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Synthesis, reactivity and biological activity of C-5 substituted uracil analogues

Syntéza a studium reaktivity a biologické aktivity C-5 substituovaných analog uracilu

Ph.D. Thesis

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Declaration of the author

I hereby declare that this Ph.D. thesis has been written by myself and using sources quoted in "References" part. Neither the thesis nor any of its substantial parts were used previously for obtaining any academic degree.

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Abstract:

The presented thesis is focused on the synthesis of various C-5 modified uracil analogues, the study of their reactivity and biological activity, especially cytotoxic activity. In the first part, the brief survey of described results for selected 5-alkoxymethyluracil analogues is performed. The second part of the presented thesis deals with the synthesis of novel uracil analogues modified at the C-5 position, the development and optimizing of procedure leading to the desired compounds, the study of biological activity and the evaluation of structure-activity relationship (SAR). This part presents the synthesis of a series of 5-[alkoxy(4-nitrophenyl)methyl)]uracil and 5-alkoxymethyluracil analogues and extended SAR studies depending on a substitution of metylene bridge directly attached at the C-5 position as well as alkoxy chain length. The last part of the presented work is focused on synthesis of pyrimidine oligodeoxynucleotides containing either substituted phenyltriazole or substituted phenylethynyl moiety at position C-5. The synthesis of such a modified oligodeoxynucleotides follows antisense approach that involves targeting of RNA within cells as a means of control of gene expression.

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Abstrakt:

Předložená disertační práce je zaměřena na syntézu 5-substituovaných derivátů uracilu, jejich reaktivitu a studium biologické aktivity, především aktivity protinádorové. V úvodní části je vytvořen přehled dosud popsaných výsledků vybraných C-5 substituovaných derivátů uracilu na poli jejich syntézy a studia biologické aktivity. Druhá část je věnována designu nových C-5 modifikovaných analog uracilu, vývoji a optimalizaci postupů vedoucích k cílovým sloučeninám, studiu biologické aktivity a odvození vztahu mezi strukturou a biologickou aktivitou (SAR studie). V této části je prezentována syntéza série derivátů odvozených od 5-[alkoxy(4-nitrofenyl)methyl)]uracilu a 5-alkoxymethyluracilu a rozsáhlá SAR studie v závislosti na typu substituce methylenového můstku bezprostředně navázaného do polohy C-5 a dále v závislosti na délce alkoxylového řetězce. V poslední části disertační práce je popsána syntéza dvou typů oligodeoxynukleotidů, z nichž první skupina je modifikována fenoltriazolovým skeletem navázaným do polohy C-5 a druhá skupina obsahuje v poloze C-5 bromofenylethynylový zbytek. Cílem takovéto modifikace je najít termicky stabilní sekvence použitelné v antisense terapii jako prostředek kontroly genové exprese.

Klíčová slova:	Uracil, biologická aktivita, protinádorová aktivita.
Počet stran:	141
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Table of contents

List of Abbreviations

A	adenine
A549	tumor cells of lung carcinoma
Ac	acetyl
AIDS	acquired immunodeficiency syndrome
BVDU	5-(2-bromovinyl)-2'-deoxyuridine
BVUr	5-bromovinyluracil
C	cytosine
CAN	ceric ammonium nitrate
CEM	subline of acute lymphoblastic leukemia
CEM-DNR-bulk	subline of acute lymphoblastic leukemia resistant on daunorubicin
DAST	diethylaminosulfur trifluoride
DCI	dicyclohexylcarbodiimide
DIPEA	diisopropylethylamin
DMAP	*N*,*N*′-dimethylaminopyridine
DMEDA	*N*,*N*′-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMTrCl	4,4′-dimethoxytrityl chloride
DNA	2’-deoxyribonucleic acid
EBV	Ebstein-Barr virus
EI MS	electron impact mass spectrometry
Et	ethyl
G	guanine
HCMV	human cytomegalovirus
HCT116p53	colorectal cancer cells wild type
HCT116p53-/-	colorectal cancer cells mutagenous
HIV	human immunodeficiency virus
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HPLC	high-performance liquid chromatography
HSV	herpes simplex virus
IVDU	5-(2-iodovinyl)-2'-deoxyuridine

K562	subline of chronic myelogeneous leukemia
K562-Tax	subline of chronic myelogeneous leukemia resistant on paclitaxel
MALDI-TOF MS	matrix assisted laser desorption ionization - time of flight mass spectrometry
MCPBA	3-chloroperoxybenzoic acid
Me	methyl
Ms	methanesulfonyl
MW	microwave irradiation
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
py	pyridine
RNA	ribonucleic acid
RP-HPLC	reversed-phase high herformance liquid chromatography
RT	room temperature
SAR	structure-activity relationship
SD	standard deviation
T	thymine
TBAF	tetrabutylammonium fluoride
TBDMS	tertbutyldimethylsilyl
TCA	trichloroacetic acid
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
U	uracil
VZV	varicella zoster virus

1. Introduction

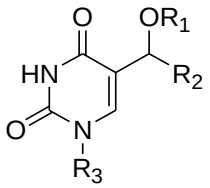
Modifications of the nucleic acids components play a significant role in the field of nucleic acids research. Nucleoside analogues find broad therapeutic applications in anti-cancer treatement or antiviral chemotherapy in particular.

In anticancer chemotherapy the huge knowledge concerning processes taking place through cell cycle has enabled to break through and understand to the mechanisms of action of many anticancer agents. 5-Fluorouracil, for instance, is one of the first and most investigated anticancer drugs, either chemically or biologically, and triggered the research of 5-substituted pyrimidine analogues.

The elucidation of the life cycle of a virus is crucial in antiviral chemotherapy. A lot of 5-substituted pyrimidine analogues capabled to weigh in the life cycle of viruses have been discovered as highly active antiviral agents. One of such a drugs with antiviral properties are 5-iodo-2´-deoxyuridine discovered in the 1960s as the first agent that is active against HSV and VZV viruses or 5-vinyl-2´-deoxyuridine indicating high activity against HSV that started studies on synthesis and biological activity of its analogues.

From these pieces of knowledge we draw inspiration to the development of new potent biological active compounds, compounds that might be more selective, more specific and much less toxic for organism.

One of those groups of investigated derivatives is a group of uracil analogues modified at the 5 position by ether or ester moiety. Since the huge amount of C-5 modified pyrimidine analogues is known, this review is focused on a group of selected compounds with specific substituent (*Figure 1*).



R_1 = Ak, Cy, Cb, Hy
R_2 = Ak, Cy, Cb, Hy
R_3 = H, Ak, Cy, Cb, Hy

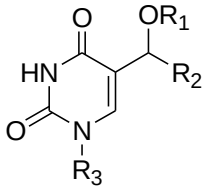
Figure 1. Investigated derivatives.

The greatest attention is paid to the studies on synthesis of selected derivatives. The brief survey of biological activity of investigated compounds is also reported. Following chapters concerning the synthesis are ranged according to the products of synthetic routes.

1.2. Synthesis

1.2.1. Synthesis of alkoxyhalogenalkyl derivatives

The most numerous and also the most investigated group in the field of research of the above mentioned derivatives is a group of alkoxyhalogenalkyl derivatives derived either from uracil or nucleosides as shows *Figure 2*. With regard to the high variability of sugar moiety all described compounds are divided into the sections according to used furanose heterocycle.

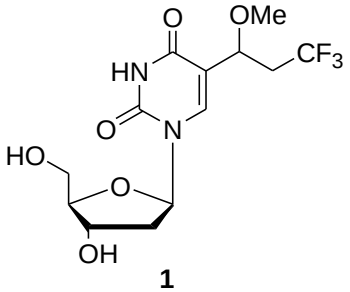


R_1 = alkyl
R_2 = halogenalkyl
R_3 = H, ribose, 2´-deoxyribose, arabinose, 2´-fluor-2´-deoxyribose, 2´-deoxy-4´-C-methyl

Figure 2. Modifications of uracil ring.

(a) 2´-deoxyuridine analogues

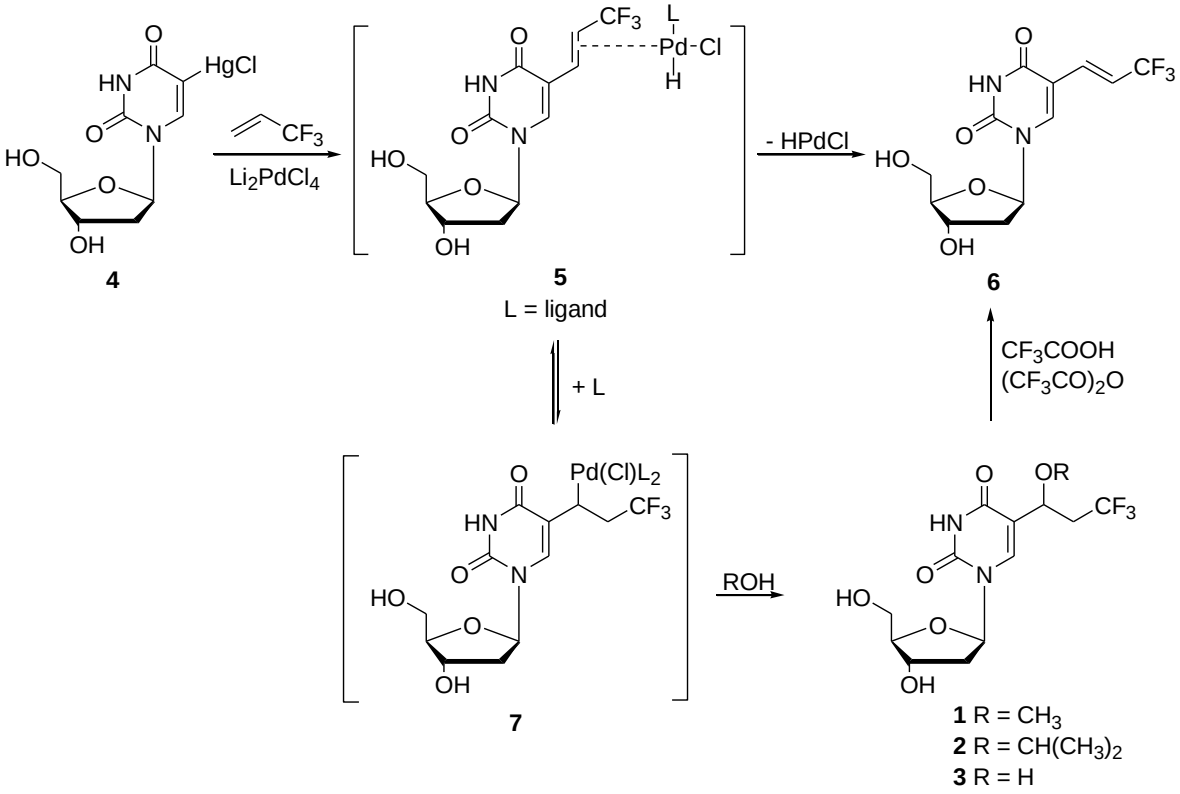
The oldest article describing 2´-deoxyuridine analogues is focused on uracil analogues modified at the 5 position by fluorine containing moiety.[1] Bases or nucleosides substituted by fluorine were investigated as potent anticancer agents since 60th. Nevertheless, many of such modified compounds were also synthesized in order to investigate their antiviral activity. As a consequence of interest in biologically active fluoro derivatives Bergstrom and co-workers performed synthesis of 5-(3,3,3-trifluoro-1-methoxypropyl)-2´-deoxyuridine **1** (*Figure 3*) as the first perfluoro derivative from the group of mentioned compounds.



1

Figure 3. 5-(3,3,3-Trifluoro-1-methoxypropyl-)-2´-deoxyuridine **1**.

The synthetic route that led to the desired fluoro compound **1** utilized known reaction[2] of organomercuri intermediate 5-chloromercuri-2′-deoxyuridine and palladium catalyst. The reaction carried out in methanol afforded 17% of *(E)*-5-(3,3,3-trifluro-1-propenyl)-2′-deoxyuridine **6** and 36% of 5-(3,3,3-trifluoro-1-methoxypropyl)-2′-deoxyuridine **1** (*Scheme 1*). Shortly after publishing successful synthesis of trifluoro nucleoside **1** Bergstrom and co-workers reported a presumed mechanism of the formation of this fluoro compound **1** (*Scheme 1*).[3] In addition, they focused their research on isopropyloxy analogue **2** as well.

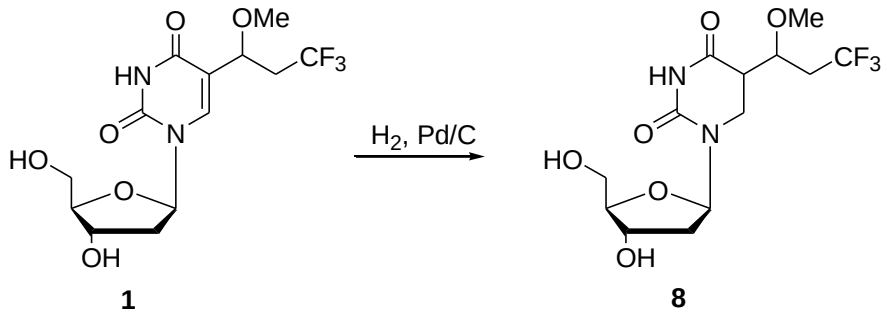


Scheme 1. Synthesis of 5-(3,3,3-trifluoro-1-methoxypropyl)-2′-deoxyuridine **1** and 5-(3,3,3-trifluoro-1-(2-propyloxy)prop-1-yl)-2′-deoxyuridine **2.**

Reaction of 3,3,3-trifluoropropen with 5-chloromercuri-2′-deoxyuridine **4** in methanol gave two major products *(E)*-5-(3,3,3-trifluro-1-propenyl)-2′-deoxyuridine **6** and derivative **1** in approximately 1:2 ratio. Furthermore, authors also carried out the synthesis in other solvents such as *N,N′*-dimethylformamide, 2-propanol or acetonitrile and found out that the using of solvents unlike the methanol decreased yields of C-5 substituted products. The utilization of 2-propanol, for instance, afforded unsaturated derivative **6** in 8% yield and 5-(3,3,3-trifluoro-1-(2-propyloxy)prop-1-yl)-2′-

deoxyuridine **2** in 12% yield. Moreover, fluoro compound **1** can be converted to the propenyl derivative **6** by treating with mixture of trifluoroacetic acid and trifluoroacetic acid anhydride. Interestingly, third product was isolated from the reaction mixture when similar reactions of 5-chloromercuri-2´-deoxyuridine **4** with 3,3,3-trifluoropropen were made. This 5-(3,3,3-trifluoro-1-hydroxypropyl)-2´-deoxyuridine **3** was obtained in 38-40% yields but authors were not able to explain reasons of the formation of hydroxyl derivative **3**.

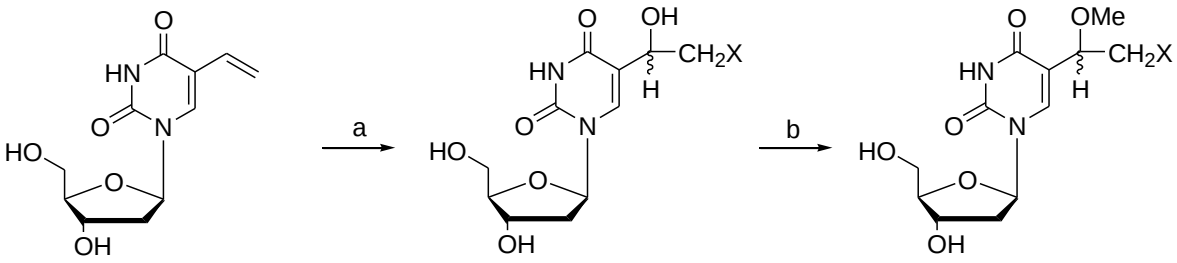
Attempt at hydrogenolysis of the methoxy group of methoxy derivative **1** using H_2 over Pd/C afforded 5-(3,3,3-trifluoro-1-methoxyprop-1-yl)-5,6-dihydro-2´-deoxyuridine **8** (*Scheme 2*).



1 **8**

Scheme 2. Synthesis of 5-(3,3,3-trifluoro-1-methoxyprop-1-yl)-5,6-dihydro-2´-deoxyuridine **8**.

A large range of alkoxyhalogenalkyl 2´-deoxyuridine nucleosides was successfully performed by Kumar and co-workers over the years 1989-1994.[4,5,6,7,8] Almost all of these compounds were synthesized in order to evaluate their biological activity, antiviral activity above all. In 1989 Kumar and co-workers reported among others two 5-(methoxy-2-haloethyl)-2´-deoxyuridines **12** and **13** (*Scheme 3*).[4] Their synthesis was based on the addition of HOX (X = Br, Cl) to the vinyl moiety of 5-vinyl-2´-deoxyuridine **9**. The reaction was carried out in aqueous dioxane and hydroxybromoethyl **10** and hydroxychloroethyl **11** derivatives were obtained in 70% and 60% yields, respectively. Subsequent treatment of hydroxyderivatives **10** and **11** with methanolic sulfuric acid gave corresponding desired 5-(methoxy-2-haloethyl) derivatives **12** and **13** in 93 and 98% yields, respectively. None of the separation method for two diastereomers was described in this article.

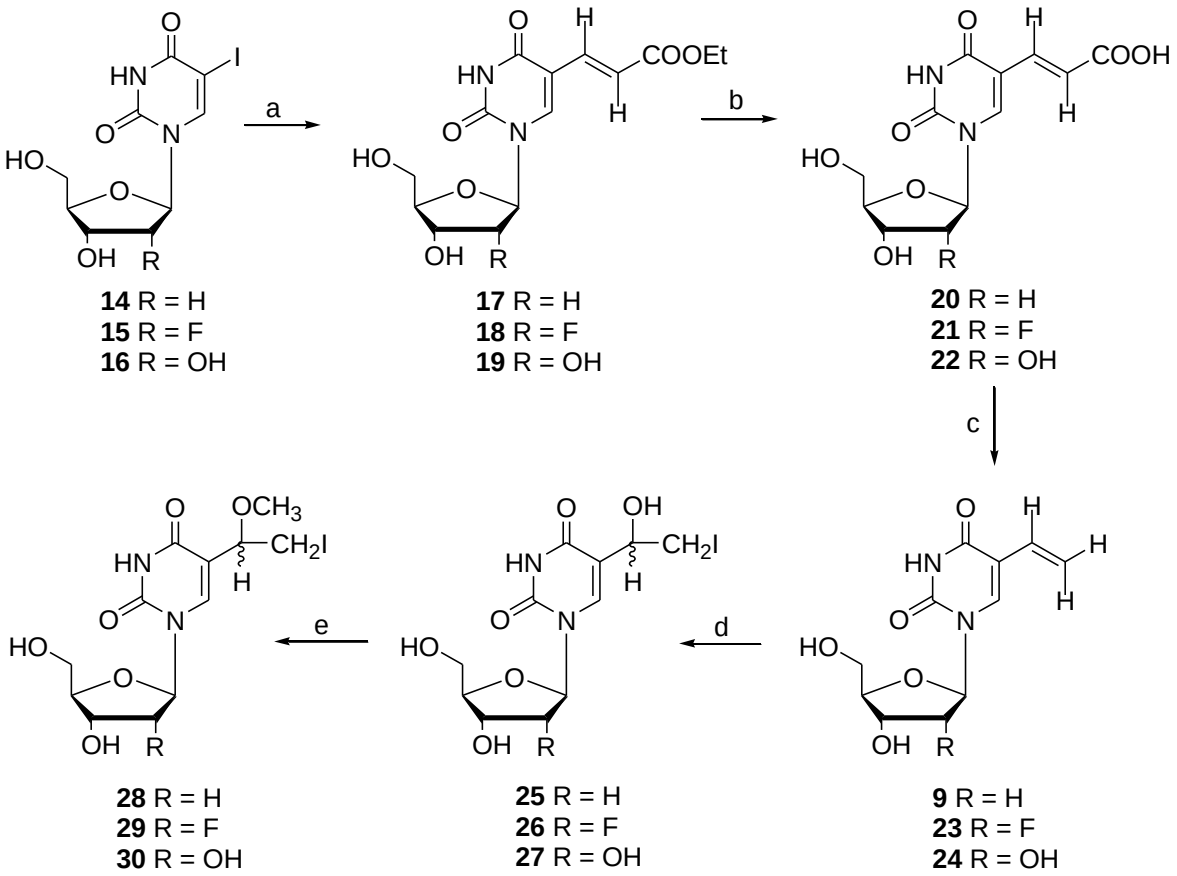


9 **10** X = Br **11** X = Cl **12** X = Br **13** X = Cl

(a) *N*-bromosuccinimide (**10**), *N*-chlorosuccinimide (**11**), dioxane-water (3:7, *v/v*), acetic acid, 25°C; NaOH (b) H_2SO_4-MeOH, 25°C

Scheme 3. Synthesis of 5-(methoxy-2-haloethyl)-2′-deoxy-uridines **12** and **13**.

A year later Kumar and co-workers extended their research also to the modification of sugar portion of nucleosides.[5] They prepared iodomethoxy derivatives of 2′-deoxyuridine **28**, 2′-fluoro-2′-deoxyuridine **29** and uridine **30** (*Scheme 4*). Authors utilized the known palladium acetate-triphenylphosphine-catalyzed reaction of 5-iodo-2′-deoxyuridine with vinyl acetate for preparation of 5-vinyl-2′-deoxyuridine **9**.[9] However, attempts to prepare 2′-fluoro-2′-deoxyuridine **23** and uridine analogue **24** by this method were unsuccessful. Hence, 5-vinyl derivatives **9, 23** and **24** were prepared by three-step palladium-catalyzed synthesis of 5-iodo-2′-fluoro-2′-deoxyuridine **15** and 5-iodouridine **16** with ethyl acrylate with subsequent alkaline hydrolysis and decarboxylation. Iodination of 5-vinyl analogues **9, 23** and **24** with iodine in the presence of the iodic acid as an oxidizing agent afforded 5-(1-hydroxy-2-iodoethyl)-2′-deoxyuridine **25** (59%), 5-(1-hydroxy-2-iodoethyl)-2′-fluoro-2′-deoxyuridine **26** (72%) and 5-(1-hydroxy-2-iodoethyl)-uridine **27** (65%) in their diasteroisomeric mixture. Finally, the treatment of hydroxyderivatives **25-27** with methanolic sulfuric acid gave desired 5-(1-methoxy-2-iodoethyl) nucleosides **28-30** in 81-94% yields. All of these three compounds were obtained as a mixture of two diastereomers without further successful separation.



(a) CH_2=CHCOOEt, $(Ph_3P)_2PdCl_2$, Et_3N; (b) 0.5 N KOH; (c) DMF, Et_3N, 100°C; (d) I_2, KIO_3, H_2O, 5 N H_2SO_4, 55°C; (e) 5 N H_2SO_4, MeOH

Scheme 4. Synthesis of 5-(1-methoxy-2-iodoethyl) nucleosides **28**-**30**.

In order to develop new potential tumor localization agents, Iwashina and co-workers investigated radiolabelled 5-(1-methoxy-2-iodoethyl) nucleoside **31** (*Figure 4*).[10] They reported radioiodination of 5-(1-methoxy-2-iodoethyl)-2′-deoxyuridine **28** by the isotope exchange with utilization of pivalic acid melt method.

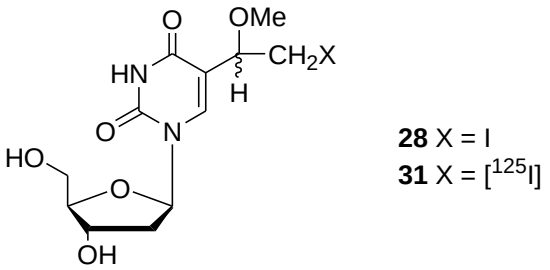
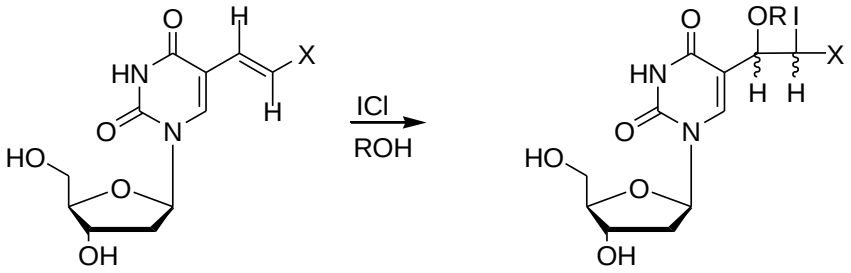


Figure 4. [^{125}I] radiolabelled 5-(methoxy-2-iodoethyl)-2′-deoxy-uridine **31**.

Among the above mentioned 5-(1-methoxy-2-iodoethyl) nucleosides, Kumar and co-workers also reported the synthesis of another alkoxy derivatives - 5-(1-alkoxy-2-iodoethyl) and 5-(1-ethoxy-2,2-diiodoethyl)-2′-deoxyuridine analogues **33-36** (*Scheme 5*).[6] The reaction of (*E*)-5-(2-iodovinyl) **32** and 5-vinyl-2′-deoxyuridine **9** with iodine monochloride and alcohols such as ethanol, 2-fluoroethanol or 2,2,2-trifluoroethanol afforded appropriate products, 5-(1-ethoxy-2,2-diodoethyl) **33** and 5-(1-alkoxy-2-iodoethyl)-2′-deoxyuridines **34-36** in 33-90% yields. Each of these four products **33-36** formed mixture of two diastereomers in a ratio 1:1.

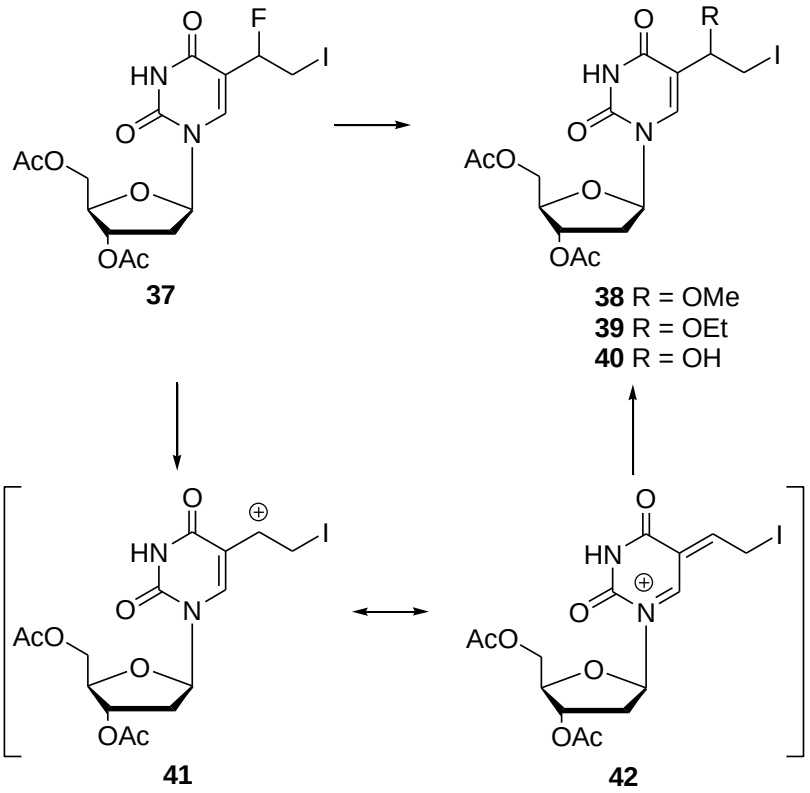


32 X = I
9 X = H

33 R = Et, X = I
34 R = Et, X = H
35 R = CH_2CH_2F, X = H
36 R = CH_2CF_3, X = H

Scheme 5. Synthesis of 5-(1-alkoxy-2-iodoethyl) **34-36** and 5-(1-ethoxy-2,2-diiodoethyl)-2′-deoxyuridine **33**.

Within the synthesis of 5-(1-fluoro-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **37**, by-products such as 5-(1-methoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **38** and 5-(1-ethoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **39** were identified (*Scheme 6*).[7] 5-(1-Hydroxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **40** was treated with DAST (Et_2NSF_3) at -40°C in anhydrous dichloromethane and the reaction afforded 5-(1-fluoro-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **37** and also the respective 5-(1-methoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **38** and 5-(1-ethoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **39** as major products. Authors suggested a mechanism for the formation of methoxy **38** and ethoxy **39** derivatives, which was based on decomposition of 5-(1-fluoro-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **37** to a carbonium cation intermediates **41** and **42** at 25°C. Consequently, the cation **41** reacted with methanol, ethanol or water to afford alkoxy derivatives **38** and **39**. Author presumed that the nucleosides **38** and **39** are formed during the silica gel column chromatography, where the mixture of methanol, chloroform and ethanol was used as an eluent.



Scheme 6. Synthesis of 5-(1-methoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **38** and 5-(1-ethoxy-2-iodoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **39**.

Furthermore, authors described the reaction of the 5-(1-hydroxy-2-chloroethyl) **43** and 5-(1-hydroxy-2-bromoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **44** with thionyl bromide, which provided 5-(1-ethoxy-2-chloroethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **45** and 5-(1-ethoxy-2-bromoethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **46** (*Figure 5*).

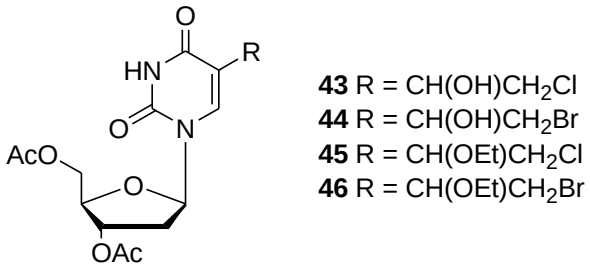
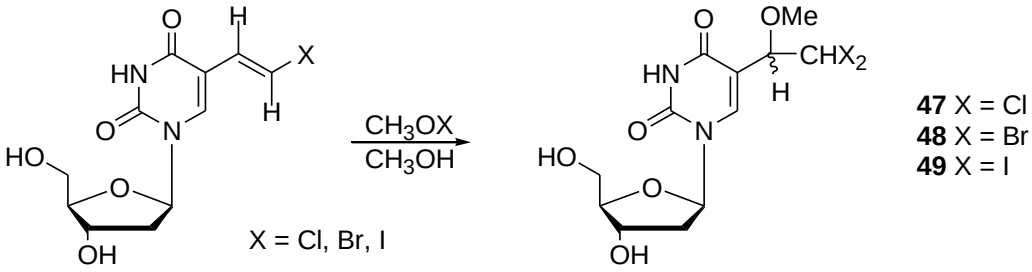


Figure 5. 5-(1-Hydroxy(or ethoxy)-2-haloethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridines **43-46**.

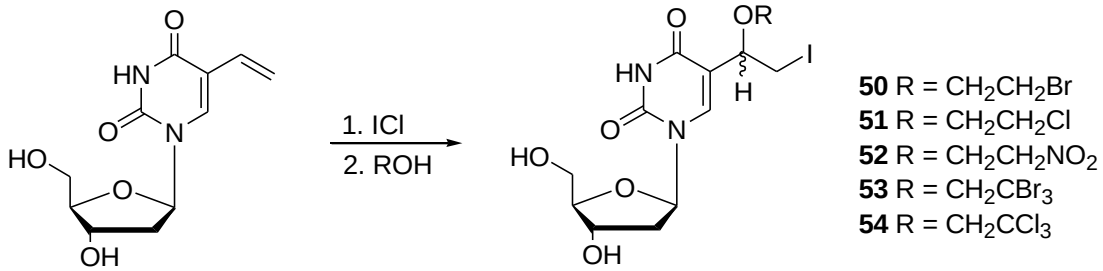
Additional halogen introduction to ethyl moiety at the C-5 position of uracil base led to the dihalogenderivatives that was reported also by Kumar and co-workers.[8] The required 5-(1-methoxy-2,2-dihaloethyl)-2′-deoxyuridines **47-49** (*Scheme 7*) were

prepared by the additional reaction of CH_3OX, where X can be either Cl, Br or I, to the vinyl moiety of (*E*)-5-(2-halovinyl)-2′-deoxyuridine.



Scheme 7. 5-(1-Methoxy-2,2-dihaloethyl)-2′-deoxyuridines **47-49**.

Rai and co-workers developed an efficient synthesis of 5-[1-(2-halo(or nitro)ethoxy-2-iodoethyl]-2′-deoxyuridines **50-54** (*Scheme 8*) and evaluated their antiviral activity.[11] For this purpose 5-vinyl-2′-deoxyuridine **9** was used as the starting compound. The regiospecific reaction of the 5-vinyl-2′-deoxyuridine **9** with iodine monochloride in the presence of various alcohols afforded 2′-deoxynucleosides **50-54** in 24-52% yields.



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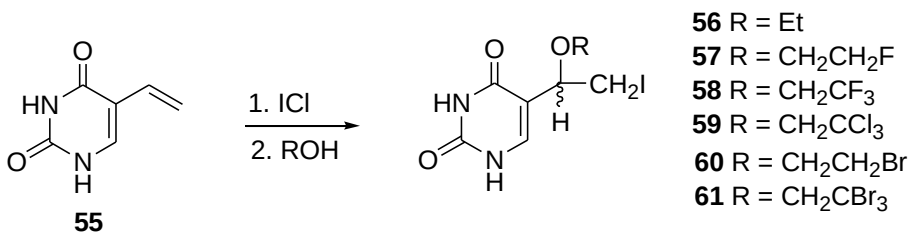
Scheme 8. Synthesis of 5-[1-(2-haloethyl(or nitro)-ethoxy-2-iodoethyl)]-2′-deoxyuridines **50-54**.

(b) uracil analogues

The syntheses of some of the above mentioned 2′-deoxyuridine analogues were also described for modified uracil derivatives. First group of these derivatives is represented by alkoxyiodoethyl derivatives **56-59** prepared by the reaction of 5-vinyluracil **55** with iodine monochloride (*Scheme 9*).[6] The reaction was carried out in the presence of ethanol, 2-fluorethanol, 2,2,2-trifluorethanol or 2,2,2-trichloroethanol to form appropriate 5-(1-ethoxy-2-iodoethyl) **56**, 5-[1-(2-fluoroethoxy)-2-iodoethyl] **57**, 5-[1-

(2,2,2-trifluoroethoxy)-2-iodoethyl] **58** and 5-[1-(2,2,2-trichloroethoxy)-2-iodoethyl] **59** uracil analogues.



Scheme 9. Synthesis of alkoxyuracil analogues **56-61**.

Also Rai and co-workers applied the reaction of vinyl derivative with iodine monochloride at the uracil analogue **55** and prepared series of 5-[1-(2-haloethoxy-2-iodoethyl)]uracils **60-61** (*Scheme 9*).[11] The utilization of the 5-vinyl uracil **55** for the regiospecific addition of iodone monochloride in the presence of various alcohols led to the synthesis of 5-[1-(2-bromoethoxy-2-iodoethyl)]uracil **60** and 5-[1-(2,2,2-tribromoethoxy)-2-iodoethyl)]uracil **61**.

The similar reaction was already used 14 years earlier by Kumar and co-workers. The authors published synthesis of 5-(methoxy-2-haloethyl)uracils **62-64** (*Figure 6*).[12] The preparation of alkoxyhaloethyl derivatives **62-64** was based on the addition of HOX (X=Br, Cl) or ICl to the 5-vinyluracil **55** and subsequent treatment with methanolic sulfuric acid.

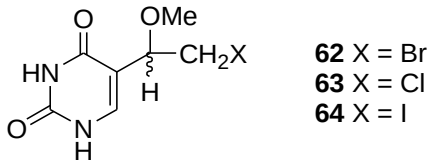
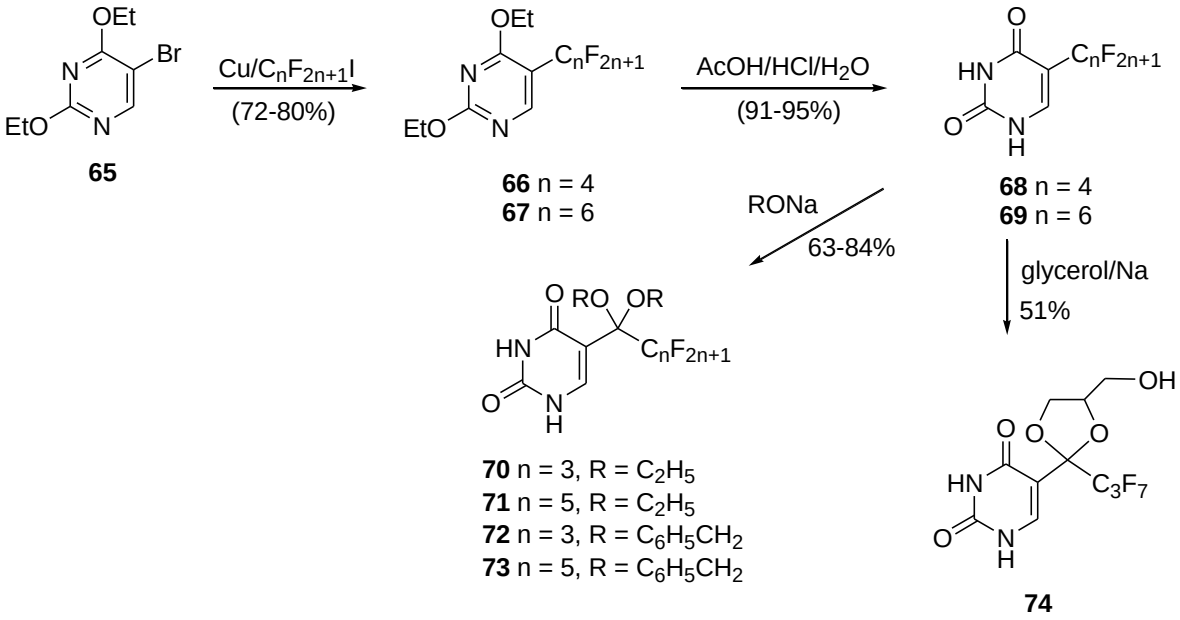


Figure 6. 5-(Methoxy-2-haloethyl)uracils **62-64**.

In 2002 Ismail and co-workers published interesting synthesis of ethoxy-substituted 5-(perfluoroalkyl)pyrimidines and their regioselective transformations.[13] Some of the fluorine-containing pyrimidine analogues are potent antitumour and antiviral agents. Within this context, authors of the mentioned publication evolved efficient synthetic route leading to the perfluoralkyl derivatives (*Scheme 10*).

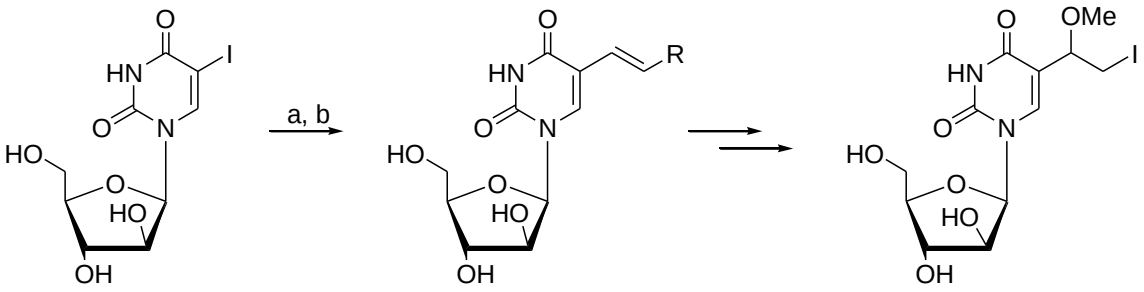


Scheme 10. Synthesis of perfluoro derivatives **70**-**74**.

The reported synthesis started with the treatment of 5-bromo-2,4-diethoxypyrimidine **65** with either perfluorobutyl or perfluorohexyl iodide in the presence of activated copper bronze in DMSO. This reaction afforded 5-(perfluoroalkyl)pyrimidines **66** and **67** in high yields. Subsequent acid hydrolysis of **66** and **67** provided 5-(perfluoroalkyl)pyrimidines **68** and **69**. The perfluoroalkyl derivatives **68, 69** readily undergo nucleophilic attack of alkoxide ions and form various alicyclic or cyclic acetals **70**-**73** and **74**, respectively, according to applied alcohols.

(c) uridine and arabinofuranosyl analogues

5-Substituted uracil nucleosides bearing ribose or arabinose were also prepared. Johar and co-workers described the synthesis of 1-*β*-*D*-2′-arabinofuranosyl-5-(1-methoxy-2-iodoethyl)uracil **79** (*Scheme 11*) in a recent article[14] as well as Kumar and co-workers in 1992.[15]

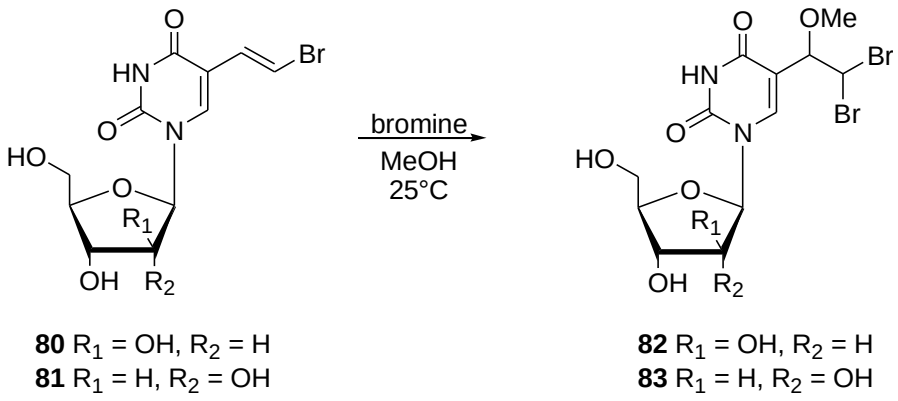


(a) CH_2=CHCOOEt, $(Ph_3P)PdCl_2$, Et_3N; (b) 0.5 N KOH

Scheme 11. Synthesis of 1-*β*-*D*-2′-arabinofuranosyl-5-(1-methoxy-2-iodoethyl)uracil **79**.

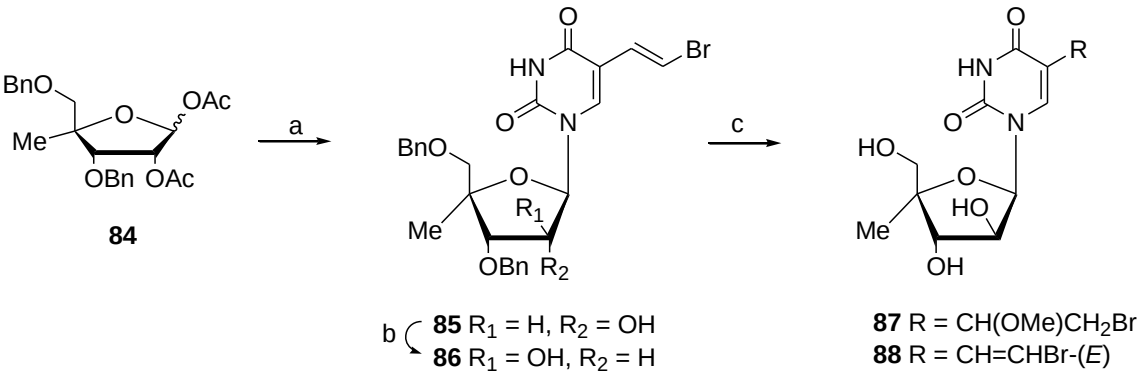
The synthesis was based on the reaction of 1-*β*-*D*-2′-arabinofuranosyl-5-iodouracil **75** with ethylacrylate in the presence of Pd catalyst and TEA. Subsequent alkaline hydrolysis of **76** afforded the (*E*)-5-(2-carboxyvinyl) derivative **77**. 5-Vinyl-arabinouridine **78**, obtained by decarboxylation of **77**, was reacted with iodine in the presence of oxidizing agent, iodic acid, and afforded 1-*β*-D-2′-arabinofuranosyl-5-(1-hydroxy-2-iodoethyl)uracil that was treated with methanolic sulfuric acid and afforded required methoxy nucleoside **79**.

The regiospecific addition of bromine in methanol to the vinyl substituent of (*E*)-5-(2-bromovinyl)arabinouridine **80** and uridine **81** afforded 1-*β*-D-arabinofuranosyl-5-(2,2-dibromo-1-methoxyethyl)uracil **82** and uridine **83**.[16]



Scheme 12. Synthesis of 1-*β*-D-arabinofuranosyl-5-(2,2-dibromo-1-methoxyethyl)uracil **82** and uridine **83**.

Within a searching for new antiviral agents, 4′-*C*-methyl-pyrimidine nucleosides were synthesized (*Scheme 13*) and their biological activity was evaluated.[17]



(a) silylated BVUr, TMSOTf/MeCN, then K_2CO_3/MeOH; (b) MSCl/pyridine, then 1N NaOH/EtOH-H_2O (3:1); (c) BBr_3/CH_2Cl_2; MeOH **(87)**; $NaHCO_3$ **(88)**.

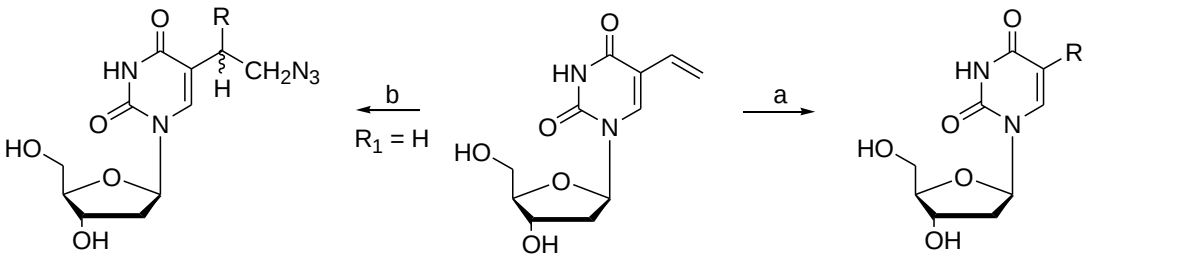
Scheme 13. Synthesis of methoxy derivative **87**.

Firstly, 4′-*C*-methyl-D-ribose **84** was prepared by the procedure described earlier.[18] After that, 5-bromvinyluracil was silylated and reacted with protected ribose **84** in the presence of TMSOTf as a Lewis acid. It followed a deacetylation with anhydrous K_2CO_3 in MeOH after ribosylation that provided di-*O*-benzylated nucleoside **85** in 73% yield. For the change of the configuration at 2′-C, derivative **85** was converted to its mesylate and treated with NaOH in EtOH-H_2O to afford 4′-*C*-methylnucleoside **86** in 58% yield. Finally, nucleoside **86** was debenzoylated with BBr_3 in CH_2Cl_2 at -78%. A quenching of reaction with MeOH led to unexpected methoxy derivative **87**. On the other hand the quenching with saturated $NaHCO_3$ solution gave target derivative **88**.

The preparation of 5-(1-methoxy-2-iodoethyl)uridine **30** is described above (*Scheme 4*, page 15).[5]

1.2.2. Synthesis of alkoxyazidoalkyl derivatives

Among the additional reactions of HOX or CH_3OX (X = Cl, Br, I) to the 5-vinyl-2′-deoxyuridine Kumar and co-workers performed the regiospecific addition of halogencyanamides (X-NHCN) (*Scheme 14*).[19]



(a) *N*-chlorosuccinimide, NH_2CN, CH_3CN, 0-25°C; (b) ceric ammonium nitrate, NaN_3, CH_3CN-H_2O, -5°C (OH); ceric ammonium nitrate, NaN_3, dry CH_3CN, -15°C (OH) and (OCH_3)

Scheme 14. Synthesis of 5-(1-methoxy-2-azidoethyl)-2′-deoxyuridine **93**.

