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Synthesis of analogs of forodesine HCl, a human purine nucleoside phosphorylase inhibitor—Part II

Vivekanand P. Kamath *, Jie Xue, Jesus J. Juarez-Brambila

BioCryst Pharmaceuticals Inc., 2190 Parkway Lake Drive, Birmingham, AL 35244, United States

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ABSTRACT

Forodesine HCl is being investigated as a potential therapeutic target for the control of T-cell proliferation. During the filing process for a New Drug Application (NDA) it became evident that there was a need to synthesize some stereo-isomers of forodesine HCl. Herein we present the synthesis of these three novel compounds (**2**–**4**).

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Purine nucleoside phosphorylase (PNP) is a purine metabolizing enzyme that catalyzes the reversible phosphorylysis of purine nucleosides such as 2′-deoxyguanosine (dGuo) to the respective base and deoxyribose-α-1-phosphate.[1–4] The importance of PNP to the integrity of the immune system became apparent when children lacking the PNP enzyme developed severe T-cell immunodeficiency.[5]

The unique sensitivity of human T-cells to PNP deficiency is attributed to a relatively high level of kinase and a low level of nucleotidase activity compared to other cell types. This unique enzyme ratio is especially characteristic of immature T-cells. Based upon these observations, several novel classes of PNP inhibitors have been designed for the treatment of T-cell mediated diseases such as cutaneous T-cell lymphoma (CTCL) and acute lymphoblastic leukemia (ALL) and other autoimmune disorders such as psoriasis, Crohn's disease and rheumatoid arthritis. One such compound investigated by BioCryst Pharmaceuticals Inc. is forodesine HCl, a PNP inhibitor which is currently in advanced stages of clinical trials (see Fig. 1).[6,7]

During the filing process for a New Drug Application (NDA) it became evident that there was a need to synthesize some stereo-isomers of forodesine HCl. In our earlier communication we described the synthetic route for making forodesine HCl.[8] The process involved using both basic and acidic conditions. Under these conditions the risk of epimerization at the stereogenic centres would be substantial. Hence it was necessary to synthesize the stereo-isomers at C-2′, C-3′ and C-4′ positions. The synthesis of the L-enantiomer of fordesine HCl has been described in the literature by Schramm and co-workers.[9] The structures of the stereo-isomers are shown (see Fig. 2).

As part of the NDA filing process there is a need to establish a validated commercial route for large scale manufacturing. FDA regulations require identification, isolation, characterization and validation of analytical methods for all possible impurities observed in the process. Comparison of the HPLC chromatograms of the parent compound with those of the stereo-isomers and other impurities supports establishment of the purity of the final product.

In Part I of our Letter we described the synthesis of some impurities which were identified as by-products of the commercial process.[10] In this Letter we describe the synthesis of the stereo-isomers, compounds **2**–**4**.

Scheme 1, describes the synthesis of compound **2**, starting from the previously reported compound **5**.[11] Treatment of **5** with *N*-chlorosuccinimide for 1 h followed by reaction with DBU gave the imine, **6** as a syrup in 65% yield.[14] The unsaturated compound was reduced using a macroporous polystyrene-bound equivalent

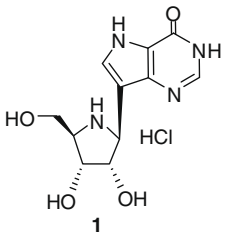

Figure 1. Structure of forodesine HCl.

* Corresponding author. Tel.: +1 205 444 4600; fax: +1 205 444 4640.
E-mail address: vkamath@biocryst.com (V.P. Kamath).

