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Nucleoside Synthesis Mediated by Glycosyl Transferring Enzymes

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In recent years, enzymatic methodology has become a standard technique for the synthesis of a wide variety of enantiomerically pure precursors and target molecules. Reports on synthesis of nucleosides involving glycosyl transferring enzymes (covering literature up to October 1998) are summarized and discussed in this review.

INTRODUCTION

Nucleosides and their derivatives form the major components of natural products and are involved in many different kinds of biochemical processes, notably the storage and transfer of genetic information. Recently, several nucleosides have been found to possess antiviral activity against HIV-I (*1–5*), herpes viruses, and influenza A and B viruses. Indeed, the importance of this class of compounds is indicated by the fact that among the eight synthetic clinically approved anti-HIV drugs, seven are nucleoside analogs (*1*). In addition to the antiviral activity of nucleosides, the emergence of antisense and antigene oligonucleotides as potential and selective inhibitors of gene expression (*6–9*) has stimulated much interest in this class of compounds. Though the number of publications dealing with the synthesis of nucleosides and their analogs is extensive and growing at a rapid rate, two basic problems have been emphasized, *i.e.*, manipulation of the multiple functionalities and the absence of stereocontrol when condensing a 2-deoxy carbohydrate with a nucleobase (*10*). Enzymes have been widely used in synthetic manipulations on carbohydrates (*11*), but these reagents have not much been exploited in carrying out similar manipulations for stereocontrolled synthesis of nucleosides (*12*). A brief review on enzyme-mediated synthesis of nucleosides has appeared as part of a general review on "Enzyme Catalysis in Synthetic Carbohydrate Chemistry" in 1991 (*11*). Again, an overview on the use of enzymatic methods for the synthesis of antiviral nucleosides has been published by Hutchinson (*4*) in 1990. No exclusive, updated review has thus far been published covering all the aspects of glycosyl-transferring enzyme-mediated synthesis of nucleosides, which has encouraged us to write this review. This review covering literature reports up to

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October 1998 focuses mainly on the synthesis of nucleosides mediated by glycosyl transferring enzymes, together with interesting examples of nucleoside modifications and resolution of enantiomeric mixtures of biologically active carbocyclic nucleoside analogs.

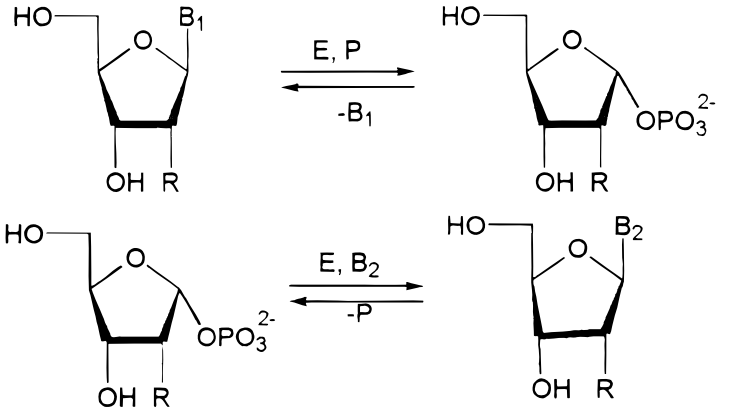
GLYCOSYL TRANSFER REACTIONS

Synthesis of nucleosides has been achieved by various chemical methods (*13*,*14*). However, chemical synthesis often involves difficult and time-consuming multistep processes, *i.e.*, suitable protecting groups are often required on the heterocyclic base and/or on the sugar residue to allow modification of naturally occurring nucleosides. These protecting groups must be removed at a later stage resulting in an overall lower yield. Other problems often faced during nucleoside synthesis include control of configuration at the anomeric center and the regioselective glycoside formation at one of several possible nucleophilic positions in the heterocyclic bases. The enzymatic syntheses of both natural and unnatural nucleosides reported in recent years offer advantages over the chemical methods, as protecting groups are not usually required and highly stereospecific reactions occur. Two main classes of enzymes, nucleoside phosphorylases and *N*-deoxyribosyltransferases, have mainly been used in the synthesis of nucleosides by mediating the transfer of glycosyl residues to acceptor bases. In addition, an immobilized enzyme from thymine-dependent *Escherichia coli* mutant cells and phosphopentomutase coupled with nucleoside phosphorylase have proven successful.

