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New and Facile Synthesis of 5,6,7,8-Tetrahydro-5-deaza-5-thiapterins *via* the Aliphatic *S–N* Type Smiles Rearrangement

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5,6,7,8-Tetrahydro-5-deaza-5-thiapterins, (1a, 1d, and 1e), were conveniently synthesized by the thermal condensation of 5-bromo-6-chloroisocytosine (5) with cysteamines (6a—c) *via* the aliphatic *S-N* type Smiles rearrangement in ethanolic pH 7.0 buffer solution.

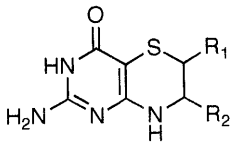
Keywords 5,6,7,8-tetrahydro-5-deaza-5-thiapterin; synthesis; thermal condensation; 5-bromo-6-chloroisocytosine; cysteamine; Smiles rearrangement

5,6,7,8-Tetrahydro-5-deaza-5-thiapterins (**1**) are the thia analogs of biologically important tetrahydropterins. Benkovic *et al.*[1] have reported that the reaction of diethyl chloromalonate with cysteamines (*e.g.* **6**) gives 5,6-dihydro-2-ethoxycarbonyl-1,4-thiazin-3-ones, which are alkylated exclusively at the lactam oxygen with triethyloxonium tetrafluoroborate and are subsequently condensed with guanidine to give the corresponding tetrahydro-5-deaza-5-thiapterins (**1a—c**). They have also demonstrated that compounds **1a—c** are good inhibitors of rat liver phenylalanine hydroxylase (see Chart 1).

In our research program on the synthesis and biological evaluation of the thia analogs of tetrahydrofolic acid, *e.g.*, 5,6,7,8-tetrahydro-5-deaza-5-thiafolic acid (**1f**),[2] we required a more convenient and versatile method for the construction of the 5,6,7,8-tetrahydro-5-deaza-5-thiapterin ring system, because Benkovic's procedure appears not to be applicable to the preparation of **1f** with a highly functionalized side-chain.

Our strategy for the construction of the target ring system involves annulation of the 2,3-dihydro-1,4-thiazine ring employing appropriately substituted isocytosine derivatives as a starting material. In this paper, we describe the facile synthesis of tetrahydro-5-deaza-5-thiapterins (*e.g.* **1**) through a hitherto-unknown aliphatic *S-N* type Smiles rearrangement. It should be applicable to the synthesis of **1f**.

Fenner *et al.* have reported that the condensation of



1

a : R_1=R_2=H

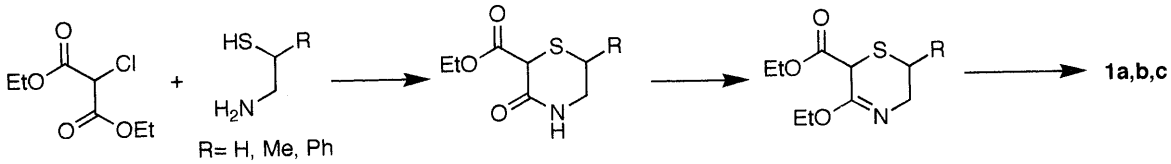
b : R_1=Me, R_2=H

c : R_1=Ph, R_2=H

d : R_1=CH_2OH, R_2=H

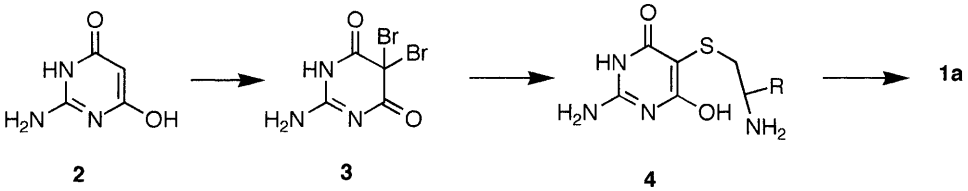
e : R_1=H, R_2=CH_2OH

f : R_1= H_2CHN-C$_6$H$_4$-$CONHCH(CO_2H)CH_2CH_2CO_2H$, R_2=H



R= H, Me, Ph

Chart 1



2 **3** **4**

a : R= H

b : R=CO_2Et

Chart 2

5-halogenobarbituric acids, prepared by the halogenation of barbituric acids, with *o*-aminothiophenol followed by acid-catalyzed cyclization results in the formation of 1,5-dihydro-5-deaza-5-thiaisoalloxazines.[3] Our first attempt at the synthesis of **1** involved application of Fenner's methodology to 6-hydroxyisocytosine (**2**) as depicted in Chart 2.

