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Fragment-based approach to novel bioactive purine derivatives

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Abstract: Using purine as a scaffold, the methods for preparation of novel 2-aminopurine and purine derivatives substituted at position C^6 by the fragments of natural amino acids, short peptides, and *N*-heterocycles, including enantiopure ones, have been proposed. The methods for determination of the enantiomeric purity of the obtained chiral compounds have been developed. Conjugates exhibiting high antimycobacterial or antiherpesvirus activity against both laboratory and multidrug-resistant strains were revealed among the obtained compounds.

Keywords: amines; amino acids; antitubercular activity; antiviral activity; bioactive molecules; coupling reactions; drug design; Mendeleev-21; nucleophilic substitution reactions; organic synthesis; stereochemistry.

Introduction

Purine and other nucleobases are part of the most important biomolecules (DNA, RNA, ATP, NAD coenzyme, alkaloids, etc.) [1, 2]. Purines and purine-based nucleosides play a unique role in the metabolism of living organisms, therefore the synthesis and study of purine conjugates is of great interest for designing efficient medicinal agents based thereof [3]. Purine-based compounds may be isolated either from natural sources [1] or may be of synthetic origin [2, 3]. At present, a large diversity of purine-based medicinal agents has been introduced into clinical practice. In particular, efficient antiviral agents, including the well-known antiherpesvirus agent Acyclovir, as well as Tenofovir and Abacavir used in the treatment of HIV infections are constructed from nucleoside analogues [4–8]. A great number of anticancer agents, including antineoplastic drugs Fludarabine and Nelarabine used for treatment of hematological malignancies, have been designed on the basis of modified purine nucleosides and other purine-based compounds [9–15]. It has been also found that various purine derivatives exhibit high antibacterial activity, including inhibitory effect against *Mycobacterium tuberculosis* [16–25].

In last years, our research is aimed at preparation of novel C^6-substituted purine derivatives and search for bioactive substances among the synthesized purine conjugates. Our intentions have been based on the

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fragment-based approach involving the combination of various fragments of bioactive molecules, such as natural amino acids, short peptides, and *N*-heterocycles, in one structure based on a purine scaffold.

Modification of bioactive compounds by introducing an amino acid fragment in their structure is one of the most common approaches applied to achieve optimal pharmacokinetic and pharmacodynamic properties of potential therapeutic agents [26–30]. There are some literature examples of purine conjugates with amino acids which were tested for their antiviral [31–34] and antimycobacterial activity [35, 36]. Another approach proposed by us consists in conjugation of purines and *N*-heterocycles, including chiral ones, in a single structure. The rationale is based on the fact that such *N*-heterocycles as quinoline, benzoxazine, etc., and especially their fluorinated derivatives are part of the molecules of a large number of biologically active compounds and pharmaceuticals. It is also known that the presence of fluorine atoms in molecules of biologically active compounds promotes their high permeability through biomembranes and stronger binding to target proteins [37, 38]. The synthesis of purine conjugates containing the residues of heterocyclic amines at position C^6 and their inhibitory activity against various types of kinases were reported [39, 40].

In this review, we summarize the results of our investigations on the synthesis and study of bioactive purine conjugates obtained at the Postovsky Institute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences.

Search for efficient antimycobacterial agents among purine conjugates with amino acids and short peptides

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (Mbt) [41, 42]. TB is a major global health problem. It is one of the leading causes of death worldwide (about 1.4 million people were died in 2018) [41]. At the same time, new strains of mycobacteria that are resistant to the action of known drugs are constantly emerging, which causes 30% of deaths attributed to antimicrobial resistance [42]. Therefore, the search for new antimycobacterial agents that differ in the mechanism of action from known drugs and are active against resistant strains seems to be an extremely important task.

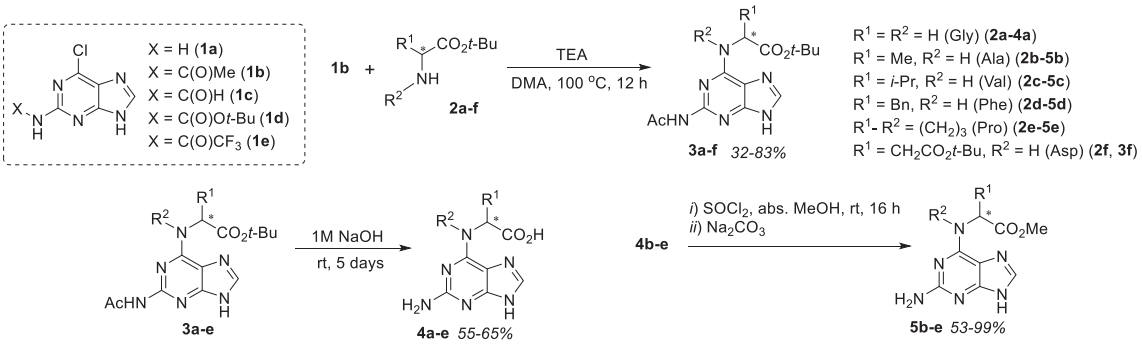
Information on the main groups of purine derivatives with antitubercular activity has been summarized by us in a review [43]. For some compounds (mainly for adenosine derivatives), mechanisms of antimycobacterial action are understood; that is, for example, inhibition of adenosine kinase, a key enzyme in the mycobacteria metabolism [19, 44], or inhibition of the MbtA enzyme involved in the biosynthesis of mycobactins responsible for the transport of iron in mycobacteria [45]. At the same time, for the majority of purine derivatives exhibiting antimycobacterial activity, the mechanism of action remains unclear. Presumably, it may be associated with the inhibition of mycobacterial glutamine synthetase [46] or dihydrofolate reductase [47].

Synthesis

In general case, the synthesis of novel purine conjugates with natural amino acids and short peptides involves the nucleophilic substitution reaction of chlorine in 6-chloropurine, 2-amino-6-chloropurine or its *N*-acyl derivatives with amino esters followed by the removal of protecting ester group to provide further transformations.

At first, we developed the methods for synthesis of 2-aminopurine conjugates containing the residues of natural amino acids (Gly, Ala, Val, Phe, Pro, and Asp) at position C^6 of purine core (Scheme 1) [48, 49]. An important structure requirement was the presence of free functional groups in the molecules of the target compounds: a carboxyl group in the amino acid fragment and an N^9H group in the purine cycle, which is necessary for further modifications.

Conjugates of 2-acetamidopurine with *tert*-butyl esters of amino acids (compounds **3a–f**) were obtained in 32–83% yields by the reaction of nucleophilic substitution of chlorine in 2-acetamido-6-chloropurine (**1b**) with



Scheme 1: Structures 2-amino-6-chloropurine (**1a**) and 2-acylamino-6-chloropurines **1b–e**; synthesis of compounds **3a–f**, **4a–e**, and **5b–e**.

tert-butyl esters **2a–f** [dimethylacetamide (DMA), triethylamine (TEA), 100 °C] (Scheme 1) [48, 49]. The need to use protection for the 2-amino group of purine is due to the fact that unprotected 2-amino-6-chloropurine (**1a**) is not subjected to the nucleophilic substitution under the indicated conditions; this is apparently caused by the positive mesomeric effect of amino group. The highest yield (83%) was observed for compound **3e** (derivative of (*S*)-proline); the lowest, for compound **3f** (derivative of (*S*)-aspartic acid). When choosing the optimal protecting group, we carried out nucleophilic substitution of chlorine in compounds **1b–e** containing acetyl, formyl, Boc, and trifluoroacetyl protecting groups, respectively, with *tert*-butyl (*S*)-phenylalaninate [(*S*)-**2d**] (Scheme 1). It was found that in the case of compounds **1c–e**, the protecting groups were removed during the synthesis, thus reducing the reaction yields (35–50%). When 2-acetamido-6-chloropurine (**1b**) was used as the starting compound, the protecting group was retained during nucleophilic substitution of chlorine (yield of **3d** was 65%) [48].

When obtaining compounds **3b–f**, the main attention was paid to the synthesis of derivatives of natural (*S*)-amino acids, while derivatives of (*R*)- and (*RS*)-series were synthesized and used as reference compounds in HPLC analysis of the enantiomeric composition of the products [49]. The enantiomeric composition of compounds (*S*)-**3b–f** was determined using reversed-phase chiral HPLC. It has been shown that in the case of derivatives of (*S*)-alanine, (*S*)-valine, (*S*)-proline, and (*S*)-phenylalanine, the synthesis of compounds (*S*)-**3b,c,e** (>99% *ee*) and (*S*)-**3d** (96% *ee*) proceeds without significant racemization; while synthesis of the (*S*)-aspartic acid derivative [(*S*)-**3f**, 50% *ee*] was accompanied by substantial racemization.