Glycosyl Transfer Reactions Catalyzed by Nucleoside Phosphorylases

Nucleoside phosphorylases catalyze the reversible phosphorolysis of ribo- and deoxyribonucleosides leading to α-D-ribose- or deoxyribose-1-phosphate (R-1-P) with the release of a nucleobase (*15*). The presence of another nucleobase results in the formation of a new nucleoside; both pyrimidine and purine nucleoside phosphorylases are known. The overall result of nucleoside phosphorylase catalysis is the transfer of the ribose or the deoxyribose moiety of a readily available nucleoside to different purine or pyrimidine nucleobases or analogs thereof through the intermediacy of R-1-P. These transformations have either been accomplished by employing isolated enzymes (*16*) or whole cells of microorganisms containing high percentages of the required enzyme (*17–21*). The deleterious effect of the other enzymes present in whole cells can be neutralized by conducting the reactions at 60°C, a temperature at which the nucleoside phosphorylases maintain more than 70% of their activities (*22*,*23*).

Two basic procedures have generally been employed to carry out the glycosyl transfer reactions in the presence of nucleoside phosphorylase:(a) isolation of R-1-P and its subsequent use as a glycosyl donor to acceptor nucleobases (Scheme 1) (*24*,*25*), and (b) exchange of one nucleobase for another in one-pot procedures in the presence of a catalytic amount of inorganic phosphate (Scheme 2). Sometimes, the released nucleobase from the glycosyl donor binds more strongly with the enzyme than the acceptor base, which results in competitive inhibition (*26*). The problem of inhibition can be solved by using a coupled enzymatic system, *e.g.*, pyrimidine and purine nucleoside phosphorylase together with a pyrimidine nucleoside as the glycosyl

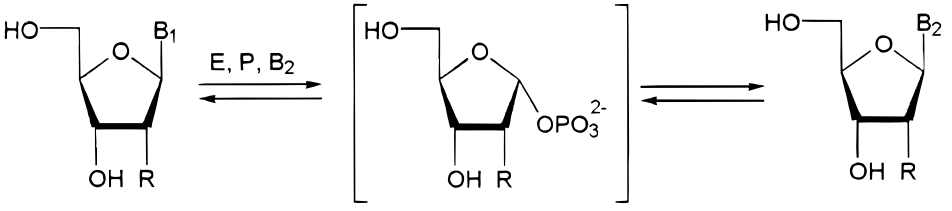


$B_1 = B_2$ = purine or pyrimidine; E = nucleoside phosphorylase
P = inorganic phosphate; R = H or OH

SCHEME 1

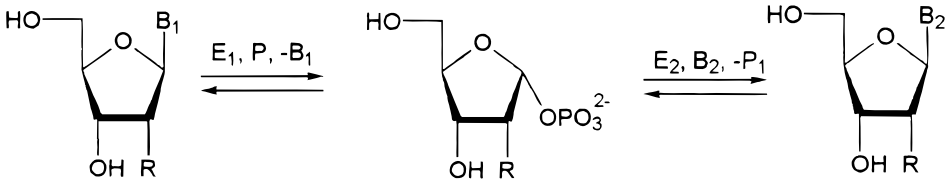
donor and a purine base as the acceptor, since the released pyrimidine base does not inhibit the purine nucleoside phosphorylase (Scheme 3) (*27–30*).

Hennen and Wong (*31*) have used activated purine derivatives, prepared by alkylation at *N*-7 as the glycosyl donor to carry out direct purine to purine exchange reactions without isolation of R-1-P, particularly suitable alkylated nucleosides are methyl nucleosides. The liberated *N*-7-methyl purines precipitate out of the solution and the reaction is almost irreversible. The effectiveness of this approach was demonstrated by a one-pot synthesis of virazole, a broad spectrum antiviral agent using 7-methylguanosine as glycosyl donor and 1,2,4-triazole-3-carboxamide (TCA) as an acceptor base (*31*).



