THESIS ON NATURAL AND EXACT SCIENCES B78

Synthesis of 4′-substituted 2′, 3′-dideoxynucleoside analogues

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology, has not been submitted for any degree.

Artur Jõgi

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4′-asendatud 2′, 3′-dideoksünukleosiidi analoogide süntees

ARTUR JÕGI

CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to by Roman numerals within the text:

I. A. Paju, M, Laos, **A. Jõgi**, M. Päri, R. Jäälaid, T. Pehk, T. Kanger, M. Lopp, Asymmetric synthesis of 2-alkyl-substituted 2-hydroxyglutaric acid γ-lactones. *Tetrahedron Lett.* **2006**, 47, 4491-4493.

II. **A. Jõgi**, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, T. Kanger, M. Lopp, Asymmetric synthesis of 2-aryl-5-oxotetrahydrofuran-2-carboxylic acids. *Synthesis*, **2006**, 18, 3031-3036.

III. **A. Jõgi**, M. Ilves, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, M. Lopp, Asymmetric synthesis of 4′-C-benzyl-2′,3′-dideoxynucleoside analogues from 3- benzyl-2-hydroxy-2-cyclopenten-1-one. *Tetrahedron: Asymmetry*, **2008**, 19, 628-634.

IV. **A. Jõgi**, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, M. Lopp, Synthesis of 4′-aryl-2′,3′-dideoxynucleoside analogues. Submitted to *Tetrahedron*.

ABBREVIATIONS

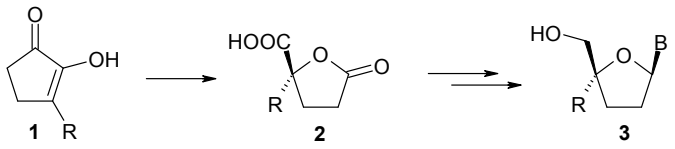
Ac	acetyl
ADP	adenosine diphosphate
AIBN	2′,2′-azobisisobutyronitrile
AIDS	acquired immune deficiency syndrome
AMP	adenosine monophosphate
ATP	adenosine triphosphate
AZT	3′-azido-2′,3′-dideoxythymidine
Bn	benzyl
BSA	N,O-bis(trimethylsilyl)-acetamide
*t*Bu	*tert*-butyl
Bz	benzoyl
CCRF-CEM	human T-cell leukaemia
CPT	camptothecin
DAST	diethylaminosulfur trifluoride
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DET	diethyltartrate
DIBAH	diisobutylaluminium hydride
DMFA	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
EI	electron ionization
ELISA	enzyme-linked immunosorbent assay
Et	ethyl
FDA	Food and Drug Administration
GC	Gas chromatography
HBV	hepatitis B
HCV	hepatitis C
Hep3B	human hepatoma
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
HPLC	high-performance liquid chromatography
HSV	herpex simplex virus
HTLV	human T-lymphotropic virus
IR	infra-red
MCF-7	human breast carcinoma
MCPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MoOPH	MoO_5•pyridine•HMPA
Ms	methanesulfonyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	nuclear magnetic resonance

TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TBHP	*tert*-butylhydroperoxide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMSOTf	trimethylsilyltriflate
Tr	triphenylmethyl
Ph	phenyl
*i*Pr	isopropyl
RNA	ribonucleic acid

INTRODUCTION

Most of biologically active compounds are chiral. The asymmetric synthesis is an important tool to prepare the compounds in order to develop modern drugs. The importance of stereoselective processes in the synthesis of pharmaceutical compounds has been recognized only recently. Thus, pharmaceutical industry needs efficient processes - it means that the most important parameters of the asymmetric synthesis to be taken into account are stereoselectivity of the conversion, yield of the product, price of reagents and simplicity of procedure. In the literature there are many examples of the asymmetric synthesis of different pharmaceutical compounds,[1] however, the number of good industrial asymmetric processes is limited.[2]

The present work is devoted to the development of a short and highly enantioselective method for the synthesis of nucleoside analogues **3**, which are known to be potential anticancer and antiviral agents. The present study starts from the assumption that the enantiopure γ-lactone acids **2** may be used as key intermediates for the preparation of different biologically active compounds. These γ-lactone acids **2** may be obtained from achiral 3-alkyl-2-hydroxy-2-cyclopenten-1-ones **1**, according to the method, which was developed at the Department of Chemistry of Tallinn University of Technology.[3] The method affords γ-lactone acids **2** have been prepared with good yield (up to 75% and high stereoselectivity (*ee* >96%).[4,5,6] (Scheme 1).



R = alkyl or aryl group containing substituent
B = purine or pyrimidine ring containing base

Scheme 1. Asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones and application of the latter to the synthesis of nucleoside analogues

This work deals with the following topics: synthesis of the substrates (various 3-substituted-2-hydroxy-2-cyclopenten-1-ones **1** with different alkyl and phenyl substituents in the ring) for asymmetric oxidation, asymmetric oxidation of new substrates to obtain new lactone acids (key intermediates for nucleoside analogues) and synthesis of nucleoside analogues from lactone acids.

In the first part of the Literature Overview nucleoside analogues have been classified according to structure and activity. The second part is focused on methods of synthesis of different 3-substituted-2-hydroxy-2-cyclopenten-1-ones. In

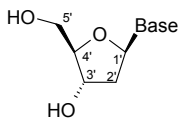
the third part some aspects of the asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones are discussed.

This work aims at developing a new route for 4'-substituted nucleoside analogues **3** *via* alkyl (benzyl) - and aryl-substituted γ-lactone acids **2** and 3-alkyl (benzyl) - and 3-aryl-2-hydroxy-2-cyclopenten-1-ones and at elucidating the potency of the compounds to act as cytotoxic anticancer drugs.

1. LITERATURE OVERVIEW

1.1. Nucleoside analogues

Nucleosides (Fig. 1) are fundamental building blocks of biological systems.



Base = Thymine
Adenine
Guanine
Cytosine
Uracil

Figure 1. The structure of 2'-deoxynucleoside

The compounds are present in cells as structural fragments of RNA and DNA and as the main constituents of mono- (AMP), di- (ADP), and triphosphates (ATP). The phosphorylated nucleosides are intermediates for the synthesis of nucleic acids.[7,8] (Fig. 2).

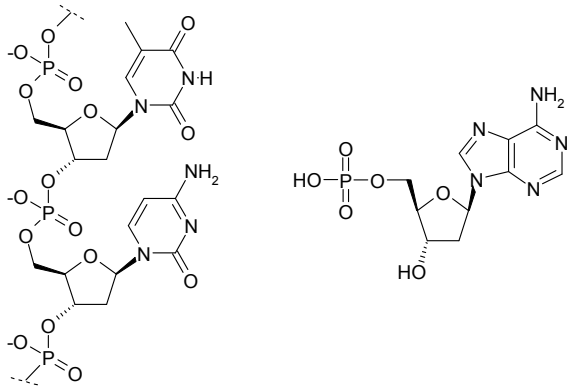


Figure 2. The structures of DNA and AMP

Nucleosides are the important intermediates in the metabolism of infected cells. The action of most drugs on viruses consists in the inhibition of viral enzymes - reverse transcriptases and proteases. Nucleoside analogues, which are good substrates for cellular kinases, but resistant to other host enzymes such as phosphorylases, are also promising candidates of therapeutic agents.
Each nucleoside consists of two components – a ribose and a base. The nucleoside analogues are, in general, classified according to differences in the structural fragment of the ribose furanose ring (1 and 2, Fig. 4), hydroxymethyl chain (3, Fig. 3), or base (4, Fig. 3). In the present thesis mainly the modifications made in the ribose ring are considered.

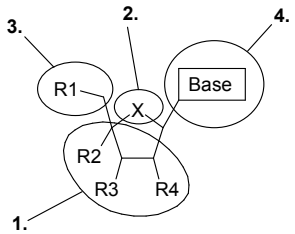


Figure 3. The classification of nucleoside analogues according to the modifications of structural fragments

1.1.1. Furanose ring substituted nucleoside analogues

1.1.1.1. 2′,3′-Substituted nucleoside analogues

Substitution in the ribose ring was one of the first strategies to improve the properties of the natural nucleosides.[9] The first active nucleoside analogue structures of against HIV were discovered in the late 1980-s.[10] At the beginning of the 1990s there was already one FDA-approved drug available to treat HIV (AZT or zidovudine **4**). After zidovudine several nucleoside analogues appeared on the market as drugs, such as didanosine **5** in 1991, zalcitabine **6** in 1992, stavudine **7** in 1994 (Fig. 4).[11]

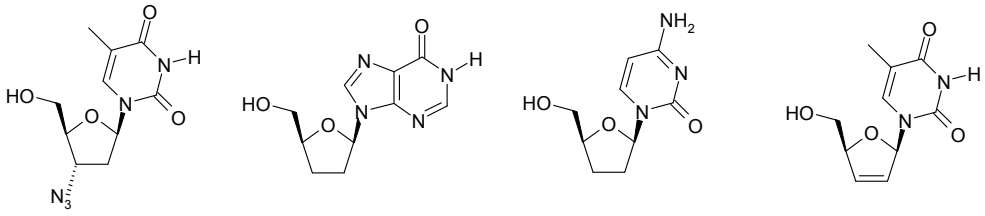


Figure 4. The structures of the nucleoside analogues acting as anti-HIV drugs

Most typically the nucleoside analogues inhibit a reverse transcriptase as a triphosphate. The triphosphate is attached to the growing DNA chain. Since 2,3-dideoxy sugars do not have the OH function in the 3′ position of the sugar ring, the nucleic acid chain cannot be extended any further. The side effects and high doses of drugs and resistance of viruses induced scientists to introduce substituents other than OH into the structure of a nucleoside analogue. As a result, different 2′- and 3′-substituted nucleoside analogues **4-6, 8-17** were synthesized (Fig. 5). The unsaturated nucleoside analogues **7, 23-34** and bicyclic nucleoside analogues **20-22**

represent a novel direction in the development of the nucleoside analogues structures. Stavudine **7** (Fig. 5) is the first compounds bearing a double bond, which was approved by the FDA and is used today as a drug against HIV.[12]

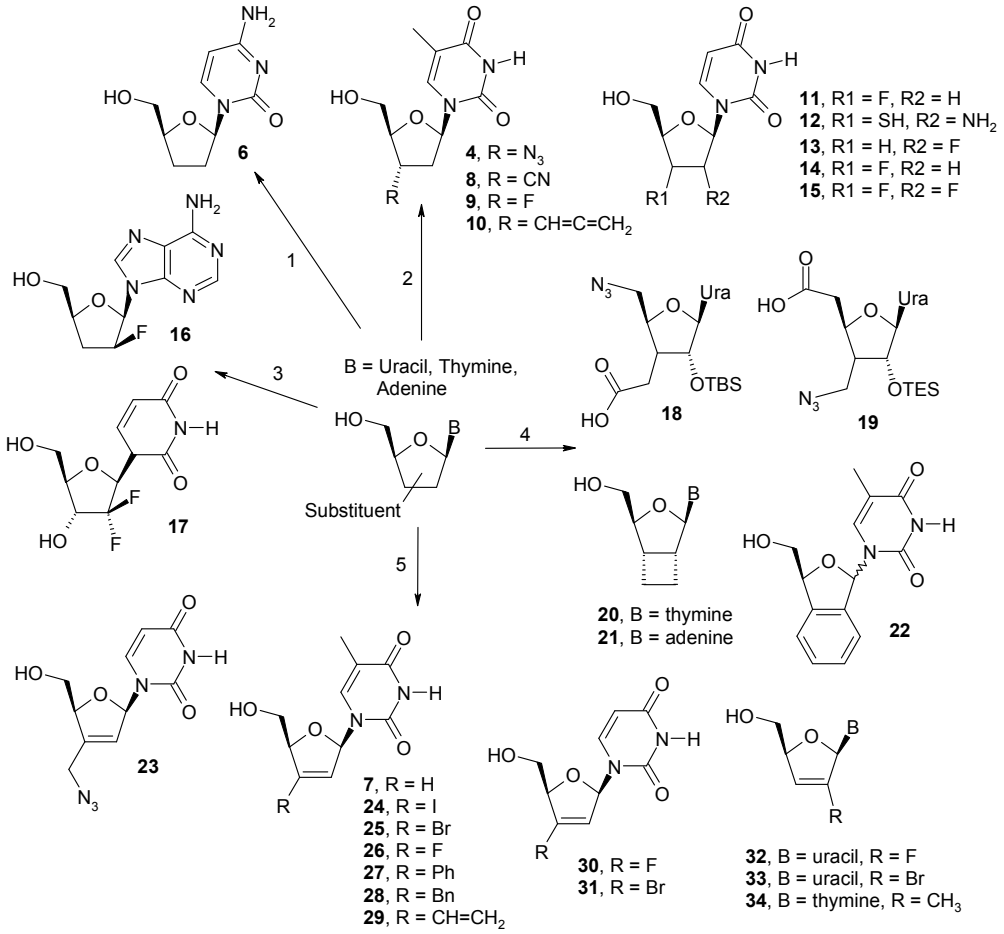
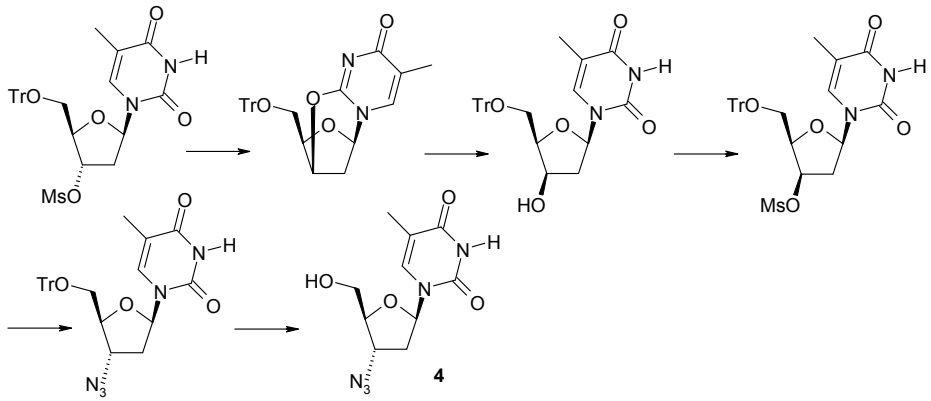


Figure 5. The structure of 2',3'-substituted nucleoside analogues

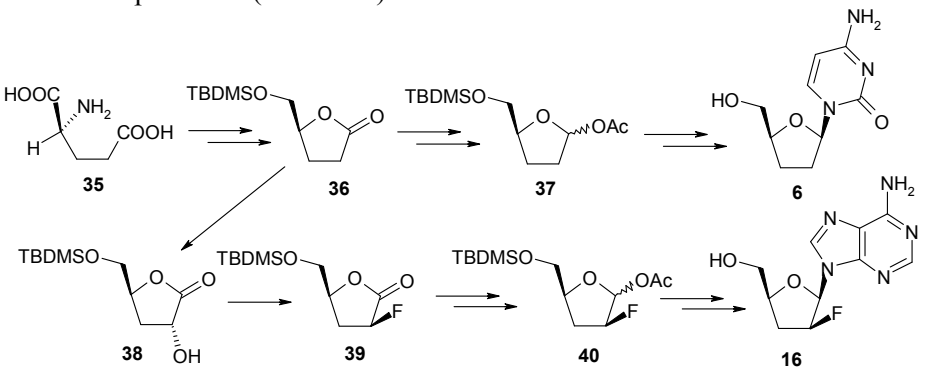
Among all nucleoside analogues outstanding position is hold by AZT. This nucleoside analogue was first prepared in 1963 by Horwitz *et al*.[13] as an anticancer agent, but at the end of the 1980s it found wide application as an anti-HIV drug. Today the compound is still on the market as the main commercial drug to treat HIV.

The synthesis of AZT starts from the natural 2'-deoxythymidine. The 3′-hydroxy group of thymidine was inverted to give a 3′-β-hydroxy group *via* a 2′,3′-anhydro compound, followed by the activation of the 3′-β-hydroxy group and its displacement with an azide group, to afford AZT **4** (Scheme 2).



Scheme 2. Synthesis of AZT

The synthesis of 2′,3′-substituted nucleoside analogues depends mainly on the choice of the starting compound. The methods of introduction and removing of the functional groups of the furan ring is generally similar to both approaches.[14]
In many cases the preparation of nucleoside analogues starts from *L*-glutamic acid **35**. A key intermediate is a lactone derivate **36**,[15] which is suitable to be used for the introduction of different functional groups into the ribose ring.
This approach has been employed by several researchers. For example, in order to introduce a hydroxyl group into the 2′-position Choudhury *et al*.[16] used a molybdenum complex MoOPH to afford compound **38**. The hydroxyl group was replaced by the fluoro group using diethylaminosulphur trifluoride DAST, which afforded compound **39** (Scheme 3).

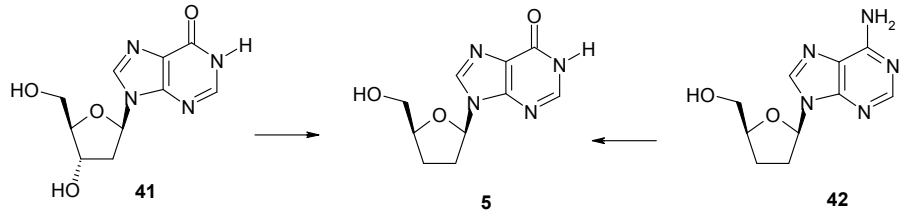


Scheme 3. Synthesis of nucleoside analogues starting from natural L-glutamic acid

In the preparation of nucleosides the last step is coupling of ribose ring with an activated base. The widely used method for that purpose is a Vorbrüggen method.[17] In the latter method a silylated base (usually purine or pyrimidine) is coupled through the S_N1 reaction with a protected and activated ribose derivative in the presence of a Lewis acid, such as tin(IV) chloride or trimethylsilyl trifluoromethanesulfonate TMSOTf. As leaving groups at the ribose ring acetate,

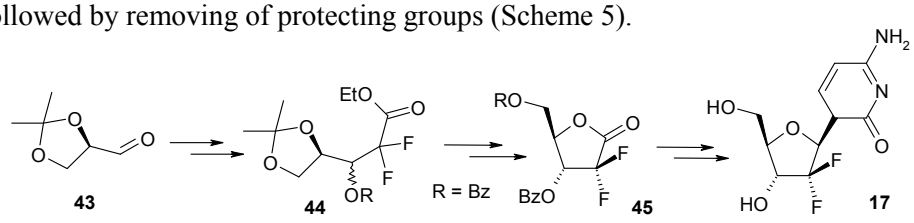
methoxy and bromide groups have been used. Thus, the coupling of the silylated cytosine with an unsubstituted ribose acetate **37** gave zalcitabine **6**. When using a fluoro derivative **40** nucleoside **16** was obtained (Scheme 3).

The synthesis of 2′,3′-dideoxyinosine (didanosine) **5** can be accomplished by removing the 3′-hydroxy group from the ribose ring. According to Webb *et al*.[18] the 5′-hydroxyl group of natural 2′-deoxyinosine **41** was selectively benzoylated, followed by deoxygenation at the 3′-position *via* thioimidazolide. Deprotection of 5′-O-benzoyl-2′,3′-dideoxyinosine gave the target compound **5**. Alternatively, didanosine **5** can be obtained by enzymatic deamination of compound **42** with adenosine deaminase (Scheme 4).



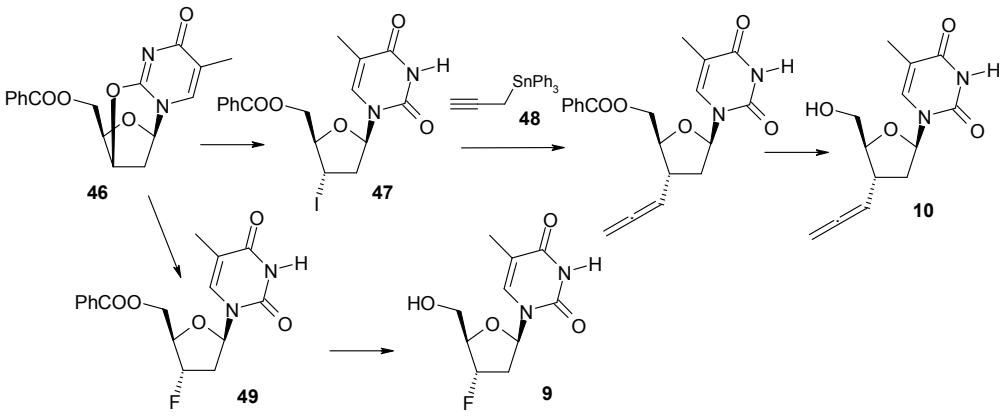
Scheme 4. Synthesis of didanosine

Another way of preparing nucleoside analogues from simple chiral natural compound *D*-glyceraldehyde **43** is demonstrated in the synthesis of a known anticancer agent, 2′-deoxy-2′,2′-difluorocytidine (gemcitabine) **17**.[19] In this case all necessary functional groups are introduced before the formation of the ribose ring. The first important intermediate is a linear compound **44** having two fluoro groups. After cyclization, the compound **45** is formed.[20] The final nucleoside analogue **17** is, again, obtained as described above, after the introduction of a base (cytidine) followed by removing of protecting groups (Scheme 5).



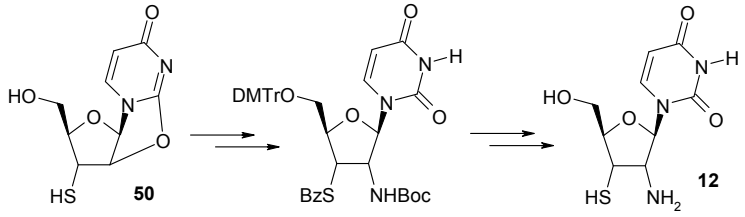
Scheme 5. Synthesis of 2'-deoxy-2',2'-difluorocytidine (gemcitabine)

The next possibility for preparation of nucleoside analogues by replacement of the functional groups in the ribose ring is outlined in the synthesis of 3′-allenyl-2′,3′-dideoxythymidine **10**, a non-polar analogue of AZT, according to Becouarn *et al*.[21]. The allenyl group was introduced to the position 3′ by using the free radical reaction between triphenyl-2-propynyl stannane **48** and iodinated nucleoside **47** (Scheme 6). The 2,3′-anhydro linkage of **46** was opened by using DAST or HF/AlF_3, affording 3′-fluoro substituted nucleoside **49**. The target nucleoside **9** was obtained after removing the protective groups (Scheme 6).[22]



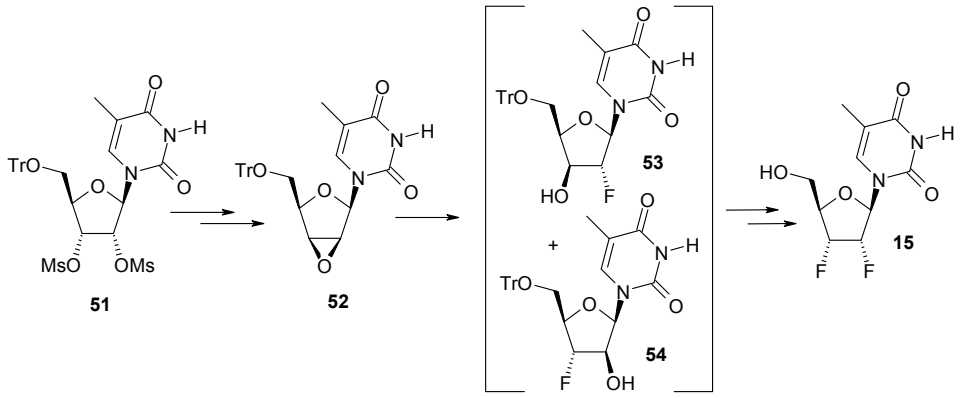
Scheme 6. Synthesis of 3′-substituted nucleoside analogues from 2′,3′- anhydro nucleoside

The introduction of an amino group at the 2’ position may be accomplished by replacement of 2′-hydroxy group as demonstrated by Dai and Piccirilli[23] in the preparation of 2′,3′-dideoxy-2′-amino-3′-thiouridine **12**, starting from 2,2′-anhydrouridine **50** (Scheme 7).



Scheme 7. Synthesis of 2′,3′-dideoxy-2′-amino-3′-thiouridine

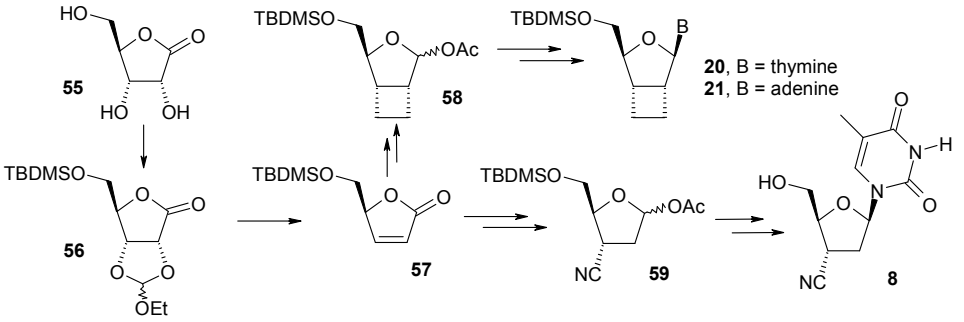
Epoxides are attractive intermediates in the modification of the ribose ring. Thus, epoxide **52** was obtained from **51** (Scheme 8).



Scheme 8. Synthesis of a 2′,3′-difluoro nucleoside analogue

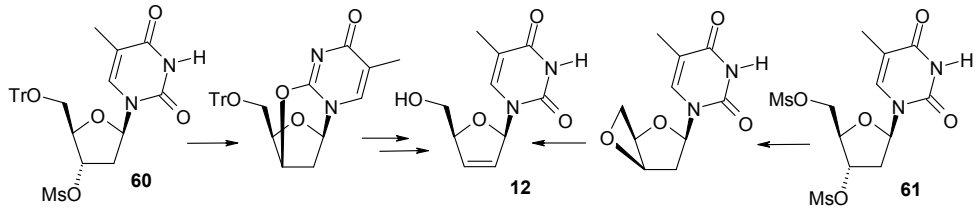
The treatment of **52** with KHF_2 gave a mixture of fluorohydrines **53** and **54**, which, without separation, were treated with DAST to afford, after removing of the protecting groups, the target 2′,3′-difluoro-2′,3′-dideoxythymidine **15** (Scheme 8).[22]

Another approach to introduce the functional groups into the 2′- and 3′-positions is *via* the generation of a double bond in the ribose ring. Compound **57** is a typical key intermediate for that purpose. The preparation of **57** can be accomplished employing several methods. One of the methods[14] is pyrolysis of a bicylic ortho ester **56** obtained from *D*-ribonolactone **55**. The introduction of the functional groups may be done *via* the hydrocyanation of **57** and reduction of the lactone group, followed by ordinary reaction sequence, described earlier, affording compound **8**. The bicyclic derivative **58** was obtained from alkene **57** by photochemical reaction between ethylene and **57**. After the introduction of the base into **58** 3-oxabicyclo[3.2.0]heptane nucleoside analogues **20-21** were obtained (Scheme 9).[24]



Scheme 9. Synthesis of different nucleoside analogues from double bond containing intermediates

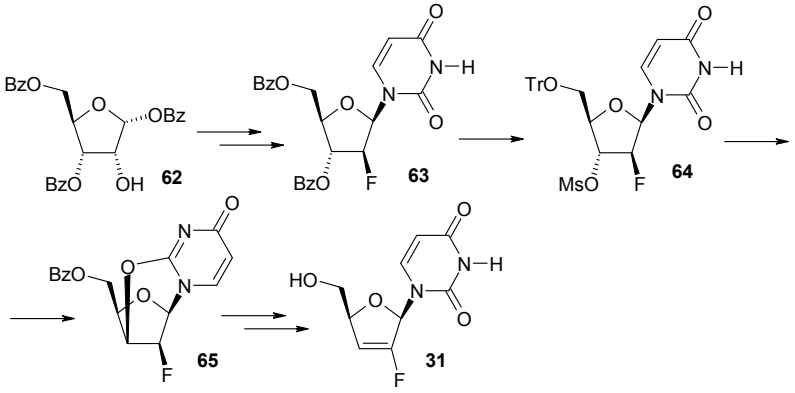
One of the most widely used methods to introduce a double bond into the ribose ring is the elimination of the mesyl group by using bases such as sodium hydroxide or -methoxide and potassium tert-butoxide.
Thus, Horwitz *et al*.[12] prepared stavudine **7** *via* the base-catalyzed elimination reaction of **60** or **61** with potassium tert-butoxide in dimethyl sulfoxide, in high yield (Scheme 10).



