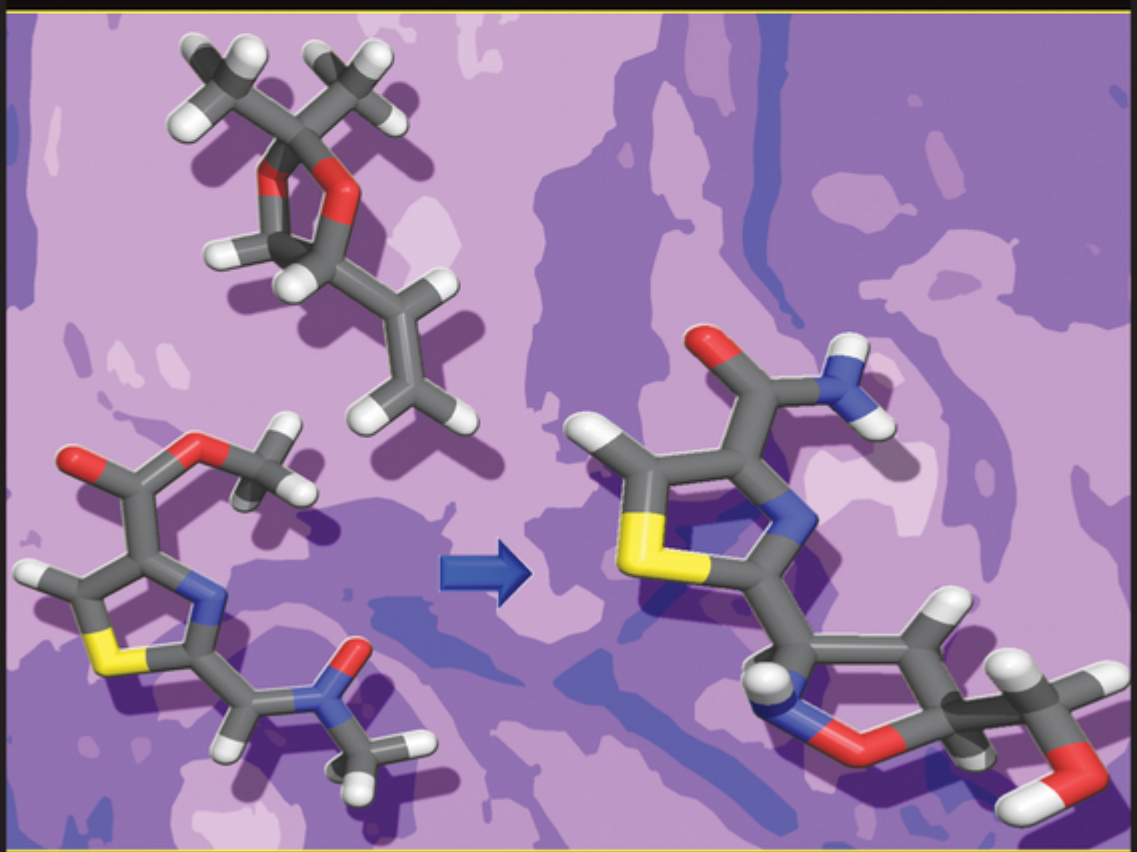
Chemical Synthesis of Nucleoside Analogues

EDITED BY

Pedro Merino

WITH A FOREWORD BY

Alberto Brandi, University of Florence



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CHEMICAL SYNTHESIS OF NUCLEOSIDE ANALOGUES

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Edited by

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To my sons Pedro, Javier, and Ignacio

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FOREWORD

Nucleosides and nucleotides are implicated in all aspects of cellular life—metabolic regulation, catalysis, energy supply, and the storage of genetic information—through the nucleic acids. This broad and important role suggests that the chemistry of this class of compounds represents a foremost research topic in bioorganic and medicinal chemistry. Since the two final decades of the twentieth century it has been clear that the synthesis of modified nucleosides and nucleoside analogues would be the key to understanding the cellular functions and mechanisms involved in a variety of diseases. In fact, research has resulted in several important successes, as many anticancer and antiviral drugs have been produced as nucleoside analogues, in addition to several other compounds employed as diagnostic tools.

