convergence, two of the "chlorine" sites on each dimer (Cl_a and Cl_b in Figure 1) had refined occupancies near 1.00, while the other two (Cl/C_a and Cl/C_b in Figure 1) had refined occupancies near 0.85. Mixed scattering factors (50% Cl and 50% C) were then calculated and used with fixed occupancies of 1.00 for the Cl/C sites in all subsequent structure factor calculations.

Unit-weighted full-matrix least-squares refinement which utilized anisotropic thermal parameters for all 56 nonhydrogen atoms converged to $R_1 = 0.048$ and R_2 (weighted, based on F)[29] = 0.052 for 4339 independent reflections having $2\theta_{MoK\alpha} < 43°$ and $I > 3\sigma(I)$; similar refinement cycles with the more complete ($2\theta_{MoK\alpha} < 55°$) data set gave $R_1 = 0.056$ and $R_2 = 0.061$ for 6542 absorption-corrected reflections having $I > 3\sigma(I)$. These and all subsequent structure factor calculations employed recent tabulations of atomic form factors[28b] and anomalous dispersion corrections[28c] to the scattering factors of the Ta and Cl atoms. The final cycles[10] of empirically weighted[30] full-matrix least-squares refinement which utilized anisotropic thermal parameters for all nonhydrogen atoms converged to $R_1 = 0.056$ and $R_2 = 0.070$ for 6542 independent reflections having $2\theta_{MoK\alpha} < 55°$ and $I > 3\sigma(I)$. The four crystallographically independent hydride atoms did not appear in the final difference Fourier.

(30) Empirical weights were calculated from the equation $\sigma = \sum_0^3 a_n|F_o|^n = ((6.66-1.57) \times 10^{-2})|F_o| + (9.87 \times 10^{-5})|F_o|^2 - (6.82 \times 10^{-8})|F_o|^3$, the a_n being coefficients derived from the least-squares fitting of the curve $||F_o| - |F_c|| = \sum_0^3 a_n|F_o|^n$, where the F_c values were calculated from the fully-refined model using unit weighting and an $I > 3\sigma(I)$ rejection criterion.

All calculations were performed on a Data General Eclipse S200 computer with 64K of 16-bit words, a floating-point processor for 32- and 64-bit arithmetic, and versions of the Nicolet E-XTL interactive crystallographic software package as modified at Crystalytics Co.

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Registry No. $[TaCp''Cl_2H]_2$, 74153-79-0; $[TaCp'Cl_2H]_2$, 74153-80-3; $[TaCp'Br_2H]_2$, 81389-10-8; $Ta_2Cp'_2Cl_3Hz_2(CH_2CMe_3)$, 81389-09-5; $Ta_2Cp''_2Cl_3H_2(CH_2CMe_3)$, 81389-08-4; $Ta_2Cp'_2Cl_3H_2(CH_2SiMe_3)$, 81389-07-3; $Ta_2Cp'_2Cl_3H_2(Me)$, 81389-06-2; $Ta_2Cp''_2Cl_3H_2(Me)$, 81389-05-1; $Ta_2Cp'_2Cl_3H_2(CH_2CH_3)$, 81389-04-0; $Ta_2Cp'_2Cl_5H$, 74153-82-5; $TaCp''Cl_2(CH_2CMe_3)(H)$, 81389-03-9; $TaCp''Cl_2$(propylene), 71453-85-5; $TaCp''Cl_2$(styrene), 71414-50-1; $TaCp''Cl_2Np_2$, 71228-87-0; $TaCp'Cl_2Np_2$, 81389-02-8; $TaCp'Br_2$(propylene), 81389-01-7; $TaCp'Cl_2$(propylene), 81389-00-6; $TaCp'Cl_3Np$, 81388-99-0; $TaCp''Cl_3Np$, 68087-41-2; $TaCp''Cl_3$(propyl), 81388-98-9.

Supplementary Material Available: Crystal structure analysis report and listings of anisotropic thermal parameters for nonhydrogen atoms (Table II) and observed and calculated structure factors from the final cycle of least-squares refinement for $Ta_2(\eta^5\text{-}C_5Me_4Et)_2Cl_3H_2(Me)$ (38 pages). Ordering information is given on any current masthead page.

Synthesis and Crystal Structure of an Analogue of 2-(α-Lactyl)thiamin, Racemic Methyl 2-Hydroxy-2-(2-thiamin)ethylphosphonate Chloride Trihydrate. A Conformation for a Least-Motion, Maximum-Overlap Mechanism for Thiamin Catalysis[1]

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Contribution from the Biocrystallography Lab, VA Medical Center, Box 12055, Pittsburgh, Pennsylvania 15240, the Department of Crystallography, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, and the Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1. *Received July 23, 1981*

Abstract: Methyl 2-hydroxy-2-(2-thiamin)ethylphosphonate, phosphalactylthiamin, is a phosphonate analogue of 2-(1-carboxy-1-hydroxyethyl)thiamin, the initial intermediate in the thiamin-catalyzed decarboxylation of pyruvic acid. Crystal-structure analysis of phosphalactylthiamin reveals that the thiamin portion of the molecule assumes the **S** conformation that is characteristic of other C(2)-substituted thiamins. However, in contrast to previously studied derivatives the conformation of the phosphalactyl substituent is unique in that its hydroxyl is in close contact with the bridging methylene instead of the thiazolium ring sulfur and the bond to the phosphonate is oriented perpendicular to the ring plane. This structural feature is also consistent with the NMR spectrum. The structure suggests that the principles of least motion and maximum orbital overlap can be applied to thiamin catalysis of the decarboxylation of pyruvate since the observed structure conforms to theoretical expectations for 2-(α-lactyl)thiamin diphosphate. Phosphalactylthiamin has the empirical formula $C_{15}H_{24}N_4O_5PS{\cdot}Cl{\cdot}3H_2O$. The crystal examined was monoclinic, $P2_1/c$, $a = 9.916$ (8) Å, $b = 16.840$ (1) Å, $c = 15.786$ (1) Å, $\beta = 119.45$ (4)°, $V = 2295$ Å^3, $Z = 4$. The structure was solved by direct methods and refined by full-matrix least squares to an R value of 0.067 for 1587 structure factor amplitudes measured with Mo Kα radiation on an Enraf-Nonius automatic diffractometer.