The condensation of 5,5-dibromo-5,6-dihydro-6-oxoisocytosine (**3**),[4] prepared preferentially from **2** by bromination using two equimolar amounts of *N*-bromosuccinimide, with cysteamine hydrochloride (**6a**) in ethanol at 70 °C gave 5-(2-aminoethyl)thio-6-hydroxyisocytosine (**4a**) as the hydrobromide salt. In this reaction, direct formation of **1a** was not observed. Despite many attempts, the intramolecular cyclization of **4a** leading to **1a** was unsuccessful under mild conditions but rather required drastic conditions, *i.e.*, when **4a** was heated in hexamethyldisilazane and diethylene glycol dimethyl ether in the presence of a catalytic amount of ammonium sulfate at 140 °C overnight, **1a** was obtained in 71% yield. The structure of **1a** was confirmed by comparison with an authentic sample prepared according to Benkovic's procedure. Employment of an analogous silylation–amination procedure[5] in the cyclization of 5-(2-amino-2-ethoxycarbonylethyl)thio-6-hydroxyisocytosine (**4b**), available from the reaction of **3** with L-cysteine ethyl ester, resulted in the recovery of the starting material, accompanied with the formation of small amounts of some decomposition products. Thus, this methodology under drastic conditions is limited to the synthesis of simply functionalized derivatives of **1**.

Among various types of the Smiles rearrangement,[6] the *S-N* type one is usually observed in the (*β*-amino substituted aryl)arylsulfide system and can be efficiently utilized for the synthesis of phenothiazines and their analogs. Although the *S-N* type Smiles rearrangement is commonly catalyzed by a base, there are several precedents for acid-catalyzed rearrangement in systems containing azaheterocycles.[7,8]

When **6a** was allowed to react with 5-bromo-6-chloroisocytosine (**5**)[9] in ethanol containing excess triethylamine under reflux for 24 h, an unexpected 6-chloroisocytosine (**7**) was obtained in 39% yield as a result of the reductive debromination of **5**, the mechanism of which is not clear at present.

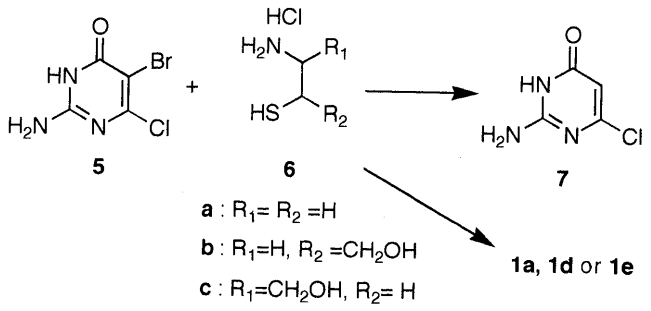


Chart 3

Contrary to our expectation, the above result indicates that a strongly basic condition is not adequate for nucleophilic attack of thiolate ion at the 6-position of **5**. Employment of a strongly acidic condition, however, also seems to be unfavorable owing to the decreased nucleophilicity of the amino and thiol groups in **6a**.

On the basis of the above considerations, systematic experiments were carried out to find the most suitable conditions for the condensation of **5** with **6** by using various pH buffer solutions. Among the buffer solutions examined, neutral or weakly basic buffer solution (pH 7—8) was found to be favorable for the formation of **1a** *via* the condensation of **5** with **6a** followed by the Smiles rearrangement. Thus, the preparation of **1a** was achieved in 50% yield by the reaction of **5** with excess **6a** in ethanolic pH 7.0 buffer solution at 80 °C under argon.

In a similar manner, 6- and 7-hydroxymethyl-5,6,7,8-tetrahydro-5-deaza-5-thiapterins (**1d** and **1e**) were obtained in moderate yields upon treatment of **5** with 3-amino-2-mercapto-1-propanol hydrochloride (**6b**)[10] and its positional isomer (**6c**),[11] respectively, in ethanolic buffer solution.

Although attempts to isolate the intermediary sulfide (**8**) in a pure state in the reaction of **5** with **6a** were unsuccessful because of its instability under the thermal conditions, compound **8** was obtained as a crude product. The structure of **8** was assigned on the basis of spectral data [MS *m/z*: 204 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 2.85 (2H, t, J=6 Hz, SCH_2), 2.96 (2H, br t, J=6 Hz, CH_2NH_2)] and was confirmed by conversion into **1a** under the conditions employed in the reaction of **5** with **6a** and by acetylation with acetic anhydride to give the corresponding *N*-acetyl derivative [NMR (DMSO-d_6) δ: 1.83 (3H, s, $COCH_3$), 3.13 (2H, t, J=6 Hz, SCH_2), 3.31 (2H, dt, J=6 and 7 Hz, CH_2NH)]. Thus, the reaction sequence for the formation of **1a** is considered to involve the Smiles rearrangement of the initially formed sulfide **8**, followed by spontaneous cyclization of the resulting thiol **9** to **1a**, accompanied with the loss of hydrogen bromide as shown in Chart 4.

