1 Biocatalytic Methodologies for Selective Modified Nucleosides

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1. INTRODUCTION

Nucleosides are fundamental building blocks of biological systems that are widely used as therapeutic agents to treat cancer, fungal, bacterial, and viral infections.[1] Since the latter 1980s, nucleoside analogues have been investigated with renewed urgency in the search for agents effective against the human immunodeficiency virus (HIV) and to use as a more effective treatment for other viral infections. This has resulted in an explosion of synthetic activity in the field of nucleosides, and consequently, extensive modifications have been made to both the heterocyclic base and the sugar moiety to avoid the drawbacks shown by nucleosides or analogues in certain applications.

The intense search for clinically useful nucleoside derivatives has resulted in a wealth of new approaches to their synthesis, and most important, their enantioselective synthesis. Thus, especially for organic chemists, biocatalytic methods have been recognized as practical procedures in the nucleoside area.[2] For the manipulation of protecting groups, the use of biocatalysts in organic synthesis has become an attractive alternative to conventional chemical methods, due to their simple feasibility and high efficiency. In general, these catalysts are inexpensive and satisfy increasingly stringent environmental constraints. Due to these advantages, biocatalyzed reactions are playing an increasing role primarily in the preparation of nonracemic chiral biologically active compounds not only in the laboratory but also in industrial production, in which enzyme-catalyzed chemical transformations are in great demand in the pharmaceutical and chemical industries.[3] In addition, enzyme-catalyzed reactions are less hazardous, less polluting, and less energy consuming than are conventional chemistry-based methods.

Chemical Synthesis of Nucleoside Analogues, First Edition. Edited by Pedro Merino.

The synthetic potential of enzymes related to nucleoside synthesis has been applied profusely, especially since the introduction of organic solvent methodology. It is our aim in this chapter to cover the literature of the last decade or so relative to nucleosides with selected examples because of special significance. Our desire is to show a range of examples that cover nucleoside analogue syntheses through enzymatic procedures and to summarize and offer an easily accessible visual reference review. Due to the vastness of the bibliographic material related to nucleosides, we do not cover other enzymatic processes, such as preparation of nucleoside antibiotics using microorganisms,[4] nucleoside syntheses mediated by glycosyl transfer,[5] or halogenation enzymes.[6]

Most enzymatic reactions, just like those included here, are performed by a small number of biocatalysts. With the passing of time, their nomenclature has changed in an effort to unify criteria and to refer to a given enzyme by only one name. Table 1 lists the enzymes mentioned in this review, sorted alphabetically. These

Table 1. Enzymes Commonly Used in Biocatalytic Processes Shown in This Review

Accepted Name (Abbreviation) Other Denominations
Adenosine deaminase (ADA)
Adenylate deaminase (AMPDA)
5′-adenylic acid deaminase, AMP deaminase
***Candida antarctica* lipase A (CAL-A)**
lipase A
***Candida antarctica* lipase B (CAL-B)**
Novozym-435, SP-435, lipase B
***Candida rugosa* lipase (CRL)**
Candida cylindracea lipase (CCL)
ChiroCLEC BL
Lipase M (from *Mucor javanicus*)
Lipozyme
Mucor miehei lipase, Lipozyme IM
***Pseudomanas cepacia* lipase (PSL)**
Pseudomonas sp. lipase, *Pseudomonas fluorescens* lipase (PFL), PCL, lipase P, lipase PS, LPS, amano PS, amano lipase PS
Burkholderia cepacia
Pig liver esterase (PLE)
Porcine pancreas lipase (PPL)
Subtilisin
***Thermomyces lanuginosa* lipase (TL IM)**
Lipozyme TL IM

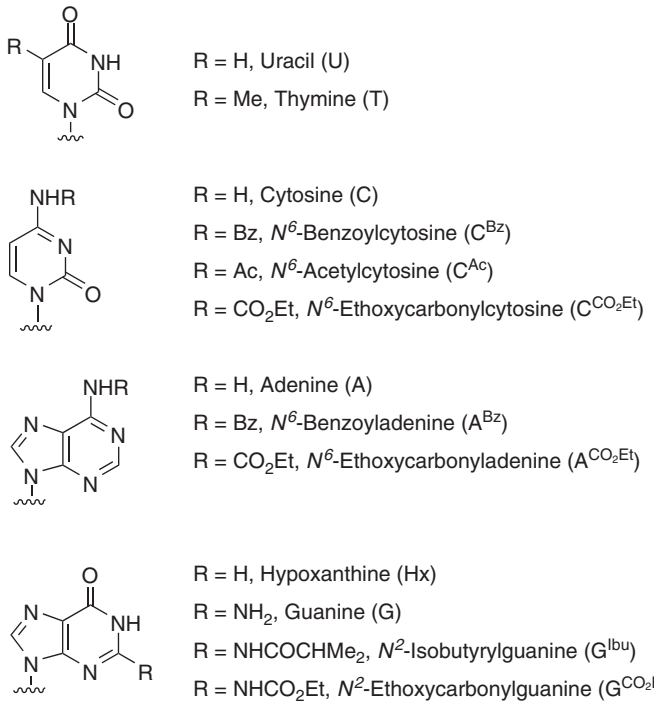


Figure 1. Pyrimidine and purine bases, their more common protected versions used in this review, and their abbreviations.

are cited as in the original papers to facilitate checking the original work, together with their corresponding new denominations.

To simplify the schemes, Figure 1 collects the common abbreviations of nucleoside bases, their protected version used in this chapter, and their structures.

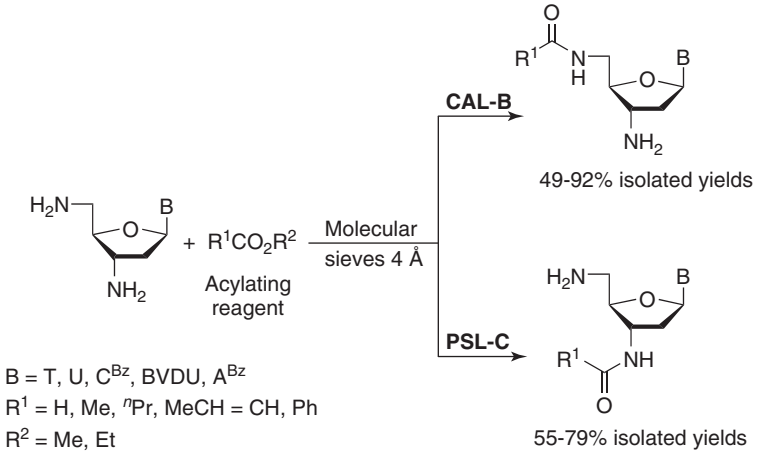
2. TRANSFORMATIONS ON THE SUGAR MOIETY

Modification of nucleosides via enzymatic acylation has been one of the most extensively used methodologies over recent years, since in some cases a simple acylation of one of the hydroxyl groups in a nucleoside can result in an increase in their biological activity compared with that of the unmodified derivative.[7]

2.1. Enzymatic Acylation/Hydrolysis

An interesting family of nucleoside analogues is that of the amino sugar nucleosides, since they possess anticancer, antibacterial, and antimetabolic activities.[8] Gotor, Ferrero, and co-workers have synthesized, through short and convenient syntheses, pyrimidine 3′,5′-diamino analogues of thymidine (T),[9] 2′-deoxyuridine

(dU),[9] 2′-deoxycytydine (dC^{Bz}),[10] (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU, Brivudin),[11] and the purine 3′,5′-diamino analogue of 2′-deoxyadenosine (dA^{Bz}).[10] Regioselective protection of one of the primary amino groups situated in the 3′- or 5′-position is a very difficult task, since traditional chemical methods do not distinguish between them, and moreover, there are other reactive points on the molecule, such as the nitrogen atoms on the bases. They report the regioselective enzymatic acylation of the amino groups in the sugar moiety of pyrimidine and purine 3′,5′-diaminonucleosides.[9,12] This enzymatic strategy made it possible for the first time to regioselectively synthesize $N^{3'}$- or $N^{5'}$-acylated pyrimidine and purine 3′,5′-diamino nucleoside derivatives by means of a very simple and convenient procedure using immobilized *Pseudomonas cepacia* lipase (PSL-C) or *Candida antartica* lipase B (CAL-B) as a biocatalyst, respectively (Scheme 1).

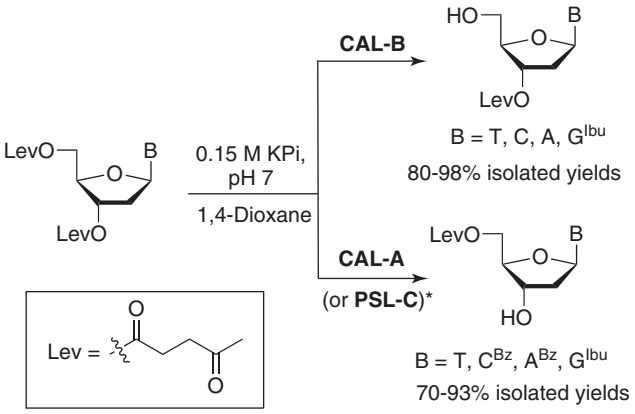


Scheme 1.

Although oxime esters are good acylating agents in regioselective enzymatic acylations of nucleosides,[13] nonactivated esters such as alkyl esters are used since amines are much more nucleophilic than alcohols, and they react nonenzymatically with oxime esters. To confer versatility to this enzymatic reaction, other acyl moieties besides acetyl, such as formyl, alkyl, alkenyl, or aryl, are introduced.

An efficient new approach to the synthesis of oligonucleotides via a solution-phase *H*-phosphonate coupling method has been reported.[14] It is particularly suitable when multikilogram quantities of oligonucleotides are required and is an alternative method of choice to traditional solid-phase synthesis. The key building blocks for solution-phase oligonucleotide synthesis are 3′- and/or 5′-protected nucleosidic monomers. Among the limited protecting groups available, the levulinyl group is frequently chosen to protect the 3′- and/or 5′-hydroxyl of the nucleosides, since this group is stable to coupling conditions and can be cleaved selectively without affecting other protecting groups in the molecule. Until recently, the preparation of these building blocks has been carried out through several tedious chemical protection and deprotection steps.

To avoid this classical approach, Gotor, Ferrero, and co-workers report,[15] for the first time, a short and convenient synthesis of 3′- and 5′-*O*-levulinyl-2′-deoxynucleosides from the corresponding 3′,5′-di-*O*-levulinyl derivatives by regioselective enzymatic hydrolysis, avoiding several tedious chemical protection–deprotection steps (Scheme 2).



*PSL-C was used for di-Lev-dG^{Ibu} since **CAL-A** did not catalyze the hydrolysis.

Scheme 2.

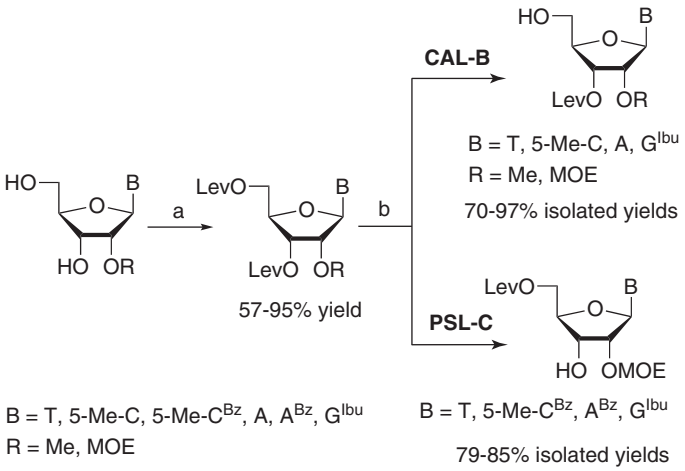
Thus, CAL-B selectively hydrolyzes the 5′-levulinate esters, furnishing 3′-*O*-levulinyl-2′-deoxynucleosides in >80% isolated yields. On the other hand, PSL-C and *Candida antarctica* lipase A (CAL-A) exhibit the opposite selectivity toward hydrolysis at the 3′-position, affording 5′-*O*-levulinyl derivatives in >70% yields.

A similar hydrolysis procedure has been extended successfully to the synthesis of 3′- and 5′-*O*-levulinyl-protected 2′-*O*-alkylribonucleosides (Scheme 3). This work demonstrates for the first time application of commercial CAL-B and PSL-C toward regioselective hydrolysis of levulinyl esters with excellent selectivity and yields. It is noteworthy that protected cytidine and adenosine base derivatives are not adequate substrates for enzymatic hydrolysis with CAL-B, whereas PSL-C is able to accommodate protected bases during selective hydrolysis.

In addition, they also report improved synthesis of dilevulinyl esters using a polymer-bound carbodiimide as a replacement for dicyclohexylcarbodiimide (DCC), thus considerably simplifying the workup for esterification reactions (Scheme 4).

Another efficient synthesis of 3′- and 5′-*O*-levulinyl-2′-deoxy- and 2′-*O*-alkylribonucleosides is described from parent nucleosides using enzyme-catalyzed regioselective acylation in organic solvents (Scheme 5).[16]

Several lipases are screened in combination with acetonoxime levulinate as an acylating agent. PSL-C is selected for acylation of the 3′-hydroxyl group in nucleosides, furnishing 3′-*O*-levulinylated products in excellent yields. Similarly, CAL-B provides 5′-*O*-levulinyl nucleosides in high yields. Base-protected cytidine and

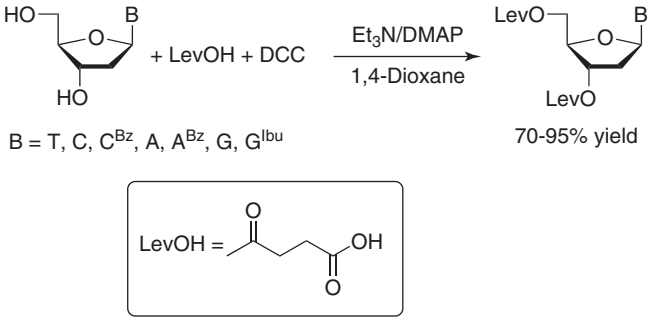


a. LevOH, DCC, Et_3N, DMAP, 1,4-dioxane

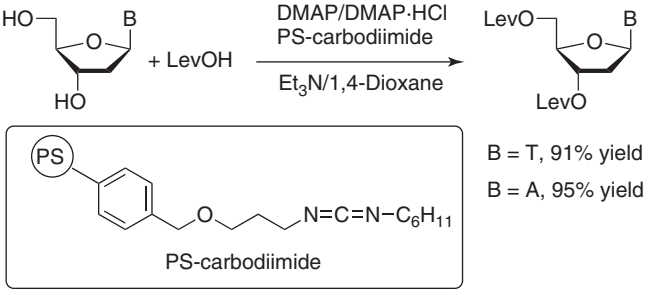
b. 0.15 M KPi (pH 7), 1,4-dioxane

Scheme 3.