Thiamin (TH), in the form of the diphosphate ester, is a coenzyme in a number of metabolically important enzymes that catalyze the decarboxylation of α-keto acids and the transfer of aldehyde or acyl groups.[2] Although the general outline of the catalytic mechanism has been known for over two decades,[3] many details of the mechanism in both enzymatic and nonenzymatic systems remain to be elucidated. In an effort to obtain information pertaining to these details, structures have been determined for thiamin and its derivatives and analogues. The derivatives that have been of particular interest are the C(2)-substituted thiamins,

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(1) The crystal structure analysis was done by August Turano in partial fulfilment of the Ph.D. degree, University of Pittsburgh, Pittsburgh, PA.
(2) For a general review of thiamin catalysis, see: Krampitz, L. O. *Annu. Rev. Biochem.* **1969**, *38*, 213. A detailed review of the structural and mechanistic aspects of thiamin catalysis is given by Gallo et al., (Gallo, A. A.; Mieyal, J. J.; Sable, H. Z. *Bioorg. Chem.* **1978**, *4*, 147–177).
(3) Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719–3726.

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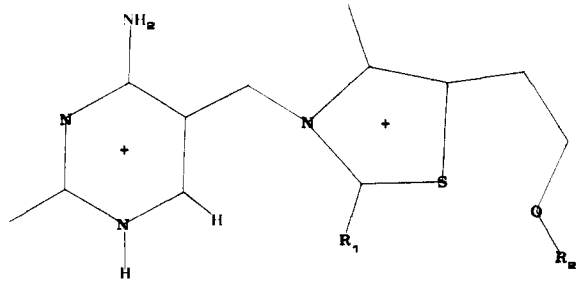
Chart I



	R_1	R_2
TH	H	H
LT	$-C(OH)(CH_3)CO_2^-$	H
PLT	$-C(OH)(CH_3)P(O)_2^- OCH_3$	H
LTDP	$-C(OH)(CH_3)CO_2^-$	$-P(O)_2^- OP(O)_2^- OH$
PLTDP	$-C(OH)(CH_3)P(O)_2^- OCH_3$	$-P(O)_2^- OP(O)_2^- OH$

which are stable intermediates in the reaction pathway. To date the α-hydroxyethyl[4] and the α-hydroxybenzyl[5] derivatives have been examined. In these compounds occurrence of common structural features indicates the existence of significant molecular properties. These compounds exhibit a similar conformation of their aromatic rings with respect to the bridging methylene in spite of the availability of other sterically favorable conformations. This conformation differs drastically from that which characterizes unsubstituted thiamin. A second characteristic feature of these thiamin derivatives is the conformation of the C(2) substituent. Although there is considerable rotational freedom, all of the derivatives have a nearly coplanar disposition of the C(2α) hydroxyl bond with respect to the thiazolium ring and a very short intramolecular separation between the ring sulfur and the substituent hydroxyl. Because of these consistent conformational preferences, it was of interest to examine the structure of the initial intermediate formed in the reaction with pyruvate in order to see whether the same stabilizing forces predominate even though it is more crowded because of the carboxylate group. Although the 2-(α-lactyl)thiamin compound (LT) has been prepared,[6] crystals suitable for X-ray structure analysis have not yet been obtained. Accordingly, an analogue was investigated (see Chart I).

Methyl acetylphosphonate is a powerful inhibitor of pyruvate dehydrogenase.[7] Experimental evidence suggests that this type of compound can form an adduct structure with thiamin diphosphate at the active site of an enzyme.[8,9] A synthetic version of the thiamin diphosphate–methyl acetylphosphonate adduct has been prepared and characterized as racemic methyl 2-hydroxy-2-(2-thiamin diphosphate)ethylphosphonate[8] (PLTDP). This is a structural and electrostatic analogue of 2-(α-lactyl)thiamin diphosphate (LTDP). Methyl acetylphosphonate similarly adds to thiamin to form methyl 2-hydroxy-2-(2-thiamin)ethylphosphonate (PLT), which serves as a structural and electrostatic analogue of 2-(α-lactyl)thiamin (LT)[2] in which the planar carboxylate is replaced by a tetrahedral phosphonate.

In this paper we report the preparation of (PLT) and its X-ray crystal structure. The structure serves as a basis for a proposal that a least-motion, maximum orbital overlap mechanism can be applied to thiamin diphosphate dependent enzymatic catalysis.

Experimental Section

Synthesis of Methyl 2-Hydroxy-2-(2-thiamin)ethylphosphonate Chloride Hydrate. Thiamin chloride hydrochloride (4.63 g) was suspended in 200 mL of methanol (−5 °C) containing 8.79 g of sodium methyl acetylphosphonate. The solution was kept under dry nitrogen and stirred with a magnetic stirrer. Sodium methoxide (630 mg of sodium in 100 mL of methanol) was added over a 10-min period after which time the solution became clear. After 30 min of additional stirring, hydrogen chloride (generated by adding concentrated hydrochloric acid dropwise into concentrated sulfuric acid) was bubbled into the reaction solution until the solution was acidic (to litmus paper).

The solution was filtered and the filtrate concentrated in vacuo to 75 mL. The resulting precipitate (thiamin) was removed by filtration. The solution was concentrated to 50 mL and stored at −15 °C overnight. The resulting precipitate was collected and found to be a 1:9 mixture of thiamin and (PLT) by ^{1}H NMR analysis of the signals for the 6′ protons.