$B_1 = B_2$ = purine or pyrimidine; E = nucleoside phosphorylase
P = inorganic phosphate; R = H or OH

SCHEME 2



B_1 = pyrimidine; B_2 = purine
E_1 = pyrimidine nucleoside phosphorylase
E_2 = purine nucleoside phosphorylase
P = inorganic phosphate; R = H or OH

SCHEME 3

Nucleoside phosphorylases have been found to accept a wide range of nucleoside analogs as substrates with modifications in both the base and the glycosyl components. Table 1 summarizes the synthesis of nucleosides with natural and modified purine and pyrimidine bases, while Table 2 gives the synthesis of nucleosides with modified glycosyl moieties. Most of these reactions have been carried out in one-pot without isolation of the intermediate sugar phosphate, although involvment of the sugar phosphate intermediate has been shown (*22*,*32*). The use of unnatural nucleobases has met with much success and a variety of 3-deazapurine nucleosides have been synthesized by transfer of the pentosyl moiety from appropriate pyrimidine nucleoside in the presence of a coupled enzymatic system (*27*). With most of the 3-deazapurines, the products obtained were pentosylated exclusively at the 1-position. Some of the nucleobases containing an azido group at the 4-position afforded 1- and 3-glycosylated isomers (*27*). 3-Methylguanine does not act as a substrate for purine nucleoside phosphorylase (*33*).

Mikhailopulo *et al.* (*34*) have used whole cells (*E. coli* BMT-1D/1A) which contain a highly active purine nucleoside phosphorylase for transferring the pentosyl moiety from guanosine or 2′-deoxyguanosine to 1- and 3-deaza and 1,3-dideazapurines and proved that N-1 or N-3 are not essential for transglycosylation. Whole cells of various bacteria (including *Enterobacter aerogenes*, *Escherichia coli*, *Erwinia herbicola*, and *Aeromonas salmonicida*), containing high percentage of nucleoside phosphorylase are capable of transferring the arabinofuranosyl moiety from arabinofuranosyluracil to adenine (*17*). Kalinichenko *et al.* (*35*) have used a suspension of glutaraldehyde treated *E. coli* BM-11 cells in potassium phosphate solution for the synthesis of an anti-herpes drug, brivudin [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] using 2′-deoxyguanosine or thymidine as glycosyl donor and (*E*)-5-(2-bromovinyl)uracil as the acceptor. Of the 424 strains of microorganisms belonging to 39 genera examined, *Bacillus megaterium* AJ 3284 was identified as the best candidate for transferring the ribosyl moiety from adenosine to 1,2,4-triazole-3-carboxamide for the synthesis

TABLE 1

Nucleoside Phosphorylase Catalysed Glycosylation of Various Heterocycles

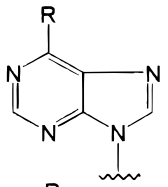
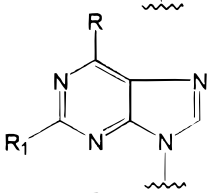
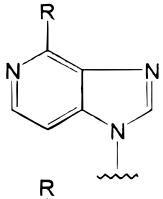
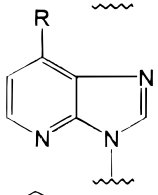
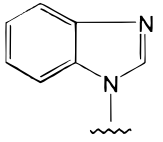
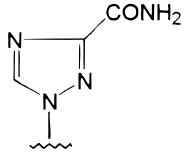
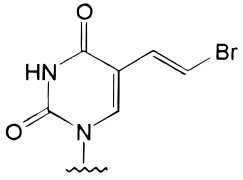
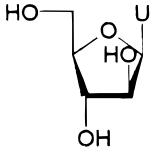
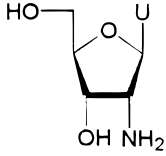
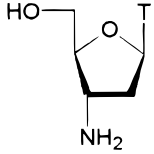
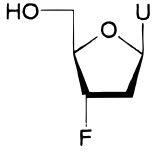
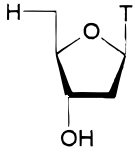
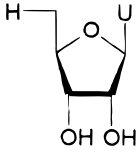
Heterocyclic base (Acceptor)	Donor	Yield (%)	References
	*ara*-uridine	76	17
	7-methylpurine-riboside	100	31
	uridine	9-92	28-30
	thymidine	81	28
	inosine	59	29
	*ara*-uridine	83	20
		—	22
	uridine	18-72	27
	thymidine	11-85	27
	guanosine	1-2	34
	2'-deoxyguanosine	52-90	34
	guanosine	56-73	34
	2'-deoxyguanosine	54-75	34
	guanosine	89	34
	2'-deoxyguanosine	90	34
	7-methylguanosine	44	31
	adenosine	14	36
	inosine	75	26
	thymidine	—	35
	2'-deoxyguanosine	—	35

