Facile Synthesis of 7,8-Dimethyl-10-D-Ribitylpyrimido-[5,4-*b*][1,4]-benzothiazine-2,4(1*H*,3*H*)-dione, a Deaza-thia Analog of 1,5-Dihydroriboflavin

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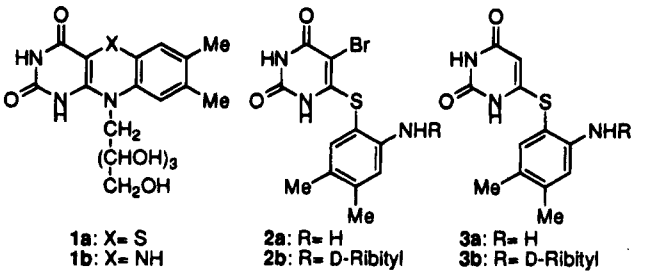
Recently, we have documented that the 5-deaza-5-thia analog of 5,6,7,8-tetrahydrofolic acid behaves as a highly selective inhibitor of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase to exhibit a high cytotoxicity toward human epidermoid carcinoma KB cells and human non-small cell lung carcinoma A 549.[1] The present results directed our attentions to synthesize deaza-thia analogs of other reduced coenzymes.

10-Substituted 7,8-dimethylpyrimido[5,4-*b*][1,4]benzothiazine-2,4(1*H*,3*H*)-diones (cf. **1a**) are of chemical and biological interest in view of the deaza-thia analogs of reduced flavins, *e.g.*, 1,5-dihydroriboflavin (**1b**), $FMNH_2$, and $FADH_2$, which play critical roles as a cofactor in biological redox systems. Along this line, the first synthesis of 10-D-ribityl derivative (**1a**) as the 5-deaza-5-thia analog of **1b** has been accomplished by Hemmerich et al. in 1976,[2a] which involves intramolecular cyclization of 6-[[*N*-(3,4-dimethylphenyl)-*N*-D-ribityl]amino]uracil with sulfur dichloride. This synthetic method, however, required the protection of hydroxyl groups in the sugar moiety during the cyclization and was inadequate for the preparation of 5-deaza-5-thia analogs of $FMNH_2$ and $FADH_2$, since the efficiency of the thiazine-ring formation was a very low (34%).

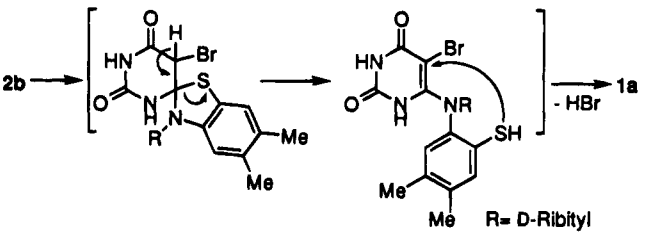
In a previous paper,[3] we have demonstrated a new and versatile method for the construction of 10*H*-pyrimido-[5,4-*b*][1,4]benzothiazine-2,4(1*H*,3*H*)-dione ring system involving S → N type Smiles rearrangement of 6-[(2-aminophenyl)thio]-5-bromouracils (cf. **2**). The rearrangement and subsequent cyclization proceed smoothly under mild conditions without any acid- or base-catalysts. As an example of the successful application of this synthetic methodology, we report herein facile synthesis of **1a**, which provides a clue to the preparation of the 5-deaza-5-thia analogs of $FMNH_2$ and $FADH_2$.

The starting 6-[(2-amino-4,5-dimethylphenyl)thio]-5-bromouracil (**2a**) was obtained in 91% yield after stirring a suspension of 5-bromo-6-chlorouracil[4] and 2-amino-4,5-dimethylthiophenol in methanol containing pH 7.0 phosphate buffer at room temperature for 2 h. The structural proof of **2a** rests upon its microanalytical results and spectral data. For example, its ^{1}H-NMR spectrum showed a broad signal (δ 3.70 ppm) which is assignable to the primary amino protons on the benzene ring, together with signals arising from two amide groups in the uracil ring, two phenyl-ring protons, and two methyl groups.

