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Invited Review

Chemoenzymatic Transformations in Nucleoside Chemistry

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Summary. The synthetic potential of enzymes in connection with nucleoside analogue preparations is described.

Keywords. Enzymes; Nucleosides; Chemoenzymatic transformations.

Introduction

During the last quarter of the century almost no other aspect of organic synthesis has received as much attention as the preparation of enantiomerically pure compounds. The synthesis of optically active materials is an important task and represents a challenge to synthetic organic chemists. Increasing interest in the understanding of biological processes and the general recognition that chirality plays a crucial role in nature fostered a tremendous effort in enantioselective synthesis. Nowadays, the choice of a racemic synthesis over the development of an enantiopure compound must be justified, since opposite enantiomers interact differently within an organism and can display various activities. Thus, each country requires investigation into the bio-availability and pharmacological effect of a new chiral drug, and its final approval is based on complete background information for each enantiomer.

The chemistry of natural nucleosides and their analogues has been thoroughly studied due to their potential as fungicidal, antitumour, and antiviral agents [1]. Consequently, extensive modifications have been made to both the heterocyclic base and the sugar moiety in order to avoid the drawbacks shown by nucleosides or analogues in certain applications. Since the late eighties, nucleoside analogues have been investigated with renewed urgency in the search for agents effective against the Human Immunodeficiency Virus (HIV), the causative agent of AIDS. More effective treatment has also been sought for other viral infections, in particular those caused by Herpes Simplex Virus (HSV types 1 and 2), Varicella Zoster Virus (VZV), Human Cytomegalovirus (HCMV), and *Epstein-Barr* Virus

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(EBV), which can prove lethal to AIDS patients and other immunocompromised individuals. This has resulted in an explosion of synthetic activity in the field of nucleosides, with special interest in their asymmetric syntheses, and in the discovery of a number of derivatives with potential antiviral activity.

For organic chemists, enzyme-catalyzed reactions have become standard procedures for the synthesis of enantiomerically pure compounds due to their simple feasibility and high efficiency. In general, these catalysts are inexpensive and in many cases able to fit to a wide range of substrate structures. Moreover, biocatalysts are ecologically beneficial natural catalysts. Due to these advantages, it is to be expected that biocatalyzed reactions will play an increasing role primarily in the preparation of nonracemic chiral biologically active compounds in the laboratory as well as in industrial production.

A number of extensive general reviews [2], special issues of periodicals [3], or specialized books [4] have been published during the last two decades on the subject of biocatalysis in organic synthesis. This is due to the explosive development and success of this methodology in the area of the synthesis of

Table 1. Enzymes commonly used in biocatalytic processes

Accepted name	Abbreviation	Other denominations
Candida rugosa lipase	CRL	*Candida cylindracea* lipase (CCL)
Candida antarctica lipase	CAL	SP-435, lipase B, CAL-B
Pseudomanas capacea lipase	PSL	*Pseudomonas sp.* lipase, *Pseudomonas fluorescens* lipase (PFL), PCL, lipase P, lipase PS, Amano PS, KWI-56
Lipase M		
Porcine pancreas lipase	PPL	
Pig liver esterase	PLE	
Acetyl esterase	AE	
α-Chymotrypsin	α-CT	
Subtilisin		
Penicillin G acylase	PGA	
Adenosine deaminase	ADA	
Adenilic acid deaminase	AMPDA	
Penicillin amidase	PA	
Cytidine deaminase	CDA	
Papain		
Wheat germ lipase	WGL	
Aspergillus niger lipase	ANL	
Butyrylcholine esterase	BCE	
β-Galactosidase		
Cyclase (*Aplysia californica*)		
Nucleoside oxidase	FERM BP-2252	
FDP aldolase		
Phosphatase		
Phosphorylase		
CloneZyme ESL-001-02	ELS-001-02	

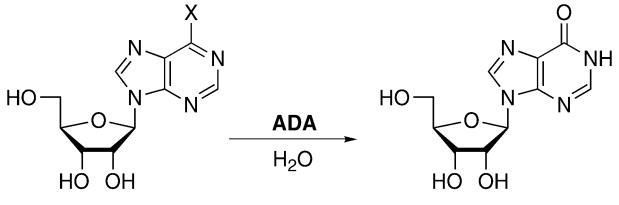
natural products, pharmaceuticals, and agrochemicals. These reviews and monographs do not usually focus their interest on the target molecule, but rather on the structure of the substrate. Moreover, although there are several review articles dealing with natural products such as carbohydrates [5] and steroids [6], little has been reported on biotransformations in the field of nucleosides, especially from the point of view of enzymatic reactions, except for a review article about enzyme protecting groups restricted to nucleosides containing the natural pentanofuranose moiety [7] and two recent reviews, one dealing with the enzymatic preparation of nucleoside antibiotics using microorganisms [8]; and the other with nucleoside synthesis mediated by glycosyl transferring enzymes [9].

The synthetic potential of enzymes related to nucleoside synthesis has been applied increasingly, especially since the introduction of organic solvent methodology. It is the aim of this report to cover the literature from the beginning of the 80s up to the end of 1999 with respect to chemoenzymatic transformations in conventional nucleosides. It is the intention of the authors to show a range of examples that cover nucleoside analogue syntheses through enzymatic procedures. Due to the vastness of the bibliographic material related to nucleosides, we will focus our attention especially on those processes which involve lipases, esterases, and proteases. Thus, the review will not cover carbocyclic nucleosides or C-nucleosides.

Most enzymatic reactions, just like those shown in this review, are mediated by a small number of biocatalysts. With the passing of time, their nomenclature has changed in an effort to unify criteria and refer to a given enzyme by only one name. Table 1 lists the enzymes mentioned in this review. These are cited as in the original papers to facilitate checking the original work, together with their corresponding modern denominations.

Enzymatic Hydrolysis on the Base Moiety

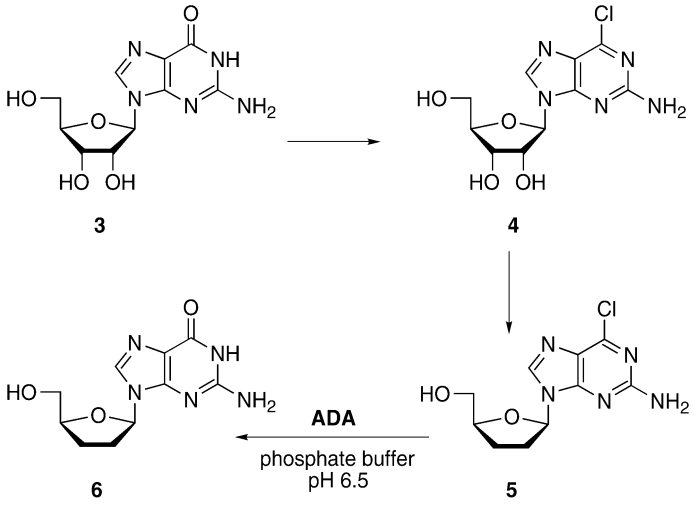
The first enzymatic hydrolysis of 6-subtituted purine riboses **(1)** was performed by *Wolfenden* [10] in 1966 in which adenosine deaminase (ADA) catalyzed the hydrolysis of 6-hydrazinopurine ribose, 6-chloropurine ribose, 6-methylaminopurine ribose, and 6-methoxypurine ribose at limiting velocities very similar to that for adenosine itself (Scheme 1). Each of these compounds, when incubated with the purified enzyme at *pH* 6.5, was completely converted to inosine (**2**). A common rate of hydrolysis for such a variety of leaving groups strongly suggests that the



1 **2**

$X = NH_2, NHNH_2, Cl, NHMe, OMe$

Scheme 1



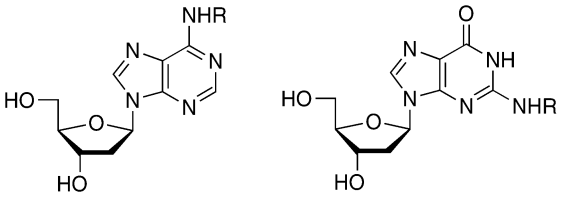
Scheme 2

same intermediate from which the original 6-substituent has been completely displaced is present in all cases. None of these reactions is detectably reversible at *pH* 6.5 or 9.

Due to its ability to inhibit the cytopathic effect of HIV, 2′,3′-dideoxyguanosine (**6**), synthesized from the readily available guanosin (**3**), is an important target molecule. Its synthesis [11] has been performed using commercially available mammalian ADA in the key step (Scheme 2). 2′,3′-Dideoxynucleoside **5** was obtained in 40% overall yield from **4** following the cyclic thionocarbonate methodology. An additional advantage of this approach is that it can be readily scaled up to gram quantities.

Despite the relatively weak reactivity of the exocyclic amino group of 2′-deoxyadenosine or 2′-deoxyguanosine under the conditions for nucleotide bond formation, its selective protection is an unavoidable step in oligonucleotide synthesis. Thus, *Galunsky et al*. [12] have reported that the phenylacetyl group can be successfully used as an enzyme cleavable aminoprotecting group of 2′-deoxyadenosine (**9**) and 2′-deoxyguanosine (**10**). These derivatives, **7** and **8**, were subjected to deprotection, catalyzed by free or immobilized penicillin amidase (PA), yielding adenosine and guanosine derivatives in 80% and 75% yield, respectively (Scheme 3). The data suggest that PA would be effective in multiple deprotection of oligonucleotides containing N-phenylacetylated purine nucleobases under mild conditions. Moreover, the acceptable deprotection times (15–150 minutes) support this conclusion.

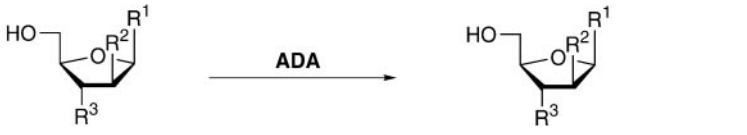
Adenosine deaminase (ADA) catalyzes the conversion of adenosine into inosine and is physiologically important in purine metabolism. Taking advantage of this natural process, ADA has been applied in the syntheses of guanosine and inosine nucleosides from the corresponding 2,6-diamino (**12**) or 6-aminopurine nucleosides (**11**), respectively [13] (Scheme 4). ADA specifically catalyses the oxidation of a particular amino group present in the base moiety of the nucleoside, leaving the amino group of a 2′-amino sugar moiety intact as *e.g*. in compound **14** [14].



7: $R = COCH_2Ph$ **8:** $R = COCH_2Ph$

9: $R = H$ (80%) **10:** $R = H$ (75%)

Scheme 3

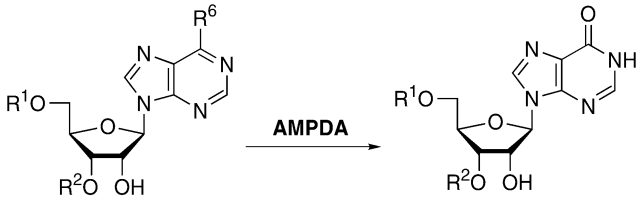


11: $R^1 = H$, $R^2 = B$, $R^3 = OH$ or CH_2OH **13:** $R^1 = H$, $R^2 = B^1$, $R^3 = OH$ or CH_2OH

12: $R^1 = B$, $R^2 = NH_2$, $R^3 = OH$ **14:** $R^1 = B^1$, $R^2 = NH_2$, $R^3 = OH$

B = 2-aminoadenine B^1 = adenine

Scheme 4



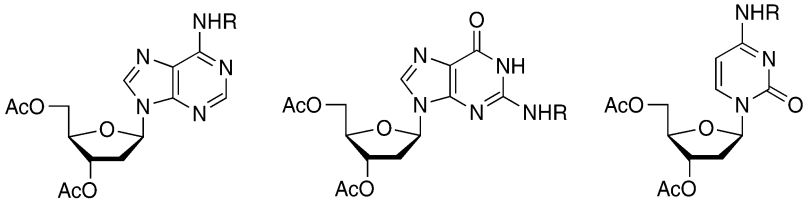
15 **16**

$R^1 = H, HPO_3$, $R^2 = H$, $R^1R^2 = HPO_2$, $R^6 = NH_2$, OMe, Cl

Scheme 5

Margolin et al. [15] have recently used adenylic acid deaminase (AMPDA) from *Aspergillus niger* in the synthesis of 6-oxopurine nucleoside in quantitative yields. This enzyme shows much broader substrate specificity and has been used for the deamination of several derivatives of adenosine **15**, including phosphorylated and cyclic analogues on a preparative scale, as well as for the dechlorination and demethylation of the purine ribosides to give the corresponding optically active 6-oxopurine derivatives **16** (Scheme 5).

