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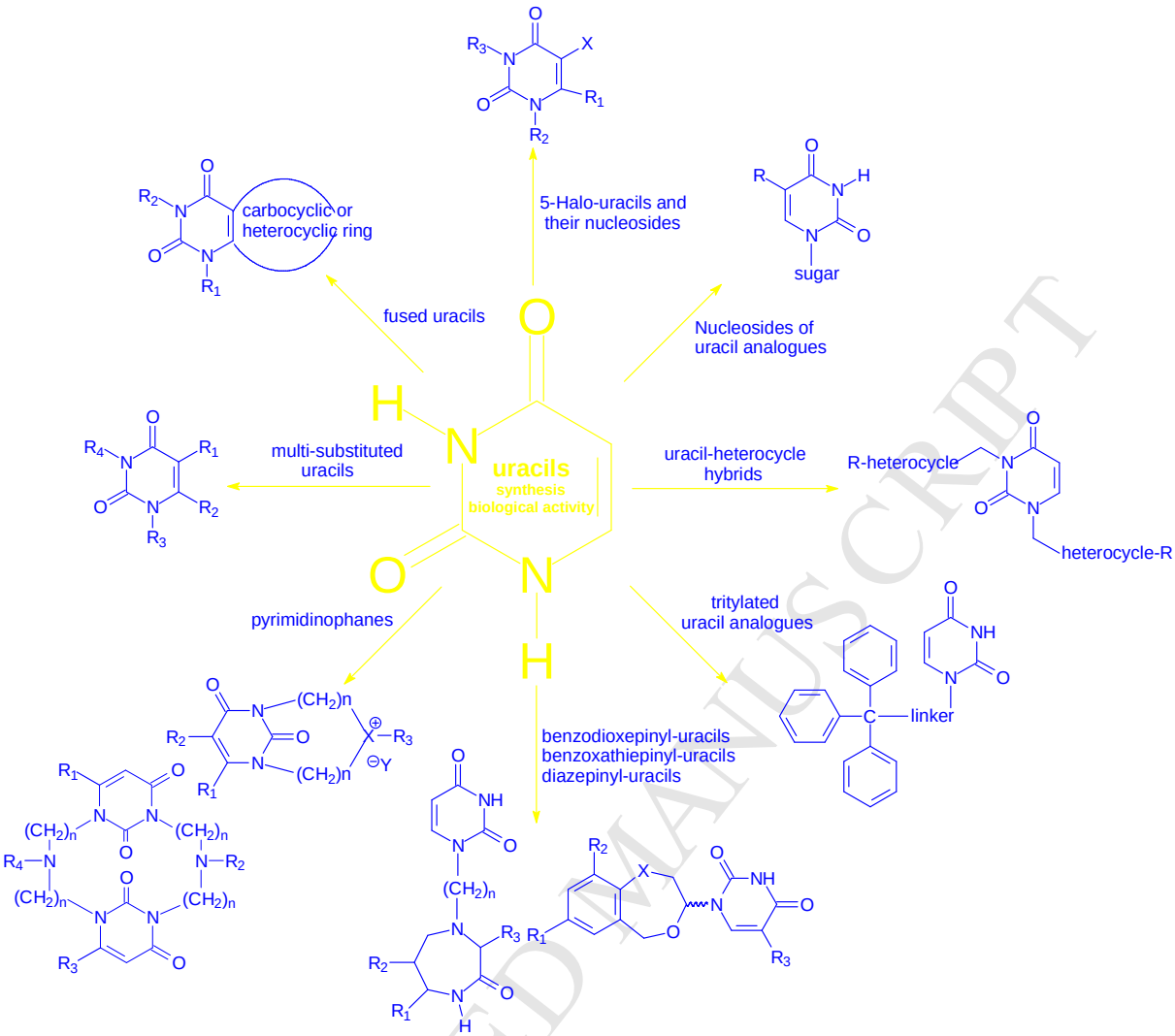

In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications.

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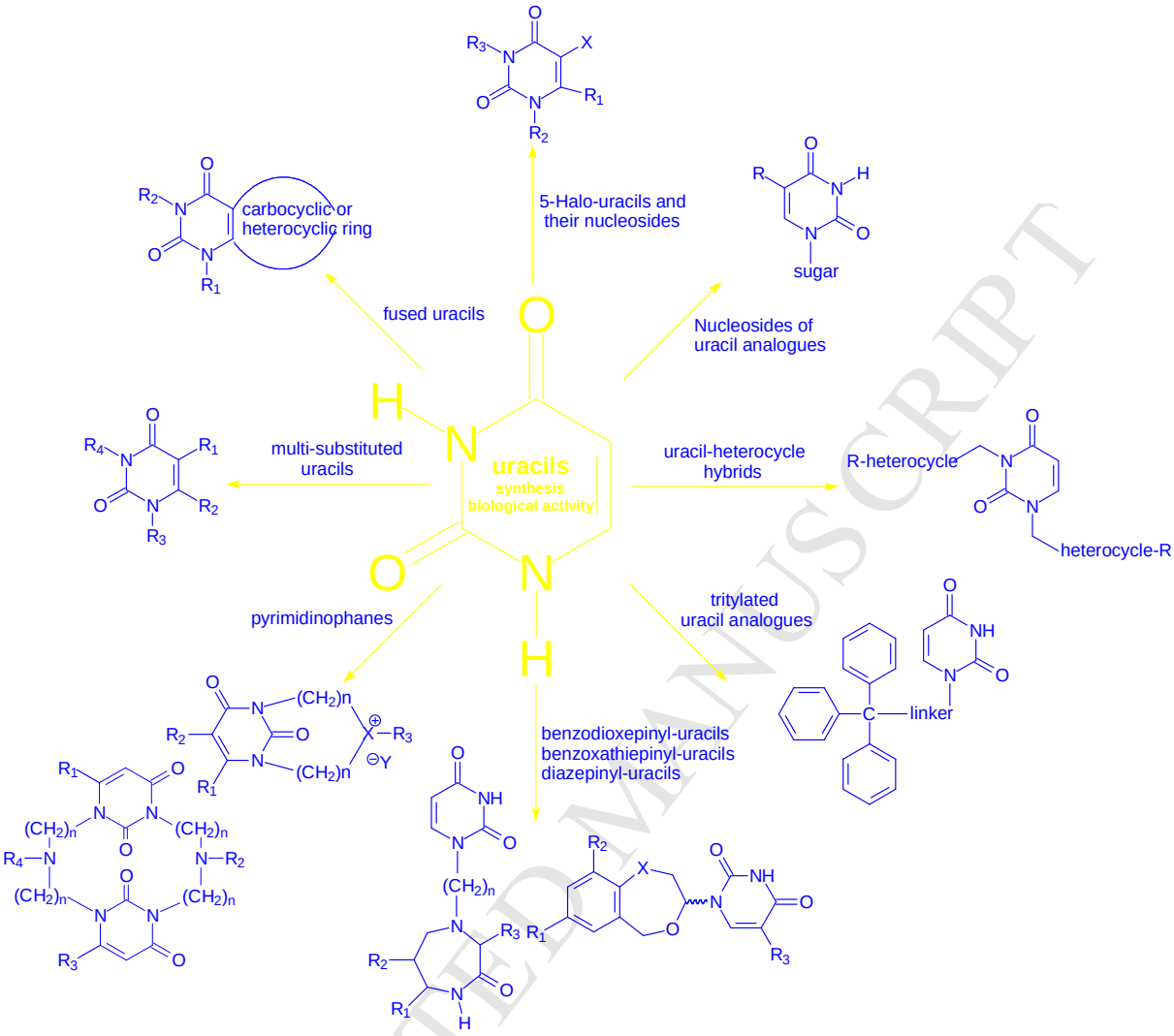
Abstract

This review article is an effort to summarize recent developments in researches providing uracil derivatives with promising biological potential. This article also aims to discuss potential future directions on the development of more potent and specific uracil analogues for various biological targets. Uracils are considered as privileged structures in drug discovery with a wide array of biological activities and synthetic accessibility. Antiviral and anti-tumor are the two most widely reported activities of uracil analogs however they also possess herbicidal, insecticidal and bactericidal activities. Their antiviral potential is based on the inhibition of key step in viral replication pathway resulting in potent activities against HIV, hepatitis B and C, the herpes viruses etc. Uracil derivatives such as 5-fluorouracil or 5-chlorouracil were the first pharmacological active derivatives to be generated. Poor selectivity limits its therapeutic application, resulting in high incidences of gastrointestinal tract or central nervous toxicity. Numerous modifications of uracil structure have been performed to tackle these problems resulting in the development of derivatives exhibiting better pharmacological and pharmacokinetic properties including increased bioactivity, selectivity, metabolic stability, absorption and lower toxicity. Researches of new uracils and fused uracil derivatives as bioactive agents are related with modifications of substituents at N^1, N^3, C^5 and C^6 positions of pyrimidine ring. This review is an endeavor to highlight the progress in the chemistry and biological activity of the uracils, predominately after the year 2000. In particular are presented synthetic methods and biological study for such analogs as: 5-

fluorouracil or 5-chlorouracil derivatives, tegafur analogs, arabinopyranonucleosides of uracil, glucopyranonucleosides of uracil, liposidomycins, caprazamycins or tunicamycins, tritylated uridine analogues**,** nitro or cyano derivatives of uracil, uracil-quinazolinone, uracil-indole or uracil-isatin-conjugates, pyrimidinophanes containing one or two uracil units and nitrogen atoms in bridging polymethylene chains etc. In this review is also discussed synthesis and biological activity of fused uracils having uracil ring annulated with other heterocyclic ring.

Keywords: uracils, fused uracils, 5-fluorouracil, anticancer, antiviral, antibacterial

Graphical abstract



1. Introduction

The nitrogen heterocycles in general and pyrimidines in particular are found in several biologically active natural products and depict considerable therapeutic potential. The pyrimidine is one of the most prominent structures found in nucleic acid chemistry. Uracil is a common and naturally occurring pyrimidine derivative and one of the four nucleobases in the nucleic acid of RNA. In RNA, uracil binds to adenine via two hydrogen bonds. In DNA, the uracil nucleobase is replaced by thymine. Uracil can be considered as a demethylated form of thymine. Studies reported in 2008, based on $^{12}C/^{13}C$ isotopic ratios of organic compounds found in the Murchison meteorite, suggest that uracil was formed extraterrestrially [1]. In

2009, NASA scientists reported having reproduced uracil from pyrimidine by exposing it to UV under space-like conditions. This suggests that one possible natural original source for uracil in the RNA world could have been panspermia [2]. In 2012, an analysis of data from the Cassini mission orbiting in the Saturn system showed that Titan's surface composition may include uracil [3].

Uracil undergoes amide-imidic acid tautomeric shifts because any nuclear instability the molecule may have from the lack of formal aromaticity is compensated by the cyclic-amidic stability (Fig. 1). The amide tautomer is referred to as the lactama structure, while the imidic acid tautomer is referred to as the lactim structure. These tautomeric forms are predominant at pH 7. The lactam structure is the most common form of uracil. Uracil is a weak acid.

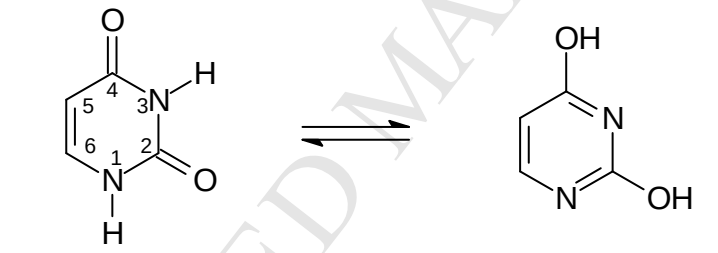
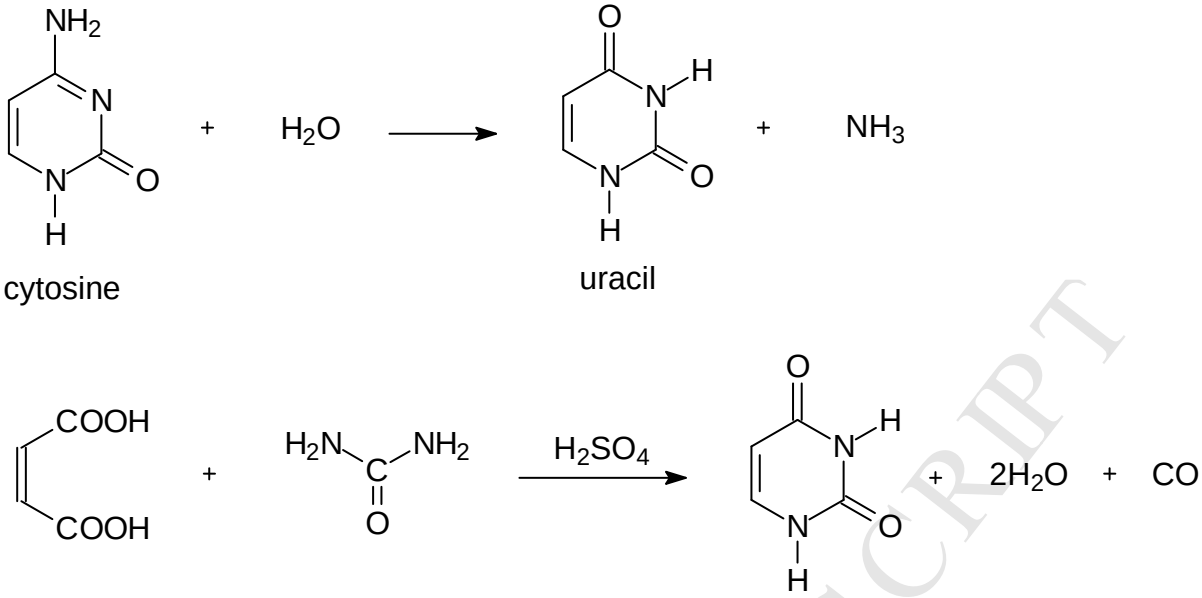


Fig. 1. Structure of uracil with numbering system. Uracil tautomers.

In RNA, uracil binds with a ribose sugar to form the ribonucleoside uridine. When a phosphate attaches to uridine, uridine 5'-monophosphate is produced. There are many laboratory syntheses of uracil available. The first reaction is the simplest of the syntheses, by adding water to cytosine to produce uracil and ammonia (Scheme 1) [4]. The most common way to synthesize uracil is by the condensation of maleic acid with urea in fuming sulfuric acid (Scheme 1) [5].



Scheme 1. Synthesis of uracil from cytosine and by the condensation of maleic acid with urea.

Uracils are considered as privileged structures in drug discovery with a wide array of biological activities, synthetic accessibility and ability to confer drug like properties to the compound libraries appended on them at N^1, N^3, C^5 and C^6 positions [6]. Antiviral and anti-tumor are the two most widely reported activities of uracil analogs [7]; however they also possess herbicidal, insecticidal and bactericidal activities [8]. In the past two decades, a variety of synthetic methods have been employed for the preparation of functionalized uracils. The quantity of papers describing the synthesis and biological activity of uracil derivatives is tremendous. We chose these publications which in our opinion show the main directions of research of uracil derivatives nowadays. We divided the next section, describing different derivatives of uracil, into eight chapters:

2.1. 5-Halo-uracils and their nucleosides

2.2. Nucleosides of uracil analogues

2.3. Uracil-heterocycle hybrids

2.4. Tritylated uracil analogues

2.5. Benzodioxepinyl-uracils, benzoxathiepinyl-uracils and diazepinyl-uracils

2.6. Pyrimidinophanes containing one or two uracil units and bridging polymethylene chains

2.7. Other substituted uracils

2.8. Fused uracils

However, we had a problem to classify specified uracil derivative to a given chapter because in most cases described compounds possessed few substituents in uracil ring.

2. Design strategies

2.1. 5-Halo-uracils and their nucleosides

5-Fluorouracil (5-FU) is antimetabolite of the pyrimidine analog type and a well-known anti-tumor agent which has been widely used in the treatment of solid tumors such as colon or breast cancers [9]. Because 5-fluorouracil is similar in shape to uracil, but does not perform the same chemistry as uracil, the drug inhibits RNA replication enzymes, thereby eliminating RNA synthesis and stopping the growth of cancerous cells. Although 5-FU has had clinical success as a single agent, it has been modified by different ways to synthesize its derivatives which may improve its therapeutic index because of its well-known side effects such as short half-life, wide distribution, low selectivity, and various toxic side effects. Recently chemists have paid more attention to the conjugates of 5-FU with a wide spectrum of low or high-molecular-weight carriers (Fig. 2).

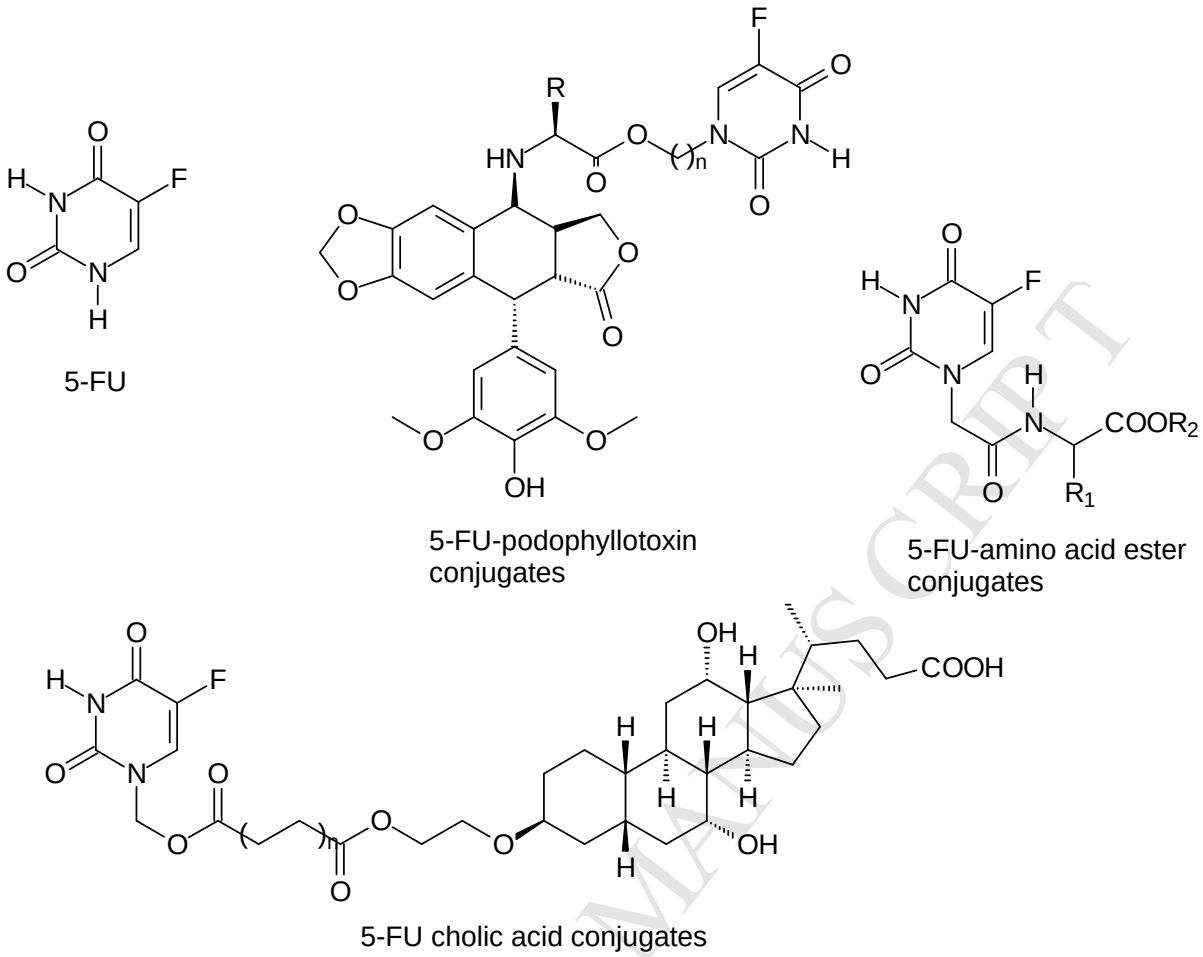
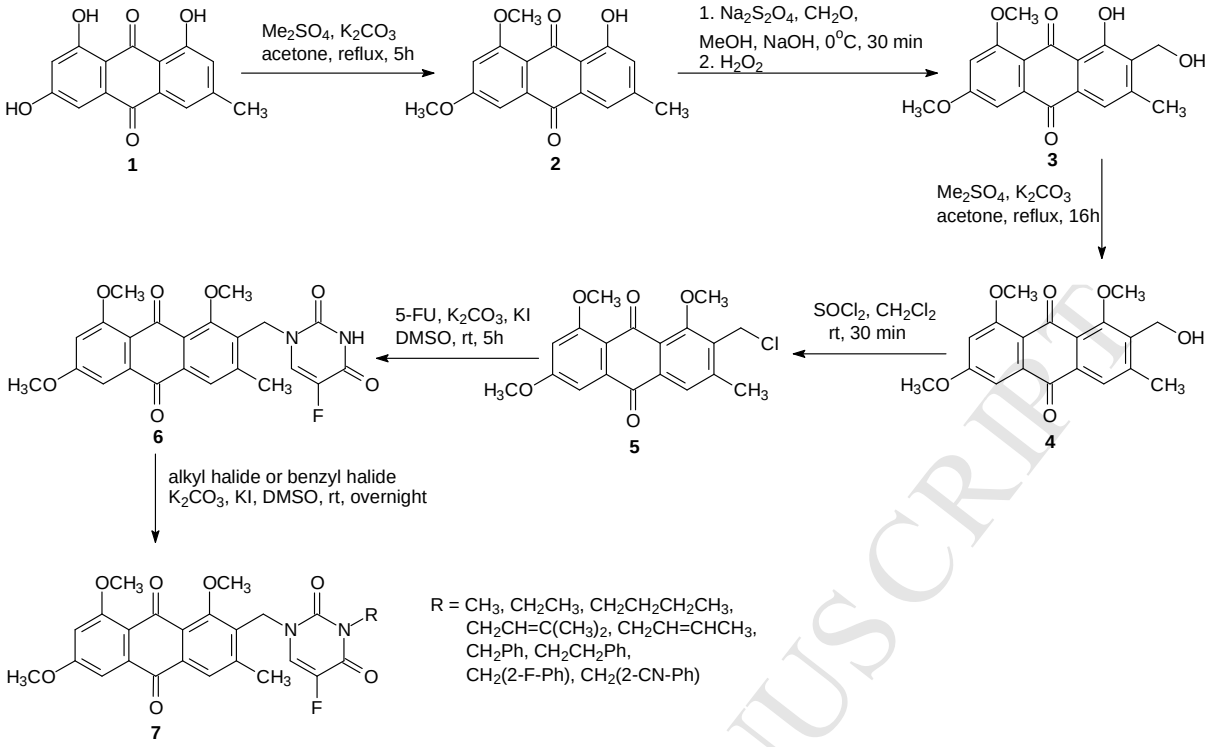


Fig. **2**. Structures of 5-FU and its conjugates.

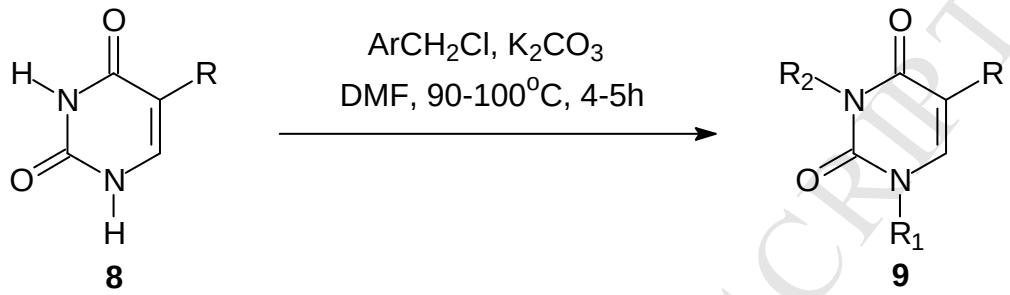
A series of conjugates of 5-fluorouracils (5-FU) and emodin were synthesized by coupling trimethyl emodin with N^1, N^3 dialkylated 5-FU [10]. 1-Hydroxy-2-(hydroxymethyl)-6,8-dimethoxy-3-methylanthracene-9,10-dione **3** was prepared using emodin **1** as starting material via methylation and modified Marschalk reaction (Scheme 2). The synthesis of intermediate **4** involved the methylation of **3** with dimethyl sulfate in the presence of anhydrous K_2CO_3. Chlorination of **4** with thionyl chloride led to the compound **5**. Subsequently, alkylation of 5-FU with intermediate **5** in the presence of K_2CO_3 and KI gave N^1-substituted 5-FU **6**. A final alkylation of intermediate **6** and the appropriately alkyl halides or benzyl halides yielded the target conjugates **7** (Scheme 2).



Scheme 2. Synthesis of 5-fluorouracil and emodin conjugates **7**.

The 5-FU moiety contained various substituents at N^3 – position were linked to the 2-position of trimethyl emodin via a methylene linkage. Their cytotoxity against three cancer cell lines and one noncancerous cell were studied [10]. The in vitro cytotoxicity of the conjugates **7** were evaluated over tumor cell lines HO-8910 (human ovarian cancer cells), SGC-7901 (human gastric cancer cells), and HepG2 (human liver caner cells), in comparison with emodin and 5-FU. Cytotoxicity of some conjugates was also evaluated against one normal cell line: 293 (human embryonic kidney cells). The results revealed that some of conjugates exhibited better or comparable in vitro anti-tumor activity to 5-FU and emodin and low toxicity in normal cell. Compound **7** (R = CH_2(2-CN-C_6H_4)) was shown to have a broad spectrum of anti-tumor activity against the tested tumor cell lines and much lower toxic activity toward normal cell compared to emodin. The structure-activity relationship study showed N^3-aromatic substituent was important for their cytotoxic activity [10].

Arutynyan and co-workers [11] prepared N^1-mono and N^1,N^3-bis-substituted 5-fluoro, 5-bromo, 5-iodouracils. Heating uracils **8** with the corresponding benzylhalides in the presence of K_2CO_3 produced the target pyrimidines **9** as mixtures of mono- and bis-derivatives that were separated by treatment with KOH solution (Scheme 3).