Scheme 1



Scheme 2



Regioselective ribitylation at the primary amine in **2a** leading to 5-bromo-6-[[4,5-dimethyl-2-(D-ribitylamino)-phenyl]thio]uracil (**2b**) was accomplished in 74% yield by treatment with excess amounts of D-ribose and sodium cyanoborohydride in methanol at 50 °C. During this reaction, the reductive debromination of **2a** also occurred to give 6-[(2-amino-4,5-dimethylphenyl)thio]uracil (**3a**).

The sulfide (**2b**) was stable in refluxing ethanol but underwent reductive debromination[5] in warming *N,N*-dimethylformamide at 130 °C to give 6-[[4,5-dimethyl-2-(D-ribitylamino)phenyl]thio]uracil (**3b**). In contrast to these facts, use of methoxyethanol as a solvent led to the occurrence of S → N type Smiles rearrangement of **2b** as shown in Scheme 2 to give **1a** on further substitution of the bromine atom, the UV-visible spectra of which were superimposed with those of the authentic compound reported by Hemmerich et al.[2a] Simple after-treatment, *i.e.*, evaporation to dryness followed by trituration with acetone, allowed isolation of **1a** in 82% in pure state. The dihydroflavin (**1b**) (oxidation-potential: $E'_0 = -0.19$ V in pH 7.0)[6] is oxidized with ease on exposure to air, especially in a solution, to give the corresponding flavin. On the other hand, the thia analogue (**1a**) ($E^{ox}_{1/2} = +0.60$ V *vs* SCE in dry acetonitrile) was stable even in a solution under aerobic conditions, *e.g.*, no change of **1a** was observed after stirring its methanol solution for 1 week.

Experimental Section

Melting points are uncorrected. ^{1}H- and ^{13}C- NMR spectra were obtained at 400 MHz. Elemental analyses were performed by the microanalytical laboratory of our university. Column chromatography was performed on silica gel (Wakogel C-300). Anhydrous solvents were distilled and stored over activated 4 Å sieves before use. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

Preparation of 6-[(2-Amino-4,5-dimethylphenyl)thio]-5-bromouracil (2a). A solution of 2-amino-5,6-dimethylbenzothiazole (Aldrich, 97% purity) (800 mg, 4.5 mmol) in 50% aqueous methoxyethanol (14 mL) containing potassium hydroxide (5.04 g, 90.0 mmol) was refluxed overnight under argon. The reaction mixture was diluted with water (100 mL), adjusted to

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(3) Maki, Y.; Sako, M.; Tanabe, M.; Suzuki, M. *Synthesis* **1981**, 462.
(4) Pfleiderer, W.; Deiss, H. *Isr. J. Chem.* **1968**, *6*, 603.
(5) Sako, M.; Suzuki, M.; Tanabe, M.; Maki, Y. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3114.
(6) Williams, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 7752.

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pH 4.0 with 1 N HCl, and then extracted with chloroform (200 mL). The extract was washed well with brine and evaporated to dryness. The residual oil (780 mg) was added to the stirred suspension of 5-bromo-6-chlorouracil (451 mg, 2.0 mmol) in methanol (30 mL) containing 0.25 M pH 7.0 phosphate buffer (10 mL). After stirring the mixture at room temperature for 2 h, the resulting precipitate (624 mg, 91%) was collected and recrystallized from methanol to give **2a** as colorless needles: mp 232–235 °C dec; mass *m/z* (rel intensity) 343 and 341 ($M^+ + 1$ and $M^+ - 1$, 22), 262 ($M^+ - Br$, 100), 205 (28), 191 (14), 152 (22); IR (KBr) 1702, 1673 cm^{-1}; UV (MeOH, ϵ) λ_{max} 293 (1.48×10^4), 245 (sh, 9.4×10^3), 220 (2.7×10^4) nm; ^{1}H-NMR (DMSO-d_6) δ 2.11 (3H, s), 2.18 (3H, s), 3.70 (2H, br), 6.65 (1H, br), 6.72 (1H, s), 7.15 (1H, s), 11.55 (1H, br. s); ^{13}C-NMR (DMSO-d_6) δ 18.1, 19.6, 92.7, 102.1, 117.0, 125.4, 137.6, 142.0, 149.3, 149.3, 151.0, 158.6. Anal. Calcd for $C_{12}H_{12}N_3O_2SBr \cdot {}^1/_6H_2O$: C, 41.76; H, 3.60; N, 12.17. Found: C, 41.63; H, 3.54; N, 12.16.