In nucleotide chemistry the protection and deprotection of the amino has to be carried out under mild conditions and with a high degree of selectivity. An enzymatically removable protecting group such as phenylacetamide meets these requirements. Thus, *Waldmann et al.* [16] have shown that the phenylacetamides of 3′,5′-di-O-acetylated 2′-deoxyadenosine **17** and the respective guanosine derivative



17: R = $COCH_2Ph$ **18:** R = $COCH_2Ph$ **19:** R = $COCH_2Ph$

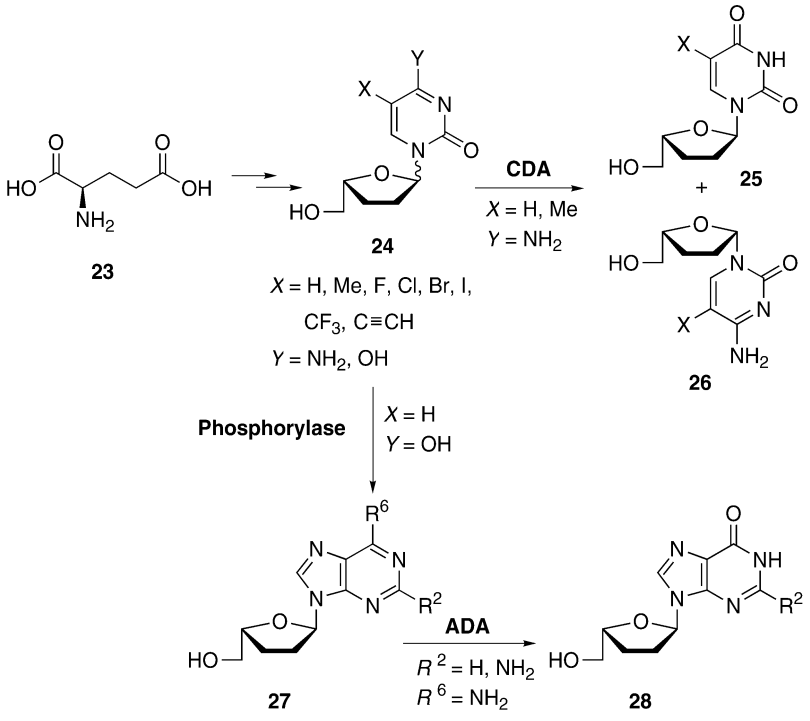
20: R = H (50%) **21:** R = H (69%) **22:** R = H (50%)

Scheme 6

18 can be prepared in a straightforward way in high yields by acylation of the corresponding amines with phenylacetic anhydride in pyridine. If they are treated with penicillin G acylase (PGA) at *pH* 7.8 in phosphate buffer, the phenylacetamido group is cleaved to deliver the selectively deprotected purine nucleobases **20** and **21** (Scheme 6). In addition, the respective protected cytidine derivative **19** is converted to the selectively deblocked pyrimidine nucleoside **22**. The conversion of the substrates is quantitative, but due to losses during work-up, the products are isolated in the yields shown in Scheme 6. The enzymatic attack on the phenylacetamides occurs with complete chemoselectitivity, leaving the more reactive esters in the carbohydrate part intact. Moreover, the reaction conditions are so mild that no undesired side reaction occurs. This new protecting group technique represents the first example for an enzymatic unmasking of amino functions in the nucleoside field. The same research team [17] has also applied this methodology to remove the phenylacetyl protecting group from oligonucleotides both in solution and on solid support.

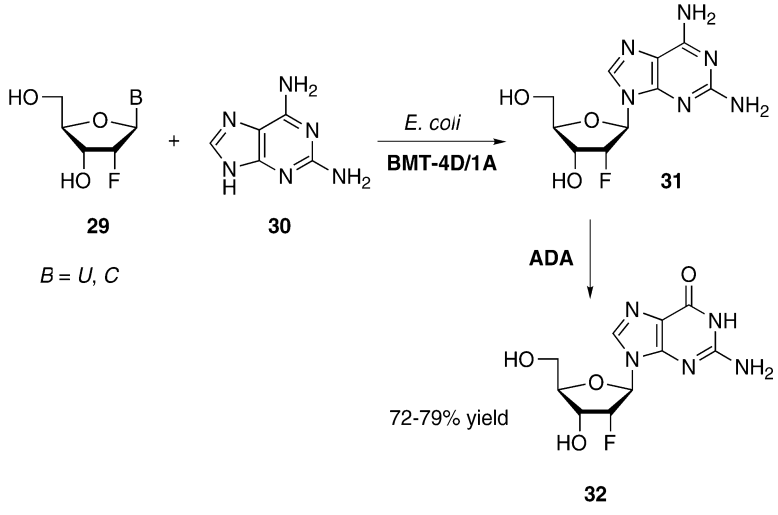
The search for novel nucleoside structures for use as antiviral agents has lead *Van Draanen* and *Koszalka* [18] to centre their interest in α-*L*-nucleosides because of their unnatural configuration at C-4′. Their work shows the preparation and evaluation of a series of novel purine and pyrimidine α-*L*-2′,3′-dideoxynucleosides. The synthesis of pyrimidine α-*L*-2′,3′-dideoxynucleosides was effected in a straightforward manner from *D*-glutamic acid (**23**) giving rise to the target nucleosides as α,β-anomeric mixtures (**24**). The α,β-anomeric mixtures **24** were separated either by preparative HPLC or, when the uracil and thymidine analogues were used, by enzymatic resolution as shown in Scheme 7. Thus, cytidine deaminase (CDA) deaminated the α-anomer **25** much more rapidly than the β-anomer **26**. By taking advantage of this enzymatic selectivity, pure α-*L*-2′,3′-dideoxyuridine and α-*L*-3′-dideoxythymidine were prepared. These compounds were easily separated from the unreacted β-cytidine analogues by flash chromatography. The purine dideoxynucleosides **27** were synthesized by a phosphorylase-catalyzed enzymatic *trans*-ribosylation from α-*L*-dideoxyuridine. Finally, the hypoxanthine and guanine analogues **28** were prepared by treatment of the corresponding adenine and diaminopurine analogues **27** with adenosine deaminase (ADA).

As a part of continuing interest in fluorodeoxy nucleosides and nucleotides, *Mikhailopulo et al*. [19] have reported on the enzymatic synthesis of 2′-deoxy-2′-fluoroguanosine (**32**) and its antiviral activity in chick embryo cells infected with



Scheme 7

Influenza Virus FPV/Rostock/34 (H7N1) and Herpes Simplex Virus (HSV) type 1 (1C strain). An enzymatic transglycosylation of 2,6-diaminopurine (**30**) using the glutaraldehyde-treated whole cells of *E. coli* BMT-4D/1A as a biocatalyst and 2′-deoxy-2′-fluorouridine or -cytidine **29** as a donor of the glycosyl moiety followed by an enzymatic deamination of the intermediate 2,6-diaminopurine glycoside **31** afforded the fluoro compound **32** in 72–79% combined yield (Scheme 8).

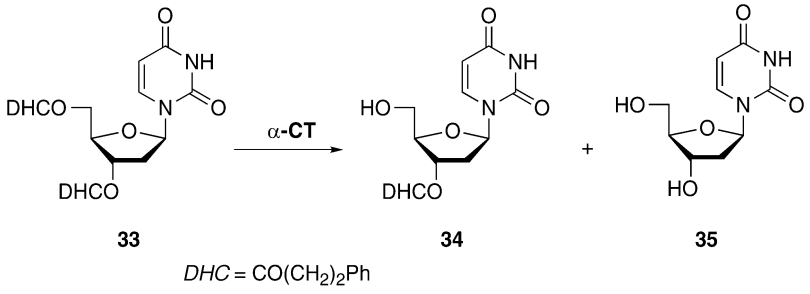


Scheme 8

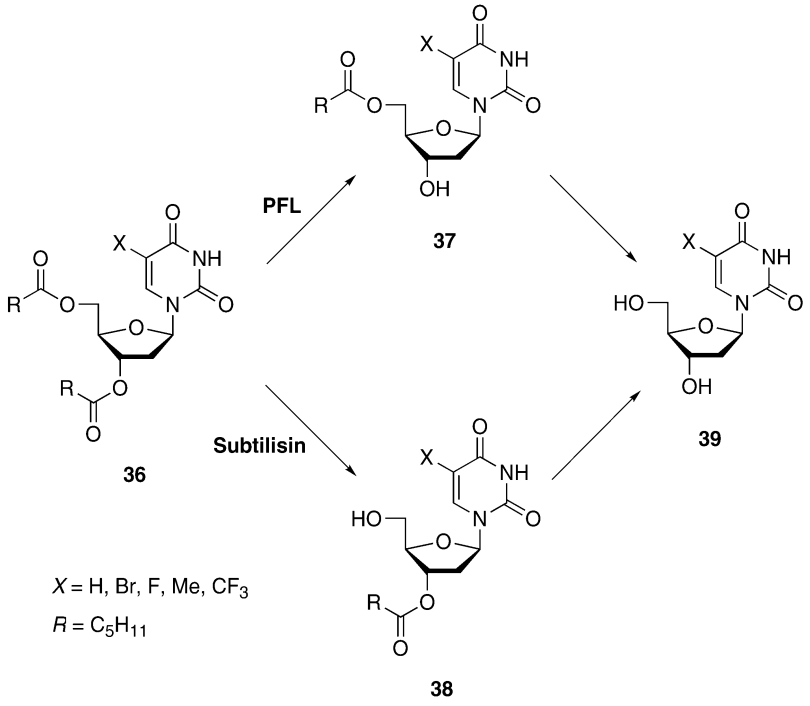
Enzymatic Hydrolysis on the Sugar Moiety

Enzyme-catalyzed deacylations have been applied as a method for the selective removal of one or more acyl groups from polyacylated nucleosides. Early studies [20] have focused on the use of the dihydrocinnamoyl (*DHC*) group as an enzyme-labile protecting group removable by α-chymotrysin (α-CT). Although the enzyme showed an interesting tendency to attack preferably the 5′-position of uracil derivative **33** giving rise to protected nucleoside **34** (Scheme 9), this approach has not been further exploited.