In the course of many trials for the synthesis of **1a** using other isocytosine derivatives *via* the Smiles rearrangement, the reaction of 5-bromo-6-chloro-1,N^2-ethenoisocytosine (**10**)[12] with **6a** in the basic medium was confirmed to involve the Smiles rearrangement.

When **10** was reacted with **6a** in the presence of excess triethylamine under reflux for 40 h, a 1,N^2-etheno derivative of **1a** (**11**) was produced in 37% yield together with some by-products. Analogous treatment of **10** with

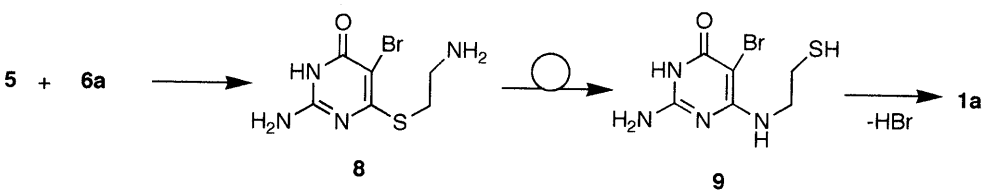


Chart 4

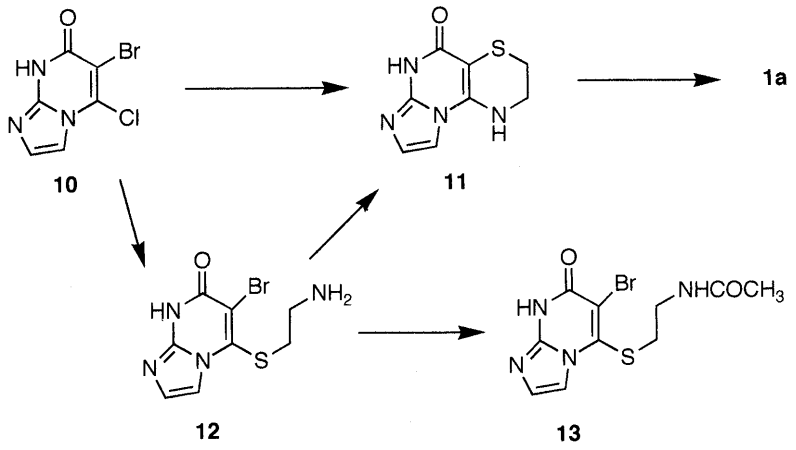


Chart 5

6a at room temperature allowed isolation of the sulfide intermediate (**12**) in 50% yield. The structure of **12** was confirmed by its conversion into the *N*-acetyl derivative (**13**), which was characterized by NMR spectral analysis, *i.e.*, the methylene protons adjacent to the acetamide group appeared as well resolved signals at δ 3.27 ppm (t) in the ^{1}H-NMR spectrum (DMSO-d_6 + D_2O), showing a lower field shift as compared with the aminomethylene protons in **12**. Upon heating **12** in ethanol, conversion into **11** occurred even without any additive, although addition of triethylamine accelerated the rearrangement followed by the intramolecular cyclization to **11**. Unmasking of the etheno moiety was achieved by silver acetate–iodine addition to the etheno double bond[13] and subsequent alkaline hydrolysis, leading to **1a** in a low yield (10%). This result demonstrates the occurrence of the base-catalyzed Smiles rearrangement in the formation of **11**. This method, however, is less favorable for the synthesis of **1** in view of the multi-step procedure and low total yield.

In conclusion, the present results provide novel examples of *S-N* type Smiles rearrangement in the β-aminoethylarylsulfide system. In particular, the use of pH 7 buffer solution is effective for the synthesis of the tetrahydro-5-deaza-5-thiapterin ring system **1**. Extension of the present method to the preparation of the biologically interesting thia-analog of tetrahydrofolic acid, **1f**, is in progress.

