Novel S-Alkylation Products from "Isolated Thiamin Ylide" via Thiaminium Neothiaminthiolate Ion Pair

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The ambident anion character of thiamin ylide was demonstrated by alkylation reactions of the "isolated thiamin ylide" under neutral or basic conditions. Alkylation with alkyl halides yielded *S*-alkyl derivatives of the yellow form of thiamin (pyrimidopyrimidine derivatives) instead of 2-alkylthiamin. These and previous results provide chemical evidence that thiamin ylide and thiaminium neothiaminthiolate (8) undergo a facile interconversion in EtOH.

The reaction of thiamin 1 toward a base is one of the major problems in thiamin chemistry. The neutralization reaction of 1 depends on the nature and mole ratio of the base as well as the solvent. Under aqueous alkaline conditions, treatment of 1 with 2 mol of NaOH (with respect to thiamin monochloride, TH^+Cl^-) gives the Na salt of thiamin thiolate (2, TS^-Na^+), while in the presence of 2 mol of NaOEt in EtOH, 1 yields the Na salt of the yellow form of thiamin (3, YF^-Na^+). These compounds have been isolated or spectroscopically characterized (Scheme I).[1]

When neutralization of 1 is attempted with an equimolar amount of base, a problem arises. Maier and Metzler[2] reported first the reaction of 1 with an equimolar amount of NaOMe, where they proposed the isolation of the tricyclic form 4 (dihydrothiochrome), whose chemical properties remain unexplored. However, a different structure was proposed by Risinger et al.[3] for the same compound: a tetracyclic derivative 5 was assigned in which a hydroxyethyl group added intramolecularly to the thiazoline double bond of 4. In 1978, Takamizawa et al.[4] synthesized compound 5, whose structure was determined by X-ray analysis. Doughty et al.[5] carried out the neutralization of 4-ethoxycarbonyl (instead of hydroxyethyl) substituted thiamine with NEt_3 and obtained the dihydrothiochrome derivative 6 without intramolecular Michael addition of the thiazole 5-substituent.

We assigned the equimolar NaOEt neutralized product of 1 as thiamin ylide 7,[6] instead of 4 or 5, although the melting point of 7 was almost the same as that reported by Maier et al. Poor reproducibility of the experiment prevented us from further examining the salt-free product. However, 7 could be isolated in high yield as a mixture with NaCl and remained stable under N_2. The structural determination was based on spectroscopic data and chemical behavior. Treatment of the isolated thiamin ylide 7 with weak acids such as an aromatic carboxylic acid or thiophenol yielded thiamin, and reaction with NaOEt afforded the yellow form of thiamin 3. These results clearly indicated that 7 is an intermediate between thiamin and the yellow form of thiamin. Hopmann et al.[7] reported the same conclusion from their kinetic studies. There is a possibility that the "isolated thiamin ylide" might be partially or predominantly in the form of the "thiaminium neothiaminthiolate" ion pair in the solid or solution state. Carbanion reactivity toward a number of electrophiles, such as aldehydes and disulfides in addition to proton donors under neutral conditions indicates that the reactivity of the isolated ylide is the same as those of the in situ generated thiamin ylides.[8,9] The most important chemical property of 7 is the disproportionation to form ion pair 8 under nonacidic condition. Maier et al.[2] described the observation of disproportionation properties with their isolated neutralized product of thiamin to which the dihydrothiochrome structure 4 was assigned.

The disproportionation properties of 7 indicate that thiamin ylide has an ambident anionic character; that is,

