127. A New Convergent Synthesis of Thiamine Hydrochloride

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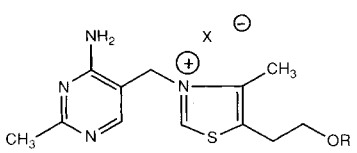
Dedicated to Dr. *Otto Isler* on the occasion of his 80th birthday

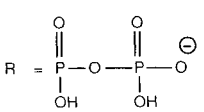
(1.VI.90)

Thiamine hydrochloride (**1a**; 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride; vitamin B_1) has been synthesized in excellent yield by condensation of 3-mercapto-4-oxopentyl acetate (**5a**) with 3,4-dihydro-7-methylpyrimido[4,5-*d*]pyrimidine (**4**) in formic acid. The two intermediates **5a** and **4** are prepared from 3-chloro-4-oxopentyl acetate (**3**) and 4-amino-2-methyl-5-(aminomethyl)-pyrimidine (*Grewe* diamine; **2a**), respectively.

1. Introduction. – Thiamine **1** (vitamin B_1) is a member of the vitamin B complex group. In its function as a dietary requirement, it continues to be in great commercial demand for use as feed/food additive and for pharmaceutical applications [1]. Thiamine, in the form of the diphosphate ester **1b**, is a coenzyme involved in a number of essential metabolic pathways [2]. A deficiency of thiamine in the diet leads to a deterioration of the nervous system – the so-called beriberisyndrome [3].



1a $X = Cl^{\ominus} \cdot HCl$, $R = H$ — Thiamine hydrochloride[1]



b $R =$ — Thiamine diphosphate

c $X = HCO_2^{\ominus} \cdot 2(HCO_2H)$, $R = Ac$ — Thiamine formate

Thiamine is widely distributed in nature. It occurs, in relatively high concentration, in seeds, most notably rice and cereal grains, and in small amounts it is present in plants and animal tissues [4].

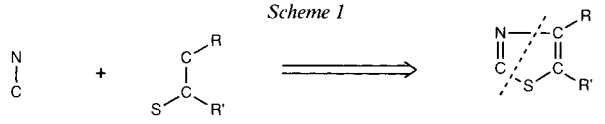
[1]) Commercial form.

Several syntheses of thiamine **1** have been published, and basically two general methods have evolved over the years [5]. The first approach is based upon the construction of the thiazole ring on a preformed pyrimidine intermediate [6].

The second general method consists of separate syntheses of the pyrimidine and thiazole rings followed by appropriate coupling of the two heterocycles [7].

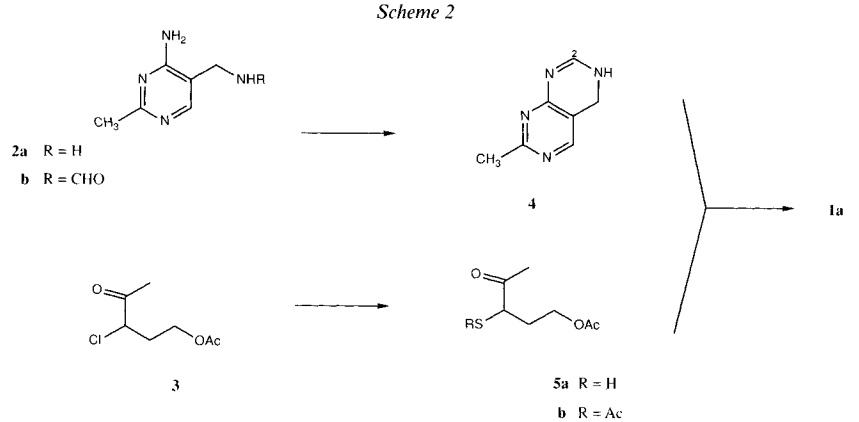
2. Results and Discussion. – Despite the fascinating array of methods for construction of the thiazole nucleus [8], only few examples of cyclizations according to the C–C–S + N–C synthetic scheme have been reported in the literature *(Scheme 1)*.

Scheme 1



Based on this approach, we have now developed a new and convergent route to thiamine hydrochloride (**1a**), involving the condensation of the α-mercaptoketone **5a** (or its derivative **5b**) with the dihydropyrimido[4,5-*d*]pyrimidine **4** as depicted in *Scheme 2*. In this transformation, the C(2)-atom of the thiazole ring of thiamine **1a** corresponds to the C(2)-atom of the starting 3,4-dihydro-7-methylpyrimido[4,5-*d*]pyrimidine (**4**).

