Biocatalytic Selective Modifications of Conventional Nucleosides, Carbocyclic Nucleosides, and *C*-Nucleosides

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Contents

I. Introduction

Nucleosides are fundamental building blocks of biological systems that show a wide range of biological activity.[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, mainly due to enzymatic degradations.

Since the latter part of the 1980s, nucleoside analogues have been investigated with renewed urgency in the search for agents effective against the human immunodeficiency virus (HIV), the causative agent of the AIDS epidemic, in addition to more effective treatment for other viral infections 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 incidence in their asymmetric syntheses, and in the discovery of a number of derivatives with potent antitumor and antiviral activities. One important discovery has been that replacement of the oxygen in the sugar portion of the nucleoside with a methylene unit results in carbocyclic nucleoside analogues which are highly resistant to phosphorylases.

The intense search for clinically useful nucleoside derivatives has resulted in a wealth of new approaches for their synthesis and most importantly their enantioselective synthesis. Thus, especially for organic chemists, enzymatic- or microbial-catalyzed reactions are becoming standard procedures to synthesize enantiomerically pure compounds due to their simple feasibility and high efficiency. In general, these catalysts are inexpensive and 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.

The synthetic potential of enzymes related to nucleoside synthesis has been applied profusely, especially since the introduction of organic solvent methodology. It is the aim of this report to cover the literature of the past decade relative to conventional nucleosides with selected examples included for historical reference or because of special significance (two review articles have already appeared: one covering this field in depth[2] and another dealing with enzyme protecting groups but restricted only to nucleosides containing the natural pentanofuranose moiety[3]) and the last 20 years of carbocyclic nucleosides and *C*-nucleosides related to chemoenzymatic transformations. With this review, the desire of the authors is to show a range of examples that cover nucleoside analogue syntheses through enzymatic procedures and thus to offer an easily accessible, visual, reference review. Due to the vastness of the bibliographic material related to nucleosides, we will focus our attention especially on those processes that involve lipases, esterases, and proteases. Thus, the review will not cover other enzymatic processes such as preparation of nucleoside antibiotics using microorganisms[4] or nucleoside synthesis mediated by gly-

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Miguel Ferrero was born in León, Spain, in 1964. He received his Licenciature (1987) and Ph.D. (1992) degrees in Chemistry from Universidad de Oviedo under the supervision of Professor J. Barluenga and Professor F. Palacios. His research involved the λ^5-phosphazenes: their preparation, reactivity, and use as intermediates in organic synthesis. After leaving Oviedo, Dr. Ferrero spent two and a half years as a postdoctoral fellow at University of California, Riverside, with Professor W. H. Okamura working in the synthesis and structural–function studies of vitamin D_3 analogues. In July 1995, he moved back to Universidad de Oviedo and joined the research group of Professor V. Gotor. In 1997 he received the IV "Dr. Severo Ochoa" Award of the Fundación Príncipe de Asturias for a project in vitamin D_3 and neurodegenerative diseases. At present, he is in charge of the natural product research line in Professor's Gotor group. His current research focuses on the chemoenzymatic synthesis of vitamin D_3 analogues, nucleosides/nucleotides, and quinic/shikimic acid derivatives, including enzymatic mechanism studies and molecular-mechanics calculations of new antisense oligonucleotides. Also, he is involved in several projects related with applied research in connection with several companies.

Vicente Gotor was born in 1947 in Calatayud, Spain. He received his Ph.D. degree from Universidad de Zaragoza in 1974. After leaving Zaragoza, Dr. Gotor carried out two years of postdoctoral studies at Max Planck Institut für Kohlenforschung (Mülhein/Rhur, Germany) in the area of organometallic chemistry. He joined the Chemistry Faculty at Universidad de Oviedo as Assistant Professor in 1977; he assumed his current position as Professor of Organic Chemistry at the same institution in 1982. His research fields include the areas of heterocyclic and bioorganic chemistry. He worked in heterocyclic chemistry until 1988. In this year, he started work in the field of biotransformations. Specific areas of his research interest are enzymatic amidation reactions with hydrolases, enzymatic chemoselective transformations on natural products, biotransformations with oxynitrilases and oxidoreductases, and chiral recognition with azamacrocycles. Additionally, applied research is carried out in collaboration with several pharmaceutical industries. He was Research Vice-Chancellor of the Universidad de Oviedo for four years until June 2000. At present, he is the leader of the Bioorganic group in the Chemistry Faculty of Universidad de Oviedo.

cosyl transferring enzymes (e.g., nucleoside phosphorylases or *N*-deoxyribosyltransferases).[5]

Most enzymatic reactions, just like those shown in this review, are performed 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 sorted alphabetically. These are cited as in the original papers to facilitate checking the original work, together with their corresponding new denominations.

To simply the schemes, Figure 1 connects the common abbreviations to the nucleoside bases used in this article.

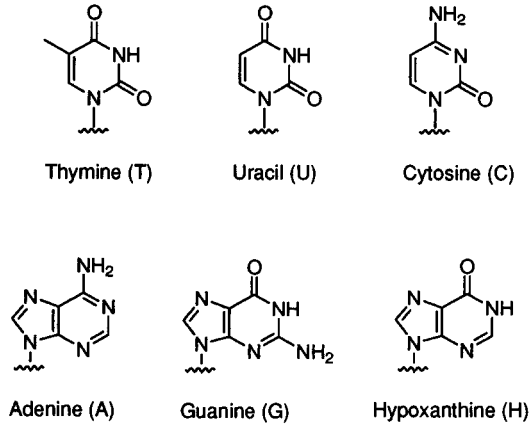


Figure 1. Pyrimidine and purine bases used in this review with their abbreviaton in brackets.

II. Conventional Nucleosides

A. Transformations Mediated by Enzymatic Hydrolysis

1. Enzymatic Hydrolysis on the Base Moiety

2′,3′-Dideoxyguanosine (**4**), synthesized from readily available guanosine (**1**), is an important target molecule due to its ability to inhibit the cytopathic effect of HIV. Its synthesis[6] has been performed using commercially available mammalian adenosine deaminase (ADA) in the key step (Scheme 1). 2′,3′-Dideoxynucleoside **3** was obtained in 40% overall yield from **2** following the cyclic thionocarbonate

Scheme 1

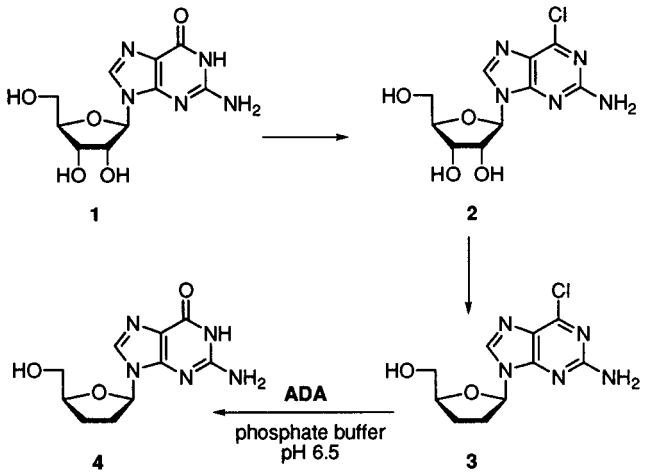


Table 1. Enzymes Commonly Used in Biocatalytic Processes Shown in This Review

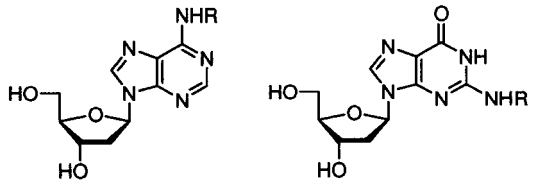
acepted name (abbreviation)	other denominations
acetone liver beef powder (ALBP)	
acetyl esterase (AE)	
Acinetobacter NCIB 9871	
adenilic acid deaminase (AMPDA)	
adenosine deaminase (ADA)	
alkaline phosphatase	
Beauveria sulfurescens	
BMT-4D/1A (from *E. coli*)	
Candida antarctica lipase (CAL)	SP-435, lipase B, CAL-B
Candida rugosa lipase (CRL)	*Candida cylindracea* lipase (CCL)
chirazyme	
Chromobacterium viscosum lipase (CVL)	
CloneZyme ESL-001−02 (ESL-001−02)	
cytidine deaminase (CDA)	
electric eel acetyl cholinesterase (EEAC)	
ENZA-1	*Rhodococcus equi* NCIB 40213
ENZA-20	*Pseudomonas solanecearum* NCIB 40249
ENZA-22	*Pseudomonas fluorescens*
ENZA-25	*Aureobacterium species*
horse liver alcohol dehydrogenase (HLADH)	
KWI-56 (from *Pseudomonas sp.*)	
lipase AY-30 (from *Candida cylindracea*)	
lipase M (from *Mucor javanicus*)	
lipase MY	
lipase SP-525	
lipase type I	
lipase type VII	
lipase type XIII	
lipoprotein lipase (LPS)	
lipozyme (from *Pseudomonas sp.*)	*Mucor miehei* lipase
L.PGS (lipase genetically modified form *Plant Genetic System*)	
Pseudomanas cepacia lipase (PSL)	*Pseudomonas* sp. lipase, *Pseudomonas fluorescens* lipase (PFL), PCL, lipase P, lipase PS, LPS, Amano PS, Amano lipase PS
Mucor sp.	
naproxen esterase	
pancreatin	
penicillin amidase (PA)	
penicillin G acylase (PGA)	
phosphorylase	
phosphohydrolase (from *Crotalus atrox* venom)	
pig liver esterase (PLE)	
porcine pancreas lipase (PPL)	
Rhizopus delemar lipase (RDL)	
SAM-2 (hydrolase from *Pseudomonas sp.*)	
savinase	
subtilisin	
subtilisin 8350 (mutant)	
subtilisin 8397 (mutant)	
timidine kinase (from HSV-1)	
wheat germ lipase (WGL)	

methodology. An additional advantage of this approach is that it can be readily scaled up to gram quantities.

Galunsky and co-workers[7] reported that the phenylacetyl group can be successfully used as an enzyme-cleavable amino protecting group of 2′-deoxyadenosine (**7**) and 2′-deoxyguanosine (**8**). These derivatives, **5** and **6**, were subjected to deprotection, catalyzed by free or immobilized penicillin amidase (PA), yielding adenosine and guanosine derivatives in 80% and 75% yield, respectively (Scheme 2). The data suggests 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 min) support this conclusion.

Adenosine deaminase (ADA) catalyzes the conversion of adenosine into inosine and is physiologically

Scheme 2

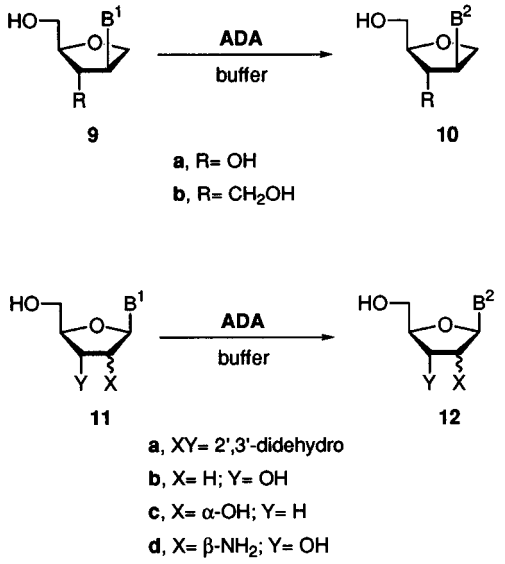


5, R= COBn **6**, R= COBn

7, R= H (80%) **8**, R= H (75%)

important in purine metabolism. Taking advantage of this natural process, ADA has been applied in the syntheses of guanosine nucleoside **10** or **12** from 6-aminopurine nucleoside **9** or **11**, respectively (Scheme 3).[8] ADA specifically catalyzes the oxidation of a particular amino group present in the base moiety of the nucleoside, leaving the amino group of

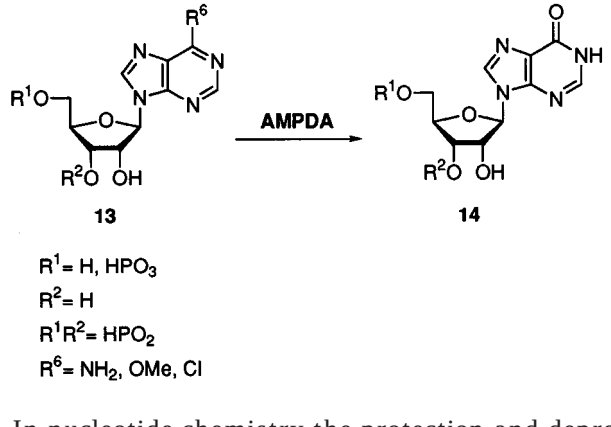
Scheme 3



a 2′-amino sugar moiety intact as, e.g., in compound **11d**.[9]

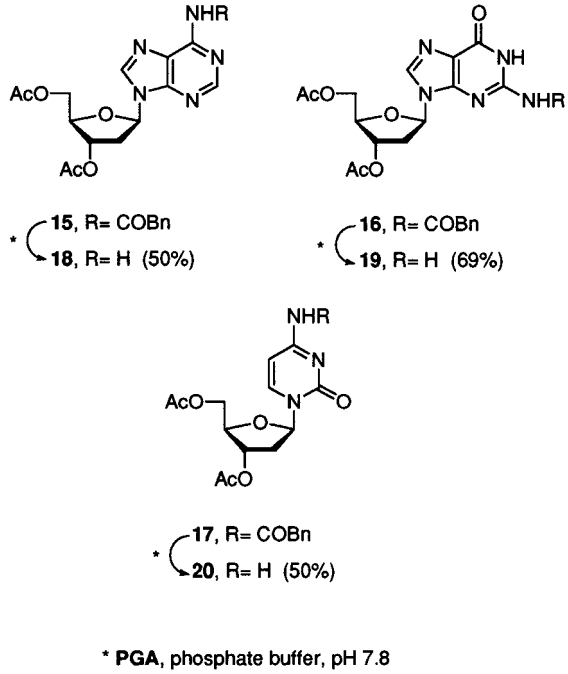
Margolin and co-workers[10] recently used adenylic acid deaminase (AMPDA) from *Aspergillus niger* in the synthesis of 6-oxopurine nucleoside in quantitative yields. This enzyme shows much broader substrate specificity and has been used for the deamination of several derivatives of adenosine **13**, 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 **14** (Scheme 4).

Scheme 4



In nucleotide chemistry the protection and deprotection of the amino functions present in its structures has to be carried out under the mildest conditions and with a high degree of selectivity. An enzymatically removable protecting group such as the phenylacetamide meets these requirements. Thus, Waldmann and co-workers[11] showed that the phenylacetamides of 3′,5′-di-*O*-acetylated 2′-deoxyadenosine **(15)** and the respective guanosine derivative **16** can be prepared in a straightforward way, with high

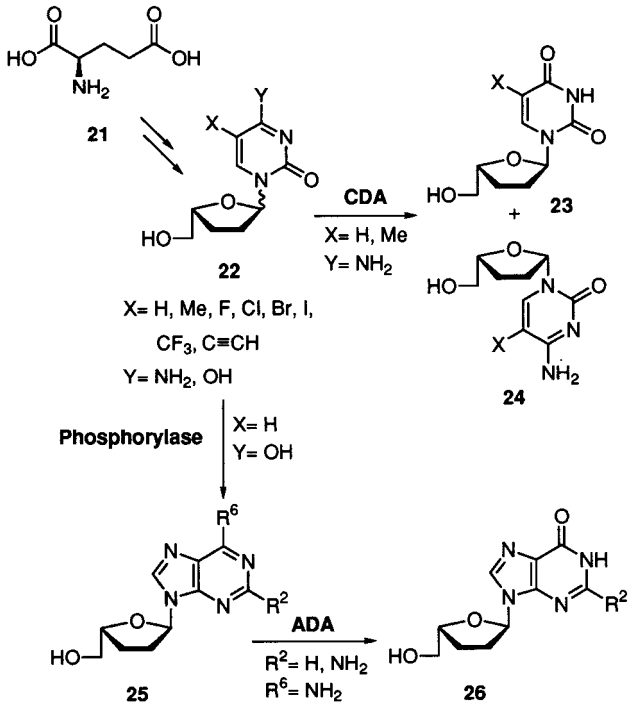
Scheme 5



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 off to deliver the selectively deprotected purine nucleobases **18** and **19** (Scheme 5). In addition, the respective protected cytidine derivative **17** is converted to the selectively deblocked pyrimidine nucleoside **20**. The conversion of the substrates is quantitative, but due to losses during work up, the products are isolated in the yields shown in Scheme 5. The enzymatic attack on the phenylacetamides occurs with complete chemoselectivity, leaving the more reactive esters in the carbohydrate part intact. Moreover, the reaction conditions are so mild that no other 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[12] 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 led Van Draanen and Koszalka[13] to center 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 (**21**), giving rise to the target nucleosides as α,β-anomeric mixtures (**22**). The α,β-anomeric mixtures **22** were separated either by preparative HPLC or, when the uracil and thymine analogues were used, by enzymatic resolution as shown in Scheme 6, because they were impractical or not possible. Thus, cytidine deaminase (CDA) deaminated the α-anomer **23** of these compounds much more rapidly than the β-anomer **24**. By taking advantage of this enzymatic selectivity, pure

Scheme 6



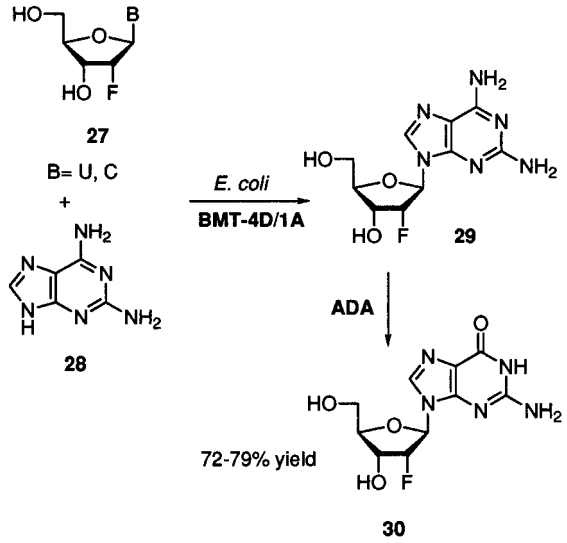
α-L-2′,3′-dideoxyuridine and α-L-3′-dideoxythymidine were prepared. These compounds were easily separated from the unreacted β-cytidine analogues by flash chromatography. The purine dideoxynucleosides **25** were synthesized by a phosphorylase-catalyzed enzymatic *trans*-ribosylation from α-L-dideoxyuridine. Finally, the hypoxanthine and guanine analogues **26** were prepared by treatment of the corresponding adenine and diaminopurine analogues **25** with adenosine deaminase (ADA).

As a part of continuing interest in fluorodeoxy nucleosides and nucleotides, Mikhailopulo and coworkers[14] reported the enzymatic synthesis of 2′-deoxy-2′-fluoroguanosine (**30**) and its antiviral activity in chick embryo cells infected with influenza virus FPV/Rostock/34 (H7N1) and herpes simplex virus (HSV) type 1 (1C strain). An enzymatic transglycosylation of 2,6-diaminopurine (**28**) using the glutaraldehyde-treated whole cells of *E. coli* BMT-4D/1A as a biocatalyst and 2′-deoxy-2′-fluoro-uridine or -cytidine **27** as a donor of the glycosyl moiety, followed by an enzymatic deamination of intermediate 2,6-diaminopurine glycoside **29**, afforded the fluoro compound **30** in 72−79% combined yield (Scheme 7).

