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 triethylorthocarbonate and are subsequently condensed with guanidine to give the corresponding tetrahydro-5-deaza-5-thiapterins (1a–e). They have also demonstrated that compounds 1a–e 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...
5-halogenobarbituric acids, prepared by the halogenation of barbituric acids, with α-aminothiophenol followed by acid-catalyzed cyclization results in the formation of 1,5-dihydro-5-deaza-5-thiaisoalloxazines. Our first attempt at the synthesis of 1 involved application of Fenner’s methodology to 6-hydroxisocytosine (2) as depicted in Chart 2.

The condensation of 5,5-dibromo-5,6-dihydro-6-oxoisocytosine (3), prepared preferentially from 2 by bromination using two equimolar amounts of N-bromo-succinimide, with cysteamine hydrochloride (6a) in ethanol at 70°C gave 5-(2-aminoethyl)thio-6-hydroxisocytosine (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 hexamethydisilazane 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 in the cyclization of 5-(2-amino-2-ethoxy-carbonylthyl)thio-6-hydroxisocytosine (4b), available from the reaction of 3 with t-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, the S-N type is usually observed in the (β-amino substituted aryl)aryl sulfide 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.

When 6a was allowed to react with 5-bromo-6-chloroisocytosine (5) 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.

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-thiaterpenes (1d and 1e) were obtained in moderate yields upon treatment of 5 with 3-amino-2-mercaptop-1-propanol hydrochloride (6b) and its positional isomer (6c), 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+) 1H-NMR (DMSO-d$_6$): δ: 2.85 (2H, t, J = 6 Hz, SCH$_2$), 2.96 (2H, br t, J = 6 Hz, CH$_2$NH$_2$)]. It 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 Hz, CH$_2$-NH)]. 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) 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
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 5.327 ppm (d) in the 1H-NMR spectrum (DMSO-d6 + D2O), 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 bond13 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-aminomethylenesulfide system. In particular, the use of pH 7 buffer solution is effective for the synthesis of the tetrahydro-5-deaza-5-thiapterin ring system I. Extension of the present method to the preparation of the biologically interesting thia-analog of tetrahydrofolic acid, IF, 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 1600 FT-IR spectrometer; UV absorption spectra with a Shimadzu 260 spectrophotometer; 1H-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 Silica gel 60 F254 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-hydroxy-isocytosine (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-nitrosoundeoxycytosine19 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 NaHCO3, the resulting precipitate was collected and washed with cold water to give 4a (0.32 g, 89%). Rf = 0.05 (CHCl3:MeOH:AcOH = 16:6:3); mp > 300°C (dec.). Anal. Calc. for C19H18N6O4S: 1.4H2O·C: 3.84; H: 5.12; N: 27.13; Found: C: 34.95; H: 4.97; N: 27.16. MS m/z (%): 184 (M+H+O, 25), 127 (67), 43 (100). IR νmax cm−1: 3434 (NH), 1656 (C=O). UV λmax nm (ε): 267.8 (2.7x105), 213.8 (2.59x105). 1H-NMR (DMSO-d6): δ: 2.58 (2H, t, J = 5 Hz, SCH2), 2.85 (2H, m, C3H2NH2), 7.54 (2H, br, NH2), 8.04 (2H, br, NH2), 11.09 (1H, br, NH).

