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Novel inhibitors of *Mycobacterium tuberculosis* growth based on modified pyrimidine nucleosides and their analogues

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Abstract. The review summarizes data on the synthesis and antituberculosis activity of pyrimidine nucleoside derivatives and their analogues. Enzymes from *M. tuberculosis* as promising targets for prototypes of new-generation drugs are considered. Nucleosides as inhibitors of drug-resistant *M. tuberculosis* strains are characterized. The bibliography includes 101 references.

I. Introduction

Tuberculosis (TB) is one of the major concerns of modern health care. According to estimates, one-third of the world's population is infected with *M. tuberculosis*, and some 2 million people die annually of it. It is the second leading cause of death from infectious diseases worldwide after the acquired immunodeficiency syndrome (AIDS). Tuberculosis might have already affected early hominids.[1] Since the discovery by Robert Koch in 1882 of the causative agent of the disease, the *M. tuberculosis* bacillus, the development of medications for anti-TB therapy has been one of high-priority tasks of pharmacology and medicinal chemistry. In the latent form, this disease is asymptomatic and not infectious. Only 5%–10% of infected individuals develop active disease during their life time.

More than 95% of the TB cases and 98% of deaths occur in developing countries. Tuberculosis is the main opportunistic infection in HIV-infected persons, which substantially increases the risk of progression of HIV infection and death. The HIV infection increases the chance of activation of latent *M. tuberculosis*[2] and causes the rapid progression of the disease soon after (re)infection.[3] Tuberculosis can be caused not only by *M. tuberculosis* but also by other microorganisms, in the first place by the mycobacteria *M. avium* and *M. bovis* [the latter served as the basis for the design of the bacillus Calmette-Guérin (BCG) for vaccination against TB, which shows higher than 98% homology with *M. tuberculosis*].[4] These mycobacteria are often found in HIV-infected individuals.[5,6]

Most of the currently used drugs were designed in the 1950–1960s. According to the present-day classification, antituberculosis drugs are divided into two lines depending on the tolerance and clinical efficacy. First-line drugs include isoniazid, rifampicin, streptomycin, pyrazinamide and ethambutol. Second-line drugs include ethionamide, protionamide, cycloserine, amikacin, capreomycin, kanamycin, ofloxacin, ciprofloxacin and *p*-aminosalicylic acid. Since second-line drugs are more toxic, they are used only for the treatment of patients with resistance to first-line drugs. Figure 1 gives a schematic view of the mycobacterium and traditional targets for antituberculosis medications, whose mechanism of action involves the inhibition of various biological processes, such as the protein, nucleic acid and cell wall biosynthesis or the cell membrane potential change.[7]

Modern antituberculosis therapy relying on multicomponent drug compositions is a prolonged course (at least 6–9 months). The TB treatment regimens often involve derivatives of natural products belonging to different classes of organic compounds, such as phenols, quinones, peptides, carbohydrates, steroids, alkaloids, terpenes and so one (see reviews [8,9]). Many new compounds are currently undergoing clinical trials (see reviews [10–12]).

The drug design for the treatment of tuberculosis is complicated due to the development of drug-resistant *M. tuberculosis* strains: practically incurable extensively drug-resistant (XDR) strains and multidrug-resistant (MDR) strains, which are barely affected by standard chemotherapy regimens. Evidently, there is the urgent need to design radically new drugs acting on new targets and active against resistant strains.

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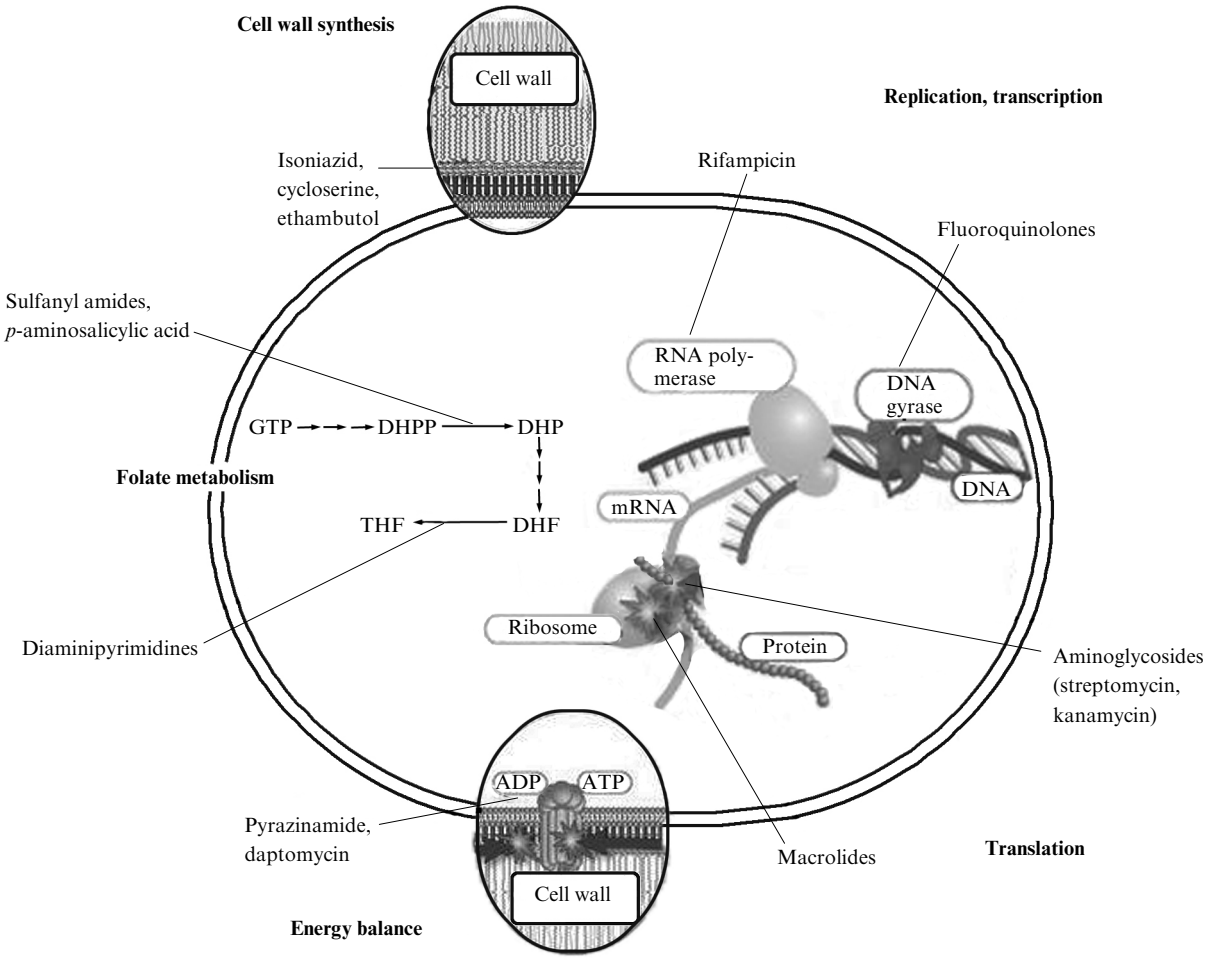


Figure 1. Targets for antituberculosis drugs.
GTP is guanosine triphosphate, DHPP is dihydropterin pyrophosphate, DHP is dihydropterin, DHF is dihydrofolate, THF is tetrahydrofolate.

The therapy of viral infections and cancer diseases is often based on the use of derivatives of natural nucleosides.[13] However, the antituberculosis activity of the latter was unknown until recently. It is only in the past decade that several groups of modified nucleosides, which exhibited considerable antituberculosis activity in experimental models, were documented. These compounds were prepared using a great variety of modern methods of bioorganic chemistry.

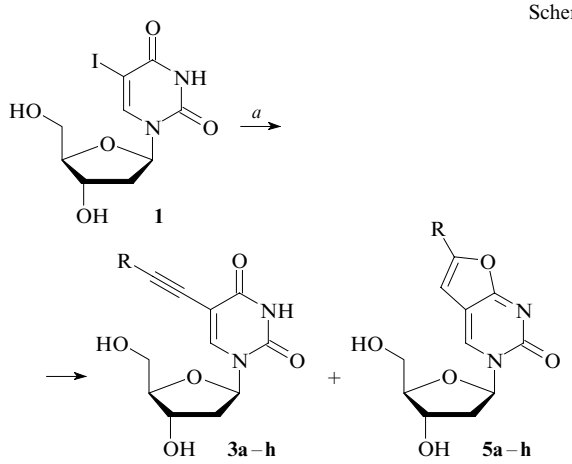
The present review considers methods for the synthesis and antituberculosis activity of modified pyrimidine nucleosides and their analogues. This area of investigation is in its infancy and has attracted attention of a rather narrow range of researchers. We restricted ourselves to the description of pyrimidine nucleoside derivatives, because the antituberculosis activity of purine nucleoside derivatives has been considered in detail in reviews concerning mainly the synthesis and investigation of analogues of sulfamoyladenosine, which inhibits the biosynthesis of siderophores mediating the acquisition of iron essential for the survival of mycobacteria.[14, 15]

II. Pyrimidine nucleoside derivatives

The first work in this field was published in 2005 by the Canadian research team headed by Kumar.[16] This group synthesized pyrimidine nucleoside derivatives containing long-chain hydrocarbon substituents at the C(5) atom of the nucleobase and characterized their antimycobacterial activity.

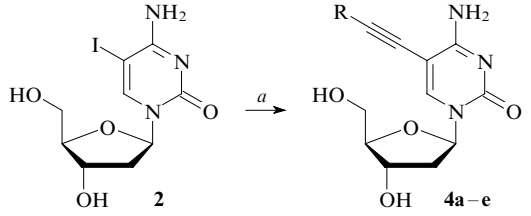
An alkynyl moiety was introduced at position 5 of the pyrimidine base according to the Sonogashira method [17–20] generally with the use of homogeneous platinum-group catalysts. Organometallic complexes of palladium soluble in dipolar organic solvents have found particularly wide application. The mechanism of the Sonogashira cross-coupling was described in detail.[17–20] This method was used to synthesize modified 5-alkynylated nucleosides of the uridine (**3a–h**) (Scheme 1) and cytidine series (**4a–e**) (Scheme 2) starting from iodo derivatives **1** and **2**, respectively.[16] The Sonogashira method is convenient for the preparation of modified nucleosides; however, the synthesis is complicated by the formation of bicyclic furopyrimidine analogues **5a–h** as by-products.[21]

Scheme 1



R = H (**a**), Me (**b**), Pr^n (**c**), n-C_5H_{11} (**d**), n-C_8H_{17} (**e**), n-$C_{10}H_{21}$ (**f**), n-$C_{12}H_{25}$ (**g**), Ph (**h**); (*a*) HC≡CR, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF

Scheme 2



R = Pr^n (**a**), n-C_5H_{11} (**b**), n-C_8H_{17} (**c**), n-$C_{10}H_{21}$ (**d**), Ph (**e**); (*a*) HC≡CR, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF

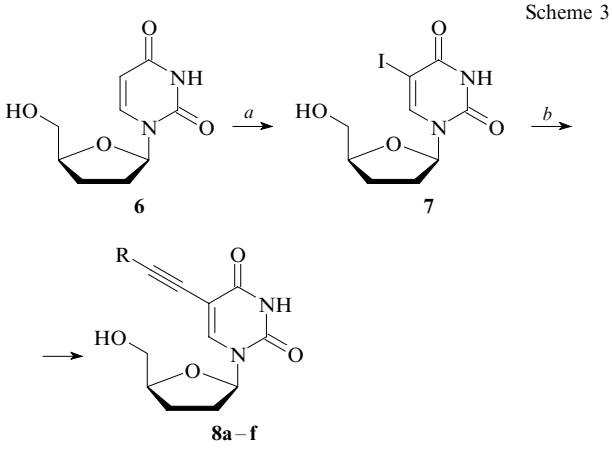
Among the synthesized compounds, note pyrimidine nucleoside derivatives exhibiting the highest activity against the mycobacteria *M. bovis* and *M. avium*: 5-dodecynyl-2′-deoxyuridine (**3f**), 5-tetradecenyl-2′-deoxyuridine (**3g**) and 5-dodecynyl-2′-deoxycytidine (**4d**). The antimycobacterial efficacy of these compounds was found to depend on the length of the hydrocarbon substituent. It is noteworthy that 2′-deoxynucleoside derivatives are much more effective than the corresponding ribo analogues, whereas bicyclic 6-alkyl-3-(2-deoxy-β-D-ribofuranosyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-ones **5** do not inhibit the mycobacterial growth at all. Therefore, it was shown that it is the presence of a long aliphatic substituent at position 5 of the pyrimidine base of 2′-deoxyribonucleoside that is responsible for high antibacterial activity of this type of compounds.

2′,3′-Dideoxynucleosides containing halogen atoms, alkyl and alkynyl substituents at position 5 of the pyrimidine base, as well as their 5-chloro-4-thio- and 3′-fluorouridine analogues, were synthesized to study the relationship between the structure of such modified nucleosides and their activity against mycobacteria.[22] Modified nucleosides of the purine series were also synthesized; however, they did not show notable biological activity.

The synthesis of 5-alkynyl derivatives of 2′,3′-dideoxyuridine is shown in Scheme 3.[22]

The reaction of 2′,3′-dideoxyuridine (**6**) with iodine monochloride and sodium azide in acetonitrile afforded 5-iodo-2′,3′-dideoxyuridine (**7**). 5-Alkynyl derivatives of 2′,3′-dideoxyuridine **8a**–**f** were synthesized by the condensation of synthon **7** with a series of terminal alkynes.

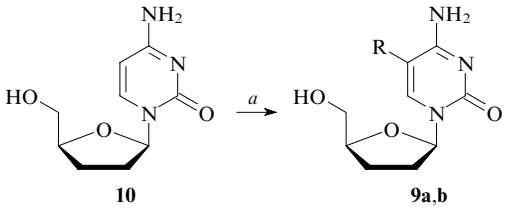
Scheme 3



R = Pr^n (**a**), n-C_8H_{17} (**b**), n-$C_{10}H_{21}$ (**c**), n-$C_{11}H_{23}$ (**d**), n-$C_{12}H_{25}$ (**e**), Ph (**f**); (*a*) ICl, NaN_3, MeCN, 0–25 °C; (*b*) HC≡CR, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF

5-Iodo- and 5-bromo-2′,3′-dideoxycytidines (**9a**,**b**) were synthesized starting from 2′,3′-dideoxycytidine (**10**) by the reaction with *N*-iodosuccinimide and *N*-bromosuccinimide (NBS), respectively (Scheme 4).[22]

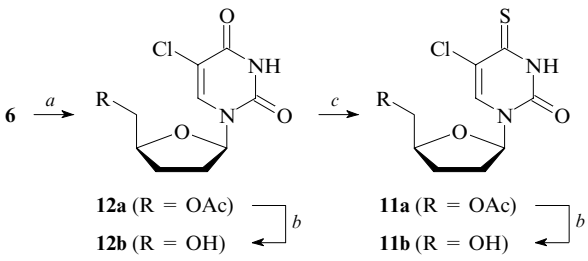
Scheme 4



R = I (**a**), Br (**b**); (*a*) N-iodo (for **9a**) or *N*-bromosuccinimide (for **9b**), DMF, 25 °C

4-Thio derivatives, such as 5′-*O*-acetyl-5-chloro-4-thio-2′,3′-dideoxyuridine (**11a**) and 5-chloro-2′,3′-dideoxy-4-thiouridine (**11b**), were synthesized (Scheme 5) by the reaction of acetylated 5-chloro-2′,3′-dideoxyuridine (**12a**) with Lawesson's reagent[23] [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide] in 1,4-dioxane under heating followed by the removal of the acetyl protecting group with an ammonia solution in methanol.