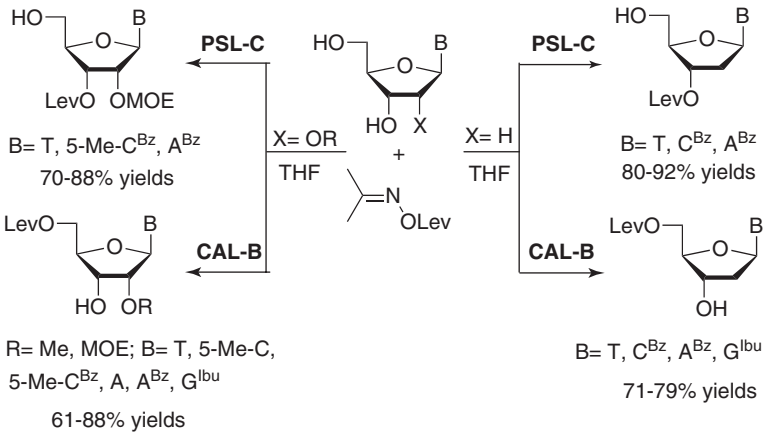
Normal procedure



Improved procedure



Scheme 4.



Scheme 5.

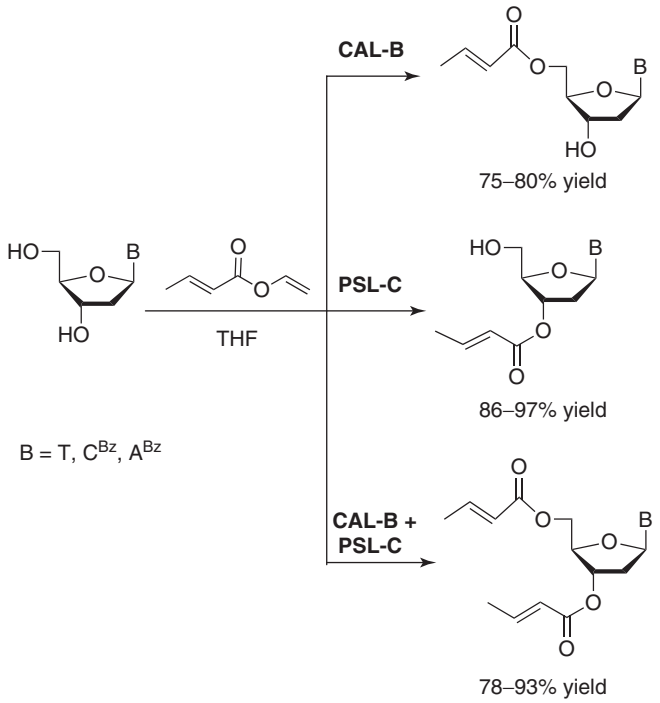
adenosine analogues are good substrates for lipase-mediated acylations. Thus, *N*-benzoyl-3′-*O*-levulinyl cytidine and adenosine derivatives are obtained in good yield, overcoming the limitations of the original hydrolysis protocol.[15] The new and improved method is shorter than the one described earlier, allowing greater atom efficiency and lowering the cost of enzyme and reagents via recycling. To demonstrate the industrial utility of this method, 3′-*O*-levulinyl thymidine and N^2-isobutyryl-5′-*O*-levulinyl-2′-deoxyguanosine are synthesized on a 25-g scale.[17] Additionally, PSL-C is reused to make the processes even more economical.[18]

The crotonyl group is present in different biological active compounds, such as antitumor agents COTC [2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-cyclohexenone][19] and COMC (2-crotonyloxymethyl-2-cyclohexenone).[20] The activity of this type of derivative can be ascribed to the presence of the α,β-unsaturated ester, which can undergo Michael-type additions of nucleophiles within an enzyme. However, introduction of this moiety on nucleosides has rarely been studied.[21] Previously, 3′-amino-5′-crotonylamino-3′,5′-dideoxythymidine was synthesized,[9] and preliminary biological studies have shown that it inhibits the in vitro replication of HIV-1 and HIV-2.[22] Due to the fact that this compound cannot be 5′-phosphorylated, it may suffer a Michael-type addition from a specific enzyme. Moreover, the presence of this moiety on nucleosides would afford excellent starting compounds for the synthesis of β-amino acid analogues of potential interest.[23]

Nevertheless, the synthesis of *O*-crotonyl derivatives is not trivial because under normal conditions, using a base-catalyzed process with crotonyl chloride, mixtures of desired compounds and β/γ-unsaturated analogues are obtained, due to deconjugation of the double bond.

Regioselective syntheses of several *O*-crotonyl-2′-deoxynucleoside derivatives using biocatalytic methodology has been reported[24] (Scheme 6).

While CAL-B affords 5′-*O*-acylated compounds, PSL-C provides the 3′-*O*-crotonylated analogues. Since classical chemical approaches did not work



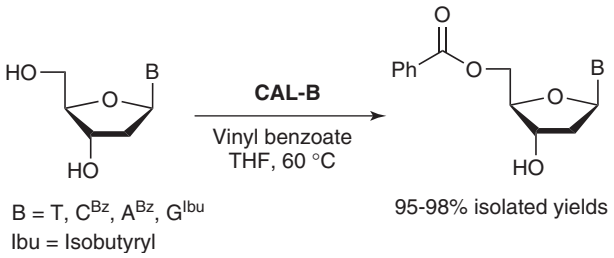
Scheme 6.

appropriately due to side isomerization reactions, a mixture of both lipases is used to achieve a useful synthetic route toward diacylated nucleosides.

Benzoylation remains one of the methods used most frequently for the protection of hydroxyl and amino functions in nucleoside and nucleotide chemistry.[25] The selective manipulation of hydroxyl over amino groups of nucleobases is an important reaction in oligonucleotide synthesis. The classical method of benzoylation of the hydroxyl group in nucleosides with benzoyl chloride or benzoic anhydride provides nonselective reactions. Other mild benzoylating reagents are reported for this purpose; however, lower selectivity has been observed toward the acylation of different hydroxyl functions.

To overcome these problems, a mild and efficient procedure for the selective benzoylation of 2′-deoxynucleosides through direct enzymatic acylation with vinyl benzoate, a commercially available reagent, is reported (Scheme 7).[26] CAL-B is selected, due to its well-demonstrated selectivity in the transesterification of the 5′-hydroxyl group and ability to recycle.

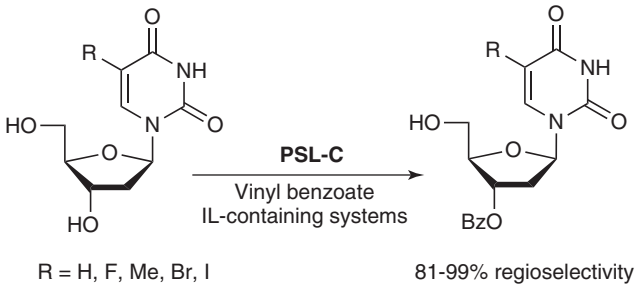
To demonstrate the suitability of the reaction for industrial applications, both the acylating agent and the enzyme were reused for subsequent reactions. The recycled CAL-B maintained total selectivity toward acylation of the 5′-OH, with the exception of the longer reaction rate. On the other hand, benzoylation with recycled vinyl benzoate gives results identical to those obtained using fresh acylating agent.



Scheme 7.

Experiments on the large-scale acylation of nucleosides are carried out on a 5- and 25-g scale of the starting material. Excellent results are obtained with CAL-B catalyzing the acylation process, with total selectivity furnishing 5′-*O*-benzoylated derivatives in quantitative yields.

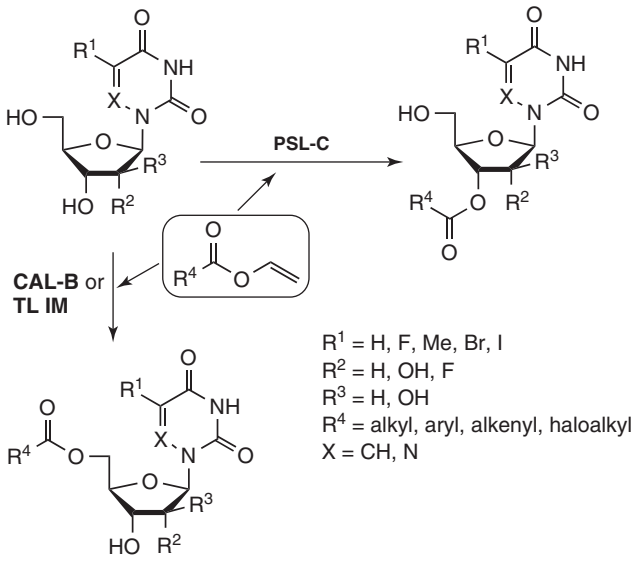
Ionic liquid-containing systems offer new opportunities for the enzymatic acylation of nucleosides.[27] In PSL-C-mediated benzoylation of floxuridine and its analogues (Scheme 8), enzyme performance, including enzyme activity and 3′-regioselectivity, are enhanced significantly using $[C_4MIm]PF_6$-containing systems, up to excellent conversions (>99%) and good-to-excellent 3′-regioselectivity (81–99%). It is observed that enzyme performance depends not only on the anion of ionic liquid (IL), but also on the cation, and that a proper combination of the cation and anion is critical to allow the enzyme to exhibit excellent performances. The optimal IL content in IL-containing systems is 5% v/v.



Scheme 8.

Highly regioselective acylations at 3′ or 5′ of fluorouridine, floxuridine, 6-azauridine, and their derivatives are performed using PSL-C or CAL-B/lipase from *Thermomyces lanuginosa* (TL IM), respectively (Scheme 9).[28]

The effects of some crucial factors on the enzymatic processes are examined. The optimum reaction medium, molar ratio of nucleoside to vinyl ester, reaction temperature, and enzyme dosage are investigated. A great variety of acyl donors are tested, from alkyl to aryl or alkenyl or haloalkyl chains on the vinyl ester.



Scheme 9.

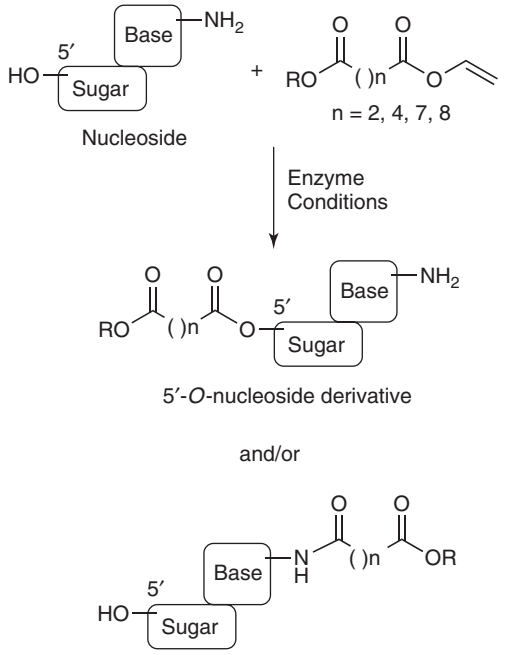
Efficient protocols for the selective synthesis of polymeric prodrugs of ribavirin, ddI, cytarabine, acyclovir, 5-fluorouridine, or aracytosine are developed using different biocatalysts: CAL-B, Lipozyme, or PSL-C (Scheme 10).[29]

Transesterifications are performed using vinyl carboxylates ranging from 4 to 10 carbon atoms. A series of analogues are prepared, with high acylation regioselectivity at 5′-OH when CAL-B or Lipozyme biocatalysts are used. However, PSL-C in DMSO selectively acylates the amino group. The influence of reaction parameters, including enzyme, solvents, molar ratio of substrates, reaction time, carbon length of the acyl donor, and reaction temperature, are investigated in detail.

Synthesis of lobucavir prodrug requires regioselective coupling of one of the two hydroxyl groups of lobucavir with valine (Scheme 11).[30]

Either hydroxyl group of lobucavir could be selectively aminoacylated with valine by using enzymatic reactions. One of them is obtained in 83–87% yield by selective hydrolysis of di-*O*-valinyl derivative with lipase M from *Mucor javanicus* or lipase from *Candida cylindracea* (CCL). The final active intermediate for lobucavir prodrug is prepared by transesterification of lobucavir using ChiroCLEC BL (61% yield), or more selectively by using PSL-C (84% yield).

Ribavirin is a powerful antiviral agent used to treat hepatitis C. Although this therapy is very effective in eradicating hepatitis C virus, it has several side effects. It was suggested that the administration of ribavirin in the form of a prodrug might improve its pharmacokinetic profile and reduce side effects. Indeed, a series of preclinical evaluations demonstrated that the bioavailability and variability of the alanine ester of ribavirin are improved compared to those of ribavirin. To satisfy the prodrug requirements to be used in toxicological studies, formulation development,



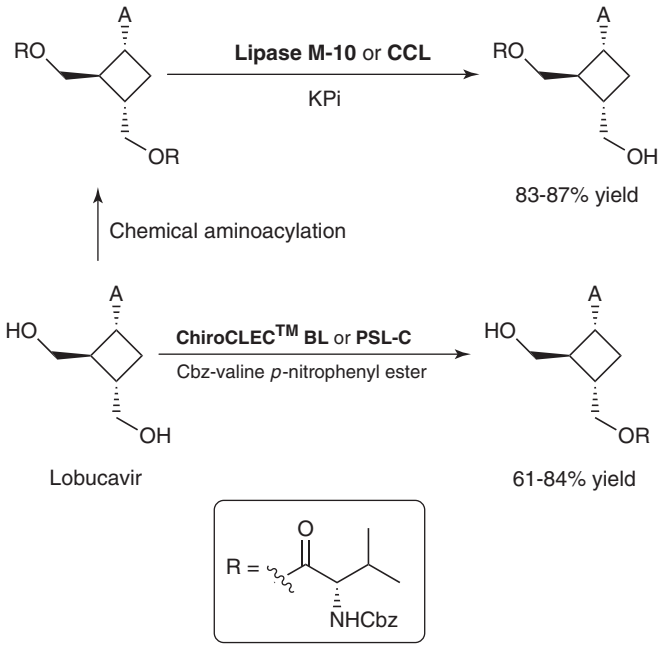
a. Ribavirin, **CAL-B**, acetone, 50 °C
b. ddI, **Lipozyme**, acetone
c. Cytarabine, **CAL-B** or **Lipozyme**, acetone
d. Acyclovir, **PSL-C**, acetone or DMSO
e. 5-Fluorouridine, **CAL-B**, THF
f. Ribavirin or *ara*-cytosine, **CAL-B**, acetone

Scheme 10.