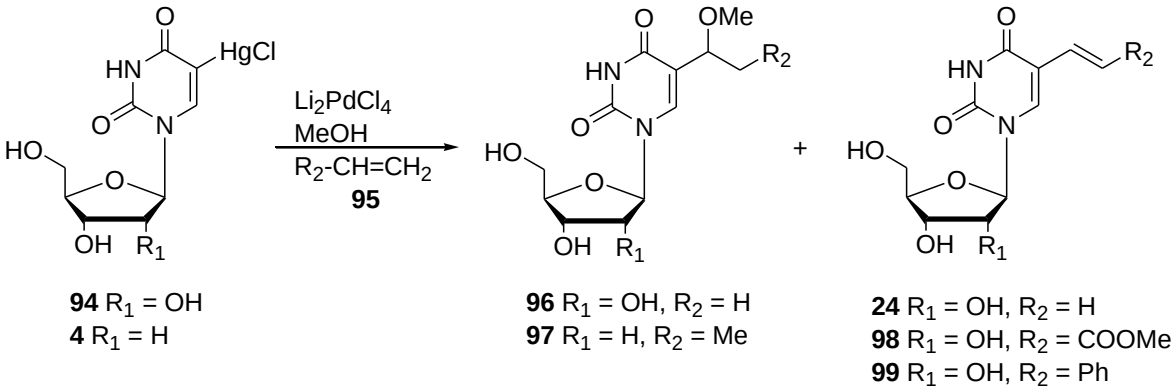
They described a synthesis of a new 5-(1-cyanamido-2-chloroethyl)-2′-deoxyuridine **90**. In addition, the reaction of 5-vinyl-2′-deoxyuridine **9** with *N*-chlorosuccinimide and cyanamide affording 5-(1-cyanamido-2-chloroethyl)-2′-deoxyuridine **90** was accompanied with formation of mixture of by-products such as (*E*)-5-(2-chlorovinyl)-2′-deoxyuridine **89** and 5-(1-methoxy-2-chloroethyl)-2′-deoxyuridine **13**. 5-Vinyl-2′-deoxyuridine **9** can also undergo reaction with ceric ammonium nitrate and sodium azide and gave 5-(1-hydroxy-2-azidoethyl)-2′-deoxyuridine **92** in 32% yield. This reaction was carried out in CH_3CN-H_2O mixture of solvents. When dry acetonitrile was used and reaction was quenched with methanol, 5-(1-methoxy-2-azidoethyl)-2′-deoxyuridine **93** was obtained in 25% yield.

1.2.3. Synthesis of alkoxyalkyl derivatives

The C-5 modified pyrimidine nucleosides with the short alkyl substituent are in the center of indispensable interest from early 70′s due to the potential chemotherapeutic and antiviral properties. It was published, for instance, that 5-ethyluracil may undergo incorporation into bacterial DNA[20] or 5-ethyl-2′-deoxyuridine readily replaces thymidine in bacteriophage DNA.[21]

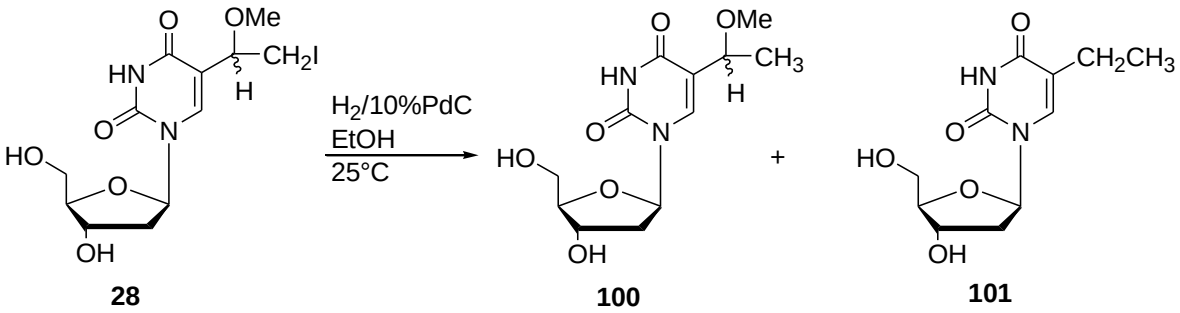
Also Bergstrom and co-workers targeted the alkyl modification at the position 5 of pyrimidine analogues.[22] They synthesized 5-(1-methoxyethyl)uridine **96** (*Scheme 15*) with the view of the transformation to 5-ethyl analogue. For this purpose they used above mentioned reaction of organomercuri nucleoside **94** and converted it to

organopalladium analogues *via* the reaction of 5-chloromercuriuridine **94** with 0.1 M palladium catalyst and ethylene in methanol. Surprisingly, the major product of the reaction was methoxy derivative **96** in 39% yield instead of expected 5-vinyluridine **24**. Other synthetic route used 2´-deoxyuridine organomercuri derivative **4** for the reaction with propylene, instead of ethylene, in the presence of Li_2PdCl_4 carried out in methanol. This reaction gave 5-(1-methoxypropyl)-2´-deoxyuridine **97** as one of products that has not been separated from the mixture.



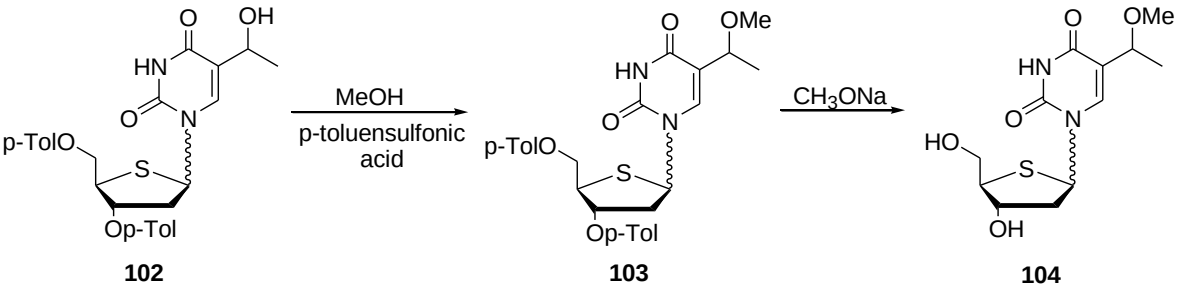
Scheme 15. Synthesis of methoxyalkyl derivatives **96** and **97**.

5-(1-Methoxyethyl)-2´-deoxyuridine **100** was prepared by Kumar and co-workers, however, they reported different synthetic route leading to desired nucleoside **100**, which differs from the above mentioned reaction of uridine analogue **96** (*Scheme 16*).[23] 5-(1-Methoxy-2-iodoethyl)-2´-deoxyuridine **28** was used and reacted with hydrogen gas in the presence of 10% Pd/C in ethanol at 25°C. This reaction gave the major product 5-(1-methoxyethyl)-2´-deoxyuridine **100** in 26% yield accompanied with 5-ethyl-2´-deoxyuridine **101** in 13% yield.



Scheme 16. Synthesis of 5-(1-methoxyethyl)-2´-deoxyuridine **100**.

Because of high activity of (*E*)-5-(2-bromovinyl)-2′-deoxy-4′-thiouridine against HSV-1, HSV-2 and varicella zoster virus, the group of 2′-deoxy-4′-thionucleosides became very investigated.[24] In this context, a series of 5-substituted 2′-deoxy-4′-thiopyrimidine nucleosides were synthesized by Rahim and co-workers in order to evaluate their antiviral activity.[25] One of these compounds is 2′-deoxy-5-(1-methoxyethyl)-4′-thiouridine **104** (*Scheme 17*). Desired methyl ether **103** was obtained by the methylation of 2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-5-(1-hydroxymethyl)-4′-thiouridine **102** with methanol in the presence of *p*-toluensulfonic acid. Subsequent treatment with sodium methoxide gave deprotected thiouridine **104**.



Scheme 17. Synthesis of 2′-deoxy-5-(1-methoxyethyl)-4′-thiouridine **104**.

Jones and co-workers focused their attention among others on alkyl ethers bearing longer chain.[26] Within the study of some chemical properties of 5-vinyluracil they successfully synthesized 5-(1-butoxyethyl)uracil **105** and 5-(1-butoxyethyl)-2′-deoxyuridine **106** (*Figure 7*). If 2′-deoxy-5-vinyluridine was reacted with butan-1-ol in the presence of trifluoroacetic acid at 55°C mixture of diastereomers of 5-(1-butoxyethyl)-2′-deoxyuridine **106** was obtained. If nearly saturated HCl in dioxane at 75°C was used, only a traces of nucleoside **106** was formed and 5-(1-butoxyethyl)uracil **105** was obtained as a major product.

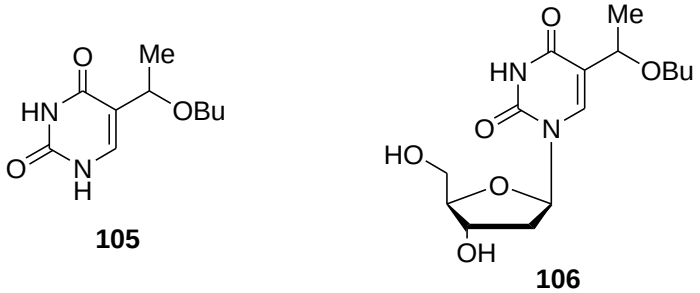
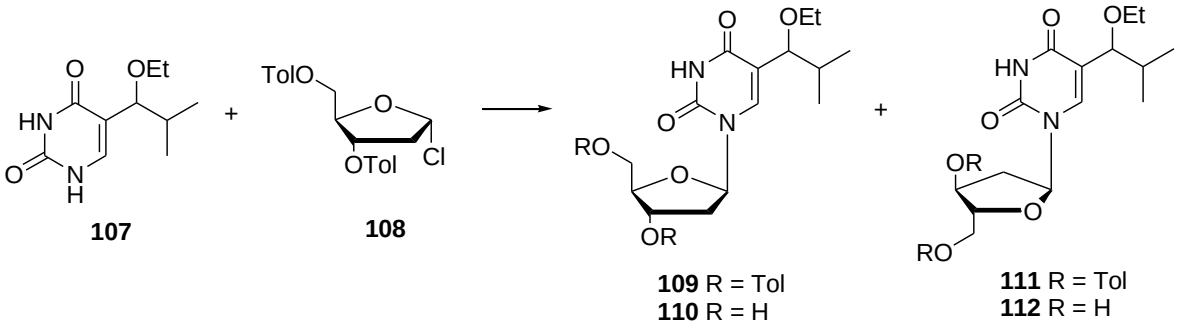


Figure 7. 5-(1-Butoxyethyl)uracil **105** and 5-(1-butoxyethyl)-2′-deoxyuridine **106**.

Another changes at the C-5 position of pyrimidine analogues led to the anomeric 5-alkyl derivatives **110** and **112**, which were synthesized in early 80´s (*Scheme 18*).[27] This preparation was based on the condensation of 5-(1-ethoxy-2-methylprop-1-yl)uracil **107** with 2´-deoxy-3,5-di-O-toluoyl-α-D-ribofuranosyl chloride **108**. Initially uracil ring was protected by the silylation with hexamethylendisilazane. Subsequently, this modified uracil was reacted with protected 2´-deoxyribose in the presence of $SnCl_4$. Finally, protected α and β anomers **111** and **109** were treated with methanolic solution of sodium methoxide and nucleosoides **112** and **110** were obtained.



107 **108**

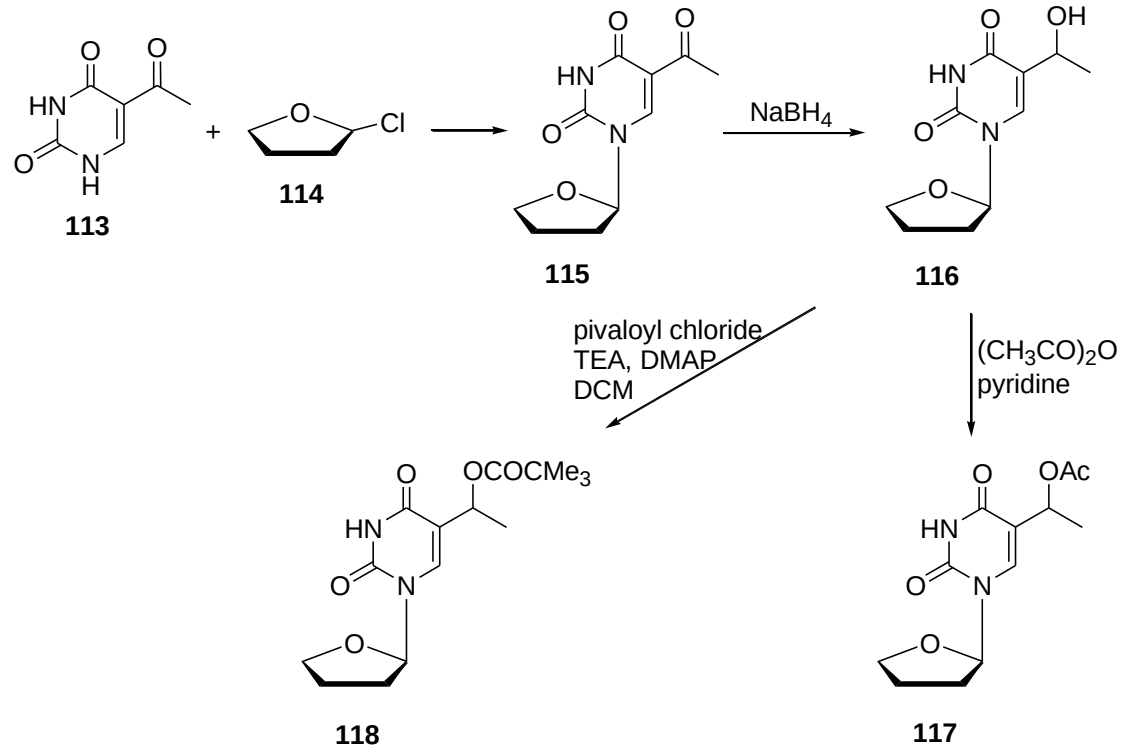
109 R = Tol
110 R = H

111 R = Tol
112 R = H

Scheme 18. Synthesis of β and α anomer of 5-(1-ethoxy-2-methylprop-1-yl)-2´-deoxyuridine.

1.2.4. Synthesis of acyloxy derivatives

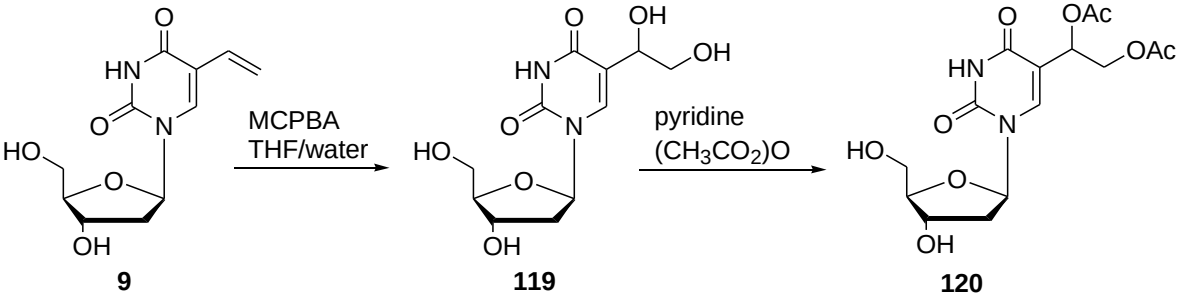
The substitution at the 5 position of pyrimidine ring by acyloxy moiety provides another group of derivatives. Some of these compounds were synthesized as 1-(tetrahydrofuran-2-yl) pyrimidine analogues.[28] The using of such an untypical furanose ring should avoid complications with a protection of hydroxyl groups of 2´-deoxyribose during the development of appropriate method for acetylation of hydroxyl group. The acyloxy derivatives **117** and **118** were synthesized in a few steps (Scheme 19). Firstly, 5-acetyluracil **113** was silylated with hexamethylendisilazane containing trimethylsilyl chloride. After that, silylated acetyluracil was coupled with 2-chlorotetrahydrofuran **114** to afford 5-acetyl-1-(tetrahydrofuran-2-yl)uracil **115**. Subsequent reduction of oxo group using sodium borohydride gave 5-(1-hydroxyethyl)-1-(tetrahydrofuran-2-yl)uracil **116**. Final acetylation of hydroxyl group of derivative **116** with acetic anhydride in pyridine afforded 5-(1-acetyloxyethyl)-1-(tetrahydrofuran-2-yl)uracil **117**. In addition, treatment of hydroxyl derivative **116** with pivaloyl chloride in the presence of triethylamine and *N,N*-dimethylaminopyridine gave pivalate ester **118**.



113 **114** **115** **116** **118** **117**

Scheme 19. Synthesis of 5-(1-acyloxyethyl)-1-(tetrahydrofuran-2-yl)uracils **117** and **118**.

An oxidation of 5-vinyl-2′-deoxyuridine **9** were also studied (*Scheme 20*).[26] Authors used *m*-chloroperbenzoic acid as an oxidizing agent and observed its influence on the reactivity of vinyl substituent in the presence or absence of water. The reaction performed in the absence of water should give expected epoxide. Nevertheless, authors observed ring opening, however, the product has not been fully characterized. As long as water was used 2′-deoxy-5-(1,2-dihydroxyethyl)uridin **119** was obtained. This dihydroxy derivative **119** was characterized after the transformation to the acetyl analogue **120** using acetanhydride in pyridine.

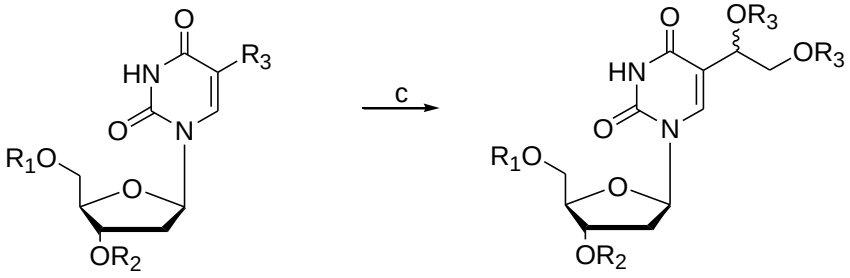


9 **119** **120**

Scheme 20. Synthesis of 5-(1,2-diacetoxyethyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine **120**.

1.2.5. Synthesis of oligonucleotide intermediates

Modified oligonucleotides are powerful tools in nucleic acid research and their synthesis has become important part of bioorganic and medicinal chemistry. One part of oligonucleotide chemistry associated with this review is focused on studies of action of 5-formyl-2´-deoxyuridine, which is one of the oxidative thymidine lesions of DNA formed by the ionizing radiation. For this purpose several methods for the preparation of appropriate intermediates for the synthesis of oligodeoxynucleotides containing 5-formyl-2´-deoxy-uridine were published. Sugiyama and co-workers reported seven-step synthesis of phosphoramidite **127** (*Scheme 21*, reaction conditions 1.) starting with easily available 5-iodo-2´-deoxyuridine **14**.[29] First two steps of synthesis involve protection of 3´,5´-dihydroxyl groups with TBDMS group followed by Pd-catalyzed coupling reaction with vinyl acetate. This procedure led to protected 5-vinyluridine **122** in 68% yield. The oxidation based on using OsO_4 with consequent acetylation with acetanhydride in pyridine gave nucleoside **124**. Target phosphoramidite **127** was obtained after standard phosphoramidite synthesis started with protected 5´-OH group with dimethoxytrityl chloride and final phosphitylation.



a **14** R_1, R_2 =H, R_3 = I
b **121** R_1, R_2 =TBDMS, R_3 = I
122 R_1, R_2 =TBDMS, R_3 = vinyl

d **123** $R_1 = R_2$ = TBDMS, R_3 = H
e **124** $R_1 = R_2$ = TBDMS, $R_3 = COCH_3$
f **125** $R_1 = R_2$ = H, $R_3 = COCH_3$
g **126** R_1 = DMTr, R_2 = H, $R_3 = COCH_3$
127 R_1 = DMTr, $R_2 = P(N\text{-}iPr)OCH_2CH_2CN$, $R_3 = COCH_3$

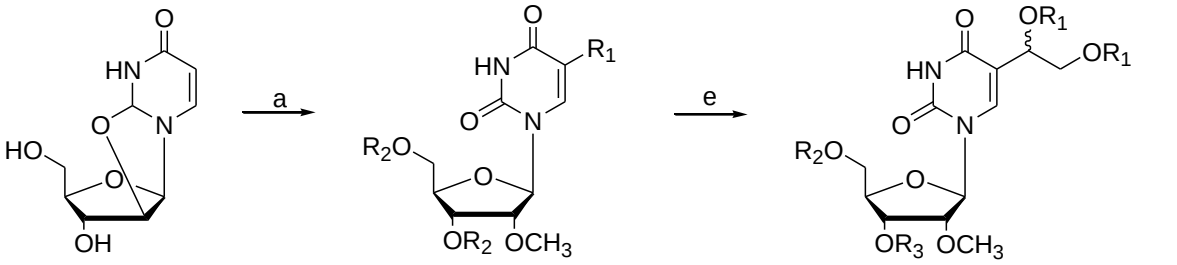
1.[29] (a) TBDMSCl, imidazole, pyridine, 33h, 99%; (b) vinyl acetate, $Pd(OAc)_2$, PPh_3, Et_3N, DMF, 70%, 16 h, 68%; (c) OsO_4, 4-methylmorpholine-*N*-oxide, acetone-H_2O-t-BuOH (4:1:1), 15 h, 44%; (d) Ac_2O, pyridine, 44 h, 96%; (e) TBAF, THF, 14 h, 75%; (f) DMTrCl, DMAP, Et_3N, pyridine, 22 h, 78%; (g) $P(N\text{-}iPr_2)_2OCH_2CH_2CN$, tetrazole, 2.5 h, quant.

2.[30] (a) TBDMSCl, imidazole, DMF, over night; (b) 5 mol% $Pd(MeCN)_2Cl_2$, $Bu_3SnCH{=}CH_2$ (1.5 eq), MeCN, 80°C; (c) *cat.* OsO_4, NMO (2.5 eq), acetone-H_2O-*t*-BuOH (4:1:1); (d) Ac_2O (4 eq), py; (e) TBAF (3eq), AcOH (2 eq), THF; (f) DMTCl (1.5 eq), py; (g) $[(i\text{-}Pr)_2N]_2POCH_2CH_2CN$ (1.8 eq), DCI (0.7 eq), MeCN-CH_2Cl_2 (1:10)

Scheme 21. Synthesis of phosphoramidite **127**.

Latter on Kittaka and co-workers reported synthesis of phosphoramidite **127** under different conditions.[30] First of all, protected 5-iodo-2′-deoxyuridine **121** was subjected to Stille coupling reaction with tributyl(vinyl)tin using $Pd(MeCN)_2Cl_2$ as a catalyst (*Scheme 21*, reaction conditions 2.). This coupling reaction was followed by the oxidation of vinyl group of nucleoside **122** by OsO_4 and acetylation of vicinal diol **123**. After deprotection of 3′,5′-bis-*O*-TBDMS groups, 5′-hydroxyl group was dimethoxytritylated and 3′-hydroxyl group phosphitylated to afford phosphoramidite **127**. The final phosphoramidite **127** was incorporated into oligodeoxynucleotide sequences *via* solid-phase synthesis by using automated DNA synthesizer.

Modified oligonucleotides can also serve as a tool for the investigation of interactions between NF-κB proteins (NF-κB is a protein complex that controls the transcription of DNA, plays a key role in regulating the immune response to infection). This study was reported by Kittaka and co-workers[31] and described an interaction between mentioned proteins and modified oligonucleotides, in which thymidine is replaced by a 5-formyl derivative. A phosphoramidite **138** for oligonucleotide synthesis was prepared from O^2-2′-cyclouridine **128** by multistep synthesis (*Scheme 22*).

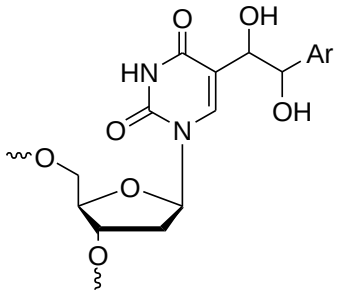


(a) $B(OCH_3)_3$, $CH(OCH_3)_3$, Na_2CO_3, MeOH, 150°C[32]; (b) I_2, (0.6 eq), CAN (0.5 eq), AcOH, 80°C; (c) TBDMSCl (3.5 eq), imidazole (5 eq), DMF; (d) 5 mol% $Pd(CH_3CN)_2Cl_2$, $Bu_3SnCH{=}CH_2$, CH_3CN, 80°C; (e) OsO_4, NMO (2.5 eq), acetone-H_2O-*t*BuOH (4:1:1); (f) Ac_2O (4 eq), py; (g) TBAF (2 eq), AcOH (2 eq), THF; (hi) DMTCl (1.5 eq), py; (i) $[(iPr)_2N]_2POCH_2CH_2CN$ (1.8 eq), DCI (0.7 eq), CH_3CN-CH_2Cl_2 (1:10)

Scheme 22. Synthesis of phosphoramidite **138**.

Firstly, O^2-2′-cyclouridine **128** was selectively methylated at 2′-*O* atom and subsequently iodinated at the 5 position with CAN-I_2 in AcOH to give nucleoside **130** in 74% yield. After that, the quantitative protection of 3′,5′-diol **130** by TBDMS groups afforded nucleoside **131**. Subsequently, this derivative **131** was subjected to Stille coupling reaction with tributyl(vinyl)tin using $Pd(CH_3CN)_2Cl_2$ as a catalyst followed by oxidative reaction using *cat.* OsO_4/NMO to afford dihydroxy derivative **134** in 77% yield in two steps. The desired phosphoramidite **138** was consequently obtained after acetylation of the vicinal diol **134**, subsequent selective deprotection of 3′,5′-dihydroxyl groups (**136** in 96% yield) and finally dimethoxytritylation of the 5′-hydroxyl group (**137** in 89% yield) followed by 3′-*O*-phosphitylation in 90% yield.

Aryl moiety containing phosphoramidite was synthesized by Ding and co-workers.[33] Authors of this article published synthesis and utilization of oligonucleotide **139** (*Figure 8*) as a hole migration probe. This compound should serve as a molecular probes that facilitate selective detection of excess electron transfer or hole migration in DNA using gel electrophoresis.

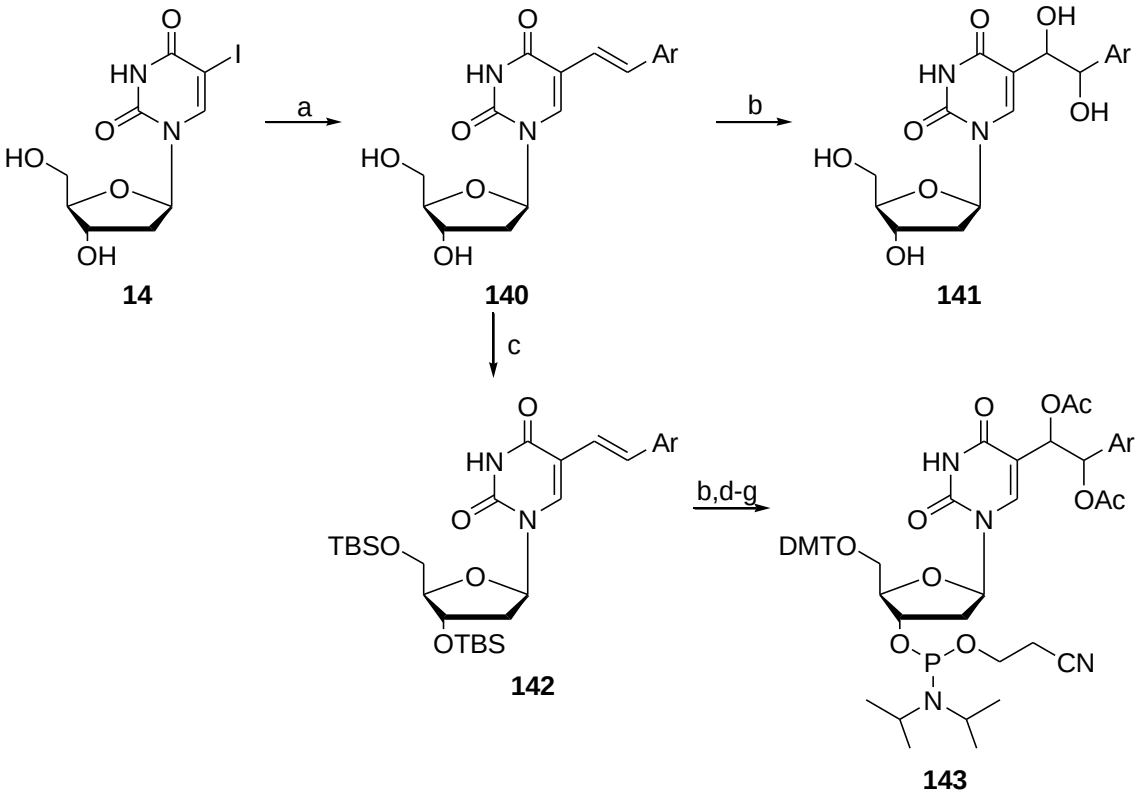


139

Figure 8. Oligonucleotide **139**.

Synthesis of desired phosphoramidite **143** (*Scheme 23*) started with Pd-catalyzed cross-coupling of 5-iodo-2′-deoxyuridine **14** and corresponding styrene to afford nucleoside **140**. The oxidation of alken **140** with OsO_4 led to the mixture of diastereomers of vicinal diols **141**. For introduction to the oligodeoxynucleotide **139**, the dihydroxynucleoside **141** was converted to the corresponding phosphoramidite. Firstly, the hydroxyl groups of deoxyribofuranosyl moiety were silylated and gave protected nucleoside **142**. Subsequently, alken **142** was reacted with OsO_4 to afford protected vicinal diol. Free hydroxyl group attached to the side chain at the 5 position of uracil ring were acetylated and protected groups at sugar ring were removed by the reaction with TBAF. Finally, 5′-hydroxyl group were tritylated and 3′-hydroxy group converted

to the corresponding phosphoramidite **143**. The phosphoramidite prepared by the above mentioned reaction was incorporated into 12-mer oligodeoxynucleotide *via* automated solid-phase synthesis.



(a) 3,4,5-trimethoxystyrene, Pd(0); (b) OsO_4; (c) TBSCl; (d) Ac_2O; (e) TBAF; (f) DMTCl; (g) phosphytilation

Scheme 23. Synthesis of phosphoramidite **143**.

1.2.6. Synthesis of bisheterocyclic derivatives

Sarfati and co-workers published interesting and facile synthesis of C-5 alkylated 2´-deoxyuridine and uridine derivatives.[34] The C-5 position can be substituted by glycosides of either 2-acetamido-2-deoxy-*β*-*D*-glucopyranose or *α*-*D*-mannopyranose. All the products **144-147** (*Figure 9*) were formed as by-products of palladium catalyzed addition reaction of alkenes to C-5-mercuriated deoxyuridines.

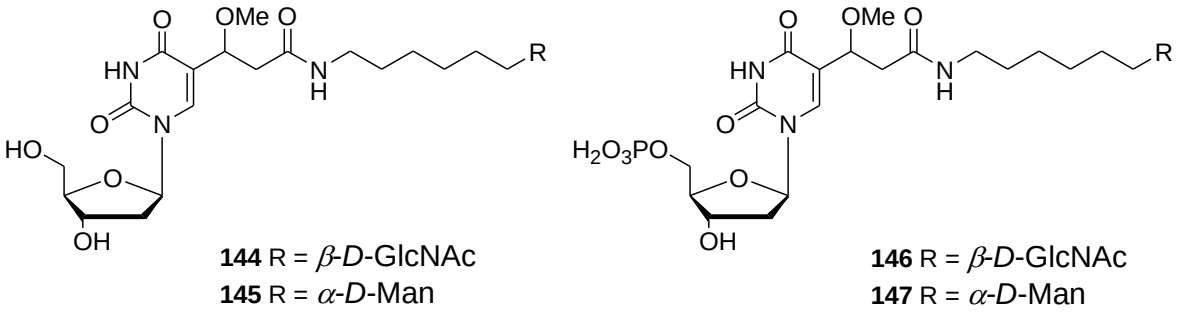
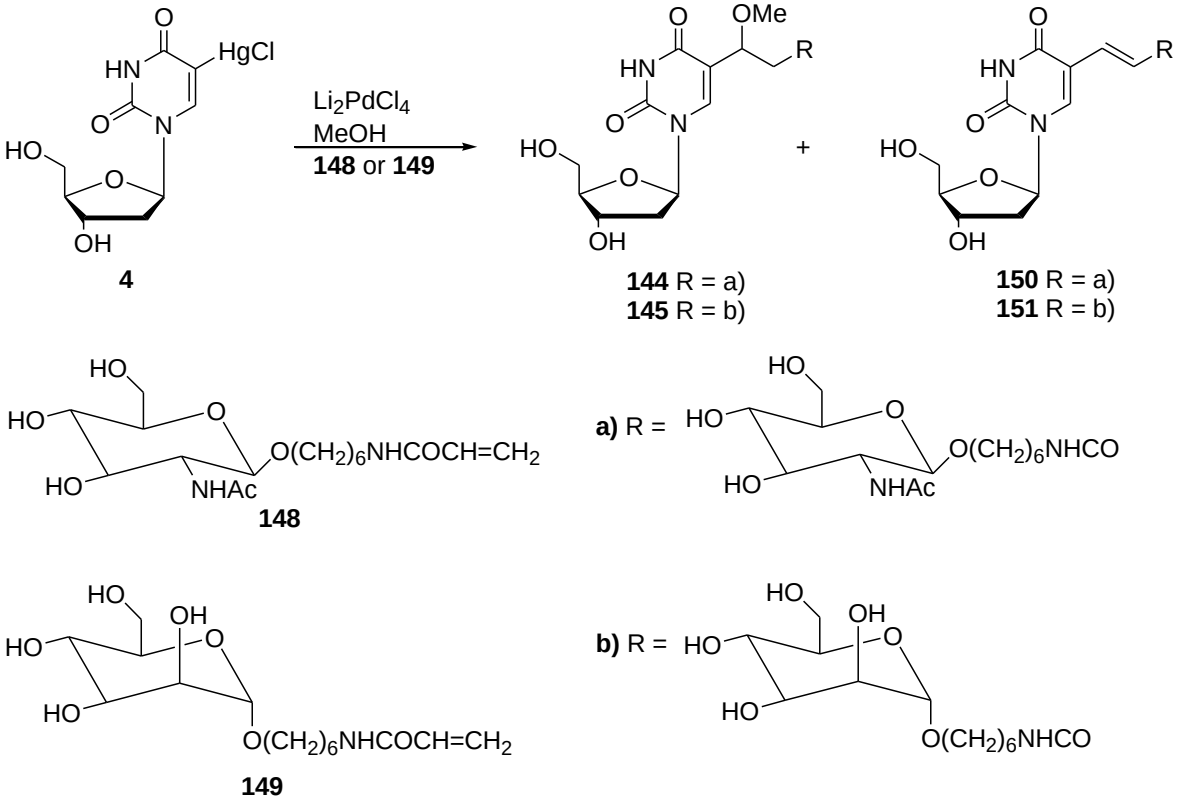


Figure 9. 2′-Deoxyuridine derivatives **144-147**.

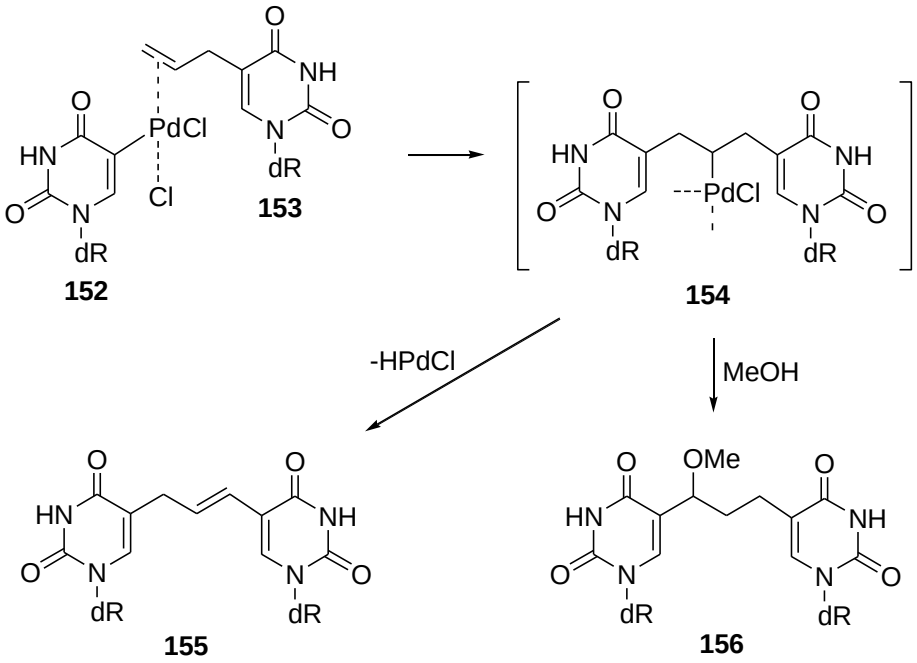
The synthesis of derivatives **144** and **145** started with condensation reactions of alkenes **148** or **149** with 5-chloromercuri-2′-deoxyuridine **4** in the presence of palladium catalyst (*Scheme 24*).



Scheme 24. Synthesis of 2′-deoxyuridine derivatives **144-145**.

The vinyl derivatives **150** and **151** were obtained as major products, however methoxy derivatives **144** and **145** were also detected in the modest yields. The monophosphate derivatives **146** and **147** were formed by the similar reaction of mercuriated 2′-deoxyuridine monophosphate used instead of 5-chloromercuri-2′-deoxyuridine **4**.

Almost 10 years earlier, Bergstrom and co-workers published the synthesis based on the same reaction – the Heck cross-coupling reaction of alkene with organometalic derivative.[35] Nevertheless, they coupled two pyrimidine nucleosides (*Scheme 25*). Firstly, 5-(chloromercuri)-2´-deoxyuridine was converted to its reactive palladium intermediate **152** by the reaction with 20 mol. % of Li_2PdCl_4 in methanol. Consequently, allyl chloride **153** reacted with this intermediate **152** and gave (*E*)-5-[3-(2´-deoxyuridin-5-yl)-1-propen-1-yl]-2´-deoxyuridine **155** as a major product and the by-product 5-[3-(2´-deoxyuridin-5-yl)-1-methoxyprop-1-yl]-2´-deoxyuridine **156**.

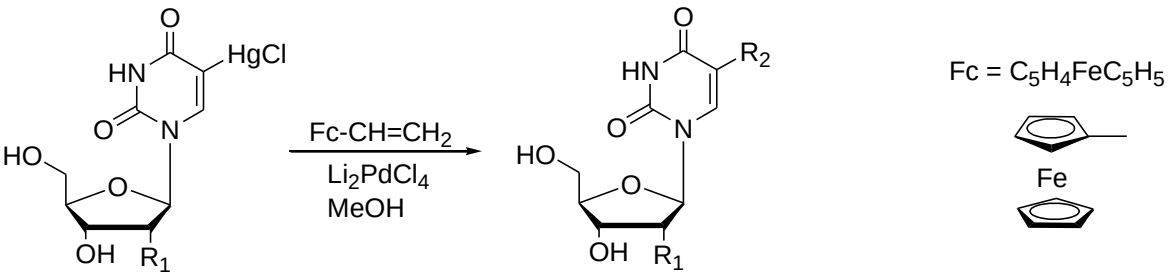


Scheme 25. Synthesis of 5-[3-(2´-deoxyuridin-5-yl)-1-methoxyprop-1-yl]-2´-deoxyuridine **156**.

1.2.7. Synthesis of metallocenonucleosides

The first "metallocenonucleosides" were synthesized and characterized by Meunier and co-workers in 1991.[36] The term "metallocenonucleosides" was derived from nucleosides containing a metallocenic moiety and these compounds were prepared in order to study their chemical as well as cytotoxic properties (*Scheme 26*). The reported work was focused on the group of nucleosides of the formula: a) Ns-CH=CH-Fc and b) Ns-CH_2-CH_2-Fc, where Ns (= nucleoside) is either uridine (derivatives **161**, **162**) or 2´-deoxyuridine (derivatives **158**, **159**) and Fc is the abbreviation of ferrocene of molecular formula $C_5H_4FeC_5H_5$. Throughout the reaction of 5-(chloromercuri)-nucleosides **4** or **94**

with ethynylferrocene, also methoxyderivatives **157** or **160** were formed among the nucleosides **158**, **159**, **161** and **162**.



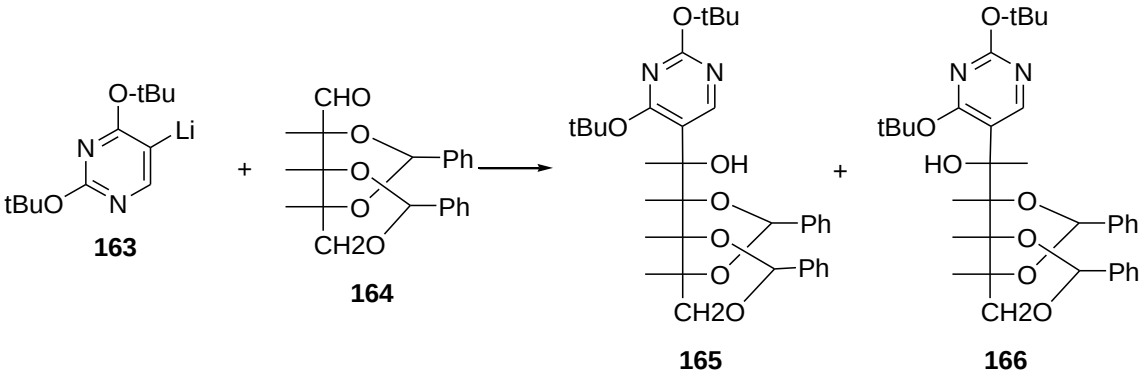
4 R_1 = H
94 R_1 = OH

157 R_1 = H, R_2 = CH(OMe)CH_2Fc
158 R_1 = H, R_2 = CH=CH-Fc
159 R_1 = H, R_2 = CH_2CH_2Fc
160 R_1 = OH, R_2 = CH(OMe)CH_2Fc
161 R_1 = OH, R_2 = CH=CH-Fc
162 R_1 = OH, R_2 = CH_2CH_2Fc

Scheme 26. Synthesis of "metallocenonucleosides" **157** and **160**.

1.2.8. Synthesis of pseudouridines

Pseudouridine is a C-glycosid isomer of uridine and plays an important role in proteosynthesis. In organism pseudouridine is biosynthesized from uridine *via* the action of pseudouridine synthases. Nevertheless, the specific roles of pseudouridines are still subject of many researches. In order to study pseudouridine analogues many of them were synthesized since 1961.[37,38,39,40] All of these works achieved the synthesis of pseudouridines, however the yields were not quite satisfactory. As late as 1971 Lerch and co-workers arranged reaction conditions and published advanced studies on the synthesis of pseudouridine (*Scheme 27*).[41] They utilized the 2,4-di-*tert*-butoxypyrimidin-5-yllithium **163** and reacted it with 2,4:3,5-di-*O*-benzylidene-*aldehydo*-D-ribose **164**. The reaction carried out in tetrahydrofuran afforded the mixture of allo and altro isomers of 5-(2,4:3,5-di-*O*-benzylidene-D-pentahydroxypentyl)-2,4-di-*tert*-butoxy-pyrimidine **165** and **166**, respectively. A complete separation using preparative TLC afforded allo-isomer **165** in 25% yield and altro isomer **166** in 37% yield.



Scheme 27. Synthesis of 5-(2,4:3,5-di-*O*-benzylidene-D-pentahydroxypentyl)-2,4-di-*tert*-butoxy-pyrimidine **165** and **166**.

Subsequent cyclization of both isomers in hydrochloric acid gave α- and β-furanose forms of pseudouridine **167** and **168**, respectively (*Figure 10*).

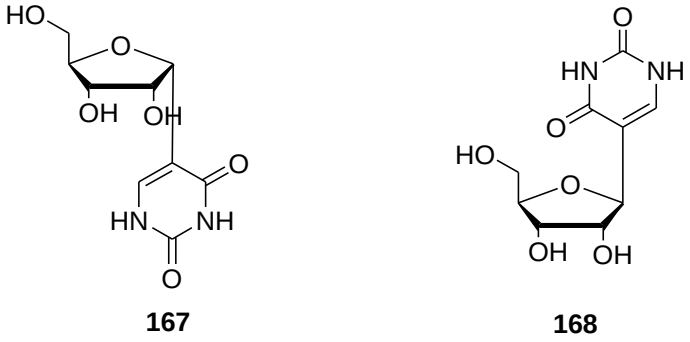


Figure 10. α- and β-pseudouridine **167** and **168**.

Other studies on synthesis of pseudouridine analogues were made by Lee and co-workers 20 years later.[42] The 5′-modified pseudouridine **169** and secopseudouridines **170** and **171** were prepared *via* the ring cleavage of sugar moiety (*Figure 11*).

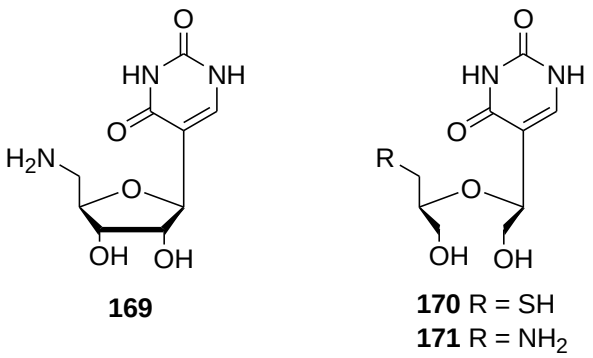


Figure 11. 5′-Modified pseudouridine **169** and secopseudouridines **170, 171**.

1.3. Biological activity

A number of the above mentioned compounds were synthesized in order to evaluate their biological activity. Most of them were investigated with a view of development of new potent and selective either antiviral or cytotoxic agents. Moreover, antibacterial activity of some of these derivatives has been also studied. Some of tested compounds have shown indispensable results and their brief survey is introduced below.

1.3.1. Antiviral activity

Shortly after discovery of antiviral activity of 5-ethyl-2′-deoxyuridine[43] another C-5 modified analogues were synthesized and studied as potent antiviral agents. Some of hereby prepared compounds were 5-(1-methoxy-2-bromoethyl)-2′-deoxyuridine **12**, 5-(1-methoxy-2-chloroethyl)-2′-deoxyuridine **13**[4,44] and 5-(1-methoxy-2-iodoethyl)-2′-deoxyuridine **28**[5] (*Figure 12*). These methoxyhaloethyl uridines were tested against *herpes simplex virus type 1* (HSV-1) and their activity was compared with antiviral activity of acyclovir and BVDU. The bromo derivative **12** exhibited greater activity than corresponding chloro analogue **13**. Nevertheless, antiviral activity was weaker in comparison with acyclovir or BVDU. The most active iodo derivative **28** exhibited an antiviral activity approaching that of IVDU and acyclovir.

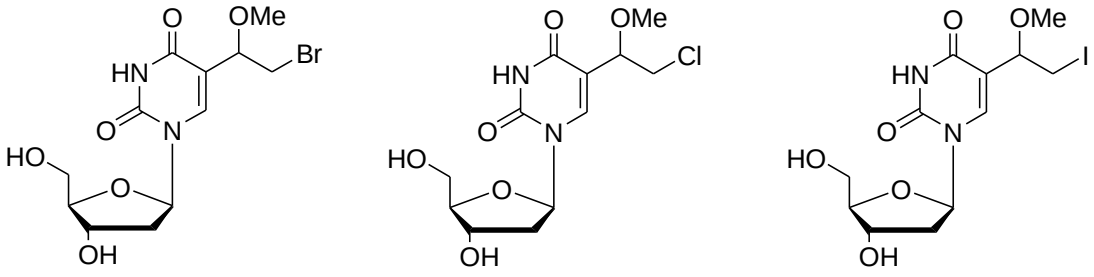


Figure 12. Methoxy derivatives **12**, **13** and **28**.

The introduction of another halo atom led to the preparation of 5-(1-methoxy-2,2-dihaloethyl)-2′-deoxyuridines **47-49** (*Figure 13*).[8] All of these compounds were subjected to *in vitro* antiviral testing against HSV-1, HSV-2, VZV (*Varicella zoster virus*), HCMV (*human cytomegalovirus*) and EBV (*Ebstein-Barr virus*) and compared with activity of 5-(1-hydroxydihaloethyl) analogues. In general, hydroxyl derivatives

were more active than methoxy derivatives **47**-**49** against HSV-1, HSV-2, VZV and EBV. All of investigated derivatives **47**-**49** were inactive against HCMV.

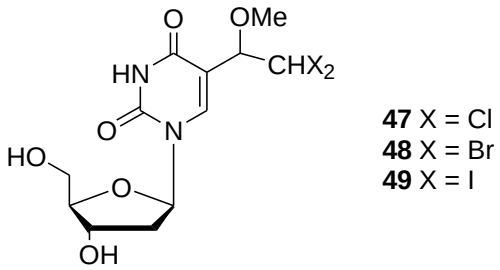
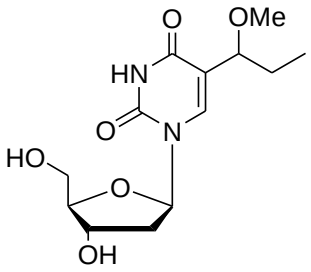


Figure 13. 5-(1-Methoxy-2,2-dihaloethyl)-2′-deoxyuridines **47**-**49**.