Experimental

All melting points (uncorrected) were determined on a Yanagimoto micro-hot stage apparatus. Elemental analyses were performed by the microanalytical laboratory of our university. Spectroscopic measurements for the structural assignment of the reaction products were performed with the following instruments: IR spectra with a Perkin Elmer 1640 FT-IR spectrometer; UV absorption spectra with a Shimadzu 260 spectrophotometer; ^{1}H-NMR spectra with JEOL JNM-GX 270 (270 MHz) and JNM-EX400 (400 MHz) FT-NMR spectrometers using tetramethylsilane as an internal standard; mass spectra (MS) and high-resolution mass spectra (HR-MS) with a JEOL JMS-D 300 machine operating at 70 eV. Thin-layer chromatographic (TLC) analyses were carried out on precoated Silicagel 60 F_{254} plates (Merck, Art 5715). Column chromatography was accomplished by using silica gel (Wakogel C-300).

5,5-Dibromo-5,6-dihydro-6-oxoisocytosine (3) *N*-Bromosuccinimide (7.02 g, 39 mmol) was added to a stirred suspension of 6-hydroxyisocytosine (**2**) (2.48 g, 19.5 mmol) in dry *N,N*-dimethylformamide (DMF) (300 ml), and stirring was continued at room temperature for 1 h. After removal of the solvent under reduced pressure, the resulting residue was triturated with EtOH. The precipitated mass was collected by filtration and washed well with EtOH to give **3** (4.50 g, 81%). Compound **3** was identical with an authentic sample prepared by the bromination of 6-hydroxy-5-nitrosoisocytosine[3] and was used in the next reactions without further purification.

5-(2-Aminoethyl)thio-6-hydroxyisocytosine (4a) A suspension of **3** (1.22 g, 4.3 mmol) and cysteamine hydrochloride (**6a**) (0.73 g, 6.4 mmol) in EtOH (90 ml) was heated at 70 °C for 2 h. The precipitated mass was collected by filtration and washed with EtOH to give **4a** as the HBr salt (0.68 g, 56%). After neutralization of an aqueous solution (35 ml) of the salt (0.50 g, 1.77 mmol) with $NaHCO_3$, the resulting precipitate was collected and washed with cold water to give **4a** (0.32 g, 89%). Rf=0.05 ($CHCl_3$: MeOH : AcOH = 16 : 6 : 3), mp >300 °C (dec.). *Anal.* Calcd for $C_6H_{10}N_4O_2S \cdot 1/4H_2O$: C, 34.89; H, 5.12; N, 27.13. Found: C, 34.95; H, 4.97; N, 27.16. MS *m/z* (%): 184 ($M^+ - H_2O$, 25), 127 (67), 43 (100). IR ν_{max}^{KBr} cm^{-1}: 3424 (NH), 1656 (C=O). UV λ_{max}^{MeOH} nm (ε): 267.8 (2.76×10^3), 213.8 (2.59×10^4). ^{1}H-NMR (DMSO-d_6) δ: 2.58 (2H, t, J=5 Hz, SCH_2), 2.85 (2H, m, CH_2NH_2), 7.54 (2H, br, NH_2), 8.04 (2H, br, NH_2), 11.09 (1H, br, NH).

5-(2-Amino-2-ethoxycarbonylethyl)thio-6-hydroxyisocytosine (4b) A suspension of **3** (720 mg, 2.5 mmol) and L-cysteine ethyl ester hydrochloride (780 mg, 4.2 mmol) in EtOH (5 ml) was heated at 80 °C for 2 h. The precipitated mass was collected and washed with EtOH to give the HBr salt of **4b** (533 mg, 60%). After neutralization of an aqueous solution (50 ml) of the salt with $NaHCO_3$, the resulting precipitate was collected and washed with cold water to give **4b** (317 mg, 77%) as a colorless powder. Rf=0.21 ($CHCl_3$: MeOH : AcOH = 16 : 6 : 3). mp >300 °C (dec.). *Anal.* Calcd for $C_9H_{14}N_4O_4S \cdot H_2O$: C, 36.98; H, 5.52; N, 19.18. Found: C, 37.03; H, 5.46; N, 19.34. MS *m/z* (%): 245 ($M^+ - Et$, 3), 174 (84), 126 (22), 44 (100). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1738 (C=O), 1656 (C=O). UV ν_{max}^{MeOH} nm (ε): 258.6 (3.95×10^3), 210.0 (6.73×10^3). ^{1}H-NMR (DMSO-d_6) δ: 1.23 (3H, t, OEt), 2.84 (2H, m, SCH_2), 4.16 (3H, m, $CHNH_2$ and OEt), 7.54 (2H, br, NH_2), 8.69 (2H, br, NH_2), 11.16 (1H, br, NH).

