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

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ABSTRACT

Forodesine HCl is being investigated as a potential therapeutic target for the control of T-cell proliferation. During our ongoing process development work on forodesine HCl several novel compounds were identified as possible impurities in the process. Herein we present the synthesis of three novel compounds (**2**–**4**).

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Purine nucleoside phosphorylase (PNP) enzyme has been widely investigated due to its importance in the purine salvage pathway. PNP catalyzes the reversible phosphorolysis of ribonucleosides and 2′-deoxyribonucleosides of guanine and hypoxanthine as well as many other related nucleosides analogs.[1]

$$\beta\text{-Nucleoside} + PO_4 \leftrightarrow \text{Base} + \alpha\text{-ribose 1-phosphate}$$

The relationship between PNP deficiency and certain types of immunological diseases such as T-cell cancer, rheumatoid arthritis, psoriasis and other autoimmune diseases has made this an attractive therapeutic target. Biochemical evidence suggests that the mammalian PNP enzyme is a trimer made up of three identical units of 32 kDa. The protein sequence analysis has shown that the human PNP is about 25% identical to members of the mammalian purine nucleoside phosphorylase (PNP) family of trimeric enzymes.[2,3]

In the past decade, various groups have identified several potent inhibitors of PNP. Schramm and co-workers have investigated the kinetic isotope effects on the PNP enzyme to predict the transition-state structure.[4,5] This has led to the development of several novel classes of inhibitors that are extremely potent against PNP. These compounds are the aza-C-nucleosides, and one of them, forodesine HCl is currently in clinical trials.

The convergent synthesis of forodesine HCl was first reported by Tyler and co-workers.[6–9] In this process the lithiated deaza-hypoxanthine (**15**) was coupled to the imine (**8**)[7a] followed by purification of the intermediate using column chromatography. Subsequent deprotection steps of the coupled intermediate gave forodesine HCl (see Fig. 1).

The process described in the literature generated small quantities of material for toxicology studies.[6–9] Hence there was a need to optimize the process conditions and implement a feasible route for large scale manufacturing of fordesine HCl. During the course of this process development some unique compounds were isolated. The structures of these compounds were determined by NMR, prep HPLC and LC–MS. These compounds have been identified as by-products of the process and have to be documented in the new drug application (NDA). To understand the nature of these compounds they were synthesized, analyzed and tested for any activity against PNP enzyme. This paper describes the synthesis of these isolated compounds **2**–**4** (structures as shown in Fig. 2).

As outlined in Scheme 1, compound **6**[9] (obtained by quenching the lithiated deaza-hypoxantine (**15**) with water) was treated with *n*-BuLi (1.6 M solution in hexanes) in anhydrous THF at −35 °C,

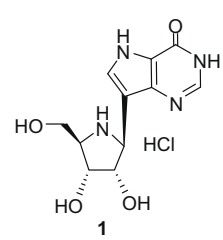

Figure 1. Structure of forodesine HCl.

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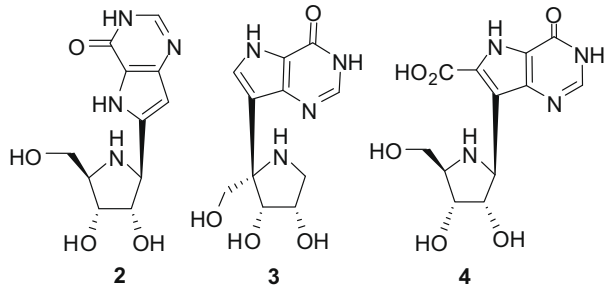
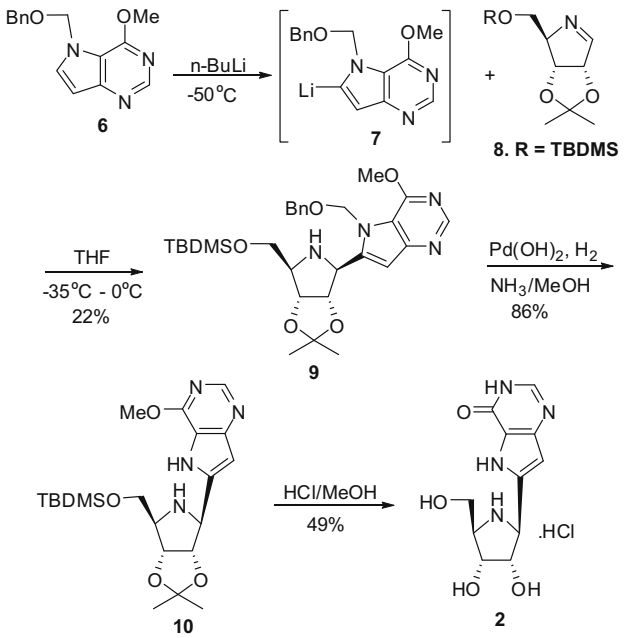