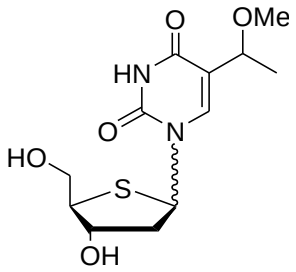
Another C-5 substituted 2′-deoxyuridine analogue **97** (*Figure 14*) was investigated as a potent antiviral agent against HSV-1, HSV-2 and HCMV.[23] This 5-(1-methoxyethyl)-2′-deoxyuridine **97** was equiactive to 5-ethyl-2′-deoxyuridine (EDU) against both HSV-1 and HSV-2 and less active against HCMV than EDU and ganciclovir.



97

Figure 14. 5-(1-Methoxyethyl)-2′-deoxyuridine **97**.

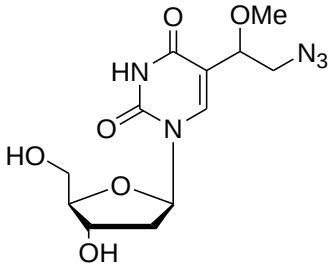
The discovery of (*E*)-5-(2-bromovinyl)-2′-deoxy-4′-thiouridine (4′-S-BVDU) as a highly active agent against HSV-1, HSV-2 and VZV[24] inspired chemists to the synthesis of the group of 2′-deoxy-4′-thionucleosides.[25] In this context anomeric 2′-deoxy-5-(1-methoxyethyl)-4′-thiouridine **104** (*Figure 15*) was prepared and its antiviral activity was evaluated. However, this thio derivative **104** did not show any significant activity.



104

Figure 15. 2′-Deoxy-5-(1-methoxyethyl)-4′-thiouridine **104**.

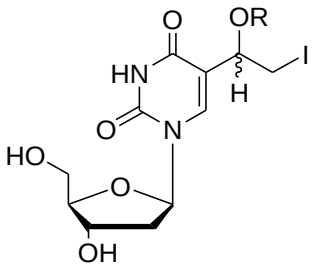
Among mentioned derivatives azido nucleoside **93** (*Figure 16*) was prepared as well in order to determine antiviral activity against HSV-1, HSV-2, VZV and HCMV.[19] Neither this compound exhibited significant antiviral properties.



93

Figure 16. 5-(1-Methoxyazidoethyl)-2′-deoxyuridine **93**.

Recent research dealing with new antiviral agents has been focused on study of antiviral activity of 5-[1-(2-halo(or nitro)ethoxy-2-iodoethyl)]-2′-deoxyuridines **50**-**54** (*Figure 17*).[11] These nucleosides were evaluated *in vitro* for the inhibitory activity against thymidine-kinase (TK) positive and negative strains of herpes simplex virus type-1. All of these 2′-deoxyuridine analogues exhibited weak anti-HSV-1 activity.



50 R = CH_2CH_2Br
51 R = CH_2CH_2Cl
52 R = $CH_2CH_2NO_2$
53 R = CH_2CBr_3
54 R = CH_2CCl_3

Figure 17. 5-[1-(2-halo(or nitro)ethoxy-2-iodoethyl)]-2′-deoxyuridines **50**-**54**.

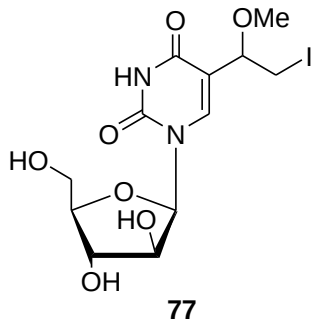
1.3.2. Cytotoxic activity

Only few derivatives were tested for their anticancer properties. The cytotoxic activity for derivatives **12**, **13** and **28** (*Figure 12*) were determined by an *in vitro* L1210 assay.[4,5] However, the comparison of results of investigated compounds with reference compound melphalan showed lower activity.

1.3.3. Antibacterial activity

The recurrence of chronic infectious disease tuberculosis has initiated research on new classes of antimycobacterial agents. The exigency of new drugs were also caused by multidrug-resistant tuberculosis strains, which are resistant to the most widely used agent either Isoniazid or Rifampicin and the needfulness of new highly active compounds is increasing. Tuberculosis is caused by species of the genus *Mycobacterium,* for instance, *Mycobacterium tuberculosis*, *Mycobacterium avium* and *Mycobacterium bovis.*

Recently it was published the study on the effect of arabinofuranosyl analogues against *Mycobacterium.*[14] A series of 1-*β*-D-2′-arabinofuranosyl pyrimidine nucleosides was prepared in order to evaluate their antimycobacterial activity. The methoxyiodoethyl pyrimidine nucleoside **77** (*Figure 18*) was also synthesized among others. Nevertheless, this nucleoside did not prove any significant antimycobacterial activity.



77

Figure 18. Methoxyiodoethyl pyrimidine nucleoside **77**.

In addition to this, nucleosides containing dodecynyl moiety instead of alkoxyhaloethyl proved significant activity. The introduction of longer alkynyl chains might be a successful way to obtain potential active antimycobacterial drugs.

1.4. Conclusion

This review attempted to summarize all available information on synthesis and biological activity of selected C-5 substituted pyrimidine derivatives. A lot of authors reported facile and successful synthesis, used large range of methods leading to desired compounds and also highlighted the ineffectual synthetic routes.

Most of published derivatives were biologically inactive; some of them exhibited weak activity. However, all of these results significantly and invaluably contributed to the development of new potent antiviral, cytotoxic or antibacterial agents and elucidate possible structure-activity relationship.

2. Aims of the work

Modifications at the C-5 position of pyrimidine analogues form an important part of nucleic acid research. The number of these derivatives is highly increasing especially in order to study their biological activity. Also the main goal of this thesis is the study of synthesis and biological activity evaluation of new compounds based on C-5 modifications of uracil analogues.

The reported thesis is divided into three main parts. The first one is focused on studies of synthesis, reactivity and biological activity of 5-[(4-nitrophenyl)methyl)]uracil analogues. The main goals of this part were:

- The synthesis of diverse range of aliphatic 5-[alkoxy(4-nitrophenyl)methyl)]uracils.
- The transformation of selected 5-[alkoxy(4-nitrophenyl)methyl)]uracils to their corresponding nucleoside analogues.
- To develop an efficient method for separation of diastereomers.
- To evaluate the cytotoxic and antibacterial activity of prepared derivatives.

The second part of the thesis is focused on studies of synthesis of various 5-alkoxymethyluracil analogues and evaluation of their biological activity. The main aims of this project can be summarized by the following terms:

- The studies of synthesis of various aliphatic 5-alkoxymethyluracils and the comparison with known results.
- The development of the efficient ribosylation of 5-alkoxymethyluracils and preparation of corresponding nucleosides.
- The evaluation of cytotoxic and antibacterial activity of the prepared compounds, the comparison with 5-[(4-nitrophenyl)methyl)]uracil analogues and the SAR study of prepared compounds.

The last part of the presented work is focused on the synthesis of pyrimidine oligodeoxynucleotides containing either substituted phenyltriazole or substituted phenylethynyl moiety attached to the C-5 position and the hybridization studies of the prepared oligodeoxynucleotides.

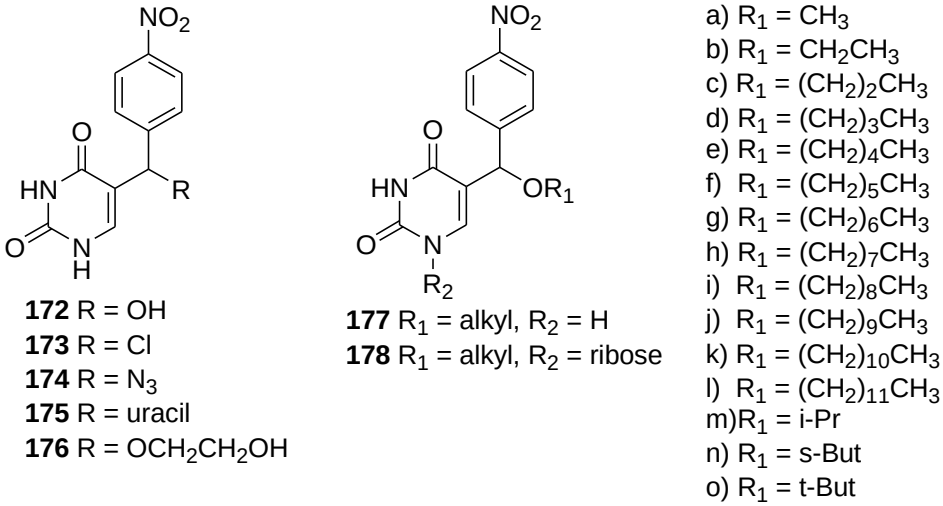
The aims of the project were:

- The preparation of uracil nucleoside with phenol-substituted triazole attached to the 5-position by Cu(I)-assisted azide-alkyne cycloadditions.
- The preparation of 4-bromophenylethynyluracil analogue.
- The synthesis of corresponding phosphoramidites to afford building blocks for oligodeoxynucleotides synthesis.
- The incorporation of phosphoramidites to oligodeoxynucleotide sequences using standard automated solid phase DNA synthesis.
- The hybridization studies of the prepared oligodeoxynucleotides.

3. Results and discussion

3.1. Studies on synthesis and biological activity of various 5-[(4-nitrophenyl)methyl)]uracil analogues

The first part of the reported thesis is focused on synthesis of 5-[(4-nitrophenyl)methyl)]uracil analogues (*Figure 19*) and study of their biological activity.



172 R = OH
173 R = Cl
174 R = N_3
175 R = uracil
176 R = OCH_2CH_2OH

177 R_1 = alkyl, R_2 = H
178 R_1 = alkyl, R_2 = ribose

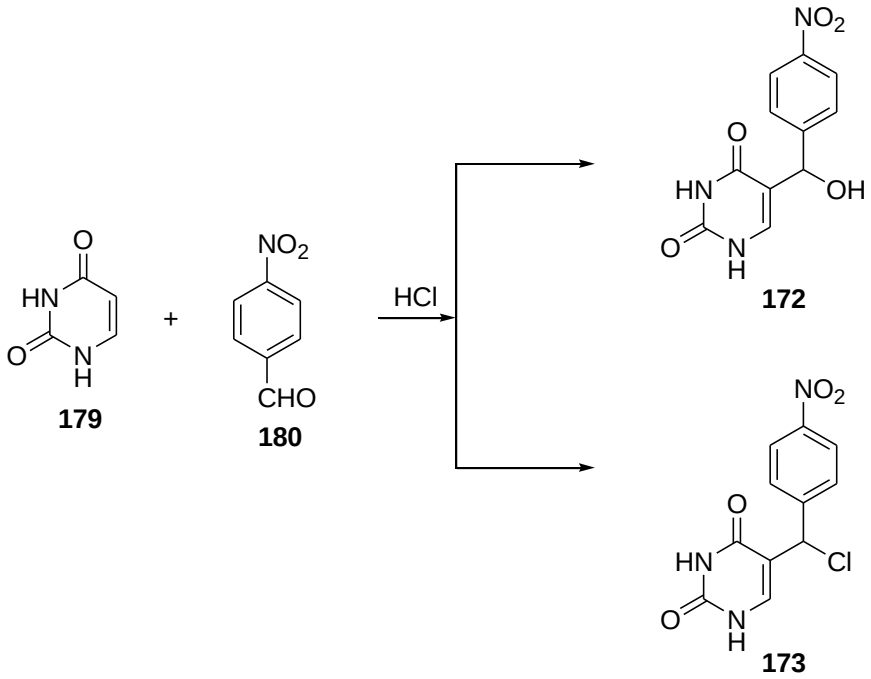
Figure 19. Summary of prepared 5-[(4-nitrophenyl)methyl)]uracil analogues.

The 4-nitrophenyl group was chosen due to several factors. Derivatives with this bulky substituent were primarily prepared in order to evaluate an effect of structure on biological activity, especially on cytotoxicity. Moreover, high potential of nitrophenyl group is related to reactions of nitro group leading to synthesis of interesting heterocyclic rings, indole or quinolone for instance, which might influence biological properties of prepared uracil analogues. Furthermore, introduction of heterocyclic system with fluorescence properties (such as quinolones) into specific oligonucleotide *via* substitution of uracil nucleobase might lead to research of new fluorescent probes usable in various biological studies. Nitro group also offers a diverse range of reactions, which might lead to the replacement of nitro functional group with large number of others (e.g. amino, subsequently with cyano, halo, etc.). Finally, none of available literature has introduced 4-nitrophenyl moiety attached to the C-5 position of uracil ring. Only a few studies describe synthesis of N-1 and N-3 modified 4-nitrophenyluracil

analogues.[45,46,47,48,49,50] Therefore, the main intention of this part of the thesis was to investigate the influence of bulky 4-nitrophenyl group attached to the 5 position of uracil ring on biological activity.

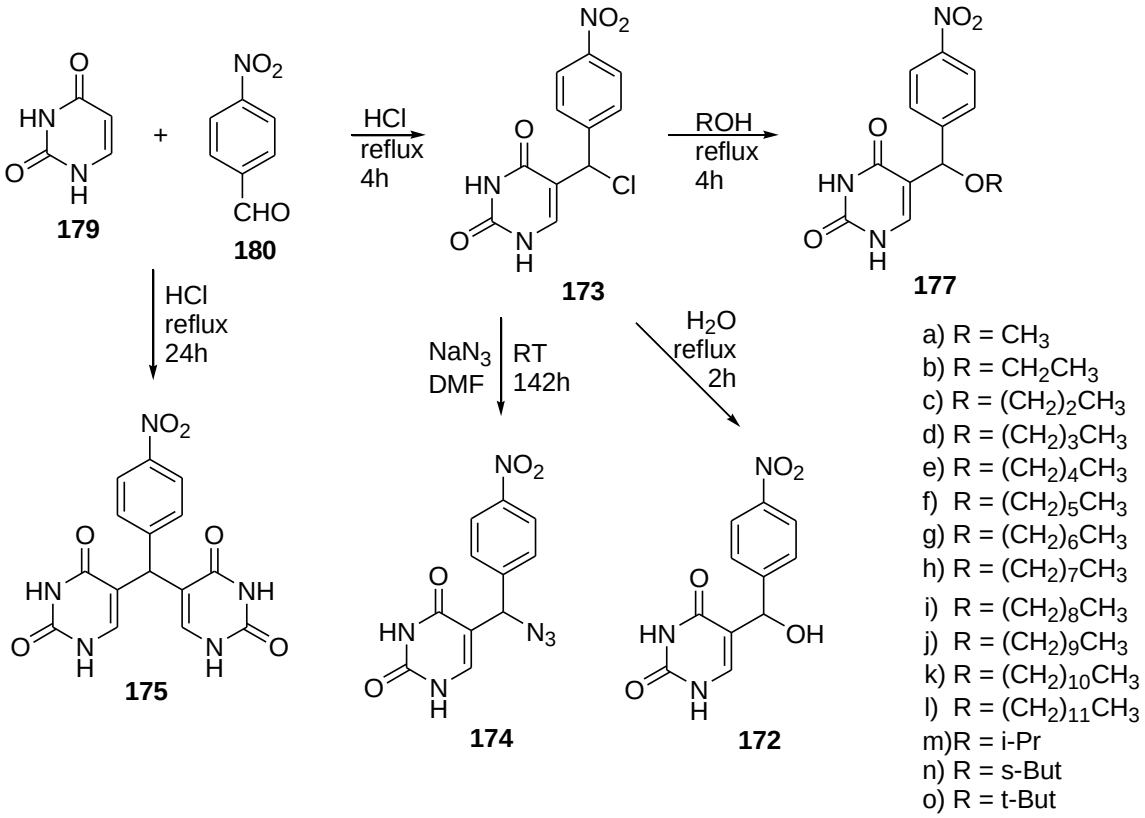
3.1.1. Synthesis and reactivity of 5-[(chloro(4-nitrophenyl)methyl)]uracil

As a result of inspiration by an acid catalyzed hydroxyalkylation of uracil published by Lam and co-worker,[51] the reaction of uracil **179** and equivalent amount of *p*-nitrobenzaldehyde **180** was carried out (*Scheme 28*). However, my attempt to reproduce the described synthesis led to a different conclusion. According to the observation of authors[51] the reaction of uracil **179** with aromatic aldehyde containing electron-deficient ring (*p*-nitrobenzaldehyde **180** in this case) in concentrated hydrochloric acid affords the arylhydroxymethyluracil **172**. In my hands, the reaction did not lead to hydroxyderivative **172** but afforded 5-[chloro(4-nitrophenyl)methyl)pyrimidine-2,4(1*H*,3*H*)-dione **173** in 68% yield.



Scheme 28. Reaction of uracil **179** and *p*-nitrobezaldehyde **180**.

The structure of chloroderivative **173** was confirmed by the series of substitution reactions (*Scheme 29*). These reactions led to the preparation of chiral compounds that were isolated and tested for their biological properties in their racemic form.



Scheme 29. Synthesis of uracil analogues **172 - 177**.

Firstly, the chloro derivative **173** was reacted with diverse range of aliphatic alcohols to afford 5-[alkoxy(4-nitrophenyl)methyl]uracils **177a-o**.[a] These relatively simple reaction conditions led to the series of modified nucleobases with different length of the alkyl side chain **177a-l** including some branched chains **177m-o** in 36-98% yields. The lower yields of some alkoxyderivatives **177h-l** were caused by the complicated isolation of products from higher boiling alcohols.

While the reaction of the equivalent amount of uracil **179** and *p*-nitrobenzaldehyde **180** in the concentrated hydrochloric acid led to the substituted uracil **173**, the reaction of uracil **179** with 0.5 equivalents of aldehyde **180** gave polynuclear analogue **175** in 85% yield. This 5,5´-[(4-nitrophenyl)methylene]bis[pyrimidine-2,4(1*H*,3*H*)-dione] **175** was already prepared by the nitration of 5,5´-phenylmethylene-bis-uracil and patented in the class of anti-ictogenic or anti-epileptogenic agents.[52] Since the preparation of

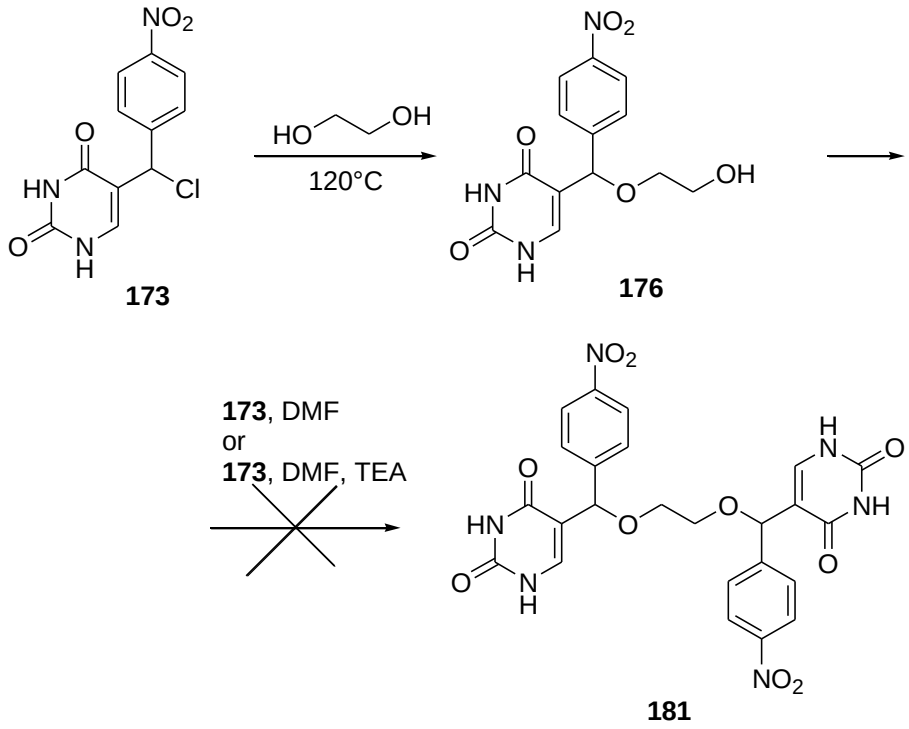
[a] The synthesis of chloroderivative **173** and alkoxyderivatives **177a-h** was already mentioned in supporting material of application for RNDr. degree.

bisderivative **175** is different from published synthesis,[52] this procedure is also presented in the experimental part.

The chloro group of the compound **173** was also substituted with the hydroxyl group by the heating of the derivative **173** in water at 100°C for 2 hours to give 5-[hydroxy(4-nitrophenyl)methyl]uracil **172** in quantitative 98% yield.

Moreover, the reaction of nucleobase **173** with sodium azide performed in DMF afforded 5-[azido(4-nitrophenyl)methyl]uracil **174** in 80% yield. The azido group of derivative **174** might be further used for synthesis of triazole heterocyclic compounds *via* azide-alkyne click reaction. The triazole products readily associate with biological targets, through hydrogen bonding and dipole interactions.

Finally, reaction of chloroderivative **173** with ethylene glycol at 120°C gave 5-[(2-hydroxyethoxy)(4-nitrophenyl)methyl]uracil **176** in 84% yield (*Scheme 30*).



Scheme 30. Synthesis of compound **176**.

In contrast to previous procedure described for derivatives **177a-o**, the precipitation of hydroxyethylether **176** from the reaction mixture required cooling to -20°C. Furthermore, hydroxyl derivative **176** was reacted with chloro derivative **173** with aim to synthesize the bis-uracil derivative **181**. Reaction was performed in DMF at various temperatures (RT → 130°C) in the presence of TEA as well as without TEA. Neither

temperature nor presence of TEA influenced formation of desired bisuracil derivative **181**. Further studies have not been carried out, because the synthesis of polynuclear uracil derivatives was not the main subject of interest of the presented thesis.

3.1.2. Biological activity of 5-[(alkoxy(4-nitrophenyl)methyl)]uracil analogues

The prepared compounds **173-175** and **177** were tested for their cytotoxic activity *in vitro* against cancer cell lines CEM, K562, their drug resistant counterparts CEM-DNR-bulk and K562-tax, and A549, HCT116p53 and HCT116p53-/- cell lines. Cytotoxic activity screening was performed by Laboratory of experimental medicine. All results are summarized in *Table 1*.

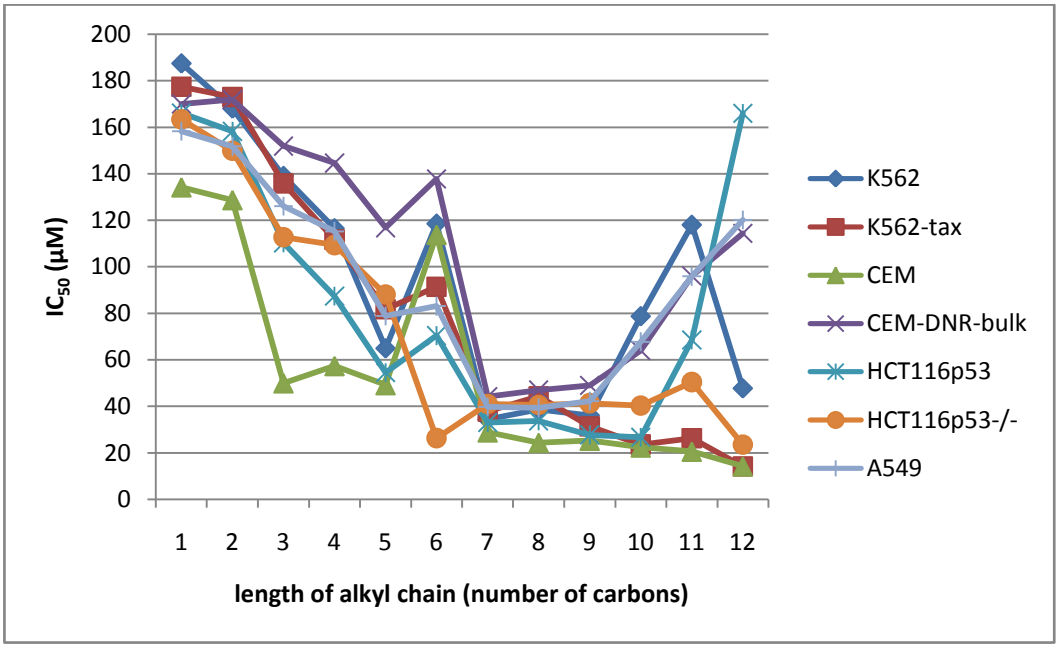
Table 1: Summary of cytotoxic activity (relative IC_{50}, μM).[a]

Entry	substituent	K562	K562-tax	CEM	CEM-DNR-bulk	HCT116p53	HCT116p53-/-	A549
173	Cl	182,5	153,6	170,3	131,4	213,5	171,4	186,5
174	N_3	177,6	139,4	141,7	114,8	192,3	155,2	195,0
175	uracil-5-yl	178,1	176,6	152,5	165,3	185,2	159,1	151,8
177a	methoxy	187,4	177,3	134,1	169,9	166,1	163,3	158,2
177b	ethoxy	168	172,9	128,6	171,8	158,2	149,8	151,7
177c	propoxy	139	135,8	49,9	151,8	110,2	112,7	126
177d	butoxy	116,5	111,6	57,3	144,5	87,4	109,3	115,2
177e	pentyloxy	64,9	81,7	49,1	116,7	54,5	88	78,8
177f	hexyloxy	118,5	91,3	113,7	137,7	70,4	26,3	83
177g	heptyloxy	34,4	37,7	28,8	44,1	33	41	39,7
177h	octyloxy	38,7	44,2	24,3	46,9	33,7	40,5	39,3
177i	nonyloxy	36,1	31,3	25,2	48,9	27,7	41,3	42,2
177j	decyloxy	78,7	23,5	22,3	63,9	26,7	40,3	67,7
177k	undecyloxy	118	26,2	20,5	96	68,5	50,4	95,8
177l	dodecyloxy	47,7	14,1	14,2	114,3	166	23,5	120,1
177m	i-propoxy	172,4	172,7	124,7	169,6	155,1	143,5	148,5
177n	s-butoxy	148,4	161,1	103,0	165,3	136,7	141,5	137,8
177o	t-butoxy	175,0	175,5	131,1	162,8	178,5	158,5	161,5

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.

While derivatives **173-175** and alkoxyderivatives with branched side chain **177m-o** exhibit no significant activity against any of tested cancer cell lines, the activity of alkoxyderivatives **177a-l** exhibits interesting dependence on the length of alkyl chain. All those activities affected by the variability of alkyl chain are illustrated in *Graph 1*. Derivatives **177** with longer alkyl chains exhibited relatively higher cytotoxic activity in CEM and K562-tax and HCT 116p53-/- cells. In cell lines A549, CEM-DNR-bulk, K562 and HCT116p53, the cytotoxic activity increases up to 9 carbons alkyl side chain and then decreases with longer alkyl chain.

Graph 1. Cytotoxic activity of compounds **177** as a function of the chain length in the diverse range of cell lines.



Furthermore, three of the most active compounds **177f-h** were studied in more details to describe their effect on cell cycle alteration and nucleic acid synthesis using model CEM cell line (*Figure 20*, *21*). Analyses were performed at equiactive concentrations corresponding to 1x IC_{50} and 5x IC_{50}. *Figure 20* demonstrates that the cytotoxic activity of alkoxyderivatives **177f-h** was accompanied by rapid inhibition of DNA synthesis at concentration 5x IC_{50}. Interestingly, at concentration 1x IC_{50} inhibition of DNA synthesis is significant only for derivative **177f**. Moreover, there was an apparent increase of RNA synthesis in compounds **177g** and **177h** but the inhibition in **177f** at 1x IC_{50}, while in 5x IC_{50} the total RNA synthesis was inhibited in cells treated with any of the compounds.

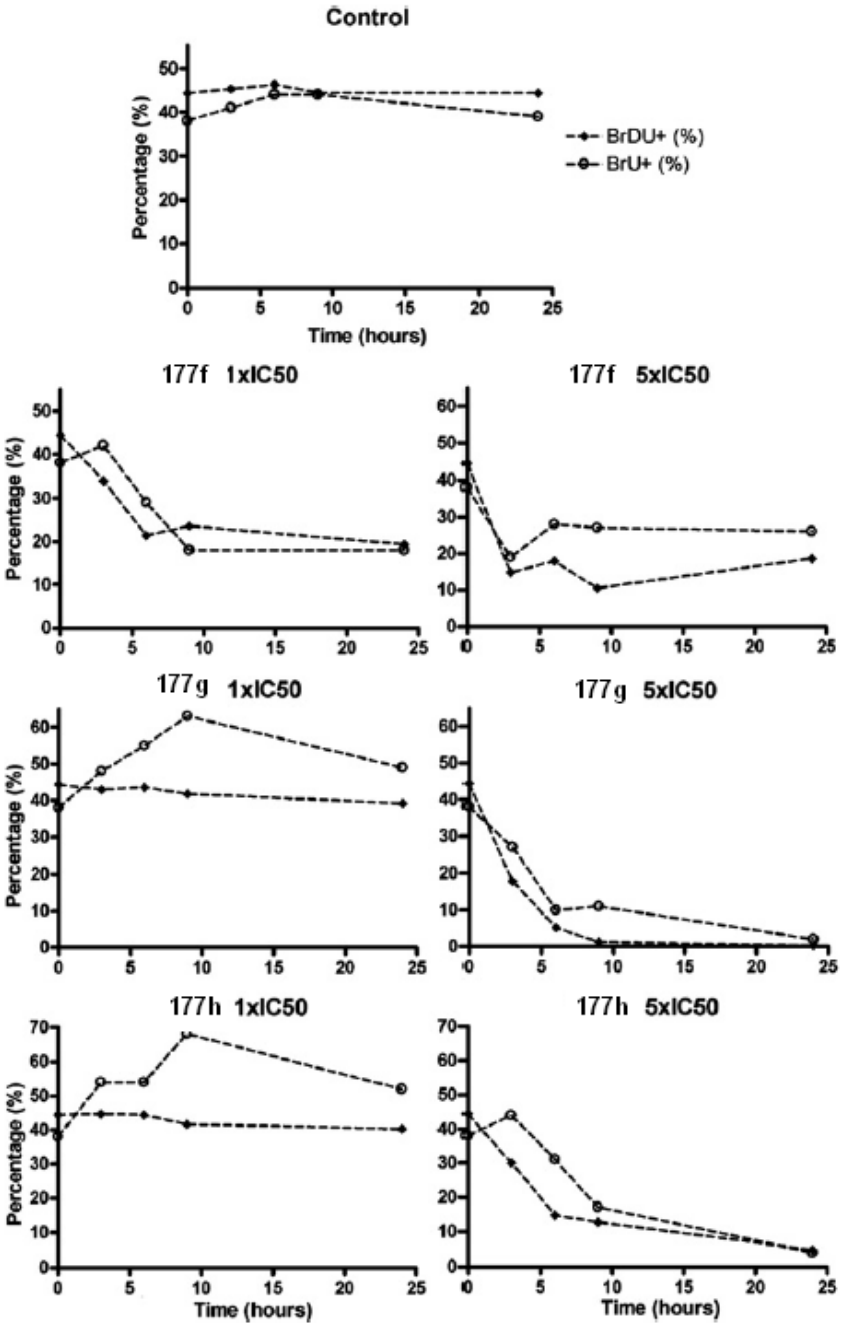


Figure 20. Summary of RNA/DNA analysis for CEM cancer cell line treated with compounds **177f-h**. Data are expressed as a percentage of positive cells in the total cellular population.

Moreover, compounds **177f-h** induced apoptosis at 5x IC_{50}, but do not cause any significant cell cycle alterations in treated CEM cells (*Figure 21).* Interestingly, at concentration 1x IC_{50} only **177f** caused significant apoptosis within 24 h.

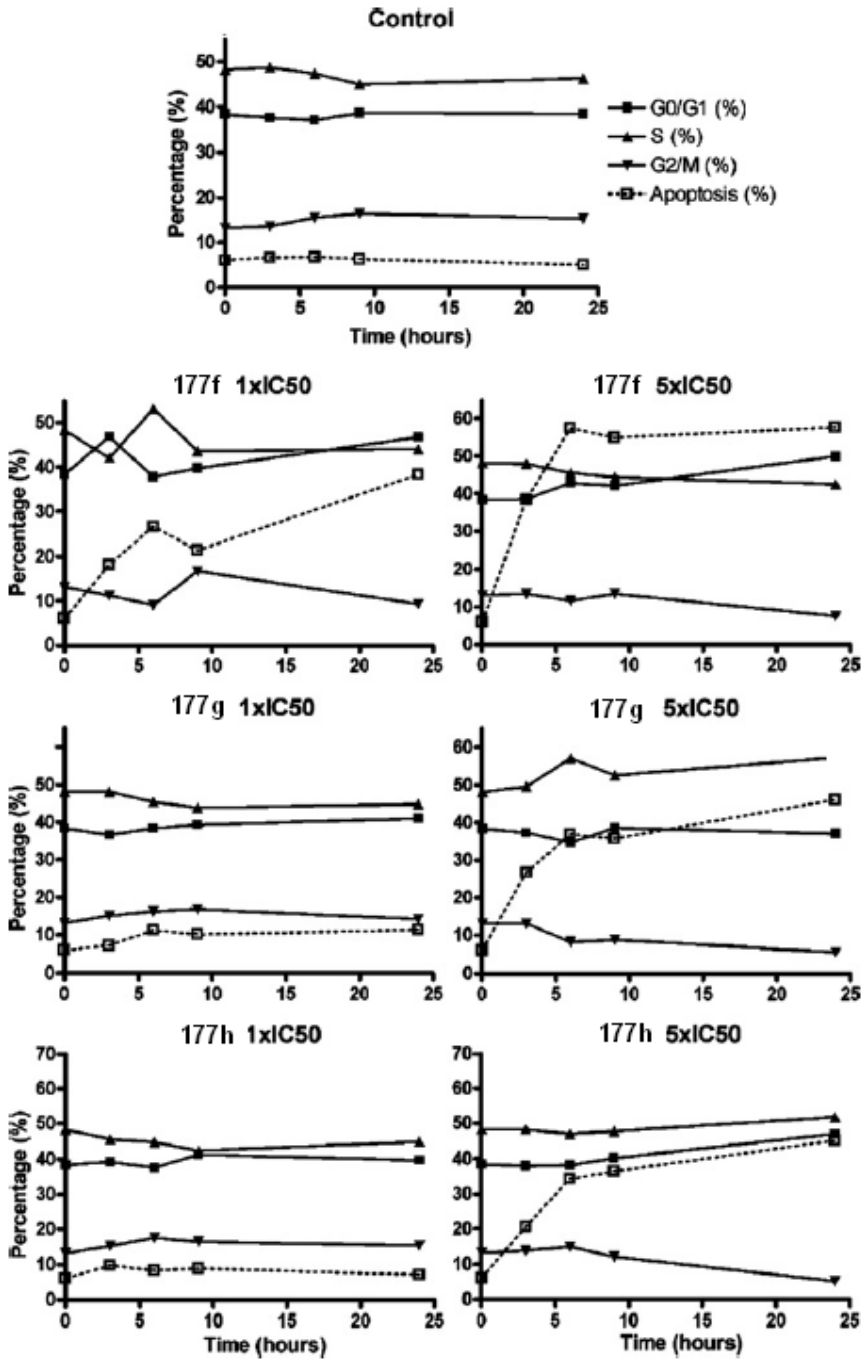
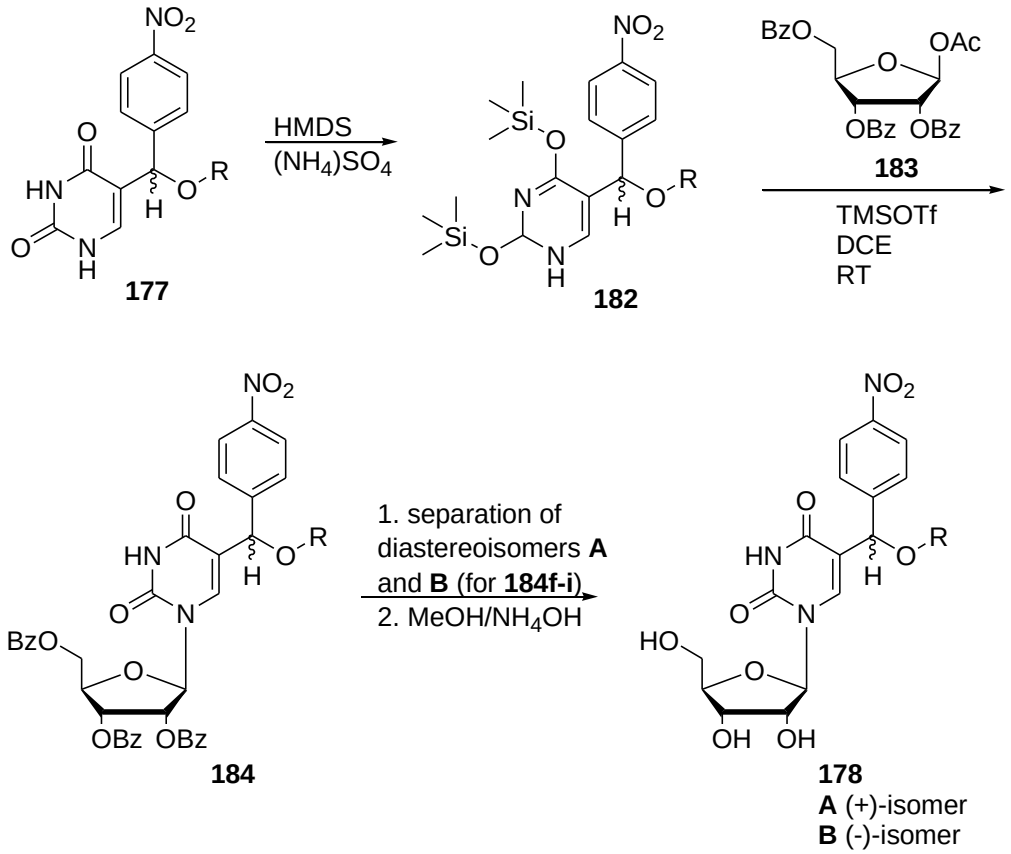


Figure 21. Summary of conventional cell cycle and apoptosis analysis for CEM cancer cell line treated with compounds **177f-h**. Data are expressed as a percentage of cells with corresponding DNA content in the total cellular population.

3.1.3. Synthesis of 5-[(alkoxy(4-nitrophenyl)methyl)]uridines

Despite the fact that the above mentioned 5-[(alkoxy(4-nitrophenyl)methyl)]uracils **177** showed an interesting relationship between the structure and the cytotoxic activity

(see *Table 1*, page 47), the values of IC_{50} remained beyond micromolar concentrations. Thereupon, alkoxyderivatives **177** were converted into their corresponding ribonucleosides **178** (*Scheme 31*) in order to improve the solubility and increase their possibility to interact with the enzymes responsible for nucleic acids transformation and/or biosynthesis. Moreover, the above mentioned alkoxy derivatives **177** were prepared in their racemic form and their conversion to corresponding diastereomeric nucleosides **184** appeared to be the suitable option for isomers separation.



a) R = CH_3
b) R = CH_2CH_3
c) R = $(CH_2)_2CH_3$
d) R = $(CH_2)_3CH_3$
e) R = $(CH_2)_4CH_3$
f) R = $(CH_2)_5CH_3$
g) R = $(CH_2)_6CH_3$
h) R = $(CH_2)_7CH_3$
i) R = $(CH_2)_8CH_3$
j) R = $(CH_2)_9CH_3$
k) R = $(CH_2)_{10}CH_3$
l) R = $(CH_2)_{11}CH_3$
m) R = i-Pr
n) R = s-But
o) R = t-But

Scheme 31. Synthesis of ribonucleosides **178**.

Firstly, all of prepared derivatives **177** were converted into their corresponding protected nucleosides **184** using the Vorbrüggen method.[53] This synthesis was based on reaction of silylated nucleobases **182** with the protected ribose **183** in the presence of Lewis acid. The reaction was initiated by boiling of nucleobases **177** in excess of

HMDS with the presence of catalytic amount of ammonium sulphate. Hereby silylated nucleobases **182** were reacted with equivalent amount of benzoylated ribose **183** in the presence of 1.1 equivalents of TMSOTf at room temperature to afford protected ribonucleosides **184**. Subsequent separation and final treatment of ribonucleosides **184** with methanolic ammonia solution at room temperature afforded the nucleosides **178**.

The most complicated step of this synthesis was separation of diastereomers **184**, which was not simple due to their similar physical properties. Primary TLC and HPLC-MS analyses showed that the protected ribonucleosides **184** exhibit better properties for separation then free nucleosides **178** in general. Therefore, isomers **A** and **B** were obtained from ribonucleosides **184** first and then deprotected separately.

Considering a difficult separation of single diastereomers I decided to separate derivatives **184f-i** derived from nucleobases **177f-i** exhibiting the most interesting cytotoxic activity against all cancer cell lines (see *Table 1*, page 47). The nucleosides **184a-e** and **184m-n** were obtained in their diastereomeric mixture. Nucleosides **184j-l** and **183o** have not been synthesized yet.

Firstly, I decided to try common silica gel column chromatography and find suitable conditions for such a separation. The separation was very sensitive to the presence of water and furthermore a carefully chosen gradient of the mobile phase was one of the most crucial factors. The methanol in chloroform (0-5%) was found as the most powerful mobile phase for separation by silica gel column chromatography and protected nucleosides **184f-i** were separated to give two diastereomers **A** (eluted as the first) and **B** (eluted as the second) from each diastereomeric mixture.

Since the R_f differences of both isomers of compounds **184f-i** on TLC with normal phase vary from 0.02 to 0.04, I attempted to find out the conditions for the separation by using reverse phase. Hence, diastereomeric mixture was tried to be separated by the use of HPLC chromatography with the reverse phase. However, this attempt failed, because I did not find suitable conditions for this separation.

Data obtained from the HPLC analyses indicate that the ribonucleosides **184** are formed in mixture with almost equivalent ratio of both isomers. Moreover, this can be also confirmed by NMR analyses for diastereomers mixtures of free nucleosides **178**. ^{13}C NMR spectra showed pair of signals for almost each carbon with a few of exceptions, when the signals overlap. On the other hand, ^{1}H NMR records showed overlapping signals in most cases and only broad peak for chiral proton suggests the

presence of diastereomeric mixture. It can be observed as a slight displacement of signals in a few exceptions that is expressed as multiplet.

3.1.4. Biological activity of 5-[(alkoxy(4-nitrophenyl)methyl)]uridines

The prepared compounds **178** were tested for their cytotoxic activity *in vitro* against cancer cell lines CEM, K562, their drug resistant counterparts CEM-DNR-bulk and K562-tax, and A549, HCT116p53 and HCT116p53-/- cell lines. All results are summarized in *Table 2*.

The introduction of ribose moiety into 5-substituted uracil analogues (compounds **178f-i**) did not bring significant increase of cytotoxic activity against cancer cell lines in comparison to the free bases (see *Table 1, page 47*). On the other hand, derivatives **178i** exhibited activity in micromolar concentrations against CEM and HCT116p53 cell lines and are perspective for future studies.

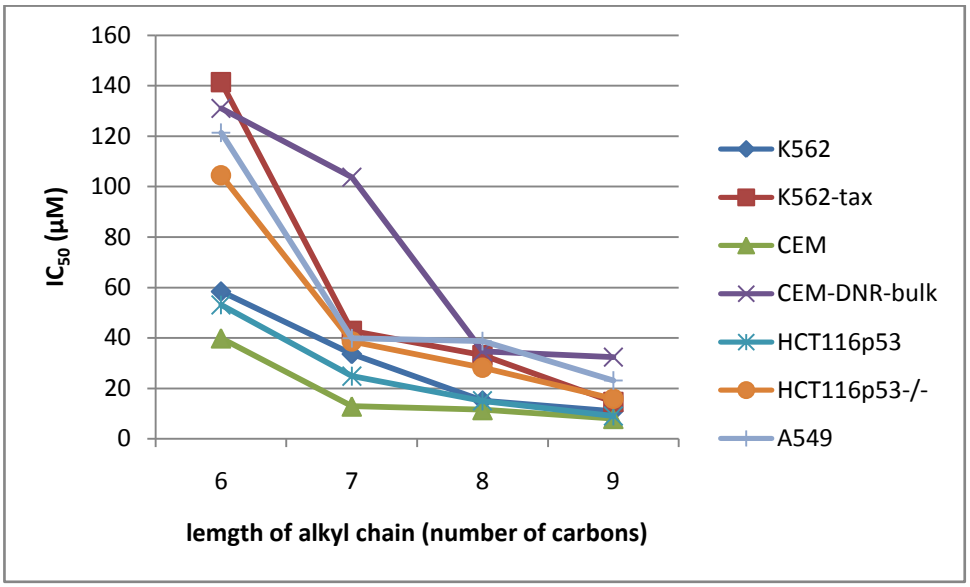
Graphs 2 and *3* illustrate the trend of increasing activity with longer alkyl chain and also show that the activity of nucleosides **178f-i** is higher in drug sensitive than in drug resistant cancer lines and is independent of the chirality of the molecule.

Table 2: Summary of cytotoxic activity (relative IC_{50}, μM).[a]

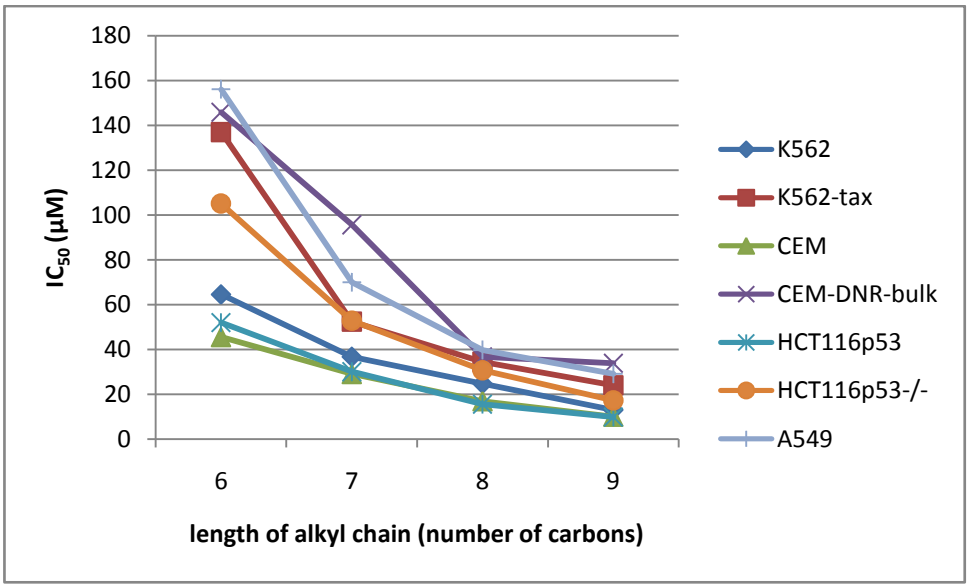
Entry	substituent	K562	K562-tax	CEM	CEM-DNR-bulk	HCT116p53	HCT116p53-/-	A549
178f (+)	hexyloxy	58,4	141,4	39,8	131,0	53,0	104,4	121,3
178g (+)	heptyloxy	33,6	42,8	12,8	103,7	24,8	38,5	39,8
178h (+)	octyloxy	15,1	33,1	11,4	34,6	15,0	28,1	38,7
178i (+)	nonyloxy	10,9	14,6	**7,9**	32,3	**9,0**	15,8	23,1
178f (-)	hexyloxy	64,5	136,9	45,5	145,8	51,9	105,0	156,0
178g (-)	heptyloxy	36,7	52,3	29,0	95,6	30,0	52,8	69,9
178h (-)	octyloxy	24,7	34,4	16,9	36,6	15,5	30,7	39,7
178i (-)	nonyloxy	13,0	23,9	10,0	33,8	**9,8**	17,3	29,1

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.

Graph 2: Cytotoxic activity of c**o**mpounds **178f-i** (+) isomers as a function of chain length in the diverse range of cell lines.



Graph 3: Cytotoxic activity of compounds **178f-i** (-) isomers as a function of chain length in the diverse range of cell lines.



Furthermore, derivatives **178f-i** were tested for their antimicrobial activity against standard reference gram-positive and gram-negative bacterial strains (*Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* CCM 3953, *Escherichia coli* CCM 3954 and *Pseudomonas aeruginosa* CCM 3955*)* and against gram-positive and gram-negative bacteria (methicillin resistant *Staphylococcus aureus* - MRSA, *Staphylococcus*

haemolyticus, *Escherichia coli* and *Pseudomonas aeruginosa*) with resistance to fluoroquinolones used in clinical practice. Only the octyl and nonyl derivatives **178h**, and **178i** showed slight activity against *Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* CCM 3953, *Staphylococcus aureus* MRSA and *Staphylococcus haemolyticus* (*Table 3*).

