HETEROCYCLES, Vol.53, No.7, 2000, p.1551 - 1557, Received, 23rd February, 2000

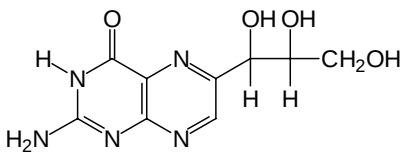
SYNTHESIS OF 2-ETHYLTHIO-6-(3-HYDROXY-1,2-*O*-ISOPROPYLIDENEPROPYL)PTERIDIN-4(3*H*)-ONE

Yonghan Kang,*[1] Seungjin Kim,[1] Youngchan Myoung,[2] and Daejin Baek[3]

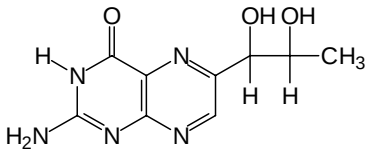
[1]Department of Chemistry, Hanyang University, Ansan, Kyunggi-do 425-791, Korea, [2]Department of Chemical Science and Technology, Agency for Technology and Standards, MOCIE, Kwacheon, Kyunggi-do, 427-010, Korea, [3]Department of Chemistry, Hanseo University, Seosan, Chungnam 356-820, Korea

Abstract - A strategy has been described for the synthesis of 2-ethylthio-6-(3-hydroxy-1,2-*O*-isopropylidenepropyl)pteridin-4(3*H*)-one, which can be used as a useful intermediate for the conversion of neopterin to biopterin.

Pterin derivatives are natural materials biosynthesized from guanosinetriphosphate (GTP) in biological systems and can be isolated in all living things. Biopterin is reported as 6-(D-*erythro*-1,2-dihydroxypropyl)pterin isolated from human urine.[1] Its biosynthetic pathways have drawn attention since they play an important role in amino acid hydroxylation as a tetrahydroform. The atypical phenylketouria disease is caused by a defect in the biosynthetic process for tetrahydrobiopterin.[2,3] Neopterin has been identified as 6-(D-*erythro*-1,2,3-trihydroxypropyl)pterin in the larva of bee, royal jelly[4,5] and human urine.[6] Wachter and his coworkers report that the urine of tumor patients had a higher fluorescent composition than normal and this was linked to a neopterin.[7] It is assumed that neopterin is produced in large amounts specifically from macrophages upon stimulation with interferon-gamma.[8] Recently, the study of the relation between cancer and neopterin and its application to cures for cancer or viral diseases have been the focus of many studies.



Neopterin



Biopterin

Neopterin is synthesized by the Viscontini reaction of 2,5,6-triaminopyrimidin-4(3*H*)-one (**1**) with D-arabinose phenylhydrazone, with more than 80% yield,[9] biopterin was obtained from the reaction of **1**

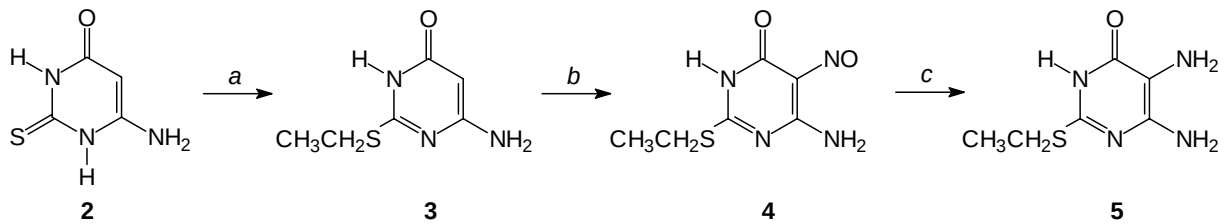
with 5-deoxy-L-arabinose phenylhydrazone in 40% yield.[10] In general, a high yield of sugar hydrazone was produced from the simple reaction of sugar with phenylhydrazine, however, 5-deoxy-L-arabinose phenylhydrazone was obtained through several steps.