Scheme 5

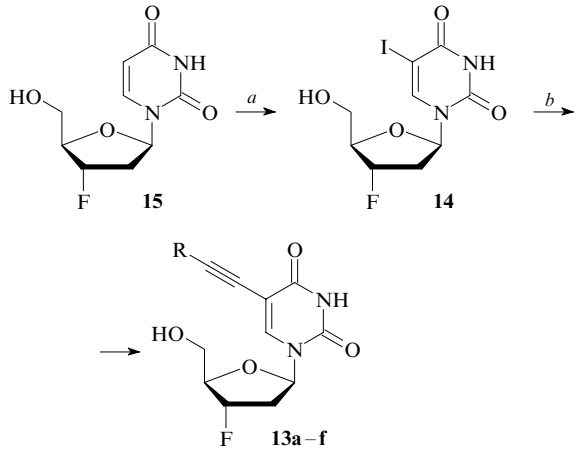


(*a*) 1) Ac_2O, Py (Py is pyridine); 2) *N*-chlorosuccinimide (NCS), Py, 90 °C; (*b*) NH_3, MeOH, 0 °C; (*c*) Lawesson's reagent, 1,4-dioxane

5-Alkynyl derivatives of 3′-fluoro-2′,3′-dideoxynucleosides **13a**–**f** were synthesized by the Sonogashira reaction[17] catalyzed by palladium and copper salts (Scheme 6). The starting 3′-fluoro-5-iodo-2′,3′-dideoxyuridine (**14**) was pre-

pared according to the above-described method from 3′-fluoro-2′,3′-dideoxyuridine (**15**).

Scheme 6



R = Pr^n (**a**), n-C_8H_{17} (**b**), n-$C_{10}H_{21}$ (**c**), n-$C_{11}H_{23}$ (**d**), n-$C_{12}H_{25}$ (**e**), Pr^nC_6H_4 (**f**); (*a*) ICl, MeOH, 60 °C; (*b*) HC≡CR, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF

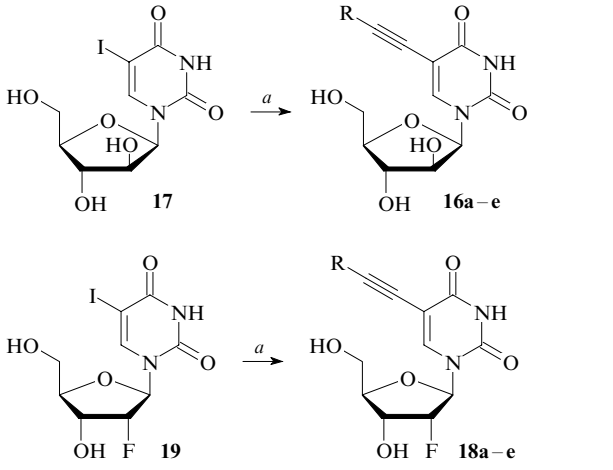
Compounds **9** and **12** did not inhibit the growth of *M. tuberculosis*, and derivatives **11** exhibited insignificant antituberculosis activity [the minimum inhibitory concentration that inhibits the growth of 90% of bacteria resulting in bacterial death (MIC_{90}) is 50–100 μg ml^{-1}]. 3′-Fluoro-2′,3′-dideoxy-5-decynyluridine (**13b**), 3′-fluoro-2′,3′-dideoxy-5-dodecynyluridine (**13c**) and 3′-fluoro-2′,3′-dideoxy-5-tridecynyluridine (**13d**) showed moderate activity (MIC_{90} = 10–50 μg ml^{-1}) compared to the corresponding dideoxynucleosides **8b**–**d**. The introduction of the fluorine atom at position 3′ of molecule **8e**, *i.e.*, the synthesis of 3′-fluoro-2′,3′-dideoxy-5-tetradecynyluridine (**13e**), considerably improved the ability of the resulting compound to inhibit the growth of *M. tuberculosis* and *M. bovis* in model systems (MIC_{90} = 1–2 μg ml^{-1}). The examination of the ability to inhibit the mycobacterium *M. avium* showed that compounds **8b**–**e**, **13c** and **13e** have considerable activity (MIC_{90} = 10–100 μg ml^{-1}).

In addition to the above-described experiments, Kumar and co-workers [22] studied the ability of the most promising compounds (**8c** and **13e**) to inhibit rifampicin-resistant strains of mycobacteria. The tests showed that the activity of these compounds against such strains remained unchanged and was retained at MIC_{90} = 1–2 μg ml^{-1}, which is comparable with the characteristics of the currently used drugs. The investigation of the structure–activity relationship for the synthesized compounds showed that the presence of substituents at the C(5) atom and the modification at position 3′ of the carbohydrate moiety in pyrimidine nucleosides play an important role and affect the antibacterial activity.

In continuation of these studies, the carbohydrate moiety of nucleosides was modified at position 2′. Arabinonucleosides and 2′-fluorine-substituted ribonucleosides containing various substituents at position 5 of the pyrimidine ring, including long-chain alkynyl substituents, alkenyl and haloalkenyl groups, halogen atoms, *etc.* were synthesized.[24] It is known that some pyrimidine arabinonucleosides, 1-(β-D-arabinofuranosyl)pyrimidines, exhibit antiviral and antitumour activities.[25,26] Among such nucleosides, 1-(β-D-arabinofuranosyl)-5-prop-1-ynyluracil (netivudine) is widely known and proved to be most efficient in the treatment of infections caused by the *Varicella zoster* virus. This compound has a long half-life in blood plasma and a number of other good pharmacokinetic parameters.[27] Kumar and co-workers [24] suggested that a compound containing simultaneously a substituent at position 5 and the fluorine atom at position 2′ of the nucleoside would be an efficient substrate of enzymes from mycobacteria.

The target compounds, 1-(β-D-arabinofuranosyl)-5-alkynyluracils **16a**–**e**, were synthesized in one step (Scheme 7) by the palladium-catalyzed addition of terminal alkynes to 1-(β-D-arabinofuranosyl)-5-iodouracil (**17**) in dimethylformamide. 1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-5-alkynyluracils **18a**–**e** were synthesized from fluoro derivative **19** according to the same procedure.

Scheme 7

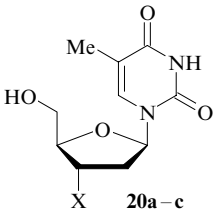


R = n-C_5H_{11} (**a**), n-C_8H_{17} (**b**), n-$C_{10}H_{21}$ (**c**), n-$C_{11}H_{23}$ (**d**), n-$C_{12}H_{25}$ (**e**); (*a*) HC≡CR, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF

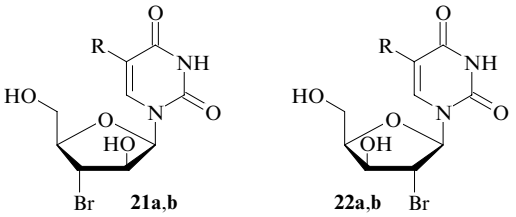
All the synthesized compounds were studied as inhibitors of the growth of the mycobacteria *M. tuberculosis*, *M. bovis* and *M. avium*. The most active arabinofuranose analogue contains the dodecynyl substituent at position 5 (compound **16c**) (MIC_{90} = 1–5 μg ml^{-1}), whereas the most active 2′-fluoro-2′-deoxyuridines are those containing dodecynyl (**18c**) and tridecynyl (**18d**) moieties (MIC_{90} = 1–5 μg ml^{-1}). It is interesting that compound **18d** proved to be much more active against all types of mycobacteria than arabino derivative **16d** containing the same substituent at the C(5) atom (MIC_{90} = 10–50 μg ml^{-1}). This fact suggests that the fluorine atom at position 2′ of nucleosides plays an important role in the manifestation of antituberculosis activity.[24]

Further attempts to prepare new antituberculosis agents resulted in the synthesis of 3′-halogen-substituted 2′,3′-dideoxyribofuranosylthymines **20a**–**c**, as well as arabinofuranosyluracil and -thymine **21a**,**b**; in addition, 2-bromo-substituted xylofuranosyluracil and -thymine **22a**,**b** were synthesized.[28] Among such nucleoside analogues, 3′-bromo-, 3′-chloro- and 3′-iodo derivatives of 3′-deoxythymidine **20a**–**c** are of most interest. Their antituberculosis properties depend on the nature of the introduced halogen atom.

Structures **20**–**22**



20a–**c** X = Br (**a**), Cl (**b**), I (**c**)

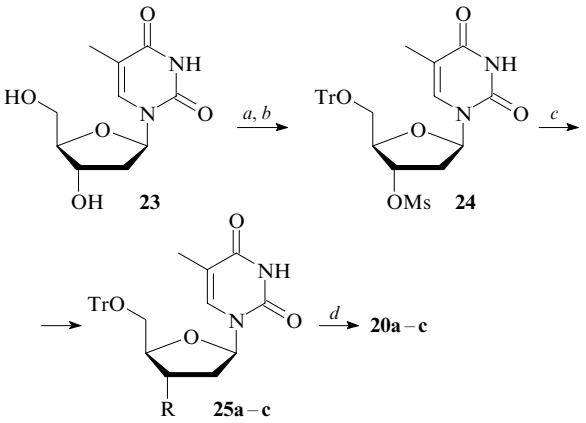


21a,**b** **22a**,**b**

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

The synthesis of 3′-halogen-substituted derivatives **20a**–**c** involved the protection of the 5′-hydroxy group of thymidine (**23**) with a trityl group (Tr) followed by the treatment with mesyl chloride (MsCl) to form activated compound **24** (Scheme 8). This resulted in the selective introduction of substituents at the required position 3′ of the 2′-deoxyribose moiety. The removal of the protecting group in halogen derivatives **25a**–**c** afforded target products **20a**–**c**.

Scheme 8

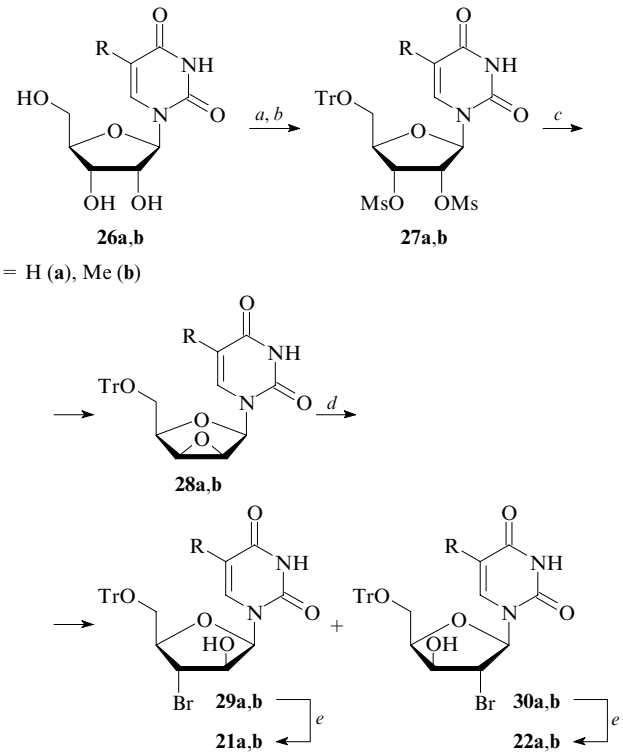


R = Br (**a**), Cl (**b**), I (**c**); (*a*) TrCl, DMAP, Py, 80 °C; (*b*) MsCl, 0–5 °C; (*c*) LiBr or LiCl, MeCN, stirring; or NaI, DME; (*d*) 80% AcOH, 90 °C; DMAP is 4-dimethylaminopyridine; DME is dimethoxyethane

Bromo derivatives **21a**,**b** and **22a**,**b** were synthesized (Scheme 9) from uridine (**26a**) or 2′-hydoxythymidine (**26b**) using the same combination of the trityl and mesyl groups. Mesyl derivatives **27a**,**b** were transformed into epoxy compounds **28a**,**b**, whose treatment with ammonium bromide in ethanol gave a mixture of products **29** and **30**. The subsequent removal of the trityl protecting groups yielded target compounds **21a**,**b** and **22a**,**b**.

This reaction gives 3′-bromo derivatives as the major products, because the conformation of the carbohydrate moiety favours the attack of the halide ion predominantly on the C(3′) atom of the carbohydrate moiety. The close arrangement of the C(2′) atom and the electron-deficient anomeric centre leads to the opening of the epoxide ring from the side of the C(3′) atom with retention of the oxygen atom at position 2′ of the arabinose moiety.

Scheme 9



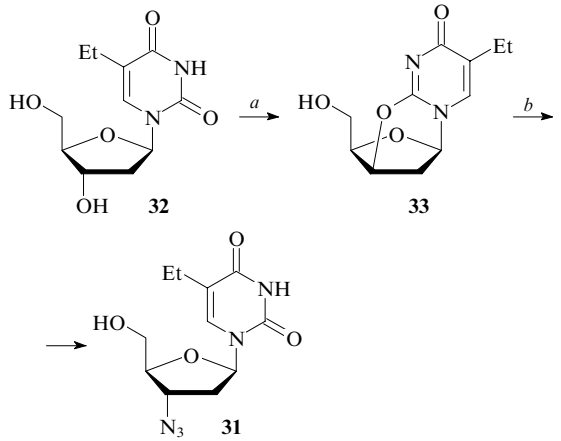
(*a*) TrCl, DMAP, Py, 80 °C; (*b*) MsCl, Py, 0–5 °C; (*c*) 1 M NaOH in an acetone–water mixture (1 : 1); (*d*) NH_4Br, EtOH; (*e*) 80% AcOH, 90 °C

3′-Bromo-3′-deoxythymidine (**20a**) efficiently suppressed the growth of both wild-type *M. tuberculosis* (MIC_{50} = 5 μg ml^{-1}) and its resistant strains (MIC_{50} = 5–10 μg ml^{-1}). By contrast, 3′-chloro-3′-deoxythymidine (**20b**) exhibited moderate activity (MIC_{50} = 50 μg ml^{-1}), whereas iodo derivative **20c** had no effect on the growth of mycobacteria. More recently, 1-(3-bromo-3-deoxy-β-D-arabinofuranosyl)uracil (**21a**) and 1-(3-bromo-3-deoxy-β-D-arabinofuranosyl)thymine (**21b**) were synthesized reasoning from the high activity of compound **20a**. The presence or the absence of the methyl group at position 5 of the pyrimidine base plays a key role in the manifestation of antituberculosis activity. Thus, uracil derivative **21a** did not inhibit the growth of *M. tuberculosis*, whereas thymine derivative **21b** proved to be highly active (MIC_{50} = 1 μg ml^{-1}). The inhibitory activity of 1-(2-bromo-2-deoxyxylofuranosyl)thymine (**22b**) is weaker than that of compound **21b** (MIC_{50} = 25 μg ml^{-1}).