Table 3. Antimicrobial activity of compounds **178h-i** [MIC (μM)].

Entry	*Enterococcus faecalis* CCM 4224	*Staphylococcus aureus* CCM 3953	*Staphylococcus aureus* (MRSA)	*Staphylococcus haemolyticus*
178hA	200	100	100	200
178iA	50	50	50	100
178hB	200	100	100	200
178iB	50	50	50	100

3.1.5. Conclusion

In this part of thesis diverse range of 5-[(4-nitrophenyl)methyl)]uracil analogues was synthesized in order to evaluate their biological properties. Substitution reactions of chloroderivative **173** led to the series of alkoxyderivatives **177** as well as hydroxyderivatives **172**, **176**, azidoderivative **174** and bis-uracil compound **175**. Efficient ribosylation of nucleobases **177** afforded protected nucleosides **184** in their diastereomeric mixtures, where two diasteroisomers from each mixture **184f-i** were isolated using silica gel column chromatography. These isomers along with non-separated mixtures **184a-e** and **184m-n** were deprotected and gave ribonucleosides **178**.

All prepared compounds were submitted to the testing for their cytotoxic activity against cancer cell lines CEM, K562, their drug resistant counterparts CEM-DNR-bulk and K562-tax, and A549, HCT116p53 and HCT116p53-/- cell lines. Activity results indicate that none of prepared compound exhibit significant cytotoxic activity, nevertheless, cytotoxic activity alkoxyderivatives **177** and their nucleoside analogues **178** exhibit interesting dependence on the length of alkyl chain. Therefore, the connection of cytotoxic activity with higher lipophilic alkyl chain might be promising for future study. Moreover, compounds **177f-h** inhibit the synthesis of both DNA and

RNA and induce apoptosis at concentration 5x IC_{50} in treated CEM cells. Interestingly, derivative **177f** caused significant apoptosis within 24 h at concentration 1x IC_{50}. The activity of nucleosides **178f-i** is higher in drug sensitive than in drug resistant cancer lines and is independent of the chirality of the molecule.

Furthermore, the presence of nitrog roup offers a large number of possibilities for derivatization of prepared compounds. Nitro group might serve for building of new heterocyclic rings, indole or quinolone for instance, with potential biological properties. Moreover, the introduction of heterocyclic system with fluorescence properties (such as quinolones) into specific oligonucleotide *via* substitution of uracil nucleobase might lead to research of new fluorescent probes applicable in various biological studies.

3.2. Studies on synthesis and biological activity of various 5-alkoxymethyluracil analogues

The second part of the reported thesis is focused on synthesis of diverse range of 5-alkoxymethyluracil analogues (*Figure 22*) and study of their biological activity. The primary purpose was the synthesis of ethers **185** and **186** in order to compare biological activity of prepared compounds with previously mentioned nitro analogues **177** and **178** (*Figure 19*, page 44) and thus to evaluate the effect of the substitution of methylene bridge direct attached to the C-5 position of uracil ring.

Furthermore, studies were extended to synthesis of acyclic nucleosides **187** and **188** and nucleoside **189** containing potentially biologically active 2,3-dihydroxy-1-propoxy moiety placed on purine as well as pyrimidine nucleobases.[54,55,56,57] Uracil derivatives substituted with this moiety were tested only for antiviral activity against HSV-1 and HSV-2 with no significant activity.[54] Anticancer activity neither of purine nor pyrimidine analogues substituted with 2,3-dihydroxy-1-propoxy moiety has been studied yet. Therefore we decided to develop synthetic approaches for preparation of uracil and uridine analogues **187-189** bearing variously substituted 2,3-dihydroxy-1-propoxy moiety at the C-5 position to evaluate anticancer activity.

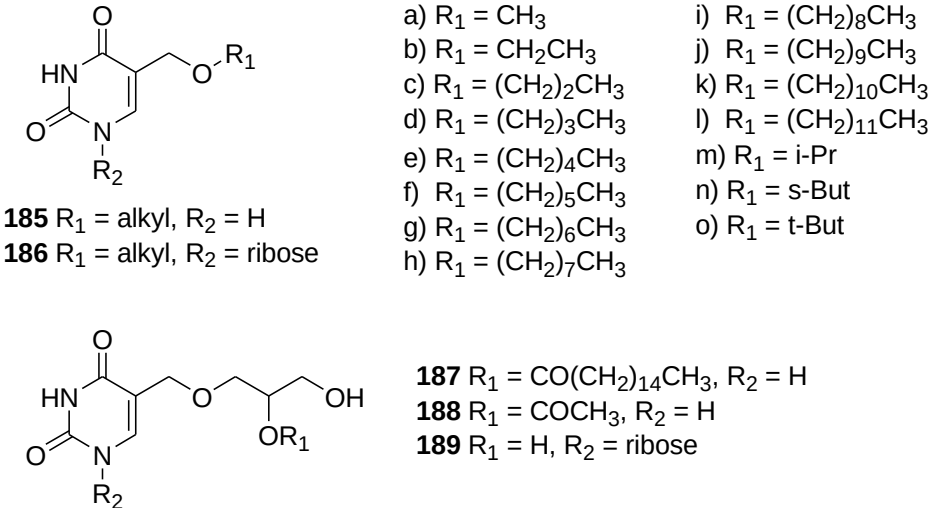
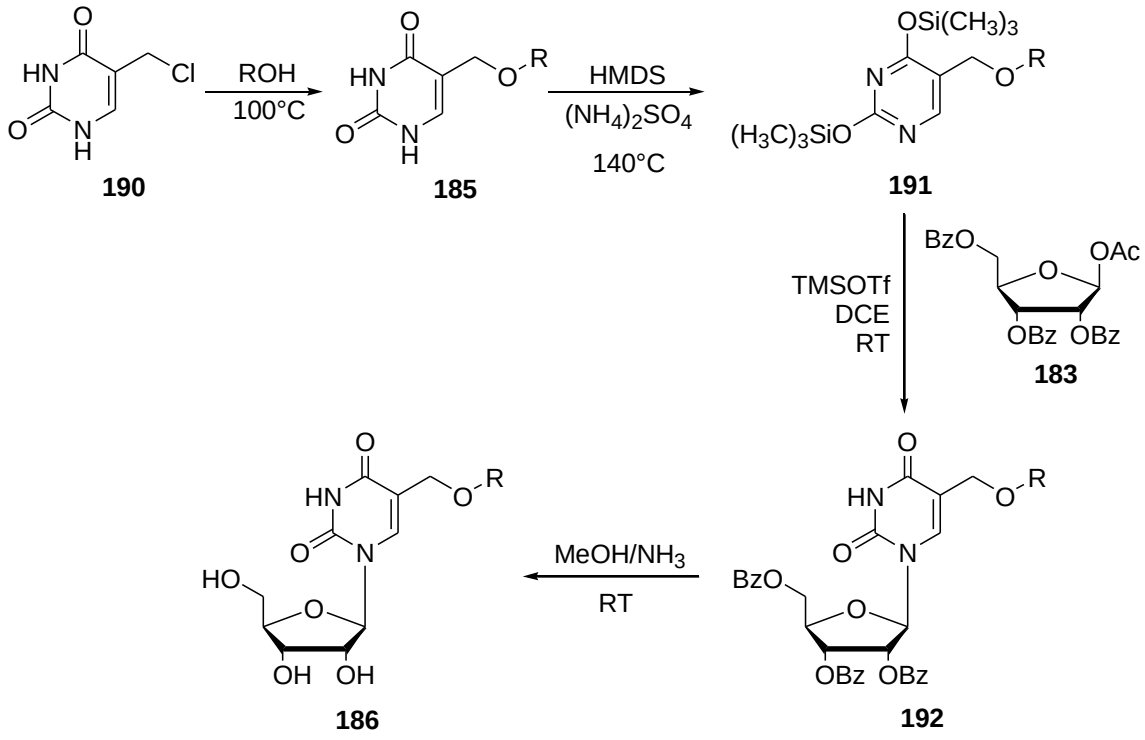


Figure 22. Summary of prepared 5-alkoxymethyluracil analogues.

3.2.1. Synthesis of 5-alkoxymethyluracil analogues

Various 5-alkoxymethyluracil analogues were studied over the last 60 years in connection with either their synthesis or their use in study of nucleic acid metabolism. Despite the fact that many published studies describe synthesis and biological activity of various 5-alkoxymethyluracils **185a-o** (*Figure 22*) with alkyl chain length C_1-C_{12} (except C_9 and C_{11}), 5-alkoxymethyluridines with alkyl chain longer than C_2 were not studied. In addition to this, any anticancer studies of 5-alkoxymethyluracil analogues **185, 186** have not been reported up to now therefore this study notably contributes to the progress of 5-alkoxymethyluracil analogues investigation.

A series of 5-alkoxymethyluracils analogues **185, 186** were prepared *via* nucleophillic substitution of 5-chloromethyluracil **190** (*Scheme 32*).



Scheme 32. General synthetic route of compounds **186.**

This starting material **190** was synthesized *via* known and described reaction[58,59] from 5-hydroxymethyluracil. Subsequently, this reactive intermediate was heated at 100°C in various aliphatic linear alcohols C_1-C_{12} as well as some branched alcohols such as isopropanol, *s*-butanol and *t*-butanol. Only two of the series of compounds **185**, 5-nonyloxymethyluracil **185i** and 5-undecyloxymethyluracil **185k**, have not been described yet and therefore their preparation is included in experimental section.

All prepared alkoxymethyluracils **185** were silylated in HMDS with addition of $(NH_4)_2SO_4$ as a catalyst at 140°C. Silylated derivatives **192** were coupled with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** using trimethylsilyl trifluoro-methanesulfonate as Lewis acid catalyst. This Vorbrügen method[53] led to the synthesis of protected nucleosides **192**, which were successfully purified using silica gel column chromatography to afford nucleosides **192** in 34-61% yields. Treatment of benzoylated nucleosides **192** with an access of methanolic ammonia solution gave a series of fifteen alkoxynucleosides **186**, where derivatives **186c-o** are newly synthesized compounds.

3.2.2. Biological activity of 5-alkoxymethyluracil analogues

5-Alkoxymethyluracils analogues **185, 186** were tested under *in vitro* conditions for their cytotoxic activity against cancer cell lines CEM, K562, their drug resistant counterparts CEM-DNR-bulk and K562-tax, and A549, HCT116p53 and HCT116p53-/- cell lines. All results are reported in *Table 4*.

Firstly, the cytotoxic activity of modified nucleobases **185** was investigated against diverse range of cancer cells. While derivatives with branched side chain **185m-o** exhibit no significant activity against any of tested cancer cell lines, derivatives **185a-l** show interesting dependence on the length of alkyl chain. All those activities affected by variability of alkyl chain are illustrated in *Graph 4*.

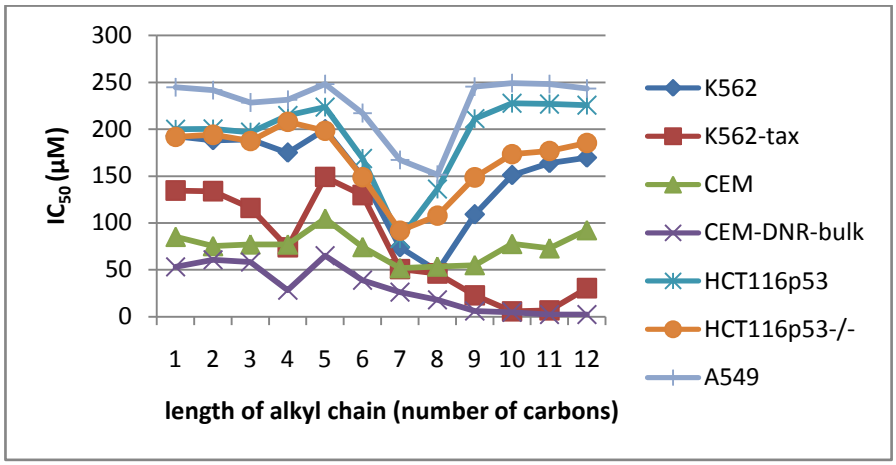
It is evident that the maximum cytotoxicity is observed for derivatives with the length of alkyl chain between 6-9 carbons excepting drug resistant lines K562-tax and CEM-DNR-bulk. Another carbon addition leads to significant decrease of cytotoxic activity. The most interesting results were obtained for drug resistant leukemia cancer cell lines (CEM-DNR-bulk and K562-tax), where an activity is approaching micromolar concentration of IC_{50} (CEM-DNR-bulk, derivative **185k**: $IC_{50} = 2.4\mu M$).

Table 4. Summary of cytotoxic activity (relative IC_{50}, μM).[a]

	substituent	K562	K562-tax	CEM	CEM-DNR-bulk	HCT116p53	HCT116p53-/-	A549
185a	methoxy	192,6	134,4	85,2	53,1	200,0	191,5	244,6
185b	ethoxy	188,1	133,7	75,3	60,7	200,3	193,8	241,7
185c	propoxy	189,2	115,9	77,1	58,4	196,6	187,2	228,4
185d	butoxy	174,9	74,0	77,1	28,3	214,4	207,6	231,4
185e	pentyloxy	199,7	148,8	104,7	65,1	223,4	198,2	248,1
185f	hexyloxy	149,4	129,7	74,3	39,0	168,7	148,6	217,1
185g	heptyloxy	74,1	50,8	51,8	26,2	83,3	91,7	167,2
185h	octyloxy	48,8	46,2	53,5	18,0	136,1	107,8	151,4
185i	nonyloxy	109,1	22,8	55,0	**6,1**	211,1	148,5	245,5
185j	decyloxy	151,0	**9,3**	77,7	**4,9**	227,4	173,1	249,1
185k	undecyloxy	163,8	**6,8**	73,0	**2,4**	226,7	176,5	248,0
185l	dodecyloxy	169,5	30,5	92,4	**2,4**	225,5	185,1	243,3
185m	i-propoxy	176,5	131,9	85,2	55,3	190,5	192,8	228,8
185n	s-butoxy	188,8	131,3	85,9	42,1	191,8	175,6	243,2
185o	t-butoxy	162,6	163,7	102,9	132,1	185,9	215,5	160,6
186a	methoxy	181,5	194,6	92,2	180,6	210,1	183,8	160,7
186b	ethoxy	180,1	183,0	98,0	170,1	220,5	187,4	165,4
186c	propoxy	180,3	180,4	100,8	178,6	229,2	177,5	147,5
186d	butoxy	203,8	196,8	93,3	155,4	210,7	193,5	181,2
186e	pentyloxy	192,2	180,1	100,2	174,6	199,5	184,5	180,6
186f	hexyloxy	138,3	173,0	109,6	173,5	204,1	174,8	166,5
186g	heptyloxy	134,5	167,3	81,3	176,2	195,8	142,6	166,1
186h	octyloxy	70,2	153,3	82,4	137,9	165,1	117,0	138,2
186i	nonyloxy	38,8	109,6	52,5	62,7	144,6	118,4	104,3
186j	decyloxy	13,6	94,5	35,3	54,0	97,5	64,9	41,8
186k	undecyloxy	13,6	74,0	28,0	82,2	121,5	90,4	17,8
186l	dodecyloxy	35,1	72,1	31,2	88,6	117,5	84,9	41,4
186m	i-propoxy	179,6	183,0	103,2	173,7	205,6	196,8	168,9
186n	s-butoxy	207,9	192,2	94,8	172,8	215,8	199,3	180,4
186o	t-butoxy	192,4	186,1	83,1	208,3	205,7	192,5	177,2

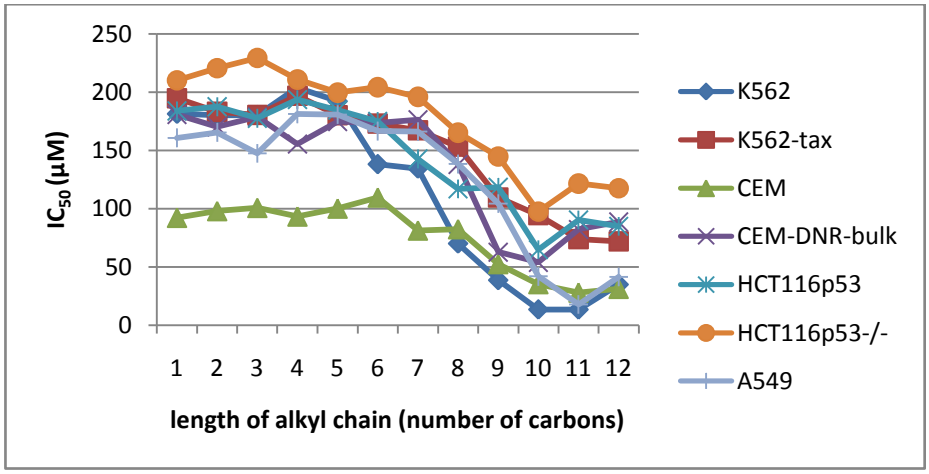
[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.

Graph 4. Cytotoxic activity of compounds **185** as a function of chain length in the diverse range of cell lines.



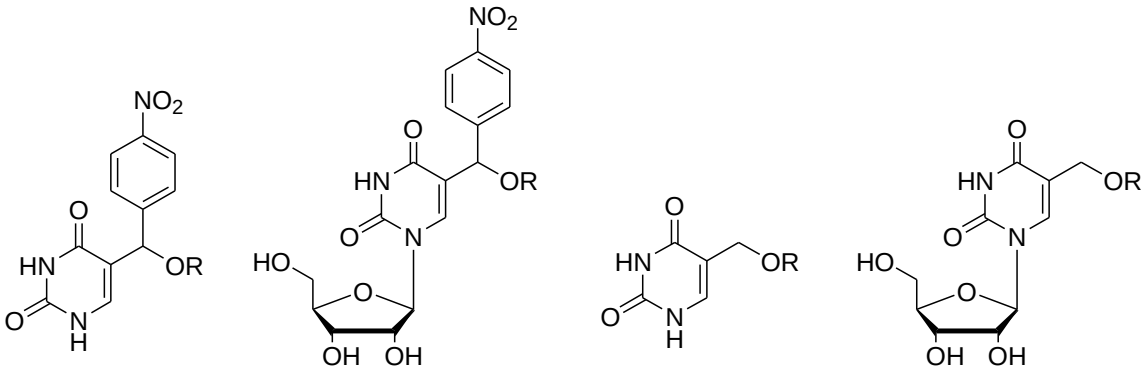
The modified nucleobases **185** were further transformed to corresponding nucleosides **186**. The activities of nucleosides **186** are summarized in *Table 4* and illustrated in *Graph 5*. In contrast to previous bases **185** it is apparent that the most active nucleosides **186** are the ones with longer alkyl chain containing 9-11 carbons. While the activity of nucleosides **186a-h** against A549, K562 and both colorectal carcinoma cell lines is approaching to activity of bases **185a-h**, nucleosides **186i-l** exhibit much better activity than bases **185i-l** on these cell lines. The most interestingly, a comparison of cytotoxic activity of nucleosides **186** and bases **185** against drug resistant leukemia cancer cell lines (CEM-DNR-bulk and K562-tax) indicates significantly decreased activity of nucleosides **186**.

Graph 5. Cytotoxic activity of compounds **186** as a function of chain length in the diverse range of cell lines.



3.2.3. Structure-cytotoxic activity relationship study

A structure-activity relationship of nucleobases **177** and **185** and their nucleoside counterparts **178** and **186** (*Figure 23*) was systematically studied using *in vitro* cytotoxic activity screening on representative cancer cell lines.



a) R = CH_3
b) R = CH_2CH_3
c) R = $(CH_2)_2CH_3$
d) R = $(CH_2)_3CH_3$
e) R = $(CH_2)_4CH_3$
f) R = $(CH_2)_5CH_3$
g) R = $(CH_2)_6CH_3$
h) R = $(CH_2)_7CH_3$
i) R = $(CH_2)_8CH_3$
j) R = $(CH_2)_9CH_3$
k) R = $(CH_2)_{10}CH_3$
l) R = $(CH_2)_{11}CH_3$
m)R = i-Pr
n) R = s-But
o) R = t-But

Figure 23. Structures of uracil analogues **177, 178, 185** and **186**.

Several conclusions can be derived from the results of the cytotoxic activity screening:

1) A comparison of cytotoxic activity of nucleobases **177** and **185** (*Table 5*) indicates that the introduction of bulky nitrophenyl moiety (nucleobases **177**) notably increases cytotoxic activity against A549 and both colorectal carcinoma cell lines. Further, the presence of nitrophenyl moiety slightly increases activity against chemosensitive CEM and K562 cell lines. On the other hand, nucleobases **185** with unsubstituted methylene bridge exhibit promising cytotoxicity against drug resistant cancer cell lines (IC_{50} = 2.4-9.3 μM). The most interestingly, nucleobases **185i-l** exhibit the unique cytotoxicity on chemoresistant CEM-DNR-bulk cell line in comparison with all screened cell lines and this activity increases with the elongation of alkyl chain. Differences between cytotoxicity of nucleobases **177** and **185** are evident especially on derivatives with longer alkyl chain.

Table 5: Summary of cytotoxic activity of uracil analogues **177** and **185** (relative IC_{50}, μM).[a]

*	K562		K562-tax		CEM		CEM-DNR-bulk		HCT116p53		HCT116p53-/-		A549	
	177	**185**	**177**	**185**	**177**	**185**	**177**	**185**	**177**	**185**	**177**	**185**	**177**	**185**
1	187,4	192,6	177,3	134,4	134	85,2	169,9	53,1	166,1	200	163,3	191,5	158,2	244,6
2	168	188,1	172,9	133,7	129	75,3	171,8	60,7	158,2	200,3	149,8	193,8	151,7	241,7
3	139	189,2	135,8	115,9	49,9	77,1	151,8	58,4	110,2	196,6	112,7	187,2	126	228,4
4	116,5	174,9	111,6	74	57,3	77,1	144,5	28,3	87,4	214,4	109,3	207,6	115,2	231,4
5	64,9	199,7	81,7	148,8	49,1	104,7	116,7	65,1	54,5	223,4	88	198,2	78,8	248,1
6	118,5	149,4	91,3	129,7	114	74,3	137,7	39	70,4	168,7	26,3	148,6	83	217,1
7	34,4	74,1	37,7	50,8	28,8	51,8	44,1	26,2	33	83,3	41	91,7	39,7	167,2
8	38,7	48,8	44,2	46,2	24,3	53,5	46,9	18	33,7	136,1	40,5	107,8	39,3	151,4
9	36,1	109,1	31,3	22,8	25,2	55	48,9	**6,1**	27,7	211,1	41,3	148,5	42,2	245,5
10	78,7	151	23,5	**9,3**	22,3	77,7	63,9	**4,9**	26,7	227,4	40,3	173,1	67,7	249,1
11	118	163,8	26,2	**6,8**	20,5	73	96	**2,4**	68,5	226,7	50,4	176,5	95,8	248
12	47,7	169,5	14,1	30,5	14,2	92,4	114,3	**2,4**	166	225,5	23,5	185,1	120,1	243,3

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.
***** Number of carbons of the side alkyl chain.

2) The cytotoxic activity screening of uracil analogues **185** and **186** (*Table 6*) enabled to evaluate the effect of ribose moiety of nucleosides **186** compared with nucleobases **185**. Data presented in *Table 6* indicate that the introduction of ribose moiety explicitly decreases cytotoxicity against drug resistant K562-tax and CEM-DNR-bulk cell lines, nucleobases **185** are more active against these drug resistant cell lines then their nucleosides counterparts **186**. On the other hand, introduction of ribose moiety increases the cytotoxicity against solid tumor cell line A549. This effect further depends upon length of alkyl chain. The elongation of alkyl chain significantly increases the cytotoxicity. Furthermore, while the activity of nucleobases **185** and their nucleoside counterparts **186** with alkyl chain up to 5 carbons is almost equipotent against chemosensitive K562, CEM and both colorectal carcinoma cell lines, the elongation of alkyl chain (9-12 carbons) causes an increasing of nucleosides **186** cytotoxicity.

Table 6: Summary of cytotoxic activity of uracil analogues **185** and **186** (relative IC_{50}, μM).[a]

*	K562		K562-tax		CEM		CEM-DNR-bulk		HCT116p53		HCT116p53-/-		A549	
	185	**186**	**185**	**186**	**185**	**186**	**185**	**186**	**185**	**186**	**185**	**186**	**185**	**186**
1	192,6	181,5	134,4	194,6	85,2	92,2	53,1	181	200	210	192	183,8	244,6	160,7
2	188,1	180,1	133,7	183	75,3	98	60,7	170	200	221	194	187,4	241,7	165,4
3	189,2	180,3	115,9	180,4	77,1	101	58,4	179	197	229	187	177,5	228,4	147,5
4	174,9	203,8	74	196,8	77,1	93,3	28,3	155	214	211	208	193,5	231,4	181,2
5	199,7	192,2	148,8	180,1	105	100	65,1	175	223	200	198	184,5	248,1	180,6
6	149,4	138,3	129,7	173	74,3	110	39	174	169	204	149	174,8	217,1	166,5
7	74,1	134,5	50,8	167,3	51,8	81,3	26,2	176	83,3	196	91,7	142,6	167,2	166,1
8	48,8	70,2	46,2	153,3	53,5	82,4	18	138	136	165	108	117	151,4	138,2
9	109,1	38,8	22,8	109,6	55	52,5	**6,1**	62,7	211	145	149	118,4	245,5	104,3
10	151	13,6	**9,3**	94,5	77,7	35,3	**4,9**	54	227	97,5	173	64,9	249,1	41,8
11	163,8	13,6	**6,8**	74	73	28	**2,4**	82,2	227	122	177	90,4	248	17,8
12	169,5	35,1	30,5	72,1	92,4	31,2	**2,4**	88,6	226	118	185	84,9	243,3	41,4

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.
***** Number of carbons of the side alkyl chain.

3) The effect of ribose moiety can be further observed when cytotoxic activity of uracil analogues **177** and **178** is compared (*Table 7*).

Table 7: Summary of cytotoxic activity of uracil analogues **177** and **178** (relative IC_{50}, μM).[a]

*	K562		K562-tax		CEM		CEM-DNR-bulk		HCT116p53		HCT116p53-/-		A549	
	177	**178**	**177**	**178**	**177**	**178**	**177**	**178**	**177**	**178**	**177**	**178**	**177**	**178**
6	118,5	58,4	91,3	141,4	113,7	39,8	137,7	131	70,4	53	26,3	104,4	83	121,3
7	34,4	33,6	37,7	42,8	28,8	12,8	44,1	103,7	33	24,8	41	38,5	39,7	39,8
8	38,7	15,1	44,2	33,1	24,3	11,4	46,9	34,6	33,7	15	40,5	28,1	39,3	38,7
9	36,1	10,9	31,3	14,6	25,2	**7,9**	48,9	32,3	27,7	**9**	41,3	15,8	42,2	23,1

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.
***** Number of carbons of the side alkyl chain.

Since cytotoxicity of (+) and (-) isomer **178** is almost equipotent, data presented in *Table 7* are related only to (+) isomer. The single-valued effect of ribose moiety in terms of increases of cytotoxicity can be observed from data related to chemosensitive CEM, HCT116p53 and K562 cell lines. Nucleosides **178i** proved the most interesting activity against CEM and HCT116p53 cell lines, where the activity reaches to micromolar concentrations (IC_{50} = 7.9 - 9.0 μM).

4) The outcome of nucleosides **178** and **186** cytotoxicity screening summarized in *Table 8* demonstrates the effect of nitrophenyl moiety. Since cytotoxicity of (+) and (-) isomer **178** is almost equipotent, data presented in *Table 8* are related only to (+) isomer. Explicitly, nitrophenyl moiety has significantly positive effect on increasing of cytotoxic activity. Nucleosides **178** exhibit higher cytotoxicity against all screened cell lines.

Table 8: Summary of cytotoxic activity of uracil analogues **178** and **186** (relative IC_{50}, μM).[a]

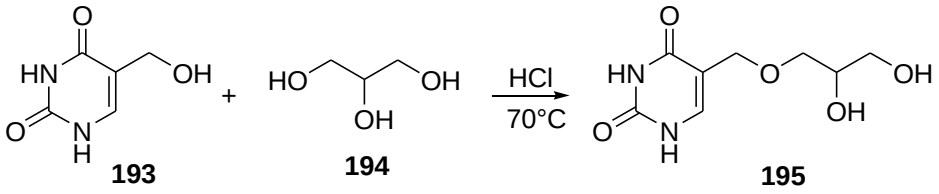
*	K562		K562-tax		CEM		CEM-DNR-bulk		HCT116p53		HCT116p53-/-		A549	
	178	**186**	**178**	**186**	**178**	**186**	**178**	**186**	**178**	**186**	**178**	**186**	**178**	**186**
6	58,4	138,3	141,4	173	39,8	110	131	174	53	204	104,4	174,8	121,3	166,5
7	33,6	134,5	42,8	167,3	12,8	81,3	103,7	176	24,8	196	38,5	142,6	39,8	166,1
8	15,1	70,2	33,1	153,3	11,4	82,4	34,6	138	15	165	28,1	117	38,7	138,2
9	10,9	38,8	14,6	109,6	**7,9**	52,5	32,3	62,7	**9**	145	15,8	118,4	23,1	104,3

[a] Average values of IC_{50} from 3 to 4 independent experiments with SD ranging from 10 to 25% of the average values.
***** Number of carbons of the side alkyl chain.

To conclude this part, the cytotoxic activity screening of uracil analogues **177**, **178**, **185** and **186** shows activity dependence on nitrophenyl and/or ribose moiety and the length of alkyl chain. The most active compounds from investigated series are nucleobases **185i-l** that exhibit significant cytotoxicity against drug resistant CEM-DNR-bulk cell line (IC_{50} = 2.4 - 6.1 μM) and nucleosides **178**.

3.2.4. Studies on reactivity of 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil

The next part of thesis is focused on synthesis of uracil acyclic nucleosides derived from 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil **195** (*Scheme 33*). Compound **195** was synthesized from 5-hydroxymethyluracil **193** and glycerol **194** in the presence of hydrochloric acid according to known procedure[54] in its enantiomeric mixture.



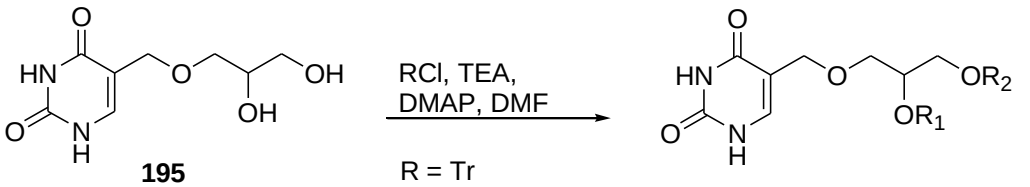
193 **194** **195**

Scheme 33. Synthesis of 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil **195.**

3.2.4.1. Synthesis of esters of 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil

To evaluate an effect of the modification of dihydroxy derivative **195** on biological activity, the secondary hydroxyl group of **195** was converted to acetyl and palmitoyl ester. These two derivatizations were selected in order to observe the effect of both introduction of ester functional group and lipophilicity.

For this reaction purpose, primary hydroxyl group of derivative **195** was protected *via* ether formation (*Scheme 34*).



195

196 R_1 = H, R_2 = Tr
197 R_1 = R_2 = Tr
198 R_1 = H, R_2 = TBDMS
199 R_1 = R_2 = TBDMS

Scheme 34. Protection of primary hydroxyl group of derivative **195**.

Firstly, dihydroxy derivative **195** was reacted with 1.5 equivalent of triphenylmethyl chloride in the presence of 0.5 equivalent of DMAP. Reaction performed in DMF at RT gave mixture of trityl and ditrityl product **196** and **197**, respectively. Decreasing of

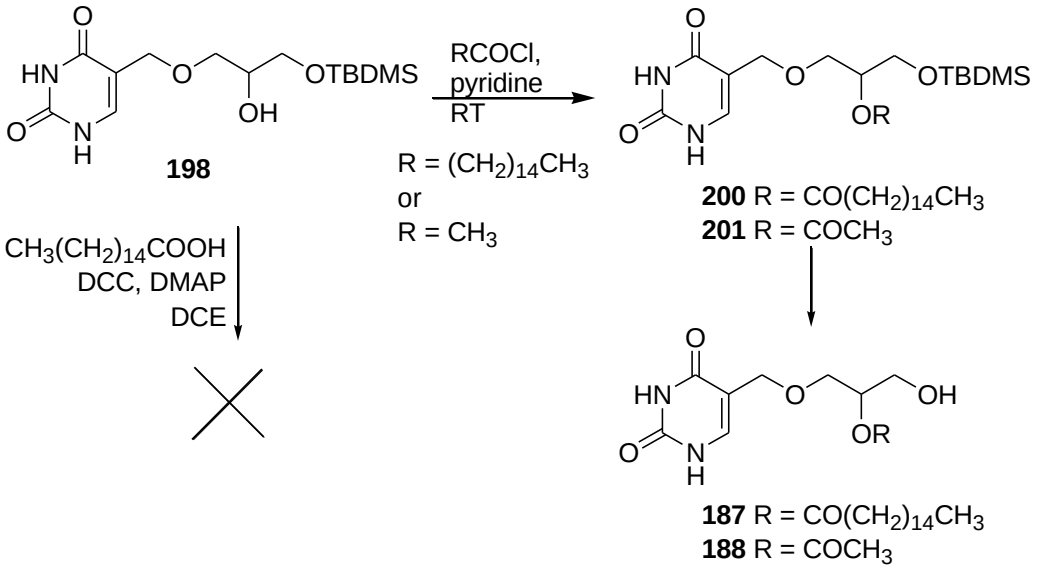
triphenylmethyl chloride led to the mixture of monosubstituted product **196** and starting compound **195**.

Considering these complications occuring already in the first reaction step, alternative TBDMS protecting group was chosen. Compound **195** upon treatment with TBDMS chloride gave desired monosubstituted derivative **198**. However, neither this reaction got along without problems. There were several factors influencing both reaction rate as well as yield of the reaction. Attempts at the best conversion of primary hydroxyl group to ether formation **198** led to the extensive reaction condition studies that are summarized in *Table 9*. The reaction was performed either in DMF, pyridine, DMF/pyridine or pyridine/acetonitrile using TEA, imidazole or NaH as a base or without base. Reaction was further carried out with or without presence of DMAP as a catalyst. All presented reactions were performed at RT over night and outcome for each reaction is evident from *Table 9*. Data introduced in last column represent the approximate percentage of desired derivative **198** according to LC-MS analysis. The best results were achieved using 1.5 equivalents of TBDMSCl, 3 equivalents of TEA and 0.5 equivalent of DMAP in DMF. While using 1.1 equivalent of TBDMSCl resulted in uncompleted conversion, using more than 2 equivalents of TBDMSCl caused the formation of disubstituted derivative **199**. The next crucial factor was reaction temperature. While silylation performed at RT afforded monosubstituted product **198**, increasing of temperature over 40°C gave also disubstituted derivative **199** beside monosubstituted product **198** and so significantly decreased a reaction yield.

Table 9: Summary of reaction conditions for synthesis of derivative **198**.

reaction	solvent	TBDMSCl	base (equiv)	other reactants (equiv)	conversion
a	DMF	1.1 equiv	TEA (2)	-	8%
b	DMF	1.1 equiv	TEA (3)	DMAP (0.5)	12%
c	DMF	1.5 equiv	TEA (3)	DMAP (0.5)	90%
d	DMF	1.1 equiv	imidazole (2.5)	-	20%
e	DMF	2 equiv	imidazole (2.5)	-	30%
f	py	1.1 equiv	imidazole (2)	-	56%
g	py	3 equiv	-	DMAP (0.5)	20%
h	py/AcCN	1.1 equiv	-	-	SC
i	py/AcCN	2 equiv	-	-	9%
j	py/AcCN	2 equiv	-	DMAP (0.25)	15%

Subsequently, the reactivity of secondary hydroxyl group towards various carboxylic acids derivatives was investigated (*Scheme 35*).



Scheme 35. Synthesis of esters **187** and **188**.

Firstly, the attempt to esterify derivative **198** using palmitoyl acid *via* activation with DCC was performed, however unsuccessfully, because all performed reactions gave only starting derivative **198**. The reaction of silyl derivative **198** with 2 equivalents of DCC, 1.2 equivalents of palmitoyl acid and 0.2 equivalent of DMAP was performed in dichloroethane at RT or 0°C, respectively. Neither changes in temperature nor higher amounts of reactants led to desired derivative **200**.

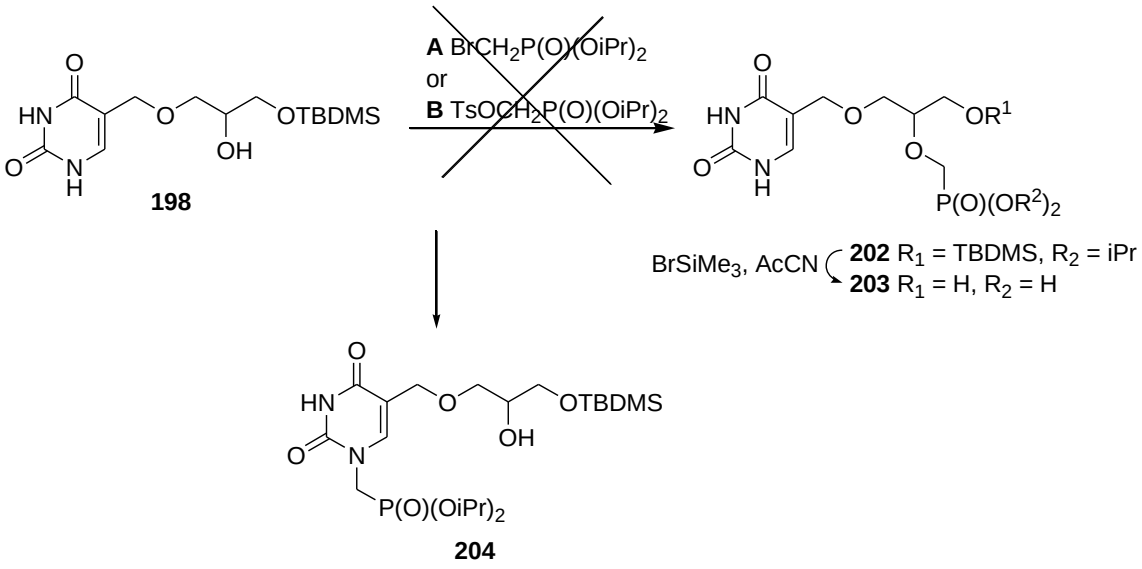
After that, the problem was solved by using much more reactive and easily available palmitoyl chloride. While reaction of **198** with 1.1 equivalents of palmitoyl chloride performed in DMF in the presence of TEA gave only traces of desired ester **200** even after few days, reaction of **198** with 4.5 equivalents of palmitoyl chloride carried out in pyridine afforded crude palmitoyl ester **200** after 2 hours in 68% yield. In effort to purify the crude product **200** by silica gel column chromatography using chloroform/methanol (9/1), protecting silyl group proved to be very sensitive towards this kind of purification and was cleaved to give derivative **187** in 41% total yield.

The same situation occurred for acetyl derivative **188**. Firstly, compound **198** was reacted with 4.5 equivalents of acetyl chloride in pyridine at 0°C-RT for 2 hours.

Reaction gave crude silyl acetyl derivative **201** in 91% yield, but consequent purification by silica gel column chromatography led to compound **188** in 79% yield.

3.2.4.2. Synthesis of phosphonate of 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil

Acyclic nucleoside phosphonates represent a group of nucleotide analogues with a broad spectrum of antiviral and cytostatic activity.[60,61,62] Therefore, I attempted to introduce this biologically active moiety into dihydroxyderivative **195** *via* an alkylation of compound **198** with appropriate phosphonate in effort to prepare phosphonate **210** in stepwise synthesis (*Scheme 36*).



Scheme 36. Synthesis of acyclic nucleoside phosphonate **203**.

Several different reaction conditions were tried in endeavor to prepare intermediate **202** and all of them are shortly summarized in *Table 10*. The reaction was performed either in dioxane or anhydrous DMF, treated either with diisopropyl (bromomethyl)phosphonate or diisopropyl [(tosyloxy)methyl]phosphonate using NaH, K_2CO_3 or *t*-BuOK as a base and various range of temperature. Alkylation by diisopropyl (bromomethyl)-phosphonate in the presence of NaH as base (reaction **a-d**) was initiated either at 0°C or RT, but none of performed reaction showed desired phosphonate **202** at this temperature even after several-days stirring. Consequently, reactions **a-d** were supported by increasing the temperature. While stirring the reaction mixture at 60°C for 6 hours did not bring any significant changes and only traces of

derivative with mass 507 $[M-H]^-$ (corresponded with molecular weight of both compound **202** and **204**) was identifed by LC-MS (reaction **c**), increasing the temperature to 100°C caused a formation of mixture of several compounds (reaction **a**, **b**). As long as the temperature was increased to 80°C, only traces of derivative with mass 507 $[M-H]^-$ were identified again (reaction **d**). On the other hand, replacement of NaH with K_2CO_3 proved better results. The reaction of diisopropyl (bromomethyl)phosphonate with **198** in the presence of K_2CO_3 at 80°C for 8 hours (reaction **e**) showed ratio of conversion approximately 3/1 (compounds **198**/**202** or **204**). Higher amount of base as well as higher temperature resulted in complex mixture of at least 6 compounds (reaction **f**). Hereafter, diisopropyl (bromomethyl)phosphonate was replaced with diisopropyl [(tosyloxy)methyl]-phosphonate and reaction temperature decreased to RT (reaction **g-j**).

The best results were achieved by using of 3 equivalents of phosphonate and 1.2 equivalents of NaH (reaction **h**), when the ratio of conversion was estimated at 65/35 % (**198**/**202** or **204**) according to LC-MS. However, isolation and purification of final product afforded yellow oil in 7% yield, which was characterized by proton NMR and MS analysis as compound **204**.

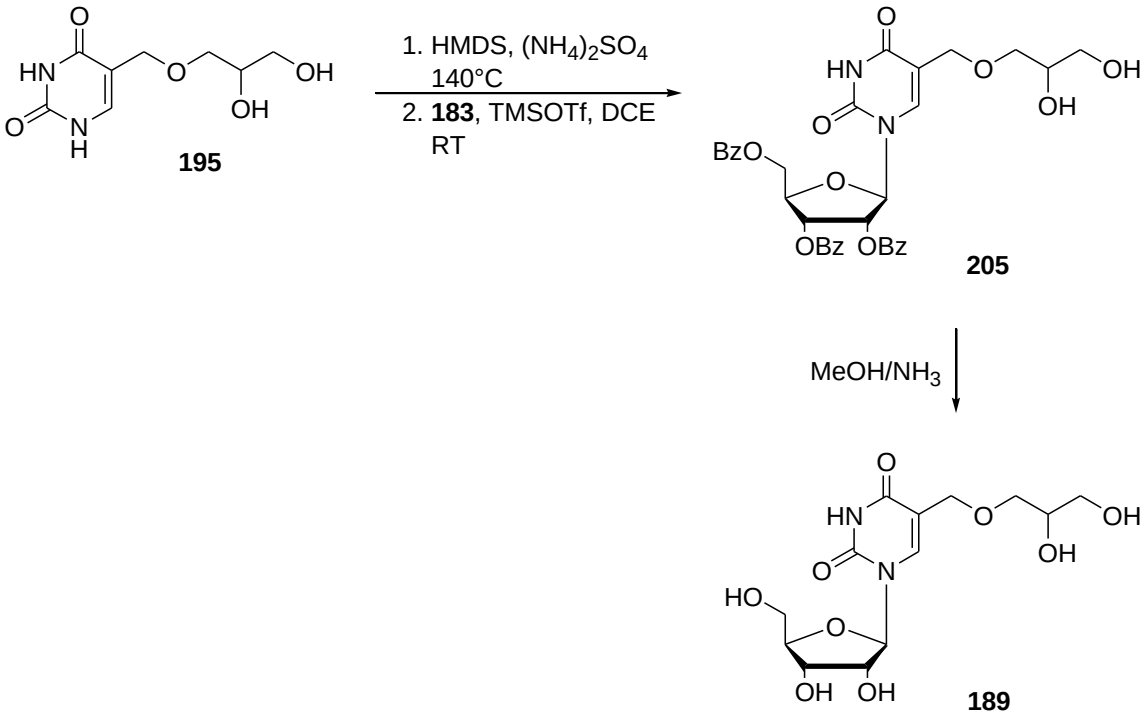
Table 10: Summary of reaction conditions for synthesis of phosphonate **202**.

reaction	phosphonate (equiv)	base (equiv)	solvent	temperature	result (LC-MS)
a	A (1.3)	NaH (1.2)	dioxane	RT→60°C→100°C	mixture of several compounds
b	A (1.3)	NaH (3)	DMF	0°C→RT→100°C	mixture of several compounds
c	A (1.3)	NaH (1.2)	DMF	RT→60°C	traces of 507 $[M-H]^-$
d	A (1.2)	NaH (3)	DMF	0°C→80°C	traces of 507 $[M-H]^-$
e	A (1.2)	K_2CO_3 (3)	DMF	0°C→80°C	30% of 507 $[M-H]^-$
f	A (2)	K_2CO_3 (6)	DMF	0°C→100°C	mixture of several compounds
g	B (1.2)	NaH (3)	DMF	0°C→RT	10% of 507 $[M-H]^-$
h	B (3)	NaH (1.2)	DMF	RT	35% of 507 $[M-H]^-$
i	B (3)	NaH (2)	DMF	RT	20% of 507 $[M-H]^-$
j	B (5)	*t*BuOK (4)	DMF	RT	15% of 507 $[M-H]^-$

To conclude this part, the secondary hydroxyl group of derivative **198** is completely unreactive toward the above mentioned reaction and the alkylation by either diisopropyl (bromomethyl)phosphonate or diisopropyl [(tosyloxy)methyl]phosphonate proceeds on N-1 of uracil ring preferentially.

3.2.4.3. Synthesis of nucleoside analogues of 5-[(2,3-dihydroxy-1-propoxy)methyl)]uracil

Dihydroxyderivative **195** was further modified by ribose moiety affording nucleoside **189** according to synthetic route presented in *Scheme 37*.



Scheme 37. Synthesis of nucleoside **189**.

Initially, compound **195** was ribosylated providing nucleoside **205** in 48% yield using the above mentioned Vorbrüggen method.[53] Thereby prepared protected ribonucleoside **205** was treated with methanolic ammonia solution at room temperature to afford the nucleosides **189**. Since enantiomeric mixture of starting derivative **195** was used, derivative **189,** afforded *via* ribose introduction, was formed as a mixture of two diastereomers with majority excess of one isomer. This fact is evident from HPLC-MS analysis that demonstrates mixture of 2 diastereomers in 8/1 ratio. Proton NMR

spectrum shows overlapped signals and thus this method is insignificant for diastereomers ratio determination. These two diastereomers were not separated and were submitted to biological activity testing as an isomers mixture.

3.2.5. Conclusion

In conclusion, a series of 5-alkoxymethyluracil analogues was synthesized in order to evaluate their chemical and biological properties. Firstly, the attention was paid to synthesis of ethers **185** and **186** with a view to compare a biological activity of prepared compounds with previous nitro analogues **177** and **178**. Modified nucleobases **185** were efficiently prepared *via* nucleophilic substitution of 5-chloromethyluracil **190** and further converted to their corresponding ribonucleosides **186** using Vorbrüggen method.

The studies were further extended to synthesis of acyclic nucleosides **187** and **188** containing modified 2,3-dihydroxy-1-propoxy moiety that were successfully prepared from dihydroxyderivative **195**. Furthermore, dihydroxyderivative **195** was efficiently ribosylated to afford nucleoside **189**. On contrary, secondary hydroxyl group of dihydroxyderivative **195** proved to be completely unreactive toward alkylation by either diisopropyl (bromomethyl)phosphonate or diisopropyl [(tosyloxy)methyl]phosphonate.

Compounds **185** and **186** were screened for their cytostatic properties against diverse range of cancer cell lines. While derivatives with branched side chain **185m-o** and **186m-o** exhibit no significant activity against any of tested cancer cell lines, derivatives **185a-l** and **186a-l** show interesting dependence on the length of alkyl chain. The most interesting results concerning to nucleobases **185** were obtained for drug resistant leukemia cancer cell lines (CEM-DNR-bulk and K562-tax), where an activity is approaching micromolar concentration of IC_{50}. On contrary, introduction of ribose moiety indicates expressive aggravation of activity of nucleosides **186** against drug resistant leukemia cancer cell lines (CEM-DNR-bulk and K562-tax) but increases the cytotoxicity against A549 cell line.

