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Pyrimidine nucleoside analogues and antitumour drugs based on them: fifty years in therapy

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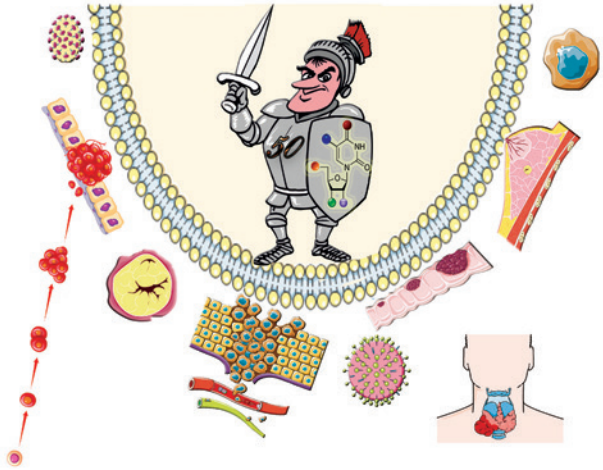
Most of the modern chemotherapeutic arsenal for the treatment of various types of cancer is based on pyrimidine nucleoside analogues. These include drugs that have been proven for decades (cytarabine, floxuridine, gemcitabine, capecitabine, azacitidine, and decitabine) as well as combinations of new antitumour agents (trifluorothymidine and tipiracil hydrochloride, decitabine and cedazuridine). New pyrimidine nucleoside analogues (doxyfluridine, tezacitabine, tiarabin, troxacitabine, *etc*.) and their depot forms (sapacitabine, MB-07133, *etc*.) are currently undergoing clinical trials for monotherapy or combination therapy for a wide range of oncological diseases. Over the past 15 years, publications have appeared describing various approaches to optimize the synthesis methods and structures of existing cancer drugs, as well as the design and synthesis of new pyrimidine nucleoside analogues with antitumour activity. This review summarizes new information and classical methods for synthesizing pyrimidine nucleoside analogues, as well as data on their antitumour activity, targets, and mechanisms of action. This review will be useful to a wide range of readers, including undergraduate and graduate students in chemistry and biology, and also specialists in chemistry, biology and medicine.



The bibliography includes 216 references.

Keywords: nucleoside analogues, anticancer activity, cytotoxicity, chemical synthesis, mechanism of action.

Contents

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1. Introduction

Natural nucleosides, in addition to their key role in the storage and transmission of genetic information, are involved in numerous cellular processes as substrates, regulators or cofactors. Nucleoside analogues with modified heterocyclic base and/or carbohydrate moiety, which have been acting as tools for affecting various pathogenic processes in the human body for more than half a century, still form the basis of antitumour and antiviral chemotherapy. Currently, 15 nucleoside analogues[1,2] are approved by the U.S. Food and Drug Administration (FDA) and are used for the treatment of cancer. The majority of the current chemotherapeutic arsenal against various types of cancer consists of drugs based on pyrimidine nucleoside analogues[3] (Fig. 1).

Although the first nucleoside drugs have been used in therapy for over fifty years, active research continues in the field of design and chemical synthesis, as well as an assessment of the anticancer potential of pyrimidine nucleoside analogues.[2,4,5] Novel pyrimidine nucleoside analogues (Fig. 2) and their depot forms[†] (Fig. 3) are currently undergoing clinical trials for use in monotherapy or combination therapy (Fig. 4) of a wide range of oncological diseases.

Nucleoside analogues are prodrugs,[‡] and require cellular metabolism to become active. After transport across the plasma membrane, nucleoside analogues undergo first phosphorylation, while heterocyclic base analogues undergo ribosylation followed by phosphorylation to afford nucleoside 5′-monophosphate analogues. 2′-Deoxynucleosides are phosphorylated by 2′-deoxycytidine kinase, thymidine kinases 1 and 2, and 2′-deoxyguanosine kinase. The main rate-limiting enzyme for activation of most approved nucleoside analogues is 2′-deoxycytidine kinase (dCK).[1] Although 2′-deoxycytidine is the preferred natural substrate for this enzyme, dCK also recognizes 2′-deoxyadenosine and 2′-deoxyguanosine as substrates. Cancer cells typically express 2′-deoxycytidine kinase at the protein level at 3–5 times higher levels than most normal cells, providing some selectivity for the action of nucleoside analogues. Thymidine kinases and 2′-deoxyguanosine kinase are base-selective, and certain modifications of the carbohydrate moiety can also interfere with the recognition of

[†] A depot form is a derivative of the active substance that ensures its slow release in the body over a long period of time.

[‡] A prodrug is a molecule that possesses no intrinsic biological activity but can be converted into a biologically active drug through various metabolic steps.

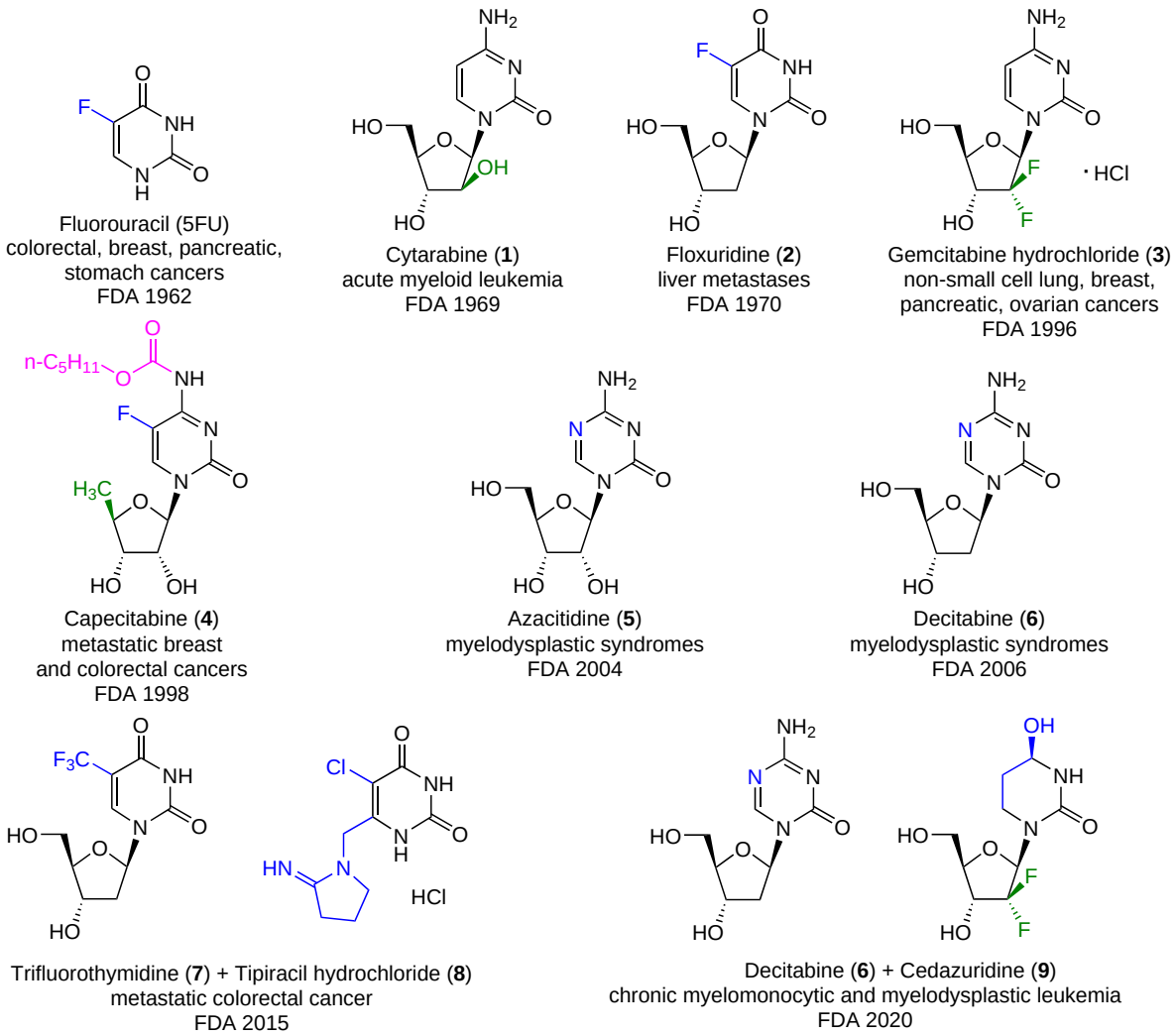


Figure 1. Pyrimidine nucleoside analogues, fluorouracil and compounds **1**–**9**, are used in cancer therapy (listed under the name and the year of approval). Pyrimidine base modifications are highlighted in blue, carbohydrate moiety modifications are highlighted in green, and fragments of the depot forms are shown in pink.

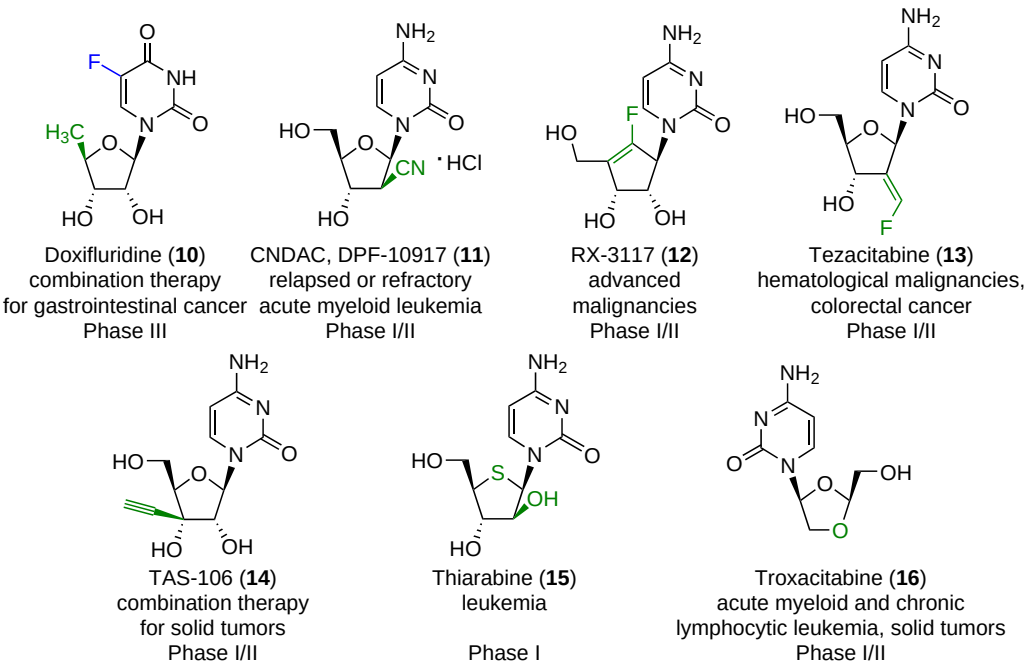


Figure 2. Pyrimidine nucleoside analogues **10**–**16**, which have undergone clinical trials at various stages as potential cancer treatments (listed under the name). Modifications of the pyrimidine base are highlighted in blue, and those of the carbohydrate moiety are highlighted in green.

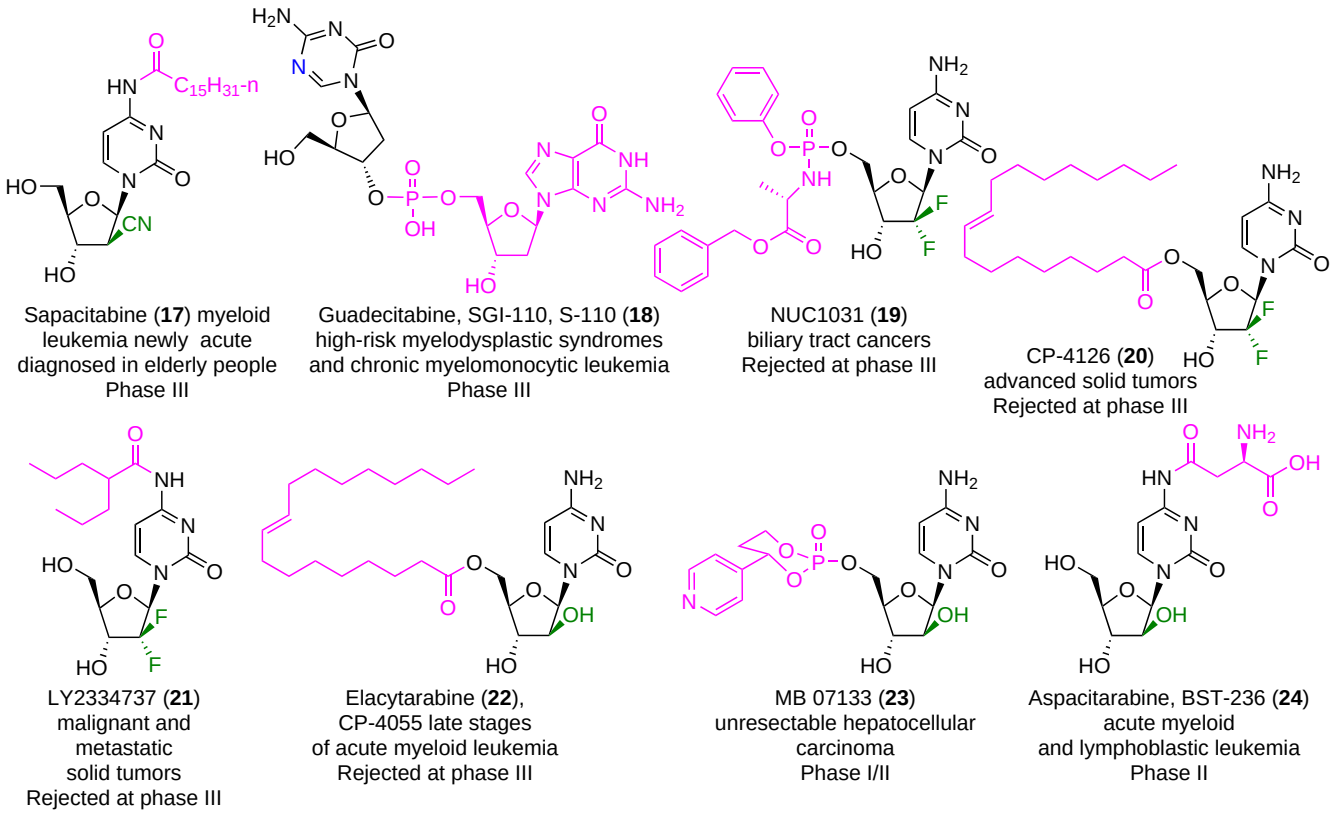
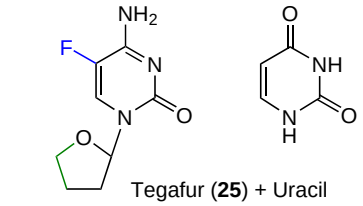
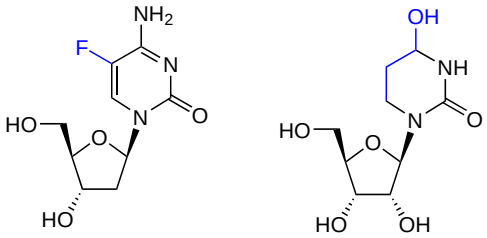


Figure 3. Depot forms and prodrugs of pyrimidine nucleoside analogues **17**–**24**, which have undergone clinical trials at various stages as potential cancer treatments (listed under the title). Pyrimidine base modifications are highlighted in blue, carbohydrate moiety modifications are highlighted in green, and fragments of the depot forms and prodrugs are shown in pink.

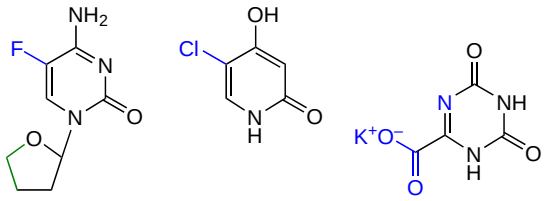
nucleosides by these kinases. Therefore, many modified nucleosides do not undergo first phosphorylation in cells. The first phosphorylation gives nucleoside 5′-monophosphates, which are converted into the corresponding nucleoside 5′-diphosphates and nucleoside 5′-triphosphates by various cellular kinases. To phosphorylate nucleoside 5′-monophosphates, the cell uses nucleoside monophosphate kinases or nucleotide kinases, which weakly distinguish between the ribose and 2′-deoxyribose fragments of the nucleotide but are specific to the nature of the base. The conversion of nucleoside



Tegafur (**25**) + Uracil
advanced gastrointestinal cancers,
aproved in Japane and Taiwan
gastric and colon cancer, combination therapy
for hepatocellular carcinoma
Phase II/III



5-Fluoro-2'-deoxycytidine (**26**) + Tetrahydrouridine (**27**)
neoplasms
Phase I/II



Tegafur (**25**) + Gimeracil + Oteracil
combination therapy with cisplatin for advanced gastric cancer
Phase III

Figure 4. Combinations containing pyrimidine nucleoside analogues **25**–**27**, currently undergoing clinical trials at various stages as potential cancer treatments (listed under the title). Pyrimidine base modifications are highlighted in blue, and carbohydrate moiety modifications are highlighted in green.

5′-diphosphates to nucleoside 5′-triphosphates is carried out by nucleoside diphosphate kinases, which are present in all cells and do not exhibit particular specificity to the types of nucleoside bases and are capable of recognizing 5′-diphosphates of ribonucleosides and 2′-deoxyribonucleosides as substrates.[6]

Nucleoside 5′-triphosphate analogues are substrates for DNA polymerases; the corresponding nucleotides are incorporated into DNA during replication or DNA synthesis during repair, leading to stalling of replication forks and chain termination.[1] These events activate various DNA damage sensors that stimulate DNA repair, arrest cell growth, and often lead to apoptosis. Most cancer cells replicate their genome more frequently than normal adult cells, which are dormant and do not actively synthesize their DNA, providing a degree of selectivity for inhibiting cancer cell growth. Some nucleoside 5′-triphosphate analogues can act as substrates for RNA polymerases; the incorporation of the corresponding nucleotides into the growing RNA chain leads to transcription termination and instability of messenger RNA (mRNA) and ribosomal RNA (rRNA).

Nucleoside and nucleotide analogues (mono-, di-, or triphosphates) can also inhibit other key cellular enzymes, providing an alternative mechanism for cell growth suppression. Such enzymes include, for example, ribonucleotide reductase (RR), which removes the 2′-OH group from the carbohydrate moiety of ribonucleoside diphosphate to form 2′-deoxyribonucleoside diphosphate *de novo*; thymidylate synthase, which catalyzes the conversion of 2′-deoxyuridine-5′-monophosphate to thymidine-5′-monophosphate using 5,10-methylenetetrahydrofolate as a source of the methyl group; uridine-cytidine kinase 2, which converts uridine and cytidine to the corresponding 5′-monophosphates; and DNA methyltransferases, which catalyze the methylation of the 5-position of cytosine bases in newly synthesized DNA strands.[7] Some nucleoside analogues exhibit anticancer activity by inhibiting cellular enzymes not involved in nucleic acid synthesis.

Thus, pyrimidine and pyrimidine nucleoside analogues, mimicking natural compounds, act as substrates and/or inhibitors for numerous enzymes involved in cellular metabolism and catabolism. Unlike normal cells, the levels of many enzymes are elevated in actively dividing cancer cells. This ensures the selectivity of drug action and makes such proteins attractive targets for cancer chemotherapy. The targets and mechanisms of action of specific pyrimidine nucleoside analogues are described in the relevant sections of this review.

Over the years of research into pyrimidine nucleoside analogues, numerous compounds have been obtained and a large number of publications have emerged that analyze a diversity of aspects of this field, from the design and chemical synthesis of new molecules to clinical observations of the use of drugs in the treatment of oncological diseases. Reviews have been published on the mechanisms of action of pyrimidine nucleoside analogues and the pathways for the development of resistance to them,[2,8] as well as on the enzymatic targets to which inhibitors are directed: ribonucleoside reductase,[9,10] uridine-cytidine kinase 2 (UCK2),[11] thymidine phosphorylase,[12] DNA methyltransferase,[7] *etc*.

Over the past 15 years, a large number of publications have appeared devoted to various approaches to optimizing the structures of known drugs intended for the treatment of oncological diseases. The design and synthesis of new pyrimidine nucleoside analogues with antitumour activity have also been described. A number of reviews provide a fairly in-depth and detailed discussion of various aspects of the chemistry and/or research on the antitumour properties of individual groups of pyridine nucleoside analogues.[5,13–17] Reviews have been published that describe in detail individual targets of action[7,9–12,18] and/or mechanisms of antitumour activity and the development of drug resistance of compounds[2,8] approved for use in practice or undergoing clinical trials.[1,19–23] Works have also been presented that summarize data on optimizing the properties of existing drugs, in particular on the creation of targeted delivery methods and depot forms.[24–26] However, in our opinion, existing reviews do not provide a comprehensive understanding of the diversity of pyrimidine nucleoside analogues with antitumour activity, their role and potential in the treatment of cancer.

This review harmoniously combines all of these aspects in a brief summary. It summarizes information regarding classical

and new methods for synthesizing pyrimidine nucleoside analogues, their antitumour activity against various cell lines, including both solid tumours and hematopoietic malignancies, as well as the targets and mechanisms of action of these compounds. Therefore, this review will be useful to a wide audience: students of chemistry and biology, researchers specializing in nucleosides, biologists, and oncologists. We hope that the information presented will serve as a basis for further research aimed at creating new therapeutic strategies in the fight against cancer.

2. Modification at the 2′ position of the carbohydrate moiety

Two drugs used in cancer therapy,[4] cytarabine (**1**) and gemcitabine (**3**), are 2′-modified pyrimidine nucleoside analogues (Fig. 5).

Both drugs were created in the last century, and during their use, data was obtained not only on the effectiveness, targets, and mechanisms of targeted anticancer action, but also on the disadvantages, *viz*., side effects on the human body, the emergence of resistance, *etc*.[27,28] Numerous studies have been described aimed at optimizing the methods of synthesizing these drugs using modern approaches, and creating analogues and derivatives to improve therapeutic properties.[5,14,17,29–33]

Gemcitabine (**3**)[14,33–37] is a 2′,2′-difluorinated cytidine analogue marketed as the hydrochloride salt under the trade name Gemzar for the treatment of cancers such as non-small cell lung cancer, ovarian cancer, bladder cancer, pancreatic cancer, and breast cancer. In recent years, the need for effective methods for producing gemcitabine has increased significantly. The synthesis of gemcitabine (**3**) was patented[38] comprising the following sequence of transformations: complete protection of the NH_2 and OH groups of cytidine with *o*-toluoyl chloride (*o*-TolC(O)Cl) followed by regioselective deprotection of position 2′, oxidation of compound **28** with pyridinium dichromate (PDC), fluorination with diethylaminosulfur trifluoride (DAST) in the HF–pyridine (Py) system, and removal of the protecting groups with sodium methoxide (Scheme 1).

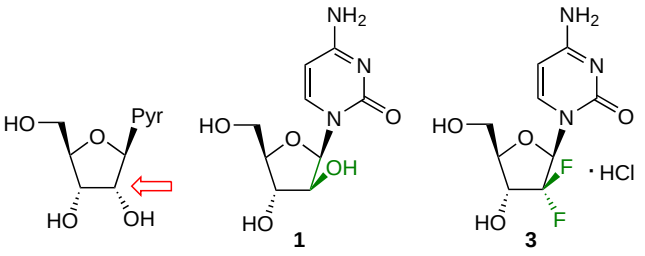
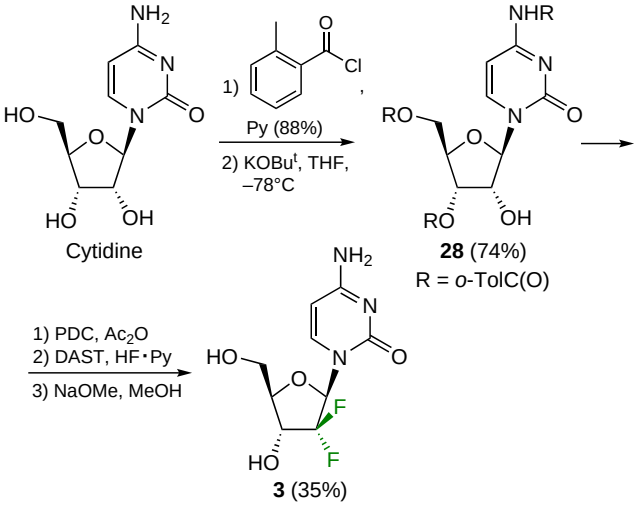


Figure 5. General structure of pyrimidine (Pyr) nucleosides (left, the arrow indicates the 2′-position of the carbohydrate moiety) and the structures of analogues **1** and **3** modified at this position.

Scheme 1

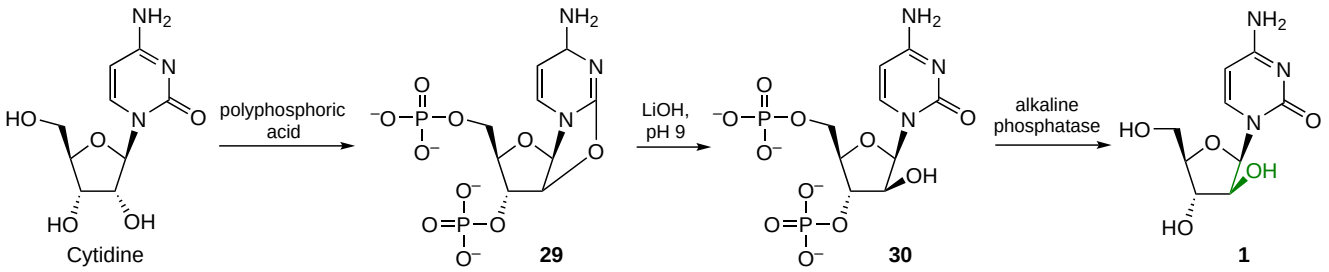


Enzymatic deamination of gemcitabine (**3**), rapid systemic clearance, and the emergence of chemoresistance limit the use of this drug. In recent years, data have been published on various strategies for creating gemcitabine (**3**) prodrugs with the aim of improving its pharmacokinetic properties, increasing efficiency and safety, as well as on the possibilities of using various strategies for its targeted delivery.[13,32,39,40] Three prodrugs of gemcitabine (**3**), known under the commercial codes NUC1031 (**19**), CP-4126 (**20**), and LY2334737 (**21**) (see Fig. 3), did not show sufficient benefits in clinical trials to warrant their recommendation for use.[13]

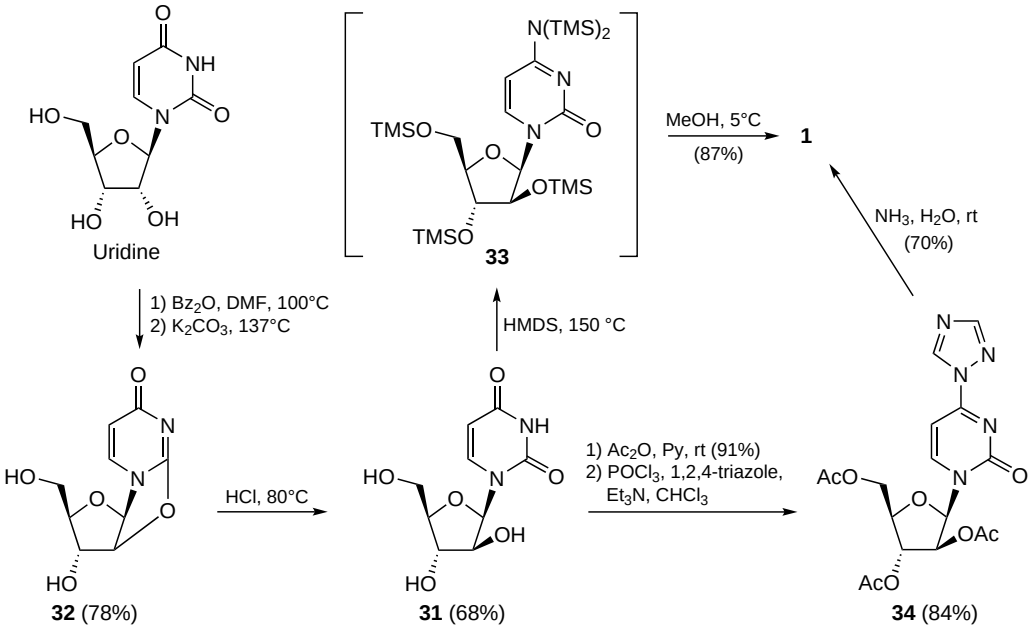
1-β-*D*-Arabinofuranosylcytosine (cytarabine, ara-C, **1**)[41] (see Fig. 5) is one of the most potent drugs for the treatment of human acute myeloid leukemia, erythromyelosis, neuroleukemia and non-Hodgkin′s lymphoma.[42–44] Cytarabine (**1**) was first synthesized by the Dekker′s group[45] in 1959 (Scheme 2). Cytidine reacted with polyphosphoric acid to give 3′,5′-*O*-diphosphorylated 2,2′-*O*-cyclocytidine **29**, which was hydrolyzed with lithium hydroxide (pH 9) to afford cytarabine-3′,5′-diphosphate **30**. To obtain the target product **1**, the phosphate groups were then removed by enzymatic hydrolysis with alkaline phosphatase.

