A Convenient Synthesis of 1′-Alkyl- and 1′-Benzylthiaminium Salts

Khashayar KARIMIAN*, Farajollah MOHANAZADEH

Laboratory of Bioorganic Chemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran 13145, Iran

A convenient and efficient method for the quaternization at N-1′ of thiamin and 2-(1-hydroxyethyl)-thiamin with various alkyl halides and benzyl bromide is presented. Quaternization of 2-(1-ethoxycarbonyl-1-hydroxyethyl)-thiamin was unsuccessful, resulting in the release of ethyl pyruvate and the formation of quaternized thiamin. The *N-1′*-derivatized thiaminium salts were found to possess drastically different physical properties as compared to their parent heterocycles.

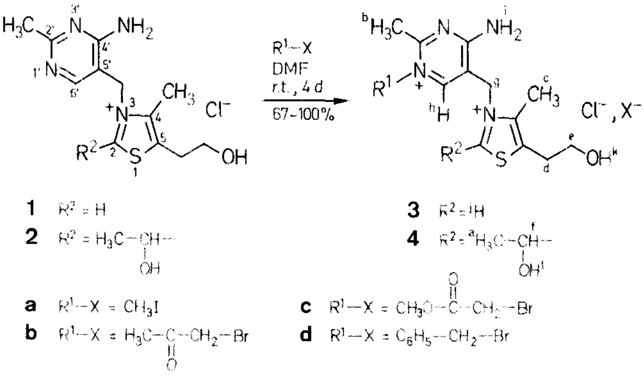
Chemical modification of coenzymes may serve not only to elucidate their mechanism of action, but also to provide information on the nature of the active sites of their respective enzymes. Since the total synthesis of thiamin nearly fifty years ago[1], the role of the pyrimidine moiety of thiamin (vitamin B_1) and its derivatives in the coenzyme's biochemical functions has been a subject of controversy[2-5].

Thiamin analogs devoid of the 4′-NH_2 group are inactive *in vivo*[6]. Nevertheless, simple thiazolium salts are capable of generating the negative charge required for activity at C-2, thereby mimicking thiamin-catalyzed reactions *in vitro*[2]. Whether the 4′-NH_2 group is merely required for binding of thiamin pyrophosphate to the active site by hydrogen bonding, plays a role in presumed conformational changes that may be a prerequisite to the biochemical activity of the coenzyme[7], or participates in proton abstraction[4] is not clear at the present time. In fact, N-1′ was only recently established unequivocally as the site of protonation[8] and methylation[9] using ^{15}N-NMR spectroscopy.

In this context and in continuation of our studies on thiamin and thiazolium compounds[10,11] it became necessary to prepare a variety of 1′-alkyl- and 1′-benzylthiaminium salts. The available procedures suffer from different shortcomings in that an earlier reported method[7] affords only low yields[9,12] and a more recent approach is limited to the preparation of 1′-methylthiamin[9] and uses the highly mutagenic dimethyl sulfate[13].

We now report that the synthesis of a variety of 1′-alkyl- and 1′-benzylthiaminium salts (**3**) may be effected conveniently and in high yields by reaction of thiamin (monochloride) (**1**) with the respective halide in dry dimethylformamide. This method is also applicable to the synthesis of 1′-alkyl- and 1′-benzyl derivatives of 2-(1-hydroxyethyl)-thiamin (HET, **2**).

Being the direct precursor of acetyl-CoA, HET constitutes the most important metabolite of thiamin in biological systems[14].



The monochlorides **1** and **2** may be prepared from the corresponding chloride hydrochlorides by treatment with one molecular equivalent of aqueous sodium hydroxide in ethanol, and then submitted to the alkylation. They may also be generated *in situ* using 0.5 molecular equivalents of anhydrous potassium carbonate in dimethylformamide; however, in this case the yields of quaternized products **3** or **4** are ~ 20 % lower and the use of excess base (> 0.5 molecular equivalents) results in fragmentation of thiamin derivatives **3** or **4** into the *N-1′*-quaternized pyrimidine and the thiazole part of the molecule (in accord with a proposal[12] on the base-catalyzed fragmentation of 1′-methylthiamin).

