NAD(P)$^+$-NAD(P)H Models. 53. Proximity Effect of Intramolecular Heteroatom on Reaction Rate

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NAD(P)H-model compounds that have a sulfur or oxygen substituent in the molecule have been synthesized and kinetics for the reduction of both substituted and unsubstituted α,α,α-trifluoroacetophenones with these model compounds are studied. Reduction with the heteroatom-containing model compounds proceeds at a slower rate than the reduction with a model without any heteroatom substituents. Furthermore, the dependency of the kinetic isotope effect on the electron-deficiency of the substrate differs. Kinetic parameters reveal that the retardation caused by the heteroatom is due to the loss of entropy in the initial electron-transfer process. The enthalpic term favors the reduction with the heteroatom-containing model.

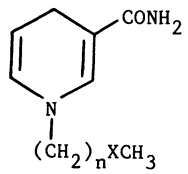
Biomimetic reaction systems for reductions which are catalyzed by NAD(P)H-dependent dehydrogenases have been extensively studied for their reaction mechanisms and applications to organic syntheses.[1] Since the true reductant in this redox system is the 1,4-dihydronicotinamide moiety of NAD(P)H, almost all researches have been concentrated on the chemistry of 1,4-dihydropyridine derivatives. After we found that magnesium ion in dry acetonitrile is a good catalyst in a mimetic system,[2] the requirements for substrates that can be subjected to mimetic reduction have been lessened extensively. However, the enzymic redox system is still much superior to the corresponding mimetic one in the sense that the former system reduces a far wider variety of substrates than the latter. There is no doubt that the difference in the reactivity between enzymic and mimetic systems lies in complex constituents of the enzyme, which supply the essential functional groups for catalysis and, at the same time, construct a favorable environment for the reaction. Therefore, in order to extend the mimetic reaction system, we have to take into account not only the chemical constitution at the reaction center but also environmental effects. There are a lot of positively or negatively polarized groups around the active site of the enzyme, and these groups may assist the transfer of (net) hydride. It is also known that, for some liver alcohol dehydrogenases, a zinc ion is essential for the reaction, and this zinc ion, which is ligated by one molecule of water, is tetrahedrally ligated by cysteinyl sulfurs and histidinyl nitrogens.[3] The zinc ion is supposed to activate substrates as a Lewis acid by ligating onto the carbonyl oxygen of the substrate ketone.[4] However, there is some doubt whether the pentacoordinated zinc ion at the reaction center can retain its catalytic activity as a Lewis acid. At least in a mimetic system, a metal ion with a chelating reagent was not a catalyst.[5]

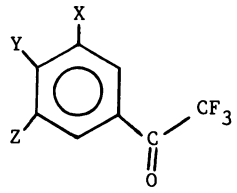
As a trial to introduce some environmental effects into a mimetic reaction system, we synthesized NAD(P)H models that have sulfur or oxygen functional groups as substituents on an alkyl chain at the N_1 position of 1,4-dihydronicotinamide and studied the effects of heteroatoms on the kinetics in the presence or absence of magnesium ion.

It should be mentioned that magnesium ion in the mimetic system is a catalyst for some substrates,[2] but an inhibitor for others.[6] For a third class of substrates, it may be a catalyst or an inhibitor depending on its concentration.[7] Such complex behavior is best interpreted in terms of a multi-step mechanism with the initial electron transfer from the dihydropyridine moiety to the substrate, and of acceleration of this electron-transfer process by magnesium ion.[8]

Results

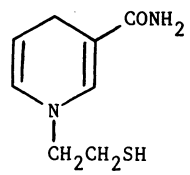
Six 1-[(ω-methylthio)alkyl]-1,4-dihydronicotinamides (S2NAH, S3NAH, S4NAH, S5NAH, S6NAH, and S8NAH) and one 1-[(ω-methoxy)alkyl]-1,4-dihydronicotinamide (O2NAH) were synthesized as model compounds with a heteroatom in the molecule. Two 1-alkyl-1,4-dihydronicotinamides (PNAH and ONAH) were employed as standards.





