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Chemoenzymatic arabinosylation of 2-aminopurines bearing the chiral fragment of 7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazines

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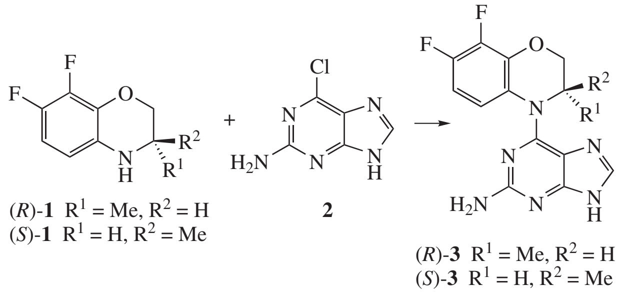
Chemoenzymatic transglycosylation of 2-aminopurine conjugates with enantiomerically pure 7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazines under the action of recombinant *E. coli* purine nucleoside phosphorylase afforded the corresponding arabinofuranosides, yields of the target compounds being dependent on both the structure and configuration of the fragment of heterocyclic amine attached at C-6 position of purine moiety.

Purine nucleosides with C-6-amino substituents comprise a potent family of adenosine receptor agonists,[1–4] antimetabolites[5,6] and other biologically active compounds.[7–9] Some of nucleosides with high therapeutic activity are related to the arabinose derivatives (for example, antitumor agents Fludarabine and Nelarabine). It has been recently reported that purine derivatives containing the fragments of heterocyclic amines at 6-position exhibit an *in vitro* cytotoxic activity.[10] However, there are only a few examples of preparation of 2-aminopurines conjugated with chiral heterocyclic amines.[11–13] To the best of our knowledge, purine nucleosides bearing fragments of chiral heterocyclic amines at C-6 position are lacking. Therefore, the development of synthetic approaches to novel purine conjugates with chiral amines and their nucleosides is an urgent task of medicinal chemistry.

The purpose of our research was to elucidate an opportunity for chemoenzymatic synthesis of arabinofuranosides based on conjugates of 2-aminopurine with enantiomers of 7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine [(*R*)-**1** and (*S*)-**1**]. Benzoxazine derivatives are of special interest as they demonstrate a wide spectrum of biological activity.[14] 2-Aminopurine conjugates, in which the difluorobenzoxazine moiety is directly attached at C-6 position (compounds **3**) or *via* 6-aminohexanoyl linker (compounds **4**), were chosen as the objects for chemoenzymatic arabinosylation. It has been suggested that the use of such structures will enable to establish the main factors determining peculiarities of enzymatic arabinosylation. Since the glycosylation process is highly stereospecific, compounds **3** and **4** were used in enantiomerically pure forms.

Majority of 6-amino derivatives of 2-aminopurine with free NH group at the 9-position were obtained from 2-amino-6-chloropurine **2** or its analogues and aliphatic or aromatic amines.[10,15–21] To synthesize enantiomers of compounds **3**, we coupled compound **2** with amines **1** in trifluoroethanol (TFE) in the presence of TFA (Scheme 1) similar to the described method.[22,23] Yields of enantiopure (*ee* > 99.5% according to chiral HPLC) *N*-(2-aminopurin-6-yl)benzoxazines (*R*)-**3** and (*S*)-**3** were 26%.[†] The reaction was not accompanied by racemization: enantiomeric purity of the products (*R*)-**3** and (*S*)-**3** was the same as that of the starting amines (*R*)-**1** and (*S*)-**1**.



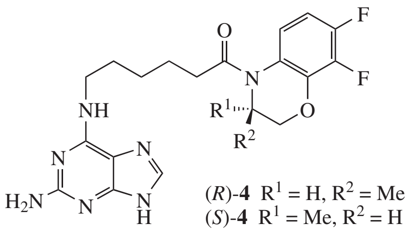
(*R*)-**1** R^1 = Me, R^2 = H
(*S*)-**1** R^1 = H, R^2 = Me

2

(*R*)-**3** R^1 = Me, R^2 = H
(*S*)-**3** R^1 = H, R^2 = Me

Scheme 1 *Reagents and conditions*: **2** (0.5 equiv.), TFA (2.5 equiv.), TFE, Δ, 17 h.