In a recent report,[15] the behavior of ADA was studied on the acetates of adenosine **31**, 2′-deoxyadenosine **32**, and 3′-deoxyadenosine **33** since this enzymatic approach could constitute a viable method for the preparation of selectively acetylated inosines and deoxyinosines. Thus, all the acetates **31a**−**e**, **32a**−**c**, and **33a**−**c** were prepared by lipase-catalyzed reactions using CAL or PSL working both in acetylation or hydrolysis modes. As a conclusion, only the acetates with a free 5′-hydroxy group were deaminated by ADA, confirming the crucial role of 5′-OH for the enzyme activity.

It had been reported[16] that nucleoside derivative **34** is a substrate for ADA. This property was used

Scheme 7



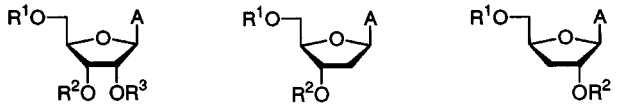
for the enzyme-catalyzed synthesis of 2-aza-2′-deoxyinosine (**35**) on a preparative scale. Those derivatives were converted into the corresponding phosphoramidites and were employed in solid-phase oligonucleotide synthesis.

2. Enzymatic Hydrolysis on the Sugar Moiety

Enzyme-catalyzed deacylations have been applied as a method to selectively remove one or more acyl groups from polyacylated nucleosides. Thus, complementary results were obtained depending on which enzyme was used.[17] When a lipase from porcine pancreas (PPL) in phosphate buffer was the catalyst for deacylation of 3′,5′-di-*O*-acetylthymidine (**36**), the 5′-*O*-acetyl group was selectively attacked leading to 3′-*O*-acetylthymidine (**37**) in very good yield (98%). In contrast, if *Candida cylindracea* lipase (CCL) was used in the catalysis, the 3′-ester (giving compound **38**) was hydrolyzed three times faster than the 5′-ester (giving compound **39**) (Scheme 10).

The regioselective hydrolysis of tri-*O*-acylated esters **40** has been carried out with pig liver esterase

Scheme 8

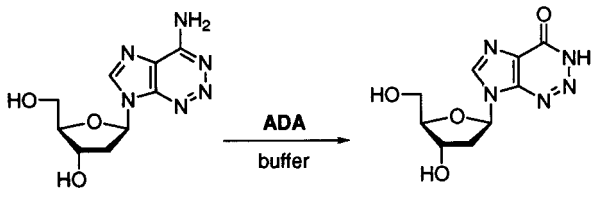


31
a, R^1= Ac; R^2= R^3= H
b, R^1= H; R^2= R^3= Ac
c, R^1= R^3= H; R^2= Ac
d, R^1= R^2= H; R^3= Ac
e, R^1= R^2= R^3= Ac

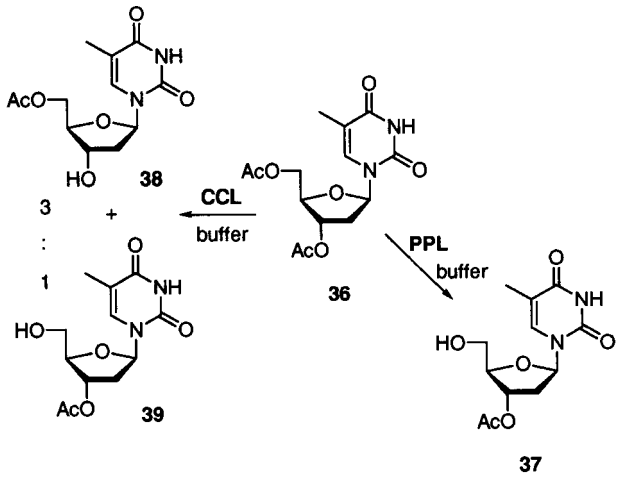
32
a, R^1= Ac; R^2= H
b, R^1= H; R^2= Ac
c, R^1= R^2= Ac

33
a, R^1= Ac; R^2= H
b, R^1= H; R^2= Ac
c, R^1= R^2= Ac

Scheme 9

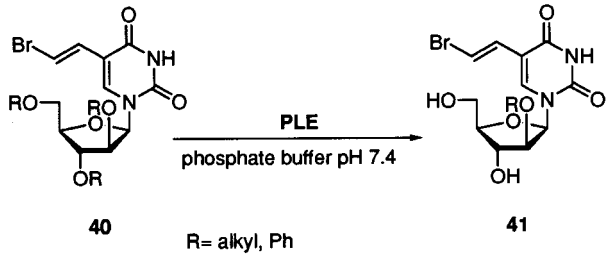


Scheme 10



(PLE) in phosphate buffer with ethanol as cosolvent leading to 2′-*O*-monoacylated nucleosides **41** in high yield (Scheme 11).[18] The markedly retarded rates of

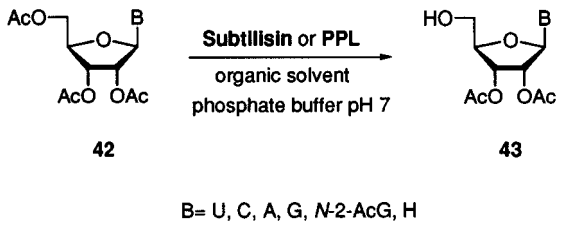
Scheme 11



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.

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 **42** to give 2′,3′-di-*O*-acylribonucleosides **43** in 40−92% yield (Scheme 12).[19] The

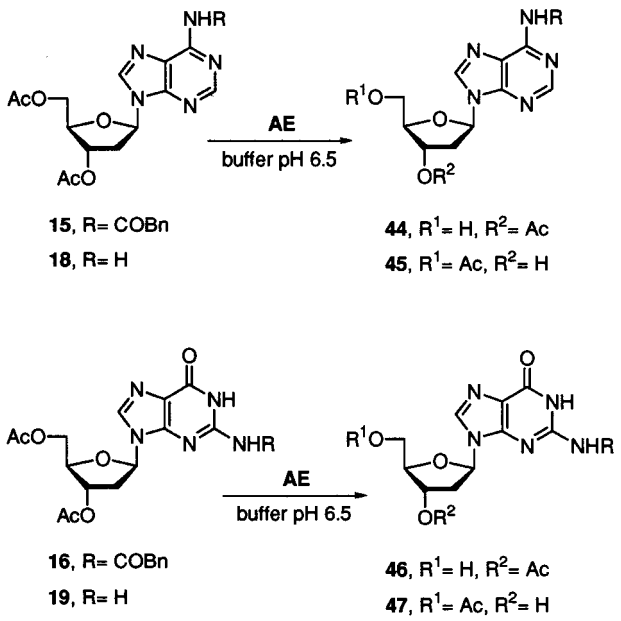
Scheme 12



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 **15**, **16**, **18**, and **19**.[11] Thus, the adenosine and guanosine derivatives **18** and **19,** which do not carry *N*-protecting groups in the nucleobases, are deprotected at the secondary 3′-OH to give nucleoside derivatives **45** and

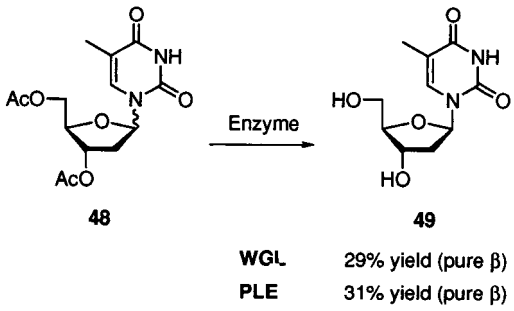
Scheme 13



47, respectively (Scheme 13). However, if the amino groups present in the purine bases are masked as phenylacetamides, as with compounds **15** and **16**, the regioselectivity displayed by the enzyme is reversed and the primary 5′-OH groups of the nucleosides are liberated to give base-protected nucleosides **44** and **46**, respectively.

Wengel and co-workers[20] reported the first attempt to use biotransformations to solve the basic problem of anomer separation in nucleoside chemistry when a convergent strategy has been used. Thus, lipase-catalyzed deacylations of an anomeric mixture of peracetylated 2′-deoxyribofuranosyl thymine (**48**) are shown in Scheme 14. Generally, the diastereoselec-

Scheme 14



tivity 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 deacetylation of **48** affording the pure β-anomer thymidine (**49**β) as the only completely deprotected nucleoside product.

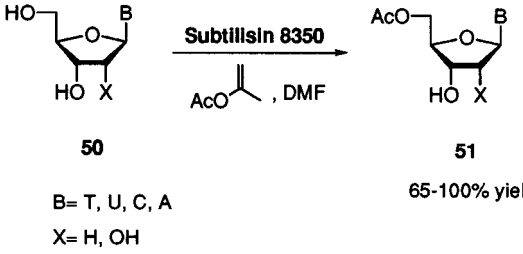
B. Regioselective Enzymatic Protections

1. Enzymatic Protections through Acylations

Selective modification of nucleosides which contain several functional groups with very similar chemical reactivities is an interesting subject of study and is a fundamental challenge to organic chemists. Thus,

highly regioselective acetyl transfer reactions to the carbohydrate moiety of nucleosides have been carried out by employing a subtilisin mutant[21] (subtilisin 8350 or even the much more stable subtilisin 8397[22]) 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 **50**, giving the 5′-*O*-acylated derivatives **51** with high yields (Scheme 15). The high regio-

Scheme 15

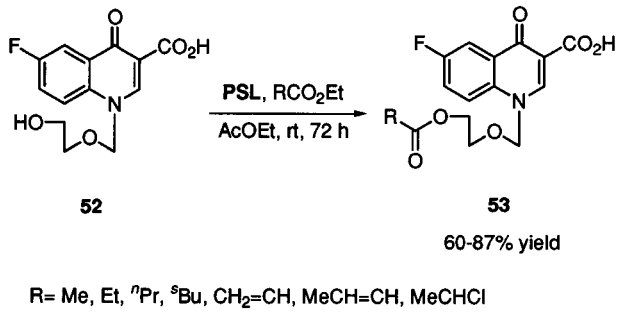


selectivity of the mutant enzyme was attributed to the fact that it binds the reaction transition state more strongly than does the parent enzyme.

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 **51** (of uridine, *N*-4-anisoylcytidine, adenosine, and *N*-6-benzoyladenosine) in 67−82% yield.[23]

As a part of a program to design new regioselective enzymatic transformations of nucleosides, Gotor and co-workers started with the lipase-mediated acylation of acyclonucleosides (Scheme 16).[24] Thus, acyclo-

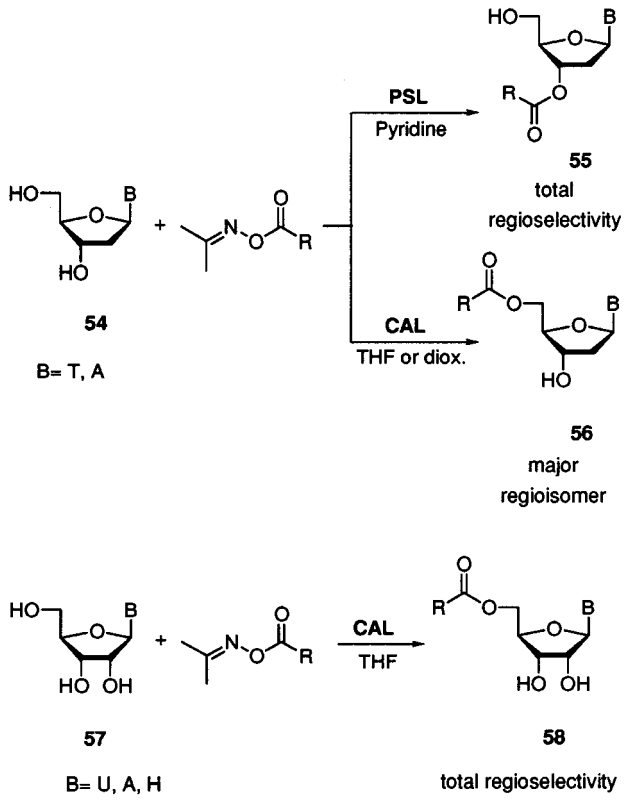
Scheme 16



nucleosides **53** 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 **52**. This was the first example of the use of enzymes in acyclonucleoside chemistry that allowed a facile preparation of prodrugs **53** of 4-quinolone acyclonucleosides which were tested as antiviral agents against a wide variety of assay systems.[25]

In addition, the same authors used oxime esters as irreversible acyl transfer agents in the regioselective acylation of 2′-deoxynucleosides **54** on the secondary hydroxyl group (Scheme 17), instead of the more chemically reactive primary alcohol, using PSL in pyridine.[26] This procedure is very versatile because

Scheme 17



Keys:

A R= Me, ^{n}Pr, $Me(CH_2)_6$, $Me(CH_2)_8$, CH_2=CH, MeCH=CH, Ph, $ClCH_2$

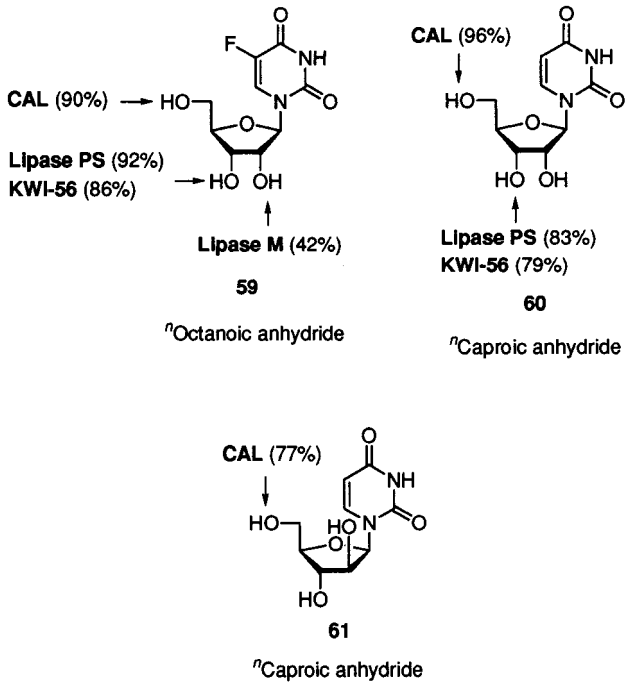
B CH_2NHP, CH_2CH_2NHP; P= Cbz, Boc

C R= OMe, OBn, OCH=CH_2, OCH_2-CH=CH_2

acylation with various oxime esters bearing saturated or unsaturated chains yields exclusively the 3′-*O*-acyl derivatives **55**. 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- (**54**) and ribonucleosides (**57**).[27] 2′-Deoxynucleosides such as thymidine and 2′-deoxyadenosine (**54**) were acylated with oxime esters (key A, bottom of Scheme 17) carrying saturated, unsaturated, aromatic, and functionalized chains, giving 5′-*O*-acylated compounds (**56**) together with small quantities of the 3′-*O*-acylated regioisomer **55**. Uridine, adenosine, and inosine as representative ribonucleosides (**57**) were acylated exclusively at the 5′-hydroxyl group (**58**) by using the same methodology.

Also, the same group 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 (key B, Scheme 17).[28] It is noteworthy that in contrast to other methods reported, the exocyclic amino function in adenine nucleosides remains unaffected. Amino acids protected with benzyloxycarbonyl groups are better substrates than those derivatized as *tert*-butyloxycarbonyl ones. With respect to α-branched amino acids, neither L nor D seems to fit the steric requirements of the enzyme's active sites.

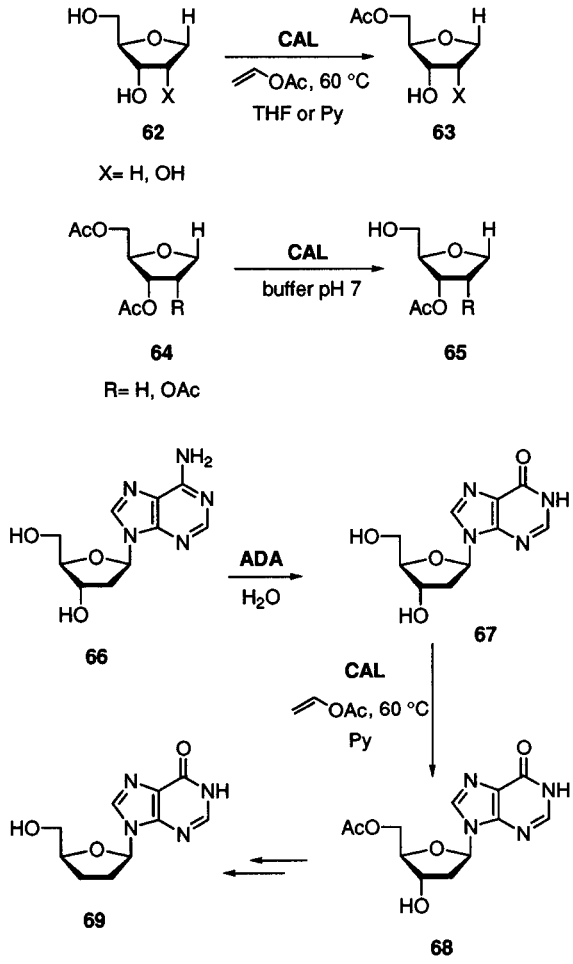
Scheme 18



To decrease toxicities and improve activities, chemical modifications of nucleosides, including protection, have been studied. Thus, Ozaki and co-workers[29] used enzymatic transesterification reactions to prepare acyl-protected derivatives of 5-fluorouridine (**59**, Scheme 18). 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 **59** (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 with excellent yield. Although several solvents were checked, THF was found to be best for this reaction. Furthermore, selective protection of uridine **60** and arabinosyluracil **61** was also investigated. In general, the acylation of these compounds proceeded faster than the acylation of **59** under the same conditions with *n*-caproic anhydride. As shown for the fluoro derivative **59**, 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 antiretroviral activity against HIV. Among these, 2′,3′-dideoxyinosine (ddI, **69**) has been approved for alternative treatment to AZT. Santaniello and co-workers[30] 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 **62** has been achieved in the presence of CAL in organic solvents (Scheme 19). Also, the enzymatic hydrolysis of the triacetate and diacetate **64** in the presence of CAL in aqueous buffer at pH 7 or in water-saturated chloroform was performed and found to be complementary to the lipase-catalyzed acylation in organic solvents. With these results in hand, they developed a new chemoenzymatic synthesis of 2′,3′-dideoxyinosine **69** starting from the 5′-acetate **68** enzymatically prepared by the transacetylation procedure. This starting material **67** was relatively expensive, but it was prepared from readily available 2′-deoxyadenosine (**66**) by a deamination reaction efficiently catalyzed by adenosine deaminase (ADA). The overall sequence for the chemoenzymatic synthesis of ddI is depicted in Scheme 19.