TABLE 2

Nucleoside Phosphorylase Catalysed Synthesis of Sugar Modified Nucleosides

Sugar modified nucleoside (Donor)	Acceptor	Yield (%)	References
	substituted purine	8-92	17,20
	substituted purine	32-87	38,39
	5-halouracil adenine guanine	7-29 46 20.5	40 41 41
	substituted purine	—	43
	3-deazapurine	17	27
	3-deazapurine	22	27

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

of ribavirin/virazole (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (*36*). In addition to nucleosides, nucleotides can also act as pentosyl donors and various purine ribo- and deoxyribonucleotides have been synthesized by transferring the pentosyl moiety from ribo- and deoxyribonucleotides to purine bases in the presence of washed cells of *Aerobacter aerogens* and *Escherichia coli* var. *communior* (*37*).

The synthesis of sugar modified nucleosides has been carried out by the use of modified glycosyl donors. Arabino- (*17,20*) and 2′-amino-2′-deoxyribonucleosides (*38,39*) have been obtained enzymatically in good yields, although the synthesis of 3′-amino-2′,3′-dideoxy-5-halouridine from 3′-amino-2′,3′-dideoxythymidine and the corresponding 5-halouracil in the presence of thymidine phosphorylase afforded very low yield (*40*). Zaitseva *et al.* (*41*) have synthesized 3′-amino-2′,3′-dideoxyadenosine and 3′-amino-2′,3′-dideoxyguanosine from the corresponding thymidine or uracil as glycosyl donor and adenine or guanine, respectively, as acceptor in the presence of glutaraldehyde-treated cells of *E. coli* BM-11 in acceptable yields. The low yields obtained in all these reactions involving the synthesis of 3′-amino-2′, 3′-dideoxyribonucleosides may be due to the absence of the 3′-hydroxy group which is important in the binding of substrates to the enzyme (*42*). Various 2′, 3′-dideoxy-3-fluororibonucleosides of purines have been synthesised in good yields by glycosyl transfer from 2′,3′-dideoxy-3′-fluorouridine to different 2-, 6-, and 8-substituted purines in the presence of purified purine nucleoside phosphorylase and thymidine phosphorylase (*43*). Recently, Fathi *et al.* (*44*) have used nucleoside phosphorylase for the synthesis of a wide range of 2′,5′-dideoxynucleosides, including 6-substituted purine, pyrazolo[3,4-d] pyrimidine and 1-deazapurine derivatives and have evaluated their specificity toward cleavage by bacterial *versus* mammalian purine nucleoside phosphorylases.

Recently, nucleoside phosphorylases have been used for the synthesis of nucleosides with modifications, both in the sugar and base moieties by using sugar modified glycosyl donors and modified nucleobases as acceptors. Thus, Chae *et al.* (*45*) have synthesized 2′,5′-dideoxy-6-thioguanosine and 5′-deoxy-6-thioguanosine using 2′,5′-dideoxythymidine and 5′-deoxyadenosine as glycosyl donors and 6-thioguanine as acceptor base in the presence of purine and pyrimidine nucleoside phosphorylases. They have also synthesized 2′,5′-dideoxy-6-mercaptopurine riboside and 5′-deoxy-6-mercaptopurine riboside by using 6-mercaptopurine nucleobase instead of 6-thioguanine as the acceptor base.

Glycosyl Transfer Reactions Catalyzed by N-*Deoxyribosyltransferases*

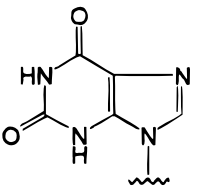
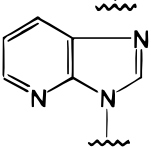
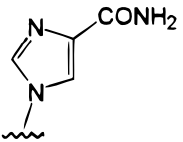
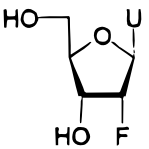
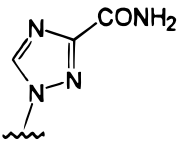
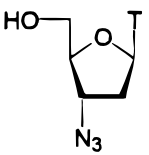
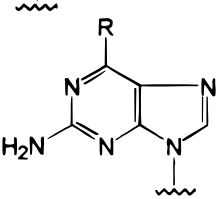
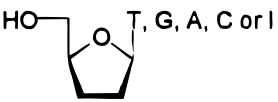
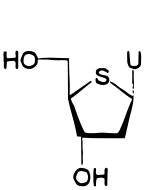
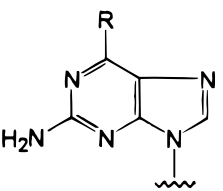
N-Deoxyribosyltransferases present in some organisms have been employed in the syntheses of deoxyribonucleosides and their analogs. Two types of *N*-deoxyribosyltransferases have been identified: type I enzymes catalyze the transfer of 2-deoxyribose between two purine bases and type II catalyze the transfer of 2-deoxyribose between pyrimidine or purine bases (*46*). *N*-Deoxyribosyltransferases provide an alternative to the nucleoside phosphorylases and are highly regioselective (*N*-1 glycosylation in pyrimidines and *N*-9 in purines), as well as stereoselective (β-anomers are exclusively formed).

