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Interconvertible (*Z*/*E*)-Stereoisomers of a Vitamin B_6 Coenzyme Analog Derived from Pyridoxal 5'-Phosphate and Rhodanine

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Abstract: The *Knoevenagel* condensation product of pyridoxal 5'-phosphate (**1**) with rhodanine (**2**) was prepared and identified as (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl] methylene]-2-thioxo-4-thiazolidinone (**3**). The labile (*E*)-stereoisomer **4** was obtained in a crude form. Its association with the nucleobase adenine was prepared suggesting an affinity to nucleic acids.
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INTRODUCTION

Besides its well known ability to form *Schiff* bases with amino acids and various primary amines, pyridoxal 5'-phosphate (**1**)[1] tends to give cyclic condensation products with certain polyfunctional amino acids, like cysteine,[2,3] histidine,[2,3] and 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA).[4] Furthermore, reactivity of pyridoxal in the *Knoevenagel*[5,6] condensation has been reported with glycine methylester.[7]

The use of *p*-dimethylaminobenzalrhodanine for the detection of silver was introduced by Feigl.[8] More recently Escobar Godoy and Guiraúm Pérez[9] observed a *Knoevenagel* condensation of pyridoxal with the active methylene heterocycle rhodanine (2-thioxo-4-thiazolidinone) (**2**), and used this pyridoxal analog 5-[3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (pyridoxylidene-rhodanine) (**5**) for the spectrophotometric quantification of silver. These preceding findings stimulated the investigation of the reaction between **1** and **2**. The structure of the reaction product was expected to be (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (**3**), or trivially named (*Z*)-5'-*O*-phosphono-pyridoxylidenerhodanine.

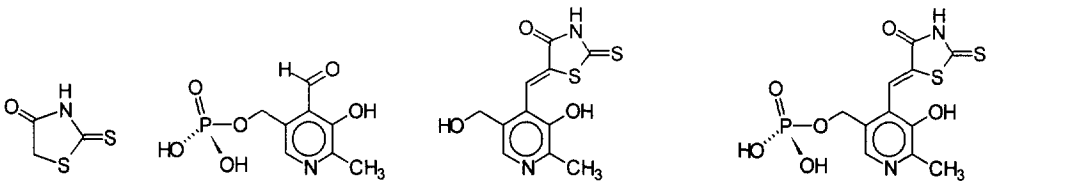


FIG. 1. Chemical structures of rhodanine, PLP, and two vitamin B_6 *Knoevenagel* condensation products.

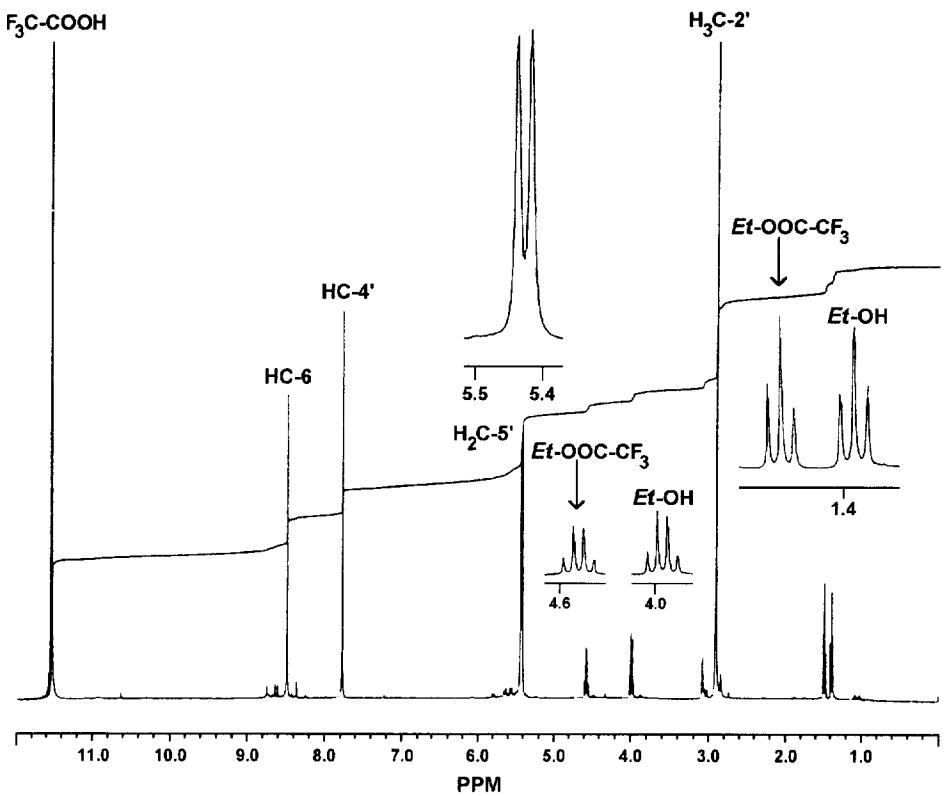


FIG. 2. *Fourier*-transformed 400 MHz ^{1}H NMR spectrum of the *Knoevenagel* condensation product of **1** and **2** dissolved in TFA-*d*. The hydrogen atoms are numbered according to the nomenclature for **1** used in literature.[10-14,16-23] Ethanol contained in the substance is responsible for a triplet and a quartet, noted as *Et*-OH. The triplet and the quartet from the solvent artifact ethyl trifluoroacetate are depicted as *Et*-OOC-CF_3.

Interestingly the imide-like proportion of the rhodanine-derived part of the molecule promises an affinity to adenine nucleobases of nucleic acids because of imitating thymidylic acid (thymidine 5'-monophosphate) counterparts. This implicates various possibilities of this new compound to interfere with gene regulation and viral replication processes.