Scheme I. Reactivity of Thiamin toward Base

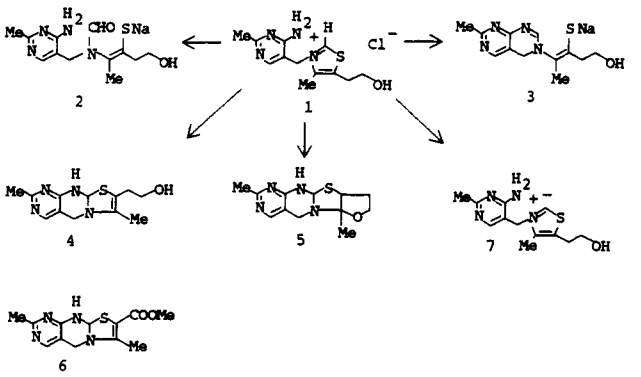

Table I. Reaction of 7 with RX in the Presence of NaOEt

9	R	yield, %
9a	Me	91
9b	Et	83
9c		80

(1) (a) Zima, O.; Williams, R. R. *Chem. Ber.* **1940**, *73*, 941. (b) Asahi, Y.; Mizuta, E. *Talanta* **1972**, *19*, 567.
(2) Maier, G. D.; Metzler, D. E. *J. Am. Chem. Soc.* **1957**, *79*, 4386–6583.
(3) Risinger, G. E.; Breaux, E. J.; Hsieh, H. H. *J. Chem. Soc., Chem. Commun.* **1968**, 841.
(4) Siro, M.; Nakai, H.; Makino, I.; Takamizawa, A. *Acta Crystallogr., sect. B* **1978**, 1334, 3424.
(5) (a) Doughty, M. B.; Lawrence, D. S. *J. Chem. Soc., Chem. Commun.* **1985**, 484. (b) Doughty, M. B.; Risinger, G. E. *Ibid.* **1985**, 1551.
(6) Sugimoto, H.; Hirai, K. *Tetrahedron Lett.* **1985**, *26*, 883.
(7) Hopmann, R. F. W. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 32.
(8) Sugimoto, H.; Hirai, K. *Heterocycles* **1987**, *26*, 13.
(9) One of the referees pointed out that thiamin ylide is a high-energy structure and should not survive EtOH washing during isolation, judging form the reported pK_a (=18) of thiamin. However, a survey of the literature reveals that the pK_a of thiamin ranges between 9 and 18.[10] Jencks's data[10a] were some of the extreme. Generally, the pK_a of thiamin is evaluated by the kinetic procedures of H–D exchanges of thiamin under acidic aqueous solution, which need the assumption of the rate constant for the proton transfer of thiamin ylide. Dubois et al.[11] suggested that the pK_a of thiamin at neutral solution was 9.7. Thus, the problem of pK_a of thiamin, which was discussed in a recent review,[17a] remains unresolved.
(10) (a) Washbaugh, M. W.; Jencks, W. P. *Biochemistry* **1988**, *27*, 5044. (b) Zoltewicz, J. A.; Urey, G. *J. Org. Chem.* **1980**, *45*, 2104.
(11) Chahine, J.-M. E. H.; Dubois, J.-E. *J. Chem. Soc., Perkin Trans 2* **1988**, 1409. These authors propose nucleophilic attack of OH^- toward the C-2 carbon of the thiazolium ring of thiamin at pH < 11 and abstraction of the C-2 proton at pH > 11 but gave no explanation about the pH dependency of the role of hydroxide anion. In neutral solution they assumed the equilibrium of deprotonation of thiamin ($K = 1.95 \times 10^{-10}$ M, hence, $pK_a = 9.70$), though they assigned the neutralized species to be pseudo-B_1 instead of thiamin ylide.

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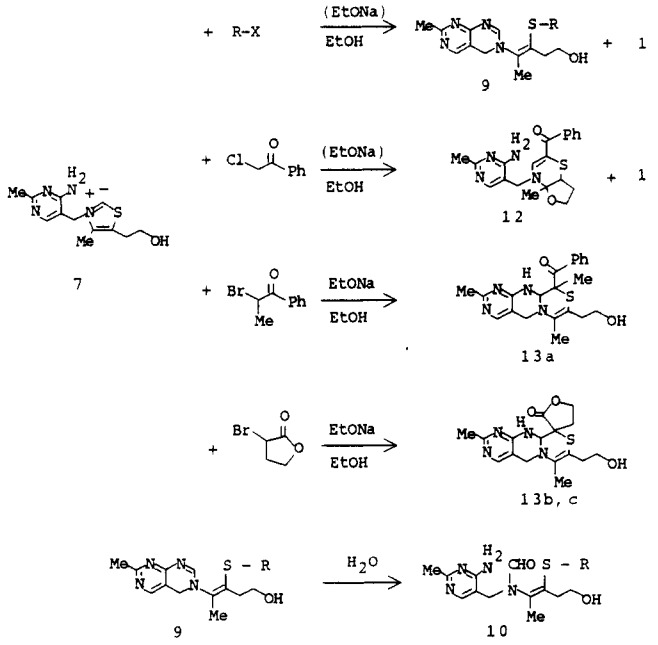
Scheme II. Alkylation Reaction of Isolated Thiamin Ylide



it exists as both carbanion and thiolate anion. The carbanion reactivity has been well studied with respect to the mechanism of the enzymatic behavior of thiamin. However, several reactions have been reported where the derivative of the yellow form of thiamin was formed by the reaction of in situ generated thiamin ylide with an electrophile. Zima and Williams[1] isolated the Na salt of the yellow form **3**. Kasahara[12] reported the formation of neocyanothiamin (**9**, R = CN). The dimeric form of the free yellow form has been reported,[13] but the chemistry of the yellow form of thiamin has not been related to the mechanistic study. In kinetic studies, the yellow form of thiamin has been proposed as an intermediate for the alkali-induced transformation of **1**; however, the cationic species is usually assumed to be the Na cation or it is not explicitly taken into consideration.