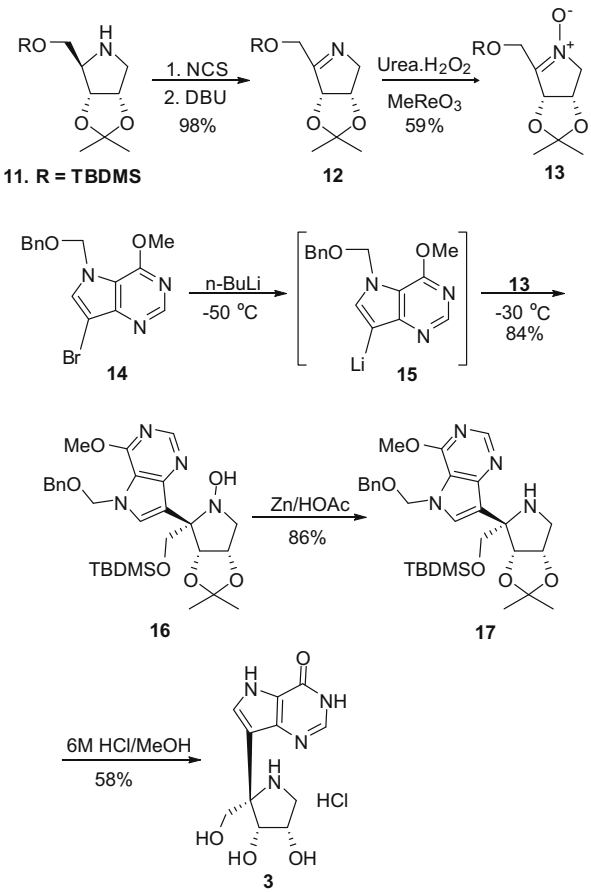
Figure 2. Structure of analogs of forodesine HCl.



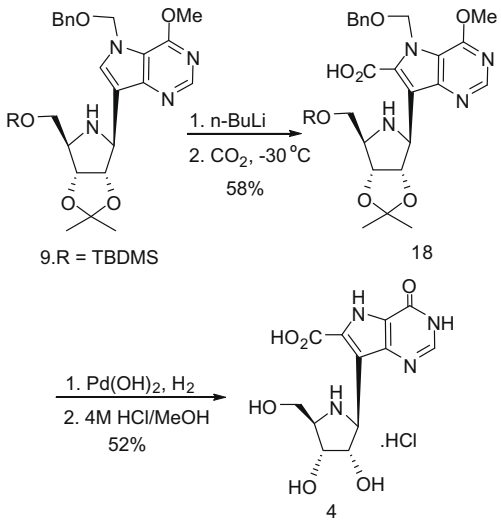
Scheme 1.

followed by addition of the imine, **8**[7a] (pre-dissolved in dry THF). The reaction was stirred for 10 min and then warmed up to 0°°C and stirred at this temperature for 1 h to furnish **9** in 22% yield. Hydrogenation of the intermediate using $Pd(OH)_2$ in methanol pre-saturated with ammonia gave 10 in 86% yield. Finally, acid hydrolysis of **10** in concd HCl/MeOH under reflux conditions for 48 h, furnished a white solid. The solid was recrystallized in water/ethanol mixture to give compound **2**, in 49% yield.[13]