The present volume provides an analysis of the chemical syntheses of nucleoside analogues that address systematically almost all the diverse classes of nucleoside analogues, giving a picture of the state of the art and of the new directions that research in this field is undertaking. The selection of topics in this volume correctly reflects the dynamism of investigations in progress in the area of nucleosides and nucleotides and draws attention to the expanding opportunities for medicinal chemistry. The scientific credentials of the editor and the contributors, all well-recognized international experts in the field, assure readers of the quality of the work.

This important volume will be of interest to a wide audience of multidisciplinary researchers in organic, bioorganic, and medicinal chemistry, and will be especially helpful for inexpert researchers who are entering the field with fresh enthusiasm to put forth new ideas.

ALBERTO BRANDI

University of Florence

PREFACE

Nucleosides are the building blocks required in constructing nucleic acids. As we learned in school, billions of combinations are possible using only four different nucleosides, and thus the genetic code is generated, carrying all our past and future features, behavior, and illnesses. But nucleosides also take part in many other biological processes, and they can be found as discrete molecules not closely related to nucleic acids. This is the case for cyclic AMP, a second messenger involved in many biological processes, and for the well-known ATP, a coenzyme used by cells for intracellular energy transfer, or *S*-adenosylmethionine, the "natural" methylating agent employed for a great variety of living beings and required for cellular growth.

In all cases, either forming a part of nucleic acids or as discrete entities, nucleosides are composed of a sugar residue of furanose (either D-ribose or 2-deoxy-D-ribose) and a heterocyclic base (adenine, guanine, thymine, cytosine, uracil, and the more recently discovered methylcytosine). Only combinations of these components are found in nature, with very few exceptions. However, for many years synthetically modified nucleosides have proven their valuable utility as therapeutic drugs. Among other uses they have been employed as antiviral and antitumoral agents with great success, although they often cause important and undesirable side effects. For this reason it is still a challenge to develop new nucleoside analogues that can be used as therapeutic drugs while minimizing side effects. The only access to nucleoside analogues is chemical synthesis, and a great effort is still necessary to develop new routes to a wide variety of modified nucleosides. Indeed, such modifications can assist in several ways, including modification of the heterocyclic base and changes at the furanose ring. The former is somewhat limited, because to achieve some biological activity the ability to form hydrogen bonds must be retained. Accordingly, the number of heterocycles that can be used as alternative bases is limited even though there is a considerable catalogue of possibilities. Modifications at the furanose ring are more versatile. It is possible to eliminate hydroxyl groups (deoxynucleosides), to change their configuration or even to exchange other substituents, as in the case of the well-known anti-HIV agent AZT. It is also possible to replace the furanose ring by a different heterocyclic ring (aza-nucleosides, thionucleosides, and dioxolanyl, oxathiolanyl, and isoxazolidinyl nucleosides are good examples), by a carbocyclic ring (carba-nucleosides), and by an acyclic chain (acyclic nucleosides). These modifications have important effects on the conformation of nucleosides that can also be achieved by introducing conformational restrictions, as in the case of constrained nucleosides and spironucleosides. Additional modifications consist of replacing the C–N nucleosidic bond by a hydrolytically stable C–C bond as in the case of *C*-nucleosides,

moving the base moiety at the furanose ring to a different position (isonucleosides) and synthesizing the enantiomers of natural nucleosides (L-nucleosides). All these modifications, should, however, preserve a relative disposition between the base moiety and the hydroxymethyl group, which is crucial for biological activity; very few exceptions are found in which such a group does not seem to be necessary. The hydroxymethyl group is required for further phosphorylation of the nucleoside analogue to form the corresponding nucleotide, which is the biologically active form. This phosphorylation should be carried out by kinases, a family of enzymes that is usually very selective, and therefore is not always easy to achieve for modified compounds. Thus, the preparation of phosphonated analogues and pronucleotides capable of generating phosphorylated analogues in situ, is of great importance. In addition, nucleoside analogues can form a part of more complex structures, as is true of nucleoside antibiotics. They can also be used as monomers in the construction of novel peptide nucleic acids, promising molecules for the treatment of autoimmune diseases and allergies and for use as molecular probes.

These transformations are somewhat different and require different synthetic approaches. In this book a collection of various possibilities are presented that have been studied by the authors in accessing the great variety of nucleoside analogues. Without their effort it would not be possible to have at our disposal a huge arsenal of nucleoside analogues to be tested as new therapeutic drugs. Chemical synthesis is the invaluable tool that makes possible our opportunity to fight against such important illnesses as antiviral and bacterial infections, malaria, cancer, and autoimmune diseases. We sincerely thank all synthetic chemists who are dedicating their professional lives to this mission, often without the recognition by society that they deserve.