5-(2-Amino-2-ethoxycarbonylthio)-6-hydroxyisocytosine (4b) A suspension of 3 (720 mg, 2.5 mmol) and 1-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 NaHCO3, the resulting precipitate was collected and washed with cold water to give 4b (317 mg, 77%) as a colorless powder. Rf = 0.21 (CHCl3:MeOH:AcOH = 16:6:3); mp > 300°C (dec.). Anal. Calc. for C19H18N6O4S·H2O·C: 3.68; 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 νmax cm−1: 3400 (NH), 1738 (C=O), 1656 (C=O). UV λmax nm (ε): 258.6 (3.85x105), 210.0 (6.73x105). 1H-NMR (DMSO-d6): δ: 1.23 (3H, t, OEt), 2.84 (2H, m, SCH2), 4.16 (3H, m, C3H2NH2 and OEt), 7.54 (2H, br, NH2), 8.09 (2H, br, NH2), 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 H2SO4 was heated under reflux for 7 h. TLC analysis of the reaction mixture showed the presence of decomposition of 4a under the conditions employed and no formation of the desired 3,6,7,8-tetrahydro-5-deaza-5-thiapterin (1a), which could be prepared by using Benckovic’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 hexamethylsilazane (0.45 mL, 1.77 mmol) and diethylglycol 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 (CHCl3:MeOH = 20:1) to isolate 1a (78 mg, 71%). Rf = 0.19 (CHCl3:MeOH = 5:1); mp 275–278°C [lit.11 mp 288–293°C (dec.)]. Anal. Calc. for C19H18N6OS·7H2O·C: 34.41; H: 5.19; N: 26.75. Found: C: 34.64; H: 4.91; N: 26.71. MS m/z (%): 184
7-Hydroxymethyl-5,6,7,8-tetrahydro-5-deaza-5-thiapterin (1ε) A mixture of 5 mg (0.03 mmol) and 2-amino-3-mercaptop-1-propanol hydroiodide (6ε) (25 mg, 1.5 mmol) was heated under conditions analogous to those used in the preparation of 5. After removal of the solvent, the residue was subjected to column chromatography and eluted with CHCl₃-MeOH-AcOH (200:10:1) to afford 1ε (43% yield, 0.037 mmol). Rf = 0.13 (CHCl₃-MeOH = 5:1). HR-MS Caled for C₂₄H₂₃N₃O₂S: 321.1452. Found: 321.1455 (M⁺). MS m/z (%): 214 (M⁺, 81), 183 (M⁺ - CH₂OH, 100), 150 (21), 43 (32). IR v cm⁻¹: 3286 (NH), 1650 (C=O). UV v nm (esp): 303.6 (3.3 x 10⁴), 268.4 (5.3 x 10⁴), 224.2 (2.2 x 10⁴). H-NMR (DMSO-d₆): δ 2.67 (1H, d, J = 3, 13 Hz, CH₂), 2.72 (1H, dd, J = 5, 13 Hz, CH₂), 3.39 (1H, dd, J = 6, 11 Hz, CH₂-C₂H₂), 3.47 (1H, dd, J = 7, 11 Hz, C₂H₂-C₂H₂), 3.58 (1H, m, CH₂), 4.09 (1H, br, OH), 6.81 (2H, br, NH₂), 6.52 (2H, br, NH), 10.09 (1H, br, NH). 5-Bromo-6-chloro-1,7,8-etnoethioinosine (10) A solution of chloroacetaldehyde (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 residue was subjected to column chromatography with AcOEt:MeOH (30:1) and then CHCl₃-MeOH (20:1) to afford 10 (2.35 g, 72%). Rf = 0.21 (AcOEt:MeOH = 10:1), mp 230°C (MeOH). Anal. Caled for C₁₄H₁₁BrClN₂O₃: C, 49.0; H, 1.72; N, 16.5; MS m/z (%): 239 (M⁺, 100), 187 (24), 185 (26), 174 (26), 164 (8), UV v nm (esp): 301.8 (2.8 x 10⁴), 242.4 (2.2 x 10⁴). H-NMR (DMSO-d₆): δ 7.4 and 7.5 (each 1H, each d, J = 8 Hz, each d). 1.72 (1H, br, NH) and 5-bromo-6-chloro-3,7-etnoethioinosine (393 mg, 12%). Rf = 0.5 (AcOEt:MeOH = 10:1), mp 219°C (MeOH). Anal. Caled for C₁₄H₁₁BrClN₂O₃: C, 49.0; H, 1.22; N, 16.5. Found: C, 49.16; H, 1.26; N, 16.4. MS m/z (%): 249 (M⁺, 100), 220 (76), 207 (100), 185 (100), UV v nm (esp): 308.8 (3.4 x 10⁴), 226.6 (8.0 x 10⁴). H-NMR (DMSO-d₆): δ 7.60 and 7.70 (each 1H, each d, J = 2 Hz, ethynyl protons), 11.17 (1H, br, NH). 