More recently, a series of nucleosides containing the ethyl substituent or a halogen (Cl, Br) atom at position 5 and those modified by different substituents (N_3, NH_2, F, I) at the C(3′) atom of the carbohydrate moiety were prepared.[29]

3′-Azido-2′,3′-dideoxy-5-ethyluridine (**31**) was synthesized by the facile one-pot method using the reaction of 2′-deoxy-5-ethyluridine (**32**) with triphenylphosphine and diisopropyl azodicarboxylate (DIAD) followed by the treatment of 2,3′-anhydro derivative **33** with sodium azide (Scheme 10).

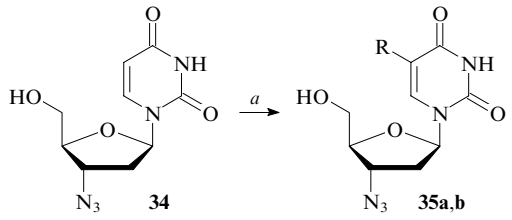
Scheme 10



(*a*) PPh_3, DIAD, MeCN, from −15 °C to 0 °C; (*b*) NaN_3, DMF, 125 °C

The chlorination and bromination of 3′-azido-2′,3′-dideoxyuridine (**34**) at position 5 was performed in one step using *N*-chloro- and *N*-bromosuccinimide to form products **35a**,**b**, respectively (Scheme 11).

Scheme 11



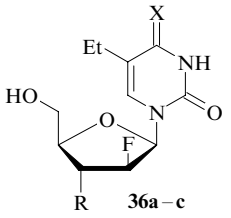
R = Cl (**a**), Br (**b**); (*a*) NCS, Me_2O-H_2O, 45 °C or NBS, DMF, 76 °C

The investigation of the ability of the synthesized compounds to inhibit the mycobacterial growth *in vitro* showed that the ethyl substituent at position 5 and the azido group at the C(3′) atom play a decisive role in the inhibitory activity of this series of nucleosides. 3′-Azido-2′,3′-dideoxy-5-ethyluridine (**31**) exhibited considerable inhibitory activity against *M. tuberculosis* (MIC_{50} = 10 μg ml^{-1}) comparable with that of the antibiotic cycloserine.[29] 5-Chloro and 5-bromo derivatives of 3′-azido-2′,3′-dideoxyuridine **35a**,**b** also suppressed the growth of *M. tuberculosis* but only at high concentrations ($MIC_{50} \geqslant 100$ μg ml^{-1}).

Recently, a new series of 5-ethyl and 5-hydroxymethyl derivatives of pyrimidine nucleosides **36**–**38** were synthesized and their antimycobacterial activity was investigated.[30]

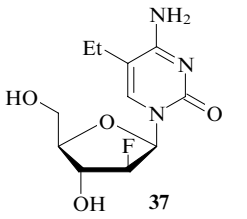
These compounds are of interest because 5-ethyl- and 5-hydroxymethyl-2′-deoxyuridines are non-mutagenic natural analogues of thymidine. Certain modifications in the carbohydrate moiety have an effect on the biological activity.[31] It was shown that 5-ethyl-2′-fluoro-2′-deoxyuridine effectively inhibits herpes and hepatitis B viruses.[32] 2′-Deoxy-5-hydroxymethyluridine inhibits smallpox vaccine and herpes viruses. The latter compound suppresses the growth of different types of tumour cells.[33]

Structures **36**–**38**

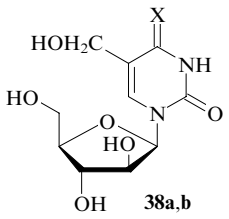


36a–**c**

Compound **36**	X	R	MIC_{50} /μg ml^{-1}
36a	O	OH	inactive
36b	S	OH	50
36c	O	Br	5



37

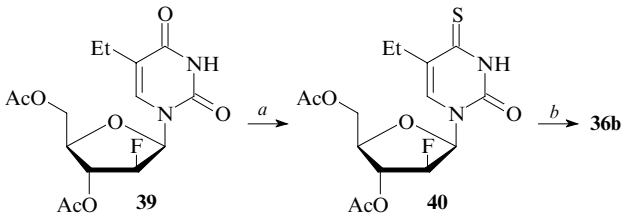


38a,**b**

X = O (**a**), S (**b**)

The replacement of the oxygen atom at C(4) of 1-(3,5-di-*O*-acetyl-2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-ethyluracil (**39**) by sulfur was accomplished using Lawesson's reagent[23] followed by the removal of acetyl groups in product **40** to prepare target compound **36b** (Scheme 12).

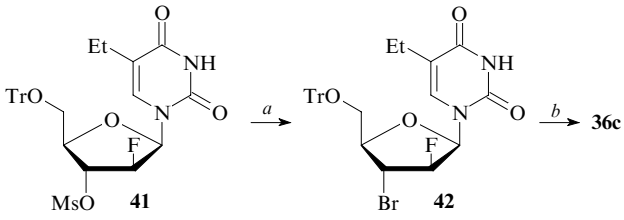
Scheme 12



(*a*) Lawesson's reagent, 1,4.-dioxane; (*b*) 24% NH_3

1-(3-Bromo-2-fluoro-2,3-dideoxy-β-D-arabinofuranosyl)-5-ethyluracil (**36c**) was synthesized from 1-(2-fluoro-3-*O*-mesyl-5-*O*-trityl-2-deoxy-β-D-arabinofuranosyl)uracil (**41**) by the treatment with sodium bromide (Scheme 13). The subsequent removal of the trityl groups in bromo derivative **42** afforded target dihalogen-substituted arabinonucleoside **36c**.

Scheme 13



(*a*) NaBr, DMF, 110 °C; (*b*) 80% AcOH, 90 °C

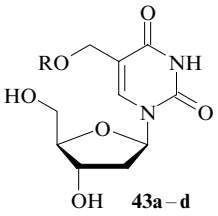
The antimycobacterial activity of a new class of 5-ethyl, 5-hydroxymethyl and 5-methoxymethyl analogues of pyrimidine nucleosides was studied *in vitro* in the *M. avium*, *M. bovis* and *M. tuberculosis* strains. Among compounds containing the ethyl group at position 5 and the fluorine atom at C(2′) of the carbohydrate moiety, compound **36c**

proved to be the most efficient inhibitor of mycobacterial growth (MIC_{50} = 5 μg ml^{-1}). The inhibitory properties of derivative **36c** are comparable with those of arabinonucleoside **21b**. Based on this fact it was concluded that the replacement of the fluorine atom at position 2′ by the OH group and of the ethyl group by methyl in the pyrimidine base does not lead to serious changes in the antituberculosis activity of pyrimidine nucleoside analogues. It is interesting that 5-ethyl-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)cytosine (**37**) displayed weak antimycobacterial activity (MIC_{99} = 50 μg ml^{-1}), whereas uridine analogue **36a** did not show activity at all.

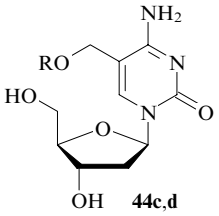
When comparing the inhibitory activity of compounds containing the hydroxymethyl substituent at position 5, noteworthy is the difference in the properties of 1-(β-D-arabinofuranosyl)-5-hydroxymethyluracil (**38a**) and its 4-thio analogue **38b**. Uridine derivative **38a** did not have antituberculosis activity, whereas **38b** proved to be the most active compound in this series (MIC_{50} = 0.5 μg ml^{-1}).

Recently, a new series of 5-modified derivatives of 2′-deoxyuridine and 2′-deoxycytidine **43**–**48** was synthesized and these compounds were shown to be able to inhibit the growth of *M. tuberculosis*.[34,35] Compounds belonging to this class contain a long-chain alkoxymethyl (**43a**–**d**, **44c**,**d**, **47a**–**d**, **48a**–**c**) or alkyltriazolylmethyl (**45a**–**c**, **46a**–**c**) substituent at position 5 of the pyrimidine base. Derivatives **47a**–**d** and **48a**–**c** include also modifications in the carbohydrate moiety. 3′,5′-Di-*O*-acetyl-2′-deoxy-5-bromomethyluridine (**49**), which can be easily prepared by the radical bromination of thymidine acetate according to Barwolff and Langen,[36] was used as the staring compound for the synthesis of most of 5-modified nucleosides. Acetylated 5-alkoxymethyl-2′-deoxyuridines **50a**–**d** were produced by the condensation of bromide **49** with the corresponding alkan-1-ol according to a facile and efficient procedure developed earlier (Scheme 14).[37,38] The deblocking of synthons **50a**–**d** gave 2′-deoxyuridine derivatives **43a**–**d**. Analogues of 2′-deoxycytidine **44c**,**d** were synthesized according to Divakar and Reese[39] by the condensation of synthons **50c**,**d** prepared *in situ* with tris(1,2,4-triazolyl)phosphoryl[40] followed by the treatment with an aqueous solution of ammonia.

Structures **43**–**48**

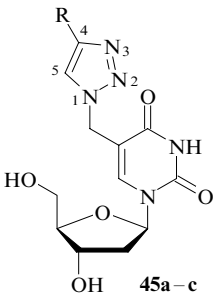


43a–**d**

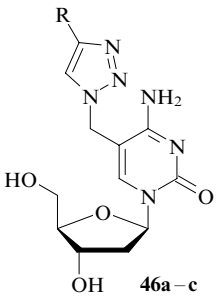


44c,**d**

R = n-$C_{10}H_{21}$ (**a**), n-$C_{11}H_{23}$ (**b**), n-$C_{12}H_{25}$ (**c**), n-$C_{14}H_{29}$ (**d**)

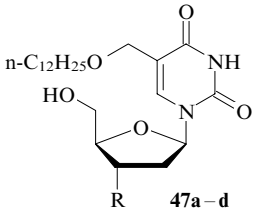


45a–**c**



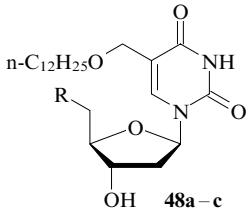
46a–**c**

R = n-C_8H_{17} (**a**), n-$C_{10}H_{21}$ (**b**), n-$C_{12}H_{25}$ (**c**)



47a–**d**

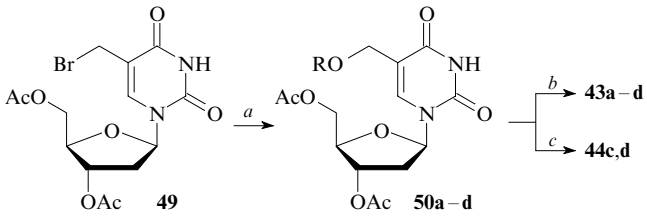
R = N_3 (**a**), NH_2 (**b**), NEt_2 (**c**), NHEt (**d**)



48a–**c**

R = I (**a**), N_3 (**b**), NH_2 (**c**)

Scheme 14

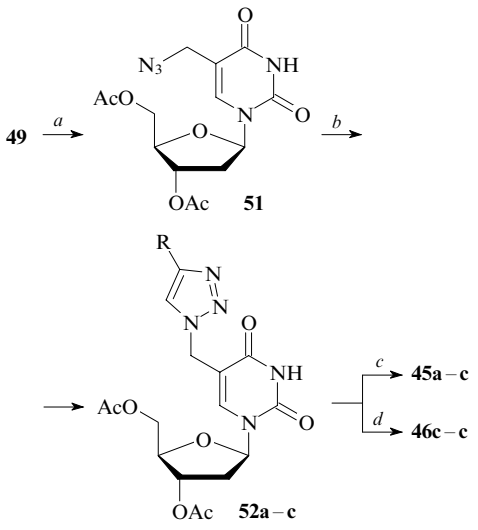


R = n-$C_{10}H_{21}$ (**a**), n-$C_{11}H_{23}$ (**b**), n-$C_{12}H_{25}$ (**c**), n-$C_{14}H_{29}$ (**d**); (*a*) n-$C_nH_{2n+1}OH$ (*n* = 10–12, 14), DMF, 37 °C; (*b*) 24% NH_3; (*c*) 1) 1,2,4-triazole, Et_3N, $POCl_3$, MeCN; 2) 34% NH_3, 1,4-dioxane, 4 °C

The 1,3-dipolar cycloaddition of organic azides and alkynes catalyzed by copper(I) salts is widely used in organic synthesis, in particular, for the preparation of new drugs. The resulting 1,2,3-triazole moieties (in compounds **45** and **46**) not only serve as linkers but also can improve the binding of the resulting compounds to biological targets due apparently to the formation of additional hydrogen bonds and dipole–dipole interactions.[41,42]

5-Alkyltriazolylmethyl derivatives of 2′-deoxyuridine **45a**–**c** (Scheme 15) were prepared using the click chemistry method developed by Lee and co-workers.[43]

Scheme 15



R = n-C_8H_{17} (**a**), n-$C_{10}H_{21}$ (**b**), n-$C_{12}H_{25}$ (**c**); (*a*) NaN_3, DMF, 50 °C; (*b*) C_nH_{2n-2} (*n* = 10, 12, 14), $CuSO_4 \cdot 5H_2O$, sodium ascorbate, CH_2Cl_2; (*c*) 24% NH_3; (*d*) 1) 1,2,4-triazole, Et_3N, $POCl_3$, MeCN; 2) 34% NH_3, 1,4-dioxane, 4 °C

The starting 5-azidomethyl-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**51**) was produced by the azidation of 5-bromomethyluridine **49** according to a procedure described in the studies.[38,44] The 1,3-dipolar cyclocondensation of azide **51** with appropriate alkynes in a two-phase dichloromethane–water system catalyzed by Cu(I), which was prepared *in situ* from $CuSO_4 \cdot 5H_2O$ and sodium ascorbate, afforded 5-alkyltriazolylmethyl derivatives of 3′,5′-di-*O*-acetyl-2′-deoxyuridine **52a–c**. The removal of acetyl groups by the treatment with ammonia gave 2′-deoxyuridines **45a–c**. 5-Alkyltriazolylmethyl derivatives of 2′-deoxycytidine **46a–c** were synthesized by analogy with compounds **44** using the condensation of synthons **52a–c** with tris(1,2,4-triazolyl)-phosphoryl.[39]

The antituberculosis activity was tested in two *M. tuberculosis* strains: the laboratory strain H37Rv and the resistant strain (resistant to five first-line antituberculosis medications) MS-115. Compounds with the chains length of 10 or 12 carbon atoms proved to be most efficient; nucleoside analogues containing shorter substituents weakly inhibited mycobacterial growth. On the whole, 2′-deoxycytidine derivatives are less active than 2′-deoxyuridine derivatives. The modification of the carbohydrate moiety gave compounds substituted at position 3′ (**47a–d**) or 5′ (**48a–c**), which showed high activity but remained highly toxic to the *Vero* cell culture.

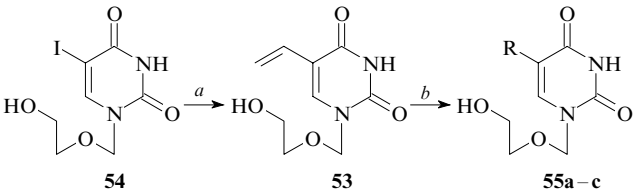
Modified nucleosides with alkyltriazolylmethyl substituents (**45a–c**, **46a–c**) exhibited high activity and were not toxic (CD_{50} > 100 μg ml^{-1}). 5-Dodecyloxymethyl-2′-deoxyuridine (**43c**), 5-(4-decyl-1,2,3-triazol-1-yl)methyl-2′-deoxyuridine (**45b**) and 5-(4-dodecyl-1,2,3-triazol-1-yl)methyl-2′-deoxycytidine (**46c**) displayed the highest antituberculosis activity. These compounds inhibited the growth of the standard laboratory *M. tuberculosis* strain H37Rv (MIC_{99} = 20, 10 and 10 μg ml^{-1}, respectively) and the resistant MDR strain of *M. tuberculosis* MS-115 (MIC_{99} = 50, 10 and 10 μg ml^{-1}, respectively) at concentrations comparable with those for the currently used drugs.