Scheme 10. The double bond formation via the base-catalyzed elimination

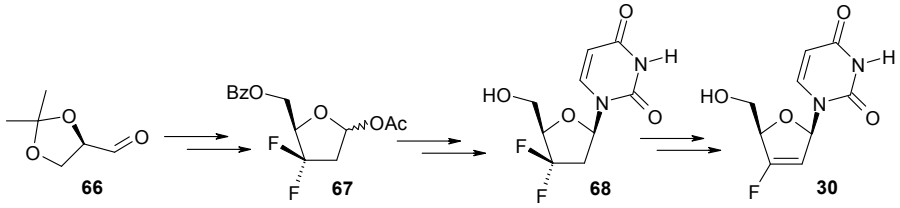
Martin *et al*.[25] used the treatment of **65** with sodium hydroxide to obtain, after deprotection, the target nucleoside **31**. Compound **65** was prepared by a sequence

of reactions, starting from the *D*-ribose derivative **62**. The fluoro group was introduced into the position 2' by treatment of **62** with KHF_2 and HF. The N-glycosylation of the halide-sugar with a silylated uracil gave compound **63**. The treatment of compound **63** with trityl chloride and then by methanesulfonyl chloride gave compound **65**, which upon treatment with an aqueous sodium hydroxide gave the target anhydronucleoside **31** (Scheme 11).



Scheme 11. Synthesis of 2'-fluoro-2',3'-unsaturated nucleoside analogue

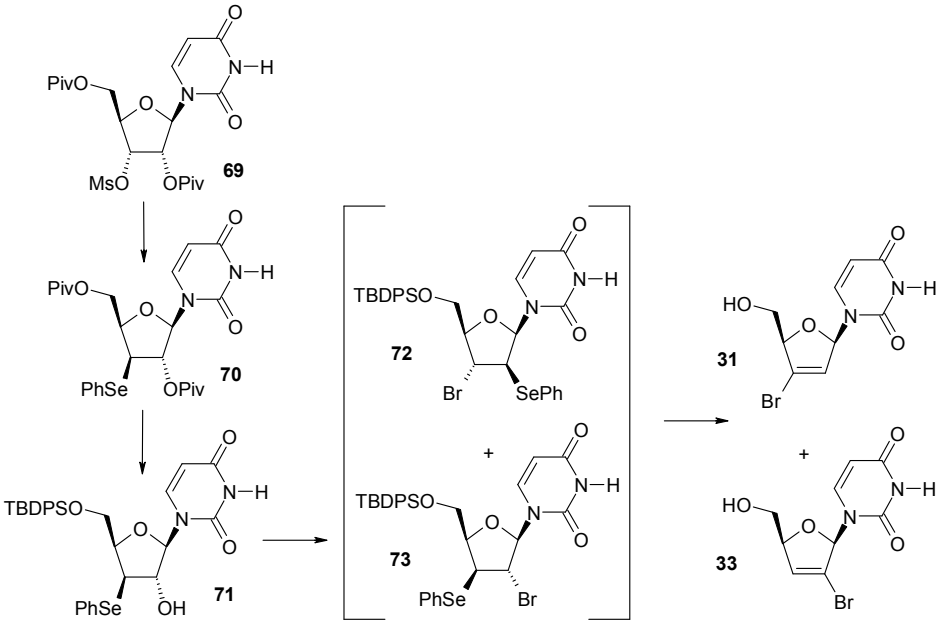
Zhou *et al*.[26] synthesized 3′-fluoro 2′,3′-unsaturated nucleosides as compounds having anti HIV-1 and HBV activity, starting from protected *D*-glyceraldehyde **66**. The 3′,3′-difluoro nucleoside **68** was prepared from **67** and then treated with *t*-BuOK to give the desired 3′-fluoro-unsaturated nucleoside **30** (Scheme 12).



Scheme 12. Synthesis of 3'-fluoro unsaturated nucleoside analogues

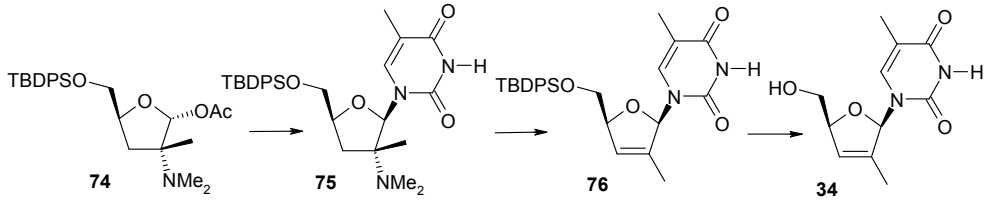
Diphenylselenide is a suitable reagent for the generation of the double bond in the ribose ring. Thus, selenide **70** was obtained by the Walden inversion at the a 3′-position of 3′-O-mesyl derivative **69** with $(PhSe)_2$ and $NaBH_4$.[27] The conversion of compound **70** into the β-hydroxyselenide **71** was effected in almost quantitative yield by deacylation, followed by the selective silylation reaction of the primary hydroxyl group. When the nucleoside **71** was brominated in CCl_4 with $SOBr_2$ in the presence of imidazole, a mixture of β-hydroxyselenide intermediates **72** and **73** was obtained. The mixture was subjected to selenoxide elimination to provide the corresponding bromovinyl nucleosides **31** and **33** after deprotection (Scheme 13). These compounds serve as versatile synthons for the preparation of anti-HIV

candidates, 2′-C- and 3′-C-branched 2′,3′-unsaturated nucleosides through palladium-catalyzed cross coupling and halogen-lithium exchange reactions.[28]



Scheme 13. Synthesis of 2'- and 3'-bromo-2',3'-unsaturated nucleoside analogues

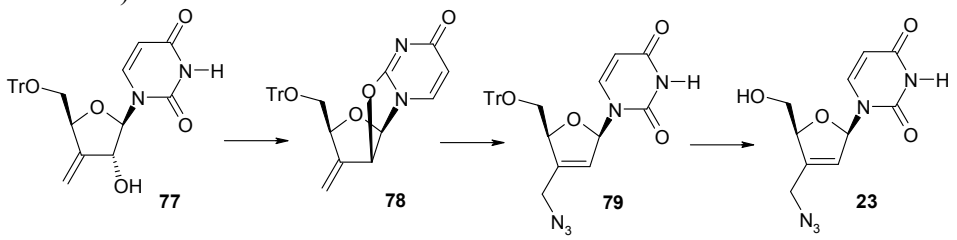
The double bond in the furanose ring may be formed by Cope elimination and by hetero-Cope rearrangement. Chiacchio *et al.*[29] reported a route to the 2′-*C*-methyl analogue of stavudine **34**, which included the Cope elimination as a key reaction (Scheme 14). After the N-glycosylation of acetate **74** by silylated thymine, the corresponding β-nucleoside analogue **75** was obtained. The elimination of the N-dimethylamino group was performed according to the Cope elimination by treating it with MCPBA to form **76**, followed by the deprotection of the primary hydroxyl group. Thus, the target nucleoside **34** in acceptable yield was obtained (Scheme 14).



Scheme 14. Synthesis of 2'-C-methyl analogue of stavudine

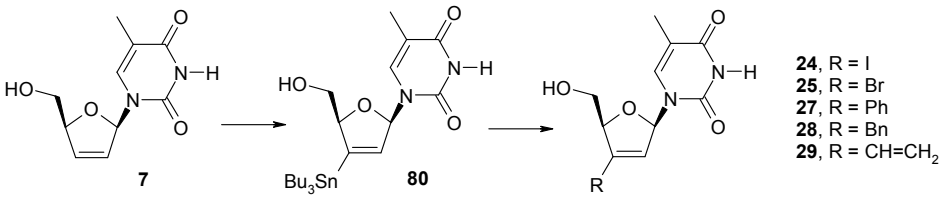
Czernecki *et al.*[30] described the synthesis of various 3′-*C*-branched 2′,3′-unsaturated pyrimidine nucleosides *via* the hetero-Cope rearrangement. Starting

from 2′-*C*-methylidene derivative **77**, an intramolecular reaction was carried out to furnish the 2′,3′-anhydro nucleoside **78**. Heating of the **78** in the presence of lithium azide resulted in the formation of the corresponding azido derivative **79**, followed by the deprotection of the 5′-position to afford the target nucleoside **23** (Scheme 15).



Scheme 15. Synthesis of 3′-C-azidomethyl-2′,3′-unsaturated pyrimidine nucleosides

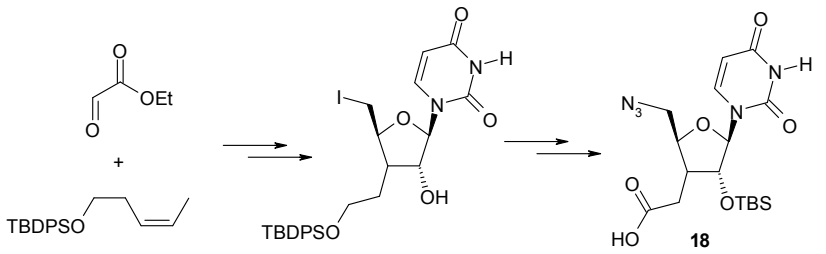
Tanaka *et al*. suggested a method for the preparation of 3′-substituted unsaturated nucleoside analogues, starting from stavudine **7**.[31] Trans-alkoxylation of the OH group of stavudine **7** with Bn_3SnOMe and the subsequent migration of an anionic O→C stannyl group gave a 3′-tributylstannyl substituted nucleoside analogue **80**. Substitution of the tributylstannyl group affords access to different nucleoside analogues **24-29**. Thus, phenyl, benzyl and vinyl groups were introduced into the 3′-position by using this method. Additionally, 3′-iodide and 3′-bromide substituted compounds **24-25** are themselves intermediates for the synthesis of other 3′-C-branched 2′,3′-unsaturated nucleosides (Scheme 16).[32]



Scheme 16. Synthesis of 3′-substituted unsaturated nucleoside analogues

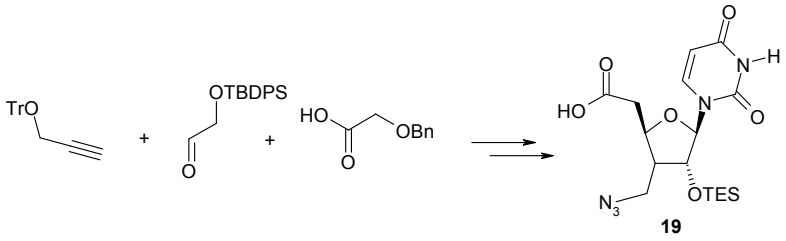
A novel direction for the preparation of nucleoside analogues is to start from the simple organic molecules, which, compared with natural starting compounds, make the process more flexible. Also, the methods can be better optimized and may provide access to both enantiomers, when achiral starting compounds and a chiral catalyst are used. Below some recent examples will be presented.

Rozners *et al*.[33] suggested a method for the preparation of 3′,5′-C-branched uridine azido acids **18** from simple starting materials. The synthesis of **18** was accomplished by using a sequence of stereoselective ene and iodolactonization reactions (Scheme 17).



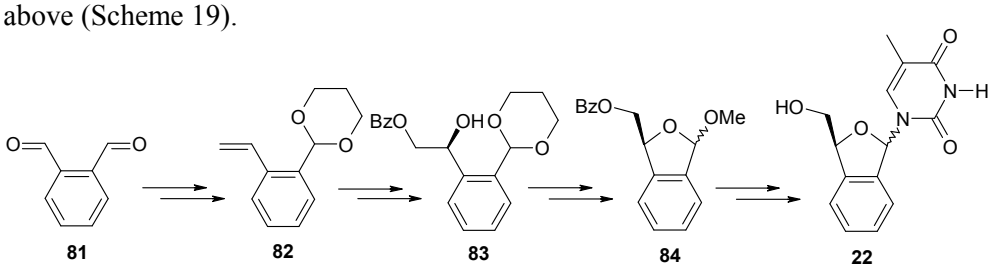
Scheme 17. Synthesis of 3′-C and 3′,5′-C-branched nucleoside analogues

To prepare compound **19** the stereoselective aldehyde alkynylation, the Ireland-Claisen rearrangement and iodolactonization have been used (Scheme 18).[34]



Scheme 18. Synthesis of 3′-C and 3′,5′-C-branched nucleoside analogues

Ewing *et al*.[35,36] prepared benzo[*c*]furan core containing nucleoside analogues. Starting from phthalaldehyde **81** followed by its selective protection and Wittig homologation, the corresponding styrene intermediate **82** was obtained. After asymmetric dihydroxylation of **82** by the Sharpless reagent and benzoylation of the primary hydroxyl group, compound **83** was obtained. Cyclization and methylation of **83** afforded a 1,3-dihydrobenzo[*c*]furan derivative **84**, which was transformed to the corresponding nucleoside analogues **22** by the Vorbrüggen method as described above (Scheme 19).



Scheme 19. Synthesis of benzo[c]furan core containing nucleoside analogues

In summary, functionalization in the ribose ring was one of the first approaches to prepare nucleoside analogues. It has afforded a series of anti-HIV drugs, such as zidovidine, zalcitabine, didanosine, and later stavudine, which were approved by the FDA in the 1990s.

According to the literature, there may be distinguished five approaches to preparing the compounds.

The first approach uses a natural chiral compound (amino acid, a sugar etc) as a basic unit. Usually the replacement of the OH-group is used in order to introduce different functional groups into the ribose ring. The last step is the coupling of the ribose ring with the corresponding base by using the Vorbrüggen method.

The second approach uses a double bond functionalization. For formation and functionalization of the double bond different synthetic methods are used.

The third approach employs the derivatization of the structure of ribose ring after the base has been introduced to it.

The fourth approach uses the modification of chiral starting compounds and the introduction of all necessary functional groups of a nucleoside analogue before cyclization and the ribose ring formation.

The fifth approach uses simple unnatural starting compounds and applies usually chiral catalysts to generate chirality. This approach may give access to both enantiomers of the nucleosides.

The classification made above is quite arbitrary. Many methods of the synthesis are combinations of some, or even all of the approaches, discussed above.

1.1.1.2. 4′-Substituted nucleoside analogues

It is known that viruses (HIV-1 virus) may become resistant to drugs (nucleoside analogues) by developing enhanced ability to excise the analogue after its incorporation. It is the reason why new and new generations of drugs have been developed. One of the perspective directions in the search for more selective and effective compounds is to introduce substituents into the 4′ position of the ribose ring. It is already known that 4′-C-methyl and 4′-C-ethyl thymidines **87-88** (Fig. 6) effectively block the replication of HIV-1 and, which is more important, these compounds are effective against many drug-resistant reverse transcriptase variants.[37]

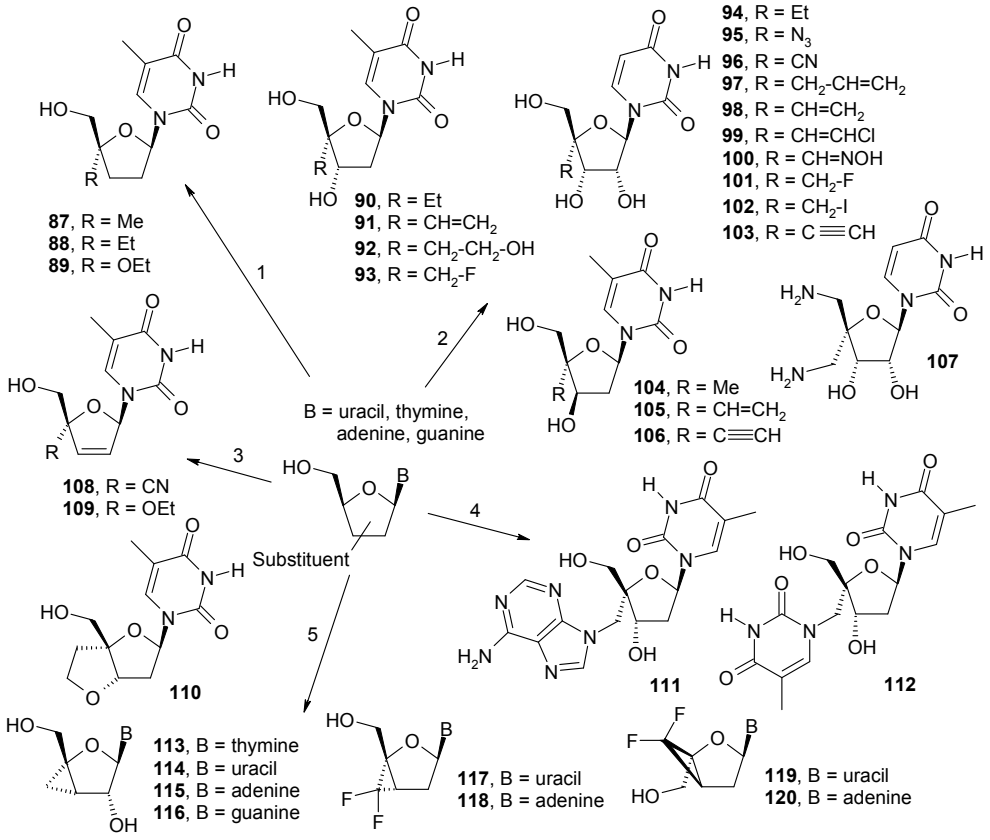
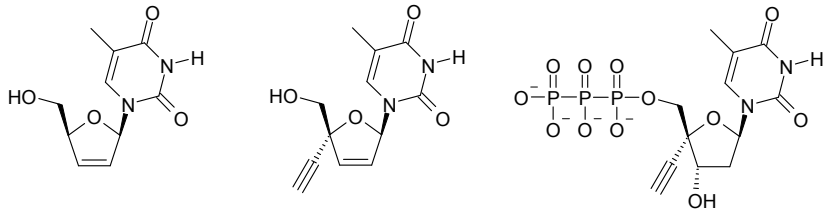


Figure 6. The structures of different 4′-substituted nucleoside analogues

Thus, the anti-HIV-1 activity of 2′,3′-didehydro-2′,3′-dideoxy-4′-ethynylthymidine **85** (Fig. 7) is 5- to 10-fold higher than that of its parental compound stavudine **7** (Fig. 7), while its cellular and mitochondrial toxicity are lower.[38] Nucleoside

analogue **85** is also active against a variety of drug-resistant HIV-1 mutants under at non-cytotoxic concentrations.



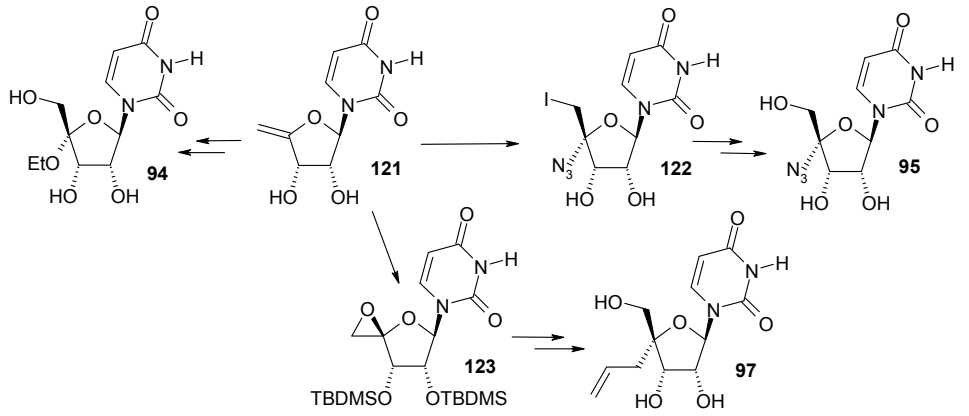
7, stavudine **85**, 4'-ethynylstavudine **86**, 4'-ethynyl thymidine triphosphate

Figure 7. The structures of stavudine, 4'-ethynylstavudine and 4'-ethynyl thymidine triphosphate

The nucleoside analogues may be used in medicine as free nucleosides or as phosphates. Thus, the anti-HIV activity of 4′-ethynyl substituted nucleosides triphosphate **86** is based on its ability to be a substrate for HIV-1 transcriptase, serving as a DNA chain terminator, and it inhibited the DNA transcriptase activity more efficiently than stavudine triphosphate. [39,40]

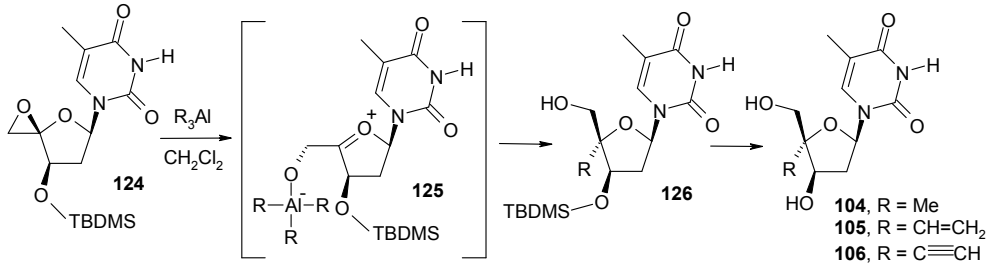
The structure of different 4′-substituted nucleoside analogues can be classified according to substitution in the ribose ring (Fig.6). The most simple examples of those analogues are 4′-substituted 2′,3′-dideoxynucleoside compounds **87-89** (Direction 1, Fig. 6). Much larger group of nucleosides analogues represents 4′-substituted 2′-deoxynucleoside analogues: **90-93**, **104-106** and 4′-substituted nucleoside analogues: **94-103**, **107** (direction 2, Fig. 6). 4′-Substituted 2′,3′-dideoxy nucleoside analogues **108-109** bear a double bond in ribose ring (direction 3, Fig. 6). Interesting structures are bisheaded nucleoside analogues **111-112** (direction 4, Fig. 6). 3′,4′-Substituted bicyclic compounds **113-120** represent a novel group of 4′-substituted nucleoside analogues (direction 5, Fig. 6).

One of the first synthetic methods for preparation of 4′-substituted nucleoside analogues uses the dehydrohalogenation of 5′-halonucleosides by DBN or sodium methoxide, to afford 4′-alkene compound **121**.[41]
The subsequent step is a regio and stereospecific addition of iodine azide to the 4′-double bond of **121**. As a result, 4′-azido nucleoside analogue **122** is obtained.[42] Addition of the iodine to ethanol solution of **121** introduced 4′-ethoxy group to afford 4′-ethoxyuridine **94**. The protection of compound **121** with TBDMS chloride followed by the reaction with dimethyldioxirane gave β-epoxide **123**.[43] The treatment of epoxide with allyl trimethylsilane and deprotection with TBAF afforded 4′-allyluridine **97** (Scheme 20).



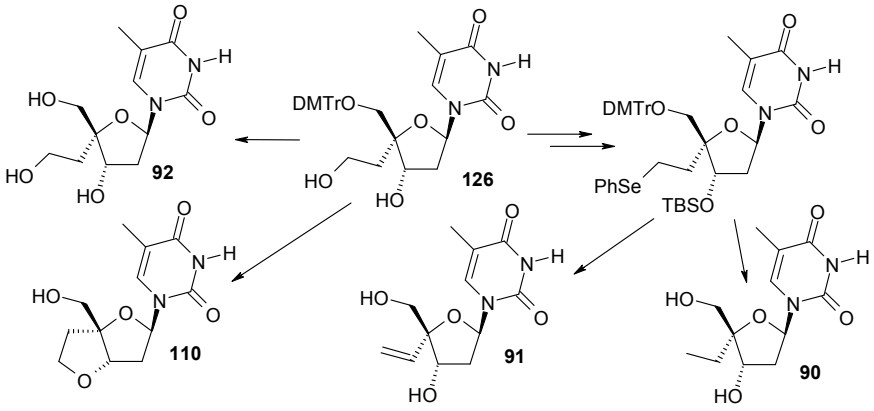
Scheme 20. Synthesis of 4′-azido-, 4′-ethoxy- and 4′-allyluridines

Later this method was applied by Haraguchi *et al*.[44] to the preparation of 4′-substituted thymidines **104-106**. The key compound 4′,5′-epoxide **124** was allowed to react with trialkylaluminium to afford compound **126** *via* intermediate **125** (Scheme 21).



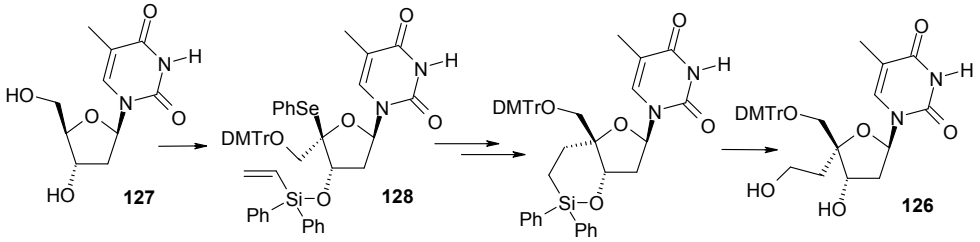
Scheme 21. Introduction of methyl, vinyl and ethynyl groups into the 4′-position of a thymine nucleoside

4′-Hydroxyethyl nucleoside analogue **126** is a well-known intermediate for the preparation of a series of 4′-substituted nucleoside analogues **90-92** and **110** (Scheme 22).[45,47]



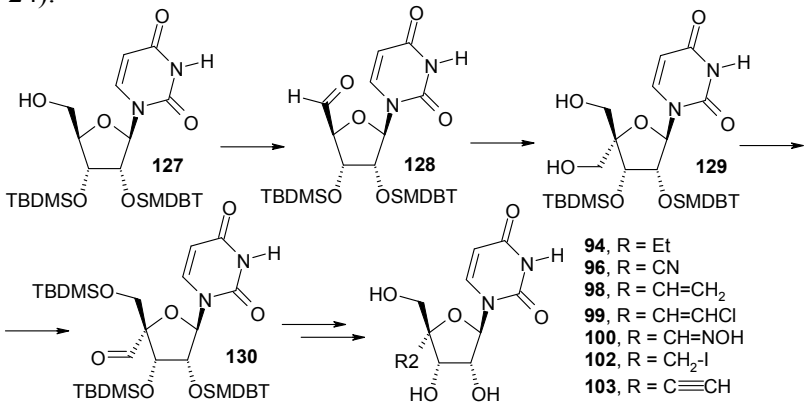
Scheme 22. Synthesis of 4'-substituted nucleoside analogues

Compound **126** was obtained from thymidine **127**. The radical reaction of **128** with Bu_3SnH and AIBN, [46] followed by the Tamao oxidation gave selectively the derivate **126** (Scheme 23).[47]



Scheme 23. Synthesis of 4'-hydroxyethyl nucleoside analogue

4′-Substituted uridines were prepared according to Scheme 24 from **129**.[48] Deprotection of the compound **129** with TBAF afforded 4′-hydroxymethyl uridine (Scheme 24).