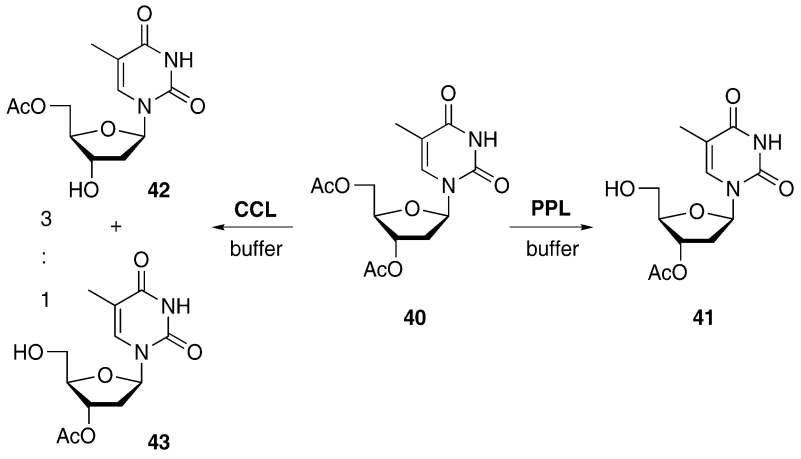
Pseudomonas fluorescens lipase (PFL) and the protease subtilisin have been used to effect the regioselective deacylation of several 2′-deoxy-5-substituted uracil nucleosides (**36**) in phosphate buffer with *DMF* as cosolvent [21]. Thus, PFL preferably removes the acyl group on the secondary hydroxyl function leading to



Scheme 9



Scheme 10

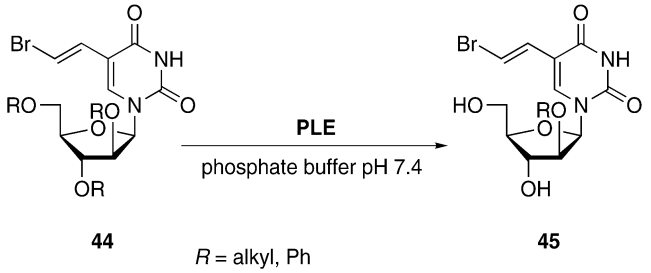


Scheme 11

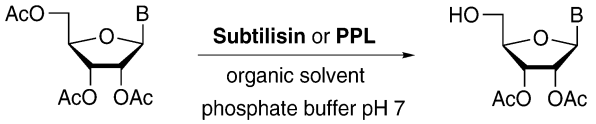
the 5′-esters **37** in reasonably good yields. In contrast, subtilisin shows preference for 5′-acyl group cleavage, rendering the 3′-esters **38** (Scheme 10). With both enzymes, the completely deprotected nucleosides **39** have also been obtained.

Complementary results were obtained depending on the enzyme used [22]. Thus, when a lipase from porcine pancreas (PPL) in phosphate buffer was the catalyst for deacylation of 3′,5′-di-O-acetylthymidine (**40**), the 5′-O-acetyl group was selectively attacked leading to 3′-O-acetylthymidine (**41**) in very good yield (98%). In contrast, if *Candida cylindracea* lipase (CCL) was used in the catalysis, the 3′-ester (giving compound **42**) was hydrolyzed three times faster than the 5′-ester (giving compound **43**) (Scheme 11).

The regioselective hydrolysis of tri-O-acylated esters **44** has been carried out with pig liver esterase (PLE) in phosphate buffer with ethanol as cosolvent leading to 2′-O-monoacylated nucleosides **45** in high yield (Scheme 12) [23]. The markedly retarded rates of hydrolysis of the 2′-O-acyl esters of these arabinonucleosides suggest that they might function as slow-release lipophilic prodrugs with long serum lifetimes. However, they have greater aqueous solubility than the triesters and more stable pharmacokinetic properties than the fully deprotected arabinonucleosides. Similarly, it has been reported [24] that subtilisin acts on 9-(2′,3′,5′-tri-O-acyl-β-*D*-arabinofuranosyl)-adenosine affording the 3′,5′-diol derivative.



Scheme 12



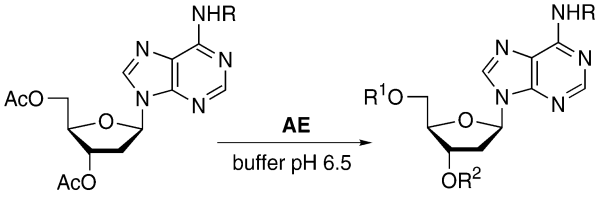
46 **47**

B = *U*, *C*, *A*, *G*, N-2-AcG, hypoxanthin-9-yl

Scheme 13

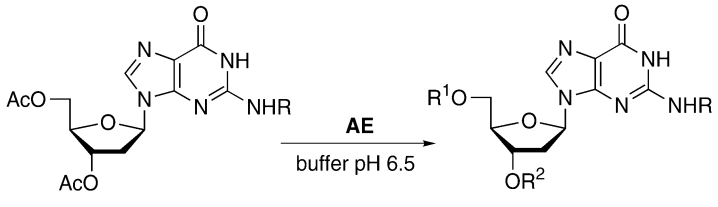
Subtilisin in phosphate buffer, with or without organic cosolvents (*DMF* or dioxane) selectively hydrolyzed the 5′-position of purine and pyrimidine tri-O-acylated esters **46** to give 2′,3′-di-O-acylribonucleosides **47** in 40–92% yield (Scheme 13) [25]. The least expensive crude lipase from porcine pancreas (PPL) also catalyzed the deacetylation, but resulted in poorer selectivity and a slower reaction rate.

The enzyme acetyl esterase (AE) from the flavedo of oranges, chemo- and regioselectively removes acetyl groups from purine nucleoside derivatives **17**, **18**, **20**, and **21** [16]. Thus, the adenosine- and guanosine derivatives **20** and **21**, which do not carry N-protecting groups in the nucleobases, are deprotected at the secondary 3′-OH to give nucleoside derivatives **49** and **51**, respectively (Scheme 14). However, if the amino groups present in the purine bases are masked as phenylacetamides, as with compounds **17** and **18**, the regioselectivity displayed by the enzyme is reversed, and the primary 5′-OH groups of the nucleosides are liberated to give base-protected nucleosides **48** and **50**, respectively.



17: R = $COCH_2Ph$
20: R = H

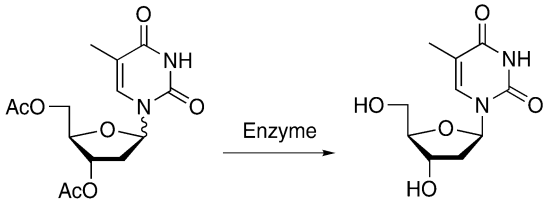
48: R^1 = H, R^2 = Ac
49: R^1 = Ac, R^2 = H



18: R = $COCH_2Ph$
21: R = H

50: R^1 = H, R^2 = Ac
51: R^1 = Ac, R^2 = H

Scheme 14



52 **53**

WGL	29% yield (pure β)
PLE	31% yield (pure β)

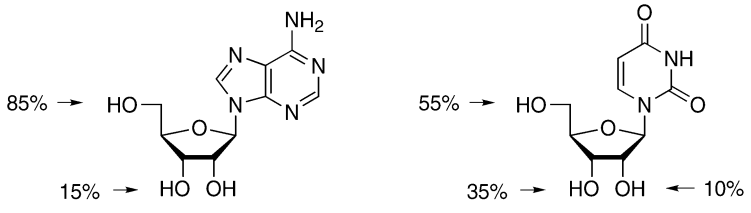
Scheme 15

Wengel et al. [26] have reported the first attempt to use biotransformations to solve the basic problem of anomer separation in nucleoside chemistry when a convergent strategy is used. Thus, lipase-catalysed deacylations of an anomeric mixture of peracetylated 2′-deoxyribofuranosyl thymine (**52**) are shown in Scheme 15. Generally, the diastereoselectivity was more pronounced in pure phosphate buffer than in phosphate buffer containing 10% of *DMF*. Wheat germ lipase (WGL) and porcine liver esterase (PLE) catalyzed diastereoselective the deacetylation of **52** affording the pure β-anomer thymidine (**53**β) as the only completely deprotected nucleoside product.

Enzymatic Protection of Nucleosides by Acylations

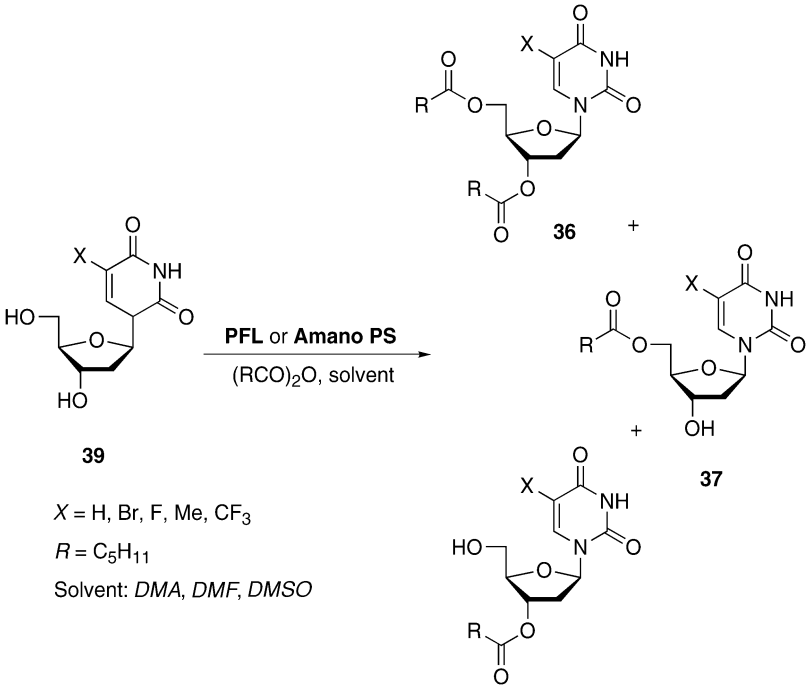
Selective modification of nucleosides with several functional groups of very similar chemical reactivities is an interesting subject of study and a fundamental challenge to organic chemists. Thus, *Klibanov et al.* [27] have studied the catalytic activity of the protease subtilisin, using an activated ester like trichloroethyl butyrate to avoid the reversibility of the process, in the regioselective esterification of adenosine (**54**) and uridine (**55**) in anhydrous *DMF*. The regioselectivity of the enzymatic transesterification is not that good, but the hydroxyl group at the 5′-position of nucleosides **54** and **55** was preferentially acylated (Scheme 16). An important fact was that the purity of subtilisin is not essential for either the yield or the regioselectivity of the enzymatic acylation.

Acylation of 2′-deoxy-5-substituted uridine nucleosides **39** with acid anhydrides in the presence of PFL in organic solvents [28] showed only moderate



54 **55**

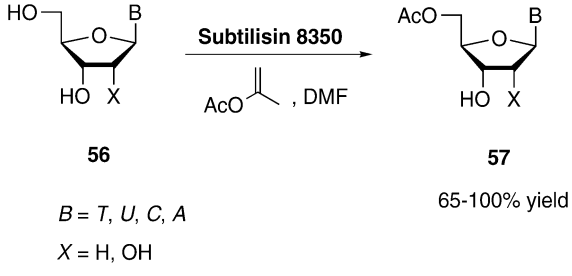
Scheme 16



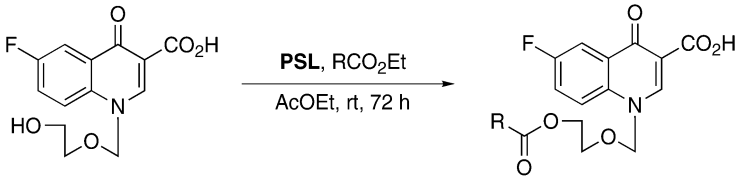
Scheme 17

selectivity towards the 5′-hydroxyl group, giving rise to derivatives **37** in addition to diprotected compound **36**. When Amano PS was used instead [29], the 3′-hydroxyl function was acylated almost exclusively yielding nucleoside derivative **38** (Scheme 17).