S2NAH: X = S, $n = 2$ **1a**: X = Y = Z = H
S3NAH: X = S, $n = 3$ **1b**: X = Z = H, Y = Cl
S4NAH: X = S, $n = 4$ **1c**: X = CF_3, Y = Z = H
S5NAH: X = S, $n = 5$ **1d**: X = NO_2, Y = Z = H
S6NAH: X = S, $n = 6$ **1e**: X = NO_2, Y = Cl, Z = H
S8NAH: X = S, $n = 8$ **1f**: X = CF_3, Y = H, Z = NO_2
O2NAH: X = O, $n = 2$
PNAH: X = CH_2, $n = 1$
ONAH: X = CH_2, $n = 6$

Substituted and unsubstituted α,α,α-trifluoroacetophenones (**1a—1f**) were prepared as substrates for the reduction. A compound with a free mercapto group (SHNAH) was also prepared, but could not be used in the kinetic studies because of its instability. First-order rate constants were measured by observing the decrease in the intensity at around 350 nm (an absorption characteristic of the dihydropyridine moiety) on a



SHNAH

spectrophotometer. The observed rate constants were corrected for the rate constant for the magnesium ion-catalyzed decomposition of each model compound. The second-order rate constant, k, was calculated by dividing the corrected rate constant by the concentration of the substrate and the results are summarized in Table 1.

Kinetic parameters for some pairs of reactants were calculated from the temperature dependency of the rate constants, and the results are listed in Table 2.

Kinetics were also studied with S2NAH-4-*d*, and the kinetic deuterium isotope effect was calculated using Steffens and Chipman's equation.[9] The values are listed in Table 3. We have confirmed that the accuracy of the value obtained by this procedure is as high as that obtained from the calculation with the dideuterated compound.[8a]

Table 1. Second-order rate constants for the reduction of α,α,α-trifluoroacetophenone and its derivatives with NAD(P)H models[a]

No.	Substrate	Model	Temp °C	10k, dm^3 mol^{-1} s^{-1} without metal ion	with Mg^{2+}	with Zn^{2+}
1	**1a**	S2NAH	30	c)	0.0786±0.0010[d]	
2	**1a**	S2NAH	40	c)	0.167±0.0029[d]	
3	**1a**	S2NAH	50	b)	0.270±0.0046[d]	b)
4	**1a**	S2NAH	60	c)	0.561±0.018[d]	
5	**1b**	S2NAH	50	b)	0.660±0.013[d]	b)
6	**1c**	S2NAH	50	0.222±0.0017[d]	1.22±0.033[d]	c)
7	**1d**	S2NAH	30	c)	1.04±0.031[d]	
8	**1d**	S2NAH	40	c)	1.84±0.021[d]	
9	**1d**	S2NAH	50	0.995±0.058	3.21±0.093[d]	c)
10	**1d**	S2NAH	60	c)	5.30±0.070[d]	
11	**1e**	S2NAH	50	b)	4.74±0.0055[d]	c)
12	**1f**	S2NAH	30	c)	2.68±0.016[d]	
13	**1f**	S2NAH	40	c)	5.49±0.021[d]	
14	**1f**	S2NAH	50	15.6±0.40	6.76±0.040[d]	c)
15	**1f**	S2NAH	60	c)	11.1±0.18[d]	
16	**1d**	S3NAH	50	2.03±0.054	4.38±0.090	1.77±0.065
17	**1d**	S4NAH	50	2.40±0.048	5.83±0.11	c)
18	**1d**	S5NAH	50	2.71±0.060	6.81±0.16	2.48±0.060
19	**1d**	S6NAH	50	3.35±0.040	8.01±0.053	c)
20	**1d**	S8NAH	30	c)	2.71±0.079	c)
21	**1d**	S8NAH	40	c)	4.64±0.033	c)
22	**1d**	S8NAH	50	3.39±0.045	8.20±0.11	3.38±0.050
23	**1d**	S8NAH	60	c)	14.3±0.082	c)
24	**1a**	O2NAH	50	b)	0.322[e]	
25	**1b**	O2NAH	50	b)	0.669[e]	
26	**1c**	O2NAH	50	0.295[e]	1.73[e]	
27	**1d**	O2NAH	50	1.46±0.050	4.10±0.055	c)
28	**1e**	O2NAH	50	1.46[e]	4.89[e]	
29	**1f**	O2NAH	50	1.89[e]	6.27[e]	
30	**1d**	PNAH	50	2.89±0.062[d]	6.43±0.13[d]	1.51±0.040
31	**1d**	ONAH	50	3.29±0.090	8.07±0.0789	c)

a) Errors are standard deviations. b) Too slow to be followed. c) Not observed. d) Data from ref. 8a. e) Data from one kinetic run.