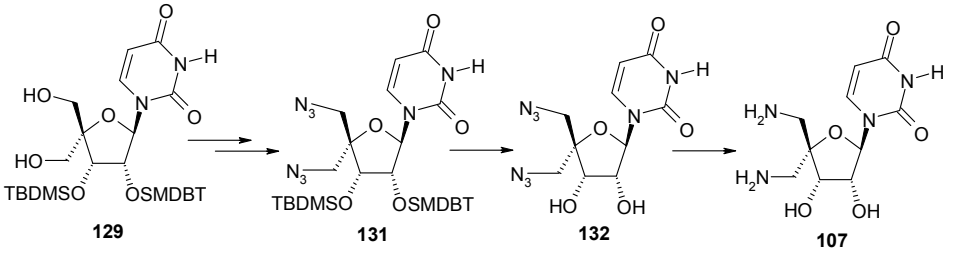


Scheme 24. Synthesis of a 4'-hydroxymethyl nucleoside analogue for the preparation of 4'-substituted uridines

The selective protection of the 4′-α-hydroxymethyl group of compound **129** was achieved by treatment with dimethoxytrityl choride, followed by protection of the 4′-β-hydroxymethyl group with TBDMS chloride. Removal of the dimethoxytrityl group with acetic acid followed by the Swern oxidation gave the aldehyde derivative **130**. The condensation of the aldehyde function in compound **130** with hydroxylamine gave the corresponding oxime, which was dehydrated with acetic anhydride to give nitrile. The total deprotection with TBAF gave 4′-α-cyanouridine **96** (Scheme 24).

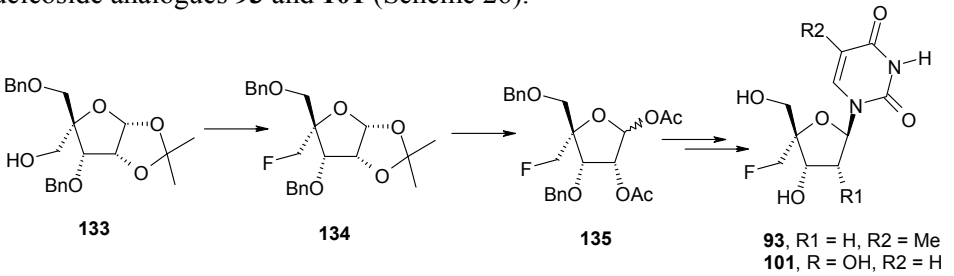
Alternatively, treatment of **130** with methylene triphenylphosphorane under the Wittig conditions followed by the deprotection with TBAF gave 4′-α-vinyl-uridine **98.** Also, intermediate **130** was condensed with a chloromethylene triphenylphosphorane Wittig reagent, followed by the dehydrohalogenation with butyl lithium to provide compound **99**. The deprotection with TBAF was conducted to afford the target molecule, 4′-α-ethynyluridine **103** (Scheme 23).[49]
The 4′-hydroxymethyl group itself provides an additional functional group for substitution in order to get various 4′-nucleoside analogues.

Yu *et al*.[50] prepared 4′,4′-*C*-diaminomethyl uridine analogue by using intermediate **129** as starting compound. Compound **131** was obtained from **129** in 5 steps. After protection of the hydroxyl groups at the 2′ and 3′ positions and treatment with lithium azide diazido nucleoside **132** was obtained. Diamino nucleoside **107** was prepared from compound **132** by reduction with hydrogen (Scheme 25).



Scheme 25. Synthesis of 4',4'-C-diaminomethyl uridine analogue

Kitano *et al*.[51] suggested a method for the preparation of 4′-fluoromethyl nucleoside analogues **93** and **101** (Scheme 26).

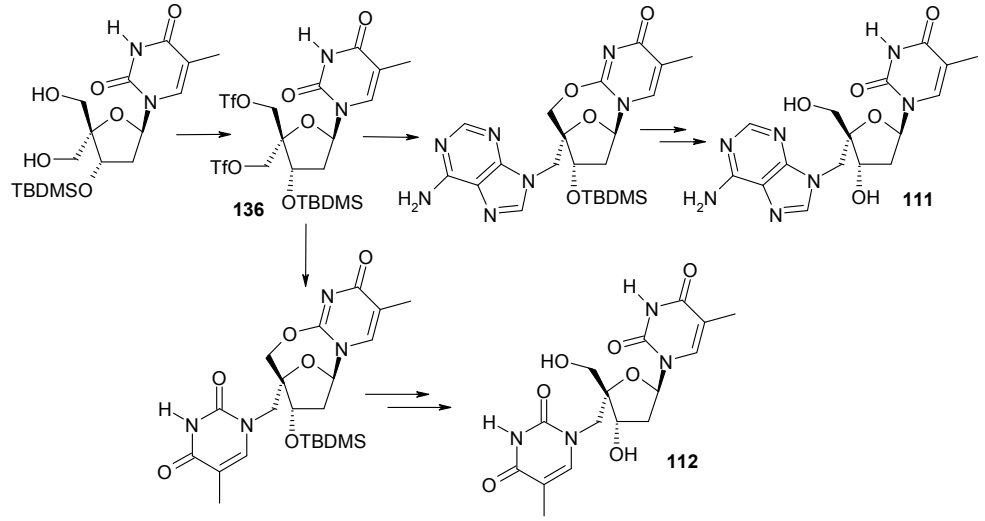


Scheme 26. Synthesis of 4'-C-fluoromethyluridines and 4'-C-fluoromethyl-2'-deoxyuridines

The synthetic intermediate **133** was initially treated with DAST to afford compound **134**. The acetolysis of **134** gave **135**. The glycosylation of **135** with silylated uracil or thymine and removal of the protective groups gave 4′-C-fluoromethyluridine **101**. In case of 4′-C-fluoromethylthymidine the hydroxy group at the position 2′ was removed with BBr_3 in CH_2Cl_2 followed by quenching with MeOH, to yield 4′-C-fluoromethyl-2′-deoxythymidine **93** (Scheme 26).

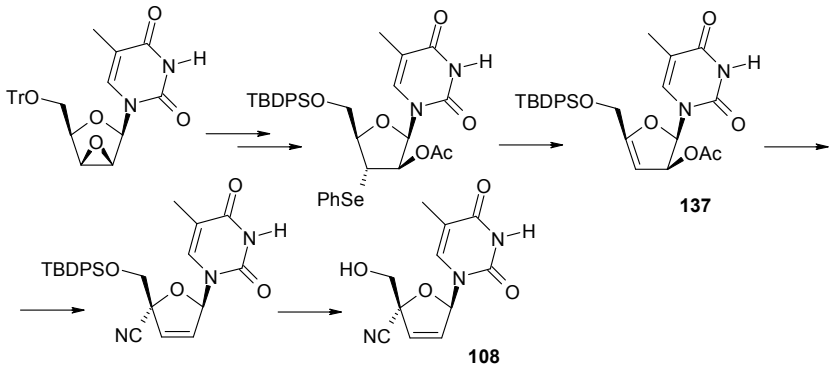
A 4′-hydroxymethyl substituted nucleoside analogue is a starting compound in the synthesis of bisheaded nucleoside analogues. 4′-Thymine or adenine substituted nucleoside analogues are new compounds in the family of nucleosides.

Wu *et al*.[52] developed a method for the synthesis of bisheaded nucleosides with thymine and adenine, compounds **111** and **112**. The adenine or the thymine ring was introduced into the sugar ring by coupling with the triflate derivative **136** (Scheme 27).



Scheme 27. Synthesis of bisheaded nucleosides with thymine and adenine bases

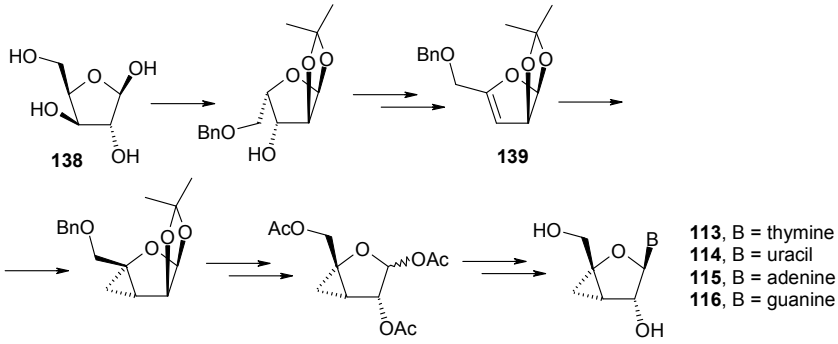
The introduction of the double bond between the 3′ and 4′ carbon atoms of compound **137** is an important method for the synthesis of several unsaturated nucleoside analogues.[53] Haraguchi *et al*. synthesized 4′-cyano-2′,3′-didehydro-2′,3′-dideoxythymidine **108** by using allylic substitution to the 3′,4′-unsaturated nucleoside **137** having a leaving group at the 2′-position with cyanomethylsilane in the presence of $SnCl_4$ (Scheme 28).[54]



Scheme 28. Synthesis of 4′-cyano-2′,3′-didehydro-2′3′-dideoxythymidine

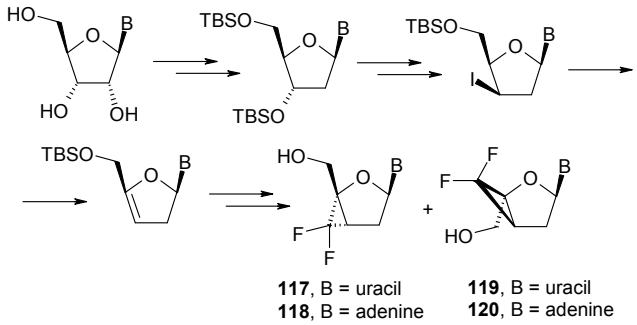
To synthesize of 3′,4′-bicyclic nucleoside analogues a double bond intermediate **139** is used. The base is introduced in the last step after bicyclic ring has been formed.

Gagneron *et al*.[55] prepared the bicyclic nucleoside analogues **113-116** starting from *L*-xylose **138**. The 2-oxabicyclo[3.1.0]hexane template was obtained from **139** by the cyclopropanation reaction (Scheme 29).



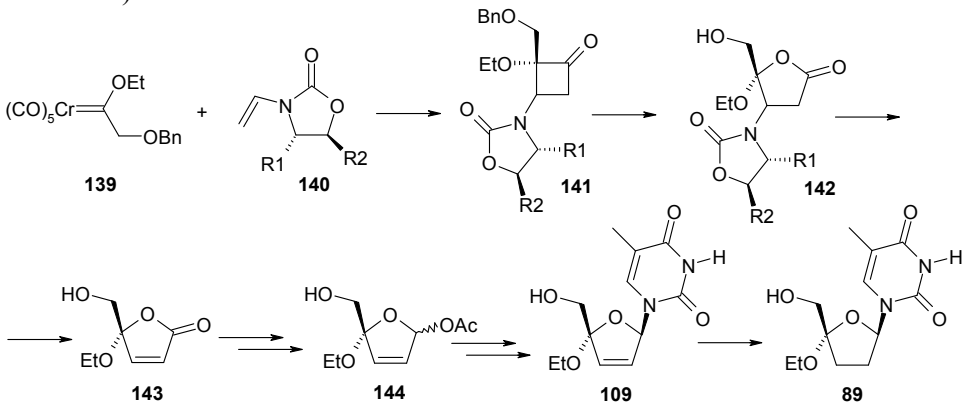
Scheme 29. Synthesis of 2-oxabicyclo[3.1.0]hexane template containing nucleoside analogues

The bicyclic template-containing fused-ring 2,2-difluorocyclopropane nucleoside analogues **122** and **123** were prepared by Nowak *et al*.[56,57] In addition, the diastereomeric compounds **122** and **123** were also separated and characterized (Scheme 30).



Scheme 30. Synthesis of difluorocyclopropane-fused nucleoside analogues

Asymmetric total synthesis of nucleoside analogues is a novel approach to nucleoside analogues. Hegedus *et al*.[58,59] synthesized 4′-ethoxy nucleoside analogues by photolysis of optically active ene carbamates **140** with the chromium carbene complex **139**, resulting in optically active cyclobutanone **141**. The Baeyer-Villiger oxidation of compound **141** gave lactone **142**. The oxazolidinone group was removed using TBAF to afford compound **143**. The base was introduced to the ribose ring by using the Vorbrüggen coupling to acetate **144** as described earlier. The reduction of the double bond of nucleoside **109** afforded nucleoside **89** (Scheme 31).



Scheme 31. Synthesis of 4'-ethoxy nucleoside analogues

In summary, 4′-substituted nucleoside analogues are promising anti-HIV compounds, especially, against drug-resistant HIV reverse transcriptase. The methods of synthesis of these compounds may be classified according to the key intermediate for synthesis or according to the starting compound used.

The first key intermediate **121** contains a double bond between the 4′ and 5′ carbon atoms. Substituent is added *via* a reaction with the double bond. In such a way simple groups, like azido, ethoxy etc are introduced in 4′-position.

The second important key intermediate **123** contains 4′,5′-epoxide group and is obtained from **121**. The reaction with the epoxide function is used to introduce the 4′-substitution into ribose ring.

4′-Hydroxyethyl substituted compound **126** is the third intermediate, providing a large number of 4′-substituted nucleoside analogues.

4′-Hydroxymethyl substituted intermediate **129** is transformed to aldehyde **130** and then used for the synthesis of many 4′-substituted analogues.
Intermediates **137** and **139** contain a double bond between the 3′ and 4′ carbon atoms. These are the starting compounds for the synthesis of a series of 4′-substituted 2′,3′-unsaturated and for the preparation of 3′,4′-bicyclic nucleoside analogues as well.

A novel approach in the preparation of 4′-substituted nucleoside analogues is asymmetric total synthesis. The advantages of the asymmetric synthesis is access to both nucleoside enantiomers, use of chiral catalytic processes and cheap simple unnatural starting materials.

1.1.2. Carbocyclic nucleoside analogues

The carbocyclic nucleosides are structural analogues of natural and synthetic nucleosides in which the ribose oxygen has been replaced by the methylene group. The structure of the cyclopentane ring is chemically much more stable than that of the ribose hemiacetal, and also more resistant to enzymes. Very often the compounds exhibit biological activity. The first members of this family of compounds are aristeromycin **145** and neoplanocin **146**, were found in nature and described in 1966. The compounds have an antibiotic and antitumor activity, respectively (Figure 8).[60] Many other biological activities such as antiparasitic, antiarthritic etc. were also found for these compounds.

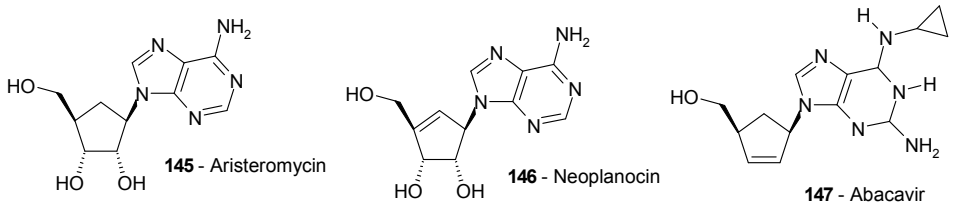


Figure 8. The structures of carbocyclic nucleosides

Some carbocyclic nucleoside analogues are active against HIV. Abacavir **147**, which was approved by FDA in 1998 (Fig. 8), is a reverse transcriptase inhibitor and is used for the treatment of AIDS. So far, many carbocyclic nucleoside analogues have been prepared and tested for antiviral activity.

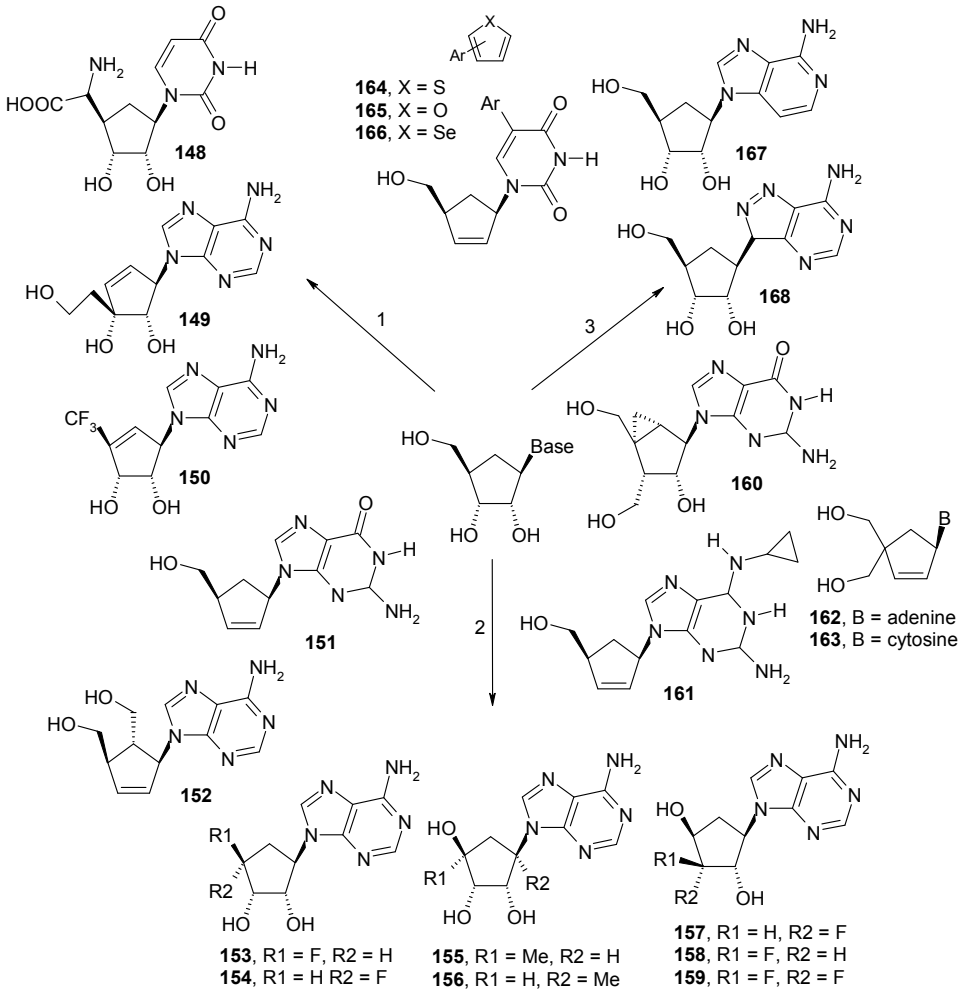


Figure 9. The structures of some carbocyclic nucleoside analogues

Carbocyclic nucleosides can be classified according to their structure, similarly to that for the ribose ring containing nucleoside analogues, to three main groups: modified in base, in cyclopentane ring or in hydroxymethyl group (Fig. 9).

The first group of compounds has a modified hydroxymethyl group (direction 1, Fig. 9). As examples compounds **148**[61], **149**[62] and **150**[63] are presented. In the case of compound **149** the 4′-hydroxyethyl group of the ribose is shifted to the position C3′.

In resent publications the introduction of a fluoro atom to the ring has been described. Thus, compounds **153-154**,[64] **155-156**[65] and **157-159**[66] have been synthesized.

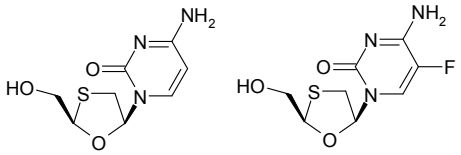
The second group of compounds has a modified carbocyclic ring (direction 2, Fig. 8). As examples compounds **160**[67] and **162-163**[68] are presented. Carbovir **151**[69] and compounds **146**[71] and **152**[70], which have found to exhibit anti-HIV activity, also belong to that group.

The modification of the base structure is the third approach to the carbocyclic nucleosides (direction 3, Fig. 9). The structures of compounds **164-166** with an aromatic substitution in the base,[60] the 3-deazapurine ring contained compound **167**[71] and carbocyclic C-nucleoside **168**[72] are presented.

Search for new carbocyclic bioactive structures is still a promising direction of the synthesis of nucleoside analogues. From the chemical point of view the carbocyclic ribose analogues in these nucleosides represent usually chiral 1,2,3,4-substituted cyclopentanes with strictly controlled stereochemistry. For the synthesis of latter the methods of the asymmetric synthesis may be applied.

1.1.3. Nucleoside analogues containing a heteroatom in the ribose ring

One of modern approaches in the synthesis of nucleoside analogues is the synthesis of heteroatom-containing nucleoside analogues in the ribose ring. This approach afforded a new drug lamivudine **169** which was approved by the FDA in 1995 for use against HIV in combination with zidovudine **4**. Also, emtricitabine **170** was approved by the FDA in 2003 (Fig. 10).[10]



169, Lamivudine **170**, Emtricitabine

Figure 10. The structures of heteroatom containing nucleoside analogues with anti-HIV activities

The nucleoside analogues containing heteroatom in the ribose ring may be classified into four main groups according to the nature of replacement of oxygen or carbon by heteroatom in the ribose ring: by replacement of oxygen by a sulfur atom (direction 1, Fig 11.), by nitrogen atom (direction 2, Fig 11.), by phosphorus

atom (direction 3, Fig 11.), and oxygen and carbon by two heteroatoms (direction 4, Fig 11.).

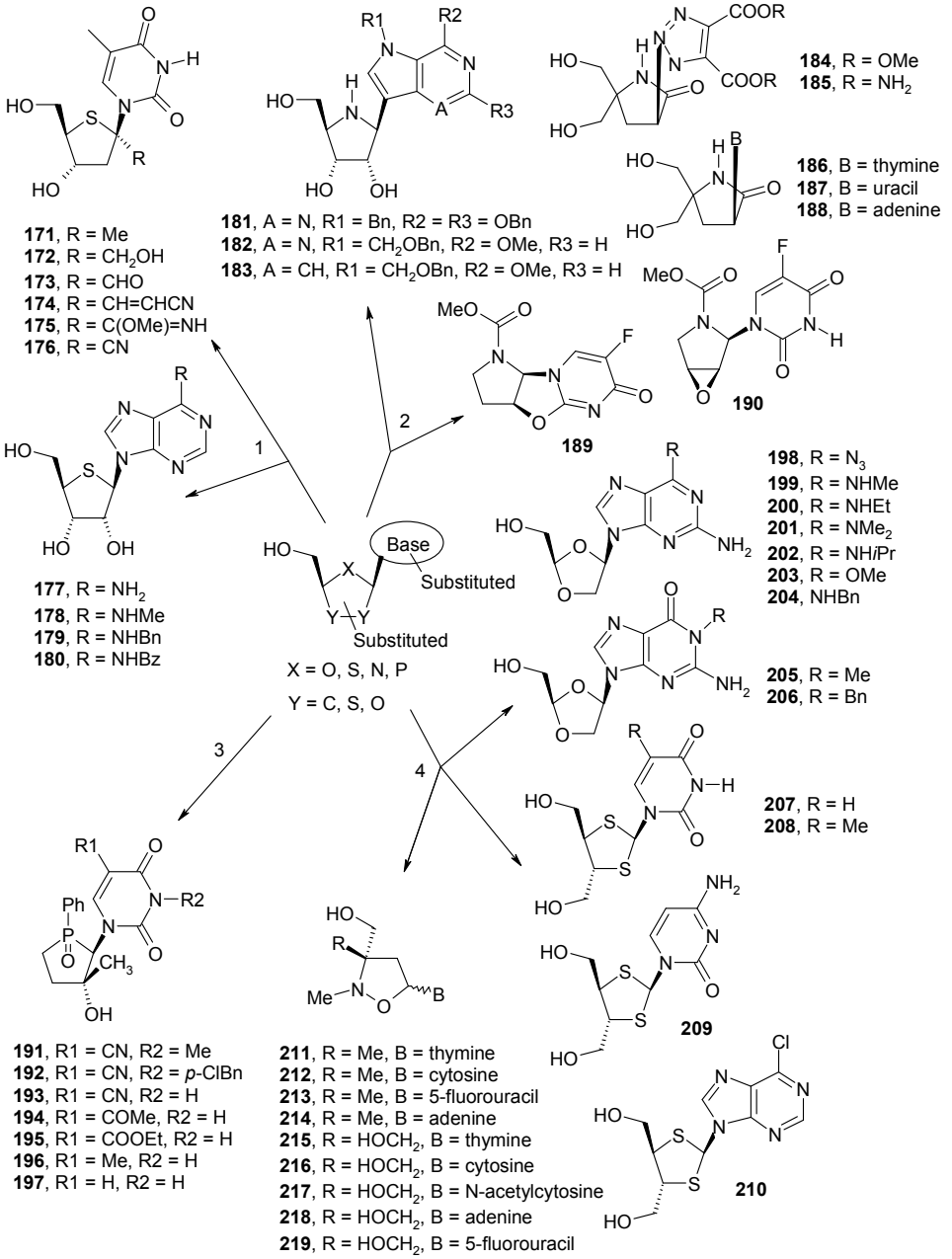
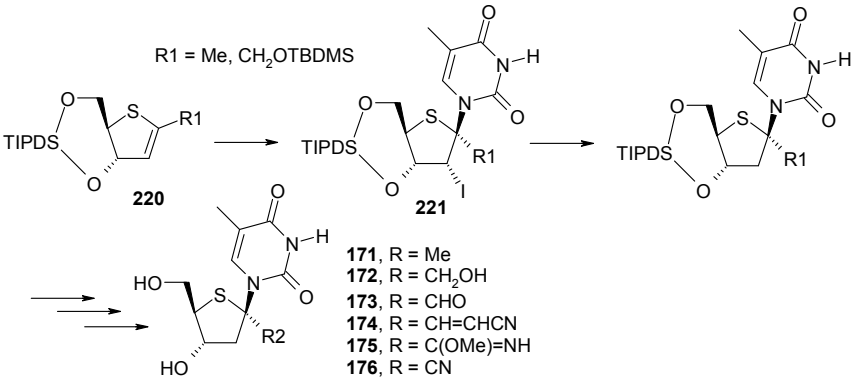


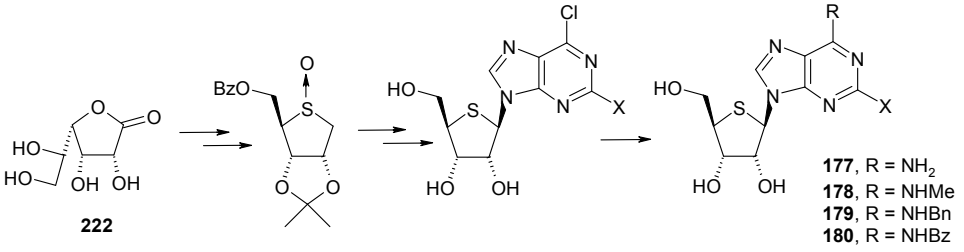
Figure 11. The structure of nucleoside analogues containing heteroatom(s) in the ribose ring

Thionucleoside analogues, 1′-carbon-substituted 4′-thiothymidines **171-176** (direction 1, Fig. 11), were synthesized by Haraguchi *et al.*[73,74]. The key step of the method is the N-iodosuccinimide-initiated electrophilic glycosidation between a silylated thymine and 1-carbon-substituted 4-thioglycals **220** to afford stereoselectively the corresponding β-anomers **221**. Tin radical-mediated removal of the 2′-iodine atom from these compounds provided the corresponding 1′-branched 4′-thiothymidine derivatives **171-176** in good yield. 1′-methyl-4′-thiothymidine **171** showed anti-HSV activity, but it was much lower than that of the parent compound, 4′-thiothymidine (Scheme 32).



Scheme 32. Synthesis of 1′-carbon-substituted 4′-thiothymidines

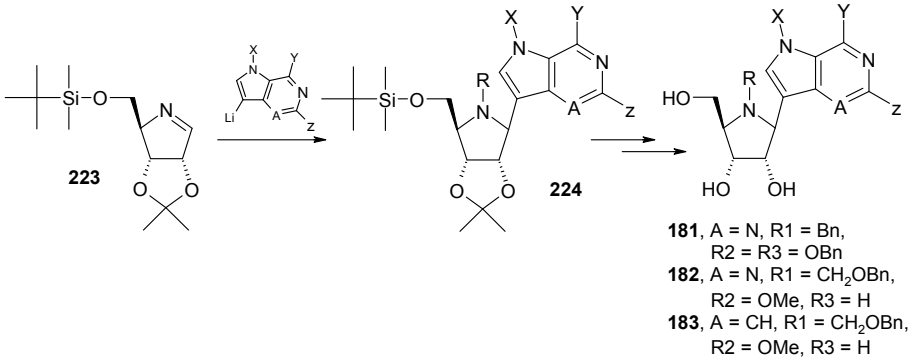
Jeong *et al.*[75] synthesized N^6-substituted-4′-thioadenosines **177-180** (direction 1, Fig. 11) starting from *D*-gulonic-γ-lactone **222**, and tested the compounds on human A_3 and other subtypes of adenosine receptors (Scheme 33).