Enantiopure conjugates of 2-aminopurine (*R*)-**4** and (*S*)-**4** linked with the chiral fragment of (*R*)- and (*S*)-7,8-difluoro-3-methyl-



(*R*)-**4** R^1 = H, R^2 = Me
(*S*)-**4** R^1 = Me, R^2 = H

[†] *(3*R*)-4-(2-Aminopurin-6-yl)-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine* (*R*)-**3**. 2-Amino-6-chloropurine **2** (0.21 g, 1.24 mmol) and TFA (0.47 ml, 6.20 mmol) were added to a solution of amine (*R*)-**1** (0.46 g, 2.48 mmol) in CF_3CH_2OH (8 ml). The reaction mixture was refluxed for 17 h, and then evaporated to dryness. The residue was treated with MeOH (10 ml), filtered through a bed of basic alumina and eluted with MeOH (10×10 ml). The methanolic solution was evaporated to dryness and the residue was purified by flash column chromatography (SiO_2, gradient 0 → 10% MeOH in $CHCl_3$). Yield 0.10 g (26%), amorphous solid, $[\alpha]_D^{20}$ +17.6 (*c* 0.8, MeOH), *ee* >99.5%, HPLC (Kromasil Cellucoat, MeCN–H_2O, 25:75): τ 14.8 min. HRMS (ESI), *m*/*z*: 319.1112 $[M+H]^+$, (calc. for $C_{14}H_{13}F_2N_6O$, *m*/*z*: 319.1113).

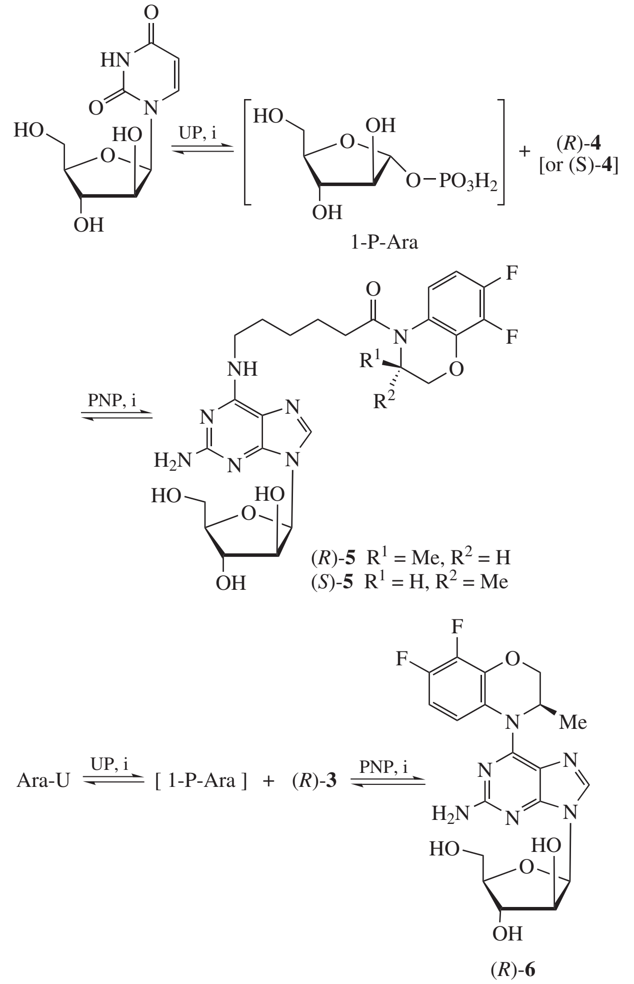
*(3*S*)-4-(2-Aminopurin-6-yl)-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine* (*S*)-**3**. Yield 0.10 g (26%), amorphous solid, $[\alpha]_D^{20}$ –17.7 (*c* 1.0, MeOH), *ee* >99.5%, HPLC (Kromasil Cellucoat, MeCN–H_2O, 25:75): τ 16.3 min. HRMS (ESI), *m*/*z*: 319.1114 $[M+H]^+$ (calc. for $C_{14}H_{13}F_2N_6O$, *m*/*z*: 319.1113).