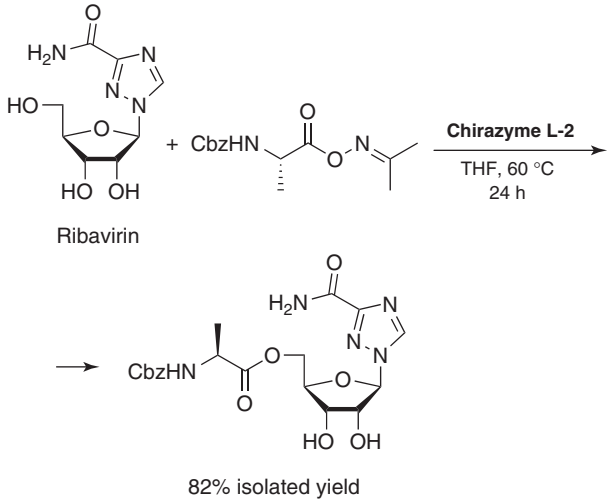
and early clinical trials, efficient synthesis of 5′-*O*-alanylribavirin has been reported (Scheme 12).[31]

The final ester is synthesized via CAL-B-catalyzed acylation of ribavirin with the oxime ester of L-Cbz-Ala in anhydrous THF. The reaction was highly regioselective, resulting in the exclusive acylation of the 5′-hydroxyl. The process is also scaled-up on a pilot-plant scale to produce 82 kg of final ester in three batches in an average isolated yield of 82%.

The protection of hydroxyl groups as esters is one of the oldest and most frequently used strategies in the synthesis of nucleosides. Acetyl and benzoyl protecting groups are prized because they can be removed by alkaline hydrolysis without cleaving the glycosidic bond in nucleosides.[25b] Protecting groups that can be removed under milder acid conditions or even under neutral conditions are of considerable value. The merits of acetal groups such as THP and THF lies in their stability under a variety of conditions, such as basic media, alkyl lithiums, metal hydrides, Grignard reagents, oxidative reagents, and alkylating or acylating reagents and cleavage under mild acidic conditions or heating.[32]



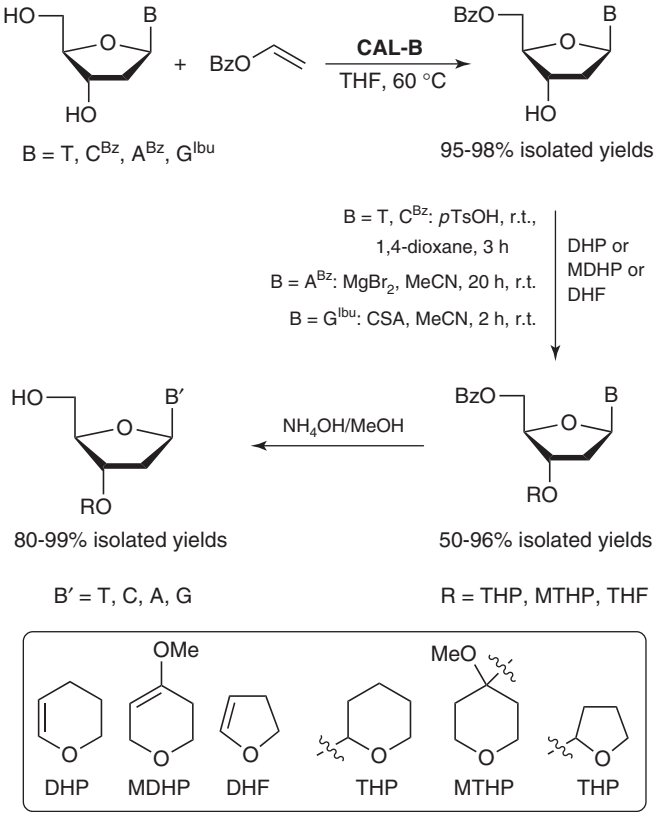
Scheme 11.



Scheme 12.

Numerous methods have been reported for the tetrahydropyranylation of alcohols.[33] However, protocols for the synthesis of 3′-*O*-THP and 3′-*O*-THF ethers of 2′-deoxynucleosides are not efficient. Often, dimethoxytrityl (DMTr) and silyl protecting groups are employed as blocking groups for the 5′-hydroxyl group during the synthesis of 3′-*O*-acetal. These reagents have scale-up limitations, due to the higher cost and corrosive nature of the silyl chloride.

A protocol is developed[34] based on the regioselective synthesis of 5′-benzoyl derivatives of 2′-deoxynucleosides catalyzed by CAL-B. It is a simple and convenient synthetic strategy for the preparation of tetrahydropyranyl, 4-methoxytetrahydropyranyl, and tetrahydrofuranyl ethers of 2′-deoxynucleosides, which are useful building blocks for nucleic acid chemistry and thus a significant improvement over reported methods (Scheme 13).



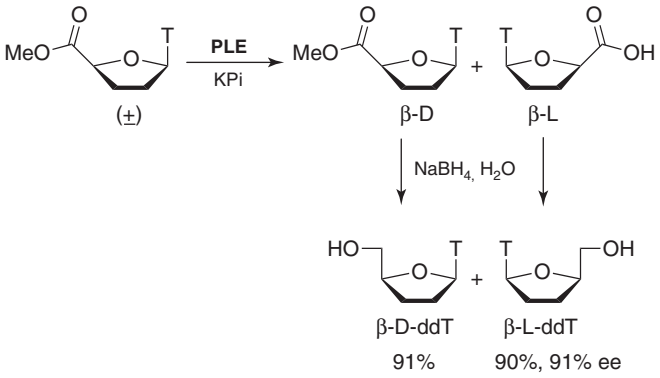
Scheme 13.

After enzymatic benzoylation, tetrahydropyranylation, and tetrahydrofuranylation at the 3′-hydroxyl group of 2′-deoxynucleosides with *p*-toluenesulfonic acid, $MgBr_2$, or camphorsulfonic acid as catalysts, deprotection of the 5′-*O*-benzoyl group furnishes 3′-*O*-acetal-protected 2′-deoxynucleosides. The three-step process enables large-scale synthesis of protected nucleosides.

2.2. Resolutions of Racemic Mixtures

Until recently, only nucleosides possessing the natural β-D-configuration have been studied as chemotherapeutic agents, due to their easy access. However, the discovery of lamivudine (L-2′,3′-dideoxy-3′-thiacytidine, 3TC), the first compound with the unnatural β-L-configuration approved by the U.S. Food and Drug Administration (FDA) for use in combination therapy against human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV), has sparked tremendous interest in the synthesis of β-L-nucleosides.[35] As a result, several L-nucleosides are undergoing clinical trials as potential antiviral or antitumor agents.[36] Favorable features of L-nucleosides include lower toxicity while maintaining antiviral activity comparable and sometimes greater than that of their D-counterparts, and higher metabolic stability. In addition, the L-series nucleosides are also of interest as precursors to nuclease-stable L-oligonucleotides.[37] The tremendous therapeutic potential of L-nucleosides has stimulated interest in their synthesis. Formation of a mixture of D- and L-nucleoside is a common occurrence during the synthesis of these compounds. This results in a challenging separation of the racemic mixtures.

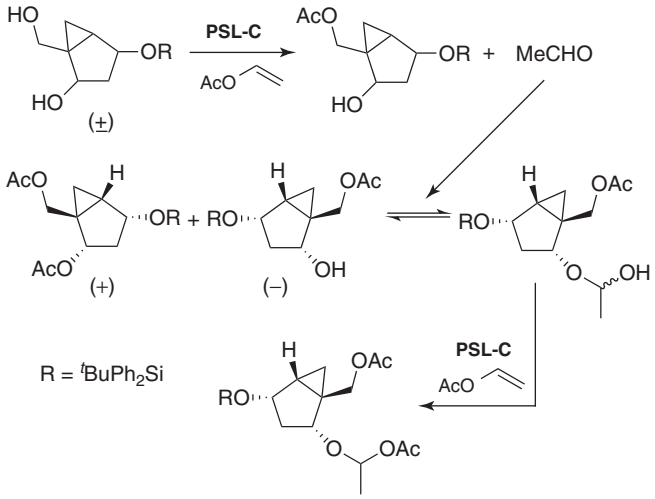
One of theses examples is the new method[38] for the synthesis of 2′,3′-dideoxynucleoside analogues (Scheme 14). Thus, electrochemical activation of 2-substituted furans, followed by coupling with a pyrimidine or purine base, gives planar furyl nucleosides as key intermediates, which are hydrogenated *cis*-selectively to give the corresponding β-2′,3′-dideoxynucleosides as racemic mixtures. An enzymatic kinetic resolution gives rise to β-D- and β-L-configured derivatives in high optical purity. This is exemplified by the synthesis of β-D- and β-L-3′-deoxythymidine.



Scheme 14.

The bicyclo[3.1.0]hexane scaffold can lock the conformation of a carbocyclic nucleoside into one of the two antipodal (north or south) conformations typical of conventional nucleosides that normally exist in a rapid two-state equilibrium in solution. A general practical method to access bicyclo[3.1.0]hexane pseudosugar for the north antipode via a lipase-catalyzed double-acetylation reaction has been

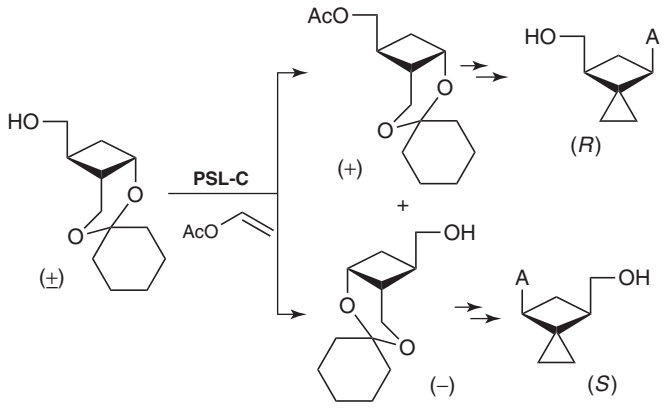
reported[39] which successfully resolves a diol precursor into enantiomerically pure (+)-diacetate and (−)-monoacetate (Scheme 15).



Scheme 15.

The former diacetate is converted to the conformationally locked (north)-carbocyclic guanosine derivative. The most attractive features of this synthesis are that a relatively complex synthon is obtained from simple and inexpensive starting materials and that the resulting racemic mixture is resolved successfully by PSL-C. During the lipase-catalyzed resolution, the presence of an unusual acetal-forming reaction that consumes small amounts of the unreactive (−)-monoacetate is detected. This side reaction is also enzyme-catalyzed and is triggered by the by-product acetaldehyde generated during the reaction.

The preparative-scale chemoenzymatic resolution of novel (*R*)- and (*S*)-spiro[2.3]hexane nucleosides has been described (Scheme 16).[40] The key step

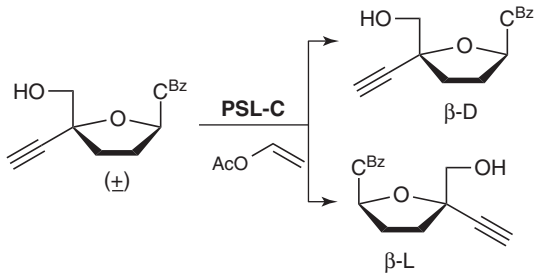


Scheme 16.

involves the PSL-C-catalyzed resolution of racemic compound, synthesized in seven steps, starting from diethoxyketene and diethyl fumarate, to give (+)-acetate and (−)-alcohol.

The (+)-acetate and (−)-acetate corresponding compounds are converted to (*R*)- and (*S*)-9-(6-hydroxymethylspiro[2.3]hexane)-4-adenine, respectively, through condensation with 6-chloropurine, followed by ammonolysis that readily provides novel spiro[2.3]hexane nucleosides in high optical purity. Among the several lipases studied, PSL-C gives the highest optical and chemical yield on a multigram scale.

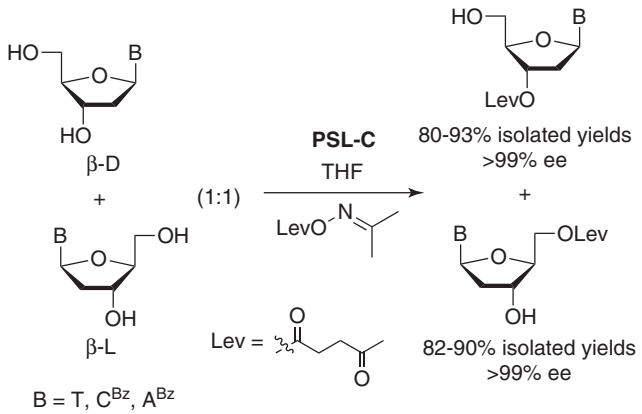
4′-*C*-Ethynyl-2′-deoxynucleosides belong to a novel class of nucleoside analogues endowed with potent activity against a wide spectrum of HIV viruses, including a variety of resistant clones. Although favorable selectivity indices are reported for several of these analogues, some concern still exists regarding the 3′-OH group and its role in cellular toxicity. To address this problem, the 3′-OH group in 4′-*C*-ethynyl-2′-deoxycytidine is removed. This compound is chosen because of its combined high potency and low selectivity index. Removal of the 3′-OH is not straightforward; it requires a different synthetic approach from the one used to synthesize the parent compound.[41] Starting with glycidyl-4-methoxyphenyl ether, the target 4′-*C*-ethynyl-2′,3′-dideoxycytidine analogue in its racemic form is obtained after 13 steps. Then the lipase-catalyzed resolution of the latter racemic mixture gives β-D-dideoxyribo and β-L-dideoxyribo (Scheme 17), which can be separated, being the key step in achieving synthesis of the corresponding 5′-triphosphates to use in the biological assays.



Scheme 17.

Gotor, Ferrero, and co-workers report for the first time a practical synthesis of β-L-3′- and β-L-5′-*O*-levulinyl-2′-deoxynucleosides through enzymatic acylation and/or hydrolysis processes.[42] The opposite selectivity during acylation exhibited by PSL-C with β-D- and β-L-nucleosides furnishes acylated compounds that have different R_f values. As a consequence, isolation of both products is achieved by simple column chromatography, allowing the parallel kinetic resolution of D/L nucleosides (Scheme 18).