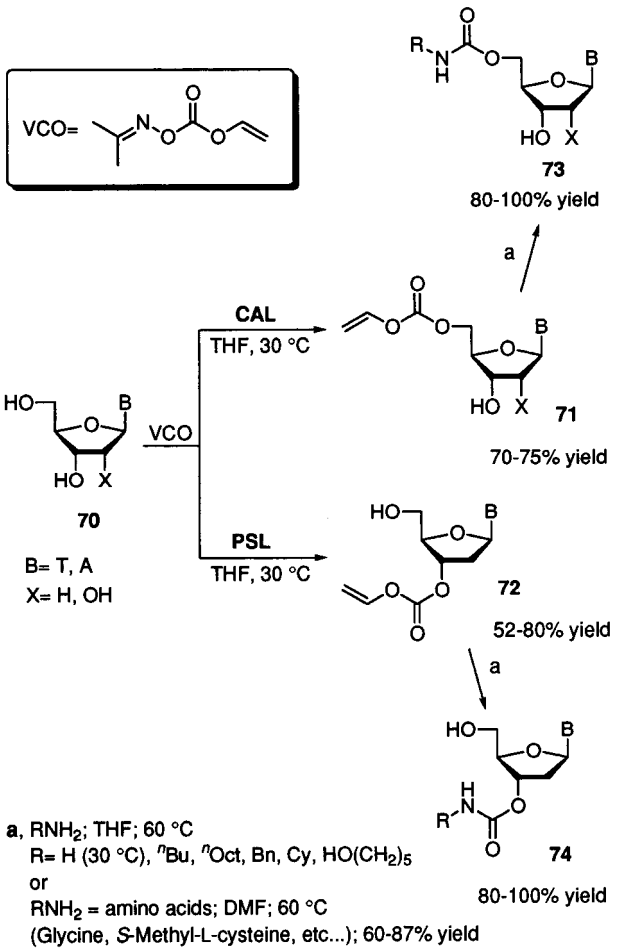
Scheme 19



2. Enzymatic Protections through Alkoxycarbonylations

An important and synthetically relevant transformation in nucleosides 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 17 (key C) summarizes a general, new, and simple procedure for the synthesis of pyrimidine and purine 3′-carbonates **55** from 2′-deoxynucleosides **54** using PSL and *O*-[(alkoxy)-carbonyl]oxime.[31] 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 **56** and **58** of 2′-deoxyribonucleosides and ribonucleosides could be obtained by enzymatic alkoxycarbonylation with

Scheme 20



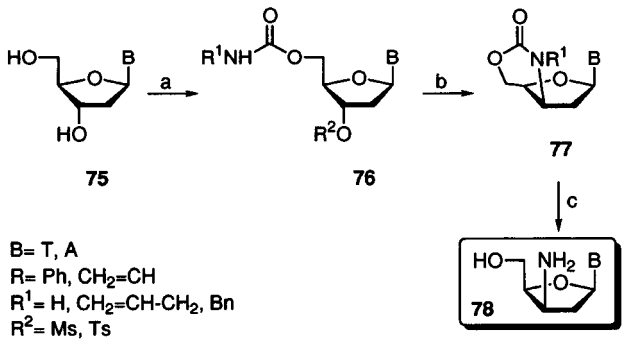
CAL and oxime carbonates,[32] the latter being easily prepared from chloroformates. Ribonucleosides **57** yield two kinds of 5′-*O*-carbonates **58**, depending on whether the alkoxy or the acetone oxime moiety acted as the leaving group. In the case of 2′-deoxynucleosides, the leaving group was always the acetonoxime moiety, giving rise to the regioselective formation of the corresponding 5′-*O*-alkyl carbonates together with small amounts of 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 increasing permeation through biological membranes. Bearing 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 and coworkers 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 20).[33] The key step is the enzymatic synthesis of 5′-*O*-vinyloxycarbonylnucleosides **71** or 3′-*O*-vinyloxycarbonylnucleosides **72** in a regioselective way using CAL or PSL catalysis, respectively. In a second aminolysis step, these carbonates yield the corresponding urethanes **73** and **74**. This methodology allows the assembly of ammonia, amines, amino alcohols, and L-amino acids to 2′-deoxynucleosides (to 3′- or 5′-positions) or ribonucleosides (5′-position).[34]

In view of the previous results, it is believed that nucleosides with different orientations of the hydroxyl groups could be an interesting subject of study with respect to the preference of some lipases according to the geometry of the substrate employed.[35] Thus, 5′-*O*-acyl- and 5′-*O*-alkoxycarbonyl derivatives of α-, anhydro-, *xylo*-, and *arabino*-nucleosides are obtained through a lipase-mediated reaction with CAL by using acetoxime butyrate or butyric anhydride together with benzyloxycarbonyl-*O*-acetoxime as acylating or alkoxycarbonylating agents. Also, as a complement for the synthesis of the highly hindered 3′-*O*-acetyl α-thymidine and *xylo*-thymidine,[36] the same authors prepared the 5′-Cbz derivatives as previously described and then chemically carried out an acylation reaction in the 3′-OH free hydroxyl group giving rise to full protected nucleosides, since the benzyloxycarbonyl group could be easily removed.

Aminosugar nucleosides are known to possess strong antibacterial, anticancer, and biosynthetic inhibitory properties. Because of this, considerable effort has been devoted to the preparation of this kind of compound. Most of the procedures are strongly dependent on the nature of the nitrogenated base present in the starting nucleoside. Schemes 21 and

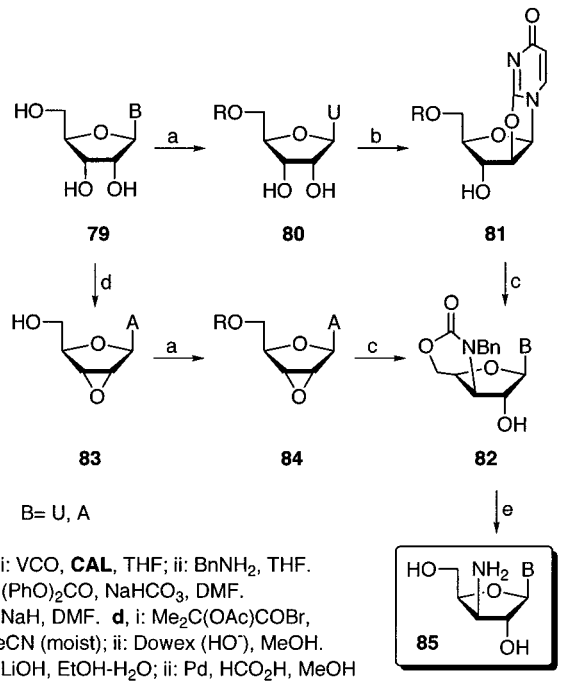
Scheme 21



22 show novel and general chemoenzymatic procedures to obtain the 3′-amino-*xylo*-nucleosides[37] 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 **76** or **81**/**84**. The methodology presented is applicable to 2′-deoxyribonucleosides (Scheme 21) and ribonucleosides (Scheme 22), regardless of their nitrogenated base.

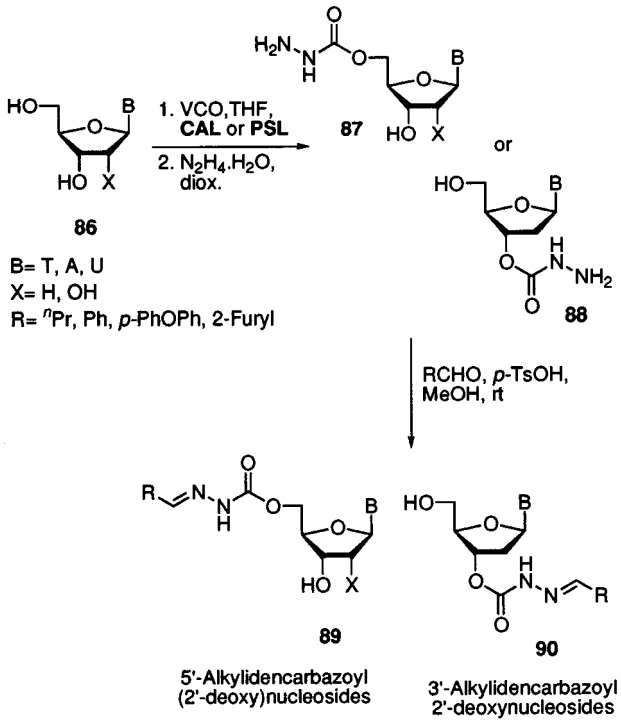
A chemoenzymatic procedure for the synthesis of 3′- and 5′-carbazoyl nucleoside derivatives prepared

Scheme 22



for the first time is shown in Scheme 23.[38] This process involves the regioselective enzymatic alkoxycarbonylation of nucleosides **86** and the subsequent transformation with hydrazine into novel carbazoyl nucleoside derivatives **87** or **88**. Taking into account previously reported data (relative to nucleoside, hydrazone, carbazate, and aryloxyphenoxypropionate derivatives), 3′-alkylidencarbazoyl 2′-deoxynucleosides (**90**), 5′-alkylidencarbazoyl 2′-deoxynucleosides, and 5′-alkylidencarbazoyl ribonucleosides (**89**), emerge as interesting targets, since they combine structural features found in both therapeutic nucleoside derivatives and fungicide/herbicide nucleoside analogues.

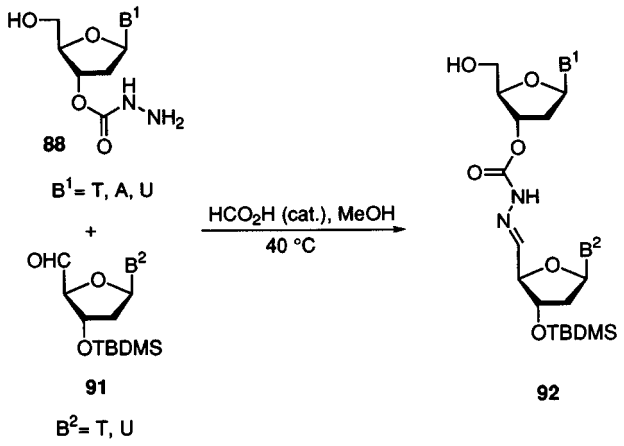
Scheme 23



Modulation of gene expression by antisense technologies requires the development of modified oligonucleotides possessing enhanced cellular uptake, 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 past 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 potential therapeutic application of the antisense strategy. 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 nonionic, hydrolytically stable, and nonchiral.

The synthesis of backbone-modified dinucleotide analogues **92** is shown in Scheme 24,[39] in which the

Scheme 24



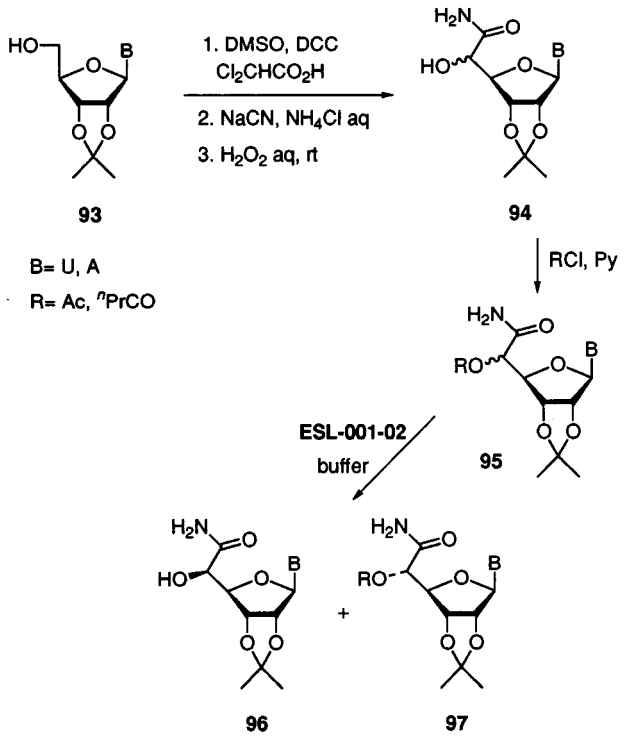
natural phosphodiester linkage is replaced by a 3′−5′-carbazoyl linkage. The bridge was formed through a coupling reaction between an appropriate 3′-carbazoyl nucleoside analogue **88** and an aldehyde nucleoside derivative **91**. It is noteworthy that starting nucleosides could be common materials to obtain the 3′-carbazoyl nucleoside derivatives by means of a simple, previously described chemoenzymatic procedure as well as obtaining the aldehyde nucleoside by way of an oxidation reaction.

These lipase-catalyzed alkoxycarbonylation processes have been studied in depth from the point of view of the influence of the carbonate on the regioselectivity in order to understand the behavior of the enzymes.[40]

C. Nucleoside Enzymatic Resolutions

Wang and co-workers[41] reported a one-pot preparation of a mixture of α-L-taluronamide **96** and β-D-alluronamide **97** nucleoside derivatives (Scheme 25). Such derivatives can be considered as intermediates to uracil polyoxin C, capuramycin, and other nucleoside antibiotics. Nucleoside derivatives **96** and **97**

Scheme 25



were prepared via a one-pot reaction sequence of oxidation, nucleophilic addition, and hydrolysis of **93** to give hydroxyamide **94**. The resulting diastereoisomers, which are difficult to separate by conventional chromatography, were resolved by stereoselective deacylation of their 5′-*O*-acylated derivatives **95** 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.

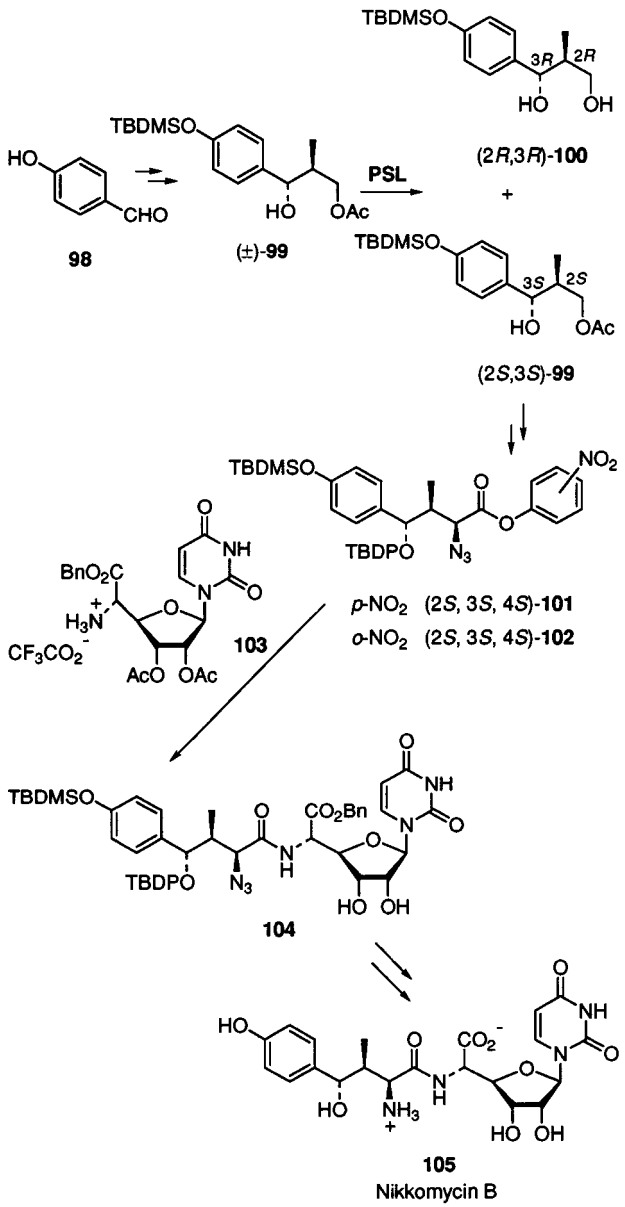
A stereoselective synthesis of the versatile chiral synthon (2*S*,3*S*)-**99** possessing two stereogenic centers was achieved using a chemoenzymatic method,[42] starting from *p*-hydroxybenzaldehyde (**98**). The enzymatic resolution of the racemic mixture of intermediate **99** using lipase Amano PS gave enantiomerically pure substrate (2*S*,3*S*)-**99** (Scheme 26). The conversion of the later into the homochiral intermediates (2*S*,3*S*,4*S*)-**101** and (2*S*,3*S*,4*S*)-**102**, corresponding to the *N*-terminal amino acid moiety of nikkomycin B, and their reaction with **103**, the corresponding part of the *C*-terminal nucleoside amino acid, produced an important intermediate **104** for the synthesis of nikkomiycin B (**105**).

III. Carbocyclic Nucleosides

A. Three-Member Ring: Precursor Enzymatic Acylation

Shibuya and co-workers[43] synthesized conformationally constrained analogue **109** and its enantiomer *ent*-**109** (Scheme 27) from 9-(difluorophosphonopentyl)guanines, which is a multisubstrate analogue inhibitor of purine nucleoside phosphorylase. They were prepared from optically active *trans*-1-(diethoxyphosphyl)difluoromethyl-2-hydroxymethylcyclopropanes (+)-**107** and (−)-**107**, which were obtained

Scheme 26



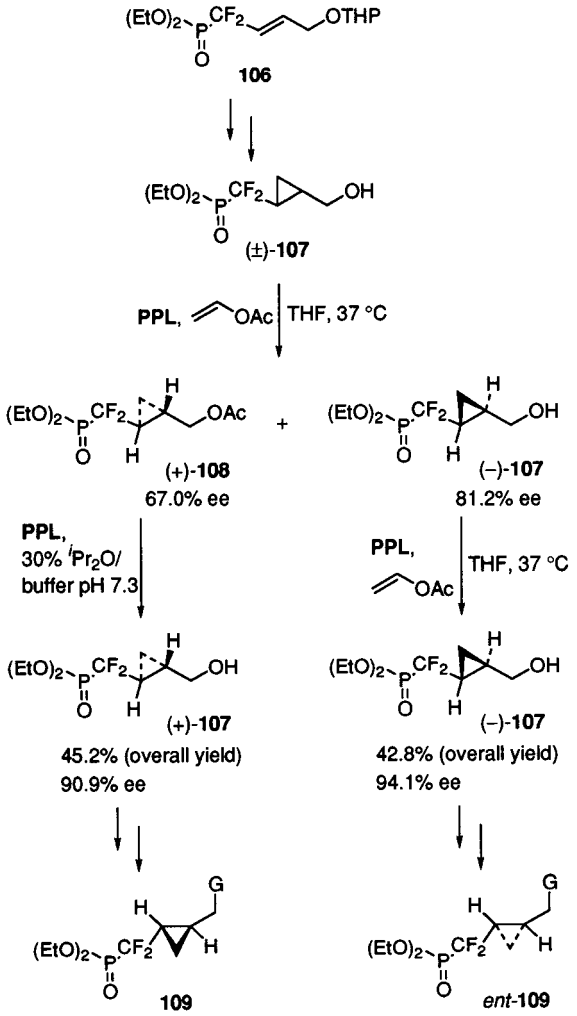
through a facile synthetic method from olefin **106**. Enzymatic double resolution was applied to obtain (+)-**107** and (−)-**107** with high enantiomeric purity. Among several lipase-catalyzed transesterifications with vinyl acetate, PPL in THF at 37 °C showed the best results, though modest (E = 12.3). The acetate (+)-**108** (67.0% ee) and the alcohol (−)-**107** (81.2% ee) obtained from the first resolution were separately submitted to the second kinetic resolution with PPL. The hydrolysis of the acetate (+)-**107** was carried out with phosphate buffer pH 7.3 to give the alcohol (+)-**2** with 90.9% ee in 45.2% overall yield. An alcohol (−)-**107** with high enantiomeric purity (94.1% ee) could be prepared in 42.8% overall yield by re-treatment of the alcohol (−)-**107** of 81.2% ee with vinyl acetate in THF under the same conditions as above.