RESULTS

The NMR Spectra of the Reaction Product of 1 and 2

^{1}H NMR Spectrum. The proton magnetic resonance spectrum was examined in TFA-*d*. One characteristic feature of the spectrum shown in Fig. 2 was a phosphorus-hydrogen spin-spin coupling between the protons of the 5'-methylene group and the *ortho*-phosphoric acid monoester phosphorus with a vicinal coupling constant $|^3J(^{31}P,^1H)| = 8.0$ Hz, which appears to be in agreement with other coupling constants for comparable derivatives of **1** reported in literature.[10-14]

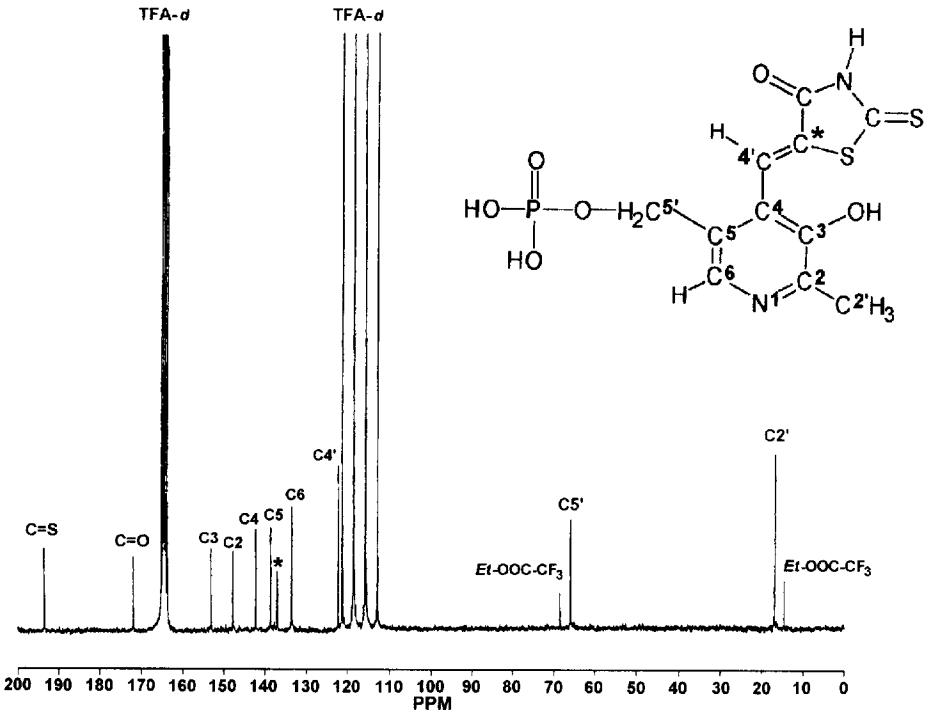


FIG. 3. *Fourier*-transformed fully proton-decoupled 100 MHz ^{13}C NMR spectrum of the *Knoevenagel* condensation product of **1** and **2** dissolved in TFA-*d*. Eight carbon atoms are numbered according to the nomenclature for **1** used in literature.[10-14,16-23] The additional carbon atoms are designated C=S, C=O, and the former C-5 of the thiazolidine moiety is depicted with a star (*). The solvent artifact ethyl trifluoroacetate is depicted as *Et*-OOC-CF_3.

The two triplets and two quartets are contributed to ethanol and trifluoroacetic acid ethyl ester (ethyl trifluoroacetate). This has been established by imitating the solvent conditions. For that purpose a mixture of ethanol and TFA-*d* was examined by proton NMR (data not shown). The straightforward esterification of TFA-*d* with ethanol, yielding ethyl trifluoroacetate (δ 1.42 ppm, *t*, CH_3; δ 4.49 ppm, *q*, CH_2; $|^3J(^1H,^1H)|$ = 7.3 Hz) and water, has been observed. Olah *et al.* are cited[15] for proton NMR data on trifluoroacetic acid ethyl ester. Because the ^1H NMR spectrum was recorded immediately after dissolving the substance in TFA-*d*, both ethanol (δ 1.38 ppm, *t*, CH_3; δ 3.99 ppm, *q*, CH_2; $|^3J(^1H,^1H)|$ = 7.0 Hz) and ethyl trifluoroacetate (δ 1.48 ppm, *t*, CH_3; δ 4.57 ppm, *q*, CH_2; $|^3J(^1H,^1H)|$ = 7.3 Hz) emerged simultaneously. Since the ^{13}C NMR spectrum was consecutively recorded with the identical solution it was therefore subjected to unintended incubation. This resulted in quantitative conversion of ethanol to ethyl trifluoroacetate.

^{13}C NMR Spectrum. The fully proton-decoupled ^{13}C NMR spectrum recorded in *Fourier* transform mode in TFA-*d* is shown in Fig. 3. This spectrum is in agreement with the expected vitamin B_6-related structure.[16-23] Surprisingly the signals for the C-4' carbon and for the joining non-aromatic carbon consist of two singlets. This may be due to the existence of molecular subspecies, a higher protonated form in the solvent used with all acidic protons replaced by deuterons.