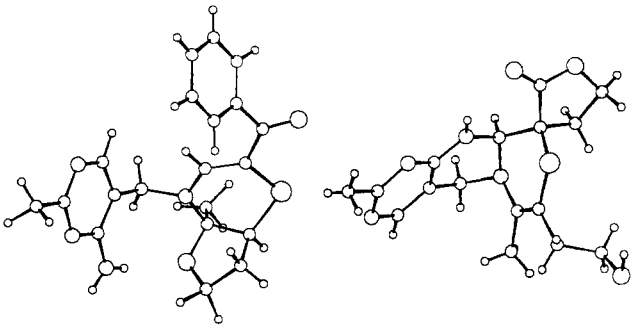
We carried out electrophilic reactions of the isolated thiamin ylide with alkylating agents under neutral or basic conditions and found the formation of derivatives of the yellow form of thiamin.[14] We report here a new reactivity of thiamin ylide.

Results and Discussion

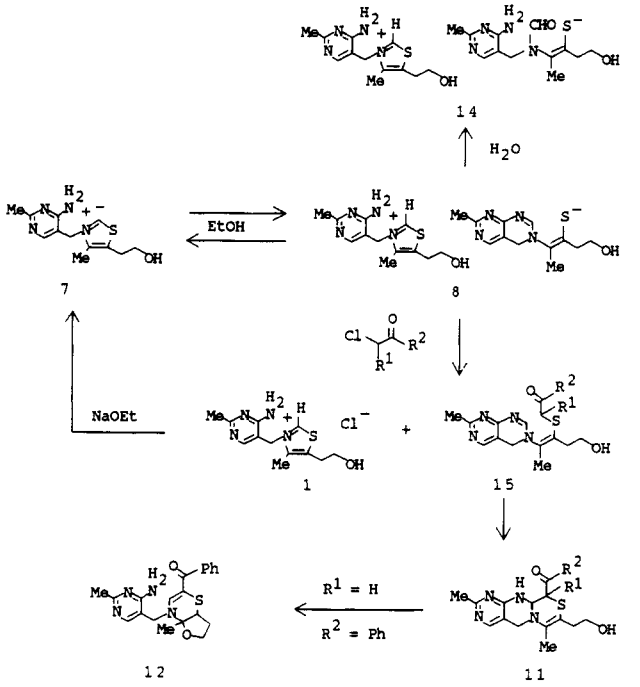
The results of alkylation of isolated thiamin ylide are summarized in Scheme II. Reaction of **7** with alkyl or aryl halides in EtOH gave thiamin **1** and 2-methyl-6-(3-(substituted thio)-5-hydroxy-2-penten-2-yl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (S-substituted yellow form, **9**) in almost quantitative yields. When the reaction was carried out in the presence of an equimolar amount of NaOEt, only **9** was obtained in high yield (Table I).

The structural proof of **9** is based on spectroscopic data and the chemical behavior of these compounds. Treatment of **9** with H_2O or SiO_2 resulted in the formation of ring-opened known compounds **10**. No C2-substituted thiamin was obtained. This alkylation reaction affords a novel and versatile method for preparing derivatives of the yellow form. Risinger and Hsieh[15] reported the reaction of **1** with phenacyl chloride in the presence of base to obtain yellow

Scheme III. PLUTO Drawing of 12 and 13c



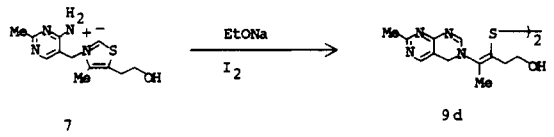
Scheme IV. Reaction Course of the Alkylation of Thiamin Ylide



crystals, which were identified as the phenacyl derivative of **9** (R = phenacyl). The reaction of **7** with phenacyl chloride in EtOH under neutral conditions gave yellow crystals **12** in addition to **1**. When the same reaction was carried out in the presence of NaOEt, the product **12** was isolated in 46% yield. The NMR spectra of **12** showed δ at 7.10 (1 H) and 6.50 (2 H, NH_2). Furthermore, this product was stable toward H_2O. In light of these data, the product was not a derivative of the yellow form of thiamin. The chemical structure was finally determined by X-ray analysis, as shown in Scheme III. Product **12** was determined to be an enantiomeric mixture, and the X-ray data of one isomer are given.