B. Four-Member Ring

1. Precursor Enzymatic Hydrolyses

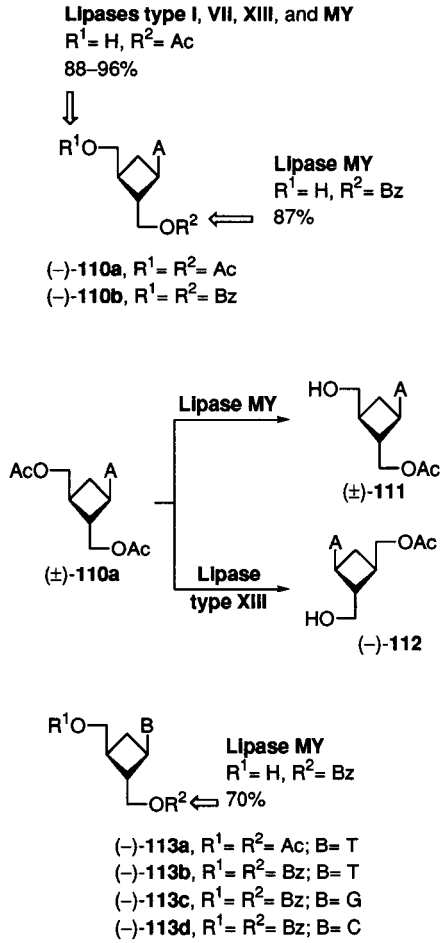
The Japanese group of Katagiri and Yamaguchi are interested in the structures and functions of oligo-

Scheme 27



nucleotides containing carbocyclic oxetanocins from the point view of antisense strategy and examined the selective protection of the two primary hydroxy groups requisite for the synthesis of the oligonucleotides. Thus, highly regio- and enantioselective deacylation of carbocyclic 3′,5′-di-*O*-acyloxetanocins using lipases were done,[44] which provides not only an effective protection for one of the two hydroxy groups of carbocyclic oxetanocins but also a method for kinetic resolution of racemic carbocyclic oxetanocins. Hydrolysis of carbocyclic 3′,5′-di-*O*-acetyloxetanocin A [(−)-**110a**] (Scheme 28) was examined using various lipases in 10% phosphate buffer (pH 7.0)−acetone at 36 °C. Among several lipases, lipase MY was the most effective for the hydrolysis. Interestingly, the di-*O*-benzoylated compound [(−)-**110b**] was hydrolyzed more readily by lipase MY to give the 3′-*O*-benzoylated product in 87% yield. This was the first example to show that an *O*-benzoylated compound is more susceptible than the corresponding *O*-acetyl derivative to the lipase-catalyzed hydrolysis. To clarify the enantioselectivity of this lipase-mediated hydrolysis, deacetylation of (±)-**110a** was carried out using lipase MY and type XIII. When (±)-**110a** was treated with lipase MY, 3′-*O*-acetylated compound (±)-**111** was obtained with high regioselectivity and in 75% yield. In contrast, hydrolysis of (±)-**110a** with lipase XIII gave (−)-**112** with 90% enantio-

Scheme 28

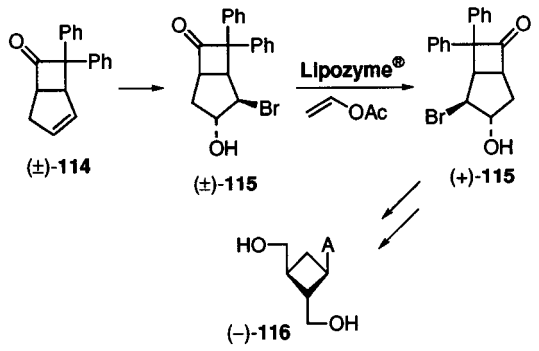


selectivity in 48% yield. To design the complementary oligonucleotide to that of carbocyclic oxetanocin A, deacylation of carbocyclic 3′,5′-di-*O*-acylated oxetanocin T [(−)-**113a**,**b**] was examined. Thymine derivatives (−)-**113a**,**b** were more resistant to the hydrolysis by lipase in comparison with adenine derivatives (−)-**110**. As in the case of (−)-**110b**, the dibenzoylated compound (−)-**113b** was hydrolyzed more readily to give the monobenzoylated compound in 70% yield. Also, using similar enzymatic hydrolysis with lipase MY and oxetanocins possessing guanine and cytosine, **113c** and **113d** were synthesized, respectively, by the same group.[45] Oligonucleotides were obtained, and their hybridization properties were examined employing these four enantiomeric carbocyclic oxetanocins derived from adenosine (**110b**), thymine (**113b**), guanosine (**113c**), and cytosine (**113d**).

2. Precursor Enzymatic Acylations

The anti-herpes agent carbocyclic oxetanocin A [(−)-**116**, Scheme 29] has been prepared from the bicyclic ketone **114** via resolution of the bromohydrin **115** using an enzyme-catalyzed transesterification reaction.[46] The bicyclic ketone **114** is readily prepared by cycloaddition of cyclopentadiene and diphenylketene. Treatment of this ketone with *N*-bromoacetamide in aqueous acetone provided the bromohydrin (±)-**115**. This was resolved by acylation using vinyl acetate and immobilized *Mucor miehei* lipase (Lipozyme), and the optically pure bromohydrin (+)-

Scheme 29



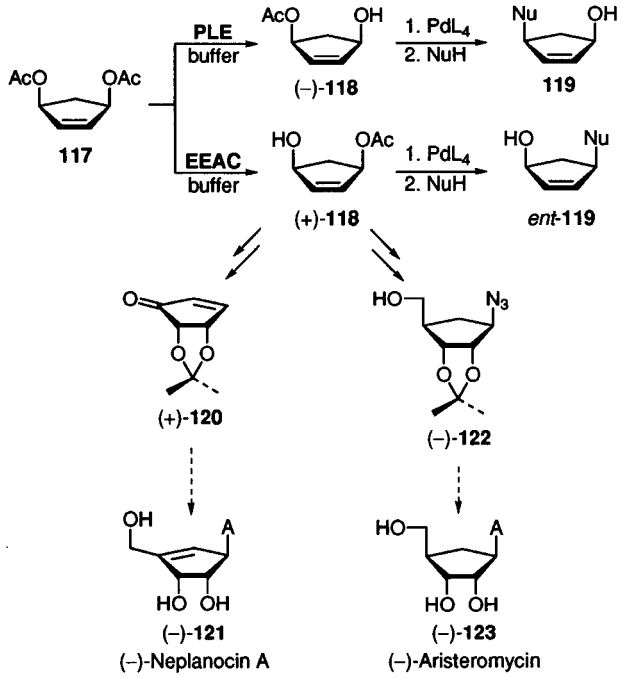
115 (42% yield) was converted through several steps in carbocyclo nucleoside (−)-**116**.

C. Five-Member Ring

1. Precursor Resolution by Enzymatic Hydrolysis or Acylation

a. Using *cis*-Cyclopent-2-en-1,4-diol and Related Structures. Laumen and Schneider[47] synthesized, through enzymatic hydrolysis of prochiral diacetate **117** (Scheme 30), (1*S*,4*R*)-4-hydroxy-2-

Scheme 30



cyclopentenyl [(−)-**118**] and (1*R*,4*S*)-4-hydroxy-2-cyclopentenyl [(+)-**118**] derivatives as versatile building blocks for cyclopentanoid natural products. Although the method was designed mainly to prepare Corey lactone and its derivatives as useful intermediates in prostaglandin synthesis, it became one of the best synthons for the preparation of carbocyclic nucleosides. Using exclusively enzymatic processes, derivative (+)-**118** was obtained through a hydrolase enzyme called acetyl-cholinesterase (from electric eel) (EEAC).[48] Exposure of *meso*-diester **117** to EEAC affords in 94% yield (+)-**118** with an ee of 96% after one recrystallization. These results complement the findings of the previous authors who were able to enzymatically prepare (−)-**118** (98% ee after recrystallization, 59%yield) but were considerably less successful in their attempt at (+)-**118** (50% ee, 82%yield). However, immobilized acetyl cholinesterase has been found equally effective and offers the additional advantage of reusability.

Synthetic applications of these enantiomerically pure synthons were carried out by Deardorff and coworkers.[49] Thus, retrosynthetic analysis made on neplanocin A (**121** in Scheme 30) has shown that cyclopentenone **120** is the ideal synthetic precursor. On the basis of this finding, they described the homochiral preparation of enone (+)-**120** from optically inactive starting material. The desired antipodal form of the starting material, (3*R*,5*S*)-3-acetoxy-5-hydroxycyclopent-1-ene [(+)-**118**], is accessible via a stereoselective hydrolysis of its parent diester **117**. Compound **118** is prepared in high optical (>99% ee) and chemical (94%) yields by using the commercially available EEAC in buffered media. Successive treatment with *N*-methylmorpholine *N*-oxide and catalytic OsO_4 gave only the triol derivative, which was protected as acetonide with *p*-TsOH in acetone and then oxidized with pyridinium chlorochromate, which led to the fortuitous β-elimination of acetic acid to afford the conjugate enone (+)-**120** in 59% overall yield based on **118**.

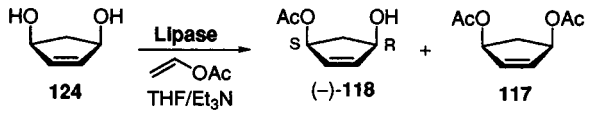
The same laboratory[50] proposes the enantioselective preparation of functionalized cyclopentanoids via a common chiral (π-allyl)palladium complex as a potentially useful intermediate to new routes to nucleoside analogues. The attractiveness of this procedure is enhanced by the ready availability of optically active starting material. Either antipodal form (+)-**118** or (−)-**118** is procurable in gram quantities via a stereoselective enzymatic hydrolysis of *meso* diester **117**. The (1*S*,4*R*)-*cis*-4-acetoxy-1-hydroxycyclopent-2-ene [(+)-**118**] is secured in >99% ee by exposure of **117** to EEAC, while the enantiomer is obtained in 98% ee by the action of PLE. A subsequent palladium(0)-catalyzed substitution reaction affords optically active *cis*-4-substituted-2-cyclopenten-1-ols **119** and *ent*-**119**.

Also, a two-step reaction sequence to homoallylic nitro compounds from allylic alcohols has been presented.[51] This methodology serves as a centerpiece for the synthesis of an important carbocyclic nucleoside intermediate. Truly relevant to this study is the stereo- and regiospecific nitromethylation of the optically pure starting material (+)-**118**. The undertaking began with the ethoxycarbonilation of (+)-**118**, followed by the nitromethylation step. Finally, the *cis*-dihydroxylation−protection sequence afforded the expected acetonide (−)-**122** (Scheme 30). This compound has been used as advanced nucleoside precursor in the formal synthesis of the notable carbocyclic nucleoside (−)-aristeromycin A (**123**).

In addition to enzymatic hydrolysis, lipase-catalyzed transesterification is a very useful option to asymmetrize *meso*-cyclopentane diols. Thus, lipase-catalyzed transesterifications of the substrate **124** with vinyl acetate were performed in THF−Et_3N as standard conditions. Pancreatin[52] and the lipase from

Mucor sp.[53] were the enzymes of choice. The most relevant results are collected in the table of Scheme 31.

Scheme 31

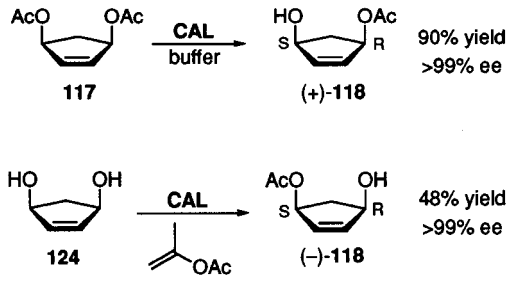


Lipase	Yield (%)	[α][a]	Yield (%)	ee (%)
Pancreatin	65	-66.0°	32	99
Mucor sp.	85	-62.0°	10	94

a. Reference: $[\alpha]_D^{20}$ = -69.6° (c 1.00, $CHCl_3$) is >99% ee

As mentioned previously, the synthetic potential of enzymes is fully realized in the asymmetrization of prochiral or *meso* substrates, which can be completely processed to a single enantiomer. Thus, the enzymatic asymmetrizations of *meso*-2-cycloalken-1,4-diols or the corresponding diacetates utilizing CAL in organic or aqueous media have been reported (Scheme 32).[54] The diacetate **117** was hydrolyzed

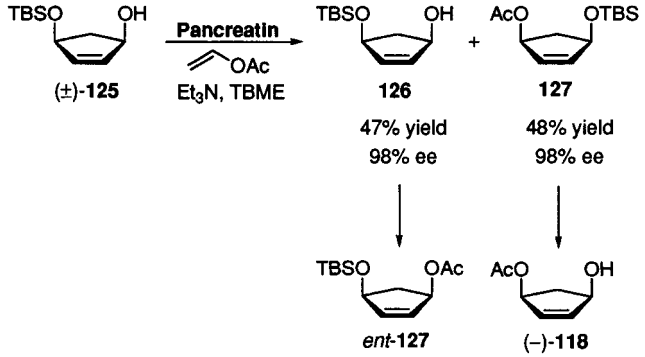
Scheme 32



with CAL (pH 8.0 phosphate buffer, 25 °C for 2 h) to produce monoacetate (+)-**118** in 90% yield. The optical purity was determined to be >99% based on the observed optical rotation. Similar results were obtained for CAL-catalyzed transesterification of *meso* diol **124** with isopropenyl acetate in organic media at 50 °C in 2 h. The monoacetate (−)-**118** was produced in 48% yield, and the optical purity was determined to be >99%.

In the resolution of *cis*-2-cyclopenten-1,4-diol, many procedures give rise to significant amounts (30−40%) of diacetate. Another observation is that substituents on the cyclopentenyl ring system improved the yield. For these reasons, Curran and Hay[55] were interested in a process to generate optically active *cis*-2-cyclopenten-1,4-diol derivatives in which both resolved materials could be used to prepare identical carbocyclic isomers. Such a process would require that the enantiomeric purity of each product from the resolution would need to be >95%. Thus, they communicate the resolution of *cis*-4-*O*-TBS-2-cyclopenten-1,4-diol [(±)-**125**, Scheme 33] using different enzymes in *tert*-butylmethyl ether (TBME) with isopropenyl or vinyl acetate as acylating agent. As can be seen in the scheme, pancreatin in TBME/Et_3N with vinyl acetate gave the best result and met their criterion for synthesis. Alcohol **126** was converted into acetate *ent*-

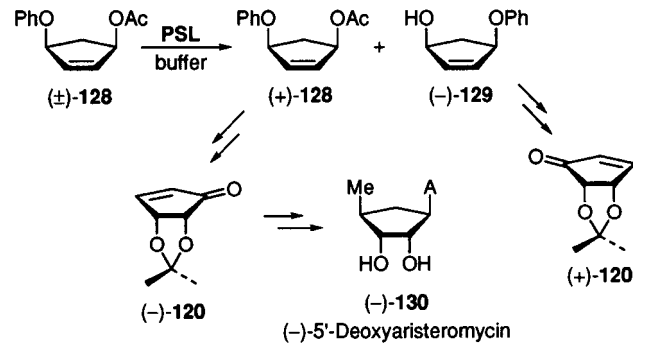
Scheme 33



127 in 98% yield, and the acetates were adequately separated by GC chiral column to assess optical purities, which were both >98%. Desilylation of **127** provided acetate (−)-**118**, which together with alcohol **126** allows for their conversion into analogues of isomeric carbocyclic compounds.

(−)-5′-Deoxyaristeromycin (−)-**130**, which showed an antiviral activity spectrum characteristic of that of *S*-adenosyl-L-homocysteine hydrolase inhibitors, has been prepared in 13 steps beginning with cyclopentadiene (Scheme 34).[56] The key step was the

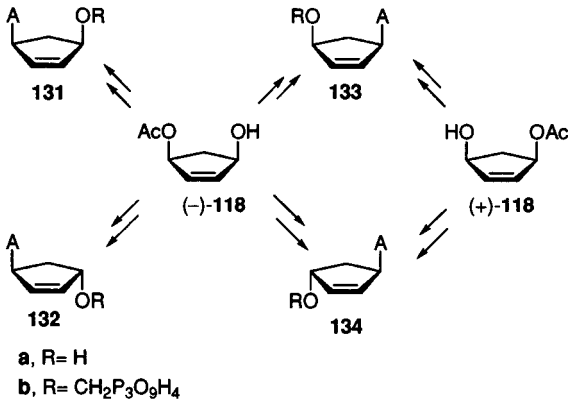
Scheme 34



enzymatic hydrolysis of a racemic mixture of (±)-**128** by means of PSL. The resulting derivatives (+)-**128** and (−)-**129** were easily separated. Then, conversion of (+)-**128** into enone (−)-**120** in high optical purity was carried out in 41% overall yield from cyclopentadiene. Enantiomer (+)-**120** was synthesized from (−)-**129** following a similar sequence of reactions. Starting with (−)-**120**, a synthesis of (−)-5′-deoxyaristeromycin [(−)-**130**] was designed.

Theil and co-workers studied the synthesis of stereoisomeric carbocyclic (2′,3′-dideoxy-2′,3′-didehydro)-5′-noradenosine derivatives[57] and their triphosphate analogues[58] in enantiomerically pure form (Scheme 35). The key feature of the new method consists of regiocontrolled switching of the functional groups of the enantiomerically pure allylic acetate (−)-**118** and using different types of stereocontrolled nucleophilic substitutions. Monoacetate (−)-**118** can be prepared by lipase-catalyzed transesterification of *cis*-cyclopent-2-ene-1,4-diol **124** or by hydrolysis of the corresponding diacetate **117** with PLE. Using the monoacetate **118** as starting material, it was possible to prepare the four possible stereoisomeric carbocyclic nucleoside analogues (R = H, two enantiomeric pairs

Scheme 35

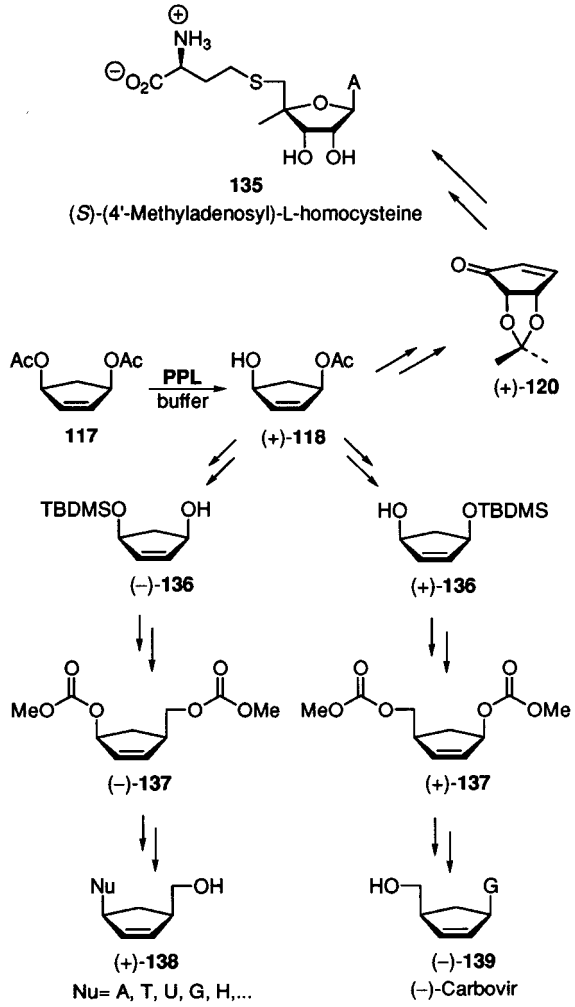


of diastereoisomers **131**/**133** and **132**/**134**). Due to the symmetry of (−)-**118**/(+)-**118**, the enantiomeric monoacetate (+)-**118** can serve as starting material in a similar way. To test the biological activity of inhibiting a target enzyme like HIV reverse transcriptase, the nucleoside analogues required activation by the corresponding triphosphates. Due to the biological activity shown by diphosphorylphosphonates of carbocyclic 5′-nornucleoside analogues (R= $CH_2P_3O_9H_4$), these were prepared through a diphosphorylation of the corresponding phosphonates.