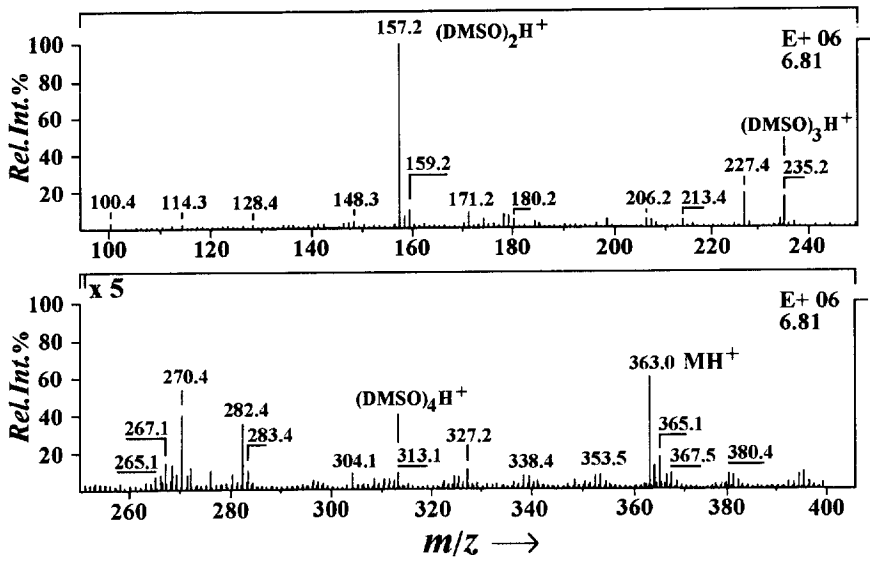


FIG. 4. Fast atom bombardment mass spectrum of the *Knoevenagel* reaction product of **1** and **2**.

In addition, two signals attributed to solvent artifacts from the ethanol contained in the substance can be detected. In trifluoroacetic acid ethanol is esterified completely, yielding ethyl trifluoroacetate[24] with δ 14.44 ppm (*s*, H_3C) and 68.40 ppm (*s*, H_2C).

^{31}P NMR Spectrum. The proton-undecoupled *Fourier*-transformed 161.977 MHz ^{31}P NMR spectrum of the reaction product gave a singlet with δ - 4.24 ppm. The spin-spin coupling of the phosphorus with the two vicinal C-5' hydrogens should create a triplet splitting of the resonance signal, as it has been observed in previous ^{31}P NMR spectra of **1**.[25-27] The appearance of the ^{31}P resonance singlet could be ascribed to strongly acidic conditions in TFA-*d*. Paramagnetic trace impurities are another possible reason, since no chelating agents were added. The proton-decoupled ^{31}P NMR spectroscopy gave a singlet with the identical chemical shift.

The UV/VIS Absorption Spectrum in Water of the Reaction Product of 1 and 2

The data are given in experimental section. Since the reaction product is assumed to be of polyionic nature, and in water will participate in complex dissociation equilibria, the *Bouguer–Lambert–Beer* law cannot be applied here. For this reason the absorbance *A* (1%/1cm) is calculated for a sample with a mass concentration of 1% (*w/v*) and a layer thickness of 1 cm. The spectrum was recorded at a sample mass concentration of 0.005% (*w/v*) and the absorbance values measured did not exceed 1.96 absorbance units over a wavelength range from 240 nm to 600 nm. The scanning speed was 750 nm/min. For the interpretion of the spectral properties corresponding literature[28,29] is cited.

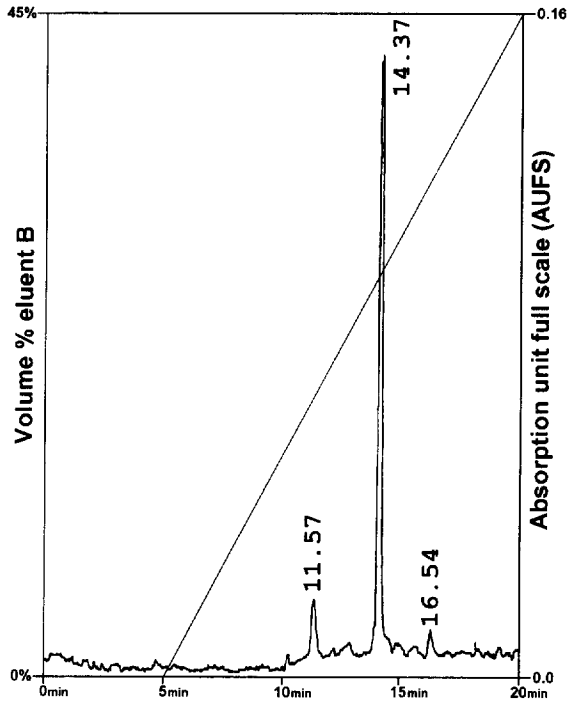


FIG. 5. RP 18 HPLC elution profile of the crude synthetic product **3** with UV absorbance monitored at a wavelength of 215 nm. The retention times are uncorrected and given as recorded.

FAB Mass Spectrometry of the Reaction Product of 1 and 2

Because of their insufficient volatility electron impact spectra of phosphorylated B_6 vitamins are not to be obtained directly. Therefore the field desorption mass spectrometry[30-32] is suitable for them. FAB ionization technique has been chosen for our purpose. About 1 μg of **3** was dissolved in the matrix DMSO. The relative peak intensity is referred to the intensity of the matrix cation *m/z* 157.2 $[(DMSO)_2H^+]$ which is set on 100%. The spectrum (Fig. 4) reveals significant fragmentation of MH^+. The loss of *ortho*-phosphoric acid H_3PO_4 (calculated for *m/z* 97.98) *m/z* 363.0 → *m/z* 265.1 (Δ *m/z* 97.9) is one suggested degradation.