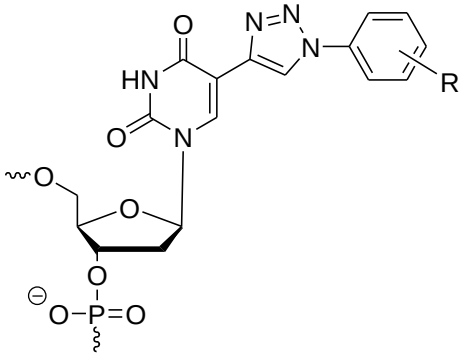
A comparison of cytotoxic activity of bases **177** and **185** indicates that nucleobases **177** exhibits significantly lower activity against CEM-DNR-bulk line but are significantly more active against A549 and both colorectal carcinoma cell lines. Furthermore, cytotoxic activity of nucleosides **178** and **186** show that the nucleosides **178** containing bulky nitrophenyl moiety exhibit significantly higher cytotoxicity than their alkoxymethyluracil analogues **186** against all sreened cell lines.

The evidence of increasing cytotoxic activity specifically in drug resistant cell lines but not in drug sensitive counterparts with growing length of alkyl chain might indicate interaction of long lipophilic groups with multidrug resistance transporters such as P-glycoprotein or the multidrug resistance-associated protein-1.

3.3. Synthesis of modified oligodeoxynucleotides

Modified oligonucleotides are an invaluable tool for study of DNA structure and recognition and have found a large number of applications in the field of antisense therapy,[63,64] antigene therapy[65,66] or DNA detection and sequencing.[67,68]

Presented chapter concerning to modified oligodeoxynucleotides is divided into two parts according to different approaches. The main aim of the first part was synthesis of oligodeoxynucleotides **ON2-ON5** (*Figure 24*) following antisense approach[63] that involves targeting of RNA within cells as a means of control of a gene expression. It has already been found that π-π-stacking between two or more phenyltriazoles in the major groove increase the thermal stability of a DNA:RNA duplex.[69] Therefore, the 9-mer oligodeoxynucletides **ON2-ON5** were synthesized in order to evaluate the effect of hydroxyl group attached to the phenyl moiety on the duplex stability and compare it with unmodified phenyl analogues **ON6** and **ON7**.[69]



X R = OH, *p*-substitution
Y R = OH, *m*-substitution
Z R = H

ON1 5´-d(GTG TTT TGC)-3´
ON2 5´-d(GTG T**X**T TGC)-3´
ON3 5´-d(GTG **XXX X**GC)-3´
ON4 5´-d(GTG T**Y**T TGC)-3´
ON5 5´-d(GTG **YYY Y**GC)-3´
ON6 5´-d(GTG T**Z**T TGC)-3´
ON7 5´-d(GTG **ZZZ Z**GC)-3´

Figure 24. Summary of oligodeoxynucleotides **ON1-ON7**.

The second approach followed the idea of postsynthetic labeling of DNA.[70] Current studies present interesting method of postsynthetic labeling of alkynemodified DNA, which is based on Huisgen cycloaddition reaction of terminal alkynes attached to the strand of DNA and azides attached to fluorescently tagged building block.[71,72] Therefore, the oligonucleotide **ON9** was synthesized with a view to postsynthetic conversion of bromo group to azide (**ON10**) (*Figure 25*). This azidomodified DNA might be further reacted with various fluorescently tagged building blocks bearing alkyne.

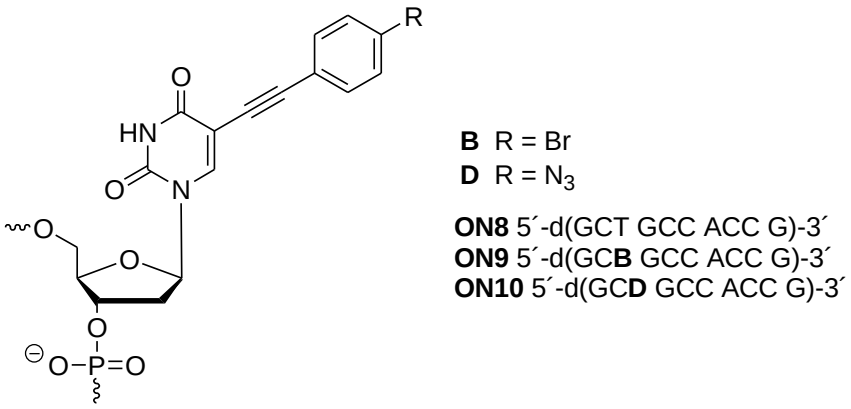


Figure 25. Summary of oligodeoxynucleotides **ON8-ON10**.

The synthesis of oligodeoxynucleotides is based on the four-step process which utilizes the reaction of deoxynucleoside phosphoramidites with deoxynucleoside or oligodeoxynucleotide attached to the solid-phase (*Figure 26*).

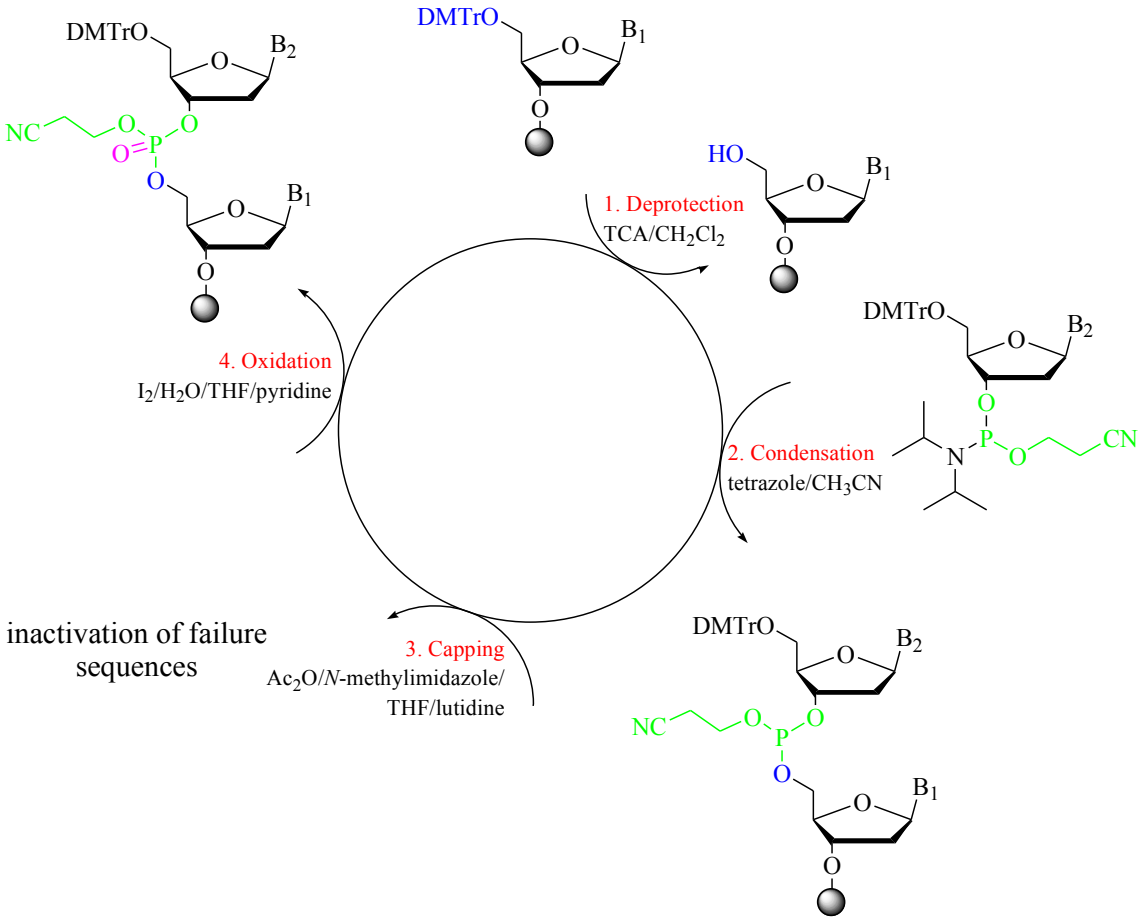


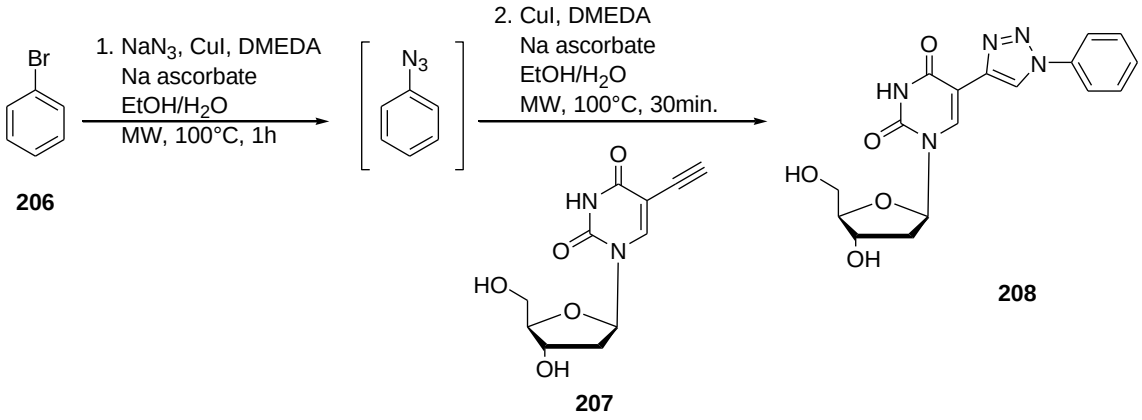
Figure 26: Basic steps in a cycle of nucleotide addition by the phosphoramidite method.

Initially the 5´-*O*-dimethoxytrityl (DMTr) group is removed from a deoxynucleoside linked to the polymer support. Detritylation is carried out with dichloroacetic or trichloroacetic acid in dichloromethane. The next step is elongation of a growing oligodeoxynucleotide, occurring *via* the initial formation of a phosphite triester internucleotide bond. This reaction product is first treated with a capping agent designed to esterify failure sequences and cleave phosphite reaction products on the heterocyclic bases. This is carried out using a mixture of acetic anhydride/2,6-lutidine and *N*-methylimidazole each in tetrahydrofuran (THF). The nascent phosphite internucleotide linkage is then oxidized to the corresponding phosphotriester. Oxidation of the intermediate phosphite to the phosphate triester is achieved with iodine and water in THF. Pyridine or 2,6-lutidine is added to neutralize the hydrogen iodide liberated. Further repetitions of this four-step process generate the oligodeoxynucleotide of desired length and sequence. The final product is cleaved from the solid phase by treatment of the support with concentrated ammonium hydroxide and free base with the β-cyanoethyl phosphate protecting groups is obtained. Synthesis by this method is carried out in the 3´→5´ direction.

3.3.1. Synthesis of triazole derivatives

This part of thesis is focused on synthesis of oligodeoxynucleotides **ON2-ON5** containing monomers **X** and **Y** (*Figure 24*, page 74). These derivatives are modified with triazole ring attached to the 5 position of 2´-deoxyuridine further substituted with *p*- or *m*-phenol. The duplex stability of **ON1-ON5** was investigated in order to evaluate an effect of hydroxyl group on duplex stability compared with oligodeoxynucleotides **ON6** and **ON7** with unsubstituted phenyl ring (monomer **Z**) that has been prepared previously.[69]

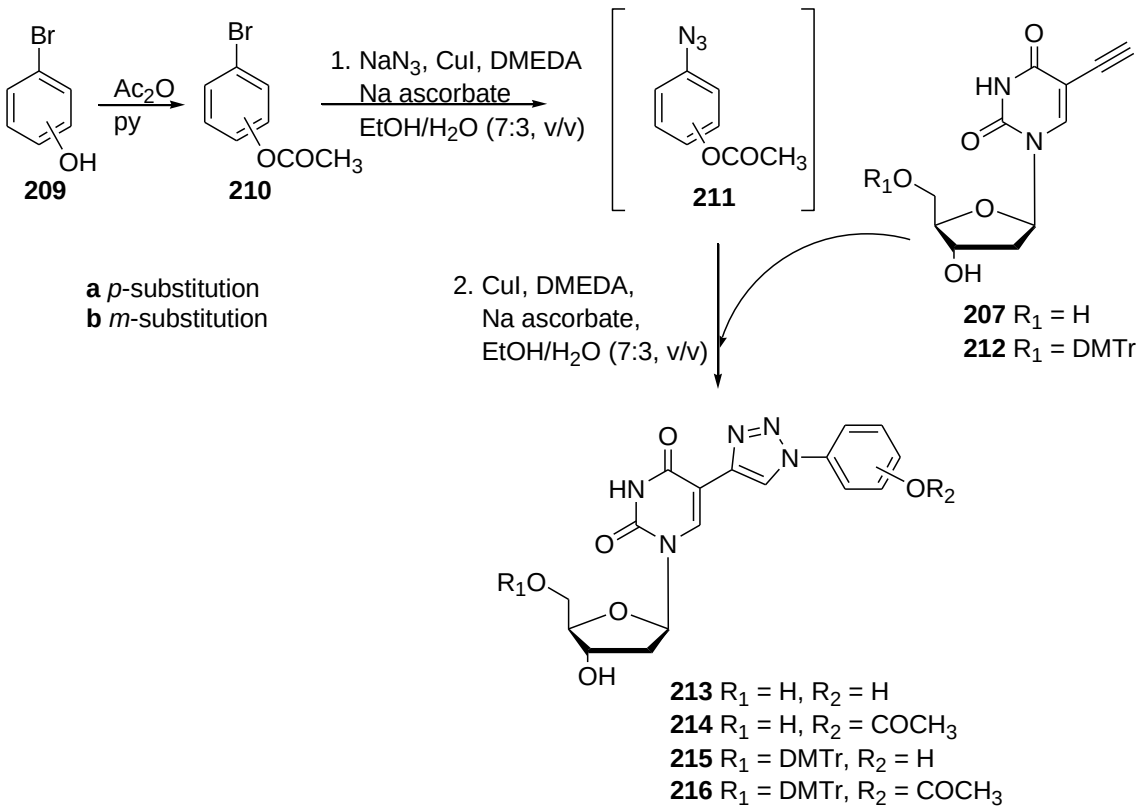
The primary strategy of synthesis was inspired by reaction already described for synthesis of derivative **208** (*Scheme 38*) based on one-pot procedure providing phenylazide from phenylbromide with subsequent Huisgen [3+2]cycloaddition reaction between alkyne **207** and azide.[69] This one-pot procedure is highly advantageous way due to the short reaction times and high yields.



Scheme 38. Synthesis of nucleoside **208**.

The using of bromophenol required protection of hydroxyl group in order to prevent from failure sequences during the oligodeoxynucleotide synthesis. This protecting group must be sufficiently stable during all synthetic steps but easily removable after oligodeoxynucleotide synthesis.

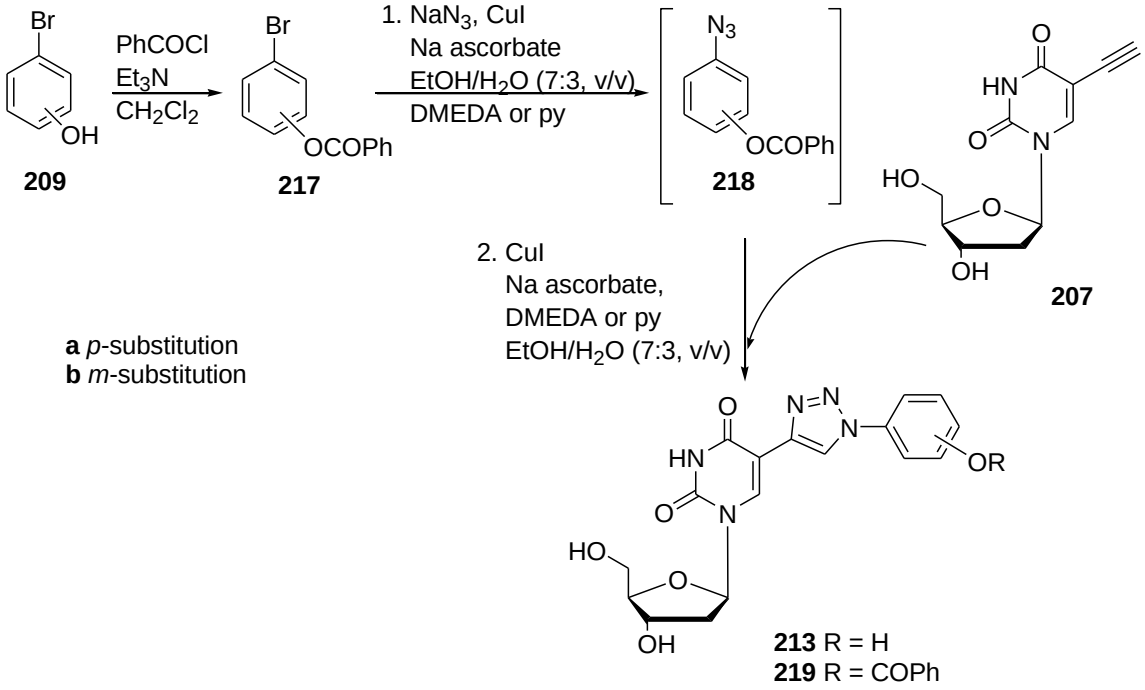
First attempts at protection were aimed to acetylation by reason that this group is easily removable after the synthesis of oligodeoxynucleotide. For this purpose the starting bromophenols **209** were acetylated to afford compounds **210** (*Scheme 39*).



Scheme 39. Reactivity of acetylbromophenols **210.**

The one-pot reaction of acetylbromophenol **210a** with sodium azide and 5-ethynyl-2′-deoxyuridine **207** prepared from commercially available 5-iodo-2′-deoxyuridine according to known procedure[73] gave click products with free hydroxyl group **213a** instead of protected compounds **214a**, though. This might be a consequence of the presence of alkaline *N,N′*-dimethylethylenediamine. Simultaneously, the one-pot reaction of acetylbromophenol **210a** with sodium azide and 5′-*O*-(4,4′-dimethoxytrityl)-5-ethynyl-2′-deoxyuridine **212** was carried out. However, this reaction also gave unprotected derivative **215a** instead of the desired nucleoside **216a**.

Afterwards, more stable benzoyl protecting group was tested (*Scheme 40*).

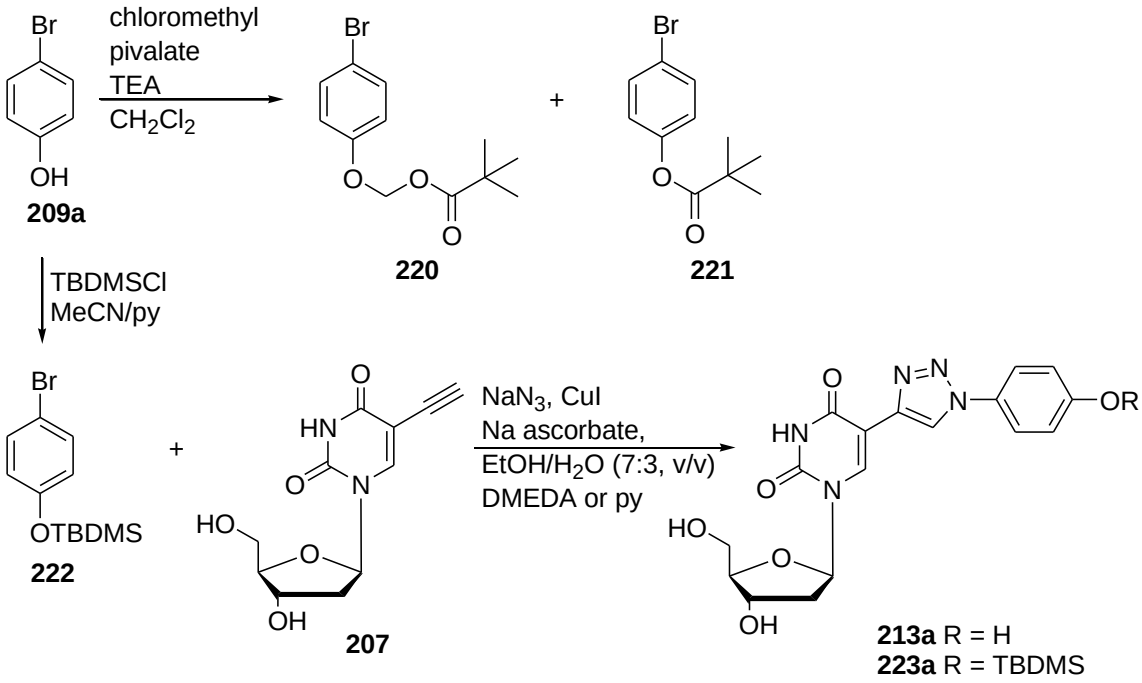


Scheme 40. Reactivity of benzoylbromophenols **217**.

Benzoylated bromophenol **217a** were reacted with sodium azide in the presence of *N,N′*-dimethylethylenediamine and subsequently with 5-ethynyl-2′-deoxyuridine **207**. Nevertheless, even this one-pot reaction resulted in the nucleoside **213a** with free hydroxyl group instead of protected derivative **219a**. While the presence of *N,N′*-dimethylethylenediamine caused deprotection of hydroxyl group, a milder base such as pyridine turned out to be insufficient for the formation of azides **218**.

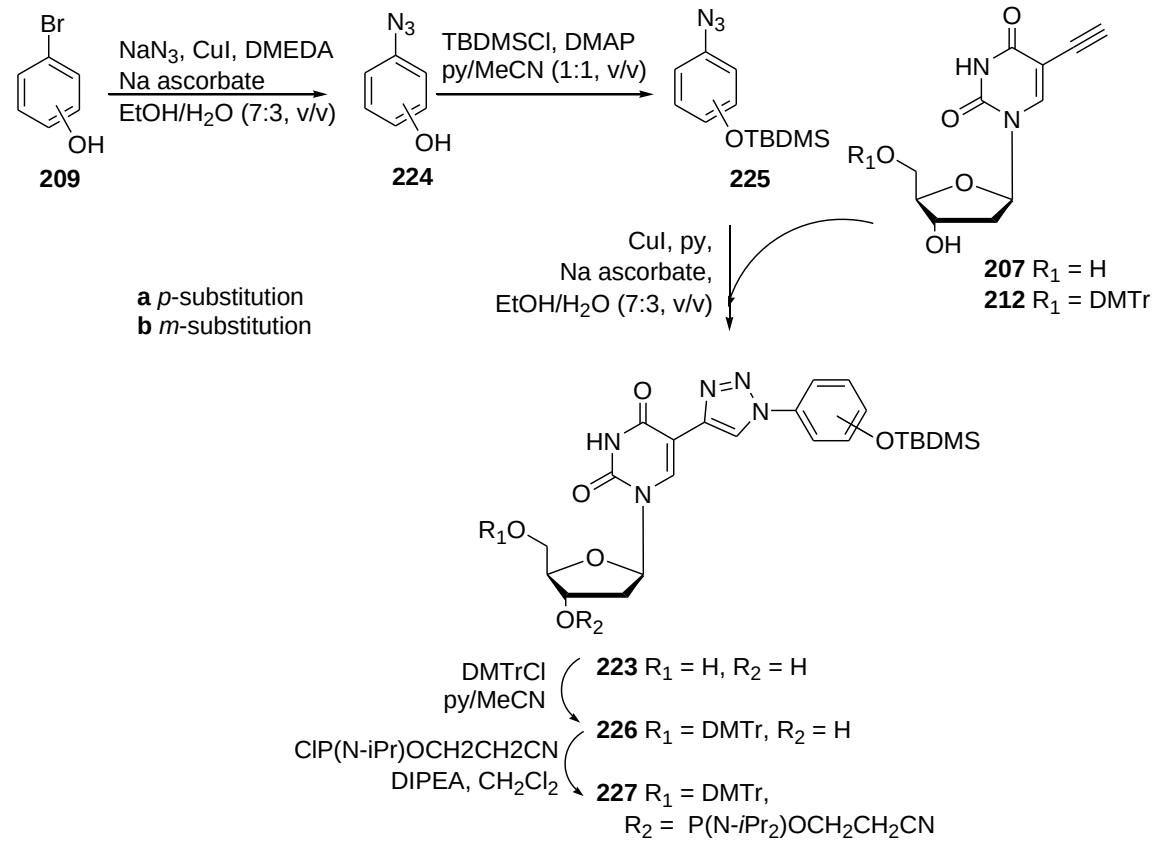
The above mentioned results led to a searching for more stable protecting group towards *N,N′*-dimethylethylenediamine, which seems to be essential for the formation

of the azide. Since ether bond should be more stable then ester bond, I concentrated my efforts on preparation of intermediates with ether formation (*Scheme 41*). Attempt to prepare pivalate derivative **220** failed due to the obtaining the mixture of two compounds **220** and **221**, where an unadvisable product **221** was in excess. On the other hand, the silylation of *p*-bromophenol **209a** was much more efficient and silylated derivative **222** was afforded. Nevertheless, the next step was not successful. The one-pot click reaction performed in the presence of *N,N´*-dimethylethylenediamine gave unprotected nucleoside **213a**, while using of pyridine was not sufficient for the formation of azide.



Scheme 41. Synthesis of protected bromophenols **220** and **222** *via* ether formation.

Finally, the strategy was changed and target compounds **227** have been obtained as shows *Scheme 42*. This strategy was based on the formation of azides **224** in a first step with subsequent protection of hydroxyl group leading to derivatives **225**. Azides **225** were further reacted with 5-ethynyl-2´-deoxyuridine **207** in next step.



Scheme 42. General synthetic route leading to phosphoramidites **227.**

Azides **224a** and **224b** were prepared from bromoderivatives **209** according to known procedure[74] in 72% and 78% yields, respectively. These azides **224** were further treated with *t*-butyldimethylsilyl chloride in the presence of 4-dimethylaminopyridine in pyridine/acetonitrile to afford silylated *p*- and *m*-azides **225** in 90% and 81% yields, respectively.

Huisgen [3+2] cycloadition of sylilated compounds **225** with 5-ethynyl-2´-deoxyuridine **207** gave corresponding triazole substituted nucleosides **223**. Subsequent tritylation of **223** with DMTrCl afforded nucleosides **226**. Moreover, nucleosides **226** were also prepared by one-step procedure based on reaction of 5´-*O*-(4,4´-dimethoxytrityl)-5-ethynyl-2´-deoxyuridine **212** and sylilated compounds **225**. This procedure proved higher yields.

As evidence by above mentioned reactions, conditions used in the previous work for the preparation of the similar compounds[69] had to be changed. Previous work utilized one-pot reaction, microwave irradiation and *N,N´*-dimethylethylenediamine as a base that turned out to be improper for mentioned reactions due to the formation of mixture of several compounds and too low yields. In my hand, reaction was performed in two-

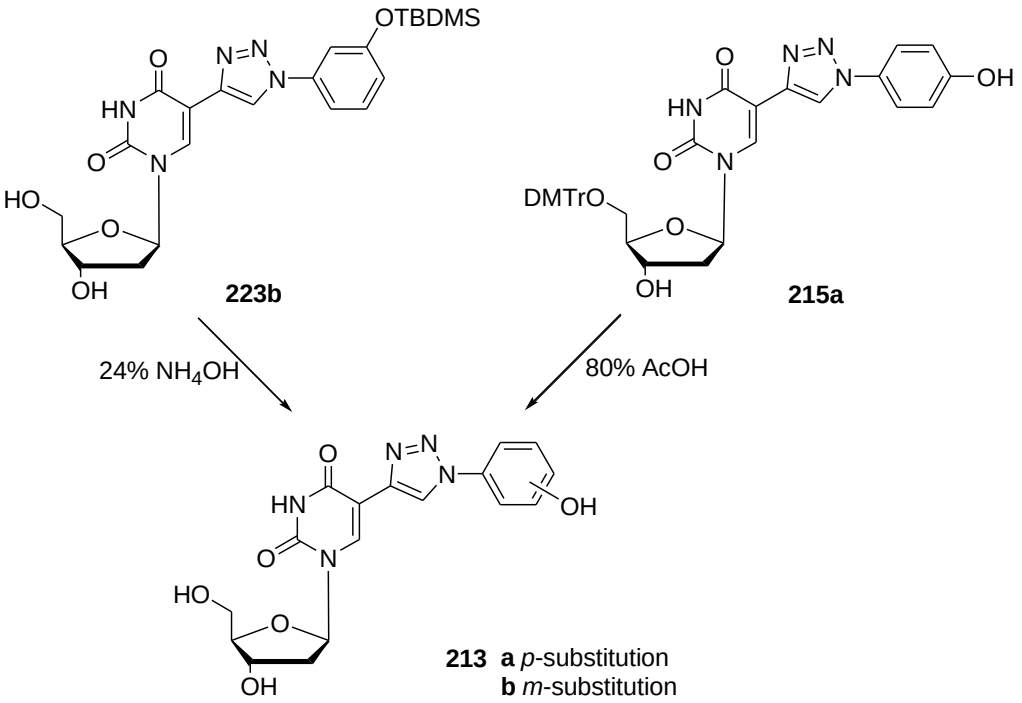
steps procedure. And further, decrease of temperature, increase of reaction time and replacing of *N,N´*-dimethylethylenediamine with pyridine gave nucleosides **226** in yields 53-78%.

Finally, phosphitylation of nucleosides **226** with 2-cyanoethyl-*N,N´*-(diisopropyl)-phosphoramidochloridite in the presence of diisopropylethylamin gave phosphoramidites **227**. These final nucleotides had to be purified by silica gel column chromatography (pre-equilibrated with 5% pyridine) using acetone in dichloromethane (2-5%). Since nucleotides **227** are very unstable, purification was the crucial step. Despite of instability of amidites, *p*- and *m*-amidites **227** were obtained in satisfactory 74% and 70% yields, respectively.

Both gained nucleotides **227** were incorporated into 9-mer oligodeoxynucleotides **ON2-ON5** *via* solid-phase synthesis using automated DNA synthesizer (*Figure 24*, page 74). Several different reaction conditions for the synthesis of mentioned oligodeoxynucleotides **ON2-ON5** were performed. Every experiment was carried out in acetonitrile with coupling time between 20-40 minutes and either tetrazole or pyridinium hydrochloride as the activator. It was found that the best way is using of pyridinium hydrochloride with coupling time 30 minutes. Increasing of coupling time to 40 minutes had no effect. The final product was cleaved from the solid phase by treatment of the support with concentrated aqueous ammonia. This treatment also removed protecting silyl group of phenol. The structure of all prepared oligodeoxynucleotides were confirmed by MALDI-MS and purity controlled by RP-HPLC.

Above described synthesis afforded oligodeoxynucleotide **ON2** containing *p*-derivative in 66% yield. Same reaction performed with *m*-derivative gave oligodeoxynucleotide **ON4** in 54% yield. The four consecutive incorporations of *p*-derivative furnished sequence **ON3** in 18% yield. The coupling efficiency monitored *via* measuring of intensity of orange colour from the liberated dimethoxytrityl cation noted decreasing of yield with every other incorporation. Ability of four consecutive incorporations of *m*-derivative to sequence **ON5** was even worse. The oligodeoxynucleotide **ON5** was prepared in 14% yield. However, owing to low yield and the presence of a large number of impurities (failure sequences), we were not able to find conditions for RP-HPLC purification and have not been able to get pure **ON5** yet.

Finally, monomers **213a** and **213b**, required for estimation of extinction coefficients[b], were prepared from intermediates of previous syntheses **223b** and **215a** (*Scheme 43*). Silyl protecting group of derivative **223b** were removed by treatment with methanolic ammonia solution to afford nucleoside **213b**. Nucleoside **215a** under treatment with acetic acid gave compound **213a**.



Scheme 43. Synthesis of nucleosides **221**.

3.3.1.1. Hybridization experiments of triazole derivatives

The series of prepared oligodeoxynucleotides **ON2-ON4** were submitted to hybridization studies, which were performed by UV spectroscopy. The oligonucleotides **ON2-ON4** were mixed with complementary DNA and RNA sequences and melting temperatures for all duplexes were determined (*Table 11*). Furthermore, all results were compared with previously reported conclusions[69] for monomer **Z** incorporated into oligodeoxynucleotides **ON6-ON7** (*Figure 24,* page 74). When the central thymidine was replaced with monomers **X** and **Y**, a clear destabilization was observed in the case

[b] Extinction coefficients are used to determine oligonucleotide concentrations. It is defined as the absorbance at 260 nm of a 1 M aqueous solution measured at 20 °C in an optical cell with 1 cm pathway (Lambert-Beer's law).

of DNA as well as RNA. The replacing of four thymidines by monomer **X** showed destabilization in the case of DNA (ΔT_m = -1.1°C), however, stabilization for RNA (ΔT_m = +4.8°C).

Table 11: Hybridization data for DNA:DNA and DNA:RNA duplexes[a]

		T_m (ΔT_m)/°C[b]			
		DNA	RNA	[c]DNA	[c]RNA
ON1	5′-d(GTG TTT TGC)-3′	33.3	32.0	33.0	31.0
ON2	5′-d(GTG T**X**T TGC)-3′	28.3 (-5.0)	30.4 (-1.6)	-	-
ON3	5′-d(GTG **XXX X**GC)-3′	29.1 (-1.1)	51.1 (+4.8)	-	-
ON4	5′-d(GTG T**Y**T TGC)-3′	27.8 (-5.5)	30.4 (-1.6)	-	-
ON6	5′-d(GTG T**Z**T TGC)-3′	-	-	28.0 (-5.0)	29.0 (-2.0)
ON7	5′-d(GTG **ZZZ Z**GC)-3′	-	-	32.0 (-0.2)	51.5 (+5.1)

[a] Target sequence 3′-d(CAC AAA ACG)-5′ and 3′-r(CAC AAA ACG)-5′.
[b] Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a medium salt buffer (Na_2HPO_4 (5 mM), NaCl (100 mM), EDTA (0.1 mM), pH 7.0) using 1.5 µM concentrations of each strand. Values in brackets show the changes in melting temperature for each modification relative to the reference strand **ON1**.
[c] Results for monomer **Z** from previously reported work.[69]

Furthermore, the mismatch studies have been carried out. The oligonucleotide with four stacking triazoles **ON3** was mixed with DNA and RNA complements containing the three possible single mis-matches in a central position and the melting temperatures of the mis-matched duplexes were determined (*Table 12* and *13*).

Table 12: Mismatch studies with DNA

		T_m (ΔT_m)/°C[a]	
		ON3 5′-d(GTG **XXX X**TG)-3′	[b]**ON7** 5′-d(GTG **ZZZ Z**TG)-3′
Match strand	3′-d(CAC AAA ACG)-5′	28.7	32.0
A:C mismatch	3′-d(CAC ACA ACG)-5′	12.6 (-16.1)	13.0 (-19.0)
A:G mismatch	3′-d(CAC AGA ACG)-5′	18.6 (-10.1)	16.0 (-16.0)
A:T mismatch	3′-d(CAC ATA ACG)-5′	17.6 (-11.1)	16.5 (-15.5)

[a] Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a medium salt buffer (Na_2HPO_4 (5 mM), NaCl (100 mM), EDTA (0.1 mM), pH 7.0) using 1.5 µM concentrations of each strand. Values in brackets show the changes in Tm values relative to the matched duplex. ¨
[b] Results for monomer **Z** from previously reported work.[69]

Table 13: Mismatch studies with RNA

		T_m (ΔT_m)/°C[a]	
		ON3	[b]**ON7**
		5′-d(GTG **XXX X**TG)-3′	5′-d(GTG **ZZZ Z**TG)-3′
Match strand	3′-r(CAC AAA ACG)-5′	50.9	51.5
A:C mismatch	3′-r(CAC ACA ACG)-5′	26.5 (-24.4)	24.0 (-27.5)
A:G mismatch	3′-r(CAC AGA ACG)-5′	41.1 (-9.8)	42.0 (-9.5)
A:U mismatch	3′-r(CAC AUA ACG)-5′	28.7 (-22.2)	31.0 (-20.5)

[a,b] see Table 12

The significant decrease in T_m was observed in all cases except the case, where A is replaced by G in RNA:DNA mixed duplex. Similar observations were reported earlier,[69] which means that the presence of hydroxyl group at the phenyl moiety has scarcely any influence on the duplex stability in compare with unsubstituted phenyl moiety on the triazole ring. The same conclusion can be observed from modeling of duplexes of oligodeoxynucleotides **ON3** and **ON7** that was created by Nicolai Krog Anderesen and is presented in *Figure 27*.

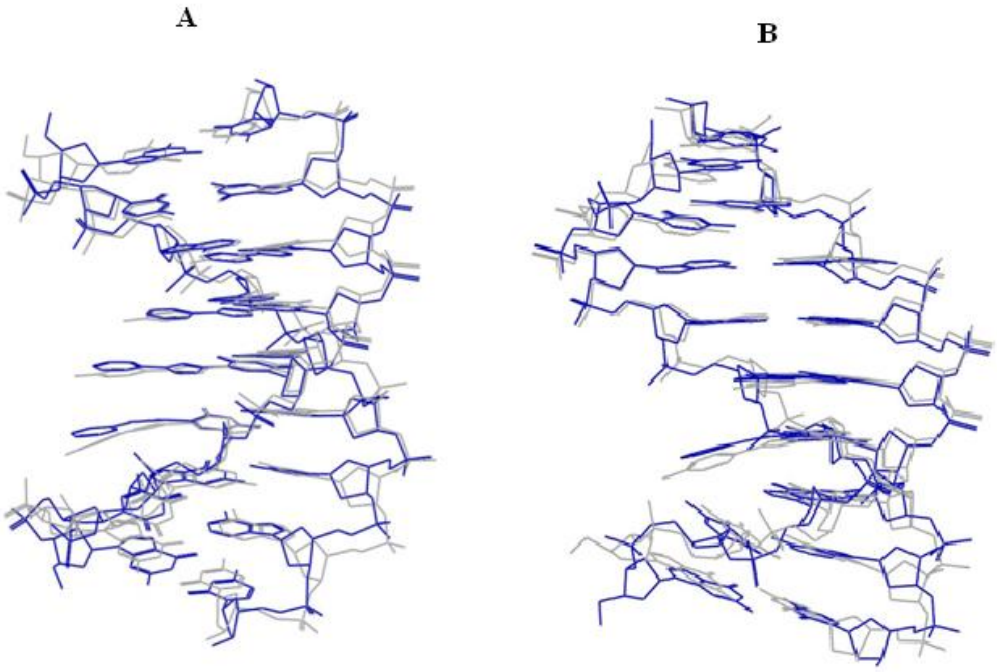


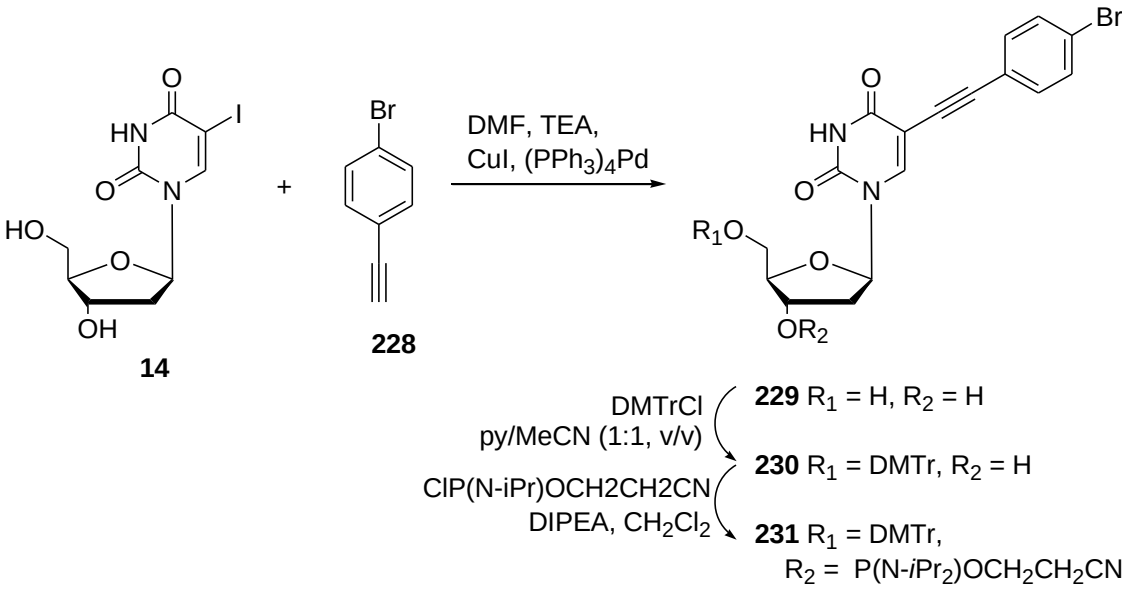
Figure 27. Modeling of DNA duplexes of oligodeoxynucleotide **ON3** and **ON7**.
Both pictures **A** and **B** present 9-mer sequence of DNA, where one strand is modified either by monomer **X** (grey structure) or monomer **Z** (blue structure). Thus, the grey structure represent DNA derived from oligodeoxynucleotide **ON3** and blue structure DNA derived from oligodeoxynucleotide **ON7**. Both pictures differ only in line of vision.

The oligonucleotide sequence used was investigated through a series of force field simulations and built as a standard B-type duplex. These modeling results indicate a resemblance of DNA duplexes containing **ON3** and **ON7**.

3.3.2. Synthesis of bromophenylethynyl derivatives

This part of the thesis is focused on synthesis of oligodeoxynucleotide **ON9** containing monomer **B** (*Figure 25,* page 75) with subsequent postsynthetic substitution of bromo group with azide leading to oligodeoxynucleotide **ON10**.

The synthesis of desired phosphoramidite **231** (*Scheme 44*) started with the Sonogashira coupling reaction[73] of 5-iodo-deoxyuridine **14** with 1-bromo-4-ethynylbenzene **228** to afford nucleoside **229** in quantitative yield. The easy obtaining of pure compound **229** in high yield without further purification was based on the insolubility of this derivative **229** and solubility of impurities in dichloromethane.

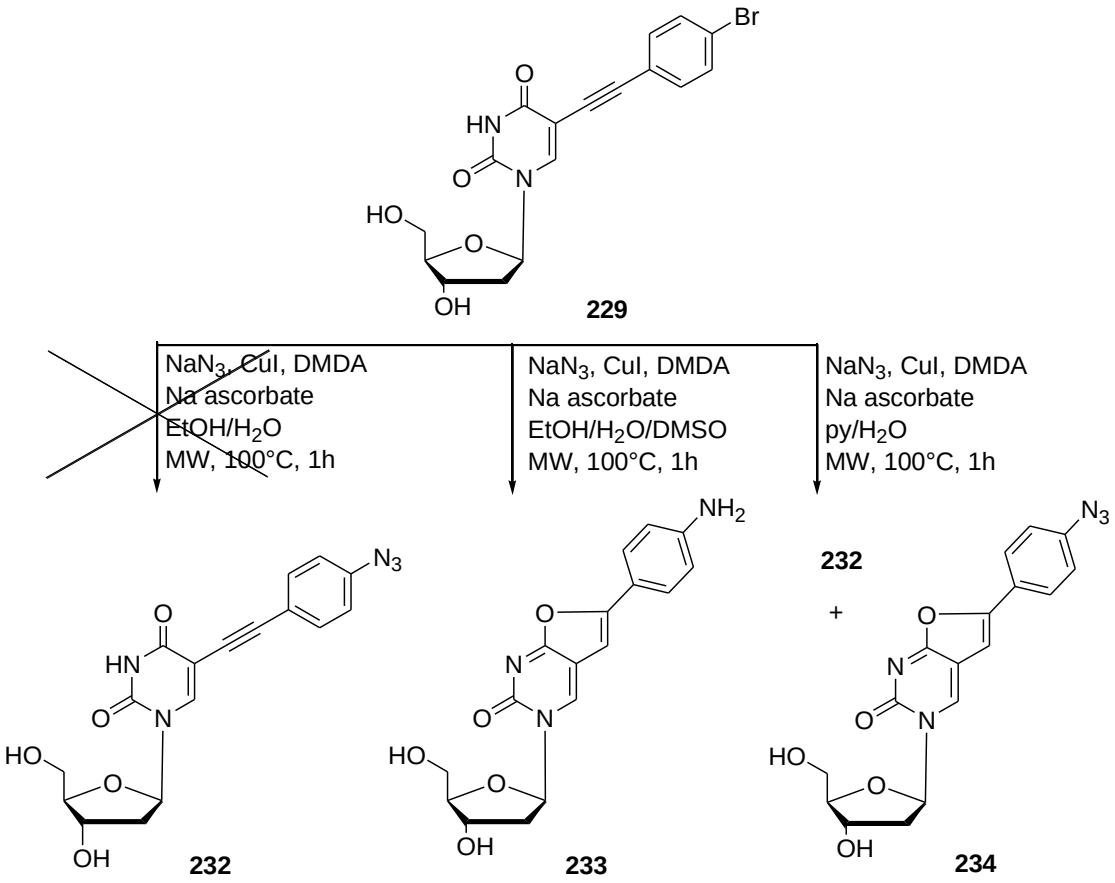


Scheme 44. General synthetic route leading to nucleotide **231.**

The next step was protection of 5´-hydroxyl group with 4,4´-dimethoxytrityl moiety leading to dimethoxytrityl derivative **230** in 61%. Synthetic route has been followed by preparation of desired phosphoramidite **231** by the standard 3´-*O*-phosphytilation using 2-cyanoethyl-*N,N´*-(diisopropyl)-phosphoramidochloridite in dichloromethane.

The intermediate **231** was further incorporated into 10-mer sequence – 5´-d(GC**B** GCC ACC G)-3´ (**ON9)**, where **B** represents modified nucleotide. Oligonucleotide **ON9** was synthesized using automated solid phase DNA synthesizer in different scales[c]. The first oligonucleotide synthesis was performed in 0.2 μmol scale and the second one in 1 μmol scale. In both cases, either 0.2 μmol scale or 1 μmol scale, phosphoramidite **231** proved very good coupling efficiency[d] and overall yield was more than 95%. Pyridinium hydrochloride was used as an activator with 20 minutes coupling time.

Furthermore, I attempted to convert the nucleoside **229** to appropriate azide **232** (*Scheme 45*).



Scheme 45. Synthesis of azide **232.**

The most difficult point had to be solved during this synthetic route was low solubility of nucleoside **237** in common solvents such as DMF, DMSO, DCM, MeCN, H_2O,

[c] Scale of synthesis refers to the amount of starting CPG (controlled-pore glass) support-bound monomer used to initiate the DNA synthesis.

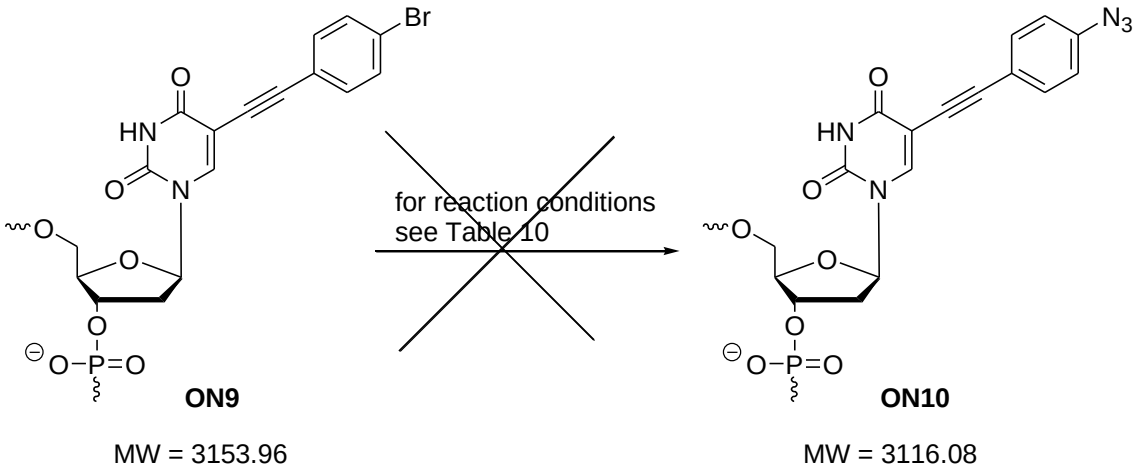
[d] Coupling efficiency is a measure of the DNA synthesizer´s ability to couple each new monomer to the growing chain.

MeOH, etc. When the mixture of bromo derivative **229** in EtOH/H_2O (7:3, *v/v*) in the presence of sodium azide (1 mol eq.), sodium ascorbate (0.05 mol eq.), copper iodide (0.1 mol eq.) and *N,N´*-dimethylethylenediamine (0.15 mol eq.) was subjected to microwave irradiation at 100°C for 1 hour only the starting compound **229** was obtained. Addition of DMSO to the reaction mixture and increasing time of microwave irradiation led to obtaining of mixture of two compounds – desired azide **232** and starting material **229**. Unfortunately, finding of efficient conditions for separation has been unsuccessful, mainly due to the low solubility of both compounds **229** and **232**.