III. Acyclic and carbocyclic analogues of pyrimidine nucleosides

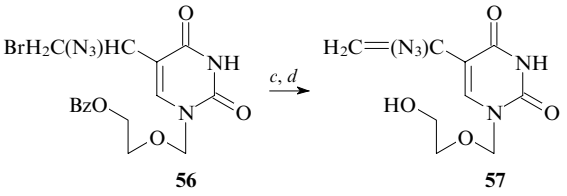
Nucleoside analogues of the pyrimidine series, in which the cyclic pentafuranose moiety is replaced by an acyclic (for example, alkoxyl) group, is another type of promising antituberculosis agents.[45] Due to the flexibility of the acyclic moiety, the three-dimensional structure of the compound can be easily adapted to the interaction with the enzyme active site. Hence, the analogue can act as a substrate or an inhibitor of enzymatic reactions like (deoxy)ribosides. It was also shown that this type of compounds is not subjected to phosphorolysis in living organisms,[30,46] whereas nucleosides are cleaved to the corresponding pyrimidine bases by bacterial nucleoside phosphorylases.[47,48] The synthesis and antituberculosis activity of a number of acyclic nucleoside analogues containing different substituents at position 5 (1-azido-2-haloethyl, where halogen is Cl, Br or I, 1-azidovinyl, alk-1-ynyl, *etc.*) were described in 2007.[45] These substituents were chosen based on the fact that 2′-deoxyuridine derivatives containing identical substituents exhibited moderate (in the case of azides) and high (in the case of alkynyl derivatives) antituberculosis activity.[49] The (2-hydroxyethoxy)methyl substituent was taken as a prototype of the acyclic moiety, which made it possible to design the effective antiherpetic drug Acyclovir.[50]

New nucleosides were synthesized starting from 1-(2-hydroxyethoxymethyl)-5-vinyluracil (**53**), which was prepared from iodo derivative **54**. The reaction of vinyluracil **53** with *N*-bromo(chloro)succinimide or iodine chloride and sodium azide afforded products **55a–c** (Scheme 16).[45] The reaction of benzoylated derivative **56** with potassium *tert*-butoxide in tetrahydrofuran followed by the removal of the benzoyl group produced target azidovinyl analogue **57** (see Scheme 16). The presence of the azido group at position 1 of the substituent plays a substantial role in the ability of the compound to inhibit mycobacterial growth.

Scheme 16



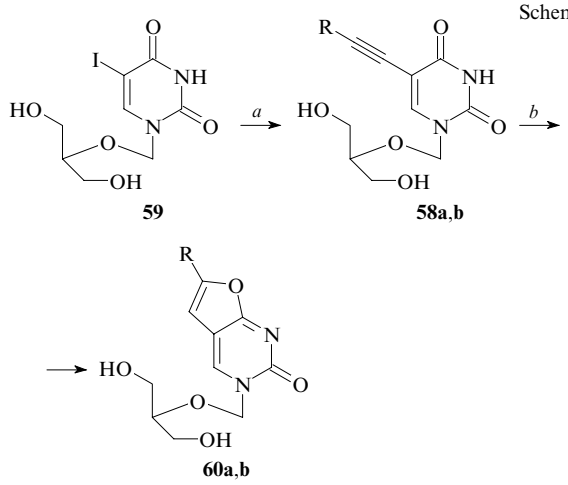
R = $CH(N_3)CH_2Br$ (**a**), $CH(N_3)CH_2I$ (**b**), $CH(N_3)CH_2Cl$ (**c**)



(*a*) $Pd(OAc)_2$, Ph_3P, Et_3N, $AcOCH{=}CH_2$, 70 °C;
(*b*) NBS or NCS, NaN_3, DMF–H_2O, 0 °C or ICl, NaN_3, MeCN;
(*c*) Bu^tOK, THF; (*d*) NH_3–MeOH, 0–25 °C

1-[(2-Hydroxy-1-hydroxymethyl)ethoxymethyl]-5-decynyluracil (**58a**) and 1-[(2-hydroxy-1-hydroxymethyl)ethoxymethyl]-5-dodecynyluracil (**58b**) were synthesized by the Sonogashira reaction of 1-[(2-hydroxy-1-hydroxymethyl)-ethoxymethyl]-5-iodouracil (**59**) with dec-1-yne or dodec-1-yne, respectively (Scheme 17).[17] The cyclization of 5-alkynylated nucleoside analogues **58a**,**b** in the presence of

Scheme 17



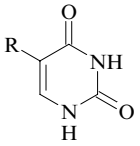
R = n-C_8H_{17} (**a**), n-$C_{10}H_{21}$ (**b**); (*a*) dec-1-yne or dodec-1-yne, $Pd(PPh_3)_4$, CuI, Pr^i_2EtN, DMF, 25 °C; (*b*) CuI, Et_3N, MeOH

copper(I) iodide and triethylamine in methanol gave the corresponding furopyrimidine derivatives **60a**,**b**.

The investigation of the antituberculosis activity of the new compounds showed that 5-(1-azido-2-chloroethyl)-1-(2-hydroxy)ethoxymethyluracil (**55c**) and alkynylated analogues **58a**,**b** exhibit considerable activity against all three types of mycobacteria under study (*M. tuberculosis*, *M. bovis*, *M. avium*) (MIC_{50} = 10 μg ml^{-1}) as opposed to 2′-deoxyuridines **3e**,**f** containing similar substituents. Bicyclic analogues **60a**,**b** showed moderate efficacy (MIC_{50} = 50 and 10 μg ml^{-1}, respectively).

5-Modified uracils **61a**–**f** containing long-chain alkynyl substituents did not show antituberculosis activity.[51]

Structures **61**



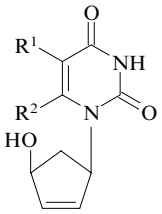
61a–**f**

R = C≡CPr^n (**a**), C≡CC$_5$H$_{11}$-n (**b**), C≡CC$_8$H$_{17}$-n (**c**), C≡CC$_{10}$H$_{21}$-n (**d**), C≡CC$_{11}$H$_{23}$-n (**e**), C≡CC$_{12}$H$_{25}$-n (**f**), C≡CPh (**g**), C≡CC$_6$H$_4$Pr^n (**h**), C≡CC$_6$H$_4$C$_5$H$_{11}$-n (**i**)

Compound **61g**–**i** containing the phenylalkynyl or *p*-alkylphenylalkynyl substituent at position 5 exhibited notable activity (MIC_{50} = 1–5 μg ml^{-1}). These compounds were synthesized using one of modifications of the Sonogashira reaction of 5-iodouracil with the corresponding alkynes catalyzed by palladium salts.[52]

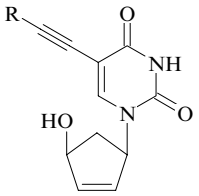
Carbocyclic analogues belong to another class of promising nucleoside derivatives. These compounds exhibit a broad spectrum of biological activities. Due to the structural similarity to nucleosides, these compounds are recognized by many enzymes and receptors. The antiviral and antitumour activity of carbocyclic nucleosides was documented.[53] Therefore, it was of interest to investigate these compounds as inhibitors of *M. tuberculosis*.

Structures **62**, **63**



62a–**c**

R^1 = H: R^2 = H (**a**), Me (**b**); R^1 = I, R^2 = H (**c**)

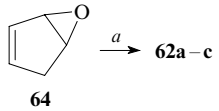


63a–**c**

R = n-C$_8$H$_{17}$ (**a**), n-C$_{10}$H$_{21}$ (**b**), n-C$_{12}$H$_{25}$ (**c**)

Recently, the synthesis and antituberculosis activity of a new series of carbocyclic analogues of 5′-noruridine **62a**–**c** and **63a**–**c** were reported.[54] The former group of compounds includes derivatives of uracil, 6-methyluracil and 5-iodouracil (**62a**–**c**) containing one and the same 4-hydroxycyclopent-2-enyl moiety. Racemic mixtures of carbocyclic nucleoside analogues were synthesized by the condensation of 3,4-epoxycyclopentene (**64**) with the corresponding nucleobase using Trost's method (Scheme 18).[55] The latter group includes 5-alkynyl derivatives of uracil **63a**–**c** containing the 4-hydroxycyclopent-2-enyl moiety. These compounds were generated from iodo derivative **62c** by the above-described Sonogashira reaction.[17]

Scheme 18



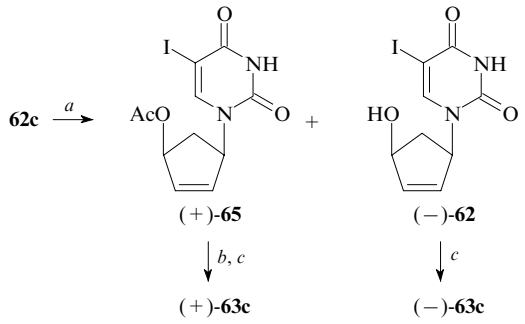
64

(*a*) uracil, 6-methyluracil or 5-iodouracil, Pd(PPh$_3$)$_4$, THF–DMF

These compounds exhibited antituberculosis activity against both the laboratory *M. tuberculosis* strain (H37Rv) and the MDR *M. tuberculosis* strain (MS-115). All these compounds, except for 1-(4-hydroxycyclopent-2-enyl)-5-tetradec-1-ynyluracil (**63c**), showed moderate inhibitory activity in the laboratory strain H37Rv (MIC_{99} = 20–40 μg ml^{-1}). A mixture of enantiomers of compound **63c** fully suppressed the mycobacterial growth with MIC_{99} = 10 μg ml^{-1}. It was of interest to study the activity of a mixture of *rac*-**63c** and of enantiomers (+)-**63** and (−)-**63** against the MDR strain (MS-115) resistant to isoniazid, rifampicin, ethambutol and pyrazinamide.

The enantiomers were synthesized[54] by the facile enzymatic reaction (using Amano lipase PS) according to a procedure described earlier.[56] This method enables the selective removal of the acetyl group only in one of the enantiomers of compound **65**, resulting in the formation of a mixture of compounds (+)-**65** and (−)-**62** (Scheme 19). The latter can be easily separated by chromatography. Then acetate (+)-**65** was transformed into enantiomer (+)-**62**, and enantiomers (+)-**63** and (−)-**63** were prepared from isomers (+)-**62** and (−)-**62**, respectively.

Scheme 19



(*a*) Amano lipase PS, AcOCH=CH$_2$; (*b*) K$_2$CO$_3$, MeOH; (*c*) HC≡CC$_{12}$H$_{25}$-n, CuI, Pr$^i_2$EtN, Pd(PPh$_3$)$_4$, DMF

A mixture of enantiomers of *rac*-**63**, as well as individual components, inhibited the growth of the resistant strain MS-115 (MIC_{99} = 10 μg ml^{-1}). It was also shown that enantiomer (−)-**63c** has higher activity against the standard strain (H37Rv) (MIC_{99} = 5 μg ml^{-1}).

IV. Directed synthesis of nucleoside inhibitors of enzymes from mycobacteria

For some pyrimidine nucleoside derivatives evaluated as inhibitors of mycobacterial growth, cell targets were identified.[57, 58] They are presented in Fig. 2. These cell targets are thymidylate synthase and thymidine monophosphate kinase (TMP kinase, TMPK) catalyzing the biosynthesis of thymidine 5′-mono- and diphosphates.

1. Inhibitors of thymidine monophosphate kinase

a. Bicyclic and aryl derivatives of thymidine

Thymidine monophosphate kinase is one of key enzymes in DNA synthesis necessary for the growth and survival of organisms. This enzyme catalyzes the reversible phosphorylation of thymidine monophosphate (dTMP) to thymidine diphosphate (dTDP) using ATP as the preferred phosphate donor (see Fig. 2). In turn, dTMP is produced in cells either from thymidine by the thymidine kinase-catalyzed reaction or from uridine monophosphate (dUMP) by the thymidylate synthase-catalyzed reaction.[58] Human thymidine monophosphate kinase ($TMPK^{h}$) and thymidine monophosphate kinase from *M. tuberculosis* ($TMPK^{tb}$) were well characterized both at biochemical and structural levels.[59–63] Taking into account the differences in the three-dimensional structures of $TMPK^{h}$ (Refs 64 and 65) and $TMPK^{tb}$ and a low level of amino acid sequence homology (22%), the development of selective inhibitors of the enzyme from *M. tuberculosis* is a challenging problem.

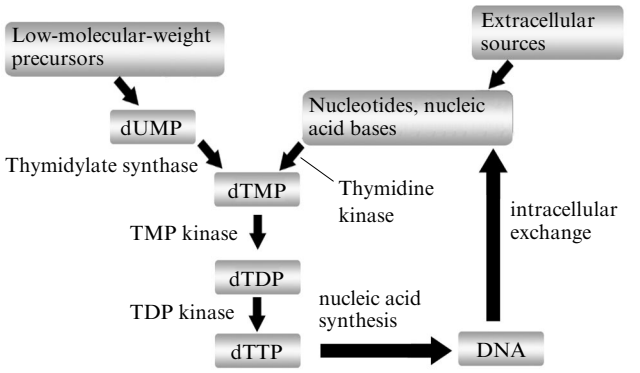
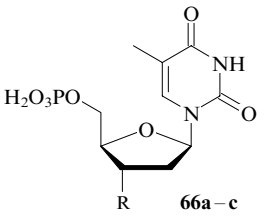


Figure 2. Metabolism of thymidine nucleotides (for abbreviations, see the text).

In 2003, Van Calenbergh and co-workers[66] reported on the synthesis of 5′-monophosphates of new 3′-modified derivatives of thymidine, which showed high inhibitory activity against $TMPK^{tb}$: 3′-aminomethyl- (**66a**), 3′-azidomethyl- (**66b**) and 3′-fluoromethyl-3′-deoxythymidine 5′-monophosphates (**66c**).

Structures **66**



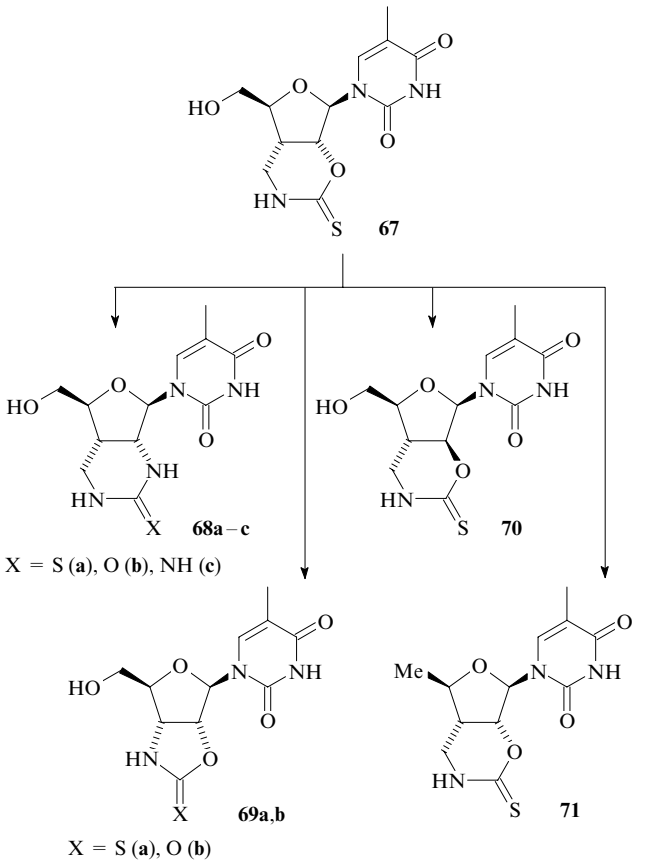
66a–c

R = CH_2NH_2 (**a**), CH_2N_3 (**b**), CH_2F (**c**)

The $TMPK^{tb}$ inhibition constants (K_i) for derivatives **66a–c** are 10, 10.5 and 15 μmol litre^{-1}, respectively. Nucleoside 5′-monophosphates are substrates of the enzymatic reaction. It was also shown that the corresponding nucleosides have high affinity for the enzyme.

