The Correct Structures of "Dihydrothiamine". Resolution of a Long-Standing Controversy

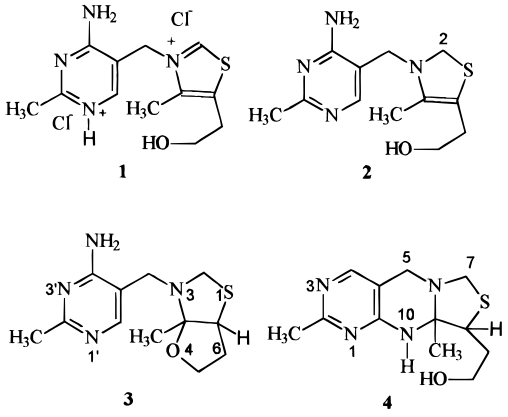
John A. Zoltewicz,* Carlton D. Dill, and Khalil A. Abboud

Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200[⊗]

Reduction of thiamin (**1**) by a borohydride in water first gives bicyclic perhydrofuro[2,3-*d*]thiazole **3**. Heating **3** in water generates fused tricyclic pyrimido[4,5-*d*]thiazolo[3,4-*a*]pyrimidine **4**. X-ray crystal structures indicate the stereocenters are *cis* in **3** and *cis-cis* in **4**. The kinetics of reduction of both **3** and **4** to tetrahydrothiamin **5** by additional aqueous borohydride show that **3** is the kinetic product of the reduction of **1** while **4** is the thermodynamic product produced from **3** in an acid-catalyzed ring-opening ring-closing isomerization process. Several structural assignments of "dihydrothiamine" presented some 40 years ago are incorrect. Subsequent reports based on the incorrect structures need to be reinterpreted.

In 1950 Karrer and Krishna reported that the reduction of thiamin[1] (vitamin B_1, **1**) using lithium aluminum hydride gave the sparingly water soluble thiazoline **2** (mp 150 °C) by the addition of hydride ion to position 2 of the thiazolium ring.[2−4] In 1957 Bonvicino and Hennessy reported the same structure **2** (mp 151 °C) but prepared the material by trimethoxyborohydride reduction of **1** at a reduced temperature in methanol−water. They also claimed that merely heating this dihydro material in water gave an isomer, mp 175 °C, to which they assigned structure **3**, a substituted bicyclic perhydrofuro[2,3-*d*]thiazole.[5] About the same time, scientists at the Takeda laboratories were preparing several dihydrothiamins by a number of routes, including hydride reduction of the vitamin and cyclization reactions that also made derivatives of the reduced materials. They reported *three* "dihydrothiamines": normal (mp 150 °C), *iso* (mp 160 °C), and *pseudo* (mp 175 °C). None of these was said to be the simple hydride adduct **2**. Instead, normal and *iso* were postulated to be ring-fused **3** having *trans* and *cis* stereochemistry, respectively, and the *pseudo* was said to be the fused tricyclic pyrimido[4,5-*d*]thiazolo[3,4-*a*]pyrimidine **4**. Oxidation of any of these three gave back **1**.[6−10] The IR spectra of the normal and *iso* materials *in solution* (chloroform) were reported to be essentially identical;[7] they matched that of Bonvicino and Hennessy.[5] The stereochemistry about the several chiral centers in **3** and **4** was never published.

Recently, we obtained from Dr. Bonvicino the same sample of dihydrothiamin that he prepared by the hydride reduction route during his graduate studies, a sample that generated the 1957 publication.[5] A proton NMR spectrum of this partially soluble old material in $CDCl_3$ indicated it was a mixture of substances, not including **2**. The obvious presence of diastereotopic protons, indicative of one or more chiral centers, immediately invalidated the originally proposed achiral structure **2** but did not eliminate the possibility that structural changes might have taken place in the solid over the many elapsed years.



The following work provides a resolution to the old controversy concerning the structures of dihydrothiamin, nicely outlined in detail in Beilstein.[11] X-ray crystal structures were obtained for **3** with its *cis* stereochemistry and **4** with *cis-cis* geometry. Kinetic studies making use of hydride reducing agents in the presence of dihydro **3** and **4** gave tetrahydrothiamin **5**[5,12] in aqueous buffers and provided information to show that **3** is the kinetic product of the hydride reduction of **1** while **4** is the thermodynamic product. While chiral **3** is homogenous in solution, **4** with three stereocenters, one of which is a nitrogen invertomer, exists as a mixture of diastereomers under some conditions. "Normal dihydrothiamine" (mp 150 °C), said to be *trans* **3**, is *cis* **3** and the mysterious and unstable "*iso*-dihydrothiamine" (mp 160 °C), claimed to be *cis* **3**, is likely to be only a polymorphic form of *cis* **3** with a higher melting point.

Results and Discussion

Two isomers of dihydrothiamin were prepared in accordance with the method of Bonvicino and Hennessy.[5] The lower melting isomer (149−151 °C), which we now show to have structure **3**, was made by the mild reduction of a neutralized aqueous-methanolic solution of thiamin chloride hydrochloride using $NaBH(OCH_3)_3$ at −12 °C,

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

(1) Zoltewicz, J. A.; Uray, G. *Bioorg. Chem.* **1994**, *22*, 1.
(2) Compounds reported in the literature are conveniently identified by their melting points.
(3) Karrer, P.; Krishna, H. *Helv. Chim. Acta* **1950**, *33*, 555.
(4) Karrer, P.; Krishna, H. *Helv. Chim. Acta* **1952**, *35*, 459.
(5) Bonvicino, G. E.; Hennessy, D. J. *J. Am. Chem. Soc.* **1957**, *79*, 6325.
(6) Iwatsu, T. *Yakugaku Zasshi* **1955**, *75*, 677.
(7) Matsukawa, T.; Hirano, H.; Iwatsu, T.; Yurugi, S. *J. Vitaminol.* **1957**, *3*, 213.
(8) Matsukawa, T.; Hirano, H.; Iwatsu, T.; Yurugi, S. *J. Vitaminol.* **1957**, *3*, 218.
(9) Matsukawa, T.; Iwatsu, T. *J. Vitaminol.* **1955**, *1*, 305.
(10) Hirano, H.; Iwatsu, T.; Yurugi, S. *Yakugaku Zasshi* **1957**, *77*, 241.
(11) *Beilsteins Handbuch der Organische Chemie, Supplement III/IV, Vol. 27/3*; Springer-Verlag: New York, 1983.
(12) Hirano, H. *Yakugaku Zasshi* **1958**, *78*, 1387.

