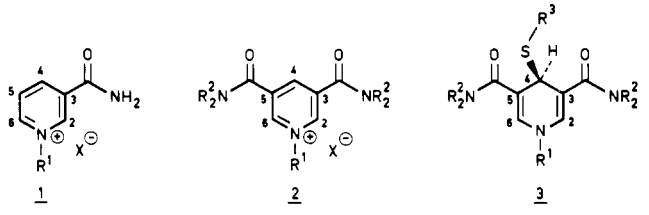
Observation of an Intimate Ion Pair Intermediate in the Ionization of Thio Addition Products of NAD(P)$^+$ Analogues. Implications for 3-Phosphoglyceraldehyde Dehydrogenase

B. J. van Keulen and Richard M. Kellogg*

Contribution from the Department of Organic Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands. *Received September 12, 1983*

Abstract: Phenyl, benzyl, 4-methoxyphenyl, and 4-nitrophenyl thiolates add to *N*-methylpyridinium salts derived from pyridine-3,5-dicarboxylic acid to afford regioselectively the corresponding 4-thio-substituted 1,4-dihydropyridines. In this manner a series of chiral bridged derivatives **4a-d** was obtained from pyridinium salt **5**. In a similar manner several derivatives of structurally more simple 3,5-bis[(ethylamino)carbonyl]- (**9a**) and 3,5-bis[(diethylamino)carbonyl]-1-methylpyridinium salts (**9b**) were synthesized. These products in (deuterio) chloroform solution have ^{1}H NMR, ^{13}C NMR, and UV spectra consistent with a covalent formulation. However, especially for the case of the phenylthio-substituted 1,4-dihydropyridines, a pronounced downfield shift of the 4-H and 4-C of the pyridine ring occurs in deuteriomethanol solution (chosen as polar solvent), whereas the 2,6-H and -C as well as the NCH_3 are shifted to a lesser extent. These effects are most pronounced in the ^{13}C NMR spectra. For the case of 4-nitrophenyl thiolate ionization to the corresponding pyridinium thiolate clearly occurs. However, on the basis of the lack of thiolate exchange, peculiarities of the ^{13}C NMR spectra (especially the selective shift at the 4-position of the pyridine ring), and arguments derived from ultraviolet spectroscopy it is concluded that for the other cases a tightly associated ion pair (intimate ion pair) is being formed. For formation of this intimate ion pair the amide nitrogen must bear a proton substituent to hydrogen bond with the freed thiolate; dialkyl-substituted amide derivatives ionize cleanly in deuteriomethanol to the pyridinium thiolates. Some details of the structure of the intimate ion pair are discussed. A possibility for the relevance of these observations to the chemistry at the active site of the enzyme 3-phosphoglyceraldehyde dehydrogenase is presented.

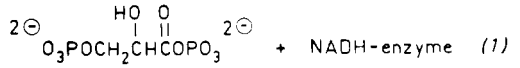
Nicotinamidium ion (**1**), the functional entity of NAD(P)$^+$ coenzyme, accepts "hydride" and transfers it via its 1,4-dihydro form, NAD(P)H. An impressive effort has gone into the uncovering of mechanisms involved in this, at first blush simple appearing, shuttle function of **1**.[1] On common chemical grounds,



1 R^1 = alkyl; **2** R^1 = alkyl; R^2 = H, alkyl; **3** R^3 = alkyl, aryl

one expects **1** as well as pyridinium ions of related structure to have an affinity for addition (and perhaps also subsequent transfer) of anionic species other than "hydride". Indeed $-CN$,[2] $-SR$,[3] $-CH_2X$, and $-CHX_2$ (X = anion stabilizing group),[4] $-OR$ and $-NR_2$,[5] and $-PO(OR)_2$ (via an Arbusov reaction)[3b] all add to pyridinium ions related to **1**. The remarkably ubiquitous reduction of pyridinium salts to 1,4-dihydropyridines by sodium dithionite (the mechanism is not certain) also deserves mention.[6] The regioselectivity of these 1,2-, 1,4-, or 1,6-additions is often chiefly or exclusively 1,4 although occasional exceptions, caused by a variety of reasons, are known. For the case of thiolate addition to the symmetrical pyridinium salts **2**, we have shown that virtually exclusively 1,4-addition products **3** are formed. These adducts will transfer thiolate to activated substrates in a subsequent step.[3b]

The thiolate addition products described above could be relevant to the enzymic conversion of 3-phosphoglyceraldehyde and 3-phosphoglyceroyl phosphate (eq 1).[7] The NAD$^+$-dependent



(1)

enzyme 3-phosphoglyceraldehyde dehydrogenase carries out this conversion. This enzyme has at the active site an active cysteine (Cys-149) that adds at some stage to the aldehyde group of 3-phosphoglyceraldehyde to form a hemithioacetal that is oxidized enzymatically to the thioester by NAD$^+$. The resulting thioester is subsequently converted to the acyl phosphate. This is shown schematically in Scheme I. A peculiarity of this enzyme is its *yellow* color in the NAD$^+$-bound form. A number of years ago Krimsky and Racker[8a] suggested that addition of a sulfur nucleophile (cysteine) to NAD$^+$ could be the cause of this yellow

(1) General review: Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman: San Francisco, 1979; pp 311–357.

(2) (a) Wallenfels, K.; Schüly, H. *Justus Liebigs Ann. Chem.* **1959**, *621*, 86. See also, for example: (b) Foster, R.; Fyfe, C. A. *Tetrahedron* **1969**, *25*, 1489. (c) Lindquist, R. N.; Cordes, E. H. *J. Am. Chem. Soc.* **1968**, *90*, 1269. (d) Lovesey, A. C. *J. Med. Chem.* **1969**, *12*, 1018. (e) Kavâlek, J.; Lyčka, A.; Machâček, V.; Šterba, V. *Collect. Czech. Chem. Commun.* **1975**, *40*, 1932. (f) Review: Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; Benjamin Press: New York, 1966; Vol. 2.

(3) (a) Wallenfels, K.; Hofmann, D.; Schüly, H. *Justus Liebigs Ann. Chem.* **1959**, *621*, 188. (b) Piepers, O.; Kellogg, R. M. *J. Chem. Soc., Chem. Commun.* **1980**, 1147.

(4) See, for example: (a) Kröhnke, E.; Ellegast, K.; Bertram, E.; *Justus Liebigs Ann. Chem.* **1956**, *600*, 176, 198. (b) Kröhnke, E.; Vogt, I. *Justus Liebigs Ann. Chem.* **1956**, *600*, 211. (c) Doering, W. v. E.; McEwen, W. E. *J. Am. Chem. Soc.* **1951**, *73*, 2104. (d) Wenkert, E.; Reynolds, G. D. *Synth. Commun.* **1973**, 241. (e) Steglich, W.; Höfle, G. *Chem. Ber.* **1969**, *102*, 1129. (f) Kavâlek, J.; Lyčka, A.; Machâček, V.; Šterba, V. *Collect. Czech. Chem. Commun.* **1976**, *41*, 67. (g) Zoltewicz, J. A.; Helmick, L. S.; O'Halloran, J. K. *J. Org. Chem.* **1976**, *41*, 1308.

(5) For indications of the various possibilities for alkoxide and other nucleophilic additions, see: (a) Wenkert, E.; Dave, K. G.; Haglid, F.; Lewis, R. G.; Oishi, T.; Stevens, R. V.; Terashima, M. *J. Org. Chem.* **1968**, *33*, 747. (b) Damji, S. W. H.; Fyfe, C. A. *J. Org. Chem.* **1979**, *44*, 1757. (c) Reference 4g.

(6) (a) Eisner, U.; Kuthan, J. *Chem. Rev.* **1972**, *72*, 1. (b) de Vries, J. G.; Kellogg, R. M.; *J. Org. Chem.* **1980**, *45*, 4126.

(7) See, for example: (a) Rossman, M. G.; Liljas, A.; Brändén, C. I.; Banazak, L. J. In "The Enzymes"; Boyer, P. D., Ed.; Academic Press: New York, 1975; pp 61–102. (b) Reference 1, pp 322–330.

(8) (a) Krimsky, I.; Racker, E. *Science (Washington, D.C.)* **1955**, *122*, 319. (b) Racker, E.; Krimsky, I. *Nature (London)* **1952**, *169*, 1043. See, for example also: (c) Herriott, J. R.; Camerman, A.; Deranleau, D. A. *J. Am. Chem. Soc.* **1974**, *96*, 1584. (d) Behr, J.-P.; Lehn, J. M. *Helv. Chim. Acta* **1980**, *63*, 2112 and references therein.