On similar lines (Scheme 2) compound **11**[9] was treated with *N*-chlorosuccinimide (NCS) to give the chlorinated compound, which upon treatment with DBU at rt for 1 h gave the ketimine **12** in quantitative yields.[10] Treatment of the ketimine, **12** with urea hydrogen peroxide and methyltrioxorhenium in MeOH at rt for 1 h followed by purification by column chromatography gave the desired nitrone **13** in 59% yield.[11] As reported earlier in the literature, 9-bromo deazahypoxanthine was lithiated using *n*-BuLi (1.6 M solution in hexanes) in TBME at −30 °C followed by addition of the nitrone 13. The reaction was warmed to 0 °C and stirred for 1.5 h. The reaction mixture was worked up and purified by column chromatography to give **16** in 84% yield. Reduction of **16** using zinc dust in acetic acid furnished compound **17** in 86% yield.[12] Acid hydrolysis using 6 M HCl/MeOH under reflux conditions for 20 h furnished compound **3** as a white solid. The solid was recrystallized in water/ethanol mixture to give compound **3**, in 58% yield.[14]



Scheme 2.



Scheme 3.

Scheme 3 describes the synthesis of compound **4**. Compound **9** (obtained from coupling of the imine (**8**) with the lithiated deazahypoxanthine, **15**) was treated with *n*-BuLi (1.6 M solution in hexanes) in dry THF at −30 °C and stirred for 20 min. Dry ice was added slowly so that the internal temperature was maintained below −30 °C. The reaction mixture was stirred for 30 min at this temperature and then warmed to 0 °C and continued stirring for 1 h. Workup of the reaction followed by column chromatography

gave the desired product, **18** in 58% yield. Finally, hydrogenation and acid hydrolysis of the intermediate followed by crystallization gave **4** as a white solid. The solid was recrystallized in water/ethanol mixture to give compound **4**, in 52% yield.[15]

