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Synthesis and antimycobacterial activity of purine conjugates with (*S*)-lysine and (*S*)-ornithine

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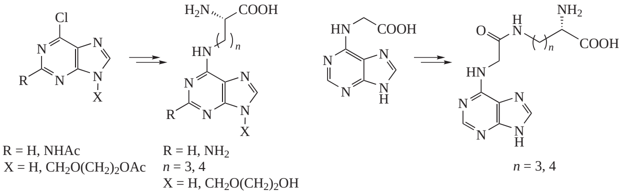
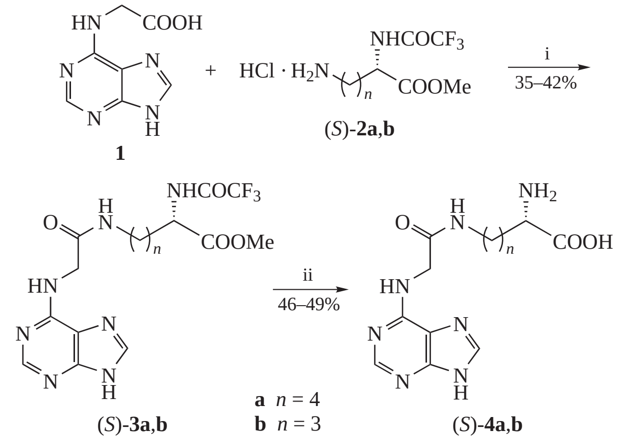
Novel purin-6-yl, 2-aminopurin-6-yl and *N*-(purin-6-yl)glycyl derivatives of (*S*)-lysine and (*S*)-ornithine with free α-functional groups, as well as compounds containing N^9-(2-hydroxyethoxy)methyl substituent in the purine core have been synthesized and tested for their inhibitory effect against *Mycobacterium tuberculosis* strains. Among the synthesized conjugates, one compound exhibiting a significant level of antimycobacterial activity has been found.



In recent years, much attention has been paid to the structural modification of natural compounds in order to obtain efficient therapeutic agents. Synthesis of purine derivatives is of particular interest since they possess diverse types of biological activity, including antiviral, antitumor, antimycobacterial, and others.[1–9] Obviously, this is due to the crucial role of purine bases being a part of the most important biomolecules: DNA, RNA, ATP, nicotinamide adenine dinucleotide (NAD) coenzyme, alkaloids, *etc*.[10,11]

Recently, we have obtained conjugates of purine and 2-aminopurine with natural amino acids and short peptides.[12–15] Some compounds of this series proved to exhibit significant antimycobacterial activity.[14,15] As a continuation of this research, we have herein obtained novel derivatives of natural diamino acids, (*S*)-lysine and (*S*)-ornithine, bearing the purin-6-yl or 2-aminopurin-6-yl fragment attached to the ω-amino group and retaining free α-amino and carboxylic groups, and studied their inhibitory effect against *Mycobacterium tuberculosis* strains. It is known that compounds containing an unsubstituted α-amino acid moiety are capable of permeation through the cell membrane *via* active transport by specific carrier proteins, which affects the bioavailability of such compounds and determines their biological activity.[16,17]

To synthesize novel conjugates of *N*-(purin-6-yl)glycine **1** with diamino acids, we have used the selectively protected derivatives, methyl N^α-trifluoroacetyl-(*S*)-lysinate and (*S*)-ornithinate (*S*)-**2a**,**b**[†] as starting materials (Scheme 1). Coupling between compounds **1** and (*S*)-**2a**,**b** was carried out in a DMSO–DMF mixture at room temperature in the presence of *N*,*N'*-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA) to afford *N*-trifluoroacetyl derivatives (*S*)-**3a**,**b** in a moderate yield. No racemization of the chiral center of the diamino acid fragment was observed, which was confirmed by the chiral HPLC analysis.[‡] Subsequent removal of both protection groups (methyl ester and *N*-trifluoroacetyl) by the action of 0.25 N LiOH led to the target compounds (*S*)-**4a**,**b** in the form of hydrates.[§]



Scheme 1 *Reagents and conditions*: i, DCC, HOBt, DIPEA, DMSO–DMF, room temperature, 2 days; ii, 0.25 N LiOH, room temperature, 1 day.