Subsequently, other synthetic approaches to cytarabine (**1**) were proposed, some of which are described in Chapter 2 of the monograph *Profiles of Drug Substances Excipients and Related Methodology*,[41] dedicated to this drug. 1-β-*D*-Arabinofuranosyl-uracil (**31**) was the key intermediate in two other synthetic routes that allow the preparation of cytarabine (**1**) in kilogram quantities. It was obtained from uridine by a two-step protocol through the formation of 2,2′-anhydrouridine **32** (Scheme 3). Next, using one method, compound **31** was treated with hexamethyldisilazane (HMDS) under pressure at high

Scheme 2



Scheme 3



TMS is trimethylsilyl, rt is room temperature

temperature. In the final step, the reaction of intermediate **33** with methanol gave the target cytarabine (**1**) in a high yield of 50 g.[46]

Another synthetic strategy[47] used the acyl-substituted 1,2,4-triazole derivative **34**, which served as an intermediate, formed through sequential acylation and substitution of the oxygen atom at the 5-position of the uracil moiety with a triazole ring (see Scheme 3). Cytarabine (**1**) was obtained in kilograms after deprotection of compound **34** with ammonia.

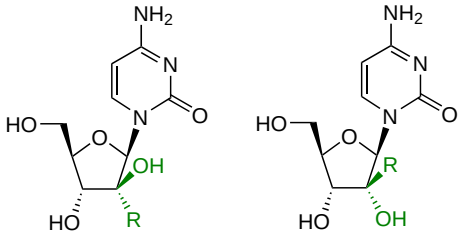
The effectiveness of cytarabine (**1**) is limited by a number of disadvantages such as a short half-life in plasma (partly due to deamination to inactive 1-(arabinofuranosyl)uracil by cytidine deaminase[48]), low affinity for the key enzyme of the metabolic pathway — deoxycytidine kinase,[49] the development of resistance to this drug[50] and lack of activity against solid tumours. In an attempt to overcome these challenges, various types of depot forms and delivery systems of cytarabine (**1**) have been developed that can increase the half-life, improve stability, and provide targeted delivery of the drug to tumour cells.[1,30,51]

Proposed depot forms of cytarabine (**1**) include elacitarabine (CP-4055, **22**),[52] which has entered phase III clinical trials as a drug for the treatment of leukemia, as well as the agent MB07133 (**23**)[1] and aspacytarabine (**24**)[53] (see Fig. 3). A randomized phase III clinical trial in patients with acute myeloid leukemia (AML) found no significant differences in overall survival or adverse events between patients receiving elacitarabine (**22**) and the control group.[54] However, a lower dose of elacitarabine (**22**) in combination with idarubicin resulted in complete remission of the disease in 40% of cases.[55] Thus, despite the disappointing results of phase III studies of elacitarabine as a monotherapy for leukemia,[54] this drug may be useful in combination with other drugs or in patients with refractory disease.

Synergism has been revealed in combinations of elacitarabine with gemcitabine, irinotecan and topotecan, one of the targets of which is topoisomerase I, as well as the additive nature of taking this drug with idarubicin and cloretazine, which act by other mechanisms.[24,56] In addition to prodrugs, cytarabine (**1**) delivery systems are being actively developed for the treatment of various types of cancer. A liposomal form of cytarabine (DepoCyt, Pacira Pharmaceuticals, New Jersey, USA) is approved for the treatment of lymphomatous meningitis and is an example of the successful application of nanotechnology in oncology.[57]

Another trend for optimizing the anticancer properties of cytarabine (**1**) is the synthesis of 2′-alkyl analogues **35a**,**b**, **36a**,**b**.

Structures 35, 36

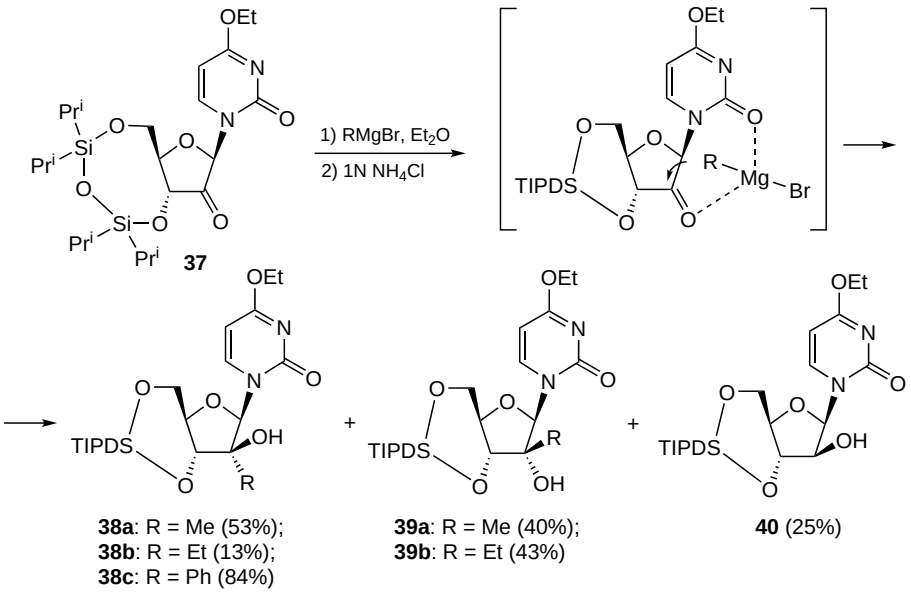


R = Me (**a**), Et (**b**)

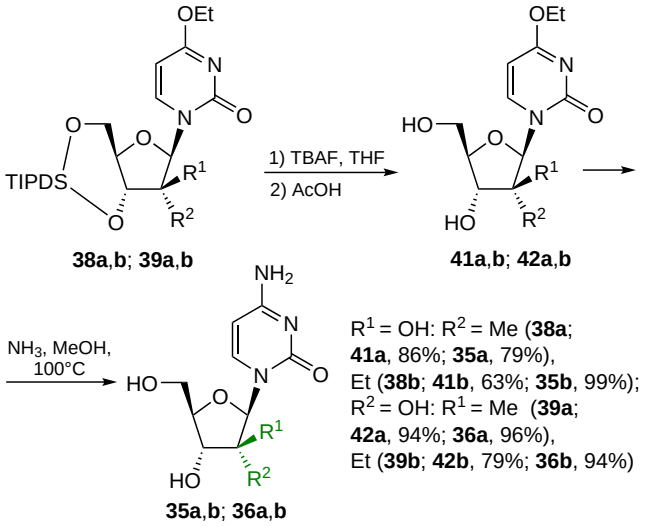
The synthesis of these compounds commenced with the treatment of 3′,5′-protected 2′-keto-nucleoside **37** with alkyl magnesium bromide in diethyl ether while cooling under argon (Scheme 4).[58] The use of methyl magnesium bromide at –78°C led to a mixture of epimers **38a** and **39a**, while ethyl magnesium bromide at –50°C afforded a mixture of three nucleoside analogues (**38b**, **39b**, and **40**). Upon treatment of compound **37** with phenyl magnesium bromide at –78°C, the major product **38c** was isolated in 84% yield, which is apparently due to steric hindrance when using a bulkier substituent.

Removal of the tetraisopropyldisiloxanyl-1,3-diyl (TIPDS) protecting group in compounds **38a**,**b** with tetra-*n*-butylammonium fluoride (TBAF) in THF followed by heating of intermediates **41a**,**b** in methanol saturated with NH_3 at 100°C in a sealed vial gave the corresponding 2′-alkyl-1-(arabinofuranosyl)cytosines **35a**,**b** (Scheme 5).[58] Under similar conditions, compounds **39a**,**b** were deprotected to afford 4-ethoxy-substituted 2′-methyl- (**42a**) and 2′-ethyl-β-*D*-ribofuranosylpyrimidin-2(1*H*)-ones (**42b**) in 94% and 79% yield, respectively. Ammonolysis of compounds **42a**,**b** under the conditions described above furnished 2′-*C*-methyl- (**36a**) and 2′-*C*-ethyl derivatives (**36b**) in high yields.

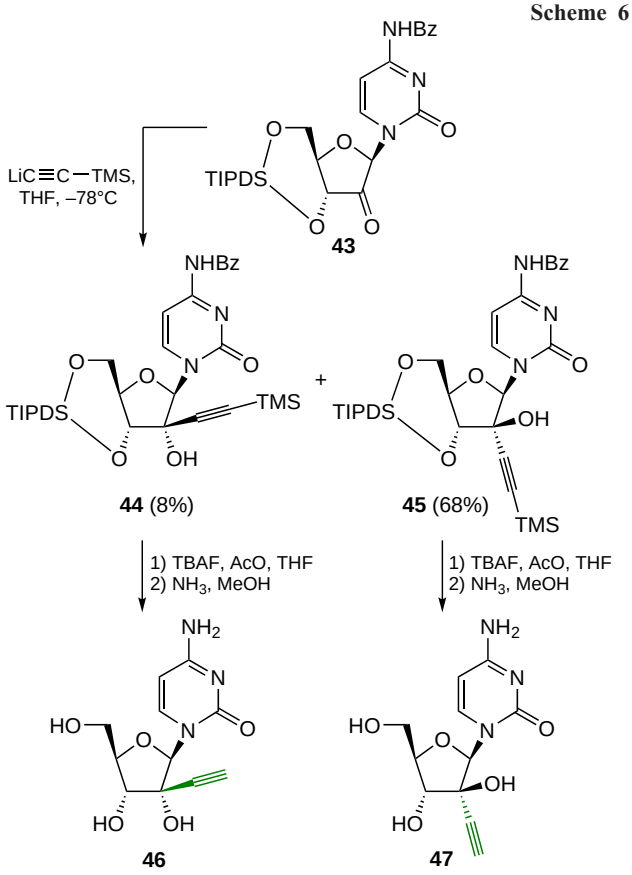
Scheme 4



Scheme 5



Scheme 6

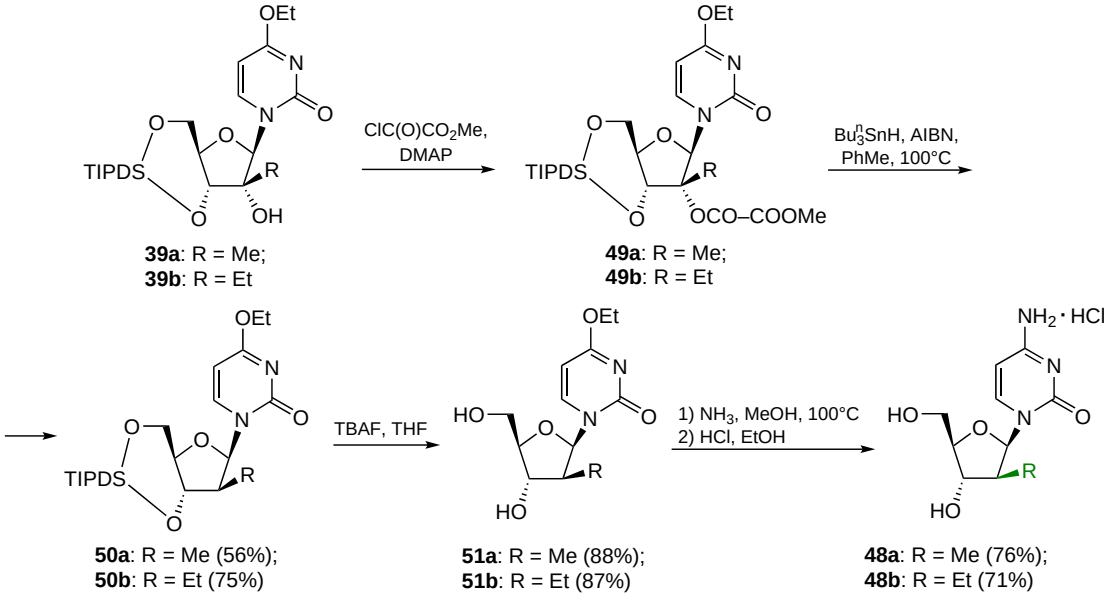


In addition to the above-mentioned 2′-alkylated analogues of cytarabine (**1**), derivatives with an ethynyl substituent at this position were synthesized. Two approaches to their preparation were proposed. Thus, the reaction of 1-[3′,5′-*O*-[1,1,3,3-tetraisopropyldisiloxane-1,3-diyl]-β-*D*-erythrofuranosyl]uracil and LiC≡CR gave predominantly the α-adduct.[59] Treatment of the N^4-benzoylcytosine derivative **43** with LiC≡CTMS in THF at –78°C led to a mixture of β- (**44**) and α-adducts (**45**), which was separated by column chromatography on silica gel, eluting with ethyl acetate in hexane with a concentration gradient (Scheme 6).[60] The silyl protection in the resulting intermediates was removed in the usual manner and the corresponding free nucleosides **46** and **47**, respectively, were isolated.

The synthesis of 2′-deoxy-2′-*C*-methyl- and 2′-deoxy-2′-*C*-ethylcytidines **48a**,**b** is described. The hydroxyl group in the starting nucleosides **39a**,**b** was deoxygenated with methyl oxalyl chloride in acetonitrile in the presence of two equivalents of 4-dimethylaminopyridine (DMAP). Thus obtained 2′-*O*-methyloxalyl esters **49a**,**b** were heated without further purification in toluene at 100°C for 1 h in the presence of tri-n-butylstannane and azabis(isobutyronitrile) (AIBN) to give 2′-deoxy derivatives **50a**,**b** in moderate yields. These compounds were then treated with TBAF in THF at room temperature to form nucleosides **51a**,**b** in high yield. Further heating at 100°C of a solution of nucleosides **51a**,**b** in methanol saturated with NH_3 in sealed tubes for 2 days, followed by treatment of the residues dissolved in ethanol with a 2 M HCl solution gave the target products **48a**,**b** in the form of hydrochlorides (Scheme 7).[61,62]

When nucleoside **38a** was deoxygenated under the conditions shown in Scheme 7, the corresponding ester was isolated in very low yield, apparently due to steric hindrance during esterification. For this reason, 2′-deoxy-2′-*C*-methylcytidine (**52**) was obtained using a less bulky protecting group (Scheme 8).[62] Thus,

Scheme 7



39a: R = Me;
39b: R = Et

49a: R = Me;
49b: R = Et

50a: R = Me (56%);
50b: R = Et (75%)

51a: R = Me (88%);
51b: R = Et (87%)

48a: R = Me (76%);
48b: R = Et (71%)

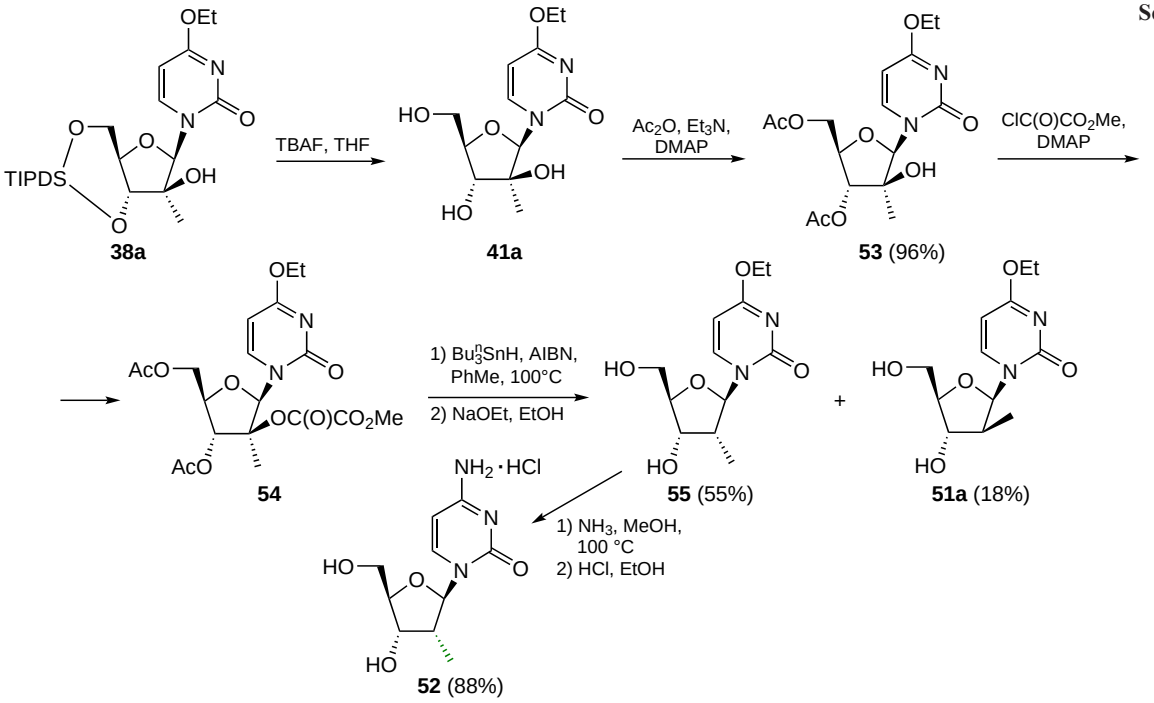
nucleoside **38a** at room temperature was treated with TBAF in THF, which furnished 4-ethoxy-1-(2′-*C*-methyl-β-*D*-arabinofuranosyl)-2(1*H*)-pyrimidinone (**41a**), which was acetylated with acetic anhydride in the presence of triethylamine and DMAP. In this case, 3′,5′-di-*O*-acetyl derivative **53** was obtained in high yield, which was condensed with methyl oxalyl chloride in the presence of two equivalents of DMAP. The resulting 2′-*O*-methyloxalyl ester **54** was heated without further purification at 100°C in toluene for 1 h in the presence of tri-*n*-butylstannane and AIBN. Subsequent treatment of the deoxygenation product with sodium ethoxide in absolute ethanol gave a mixture of nucleosides **51a** and **55**, which were chromatographically separated to give the corresponding products in individual form. Ammonolysis of nucleoside **55** and subsequent treatment of the residue with a solution of HCl in ethanol afforded the hydrochloride of the target (2′*R*)-2′-deoxy-2′-*C*-methylcytidine (**52**) in high yield (see Scheme 8).[62]

(2′*S*)-2′-*C*-Methylthymidine **56** was obtained by acid hydrolysis of 4-ethoxy-5-methyl-1-(2′-deoxy-2′-*C*-methyl-β-D-arabinofuranosyl)-2(1*H*)-pyrimidinone (**57**) in an aqueous–alcoholic medium (Scheme 9).[62]

The synthetic approach to 1-(2′-deoxy-β-*D*-arabinofuranosyl)-cytosines with azide (**58**) and amino groups (**59**) at position 2′ involved the condensation of 5-*O*-benzoyl-3-*O*-acetyl-2-azido-3-deoxy-*D*-arabinofuranosyl chloride[63] with trimethylsilylated cytosine. This gave a mixture of α- and β-anomers, with the isolated yield after chromatographic separation of 9 and 39%, respectively. The protecting groups were removed by treatment with K_2CO_3 in methanol to afford the target 1-(2′-azido-2′-deoxy-β-*D*-arabinofuranosyl)cytosine **58**.[63] Reduction of the latter on 5% Pd/C gave the 2′-amino-2′-deoxy derivative **59** in 83% yield.[64]

To introduce an azido group at the 2′ position of uridine in an alternative method for the synthesis of azido derivative **58**, the

Scheme 8



38a

41a

53 (96%)

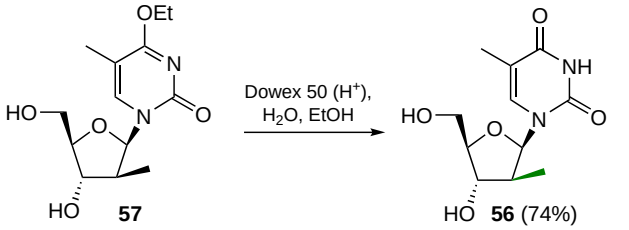
54

55 (55%)

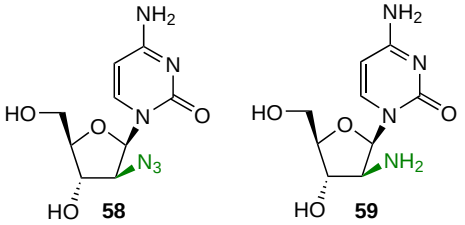
51a (18%)

52 (88%)

Схема 9



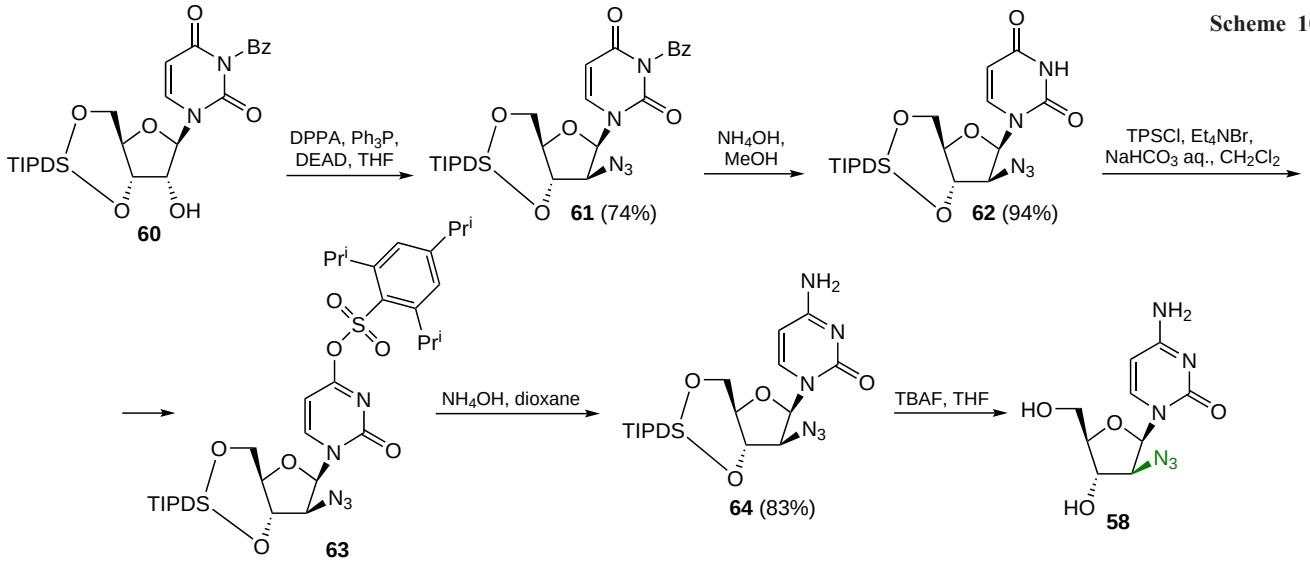
Structures 58, 59



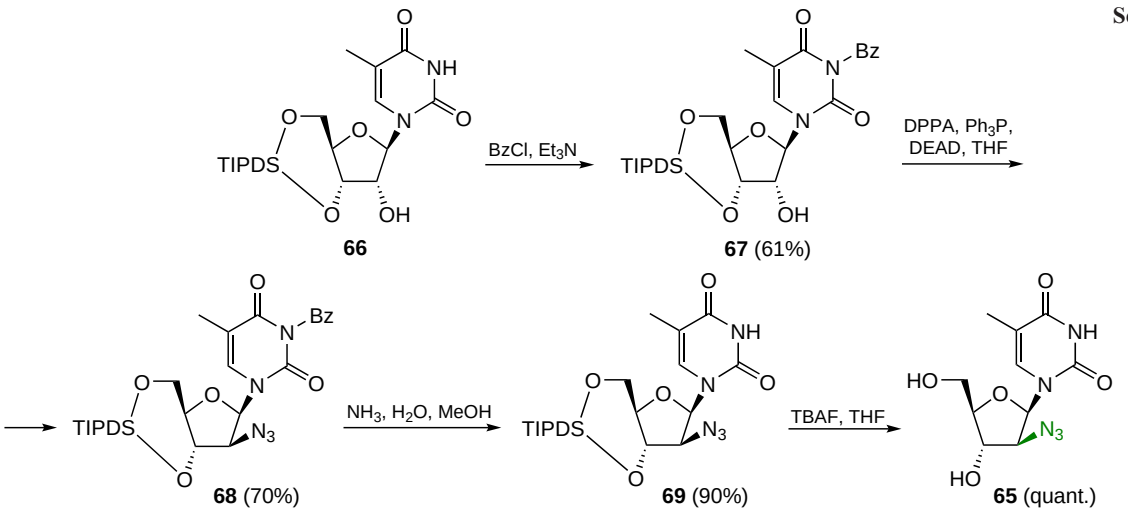
nucleophilicity of the carbonyl oxygen atom at position 2 was reduced in the first step. For this purpose, benzoyl protection of the N^3 group was used.[65] Treatment of protected uridine **60** with diphenyl phosphorazidate (DPPA), triphenylphosphine, and diethyl azodicaboxylate (DEAD) afforded N^3-benzoyl-1-(2′-azido-2′-deoxy-3′,5′-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-β-*D*-arabinofuranosyl)uracil (**61**) in moderate yield (Scheme 10).[66] After its debenzoylation with NH_3 in methanol, 1-(2′-azido-2′-deoxy-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-arabinofuranosyl)uracil (**62**) was obtained in high yield. Compound **62** was converted to the O^4-triisopropylbenzenesulfonate derivative **63** by treatment with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in dichloromethane and, without further purification, was treated with NH_4OH in dioxane to give the cytosine derivative **64** (yield 83% based on compound **62**). Deprotection of nucleoside **64** with TBAF in THF gave product **58** in quantitative yield as the hydrochloride.