Both purified and crude extracts of *N*-deoxyribosyltransferases have been employed for nucleoside synthesis and a reasonable amount of variation in the donor nucleoside and acceptor heterocyclic base is tolerated (Table 3). Holguin-Hueso and Cardinaud (*47*) have synthesized 9-(2′-deoxy-β-D-ribosyl) xanthine in 80% yield for the first time by using thymidine as the glycosyl donor and xanthine as acceptor in the presence of *N*-deoxyribosyltransferase extracted from *Lactobacillus helveticus*. A crude enzyme preparation from *L. leichmanii* has been used for the transfer of 2-deoxyribose from thymidine to 1-deazapurine (*48*) and 4(5)-imidazolecarboxamide (*49*) leading to 9-(2′-deoxy-β-D-ribofuranosyl)-1-deazapurine in 76% yield and 1-(2′-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide in 56% yield, respectively.

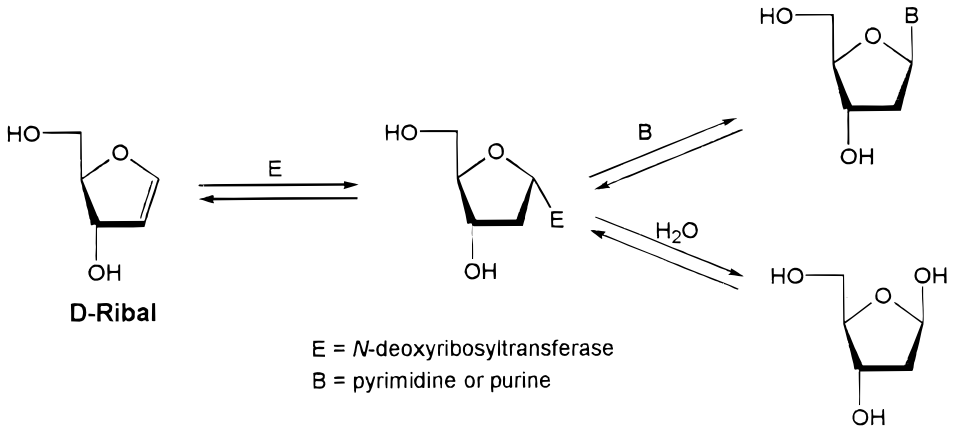
A series of 3′-azidopurine nucleosides have been synthesised by glycosyl transfer from 3′-azido-3′-deoxythymidine (AZT) to various substituted purines in the presence of a cell free extract of *E. coli* (*50*). Syntheses of 2′,3′-dideoxynucleosides have been carried out with the *N*-deoxyribosyltransferase either from *L. helveticus* or from *L. leichmanii*. Both purine and pyrimidine 2′,3′-dideoxynucleosides are substrates for the enzyme and a variety of substituted purines have been used as acceptors for the synthesis of 2′,3′-dideoxynucleosides (*51–53*). Carson and Wasson (*51*) have demonstrated that *N*-deoxyribosyltransferase is stable at 50°C for at least 2 h and the transfer reaction from 2′,3′-dideoxyribosyl nucleoside as a donor proceeded faster at this temperature than at 37°C. It has also been noticed that 2′,3′-dideoxyribosyl transfer proceeds faster than 2′-deoxyribosyl transfer under comparable conditions (*51*). A series of 3′-azido-2′,3′-dideoxyribonucleosides containing 2-amino-6-substituted purines have been synthesized by transfer of the 3-azido-2,3-dideoxyribosyl moiety from AZT to a series of 2-amino-6-substituted purine bases in the presence of *N*-deoxyribosyltransferase expressed in *E. coli* (*54*). These results are in contrast to those of Carson and Wasson (*51*) who failed to isolate any product by using AZT as deoxyribosyl donor and adenine as acceptor in the presence of *N*-deoxyribosyltransferase purified from *L. helveticus*. Recently, Koszalka *et al.* (*55*) have extended the scope of the use of the *N*-deoxyribosyltransferase for the synthesis of nucleosides bearing thiosugars. Thus, a series of virucidal nucleosides have been synthesized by thioglycosyl transfer from 2′-deoxy-4′-thiouridine to 6-substituted 2-aminopurines in the presence of *N*-deoxyribosyltransferase. The preparation of nucleosides by the deoxyribosyltransferase method is well suited for the preparation of radiolabeled compounds and a crude enzyme preparation from *L. helveticus* has been used to synthesize 2-[^{14}C]-2′-deoxyribofuranosyl-5-trifluoromethyluracil (*56*) and [2′,3′-^{3}H]-2′,3′-dideoxyribofuranosylpurines (*51*). Smar *et al.* (*57*) found that D-ribal serves as a substrate for *N*-deoxyribosyltransferase and incubation with adenine or other purines or pyrimidines led to the formation of the corresponding 2′-deoxyribonucleosides (Scheme 4). In the absence of nucleobases, the enzyme caused hydration of D-ribal leading to the formation of 2-deoxyribose. It has been found that the 2′-deoxyribosyltransferase (EC 2.4.2.6 transferase) has tolerance for the base 1,2,4-triazole-3-carboxamide and the glycosyl donor, 2′-deoxy-2′-fluorouridine, thus providing an efficient synthetic route to the preparation of analogs of ribavirin, *i.e.*, 2′-deoxy-2′-fluororibavirin (*58*) (Table 3). This enzyme may find applications in the synthesis of various analogs of ribavirin for the evaluation of their antiviral activity.