Reversed Phase C 18 HPLC of the Reaction Product of 1 and 2

For a chromatographic analysis a crude synthesis product **3** was used without further purification. An UV detector monitored absorbance at a wavelength of 215 nm. Eluent A was 0.1% (*v/v*) TFA, eluent B acetonitrile with 0.1% (*v/v*) TFA and the flowrate was 0.8 ml/min using a gradient from 0% B → 45% B in 15 min (Fig. 5). The identity of the UV absorbing material at a retention time of 11.57 min has not been established, although it is believed to represent either a stereoisomer or a higher protonated form of the main UV absorbing species eluting at 14.37 min. The peak at a retention time of 16.54 min was identified[9] as **5** by evaluation of comparative runs with **5**, **2**, pyridoxal, and **1** (data not shown).

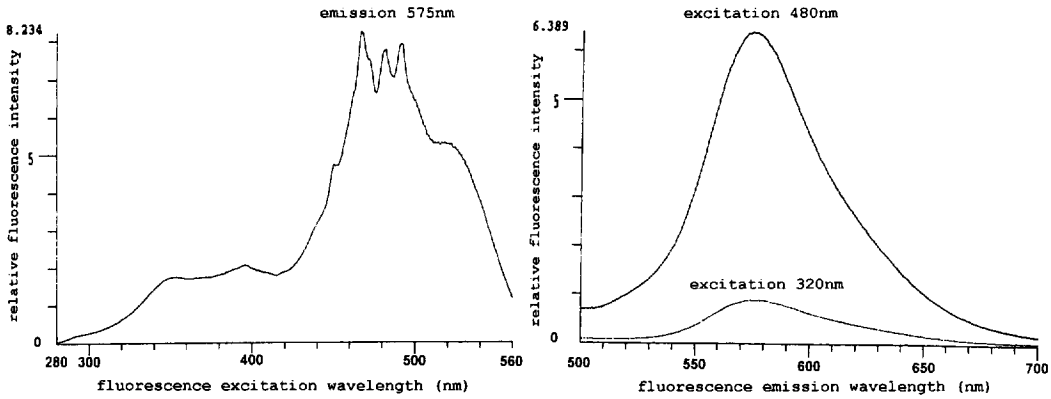


FIG. 6. Fluorescence excitation and emission spectra in anhydrous DMSO of the complex formed between the designated (*E*)-stereoisomer of 5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (**4**) and a molar excess of the nucleobase adenine.

The Equilibration Product of 3

Following titration of (*Z*)-5'-*O*-phosphono-pyridoxylidenerhodanine (**3**) which has been dissolved in 10 M hydrochloric acid with sodium bicarbonate to pH 6, a red salt-containing precipitate was obtained. 75 mg of dry product have been extracted with anhydrous DMSO yielding a cherry red solution, nearly free of coprecipitated anorganic salts like sodium chloride and sodium bicarbonate. This solution is stable under anhydrous conditions. Addition of water to this solution changes colour from red to yellow. Since DMSO is reasonably hygroscopic, this effect proceeds if the DMSO solution is left without proper humidity exclusion.

^{1}H NMR Spectrum in DMSO-d_6. Proton NMR gave four singlets. The missing of phosphorus-hydrogen spin-spin coupling is contributed to the existence of the (*E*)-stereoisomer **4** as sodium salt oligohydrate. This created displacement of hydrate water in the DMSO-d_6 solution and induced peak broadening.

Fully Proton-Decoupled ^{13}C NMR Spectrum in DMSO-d_6. Data are given in synthesis section. The resonances of C-2', C-4', C-2, C-3, and C=S were doubled. Remarkably high was the shielding of C-3 which is not often observed for derivatives of **1**. Also shielded, although more than expected for the solvent change were C-6, C=O, and C=S. The assignment of the NMR resonances to certain carbon atoms followed well known rules in literature[16-23] as well as our own reference spectra of related vitamin B_6 derivatives (data not shown).

The Equilibration Product of a Mixture of Adenine with 3

A 6 : 1 molar mixture of adenine (formulated as tautomer 9*H*-purin-6-amine) with (*Z*)-5'-*O*-phosphono-pyridoxylidenerhodanine (**3**) was suspended in 10 M hydrochloric acid. The suspension was brought to pH 6 with portions of sodium bicarbonate. A red salt-containing precipitate was formed and could be isolated.