Table 2. Second-order rate constants for the reduction of α,α,α-trifluoroacetophenone and its derivatives with 1-[(2-methylthio)ethyl]-1,4-dihydronicotinamide-4-*d* (S2NAH-*d*) and kinetic deuterium isotope effects[a]

Substrate	10k dm^3 mol^{-1} s^{-1}[b] without Mg^{2+}	with Mg^{2+}	k^H/k^D[c] without Mg^{2+}	with Mg^{2+}
1a		0.178±0.0075		3.11
1c	0.157±0.0018		2.43	
1d	0.704±0.018	2.12±0.039	2.41	3.13
1e		2.92±0.023		4.31
1f	10.2±0.17	3.99±0.049	3.29	5.51

a) At 50 °C in acetonitrile. b) Errors are standard deviations. c) Error is less than ±10%.

Table 3. Kinetic parameters for the reduction of 3-nitro-α,α,α-trifluoroacetophenone with NAD(P)H model compounds[a)]

Substrate	Model	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta G^{\ddagger}$
		kJ mol^{-1}	J K^{-1} mol^{-1}	kJ mol^{-1}[b)]
1a	S2NAH	56.0	−151.2	104.9±0.060
1d	S2NAH	48.5	−155.5	98.6±0.053
1d	S8NAH	49.2	−145.7	96.2±0.021
1f	S2NAH	40.4	−173.5	96.6±0.098

a) Values at 50°C. b) Errors are standard deviations.

Water in the solvent acetonitrile strongly influences the results in these kinetic studies. Particular attention was paid to this point and the water content of the reaction system was measured before and after each kinetic run and was always less than 0.075% v/v even in the presence of magnesium perchlorate.[10)]

Discussion

Hammett plots for the reduction of various substrates with S2NAH and O2NAH in the presence and absence of magnesium ion are illustrated in Fig. 1. In the reductions with S2NAH, a straight line with ρ=2.62 (correlation coefficient r=0.998) is obtained when magnesium ion is absent, but the plot for the reduction in the presence of magnesium ion is composed of two straight lines, one of which has ρ=1.50 (r=0.999) and the other has ρ=0.768 (r=1.000).[11)] The corresponding plots for the reduction with O2NAH have essentially the same tendency; ρ=2.55 (r=1.000) for the reduction without magnesium ion, and ρ= 1.59 (r=0.995) and 0.436 (r=0.990) for the reduction with magnesium ion.

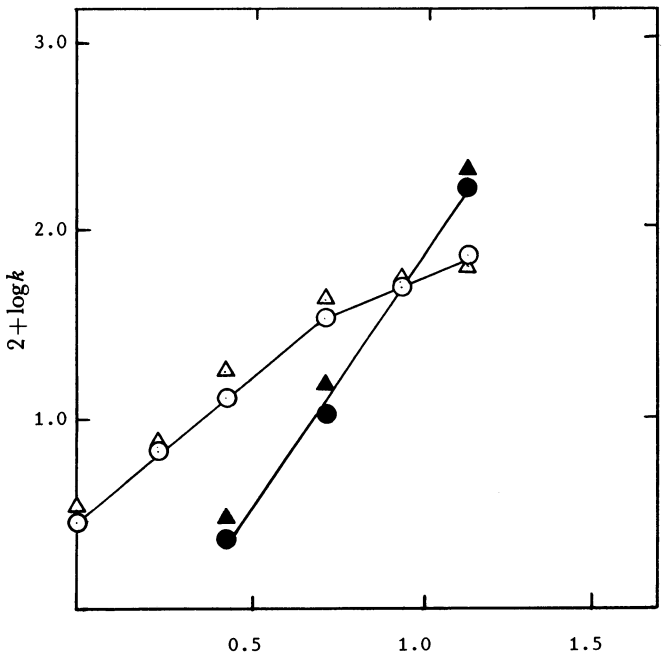


Fig. 1. Hammett plots for the reduction with S2NAH in the presence (○) and absence (●) of magnesium ion and with O2NAH in the presence (△) and absence (▲) of magnesium ion.