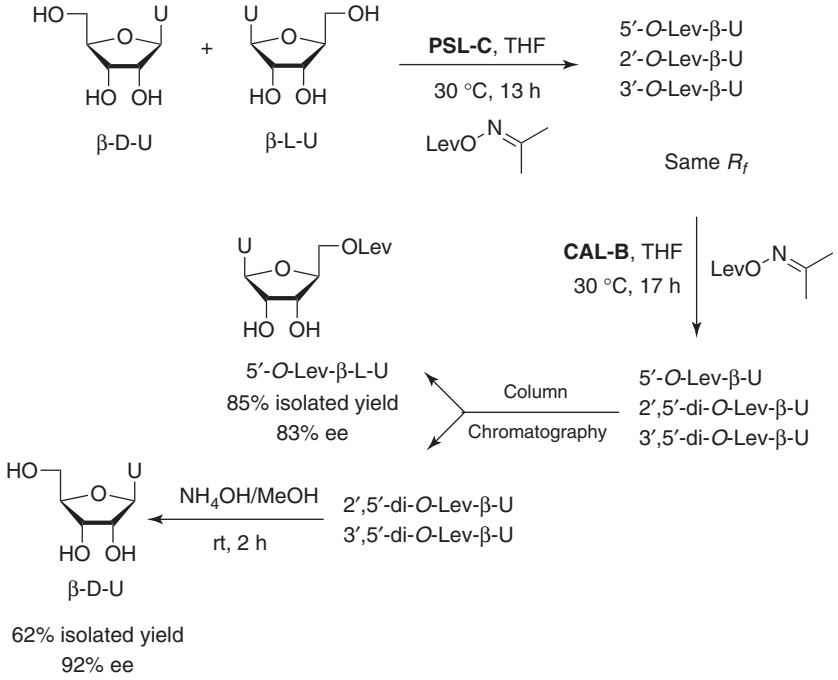
They extend the procedure to β-D/L-mixtures of ribonucleosides[43] due to their applications in the synthesis of therapeutic aptamers.[44] Therefore, they investigate the enzymatic acylation of β-D- and β-L-ribonucleosides with CAL-B and PSL-C and its application in the resolution of racemic mixtures. PSL-C also



Scheme 18.

exhibits total selectivity toward the acylation of the 5′-hydroxyl group of β-L-uridine, whereas a mixture of acylated derivatives is obtained during bioacylation of β-D-uridine. Thus, a double-sequential parallel kinetic resolution catalyzed by PSL-C and CAL-B is developed to separate the β-D/L-uridine racemic mixture.

Among the approaches tested, the route specified in Scheme 19 provides the most convenient strategy. Treatment of β-D/L-uridine with acetonoxime levulinate



Scheme 19.

in the presence of PSL-C at 30 °C in THF furnishes multiple inseparable monoacylated products with the same R_f. They attribute the 5′-*O*-levulinylation of the L-isomer due to the expected selectivity observed with PSL-C, whereas secondary hydroxyl groups of the D-isomer are acylated in a nonselective manner with PSL-C. To accomplish the separation of these products, they carry out a second acylation step catalyzed by CAL-B. Since this lipase is regioselective toward the 5′-hydroxyl group of the D-nucleosides, 2′- and 3′-*O*-acyl derivatives are transformed into 2′,5′- and 3′,5′-di-*O*-levulinyl nucleosides while the 5′-*O*-levulinyl compound remained unreacted. The diacylated products and 5′-*O*-Lev-β-L-U are easily separated by column chromatography. Subsequently, the diacyl derivatives are treated with aqueous ammonium in MeOH to give the corresponding nucleoside β-D-U, which is obtained with a satisfactory 92% ee, determined by transformation into 5′-*O*-Lev-β-D-U. The chiral HPLC analysis of 5′-*O*-Lev-β-L-U reveals 83% ee during acylation with PSL-C.

An efficient biocatalytic procedure is described[45] to obtain chiral nonracemic N,O-nucleoside derivatives consisting of a lipase-catalyzed resolution of the corresponding racemates in organic solvent. Despite the low enantioselectivity shown by the lipases investigated, single enantiomers of (+)-thymine and (+)-cytosine derivatives are obtained in high enantiomer purity by developing a lipase-catalyzed double-sequential kinetic resolution route (Scheme 20).

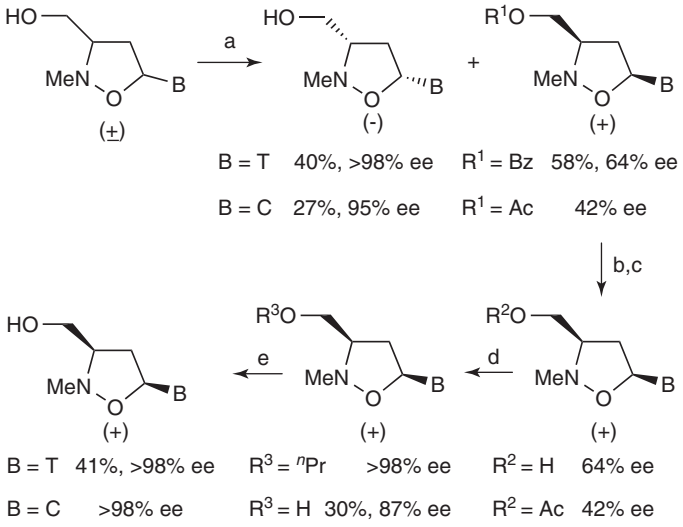
Enantioselective esterification of thymine and cytosine derivatives is investigated by comparing the efficiency of different lipases and acyl donors. Since esterifications occur with low enantioselectivity ($E \leq 14$) with all the lipases considered, for preparative purposes, a double-sequential kinetic resolution is achieved using Lipozyme IM as the best catalyst. This approach enables to obtain all the enantiomers with >95% ee.

2.3. Asymmetrizations and Desymmetrizations

The selectivity of CAL-B is used in the manipulation of a diastereotopic furanose diol as the key step in the synthesis of a novel bicyclo-3-amino-3-deoxyfuranose derivative, which is an important intermediate for the synthesis of bicyclic analogues of AZT (Scheme 21).[46] The asymmetrization of the diol has been achieved with the preferred formation of a monoacylated product with 100% diastereoselectivity. Further, this approach also has advantages with respect to high stereoselectivity and better yields obtained through ecofriendly routes.

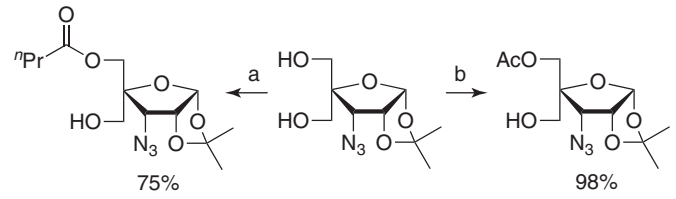
The same group also studies the inverse enzymatic process, which is the enzymatic hydrolysis reaction.[47] Lipozyme TL IM catalyzes the deacylation of 4-*C*-acyloxymethyl-3,5-di-*O*-acyl-1,2-*O*-(1-methylethylidene)-β-L-threopentofuranose to form 3,5-di-*O*-acyl-4-*C*-hydroxymethyl-1,2-*O*-(1-methylethylidene)-α-D-xylopentofuranose in a highly selective and efficient manner (Scheme 22).

The rate of lipase-catalyzed deacylation of tributanoyl furanose is 2.3 times faster than the rate of deacylation of the triacetyl furanose derivative. To confirm the structure of the lipase-catalyzed deacylated product, it is converted to a bicyclic sugar derivative which can be used for the synthesis of bicyclic nucleosides of



a. **Lipozyme IM**, vinyl benzoate (B = T); vinyl acetate (B = C)
b. Preparative chromatography (B = T, C)
c. Chemical hydrolysis (B = T); none (B = C)
d. **Lipozyme IM**, vinyl propionate (B= T); **Lipozyme IM**, ^{n}BuOH (B = C)
e. Chemical hydrolysis (B = T); Crystallization (B = C)

Scheme 20.

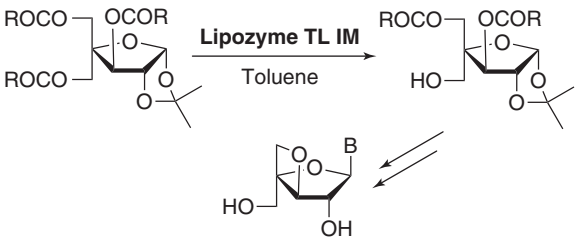


a. **CAL-B**, toluene, 2,2,2-trifluoroethyl butyrate
b. **CAL-B**, toluene, vinyl acetate

Scheme 21.

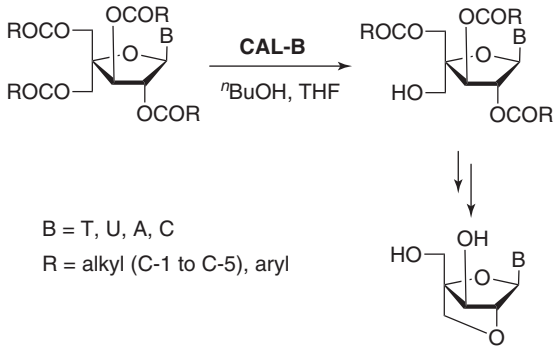
importance in the development of novel antisense and antigene oligonucleotides. Further, it is established that the monohydroxy product of the lipase-catalyzed reaction is the result of selective deacylation of the 4-*C*-acyloxymethyl moiety in the substrate and not of any acyl migration process.

A CAL-B catalytic methodology is developed[48] for selective deacylation of one of the acyloxy functions involving a primary hydroxyl group over the other acyloxy



Scheme 22.

functions involving primary and secondary alcohol groups in 4′-*C*-acyloxymethyl-2′,3′,5′-tri-*O*-acyl-β-D-xylofuranosylnucleosides (Scheme 23).

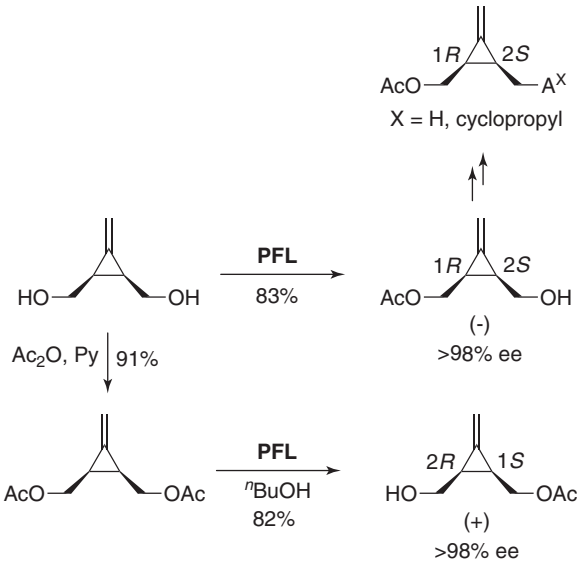


Scheme 23.

Optimization of the biocatalytic reaction reveals that tetra-*O*-butanoyl-β-D-xylofuranosyl nucleosides are the best substrates for the enzyme. The possibility of acyl migration during enzymatic deacylation reactions is ruled out by carrying out biocatalytic deacylation reactions on mixed esters of 4′-*C*-hydroxymethyl-2′,3′,5′-tri-*O*-acetyl-β-D-xylofuranosylnucleosides. The methodology developed is used for the efficient synthesis of xylo-LNA monomers T, U, A, and C in good yields.

The enzymatic desymmetrization of methylenecyclopropane diol or its corresponding diacetate derivative, generated from a [2 + 1]-cycloaddition between dioxepin and methylchlorocarbene, has been described[49] (Scheme 24).

After screening five commercial lipases, the two enantiomers of acetic acid 2-hydroxymethyl-3-methylenecyclopropylmethyl ester are obtained in high yields and excellent enantioselectivities using *Pseudomonas fluorescens* lipase (PFL) or porcine pancreas lipase (PPL) in organic solvent. The stereostructure of the desymmetrization products is established by x-ray analysis. Using these enantiopure building blocks, a synthesis of novel nucleoside analogues is also reported.



Scheme 24.

2.4. Separation of Mixtures

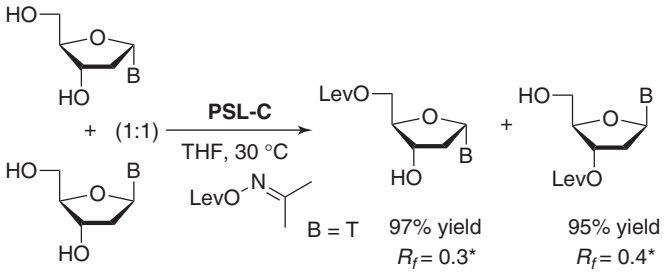
A majority of nucleosides or oligonucleotides with therapeutic applications are β-nucleoside analogues or oligonucleotides assembled from β-nucleosides. The principal reason for the use of β-nucleosides lies in their availability from natural sources and well-established synthetic routes. On the contrary, the use of α-nucleosides in therapeutics has been limited because they are unnatural and difficult to synthesize in a pure state. Despite these limitations, α-nucleosides continue to be of general interest because of their unique properties. Furthermore, the α-oligonucleotides and their derivatives have been found to be attractive agents for diagnostic applications via the antisense or antigene mode of action. These oligonuclotides have shown good hybridization properties toward DNA or RNA with stability against nuclease degradation.

Due to the ongoing interest in the utilization of α-nucleosides for therapeutic applications, the development of efficient methods for the synthesis of α-nucleoside monomers is an important endeavor. Several methods have been reported in the literature for the synthesis of α-nucleosides. However, these methods often result in a mixture of α- and β-anomers that are tedious to separate. The separation of synthetically derived α/β-nucleosides remains an underexplored area of research.

Several lipase-catalyzed methodologies have been described for the separation of α/β-anomeric nucleosides.[13b,50] One of them is the selective acylation of α-thymidine using acetonoxime butyrate catalyzed by PSL and CAL-B. Additional examples in the literature are the lipase-catalyzed diastereoselective deacetylation

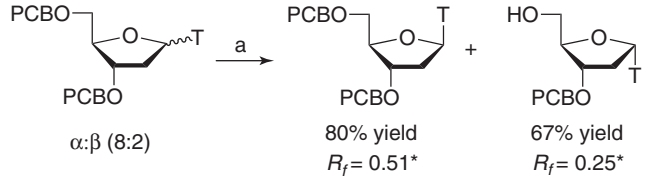
of an anomeric mixture of peracetylated α/β-thymidine and the separation of α/β-L-2′,3′ -dideoxynucleosides using cytidine deaminase (CDA). All of them are around 20 years old and are not described here.