Alkaline hydrolysis (1M NaOH, room temperature, 5 days) of compounds **3a–e** led to the removal of *N*-acetyl and ester groups to afford products **4a–e** (Scheme 1) [49]. Compounds **4b–e** were subjected to pre-column derivatization (abs. MeOH, $SOCl_2$) to form corresponding methyl esters **5b–e** (Scheme 1) followed by normal-phase chiral HPLC. It has been shown that in all cases, enantiomeric excess of esters **5b–e** is not less than 99%, which indicates the absence of racemization at the stage of alkaline hydrolysis. It should be noted that when alkaline hydrolysis was carried out at 60 °C for 2–3 h, partial racemization of the (*S*)-valine [(*S*)-**5c**] and (*S*)-phenylalanine [(*S*)-**5d**] derivatives was observed, 86 and 80% *ee*, respectively [49].

Starting from acids **4a** and (*S*)-**4b–e** with free N^9H group in the purine fragment, the chemo-enzymatic transglycosylation reaction was carried out in the presence of recombinant *Escherichia coli* purine nucleoside phosphorylase to afford corresponding modified nucleosides: ribosides **6a** and (*S*)-**6b–e**, 2′-deoxyribosides **7a** and (*S*)-**7b–e**, and arabinosides **8a** and (*S*)-**8b–e** in 70–88% yield (conversion reached 90–98%) (Fig. 1) [50]. The possibility of transglycosylation of compounds **3a**, (*S*)-**3b–e**, and (*R*)-**3c** containing protecting groups was also evaluated; it has been shown that all of them, with the exception of the (*S*)-proline derivative (*S*)-**3e**, are able to form the corresponding nucleosides [51].

The presence of a free carboxyl group in the amino acid moiety of purine conjugates enables their further modification by the fragments of amines or amino acids. We chose *N*-(2-acetamidopurin-6-yl)glycine (**9**), which was obtained from compound **3a** as a result of selective removal of the ester group (Scheme 2) [52], and

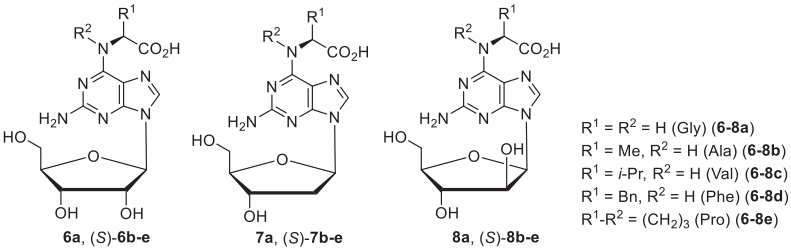
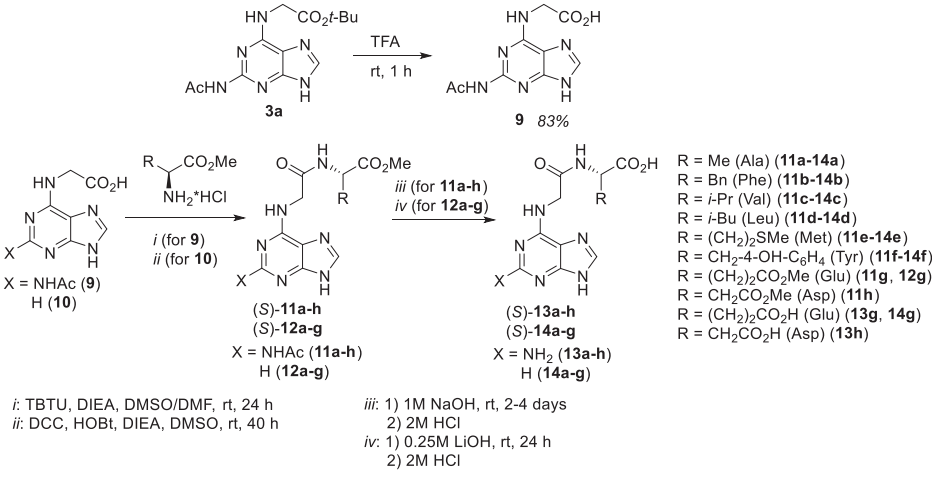


Fig. 1: Structures of modified nucleosides **6a–e**, **7a–e**, and **8a–e**.

N-(purin-6-yl)glycine (**10**) prepared as described in [53] as the objects for such modification. It should be noted that the choice of compounds **9** and **10** was determined by their pronounced inhibitory activity against Mbt (see below subsection "Antimycobacterial activity") [52].

Coupling of compounds **9** and **10** with methyl (*S*)-amino ester hydrochlorides was carried out in the presence of various coupling agents according to the classical methods of peptide chemistry (Scheme 2) [52, 54]. In the case of *N*-(2-acetamidopurin-6-yl)glycine (**9**), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) was used as a coupling agent; in the case of *N*-(purin-6-yl)glycine (**10**), we used *N*,*N*′-dicyclohexylcarbodiimide (DCC) in combination with 1-hydroxybenzotriazole (HOBt). All the reactions were carried out in the presence of *N*,*N*-diisopropylethylamine (DIEA) as a base [52]. The synthesis of derivatives of (*S*)-tyrosine (compounds (*S*)-**11f** and (*S*)-**12f**) in both cases was carried out in the presence of TBTU [54]. The 2-acetamidopurine conjugates (*S*)-**11a–h** were obtained in high yields (66–82%); purine conjugates (*S*)-**12a–g**, in moderate yields (30–51%). Our attempts to synthesize compounds (*S*)-**11a–h** starting from *N*-(2-aminopurin-6-yl)glycine (**4a**) containing an unprotected amino group did not lead to a satisfactory result. A comparison of the yields of compounds (*S*)-**11f** (70%) and (*S*)-**12f** (51%) obtained using the same coupling agent allows us to conclude that lower yields of products (*S*)-**12a–g** are related not to the use of DCC instead TBTU, but with a difference in the reactivity of the starting compounds **9** and **10**. The absence of racemization at the stage of coupling was proved using reversed-phase chiral HPLC [54].

Compounds (*S*)-**11a–h** and (*S*)-**12a–g** were subjected to alkaline hydrolysis in order to remove protecting groups. *N*-(2-Aminopurin-6-yl) dipeptides (*S*)-**13a–h** were obtained in 63–95% yield; *N*-(purin-6-yl) dipeptides (*S*)-**14a–g**, in 45–68% yield (Scheme 2) [52, 54]. Alkaline hydrolysis of 2-acetamidopurine derivatives (*S*)-**11a–h** was carried out under more drastic conditions (1M NaOH, 2–4 days) than in the case of purine derivatives (*S*)-**12a–g** (0.25M LiOH, 24 h), which was associated with the need of additional removal of *N*-acetyl group.



Scheme 2: Synthesis of compounds **9**, **11,13a–h**, and **12,14a–g**.

The approach used for the synthesis of compounds (*S*)-**13a–h** and (*S*)-**14a–g** proved to be unsuitable for the synthesis of (*S*)-serine derivatives (*S*)-**13i** and (*S*)-**14i** because of the involvement of aliphatic hydroxy group in the side processes during the coupling reaction [54]. Therefore, an alternative approach based on the nucleophilic substitution of chlorine in 2-amino-6-chloropurine (**1a**) or in 6-chloropurine (**1f**) with the enantiopure glycyl-(*S*)-serine dipeptide was proposed for the synthesis of these compounds. The reaction was carried out under reflux in an aqueous solution of sodium carbonate; the yields of (*S*)-**13i** and (*S*)-**14i** were 54 and 78%, respectively (Scheme 3) [54].

Analysis of enantiomeric purity of compound (*S*)-**14i** was carried out by reversed-phase chiral HPLC of its methyl ester (*S*)-**15** (Scheme 3) in comparison with specially prepared racemic ester (*RS*)-**15**. Enantiomeric excess (*ee*) of ester (*S*)-**15** more than 99% indicates the absence of racemization during nucleophilic substitution.

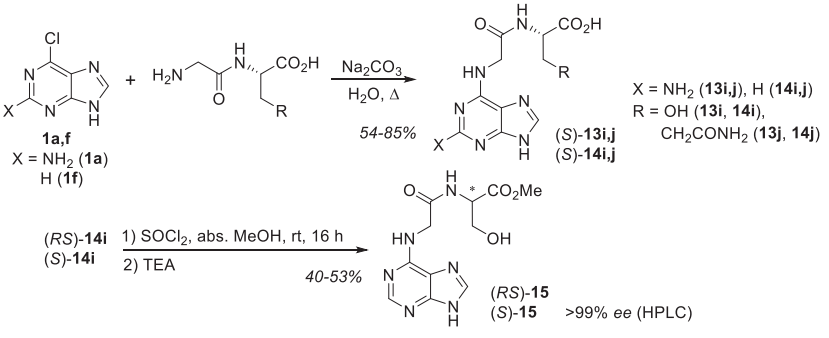
The above approach was also applied to obtain the (*S*)-glutamine derivatives (*S*)-**13j** and (*S*)-**14j**; the yields of the target compounds were 66 and 85%, respectively (Scheme 3) [54].