Neopterin and biopterin are formed by the respective addition of 1,2,3-trihydroxypropyl group and 1,2-dihydroxypropyl group to the 6-position of the pterin. Therefore, the conversion of the terminal hydroxymethy group to the methyl group in neopterin is strongly sought to improve the synthetic process of biopterin. The selective protection to the 1,2-dihydroxyl groups, the secondary alcohols, is necessary for the conversion of neopterin into biopterin. There were a few attempts to protect the two secondary hydroxyl groups of the neopterin derivatives.[11-13] However, the trials failed because the primary 3-OH in the 1,2,3-trihydroxypropyl group has a higher chemical reactivity than the 1,2-dihydroxyl groups.

In general, pterin [2-aminopteridin-4(3*H*)-one] derivatives are insoluble in water or organic solvents. To induce to selective protecting group at the 1,2-dihydroxyl group of secondary alcohol in neopterin, a 2-ethylthiopterin derivative related to neopterin was synthesized. 2-Alkylthiopteridin-4(3*H*)-one provided better solubility than ordinary pterins and could be converted easily to the corresponding pterins by replacement reaction of 2-alkylthio group with amine nucleophiles.[14]

6-Amino-2-ethylthiopyrimidin-4(3*H*)-one (**3**) was prepared from the reaction of 6-amino-2(1*H*)-thio-pyrimidin-4(3*H*)-one (**2**)[15] with diethyl sulfate in KOH(*aq*) in 70% yield. The treatment of **3** with sodium nitrite in acidic medium provided 6-amino-2-ethylthio-5-nitrosopyrimidin-4(3*H*)-one (**4**) as a dark blue powder in 82% yield. We obtained 5,6-diamino-2-ethylthiopyrimidin-4(3*H*)-one (**5**), from the reduction of nitrosopyrimidine (**4**) with hydrosulfite as a colorless needle in 60 % yield (Scheme 1).