The quaternization reaction was found to be less effective with increasing degree of substitution at the electrophilic carbon. Ethyl iodide, for example, gave 1′-ethylthiamin in 28 % yield and no alkylation product could be obtained with 2-iodopropane. Also, attempted quaternization of 2-(1-ethoxycarbonyl-1-hydroxyethyl)-thiamin (CEHET)[15] was unsuccessful, resulting in the formation of ethyl pyruvate and *N-1′*-quaternized thiamin. This may be ascribed to facile release of ethyl pyruvate from CEHET[16].

Further variation of compounds **3** and **4** may be achieved by introducing *N-1′* substituents R^1 having reactive groups such as carbonyl. However, such substitution reactions are

Table 1. Thiaminium Salts **3** and **4** Prepared

Product	Yield [%][a]	m.p. [°C]	Molecular Formula[b]
3a	100	231–233	$C_{13}H_{20}ClIN_4OS$ (442.2)
3b	94	202–204	$C_{15}H_{22}BrClN_4O_2S$ (437.1)
3c	89	206–207	$C_{15}H_{22}BrClN_4O_3S$ (453.3)
3d	95	242–243	$C_{19}H_{24}BrClN_4OS$ (471.2)
4a	80	208–209	$C_{15}H_{24}ClIN_4O_2S$ (486.1)
4b	80	174–177	$C_{17}H_{26}BrClN_4O_3S$ (481.2)
4c	67	166–168	$C_{17}H_{26}BrClN_4O_4S$ (496.9)
4d	80	193–194	$C_{21}H_{28}BrClN_4O_2S$ (515.1)

[a] Based on thiamin hydrochloride (**1** · HCl or **2** · HCl).
[b] Satisfactory microanalyses were obtained for all compounds reported here: C ±0.21, H ±0.29, N ±0.18.

Table 2. ^{1}H-NMR Data (Solvent/DSS^a$_{int}$) of Thiaminium Salts **3** and **4**

Compound	Solvent	δ [ppm] and J for Protons a–I and Protons of the Group R^1 (see Formula Scheme)								
		a (d, 3H)	b (s, 3H)	c (s, 3H)	d (t, 2H)	e (t, 2H)	f (q, 1H)	g (s, 2H)	h (s, 1H)	i (d, 2H)
3a	D_2O		2.56	2.70	3.19 (5.9 Hz)	3.89 (5.9 Hz)		5.57	8.13	
	DMSO-d_6		2.58	2.70	3.14 (4.5 Hz)	3.72 (4.5 Hz)		5.58	8.51	8.80; 9.29
3b	D_2O		2.39	2.58	3.19 (5.8 Hz)	3.90 (5.8 Hz)		5.61	7.98	
3c	D_2O		2.55	2.67	3.19 (5.8 Hz)	3.89 (5.8 Hz)		5.61	8.10	
3d	D_2O		2.45	2.76	3.16 (5.6 Hz)	3.88 (5.6 Hz)		5.60	7.69	
	DMSO-d_6		2.54	2.64	3.11 (5.1 Hz)	3.70 (5.1 Hz)		5.66	8.60	9.13; 9.53
4a	D_2O	1.72 (5.6 Hz)	2.43	2.66	3.19 (5.9 Hz)	3.85 (5.9 Hz)	5.45 (5.6 Hz)	5.55	7.39	
	DMSO-d_6	1.55 (6.1 Hz)	2.36	2.62	3.09 (5.4 Hz)	3.80 (5.4 Hz)	5.38 (6.1 Hz)	5.53	7.55	8.67; 9.40
4b	D_2O	1.68 (6.4 Hz)	2.32	2.54	3.16 (5.6 Hz)	3.89 (5.6 Hz)	5.44 (6.4 Hz)	5.58	7.42	
4c	D_2O	1.69 (6.5 Hz)	2.41	2.63	3.17 (5.8 Hz)	3.90 (5.8 Hz)	5.49 (6.5 Hz)	5.58	7.53	
4d	D_2O	1.60 (6.1 Hz)	2.26	2.76	3.10 (5.6 Hz)	3.86 (5.6 Hz)	5.45 (6.1 Hz)	5.54	6.75	
	DMSO-d_6	1.58 (6.0 Hz)	2.35	2.58	3.07 (5.1 Hz)	3.73 (5.1 Hz)	5.53 (6.0 Hz)	5.74	7.88	9.04; 9.63