The sulfide **2a** was also obtained by use of bis(2-amino-4,5-dimethylphenyl) disulfide as a starting material: a mixture of the disulfide (304 mg, 1.0 mmol) and triphenylphosphine (289 mg, 1.1 mmol) in dioxane (7 mL)[7] containing 0.1 M pH 4.0 phosphate buffer (3 mL) was stirred at room temperature for 2 h. The mixture was added to the stirred suspension of 5-bromo-6-chlorouracil (451 mg, 2.0 mmol) in methanol (15 mL) containing 0.25 M pH 7.0 phosphate buffer (5 mL). After stirring the mixture at room temperature for 2 h, the resulting crystalline product (606 mg, 97%) was collected by suction. The product was identical in every respect with the compound described above.

Preparation of 5-Bromo-6-[4,5-dimethyl-2-(D-ribitylamino)phenyl]thiouracil (2b). A mixture of **2a** (342 mg, 1.0 mmol), D-ribose (300 mg, 2.0 mmol), and sodium cyanoborohydride (95% purity) (113 mg, 1.7 mmol) in methanol (50 mL) was heated at 50 °C overnight. The resulting precipitate was collected by suction and washed with water and then with methanol to give **2b** (353 mg, 74%) as a colorless powder: mp 210–211 °C (from methanol); mass *m/z* (rel intensity) 478 and 476 (M^+ and $M^+ - 2$, 8), 369 (25), 277 (88) and 185 (100); IR (KBr) 1704, 1668 cm^{-1}; UV (MeOH, ϵ) λ_{max} 294 (1.3×10^4), 255 (1.2×10^4), 222 (3.0×10^4) nm; ^{1}H-NMR (DMSO-d_6) δ 2.12 (3H, s), 2.22 (3H, s), 3.06 (1H, dd, J = 13 and 8), 3.3–3.7 (5H, m), 3.80 (1H, m), 4.37 (1H, t, J = 5), 4.70 (1H, br), 4.75 (1H, br), 4.86 (1H, br), 6.65 (1H, s), 7.15 (1H, s); ^{13}C-NMR (DMSO-d_6) δ 18.6, 20.5, 45.9, 63.5, 69.9, 73.1, 73.7, 113.7, 125.4, 138.7, 142.3, 149.4, 159.9; Anal. Calcd for $C_{17}H_{22}N_3O_6SBr \cdot {}^1/_2H_2O$: C, 42.07; H, 4.78; N, 8.66. Found: C, 42.17; H, 4.54; N, 8.77.

After evaporation of the filtrate to dryness, the resulting residue was subjected to column chromatography and eluted with chloroform–methanol (20:1) to isolate a trace amount of **2a** and 6-(2-amino-4,5-dimethylphenyl)thiouracil (**3a**) (6 mg, 5%): mass *m/z* (rel intensity) 263 (M^+, 100), 230 ($M^+ - SH$, 30), 205 (42), 203 (74); IR (KBr) 1719, 1654 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.10 (3H, s), 2.17 (3H, s), 4.50 (1H, s), 5.35 (2H, br), 6.69 (1H, s), 7.06 (1H, s), 10.87 (1H, br), 11.45 (1H, br).

Preparation of 7,8-Dimethyl-10-D-ribitylpyrimido[5,4-*b*][1,4]-benzothiazine-2,4(1*H*,3*H*)-dione (1a). A suspension of **2b** (44.9 mg, 0.09 mmol) in methoxyethanol (2 mL) was heated at 120 °C under argon. During the reaction, the color of the solution was turned to green and then pale yellow. After continuation of the stirring for 2 h, the reaction mixture was evaporated to dryness and triturated with acetone. The resulting precipitate was collected by suction to give **1a** (30.5 mg, 82%) as an analytically pure product: mp 190–195 °C (lit.[2b] mp 170–173 °C); mass *m/z* (rel intensity) 396 ($M^+ + 1$, 36), 277 (38), 185 (100); IR (KBr) 1699, 1627 cm^{-1}; UV (ϵ) λ_{max} 370 (1.8×10^3), 327 (2.5×10^3), 287 (9.3×10^3), 254 (2.0×10^4), 225 (1.7×10^4) nm in MeOH; 345 (2.0×10^3), 283 (1.0×10^4), 247 (1.8×10^4) nm in pH 2.0; 316 (3.8×10^3), 290 (sh 8.5×10^3), 260 (2.9×10^4) nm in pH 13.0; ^{1}H-NMR (DMSO-d_6) δ 2.10 (3H, s), 2.14 (3H, s), 3.5–4.1 (7H, m), 4.2 (1H, br. s), 4.6 (1H, br. s), 5.0 (1H, br. s), 5.2 (1H, br. s), 6.79 (1H, s), 7.03 (1H, s), 11.03 (1H, br), 11.37 (1H, br); ^{13}C-NMR (DMSO-d_6) δ 18.1, 19.1, 51.6, 63.3, 69.7, 72.6, 73.3, 81.3, 118.0, 119.0, 127.3, 132.8, 135.3, 139.4, 149.4, 149.8, 159.8; HR-FAB MS *m/z* $[M + H]^+$ 396.1253 (calcd for $C_{17}H_{22}N_3O_6S$: $[M + H]^+$ 396.1229).