(9) Addition of alkyl and aryl thiolates to **5** and other related pyridinium ions that we have investigated occurs regioselectively at the 4-position; the addition of alkyl thiolates to **1** in liquid ammonia is thought, on the other hand, to give a mixture of 1,4 and 1,6 adducts.[4g]

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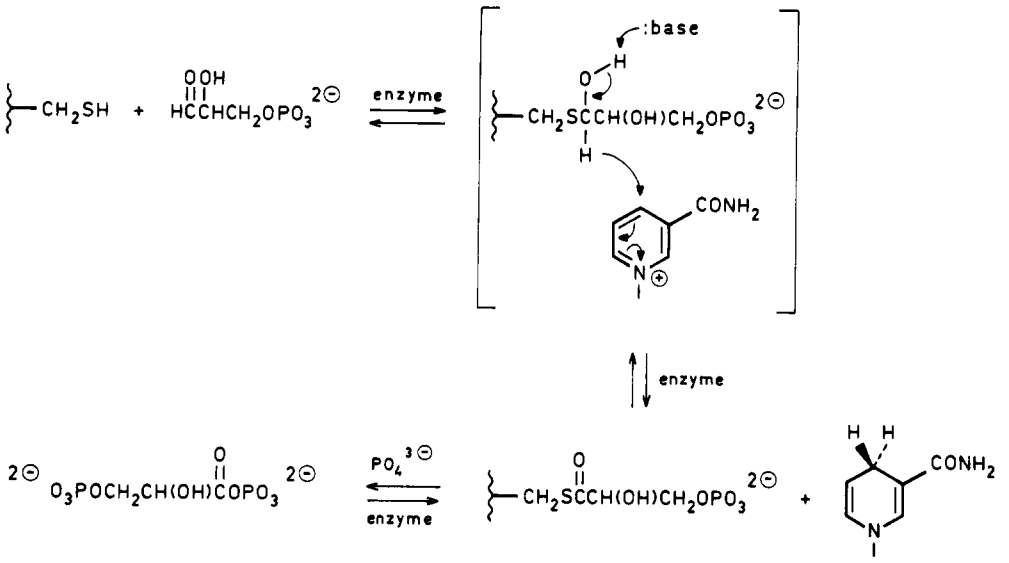
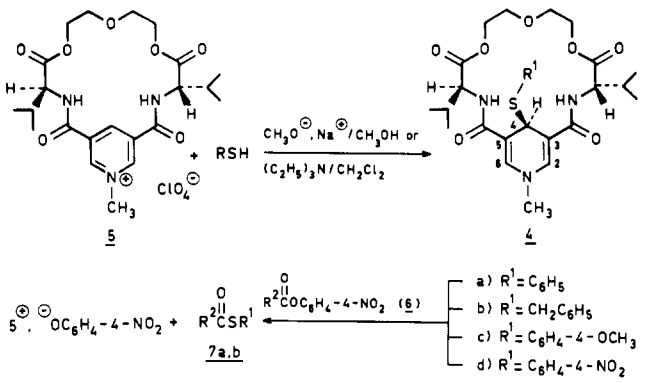
Scheme I



Scheme II



color. An alternative explanation offered in recent years is that the yellow color is the result of a NAD^+–tryptophan charge-transfer interaction in the enzyme.[8b,c] The availability of *yellow* adducts **3** and related compounds gave the incentive to study the chemistry of thio-substituted 1,4-dihydropyridines in more detail. These compounds are interesting in their own right, and, moreover, we considered that study of their chemistry could provide information pertinent for understanding 3-phosphoglyceraldehyde dehydrogenase.

Results

The bridged chiral thio-substituted 1,4-dihydropyridines **4a-d** are derived from the pyridinium salt **5** as shown in Scheme II. The choice of **5** lay in the fact that we have already established[10,11] that the 1,4-dihydropyridine obtained on reduction of **5** is extremely efficient in the enantioselective reductions of some carbonyl compounds. With this experience in hand we wished to examine also the enantioselective transfer of entities other than hydride. The thiolate additions carried out under conditions described in the Experimental Section provided good yields of **4a–d**, which were characterized by spectral means. All of these compounds are fairly sensitive to oxidation and decompose readily on exposure to light.[12]

The symmetry properties of these adducts should be noted with a view to the subsequent discussion. The pyridinium salt **5** has a C_2 symmetry axis, and both faces of the pyridinium ring are equivalent (homotopic). The adducts **4a-d** do not possess this symmetry axis; this division of the molecule into "right" and "left" halves is regularly revealed in the ^{13}C NMR spectra and is of considerable use for peak and structural assignments.

As shown in Scheme II **4a,b** will transfer thiolate to activated esters **6** (R^2 = L-$C_6H_5CH_2CH(NHCO_2CH_2C_6H_5)$) to form the corresponding thioesters **7**. This reaction is in fact a thio analogue of the carboxyl to aldehyde reduction by NADH (eq 1, to the left). The scope and chiral aspects of these reactions, which we have examined extensively, will be reported separately. We comment here on observations relevant to an unusual bonding phenomenon in these molecules. At 25 °C the rate of thiolate transfer from **4b** in *methanol* or *acetonitrile* is at least 10^4 times faster than in *chloroform* solution. The respective second-order rate constants are 1.07 L mol^{-1} s^{-1} in CH_3CN and $< 6.67 \times 10^{-4}$ L mol^{-1} s^{-1} in $CHCl_3$. A study of the spectroscopic behavior of **4a** and other thio-substituted 1,4-dihydropyridines has provided evidence for an intermediate, the reactivity of which is the cause of this large solvent effect.

In Figure 1 the ^1H NMR spectra of **4a** in $CDCl_3$ (the spectrum in CD_2Cl_2, not shown, is virtually equivalent) and CD_3OD are shown together with the spectrum of the pyridinium ion $\mathbf{5}^+$,-ClO_4^-).[13b] The spectrum of **4a** in CD_3SOCD_3 (Table I) resembles closely that in CD_3OD as do the spectra (not listed) in CD_3CN and CD_3COCD_3. In Figure 2 the corresponding ^{13}C NMR spectra are shown and the spectrum in CD_3COCD_3 is listed in Table I. The temperature-dependent behavior at position 4 of the dihydropyridine ring is shown in insets. In CD_3OD, but not $CDCl_3$, **4a** undergoes temperature-dependent changes at H-4; no other changes of consequence are seen. In the ^{13}C spectra both C-4 and C-2,6 are subject to reversible temperature-dependent changes

(10) Jouin, P.; Troostwijk, C. B.; Kellogg, R. M. *J. Am. Chem. Soc.* **1981**, *103*, 2091. The synthesis of the pyridinium salts used here is described in this publication; full procedures will appear separately.[11]

(11) Talma, A.; Jouin, P.; de Vries, J. G.; Troostwijk, C. B.; Werumeus Buning, G.; Waninge, J.; Visscher, J.; Kellogg, R. M., submitted for publication.

(12) For an extensive discussion of the effect of light on dihydropyridines, see: (a) van Bergen, T. J.; Hedstrand, D. M.; Kruizinga, W. H.; Kellogg, R. M. *J. Org. Chem.* **1979**, *44*, 4953. See also subsequent work, for example: (b) Nakamura, K.; Yasui, S.; Ohno, A.; Oka, S. *Tetrahedron Lett.* **1983**, *24*, 2001.

(13) (a) For spectral data on 1,4- and 1,2-dihydropyridines of related structure, see, for example: van Bergen, T. J.; Mulder, T.; van der Veen, R. A.; Kellogg, R. M.; *Tetrahedron* **1978**, *34*, 2377. Thio-substituted 1,2-dihydropyridines have also been made and the spectra taken, see ref 3b and: Piepers, O. Ph.D. Thesis, Groningen, 1981. (b) Solubility problems limited the solvent choice. Although **4a** dissolved well in $CDCl_3$ it is virtually insoluble in C_6D_6 and CCl_4. For $\mathbf{5}^+,ClO_4^-$ CD_3CN was the only useful solvent. To ensure no serious solvent-dependent shifts occur with pyridinium ions, several structurally analogous pyridinium salts that are soluble also in $CDCl_3$ and Me_2SO were examined. The ^1H ring absorptions are solvent dependent: $\mathbf{9a}^+,ClO_4^-$ absorps at δ 9.52 (H-2,6) and 9.30 (H-4) in Me_2SO whereas $\mathbf{9c}^+,ClO_4^-$ absorps at δ 8.73 (H-2,6) and 8.13 (H4) in $CDCl_3$ (δ 8.90 and 8.65 for $\mathbf{5}^+,ClO_4^-$ in CD_3CN). There is virtually no change, however, in the ^{13}C NMR spectra. For $\mathbf{9a}^+,ClO_4^-$ in Me_2SO the ring carbons absorp at δ 135.6 (C-3,5), 141.6 (C-4), and 147.7 (C-2,6); for $\mathbf{9c}^+,ClO_4^-$ in $CDCl_3$ these absorptions are, respectively, δ 138.5, 140.8, and 144.5 (for $\mathbf{5}^+,ClO_4^-$ in CD_3CN δ 135.4, 142.1, and 147.9).

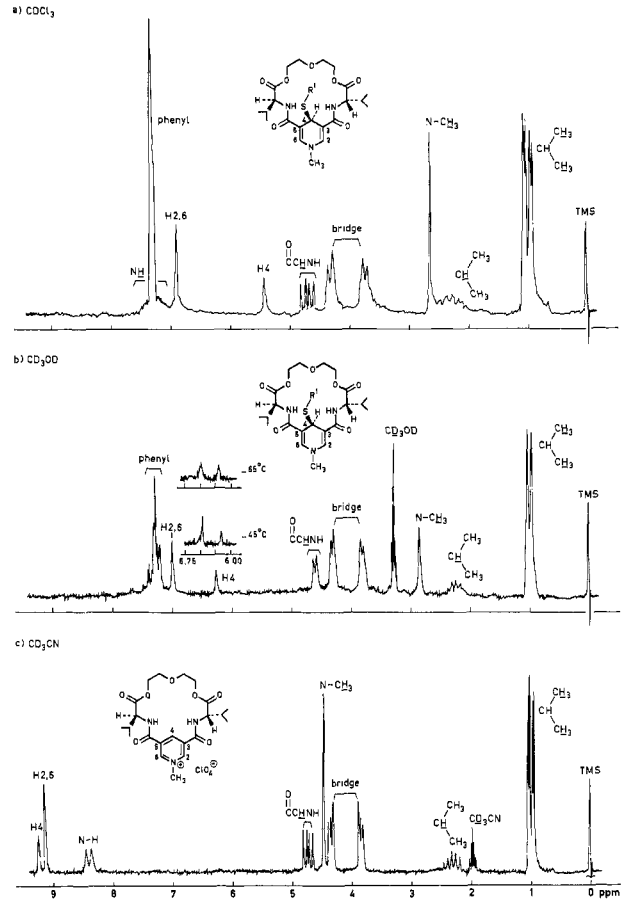
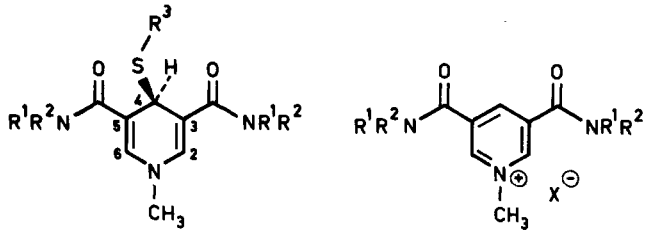


Figure 1. ^1H NMR spectra at 100 MHz of **4a** at 25 °C in (a) $CDCl_3$ and (b) CD_3OD. Spectrum c is of $5^+,ClO_4^-$ in CD_3CN at 20 °C.

at C-4 and to a lesser extent at C2,6 as shown. Additional ^1H and ^{13}C NMR data for **4a–d**, **5**, **8a–c**, and **9a,b** are collected in



8 **9a-c**

a) $R^1 = C_2H_5; R^2 = H; R^3 = C_6H_5$

b) $R^1 = C_2H_5; R^2 = H; R^3 = CH_2C_6H_5$

c) $R^1 = R^2 = C_2H_5; R^3 = C_6H_5$

Table I and the complete spectra are given in the Experimental Section. Peak assignments are based on symmetry arguments, comparison with other dihydropyridines,[13] and the multiplicity and magnitude of ^{13}C–H couplings.