Compounds that interfere with the transmethylases have potential for therapeutic intervention in the viral replication process. Johnson and co-workers[59] described the synthesis of 4-*C*-substituted-D-ribose derivatives as well as that of the *S*-(4′-methyladenosyl)-L-homocysteine by a chemoenzymatic strategy beginning from cyclopentadiene as good potential inhibitors of transmethylases. The implementation of this strategy required a convenient route to 4-substituted riboses, and a chemoenzymatic approach was the best option because it allowed access to both the D and L series. The stereoselective strategy involving the readily available enantiopure carbocyclic precursor (+)-**120** and its application to the synthesis of *S*-(4′-methyladenosyl)-L-homocysteine (**135**) is summarized in Scheme 36. For the synthesis of enone (+)-**120**, the very inexpensive crude PPL was used. Also, the same key compound (+)-**118** was used as the starting point for the synthesis of (+)- and (−)-carbovir analogues.[60] Enantiomeric alcohols (−)-**136** and (+)-**136** were synthesized from monoacetate (+)-**118** in two and four steps, respectively. The different carbovir analogues (+)-**138** were obtained from (−)-**136** by nucleophilic substitutions with different nucleophiles in derivative (−)-**137**. A similar procedure from (+)-**136** yielded (+)-**137** and then carbovir (−)-**139**.

Using synthon precursor **117**, several aristeromycin analogues have been designed through chemoenzymatic routes (Scheme 37).[61] Thus, treatment of the diacetate **117** with PSL gave monoalcohol (+)-**118**, which was used to prepare 5′-noraristeromycin (**140**) and (−)-7-deaza-5′-noraristeromycin (**141**). On the other hand, subjecting **117** to treatment with PLE led to an efficient synthesis of substituted cyclopentane precursor (−)-**118**, which can be converted into homoaristeromycin (**142**). The same monoacetate (−)-**118** was transformed into the methylated derivative

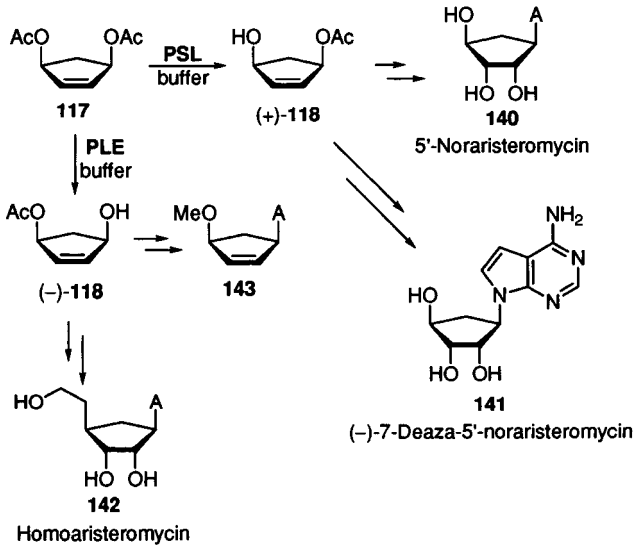
Scheme 36



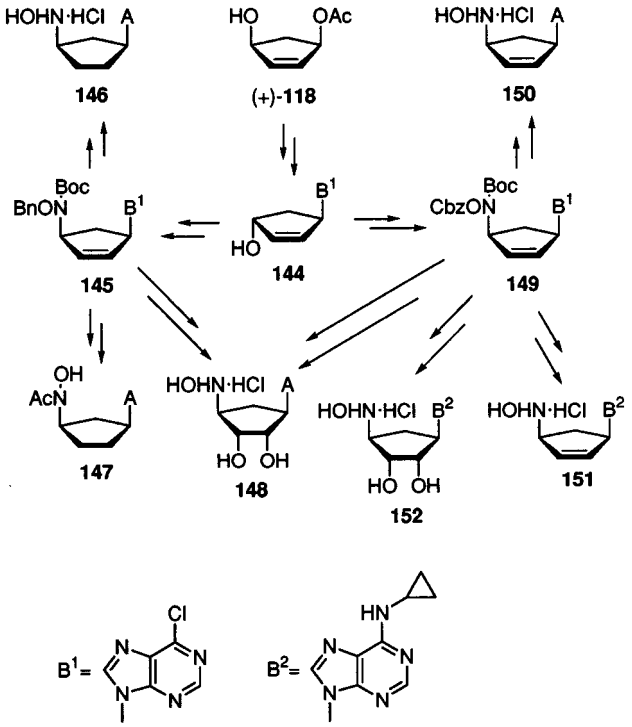
143 of 5′-noraristeromycin.[62] To determine if the potent antiviral properties of 5′-noraristeromycin reside in one of its enantiomers, syntheses of both were carried out.[63] Thus, a five-step route to (+)-**140** was described from (+)-**118**, while the synthesis of (−)-**140** has been previously reported.[61]

Recognizing the need for a better understanding of the structure−activity relationships among nucleo-

Scheme 37



Scheme 38

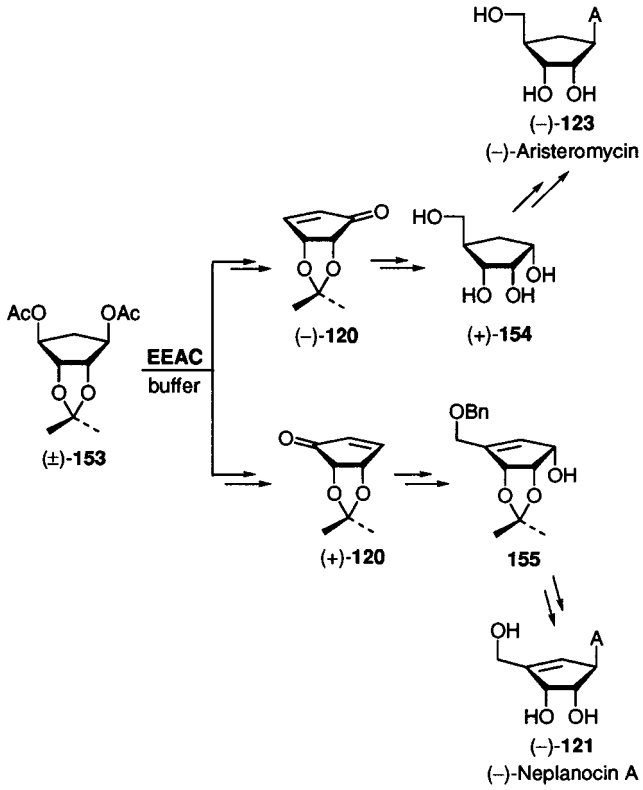


sides, Mulvihill and Miller[64] postulated that a hydroxylamine moiety at the 4′-position could serve as an isostere to hydroxymethyl. Thus, they disclose the synthesis of six novel enantiomerically pure hydroxylamine carbanucleosides **146**−**148** and **150**−**152** (Scheme 38). Initially, target molecules **146**−**148** were envisioned to be derived from one advanced intermediate, **145**. The synthesis of enantiomerically pure **145** began with the allylic alcohol (+)-**118**, readily prepared from diacetate **117** following the Deardorff enzymatic methodology applied to gram quantities.[65] Having demonstrated that novel hydroxylamine analogues **146**−**148** were stable isolable compounds in deprotected form, the project was aimed toward the syntheses of other unique but related hydroxylamine carbanucleosides **150**−**152** by also utilizing a common advanced intermediate **149**.

Due to the importance of desymmetrization of *meso*-cyclopentenediol **124**, Kalkote, Ravindranathan, and co-workers[66] successfully demonstrated its utility through irreversible transesterification by a parameter-optimization approach. Thus, diol **124** was monoacylated with vinyl acetate in the presence of Chirazyme in TBME at 4 °C to afford (−)-**118** in >60% yield with >98% ee. The results indicate a strong possibility of exploitation of Chirazyme, which is available commercially in bulk, for the development of economically viable technology for large-scale production of (−)-**118**.

It was supposed that compound **154** (Scheme 39) was an intermediate in the biosynthesis of aristeromycin (**123**). Parry, Johnson, and co-workers[67] synthesized the enantiomerically pure cyclopentanetetrol (+)-**154** to demonstrate its role in the biosynthesis. Thus, administration of doubly labeled forms of D-glucose to the fermentation broth of *Streptomyces citricolor* allowed the isolation of the tetrol (+)-**154** using isotope dilution methods, and through this way

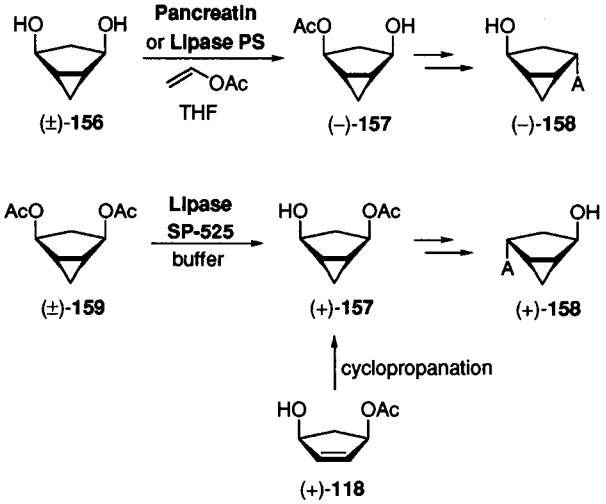
Scheme 39



evidence was provided for the intermediacy of a 4*β*-hydroxymethyl-1α,2α,3α-trihydroxycyclopentanetriol (**154**) in the biosynthesis of the nucleoside antibiotic aristeromycin [(−)-**123**]. The enantioselective synthesis of **154** was accomplished from the known[68] cyclopentenone derivative (−)-**120**, which was obtained from (±)-**153** through enzymatic hydrolysis with EEAC at 25 °C. Likewise, neplanocin A (**121**), a naturally occurring but scarce analogue of the nucleoside adenosine, is a compound with significant antitumor as well as antiviral activity and has high interest as a clinical candidate. Due to these properties, a total synthesis[69] has been reported using cyclopentadiene as starting material (Scheme 39). Thus, racemic diacetate (±)-**153** was obtained, which was transformed into (+)-**120**[70] through a chemoenzymatic procedure using enzymatic hydrolysis with EEAC. With enone **120** readily available, the most direct route to ketone **155** appeared only to require introduction of the benzyloxymethyl moiety and a subsequent oxidative rearrangement of the tertiary allylic alcohol. Successive transformations gave (−)-neplanocin A (**121**) in 11% overall yield in 14 steps from cyclopentadiene.

b. Using Bicyclo[3.1.0]hexane Structures. Carbocyclic nucleoside analogues (−)-**158** and (+)-**158** were of interest as substrates to investigate the structure and properties of an allosteric inhibitory site of adenylyl cyclase (Scheme 40).[71] Their synthesis was carried out through the lipase-catalyzed enantioselective transesterification of the diol (±)-**156** with vinyl acetate in THF−triethylamine in the presence of Pancreatin or lipase PS yielding (*S*)-monoacetate (−)-**157** in high chemical yield and in almost enantiomerically pure form. Although lipases show the same stereopreference under conditions of trans-

Scheme 40

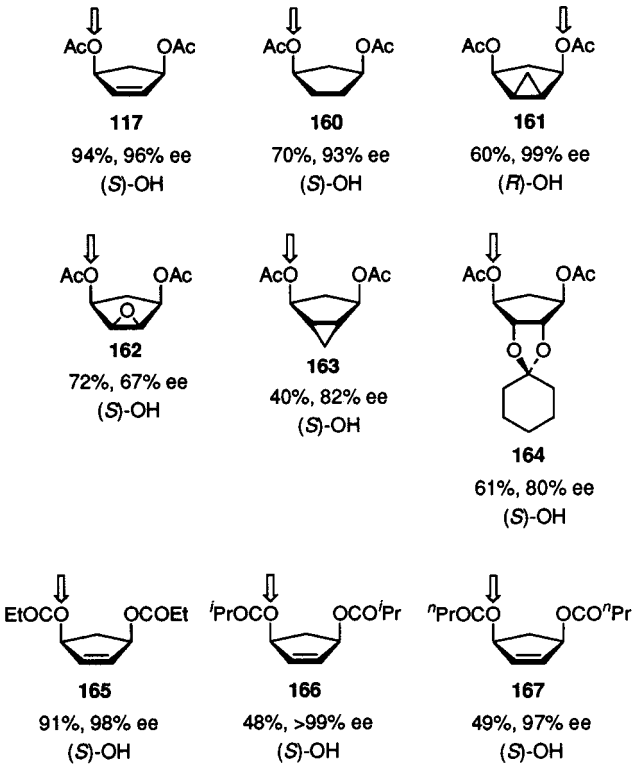


esterification and hydrolysis, none of the previous lipases were able to catalyze the hydrolysis of the diacetate (±)-**159**. Testing several lipases it was found that lipase SP-525 from Novo was the enzyme of choice to hydrolyze the diacetate **159** to furnish the chiral monoacetate (+)-**157** in 70% yield with >99% ee. Alternatively, (+)-**157** was prepared by cyclopropanation of the enantiomerically pure monoacetate (+)-**118**. By an enantiodivergent approach, the monoacetate (−)-**157** was used both to synthesize bicyclonucleoside (−)-**158** as well as to prepare the corresponding enantiomeric nucleoside analogue (+)-**158**. Also, to shorten the synthetic scheme and to improve the overall chemical yield of the adenine derivative (+)-**158**, another synthetic pathway was performed starting from the enantiomerically pure monoacetate (+)-**157**. Studies about both enzymatic and cyclopropanation processes have already been made.[72]

Enantiomerically pure cyclopentanoids are important building blocks for the preparation of carbocyclic nucleosides as was shown previously. Deardorff and co-workers[48] showed the use of the enzyme EEAC to resolve 1,3-*meso*-diesters as **117**, even at 10-g scale.[65] This result prompted them to explore further the scope and limitations of using EEAC for the preparation of chiral, nonracemic cyclopentanoids. They reported[73] on the remarkable substrate enantioselectivity that EEAC had exhibited toward a series of structurally related *meso*-diesters derived from 1,3-cyclopentanediols **117**, **160**−**164**, as well as structural variants in the acyl moieties **117**, **165**−**167** (Scheme 41). Details of the enzyme's substrate specificities and stereoselectivities gleaned from this study may eventually prove useful in the development of an experimental active-site model of predictive merit.

c. Using 3,5-*cis*-Diacetoxy-4-*trans*-benzyloxymethylcyclopentene. LeGrand and Roberts[74] reported a formal synthesis of both enantiomers of aristeromycin (Scheme 42). They started from the hydrolysis of the diester (±)-**168** by PPL giving rise to monoester (+)-**169** (92% yield, >95% ee). Subsequent transformations led to a mixture of products **170** and **171**. The latter was converted into aminotriol (−)-**172**, which has been previously described,

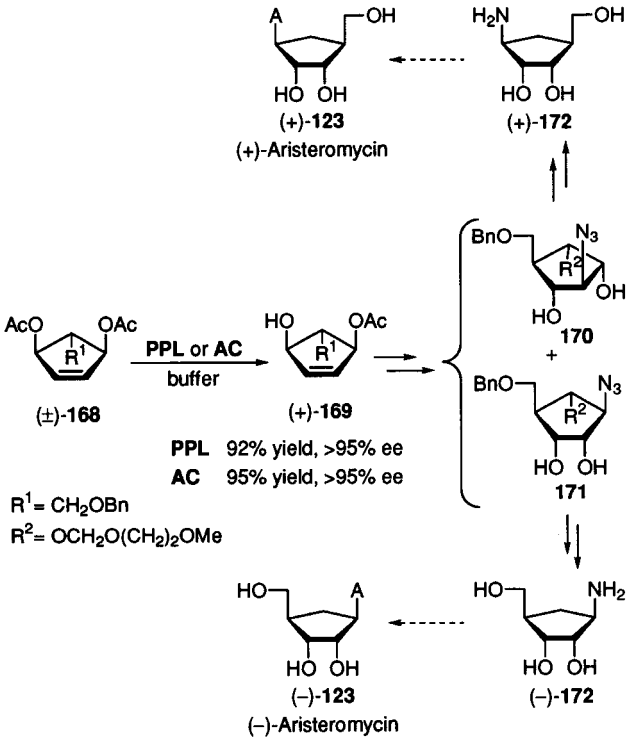
Scheme 41



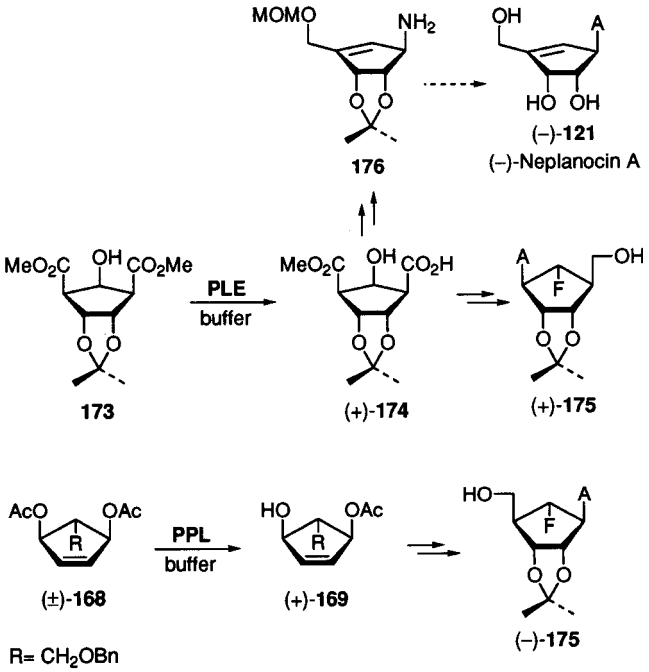
and used to synthesize (−)-aristeromycin [(−)-**123**]. Similarly, compound **170** was transformed into the enantiomer of the above-mentioned aminotriol, derivative (+)-**172**, and used to obtain the (+)-aristeromycin [(+)-**123**].

d. Using 1,3-Cyclopentanedicarboxylates. Zemlicka and co-workers[75] reported the hydrolysis of the *meso*-diester **173** with PLE (Scheme 43). On the basis of X-ray data alone, they misassigned the structure

Scheme 42



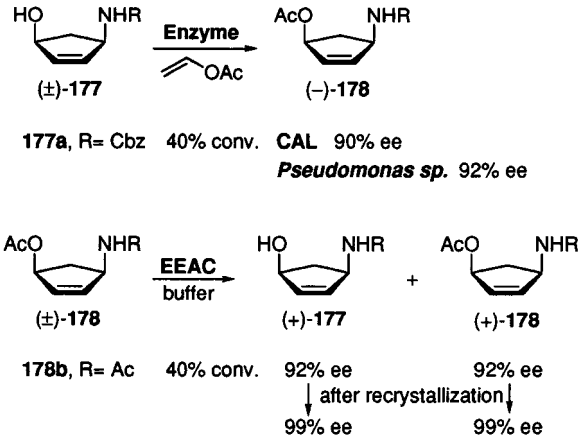
Scheme 43



of the product of the PLE-catalyzed hydrolysis as the enantiomer of monoester (+)-**174**. Later, Roberts' group[76] confirmed the structure of (+)-**174**, converting this product into the known fluorine-containing carbocyclic nucleoside (+)-**175**. Additional proof was the preparation through a different starting material, compound **168**, of its enantiomer (−)-**175**. Thus, they described a series of stereocontrolled reactions starting from (+)-**169**, which was obtained from enzymatic hydrolysis of **168** with PPL, to give carbocyclic nucleoside (−)-**175**. To provide more convincing evidence that the product obtained on PLE-catalyzed hydrolysis of **173** is compound (+)-**174** and not its enantiomer, they reported the preparation of amine intermediate **176** en route to (−)-neplanocin A using two different approaches, one from the monoester (+)-**174**[77] and a complementary one from D-ribose.[78]

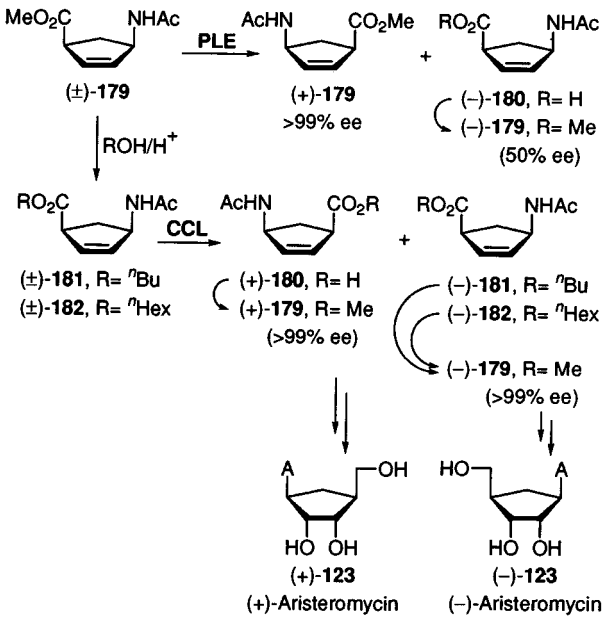
e. Using *cis*-4-Aminocyclopent-2-en-1-ol. Since 4-aminocyclopent-2-enol precursors can serve as chiral building blocks for D- and L-carbocyclic nucleoside derivatives, the need for a concise, inexpensive route to them was recognized. Miller and co-workers[79] reported a successful enantiodivergent synthesis of 4-aminocyclopent-2-enols using enzymatic procedures, which alleviate the need for a chiral auxiliary. Thus, (±)-*cis*-4-aminocyclopent-2-en-1-ols (±)-**177** or their corresponding acetates (±)-**178** were kinetically resolved by enzymatic acetylation or hydrolysis, respectively (Scheme 44). For example, enzymatic acetylation of *cis*-*N*-(benzylcarbamoyl)-4-aminocyclopent-2-enol [(±)-**177a**] with CAL and *Pseudomonas sp*. lipase gave the corresponding acetate (−)-**178** in 90% and 92% ee, respectively, after 40% conversion. Enzymatic hydrolysis of *cis*-*N*-acetyl-4-aminocyclopent-2-enol 1-*O*-acetate (±)-**178b** with EEAC was successful in providing both *cis*-*N*-acetyl-4-aminocyclopent-2-enols (+)-**177b** and (+)-**178b** in 92% ee (99% ee after a single recrystallization) after 40% conversion.