Intramolecular Cyclization of 4 A solution of **4a** (20.3 mg, 0.1 mmol) in DMF (3 ml) containing a catalytic amount of concentrated H_2SO_4 was heated under reflux for 7 h. TLC analysis of the reaction mixture showed the occurrence of decomposition of **4a** under the conditions employed and no formation of the desired 5,6,7,8-tetrahydro-5-deaza-5-thiapterin (**1a**), which could be prepared by using Benkovic's procedure. To a solution of **4a** (0.1 mmol) in DMF (3 ml) was added two equimolar amounts of tri-*n*-propylamine, and the mixture was heated under reflux for 7 h. The starting material **4a** was recovered unchanged.

A suspension of **4a** (120 mg, 0.6 mmol) in hexamethyldisilazane (0.45 ml, 1.77 mmol) and diethylene glycol dimethyl ether (4 ml) containing ammonium sulfate (7.8 mg, 0.06 mmol) was heated at 140 °C under argon overnight. After treatment of the reaction mixture with MeOH (50 ml) at room temperature for 10 min and subsequent removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography ($CHCl_3$: MeOH = 20 : 1) to isolate **1a** (78 mg, 71%): Rf=0.19 ($CHCl_3$: MeOH = 5 : 1), mp 275—278 °C [lit.[1] mp 288—293 °C (dec.)]. *Anal.* Calcd for $C_6H_8N_4OS \cdot 7/5H_2O$: C, 34.41; H, 5.19; N, 26.75. Found: C, 34.64; H, 4.91; N, 26.71. MS *m/z* (%): 184

(M^+, 100), 169 (16), 139 (14), 43 (27). IR ν_{max}^{KBr} cm^{-1}: 3411 (NH), 3344 (NH), 1686 (C=O). UV ν_{max}^{MeOH} nm (ε): 307.0 (6.21×10^3), 270.0 (5.74×10^3), 224.8 (1.76×10^4) [lit.[1)] (pH 6.8) nm (ε): 302 (8.2×10^3), 266 (5.6×10^3), 223 (2.1×10^4)]. ^{1}H-NMR (DMSO-d_6) δ: 2.74 (2H, dd, J=5, 6 Hz, C_6-H), 3.49 (2H, m, C_7-H), 6.03 (2H, br, NH_2), 6.64 (1H, br, N_8H), 10.07 (1H, br, N_3H) and the recovered **4a** (6 mg, 15%).

A suspension of **4b** (28 mg, 0.1 mmol) and ammonium sulfate (2.6 mg, 0.2 mmol) in hexamethyldisilazane (0.2 ml, 0.95 mmol) was heated under conditions analogous to those used in the case of **4a**. TLC analysis of the reaction mixture after treatment with MeOH showed partial decomposition of **4b** to give a complex mixture, with substantial recovery of **4b**.

Thermal Condensation of 5-Bromo-6-chloroisocytosine (5) with Cysteamine (6a) A suspension of **5** (44.9 mg, 0.2 mmol), prepared from 6-chloroisocytosine (**7**),[9)] and **6a** (22.7 mg, 0.24 mmol) in EtOH (5 ml) containing triethylamine (0.07 ml, 0.5 mmol), was refluxed under argon for 24 h. TLC analysis of the reaction mixture showed the formation of a single product. After removal of the solvent under reduced pressure, the resulting residue was chromatographed with $CHCl_3$–MeOH (20 : 1) to afford **7** (11.4 mg, 39%), which is the starting material for the preparation of **5**.

A solution of **5** (11.2 mg, 0.05 mmol) and **6a** (28.5 mg, 0.25 mmol) in 0.5 M phosphate buffer, pH 7.0, 8.0, 9.0, 10.0, or 11.0 (3 ml) containing EtOH (3 ml) was heated at 80 °C. Product analysis of the reaction mixtures after 12 h was carried out by using TLC densitometry (eluent, $CHCl_3$: MeOH = 5 : 1; detector, 307 nm). The yields of **1a** were as follows, 49% (pH 7.0), 41% (8.0), 26% (9.0), 30% (10.0), and 19% (11.0).

A solution of **5** (560 mg, 2.5 mmol) and **6a** (1.4 g, 12.5 mmol) in 0.5 M phosphate buffer, pH 7.0 (50 ml) and EtOH (50 ml) was heated under argon at 80 °C. When **5** was no longer detectable (monitored by TLC, for 12 h), the reaction mixture was evaporated and the residue was purified by column chromatography with $CHCl_3$–MeOH (20 : 1) and then $CHCl_3$–MeOH–AcOH (100 : 5 : 1), to give **1a** (227 mg, 50%) and 6-(2-aminoethyl)thio-5-bromoisocytosine (**8**) (198 mg) as a crude powder. MS *m/z* (%): 204 (16), 184 (M^+ − HBr, 100), 139 (31). ^{1}H-NMR (DMSO-d_6) δ: 2.85 (2H, t, J=6 Hz, SCH_2), 2.96 (2H, br t, J=6 Hz, $C\underline{H}_2NH_2$), 6.40 (2H, br, NH_2).

