Synthesis of 2-Amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine from 2-Amino-4-hydroxyl-6-methylpyrimidine

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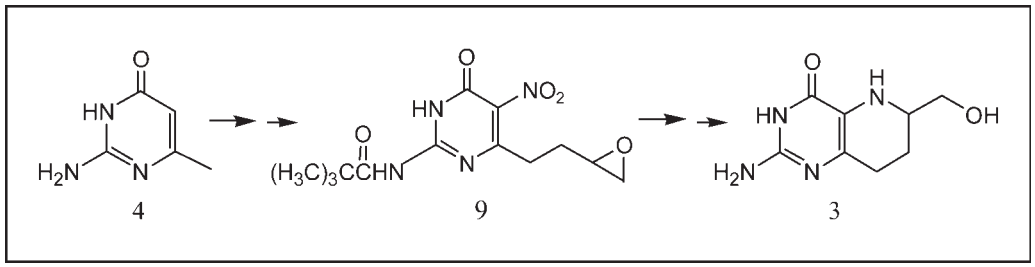
Received March 3, 2009
DOI 10.1002/jhet.197
Published online 4 November 2009 in Wiley InterScience (www.interscience.wiley.com).



The synthesis of 2-amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidine **3** is described from 2-amino-6-methyluracil **4** through the crucial step of 2-pivaloyl protecting and cyclization. The assignment of the structure of **3** was performed by its spectral data, the ^{1}H NMR, ^{13}C NMR, gHMQC, and HRMS spectra.

J. Heterocyclic Chem., **46**, 1151 (2009).

INTRODUCTION

The requirement for folic acid in the metabolism of one carbon units is well established [1]. The coenzyme forms are actually their reduced products, mostly 5,6,7,8-tetrahydro derivatives, whose nitrogen atom at position 5 is reactive site of those molecules in C_1-unit metabolism [2]. The inhibitors of these enzymes will cause a deficiency of tetrahydrofolate (Fig. 1) cofactors, which will result in blocks in the synthesis of pyrimidine, purines, and protein. Consequently, these blocks will affect both DNA synthesis and cell division. Thus, the enzymes in folate cycle have been recognized as an attractive target for cancer chemotherapy [3,4].

RESULTS AND DISCUSSION

As part of a program focused on the design and synthesis of inhibitors of methionine synthase [5], access to 2-amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidine (**3**) was required as the intermediate of the preparation of 8-deaza 5,6,7,8-tetrahydrofolic acid (**2**) and their derivatives. Although the structure of **3** is not complicated, the direct synthesis of the 8-deaza-5,6,7,8-tetrahydropterdine and derivatives has not been reported. Limited reports describe the synthesis of N^5-substituted derivatives of **2** from a hydrogenation of 8-deazafolic acid (**1**) [6–8]; however, the yields are quite low. In this article, we report a successful synthesis of the 6-substituted 8-deazatetrahydropteridine starting from commercially available 2-amino-6-methyluracil.

Initial attempts to get access to the target compound **3** using 2-acetamide-5-nitro-6-methyluracil (**6**) were investigated. 2-Amino-5-nitro-6-methylpyrimidin-4-one **(5)** was prepared in 91% yield by reaction of 2-amino-6-methyluracil **(4)** with HNO_3-P_2O_5 under 0°C (Scheme 1) [9]. Compound **5** was reacted with Ac_2O to give the corresponding 2-acetamide (**6**). Attempted the transformation of 2-acetamide-5-nitro-6-methyluracil (**6**) into **7** expecting the successive cyclization of the resulting adduct by nucleophilic attack of the 5-amino group obtained from 5-nitro group. However, the reactions of **6** with 3-chloro-1,2-epoxypropane or 3-bromo-1,2-epoxypropane in different base conditions failed to yield any product **7**, a large percent of starting material (**5**) being recovered. The failure reason could be less stable acetyl group, and the pivaloyl group was selected to improve the stability of the protect group at 2-position.