S0022-3263(97)00782-2 CCC: $14.00

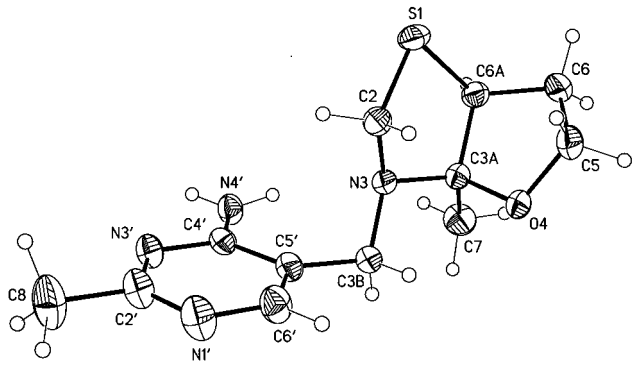


Figure 1. X-ray structure of perhydrofuro[2,3-*d*]thiazole **3**.

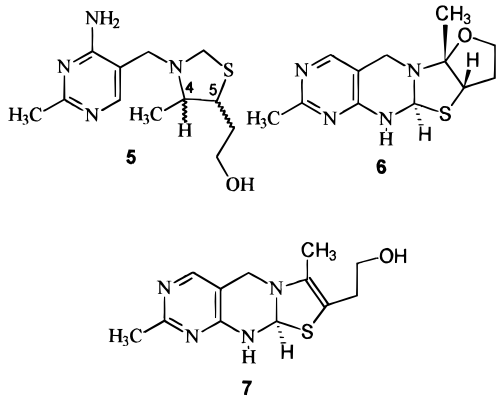
and the higher melting isomer (173−175 °C), **4**, was prepared from the lower melting substance **3** by dissolving it in hot water, adding Na_2CO_3, and extracting it into $CHCl_3$. Both preparations attempted to reproduce the original work.[5]

Crystal Structure of 3. Slow evaporation of a solution of **3** in ethyl acetate gave crystals that led to the X-ray generated structure in Figure 1 having a unit cell containing both of the enantiomers; it is a racemic compound and not a racemic mixture or conglomerate.[13] The pair of *cis*-fused five-membered rings has the bicyclic perhydrofuro[2,3-*d*]thiazole structure and form concave dihedral angles of 110.8° (N_3-C-O_4) and 113.6° (S_1-C-C_6). The thiazolidine methyl group and the methinyl hydrogen atom are slightly staggered and both are directed outside and above the envelope of the fused rings while one hydrogen atom on each of the N−**CH_2**−S and O−**CH_2**−CH_2 methylene units is directed inside the envelope. The two bridging Py−**CH_2**−N hydrogen atoms have different environments; one of these is located closer to the thiazolidine methyl group than the other by 0.69 Å while the second hydrogen is nearer by 0.93 Å to the pyrimidine proton, distances of significance in the subsequent NOE experiments. One hydrogen atom of the NH_2 group is directed toward the N-atom of the thiazolidine ring, forming a bent H-bond with a distance of 2.29 Å between the connected hydrogen and nitrogen sites. The resultant six-membered ring is a common feature of crystal structures.[14]

The molecular structure of **3** has some resemblance to the crystal structure of tetracyclic **6** (mp 175 °C), formed by simple deprotonation of **1** under mild conditions. Again, the *cis* perhydrofuro[2,3-*d*]thiazole unit is present among the four fused rings formed as a consequence of the addition of the amino group of the free base of **1** to the 2 position of the thiazolium ring to produce tricyclic **7** (dihydrothiochrome) followed by additional cyclization to give the two fused five-membered rings.[15,16]

NMR Analysis of 3. The structure of **3** in the solid state and in $CDCl_3$ solution is the same. Figure 2 contains chemical shift and stereochemical data and the results of NOE investigations.

A COSY analysis indicated that the closely spaced, unsymmetrical, two proton multiplet at 4.03 and 4.05 ppm was coupled with both of the high field **CH_2**−CH_2−O protons at 2.41 and 2.09 ppm and therefore they were assigned to CH_2-**CH_2**$-O$ group. The signal at 2.41 ppm also was coupled to the methinyl site at 3.78 ppm.



Distinguishing between the two remaining methylene groups was accomplished by adding **1** to D_2O, first allowing the thiazole proton N=C**H**−S to exchange to N=C**D**-S,[17,18] prior to reduction with $NaBH(OCH_3)_3$ in D_2O-CH_3OD to give N−C**DH**−S. The deuterium labeled compound showed a loss of geminal coupling at this partially exchanged methylene; the associated doublets (J = 8 Hz) at 3.78 and 3.62 ppm collapsed to apparent singlets. Therefore, the more highly coupled geminal methylene protons (14 Hz) at 3.92 and 3.62 ppm that did not undergo isotope exchange correspond to the bridging Py−C**H_2**−N unit. Moreover, the methinyl multiplet at 3.78 ppm now was largely isotope exchanged (>85%), thereby causing the signal at 2.41 ppm which is associated with the proton of the **CH_2**−CH_2−O unit *anti* to it to lose some of its multiplicity. Isotope exchange at the methinyl site is an observation of some significance when considering subsequently the mechanism of ring formation.