8 **9**

R = H, F, Br, I
R_1 = CH_2(2',5'-Me_2)Ph, CH_2(2',4'-Me_2)Ph, CH_2(2'-Cl)Ph, CH_2(4'-Cl)Ph
R_2 = H, CH_2(2',5'-Me_2)Ph, CH_2(2',4'-Me_2)Ph, CH_2(2'-Cl)Ph, CH_2(4'-Cl)Ph

Scheme 3. Synthesis of N^1-mono and N^1,N^3-bis-substituted 5-fluoro-, 5-bromo, 5-iodouracils **9**.

The toxicity, anti-tumor, and antibacterial properties of the synthesized compounds were investigated. Chemotherapy tests found that the majority of the studied compounds exhibited a statistically significant antitumor activity for sarcomas 45 and 37. It was established that 5-fluoro- and 5-iodouracils exhibited more pronounced anti-tumor properties than 5-bromouracil derivatives [11].

6-Amino-5-chlorouracil and 6-amino-5-bromouracil were the first thymidine phosphorylase inhibitors to be generated. However, their relatively less favourable IC_{50} values did not allow them to be developed into drug candidates. In the year 2000, a very potent inhibitor namely 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride (TPI) (Fig. 3) was discovered by Fukushima et al. [12]. Thymidine phosphorylase (TP) is an enzyme that catalyses the reversible phosphorolysis of pyrimidine nucleosides. Besides the regulation of pyrimidine nucleosides, TP can also induce the phosphorolysis of several pyrimidine

nucleoside derivatives. Some nucleoside derivatives such as capecitabine and 5'-deoxy-5-fluorouridine were designed as prodrugs and they are being converted to the corresponding parent drugs by TP. It has been reported in literature that TP also possesses other functions related to cancer biology: it can stimulate tumor angiogenesis, induce tumor metastasis and promote tumor growth by preventing apoptosis. Therefore, TP is a target for developing inhibitors that may be used as therapeutic agents for chemotherapy.

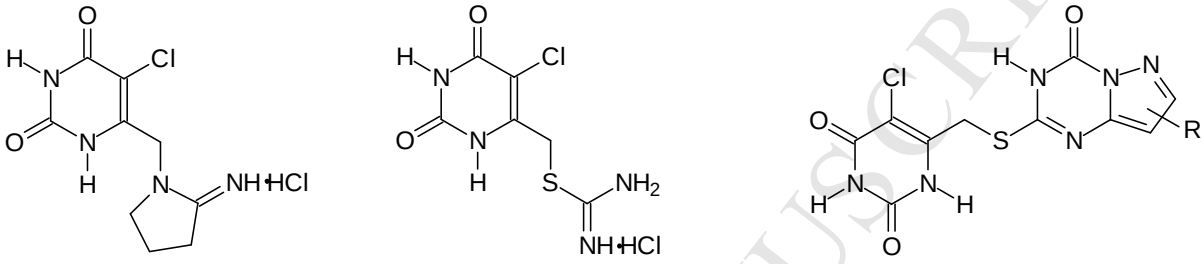
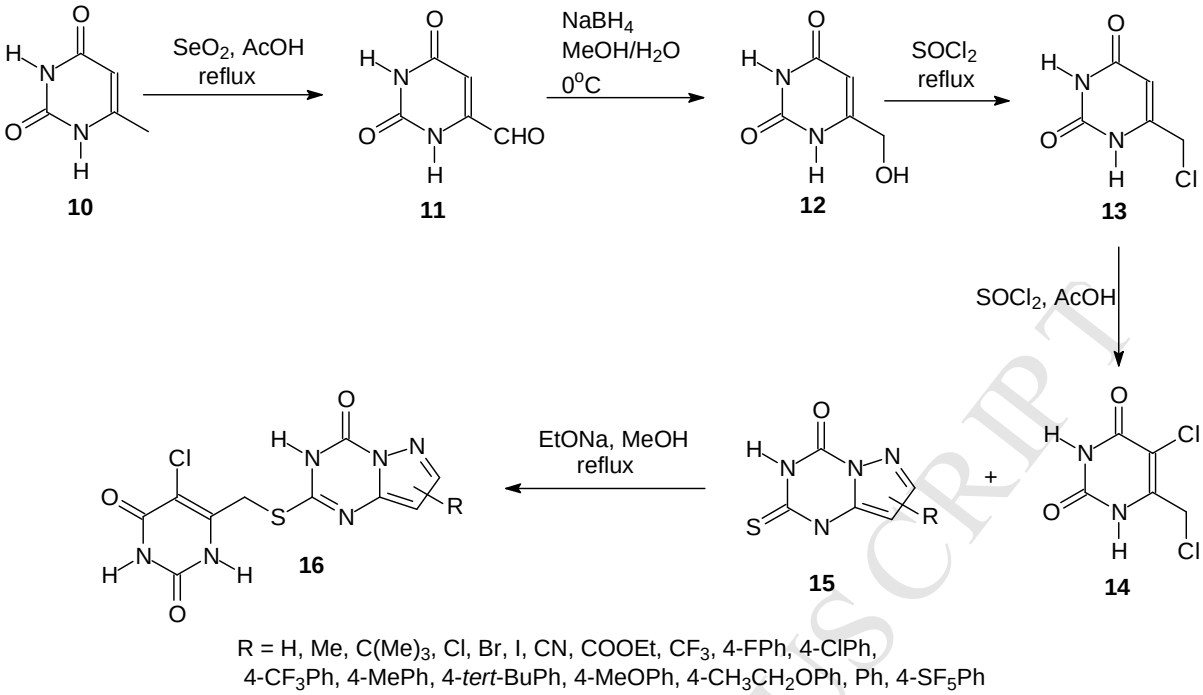


Fig. **3**. Examples of 5-chlorouracil analogs - thymidine phosphorylase inhibitors.

Sun and co-workers [13] designed 5-chlorouracil-linked-pyrazolo[1,5-*a*][1,3,5]triazines as new thymidine phosphorylase inhibitors. The intermediate 5-chloro-6-chloromethyluracil **14** was synthesized by 4-step reaction (Scheme 4).

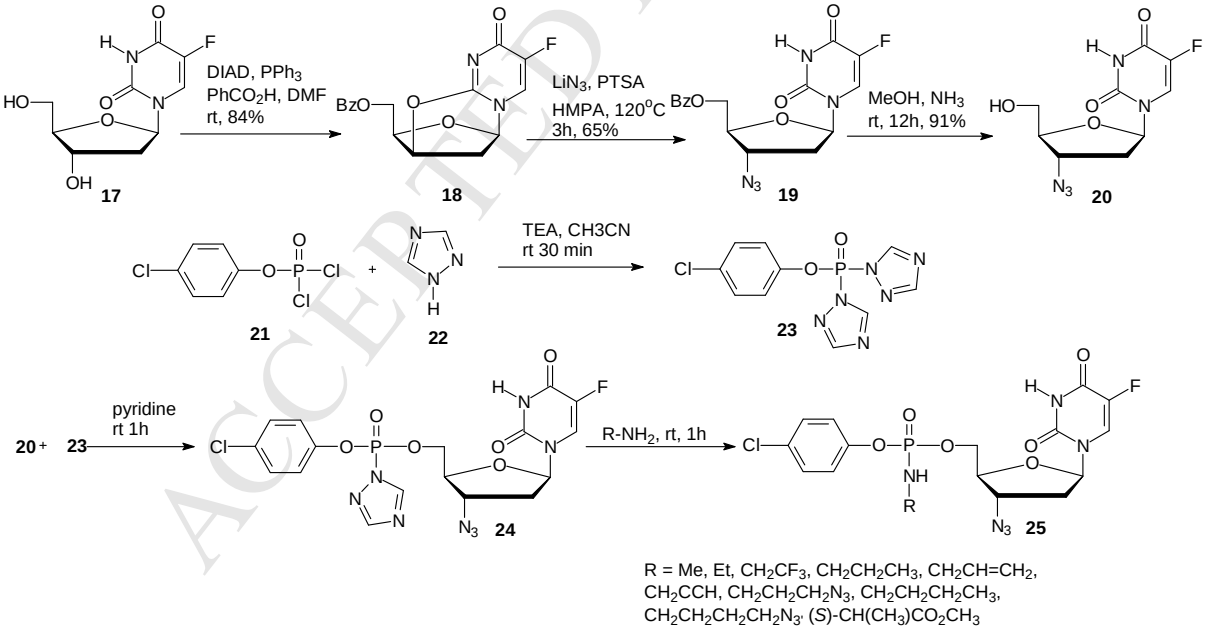


Scheme 4. Synthesis of 5-chloro-6-chloromethyluracil **14** and 3*H*-2-(5-chlorouracil-6-methylthio)pyrazolo[1,5-*a*]-1,3,5-triazin-4-ones **16**.

A series of second bicyclic intermediates, namely pyrazolo[1,5-*a*][1,3,5]triazin-2-thioxo-4-one **15**, was obtained from various substituted 3-aminopyrazoles. These two intermediates **14** and **15** were coupled finally in the presence of sodium ethoxide and methanol to yield the desirable target compounds **16** (Scheme 4). The methylthio coupling spacer was found to be suitable in enabling the interaction of the two fragments at the active site and allosteric site of the enzyme. The best coupled compound **16** (R = 4-SF_5-phenyl) inhibited the thymidine phosphorylase with an IC_{50} value as low as 0.36+/-0.1 μM [13]. In addition, this compound demonstrated a mixed-type of enzyme inhibition kinetics, thus suggesting that it might indeed potentially bind at two different sites on the enzyme.

The introduction of fluorine atoms into organic molecules usually promotes dramatic changes in their biological properties. This strategy has been used to synthesize biologically active fluorinated nucleotides and nucleosides. Lewandowska and co-workers [14]

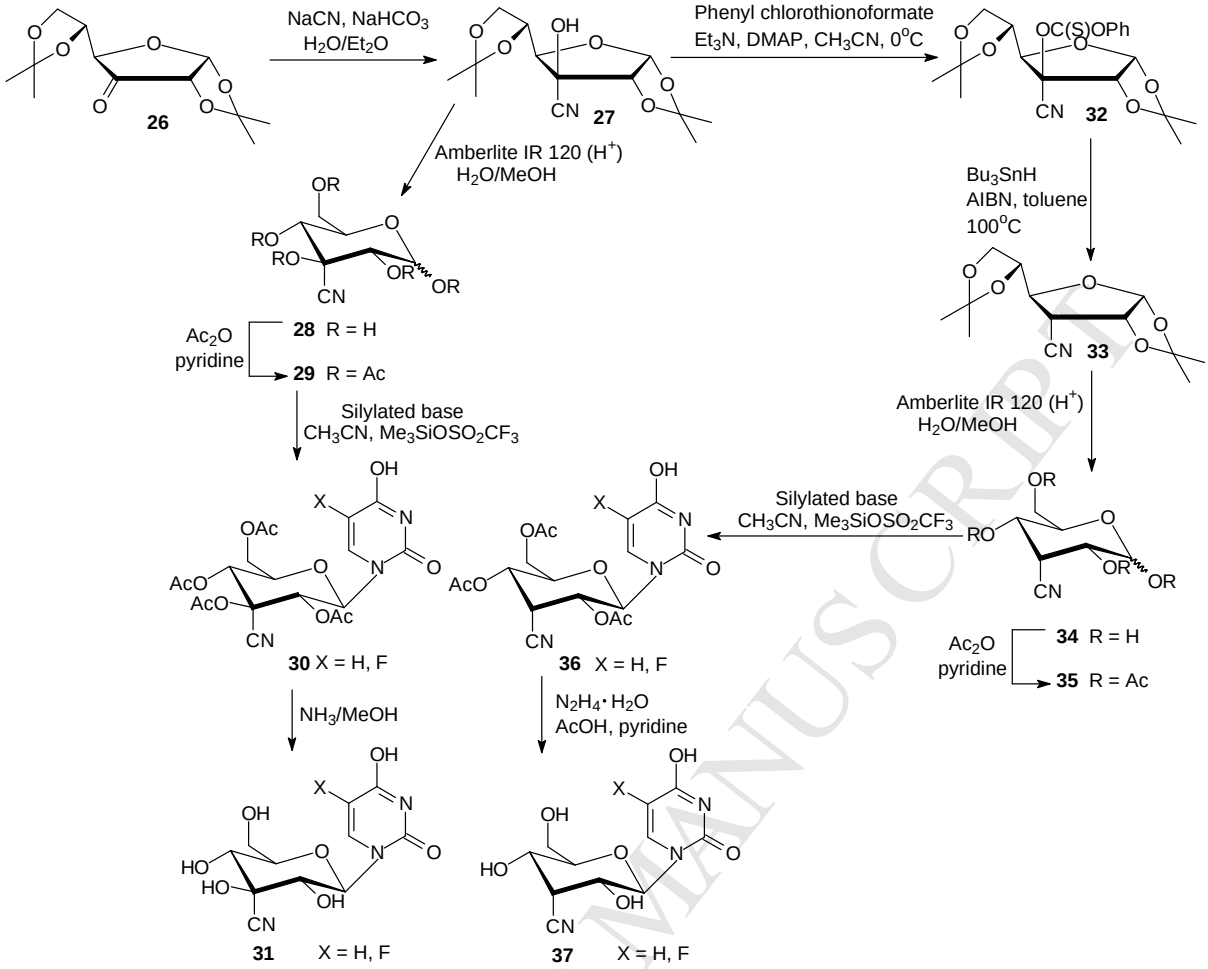
synthesized a series of 4-chlorophenyl *N*-alkyl phosphoramidates of 3'-azido-2',3'-dodeoxy-5-fluorouridine **25** by phosphorylation of 3'-azido-2',3'-dideoxy-5-fluorouridine **20** with 4-chlorophenyl phosphoroditriazolide **23** followed by a reaction with appropriate amine (Scheme 5). 5-Fluoro-2'-deoxyuridine **17** was converted into 2,3'-anhydro-5'-*O*-benzoyl-5-fluoro-2'-deoxyuridine **18** by a one-pot transformation involving Mitsunobu reaction. Ring opening of derivative **18** with lithium azide in hexamethylphosphoramide (HMPA) in the presence of *p*-toluenesulfonic acid (PTSA) afforded uridine **19**. 5'-*O*-benzoyl group was removed from compound **19** by treatment with methanolic ammonia to give **20**. 4-Chlorophenyl phosphoroditriazolide **23** was prepared by reaction of 4-chlorophenyl phosphorodichlorate **21** with 1,2,4-triazole **22** in the presence of triethylamine. Reaction of compound **23** with **20** in the presence of pyridine afforded reactive intermediate **24** which was treated in situ with appropriate amine to give the desired products **25** in 67-86% yield (Scheme 5).



Scheme 5. Synthesis of 4-chlorophenyl *N*-alkyl phosphoramidates of 3'-azido-2',3'-dodeoxy-5-fluorouridine **25**.

4-Chlorophenyl phosphoroditriazolide **23** as a phosphorylating agent was more selective than its dichloro counterpart and its use did not result in the formation of symmetrical (5-5')dinucleoside phosphates. Prepared compounds **25** were evaluated for their cytotoxic activity in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7) using the sulforhodamine B(SRB) assay. The highest activity in all the investigated cancer cells was displayed by phosphoramidate **25** (R = CH_2CH_3) with the *N*-ethyl substituent and its activity was much higher than of the parent nucleoside. Also compound **25** (R = CH_2CCH) with the *N*-propargyl substituent exhibited good activity in all the used cell lines [14].

Kiritsis et al. [15] described the total and facile synthesis of 3'-*C*-cyano and 3'-*C*-cyano-3'-deoxy pyrimidine pyranonucleosides. Reaction of 3-keto glucoside **26** with sodium cyanide gave the desired precursor 3-*C*-cyano-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **27** (Scheme 6). Hydrolysis followed by acetylation led to the 1,2,3,4,6-penta-*O*-acetyl-3-*C*-cyano-D-glucopyranose **29**. Compound **29** was condensed with silylated 5-fluorouracil or uracil and deacylated to afford the 1-(3'-*C*-cyano-β-D-glucopyranosyl)nucleosides **31**.



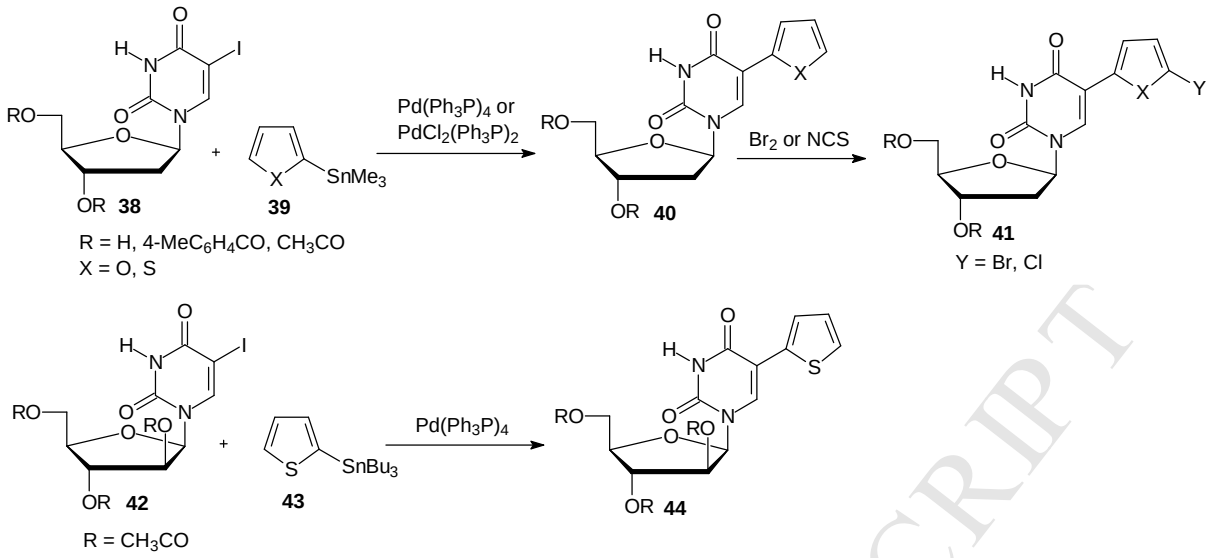
Scheme 6. Synthesis of 3'-*C*-cyano and 3'-*C*-cyano-3'-deoxy pyrimidine pyranonucleosides **31** and **37**.

Routine deoxygenation at position 3' of cyanohydrin **27**, followed by hydrolysis and acetylation led to the 3-*C*-cyano-3-deoxy-1,2,4,6-tetra-*O*-acetyl-D-allopyranose **35**. Coupling of sugar **35** with silylated pyrimidines and subsequent deacetylation yielded the 1-(3'-*C*-cyano-3'-deoxy-β-D-allopyranosyl)nucleosides **37**. It was found that **31** (X = F) was endowed with a pronounced anti-proliferative that was only 2- to 8-fold less potent than that shown for the 5-fluorouracil. None of the compounds showed activity against a broad panel of DNA and RNA viruses [15].

2.2. Nucleosides of uracil analogues

Nucleosides and nucleotides analogs play a pivotal role in antiviral and anticancer therapy. They are structurally related to the natural nucleosides bearing modifications at the base and/or at the sugar moieties. Numerous nucleosides and nucleotides analogues have been developed for medicinal purposes. Many of which have been used in the clinic for the treatment of human immunodeficiency virus (HIV), hepatitis B virus (HBV), herpes simplex virus (HSV), hepatitis C virus (HCV) and various cancers as anticancer and antiviral drugs. The preparation of molecules that mimic the structures of nucleic acids or their building blocks has provided many therapeutically useful compounds.

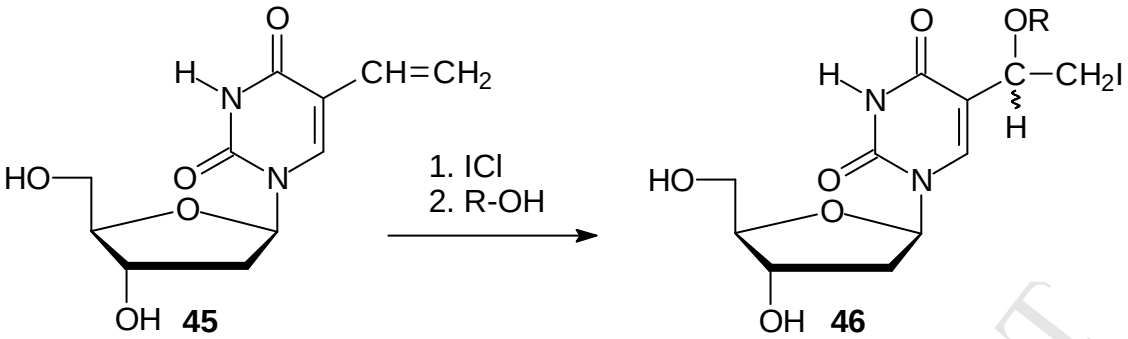
Wigerinck et al. [16] reported that uracil nucleosides bearing thienyl or furanyl substituents exhibit interesting biological properties. The coupling reactions between 5-iodo-2'-deoxyuridine **38** and the trialkylstannyl derivatives of the heterocycles **39** were carried out in a coordinating solvent (THF, dioxane) and a Pd(II) catalyst was needed (Scheme 7). When a non-coordinating solvent (toluene) was used, Pd (0) catalyst was used. 5-(Thien-2-yl)-1-(*β*-D-arabinofuranosyl)uracil **44** was obtained in the same way. The protective groups were removed with ammonia in methanol. Halogenation of the furan-2-yl or thien-2-yl substituents with Br_2 in CCl_4 and *N*-chlorosuccinimide in pyridine gave 5-halogenated derivatives **41** (Scheme 7).



Scheme 7. Synthesis of 5-(thien-2-yl)- and 5-(furan-2-yl)-2'-deoxyuridines **40** and their 5-halogenated analogues **41**. Synthesis of 5-(thien-2-yl)-1-(*β*-D-arabinofuranosyl)uracil **44**.

5-(Thien-2-yl)- and 5-(furan-2-yl)-2'-deoxyuridines **40** and 5-(thien-2yl)-1-(*β*-D-arabinofuranosyl)uracil **44** have been found to exhibit marked activity against herpes simplex virus type 1 (HSV-1). Substitution of the heterocyclic ring of 5-(thien-2-yl)-2'-deoxyuridine has resulted in 5-halogenated analogues **41**, equipotent to (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU, brivudin) against HSV-1 and significantly active against varicella-zoster virus (VZV) [16].

Rai et al. [17] prepared of 5-[1-(2-haloethyl(or nitro)ethoxy-2-iodoethyl)]-2'-deoxyuridines **46** using 5-vinyl-2'-deoxyuridine **45** as starting compounds (Scheme 8). The regiospecific reaction of **45** with iodine monochloride and an alcohol provided the target compounds **46**. The products **46** were prepared as mixture of two diastereoisomers in ratio 1:1, which differ in configuration at the 1 position of the 5-substituent.

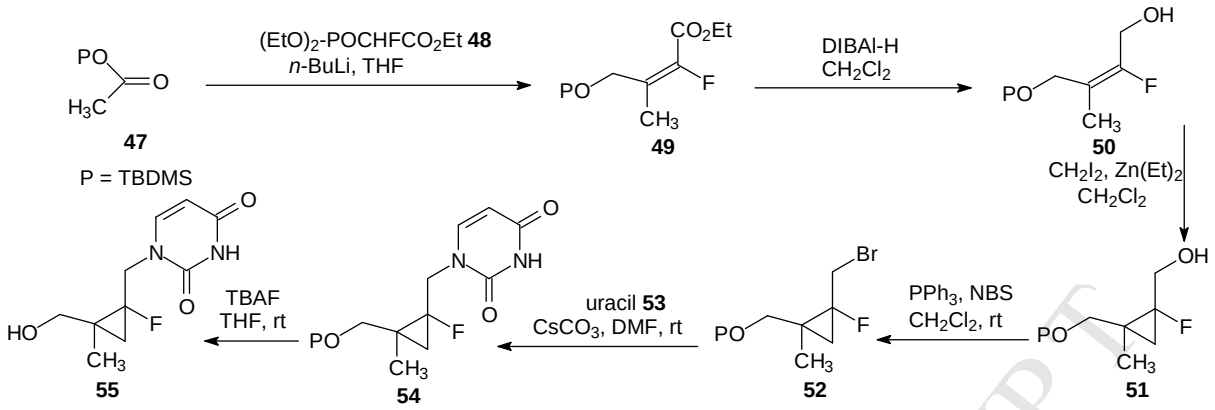


R = CH_2CH_2Br, CH_2CH_2Cl, $CH_2CH_2NO_2$
CH_2CBr_3, CH_2CCl_3

Scheme 8. Synthesis of 5-[1-(2-haloethyl(or nitro)ethoxy-2-iodoethyl)]-2'-deoxyuridines **46**.

These analogs were evaluated in vitro for inhibitory activity against thymidine-kinase (TK) positive and negative strains of herpes simplex virus type-1. The compounds **46** were either weak or non-inhibitory to HSV-1 replication. All compounds **46** exhibited low host cell cytotoxicity [17].