Since the (*S*)-glutamic acid derivatives (*S*)-**13g** and (*S*)-**14g** (Scheme 2) exhibited the highest inhibitory effect against Mbt (see subsection "Antimycobacterial activity") in the series of the obtained *N*-(2-aminopurin-6-yl) and *N*-(purin-6-yl) dipeptides [52], we decided to perform studies on the structural modification of these conjugates (Fig. 2) in order to obtain more active compounds.

Starting from *N*-(purin-6-yl)-*ω*-amino acids **16a–c** obtained by the known method [55], we synthesized structural analogues of compound (*S*)-**14g**, in which the glycine fragment was replaced by the *β*-alanine (compound (*S*)-**18a**), *γ*-aminobutyric acid [(*S*)-**18b**], and *ε*-aminocaproic acid fragments [(*S*)-**18c**] (Scheme 4) [54]. These conjugates were obtained in a similar way as the *N*-(purin-6-yl)glycine derivatives **14a–g** by coupling of compounds **16a–c** with dimethyl (*S*)-glutamate hydrochloride followed by alkaline hydrolysis of the coupling products (*S*)-**17a–c**. Enantiomeric purity (>99% *ee*) of the obtained compounds was monitored by reversed-phase chiral HPLC [54].

It is known that 1,2-ethylenediamine is an important pharmacophore fragment present in the structures of many antimycobacterial agents [56–58]. Therefore, another direction of structural modification was the replacement of the glycine residue with a 1,2-ethylenediamine fragment. We obtained compounds (*S*)-**21a–d**, in which the residue of (*S*)-aspartic or (*S*)-glutamic acid was attached to the purine fragment through the 1,2-ethylenediamine linker at the *β*- or *γ*-carboxyl group, respectively (Scheme 5) [59]. The nucleophilic substitution of chlorine in 2-acetamido-6-chloropurine (**1b**) or 6-chloropurine (**1f**) with *N*-Boc-1,2-ethylenediamine and subsequent removal of the Boc-protection led to purines **19a,b** containing 1,2-ethylenediamine moiety at position C^6. The coupling of these compounds with *α*-*tert*-butyl *N*-Boc-(*S*)-aspartate or *N*-Boc-(*S*)-glutamate by the method of mixed anhydrides resulted in products (*S*)-**20a–d**, the sequential treatment of which with trifluoroacetic acid (TFA) and 1M NaOH was accompanied by the removal of the protecting groups and the formation of the target compounds (*S*)-**21a–d** (Scheme 5) [59]. It has been demonstrated that synthesis of the target compounds (*S*)-**21a–d** is not accompanied by racemization [59].

Compounds (*S*)-**21a–d** are poorly soluble in water; therefore, to increase water solubility, we modified these compounds by introducing the (2-hydroxyethoxy)methyl substituent at position N^9 of the purine cycle



Scheme 3: Synthesis of compounds (*S*)-**13i,j**, (*S*)-**14i,j**, and (*RS*)-**15**, (*S*)-**15**.

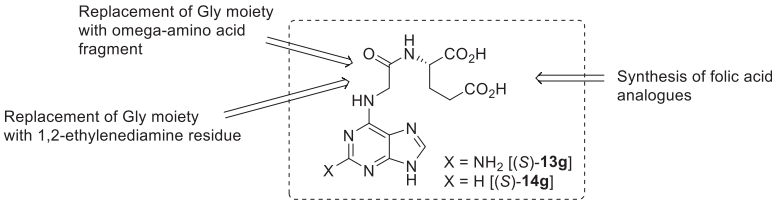
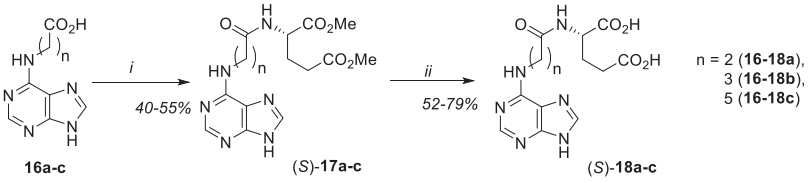


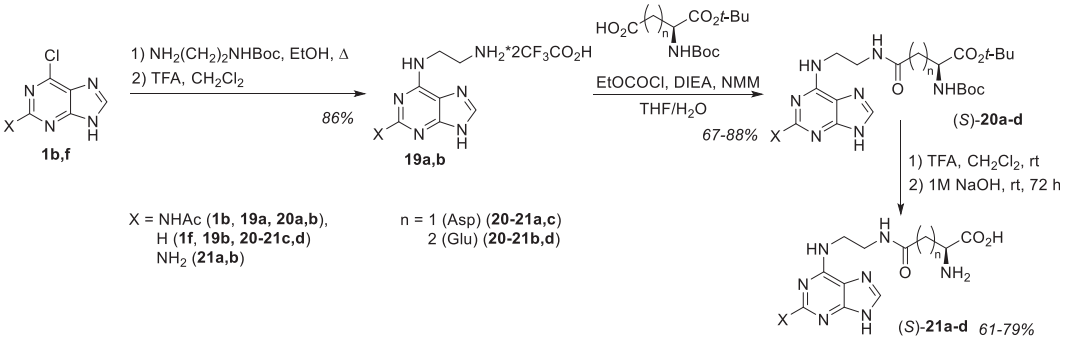
Fig. 2: Directions of structural modification of conjugates (*S*)-**13g** and (*S*)-**14g**.



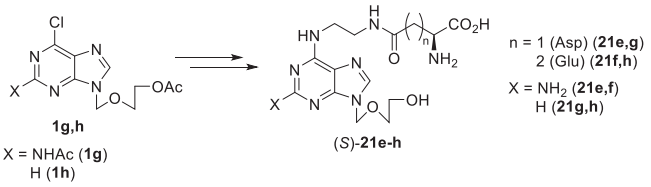
Scheme 4: Synthesis of compounds (*S*)-**18a–c**.

[59]. To prepare conjugates (*S*)-**21e–h**, we used the abovementioned synthetic sequence applied for (*S*)-**21a–d** (Scheme 5), 2-acetamido-9-(2-acetoxyethoxy)methyl-6-chloropurine (**1g**) and 9-(2-acetoxyethoxy)methyl-6-chloropurine (**1h**) [60] being the starting substances (Scheme 6). Nucleophilic substitution of the chlorine atom in compounds **1g,h** with *N*-Boc-1,2-ethylenediamine followed by removal of the Boc-protection, coupling with *α-tert*-butyl (*S*)-aspartate and (*S*)-glutamate, and further removal of protecting groups (*tert*-butyl ester and acetyl) resulted in conjugates (*S*)-**21e–h**. These compounds are highly soluble in water and poorly soluble in organic solvents [59].

Another direction of structural modification of 2-aminopurine and purine conjugates with (*S*)-glutamic acid (compounds (*S*)-**13g** and (*S*)-**14g**) is the replacement of the glycine residue with a fragment of *p*-aminobenzoic acid or *N*-(2-aminoethyl)-*p*-aminobenzoic acid [61]. Compounds obtained as a result of such a replacement can be considered as structural analogues of folic acid [62]. We carried out nucleophilic substitution of chlorine in 2-amino-6-chloropurine (**1a**) and 6-chloropurine (**1f**) with *p*-aminobenzoic acid (**22a**) under reflux in aqueous sulfuric acid, or *tert*-butyl *N*-(2-aminoethyl)-*p*-aminobenzoate (**22b**) under reflux in ethanol or *n*-butanol, followed by removal of the ester group (Scheme 7). Coupling of compounds **23a–d** with dimethyl (*S*)-glutamate hydrochloride followed by alkaline hydrolysis led to conjugates (*S*)-**24a–d** (Scheme 7) [61]. We also synthesized 2-aminopurine conjugate **23e** via nucleophilic substitution of the chlorine atom in 2-amino-6-chloropurine (**1a**) with methyl *N*-glycyl-*p*-aminobenzoate (**22c**) in 37% yield (Scheme 7). We failed to remove the ester group (for the subsequent coupling with dimethyl (*S*)-glutamate), since alkaline hydrolysis led to the breakage of the amide bond between the glycine and *p*-aminobenzoic acid fragments [61].



Scheme 5: Synthesis of compounds (*S*)-**21a–d**.