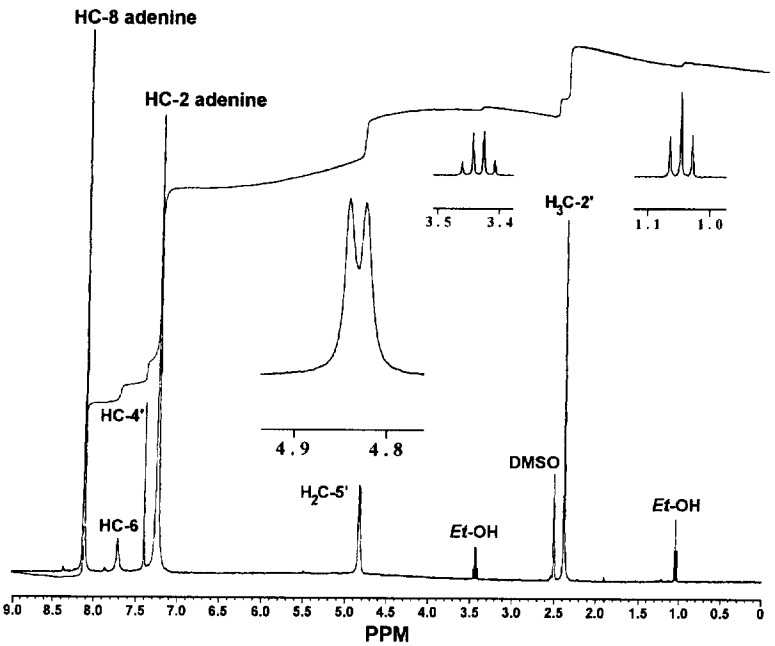


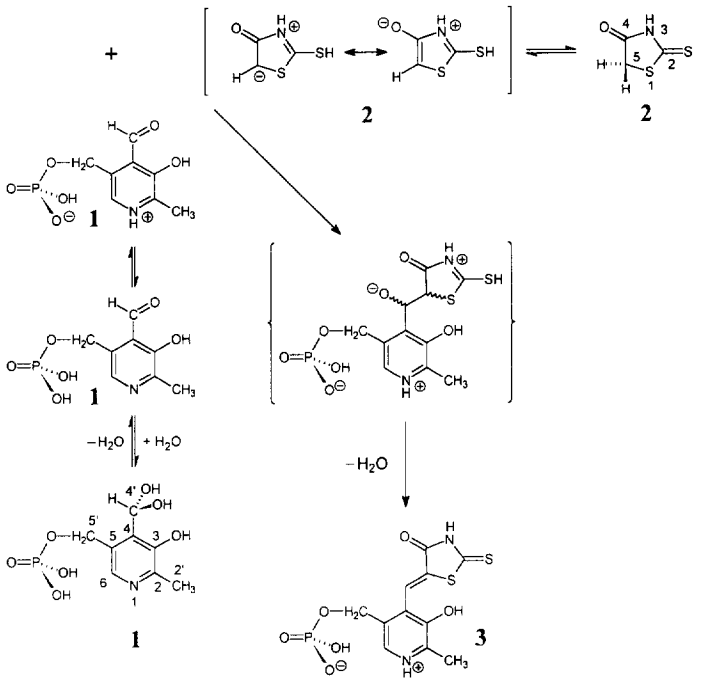
FIG. 7. The *Fourier*-transformed 400 MHz ^{1}H NMR spectrum in DMSO-d_6 of the complex formed between the designated (*E*)-stereoisomer of 5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (**4**) and a molar excess of the nucleobase adenine, prepared according to the instruction given in synthesis section.

70 mg of dry product have been extracted either with DMSO or DMSO-d_6 yielding a solution nearly free of sodium chloride and sodium bicarbonate. For this solution the preceding remarks are valid.

UV/VIS Electronic Absorption Spectrum. The red DMSO solution showed a low intensity VIS electronic absorption maximum at a wavelength of 525 nm. An aqueous solution of the product showed an UV/VIS spectrum very comparable to that of **3** in water.

Fluorescence Excitation and Emission Spectra.[33] The DMSO solution defined previously showed a strong fluorescence emission at a wavelength of 575 nm, both under monochromatic excitation at 320 nm and 480 nm as shown in Fig. 6. The spectra are uncorrected and the *y*-axis is expressed in terms of relative fluorescence intensity units.

^{1}H NMR Spectrum in DMSO-d_6. Proton NMR gave strong singlets of two protons of adenine. The proton at C-2 is shielded more than expected, differing from chemical shifts in literature[34,35] (adenine in DMSO-d_6). The four resonances of 5'-*O*-phosphono-pyridoxylidenerhodanine confirm that no covalent chemical change occured during the isolation procedure. The ^{31}P-^{1}H coupling secures complete phosphorylation. Analyzing the integration curve, a molar ratio of 9 : 1 of adenine to 5'-*O*-phosphono-pyridoxylidenerhodanine is recognized.



(*Z*)-5-[[3-Hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone, (*Z*)-5'-*O*-Phosphono-pyridoxylidenerhodanine (**3**)

FIG. 8. The proposed *Knoevenagel* mechanism for the reaction between **1** and **2**.

The triplet at δ 1.05 ppm and the quartet at δ 3.43 ppm, $|^3J(^1H,^1H)| = 7.0$ Hz, result from ethanol added during isolation procedure. Uncompletely deuterated DMSO corresponds to the quintet at δ 2.50 ppm. The spectrum shown in Fig. 7 is proof for the non-covalent character of a possible complex between the concerning components.

Fully Proton-Decoupled ^{13}C NMR Spectrum in DMSO-d_6. Resonances of C-2', C-5', *RR'*C-S*R''*, C-3, C-5, and C-6 were detectable for 5'-*O*-phosphono-pyridoxylidenerhodanine. For unknown reasons we could not detect the signals of C-4', C-2, C-4, C=O, and C=S. Remarkably high, again, were the shieldings of C-3 and C-6. The residual NMR absorbancies were comparable to **4**. The assignment of five carbons of adenine followed the literature[36] values.

DISCUSSION

NMR and FAB MS data confirm the structure of the reaction product between **1** and **2** as isolated from synthesis to be (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (**3**). A trivial name is (*Z*)-5'-*O*-phosphono-pyridoxylidenerhodanine. Considering the structural features of educts and product the *Knoevenagel*[5,6] condensation appears to be involved in reaction (Fig. 8).