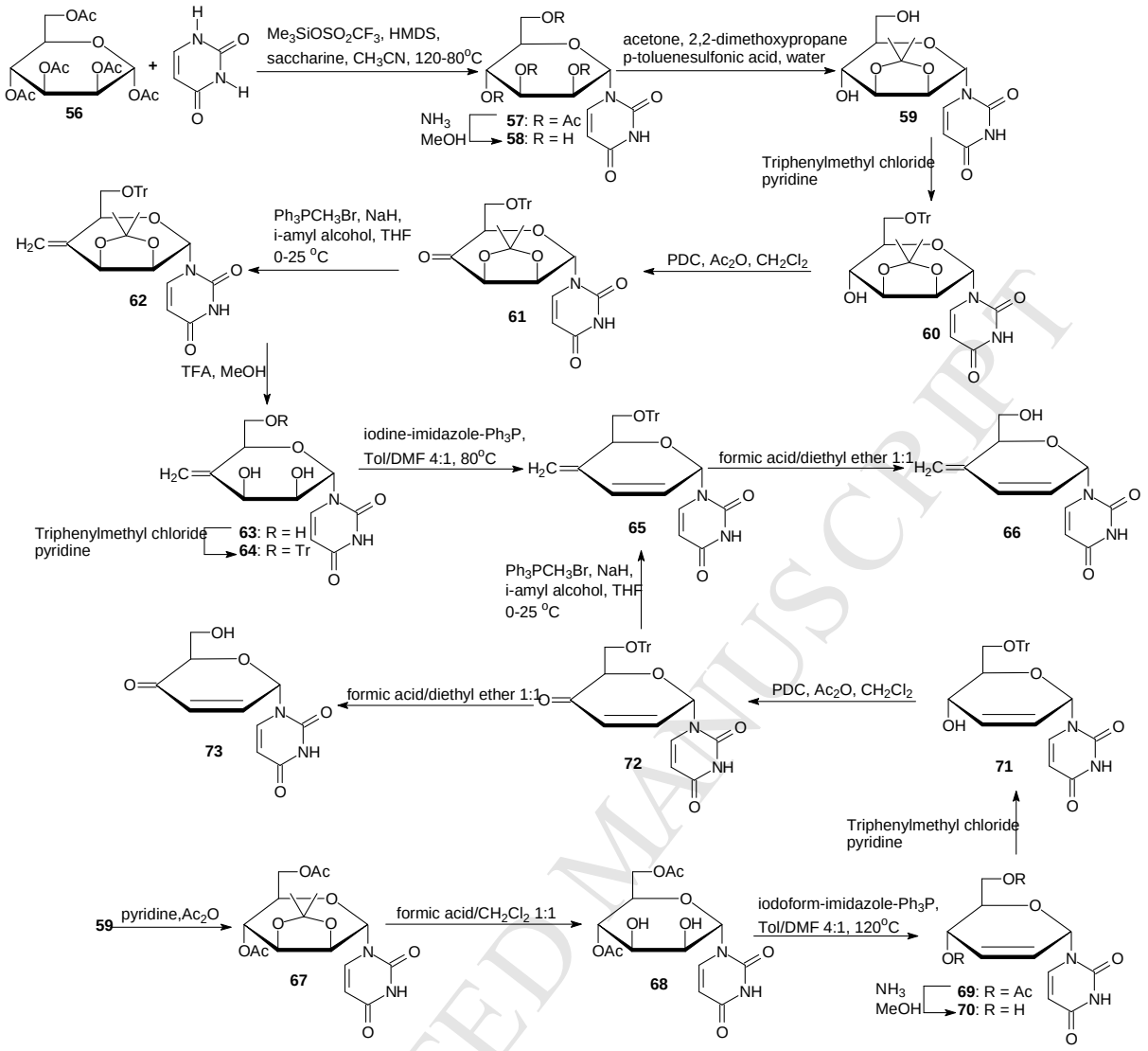
Kim and Hong [18] synthesized a series of fluorocyclopropyl nucleoside **55** starting from acetol using Simmons-Smith reaction as a key step (Scheme 9). A number of nucleosides comprising the cyclopropyl sugar moiety have been synthesized as conformationally constrained analogues of acyclonucleosides. Regarding cyclopropyl derivatives, several structural modifications have been made with the purpose of improving or enhancing the antiviral activity of some of them [18]. Methylene-spacered cyclopropyl nucleosides have shown potent antiviral activity, particularly against the human cytomegalovirus (HCMV) [18]. As a part of a searching for antiviral agents, novel classes of nucleosides comprising cyclopropyl backbone and trisubstituted cyclopropyl nucleosides with a fluorine group at 1'-position were designed and synthesized to evaluate them against various viruses because fluorine group might act as a hydrogen bonding acceptor at the active site of their target enzyme [18].



Scheme 9. Synthesis of fluorocyclopropyl bromide **52** and next fluorocyclopropyl nucleoside **55**.

Compound **49** was subjected to reduction conditions using diisobutylaluminium hydride (DIBAL-H) to afford the fluoroallylic alcohol **50**, which then underwent a Simmons-Smith reaction with $Zn(Et)_2/CH_2I_2$ to give compounds **51**. The sugar moiety was alkylated via a nucleophilic substitution reaction by converting the allylic alcohols **51** to the allylic bromide **52** and by sequential addition of NBS to a solution of the alcohols and triphenylphosphine. The condensation of the allylic bromide **52** with the uracil **53** in DMF with cesium carbonate as a basic catalyst afforded the nucleoside derivative **54**. The deprotection of the *tert*-butyldimethylsilyl grup (TBDMS) using tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave the desired fluorocyclopropyl nucleoside **55** (Scheme 9). Prepared compounds were tested against several viruses such as the HIV (MT-4 cells), HSV-1 (herpes simlex virus type 1: CCL-81), HSV-2 (herpes simplex virus type 2; CCL-81) cells, and HCMV (human cytomegalovirus; AD-169). The uracil analogue **55** showed moderate anti-HCMV activity (10.61 μg/mL in AD-169) [18]. The *cis*-like uracil analogue **55** showed higher anti-HCMV activity compared with the *trans*-like derivative, indicating that this virus might allow the sugar moiety to serve as a template for phosphorylation as well as for DNA polymerase, which is unlike other viruses.

Agelis et al. described [19] the total and facile synthesis of the unsaturated and exomethylene pyranonucleoside analogues, 1-(2,3,4-trideoxy-4-methylene-6-*O*-trityl-α-D-glycero-hex-2-enopyrano)uracil **66**, 1-(2,3-dideoxy-α-D-glycero-hex-2-enopyranosyl-4-ulose)uracil **72** and 1-(2,3,4-trideoxy-4-methylene-α-D-glycero-hex-2-enopyrano)uracil **73**. Commercially available 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose **56** was condensed with silylated uracil, deacetylated and acetalated to afford 1-(2,3-*O*-isopropylidene-α-D-mannopyranosyl)uracil **59** (Scheme 10). Two different synthetic routes were investigated for the conversion of **59** into the olefinic derivative **65**. Although the two procedures are quite similar with respect to yields and final products, the second also leads to the keto-2',3'-unsaturated analogue **73**.

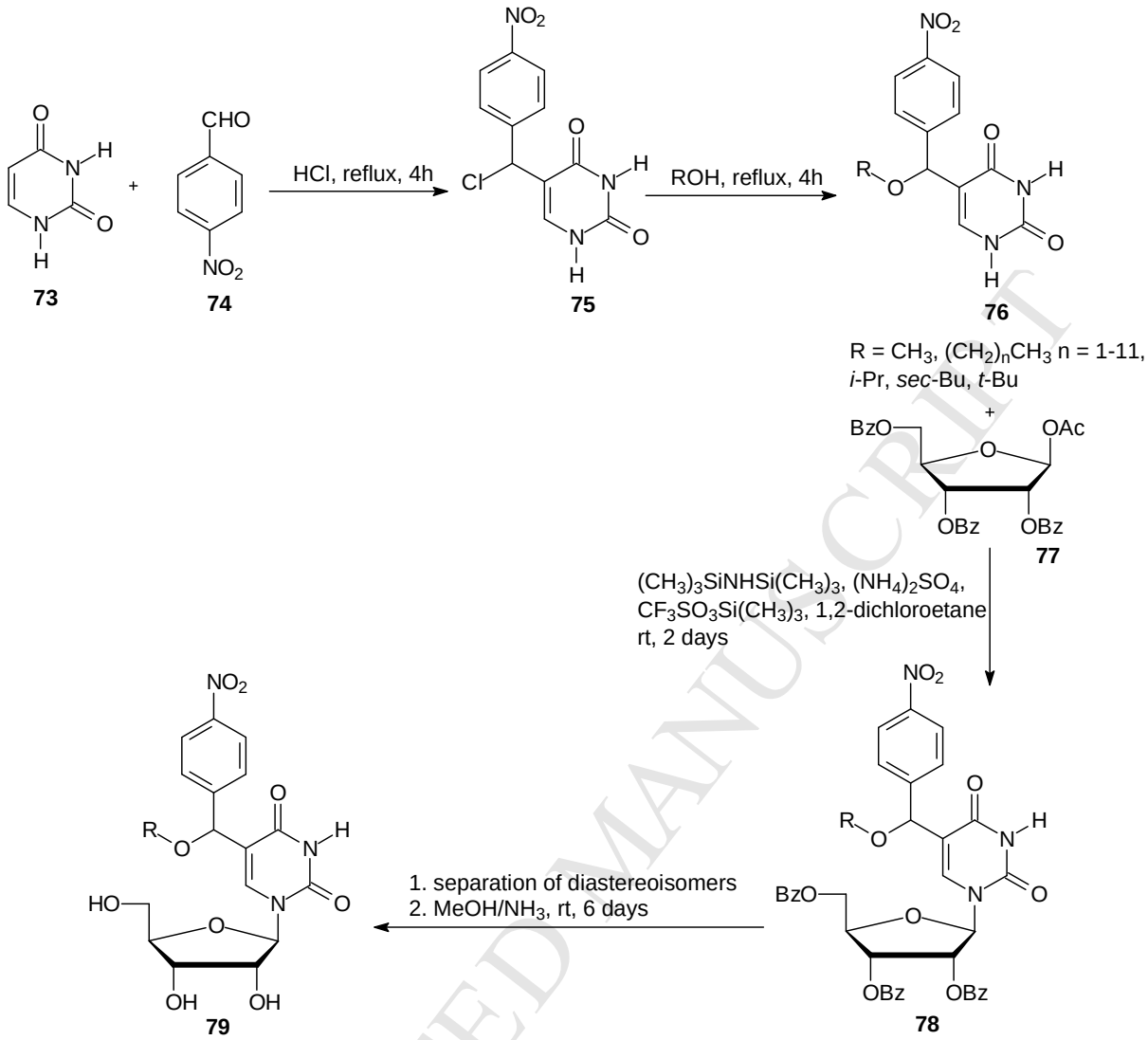


Scheme 10. Synthesis of the unsaturated exomethylene **66** and keto pyranonucleosides **73**.

All new analogues were evaluated for their anticancer and antiviral activities using several tumor cell lines and gastrointestinal rotavirus. The antiviral properties of the nucleoside analogues were examined using a colon adenocarcinoma Caco-2 cell line infected with gastrointestinal rotavirus as a model virus and AZT drug as positive control. The cytotoxicity (CC_{50}) of the new compounds was measured on normal human intestinal cell line (H4) and on a series of human tumor cell, such as human colonic adenocarcinoma cell line (Caco-2), skin melanoma cell line, and epithelial breast cancer cell line (MCF-7). All of the compounds showed direct antiviral effect against rotavirus infectivity. Moreover, uracil **65** was found to

be potent in MCF-7 breast carcinoma cell line. Uracil **65** being sufficiently cytotoxic on carcinoma cells and also highly selective was identified as a lead compound for further studies. All new molecules inhibited the growth of Caco-2 cells and showed direct antiviral effect as they were able to inhibit rotavirus action [19].

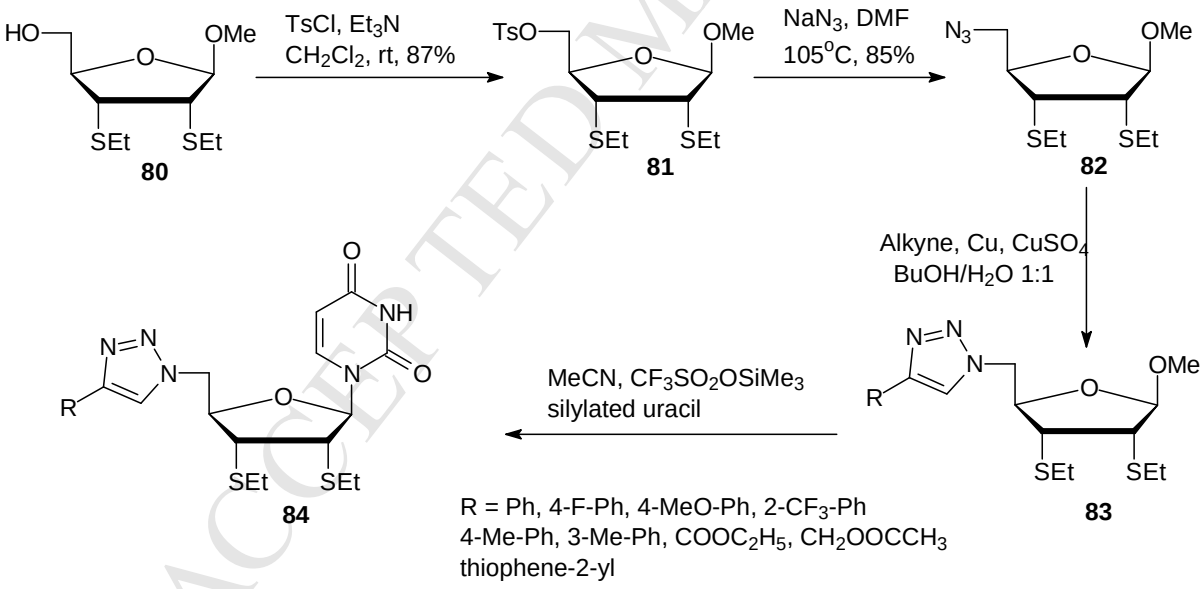
Brulikova and co-workers [20] produced uridine analogues modified at the 5-position with the 5-[alkoxy-(4-nitrophenyl)-methyl] moiety **76** (Scheme 11). Bases **76** with the highest activity were transformed into the corresponding nucleosides **79**. The Vorbruggen method which utilizes silylated nucleobases and strong Lewis acids was used. First the starting compounds were silylated, next silylated uracils reacted with protected sugar **77** in the presence of TMSOTf to afford benzoylated ribonucleosides **78** that were formed as a mixture of two diastereoisomers. The diastereoisomers were separated and treatment of ribonucleosides with methanolic ammonia afforded the nucleosides **79**.



Scheme 11. Synthesis of 5-(alkoxy-(4-nitrophenyl)methyl)uracils **76** and their nucleosides **79**.

Bases **76** and nucleosides **79** were tested for their cytotoxic activity in vitro against cancer cell lines including drug sensitive (CEM and K-562) as well as drug resistant (CEM-DNR-B and K-562 TAX) cell lines and A549 cells as representative of solid tumors. The cytotoxic activity was slightly increased in some cases by transformation of bases to nucleosides. The activity increases with chain length in both anticancer as well as antimicrobial activity. The cytotoxic activity of the nucleosides was not due to cell cycle alterations, DNA and/or RNA synthesis [20].

Yu and co-workers [21] designed a series of 2',3'-diethanethio-2',3',5'-trideoxy-5'-triazoloribonucleosides of uracil and evaluated their antitumor activity. Compound **80** was prepared from D-xylose via a cascade reaction and next it was converted into tosylate **81** followed by treatment with sodium azide to give azido ribofuranoside derivative **82** (Scheme 12). Under Huisge-Sharpless azide-alkyne cycloaddition reaction conditions, conversion of the azido group in **82** into the triazole ring with phenyl acetylene was carried out with CuCl, CuBr or CuI as the catalyst. The yield for the products **83** was higher when Cu-$CuSO_4$ was used to activate this 1,3-dipolar cycloaddition reaction at room temperature. Under the Silyl-Hilbert-Johnson glycosylation conditions, derivatives **83** were treated with trimethylsisilylated uracil in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give the nucleosides **84** (Scheme 12).

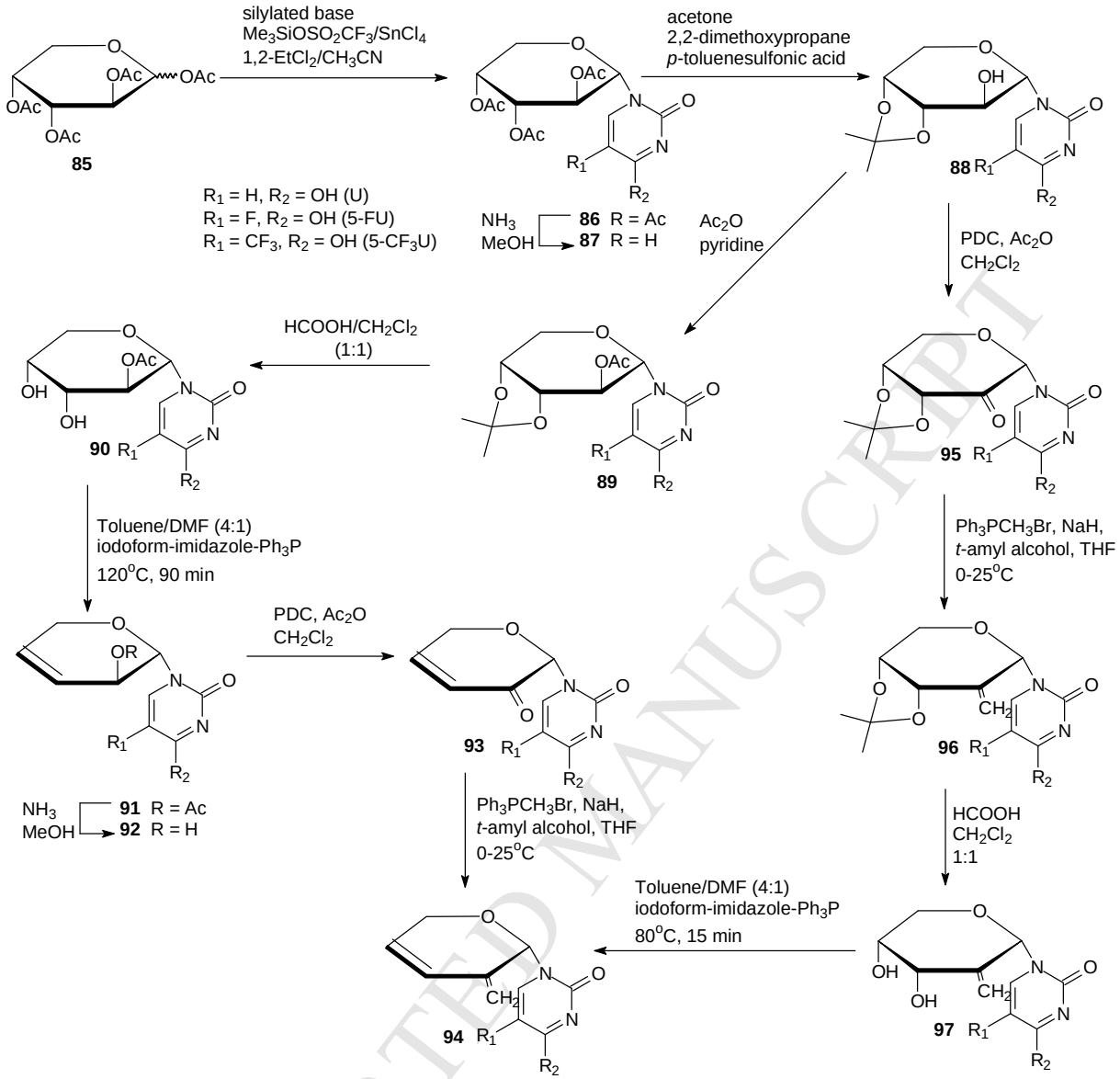


Scheme 12. Synthesis of 2',3'-diethanethio-2',3',5'-trideoxy-5'-triazoloribonucleosides of uracil **84**.

The antitumor activity of these novel nucleosides was tested *in vitro* towards the following human cancer cell lines: human hepatocellular liver carcinoma cell line ($HepG_2$), two types of

non-small cell lung cancer: lung adenocarcinoma (LAC) and squamous subdivision of epithelial cells (A549), and human cervical carcinoma cell line (Hela). The compounds **84** with aromatic substituted trazole rings showed significantly improved activity towards a broad range of tumor cell lines and those without arene substitutes were inactive. The molecular structure analysis indicated the potential coplanar relationship between the phenyl moiety and the triazole ring which results in the conjugate effects. These results suggested that the conjugation effects of the triazole ring with the aromatic system are essential for bioactivity [21].

Tzioumaki et al. [22] described the synthesis of pyrimidine unsaturated keto and exomethylene arabinopyranopyranonucleoside analogs as potential antitumor and antiviral agents. Commercially available 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose **85** was condensed with silylated uracil (U), 5-fluorouracil (5-FU) or 5-(trifluoromethyl)uracil (5-CF_3U), deacetylated and acetylated to afford 1-(3,4-*O*-isopropylidene-α-D-arabinopyranosyl)pyrimidine analogs **88** (Scheme 13).

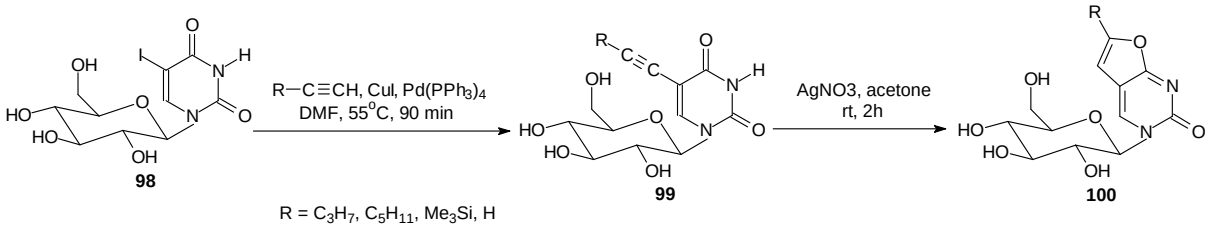


Scheme 13. Synthesis of pyrimidine unsaturated keto and exomethylene arabinopyranopyranonucleoside analogs **93**, **94** and **97**.

The conversion of compounds **88** into the new 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)nucleoside derivatives of uracils **94** was described [22]. This reactions resulted also to the 2-keto-3,4-unsaturated analogs **93**. The new analogs did not show inhibition of DNA and RNA virus replication in cell culture. With the exception of the human T-lymphocyte CEM cells that were inhibited by the 5-fluorouracil derivatives at higher micromolar concentrations, the murine leukemia L1210, the murine mammary carcinoma

FM3A, and the human cervix carcinoma HeLa cells were inhibited in their proliferation by some compounds at concentrations that were in the lower micromolar range. The 2'-ketonucleoside derivatives **93** were found to be more cytostatic than the corresponding 2'-exomethylene nucleosides **94**. The 5-fluorouracil unsaturated ketoderivative **93** (R_1 = F, R_2 = OH) and the exomthylene derivatives **94** (R_1 = F, R_2 = OH) and **97** (R_1 = F, R_2 = OH) showed antiproliferative activity in the lower micromolar range. Experimental evidence revealed that these compounds may act as novel types of 5-fluorouracil releasing prodrugs, and points to thymidylate synthase as target for their cytostatic action [22].

Novel 5-alkynyl and alkylfurano[2,3-*d*]pyrimidine glucopyranonucleosides have been synthesized and studied by Kantsadi et al. [23] as inhibitors of glycogen phosphorylase (GP). Glycogen phosphorylase catalyzes the first step of intra-cellular degradation of glycogen to Glc-1-P [24]. As such, GP has a pivotal role in glucose homeostasis and over last decade it has been validated as an important target for structure-assisted design of hypoglycaemic agents [25-27]. The efficacy of such inhibitors on blood glucose control and hepatic glycogen balance has been confirmed from biological studies [28-30]. All inhibitors were designed to mimic the contacts of glucose that localize the closed position of the 280s loop that blocks the entrance to the active site. The most potent inhibitors for the catalytic site have hydrophobic groups pointing towards the direction of β-pocket [25]. The synthesis of the 5-alkynyl pyrimidine glucopyranonucleosides is illustrated in Scheme 14. The introduction of 5-alkynyl groups was performed by reaction of terminal alkynes with the nucleoside 1-(β-D-glucopyranosyl)-5-iodouracil **98** by a Pd(0)-mediated reaction, using the Sonogashira conditions and yielded 5-ethynyluracil derivatives **99** [23]. Fused bicyclic pyrimidine pyranonucleosides **100** were prepared by electrophilic cyclization catalyzed by $AgNO_3$.



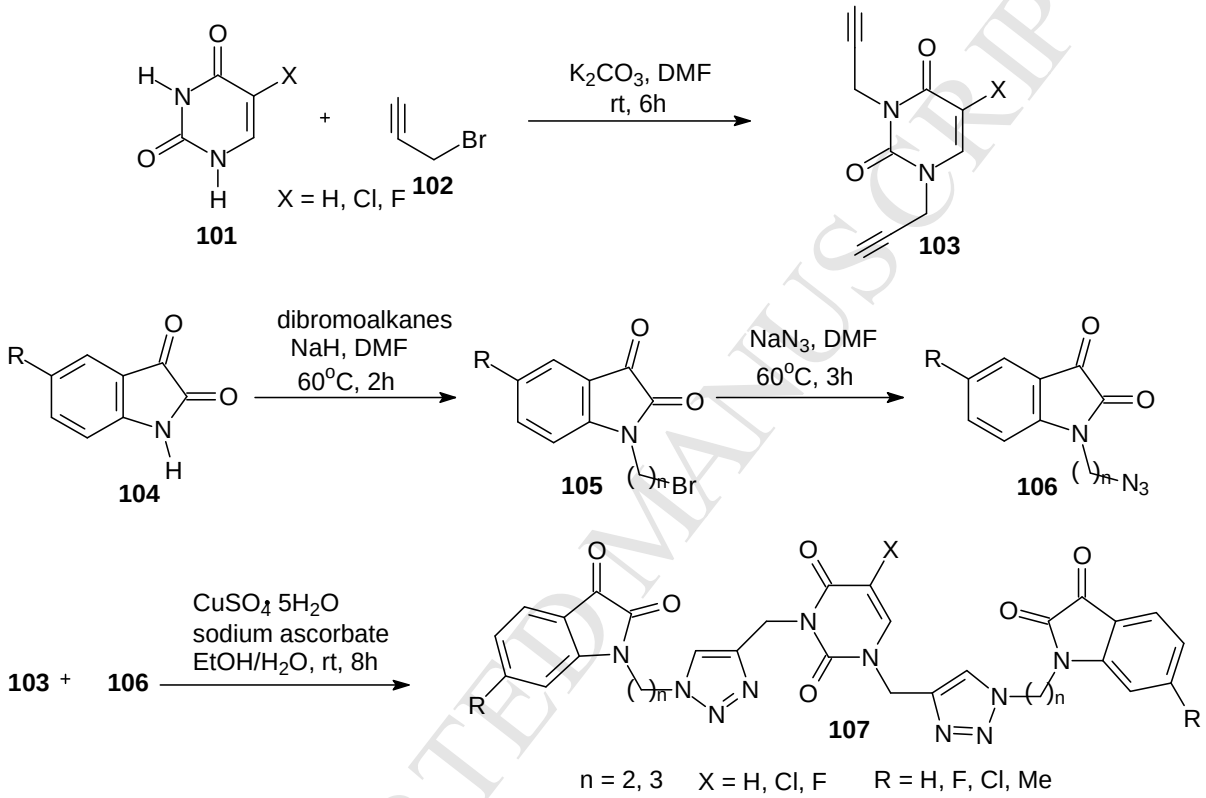
Scheme 14. Synthesis of 5-alkynyl and alkylfurano[2,3-*d*]pyrimidine glucopyranonucleosides **99** and **100**.