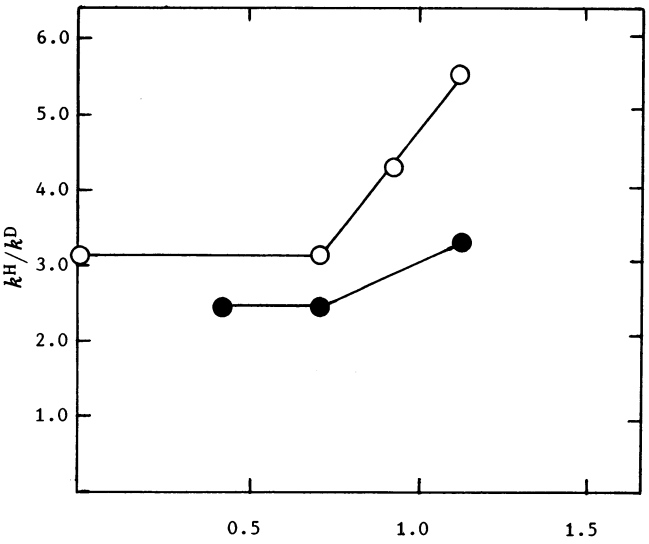


Fig. 2. Plotts of kinetic isotope effects in the reduction with S2NAH against σ-values of substituents in the presence (○) and absence (●) of magnesium ion.

The present results with heteroatom-containing models[12)] are a striking contrast to the free energy relationship exerted by PNAH, where the reduction with magnesium ion gave a straight line but that without magnesium ion appeared as a bent line.[8a)]

The dependency of kinetic isotope effects on the substituent σ-value for the reduction with S2NAH again differs from that with PNAH. That is, as plotted in Fig. 2, the isotope effect for the former reduction increases, regardless of the presence or absence of magnesium ion, as the electron-deficiency of the substrate becomes large. On the other hand, in the reduction with PNAH, the kinetic isotope effect remained constant when the reduction was run in the presence of magnesium ion, whereas in its absence the value increased to an asymptote.[8a)] For each reductant, the σ-value where the slope of the line changes in the plot of the kinetic isotope effect corresponds exactly with the position where the change in slope is seen in the Hammett plot. Thus, it is apparent that the change in slope reveals a change in the rate-determining step, or the reduction is composed of several steps.

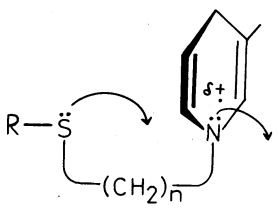
Comparison of activation parameters for the reductions of **1a** (a weakly electron-deficient substrate), **1d** (a moderately electron-deficient substrate), and **1f** (a strongly electron-deficient substrate) with S2NAH in the presence of magnesium ion reveals that the entropy of activation for the reduction of **1f** is much smaller than those for the reductions of **1a** and **1d**. If the reduction proceeded through a one step hydride-transfer mechanism, Hammond's posturation proposes that the transition state for a strongly electron-deficient substrate should resemble the reactant more than the transition state for a less electron-deficient substrate, or the entropy of activation for the reduction of **1f** should be larger than those for the reductions of **1a** and **1d**. On the other hand, if the electron-transfer

step constitutes a major part of the rate-determining step, the stability of the resulting radical ion pair might play an important role in determining the activation parameter, and the entropy of activation may be smaller for **1f** than for **1a** or **1d**. This is a conclusion which comes out of the fact that a strongly electron-deficient substrate in a radical ion pair can keep an electron in its own counterpart more tightly than a less electron-deficient substrate.

Nevertheless, the reduction is accompanied by a certain amount of kinetic isotope effect. Thus, the transition state of the rate-determining step of the reduction seems to be such that the transfer of an electron is accompanied by the loosening of C_4–H bond in the reductant.