The mixture was recrystallized from 3 N hydrochloric acid in ethanol (2 g in 50 mL) producing 1 g of (PLT) as fine white crystals. The crystals were dissolved in water and freeze-dried to remove residual ethanol as well as water. Analysis (Galbraith Laboratories) for $C_{15}H_{25}N_4O_5PS\cdot Cl_2\cdot H_2O$: C, H, N, P. Spectral data: 90-MHz ^{1}H NMR (.01 M DCl), chemical shifts relative to internal DSS in deuterium oxide δ 2.00 (d, $^3J_{P-H}$ = 12.3 Hz, 3 H, CH_3CP), 2.42 (s, 3 H, C(4)CH_3), 2.61 (s, C(2)CH_3), 3.11 (t, 2 H, C(5)CH_2), 3.65 (d, 3 H, $^3J_{PH}$ = 10 Hz, $POCH_3$), 3.91 (t, 2 H, C(5)CH_2CH_2O), 5.68, 6.26 (dd, $^2J_{HH}$ = 17.3 Hz, 2 H, C(5′)CH_2N(3)), 7.39 (s, 1 H, C(6′)H). The spectrum has been analyzed in analogy to that of lactylthiamin.[6] ^{13}C NMR, ^{31}P NMR, and UV spectra were also consistent with the structure.[10]

Crystal Data. Colorless, tabular crystals of phosphalactylthiamin (PLT) grew from a 0.1 N HCl and acetone mixture (1:9 by volume) at 4 °C. They are monoclinic with space group $P2_1/c$ as determined by Weissenberg photography. The crystal (0.4 × 0.06 × 0.06 mm) used in the analysis was mounted perpendicular to the 100 face. The cell parameters were determined from a least-squares fit of the setting angles for 24 strong reflections centered on the Nonius-Cad4 autodiffractometer. Integrated intensities were collected in the $\omega/2\theta$ mode to a sin θ limit of 0.55. Due to the poor diffracting quality of the crystal, only 1933 out of 5950 reflections had intensities greater than $3\sigma(I)$. Standard reflections, which were measured after every 100 reflections, remained within ±9% of their initial values. The data were corrected for these fluctuations by using Shiono's[11] scaling program, which employs a four-point interpolation algorithm. The standard Lorentz and polarization factors were applied along with absorption corrections based on Howell's analytical expression for polyhedra.[12] The minimum and maximum absorption corrections were 1.039 and 1.052, respectively. No extinction correction was applied. Crystal data are summarized in Table I.

Table I. Crystal Data

formula	$C_{15}H_{24}N_4O_5PS\cdot Cl\cdot 3H_2O$
temp, °C	25
a, Å	9.916 (8)
b, Å	16.840 (1)
c, Å	15.786 (1)
β, deg	119.45 (4)
V, Å^3	2295.3
Z	4
D_{calcd}, g/cm^3	1.426
D_{obsd}, g/cm^3	1.402
space group	$P2_1/c$
μ, cm^{-1}	3.696
Mo Kα	0.7107
M_r	492.7
F_{000}	964

Structure Determination and Refinement

The application of MULTAN[13] yielded the positions of 24 of the 30 nonhydrogen atoms comprising the structure. A subsequent structure factor calculation with isotropic B (2.63 Å^2) derived from a Wilson plot gave an R_F = 0.367 for the observed data ($R_F = \sum||F_o| - |F_c||/\sum|F_o|$). Isotropic refinement using full-matrix least-squares techniques followed by difference Fourier syntheses revealed the remaining atomic positions in the molecule. Two of the three water molecules were easily located and entered into the refinement. At this stage all heavy atoms were refined anisotropically to an R_F = 0.110. Disordering was indicated for the third water molecule, since two sites kept appearing at likely positions

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Table II. Atomic Coordinates of PLT[a]

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1')	10327 (8)	2711 (4)	397 (6)	36 (5)	42 (5)	53 (5)	4 (3)	25 (4)	8 (4)
C(2')	11825 (10)	2631 (6)	687 (7)	35 (6)	52 (6)	50 (6)	−10 (4)	23 (5)	−2 (5)
N(3')	12672 (8)	2075 (5)	1278 (6)	29 (4)	54 (5)	57 (5)	−4 (4)	28 (4)	3 (4)
C(4')	11995 (9)	1550 (5)	1587 (6)	39 (5)	40 (5)	36 (5)	2 (4)	23 (4)	−5 (4)
C(5')	10356 (9)	1585 (5)	1240 (6)	26 (4)	43 (5)	45 (5)	0 (4)	22 (4)	−7 (4)
C(6')	9613 (11)	2186 (6)	666 (6)	31 (5)	55 (6)	46 (5)	−7 (5)	25 (4)	−2 (5)
C(2'α)	12533 (14)	3199 (8)	317 (11)	48 (6)	80 (9)	81 (9)	−1 (6)	36 (7)	25 (7)
C(3,5')	9615 (9)	962 (6)	1554 (7)	30 (4)	37 (5)	48 (5)	3 (4)	25 (4)	−4 (4)
S	5224 (2)	1433 (1)	595 (2)	29 (1)	55 (1)	45 (1)	2 (1)	19 (1)	−2 (1)
C(2)	7126 (9)	1415 (5)	1409 (6)	38 (4)	35 (4)	48 (5)	−3 (4)	29 (4)	3 (4)
N(3)	7914 (7)	1056 (4)	1031 (5)	27 (3)	34 (4)	47 (5)	1 (3)	21 (3)	1 (3)
C(4)	7020 (10)	788 (5)	102 (6)	43 (5)	37 (5)	40 (5)	1 (4)	22 (4)	0 (4)
C(4α)	7713 (15)	371 (8)	−420 (9)	58 (7)	79 (9)	55 (7)	8 (6)	36 (6)	−17 (6)
C(5)	5493 (10)	949 (5)	−262 (6)	42 (5)	36 (5)	48 (5)	−7 (4)	24 (4)	−8 (4)
C(2α)	7738 (9)	1743 (5)	2424 (7)	30 (4)	40 (5)	57 (6)	−11 (4)	25 (4)	−10 (4)
N(4'α)	12882 (10)	1007 (5)	2181 (6)	27 (4)	46 (5)	63 (5)	−3 (4)	23 (4)	0 (5)
C(5α)	4151 (12)	792 (6)	−1240 (7)	44 (5)	56 (6)	46 (6)	1 (5)	20 (5)	−1 (5)
O(2α1)	9334 (7)	1975 (5)	2819 (5)	33 (3)	67 (5)	84 (5)	−14 (3)	29 (3)	−20 (4)
C(5β)	3658 (16)	1468 (8)	−1914 (9)	60 (7)	78 (9)	58 (7)	−5 (7)	19 (6)	17 (7)
O(5γ)	3211 (11)	2114 (6)	−1521 (8)	84 (7)	70 (6)	101 (7)	5 (5)	40 (5)	27 (5)
C(2α2)	6744 (13)	2428 (7)	2390 (10)	49 (6)	52 (7)	61 (7)	1 (5)	33 (6)	−7 (5)
O(2γ)	8583 (8)	296 (5)	3026 (5)	55 (4)	64 (5)	53 (4)	14 (3)	23 (4)	9 (3)
P(2β)	7628 (3)	961 (1)	3194 (2)	36 (1)	51 (1)	39 (1)	−3 (1)	19 (1)	−8 (1)
O(2β1)	8415 (9)	1219 (5)	4215 (5)	76 (5)	85 (5)	46 (4)	−27 (4)	33 (4)	−18 (4)
O(2β2)	5999 (7)	706 (4)	2759 (5)	45 (4)	55 (4)	80 (5)	−13 (3)	42 (4)	2 (3)
C(2δ)	9663 (25)	−173 (12)	3727 (12)	128 (15)	113 (14)	57 (9)	55 (12)	13 (9)	8 (9)
Cl	10446 (4)	3627 (2)	3664 (3)	75 (2)	68 (2)	114 (3)	−15 (2)	51 (2)	−12 (2)
O(W1)	6048 (13)	2985 (6)	−354 (8)	0.12					
O(W2)	12276 (14)	−431 (7)	2912 (9)	0.13					
O(W3A)	5365 (41)	−385 (18)	3617 (25)	0.13					
O(W3B)	6287 (58)	323 (26)	4984 (35)	0.13					
H(2'α1)	135 (1)	326 (7)	567 (8)	0.06					
H(2'α2)	122 (1)	372 (7)	374 (8)	0.06					
H(2'α3)	121 (1)	322 (7)	−286 (9)	0.06					
H(4α1)	702 (1)	105 (7)	−898 (8)	0.06					
H(5α1)	433 (1)	399 (6)	−158 (7)	0.05					
H(5α2)	337 (1)	557 (6)	−106 (7)	0.05					
H(5γ)	270 (2)	201 (9)	−133 (1)	0.08					
H(4α2)	832 (1)	665 (6)	−569 (7)	0.06					
H(3,5'1)	989 (8)	101 (4)	224 (6)	0.03					
H(3,5'2)	984 (9)	515 (5)	141 (6)	0.03					
H(4α3)	84 (1)	−47 (7)	−27 (7)	0.06					
H(5β1)	45 (1)	184 (6)	−191 (6)	0.05					
H(5β2)	27 (1)	144 (6)	−241 (8)	0.05					
H(2α)	91 (1)	247 (7)	282 (8)	0.06					
H(1')	98 (1)	310 (6)	−11 (7)	0.04					
H(4'α1)	139 (1)	10 (5)	247 (6)	0.05					
H(4'α2)	125 (1)	70 (7)	225 (8)	0.05					
H(2δ1)	100 (1)	−66 (9)	35 (1)	0.10					
H(2δ2)	93 (2)	−31 (11)	35 (1)	0.10					
H(2δ3)	105 (2)	14 (9)	41 (1)	0.10					
H(2α1)	70 (1)	248 (6)	306 (8)	0.05					
H(2α2)	57 (1)	219 (7)	211 (8)	0.05					
H(2α3)	68 (1)	287 (7)	207 (7)	0.05					