However, an efficient and high-yield protocol for the synthesis and separation of 3′- and/or 5′-protected α-2′-deoxynucleosides is described[51] through regioselective acylation–deacylation processes catalyzed by enzymes, which allow the separation of α/β-nucleosides frequently obtained during the synthesis of nucleosides utilizing glycosylation protocols.[52] Thus, PSL-C is highly chemo- and regioselective toward the 3′-position of β-2′-deoxynucleoside derivatives, whereas PSL-C displays opposite selectivity toward the 5′-position for the corresponding α-anomer (Scheme 25).



Scheme 25.

The usefulness of this protocol is clearly demonstrated by the separation of α/β-nucleosides for two industrial projects. The first project is the successful application of this protocol to a convenient separation of α/β-mixture of thymidine derivatives from an industrial waste stream (Scheme 26).

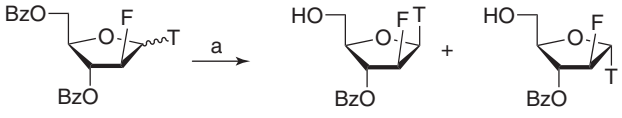


a. 0.15 M KPi (pH 7), 1,4-dioxane, 60 °C;
PSL-C, 164 h; **CAL-B**, 104 h

Scheme 26.

In particular, the isolation of α-thymidine from industrial waste is of paramount importance considering the commercial value[53] of this product, now accessible in two simple steps. The waste mother liquor represents approximately 5% of the total β-thymidine production via the glycosylation procedure. This protocol permits the environmentally benign biodegradation of industrial waste and transforms it into two valuable products: (1) α-thymidine, which is the key raw material for the synthesis of α-nucleosides and oligonucleotide analogues as therapeutics, and (2) β-thymidine, which is a starting material for the synthesis of AZT.

Furthermore, this technique is also applied for the separation of anomeric mixture of 2′-deoxy-2′-fluoro-α/β-arabinonucleosides (FANA), which are useful building blocks for the antisense constructs (Scheme 27). It is found that PSL-C has different substrate specificity for α- and β-FANA-nucleosides, where both are hydrolyzed at the 5′-hydroxyl groups with comparable rates.



α:β (4:6)

82% yield
R_f = 0.20*

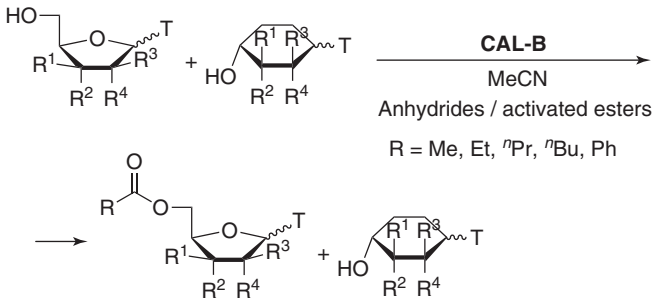
87% yield
R_f = 0.13*

*TLC eluent: 10% ^{i}PrOH/CH_2Cl_2 (v/v)

a. 0.15 M KPi (pH 7), 1,4-dioxane, 60 °C; **PSL-C**, 165 h

Scheme 27.

CAL-B immobilized on lewatite selectively acylates the primary hydroxyl group of the furanosyl nucleoside in a mixture of 1-(α-D-arabinofuranosyl)thymine and 1-(α-D-arabinopyranosyl)thymine (Scheme 28).[54]



α-D-arabinofuranosyl / α-D-arabinopyranosyl thymine

R^1,R^4 = H; R^2,R^3 = OH

β-D-xylofuranosyl / β-D-xylopyranosyl thymine

R^1,R^4 = OH; R^2,R^3 = H

Scheme 28.

This selective biocatalytic acylation of furanosyl nucleoside allows easy separation of arabinofuranosyl thymine from an inseparable mixture with arabinopyranosyl thymine. The primary hydroxyl-selective acylation methodology of arabinonucleoside is also used successfully for the separation of 1-(β-D-xylofuranosyl)thymine and 1-(β-D-xylopyranosyl)thymine from a mixture of the two, demonstrating the generality of the enzymatic methodology for the separation of furanosyl and pyranosyl nucleosides.

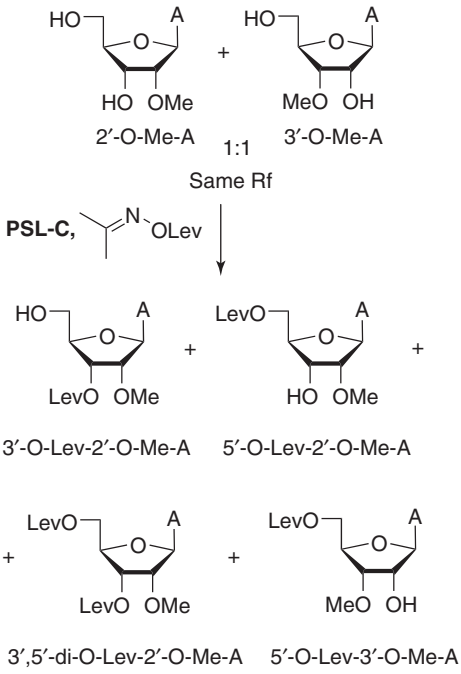
In recent years, several oligonucleotide-based drugs have entered human clinical trials for the treatment of a variety of viral, infectious, and cancer-related diseases.[55] Among these, 2′-*O*-methylribonucleotides have been used extensively as a second-generation chemistry to elicit high nuclease resistance, cellular uptake, and improved binding affinity for the RNA target. Additionally, methylribonucleotides have found applications in studying pre-mRNA splicing, examining the structures of spliceosomes, and preparing nuclease-resistant hammerhead ribozymes. Also, 2′-*O*-alkylribonucleosides are present in RNAs as minor components.

The importance of methylribonucleotides, which constitute a significant portion of the raw material cost in the preparation of oligonucleotides, has stimulated a widespread effort toward the synthesis of 2′-*O*-methylated nucleosides as key building blocks using several synthetic approaches. Although some of these strategies have been implemented successfully for the production of pyrimidine-containing 2′-*O*-methyl nucleosides, these protocols are much less efficient for purine analogues.[56] In the latter case, the problems derive from the inherent reactivity of purine bases toward alkylation and the need for selective protection of the 3′- and/or 5′-hydroxyl groups.

The increasing demand for 2′-*O*-methylribonucleosides, due to its incorporation in oligonucleotide-based therapeutics and the interesting profile of 3′-*O*-methylribonucleosides, were motivations to develop an approach where both products could be harvested from a single process that is environmentally safe and scalable (Scheme 29).[57]

Thus, efficient separation of a 1:1 mixture of 2′/3′-*O*-methyladenosine regioisomers is carried out by selective enzymatic acylation using PSL-C in combination with acetonoxime levulinate as an acyl donor. The 3′-hydroxyl group of 2′-*O*-methyladenosine is acylated with high selectivity (ca. 70%), whereas an equal amount of 3′-*O*-methyladenosine in the same solution resulted in minor acylation of 5′-hydroxyl group (ca. 8%). The differential behavior of both regioisomers toward enzymatic acylation allows a separation protocol. Upon extraction of the acylated products, the 3′-*O*-methyladenosine is isolated in 81% yield and 97% purity from the aqueous layer. Hydrolysis of acylated products in organic layer furnished 2′-*O*-methyladenosine in 67% yield and 99% purity.

The separation process is applied successfully to the crude reaction mixture of methylated products (ca. 3:1 of 2′/3′-*O*-methyladenosine) on a 5-g scale. Also reported is the use of methyl *p*-toluenesulfonate as a safe reagent for the 2′-*O*-methylation of adenosine (Scheme 30). Thus, an economical methodology enabling the synthesis and efficient separation of the isomeric mixture of



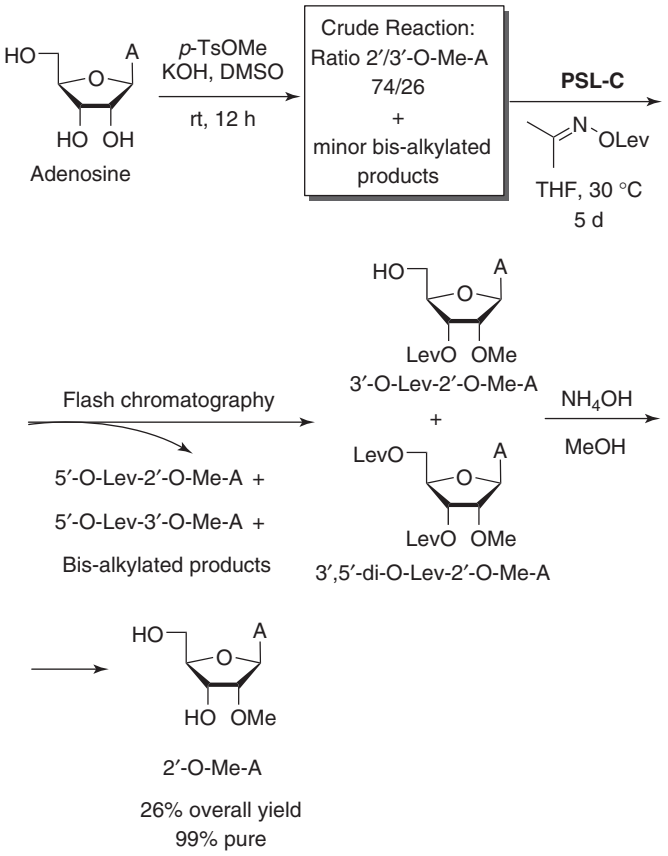
Scheme 29.

2′/3′-*O*-methyladenosine formed during the direct methylation of inexpensive adenosine has been described for the first time.

2.5. Preparation of Nucleoside Derivatives

As modified oligonucleotides have become a major field of investigation for chemists, methods for their suitable protection and deprotection for the synthesis of nucleoside monomers have become equally important. Among the plethora of synthetic tools available to chemists, application of biocatalysts in organic chemistry has become one of the most attractive alternatives to conventional chemical methods.

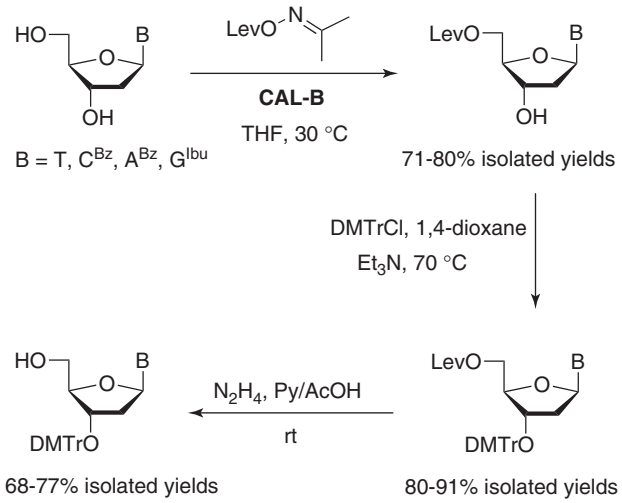
One of these monomers consists of 3′-*O*-dimethoxytrityl-protected nucleosides, which are valuable building blocks for the assembly of oligonucleotides. Use of the DMTr group for protection of the primary 5′-hydroxyl group in nucleoside and oligonucleotide synthesis is well established.[58] By contrast, efficient methods for the preparation of 3′-*O*-DMTr-protected nucleosides are not available. To perform an inverse (5′→3′) oligodeoxyribonucleotide synthesis,[59] large quantities of 3′-*O*-DMTr-protected nucleosides are needed. Interestingly, the recently approved drug Macugen,[60] a vascular endothelial growth factor antagonist, possesses a 3′-thymidine residue that is inverted.[61] The incorporation of inverted residue in Macugen is accomplished via 3′-*O*-DMTr-protected thymidine.



Scheme 30.

The overall approach for the syntheses of 3′-*O*-DMTr derivatives is outlined in Scheme 31, which is an easy, efficient, and scalable chemoenzymatic strategy for the synthesis of 3′-*O*-dimethoxytrityl-2′-deoxynucleosides. A key feature of this approach is the regioselective synthesis of 5′-*O*-levulinyl-2′-deoxynucleosides through enzymatic acylation in the presence of CAL-B. In addition, it was observed that the deblocking of the levulinyl group from the 5′-position is perfectly compatible with conventional base-protecting groups. To demonstrate the scalability of this method, 3′-*O*-dimethoxytritylthymidine is synthesized on a 25-g scale.

Chemically modified nucleosides are widely used as therapeutic agents to treat cancer and fungal, bacterial, and viral infections.[62] Similarly, carbohydrates are of paramount importance in intercellular recognition, bacterial and viral infection processes, and inflammation events, making them an attractive target for drug development.[63] Consequently, a conjugate of these two classes of molecules may offer an avenue to the design and development of novel therapeutics with improved biological functions. The use of a phosphate ester linkage for conjugation is widely practiced because of its natural occurrence in cellular physiology and as a backbone



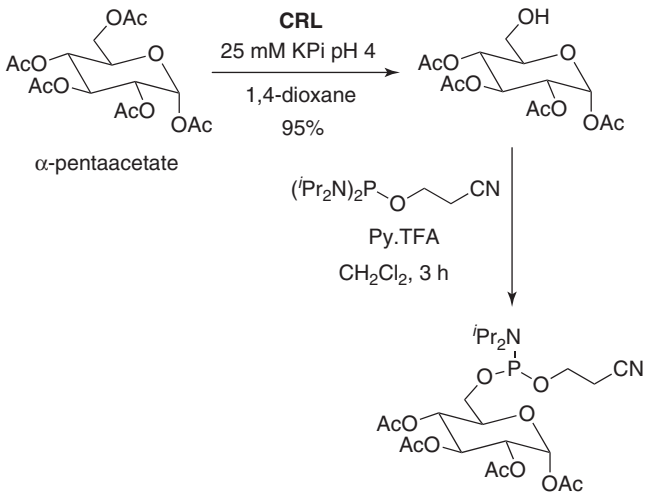
Scheme 31.

for DNA and RNA molecules. The culmination of these attributes motivated the use of natural phosphate linkage to conjugate a biologically active nucleoside with a carbohydrate unit, anticipating that it could modulate the therapeutic effects of known nucleosides in a favorable manner.