Kinetic experiments have shown that most of these compounds were low micromolar inhibitors of the enzyme. The best inhibitor was 1-(*β*-D-glucopyranosyl)-5-ethynyluracil **99** (*K*i = 4.7 mM) [23]. Crystallographic analysis of compounds **99** and **100** in complex with GP revealed that inhibitors with a long 5-alkynyl group exploited interactions with *β*-pocket of the active site and induced significant conformational changes of the 280s loop compared to GP in complex with compounds with a short 5-alkynyl group. The results highlight the importance in the length of the aliphatic groups used to enhance inhibitory potency for the exploitation of the hydrophobic *β*-pocket. The best of the inhibitors had also a moderate effect on glycogeneolysis in the cellular lever with an IC_{50} value of 291.4 μM.

2.3. Uracil-heterocycle hybrids

The incorporation of heterocyclic compounds, for example 1,2,3-triazoles as attractive linker units between two pharmacophores to give bifunctional drugs, have become interestingly useful and important in constructing bioactive molecules. Kumar et al. [31] reported the synthesis of uracil-isatin hybrids *via* azide-alkyne cycloadditions and their cytotoxic evaluation against three human cancer cell lines *viz*. HeLa (cervix), MCF-7 (breast) and DU145 (prostate) using MTT assay. The synthetic protocol involved an initial di-alkylation of C-5 substituted uracil **101** using propargyl bromide **102** to yield the

corresponding dipropargylated precursor **103** as shown in Scheme 15. The synthesis of *N*-alkyl azido isatin derivatives **106** was based on initial base-assisted *N*-alkylation of substituted isatins with dibromoalkane and subsequent reaction with sodium azide resulting in second precursor **106**. The target compounds **107** were synthesized by utilizing azide-alkyne cycloaddition reaction of precursors **103** and **106** (Scheme 15).

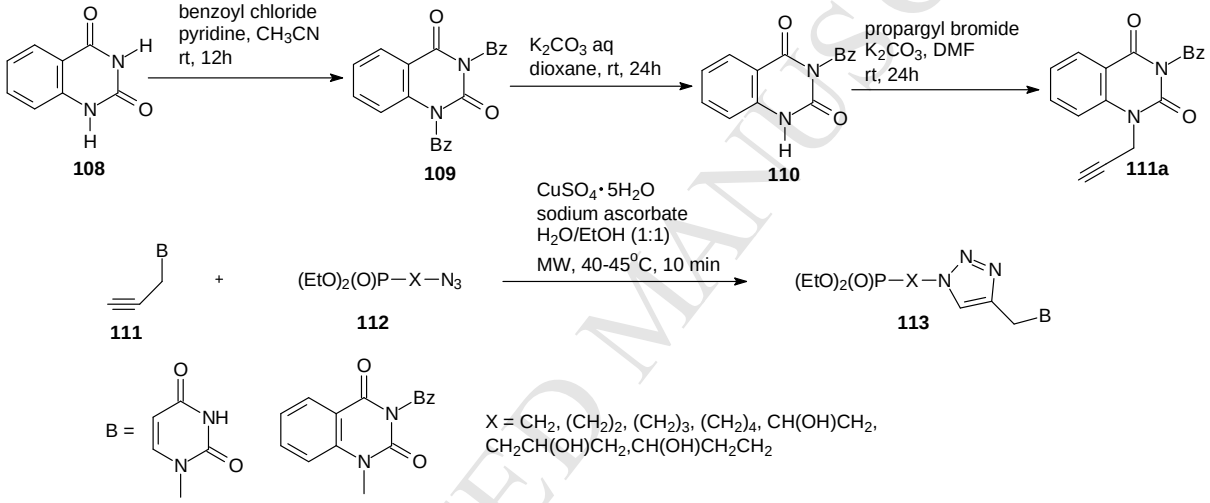


Scheme 15. Synthesis of 1,3-dipropargylated uracils **103**, *N*-alkyl azido isatins **106** and uracil-isatin-conjugates **107** using click chemistry.

The evaluation studies revealed the dependence of cytotoxicity on C-5 substituents on both uracil and isatin as well as the alkyl chain length with marked preference of Cl substituent over H, F and CH_3 along with longer alkyl chain length (*n* = 3). The cytotoxic profiles revealed that the compounds **107** (X = Cl, R = H) and **107** (X = H, R = Cl) have shown lowest IC_{50} values, 18.21 and 13.90 μM respectively, among the test compounds against DU 145

[31]. Most of the synthesized conjugates exhibited considerable selectivity against MCF-7 and DU 145 cell lines.

Głowacka and co-workers [32] produced nucleoside analogues having uracils connected to the 1,2,3-triazole ring by a methylene linker. The 1,2,3-triazoloacyclonucleotides **113** has been obtained from diethyl azidomethyl-, 2-azidoethyl-, 3-azidopropyl-, 4-azidobutyl-, 2-azido-1-hydroxyethyl-, 3-azido-2-hydroxypropyl- and 3-azido-1-hydroxypropylphosphonates **112** and N^1-propargyl uracils **111** via 1,3-dipolar cycloadditions carried out under microwave irradiation (Scheme 16).

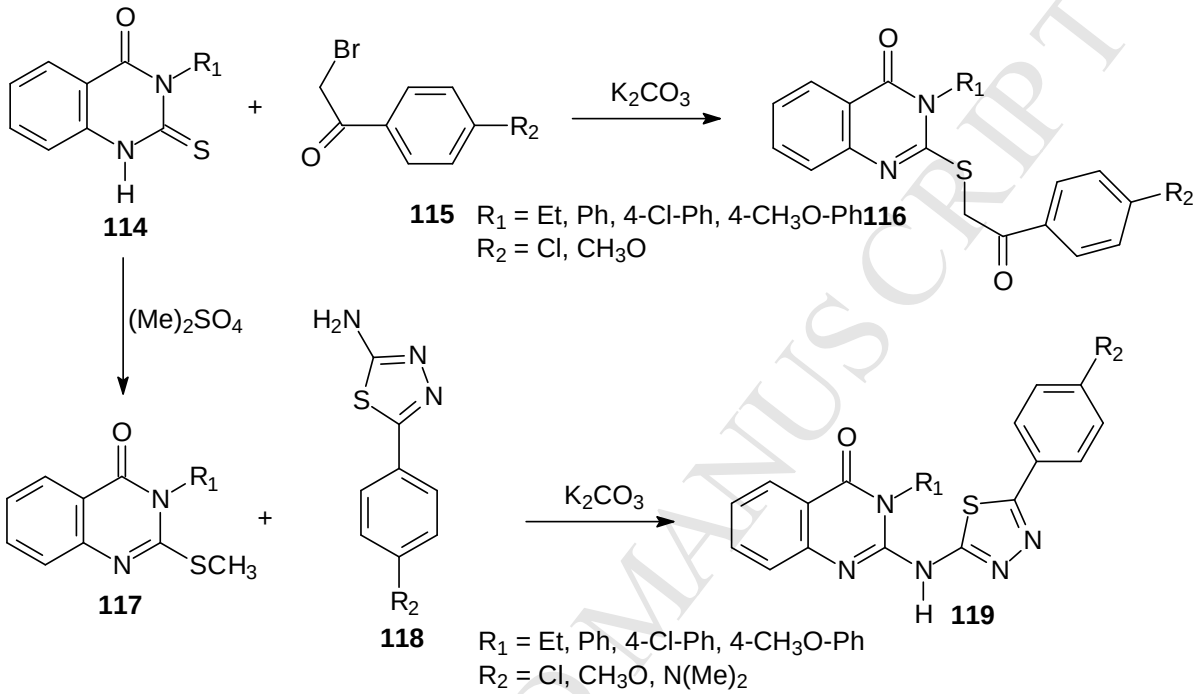


Scheme 16. Synthesis of 1,2,3-triazoloacyclonucleotides of uracil **113**

All compounds were evaluated in vitro for activity against a broad variety of DNA and RNA viruses and cytostatic activity against murine leukemia L1210, human T-lymphocyte CEM and human cervix carcinoma HeLa cells. Several compounds of **113** were found to be the most active towards T-lymphocyte CEM cell proliferation [32].

Gawad et al. [33] described the synthesis of 3-substituted quinazolin-4(3*H*)-ones and 3,4-dhydro-quinazolin-2(1*H*)-one derivatives and their biological evaluation as antitumor agents. 3-Substituted-2-thioxo-2,3-dihydro-quinazolin-4(1*H*)-ones **114** reacted with the appropriate phenacyl bromides **115** to yield 3-substituted-quinazolin-4(3*H*)-ones **116** (Scheme

17). On the other hand, the reaction of **114** with dimethyl sulphate in ethanolic sodium hydroxide afforded the 2-methylthio derivatives **117**. Nucleophilic displacement of the $–SCH_3$ function of **117** by 2-amino-5-aryl-1',3',4'-thiadiazoles **118**, produced 3-substituted-quinazolin-4(3*H*)-ones **119** (Scheme 17).



Scheme 17. Synthesis of quinazolinones derivatives **116** and **119**.

The synthesized compounds were subjected to the NCI's disease-oriented human cell lines screening assay to be evaluated for their *in-vitro* antitumor activity. They were used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely; leukemia, non small cell lung cancer, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells. Quinazolin-4(3*H*)-ones **116** (R_1 = 4-CH_3O-Ph, R_2 = Cl) and **116** (R_1 = 4-Cl-Ph, R_2 = CH_3O) which represent fused thiouracil system are broad-spectrum antitumors showing effectiveness toward numerous cell lines that belong to different tumor subpanels. Those two quinazoline analogues could be considered as useful templates for future development to obtain more potent antitumor agents [33].

2.4. Tritylated uracil analogues

Tritylated deoxyuridine analogues and tritylated acyclic uridine analogues (Fig. 4) are able to inhibit the *Plasmodium falciparum* dUTPase (*Pf*dUTPase) enzyme selectively in low micromolar range [34]. The parasite *Plasmodium falciparum*, transmitted to the human host through the female mosquito, is the main cause of severe clinical malaria. The ubiquitous enzyme dUPT nucleotidohydrolase (dUTPase) catalyses the hydrolysis of dUTP to dUMP and can be considered as the first line of defence against incorporation of uracil into DNA. Inhibition of this enzyme results in over-incorporation of uracil into DNA, leading to DNA fragmentation and cell death and is therefore lethal. By taking advantage of structural differences between the human and *Plasmodium* dUTPase, selective inhibitors of the enzyme can be designed and synthesized with the aim of being developed into novel anti-parasitic drugs.

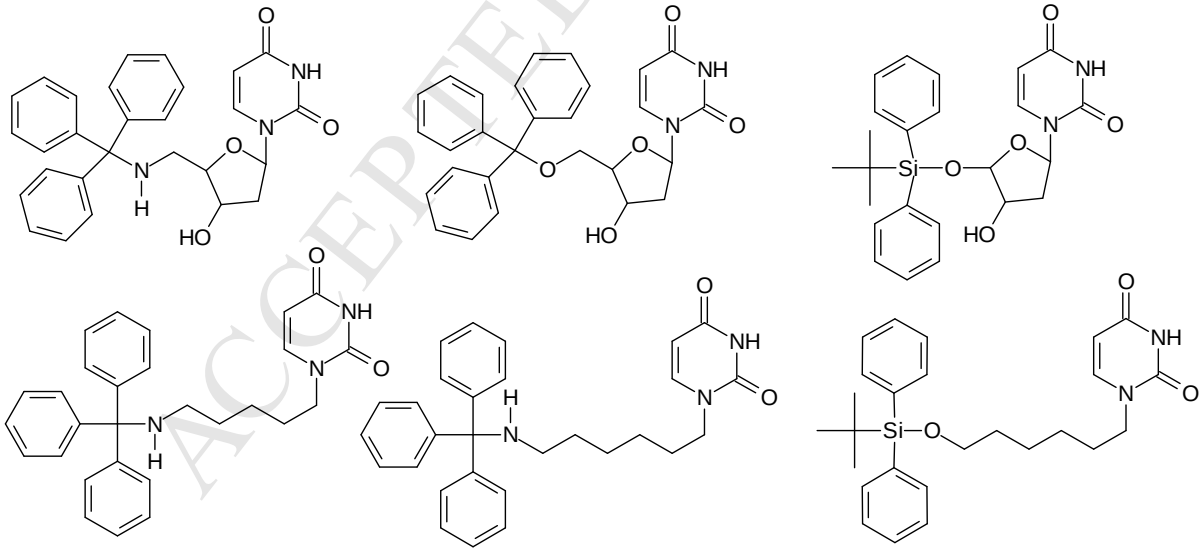
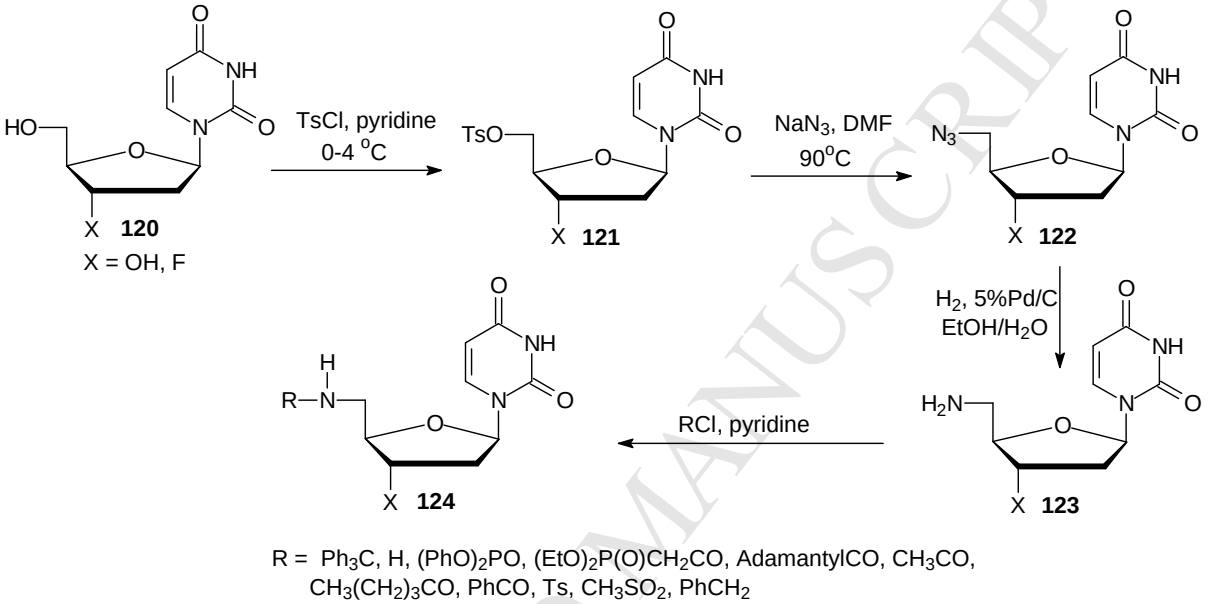


Fig. 4. Tritylated deoxyuridine analogues and tritylated acyclic uridine analogues – *Pf*dUTPase inhibitors.

Nguyen and co-workers [35] described the successful synthesis of a variety of analogues of dUMP in which the substituents are introduced at 3'- and 5'-positions, together with variation in the heteroatom at the 5'-position. Preparation of 5'-aminodeoxyuridines **124** was achieved by selective tosylation of the 5'-position of deoxyuridine **120**, displacement with azide, and hydrogenation, following a reaction with Ph_3CCl (Scheme 18).



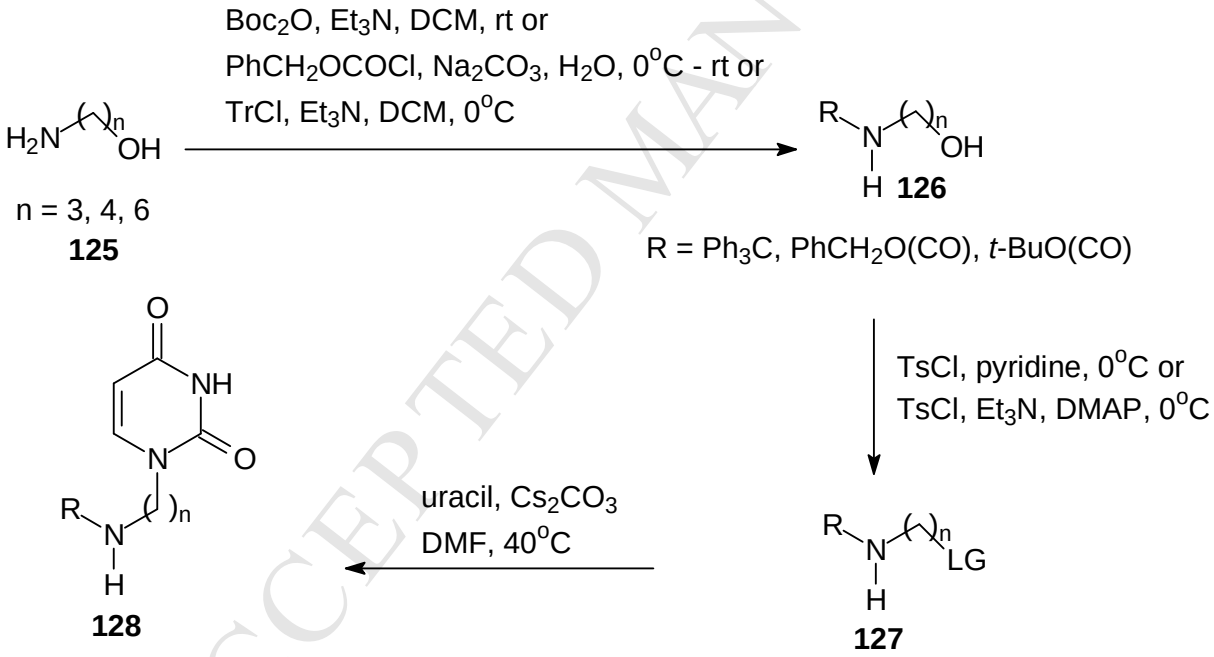
R = Ph_3C, H, $(PhO)_2PO$, $(EtO)_2P(O)CH_2CO$, AdamantylCO, CH_3CO, $CH_3(CH_2)_3CO$, PhCO, Ts, CH_3SO_2, $PhCH_2$

Scheme 18. Synthesis of tritylated deoxyuridine analogues **124**.

The compounds were assayed against recombinant *Plasmodium falciparum* and *Leishmania major* enzymes and the human enzyme to give a measure of selectivity [35]. The compounds were also tested in vitro against the intact parasites *P. falciparum* and *L. donovani*. A number of potent and selective inhibitors of the *P. falciparum* dUTPase that show drug-like properties and represent good leads for future development were identified. The best inhibitors was the compound 5'-tritylamino-2',5'-dideoxyuridine **124 (**X = OH) (*K*i = 0.2 μM) with selectivity greater than 200-fold compared to the human enzyme. The correlation observed between the inhibition of the enzyme and the inhibition of the parasite growth in vitro demonstrates that

the *P. falciparum* dUTPase constitutes a valid and attractive novel target for the development of much-needed new antimalarial drugs [35].

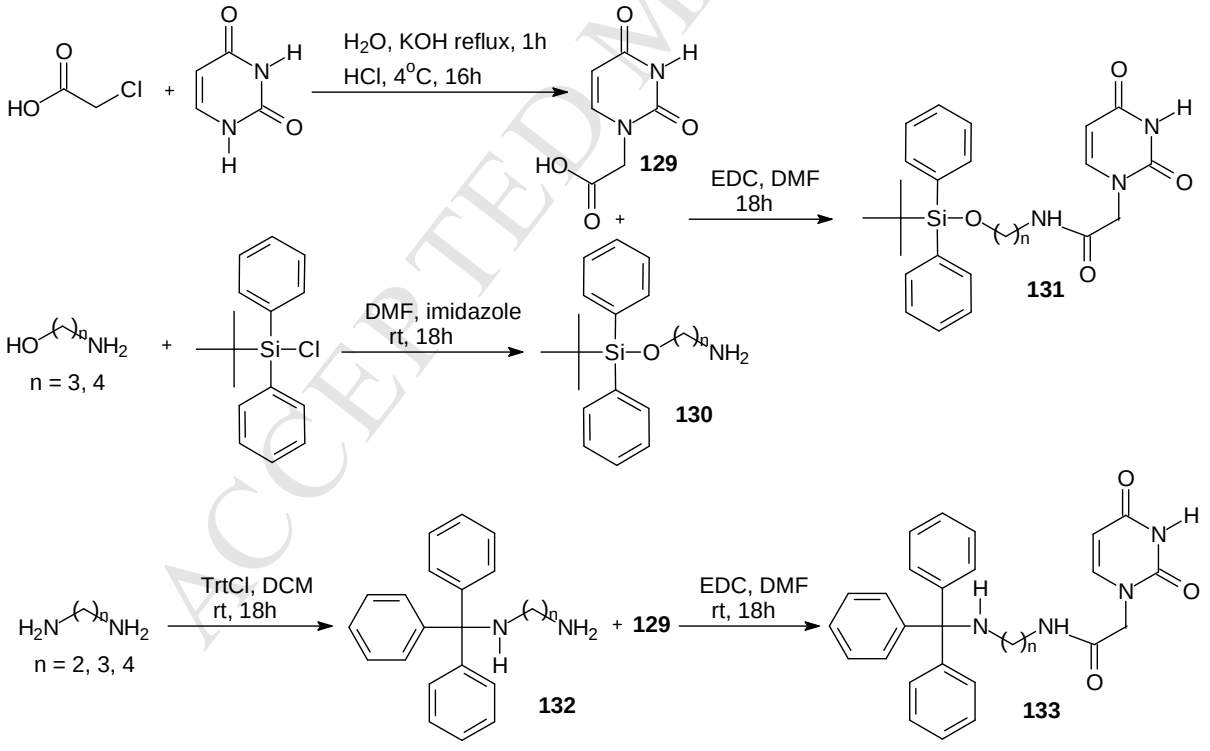
Gilbert at al. [36] reported in 2006 the discovery of novel uracil-based acyclic compounds as inhibitors of dUTPase. Compounds were assayed against both *P. falciparum* dUTPase and intact parasites. A good correlation was observed between enzyme inhibition and cellular assays. Acyclic uracil derivatives were identified that showed greater or similar potency and in general increased selectivity compared to previously reported inhibitors [35]. The most active compound reported against the *P. falciparum* enzyme had a K_i of 0.2 μM. Preliminary ADME studies indicated that some of the lead compounds are drug-like molecules [36].



Scheme 19. Synthesis of acyclic nucleoside analogues **128** as inhibitors of *Plasmodium falciparum* dUTPase.

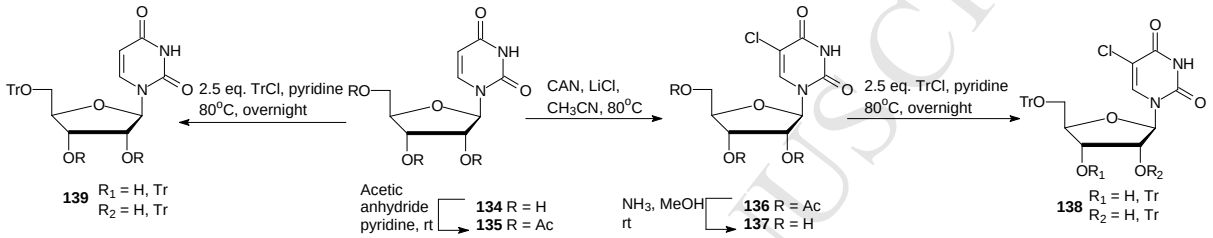
Tritylaminopropyl and tritylaminohexyl analogues **128** (Scheme 19) could be obtained by direct coupling of uracil with suitably activated aliphatic fragment. Gilbert et al. have shown that tritylated deoxyuridine analogues and then subsequently tritylated acyclic uridine

analogues were able to inhibit the *P. falciparum* dUTPase enzyme selectively in the low micromolar range [35, 36]. These compounds also inhibited parasite growth in the low micromolar range. The structures of previously discovered selective inhibitors of the *Pf*dUTPase (Fig. 4) were modified in 2009 by McCarthy et al. by insertion of an amide bond [34]. A series of tritylated uracil acetamide derivatives were synthesized and assessed for inhibition of the enzyme and parasite growth *in vitro* [34]. These compounds were weak inhibitors of the *Pf*dUTPase. The synthesis of the *tert*-butyl diphenyl silyl (TBDPS) uracil acetamides **131** was carried out by coupling the TBDPS protected amino alcohols **130** to 1-carboxymethyl uracil **129** using EDC as a coupling reagent (Scheme 20). The tritylamino analogues were synthesized by mono-tritylation of the relevant diamines **132** followed by coupling to 1-methylcarboxyuracil **129** again using EDC as a coupling reagent.



Scheme 20. Synthesis of TBDPS uracil acetamide derivatives **131** and tritylamino uracil acetamide derivatives **133**.