PEDRO MERINO

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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.

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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

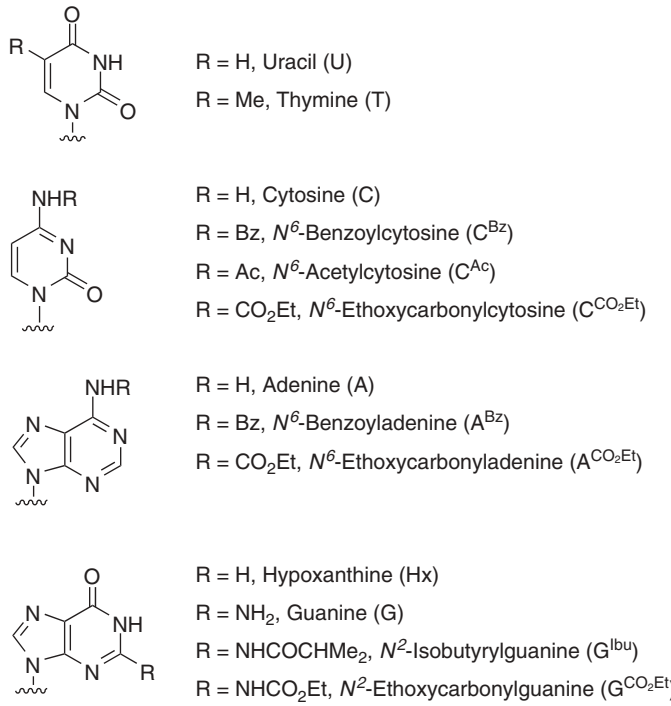


Figure 1. Pyrimidine and purine bases, their more common protected versions used in this review, and their abbreviations.

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.

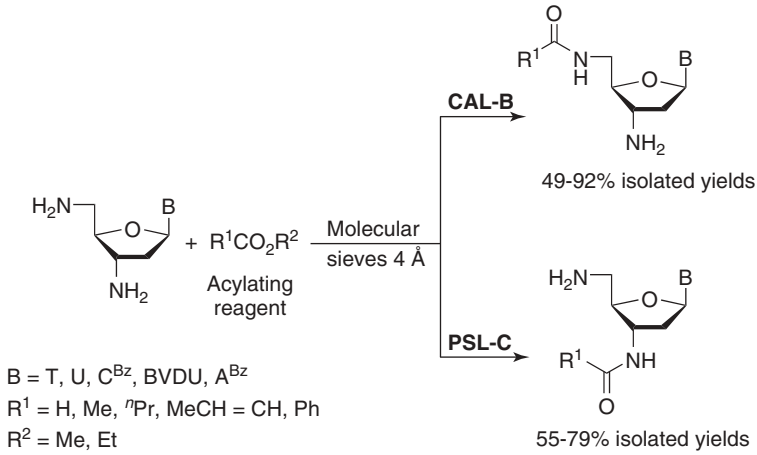
2. TRANSFORMATIONS ON THE SUGAR MOIETY

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).

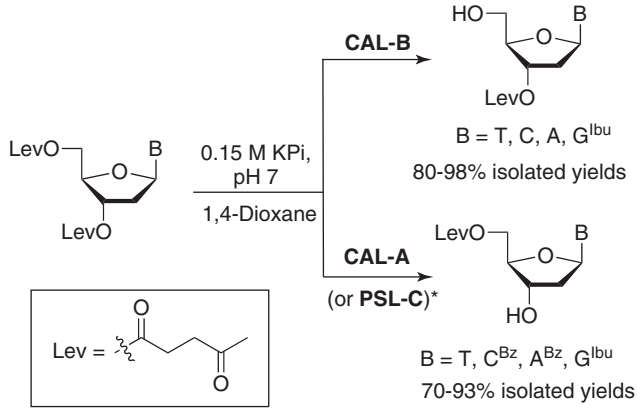


Scheme 1.

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.