Scheme 44



f. Using *cis*-4-Acetamidocyclopent-2-ene Carboxylate. Csuk and Dörr[80] in their research about inhibitors of *S*-adenosylmethionine synthetase became interested in both enantiomers of aristeromycin, the carbocyclic analogue of adenosine. The strategy selected included an efficient separation of the respective enantiomers at an early stage of the synthetic scheme by means of an enzymatic process. The key intermediate in this approach was (±)-**179**, which can be obtained in large quantities (Scheme 45). Their results for the reaction of (±)-**179** with PLE

Scheme 45

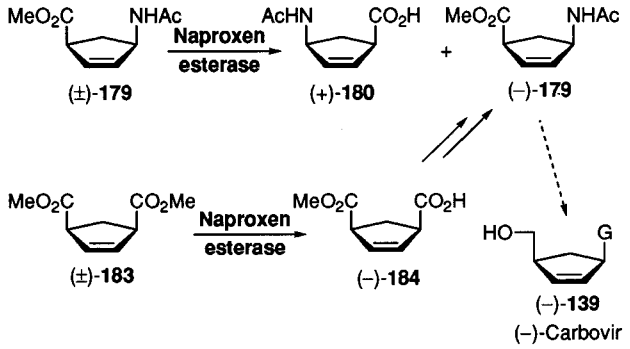


are in striking contrast to previous findings,[81] and although the unreacted ester (+)-**179** was enantiomerically pure, the acid (−)-**180** was esterified to find the ee of ester (−)-**179**, which turned out to be approximately 50%. Due to the unsuccessful enzymatic resolution with PLE, (±)-**179** was transesterified with *n*-butanol and *n*-hexanol to the corresponding alkyl carboxylates (±)-**181** and (±)-**182**, respectively. Then, enzymatic hydrolysis with CCL afforded the unreacted esters (−)-**181**, (−)-**182**, and the enantiomerically pure acid (+)-**180** checked by transformation in the corresponding methyl esters (−)-**179**

and (+)-**179**. Later, the same authors[82] completed the synthesis from (+)-**179** and (−)-**179** to (+)-aristeromycin and (−)-aristeromycin, respectively.

The same key intermediate (±)-**179** was used to formally prepare (−)-carbovir [(−)-**139**].[83] Scheme 46

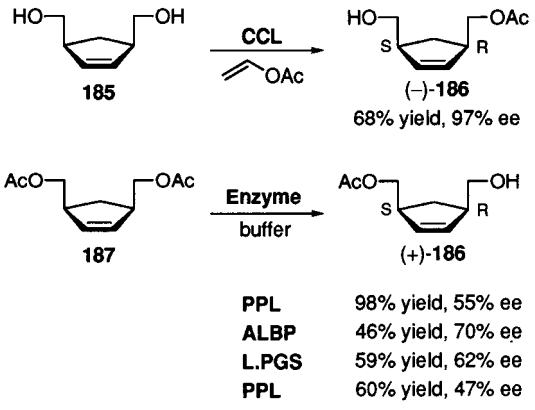
Scheme 46



shows the resolution of (±)-**179** with naproxen esterase to yield 40% of (−)-**179** with 94% ee. In addition to this approach, ester (−)-**179** can also be synthesized in high enantiomeric excess via an enzymatic enantiotopic hydrolysis of the *meso* diester **183** also using naproxen esterase.

g. Using *meso*-1,3-Bis(hydroxymethyl)cyclopentane and Related Structures. In the synthesis of carbocyclic analogues of nucleosides, *cis*-cyclopentene-1,3-dimethanol monoacetates (−)-**186** and (+)-**186** are useful intermediates as the hydroxymethyl group can be easily converted into the carboxylic acid and further into the amine. Thus, the availability of (−)-**186** and (+)-**186**, which contain two stereogenic carbons, in an enantiomerically pure form is essential. Mekrami and Sicsic[84] performed the asymmetrization by enzymatic acetylation of the diol **185** or enzymatic hydrolysis of the diacetate **187** (Scheme 47). They tried several lipases and esterases for both

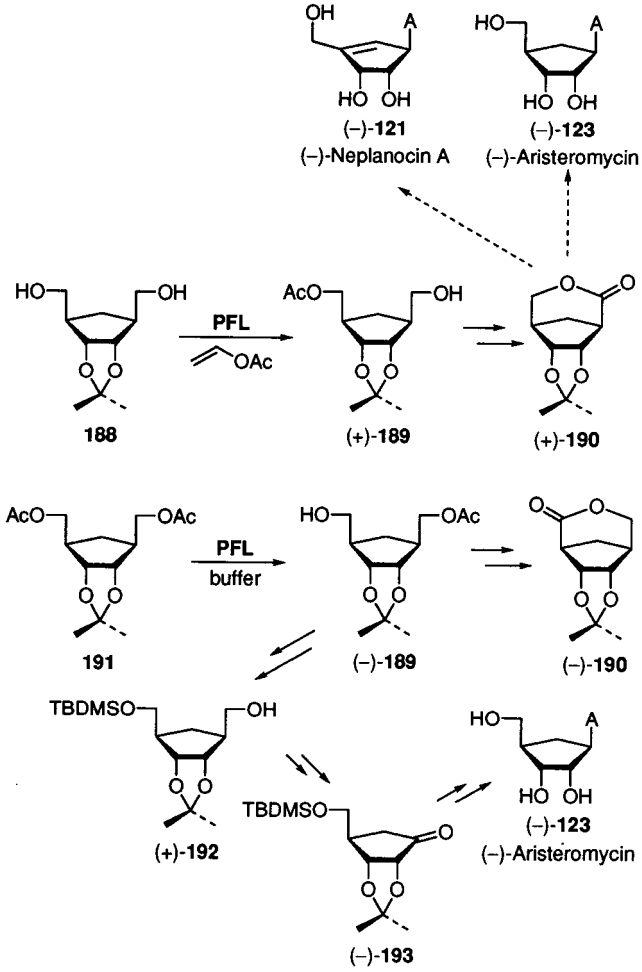
Scheme 47



proccesses. Only the synthesis catalyzed by CCL had both a good chemical yield and an excellent ee of 97%. In the cases of hydrolysis, only PPL gave exclusively monohydrolysis, but the ee of the monoacetate produced is not satisfactory. The best ee is obtained with ALBP (acetone liver beef powder) and L.PGS (lipase genetically modified from Plant Genetic System), but the chemical yields of the monoacetate are low. It was remarkable that the preferentially reactive group is attached in all cases to the carbon that has the *R*-configuration. In another communication[85] they used PPL in the enzymatic hydrolysis, but the chemical yield was not good and the ee was just just 47%.

Sakai and co-workers[86] described the synthesis of (−)-aristeromycin [(−)-**123**] via PFL-catalyzed asymmetric hydrolysis of the *meso*-diacetate **191** or transesterification of the *meso*-diol **188** (Scheme 48). Thus,

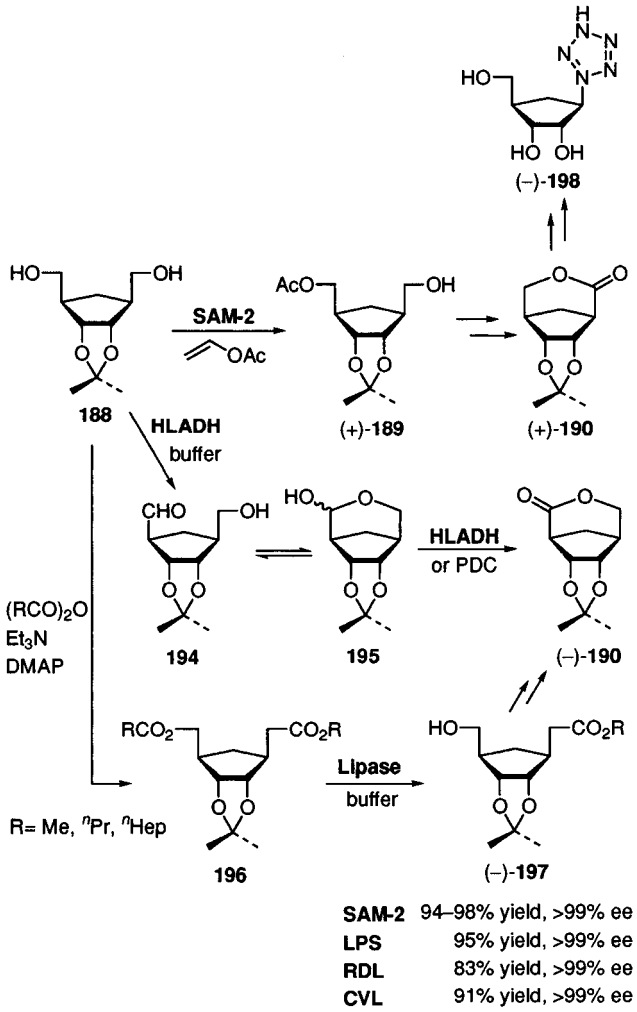
Scheme 48



hydrolysis of **191** using PFL in a phosphate buffer afforded the monoacetate (−)-**189** in 71% yield (>99% ee). On the other hand, PFL-catalyzed transesterification of **188** with vinyl acetate yielded the monoacetate (+)-**189** in 81% yield (>99% ee). Both reactions were carried out on the gram scale. From (+)-**189** it was possible to prepare lactone (+)-**190**, a precursor in the formal syntheses of (−)-aristeromycin (**123**) and (−)-neplanocin A (**121**). Furthermore, (−)-**189** with an undesirable configuration for the synthesis of (−)-aristeromycin can be converted into this following the route that goes through alcohol (+)-**192** and ketone (−)-**193**.

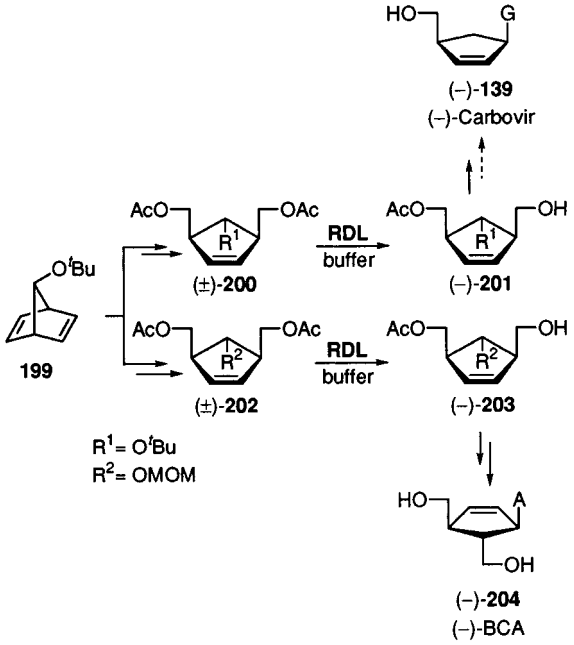
Kobe and co-workers[87] proposed compound **188** (Scheme 49) as an alternative precursor to Ohno's lactone, **190**. A comparison has been made between oxidoreductase (HLADH) and hydrolase (SAM-2, LPS, RDL, CVL) catalyzed transformations of *meso*-cyclopentane-1,3-dimethanol derivatives **188** and **196**

Scheme 49



in order to develop a synthetically useful procedure for enantiomerically pure carbocyclic ribonucleoside synthons, namely, (+)-**190** of proper absolute configuration. In the first case, HLADH-catalyzed oxidation of **188** resulted mainly in lactol **195**, which was further oxidized chemically rather than enzymatically to give lactone (−)-**190** with undesired absolute configuration as well as insufficient chemical (25%) and optical (74%) yields. On the other hand, SAM-2 lipase-catalyzed transesterification of **188** with vinyl acetate yielded the chiral half ester (+)-**189** with excellent chemical (89%) and optical (>99%) yields and a proper absolute configuration for further elaboration to the target lactone (+)-**190**. In addition, several lipases catalyzed hydrolysis of diesters **196**, providing enantiocomplementary half esters (−)-**197** of excellent optical purities (>96% ee), whose absolute configuration correlated with lactone (−)-**190**. This group,[88] using a methodology parallel to Sakai's work (Scheme 48) and just changing the enzyme to lipase SAM-2, have also prepared both enantiomers of Ohno's lactone, namely, (+)-**190** and (−)-**190**. Alternatively, using immobilized PLE and following the asymmetrization described by Ohno, subjected to certain modifications on a synthetic scale with respect to the original small-scale experiment, lactone (+)-**190** was obtained and then transformed into carbocyclic tetrazole *C*-nucleoside **198**.

Scheme 50

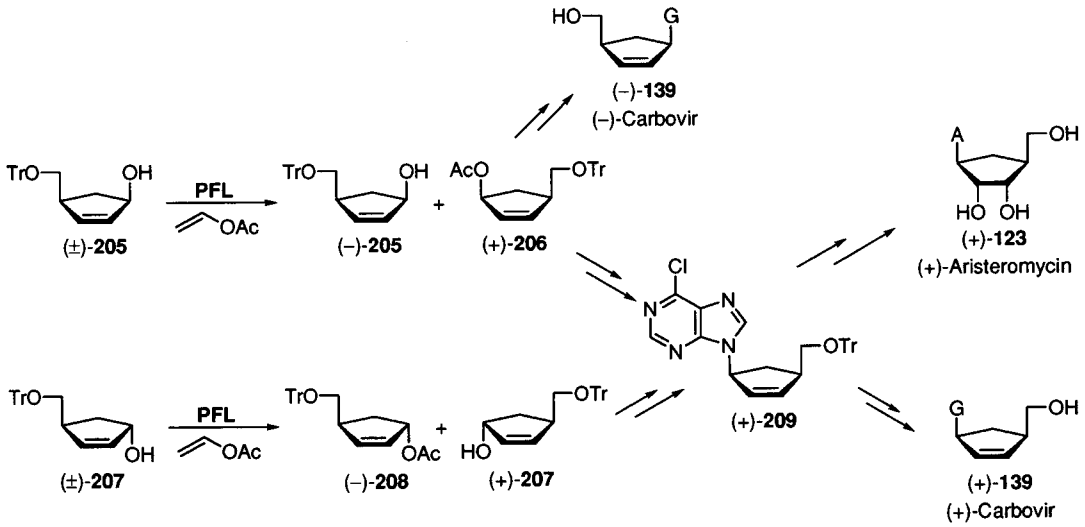


Tanaka and co-workers[89] used asymmetric hydrolysis with *Rhizopus delemar* lipase (RDL) to prepare antiviral carbocyclic nucleosides (Scheme 50). Thus, 7-substituted norbornadiene **199** was stereoselectively converted into the *meso*-3,5-bis(acetoxymethyl)cyclopentenes **200** and **202** by a three-step sequence of ozonolysis, reduction, and acetylation. RDL asymmetric hydrolysis of *meso*-acetates **200** and **202** afforded the monoalcohols (−)-**201** and (−)-**203**, respectively, with high enantiomeric purities (>95%) in good yields (95%). The monoalcohols (−)-**201** and (−)-**203** obtained have been applied to the formal synthesis of antiviral carbocyclic nucleoside (−)-carbovir [(−)-**139**] and the total synthesis of carbocyclic nucleoside derivative (−)-BCA [(−)-**204**].

h. Using *cis*- and *trans*-4-Hydroxycyclopent-2-enylmethanol. Roberts and Shoberu[90] described a method for the preparation of chiral synthons for the production of various carbocyclic nucleosides (Scheme 51). Thus, reaction of trityloxymethylcyclopentenol (±)-**205** with vinyl acetate using PFL as catalyst gave a 98% yield of equal amounts of the readily separated alcohol (−)-**205** and the acetate (+)-**206**. Both compounds showed excellent optical purities (>95%). Similarly, alcohol (±)-**207** was acetylated using PFL and vinyl acetate. Roughly equal quantities of the alcohol (+)-**207** and the acetate (−)-**208** (combined yield 100%, 74% ee for both compounds) were obtained. Compounds (+)-**206** and (+)-**207** are extremely useful building blocks for the preparation of optically active carbocyclic nucleosides. Both trityl derivatives were converted by separate routes into the 2′,3′-dideoxydidehydronucleoside (+)-**209**. The later was transformed with high yield into (+)-aristeromycin **123** or (+)-carbovir **139**. The same group[91] also designed a route to (−)-carbovir using the enzymatic asymmetrization of alcohol (±)-**205** through the acetate (+)-**206**.