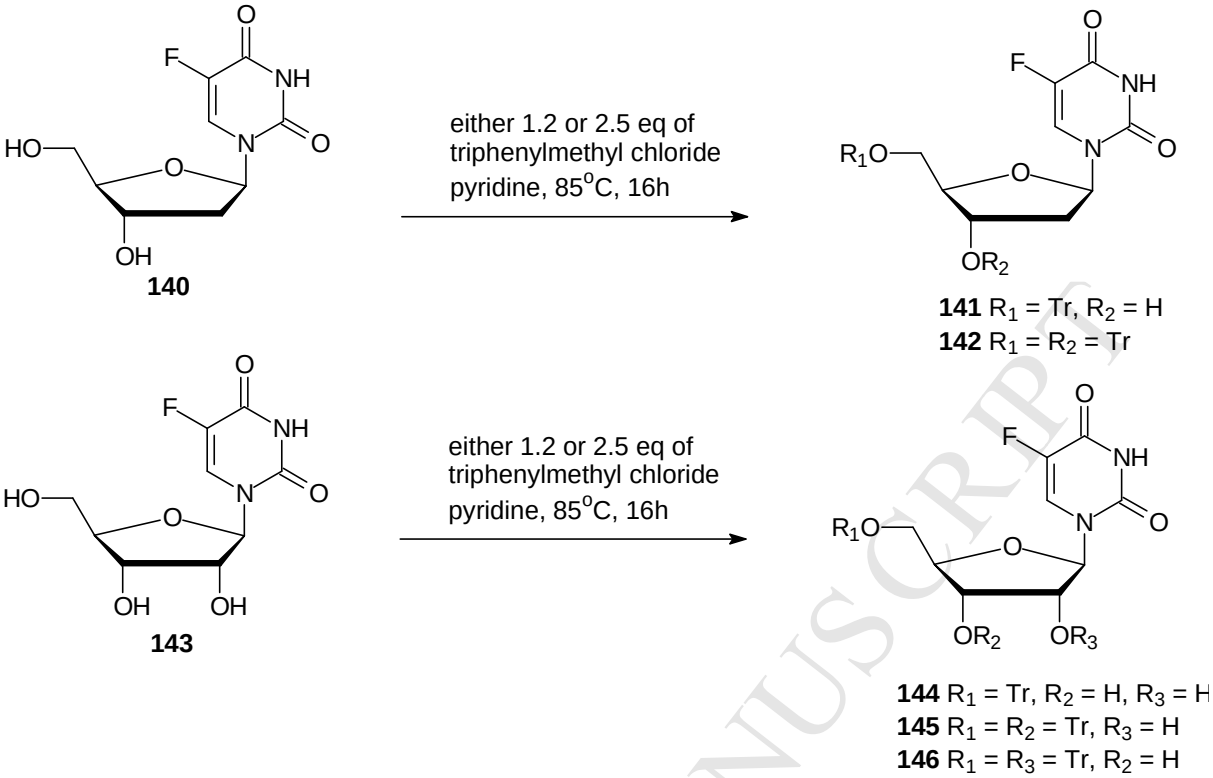
Chatelain et al. [37] synthesized triphenylmethyl alkylated nucleoside of uracil and 5-chlorouracil. Trityl moieties were attached at various positions of the sugar ring. Synthesis of 5-chlorouridine **137** was attempted on acetylated uridine **135** using ceric(IV) ammonium nitrate (CAN) and LiCl (Scheme 21). Compound **136** was deprotected to afford uridine **137**. Tritylation of unprotected ribonucleosides of uridine **134** and chlorouridine **137** was done using triphenylmethyl chloride, affording the 5'-monotritylated, the 2', 5'- and 3',5'-bistritylated analogues **138** and **139**.



Scheme 21. Synthesis of tritylated-5-chlorouridines **138** and tritylated-uridines **139**.

Prepared analogs were evaluated for their *in vitro* antiviral activities against the dengue virus (DENV) and yellow fever virus (YFV). The most selective inhibitor was 3',5'-bis-O-tritylated-5-chlorouridine **139** (R_1 = Tr, R_2 = H) affording a selectivity index of over 90. These lipophilic compounds are exerting their effect through inhibition of the RNA polymerase of flaviviruses. The finding of these lipophilic structures should stimulate the interest for structure-activity research [37].

Saudi and co-workers [38] synthesized a series of nucleoside analogues of 3',5'-bistritylated uridine. Mono or bis-tritylation of 5-fluoro-2'deoxyuridine **140** and 5-fluorouridine **143** provided the compounds **141**, **142**, **143**, **144** and **145**, respectively (Scheme 22).



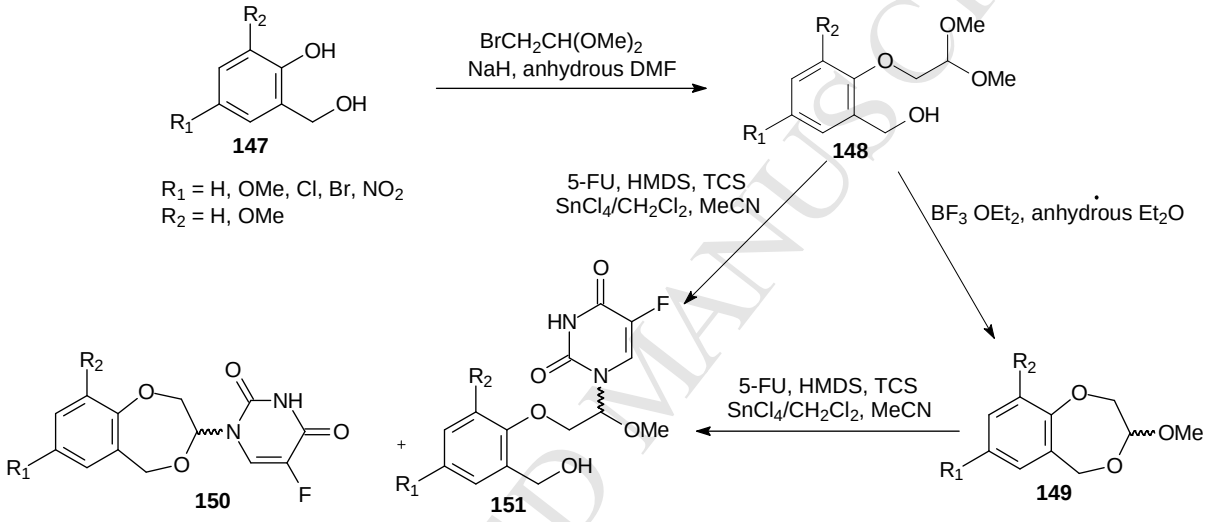
Scheme 22. Synthesis of tritylated derivatives of 5-fluoro-2'-deoxyuridine **141**, **142** and 5-fluorouridine **144**, **145** and **146**.

Prepared compounds were evaluated for their *in vitro* antiviral activities against dengue fever virus and yellow fever virus. Among the new series of derivatives, 3',5'-di-*O*-trityl-5-fluoro-2'-deoxyuridine **142** ($R_1 = R_2 = Tr$) was the most efficient in this series and inhibited both yellow fever virus and dengue virus replication with a 50% effective concentration (EC_{50}) of 1 μg/mL without considerable cytotoxicity. The other fluorinated derivatives proved more toxic [38].

2.5. Benzodioxepinyl-uracils, benzoxathiepinyl-uracils and diazepinyl-uracils

The synthesis of a wide range of uracil derivatives linked to saturated seven-membered moieties through *N*-1 of uracil ring was carried out and this methodology allowed on access to non-classical nucleosides with a 1,4-dioxepanyl group as the "sugar" moiety. 1-

(2,3-Dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracils have proved to be good antiproliferative agents against the MCF-7 human breast cancer cell line [39]. Such compounds accumulate the cancerous cells in the G_0/G_1 phase. Therefore, Saniger et al. [40] completed the synthesis and biological evaluation of cyclic **150** and acyclic **151** 5-FU *O*,*N*-acetals starting from several salicyl alcohols **147** (Scheme 23). They have investigated the Lewis acid-promoted transformation of the acyclic *O*,*N*-acetals **148** to the corresponding cyclic *O*,*N*-acetals **150**.

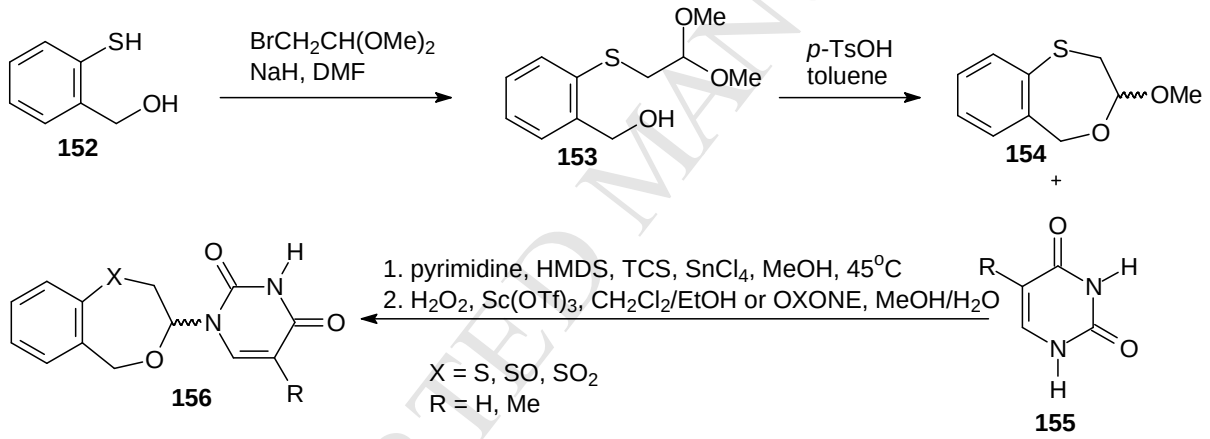


Scheme 23. Synthesis of cyclic **150** and acyclic **151** 5-FU *O*,*N*-acetals.

All the compounds **150** and **151** exhibited an IC_{50} against the MCF-7 human breast cancer cell line in the micromolar range, among which the nitro derivative **151** ($R_1 = NO_2$, $R_2 = H$) was found to be the most cytotoxic. They have demonstrated that compound **151** ($R_1 = R_2 = H$) induced apoptosis and G_0/G_1 cell cycle arrest in the MCF-7 human breast cancer cell line, whereas **151** ($R_1 = NO_2$, $R_2 = H$) seems to act as the prodrug Ftorafur on the basis that both compounds accumulate the tumoural cells in the S phase of the cell cycle [40].

On the basis of molecular variations on isosteric replacements from the prototype 1-(2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil, Nunez and co-workers [41] prepared a series of 3-(2,3-dihydro-5*H*-1,4-benzoxathiepin-3-yl)-uracil or thymine *O*, *N*-acetals. The

commercially available *o*-(hydroxymethyl)thiophenol **152** was selective alkylated using bromoacetaldehyde dimethyl acetal in the presence of sodium hydride (Scheme 24). Subsequent cyclization of the resulting hydroxyacetal **153** using *p*-toluenesulfonic acid produced the sulfur-containing acetal **154**. The last step was the condensation reaction between the seven-membered acetal **154** and the natural pyrimidine bases uracil and thymine **155**. For this procedure Nunez et al. used tin(IV) chloride in dichloromethane, trimethylchlorosilane (TCS) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in acetonitrile. The oxidation of sulfides to sulfoxides or sulfones was made using hydrogen peroxide in the presence of scandium trifluoromethansulfonate or potassium peroxymonosulfate (OXONE) (Scheme 24).



Scheme 24. Synthesis of 3-(2,3-dihydro-5*H*-1,4-benzoxathiepin-3-yl)-uracil or thymine *O, N*-acetals **156**.

O,N-Acetals **156** have been screened for their anticancer acivity. They were assayed for their *in vitro* antiproliferative activity against the MCF-7 cell line. Some of them were found to be inhibitors of the MCF-7 cell growth [41].

Mravljak and co-workers [42] described new inhibitors of the bacterial transferase MraY. A scaffold strategy based on the diazepanone central core of liposidomycins, natural inhibitors of MraY has been developed. Several families of natural antibiotics including

liposidomycins, caprazamycins or tunicamycins (Fig. 5) have been identified which display high *in vitro* inhibition of the MraY enzymatic activity, but modest antibacterial activity probably due to their high hydrophilicity limiting their passive diffusion through membranes [43].

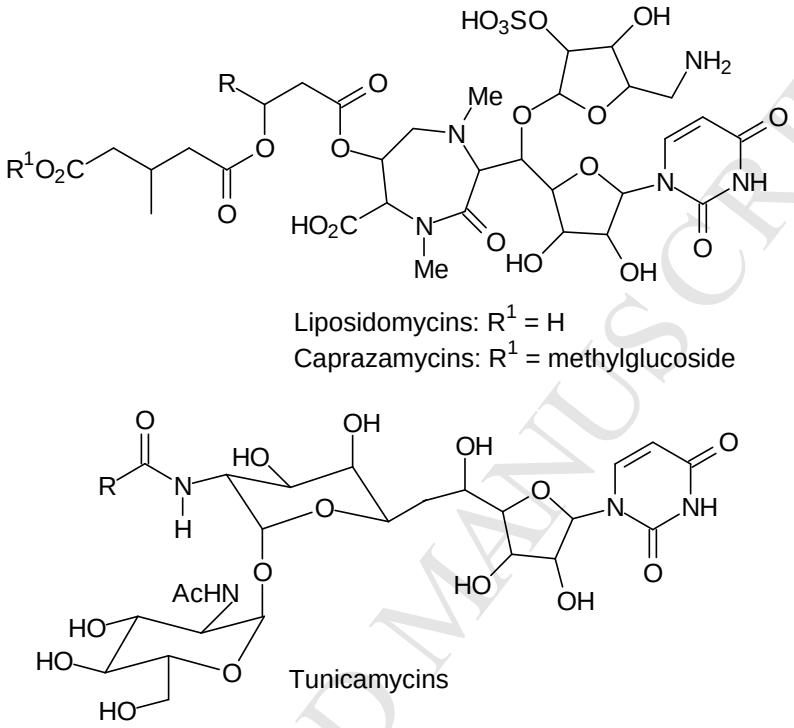
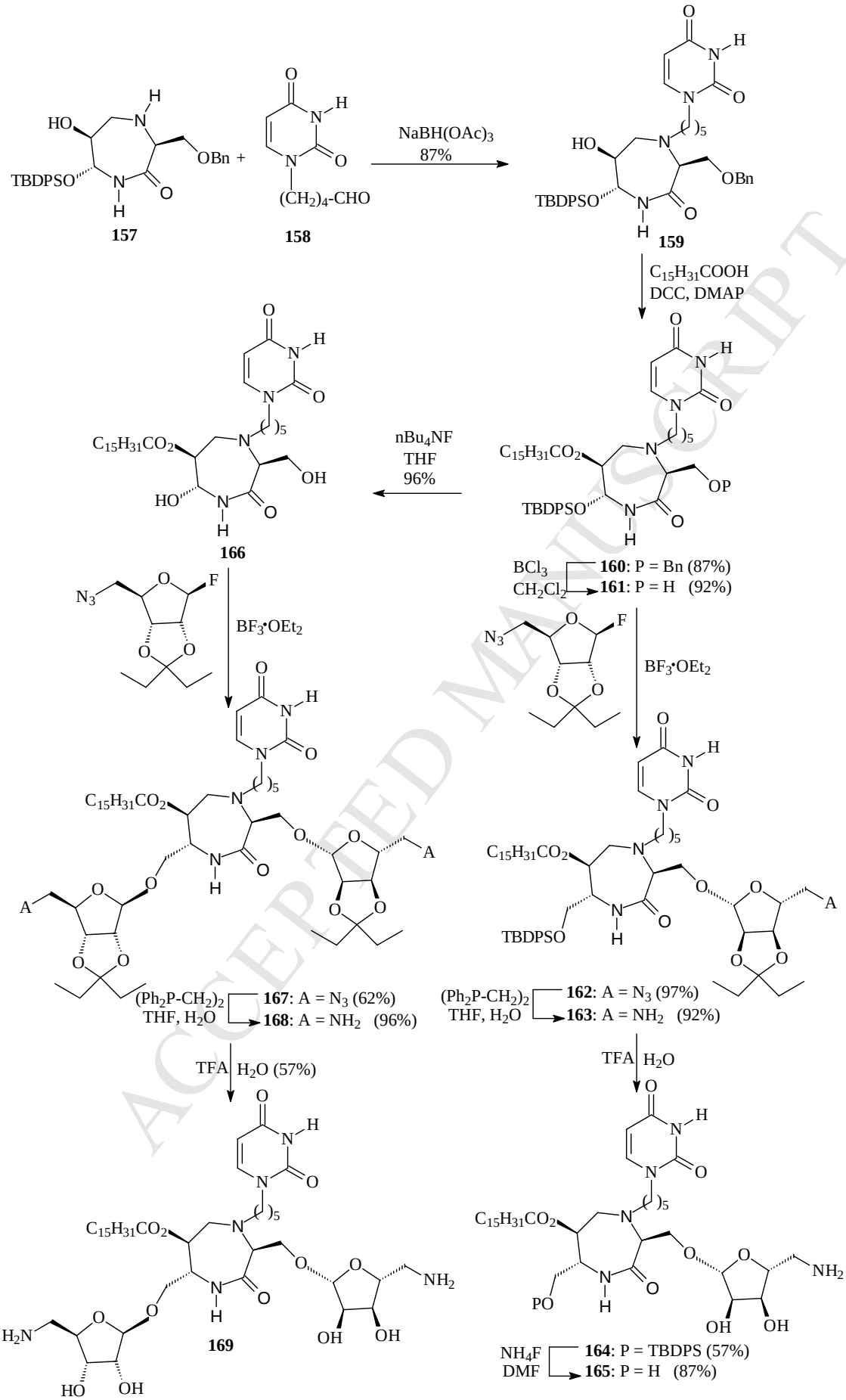


Fig. **5**. Uracil derivatives - natural inhibitors of MraY.

A scaffold strategy involved the introduction of key structural fragments required for biological activity on enantiopure diazepanones **157** by reductive amination, estrification and glycosylation (Scheme 25). Biological evaluation of these compounds on MraY enzyme revealed interesting inhibitory activity for compounds **165** or **169** displaying three fragments on the scaffold: a palmitoyl chain, an aminoribose part and an alkyluracil moiety. The inhibitors were also evaluated on MurG [42].



Scheme 25. Synthesis of inhibitors with three **165** or four **169** key fragments from diazepanone **157**.

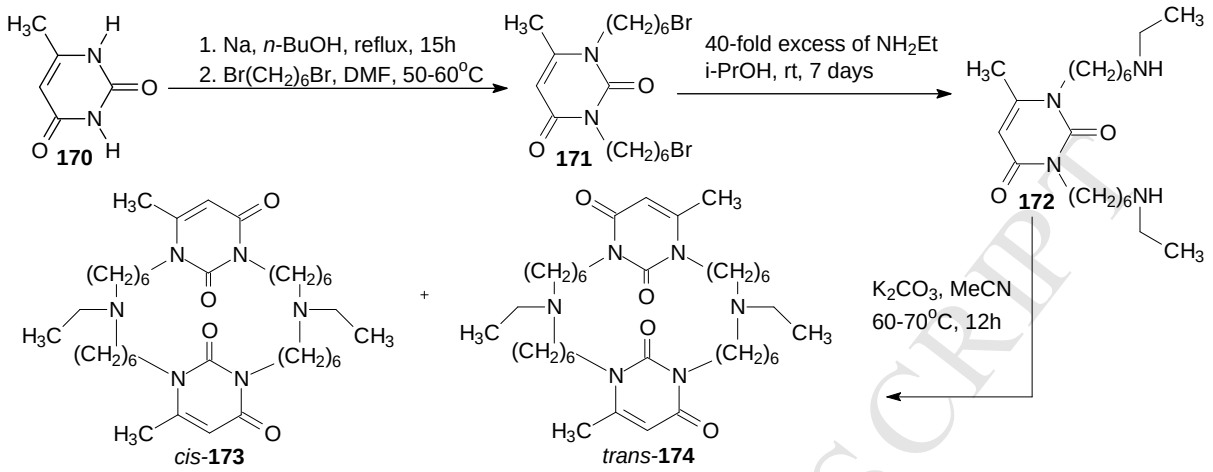
Biological evaluation of the resulting inhibitors on both MraY and MurG activities resulted in IC_{50} values in the 100 μM range for the best compounds and showed that among the introduced fragments, three of them are important for activity since their absence led to lower activities, while the presence of a second aminoribose leads to weaker inhibitors. Interestingly the biological results suggested that the presence of a hydrophobic residue on the primary alcohol function of the scaffold is preferable to that of a free alcohol for MraY inhibition. Furthermore, a free primary amine on the ribose seemed to be crucial to inhibit the MurG activity. Therefore, this study led to reaching some insight into the requirements for inhibition of the trans-membrane protein MraY [42].

2.6. Pyrimidinophanes containing one or two uracil units and bridging polymethylene chains

The synthesis of macrocyclic compounds containing pyrimidine rings, pyrimidinophanes, is rapidly developing field of pyrimidines chemistry. These macrocycles can contain different number of pyrimidine units linked to each other by hydrocarbon or polyether spacers through either the N-1 and N-3 atoms or carbon atoms of pyrimidine rings or substituents at pyrimidine rings. In most of known pyrimidinophanes uracil derivatives fragments were introduced as pyrimidine units because the chemical features of uracils allow to vary the structure of the macrocyclic compounds synthesized.

Semenov and co-workers [44] described the reactions of 1,3-bis(α,ω–bromoalkyl)-6-methyluracils **171** with 1,3-bis(α,ω–ethylaminoalkyl)-6-methyluracils **172** (Scheme 26). These reactions afforded pyrimidinophanes containing one or two uracil units and nitrogen atoms in bridging polymethylene chains. 1-Mono- or 1,3-bis(aminoalkyl)uracils were

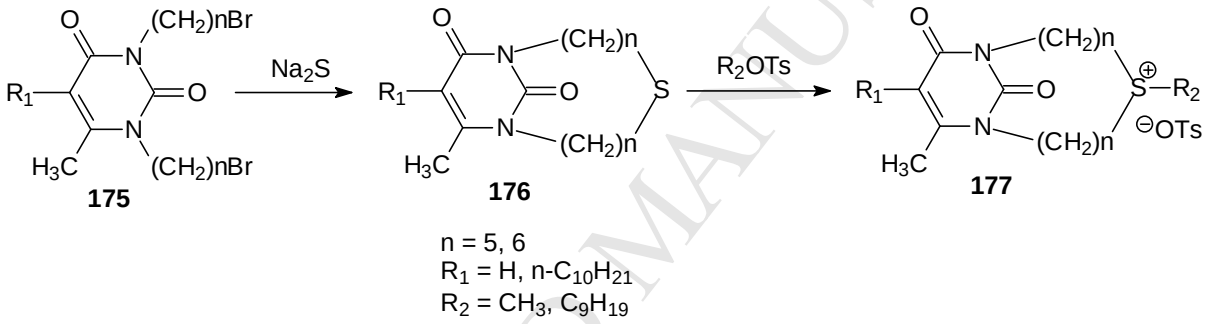
prepared by amination of 1-mono- or 1,3-bis(bromoalkyl)uracils with a considerable excess of appropriate amine.



Scheme 26. The example of synthesis of pyrimidinophanes with six methylene groups with *cis*-**173** and *trans*-**174** arragement of the carbonyl groups.

Quaternization of the bridging nitrogen atom with *o*-nitrobenzyl bromide, benzyl bromide, *n*-decyl bromide gave rise to water-soluble pyrimidinophanes. The *in vitro* antibacterial and antifungal activity of the synthesized pyrimidinophanes was investigated against several pathogenic representative Gram-negative bacteria (*Pseudomonas aeruginosa* 9027 and *Escherichia coli* F-50), Gram-positive bacteria (*Staphylococcus aureus* 209p, *Bacillus subtilis* 6633 and *Enterococcus faecalis* ATCC 8043), pathogenic fungi (*Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes var*. *gypseum* 1773 and *Aspergillus fumigatus* AF-27) and yeast (*Candida Albicans* 885-653). Antibacterial and antifungal activity of pyrimidinophanes increases with the increase of polymethylene $N_{(pyr)}$-N-chain length and dramatically increases upon the introduction of *n*-decyl substituent at nitrogen atoms in spacers. Pyrimidinophanes with 5 and 6 methylene groups in $N_{(pyr)}$-N-chain and *n*-decyl substituent showed significant bacteriostatic, fungistatic, bactericidal, fungicidal activity which comparable with standard antibacterial and antifungal drugs [44].

Nikolaev et al. [45] synthesized pyrimidinophanes containing one 5(6)-alkylsubstituted uracil moiety and a 10- or 12-methylene bridge including a sulfur atom. Pyrimidinophanes **176** with bridging S atom were synthesized using 1,3-bis(ω-bromoalkyl)uracils **175** with sodium sulfide as the cyclizing agent (Scheme 27). The bridging S atom of macrocycles was converted into a sulfonium ion by reaction of pyrimidinophanes **176** with the methyl or nonyl esters of *p*-toluenesulfonic acid. Amphiphilic pyrimidinophanes **177** with $S^{+}CH_3$ and $S^{+}C_9H_{19}$ groupings in macrocycles with 10 and 12 methylene groups were prepared (Scheme 27).

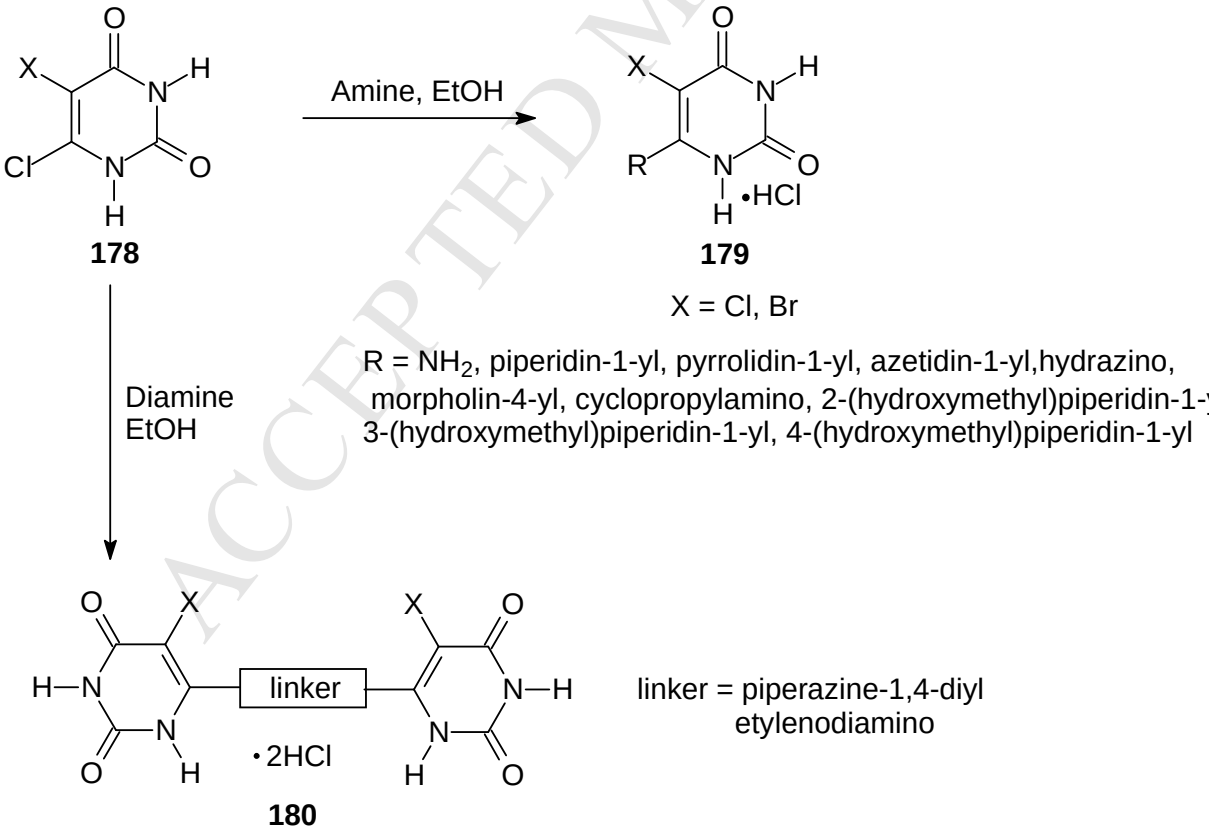


Scheme 27. Synthesis of pyrimidinophanes **177** containing one 5(6)-alkylsubstituted uracil moiety and a 10- or 12-methylene bridge including a sulfur atom.