Highly regioselective acetyl transfer reactions to the carbohydrate moiety of nucleosides have been carried out by employing a subtilisin mutant [30] (subtilisin 8350, or even the much more stable subtilisin 8397 [31]) obtained *via* site-specific mutations in anhydrous *DMF*. This mutant enzyme transfers the acetyl group from isopropenyl acetate to the primary hydroxyl groups of various ribonucleosides and 2′-deoxyribonucleosides **56** giving the 5′-O-acylated derivatives **57** in high yields (Scheme 18). The high regioselectivity of the mutant enzyme was attributed to the fact that it binds the reaction transition state more strongly than does the parent enzyme.



Scheme 18



58 **59**

60-87% yield

R = Me, Et, ^{n}Pr, ^{s}Bu, CH_2=CH, MeCH=CH, MeCHCl

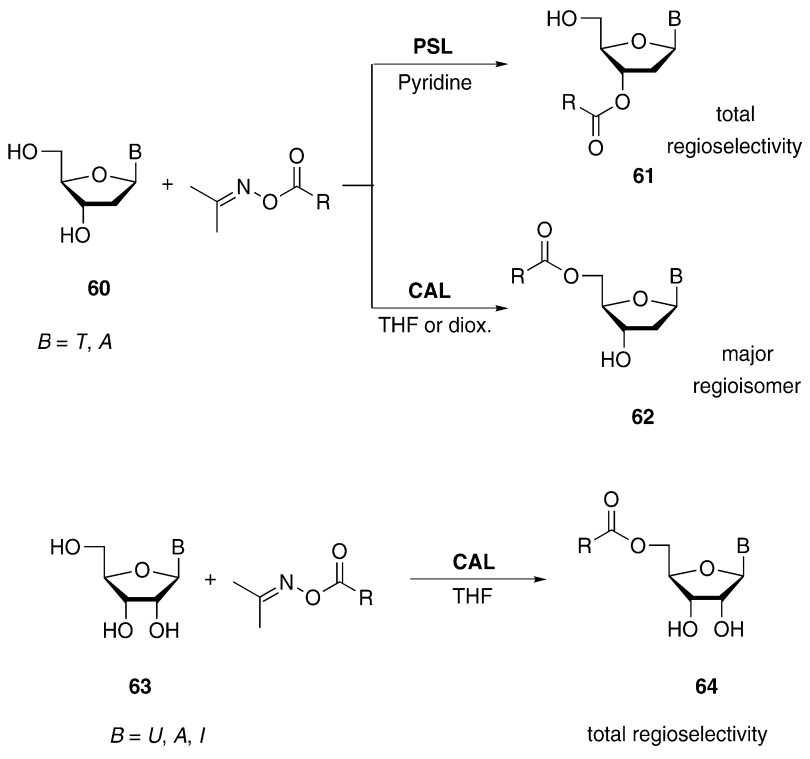
Scheme 19

The reactivity and selectivity of subtilisin was later increased by changing the acylating agent and the solvent from trichloroethyl butyrate in *DMF* to trifluoroethyl butyrate in anhydrous pyridine, thus affording 5′-O-acylated derivatives **57** (uridine: N-4-anisoylcytidine adenosine N-6-benzoyladenosine) in 67–82% yield [32].

As a part of a program to design new regioselective enzymatic transformations of nucleosides, *Gotor et al.* have started with the lipase-mediated acylation of acyclonucleosides (Scheme 19) [33]. Thus, acyclonucleosides **59** bearing different substituents in the 2-hydroxyethoxymethyl chain were prepared in almost quantitative yields using *Pseudomonas cepacea* lipase (PSL) and several ester derivatives through a transesterification reaction from precursors **58**. This was the first example of the use of enzymes in acyclonucleoside chemistry that allowed a facile preparation of prodrugs **59** of 4-quinolone acyclonucleosides which were tested as antiviral agents against a wide variety of assay systems [34].

In addition, the same authors have used oxime esters as irreversible acyl transfer agents in the regioselective acylation of 2′-deoxynucleosides **60** on the secondary hydroxyl group (Scheme 20) instead of the chemically more reactive primary alcohol using PSL in pyridine [35]. This procedure is very versatile because acylation with various oxime esters bearing saturated or unsaturated chains, yields exclusively the 3′-O-acyl derivatives **61**. However, N-acylation of 2′-deoxyadenosine is not observed. Similarly, *Candida antarctica* lipase (CAL) showed high regioselectivity toward the primary hydroxyl group of both deoxy- (**60**) and ribonucleosides (**63**) [36]. 2′-Deoxynucleosides such as thymidine and 2′-deoxyadenosine, (**60**) were acylated with oxime esters carrying saturated, unsaturated, aromatic, and functionalized chains, giving 5′-O-acylated compounds (**62**) together with small quantities of the 3′-O-acylated regioisomer **61**. Uridine, adenosine, and inosine as representative ribonucleosides (**63**) were acylated exclusively at the 5′-hydroxyl group (**64**) by using the same methodology. Furthermore, these authors have studied in depth several factors that affect this kind of enzymatic process, such as the alcohol inhibition and specificity of CAL in organic solvents [37], as well as solvent effects on equilibrium and individual rate constants [38].

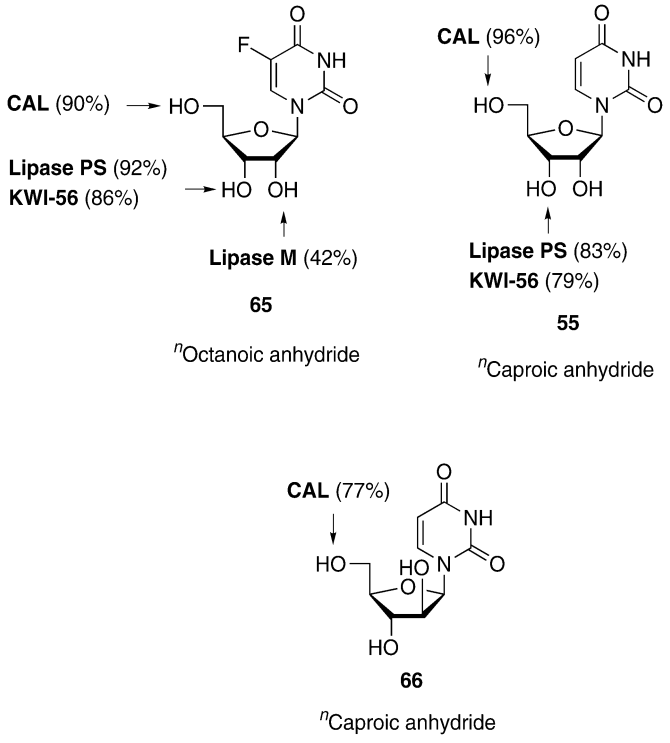
To decrease toxicities and improve activities, chemical modifications of nucleosides, including protection, have been studied. Thus, *Ozaki et al.* [39] have used enzymatic transesterification reactions to prepare acyl-protected derivatives of



Scheme 20

5-fluorouridine (**65,** Scheme 21). They used anhydrides (*n*-octanoic anhydride showed the highest selectivity) and lipase PS (a lipase from *Pseudomonas sp*., Amano) or lipase KWI-56 (a lipase from *Pseudomonas sp*., Kurita water industries Ltd.) to almost exclusively acylate the 3′-position in this nucleoside derivative **65** (isolated yields in parentheses). Additionally, lipase M (from *Mucor javanicus*, Amano) acylated the 2′-hydroxyl group. However, CAL acylated the 5′-OH to give the corresponding derivative in excellent yield. Although several solvents were checked, *THF* was found to be suited best for this reaction. Furthermore, selective protection of uridine **55** and arabinosyluracil **66** was also investigated. In general, the acylation of these compounds proceeded faster than the acylation of **65** under the same conditions with *n*-caproic anhydride. As shown for the fluoroderivative **65**, the 3′-OH of uridine was acylated by lipase PS and KWI-56, and the 5′-OH was acylated by CAL. The 5′-OH of arabinosyluracil was selectively protected by CAL.

2′,3′-Dideoxynucleosides have been introduced as chemotherapeutic agents with anti-retroviral activity against HIV. Among these, 2′,3′-dideoxyinosine (DDI, **74**) has been approved for alternative treatment to AZT. *Santaniello et al.* [40] have described a chemoenzymatic synthesis of DDI based on enzymatic acylation studies of inosine and 2′-deoxyinosine. Thus, the selective acylation of the hydroxyl groups of the nucleosides inosine and 2′-deoxyinosine **67** has been achieved in presence of CAL in organic solvents (Scheme 22). Also, the enzymatic

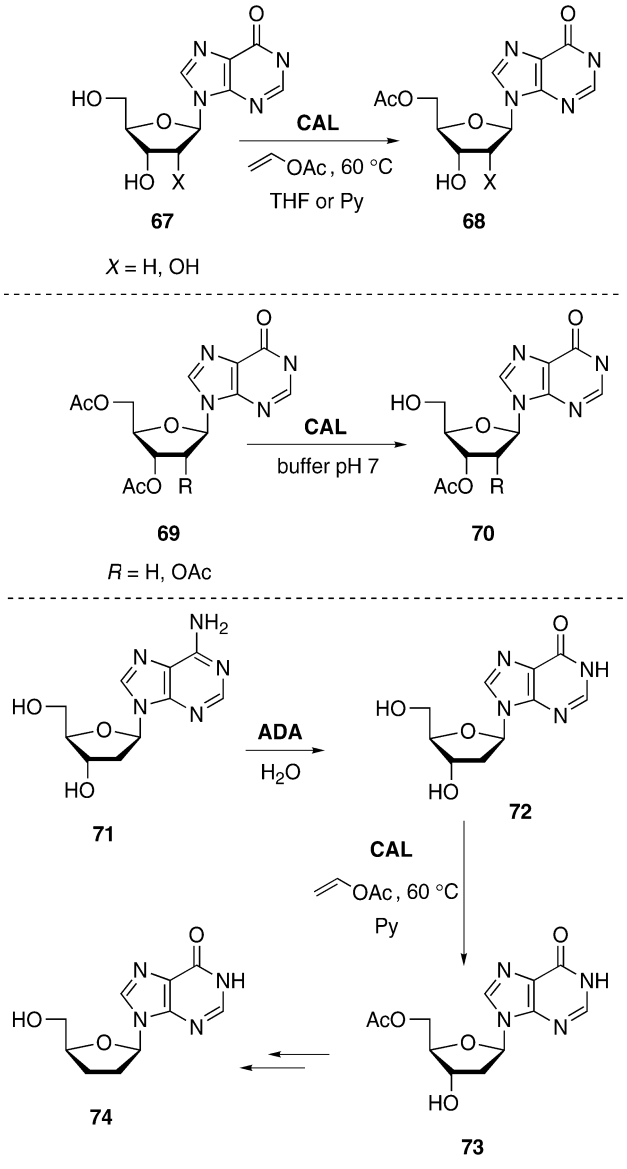


Scheme 21

hydrolysis of the triacetate and diacetate **69** in presence of CAL in aqueous buffer at $pH = 7$ or in water-saturated chloroform was performed and was found to be complementary to the lipase-catalysed acylation in organic solvents. With these results in hand, they developped a new chemoenzymatic synthesis of 2′,3′-dideoxyinosine **74** starting from the 5′-acetate **73** enzymatically prepared by the transacetylation procedure. Starting material **72** which is relatively expensive, was prepared from readily available 2′-deoxyadenosine (**71**) by a deamination reaction efficiently catalyzed by adenosine deaminase (ADA). The overall sequence for the chemoenzymatic synthesis of DDI is depicted in Scheme 22.