The reaction of a secondary α-halo ketone with **7** was different from that of a primary α-halo ketone, such as phenacyl chloride. The reaction of **7** with α-bromopropiophenone afforded yellow products **13a**. The NMR spectrum of **13a** suggested the formation of a (pyrimidopyrimidino)thiazine skeleton; however, no crystalline products were isolated. The structure was determined by spectroscopic comparison with those of **13b** or **13c**, which are discussed below. With α-bromo-γ-butyrolactone as an electrophile, two products were obtained, whose NMR spectra indicate that they were ca. 1:1 diastereomeric isomers. The spectra exhibit the presence of NH and CH protons. The structure of one of the diastereoisomers was determined by X-ray analysis and is shown in Scheme III.

(12) Kasahara, S. *Chem. Pharm. Bull.* **1960**, *8*, 340.
(13) Risinger, G. E.; Parker, P. N.; Hsieh, H. H. *Experientia* **1965**, 434.
(14) Sugimoto, H.; Hirai, K. *Heterocycles* **1988**, *27*, 877.
(15) Risinger, G. E.; Hsieh, H. H. *Chem. Ind.* **1966**, 1600.



7 9d

Figure 1.

The reaction pathway for the alkylation of 7 is shown in Scheme IV. In neutral water, ion pair 8 (thiaminium neothiaminthiolate, TH^+YF^-) is converted further into ion pair 14 (thiaminium thiaminthiolate, TH^+TS^-) via ring opening of the pyrimidopyrimidine ring by H_2O.[8] The results of alkylation of 7 indicate that this ion pair 8 is directly trapped by RX to afford 1 and 9, the former reverting to thiamin ylide in the presence of NaOEt. The formation of 12 or 13 occurred via the intermediate formation of 9 type derivatives. In this pathway, we assume product 12 was formed via 15. Intramolecular Michael addition of 15 takes place due to the enhanced acidity of the methylene protons. Retro-Michael reaction of 11 gives rise to the pyrimidine ring followed by addition of the hydroxyethyl group, resulting in the formation of 12. If the carbon atom adjacent to the benzoyl moiety has no hydrogen, then no retro-Michael reaction would occur; thus it should be possible to isolate tricyclic product 11. To check this hypothesis, we tried the reaction of 7 with secondary α-halo ketone as discussed above.

Isolation of 9 indicates that 7 reacted with RX as the thiolate anion. We have reported that the most characteristic feature of 7 is its disproportionation properties; that is, 7 changes into ion pair 8 in a protic solvent even under neutral conditions.[16] The formation of S-alkylation products is consistent with rapid equilibrium between 7 and 8.

The disproportionation character of 7 under neutral conditions indicates that it has a chemically ambident anionic character (C^- vs S^-) and its reactivity toward electrophiles depends upon the solvent. Thus, a number of derivatives of 9 have been reported, but the chemical meaning of the yellow form of thiamin with regard to the coenzyme or noncoenzyme mechanism of thiamin is not fully understood.[17] Many hypotheses have been proposed on the role of the aminopyrimidine ring in the coenzymatic or noncoenzymatic reaction mechanism of thiamin.[19] Our results indicate that it plays an important role in the disporportionation step of thiamin ylide in producing an ion pair of the yellow form 8.

As a synthetic application of the in situ prepared ion pair of thiamin 8, we tried oxidation of 8 to obtain the disulfide of yellow form of thiamin (**9d** or neothiamin disulfide; Figure 1). Risinger et al.[13] reported the isolation of **9d** by the reaction of 1 with base (NaOMe) followed by workup using H_2O. Treatment of 7 with 1 equiv of NaOEt in EtOH followed by the addition of I_2 solution yielded a colorless suspension. Workup without using H_2O gave a colorless product that contained inorganics (NaI and NaCl) but the spectroscopic data clearly indicated the formation of **9d**.

Further studies on the reactivity of the isolated 7 are now in progress.