The successful synthesis route of **3** using the more stable pivaloyl group was shown in Scheme 2. The 2-amino-5-nitro-6-methyluracil (**5**) was pivaloylated by pivaloyl chloride in acetonitrile or pyridine to give the corresponding 2-pivaloylamide (**8**) [10], which was then successfully epoxyalkylated in 6 position with 3-chloro-

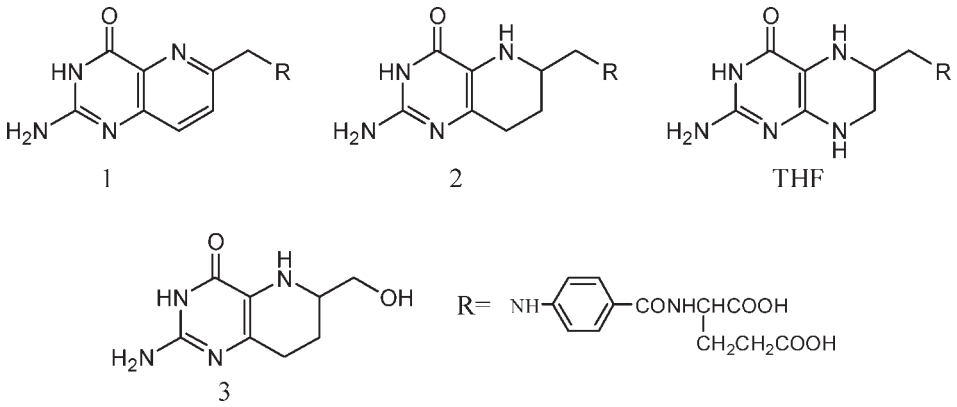


Figure 1. Structures of tetrahydrofolate (THF), compounds **1**, **2**, and **3**.

1,2-epoxypropane and KI in ethanolic sodium ethoxide to form the key intermediate **9** [11]. 2-Pivaloylamino-5-nitro-6-(3,4-epoxybutyl)uracil (**9**) was reduced by sodium dithionite under refluxing for 7 h to afford 2,5-diamino-6-(3,4-epoxybutyl)uracil (**10**). The next crucial step was the cyclization of **10** to give compound **3**. This was carried out by treatment of **10** with BF_3-etherate in dichloromethane to give ca. 1:1(high performance liquid chromatography) mixture R and S isomer of 2-amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-*d*] pyrimidine (**3**) in 37% yield. The structure of product was confirmed by its spectral data, the ^{1}H NMR, ^{13}C NMR, gHMQC, and HRMS spectra.

CONCLUSION

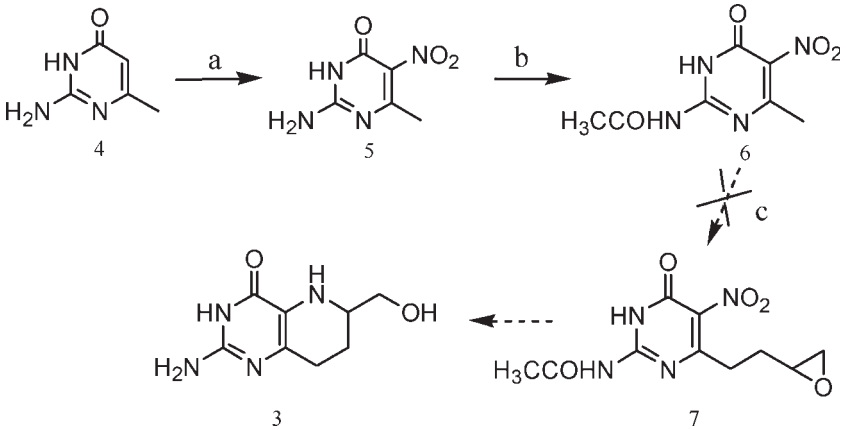
In conclusion, we have developed a new and general approach to the synthesis of 2-amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine, which is an interesting biological model and synthetic precursor used in synthesis of the modified tetrahydrofolic acid derivatives. The procedure described in this article should be a convenient means for the preparation of such analogues due to its simplicity.