1-(3-Aminomethyl-3-deoxy-2-*O*,6-*N*-thiocarbonyl-β-D-ribofuranosyl)thymine (**67**) containing the thiourethane moiety is another specific inhibitor of $TMPK^{tb}$ (K_i = 3.5 μmol litre^{-1}).[67] Modifications of compound **67** gave bicyclic nucleoside analogues **68–71**.[68]

Structures **67–71**



67

68a–c

X = S (**a**), O (**b**), NH (**c**)

70

69a,b

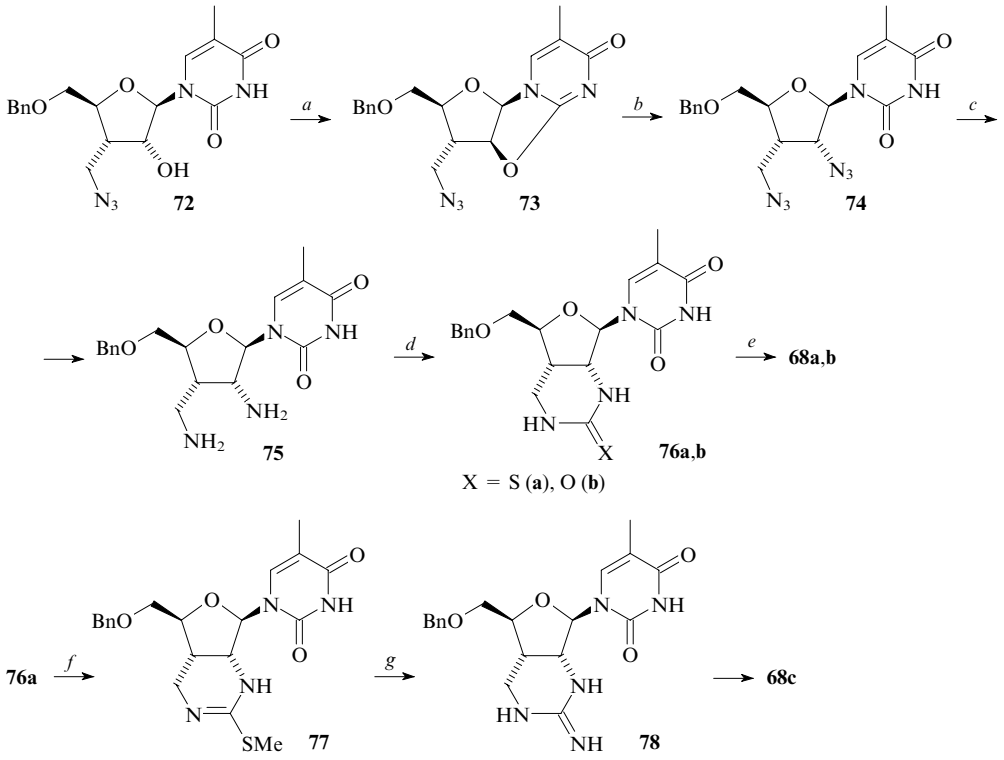
X = S (**a**), O (**b**)

71

First, bicyclic derivatives **68a–c** with a six-membered ring, in which the thiourethane moiety is replaced by thiocarbamide, carbamide and guanidine, respectively, were investigated. Another type of modifications includes bicyclic nucleosides **69a,b** with a five-membered ring. Nucleoside **70** was synthesized with the aim of studying the effect of analogues having an inverted configuration at position 2′ of the carbohydrate moiety on the binding to the enzyme. Recently, it was shown that, being a potential inhibitor of the enzyme $TMPK^{tb}$, compound **67** does not inhibit mycobacterial growth, which is attributed to low permeability of the cell membrane to polar molecules. In attempting to overcome this barrier, nucleoside analogue **71** methylated at position 5′ was synthesized.[68]

The synthesis of compounds **68a–c** from 3′-azidomethyl-5′-*O*-benzyl-2′-hydroxy-3′-deoxythymidine (**72**) involved the transformation of the latter into anhydronucleoside **73** as the first step (Scheme 20). The cleavage of the anhydro ring with sodium azide afforded bis(azido) derivative **74**. The reduction of both azido groups was performed by the catalytic hydrogenation (Pd/C) without deprotection of position 5′. The six-membered ring was closed by treating compound **75** with *N*,*N*′-carbonyldiimidazole or its thiocarbonyl analogue in tetrahydrofuran. The protecting group in compounds **76a,b** was removed with BCl_3. Thiourethane derivative **76a** was used to synthesize guanidine analogue **68c** by the methylation of the latter with iodomethane followed by the treatment of product **77** with

Scheme 20

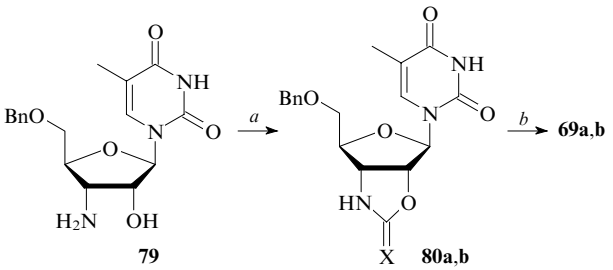


(*a*) Trifluoromethanesulfonyl chloride, DMAP, CH_2Cl_2; (*b*) NaN_3, $PhCO_2H$, DMF; (*c*) Pd/C, H_2, MeOH; (*d*) *N*,*N*′-thiocarbonyldiimidazole, THF (for **77a**); *N*,*N*′-carbonyldiimidazole, THF (for **77b**); (*e*) BCl_3, CH_2Cl_2; (*f*) MeI, MeOH; (*g*) NH_3–MeOH, 100 °C

ammonia at 100 °C and the removal of the protecting group in compound **78**.

Bicyclic nucleoside analogues **69a**,**b** with a five-membered ring were prepared from 3′-amino-5′-*O*-benzyl-2′-hydroxy-3′-deoxythymidine (**79**) (Scheme 21). The ring closure and the removal of protecting groups in compounds **80a**,**b** were accomplished under conditions identical to those used in the synthesis of six-membered nucleoside analogues.

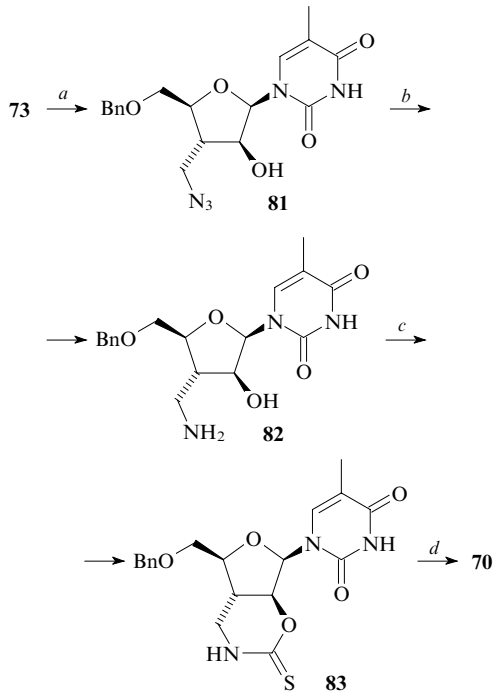
Scheme 21



X = S (**a**), O (**b**); (*a*) *N*,*N*′-thiocarbonyldiimidazole, THF (for **80a**) or *N*,*N*′-carbonyldiimidazole, THF (for **80b**); (*b*) BCl_3, CH_2Cl_2, −78 °C

Compound **70** having an inverted configuration at the C(2) atom of the ribose moiety (compared to derivatives **68**, **69** and **71**) was prepared using anhydronucleoside **73** (Scheme 22). The strategy of the synthesis through intermediates **81**–**83** repeats the scheme of the synthesis of nucleoside analogues **68a**–**c**.

Scheme 22

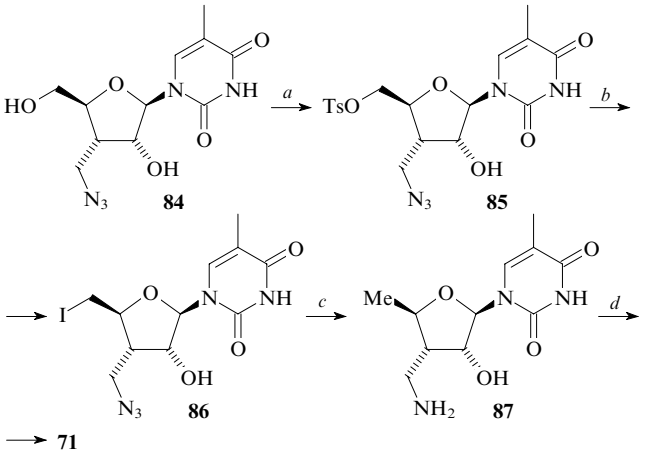


(*a*) NaOH, 1,4-dioxane, EtOH–H_2O; (*b*) Pd/C, H_2, MeOH; (*c*) *N*,*N*′-thiocarbonyldiimidazole, THF; (*d*) BCl_3, CH_2Cl_2, −78 °C

Since the attempts to transform compound **67** into 5′-deoxy derivative **71** failed, an alternative procedure for the synthesis of this compound was developed. The key step

involves the simultaneous reduction of the iodomethyl and azido group followed by the ring closure (Scheme 23).

Scheme 23



(*a*) TsCl, Py; (*b*) NaI, Bu^n_4NI, Me_2CO, 60 °C; (*c*) Pd/C, H_2, MeOH; (*d*) *N*,*N*′-thiocarbonyldiimidazole, THF

The selective tosylation of the 5′-hydroxy group of compound **84** was followed by the reaction of 3′-azidomethyl-2′-hydroxy-5′-*O*-tosyl-3′-deoxythymidine (**85**) with sodium iodide to produce 3′-azidomethyl-2′-hydroxy-5′-iodothymidine (**86**). The simultaneous reduction of the azido and iodomethyl groups by means of catalytic hydrogenation afforded 3′-aminomethyl-5′-methyl-2′-hydroxy-3′,5′-dideoxythymidine (**87**). The reaction of the latter with thiocarbonyldiimidazole gave target derivative **71**.

A comparison of the antimycobacterial activity of the synthesized bicyclic nucleosides against *M. bovis* BCG showed that thio derivatives **69a** (MIC_{99} = 500 μg ml^{-1}) and **71** (MIC_{99} = 100 μg ml^{-1}) are most active. These compounds are also the most effective inhibitors of $TMPK^{tb}$ (K_i = 8 and 2.3 μmol $litre^{-1}$, respectively). Apparently, this is attributed to strong hydrophobic interactions with the enzyme due to the lipophilicity of the sulfur atoms. Arabino derivative **70** showed a considerable decrease in the affinity for the enzyme, whereas the activity of bicyclic derivative **69a** containing a five-membered ring proved to be twice as low as that of its six-membered analogue **67**.

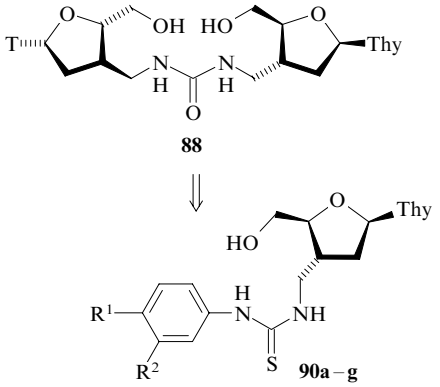
The removal of the 5′-hydroxy group from compound **67** substantially reduced the polarity of 5′-methyl derivative **71**. It was expected that this would decrease the activity of the resulting compound due to the loss of hydrogen bonds between the inhibitor and the active-site amino acid residues. However, it appeared that the activity of the resulting compound was doubled, apparently, due to the enhancement of the hydrophobic interaction between the 5′-methyl group of molecule **71** and the active site of the enzyme.[68]

In 2007, this Belgian research team published [69] data on new compounds, modified pyrimidine nucleosides, exhibiting specific inhibitory activity against $TMPK^{tb}$. Among them was dinucleoside **88**, which showed considerable inhibition of $TMPK^{tb}$ (K_i = 37 μmol $litre^{-1}$) and did not suppress the activity of $TMPK^h$.

The high selectivity of compound **88** stimulated the synthesis of thymidine derivatives containing aromatic moieties, which can occupy the binding site of the second thymidine residue [69] in the active site of $TMPK^{tb}$.

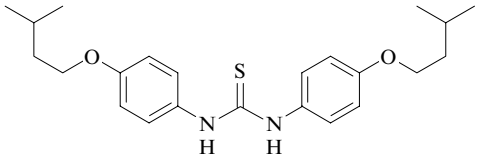
The structure of thiocarlide (**89**),[70, 71] which efficiently blocks *M. tuberculosis*, prompted researchers to use a thiourea linkage instead of the urea one. This resulted in the synthesis of 3′-arylthiourethane derivatives of thymidine **90a**–**g**.