Scheme 33. Synthesis of N^6-substituted-4′-thioadenosines

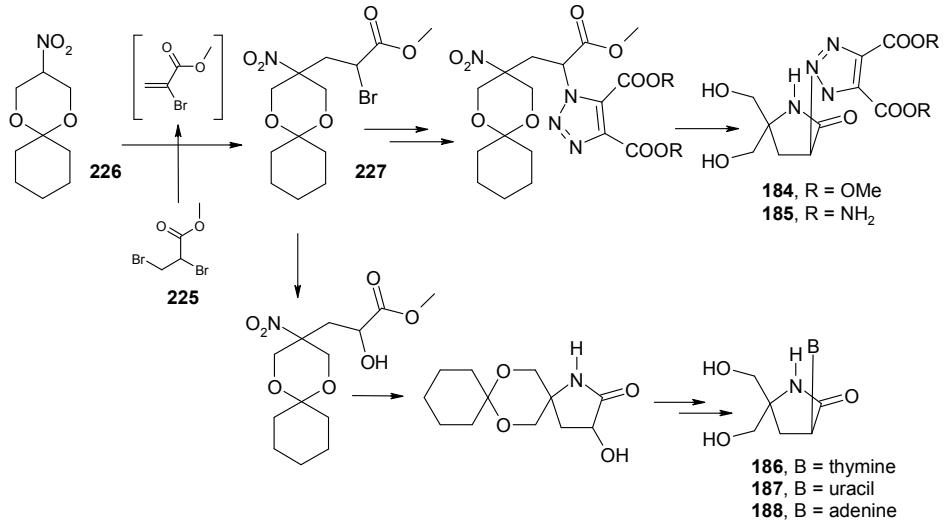
The azanucleosides having the structure of immucillins are extremely powerful inhibitors of human purine nucleoside phosphorylase, protozoan nucleside hydrolases and purine phosphoribosyltransferases. The immucillins have become target compounds for drug design because of an expected selective proliferation control on T-cells. Evans *et al.*[76,77] described a method of preparation of the aza-C-nucleoside analogues. The key step of the synthesis is addition of 9-lithio-9-deazapurine to the carbohydrate-derived cyclic imine **223** that affords **224** in

excellent yield. Using this method a series of immucillin analogues **181-183** has been synthesized (Scheme 34).



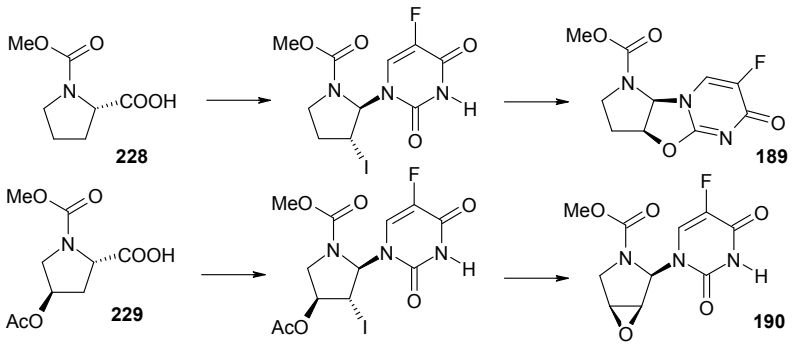
Scheme 34. Synthesis of the analogues of immucillin

Mironiuk-Puchalska *et al*.[78] prepared branched-chain (±)-azaisonucleosides *via* the Michael addition of 5-nitro-2,2-pentamethylene-1,3-dioxane **226** to methyl 2-bromoacrylate, which was generated in situ from methyl 2,3-dibromopropanoate **225** and triethylamine to afford α-bromo-γ-nitroester **227**. This compound is readily converted into 5,5-bis(hydroxy-methyl)pyrrolidine nucleoside analogues **184-188** (Scheme 35).



Scheme 35. Synthesis of branched-chain (±)-azaisonucleosides

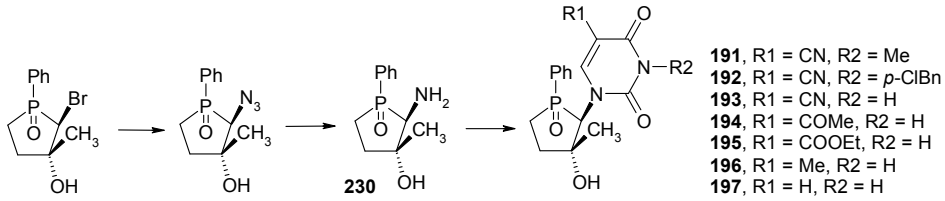
Boto *et al*.[79] synthesized azanucleosides **189-190** by a "one-pot" synthesis procedure from the corresponding proline derivatives **228** or **229** (Scheme 36).



Scheme 36. Synthesis of azanucleosides from proline and hydroxyproline

The synthesis of phosphonucleoside analogues is a relatively new approach in the chemistry of nucleoside analogues. The phospha-sugar molecules have aroused interest due to their physicochemical properties as well as potential biological activity.

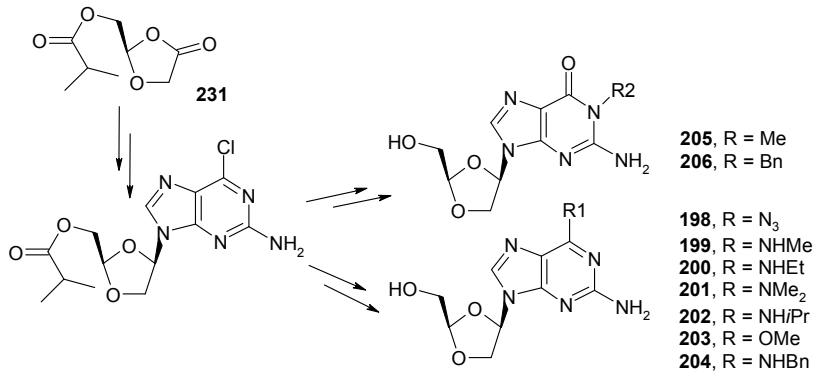
Yamashita *et al.*[80] described a method for preparation of several novel 2-(5′-substituted)uracil deoxy phospha-sugar pyrimidine nucleosides **191-197** (direction 3, Fig. 11) in racemic form by treatment of 2-aminophospholane 1-oxide **230** with several carbonylacrylamides (Scheme 37).



Scheme 37. Synthesis of 2-(5'-substituted)uracil deoxy phospha-sugar pyrimidine nucleosides

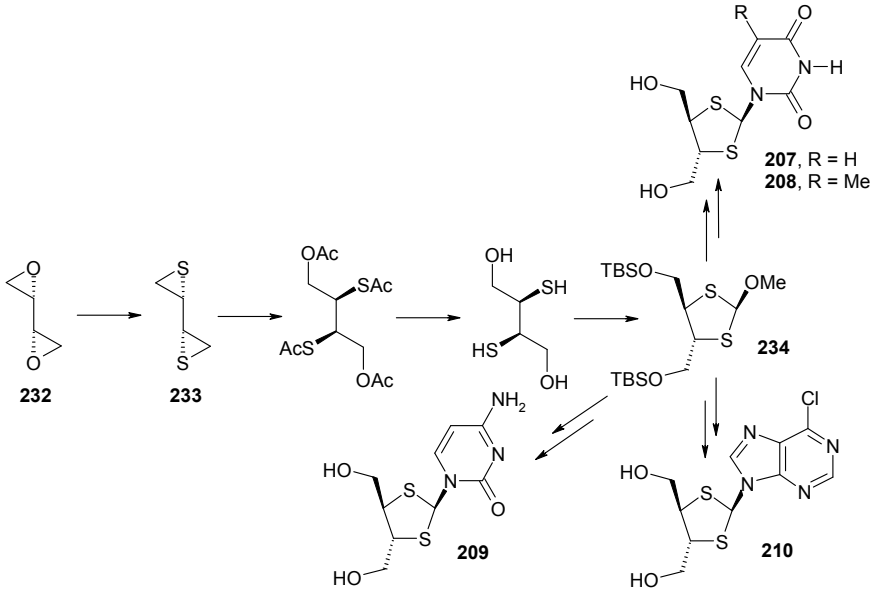
The synthesis of the nucleoside analogues that bear two heteroatoms in the furanose ring is a logic continuation of that of heteroatom-containing derivatives. Narayanasamy *et al.*[81] synthesized 1,3-dioxolane analogues **198-206** (direction 4, Fig. 11) starting from **231** (Scheme 38), which were modified at the C6 position of the purine.

The presence of alkyl amino groups at the C6 position of the purine ring increased the antiviral potency of the compound in several times. The most potent antiviral compound was found to be 1,3-dioxolane-2-amino-6-aminoethyl- purine **200** (Scheme 38).



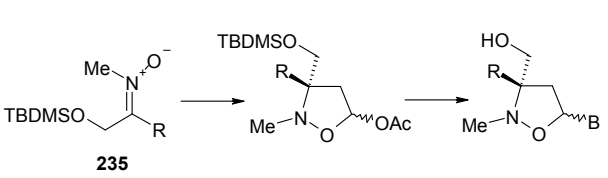
Scheme 38. Synthesis of 1,3-dioxolane ring containing nucleosides

Brånalt and Kvarnström[82] reported a method for the synthesis of [4,5-bis(hydroxymethyl)-1,3-dithiolan-2-yl]nucleosides **207-210** (Fig. 11). 1,2:3,4-diepoxybutane **232** as a starting compound was reacted with potassium thiocyanate to give 1,2:3,4-diepithiobutane **233**. The opening of the thirane ring with acetate followed by deacetylation gave 2,3-dithiothreitol, which was silylated and treated with trimethyl orthoformate to give 2-methoxy-1,3-dithiolane **234**. The condensation of this compound with silylated thymine, uracil, N4-benzoylcytosine and 6-chloropurine using a modified Vorbrüggen procedure, followed by deprotection, gave nucleoside analogues **207-210** (Scheme 39). Unfortunately, all compounds were inactive against HIV *in vitro*.



Scheme 39. Synthesis of [4,5-bis(hydroxymethyl)-1,3-dithiolan-2-yl]nucleosides

Chiacchio *et al*.[83] described the synthesis of 4′-α-C-branched N,O-nucleosides **211-219**, based on the 1,3-dipolar cycloaddition of nitrones **235** with vinyl acetate followed by the coupling with silylated nucleobases (Scheme 40). The compounds **211-219** obtained were evaluated for their activity against HSV-1, HSV-2 and HTLV-1. Compound **219** showed a moderate apoptotic activity in Molt-3 cells.



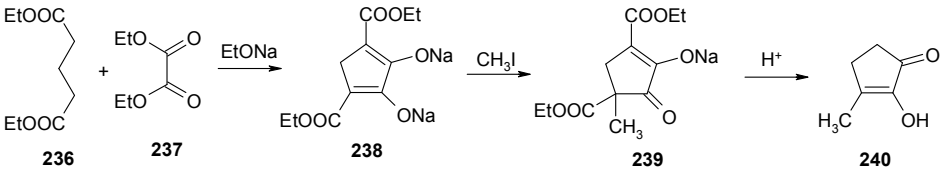
211, R = Me, B = thymine
212, R = Me, B = cytosine
213, R = Me, B = 5-fluorouracil
214, R = Me, B = adenine
215, R = CH_2OH, B = thymine
216, R = CH_2OH, B = cytosine
217, R = CH_2OH, B = N-acetylcytosine
218, R = CH_2OH, B = adenine
219, R = CH_2OH, B = 5-fluorouracil

Scheme 40. Synthesis of 4'-α-C-branched N,O-nucleosides

The heteroatoms present in ribose represent an important class of nucleoside analogues. In the early 1990s it was reported that a simple replacement of oxygen in the ribose ring with sulfur atom leads to potential antiviral and antitumor nucleosides. This observation induced researchers to synthesize different heteroatom-containing nucleoside analogues. As a result two anti-HIV drugs lamivudine and emtricitabine have been synthesized and have already approved by FDA.

1.2. Synthesis of substrates for the asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones

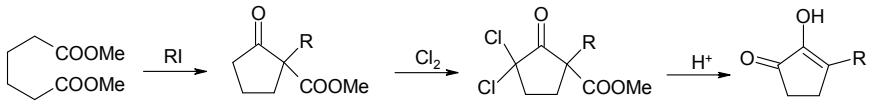
One of the earliest methods for the preparation of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones was described by Hesse and Böckmann.[84] Thus, the methyl derivative **240**, was prepared from glutaric ester **236** ethyloxalate **237** and methyl iodide. Ethylglutarate **236** was coupled with ethyloxalate **237** in the presence of sodium ethylate to afford sodium salt **238**. Alkylation of **238** with methyl iodide afforded diester **239**. Hydrolysis and decarboxylation of **239** using a 20% solution of H_2SO_4 under reflux gave the target compound **240** (Scheme 41).



Scheme 41. Synthesis of 3-methyl-2-hydroxy-2-cyclopenten-1-one from glutarate

McFearin[85] used different glutarate and oxalate esters for the preparation of 3-methyl-2-hydroxy-2-cyclopenten-1-one **240**. Ito *et al*.[86,87] improved the above method (Scheme 41) by using NaH as a base to couple **236** and **237**, and obtained compound **238** in 84% yield. The 81% yield of compound **239** was achieved by refluxing compound **238** and methyl iodide in acetonitrile for 16 hours in the presence of potassium carbonate. The yield of hydrolysis and decarboxylation step was also improved by using a mixture of concentrated hydrochloric and acetic acids (1:1). Thus, refluxing **239** in the mixture of acids for 3 hours gave compound **240** in 80% yield. The method can be used to prepare other alkyl-substituted substrates. Thus, Strunz and Lal[88] used different halides – methyl- and ethyl iodides, benzyl- and allylbromides for this purpose. (Scheme 42) Using a 42% solution of aqueous phosphoric acid the yield of hydrolysis was improved and the final 3-alkyl-2-hydroxy-2-cyclopenten-1-ones were obtained in 44-80% overall yield.

Leir[89,90] reported a method of preparation of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones **240, 244a-f** from dimethyl adipate **241** in 65-70% overall yield. The Dieckmann cyclization of **241** followed by alkylation with different halides in DMFA afforded compounds **242a-f**. Dichloro keto esters **243a-f** were obtained from **242a-f** by chlorination.



241

242a, R = Me
242b, R = Et
242c, R = Bn
242d, R = *n*Pr
242e, R = *n*Bu
242f, R = *sec*Bu

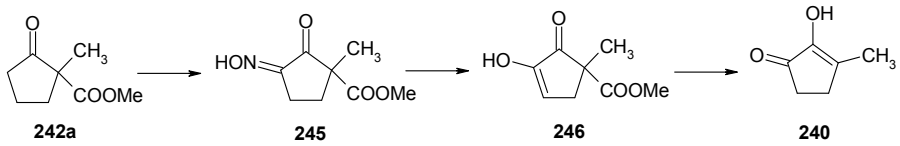
243a, R = Me
243b, R = Et
243c, R = Bn
243d, R = *n*Pr
243e, R = *n*Bu
243f, R = *sec*Bu

240, R = Me
244b, R = Et
244c, R = Bn
244d, R = *n*Pr
244e, R = *n*Bu
244f, R = *sec*Bu

Scheme 42. Synthesis of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones from adipate

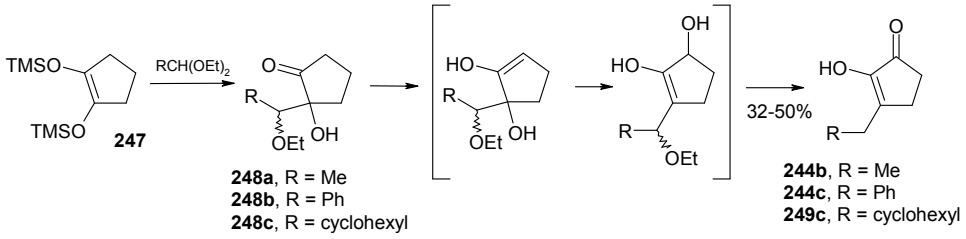
Instead of chlorine, bromine can also be used, giving the dibromide of esters **242a-f**. The last step is hydrolysis and decarboxylation of **243a-f** to afford the target compounds **240, 244b-f** in 26-65% overall yield form dimethyl adipate (Scheme 42).

Instead of chlorination of ester **242a** (Scheme 42) Sato *et al*.[91,92] used nitrite in order to introduce a carbonyl group into cyclopentane ring. However, lower yield were obtained if compared with the method of chlorination. Thus, methyl ester **242a** was treated with n-butyl nitrite resulting in oxime **245**. This compound was hydrolyzed and decarboxylated, to afford the final compound 3-methyl-2-hydroxy-2-cyclopenten-1-one **240** in 35% yield (Scheme 43).



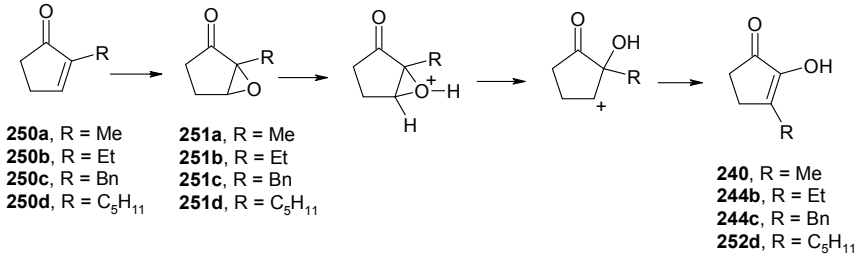
Scheme 43. Synthesis of 3-methyl-2-hydroxy-2-cyclopenten-1-one by α-oximation

The method of α-oximation of ketones was later investigated by Mohammed and Nagendrappa,[93] who suggested a convenient procedure for the preparation of NOCl in situ, using a combination of Me_3SiCl and isoamyl nitrite.
Pattenden and Teague[94] and later Gao and Burnell[95] prepared 3-alkyl-2-hydroxy-2-cyclopenten-1-ones **244b-c, 249c** from 1,2-bis(trimethylsilyloxy)cyclopentene **247.** The Lewis acid-mediated reaction of **247** with acetals derived from a variety of aldehydes gave compounds **248a-c**. The treatment of **248a-c** with Amberlyst 15 resin in THF afforded final products **244b-c** and **249c** in 32-50% yield (Scheme 44).



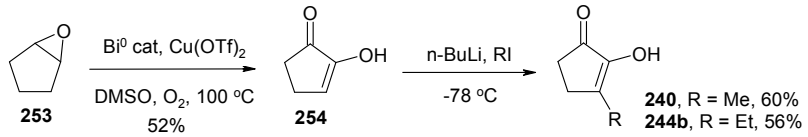
Scheme 44. Synthesis of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones from bis(trimethyl-silyloxy)cyclopentene

Barco *et al*.[96] described the synthesis of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones **240, 244b-c** and **252d** in 52-70% yield. ´The method was based on the acid-catalysed rearrangement of 2,3-epoxy-2-alkylcyclopentanones **251a-d**, which are readily available from the alkaline hydrogen peroxide oxidation of the corresponding 2-alkyl-2-cyclopenten-1-ones **250a-d** (Scheme 45).



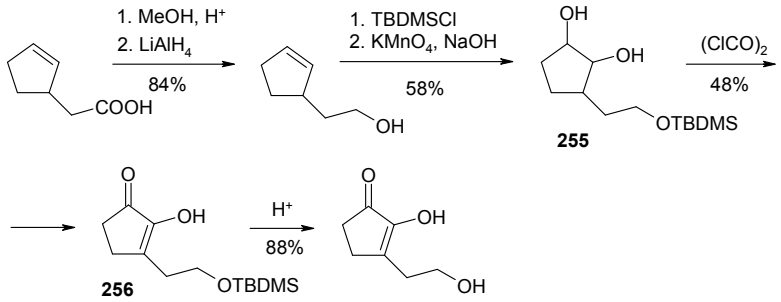
Scheme 45. Synthesis of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones from 2-substituted-2-cyclopenten-1-one

Antoniotti *et al*.[97] prepared 3-methyl and 3-ethyl-2-hydroxy-2-cyclopenten-1-ones **240** and **244b** from the commercially available cyclopentene oxide **253**. The oxidation of **253** was carried out using oxygen and a mixed catalyst consisting 6 mol% of bismuth(0) and 7 mol% of copper(II)triflate to afford 2-hydroxy-2-cyclopenten-1-one **254** in 52% yield. The treatment of **254** with two equivalent of n-butyllithium at -78 °C, followed by the addition of four equivalents of methyl- or ethyl- iodide afforded the final product **240** or **244b**, in 56-60% yield, respectively (Scheme 46).



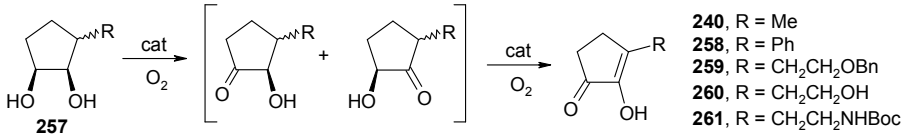
Scheme 46. Synthesis of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones from cyclopentene oxide

A direct method for the synthesis of cyclopentan-1,2-dione is the oxidation of cyclopentan-1,2-diols. For example, a procedure using the Swern oxidation of diol **255** resulted in diketone **256** with moderate yield (Scheme 47).[98]



Scheme 47. Synthesis of 3-hydroxyethyl-2-hydroxy-2-cyclopenten-1-one from 2-cyclopenten-1-acetic acid

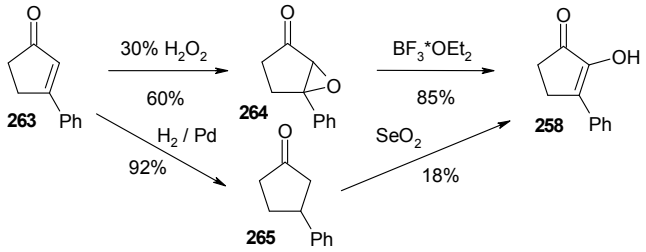
Recently a catalytic aerobic oxidation method[99] for the preparation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones **240-252** from the corresponding 3-substituted cyclopentan-1,2-diols **257** up to 76% yield, using a heterogeneous Pt/C catalyst was developed (Scheme 48).



Scheme 48. Synthesis of 3-substituted-2-hydroxy-2-cyclopentene-1-ones starting from the cyclopentane-1,2-diols

Synthesis of 3-aryl-2-hydroxy-2-cyclopenten-1-ones is a special target for chemists because these compounds are not available from electrophilic substitutions that were described above.

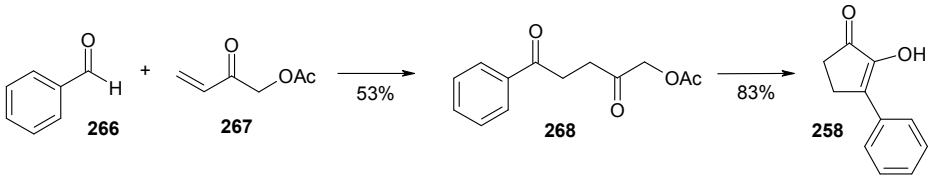
House and Wasson[100] synthesized 3-phenyl-2-hydroxy-2-cyclopenten-1-ones starting from the 3-phenyl-2-cyclopenten-1-one **258**. The epoxidation of **263** using the hydrogen peroxide solution gave epoxide **264** in 60% yield. 3-Phenyl-2-hydroxy-2-cyclopenten-1-one **258** was formed in the presence of BF_3•Et_2O in 85% yield (Scheme 49).



Scheme 49. Synthesis of 3-phenyl-2-hydroxy-2-cyclopenten-1-one from 3-phenyl-2-cyclopenten-1-one

Alternatively, 3-phenyl-2-hydroxy-2-cyclopenen-1-one **258** was prepared by the reduction of **263** with hydrogen on the palladium catalyst in 92% yield. The oxidation of **265** with SeO_2 gave the target compound **258**, but only in 18% yield (Scheme 49).

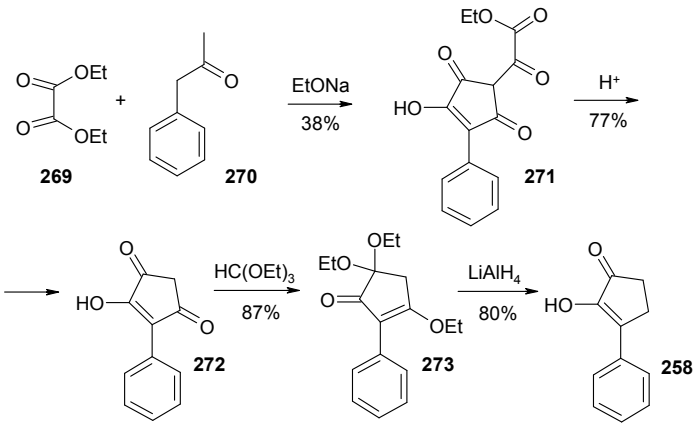
Stetter and Schlenker[101] reported a method for the preparation of 3-alkyl- and 3-aryl-2-hydroxy-2-cyclopenten-1-ones starting from the corresponding aldehydes. Thus, benzaldehyde **266** was condensed with 1-acetoxy-3-buten-2-one **267** to afford compound **268** in 53% yield. Cyclization of **268** with NaOEt gave the target 3-phenyl-2-hydroxy-2-cyclopenten-1-one **258** in 83% yield (Scheme 50).



Scheme 50. Synthesis of 3-phenyl-2-hydroxy-2-cyclopenten-1-one from benzaldehyde

Van Brussel *et al.*[102] prepared 3-alkyl- and 3-phenyl-2-hydroxy-2-cyclopenten-1-ones starting from 4-hydroxy-5-alkyl-4-cyclopenten-1,3-diones (or 4-hydroxy-5-phenyl-4-cyclopenten-1,3-diones) by treatment with triethylorthoformiate, followed by reduction with $LiAlH_4$. This method was further used by Norman *et al.*[103] for preparation of cyclopentanediones and -triones. Thus, synthesis of 3-phenyl-2-hydroxy-2-cyclopenten-1-one **258** started from the coupling of phenylacetone **270** and diethyloxalate **269** to afford intermediate **271**, which was hydrolyzed under the acidic conditions to give compound **272** in 77% yield. Refluxing of triethylorthoformiate with compound **272** lead to compound **273** in

87% yield. Reduction of **273** with $LiAlH_4$ gave the target 3-phenyl-2-hydroxy-2-cyclopenten-1-one **258** in 80% yield (Scheme 51).



Scheme 51. Synthesis of 3-phenyl-2-hydroxy-2-cyclopenten-1-one from phenylacetone

In conclusion it should be pointed out that there is an abundance of different methods for the synthesis of 3-alkylsubstituted-2-hydroxy-2-cyclopenten-1-ones. Many of those result in acceptable yield and are easy to perform. However, there is no method which is considerably superior to the others. To prepare 3-alkyl-2-hydroxy-2-cyclopenten-1-ones the condensation using adipate and glutarate may be used. In laboratory conditions the methods using diol oxidation can also be employed. However, methods to obtain 3-aryl-2-hydroxy-2-cyclopenten-1-ones are quite rare.

The first method of condensation employs the Stetter reaction of benzaldehyde and acetoxymethylvinyl ketone.

