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Conformations and Rotational Barriers in NADH and NAD^+ Analogues. A Dynamic NMR and Molecular Mechanics Investigation

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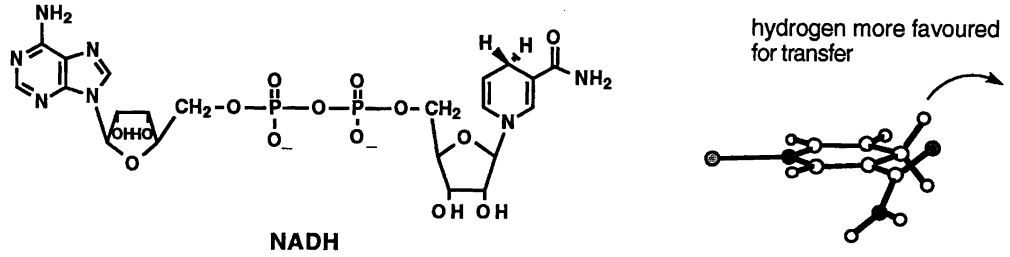
The conformational preferences and rotational barriers about both the ring–amide bond and the C–N bond in NADH and NAD^+ analogues have been investigated by dynamic NMR spectroscopy and molecular mechanics calculations with the intention to clarify the stereodynamic situation in these types of cross-conjugated amide. The results can be summarized as follows. (i) The primary amides an thioamides are planar or nearly planar and possess ring–amide barriers ($\Delta G^{\ddagger} \approx 4$–7 kcal mol^{-1}) over the 90° twisted state. (ii) The *cis* conformation is strongly preferred for NADH analogues, but in the indole analogue **6b** the two conformations differ by only ca. 0.3 kcal mol^{-1}. (iii) The tertiary amides are *twisted* in the ground state and have steric barriers ($\Delta G^{\ddagger}$ = 6–10 kcal mol^{-1}) over the planar state. (iv) The C–N barriers are considerably larger, ($\Delta G^{\ddagger}$ = 11–17 kcal mol^{-1} for amides, and 12–21 kcal mol^{-1} for thioamides). (v) The barrier heights are governed by the efficiency of the cross-conjugation in the ground and transition states, and by the steric interactions in the planar conformations. The exchange process observed in NMR for NADH by Redfield *et al.* is due to C–N rotation and not to amide group rotation.

The NADH/NAD^+ coenzyme systems are involved in many important biological reduction–oxidation reactions. Mechanistic studies of such enzyme-catalysed reactions cover both experimental and theoretical studies.[1,2] The reduction is usually described as a hydride transfer from the 4-position of the dihydronicotinamide moiety, although electron-transfer or charge-transfer mechanisms has been proposed in certain model systems.[3,4] In the transition state for such hydride-transfer reactions the dihydropyridine is believed to assume a flat boat conformation with the amide group twisted out of the mean plane of the ring such that the carbonyl oxygen is on the same side as the hydrogen that is transferred (Fig. 1).[5–7] This raises the question of the conformations and rotational barriers in NADH (**I**) and NAD^+ (**II**) and their analogues (Scheme 1).

Free NADH analogues are found to be predominantly *cis* in solution,[7] and in the crystalline state.[8,9] Diffraction studies, however, reveal preferred *trans* conformation and considerable boat puckering of NADH when bound to certain enzymes.[2,10] NAD^+ and model compounds are varyingly reported as planar and twisted by ca. 30°.[11] *Ab initio* computations with various basis sets of both reduced and oxidized analogues give *cis* planar as the most stable conformer in both NADH and NAD^+.[12] The barriers for type **II** rotation were calculated as 3.1–



NADH

Fig. 1. The structure of NADH and the proposed conformation of the amide group in the transition state for hydride transfer.

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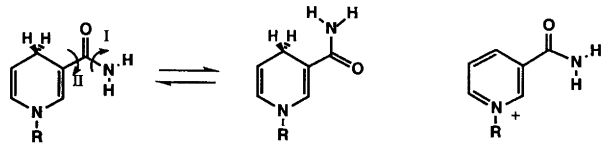


trans-I *cis*-I II

Scheme 1.