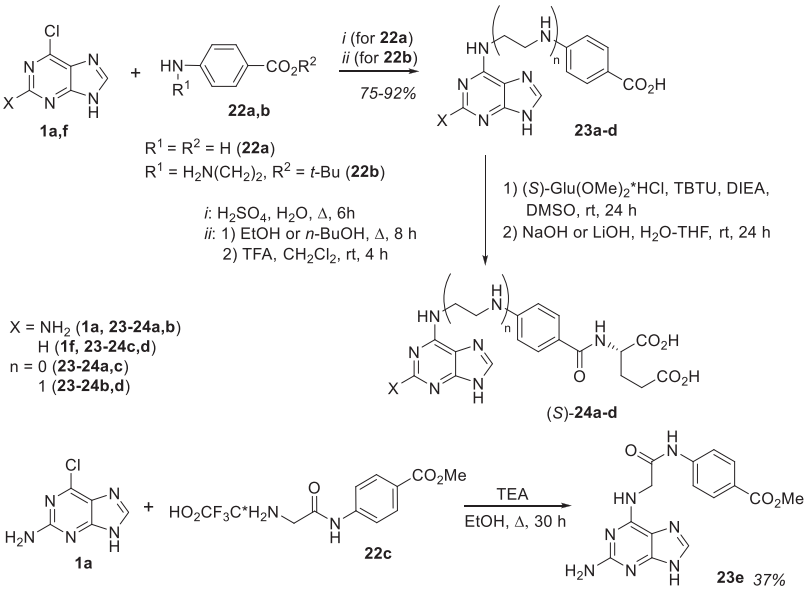
Scheme 6: Synthesis of compounds (*S*)-**21e–h**.

We have developed methods for the synthesis of conjugates of 2-aminopurine and purine with diamino acids, (*S*)-lysine and (*S*)-ornithine. It is known that compounds containing unsubstituted alpha-amino and carboxyl groups in their structure are able to pass through cell membranes via specific carrier proteins, which determines the active transport and, in some cases, the selectivity of these compounds [63, 64]. In particular, conjugates of 2-aminopurine and purine with (*S*)-aspartic and (*S*)-glutamic acid (*S*)-**21a–h** (Schemes 5 and 6) [59] are characterized by such structural features. Another way to obtain compounds containing free alpha-amino and carboxyl groups is modification of the purine cycle with diamino acid fragments through interaction of a purine derivative with N^{α}-protected diamino acids followed by removal of the protecting group. We have demonstrated the possibility of synthesizing the target compounds using N^{α}-trifluoroacetyl, N^{α}-Boc, and N^{α}-Cbz protecting groups [49, 65].

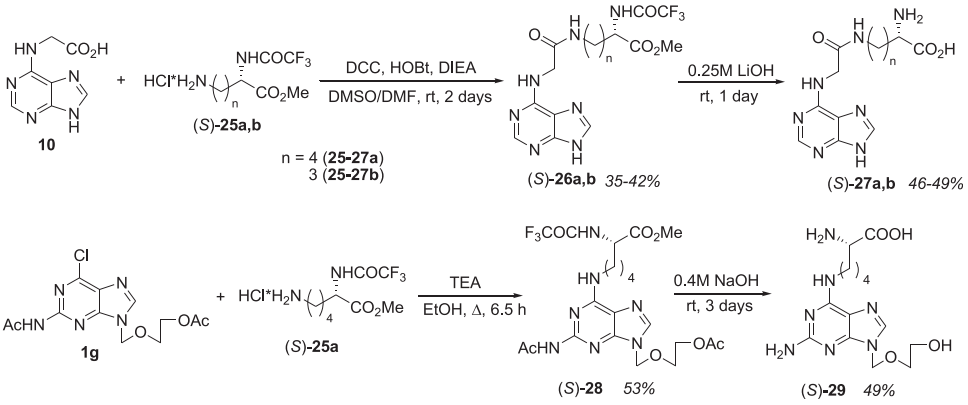
Coupling of *N*-(purin-6-yl)glycine (**10**) with methyl N^{α}-trifluoroacetyl-(*S*)-lysinate [(*S*)-**25a**] or N^{α}-trifluoroacetyl-(*S*)-ornithinate [(*S*)-**25b**] in the presence of DCC, HOBt, and DIEA followed by alkaline hydrolysis resulted in conjugates (*S*)-**27a,b** (Scheme 8) [65]. Enantiomeric purity of the obtained compounds was confirmed by reversed-phase chiral HPLC.

N^{α}-Trifluoroacetyl derivative of (*S*)-lysine [(*S*)-**25a**] was also used for the synthesis of 2-aminopurine conjugate (*S*)-**29** containing the (2-hydroxyethoxy)methyl substituent at position N^{9} (Scheme 8) [65]. Nucleophilic substitution of chlorine in 2-acetamido-9-(2-acetoxyethoxy)methyl-6-chloropurine (**1g**) with compound (*S*)-**25a** was carried out in refluxing ethanol in the presence of TEA and afforded compound (*S*)-**28** in 53% yield. The subsequent alkaline hydrolysis (0.4M NaOH) led to the simultaneous removal of four protecting groups (ester, *N*-trifluoroacetyl, *N*-acetyl and *O*-acetyl) and the formation of conjugate (*S*)-**29** in 49% yield.

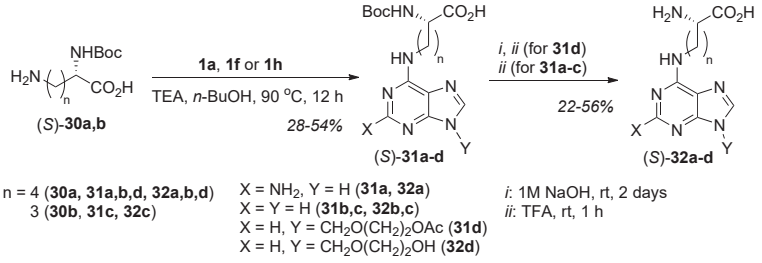
N^{α}-Boc-(*S*)-Lysine [(*S*)-**30a**] and N^{α}-Boc-(*S*)-ornithine [(*S*)-**30b**] were used as nucleophiles in the nucleophilic substitution of the chlorine atom in 2-amino-6-chloropurine (**1a**), 6-chloropurine (**1f**), and 9-(2-acetoxyethoxy)-methyl-6-chloropurine (**1h**) (Scheme 9) [65]. The reaction was carried out in *n*-butanol at 90 °C in the presence of TEA and afforded compounds (*S*)-**31a–d** in 28–54% yield. The subsequent treatment of compounds



Scheme 7: Synthesis of compounds (*S*)-**24a–d**.



Scheme 8: Synthesis of compounds (*S*)-**27a,b** and (*S*)-**29**.



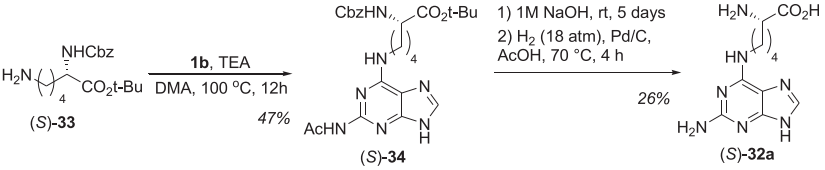
Scheme 9: Synthesis of compounds (*S*)-**32a–d**.

(*S*)-**31a–d** with TFA resulted in the removal of N^{α}-Boc protection; the target conjugates, 2-aminopurine–(*S*)-lysine [(*S*)-**32a**], purine–(*S*)-lysine [(*S*)-**32b**], and purine–(*S*)-ornithine [(*S*)-**32c**] were obtained in 54–56% yield. The synthesis of conjugate of 9-(2-hydroxyethoxy)methylpurine with (*S*)-lysine [(*S*)-**32d**] included an additional step of alkaline hydrolysis of compound (*S*)-**31d** in order to remove the *O*-acetyl protection; the total yield after hydrolysis and TFA treatment was 22%.

The synthesis of N^{ε}-(2-aminopurin-6-yl)-(*S*)-lysine [(*S*)-**32a**] was also carried out starting from *tert*-butyl N^{α}-Cbz-(*S*)-lysinate [(*S*)-**33**] (Scheme 10) [49]. The nucleophilic substitution of chlorine in 2-acetamido-6-chloropurine (**1b**) led to the product (*S*)-**34**; further alkaline hydrolysis resulted in the removal of the *N*-acetyl and ester protecting groups. The subsequent hydrogenolysis (Pd/C, AcOH) was carried out to remove the N^{α}-Cbz group; in this case, the total yield of conjugate (*S*)-**32a** in three stages was 12% [49]. At the same time, the total yield of this compound in the synthesis starting from N^{α}-Boc-(*S*)-lysine [(*S*)-**30a**] was 15% (Scheme 9) [65]. Despite the close yields, the approach shown in Scheme 9 seems to be more convenient because of the smaller number of stages and, accordingly, less complexity.

Antimycobacterial activity

We tested more than 75 conjugates of 2-aminopurine and purine with amino acids, short peptides and other compounds for their antimycobacterial activity in vitro against both the laboratory strains (*M. tuberculosis*



Scheme 10: Synthesis of compound (*S*)-**32a**.