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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13. A cold solution of *n*-BuLi (1.6 M, 208 mL) was added slowly to a solution of **6** (81.6 g, 303 mmol) in dry THF at −35 °C. Upon completion of the reaction as indicated by TLC a solution of **8** (66.9 g, 233.1 mmol) in dry THF was added at −35 °C. After the last addition the reaction was warmed to 0 °C and stirred for 1 h. Evaporation of the solvent followed by column chromatography gave **9** (28.4 g, 22%). The syrup was suspended in MeOH (300 mL), pre-saturated with ammonia followed by addition of $Pd(OH)_2$ and hydrogenated at 150 psig. The catalyst was filtered over Celite pad and washed with copious amount of MeOH (100 mL). Evaporation of the solvent gave **10** (22.2 g, 86%). The solid was dissolved in 1:1 (v/v) ratio of MeOH/concd HCl (208 mL) and refluxed for 48 h. Evaporation of the solvent gave a solid residue. The solid was redissolved in water and decolorized with activated carbon. The mixture was filtered, washed with water and then concentrated to a solid. The solid was recrystallized with water/ethanol mixture to furnish **2** (6.1 g, 49%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 7.92 (s, 1H, H-2), 6.68 (s, 1H, H-9), 4.78 (d, J = 9 Hz, H-2′), 4.65 (m, 1H, H-3′), 4.34 (t, 1H, H-4′), 3.86 (dd, 1H, J = 6.0, 8.6 Hz, H-6′), 3.80 (m, 1H, H-5′); ^{13}C NMR (75.5 MHz, D_2O) δ 155.8, 145.0, 143.4, 134.3, 119.4, 104.1, 74.2, 71.3, 66.4, 59.1, 58.5. HRMS: Calcd for $C_{11}H_{15}N_4O_4$ ($M+H^+$): 267.1082; found: 267.1080.
14. To a solution of **11** (100 g, 348 mmol) in toluene (500 mL) was added *N*-chlorosuccinimide (60.4 g, 452 mmol). After stirring for 1 h the solids were filtered off and DBU (57 mL, 381 mmol) was added to the filtrate. The reaction mixture was stirred for 1 h and then purified by column chromatography to furnish **12** (100 g,) as a syrup. Urea hydrogen peroxide (98.2 g, 1044 mmol) and methyltrioxorhenium (1.73 g, 6.96 mmol) was added to a solution of **12** in MeOH (660 mL). The reaction mixture was stirred for 1 h, filtered and concentrated. The residue was purified by column chromatography to furnish the nitrone, **13** (62.4 g, 59%). 9-Bromo deazahypoxanthine, **14** (87.1 g, 250 mmol) was lithiated using *n*-BuLi (164 mL) in dry TBME (1.6 L) at −30 °C. The nitrone, **13** dissolved in TBME (300 mL) was added slowly to the reaction at −30 °C over 20 min. and then warmed to 0 °C and stirred for 1.5 h. Chromatography of the crude product gave **16** (95.8 g, 84%) as a syrup. Reduction of **16** using zinc dust (110.6 g, 1691 mmol) in acetic acid (400 mL) and stirring for 1 h at rt gave **17** (90 g, 86%) as a oil. Finally, acid hydrolysis using 6 M HCl/MeOH (300 mL) under reflux conditions for 20 h followed by workup (as described for compound **2**) and crystallization furnished **3** (25.2 g, 58%) as a white solid. ^{1}H NMR: (DMSO-d_6) δ 12.38 (d, 1H, J = 3.0 Hz, H-7), 12.18 (d, 1H, J = 3.0 Hz, H-1), 9.56 (br, 1H, H-1′), 9.16 (br, 1H, H-1′), 7.92 (d, 1H, J = 3.0 Hz, H-2), 7.64 (d, 1H, J = 3.0 Hz, H-8), 5.81 (br, 1H, OH), 5.47 (br, 1H, OH), 5.28 (br, 1H, OH), 4.66 (d, 1H, J = 6.0 Hz, H-4′), 4.18 (m, 1H, H-3′), 4.13 (d, 1H, J = 12.0 Hz, H-6′), 4.01 (d, 1H, J = 12.0 Hz, H-6′), 3.60 (m, 1H, H-2′), 3.09 (m, 1H, H-2′); ^{13}C NMR (75.5 MHz, D_2O) δ 155.8, 143.6, 142.8, 127.7, 118.4, 110.6, 76.2, 72.4, 70.2, 63.4, 48.6. HRMS: Calcd for $C_{11}H_{15}N_4O_4$ ($M+H^+$): 267.1082; found: 267.1085.
15. A cold solution of *n*-BuLi (2.5 M, 150.8 mL) was added slowly to a solution of **9** (95 g, 171.5 mmol) in dry THF (1.8 L) at −30 °C. Upon completion of the reaction as indicated by TLC, dry ice (37.7 g, 858 mmol) was added slowly to the reaction mixture. at −30 °C. The reaction was warmed to 0 °C and stirred for 1 h. Evaporation of the solvent followed by column chromatography gave **18** (60.0 g, 58%). The syrup was suspended in MeOH (700 mL), pre-saturated with ammonia followed by addition of $Pd(OH)_2$ and hydrogenated at 150 psig. The catalyst was filtered over a Celite pad followed by evaporation of the filtrate to furnish a solid residue. Acid hydrolysis using 4 M HCl/MeOH (300 mL) under reflux conditions for 2 h furnished **4** (16.8 g, 52%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6 + 3 drops DCl) δ 8.27 (s, 1H, H-2), 5.22 (d, 1H, J = 7.77 Hz, H-2′), 4.62 (dd, 1H, J = 5.04, 7.74 Hz, H-3′), 4.28 (t, 1H, H-4′), 3.80 (m, 2H, H-6′), 3.59 (m, 1H, H-5′); ^{13}C NMR (75.5 MHz, D_2O) δ 161.8, 153.2, 143.3, 140.8, 128.1, 119.5, 112.8, 73.1, 69.8, 64.9, 58.1, 56.2. HRMS: Calcd for $C_{12}H_{14}N_4O_6$ ($M+H^+$): 310.2710; found: 310.2714.