Antimicrobal activity against *Pseudomonas aeruginosa* 9027, *Escherichia coli* F-50, *Staphylococcus aureus* 209p, *Bacillus cereus* 8035, and *Enterococcus faecalis* ATCC 8043 was measured. Amphiphilic pyrimidinophanes **177** with 5-decyl-6-methyluracil moieties had high levels of bacteriostatic activity against Gram-positive bacteria. Introduction of a lipophilic substituent into the uracil moiety increases the bacteriostatic activity of this type of pyrimidinophane. The minimum inhibitory concentration of the macrocycle containing a methyl group in the sulfonium group against *Staphylococcus aureus* was 0.75 μg/ml [45].

2.7. Other substituted uracils

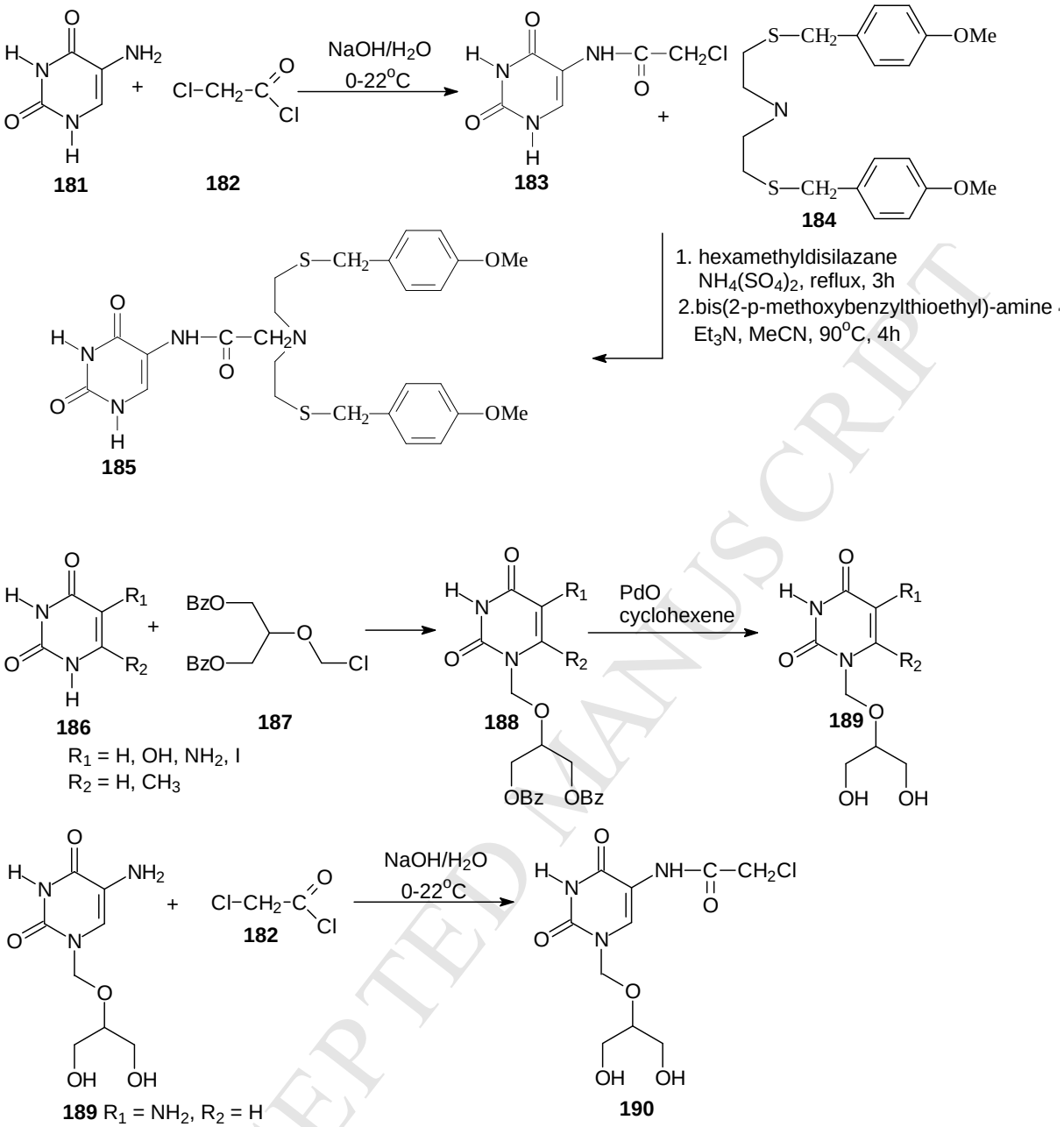
Numerous modifications of the uracil structure have been performed which resulted in the development of derivatives exhibiting better pharmacological and pharmacokinetic properties including increased bioactivity, selectivity, metabolic stability, absorption and lower toxicity. For example, 6-amino-5-chlorouracil is known as a potent inhibitor of thymidine phosphorylases (TP). The efforts to enhance the inhibitory activity of this uracil derivative by substitution of the amino group with different amines were made. 5,6-Disubstituted uracils with significant inhibitory activity against human and *Escherichia coli* TP were reported by Nenecka et al [46]. They employed a simple method for the synthesis of target uracils **179** and **180** based on a selective nucleophilic substitution of the chlorine atom at the position 6 of 5,6-dichlorouracil or 5-bromo-6-chlorouracil by various amines and diamines (Scheme 28).



Scheme 28. Synthesis of 5,6-disubstituted uracils **179** and bis-uracil derivatives **180**.

5-Bromo-bis-uracil derivatives with piperazine-1,4-diyl and ethylenediamino linkers were identified as the most potent inhibitors of human TPs and *E.coli* enzyme in this study. Authors of this study supposed that one of the nitrogen atoms in the linker interacts with the serine oxygen of the enzyme (S117), and enhancing the inhibitory activity of bis-uracil derivatives **180**. The second uracil moiety could enhance the probability of interaction with the enzyme [46].

Noll and co-workers [47] examined the antiproliferative activity on human tumor cell lines of a series of modified uracil derivatives and their corresponding acyclonucleosides. They synthesized of a series of uracil derivatives, differing in properties of C5-positioned side chains, like chemical reactivity and bulkiness. Most of these compounds were also prepared in a form of N^1 – substituted acyclic uridine analogues, to explore the impact of acyclic sugar analogues on biological activity. 5-(Chloroacetyl)aminouracil **183** was synthesized by reaction of 5-aminouracil **181** and chloroacetyl chloride **182** (Scheme 29). The acetylaminouracil **185** was prepared by direct condensation of the persilylated uracil **183** with amine **184**.

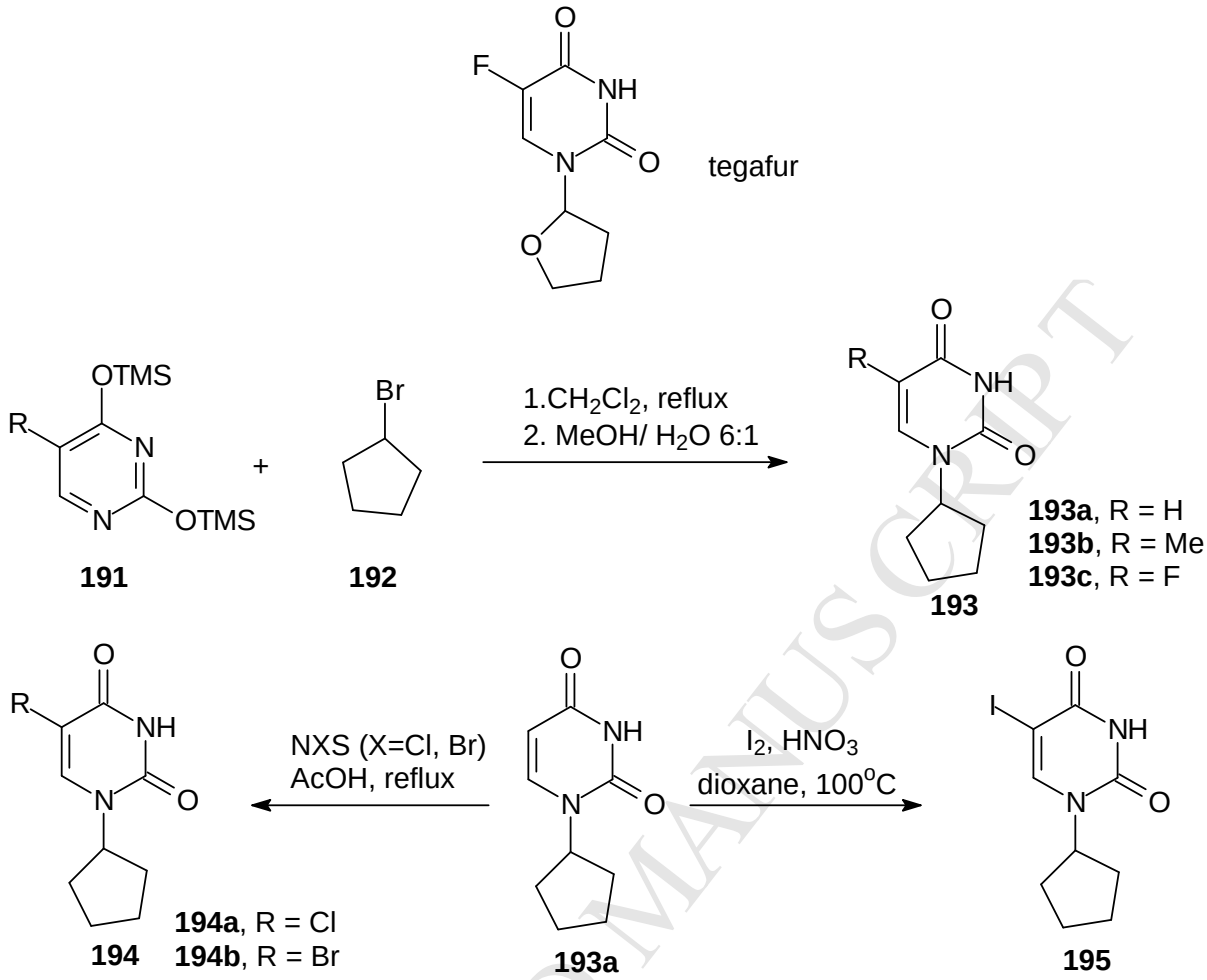


Scheme 29. Synthesis of C5-substituted uracil derivatives **183** and **185** and uridine derivatives **189** and **190**.

Synthesized compounds have been screened for their anticancer acivity. They were assayed for their *in vitro* antiproliferative activity against HeLa, MiaPaCa-2, SW620, MCF-7 and H460 cells. Modification of the N^1-position of the uracil molecule was accomplished by direct condensation of the appropriate uracils **186** with 1,3-dibenzyloxy-2-chloro-methoxypropane

187 (Scheme 29). Comparison of structure-activity relationship revealed the importance of chemical reactivity of the substituent attached to the C5-position of uracil for the biological activity of studied compounds. The results obtained for the most active compounds, 5-(chloroacetylamino)uracil **183** and its acyclic sugar analogue **190**, suggested that formation of a covalent bond between reactive substituent and several possible targets within the thymidylate synthase mechanism (sulphur of the cysteine residue, basic part of the enzyme, *N*,*N*-methylene tetrahydrofolate or its reactive iminium forms) is the most probable mode of action. C5-substituted uracil derivative **185** (5-[bis-(2-*p*-methoxybenzylthioethyl)amine]acetylaminouracil) exhibited high antiproliferative activity against HeLa and MiaPaCa-2 cell lines [47].

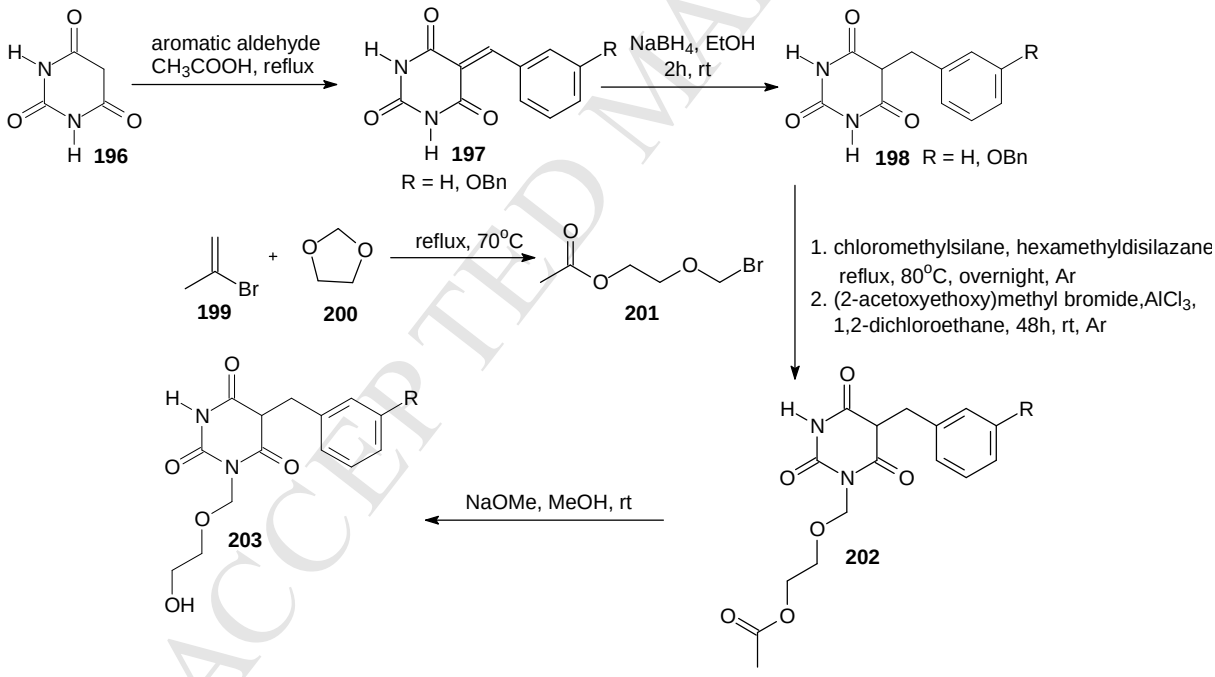
The ANN (artificial neutral networks) – QSAR (quantitative structure-activity relationship) model developed was used to predict the biological activity of some cyclopentylpyrimidine derivative analogues of tegafur (Scheme 30), a largely known anticancer drug [48]. Gonzalez-Diaz and co-workers predicted the biological activity only for simple analogues from which CH_3, F, Cl, Br, and I substituted at 5-carbon of the uracil skeleton was selected. This selection was based on the simplicity and synthetic accessibility. Compounds **193** were prepared by condensation of the trimethylsilylated base **191** (uracil, thymine, and fluorouracil) with bromocyclopentane **192**. Compouds **194** and **195** were prepared from **193a** using *N*-chlorosuccinimide or *N*-bromosuccinimide in acetic acid, or I_2 in nitric acid and dioxane (Scheme 30).



Scheme 30. Synthesis of cyclopentylpyrimidine derivative analogues of tegafur.

The predictions coincided with the biological test result where all the compounds showed detectable biological activity in the three studied cellular lines namely L 1210/0. Molt4/C8, and CEM/0. The most interesting activity on the leukemia line L1210/0 was presented by the F-tegafur analogue **193c**. However, results in the human lymphocyte lines were more discrete. Interestingly, the Br-tegafur analogue **194b** resulted more selective for the human lymphocyte lines than the leukemia line. This result confirmed the potentialities of the MARCH-INSIDE approach to model biological data and guide drug discovery in bioorganic medicinal chemistry [48].

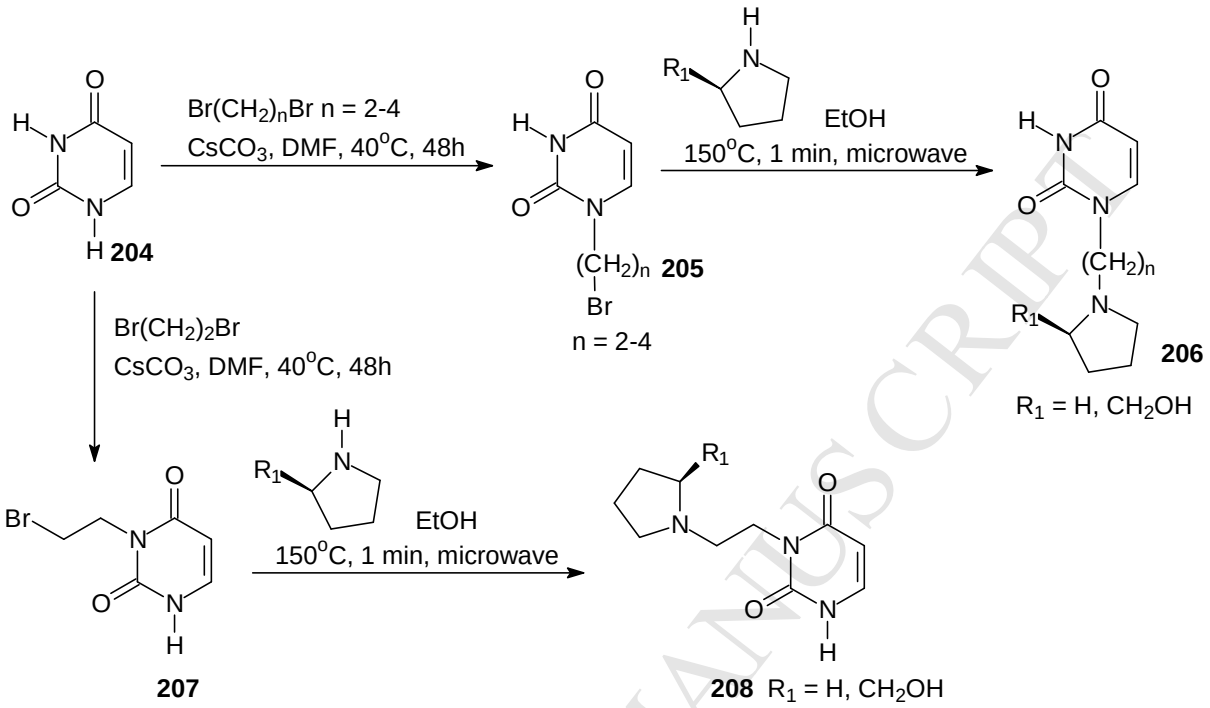
Cui and co-workers [49] produced uracil derivatives as potential *Pf*PNP inhibitors. *Plasmodium falciparum* purine nucleoside phosphorylase *Pf*PNP has a central role in purine salvage and inhibitors of the enzyme have been shown to have antiplasmodial activity. The first series of compounds was based on known human uridine phosphorylase inhibitors whilst the second series of compounds were uracil equivalents of purine-based PNP transition state inhibitors. The first series of compounds were prepared as shown in Scheme 31. Barbituric acid **196** was condensed with aromatic aldehyde to give the derivatives **197**. The compounds **197** were reduced with sodium borohydride. (2-Acetoxyethoxy)methyl bromide **201** was prepared by reaction of acetyl bromide **199** and 1,3-dioxolane **200**. Compound **198** (R = OBn) was first silylated and then stirred with the bromide **201** to give the required ester **202**. The ester **202** was deprotected to give the final product **203**.



Scheme 31. Synthesis of the first series of inhibitors of human uracil phosphorylase **198** and **203**.

The second series of inhibitors **206** and **208** was prepared as shown in Scheme 32 [49]. Uracil **204** reacted with 1,2-dibromoethane, 1,3-dibromopropane or 1,4-dibromobutane in presence

of $CsCO_2$. The dibromoalkanes could alkylate either N^1 or N^3 of the uracil **204**. Compounds **205** or **207** were then reacted with pyrrolidine to give the final compounds **206** or **208**.

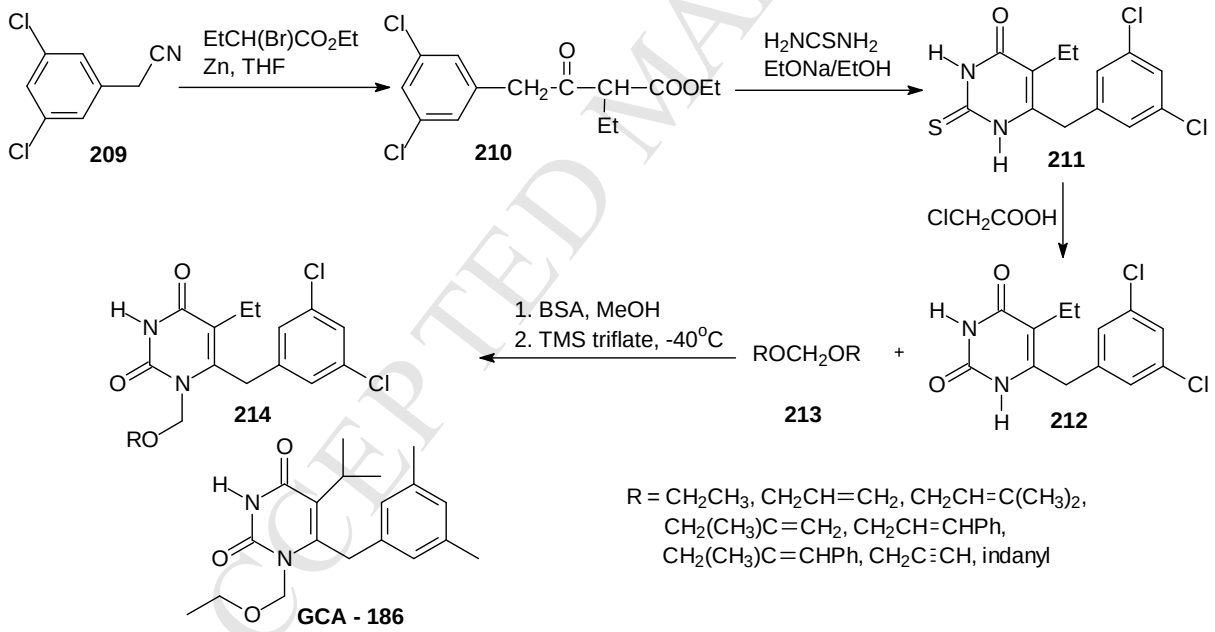


Scheme 32. Synthesis of the second series of inhibitors of human uracil phosphorylase **206** and **208**.

These two series of compounds were assayed for inhibition of both *Pf*PNP activity and growth of *P. falciparum*. The transition state analogues were found to be moderate inhibitors of *Pf*PNP (most potent compound, *K*i = 6 μM) [49].

In the recent years, the HIV-1 non-nucleoside reverse transcriptase inhibitors have been of great interest for the design of drugs against HIV-1 reverse transcriptase, the enzyme being an important factor in the development of the disease Acquired Immuno Deficiency Syndrome (AIDS). 6-(3,5-Dimethylbenzyl)-1-(ethoxy-methyl)-5-isopropyluracil(GCA-186) has been investigated as a drug candidate (Scheme 33) [50]. The two methyl groups on the 6-benzyl moiety have been shown to improve the binding stability of the drug to the NNRT1-binding site in reverse transcriptase of drug mediated mutant HIV-1 viruses. The two methyl

substituents on GCA-186 were though to undergo metabolism by the cytochrom P450 system in the liver. Pedersen and co-workers [50] described the HIV-1 inhibitors closely related to GCA-186. The methyl groups were replaced with isosteric chloro-atoms to avoid metabolism due to the two methyl groups. Reaction of 3,5-dichlorophenylacetonitrile **209** with the zinc organometallic reagent from ethyl 2-bromobutyrate gave ethyl 4-(3,5-dichlorophenyl)-2-ethyl-3-oxobutyrate **210** which was reacted with thiourea in the presence of sodium ethoxide to give the 2-thiouracil **211** (Scheme 33). Desulfuration of **211** with aqueous chloroacetic acid afforded 6-(3,5-dichlorobenzyl)-5-ethyluracil **212**. The uracil **212** was silylated with *N*,*O*-bis-(trimethylsilyl)acetamide (BSA) and then treated with bis oxysubstituted methanes **213** under the Vorbruggen conditions using trimethylsilyl trifluoromethanesulfonate (TMS-triflate) as a catalyst to give the corresponding compounds **214** (Scheme 33).



Scheme 33. Synthesis of 6-(3,5-dichlorolbenzyl)- analogues of uracil **214**.

The isosteric chloro derivatives show tenfold less activity against HIV-1 than their corresponding methyl derivatives. This showed that chlorine is not the substituents of choice

for replacing the methyl groups in GCA-186 in order to reduce its biodegradable properties [50].

Mager and co-workers [51a] investigated 5,6-substituted 1-[(2-hydroxyethoxy)methyl]uracils (Fig. 6). The dioxypyrimidine ring of these uracils is an extended "partial π system" with ring distortion mainly due to the N^1 atom. On basis of PM3-MM+ geometry optimization authors suggested a lipophilic "butterfly-like" orientation which was also found in other non-nucleoside inhibitors that interact with the HIV-1 reverse transcriptase. The synthesis of 5,6-substituted 1-[(2-hydroxyethoxy)methyl]uracils was described in 1992 [51b].

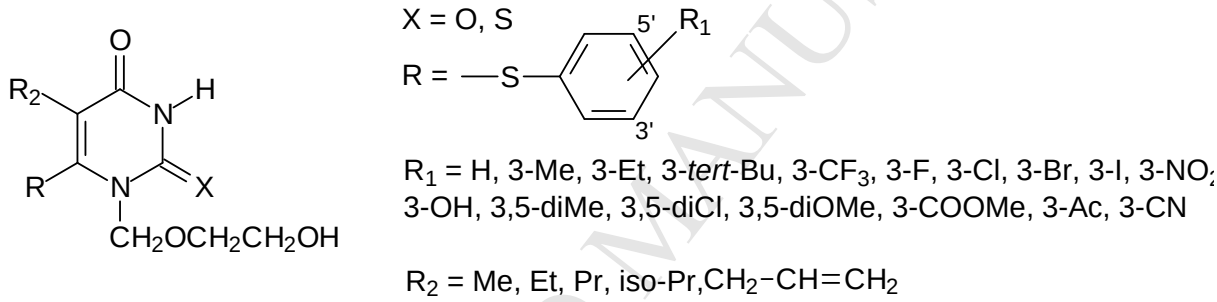


Fig. **6**. Basic structure of 5,6-substituted 1-[(2-hydroxyethoxy)methyl]uracils.