Enzymatic Protection of Nucleosides by Alkoxycarbonylations

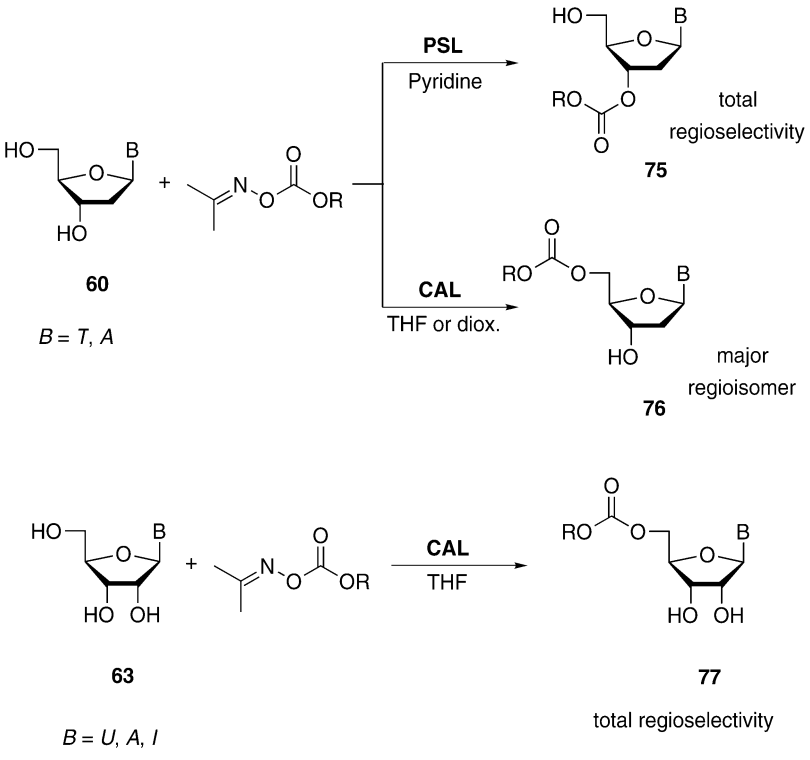
An important and synthetically relevant transformation in nucleoside chemistry is the selective alkoxycarbonylation of the sugar moiety in order to obtain nucleoside carbonates, which play an important role in the synthesis of oligonucleotides, and other derivatives assayed in medicine, such as dinucleoside carbonates. Scheme 23 summarizes a general, new, and simple procedure for the synthesis of pyrimidine and purine 3′-carbonates **75** from 2′-deoxynucleosides **60** using PSL and O-alkoxycarbonyl oxime [41]. In this method, no previous protection of the primary hydroxyl group is necessary, as has been traditionally described for the preparation of these compounds. 5′-O-Carbonates **76** and **77** of ribonucleosides and 2′-deoxyribonucleosides could be obtained by enzymatic alkoxycarbonylation with



Scheme 22

CAL and oxime carbonates [42], the latter being easily prepared from chloroformates. Ribonucleosides **63** yield two kinds of 5′-O-carbonates **77**, depending on whether the alkoxy or the acetone oxime moiety acted as the leaving group. In the case of 2′-deoxynucleosides, the leaving group was always the acetonoxime moiety, giving rise to the regioselective formation of the corresponding 5′-O-alkyl carbonates together with small amounts of the 3′-O-regioisomer and diacylated compounds.

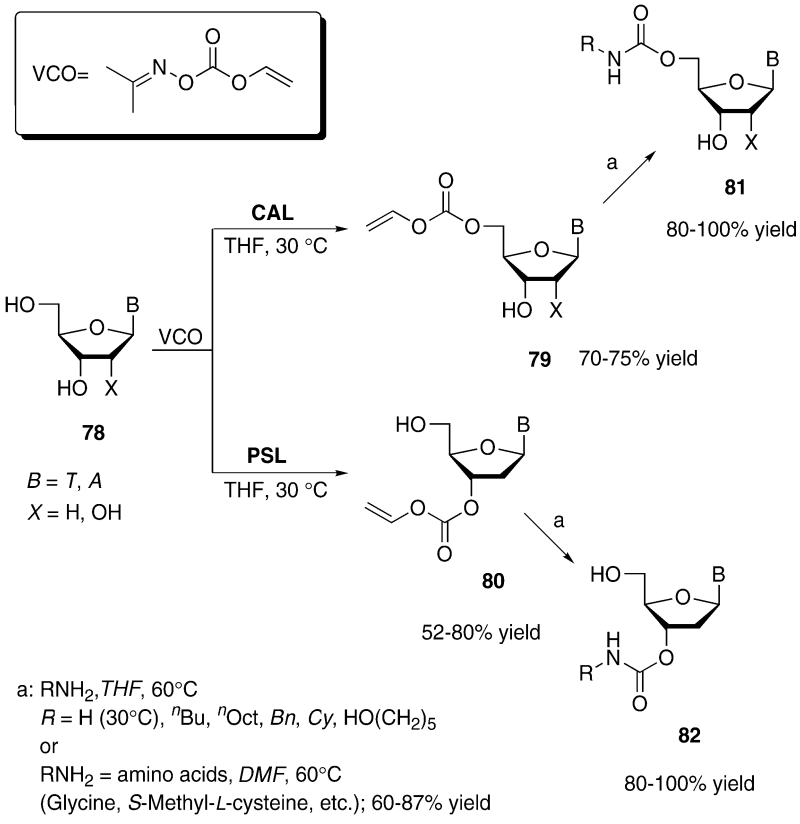
One of the most widespread groups in chemotherapy is the carbamate moiety which occurs, among others, in several classes of antitumorous products, and is also used for the increase of permeation through biological membranes. Bearing



Scheme 23

this in mind, and taking into account the interesting features shown by the carbamate moiety in the nucleoside field, such as phosphate simulation ability and chemical and enzymatic stability, *Gotor et al.* were interested in proposing a pathway which would allow them to synthesize these nucleoside carbamates through a simple, generic procedure. Testing of various lipases revealed them to be unable to catalyze the reaction between vinyl carbamates and nucleosides. One feasible explanation might be that some carbamates have been shown to be good inhibitors of many serine hydrolases. Taking this into account, it was thought appropriate to plan the synthesis in a two-step procedure (Scheme 24) [43]. The key step is the enzymatic synthesis of 5′-O-vinyloxycarbonylnucleosides **79** or 3′-O-vinyloxycarbonylnucleosides **80** in a regioselective way using CAL or PSL catalysis, respectively. In a second aminolysis step these carbonates yield the corresponding urethanes **81** and **82**. This methodology allows the assembly of ammonia, amines, amino alcohols, and *L*-amino acids to 2′-deoxynucleosides (3′- or 5′-positions) or ribonucleosides (5′-position) [44].

In view of the previous results, it is believed that nucleosides with different orientations of the hydroxyl groups (**83**–**88**) could be an interesting subject of study with respect to the preference of some lipases according to the geometry of the substrate employed [45]. Thus, 5′-O-acyl and 5′-O-alkoxycarbonyl derivatives of α-, anhydro-, xylo-, and arabino-nucleosides are obtained through a lipase-

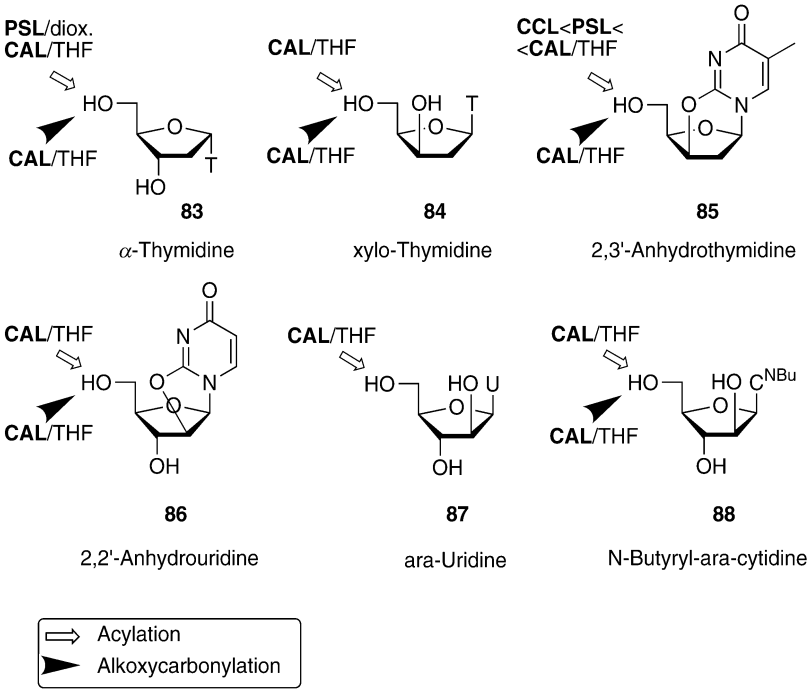


Scheme 24

mediated reaction with CAL by using acetoxime butyrate or butyric anhydride, together with benzyloxycarbonyl-O-acetoxime as acylating or alkoxycarbonylating agents (Scheme 25).

For the synthesis of the highly hindered 3′-O-acetyl α-thymidine **91** and xylo-thymidine **94** [46], the same authors prepared the 5′-*Cbz*-derivatives **89** and **92** as previously described, and then chemically carried out an acylation reaction in the 3′-OH free hydroxyl group giving rise to fully protected nucleosides **90** and **93**, since benzyloxycarbonyl group could be easily removed (Scheme 26).

Aminosugar nucleosides are known to possess strong antibacterial, anticancer, and biosynthetic inhibitory properties. Therefore, considerable effort has been devoted to the preparation of this kind of compounds. Most of the procedures depend strongly on the nature of the nitrogenated base present in the starting nucleoside. Schemes 27 and 28 show novel and general chemoenzymatic procedures to obtain the 3′-amino-xylo-nucleosides [47] in both 2′-deoxynucleosides and ribonucleosides. The synthetic scheme is based on the 5′-directed intramolecular nucleophilic substitution at the 3′-activated position of the nucleoside. The approach of the incoming group to this position takes place both regio- and stereoselectively from the most hindered face of the nucleoside **96** or **101/104**. The methodology presented is applicable to 2′-deoxyribonucleosides (Scheme 27) and ribonucleosides (Scheme 28), regardless of their nitrogenated base.

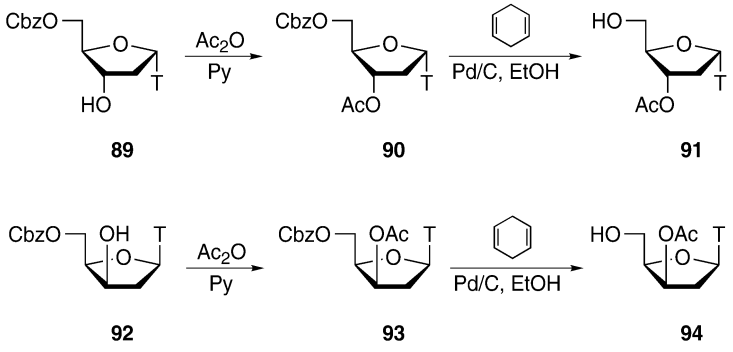


83 α-Thymidine
84 xylo-Thymidine
85 2,3'-Anhydrothymidine
86 2,2'-Anhydrouridine
87 ara-Uridine
88 N-Butyryl-ara-cytidine

Scheme 25

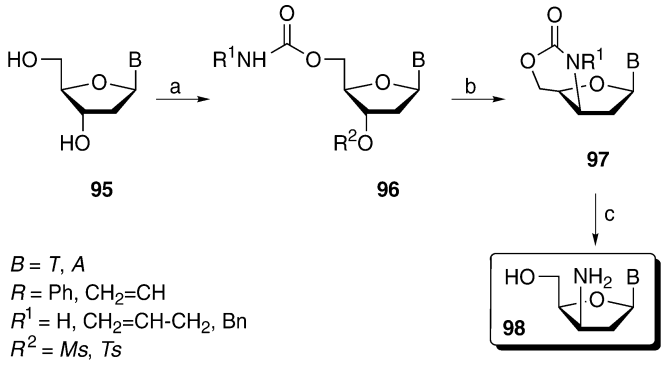
A chemoenzymatic procedure for the synthesis of 3′- and 5′-carbazoyl nucleoside derivatives prepared for the first time is shown in Scheme 29 [48]. This process involves the regioselective enzymatic alkoxycarbonylation of nucleosides **106** and the subsequent transformation with hydrazine into novel carbazoyl nucleoside derivatives **107** or **108**. Taking into account previously reported data, 3′-alkylidencarbazoyl-2′-deoxynucleosides (**110**) as well as 5′-alkylidencarbazoyl-2′-deoxynucleosides and 5′-alkylidencarbazoyl ribonucleosides (**109**) emerge as interesting targets combining structural features found in both therapeutic nucleoside derivatives and fungicide/herbicide nucleoside analogues.