9.2 kcal mol^{-1} for 1-methylnicotinamide and 4.8–12.1 kcal mol^{-1} for the 1,4-dihydro derivative, depending upon the basis set, but the larger barrier was always found for the 1,4-dihydro derivatives.

Redfield and coworkers studied the conformations of NADH with proton NMR at various temperatures.[13,14] They assigned, tentatively, the observed symmetrical decoalescence of the amide protons to slow rotation of the bond joining the ring with the carboxamide group (process **II** in Scheme 1) rather than rotation of the C–N amide bond (process **I**). A similar interpretation was proposed by Fischer *et al.*[15] The behaviour of the decoalescence observed by Tropp and Redfield,[13] and comparison with similar vinylogous urea systems led us to believe that the observed process is actually the C–N rotation. The barrier height, $\Delta G^{\ddagger} = 13.3$ kcal mol^{-1}, is of the expected order of magnitude for such a cross-conjugated system. Furthermore, freezing of the C–N barrier implicates the symmetric decoalescence of only the NH protons, as was observed, whereas slow C–C rotation is expected to have profound influence on the nearby ring protons and lead to symmetrical decoalescence only for the special case of 1:1 population. The freezing of both of these rotations would unequivocally settle this dispute.

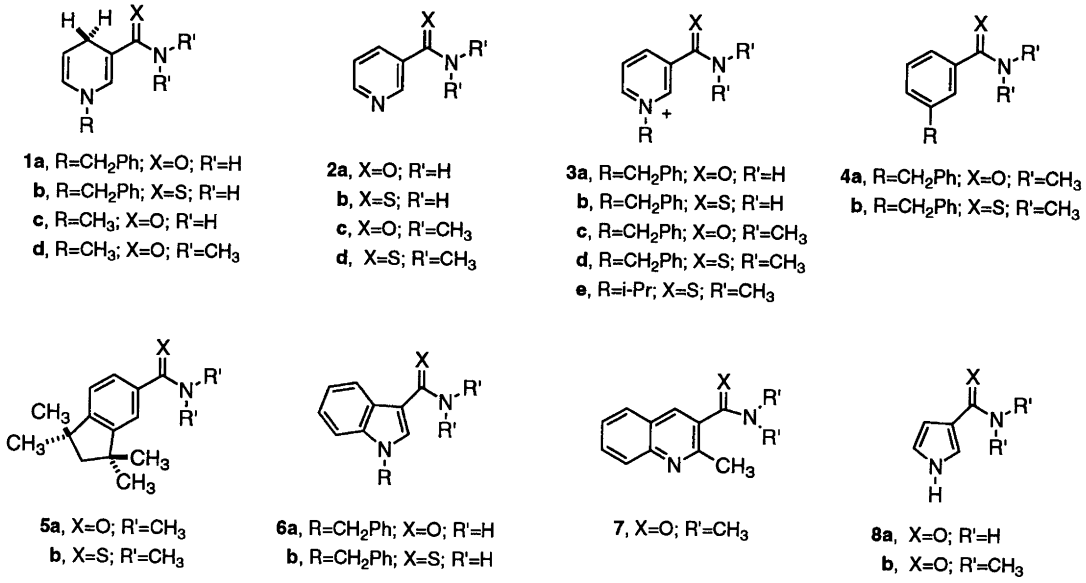
We have synthesized a number of NADH/NAD$^+$ analogues in order to shed light on the conformational situation from a static as well as a dynamic point of view (Scheme 2). The study includes thioamide and *N,N*-dimethyl derivatives, thus changing both steric and electronic conditions.

Results

Rotation around the bond joining the amide group with the (dihydro)pyridine ring. The first targets for our study were compounds **1–3**. Compounds **1a** and **1b** dissolved in various solvents were studied at temperatures down to $-150\,^{\circ}C$ by both ^1H and ^{13}C NMR spectroscopy. There was no indication of any selective broadening in this temperature interval due to rotation around the ring–amide bond. Since small chemical shift differences between the sites for all signals are very unlikely (see below), this behaviour is interpreted as either a biased conformational equilibrium ($\Delta G^{\circ} \geq 2.0$ kcal mol^{-1}) and/or as being due to low rotational barriers. It is known that the *cis* conformer predominates in NADH analogues in solution and biased conformer populations are also our preferred interpretation for **1a** and **1b**.