H37Rv, *Mycobacterium avium*, and *Mycobacterium terrae*) and multidrug-resistant *M. tuberculosis* strain isolated from a tuberculosis patient in the Ural region (Russia). Biological testing was performed at the Ural Research Institute of Phthisiopulmonology (Ekaterinburg, Russia). The structural formulas of the most active compounds and their minimum inhibitory concentrations (MICs, μg/mL) are shown in Fig. 3.

Among purine conjugates with natural amino acids, *N*-(2-acetamidopurin-6-yl)glycine (**9**) exhibited the highest antimycobacterial activity against all studied strains (MIC 0.7 μg/mL) [52]. At the same time, *N*-(purin-6-yl)glycine (**10**) possessed low activity against *M. tuberculosis* H37Rv (MIC 6.25 μg/mL).

Conjugates of purines with glycyl-(*S*)-glutamic acid (compounds (*S*)-**13g** and (*S*)-**14g**; MIC 0.7 μg/mL) and glycyl-(*S*)-phenylalanine (compound (*S*)-**12b**, MIC 1.5 μg/mL) exhibited the highest inhibitory effect against *M. tuberculosis* H37Rv among other studied conjugates with dipeptides [52]. All compounds with high antimycobacterial activity were also tested for their cytotoxic activity against human embryonic fibroblasts (HEFs) in the MTT assay [52]. It has been shown that these compounds are almost nontoxic (IC_{50} > 50 μM), the calculated selectivity index (SI = IC_{50}/MIC) being more than 25 [52]. Of particular interest is their activity against sustainable multidrug-resistant (Rifampin and Isoniazid resistant) *M. tuberculosis* strain. Purine conjugates with glycyl dipeptides containing the fragment of (*S*)-methionine (compounds (*S*)-**11e** and (*S*)-**13e**), (*S*)-phenylalanine [(*S*)-**13b** and (*S*)-**14b**], and (*S*)-alanine [(*S*)-**13a**] possessed from moderate to low antimycobacterial activity (MIC 3.1–6.25 μg/mL), while other tested compounds were inactive.

2-Aminopurine and purine conjugates with (*S*)-aspartic acid attached via a 1,2-ethylenediamine linker (Fig. 3, compounds (*S*)-**21a** and (*S*)-**21c**) [59], which can be considered as structural analogues of compounds (*S*)-**13g** and (*S*)-**14g**, exhibited high antimycobacterial activity. The MIC values for these compounds are 1.5 and 0.7 μg/mL, respectively. At the same time, compounds (*S*)-**21b,d** containing (*S*)-glutamic acid (Scheme 5), and compounds (*S*)-**21e-h** with (2-hydroxyethoxy)methyl substituent at position N^9 (Scheme 6) were inactive against *M. tuberculosis* H37Rv strain [59].

Other approaches used for structural modification of bioactive conjugates (*S*)-**13g** and (*S*)-**14g** did not lead to compounds with antimycobacterial activity. Analogues of conjugate (*S*)-**14g**, in which the glycine fragment is replaced by *ω*-amino acid residues (compounds (*S*)-**18a–c**, see Scheme 4) and folic acid analogues (*S*)-**24a–d** (Scheme 7) turned out to be inactive against *M. tuberculosis* H37Rv strain [54, 61]. At the same time, compound **23e**, an intermediate in the synthesis of folic acid analogues exhibited high antimycobacterial activity (MIC 1.5 μg/mL) [61].

Among conjugates of 2-aminopurine and purine with diamino acids, N^{ε}-[2-amino-9-(2-hydroxyethoxy)-methylpurin-6-yl]-(*S*)-lysine (Fig. 3, compound (*S*)-**29**, MIC 0.7 μg/mL) is characterized by high antimycobacterial activity [65]. Other purine conjugates with (*S*)-lysine (compounds (*S*)-**26a**, (*S*)-**27a**, (*S*)-**32a,b,d**;

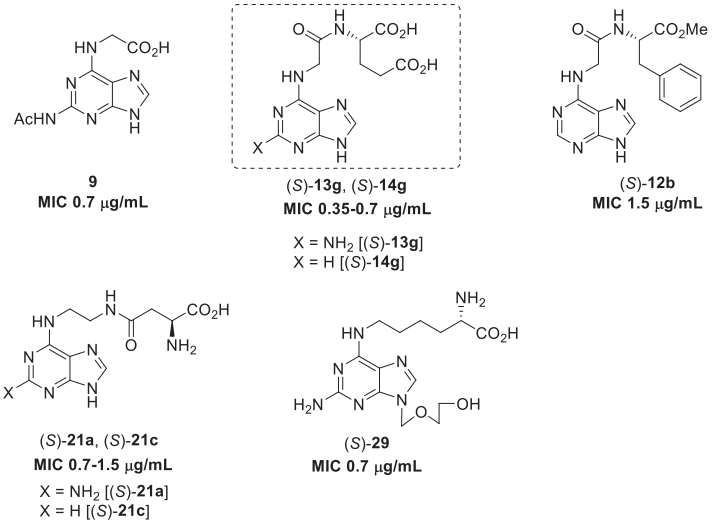


Fig. 3: Structures of purine conjugates with high antimycobacterial activity.

see Schemes 8 and 9) exhibited low antimycobacterial activity (MIC 6.25 μg/mL), while derivatives of (*S*)-ornithine (compounds (*S*)-**27b** and (*S*)-**32c**; Schemes 8 and 9) were inactive.

We also studied the in vitro antimycobacterial activity of 6-chloropurines **1a–e** (Scheme 1) [66]. It has been found that 2-amino-6-chloropurine (**1a**), 2-acetamido-6-chloropurine (**1b**), and 2-formamido-6-chloropurine (**1c**) exhibit high inhibitory effect against mycobacteria strains (MIC 0.7–1.5 μg/mL). These compounds also exhibit low cytotoxicity against HEF cells (MTT test, CC_{50} > 50 μM).

Search for efficient anti-herpesvirus agents among purine conjugates with *N*-heterocycles

Diseases caused by the herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) are extremely widespread [67, 68]. According to the latest estimates of the World Health Organization, 67% of the world's population under age 50 (about 3.7 billion people) are infected with HSV-1 [67], which can remain in a latent state for a long time [69]. HSV is one of the significant causes of death among immunocompromised patients [70]. In addition, the relationship between the presence of anti-HSV IgM antibodies in the body, a sign of reactivated herpesvirus infection, with the risk of Alzheimer's disease has been found [71].

The first-line chemotherapy drugs most effective for the treatment of HSV-caused infections are a modified nucleoside Acyclovir (zovirax, 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one), its metabolic prodrug Valacyclovir and a structural analogue Famciclovir [67]. As is known, the molecular mechanism of antiviral action of these drugs consists in stepwise phosphorylation with the formation of mono-, di- and then triphosphates that by competing with natural nucleotides selectively inhibit the activity of the herpetic DNA polymerase. Moreover, by integrating into the elongating chain of viral DNA, they interrupt its synthesis. Due to the fact that at the first stage the phosphorylation of acyclovir and its analogues leading to corresponding monophosphates is catalyzed by the virus thymidine kinase, and the subsequent two stages are catalyzed by cellular enzymes, the formation of active metabolites (triphosphates of modified nucleotides) is possible only in the infected cell. One of the factors limiting the use of this group of drugs is the development of drug resistance in the HSV, especially in immunocompromised patients, including HIV-infected persons [72].

In this regard, the search for new antiviral agents with a mechanism of anti-HSV-1 action different from the action of known drugs is highly topical. Our idea was to synthesize 2-aminopurine and purine conjugates containing fragments of various *N*-heterocycles attached directly to the purine scaffold at position C^6 or via an amino acid linker, and to search for compounds with anti-herpesvirus activity among them.

Synthesis

The main synthetic strategy used for preparation of novel purine conjugates with *N*-heterocycles consists in the nucleophilic substitution of chlorine in 6-chloropurine and 2-amino-6-chloropurine with various heterocyclic amines as nucleophiles. We used both chiral *N*-heterocycles **35–40** and achiral ones **41**, **42** (Fig. 4). Chiral *N*-heterocycles **35–40** were used both in the enantiopure form and as racemates. Racemic and achiral *N*-heterocycles are commercially available or were obtained by the known procedures. Individual (*R*)- and (*S*)-enantiomers of amines **35–40** were prepared via acylative kinetic resolution of their racemates with chiral acyl chlorides [73, 74].