Scheme 2



First experiments were performed using the acetate **5b**, easily obtained in over 80% yield by reaction of chloro-acetate **3** with potassium thioacetate in MeOH.

Compound **4** was prepared by heating *Grewe* diamine **2a** in 1 equiv. of dimethylformamide dimethyl acetal[2]) (DMF-DMA) [9] (90% yield) or, alternatively, in an excess of triethyl orthoformate with a catalytic amount of TsOH (71% yield).

Based on the assumption *(vide supra)* that the free thiol derived from **5b** would add to the C(2)-position of **4**, and that the resulting intermediate would, after subsequent cyclization-aromatization, give the thiamine hydrochloride **1a**, we first treated the combined reactants **5b** and **4** at 0° with 2 equiv. of a 5% solution of LiOH in EtOH followed

[2]) The yield of pure isolated pyrimidine **4** decreases notably, if an excess of DMF-DMA is used in the reaction.

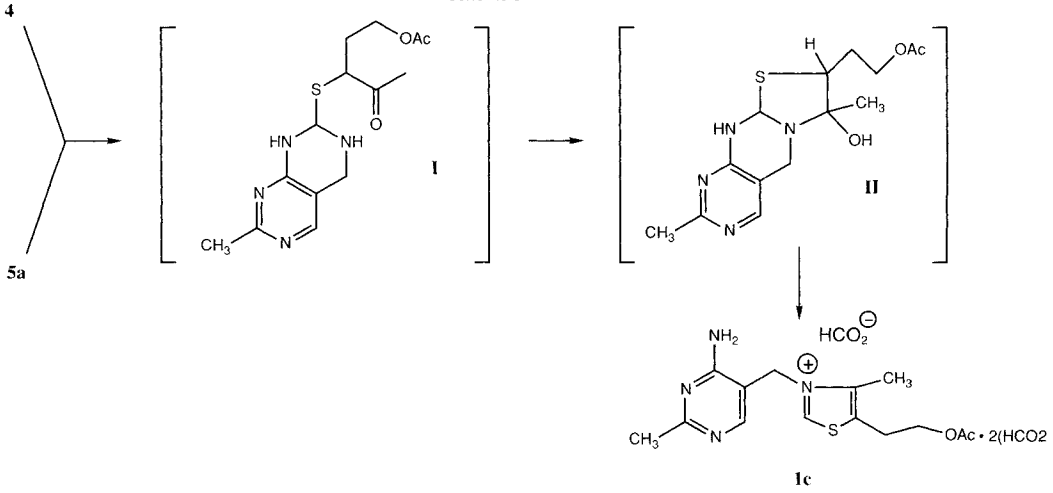
by acidification with ethanolic HCl. This procedure afforded recrystallized pure **1a** in 45% yield along with aldehyde **2b** and *Grewe* diamine **2a** as major side-products[3]). After considerable investigation, it was found that the yield of pure **1a** increased to 74%, when the addition of the free thiol[4]) obtained from **5b** to the pyrimidine **4** was carried out in HCOOH.

At this point, it was felt that the use of isolated mercapto-acetate **5a** in the condensation step might be advantageous.

Accordingly, **5a** was prepared in 75% yield by reacting chloro-acetate **3** with 1 equiv.[5]) of anhydrous potassium hydrogen sulfide in MeOH [11]. Addition of **5a** to a solution of dihydropyrimido[4,5-*d*]pyrimidine **4** in HCOOH at room temperature provided pure **1a** in high yield [12].

Evaporating HCOOH after the condensation step allowed us to isolate an intermediate which can be converted to **1a** by treatment with ethanolic HCl. Based on analytical and spectral data, this intermediate was assigned the structure of *O*-acetylthiamine formate **1c** *(Scheme 3)*.

Scheme 3



In an additional experiment, the condensation of **4** with **5a** was carried out in deuterated formic acid, and the reaction was followed by ^{1}H-NMR spectroscopy. While the decrease of the starting materials **5a** and **4** and the building-up of compound **1c** could be easily followed, no intermediates could be detected in appreciable amount. However, it is reasonable to assume that the first step of the condensation involves the acid-catalyzed addition of thiol **5a** to the C(2)-atom of **4** to form intermediate **I** which then cyclizes and leads, possibly *via* intermediate **II**, to *O*-acetylthiamine formate **1c** *(Scheme 3)*.