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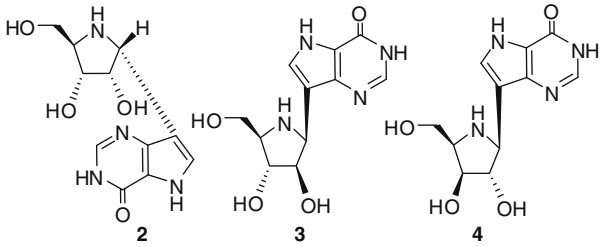
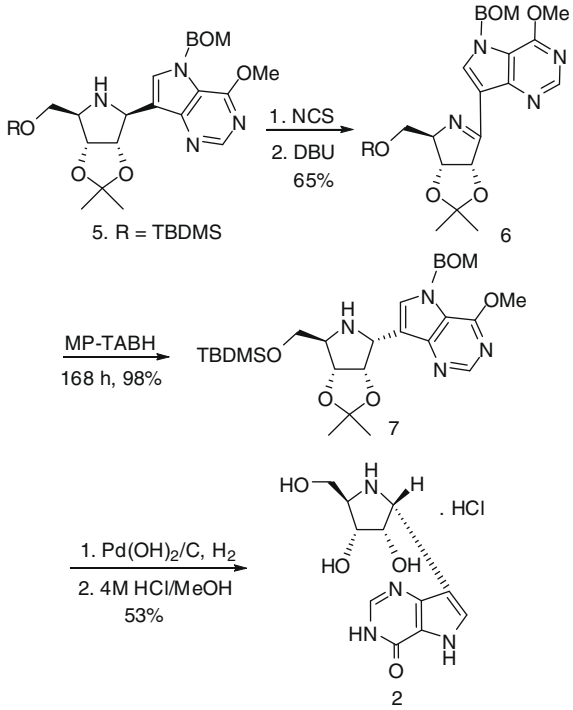
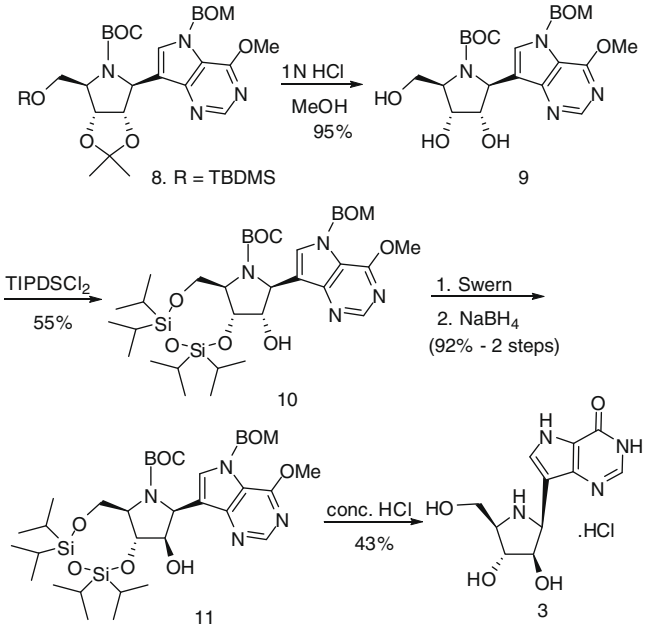


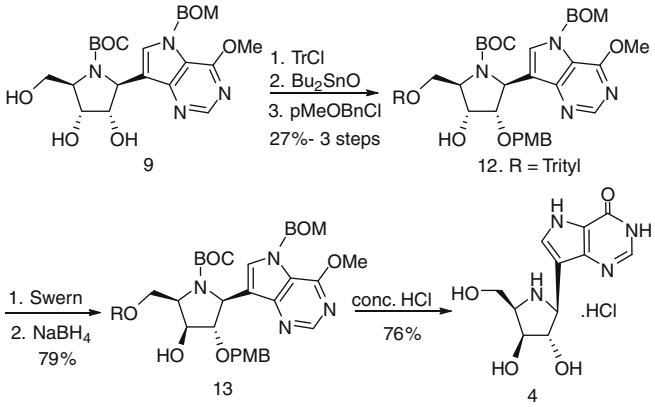
Figure 2. Structures of stereo-isomers of forodesine HCl.



Scheme 1.



Scheme 2.



Scheme 3.

tetraalkylammonium triacetoxyborohydride (MP-TABH) reagent [purchased from Biotage, 1.8–2.4 mmol/g] with stirring for one week at rt, to furnish compound **7** in 98% yield. Hydrogenation of the intermediate using $Pd(OH)_2/C$ in MeOH pre-saturated with ammonia followed by acid hydrolysis using 4 M HCl/MeOH under reflux conditions for 2 h, furnished a solid. The solid was recrystallized in water/EtOH mixture to give the desired compound **2**, in 53% yield.[14] The structure of compound **2** was further supported by X-ray crystallography data.