[a] Positional parameters are given as fractional coordinates of the unit cell axes; nonhydrogen atoms $\times 10^4$, hydrogen atoms $\times 10^3$. Anisotropic thermal factors have the form $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^* \cos\gamma^*U_{12} + 2hla^*c^* \cos\beta^*U_{13} + 2klb^*c^* \cos\alpha^*U_{23})]$, coefficients $\times 10^3$. Isotropic thermal parameters have the form $\exp[-8\pi^2U(\text{iso}) \sin^2\theta/\lambda^2]$. All anisotropic U_{ij} values are $\times 10^3$. Estimated standard deviations in parentheses are for the least significant figure.

in the difference maps, but crystal density measurements indicated the presence of only three solvent molecules. Isotropic temperature factors were held constant on the two possible solvent positions (set to 10.4 Å^2, the average B for the two fully occupied water molecules) and the occupancies were refined. The sum of the occupancy factors for the solvent positions O(W3A) (0.45) and O(W3B) (0.50) is near unity (0.95), and in either position favorable contacts with neighbors are made. Hydrogen atom positions (except those in the water molecules) were derived from difference Fourier syntheses and their inclusion reduced R_F to 0.081. Weights for the structure factors were determined by analysis of variance.[14] Atomic scattering factors for the nonhydrogen atoms and Stewart's H scattering factors were taken from ref 15; for atoms Cl^-, P, and S the anomalous components were included in the calculation.

For reduction of the disagreement between observed and calculated structure factors arising from the disordered solvent molecule, the 153 reflections with the highest F_{calcd} contribution (>20%) from OW3 were then removed. Refinement of structural parameters was done with a 0.4 sin θ cutoff, which eliminated 193 highest order reflections that barely exceeded the 3σ criterion. Further anisotropic refinement yielded R_F of 0.067 for a total of 1587 reflections. The estimated standard deviation for an observation of unit weight, $\sum|\sum(F_o - F_c)|/(NO - NV)$ was 0.9717, and a final difference map showed no significant peaks. The final atomic coordinates are listed in Table II. Structure factors are available as supplementary material.

Description of the Molecular and Crystal Structure

A schematic representation of the molecule in Figure 1 contains bond distances, angles, and atom designations. PLT is an ionic molecule having positively charged pyrimidinium and thiazolium

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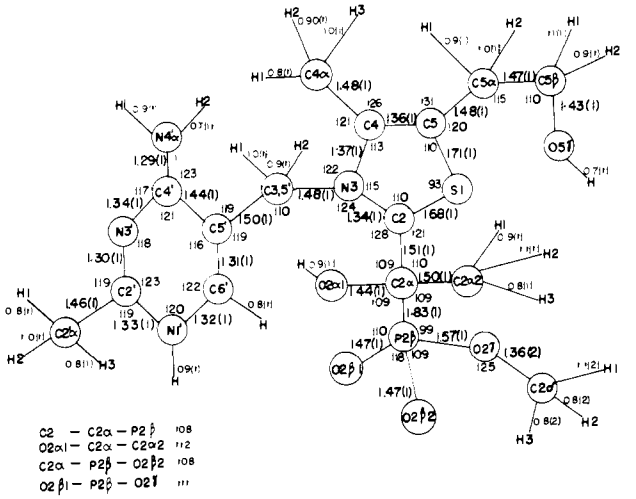

Figure 1. Schematic diagram of phosphalactylthiamin showing bond distances in angstroms, bond angles in degrees, and the atom designations. The esds for the bond lengths are given in parentheses. The bond angle data are given only for those angles involving nonhydrogen atoms. The numeric labels for the hydrogen atoms are identical with those atoms to which they are bonded. If more than one hydrogen is bonded to the same atom, a sequential suffix number is appended to the label.