TABLE 3

N-Deoxyribosyltansferase Catalysed Synthesis of Nucleosides

Donor	Acceptor	Yield(%)	References
Thymidine		80	47
Thymidine		76	48
Thymidine		56	49
		25	58
		≤50	50,54
	substituted purine bases	—	51-53
		—	55

T = thymin-1-yl, G = guanin-1-yl, A = adenin-9-yl , C = cytosin-1-yl,
I = inosin-9-yl, U=uracil-1-yl



SCHEME 4

Glycosyl Transfer Reactions Catalyzed by Immobilized Enzymes

Holy and Votruba (*59*) have used immobilized bacterial cells (a thymine-dependent mutant of *E. coli* encapsulated in permeable alginate gel) to carry out a transdeoxyribosylation reaction. Several purine and pyrimidine nucleosides were prepared using 2′-deoxyuridine as glycosyl donor and substituted purine and pyrimidine bases as acceptors in 11–75% yield. The advantages of immobilization of bacterial cells are that they can be stored in cold for several days without any apparent loss of catalytic-activity and can be used repeatedly (3–4 times) for the same reaction. The biocatalyst looses its regioselectivity when ethyl 5-amino-imidazole-4-carboxylate is used as glycosyl acceptor and 2′-deoxyuridine as donor leading to two isomers, *i.e.*, ethyl 5-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl) imidazole-4-carboxylate (18%) and ethyl 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl) imidazole-5-carboxylate (35%) (*60*). Additionally, the immobilized cells have also been used for the transfer of 2,3-dideoxyribofuranosyl moiety of 2′,3′-dideoxyuridine to 6-methylpurine (*61*) (Table 4).

Glycosyl Transfer Reactions Catalyzed by Phosphopentomutase Coupled with Nucleoside Phosphorylase

A new route to enzymatic synthesis of nucleosides involves the coupling of nucleoside phosphorylase with phosphopentomutase (Scheme 5). This approach has the advantage that the used pentose-5-phosphate (R-5-P) is easier to prepare and is more stable than α-pentose-1-phosphate (R-1-P). R-1-P is generated *in situ* from R-5-P, which then serves as a substrate for the nucleoside phosphorylase. The process, however, is limited by the specificity of the mutase. Initial studies indicate that the enzyme accepts D-ribose-5-phosphate and D-arabinose-5-phosphate as substrates in addition to its natural substrate 2-deoxyribose-5-phosphate (*62*). Thus, synthesis of a variety of ribo-, arabino-, and 2′-deoxyribonucleosides have been achieved by the use of phosphomutase coupled with thymidine or purine phosphorylase (*62*). Recently,