Structures **88**, **90**



T is thymine; $R^1 = R^2$ = H (**a**), R^2 = H; R^1 = Cl (**b**), OMe (**c**), Me (**d**); $R^1 = R^2$ = Cl (**e**); R^1 = OBn, R^2 = H (**f**); R^1 = Cl, $R^2 = CF_3$ (**g**)

Structure **89**

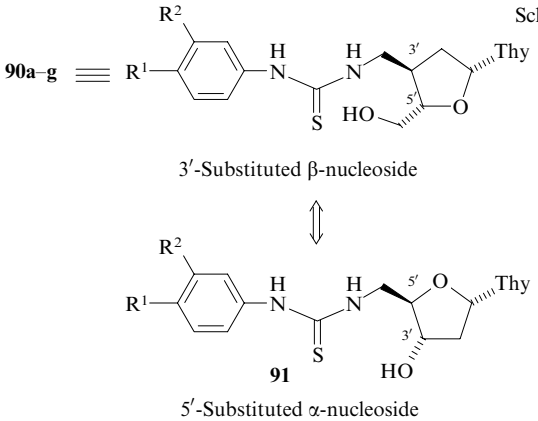


89 (Thiocarlide)

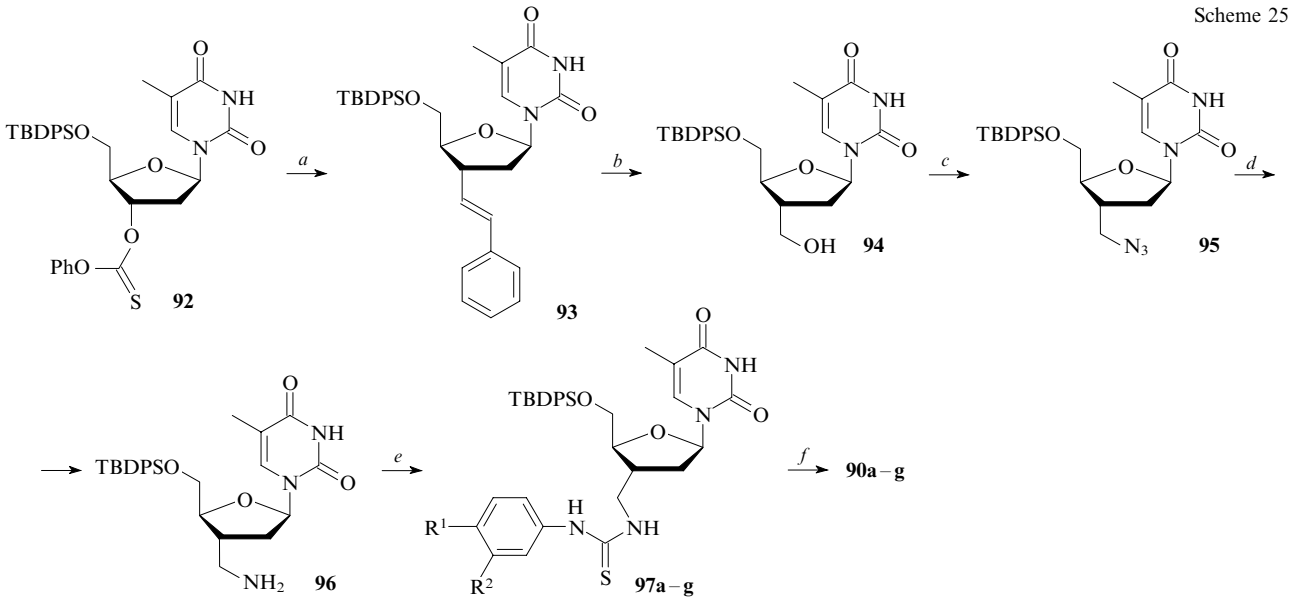
The results of the modelling of the binding of dinucleoside **88** to its target ($TMPK^{tb}$) suggest that the second monomer is located in the site generally occupied by the phosphate donor, which causes the rotation of the carbohydrate moiety by 180° compared to the orientation of the natural substrate dTMP.

By analogy, it can be suggested that thiourea derivatives of β-thymidine bind to the dTMP site in such a way that the aromatic substituent is located in the phosphate donor site and the nucleobase lies under the carbohydrate moiety.[67] It was shown that compound **88** is a competitive inhibitor of the enzyme (K_i = 15.0 μmol $litre^{-1}$). Based on this fact, it was hypothesized that 5′-*N*²-arylthiourea derivatives of 5′-deoxy-α-thymidine (**91**) and 3′-*N*²-arylthiourea derivatives of 3′-deoxy-β-thymidine (**90**) can bind to $TMPK^{tb}$ in the

Scheme 24



Scheme 25



(*a*) $Bu^n_3SnCH{=}CHPh$, AIBN, benzene; (*b*) 1) 4-methylmorpholine, OsO_4, 1,4-dioxane; 2) $NaIO_4$; 3) $NaBH_4$, $EtOH-H_2O$; (*c*) 1) MsCl, Py, 0 °C; 2) NaN_3, DMF, 60 °C; (*d*) Pd/C, H_2, MeOH; (*e*) 3-R^2-4-R^1-phenyl isothiocyanate, DMF, 0 °C; (*f*) Bu^n_4NF, THF; TBDPS = Bu^tPh_2Si

same fashion because they have similar three-dimensional structures (Scheme 24).

β-D-Thymidine derivatives **90** were the first to be synthesized. Van Daele *et al.*[69] developed a more efficient procedure for the introduction of substituents compared to the method proposed earlier[72,73] (Scheme 25). The starting compound **92**, which was prepared according to the method developed by Chu *et al.*,[74] was treated with β-(tributylstannyl)styrene and 2,2′-azobisisobutyronitrile (AIBN) in benzene.

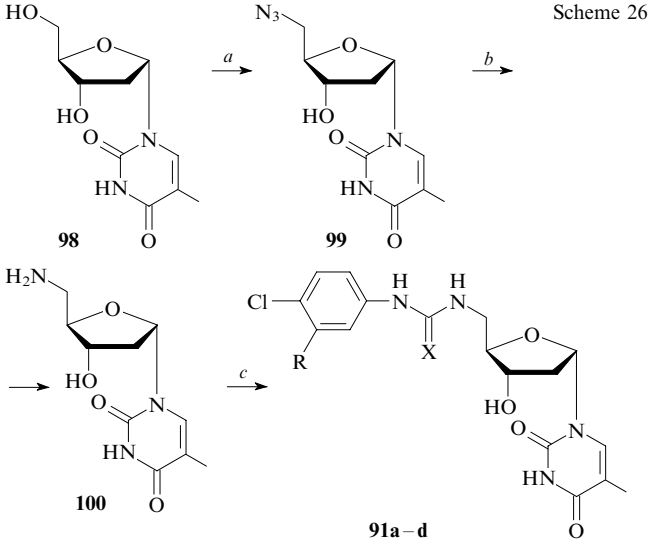
The next step involved the *cis*-dihydroxylation of compound **93** with osmium tetroxide and 4-methylmorpholine followed by the cleavage of diol with sodium periodate giving unstable aldehyde. The latter was immediately reduced with sodium borohydride in aqueous ethanol to form compound **94**. After the mesylation of the hydroxy group in the latter compound, the azido group was introduced using sodium azide in dimethylformamide. The hydrogenation of the azido group in compound **95** over palladium afforded amino derivative **96**. This intermediate was introduced into the reaction together with various isothiocyanates, and target products **90a–g** were obtained after the removal of the silyl protecting group.

5′-Substituted derivatives of α-thymidine **91a–d** were synthesized starting from commercially available α-thymidine (**98**) through intermediate derivatives **99** and **100** (Scheme 26).

The scheme of the transformation involves reactions similar to the stages *c*, *d* and *e* presented in Scheme 25. It should be noted that a facile method was developed for the selective mesylation of thymidine at the 5′-hydroxy group using the short-term treatment with mesyl chloride in pyridine at 0 °C.

For compound **90a** containing the unsubstituted phenyl group, K_i = 69 μmol litre^{-1}, which is indicative of its weaker binding to the enzyme $TMPK^{tb}$ compared to dinucleoside **88**. To optimize the inhibitory activity of aryl derivatives, Van Daele *et al.*[69] employed the Topliss approach based on the correlation between the properties

Scheme 26



R = Cl: X = S (**a**), O (**b**); R = CF_3: X = S (**c**), O (**d**);
(*a*) 1) MsCl, Py, 0 °C; 2) NaN_3, DMF, 60 °C; (*b*) H_2, Pd/C, MeOH;
(*c*) aryliso(thio)cyanate, DMF or amine, *N*,*N*′-carbonyldiimidazole, DMF

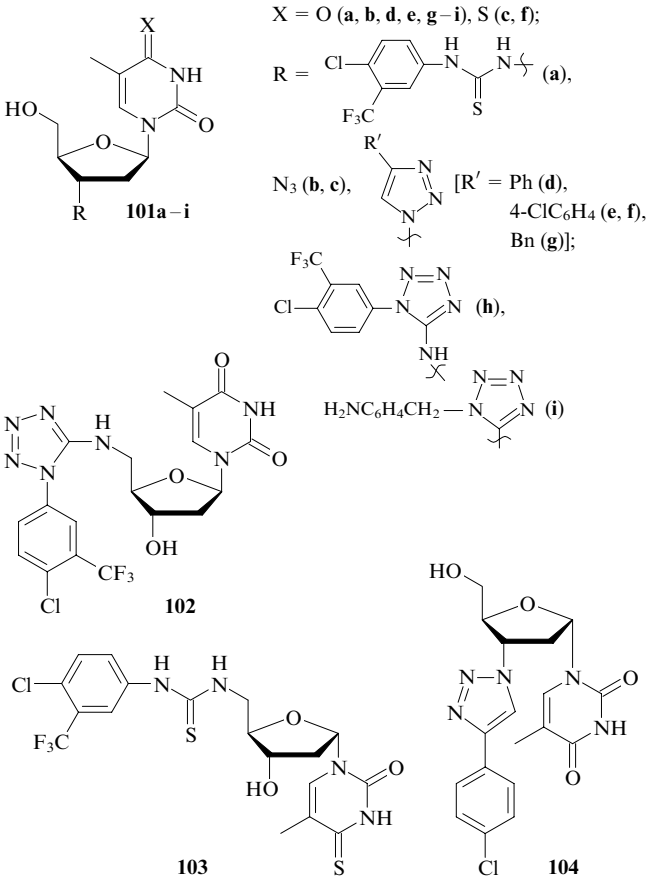
(lipophilic, electronic and steric) of the substituents introduced into the molecule and the biological activity.[75] The introduction of one or two chlorine atoms (compounds **90b**,**e**) leads to a three- and ninefold increase, respectively, in the inhibitory activity of these compounds compared to derivative **90a**. On the other hand, the 4-methoxy- and 4-methyl substituents (**90c**,**d**) cause only a slight increase in the inhibitory activity (by a factor of 1.5–2). 4-Chloro-3-trifluoromethylphenyl derivative **90g** (K_i = 5.0 μmol litre^{-1}) proved to be the best inhibitor of $TMPK^{tb}$. This compound almost did not suppress human thymidine monophosphate kinase (K_i > 1000 μmol litre^{-1}) and, like other analogues of this series, showed low toxicity in the *Vero* cell culture.

A wide range of compounds containing various substituents in the phenylthiourea moiety were synthesized. However, the same 4-chloro-3-trifluoromethylphenyl and 3,4-dichlorophenyl derivatives of nucleosides remained to be effective and highly selective. The 5′-(4-chloro-3-trifluoromethyl)phenylthiourea derivative of 5′-deoxy-α-thymidine (**91c**) proved to be the most effective currently known inhibitor of the enzyme (K_i = 0.6 μmol litre^{-1}).

A comparison of α- and β-phenylthiourea derivatives showed that the introduction of lipophilic and electron-withdrawing substituents results in the formation of the most active inhibitors of TMPK^{tb}. In both series, the same dependence of K_i on the nature of the substituent was observed, α-derivatives being approximately 5 times more active than the corresponding β analogues. The compounds exhibiting the highest inhibitory activity against the enzyme TMPK^{tb} were tested in the *M. tuberculosis* strain H37Rv and showed moderate activity (MIC_{99} = 20–100 μg ml^{-1}).

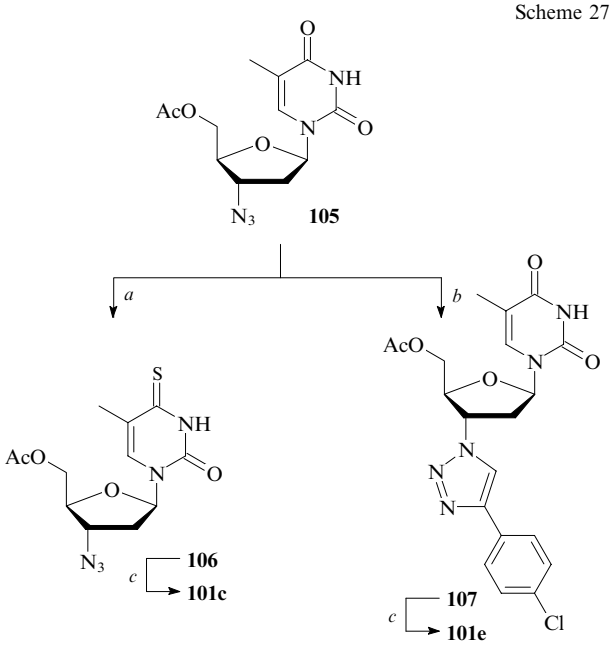
Based on the structure of nucleosides **90g** and **91c** exhibiting considerable inhibitory activity against TMPK^{tb}, the research team headed by Van Calenbergh synthesized new α- and β-thymidine analogues **101**–**104** containing different substituents in the carbohydrate moiety, including those with the triazole or tetrazole ring as a linker.[76, 77]

Structures **101**–**104**



Since some 4-thio derivatives of thymidine exhibit antimycobacterial activity against the *M. bovis* and *M. tuberculosis* strains,[22] the oxygen atom at C(4) of the nucleobase was replaced by the more lipophilic sulfur atom according to a standard procedure using Lawesson's reagent.[23] This method was employed to synthesize thio derivative of thymidine **101c** starting from 5′-*O*-acetyl-3′-azido-3′-deoxythymidine (**105**) through the intermediate formation of compound **106** (Scheme 27).

Scheme 27

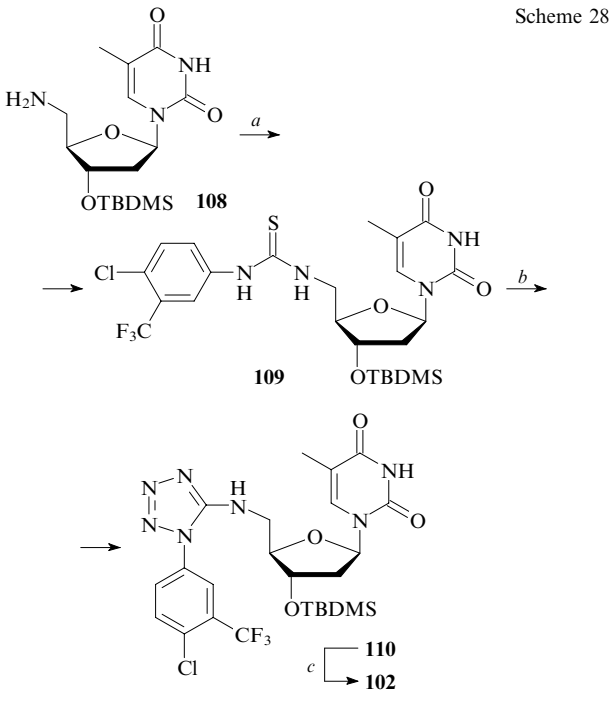


(*a*) Lawesson's reagent, PhMe, 80 °C; (*b*) 4-ClC$_6$H$_4$C≡CH, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O–Bu^{t}OH (2 : 1), rt; (*c*) NH_3–MeOH

Thymidine analogues **101d**,**e** containing the aryltriazolyl group at position 3′ of the carbohydrate moiety were prepared by the catalytic reaction of the corresponding arylalkyne with azide **105** followed by the removal of the acetyl protecting group in compound **107** using ammonia in methanol (see Scheme 27).

The synthesis of the tetrazole analogue of thymidine **102** involved the condensation of 5′-amino-3′-*O*-*tert*-butyldime-

Scheme 28



(*a*) 4-chloro-3-trifluoromethylphenyl isothiocyanate, DMF, 0 °C → rt; (*b*) NaN_3, $HgCl_2$, Et_3N, DMF, 0 °C → rt; (*c*) 1 M Bu^n_4NF, THF

thylsilyl-5′-deoxythymidine (**108**) with 4-chloro-3-trifluoromethylphenyl isothiocyanate as the first step to produce the corresponding arylthiourea derivative **109** (Scheme 28). Then the thiourea group was replaced by tetrazole by the treatment with sodium azide in the presence of mercury chloride and triethylamine followed by the removal of the TBDMS protecting group in derivative **110**.