Nicotinamide salts **3a** and **3b** are expected to have very low barriers to rotation and are less soluble in solvents suitable for low-temperature NMR spectral experiments and little effort has been devoted to them.

N,N-Dimethyl substituted aromatic amides and thioamides are significantly twisted in both crystal[16] and solution.[17] If this is true also for our model compounds **3** and if these compounds have a substantial barrier through the planar state, then such a conformation can be unveiled by introducing a prochiral sensor such as the benzyl group into the molecule. Compound **3d** dissolved in [$^2H_4$]methanol showed selective broadening of the methylene signal below $-80\,^{\circ}C$ and the appearance of an AB quartet at $-95\,^{\circ}C$. Unfortunately, all signals were



1a, R=CH_2Ph; X=O; R'=H
b, R=CH_2Ph; X=S; R'=H
c, R=CH_3; X=O; R'=H
d, R=CH_3; X=O; R'=CH_3

2a, X=O; R'=H
b, X=S; R'=H
c, X=O; R'=CH_3
d, X=S; R'=CH_3

3a, R=CH_2Ph; X=O; R'=H
b, R=CH_2Ph; X=S; R'=H
c, R=CH_2Ph; X=O; R'=CH_3
d, R=CH_2Ph; X=S; R'=CH_3
e, R=i-Pr; X=S; R'=CH_3

4a, R=CH_2Ph; X=O; R'=CH_3
b, R=CH_2Ph; X=S; R'=CH_3

5a, X=O; R'=CH_3
b, X=S; R'=CH_3

6a, R=CH_2Ph; X=O; R'=H
b, R=CH_2Ph; X=S; R'=H

7, X=O; R'=CH_3

8a, X=O; R'=H
b, X=O; R'=CH_3

Scheme 2.

very broad at this temperature and the CH_2-signal was partly hidden in the solvent OH signal. At lower temperatures the sample crystallized. A barrier around 9.0 (0.5) kcal mol^{-1} could be estimated. Compound **3e** did not show any significant exchange broadening of the i-Pr methyl signals.

Thus, very little information was obtained from the most closely related analogues. In order to extend the accessible temperature range, the non-ionic compounds **4** were synthesized. No evidence of any rate process was, however, observed down to $-160°C$. Since we had earlier observed vanishing chemical shift differences in related systems we further modified the molecule as **5**, in which incorporation of the prochiral group in a cyclic system enhances the sensitivity.[18] Compound **5b** accordingly showed selective broadening and decoalescence of the methyl signals in both ^1H and ^{13}C spectra. Four resolved methyl signals were observed below $-116°C$ in the ^{13}C NMR spectrum. Bandshape analysis gave $\Delta G^{\ddagger} = 8.8$ kcal mol^{-1} at $-110°C$. Very similar behaviour was observed for **5a** but the signals were not resolved at the low temperature limit. Fig. 2 shows the methyl and methylene signals for both **5a** and **5b** at various temperatures. At $-163°C$, selective broadening of both methyl and methylene signals of **5a** was observed. From spectra run at both 300 and 500 MHz, a barrier of about 5.8(0.5) kcal mol^{-1} at $-163°C$ was estimated.

The most crucial experiment remained: how to design an acceptable analogue of NADH? The requirements that have to be met are (i) the steric interactions with the flanking groups in the *cis* and *trans* conformers should be as similar as possible in order to give a less biased equilibrium and (ii) the electronic effects should be as similar as possible to the vinylogous urea system of NADH. The indole derivatives **6** seem reasonably to meet these criteria.[19] The ambient temperature NMR spectrum of **6b** in [$^2H_6$]dimethyl ether–[$^2H_2$]dichloromethane (6:4) showed a broad signal near coalescence at δ 7.7 for the NH pro-

