The Synthesis of C-6′-Methylthiamin and C-6′-Ethylthiamin

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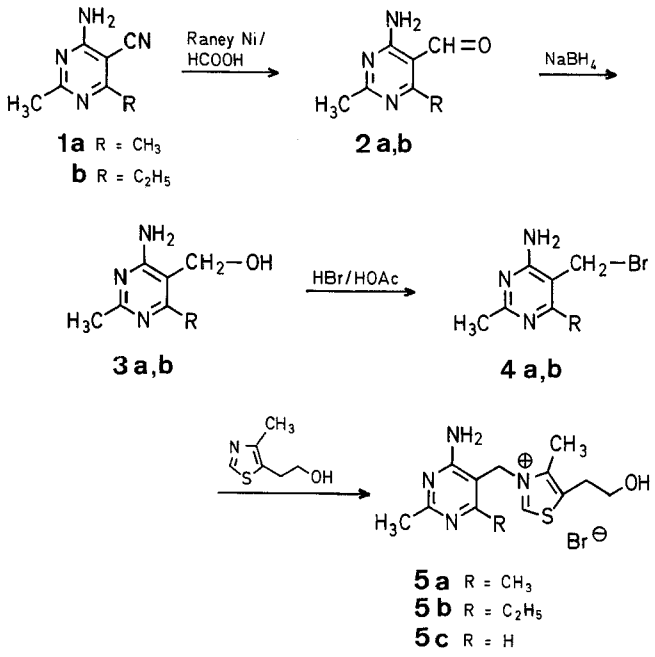
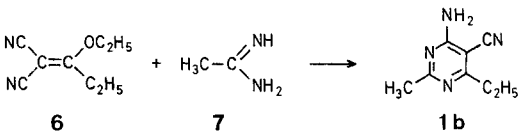
It is now well documented that thiamin (**5c**; vitamin B_1) can assume two major conformations of the two aromatic rings with respect to the bridge methylene group[1,2,3]. It is of particular importance, that the putative intermediates during the enzymatic reactions that require **5c** (in its diphosphate coenzyme form) have a substituent at C-2 of the thiazolium ring[4], very likely forcing the molecule to assume a conformation only rarely found in unsubstituted thiamins. In order to test the possible kinetic effect of such unusual conformations on model enzyme reactions, we have synthesized C-6′-methylthiamin **(5a)** and C-6′-ethylthiamin **(5b)**. Such alkyl substituents, while affecting the conformation, should have no significant inductive effects on the nucleophilic reactivity of the C-2 position of the thiazolium ring. In addition, the C-6′-alkyl thiamins should be useful in metabolic studies (since the alkyl group should increase vitamin uptake by a variety of cells) and in enzymological studies on the enzymes interconverting and utilizing thiamin phosphates. A preparation of **5a** had been reported[5] by a method very different from the present one. No reports on the synthesis of **5b** have appeared. The approach herein employed provides a general method for the synthesis of C-6′-substituted thiamin analogues.



The most difficult step of the above Scheme is the reduction of the nitrile **1** to the aldehyde **2.** Of some 15 procedures attempted, the one quoted below was the only satisfactory one. The mass spectral analysis of all the relevant intermediates, as well as the ^{1}H-N.M.R. spectral data (in analogy with well substantiated data on **5c**[2]) and microanalyses were totally consistent with the structures. In addition, it was found that **4** was highly susceptible to nucleophilic displacement of bromide at the bromomethyl carbon by methanol or ethanol.

The commercial availability of 5-(2-hydroxy)-ethyl-4-methylthiazole makes this sequence simpler compared to the former synthesis of **5a**[5]. The pyrimidine **1a** was prepared by known methods[5,6] and pyrimidine **1b** as shown below.



6 **7** **1b**

All reagents were of the highest quality commercially available and unless otherwise stated were used without purification. All N.M.R. data were taken on a JEOL PS-FT 100 MHz instrument.

4-Amino-2,6-dimethyl-5-formylpyrimidine[7] (2a):

Pyrimidine **1a** (2.42 g, 16.4 mmol) is dissolved in 75% aqueous formic acid (55 ml). Raney nickel (4.4 g) is added and the slurry refluxed for 20 h. The supernatant liquid is then centrifuged to remove the Raney nickel and the solvent from the supernatant liquid is evaporated under reduced pressure to give a yellow oil (1.65 g, 67%). The crude product **2a** is purified on a Florisil column with ethyl acetate as eluant to give the pure product as yellowish crystals; yield: 0.56 g (24%); m.p. 190–192 °C.