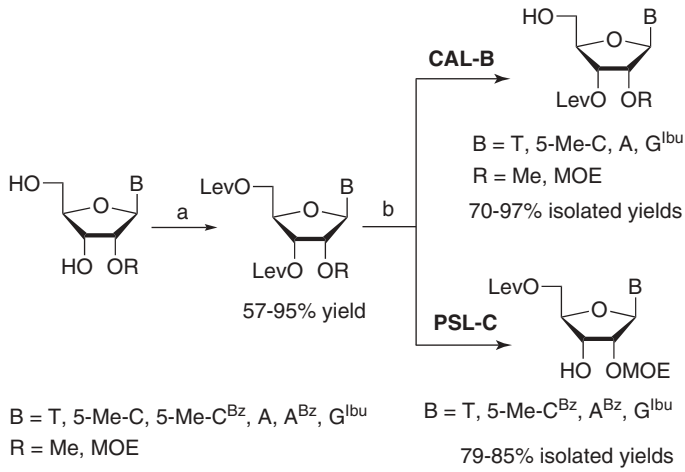
Thus, CAL-B selectively hydrolyzes the 5′-levulinate esters, furnishing 3′-*O*-levulinyl-2′-deoxynucleosides in >80% isolated yields. On the other hand, PSL-C and *Candida antarctica* lipase A (CAL-A) exhibit the opposite selectivity toward hydrolysis at the 3′-position, affording 5′-*O*-levulinyl derivatives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

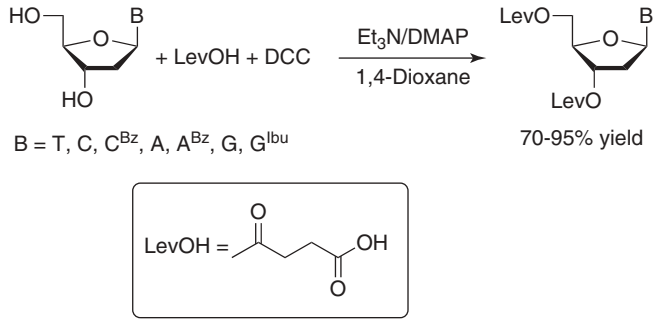


a. LevOH, DCC, Et_3N, DMAP, 1,4-dioxane

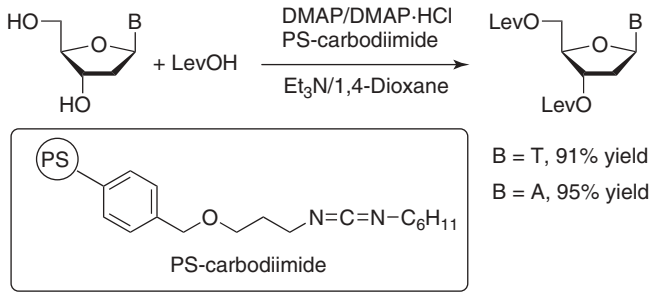
b. 0.15 M KPi (pH 7), 1,4-dioxane

Scheme 3.

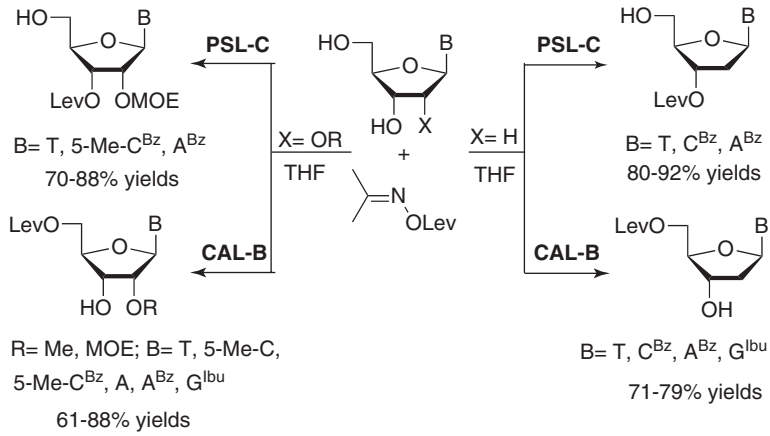
Normal procedure



Improved procedure



Scheme 4.



Scheme 5.