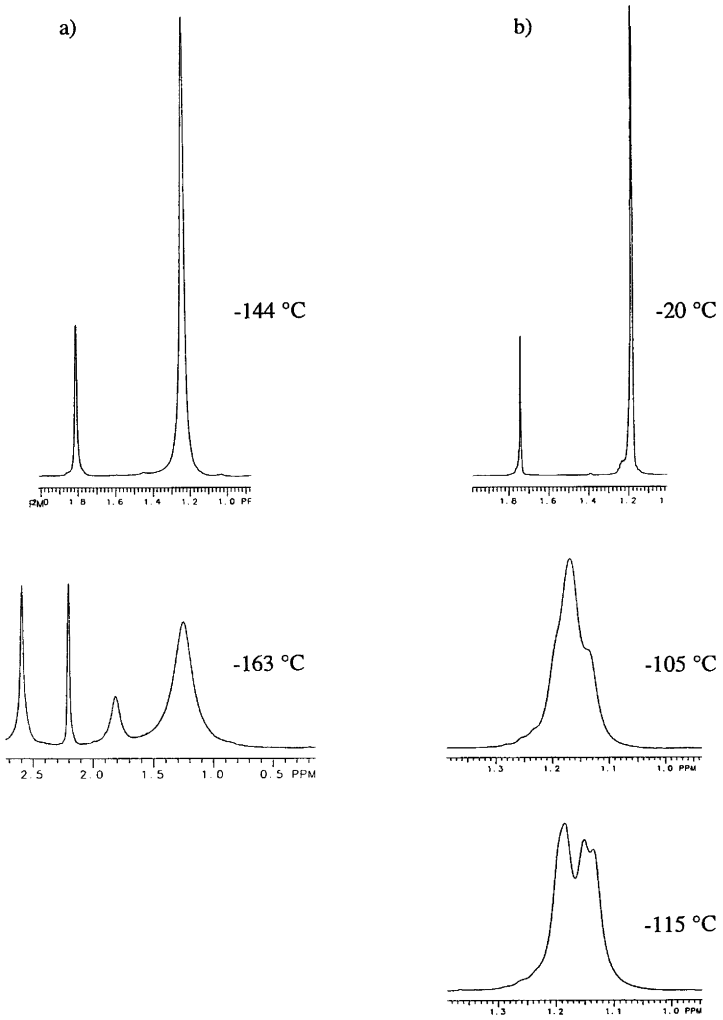


Fig. 2. Part of the ^1H NMR (300 MHz) spectra of **5a** (a) and **5b** (b). The methyl (δ 1.24) and methylene (δ 1.82) signals of **5a** are selectively broadened at $-163°C$ compared with the other signals, which originate from *N*-methyl (δ 2.60) and residual protons from the solvent ([$^2H_8$]toluene, δ 2.20).

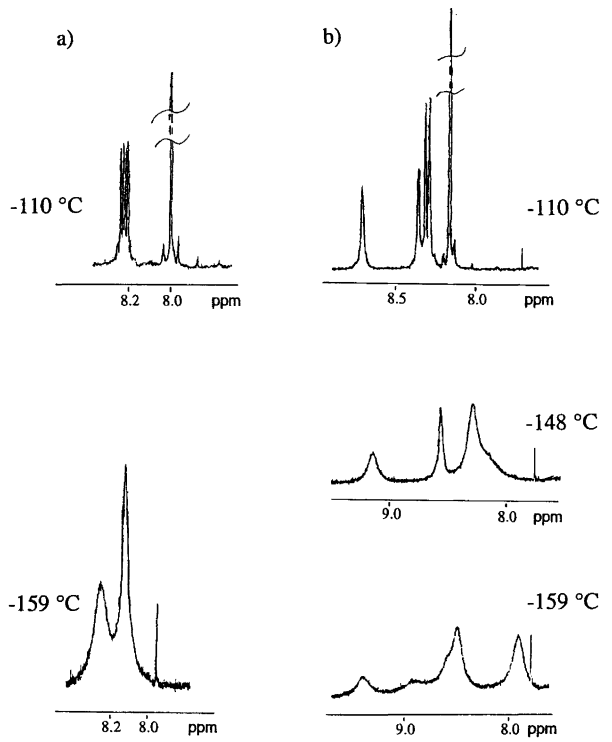


Fig. 3. Part of the ^{1}H NMR (300 MHz) spectra of **6a** (a) and **6b** (b).