$C_7H_9N_3O$	calc.	C 55.62	H 6.00
(151.1)	found	55.43	6.12

M.S.: $m/e = 152$ ($M^+ + 1$, 100%).

^{1}H-N.M.R. (D_2O/TSP: sodium 3-trimethylsilylpropanoate): $\delta = 2.51$ (s, 3H); 2.68 (s, 3H); 10.35 ppm (s, 1H).

4-Amino-2,6-dimethyl-5-hydroxymethylpyrimidine (3a):

Compound **2a** (0.55 g, 3.64 mmol) is dissolved in 5/1, v/v ethanol/water (6 ml). Sodium borohydride (0.138 g, 3.64 mmol) is added slowly[8] and the mixture is stirred for 30 min. The reaction is quenched by the addition of 6 normal sodium hydroxide (5 ml), neutralized with 2 normal hydrochloric acid, and the solvent is evaporated under reduced pressure to give an oil (0.56 g, 100%). Purification on a short Florisil column, and then by preparative T.L.C. (silica gel, ethyl acetate) provide the product as an oil; yield: 0.26 g (52%).

M.S.: $m/e = 154$ ($M^+ + 1$, 100%); 136 (8%).

^{1}H-N.M.R. (D_2O/TSP): $\delta = 2.38$ (s, 6H); 4.63 ppm (2H).

4-Amino-5-bromomethyl-2,6-dimethylpyrimidine Hydrobromide (4a·HBr):

Compound **3a** (300 mg, 2 mmol) is dissolved in glacial acetic acid (5 ml); 20% anhydrous hydrogen bromide is added and the mixture is refluxed for 2 h. The solvent is evaporated and, after washing the residue with anhydrous ether, white crystals result; yield: 0.6 g (100% based on hydrobromide, but very likely it exists as a hydrate), m.p. 210–215 °C (decomposition). The compound **4a** was employed without further purification in the subsequent reaction.

M.S.: $m/e = 216$ ($M^+ + 1$, 53%); 218 (51%).

^{1}H-N.M.R. (D_2O/TSP): $\delta = 2.49$ (s, 3H); 2.53 (s, 3H); 4.65 ppm (s, 2H).

C-6′-Methylthiamin (5a):

Compound **4a**·HBr (0.56 g, 1.9 mmol) is dissolved in *n*-butanol (2 ml) and 5-(2-hydroxy)-ethyl-4-methylthiazole (0.6 ml) is added[9]. The mixture is refluxed for 3 h. After cooling, the precipitate settles out. The mixture is centrifuged and the solid is washed with absolute ethanol and dried to give the product as light brown crystals; yield: 103 mg (15%). Recrystallization from water/ethanol gives yellowish crystals; m.p. 246.5–247 °C (uncorrected, Lit.[5], m.p. 236–239 °C).

$C_{13}H_{20}Br_2N_4OS$	calc.	C 35.47	H 4.58
(440.2)	found	35.49	4.39

^{1}H-N.M.R. (D_2O/TSP): $\delta = 2.37$ (s, 3H); 2.64 (s, 3H); 3.20 (t, 2H, $J = 6$ Hz); 3.88 (t, 2H, $J = 6$ Hz); 5.46 ppm (s, 2H).

Ethoxypropylidenemalononitrile **(6)** is synthesized from malononitrile and triethyl orthopropanoate as described in the literature[10].

4-Amino-5-cyano-6-ethyl-2-methylpyrimidine (1b):
To a solution of acetamidine hydrochloride (7 · HCl; 3.78 g, 0.04 mol) in absolute ethanol (50 ml) is added an ice-cold ethanolic solution of sodium ethoxide (2.76 g, 0.04 mol). After stirring for 15 min, the sodium chloride is filtered off. To the filtrate is added an ice-cold solution of ethoxypropylidenemalononitrile (**6**; 6 g, 0.04 mol) in absolute ethanol (80 ml)[11]. A white precipitate forms immediately. After 1 h, the precipitate is filtered, washed with absolute ethanol, and recrystallized from methanol to give the product; yield: 3.2 g (50%); m.p. 193–194 °C.