Several modified nucleosides (AZT, d4T, 3TC, ddI, ddC, and abacavir) have been approved by the FDA as anti-HIV drugs. Although these drugs are effective for the treatment of HIV, some limitations, such as toxicity, short half-life, and dependence on cellular enzymes, preclude their wider use. To overcome these limitations and to improve the therapeutic potential of these nucleosides, several phosphate-based prodrug strategies have been developed.[64] The prodrug approach appears to be a promising way to improve the anti-HIV activity of the approved nucleosides, to reduce their cellular toxicity, to enhance cellular uptake, and to prevent viral resistance.[65]

Among the various protocols available for conjugation, phosphoramidite chemistry offers the best yields. Tetraacetylphosphoramidite (Scheme 32)[66] is envisioned as the key building block for synthesis of the target nucleoside–carbohydrate prodrugs, for several reasons. First, the phosphoramidite chemistry offers excellent yield during solution-phase coupling of various nucleosides. Second, phosphoramidite compounds are reasonably stable and easy to handle during synthesis. Third, the glucose–amidite would be an ideal molecule offering a versatile conjugation unit for attachment of glucose to a wide range of nucleosides. Thus, the synthesis of glucosyl phophoramidite is accomplished in a few steps via a regioselective enzymatic hydrolysis of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose.

Commercial *Candida rugosa* lipase (CRL) was found to be an efficient catalyst for both regio- and chemoselective deacetylation of the primary hydroxyl group in the peracetylated α-D-glucose, furnishing the 6-OH derivative in excellent yield after crystallization from diethyl ether/*n*-hexane (4:1). Synthesis



Scheme 32.

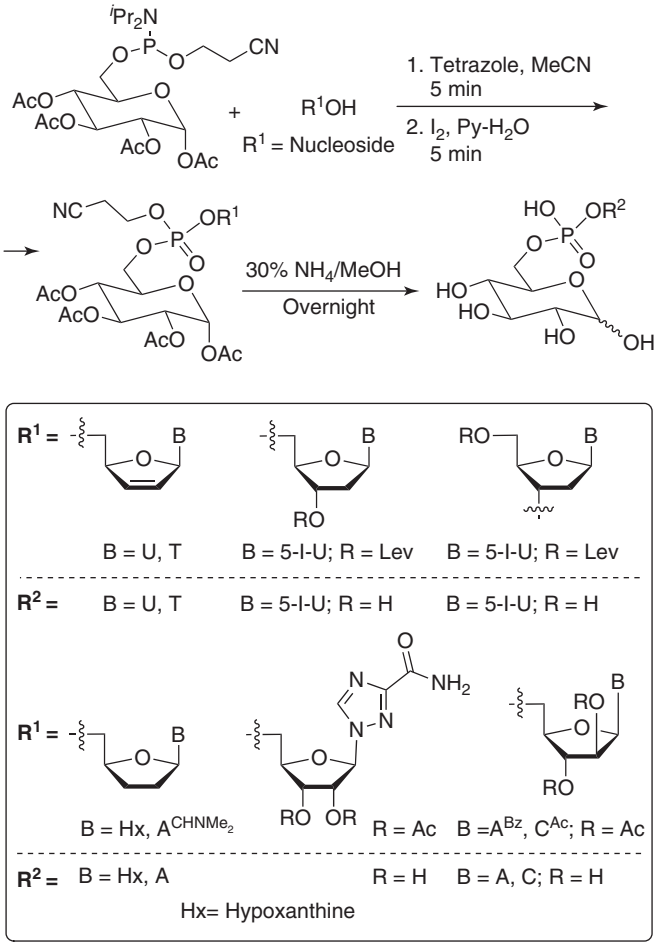
of the final phosphoramidite is carried out by phosphitylation of corresponding tetraacetyl alcohol with 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphoramidite and pyridinium trifluoroacetate as an activator in high yield.

The conjugation[67] of glucosyl phosphoramidite is done using standard amidite coupling conditions. Phosphoramidite is first activated with 1*H*-tetrazole, and the resulting intermediate was oxidized in situ using an iodine/pyridine/water solution to give the phosphotriester derivative in 39–49% isolated yields after flash chromatography. Following this procedure, d4U, d4T, ddI, IdU, ddA, virazole, ara-A, and ara-C glucose conjugates are synthesized (Scheme 33). Several of the nucleoside precursors shown in Scheme 33 are prepared using biocatalytic methodology.

Abasic sites are one of the most common cellular DNA damage areas in the genome. They result from cleavage of the glycosidic bond, which can occur spontaneously under physiological conditions or during the intermediate steps of base excision repair when DNA is exposed to various endogenous and exogenous damaging agents.[68] Therefore, studies of repair of DNA are important for maintaining the integrity of the genome.[69] Invivo, the abasic sites are believed to be repaired by the action of cellular endonucleases.[70]

Several oligonucleotide analogues containing abasic site have been synthesized to determine the structural basis of these lesions, which is responsible for their distinct biochemical effects.[71] In addition, abasic sugars were introduced into therapeutic siRNAs to inhibit degradation by nucleases.[72]

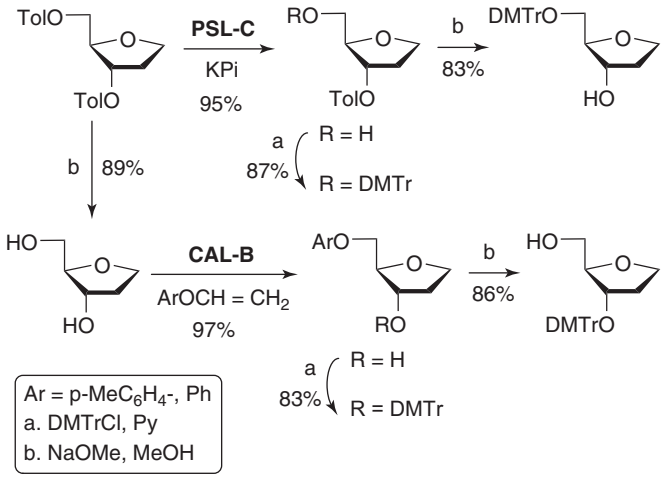
The widespread use of furanoid glycals as key intermediates for the synthesis of interesting biological compounds[73] has aroused considerable interest in scalable and cost-efficient procedures for their preparation. Most synthetic methods employed 2-deoxy-D-ribose as a starting material and imply loss of atoms.[74] Clearly, these protocols are not atom-efficient, as a good portion of the molecule is sacrificed to



Scheme 33.

obtain 1,2-dideoxy-D-ribose. To overcome these limitations, a green protocol for the preparation of 3- and 5-*O*-DMTr-1,2-dideoxy-D-ribose is reported (Scheme 34).[75]

CAL-B and PSL-C catalyze the regioselective hydrolysis of di-*O*-toluoyl-1,2-dideoxy-D-ribose, furnishing the 3-*O*-toluoyl derivative in quantitative yield. Meanwhile, both lipases exhibit complementary behavior in the acylation reaction of 1,2-dideoxy-D-ribose, providing the opposite regioisomer exclusively. The presence of electron-donating substituents in the *para*-position of the benzoate vinyl ester decreases the rate of enzymatic acylation. However, reaction with the electron-withdrawing group in the acylating agent proceeds at a faster rate. The protocol described offers a fast, reliable, and scalable route to orthogonally protected carbohydrate building blocks in excellent overall yields following an atom-efficient and low environmental impact protocol without sacrificing the stereo- and regiocontrol requirements of modern carbohydrate chemistry. Specially, the 3-*O*-DMTr



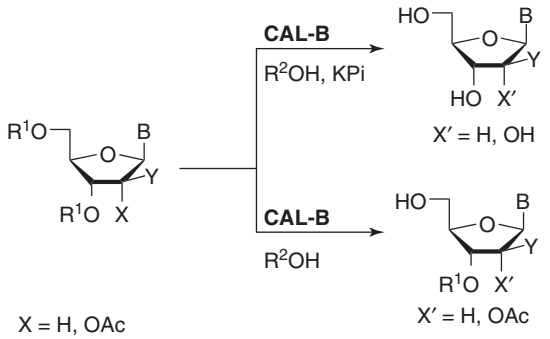
Scheme 34.

precursor is very useful when reverse amidite is required for therapeutic oligonucleotides synthesis. In addition, the enzyme has been reused to make the process more economical for industrial applications.

In the synthesis of modified nucleosides, esters are currently used to protect ribose hydroxyl groups. As their subsequent removal is carried out by treatment with ammonia or methoxide, reactive groups present either in the ribose or in the base may interfere. Thus, a mild procedure for removal of acyl groups is an alternative route of synthetic value. Although enzymatic techniques appear to be very attractive for such a purpose, due to their well-known mildness and efficiency,[76] little attention has been paid to hydrolase-catalyzed deacylation of ribonucleosides.

In this context, Iglesias, Iribarren, and co-workers[77] have investigated two different enzymatic procedures to achieve full deacetylation of nucleosides: lipase-catalyzed alcoholysis and hydrolysis (Scheme 35).

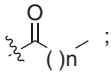
Simple and mild enzymatic transformations have been described for full removal of acetates as well as regioselective conditions for the production of compounds, in good yields, derived from 2′,3′,5′-tri-*O*-acetyluridine, 2′,3′,5′-tri-*O*-acetyl-2′-*C*-methyluridine, base-labile 6-chloro-2′,3′,5′-tri-*O*-acetylpurine riboside, 2-amino-6-chloro-2′,3′,5′-tri-*O*-acetylpurine riboside, 3′,5′-di-*O*-acetyl-2′-deoxynucleosides, 2′,3′,5′-tri-*O*-acetylcytidine, 4-*N*-acetyl-2′,3′,5′-tri-*O*-acetylcytidine, and a set of 3-deazauridine and 6-azauridine peracylated derivatives. Among the enzymes tested, CAL-B is the biocatalyst that gives the best results. In some cases, the alcohol employed in the biotransformation affects the rate of the enzymatic reaction and the yield of the products obtained, but in all cases only one regioisomer is formed. It is of note that this mild and simple enzymatic technique represents a convenient procedure for the removal of acetyl groups from base labile halogenated nucleosides.



X = H, OAc

Y = H, Me

B = T, U, C, C^{Ac}, A, G, 6-Cl-purine, 2-amino-6-Cl-purine, 3-deaza-U, 6-aza-U

R^1 =  ; n = 0, 2, 4

R^2 = Et, ^{n}Pr, ^{n}Bu

Scheme 35.

2.6. Alkoxycarbonylations and Hydrolysis

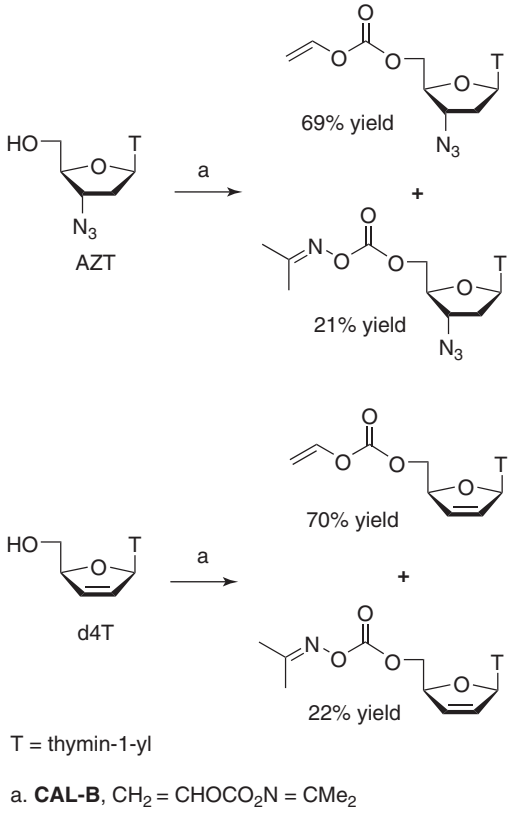
Lipases are one of the most used biocatalysts, due to their versatility in accepting a wide range of nucleophiles (alcohols, amines, thiols, water, etc.) and carbonylating agents (esters, anhydrides, carbonates, etc.).[76] The enzymatic alkoxycarbonylation reaction has been studied very little,[78] despite the biological relevance shown by carbonates and carbamates.[79] It is noteworthy that through these processes it is possible to introduce functionalities selectively, which act as protected or activated groups in alcohols and amines. In the last case, reactions are irreversible since carbamates are not substrates to lipases.

In the treatment of acquired immunodeficiency syndrome (AIDS),[80] many nucleosides have been recognized as potent and selective inhibitors of the replication of HIV. Although zidovudine (AZT) was the first compound approved by the FDA, it is still one of the most potent agents active against HIV. Stavudine (d4T) showed a clinical benefit superior to that of zidovudine.[81] On the other hand, serious side effects are associated with the administration of AZT and d4T and often require cessation of treatment. The search for new combinations of compounds with improved selectivity, lipophilicity, and efficiency that could overcome problems of resistance as well as toxicity is a field of great interest.

Based on this strategy, a new series of homo- and heterodimers of AZT and d4T, which contain carbonate and carbamate linkages, has been prepared.[82] Several arguments support this work: (1) As long as the linkage between the nucleosides (AZT and d4T) is not hydrolyzed extracellularly, the delivery and bioavailability

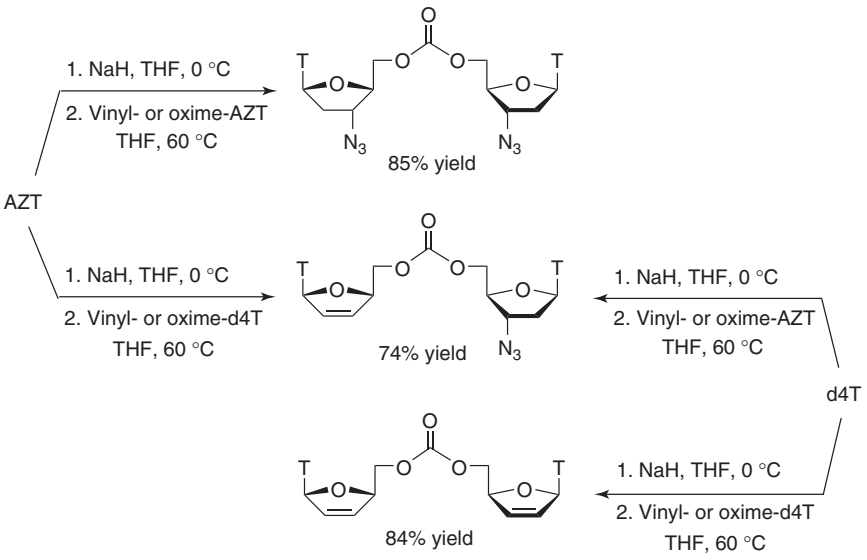
might be enhanced, depending on the lipophilic character of these new models; (2) some synergetic effects on the inhibition of HIV replication could be expected; and (3) depending on the nature of the chemical bond between the two nucleosides, intracellular hydrolysis could regenerate the two nucleosides in the cytoplasm. Owing to these facts, the dimers cited could be considered as prodrugs.