Nucleophilic substitution of the chlorine atom in 2-amino-6-chloropurine (**1a**) and 6-chloropurine (**1f**) with amines **35**, **36**, **38–41** was carried out under different conditions (Scheme 11) [75, 76]. For preparation of the target compounds, we used two known methods: heating in water under reflux in the presence of sulfuric acid (method ***A***) [77, 78] and heating in 2,2,2-trifluoroethanol (TFE) under reflux in the presence of TFA (method ***B***) [79–81]. That is nucleophilic substitution of chlorine with racemic amines **35**, **36**, **38–40**, and amine **41** was

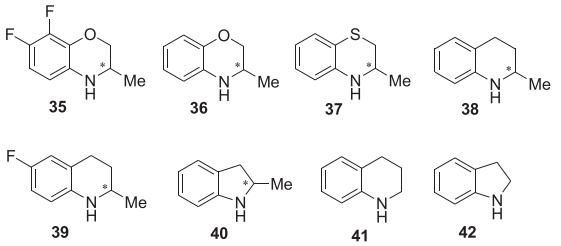


Fig. 4: Structures of *N*-heterocycles **35–42**.

carried out under conditions of acidic catalysis; in this case, it was important to choose such an amount of acid that purine was in the protonated form and the amine, in the free form. The nucleophilic substitution of chlorine in 2-amino-6-chloropurine (**1a**) was carried out in water under reflux in the presence of 0.9 equiv. sulfuric acid (method ***A***) to afford the target products (compounds **43–45a**, and **47–48a**) in 30–88% yields. The highest yield was observed in the reaction with racemic 2-methylindoline (**40**) resulting in product **47a**; the lowest, in the case of 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine (**35**).

The reaction of 6-chloropurine (**1f**) with racemic amine **40** according to method ***A*** (Scheme 11) afforded product (*RS*)-**47b** in high yield (91%), while yields in the reaction 6-chloropurine (**1f**) with other amines under these conditions were extremely low (less than 10%). Reducing the amount of sulfuric acid to 0.5 equiv. allowed obtaining compounds **43–45b**, and **48b** in moderate yields (up to 48%) [75]. We assume that in the case of the nucleophilic substitution reaction in 6-chloropurine (**1f**), low yields of the reaction products are caused by the side reaction resulting in a purine system destruction under acidic conditions. It is known that purine derivatives without substituents at position C^2 tend to decompose in acidic medium with the pyrimidine or imidazole ring opening leading to the formation of 4-aminoimidazole [82–85] or 4-aminopyrimidine derivatives [86, 87].

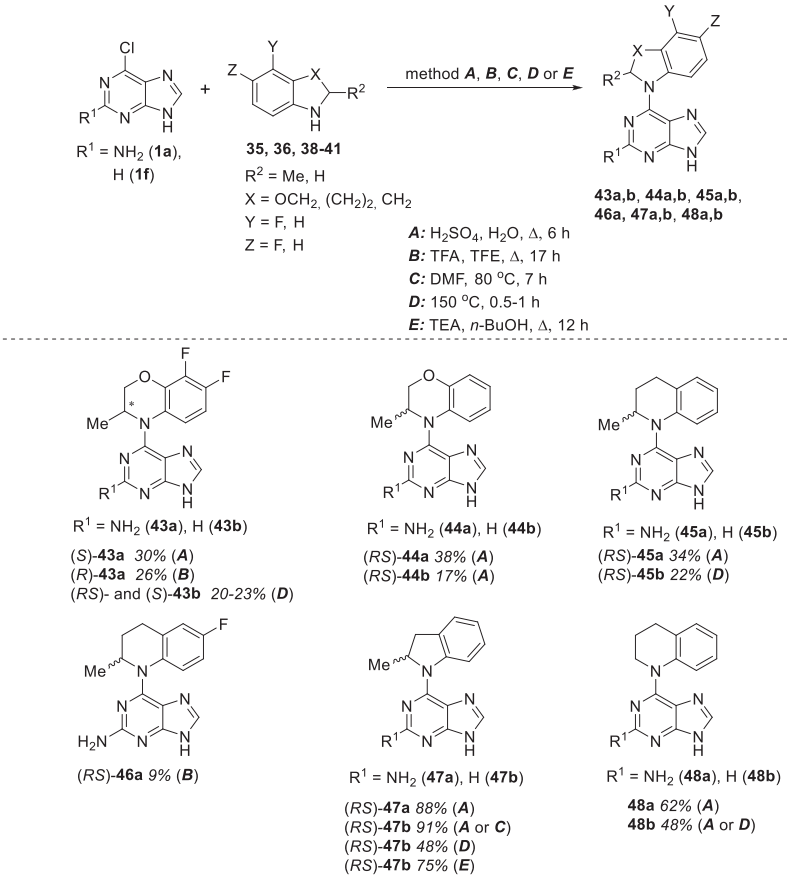
Interaction of 2-amino-6-chloropurine (**1a**) with fluorine-containing amines (*R*)-**35**, (*S*)-**35**, and (*RS*)-**39** carried out in TFE in the presence of TFA under reflux (Scheme 11, method ***B***) afforded compounds (*R*)-**43a** (26%), (*S*)-**43a** (26%) [76] and (*RS*)-**46** (9%) [75], while non-fluorinated amines were found to be unreactive under these conditions [75].

Nucleophilic substitution of chlorine in 6-chloropurine (**1f**) was also carried out in DMF at 80 °C (Scheme 11, method ***C***). In the case of amine (*RS*)-**40**, product (*RS*)-**47b** was obtained in high yield (91%) as in the reaction carried out under acidic catalysis; in the case of amine **41**, the yield of product **48b** was lower (34%); amine **38** does not react under these conditions [75].

Nucleophilic substitution in 6-chloropurine (**1f**) was also carried out without any catalyst and solvent, in the presence of threefold excess of a nucleophile (amines **35**, **38**, **40** and **41**) under heating at 150 °C (Scheme 11, method ***D***). In this case, compound **48b** was obtained in the same yield (48%) as under conditions of acidic catalysis (method ***A***); the yields of products (*RS*)-**43b**, (*S*)-**43b**, and (*RS*)-**45b**, were higher (20–23% compared to 10–17%), and the yield of compound (*RS*)-**47b** was 48%, which is significantly lower than in the methods ***A*** or ***C*** [75].

It should be noted that the reaction under conditions of basic catalysis, in *n*-butanol in the presence of TEA (Scheme 11, method ***E***) in most cases did not lead to the desired products, even when amines–nucleophiles were taken in a large excess; an exception was the interaction of 6-chloropurine (**1f**) with racemic amine **40** resulting in product **47b** in 75% yield.

Thus, we have shown that the result of nucleophilic substitution of the chlorine atom in 2-amino-6-chloropurine or 6-chloropurine with the studied heterocyclic amines under the conditions of either acidic or basic catalysis or in the absence of catalyst, significantly depends on the structure of nucleophile, as well as on the presence of amino group at position 2 of the purine fragment. The generally preferable procedure for the synthesis of purin-6-yl derivatives of *N*-heterocycles in satisfactory yields was the reaction in the absence of



Scheme 11: Synthesis of compounds **43–45a,b, 46a, 47–48a,b**.

catalysts, while 2-aminopurin-6-yl derivatives were best obtained in the reaction of 2-amino-6-chloropurine with heterocyclic amines in water in the presence of sulfuric acid.

Using chiral HPLC, we found the conditions for enantiomeric separation for (*RS*)-**43a** and (*RS*)-**43b** mixtures [75]. The enantiomeric purity of compound (*S*)-**43a** obtained in method ***A***, and (*S*)-**43b** obtained under heating at 150 °C without solvent (method ***D***) was determined. In both cases, the value of enantiomeric excess (>99% *ee*) indicates that the proposed synthetic approaches are not accompanied by racemization [75].

We have also proposed synthetic approaches to novel 2-aminopurine and purine conjugates containing fragments of *N*-heterocycles attached at position 6 of purine core via a linker, a fragment of omega-amino acid [88, 89]. Specifically, it has been reported that the 6-aminohexanoyl moiety is used as a linker/spacer in the synthesis of some bioactive purine-based compounds [90, 91].

At first, we synthesized 2-aminopurine conjugates **51a–e** starting from *N*-phthaloyl derivatives **49a–e** obtained as a result of acylation of amines **35–39** with *N*-phthalimidohexanoyl chloride in dichloromethane at room temperature in the presence of *N*,*N*-diethylaniline (Scheme 12) [88]. Amines **35–39** were used both as racemates and in the form of (*R*)- and (*S*)-enantiomers. Removal of the phthaloyl protection by hydrazinolysis in refluxing ethanol followed by the nucleophilic substitution of chlorine in 2-acetamido-6-chloropurine (**1b**) in DMA in the presence of TEA at 100 °C gave compounds **50a–e**. The subsequent alkaline hydrolysis for removal of acetyl protecting group resulted in conjugates **51a–e**. By the example of transformations of amine (*R*)-**37**, we demonstrated the possibility of preparation of the target conjugates without using *N*-acetyl protecting group in 2-amino-6-chloropurine. Thus, treatment of compound (*R*)-**49c** with hydrazine hydrate followed by interaction with 2-amino-6-chloropurine (**1a**) afforded conjugate (*R*)-**51c** in 92% yield (Scheme 12) [89].