Table 1 shows that the reduction with S2NAH or O2NAH is slower than the reduction with PNAH regardless the presence or absence of magnesium ion. The retardation exerted by a heteroatom, however, becomes less important as the number of methylene groups, n, becomes large, and comes to an asymptote after n=6. The asymptotic value is equivalent to the reduction rate with ONAH. Activation parameters for the reduction of **1d** with S2NAH and S8NAH, as listed in Table 3, show that the reduction with the former model is rather preferred by the enthalpic term but the larger contribution of the entropic term results in the retardation of reduction with the former model.

Consequently, the retardation of the reduction caused by the intramolecular heteroatom can be accounted for by a major contribution of the initial process, in which at least a partial positive charge is developed in the reductant without affording a large kinetic deuterium isotope effect (or without dissociating the hydrogen nucleus greatly from the carbon at the 4-position), to the rate-determining step of the reduction. The intermediate must be one which is stabilized, in terms of enthalpy, by the proximity effect of an intramolecular hetero-atom, and the stabilization becomes more important as the electron deficiency of the substrate increases. The structure of the transition state for the initial step is, therefore, best expressed as a one-electron-transfer type of complex between the model compound and a substrate, the cation radical part of which is stabilized by participation by an electron pair on the heteroatom as illustrated in Scheme I. By the participation of an electron pair on the heteroatom, the partial positive charge in the dihydropyridine ring which develops as an electron in this ring is transferred onto the substrate, is stabilized in terms of enthalpy. However, for the electron pair to stabilize the electron-deficient dihydropyridine ring, the side-chain must be kept from free rotation, which causes a decrease in entropy. As the number of spacer methylene groups becomes larger, such participation of a heteroatom becomes less important because the entropic loss becomes too large, and the rate constant for the reduction comes to an asymptotic value. Since the activation energies for the initial electron-transfer process in the reduction with the heteroatom-substituted models are higher than those with PNAH and ONAH, the priority of this process in the rate-determining step of the reduction with a heteroatom-substituted model still remains even with the catalytic assistance of magnesium ion, keeping the kinetic isotope effects for these reductions small.



Scheme 1.

It is noteworthy that an inspection with a CPK-model shows that the sulfur or oxygen atom in S2NAH and O2NAH, respectively, is close to the dihydropyridine ring nitrogen when the dihydropyridine ring and the heteroatom are set at the *syn* position.

Table 1 shows that zinc ion retards the reduction in contrast to the catalytic effect of magnesium ion. However, the retardation diminishes as the alkyl chain becomes longer. The inhibitory effect of zinc ion can be accounted for if zinc ion has a higher efficiency than magnesium ion in stabilizing the charge-transfer ternary complex given by the initial one electron-transfer process.[6c,d] Since zinc ion is softer than magnesium ion as a Lewis acid, the former is better trapped by sulfur than the latter.[13] At the same time, the sulfur atom has a stronger affinity for the zinc ion than for the dihydropyridine ring. When the sulfur atom is set far from the reaction center as in the case of S8NAH, therefore, zinc ion is less involved in the reaction (the interaction between the dihydropyridine ring and the substrate) and the rate increases to the magnitude available without metal ion.

Although, unfortunately, it has been confirmed that intramolecular heteroatoms in the NAD(P)H model compounds decrease the reactivity, the interference is caused by the entropic term. The above conclusion, therefore, proposes that, if the heteroatom in a molecule is fixed at a proper position in the reactant, like atoms in a reaction pocket of an enzyme, there is no entropic interference and the increase in the reaction rate due to enthalpic assistance is expected. Enhanced reactivity caused by the participation of a proximate negative charge has been reported for reduction in micellar system.[14]

Experimental

Melting points were not corrected. UV spectra and VPC were recorded on a Union Giken SM-401 spectrophotometer and Hitachi 633-50 chromatograph, respectively. JEOL JNMFX-100 and Hitachi R-22 spectrometers were employed

for NMR spectroscopy.

