin-9-yl, guanin-9-yl or*N*-2-acetylguanin-9-yl

SCHEME 9



SCHEME 10

CONCLUSION

Although enzyme-catalysed glycosyl transferring reactions so far have been the predominant type of biotransformations on nucleosides, the number of reports describing enzyme-mediated protections and deprotections has been increasing in the last few years, however limited so far to acylation and deacylation reactions. As some of these enzymatic protecting group strategies allow high-yielding syntheses under mild conditions of regioselectively protected nucleosides, they should find future applications in nucleoside chemistry and research in this direction should be continued.

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