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Synthesis and antimycobacterial activity of novel purin-6-yl and 2-aminopurin-6-yl conjugates with (*S*)-aspartic and (*S*)-glutamic acids

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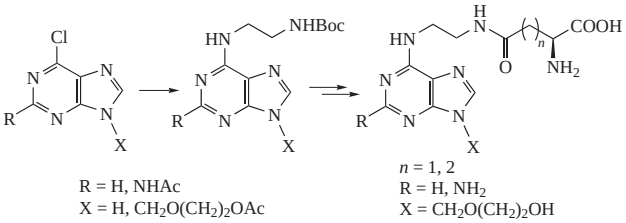
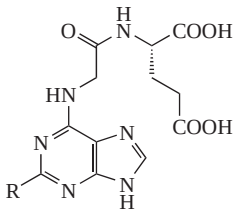
Novel purine and 2-aminopurine conjugates containing fragments of (*S*)-aspartic and (*S*)-glutamic acids with free α-functional groups attached *via* a 1,2-ethylenediamine linker at 6-position of the purine core, as well as their analogues with N^9-(2-hydroxyethoxy)methyl substituent have been synthesized. Among the conjugates obtained, compounds exhibiting a significant antimycobacterial activity have been found.

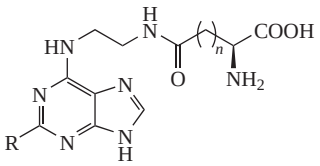


During recent decades, a considerable attention has been paid to structurally diverse purine derivatives possessing various types of biological activity.[1–3] Among them, compounds exhibiting antiviral,[4–8] antitumor,[9–12] antimycobacterial[13–16] and other types of biological activity were found.

Recently, we have studied the *in vitro* antimycobacterial activity of novel purin-6-yl derivatives of dipeptides and amino acids.[17,18] Within this family, purin-6-yl and 2-aminopurin-6-yl conjugates with glycyl-(*S*)-glutamic acid (compounds **1a**,**b**) show the highest inhibitory activity against both laboratory strains of *Mycobacterium tuberculosis* and clinical isolates of multidrug-resistant (MDR) *M. tuberculosis* strain.[18] As a continuation of this research, we have herein obtained a number of novel purine and 2-aminopurine conjugates, in which the fragment of (*S*)-aspartic or (*S*)-glutamic acid is attached to C-6 of the purine ring system *via* 1,2-ethylenediamine residue (compounds **2a**–**d**) and have studied their antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ and other strains.



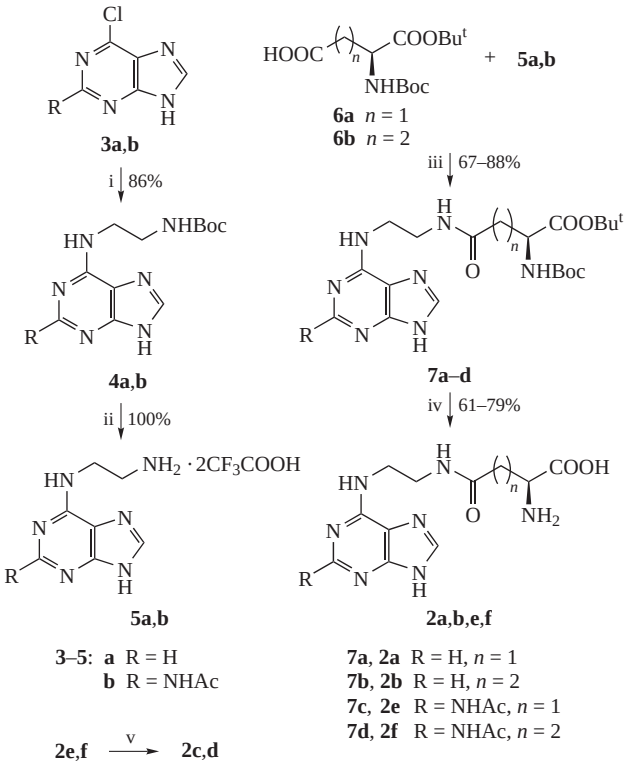
1a R = H
1b R = NH_2



2a R = H, *n* = 1
2b R = H, *n* = 2
2c R = NH_2, *n* = 1
2d R = NH_2, *n* = 2

Incorporation of amino acid residutes is a common approach to optimize the structure of biologically active compounds, which provides the targeted transport, reduced toxicity, and other improvements in pharmacological properties.[19–21] Note that 1,2-ethylenediamine is another important pharmacophore fragment of antituberculosis (anti-TB) agents.[14,22–25]