3,4-dihydro-2*H*-[1,4]benzoxazines, respectively, *via* the 6-aminohexanoyl residue were previously obtained by our research team.[24]

Conversion of compounds **3** and **4** to the corresponding 9-β-D-arabinofuranosides **5** and **6** was performed in the presence of recombinant *E. coli* purine nucleoside phosphorylase (PNP) and α-D-arabinofuranosyl-1-phosphate (1-P-Ara) as a donor of sugar moiety generated *in situ* from 1-β-D-arabinofuranosyluracil (Ara-U) by action of recombinant uridine phosphorylase (UP) *E. coli* (Scheme 2). We have already reported the highly efficient synthesis of nucleosides using the nucleoside phosphorylases; these reactions usually proceed in buffered solutions under mild conditions (55 °C, pH 7.0).[25,26] Furthermore, it is important to note that only the β-anomers of nucleosides are obtained through this enzymatic reaction.

It was found that the efficiency of transglycosylation depended on the structure and stereo configuration of a substituent in the C-6 position of purine. Thus, arabinosylation of compounds (*R*)-**4** and (*S*)-**4** (Scheme 2), in which a bulky heterocyclic moiety is separated from purine entity by a spacer, proceeded rapidly and was distinguished by high conversion (89–91% in 6 h) (Figure 1). Yields of isolated arabinosides (*R*)-**5** and (*S*)-**5** were 65 and 46%, respectively (chemical purity was more than 96% according to reversed-phase HPLC).[‡] Configuration of the chiral center in the residue of heterocyclic amine in compound **4** did not significantly affect the substrate-specific properties of PNP.

Note that glycosylation of (*R*)-enantiomer of compound **3** with a bulky substituent attached directly at the 6-position proceeded much slower. Conversion of nucleobase (*R*)-**3** to arabinoside (*R*)-**6** was only 4.19% after 24 h (Figure 1). However, when reaction was carried out for 15 days, the yield of nucleoside (*R*)-**6** reached 43%.[§]



Scheme 2 *Reagents and conditions*: i, KH_2PO_4, H_2O–DMSO (96:4), 55 °C.

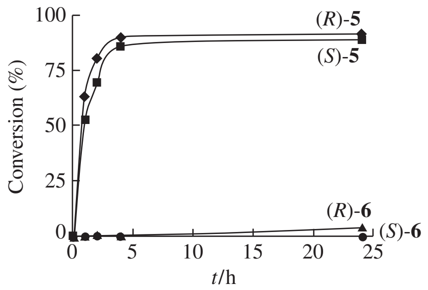


Figure 1 The time-dependence of conversion of nucleobases **3** and **4** to arabinosides **6** and **5**, respectively [nucleobase (2 μM), Ara-U (3 μM), UP (5 IU), PNP (5 IU), pH 7.0, 55 °C].

At the same time, (*S*)-enantiomer of nucleobase **3** did not undergo arabinosylation at all; none of the product was detected after 9 days (LC-MS data). So, this fact demonstrates once again a significant dependence of the result of enzymatic reaction on a substrate stereo configuration, particularly in the case when the chiral center of the nucleobase derivative is located close to the reaction site.

Thus, we for the first time carried out the chemoenzymatic transglycosylation of 2-aminopurines conjugated with enantiopure 7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazines and their derivatives under the action of recombinant *E. coli* purine nucleoside phosphorylase. The outcome of transglycosylation depended substantially on both the structure of a modified nucleobase and its configuration. For successful arabinosylation it is preferable to use 2-aminopurine derivatives in which a bulky substituent is located far from the reactive purine system.

[‡] *(3*R*)-4-{6-[2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl]aminohexanoyl}-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine* (*R*)-**5**. UP (145 units) and PNP (145 units) were added to a solution of compound (*R*)-**4** (20 mg, 46 μmol) and Ara-U (21 mg, 87 μmol, 1.5 equiv.) in 5 mM KH_2PO_4 buffer solution (pH 7.0, 23 ml). A mixture was gently stirred at 55 °C for 7 days. Then the warm reaction mixture was extracted by $CHCl_3$ (3×30 ml). The extract was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (SiO_2, gradient 0 → 7% EtOH in $CHCl_3$). Yield 17 mg (65%), amorphous solid, $[\alpha]_D^{20}$ +6 (*c* 0.2, DMF). HPLC (Ascentis® Express C18, 0.1% aqueous TFA → 0.1% aqueous TFA–70% aqueous MeCN, 1:1): τ 16.2 min (96.6%) [(*R*)-**5**], τ 17.1 min (3.4%) [(*R*)-**4**]. HRMS (ESI), *m/z*: 564.2297 $[M+H]^+$ (calc. for $C_{25}H_{32}O_5N_7F_2$, *m/z*: 564.2382).