Scheme 1

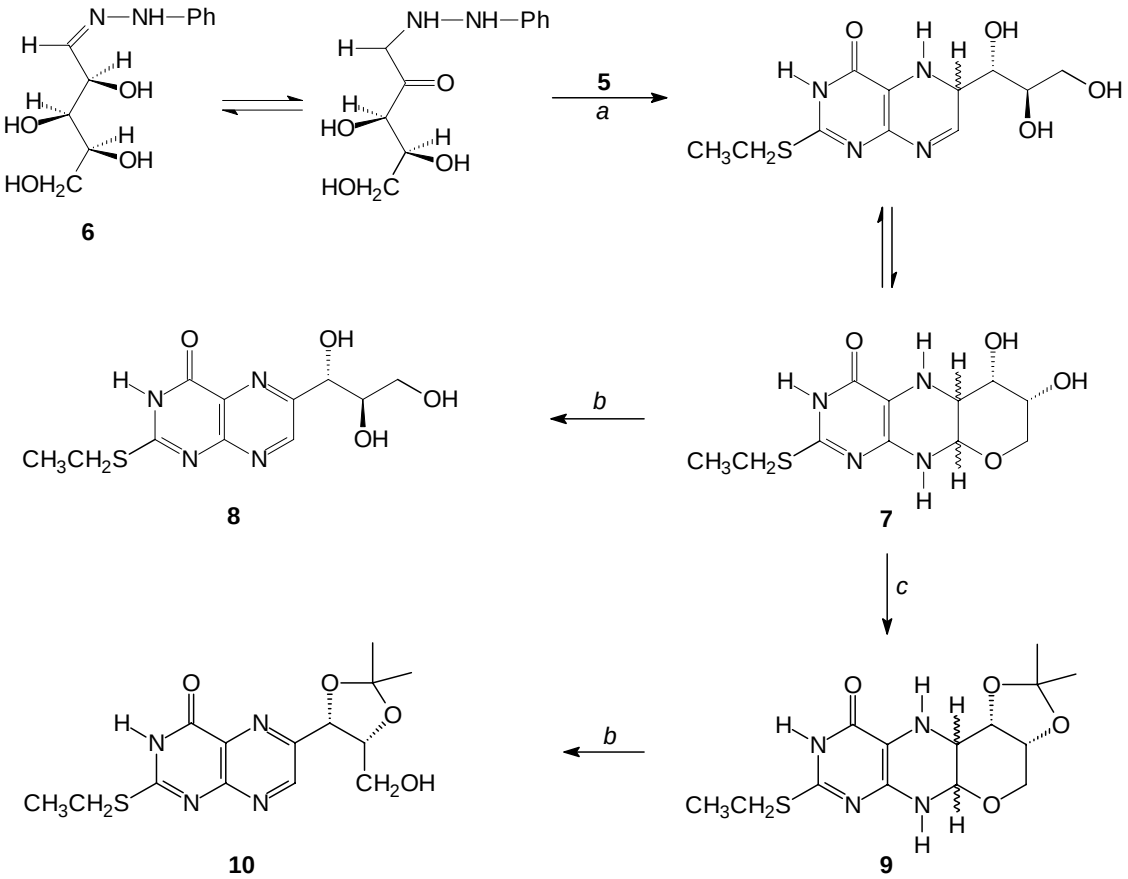


a: 10% KOH(*aq*), $(CH_3CH_2O)_2SO_2$ *b*: i) $NaNO_2$ / H_2O ii) AcOH *c*: $Na_2S_2O_4$, 60-70 ^oC

5,6-Diamino-2-ethylthiopyrimidin-4(3*H*)-one (**5**) was reacted with the D-arabinose phenylhydrazone (**6**), which was obtained from the reaction of D-arabinose with phenylhydrazine in acidic medium by a method similar to the reported procedure,[16] and the diastereomeric mixture of 8-ethylthio-3,4,4a(*R*,*S*),5,10,10a(*S*,*R*)-hexahydro-3,4-dihydroxy-2*H*-pyrano[3,2-*g*]pteridin-6(7*H*)-one (**7**) was separated (Scheme 2). The ^{1}H NMR spectrum of **7** showed the presence of two D_2O exchangable N10-H peaks[17] at 7.74 (J = 4.8

Hz) and 7.24 (J = 4.0 Hz) ppm, which were not found in the compound (**8**). Diastereomeric mixture (**7**) was converted to 2-ethylthio-6-(D-*erythro*-1,2,3-trihydroxypropyl)pteridin-4(3*H*)-one (**8**) by handling with O_2 in a pH 10 buffer solution in 64% yield. In the ^{1}H NMR spectrum of **8**, the aromatic proton at C7 was identified at 8.70 ppm. Without separation of the diastereomeric mixture, **7** was reacted with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid as a catalyst, and the ^{1}H NMR spectrum of the isolated product (**9**) showed the isopropylidene group (1.25 and 1.43 ppm) and N10-H peaks (δ 7.43 and 7.81 ppm). Compound (**9**), therefore, was expected to be 8-ethylthio-3,4,4a(*R*,*S*),5,10,10a(*S*,*R*)-hexahydro-3,4-*O*-isopropylidene-2*H*-pyrano[3,2-*g*]pteridin-6(7*H*)-one (Scheme 2).

Scheme 2



The oxidation of compound (**9**) was carried out by a procedure similar to the preparation of **8**. The obtained product (**10**) was characterized as 2-ethylthio-6-(D-*erythro*-3-hydroxy-1,2-*O*-isopropylidene-propyl)pteridin-4(3*H*)-one by the ^{1}H NMR spectrum and elemental analysis.

EXPERIMENTAL

General Procedures. All chemicals used were purchased from commercial sources with an analytical grade. The solvents were purified by distillation and the other reagents were used without further purification. ^{1}H-NMR spectra were measured at 300 MHz using a Varian Unity Plus 300 spectrometer. The chemical shift values are reported as δ downfield from TMS as an internal standard. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Elemental analyses were performed by Fisons EA 1108.