The presence of isotopes in the N−**CDH**−S group resulted in an interesting long range four-bond spectral change. This newly formed chiral center, present equally in both configurations, caused the bridging methylene protons (Py−**CH_2**−N) to assume slightly different chemical shifts; the doublet for each proton now was split into two additional lines with separations on the order of 2−4 Hz, giving an apparent doublet of doublets. However, the magnitude of the new separation was different for each of the methylene protons, and the magnitude also changed with the field strength (300 vs 500 MHz), showing that the new multiplicity was not due to spin coupling but rather to differential shielding by the chiral center.

NOE experiments on a $CDCl_3$ solution established the environment about the Py−CH_2−N grouping. On irradiation of the aromatic proton signal at 7.96 ppm, only *one* signal (3.62 ppm, 14 Hz) associated with one of the two protons of the Py−**CH_2**−N bridge was enhanced (5.2%). But on irradiation of the thiazolidine methyl signal at 1.56 ppm, the other proton of this methylene bridge at 3.92 ppm was enhanced (8.7%), along with the methinyl multiplet at 3.78 ppm (13%). The different NOE values for the methylene protons indicate there is likely to be a preferred conformation, one that may make use of H-bonding between the amino group and the tertiary nitrogen atom, as in the crystal.

(13) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; J. Wiley & Sons: New York, 1994.
(14) Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120.
(15) Takamizawa, A.; Hirai, K.; Ishiba, T.; Makino, I. *Chem. Pharm. Bull.* **1971**, *19*, 759.
(16) Siro, M.; Nakai, H.; Makino, I.; Takamizawa, A. *Acta Crystallogr.* **1978**, *B34*, 3424.
(17) Kemp, D. S.; O'Brien, J. T. *J. Am. Chem. Soc.* **1970**, *92*, 2554.
(18) Clarke, G. M.; Sykes, P. *J. Chem. Soc.* **1967**, 1411.

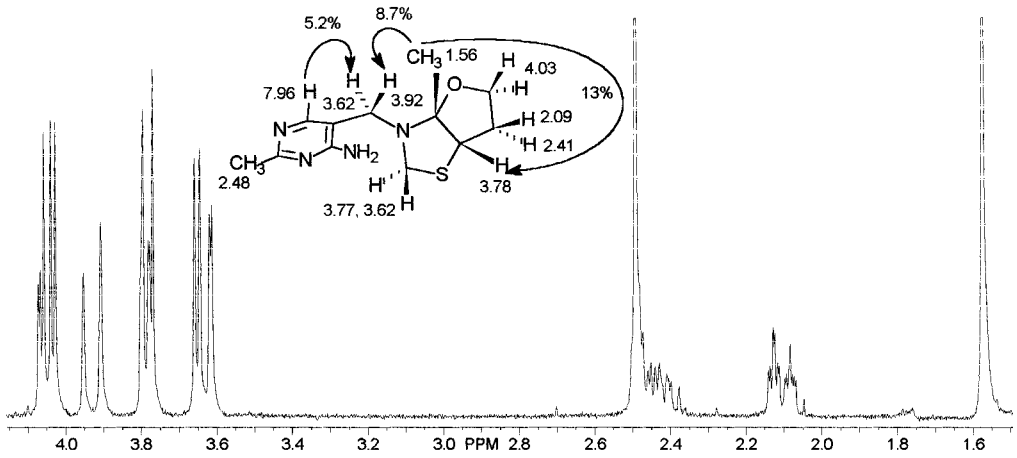


Figure 2. Proton NMR spectrum at 300 MHz of perhydrofuro[2,3-*d*]thiazole **3** in $CDCl_3$. The pyrimidine proton at 7.96 and the amino protons at 5.8 ppm are not included. Numbering corresponds to that in the X-ray structure. The arrows and percentages designate NOE values.

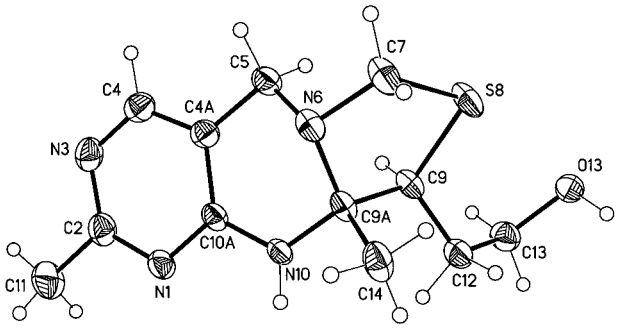


Figure 3. X-ray structure of pyrimido[4,5-*d*]thiazolo[3,4-*a*]pyrimidine **4**.

The APT ^{13}C spectrum of **3** contained signals for four carbons of the CH and CH_3 types and eight of the C and CH_2 types, in agreement with the proposed structure. The O−**C**−N carbon had a shift of 104.3 ppm making it distinguishable from the equivalent atom (N−**C**−N) found in isomer **4** at 84 ppm.

Crystal Structure of 4. A crystal grown from chloroform has molecular structure **4**, containing three fused rings that do not include the hydroxyethyl group. The central six-membered ring was formed by the addition of the pyrimidyl amino group to the C-methyl site of the reduced thiazolium ring; the tertiary nitrogen atom in this ring resides outside the plane formed by the other atoms in the ring, Figure 3. The three stereocenters defined by atoms NCC give rise to the *cis*−*cis* diastereomer. The five-membered ring has a puckered envelope-like conformation.

NMR Analysis of 4 and Its Diastereomers. The proton spectrum of the compound **4** (mp 175 °C) dissolved in alkaline water in order to suppress any pH-dependent isomerization, Figure 4, shows sharp signals and is consistent with the presence of just a single structure or a set of rapidly equilibrating structures at ambient temperatures, except for the presence of a small methyl peak at 1.46 ppm believed to be due to a diastereomer. The same compound when dissolved in unbuffered water gives signals that are less well resolved, possibly indicative of signal averaging due to isomerization, including minor peaks among the multiplets. Spectra of **4**, especially when dissolved in $CDCl_3$, show a number of small peaks in addition to the main spectrum, again suggesting the possibility of the presence of a diastereomer.