Prolongation of the reaction time did not increase the yield of **1a** in this reaction.

Conversion of 8 into 1a A solution of **8** (a crude sample, 83.2 mg) in 0.5 M phosphate buffer, pH 7.0 (10 ml) and EtOH (5 ml) was heated under argon at 80 °C for 32 h. TLC analysis of the mixture showed the formation of **1a**. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography and eluted with $CHCl_3$–MeOH–AcOH (200 : 10 : 1) to afford **1a** (23.2 mg), which was identical with an authentic sample.

Acetylation of 8 A solution of **8** (a crude sample, 60 mg) in pyridine (10 ml) and acetic anhydride (5 ml) was stirred at room temperature for 12 h. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography and eluted with $CHCl_3$–MeOH–AcOH (200 : 10 : 1) to afford 6-(2-acetylaminoethyl)thio-5-bromoisocytosine (8 mg). Rf=0.14 ($CHCl_3$: MeOH = 5 : 1). MS *m/z* (%): 306 and 308 (M^+, 1), 227 (M^+ − Br, 7), 221 and 223 (M^+ − 86, 7), 169 (67), 86 (31), 43 (100). IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O). UV ν_{max}^{MeOH} nm: 306.8, 239.4, 205.8. ^{1}H-NMR (DMSO-d_6) δ: 1.83 (3H, s, $COCH_3$), 3.13 (2H, t, J=6 Hz, SCH_2), 3.31 (2H, br dt, J=6, 7 Hz, $C\underline{H}_2NH$), 6.78 (2H, br t, NH), 8.04 (1H, br, CONH).

6-Hydroxymethyl-5,6,7,8-tetrahydro-5-deaza-5-thiapterin (1d) Under the conditions analogous to those used in the case of **1a**, a mixture of **5** (171 mg, 0.76 mmol) and 3-amino-2-mercapto-1-propanol hydrochloride (**6b**) (550 mg, 3.83 mmol), prepared by the reduction of isocysteine ethyl ester,[10)] was heated in 0.5 M phosphate buffer, pH 7.0 (40 ml) and EtOH (40 ml). After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography and eluted with $CHCl_3$–MeOH–AcOH (200 : 10 : 1) to afford **1d** (61 mg, 37%). Rf=0.14 ($CHCl_3$: MeOH = 5 : 1), mp 258—260 °C (MeOH). *Anal.* Calcd for $C_7H_{10}N_4O_2S\cdot3/10MeOH\cdot1/10H_2O$: C, 38.85; H, 5.00; N, 24.83. Found: C, 38.80; H, 5.06; N, 24.81. MS *m/z* (%): 214 (M^+, 100), 184 (62). IR ν_{max}^{KBr} cm^{-1}: 3389 (NH and OH), 1675 (C=O). UV ν_{max}^{MeOH} nm (ε): 306.8 (8.7×10^3), 268.4 (5.8×10^3), 224.4 (2.2×10^4). ^{1}H-NMR (DMSO-d_6) δ: 2.91—2.94 (1H, m, C_6-H), 3.32—3.40 (2H, m, C_7-H_2), 3.42 (1H, dd, J=6, 12 Hz, C_6-$C\underline{H}_2OH$), 3.47 (1H, dd, J=3, 12 Hz, C_6-$C\underline{H}_2OH$), 4.96 (1H, br, OH), 6.00 (2H, br, NH_2), 6.58 (1H, br, N_8H), 10.06 (1H, br, N_3H).