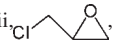
EXPERIMENTAL

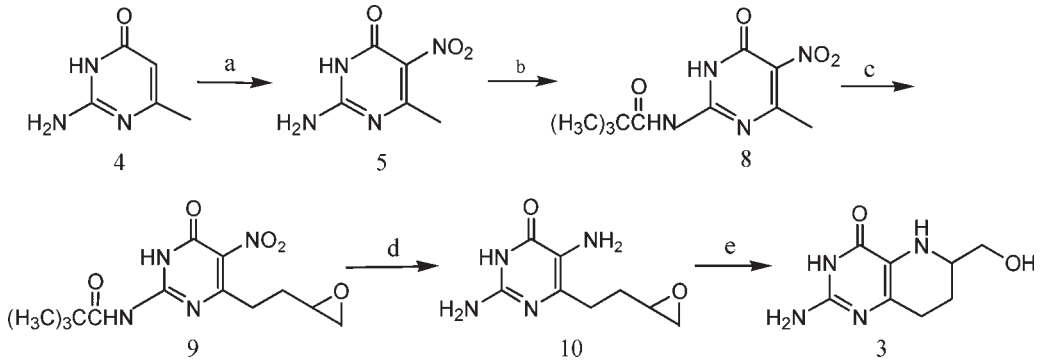
Melting points (uncorrected) were determined with an X_4-type apparatus. ^{1}H and ^{13}C NMR spectra were recorded on JNM-AL-300 or a varian INOVA-500 spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on a VG-ZAB-HS spectrometry. Infrared spectra were recorded with Avatar 360 FT-IR and reported in cm^{-1}. Silica gel (0.40–0.64 mm) was used for column chromatography.

2-Amino-5-nitro-6-methylpyrimidin-4-one (5). To a suspension of fuming nitric acid (6.5 mL, 15.5 mmoles) and P_2O_5 (2.2 g, 15.5 mmoles) 2-amino-6-methylpyrimidin-4-one (1.25g, 10 mmoles) was added at −5 to 0°C. The reaction mixture was stirred for 4 h and poured into ice water (10 mL). The resulting solid was filtered and recrystallized from ethanol to give 2-amino-5-nitro-6-methylpyrimidin-4-one as light yellow crystal (1.55 g, 91%), mp 250°C (dec). ^{1}H nmr (300 MHz, DMSO-d_6): δ 2.29 (s, 3H), 11.78 (s, 2H), 11.81 (s, 1H); ^{13}C nmr (75 MHz, DMSO-d_6): δ = 16.7, 127.2, 149.1, 154.2, 156.4; ms: m/z 170.

Scheme 1. Reagents and conditions: a, HNO_3, P_2O_5, −5 to 0°C, 4 h; b, Ac_2O, 80 to 90°C, 5 h; c: i, NaOEt, EtOH. ii, 3-bromo-1,2-epoxypropane.



Scheme 2. Reagents and conditions: a, HNO_3, P_2O_5, −5 to 0°C, 4 h; b, $(CH_3)_3COCl$, acetonitrile/pyridine, 80 to 90°C, 4 h; c: i, CH_3CH_2ONa, CH_3CH_2OH, 0.5 h. ii,  , KI, DMF, 80 to 90°C, 3 h; d, $Na_2S_2O_4$, CH_3CH_2OH, 80°C, 7 h; e, BF_3-etherate, CH_2Cl_2, room temperature, 10 h.



2-Acetamide-5-nitro-6-methylpyrimidin-4-one (6). 2-Amino-5-nitro-6-methyl pyrimidin-4-one (2.0 g, 11.8 mmoles) was added to acetic anhydride (15 mL) and refluxed for 5 h. The reaction mixture was cooled to 0°C. The resulting solid was filtered and washed with ethyl acetate and petrol to give the product **6** as a light yellow (2.0 g, 80%), mp > 250°C (dec). ^{1}H nmr (500 MHz, DMSO-d_6): δ 2.19 (s, 3H), 2.33 (s, 3H), 12.10–12.30 (m, 2H); ^{13}C nmr (125 MHz, DMSO-d_6): δ 20.9, 23.9, 133.4, 150.5, 153.1, 161.5, 174.2; ms: m/z 213.0292.