To prepare homo- and heterodimer carbonates, vinyl and oxime esters derived from AZT and d4T are used (Scheme 36). AZT and d4T are converted into 5′-*O*-alkoxycarbonylated derivatives by reaction with acetone *O*-[(vinyloxy)carbonyl]oxime acting as an alkoxycarbonylating reagent and CAL-B as a biocatalyst. In addition, 5′-*O*-acetonoximecarbonylated nucleosides are obtained as minor compounds. Vinyl carbonates as well as oxime carbonates can be used as intermediates (90% and 92% isolated combined yields, respectively) in the next step when they react with AZT or d4T.



Scheme 36.

Then AZT reacts with NaH in THF at 0 °C, giving an alkoxide which subsequently reacts with activated nucleosides (Scheme 37). After workup and

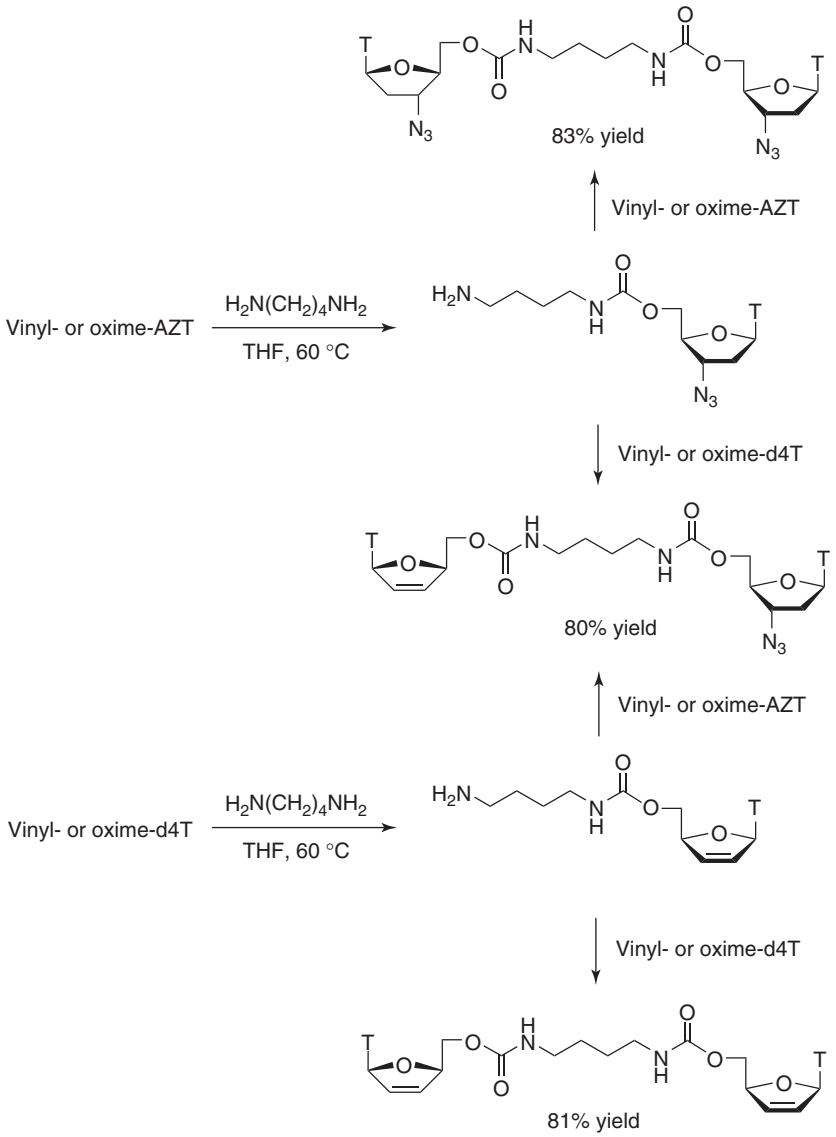


Scheme 37.

purification on silica gel chromatography column, the homodimer AZT–AZT is isolated in an 85% yield. The heterodimer AZT–d4T is prepared by reaction of the alkoxide from either AZT or d4T. The process is carried out by reaction of the AZT alkoxide with activated derivatives of d4T or by reaction of d4T alkoxide with activated derivatives of AZT, in all cases in an isolated yield of 74%. Finally, the homodimer d4T–d4T is prepared following the same procedure with an isolated yield of 84%.

5′-*O*-Carbamate-AZT and 5′-*O*-carbamate-d4T are obtained by condensation of 5′-*O*-vinylcarbonate–AZT (or 5′-*O*-oximecarbonate–AZT) or 5′-*O*-vinylcarbonate–d4T (or 5′-*O*-oximecarbonate–d4T) with 1,4-diaminobutane in THF at 60 °C, respectively (Scheme 38). Then those amino carbamates are condensed with activated carbonates derived from AZT and d4T, or with a mixture of both, giving place to homodimer dicarbamate AZT–AZT in an 83% isolated yield, heterodimer dicarbamate AZT–d4T in an 80% yield, and homodimer d4T–d4T in an 81% yields.

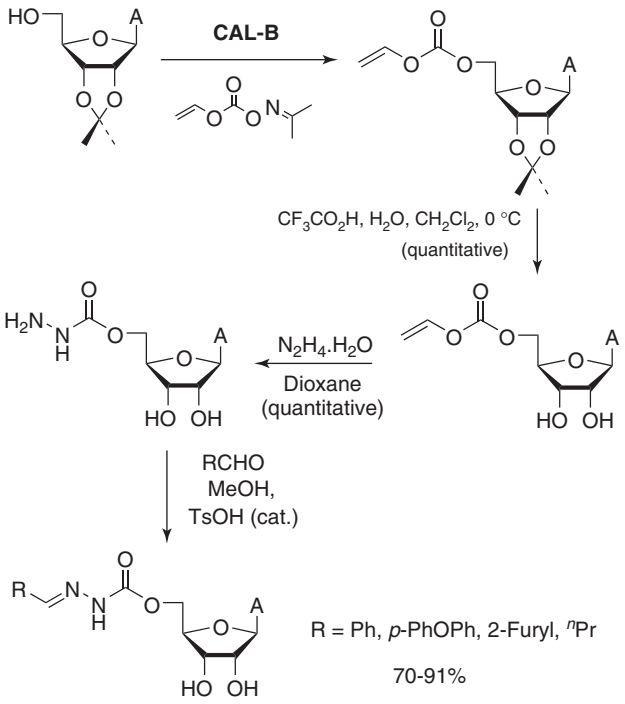
Ferrero and Gotor have a program devoted to a study of the synthesis of nucleoside[83] and nucleotide[84] derivatives prepared through a chemoenzymatic procedure. Both of these 3′- and 5′-alkylidencarbazoylnucleoside analogues are promising precursors for novel types of therapeutic nucleoside derivatives. The first step is the enzymatic alkoxycarbonylation reaction, which takes place with high regioselectivity and yield toward the 3′- or 5′-position, depending on the conditions. The carbonate nucleosides then react with hydrazine to give carbazoyl nucleoside analogues, which through reaction with aldehydes provide the corresponding alkylidencarbazoyl nucleoside derivatives. When applied to adenosine, this general procedure failed because of the low solubility of this nucleoside in the solvents



Scheme 38.

required for the enzymatic process. As a consequence, this is a limiting step in the preparation of the corresponding nucleoside derivatives (Scheme 39).

To address this limitation,[85] a commercially available 2′,3′-protected adenosine derivative is used. This alternative pathway led to an excellent yield of 5′-alkylidencarbazoyl adenosine analogues, through the formation of vinylcarbonate and/or oximecarbonate of 2′,3′-protected adenosine, hydrazinolysis of which give vinylcarbonate, which is transformed in carbazoyl nucleoside. This finally



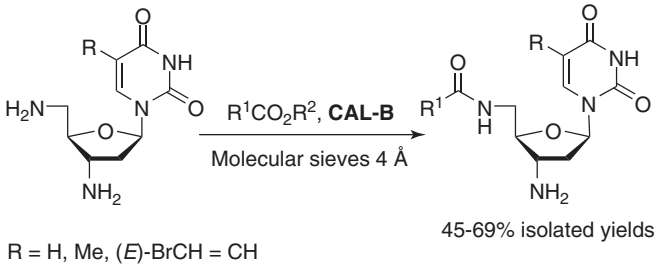
Scheme 39.

reacted with different aldehydes to yield 5′-alkylidencarbazoyl adenosine analogues.

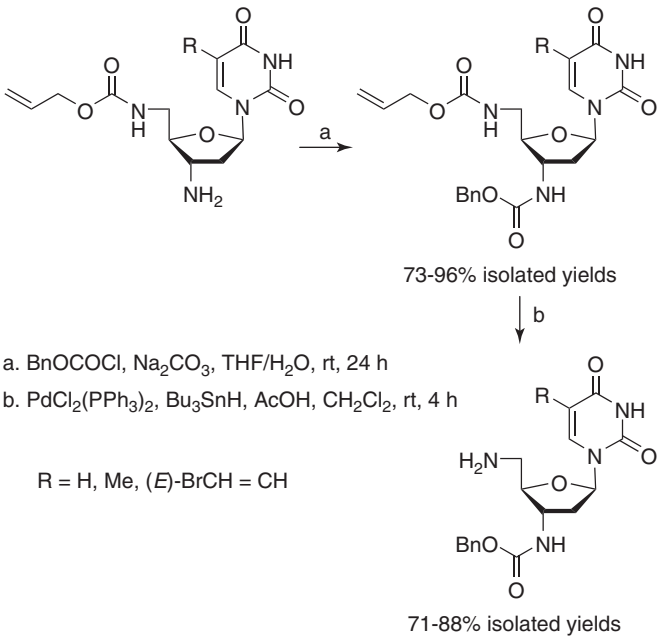
In recent years, the search for new nucleoside derivatives using enzymatic clean, simple, and efficient methodology has received a great deal of attention from organic chemists. In this context, oxime carbonates have been used with lipases for direct and selective protection of natural nucleosides.

This regioselective process would be more difficult in the case of two primary amines, due to the major nucleophilic character of the amino group compared to the hydroxyl group. The first regioselective enzymatic alkoxycarbonylation of primary amino groups[86] is reported in pyrimidine 3′,5′-diaminonucleoside derivatives to synthesize novel pyrimidine aminonucleosides regioselectively protected as carbamates. CAL-B catalyzes this reaction with nonactivated homocarbonates, allowing the selective synthesis of several *N*-5′-carbamates, including (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) analogues, with moderate-to-high yields, whereas PSL-C affords mixtures of alkoxycarbonylated regioisomers (Scheme 40).

Since direct enzymatic methodology did not allow for *N*-3′-alkoxycarbonylated nucleoside derivatives in good yields, an orthogonal protection scheme is designed (Scheme 41). The strategy starts with protection of the 5′-NH_2 group as allyloxycarbamate. Treatment of the later compounds with benzyloxycarbonyl chloride at room temperature affords dicarbamate nucleosides with high efficiency.



Scheme 40.

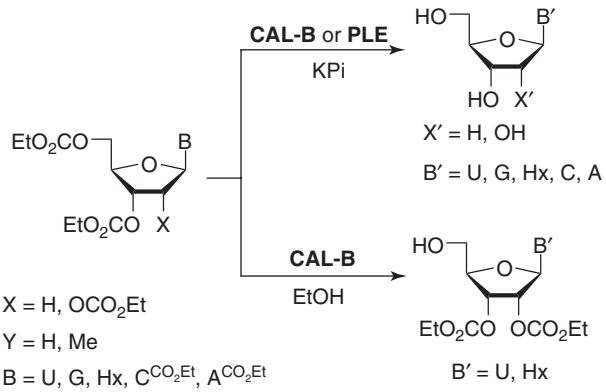


Scheme 41.

Then, selective deprotection of the allyloxycarbonyl group gives place to *N*3′-Cbz-protected derivatives through a short and efficient chemoenzymatic route.

Carbonates display a wide range of applications in synthetic, polymer, and medicinal chemistry.[87] Whereas in the former field, carbonates are employed as protective groups for diols and poliols, in the latter the lipophilic nature of carbonates can afford prodrugs of pharmacological active compounds. Therefore, the use of carbonates in nucleoside chemistry offers an interesting potential. However, the use of alkyl carbonates as protective groups of nucleosides has been very restricted, because their removal requires a strong basic medium, frequently not compatible

with the complex structure of such molecules. To avoid this limitation, the report[88] on the removal of carbonates by hydrolases in nucleoside alkyl carbonates is of great interest (Scheme 42).



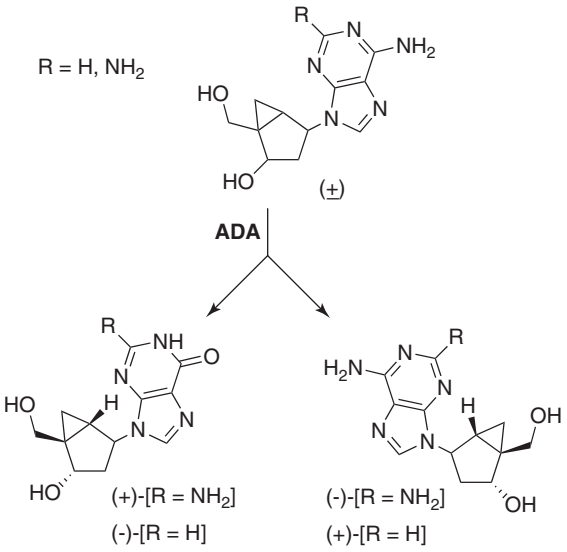
Scheme 42.

A set of mono-, di-, tri-, and tetraalkoxycarbonylated nucleosides is tested to assess their enzymatic hydrolysis. All the alkoxycarbonyl groups of the substrates, from both carbonate and carbamate functions, are quantitatively hydrolyzed using pig liver esterase (PLE) at pH 7 and 60 °C, regardless of the nucleoside base. Quantitative full alkoxycarbonyl group removal is also reached by CAL-B under mild conditions, but in this case, longer reaction times are required. Thus, PLE appears as a useful catalyst for the mild and quantitative deprotection of nucleoside carbonates and carbamates in the synthesis of modified nucleosides.