Table III. Torsion Angles for PLT

	ϕ_T	ϕ_P	$\phi_{s\alpha}$	$\phi_{s\beta}$
F Conformation				
av abs value (16 structures)	6.8	83.8	76.4	70.0
(rms deviation)	(4.8)	(6.7)	(19.3)	(28.5)
S Conformation				
this structure	−99.5	−173.6	80.0	−60.2
2-(α-hydroxyethyl)-thiamin[4]	−100.3	−145.6	81.8	30.1
2-(α-hydroxybenzyl)-thiamin[5a]	92.7	−167.3	3.3	63.4
2-(α-hydroxybenzyl)-oxythiamin[5b]	92.9	−165.9	91.8	−48.9

rings and a negatively charged phosphonate. The net positive charge on the molecule is balanced by a chloride ion in the crystal structure. There are no unusual bond lengths in this structure; however, a critical evaluation is not possible because of the large standard deviations for the distances and angles. Both of the aromatic rings in the compound are planar to within the experimental limits of error. The equations of the least-squares planes though the rings and the displacements of the relevant atoms from the planes are available as supplementary material.

The conformation of the molecule is best expressed in terms of the torsion angles about the bonds from the methylene bridge carbon to the rings. ϕ_T and ϕ_P specify the angles about the bonds to the thiazolium and pyrimidinium rings, respectively.[16] The values for these angles are presented in Table III along with values for other thiamin structures. A comparison reveals that the conformation is similar to that of other C2-substituted thiamins. Although the conformation with respect to the methylene bridge is similar to that of the other derivative structures, the conformation of the substituent itself does not possess the characteristics observed in previous structures. In particular, the nearly coplanar disposition of the S(1)–C(2) and the C(2α)–O(2α1) bonds and the resulting close contacts between the sulfur and oxygen atoms are not present in this structure. The torsion angles about the

(16) The torsion angle ϕ_T = C(5′)–C(3,5′)–N(3)–C(2), ϕ_P = N(3)–C(3,5′)–C(5′)–C(4′), $\phi_{s\alpha}$ = S–C(5)–C(5α)–C(5β), and $\phi_{s\beta}$ = C(5)–C(5α)–C(5β)–O(5γ). An S conformation is specified by torsion angles ($\phi_T \sim \pm 100°$, $\phi_P \sim \pm 150°$). An F conformation is specified by $\phi_T \sim 0°$, $\phi_P \sim \pm 90°$. For more complete details, see: footnote 13, in ref 5a, and footnote 4 in Shin et al. (Shin, W.; Pletcher, J.; Sax, M.; Blank, G. *J. Am. Chem. Soc.* **1979**, *101*, 2462–2469).

Table IV. Torsion Angles for the C(2) Substituent

atoms	angle, deg
O(2α1)-C(2α)-C(2)-S	−155.9
C(2α2)-C(2α)-C(2)-S	−31.9
P(2β)-C(2α)-C(2)-S	86.0
H(2α)-O(2α1)-C(2α)-C(2)	113.2

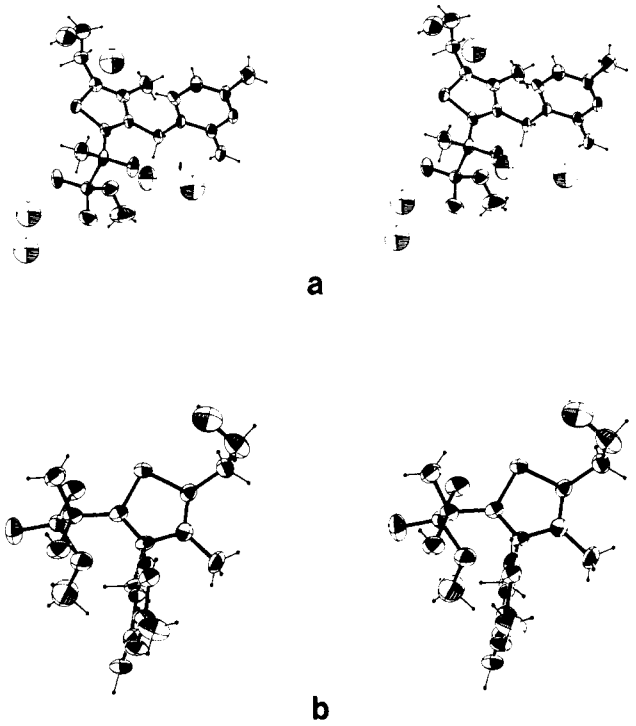

Figure 2. (a) Stereoscopic drawing of a single PLT molecule including the chloride ion and water molecules. (b) Stereoscopic view of molecule seen normal to thiazolium ring for comparison with structures of other C(2) derivatives.[4,5]

C(2)–C(2α) bond, which are listed in Table IV, indicate that the C(2) substituent is oriented so as to maximize the displacements of the C(2α) substituents from the plane of the thiazolium ring with the phosphonate group directed nearly perpendicular to the ring. The C(2α2) methyl group, which is now close to the sulfur, minimizes its unusually close contact with sulfur by straddling the sulfur with two of its hydrogens, but the closest contact with the C(2) substituent is between the hydroxyl and the C(3,5′) methylene (Figure 2).

Molecular Packing of PLT. The molecular packing can be discerned from the hydrogen bonding and other close contacts listed in Table V and from the packing diagram as viewed down the *b* axis in Figure 3. The reason for the generally poor diffracting quality of the crystal is apparent from the overall pattern of weak and unsatisfied hydrogen bonds. This is particularly evident in the two alternative positions assumed by water molecule O(W3). O(W3A) has one strong and one moderate hydrogen bond. The alternative position, O(W3B), only participates in several weak bonds, although the contact with O(W1) provides a stronger alternate interaction with O(W1) than the one from O(W1) to O(2β1). O(W2) seems to be the most strongly bound water molecule, but it does not utilize its full hydrogen-bonding potential. Even the chloride ion forms only one weak and two moderate hydrogen bonds. The hydrogen bonding of the thiamin molecule for the most part follows the expected patterns. Both 4′-amino hydrogens and N(1′)–H participate as hydrogen-bond donors with the latter forming a stronger bond as expected.[17] However, N(3′) does not accept any hydrogen bonds. An unusual

(17) Jardetzky, O.; Papas, P.; Wade, N. G. *J. Am. Chem. Soc.* **1963**, *85*, 1657–1658.