Attention is drawn to several aspects of these spectra. First, the spectra are in accord with the 1,4-dihydropyridine structural assignments, i.e., addition of thiolate to the 4-position of the pyridinium ion precursor. Consider, for example, the ^1H NMR spectrum of **4a**. This has two absorptions for the three ring protons in the ratio 1:2; the vinylic 2,6-protons are, as expected, at lower field. A 1,2-dihydropyridine will for reasons of symmetry have three absorptions; the two vinylic protons are separated usually by 0.4–0.5 ppm (H6 farthest downfield) and are coupled, $J = 2$–3 Hz.[13] In fact H2,6 are diastereotopic in **4a** but this is not revealed in the ^1H NMR spectra; in the ^{13}C NMR this effect is seen in the absorptions for C3,5 (Figure 2 and Table I). The series **8** in which the macrocyclic bridge is absent have the expected sym-

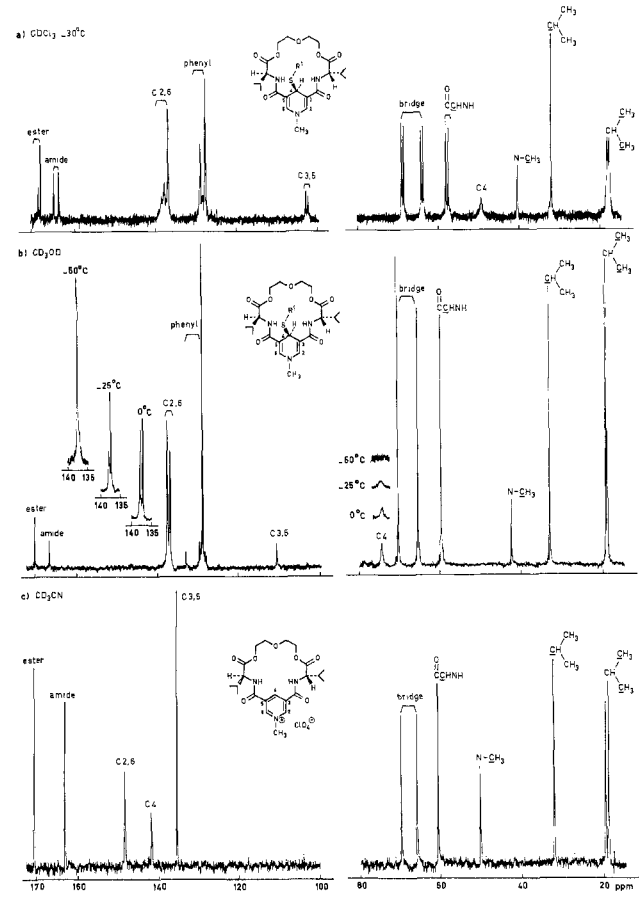


Figure 2. ^{13}C NMR spectra at 90.52 MHz of **4a** in (a) $CHCl_3$ at −30 °C and (b) in CD_3OD at 25 °C (see also temperature insets). Spectrum c is of $5^+,ClO_4^-$ in CD_3CN at 20 °C.

metry in the ^{13}C NMR and ^1H NMR spectra. The ^{13}C NMR chemical shifts in $CDCl_3$ are consistent with those expected from covalent structures. Let us use **4a** again for purposes of illustration. From ^{13}C increment tables[14] the shift for C4, which is by far the most sensitive structural probe, should be roughly 52 ppm downfield relative to Me_4Si; a value of 49.7 ppm is found.

It is obvious that both the ^1H and the ^{13}C spectra are solvent dependent. This solvent dependence is concentrated in the dihydropyridine ring. In the ^1H NMR spectra H-4, H-2,6, and NCH_3 in the more polar solvent CD_3OD (spectrum b, Figure 1) shift downfield in the direction of the absorption positions for pyridinium salt **5** (spectrum c). The ^{13}C NMR spectra (Figure 2) reveal even more striking behavior; C-4 shifts 22.7 ppm downfield in CD_3OD (spectrum b) compared to $CDCl_3$ (spectrum a). C-4 in pyridinium salt (**5**) is, however, 92.4 ppm downfield relative to the position of C-4 in **4a** in $CDCl_3$. More modest but unambiguous downfield shifts of C-2,6 and NCH_3 in **4a** also occur in CD_3OD. This behavior is not a direct consequence of the presence of the unique bridge in **4a**; compound **8a**, analogous in structure to **4a** save for the bridge, undergoes, for example, a 29.9 ppm downfield shift of C4 on change of solvent from $CDCl_3$ to CD_3OD (Table I). Nor is this behavior due to the use of a protic solvent. CD_3OD is convenient because the majority of the compounds are soluble in this medium; however, as seen from Table I very similar NMR behavior is found for **4a** in CD_3COCD_3 and CD_3SOCD_3.

If for purposes of discussion the pair **4a** and **8a** is considered the following points can be made: both compounds are recovered unchanged on removal of solvent, be it either $CDCl_3$ or CD_3OD (the compounds must first be redissolved in CH_3OH to wash

(14) Hesse, M.; Meier, H.; Zech, B. "Spektroskopische Methoden in der organischen Chemie"; Georg Thieme Verlag: Stuttgart, 1979; pp 230–237. This is only a crude calculation based purely on C-4 being substituted with two vinyl groups and one sulfur substituent with no further corrections.

Table I. ^1H and ^{13}C NMR Chemical Shifts[a] of Various 4-Thio-Substituted 1,4-Dihydropyridines and Corresponding Pyridinium Salts

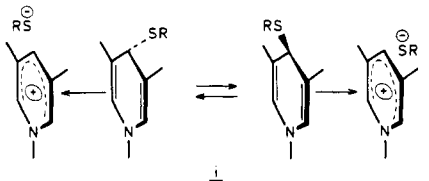
	solvent (temp, °C)							
	$CDCl_3$		CD_3OD		other		^1H	^{13}C
compd	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	$\Delta\delta_{CD_3OD-CDCl_3}$	$\Delta\delta_{CD_3OD-CHCl_3}$
4a	(20)	(−30)	(20)	(20)	CD_3SOCD_3 (20)	CD_3COCD_3 (20)		
2,6 (H/C)	6.80	138.1/138.8	7.22	138.0/136.8	7.37	137.7/137.2	0.42	~0
3,5 (C)		102.1/102.8		110.3		109.6		6
4 (H/C)	5.38	49.7	6.48	72.4	6.60	71.2	1.10	22.7
NCH_3 (H/C)	2.57	40.5	3.05	42.5	3.18	42.1	0.48	1.7
4b	(20)	(20)	(20)			CD_3CN (−20)[b]		
2,6 (H/C)	7.26/7.13	138.2/137.8	7.13			140.5/139.6	~−0.07	2
3,5 (C)		104.4/103.1				107.0/102.8		~0
4 (H/C)	5.05	42.7	6.23			43.9	1.18	~0
NCH_3 (H/C)	3.10	41.3	3.21			42.0	0.11	~0
4c	(20)	(−5)	(20)	(20)				
2,6 (H/C)	6.65	138.0	6.93	139.6			0.28	1.6
3,5 (C)		102.5/103.4		106.7				~3
4 (H/C)	5.08	49.0	6.03	61.9			0.95	12.9
NCH_3 (H/C)	2.58	40.6	2.86	41.5			0.28	0.9
4d	(20)		(20)					
2,6 (H)	7.66	*c*	9.47	*c*			1.81	
4 (H)	7.47[d]		9.29				1.82	
NCH_3 (H)	3.37		4.57				1.20	
5$^+$,ClO_4^-					CD_3CN (20)[b]			
2,6 (H/C)					8.90	147.9		
3,5 (C)						135.4		
4 (H/C)					8.65	142.1		
NCH_3 (H/C)					4.40	49.9		
8a	(20)	(20)	(20)	(20)				
2,6 (H/C)	7.00	135.9	7.72	136.8			0.72	~0
3,5 (C)		105.5		115.9				5.4
4 (H/C)	5.90	62.3	7.06[d]	92.2			~1.16	29.9
NCH_3 (H/C)	2.72	41.0	3.34	44.1			0.62	3.1
8b	(20)	(20)	(20)	(−30)				
2,6 (H/C)	7.09	137.7	7.25	139.2[d]			0.16	~2
3,5 (C)		105.2		105.4				0
4 (H/C)	5.16	42.8	6.80	42.6			1.64	~0
NCH_3 (H/C)	3.10	41.3	3.45	41.2			0.35	~0
9a$^+$,ClO_4^-					CD_3SOCD_3 (20)	CD_3COCD_3/CD_3CN (20)[b]		
2,6 (H/C)					9.52	147.4		
3,5 (C)						135.6		
4 (H/C)					9.30	141.6		
NCH_3 (H/C)					4.60	49.9		
8c								
2,6 (H)	7.56		9.0				1.44	
4 (H)	7.06[d]		8.42				1.36	
NCH_3 (H)	3.69		4.26				0.57	
9b					CD_3CN (20)			
2,6 (H)					8.68			
4 (H)					8.25			
NCH_3 (H)					4.40			
10								
2,6 (H/C)	7.13	139.6/138.7	7.45/7.25	142.2/139.4			~0.2	~2
3,5 (C)		100.3/100.1		102.2/100.4				~1
4 (H/C)	5.03	25.5	5.23	25.6			0.2	0
NCH_3 (H/C)	3.23	41.5	3.42	42.0			0.19	0.5

[a] All chemical shifts relative to Me_4Si internal standard; concentrations roughly 0.1 M but chemical shifts have not been corrected to infinite dilution. [b] Not soluble in $CDCl_3$ or CD_3OD. [c] Too unstable for measurement of pulsed ^{13}C NMR spectrum. [d] Poorly resolved, estimated chemical shift.

protium back onto the amide nitrogens). No deuterium exchange with hydrogens bonded to carbon is detected by ^1H NMR even after several hours in CD_3OD. Reversible dimerization is excluded; **4a**, molecular weight 559.3, has an osmometrically determined molecular weight of 525.3 in $CHCl_3$ and 559.7 in CH_3COCH_3 (experimental difficulties prevented the use of CH_3OH).[15] Finally, the spectra in CD_3OD, for reasons mentioned in the previous paragraph, are inconsistent with 1,2-dihydropyridine structures. Reversible positional isomerization is *not* the cause of these shifts.