6-Chloropurine **3a** and 2-acetamido-6-chloropurine **3b** were used as starting materials in the synthesis of the target compounds **2a** and **2b** (Scheme 1). Nucleophilic substitution of 6-positioned chlorine atom by the action of *N*-Boc-1,2-ethylenediamine (by analogy to the published procedure[26]) followed by removal of



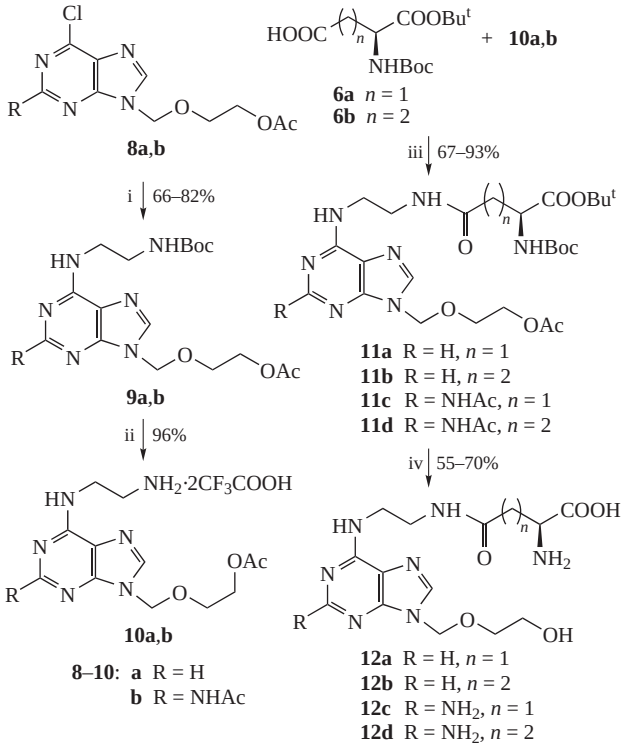
Scheme 1 *Reagents and conditions:* i, $H_2N(CH_2)_2NHBoc$, EtOH, Δ, 8 h; ii, TFA, CH_2Cl_2, room temperature, 4 h; iii, EtOCOCl, *N*-methylmorpholine, DIPEA, THF–H_2O, –10 °C to room temperature; iv, TFA, CH_2Cl_2, room temperature; v, 1 N NaOH, room temperature, 72 h.

N-Boc protection group in compounds **4a**,**b** on treatment with TFA afforded compounds **5a** and **5b** (as bis-hydrotrifluoroacetates) in high yields.†

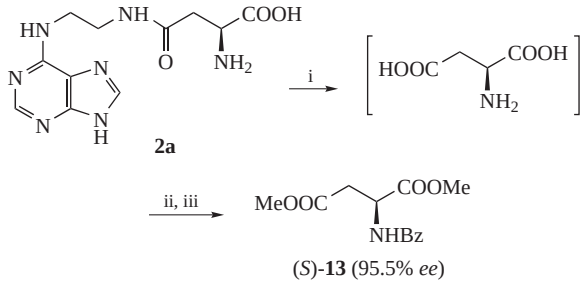
As the next step, amines **5a**,**b** were coupled with selectively protected derivatives, α-*tert*-butyl *N*-Boc-(*S*)-aspartate **6a** and *N*-Boc-(*S*)-glutamate **6b**[27] by the mixed anhydride method in the presence of ethyl chloroformate (see Scheme 1). Removal of protecting groups in the amino acid fragment of compounds **7a**–**d** by the treatment with TFA afforded the target purin-6-yl conjugates **2a**,**b** and 2-acetamidopurin-6-yl derivatives **2e**,**f**. Alkaline hydrolysis of compounds **2e**,**f** was effective to remove the acetyl group, thus giving 2-aminopurine conjugates **2c** and **2d** in 85 and 47% yields, respectively.