Materials. Acetonitrile was refluxed over calcium hydride for several hours, then distilled from the refluxing pot under an argon atmosphere before use. Magnesium and zinc perchlorates were dried over phosphorus pentaoxide for 1 d under reduced pressure at 100 °C. The amount of water in kinetic solutions was determined on VPC (30% bis(2-cyanoethyl) ether, 1 m, 40 °C) before and after the kinetic measurement and was found to be less than 0.075% v/v and 0.094% v/v for the magnesium and zinc perchlorate solutions, respectively.

α,α,α-Trifluoroacetophenone (**1a**), 4-chloro-α,α,α-trifluoroacetophenone (**1b**), 3-trifluoromethyl-α,α,α-trifluoroacetophenone (**1c**), 3-nitro-α,α,α-trifluoroacetophenone (**1d**), 4-chloro-3-nitro-α,α,α-trifluoroacetophenone (**1e**), and 3-nitro-5-trifluoromethyl-α,α,α-trifluoroacetophenone (**1f**) were prepared according to procedures in the literature.[8a]

1-Propyl-1,4-dihydronicotinamide (PNAH) was prepared according to a procedure in the literature.[15] 1-Octyl-1,4-dihydronicotinamide (ONAH) was synthesized similarly. 1-[(2-Methylthio)ethyl]-1,4-dihydronicotinamide (S2NAH), 1-[(3-methylthio)propyl]-1,4-dihydronicotinamide (S3NAH), and 1-[(2-methoxy)ethyl]-1,4-dihydronicotinamide (O2NAH) were prepared in the same manner by the reduction of the corresponding pyridinium salt.[16]

1-[(8-Methylthio)octyl]-1,4-dihydronicotinamide (S8NAH). A solution of nicotinamide (0.48 g) and 10-fold excess of 1,8-dibromooctane in 10 cm^3 of acetonitrile was heated at 60 °C for 1 d to obtain 1-(8-bromooctyl)-3-carbamoylpyridinium bromide in 72% yield after recrystallization from acetonitrile. The pyridinium salt was subjected to methylthiolation with sodium methanethiolate in water. The resulting 1-[(8-methylthio)octyl]-3-carbamoylpyridinium bromide (92% yield) was reduced with sodium dithionite to yield yellow crystals of S8NAH in 54% yield after recrystallization from ethanol.

1-[(4-Methylthio)butyl]-1,4-dihydronicotinamide (S4NAH), 1-[(5-methylthio)pentyl]-1,4-dihydronicotinamide (S5NAH), and 1-[(6-methylthio)hexyl]-1,4-dihydronicotinamide (S6NAH) were synthesized similarly.

UV and NMR spectra as well as the results of elemental analyses are listed in Table 4.

2-(Methoxymethylthio)ethanol. A solution of 31.3 g of 2-mercaptoethanol (0.40 mol) in 400 cm^3 of ethanol was added to a solution of sodium ethoxide (0.44 mol) in 40 cm^3 of ethanol under a nitrogen atmosphere. The mixture was cooled to 0 °C, and to this mixture 35.4 g of chloromethyl methyl ether (0.44 mol) was added dropwise. The reaction mixture was stirred for an hour, then the precipitate was filtered off and the solvent was evaporated from the filtrate. The residue was distilled (bp 58—60 °C/26.6 Pa) to give 35.9 g of 2-(methoxymethylthio)ethanol as an oil (73.5% yield). ^1H NMR in $CDCl_3$ (δ from TMS) 2.81 (t, 2H), 3.13 (bs, 1H), 3.42 (s, 3H), 3.78 (t, 2H), and 4.67 (s, 2H).

2-(Methoxymethylthio)-1-chloroethane. Into a stirred mixture of 33.0 g of 2-(methoxymethylthio)-1-ethanol (0.27 mol) and 23.7 g of pyridine (0.30 mol) at 0 °C, 33.7 g of thionyl chloride (0.28 mol) was added dropwise. It took about 1 h. After the reaction, the mixture was warmed to room temperature and organic materials were extracted with ether. The organic layer was washed with water and dried over sodium sulfate, then the solvent was evaporated. The residue was distilled (bp 43 °C/266 Pa) to give 2-(methoxymethylthio)-1-chloroethane in 64% yield (24.2 g). ^1H NMR in $CDCl_3$ (δ from TMS) 2.93 (t, 2H), 3.37 (s, 3H), 3.71 (t, 2H), and 4.67 (s, 2H). Found: C, 33.95; H, 6.72%. Calcd for C_4H_9OSCl: C, 34.15; H, 6.46%.