Experimental Section

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were measured at 200 or 90 and 50 MHz, respectively. The X-ray analysis data of **12** and **13b** and the NMR spectra of **7**, **9**, and **10** are summarized in the supplementary material (see paragraph at end of paper).

Preparation of NaCl-Containing Thiamin Ylide 7. Isolation of salt-free thiamin ylide has been described.[6] Thiamin monochloride 1 was prepared according to the method described by Uray.[21] A suspension of 15.6 g (51.8 mmol) of 1 in 30 mL of EtOH was cooled to −10 °C under N_2. To this, a solution of NaOEt in EtOH that was prepared from 1.20 g of Na in 20 mL of EtOH was added dropwise with efficient stirring over about 3 h. When NaOEt solution was mixed with 1, the mixture immediately turned yellow locally. This color faded to colorless if the mixture was stirred without adding base solution. At the end of the addition of base, the yellow color remained. After NaOEt solution was added, the mixture, which had turned red, was further stirred for 30 min at −10 to −5 °C. Next the mixture was filtered in a drybox (this was absolutely necessary), and the remaining orange–red product was washed successively with cold EtOH and then cold ether followed by drying under reduced pressure to afford 13.8 g of colorless 7. Elemental analysis showed that the product was a 1:1.18 mixture of thiamin ylide and NaCl; that is, 41.4 mmol (80.0%) of 7 was obtained. The ylide–NaCl ratio is always around 1:1.2 by our method. Although 7 is hygroscopic, it can be stored for several months under N_2 without any significant decomposition at room temperature; ^1H NMR (DMSO-d_6) δ 1.62 (s, 3 H, Me), 2.29 (s, 3 H, Pm-Me), 2.30 (m, 2 H, CH_2CH_2OH), 3.35 (m, 2 H, CH_2OH), 4.08 (d, J = 15 Hz, 1 H, $PmCH_2$), 4.23 (d, J = 15 Hz, 1 H, $PmCH_2$), 4.64 (br, 1 H, OH), 6.38 (s, 1 H), 7.43 (s, 1 H), 7.86 (s, 1 H, PmH); ^{13}C NMR (DMSO-d_6) δ 10.5, 25.2, 31.2, 42.1, 60.3, 108.3, 131.5, 149.5, 158.3, 165.2. The NMR spectra of 7 are the same as those of salt-free thiamin ylide.[6] Anal. Calcd for $C_{12}H_{16}N_4OS\cdot1.18NaCl$: C, 43.24; H, 4.38; S, 9.62. Found: C, 43.09; H, 4.49; N, 16.51; S, 9.73. The second lot of 7 was $TY\cdot n$NaCl, where n = 1.25.

Reaction of 7 with MeI under Neutral Conditions. To a cooled (−20 °C) suspension of 520 mg (1.56 mmol) of 7 in 10 mL of EtOH was added 0.15 mL of MeI. The mixture was stirred for 20 min at −20 °C, then the cooling bath was removed, and stirring was continued for 3 h. The reaction mixture was separated by filtration, and 310 mg (about 43%) of 1 was obtained, which was a mixture with inorganic materials. The NMR and IR spectra of this product are identical with those of pure 1. The filtrate was concentrated and gave 430 mg (50%) of 3-methyl-6-(5-hydroxy-3-(methylmercapto)-2-penten-2-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine (**9a**) as an oil: ^1H NMR ($CDCl_3$) δ 2.03 (s, 3 H, Me), 2.20 (s, 3 H, SMe), 2.57 (s, 3 H, Pm-Me), 4.68 (s, 2 H, Pm-CH_2), 7.30 (s, 1 H, Pm-C7H), 8.07 (s, 1 H, Pm-C4H); ^{13}C NMR ($CDCl_3$) δ 15.1 (Me), 17.0 (Me), 25.6 (Pm-Me), 33.6 (CH_2CH_2OH), 43.8 (Pm-CH_2), 60.0 (CH_2OH), 110.0 (Pm-C4a), 132.7, 134.7, 153.5, 157.3, 160.8, 168.3; UV λ_{max} (EtOH) 245 (s), 335; mass, m/z (rel intensity) 278 (M^+, 5), 263 (14), 249 (18), 147 (100), 136 (82), 122 (60). Horman[18] reported that the fragment with m/z 147 was the base peak of 3.