A COSY analysis (DMSO-d_6) allowed all the protons to be assigned, Figure 4. The structure is consistent with that found in the solid state. The multiplet at 1.69 ppm due to one of the protons of the **CH₂**−CH_2−O methylene group was coupled to the methinyl proton at 3.43 ppm; the other **CH₂**−CH_2−O multiplet at 2.18 ppm was coupled to both of the mutually coupled one proton multiplets at 3.58 and 3.70 ppm, the latter two being assigned to the CH_2−**CH₂**−O group. These **CH₂**−O protons are upfield relative to those in **3**. The pair of doublets (9 Hz) at 4.16 and 3.84 ppm were assigned to the N−**CH₂**−S methylene group on the basis of their small coupling constant;[19] they are shifted downfield relative to those for the equivalent group in **3**. The Py−**CH₂**−N bridge protons at 3.98 and 3.88 ppm are also shifted downfield with a coupling constant, 16 Hz, similar to the value of 14 Hz found for **3**.

NOE experiments, Figure 4, allowed the stereochemistry to be assigned. Irradiation of the thiazolidine methyl group at 1.45 ppm of a sample (D_2O) highly enriched in a single diastereomer by recrystallization caused enhancement of five signals at 4.16 (11%), 3.98 (6.5%), 3.43 (3.9%), 2.18 (4.3%), and 1.69 ppm (3.9%) corresponding to one *syn* proton in the methylene groups of N−**CH₂**−S and Py−**CH₂**−N, as well as the methinyl site and two protons in the **CH₂**−CH_2−O methylene unit, respectively. Irradiation of the methinyl proton caused a 5.5% enhancement of the Py−**CH₂**−N proton *syn* to it at 3.88 ppm. As expected, irradiation of the pyrimidyl proton generated NOEs (3.1%) in *both* of the adjacent methylene protons, in contrast with the observations for **3** where only a single site was enhanced.

The ^{13}C APT spectrum again demonstrated four CH and CH_3 types and eight C and CH_2 types, in agreement with structure **4**. Also, the N−**C**−N carbon was present at 84 ppm, 20 ppm higher than the analogous carbon (N−**C**−O) in **3**. These two signals at 104 and 84 ppm stand apart and therefore serve as convenient indicators of **3** and **4**, respectively.

Conditions for the Conversion of 3 to 4. The proton spectra of **3** and **4** separately dissolved in acidic phosphate buffer (pD = 6.25) at room temperature indicated that **3** after 22 h had converted to **4** with deuterium exchange of both the thiazolidine methyl and the methinyl protons; **4** experienced hydrogen−deute-

(19) Leeper, F. J.; Lowe, P. N. *Heterocycles* **1983**, *20*, 65.

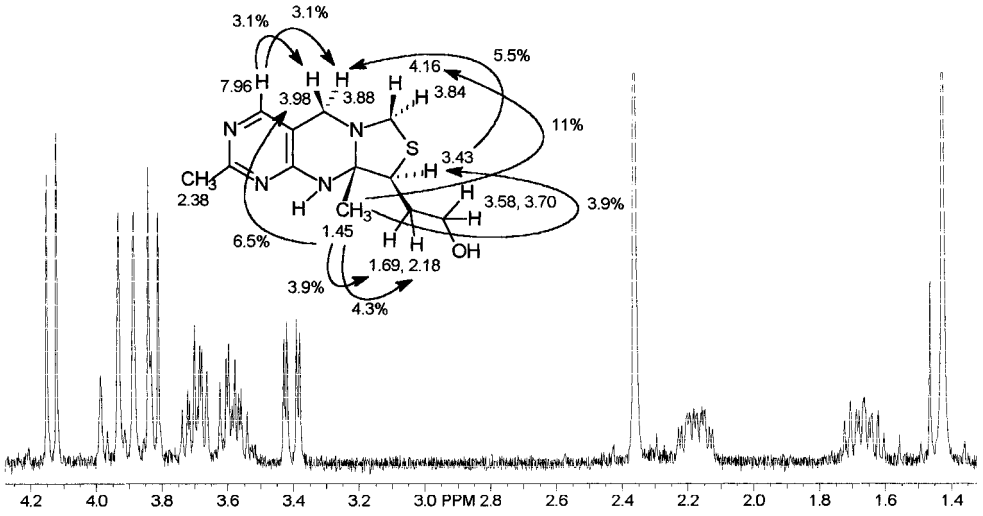


Figure 4. Proton NMR spectrum at 300 MHz of pyrimido[4,5-*d*]thiazolo[3,4-*a*]pyrimidine **4** in D_2O containing carbonate, not including the aromatic proton at 7.94 ppm. The minor methyl peak at 1.46 ppm is due to a diastereomer. The X-ray structure contains the same numbering pattern. The arrows and percentages designate NOE values.

rium exchange at the same two sites but otherwise remained unchanged structurally but several new peaks did appear, most noticeably at 3.88 (d) and 4.20 (d) ppm, again suggesting the presence of a stereoisomer.

In alkaline carbonate (pD = 9.77) at the same temperature and after the same time **3** was only partially converted to **4** while a separate sample of **4** was completely unchanged. Therefore, both isomers must form a common set of intermediates to allow isotope exchange to take place at the same sites in an acid dependent process, likely by way of opening of the fused rings in order to generate sites of unsaturation.

Kinetics of Trapping with Hydride. Knowing that further reduction of both dihydro isomers resulted in the formation of tetrahydro **5**,[5,18–20] and that hydride is a carbocation trapping agent,[21] the reactivity of each isomer toward hydride was examined in order to obtain information about the conditions required to generate the expected ring-opened intermediate.

Tetrahydro **5** exists in *cis* and *trans* diastereomeric forms that are distinguishable by proton NMR. The thiazolidine methyl substituent appears as a pair of doublets ($J = = 6$ Hz) at 1.30 ppm (minor, *trans*[19]) and 1.05 ppm (major, *cis*[19]). Their formation served as a convenient way to follow the reduction process.