2-Pivaloylamino-5-nitro-6-methyluracil (8). A mixture of 1.5 g (8.8 mmoles) of **5** and 3.8 mL (30.8 mmoles) of pivaloyl chloride in acetonitrile or pyrimidine (20 mL, v/v: 5/2) was stirred and refluxed for 4 h. The solution was evaporated to dryness, and the residue was recrystallized in 10% ammonia in water to give 1.4 g (63%) of **8**. mp 211–214°C. ^{1}H nmr (300 MHz, DMSO-d_6): δ 1.22 (s, 9H), 2.29 (s, 3H), 10.0 (br, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.7, 26.1, 27.0, 133.4, 151.9, 167.4, 181.5; esi-ms: $[M+H]^+$ *m*/*z* 254.9.

2-Pivaloylamino-5-nitro-6-(3,4-epoxybutyl)uracil (9). To a solution of 0.83 g (3.27 mmoles) of **8** in 7 mL of dry ethanol, 3.5 mL of ethanolic sodium ethoxide was added (1 N), and the mixture was reluxed for 0.5 h. The solvent was removed in vacuo, and the residue was added to 6 mL of dry *N*,*N*-dimethyformamide (DMF). To the solution, 0.35 ml (4.48 mmoles) of 3-chloro-1,2-epoxypropane and 0.22 g (1.33 mmoles) of KI was added. The resulting solution was refluxed for 3 h. Then the solvent was removed in vacuo, and the residue was suspended in water and extracted with ethyl acetate. The organic layers were pooled, dried over anhydrous sodium sulfate, and concentrated to a small volume. The residue was purified by preparative thin layer chromatograph (silica gel). Elution with $CHCl_3$—CH_3OH (9:1) gave pure **9** (0.25 g, 25%) as yellow oil. ^{1}H nmr (300 MHz, $CDCl_3$): δ 1.26 (s, 9H), 2.27–2.44 (m, 4H), 3.41–3.53, 3.66–3.81 (m, 1H), 4.10–4.26 (m, 1H), 4.49–4.56 (m, 1H); esi-tof-ms: $[M+H]^+$ *m*/*z* 311.0.

2,5-Diamino-6-(3,4-epoxybutyl)uracil (10). To the solution of 0.6 g (1.94 mmoles) of **9** in 30 ml of 95% ethanol, 3.36 g (19.3 mmoles) of sodium dithionite was added. The mixture was heated at 80°C for 7 h and filtered after cooling to room temperature. The filtrate was evaporated to dryness under reduced pressure, and the residue was purified by preparative thin layer chromatograph (silica gel). Elution with $CHCl_3$—CH_3OH (20:1) gave pure **10** (0.17 g, 45%) as oil. ^{1}H nmr (300 MHz, $CDCl_3$): δ 2.17–2.34 (m, 2H), 3.17, 3.21 (d, 2H), 3.37, 3.40 (d, 1H), 3.49, 3.53 (d,1 H), 4.09–4.17 (m, 1H), 4.25 (s, 2H), 5.45 (s, 2H), 8.88 (s, 1H); esi-tof-ms: $[M+H]^+$ *m*/*z* 197.1.

2-Amino-4-hydroxyl-6-hydroxymethyl-5,6,7,8-tetrahydropyrido[3,2-*d*]pyrimidine (3). To a solution of 0.24 g (0.86 mmoles) of **10** in 20 mL of dry dichloromethane in a dry and nitrogen flushed flask, the BF_3-etherate (0.11 mL, 0.86 mmoles) was added dropwise. The mixture was stirred for 10 h at room temperature in the dark, quenched by adding water, and extracted with dichloromethane. The extracts were pooled and dried over anhydrous $MgSO_4$. After the evaporation of solvent to a small volume, the residue was purified by column chromatography (silica gel) to give pure **3** (0.089 g, 37%) as yellow oil. ^{1}H nmr (300 MHz, DMSO): δ 1.09–1.13 (m, 2H), 3.07–3.29 (m, 2H), 3.52, 3.56 (d, 1H, J = 14 Hz), 4.00, 4.04 (d, 1H, J = 14 Hz), 4.12 (br, 1H); ^{13}C nmr (75 MHz, DMSO): δ 22.4, 44.7, 45.1, 57.2, 126.9, 152.1, 155.0, 162.2; esi-hrms: $[M+H]^+$ *m*/*z* 197.10251 (error (ppm): −4.02).

Acknowledgments. We thank the National Sciences Foundation of China (20672008) and 985 Program of Ministry of Education of China for financial support.

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