α-Anomer **103** showed the highest activity against $TMPK^{tb}$. The data on the inhibitory activity of the compounds under consideration against $TMPK^{tb}$ are given below.

Compound	K_i /μmol litre^{-1}	Compound	K_i /μmol litre^{-1}
101a	2.8	**101g**	2.7
101b	28	**101h**	45
101c	> 100	**101i**	2.3
101d	4.2	**102**	73
101e	2.1	**103**	0.17
101f	15	**104**	35

The most effective compound, 5′-(4-chloro-3-trifluoromethylphenylaminothiocarbonylamino)-2′,5′-dideoxy-4-thio-α-thymidine (**103**), showed $K_i = 0.17$ μmol litre^{-1} for $TMPK^{tb}$, and it fully suppressed the growth of the mycobacetrium *M. bovis* at a concentration of 25 μg ml^{-1}. The replacement of the 3′-azido group in azidothymidine **101b** by the 4-chloro-3-trifluoromethylphenylaminothiocarbonylamine substituent (**101a**) led to a tenfold increase in the activity. 3′-(4-Phenyltriazol-1-yl)-3′-deoxythymidine (**101d**) produced by the click reaction [78] of azidothymidine with phenylacetylene proved to be much more active than azidothymidine **101b**.

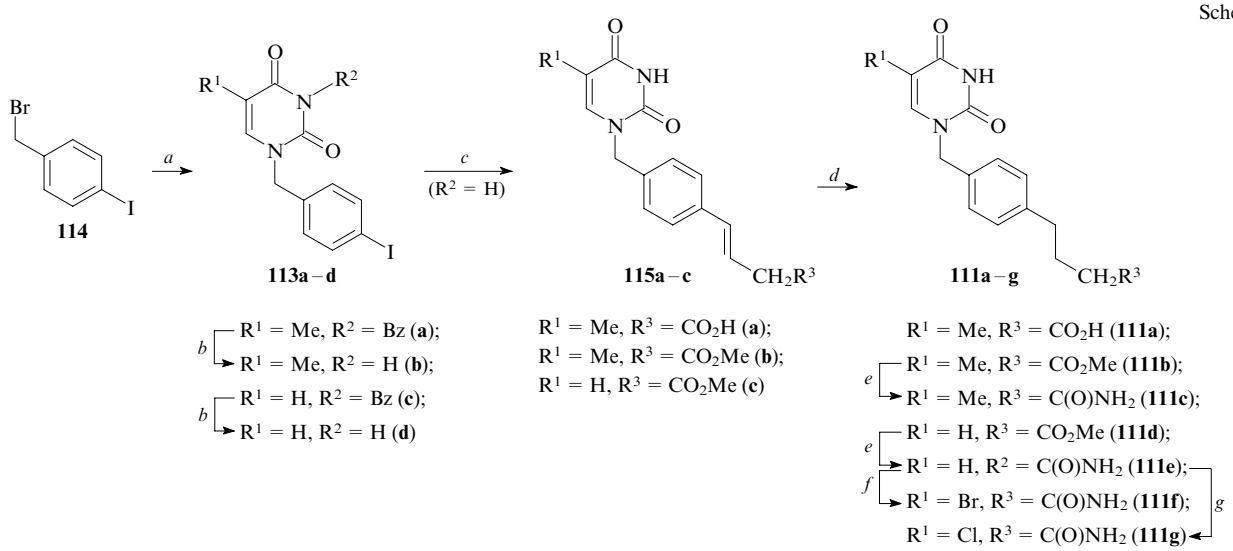
The introduction of the chlorine atom at the *para* position of the phenyl group of compound **101e** or the introduction of the methylene group between the triazole and phenyl rings (**101g**) resulted in a moderate increase in the activity. In the series of 3′-modified analogues of thymidine, the replacement of the oxygen atom at position 4 by sulfur led mainly to a considerable decrease in the inhibitory activity. Thus, the inhibition constant is > 100 μmol litre^{-1} for 3′-azido-3′-deoxy-4-thiothymidine (**101c**), whereas this constant for 3′-[4-(4-chlorophenyl)-1,2,3-triazol-1-yl]-3′-deoxy-4-thiothymidine (**101f**) is 7 times smaller than that of 4-oxo analogue **101e**. It should be noted that compounds **101h** and **101i** containing the 1,5-disubstituted tetrazole moiety considerably differ in the ability to inhibit $TMPK^{tb}$. Aminobenzyltetrazole derivative **101i**, in which the tetrazole ring is directly bound to the carbohydrate moiety, showed better activity compared to compound **101h**, in which the tetrazole substituent is attached through the NH linkage. It is noteworthy that the introduction of the tetrazole substituent at position 5′ of the nucleoside through the NH bridge did not lead to a considerable improvement of the inhibitory activity (compound **102**).

b. Arylpyrimidines

A French research group characterized arylpyrimidines **111a–g** (Scheme 29) and **112a–d** as inhibitors of $TMPK^{tb}$ (Scheme 30).[79] A facile method was developed for the synthesis of *para*-substituted benzylpyrimidine derivatives by the Heck [80] and Sonogashira [17] reactions catalyzed by palladium complexes. The Heck reaction was carried out using the $Pd(OAc)_2-P(o\text{-}Tol)_3$ catalytic system (o-Tol = 2-MeC_6H_4); the catalytically active Pd^0 complex with phosphine was formed *in situ*. The Sonogashira reaction was performed with the use of two catalysts, tetrakis(triphenylphosphine)palladium(0) and copper halide (iodide). In the Heck and Sonogashira reactions, the palladium complex activates aryl halide *via* the oxidative addition at the halogen–carbon bond. In both reactions, triethylamine served as a strong base neutralizing hydrogen iodide that was eliminated.

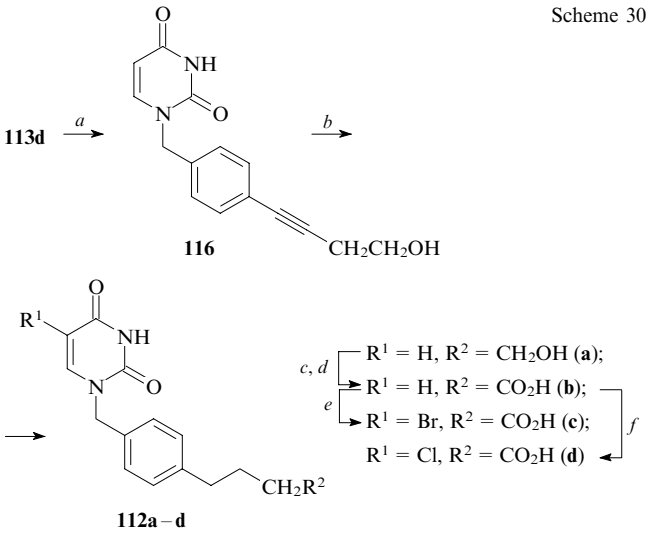
Starting compounds **113b,d** were synthesized by the N^1-benzylation of substituted uracil or thymidine with 4-iodobenzyl bromide (**114**) followed by the removal of the benzoyl group at the N^3 atom of derivatives **113a,c** (see Scheme 29). The introduction of the Bz protecting group ensured the regioselectivity of the reaction. The reduction of double and triple bonds in intermediates **115a–c** and **116** (see Schemes 29 and 30) was accomplished by catalytic

Scheme 29



(*a*) N^3-benzoylthymine (R^1 = Me) or N^3-benzoyluracil (R^1 = H) (R^1 = H), K_2CO_3, DMF; (*b*) 36% NH_4OH, MeOH;
(*c*) but-3-enoic acid ($R^3 = CO_2H$) or methylbut-3-enoate ($R^3 = CO_2Me$), $Pd(OAc)_2$, $P(o\text{-}Tol)_3$, Et_3N, MeCN; (*d*) H_2/Pd, MeOH;
(*e*) NH_3–MeOH (saturated); (*f*) Br_2, CCl_4, Py; (*g*) NCS, Ac_2O, AcOH, 60 °C

Scheme 30



(*a*) but-3-yn-1-ol, $Pd(PPh_3)_4$, CuI, Et_3N, CH_2Cl_2, 50 °C; (*b*) H_2/Pd, MeOH; (*c*) pyridinium dichromate, Bu^tOH, Ac_2O, CH_2Cl_2; (*d*) 2 M NaOH, MeOH; (*e*) Br_2, CCl_4, Py; (*f*) NCS, Ac_2O, AcOH, 60 °C

hydrogenation in the presence of carbon-supported palladium. Amides **111c**,**e** were produced from esters **111b**,**d** by ammonolysis in methanol. The oxidation of alcohol **112a** to acid **112b** was performed in two steps. Thus, the oxidation was carried out using pyridinium dichromate in the presence of *tert*-butanol and acetic anhydride [81] followed by acid- or base-catalyzed hydrolysis.

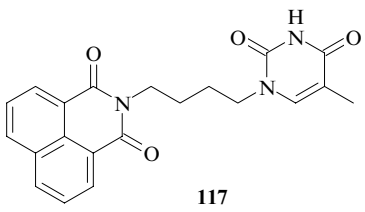
The chlorination of compounds **111e** and **112b** with *N*-chlorosuccinimide in the presence of acetic anhydride in acetic acid under heating or the bromination with bromine in the presence of pyridine in tetrachloromethane according to a procedure proposed earlier [82] afforded the corresponding 5-halogen derivatives **111f**,**g** and **112c**,**d**.

The structures possessing the most pronounced inhibitory activity against recombinant $TMPK^{tb}$ were found by introducing various substituents and varying the chain length of the linker. These are compounds **111c**,**f**,**g** and **112b**,**c**,**d**. Of halogen derivatives **111f**,**g** and **112c**,**d**, only compounds **112c**,**d** containing the butanoate substituent exhibited the highest activity against $TMPK^{tb}$ (K_i = 10 and 6.5 μmol litre^{-1}). In this series of inhibitors of $TMPK^{tb}$, compounds **111e**,**f** and **112c**,**d** suppressed the growth of *M. bovis* in a moderate degree ($MIC_{50} \geqslant$ 45 μg ml^{-1}).

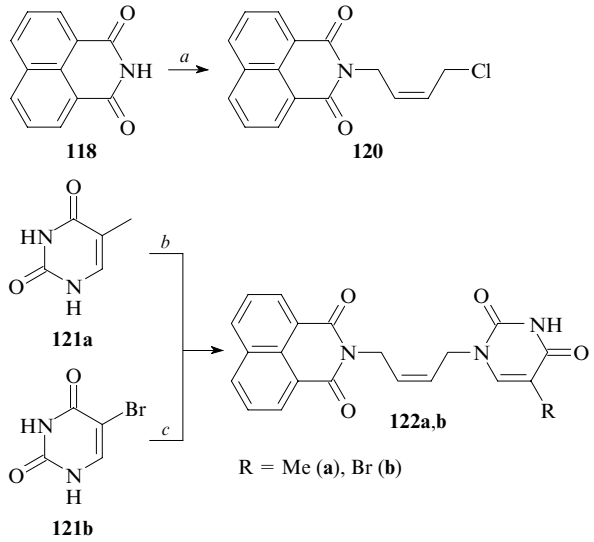
Successful studies [83, 84] aimed at the search for new inhibitors of $TMPK^{tb}$ were devoted to nucleoside analogues based on 1-[4-(1,8-naphthalimido)butyl]thymine (**117**) (K_i = 42 μmol litre^{-1}), which has such a structure that the thymine moiety, the spacer and the introduced 1,8-naphthalimide substituent can be modified.[83]

The synthesis was accomplished by treating 1,8-naphthalimide (**118**) with sodium hydride in dry dimethylformamide followed by the reaction with dichlorobutene (**119**) (Scheme 31). The reaction of the resulting chloro derivative **120** with thymine (**121a**) or 5-bromouracil (**121b**) in the presence of sodium iodide in acetonitrile afforded target compounds **122** in good yields.

Structure **117**



Scheme 31

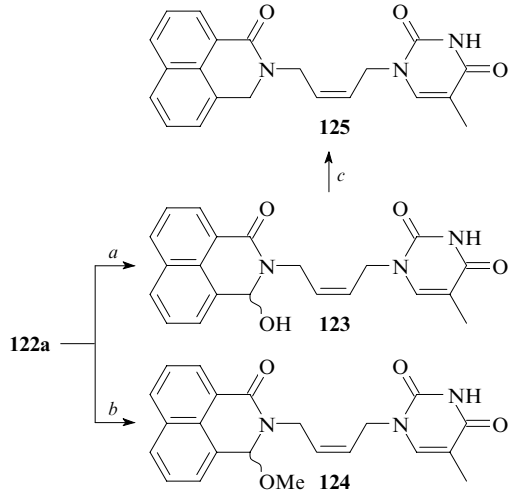


(*a*) 1) NaH, DMF, 0 °C, 30 min; 2) *cis*-$ClCH_2CH{=}CHCH_2Cl$ (**119**), 80 °C, 5 h; (*b*) 1) *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, 80 °C; 2) **120**, NaI, MeCN, 80 °C, 8 h; (*c*) 1) hexamethyldisilazane (HMDS), $(NH_4)_2SO_4$ (Cat.), 150 °C; 2) **120**, NaI, MeCN, 80 °C, 8 h

Derivatives **122a**,**b** containing the (*Z*)-butenyl linker proved to be almost 10 times more effective than the starting compound **117** (K_i = 1.9 and 1.1 μmol litre^{-1}, respectively).

It was of interest to find out the effect of the reduction of the carbonyl group of the naphthalimide moiety in the structure of compound **122a**. The reduction of the latter with sodium borohydride in ethanol followed by the treatment with sodium hydroxide (Scheme 32) gave the corresponding hydroxy derivative **123**. The reduction of the reaction mixture diluted with methanol produced the corresponding methoxy derivative **124**. The reduction of hydroxy

Scheme 32



(*a*) 1) $NaBH_4$, EtOH, 55 °C; 2) 3 M HCl; 3) 1 M NaOH; (*b*) 1) $NaBH_4$, EtOH, 55 °C; 2) 3 M HCl, MeOH; (*c*) $NaBH_4$, TFA

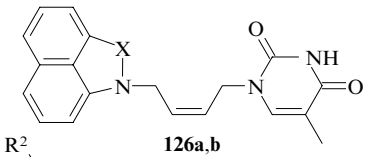
derivative **123** with $NaBH_4$ followed by the treatment with trifluoroacetic acid TFA yielded compound **125**.[85]

The hydroxy and methoxy derivatives proved to be three times less active inhibitors of $TMPK^{tb}$ than the starting compound **122a**, whereas compound **125** showed higher activity (K_i = 1.4 μmol litre^{-1}).

The ability of the most effective inhibitors of $TMPK^{tb}$ to suppress mycobacterial growth was examined by different methods. All the tested compounds did not inhibit the growth of *M. bovis* BCG, the laboratory *M. tuberculosis* strain H37Rv and four clinical isolates. The authors suggested that these compounds either cannot penetrate the bacterial cell wall and reach the target enzyme or their concentration is insufficient to exert a considerable effect on the mycobacterial growth.