On reduction of **3** with $NaBH_4$ in aqueous solution, one tetrahydro diastereomer was formed consistently and preferentially in a 3:2 (*cis*/*trans*) ratio. However, **4** did not react with $NaBH_4$ in aqueous carbonate but it did undergo reduction with $NaBH_3CN$ in the more acidic phthalic acid buffer (pD = 4.2). Tetrahydrothiamin was produced from **4** in a 9:11 (*cis*/*trans*) ratio, the other diastereomer now predominating slightly. Curiously, $NaBH_4$ reduction of **1** consistently gave the *cis* product as the major diastereomer (2.5:1) as reported[18,19] and in our hands as well (3.1:1), in contrast to our observations with this same hydride and **3** that gave a smaller ratio.

Under our conditions the rate of reaction of both hydrides with water is negligible,[22,23] although some bubbles were always evolved on mixing. The cyano hydride is some 10^8 times less reactive than the parent hydride toward water.[23] No attempt was made to control the ionic strength of the medium, which generally was high from the buffer, present in much higher concentration than the hydride in order to prevent increases in the pD value. Pseudo-first-order rate constants, k_{obs}, were obtained by following changes in the proton peak intensities of the closely spaced thiazolidine methyl groups in the starting material and products.

Table 1. Concentrations and Rate Constants for the Reduction of Dihydro 3 and 4 to Tetrahydrothiamin 5 by Borohydrides in Aqueous Buffers at 25 °C

substrate	[**3**] or [**4**], M	[hydride], M	pD	k_{obs} (s^{-1})	k_3 or k_2
3	1.1×10^{-2}	6.4×10^{-2} [a]	9.77[c]	2.45×10^{-4}	2.6×10^{7} [h]
3	1.1×10^{-2}	2.9×10^{-2} [a]	9.77[c]	1.36×10^{-4}	2.3×10^{7} [h]
3	2.1×10^{-2}	5.6×10^{-2} [a]	10.71[d]	1.71×10^{-5}	1.6×10^{7} [h]
4	2.3×10^{-2}	6.4×10^{-2} [b]	6.25[e]	5.07×10^{-4}	90[i]
4	1.2×10^{-2}	6.4×10^{-2} [b]	5.75[f]	2.19×10^{-4}	123[i]
4	1.0×10^{-2}	3.0×10^{-2} [b]	5.75[f]	1.68×10^{-4}	94[i]
4	1.2×10^{-2}	9.1×10^{-2} [b]	5.75[f]	2.12×10^{-4}	119[i]
4	3.7×10^{-2}	9.1×10^{-2} [b]	6.25[g]	5.56×10^{-4}	99[i]

[a] $NaBH_4$. [b] $NaBH_3CN$. [c] Buffer 1. [d] Buffer 2. [e] Buffer 3. [f] Buffer 4. [g] Buffer 5. [h] k_3 M^{-2} s^{-1}. [i] k_2 M^{-1} s^{-1}

Table 1 summarizes the kinetic results using carbonate buffer with $NaBH_4$ for **3**, and phosphate and acetate buffers with $NaBH_3CN$ for **4**. Attempted reduction of **3** with $NaBH_3CN$ at high pD resulted in only a meager amount of **5** along with ring degradation products. The data show that (1) the rate of reduction of **3** was first-order in hydride ion concentration, but the rate for **4** was independent of the hydride concentration, and (2) the rates for both substrates were clearly first-order in hydronium ion concentration. For **3**, the apparent third-order rate constant, k_3, was calculated making use of eq 2. The third entry in Table 1 for **3** shows a k_3 value 35% smaller than the average of the first two, perhaps because the initial ionic strength was twice as large or because some **2** was present at the higher pD as indicated by a consideration of the following mechanism.

With substrate **4** the apparent second-order rate constant, k_2, was calculated using eq 3.

(20) Bau, R.; Hyun, Y.-S.; Lim, J.; Choi, H.; Yannopoulos, A.; Hadjiliadis, N. *Inorg. Chim. Acta* **1988**, *150*, 107.
(21) Brown, H. C.; Bell, H. M. *J. Org. Chem.* **1962**, *27*, 1928.
(22) Schlesinger, H. I.; Brown, H. C.; Hoekstra, H. R.; Rapp, L. R. *J. Am. Chem. Soc.* **1953**, *75*, 199.
(23) Lane, C. F. *Synthesis* **1975**, 135.

$$k_3 = k_{obs}/[D_3O^+][NaBH_4] \quad (2)$$

$$k_2 = k_{obs}/[D_3O^+] \quad (3)$$

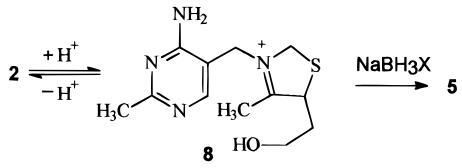
Mechanism of Ring-Opening and Ring-Closing. Hydrogen−deuterium isotope exchange took place at the same methyl and methinyl sites of the thiazolidine ring in **3** and **4**, and hydride ion reduction of each gave the same product **5**, suggesting a common set of ring-opened intermediates for the reduction of both of these to **5** in a pH dependent process, Scheme 1. Thus, the mechanism appears to involve protonation of the oxygen atom in **3** and a nitrogen atom in **4** to give iminium ion **8** resulting from ring cleavage. The iminium ion then can undergo a number of reactions: (a) isotope exchange at the methinyl and the thiazolidine methyl sites by deprotonation of **8** to an enamine such as **2** in the case of isotope exchange at the methinyl site, and this then is followed by deuteronation to give back **8** now isotopically labeled or (b) be trapped by hydride to give tetrahydro product **5**[18] or (c) ring close in an intramolecular reaction to form either **3** or **4** following the loss of a proton.

The *cis*/*trans* ratio for **5** is not the same starting from aromatic **1** (2.5−3.1 to 1) and from dihydro **3** (3/2) or **4** (9/11). The former two reactants make use of $NaBH_4$ and the latter $NaBH_3CN$. Although the ratios are similar starting from **3** and **4**, these values are clearly smaller than that beginning with **1**,[18] showing that more *trans* tetrahydro material is generated from the two dihydro isomers. The reason for the larger *cis*/*trans* ratio for **5** from **1** than from **3** with the same hydride donor is not apparent. However, under the conditions starting from **1**, reducible tricyclic enamine **7** is present,[24−27] providing another possible route to reduced material, one in which the order of addition of the two hydrogen atoms is likely to be reversed.