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[3]) Although the hydrolysis of compound **4** is rather slow in H_2O, it is greatly accelerated under acidic or basic conditions.

[4]) Under very strong acidic conditions, the α-mercapto-ketone obtained from **5b** decomposes to cyclic thioethers [10].

[5]) Initial experiments showed that even a small excess of sodium or potassium hydrogen sulfide was detrimental to the yield of **5a**. The crude reaction mixture was contaminated by a substantial amount of 4-oxopentyl acetate, which probably arises from reduction of **5a** by excess hydrogen-sulfide ions.

We thank our colleagues from the *Central Research Units, F. Hoffmann-La Roche Ltd.*, for IR spectra (Dr. *L. Chopard*, Mr. *A. Bubendorf*), NMR spectra (Dr. *W. Arnold*), UV spectra (Dr. *M. Grosjean*), mass spectra (Dr. *W. Vetter*, Mr. *W. Meister*), and elemental analyses (Dr. *A. Dirscherl* †). We thank also Dr. *W. Wessely* (Quality Control) for the quantitative determination of the free HCOOH in compound **1c** and of the H_2O content in the thiamine-hydrochloride samples.

Experimental Part

General. Chloro-acetate **3** and *Grewe* diamine **2a** were obtained[6]) from *F. Hoffmann-La Roche AG*, D–Grenzach-Wyhlen. All solvents used were of the highest purity grade. All commercially available chemical reactants were of 'reagent-grade' and used as provided by the supplier without purification, except described otherwise. Anal. TLC on precoated plates (2.5 × 10 cm) SiO_2 *60F-254*, layer thickness 0.25 mm (*E. Merck & Co.*, D–Darmstadt). M.p. were taken on a *Büchi 510* apparatus and are not corrected. UV spectra (in EtOH containing 5% H_2O) were recorded on a *Uvikon 810* instrument; λ_{max} in nm ($\log \varepsilon$). IR spectra (in cm^{-1}) were obtained on a *Nicolet 170-SX FT-IR* spectrometer. ^{1}H-NMR spectra were recorded on a *Brucker AC-250* at 250 MHz. Chemical shift δ of signal centers or ranges are reported in ppm downfield from TMS as internal standard, coupling constants J in Hz. Mass spectra were obtained on a *MS 9* (*AEI*, GB-Manchester) for EI at 70 eV and on a *MS 902* (*AEI*, GB–Manchester) for FAB at 7 keV (ionising gas: Xe). Data are given as *m/z* (%).

1. *3-Mercapto-4-oxopentyl Acetate* (**5a**). Anh. KSH (7.22 g, 0.1 mol) was suspended in 50 ml of abs. MeOH. The mixture was cooled to 0° in an ice-bath and *3-chloro-4-oxopentyl acetate* (**3**; 17.9 g, 0.1 mol), previously dissolved in 50 ml of abs. MeOH, was added dropwise in order to maintain the temp. in the mixture between 0 and 5°. After complete addition, stirring was continued at r.t. for 1 h, while a slow stream of N_2 was passed through the mixture to remove residual H_2S. The precipitated KCl was filtered off and the solvent evaporated under reduced pressure. The residue was taken up in 50 ml of CH_2Cl_2 and the insoluble material removed by filtration. Evaporation of the solvent *in vacuo* at 30° gave 14.9 g of slightly yellow liquid. Bulb-to-bulb distillation of the crude mixture at 120°/0.3 mm yielded 12.95 g (0.07 mol, 73.5%) of **5a** as a colourless liquid[7]). IR (film): 2960*w*, 2550*w*, 1740*s*, 1715*s*, 1370*m*, 1245*s*, 1050*m*. ^{1}H-NMR ($CDCl_3$): 1.74 (*d*, J = 12, SH); 1.95–2.25 (*m*, CH_2); 2.05 (*s*, AcO); 2.35 (*s*, Me); 3.42 (*td*, J = 12, 5.7, SCH); 4.2 (*t*, J = 5.7, CH_2O). EI-MS: 134 (2), 116 (36), 74 (21), 73 (58), 43 (100). Anal. calc. for $C_7H_{12}O_3S$ (176.23): C 47.71, H 6.86, S 18.19; found: C 47.94, H 6.95, S 17.24.