Note that conjugates **2a**–**d** are poorly soluble in water; therefore, to increase water solubility, we have suggested to obtain N^9-substituted conjugates containing (2-hydroxyethoxy)methyl substituent. For this, derivatives **8a** and **8b** chosen as starting materials (Scheme 2) were synthesized by analogy with the known methods.[28,29] Nucleophilic substitution of the chlorine atom in compounds **8a**,**b** by the action of *N*-Boc-1,2-ethylenediamine proceeded smoothly in EtOH in the presence of TEA followed by removal of the Boc-protection group with TFA, thus resulting in amines **10a**,**b** in the form of bis-hydrotrifluoroacetates. Coupling of amines **10a**,**b** with acids **6a** and **6b** by the mixed anhydride method in the presence of ethyl chloroformate led to amides **11a**–**d**. Removal of protecting groups in compounds **11a**–**d** was carried out by subsequent treatment with TFA (removal of the *N*-Boc and OBu^t groups) and 1 N NaOH (removal of the acetyl group) to afford the target compounds **12a**–**d** in moderate yields. Conjugates of purine **12a**,**b** and 2-aminopurine **12c**,**d** are well soluble in water, while they are scarcely soluble in most organic solvents.

We have verified that in the course of syntheses racemization of the chiral center in the amino acid residue did not occur, as exemplified by the behavior of compound **2a**. To establish it, we carried out acidic hydrolysis of compound **2a** on heating in 6 N HCl in the presence of 0.1% phenol (Scheme 3), similarly to the reported approach.[30] The resulting (*S*)-aspartic acid was treated sequentially with thionyl chloride in MeOH and benzoyl chloride in dichloromethane to afford known dimethyl *N*-benzoyl-(*S*)-aspartate **13**, which was analyzed by the chiral HPLC. Starting from the enantiopure (*S*)-aspartic acid and its racemate, the reference compounds (*S*)-**13** and (*RS*)-**13** were synthesized for HPLC analysis. The enantiomeric excess (*ee*) of compound **13** obtained from purine conjugate **2a** was 95.5% (according to chiral HPLC). In a control experiment, treatment of authentic (*S*)-aspartic acid with 6 N HCl followed by its conversion to compound **13**, gave the product with 94.8% *ee*.



Scheme 2 *Reagents and conditions:* i, $H_2N(CH_2)_2NHBoc$, TEA, EtOH, Δ, 6 h; ii, CF_3CO_2H, room temperature, 1.5 h; iii, EtOCOCl, *N*-methylmorpholine, DIPEA, THF, −10 °C to room temperature; iv, TFA, room temperature, 1.5 h, then 1 N NaOH, room temperature, 1–3 days.



Scheme 3 *Reagents and conditions:* i, 6 N HCl, PhOH, 110–115 °C, 24 h; ii, MeOH, $SOCl_2$; iii, BzCl, TEA, CH_2Cl_2, 0 °C to room temperature, 2 h.

The synthesized purine conjugates **2a**–**d** and **12a**–**d** were tested *in vitro* for antimycobacterial activity against a laboratory *M. tuberculosis* $H_{37}Rv$ strain.‡ Purine conjugate **2a** and 2-aminopurine one **2c** bearing fragment of (*S*)-aspartic acid exhibit a significant inhibitory activity against *M. tuberculosis* $H_{37}Rv$ strain (the minimum inhibitory concentration, MIC, was 0.7 and 1.5 μg ml^{-1}, respectively), whereas other tested compounds **2b**,**d** and **12a**–**d** demonstrated a low level of antimycobacterial activity (MIC = 12.5 μg ml^{-1}). Conjugates **2a** and **2c** were also tested against laboratory strains *M. avium* and *M. terrae*, as well as MDR-TB strain isolated from patients of the Ural region.‡ Compounds **2a** and **2c** inhibit the growth of all studied strains with MIC = 0.7 and 1.5 μg ml^{-1}, respectively.

In summary, we have developed a synthetic approach to novel purine and 2-aminopurine derivatives equipped with (*S*)-aspartic and (*S*)-glutamic acid residues with free α-functional groups, as well as their N^9-(2-hydroxyethoxy)methyl derivatives. The synthetic sequence is not accompanied by racemization of the amino acid chiral centre. Two of the synthesized compounds have demonstrated a significant antimycobacterial activity against laboratory and multidrug-resistant *M. tuberculosis* strains.

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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.2017.11.002.

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† For procedures and characteristics of the products, see Online Supplementary Materials.

‡ For details, see Online Supplementary Materials.

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