1-[2-(Methoxymethylthio)ethyl]-3-carbamoylpyridinium Chloride. A mixture of 16.9 g of 2-(methoxymethylthio)-1-chloroethane (0.12 mol) and 13.4 g of nicotinamide (0.11 mol) in 12 cm^3 of ethanol was refluxed for 20 h. After the reaction, the precipitate was filtered and washed with ether and ethanol to give crude 1-[2-(methoxymethylthio)ethyl]-3-carbamoylpyridinium chloride in 95.6% yield. Recrystallization from methanol–ether gave the pure salt as white crystals in 59.8% yield (based on nicotinamide). ^1H NMR DMSO-d_6 (δ from TMS) 3.16 (s, 3H), 3.32 (t, 2H), 4.71 (s, 2H), 4.92 (t, 2H), 8.0—8.4 (m, 2H), 9.05 (s, 2H), and 9.18 (dd, 1H). Found: C, 44.59; H, 5.69; N, 10.25%. Calcd for $C_{10}H_{15}ClO_2S$: C, 45.70; H, 5.77; N, 10.66%.

1-(2-Mercaptoethyl)-3-carbamoylpyridinium Chloride (SHNA$^+$).[17] A solution of 26.3 g of 1-[2-(methoxymethylthio)ethyl]-3-carbamoylpyridinium chloride (0.1 mol) in 400 cm^3 of water-acetonitrile (1:1 v/v) was poured into a solution of $HgCl_2$ in 400 cm^3 of water-acetonitrile (1:1 v/v) to precipitate white solid. After 24 h, the solid was filtered and washed with acetonitrile and water. Thus obtained mercury-complex of the pyridinium salt was placed in 250 cm^3 of water and H_2S gas was bubbled through the water layer for 3 h. The resulted HgS was filtered off, and the filtrate was freeze-dried to give 1-(2-mercaptoethyl)-3-carbamoylpyridinium chloride in 40% yield after recrystallization from methanol–ether mixture. ^1H NMR in DMSO-d_6 (δ from TMS) 2.8—3.4 (m, 3H), 4.87 (t, 2H), 7.9—8.4 (m, 2H), 8.92 (s, 2H), 9.18 (dd, 1H), and 9.72 (s, 1H).

1-(2-Mercaptoethyl)-1,4-dihydronicotinamide (SHNAH). A solution of 1.01 g of SHNA$^+$ (5 mmol) in 90 cm^3 of water was added to a mixture of CH_2Cl_2 (400 cm^3) and buffered aqueous solution of sodium dithionite (21 g of $Na_2S_2O_4$, 37 g of $NaHPO_4 \cdot 2H_2O$, 150 cm^3 of 1 mol dm^{-3} aq-NaOH, and 150 cm^3 of water; pH=7.5) dropwise with vigorous stirring under an argon atmosphere. After 40 min, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined dichloromethane layer was washed with water and dried over sodium sulfate. The evaporation of the solvent from the dried solution gave 1-(2-mercaptoethyl)-1,4-dihydronicotinamide (SHNAH) as a yellow oil in 68.4% yield (0.63 g). UV in CH_3CN; λ_{max} 354 nm. When this compound was kept in a freezer at −20 °C overnight, an absorption at 303 nm appeared suggesting that the decomposition had begun under such conditions. ^1H NMR in $CDCl_3$ (δ from TMS) 1.40 (t, 1H), 2.70 (dt, 2H), 3.0—3.5 (m, 4H), 4.83 (dt, 1H), 5.4—5.9 (m, 3H), and 7.00 (d, 1H).

Kinetic Procedures. A kinetic solution was prepared under an atmosphere of argon and placed in a 1 cm UV cell which had a silicone rubber stopper. The compartment for UV cells was kept within ±0.05 °C of an appropriate temperature through the kinetic run.