In the first step of the synthesis of 1-(2′-azido-2′-deoxy-β-*D*-arabinofuranosyl)thymine (**65**), N^3-benzoyl derivative **67** was obtained from 1-[3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]thymine (**66**) by treatment with benzoyl chloride in the presence of triethylamine in dichloromethane (Scheme 11).[66] Nucleoside **67** was treated with diphenyl phosphorazidate, triphenylphosphine, and diethyl azodicarboxylate in THF at room temperature to afford intermediate **68**, which was purified by silica gel column chromatography in 70% isolated yield. Debenzoylation of compound **68** was achieved with a methanolic NH_3 solution, and the reaction mixture was then treated with a THF solution of TBAF, affording the target product **65** in quantitative yield over two steps.[66]

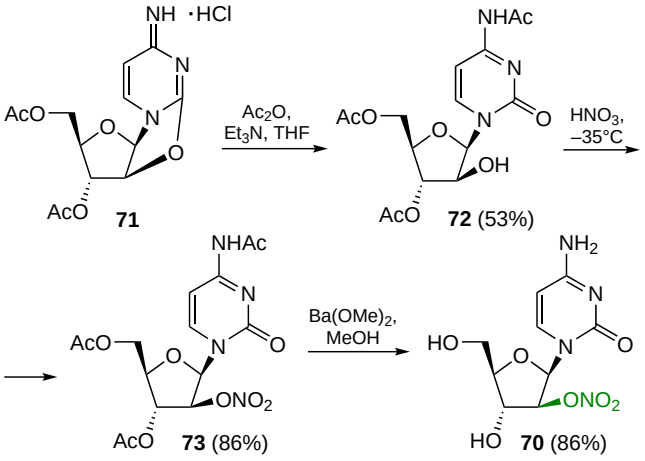
Scheme 10



Scheme 11



Scheme 12

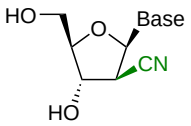


In order to overcome the susceptibility of cytarabine (**1**) to enzymatic deamination and thereby prevent its deactivation, 2′-*O*-nitro-β-*D*-arabinofuranosylcytosine (**70**) was prepared.[67] The first step of the reaction sequence shown in Scheme 12 comprised treating the starting 2,2′-anhydro-1-(3′,5′-di-*O*-acetyl-β-*D*-arabinofuranosyl)cytosine hydrochloride (**71**) with acetic anhydride in THF in the presence of triethylamine, which led to 1-(3′,5′-di-*O*-acetyl-β-*D*-arabinofuranosyl)-N^4-acetylcytosine (**72**) in moderate yield. Esterification of compound **72** was accomplished with concentrated nitric acid at a temperature from –30 to –35°C, yielding 1-(3′,5′-di-*O*-acetyl-2′-*O*-nitro-β-*D*-arabinofuranosyl)-N^4-acetylcytosine (**73**). Deacetylation of compound **73** with barium methoxide in methanol gave the target nucleoside, 1-(2′-*O*-nitro-β-*D*-arabinofuranosyl)cytosine (**70**), in high yield.

The synthesis of 1-(2′-*C*-cyano-2′-deoxy-β-*D*-arabinofuranosyl)cytosine (CNDAC, **11**) was started by treating N^4-acetyl-1-[3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-erythrofuran-2-ylosyl]cytosine (**74**) with sodium cyanide in the presence of $NaHCO_3$ in a diethyl ether–water mixture (Scheme 13).[68] This afforded epimeric 2′-cyanohydrins **75**, which were then reacted with phenyl chlorothiocarbonate in the presence of DMAP in acetonitrile. Deoxygenation of intermediate **76** was carried out by treatment with tri-n-butylstannane and 2,2′-azobis(isobutyronitrile) in toluene at 100°C. According to NMR spectroscopy data (nuclear Overhauser effect (NOE) experiments), radical deoxygenation proceeds stereospecifically to form arabinonucleoside **77**, apparently due to the steric hindrance at the β-position. Desilylation of compound **77** with TBAF in the presence of acetic acid in THF afforded the N^4-acetyl derivative **78** in high yield. Further treatment of the reaction mixture with HCl in MeOH at room temperature yielded 1-(2′-*C*-cyano-2′-deoxy-1-β-*D*-arabinofuranosyl)cytosine (**11**) as hydrochloride.[68]

Under similar conditions (see Scheme 13), 1-(2′-*C*-cyano-2′-deoxy-β-*D*-arabinofuranosyl) derivatives of uracil (**79**) and thymine (**80**) were obtained.[69]

Structures 79, 80

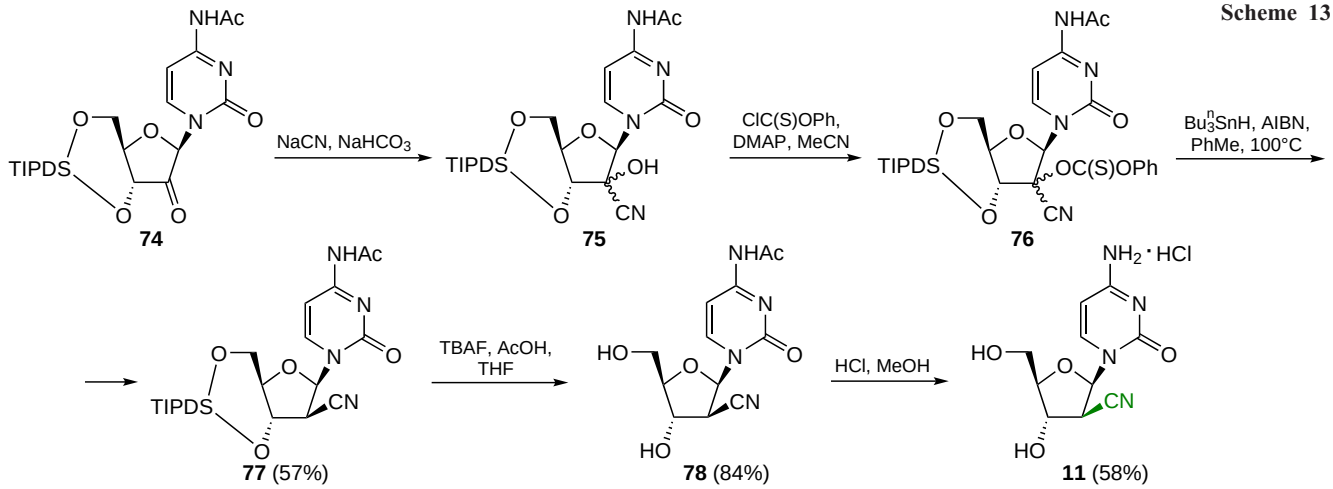


Base = uracyl (**79**), thymine (**80**)

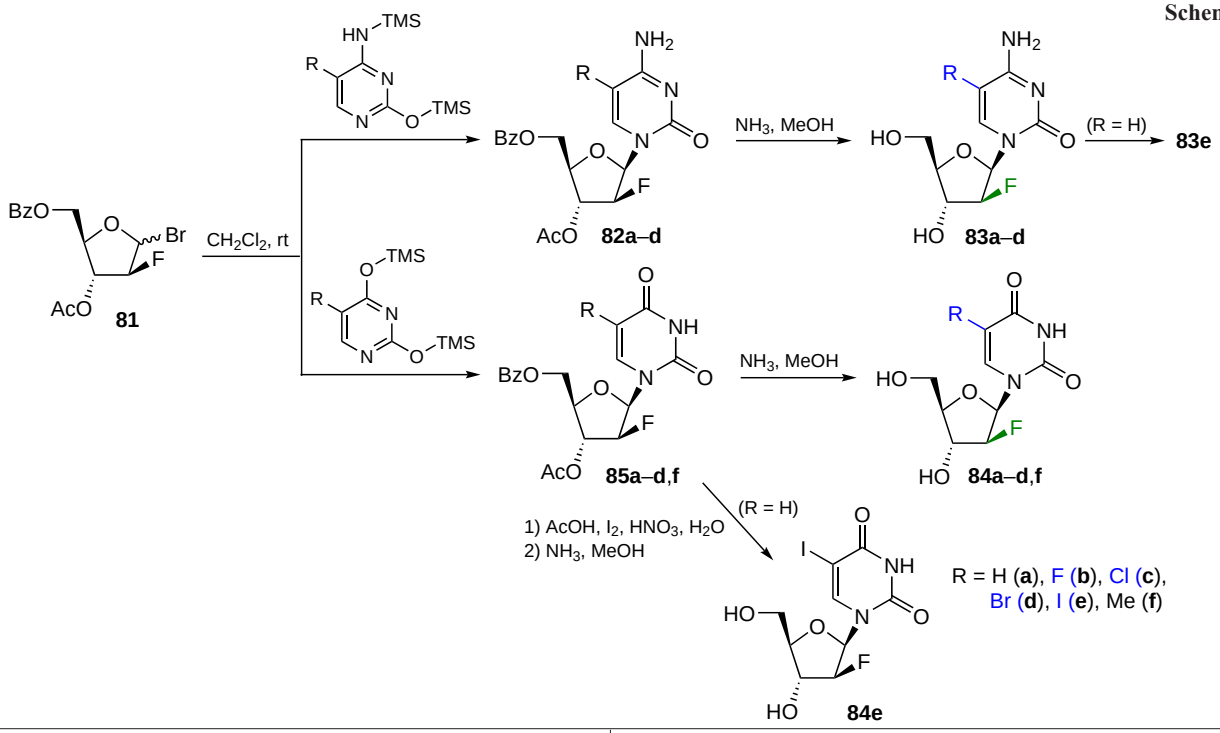
Condensation of 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-*D*-arabinofuranosyl bromide (**81**)[70] with trimethylsilylcytosine or its 5-halo derivatives afforded protected nucleosides **82a–d**, which were deprotected by ammonolysis to 2′-*F*-ara-*C*-analogues **83a–d** (Scheme 14).[71] The 5-iodo derivative **83e** was formed by iodination of compound **83a** under the following conditions: AcOH, HIO_3, I_2, CCl_4, H_2O, 40–50°C. Products **84a–d**,**f** were obtained by condensation of bromide **81** with the corresponding trimethylsilyluracils and subsequent deprotection of compounds **85a–d**,**f**. Iodination of nucleoside **85a** (R = H) followed by ammonolysis afforded analogue **84e** (see Scheme 14).[71]

When a double bond is introduced into the 2′ position of 2′-deoxycytidine, it forms, together with the secondary 3′-hydroxyl group, an allyl alcohol system, which is also found in a number of nucleoside antibiotics, including angustmycin A and neplanocin A. This structural feature may play an important role in the manifestation of biological activity due to increased reactivity and/or conformational fixation of the carbohydrate moiety. To test this hypothesis, 2′-deoxy-2′-methylidenecytidine (DMDC, **86a**) was synthesized.[72] The starting 2′-ketonucleoside **37** was treated with an excess of methylenetriphenylphosphorane, previously obtained by the reaction of potassium hydride with methyltriphenylphosphonium bromide in dimethyl sulfoxide (Scheme 15). This resulted in the formation of 2′-methylidene nucleoside **87**, desilylation of which by TBAF in THF afforded derivative **88** in high yield. Compound **88** was then converted by ammonolysis to the target nucleoside **86a**, which was isolated as the hydrochloride.[72]

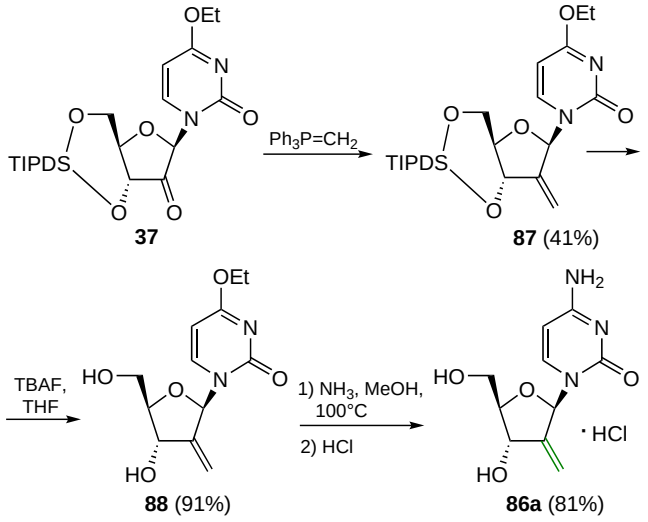
Scheme 13



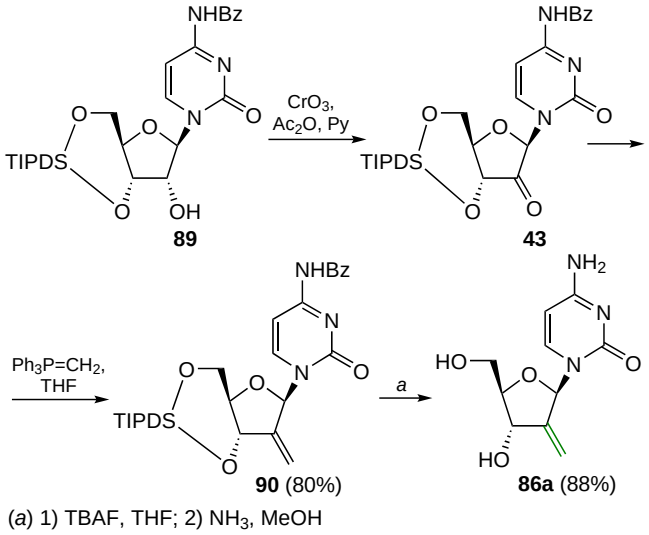
Scheme 14



Scheme 15



Scheme 16



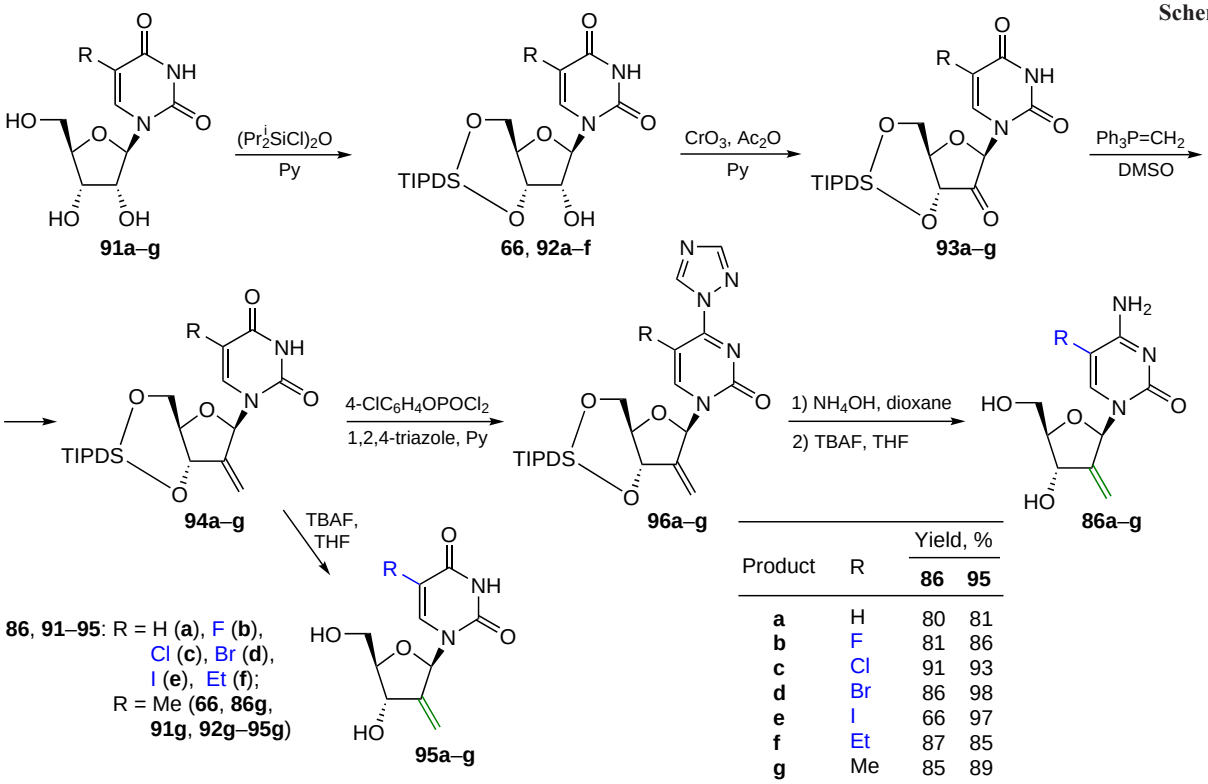
An alternative method for the synthesis of compound **86a** is shown in Scheme 16. The starting *N*^{4}-benzoyl-1-[3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]cytosine (**89**) was oxidized[73] with the CrO_3–Py–Ac_2O system (molar ratio 1 : 2 : 1) in dichloromethane, which led to the formation of the 2′-keto derivative **43**, which was condensed with methylenetriphenylphosphorane in THF. In this case, compound **90** was isolated, and its deprotection with TBAF in THF followed by treatment with NH_3 in MeOH gave the target nucleoside **86a**.[74]

Derivatives of 2′-deoxy-2′-methylideneuridine and -cytidine containing various substituents at position 5 of the pyrimidine ring were also obtained. The synthesis of such products, as shown in Scheme 17, comprised an initial conversion of the starting nucleosides **91a–g** under the action of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to the corresponding 5-substituted 1-[3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]uracils **92a–f** and the above-described analogue **66** (R=Me). These compounds were then oxidized with the CrO_3-Py–Ac_2O system (molar ratio 1 : 2 : 1) in dichloromethane.[73] The resulting 2′-keto derivatives **93a–g** were condensed with methylenetriphenylphosphorane in THF, leading to 2′-methylidene derivatives **94a–g**, which were treated with TBAF in THF to afford the target uridines **95a–g**.[74,75]

2′-Deoxy-2′-methylidenecytidine **86a** and its analogues **86b–g** (see Scheme 17) were synthesized from 2′-methylidene derivatives of uridine **94a–g**. The latter were reacted with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine, and then the resulting 4-triazolyl derivatives **96a–g** were treated with NH_4OH followed by deprotection with TBAF in THF to afford the target products **86a–g** in high yields.

In an alternative approach to compounds **94** with a 2′-exomethylene group, 3′,5′-silylene acetals **66** and **92a** were first obtained in 76–84% yields, as described above; the 2′-OH

Scheme 17



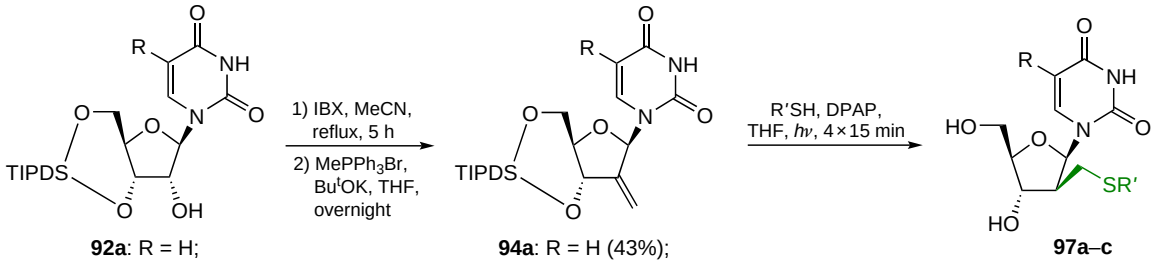
Product	R	Yield, %	
		86	**95**
a	H	80	81
b	F	81	86
c	Cl	91	93
d	Br	86	98
e	I	66	97
f	Et	87	85
g	Me	85	89

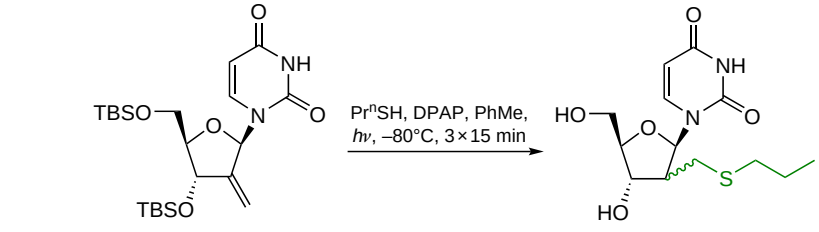
groups in these compounds were then oxidized with 2-iodoxybenzoic acid (IBX). Subsequent Wittig methylenation afforded the desired olefins **94a**,**g** (Scheme 18, reaction (1)). Compounds **94a**,**g** were treated with the appropriate mercaptans in the presence of 2,2-dimethoxy-2-phenylacetophenone (DPAP) at room temperature under ultraviolet light irradiation. Derivatives **97a**–**c** were obtained in moderate yields; in all cases, mixtures of diastereomers were observed, with the *D*-arabino form predominating.[76]

The use of *tert*-butyldimethylsilyl protection of the 5′- and 3′-hydroxy groups of uridine to obtain its 2′-alkylsulfanylmethyl derivatives is also described (see Scheme 18, reaction (2)). Carrying out the process at –80°C significantly increased the product yield and improved diastereoselectivity compared to a similar protocol at room temperature. The authors note that TIPDS protection is preferable, since the alternative method afforded the starting 3′,5′-di-*O*-*tert*-butyldimethylsilyl nucleoside **98** in low yield, and the main reaction product was the regioisomeric 2′,5′-di-*O*-*tert*-butyldimethylsilyl isomer.[77]

Using the Horner–Wittig reaction (or Julia–Lythgoe reaction), 2′-ketonucleoside **37** was converted into a mixture of easily separated fluorovinyl sulfones **99** (predominant product) and **100** (by-product). Next, two equivalents of tri-*n*-butylstannane were added to these compounds to obtain (fluorovinyl)stannanes **101** and **102** in good yields. Analysis of the crude reaction mixtures by ^{19}F NMR spectroscopy confirmed the selectivity of the reaction, showing the absence of isomer **101** in product **102** and *vice versa*. Then (*E*)-2′-deoxy-2′-(fluoromethylidene)cytidine (**13**) was synthesized directly from compound **101** by treating it with a CsF–NH$_3$–MeOH mixture.

Scheme 18





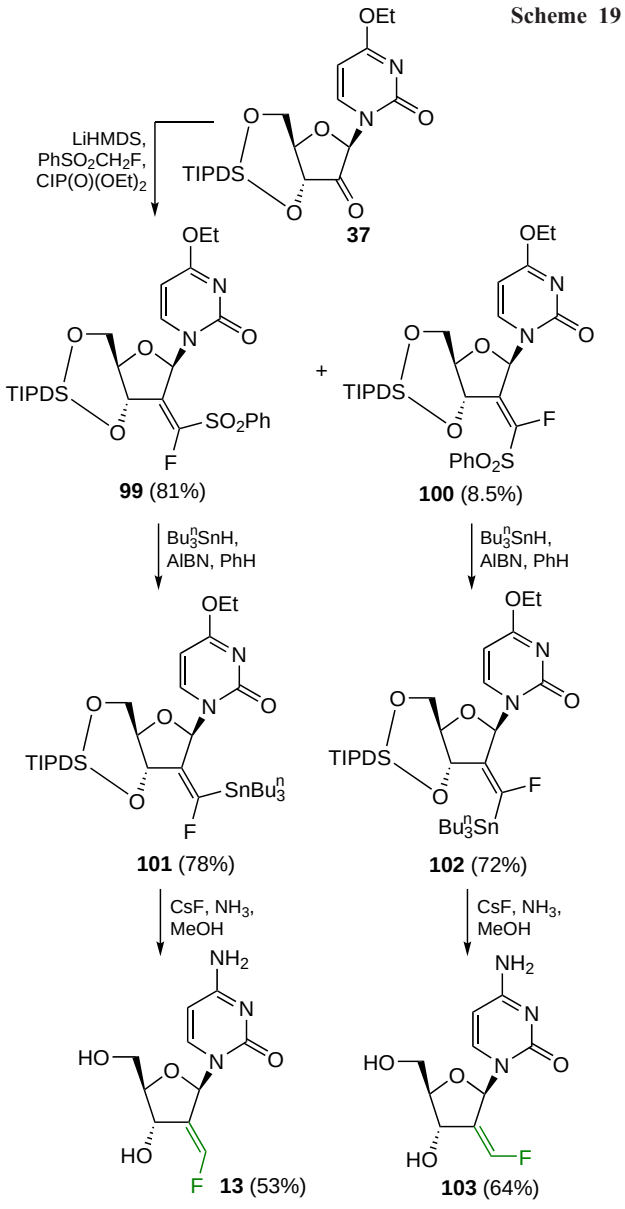
TBS is *tert*-butyldimethylsilyl

(*Z*)-2′-Deoxy-2′-(fluoromethylidene)cytidine (**103**) was prepared from compound **102** under similar conditions (Scheme 19).[78]

Uridine was converted to 5′-*O*-TBDPS-2,2′-*O*-anhydrouridine **104** (TBDPS is *tert*-butyldiphenylsilyl), which was sequentially treated with 1,1′-carbonyldiimidazole and *O*-benzylhydroxylamine (Scheme 20).[79,80] The resulting 3′-*O*-(benzyloxyamino)carbonyl derivative without purification was converted into the 2′-*N*-benzyloxyamino-3′-*O*-carbonyl compound **105** in good yield. Treatment of this compound with cesium carbonate in methanol effectively cleaved the cyclic carbamate moiety to afford 2′-*O*-benzyloxyamino-2′-deoxyuridine **106**. However, the latter did not give the desired 2′-hydroxylamino derivative **107** under hydrogenation conditions.

In an alternative method, hydrogenation of precursor **105** under the same conditions gave hydroxylamine **108**. After protecting its *N*-hydroxy group with a tetrahydrofuran-2-yl (2-THF) moiety, compound **109** was obtained. The 5′-*O*-silyl group in this compound was removed with TBAF. Cleavage of the cyclic carbamate moiety in derivative **110** with $CsCO_3$ in MeOH afforded analogue **111**. Cleavage of tetrahydrofuranyl protecting group in the presence of 10% HCl in EtOH furnished the desired 2′-hydroxylamino-2′-deoxyuridine (2′-DHAU, **112**) as the hydrochloride (see Scheme 20).[79,80]

Scheme 19



Cytidine analogue **113** was obtained from compound **114**, in which the 3′-hydroxy group was first protected with TBS to give derivative **115** (Scheme 21).[79,80] From the latter, by a standard method, which involved the reaction with 2,4,6-triisopropylbenzenesulfonyl chloride in acetonitrile, O^4-triisopropylbenzenesulfonate was obtained and converted without further purification into nucleoside **116** by treatment with NH_4OH. The protecting groups of compound **116** were removed in a mixture of conc. HCl and MeOH to give 2′-hydroxylamino-2′-deoxycytidine (**113**) as dihydrochloride.