*(3*S*)-4-{6-[2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl]aminohexanoyl}-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]benzoxazine* (*S*)-**5**. UP (115 units) and PNP (145 units) were added to a solution of compound (*S*)-**4** (25 mg, 58 μmol) and Ara-U (21 mg, 87 μmol, 1.5 equiv.) in 5 mM KH_2PO_4 buffer solution (pH 7.0, 23 ml). A mixture was gently stirred at 55 °C for 7 days. Then the warm reaction mixture was extracted by $CHCl_3$ (3×30 ml). The extract was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (SiO_2, gradient 0 → 7 % EtOH in $CHCl_3$). Yield 15 mg (46%), amorphous solid, $[\alpha]_D^{20}$ +50 (*c* 0.2, DMF). HPLC (Ascentis® Express C18, 0.1% aqueous TFA → 0.1% aqueous TFA–70% aqueous MeCN, 1:1): τ 15.6 min (96.9%) [(*S*)-**5**]. HRMS (ESI), *m/z*: 564.2300 $[M+H]^+$ (calc. for $C_{25}H_{32}O_5N_7F_2$, *m/z*: 564.2382).

[§] *(3*R*)-4-[2-Amino-9-(β-D-arabinofuranosyl)purin-6-yl]-7,8-difluoro-3-methyl-3,4-dihydro-2*H*-[1,4]-benzoxazine* (*R*)-**6**: UP (180 units) and PNP (290 units) were added to a solution of compound (*R*)-**3** (25 mg, 80 μmol) and Ara-U (29 mg, 120 μmol) in 5 mM KH_2PO_4 buffer solution (pH 7.0, 40 ml). The reaction mixture was gently stirred at 55 °C for 15 days, and then concentrated under reduced pressure to a volume of 2 ml. Resulting solution was subjected to flash column chromatography on reversed-phase octadecyl–Si, 100 polyol (0.03 mm, 30×120 mm) using a linear gradient EtOH–H_2O 10:90 → EtOH–H_2O 40:60 as an eluent. Yield 15 mg (43%), amorphous solid, $[\alpha]_D^{20}$ +50 (*c* 0.3, DMF). HRMS (ESI), *m/z*: 451.1474 $[M+H]^+$ (calc. for $C_{19}H_{20}O_5N_6F_2$, *m/z*: 451.1546).

For more details, see Online Supplementary Materials.

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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.2016.01.003.

References

1 T. D. Ashton, S. P. Baker, S. A. Hutchinson and P. J. Scammells, *Bioorg. Med. Chem.*, 2008, **16**, 1861.

2 S. M. Devine, A. Gregg, H. Figler, K. McIntosh, V. Urmaliya, J. Linden, C. W. Pouton, P. J. White, S. E. Bottle and P. J. Scammells, *Bioorg. Med. Chem.*, 2010, **18**, 3078.

3 R. Narlawar, J. R. Lane, M. Doddareddy, J. Lin, J. Brussee and A. P. Ijzerman, *J. Med. Chem.*, 2010, **53**, 3028.

4 X. Hou, M. S. Majik, K. Kim, Y. Pyee, Y. Lee, V. Alexander, H.-J. Chung, H. W. Lee, G. Chandra, J. H. Lee, S. Park, W. J. Choi, H. O. Kim, K. Phan, Z.-G. Gao, K. A. Jacobson, S. Choi, S. K. Lee and L. S. Jeong, *J. Med. Chem.*, 2012, **55**, 342.

5 S. R. Ross, D. McTavish and D. Faulds, *Drugs*, 1993, **45**, 737.

6 P. L. Bonate, L. Arthaud, W. R. Cantrell, Jr., K. Stephenson, J. A. Secrist, III and S. Weitman, *Nat. Rev. Drug Discov.*, 2006, **5**, 855.