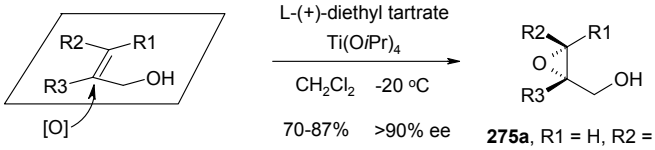
The use of the second method, which employs oxalate and phenylacetone may also be considered. Unfortunately, the use of the latter compound in the lab has several restrictions and, therefore, cannot be widely used.

The third method is not competitive because of the high cost of the starting compounds.

1.3. Asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones

The first practical method of asymmetric epoxidation was described by Katsuki and Sharpless.[104] They used diethyl tartrate (DET), titanium tetraisopropoxide $Ti(OiPr)_4$ and tert-butyl hydroperoxide (*t*BuOOH) for the asymmetric oxidation of allylic alcohols **274a-e** (Scheme 52). DET and $Ti(OiPr)_4$ form a large, sterically hindered complex with allylic alhohol derivatives, which direct the stereocontrolled

addition of oxygen to the double bond. Epoxides **275a-e** were obtained in 70-87% yield and with high enantiopurity (*ee* > 90 %, Scheme 52).



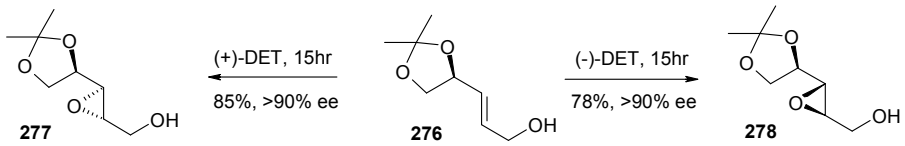
274a, R1 = H, R2 = R3 = Ph
274b, R1 = Me, R2 = $(CH_3)_2C$=CH-$(CH_2)_2$, R3 = H
274c, R1 = H, R2 = nC_9H_{19}, R3 = H
274d, R1 = nC_9H_{19}, R2 = R3 = H
274e, R1 = R2 = H, R3 = cyclohexyl

275a, R1 = H, R2 = R3 = Ph
275b, R1 = Me, R2 = $(CH_3)_2C$=CH-$(CH_2)_2$, R3 = H
275c, R1 = H, R2 = nC_9H_{19}, R3 = H
275d, R1 = nC_9H_{19}, R2 = R3 = H
275e, R1 = R2 = H, R3 = cyclohexyl

Scheme 52. Asymmetric epoxidation of allylic alcohols

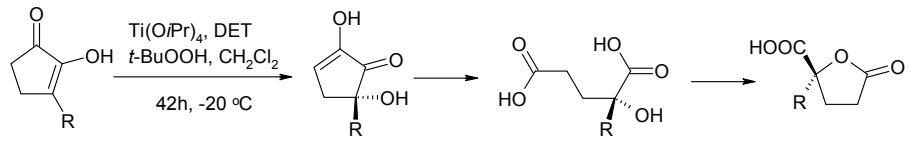
In asymmetric oxidation many different ligands with various metal catalysts have been used while dialkyl tartrates and tartramides with titanium have showed the best results.[105]

Depending on the configuration of the tartrate used it is possible to direct the stereochemistry of the epoxide.[106,107] In the case of (+)-diethyltartrate epoxide **277** is formed from **276** with 85% yield and >90% *ee*. When (-)-diethyltartrate is used, epoxide **278** is obtained with the a similar yield and stereoselectivity, but in the opposite configuration (Scheme 53).



Scheme 53. The dependence of the absolute configuration of epoxide on the configuration of the enantiomer of diethyl tartrate in the complex

The oxidation of different 3-alkyl-2-hydroxy-2-cyclopentan-1-ones **240, 244b, 259** and **260** with the Sharpless complex was investigated in our department (Scheme 54).[5,6]



240, R = Me
244b, R = Et
259, R = CH_2CH_2OBn
260, R = CH_2CH_2OH

279a, R = Me
279b, R = Et
279c, R = CH_2CH_2OBn
279d, R = CH_2CH_2OH

280a, R = Me
280b, R = Et
280c, R = CH_2CH_2OBn
280d, R = CH_2CH_2OH

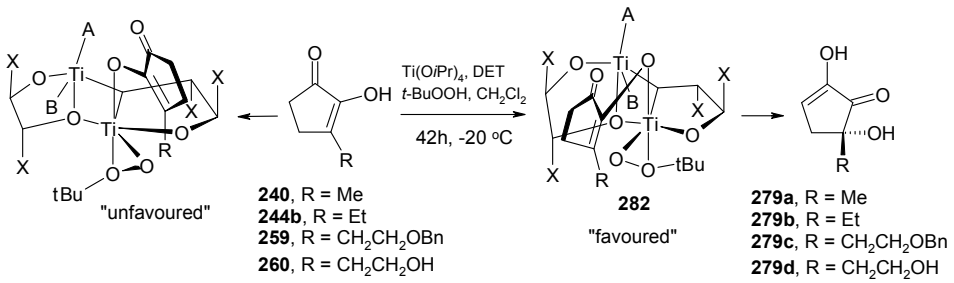
281a, R = Me
281b, R = Et
281c, R = CH_2CH_2OBn
281d, R = CH_2CH_2OH

Scheme 54. Asymmetric oxidation of 3-alkyl-2-hydroxy-2-cyclopentene-1-ones

Two major oxidation products viz. primary hydroxylation products **279a-d** and derivatives of more oxygenated, ring-cleaved, hydroxylation products **280a-d** were found to form. After lactonization, these were finally isolated as lactone acids **281a-d** (Scheme 54).

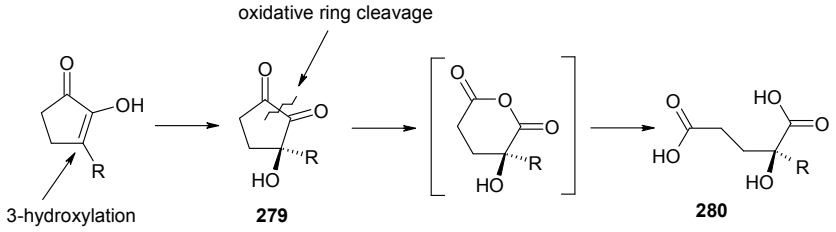
The stereoselectivety of the oxidation of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones using a Ti(O*i*Pr)$_4$-DET-complex depends on the ratio of Ti(O*i*Pr)$_4$ to DET in the complex and the amount of *t*-BuOOH. The oxidation of compound **240** (Scheme 54) at a ratio of Ti(O*i*Pr)$_4$:DET:*t*BuOOH of 1.0:1.6:1.5 affords lactone acid **281a** in 44% yield and 95% *ee*.[5]

The high stereoselectivity of the asymmetric oxidation of **240, 244b, 259** and **260** is connected with the formation of a „favoured“ conformation complex **282** between the substrate, Ti(O*i*Pr)$_4$ and DET. The oxidation of the "favoured" complex with *t*BuOOH at the double bond resulted in directed stereoselective primary oxidation products **279a-d** (Scheme 55).[5]



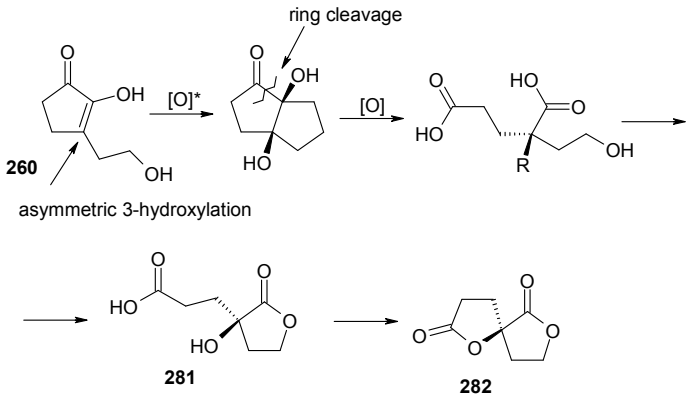
Scheme 55. An enantioface selection of the asymmetric oxidation of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones.

The hydroxylated product **279** oxidizes according to the Baeyer –Villiger reaction[108] to afford anhydride, which upon at hydrolysis leads to hydroxy diacids **280** (Scheme 56).



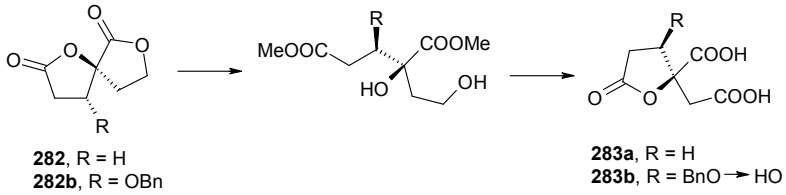
Scheme 56. The oxidative ring cleavage of 3-alkyl-2-hydroxy-2-cyclopenten-1-ones

In the case of 3-hydroxyalkyl-2-hydroxy-2-cyclopenten-1-one **260** hydroxylated at the same conditions the ring cleavage product **281** is cyclized to spirodilactone **282** (Scheme 57).[6]



Scheme 57. Synthesis of spirodilactone

The asymmetric synthesis of spirodilactones has been widely applied to the synthesis of homocitric acid derivatives. A simple method for the synthesis of enantiopure homocitric acid γ-lactone **283a** and its 4-hydroxy analogue **283b**, starting from spiro-γ-dilactone **282** or **282b** up to 74% isolated yield was reported (Scheme 58).[4]



Scheme 58. Synthesis of homocitric acid γ-lactone and its derivatives

Spirodilactones are starting lactones for the synthesis of sterically hindered nucleoside analogues.

The recently developed method for the asymmetric oxidation of 3-alkyl-2-hydroxy-2-cyclopentan-1-ones using Sharpless catalyst is one of the most convenient methods to afford substituted γ-lactone acids in good yield and high stereoselectivity. The method is acceptable for a large scale synthesis and may have application in industry. In the present work, the substituted γ-lactone acids served as starting compounds (key intermediates) for the synthesis of nucleoside analogues.

2. AIMS OF THE STUDY

Nucleoside analogues are in general prepared from natural compounds such as sugars, amino acids or nucleosides. This approach, however, does not satisfy needs of the pharmaceutical chemistry as many analogues of nucleosides cannot be prepared from a limited number of natural starting compounds. A more flexible approach is to use asymmetric synthesis, starting from simple achiral molecules. Also, asymmetric synthesis usually affords both enantiomers of the compound. In the field of nucleoside analogue synthesis there are only few examples of application of asymmetric total synthesis. Because of inconvenience of preparing analogues substituted in the 4′ position of the ribose ring starting from natural compounds, these analogues are quite rare. According to our knowledge no examples describing asymmetric synthesis of 4′-arylsubstituted nucleoside analogues has been reported.

Making use of the availability of chiral 2-substituted γ-lactone acids from asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones, this work aims at developing a method for the synthesis of 4′-substituted nucleoside analogues.

To achieve the aim the specific problems need to be solved. Hence, the goals of the present work are:

- to develop a method for the synthesis of alkyl (benzyl) and aryl 3-substituted-2-hydroxy-2-cyclopenten-1-ones
- to investigate the asymmetric oxidation of several 3-substituted-2-hydroxy-2-cyclopenten-1-ones in order to find a method for the synthesis of chiral 2-substituted γ-lactone acids
- to develop a pathway to preparation of 4′-substituted nucleoside analogues from γ-lactone acids

The preliminary determination of the biological activity of the synthesized 4′-substituted nucleoside analogues may lead to a further modifications of the structure of the compounds and the broadening the synthetic methods for their preparation.

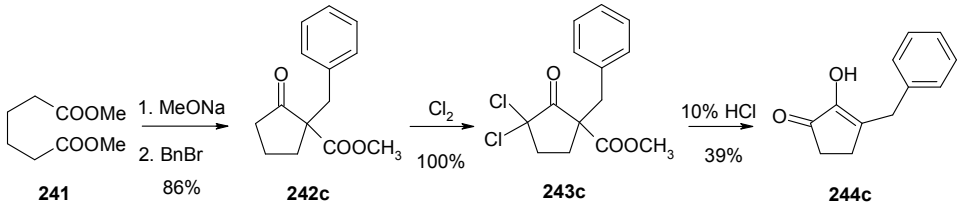
3. RESULTS AND DISCUSSION

3.1. Synthesis of 3-substituted-2-hydroxy-2-cyclopenten-1-ones (Articles II, III)

In the synthesis of 4′-substituted nucleoside analogues the key intermediates – chiral substituted γ-lactone acids are prepared by the asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones. Asymmetric oxidation will require a variety of substrates – 3-substituted-2-hydroxy-2-cyclopenten-1-ones.

3.1.1. Synthesis of 3-benzyl-2-hydroxy-2-cyclopenten-1-one (Article III)

As seen from the literature overview, there are several methods for the synthesis of 3-benzyl-2-hydroxy-2-cyclopenten-1-ones. We have to consider also the scale of the synthesis. So, to perform preparative or industrial-scale synthesis effective and cheap methods are needed. In the present work, synthesis of the title compounds was accomplished according to the procedure described by Leir *et al*.[89] The Dieckmann cyclization of dimethyl adipate **241**, using sodium methoxide, followed by alkylation with benzyl bromide gave compound **242c** in 86% yield. The next step was chlorination of **242c** by chlorine. Dichloro derivative **243c** was obtained in quantitative yield. Hydrolysis of **243c** by reflux with a solution of 10% hydrochloric acid afforded the target compound **244c** with 39% yield (Scheme 59). The advantage of this method consists in the use of cheap dimethyl adipate as a starting compound and of only three synthesis steps to achieve the target compound. The weakness of the method is the use of toxic and hazardous chlorine that requires taking additional safety measures. The method may be applied to industrial synthesis after the hydrolysis step has been improved.

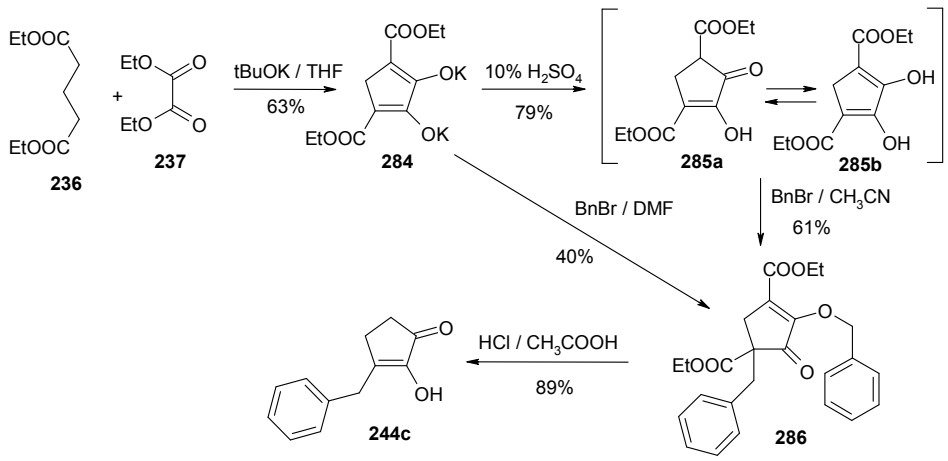


241 **242c** **243c** **244c**

Scheme 59. Synthesis of 3-benzyl-2-hydroxy-2-cyclopenten-1-one from dimethyl adipate

In order to find a more convenient method for the preparation of 3-benzyl-2-hydroxy-2-cyclopenten-1-one **244c**, the method starting from glutarate was tested

(Scheme 60). The coupling of diethyl glutarate **236** and diethyl oxalate **237** was realized using potassium tert-butoxide in THF.[109] At these conditions dipotassium salt **284**, which can easily be removed from the reaction mixture, was formed in 63% yield. It offers two possibilites for the synthesis of compound **286**: first, a direct alkylation of disalt **284** with benzyl bromide and, second, hydrolysis of dipotassium salt **284** followed by alkylation. The first route was performed in DMF giving the dialkylated product **286** in 40% yield. The modest yield may be due to the poor solubility of **284** in DMF. The second approach starts with hydrolysis of dipotassium salt **284** with a solution of 10% sulfuric acid, to afford intermediate **285** as a mixture of **285a**:**285b** (the ratio depends on the solvent, e.g in methanol the ratio is 1:1) in 79% yield. This tautomeric mixture was alkylated with benzyl bromide in the presence of an excess of potassium carbonate in acetonitrile, affording **286** in 61% yield. In both cases the last steps are the hydrolysis and decarboxylation of **286**.



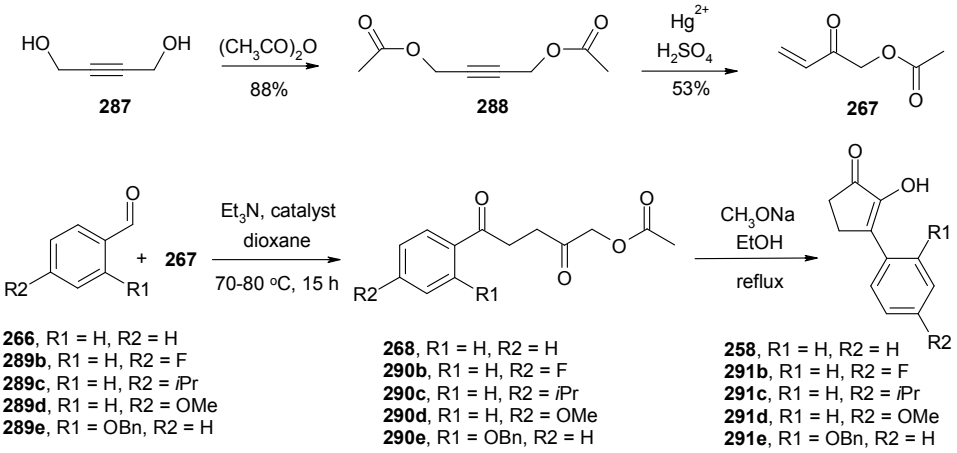
Scheme 60. Synthesis of 3-benzyl-2-hydroxy-2-cyclopenten-1-one from diethyl glutarate

Hydrolysis of **286** using concentrated hydrochloric acid afforded the target compound **244c** in 30% yield. A mixture of concentrated hydrochloric and acetic acids (1:1), which improved the solubility of the compounds, gave **244c** in 89% yield. In ten-gram scale by improving of the isolation steps of compounds **284** and **285a:285b**, and decarboxylation process, using compound **286** without purification, the overall yield of **244c** was increased to 43%.

Both methods employ cheap reagents. The first method is more straightforward. However, due to the simplicity of separation of dipotassium salt **284** from the reaction mixture, the second method may also be considered competitive.

3.1.2. Synthesis of 3-aryl-2-hydroxy-2-cyclopenten-1-ones (Article II)

As we can see from the Literature Overview, the number of methods for the preparation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones is limited. We chose the Stetter[101] method, which starts from commercially available benzaldehyde **266** or its derivatives **289b-c**, and 2-butyn-1,4-diol **287** (Scheme 61). The intermediate diacetate **288** was prepared from 2-butyn-1,4-diol **287** in 88% yield.



Scheme 61. Synthesis of 3-aryl-2-hydroxy-2-cyclopenten-1-ones

The intermediate 1-acetoxy-3-buten-2-one **267** was synthesized from **288** in 53% yield, using mercury sulphate as a catalyst. The Stetter coupling of **267** with benzaldehydes **266** and **289b-e** using 3-benzyl-5-(2-hydroxyethyl)-4-methyl-thiazolium chloride as a catalyst gave the intermediate 2,5-dioxo-5-phenylpentyl esters **268** and **290b-e** in 48-71% yield. The highest yield of **268** was obtained using the unsubstituted benzaldehyde **266** (71%, Table 1, entry 1) and the lowest yield of **290d**, in the case of the *p*-OMe substituted benzaldehyde derivative **289d** (48%, Table 1, entry 4).

Table 1. Synthesis of 3-aryl-2-hydroxy-2-cyclopenten-1-ones

Entry	Substrate 266, 289	Substituent		Yield, %	
		R1	R2	268, 290	258, 291
1	a	H	H	71	40
2	b	H	F	68	72
3	c	H	*i*Pr	53	54
4	d	H	OMe	48	52
5	e	OBn	H	66	31

The cyclization of **268** and **290b-e** using sodium methoxide in ethanol at reflux gave the target 3-aryl-2-hydroxy-2-cyclopenten-1-ones **258** and **291b-e** in 31-72% yield. The *p*-F-Ph derivative **291b** was cyclizised with the highest yield (72%) and *o*-BnO-Ph **291e**, with the lowest one (31%).

In summary, substrates for asymmetric oxidation be easily obtained from simple compounds and, if necessary, the methods used can be scaled up. Thus, 3-benzyl-2-hydroxy-2-cyclopenten-1-one is obtained from both glutarate and adipate in satisfactory yield. 3-Aryl-2-hydroxy-2-cyclopenten-1-ones are obtained starting from benzaldehyde and 1-acetoxy-3-buten-2-one. The weakness of the latter method is the use of toxic mercury salt and low yields at different steps of cyclization. However, the method needs to be improved prior to its industrial application.

3.2. Asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones (Articles I, II, III)

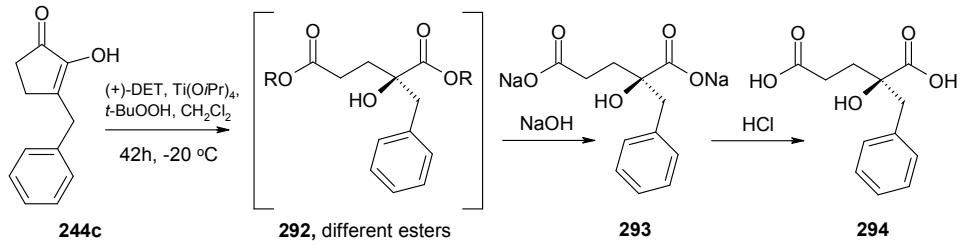
The asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones affords 2-substituted-5-oxotetrahydrofuran-2-carboxylic acids, which are the key intermediates for the synthesis of 4′-substituted nucleoside analogues in the present work.

3.2.1. Asymmetric oxidation of 3-benzyl-2-hydroxy-2-cyclopenten-1-one (Article I)

For the asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones the Ti(O*i*Pr)$_4$-DET-*t*BuOOH complex[5] was used. The absolute configuration of the γ-lactone acid formed in the oxidation reaction is determined by the absolute configuration of the diethyltartrate present in the complex. In the case of *L*-(+)-DET *R*-2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid **298** is formed, while *D*-(-)-DET affords the corresponding *S*-enantiomer **299**.[5] To elucidate the stereoselectivity of the asymmetric oxidation process both enantiomers of γ-lactone acid were synthesized (Schemes 62 and 64) and the enantiopurity of the oxidation product was measured using HPLC with a chiral column.

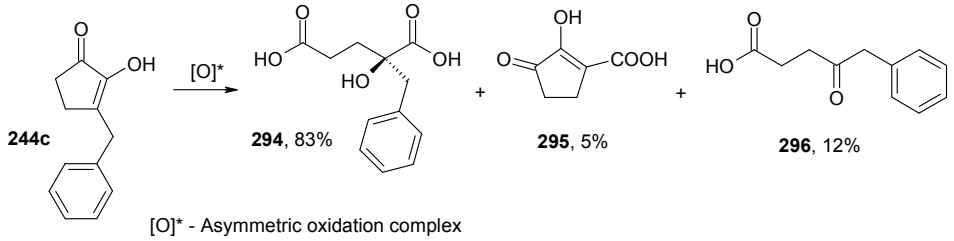
For the double oxidation of 3-benzyl-2-hydroxy-2-cyclopenten-1-one **244c** the ratio of the substrate **244c** to reagents in the oxidation complex Ti(O*i*Pr)$_4$:DET:*t*BuOOH was 1:1:1.6:2.5. As two oxidations, viz. the 3-hydroxylation and the oxidative ring cleavage proceed, at least two equivalents of *t*BuOOH are needed. In this work, usually 2.5 equivalents of *t*BuOOH were used.[5]

In order to hydrolyze the esters formed during the asymmetric oxidation (products **292)** of 3-benzyl-2-hydroxy-2-cyclopenten-1-one **244c**, the reaction mixture was sequentially treated with a base and an acid. This afforded first disodium salt **293**, and then, after acidification, diacid **294** (Scheme 62).



Scheme 62. Asymmetric oxidation and the work-up of the reaction products

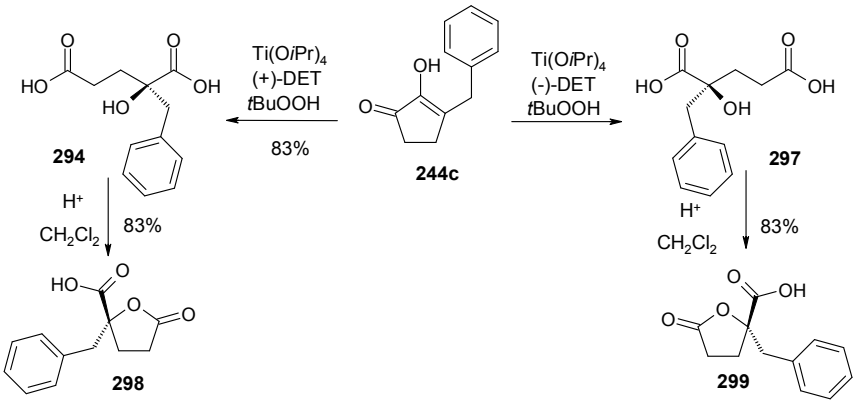
In a 1 mmol synthesis diacid **294** was isolated in 83% yield, together with by-products **295** and **296** in 5% and 12% yields, respectively. The formation of compound **295** may be associated with the benzylic oxidation of the substrate. Ketoacid **296**, which was formed in 12% yield, is a usual by-product of the present asymmetric oxidation process (Scheme 63).



Scheme 63. The products of the asymmetric oxidation of 3-benzyl-2-hydroxy-2-cyclopenten-1-one

(*R*)-Dicarboxylic acid **294** was separated from the reaction mixture by chromatography on silica gel. (*R*)-Dicarboclylic acid **294** is not stable and is cyclized on handling and standing. The separated dicarboxylic acid was cyclized into (*R*)-2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid **298**, using HCl in CH_2Cl_2 in 83% yield and >96% *ee* (Scheme 64).

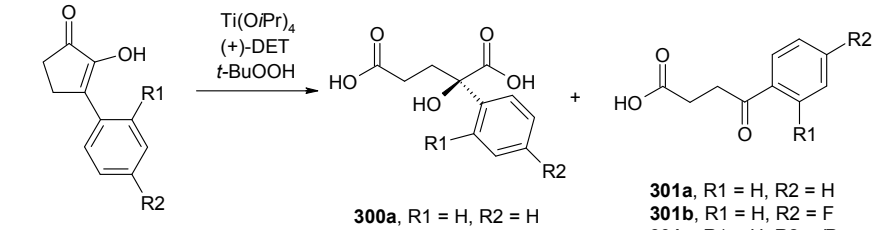
Using *D*-(-)-DET and the same reaction conditions (*S*)-dicarboxylic acid **297** and the corresponding (*S*)-2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid **299** were obtained in the same yield and with the same enantiopurity. (Scheme 64). After recrystallization the *ee* of compounds **298** and **299** increased to ≥99%.



Scheme 64. Synthesis of the both enantiomers of 2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid from the same starting compound

3.2.2. Asymmetric oxidation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones (Article II)

According to our synthesis strategy 5-phenyl-substituted γ-lactone carboxylic acids were selected to be the key intermediates for the synthesis of 4′-phenylsubstituted nucleoside analogues. Moreover, these phenyl-substituted γ-lactones are bioactive compounds themselves: some of them exhibit anticancer activity[110,111] and depressive action on the central nervous system[112,113,114]. In the literature the phenyl substituted γ-lactones carboxylic acids have been prepared as racemates. So far, no convenient method for the asymmetric synthesis of these compounds has been described.