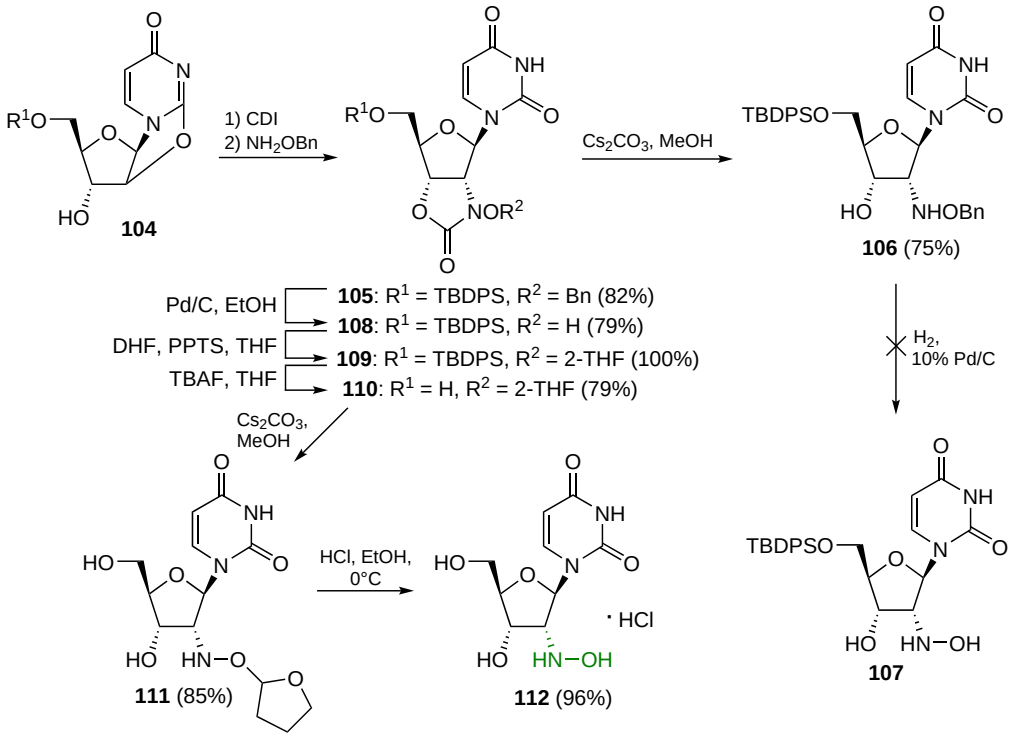
Among analogues of pyrimidine nucleosides modified at the 2′-position of the carbohydrate moiety, the synthesis of which is described in this Section, the compounds listed in Table 1 showed the most significant cytotoxicity against various tumour cell cultures.

The mechanisms of biological action of these drugs have been studied. For example, cytarabine (**1**) (see Fig. 1) enters cells primarily *via* the ENT1 transporter and is then sequentially phosphorylated by deoxycytidine kinase, deoxycytidylate kinase, and nucleoside diphosphate kinase (NDPK).[2] The effectiveness of this drug is directly related to the expression of these enzymes in the cell. The corresponding nucleotide is incorporated into the nascent DNA, leading to single-strand breaks, stalling the replication fork, and ultimately cell death.[2]

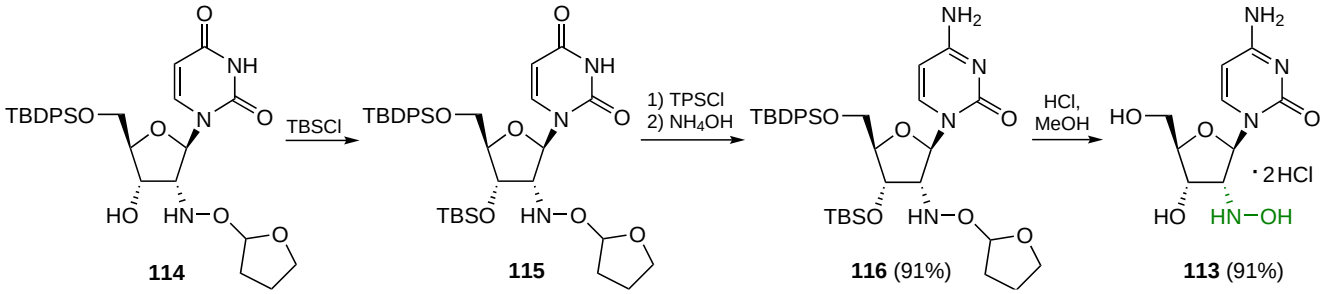
Gemcitabine (**3**) (see Fig. 1) enters cells primarily through the ENT1 and CNT3 transporters. Upon entering the cell, it is phosphorylated to the corresponding 5′-monophosphate (by dCK), 5′-diphosphate (by nucleotide monophosphate kinase (NMPK)) and 5′-triphosphate (by NDPK). Gemcitabine 5′-diphosphate inhibits ribonucleotide reductase, and its 5′-triphosphate terminates DNA synthesis, similar to cytarabine 5′-triphosphate. Furthermore, phosphorylated forms of gemcitabine can inhibit 3′-5′ exonuclease, which removes some of the nucleotide analogues incorporated into DNA, enhancing their anticancer activity. These and other targets, as well as the mechanisms of action of gemcitabine (**3**), are described in detail.[2]

2′-*C*-cyano-2′-deoxy-1-β-*D*-arabinopentofuranosylcytosine (CNDAC, **11**) (see Fig. 2) and its prodrug form sapacitabine (**17**) (see Fig. 3),[88] intended for oral administration,[20,89] have demonstrated clinical activity against AML.[90–92] The mechanism of action of CNDAC differs from that of other deoxycytidine analogues.[20] Following intracellular phosphorylation, the corresponding nucleotide is incorporated into the DNA chain; however, unlike cytarabine (**1**) and gemcitabine (**3**), this incorporation does not lead to inhibition of DNA replication, stalling of the replication fork, or arrest of the cell in S phase. Following incorporation of the next 2′-deoxynucleotide into DNA, instability of compound **11** causes β-elimination, resulting in conversion to 2′,3′-didehydro-2′,3′-dideoxy-2′-*C*-cyanocytidine (CNddC), which is virtually the 3′-terminal DNA chain terminator. The resulting single-strand breaks cannot be repaired by ligation and are converted to double-strand breaks during subsequent DNA replication.[93] Thus, after incubation of leukemic cells with cytostatic concentrations of drug **11**, inhibition of the cell cycle in the S phase and its arrest in the G2 phase occur.[94] Acquired resistance to CNDAC is primarily mediated by a decrease in the activity of deoxycytidine kinase, which catalyzes the first step of phosphorylation of this compound.[95] The sensitivity of leukemia cells to CNDAC (**11**) is also critically dependent on the level of the SAMHD1 protein.

Scheme 20



Scheme 21



This protein is a triphosphohydrolase that breaks down nucleoside triphosphates into deoxyribonucleosides and inorganic triphosphate.[95] Triphosphorylated forms of some anticancer nucleoside analogues, in particular cytarabine (**1**) and CNDAC (**11**), are substrates of this enzyme,[95,96] therefore high levels of SAMHD1 [§] predetermine the low efficacy of therapy with these drugs in acute myeloid leukemia, acute lymphoblastic leukemia and Hodgkin's lymphoma.[97–99]

Tezacitabine (**13**) (see Fig. 2), being a structural analogue of deoxycytidine, like gemcitabine (**3**), is poorly susceptible to metabolism by cytidine deaminase.[100] Tezacitabine **13** is an antimetabolite inhibitor of human ribonucleotide reductase (hRR),[9] which consists of three subunits: hRRM1, hRRM2, and p53R2. The 5′-diphosphate of tezacitabine irreversibly inhibits the hRRM1 subunit, and its 5′-triphosphate is a terminator substrate for DNA polymerases.[100] Clinical evaluation of tezacitabine (**13**) included phase I and II studies, both as a monotherapy of refractory solid tumours and in combination with other agents, *viz*., cisplatin (for the treatment of gastrointestinal cancer) and 5-fluorouracil (for the treatment of advanced solid tumours). In all three cases, the trials did not progress beyond phase II due to lack of efficiency.

2′-Deoxy-2′-methylidenecytidine (DMDC, **86a**) is also a structural analogue of deoxycytidine and is activated by intracellular phosphorylation to the corresponding 5′-diphosphate and 5′-triphosphate. The diphosphate inhibits the hRRM1 subunit, while the triphosphate inhibits DNA biosynthesis. These two nucleotide metabolites act in combination to cause DNA strand breaks, leading to apoptosis. Compound **86a** is resistant to cytidine deaminase and has proven effective in xenograft cancer models where cytidine deaminase activity is high and the response to gemcitabine (**3**) is typically low. Phase I trials of DMDC as a solid tumour treatment revealed high levels of hematological toxicity, which suspended its development as a chemotherapeutic agent.[101] Subsequently, analogues **58**, **86a** and **86b** demonstrated significant cytotoxic effects against T-cell leukemias in cell cultures and animal models.[87]

Thus, modification of the 2′-pyrimidine nucleoside position has proven its effectiveness and led to the development of several drugs. Currently, active research is underway to create depot forms and prodrugs to improve the pharmacokinetic properties, efficiency, and safety of existing drugs, as well as to explore various targeted delivery strategies and synthesize new analogues.

[§] SAMHD1 is a human protein 1 containing the SAM and HD domains.

Table 1. Inhibition of tumour cell proliferation *in vitro* by pyrimidine nucleosides modified at position 2′ and a reference drug.

Compound	IC_{50}, μM (cell culture)	Ref.
1	0.05–0.4 (L1210), 0.99 (B16), 0.02 (P388), 0.2 (S-180), 0.006–1.3 (CCRF CEM), 0.01–0.25 (MOLT4), >0.40 (HL60), 13.2 (K562), 1.28–1.9 (U937), 2.1–32.1 (T24), 18.5 (MKN28)	74, 75
3	0.009–41 (KB), 0.44 (HT-29), 0.007 (NCI-H69)	81, 82
11	0.93 (L1210), 0.14–8.3 (MKN1, MKN7, MKN28, MKN74), 15.2–19.0 (PC3, PC8, PC9), 0.93 (SW480)	68, 83
13	0.058 (L1210), 0.03–0.09 (HS683, J889H, SK-N-MC), 0.015–0.026 (MCF7, MDA-MB-231, MDA-MB- 468, MDA-MB-435)	78, 84, 85
48a	0.94 (L1210), 0.54 (CCRF CEM), 0.12 (MOLT4), 2.35 (HL60), 2.2 (K562), 7.94 (U937), 293 (PC10), >360 (SW480), 3.97 (T24)	61, 62
58	0.6 (L1210), 0.07 (MOLT4), 0.2 (HeLa), 0.56 (T24), 1.95 (CCRF CEM), 6.92 (KOB), 18 (MT-1), 18.6 (ST1), 0.35 (MT-4), 2.19 (MOLT4),	63, 86, 87
59	4 (L1210), 30 (HeLa), <200 (CCRF CEM)	63, 86
70	0.6 (CCRF CEM), 0.4 (MOLT4)	67
83a	0.2 (L5178Y), 0.2 (P815)	71
83b	1.9 (L5178Y), 1.5 (P815)	71
83c	5.0 (L5178Y), 3.6 (P815)	71
84b	3.8 (L5178Y), 2.7 (P815)	71
84c	5.0 (L5178Y), 12.1 (P815)	71
84d	2.8 (L5178Y), 3.6 (P815)	71
84e	2.4 (L5178Y), 2.2 (P815)	71
86a	0.3–1.5 (L1210), 0.4 (P388), 1.5 (S-180), 0.03–0.61 (CCRF CEM), 0.11–0.17 (MOLT4), 2.01 (KOB), 3.19 (MT-1), 2.1 (ST1), 2.36 (MT-4), 0.17 (HL60), 1.84 (U937), 4.2 (PC13), 1.5 (PC36), 10.1–15.9 (SW480), 5.9 (KB), 34.7 (KATO III), 12.1(TE2), 4.6–15.5(T24)	72, 74, 75, 87
86b	1.2 (L1210), 0.6 (P388), 1.5 (S-180), 0.05–0.83 (CCRF CEM), 0.12–0.58 (MOLT4), 1.53 (KOB), 3.28 (MT-1), 2.91 (ST1), 4.5 (MT-4), 0.2 (HL60), 1.6 (U937), 5.5 (PC13), 0.97 (PC36), 17.1 (SW480), 5.5 (KB), 32.3 (KATO III), 7.0(TE2), 3.7(T24)	74, 75, 87
97a	3.7 (SCC-VII)	76
97b	4.7 (SCC-VII)	76
97c	7.5 (SCC-VII)	76
97d	9.8 (SCC-VII)	76
103	3.9 (L1210)	78
112	1.58 (L1210), 1.99 (KB), 1.73 (CCRF CEM), 5.76 (HL60), 2.77 (K562), 8.93 (Colo320DM), 4.00 (MiaPaCa-2), 2.53 (HT1080), 1.30 (MCF7), 0.89 (A375), 1.47 (HuH7)	79, 80
5FU	2.5 (L1210), 20 (B16), >300 (CCRF CEM), 29 (MOLT4), 10 (HL60), 27 (U937), >760 (PC10), 77 (PC14), 82 (PC36), 93 (KB), 28.53 (KATO III), 25.4 (SW480), 30 (TE2), 47 (T24)	74

Notes. Value interval means summary data from different publications; IC_{50} is half-maximal inhibitory concentration. Here and in Tables 2 and 3, the following designations of murine cell lines are used: L1210 is lymphocytic leukemia; P388 is lymphoid neoplasm; P815 is mastocytoma; S-180 is sarcoma; SCC-VII is squamous cell carcinoma; L5178Y is lymphoma; designations of human cell lines: MiaPaCa-2 is pancreatic adenocarcinoma; HT1080 is fibrosarcoma; MCF7 is breast adenocarcinoma; HuH7 is hepatoma; CCRF CEM and MOLT4 are T-cell acute lymphoblastic leukemia; MT-4, KOB, MT-1 and ST1 are T-cell adult leukemia; Raji and BJAB are non-Hodgkin lymphomas; K562 is chronic myeloid leukemia; HL60 is promyelocyte leukemia; U937 is histiocytic lymphoma; MKN1, MKN7, MKN28 and MKN74 are gastric adenocarcinomas; PC3 is prostatic cancer; PC8, PC9 and PC10 are squamous cell lung carcinoma; PC13 is large cell lung carcinoma; NCI-H69 is small cell lung carcinoma; PC36 и A375 are melanomas; KB is epidermoid oral cavity carcinoma; KATO III is gastric carcinoma; SW480, HT-29 and Colo320DM are colon adenocarcinomas; TE2 is esophageal adenocarcinoma; T24 is bladder transitional cell carcinoma; HS683 and J889H are glioblastomas; SK-N-MC is neuroblastoma; SW480 is colon adenocarcinoma.

3. Pyrimidine nucleoside analogues modified at the 3′ position of the carbohydrate moiety

3′-Substituted pyrimidine nucleoside derivatives have attracted the interest of scientists for several decades. To date, many nucleoside analogues have been synthesized; however, unlike the compounds presented in the previous Section, none of them have been approved for cancer treatment (Fig. 6).

3′-Ethynyl-substituted nucleosides **14**, **117–120** were synthesized by condensation of sugar **121**, obtained in six steps from *D*-xylose,[102,103] with silylated nitrogenous bases under Vorbruggen reaction conditions.[104] Bis(trimethylsilyl)cytosine was reacted with compound **121** in the presence of tin tetrachloride in acetonitrile (Scheme 22). The target 2′,3′,5′-tri-*O*-benzoyl-3′-ethynylcytidine (**122**) was obtained as the major product in high yield.[103] Condensation of sugar **121** and the

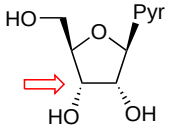
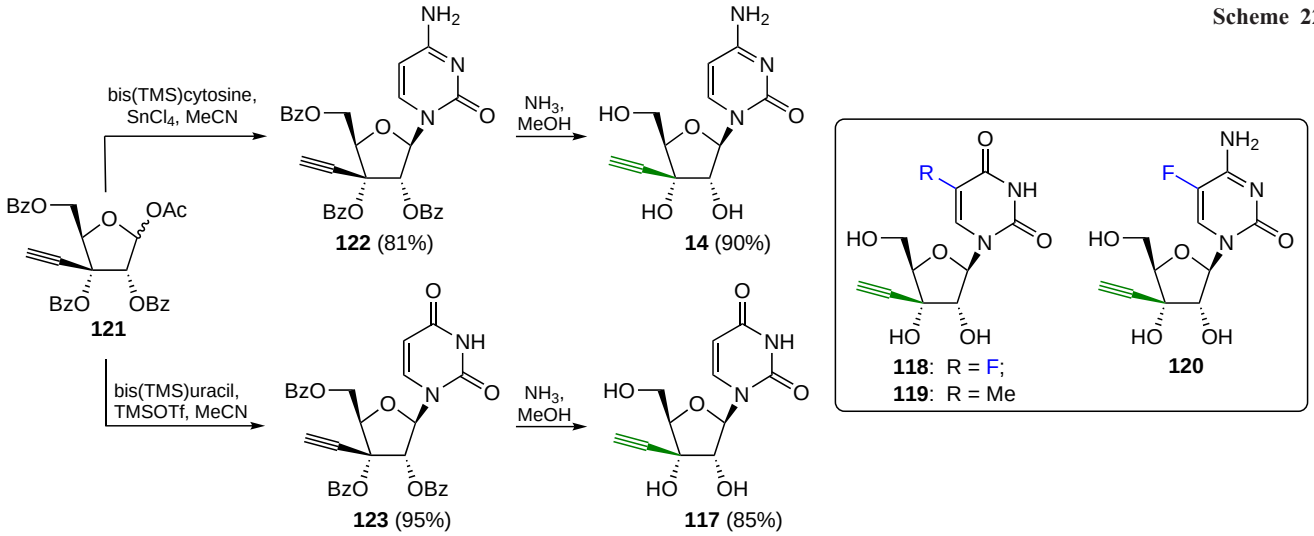
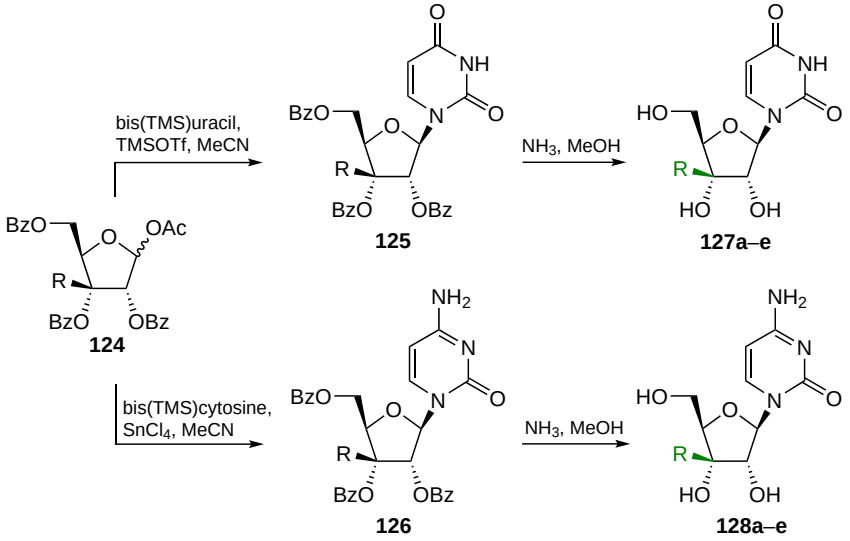


Figure 6. General structure of pyrimidine nucleosides. The arrow indicates the position of the 3′ carbohydrate moiety.

Scheme 22



Scheme 23



Product	R	Yield, %	
		127	**128**
a	C≡CMe	81	72
b	C≡CEt	97	68
c	CH=CH$_2$	98	86
d	Et	90	89
e	cyclopropyl	83	94

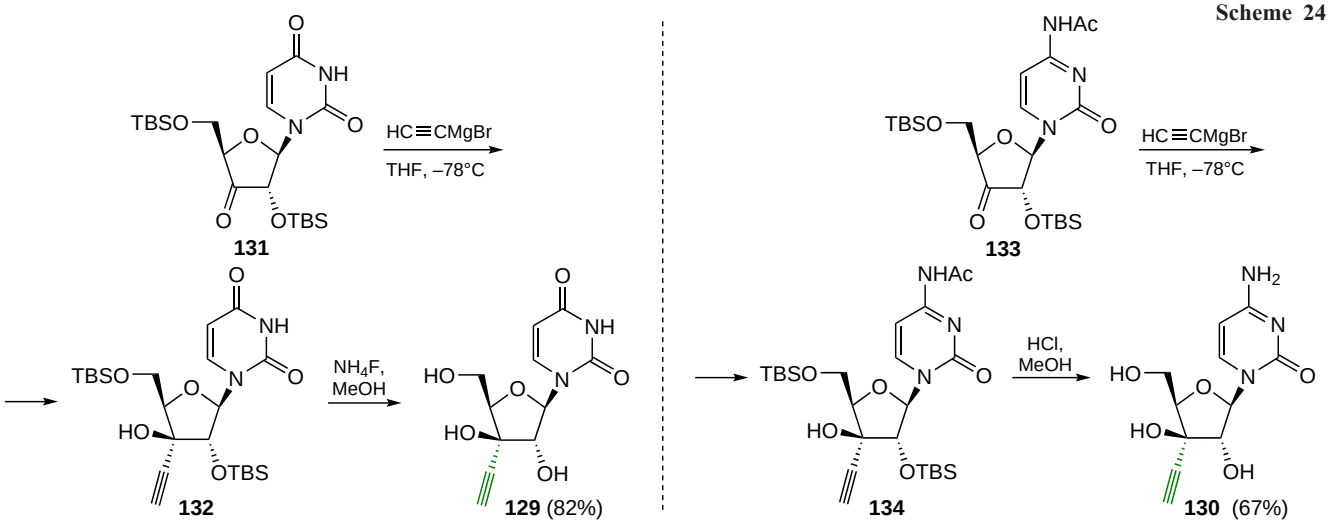
corresponding pertrimethylsilylated nucleobases (5-fluorocytosine, 5-fluorouracil, thymine) under the same conditions afforded the corresponding protected nucleoside precursors **118**–**120** (the structures are shown in the lower part of Scheme 22) in high yields. The reaction of bis(trimethylsilyl) uracil under the conditions described above yielded a mixture of N^1- and N^3-nucleosides in yields of 58% and 20%, respectively. However, if TMSOTf (TfO is trifluoromethanesulfonate (triflate)) was used as the Lewis acid instead of tin tetrachloride, the yield of product **123** reached 95%. To remove the protecting groups, nucleosides **122** and **123** were treated with ammonia in methanol at room temperature, and 3′-*C*-ethynyl nucleosides **14** (Ref. 105) and **117** were isolated in crystalline form in high yields. Compounds **118**–**120** were prepared in a similar manner.

A further development of this area was the synthesis of uridine and cytidine derivatives containing propyn-1-yl, butyn-1-yl, vinyl, cyclopropyl, and ethyl groups at position 3′.[103] The synthesis of such compounds, shown in Scheme 23, involved the condensation of silylated uracil or cytosine with acylated sugar **124** in acetonitrile in the presence of TMSOTf (in the case of uracil) or $SnCl_4$ (in the case of cytosine), followed by deprotection of intermediates **125** and **126** with NH_3 in methanol. This gave 3′-*C*-substituted uridine derivatives **127a**–**e** (yield 81–98%) and cytidine derivatives **128a**–**e** (yield 68–94%).

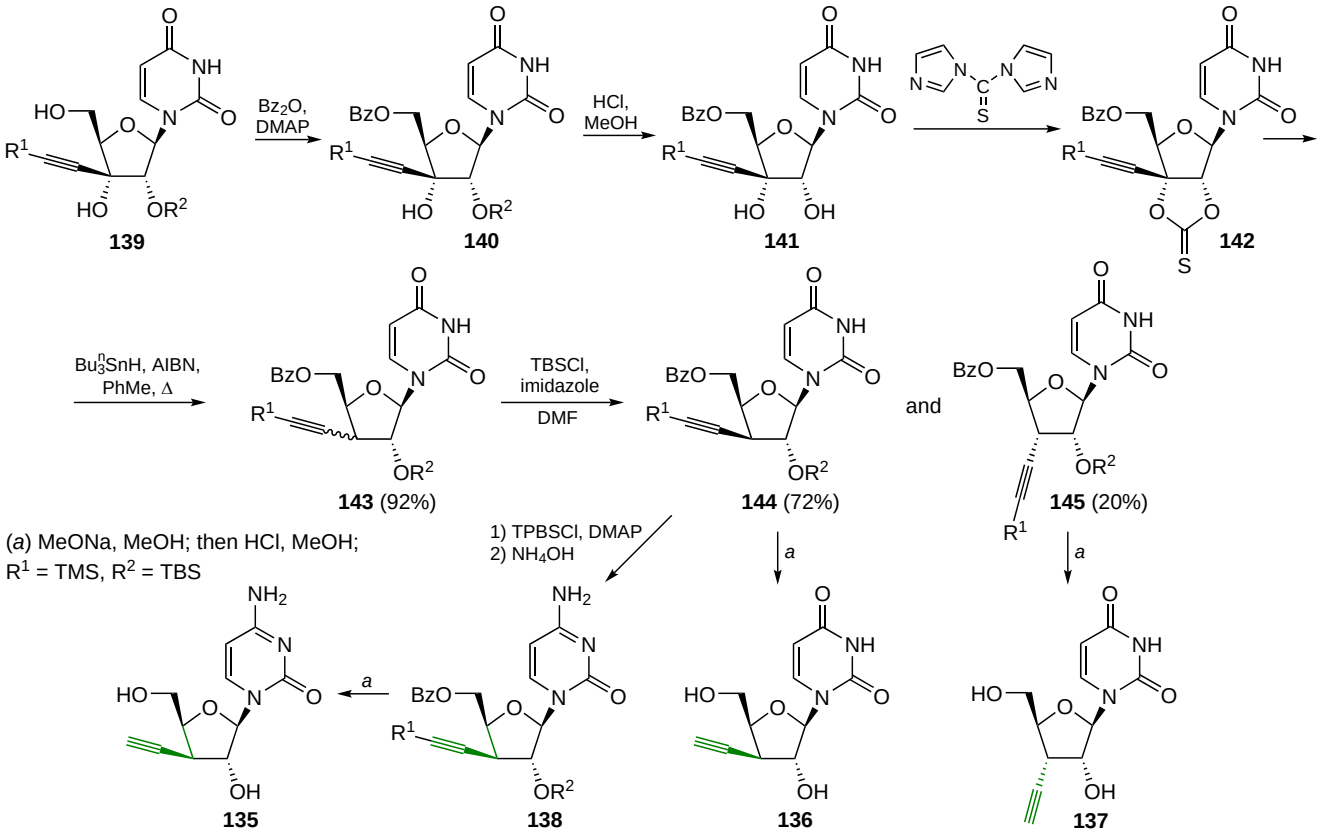
1-(3′-*C*-ethynyl-α-*D*-xylo-furanosyl)pyrimidines **129** and **130** (Scheme 24), which are 3′-epimers of compounds **14** and **117**, were also synthesized. The reaction of the 2′,5′-di-*O*-*tert*-butyldimethylsilyl-3′-ketouridine derivative (**131**) with HC≡CMgBr in THF at –78°C gave the *xylo*-adduct **132** with high stereoselectivity.[106,107] Similarly, the N^4-acetyl-3′-ketocytidine derivative **133** was converted to xylofuranoside **134**. Removal of the protecting groups in compounds **132** and **134** under standard conditions led to the corresponding 3′-*C*-ethynyl-β-*D*-xylofuranosyl derivatives of uracil (**129**) and cytosine (**130**).[103]

To determine the role of the 3′-hydroxyl group of compounds **14** and **117** in cytotoxicity, their 3′-deoxy analogues **136**–**138** were obtained (Scheme 25). In the starting compound **139** (Ref. 108), the 5′-hydroxy group was protected by the action of benzoic anhydride in acetonitrile in the presence of DMAP, which gave derivative **140**. Subsequent acid hydrolysis of the 2′-*O*-*tert*-butyldimethylsilyl group with a solution of HCl in MeOH afforded compound **141** (yield 56%). Treatment of this intermediate with 1,1′-thiocarbonyldiimidazole in acetonitrile afforded 2′,3′-cyclic thiocarbonate **142**, which was refluxed without further purification in the presence of tri-*n*-butylstannane and AIBN in toluene. This afforded an inseparable mixture of epimeric 3′-deoxy derivatives **143** in a ratio of ~4:1 in a high

Scheme 24



Scheme 25



yield. The mixture was treated with *tert*-butylchlorodimethylsilane in the presence of imidazole in DMF and then separated by silica gel column chromatography. This furnished protected nucleosides **144** and **145**, with the former predominating. Compound **144** was converted to the cytosine derivative **138** by reaction with 2,4,6-triisopropylbenzenesulfonyl chloride in acetonitrile in the presence of DMAP, followed by treatment with NH_4OH. Removal of the protecting groups in epimers **144** and **145** afforded the target nucleosides **136** and **137** in quantitative yields.[60]

A study of the antitumour activity of compounds **14** and **117**–**120** *in vitro* showed compounds **14** and **117** to be the most potent inhibitors of the proliferation of L1210 and KB tumour cells (Table 2).[103] The activity of these derivatives **14** and **117** was also assessed against 36 human solid tumour cell lines. Cytidine analogue **14** demonstrated efficiency against 22 cell lines, with IC_{50} values ranging from nanomolar to subnanomolar, and was inactive against two cell lines. At the same time, the uridine analogue **117** had strong inhibitory activity against 12 cell lines with IC_{50} values in the subnanomolar range and was ineffective against six cell lines.[103]

In vitro studies of the antitumour activity of compounds **127a**–**e** and **128a**–**e** revealed that they either inhibited the growth of L1210 and KB cell lines 3–4 orders of magnitude worse than analogues **14** and **117**, or were completely inactive. Evaluation of the efficiency of nucleoside analogues **136**–**138** in suppressing L1210 and KB cell growth showed that these compounds had no activity at concentrations below 350 μM.[60]

Table 2. Inhibition of tumour cell proliferation *in vitro* by 1-(3′-*C*-ethynyl-β-*D*-ribofuranosyl)nucleosides.