Are these solvent-dependent shifts the result of reversible but incomplete ionization to a pyridinium thiolate, the rates of dissociation and recombination of which being fast on the NMR time scale? This possibility is certainly not supported by the observation that added benzenethiol has no observable effect on the ^1H chemical shifts of **4a** dissolved in CD_3OD. One would have predicted that excess benzenethiol should shift the H4 resonance upfield by shifting the equilibrium from solvent-separated ion pairs to covalent adduct. However, conversion to an ionic form can certainly occur. Compound **4d**, which is prepared by $(C_2H_5)_3N$-catalyzed addition of 4-$NO_2C_6H_4SH$ to **5**$^+$,ClO_4^- in $CHCl_3$ or CH_2Cl_2, clearly ionizes in CD_3OD to **5**$^+$,$^-SC_6H_4NO_2$) as revealed by the ^1H NMR shifts, which approach closely those of the

(15) We considered the possibility of the formation of a dimer that might be formulated as i.



i

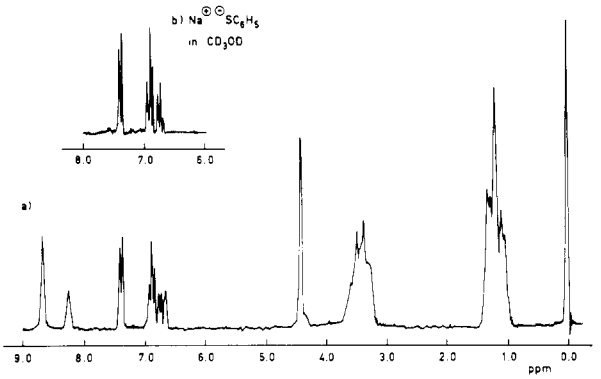


Figure 3. ^1H NMR spectra at 200 MHz of (a) **8c** in CD_3OD (1.6×10^{-1} M/L) and (b) sodium benzylthiolate in CD_3OD (1.4×10^{-1} M/L).

corresponding $\mathbf{5b}^+,ClO_4^-$. The more acidic 4-nitrophenyl thiolate is released in an ionization process whereas the less acidic phenyl, benzyl, and 4-methoxyphenyl thiolates fail to display this kind of ionization (Table I).

A telling observation (Scheme III) is that even after standing 12 h in CD_3OD a 1:1 mixture of **4b** and **8a** undergoes *no* detectable exchange of the benzyl and phenyl thiolate groups. The spectra of **4b** and **8a** underwent, however, the anticipated solvent-induced shifts. *Irreversible* exchange does occur when **8a** is treated with sodium benzyl thiolate. In this case the more basic external nucleophile displaces readily less basic phenyl thiolate. The thio-substituted 1,4-dihydropyridines **4b** and **8a** are not sources of free thiolate in a polar solvent. A pyridinium thiolate should be a source of free thiolate. These exchanges differ fundamentally, of course, from the strongly exothermic acylations previously described,[3b] which may well be initiated by initial attack at sulfur.

The $J_{^{13}C-H}$ coupling constants at C-4 also indicate the absence of ionization to pyridinium thiolate. For **4a**, which was carefully measured at 90.52 MHz, $J_{^{13}C-H}$ in $CDCl_3$ at −30 °C in 149.6 Hz and in CD_3OD at 20 °C $J_{^{13}C-H}$ is 152.0 Hz. For $\mathbf{5}^+,ClO_4^-$ at 20 °C (in CD_3CN) $J_{^{13}C-H}$ for C-4 is 180 Hz, consistent with the sp^2 hybridization. The pronounced solvent-induced shifts, especially at C-4 of thio-substituted 1,4-dihydropyridines, *is not associated with a large degree of rehybridization at this position.*

The solvent-induced shifts, strongest at C-4, are associated also with a temperature-dependent process. This is observed clearly in Figure 1b where H-4 is seen to resolve into two peaks of unequal intensity on lowering of the temperature. There are no other noteworthy changes in the spectrum. In the ^{13}C spectrum of **4a** shown in Figure 2b, we could not detect splitting into two new peaks (see caption for Figure 2) although various line-broadening effects were seen. Although an extensive study has not been made, the rates of these temperature-dependent processes appear to decrease in the order of increasing basicity C_6H_5S > 4-$CH_3OC_6H_4S$ > $C_6H_5CH_2S$ (4-nitrophenyl thiolate is so acidic that the **4d** simply ionizes in polar solvent). Attempts to obtain additional NMR data with compounds with more basic thiolate substituents have been frustrated by decomposition that sets in as the temperatures are raised to obtain coalescence.

In order to probe further into the effect of structure on these solvent-induced changes the effect of substitution at the amide nitrogens was examined. In **9c** the amide nitrogens are tertiary but the 4-position of the pyridinium ion is still accessible for addition as judged from Corey–Pauling–Kolthum molecular models. Phenyl thiolate adds regioselectivity when the addition is carried out *in* CH_2Cl_2 (not CH_3OH in which the required covalent adducts do not form). The adduct is not stable and decomposed during the time required for acquisition of a pulsed ^{13}C NMR spectrum. The ^1H NMR spectrum (Table I) is that expected for a covalent compound whereas in CD_3OD the ^1H NMR spectrum (Table I, Figure 3a) must be that of the ionic *pyridinium thiolate.* This conclusion is based on the characteristic downfield shifts of H-2,6, H-4, and the NCH_3, and the relatively broad absorption for *phenyl thiolate* in Figure 3a, which closely resembles that for sodium benzyl thiolate in CD_3OD (Figure 3b).

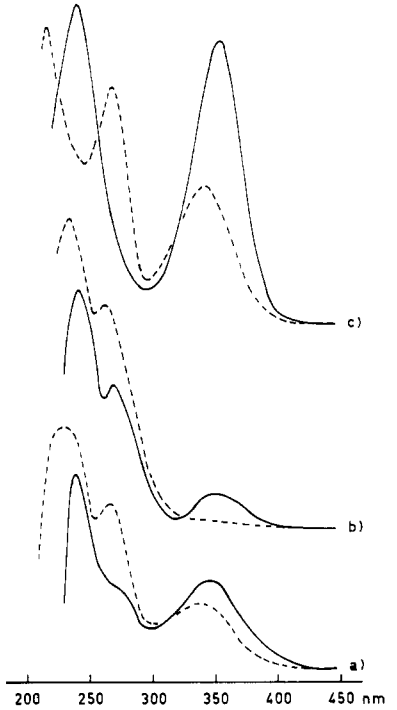
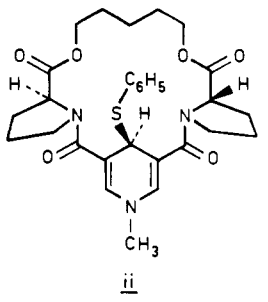


Figure 4. Ultraviolet spectra of **4a**, **8c**, and **10** at 25 °C: Spectrum a is of **4a** (6.67×10^{-4} M/L) (—) CH_2Cl_2, (---) CH_3OH; spectrum b is of **8c** (3.33×10^{-4} M/L) (—) CH_2Cl_2, (---) CH_3OH; spectrum c is of **10** (6.67×10^{-4} M/L) (—) $CHCl_3$, (---) CH_3OH.

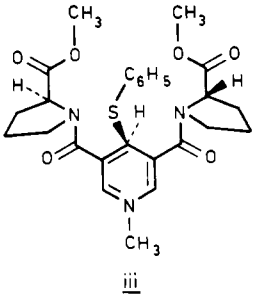
These phenyl absorptions are quite sharp for **8c** in $CDCl_3$ and for **4a**, **8a**, etc. in CD_3OD. The contrast in behavior in CD_3OD of **8c**, which bears no amide hydrogens, and other compounds described here, points to an influence of the carboxamide groups.[16]

Independent support for the NMR observations can be obtained by ultraviolet spectroscopy. In Figure 4 the ultraviolet spectra in $CHCl_3$ and CH_3OH for **4a**, **8c**, and **10** are given. The latter compound, derived by addition of CN to $\mathbf{5}^+,ClO_4^-$,[2a] serves as a covalent model, for it undergoes no significant solvent-induced shifts in the NMR spectra (Table I). The ultraviolet spectrum of **10** (spectrum c) shows both in $CHCl_3$ and CH_3OH the characteristic long wavelength band of a 1,4-dihydropyridine around 350 nm (the position depends somewhat on solvent),[2a,6a,17] but in CH_3OH a second band at 272 nm appears. This has been attributed to pyridinium salt in other compounds[2a] but as seen for the NMR spectra (Table I) this cannot be true here. Com-

(16) We have prepared ii and iii from the corresponding pyridinium salt derivatives prepared in conjunction with other research.[10,11] These addition



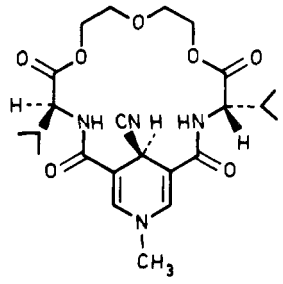
ii



iii

products could be characterized by ^1H NMR spectroscopy as the 1,4-dihydro derivatives shown (the spectra are given in the Experimental Section) but the compounds were too unstable to permit further characterization. In CD_3OD the corresponding *pyridinium thiolates*, recognizable by a broad phenyl thiolate absorption, are formed immediately on dissolution. These appear, however, to be in equilibrium with a small amount of the covalent form, calculated from ultraviolet spectra as maximally 25% for ii or iii. The two forms are in rapid equilibrium, which leads to averaged ^1H NMR shifts for H-4 and H-2,6. Owing to the pronounced instability of the compounds and the availability of only limited amounts of material they have not been investigated further.