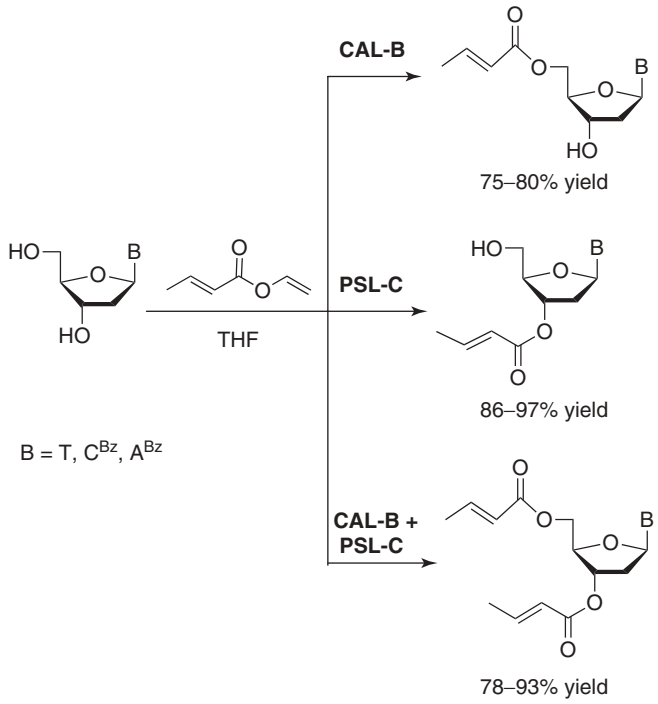
adenosine analogues are good substrates for lipase-mediated acylations. Thus, *N*-benzoyl-3′-*O*-levulinyl cytidine and adenosine derivatives are obtained in good yield, overcoming the limitations of the original hydrolysis protocol.[15] The new and improved method is shorter than the one described earlier, 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



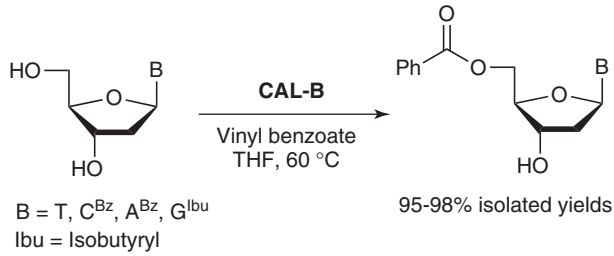
Scheme 6.

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.

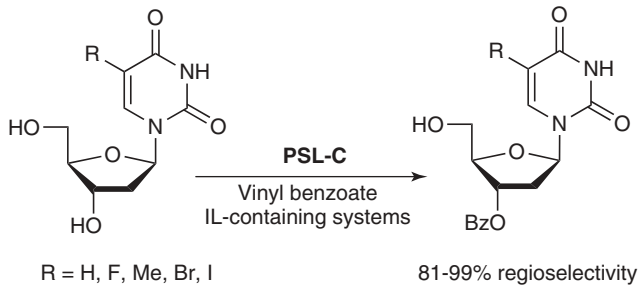
To demonstrate the suitability of the reaction for industrial applications, both the acylating agent and the enzyme were reused for subsequent reactions. The recycled CAL-B maintained total selectivity toward acylation of the 5′-OH, with the exception of the longer reaction rate. On the other hand, benzoylation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

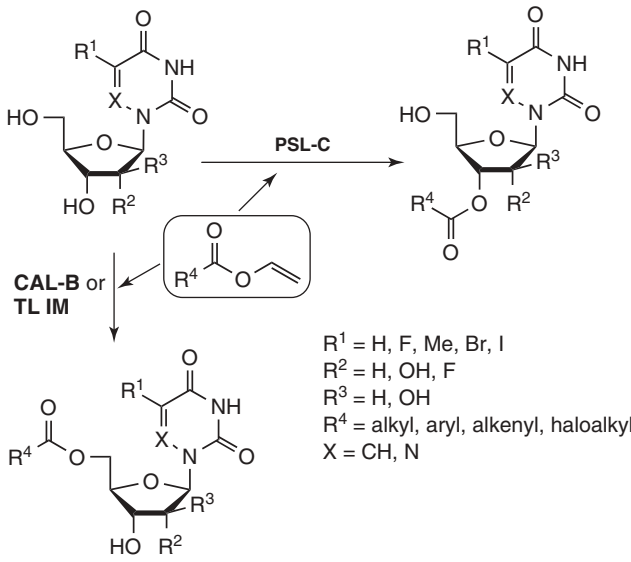
Ionic liquid-containing systems offer new opportunities for the enzymatic acylation of nucleosides.[27] In PSL-C-mediated benzoylation of floxuridine and its analogues (Scheme 8), enzyme performance, including enzyme activity and 3′-regioselectivity, are enhanced significantly using $[C_4MIm]PF_6$-containing 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.