6-Amino-2-ethylthiopyrimidin-4(3*H*)-one (3)

To a well-stirred solution of 6-amino-2(1*H*)-thiopyrimidin-4(3*H*)-one (**2**)[15] (25 g, 0.17 mol) in 10% aqueous KOH solution (100 mL), diethyl sulfate (25 mL, 0.17 mol) at ambient temperature was added dropwise. After being stirred for 2 h at ambient temperature, the precipitated solids were filtered off, washed with water and ethanol, and recrystallized from ethanol to give **3** as a colorless needle (20.5 g, 70%): mp 205-206 ^{o}C; ^{1}H NMR (DMSO-d_6) 1.25 (3H, t, J = 7.14 Hz, -SCH$_2$-*CH$_3$*), 3.02 (2H, q, J = 7.32 Hz, -S*CH$_2$*-CH$_3$), 4.88 (1H, s, C5-H), 6.41 (2H, br s, C6-NH$_2$), 11.22 (1H, br s, N3-H); Anal. Calcd for $C_6H_9N_3OS$: C, 42.09; H, 5.30; N, 24.54. Found C, 41.72; H. 5.29; N, 24.41.

6-Amino-2-ethylthio-5-nitrosopyrimidin-4(3*H*)-one (4)

Compound (**3**) (20 g, 0.12 mol) and $NaNO_2$ (5.1 g, 0.12 mol) were suspended in water (350 mL) and treated with acetic acid (16 mL). The reaction mixture was stirred at ambient temperature for 8 h. The precipitated solids were filtered off and washed with water and ethanol, and recrystallized from water to give **4** as a dark blue powder (19.7 g, 82%): mp 198 ^{o}C (decomp); ^{1}H NMR (DMSO-d_6) 1.29 (3H, t, J = 7.14 Hz, -SCH$_2$-*CH$_3$*), 3.15 (2H, q, J = 7.32 Hz, -S*CH$_2$*-CH$_3$), 9.05 and 11. 24 (2H, 2 s, C6-NH$_2$), 12.67 (1H, br s, N3-H); Anal. Calcd for $C_6H_8N_4O_2S$: C, 35.99; H, 4.03; N, 27.98. Found C, 35.76; H, 4.33; N, 27.71.

5,6-Diamino-2-ethylthiopyrimidin-4(3*H*)-one (5)

To a suspension of compound (**4**) (10 g, 0.05 mol) in water (400 mL) at 60-70 ^{o}C, $Na_2S_2O_4$ (10 g, 0.06 mol) was added until a clear solution was obtained. The resulting solution was cooled in an ice bath. The precipitated solids were filtered off and washed with water and ethanol, and recrystallized from water to give **5** as a colorless needle (5.66 g, 60%): mp 172 ^{o}C (decomp); ^{1}H NMR (DMSO-d_6) 1.23 (3H, t, J = 6.39 Hz, -SCH$_2$-*CH$_3$*), 3.01 (2H, q, J = 7.30 Hz, -S*CH$_2$*-CH$_3$), 5.6 (2H, br s, C5-NH$_2$), 6.82 (2H, br s, C6-NH$_2$), 11.02 (1H, br s, N3-H); Anal. Calcd for $C_6H_{10}N_4OS$: C, 38.70; H, 5.41; N, 30.08. Found C, 38.60;

H, 5.49; N, 29.79.

8-Ethylthio-3,4,4a(*R*,*S*),5,10,10a(*S*,*R*)-hexahydro-3,4-dihydroxy-2*H*-pyrano[3,2-*g*]pteridin-6(7*H*)-one (7).