(17) van Bergen, T. J., Kellogg, R. M. *J. Am. Chem. Soc.* **1972**, *94*, 8451.



10

pound **4a** shows quite similar ultraviolet behavior but here the NMR spectra reveal changes in bonding in the ring. On the other hand, **8c** (spectrum b) clearly—exactly as concluded from the NMR spectrum—ionizes in CH_3OH to pyridinium thiolate as shown by the complete loss of the characteristic 1,4-dihydropyridine band. In none of the spectra were we able to locate a charge-transfer absorption although a very weak or buried adsorption would have escaped detection.

Discussion

In the 1950s Wallenfels and co-workers[2a,3a] established beyond any reasonable doubt that anions other than hydride can add to nicotinamidium ion **1** to form covalent adducts. The tendencies of these adducts to ionize as well as thermodynamic vs. kinetic control in the position of addition were shown to be affected by the solvent. These investigations were based almost exclusively on elegant use of ultraviolet spectroscopy since advanced NMR techniques were not available.

This possibility that covalent and ionic structures (and intermediates in between) exist side by side raises experimental problems that can be examined probably most profitably with the aid of NMR techniques. We are cognizant of the problems involved in correlating either ^1H or ^{13}C NMR shifts with charge densities[18] and extracting bonding information from these observations. We are also aware that the questions of temperature dependence of the ^{13}C and ^1H NMR spectra as well as the effect of change of basicity of the thiolates have been examined only qualitatively. Nevertheless, we feel that the bulk of the evidence, taken together, leads to a reasonable hypothesis.

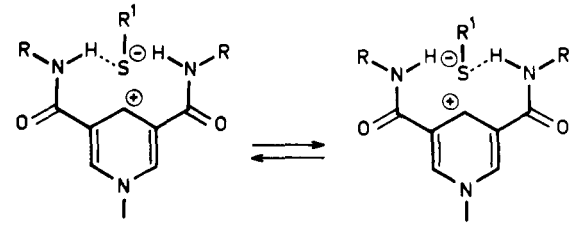
In our opinion the available evidence is not in accord with a rapid and reversible ionization to pyridinium thiolates. In addition to the lack of thiolate exchange (Scheme III), the relative restriction of the downfield shifts in CD_3OD to the 4-position rather than the 2,6-position, as revealed by ^{13}C NMR spectroscopy, is not in accord with a reversible ionization. On the basis of a crude calculation in which the difference in solvents used for the pyridinium salts is ignored as well as difference in anions, C-4 in **4a** shifts 24.6% of the maximum possible, i.e., pyridinium salt, downfield and C-2,6 virtually not at all. For **8a** C-4 shifts 60.5% of the maximum possible and C-2,6 again virtually not all. If an allyl carbonium ion is used as model, roughly equal shifts should be expected for both C-4 and C-2,6 on ionization.[18]

Because of the range of structures investigated we do not believe that important conformational effects arising from the presence of a bridge can be invoked. The differences in behavior between the series **8** and **4** should have been greater were this the case. In particular, the close analogy in behavior between **4a** and **8a** points to the absence of significant bridge-induced conformational effects or to effects caused by differing orientations of the carboxamide groups.

We believe that the observations reported here are best explained in terms of two species in equilibrium with each other. The temperature-dependent behavior of H-4 in **4a** as shown in Figure 1b is the strongest argument for this assertion. One component is the covalently bound compound. The other component must have some polar characteristics judging from the NMR behavior; this component is stabilized in polar solvents.

(18) See for example: Farnum, D. G. *Adv. Phys. Org. Chem.* **1975**, *11*, 123.

An explanation consistent with the above conclusions is that in polar solvents the covalent structure is in equilibrium with an ion pair **11**.[19a] Ion pairs are, of course, virtually obligatory



11

intermediates in solvent-assisted transitions from covalent to ionic structures (pyridinium thiolates in the present case). The ion pair here must, however, be a special one indeed since the change in bonding effects seems to be strongest at C-4, the thiolate remains bonded to C-4 without exchange, and no significant rehybridization can be detected at C-4. These properties are those expected for *intimate ion pairs*, species that have often been postulated but seldom observed spectroscopically.[19a-i] An *intimate ion pair*, following the suggestions made by Sneen,[19f] has the leaving group still so closely associated with the carbon atom that a molecule of solvent has not yet penetrated between the incipient cation and anion nor has the carbon atom yet undergone the necessary rehybridization to sp.[2] A solvent-separated ion pair (and we do not know what types of ion pairs may be present in pyridinium thiolates) should have the properties of an ion[19a-g] and should reasonably be expected to exchange thiolate. The rather special characteristics of some ion pairs formed with pyridine have been thoroughly discussed and elegantly applied by Steglich and Höfle.[4c]

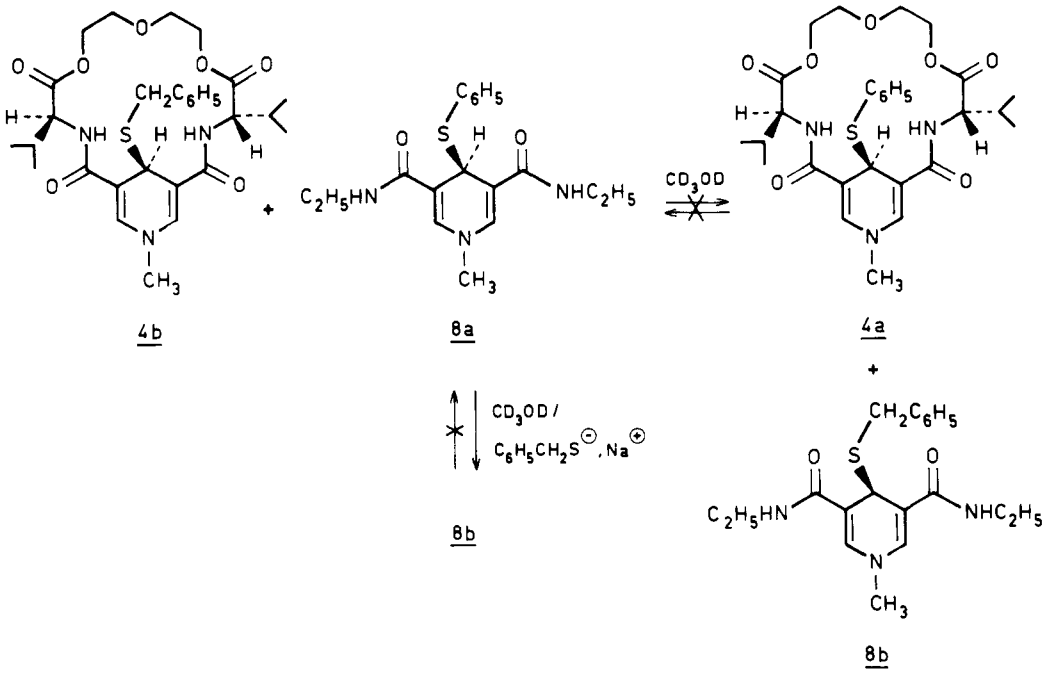
The amide hydrogen must have a central role, namely, hydrogen bonding, in the stabilization of **11**. The behavior of the tertiary amide derivative **8c** accords fully with this interpretation; in the absence of these hydrogens ionization to pyridinium thiolate occurs in polar medium. In **11** two isoenergetic structures involving *one* hydrogen bond between thiolate and the highly proximate amide hydrogens are indicated. An alternative possibility is that both amide hydrogens bond simultaneously to the thiolate as it is released. In accord with the supposition of hydrogen bonding, **4** and **8a,b** have (Experimental Section) in CH_3CN infrared absorptions in the 3670–3730-cm^{-1} range. These are most reasonably assigned to a non-hydrogen-bonding amide absorption that arises from the considerable steric shielding from solvent in these 1,3,4,5-tetrasubstituted systems. These protons should be readily available for internal hydrogen bonding.