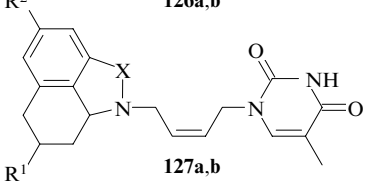
Another series of modifications involves the replacement of the naphthalimide moiety by naphtholactam (derivative **126a**) or naphthosultam (**126b**).[85] The synthesis of these compounds was analogous to that described above. The naphthosultam derivative showed very high inhibitory activity (K_i = 0.27 μmol litre^{-1}) and is one of the most effective compounds not only in this series but also of all known inhibitors of $TMPK^{tb}$. These compounds are poorly soluble in water. To increase the solubility, the aromatic rings were modified by amino groups. The planar structure of the moieties in the molecule (thymine, naphtholactam and naphthosultam) favourable for stacking interactions may also be responsible for low solubility. Hence, the goal was to reduce the planarity of substituents. Since the thymine moiety and the CO and SO_2 groups in the naphthalene moiety are necessary for the interaction with the enzyme active site, the main modifications were introduced into the naphthalene moiety. This resulted in the suggestion of dihydro derivatives **127a**,**b** of basic compounds **126a**,**b**.

Structures **126**, **127**



126a,**b**

X = C(O) (**a**), $S(O)_2$ (**b**)



127a,**b**

X = C(O) (**a**), $S(O)_2$ (**b**); R^1, R^2 = H, NH_2

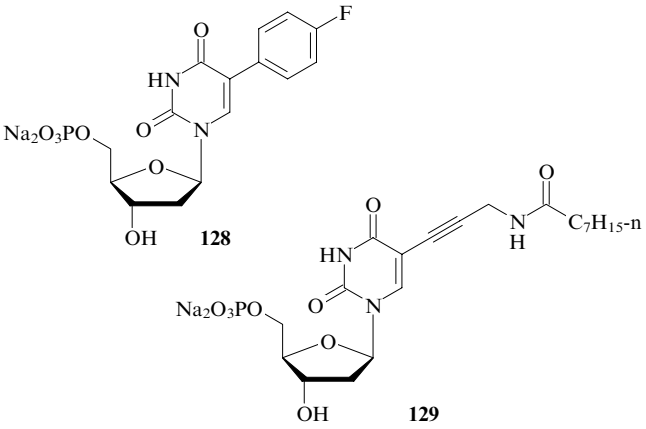
Studies aimed at determining the inhibitory activity of this series of compounds against $TMPK^{tb}$ showed that the activity of amino derivatives **127** is not higher than that of the starting compounds **126a**,**b**. Thus, the dihydro derivatives of naphtholactam and naphthosultam proved to be two times less active. These compounds did not inhibit the growth of *M. bovis* even at high concentrations (160 μg ml^{-1}).

2. Inhibitors of thymidylate synthase

The synthesis of thymidine monophosphate in living organisms is commonly catalyzed by the enzyme thymidylate synthase (ThyA).[86] However, it is known that some microorganisms do not possess genes encoding ThyA but have no deficiency of dTMP, which is attributed to the presence of the isoenzyme revealing no structural similarity to ThyA and abbreviated as ThyX.[87] It is known that *M. tuberculosis* strains contain both ThyA and ThyX, the latter being absolutely necessary for mycobacteria to maintain the life cycle in macrophages. Since macrophages serve as a reservoir of *M. tuberculosis* in the latent form of the disease, the inhibitors of ThyX can be prototypes of drugs having an effect on the latent form. An analysis of the crystal structure of the enzyme ThyX (Refs 88 and 89) supports these prospects.[90]

In 2011, a Belgian research group synthesized modified pyrimidine nucleotides, in which position 5 of the nucleobase was modified by aryl or alkynyl substituents.[91] The authors succeeded in finding two nucleotides, 5-(4-fluorophenyl)-2′-deoxyuridine (**128**) and 5-(3-octanoylaminoprop-1-ynyl)-2′-deoxyuridine 5′-monophosphates (**129**), which selectively inhibit mycobacterial ThyX, the main enzyme ThyA remaining virtually intact.

Structures **128**, **129**



128

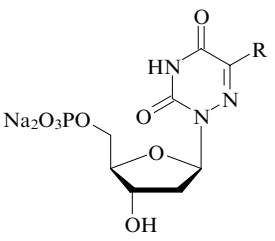
129

It should be noted that the phosphorylation of nucleosides containing bulky groups at position 5 of the nucleobase presented difficulties. Thus, the commonly emplolyed modified Yoshikawa phosphorylation procedure[92] ($POCl_3$, proton sponge, $P(O)(OMe)_3$) gave low yields (8%). Hence, some nucleotides were synthesized according to methods proposed by Sowa and Ouchi,[93] Mitsunobu *et al.*[94] and Tener[95] (in the last-mentioned case, a combination of acetyl and trityl protecting groups is used).

Nucleotide **128** with an aryl substituent exhibited pronounced inhibitory effect against ThyX (75% at a concentration of 50 μmol litre^{-1}). This compound showed 1% suppression of the enzyme ThyA at the same concentration. Second nucleotide **129** inhibited the enzymes ThyX and ThyA by 92% and 15%, respectively, under the same conditions.

In 2012, this research group synthesized 5-modified 6-azauridine 5′-monophosphates **130**–**133**, which belong to another group of inhibitors of mycobacterial enzymes

Structures **130**–**133**



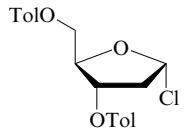
130a–**d**, **131**–**133**

130: R = H (**a**), Me (**b**), Pr^i (**c**), n-C_8H_{17} (**d**); R = 4-FC_6H_4 (**131**), n-$C_7H_{15}C(O)NHCH_2C{\equiv}C$ (**132**), n-$C_7H_{15}C(O)NHCH_2C(O)CH_2$ (**133**)

involved in thymidine metabolism (thymidylate synthase and thymidine monophosphate kinase).[96] These compounds can presumably inhibit the FAD-dependent biosynthesis of thymidine monophosphate from uridine monophosphate (FAD is flavin adenine dinucleotide).

The starting nucleoside analogues were produced by the glycosylation procedure proposed by Verbrüggen *et al.*[97] The corresponding 6-azauracil reacted with α-chloro-2-deoxy-3,5-di-*O*-toluoyl-1-ribofuranose (**134**).

Structure **134**

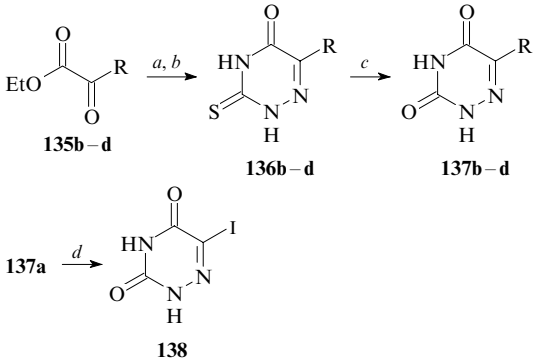


134 Tol = 4-MeC$_6$H$_4$C(O)

Analogues of 6-azauracil were prepared by treating keto esters **135b–d** with thiosemicarbazide in ethanol (Scheme 33) followed by the cyclization in the presence of an aqueous solution of sodium hydroxide.

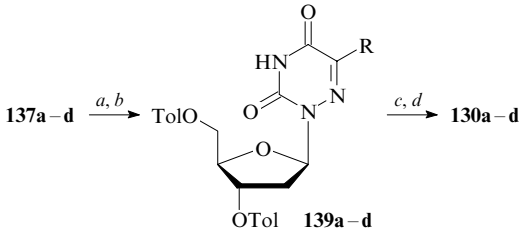
The further transformation of 2-thio derivatives **136b–d** into target compounds **137b–d** was accomplished in an alkaline solution of hydrogen peroxide. 5-Iodo-6-azauracil (**138**) was prepared by the iodination of commercially available 6-azauracil (**137a**) according to Chang's method.[98] The condensation of 6-azauracils **137a–d** with chlorocarbohydrate **134** was carried out in the presence of CuI as the catalyst according to Freskos's procedure.[99] Then the toluoyl groups in intermediates **139a–d** were removed followed by the phosphorylation of the 5′-hydroxy group to form target products **130a–d** (Scheme 34). The glycosylation reaction proceeded with no stereospecificity, which is unusual this method.

Scheme 33



R = H (**a**), Me (**b**), Pr^i (**c**), n-C$_8$H$_{17}$ (**d**); (*a*) thiosemicarbazide, EtOH, 90 °C; (*b*) NaOH, H$_2$O; (*c*) 35% H$_2$O$_2$, 1 M NaOH; (*d*) I$_2$, KI, NaOH, H$_2$O

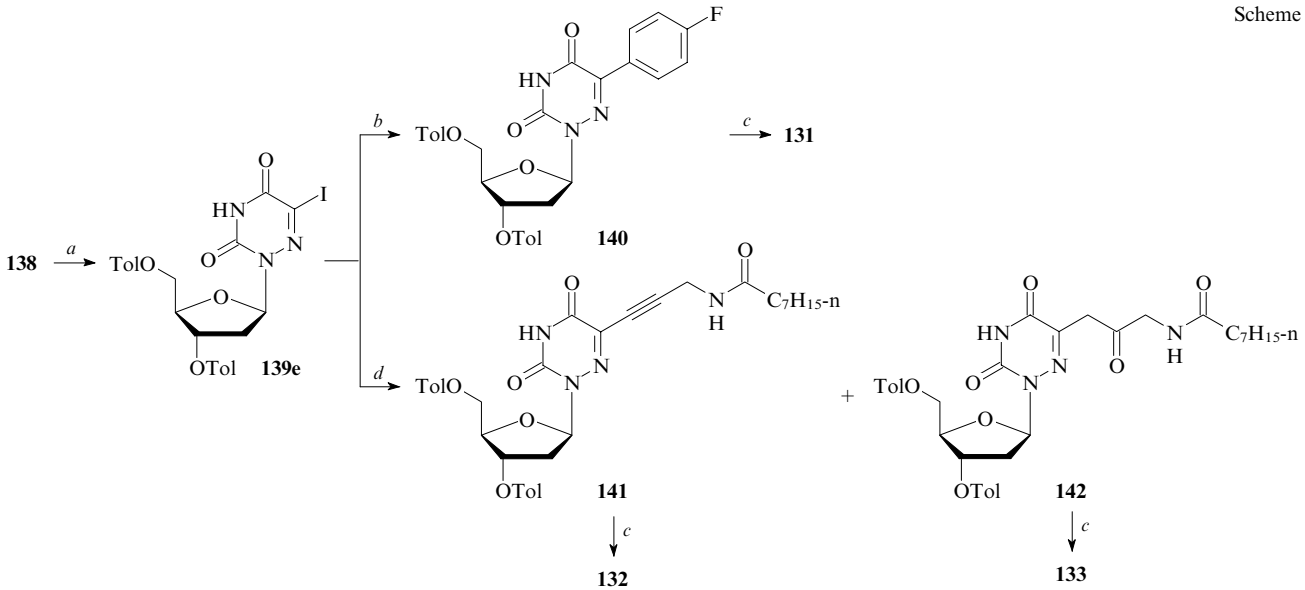
Scheme 34



R = H (**a**), Me (**b**), Pr^i (**c**), n-C$_8$H$_{17}$ (**d**); (*a*) HMDS, (NH$_4$)$_2$SO$_4$; (*b*) 134, CuI, Me$_3$SiCl, CHCl$_3$; (*c*) 0.1 M MeONa, MeOH; (*d*) POCl$_3$, 'proton sponge' [1,8-bis(dimethylamino)naphthalene], P(O)(OMe)$_3$, 0 °C

The same approach was employed to synthesize 5-(4-fluorophenyl)-6-aza derivative **131** and 5-(prop-1-ynyl)-6-aza derivative **132** (Scheme 35). Target phosphate **131** was prepared by the Suzuki reaction[100] followed by the phosphorylation of intermediate **140**, whereas phosphate **132** was synthesized by the Sonogashira reaction.[18,101] Intermediates **141** and **142** were separated by means of chromatography and phosphorylation. Therefore, compound **133**

Scheme 35



(*a*) 1) HMDS, (NH$_4$)$_2$SO$_4$, Δ; 2) **134**, CuI, Me$_3$SiCl, CHCl$_3$, rt; (*b*) 1) 4-FC$_6$H$_4$B(OH)$_2$, Pd(PPh$_3$)$_4$, copper(I) thiophene-2-carboxylate, THF, 50 °C; 2) 0.1 M MeONa, MeOH, rt; (*c*) POCl$_3$, 'proton sponge', P(O)(OMe)$_3$, 0 °C; (*d*) 1) *N*-(prop-2-ynyl)octanamide, [Pd$_2$(dba$_3$)], PPh$_3$, CuI, Et$_3$N, DMF, rt; 2) 0.1 M MeONa, MeOH, rt; dba is dibenzylideneacetone

was finally obtained as a by-product in the Sonogashira reaction *via* the hydrogenation of the triple bond in the introduced substituent.

Experiments with ThyX showed that only derivatives **130a** and **131** exhibit weak inhibitory activity (30%–40% at 50 μmol litre^{-1}). None of the 6-aza derivatives were active against ThyA. Besides, it was found that this type of compounds can interact with the enzyme TMPK^{tb} serving either as a substrate or an inhibitor. Consequently, these compounds hold promise because they can interact with different enzyme targets.

The replacement of the carbon atom at position 6 in the natural substrate dUMP by the nitrogen atom leads to weakening of the inhibitory effect against ThyX. The introduction of aryl and alkyl substituents at position 5 resulted in the complete loss of activity, whereas the activity of derivative **132** containing the 3-(octanoylamino)propynyl substituent in the side chain was retained. On the whole, this group of compounds showed moderate inhibition only against the enzyme ThyX. None of the synthesized compounds inhibited ThyA and TMPK^{tb}.

V. Conclusion

Derivatives of natural nucleosides belong to a promising class of organic compounds, among which new antituberculosis agents are searched for. Table 1 includes five compounds, which are considered in the present review and which showed the highest *in vitro* activity against *M. tuberculosis*. The antimycobacterial activity of these compounds is comparable with the activity of the currently used medications, for which MIC varies from 0.5 (isoniazid) and 2–20 (cycloserine, ofloxacin, ethambutol) to 100 μg ml^{-1} (pyrazinamide).

For several classes of modified nucleosides of the pyrimidine series, the mechanism of antituberculosis activity was studied in detail. Noteworthy are the studies aimed at searching for nucleoside derivatives active against mycobacterial thymidine monophosphate kinase and thymidylate synthase. An important advantage of inhibitors of these enzymes is their exclusively high selectivity. The results of the studies devoted to the design of compounds, which are active also against the MDR strain of *M. tuberculosis* (MS-115) suggest that such nucleoside derivatives can be prototypes of drugs of the future. Evidently, data on the mechanism of action of this young class of inhibitors on mycobacteria are scarce. Hence, studies on the directed search for new compounds suppressing mycobacterial growth are very promising.

Table 1. Five pyrimidine nucleoside analogues exhibiting the highest activity in the *M. tuberculosis* strain.

Compound	Name	MIC_{99} /μg ml^{-1}	Ref.
13e	3′-fluoro-2′,3′-dideoxy-5-(tetradec-1-ynyl)uridine	1–2	22
16c	1-(β-D-arabinofuranosyl)-5-(dodec-1-ynyl)uracil	1–5	24
38b	1-(β-D-arabinofuranosyl)-4-thio-5-hydroxymethyluracil	0.5	30
45b	5-[(4-decyl)-1,2,3-triazol-1-yl]-methyl-2′-deoxyuridine	10	35
(−)-**63c**	1-(4-hydroxycyclopent-2-en-1-yl)-5-(tetradec-1-ynyl)uracil	5	54

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