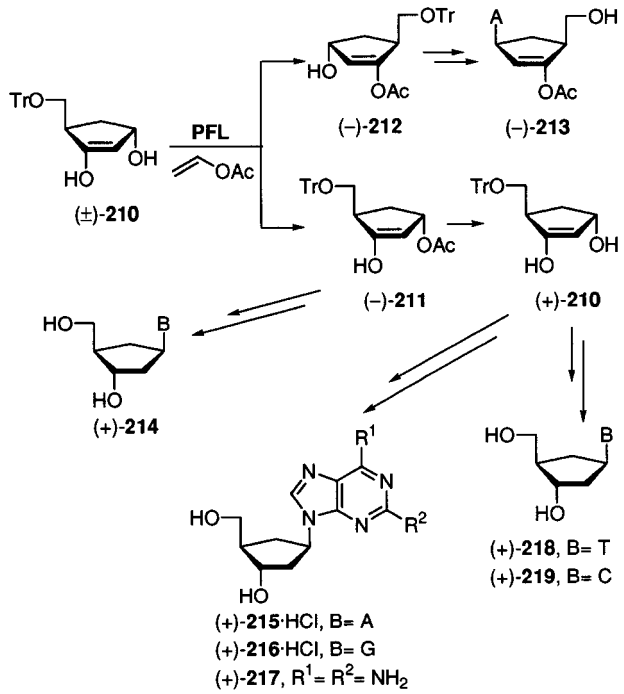
i. Using *cis*-1,3-Cyclopentanediol Derivatives. The enzyme-catalyzed acetylation of racemic deriva-

Scheme 51



tives similar to (±)-**210** (different substituents from trityl moiety) in vinyl acetate was greatly influenced by the size of the protecting groups at the additional *trans*-4-hydroxymethyl function (Scheme 52).[92] The highest regio- and enantioselectivities were obtained using the diol substrate protected with the bulky triphenylmethyl group. Thus, the racemic diol (±)-**210** was easily resolved by enzymatic transesterification. PFL in vinyl acetate catalyzed the acetylation of (±)-**210** to give, in excellent yield, roughly equal amounts of two compounds which were the regioisomeric monoacetates (−)-**211** and (−)-**212**. Both compounds were used by the same group[93] to prepare enantiomerically pure carbocyclic 2′-deoxyribonucleosides. Thus, enantiomerically pure acetate (−)-**212** was the starting compound to get carbocyclic nucleoside (−)-**213**. In analogy to this conversion, the monoacetate (−)-**211** of the natural series was converted to the carbocyclic 2′-deoxyribonucleoside (+)-**214**. Using the derivative (−)-**211** as a starting point, diol (+)-**210** was prepared by simple deprotection of the secondary alcohol. The latter was used as a key intermediate to obtain purine carbocyclic nucleoside (+)-**215**·HCl derived from adenine, (+)-**216**·HCl derived from guanine, and diamino derivative (+)-**217** as well as pyrimidine carbocyclic nucleosides (+)-**218** derived from thymine, and (+)-**219** derived from cytosine.

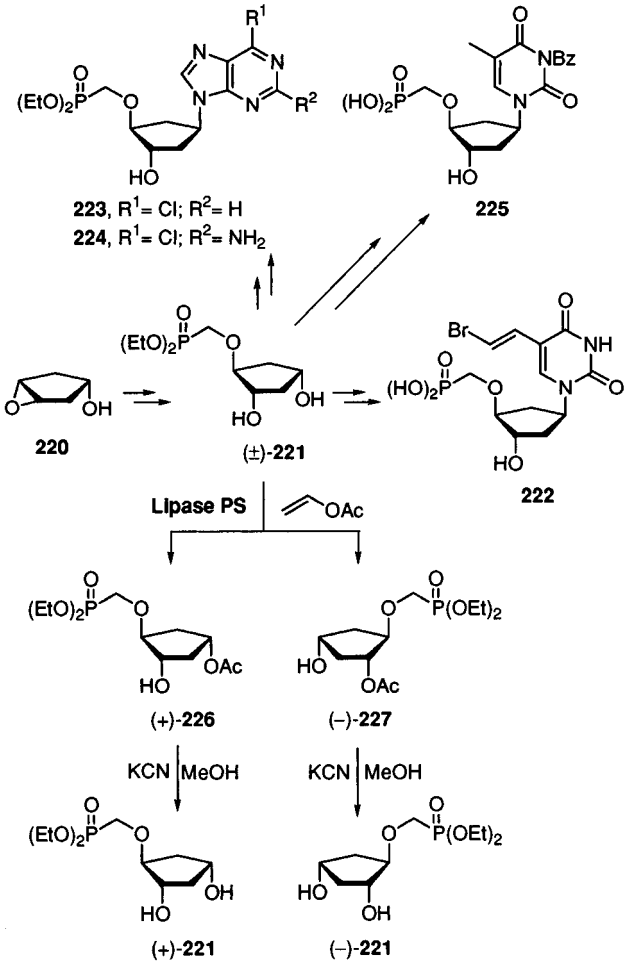
Scheme 52



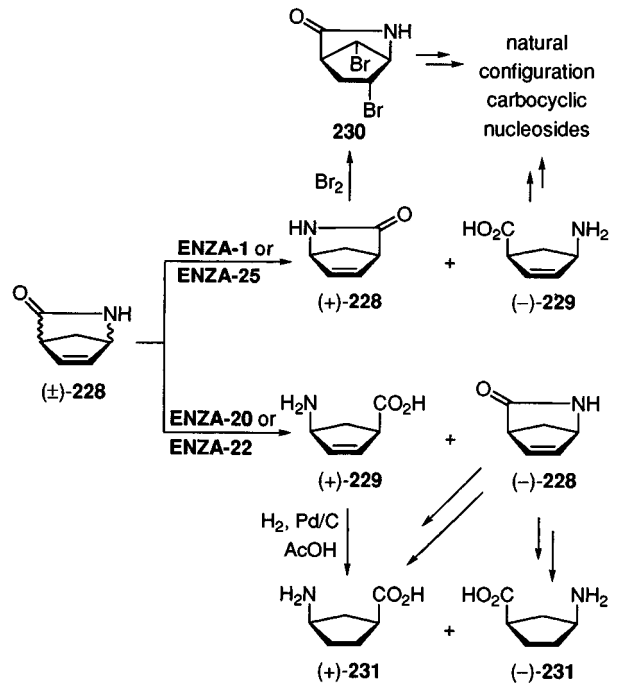
Roberts and co-workers prepared an isostere of carbocyclic 5-bromovinyldeoxyuridine[94] (C−BVDU) **222** and nucleotide minics[95] **223**, **224**, and **225** from diol (±)-**221**, which was synthesized from epoxy alcohol **220**, to check their activity against HSV (Scheme 53). They have also proposed the resolution of optically active dextrorotatory diol (+)-**221** and levorotatory diol (−)-**221** by means of enzymatic transesterification with lipase PS in vinyl acetate affording equal amounts of the acetates (+)-**226** and (−)-**227**. Both compounds were deacetylated with potassium carbonate in methanol to give the enantiomeric diols (+)-**221** and (−)-**221**. Additionally, the absolute configurations of the enantiomers were deduced by CD spectroscopy. The optically active diols (+)-**221** and (−)-**221** were not converted into optically active derivatives since the racemic mixtures were shown to be inactive against herpes simplex virus-1 in vitro.

j. Using 2-Azabicyclo[2.2.1]hept-5-en-3-one. The azabicyclo **228** is a versatile intermediate in the synthesis of carbocyclic nucleosides. Roberts and co-workers[96] described the use of two distinct whole-cell biocatalysts in the kinetic resolution of the racemic azanorbornenone **228** to generate both enantiomers with very high optical purities (>98%). Thus, in Scheme 54, enantiospecific and enantiocomplementary hydrolyses of compound **228** were catalyzed by whole-cell preparations of microbial strains ENZA-1 (*Rhodococcus equi* NCIB 40213) and ENZA-20 (*Pseudomonas solanacearum* NCIB 40249). Noteworthy was the low concentration of enzyme, the high concentration of the substrate, and the speed of the biotransformation. Furthermore, the amino acids (+)-

Scheme 53

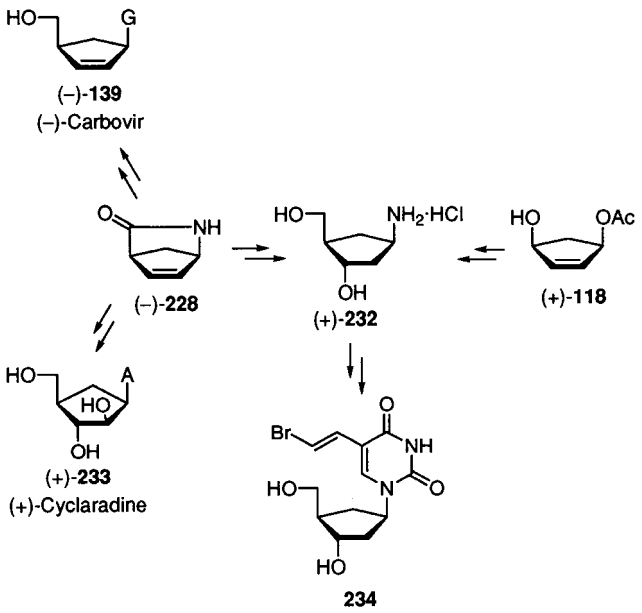


Scheme 54



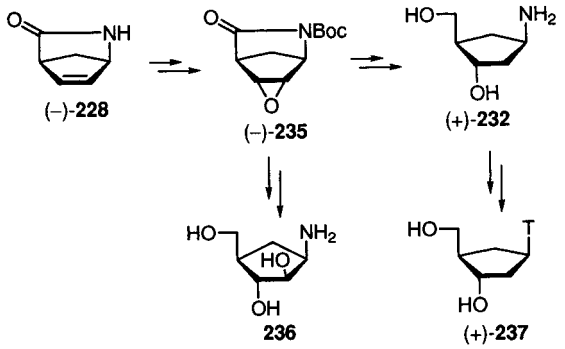
229 and (−)-**229** were also available from this simple biotransformation at high optical purity, this strategy being more convenient than that previously reported using an esterase.[81] Progressing from this, the same research group[97] described alternative microbial strains with lactamase capability having improved properties such as stability and degree of selectivity: ENZA-22 (*Pseudomonas fluorescens*) and ENZA-25 (*Aureobacterium species*). From the whole-cell preparations, lactamases were isolated and immobilized. These processes could be used at the multikilogram scale. To utilize the unwanted enantiomer (+)-**228**, an inversion process was developed via bromination, then there was a skeletal rearrangement to compound **230**. Thus, derivatives (+)-**228** and (−)-**229** can be used to prepare different natural configuration carbocyclic nucleosides. Through a similar procedure it was possible to obtain the unnatural enantiomer of the intermediate, which led to several unnatural configuration carbocyclic nucleosides. Another application by the same group[98] was the synthesis of the enantiomerically pure (−)-*cis*-3-aminocyclopentane carboxylic acid (−)-**231**, a GABA agonist, from (−)-**228** or its enantiomer (+)-**231** either from (+)-**229** or (−)-**228**. In addition, the use of the (−)-enantiomer of the lactam **228** as the precursor of the anti-HIV agent (−)-carbovir has been described in full (Scheme 55).[91] A method for the

Scheme 55



stereospecific synthesis of the 4-amino-2-hydroxy-1-hydroxymethylcyclopentane [(+)-**232**] was described starting from lactam (−)-**228** by the Glaxo research group.[99] These short and efficient syntheses provide rapid access to key intermediate (+)-**232** in the construction of 2′-deoxy carbocyclic nucleosides. The aminodiol (+)-**232** was prepared alternatively from monoester (+)-**118**, which was prepared in kilogram quantities in their pilot plant. Moreover, a highly efficient synthesis of the antiviral agent (+)-cyclaradine [(+)-**233**], carbocyclic arabinofuranosyladenine, having anti-HSV activity used (−)-**228** as a starting material.[100] Likewise, Wyatt and co-workers at Glaxo developed an optically pure and high-yielding synthesis of carbocyclic nucleoside analogue carba-BVDU **234** from aminodiol (+)-**232**.[101]

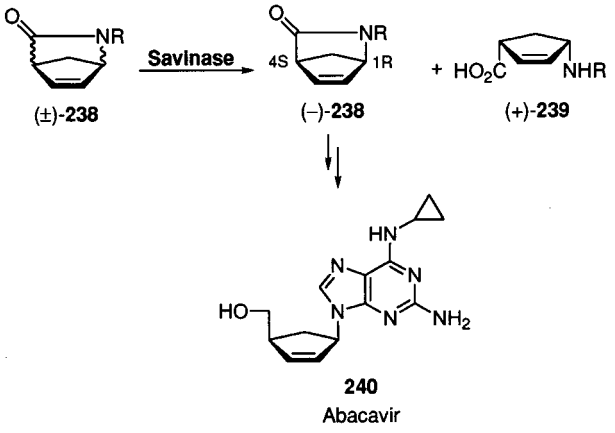
Scheme 56



Domínguez and Cullis[102] proposed the use of 5,6-epoxy-exo-2-azabicyclo[2.2.1]heptan-3-one (−)-**235** as a versatile intermediate in the synthesis of 2′-deoxy-, 3′-deoxy, and *ara*-cyclopentyl carbocyclic nucleosides. This epoxide and its enantiomer are synthesized enzymatically, as shown previously. Starting from (−)-**235**, it is possible to prepare carbocyclic 2′-deoxyribose analogue (+)-**232**. Additionally, the epoxide (−)-**235** can be converted into carbocyclic *ara*-ribose analogue **236**. Compound (+)-**232** was transformed into the corresponding (+)-carbocyclic thymidine derivative (+)-**237**.

An important step in the manufacture of abacavir **240**, an aminopurine nucleoside analogue that is a selective and potent reverse transcriptase inhibitor, is the preparation of enantiomerically pure *N*-substituted γ-lactams (−)-**238** (Scheme 57).[103] Activating

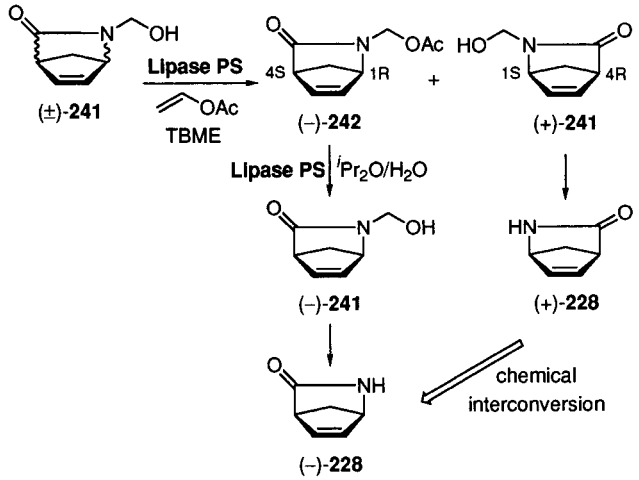
Scheme 57



the lactam ring with acyl protecting groups such as Boc or Ac would allow us to seek a conventional hydrolytic enzyme, rather than needing a specialized γ-lactamase, to hydrolyze the lactam bond of **238** enantioselectively. Savinase, inexpensive and available in bulk, was selected among a number of hydrolytic enzymes (esterases, lipases, proteases) because of being highly enantioselective [ee of (−)-**238** better than 99%], and (−)-**238** was isolated in good chemical yield (84%). This has formed the basis for the development of a simple and scalable process for preparation of enantiomerically pure *N*-substituted γ-lactams.

Hongo and co-workers[104] proposed another alternative to the whole-cell process of Roberts with the use of a lipase to resolve the enantiomeric mixture of (±)-

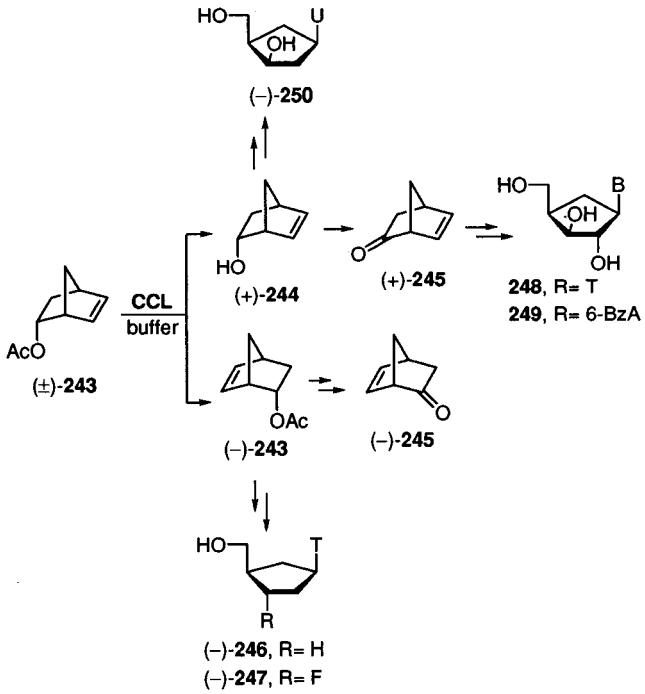
Scheme 58



228 with no production of the amino acid (Scheme 58). Thus, the racemic (±)-**241** was prepared easily from racemate (±)-**228** and paraformaldehyde. The subsequent transesterification with vinyl acetate and lipase PS in *tert*-butyl methyl ether (TBME) gave acetate (−)-**242** (38% yield, 94% ee) and the recovery of (+)-**241** (40% yield, 89% ee). The (+)-**241** was purified by recrystallization (>99% ee), and the hydrolysis of (−)-**242** using lipase PS gave *N*-hydroxymethyl form (−)-**241** in >99% ee. The (+)-**241** and (−)-**241** were converted conveniently into the (+)-**228** and (−)-**228** by removal of the *N*-hydroxymethyl group under basic conditions. This strategy has become more important since Palmer and McCague[105] reported the interconversion of one enantiomer of **228** into the other.

k. Using Norbornane-type Framework. Griengl and co-workers[106] reported the large-scale preparation of (+)- and (−)-*endo*-norbornenol by enzymatic hydrolysis (Scheme 59). Thus, they found that CCL

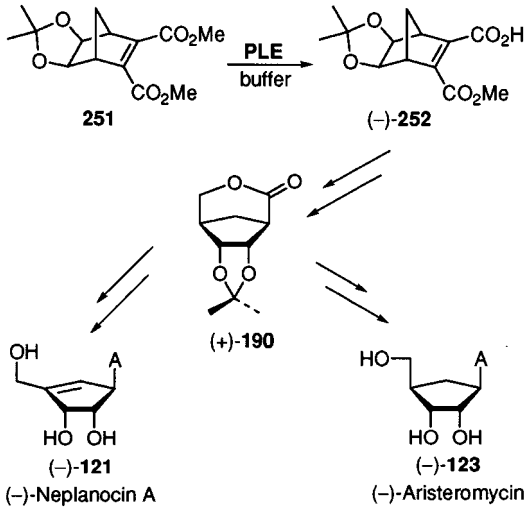
Scheme 59



catalyzed the enzymatic hydrolysis of *endo*-norbornenyl acetate (±)-**243** with high chemical and optical yield for both (+)-*endo*-norbornenol (+)-**244** and (−)-*endo*-norbornenyl acetate (−)-**243**. To accomplish this, the enzymatic hydrolysis was stopped when a conversion of 40% was reached. This furnished the alcohol (+)-**244** with 90% ee. The recovered acetate **243**, enriched in its (−)-enantiomer, was again subjected to enzymatic hydrolysis until an additional conversion of 20% was obtained. The remaining unreacted ester (−)-**243** was shown to be >96% ee. Verification of the absolute configuration was performed by oxidation of both enantiomeric alcohols **244** yielding (+)-norbornenone (+)-**245** and (−)-norbornenone (−)-**245**. The same group[107] has extended the study to other enzymatic resolutions of norbornane-type esters using lipases. Likewise, (−)-carba-2′,3′-dideoxythymidine [(−)-**246**] and (−)-carba-2′,3′-dideoxy-3′-fluorothymidine [(−)-**247**] were prepared from (±)-*endo*-5-norbornenyl acetate **243**.[108] The same strategy was applied to the synthesis of carbanucleosides derived from thymine (**248**) and from adenine (**249**).[109] Also, starting from the acetate of (+)-**244**, derivative (−)-**250** was synthesized in a six-step sequence.[110]

Ohno and co-workers[111] reported an efficient synthesis of the carbocyclic nucleosides (−)-aristeromycin [(−)-**123**] and (−)-neplanocin A [(−)-**121**] (Scheme 60).