3. BIOTRANSFORMATIONS THAT MODIFY THE BASE

A systematic study of the role of the sugar ring in the process of recognition and binding of nucleosides, nucleotides, and oligonucleotides to their target enzymes has been undertaken.[89] The picture that is emerging from these studies shows that the majority of enzymes appear to have strict conformational requirements for substrate binding with the furanose ring in a well-defined shape. In particular, methanocarbanucleosides built on a rigid bicyclo[3.1.0]hexane template have been instrumental in defining the role of sugar puckering in nucleosides and nucleotides by stabilizing the active receptor-bound conformation and thereby identifying the biologically favored sugar conformer.

The synthetic approach to these conformationally locked carbocyclic nucleosides is not simple and relies on the availability of the chiral synthon that constitutes the sugar moiety (Scheme 43).[90] The overall yields of amino and diamino derivatives obtained from two extremely simple and cheap starting materials, such as



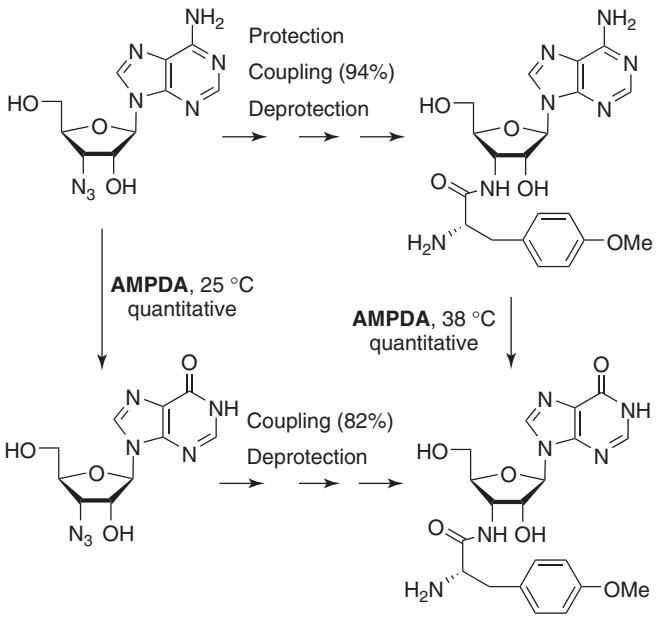
Scheme 43.

acrolein and ethyl acetoacetate, are 15% and 19%, respectively. This is remarkable considering the complexity of the resulting structures with four asymmetric carbons.

The key step on the syntheses is the enzymatic resolution of an aqueous solution of bicyclonucleosides with adenosine deaminase (ADA). The two purine analogues built on the complex bicyclo[3.1.0]hexane platform are resolved efficiently with remarkable enantioselectivity by ADA.

To study the ribosomal peptidyl transfer, puromycin analogues are of interest in which adenine has been replaced by hypoxanthine. Inosine puromycin analogues from 3′-azidodeoxyadenosine derivatives for the quantitative transformation of the N-heterocycle are synthesized using adenylate deaminase (AMPDA) (Scheme 44).[91] The amino acid coupling was carried out under Staudinger–Vilarrasa conditions in 94% yield starting with protected azide and in 82% yield using unprotected azide: thus, in the presence of two hydroxyls and a lactam function.

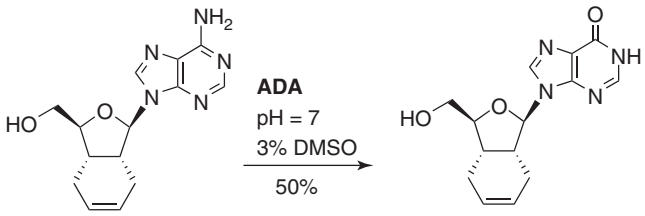
A chiral synthesis of a series of hexahydroisobenzofuran (HIBF) nucleosides is accomplished via glycosylation of a stereo-defined (*syn*-isomer) sugar motif with the appropriate silylated bases.[92] All nucleoside analogues are obtained in 52–71% yield as a mixture of α- and β-anomeric product, increasing the breadth of the novel nucleosides available for screening. The structure of the novel bicyclic HIBF nucleosides is established by a single-crystal x-ray structure of the β-HIBF thymine analogue. Furthermore, the sugar conformation for these nucleosides is established as N-type. Among the novel HIBF nucleosides synthesized, 25 compounds have been tested as an inhibitor of HIV-1 in human peripheral blood mononuclear (PBM) cells, and seven were found to be active (EC_{50} = 12.3 – 36.2 μM). Six of these



Scheme 44.

compounds are purine analogues, with β-HIBF inosine analogue being the most potent (EC_{50} = 12.3 μM) among all compounds tested. The striking resemblance between didanosine (ddI) and β-HIBF inosine analogue may explain the potent anti-HIV activity.

This inosine compound is obtained via enzymatic deamination of the corresponding adenosine analogue (Scheme 45). This reaction is slower than the deamination of natural adenosine reported and afforded only a 50% yield of the desired product after 72 h. Authors attribute this sluggish reactivity to the lipophilic nature of the HIBF nucleoside. Nonetheless, it is remarkable to observe that the presence of the cyclohexene ring is not incompatible with recognition by the enzyme. Recognition as a substrate for adenosine deaminase further confirms that the 5′-hydroxyl group was accessible to the active site in enzyme for its transformation.[93]



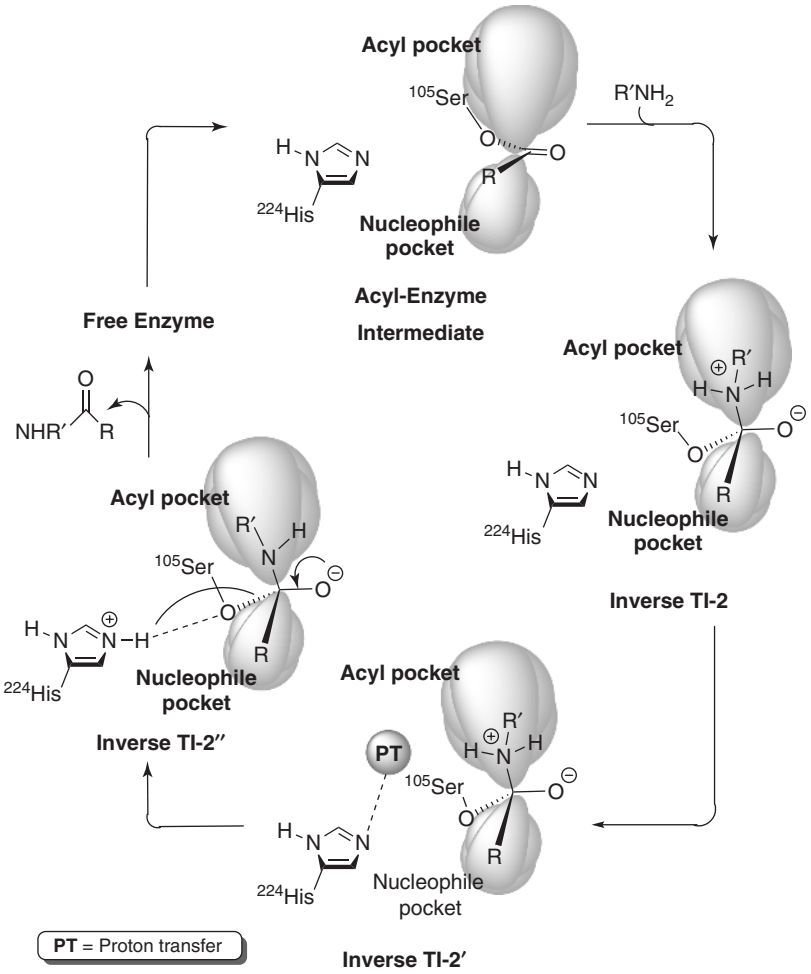
Scheme 45.

4. MOLECULAR MODELING

Due to the cleanness, simplicity, and efficiency of enzymatic reactions, they are often the best route to complex molecules such as nucleoside analogues, which are drug candidates for several diseases. Modifications of the sugar moiety are important sources of new compounds, with promising chemotherapeutic properties. One sugar modification is the lipase-catalyzed regioselective acylation of natural 2′-deoxy- and ribonucleosides with vinyl and oxime esters, and 3′,5′-diamino-2′,3′,5′-trideoxynucleosides using nonactivated esters, as described previously in several examples in this chapter. One of the most useful lipases, CAL-B, shows high regioselectivity for the functional group at the ribose 5′-position for many different derivatives. The molecular basis of this regioselectivity is analyzed in depth[94] for CAL-B-catalyzed regioselective acylation of natural thymidine with oxime esters, and also regioselective acylation of an analogue, 3′,5′-diamino-3′,5′-dideoxythymidine, with nonactivated esters. In both cases, acylation favors the less hindered 5′-position up to 80-fold over the 3′-position. Computer modeling of phosphonate transition-state analogues for acylation of thymidine suggests that CAL-B favors acylation of the 5′-position because this orientation allows the thymine ring to bind in a hydrophobic pocket and forms stronger key hydrogen bonds as compared to acylation of the 3′-position.

On the other hand, computer modeling of phosphonamidate analogues of the transition states for acylation of either the 3′- or 5′-amino groups in 3′,5′-diamino-3′,5′-dideoxythymidine shows similar orientations and hydrogen bonds and thus does not explain the high regioselectivity. However, computer modeling of inverse structures, where the acyl chain binds in the nucleophile pocket, and vice versa, does rationalize the regioselectivity observed. The inverse structures fit the 5′-intermediate thymine ring, but not the 3′-intermediate thymine ring, in the hydrophobic pocket and form a weak new hydrogen bond between the carbonyl *O*-2 of the thymine and the nucleophile amine only for the 5′-intermediate. A water molecule may transfer a proton from the ammonium group to the active-site histidine. As a test of this inverse orientation, the acylation of thymine and 3′,5′-diamino-3′,5′-dideoxythymidine has been compared with butyryl acyl donors and with the isosteric methoxyacetyl acyl donors. Both acyl donors reacted at equal rates for thymidine, but the methoxyacetyl acyl donor reacted four times faster than the butyryl acyl donor for 3′,5′-diamino-3′,5′-dideoxythymidine. This faster rate is consistent with an inverse orientation for 3′,5′-diamino-3′,5′-dideoxythymidine, where the ether oxygen of the methoxyacetyl group can form a hydrogen bond similar to that of the nucleophilic amine. This combination of modeling and experiments suggests such lipase-catalyzed reactions of apparently close substrate analogues, as alcohols and amines may follow different pathways. The alternative mechanism for amines is shown in Scheme 46.

As it has been shown, hydrolases, and more specifically, lipases, can regioselectively acylate primary hydroxyl groups in the presence of secondary hydroxyl groups in nucleosides. For example, CAL-B catalyzes the selective enzymatic acylation of the primary hydroxyl in several 2′-deoxynucleosides. In special cases,



Scheme 46.

chemical methods can favor reactions at a secondary over a primary hydroxyl group. Also, PSL-C catalyzes the regioselective acylation of the more hindered secondary 3′-position in β-D-2′-deoxynucleosides, as has been shown. The explanation of this "abnormal" behavior is the focus of a paper[95] that identifies the molecular basis of this selectivity using computer-aided molecular modeling.

Surprisingly, PSL-C favors acylation of the secondary hydroxyl at the 3′-position over the primary hydroxyl at the 5′-position in 2′-deoxynucleosides by up to >98:1. Molecular modeling found catalytically productive tetrahedral intermediate analogues for both orientations. However, acylation of 3′-hydroxyl places the thymine base in the alternative hydrophobic pocket of PSL-C's substrate-binding site, where it can hydrogen-bond to the side-chain hydroxyls of Tyr23 and Tyr29 and the main-chain carbonyl of Leu17. Conversely, acylation of the 5′-hydroxyl

leaves the thymine base in the solvent, where there is no favorable binding to the enzyme. It is proposed that these remote stabilizing interactions between the thymine base and the PSL-C's substrate-binding site stabilize the 3′-acylation transition state and thus account for the unusual regioselectivity.

5. CONCLUDING REMARKS

Chemoenzymatic approaches to the synthesis of nucleosides have been demonstrated to be a powerful tool to generate a great variety of natural and nonnatural analogue structures with a wide structural diversity. The number of reports describing enzyme-mediated hydrolysis, protections, and resolutions has been increasing in the past decade, not only applied to laboratory scale but also in industrial production. This is due to the favorable features of biocatalytic systems, especially the selectivity. With the development of modern biotechnology, we predict that enzymes will become available more cheaply and will enable industrial preparation of nucleoside derivatives via biotransformations. As the area continues to grow, new enzymatic procedures for the synthesis of many natural and unnatural nucleosides will continue to be developed. It is believed that more effective synthetic strategies based on combined chemical and enzymatic methods will have to be developed to tackle the new generation of problems associated with nucleosides, particularly those involved in modifications in both the heterocyclic base and the sugar moiety, to avoid the drawbacks shown in certain applications, such as agents effective against viral infections or cancer, or applications related antisense oligonucleotide preparation. Moreover, biocatalysts are ecologically beneficial natural catalysts that offer the opportunity to carry out highly chemo-, stereo-, and regioselective synthesis of nucleosides, which could not be performed by classical chemical methodologies. Recently, molecular modeling is helping to explain the selectivity shown by several biocatalysts. The aim of this tool is to predict which catalyst would be best suited for a particular substrate, thus avoiding investing so much time and money in screening.

Acknowledgments

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ABBREVIATIONS

A	adenine
AIDS	acquired immunodeficiency syndrome
ara-A	arabinoadenosine
ara-C	arabinocytosine
AZT	zidovudine
C	cytosine

Cbz	benzyloxycarbonyl
DCC	dicyclohexylcarbodiimide
ddA	2′,3′-dideoxyadenosine
ddC	2′,3′-dideoxycytosine
ddI	2′,3′-dideoxyinosine
d4T	2′,3′-dideoxy-2′,3′-didehydrothymidine (stavudine)
d4U	2′,3′-dideoxy-2′,3′-didehydrouridine
DHF	dihydrofuran
DHP	dihydropyran
DMSO	dimethylsulfoxide
DMTr	dimethoxytrityl
FDA	U.S. Food and Drug Administration
G	guanine
HBV	hepatitis B virus
HIBF	hexahydroisobenzofuran
HIV	human immunodeficiency virus
IdU	5-yodo-2′-deoxyuridine
IL	ionic liquid
MDHP	methoxydihydropyran
MTHP	methoxytetrahydropyran
PBM	peripheral blood mononuclear
Py	pyridine
T	thymine
THF	tetrahydrofuran
THP	tetrahydropyran
3TC	lamivudine

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