Cyclization to form a five-membered ring, here 5-*exo-trig*, generally is much faster than closure to a six-membered ring, 6-*exo-trig*.[28] Moreover, the pyrimidyl amino group should undergo some rotation and rehybridization on addition to give the six-membered central ring and this change should contribute to the energy barrier; in the microscopic reverse ring-cleavage process, the transition state is expected to adopt an E1cb-like, antiparallel geometry to generate **8**. Hence, **3** is a kinetic product and **4** is a thermodynamic product of cyclization. Cyclization back to **3** is faster than hydride reduction and so there is a kinetic dependence on the $[BH_4^-]$, reduction becoming the rate-limiting step in the forward direction to yield **5**. By contrast, cyclization back to **4** is so slow that trapping by the less reactive BH_3CN^- is more rapid. Thus, in the forward process involving the reduction of **4** to **5**, ring-opening of protonated **4** becomes the rate-limiting step. At low pH any conversion of **4** to **3** by way of the open-chain intermediate **8** should be rapidly reversible, giving back the lower energy **4**.

There is some question about where to locate the site of protonation in **4**. In the case of **1** protonation is known to take place at the pyrimidyl ring nitrogen atom shown in **1**.[29] But we favor protonation at the amino nitrogen

Scheme 1



atom of **4** that leaves as an amino group on ring-cleavage rather than protonation at a nitrogen atom of the pyrimidine ring. This protonation avoids the formation of a high energy imino tautomer of the amino pyrimidine on departure. However, the favored protonation process requires the addition to **8** of the weakly basic[30,31] and weakly nucleophilic amino group in the forward direction. But a similar addition of the amino group to the 2 position of the thiazolium ring to give tricyclic **7** has been advanced.[25−27]

Ring opening to iminium ion **8** followed by ring closure may change the stereochemistry about the thiazolidine methyl group, giving *cis*−*trans* isomers about the CC bond. Inversion of configuration at the stereogenic tertiary nitrogen atom is expected to be facile, leading to equilibrating *cis* and *trans* ring-fused structures.[32] Likely, all four diastereomers are formed on the initial conversion of **3** to **4** as suggested by NMR spectra, clearly showing a set of minor peaks that are consistent with the presence of another diastereomer. Recrystallization enriches that set of diastereomers having the two chiral carbon centers *cis* as found in the crystal structure.

"Normal and *Iso*-dihydrothiamine". We were not able to repeat the reported (no yield) conversion of the lower melting (150 °C) "normal" to the higher melting "*iso*" (160 °C) dihydro material by adding 10% NaOH to an *ethanolic* solution of the 150 °C substance followed by recrystallization from ethanol.[9] But we were able to generate a material (60%) that melted at 160 °C by adding 10% aqueous hydroxide to a *methanolic* solution of the lower melting substance resulting in the rapid formation of a precipitate. Recrystallization avoided the use of a hydroxylic solvent that might be conducive to a rearrangement. This solid on addition to the 150 °C substance gave a mixed melting range between that of the lower and the higher melting (160 °C) compounds as originally reported.[6,9] But the NMR spectra of the two materials separately dissolved in $CDCl_3$ were identical. All attempts to grow a crystal for X-ray analysis caused reversion of the higher back to the lower melting material.

Conclusions and Consideration of the Old Literature Bonvicino and Hennessy misidentified both isomers of dihydrothiamin, attributing structure **2** to the 151 °C isomer (now known to be **3**) and the structure **3** to the material melting at 175 °C that we now know as **4**.

The Japanese scientists correctly identified the 175 °C material as **4** but misidentified the structure of the materials melting at 150 °C and 160 °C, considering them to be the *trans* and *cis* diastereomers of **3**, respectively. We believe both of these latter two materials to be polymorphic forms of *cis* **3**, one as the racemic compound

(24) Maier, G. D.; Metzler, D. E. *J. Am. Chem. Soc.* **1957**, *79*, 4387.
(25) Chahine, J. M. E. H.; Dubois, J. E. *J. Chem. Soc., Perkin Trans. 2* **1989**, 25.
(26) Chahine, J. M. E. H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1045.
(27) Washabaugh, M. W.; Yang, C. C.; Hollenbach, A. D.; Chen, P. *Bioorg. Chem.* **1993**, *21*, 170.
(28) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.
(29) Cain, A. H.; Sullivan, G. R.; Roberts, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 6423.
(30) Schellenberger, A. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1024.
(31) Schellenberger, A. *Chem. Ber.* **1990**, *123*, 1489.
(32) Crabb, T. A.; Katritzky, A. R. *Adv. Heterocycl. Chem.* **1984**, *36*, 1.

(mp 150 °C) and other as the metastable racemic conglomerate (mp 160 °C).[13]

The correct structure of "dihydrothiamine" as enamine **2** generally has been accepted. Therefore, those reports[33−37] claiming **2** need to be reevaluated.

Experimental Section

General. Thiamin chloride hydrochloride, $NaBH(OCH_3)_3$, $NaBH_4$, $NaBH_3CN$, CD_3CO_2D, and CD_3CO_2Na were commercially available and were used without further purification. NMR data were collected on either a 300 or a 500 MHz instrument.

Dihydrothiamine Prepared by Dr. G. Bonvicino in the 1950's. The needles of our 40-year old sample had largely changed to powder, the combustion analysis was essentially correct for the original empirical formula for dihydrothiamin, and the melting point, curiously, was virtually unchanged (150−155 °C). Mixed melting of equal amounts of the old sample and **3** gave a substantial depression (135−142 °C) but mixed melting with **4** gave no depression (151−156 °C). The melting range of an equimolar mixture of authentic **3** and **4** was 135−142 °C. Partial extraction into $CDCl_3$, leaving some unidentified yellow residue, showed the presence of dissolved **4** by proton NMR. The old material now consisted of some **3**, mostly **4**, and possibly a third substance that was not identified. It is likely that **4** originated in the solid state over the years because the sample when added to D_2O gave the spectrum of **4** without deuterium incorporation at the methinyl site, indicating that protonation of enamine **2** and cyclization had taken place prior to dissolution of the old solid in D_2O. Anal. Calcd for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.81; N, 21.03. Found: C, 53.62; H, 6.81; N, 20.90.