258, R1 = H, R2 = H
291b, R1 = H, R2 = F
291c, R1 = H, R2 = *i*Pr
291d, R1 = H, R2 = MeO
291e, R1 =BnO, R2 = H

300a, R1 = H, R2 = H
300b, R1 = H, R2 = F
300c, R1 = H, R2 = *i*Pr
300d, R1 = H, R2 = MeO
300e, R1 =BnO, R2 = H

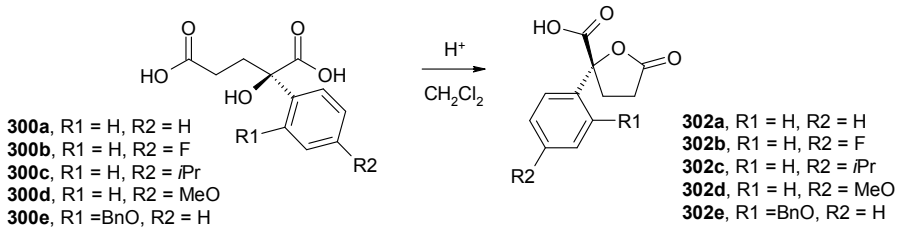
301a, R1 = H, R2 = H
301b, R1 = H, R2 = F
301c, R1 = H, R2 = *i*Pr
301d, R1 = H, R2 = MeO
301e, R1 =BnO, R2 = H

Scheme 65. Asymmetric oxidation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones

The asymmetric oxidation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones offers an excellent possibility for the preparation of chiral enantiomeric aryl-substituted γ-lactone acids. Following the general strategy, different 3-aryl substituted 2-hydroxy-2-cyclopenten-1-ones **258** and **291b-e** were synthesized and subjected to the asymmetric oxidation under usual conditions.[5]

Dicarboxylic acids **300a-e** were obtained together with a certain amount of **301a-e** as by-products (Scheme 65).
Under acidic treatment the dicarboxylic acids **300a-e** are cyclisized into the corresponding γ-lactone acids **302a-e** (Scheme 66). The yield and selectivity of the asymmetric oxidation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones depends substantially on the substituents in the phenyl ring. Thus, **258** was oxidized at standard conditions: **258**:Ti(O*i*Pr)$_4$:(+)-DET:*t*BuOOH = 1.0:1.0:1.6:2.5.

Surprisingly enough, the yield of γ-lactone acid **302a** was considerably lower than that of alkyl-substituted substrates (only 36%, Table 2, Scheme 66). In addition, also the amount of the by-product ketoacid **301a** was higher (16%) than that for alkyl substrates. And, which is the most important, the enantiopurity of the process (determined from lactone acid **302a)** was lower (86%, Table 2) than in the case of 2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid (ee >96%). At the same time, during the asymmetric oxidation process the starting compound **258** was not completely consumed and remained in the reaction mixture (48%).



300a, R1 = H, R2 = H
300b, R1 = H, R2 = F
300c, R1 = H, R2 = *i*Pr
300d, R1 = H, R2 = MeO
300e, R1 =BnO, R2 = H

302a, R1 = H, R2 = H
302b, R1 = H, R2 = F
302c, R1 = H, R2 = *i*Pr
302d, R1 = H, R2 = MeO
302e, R1 =BnO, R2 = H

Scheme 66. Lactonization of (R)-2-hydroxy-2-aryl-pentanedioic acids

This interesting phenomenon motivated us to elucidate whether it is possible to improve the yield and enantioselectivity of the synthesis of phenyl-γ-lactone acid **302a** by changing the conditions of the asymmetric oxidation.

First, the reaction time was prolonged, but this resulted, only in a small increase of the yield with the same enantiopurity. (Table 2, Entry 2).
The higher amount of the Ti(O*i*Pr)$_4$/(+)-DET/*t*BuOOH complex leads to lower yield and selectivity (Table 2, Entry 3). The higher degree of dilution of the reaction medium increased yield (43%), but decreased enantiopurity (78% *ee*) (Table 2, Entry 4).

Table 2. Asymmetric oxidation of 3-phenyl-2-hydroxy-2-cyclopenten-1-one at different reaction conditions

Entry	Reaction time (h)	Conc. of 258 (mg/ml)	Complex ratio: 258: $Ti(OiPr)_4$:(+)-DET: *t*BuOOH	Lactone acid 302a yield, % (*ee*, %)	Ketoacid 301a yield, %
1	66	22	1.0 : 1.0 : 1.6 : 2.5	36 (86)	16
2	113	22	1.0 : 1.0 : 1.6 : 2.5	38 (86)	18
3	115	22	1.0 : 3.0 : 3.6 : 4.5	32 (65)	17
4	114	11	1.0 : 1.0 : 1.6 : 2.5	43 (78)	21

The enantiomers of 2-phenyl-5-oxotetrahydrofuran-2-carboxylic acid were not separated on the chiral HPLC column we had in hands. So, the enantiomeric purity of (*R*)-2-phenyl-5-oxotetrahydrofuran-2-carboxylic acid **302a** was determined from the ^{1}H NMR-spectra of (1*R*, 2*S*, 5*R*)-(–)-menthol ester of **302a**.[5]

The found conditions (Table 2, Entry 2) were used for the oxidation of other 3-aryl-2-hydroxy-2-cyclopenten-1-ones (Scheme 66). Under the slightly modified conditions (Table 2, entry 2) different *p*-phenyl and *o*-phenyl substituted 3-aryl-2-hydroxy-2-cyclopenten-1-ones **291b-e** were subjected to asymmetric oxidation. The obtained results are presented in Table 3. The enantiomeric purity of (*R*)-2-aryl-5-oxotetrahydrofuran-2-carboxylic acids was determined using a chiral phase HPLC. According to the data obtained, the *p*-substituent influences both the yield of the products and the enantioselectivity of the process. The yield of lactone acid was the highest in case of **302e** (57%, entry 5, Table 3), while the enantioselectivity was the best in case of compounds **302a** and **302b** (both 86% ee, Table 3, entries 1 and 2).

Table 3. Asymmetric oxidation of 3-aryl-2-hydroxy-2-cyclopenten-1-ones

Entry	Compound 258, 291b-e	Substituent		Lactone acid 302 yield, %	Lactone acid 302 *ee*%	Ketoacid 301 yield %
		R1	R2			
1	a	H	H	38	86	16
2	b	H	F	43	86	24
3	c	H	*i*Pr	42	72	23
4	d	H	OMe	52	50	34
5	e	OBn	H	57	81	14

It was observed that the yield and *ee* of the *p*-F-group were quite similar to those of unsubstituted phenyl substrates, the respective values being 38% *vs* 43% and *ee* 86% *vs* 86% (Table 2, entries 1 and 2). The strongest electron donating OMe-group affords a higher yield of **302d** (52%) and the lowest stereoselectivity (*ee* 50%; Table 3, entry 4). In all cases the strongest electron donating group causes also the highest extent of the decarboxylated byproduct **301d**. When correlating the yield of

the products and the stereoselectivity of the process with σ+ constants[115] of the substituents, a strong dependence of these values on the electron donating abilities of the groups was established. The correlation of the enantioselectivity of **302a-d** with σ+ constants R^2=0,989 is the best (Figure 12).
Interestingly, the yields of by-products, ketoacids **301a-d**, display the same tendency towards the resonance σ+ constants of the *p*-substituted groups than the lactone acids **302a-d**, (Fig. 12).

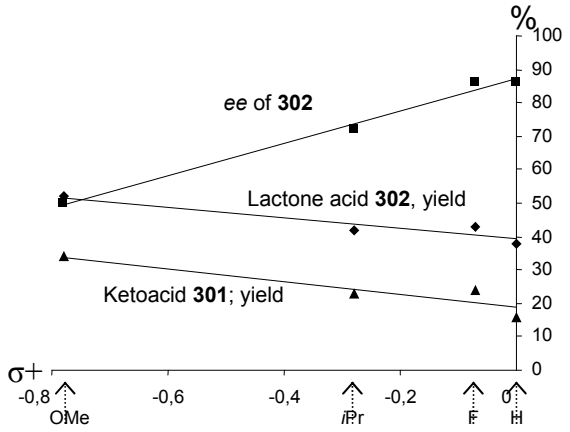


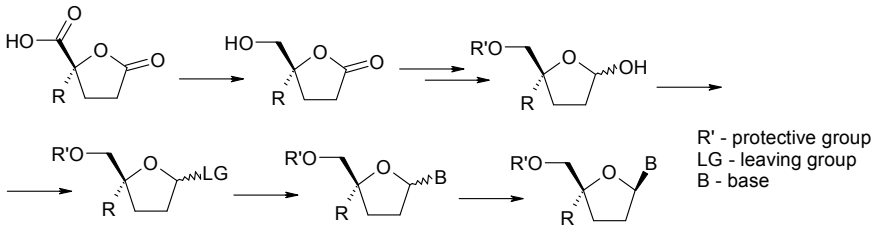
*Figure 12. The correlation between the yield of γ-lactone acids **302a-d**, enantiopurity of **302a-d** and the yield of ketoacids **301a-d** with those of the σ^+ constants of the p-substituents[115]*

The effects of the *p*-substituent in the phenyl ring in phenylallylic alcohols have been previously investigated using the epoxidation reaction with the same Ti-complex. The authors observed that the reaction was faster in the case of electron-donating groups.[115] The results obtained in this work are in good accordance with those results and indicate that a faster reaction reduces enantioselectivity and increases the rate of the side reaction.

It may be concluded that using asymmetric oxidation method starting from achiral 3-substituted-2-hydroxy-2-cyclopenten-1-ones chiral 2-substituted-5-oxo-tetrahydrofuran-2-carboxylic acids can be prepared. Benzyl substitution γ-lactone acids can be obtained in good yield (up to 83%) and with high stereoselectivity (*ee* >96%). The yield of aryl substituted γ-lactone acids is lower (38-57%) and their enantioselectivity moderate to good (50-86%). In both cases, the enantiopurity of γ-lactone acids can be improved by a single recrystallization, affording lactone acids in >99% *ee*. Thus, the method of asymmetric oxidation is suitable for preparing the key intermediates 2-substituted-5-oxo-tetrahydro-furan-2-carboxylic acids (γ-lactone acids), for the syntheses of nucleoside analogues.

3.3. Synthesis of 4′-substituted 2′, 3′-dideoxynucleoside analogues

2-Substituted-5-oxo-tetrahydrofuran-2-carboxylic acids (γ-lactone acids) are the key intermediates for the preparation of 4′-substituted 2′,3′-dideoxynucleoside analogues. The starting compounds are readily available in high enantiopurity (>99% *ee*) when the methods described in the previous chapter were used. The structure of substituents and their configuration in the ribose ring of the starting compounds is expected not to change during the subsequent chemical transformations, but will be transformed to the structure of nucleoside analogues.
We proposed that the reaction sequence presented in Scheme 67 should be successful, giving the desired nucleoside analogues.

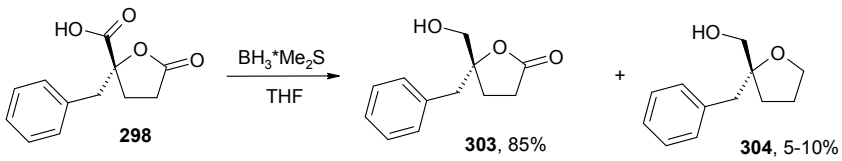


Scheme 67. A strategy of preparation of 4′-substituted nucleoside analogues

3.3.1. Synthesis of 4′-benzyl-2′,3′-dideoxynucleoside analogues (Article III)

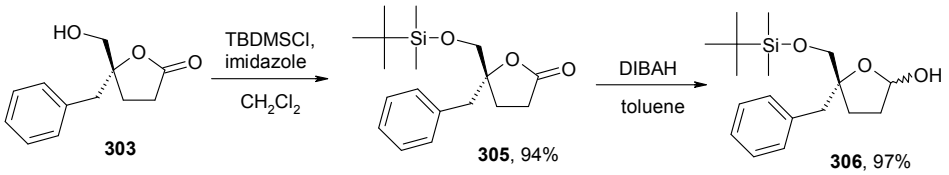
Both enantiomers of 4′-benzyl-2′,3′-dideoxynucleoside analogues were synthesized from the corresponding enantiomers of 2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acids using the proposed reaction sequence.

The first step, reduction of the carboxyl group of (R)-2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid to a primary alcohol was accomplished by a literature based method[15,23] using borane complex in dimethylsulfide. Lactone alcohol **303** as the main product was obtained in 85% yield. Compound **304** as a by-product of reduction (double reduction) was also isolated in 5-10% yield and characterized by spectra (Scheme 68).



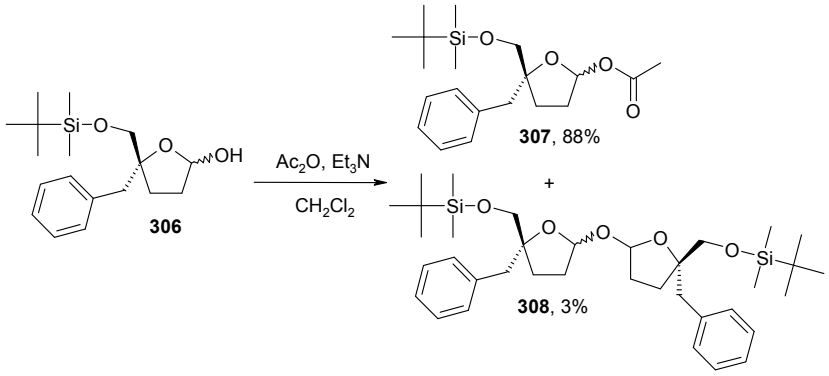
Scheme 68. Synthesis of (R)-5-benzyl-5-hydroxymethyl-dihydro-furan-2(3H)-one

In the next step the hydroxyl group of lactone **303** was protected with *tert*-butyldimethylsilyl group using TBDMSCl and imidazole,[24] affording the TBDMS derivative **305** in 94% yield. Reduction of the lactone group was performed using DIBAH to afford lactol **306** in 97% yield. Lactol **306** was isolated as a mixture of two diastereomers, the ratio of 2*R*:2*S* diastereomers was 1.9:1.0. (Scheme 69).



Scheme 69. Synthesis of (R)-5-benzyl-5-(tert-butyl-dimethyl-silyloxymethyl)-tetrahydro-furan-2-ol

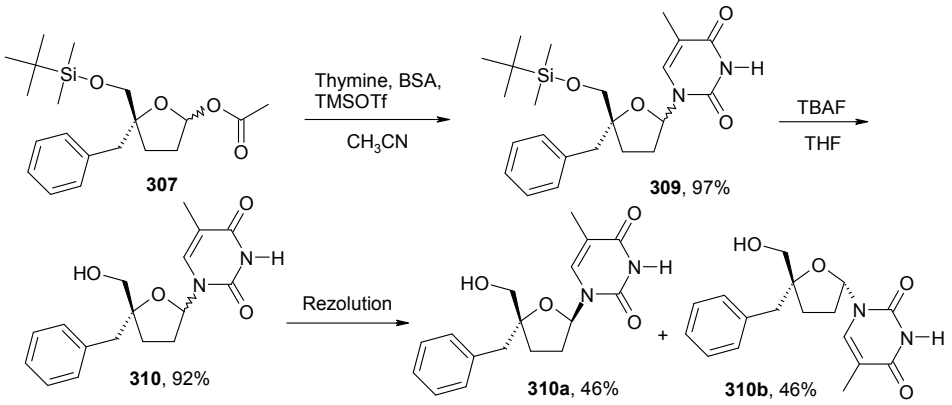
Introduction of a base to the ribose ring can be realized by using different methods.[17] The leaving group LG in ribose may be the chloro, bromo, methoxy, ethoxy, acetyl, thiophenyl or benzoyl group. Coupling of a silylated base with the ribose derivative using Lewis acid catalysis results in the nucleoside analogue. In this work, the acetyl group was always used as a leaving group. Thus, lactol **306** was transformed to acetate **307** with acetic anhydride and triethylamine in 88% yield. The 2*R*:2*S* ratio of diastereomers of **307** was 1.7:1.0. Without using a solvent the acetal dimer **308** was formed as a by-product in 14% yield. When CH_2Cl_2 was used as a solvent at the lactol concentration of 1.0 mmol/mL, the amount of the by-product decreased down to 3% (Scheme 70).



Scheme 70. Synthesis of (R)-5-Benzyl-5-(tert-butyl-dimethyl-silyloxymethyl)-tetrahydro-furan-2-yl acetate

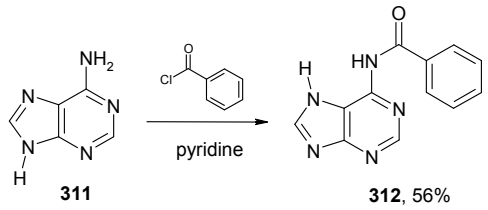
In the experiments the pyrimidine base thymine and the purine base adenine were used. Thymine was first silylated with N,O-bis(trimethylsilyl)-acetamide and then coupled with ribose acetate **307** using trimethylsilyltriflate as a Lewis acid in acetonitrile, to give a nucleoside analogue derivative **309** in 97% yield. The ratio 2*R*:2*S* of diastereomers in **307** measured by NMR was found to be 1.3:1.0. The last

step, removing the protective silyl group was accomplished using tetrabutylammonium fluoride, which resulted in the mixture β- and α- anomers of nucleoside analogue **310** in 92% yield. The anomers were separated by column chromatography on silica gel affording separately β-anomer **310a** and α-anomer **310b** in approximately equal amounts (Scheme 71).



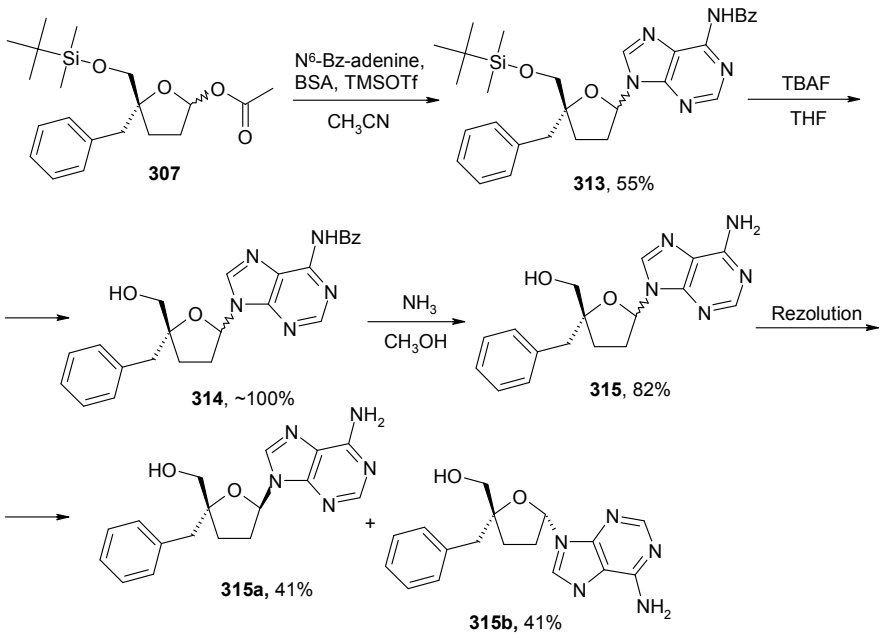
Scheme 71. Synthesis of 1-(4'-benzyl-2',3'-dideoxy-D-ribo-pentofuranosyl)-thymines

For the preparation of adenine nucleoside analogues the same procedure as in the case of thymine was used. Precedingly the amino group in the 6th position of adenine was protected by benzoyl protective group.[116] N^6-benzoyl-protected adenine **312** was obtained from adenine **311** and benzoylchloride in 56% yield (Scheme 72).



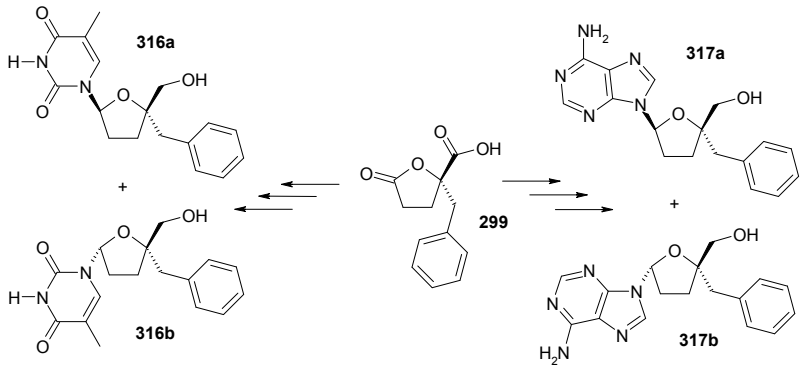
Scheme 72. Synthesis of N^6-benzoyl-protected adenine

The reaction of acetate **307** with silylated N^6-benzoyl-protected adenine in acetonitrile using TMSOTf as a Lewis acid gave the silylated nucleoside analogue **313** in 55% yield. After the removal of the silyl protecting group by TBAF in THF compound **314** was obtained in almost quantitative yield. The target nucleoside **315** was obtained after removal of the N-benzoyl protecting group with saturated ammonia in methanol, affording a mixture of β- and α- anomers of nucleoside analogues **315** in 82% yield. The β- and α-anomers were easily separated by column chromatography, resulting in β-anomer **315a** and α-anomer **315b** at a ratio of 1:1 (Scheme 73).



Scheme 73. Synthesis of 9-(4'-benzyl-2',3'-dideoxy-D-ribo-pentofuranosyl)-adenine

For the preparation of the other enantiomer of the nucleoside analogues the same reaction sequence was repeated using (*S*)-2-benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid **299** (Scheme 64). In the case of thymine the analogues **316a** (β-anomer) and **316b** (α-anomer) were obtained from 299 in 59% overall yield. The corresponding adenine nucleoside analogues **317a** (β-anomer) and **317b** (α-anomer) were obtained from **299** in 33% overall yield (Scheme 74).

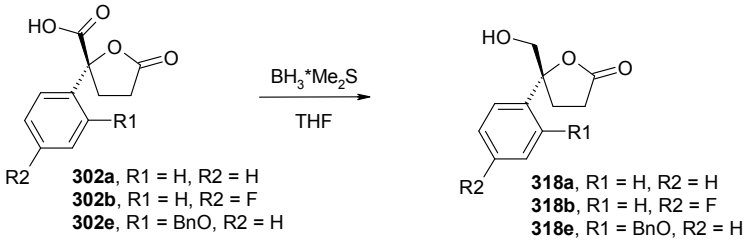


Scheme 74. Synthesis of 4'-substituted 2',3'-dideoxynucleoside analogues, starting from (S)-2-benzyl-5-oxo-tetrahydrofuran-2-carboxylic acid

3.3.2. Synthesis of 4′-aryl-2′,3′-dideoxynucleoside analogues (Article IV)

4′-Aryl-2′,3′-dideoxynucleoside analogues were synthesized from the corresponding (*R*)-2-aryl-5-oxo-tetrahydro-furan-2-carboxylic acids **302a** (unsubstituted phenyl)**, 302b** (*p*-fluoro phenyl), **302e** (*o*-benzyloxyphenyl). We expected that the substituents in the phenyl ring may influence the biological activity of the 4′-aryl nucleoside. The electronegativity of the phenyl ring together with the high electronegativity of the fluoro group in the *para*-position should influence the electron density of the ribose ring. A large lipophilic benzyloxy group in the *orto*-position should increase the interaction of the compound with membranes and other lipid-containing substructures of tissues and cells.
4′-Aryl-2′,3′-dideoxynucleoside analogues with thymine base were synthesized in six steps starting from (*R*)-2-aryl-5-oxo-tetrahydro-furan-2-carboxylic acids **302a,b,e**, using a similar synthetic scheme as in case of 4'-benzyl nucleosides.

The first step, reduction of the carboxyl group of (*R*)-2-aryl-5-oxo-tetrahydrofuran-2-carboxylic acids **302a,b,e** was accomplished using the borane complex in dimethylsulfide. The corresponding hydroxylactones **318a,b,e** were formed in 44-88% yield (Scheme 75, Table 4).



302a, R1 = H, R2 = H
302b, R1 = H, R2 = F
302e, R1 = BnO, R2 = H

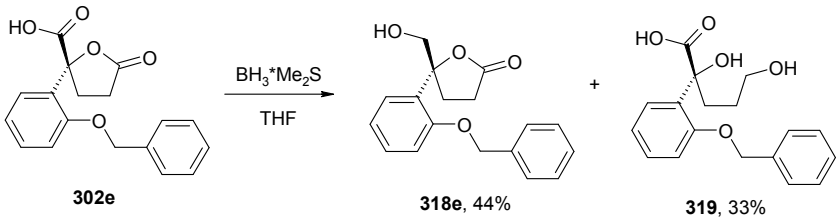
318a, R1 = H, R2 = H
318b, R1 = H, R2 = F
318e, R1 = BnO, R2 = H

Scheme 75. Synthesis of (R)-5-aryl-5-hydroxymethyl-dihydrofuran-2(3H)-ones

Table 4. Synthesis of (R)-5-aryl-5-(tert-butyl-dimethyl-silyloxymethyl)-tetrahydro-furan-2-ols

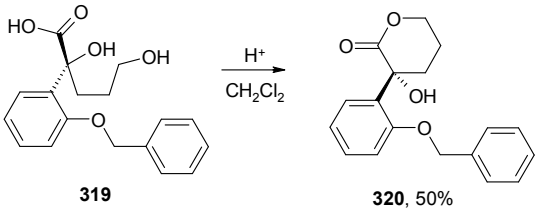
Entry	Compound 302	Substituent		318 yield %	321 yield %	322	
		R1	R2			Yield, %	Ratio, 2*R*:2*S*
1	a	H	H	86	100	92	3.8 : 1.0
2	b	H	F	84	93	93	4.0 : 1.0
3	e	OBn	H	44	78	88	2.6 : 1.0

Compounds **318a,b** were obtained in similar yields 84-86%, but the yield of **318e** yield was only 44%. It is accounted for the formation of the by-product **319** in 33% yield. The large benzyloxy substituent in the *orto*-position may be the reason why reduction of **302e** occurs in parallel in two ways: towards the predicted hydroxylactol **318e** and the linear dihydroxyacid **319** (Scheme 76).



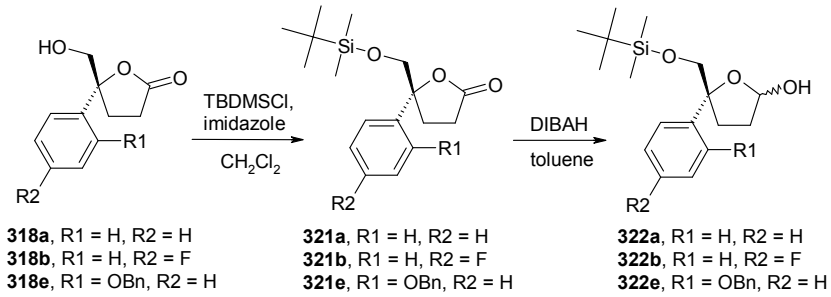
Scheme 76. The reduction of (R)-5-oxo-2-(2-benzyloxyphenyl)-tetrahydrofuran-2-carboxylic acid by the borane complex

The cyclization of **319** by hydrochloric acid in CH_2Cl_2 gave δ-lactone **320** in 50% yield (Scheme 77).



Scheme 77. The cyclization of (R)-2-(2-benzyloxy-phenyl)-2,5-dihydroxy-pentanoic acid

The next step, the protection of the hydroxyl group with the TBDMS group was performed using TBDMSCl and imidazole in CH_2Cl_2. Compounds **321a,b,e** were formed from **318a,b,e** in 78-100% yield (Scheme 78, Table 4).