Modulation of gene expression by antisense technologies requires the development of modified oligonucleotides possessing enhanced cellular uptake,



89 90 91
92 93 94

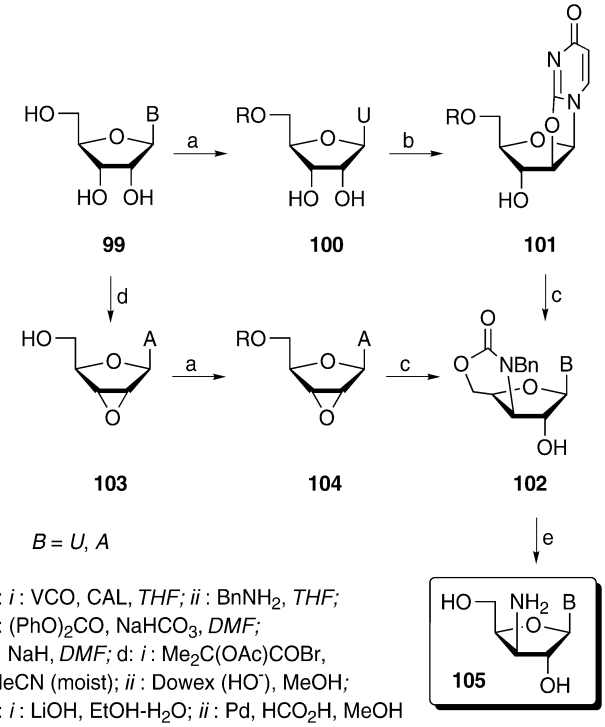
Scheme 26



a: *i* : $ROCO_2N{=}CMe_2$, CAL, *THF*; *ii* : R^1NH_2, *THF*; *iii* : R^2 Cl, *Py;*
b: NaH, *THF;* c: *i* : LiOH, EtOH-H_2O; *ii* : Pd, HCO_2H, MeOH

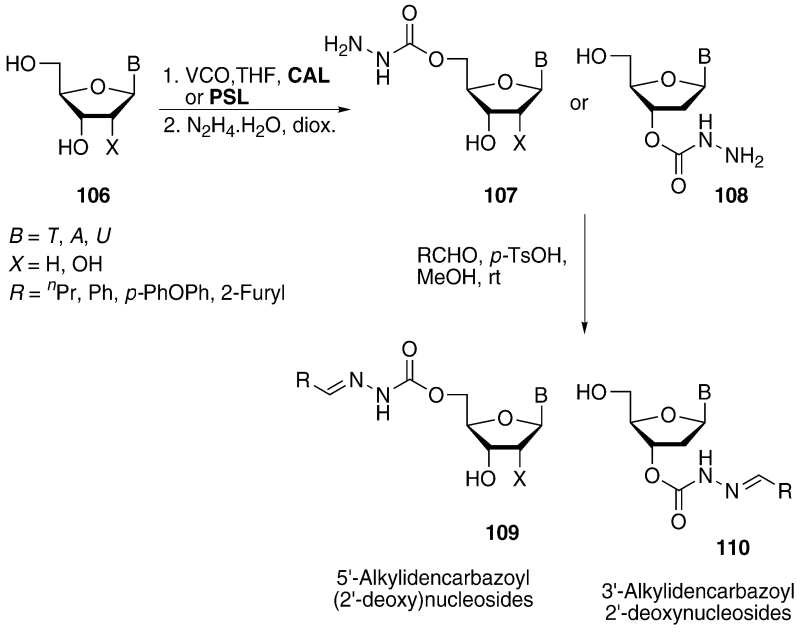
Scheme 27

resistance toward degradation by nucleases, and appropriate hybridization to target natural oligonucleotides. Consequently, these modified oligonucleotides are now being actively investigated as a new generation of pharmaceuticals. In the last decade, a great deal of effort has been directed toward the synthesis of analogues with an altered phosphodiester linkage, and one of the most important modifications is the complete substitution of the phosphate internucleoside bridge. Thus, several properties of the natural oligonucleotides have been improved for the



a: *i* : VCO, CAL, *THF; ii* : $BnNH_2$, *THF;*
b: $(PhO)_2CO$, $NaHCO_3$, *DMF;*
c: NaH, *DMF;* d: *i* : $Me_2C(OAc)COBr$,
MeCN (moist); *ii* : Dowex (HO^-), MeOH*;*
e: *i* : LiOH, EtOH-H_2O; *ii* : Pd, HCO_2H, MeOH

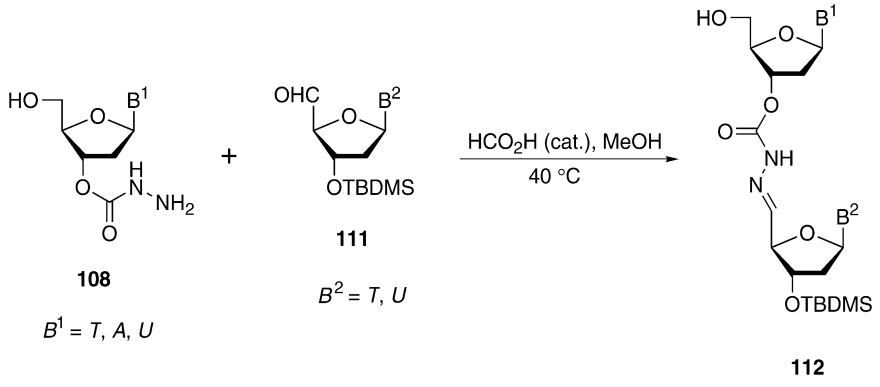
Scheme 28



Scheme 29

potential therapeutic application of the antisense strategy. Up to date there has been no investigation of backbone linkages that contain carbazoyl groups, and yet this type of linkage appears to have properties that make it an attractive surrogate for the phosphodiester linkage, being non-ionic, hydrolytically stable, and non-chiral.

The synthesis of backbone modified dinucleotide analogues **112** is shown in Scheme 30 [49], in which the natural phosphodiester linkage is replaced by a 3′–5′ carbazoyl linkage. The bridge was formed through a coupling reaction between an appropriate 3′-carbazoyl nucleoside analogue **108** and an aldehyde nucleoside derivative **111**. It is noteworthy that the starting nucleosides could be common materials to obtain the 3′-carbazoyl nucleoside derivatives by means of a simple previously described chemoenzymatic procedure, the aldehyde nucleoside could be obtained by an oxidation reaction.



Scheme 30

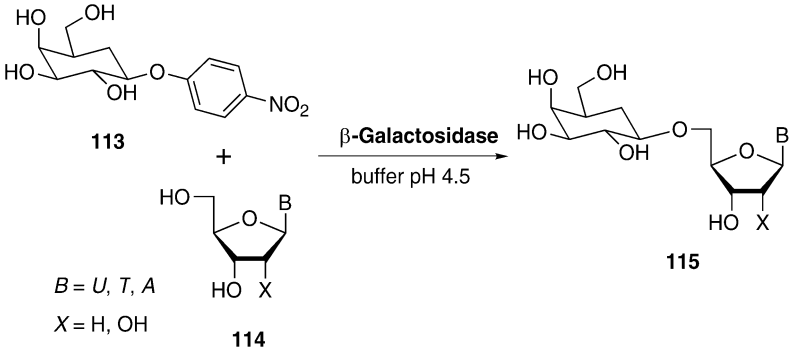
Gotor et al. have studied these lipase-catalyzed alkoxycarbonylation processes in depth from the point of view of the influence of the carbonate on the regioselectivity [50] in order to understand the behaviour of the enzymes.

Enzymatic Protections by β-Galactosidase

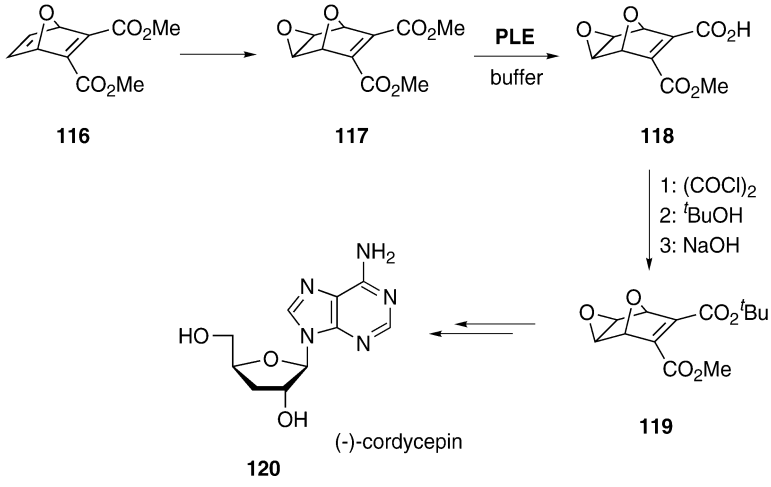
The synthesis of various galactose containing disaccharide nucleosides **115** has been achieved by utilizing the transglycosilation potential of β-galactosidase from *Aspergillus oryzae* [51] (Scheme 31). Thus, using *p*-nitrophenyl-β-*D*-galactoside **113** as galactosyl donor, 2-deoxyuridine, uridine, thymidine, and adenosine have proven to be useful acceptors for enzyme-catalyzed disaccharide nucleoside formation. The regiochemistry of the products **115** showed that only 5′-protected derivatives were formed.