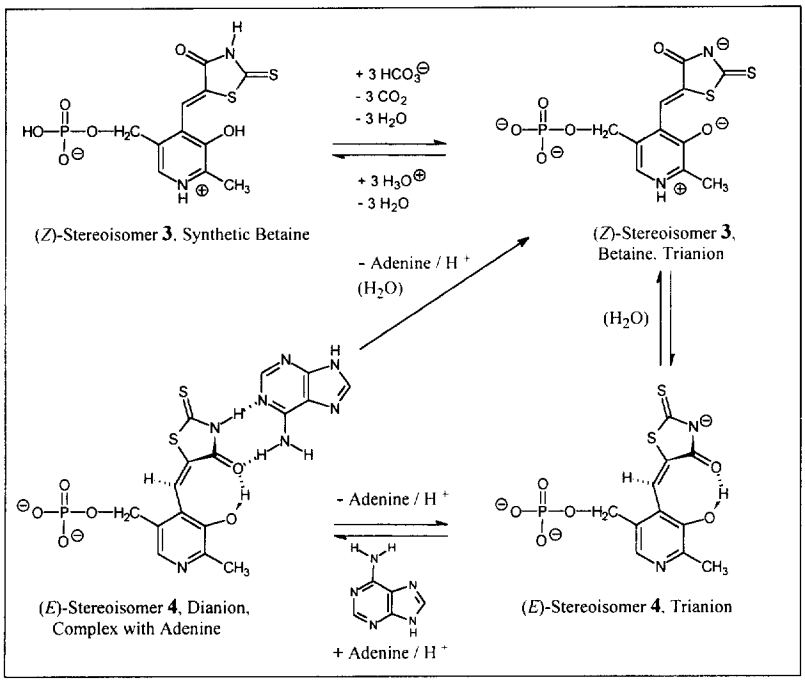


FIG. 9. The (*Z*/*E*)-stereoisomerism of 5'-*O*-phosphono-pyridoxylidenerhodanine. The (*E*)-stereoisomer **4** is presumably stabilized by an intramolecular hydrogen bonding.

Since only one (*Z*/*E*)-stereoisomer has been observed in NMR spectra performed in TFA-*d*, the (*Z*)-configuration is deduced for the synthetic compound **3**. The (*E*)-stereoisomer **4** would be sterically hindered by the *ortho*-phosphoric acid monoester moiety and the phenolic oxygen in competition with the C=O group of the 2-thioxo-4-thiazolidinone ring. The (*E*)-stereoisomer **4** is expected to be thermodynamically and kinetically less stable than the (*Z*)-stereoisomer **3**.

Nevertheless, attempts were made to produce significant amounts of the presumably existing (*E*)-stereoisomer **4**. An empirically developed procedure is described in the synthesis section. The evolving product was subjected to proton and carbon NMR spectroscopy. For several reasons we suggest this product **4** to represent the (*E*)-stereoisomer of 5'-*O*-phosphono-pyridoxylidenerhodanine. The proton NMR spectrum rules out unequivocally any change in the atomic composition. No hydrolysis occured during the isolation procedure. The carbon NMR spectrum of **4** reveals that several carbons are more shielded than expected. The significant shielding of C-6 interpretes[16-23] for a deprotonated nitrogen in its proximity. The high shielding of C-3 shows that the phenolic oxygen is protonated. Furthermore, we attribute this shielding of more than 10 ppm compared to common data[16-23] for comparable compounds to an involvement of the undissociated phenolic group in hydrogen bonding. Additionally the appreciable shielding of the C=O and C=S resonances is interpreted both as an involvement of the C=O group in hydrogen bonding and as participation of a resonance structure for the C=S group. The latter arises by deprotonation of the imide-like 2-thioxo-4-thiazolidinone system. Summarizing the available data on 5'-*O*-phosphono-pyridoxylidenerhodanine, we would like to propose the reaction scheme in Fig. 9.

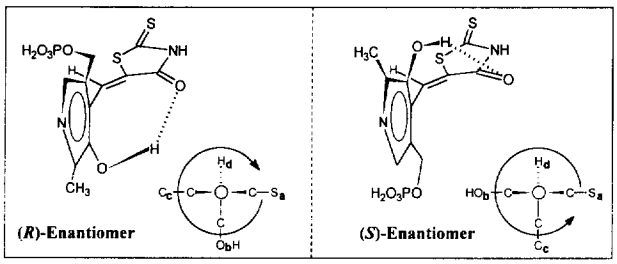


FIG. 10. Theoretically deduced enantiomeric atropisomers of (*E*)-5'-*O*-phosphono-pyridoxylidenerhodanine (**4**). Provided that in the (*E*)-isomers the two ring systems are rotationally restricted, two perpendicular disymmetric planes are created, neither of which can be bisected by a plane of symmetry. In spite of lacking an asymmetric carbon, these atropisomers should be optically active under certain conditions.

Ionic forms of **3** (yellow) and isomer **4** (red) are isolated by synthesis from **1** and **2**. For the isolation procedure of the (*E*)-isomer the cascade in Fig. 9 is postulated. Isomer **4** is characterized by an intramolecular hydrogen bonding, and is stable in DMSO. This can be clearly understood because the dipolar aprotic solvent DMSO is known to stabilize hydrogen bondings. The rearrangement of **4** to **3** occurs by dissolving the (*E*)-isomer in water.

We decided to examine the possibility to stabilize the designated (*E*)-stereoisomer **4** by addition of complexing agents. Bearing in mind the mimicry of the nucleobase thymine by the imide-like system of 5'-*O*-phosphono-pyridoxylidenerhodanine, we have chosen its well known counterpart adenine as a complex participant. Adenine was used in molar excess because we intended to drive possible complexation equilibria on the product side. The isolation of a salt-containing crude preparation is outlined in synthesis section. Again, any covalent change in atomic composition can be ruled out by proton NMR spectroscopy. Carbon NMR spectroscopy shows significant shielding of C-3 and C-6. Analogously to the single (*E*)-stereoisomer **4** it is inferred that the pyridine nitrogen is unprotonated, the phenolic group is undissociated and involved in hydrogen bonding. Thus, we propose the existence of a hydrogen bonded complex of **4** with adenine (Fig. 9) which is stable in anhydrous DMSO. The hypothetical existence of two optically active enantiomeric atropisomers of the (*E*)-diastereoisomer is suggested (Fig. 10), if the energy barrier between the enantiomorph atropisomers is high enough to prevent racemization. Our speculations must be experimentally verified, to prove the existence of the hypothetical [*R*-(*E*)]-5'-*O*-phosphono-pyridoxylidenerhodanine and [*S*-(*E*)]-5'-*O*-phosphono-pyridoxylidenerhodanine.