Scheme 78. Synthesis of (R)-5-aryl-5-(tert-butyl-dimethyl-silyloxymethyl)-tetrahydro-furan-2-ols

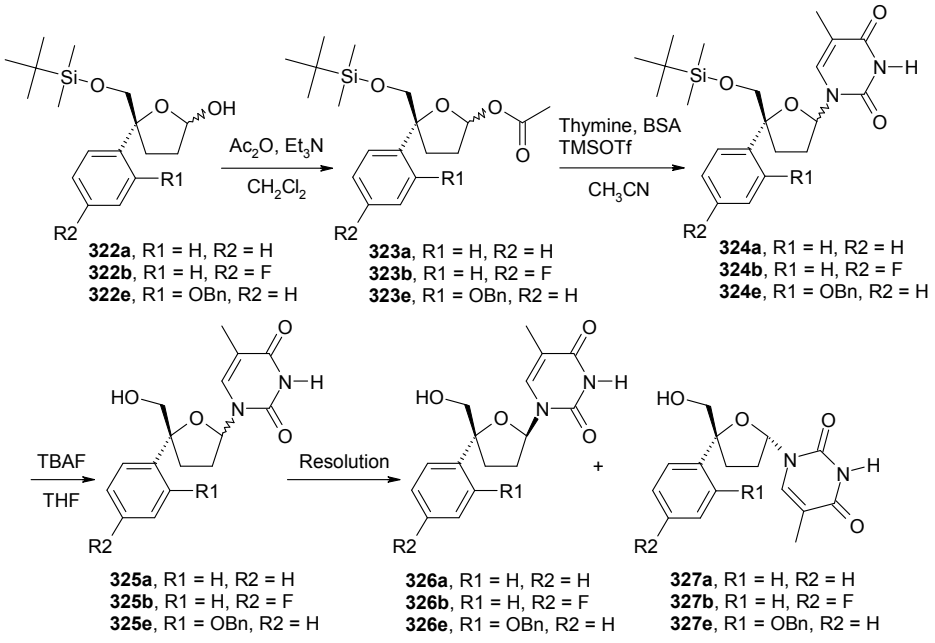
Reduction of the lactone group was performed using DIBAH to afford lactols **322a,b,e** in 88-93% yield (Scheme 78, Table 4). The 2*R*:2*S* ratio of diastereomers of lactols **322a,b,e** was different (Table 4). In the case of a large *orto*-benzyloxy-substituted phenyl derivative **322e**, the 2*R*: 2*S* ratio was 2.6:1.0, being 3.8:1.0 in the case of unsubstituted phenyl group derivative **322a**. Interesting effect to the anomeric OH has *para*-fluoro-phenyl derivative **322b**. In the fresh solution of **322b** the ratio of 2*R*:2*S* was 1.0:1.3, but after 12 hours this was 4.0:1.0 (Table 4).

Lactols **322a,b,e** were transformed to the corresponding acetates **323a,b,e** by using the same conditions as in the case of the synthesis of benzyl-substituted nucleosides. Treatment of lactols **322a,b,e** with acetic anhydride in CH_2Cl_2 in the presence of triethylamine gave acetates **323a,b,e** in 67-90% yield (Scheme 79, Table 5).

Table 5. Synthesis of 4'-aryl-substituted 2',3'-dideoxynucleoside analogues with thymine

Entry	Compound 322	Substituent		323		324	
		R1	R2	Yield %	Ratio, 2*R*:2*S*	Yield %	Ratio, 2*R*:2*S*
1	a	H	H	90	3.8 : 1.0	87	1.0 : 1.0
2	b	H	F	81	3.1 : 1.0	100	1.0 : 1.1
3	e	OBn	H	67	1.7 : 1.0	91	1.3 : 1.0

In case of unsubstituted phenyl derivative **323a** the ratio of diastereomers 2*R*:2*S* remained unchanged (2*R*:2*S* = 3.8:1.0), being lower in the case of *para*-fluoro phenyl derivative **323b** (2*R*:2*S* = 3.1:1.0) and higher in the case of *orto*-benzyloxy-substituted derivative **323c** (2*R*:2*S* = 1.7:1.0) than in the case of the corresponding lactols **7a-c** (compare the data presented in Tables 4 and 5).



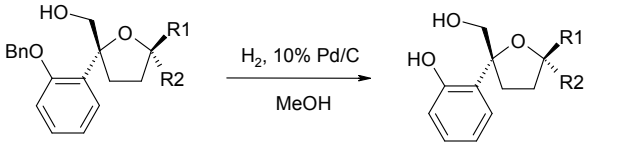
Scheme 79. Synthesis of 4'-aryl-substituted 2',3'-dideoxynucleoside analogues with thymine

Thymine was introduced to the arylsubstituted ribose ring as in the case of benzyl derivatives. First thymine was silylated with BSA and then coupled with acetates **323a,b,e** in acetonitrile using TMSOTf as a Lewis acid, to afford silylated

nucleosides **324a,b,e** in 87-100% yield (Scheme 79, Table 5). Interestingly enough, the ratio of 2*R*:2*S* is similar in the case of all compounds but is different from that for acetates: **324b** 1.0:1.1; **324a** 1.0:1.0 and **324e** 1.3:1.0 (Table 5).

The last step removing of the protective silyl group with TBAF afforded a mixture of β- and α- anomers of nucleoside analogues **325a,b,e** in 90-100% overall yield. The β- and α- anomers were separated by column chromatoghraphy, affording separately β-anomers **326a,b** and α-anomers **327a,b** at an approximately 1:1 ratio; in the case of **326e** and **327e** the ratio was 1.34:1 (Scheme 79).

In the case of compounds **326e** and **327e** the benzoyl group was additionally removed using H_2 on a Pd catalyst to give the corresponding nucleoside analogues **328** and **329** in quantitative yield (Schemes 80).



326e, R1 = Thymine, R2 = H
327e, R1 = H, R2 = Thymine

328, R1 = Thymine, R2 = H
329, R1 = H, R2 = Thymine

Scheme 80. Synthesis of 4'-(2-hydroxyphenyl)-2',3'-dideoxythymidines

In conclusion, both benzyl-substituted and aryl-substituted nucleoside analogues may be synthesized using the reaction sequence proposed above, starting from 2-substituted-5-oxo-tetrahydrofuran-2-carboxylic acid. The sequence is acceptable also for both thymine and adenine bases. The same procedure is suitable for the synthesis of enantiomeric analogues.
As a result, 16 enantiomerically pure nucleoside analogues were prepared.
All synthesized 4′-substituted nucleoside analogues were tested for antitumor (cytotoxic) activity.

3.4. Biological activity of 4′-substituted 2′,3′-dideoxynucleoside analogues

The biological activity of compounds was determined at InBio Ltd. The nucleoside analogues **310a,b** and **315a,b** at concentration of 0.1 μM to 1000 μM were tested for antitumor activity on the MCF-7 (breast carcinoma), Hep3B (hepatoma) and CCRF-CEM (T-cell leukaemia) tumor cells upon a 48-hour incubation (Appendix, Table 1). The cytotoxic activity of Test results reveal that the analogues **310a,b** and **315a,b** have a certain cytotoxic activity at concentrations of 100 μM and above. It was found that 48 hours were not enough to elucidate activity.

In the next experiments the cell cycle was analyzed (Appendix. Tables 2, 3, 4). The test showed that the nucleoside analogues have a certain effect on the cell cycle of MCF-7 and Hep3B cells, though at relatively high concentrations (100 μM and above; Appendix, Tables 2, 3). The negative effect of nucleosides **310a,b** and **315a** on the DNA synthesis phase (S phase) is well demonstrated on MCF-7 cells (Appendix, Table 2). On Hep3B cells, the S phase is inhibited by nucleosides **310a,b** and **315b** at higher concentrations (Appendix, Table 3). The cell cycle of CCRF-CEM cells seems not to be sensitive to the treatment by nucleoside analogues (Appendix, Table 4).

As the analysis of the cell cycle shows, the tested nucleoside analogues were more active in the case on MCF-7 and Hep3B cells. For that reason the following biological tests were performed on the MCF-7 and Hep3B tumor cells (Appendix, Tables 4, 5). Taking into account the previous conclusions made above, the nucleoside analogue **310b** (as the most effective compound tested) and gemcitabine containing drug Gemzar® (in the clinical use against breast cancer) were tested on the MCF-7 and Hep3B tumor cells for 48, 72 and 140 hours. The compounds revealed cytotoxic activity on both cell lines. Using a longer incubation time, the activity of the tested compound was better expressed. Interestingly enough, in case of Gemzar® the cytotoxic effect does not depend on the concentration of the compounds. So, the activity of compounds with concentrations of 1, 10 and 100 μM is almost the same.
Table 12 summarizes the results of biological evaluation of natural 4′-benzyl substituted nucleoside analogues **310a,b** and its enantiomers **316a,b**, as well as the corresponding adenine derivatives **317a,b** on MCF-7 tumor cells. The tested compounds showed a significant cytotoxic activity after 6 day of incubation at a concentration of 100 μM. This effect becomes more evident after 9 days of incubation. Compound **317a** exibited the highest cytotoxic activity. The activity of the α-anomers of nucleosides **310b**, **316b** and **317b** is negligible, contrary to that of β-anomers **310a**, **316a** and **317a**. The "natural" enantiomers of 4′-benzyl substituted nucleoside analogues **310a,b** containing thymine were more active than the "unnatural" enantiomers **316a,b** (Appendix, Table 7).

The cytotoxic activity of synthesized 4′-aryl-2′,3′-dideoxynucleoside analogues **326a,b,e, 327a,b,e, 328** and **329** was also tested on MCF-7 cells for 3, 6 and 9 days and compared with that of Gemzar® (Appendix, Table 8). The activity of unsubstituted phenyl nucleoside analogues **326a** and **327a** as well as of *para*-F-phenyl nucleoside analogues **326b** and **327b** was negligible.
The *ortho*-benzyloxophenyl group contained nucleoside analogues **326e** and **327e** which were more active on MCF-7 cells than *ortho*-hydroxyphenyl nucleoside analogues **328** and **329**. Analogues **328** and **329** having no large lipophilic benzyl group lose activity. The activity of *ortho*-hydroxyphenyl compounds was similar to that of unsubstituted and *para*-F-substituted 4′-phenyl nucleoside analogues **326a,b** and **327a,b**.
The cytotoxic activity of compounds **326e** and **327e** (100 μM) on MCF-7 cells during a 9-day incubation was similar to that of Gemzar® (1-100 μM). It is noteworthy that the activity of Gemzar® reached maximum after 9 days of incubation, while analogue **326e** exibited higher activity than Gemzar® already after 3 days of treatment (Appendix, Table 8).

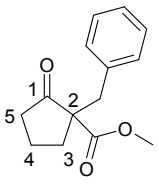
In conclusion, all the synthesized nucleoside analogues exhibit a certain cytotoxic activity at concentrations 100 μM and higher. The cytotoxic effect is more pronounced after 9 days of incubation. β-anomers proved to be more active than α-anomers. The cytotoxic activity of 4′-(2-benzyloxy)-phenyl-2′,3′-dideoxythymidine on MCF-7 tumor cells was the highest.

4. EXPERIMENTAL

4.1. Synthesis

General information:
Commercial reagents were obtained from Aldrich and were used as received. CH_2Cl_2 was distilled from CaH_2 and stored over 3Å molecular sieve pellets before use. DMF and acetonitrile were distilled from CaH_2, THF from $LiAlH_4$ and toluene from Na before use. The petroleum ether fraction bp 40-60 ^oC was used. All reactions sensitive to oxygen or moisture were conducted under argon atmosphere in oven-dried glassware. The TLC analysis was performed using DC-Alufolien Kieselgel 60 F254 (Merck) and Silufol® UV 254 silica gel plates. Merck Silica gel 60 (0.063-0.200 mm) and Chemapol silica gel L 40/100 were used for column chromatography. Melting points were determined using VEB Nombinat Nagema K8 (Germany) apparatus. Optical rotations were obtained using a A. Krüss Optronic GmbH polarimeer P 3002. Enantiomeric purity was determined on a LKB liquid chromatograph with a Uvicord UV detector, using a Daicel Chiracel ODH chiral column. IR spectra were recorded on a Perkin-Elmer Spectrum BX FTIR spectrometer. ^1H and ^{13}C NMR spectra were determined using deuterated solvents ($CDCl_3$, δ = 7.27 and 77.00, CD_3OD, δ = 3.30 and 49.00, or DMSO-d6, δ = 2.50 and 39.50) on a Bruker AMX-500 spectrometer. Mass spectra were measured on a Hitachi M80B spectrometer using the EI (70 eV) mode. Elemental analyses were performed on a Perkin-Elmer C, H, N, S-Analyzer 2400.

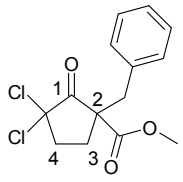
1-Benzyl-2-oxo-cyclopentanecarboxylic acid methyl ester (compound **242c**, Scheme 59).
To a stirred mixture of sodium methoxide (2.86 g, 0.053 mol) in DMF (12.5 mL) 8.7g (0.05 mol) of dimethyladipate was added. Methanol and DMF were distilled from the reaction mixture under vacuum (160-170 mmHg) and the crude was cooled to 40 ^oC. The solution of benzyl bromide (10.3 g, 0.060 mol) in DMF (7.5 mL) was added with stirring. The temperature of the reaction mixture was maintained at 40-45 ^oC with external cooling. The precipitated NaBr was removed by filtration, water (10 mL) was added and the mixture was extracted with ether (3x5 mL). The combined extracts were dried over $MgSO_4$. After solvent evaporation in vacuo, the residue was purified by column chromatography (petroleum ether/acetone=10:2) to give compound **242c** (9.98g, 86%) as colourless syrup.



IR (KBr): 3063, 3030, 2955, 1753, 1728, 1604, 1496, 1454, 1435, 1266, 1237, 1163, 1144, 704 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$) δ 7.26-7.18 (m, 3H, *m,p*-Bn), 7.12-7.10 (m, 2H, *o*-Bn), 3.70 (s, 3H, OMe), 3.20 and 3.10 (2d, *J* = 13.8

Hz, 2H, CH_2-Ph), 2.44-2.32 (m, 2H, H-3 (2.41) and H-5 (2.35)), 2.07-1.82 (m, 3H, H-5 (2.03), H-3 (1.96) and H-4 (1.87)), 1.63-1.55 (m, 1H, H-3).
^{13}C NMR (125 MHz, $CDCl_3$) δ 214.6 (C-1), 171.3 (COO), 136.4 (*s*-Bn), 130.0 (*o*-Bn), 128.3 (*m*-Bn), 126.7 (*p*-Bn), 61.4 (C-2), 52.5 (OMe), 39.0 (C-4), 38.2 (CH_2-Ph), 31.6 (C-5), 19.3 (C-3).

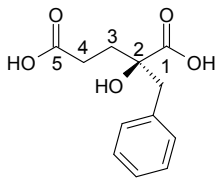
1-Benzyl-3,3-dichloro-2-oxo-cyclopentanecarboxylic acid methyl ester (compound **243c**, Scheme 59).
Through the solution of **242c** (4.64 g, 0.02 mol) in acetic acid (23.5 mL) chlorine (0.042 mol) was bubbled rapidly until the starting compound disappeared (controlled by GC). Chlorine was obtained by the reaction of sodium permanganate with concentrated hydrochloric acid. After the reaction was completed, the acetic acid was evaporated, giving compound **243c** (6.02 g, 100%) as a colourless syrup.
IR (KBr): 3064, 3031, 2955, 1777, 1737, 1604, 1496, 1455, 1436, 1237, 1152, 851, 826, 733, 702 cm^{-1}.



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.24 (m, 3H, *m,p*-Bn), 7.12-7.10 (m, 2H, *o*-Bn), 3.77 (s, 3H, OMe), 3.33 and 3.24 (2d, J = 13.8 Hz, 2H, CH_2-Ph), 2.61 (ddd, J = 6.9, 8.6 and 14.4 Hz, 1H, H-4), 2.50 (ddd, J = 5.0, 6.9 and 13.6 Hz, 1H, H-3), 2.30 (ddd, J = 5.0, 6.7 and 14.4 Hz, 1H, H-4), 2.15 (ddd, J = 6.7, 8.6 and 13.6 Hz, 1H, H-3).
^{13}C NMR (125 MHz, $CDCl_3$) δ 198.5 (C-1), 169.5 (COO), 134.9 (*s*-Bn), 130.1 (*o*-Bn), 128.5 (*m*-Bn), 127.4 (*p*-Bn), 83.5 (C-5), 59.2 (C-2), 53.3 (OMe), 41.9 (C-4), 41.0 (CH_2-Ph), 27.5 (C-3).
MS (EI, 70eV): m/z (%) = 301 (5.5, M^+), 272 (3.4), 251 (10.7), 205 (6.4), 175 (8.5), 141 (5.8), 116 (27.8), 91 (100.0, Bn^+).

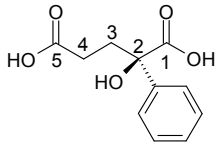
(R)-2-Benzyl-2-hydroxy-pentanedioic acid (compound **294**, Scheme 63, 64).
$Ti(OiPr)_4$ (0.3 mL, 1 mmol) was added to CH_2Cl_2 (6 mL) and 4Å powdered molecular sieves (100 mg) under argon atmosphere and the reactor was cooled to -20…-25 ^{o}C using the CO_2/CCl_4 freezing-mixture. (+)-DET (0.27 mL, 1.6 mmol) was added and the mixture was stirred for 15 min. The solution of 3-benzyl-2-hydroxy-2-cyclopenten-1-one **244c** (1 mmol) in CH_2Cl_2 (2.0 mL) was added and the reaction mixture was stirred for 30 min. Then TBHP (0.4 mL, 2.5 mmol, 6.25 M solution in decane) was added and the reactor was kept at -20 ^{o}C for 114 hours.
When the reaction was completed, water (6.0 mL) was added and the mixture was intensively stirred at room temperature for 1 hour. Then, 1.2 mL of NaOH solution was added (1.5 g NaOH + 0.25 g NaCl + 4.5 mL distilled water) to the reaction mixture and the latter was again intensively stirred at room temperature for an additional 1 hour. The CH_2Cl_2 layer was removed and the mixture acidified with a 1M HCl solution (pH=2) and extracted with EtOAc 3-5 times. The combined extracts were dried over $MgSO_4$ and the solvents were evaporated on a rotary evaporator. The residue was purified by flash chromatography (Chemapol silica gel L40/100), petroleum ether-acetone, 10:2, to give **294** (0.198g, 83%).

IR (KBr): 3449, 3031, 2950, 2623, 1728, 1605, 1495, 1454, 1415, 1266, 1228, 1189, 1111, 915, 875, 773, 740, 700 cm^{-1}.



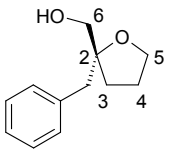
^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.24 (m, 4H, *o*,*m*-Bn), 7.23-7.21 (m, 1H, *p*-Bn), 3.12 and 2.95 (2d, J = 13.8 Hz, 2H, CH_2-Ph), 2.60-2.40 (m, 2H, H-4), 2.34-2.26 and 2.14-2.08 (m, 2H, H-3).
^{13}C NMR (125 MHz, $CDCl_3$) δ 179.1 (C-5), 179.0 (C-1), 134.9 (*s*-Bn), 130.2 (*m*-Bn), 128.3 (*p*- Bn), 127.1 (*o*- Bn), 77.2 (C-2), 45.2 (*C*H_2-Ph), 33.2 (C-3), 28.7 (C-4).

(R)-2-Hydroxy-2-phenyl-pentanedioic acid (compound **300a**, Scheme 65).
Compound **300a** was obtained from 3-phenyl-2-hydroxy-2-cyclopenten-1-one **258** (1 mmol) in 36% yield analogously to the procedure described above.
$[\alpha]_D^{21}$= -10.0 (c = 1.69, MeOH); $[\alpha]_D^{21}$= -9.2 (c = 1.69, CH_3COCH_3);
IR (KBr): 3382, 2937, 2634, 1704, 1600, 1497, 1447, 1426, 1301, 1246, 1186, 1121, 942, 891, 720, 696 cm^{-1}.



^1H NMR (500 MHz, $CDCl_3$ + CD_3OD) δ 7.57 (d, J = 7.7 Hz, 2H, *o*-Ph), 7.30 (t, J = 7.7 Hz, 2H, *m*-Ph), 7.24 (t, J = 7.7 Hz, 1H, *p*-Ph), 2.52-2.43 (m, 1H, H-3), 2.41-2.30 (m, 3H, H-3, H-4).
^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD) δ 176.2 (C-5), 176.1 (C-1), 141.0 (*s*-Ph), 127.9 (*m*-Ph), 127.4 (*p*-Ph), 125.1 (*o*-Ph), 77.1 (C-2), 34.2 (C-3), 28.6 (C-4).

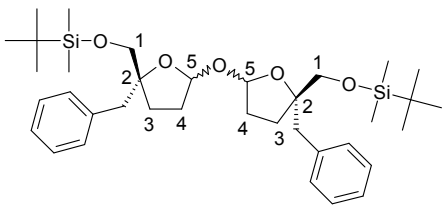
((R)-2-Benzyl-tetrahydrofuran-2-yl)-methanol (compound **304**, Scheme 68).
To the solution of **298** (0.880 g, 4.0 mmol) in THF (3.0 mL) at 0 ^oC a BH_3•Me_2S complex in THF (0.52 mL, 4.8 mmol) was added dropwise over a period of 5 min. The reaction mixture was stirred at room temperature for 2 hours and then treated with methanol (1.0 mL) by cooling. The mixture was concentrated in vacuum and purified by column chromatography (petroleum ether/acetone = 10:1 to 10:3) affording compound **304** (69mg, 9%) as a by-product.
IR (KBr): 3422, 3062, 3028, 2957, 2872, 1604, 1495, 1454, 1084, 1051, 738, 702 cm^{-1}.



^1H NMR (500 MHz, $CDCl_3$) δ 7.32-7.21 (m, 5H, o,*m*,*p*-Bn), 3.83-3.74 (m, 2H, H-5), 3.50 and 3.44 (2d, J = 11.4 Hz, 2H, H-6), 2.86 and 2.84 (2d, J = 11.4 Hz, 2H, CH_2-Ph), 1.85-1.79 (m, 2H, H-4 and H-3), 1.75-1.69 (m, 1H, H-3), 1.67- 1.61 (m, 1H, H-4).
^{13}C NMR (125 MHz, $CDCl_3$) δ 137.5 (*s*-Bn), 130.4 (*o*-Bn), 127.9 (*m*-Bn), 126.2 (*p*-Bn), 85.5 (C-2), 68.4 (C-5), 67.1 (C-6), 42.0 (*C*H_2-Ph), 31.0 (C-3), 26.2 (C-4).

Oxybis[((R)-2-benzyltetrahydrofuran-5,2-diyl)methyleneoxy]-bis[*tert*-butyl(dimethyl)-silane] (compound **308**, Scheme 70).
To the mixture of compound **306** (0.323g, 1.0 mmol) and Et_3N (0.418 mL, 3.0 mmol) in CH_2Cl_2 (1.0 mL) acetic anhydride (0.283 mL, 3.0 mmol) was added dropwise at 0 ^oC. The reaction mixture was stirred overnight at room temperature.

Water (5 mL) was added and the mixture extracted with EtOAc (4x5mL). The combined extracts were dried over $MgSO_4$. After solvent evaporation in vacuum the residue was purified by column chromatography (petroleum ether/acetone = 20:1) to afford compound **308** (19mg, 3%) as a by-product.



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45-7.21 (m, 4x5H, *o,m,p*-Bn), 5.53(d, *J*=4.6 Hz, 1H, H-5), 5.34(d, *J*=4.9 Hz, 1H, H-5), 5.25 (d, *J*=4.4 Hz, 1H, H-5), 5.24 (d, *J*=5.0 Hz, 1H, H-5), 3.66 (s, 2H, H-6), 3.51 and 3.42 (2d, *J* = 9.6 Hz, H-6), 3.30 and 3.26 (2d, *J* = 10.0 Hz, H-6), 3.23 and 3.16 (2d, *J* = 9.7 Hz, H-6), 3.03 and 2.99 (2d, *J* = 13.5 Hz, H-10), 2.96 and 2.91 (2d, *J* = 13.8 Hz, H-10), 2.88 and 2.85 (2d, *J* = 13.8 Hz, H-10), 3.03 and 2.99 (2d, *J* = 13.5 Hz, H-10),2.87 and 2.81 (2d, *J* = 13.5 Hz, H-10), 2.04-1.58 (m, 10H, H-3, H-4), 1.37-1.23 (m, 2H, H-4), 0.95, 0.944, 0.941, 0.924 (4s, 4x9H, H-9), 0.078, 0.070(2), 0.065, 0.046, 0.042, 0.017, 0.012 (8s, 24H, H-7).

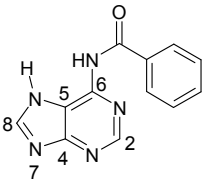
^{13}C NMR (125 MHz, $CDCl_3$) δ 138.22, 138.19, 138.06(2) (*s*-Bn), 130.75, 130.69(2), 130.56 (*o*-Bn), 127.77, 127.72(2), 127.69 (*m*-Bn), 126.02(2), 125.97, 125.94 (*p*-Bn), 102.90, 102.84 (C-5, 2-CH_2O- and 5-O- *cis*), 100.33, 100.22 (C-5, 2-CH_2O- and 5-O- *trans*), 87.27, 87.08, 87.01, 86.91 (C-2), 69.71, 69.55 (C-6, 2-CH_2O- and 5-O- *cis*), 66.16, 65.40 (C-6, 2-CH_2O- and 5-O- *trans*), 44.02, 43.90 (C-10, 2-CH_2O- and 5-O- *trans*), 42.33, 42.24 (C-10, 2-CH_2O- and 5-O- *cis*), 32.45, 32.24, 32.20(2) (C-4), 30.92, 30.49, 30.04, 30.00 (C-3), 25.92 (C-9), 18.29, 18.24(2), 18.21 (C-8), -5.35, -5.38, -5.45(2), -5.47, -5.48, -5.52, -5.58.

MS (EI, 70eV): m/z (%) = 305 (24.6), 259 (55.7), 247 (49.3), 205 (77.0), 177 (9.5), 155 (6.6), 129 (55.7), 105 (9.8), 91 (93.4, Bn^+), 85 (100.0).

N-(*9H*-Purin-6-yl)-benzamide (compound **312**, Scheme 72).

To the suspension of 464 (1.0 g, 7.4 mmol) in pyridine (5.0 mL) benzoyl bromide (2.08 g, 1.5 mmol) was added. The reaction mixture was refluxed for 2 hours. Water (10 mL) was added and the mixture extracted with CH_2Cl_2 (5x10mL). The combined extracts were dried over $MgSO_4$. After solvent evaporation in vacuum the residue was purified by column chromatography (CH_2Cl_2/methanol = 10:1) to afford compound **312** (0.99g, 56%).

IR (KBr): 3370, 3258, 3064, 1687, 1600, 1552, 1522, 1489, 1448, 1375, 1267, 707 cm^{-1}.



^{1}H NMR (500 MHz, DMSO-d6) δ 12.40 and 11.50 (2bs, NH), 8.74 (s, 1H, H-2), 8.52 (s, 1H, H-8), 8.13 (d, *J* = 7.5 Hz, 2H, *o*-Ph), 7.66 (t, *J* = 7.5 Hz, 1H, *p*-Ph), 7.57 (t, *J* = 7.5 Hz, 2H, *m*-Ph); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.6 (bs, 1H, H-7), 8.99 (bs, 1H, NH), 8.81 (s, 1H, H-2), 8.37 (s, 1H, H-8), 8.02 (d, *J* = 7.5 Hz, 2H, *o*-Ph), 7.70 (t, *J* = 7.5 Hz, 1H, *p*-Ph), 7.60 (t, *J* = 7.5 Hz, 2H, *m*-Ph).