$C_8H_{10}N_4$	calc.	C 59.24	H 6.21
(162.2)	found	58.87	6.24

M.S.: $m/e = 163$ ($M^+ + 1$, 100%).

^{1}H-N.M.R. (DMSO-d_6/TMS): $\delta = 1.20$ (t, 3 H, $J = 7$ Hz); 2.40 (s, 3 H); 2.70 (q, 2 H, $J = 7$ Hz); 7.6 ppm (br. s, 2 H).

4-Amino-6-ethyl-5-formyl-2-methylpyrimidine (2b):
Prepared from **1b** as described above for **2a**; yield: 1.6 g (67%); m.p. 118–121 °C.

$C_8H_{11}N_3O$	calc.	C 58.17	H 6.71
(165.2)	found	57.98	6.90

M.S.: $m/e = 166$ ($M^+ + 1$, 100%).

^{1}H-N.M.R. ($CDCl_3$/TMS): $\delta = 1.34$ (t, 3 H, $J = 7$ Hz); 2.51 (s, 3 H); 2.95 (q, 2 H, $J = 7$ Hz); 9.2 ppm (s, 1 H).

4-Amino-6-ethyl-5-hydroxymethyl-2-methylpyrimidine (3b):
Prepared from **2b** as described above for **3a**; yield: 0.5 g (89%); white crystals; m.p. 170–173 °C (decomposition).

M.S.: $m/e = 168$ ($M^+ + 1$, 100%); 150 (8%).

^{1}H-N.M.R. (D_2O/DCl/DSS: sodium 3-trimethylsilylpropanesulfonate): $\delta = 1.21$ (t, 3 H, $J = 7$ Hz); 2.53 (s, 3 H); 2.80 (q, 2 H, $J = 7$ Hz); 4.67 ppm (s, 2 H).

4-Amino-5-bromomethyl-6-ethyl-2-methylpyrimidine (4b):
Anhydrous hydrogen bromide is employed to saturate a solution of **3b** (100 mg, 0.59 mmol) dissolved in acetic acid which has been passed through alumina and dried over molecular sieves. The mixture is refluxed for two periods of 1 h each. In between, the solution is again saturated with anhydrous hydrogen bromide. The solid that results on cooling and evaporation of the solvent is washed with ether to give white crystals; yield: 182 mg (75%); m.p. 239–245 °C (dec).

It was found that the presence of methanol or ethanol led to the formation of the 5-methoxymethyl and 5-ethoxymethyl derivatives of **4b**, respectively, in high yield, hence these solvents has to be excluded. Compound **4b** is employed without further purification in the subsequent reaction.

M.S.: $m/e = 230$ ($M^+ + 1$, 53%); 232 (51%); 150 (100%).

^{1}H-N.M.R. (D_2O/TSP): $\delta = 1.24$ (t, 3 H, $J = 7$ Hz); 2.53 (s, 3 H); 2.76 (q, 2 H, $J = 7$ Hz); 4.81 ppm (s, 2 H). These values refer to protonated **4b**.

C-6′-Ethylthiamin[9] (5b):
A solution of compound **4b** (0.47 g, 1.5 mmol) and 5-(2-hydroxy)-ethyl-4-methylthiazole (1.15 g, 8 mmol; from Aldrich Chemical Co.) in *n*-butanol (2 ml) is refluxed for 3 h. After cooling, the solid is separated by centrifugation and washed with absolute ethanol (3 × 2 ml) to give the product; yield: 370 mg (52%); m.p. 219–220 °C (uncorrected).

$C_{14}H_{22}Br_2N_4OS \cdot H_2O$	calc.	C 35.62	H 5.13
(472.2)	found	35.60	4.96

^{1}H-N.M.R. (D_2O/TSP): $\delta = 1.23$ (t, 3 H, $J = 7$ Hz); 2.57 (s, 3 H); 2.60 (q, 2 H, $J = 7$ Hz) superimposed on 2.66 (s, 3 H); 3.20 (t, 2 H, $J = 6$ Hz); 3.89 (t, 2 H, $J = 6$ Hz); 5.48 ppm (s, 2 H).

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