Dihydrothiamin 3 or *cis*-[3-(2′-Methyl-4′-amino-5′-pyrimidyl)methyl]-3a-methylperhydrofuro[2,3-*d*]-[1,3]thiazole. This procedure follows that published in 1957.[5] Thiamin chloride hydrochloride (5.00 g, 14.8 mmol), dissolved in water (15 mL) and cooled to 0 °C, was treated with 1 N NaOH (15 mL, 15 mmol) and MeOH (20 mL). After cooling to −12 °C, $NaBH(OCH_3)_3$ (2.40 g, 19.0 mmol) was added in small portions over 20 min. The mixture was warmed to room temperature, and a heavy white precipitate formed after several hours. The solid was recrystallized from EtOAc and hexanes to afford 2.37 g (8.90 mmol, 60.1% yield, mp 149−151 °C). ^{1}H NMR ($CDCl_3$): 7.96 (1H, s), 5.79 (2H, s, NH_2), 4.03 (2H, dd J = 4 and 9 Hz), 3.92 (1H, d J = 14 Hz), 3.78 (1H, dd, J = 2 and 6 Hz), 3.77 (1H, d, J = 8 Hz), 3.62 (1H, d, J = 14 Hz), 3.62 (1H, d, J = 8 Hz), 2.48 (3H, s), 2.41 (1H, m), 2.09 (1H, dtd, J = 1, 4, and 12 Hz), 1.56 (3H, s). ^{13}C NMR ($CDCl_3$): 167.1, 162.6, 154.7, 110.2, 104.3, 68.5, 55.0, 54.5, 47.7, 35.4, 25.5, 23.1. Anal. Calcd for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.81; N, 21.03. Found: C, 54.38; H, 6.71; N, 20.95.

Deuterated Dihydrothiamin 3. The method is substantially the same as that above except that the solvent contained OD in place of OH. Na_2CO_3 was used to make an alkaline solution and CH_3OD was the cosolvent. Product (0.86 mmol, 57% yield, mp 147−149 °C) had the same proton NMR ($CDCl_3$) as **3** except that the doublets at 3.77 and 3.62 ppm had collapsed to singlets, and the methinyl site at 3.78 ppm was >85% exchanged.

Dihydrothiamin 4 or *cis,cis*-9-(2-Hydroxyethyl)-2,9a-dimethyl-5,9,9a,10-tetrahydro-7*H*-pyrimido[4,5-*d*]thiazolo[3,4-*a*]pyrimidine. This procedure follows that published in 1957.[5] The white solid was recrystallized from EtOAc, hexanes, and MeOH (22% yield, mp 173−175 °C). ^{1}H NMR (D_2O): 7.94 (1H, s), 4.16 (1H, d J = 9 Hz), 3.98 (1H, d J = 16 Hz), 3.88 (1H, d, J = 16 Hz), 3.84 (1H, d, J = 9 Hz), 3.72 (1H, m), 3.62 (1H, m), 3.43 (1H, d, d, J = 3 and 12 Hz), 2.38 (3H, s), 2.18 (1H, m), 1.69 (1H, m), 1.45 (3H, s). ^{13}C NMR (D_2O): 170.7, 161.6, 156.2, 111.4, 84.1, 65.4, 57.5, 55.7, 48.6, 38.0, 28.4, 25.5. Anal. Calcd for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.81; N, 21.03. Found: C, 53.84; H, 6.89; N, 20.80.

General Procedure to Observe H−D Isotope Exchange. Two samples of **3** and **4** (4.0 mg) in four NMR tubes were dissolved in 0.1 mL of CD_3OD. Buffers (1 and 3) below were added. After 22 h at room temperature their ^{1}H NMR spectra were recorded. Observations are reported in the text.

Tetrahydrothiamin 5.[5,18] The reductions to tetrahydro **5** were accomplished by dissolving 50 mg (0.19 mmol) of **3** or **4** in 4 mL of MeOH and adding borax buffer and a molar excess of $NaBH_4$ along with **3** or phthalate buffer and excess $NaBH_3CN$ with **4**. The reactions were monitored by TLC on silica gel. Tetrahydrothiamin was isolated by extracting the aqueous mixtures with chloroform, then drying over K_2CO_3 and evaporating to afford the white product consisting of a mixture of diastereomers, mp from **3** 122−127 °C and from **4** 120−125 °C (lit.[18] 129−131 °C for the mixture and 145 °C for purified material[5]). The average yield for several experiments was 75%. Anal. Calcd for $C_{12}H_{20}N_4OS.1/3H_2O$ (unrecrystallized raw product) C, 52.53; H, 7.59; N, 20.42. Found: C, 52.27; H, 7.93; N, 20.27.

The original preparation of **5** from **1** with $NaBH_4$ under alkaline conditions[18] was repeated at 0° C and at room temperature. The unrecrystallized product had a *cis*/*trans* ratio of 3.1:1 (lit.[18] 2.5:1). Under these conditions with the intermittent addition of the hydride, the solution had a yellow color indicative of the presence of **7** and its bicyclic sulfide ring-opened derivative.[24] Attempts to reduce **1** with $NaBH_3CN$ in water resulted in the precipitation of a slightly soluble borohydride salt.

Buffers Used in Kinetic Runs with Hydride in D_2O. Buffer (1): pD 9.77; carbonate 0.25 M, bicarbonate 0.25 M; (2): pD 10.71; carbonate 0.625 M, bicarbonate 0.125 M; (3): pD 6.25 deuterophosphate 0.050 M, dideuterophosphate 0.10 M; (4): pD 5.75; acetate 0.75 M, acetic acid 0.15 M; (5): pD; 6.25 acetate 0.75 M, acetic acid 0.050 M.