Methyl N^α-trifluoroacetyl-(*S*)-lysinate (*S*)-**2a** was also used as the starting compound in the synthesis of 2-aminopurine conjugate containing N^9-(2-hydroxyethoxy)methyl substituent (Scheme 2). Nucleophilic substitution of the chlorine atom in

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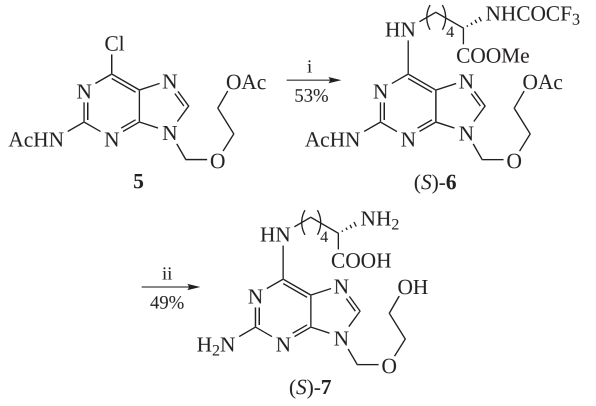
[†] Methyl N^α-trifluoroacetyl-(*S*)-lysinate and (*S*)-ornithinate hydrochlorides (*S*)-**2a**,**b** were obtained from N^α-Cbz-(*S*)-lysine and N^α-Cbz-(*S*)-ornithine as a result of treatment with trifluoroacetic anhydride in refluxing trifluoroacetic acid (TFA) followed by treatment with $SOCl_2$ in dry methanol. For procedures and characteristics of the products, see Online Supplementary Materials.

[‡] For HPLC data, see Online Supplementary Materials.

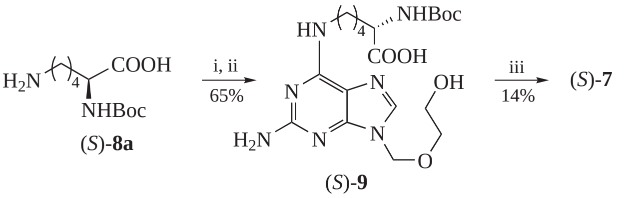
[§] For procedures and characteristics of the products, see Online Supplementary Materials.



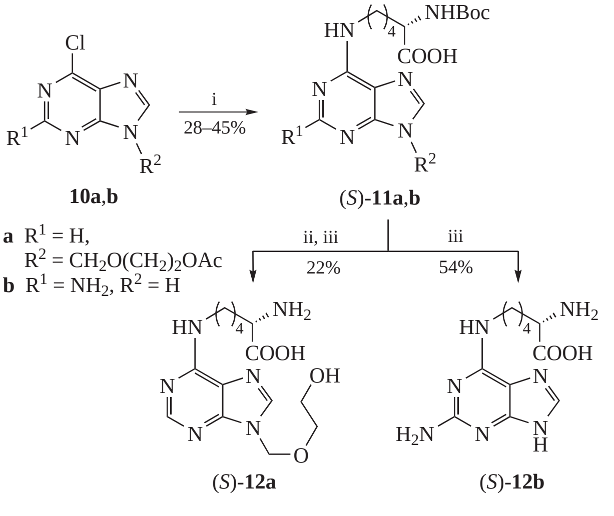
Scheme 2 *Reagents and conditions*: i, (*S*)-**2a**, Et_3N, EtOH, reflux, 6.5 h; ii, 0.4 N NaOH, room temperature, 3 days.

2-acetamido-9-(2-acetoxyethoxy)methyl-6-chloropurine **5**[¶] proved to proceed smoothly by the action of compound (*S*)-**2a** in refluxing EtOH in the presence of Et_3N followed by removal of all protection groups by the alkaline hydrolysis, thus resulting in conjugate (*S*)-**7**.

An alternative approach to prepare conjugate (*S*)-**7** can be applied starting from another lysine derivative, N^{α}-Boc-(*S*)-lysine (*S*)-**8a** (Scheme 3). Nucleophilic substitution of the chlorine atom in compound **5** by the action of compound (*S*)-**8a** in BuOH at 90 °C followed by removal of *N*-acetyl protection group by the alkaline hydrolysis has afforded compound (*S*)-**9** in good yield. However, its subsequent treatment with TFA has resulted in the target compound (*S*)-**7** in low yield. Similar synthetic sequence has been applied to obtain N^9-substituted purine conjugate (*S*)-**12a** and 2-aminopurine conjugate (*S*)-**12b** (Scheme 4). Note that we have already obtained compound (*S*)-**12b** in three steps starting from *tert*-butyl N^{α}-Cbz-(*S*)-lysinate,[13] but in a lower yield.