On similar lines (Scheme 2) compound **8**[11] was treated with 1 N HCl in MeOH and heated to 40 °C for 24 h to furnish **9** in 95% yield. The intermediate was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) that would allow the hydroxyl groups, O-6′ and O-4′ to be bridged together to furnish **10** in 55% yield. Swern oxidation, followed by reduction of the ketone using NaBH4 furnished **11** in 92% yield (two steps).[12] Finally, acid hydrolysis was carried out using concd HCl under reflux conditions for 1 h followed by workup of the reaction mixture to furnish a solid. The solid was recrystallized in water/EtOH mixture to give compound **3** as a white solid in 43% yield.[15]

Scheme 3 describes the synthesis of compound **4**. Compound **9** was treated with trityl chloride to protect the primary hydroxyl at O-6′ position. The intermediate was subjected to standard stannylation chemistry followed by addition of *p*-MeOBnCl. The reaction mixture was purified by column chromatography to isolate compound **12** (27%—three steps).[13] Swern oxidation, followed by reduction of the ketone using NaBH4 gave **13** in 79% yield. Finally, acid hydrolysis was carried out using concd HCl under reflux conditions. Workup of the reaction mixture gave a solid residue. The solid was recrystallized in water/EtOH mixture to give compound **4** in 76% yield as a white solid.[16]

The final compounds were tested for PNP activity. The IC_{50} values were observed to be in the range of 100–300 nM and although the compounds did show some activity it was not as potent as the parent compound, forodesine HCl.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.018.