Compound	IC_{50}, μM (cell culture)	Ref.
14	0.016 (L1210), 0.028 (KB), 0.21 (MKN-28), 0.0088 (MKN-45), 0.031 (KATO III), 0.024 (NUGC-4), 0.047 (ST-KM), 0.021 (KKLS), 0.15 (STSA-1), 0.17 (NAKAJIMA), 0.02 (Colo320DM), 0.0082 (HCT-15), >1.9 (SW-48), 0.26 (SW-480), 0.090 (PC-8), 0.11 (PC-9), 0.16 (PC-10), 0.045 (QG-56), 0.21 (QG-95), 0.032 (Lu-65), 0.13 (QG-90), 0.39 (QG-96), 0.12 (NCI-H-82), 0.043 (NCI-H-417), 0.024 (MDA-MB-321), 0.16 (YMB-1-E), 0.069 (MCF-7), >1.9 (PANC-1), 0.015 (Mia-PaCa-2), 0.028 (T24), 0.12 (KK-47), 1.0 (HOS), 0.15 (MG-63), 0.033 (A-431), 0.073 (HT-1080), 0.026 (A 375), 0.047 (SK-MEL-28)	60, 103
117	0.13 (L1210), 0.029 (KB), 0.05 (MT-4), 0.065 (MKN-28), 0.052 (MKN-45), 0.031 (KATO III), 0.22 (NUGC-4), 0.086 (ST-KM), 0.23 (KKLS), 1.9 (STSA-1), 0.78 (NAKAJIMA), 0.06 (Colo320DM), 0.075 (HCT-15), >1.9 (SW-48), 0.34 (SW-480), 0.33 (PC-8), 0.19 (PC-9), >1.9 (PC-10), 0.15 (QG-56), 0.28 (QG-95), 0.089 (Lu-65), 1.0 (QG-90), 0.38 (QG-96), 0.23 (NCI-H-82), 0.11 (NCI-H-417), >1.9 (MDA-MB-321), >1.9 (YMB-1-E), 0.2 (MCF-7), >1.9 (PANC-1), 0.054 (Mia-PaCa-2), 0.21 (T24), 0.089 (KK-47), 1.8 (HOS), >1.9 (MG-63), 0.29 (A-431), 0.16 (HT-1080), 0.021 (A 375), 0.086 (SK-MEL-28)	60, 103, 108
118	2.5 (L1210), 1.4 (KB)	103
119	>300 (L1210), >300 (KB)	103
120	0.53 (L1210), 0.46 (KB)	103

Notes. Human cell line designations: MKN45, ST-KM, NUGC-4, KKLS, STSA-1 and NAKAJIMA are gastric adenocarcinomas; HCT-15 and SW48 are colon adenocarcinomas; QG-56 and QG-95 are squamous lung carcinomas; Lu-65 is large cell lung carcinoma; QG-90, QG-96, NCI-H-82 and NCI-H-417 are small cell lung adenocarcinomas; MDA-MB-321, YMB-1-E are breast adenocarcinomas; PANC-1 is pancreatic adenocarcinoma; KK-47 is bladder transitional cell carcinoma; HOS and MG-63 are osteosarcomas; A-431 is leiomyosarcoma; SK-MEL-28 is oral cavity carcinoma.

Other analogues of ribo-, arabino- and deoxyribonucleosides containing various substituents at position 3′ (methyl, fluoromethyl, hydroxymethyl, aminomethyl, azidomethyl and other groups) were also obtained;[109] however, they also did not exhibit noticeable cytotoxic properties *in vitro* even at the highest concentration (100 μM).

In vivo studies of compounds **14** (0.25 mg/kg dose) and **117** (2 mg/kg) in various human tumours as xenografts in nude mice demonstrated a more potent antitumour effect of these analogues compared to 5-fluorouracil (15 mg/kg).[103] Each of the doses for **14**, **117**, and FU used in this study represented the respective maximum nontoxic dose.[103,105]

The most interesting representative of the 3′-modified pyrimidine nucleoside analogues is 1-(3′-*C*-ethynyl-β-*D*-ribofuranosyl)cytosine (3′-ethynylcytidine (ECyd), TAS-106, **14**) (see Fig. 2), which has demonstrated antiproliferative activity both *in vitro* and *in vivo*.[103,110–114] The cytotoxic mechanisms of compound **14** have been shown to be primarily related to the inhibition of RNA synthesis.[110,111,115] This nucleoside analogue is converted by uridine cytidine kinase (UCK, EC 2.7.1.48 enzyme classification) to 3′-ethynylcytidine-5′-monophosphate,[116] which is then phosphorylated to 3′-ethynylcytidine-5′-diphosphate (ECDP) and finally to 3′-ethynylcytidine-5′-triphosphate. The UCK family consists of two enzymes, UCK1 and UCK2 (Ref. 117), and catalyzes the phosphorylation of uridine and cytidine to uridine 5′-monophosphate and cytidine 5′-monophosphate, respectively. These enzymes also catalyze the phosphorylation of several nucleoside analogues, such as 6-azauridine, 5-fluorouridine, and 5-methylcytidine.[118,119] UCK2 has been shown to be responsible for the phosphorylation of ECyd (**14**) under biological conditions.[120,121] 3′-Ethynylcytidine, which contains a covalently linked ethynyl group, exhibits pronounced cytotoxicity and radiosensitizing activity. As an analogue of cytidine, in the form of its 5′-triphosphate, it acts as a non-selective competitive inhibitor of the biosynthesis of all three RNA polymerases, thereby suppressing RNA synthesis.[21,122] 3′-*C*-Ethynylcytidine is an effective inhibitor of proliferation of >40 types of cultured cancer cells, as well as human solid tumours xenografted into mice.[21,116] This drug has undergone phase I and phase II clinical trials under the commercial codes NCT00752011 and NCT00737360.[21] Phase I trials concluded that ECyd (**14**) can be administered either as an infusion or as a bolus injection, and that the main dose-limiting side effect is its neurotoxicity.[123–125] Phase II clinical trials showed no significant benefit from monotherapy with this drug. Further studies were ceased due to lack of efficiency and the occurrence of side effects.[126,127]

4. Pyrimidine nucleoside analogues modified at the 4′-position of the carbohydrate moiety

The literature describes the synthesis of pyrimidine nucleoside analogues modified at the 4′ position of the carbohydrate moiety (Fig. 7).

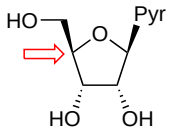
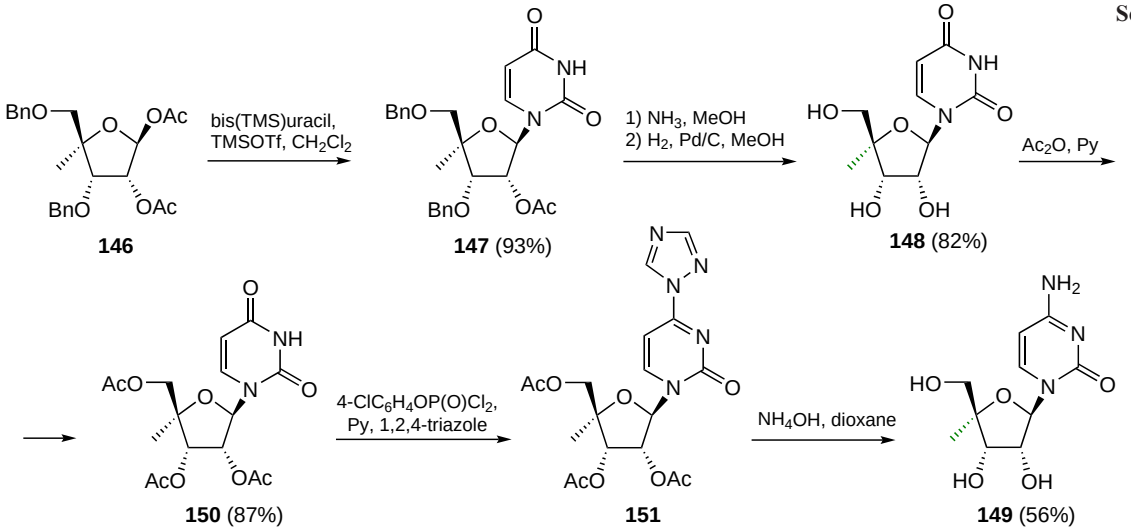


Figure 7. General structure of pyrimidine nucleosides. The arrow indicates the 4′ position of the carbohydrate moiety.

4.1. 4′-Methyl pyrimidine nucleosides

The synthesis of 4′-C-methyl derivatives consisted of the condensation of 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-methyl-β-*D*-ribofuranose (**146**) with silylated uracil under the conditions of the Vorbruggen method (TMSOTf, dichloromethane, room temperature, 2 h), leading to 2′-*O*-acetyl-3′,5′-di-*O*-benzyl-4′-*C*-methyluridine (**147**), the yield of which was 93% after chromatographic purification (Scheme 26).[128] Deacetylation of compound **147** with saturated methanolic ammonia and subsequent debenzoylation by hydrogenolysis on Pd/C in methanol afforded 4′-*C*-methyluridine (**148**). To obtain 4′-*C*-methylcytidine (**149**), nucleoside **148** was converted to 2′,3′,5′-tri-*O*-acetyl-4′-*C*-methyluridine (**150**), which was treated with 4-chlorophenyl phosphorodichloridate in the presence of

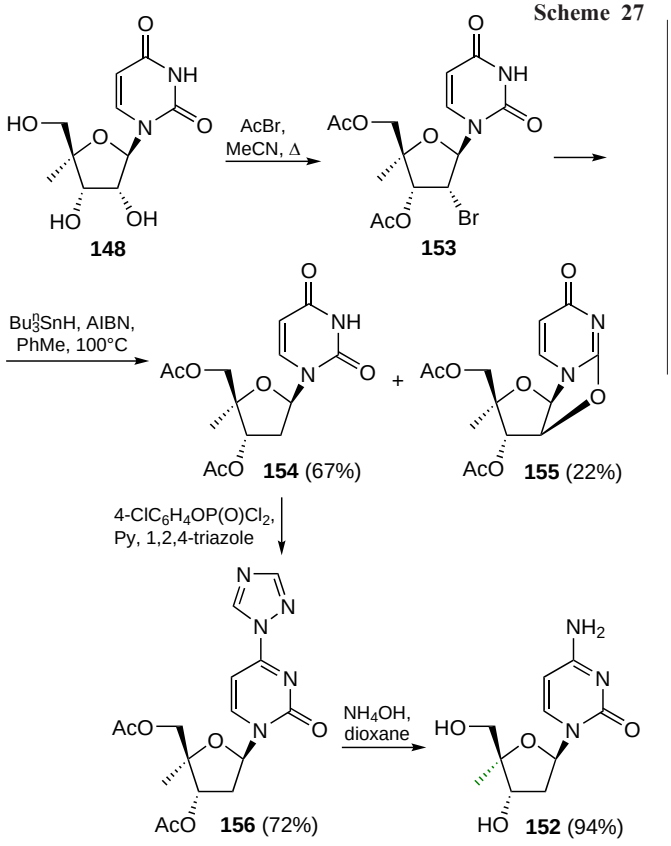
Scheme 26



1,2,4-triazole in pyridine. This gave 1-(2′,3′,5′-tri-*O*-acetyl-4′-*C*-methyl-β-*D*-ribofuranosyl)-4-(1,2,4-triazol-1-yl)-1*H*-pyrimidin-2-one (**151**) (yield 91%), which was dissolved in a mixture of NH_4OH-dioxane and stirred for 3 h at room temperature to afford the target 4′-*C*-methylcytidine (**149**) in moderate yield.[128]

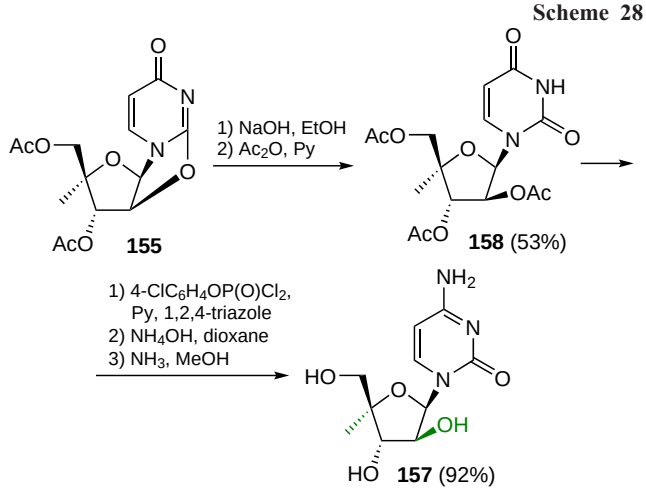
The synthesis of 2′-deoxy-4′-*C*-methylcytidine (**152**) commenced with the treatment of the starting 4′-C-methyluridine (**148**) with excess acetyl bromide in boiling acetonitrile, which furnished 2′-bromo-2′-deoxy derivative **153** (Scheme 27).[129] Heating a solution of compound **153** in toluene in the presence of tributylstannane and AIBN gave a mixture of 3′,5′-di-*O*-acetyl-2′-deoxy-4′-*C*-methyluridine (**154**) and 3′,5′-di-*O*-acetyl-4′-*C*-methyl-2,2′-anhydrouridine (**155**), with the former predominating. Using the methods described above, nucleoside **154** was converted first to triazolide **156** and then to the target 2′-deoxy-4′-*C*-methylcytidine (**152**).

Scheme 27



The reaction sequence used to prepare 1-(4′-*C*-methyl-β-*D*-arabinofuranosyl)cytosine (**157**) is shown in Scheme 28.[129] The starting 3′,5′-di-*O*-acetyl-4′-*C*-methyl-2,2′-anhydrouridine (**155**) was treated sequentially with 1N NaOH in ethanol and acetic anhydride in pyridine to afford 1-(2′,3′,5′-tri-*O*-acetyl-4′-*C*-methyl-β-*D*-arabinofuranosyl)uracil (**158**) in moderate yield. The latter was treated with 4-chlorophenyl phosphorodichloridate in the presence of 1,2,4-triazole, followed by reaction with NH_4OH-dioxane and then with NH_3 in MeOH gave the desired nucleoside **157** in high yield.[129]

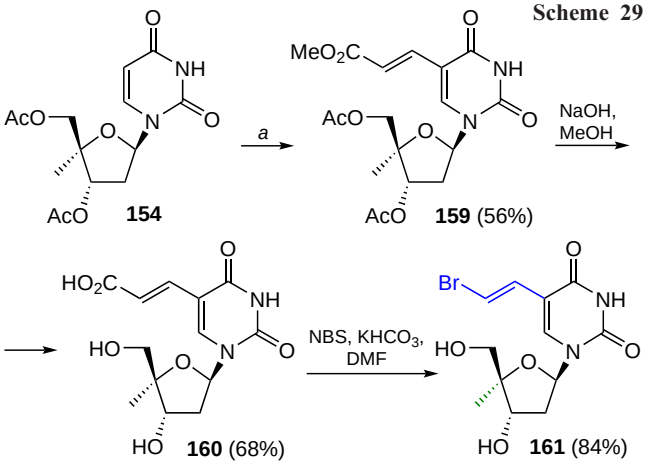
Scheme 28



Iodination of the 5-position of the uracil residue in compound **154** with iodine in the presence of cerium(IV)-ammonium nitrate (CAN)[130] followed by the Heck reaction with methyl acrylate afforded the 5-methylacrylate derivative **159** in 56% yield. Compound **159** was hydrolyzed under alkaline conditions; acidification of the reaction mixture with HCl afforded carboxylic acid **160**, which was purified using reverse-phase octadecylsilyl (ODS) ion-exchange resin column chromatography. Final treatment of compound **160** with anhydrous $KHCO_3$ and *N*-bromosuccinimide (NBS) in DMF[131] gave the target product **161** in high yield (Scheme 29).[132]

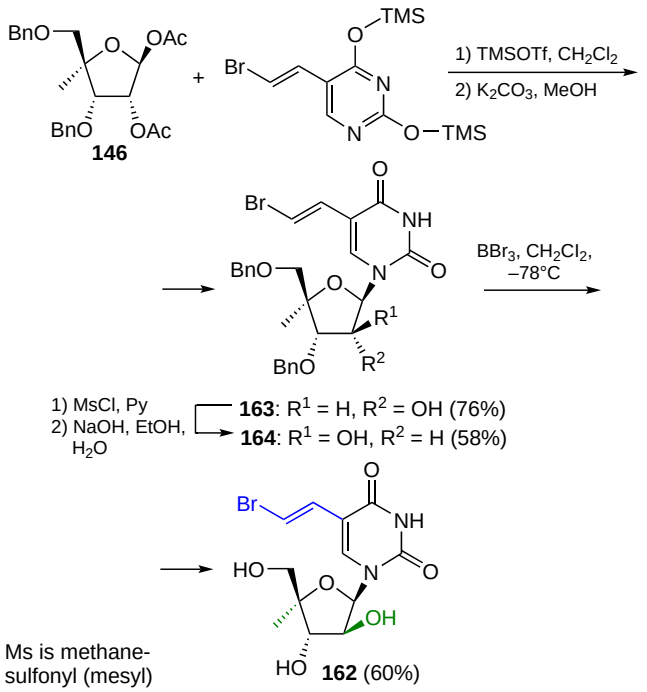
The synthetic route to 5-(2-bromovinyl)-1-(4′-*C*-methyl-β-*D*-arabinofuranosyl)uracil (**162**)[132] is shown in Scheme 30. Di-*O*-benzylated ribonucleoside **163** was prepared by condensation of protected 4-*C*-methyl-*D*-ribose **146** with silylated

Scheme 29



(*a*) 1) I_2, CAN, MeCN; 2) $CH_2{=}CHCO_2Me$, $Pd(OAc)_2$, PPh_3, Et_3N, dioxane

Scheme 30

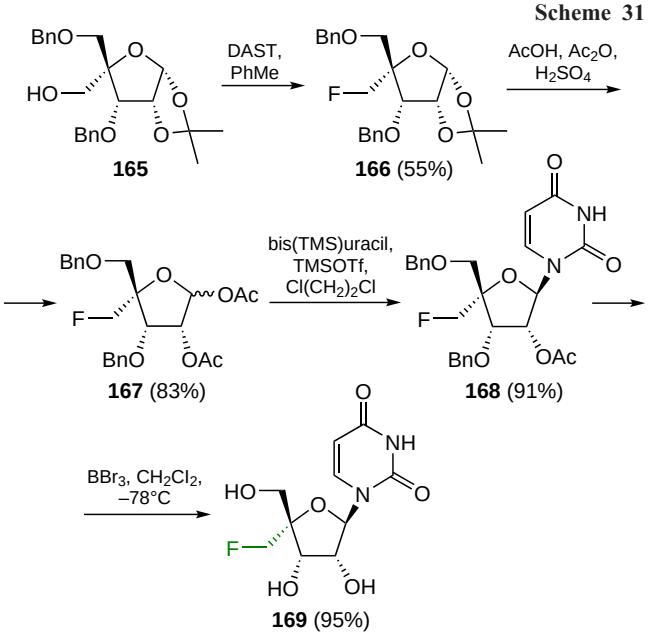


5-bromovinyluracil in the presence of TMSOTf followed by treatment with potassium carbonate in methanol (yield 76% over two steps). Compound **163** was converted to an intermediate mesylate, which, when treated with an aqueous alcoholic NaOH solution, gave arabinonucleoside **164**. Debenzoylation of the latter with BBr_3 in dichloromethane at –78°C and subsequent quenching with a saturated Na_2CO_3 solution afforded the target analogue **162** in moderate yield.

4.2. 4′-Fluoromethyl pyrimidine nucleosides

The preparation of nucleoside analogues with a fluoromethyl group at the 4′-position has been reported.[133] The starting protected 4-*C*-hydroxymethyl-*D*-ribofuranose (**165**) was treated with DAST in toluene at 60°C to give the corresponding 4′-fluoromethyl derivative **166** in moderate yield. Subsequent acetolysis of this compound gave a mixture of α- and β-anomers of 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-4-*C*-fluoromethylribofuranose (**167**), which was condensed in 1,2-dichloroethane

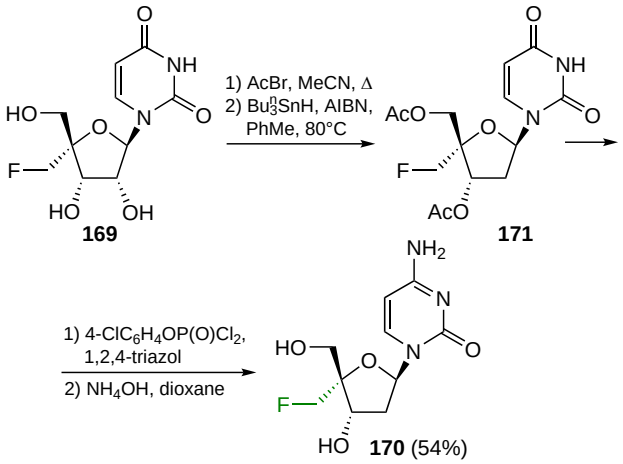
Scheme 31



with silylated uracil in the presence of TMSOTf. After chromatographic purification, 2′-*O*-acetyl-3′,5′-di-*O*-benzyl-4′-*C*-fluoromethyluridine (**168**) was isolated in high yield. Compound **168** was treated with BBr_3 in dichloromethane at –78°C to afford 4′-C-fluoromethyluridine (**169**) in 95% yield (Scheme 31).[133]

The first step in the synthesis of 2′-deoxy-4′-fluoromethylcytidine (**170**), shown in Scheme 32, involved treating ribonucleoside **169** with acetyl bromide in boiling acetonitrile and subsequent heating in the presence of tributylstannane and AIBN in toluene at 80°C to afford 2′-deoxy-3′,5′-di-*O*-acetyl-4′-fluoromethyluridine (**171**). Compound **171** was treated sequentially with 4-chlorophenyl phosphorodichloridate, then with 1,2,4-triazole and NH_4OH in dioxane. This gave 2′-deoxy-4′-fluoromethylcytidine (**170**) in 54% yield.[133]

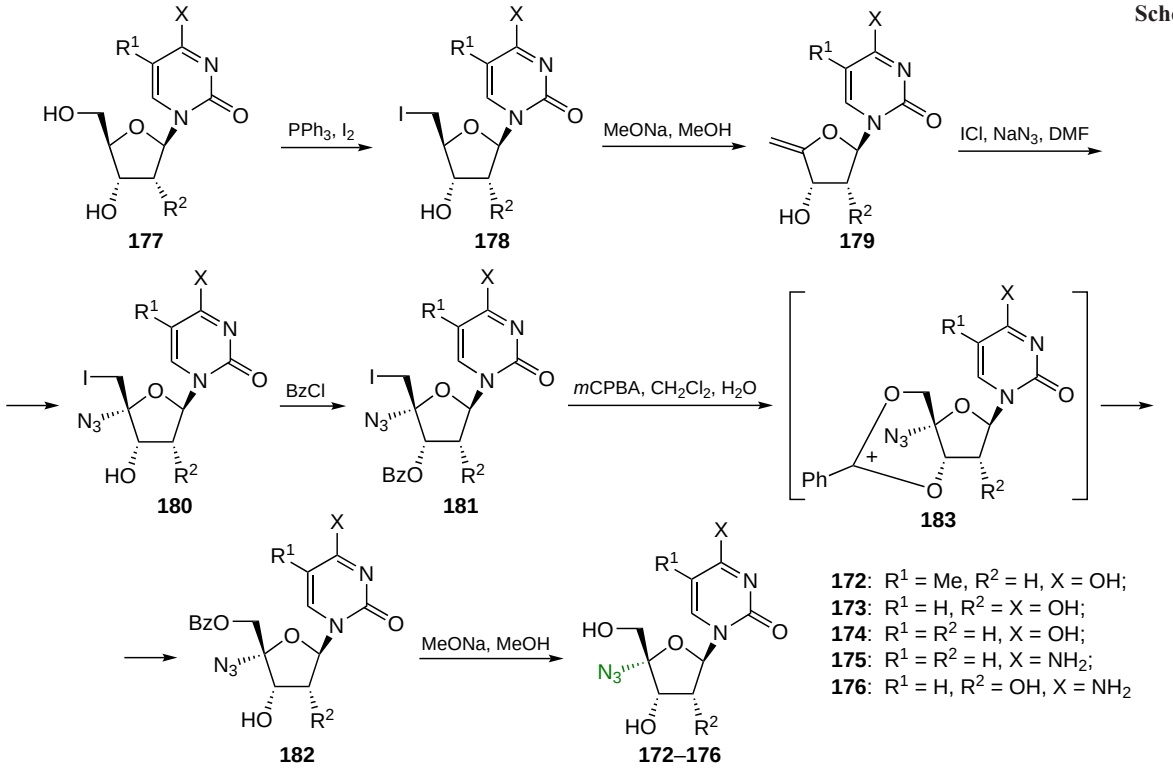
Scheme 32



4.3. 4′-Azidopyrimidine nucleosides

4′-*C*-Azido-β-*D*-ribofuranosyl nucleosides and their 2′-deoxy derivatives **172**–**176** were prepared in several steps (Scheme 33).[134] The synthesis of such analogues began with the conversion of the starting nucleosides **177** to 5′-deoxy-5′-iodo derivatives **178** using the PPh_3–I_2 system. Subsequent treatment with sodium methoxide in methanol afforded compounds **179**, which were reacted with ICl and sodium azide in DMF. This