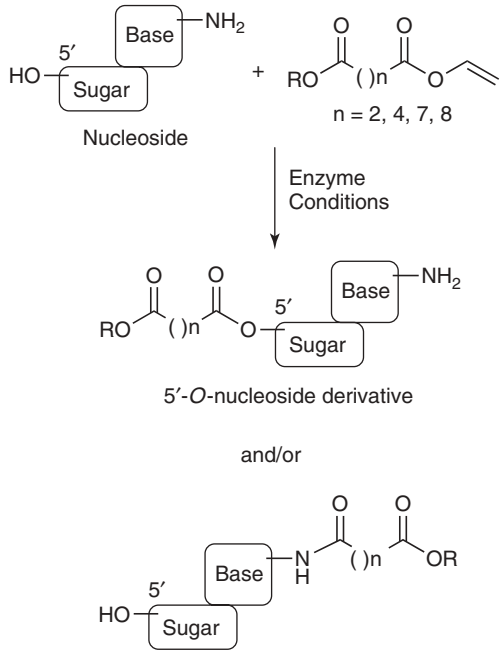
Efficient protocols for the selective synthesis of polymeric prodrugs of ribavirin, ddI, cytarabine, acyclovir, 5-fluorouridine, or aracytosine are developed using different biocatalysts: CAL-B, Lipozyme, or PSL-C (Scheme 10).[29]

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,



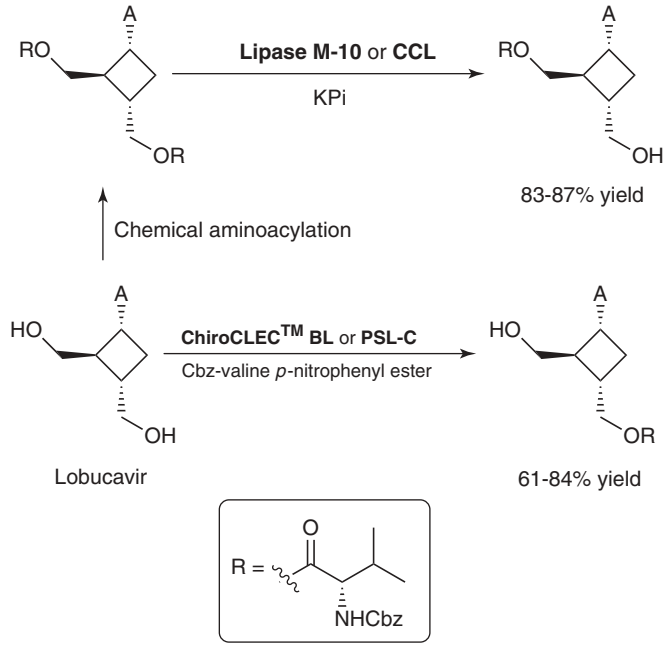
a. Ribavirin, **CAL-B**, acetone, 50 °C
b. ddI, **Lipozyme**, acetone
c. Cytarabine, **CAL-B** or **Lipozyme**, acetone
d. Acyclovir, **PSL-C**, acetone or DMSO
e. 5-Fluorouridine, **CAL-B**, THF
f. Ribavirin or *ara*-cytosine, **CAL-B**, acetone

Scheme 10.

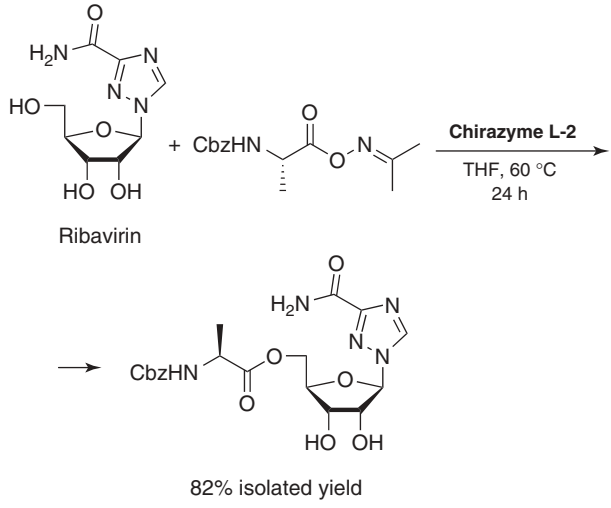
and early clinical trials, efficient synthesis of 5′-*O*-alanylribavirin has been reported (Scheme 12).[31]

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.