7-Hydroxymethyl-5,6,7,8-tetrahydro-5-deaza-5-thiapterin (1e) A mixture of **5** (69 mg, 0.3 mmol) and 2-amino-3-mercapto-1-propanol hydrochloride (**6c**) (215 mg, 1.5 mmol), prepared by the reduction of cysteine ethyl ester,[11)] was heated in 0.5 M phosphate buffer, pH 7.0 (20 ml) and EtOH (20 ml) under conditions analogous to those used in the case of **1d**. After removal of the solvent, the resulting residue was subjected to column chromatography and eluted with $CHCl_3$–MeOH–AcOH (200 : 10 : 1) to afford **1e** (43 mg, 67%). Rf=0.13 ($CHCl_3$: MeOH = 5 : 1). HR-MS Calcd for $C_7H_{10}N_4O_2S$: 214.0525. Found: 214.0540 (M^+). MS *m/z* (%): 214 (M^+, 81), 183 ($M^+ - CH_2OH$, 100), 150 (21), 43 (32). IR ν_{max}^{KBr} cm^{-1}: 3286 (NH), 1650 (C=O). UV ν_{max}^{MeOH} nm (ε): 303.6 (8.3×10^3), 268.4 (5.3×10^3), 224.2 (2.2×10^4). ^{1}H-NMR (DMSO-d_6) δ: 2.67 (1H, dd, J=3, 13 Hz, C_6-H), 2.72 (1H, dd, J=5, 13 Hz, C_6-H), 3.39 (1H, dd, J=6, 11 Hz, C_7-CH_2OH), 3.47 (1H, dd, J=7, 11 Hz, C_7-CH_2OH), 3.58 (1H, m, C_7-H), 4.90 (1H, br, OH), 6.01 (2H, br, NH_2), 6.52 (1H, br, N_8H), 10.09 (1H, br, N_3H).

5-Bromo-6-chloro-1,N^2-ethenoisocytosine (10) A solution of chloroacetoaldehyde (40% aqueous solution, 26 ml, 132 mmol) and **5** (2.96 g, 13 mmol) in 30% aqueous EtOH (500 ml) was stirred at 80 °C for 36 h. After removal of the solvent, the resulting residue was subjected to column chromatography with AcOEt–MeOH (30 : 1) and then $CHCl_3$–MeOH (20 : 1) to afford **10** (2.35 g, 72%), Rf=0.21 (AcOEt : MeOH = 10 : 1), mp 230 °C (MeOH). *Anal.* Calcd for $C_6H_5BrClN_3O$: C, 29.00; H, 1.22; N, 16.91. Found: C, 28.71; H, 1.37; N, 16.91. MS *m/z* (%): 249 (M^+, 100), 187 (24), 185 (26). IR ν_{max}^{KBr} cm^{-1} : 3420 (NH), 1645 (C=O). UV ν_{max}^{MeOH} nm (ε): 300.8 (4.7×10^3), 224.8 (2.2×10^4). ^{1}H-NMR (DMSO-d_6) δ: 7.4 and 7.5 (each 1H, each d, J=2 Hz, ethenyl protons), 12.7 (1H, br, NH) and 5-bromo-6-chloro-3,N^2-ethenoisocytosine (393 mg, 12%). Rf=0.5 (AcOEt : MeOH = 10 : 1), mp 219 °C (MeOH). *Anal.* Calcd for $C_6H_5BrClN_3O$: C, 29.00; H, 1.22; N, 16.91. Found: C, 29.16; H, 1.26; N, 16.94. MS *m/z* (%): 249 (M^+, 100), 225 (67). IR ν_{max}^{KBr} cm^{-1}: 3420 (NH), 1665 (C=O). UV ν_{max}^{MeOH} nm (ε): 308.8 (3.4×10^3), 226.6 (8.0×10^3). ^{1}H-NMR (DMSO-d_6) δ: 7.60 and 7.70 (each 1H, each d, J=2 Hz, ethenyl protons), 11.7 (1H, br, NH).

1,N^2-Etheno-5,6,7,8-tetrahydro-5-deaza-5-thiapterin (11) A mixture of **10** (224 mg, 0.9 mmol) and **6a** (339 mg, 3.0 mmol) was heated in EtOH (40 ml) containing triethylamine (0.8 ml, 5.7 mmol) at 80 °C under argon for 40 h. After evaporation of the solvent, the residue was subjected to column chromatography with $CHCl_3$–MeOH–AcOH (100 : 5 : 1) to afford **11** (70 mg, 37%). Rf=0.39 ($CHCl_3$: MeOH = 5 : 1), mp 275—276 °C (MeOH). *Anal.* Calcd for $C_8H_8N_4OS$: C, 46.14; H, 3.87; N, 26.91. Found: C, 46.19; H, 4.02; N, 26.64. MS *m/z* (%): 208 (M^+, 100), 194 (11). IR ν_{max}^{KBr} cm^{-1}: 3449 (NH), 1655 (C=O). UV ν_{max}^{MeOH} nm (ε): 309.8 (7.05×10^3), 232 (1.87×10^4). ^{1}H-NMR (DMSO-d_6) δ: 2.95 (2H, m, C_6-H), 3.71 (2H, m, C_7-H), 7.02 and 7.52 (each 1H, each d, J=1 Hz, ethenyl protons), 7.76 (1H, br, N_8H), 11.92 (1H, br, N_3H) and 6-(2-aminoethyl)thio-5-bromo-1,N^2-ethenoisocytosine (**12**) (95 mg, 33%). Rf=0.06 ($CHCl_3$: MeOH : AcOH = 16 : 6 : 3). MS *m/z* (%): 208 (M^+ − Br, 29), 79 and 81 (100). UV ν_{max}^{MeOH} nm (ε): 301.4, 231.4. ^{1}H-NMR (DMSO-d_6) δ: 2.95 (2H, t, J=7 Hz, $C\underline{H}_2NH_2$), 3.20 (2H, t, J=7 Hz, SCH_2), 7.35 and 7.65 (each 1H, each d, J=2 Hz, ethenyl protons), 9.30 (1H, br, NH). When a solution of **10** (248 mg, 1 mmol) and **6a** (340 mg, 3 mmol) in EtOH (40 ml) containing triethylamine (0.42 ml, 3 mmol) was stirred at room temperature for 44 h, **12** (184 mg) was obtained as a precipitated mass. TLC analysis of the supernatant showed the presence of small amounts of **12** and unchanged **10**. Compound **12** was unstable in refluxing EtOH, being converted gradually into **11**.