To a suspension of compound (**5**) (3.72 g, 0.02 mol) in aqueous methanol (50%, 360 mL) stirred under nitrogen, D-arabinose phenylhydrazone (**6**)[16] (6.5 g, 0.03 mol), 5 *N* HCl (6 mL), and mercaptoethanol (1 mL) were added successively, and the resulting mixture was heated at reflux for 1 h and then cooled to ambient temperature. The precipitated solids were filtered off and washed with water and ethanol to give the diastereomeric mixture (**7**) as a pale yellow solid (3.81 g, 63%), which was used in the following reaction without further purification and separation: ^{1}H NMR (DMSO-d_6) 3.30~1.24 (C4-H, C3-H, C2-H, and -SCH_2CH_3 of major and minor isomers), 3.06 (s, 4a-H of minor isomer), 3.10 (s, 4a-H of major isomer), 4.21 (br s, N5-H of minor isomer), 4.26 (d, J = 4.27 Hz, 3-OH of minor isomer, D_2O exchangable), 4.40 (br s, N5-H of major isomer), 4.52 (s, 10a-H of minor isomer), 4.62 (d, J = 4.51 Hz, 3-OH of major isomer, D_2O exchangable), 4.79 (d, J = 4.16 Hz, 4-OH of major isomer, D_2O exchangable), 4.81 (s, 10a-H of major isomer), 5.41 (d, J = 4.36 Hz, 4-OH of major isomer), 7.74 (d, J = 4.8 Hz, N10-H of major isomer), 7.24 (d, J = 4.0 Hz, N10-H of minor isomer), 12.11 (br s, N7-H).

2-Ethylthio-6-(D-*erythro*-1,2,3-trihydroxypropyl)pteridin-4(3*H*)-one (8)

A suspension of diastereomixture (**7**) (5.26 g, 17.5 mmol) in buffer solution (pH 10, 300 mL) was protected from light with aluminum foil and stirred under oxygen atmosphere until the starting material completely disappeared. The resulting solution was filtered, and then the pH of the filtrate was adjusted to 6 by addition of ambelite IR 120(H^+). After the resin was filtered out, the filtrate was evaporated *in vacuo* to dryness. Recrystallization of the residual solid from a mixture of methanol/acetone (50:600, v/v) afforded **8** as a pale yellow powder (3.3 g, 64%): mp 162 ^{o}C (decomp); ^{1}H NMR (DMSO-d_6) 1.28 (3H, t, J = 7.32 Hz, S-CH_2CH_3), 3.05 (2H, q, J = 7.32 Hz, S-CH_2CH_3), 3.42(2H, m, C3′-H_2), 3.76 (2H, m, C2′-H and C3′-OH), 4.68 (1H, d, J = 5.67 Hz, C1′-H), 4.82 (1H, s, C2′-OH, D_2O exchangable), 5.62 (1H, s, C1′-OH, D_2O exchangable), 8.70 (1H, s, C7-H), 12.4 (1H, br s, N3-H, D_2O exchangable); Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73; N, 18.78. Found C, 43.98; H, 4.97; N, 18.51.

8-Ethylthio-3,4,4a(*R*,*S*),5,10,10a(*S*,*R*)-hexahydro-3,4-*O*-isopropylidene-2*H*-pyrano[3,2-*g*]pteridin-6(7*H*)-one (9)

To a suspension of compound (**7**) (3.72 g, 0.02 mol) in dry DMF (150 mL), 2,2-dimethoxypropane (6.4 mL, 0.05 mol) and *p*-toluenesulfonic acid monohydrate (0.76 g, 4 mmol) were added, and the resulting mixture was stirred at ambient temperature for 16 h. The reaction mixture was treated with a small

amount of sodium carbonate powder. After the insoluble solids were filtered off, the filtrate was evaporated in high vacuo to dryness. Dichloromethane and water were then added to the residual solid, and the organic layer was washed with water twice. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel eluting with dichloromethane: methanol (35:1, v/v) to give **9** (4.4 g, 74%) as a pale yellow solid, which was used in the following reaction without further separation: ^{1}H NMR (DMSO-d_6) 1.28 and 1.43 (6H, 2 s, 2 x CH_3 of isopropylidene), 1.25~3.27 (C4-H, C3-H, C2-H, and -SCH_2CH_3 of major and minor isomers), 3.06 (s, 4a-H of minor isomer), 3.10 (s, 4a-H of major isomer), 4.45 (br s, N5-H of minor isomer), 4.78 (s, 10a-H of minor isomer), 4.81 (br s, N5-H of major isomer), 5.42 (s,10a-H of major isomer), 7.81 (br s, N10-H of major isomer), 7.43 (br s, N10-H of minor isomer), 11.95 (br s, N7-H).