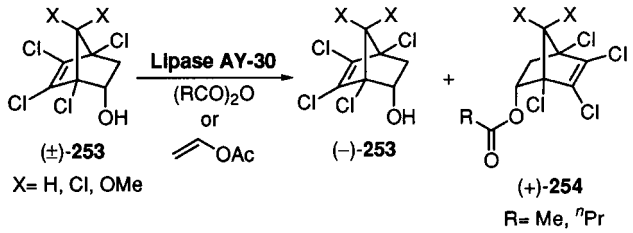
Scheme 60



They developed an enantioselective and stereocontrolled process starting from the Diels−Alder adduct **251** of cyclopentadiene and dimethyl acetylenedicarboxylate. The symmetric unsaturated dimethyl ester **251** was quantitatively hydrolyzed with PLE to yield a half-ester (−)-**252** with high optical yield (80% ee), but the optically pure material was most easily and preferably obtained by recrystallization of the *δ*-lactone (+)-**190**. Decarboxylative ozonolysis followed by chemical transformation afforded versatile chiral intermediates of cyclopentylamine and cyclopentenylamine that were converted to (−)-aristeromycin and (−)-neplanocin A, respectively.

Griengl, Faber, and co-workers[112] showed the role that the removal of the acid plays in an enzymatic acylation using anhydrides in substituted nor-

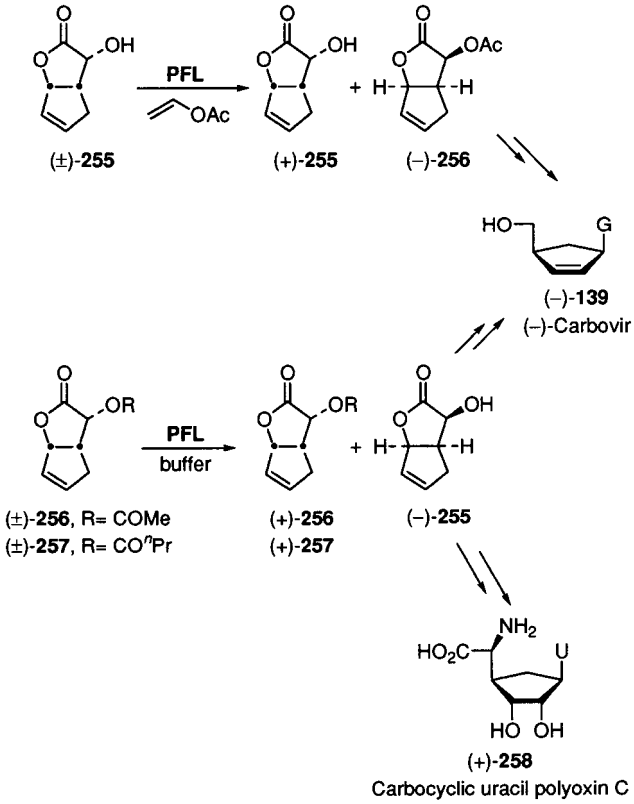
Scheme 61



bornenols. Thus, an efficient enzymatic resolution of 7,7-disubstituted-1,4,5,6-tetrachlorobicyclo[2.2.1]hept-5-en-2-ols **253** was accomplished by means of lipase AY-30 from *Candida cylindracea* in toluene. When acid anhydrides were used as acyl donors, the enantioselectivity was found to depend strongly on the reaction conditions: whereas low selectivity ($E < 20$) was observed without precautions taken in order to remove the coproduced acid, a more than 10-fold improvement was achieved with addition of a weak base ($E > 200$). Alternatively, adsorption of the biocatalyst onto Celite was equally affective ($E > 300$). Complete specificity was obtained when vinyl acetate was used as acyl donor ($E \approx 1000$). Using both compounds (−)-**253** and (+)-**254** as building blocks, it would be possible to prepare functionalized carbocyclic nucleoside analogues.

l. Using Hydroxylactones. Roberts and co-workers[113] reported that lactone (±)-**255** can be resolved using PFL and vinyl acetate (Scheme 62). At 40% conversion, the acetate (−)-**256** was obtained in a state of high optical purity (>95% ee), while at 60% conversion, the optically pure alcohol (+)-**255** could be isolated (>95% ee). The ester (−)-**256** obtained by this process was subsequently converted into the

Scheme 62

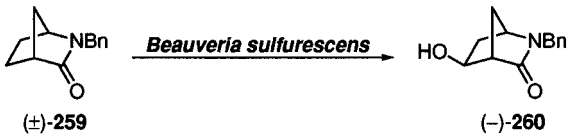


anti-HIV agent carbovir (−)-**139**. Alternatively, the corresponding butyrate (±)-**257** was hydrolyzed with PFL to give the ester (+)-**257** and the alcohol (−)-**255** and was scaled up to utilize kilogram amounts of substrate (crystallization enhanced the ee from 97 to >99%).[114] The *E* value for the butyrate ester (±)-**257** hydrolysis was typically 200, and for the acetate ester (±)-**256** there was a slightly lower value (*E* = 85−100). The later compound was converted into (−)-carbovir. Olivo and Yu[115] extended this methodology to six-membered ring analogues of the cyclopentane moiety. Likewise, optically pure hydroxy lactone (−)-**255** was used to synthesize carbocyclic uracil polyoxin C (+)-**258** through a short, practical, and asymmetric route.[116]

2. Precursor Enzymatic Oxidations

a. Biohydroxylation. Archelas and Morin[117] prepared the enantiomerically pure derivative (−)-**260**, which is a precursor of carbocyclic 2′-deoxynucleosides, through a biohydroxylation with *Beauveria sulfurescens* of the 5-exo position of the 2-azabicyclo-[2.2.1]heptane ring system (±)-**259** (Scheme 63). The latter was obtained as a racemic mixture from cyclopentadiene.

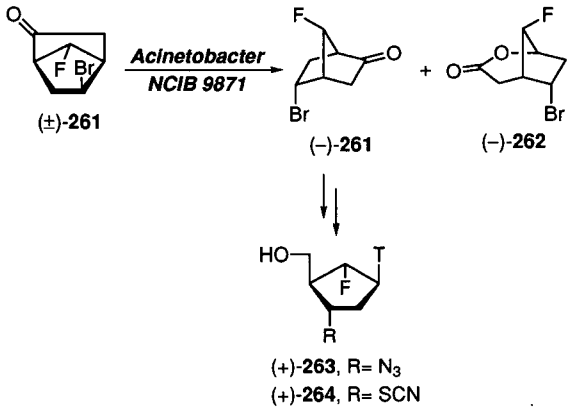
Scheme 63



(±)-**259** (−)-**260**

b. Baeyer−Villiger Oxidation. Roberts and co-workers[118] reported the kinetic resolution of the racemic ketone (±)-**261** using *Acinetobacter* NCIB 9871 (Scheme 64). Thus, racemic ketone **261** was

Scheme 64

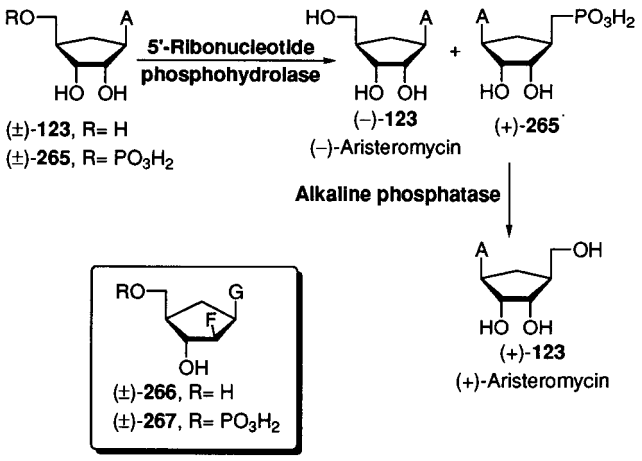


(±)-**261** (−)-**261** (−)-**262**

(+)-**263**, R= N_3
(+)-**264**, R= SCN

incubated with *Acinetobacter* NCIB 9871 until half the starting material had been consumed and the extraction afforded a mixture of ketone (−)-**261** and lactone (−)-**262**, both in a 40% of chemical yield and >95% ee. Also, the optically active norbornanone (−)-**261** was converted into the optically pure antiviral carbocyclic nucleoside (+)-**263** and nucleoside analogue (+)-**264**.

Scheme 65



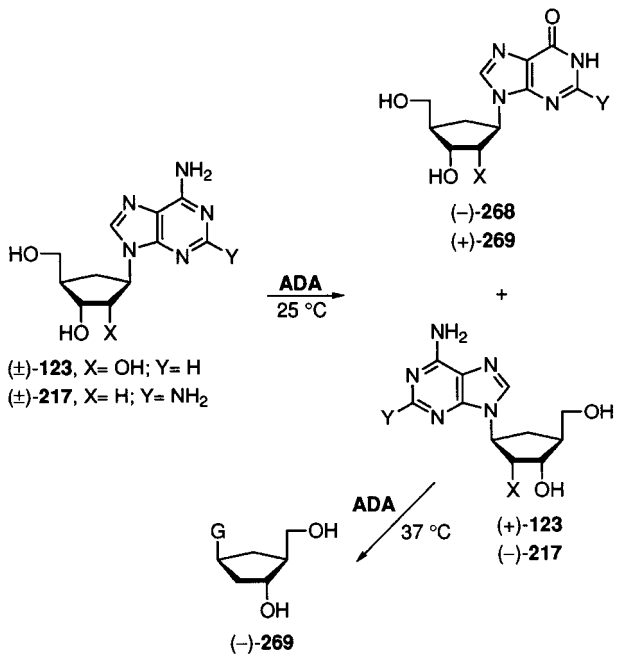
(±)-**123**, R= H
(±)-**265**, R= PO_3H_2
(−)-**123**
(−)-Aristeromycin
(+)-**265**
(+)-**123**
(+)-Aristeromycin
(±)-**266**, R= H
(±)-**267**, R= PO_3H_2

3. Enzymatic Resolutions of Racemic Mixtures

This approach was first applied to carbocyclic nucleosides by De Clercq and co-workers[119] to resolve aristeromycin (±)-**123** (Scheme 65). This racemic compound was chemically transformed into its 5′-monophosphate (±)-**265** and then treated with 5′-ribonucleotide phosphohydrolase from *Crotalus atrox* venom in buffer. The natural (−)-enantiomer was thereby selectively hydrolyzed to provide (−)-aristeromycin [(−)-**123**], while the dextrorotatory monophosphate (+)-**265** was recovered. Treatment of the later with alkaline phosphatase then gave (+)-aristeromycin [(+)-**123**]. A similar procedure was employed to resolve the potent anti-herpetic carbocyclic 2′-*ara*-fluoroguanosine (±)-**266**,[120] but the initial phosphorylation to provide the racemic 5′-monophosphate (±)-**267** was also achieved enzymatically using thymidine kinase isolated from HSV-1.

The use of adenosine deaminase (ADA) to resolve carbocyclic nucleosides was described for the first time by Secrist and co-workers (Scheme 66).[121] Thus,

Scheme 66

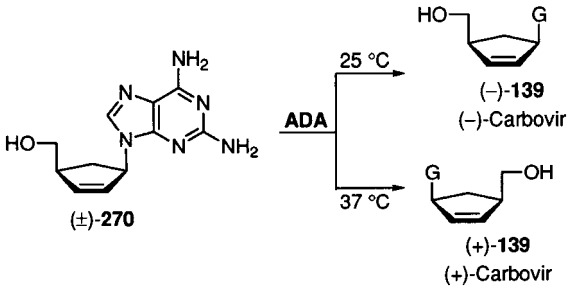


(±)-**123**, X= OH; Y= H
(±)-**217**, X= H; Y= NH_2
(−)-**268**
(+)-**269**
(+)-**123**
(−)-**217**
(−)-**269**

incubation of racemic aristeromycin (±)-**123** with ADA afforded (−)-carbocyclic inosine (−)-**268**, and the enantiomer (+)-**123** was recovered. Similarly, treatment of the racemic 2,6-diaminopurine derivative (±)-**217** with ADA provided the anti-herpetic carbocyclic 2′-deoxyguanosine (+)-**269**, and (−)-**217** was recovered. The latter was hydrolyzed slowly by ADA, but prolonged treatment with a higher concentration of the enzyme provided carbocyclic 2′-deoxyguanosine (−)-**269**.

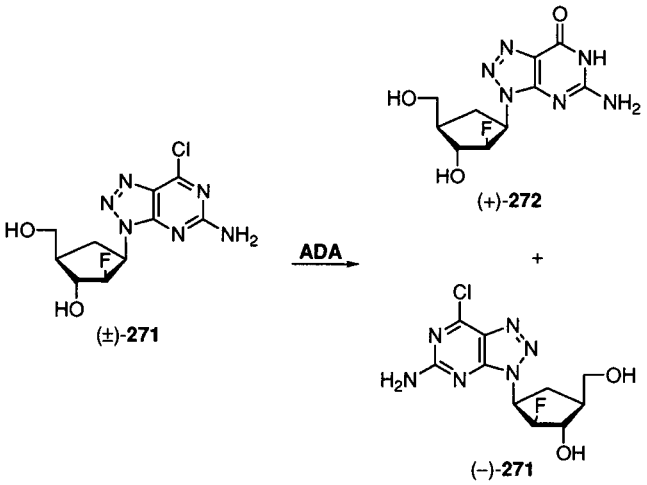
The (+)- and (−)-Carbovir can be prepared by the action of ADA[122] on racemic 2,6-diaminopurine (±)-**270** (Scheme 67).

Scheme 67



The versatility of this enzyme has been shown by the Glaxo research group[123] with the enantioselective hydrolysis of the 6-chloro derivative (±)-**271** (Scheme 68). The enzyme tolerates the 2-amino and 8-aza

Scheme 68

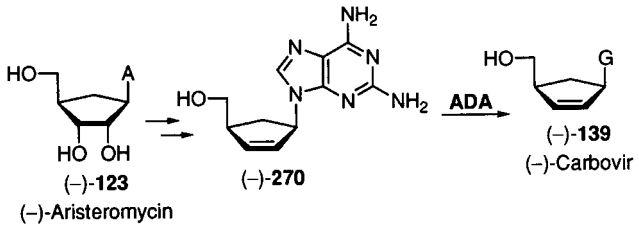


functionality in the heterocyclic base and the 2-fluoro substituent in the carbocyclic ring to afford carbocyclic 2′-*ara*-fluoro-8-azaguanosine (+)-**272** and unreacted derivative (−)-**271**.

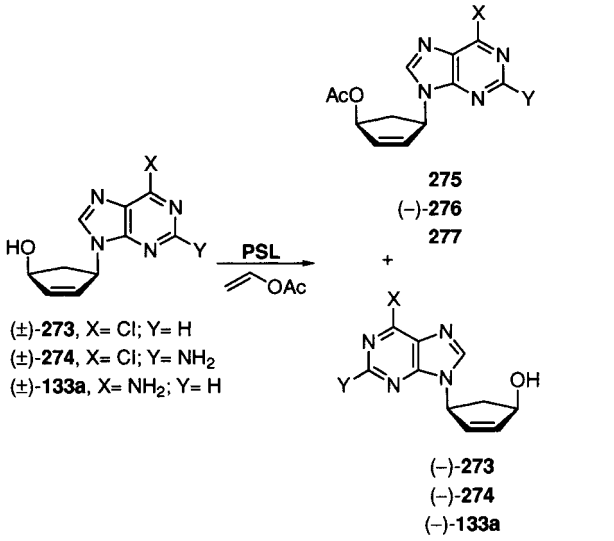
An efficient synthesis of the active enantiomer of the carbovir (−)-**139** from aristeromycin has also been reported from Glaxo's labs (Scheme 69).[124] First, the aristeromycin was transformed into the 2,6-diaminopurine **270**, which was then hydrolyzed using ADA to complete the synthesis of (−)-**139**.

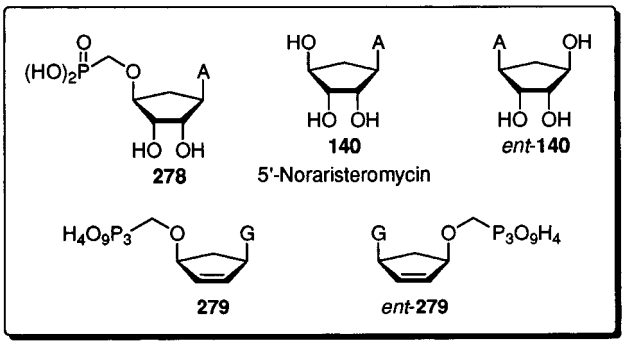
A completely different approach using lipases has been reported to resolve carbocyclic nucleosides (Scheme 70).[125] A chemoenzymatic route was proposed in which the key step was the enantioselective PSL-catalyzed acetylation. Thus, compounds of the type **273**, **274**, and **133a** were resolved using PSL

Scheme 69



Scheme 70





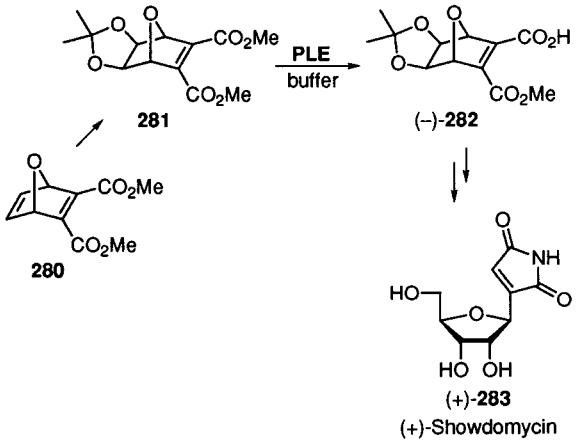
working in the esterification mode. Likewise, starting with enantiomerically pure compound **275**, it was possible to prepare the AMP-mimic **278**, while using a different method, noraristeromycin **140** can be synthesized. Similarly, the alcohol (−)-**273** furnished the enantiomer of the noraristeromycin *ent*-**140**. The same group[126] proposed additional transformations with these resolved compounds (Scheme 70). Thus, starting from the enzymatic resolution of (±)-**274** with PSL and vinyl acetate, which gave the ester (−)-**276** and the alcohol (−)-**274**, both in states of high optical purity (>90% ee), it was possible to prepare the carbovir triphosphate analogue **279** and its enantiomer *ent*-**279**, respectively.

IV. C-Nucleosides. Synthesis of Precursors by Enzymatic Hydrolysis

Natural occurring *C*-nucleosides have attracted a great deal of synthetic study because of their unique structures of *C*-glycosylated heterocycles and their interesting biological properties, such as their anti-

biotic, antiviral, and antitumor activity. Most of the synthetic approaches have been based on the utilization of natural carbohydrate precursors. However, Ohno and co-workers[127] reported a chemoenzymatic approach to the synthesis of D- and L- riboses through the optically active half ester (−)-**282** enzymatically generated starting from the Diels−Alder adduct **280** as shown in Scheme 71 and its application to the

Scheme 71



synthesis of the optically pure *C*-nucleoside showdomycin [(+)-**283**]. Thus, PLE was employed to asymmetrize diester **281** through hydrolysis on a large scale, giving rise to half-ester (−)-**282** in 96% yield.

V. Concluding Remarks

The predominant type of biotransformations on nucleosides has been glycosyl transferring reactions. However, the number of reports describing enzyme-mediated hydrolysis, protections, and resolutions has been increasing over the last two decades, especially since the introduction of organic solvent methodology. Regioselective alkoxycarbonylation processes catalyzed by enzymes is a new area of interest due to its offering great flexibility and the possibility to provide practical routes to nucleoside carbonates with potential value. Certain applications, such as agents effective against HIV and other viral infections or applications related to antisense oligonucleotide preparation, require combined chemical and enzymatic methods to develop new nucleoside derivatives, particularly those with modifications in both the heterocyclic base and the sugar moiety. Thus, the carbocyclic analogues of nucleosides have a wide field of application in antiviral chemotherapy because these compounds are not substrates of phosphorylases and have similar or superior biological activities to the conventional nucleosides. Moreover, biocatalysts are ecologically beneficial natural catalysts that offer the opportunity to carry out highly chemo-, stereo-, and regioselective syntheses of nucleosides, which could not be performed by classical chemical methodologies.

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VII. References

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