Scheme 33



afforded the target 4′-*C*-azido derivatives **180** as a mixture with the by-product 4′-epimer in a ratio of ~20 : 1.[134]

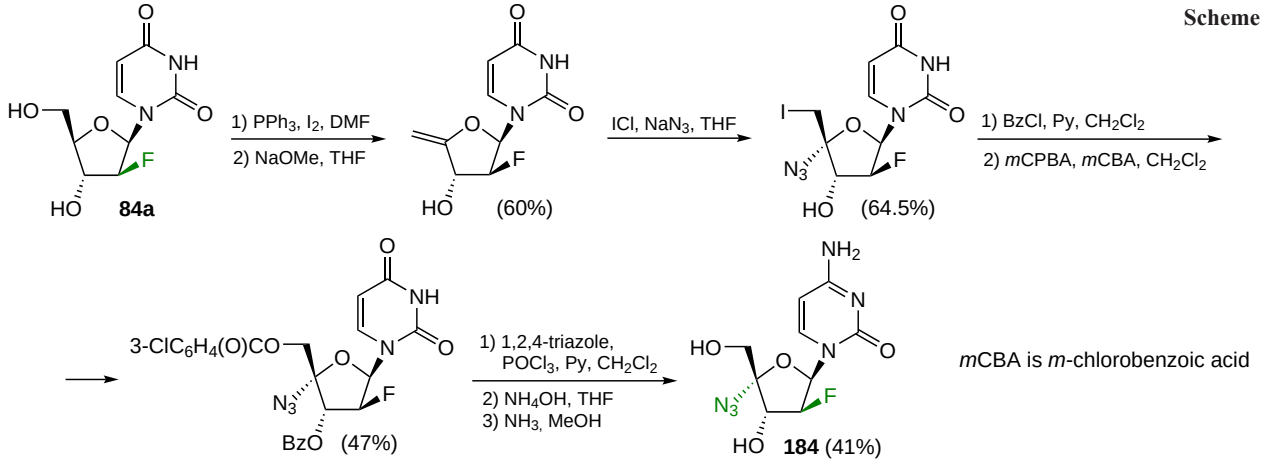
The conversion of the iodine atom at position 5′ to a hydroxyl group is the greatest challenge. The reactivity of this iodine atom is reduced if the 4′ carbon atom contains an electron-withdrawing substituent (*e.g.*, an oxygen atom). To stabilize the nucleoside, it should be converted to 3′-*O*-benzoyl derivative **181** *via* esterification, after which the iodine atom can be activated by oxidation to the corresponding hypervalent state. Treatment of 4′-*C*-azido-3′-*O*-benzoyl-5′-deoxy-5′-iodine nucleoside **181** with *m*-chloroperoxybenzoic acid (*m*CPBA) in water-saturated dichloromethane was found to yield a number of products, with 5′-*O*-benzoyl-3′-hydroxy derivative **182** being isolated as the major component of the mixture (see Scheme 33). This compound is likely formed from the 3′,5′-cyclic benzoxonium ion **183** by hydrolysis. The target nucleosides **172**–**176** were obtained in the individual state by hydrolysis of compounds **182** in the presence of a base followed by chromatographic purification.[134]

Similarly, azvudine[13] (**184**) was synthesized from 2′-deoxy-2′-fluoroarabinouridine (**84a**), obtained in three steps from 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-*D*-arabinofuranoside in 78% yield (Scheme 34).[135]

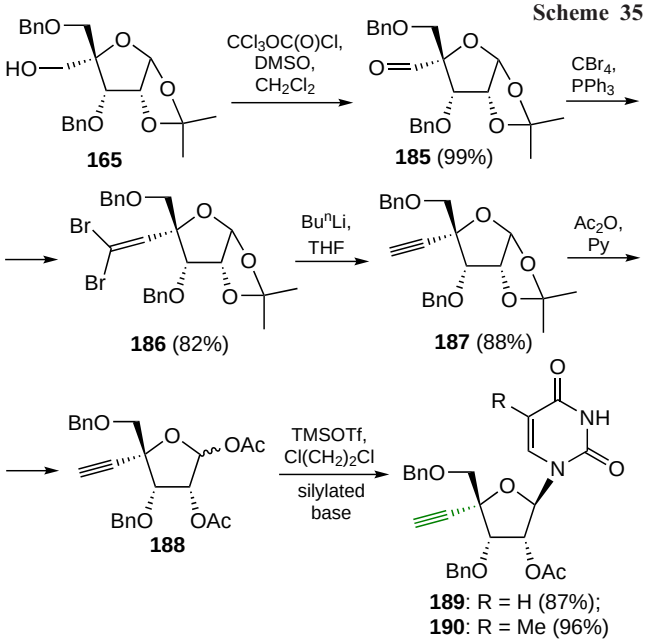
4.4. Derivatives with unsaturated hydrocarbon substituents at the 4′-position

A series of nucleosides with an acetylene group at the 4′ position of the carbohydrate moiety were obtained.[136] The synthesis of such compounds involved several steps (Scheme 35). In the first step, the starting riboside **165** (Ref. 128) was oxidized with a DMSO-diphosgene mixture in dichloromethane, which gave 4-aldehyde **185** in quantitative yield. It was treated with CBr_4 and PPh_3 under the Wittig reaction conditions to afford 4-(2,2-dibromovinyl) derivative **186**. Treatment of the latter with *n*-butyllithium in THF gave 4-ethynyl-containing carbohydrate **187**, which was hydrolyzed and then acetylated with the Ac_2O-Py system to form the 1,2-di-*O*-acetyl derivative

Scheme 34



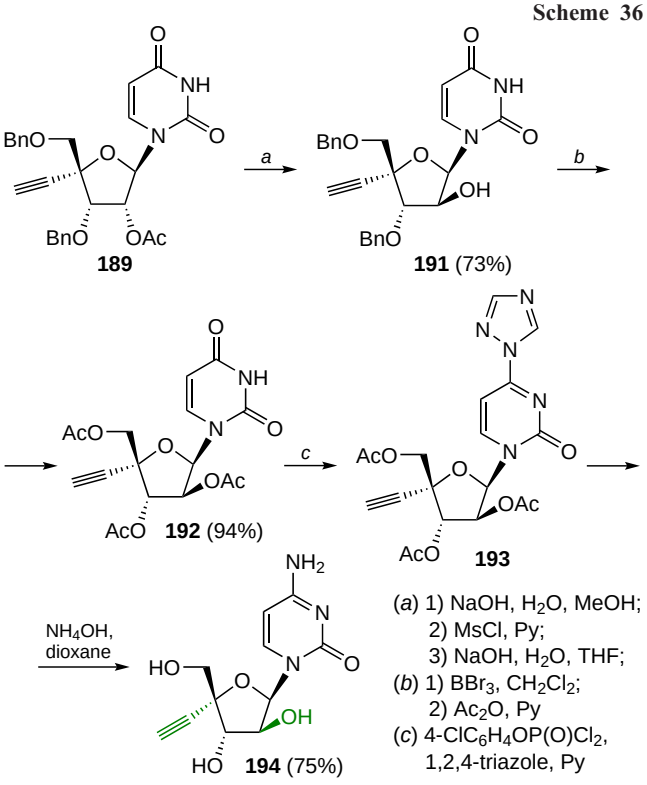
Scheme 35



188 (yield 88%). Further condensation with a silylated pyrimidine base (thymine or uracil) was carried out in the presence of TMSOTf in 1,2-dichloroethane. Uracil (**189**) and thymine (**190**) derivatives were isolated in high yields.[136]

The second step in the synthesis of the target nucleosides involved the transformation of the ribofuranosyl moiety of compound **189** into an arabinoside (Scheme 36).[136] For this purpose, nucleoside **189** was successively subjected to alkaline hydrolysis, treatment with MsCl in pyridine, and a NaOH solution in aqueous THF, which afforded 1-(4′-*C*-ethynyl-β-*D*-arabinofuranosyl)uracil (**191**) in good yield. Further reaction with BBr_3 in dichloromethane and subsequent acetylation gave the 2′,3′,5′-tri-*O*-acetyl derivative **192**, which was treated with

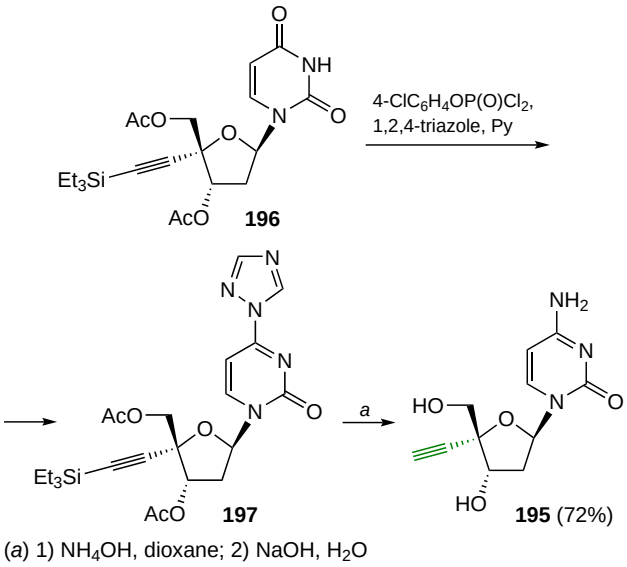
Scheme 36



4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine to form the corresponding 4-triazole derivative **193**. The latter was converted to the target 1-(4′-*C*-ethynyl-β-*D*-arabinofuranosyl)cytosine (**190**) under basic conditions.

2′-Deoxy-4′-*C*-ethynylcytidine (**195**) was synthesized from 3′,5′-di-*O*-acetyl-4′-*C*-triethylsilylethynyl-2′-deoxyuridine (**196**) (Scheme 37).[136] Starting compound **196** was treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine, which delivered the 4-triazole derivative **197**. Further steps of reaction with NH_4OH in dioxane and alkaline hydrolysis gave rise to the target 2′-deoxy-4′-*C*-ethynylcytidine (**195**) in moderate yield.

Scheme 37

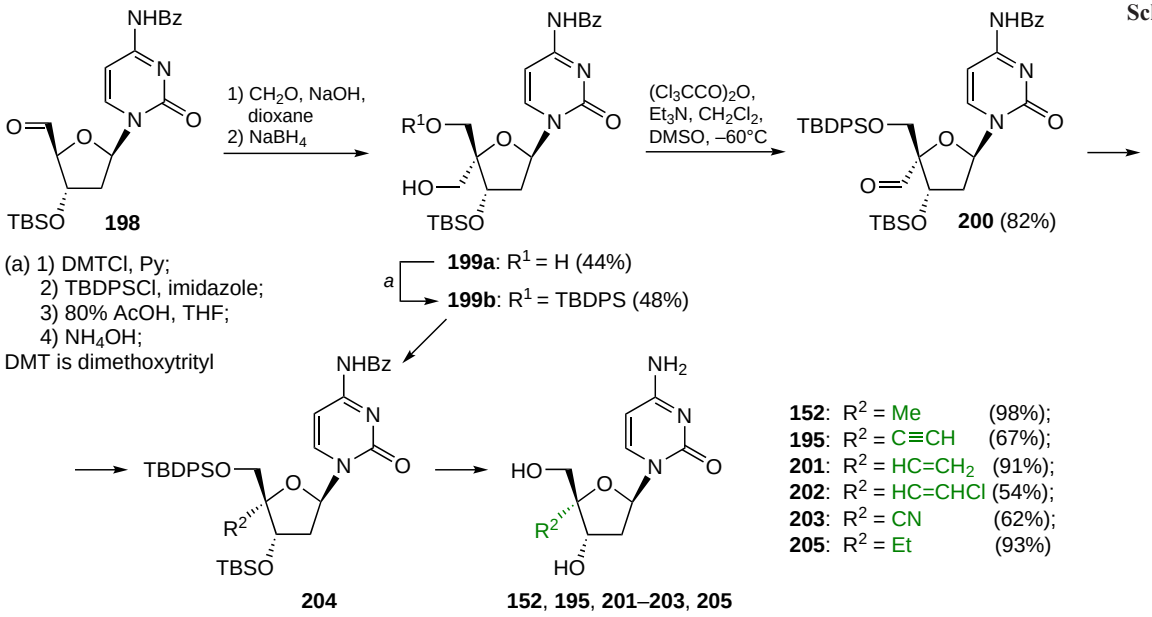


Scheme 38 shows the synthetic route to 4′-substituted 2′-deoxycytidines. Aldehyde **198** was the starting compound, which was treated with an aqueous solution of formaldehyde and NaOH in dioxane for 10 min at room temperature. Subsequent reduction of the aldehyde function at position 4′ with $NaBH_4$ afforded compound **199a**. Intermediate compound **199**, obtained by manipulation with the protecting groups, was oxidized to the corresponding 4′-C-aldehyde **200** under the Swern reaction conditions.[137] Intermediate **200** was isolated as crystals and used for the synthesis of the target nucleosides **152**, **195**, **201**–**205**.[138]

When subjected to the Wittig reaction with $Ph_3P{=}CH_2$ and $Ph_3P{=}CHCl$, compound **200** gave 4′-*C*-ethenyl and 4′-*C*-(2-chloroethenyl) derivatives **204**, ($R^2 = CH{=}CH_2$ and $R^2 = CH{=}CHCl$ respectively), with an isomer ratio of Z : E = 20 : 1; deprotection delivered the target nucleosides **201** and **202** in good yields. Compound **204** ($R^2 = CH{=}CHCl$) was further treated with *n*-butyllithium in THF at –78°C to give debenzoylated 4′-*C*-ethynyl derivative deprotection of which led to **195**.

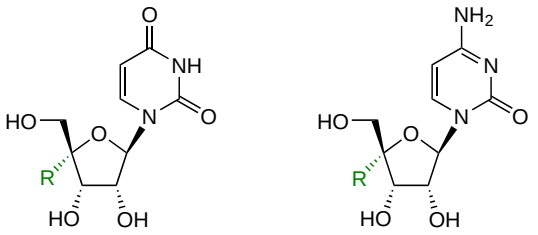
Compound **200** was converted to the oxime **204** ($R^2 = CH{=}NOH$), which was then dehydrated in a mixture of NaOAc and Ac_2O and debenzoylated with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in MeOH to form 4′-*C*-cyanide, which was deprotected to give product **203**. 4′-*C*-Methyl derivative **152** was prepared from compound **199b** using the I_2–imidazole–triphenylphosphine system *via* the 4′-*C*-iodomethyl analogue, which was hydrogenated on Pd/C in the presence of Et_3N. Hydrogenation of compound **201** on Pd/C gave the 4′-C-ethyl derivative **205** (see Scheme 38).

Scheme 38



Ribonucleoside analogues were also synthesized: 4′-*C*-ethynyluridine (**206**), 4′-*C*-cyanouridine (**207**), 4′-*C*-ethynylcytidine (**208**) and 4′-*C*-cyanocytidine (**209**).

Structures 206–209



An *in vitro* study of the antitumour activity of 4′-modified nucleosides showed that compound **170** (see Scheme 32) exhibited significant activity against CCRF-HSB-2 lymphoblastic leukemia cells and had no significant effect on the growth of KB human oral carcinoma cells (Table 3).[133] Cytosine derivatives **175** and **176** (see Scheme 33) performed good in inhibiting the growth of the A3.01 cell line, a childhood acute lymphoblastic leukemia: complete inhibition of cell growth was recorded at IC_{100} concentrations of 1.9 and 74.1 μM, respectively.[134]

Data on the *in vitro* anticancer activity of azvudine (FNC, **184**) (see Scheme 34)[13,19] are also presented in Table 3. A study of the antitumour activity of cytosine derivatives **194** and **195** against CCRF-HSB-2 and KB lymphoblastic leukemia cells showed that they exhibit significant activity in suppressing the growth of CCRF-HSB-2 cells.[133] The cytotoxicity of 4′-*C*-substituted nucleosides **152**, **195**, **201**–**203**, and **205**–**209** was studied *in vitro* using L1210 mouse lymphocytic leukemia cells and KB cells (see Table 3). 4′-Methyl-substituted 2′-deoxycytidine **152** showed significant cytotoxicity against L1210 cells with an IC_{50} value of 0.16 μM. Analogues with cyano (**203**), ethynyl (**195**), ethenyl (**201**), and ethyl (**205**) groups at this position also exhibited cytotoxicity with IC_{50} values of 0.33, 0.80, 21, and 55 μM, respectively. At the same time, the 4′-*C*-[(2*Z*)-chloroethenyl] derivative **202** showed only insignificant antiproliferative activity.

Table 3. Inhibition of tumour cell proliferation *in vitro* by pyrimidine nucleosides substituted at the 4′-position of the carbohydrate moiety and cytarabine.

Compound	IC, μM (cell culture)			Ref.
	IC_{25}	IC_{50}	IC_{100}	
152		0.13 (MT-4), 0.47 (CCRF-HSB-2), 0.16 (L1210)		129, 132, 133, 138
157		1.2 (MT-4)		129
161		1.3 (CCRF-HSB-2)		132
162		>275 (CCRF-HSB-2)		132
169		>300 (CCRF-HSB-2), 214 (KB)		133
170		1.0 (CCRF-HSB-2), >300 (KB)		133
175	0.21 (A3.01)		1.9 (A3.01)	134
176	8.2 (A3.01)		74.1 (A3.01)	134
184		0.267 (H460), 1.22 (A549), 0.2 (Raji), 0.097 (JeKo-1), 0.95 (Granta-519), 3.3 (HL-60), 4.55 (SUDHL-6), 1.74 (RL)		13
194		11.2 (CCRF-HSB-2), >375 (KB)		136
195		3.5 (CCRF-HSB-2), 141 (KB)		136
201		21 (L1210), >350 (KB)		138
202		200 (L1210), >350 (KB)		138
203		0.33 (L1210), >350 (KB)		138
205		55 (L1210), >350 (KB)		138
1		0.053 (CCRF-HSB-2), 0.99 (KB)		133

Notes. IC_{25} and IC_{100} are concentrations of the compound that inhibit cell growth by 25 and 100%, respectively. The following designations are accepted for human cell lines: H460 and A549 are non-small-cell lung cancer; Raji, JeKo-1 and Granta-519 are non-Hodgkin lymphomas; SUDHL-6 is transformed follicular lymphoma; RL is diffuse large B-cell lymphoma.

As is evident from the data in Table 3, the 4′-substituents in 2′-deoxycytidines affect cytotoxicity in the following order: Me (**152**) > CN (**203**) > C≡CH (**195**) > CH=CH_2 (**201**) > Et (**205**) > CH=CHCl (**202**). Activity is apparently related to the bulkiness of the substituents. Since 2′-deoxycytidine analogues

can be phosphorylated by deoxycytidine kinase, and their cytotoxicity depends on the sensitivity of this enzyme to such compounds. The difference in activity against L1210 and KB cell lines may be related to the action of certain activation enzymes.[138] Ribonucleoside derivatives **206**–**209** did not exhibit cytotoxic properties.

Thus, the most promising representatives in terms of the presence of antitumour activity among 4′-modified analogues of pyrimidine nucleosides at the moment are 4′-methyl-2′-deoxycytidine (**152**) and azvudine (4′-azido-2′-deoxy-2′-fluoroarabinouridine, **184**), which contains two substituents. Two more nucleoside analogues that combine a 4′-methyl substituent with other structural modifications — capecitabine (**4**) (see Fig. 1) and doxifluridine (**10**) (see Fig. 2) — are prodrugs of 5-fluorouracil and are mentioned in the next Section of this review. Based on the available literature data, it can be hypothesized that, unlike 2′-modified analogues of pyrimidine nucleosides, the introduction of one substituent at the 4′-position is not sufficient to exhibit cytotoxicity, and additional structural optimization is necessary to obtain highly active drugs of this class.

5. Modification of the heterocyclic backbone

5.1. 5-Fluorouracyl and fluoropyrimidines

The introduction of a fluorine atom into position 5 of the pyrimidine base has become one of the successful modifications of nucleosides in the development of drugs for the treatment of cancer (Fig. 8).[16]

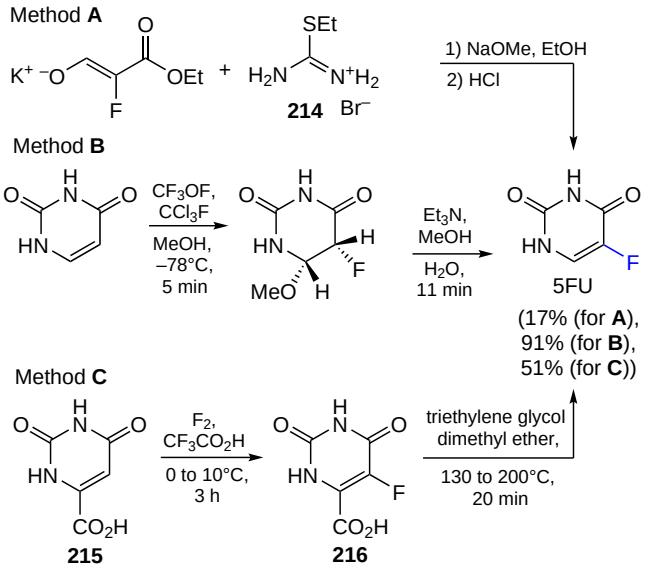
Although the antitumour activity of 5-fluorouracil, the first and best-known drug in the fluoropyrimidine class, was discovered by Heidelberger *et al.*[139] in 1957, it remains the most commonly used chemotherapy drug today. It is effective against a wide range of solid tumours, is a central component of many combination treatment regimens for intractable cancers,[140] and is also used as monotherapy when more aggressive multidrug approaches are not feasible.[141,142]

The antitumour effect of 5-fluorouracil is due to its conversion into active intracellular metabolites: 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), 5-fluoro-2′-deoxyuridine-5′-triphosphate (FdUTP), and 5-fluorouridine-5′-triphosphate (FUTP).[141,142] The metabolite FdUMP, having a high affinity for thymidylate synthase (TS), irreversibly inhibits this enzyme. Another mechanism of the cytotoxic effect of 5-fluorouracil is the interaction of FUTP with RNA polymerases and the incorporation of the corresponding nucleotide into RNA, leading to disruption of transcription, translation, and intracellular sorting of synthesized proteins.[141–143]

In 1957, a method for the synthesis of 5-fluorouracil was reported,[144] based on the interaction of ethyl pseudothiourea (**214**) with potassium 3-oxo-2-fluoro-3-ethoxypropenolate and subsequent hydrolysis (Scheme 39, Method **A**).

Two decades later, Robins *et al.*[145] presented a two-step protocol for the synthesis of 5-fluorouracil from uracil (see

Scheme 39



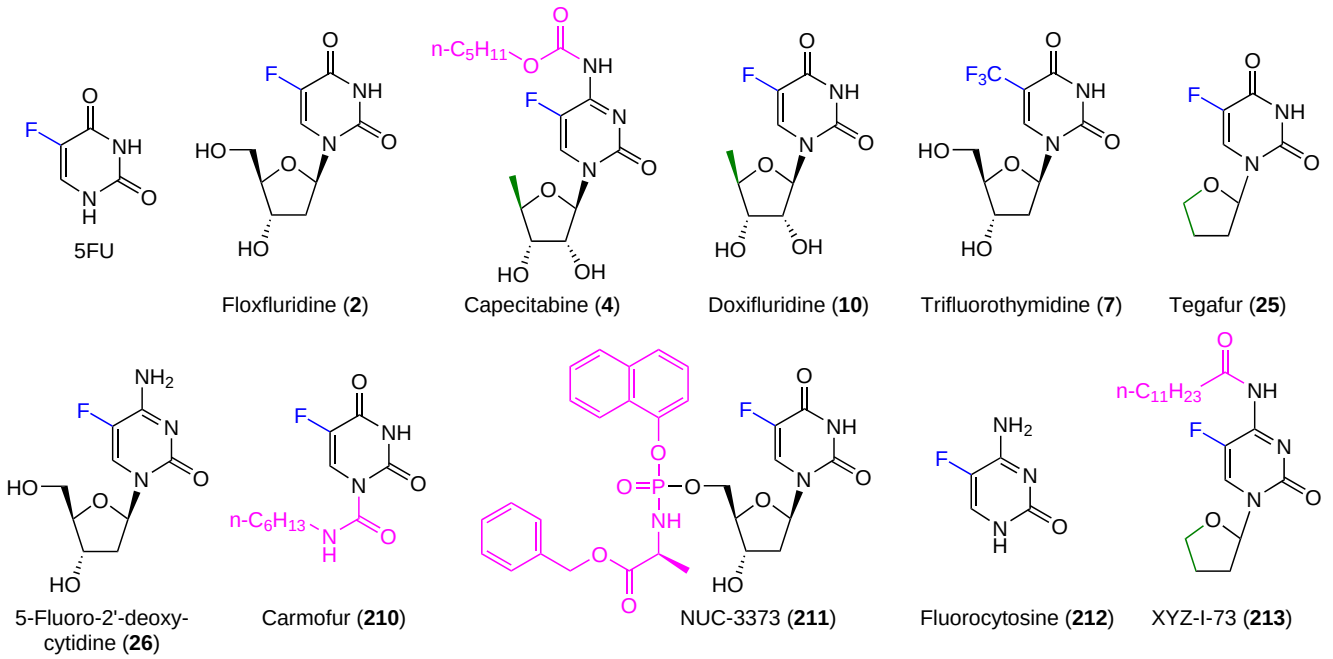
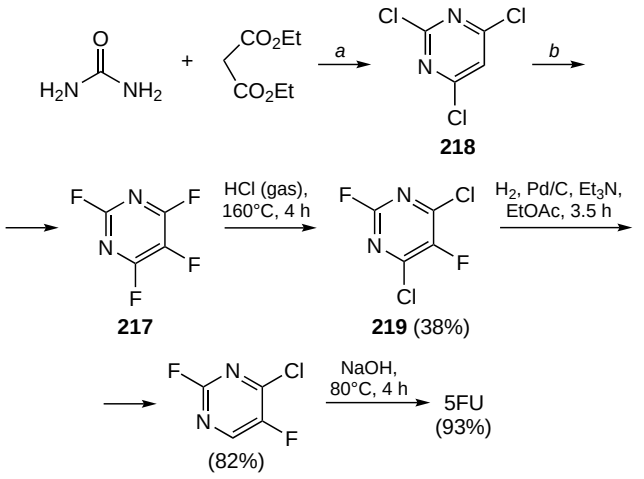


Figure 8. Structures of 5-fluoropyrimidines **2**, **4**, **7**, **10**, **25**, **26**, **210**–**213**. Modifications of the pyrimidine base are marked in blue, modifications of the carbohydrate moiety are marked in green, fragments of depot forms are marked in pink.