2-Ethylthio-6-(D-*erythro*-3-hydroxy-1,2-*O*-isopropylidenepropyl)pteridin-4(3*H*)-one (10)

Following the procedure for prepararing **8** from **7**, the product (**10**) was obtained from **9** in 68% yield: mp 110 ^{o}C (decomp); ^{1}H NMR: (DMSO-d_6) δ 1.27 (3H, t, J = 7.34 Hz, -S-CH_2CH_3), 1.41 and 1.59 (2 s, CH_3 x 2 in isopropylidene), 3.00 (4H, m, S-CH_2CH_3 and C3′-2H), 4.47 (1H, m, C2′-H), 4.68 (1H, br s, C3′ OH, D_2O exchangable), 5.36 (1H, d, J = 7.32 Hz, C1′-H), 8.60 (1H, s, C7-H), 12.6 (1H, br s, N3-H, D_2O exchangable); Anal. Calcd for $C_{14}H_{18}N_4O_4S$: C, 49.69; H, 5.36; N, 16.56. Found C, 49.36; H, 5.61; N, 16.41.

ACKNOWLEDGMENT

The author wishes to acknowledge the financial support of Hanyang University, Korea, made in the program year of 1999.

REFERENCES

1. S. Kaufmann, in ‘Unconjugated Pterins in Neurobiology’, ed. by W. Lovenberg and R. Levine, Taylor & Francis, London, 1987, p. 1.
2. S. Kaufmann, N. Holtzman, S. Milstein, I. J. Buther, and A. Krumholtz, *New Engl. J. Med*., 1975, **293**, 785.
3. T. Nagatsu, T. Yamaguchi, T. Kato, T. Sugimoto, S. Matsuura, M. Akino, I. Nagatsu, R. Iizuka, and H. Naraoyashi, *Clin. Chim. Acta*, 1981, **109**, 305.
4. H. Rembold and L. Buschmann, *Ann.*, 1963, **662**, 72.
5. H. Rembold and L. Buschmann, *Chem. Ber*., 1963, **96**, 1406.
6. A. Sakuri and M. Goto, *J. Biochem. (Tokyo)*, 1967, **61**, 142.

7. H. Wachter, K. Grassmayer, W. Gutter, A. Hausen, and G. Sallaberger, *Wien, Klin. Wochenschr*., 1972, **84**, 586.
8. L. Leohirun, P. Thuvasethakul, T. Pholcharoen, and S. Lapanant, Pteridines, 1993, **4**, 67.
9. M. Viscontini, R. Provenzale, S. Ohlgart, and J. Mallevialle, *Helv. Chim. Acta*, 1970, **53**, 1202.
10. M. Viscontini, in 'Biochemical and Clinical Aspects of Pteridines', ed. by H. Wachter, H. Ch. Curtius, and W. Pfleiderer, Walter de Gruyter, Berlin, 1984, Vol. 3, p. 19.
11. A. Kaiser and H. P. Wessel, *Helv. Chim. Acta*, 1987, **70**, 766.
12. Y. Kang, R. Soyka, W. Hutzenlaub, M. Wiesenfeldt, W. Leskopf, and W. Pfleiderer, in 'Chemistry and Biology of Pteridines 1986', ed. by B. Cooper and V. Whitehead, Walter de Gruyter, Berlin, 1986, p. 31.
13. R. Soyka and W. Pfleiderer, in 'Biochemical and Clinical Aspects of Pteridines', ed by H. Wachter, H. Ch. Curtius, and W. Pfleiderer, Walter de Gruyter, Berlin, 1985, Vol. 4, p. 33.
14. T. Sugimoto, S. Matsuura, and T. Nagatsu, *Bull. Chem. Soc. Japan*, 1980, **53**, 2344.
15. W. Traub, *Ann.*, 1904, **331**, 64.
16. T. Sugimoto and S. Matsuura, *Bull. Chem. Soc. Japan*, 1975, **48**, 3767.
17. R. Soyka, W. Pfleiderer, and R. Prewo, *Helv. Chim. Acta*, 1990, **73**, 808.