The same synthetic approach was applied to the synthesis of purine conjugates **52a,c,f,g** (Scheme 13) [88]. Phthaloyl derivatives **49 a,c,f,g** derived from amines **35**, **37**, **40**, and **41** were subjected to hydrazinolysis, the resulting primary amines (without purification) were used as nucleophiles in the nucleophilic substitution of

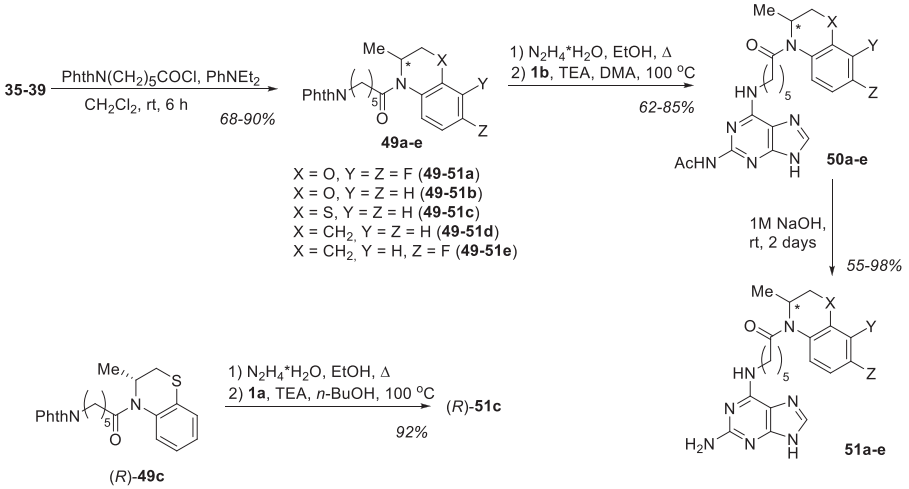
chlorine in 6-chloropurine (**1f**). We also used another protocol to obtain conjugates **52f–h**: the coupling of N^{ε}-(purin-6-yl)aminocaproic acid (**16c**) with heterocyclic amines **40–42** (Scheme 13) [88]. The reaction was carried out in the presence of TBTU and DIEA to afford conjugate **52h** (indoline derivative) in 60% yield; whereas compounds **52f,g** were obtained in lower yields (28 and 7%, respectively). Racemic 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine (**35**) and 2-methyl-1,2,3,4-tetrahydroquinoline (**38**) were not reactive under these conditions [88].

It has been found that the proposed synthetic approaches to purine and 2-aminopurine conjugates with chiral *N*-heterocycles (Schemes 12 and 13) do not lead to racemization [88, 89]. Enantiomeric purity of the obtained conjugates was confirmed by chiral HPLC [88, 89, 92].

It should be noted that compounds exhibiting significant anti-herpesvirus activity were found among the synthesized *N*-[6-(2-aminopurin-6-yl)]- and *N*-[6-(purin-6-yl)]-aminohexanoyl derivatives **51** and **52** (see subsection "Anti-herpesvirus activity"). Since *N*-[6-(purin-6-yl)]-aminohexanoyl conjugates of 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine (Scheme 13, compounds (*RS*)- and (*S*)-**52a**) were among the most active compounds against HSV-1, in order to study structure–activity relationship we extended the proposed synthetic approach to other *N*-[ω-(purin-6-yl)-aminoalkanoyl] conjugates (Scheme 14) [89]. Compounds **54a–e** were synthesized from *N*-phthaloyl derivatives **53a–e** obtained by acylation of racemic amine **35** with acyl chlorides derived from various ω-amino acids (glycine, β-alanine, γ-aminobutyric, 5-aminopentanoic, and 8-aminooctanoic acids). Treatment of compounds **53a–e** with hydrazine hydrate and subsequent interaction with 6-chloropurine (**1f**) led to products **54a–e** (Scheme 14). In most cases, the nucleophilic substitution reaction was carried out under heating in *n*-butanol in the presence of TEA (51–69% yield). However, such conditions were unsuitable for the synthesis of the β-alanine derivative **54b**; in this case, a large number of by-products were formed (the causes of this phenomenon were studied by us [89]; their discussion is beyond the scope of this review). Compound **54b** was obtained in 48% yield by heating the reagents at 120 °C without a solvent [89].

We also modified the structure of conjugate (*S*)-**51c** with high antiviral activity and prepared compound (*S*)-**56** containing the (2-hydroxyethoxy)methyl substituent at position N^9 of the purine core, which can be considered as an analogue of Acyclovir (Scheme 15) [89]. Nucleophilic substitution of chlorine in 2-acetamido-9-(2-acetoxyethoxy)methyl-6-chloropurine (**1g**) with primary amine formed after removal of the *N*-phthaloyl group of compound (*S*)-**49c** followed by alkaline hydrolysis of derivative (*S*)-**55** resulted in the target compound (*S*)-**56** in a total yield of 49% (Scheme 15).

Starting from 2-aminopurine conjugates (*R*)-**43a**, (*S*)-**43a**, and (*R*)-**51a**, (*S*)-**51a** with free N^9 position of purine core, the corresponding ribosides (*R*)-**57a**, (*S*)-**57a**, (*R*)-**58a**, and (*S*)-**58a**; 2′-deoxyribosides (*R*)-**57b**, (*S*)-**57a**, (*R*)-**58b**, and (*S*)-**58b** [93, 94]; and arabinosides (*R*)-**57c**, (*R*)-**58c** and (*S*)-**58c** [76, 95] (Fig. 5) were obtained by chemo-enzymatic transglycosylation in the presence of recombinant *E. coli* purine nucleoside phosphorylase.



Scheme 12: Synthesis of compounds **51a–e**.

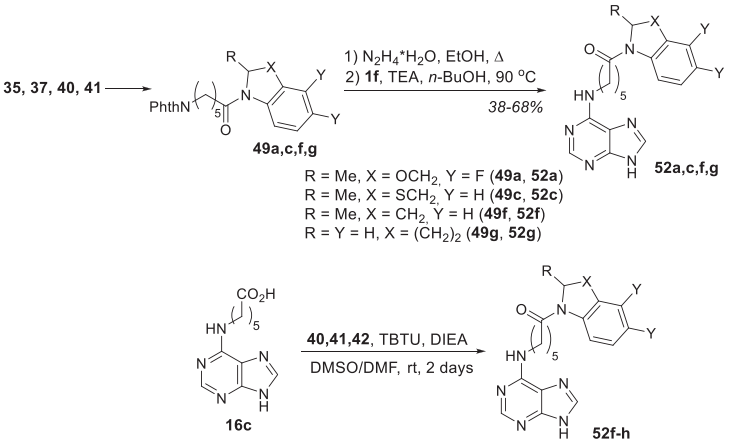
It has been noted that configuration of the chiral center of the heterocyclic fragment does not significantly affect the efficiency of ribosylation and 2′-deoxyribosylation; the yields of ribosides **57a** and **58a** were 50–71% and 32–43%, respectively; 2′-deoxyribosides **57b** and **58b** were obtained in 15% and 60–97%, respectively [93, 94]. At the same time, the efficiency of chemo-enzymatic arabinosylation of compounds (*R*)-**51a** and (*S*)-**51a**, in which the heterocyclic and 2-aminopurine fragments were separated by a linker, did not depend on the configuration of the chiral center; arabinosides (*R*)-**58c** and (*S*)-**58c** were prepared in 46–55% yields. It should be noted that arabinoside **57c** with difluorobenzoxazine fragment attached directly at the position C^6 of 2-aminopurine was obtained only as (*R*)-isomer in 43% yield, and (*S*)-enantiomer of conjugate **43a** did not enter into the arabinosylation reaction [76, 95].

Anti-herpesvirus activity

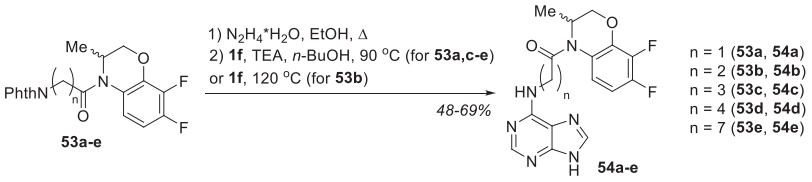
We tested more than 40 purine conjugates with *N*-heterocycles for their inhibitory activity against reference strain of HSV-1/L_2 and acyclovir-resistant HSV-1/L_2/R strain in the Vero E6 cells (see Table 1) [89, 94]. Testing was carried out at the Ivanovsky Institute of Virology of the Gamaleya Federal Research Center for Epidemiology and Microbiology (Moscow). The study of in vitro anti-herpetic activity of the obtained purine conjugates was carried out in accordance with internationally accepted cytopathogenic effect (CPE) inhibition assay [96, 97]. To evaluate the cytotoxicity expressed as the CC_{50} values of the studied compounds, the percentage of viable and non-viable cells was determined using Trypan blue exclusion assay [89].