2. *3,4-Dihydro-7-methylpyrimido[4,5-d]pyrimidine* (**4**). *From 4-amino-2-methyl-5-(aminomethyl)pyrimidine* (**2a**) *and DMF-DMA*. In a flask equipped with a *Vigreux* column and a *Liebig* condenser, **2a**[8]) (69 g, 0.5 mol) was suspended in dimethylformamide dimethyl acetal (59.6 g, 0.5 mol). The stirred suspension was slowly heated to *ca.* 80–85°, until the temp. at the head of the *Vigreux* column reached 60°[9]). The MeOH/Me_2NH mixture was then distilled off, until the mixture in the flask became a thick mass. The temp. was increased to 90° for 30 min, 250 ml of toluene were added, and the obtained suspension was further stirred for 1 h at 90°. It was then allowed to cool to r.t., filtered, and washed twice with 100 ml of hexane. The crude material was dried at 50° under reduced pressure: 69.6 g of a tan solid was obtained, which was then sublimated at 150° (oil-bath temp.) under high vacuum (0.2 mm) to give 65.5 g (0.44 mol, 88.5%) of **4** as a white solid. M.p. 173° (dec.). UV: 202 (4), 298 (3.7). IR (KBr): 3430*m* (br.), 2860*m*, 2840*s*, 1670*s*, 1620*s*, 1580*s*, 1530*s*, 1450*s*, 1210*s*. ^{1}H-NMR ((D_6)DMSO): 2.4 (*s*, Me); 4.5 (*s*, CH_2); 7.2 (br. *s*, vinyl. CH); 8.03 (*s*, arom. H); 9.9 (br. *s*, NH). EI-MS: 148 (50, M^+), 147 (100), 106 (12), 53 (17), 42 (20). Anal. calc. for $C_7H_8N_4$ (148.169): C 56.74, H 5.44, N 37.81; found: C 56.79, H 5.44, N 37.75.

From **2a** *and Triethyl Orthoformate*. In a flask equipped with a 20-cm *Vigreux* column and a *Liebig* condenser, **2a**[8]) (69 g, 0.5 mol), triethyl orthoformate (148.2 g, 1 mol), and TsOH (2.5 g)[10]) were introduced. The stirred suspension was slowly heated to *ca.* 110° so that the temp. at the head of the *Vigreux* column reached 80–85°. The EtOH was then distilled off, until the mixture in the flask became a thick mass. The temp. was maintained at 100–110° for 30 min, then 250 ml of toluene were added, and the obtained suspension was further stirred for 1 h at

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[6]) We thank Dr. *K. Goth* for generously providing us with these starting materials.

[7]) Compound **5a** should be used very rapidly after its preparation. Even storage in the cold and under Ar does not prevent its alteration.

[8]) Recrystallized from MeCN, m.p. 124–126°.

[9]) During the heating period, the suspension becomes a clear soln. at about 55°. Compound **4** precipitates, when the reaction is nearly completed.

[10]) TsOH · H_2O was dried according to standard purification procedure.

90°. It was cooled to r.t. and placed overnight in the refrigerator. The light-brown precipitate was filtered and washed twice with 50 ml of toluene. The crude material was dried at 50° under reduced pressure to give 59.3 g of a beige solid which was sublimated at 150° (oil-bath temp.) under high vacuum (0.2 mm) to yield 52.5 g (0.35 mol, 71%) of **4** as a white solid. M.p. 182° (dec.).