Nucleoside Enzymatic Resolutions

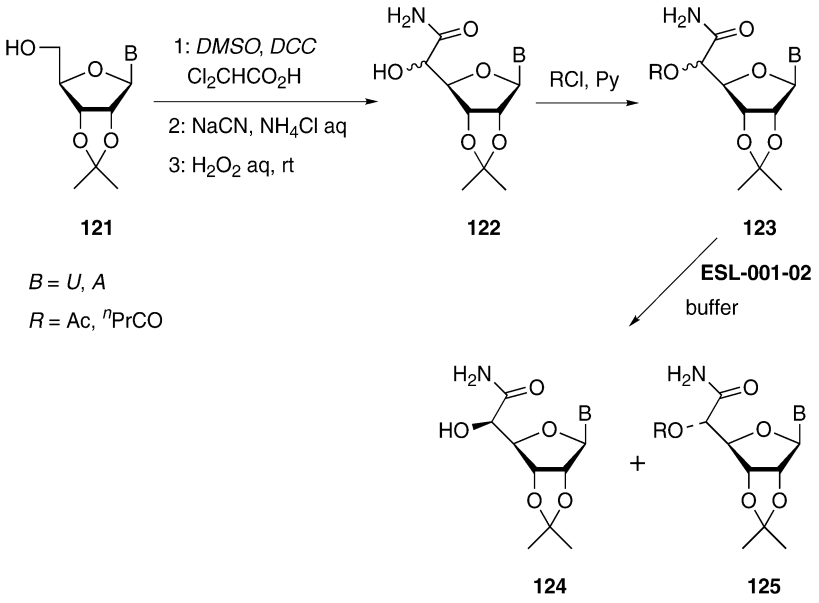
Ohno et al. [52] have reported an efficient synthesis of the nucleoside (–)-cordycepin (**120**) in an enantioselective and stereocontrolled manner by chemo-



Scheme 31



Scheme 32



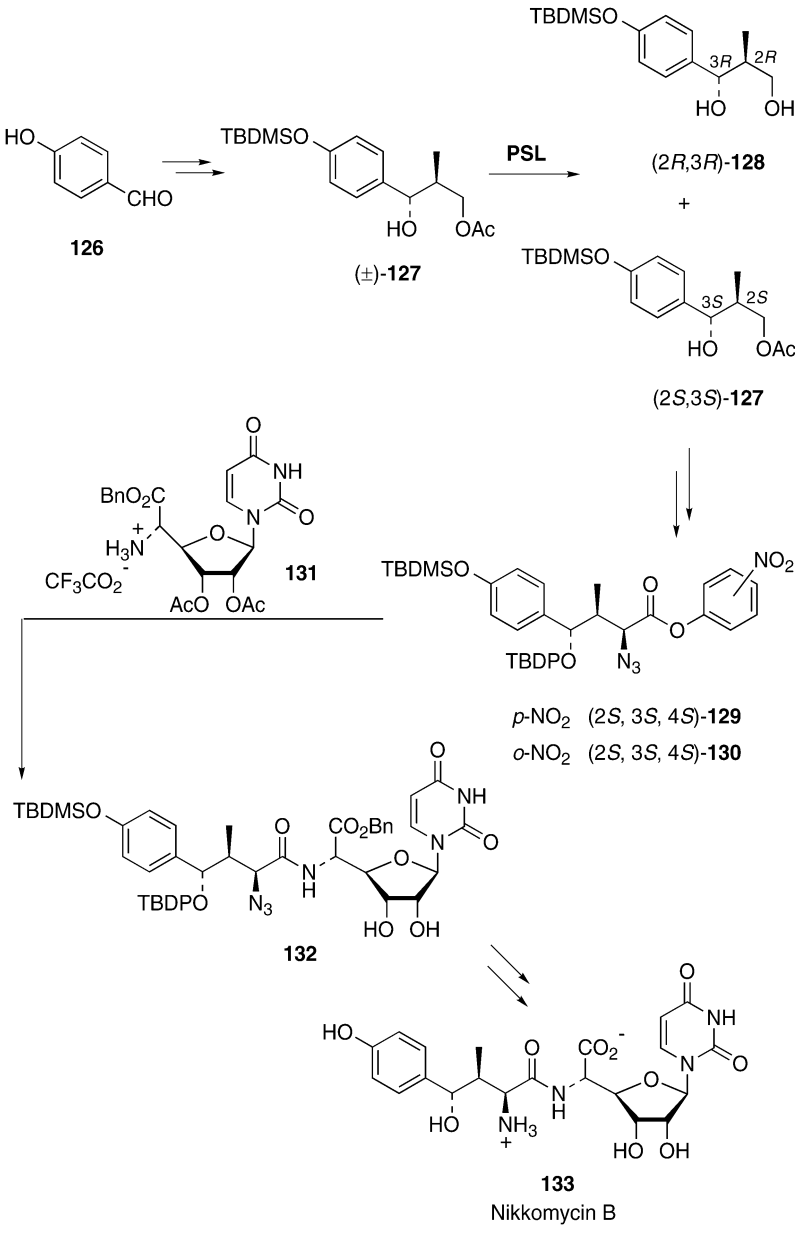
Scheme 33

enzymatic strategy, starting from the *Diels-Alder* adduct of furan and dimethyl acetylenedicarboxylate **116** (Scheme 32). The symmetric unsaturated dimethyl ester **117** was almost quantitatively hydrolyzed with pig liver esterase (PLE) to yield half-ester **118** in reasonably high optical yield. After the enantiomer conversion (from **118** to **119**), (–)-codycepin (**120**) was obtained from **119**. This is one of the first synthetic approaches to the sugar moiety of nucleosides starting from non-carbohydrate synthons.

Wang et al. [53] have reported a one pot preparation of a mixture of α-*L*-taluronamide **124** and β-*D*-alluronamide **125** nucleoside derivatives (Scheme 33). Such derivatives can be considered as intermediates to uracil polyoxin C, capuramycin, and other nucleoside antibiotics. Nucleoside derivatives **124** and **125** were prepared *via* a one-pot reaction sequence of oxidation, nucleophilic addition, and hydrolysis of **121** to give hydroxyamide **122**. The resulting diastereoisomers, which are difficult to separate by conventional chromatography, were resolved by stereoselective deacylation of their 5′-O-acylated derivatives **123** with thermophilic enzymes (from esterase/lipase CloneZyme (ESL-001) library). Themophilic enzyme ESL-001-02 exhibited good selectivity in the hydrolysis of (*R*)-C5′ substrates.

Enzymatic elaboration of the side chain in nikkomycin B

A stereoselective synthesis of the versatile chiral synthon (2*S*,3*S*)-**127** possessing two stereogenic centres was achieved using the chemoenzymatic method [54], starting from *p*-hydroxybenzaldehyde (**126**). The enzymatic resolution of the racemic mixture of intermediate **127** using lipase Amano PS gave enantiomerically pure substrate (2*S*,3*S*)-**127** (Scheme 34). The conversion of the latter into the homochiral intermediates (2*S*,3*S*,4*S*)-**129** and (2*S*,3*S*,4*S*)-**130**, corresponding to the N-terminal amino acid moiety of nikkomycin B, and their reaction with **131**, the

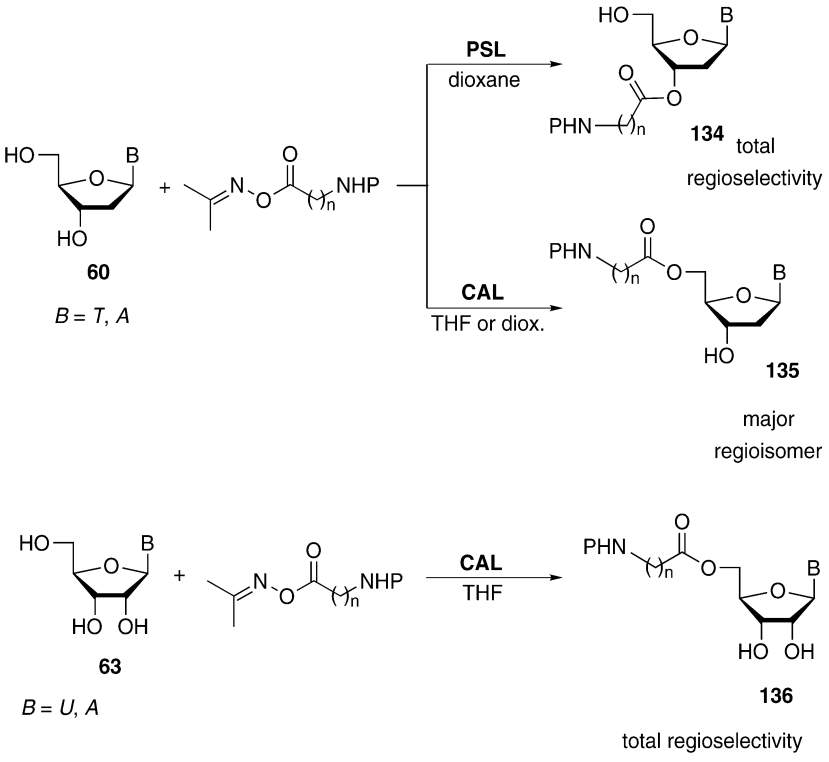


Scheme 34

corresponding part of the C-terminal nucleoside amino acid, afforded the important intermediate **132** for the synthesis of nikkomiycin B (**133**).

Enzymatic Protecting Strategies in Nucleopeptides

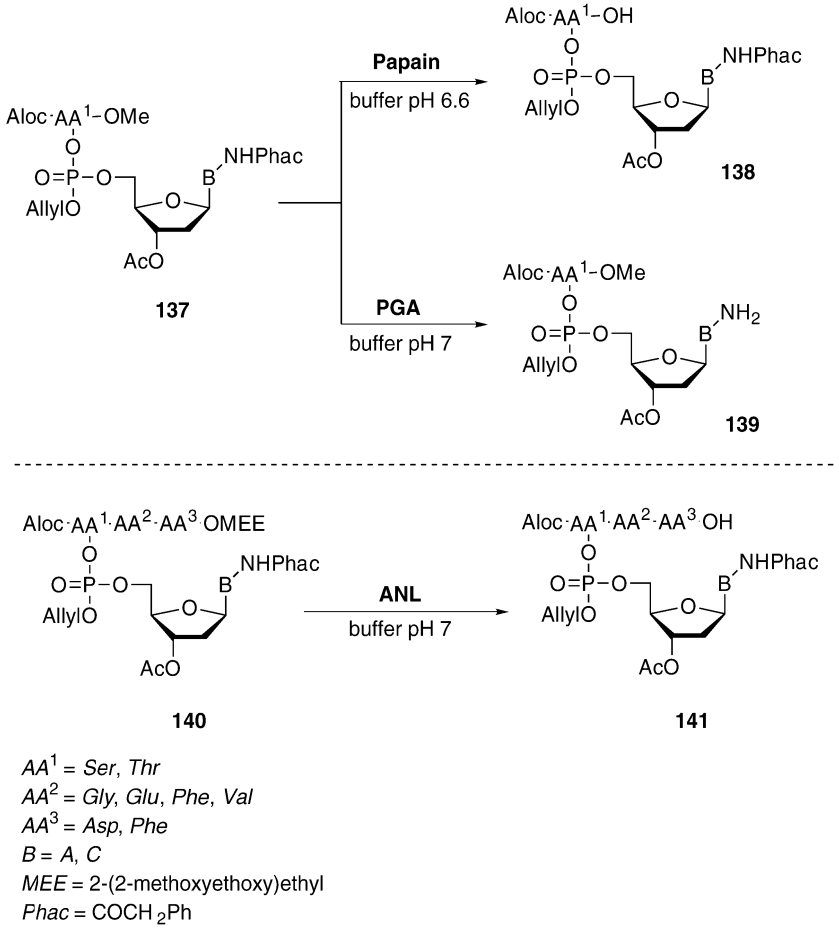
Aminoacyl nucleosides (**134**–**136**) and their oligonucleotide derivatives are compounds of high significance in biological chemistry, since it has been established that the 3′-terminus of all *t*-RNAs contains the common CCA sequence



Scheme 35

and the 3′-terminal adenosine *cis*-diol system is a site of attachment of the amino acid. Therefore, aminoacylated derivatives of oligoribonucleotides are important tools in the study of the specific incorporation of amino acid into proteins. *Gotor et al.* have applied the methodology previously described to obtain aminoacylated nucleosides in a regioselective manner by using the lipases PSL and CAL and employing N-protected acetoxime aminoacyl esters as acylating agents (Scheme 35) [55]. It is noteworthy that, in contrast to other methods reported, the exocyclic amino function in adenine nucleosides remains unaffected. Amino acids protected with benzyloxycarbonyl moieties are better substrates than those derivatized as *tert*-butyloxycarbonyl groups. With respect to α-branched amino acids, neither *L* nor *D* seems to fit the steric requirements of the enzyme's active sites.