^{13}C NMR (125 MHz, DMSO-d6) δ 166.4 (CO), 162.0 (C-6), 151.1 (C-2), 146.0 (C-8), 144.5 (C4), 132.8 (*s*-Ph), 132.6 (*p*-Ph), 128.5 (*o*-Ph), 128.4 (*m*-Ph); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 166.4 (CO), 162.7 (C-6), 152.4 (C-2), 144.2 (C-8), 143.3 (C4), 133.6 (*p*-Ph), 132.2 (*s*-Ph), 129.3 (*o*-Ph), 127.7 (*m*-Ph).
MS (EI, 70eV): m/z (%) = 238 (16.9, M^+-1), 224 (2.7), 211 (18.8), 195 (1.5), 162 (0.78), 149 (2.4), 135 (5.7), 119 (9.4),105 (100, Bz^+), 77 (70.6, Ph^+).

4.2. Biological activity

MTT CYTOTOXICITY ASSAY (Apendix, Table 1)
Procedure: The cells were grown in the presence of nucleoside analogues at a concentration ranging from 0.1 μM to 1000 μM in 24-well tissue culture plates for 48 hours. In order to increase the solubility of nucleoside analogues, DMSO was incorporated in the incubation solution at a final concentration of 0.1%. The cells were subsequently incubated with the MTT solution, and the solubilized formazan product formed by the methabolisation of MTT was spectrophotometrically quantified using an ELISA plate reader. Measurements of each concentration point were performed in triplicate wells. The results presented show the average ± standard error of the corresponding normalized background-subtracted optical density readouts.
Controls: The positive and negative controls were included in this assay, the negative controls comprising untreated cells and the positive control (included in the analysis of MCF-7 and CCRF-CEM cells) comprising cells treated with camptothecin (CPT), a well-characterised cytotoxic agent, at the concentration of 10 μM.

CELL CYCLE ANALYSIS (Appendix, Tables 2-4)
Procedure: The cells were grown in the presence of nucleoside analogues at concentrations ranging from 0.1μM to 1000μM for 48 hours. In order to increse the solubility of nucleoside analogues, DMSO was incorporated in the incubation solution at a final concentration of 0.1% (for Hep3B and CCRF-CEM cells) or 1% (for MCF-7 cells). The total DNA content in the cells was visualised by staining the cells with propidium iodide. The cell cycle distribution in the treated cell populations was subsequently analysed by flow cytometry using a FACSCalibur instrument. The results presented show the proportion of cells in the individual phases of the cell cycle (G_0/G_1, S and G_2/M).
Controls: The negative control (0.1%DMSO) included in this assay comprised untreated cells.

MTT CYTOTOXICITY ASSAY COMPARING WITH GEMZAR® (Appendix, Tables 5-8)
Procedure: The cells were grown in the presence of the corresponding nucleoside analogues at a concentrations ranging from 1 μM to 100 μM (diluted from 500 μM stock solutions) in 24-well tissue culture plates for 72 to 261 hours (3 to 9 days). In

order to increase the solubility of nucleoside analogues, DMSO was incorporated in the incubation at a final concentration of 0.1%. The cells were subsequently incubated with the MTT solution, and the solubilized formazan product formed by the metabolisation of MTT was spectrophotometrically quantified using an ELISA plate reader. Measurements of each concentration point were performed in triplicate wells. The results presented show the average ± standard error of the corresponding normalised background-substracted optical density readouts.

Controls: The positive and negative controls were included in this assay, the negative controls comprising untreated cells (with 0.1%DMSO) and the positive controls comprising cells treated with either campthothecin (10 μM) or Gemzar® (gemcitabine), both well-characterised cytotoxic agents, at concentrations ranging from 1 μM to 100 μM.

CONCLUSIONS

1. Methods for the synthesis of 3-benzyl-2-hydroxy-2-cyclopenten-1-one (starting from ethyl glutarate) and 3-aryl-2-hydroxy-2-cyclopenten-1-ones (using Stetter reaction) were developed.
2. The asymmetric oxidation of 3-benzyl- and 3-aryl-2-hydroxy-2-cyclopenten-1-ones afforded the corresponding substituted γ-lactone acids in high yield and good stereoselectivity. These compounds were proved to be good key intermediates for the synthesis of nucleoside analogues.
3. A method for the synthesis of 4′-benzyl nucleoside analogues, starting from of benzyl-substituted γ-lactone acid was developed. The adenine derivatives were synthesized in 32-33% overall yield and the thymine derivatives, in 59-61% overall yield.
4. A series of 4′-aryl substituted nucleoside analogues with thymine were prepared, starting from the corresponding γ-lactone acids in 18-62% overall yield.
5. The sixteen new nucleoside analogues were synthesized and tested for cytotoxic activity on Hep3B, MCF-7 and CCRF-CEM tumor cells. Of the compounds tested, 4′-(2-benzyloxy)-phenyl-2′,3′-dideoxythymidine exhibited the highest activity on the MCF-7 tumor cells.

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Tallinn, June 2008
Artur Jõgi

ABSTRACT

Asymmetric synthesis has been widely applied to the synthesis of natural compounds as well as the development of modern drugs. The main aim of the present work was to develop a method for the synthesis of 4′-substituted nucleoside analogues - potent anticancer and antiviral agents. Using different properties of substituents such as electronic density, lipophilicity and sterical factors, the properties of the target nucleoside analogue can be tuned.

The key intermediates for the synthesis, the chiral substituted γ-lactone acids, were obtained by the asymmetric oxidation of 3-substituted-2-hydroxy-2-cyclopenten-1-ones. Thus the asymmetric oxidation of 3-benzylsubstituted-2-hydroxy-2-cyclopenten-1-ones leads to the benzyl γ-lactone acids with good yield (83%) and high stereoselectivity (96%). The asymmetric oxidation of 3-arylsubstituted-2-hydroxy-2-cyclopenten-1-ones affords aryl γ-lactones with moderate yield (38-57%) and good stereoselectivity (81-86%). The enantiomerically highly pure compounds (>99% *ee*) may be obtained by a simple re-crystallization.

The key intermediates were transformed to the nucleoside analogues in 6-7 steps in up to 62% yield.

As a result a general strategy of the synthesis of enantiomerically pure 4′-substituted nucleoside analogues from an achiral 3-substituted-2-hydroxy-2-cyclopenten-1-ones was developed.

Using that pathway 16 new nucleoside analogues were synthesized. The anticancer activity of the compounds synthesized was tested on the MCF-7, Hep3B and CCRF-CEM tumor cells. 4′-(2-Benzyloxy)-phenyl-2′,3′-dideoxythymidine revealed the highest activity on the breast carcinoma cells.

KOKKUVÕTE

Asümmeetriline süntees on laialdaselt kasutusel looduslike ühendite sünteesil ja samuti kaasaegsete ravimite väljatöötamisel. Käesoleva töö põhieesmärgiks oli välja töötada 4′-asendatud nukleosiidi analoogide kui potentsiaalsete vähi- ja viirusevastaste ainete sünteesimeetod. Erinevate asendajate elektroonsete, hüdrofoobsete ja steeriliste omaduste arvestamisega on võimalik häälestada lõpliku nukleosiidi analoogi selliseid omadusi nagu elektrontihedus riboosi ringis, lipofiilsus ning steerilised faktorid.

Nukleosiidi analoogide sünteesi võtmeühenditeks on kiraalsed asendatud γ-laktoonhapped, mis saadi asümmeetrilise oksüdatsiooni tulemusena hea saagise ning kõrge enantioselektiivsusega lähtudes 3-asendatud-2-hüdroksü-2-tsüklopenteen-1-oonidest. Nii saadi 3-bensüül-2-hüdroksü-2-tsüklopenteen-1-oonist bensüül γ-laktoonhappe hea saagise (83%) ja kõrge stereoselektiivsusega (96%). 3-Arüül-2-hüdroksü-2-tsüklopenteen-1-oonidest saadi arüül γ-laktoonhapped rahuldava saagise (38-57%) ja hea stereoselektiivsusega (81-86%). Enantiomeerselt puhtad ühendid (>99% *ee*) saadi lähteühendite lihtsal ümberkristallimisel.

Lähtudes γ-laktoonhapetest saadi vastavad nukleosiidi analoogid 6-7 etapilise sünteesiga ja kuni 62% kogusaagisega.

Tulemusena töötati välja üldstrateegia enantiomeerselt puhaste 4′-asendatud nukleosiidi analoogide sünteesiks akiraalsetest 3-asendatud-2-hüdroksü-2-tsüklopenteen-1-oonidest.

Seda meetodit kasutades sünteesiti 16 erinevat nukleosiidi analoogi. Sünteesitud ühendite vähivastast aktiivsust testiti MCF-7, Hep3B ja CCRF-CEM vähirakkudel. Kõige aktiivsem oli 4′-(2-bensüüloksü)-fenüül-2′,3′-dideoksütümidiin rinnavähi rakkudel.

APPENDIX

*Table 1. The cytotoxity of **310a,b** and **315a,b** on MCF-7, Hep3B and CCRF-CEM cells*

Compound	MCF-7		Hep3B		CCRF-CEM	
	Average (n=3)	SEM	Average (n=3)	SEM	Average (n=3)	SEM
Growth medium	100.00	3.32	100.00	2.43	100.00	0.58
Negative Control	93.27	0.68	86.13	2.39	87.52	1.80
Positive Control	22.25	1.02			1.33	0.06
310a (0.1μM)	96.67	1.12	80.49	2.01	94.03	0.82
310a (1μM)	102.50	2.99	81.32	2.64	94.18	4.80
310a (10μM)	106.65	3.04	84.05	2.23	107.54	6.00
310a (100μM)	82.48	1.53	86.36	2.68	90.53	1.94
310a (200μM)	65.36	1.00	69.72	2.38	53.10	1.20
310b (0.1μM)	87.03	1.85	87.29	1.07	90.07	1.38
310b (1μM)	103.35	0.85	94.42	1.82	101.49	1.87
310b (10μM)	92.15	3.55	96.87	2.99	95.39	2.04
310b (100μM)	87.41	2.46	61.37	1.29	93.88	4.97
310b (1000μM)	69.05	1.41	34.85	0.50	57.01	1.23
315a (0.1μM)	106.56	3.74	92.74	3.91	100.89	5.03
315a (1μM)	98.15	1.43	93.43	1.24	97.13	0.91
315a (10μM)	93.79	0.46	94.84	1.87	90.22	2.15
315a (100μM)	88.63	1.84	65.31	3.40	88.96	1.11
315b (0.1μM)	105.32	0.42	99.98	0.98	99.23	2.74
315b (1μM)	102.23	3.35	101.56	1.32	105.02	3.82
315b (10μM)	97.87	2.81	98.83	2.45	95.50	2.62
315b (100μM)	98.19	3.64	87.64	3.74	91.77	3.32
315b (1000μM)	78.80	6.16	49.33	1.16	33.48	0.59

*Table 2. Analysis of cell cycle on MCF-7 cells by treatment with **310a,b** and **315a,b***

Compound	G_0/G_1 phase (%)	S phase(%)	G_2/M phase (%)
Negative Control	60.56	10.24	19.58
310a (0.1μM)	51.32	13.19	26.09
310a (1μM)	50.55	13.54	26.46
310a (10μM)	48.87	14.14	27.11
310a (100μM)	53.07	12.52	25.64
310a (200μM)	69.20	4.34	17.08
310b (0.1μM)	50.77	13.22	26.71
310b (1μM)	51.12	13.34	25.85
310b (10μM)	49.42	13.52	26.98
310b (100μM)	52.90	12.88	25.02
310b (1000μM)	72.48	3.32	15.19

315a (0.1μM)	53.48	13.82	24.89
315a (1μM)	53.58	13.17	25.46
315a (10μM)	53.51	13.67	25.13
315a (100μM)	71.74	5.36	14.99
315b (0.1μM)	51.40	14.43	25.19
315b (1μM)	52.80	14.15	24.52
315b (10μM)	53.16	13.74	25.47
315b (100μM)	54.04	14.08	24.63
315b (1000μM)	45.90	12.05	26.26

*Table 3. Analysis of cell cycle in Hep3B cells by treatment with **310a,b** and **315a,b***

Compound	**G_0/G_1 phase (%)**	**S phase(%)**	**G_2/M phase (%)**
Negative Control	45.51	16.01	27.13
310a (0.1μM)	44.69	16.97	28.86
310a (1μM)	45.51	17.77	26.82
310a (10μM)	42.81	17.04	31.75
310a (100μM)	50.92	17.30	25.37
310a (200μM)	50.64	9.18	23.93
310b (0.1μM)	43.19	14.68	25.79
310b (1μM)	41.89	15.00	29.45
310b (10μM)	42.46	16.06	28.58
310b (100μM)	40.88	15.03	30.26
310b (1000μM)	45.61	8.13	24.97
315a (0.1μM)	46.98	16.18	24.46
315a (1μM)	44.15	15.66	27.22
315a (10μM)	49.31	16.35	24.98
315a (100μM)	53.05	14.77	20.77
315b (0.1μM)	43.56	14.15	23.90
315b (1μM)	46.55	15.23	27.75
315b (10μM)	45.93	16.57	26.48
315b (100μM)	44.27	11.63	20.60
315b (1000μM)	43.34	9.70	33.78

*Table 4. Analysis of cell cycle in CCRF-CEM cells by treatment with **310a,b** and **315a,b***

Compound	**G_0/G_1 phase (%)**	**S phase(%)**	**G_2/M phase (%)**
Negative Control	55.70	19.41	22.21
310a (0.1μM)	54.42	19.11	24.05
310a (1μM)	54.83	19.93	22.75
310a (10μM)	54.67	19.25	23.25
310a (100μM)	54.67	20.36	23.03
310a (200μM)	45.95	24.82	26.65

310b (0.1μM)	53.82	19.72	24.14
310b (1μM)	53.35	19.40	24.96
310b (10μM)	53.94	20.27	23.54
310b (100μM)	50.08	19.80	27.49
310b (1000μM)	45.44	29.07	22.12
315a (0.1μM)	55.29	20.11	22.04
315a (1μM)	56.95	20.00	20.70
315a (10μM)	55.17	19.53	22.82
315a (100μM)	50.54	21.92	25.20
315b (0.1μM)	53.13	19.61	24.72
315b (1μM)	52.45	21.16	23.83
315b (10μM)	53.01	20.40	24.00
315b (100μM)	50.58	21.50	24.48
315b (1000μM)	43.37	22.87	26.59

*Table 5. The cytotoxity of **310b** on MCF-7 cells during 48-, 72- and 140-hour incubation*

Compound	**48 hour incubation**		**72 hour incubation**		**140 hour incubation**	
	Average (n=3)	**SEM**	**Average (n=3)**	**SEM**	**Average (n=3)**	**SEM**
Growth medium	100.00	3.31	100.00	1.28	100.00	4.78
Negative Control	100.26	1.33	90.45	3.37	86.19	6.12
Positive Control	42.11	3.85	34.49	1.71	19.88	0.32
Gemzar (1μM)	76.48	0.53	62.52	1.84	28.97	0.74
Gemzar (10μM)	82.30	1.41	64.23	0.54	27.62	0.88
Gemzar (100μM)	77.54	2.27	64.06	1.01	25.66	0.72
310b (10μM)	97.21	3.34	106.16	8.99	103.54	3.18
310b (100μM)	106.49	4.17	101.20	1.33	110.63	2.31
310b (1000μM)	78.77	1.59	63.41	0.94	39.69	1.36

*Table 6. The cytotoxity of **310b,** on Hep3B cells during a 2-, 3- and 6-day incubation*

Compound	**48 hour incubation**		**72 hour incubation**		**140 hour incubation**	
	Average (n=3)	**SEM**	**Average (n=3)**	**SEM**	**Average (n=3)**	**SEM**
Growth medium	100.00	0.85	100.00	2.77	100.00	7.45
Negative Control	79.53	4.60	109.49	0.77	92.21	1.71
Positive Control	78.12	4.29	62.30	1.50	30.21	1.59
Gemzar (1μM)	98.33	5.30	88.69	2.04	37.73	2.86
Gemzar (10μM)	84.76	4.42	83.19	2.09	35.62	1.50
Gemzar (100μM)	100.00	2.83	79.46	1.95	32.81	3.92

310b (10μM)	100.53	8.12	96.79	1.65	109.02	5.31
310b (100μM)	94.42	9.98	103.69	2.28	92.14	4.10
310b (1000μM)	64.35	2.34	78.43	3.49	50.12	4.03

*Table 7. The cytotoxity of **310a,b, 316a,b** and **317a,b** on MCF-7 cells during a 3-, 6- and 9-day incubation*

Compound	**3-day incubation**		**6-day incubation**		**9-day incubation**	
	Average (n=3)	**SEM**	**Average (n=3)**	**SEM**	**Average (n=3)**	**SEM**
Growth medium	100.00	6.96	100.00	0.92	100.00	5.65
Negative control	75.02	13.11	99.42	3.50	109.40	10.64
Positive Control	11.32	0.22	4.68	0.22	2.20	0.18
Gemzar (1μM)	66.31	1.78	39.22	5.65	25.24	1.44
Gemzar (10μM)	66.78	0.72	40.31	6.09	19.31	0.58
Gemzar (100μM)	62.59	0.59	41.34	1.06	18.95	0.48
310a (1μM)	98.49	5.09	121.84	0.77	111.23	4.13
310a (10μM)	97.50	4.36	107.87	2.21	100.40	3.54
310a (100μM)	62.26	4.45	41.17	2.56	37.03	3.61
310b (1μM)	98.25	14.26	126.86	7.32	115.31	11.57
310b (10μM)	88.92	1.80	107.62	7.45	105.23	1.46
310b (100μM)	83.83	9.87	68.35	7.31	70.47	8.01
316a (1μM)	85.94	0.29	92.23	6.01	112.52	9.10
316a (10μM)	95.02	3.27	88.22	3.21	111.90	3.89
316a (100μM)	90.98	1.87	54.68	3.69	40.20	1.77
316b (1μM)	87.30	2.31	93.05	2.89	113.88	6.05
316b (10μM)	92.37	2.63	88.32	4.17	113.89	0.49
316b (100μM)	90.80	3.18	76.97	1.11	53.53	0.68
317a (1μM)	102.30	1.33	102.35	1.95	80.58	2.71
317a (10μM)	95.58	3.16	93.50	8.02	70.28	1.28
317a (100μM)	81.99	1.28	46.40	1.71	22.53	1.94
317b (1μM)	103.51	5.95	100.40	2.56	83.17	1.58
317b (10μM)	105.98	2.72	102.65	2.90	82.36	1.01
317b (100μM)	92.26	5.57	84.87	5.66	64.44	3.96

*Table 8. The cytotoxity of **326a,b,e, 327a,b,e, 328** and **329** on MCF-7 cells during a 3-, 6- and 9-day incubation*

Compound	3-day incubation		6-day incubation		9-day incubation	
	Average (n=3)	SEM	Average (n=3)	SEM	Average (n=3)	SEM
Growth medium	100.00	1.32	100.00	1.66	100.00	1.62
Positive Control	11.41	0.74	3.93	0.94	2.68	0.36
Gemzar (1μM)	66.75	1.15	36.57	1.45	15.63	3.09
Gemzar (10μM)	64.71	1.10	35.52	1.39	15.60	1.24
Gemzar (100μM)	65.06	3.09	29.76	3.90	13.74	1.07
326a (1μM)	97.16	1.55	100.40	1.96	108.51	5.80
326a (10μM)	86.92	2.26	101.44	2.85	103.10	1.05
326a (100μM)	86.79	2.09	64.85	2.63	64.52	1.92
327a (1μM)	104.06	2.46	104.56	3.10	105.82	1.88
327a (10μM)	99.39	5.37	90.06	6.77	102.90	2.50
327a (100μM)	103.25	2.23	72.25	2.81	67.40	1.97
326b (1μM)	94.50	2.73	113.30	3.44	98.86	3.12
326b (10μM)	96.51	3.21	110.36	4.04	91.08	2.83
326b (10μM)	86.95	3.78	62.34	4.77	43.46	1.54
327b (1μM)	99.42	2.97	117.61	3.74	95.41	2.40
327b (10μM)	100.47	4.23	109.00	5.33	86.33	3.25
327b (100μM)	102.04	3.68	93.26	4.64	61.24	2.52
326e (1μM)	93.86	3.02	110.43	3.80	84.67	2.04
326e (10μM)	91.89	1.63	104.67	2.06	69.02	2.36
326e (100μM)	44.36	0.97	29.74	1.23	12.93	1.17
327e (1μM)	106.65	2.00	110.80	2.52	97.63	4.51
327e (10μM)	109.02	3.52	107.50	4.43	74.37	1.96
327e (100μM)	58.60	0.33	35.77	0.41	14.72	1.23
328 (1μM)	92.71	6.65	101.43	2.75	110.44	5.40
328 (10μM)	87.84	5.01	90.29	1.04	84.74	4.06
328 (100μM)	85.82	5.62	85.91	3.62	77.74	4.56
329 (1μM)	96.90	4.79	103.45	1.03	108.58	3.89
329 (10μM)	93.82	1.43	103.69	2.02	106.14	1.16
329 (100μM)	85.92	5.98	85.35	2.79	75.43	4.85

ARTICLE I

A. Paju, M, Laos, A. Jõgi, M. Päri, R. Jäälaid, T. Pehk, T. Kanger, M. Lopp, Asymmetric synthesis of 2-alkyl-substituted 2-hydroxyglutaric acid γ-lactones. *Tetrahedron Lett.* **2006**, *47*, 4491-4493.

ARTICLE II

A. Jõgi, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, T. Kanger, M. Lopp, Asymmetric synthesis of 2-aryl-5-oxotetrahydrofuran-2-carboxylic acids. *Synthesis*, **2006**, *18*, 3031-3036.

ARTICLE III

A. Jõgi, M. Ilves, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, M. Lopp, Asymmetric synthesis of 4′-C-benzyl-2′,3′-dideoxynucleoside analogues from 3-benzyl-2-hydroxy-2-cyclopenten-1-one. *Tetrahedron: Asymmetry*, **2008**, 19, 628-634.

ARTICLE IV

A. Jõgi, A. Paju, T. Pehk, T. Kailas, A.-M. Müürisepp, M. Lopp, Synthesis of 4′-aryl-2′,3′-dideoxynucleoside analogues. Submitted to *Tetrahedron*.

ELULOOKIRJELDUS

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August, 2004	TUT, Institute of Chemistry, researcher
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Asymmetric oxidation, synthesis of nucleoside analogues

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Master thesis	„Effects of acute and chronic antidepressant treatment on the FosB expression in the mouse brain“, Tartu University 2004. Supervisor: Professor Aleksandr Žarkovski.
Diploma thesis	„Synthesis of luminol from phthalic anhydride (application to the school conditions)”, Tartu University 2003. Supervisor: associate professor Uno Mäeorg.
Master thesis	„Synthesis of enynic compounds and their reaction in superbasic MEDA/EDA media”, Tartu University 2001. Supervisor: associate professor Uno Mäeorg.
Bachelor thesis	„Synthesis of 4-(Z,E)-octen-2-yn-1-ol and isomerization in superbasic media“, Tartu University 1999. Supervisor: Associate Professor Uno Mäeorg.

9. Main areas of scientific work

Asymmetric synthesis and applications

LIST OF THE PUBLICATIONS

Articles in journals

1. Jõgi, A., Ilves, M., Paju, A., Pehk, T., Kailas, T., Müürisepp, A.-M., Lopp, M. Asymmetric synthesis of 4′-C-benzyl-2′,3′-dideoxynucleoside analogues from 3-benzyl-2-hydroxy-2-cyclopenten-1-one. *Tetrahedron: Asymmetry*, **2008**, 19, 628-634.
2. Jõgi, A., Paju, A., Pehk, T., Müürisepp, A.M., Kanger, T., Lopp, M., Asymmetric synthesis of 2-phenyl-5-oxotetrahydrofuran-2-carboxylic acids, *Synthesis*, **2006**, 18, 3031-3036.
3. Paju, A., Laos, M., Jõgi, A., Päri, M., Jäälaid, R., Pehk, T., Kanger, T., Lopp, M., Asymmetric synthesis of 2-alkyl-substituted 2-hydroxyglutaric acid γ-lactones, *Tetrahedron Lett*., **2006**, 47, 4491-4493.
4. Jõgi, A., Mäeorg, U., Zn Mediated Regioselective Barbier Reaction of Propargylic Bromides in THF/ag.NH_4Cl Solution, *Molecules*, **2001**, 6, 964-698.
5. Jõgi, A., Mäeorg, U., Synthesis and Isomerization of Enyne-group Containing Compounds in NaEDA/EDA Media, *ARKIVOC*, **2001**, 3, 26-32.
6. Kotljarov, A., Jõgi, A., Mäeorg, S., Mäeorg, U., Behaviour of 1-butoxy-9-tetradecyne in Superbasic MEDA/EDA Media, *Proc. Estonian Acad. Sci. Chem*., **2001**, 50, 226-228.

Conference abstracts

1. Jõgi, A., Paju, A., Pehk, T., Lopp, M. Synthesis of 4′-substituted 2′,3′-dideoxynucleoside analogues and their biological activity, Baltic Polymer Symposium, BPS-2008, May 13-16, Otepää, Estonia.
2. Jõgi, A., Paju, A., Pehk, T., Siirde, K., Lopp, M. New Synthesis of Nucleoside analogues from 3-substituted-2-hydroxy-2-cyclopenten-1-ones, 15th European Symposium on Organic Chemistry, ESOC-2007, July 8-13, 2007, Dublin, Ireland.
3. Lopp, M., Jõgi, A., Paju, A., Siirde, K. New asymmetric chemical oxidation in the synthesis of chiral anticancer nucleoside analogues, *European Journal of Pharmaceutical Sciences*, 2007, 32S, S5. The 2nd BBBB Conference on Pharmaceutical Sciences, 13-15 September 2007, Tallinn-Tartu, Estonia.
4. Jõgi, A., Asymmetric synthesis and its application to synthesis new type of nucleosides, summer school for Ph.D.-students in Saaremaa, 14.08-17.08.2006, Jurna, Estonia.
5. Jõgi, A., Paju, A., Pehk, T., Müürisepp, A.-M., Kailas, T., Lopp, M. Synthesis of 3-phenyl-2-hydroxy-2-cyclopenten-1-ones and their asymmetric oxidation, International Conference on Organic Synthesis, BOS 2006, June 25-29, 2006, Tallinn, Estonia.
6. Laos, M., Jõgi, A., Paju, A., Kanger, T., Pehk, T., Lopp, M., Asymmetric synthesis of 2-benzyl- and 2-benzyloxymethyl γ-lactone acids, 14th European Symposium on Organic Chemistry, Helsinki, July 4-8 2005, Finland.

7. Paju, A., Jõgi, A., Laos, M., Pehk, T., Lopp, M., Asymmetric oxidation of 1,2-diketones - a simple route for 2-substituted 2-hydroxydiacids and the corresponding lactones, Sixth Tetrahedron Symposium, 2005, France.
8. Jõgi, A., Mäeorg, U., Synthesis of Luminol from Phthalic Anhydride, 28th Estonian Chemistry Days, Tallinn, 2002.
9. Jõgi, A., Mäeorg, U., Zn Mediated Regioselective Barbier Reaction of Propargylic Bromides in THF/ag.NH_4Cl Solution, 27th Estonian Chemistry Days, Tallinn, 2001.
10. Jõgi, A., Mäeorg, U., THe Synthesis of Enyne-group Containing Compounds and Isomerization in NaEDA/EDA Media, 26th Estonian Chemistry Days, Tallinn, 2000.
11. Jõgi, A., Talu, L., Mäeorg, U., The Synthesis of 4-(Z,E)-octen-2-yn-1-ol and Isomerization in Superbasic Media, 25th Estonian Chemistry Days, Tallinn, 1999.

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *NATURAL AND EXACT SCIENCES*

1. **Olav Kongas**. Nonlinear dynamics in modeling cardiac arrhytmias. 1998.

2. **Kalju Vanatalu**. Optimization of processes of microbial biosynthesis of isotopically labeled biomolecules and their complexes. 1999.

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