[a] DSS = Sodium 3-(trimethylsilyl)-1-propanesulfonate

restricted to acidic or neutral media because of the observed susceptibility of quaternized thiaminium salts to base-catalysed fragmentation (see above). On the other hand, since the displacement of the thiazole nucleus is known to be effected by a variety of nucleophiles[17], a large number of 2,5-dimethyl-4-aminopyrimidines possessing diverse substituents at N-1′ and various groups (the nucleophile used) bonded to 5-methylene might be prepared.

The *N-1′*-substituted thiamin derivatives reported here possess a number of interesting properties which warrant further studies on these compounds. For example, in contrast to thiamin and HET, derivatives **3** and **4** provide clear rhombic crystals from water, making them ideal candidates for crystallographic studies. Also, whereas thiamin and HET dissolve only in water, compounds **3** and **4** are also soluble in dimethyl sulfoxides, thus providing a means of studying possible modes of intramolecular hydrogen bonding in these compounds.

Thiamin chloride hydrochloride (Art. 500923) was purchased from Merck (Darmstadt, FRG). Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Microanalyses were obtained using a Perkin-Elmer 240 element analyser. ^{1}H-NMR spectra were recorded on a Varian FT-80 spectrometer.

2-(1-Hydroxyethyl)-thiamin (HET, 2) (Modification of the Procedure of Lit.[18]):

The whole reaction is performed under nitrogen. In a three-neck flask equipped with stirrer, addition funnel, and nitrogen inlet and outlet tubes are placed thiamin (chloride) hydrochloride[19] (**1** · HCl; 33.7 g, 0.1 mol) and absolute ethanol (900 ml). This suspension is cooled to 0 °C and a solution of sodium ethoxide (4.6 g, 0.2 mol) in absolute ethanol (400 ml) is added over a 5 min period. Then, acetaldehyde (28 ml) is added dropwise and stirring is continued for 6 h at 0 °C. The mixture is then adjusted to pH 3 by the addition of conc. hydrochloric acid/ethanol (1:1) at 0 °C. The resultant pale yellow suspension is filtered through a glas-frit Büchner funnel and the filtrate is concentrated to half its original volume using a rotavapor (25 °C). The filtrate is kept at 4 °C for 24 h and the precipitated product **2** then isolated by suction and washed with a small amount of cold absolute ethanol. Treatment of the mother liquor in the same manner affords further product **2**; total yield: 35 g (93%).

As evidenced by integration of 6′-H (HET 7.4 ppm, thiamin 8.1 ppm), this material contained a small amount of thiamin (**1**). Repeated attempts to obtain pure HET (**2**) by fractional crystallization in various solvents were unsuccessful. However, purer HET may be obtained from its hydrochloride (see below).

1′-Alkylthiamins (3) and 1′-Alkyl-2-(1-hydroxyethyl)-thiamins (4) (including 1′-Benzyl Derivatives); **General Procedures**:

Thiamin (**1**) *and 2-(1-Hydroxyethyl)-thiamin* (**2**) *from their Hydrochlorides:* To a stirred suspension of thiamin hydrochloride (**1** · HCl) or 2-(1-hydroxyethyl)-thiamin hydrochloride (**2** · HCl) in ethanol is added an aqueous 0.5 molar solution (1 molecular equivalent) of sodium hydroxide. The solvent is removed at room temperature using a rotary evaporator and the resultant solid is suspended in hot ethanol. The mixture is filtered while hot. From the filtrate, product **1** or **2** is precipitated by the addition of ether; it is isolated by suction and dried in vacuum.

Thiamin (**1**); yield: ~100%; m.p. 141–151 °C, dec.

2-(1-Hydroxyethyl)-thiamin (HET, **2**): yield: 86%; m.p. 154–159 °C, dec.