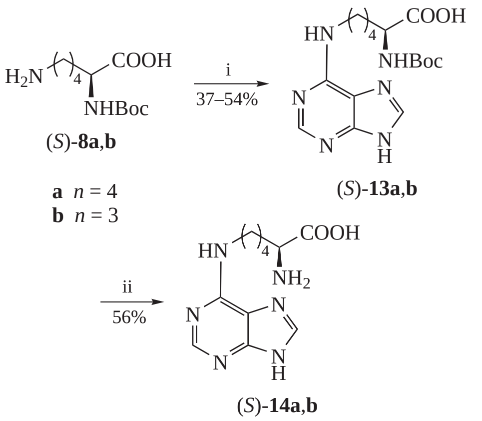


Scheme 3 *Reagents and conditions*: i, **5**, Et_3N, KI, BuOH, 90 °C, 7.5 h; ii, 1 N NaOH, room temperature, 3 days; iii, $CF_3C(O)OH$, room temperature, 1.5 h.



Scheme 4 *Reagents and conditions*: i, (*S*)-**8a**, Et_3N, KI, BuOH, 90 °C, 12 h; ii, 1 N NaOH, room temperature, 2 days; iii, $CF_3C(O)OH$, room temperature, 1 h.

We have also used N^{α}-Boc-(*S*)-lysine (*S*)-**8a** and N^{α}-Boc-(*S*)-ornithine (*S*)-**8b** as the starting materials in the synthesis of purine conjugates (*S*)-**14a**,**b** *via* nucleophilic substitution of the chlorine atom in 6-chloropurine followed by removal of *N*-Boc protection by treatment of compounds (*S*)-**13a**,**b** with TFA (Scheme 5). Note that the synthesis of racemic compounds **14a**,**b** in 76–77% yields has been reported to proceed *via* nucleophilic substitution of the chlorine atom in 6-chloropurine by the action of dihydrohalogenides of DL-lysine and DL-ornithine in refluxing aqueous potassium hydroxide.[20] However, our attempts to exploit this approach to obtain enantiopure compounds (*S*)-**14a**,**b** have failed; the reaction resulted in a rather complicated and hardly separable mixture of products. So, our strategy to use protected derivatives of diamino acids seems to be more appropriate for the synthesis of purine conjugates.



Scheme 5 *Reagents and conditions*: i, 6-chloropurine, Et_3N, KI, BuOH, 90 °C, 12 h; ii, $CF_3C(O)OH$, room temperature, 1 h.

The synthesized purine conjugates (*S*)-**3a**, (*S*)-**4a**,**b**, (*S*)-**7**, (*S*)-**12a**,**b** and (*S*)-**14a**,**b** have been tested for their antimycobacterial activity *in vitro* against laboratory *M. tuberculosis* $H_{37}Rv$ strain.[††] 2-Aminopurine–lysine conjugate (*S*)-**7** with N^9-(2-hydroxyethoxy)methyl substituent in the purine core exhibited the highest inhibitory activity against both standard *M. tuberculosis* $H_{37}Rv$ strain and *M. avium* and *M. terrae* laboratory strains (the minimum inhibitory concentration, MIC was 0.7 μg ml^{-1}), as well as multidrug-resistant *M. tuberculosis* strain[‡‡] isolated from a tuberculosis patient of the Ural region (MIC = 1.5 μg ml^{-1}). Other tested compounds have demonstrated moderate (compounds **3a**, **4a**, **12a**,**b**, and **14a**; MIC = 6.2 μg ml^{-1}) or low level of antimycobacterial activity (compounds **3b** and **14b**; MIC = 12.5 μg ml^{-1}). In general, purine conjugates with (*S*)-lysine proved to be more active than derivatives containing (*S*)-ornithine.

In summary, we have prepared novel enantiomerically pure purine, 2-aminopurine and *N*-(purin-6-yl)glycine conjugates with (*S*)-lysine and (*S*)-ornithine and have tested their inhibitory effect against mycobacteria strains. One of the compounds obtained, N^{ε}-[2-amino-9-(2-hydroxyethoxy)methyl-purin-6-yl]-(*S*)-lysine has demonstrated a significant antimycobacterial activity against both laboratory and multidrug-resistant *M. tuberculosis* strains.

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[¶] Compound **5** was synthesized by analogy with the known methods.[18,19]

[††] For details, see Online Supplementary Materials.

[‡‡] Rifampin and Isoniazid resistant *M. tuberculosis* strain.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.01.002.

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