Table V. Possible H Bonds and Other Short Contacts

A	B	C	d_{AC}, Å	d_{BC}, Å	∠ABC, deg	symmetry
N(1')	H(1')	O(2β1)	2.62	1.75	167	$x, {}^1/_2 - y, {}^1/_2 + z$
N(4'α)	H(4'α1)	O(2β2)	2.81	1.94	161	$-1 + x, y, z$
N(4'α)	H(4'α2)	O(W2)	2.87	2.24	157	x, y, z
O(2α1)	H(2α1)	Cl	3.05	2.35	134	x, y, z
O(5γ)	H(5γ)	Cl	3.15	2.47	152	$-1 + x, {}^1/_2 - y, {}^1/_2 + z$
C(6')	H(6')	O(W1)	3.32	2.58	155	x, y, z
C(3,5')	H(3,5'1)	O(2α)	2.74	2.06	125	x, y, z
C(3,5')	H(3,5'1)	O(2γ)	3.17	2.49	125	x, y, z
O(W1)	H(?)	O(5γ)	2.89			x, y, z
O(W1)	H(?)	O(2β1)	3.06			$x, {}^1/_2 - y, {}^1/_2 + z$
O(W1)	H(?)	O(W3B)	2.89			$x, {}^1/_2 - y, {}^1/_2 + z$
O(W2)	H(?)	Cl	3.07			$2 - x, -{}^1/_2 + y, {}^1/_2 - z$
O(W3A)	H(?)	O(2β2)	2.54			x, y, z
O(W2)		O(W3A)	2.70			$-1 + x, y, z$
O(W2)		O(W3B)	2.91			$2 - x, -y, 1 - z$
O(W3B)		O(W3B)	2.80			$1 - x, -y, 1 - z$
O(W3B)		O(2β1)	3.28			x, y, z
O(5γ)		S	3.15			$1 + x, y - {}^1/_2, z + {}^1/_2$
N(3')		S	3.38			$1 + x, y, z$
C(2α2)		S	2.98			x, y, z
H(2α2)		S	2.54			x, y, z
H(2α3)		S	3.18			x, y, z

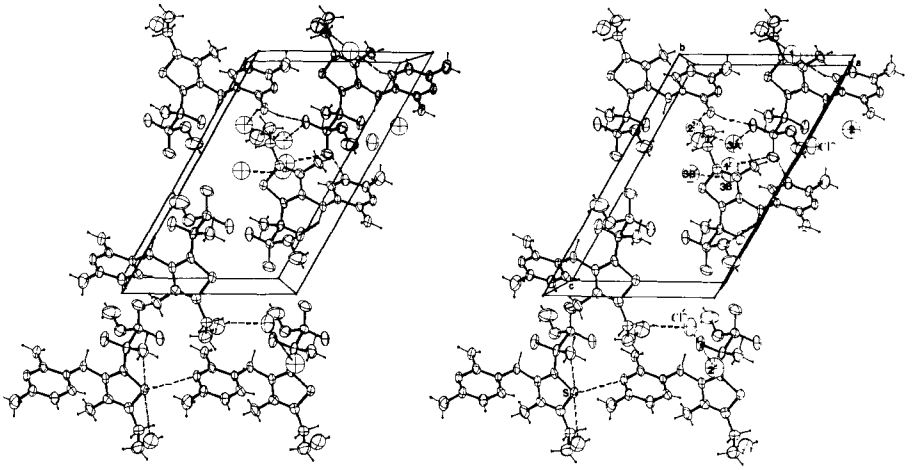

Figure 3. Stereoscopic drawing of crystal packing viewed down the *b* axis. The shaded molecule and the labeled chloride and water molecules correspond to the atoms in Table II. The atoms with primed labels indicate symmetry or translation equivalent atoms. Selected hydrogen bonds and close contacts are indicated with dotted lines. Only partial contents of the unit cell are shown to improve the clarity of the intermolecular bonding.

"hydrogen-bonding interaction" exists between the methylene bridge H(3,5'1) and both O(2α1) and O(2γ) with the former making a substantially closer contact (see Discussion).

The characteristic close contact between sulfur and electronegative atoms in the plane of the thiazolium ring seen in previous crystal structures is weaker in this structure. The intramolecular contact with O(5γ) is shorter than the sum of the van der Waals radii, but it is substantially greater than the values observed with O(2α1) and even O(5γ) in structures where it is closer to the ring plane as a result of $\phi_{5\alpha}$ being close to 0°. A second contact exists with N(3') of a translationally equivalent molecule, but this contact is not closer than the sum of the radii.

Discussion

Although the conformation of the C(2) substituent was initially surprising, subsequent analysis revealed its favorable aspects. The intramolecular S···O interaction, which has characterized the structures of all previous C(2) substituents, certainly contributed to the stability of that conformation. Evidently, in the present structure the loss of the electrostatic S···O interaction is more than offset by avoidance of deleterious steric factors and by the compensating formation of an energetically favorable interaction between a methylene bridge hydrogen and oxygens O(2α1) and O(2γ). In addition O(2α1) does participate in a favorable, but weaker, electrostatic interaction with N(3) (separation equals the sum of the van der Waals radii). The distinctive structural feature of this derivative in comparison with the previous ones is the replacement of the C(2α) hydrogen with the much bulkier phosphonate group. The presence of that group drastically restricts the rotational mobility of both the C(2) substituent and the pyrimidium ring. This enhanced restriction is apparent from space-filling models and from computer-modeling studies. Those studies also indicate that the observed conformation provides the least steric interference for the groups attached to C(2α), given the observed conformation of the thiamin rings.