While increasing of amount of sodium azide (2 mol eq.) has also led to the mixture of desired azide and starting material, increased amount of all reagents gave reduced compound - amine **233**. Probably too high amount of Cu^{I+} salt (0.5 mol eq.) took effect like a reductant.

Finally, it has been tried to use pyridine in water like a solvent due to the better solubility of bromo derivative **229** in pyridine in comparison to DMSO or DMF. Unfortunately, also this procedure led to the mixture of two compounds, desired azide **232** and compound **234**. I attempted to separate these two derivatives by silica gel column chromatography using 10% MeOH in toluene/acetonitrile (5:2, *v/v*), however, unsuccesfuly. NMR analyses showed the mixture of two compounds **232** and **234**. Further attempts at separation have not been performed yet.

Attempts at conversion of bromo group to azide at oligodeoxynucleotide level were absolutely unsuccessful (*Scheme 46*). All of them are summarized in *Table 14.*



Scheme 46. Synthesis of oligodeoxynucleotide **ON10.**

Table 14: Summary of reaction conditions for postoligosynthesis of **ON10**.

ON9 (0.01 µmol)	NaN_3 (µmol)	CuI (µmol)	$CuSO_4.5H_2O$ (µmol)	sodium ascorbate (µmol)	N,N´-DMDA (µmol)	TEA (µmol)	solvent	conditions	result
a	0.02	0.001	-	0.0005	0.0015	-	H_2O / EtOH	MW, 100°C, 30 min	3152.7
b	0.02	0.001	-	0.0005	0.0015	-	H_2O / EtOH	MW, 100°C, 10 min	3151.4
c	0.02	0.001	-	0.0005	0.0015	-	H_2O / EtOH	100°C, 60 min	3151.4
d	0.02	0.001	-	0.0005	0.0015	-	H_2O / EtOH	RT, over night	3151.4
e	0.05	-	0.01	0.02	0.02	-	H_2O / EtOH	MW, 100°C, 60 min	3152.2
f	0.5	-	0.2	0.2	1	-	H_2O / EtOH	MW, 100°C, 30 min	*
g	0.2	-	0.1	2	-	2	H_2O	MW, 100°C, 30 min	*
h	0.2	-	0.1	2	-	2	H_2O / DMSO	MW, 100°C, 30 min	*
i	0.2	-	0.1	2	-	2	H_2O / DMSO	RT, over night	*
j	0.1	-	0.02	0.5	-	0.5	H_2O	MW, 60°C, 10 min	*
k	0.1	-	0.02	0.5	-	0.5	H_2O	RT, over night	*
(0.05 µmol)									
l	1	-	0.5	10	-	10	H_2O	MW, 70°C, 15 min	3153.9
m	5	-	1	10	-	10	H_2O	MW, 100°C, 20 min	3153.3
n	5	-	2.5	5	2.5	-	H_2O / EtOH	MW, 100°C, 20 min	*

* Unreadable MS spectrum.

Several different reaction conditions according to known postoligo synthetic routes[75,76] were tried but none of them has led to desired azido-modified oligodeoxynucleotide **ON10**. In the most cases the microwave irradiation with different time and different temperature was used. On the other hand the room temperature was also tested. The *Table 14* clearly shows used reagents and their amounts and also used solvents and conditions. After each reaction, the mixture was evaporated and analyzed by MALDI-TOF MS. In some cases only the peak of starting oligodeoxynucleotide **ON9** (molecular weigth 3153.96) was detected. On the other hand, in some cases the product had to be desalted using NAP-10 column first (reaction **l** and **m**). The results of several reactions were not identified due to unreadable MS spectrum.

3.3.2.1. Hybridization experiments of bromophenylethynyl derivatives

Melting experiments were carried out for oligodeoxynucleotide **ON9** and melting temperatures were obtained from the UV melting curves at neutral pH (*Table 15*). The melting temperatures obtained for the duplex between oligonucleotide **ON9** and DNA-complement with the corresponding unmodified duplex have been compared and small destabilization has been found (ΔT_m = -1.7°C). Moreover, the extinction coefficient of compound **237** was determined (16 293 $mol^{-1}L^{-1}$).

Table 15: Melting temperatures obtained from the UV melting curves (A_{260} vs. temperature) at neutral pH = 7 (110 mM sodium buffer).

		DNA-complement 3′-d(CGA CGG TGG C)-5′ **T_m (ΔT_m)/°C** [a]
ON8	5′-d(GCT GCC ACC G)-3′	52.5
ON9	5′-d(GC**B** GCC ACC G)-3′	50.8 (-1.7)

[a] Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a medium salt buffer (Na_2HPO_4 (5 mM), NaCl (100 mM), EDTA (0.1 mM), pH 7.0) using 1.5 μM concentrations of each strand. Value in brackets shows the changes in melting temperature for each modification relative to the reference strand **ON8**.

3.3.3. Conclusion

In conclusion, nucleosides **223** were successfully prepared employing click chemistry, converted to their corresponding phosphoramidites **227** and incorporated into

the oligodeoxynucleotides **ON2-ON5**. The oligonucleotides **ON2-ON4** were mixed with complementary DNA and RNA sequences and melting temperatures for duplexes forms were obtained. While a replacing of central thymidine either with monomer **X** or monomer **Y** in oligodeoxynucleotides **ON2** and **ON4** led to a clear destabilization for both DNA and RNA duplexes, the replacing of four thymidines by monomer **X** showed significant stabilization ($\Delta T = +4.8°C$). Since the stacking of triazole analogues in the major groove led to very stable DNA:RNA duplexes, the above mentioned monomer **X** shows promising therapeutically potential *via* RNA-targeting.

Moreover, derivative **229** was successfully and efficiently incorporated into oligodeoxynucleotide **ON9** *via* standard phosphoramidite method. The reactivity of bromo group of **ON9** containing monomer **B** was further studied following the idea of postsynthetic labeling of DNA. Despite the unsuccessful postsynthetic conversion of bromo group to azide, the studies of the same reaction on the nucleoside level showed promising results. Nevertheless, the primary idea of postsynthetic substitution of bromo group with azide might be further modified. Oligonucleotide **ON9** contains also ethynylen moiety that might be further used for building up of new heterocycle and so influence a fluorescence properties of new oligodeoxynucleotide.

4. Summary

In summary, a number of novel compounds was synthesized in order to evaluate their chemical and biological properties and explore the structure-activity relationships.

Firstly, the extensive studies of available information on synthesis and biological activity evaluation of 5-alkoxymethyluracil analogues were performed and summarized in review part.

Further, the synthesis of a diverse range of 5-alkoxymethyluracil analogues bearing bulky 4-nitrophenyl group attached to the methylen bridge was reported. Substitution reactions of chloroderivative **173** led to the series of alkoxyderivatives **177** as well as hydroxyderivatives **172** and **176** and azidoderivative **174**. Efficient ribosylation of nucleobases **177** afforded protected nucleosides **184** in their diastereomeric mixtures, where two diasteroisomers from each mixture **184f-i** were isolated using silica gel column chromatography. These isomers along with non-separated mixtures **184a-e** and **184m-n** were deprotected and gave ribonucleosides **178**.

In the second part of my thesis, I focused on synthesis of 5-alkoxymethyluracil analogues. Alkoxyderivatives **185** were synthesized *via* nucleophilic substitution from chloro analogue **190**. Efficient ribosylation of nucleobases **185** using Vorbrüggen method followed with deprotection afforded nucleosides **186**.

The research of 5-alkoxymethyluracil analogues was further extended to synthesis of compounds **187** and **188** and nucleoside **189** containing modified 2,3-dihydroxy-1-propyloxy moiety. Esters **187** and **188** were prepared from dihydroxyderivative **195** after protection of primary hydroxyl group with subsequent reaction with palmitoyl and acetyl chloride, respectively. Furthemore, compound **195** was efficiently ribosylated to afford nucleoside **189**. On contrary, secondary hydroxyl group of derivative **195** proved to be completely unreactive toward alkylation by either diisopropyl (bromomethyl)phosphonate or diisopropyl [(tosyloxy)methyl]phosphonate.

Nucleobases **177** and **185** and their nucleosides analogues **178** and **186**, respectively, were screened for their cytotoxic activity *in vitro* against cancer cell lines CEM, K562, their drug resistant counterparts CEM-DNR-bulk and K562-tax, and A549 as representative of solid tumor and colorectal carcinoma HCT116p53 and HCT116p53-/- cell lines. All available outcomes indicate the interesting dependence of cytotoxic activity on the presence of nitrophenyl and/or ribose moiety and length of alkyl chain.

The most interesting results were obtained for nucleobases **185** for drug resistant leukemia cancer cell lines (CEM-DNR-bulk and K562-tax), where an activity is approaching micromolar concentration of IC_{50}. On the other hand, nucleobases **177** are significantly more active against A549 and both colorectal carcinoma cell lines then their analogues **185**. Cytotoxic activity of nucleosides **178** and **186** show that the nucleosides **178** containing bulky nitrophenyl moiety exhibit significantly higher cytotoxicity than their alkoxymethyluracil analogues **186** againts all screened cell lines. The activity of nucleosides **178f-i** is higher in drug sensitive than in drug resistant cancer lines and is independent of the chirality of the molecule. In addition, compounds **177f-h** were found to inhibit the synthesis of both DNA and RNA and induce apoptosis at concentration 5x IC_{50} in treated CEM cells. Interestingly, derivative **177f** caused significant apoptosis within 24 h at concentration 1x IC_{50}.

In the last part of my work, I concentrated my efforts on the synthesis of oligodeoxynucleotides, the study of their thermal stability and the postoligo synthesis. Firstly, compounds **223** prepared employing click chemistry was converted to their corresponding phosphoramidites **227** *via* tritylation of 5´-OH group with subsequent reaction with 2-cyanoethyl-*N,N´*-(diisopropyl)-phosphoramidochloridite and further incorporated into the oligodeoxynucleotides **ON2-ON5**. Hybridization experiments showed that the stacking of triazole analogues in the major groove led to very stable DNA:RNA duplexes. Thus the monomer **X** shows promising therapeutical potential *via* RNA-targeting within cells as a means of control of a gene expression.

Moreover, derivative **229** was synthesized by Sonogashira coupling reaction of 5-iodo-2´-uridine with 1-bromo-4-ethynylbenzene and converted to corresponding phosphormidite **231** that was successfully and efficiently incorporated into oligodeoxynucleotide **ON9**. The reactivity of bromo group of **ON9** contaning monomer **B** was further studied followed the idea of postsynthetic labeling of DNA. Results obtained within these studies did not lead to desired azido substituted oligodeoxynucleotide **ON10**, however derivative **229** can be further modified and thus significantly contribute to investigation of similar compounds.

In conclusion, diverse range of C-5 substituted uracil analogues was synthesized within presented thesis and their reactivity and biological activity was studied. Extensive SAR studies brought out significant results and outlined the next inspiration to the development of new potent biological active compounds, compounds that might be more selective and more specific in targeting of cancer cells.

5. List of publications of the author related to the thesis

1. Spáčilová, L.; Džubák, P.; Hajdúch, M.; Křupková, S.; Hradil, P.; Hlaváč, J. *Synthesis and Cytotoxic Activity of Various 5-[Alkoxy-(4-nitro-phenyl)-methyl]-uracils in their racemic form*. Bioorganic and Medicinal Chemistry Letters **2007**, *17*, 6647-6650.

2. Brulíková, L.; Džubák, P.; Hajdúch, M.; Lachnitová, L.; Kollareddy, M.; Kolář, M.; Bogdanová, K.; Hlaváč, J. *Synthesis of 5-[alkoxy-(4-nitro-phenyl)-methyl]-uridines and study of their cytotoxic activity*. European Journal of Medicinal Chemistry **2010**, *45*, 3588-3594.

3. Andersen, N.K.; Chandak, N.; Brulíková, L.; Kumar, P.; Jensen, M.D,; Jensen, F.; Sharma, P.K.; Nielsen, P. *Efficient RNA-targeting by the introduction of aromatic stacking in the duplex major groove via 5-(1-phenyl-1,2,3-triazol-4-yl)-2'-deoxyuridines*. Bioorganic and Medicinal Chemistry **2010**, *18*, 4702-4710.

4. Brulíková, L.; Hlaváč, J.; Džubák, P.; Hajdúch, M. *Synthesis and cytotoxic activity of various 5-alkoxymethyluracil analogues*. Carbohydrate Research, **2011**, submitted.

5. Brulíková, L.; Hlaváč, J. *Synthesis, reactivity and biological activity of 5-alkoxymethyluracil analogues.* Beilstein Journal of Organic Chemistry, **2011**, submitted.

6. Experimental part

6.1. Material and methods

Material and methods 1 (for derivatives 172-205): Melting points were determined on a Boetius stage and are uncorrected. NMR spectra were recorded at ambient temperature (21°C) in DMSO-d_6 solutions at 300K on a Bruker Avance 300 spectrometer and referenced to the resonance signal of DMSO (with TMS as an internal standard; chemical shifts are reported in ppm, and coupling constants in Hz). Mass spectrometric experiments were performed using a triple quadrupole mass spectrometer TSQ Quantum Access and chromatographic analysis were performed using ultra-high pressure liquid chromatograph Accela (both from Thermo Scientific, San Jose, CA, USA). HPLC experiments were performed using Dionex liquid chromatograph (P 680 HPLC Pump, PDA-100 Photodiode Array Detector). Preparative chromatography was performed with using of Sepacore chromatography system (Büchi). Purification by column chromatography was carried out using silica gel. Elemental analysis was perpormed on EA 1108 Elemental Analyser (Fisons Instrument).

Material and methods 2 (for derivatives 213-237): All commercial reagents were used as supplied. Reactions were carried out under argon or nitrogen when anhydrous solvents were used. Column chromatography was performed with Silica gel 60 (particle size 0.040–0.063 μm, Merck). NMR spectra were recorded on a Varian Gemini 2000 spectrometer or a Bruker Advance III 400 spectrometer. Values for δ are in ppm relative to tetramethylsilane as an internal standard or 85% H_3PO_4 as an external standard. Assignments of NMR-signals when given are based on 2D spectra and follow standard carbohydrate and nucleoside convention. ESI mass spectra as well as accurate mass determinations were performed on an Thermo Finnigan TSQ 700 spectrometer. Microwave heated reaction were performed on an Emrys^{TM} Creator.

6.2. Chemical synthesis

5-[Hydroxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (172)

Compound **173** (200 mg, 0.71 mmol) was refluxed in water (5 ml) for 2 hours. After cooling to RT, the precipitated solid was filtered, washed with water and dried. Yield 183mg (98%), m.p.269-271°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 5.59 (ds, 1H, CH, *J*=3.3 Hz); 5.97 (ds, 1H, OH, *J*=4.2 Hz); 7.33 (d, 1H, H-6, *J*=6.0 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.15 (d, 2H, Ar, *J*=9.0 Hz); 10.86 (ds, 1H, NH, *J*=5.4 Hz); 11.11 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 66.9, 115.0, 123.1, 127.5, 138.4, 146.4, 151.0, 151.6, 163.0. MS m/z calcd. for $C_{11}H_9N_3O_5$: 263.21, found 262.2 $[M-H]^-$. For $C_{11}H_9N_3O_5$ (263.21) calcd. C 50.20, H 3.45, N 15.96; found C 49.92, H 3.09, N 16.28.

5-[Chloro(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (173)

Uracil **179** (2.0 g, 17.8 mmol) was dissolved in hot concentrated HCl (30 ml) and *p*-nitrobenzaldehyde **180** (2.7 g, 17.8 mmol) was added. The mixture was refluxed for 4 hours. Then the precipitated solid was cooled to RT, filtered, washed with HCl and dried. Yield 3.4g (68%), m.p.246-249°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 5.59 (s, 1H, CH); 7.34 (d, 1H, H-6, *J*=6.3 Hz); 7.63 (d, 2H, Ar, *J*=8.7 Hz); 8.15 (d, 2H, Ar, *J*=8.7 Hz); 10.92 (ds, 1H, NH, *J*=6.0 Hz); 11.09 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 56.8, 111.2, 123.4, 128.8, 141.5, 146.9, 150.7, 151.0, 161.9. MS m/z calcd. for $C_{11}H_8ClN_3O_4$: 281.65, found 280.00; 282.00 $[M-H]^-$. For $C_{11}H_8ClN_3O_4$ (281.65) calcd. C 46.91, H 2.86, N 14.92; found C 47.02, H 2.47, N 14.67.

5-[Azido(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (174)

Compound **173** (200 mg, 0.71 mmol) and NaN_3 (46 mg, 0.71 mmol) was dissolved in *N*,*N*-dimethylformamide (2 ml). The mixture was stirred for 142 hours at RT and poured into 50 ml ice water. The precipitated solid was filtered, washed with water and dried. Yield 159 mg (80%), m.p. 313-314°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 5.59 (s, 1H, CH); 7.75 (s, 1H, H-6); 7.84 (d, 2H, Ar, *J*=8.4 Hz); 8.35 (d, 2H, Ar, *J*=8.7 Hz). ^{13}C NMR (75MHz, DMSO-d_6) δ 67.47, 115.66, 123.72, 128.14, 139.06, 147.00, 151.60, 152.25, 163.62. MS m/z calcd. for $C_{11}H_8N_6O_4$: 288.22, found 259.07 $[M-H]^-$. For $C_{11}H_8N_6O_4$ (-N_2 260.21) calcd. C 50.78, H 3.10, N 21.53; found C 51.02, H 2.96, N 21.38.

5,5´-[(4-nitrophenyl)methylene]bis[pyrimidine-2,4(1*H*,3*H*)-dione] (175)

Uracil **179** (2.0 g, 17.8 mmol) was dissolved in hot concentrated HCl (30 ml) and *p*-nitrobenzaldehyde **180** (1.35 g, 8.9 mmol) was added. The mixture was heated under

reflux for 24 hours. Then the precipitated solid was cooled to RT, collected by filtration, washed with HCl and dried. Yield 2.72 g (85%), m.p.>360°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 5.14 (s, 1H, CH); 6.94 (d, 2H, H-6, *J*=6.0 Hz); 7.45 (d, 2H, Ar, *J*=8.7 Hz); 8.15 (d, 2H, Ar, *J*=8.7 Hz); 10.81 (ds, 2H, NH, *J*=5.4 Hz); 11.17 (s, 2H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 39.65, 112.44, 123.95, 130.20, 140.77, 146.72, 149.52, 151.72, 164.08. MS m/z calcd. for $C_{15}H_{11}N_5O_6$: 357.28, found 356.30 $[M-H]^-$. For $C_{15}H_{11}N_5O_6$. H_2O (375.29) calcd. C 48.00, H 3.49, N 18.66; found C 48.13, H 3.57, N 18.55.

5-[(2-Hydroxyethoxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (176)

Chloro derivative **173** (500 mg, 1.78 mmol) was stirred in ethylene glycol (10 ml) at 120°C for 3 hours. The mixture was cooled down and leave in freezer at -20°C over night. The precipitated solid was filtered, washed with water and dried to give white solid. Yield 460.1 mg (84%), m.p. 114-120°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 3.36-3.58 (m, 4H, CH_2); 4.65-4.71 (m, 1H, OH); 5.41 (s, 1H, CH); 7.40 (s, 1H, H-6); 7.65 (d, *J*=8.60 Hz, 2H, Ar); 8.19 (d, *J*=8.78 Hz, 2H, Ar); 10.97 (br. s., 1H, NH); 11.15 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 65.61, 71.19, 75.47, 112.83, 123.85, 128.41, 139.81, 147.31, 149.33, 151.59, 163.59. MS m/z calcd. for $C_{13}H_{13}N_3O_6$ 307.26, found 306.27 $[M-H]^-$.

***General procedure 1 for preparation of 5-[alkoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1H,3H)-diones* (177)**

Compound **173** (4.00 g, 14.2 mmol) was refluxed in the alcohol (80 ml) for 4 hours. After cooling to RT, the precipitated solid was collected by filtration, washed with the alcohol and dried. Compounds **177i-177l** were crystallized from toluene to remove residual alcohols.

5-[Methoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177a)

Derivative **177a** was prepared from **173** according to *general procedure 1*.

Yield 3.23 g (82%), m.p. 260-263°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 3.27 (s, 3H, CH_3); 5.27 (s, 1H, CH); 7.32 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=8.4 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.95 (s, 1H, NH); 11.16 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 57.13, 77.14, 112.47, 123.85, 128.45, 139.89, 147.31, 148.99, 151.56, 163.47. MS m/z

calc. for $C_{12}H_{11}N_3O_5$: 277.23, found 276.32 $[M-H]^-$. For $C_{12}H_{11}N_3O_5$ (277.23) calcd. C 51.99, H 4.00, N 15.16; found C 51.01, H 4.11, N 16.28.

5-[Ethoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177b)

Derivative **177b** was prepared from **173** according to *general procedure 1*.

Yield 3.31 g (80%), m.p. 222-225°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 1.15 (t, 3H, CH_3, *J*=7.2 Hz); 3.37-3.55 (m, 2H, CH_2); 5.37 (s, 1H, CH); 7.30 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=9.0 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.93 (s, 1H, NH); 11.15 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 15.65, 64.69, 75.24, 112.97, 123.87, 128.44, 139.69, 147.28, 149.43, 151.56, 163.48. MS m/z calc. for $C_{13}H_{13}N_3O_5$: 291.26, found 290.32 $[M-H]^-$. For $C_{13}H_{13}N_3O_5$ (291.26) calcd. C 53.61, H 4.50, N 14.43; found C 52.99, H 4.11, N 14.03.

5-[(4-Nitrophenyl)(propoxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177c)

Derivative **177c** was prepared from **173** according to *general procedure 1*.

Yield 3.43 g (79%), m.p. 204-206°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.87 (t, 3H, CH_3, *J*=7.5 Hz); 1.54 (sex, 2H, CH_2, *J*=6.6 Hz); 3.38-3.45 (m, 2H, CH_2); 5.36 (s, 1H, CH); 7.30 (ds, 1H, H-6, *J*=5.7 Hz); 7.63 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=9.0 Hz); 10.92 (ds, 1H, NH, *J*=4.8 Hz); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 11.09, 23.04, 70.84, 75.37, 112.97, 123.87, 128.44, 139.65, 147.28, 149.46, 151.54, 163.47. MS m/z calc. for $C_{14}H_{15}N_3O_5$: 305.29, found 304.41 $[M-H]^-$. For $C_{14}H_{15}N_3O_5$ (305.29) calcd. C 55.08, H 4.95, N 13.76; found C 55.22, H 4.54, N 13.53.

5-[Isopropoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177m)

Derivative **177m** was prepared from **173** according to *general procedure 1*.

Yield 3.04 g (70%), m.p. 211-215°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 1.11 (dd, 6H, CH_3, J_1=6.0 Hz, J_2=6.9 Hz); 3.60 (hept, 1H, CH, *J*=6.0 Hz); 5.48 (s, 1H, CH); 7.28 (ds, 1H, H-6, *J*=6.0 Hz); 7.63 (d, 2H, Ar, *J*=9.0 Hz); 8.18 (d, 2H, Ar, *J*=9.0 Hz); 10.91 (ds, 1H, NH, *J*=5.7 Hz); 11.15 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 22.64, 22.76, 70.07, 72.59, 113.58, 123.84, 128.50, 139.66, 147.22, 150.00, 151.53, 163.51. MS m/z calc. for $C_{14}H_{15}N_3O_5$: 305.29, found 304.31 $[M-H]^-$. For $C_{14}H_{15}N_3O_5$ (305.29) calcd. C 55.08, H 4.95, N 13.76; found C 54.87, H 4.45, N 13.45.

5-[Butoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177d)

Derivative **177d** was prepared from **173** according to *general procedure 1*.

Yield 3.17 g (70%), m.p. 172-173°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.86 (t, 3H, CH_3, *J*=7.5 Hz); 1.32 (sex, 2H, CH_2, *J*=7.8 Hz); 1.52 (p, 2H, CH_2, *J*=8.1 Hz); 3.34-3.49 (m, 2H, CH_2); 5.35 (s, 1H, CH); 7.29 (ds, 1H, H-6, *J*=6.0 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.91 (ds, 1H, NH, *J*=5.7 Hz); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.24, 19.39, 31.84, 68.89, 75.41, 112.97, 123.85, 128.44, 139.63, 147.28, 149.45, 151.54, 163.47. MS m/z calc. for $C_{15}H_{17}N_3O_5$: 319.31, found 318.31 $[M-H]^-$. For $C_{15}H_{17}N_3O_5$ (319.31) calcd. C 56.42, H 5.37, N 13.16; found C 56.49, H 5.25, N 13.06.

5-[*sec*-Butoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177n)

Derivative **177n** was prepared from **173** according to *general procedure 1*.

Yield 3.31 g (73%), m.p. 177-180°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.80 (dt, 3H, CH_3, J_1=7.5 Hz, J_2=5.1 Hz); 1.07 (dd, 3H, CH_3, J_1=8.7 Hz, J_2=6.3 Hz); 1.32-1.57 (m, 2H, CH_2); 3.36-3.50 (m, 1H, CH); 5.47 (ds, 1H, CH, *J*=5.1 Hz); 7.32 (ds, 1H, H-6, *J*=3.3 Hz); 7.64 (d, 2H, Ar, *J*=8.4 Hz); 8.17 (d, 2H, Ar, *J*=9.6 Hz); 10.92 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.08, 19.59, 29.14, 70.54, 75.41, 112.97, 123.85, 128.44, 139.65, 147.28, 149.45, 151.54, 163.47. MS m/z calc. for $C_{15}H_{17}N_3O_5$: 319.31, found 318.42 $[M-H]^-$. For $C_{15}H_{17}N_3O_5$ (319.31) calcd. C 56.42, H 5.37, N 13.16; found C 56.21, H 5.16, N 12.99.

5-[*tert*-Butoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177o)

Derivative **177o** was prepared from **173** according to *general procedure 1*.

Yield 2.95 g (65%), m.p. 158-161°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 1.15 (s, 9H, CH_3); 5.61 (s, 1H, CH); 7.32 (ds, 1H, H-6, *J*=6.0 Hz); 7.64 (d, 2H, Ar, *J*=9.0 Hz); 8.15 (d, 2H, Ar, *J*=9.0 Hz); 10.87 (s, 1H, NH); 11.13 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 27.84, 56.79, 75.42, 112.97, 123.85, 128.44, 139.65, 147.28, 149.45, 151.54, 163.4. MS m/z calc. for $C_{15}H_{17}N_3O_5$: 319.31, found 318.32 $[M-H]^-$. For $C_{15}H_{17}N_3O_5$ (319.31) calcd. C 56.42, H 5.37, N 13.16; found C 56.41, H 5.23, N 13.14.

5-[(4-Nitrophenyl)(pentyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177e)

Derivative **177e** was prepared from **173** according to *general procedure 1*.

Yield 3.17 g (67%), m.p. 186-187°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.85 (t, 3H, CH_3, *J*=7.5 Hz); 1.25-1.30 (m, 4H, CH_2); 1.53 (p, 2H, CH_2, *J*=6.6 Hz); 3.35-3.48 (m,

2H, CH_2); 5.35 (s, 1H, CH); 7.30 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=9.0 Hz); 8.18 (d, 2H, Ar, *J*=9.0 Hz); 10.92 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.42, 22.43, 28.34, 29.42, 69.22, 75.41, 112.97, 123.85, 128.44, 139.65, 147.28, 149.45, 151.54, 163.47. MS m/z calc. for $C_{16}H_{19}N_3O_5$: 333.34, found 332.42 $[M-H]^-$. For $C_{16}H_{19}N_3O_5$ (333.34) calcd. C 57.65, H 5.75, N 12.61; found C 57.45, H 6.05, N 12.48.

5-[(Hexyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177f)

Derivative **177f** was prepared from **173** according to *general procedure 1*.

Yield 3.16 g (64%), m.p. 194-196°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=6.9 Hz); 1.20-1.35 (m, 6H, CH_2); 1.52 (p, 2H, CH_2, *J*=7.2 Hz); 3.34-3.48 (m, 2H, CH_2); 5.35 (s, 1H, CH); 7.29 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=9.0 Hz); 8.18 (d, 2H, Ar, *J*=9.0 Hz); 10.91 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.42, 22.57, 25.79, 29.69, 31.55, 69.22, 75.41, 112.97, 123.85, 128.44, 139.63, 147.28, 149.46, 151.54, 163.47. MS m/z calc. for $C_{17}H_{21}N_3O_5$: 347.37, found 346.35 $[M-H]^-$. For $C_{17}H_{21}N_3O_5$ (347.37) calcd. C 58.78, H 6.09, N 12.10; found C 58.83, H 6.45, N 12.48.

5-[(Heptyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177g)

Derivative **177g** was prepared from **173** according to *general procedure 1*.

Yield 3.70 g (72%), m.p. 183-184°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.81-0.86 (m, 3H, CH_3); 1.23-1.34 (m, 8H, CH_2); 1.52 (p, 2H, CH_2, *J*=6.9 Hz); 3.35-3.49 (m, 2H, CH_2); 5.35 (s, 1H, CH); 7.30 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.92 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.43, 22.58, 26.10, 28.98, 29.72, 31.75, 69.20, 75.41, 112.98, 123.84, 128.44, 139.63, 147.26, 149.46, 151.54, 163.45. MS m/z calc. for $C_{18}H_{23}N_3O_5$: 361.39, found 360.41 $[M-H]^-$. For $C_{18}H_{23}N_3O_5$ (361.39) calcd. C 59.82, H 6.41, N 11.63; found C 60.09, H 6.45, N 11.60.

5-[(4-Nitrophenyl)(octyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177h)

Derivative **177h** was prepared from **173** according to *general procedure 1*.

Yield 2.24 g (42%), m.p. 184-185°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.82-0.86 (m, 3H, CH_3); 1.22-1.34 (m, 10H, CH_2); 1.46-1.57 (m, 2H, CH_2); 3.35-3.48 (m, 2H, CH_2); 5.35 (s, 1H, CH); 7.30 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=8.7

Hz); 10.92 (ds, 1H, NH, *J*=6.0 Hz); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.43, 22.62, 26.11, 29.31, 29.47, 29.70, 31.80, 69.19, 75.40, 112.98, 123.82, 128.44, 139.60, 147.26, 149.46, 151.54, 163.45. MS m/z calc. for $C_{19}H_{25}N_3O_5$: 375.42, found 374.31 $[M-H]^-$. For $C_{19}H_{25}N_3O_5$ (375.42) calcd. C 60.79, H 6.71, N 11.19; found C 61.13, H 6.95, N 10.92.

5-[(4-Nitrophenyl)(nonyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177i)

Derivative **177i** was prepared from **173** according to *general procedure 1*.

Yield 2.43 g (44%), m.p. 139-143°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.82-0.85 (m, 3H, CH_3); 1.22-1.31 (m, 12H, CH_2); 1.48-1.54 (m, 2H, CH_2); 3.34-3.48 (m, 2H, CH_2); 5.34 (s, 1H, CH); 7.29 (ds, 1H, H-6, *J*=5.7 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.92 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.43, 22.62, 26.11, 28.98, 29.31, 29.47, 29.70, 31.80, 69.19, 75.40, 112.98, 123.82, 128.44, 139.60, 147.26, 149.46, 151.54, 163.45. MS m/z calc. for $C_{20}H_{27}N_3O_5$: 389.45, found 388.20 $[M-H]^-$. For $C_{20}H_{27}N_3O_5$ (389.45) calcd. C 61.68, H 6.99, N 10.79; found C 61.88, H 7.52, N 10.69.

5-[(Decyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177j)

Derivative **177j** was prepared from **173** according to *general procedure 1*.

Yield 2.87 g (50%), m.p. 186-190°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.82-0.86 (m, 3H, CH_3); 1.21-1.30 (m, 14H, CH_2); 1.47-1.54 (m, 2H, CH_2); 3.34-3.48 (m, 2H, CH_2); 5.34 (s, 1H, CH); 7.29 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.92 (s, 1H, NH); 11.14 (s, 1H, NH).^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.62, 26.11, 28.98, 29.22, 29.29, 29.51, 29.69, 31.83, 69.19, 75.41, 112.98, 123.84, 128.44, 139.60, 147.26, 149.48, 151.53, 163.45. MS m/z calc. for $C_{21}H_{29}N_3O_5$: 403.47, found 402.40 $[M-H]^-$. For $C_{21}H_{29}N_3O_5$ (403.47) calcd. C 62.51, H 7.24, N 10.41; found C 62.57, H 7.43, N 10.04.

5-[(4-Nitrophenyl)(undecyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177k)

Derivative **177k** was prepared from **173** according to *general procedure 1*.

Yield 3.50 g (59%), m.p. 153-156°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.85 (t, 3H, CH_3, *J*=6.3 Hz); 1.22-1.33 (m, 16H, CH_2); 1.48-1.54 (m, 2H, CH_2); 3.35-3.48 (m, 2H, CH_2); 5.34 (s, 1H, CH); 7.29 (s, 1H, H-6); 7.62 (d, 2H, Ar, *J*=9.0 Hz); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 10.91 (s, 1H, NH); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ

14.48, 22.64, 26.11, 29.25, 29.29, 29.53, 29.56, 29.58, 29.64, 31.84, 69.17, 75.40, 112.97, 123.84, 128.45, 139.60, 147.26, 149.46, 151.54, 163.45. MS m/z calc. for $C_{22}H_{31}N_3O_5$: 417.50, found 416.40 $[M-H]^-$. For $C_{22}H_{31}N_3O_5$ (417.50) calcd. C 63.29, H 7.48, N 10.06; found C 63.43, H 7.50, N 9.87.

5-[(Dodecyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (177l)

Derivative **177l** was prepared from **173** according to *general procedure 1*.

Yield 2.21 g (36%), m.p. 158-161°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.85 (t, 3H, CH_3, *J*=6.6 Hz); 1.24-1.27 (m, 18H, CH_2); 1.50-1.52 (m, 2H, CH_2); 3.34-3.48 (m, 2H, CH_2); 5.34 (s, 1H, CH); 7.29 (ds, 1H, H-6, *J*=6.0 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 8.17 (d, 2H, Ar, *J*=8.7 Hz); 10.92 (ds, 1H, NH, *J*=5.1 Hz); 11.14 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.45, 22.62, 26.11, 29.22, 29.29, 29.50, 29.69, 31.83, 69.19, 75.40, 112.98, 123.82, 128.44, 139.59, 147.25, 149.46, 151.54, 163.45. MS m/z calc. for $C_{23}H_{33}N_3O_5$: 431.53, found 430.20 $[M-H]^-$. For $C_{23}H_{33}N_3O_5$ (431.53) calcd. C 64.02, H 7.71, N 9.74; found C 64.19, H 8.20, N 9.46.

***General procedure 2 for preparation of 5-[alkoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1H,3H)-diones* (178)**

Nucleosides **184** were dissolved in MeOH/NH_3 solution and stirred at room temperature for 6 days. Then the mixture was evaporated, co-evaporated with methanol and purified by silica gel column chromatography using $CHCl_3$/MeOH (9/0.5).

5-[Methoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178a)

Nucleoside **178a** was prepared from **184a** (253.1 mg, 0.35 mmol) and MeOH/NH_3 solution (5 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 108.1 mg (75%), m.p. 100-104°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.28 (m, 3H, CH_3); 3.50 – 3.68 (m, 2H, H-5′); 3.82 – 4.08 (m, 3H, H-2′, H-3′, H-4′); 5.04 – 5.13 (m, 2H, 5′-OH, 3′-OH); 5.31 (m, 1H, CH); 5.38-5.44 (m, 1H, 2′-OH); 5.78 (m, 1H, H-1′); 7.60 – 7.64 (m, 2H, Ar); 8.03 – 8.05 (m, 1H, Ar); 8.20 (d, 2H, *J*=8.60 Hz); 11.45 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 57.23, 61.16, 61.20, 70.33, 70.45, 74.46, 74.53, 77.08, 77.11, 85.32, 85.36, 88.75, 88.89, 113.71, 113.79, 123.92, 123.95,

128.36, 128.39, 138.77, 138.94, 147.35, 147.38, 148.79, 148.88, 150.80, 162.49, 162.52. MS m/z calcd. for $C_{17}H_{19}N_3O_9$: 409.36, found 408.27 $[M-H]^-$.

5-[Ethoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione

(178b)

Nucleoside **178b** was prepared from **184b** (150.6 mg, 0.21 mmol) and MeOH/NH_3 solution (5 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 57.7 mg (67%); m.p. 96-101°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.09-1.20 (m, 3H, CH_3); 3.38-3.68 (m, 4H, CH_2, H-5′); 3.83-4.10 (m, 3H, H-2′, H-3′, H-4′); 5.00-5.14 (m, 2H, 5′-OH, 3′-OH); 5.35-5.45 (m, 2H, 2′-OH, CH); 5.79 (t, *J*=4.76 Hz, 1H, H-1′); 7.60 – 7.65 (m, 2H, Ar); 8.01 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.60 Hz); 11.44 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.58, 15.64, 61.33, 61.36, 64.76, 70.52, 70.64, 74.46, 74.56, 75.19, 75.22, 85.42, 85.46, 88.69, 88.75, 114.21, 114.30, 123.91, 123.94, 128.35, 128.38, 138.61, 138.72, 147.31, 147.34, 149.21, 149.29, 150.84, 162.50, 162.55. MS m/z calcd. for $C_{18}H_{21}N_3O_9$: 423.37, found 422.33 $[M-H]^-$.

5-[(4-Nitrophenyl)(propoxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178c)

Nucleoside **178c** was prepared from **184c** (277.8 mg, 0.37 mmol) and MeOH/NH_3 solution (5 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 104.6 mg (65%); m.p. 86-90°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.81-0.93 (m, 3H, CH_3); 1.46-1.63 (m, 2H, CH_2); 3.24-3.48 (m, 2H, CH_2); 3.48-3.68 (m, 2H, H-5′); 3.81-3.94 (m, 1H, H-4′); 3.95 – 4.06 (m, 2H, H-2′, H-3′); 4.97-5.18 (m, 2H, 5′-OH, 3′-OH); 5.39 – 5.44 (m, 2H, CH, 2′-OH); 5.80 (m, 1H, H-1′); 7.57-7.69 (m, 2H, Ar); 7.99 (d, 1H, Ar, *J*=6.22 Hz); 8.19 (d, 2H, Ar, *J*=8.78 Hz); 11.44 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.08, 11.14, 22.99, 61.39, 61.44, 70.55, 70.70, 70.93, 74.48, 74.51, 75.30, 75.34, 85.46, 85.51, 88.63, 88.73, 114.30, 114.43, 123.92, 123.95, 128.32, 128.36, 138.61, 138.69, 147.31, 147.35, 149.23, 149.35, 150.82, 162.47, 162.53. MS m/z calcd. for $C_{19}H_{23}N_3O_9$: 437.41, found 436.27 $[M-H]^-$.

5-[Isopropoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178m)

Nucleoside **178m** was prepared from **184m** (217.7 mg, 0.29 mmol) and MeOH/NH_3 solution (5 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 89.3 mg (70%); %); m.p. 92-97°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.09-1.16 (m, 6H, CH_3); 3.54-3.65 (m, 3H, CH, H-5′); 3.84-4.07 (m, 3H, H-2′, H-3′, H-4′); 5.01-5.11 (m, 2H, 5′-OH, 3′-OH); 5.35 – 5.40 (m, 1H, 2′-OH); 5.51-5.52 (m, 1H, CH); 5.80 (t, 1H, H-1′, *J*=6.9Hz); 7.61-7.65 (m, 2H, Ar); 7.96-7.98 (m, 1H, Ar); 8.18 (d, 2H, Ar, *J*=8.4 Hz); 11.43 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.64, 22.71, 61.45, 61.50, 70.10, 70.12, 70.61, 70.78, 72.45, 72.51, 74.46, 74.51, 85.49, 85.54, 88.56, 88.63, 114.86, 115.03, 123.90, 123.92, 128.36, 128.42, 138.72, 147.25, 149.76, 149.84, 150.84, 162.50, 162.56. MS m/z calcd. for $C_{19}H_{23}N_3O_9$: 437.41, found 436.33 $[M-H]^-$.

5-[Butoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178d)

Nucleoside **178d** was prepared from **184d** (352.0 mg, 0.46 mmol) and MeOH/NH_3 solution (6 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 194.6 mg (94%); m.p. 85-90°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.83-0.89 (m, 3H, CH_3); 1.28-1.39 (m, 2H, CH_2); 1.48-1.58 (m, 2H, CH_2); 3.36-3.38 (m, 2H, CH_2); 3.41-3.60 (m, 2H, H-5′); 3.85 – 4.08 (m, 3H, H-2′, H-3′, H-4′); 5.01-5.10 (m, 2H, 5′-OH, 3′-OH); 5.35 – 5.41 (m, 2H, CH, 2′-OH); 5.79 (t, 1H, H-1′, *J*=6.0 Hz); 7.60-7.65 (m, 2H, Ar); 7.95-7.98 (m, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.4 Hz); 11.43 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.30, 19.39, 31.80, 61.42, 61.54, 68.98, 69.03, 70.56, 70.70, 74.51, 75.33, 75.40, 85.48, 85.55, 88.66, 88.73, 114.30, 114.40, 123.94, 124.13, 128.33, 138.72, 147.32, 149.23, 150.84, 162.47, 162.55. MS m/z calcd. for $C_{20}H_{25}N_3O_9$: 451.44, found 450.27 $[M-H]^-$.

5-[*sec*-Butoxy(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178n)

Nucleoside **178n** was prepared from **184n** (325.0 mg, 0.43 mmol) and MeOH/NH_3 solution (6 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 113.7 mg (59%); m.p. 94-98°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.76-0.88 (m, 3H, CH_3); 1.05-1.13 (m, 3H, CH_3); 1.33-1.59 (m, 2H, CH_2); 3.37-3.48 (m, 1H, CH); 3.56-3.64 (m, 2H, H-5′); 3.83 – 4.05 (m, 3H, H-2′, H-3′, H-4′); 5.06-5.12 (m, 2H, 5′-OH, 3′-OH); 5.35 – 5.40 (m, 1H, 2′-OH); 5.50-5.53 (m, 1H, CH); 5.77-5.83 (m, 1H, H-1′); 7.62-7.66 (m, 2H, Ar); 7.93-8.00 (m, 1H, Ar); 8.16-8.20 (m, 2H, Ar); 11.41 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.04, 10.11, 19.58, 19.65, 29.19, 29.26, 61.51, 61.58, 70.55, 70.67, 70.75, 70.84, 72.51, 72.53, 74.46, 74.53, 74.95, 74.97,

85.52, 85.58, 88.53, 88.64, 114.61, 114.86, 123.87, 123.91, 128.51, 128.58, 138.52, 138.56, 147.29, 147.32, 149.58, 149.67, 150.81, 150.84, 162.41, 162.47. MS m/z calcd. for $C_{20}H_{25}N_3O_9$: 451.44, found 450.33 $[M-H]^-$.

5-[(4-Nitrophenyl)(pentyloxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178e)

Nucleoside **178e** was prepared from **184e** (319.5 mg, 0.41 mmol) and MeOH/NH_3 solution (6 ml) according to *general procedure 2* as a mixture of two diastereomers.

Yield 161.3 mg (84%); m.p. 60-68°C; ^{1}H NMR (300MHz, DMSO-d_6): δ 0.83-0.87 (m, 3H, CH_3); 1.23-1.29 (m, 4H, CH_2); 1.52-1.56 (m, 2H, CH_2); 3.29-3.48 (m, 2H, CH_2); 3.50-3.65 (m, 2H, H-5′); 3.85-3.88 (m, 1H, H-4′); 3.95-3.98 (m, 1H, H-3′); 4.03-4.08 (m, 1H, H-2′); 5.01-5.04 (m, 1H, 5′-OH); 5.08-5.12 (m, 1H, 3′-OH); 5.39 (br.s, 1H, CH); 5.42-5.44 (m, 1H, 2′-OH); 5.77-5.81 (m, 1H, H-1′); 7.60-7.65 (m, 2H, Ar); 7.95-7.99 (m, 1H, Ar); 8.19 (d, 2H, Ar, *J*=9.0 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ14.43, 22.46, 28.30, 28.37, 29.38, 61.41, 61.50, 69.32, 69.35, 70.55, 70.70, 74.49, 75.34, 75.38, 85.46, 85.54, 88.64, 88.73, 114.28, 114.39, 123.92, 123.95, 128.32, 128.35, 138.63, 138.74, 147.34, 149.21, 149.33, 150.82, 162.47, 162.53. MS m/z calcd. for $C_{21}H_{27}N_3O_9$: 465.46, found 464.20 $[M-H]^-$.

(+)-5-[(Hexyloxy)(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178fA)

Nucleoside **178fA** was prepared from **184fA** (171.8 mg, 0.22 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 83.1 mg (80%), m.p. 72-74°C, $[\alpha]_D^{25}$ +55.2 (c 0.077, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=7.2 Hz); 1.23-1.35 (m, 6H, CH_2); 1.49-1.58 (m, 2H, CH_2); 3.41-3.48 (m, 2H, CH_2); 3.50-3.64 (m, 2H, H-5′); 3.88-3.98 (m, 3H, H-2′, H-3′, H-4′); 5.01 (t, 1H, 5′-OH, *J*=4.8 Hz); 5.06 (d, 1H, 3′-OH, *J*=4.2 Hz); 5.35-5.38 (m, 2H, 2′-OH, CH); 5.78 (d, 1H, H-1′, *J*=5.4 Hz); 7.62 (d, 2H, Ar, *J*=9.0 Hz); 7.98 (s, 1H, Ar); 8.18 (d, 2H, Ar, *J*=8.7 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.57, 25.76, 29.64, 31.61, 61.39, 69.32, 70.54, 74.49, 75.33, 85.52, 88.73, 114.28, 123.91, 128.35, 138.75, 147.29, 149.33, 150.82, 162.53. MS m/z calcd. for $C_{22}H_{29}N_3O_9$: 479.49, found 478.21 $[M-H]^-$.

(-)-5-[(Hexyloxy)(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178fB)

Nucleoside **178fB** was prepared from **184fB** (113.5 mg, 0.14 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 60.1 mg (88%), m.p. 68-69°C, $[\alpha]_D^{25}$ -39.0 (c 0.39, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=6.9 Hz); 1.25-1.36 (m, 6H, CH_2); 1.49-1.58 (m, 2H, CH_2); 3.42-3.50 (m, 2H, CH_2); 3.52-3.56 (m, 2H, H-5′); 3.84-3.86 (m, 1H, H-4′); 3.95-3.99 (m, 1H, H-3′); 4.02-4.08 (m, 1H, H-2′); 5.02 (t, 1H, 5′-OH, *J*=4.5 Hz); 5.10 (d, 1H, 3′-OH, *J*=5.1 Hz); 5.39 (s, 1H, CH); 5.42 (d, 1H, 2′-OH, *J*=5.4 Hz); 5.81 (d, 1H, H-1′, *J*=5.1 Hz); 7.61 (d, 2H, Ar, *J*=8.7 Hz); 7.94 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.7 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.45, 22.57, 25.82, 29.64, 31.59, 61.50, 69.35, 70.70, 74.48, 75.40, 85.46, 88.64, 114.39, 123.94, 128.33, 138.63, 147.34, 149.20, 150.82, 162.46. MS m/z calcd. for $C_{22}H_{29}N_3O_9$: 479.49, found 478.25 $[M-H]^-$.

(+)-5-[(Heptyloxy)(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178g)

Nucleoside **178gA** was prepared from **184gA** (207.6 mg, 0.26 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 95.7 mg (75%), m.p. 63-65°C, $[\alpha]_D^{25}$ +7.5 (c 0.30, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=7.2 Hz); 1.23-1.32 (m, 8H, CH_2); 1.49-1.58 (m, 2H, CH_2); 3.35-3.48 (m, 2H, CH_2); 3.51-3.64 (m, 2H, H-5′); 3.88-3.98 (m, 3H, H-2′, H-3′, H-4′); 4.99-5.02 (m, 1H, 5′-OH); 5.06 (bs, 1H, 3′-OH); 5.35-5.38 (m, 2H, 2′-OH, CH); 5.79 (d, 1H, H-1′, *J*=4.8 Hz); 7.63 (d, 2H, Ar, *J*=8.7 Hz); 7.98 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.7 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.45, 22.60, 26.04, 29.01, 29.67, 31.74, 61.39, 69.30, 70.54, 74.49, 75.33, 85.54, 88.75, 114.28, 123.91, 128.35, 138.75, 147.29, 149.33, 150.82, 162.53. MS m/z calcd. for $C_{23}H_{31}N_3O_9$: 493.52, found 492.31 $[M-H]^-$.