Reaction of 7 with MeI in the Presence of Base. A suspended solution of 1.80 g (5.39 mmol) of 7 in 15 mL of EtOH was cooled to −10 °C. To this, a solution of NaOEt in EtOH that had been prepared from 132 mg (5.74 mg atom) of Na in 10 mL of EtOH was added dropwise. Next, 0.40 mL of MeI was added, and the mixture was stirred for 3 h at −10 °C to room temperature. The mixture was subjected to filtration, and the filtrate was concentrated. Purification of the crude product SiO_2 column chromatography with MeOH/AcOEt (2/3 v/v%) as eluent gave 1.40 g of oil, which showed two spots on TLC (R_f = 0.40 and 0.35 in MeOH/AcOEt = 1/2 v/v%, SiO_2). The NMR spectrum indicated that it was a 85:15 mixture of **9a** (R_f = 0.35) and **10a** (R_f

(16) Maier and Metzler[2] observed the existence of disproportionation properties with their isolated neutralized product of thiamin, which was assigned to be dihydrothiochrome (4 to TH^+ and YF^-).

(17) (a) Kluger, R. *Chem. Rev.* **1987**, *87*, 863. (b) For alkali-induced transformation of thiamin into thiamin thiolate, Hopmann et al.[7] and Horman[18] proposed a yellow form as one of the intermediates; however, for the acid-induced reverse reaction, Tee et al.[19] ruled out the yellow form as an intermediate. We have proposed a new mechanism including the crucial contribution of the ion pair of yellow form 8.[8]

(18) Horman, I. *Helv. Chim. Acta* **1984**, *67*, 1478.

(19) Tee, O. S.; Spiropoulos, G. D.; McDonald, R. S.; Geldart, V. D.; Moor, D. *J. Org. Chem.* **1986**, *51*, 2150.

(20) Jordan, F.; Chen, G.; Nishikawa, S.; Wu, B. S. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 51.

(21) Uray, G. *Monatsch. Chem.* **1982**, *113*, 1475.

= 0.40). When the whole mixture was allowed to stand for 3 days in 95% EtOH, TLC analysis showed only one spot of R_f = 0.40. Evaporation of the solvent yielded 1.40 g of *N*-[(4-amino-2-methylpyrimidin-5-yl)methyl]-*N*-(5-hydroxy-3-(methylmercapto)-2-penten-2-yl)formamide (**10a**): ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 1.90, 1.97 (2 s, 6 H, two Me), 2.37 (s, 3 H, Pm-Me), 2.55 and 3.61 (2 m, 4 H, ethylene), 4.40 (br s, 2 H, Pm-CH_2), 6.50 (br s, 2 H, NH_2), 7.77, 7.85 and 7.92 (m, 2 H, NCHO + PmH).

By the same procedure, products **9b,c** and **10b,c** were obtained. **9b**: oil; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3 H, J = 6 Hz, Me of SEt), 2.04 (s, 3 H, Me), 2.57 (s, 3 H, Pm-Me), 2.66 (m, 4 H, CH_2CH_2OH and Et), 3.83 (t, 2 H, J = 6 Hz, CH_2CH_2OH), 4.36 (br s, 1 H, OH), 4.67 (s, 1 H, Pm-CH_2), 7.21 (s, 1 H, Pm-C7H), 8.05 (s, 1 H, Pm-C4H). **9c**: mp 116–119 °C, hygroscopic; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3 H, Me), 2.54 (s, 3 H, Pm-Me), 4.70 (s, 2 H, Pm-Me), 7.40 (s, 1 H, Pm-C7H), 8.05 (s, 1 H, Pm-C4H), 7.6–9.0 (m, 3 H, Ar); IR (Nujol cm^{-1}) 1597, 1530; UV (EtOH) λ_{max} (log ϵ) 227 (4.28), 270 (s, 3.97), 334 (4.20). Anal. Calcd for $C_{18}H_{18}N_6O_5S\cdot H_2O$: C, 48.21; H, 4.50; N, 18.74; S, 7.15. Found: C, 48.36; H, 4.33; N, 18.56; S, 7.09.

10b: ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, Me of Et), 1.96 (s, 3 H, Me), 2.35 (s, 3 H, Pm-Me), 2.3–2.6 (m, 4 H, CH_2 × 2), 3.20 (m, 2 H, CH_2), 4.38 (br s, 2 H, Pm-CH_2), 6.40 (br s, 2 H, NH_2), 7.78 and 7.89 (2 s, 2 H, NCHO and Pm-CH).