The hypothesis of an intimate ion pair is in our opinion consistent with all the presently available information. We believe that the data are more in agreement with an intermediate characteristic of a solvolytic process than a charge-transfer intermediate although additional stabilization through some type of—so far unobserved—charge-transfer interaction remains a possibility.[20] Stability certainly seems directly linked to basicity of the leaving group (i.e., 4-nitrophenyl thiolate ionizes directly whereas the ion pair forms sluggishly with benzyl thiolate). There are some puzzling questions that remain at this point unanswered. In particular more information is required to describe satisfactorily the structure of the intermediate *crudely* depicted as **11**. We believe that we have correctly identified hydrogen bonding as a major stabilizing factor; we have not, however, defined the role

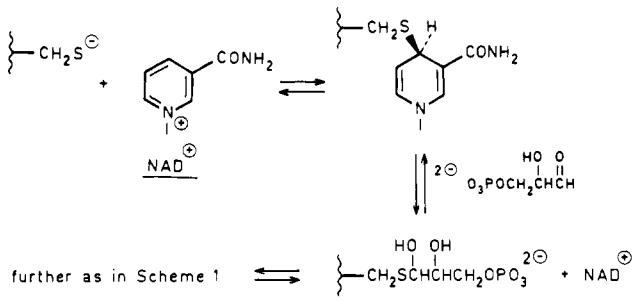
(19) See, for example: (a) Hogen-Esch, T. E.; Smid, J. *J. Am. Chem. Soc.* **1966**, *88*, 318. (b) Collins, G. L.; Smid, J. *J. Am. Chem. Soc.* **1973**, *95*, 1503. (c) Smid, J. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 112. (d) Hogen-Esch, T. E. *Adv. Phys. Org. Chem.* **1977**, *15*, 153. (e) Raber, D. J.; Harris, J. M.; Schleyer, P. v. R. In "Ions and Ion Pairs in Organic Reactions"; Szwarc, M., Ed.; Wiley: New York, 1974; Vol. 2, pp 247–374. (f) See also: Sneen, R. A. *Acc. Chem. Res.* **1973**, *6*, 46. (g) Gordon, J. E. "The Organic Chemistry of Electrolyte Solutions"; Olah, G. A., Ed.; Wiley-Interscience: New York, 1975. For typical NMR observations of ion pairs (tropylium), see: (h) Feigel, M.; Kessler, H.; Walter, A. *Chem. Ber.* **1978**, *111*, 2947. (i) Kessler, H.; Walter, A. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 773.

(20) We are indebted to Prof. E. M. Kosower for conversations regarding charge-transfer interactions.

Scheme III

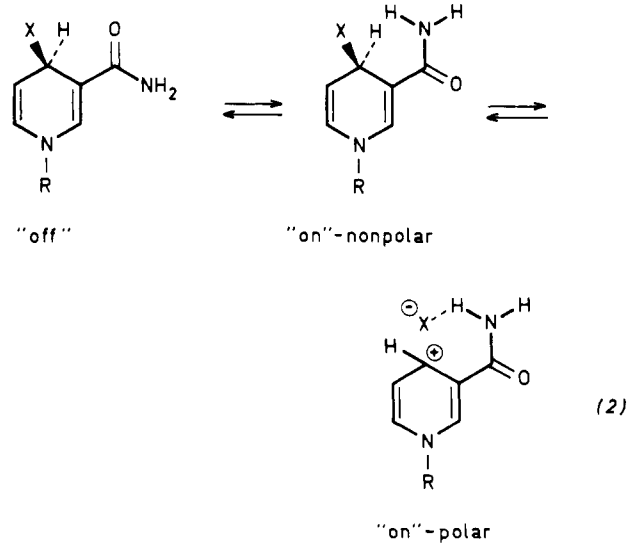


Scheme IV



of the solvent. It is attractive to speculate that a mildly nucleophilic solvent molecule (CD_3OD, CD_3CN, etc.) initiates the ion pair formation by attempted S_N2 attack at C-4 but that the process stalls at the stage of **11**, which has one or more solvent molecules still attached. The question of hybridization at C-4 (or rather apparent lack of rehybridization) can also only be resolved by additional structural information, which we hope will be available from nuclear Overhauser effects as well as two-dimensional NMR. These ambiguities are not immediately apparent, nor are they readily made so, in the crude drawing **11**. Certainly the charge separation indicated is a formalism that does not do justice to the partial bonding that must still exist between C-4 and the thiolate.

What is the significance of the observations reported here for the chemistry of 3-phosphoglyceraldehyde dehydrogenase? The most direct sort of interaction between Cys-149 and NAD^+ would be formation of a covalent adduct with thiolate bonded, we assume, at the 4-position (Scheme IV). This adduct should be yellow (the NAD^+–enzyme complex from rabbit muscle has λ_{max} 360 nm whereas for **4a**, which is representative, λ_{max} (Figure 4) lies at 340–350 nm dependent on solvent). Although, as mentioned,[8] an NAD^+–tryptophan charge-transfer interaction has been suggested as a cause of the yellow color, there appears to be no tryptophan residue close to the active site.[21] A charge-transfer interaction between thiolate and NAD^+ has also been postulated.[22] If a *covalent* adduct of NAD^+ and Cys-149 should be a true intermediate, then there must be a mechanism, as illustrated in Scheme IV, to transfer this thiolate from the dihydropyridine to the aldehyde functionality to generate the hemithioacetal. A graded transition from covalent to ionic bonding is a reasonable postulation in any such mechanism.[21c] The transition from covalent to tightly associated ion pair bonding as a function of local polarity of the surroundings is an attractive way to accomplish this. Moreover, a potential means of activation and deactivation could be present in the form of the carboxamide side arm which, one could speculate, acts as a literal "on–off" switch (eq 2) by



(2)

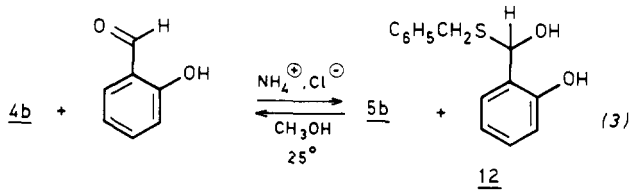
providing, or removing, by means of conformation, the possibility of hydrogen bonding from the amide to the thiolate.[23]

Although it does not prove the validity of the above speculations, the observation of the reversible equilibrium shown in eq 3, which occurs in CH_3OH but *not* $CHCl_3$ and only in the presence of a

(21) (a) See, for example, the discussion of: Harris, J. I.; Waters, M. *The Enzymes* **1976**, *13*, 1–49. (b) In thiol proteases like papein there appears to be an ion pair formed between an active site cysteine and an active site imidazole (Drenth, J. *Recl. Trav. Chim. Pays-Bas* **1980**, *99*, 185). The suggestion has been made (Polgár, L. *Eur. J. Biochem.* **1975**, *51*, 63) that a similar ion pair is involved in the system 3-phosphoglyceraldehyde dehydrogenase with NAD^+. See, however: Harigan, P. J.; Trentham, D. R. *Biochem. J.* **1971**, *124*, 573. We hope to undertake biochemical experiments to validate or reject our suggestion here.

(22) Kosower, E. M. *J. Am. Chem. Soc.* **1956**, *78*, 3497.

(23) For MINDO/3 and STO-3G calculations on the effect of the carboxamide during transfer from NADH, see: (a) Donkersloot, M. C. A.; Buck, H. M. *J. Am. Chem. Soc.* **1981**, *103*, 6549, 6554. (b) For discussions of evolutionary mechanisms for flavin enzymes with ramifications for NADH enzymes and the orientation of the carboxamide group, see: Visser, C. M. *Naturwissenschaften* **1980**, *67*, 549.



catalyst, NH_4^+, does establish at a minimum the validity of the thiolate-transfer step suggested in Scheme IV. At equilibrium about 50% of hemithioacetal **12** is present. The scope of this and other transfer reactions is being investigated.

Experimental Section

^1H NMR spectra were obtained on 60-MHz JEOL C-60 HL, Varian XL 100 MHz, and 60-MHz Hitachi Perkin-Elmer R-24B spectrometers. ^{13}C NMR spectra were obtained on either a Brucker HX-360 at 90.52 or on a Varian XL-100 at 25.16 MHz. Chemical shifts are in δ units relative to Me_4Si. Infrared spectra were recorded on a Unicam SP-2000 infrared spectrophotometer. Ultraviolet spectra were obtained on a Beckman Model 24 spectrophotometer. Melting points were determined on a Mettler FP-2 melting point apparatus, equipped with a Mettler FP-21 microscope. The melting points are not corrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

All solvents were distilled before use with the exception of analytical-grade compounds and were stored over molecular sieves 3–4 Å.

Methylene chloride and chloroform were distilled from phosphorus pentoxide. Methanol and acetonitrile (analytical grade) were purchased from E. Merck.

All chemical shifts are relative to Me_4Si at δ 0.0. Splitting patterns are designated as follows: d, doublet; t, triplet; q, quartet; m, multiplet.

The pyridinium salts **9a,b** were synthesized according to standard literature procedures.

General Procedure for the Synthesis of Thio-1,4-dihydropyridines. In the first procedure (method A) to a solution of 2 mL of sodium methoxide in methanol (0.1 N) was added 1 equiv of alkane- or arenethiol, followed by the addition of 1 equiv of pyridinium salt, under nitrogen at room temperature. The mixture was stirred for 5 min. The solvent was evaporated and the residue dissolved in CH_2Cl_2. The solution was filtered to remove precipitated $NaClO_4$. After evaporation of the solvent, the crude thio-substituted 1,4-dihydropyridines were obtained in quantitative yield. All thio-1,4-dihydropyridines were sensitive to light and air. The compounds deteriorate on standing over a period longer than 24 h. Because of the sensitivity no attempt was made to obtain elemental analyses. The structures of the compounds (at least 95% pure) were established from the NMR data.

In the second procedure (method B) to a stirred solution of 0.2 mmol of thiol and 0.2 mmol of triethylamine in CH_2Cl_2 (10 mL) at room temperature under nitrogen was added 0.2 mmol of pyridinium salt. The solution was stirred for 30 min and then washed twice with water. The organic phase was dried (Na_2SO_4), and the solvent evaporated. The essentially pure 4-thio-substituted 1,4-dihydropyridines were obtained in again essentially quantitative yield.