Recently vitamin B_6 analogs have been isolated from natural sources.[37,38] Vitamin B_6 antagonists from natural sources had been known before.[39,40] Synthetic vitamin B_6 analogs,[41,42] which frequently act as vitamin B_6 antagonists in biological test systems, have been examined ever since the structural work on the vitamin B_6 group has culminated.[1] Synthetic non-structurally related vitamin B_6 antagonists in most cases are aldehyde interceptors, or their metabolic prodrugs, like *asym*-hydrazine derivatives *RR'*N-NH_2 and carboxylic acid hydrazides *R*-CO-NH-NH_2. For example, the chemotherapeutical agents isonicotinic acid hydrazide[43] (isoniazid, INH) and 6-azauridine[44] exert vitamin B_6 antagonism *in vivo*. Eggers *et al.* have reported[45,46] **2** to be a selective inhibitor of echovirus 12 [(+)-ssRNA; *Picornaviridae*; *Enterovirus*] *in vitro*. With respect to this findings the synthetic analog of **1** presented here may be of further interest. Whether it acts as a vitamin B_6 antagonist under biological conditions remains to be elucidated.

EXPERIMENTAL

General Procedures. Pyridoxal 5'-phosphate monohydrate, rhodanine (2-thioxo-4-thiazolidinone) and deuterated dimethylsulfoxide (DMSO-d_6), deuteration grade min. 99.8%, were purchased from E. Merck AG (Darmstadt, FRG). Deuterated trifluoroacetic acid (TFA-*d*), deuteration grade min. 99.5%, and adenine, $w > 99\%$, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Any other chemicals of analytical grade were purchased from Merck. Water twice distilled from a fused silica apparatus was used for chromatography and spectroscopy.

^{1}H NMR spectra were recorded at 400.136 MHz and ^{13}C NMR spectra were recorded at 100.614 MHz on a Bruker AM-400 *Fourier* transform spectrometer (tetramethylsilane as internal standard), ^{31}P NMR spectra were recorded at 161.977 MHz and measured against 85% *ortho*-phosphoric acid as external standard. Measurements were performed at 27° C. Chemical shifts are reported in parts per million (δ) in relation to the standard. Signal multiplicities are quoted as *s* (singlet), *d* (doublet), *t* (triplet) and *q* (quartet). The mass concentration of the solutions for nuclear magnetic resonance spectroscopy was 60 mg/ml unless otherwise stated.

Fast atom bombardment mass spectrometry was performed on a Finnigan MAT 900 mass spectrometer (Finnigan MAT, Bremen, FRG) equipped with a cesium gun (20 kV, 3 μA - 4 μA), and using DMSO as matrix substance.

RP 18 HPLC was performed at room temperature with Beckman Gradient Liquid Chromatograph 334, Beckman Controller 421, Beckman pump 100A [eluent 0.1% (*v*/*v*) TFA] and Beckman pump 110A [eluent acetonitrile with 0.1% (*v*/*v*) TFA]. The column was a prepacked Bio-Rad Hi-Pore® RP-318 (250 mm • 4.6 mm) connected with a Biotronik BT3030 UV detector.

UV/VIS spectra were recorded in wavelength scan mode on Beckman DU® 65 and DU® 640 spectrophotometers at room temperature. Fluorescence excitation and emission spectra were recorded at room temperature using an Aminco Bowman AB2 luminescence spectrophotometer (SLM, Urbana, IL, USA). Background fluorescence and *Raman* scattering were corrected in all spectra.

(Z)-5'-O-Phosphono-pyridoxylidenerhodanine, (Z)-5-[[3-Hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (3). A solution of 250 mg rhodanine (2-thioxo-4-thiazolidinone) (1.88 mmol) (**2**) in 10 ml of absolute ethanol was suspended with 500 mg of pyridoxal 5'-phosphate monohydrate (1.89 mmol) (**1**). The suspension was refluxed for 10 min in a 29 mm/32 mm standard ground joint 50 ml round-bottomed flask with a *Dimroth* reflux condenser. The pyridoxal 5'-phosphate dissolved slowly and a coloured suspension was developing continuously. 10 ml of water were added in one portion and the reaction product was warmed for no more than 10 min. The mixture with the now red coloured condensate was freezed for 1 h at a temperature of -18° C. During this freezing procedure the colour of the precipitating granules brightened until it reached an orange-yellow colour. The product was collected in a filter flask (∅ 70 mm), washed with 60 ml of absolute ethanol, and dried over anhydrous calcium chloride in a vacuum desiccator; yield: 580 mg (85%) orange-yellow granules (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (**3**). In spite of vacuum drying, the substance contained ethanol and water of crystallization in not determined amounts. Calculated for