10c: mp 227–230 °C (dec);[22] ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3 H, Me), 2.27 (s, 3 H, Pm-E), 4.46 (br s, 2 H, Pm-CH_2), 6.43 (br s, 2 H, NH_2), 7.87 and 8.17 (2 s, 2 H, NCHO and Pm-CH), 7.0, 8.1, 8.8 (each m, 3 H aryl). Anal. Calcd for $C_{18}H_{20}N_6O_6S\cdot 0.5H_2O$: C, 47.26; H, 4.62; N, 18.37; s, 7.08. Found: C, 47.54; H, 4.58; N, 18.46; S, 6.85.

Reaction of 7 with Phenacyl Chloride. A suspended solution of 850 mg (second lot; 2.57 mmol) of **7** in 10 mL of EtOH was cooled to −10 °C. To this, 5.0 mL of 0.5 M NaOEt in EtOH was added dropwise followed by portionwise addition of 390 mg of phenacyl chloride. The mixture turned from yellow to red. It was stirred for 3 h at −10 °C to room temperature. Amberlyst 15 was added, and the mixture was separated by filtration. The filtrate was concentrated, and the crude product was separated by SiO_2 column chromatography with MeOH/AcOEt (1/6 v/v%) as eluent to give 450 mg (46%) of 2-benzoyl-4-(4-amino-2-methylpyrimidin-5-yl)-4a-methyl-4a,7a,6,7-tetrahydrofuro[3,2-*b*][1,4]thiazine (**12**): mp 207–210 °C; UV (EtOH) λ_{max} (log ϵ) 236 (4.32), 275 (sh, 3.86), 346 (4.13); ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 3 H, Me), 2.33 (s, 3 H, Pm-Me), 4.17 (br s, 2 H, CH_2), 6.50 (br s, 2 H, NH_2), 7.10 (m, 1 H, H), 7.20 (s, 5 H, Ph), 7.78 (s, 1 H, Pm-CH). Anal. Calcd for $C_{20}H_{22}N_4O_2S$: C, 62.07; H, 5.86; N, 14.48; S, 8.28. Found: C, 62.35; H, 5.89; N, 14.29; S, 7.96.

Reaction of 7 with α-Bromopropiophenone. A suspended solution of 675 mg of **7** (second lot; 2.04 mmol) in 5 mL of EtOH was cooled to −20 °C. To this, a solution of NaOEt in EtOH (0.5 M, 4.0 mL) and 434 mg (2.03 mmol) of α-bromopropiophenone was added. The cooling bath was removed, and the reaction mixture was stirred for 4 h. The color remained yellow, and the TLC showed the formation of a single-spot product (SiO_2, MeOH/AcOEt = 1/3). The reaction mixture was concentrated in vacuo, and the remaining crude product was separated by column chromatography (SiO_2) with MeOH/AcOEt = 1/4 as an eluant to give a foamlike product (800 mg) as a single-spot fraction. However, NMR spectroscopy indicated that the product was a mixture of the diastereoisomers of **13a** (δ 2.35 and 2.40 (Pm-Me), 5.30 and 5.37 (C11a-H), 5.95 and 5.45 (NH)). This product was again separated by SiO_2 column chromatography with MeOH/AcOEt = 1/11 as eluant to afford an oily product, which on trituration with ether gave 200 mg of **13a**: mp 106–110 °C; ^{1}H NMR ($CDCl_3$) δ 1.78 and 1.85 (2 s, 3 H × 2, C1 and C4 Me), 2.44 (s, 3 H, C9 Me), 2.4–2.6 (m, 2 H, CH_2), 2.8 (br s, 1 H, OH), 3.67 (t, J = 6 Hz, 2 H, CH_2), 4.24 and 4.52 (2 d, 2 H J = 16 Hz, C6 CH_2), 5.30 (s, 1 H, C11a H), 5.48 (br s, 1 H, NH), 7.4–7.6 (m, 3 H, Ar), 7.85 (s, 1 H, C7 H), 8.1–8.2 (m, 2 H, Ar). Anal. Calcd for $C_{21}H_{24}N_4O_2S\cdot 0.5H_2O$: C, 62.20; H, 6.21; N, 13.82; S, 7.91. Found: C, 62.53; H, 6.40; N, 13.49; S, 7.70.