3. *3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium Chloride Hydrochloride* (*Thiamine Hydrochloride*, **1a**). Compound **4** (7.4 g, 0.05 mol) was dissolved in 100 ml of HCOOH. To this slightly yellow soln., **5a** (9.25 g, 0.052 mol) was immediately added at such a rate so that the temp. did not exceed 35–40°. The mixture was further stirred for 30 min at r.t. and then 25 ml of a freshly prepared sat. soln. of HCl in abs. EtOH was added dropwise. The temp. rose to 35–36°, and the mixture was further stirred for 30 min at r.t.[11]). The crude mixture was then poured into a 500-ml flask and evaporated at 50° under reduced pressure to give 26.07 g of a green-yellow solid residue, which was taken up in 100 ml of abs. EtOH. Aq. HCl soln. (25%, 30 ml) was then added and the crude mixture heated on a steam-bath, until a clear soln. was obtained. The soln. was cooled to r.t. and placed overnight in the refrigerator. The resulting white crystals were collected and dried *in vacuo* to yield 14.56 g (86.3%) of **1a**. M.p. 245–246° (dec.). The mother-liquor was then evaporated at 50° under reduced pressure and the residue taken up in 50 ml of H_2O. The aq. phase was then washed twice with 25 ml of CH_2Cl_2 and evaporated under reduced pressure to give 3.29 g of a still slightly greenish residue, which was again taken up in 20 ml of abs. EtOH. Aq. HCl soln. (25%, 5 ml) was added and the mixture heated on a steam-bath, until a clear soln. was obtained. It was then cooled to r.t. and kept overnight in the refrigerator. The white crystals were filtered to give 1.42 g (8.4%) of **1a**. M.p. 244–245° (dec.) (combined yield[12]) of **1a**: 94.7% based on **4**).

Recrystallization. The two crops of **1a** were combined and dissolved in 100 ml of warm abs. EtOH. Aq. HCl soln. (25%, 40 ml) was added. The soln. was then allowed to cool slowly to r.t. and kept at 0° overnight. The white crystals were filtered and dried *in vacuo* at 50° to give 13.6 g (0.04 mol, 80.6%) of **1a**. M.p. 243–244° (dec.). UV: 234 (4.1), 266 (3.9). IR (KBr): 3500*m*, 3430*m*, 3340*m*, 3240*m*, 3065*s*, 2615*m*, 1660*s*, 1607*m*, 1380*m*. ^{1}H-NMR (D_2O): 2.54 (*s*, Me); 2.62 (*s*, Me); 3.19 (*t*, J = 5.8, CH_2); 3.88 (*t*, J = 5.8, CH_2O); 5.56 (*s*, 1H, CH_2N); 8.02 (*s*, 1 arom. H); proton of thiazole ring is exchanged with deuterium of D_2O. FAB-MS: 265 (100, M^+), 181 (18), 144 (30), 123 (65), 122 (65), 91 (78). Anal. calc. for $C_{12}H_{18}Cl_2N_4OS$ (337.27): C 42.74, H 5.38, N 16.61, S 9.51, Cl 21.02; found: C 42.93, H 5.28, N 16.70, S 9.61, Cl 21.17.

4. *5-(2-Acetoxyethyl)-3-[(4-amino-2-methylpyrimidin-2-yl)methyl]-4-methylthiazolium Formate* (**1c**). Compound **4** (7.4 g, 0.05 mol) was dissolved in 100 ml of HCOOH. To this slightly yellow soln., **5a** (9.25 g, 0.052 mol) was immediately added at such a rate so that the temp. did not exceed 35–40°. The mixture was further stirred for 30 min. at r.t. and then poured into a 500-ml flask. The HCOOH was evaporated at 50° under reduced pressure. The yellow oily residue was taken up in 50 ml abs. MeOH. Abs. Et_2O (200 ml) was added, and the flask was placed for 1 h in the cold. The white precipitate was filtered, washed with 50 ml of abs. Et_2O, and then dried *in vacuo* at r.t. to yield 19.84 g (0.045 mol, 89.3%) of **1c**. UV: 234 (4.1). IR (KBr): 3300*m*, 3130*m*, 2960*w*, 1740*s*, 1660*s*, 1630*s*, 1600*s*, 1240*s*, 1220*s*. ^{1}H-NMR (D_2O): 2.1 (*s*, AcO); 2.56 (*s*, Me); 2.62 (*s*, Me); 3.37 (*t*, J = 5.9, CH_2); 4.37 (*t*, J = 5.9, CH_2O); 5.55 (*s*, CH_2N); 8.01 (*s*, 1 arom. H). FAB-MS: 307 (90, M^+), 230 (25), 186 (20), 123 (65), 122 (100), 93 (40). Anal. calc. for $C_{17}H_{24}N_4O_8S$ (444.46): C 45.96, H 5.44, N 12.61, S 7.21; found: C 46.08, H 5.47, N 12.63, S 7.11.

Titration of compound **1c** indicates the presence of 2 mol-equiv. of free HCOOH. (HCOOH content: calc. 20.71; found: 20.76).

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[11]) From slightly yellow, the reaction mixture turns over to a deep green.
[12]) Yield not corrected for 1.4% of H_2O.

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