Quaternization Products **3** *and* **4**: To a stirred suspension of compound **1** or **2** (0.01 mol) in dry dimethylformamide (10 ml) (for products **3c** and **4c** 20 ml) under nitrogen is added the alkyl halide and stirring is continued for 4 days. Then, ethyl acetate (20 ml) is added, followed by ether (80 ml) to precipitate the product **3** or **4** which is isolated by suction and recrystallized twice from water/acetone.

j (s, 1H)	k (m, 1H)	l (d, 1H)	R[1]
9.85			3.87 (s, 3H)
10.0	3.40		3.89 (s, 3H)
9.74			2.55 (s, 3H); 5.39 (s, 2H)
9.75			3.85 (s, 3H); 5.18 (s, 2H)
9.75			5.44 (s, 2H); 7.45 (m, 5H)
10.16	3.43		5.54 (s, 2H); 7.43 (s, 5H) 3.79 (s, 3H)
	3.34	6.82 (5.0 Hz)	3.78 (s, 3H)
			2.40 (s, 3H); 5.32 (s, 2H)
			3.80 (s, 3H); 5.11 (s, 2H)
			5.36 (s, 2H); 7.36 (m, 5H)
	3.40	6.92 (5.2 Hz)	5.60 (s, 2H); 7.37 (m, 5H)

In an alternative procedure, thiamin (**1**) or 2-(1-hydroxyethyl)-thiamin (**2**) are generated *in situ* by the addition of anhydrous potassium carbonate (0.5 molecular equivalents) to a stirred suspension of the chloride hydrochloride **1** · HCl or **2** · HCl, respectively, in dry dimethylformamide and quaternization is performed as above. In average, the yields of products **3** and **4** are ~ 20 % lower than those obtained by the two-step procedure.

Received: April 24, 1986

* Present address: Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 1A1, Canada.

[1] Cline, J.K., Williams, R.R., Finkelstein, J. *J. Am. Chem. Soc.* **1937**, *59*, 1052.
[3] Bruice, T.C., Benkovic, S. *Bioorganic Mechanisms*, Vol. 2, W.A. Benjamin, Inc., New York, 1966, p. 181.
[3] Gallo, A.A., Sable, H.Z. *J. Biol. Chem.* **1974**, *249*, 1382.
[4] Schellenberger, A. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 51.
[5] Jordan, F., Chen, G., Nishikawa, S., Wu, B.S. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 14.
[6] Sexton, W.A. *Chemical Constitution and Biological Activity*, D. Van Nostrand Company, Inc., New York, 1950, p. 112.
[7] Jordan, F., Mariam, Y.H. *J. Am. Chem. Soc.* **1978**, *100*, 2534.
[8] Cain, A.H., Sullivan, G.R.; Roberts, J.D. *J. Am. Chem. Soc.* **1977**, *99*, 6423.
[9] Zoltewicz, J.A., Baugh, T.D. *Synthesis* **1980**, 217.
[10] Karimian, K., Mohanazadeh, F., Rezai, S. *J. Heterocycl. Chem.* **1983**, *20*, 1119; and references therein.
[11] Kluger, R., Stergipoulos, V., Gish, G., Karimian, K. *Bioorganic Chem.* **1985**, *13*, 227.
[12] Zoltewicz, J.A., Kauffman, G., Uray, G. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 7.
[13] Ramstein, J., Helene, C., Leng, M. *Eur. J. Biochem.* **1971**, *21*, 125.
[14] Lehninger, A. *Biochemistry*, Worth Publishers, Inc., New York, 1975, p. 372.
[15] Kluger, R., Chin, J., Smyth, T. *J. Am. Chem. Soc.* **1981**, *103*, 884.
[16] Crosby, J., Lienhard, G.E. *J. Am. Chem. Soc.* **1970**, *92*, 5707.
[17] Zoltewicz, J.A. *Synthesis* **1980**, 218.
[18] Risinger, G.E., Gore, W.E., Pulver, K. *Synthesis*, **1974**, 659.
[19] Lower yields of HET were obtained using other sources of thiamin (e.g. Sigma, Nutritional Biochemicals). This may be due to the degree of hydration of the thiamin used.