(4*S*,14*S*)-4,14-Diisopropyl-19-methyl-21-(phenylthio)-6,9,12-trioxa-3,16,19-triazabicyclo[15.3.1]heneicosa-17,20-diene-2,5,13,16-tetraone (4a) was prepared by method A:[10,11] ^1H NMR ($CDCl_3$) δ 0.95 (d of d, 12 H, $CH(CH_3)_2$), 2.00–2.48 (m, 2 H, $CH(CH_3)_2$), 2.57 (s, 3 H, NCH_3), 3.52–3.84 (m, 4 H, CH_2OCH_2), 4.16–4.40 (m, 4 H, $CO_2CH_2CH_2$), 4.68 (d of d, 2 H, COCHNH), 5.38 (s, 1 H, SCH), 6.80 (s, 2 H, =CHN), 7.16 (s, 5 H, C_6H_5), 6.95–7.50 (br s, 2 H, NH); ^{13}C NMR ($CDCl_3$, −20 °C) δ 17.7/17.9/18.4/18.7 (all q, $CH(CH_3)_2$), 32.3 (d, $CH(CH_3)_2$), 40.5 (q, NCH_3), 49.7 (d, $CHSC_6H_5$), 57.7/58.4 (d, COCHNH), 64.1/64.5 (t, CO_2CH_2), 68.7/69.1 (t, CH_2OCH_2), 102.1/102.8 (s, C-1, C-17), 127.9 (d, 2′,6′-CH), 129.0 (d, 4′-CH), 129.1 (d, 3′,5′-CH), 136.8 (s, 1′-CH), 138.1/138.8 (d, 18-CH, 20-CH), 164.3/165.4 (s, HNCO), 168.6/169.4 (s, CO_2); UV λ_{max} ($CHCl_3$) 247 (η 14800), 350 nm (shoulder); IR (CH_2Cl_2 solution) 3450, 3360, 3100, 2900, 1730, 1675, 1645, 1560, 1530, 1220, 1130, 1050, 975 cm^{-1}; IR in CH_3CN shows two NH absorptions at 3650 and 3400 cm^{-1}; $[\alpha]^{23}_D$ −240.2° (*c* 1.12, CH_2Cl_2); mol wt calcd 559, osmometric 559.7 (acetone), 525.3 (chloroform).

(4*S*,14*S*)-4,14-Diisopropyl-19-methyl-21-(benzylthio)-6,9,12-trioxa-3,15,19-triazabicyclo[15.3.1]heneicosa-17,20-diene-2,5,13,16-tetraone (4b) was prepared by method A: ^1H NMR ($CDCl_3$) δ 0.95 (d of d, 12 H, $CH(CH_3)_2$), 1.95–2.50 (m, 2 H, $CH(CH_3)_2$), 3.1 (s, 3 H, NCH_3), 3.50–3.84 (m, 4 H, CH_2OCH_2), 3.65 (s, 2 H, SCH_2), 4.10–4.35 (m, 4 H, CO_2CH_2), 4.67 (d of d, 2H, COCHNH), 5.05 (s, 1 H, SCH), 6.67 (d, 1 H, NH), 7.13 (s, 1 H, =CHN), 7.26 (s, 1 H, =CHN), 7.28 (s, 5 H, C_6H_5), 8.06 (d, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 18.1 (q, $CHCH_3$), 31.7 (t, SCH_2), 32.4/32.5 (d, $CHCH_3$), 41.3 (q, NCH_3), 42.7 (d, SCH), 58.3 (d, COCHNH), 63.9/64.5 (t, CO_2CH_2), 69.1/69.5 (t, CH_2OCH_2), 103.1/104.4 (s, C-1, C-17), 126.8 (d, 4′-CH), 128.1 (d, 2′,6′-CH), 128.9 (d, 3′,5′-CH), 136.8 (s, 1′-C), 137.8/138.2 (d, 18-CH, 20-CH), 164.6/165.3 (s, CONH), 169.0/169.2 (s, CO_2); UV ($CHCl_3$) λ_{max} 249 (ϵ 11900), 365 (ϵ 6000) nm; IR (CH_2Cl_2 solution) 3450, 3350, 3100–2900, 1735, 1675, 1645, 1565, 1530, 1210, 1130, 1090, 1050, 980 cm^{-1}; IR in CH_3CN shows two NH bands 3700 (weak) and 3400 cm^{-1}; $[\alpha]^{23}_D$ −172.7° (*c* 2.3, CH_3OH), $[\alpha]^{23}_{578}$ −183.9°, $[\alpha]_{546}$ −223°.

(4*S*,14*S*)-4,14-Diisopropyl-19-methyl-21-[(4-methoxyphenyl)thio]-6,9,12-trioxa-3,15,19-triazabicyclo[15.3.1]heneicosa-17,20-diene-2,5,13,16-tetraone (4c) was prepared by method A: ^1H NMR ($CDCl_3$) δ 0.95 (s br, 12 H, $CHCH_3$), 1.90–2.50 (m, 2 H, $CHCH_3$), 2.58 (s, 3 H, NCH_3), 3.40–3.80 (m, 4 H, CH_2OCH_2), 3.65 (s, 3 H, OCH_3), 4.05–4.30 (m, 4 H, CO_2CH_2), 4.55 (d of d, 2 H, COCHNH), 5.08 (s, 1 H, SCH), 6.65 (s + d, 3 H, CH, NH), 6.80 (d of d, 4 H, C_6H_4), 7.70 (d, 1 H, NH); ^{13}C NMR ($CDCl_3$, −5 °C) δ 18.0/18.2 (q, $CHCH_3$), 32.4 (d, $CHCH_3$), 40.6 (q, NCH_3), 49.0 (d, SCH), 55.0 (q, OCH_3), 58.0/58.4 (d, COCHNH), 64.0/64.5 (t, CO_2CH_2), 68.9/69.3 (t, CH_2OCH_2), 102.5/103.4 (s, 1-C, 17-C), 113.5 (d, 3′,5′-CH), 120.2 (s, 1′-C), 138.0 (d, 18-CH, 20-CH), 138.5 (d, 2′,6′-CH), 160.1 (s, 4′-C), 164.5/165.5 (s, CONH), 168.9/169.3 (s, CO_2); UV λ_{max} (CH_2Cl_2) 351 (ϵ 7600), 275 (ϵ 10700), 240 nm (ϵ 20000); $[\alpha]^{22}_D$ −272.3° (*c* 1.10, $CHCl_3$), $[\alpha]^{23}_{578}$ = −293.7°, $[\alpha]^{23}_{546}$ −371.9°.

(4*S*,14*S*)-4,14-Diisopropyl-19-methyl-21[(4-nitrophenyl)thio]-6,9,12-trioxa-3,15,19-triazabicyclo[15.3.1]heneicosa-17,20-diene-2,5,13,16-tetraone (4d) was prepared by method B: ^1H NMR ($CDCl_3$) δ 0.95 (d of d, 12 H, $CHCH_3$), 2.00–2.60 (m, 2 H, $CHCH_3$), 3.37 (s, 3 H, NCH_3), 3.60–3.90 (m, 4 H, CH_2OCH_2), 4.20–4.55 (m, 4 H, CO_2CH_2), 4.65 (d of d, 2 H, COCHNH), 7.20–8.40 (m, 7 H, NH, SCH, C_6H_4), 7.66 (s, 2 H, 18-CH, 20-CH). This compound was too unstable for measurement of a pulsed ^{13}C NMR spectrum in $CDCl_3$ or for reliable measurement of $[\alpha]_D$. The identification of the compound is based on the ^1H NMR spectral data.

(7*S*,17*S*)-25-Methyl-27-(phenylthio)-9,15-dioxa-3,21,25-triazatetracyclo[21.3.1.0^{3,7}.0^{17,21}]hepteicosa-23,26-diene-2,8,16,22-tetraone (ii) was prepared by method B: ^1H NMR ($CDCl_3$) δ 1.40–2.50 (m, 14 H), 3.15 (s, 3 H, NCH_3), 3.40–3.80 (m, 4 H, 4-CH_2, 20-CH_2), 4.00–4.34 (m, 4 H, CO_2CH_2), 4.56 (d of d, 2 H, COCHN), 6.56 (s, 1 H, SCH), 6.86 (s, 2 H, 24-CH, 26-CH), 7.24 (s, 5 H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 23.2, 23.6, 28.0, 30.4 (all t), 43.3 (q, NCH_3), 48.1 (t, NCH_2), 60.5 (d, OCCHN), 65.4 (t, CO_2CH_2), 127.0 (d), 127.3 (d), 128.5 (d), 128.6 (d), 128.9 (d), 134.6 (s, 1′-C), 165.6 (s, CON), 172.3 (s, CO_2), the 2,6-C, 3,5-*C* and 4-C resonances were too poorly resolved to be located; UV (CH_2Cl_2) 245 (ϵ 4830), 273 (ϵ 3420), 358 nm (ϵ 830).

3,5-Bis[(2′*S*)-(2′-(methoxycarbonyl)pyrrolidino)carbonyl]-1-methyl-4-(phenylthio)-1,4-dihydropyridine (iii) was prepared by method B: ^1H NMR ($CDCl_3$) δ 1.70–2.30 (m, 8 H, CH_2CH_2), 3.23 (s, 3 H, NCH_3), 3.45 (m, 4 H, NCH_2), 3.75 (s, 6 H, OCH_3), 4.49 (t, 2 H, COCHN), 6.83 (s, 2 H, 2,6-CH), 7.0–7.45 (m, 6 H, C_6H_5 + CHS); ^{13}C NMR ($CDCl_3$) δ 25.2 (t, $CHCH_2CH_2$), 29.1 (t, $CH_2CH_2CH_2$), 41.1 (q, NCH_3), 49.1 (t, CH_2CH_2N), 52.0 (q, OCH_3), 59.7 (d, COCHN), 127.4, 128.6, 128.9 (all d, C_6H_5), 134.1 (s, CS), 134.5, 134.8 (d, 2-CH, 6-CH), 166.1 (s, CON), 172.3 (s, $COOCH_3$), the C-4 and C-3,5 absorptions were too poorly resolved to be located.

1-Methyl-3,5-bis[(ethylamino)carbonyl]-4-(phenylthio)-1,4-dihydropyridine (8a) was prepared by method A: ^1H NMR ($CDCl_3$) δ 1.16 (t, 6 H, CH_2CH_3), 2.72 (s, 3 H, NCH_3), 3.34 (m, 4 H, CH_2CH_3), 5.90 (s, 1 H, CHS), 6.95 (t, 2 H, NH), 7.00 (s, 2 H, 2,6-CH), 7.26 (s, 5 H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.6 (q, CH_2CH_3), 34.5 (t, CH_2CH_3), 41.0 (q, NCH_3), 62.3 (d, CH), 105.5 (s, 3,5-C), 128.1 (d), 128.2 (d), 131.6 (s, 1′-C), 135.9 (d, 2,6-CH), 165.8 (CON); UV (CH_2Cl_2) 238 (ϵ 14000), 265 (ϵ 17700), 347 nm (ϵ 10500); IR (CH_3CN) 3600, 3450, 3100–2900 (very weak), 1665–1655, 1260, 1220, 1140, 770, 645 cm^{-1}.