6-(2-Acetamidoethyl)thio-5-bromo-1,N^2-ethenoisocytosine (13) Acetic anhydride (1.5 ml) was added to a solution of **12** (93.7 mg, 0.32 mmol) in pyridine (3 ml), and the mixture was stirred at room temperature for 10 h. After removal of the solvent, the resulting residue was subjected to column chromatography with $CHCl_3$–MeOH–AcOH (100 : 5 : 1) to afford **13** (98 mg, 93%). Rf=0.15 ($CHCl_3$: MeOH = 5 : 1). mp 217 °C (MeOH). *Anal.* Calcd for $C_{10}H_{11}BrN_4O_2S$: C, 36.27; H, 3.35; N, 16.92. Found: C, 36.20; H, 3.37; N, 16.88. MS *m/z* (%): 331 (M^+, 1), 245 (2), 86 (29), 43 (100). IR ν_{max}^{KBr} cm^{-1}: 3446 (NH), 1634 (C=O). UV ν_{max}^{MeOH} nm (ε): 301.8 (5.16×10^3), 230.6 (2.34×10^4). ^{1}H-NMR (DMSO-d_6) δ: 1.75 (3H, s, CH_3C=O), 3.17 (2H, t, J=6 Hz, SCH_2), 3.27 (2H, br dt, J=6, 7 Hz, $C\underline{H}_2NH$), 7.33 and 7.58 (each 1H, each d, J=2 Hz, ethenyl protons), 8.01 (1H, br, CONH), 12.54 (1H, br, N_3H).

Unmasking of 11 Silver acetate (82 mg, 0.99 mmol) and iodine (58 mg, 0.46 mmol) were added in small portions to a solution of **11** (20.8 mg, 0.1 mmol) in glacial acetic acid (8 ml) at room temperature. When all

the iodine had been consumed, 4% aqueous acetic acid (0.088 ml) was added to the solution. The reaction mixture was then heated at 90 °C for 5 h. After removal of the insoluble material by filtration, the filtrate was evaporated under reduced pressure. A solution of the resulting residue in MeOH (2 ml) containing NaOH (7 mg, 0.18 mmol) was stirred at room temperature overnight. After neutralization of the solution with 0.5 N HCl, the solvent was evaporated off *in vacuo*, and the residue was chromatographed with $CHCl_3$–MeOH–AcOH (100 : 5 : 1) as an eluent to give **1a** (2.3 mg, 10%).

References and Notes

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8) In a preliminary experiment, we observed that the reaction of **5** with *o*-aminothiophenol in ethanol in the presence of acid at 80 °C for 2 d resulted in the formation of 2-amino-10*H*-pyrimido[5,4-*b*][1,4]benzothiazin-4(3*H*)-one (80% yield) *via* the Smiles rearrangement. (For an alternative synthesis of this compound, see B. Roth, L. A. Schloemer, *J. Org. Chem.*, **28**, 2659 (1963)).
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12) Synthesis of ethenylated isocytosines and their unmasking have been reported by us (see M. Sako, R. Totani, K. Hirota, Y. Maki, *Chem. Pharm. Bull.*, **40**, 235 (1992)). Analogously, ethenylation of **5** was carried out by the reaction with chloroacetoaldehyde. The structure of the major product was confirmed to be 5-bromo-6-chloro-1,N^2-ethenoisocytosine (**10**) rather than its 3,N^2-etheno isomer based on a comparison of their NMR spectra with reference to the previous NMR spectral results.
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