3-(2-Hydroxyethyl)-4,9-dimethyl-1,6,11,11a-tetrahydropyrimidino[4′,5′:4,5]pyrimidino[2,1-*c*][1,4]thiazine-1-spiro-3′-(tetrahydro-2′-oxofuran) (13b,c). A suspended solution of 675 mg (second lot; 2.00 mmol) of **7** in 5 mL of EtOH was cooled to −20 °C. To this, 4.0 mL of 0.5 M solution of NaOEt in EtOH was added dropwise, and the mixture was stirred for 5 min at −20 °C, then 330 mg (2.00 mmol) of bromolactone was added, and the cooling bath was removed. The mixture was stirred for 3 h at −20 °C to room temperature, followed by concentration of the solvent. The TLC of the mixture showed the presence of two products with R_f = 0.26 and 0.19 ($CHCl_3$/MeOH = 10/1 v/v%, SiO_2). The crude product was separated by SiO_2 column chromatography with MeOH/$CHCl_3$ (1/10 v/v%) as eluent and gave 120 mg of **13b**, 280 mg of a mixture of **13b** and **13c**, and 180 mg of **13c**; the combined yield was 83%.

13b: mp 176–177 °C; UV (EtOH) λ_{max} (log ϵ) 235 (4.17), 282 (3.86); ^{1}H NMR (DMSO-d_6) δ 1.83 (s, 3 H, thiazine Me), 2.31 (s, 3 H, Pm-Me), 1.9–2.7 (m, 4 H, CH_2 × 2), 3.2–3.5 (m, 3 H, CH_2, OH), 4.14 and 4.52 (2 d, J = 16 Hz, C6 H_2), 4.2–4.5 (m, 2 H, CH_2), 5.18 (s, 1 H, C11a H), 7.51 (s, 1 H, NH), 7.89 (s, 1 H, C7 H). Anal. Calcd for $C_{16}H_{20}N_4O_3S$: C, 55.16; H, 5.79; N, 16.08; S, 9.20. Found: C, 55.13; H, 5.76; N, 15.82; S, 9.07.

13c: mp 222–223 °C; UV (EtOH) δ_{max} (log ϵ) 235 (4.17), 282 (3.86); ^{1}H NMR (DMSO-d_6) δ 1.82 (s, 3 H, thiazine Me), 2.28 (s, 3 H, Pm-Me), 2.1–2.6 (m, 4 H, CH_2 × 2), 3.1–3.5 (m, 3 H, CH_2, OH), 4.14 and 4.56 (2 d, J = 16 Hz, C6 H_2), 4.3–4.6 (m, 2 H, CH_2), 5.21 (s, 1 H, C11a H), 6.35 (s, 1 H, NH), 7.88 (s, 1 H, C7 H). Anal. Calcd for $C_{16}H_{20}N_4O_3S$: C, 55.16; H, 5.79; N, 16.08; S, 9.20. Found: C, 55.23; H, 5.76; N, 15.91; S, 9.05.

Attempted Preparation of Neothiamin Disulfide (9d). To a cooled (−15 °C) suspended solution of 1.20 g (3.60 mmol) of **7** in 20 mL of EtOH was added NaOEt solution in EtOH, which had been prepared from 88 mg (3.82 mmol) of Na in 10 mL of EtOH. This yellow suspension was oxidized by dropwise addition of a solution of 490 mg of I_2 in 60 mL of EtOH at −15 °C over 40 min. After the colorless mixture had been stirred for 30 min at −10 °C, it was separated by filtration under N_2 atmosphere. The filtrate was concentrated in vacuo, and the residue was triturated with EtOH/acetone (ca. 1/1 v/v%) to afford 480 mg of the product **9d**. No further purification was carried out. Elemental analysis indicated that it was a mixture of free **9d** and inorganic materials (NaCl and NaI); ^{1}H NMR (DMSO-d_6) δ 2.03 (s, 3 H, olefinic Me), 2.46 (s, 3 H, pyrimidyl Me), 3.43 (m, 4 H, hydroxyethyl), 4.62 (s, 2 H, CH_2), 7.25 (s, 1 H, pyrimidyl C7 H), 8.17 (s, 1 H, pyrimidyl C4 H); UV λ_{max} (EtOH) 216, 334; IR (Nujol) 5.90, 6.20, 6.31 μm.

Supplementary Material Available: X-ray analysis data of **12** and **13c** as well as NMR spectra of **7**, **9**, and **10** (16 pages). Ordering information is given on any current masthead page.

(22) (10c) Matuoka, T.; Iwatu, I. *J. Pharm. Soc. Jpn.* **1950**, *70*, 224.