(-)-5-[(Heptyloxy)(4-nitrophenyl)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178gB)

Nucleoside **178gB** was prepared from **184gB** (232.4 mg, 0.29 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 107.5 mg (76%), m.p. 71-72°C, $[\alpha]_D^{25}$ -40.0 (c 0.30, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=6.9 Hz); 1.23-1.32 (m, 8H, CH_2); 1.50-1.59 (m, 2H, CH_2); 3.47-3.52 (m, 2H, CH_2); 3.54-3.60 (m, 2H, H-5′); 3.84-3.87 (m, 1H, H-4′); 3.95-3.99 (m, 1H, H-3′); 4.02-4.08 (m, 1H, H-2′); 5.00 (t, 1H, 5′-OH, *J*=4.8 Hz); 5.09 (d, 1H, 3′-OH, *J*=4.8 Hz); 5.39-5.41 (m, 2H, CH, 2′-OH); 5.80 (d, 1H, H-1′, *J*=5.1 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 7.94 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.7 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.45, 22.57, 26.07, 28.98, 29.66, 31.72, 61.47, 69.30, 70.68, 74.45, 75.37, 85.43, 88.63, 114.34, 123.91, 128.30, 138.62, 147.31, 149.18, 150.79, 162.43. MS m/z calcd. for $C_{23}H_{31}N_3O_9$: 493.52, found 492.22 $[M-H]^-$.

(+)-5-[(4-Nitrophenyl)(octyloxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178hA)

Nucleoside **178hA** was prepared from **184hA** (228.3 mg, 0.28 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 96.0 mg (68%), m.p. 67-69°C, $[\alpha]_D^{25}$ +9.5 (c 0.32, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=7.2 Hz); 1.22-1.31 (m, 10H, CH_2); 1.49-1.58 (m, 2H, CH_2); 3.43-3.48 (m, 2H, CH_2); 3.54-3.65 (m, 2H, H-5′); 3.86-3.98 (m, 3H, H-2′, H-3′, H-4′); 5.00 (t, 1H, 5′-OH, *J*=5.1 Hz); 5.06 (m, 1H, 3′-OH); 5.36 (d, 1H, 2′-OH, *J*=5.1 Hz); 5.38 (s, 1H, CH); 5.78 (d, 1H, H-1′, *J*=4.8 Hz); 7.63 (d, 2H, Ar, *J*=8.7 Hz); 7.98 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.7 Hz); 11.43 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.61, 26.08, 29.16, 29.31, 29.66, 31.80, 61.39, 69.30, 70.54, 74.49, 75.31, 85.54, 88.75, 114.28, 123.91, 128.36, 138.74, 147.29, 149.35, 150.81, 162.53. MS m/z calcd. for $C_{24}H_{33}N_3O_9$: 507.55, found 506.23 $[M-H]^-$.

(-)-5-[(4-Nitrophenyl)(octyloxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178hB)

Nucleoside **178hB** was prepared from **184hB** (175.0 mg, 0.21 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 76.9 mg (71%), m.p. 62-64°C, $[\alpha]_D^{25}$ -36.0 (c 0.38, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=7.2 Hz); 1.23-1.32 (m, 10H, CH_2); 1.49-1.57 (m, 2H, CH_2); 3.42-3.49 (m, 2H, CH_2); 3.52-3.56 (m, 2H, H-5′); 3.83-3.87 (m, 1H, H-4′); 3.95-3.99 (m, 1H, H-3′); 4.02-4.08 (m, 1H, H-2′); 5.02 (t, 1H, 5′-OH, *J*=4.5 Hz); 5.10 (d, 1H, 3′-OH, *J*=4.8 Hz); 5.38 (s, 1H, CH); 5.41 (d, 1H, 2′-OH, *J*=5.1 Hz); 5.80 (d, 1H, H-1′, *J*=5.1 Hz); 7.61 (d, 2H, Ar, *J*=8.7 Hz); 7.95 (s, 1H, Ar); 8.18 (d, 2H, Ar, *J*=8.7

Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.48, 22.61, 26.14, 29.17, 29.31, 29.66, 31.78, 61.60, 69.33, 70.71, 74.48, 75.40, 85.46, 88.66, 114.37, 123.92, 128.33, 138.63, 147.34, 149.21, 150.82, 162.46. MS m/z calcd. for $C_{24}H_{33}N_3O_9$: 507.55, found 506.28 $[M-H]^-$.

(+)-5-[(4-Nitrophenyl)(nonyloxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178iA)

Nucleoside **178iA** was prepared from **184iA** (180.7 mg, 0.22 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 89.0 mg (79%), m.p. 62-64°C, $[\alpha]_D^{25}$ +7.7 (c 0.26, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=6.9 Hz); 1.22-1.33 (m, 12H, CH_2); 1.48-1.57 (m, 2H, CH_2); 3.42-3.50 (m, 2H, CH_2); 3.54-3.64 (m, 2H, H-5′); 3.88-3.98 (m, 3H, H-2′, H-3′, H-4′); 5.02 (t, 1H, 5′-OH, *J*=4.8 Hz); 5.07 (d, 1H, 3′-OH, *J*=4.5 Hz); 5.37-5.38 (m, 2H, 2′-OH, CH); 5.77 (d, 1H, H-1′, *J*=4.8 Hz); 7.62 (d, 2H, Ar, *J*=8.7 Hz); 7.99 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=8.7 Hz); 11.44 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.62, 26.07, 29.22, 29.35, 29.47, 29.64, 31.80, 61.38, 69.29, 70.54, 74.51, 75.31, 85.54, 88.75, 114.28, 123.91, 128.35, 138.74, 147.29, 149.35, 150.81, 162.53. MS m/z calcd. for $C_{25}H_{35}N_3O_9$: 521.57, found 520.30 $[M-H]^-$.

(-)-5-[(4-Nitrophenyl)(nonyloxy)methyl]-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (178iB)

Nucleoside **178iB** was prepared from **184iB** (186.0 mg, 0.22 mmol) and MeOH/NH_3 solution (7 ml) according to *general procedure 2*.

Yield 84.4 mg (73%), m.p. 63-64°C, $[\alpha]_D^{25}$ -32.2 (c 0.38, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.84 (t, 3H, CH_3, *J*=6.9 Hz); 1.22-1.31 (m, 12H, CH_2); 1.49-1.58 (m, 2H, CH_2); 3.44-3.47 (m, 2H, CH_2); 3.52-3.56 (m, 2H, H-5′); 3.85-3.86 (m, 1H, H-4′); 3.95-3.99 (m, 1H, H-3′); 4.02-4.08 (m, 1H, H-2′); 5.02 (t, 1H, 5′-OH, *J*=4.2 Hz); 5.10 (d, 1H, 3′-OH, *J*=5.1 Hz); 5.38 (s, 1H, CH); 5.42 (d, 1H, 2′-OH, *J*=5.7 Hz); 5.79 (d, 1H, H-1′, *J*=5.1 Hz); 7.61 (d, 2H, Ar, *J*=9.0 Hz); 7.94 (s, 1H, Ar); 8.19 (d, 2H, Ar, *J*=9.0 Hz); 11.43 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.49, 22.62, 26.14, 29.22, 29.35, 29.48, 29.66, 31.81, 61.48, 69.30, 70.70, 74.48, 75.40, 85.54, 88.64, 114.36, 123.94, 128.33, 138.63, 147.32, 149.21, 150.82, 162.46. MS m/z calcd. for $C_{25}H_{35}N_3O_9$: 521.57, found 520.26 $[M-H]^-$.

***General procedure 3 for preparation of 5-[alkoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1H,3H)-diones* (184)**

5-[Alkoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-diones **177** were heated in hexamethyldisilazane at 140°C with $(NH_4)_2SO_4$ (approximately 5 mg) for 8 hours. After that it was evaporated and residue was dissolved in anhydrous 1,2-dichloroethane. To this solution, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** and trimethylsilyl trifluoromethanesulfonate were added. This mixture was stirred at room temperature for 2 days, washed with water (20 ml) and ethyl acetate (60 ml), dried over sodium sulphate, filtered and evaporated to dryness. All derivatives **184** were purified by silica gel column chromatography using chloroform/acetonitrile (5/1, *v/v*).
Two diastereomers **A** and **B** were isolated from derivatives **184f-i** by silica gel column chromatography using methanol in chloroform (0-5%). Other derivatives **184a-e** and **184m-n** were isolated in their diastereomeric mixtures.

5-[Methoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)--pyrimidine-2,4(1*H*,3*H*)-dione (184a)

Nucleoside **184a** was prepared from 5-[methoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177a** (600 mg, 2.16 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (1.09 g, 2.16 mmol) and trimethylsilyl trifluoromethanesulfonate (430 μl, 2.38 mmol) according to *general procedure 3* as a diastereomeric mixture.
Yield 952.3 mg (61%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.16-3.17 (m, 3H, CH_3); 4.57-4.79 (m, 3H, H-5′, H-4′); 5.25 (br.s, 1H, CH); 5.94 – 6.04 (m, 2H, H-2′, H-3′); 6.22 – 6.26 (m, 1H, H-1′); 7.40-7.70 (m, 11H, Ar); 7.81-7.96 (m, 5H, Ar); 8.03 (d, 2H, Ar, *J*=9.0 Hz); 8.15 (d, 2H, Ar, *J*=9.0 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{38}H_{31}N_3O_{12}$: 721.67, found 720.07 $[M-H]^-$.

5-[Ethoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184b)

Nucleoside **184b** was prepared from 5-[ethoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177b** (420 mg, 1.44 mmol), hexamethyldisilazane (10 ml), anhydrous 1,2-dichloroethane (15 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (726.5

mg, 1.44 mmol) and trimethylsilyl trifluoromethanesulfonate (290 µl, 1.58 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 532.0 mg (50%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03-1.12 (m, 3H, CH_3); 3.38-3.52 (m, 2H, CH_2); 4.57-4.70 (m, 2H, H-5′); 4.73 – 4.80 (m, 1H, H-4′); 5.36 (br.s, 1H, CH); 5.95 – 6.01 (m, 2H, H-2′, H-3′); 6.21 – 6.24 (m, 1H, H-1′); 7.42-7.68 (m, 11H, Ar); 7.8 (s, 1H, H-6); 7.85-7.94 (m, 4H, Ar); 8.02 (d, 2H, Ar, *J*=7.2 Hz); 8.14 (d, 2H, Ar, *J*=8.1 Hz); 11.63 (s, 1H, NH). MS m/z calcd. for $C_{39}H_{33}N_3O_{12}$: 735.71, found 734.13 $[M-H]^-$.

5-[(4-Nitrophenyl)(propoxy)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184c)

Nucleoside **184c** was prepared from 5-[(4-nitrophenyl) (propoxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177c** (470 mg, 1.54 mmol), hexamethyldisilazane (10 ml), anhydrous 1,2-dichloroethane (15 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (776.9 mg, 1.54 mmol) and trimethylsilyl trifluoromethanesulfonate (310 µl, 1.69 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 710.7 mg (62%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75-0.86 (m, 3H, CH_3); 1.41 – 1.51 (m, 2H, CH_2); 3.18-3.43 (m, 2H, CH_2); 4.56-4.70 (m, 2H, H-5′); 4.71-4.82 (m, 1H, H-4′); 5.35 (br.s, 1H, CH); 5.93-6.04 (m, 2H, H-2′, H-3′); 6.23 (dd, *J*=10.98, 4.03 Hz, 1H, H-1′); 7.40-7.70 (m, 11H, Ar); 7.77-7.96 (m, 5H, Ar); 7.99-8.05 (m, 2H, Ar); 8.15 (m, 2H, Ar); 11.64 (br.s, 1H, NH). MS m/z calcd. for $C_{40}H_{35}N_3O_{12}$: 749.74, found 748.20 $[M-H]^-$.

5-[Isopropoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184m)

Nucleoside **184m** was prepared from 5-[isopropoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177m** (400 mg, 1.31 mmol), hexamethyldisilazane (10 ml), anhydrous 1,2-dichloroethane (15 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (660.9 mg, 1.31 mmol) and trimethylsilyl trifluoromethanesulfonate (265 µl, 1.44 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 485.3 mg (49%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.99-1.15 (m, 6H, CH_3); 3.55-3.66 (m, 1H, CH); 4.56-4.68 (m, 2H, H-5′); 4.70-4.82 (m, 1H, H-4′); 5.47 (br.s, 1H, CH); 5.92-6.04 (m, 2H, H-2′, H-3′); 6.23 (m, 1H, H-1′); 7.40-7.71 (m, 11H, Ar); 7.75-

7.96 (m, 5H, Ar); 8.01 (d, 2H, Ar, *J*=7.5 Hz); 8.14 (m, 2H, Ar); 11.64 (br.s, 1H, NH). MS m/z calcd. for $C_{40}H_{35}N_3O_{12}$: 749.74, found 748.13 $[M-H]^-$.

5-[Butoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184d)

Nucleoside **184d** was prepared from 5-[butoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177d** (600 mg, 1.88 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (947.9 mg, 1.88 mmol) and trimethylsilyl trifluoromethanesulfonate (374 µl, 2.07 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 922.5 mg (64%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.74-0.86 (m, 3H, CH_3); 1.18-1.35 (m, 2H, CH_2); 1.36 – 1.50 (m, 2H, CH_2); 3.22-3.49 (m, 2H, CH_2); 4.57-4.69 (m, 2H, H-5′); 4.77 (m, 1H, H-4′); 5.34 (m, 1H, CH); 5.93-6.04 (m, 2H, H-2′, H-3′); 6.24 (m, 1H, H-1′); 7.40-7.70 (m, 11H, Ar); 7.77-7.97 (m, 5H, Ar); 8.02 (d, 2H, Ar, *J*=8.23 Hz); 8.15 (m, 2H, Ar); 11.65 (br.s, 1H, NH). MS m/z calcd. for $C_{41}H_{37}N_3O_{12}$: 763.76, found 762.00 $[M-H]^-$.

5-[*sec*-Butoxy(4-nitrophenyl)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184n)

Nucleoside **184n** was prepared from 5-[*sec*-butoxy(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177n** (600 mg, 1.88 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (947.9 mg, 1.88 mmol) and trimethylsilyl trifluoromethanesulfonate (374 µl, 2.07 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 674.5 mg (47%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.65-0.84 (m, 3H, CH_3); 0.93-1.10 (m, 3H, CH_3); 1.20-1.57 (m, 2H, CH_2); 3.35-3.50 (m, 1H, CH); 4.58-4.69 (m, 2H, H-5′); 4.70-4.83 (m, 1H, H-4′); 5.43-5.48 (m, 1H, CH); 5.91-6.05 (m, 2H, H-2′, H-3′); 6.19-6.29 (m, 1H, H-1′); 7.39-7.70 (m, 11H, Ar); 7.77-7.96 (m, 5H, Ar); 7.98-8.05 (m, 2H, Ar); 8.10-8.18 (m, 2H, Ar); 11.65 (br.s, 1H, NH). MS m/z calcd. for $C_{41}H_{37}N_3O_{12}$: 763.76, found 762.00 $[M-H]^-$.

5-[(4-Nitrophenyl)(pentyloxy)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184e)

Nucleoside **184e** was prepared from 5-[(4-nitrophenyl)(pentyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177e** (600 mg, 1.80 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (908.0 mg, 1.80 mmol) and trimethylsilyl trifluoromethanesulfonate (358 μl, 1.98 mmol) according to *general procedure 3* as a diastereomeric mixture.

Yield 868.0 mg (62%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.73-0.86 (m, 3H, CH_3); 1.17 – 1.26 (m, 4H, CH_2); 1.36-1.53 (m, 2H, CH_2); 3.21-3.46 (m, 2H, CH_2); 4.57-4.70 (m, 2H, H-5´); 4.71-4.83 (m, 1H, H-4´); 5.34 (br.s, 1H, CH); 5.92-6.05 (m, 2H, H-2´, H-3´); 6.24 (m, 1H, H-1´); 7.38-7.71 (m, 11H, Ar); 7.77-7.96 (m, 5H, Ar); 8.02 (d, 2H, Ar, *J*=8.05 Hz); 8.15 (m, 2H, Ar); 11.65 (br.s, 1H, NH). MS m/z calcd. for $C_{42}H_{39}N_3O_{12}$: 777.79, found 776.13 $[M-H]^-$.

5-[(Hexyloxy)(4-nitrophenyl)methyl]-1-(2´,3´,5´-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184f)

Compound **184f** was prepared from 5-[(hexyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177f** (600 mg, 1.73 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (871.4 mg, 1.7273 mmol) and trimethylsilyl trifluoromethanesulfonate (345 μl, 1.9061 mmol) according to *general procedure 3*.

Yield of crude product **184f** (mixture of diastereomers) 852.3 mg (62%).

1. isomer **(184fA)**

Yield 189.3 mg (14%), m.p. 75-77°C, $[\alpha]_D^{25}$ +151.9 (c 0.027, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.76-0.81 (m, 3H, CH_3); 1.12-1.26 (m, 6H, CH_2); 1.37-1.47 (m, 2H, CH_2); 3.23-3.44 (m, 2H, CH_2); 4.63-4.65 (m, 2H, H-5´); 4.77-4.81 (m, 1H, H-4´); 5.34 (s, 1H, CH); 5.94-6.02 (m, 2H, H-2´, H-3´); 6.25 (d, 1H, H-1´, *J*=4.2 Hz); 7.41-7.51 (m, 6H, Ar); 7.57 (d, 2H, Ar, *J*=8.7 Hz); 7.61-7.69 (m, 3H, Ar); 7.82 (s, 1H, Ar); 7.86 (d, 2H, Ar, *J*=7.2 Hz); 7.93 (d, 2H, Ar, *J*=7.2 Hz); 8.02 (d, 2H, Ar, *J*=7.2 Hz); 8.15 (d, 2H, Ar, *J*=8.7 Hz); 11.63 (s, 1H, NH). MS m/z calcd. for $C_{43}H_{41}N_3O_{12}$: 791.82, found 790.25 $[M-H]^-$.

2. isomer **(184fB)**

Yield 167.2 mg (12%), m.p. 69-72°C, $[\alpha]_D^{25}$ +213.9 (c 0.018 , $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.81 (t, 3H, CH_3, *J*=7.2 Hz); 1.19-1.26 (m, 6H, CH_2); 1.42-

1.50 (m, 2H, CH_2); 3.23-3.45 (m, 2H, CH_2); 4.62-4.68 (m, 2H, H-5´); 4.73-4.78 (m, 1H, H-4´); 5.33 (s, 1H, CH); 5.95-6.02 (m, 2H, H-2´, H-3´); 6.21 (d, 1H, H-1´, *J*=3.6 Hz); 7.42-7.53 (m, 6H, Ar); 7.58-7.68 (m, 5H, Ar); 7.79 (s, 1H, Ar); 7.88-7.91 (m, 4H, Ar); 8.02 (d, 2H, Ar, *J*=6.9 Hz); 8.15 (d, 2H, Ar, *J*=8.7 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{43}H_{41}N_3O_{12}$: 791.82, found 790.26 $[M-H]^-$.

5-[(Heptyloxy)(4-nitrophenyl)methyl]-1-(2´,3´,5´-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184g)

Compound **1842g** was prepared from 5-[(heptyloxy)(4-nitrophenyl)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177g** (600 mg, 1.66 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (837.6 mg, 1.66 mmol) and trimethylsilyl trifluoromethanesulfonate (331 μl, 1.83 mmol) according to *general procedure 3*.

Yield of crude product **184g** (mixture of diastereomers) 954.5 mg (71%).

1. isomer **(184gA)**

Yield 372.7 mg (28%), m.p. 72-74°C, $[\alpha]_D^{25}$ +32.7 (c 0.11, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.77-0.82 (m, 3H, CH_3); 1.20-1.23 (m, 8H, CH_2); 1.38-1.46 (m, 2H, CH_2); 3.25-3.44 (m, 2H, CH_2); 4.64-4.65 (m, 2H, H-5´); 4.77-4.81 (m, 1H, H-4´); 5.34 (s, 1H, CH); 5.94-6.01 (m, 2H, H-2´, H-3´); 6.25 (d, 1H, H-1´, *J*=4.5 Hz); 7.41-7.51 (m, 6H, Ar); 7.56-7.59 (m, 2H, Ar); 7.62-7.69 (m, 3H, Ar); 7.82-7.87 (m, 3H, Ar); 7.91-7.94 (m, 2H, Ar); 8.00-8.03 (m, 2H, Ar); 8.14 (d, 2H, Ar, *J*=9.0 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{44}H_{43}N_3O_{12}$: 805.85, found 804.25 $[M-H]^-$.

2. isomer **(184gB)**

Yield 361.5 mg (27%), m.p. 65-66°C, $[\alpha]_D^{25}$ +48.6 (c 0.074, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.78-0.82 (m, 3H, CH_3); 1.19-1.26 (m, 8H, CH_2); 1.42-1.52 (m, 2H, CH_2); 3.25-3.45 (m, 2H, CH_2); 4.62-4.65 (m, 2H, H-5´); 4.73-4.78 (m, 1H, H-4´); 5.34 (s, 1H, CH); 5.95-6.02 (m, 2H, H-2´, H-3´); 6.21 (d, 1H, H-1´, *J*=3.9 Hz); 7.42-7.52 (m, 6H, Ar); 7.58-7.68 (m, 5H, Ar); 7.80 (s, 1H, Ar); 7.87-7.91 (m, 4H, Ar); 8.00-8.03 (m, 2H, Ar); 8.15 (d, 2H, Ar, *J*=8.7 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{44}H_{43}N_3O_{12}$: 805.85, found 804.25 $[M-H]^-$.

5-[(4-Nitrophenyl)(octyloxy)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184h)

Compound **184h** was prepared from 5-[(4-nitrophenyl)(octyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177h** (600 mg, 1.60 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **183** (806.3 mg, 1.60 mmol) and trimethylsilyl trifluoromethanesulfonate (319 μl, 1.76 mmol) according to *general procedure 3*.

Yield of crude product **184h** (mixture of diastereomers) 842.3 mg (67%).

1. isomer **(184hA)**

Yield 260.0 mg (21%), m.p. 69-70°C, $[\alpha]_D^{25}$ +38.5 (c 0.091, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.81 (t, 3H, CH_3, *J*=7.2 Hz); 1.16-1.23 (m, 10H, CH_2); 1.37-1.46 (m, 2H, CH_2); 3.22-3.44 (m, 2H, CH_2); 4.64-4.65 (m, 2H, H-5′); 4.76-4.81 (m, 1H, H-4′); 5.34 (s, 1H, CH); 5.94-6.01 (m, 2H, H-2′, H-3′); 6.25 (d, 1H, H-1′, *J*=4.5 Hz); 7.41-7.51 (m, 6H, Ar); 7.57 (d, 2H, Ar, *J*=8.7 Hz); 7.62-7.68 (m, 3H, Ar); 7.82 (s, 1H, Ar); 7.86 (d, 2H, Ar, *J*=7.2 Hz); 7.93 (d, 2H, Ar, *J*=7.2 Hz); 8.02 (d, 2H, Ar, *J*=7.2 Hz); 8.14 (d, 2H, Ar, *J*=8.7 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{45}H_{45}N_3O_{12}$: 819.87, found 818.27$[M-H]^-$.

2. isomer **(184hB)**

Yield 205.0 mg (16%), m.p. 60-62°C, $[\alpha]_D^{25}$ +56.8 (c 0.059, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.78-0.83 (m, 3H, CH_3); 1.18-1.26 (m, 10H, CH_2); 1.41-1.52 (m, 2H, CH_2); 3.25-3.45 (m, 2H, CH_2); 4.62-4.65 (m, 2H, H-5′); 4.73-4.78 (m, 1H, H-4′); 5.34 (s, 1H, CH); 5.95-6.02 (m, 2H, H-2′, H-3′); 6.22 (d, 1H, H-1′, *J*=3.6 Hz); 7.42-7.52 (m, 6H, Ar); 7.56-7.68 (m, 5H, Ar); 7.80 (s, 1H, Ar); 7.88-7.91 (m, 4H, Ar); 8.01 (d, 2H, Ar, *J*=7.5 Hz); 8.14 (d, 2H, Ar, *J*=8.7 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{45}H_{45}N_3O_{12}$: 819.87, found 818.29 $[M-H]^-$.

5-[(4-Nitrophenyl)(nonyloxy)methyl]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrimidine-2,4(1*H*,3*H*)-dione (184i)

Compound **184i** was prepared from 5-[(4-nitrophenyl)(nonyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione **177i** (600 mg, 1.54 mmol), hexamethyldisilazane (15 ml), anhydrous 1,2-dichloroethane (20 ml), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose

183 (777.2 mg, 1.54 mmol) and trimethylsilyl trifluoromethanesulfonate (307 µl, 1.70 mmol) according to *general procedure 3*.

Yield of crude product **184i** (mixture of diastereomers) 721.3 mg (54%).

1. isomer **(184iA)**

Yield 207.7 mg (16%), m.p. 62-63°C, $[\alpha]_D^{25}$ +90.2 (c 0.041, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.82 (t, 3H, CH_3, *J*=6.9 Hz); 1.16-1.24 (m, 12H, CH_2); 1.37-1.47 (m, 2H, CH_2); 3.23-3.44 (m, 2H, CH_2); 4.64-4.65 (m, 2H, H-5´); 4.77-4.81 (m, 1H, H-4´); 5.34 (s, 1H, CH); 5.94-6.01 (m, 2H, H-2´, H-3´); 6.26 (d, 1H, H-1´, *J*=4.5 Hz); 7.41-7.51 (m, 6H, Ar); 7.57 (d, 2H, Ar, *J*=8.7 Hz); 7.62-7.69 (m, 3H, Ar); 7.82-7.87 (m, 3H, Ar); 7.93 (d, 2H, Ar, *J*=6.9 Hz); 8.02 (d, 2H, Ar, *J*=7.2 Hz); 8.14 (d, 2H, Ar, *J*=8.7 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{46}H_{47}N_3O_{12}$: 833.90, found 832.39 $[M-H]^-$.

2. isomer **(184iB)**

Yield 213.2 mg (16%), m.p. 54-55°C, $[\alpha]_D^{25}$ +56.4 (c 0.078, $CHCl_3$); ^{1}H NMR (300MHz, DMSO-d_6): δ 0.81 (t, 3H, CH_3, *J*=6.9 Hz); 1.18-1.24 (m, 12H, CH_2); 1.42-1.51 (m, 2H, CH_2); 3.25-3.46 (m, 2H, CH_2); 4.62-4.64 (m, 2H, H-5´); 4.73-4.77 (m, 1H, H-4´); 5.34 (s, 1H, CH); 5.95-6.02 (m, 2H, H-2´, H-3´); 6.22 (d, 1H, H-1´, *J*=3.6 Hz); 7.42-7.52 (m, 6H, Ar); 7.58-7.68 (m, 5H, Ar); 7.80 (s, 1H, Ar); 7.87-7.91 (m, 4H, Ar); 8.01 (d, 2H, Ar, *J*=8.4 Hz); 8.14 (d, 2H, Ar, *J*=9.0 Hz); 11.64 (s, 1H, NH). MS m/z calcd. for $C_{46}H_{47}N_3O_{12}$: 833.90, found 832.40 $[M-H]^-$.

5-[(Nonyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (185i)

Compound **190** (500.0 mg, 3.11 mmol) was heated in 1-nonanol (25 ml) at 100°C for 4 hours. After cooling to RT, the precipitated solid was filtered, washed with the 1-nonanol and dried.

Yield 625.2 mg (75%), m.p. 210-214°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.79-0.89 (m, 3H, CH_3); 1.23 (s, 12H, CH_2); 1.46 (t, 2H, CH_2, *J*=5.95 Hz); 3.35 (t, 2H, CH_2, *J*=6.50 Hz); 4.03 (s, 2H, CH_2); 7.37 (d, 1H, H-6, *J*=5.85 Hz); 10.83 (d, 1H, NH, *J*=4.76 Hz); 11.11 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) d 14.49, 22.62, 26.20, 29.20, 29.39, 29.53, 29.70, 31.81, 64.79, 70.01, 109.80, 140.86, 151.84, 164.32. MS m/z calcd. for $C_{14}H_{24}N_2O_3$: 268.35, found 267.10 $[M-H]^-$.

5-[(Undecyloxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (185k)

Compound **190** (496.0 mg, 3.09 mmol) was heated in 1-undecanol (25 ml) at 100°C for 4 hours. After cooling to RT, the precipitated solid was filtered, washed with the 1-undecanol and dried.

Yield 612.4 mg (67%), m.p. 204-206°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, CH_3, *J*=6.31 Hz); 1.24 (br.s, 16H, CH_2); 1.41-1.53 (m, 2H, CH_2); 3.35 (t, 2H, CH_2, *J*=6.22 Hz); 4.04 (s, 2H, CH_2); 7.35 (br.s, 1H, H-6); 10.77 (br.s, 1H, NH); 11.06 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) d 14.49, 22.62, 26.20, 29.23, 29.39, 29.53, 29.56, 29.70, 31.83, 64.79, 70.02, 109.80, 140.85, 151.84, 164.32. MS m/z calcd. for $C_{16}H_{28}N_2O_3$: 296.41, found 295.13 $[M-H]^-$.

***General Procedure 4 for preparation 5-alkoxymethyl-1-(β-D-ribofuranosyl)-pyrimidine-2,4(1H,3H)-diones* (186)**

Nucleosides **192** were dissolved in MeOH/NH_3 solution and stirred at room temperature for 6 days. Then the mixture was evaporated, co-evaporated with methanol and purified by preparative chromatography using chloroform/methanol (9:0.5) to get the nucleosides **186.**

5-Propoxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186c)

Nucleoside **186c** was prepared from **192c** (390.2 mg, 0.62 mmol) and MeOH/NH_3 solution (15 ml) according to *general procedure 4*.

Yield 178.2 mg (91%), m.p. 134-136°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.85 (t, 3H, CH_3, *J*=7.41 Hz); 1.43-1.57 (m, 2H, CH_2); 3.29-3.38 (m, 2H, CH_2); 3.49-3.68 (m, 2H, H-5′); 3.81.3.88 (m, 1H, H-4′); 3.92-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.05-5.13 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.39 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 10.46, 22.28, 60.77, 64.35, 69.81, 71.17, 73.43, 84.74, 87.54, 110.61, 138.94, 150.53, 162.57. MS m/z calcd. for $C_{13}H_{20}N_2O_7$: 316.31, found 315.05 $[M-H]^-$.

5-Isopropoxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186m)

Nucleoside **186m** was prepared from **192m** (195.1 mg, 0.27 mmol) and MeOH/NH_3 solution (10 ml) according to *general procedure 4*.

Yield 91.8 mg (94%), m.p. 113-115°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.09 (d, 6H, CH_3, *J*=6.04 Hz); 3.49-3.68 (m, 3H, CH, H-5′); 3.80-3.88 (m, 1H, H-4′); 3.92-4.14 (m,

4H, CH_2, H-2′, H-3′); 5.04-5.13 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.49 Hz); 5.79 (d, 1H, H-1′); 7.90 (s, 1H, H-6); 11.37 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 22.54, 22.57, 61.45, 62.43, 70.51, 71.03, 74.05, 85.39, 88.16, 111.85, 139.09, 151.18, 163.13. MS m/z calcd. for $C_{13}H_{20}N_2O_7$: 316.31, found 315.07 $[M-H]^-$.

5-Butoxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186d)

Nucleoside **186d** was prepared from **192d** (157.7 mg, 0.25 mmol) and MeOH/NH_3 solution (10 ml) according to *general procedure 4*.

Yield 73.4 mg (91%), m.p. 125-127°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.86 (t, 3H, CH_3, *J*=7.32 Hz); 1.21-1.37 (m, 2H, CH_2); 1.40-1.54 (m, 2H, CH_2); 3.35-3.42 (m, 2H, CH_2); 3.49-3.68 (m, 2H, H-5′); 3.81.3.87 (m, 1H, H-4′); 3.92-4.13 (m, 4H, CH_2, H-2′, H-3′); 5.09 (br.s, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.32, 19.37, 31.72, 61.41, 65.01, 69.83, 70.43, 74.05, 85.38, 88.18, 111.24, 139.60, 151.16, 163.21. MS m/z calcd. for $C_{14}H_{22}N_2O_7$: 330.33, found 329.06 $[M-H]^-$.

5-*sec*-Butoxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186n)

Nucleoside **186n** was prepared from **192n** (300.2 mg, 0.47 mmol) and MeOH/NH_3 solution (15 ml) according to *general procedure 4*.

Yield 147.7 mg (96%), m.p. 120-121°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.82 (t, 3H, CH_3, *J*=7.41 Hz); 1.07 (d, 3H, CH_3, *J*=6.04 Hz); 1.31-1.53 (m, 2H, CH_2); 3.34-3.44 (m, 1H, CH); 3.48-3.68 (m, 2H, H-5′); 3.81.3.88 (m, 1H, H-4′); 3.92-4.18 (m, 4H, CH_2, H-2′, H-3′); 5.04-5.13 (m, 2H, 5′-OH, 3′-OH); 5.38 (d, 1H, 2′-OH, *J*=5.49 Hz); 5.79 (d, 1H, H-1′, *J*=5.49 Hz); 7.89 (s, 1H, H-6); 11.37 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 10.08, 19.59, 29.14, 61.47, 62.70, 70.54, 74.05, 76.01, 85.39, 88.10, 111.93, 139.13, 151.19, 163.15. MS m/z calcd. for $C_{14}H_{22}N_2O_7$: 330.33, found 329.08 $[M-H]^-$.

5-*tert*-Butoxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186o)

Nucleoside **186o** was prepared from **192o** (277.3 mg, 0.43 mmol) and MeOH/NH_3 solution (15 ml) according to *general procedure 4*.

Yield 114.6 mg (80%), m.p. 79-81°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 1.17 (s, 9H, CH_3); 3.47-3.67 (m, 2H, H-5′); 3.81.3.88 (m, 1H, H-4′); 3.91-4.09 (m, 4H, CH_2, H-2′, H-3′); 5.01-5.14 (m, 2H, 5′-OH, 3′-OH); 5.38 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.81 (d, 1H,

H-1′, *J*=5.49 Hz); 7.84 (s, 1H, H-6); 11.34 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 27.84, 56.79, 61.58, 70.65, 73.51, 74.05, 85.45, 88.10, 112.56, 138.49, 151.18, 163.03. MS m/z calcd. for $C_{14}H_{22}N_2O_7$: 330.33, found 329.11 $[M-H]^-$.

5-Pentyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186e)

Nucleoside **186e** was prepared from **192e** (152.6 mg, 0.23 mmol) and MeOH/NH_3 solution (20 ml) according to *general procedure 4*.

Yield 52.6 mg (66%), m.p. 122-125°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.80-0.90 (m, 3H, CH_3); 1.19-1.34 (m, 4H, CH_2); 1.42-1.55 (m, 2H, CH_2); 3.32-3.41 (m, 2H, CH_2); 3.49-3.69 (m, 2H, H-5′); 3.81.3.87 (m, 1H, H-4′); 3.92-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.06-5.13 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.48, 22.48, 28.40, 29.34, 61.44, 65.01, 70.15, 70.43, 74.05, 85.39, 88.20, 111.25, 139.60, 151.16, 163.21. MS m/z calcd. for $C_{15}H_{24}N_2O_7$: 344.36, found 343.19 $[M-H]^-$.

5-Hexyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186f)

Nucleoside **186f** was prepared from **192f** (315.7 mg, 0.47 mmol) and MeOH/NH_3 solution (15 ml) according to *general procedure 4*.

Yield 143.4 mg (85%), m.p. 119-121°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.85 (t, 3H, CH_3, *J*=6.40 Hz); 1.17-1.34 (m, 6H, CH_2); 1.41-1.55 (m, 2H, CH_2); 3.35-3.41 (m, 2H, CH_2); 3.48-3.70 (m, 2H, H-5′); 3.81.3.87 (m, 1H, H-4′); 3.92-4.13 (m, 4H, CH_2, H-2′, H-3′); 5.05-5.14 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.61, 25.85, 29.63, 31.65, 61.42, 65.01, 70.15, 70.43, 74.07, 85.39, 88.22, 111.24, 139.63, 151.16, 163.21. MS m/z calcd. for $C_{16}H_{26}N_2O_7$: 358.39, found 357.13 $[M-H]^-$.

5-Heptyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186g)

Nucleoside **186g** was prepared from **192g** (504.0 mg, 0.74 mmol) and MeOH/NH_3 solution (20 ml) according to *general procedure 4*.

Yield 236.7 mg (86%), m.p. 120-122°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.78-0.91 (m, 3H, CH_3); 1.24 (br.s, 8H, CH_2); 1.41-1.54 (m, 2H, CH_2); 3.35-3.41 (m, 2H, CH_2); 3.49-3.69 (m, 2H, H-5′); 3.81.3.87 (m, 1H, H-4′); 3.90-4.13 (m, 4H, CH_2, H-2′, H-3′); 5.04-5.14 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′,

J=5.49 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 13.86, 21.96, 25.50, 28.43, 29.04, 31.16, 60.79, 64.39, 69.50, 69.80, 73.43, 84.75, 87.57, 110.59, 139.01, 150.53, 162.57. MS m/z calcd. for $C_{17}H_{28}N_2O_7$: 372.41, found 371.13 $[M-H]^-$.

5-Octyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186h)

Nucleoside **186h** was prepared from **192h** (343.1 mg, 0.49 mmol) and MeOH/NH_3 solution (15 ml) according to *general procedure 4*.

Yield 188.6 mg (99%), m.p. 124-125°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.78-0.91 (m, 3H, CH_3); 1.24 (br.s, 10H, CH_2); 1.47 (br.s, 2H, CH_2); 3.35-3.41 (m, 2H, CH_2); 3.48-3.68 (m, 2H, H-5′); 3.80.3.87 (m, 1H, H-4′); 3.91-4.13 (m, 4H, CH_2, H-2′, H-3′); 5.04-5.13 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.49 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.38 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.48, 22.62, 26.20, 29.22, 29.38, 29.67, 31.80, 61.42, 65.01, 70.15, 70.43, 74.07, 85.39, 88.22, 111.24, 139.63, 151.16, 163.21. MS m/z calcd. for $C_{18}H_{30}N_2O_7$: 386.44, found 385.16 $[M-H]^-$.

5-Nonyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186i)

Nucleoside **186i** was prepared from **192i** (578.6 mg, 0.81 mmol) and MeOH/NH_3 solution (20 ml) according to *general procedure 4*.

Yield 251.5 mg (77%), m.p. 125-127°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.79-0.91 (m, 3H, CH_3); 1.24 (br.s, 12H, CH_2); 1.47 (t, 2H, CH_2, *J*=6.04 Hz); 3.36-3.42 (m, 2H, CH_2); 3.48-3.68 (m, 2H, H-5′); 3.81.3.88 (m, 1H, H-4′); 3.91-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.09 (br.s, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=4.76 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.38 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.51, 22.64, 26.20, 29.23, 29.44, 29.54, 29.67, 31.83, 61.42, 65.01, 70.15, 70.43, 74.07, 85.39, 88.20, 111.22, 139.65, 151.16, 163.22. MS m/z calcd. for $C_{19}H_{32}N_2O_7$: 400.47, found 399.19 $[M-H]^-$.

5-Decyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186j)

Nucleoside **186j** was prepared from **192j** (347.3 mg, 0.48 mmol) and MeOH/NH_3 solution (15 ml) according *to general procedure 4*.

Yield 175.3 mg (89%), m.p. 129-131°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.80-0.90 (m, 3H, CH_3); 1.24 (br.s, 14H, CH_2); 1.41-1.54 (m, 2H, CH_2); 3.34-3.39 (m, 2H, CH_2);

3.48-3.69 (m, 2H, H-5′); 3.80-3.88 (m, 1H, H-4′); 3.91-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.04-5.13 (m, 2H, 5′-OH, 3′-OH); 5.38 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.38 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.49, 22.64, 26.20, 29.25, 29.44, 29.53, 29.58, 29.67, 31.84, 61.42, 65.03, 70.17, 70.43, 74.07, 85.39, 88.23, 111.24, 139.63, 151.16, 163.21. MS m/z calcd. for $C_{20}H_{34}N_2O_7$: 414.49, found 413.18 $[M-H]^-$.

5-Undecyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186k)

Nucleoside **186k** was prepared from **192k** (161.4 mg, 0.22 mmol) and MeOH/NH_3 solution (10 ml) according to *general procedure 4*.

Yield 84.5 mg (91%), m.p. 131-133°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75-0.90 (m, 3H, CH_3); 1.24 (br.s, 16H, CH_2); 1.38-1.55 (m, 2H, CH_2); 3.34-3.40 (m, 2H, CH_2); 3.50-3.69 (m, 2H, H-5′); 3.80-3.87 (m, 1H, H-4′); 3.91-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.03-5.14 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.31 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.86, 22.00, 25.56, 28.63, 28.80, 28.92, 28.95, 28.95, 29.04, 31.20, 60.79, 64.39, 69.53, 69.80, 73.43, 84.75, 87.57, 110.59, 138.99, 150.53, 162.57. MS m/z calcd. for $C_{21}H_{36}N_2O_7$: 428.52, found 427.25 $[M-H]^-$.

5-Dodecyloxymethyl-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (186l)

Nucleoside **186l** was prepared from **192l** (530.1 mg, 0.70 mmol) and MeOH/NH_3 solution (20 ml) according to *general procedure 4*.

Yield 250.1 mg (81%), m.p. 132-133°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.79-0.90 (m, 3H, CH_3); 1.23 (br.s, 18H, CH_2); 1.40-1.53 (m, 2H, CH_2); 3.34-3.40 (m, 2H, CH_2); 3.49-3.69 (m, 2H, H-5′); 3.81-3.88 (m, 1H, H-4′); 3.92-4.12 (m, 4H, CH_2, H-2′, H-3′); 5.05-5.13 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.79 (d, 1H, H-1′, *J*=5.49 Hz); 7.93 (s, 1H, H-6); 11.39 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.48, 22.64, 26.20, 29.25, 29.26, 29.44, 29.56, 29.57, 29.60, 29.67, 31.84, 61.42, 65.03, 70.17, 70.43, 74.07, 85.39, 88.23, 111.24, 139.63, 151.16, 163.21. MS m/z calcd. for $C_{22}H_{38}N_2O_7$: 442.55, found 441.25 $[M-H]^-$.

1-[(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl]methoxy)-3-hydroxypropan-2-yl palmitate (187)

Compound **198** (267.1 mg, 0.81 mmol) was dissolved in pyridine (5 ml) and cooled to 0°C. The palmitoyl chloride (1.1 ml, 3.63 mmol) was slowly dropped to reaction mixture. The mixture was warmed to RT and stirred for 2 hours. Then it was evaporated, co-evaporated with toluene (4 x 10 ml) and methanol (1 x 10 ml). Crude product was purified by silica gel column chromatography using chloroform/methanol (9/1) to give derivative **187**.

Yield 151.2 mg (41%); m.p. 159-161°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.85 (t, 3H, CH_3, *J*=6.0 Hz); 1.23 (br.s, 26H, CH_2); 1.47-1.52 (m, 2H, CH_2); 2.26 (t, 2H, CH_2, *J*=7.2 Hz); 3.43-3.55 (m, 3H, CH_2, CH); 4.08 (s, 2H, CH_2); 4.80-4.90 (m, 1H, OH); 7.36 (s, 1H, H-6); 10.86 (br.s, 1H, NH); 11.12 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 14.46, 22.64, 25.04, 28.94, 29.00, 29.25, 29.44, 29.60, 31.84, 34.23, 60.40, 65.32, 68.92, 73.67, 109.42, 140.98, 151.81, 164.26, 173.07. MS m/z calcd. for $C_{24}H_{42}N_2O_6$: 454.60, found 453.21 $[M-H]^-$.

1-[(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl]methoxy)-3-hydroxypropan-2-yl acetate (188)

Compound **198** (200.0 mg, 0.61 mmol) was dissolved in pyridine (2 ml) and cooled to 0°C. The acetyl chloride (195 µl, 2.74 mmol) was slowly dropped to reaction mixture. The mixture was warmed to RT and stirred for 2 hours. Then it was evaporated, co-evaporated with toluene (4 x 5 ml) and methanol (1 x 5 ml). Crude product was purified by silica gel column chromatography using chloroform/methanol (9/1) to give derivative **188**.

Yield 123.7 mg (79%); m.p. 129-131°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 2.00 (s, 3H, CH_3); 3.36-3.55 (m, 5H, CH_2, CH); 4.08 (s, 2H, CH_2); 4.82-4.85 (m, 1H, OH); 7.39 (s, 1H, H-6); 10.86 (br.s, 1H, NH); 11.13 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 21.51, 60.35, 65.29, 68.75, 73.90, 109.38, 141.23, 151.84, 164.32, 170.53. MS m/z calcd. for $C_{10}H_{14}N_2O_6$: 258.23, found 259.98 $[M-H]^-$.

5-[(2,3-dihydroxy-1-propoxy)methyl)]-1-(β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (189)

Nucleosides **205** (393.6 mg, 0.60 mmol) was dissolved in MeOH/NH_3 solution (10 ml) and stirred at room temperature for 6 days. Then the mixture was evaporated, co-evaporated with methanol and purified by silica gel column chromatography using chloroform/methanol (9/3) to get the nucleosides **189.**

Yield 150.6 mg (73%); m.p. 78-80°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.26-3.46 (m, 4H, CH_2); 3.50-3.69 (m, 3H, CH, H-5′); 3.80-3.87 (m, 1H, H-4′); 3.93-4.00 (m, 1H, H-3′); 4.00-4.09 (m, 1H, H-2′); 4.12 (s, 2H, CH_2); 4.49 (t, 1H, OH, *J*=5.49 Hz); 4.67 (d, 1H, OH, *J*=4.94 Hz); 5.06-5.16 (m, 2H, 5′-OH, 3′-OH); 5.39 (d, 1H, 2′-OH, *J*=5.67 Hz); 5.78 (d, 1H, H-1′, *J*=5.31 Hz); 7.95 (s, 1H, Ar); 11.40 (br.s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6) δ 61.45, 63.58, 65.64, 70.43, 71.08, 72.56, 73.99, 85.41, 88.32, 111.22, 139.57, 151.15, 163.26. MS m/z calcd. for $C_{13}H_{20}N_2O_9$: 348.31, found 349.07 $[M+H]^-$.

***General Procedure 5 for preparation 5-alkoxymethyl-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1H,3H)-diones* (192)**

Uraciles **185** were heated in hexamethyldisilazane at 140°C with $(NH_4)_2SO_4$ (approximately 5 mg) for 8 hours. After that it was evaporated and residue was dissolved in anhydrous 1,2-dichloroethane (20 ml). To this solution, 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose **183** and trimethylsilyl trifluoromethane-sulfonate were added. This mixture was stirred at room temperature for 24 hours, washed with water (20 ml) and ethyl acetate (3 x 60 ml), dried over sodium sulphate, filtered and evaporated to dryness. Crude product was purified by preparative chromatography using chloroform/acetonitrile (5:1) to get the nucleosides **192.**

5-Methoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192a)

Nucleoside **192a** was prepared from **185a** (238.5 mg, 1.53 mmol), hexamethyldisilazane (10 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (847.7 mg, 1.68 mmol) and trimethylsilyl trifluoromethanesulfonate (415 μl, 2.29 mmol) according to *general procedure 5*.

Yield 557.4 mg (61%), m.p. 78-80°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.15 (s, 3H, CH_3); 3.90-4.04 (m, 2H, CH_2); 4.58-4.80 (m, 3H, H-5′, H-4′); 5.93-5.95 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=3.48 Hz); 7.39-7.56 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.82 (s, 1H, H-6); 7.91 (d, 2H, Ar, *J*=7.32 Hz); 7.87 (d, 2H, Ar, *J*=7.32 Hz); 8.03 (d, 2H, Ar, *J*=7.32 Hz); 11.61 (s, 1H, NH). MS m/z calcd. for $C_{32}H_{28}N_2O_{10}$: 600.57, found 599.10 $[M-H]^-$.

5-Ethoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192b)

Nucleoside **192b** was prepared from **185b** (351.4 mg, 2.07 mmol), hexamethyldisilazane (15 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (1041.8 mg, 2.0651 mmol) and trimethylsilyl trifluoromethanesulfonate (415 μl, 2.30 mmol) according to *general procedure 5*.

Yield 644.6 mg (69%), m.p. 72-75°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, 3H, CH_3, *J*=6.95 Hz); 3.30-3.38 (m, 2H, CH_2); 3.94-4.09 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-4′); 5.92-5.95 (m, 2H, H-2′, H-3′); 6.22 (d, 1H, H-1′, *J*=2.74 Hz); 7.40-7.56 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.91 (d, 2H, Ar, *J*=8.05 Hz); 7.87 (d, 2H, Ar, *J*=7.87 Hz); 8.03 (d, 2H, Ar, *J*=7.68 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{33}H_{30}N_2O_{10}$: 614.60, found 623.18 $[M-H]^-$.

5-Propoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192c)

Nucleoside **192c** was prepared from **185c** (293.8 mg, 1.60 mmol), hexamethyldisilazane (15 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (804.7 mg, 1.60 mmol) and trimethylsilyl trifluoromethanesulfonate (320 μl, 1.77 mmol) according to *general procedure 5*.