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14. To a solution of **5** (45.4 g, 82 mmol) in dry mTBE (3.0 L) at 0 °C was added NCS (16.4 g, 123 mmol) and stirred for 1 h. The solids were filtered and then the filtrate was treated with DBU (18.4 mL, 123 mmol). And stirred at rt for 3 h. Upon completion of the reaction the unsaturated product was purified by chromatography to furnish **6** (23 g, 65%). MP-triacetoxyborohydride (100 g, 240 mmol) was added to the unsaturated product dissolved in THF (300 mL) and stirred for 1 week at rt. The resin was filtered and concentrated to **7** (33 g, 98%). The syrup was suspended in MeOH (700 mL), pre-saturated with ammonia followed by addition of $Pd(OH)_2/C$ (2.5 g) and hydrogenated at 150 psig. The catalyst was filtered over Celite pad and washed with MeOH. Evaporation of the filtrate followed by acid hydrolysis using 4 M HCl (160 mL) and methanol (250 mL) under reflux conditions for 2 h furnished **2** (20.3 g, 53%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.76 (s, 1H, H-7), 9.59 (br, 1H, H-1), 8.43 (s, 1H, H-2), 7.78 (d, 1H, *J* = 3.0 Hz, H-8), 6.98 (br, 4H), 4.96 (t, 1H, *J* = 3.0 Hz, H-2′), 4.23 (dd, 1H, *J* = 9.0, 3.0 Hz, H-3′), 4.08 (t, 1H, *J* = 3.0 Hz, H-4′), 3.81 (dd, 1H, *J* = 12.0, 3.0 Hz, H-6′), 3.72 (dd, 1H, *J* = 12.0, 6.0 Hz, H-6′), 3.45 (br, 1H, H-5′); ^{13}C NMR: (DMSO-d_6, 75.5 MHz) δ 152.83, 143.15, 129.13, 117.16, 106.60, 71.76, 71.67, 62.48, 58.27, 55.30. HRMS: calcd for $C_{11}H_{15}N_4O_4$ (M+H$^+$): 267.1082; found: 267.1080.
15. To a solution of **8** (65.6 g, 100 mmol) in MeOH (260 mL) was added 1 N HCl (145 mL) and the reaction was heated to 40 °C for a period of 24 h. Upon completion of the reaction as indicated by TLC the reaction mixture was worked up to furnish **9** (47.5 g, 95%). A small portion of the sample **9** (10 g, 20 mmol) was stirred in dry DMF (50 mL) in presence of imidazole (5.45 g, 80 mmol) and $TIPDSCl_2$ reagent (7.04 mL, 22 mmol). The reaction mixture was stirred at rt for 20 h. Workup of the reaction followed by column chromatography gave **10** (8.3 g, 55%). To a solution of DMSO (2.8 mL, 39.2 mmol) in DCM (72 mL) at −70 °C was added TFAA (2.8 mL, 20.2 mmol) and stirred for 10 min. To this was added compound **10** (2.6 g, 3.5 mmol) pre-dissolved in DCM (60 mL) followed by addition of triethylamine (4.2 mL, 56.7 mmol). The reaction was stirred at that temperature for 15 min. and then allowed to warm to rt. Usual workup of the reaction furnished the crude ketone. The crude ketone was suspended in MeOH (100 mL) followed by addition of $NaBH_4$ (1.4 g, 35 mmol) and stirred at rt for 30 min. Evaporation of the solvent followed by column chromatography furnished **11** (2.4 g, 92%—two steps). Compound **11** (2.4 g, 3.22 mmol) was taken in concd HCl (5 mL) and refluxed for 1 h. The solvent was evaporated to give a solid which was then recrystallized from water/EtOH to give compound **3** (0.42 g, 43%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 7.82 (s, 1H), 7.60 (s, 1H), 4.98 (s, 1H), 4.22 (s, 1H), 4.04 (s, 1H), 3.83 (dd, *J* 12.0, 5.0 Hz, 1H), 3.72 (dd, *J* 12.0, 12.0 Hz, 1H), 3.52 (br, 1H); ^{13}C NMR: (D_2O, 75.5 MHz) δ 155.1, 143.4, 142.5, 130.2, 117.4, 105.3, 76.3, 75.9, 67.1, 59.6, 57.5. HRMS: calcd for $C_{11}H_{15}N_4O_4$ (M+H$^+$): 267.1082; found: 267.1085.
16. To a solution of compound **9** (5.0 g, 10 mmol) in DCM (30 mL) was added *N,N* di-isopropylethylamine (6.89 mL, 39.6 mmol), catalytic amount of DMAP (0.2 g) and trityl chloride (3.36 g, 12.0 mmol). The reaction mixture was stirred at rt for 16 h. Upon completion of the reaction (as indicated by TLC) the reaction mixture was evaporated to furnish the crude intermediate. The crude was taken in toluene (100 mL) followed by addition of dibutyl tin oxide (2.2 g, 8.81 mmol) and refluxed using a Dean–Stark apparatus for 1 h. This was followed by addition of tetrabutylammonium bromide (2.82 g, 8.81 mmol) and *p*-MeO benzyl chloride (21.7 mL, 159.6 mmol) and the reaction refluxed further for 4 h. Workup of the reaction followed by column chromatography gave the desired compound **12** (3.1 g, 27%—three steps). To a solution of DMSO (2.5 mL, 35 mmol) in DCM (72 mL) at −70 °C was added TFAA (2.5 mL, 18 mmol) and stirred for 10 min. To this was added compound **12** (3.1 g, 2.68 mmol) pre-dissolved in DCM (60 mL) followed by addition of triethylamine (3.7 mL, 50 mmol). The reaction was stirred at that temperature for 15 min. and then allowed to warm to rt. Evaporation of the solvent furnished the crude ketone. The crude ketone was suspended in MeOH (100 mL) followed by addition of $NaBH_4$ (1.2 g, 30 mmol) and stirred at rt for 30 min. Evaporation of the solvent followed by column chromatography furnished **13** (2.45 g, 79%—two steps). Compound **13** (2.45 g, 3.22 mmol) was taken in concd HCl (5 mL) and refluxed for 1 h. The solvent was evaporated to give a solid which was then recrystallized from water/EtOH to give compound **4** (0.62 g, 76%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 7.97 (s, 1H), 7.64 (s, 1H), 4.74 (d, *J* 5.0 Hz, 1H), 4.62 (dd, *J* 5.0, 3.0 Hz, 1H), 4.34 (dd, *J* 4.2, 3.0 Hz, 1H), 3.82–3.96 (m, 3H); ^{13}C NMR: (D_2O, 75.5 MHz) δ 155.0, 143.1, 142.7, 129.4, 117.7, 108.2, 79.4, 74.6, 62.8, 58.9, 57.1. HRMS: calcd for $C_{11}H_{15}N_4O_4$ (M+H$^+$): 267.1082; found: 267.1081.