1-Methyl-3,5-bis[(ethylamino)carbonyl]-4-(benzylthio)-1,4-dihydropyridine (8b) was prepared by method A; ^1H NMR ($CDCl_3$) δ 1.19 (t, 6 H, CH_2CH_3), 3.10 (s, 3 H, NCH_3), 3.35 (m, 4 H, NCH_2), 3.65 (s, 2 H, CH_2S), 5.16 (s, 1 H, CHS), 6.49 (d of d, 2 H, NH), 7.09 (s, 2 H, 2,6-CH), 7.31 (s, 5 H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.4 (q, CH_2CH_3), 32.5 (t, SCH_2), 34.3 (t, NCH_2), 41.3 (q, NCH_3), 42.8 (d, CHS), 105.2 (s, 3,5-C), 126.6 (d, 4′-CH), 127.9 (d, 2′,6′-CH), 128.7 (d, 3′,5′-CH), 137.0 (s, 1′-C), 137.7 (d, 2,6-CH), 165.7 (s, CON); UV (CH_2Cl_2) 234 (ϵ 20600), 267 (ϵ 18100), 348 nm (ϵ 12700).

1-Methyl-3,5-bis[(diethylamino)carbonyl]-4-(phenylthio)-1,4-dihydropyridine (8c) was prepared by method B; ^1H NMR ($CDCl_3$) δ 1.19 (t, 12 H, CH_2CH_3), 3.37 (q, 8 H, NCH_2), 3.69 (s, 3 H, NCH_3), 7.00–7.50 (m, 6 H, phenyl ring, CHS), 7.56 (s, 2 H, 2,6-CH); UV (CH_2Cl_2) λ_{max} 244 (ϵ 7750), 270 (ϵ 4670), 350 nm (ϵ 1200); compound **8c** was too unstable for measurement of the pulsed ^{13}C NMR spectrum.

(4*S*,14*S*)-4,14-Diisopropyl-19-methyl-21-cyano-6,9,12-trioxa-3,15,19-triazabicyclo[15.3.1]heneicosa-17,20-diene-2,5,13,16-tetraone

(10). Bridged pyridinium salt **5** (110 mg, 0.2 mM) was suspended in 25 mL of $CHCl_3$. To this suspension 100 mg of KCN in 25 mL of H_2O were added, and the two layers were mixed by vigourous shaking until all pyridinium salt had dissolved. The organic layer was separated, and the H_2O phase was washed with 26 mL of $CHCl_3$. The combined organic phase was dried over Na_2SO_4 and filtered. After evaporation of the solvent, the yellow, oily product **10** was isolated (95 mg, 0.2 mM, quantitative): ^1H NMR ($CDCl_3$) δ 0.95 (d of d, 12 H, $CH(CH_3)_2$), 2.05–2.50 (m, 2 H, $CH(CH_3)_2$), 3.23 (s, 3 H, NCH_3), 3.58–3.90 (m, 4 H, CH_2OCH_2), 4.06–4.48 (m, 4 H, CO_2CH_2), 4.50–4.80 (dbl d, COCHN), 5.03 (s, 1 H, NCCH), 6.56 (dbl d, 2 H, NH), 7.13 (s, 2 H, 2,6-CH); ^{13}C NMR ($CDCl_3$) δ 17.7, 18.2, 18.3 and 18.5 (all q, $CH(CH_3)_2$), 25.5 (d, NCCH), 31.8 and 32.0 (d, $CH(CH_3)_2$), 41.5 (q, NCH_3), 58.6 and 59.0 (d, COCHN), 64.1 and 64.6 (t, CO_2CH_2), 69.4 and 69.5 (t, CH_2OCH_2), 100.1 and 100.3 (s, CH), 117.5 (s, CN), 138.7 and 139.6 (d, 18,20-CH), 163.1 and 163.9 (s, CONH), 168.4 and 169.3 (s, CO_2); $[\alpha]^{23}_D$ = −160.3 (*c* 1.5, CH_2Cl_2); UV λ_{max} ($CHCl_3$) 246 (ϵ 24 000), 365 (ϵ 16 000) nm.

Preparation of a Mixture of 4b and 8a. (Benzylthio)-1,4-dihydropyridine **4b** was prepared as described. After evaporation of the solvent (CH_2Cl_2), the crude compound was taken up in 0.5 mL of CD_3OD. (Phenylthio)-1,4-dihydropyridine **8a** was then prepared (method A). After evaporation of CH_2Cl_2, the solution of **4b** in 0.5 mL of CD_3OD was added to the residue. The concentrations of both were equal (ca. 0.15 M). This mixture was analyzed by ^1H NMR spectroscopy. The spectrum was nearly identical with the superimposed spectra of **4b** and **8a**; for instance, 3.12 (s, 3 H, NCH_3, **4b**), 3.30 (s, 3 H, NCH_3, **8a**), 6.20 (br s, 1 H, 4-CH, **4b**), 6.70 (br s, 1 H, 4-CH, **8a**), 7.10 (br s, 2 H, 2,6-CH, **4b**), and 7.40 (s, 2 H, 2,6-CH, **8a**).

Deuterium exchange experiment was carried out as follows: the (benzylthio)-1,4-dihydropyridine **4b** was prepared as described and dissolved in CD_3OD, and the spectrum was recorded, first immediately after preparation of **4b** and after 2 days. The spectrum remained unchanged. CD_3OD was evaporated, the residue was taken up in $CDCl_3$, and the mixture was analyzed again by ^1H NMR. The $CDCl_3$ spectrum was identical with the spectrum of **4b** in $CDCl_3$ as described with two exceptions: no NH absorptions were present and the absorption at δ 4.67 (COCHN) was a doublet, instead of the double doublet at δ 4.67 in the original $CDCl_3$ spectrum.

***N*-(Carboxybenzoyl)phenylalanine *p*-nitrophenol ester (6)** was prepared according to the literature:[24a,c] mp 125.5–127.5 °C (lit.[24b] 126.5–127.5 °C); $[\alpha]^{23}_D$ −14.1° (*c* 1, ethyl acetate) (lit.[24b,c] $[\alpha]^{25}_D$ −8.9° (*c* 2.2, $CHCl_3$), $[\alpha]^{20}_D$ −24.7° (*c* 2, $(CH_3)_2NCHO$).

Rates of Transfer of Thiolate of 4a with 6. Pseudo-first-order rates for the liberation of *p*-nitrophenolate anion from **6** (R^2 = $C_6H_5CH_2CHNHCO_2CH_2C_6H_5$) were followed spectrophotometrically at 25 °C in CH_3CN and in 10% CH_3CN–$CHCl_3$ (v/v). A Zeiss M4 Q III spectrometer was used the cell compartment of which was thermostated at 25 °C with a Lauda$^{s15/12}$ circulating bath. A stock solution of N-protected amino ester in CH_3CN was prepared (1.5 mM/L). Freshly prepared 4-thio-1,4-dihydropyridine was dissolved in CH_3CN (0.09 mMol in 2 mL), 100 μL of this solution was added to 2.8 mL CH_3CN, and the mixture was equilibrated thermally in the cell compartment. To this solution was added 100 μL of the amino ester solution, and the mixture was mixed rapidly. The changes in absorbance vs. time were recorded. The kinetics parameters, $A_\infty - A_t$ vs. time, were plotted on semilog graph paper, half-lives were determined from the plots, the pseudo-first-order rate constant (k_{obsd}) was determined from the equation, $k_{obsd} = 0.693 t_{1/2}^{-1}$.

The rate constant from the runs in 10% CH_3CN–$CHCl_3$ (v/v) was determined as follows: the parameter A_∞ was taken equal to A_∞ as determined from the runs in CH_3CN, after 1060 min. A_t was determined, and k_{obsd} was determined from the equation $k_{obsd} = (\ln A_\infty - \ln A_t)\Delta t^{-1}$.

Transfer of Thiolate from 4b to Salicylaldehyde. 4-(Benzylthio)-1,4-dihydropyridine **4b** (0.2 mM) was prepared as described and was dissolved in 0.5 mL of CD_3OD. To this solution were added 20 μL (0.2 mM) of salicylaldehyde and 25 mg of NH_4Cl. The reaction was carried out at room temperature in a NMR tube and was monitored by ^1H NMR spectroscopy. The formation of pyridinium salt and of hemithioacetal **12** was observed. The ratio of products was calculated to be 1:1 from the integrals of the absorption of the aldehyde proton in salicylaldehyde and the characteristic absorption of the tertiary hydrogen (HOC*H*S) at 5.79 ppm of **12**. When the methanol was removed and the residue dissolved in chloroform, starting material **4b** and salicylaldehyde were reformed at the cost of **12** and pyridinium salt.

The experiment was also carried out in $CDCl_3$. After 24 h at room temperature only starting material **4b** and salicylaldehyde could be detected.

Addition of Excess Benzenethiol to 4a in CD_3OD. Compound **4a** (0.2 mM) was prepared as described above and was dissolved in 0.5 mL of CD_3OD, and the ^1H NMR spectrum was recorded. To this solution an equivalent amount (0.2 mM, 22 μL) of benzenethiol was added followed by a second equivalent. After each addition the ^1H NMR spectra were recorded.

Comparison of the three spectra revealed that H-4 and H-2,6 underwent *no* measurable chemical shift whatsoever upon addition of excess benzenethiol. This was monitored by examining both the relative shift differences between H-4 and H-2,6 as well as the absolute chemical shifts relative to Me_4Si.

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