This structural analysis suggests that the introduction of a substituent on C(2) can generate a second element of asymmetry by virtue of restricted rotation about the bonds at C(3,5'). Since a specific enantiomeric form at C(2α) exists only with a specific "enantiomeric" form at C(3,5'), it implies that the conformation of thiamin in the synthetic reaction is a stereochemical determinant of the resultant product producing essentially two of four possible isomers. Clearly on steric grounds other conformations with different pyrimidine ring orientations are equally plausible, but the rotational barriers between them appear substantial. (Theoretical studies by Jordan[18] on the considerably less bulky hydroxyethyl derivative indicate rotational barriers greater than 18 kcal.) Thus the conformations as they exist in solution are likely to be structurally similar to the synthesized molecule as observed in this crystal structure. It is also interesting that even though O(2α1) is in a position where it could accept a hydrogen bond

(18) Jordan, F. *J. Am. Chem. Soc.* **1976**, *98*, 808–813.

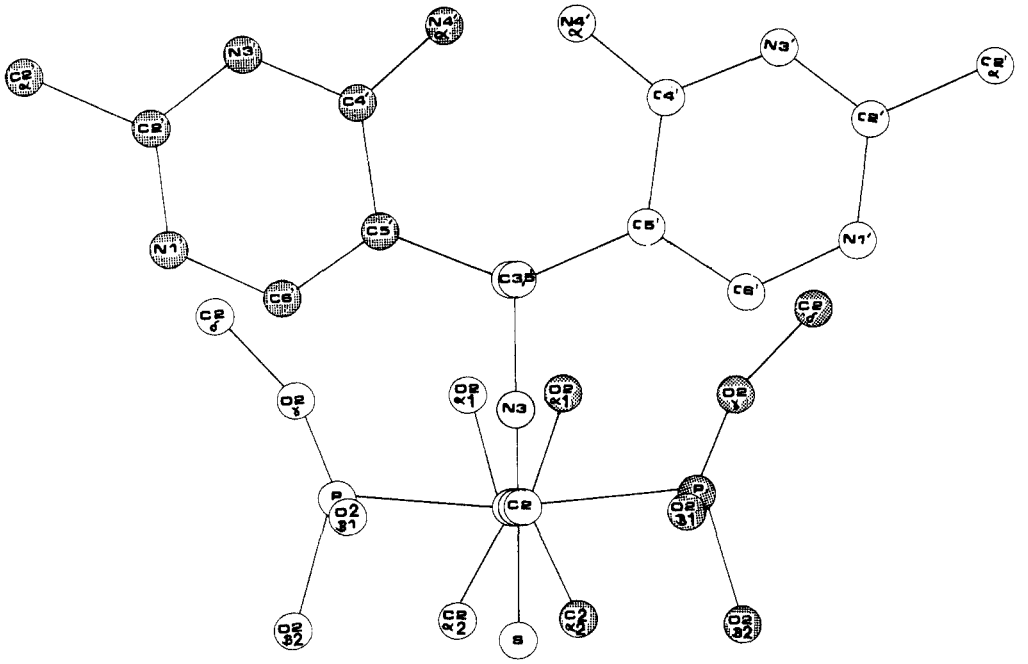

Figure 4. Projection down the C(2)–C(2α) bond of both enantiomeric forms that have been least-squares fit to the thiazolium ring. The remaining half of the thiazolium ring and the attached substituents have been removed for clarity. The noncoincident atoms from the second molecule are shaded.

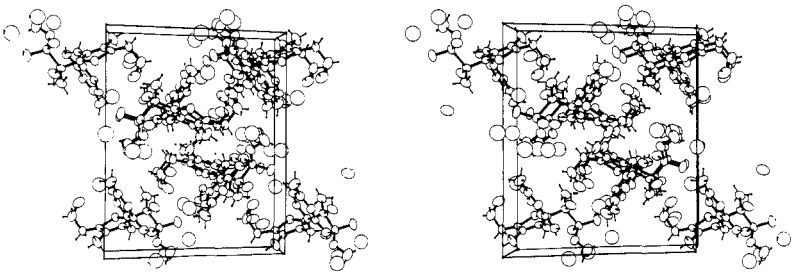

Figure 5. Stereoscopic drawing of crystal packing as viewed down the *a* axis showing the clustering of water molecules about the phosphate segment of the molecule.

from N(4′α)–H, the pyrimidine ring is not oriented in the **V** conformation that is required for such an interaction. It does not appear very likely that a change from **S** to **V** could take place after the substituent added to C(2).

If this structure truly represents the two enantiomeric forms produced in its synthesis and if the molecular rotations are as sterically hindered as suggested above, then the structure provides insight into the binding site of pyruvate dehydrogenase. The preliminary kinetic studies by Kluger and Pike[8] were consistent with both enantiomers of PLTDP binding to the active site with only one of the enantiomers undergoing the catalytic reversal of inhibitor addition. Some ideas concerning the steric environment of the binding site can be suggested from a comparison of the two enantiomers. If the thiazolium ring is taken as a constant structural feature, then the structures of the two enantiomers can be compared by performing a least-squares fit of the thiazolium rings (root-mean-square deviation, 0.005 Å) and examining the spatial relationships for the remainder of the molecule with respect to the plane containing the thiazolium ring. (The C(5) hydroxyethyl substituent and its diphosphate ester can assume identical conformations in the two enantiomers.) Figure 4 shows the relative arrangements of the pyrimidinium rings and the C(2) substituents. The close proximity of positions for O(2α1) and N4′α) between the two enantiomers is apparent. The actual distances between some of the corresponding atoms are listed in Table VI. It is evident that these alternative O(2α1) sites are much closer together than the 2.4 Å that separates alternative sites produced by interchanging O(2α1) and C(2α2). Perhaps this reduced separation for the alternative O(2α1) site is still too far removed from the basic group that must extract the proton in order to catalyze the reversal of the inhibitor addition. It is also possible that in accommodating the alternative positions for the pyrimidine ring or the phosphonate group, the positioning of the entire molecule must be adjusted slightly with a resultant increase in the distance between O(2α1) and the basic group that removes its proton.

Table VI. Comparison of R vs. S Conformers of PLT[a]

atom	Δ, Å	atom	Δ, Å
O(2α1)	1.02	C(2′α)	10.41
N(4′α)	1.43	O(2β2)	4.82
N(1′)	7.41	O(2γ)	4.74
N(3′)	5.77	C(2δ)	6.44

[a] Δ is the separation between selected atoms of the two enantiomeric conformers appearing in the crystal structure after the thiazolium rings of the two molecules were fit by a least-squares minimization.