Cluster analysis of the biological activity profile and multivariate QSAR has shown that maximization of the antiviral response and minimization of the undesired cytotoxicity is possible. Related to the C-6 thiophenyl ring substituents and to modifications at the C-5 position, the antiviral response depends on hydrogen-bonding forces, steric parameters, and electronic properties. The cytotoxicity depends on the lipophilicity and steric parameters [51a].

Illan-Cabeza et al. [52a] reported the synthesis and molecular structures of (6-amino-1-methyl-5-nitrosouracilato-N^3)-triphenylphosphine-gold(I) with interesting abilities to inhibit tumor growth in an animal model of experimental glioma. The synthesis of the precursor organic ligand 6-amino-1-methyl-5-nitrosouracil (MANU) (Fig. 7) was described earlier

[52b]. The complex [Au(MANUH$_{-1}$)PPh$_3$] was obtained by mixing [AuCl(PPh$_3$) and MANU (1:4) in a methanolic alkaline medium.

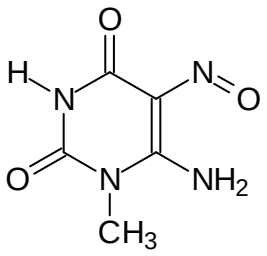
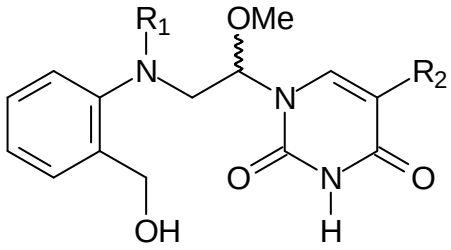


Fig. **7**. Structure of the 6-amino-1-methyl-5-nitrosouracil (MANU).

Anti-tumor properties of this compound, effects on both enzyme and non-enzyme antioxidant defense systems and the response of several biochemical biomarkers have been analyzed. After seven days of treatment, the gold compound decreased the tumor growth to *ca*. one-tenth and reduced oxidative stress biomarkers compared to animals treated with the vehicle. Also, gold compound maintained non-enzyme antioxidant defense systems as in non-tumor animals and increased enzyme antioxidant defenses, such as superoxide dismutase and glutathione peroxidase activities, and decreased catalase activity. The analysis of blood serum parameters indicated few adverse effects [52a].

Caba and co workers [53] have selected an anthranilic alcohol-derived acyclic 5-fluorouracil *O*,*N*-acetal (Fig. 8) to carry out the anti-cancer studies. Previous they reported the cytotoxic activities against the MCF-7 human breast cancer line of acyclic *O*,*N*-acetals [54]. Selected uracils showed activity as a potent growth inhibitor of the tumour cell line MCF-7 at a very low concentration.

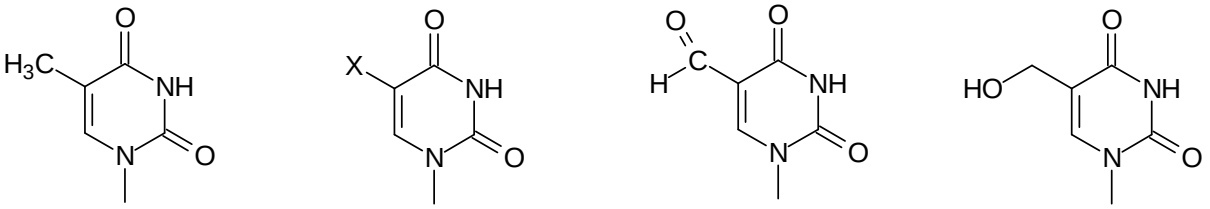


R_1 = H, PhCO, 4-NO_2-$PhSO_2$
2-NO_2-$PhSO_2$, 2-NH_2-$PhSO_2$
R_2 = H, F

Fig. 8. Acyclic *O,N*-acetals of uracil and 5-fluorouracil.

Uracil (R_1 = SO_2-C_6H_4-*o*NH_2, R_2 = F) had no activity against classic pro-apoptotic genes such as *p53*, and even induced the down-regulation of anti-apoptotic genes such as *Bd-2*. In contrast, several proapoptotic genes related with the endoplasmic reticulum (ER)-stress-induced apoptosis, such as *BBC3* and *Noxa*, appeared up-regulated. These results seemed to show that the mechanism of action and selectivity of uracils presented in Fig. 8 was via the activation of ER stress-induced apoptosis. The selective activity of this compound against tumour cells via the ER stress-induced apoptosis supposes a great advantage for future therapeutic use against breast cancer [53].

Morgan et al. [55] discussed the potential role played by human thymine DNA glycosylase (TDG) in the cytotxic effects of ClU and BrU incorporation into DNA, which can occur under inflammatory conditions and in the cytotoxicity of FU, a widely used anticancer agent (Fig. 9). They reported that TDG rapidly excises 5-halogenated uracils, exhibiting much greater activity for CpG·FU, CpG·ClU, and CpG·BrU than for CpG·T.



thymine
(5-methyl-U)

5-halo-U
X = F, Cl, Br, I

5-formyl-U

5-hydroxymethyl-U

Fig.9. Uracils removed by human thymine DNA glycosylase (hTDG).

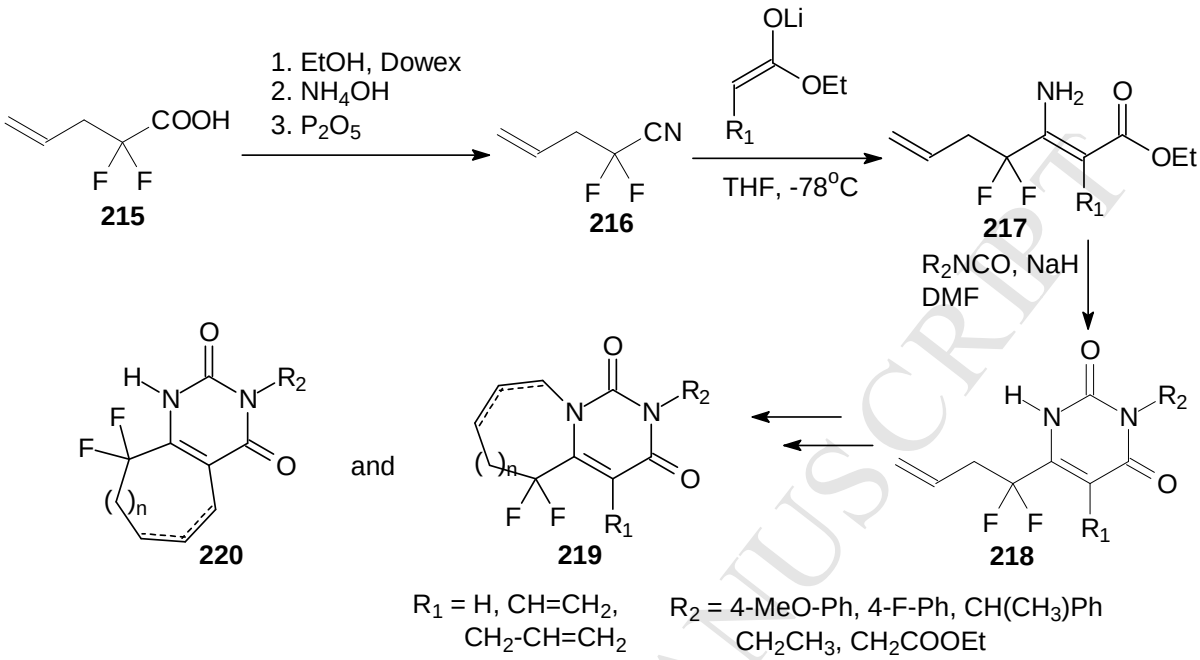
They examined the effects of altering the CpG context on the excision activity for U, T, FU, ClU, and BrU. TDG excises thymine from G·T mispairs and removes a variety of damaged bases (X) with a preference for lesions in a CpG·X context. They showed that the maximal activity (k_{max}) for G·X substrates depends significantly on the 5'base pair. For example, k_{max} decreases by 6-, 11-, and 82-fold for TpG·ClU, GpG·ClU, and ApG·ClU, respectively, as compared with CpG·ClU. For the other G·X substrates, the 5'-neighbor effects have a similar trend but vary in magnitude. The activity for G·FU, G·ClU, and G·BrU, with any 5'-flanking pair, meets and in most cases significantly exceeds the CpG·T activity [55].

2.8. Fused uracils

Fused uracils as fused heterobicyclic system, constitutes an important contribution in medicinal chemistry and a wide variety of attractive pharmacological effects was attributed to them. Fused uracils, such as pyrano[2,3-*d*]pyrimidines, pyrido[2,3-*d*]pyrimidines, thieno[2,3-d]pyrimidines, pyrazo[3,4-*d*]pyrimidines or pyrimido[4,5-*d*]pyrimidines are reported to have a wide range of biological activities such as antiallergic, antihypertensive, cardiotonic, bronchodilator, antibronchitic or anti-tumor activity [56]. Moreover, thieno[2,3-*d*]pyrimidines or pyrazo[3,4-*d*]pyrimidines are potential bioactive molecules as they bear structural analogs and isoelectronic relations to purines. In view of the above, the synthesis and biological activity of novel analogs of fused uracils is presented below.

Fustero et al. [57] described synthesis and biological evaluation of bicyclic fluorinated uracils possessing ring, which is fused at the C-5 and C-6 or N-1 and C-6 positions of the uracil moiety. Nitrile **216** was obtained from 2,2-difluoropent-4-enoic acid **215** by ethyl ester

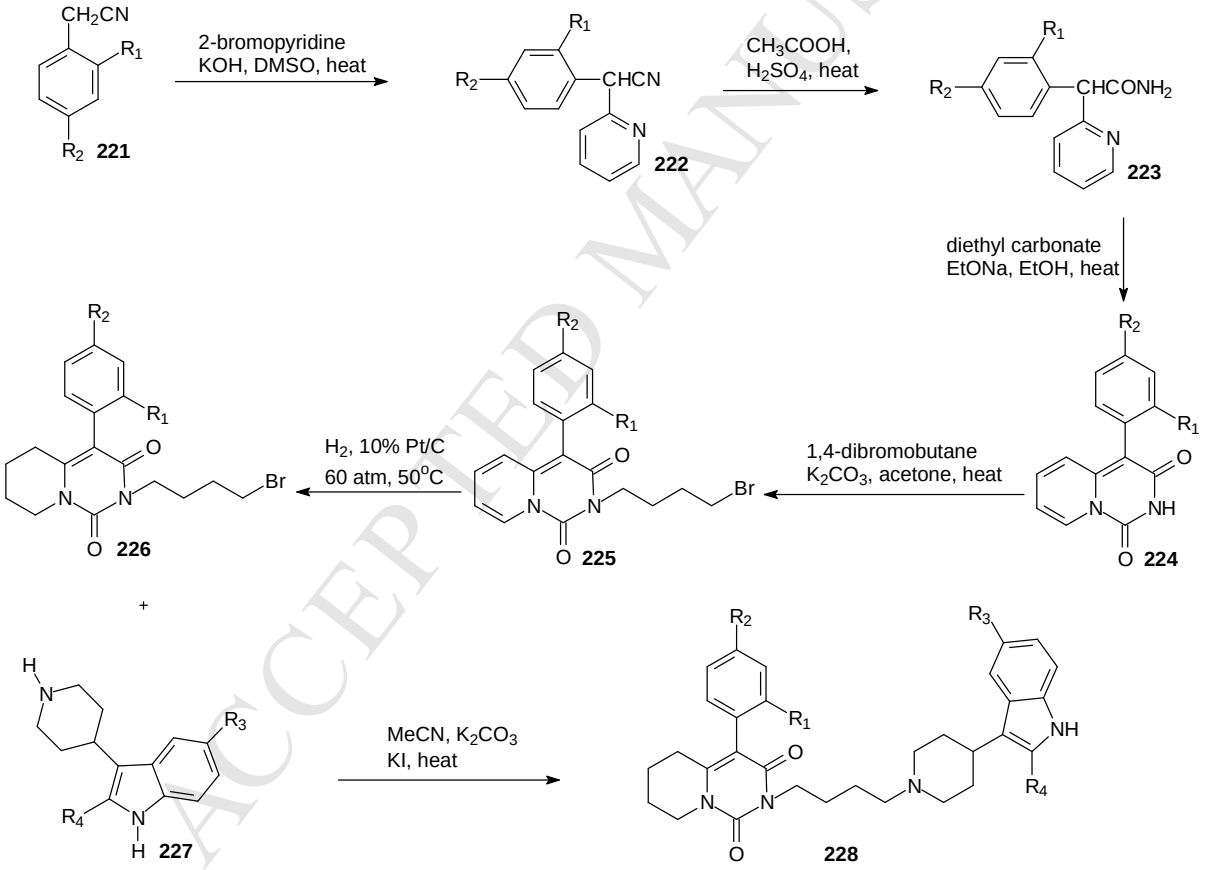
formation, transformation to the corresponding primary amide, and dehydratation (Scheme 34).



Scheme 34. Synthesis of bicyclic fluorinated uracils **219** and **220** by ring-closing metathesis (RCM) reaction.

Next, compound **216** was treated with the lithium enolates, to afford *β*-enamino esters **217**. Compounds **217** were deprotonated with NaH, and further addition of isocyanate gave uracils **218**. Uracils **218** were precursors of bicyclic uracils **219** and **220** (Scheme 34). The ring-closing metathesis (RCM) reactions of compound **218** were carried out in the presence of Grubbs' second generation catalyst and bicyclic fluorinated uracils **219** and **220** were obtained. The selective formation of olefin regioisomers in the metathesis process can be controlled according to the reaction conditions. These bicyclic uracils were tested as acaricides against *Tetranychus urticae*, a parasite of crops and common houseplants, using the commercially available miticide tebafenpyrad as reference standard. Only few compounds were active at a concentration of 4.0-5.0 μg/mL, in terms of both mortality and fecundity inhibition [57].

Herold et al. [58] synthesized derivatives of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine possessing a 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy or 2-methyl derivative. The nitriles **222** were prepared by C-arylation of the arylacetonitriles **221** with 2-bromopyridine (Scheme 35). The nitriles **222** were hydrolyzed using a mixture of sulfuric acid and acetic acid to produce amides **223**. Compounds **224** were formed by the intermolecular cyclization of **223** with diethyl carbonate. The nitrogen in the imide group of compounds **224** was alkylated with 1,4-dibromobutane to form derivatives **225**, which were hydrogenated to form compounds **226**. The target compounds **228** were obtained by the condensation of bromobutyl derivatives **226** with the piperidyl-indole **227** (Scheme 35).

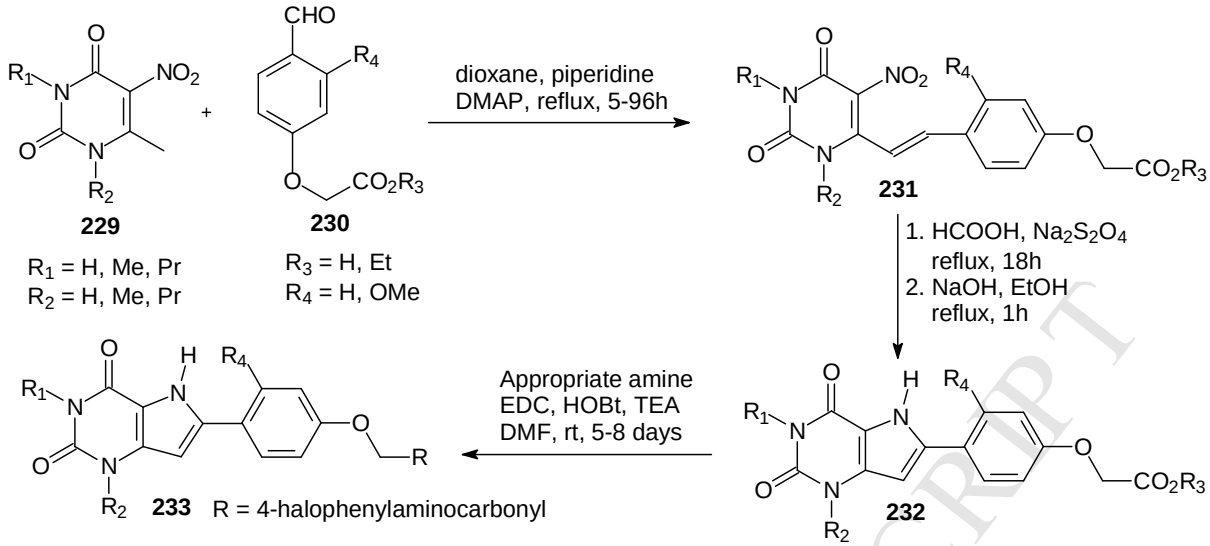


Scheme 35. Synthesis of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidines **228**.

These compounds were considered to have a double mechanism of action. In vitro receptor binding studies of synthesized ligands showed a very high to minimal binding affinity for 5-

HT_{1A} receptors, as well as a moderate to weak binding affinity for SERT [58]. The *K*i values for both 5-HT_{1A} receptor and SERT showed that compounds **228** (R_1 = R_3 = R_4 = H, R_2 = OCH_3), **228** (R_1 = R_4 = H, R_2 = R_3 = OCH_3), **228** (R_1 = F, R_2 = R_3 = R_4 = H), and **228** (R_1 = H, R_2 = F, R_3 = OCH_3, R_4 = H) had the highest affinity among new derivatives. The authors established that the presence of the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy derivative, as well as the fluorine atom at the *ortho/para* position or –OCH_3/-CH_3 group at the *para* position of the phenyl group in the aryl ring of the pyrido[1,2-c]pyrimidine system, are at an advantage with regard to binding affinity. The presence of the 2-methyl-3-(4-piperidyl)-1*H*-indole group results in a drastic affinity decrease with regard to 5-HT_{1A} receptors and a significant reduction in the SERT-inhibiting action of relevant compounds [58].

Nieto et al. [59] produced 9-deazaxanthine (9-dAX) derivatives having uracil ring fused with pyrrole ring. Along with purine nucleosides analogues, xanthines derivatives constitute one of the most widely exploited classes of adenosine receptor ligands. Selective A_{2B} receptor antagonists can play a role in important pathologies such as neurological and hypersensitive disorders, and diabetes [59]. The target was to develop of selective A_{2B} antagonists as potential anti-asthmatic agents. Some 9-deazaxanthine derivative represent a novel class of adenosine receptor ligands endowed with nanomolar affinities at *h*A_{2B} adenosine receptor. The key intermediates for the synthesis of compounds **233** were 9-dAX carboxylic acid derivatives **232**, and these were prepared by the condensation of appropriate uracil derivatives **229** with the corresponding *para*-substituted benzaldehydes **230** to afford the 6-styryluracils **231**, followed by cyclization to the 9-dAXs **232** and saponification to the free carboxylic acid (Scheme 36). Condensation of 9-dAX carboxylic acids **232** and the appropriate amines was conducted in DMF in the presence of base TEA, 1-[3-dimethylaminopropyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt).

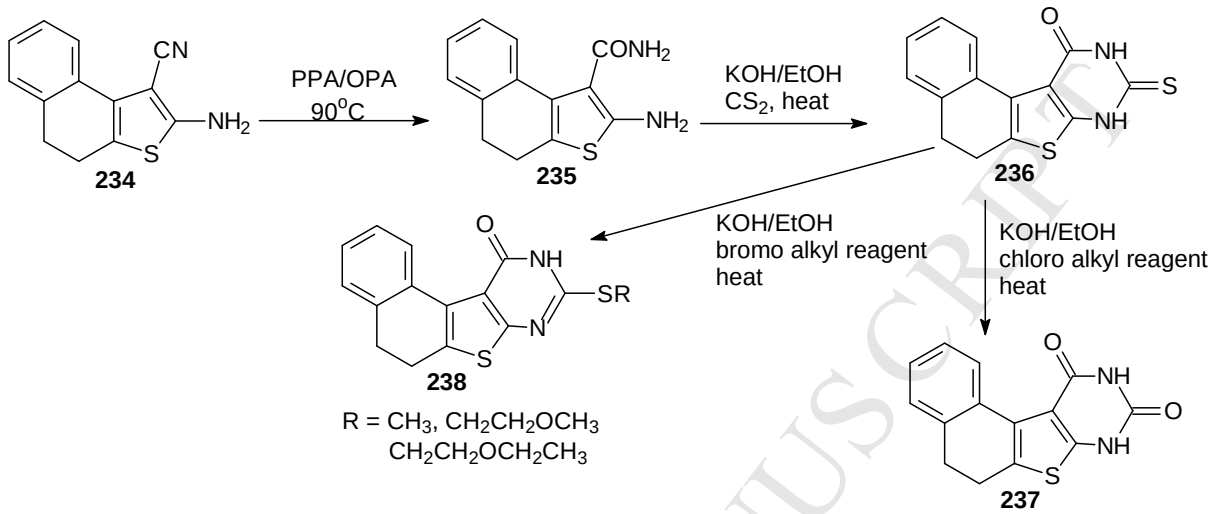


Scheme 36. Synthesis of 9-deazaxanthine derivatives (9-dAX) **233**.

These compounds were evaluated for their binding affinities for the four human recombinant adenosine receptors, A_1-A_3 subtypes. A number of the 9-deazaxanthines **233** showed moderate to high affinity for *h*A_{2B} receptors, with compound **233** (R_1 = Pr, R_2 = H, R = 4-fluorophenylaminocarbonyl, R_4 = H) showing a 32-fold selectivity for A_{2B} over A_1 and a 2750-fold selectivity for A_{2B} over A_{2A} [59].

Rashad et al. prepared uracil derivatives containing thieno[2,3-*d*]pyrimidine ring systems [60]. A large number of fused thieno[2,3-*d*]pyrimidine derivatives exhibited biological activities as potential antimicrobial, antiviral, analgesic, anti-inflammatory and anticanceractivities [60]. The synthesis of novel analogs of thieno[2,3-*d*]pyrimidine derivatives was described. Hydrolysis of the cyano group of compounds **234** was done with using of a mixture of orthophosphoric acid/polyphosphoric acid (1:1) at temperature 90^{o}C (Scheme 37). Amide **235** underwent cyclization with carbon disulfide to give the corresponding 9-thioxo derivative **236**. Alkaline treatment of compound **236** with some chloroalkyl reagents like 2-chloroethyl methyl ether, chloroacetaldehyde dimethyl acetal or 2-chloroethanol afforded product assigned to the structure of the unexpected thieno[2,3-

d]pyrimidinedione derivative **237**. When the potassium salt of compound **236** was coupled with some bromoalkyl reagents like bromoethane, 2-bromoethyl methyl ether or 2-bromoethyl ethyl ether, the corresponding S-alkyl derivatives **238**, were formed (Scheme 37).

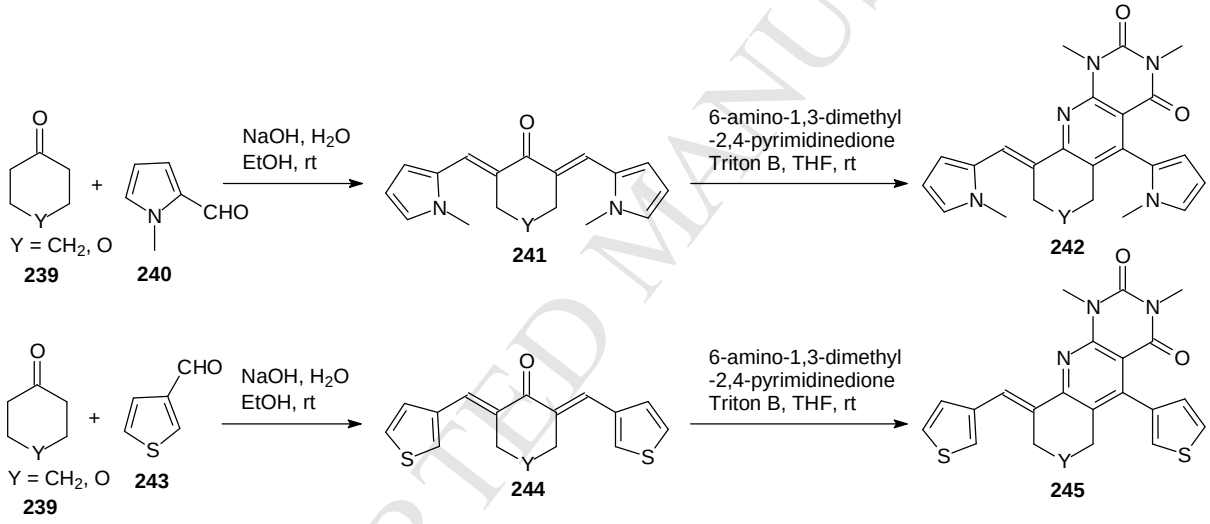


Scheme 37. Synthesis of thieno[2,3-*d*]pyrimidines **237** and **238**.

The prepared products were tested for antiviral activity against H5N1 influenza virus by determination of both EC_{50} and LD_{50} and confirmed by plaque reduction assay on MDCK cells. Compounds **235**, **236** and **237** showed the highest effect compared with the other tested compounds. High activity of thienopyrimidine derivatives **236** and **237** may be due to the structure resemblance of these compounds to thiouracil and uracil, respectively. Compound **237** has the highest anti-H5N1 activity compared with the other tested compounds with percentage of virus reduction varying from 92 at concentration of 5 μg to 98 at concentration of 40 μg [60].

Solano and co-workers [61] reported the synthesis of the fused uracils - pyrimidine monoadducts of *bis*-chalcones prepared the reaction between 6-amine-1,3-dimethyl uracil and *bis*-chalcones. Over the last years, the interest for chalcones-like compounds has been continually renewed given the diversity of their effects [62]. Their antineoplastic activity is mediated in part by induction of a programmed cell death (PCD) type 1 or apoptosis.

Chalcones and some of their biosynthetic derivatives such as flavopiridol, baicelein and quercetin, induce apoptosis in vitro [63]. Even when current anticancer drugs are used to induce apoptosis, tumor cells may evade this PCD, so it is important to develop new strategies to induce different PCDs. The first step of the synthesis consisted in the generation of α,β-unsaturated compounds **241**, these *bis* chalcones were obtained in moderate yields (40-50%) by aldolic condensation between ketones **239** and aldehydes **240** or **243**. Subsequently, the synthesis of pyrimidine mono-adducts of *bis*-chalcones **242** or **245** was performed through the Michael addition of 6-amino-1,3-dimethyl-2,4-uracil to *bis* chalcones **241** or **244** (Scheme 38).