As a result of testing the 2-aminopurine conjugates with *N*-heterocycles attached directly at position C^6, it has been shown that derivatives of 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine exhibit anti-herpetic activity. Thus, conjugate (*S*)-**43a** is characterized by significant inhibitory effect against HSV-1 strains (IC_{50} 37 μM) and moderate cytotoxicity (CC_{50} 297 μM), selectivity index (SI) = 8 [94]. (*R*)-Enantiomer of conjugate **43a** was much less active and more toxic (IC_{50} 37 μM, CC_{50} 168; SI = 2) [94]. Modification of these conjugates by introducing a riboside fragment at position N^9 (compounds **57a**) leads to a significant reduction in cytotoxicity: (*S*)-**57a**, CC_{50} > 1187 μM (SI > 32); (*R*)-**57a**, CC_{50} 856 μM (SI = 23) [94].

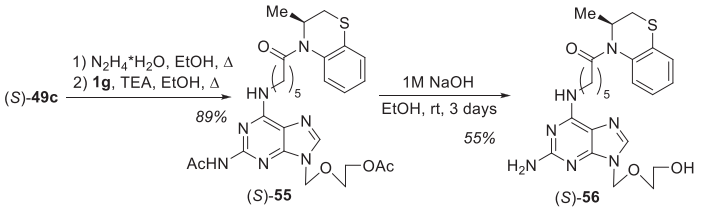
Among the studied conjugates of 2-aminopurine, compounds (*S*)-**51c** and (*S*)-**56** (derivatives of 3,4-dihydro-3-methyl-2*H*-[1,4]benzothiazine), (*R*)-**51e** and (*S*)-**51e** (containing 6-fluoro-2-methyl-1,2,3,4-tetrahydroquinoline fragment) were most active against HSV-1 strains. At the same time, the inhibitory activity of compound (*S*)-**51c** against acyclovir-resistant HSV-1/L_2/R strain was slightly reduced (IC_{50} 74 μM, SI = 5) compared to the inhibitory activity against reference HSV-1/L_2 strain (IC_{50} 37 μM, SI = 10) [89]. Compounds (*R*)-**51e** and (*S*)-**51e** were less active (IC_{50} 74 μM); however, they exhibited a lower toxicity compared to compound (*S*)-**51c** (CC_{50} ~ 760 and 365 μM, respectively); as a result, the SI values



Scheme 13: Synthesis of compounds **52a,c,f–h**.



Scheme 14: Synthesis of compounds **54a–e**.



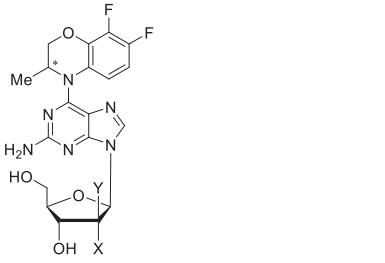
Scheme 15: Synthesis of compound (*S*)-**56**.

for these conjugates are the same and equal to 10. Modification of the structure of conjugate (*S*)-**51c** by introducing the (2-hydroxyethoxy)methyl substituent at position N^9 (compound (*S*)-**56**) led to a decrease in both the inhibitory activity (IC_{50} 74 μM) and cytotoxicity (CC_{50} 696 μM), selectivity indices being close (reference HSV-1/L_2 strain). Purine conjugate with 3,4-dihydro-3-methyl-2*H*-[1,4]benzothiazine (compound (*S*)-**52c**) also exhibited noticeable anti-herpetic activity (IC_{50} 37 μM), but was more toxic (CC_{50} 243 μM, SI = 7) [89].

It has been found that purine conjugates (*RS*)-**52a**, (*R*)-**52a**, (*S*)-**52a** and (*RS*)-**54e** containing the 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine fragment attached via "long" 6-aminohexanoyl and 8-aminooctanoyl linkers exhibit the highest inhibitory effect against HSV-1 strains (both reference and acyclovir-resistant) [89]. Noteworthy, the antiviral activity of these compounds is significantly depended on the configuration of the chiral center in the 3-methylbenzoxazine fragment. Thus, the IC_{50} value for (*S*)-enantiomer of compound **52a** was 4.6 μM being significantly lower this indicator for compound (*R*)-**52a** (IC_{50} 18 μM). Both (*S*)- and (*R*)-enantiomers **52a** exhibited close cytotoxicity in the Vero E6 cells (CC_{50} 293–316 μM), so their SI = 64 and 18, respectively ((*RS*)-**52a** SI = 32) [89].

It has been also demonstrated that structural modification of purine conjugate (*RS*)-**52a** by shortening the linker fragment does not lead to active compounds, while elongation (compound **54e**, n = 7) results in a simultaneous increase in antiviral activity (IC_{50} 2.3 μM) and cytotoxicity (CC_{50} 90 μM) (Table 1). For conjugate (*RS*)-**54e**, SI = 39 was slightly higher than that of the parent compound (*RS*)-**52a** [89].

To justify a fragment-based approach to anti-herpesvirus purine conjugates with *N*-heterocycles, i. e., the combination of two structural moieties in one molecule, we also evaluated the antiherpetic activity of such "structural components" of the most active purine conjugates **52a**, as *N*-(purin-6-yl)-6-aminohexanoic acid **16c**, amine (*RS*)-**35**, (*RS*)-4-(6-aminohexanoyl)-7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4] benzoxazine, and its phthaloyl derivative **49a**. It has been found that all these compounds were not efficient in inhibiting the development of virus-induced CPE. So, only the combination of various



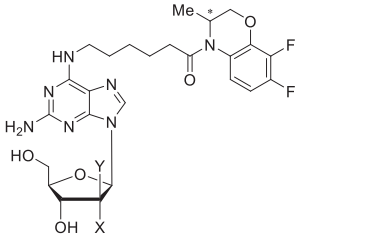


Fig. 5: Structures of modified nucleosides (*R*)-**57a–c**, (*S*)-**57a,b**, and (*R*)- and (*S*)-**58a–c**.

Table 1: Cytotoxicity and inhibitory activity of the most active conjugates against herpes simplex virus type 1 (HSV-1) strains in the Vero E6 cells.

Conjugate (Scheme no.)	CC_{50} (μM)[a]	HSV-1/L_2		HSV-1/L_2/R	
		IC_{50} (μM)[b]	SI[c]	IC_{50} (μM)	SI
(*S*)-**43a** (Scheme 11)	>296.88	37	>8	37	>8
(*S*)-**57a** (Fig. 5)	>1187.5	37	>32	37	>32
(*R*)-**57a** (Fig. 5)	855.82	37	23	37	23
(*S*)-**51c** (Scheme 12)	364.52	37	10	74	5
(*S*)-**56** (Scheme 15)	696.15	74	9	74	9
(*S*)-**52c** (Scheme 13)	243.60	37	7	37	7
(*S*)-**51e** (Scheme 12)	767.78	74	10	74	10
(*R*)-**51e** (Scheme 12)	760.00	74	10	74	10
(*S*)-**52a** (Scheme 13)	293.48	4.6	64	4.6	64
(*R*)-**52a** (Scheme 13)	316.31	18	18	18	18
(*RS*)-**52a** (Scheme 13)	293.37	9.3	32	9.3	32
(*RS*)-**54e** (Scheme 14)	89.99	2.3	39	2.3	39
Acyclovir	>444	1.73	>256	>444	1
Ribavirin	>4098	1025	>4	1025	>4
Foscarnet	>667	104	>6.4	104	>6.4

[a] CC_{50} is the cytotoxic concentration of compound required to reduce the viability of Vero E6 cells by 50%.
[b] IC_{50} is the concentrations of compounds producing 50% inhibition of the development of the virus-induced CPE relative to complete CPE in infected but untreated control cultures.
[c] SI is the selectivity index calculated as a CC_{50}/IC_{50} ratio.

fragments in one structure made it possible to obtain new biologically active compounds exhibiting significant anti-herpetic activity.

It should be noted that the absence of (2-hydroxyethoxy)methyl substituent (a pseudo-sugar residue) in position N^9 of purine in the most active compounds, as well as the activity of these compounds against the acyclovir-resistant HSV-1/L_2/R strain, indicate an alternative molecular mechanism of antiviral activity of the synthesized compounds as compared with Acyclovir. It is most likely that these compounds do not require TK-dependent activation (phosphorylation). However, elucidation of the mechanism of antiviral activity observed in a number of the synthesized compounds requires additional studies.

Conclusions

Thus, effective synthetic approaches for novel 2-aminopurine and purine conjugates with natural amino acids, short peptides, and heterocyclic amines were developed; a significant number of new compounds were obtained. Much attention was paid to the analysis of the enantiomeric purity of the obtained compounds; it has been found that the methods proposed by us make it possible to avoid racemization during the synthesis. Among the synthesized conjugates, compounds with high antimycobacterial or anti-herpetic activity and low cytotoxicity were identified. The high activity of these compounds especially against strains resistant to the action of known drugs allows us to consider them as the basis for design of new pharmaceutical agents.

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