C_{11} H_{11} N_2 O_6 P S_2, M = 362.31 g/mol. FAB MS (MH^+ calculated for m/z 362.99): m/z (rel.Int.) 363.0 (12.0%, MH^+), 227.4 (18.1%), 270.4 (8.7%), 171.2 (8.3%), 282.4 (7.1%), 267.1 (2.8%), 283.4 (2.1%), 265.1 (1.5%). ^{1}H NMR (TFA-*d*): δ 2.91 (*s*, 3 H, 2'-CH_3), 5.42 (*d*, $|^3J(^{31}P,^1H)|$ = 8.0 Hz, 2 H, 5'-CH_2-OPO_3H_2), 7.76 (*s*, 1 H, 4'-CH), 8.47 (*s*, 1 H, 6-CH). ^{13}C NMR, proton-decoupled (TFA-*d*): δ 16.83 (*s*, 2'-CH_3), 65.75 (*s*, 5'-CH_2-OPO_3H_2), 121.04, 122.13 (2 *s*, C-4'), 133.34 (*s*, C-6), 136.85, 136.91 (2 *s*, *RR'*C-S*R''*), 138.54 (*s*, C-5), 142.05 (*s*, C-4), 147.64 (*s*, C-2), 153.03 (*s*, C-3), 171.93 (*s*, C=O), 193.39 (*s*, C=S). ^{31}P NMR proton-undecoupled and proton-decoupled (TFA-*d*): δ - 4.24 (*s*, *R*-OPO_3H_2). UV/VIS (H_2O): $\lambda_{max,1}$ = 300 - 304 nm [*A* (1%/1cm) = 250 - 270], $\lambda_{max,2}$ = 346 - 358 nm [*A* (1%/1cm) = 380 - 410], $\lambda_{max,3}$ = 446 - 450 nm [*A* (1%/1cm) = 220 - 240].

Crude (E)-5-[[3-Hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (4). A mass of 70 mg (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (0.19 mmol) (**3**) was overlayed with 7.5 ml of 10 M hydrochloric acid. The resulting yellow suspension was shaken occasionally and left at room temperature for 10 min. 10 ml of water were added and the solution was immediately mixed with small portions of sodium bicarbonate until a pH value of 5.5 - 6.0 was achieved. The adding of sodium bicarbonate was stopped instantly when the colour of the solution changed from yellow to red. Addition of 5 ml of ethanol followed and the mixture was freezed for 4 h at a temperature of -18° C. The red precipitate was filtered in a G 4 sintered glass crucible. Since hygroscopic, this product was stored in a vacuum desiccator over anhydrous calcium chloride; yield: 170 mg amorphous red granules **4**, containing sodium chloride and sodium bicarbonate. ^{1}H NMR (DMSO-d_6): δ 2.38 (*s*, 3 H, 2'-CH_3), 4.68 (*s*, 2 H, 5'-CH_2-OPO_3H_2), 7.11 (*s*, 1 H, 4'-CH), 8.03 (*s*, 1 H, 6-CH). ^{13}C NMR, proton-decoupled (DMSO-d_6): δ 18.04, 19.56 (2 *s*, 2'-CH_3), 61.65 (*s*, 5'-CH_2-OPO_3H_2), 115.30, 116.87 (2 *s*, C-4'), 125.04 (*s*, *RR'*C-S*R''*), 129.04 (*s*, C-6), 130.65 (*s*, C-5), 137.85 (*s*, C-4), 141.92, 143.57 (2 *s*, C-2), 146.13, 147.18 (2 *s*, C-3), 160.80 (*s*, C=O), 181.52, 182.86 (2 *s*, C=S).

Crude Complex of Adenine with (E)-5-[[3-Hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl] methylene]-2-thioxo-4-thiazolidinone (4). A mixture of 330 mg adenine (2.44 mmol) with 150 mg (*Z*)-5-[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinyl]methylene]-2-thioxo-4-thiazolidinone (0.41 mmol) (**3**) was overlayed with 15 ml of 10 M hydrochloric acid. The resulting yellow suspension was shaken occasionally and left at room temperature for 10 min. 20 ml of water were added and the solution was immediately mixed with small portions of sodium bicarbonate until a pH value of 5.5 - 6.0 was achieved. The adding of sodium bicarbonate was stopped instantly when the colour of the solution changed from yellow to red. 10 ml of ethanol were added, and the mixture was freezed for 4 h at a temperature of -18° C. The resulting red precipitate was filtered in a G 4 sintered glass crucible. Since hygroscopic, this product was stored in a vacuum desiccator over anhydrous calcium chloride; yield: 400 mg amorphous red granules, containing sodium chloride and sodium bicarbonate. ^{1}H NMR (DMSO-d_6): δ 2.38 (*s*, 0.3 H, 2'-CH_3), 4.83 (*d*, $|^3J(^{31}P,^1H)|$ = 7.0 Hz, 0.2 H, 5'-CH_2-OPO_3H_2), 7.25 (*s*, 1 H, 2-CH adenine), 7.42 (*s*, 0.1 H, 4'-CH), 7.73 (*s*, 0.1 H, 6-CH), 8.14 (*s*, 1 H, 8-CH adenine). ^{13}C NMR, proton-decoupled (DMSO-d_6): δ 18.14 (*s*, 2'-CH_3), 62.05 (*s*, 5'-CH_2-OPO_3H_2), 115.96 (*s*, C-5 adenine), 123.91 (*s*, *RR'*C-S*R''*), 126.73 (*s*, C-6), 131.16 (*s*, C-5), 139.91 (*s*, C-8 adenine), 147.19 (*s*, C-3), 151.85 (*s*, C-2 adenine), 152.20 (*s*, C-4 adenine), 154.41 (*s*, C-6 adenine). UV/VIS

(DMSO): λ_{max} = 525 nm [*A* (1%/1cm) = 2.8 - 3.4]. Fluorescence excitation (ex) and emission (em) spectra (DMSO): λ_{ex} = 320 nm, $\lambda_{em, max}$ = 575 nm; λ_{ex} = 480 nm, $\lambda_{em, max}$ = 575 nm.

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