General Procedure for the Kinetics of Reduction of 3 and 4 with a Borohydride at 500 MHz. To **3** in 0.1 mL of CD_3OD in an NMR tube was quickly added 0.6 mL of a freshly prepared D_2O solution of carbonate- buffered $NaBH_4$, prepared by adding the solid hydride to the alkaline solution in order to minimize decomposition. Timing began upon mixing. The heights of the thiazolidine methyl peak in the starting material and the two methyl diastereomeric peaks in reduced product **5** were measured at 25 °C. (Peak heights proved to be more accurate than peak areas due to the proximity of other signals.) The logarithm of the mole fraction of **3**, ([**3**]/([**3**] + [**5**]), versus time was subjected to a least squares analysis to give the rate constant. Plots were constructed for at least two half-lives.

With **4**, phosphate and acetate buffers and $NaBH_3CN$ were employed. The same kinetic treatment was applied.

***"Iso*-Dihydrothiamine".** When 50 mg (0.19 mmol) of dihydrothiamin **3** (mp 149−151 °C) in MeOH (3 mL) was treated with 10% NaOH (5 mL) and stirred for a few minutes, a white precipitate formed. The solid was collected, carefully washed with cold water to remove excess alkali, dried, and recrystallized from EtOAc and hexanes to afford 22 mg (0.083 mmol, 43% yield, mp 156−158 °C) of product. ^{1}H, NMR ($CDCl_3$): δ 7.96 (1H, s), 5.79 (2H, s), 4.03 (2H, dd J = 4 and 9 Hz), 3.92 (1H, d J = 14 Hz), 3.78 (1H, dd, J = 2 and 6 Hz), 3.77 (1H, d, J = 8 Hz), 3.62 (1H, d, J = 14 Hz), 3.62 (1H, d, J = 8 Hz), 2.48 (3H, s), 2.41 (1H, m), 2.09 (1H, dtd, J = 1, 4, and 12 Hz), 1.56 (3H, s). Anal. Calcd for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.81; N, 21.03. Found: C, 54.27; H, 6.71; N, 21.09. When the solid was dissolved in a mixture of EtOH, hexanes, and EtOAc and allowed to stand, the resultant crystals had a lower melting point corresponding to the isomer (mp 149−151 °C) from which it was prepared.

X-ray Information. Slow solvent evaporation of a solution of **3** in EtOAc resulted in the formation of needles, mp 149−

(33) Hadjiliadis, N.; Markopoulos, J. *J. Chem. Soc., Dalton Trans.* **1981**, 1635.
(34) Risinger, G. E.; Breaux, E. J.; Hsieh, H. H. *J. Chem. Soc., Chem. Commun.* **1968**, 841.
(35) Sorensen, H. C.; Ingraham, L. L. *Biochim. Biophys. Acta* **1970**, *208*, 343.
(36) Sorensen, H. C.; Ingraham, L. L. *J. Heterocycl. Chem.* **1970**, *7*, 711.
(37) Moorthy, P. N.; Haynon, E. *J. Org. Chem.* **1977**, *42*, 879.

151 °C, that were submitted for X-ray analysis. CH_3Cl was used to grow a crystal of **4**, mp 173−175 °C.

Compound **3**: $C_{12}H_{18}N_4OS$, $M_r = 266.36$, monoclinic, $P2(1)/n$, $a = 6.3749(1)$ Å, $b = 23.1887(3)$ Å, $c = 9.724$ Å, $\alpha = 90°$, $\beta = 106.94°$, $\gamma = 90°$, $V = 1375.04(3)$ Å^3, Z = 4, $D_{calcd} = 1.287$ g cm^{-3}, Mo Kα ($\lambda = 0.71073$ C), $T = 173$ K. Compound **4**: $C_{12}H_{18}N_4OS$, $M_r = 266.36$, orthorhombic, $Pbca$, $a = 6.7405(1)$ Å, $b = 16.9618(1)$ Å, c = 23.0344(2) Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 2633.55(5)$ Å^3, $Z = 8$, $D_{calcd} = 1.344$ g cm^{-3}, Mo Kα ($\lambda = 0.71073$ Å), $T = 173$ K.

Data for both compounds were collected at 173 K on a Siemens CCD SMART PLATFORM equipped with a CCD area detector and a graphite monochromator utilizing Mo Kα radiation ($\lambda = 0.71073$ Å). Cell parameters were refined using 5969 (compound **3**) and 6943 (compound **4**) reflections from each data set. A hemisphere of data (1381 frames) was collected using the ω-scan method (0.3° frame width). The first 50 frames were remeasured at the end of data collection to monitor instrument and crystal stability (maximum correction on I was < 1%). Absorption corrections were applied based on the psi scan using the entire data sets.

Both structures were solved by the Direct Methods[38] in SHELXTL5, and refined using full-matrix least squares on F^2. The non-H atoms were refined with anisotropic thermal parameters. All of the H atoms were included in the final cycle of refinement and were riding on the atoms to which they are bonded. In each compound one of the methyl H atoms was disordered, and they were refined in riding models in two parts with 50% occupancy for each part. For compound **3**, 173 parameters were refined in the final cycle of refinement using 2658 reflections with $I > 2\sigma(I)$ to yield R_1 and wR_2 of 3.82% and 8.99%, respectively. And for compound **4**, 173 parameters were refined in the final cycle of refinement using 2280 reflections with $I > 2\sigma(I)$ to yield R_1 and wR_2 of 4.82% and 9.26%, respectively. Refinement was done using F^2.

Acknowledgment. The National Science Foundation and the University of Florida kindly provided funds for the purchase of X-ray equipment.

Supporting Information Available: Crystallographic parameters for **3** and **4** (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO970782N

(38) Sheldrick, G. M. **1995**, SHELXTL5. Siemens Analytical Instrumentation. Madison, WI.