The diastereotopic C(3,5′) hydrogens in PLT produce an AB quartet in the NMR spectrum. This spectral feature is consistent with the conformation seen in this analysis, which places the hydrogens in totally different environments. H(3,5′1) forms intramolecular "hydrogen bonds" with both O(2α1) and O(2γ) while H(3,5′,2) makes normal contacts with the C(4α)-methyl and N(4′α)-amino hydrogens. Since the spectrum for 2-(α-lactyl)-thiamin shows a similar (but smaller) splitting, we would predict a similar conformation in which the bond to the carboxyl is perpendicular to the thiazolium ring and the hydroxyl is in contact with a methylene hydrogen. Enantiomeric structures, similar to those found in PLT, are likely to predominate in LT because of similar steric interactions. The presence of a single peak for the diastereotopic C(3,5′) hydrogens in 2-(α-hydroxyethyl)thiamin is consistent with their very similar nearest neighbor environments

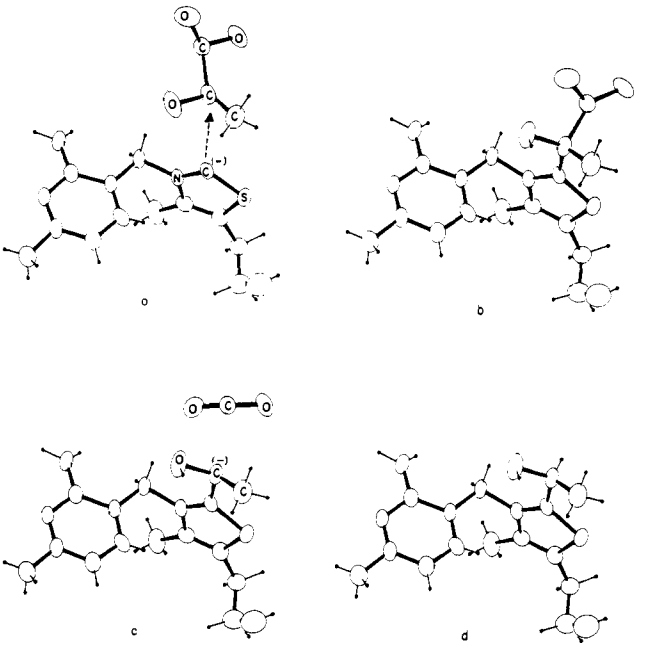

Figure 6. Diagrammatic representation of reaction pathway from pyruvate addition through decarboxylation and formation of the 2-(α-hydroxyethyl) intermediate. (a) Addition of thiazolium ylide to carbonyl of pyruvate. (b) 2-(α-Lactyl)thiamin intermediate. (c) Decarboxylation of (LT) intermediate to form (d) the 2-(α-hydroxyethyl)thiamin intermediate.

consisting of methyl groups as seen in the crystal structure.[4]

The packing diagram (Figure 5) shows the extensive clustering of the water molecules around the C(2) substituent. Because of the suggested mechanistic involvement of substrate dehydration as a source of catalysis for the decarboxylation of lactylthiamin,[19] this type of hydration could prove to be a significant observation if it is also found in lactylthiamin.

Least Motion and Maximum Orbital Overlap. The torsional relationship of the portion of (PLT) derived from thiamin to that derived from methyl acetylphosphonate can be predictive of that expected for 2-(α-lactyl)thiamin (LT) with the phosphonate group of (PLT) substituting for the carboxylate of (LT) (see Figure 6).

The most favorable geometry for formation of (LT) can be deduced. The ylide formed by ionization of the C(2) proton of thiamin contains a negative charge localized in an sp^2 orbital that is in the plane of the thiazolium ring.[2] The thiazolium ylide can thus be considered to be a planar nucleophile at C(2). This will add to the carbonyl carbon atom of pyruvate so that the plane of the carbonyl group will be perpendicular to the nucleophile plane in the lowest energy electronic conformation as is the case for other carbonyl group additions (Figure 6a). The dihedral angle between the planes containing the reacting pairs is then 90° with a choice of two conformations. One places the positive thiazolium nitrogen center of the ylide synperiplanar to the carbonyl oxygen atom of pyruvate. The other is the antiperiplanar conformation, which places the carbonyl oxygen adjacent to the positive thiazolium sulfur. Because a favorable electrostatic interaction would exist for either conformation, electrostatic attraction imposes little, if any, selectivity between the two possible conformations. However, as discussed above, the synperiplanar conformation should be favored on steric grounds. This transition state then produces (LT) in a conformation analogous to that found in this study for crystalline (PLT) (Figure 6b).

This conformation allows (LT) to decarboxylate with minimal motion (the principle of least motion[20]), since in order for carbon dioxide to leave, the σ bond between the carboxylate and the C(2α) atom must break and will be stabilized by overlap with the π system of the thiazolium ring. Maximum orbital overlap is necessary in the transition state of the developing electron pair and the π system. This state is achieved when the σ bond is in a plane perpendicular to that of the thiazolium ring (Figure 6c,d). This use of the principle of maximum overlap has been applied successfully to many other enzymatic decarboxylation reactions after its initial justification by Dunathan for pyridoxal-dependent systems.[21] The conformation of (PLT) is precisely that which would permit (LT) to decarboxylate readily.

We suggest that when pyruvate reacts with thiamin diphosphate and an enzyme proceeding through the conformation we find for PLT, it would lead to maximal catalytic efficiency since its formation and decarboxylation would be structurally correlated. This minimizes entropic restrictions on the enzymatic reaction.

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Registry No. Thiamin chloride hydrochloride, 67-03-8; sodium methyl acetylphosphonate, 69103-75-9; methyl (±)-2-hydroxy-2-(2-thiamin)-ethylphosphate chloride trihydrate, 81457-41-2.

Supplementary Material Available: A listing of the observed and calculated structure factor amplitudes and least-squares planes with atomic deviations is available (9 pages). Ordering information is given on any current masthead page.

(19) Kluger, R.; Smyth, T. *J. Am. Chem. Soc.* **1981**, *103*, 1214–1216.

(20) Hine, J. *Adv. Phys. Org. Chem.* **1977**, *15*, 1–91.

(21) Dunathan, H. C. *Adv. Enzymol. Relat. Subj. Biochem.* **1971**, *35*, 79–134.