Scheme 39, Method **B**). In this procedure, trifluoromethyl hypofluorite was used to directly introduce a fluorine atom into the uracil ring, followed by an elimination reaction in methanol in the presence of a base to yield 5-fluorouracil. An alternative approach (method **C**) involved using inexpensive and readily available orotic acid (vitamin B13, **215**) as the starting compound.[146] Its fluorination with gaseous fluorine in the presence of trifluoroacetic acid produced acid **216**, which, after decarboxylation, delivered 5-fluorouracil in gram quantities.

Another approach to 5-fluorouracil was proposed by Baasner and Klauke[147] (Scheme 40). This process involved the selective exchange of a fluorine atom for chlorine and subsequent hydrogenation. Tetrafluoropyrimidine (**217**) was synthesized in four steps from urea and diethyl malonate *via* the formation of 2,4,5-trichloropyrimidine (**218**). The substrate **217** was treated with gaseous hydrogen chloride, the dichloro derivative **219** was dechlorinated by palladium-catalyzed selective hydrogenation, and then treated with aqueous sodium hydroxide to yield 5-fluorouracil. These and other approaches to the synthesis of 5-fluorouracil and fluoropyrimidine-containing nucleoside analogs have been summarized and described in detail in review articles published in recent years.[1,14,16,148–150]

Scheme 40

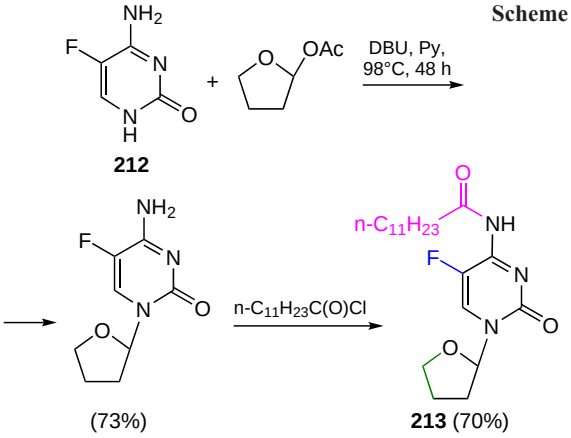


(*a*) 1) Na, MeOH; 2) $POCl_3$, $PhNMe_2$;
(*b*) 1) proton sponge, HF; 2) NaF, F_2, $CFCl_3$

The bioavailability of unmodified 5-fluorouracil after oral administration can vary from 0% to 80% not only between patients but also within the same individual. This is primarily due to differences in the activity of dihydropyrimidine dehydrogenase, the enzyme responsible for the degradation of 5-fluorouracil. Due to the unpredictability and instability of the efficacy and toxicity of oral 5-fluorouracil, this drug is typically administered intravenously.

The development of effective fluoropyrimidine-based thymidylate synthase inhibitors continued with the advent of orally administered 5-fluorouracil-containing drugs such as capecitabine (4), tegafur, and the drug S-1, a combination of tegafur (25), gimeracil, and oteracil. These drugs are prodrugs, and their cytotoxicity is due to metabolic conversion to 5-fluorouracil.[25,151] Various methods of chemical synthesis and important aspects of the biological properties of 5-fluorouracil prodrugs, including floxuridine (2), capecitabine (4), doxifluridine (10), carmofur (210), NUC-3373 (211), *etc*., as well as combinations of fluoropyrimidines (trifluorothymidine (7)+tipiracil hydrochloride (8); tegafur (25)+uracil; tegafur (25)+gimeracil+oteracil; 5-fluoro-2′-deoxycytidine (26)+tetrahydrouridine (27)) are described in detail in a review by Shelton *et al*.[1] published in 2016.

Scheme 41



Frimpong *et al*.[152] proposed a 5-fluorouracil prodrug with the commercial code XYZ-I-73 (213), obtained by introducing a tetrahydrofuran ring into 5-fluorocytosine (212) and subsequent conjugation with lauroyl chloride (Scheme 41). The antiproliferative activity of this compound was demonstrated in MiaPaCa-2, PANC-1, and BxPC-3 pancreatic cancer cell lines. Prodrug XYZ-I-73 (213) demonstrated a more pronounced cytotoxic effect ($IC_{50} = 3.6 \pm 0.4$ μM) compared to standard drugs gemcitabine (3) ($IC_{50} = 24.2 \pm 1.3$ μM) and 5FU ($IC_{50} = 13.2 \pm 1.1$ μM) and improved metabolic stability *in vitro*. It is of interest for further study as a potential drug for pancreatic cancer therapy.[152]

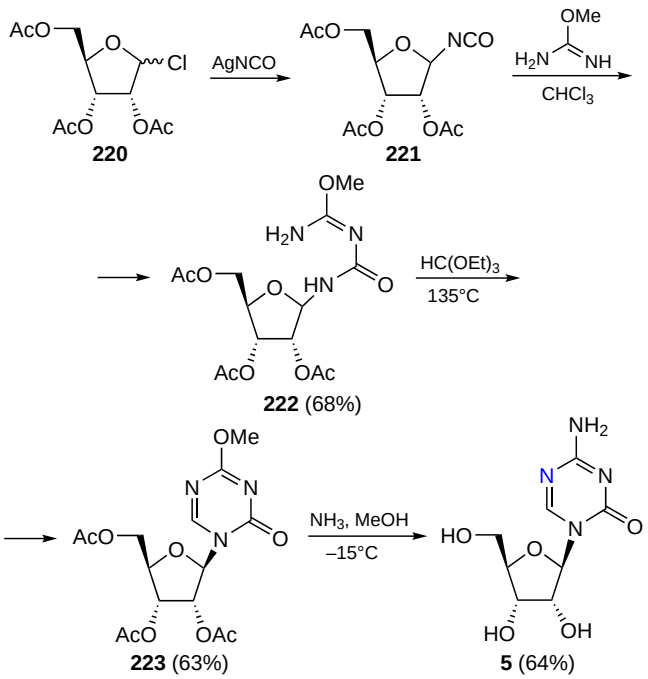
5.2. Azacitidine and decitabine

Azacitidine (**5**) and decitabine (**6**) are DNA methyltransferase (DNMT) inhibitors.[153] Both compounds are currently commercially available and approved for the treatment of myelodysplastic syndromes (MDS). Decitabine is also approved for use in patients with AML who have 20–30% blasts in the bone marrow.[154] These agents have shown excellent results in the treatment of elderly AML patients and those intolerant to more intensive therapy. Their relatively low toxicity allows them to be considered for maintenance therapy after initial treatment of AML.[155]

Azacitidine (**5**) (see Fig. 1), which has the international nonproprietary name 5-azacytidine, was isolated from the spore-forming bacteria *Streptoverticillium ladakanus*.[156] The synthesis of this compound was first described by Píškala and Šorm.[157] The authors described reaction of 1-chloro-2,3,5-tri-*O*-acetyl-*D*-ribofuranose (**220**) with silver cyanate, resulting in 2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl isocyanate (**221**), which was treated without further purification with 2-methylisourea to obtain crystalline 1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-4-methyl-isobiuret (**222**). Condensation of this compound with ethyl orthoformate at 135°C afforded 1-(2,3,5-tri-*O*-acetyl-β-*D*-ribofuranosyl)-4-methoxy-2-oxo-1,2-dihydro-1,3,5-triazine (**223**), which, when treated with ammonia in methanol, yielded azacitidine (**5**) (Scheme 42). This method uses relatively readily available starting materials, and therefore, despite its complexity and multi-step nature, it remains relevant for the industrial synthesis of azacitidine (**5**).

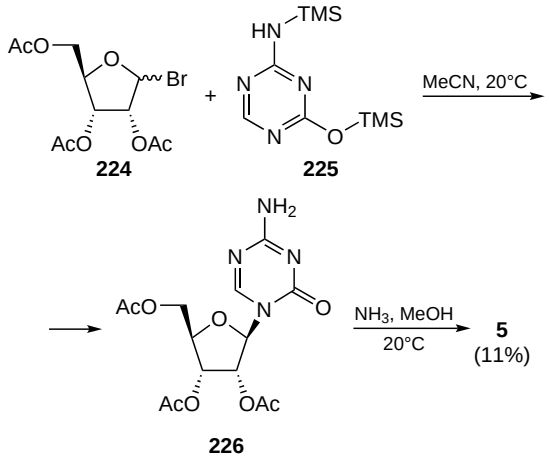
A method for the preparation of azacitidine based on the silyl version of the Gilbert–Johnson reaction was proposed by Winkley and Robins.[158] In this case, the condensation of 1-bromo-2,3,5-tri-*O*-acetyl-*D*-ribofuranose (**224**) with the

Scheme 42



trimethylsilyl derivative of 5-azacytosine **225** in anhydrous acetonitrile at room temperature gave compound **226**. Removal of acetyl residues and subsequent purification allowed the preparation of azacitidine (**5**) in 11% yield (Scheme 43). The low yield of the target product limits the application of this method on an industrial scale.

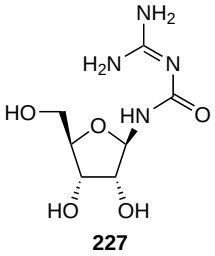
Scheme 43



The bioactivation of azacitidine begins with its phosphorylation by uridine cytidine kinase to 5-azacytidine-5′-monophosphate (5-AZA-CMP), which is converted to 5-azacytidine-5′-diphosphate (5-AZA-CDP) by cytosine nucleoside monophosphate kinase (CMPK). Nucleoside diphosphate kinase then phosphorylates 5-AZA-CDP to 5-azacytidine triphosphate (5-AZA-CTP), which is a substrate for RNA polymerases and disrupts protein synthesis. 5-AZA-CDP can be metabolized by ribonucleotide reductase to 2′-deoxyazacytidine-5′-diphosphate, the phosphorylated form of decitabine (**6**). After final phosphorylation, the decitabine-containing nucleotide acquires the ability to integrate into DNA and forms a covalent complex with DNMT, which leads to irreversible inhibition of the enzyme.

Azacitidine (**5**) has orphan drug status for the treatment of AML in Europe[159] and the United States.[160] It is also used to treat adult patients who are not candidates for hematopoietic stem cell transplantation and in the following diseases: high- or intermediate-risk MDS according to the International Prognostic Scoring System (IPSS), acute myeloid leukemia, or chronic myelomonocytic leukemia without features of MDS. Azacitidine was registered in Russia in 2010. The safety profile of this drug allows its effective use in elderly patients with comorbidities.[161,162] A disadvantage of azacitidine (**5**), which limits its scope of application, is its low stability in aqueous solutions. Azacitidine (**5**) hydrolyzes to give ribose guanylureide (**227**).[163,164]

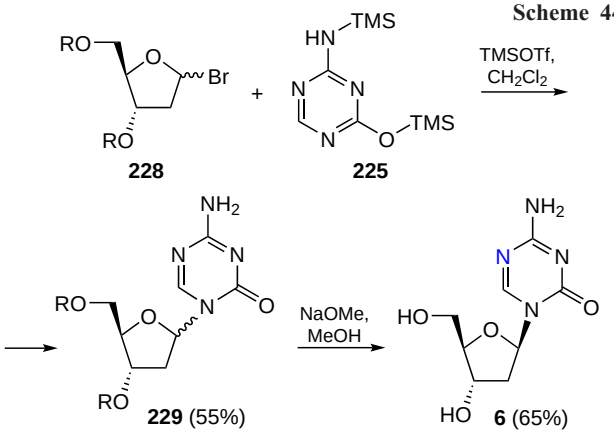
Structure 227



Decitabine (AzadC, **6**) (see Fig. 1),[165–167] or 5-aza-2′-deoxycytidine, 4-amino-1-(2-deoxy-β-*D*-erythrofuranosyl)-1,3,5-triazin-2(1*H*)-one, is an analogue of deoxycytidine. Early methods for preparing this compound gave very low yields of the β-stereoisomer. Decitabine (**6**) was first synthesized by Pliml and Šorm[168] in 1964 using a strategy similar to the synthesis of azacitidine, but the authors failed to isolate β-isomer from the mixture of anomers. Winkley and Robins[158] obtained pure β-decitabine by reacting a triacetyl-protected sugar with silylated 5-azacytosine **225**, but the yield of the product was only 7%. In this case, the α-isomer was predominantly formed, for which the yield reached 52%.

It was shown[169] that the selectivity of the glycosylation process depends on the solvent, reaction temperature, and the ratio of silylated triazine **225** to protected ribosaccharide **228** (Scheme 44). Varying these parameters leads to a change in the ratio of α- and β-glycosylated products from 1 : 1 to 1 : 3. Optimum conditions for large-scale synthesis involved the use of protected sugar **228** and silylated triazine **225** in a 1 : 1 ratio with the addition of 1.05 equiv. TMSOTf in cooled dichloromethane. It was found that in order to maintain the initial ratio of α- and β-forms and prevent isomerization of the target β-isomer into the undesirable α-anomer after completion of the reaction, immediate quenching of excess TMSOTf with an organic amine (preferably a primary one: $MeNH_2$ or $EtNH_2$)

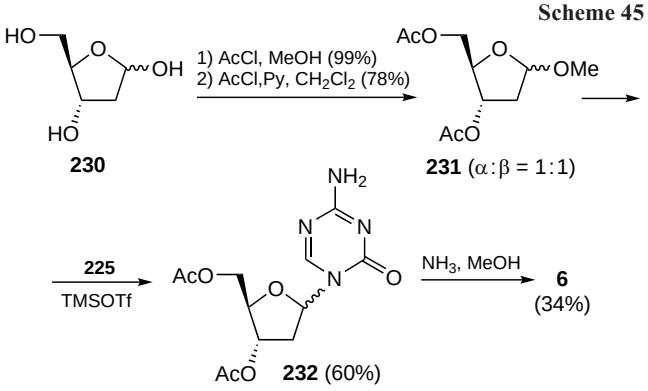
Scheme 44



at a temperature not exceeding 0°C is required.[169] As a result, protected decitabine **229** was obtained as a mixture of α- and β-isomers in a ratio of 1 : 2.7. The final step in the synthesis of decitabine (**6**) involved removing the protecting groups of compound **229** with sodium methoxide in methanol. Subsequent filtration and recrystallization from a DMSO-methanol mixture gave decitabine (**6**) in kilogram quantities.

Another method for the industrial synthesis of decitabine has been patented (Scheme 45).[170] In this strategy, 2′-deoxy-*D*-ribose **230** was treated with acetyl chloride first in methanol and then in a mixture of pyridine and dichloromethane to form the bis(acetyl)-protected sugar **231**. Subsequent condensation of this product with silylated 5-azacytosine in the presence of TMSOTf afforded a 1 : 1 mixture of α- and β-acylated compound **232**. Acetyl groups were removed with a solution of ammonia in methanol, followed by crystallization to yield decitabine (**6**) with a purity of 90–99% according to HPLC. To improve purity, the resulting product was dissolved in DMSO, filtered, washed with a mixture of methanol and ethyl acetate, and dried *in vacuo* to give crystalline decitabine (**6**) with a purity of 99.8%.

Scheme 45



The antileukemic activity of decitabine (**6**) was first demonstrated in 1968.[171] Its mechanism of action is dual: at high concentrations, a cytotoxic effect is observed, leading to cell death, and at lower concentrations, this drug affects cancer cells through DNA hypomethylation. This results in the reactivation of epigenetically suppressed genes, such as tumour suppressor genes, which may promote cell differentiation and restoration of normal cellular functions.[172,173] Clinical studies have shown the efficacy of low doses of decitabine (**6**), administrated by daily infusion for 5 days, in patients with MDS.[174]

A limiting factor in the use of decitabine is its low oral bioavailability due to rapid metabolism by cytidine deaminase (CDA) action.[175] To address this problem, another drug, cedazuridine (**9**) (see Fig. 1), was synthesized, which is an analogue of tetrahydrouridine with two fluorine atoms at the 2′ position of the carbohydrate moiety. It is a competitive inhibitor of CDA and is part of the oral combination drug (cedazuridine+decitabine).[22,23,26] The combination of decitabine and cedazuridine is FDA-approved for the treatment of intermediate- or high-risk MDS and chronic myelomonocytic leukemia.[176] The European Medicines Agency also approved the use of the combination therapeutic agent cedazuridine–decitabine, trade name Inqovi®, in 2023.[159]

In an attempt to improve the bioavailability, metabolic stability, and cellular penetration of nucleoside analogues **5** and **6**, a number of prodrugs have been obtained, some of which are shown in Fig. 9.

Structural modifications render these compounds poor substrates for cytidine deaminase. Guadecitabine (SGI-110, **18**)

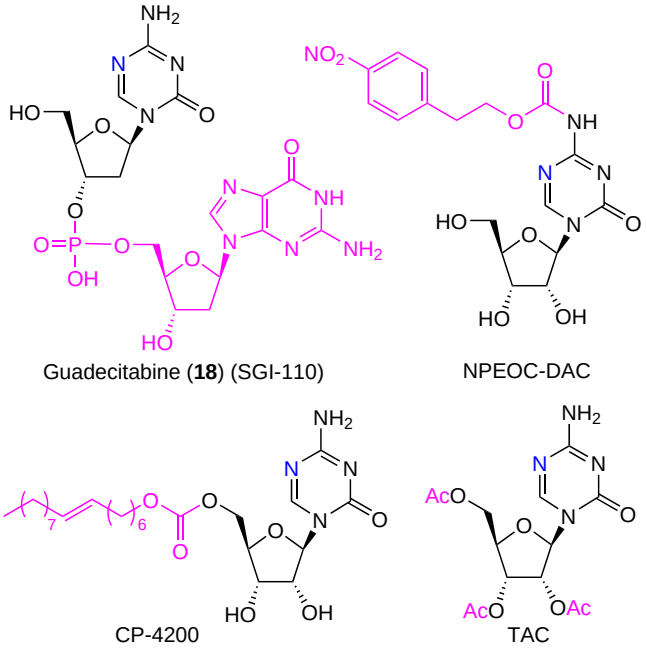


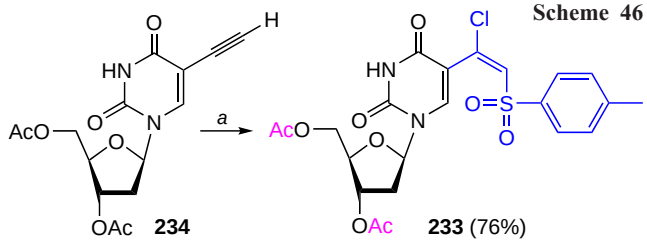
Figure 9. Examples of azacytidine and decitabine prodrugs.

(see Fig. 9), a prodrug of decitabine, has been clinically tested in combination with carboplatin for platinum-resistant ovarian cancer and hepatocellular carcinoma following sorafenib failure, and in combination with irinotecan for colorectal cancer.[177,178] Guadecitabine has been tested as a monotherapy for the treatment of high-risk myelodysplastic syndromes and chronic myelomonocytic leukemia, but it did not demonstrate a statistically significant improvement in overall survival compared with a control group of patients receiving alternative therapy.[179] Several acyl prodrugs of 5-azacytidine with increased lipophilicity and improved absorption have also been studied in clinical trials: 2′-deoxy-*N*⁴-[2-(4-nitrophenyl)ethoxycarbonyl]-5-azacytidine (NPEOC-DAC), 5-azacytidine-5′-elaidate (CP-4200), and 2′,3′,5′-triacetyl-5-azacytidine (TAC).[180]

5.3. 5-Alkynylpyrimidine nucleoside analogues

Several examples of reactions involving nucleosides with 5-alkynyl-substituent in the pyrimidine ring that yield products with antitumour activity have been reported. For example, compound **233**, containing a 1-chloro-2-sulfonylvinyl fragment, was obtained by halovinylsulfonation of 2′-deoxy-5-ethynyluridine.[181] The reaction of 3′,5′-di-*O*-acetyl-2′-deoxy-5-ethynyluridine (**234**) with $TsNHNH_2$ (Ts is *p*-toluenesulfonyl (tosyl)) in the presence of $FeCl_3 \cdot 6H_2O$ and *tert*-butyl hydroperoxide (TBHP) afforded the target product **233** in good yield (Scheme 46). Acetyl-protected (β-chloro)vinyl sulfone **233** inhibited the proliferation of murine leukemia (L1210),

Scheme 46

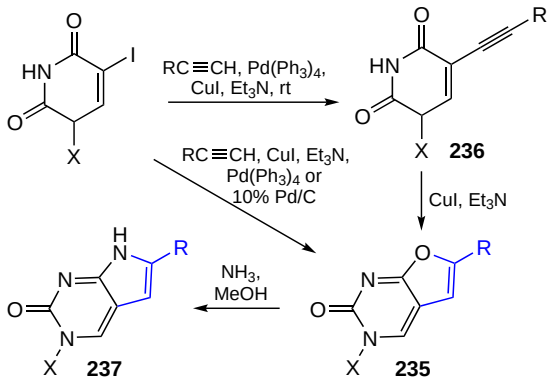


(*a*) $TsNHNH_2$, $FeCl_3$, TBHP, MeCN, 80°C, 4 h

human T lymphocyte (CEM), and human cervical carcinoma (HeLa) cells with IC_{50} values of 5.6±4.7, 11±10, and 23±8 μM, respectively.[182]

Ribonucleoside derivatives of furano[2,3-*d*]pyrimidine **235** (Ref. 183) were synthesized by Pd-catalyzed cross-coupling of 5-iodouridines with the corresponding alkynes in two steps *via* the formation of intermediate 5-alkynepyrimidines **236** or in one step.[184,185] Treatment of substrates **235** with ammonia pyrrolo[2,3-*d*]pyrimidine ribonucleosides **237** (Scheme 47).[186,187] Similarly, 5′-norcarbocyclic analogues of furano- and pyrrolo[2,3-d]pyrimidine nucleosides were obtained from 1-(4′-hydroxy-2′-cyclopenten-1′-yl)-5-iodouracil.[188,189]

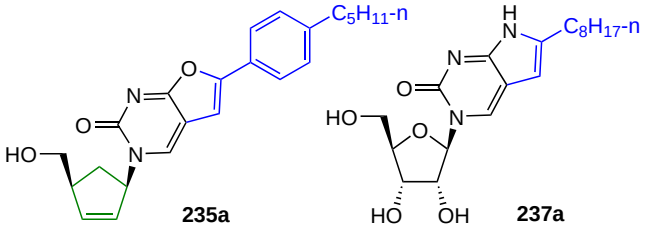
Scheme 47



X = sugar or pseudosugar; R = Alk, Ar or aralkyl

6-Octylpyrrolo[2,3-*d*]pyrimidine ribonucleoside **237a** was shown to exhibit significant selective cytotoxicity against T98G glioblastoma (IC_{50} = 0.74 μM) compared to normal MRC-5 cells (IC_{50} > 130 μM) and the human cancer cell line HeLa (IC_{50} = 17.8 μM).[167] This compound is recommended for further study as a potential key structure in the development of an anti-glioma drug. Furano[2,3-*d*]pyrimidine **235a** exhibited selective cytotoxicity against the following human cancer cells: HeLa (IC_{50} = 6.5 μM), HuTu80 (IC_{50} = 5.1 μM), KB-3-1 (IC_{50} = 8.2 μM).[189] Carbocyclic nucleoside 235a was found to induce cell death *via* the apoptotic pathway.[189]

Structures 235a, 237a

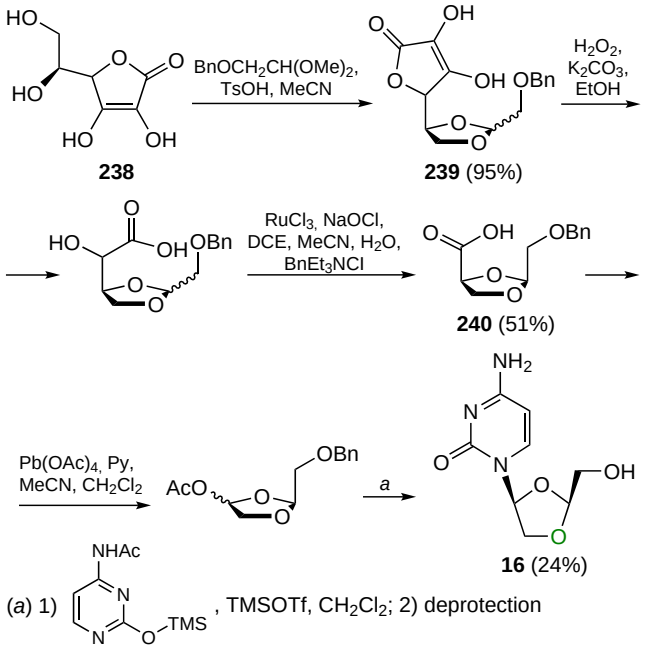


6. Troxacitabine

The successful clinical use of cytarabine (**1**) and gemcitabine (**3**), as well as the need to overcome the side effects associated with these drugs, have led to the development of new cytidine nucleoside analogues. One such analogue is troxacitabine (β-*L*-dioxolanocytidine) (**16**) (see Fig. 2).[190]

In 1992, the first method for the diastereoselective synthesis of troxacitabine was presented, using *L*-ascorbic acid (**238**) as the starting material (Scheme 48).[191] The first step involved condensation of acid **238** and benzyloxyacetaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid, affording a

Scheme 48



mixture of diastereomers **239**. This mixture was then subjected to oxidative degradation and catalytic Wolfe oxidation in the presence of benzyltriethylammonium chloride to yield intermediate **240**, which was isolated by flash chromatography. Subsequent steps of oxidative decarboxylation and *N*-glycosylation involving silylated *N*-acetylcytosine under Vorbruggen reaction conditions were completed by deprotection, resulting in the isolation of product **16** in an overall yield of 24%.[191] Other synthetic approaches to compound **16** have also been described.[1,192]

Unlike cytarabine (**1**) and gemcitabine (**3**), troxacitabine (**16**) has low affinity for cellular nucleoside transporters, and its membrane penetration occurs primarily by simple diffusion.[193] The cytotoxic metabolite of this drug is its 5′-triphosphate, which inhibits DNA biosynthesis by being a terminator for DNA polymerases. The first step in troxacitabine phosphorylation is realized by deoxycytidine kinase, and the conversion to 5′-di- and 5′-triphosphate is catalyzed by 3-phosphoglycerate kinase.[190] An important feature and advantage of compound **16** is its resistance to deamination by cytidine deaminase, resulting in a significantly longer half-life (approximately 80 h) compared to its predecessors. This is facilitated by the unique stereochemistry of troxacitabine (**16**). Natural nucleosides and all anticancer drugs based thereon have a β-*D*-configuration of the carbohydrate moiety; compound **16** is β-*L*-dioxolanylcytidine. Therefore, we are dealing with the first *L*-nucleoside analogue to exhibit antitumour activity.[194]