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Additions and Corrections

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Zhi Zhong Song and Henry N. C. Wong*. Regiospecific Synthesis of Furan-3,4-diyl Oligomers via Palladium-Catalyzed Self-Coupling of Organoboroxines.

Page 41. The spectral data of compound **29** and the proposition that **29** is deprived of any molecular symmetry mentioned in our previous paper [Song, Z. Z.; Wong, H. N. C. *J. Org. Chem.* **1994**, *59*, 33–41] are incorrect. Our new findings indicate that **29** is also a molecule with 2-fold symmetry. A detailed procedure for the preparation of **29**, as well as its pertinent data are outlined below.

4,4‴″″″-Bis(trimethylsilyl)-3,3′:4′,3″:4″,3‴:4‴,3⁗:4⁗,3⁗′:4⁗′,3⁗″:4⁗″,3⁗‴:4⁗‴,3⁗⁗-octifuran (**29**). To a solution of 4,4‴-bis(trimethylsilyl)-3,3′:4′,3″:4″,3‴-quaterfuran (**27**) (164 mg, 0.4 mmol) in dry CH_2Cl_2 (10 mL) was added a solution of BCl_3 (1.0 M) in CH_2Cl_2 (0.6 mL) under a nitrogen atmosphere at −78 °C. After 50 h, the reaction was quenched with Na_2CO_3 solution (1 M, 10 mL) and the resulting mixture was extracted with Et_2O (3 × 40 mL). The organic layer was separated and dried ($MgSO_4$), and the solvent was evaporated. The crude boroxine (43 mg, 50%) and the unreacted **27** (82 mg, 50% recovered) were separated by chromatography on silica gel (50 g, hexanes/Et_2O, 1/1). A mixture of the crude boroxine (43 mg, 0.04 mmol), 2,3-bis(bromomethyl)quinoxaline (13 mg, 0.04 mmol), and $Pd(PPh_3)_4$ (5 mg, 0.004 mmol) in MeOH–PhMe (1/1, 10 mL) was stirred for 5 min. After that Na_2CO_3 solution (1 M, 1 mL) was added, and the resulting mixture was further stirred and refluxed for 4 h. Water (30 mL) was then added, and the mixture was cooled to room temperature. The mixture was then extracted with Et_2O (3 × 20 mL), and the combined ethereal extract was dried ($MgSO_4$) and concentrated. Chromatography on a silica gel column (20 g, hexanes/Et_2O, 4/1) gave **29** (20 mg, 60% based on the boroxine intermediate) as a wax: ^{1}H NMR ($CDCl_3$) δ 0.06 (s, 18H), 7.09–7.10 (d, J = 1.7 Hz, 2H), 7.10–7.11 (d, J = 1.8 Hz, 2H), 7.16–7.17 (d, J = 1.7 Hz, 2H), 7.21–7.22 (d, J = 1.9 Hz, 2H), 7.22–7.23 (d, J = 1.7 Hz, 2H), 7.27–7.28 (d, J = 1.5 Hz, 2H), 7.32–7.33 (d, J = 1.6 Hz, 2H), 7.99–8.00 (d, J = 1.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ −0.59, 115.57, 115.77, 116.91, 117.23, 117.27, 117.31, 119.50, 120.10, 139.73, 140.35, 140.43, 141.09, 141.13, 141.29, 141.63, 148.44; MS (EI) *m/e* 674 (M^+). Anal. Calcd for $C_{38}H_{34}O_8Si_2$: C, 67.63; H, 5.08. Found: C, 67.61; H, 5.04.

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