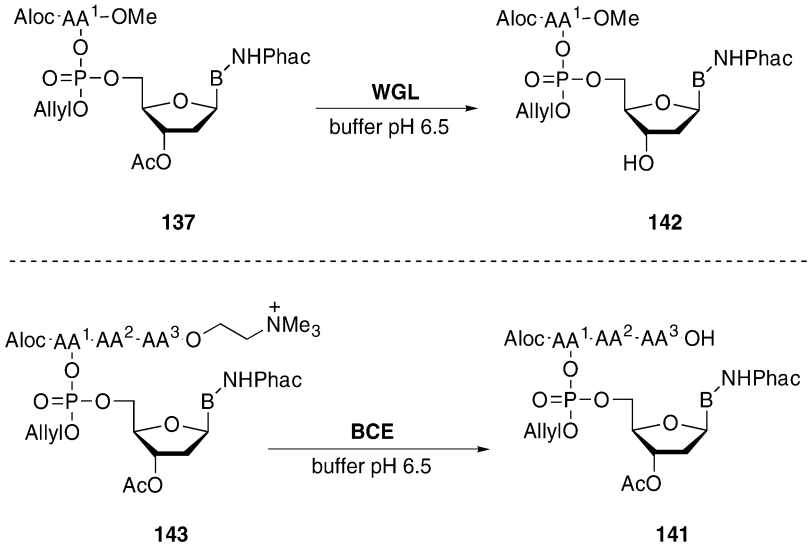
Acid- and base-labile multifunctional nucleopeptides have been selectively constructed under mild conditions by means of enzymatic protecting group techniques [56]. The nucleopeptides **137** and **140** have the nucleobases masked with enzyme-labile phenylacetamide groups and the carboxy groups of the amino acids masked as methyl or *MEE* esters (Scheme 36). From the nucleoamino acid esters **137** the C-terminal protecting groups are selectively removed with the protease papain from *Carica papaya* in a cysteine buffer at *pH* 6.6 and 37°C giving nucleopeptide **138**. Under these conditions the protease did not attack the O-acetates. However, the nucleobase in **137** is selectively N-deprotected by means of



Scheme 36

penicillin G acylase (PGA) in phosphate buffer ($pH = 7$) MeOH (80:20) at room temperature, giving rise to compound **139**. Upon treatment of the nucleopeptide acid *MEE* esters **140** with lipase from *Aspergillus niger* (ANL) in analogously phosphate buffer ($pH = 7$) acetone (90:10) a 37°C, the C-terminal carboxylic acid was smoothly deprotected giving derivative **141**. The biocatalyst tolerated the presence of purine and pyrimidine bases and different amino acids and amino acid sequences in the nucleopeptides.

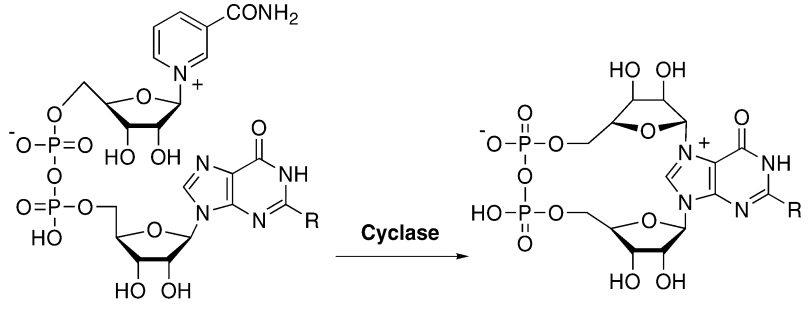
This strategy was later complemented by the same authors [57] for the hydrolysis of the 3′-O-acetyl group in nucleopeptides **137** using wheat germ lipase (WGL) in phosphate buffer at $pH = 6.5$ to give exclusively nucleopeptide **142** (Scheme 37). However, they show that the enzyme butyrylcholine esterase (BCE) from horse serum was advantageously employed to smoothly deprotect C-terminal carboxylic acids like **143** to give compound **141**. Thus, even on these multifunctional, complex conjugates, the mild enzymatic transformations occurred without any undesired side reactions.



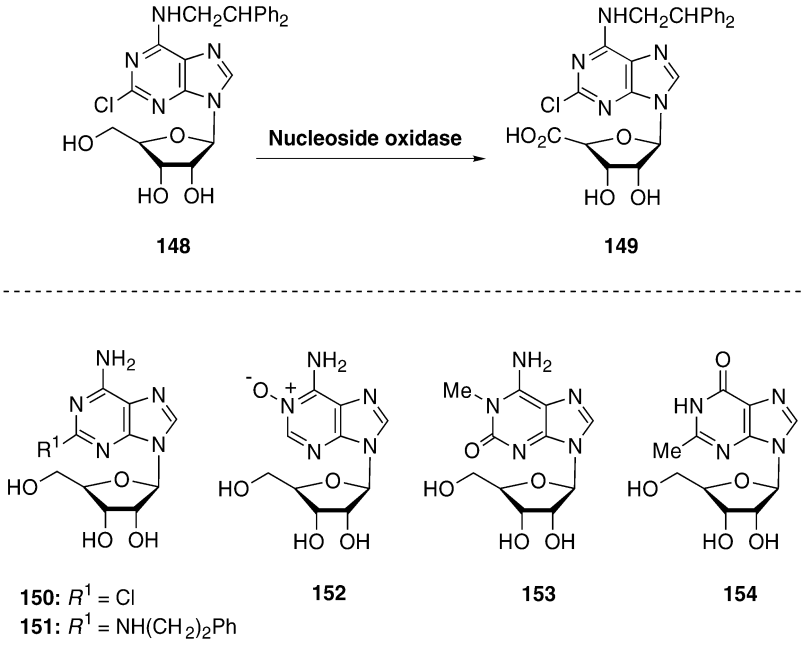
Scheme 37

Other Enzymatic Transformations

The cyclase of *Aplysia californica* [58] catalyzed an alternative mode of cyclization of nicotinamide guanine dinucleotide **144** and nicotinamide hypoxantine dinucleotide **145** to form the cyclic GDP-ribose **146** and cyclic HDP-ribose **147**, respectively (Scheme 38). In these cyclic nucleotides, the newly formed glycosyl bonds are attached onto the N-7 nitrogen of the purine rings instead of the N-1 nitrogen as in cyclic *ADP*-ribose. In addition, a series of NMR techniques were employed to deduce its chemical identity, which had been erroneously proposed previously. This alternative mode of cyclization provides one with more flexibility for the chemoenzymatic synthesis of novel analogues of cyclic *ADP*-ribose, a potent mediator of calcium mobilization in many mammalian and invertebrate tissues.



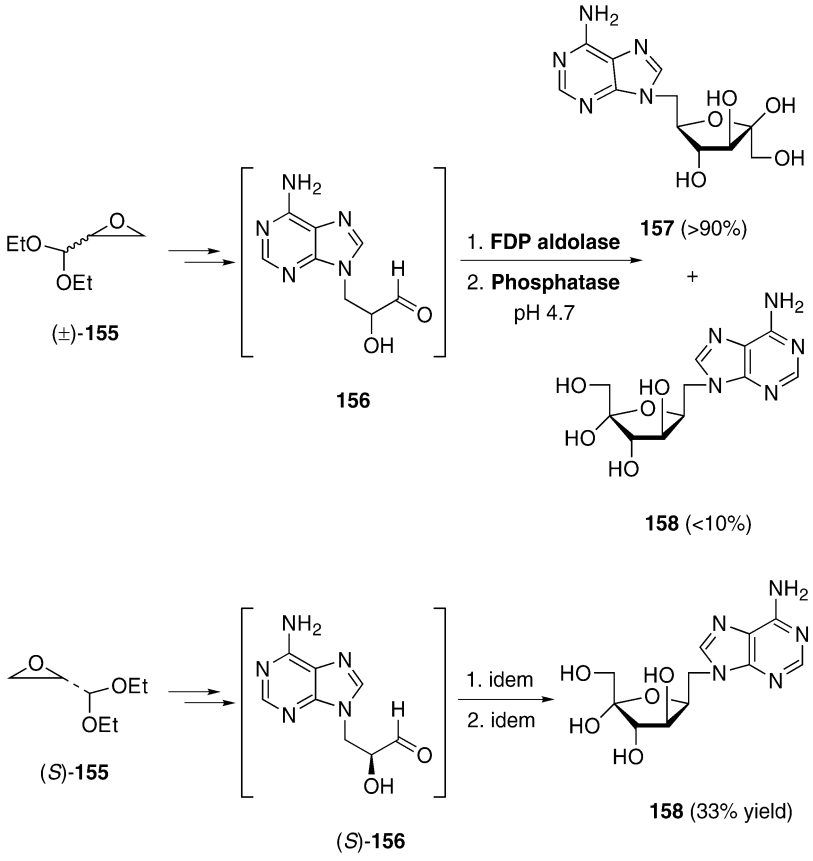
Scheme 38



Scheme 39

Enzymatic oxidation of a nucleoside precursor offers potential for an improved synthesis (in comparison with a chemical one): it is environmentally clean and avoids the need for protection of other functional groups. An effective and practical enzymatic procedure [59] for the preparation of carboxylic nucleoside derivative **149** with nucleoside oxidase in high chemical yields from hydroxyl-unprotected nucleoside **148** has been reported (Scheme 39). Substrate specificity of this enzyme was also studied through a number of nucleoside 5′-carboxylates on a preparative scale. Nucleoside oxidase from *Stenotrophomonas maltophilia* (FERM BP-2252) has been developped for the analytical determination of nucleosides (assessing food freshness), but this is the first report for preparative synthesis of nucleoside 5′-carboxylates. It has been demonstrated that the enzyme is tolerant of different functionalities on the base, especially at the 2-position, including chloro (**150**) or phenylethyl amino (**151**) groups. Furthermore, the nitrogen at position 1 can be modified to the N-oxide (**152**) or methylated in the case of methyl isoguanine (**153**). Interestingly, in the inosine series, with 2-methylinosine (**154**) as a substrate, the reaction tended to stop at the 5′-aldehyde intermediate which was not further oxidized.

A new synthetic approach to nucleoside analogues based on enzyme-catalyzed aldol condensations has been reported [60]. This enzymatic route provides a new pathway to nucleosides with novel structures at the sugar and/or the base moiety and is outlined in Scheme 40. The racemic aldehyde component **156** used in the kinetically controlled enzymatic synthesis was obtained from glycidaldehyde diethyl acetal (**155**). The latter was treated with adenine to generate 3-adenyl-2-hydroxypropanal diethyl acetal, which was deprotected to form the free aldehyde



Scheme 40

156 *in situ*. Then FDP aldolase from rabbit muscle was added, thus initiating the aldolic enzymatic process. After the reaction was complete, the phosphate moiety was cleaved with acid phosphatase to afford compound **157** in 20% yield and a minor amount of product **158** ($\leq$10%). However, in a separate synthesis of **158**, enantiomerically pure (*S*)-**156** was prepared from (*S*)-**155** and used as a substrate for the enzymatic reaction.

Concluding Remarks

Although enzyme-catalyzed glycosyl transferring reactions have been the predominant type of biotransformations on nucleosides so far, the number of reports describing enzyme-mediated hydrolyses, protections, and resolutions has been increasing in the last two decades, especially since the introduction of organic solvent methodology. As the area continues to grow, new enzymatic procedures for the synthesis of many natural and unnatural nucleosides and intermediates will continue to be developed. Regioselective alkoxycarbonylation processes catalyzed by enzymes offers a new area of interest due to great flexibility and the possibility to provide practical routes to nucleoside carbonates of potential value. 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 in order to avoid the drawbacks shown in certain applications such as agents effective against HIV and other viral infections or applications related with 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.

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