As a general procedure, both sample and reference cells were filled with a solution of substrate (and magnesium or zinc perchlorate, when necessary) in acetonitrile to obtain a difference spectrum. Then, an acetonitrile solution of a

TABLE 4. ANALYTICAL DATA FOR NAD(P)H MODEL COMPOUNDS

Model	Mp(θ_m/°C) (decomp)	λ_{max}/nm in MeCN	Anal(%)[a]			^{1}H-NMR[b]
			C	H	N	
S2NAH	71	348	54.51 (54.29	7.13 7.21	14.13 14.37)	2.11 (s, 3H) 2.60 (t, 2H) 3.13 (m, 2H) 3.29 (t, 2H) 4.72 (dt, 1H) 5.55 (bs, 2H) 5.74 (dq, 1H)
S3NAH	oil	349				1.6—2.1 (m, 2H) 2.07 (s, 3H) 2.49 (t, 2H) 3.1—3.4 (m, 4H) 4.70 (dt, 1H) 5.55 (bs, 2H) 5.73 (dq, 1H) 6.98 (d, 1H)
S4NAH	oil	351				1.4—1.9 (m, 4H) 2.08 (s, 3H) 2.48 (t, 2H) 2.9—3.3 (m, 4H) 4.69 (dt, 1H) 5.58 (bs, 1H) 7.71 (dq, 1H) 6.97 (d, 1H)
S5NAH	oil	351				1.2—1.8 (m, 6H) 2.09 (s, 3H) 2.46 (t, 2H) 2.9—3.3 (m, 4H) 4.68 (dt, 1H) 5.47 (bs, 2H) 5.69 (dq, 1H) 6.98 (d, 1H)
S6NAH	70	351	60.87 (61.36	9.14 8.73	11.01 11.07)	1.0—1.9 (m, 8H) 2.08 (s, 3H) 2.48 (t, 2H) 2.8—3.3 (m, 4H) 4.70 (dt, 1H) 5.38 (bs, 2H) 5.70 (dq, 1H) 7.00 (d, 1H)
S8NAH	74	353	63.67 (63.77	9.17 9.30	9.82 9.92)	1.0—1.9 (m, 12H) 2.48 (s, 2H) 2.9—3.3 (m, 4H) 4.71 (dt, 1H) 5.46 (bs, 2H) 5.71 (dq, 1H) 7.01 (d, 1H)
O2NAH	91	347	59.29 (59.31	7.93 7.76	15.30 15.37)	3.0—3.6 (m, 9H) 4.71 (dt, 1H) 5.64 (bs, 2H) 5.77 (dq, 1H) 7.01 (d, 1H)
ONAH	57	351	71.18 (71.13	10.12 10.12	11.79 11.79)	0.87 (t, 3H) 0.8—1.8 (m, 12H) 2.9—3.3 (m, 4H) 4.70 (dt, 1H) 5.56 (bs, 2H) 5.72 (dq, 1H) 7.01 (d, 1H)

a) Numbers in parentheses are calculated values. b) δ from TMS in $CDCl_3$.

model compound was injected into the sample cell with a syringe to start the reaction. An appropriate concentration of the substrate (and the metal ion, when necessary) was chosen to keep at least 80-fold molarity of the substrate (and the metal ion) over the concentration of the reductant. For example, for the run in entry 9 in Table 1, the concentrations were: [**1d**]=6.59×10^{-2} mol dm^{-3}, [$Mg(ClO_4)_2$]=2.58×10^{-2} mol dm^{-3}, and [S8NAH]=3.0×10^{-4} mol dm^{-3}. It was confirmed

that the rates were first-order in the substrate, first-order in the reductant, and zero-order in the metal ion.

The kinetics was followed by observing the decrease in the intensity of absorption at around 400 nm from the reductant. The spectrometer was connected to an NEC PC-8001 computer and the intensity of absorption at appropriate times was monitored over 3 half-lives through the computer. One observed absorption was the average of 80 consecutive measurements. More than 30 points were monitored for a run. The data were automatically subjected to the least-squares treatment to give a pseudo-first order rate constant. The standard deviation and correlation coefficient for each run were better than 1% and 0.9999, respectively. More than 3 runs were averaged to obtain the rate constants listed in Table 1.

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