1,7,8-Etheno-5,6,7,8-tetrahydro-5-deaza-5-thiapterin (11) A solution of 10 (224 mg, 0.9 mmol) and 6a (339 mg, 0.5 mmol) 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 CHCl₃-MeOH-AcOH (100:5:1) to afford 11 (70 mg, 37%). Rf = 0.39 (CHCl₃-MeOH = 5:1), mp 275– 276°C (MeOH). Anal. Caled for C₁₄H₁₁BrClN₂O₃: C, 46.14; H, 3.87; N, 26.21. Found: C, 46.19; H, 4.02; N, 26.6. MS m/z (%): 208 (M⁺, 100), 194 (11), IR v cm⁻¹: 3449 (NH), 1655 (C=O), UV v nm (esp): 309.8 (7.05 x 10⁴), 232.2 (1.87 x 10⁴). H-NMR (DMSO-d₆): δ 2.95 (2H, m, C₂H₂), 3.71 (2H, m, C₂H₂), 7.02 and 7.52 (each 1H, each d, J = 11 Hz, ethynyl protons), 7.76 (1H, br, NH₂), 11.92 (1H, br, NH), and 6-aminiothio-5-bromo-1,7,8-etnoethioinosine (12) (95 mg, 33%). Rf = 0.06 (CHCl₃-MeOH:AcOH = 16:6:3). MS m/z (%): 208 (M⁺, 100), 194 (11), IR v cm⁻¹: 3449 (NH), 1655 (C=O), UV v nm (esp): 301.4 (3.0 x 10⁴), 232.2 (1.87 x 10⁴). H-NMR (DMSO-d₆): δ 2.95 (2H, t, J = 7 Hz, CH₂-C₂H₂), 3.26 (2H, t, J = 7 Hz, S=CH₂), 7.35 and 7.65 (each 1H, each d, J = 2 Hz, ethynyl 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 4 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-Acetamidothio)-5-bromo-1,7,8-etnoethioinosine (13) Acetic anhydride (1.5 ml) was added to a solution of 12 (93.7 mg, 0.32 mmol) in MeOH (3 ml) and the mixture was stirred at room temperature for 10 h. After removal of the solvent, the residue was subjected to chromatography with CHCl₃-MeOH-AcOH (100:5:1) to afford 13 (98 mg, 93%). Rf = 0.15 (CHCl₃-MeOH = 5:1). mp 217°C (MeOH). Anal. Caled for C₁₄H₁₁BrC₄NO₂S: C, 36.27; H, 3.35; N, 16.92. Found: C, 36.2; H, 3.3; N, 16.88. MS m/z (%): 331 (M⁺, 1), 245 (2), 86 (39), 43 (100). IR v cm⁻¹: 3449 (NH), 1655 (C=O), UV v nm (esp): 301.8 (5.16 x 10⁴), 230.6 (2.34 x 10⁴). H-NMR (DMSO-d₆): δ 1.75 (3H, s, CH₃-C₂H₂), 3.17 (2H, t, J = 6 Hz, S=CH₂), 3.27 (2H, br, dt, J = 6, 13 Hz, CH₂-NH₂), 7.33 and 7.58 (each 1H, each d, J = 2 Hz, ethynyl protons), 8.01 (1H, br, CH₂), 12.54 (1H, br, NH). Metalation of 11 Silver nitrate (0.02 mmol, 0.99 mmol) and iodine (58 mg, 0.46 mmol) were added in small portions to a solution of 11 (70 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 CHCl₃-MeOH-AcOH (100:5:1) as an eluent to give 1a (2.3 mg, 10%).

References and Notes
7) For an example, see Y. Maki, T. Hiramitsu, M. Suzuki, Tetrahedron, 36, 2097 (1980) and preceding papers.
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-10H-pterimid[5,4-b][1,4]benzo-thiazin-4(3H)-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)).
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, ethenyllation of 5 was carried out by the reaction with chloroacetoaldehyde. The structure of the major product was confirmed to be 5-bromo-6-chloro-1,3,5-ethenoisoctosine (10) rather than its 3,5-etheno isomer based on a comparison of their NMR spectra with reference to the previous NMR spectral results.
13) R. B. Woodward, F. V. Bruchter, Jr., J. Am. Chem. Soc., 80, 209 (1958). Our unmasking method of the etheno moiety using lead tetraacetate or ammonium persulfate was not applicable to the present case because the formation of the corresponding sulfoxide of 1a was unavoidable.