Yield 437.4 mg (44%), m.p. 70-72°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.79 (t, 3H, CH_3, *J*=7.41 Hz); 1.35-1.49 (m, 2H, CH_2); 3.23-3.28 (m, 2H, CH_2); 3.95-4-07 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-4′); 5.94-5.96 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=2.93 Hz); 7.40-7.56 (m, 6H, Ar); 7.61-7.71 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.83-7.95 (m, 4H, Ar); 8.03 (d, 2H, Ar, *J*=7.32 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{34}H_{32}N_2O_{10}$: 628.63, found 627.14 $[M-H]^-$.

5-Isopropoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192m)

Nucleoside **192m** was prepared from **185m** (191.6 mg, 1.04 mmol), hexamethyldisilazane (15 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (524.8 mg, 1.04 mmol) and trimethylsilyl trifluoromethanesulfonate (210 μl, 1.16 mmol) according to *general procedure 5*.

Yield 307.7 mg (47%), m.p. 46-47°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.98-1.07 (m, 6H, CH_3); 3.48-3.60 (m, 1H, CH); 3.96-4.08 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-

4′); 5.92-5.98 (m, 2H, H-2′, H-3′); 6.22 (d, 1H, H-1′, *J*=3.11 Hz); 7.40-7.56 (m, 6H, Ar); 7.61-7.72 (m, 3H, Ar); 7.78 (s, 1H, H-6); 7.84-7.95 (m, 4H, Ar); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.59 (s, 1H, NH). MS m/z calcd. for $C_{34}H_{32}N_2O_{10}$: 628.63, found 627.11 $[M-H]^-$.

5-Butoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192d)

Nucleoside **192d** was prepared from **185d** (166.8 mg, 0.84 mmol), hexamethyldisilazane (15 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (424.5 mg, 0.84 mmol) and trimethylsilyl trifluoromethanesulfonate (170 μl, 0.94 mmol) according to *general procedure 5*.

Yield 185.1 mg (34%), m.p. 63-66°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77-0.86 (m, 3H, CH_3); 1.24 (sxt, 2H, CH_2, *J*=7.25 Hz); 1.33-1.46 (m, 2H, CH_2); 3.25-3.32 (m, 2H, CH_2); 3.95-4.07 (m, 2H, CH_2); 4.58-4.80 (m, 2H, H-5′, H-4′); 5.91-5.98 (m, 2H, H-2′, H-3′); 6.20-6.25 (m, 1H, H-1′); 7.40-7.56 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.91 (d, 2H, Ar, *J*=7.50 Hz); 7.87 (d, 2H, Ar, *J*=7.32 Hz); 8.03 (d, 2H, Ar, *J*=7.32 Hz); 11.59 (br.s, 1H, NH). MS m/z calcd. for $C_{35}H_{34}N_2O_{10}$: 642.65, found 641.14 $[M-H]^-$.

5-*sec*-Butoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192n)

Nucleoside **192n** was prepared from **185n** (303.7 mg, 1.53 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (772.9 mg, 1.53 mmol) and trimethylsilyl trifluoromethanesulfonate (420 μl, 2.32 mmol) according to *general procedure 5*.

Yield 457.2 mg (46%), m.p. 67-70°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77 (td, 3H, CH_3, *J*=7.36, 2.10 Hz); 0.95-1.04 (m, 3H, CH_3); 1.21-1.45 (m, 2H, CH_2); 3.25-3.31 (m, 1H, CH); 3.89-4.15 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-4′); 5.91-5.98 (m, 2H, H-2′, H-3′); 6.21-6.24 (m, 1H, H-1′); 7.39-7.56 (m, 6H, Ar); 7.60-7.71 (m, 3H, Ar); 7.78 (s, 1H, H-6); 7.91 (d, 2H, Ar, *J*=8.23 Hz); 7.87 (d, 2H, Ar, *J*=8.23 Hz); 8.02 (d, 2H, Ar, *J*=7.32 Hz); 11.59 (br.s, 1H, NH). MS m/z calcd. for $C_{35}H_{34}N_2O_{10}$: 642.65, found 641.14 $[M-H]^-$.

5-*tert*-Butoxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192o)

Nucleoside **192o** was prepared from **185o** (186.0 mg, 0.94 mmol), hexamethyldisilazane (15 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (473.4 mg, 0.94 mmol) and trimethylsilyl trifluoromethanesulfonate (190 μl, 1.05 mmol) according to *general procedure 5*.

Yield 248.0 mg (41%), m.p. 88-90°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (s, 9H, CH_3); 3.93-4.05 (m, 2H, CH_2); 4.56-4.81 (m, 3H, H-5′, H-4′); 5.96 (br.s, 2H, H-2′, H-3′); 6.22 (br.s, 1H, H-1′); 7.38-7.57 (m, 6H, Ar); 7.59-7.71 (m, 3H, Ar); 7.73 (s, 1H, H-6); 7.89 (t, 4H, Ar, *J*=8.23 Hz); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.57 (s, 1H, NH). MS m/z calcd. for $C_{35}H_{34}N_2O_{10}$: 642.65, found 614.04 $[M-H]^-$.

5-Pentyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192e)

Nucleoside **192e** was prepared from **185e** (145.6 mg, 0.69 mmol), hexamethyldisilazane (10 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (346.1 mg, 0.69 mmol) and trimethylsilyl trifluoromethanesulfonate (137 μl, 0.76 mmol) according to *general procedure 5*.

Yield 165.1 mg (37%), m.p. 62-64°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H, CH_3, *J*=6.77 Hz); 1.12-1.29 (m, 4H, CH_2); 1.30-1.47 (m, 2H, CH_2); 3.23-3.31 (m, 2H, CH_2); 3.94-4.07 (m, 2H, CH_2); 4.57-4.82 (m, 3H, H-5′, H-4′); 5.92-5.98 (m, 2H, H-2′, H-3′); 6.23 (br.s, 1H, H-1′); 7.37-7.57 (m, 6H, Ar); 7.59-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.08, 7.32 Hz); 8.03 (d, 2H, Ar, *J*=7.32 Hz); 11.14 (br.s, 1H, NH). MS m/z calcd. for $C_{36}H_{36}N_2O_{10}$: 656.68, found 655.14 $[M-H]^-$.

5-Hexyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192f)

Nucleoside **192f** was prepared from **185f** (341.8 mg, 1.51 mmol), hexamethyldisilazane (10 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (838.3 mg, 1.66 mmol) and trimethylsilyl trifluoromethanesulfonate (410 μl, 2.27 mmol) according to *general procedure 5*.

Yield 405.2 mg (40%), m.p. 50-51°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.75-0.90 (m, 3H, CH_3); 1.10-1.31 (m, 6H, CH_2); 1.32-1.48 (m, 2H, CH_2); 3.22-3.32 (m, 2H, CH_2); 3.95-4.07 (m, 2H, CH_2); 4.58-4.82 (m, 3H, H-5′, H-4′); 5.89-6.00 (m, 2H, H-2′, H-3′);

6.21-6.25 (m, 1H′, H-1′); 7.38-7.57 (m, 6H, Ar); 7.59-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.08, 7.32 Hz); 8.03 (d, 2H, Ar, *J*=7.32 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{37}H_{38}N_2O_{10}$: 670.71, found 669.16 $[M-H]^-$.

5-Heptyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192g)

Nucleoside **192g** was prepared from **185g** (374.9 mg, 1.56 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (787.1 mg, 1.56 mmol) and trimethylsilyl trifluoromethanesulfonate (420 μl, 2.32 mmol) according to *general procedure 5*.

Yield 526.3 mg (49%), m.p. 48-50°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H, *J*=6.59 Hz); 1.11-1.31 (m, 8H, CH_2); 1.32-1.46 (m, 2H, CH_2); 3.24-3.31 (m, 2H, CH_2); 3.95-4.06 (m, 2H, CH_2); 4.57-4.81 (m, 3H, H-5′, H-4′); 5.91-5.97 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=2.93 Hz); 7.39-7.57 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.26, 7.32 Hz); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{38}H_{40}N_2O_{10}$: 684.73, found 683.14 $[M-H]^-$.

5-Oktyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192h)

Nucleoside **192h** was prepared from **185h** (300.3 mg, 1.18 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (655.2 mg, 1.30 mmol) and trimethylsilyl trifluoromethanesulfonate (320 μl, 1.77 mmol) according to *general procedure 5*.

Yield 379.9 mg (46%), m.p. 56-57°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H, CH_3, *J*=6.68 Hz); 1.11-1.30 (m, 10H, CH_2); 1.32-1.47 (m, 2H, CH_2); 3.23-3.32 (m, 2H, CH_2); 3.92-4.10 (m, 2H, CH_2); 4.59-4.81 (m, 3H, H-5′, H-4′); 5.91-5.97 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=2.93 Hz); 7.39-7.55 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.44, 7.32 Hz); 8.02 (d, 2H, Ar, *J*=7.32 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{39}H_{42}N_2O_{10}$: 698.76, found 697.22 $[M-H]^-$.

5-Nonyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192i)

Nucleoside **192i** was prepared from **185i** (505.9 mg, 1.89 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (1046.2 mg, 2.07 mmol)

and trimethylsilyl trifluoromethanesulfonate (515 μl, 2.85 mmol) according to *general procedure 5*.

Yield 623.4 mg (46%), m.p. 58-60°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78-0.89 (m, 3H, CH_3); 1.13-1.29 (m, 12H, CH_2); 1.33-1.46 (m, 2H, CH_2); 3.23-3.32 (m, 2H, CH_2); 3.93-4.08 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-4′); 5.90-5.98 (m, 2H, H-2′, H-3′); 6.20-6.26 (m, 1H, H-1′); 7.39-7.56 (m, 6H, Ar); 7.60-7.71 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.26, 7.50 Hz); 8.02 (d, 2H, Ar, *J*=7.50 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{40}H_{44}N_2O_{10}$: 712.78, found 711.20 $[M-H]^-$.

5-Decyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192j)

Nucleoside **(192j)** was prepared from **185j (**338.1 mg, 1.20 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (604.0 mg, 1.20 mmol) and trimethylsilyl trifluoromethanesulfonate (325 μl, 1.80 mmol) according to *general procedure 5*.

Yield 425.9 mg (49%), m.p. 67-68°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.76-0.89 (m, 3H, CH_3); 1.11-1.31 (m, 14H, CH_2); 1.33-1.45 (m, 2H, CH_2); 3.23-3.32 (m, 2H, CH_2); 3.93-4.08 (m, 2H, CH_2); 4.59-4.81 (m, 3H, H-5′, H-4′); 5.90-5.98 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=2.93 Hz); 7.38-7.57 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.81 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.53, 7.23 Hz); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{41}H_{46}N_2O_{10}$: 726.81, found 725.21 $[M-H]^-$.

5-Undecyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192k)

Nucleoside **192k** was prepared from **185k** (215.9 mg, 0.73 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (367.5 mg, 0.73 mmol) and trimethylsilyl trifluoromethanesulfonate (198 μl, 1.09 mmol) according to *general procedure 5*.

Yield 181.5 mg (34%), m.p. 69-72°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77-0.90 (m, 3H, CH_3); 1.11-1.31 (m, 16H, CH_2); 1.33-1.46 (m, 2H, CH_2); 3.22-3.31 (m, 2H, CH_2); 3.92-4.08 (m, 2H, CH_2); 4.56-4.81 (m, 3H, H-5′, H-4′); 5.90-5.97 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=3.11 Hz); 7.39-7.55 (m, 6H, Ar); 7.59-7.72 (m, 3H, Ar); 7.80 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.72, 7.23 Hz); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{42}H_{48}N_2O_{10}$: 740.84, found 739.27 $[M-H]^-$.

5-Dodecyloxymethyl-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (192l)

Nucleoside **192l** was prepared from **185l** (359.3 mg, 1.16 mmol), hexamethyldisilazane (12 ml), 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose (583.9 mg, 1.16 mmol) and trimethylsilyl trifluoromethanesulfonate (315 μl, 1.74 mmol) according to *general procedure 5*.

Yield 535.4 mg (61%), m.p. 77-78°C; ^{1}H NMR (300MHz, DMSO-d_6) δ 0.83 (t, 3H, CH_3, *J*=6.50 Hz); 1.12-1.30 (m, 18H, CH_2); 1.32-1.46 (m, 2H, CH_2); 3.21-3.31 (m, 2H, CH_2); 3.92-4.09 (m, 2H, CH_2); 4.58-4.81 (m, 3H, H-5′, H-4′); 5.91-5.97 (m, 2H, H-2′, H-3′); 6.23 (d, 1H, H-1′, *J*=2.93 Hz); 7.39-7.55 (m, 6H, Ar); 7.60-7.72 (m, 3H, Ar); 7.80 (s, 1H, H-6); 7.89 (dd, 4H, Ar, *J*=12.53, 7.23 Hz); 8.02 (d, 2H, Ar, *J*=7.14 Hz); 11.60 (s, 1H, NH). MS m/z calcd. for $C_{43}H_{50}N_2O_{10}$: 754.86, found 753.56 $[M-H]^-$.

5-[(3-((*t*-butyldimethylsilyl)oxy)-2-hydroxypropoxy)methyl]pyrimidine-2,4(1*H*, 3*H*)-dione (198)

Compound **195** (100 mg, 0.46 mmol), TBDMSCl (105.0 mg, 0.70 mmol) and DMAP (28.3 mg, 0.23 mmol) were suspended in DMF and TEA (195 μl, 1.40 mmol) was added. This solution was stirred at RT over night, washed with water (15 ml) and diethyl ether (2 x 25 ml). White precipitate formed in organic layer was filtered, washed with diethyl ether and dried to give derivative **198.**

Yield 121.7 mg (80%); m.p. 206-208°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.02 (s, 6H, TBDMS); 0.84 (s, 9H, TBDMS); 3.25- 3.44 (m, 2H, CH_2); 3.45-3.51 (m, 2H, CH_2); 3.52-3.63 (m, 1H, CH); 4.09 (s, 2H, CH_2); 4.72 (d, 1H, OH, *J*=4.94 Hz); 7.41 (s, 1H, H-6); 10.85 (br.s, 1H, NH); 11.11 (br.s, 1H, NH).^{13}C NMR (75MHz, DMSO-d_6) δ -4.77, 18.54, 26.35, 64.97, 65.32, 70.71, 71.81, 109.63, 140.92, 151.84, 164.36. MS m/z calcd. for $C_{14}H_{26}N_2O_5Si$: 330.16, found 329.09 $[M-H]^-$.

Diisopropyl ((5-((3-((*tert*-butyldimethylsilyl)oxy)-2-hydroxypropoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl)phosphonate (204)

Compound **198** (54.0 mg, 0.16 mmol) in DMF (2 ml) was treated with NaH (7.7 mg, 60% in paraffin oil, 0.19 mmol) and stirred at RT for 30 min. Diisopropyl [(tosyloxy)methyl]phosphonate (171.3 mg, 0.49 mmol) was added and the resulting mixture was stirred at RT for 6 days. After that, DMF was evaporated in vacuo at 60 °C.

The residue was codistilled with toluen and MeOH. Chromatography on silica gel column using $CHCl_3$/MeOH (9/1) afforded derivative **204** as a yellow oil.

Yield 5.7 mg (7%); ^{1}H NMR (300MHz, DMSO-d_6) δ 0.03 (s, 6H, TBDMS); 0.86 (s, 9H, TBDMS); 1.22-1.26 (m, 12H, CH_3ipr); 3.34- 3.54 (m, 4H, CH_2); 3.55-3.67 (m, 1H, CH); 4.14 (s, 2H, CH_2); 4.18 (d, 2H, PCH_2, *J*=9.0Hz); 4.53-4.66 (m, 2H, CHipr); 4.70 (d, 1H, OH, *J*=6.0 Hz); 7.56 (s, 1H, H-6); 11.51 (br.s, 1H, NH). MS m/z calcd. for $C_{21}H_{41}N_2O_8PSi$: 508.62, found 507.09 $[M-H]^-$.

5-[(2,3-dihydroxy-1-propoxy)methyl)]-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidine-2,4(1*H*,3*H*)-dione (205)

Compound **195** was heated in hexamethyldisilazane (50 ml) at 140°C with $(NH_4)_2SO_4$ (approximately 5 mg) for 6 hours. After that it was evaporated and residue was dissolved in anhydrous 1,2-dichloroethane (50 ml). To this solution, 1-*O*-acetyl-2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranose **183** (2.7424 g, 5.44 mmol) and trimethylsilyl trifluoromethanesulfonate (1090 μl, 6.02 mmol) were added. This mixture was stirred at room temperature for 24 hours, washed with water (50 ml) and ethyl acetate (3 x 150 ml), dried over sodium sulphate, filtered and evaporated to dryness. Crude product was purified by preparative chromatography using chloroform/methanol (9/0.5) to get the nucleosides **205.**

Yield 1.71 g (48%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.24-3.45 (m, 4H, CH_2); 3.51-3.60 (m, 1H, CH); 4.00-4.15 (m, 2H, CH_2); 4.48 (t, 1H, OH, *J*=5.67 Hz); 4.58 - 4.80 (m, 4H, H-5′, H-4′, OH); 5.90-6.00 (m, 2H, H-2′, H-3′); 6.15-6.21 (m, 1H, H-1′); 7.39-7.56 (m, 6H, Ar); 7.60-7.71 (m, 3H, Ar); 7.83-7.94 (m, 5H, Ar); 8.01 (d, 2H, Ar, *J*=7.14 Hz); 11.63 (s, 1H, NH). MS m/z calcd. for $C_{34}H_{32}N_2O_{12}$: 660.20, found 659.11 $[M-H]^-$.

5-(1-(4-(hydroxyphenyl)-1,2,3-triazol-4-yl)-2′-deoxyuridine (213a)

Nucleoside **215a** (37.3 mg, 0.05 mmol) was dissolved in 80% AcOH (5 ml). This mixture was stirred at RT over night, evaporated, co-evaporated with ethanol (3 x 5 ml) and purified by silica gel column chromatography using MeOH/ CH_2Cl_2 (5-15%) to afford white solid.

Yield 17.4 mg (83%). ^{1}H NMR (300MHz, DMSO-d_6): δ 2.19-2.23 (m, 2H, H-2′); 3.61-3.62 (m, 2H, H-5′); 3.85-3.88 (m, 1H, H-4′); 4.29-4.30 (m, 1H, H-3′); 6.25 (t, 1H, H-1′, *J*=6.6Hz); 6.93 (d, 2H, Ar, *J*=9 Hz); 7.68 (d, 2H, Ar, *J*=9 Hz); 8.62 (s, 1H, H-6); 8.68

(s, 1H, CH triazole). ^{13}C NMR (75MHz, DMSO-d_6): δ 61.4 (C-5′); 70.7 (C-3′); 84.8 (C-1′); 87.7 (C-4′); 104.9 (C-5); 116.1 (Ar); 120.1 (C-5 triazole); 122.0 (Ar); 128.7 (C-4 triazole); 136.6 (C-6); 139.8, 149.8 (Ar); 157.9 (C-2); 161.3 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{17}H_{17}N_5O_6$: 410.1071, found 410.1079.

5-(1-(3-(hydroxyphenyl)-1,2,3-triazol-4-yl)-2′-deoxyuridine (213b)

Nucleoside **223b** (77 mg, 0.15 mmol) was dissolved in NH_4OH (10 ml, 24% aqueous solution). This mixture was stirred at RT over night, evaporated, co-evaporated with ethanol (3 x 5 ml) and purified by silica gel column chromatography using MeOH/ CH_2Cl_2 (5-15%) to afford white solid.

Yield 54 mg (91%). ^{1}H NMR (300MHz, DMSO-d_6): δ 2.22-2.25 (m, 2H, H-2′); 3.65 (bs, 2H, H-5′); 3.89-3.90 (m, 1H, H-4′); 4.33 (bs, 1H, H-3′); 5.11 (bs, 1H, 5′-OH); 5.36 (bs, 1H, 3′-OH); 6.27 (t, 1H, H-1′, *J*=6.6Hz); 6.89-6.92 (m, 1H, Ar); 7.41-7.32 (m, 3H, Ar); 8.67 (s, 1H, H-6); 8.77 (s, 1H, CH triazole). ^{13}C NMR (75MHz, DMSO-d_6): δ 40.1 (C-2′); 61.4 (C-5′); 70.7 (C-3′); 84.9 (C-1′); 87.7 (C-4′); 104.8 (C-5); 107.1, 110.5, 115.7 (Ar); 120.0 (C-5 triazole); 130.8 (C-4 triazole); 136.8 (C-6); 137.6, 140.0, 149.8 (Ar); 158.6 (C-2); 161.2 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{17}H_{17}N_5O_6$: 410.1071, found 410.1068.

5-(1-(4-(hydroxyphenyl)-1,2,3-triazol-4-yl)-5′-(4,4′-dimethoxytrityl)-2′-deoxyuridine (215a)

Compound **210** (107.5 mg, 0.50 mmol) was dissolved in EtOH/ H_2O (1 ml, 7/3, *v/v*). To this solution, NaN_3 (32.5 mg, 0.50 mmol), sodium ascorbate (5 mg, 0.03 mmol), CuI (9.5 mg, 0.05 mmol) and N,N′-dimethylenediamine (20 µl, 0.19 mmol) were added. The mixture was stirred at RT over night. Then, DMTr-nucleoside (110.9 mg, 0.20 mmol), sodium ascorbate (5 mg, 0.03 mmol), CuI (9.5 mg, 0.05 mmol) and N,N′-dimethylethylendiamine (20 µl, 0.19 mmol) were added. Reaction mixture was stirred at RT over night, evaporated and purified by silica gel column chromatography using MeOH in CH_2Cl_2 (5%) to afford yelllow solid.

Yield 58.8 mg (43%). ^{1}H NMR (300MHz, DMSO-d_6): δ 2.28-2.31 (m, 2H, H-2′); 3.14-3.27 (m, 2H, H-5′); 3.67 (s, 3H, OCH_3); 3.68 (s, 3H, OCH_3); 3.94-3.98 (m, 1H, H-4′); 4.20-4.23 (m, 1H, H-3′); 5.37 (d, 1H, 3′-OH, J = 4.2 Hz); 6.20 (t, 1H, H-1′, J = 6.3 Hz); 6.83 (dd, 4H, Ar, J_1 = 7.2 Hz, J_2 = 1.5 Hz); 6.91 (d, 2H, Ar, J = 8.1 Hz); 7.12-7.39 (m,

9H, Ar); 7.67 (d, 2H, Ar, J = 8.7 Hz); 8.38 (s, 1H, H-6); 8.64 (s, 1H, H-triazole); 9.93 (s, 1H, OH); 11.79 (s, 1H, NH). ^{13}C NMR (300 MHz, DMSO): δ 54.9 (OCH_3); 63.6 (C-5´); 70.4 (C-3´); 85.3 (C-1´); 85.7 (Ph_3C); 85.7 (C-4´); 104.9 (C-5); 113.1, 116.0 (Ar); 120.0 (C-5 triazole); 122.0, 126.6, 127.7, 127.8, 128.7, 129.5, 129.6 (Ar); 129.7 (C-4 triazole); 135.4, 135.5 (Ar); 136.1 (C-6); 144.8 (DMTr); 149.6 (C-2); 157.7, 158.0 (DMTr); 161.1 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{38}H_{35}N_5O_8$: 712.2378, found 712.2373.

5-(1-(4-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-2´-deoxyuridine (223a)

Compound **225a** (51.9 mg, 0.21 mmol) was dissolved in EtOH/ H_2O (1ml, 7:3, *v/v*). To this solution, 5-ethynyl-2´-deoxyuridine **207** (27.7 mg, 0.11 mmol), CuI (4.0 mg, 0.02 mmol) and sodium ascorbate (2.1mg, 0.01 mmol) were added. This mixture was stirred at RT over night. The next day it was evaporated, co-evaporated with toluene (2 x 5ml) and methanol (1 x 5ml). It was purified by silica gel column chromatography using MeOH in CH_2Cl_2 (0-10%).

Yield 29.5 mg (54%). ^{1}H NMR (300MHz, DMSO-d_6): δ 0.24 (s, 6H, TBDMS); 0.98 (s, 9H, TBDMS); 2.22 (t, 2H, H-2´, *J*=5.7Hz); 3.63 (bs, 2H, H-5´); 3.88 (bs, 1H, H-4´); 4.31 (bs, 1H, H-3´); 5.06 (t, 1H, 5´-OH, *J*=4.8Hz); 5.30 (d, 1H, 3´-OH, *J*=4.2 Hz); 6.27 (t, 1H, H-1´, *J*=6.3Hz); 7.04 (d, 2H, Ar, *J*=9Hz); 7.80 (d, 2H, Ar, *J*=8.7Hz); 8.66 (s, 1H, H-6); 8.77 (s, 1H, CH triazole); 11.74 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6): δ -4.58 (TBDMS); 18.0 ($(CH_3)_3C$); 25.5 (TBDMS); 61.4 (C-5´); 70.7 (C-3´); 84.9 (C-1´); 87.7 (C-4´); 104.8 (C-5); 120.1 (C-5 triazole); 120.9, 121.9, 122.0 (Ar); 130.7 (C-4 triazole); 136.7 (C-6); 139.9 (Ar); 149.7 (C-2); 155.3 (Ar); 161.1 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{23}H_{31}N_5O_6Si$: 524.1936, found 524.2603.

5-(1-(3-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-2´-deoxyuridine (223b)

Compound **225b** (300 mg, 1.20 mmol) was dissolved in EtOH/ H_2O (10ml, 7:3, *v/v*). To this solution, 5-ethynyl-2´-deoxyuridine **207** (303.4 mg, 1.20 mmol), CuI (91.6 mg, 0.48 mmol) and sodium ascorbate (238.3 mg, 1.20 mmol) were added. This mixture was stirred at RT over night. The next day it was evaporated, co-evaporated with toluene (2 x 10 ml) and methanol (1 x 10ml), dissolved in EtAc (30 ml), washed with water (30 ml) and brine (30 ml). Organic phase was dried over sodium sulfate, filtered and evaporated. Then it was dissloved in MeOH, adsorbed on silica gel and purified by silica gel column chromatography using MeOH in CH_2Cl_2 (0-10%).

Yield 319 mg (53%). ^{1}H NMR (300MHz, DMSO-d_6): δ 0.25 (s, 6H, TBDMS); 0.99 (s, 9H, TBDMS); 2.21-2.25 (m, 2H, H-2´); 3.64 (bs, 2H, H-5´); 3.89-3.90 (m, 1H, H-4´); 4.32 (bs, 1H, H-3´); 5.04-5.10 (m, 1H, 5´-OH); 5.32 (s, 1H, 3´-OH); 6.27 (t, 1H, H-1´, *J*=6.6Hz); 6.98 (d, 1H, Ar, *J*=8.4 Hz); 7.44-7.58 (m, 3H, Ar); 8.68 (s, 1H, H-6); 8.88 (s, 1H, CH triazole); 11.75 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6): δ -4.58 (TBDMS); 18.0 ($(CH_3)_3C$); 25.4 (TBDMS); 61.4 (C-5´); 70.7 (C-3´); 84.9 (C-1´); 87.7 (C-4´); 104.7 (C-5); 111.7, 113.1, 120.0 (Ar); 120.1 (C-5 triazole); 131.0 (C-4 triazole); 136.9 (C-6); 137.6, 140.1, 149.7 (Ar); 156.1 (C-2); 161.1 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{23}H_{31}N_5O_6Si$: 524.1936, found 524.1920.

(4-Azidophenoxy)(*t*-butyl)dimethylsilane (225a)

Azide **224a** (1.12 g, 8.27 mmol), TBDMSCl (3.74 g, 24.81 mmol) and DMAP (0.5 g, 4.09 mmol) were dissloved in the mixture of pyridine and acetonitrile (32 ml, 1/1, *v/v*). This solution was stirred at RT for two days, evaporated, co-evaporated with toluene (2 x 20ml) and methanol (1 x 10ml). Residue was purified by silica gel column chromatography using CH_2Cl_2 to afford yellow-brown liquid.

Yield 1.86 g (90%). ^{1}H NMR (300MHz, DMSO-d_6): δ 0.17 (s, 6H, TBDMS); 0.94 (s, 9H, TBDMS); 6.87-6.91 (m, 2H, Ar); 6.99-7.02 (m, 2H, Ar). ^{13}C NMR (75MHz, DMSO-d_6): δ -4.7 (TBDMS); 17.9 ($(CH_3)_3C$); 25.5 (TBDMS); 120.2, 121.2, 132.3, 152.5 (Ar). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{12}H_{19}N_3OSi$: 272.1190, found 272.1184. IR: 2957.5; 2931.2; 2859.5; 2122.4; 1504.5; 1472.7; 1301.5; 1258.0; 1102.4; 913.7; 839.0; 782.1 cm^{-1}.+

(3-Azidophenoxy)(*t*-butyl)dimethylsilane (225b)

Azide **224b** (1.29 g, 9.55 mmol), TBDMSCl (3.6 g, 23.88 mmol) and DMAP (583 mg, 4.77 mmol) were put to the flask under nitrogen. To this flask, pyridine/AcCN (32ml, 1:1, *v/v*) was added. This mixture was stirred at RT for two days, evaporated, co-evaporated with toluene (2 x 10ml) and methanol (1 x 10ml) and purified by silica gel column chromatography using CH_2Cl_2 to afford yellow-brown liquid.

Yield 1.92 g (81%). ^{1}H NMR (300MHz, $CDCl_3$): δ 0.28 (s, 6H, TBDMS); 1.06 (s, 9H, TBDMS); 6.56-6.57 (m, 1H, Ar); 6.67-6.74 (m, 2H, Ar); 7.26 (t, 1H, Ar, *J*=8.1 Hz). ^{13}C NMR (75MHz, $CDCl_3$): δ -4.3 (TBDMS); 18.3 ($(CH_3)_3C$); 25.8 (TBDMS); 111.1, 112.1, 116.9, 130.5, 141.2, 157.1 (Ar). MS-EI: 249.00 ($C_{12}H_{19}N_3OSi$ calcd. 249.38). IR:

3075.9, 2930.6, 2859.8, 2114.9, 1595.8, 1487.9, 1391.0, 1362.9, 1233.6, 1114.4, 963.6, 840.2 cm^{-1}.

5-(1-(4-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-5´-(4,4´-dimethoxytrityl)-2´-deoxyuridine (226a)

Nucleoside **212** (485.3 mg, 0.88 mmol) and azide **225a** (354 mg, 1.42 mmol) were dissolved in EtOH/ H_2O (10 ml, 7:3, *v/v*). To this solution, CuI (108.2 mg, 0.57 mmol), sodium ascorbate (281.3 mg, 1.42 mmol) and pyridine (3 ml) were added. This mixture was stirred at RT for 4.5 hours. Then it was evaporated, co-evaporated with toluene (2 x 10ml) and methanol (1 x 10ml). It was purified by silica gel column chromatography using MeOH in CH_2Cl_2 (0-10%).

Yield 550 mg (78%). ^{1}H NMR (300MHz, DMSO-d_6): δ 0.24 (s, 6H, TBDMS); 0.98 (s, 9H, TBDMS); 2.27-2.33 (m, 2H, H-2´); 3.22-3.25 (m, 2H, H-5´); 3.67 (s, 3H, OCH_3); 3.68 (s, 3H, OCH_3); 3.97-3.98 (m, 1H, H-4´); 4.22-4.23 (m, 1H, H-3´); 5.37 (d, 1H, 3´-OH, *J*=4.5Hz); 6.21 (t, 1H, H-1´, *J*=6.3Hz); 6.82 (dd, 4H, Ar, *J*=8.7Hz); 7.04 (d, 2H, Ar, *J*=8.9Hz); 7.15 (m, 1H, Ar); 7.21-7.29 (m, 6H, Ar); 7.39 (m, 2H, Ar); 7.77 (d, 2H, Ar, J=8.9Hz); 8.40 (s, 1H, H-6); 8.72 (s, 1H, H-triazole); 11.81 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6): δ -4.6 (TBDMS); 17.9 ($(CH_3)_3C$); 25.5 (TBDMS); 54.9 (OCH_3); 63.6 (C-5´); 70.4 (C-3´); 85.3 (C-1´); 85.7 (Ph_3C); 85.7 (C-4´); 104.8 (C-5); 113.1 (Ar); 120.1 (C-5 triazole); 120.8, 121.9, 126.5, 127.6, 127.7, 129.6, 129.7 (Ar); 130.7 (C-4 triazole); 135.4, 135.5 (Ar); 136.2 (C-6); 144.8, 149.4 (Ar); 149.7 (C-2); 155.3, 158.0 (DMTr); 161.1 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{44}H_{49}N_5O_8$: 826.3243, found 826.3213.

5-(1-(3-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-5´-(4,4´-dimethoxytrityl)-2´-deoxyuridine (226b)

Nucleoside **212** (250 mg, 0.45 mmol) and azide **225b** (179.9 mg, 0.72 mmol) were dissolved in EtOH/ H_2O (10 ml, 7:3, *v/v*). To this solution, CuI (54.5 mg, 0.29 mmol), sodium ascorbate (142.9 mg, 0.72 mmol) and pyridine (2.4 ml) were added. This mixture was stirred at RT over night. Then it was evaporated, co-evaporated with toluene (2 x 10ml) and methanol (1 x 10ml). It was purified by silica gel column chromatography using MeOH in CH_2Cl_2 (0-5%).

Yield 277 mg (76%). ^{1}H NMR (300MHz, $CDCl_3$): δ 0.25 (s, 6H, TBDMS); 1.01 (s, 9H, TBDMS); 2.31-2.57 (m, 2H, H-2´); 3.40-3.53 (m, 2H, H-5´); 3.73 (s, 6H, OCH_3); 4.06-

4.09 (m, 1H, H-4´); 4.46 (bs, 1H, H-3´); 6.33 (t, 1H, H-1´, *J*=6.6Hz); 6.79-6.91 (m, 5H, Ar); 7.12-7.43 (m, 11H, Ar); 8.53 (s, 1H, H-6); 8.57 (s, 1H, H-triazole); 9.29 (bs, 1H, Ar). ^{1}H NMR (300MHz, DMSO-d_6): δ 0.25 (s, 6H, TBDMS); 0.99 (s, 9H, TBDMS); 2.32-2.30 (m, 2H, H-2´); 3.25-3.24 (m, 2H, H-5´); 3.68-3.67 (m, 6H, OCH_3); 3.97-3.96 (m, 1H, H-4´); 4.22-4.20 (m, 1H, H-3´); 5.38-5.37 (m, 1H, 3´-OH); 6.22-6.18 (t, 1H, H-1´); 6.83 (d, 4H, Ar, *J*=8.1Hz); 6.98 (d, 1H, Ar, *J*=7.5Hz); 7.12-7.54 (m, 12H, Ar); 8.40 (s, 1H, H-6); 8.84 (s, 1H, H-triazole); 11.82 (s, 1H, NH). ^{13}C NMR (75MHz, $CDCl_3$): δ -4.3 (TBDMS); 18.3 ($(CH_3)_3C$); 25.7 (TBDMS); 55.3 (OCH_3); 63.8 (C-5´); 72.6 (C-3´); 86.0 (C-1´); 86.7 ($(Ph)_3C$); 87.0 (C-4´); 106.2 (C-5); 112.7, 113.1, 113.3, 113.4 (Ar); 120.2 (C-5 triazole); 120.3, 127.0, 127.9, 128.0, 128.2, 130.1, 130.2, 130.5, 135.7, 135.8 (Ar); 136.5 (C-6); 138.1, 139.6, 144.7, 149.7 (Ar); 156.9 (C-2); 158.6, 158.7 (DMTr); 161.2 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{44}H_{49}N_5O_8$: 826.3243, found 826.3281.

5-(1-(4-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-5´-(4,4-dimethoxytrityl)-3´-*O*-(*P*-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphinyl)-2´-deoxyuridine (227a)

Nucleoside **226a** (150 mg, 0.19 mmol) was co-evaporated with anhydrous dichloromethane (2 x 5 ml) and dissolved in the same solvent (3.8 ml). To this solution, anhydrous DIPEA (165.5 µl, 0.95 mmol) and 2-cyanoethyl-N,N´-(diisopropyl)-phosphoramidochloridite (106 µl, 0.48 mmol) were added. The mixture was stirred at RT for 2.5h. Then it was diluted with CH_2Cl_2, washed with saturated aqueous solution of sodium hydrogencarbonate (20 ml), brine (20 ml) and water (30 ml). Organic phase was dried over sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (pre-equilibrated with 5% pyridine) using aceton in CH_2Cl_2 (0-5%) to afford of white product.

Yield 113.5 mg (61%). ^{31}P NMR (121.5MHz, $CDCl_3$): δ 149.7; 150.1. ^{31}P NMR (121.5MHz, DMSO-d_6): δ 148.3; 148.6. HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{53}H_{66}N_7O_9PSi$: 1026.4321, found 1026.4308.

5-(1-(3-(*t*-butyldimethylsilyloxy)phenyl)-1,2,3-triazol-4-yl)-5´-(4,4-dimethoxytrityl)-3´-*O*-(*P*-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphinyl)-2´-deoxyuridine (227b)

Nucleoside **226b** (127 mg, 0.16 mmol) was co-evaporated with anhydrous dichloromethane (2 x 5 ml) and dissolved in the same solvent (3.2 ml). To this solution, anhydrous DIPEA (137.6 µl, 0.79 mmol) and 2-cyanoethyl-N,N´-(diisopropyl)-

phosphoramidochloridite (105.7 µl, 0.47 mmol) were added. The mixture was stirred at RT for 1h. Then it was diluted with CH_2Cl_2, washed with brine and water. Organic phase was dried over sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (pre-equilibrated with 5% pyridine) using aceton in CH_2Cl_2 (2-5%) to afford of white product.

Yield 111.7 mg (70%). ^{31}P NMR (121.5MHz, DMSO-d_6): δ 148.3; 148.7. HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{53}H_{66}N_7O_9PSi$: 1026.4321, found 1026.4322.

5-[(4-Bromophenyl)ethynyl)]-2´-deoxyuridine (229)

To the 5-iodo-2´-deoxyuridine **14** (100 mg, 0.28 mmol), 1-bromo-4-ethynyl-benzene **228** (153.4 mg, 0.85 mmol), CuI (10.8 mg, 0.06 mmol) and $(PPh_3)_4Pd$ (32.6 mg, 0.03 mmol) under nitrogen, anhydrous DMF (2 ml) and anhydrous TEA (1 ml) were added. The mixture was stirred at RT for 4 hours. It was evaporated and co-evaporated with xylene (2x 5ml). The dichloromethane was added and yellow solid was filtered and washed with CH_2Cl_2 to afford yellow product.

Yield 110.3 mg (96%); ^{1}H NMR (300MHz, DMSO-d_6): δ 2.17 (bs, 2H, H-2´); 3.61-3.64 (m, 2H, H-5´); 3.82 (bs, 1H, H-4´); 4.26 (bs, 1H, H-3´); 5.17 (bs, 1H, 5´-OH); 5.25 (d, 1H, 3´-OH, *J*=3.0Hz); 6.13 (t, 1H, H-1´, *J*=5.7Hz); 7.41 (d, 2H, Ar, *J*=7.8Hz); 7.61 (d, 2H, Ar, *J*=7.8Hz); 8.41 (s, 1H, C-6); 11.70 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6): δ 40.2 (C-2´); 60.8 (C-5´); 69.9 (C-3´); 83.8 (-C≡); 84.9 (C-1´); 87.6 (C-4´); 90.7 (-C≡); 97.8 (C-5); 121.6, 122.0, 131.8, 133.0 (Ar); 144.2 (C-6); 149.4 (C-2); 161.3 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{17}H_{15}BrN_2O_5$: 429.0057, found 429.0047.

5-[(4-Bromophenyl)ethynyl)]-5´-(4,4´-dimethoxytrityl)-2´-deoxyuridine (230)

Compound **229** (300 mg, 0.74 mmol) was co-evaporated with anhydrous pyridine (4 x 5 ml). It was suspended in the mixture of pyridine/ acetonitrile (8 ml, 1:1, *v/v*), 4,4´-dimethoxytrityl chloride (299.7 mg, 0.89 mmol) was added and mixture was stirred at RT over night. The reaction was quenched by the addition of anhydrous methanol (10 ml) and then concentrated under reduced pressure. The residue was co-evaporated with ethanol (3 x 5 ml), diluted with CH_2Cl_2 (30 ml), washed with saturated aqueous solution of sodium hydrogencarbonate (15 ml), brine (15 ml) and water (15 ml). The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure. Residue was purified by silica gel column chromatography using MeOH in CH_2Cl_2 (0-5%) to afford yellow solid.

Yield 319.2 mg (61%); ^{1}H NMR (300MHz, DMSO-d_6): δ 2.25-2.31 (m, 2H, H-2´); 3.15-3.26 (m, 2H, H-5´); 3.67 (s, 6H, OCH_3); 3.97-3.98 (m, 1H, H-4´); 4.33-4.34 (m, 1H, H-3´); 5.35 (d, 1H, 3´-OH, *J*=4.5 Hz); 6.15 (t, 1H, H-1´, *J*=6.3 Hz); 6.83 (dd, 4H, Ar, J_1 = 9 Hz, J_2 = 3Hz); 6.99 (d, 2H, Ar, *J*=6.6 Hz); 7.14-7.19 (m, 1H, Ar); 7.26-7.32 (m, 6H, Ar); 7.41-7.50 (m, 4H, Ar); 8.12 (s, 1H, H-6); 11.77 (s, 1H, NH). ^{13}C NMR (75MHz, DMSO-d_6): δ 54.9 (OCH_3); 65.5 (C-5´); 70.4 (C-3´); 83.2 (-C≡); 85.2 (C-1´); 85.9 (C-4´); 86.1 (Ph_3C); 90.8 (-C≡); 98.2 (C-5); 113.2, 121.4, 121.8, 126.7, 127.6, 127.9, 129.5, 129.6, 131.4, 132.9, 135.4, 135.5, 143.2 (Ar); 144.7 (C-6); 149.3 (C-2); 158.1 (DMTr); 161.3 (C-4). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{38}H_{33}BrN_2O_7$: 731.1363, found 731.1376.

5-[(4-Bromophenyl)ethynyl)]-5´-(4,4-dimethoxytrityl)-3´-*O*-(*P*-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphinyl)-2´-deoxyuridine (231)

The nucleoside **230** was co-evaporated with anhydrous CH_2Cl_2 (4 x 10 ml), dissolved in solution of diisopropylethylamine (1.6 ml) in anhydrous CH_2Cl_2 (6.4 ml). To this solution, 2-cyanoethyl-N,N´-(diisopropyl)-phosphoramidochloridite (110 μl, 0.49 mmol) was added. The mixture was stirred at RT for 3,5 hours, quenched by the addition of 99% ethanol (2 ml), diluted with CH_2Cl_2 (20 ml) and washed with saturated aqueous solution of $NaHCO_3$ (20 ml), brine (20 ml) and water (20 ml). The organic phase was dried over sodium sulfate, filtered and evaporated to dryness and purified by silica gel column chromatography using acetone in CH_2Cl_2 (2-5%, equilibrated with 1% pyridine). Finally it was co-evaporated with toluene (3 x 10 ml) to afford white solid. Yield 263 mg (65%). ^{31}P NMR (121.5MHz, DMSO-d_6): δ 148.18; 148.51. HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{47}H_{50}BrN_4O_8P$: 931.2442, found 931.2484.

6-(4-Amiophenyl)-3-(2´-deoxy-*β*-D-ribofuranosyl)furo-[2,3-d]-pyrimidin-2(3*H*)-one (233)

To the mixture of **229** (50 mg, 0.12 mmol) in DMF/H_2O (1ml, 1:1, *v/v*), sodium azide (40 mg, 0.62 mmol), sodium ascorbate (7 mg, 0.04 mmol), copper iodide (12 mg, 0.06 mmol) and *N,N´*-dimethylethylenediamine (12 μl, 0.11 mmol) were added. The mixture was subjected to the microwave irradiation at 100°C for 1 hour. Then it was evaporated, and purified by silica gel column chromatography using MeOH/CH_2Cl_2 (0-10%) to afford the yellow solid.

Yield 21.6 mg (48%); ^{1}H NMR (300MHz, DMSO-d_6): δ 2.05-2.43 (m, 2H, H-2′); 3.62-3.73 (m, 2H, H-5′); 3.92-3.93 (m, 1H, H-4′); 4.27 (bs, 1H, H-3′); 5.14-5.17 (m, 1H, 5′-OH); 5.29-5.30 (m, 1H, 3′-OH); 5.67 (s, 2H, NH_2); 6.21 (t, 1H, H-1′, *J*=5.7 Hz); 6.65 (d, 2H, Ar, *J*=8.1 Hz); 6.81 (s, 1H, -CH=); 7.50 (d, 2H, Ar, *J*=8.4 Hz); 8.67 (s, 1H, H-6). ^{13}C NMR (75MHz, DMSO-d_6): δ 41.2 (H-2′); 60.8 (C-5′); 69.7 (C-3′); 87.3 (C-4′); 88.1 (C-1′); 94.1 (-CH=); 107.7 (C-5); 113.7, 115.4, 126.1 (Ar); 135.7 (C-6); 150.4 (-CH=); 153.8 (Ar); 155.5 (C=O); 171.1 (C-O). HIRes ESI MS m/z calcd. for $[M+Na]^+$ $C_{17}H_{17}N_3O_5$: 366.1060, found 366.1206.

6.3. Synthesis of oligodeoxynucleotides

Oligonucleotide synthesis was carried out on an automated DNA synthesizer following the phosphoramidite approach. Synthesis of oligonucleotides **ON1-ON5** was performed on a 0.2 μmol scale by using the amidites **227**, synthesis of oligonucleotides **ON8-ON9** was performed either on a 0.2 μmol or 1 μmol scale by using the amidite **231** as well as the corresponding commercial 2-cyanoethylphosphoramidites of the natural 2′-deoxynucleosides. The synthesis followed the regular protocol for the DNA synthesizer. For compounds **227** a prolonged coupling time of 30 min was used. For compound **231** a prolonged coupling time of 20 min was used. 1*H*-Tetrazole was used as the activator. The 5′-*O*-DMT-ON oligonucleotides were removed from the solid support by treatment with concentrated aqueous ammonia at 55°C for 16 h, which also removed the protecting groups. The oligonucleotides were purified by reversed phase HPLC on a Waters 600 system using a X_{terra} prep MS C_{18}; 10 μm; 7.8 x 150 mm column; buffer A: 0,05 M triethyl ammonium acetate pH 7.4. Buffer B: MeCN/H_2O (1:1). Program used: 2 min 100% A, 100–30% A over 38 min, 10 min 100% B, 10 min 100% A. All oligonucleotides were detritylated by treatment with an 80% aqueous solution of acetic acid for 20 min, quenched with a aqueous solution of sodium acetate (3 M, 15 μL) and then addend sodium perchlorate (5 M, 15 μL) followed by acetone (1 mL). The pure oligonucleotides precipitated overnight at -20°C. After centrifugation 12,000 rpm, 10 min at 4°C, the supernatant was removed and the pellet washed with cold acetone (2 x 1 mL) and dried for 30 min under reduced pressure, and dissolved in pure water (500 μL). The concentration was determined by UV at 260 nm, and the purity confirmed by IC analysis. MALDI-TOF-MS $[M-H]^-$ gave the following results

(calcd/found): **ON2** (2880.9/2878.0); **ON3** (3316.0/ 3315.8); **ON4** (2880.9/2879.1); **ON9** (3154.0/3152.7).

6.4. Thermal denaturation experiments

Extinction coefficients of the modified oligonucleotides were estimated from a standard method but calibrated by the micromolar extinction coefficients of the monomeric compounds **213a**, **213b**, **229** and dT, which were estinated from their UV spectra (dT: ε_{260} = 8.5, **213a**: ε_{260} = 12.6, **213b**: ε_{260} = 12.2, **229**: ε_{260} = 16.3). UV melting experiments were thereafter carried out on a UV spectrometer. Samples were dissolved in a medium salt buffer containing 2.5 mM Na_2HPO_4, 5 mM NaH_2PO_4, 100 mM NaCl, and 0.1 mM EDTA at pH 7.0 with 1.5 μM concentrations of the two complementary sequences. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was increased linearly from 5 to 60 or 75°C at a rate of 0.5 or 1.0°C/min by means of a Peltier temperature programmer. The melting temperature was determined as the local maximum of the first derivatives of the absorbance versus temperature curve. The melting curves were found to be reversible. All determinations are averages of at least duplicates within ±0.5°C.

6.5. Biological activity assays

In vitro cytotoxic activity screening was performed on K562, K562-tax, CEM, CEM-DNR-bulk, HCT163p53, HCT163p53-/- and A549 cell lines. For detailed description see published results.[77,78]

7. References

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