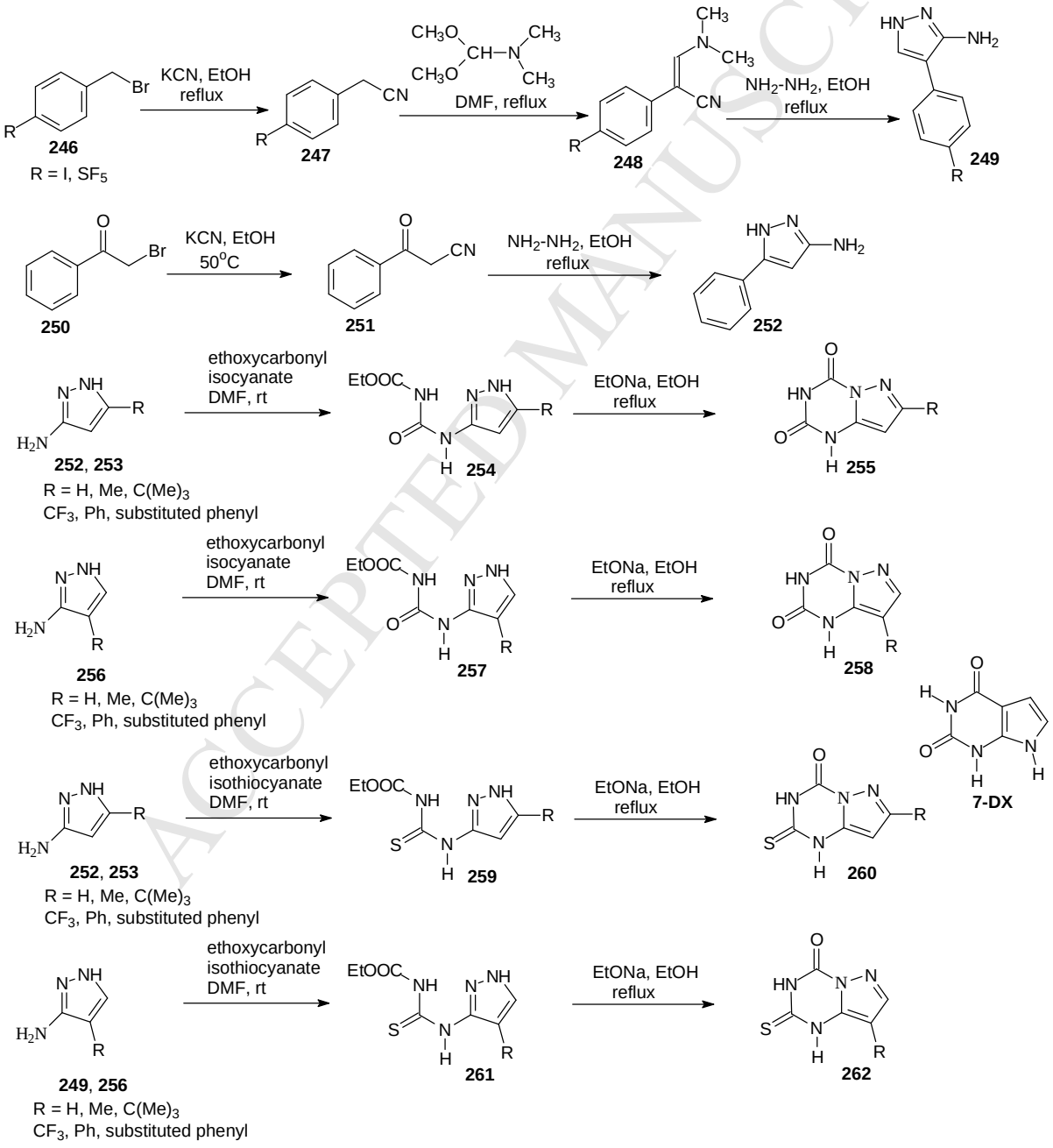
Scheme 38. Synthesis of the pyrimidine monoadducts of chalcones **242** and **245**.

The cytotoxic activity of pyrimidine monoadducts of *bis*-chalcone was assessed using the MTT (3-(4,5-dimethylthiazol-3-yl)-2,5-diphenyltetrazolium bromide) assay on; cervical cell lines HeLa, C-33 and CaLo, breast cancer MCF-7, myelogenous leukemia K-562, colorectal cancer SW480 and SW620, and on normal epithelial cell of embryonic kidney 293Q; all cell lines used had a human origin. Examined compounds displayed cytotoxicity with a massive vacuolation in different human cell lines *in vitro*. The cytotoxicity is favored by having a 1-methylpyrrol-2-yl group in the hetero-arylidene moiety. The compounds induced G1 phase

cell cycle arrest, and their cytotoxicity went without morphological and biochemical evidence of apoptotic cell death in HeLa cells. In addition, the results showed that this vacuole formation does not require de novo protein synthesis and the content vacuolar is acidic. Prepared compounds could be of pivotal importance in the study of non-apoptotic cell death with vacuole formation and could be useful in research into new autophagy inhibitors agents [61].

Sun et al. [64] constructed the fused uracils - pyrazolo[1,5-*a*][1,3,5]triazine nucleus using a reaction that annulated the 1,3,5-triazine ring onto a pyrazole scaffold. Prepared 1,3-dihydro-pyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-ones exhibited various extent of inhibitory activity against thymidine phosphorylase (TP). TP is an enzyme that promotes tumor growth and metastasis and therefore is an attractive drugable target. 7-Deazaxanthine (7DX) has been found to demonstrate inhibitory activity against TP, however its structural modifications may lead to more potent purine like TP inhibitors. The authors use 7DX as the lead compound and checked if 1,3-dihydro-pyrazolo[1,5-a][1,3,5]triazin-2,4-diones and 1,3-dihydro-pyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-ones may possess TP inhibitory activity comparable to 7DX. The pyrazolo[1,5-*a*][1,3,5]triazine scaffold has served as a good template for developing enzyme inhibitors as therapeutic agents. With appropriate structural modifications, this scaffold can be readily used to fulfill pharmacophoric requirements in the design stage of drug discovery. By reacting respectively the substituted 3-amino pyrazoles with either ethoxycarbonyl isocyanate or ethoxycarbonyl isothiocyanate: both the 2,4-dione series and the 2-thioxo-4-one series can be readily synthesized in two steps (Scheme 39). Target compounds 1,3-dihydro-pyrazolo[1,5-*a*][1,3,5]triazin-2,4-diones **255** and **258** were synthesized via a two-step synthetic scheme. The corresponding amines **252**, **253** and **256** were reacted separately with ethoxycarbonyl isocyanate at room temperature in DMF to give *N*-ethoxycarbonyl-*N'*-(pyrazol-3-yl) ureas **254** and **257**. This was followed subsequently by an intramolecular ring

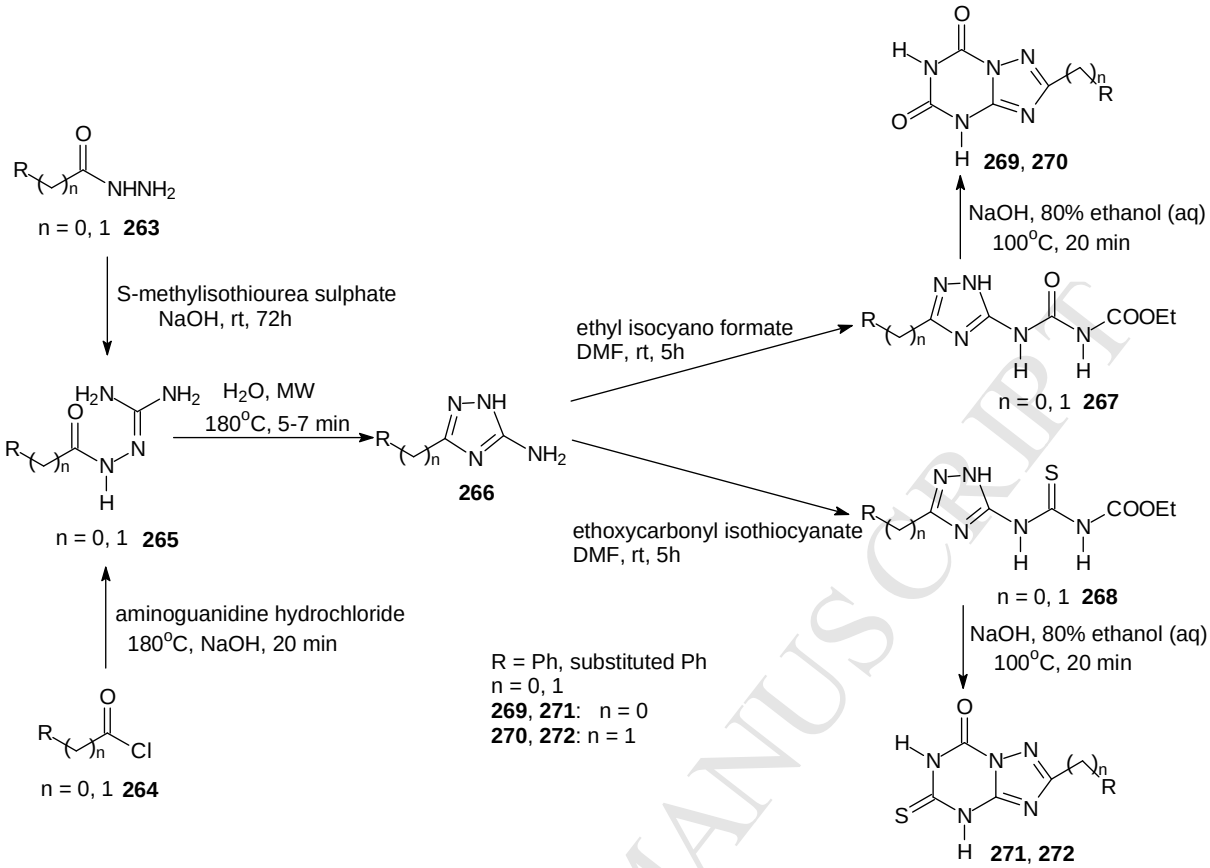
annulation reaction under the catalysis of sodium ethoxide to generate the target compounds **255** and **258**. 1,3-Dihydropyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-ones **260** and **262** were synthesized similarly by a two-step synthesis (Scheme 39). The corresponding amines **249**, **252**, **253**, and **256** were reacted separately with ethoxycarbonyl isothiocyanate at room temperature in DMF to give *N*-ethoxycarbonyl-*N'*-(pyrazol-3-yl)thioureas **259** and **261**. An intramolecular ring annulation reaction of compounds **259** and **261** was carried out under the catalysis of sodium ethoxide to produce the target compounds **260** and **262**.



Scheme 39. Synthesis of 1,3-dihydro-pyrazolo[1,5-*a*][1,3,5]triazin-2,4-diones **255**, **258** and 1,3-dihydropyrazolo[1,5-*a*][1,3,5]triazin-2-thioxo-4-ones **260** and **262**.

Prepared compounds were evaluated for inhibitory activity using a modified TP bioassay. The bioassay used recombinant *Escherichia coli* TP, expressed in *E. coli* as the enzyme and thymidine as the substrate. The bioisosteric replacement of oxygen with sulfur at position 2 of the pyrazolo[1,5-a][1,3,5]triazine scaffold was found to be essential for the TP inhibitory activity of this type of compounds. The insertion of a phenyl ring at either position 7 or 8 was enough to enhance the TP inhibition, especially when the aryl group was located at position 8. It was also found that when substituents introduced at position 4 of the attached phenyl ring, were more electron withdrawing, the IC_{50} values would decrease further. The best compound **262** (R = 4-F_5S -phenyl), which bears a para-substituted pentafluorosulfur group, showed an IC_{50} value of 0.04 μM, which was around 800 times more potent than the 7DX (IC_{50} = 32 μM) under the same bioassay conditions. In addition, **262** (R = 4-F_5S-phenyl) was found to be a non-competitive inhibitor thus suggested that it might interact with TP at a position different from the substrate binding site [64].

Bera and co-workers [65] designed and synthesized fused uracil derivatives - 1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5,7-diones **269**, **270** and its 5-thioxo analogues **271**, **272** which contained different substituents at *meta*- and/or *para*-positions of 2-phenyl or 2-benzyl ring attached to the fused ring structure (Scheme 40).

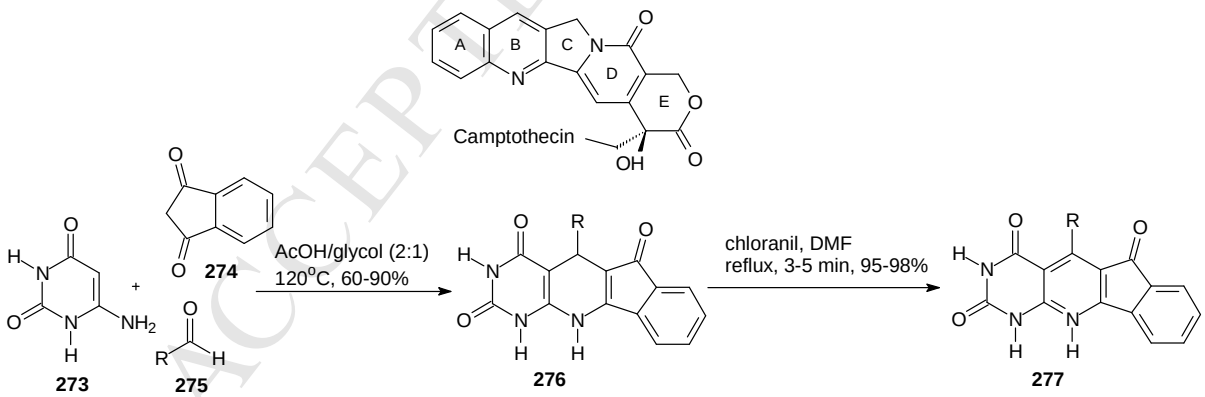


Scheme 40. Synthesis of 1,2,4-triazolo[1,3,5]triazin-5,7-diones **269**, **270** and 5-thioxo analogues **271** and **272**.

Synthetic approach was utilized to produce 5-amino-1,2,4-triazoles **266**. The two-step reaction involved the synthesis of amidoguanidines **265** from readily available substituted acid chlorides **264** or hydrazides **263**, followed by microwave-assisted cyclocondensation in water (Scheme 40). The reaction of 5-amino-1,2,4-triazoles **266** with ethyl iso-cyanoformate or ethoxycarbonyl isothiocyanate in DMF afforded the urea **267** and thiourea **268** derivatives which underwent intramolecular heterocyclization in the presence of base, resulting in the formation of target compounds **269**, **270**, **271** and **272**. The pharmacological evaluation demonstrated that 5-thioxo analogues exhibited a varying degree of inhibitory activity towards thymidine phosphorylase, comparable or better than reference compound, 7-deazaxanthine (7-DX) (IC_{50} value = 42.63 μM). Compounds **271** (R = 3,4-$Cl_2C_6H_3$) and **272**

(R = 3,4-$Cl_2C_6H_3$), those that bear di-chloro group on the phenyl ring, displayed a mixed-type of inhibitory mechanism in the presence of variable concentrations of thymidine (dThd). Several compounds were found to have a noticeable inhibitory effect on the expression of angiogenesis markers, including VEGF and MMP-9 in MDA-MB-231 breast cancer cells. These compounds constitute a new direction for design and synthesis of novel TP inhibitors with promising anti-angiogenic property [65].

Evdokimov et al. [66] prepared the camptothecin-inspired fused uracils - 1*H*-indeno[2',1':5,6]dihydropyrido[2,3-*d*]pyrimidine analogues **276** as well as their oxidized planar counterparts **277** utilizing a practical multicomponent synthetic protocol (Scheme 41). Natural-product-mimetic scaffolds were synthesized by one-step multicomponent synthesis. 1*H*-Indeno[2',1':5,6]dihydropyrido[2,3-*d*]pyrimidines **276** were accessible by refluxing 6-aminouracil **273**, indane-1,3-dione **274** and selected aldehydes **275** in a mixture of acetic acid and ethylene glycol. Dihydropyridines **276** were further chemically oxidized. Chloroanil was found to be excellent oxidizing agent, and indeneopyridines **277** precipitated from refluxing DMF solutions within a few minutes.

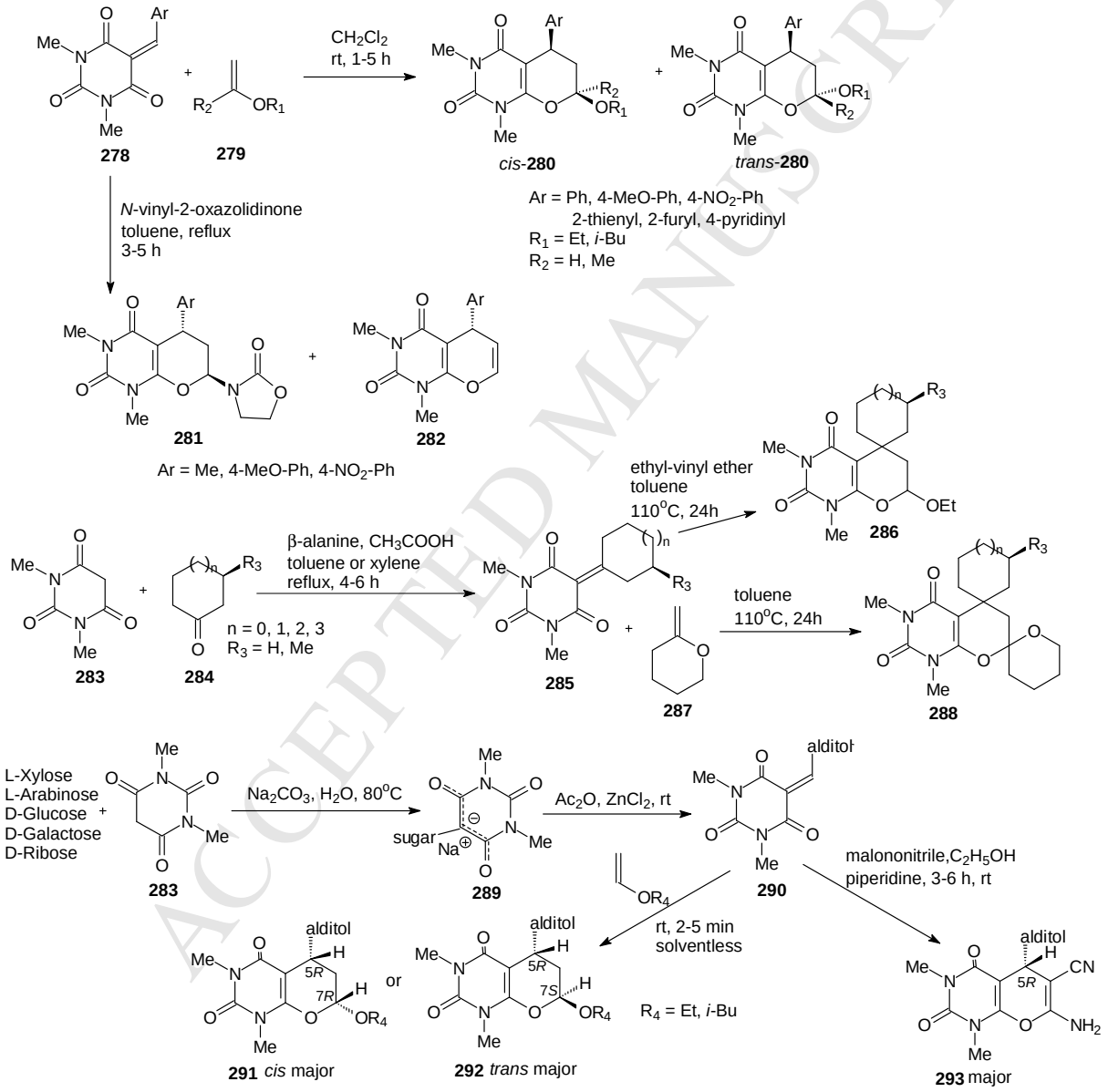


Scheme 41. Preparation of fused uracils – dihydro- **276** and indenoyrido[2,3-*d*]pyrimidines **277** by MCR protocol.

Camptothecin is natural product but due to limited water solubility and high levels of toxicity, the clinical trials aimed at evaluation of camptothecin as an anticancer drug were suspended. The synthesized compounds exhibited submicromolar antiproliferative potencies toward a panel of human cancer cell lines: HeLa human cervical cancer, Jurkat human T-cell leukemia, MCF-7 human mammary carcinoma, A-549 human NSCLC, Lovo, human colon cancer, U373 human glioblastoma, SKMEL-28 melanoma, PC-3 human prostate cancer. Biochemical experiments were consistent with the dihydropyridine library members undergoing intracellular oxidation to the corresponding planar pyridines, which then inhibit topoisomerase II activity, leading to inhibition of proliferation and cell death. The compounds **276** and **277** represent a convenient starting point for anticancer drug discovery [66].

According to our interest of uracils we also occupied in the synthesis of fused uracils of potential pharmacological activity. Pałasz et al. described convenient and efficient procedures for the preparation of fused uracils - pyrano[2,3-*d*]pyrimidine derivatives [67-71]. Pyran derivatives are common structural subunits in a variety of important natural products, including carbohydrates, alkaloids, polyether antibiotics, pheromones, and iridoids. The preparation of the compounds containing a pyran ring fused to an uracil ring poses a significant synthetic challenge. Fused uracils such as pyrano[2,3-*d*]pyrimidines can be efficiently synthesized by hetero-Diels-Alder reactions of 5-arylidene derivatives of barbituric acids with the vinyl ethers. We demonstrated that 5-arylidene-*N*,*N*-dimethylbarbituric acids **278** underwent smooth hetero-Diels-Alder reactions with enol ethers **279** to afford *cis* and *trans* diastereoisomers of 7-alkoxy-5-aryl-2*H*-pyrano[2,3-*d*]pyrimidine-2,4-diones **280** in excellent yields (84-95%) (Scheme 42) [67]. Cycloadducts with *cis*-configuration were the major products. It was also examined that, three-component one-pot reactions of *N*,*N*-dimethylbarbituric acid, aromatic and heteroaromatic aldehydes, and enol ethers in the presence of piperidine gave uracils in very good yields (87-95%) [67]. Moreover, solvent-free

hetero-Diels-Alder reactions of 5-arylidene derivatives of barbituric acids **278** with ethyl vinyl ether were investigated at room temperature and pyrano[2,3-*d*]pyrimidines **280** were obtained in excellent yields (Scheme 42) [68]. Three-component one-pot syntheses of fused uracils **280** were also performed in aqueous suspensions [68]. "On-water"cycloadditions were characterized by high diastereoselectivity in contrast to reactions carried out in homogeneous organic media.



Scheme 42. Different fused uracils - pyrano[2,3-*d*]pyrimidines of potential pharmacological activity, prepared by hetero-Diels-Alder cycloadditions.

.

Next, we introduced oxazolidin-2-one ring as substituent at 7 position of pyrano[2,3-*d*]pyrimidines by hetero-Diels-Alder cycloadditions [69]. We demonstrated that reactions of 5-arylidene-1,3-dimethylbarbituric acids **278** with *N*-vinyl-oxazolidin-2-one afforded mixtures of 2*H*-pyrano[2,3-*d*]pyrimidine-2,4-diones *trans* **281** and uracils **282** resulted from an elimination of oxazolidin-2-one (Scheme 42). Designing of structural modifications of pyrano[2,3-*d*]pyrimidine scaffold we synthesized spirouracils and dispirouracils [70]. Inverse-electron demand hetero-Diels-Alder cycloadditions of sterically hindered cycloalkylidene derivatives of *N*,*N*-dimethylbarbituric acid **285** with enol ethers or cyclic enol ether **287** were investigated (Scheme 42). Cycloalkylidene derivatives of *N*,*N*-dimethylbarbituric acid were synthesized by Knoevenagel condensation of *N*,*N*-dimethylbarbituric acid **283** with appropriate cycloalkanone **284** by refluxing in toluene or xylene in the presence of β-alanine and acetic acid as catalyst. Cycloaddition reactions were performed in toluene solution at 110^{o}C for 24h. Spirouracils **286** and dispirouracils **288** were obtained in good 78-93% yields. In our recent work we developed a convenient and efficient procedure for the preparation of fused uracils containing a sugar moiety [71]. The reaction sequence was: Knoevenagel condensation of unprotected sugars and *N*,*N*-dimethylbarbituric acid **283**, acetylation of *C*-glycosides and hetero-Diels-Alder reaction. *O*-Acetylated 1,3-dimethyl-2,4,6-trioxo-pyrimidin-5-ylidene derivatives **290** were used as new heterodienes in the synthesis of fused uracils - pyrano[2,3-*d*]pyrimidines **291** and **292** with a sugar moiety (Scheme 42). Solvent-free hetero-Diels-Alder cycloadditions of *O*-acetylated pyrimidin-5-ylidene alditols **290** with enol ethers and cyclic enol ether were investigated at room temperature. New, enantiomerically pure *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **291** and **292** with alditol moiety were obtained. The same pyrimidin-5-ylidene alditols **290** underwent conjugate Michael addition-cyclizations with malononitrile at room temperature to afford

optically active uracils **293** - diastereoisomers of pyrano[2,3-*d*]pyrimidine-6-carbonitriles with a sugar moiety (Scheme 42) [71]. All fused uracils presented on Scheme 42 have not been evaluated for their pharmacological activity yet.

3. Conclusion

Drug development has been a principal driving force in the field of medicinal chemistry. A significant attention of scientists has been paid to the development of synthesis of heterocyclic compounds containing nitrogen atoms. The uracil derivatives are one of the most researched areas in medicinal chemistry. The new uracil conjugates exhibit promising activities like anticancer, antiviral, antibacterial, antiparasitic or can act as hypoglycaemic agents. In this review, we have introduced readers to a broad range of highly functionalized uracil derivatives. This article is focused on the showing of reaction conditions, substrate scope and development of new reaction types. The structure-activity relationship of the reported uracil derivatives revealed that the choice of a suitable substitution pattern including the presence of fluorine atom or chlorine atom or some heterocyclic moieties, on the basic skeleton plays a key role in regulating the biological potential of the synthesized uracils. We sincerely hope that this article will stimulate further research in uracil derivatives synthesis and will encourage scientists to design novel approaches. The authors apologize to the researches which, for one reason or another, have not been mentioned in this review.

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