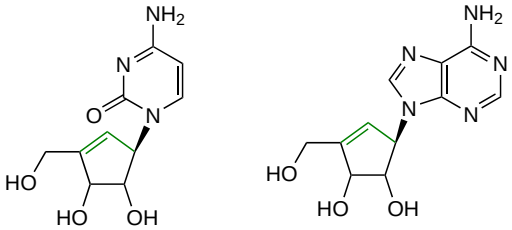
Phase II clinical trials showed that troxacitabine exhibits moderate activity against advanced and/or metastatic renal cell carcinoma. This drug has been used with varying degrees of success to treat leukemias and myelodysplastic syndromes, lymphoproliferative neoplasms and multiple myeloma, as well as pancreatic and lung cancer.[195] At maximum tolerated doses of 8 mg/m^2/day administered intravenously every day for 5 days, it caused side effects such as mucositis, rash, and hand-foot syndrome.[196] Troxacitabine was granted orphan drug designation by the FDA and the European Medicines Agency (EMA) in 2005, but it was withdrawn in 2008 at the request of the sponsor and remains unlicensed.[195]

7. Carbocyclic analogues of pyrimidine nucleosides

Replacing the tetrahydrofuran ring in nucleosides with a cyclopentane moiety produces carbocyclic nucleoside analogues.[197] Compounds of this class lack a glycosidic bond, making them more resistant to cleavage by nucleoside phosphorylases and hydrolases. The conformational similarity between the cyclopentane and tetrahydrofuran moieties allows carbocyclic nucleoside analogues to act as substrates or inhibitors of enzymes catalyzing the synthesis and conversion of natural nucleosides and nucleotides.[197]

The cytotoxic drug cyclopentenylcytosine (CPEC, **241**) was developed in 1979 as a cytosine-containing analogue of neplanocin A (**242**), a biologically active and toxic nucleoside isolated from natural sources. Among its purine and pyrimidine analogues, neplanocin A has proven to be the most potent in terms of antiviral activity and activity against murine leukemia and human tumour xenografts.[198]

Structures 241, 242



241 (CPEC) **242** (Neplanocin A)

The synthesis of (1*R*,4*R*,5*S*)-1-[4,5-dihydroxy-3-(hydroxymethyl)-2-cyclopenten-1-yl]cytosine (**241**)[¶] and a similar carbocyclic derivative of uracil was described by Lim *et al.*[199] Cyclopentenyl nucleosides were synthesized from optically pure 3-aminocyclopentene **243**, obtained according to a previously described method[200] (Scheme 49). Condensation of the starting compound **243** with ethyl-(*Z*)-2-[(ethoxycarbonyl)carbamoyl)-3-methoxyacrylate in the presence of triethylamine in ethanol gave intermediate **244**, which was then cyclized to uracil derivative **245** in 80% yield. Further hydrolysis of compound **245** with LiOH in a water–methanol mixture followed by neutralization with Dowex 50W-X8 ion exchange resin in the H^+ form yielded an intermediate carboxylic acid, which was decarboxylated without purification in the presence of copper powder in boiling quinoline to afford the protected uridine analogue **246a**. Debenzylation of the latter with boron trichloride in dichloromethane was accompanied by removal of the isopropylidene group during subsequent workup and gave (1*R*,4*R*,5*S*)-1-[4,5-dihydroxy-3-(hydroxymethyl)-2-cyclopenten-1-yl]uracil (**247**) in moderate yield.[199]

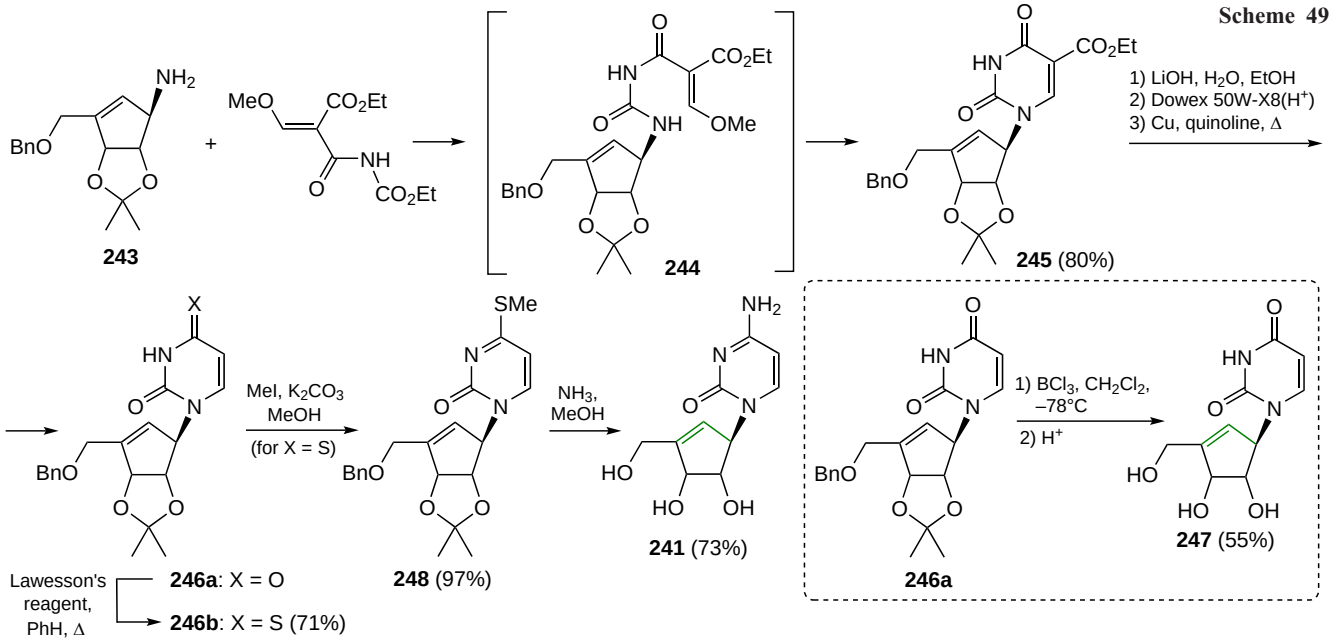
Conversion of compound **246a** (X = O) to its thio analogue **246b** (X = S) was accomplished by reacting the former with Lawesson′s reagent in benzene. Methylthio derivative **248** was obtained in high yield from compound **246b** (X = S) and methyl iodide in the presence of potassium carbonate in methanol. Finally, treatment of compound **248** with methanolic ammonia afforded the target product **241** (see Scheme 49).

The drug CPEC (**241**), in contrast to the inactive carbocyclic uracil derivative **247**, showed significant cytotoxicity against the L1210 cell line, inhibiting cell growth by 58% at a concentration of 30 μM.[199] The efficiency of compound **241** was subsequently confirmed against various tumours, including neuroblastoma, but it did not find clinical use due to neurotoxicity.[201]

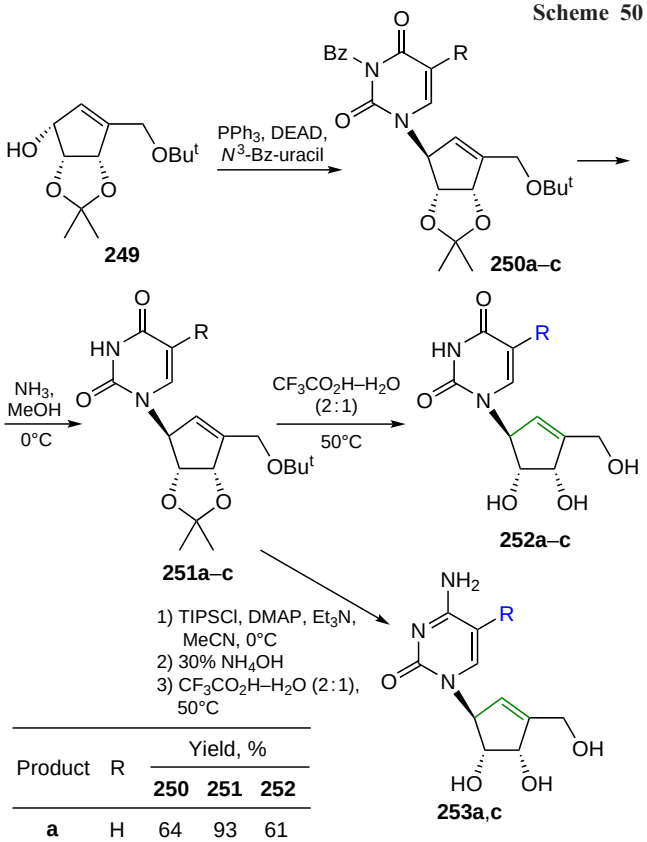
Song *et al.*[202] synthesized the optical isomer of compound **241**, as well as 5-methyl- and 5-fluoro-substituted (1′*S*,2′*R*,3′*S*)-1-[2,3-dihydroxy-4-(hydroxymethyl)-4-cyclopenten-1-yl]-pyrimidines shown in Scheme 50. The key step involved the condensation of cyclopentene **249** with *N*-benzoylated pyrimidine bases to form protected carbocyclic analogues **250a–c**. The condensation was carried out using the Mitsunobu reaction: cyclopentenyl alcohol **249** was treated with N^3-benzoyluracil,[203] N^3-benzoylthymine,[203] or N^3-benzoyl-5-fluorouracil[204] in the presence of DEAD and Ph_3P in THF at room temperature. Debenzoylation of compounds **250a–c** with saturated ammonia in MeOH delivered products **251a–c**, and subsequent removal of the protecting groups (*tert*-butyl and isopropylidene) with aqueous trifluoroacetic acid at 50°C

[¶] In this Section, the names of the compounds are given according to the IUPAC nomenclature, in contrast to the systematic and trivial names used above for nucleoside derivatives.

Scheme 49



Scheme 50

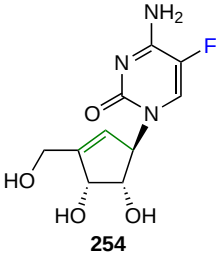


Product	R	Yield, %		
		250	**251**	**252**
a	H	64	93	61
b	Me	72	87	59
c	F	61	83	62

afforded carbocyclic analogues **252a–c**. Protected uridine analogues **251a** and 5-fluorouridine **251c** were converted to the corresponding cytosine and 5-fluorocytosine derivatives using the described method,[205] followed by deprotection to isolate the cytosine (**253a**) and 5-fluorocytosine nucleosides (**253c**).

Nucleoside analogues **252** and **253** were shown to lack cytotoxicity against CEM cells. Under the same conditions, 1-[4,5-dihydroxy-3-(hydroxymethyl)-2-cyclopenten-1-yl]-cytosine **241** exhibited high cytotoxicity (IC_{50} = 0.08 μM),

Structure 254



254

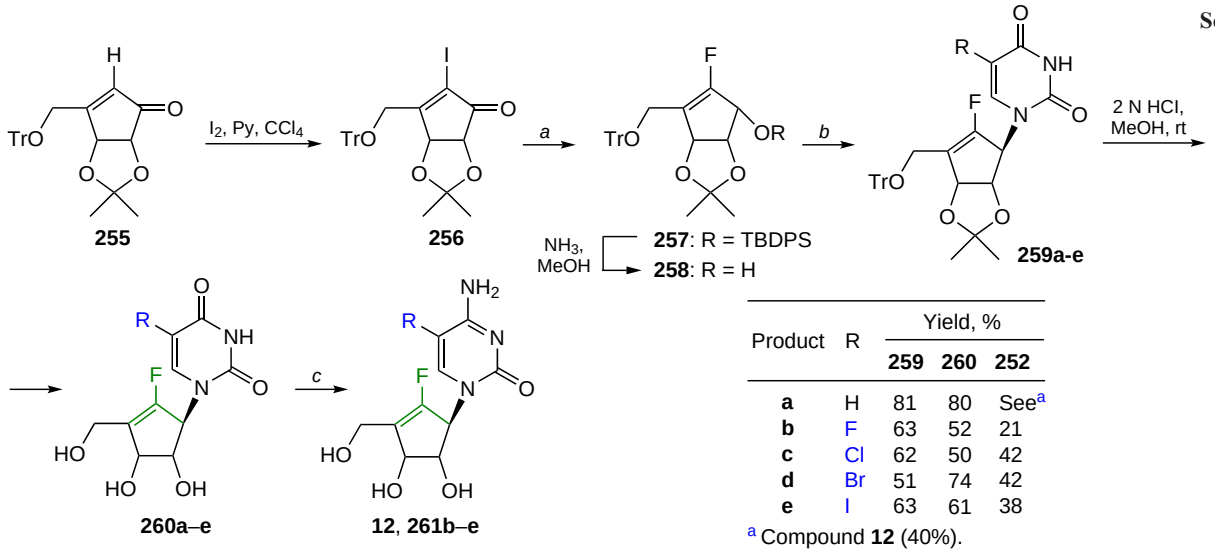
while its 5-fluoro-containing analogue **254** was found to be inactive (IC_{50} = 51.8 μM).[202]

Choi *et al.*[206] described a method for the preparation of fluorocyclopentenyl nucleoside analogues of cytidine and uridine and studied their antitumour properties. The method involved converting the key compound, cyclopentenone **255**, into a known glycosyl donor,[207] and then into the target cytosine derivative **12** (Scheme 51).

Iodination of cyclopentenone **255** with iodine in the presence of pyridine in CCl_4 proceeded slowly, but when the solvent was replaced with THF, iodocyclopentenone **256** was obtained in fairly good yield. Reduction of this compound with sodium borohydride in the presence of cerium chloride, followed by protection of the hydroxyl group with a *tert*-butyldiphenylsilyl moiety, yielded a TBDPS ester, in which electrophilic substitution of the iodine atom by fluorine was achieved by the sequential addition of Bu^nLi and *N*-fluorobenzenesulfonimide (NFSI) at –78°C. The resulting fluorocyclopentene **257** was then desilylated to form the key intermediate **258**.[207] Condensation of the latter with N^3-benzoyluracil under standard Mitsunobu reaction conditions[208] afforded uracil derivatives **259a–e**. Treatment of these compounds with hydrochloric acid solution delivered uracil derivatives **260a–e**.

The carbocyclic analogue of cytidine **12** was prepared in three steps: treatment of compound **260a** (R = H) with acetic anhydride gave the triacetate, which was converted first to the triazole derivative by reaction with $POCl_3$ and 1,2,4-triazole in the presence of triethylamine, and then to the corresponding cytosine derivative by reaction with ammonium hydroxide in 1,4-dioxane. Deacetylation of this compound with ammonia in methanol afforded the final product, 1-[(1*R*,2*S*,3*R*)-2,3-

Scheme 51



Product	R	Yield, %		
		259	**260**	**252**
a	H	81	80	See[a]
b	F	63	52	21
c	Cl	62	50	42
d	Br	51	74	42
e	I	63	61	38

[a] Compound **12** (40%).

(*a*) 1) $NaBH_4$, $CeCl_3$, MeOH, 0°C; 2) TBDPSCl, imidazole, 50°C; 3) NFSI, Bu^nLi, THF, –78°C; (*b*) N^3-Bz-uracils, PPh_3, DIAD, THF, 0°C; (*c*) 1) Ac_2O, Py, rt; 2) $POCl_3$, 1,2,4-triazole, Et_3N, MeCN, rt; 3) NH_4OH, dioxane, rt; 4) NH_3, MeOH;
Tr is triphenylmethyl (trityl), DIAD is diisopropyl azodicarboxylate

Table 4. Inhibition of tumour cell proliferation *in vitro* by a carbocyclic analogue of cytidine **12**.[209]

No.	Cell line	IC_{50}, μM
1	A549	0.76
2	HCT-116	0.18
3	SNU-6	0.31
4	MDA-MB-231	0.48
5	SK-Hep-1	0.62
6	PC-3	0.64

dihydroxy-4-(hydroxymethyl)-5-fluorocyclopent-4(5)-en-1-yl]-cytosine (**12**) with the commercial code RX-3117. 5-Substituted in the pyrimidine ring 1-[(1*R*,2*S*,3*R*)-2,3-dihydroxy-4-(1-hydroxymethyl)-5-fluoro-cyclopent-4(5)-en-1-yl]cytosines **261b–e** were obtained in a similar way (see Scheme 51).[209]

Compounds **261** exhibited no or 2–3 orders of magnitude lower antitumour activity against human cancer cells, including A549 (lung carcinoma), HCT-116 (colon carcinoma), SNU-638 (gastric adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), SK-Hep-1 (epithelial tumour of the liver and intrahepatic bile ducts), and PC-3 (prostate adenocarcinoma) than cytosine **12** with a hydrogen atom at position 5 of the pyrimidine ring. The results of the antitumour activity study for compound **12** are presented in Table 4.[209]

RX-3117 has an improved pharmacological profile compared to gemcitabine and other nucleoside analogues, high activity against various cancer cell lines and xenografts, including those resistant to gemcitabine (**3**), and excellent oral bioavailability.[206,210,211] This compound is not a substrate of cytidine deaminase. The drug′s pharmacokinetic parameters were evaluated with daily oral administration of 700 mg (5 days a week for 3 weeks). Plasma levels were found to be micromolar, which is toxic to cancer cells. It demonstrated clinical activity in bladder and pancreatic cancers.[211]

Compound **12** is taken up by equilibrating nucleoside transporters and is selectively phosphorylated by uridine-cytidine kinase **2**, which is expressed only in tumours. This drug has a dual mechanism of action: DNA damage and inhibition of DNA methyltransferase 1.[212,213]

The synthesis of a carbocyclic analogue of decitabine (cAzadC, **262**) has been reported (Scheme 52).[214] The hydroxyl groups of the starting NHBoc-cyclopentane **263** (Boc is *tert*-butoxycarbonyl) were protected with benzyl groups, and then the Boc protection was removed from the amino group of intermediate **264** using trifluoroacetic acid. Compound **265** with the free amino group was reacted with methyl (1*H*-imidazole-1-carbonyl)carbaimidate (**266**), which was prepared from isomethylurea hydrochloride **267** *via* the formation of free base **268** with potassium hydroxide and its subsequent reaction with carbonyldiimidazole (CDI). The reaction of compounds **265** and **266** yielded the carbamoylurea-cyclopentane analogue of nucleoside **269**, which was cyclized to triazine base **270** using triethyl orthoformate. The reaction of compound **270** with NH_3 in methanol and deprotection with BCl_3 in dichloromethane, followed by treatment with MeOH, column chromatography, and recrystallization from MeOH afforded the target product **262**.

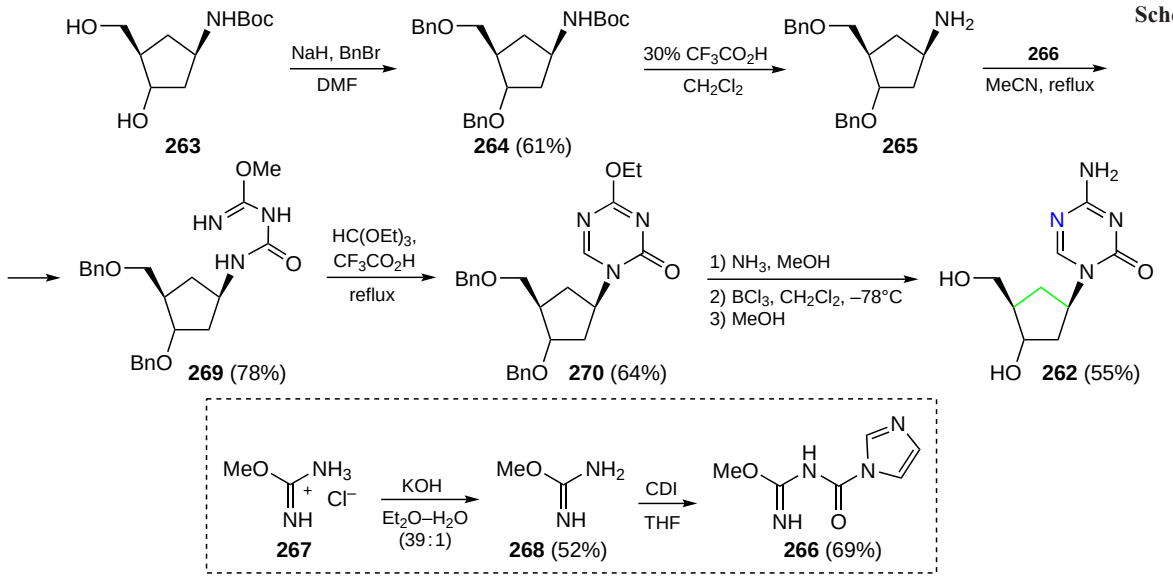
The carbocyclic analogue **262** is not only significantly more chemically stable than decitabine *per se*,[215] facilitating its administration, but also demonstrates high activity in inducing cancer cell death. Initial studies of the general toxicity of cAzadC indicate that this compound is less toxic and can be administered at significantly higher doses than decitabine.[216]

Methods for the chemical synthesis and study of the anticancer activity of C2′,C4′-bridged bicyclic nucleosides derived from norbornane, as well as C3′,C5′-bridged carbocyclic *L*-nucleosides and pyrimidine nucleoside analogues with various heteroatoms (nitrogen, sulfur, selenium, *etc*.) replacing the oxygen atom in the tetrahydrofuran ring, are described in detail by Guinan *et al*.[17]

8. Conclusion

Pyrimidine nucleoside analogues remain an important and promising class of anticancer drugs. In the decades since the discovery of the first representatives of this class, significant advances have been made in understanding their mechanisms of action, optimizing their structure, and expanding their clinical applications. Both classical pyrimidine nucleoside analogue-based drugs — 5-fluorouracil and cytarabine — and modern drugs (*e.g*., gemcitabine, decitabine, and capecitabine) have made significant contributions to improving survival and quality of life in patients with various types of malignant neoplasms.

Scheme 52



Literature data show that the effectiveness of pyrimidine nucleoside analogues stems from their ability to affect DNA replication and RNA biosynthesis, as well as nucleotide metabolism, leading to tumour cell growth arrest and death. However, despite their antitumour activity, these drugs also exhibit significant toxicity, limiting their clinical use. The development of drug resistance is also a serious problem, requiring a constant search for new ways to overcome it.

Current research in the field of pyrimidine nucleoside analogues aims to address a number of issues. Researchers are actively working to develop new drugs with increased selectivity, improved bioavailability, and the ability to overcome drug resistance mechanisms. Approaches to modifying the structure of the heterocyclic base, altering the carbohydrate moiety, and the synthesis of the prodrug forms allow the creation of compounds with a more favorable pharmacological profile and reduced toxicity. The development of targeted drug delivery technologies, including the use of nanoparticles and antibody conjugates, also opens up new opportunities for increasing the efficiency and selectivity of pyrimidine nucleoside analogues.

In conclusion, it can be noted that pyrimidine nucleoside analogues continue to play an important role in modern cancer therapy. Continued intensive research in this area should lead to the development of effective therapeutic methods for cancer control. Further efforts to develop new cytotoxic agents, optimize existing drugs, and understand the mechanisms of drug resistance will contribute to improved treatment outcomes and enhance patient quality of life. Data accumulated over years of development and clinical use of pyrimidine nucleoside analogues can be used as a solid foundation for future research in anticancer drug design.

This review was financially supported by the Russian Science Foundation (Project No. 23-64-10018).

9. List of abbreviations and designations

The following abbreviations and designation are used in the review:

AIBN — azobis(isobutyronitril),
AML — acute myeloid leukemia,
ara-C — 1-β-*D*-arabinofuranosylcytosine (cytarabine),
5-AZA-CDP — 5-azacytidine-5′-diphosphate,
5-AZA-CMP — 5-azacytidine -5′-monophosphate,
5-AZA-CTP — 5-azacytidinetriphosphate,
AzadC — 5-aza-2′-deoxycytidine (decytabine),
Boc — *tert*-butoxycarbonyl,
CAN — cerium(IV)-ammonium nitrate,
cAzadC — carbocyclic analogue of decytabine,
CDA — cytidindesaminase,
CDI — carbonyldiimidazole,
CMPK — cytosine nucleoside monophosphate kinase,
CNDAC — 2′-deoxy-2′-*C*- cyanocytidine,
CNddC — 2′,3′-didehydro-2′,3′-dideoxy-2′-*C*-cyanocytidine,
CPEC — cyclopentenylcytosine,
DAST — diethylaminosulful trifluoride,
DBU — 1,8-diazabicyclo[5.4.0]undec-7-ene,
dCK — deoxycytidine kinase,
DEAD — diethylazodicarboxylate,
2′-DHAU — 2′-hydroxylamino-2′-deoxyuridine,
DIAD — diisopropylacetylenedicarboxylate,
DMAP — 4-dimethylaminopyridine,
DMDC — 2′-deoxy-2′-methylydenecytidine,
DNMT — DNA-methyltransferase,
DPPA — diphenylphosphoryl azide,
DPAP — 2,2-dimethoxy-2-phenylacetophenone,
ECyd — 3′-ethynylcytidine,
ECDP — 3′-ethynylcytidine-5′-diphosphate,
EMA — European Medicines Agency,
FDA — U.S. Food and Drug Administration,
FNC — azvudine,
FdUMP — 5-fluoro-2′-deoxyuridine-5′-monophosphate,
FdUTP — 5-fluoro-2′-deoxyuridine-5′-triphosphate,
5-FU — 5-fluorouracyl,
FUTP — 5-fluorouridine-5′-triphosphate,
IBX — 2-iodoxybenzoic acid,
IC_{50} — half-maximal inhibitory concentration,
IPSS — international prognostic scoring system,
HMDS — hexamethyldisilazane,
hRR — human ribonucleotide reductase
mCBA — *m*-chlorobenzoic acid,
mCPBA — *m*-chloroperoxybenzoic acid,
MDS — myelo-dysplastic syndrome,
Ms — methanesulfonyl (mesyl),
NBS — *N*-bromosuccinimide,
NDPK — nucleotide diphosphate kinase,
NFSI — *N*-fluorobenzenesulfonimide,
NMPK — nucleotide monophosphate kinase,
NPEOC-DAC — 2′-deoxy-N^4-[2-(4-nitrophenyl) ethoxycarbonyl]-5-azacytidine,
PDC — pyridinium dichromate,
Py — pyridine,
Pyr — pyrimidin-1-yl,
RR — ribonucleotide reductase,
rt — room temperature,
SAMHD1 — SAM domain and HD domain-containing human protein 1,
TAC — 2′,3′,5′-triacetyl-5-azacytidine,
TBAF — tetra-*n*-butylammonium fluoride,
TBDPS — *tert*-butyldiphenylsilyl,
TBHP — *tert*-butylhydroperoxide,
TBS — *tert*-butyldimethylsilyl,
2-THF — tetrahydrofuran-2-yl,
TIPDS — tetraisopropyldisiloxane-1,3-diyl,
TMS — trimethylsilyl,
TfO — trifluoromethanesulfonate,
TPSCl — triisopropylbenzenesulfonyl chloride,
Ts — *p*-toluenesulfonyl (tosyl),
Tol — tolyl,
Tr — triphenylmethyl (trityl),
TS — thymidilate synthase,
UCK — uridine-cytidine kinase.

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