7 J. C. Bressi, J. Choe, M. T. Hough, F. S. Buckner, W. C. Van Voorhis, C. L. M. J. Verlinde, W. G. J. Hol and M. H. Gelb, *J. Med. Chem.*, 2000, **43**, 4135.

8 M. C. Long and W. B. Parker, *Biochem. Pharmacol.*, 2006, **71**, 1671.

9 M. Tuncbilek, E. B. Guven, T. Onder and R. C. Atalay, *J. Med. Chem.*, 2012, **55**, 3058.

10 Z. Demir, E. B. Guven, S. Ozbey, C. Kazak, R. C. Atalay and M. Tuncbilek, *Eur. J. Med. Chem.*, 2015, **89**, 701.

11 F. B. Pallier and J. L. Haesslein, *French Patent, 2851248, C07D*, 2004 (*Chem. Abstr.*, 2004, **141**, 206968).

12 G. Chen, T. D. Cushing, B. Fisher, X. He, K. Li, Z. Li, L. R. McGee, V. Pattaropong, P. Faulder, J. L. Seganish and Y. Shin, *PCT Patent, WO 2009158011, C07D*, 2009 (*Chem. Abstr.*, 2009, **152**, 119631).

13 C. Guibourdenche, S. Hintermann, K. Hurth, S. Jacquier, C. Kalis, H. Moebitz and N. Soldermann, *PCT Patent, WO 2014128612, C07D*, 2014 (*Chem. Abstr.*, 2014, **161**, 433034).

14 J. Ilaš, P. Š. Anderluh, M. S. Dolenc and D. Kikelj, *Tetrahedron*, 2005, **61**, 7325.

15 P. Imbach, H.-G. Capraro, P. Furet, H. Mett, T. Meyer and J. Zimmermann, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 91.

16 A. Holý, I. Votruba, E. Tloušťová and M. Masojídková, *Collect. Czech. Chem. Commun.*, 2001, **66**, 1545.

17 L.-K. Huang, Y.-C. Cherng, Y.-R. Cheng, J.-P. Jang, Y.-L. Chao and Y.-J. Cherng, *Tetrahedron*, 2007, **63**, 5323.

18 A. Thalassitis, D. J. Hadjipavlou-Litina, K. E. Litinas and P. Miltiadou, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6433.

19 Y. L. Hu, X. Liu and M. Lu, *J. Mex. Chem. Soc.*, 2010, **54**, 74.

20 S. M. Devine, A. Gregg, H. Figler, K. McIntosh, V. Urmaliya, J. Linden, C. W. Pouton, P. J. White, S. E. Bottle and P. J. Scammells, *Bioorg. Med. Chem.*, 2010, **18**, 3078.

21 A. Yu. Vigorov, V. P. Krasnov, D. A. Gruzdev, A. A. Men'shikova, A. M. Demin, G. L. Levit and V. N. Charushin, *Mendeleev Commun.*, 2014, **24**, 35.

22 H. J. Whitfield, R. J. Griffin, I. R. Hardcastle, A. Henderson, J. Meneyrol, V. Mesguiche, K. L. Sayle and B. T. Golding, *Chem. Commun.*, 2003, 2802.

23 B. Carbain, C. R. Coxon, H. Lebraud, K. J. Elliott, C. J. Matheson, E. Meschini, A. R. Roberts, D. M. Turner, C. Wong, C. Cano, R. J. Griffin, I. R. Hardcastle and B. T. Golding, *Chem. Eur. J.*, 2014, **20**, 2311.

24 V. P. Krasnov, D. A. Gruzdev, E. N. Chulakov, A. Yu. Vigorov, V. V. Musiyak, T. V. Matveeva, A. A. Tumashov, G. L. Levit and V. N. Charushin, *Mendeleev Commun.*, 2015, **25**, 412.

25 I. D. Konstantinova, K. V. Antonov, I. V. Fateev, A. I. Miroshnikov, V. A. Stepchenko, A. V. Baranovsky and I. A. Mikhailopulo, *Synthesis*, 2011, 1555.

26 I. V. Fateev, K. V. Antonov, I. D. Konstantinova, T. I. Muravyova, F. Seela, R. S. Esipov, A. I. Miroshnikov and I. A. Mikhailopulo, *Beilstein J. Org. Chem.*, 2014, **10**, 1657.

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