tons, a singlet at δ 8.0 for the 2-proton (a doublet at −83 °C, J = 1.6 Hz) and a multiplet at δ 8.2 for the 4-proton. Below −120 °C all these signals selectively broadened and eventually decoalesce and at −163 °C giving rise to partly overlapping signals from two conformers with a population ratio of 76:24 (Fig. 3). Considering the temperature dependence of the chemical shift of the 2-H and 4-H signals we assign the major form to the *cis* conformer. The amide analogue **6a** underwent only weak broadening of the 2-H and 4-H signals but remained unresolved at −160 °C.

Rotation around the amide C–N bond. Rotational barriers around the C–N bond in compounds **1–6** are also given in Table 1. Both primary and tertiary amides and thioamides show a symmetrical decoalescence of the N–H or N–CH_3 signals and left all other signals unaffected as expected for the freezing of this rotation. The N–H protons of **3b** gave rise to two signals at ambient temperature. On raising the temperature the signals approached one another and merged to one signal below coalescence, thus making the determination of the rate constant impossible.

The effect of solvent polarity and hydrogen bonding was studied for the nicotinamides **2a** and **2c**. Toluene and chloroform have similar polarity but chloroform can act as a hydrogen-bond donor, whereas DMSO is highly polar and a hydrogen-bond acceptor.

Molecular mechanics computations. We have recently parametrized an MM2 force field for cross conjugated amides.[20] In order to be able to handle systems of the types shown in Scheme 2 the amide group must be incorporated into the π-electron SCF calculations generating torsional force constants (V_2) which are sensitive to

Table 1. Rate constants, free energies of activation for rotation and conformer populations in some NADH/NAD^+ analogues.

	Ar–$CXBR_2$				C–N		
Compound	Solvent[a]	p_{cis}	k/s^{-1} (T/K)	$\Delta G^{\ddagger}$/kcal mol^{-1}	Solvent[a]	k/s^{-1} (T/K)	$\Delta G^{\ddagger}$/kcal mol^{-1}
1a	A	(>0.98)	–	–	B	167 (239)	11.4
1b	K	(>0.98)	–	–	B	90 (251)	12.4
2a	–	–	–	–	H	400 (280)	13.0
2a	–	–	–	–	B	172 (296)	14.3
2a	–	–	–	–	G	270 (348)	16.6
2c	–	–	–	–	H	225 (311)	14.9
2c	–	–	–	–	B	58 (314)	15.9
2c	–	–	–	–	G	37 (314)	16.2
3a	–	–	–	–	G	87 (336)	16.8
3b	–	–	–	–	G	–	–[c]
3c	–	*b*	–	–	G	42 (336)	17.3
3d	C	*b*	–	[9.0 (5)]	G	90 (418)	21.0
5a	D	*b*	–	5.8 (5)	H	180 (290)	14.0
5b	E	*b*	11 (169)	8.9			
5b	H	*b*	8.5 (163)	8.7	H	135 (357)	17.5
6a	F	–	–	–	B	67 (249)	12.4
6b	I	0.78	600 (118)	5.2[d]	I	150 (298)	14.5
7	–	–	–	–	H	190 (348)	16.9

[a]Solvents: A, $(CD_3)_2O$–CD_3OD (7:3); B, $CDCl_3$; C, CD_3OD; $(CD_3)_2O$–[$^2H_8$]toluene (1:1); E, $(CD_3)_2O$; F, $(CD_3)_2O$–CD_3OD–CD_2Cl_2 (5:2:5); G, [$^2H_6$]DMSO; H, [$^2H_8$]toluene; I, $(CD_3)_2O$–CD_2Cl_2 (1:1); K, $CDCl_3$–$CHCl_2F$ (1:1). [b]Twisted in the ground state; conformers are enantiomers. [c]Vanishing chemical difference at T_c. [d]From minor to major conformer.

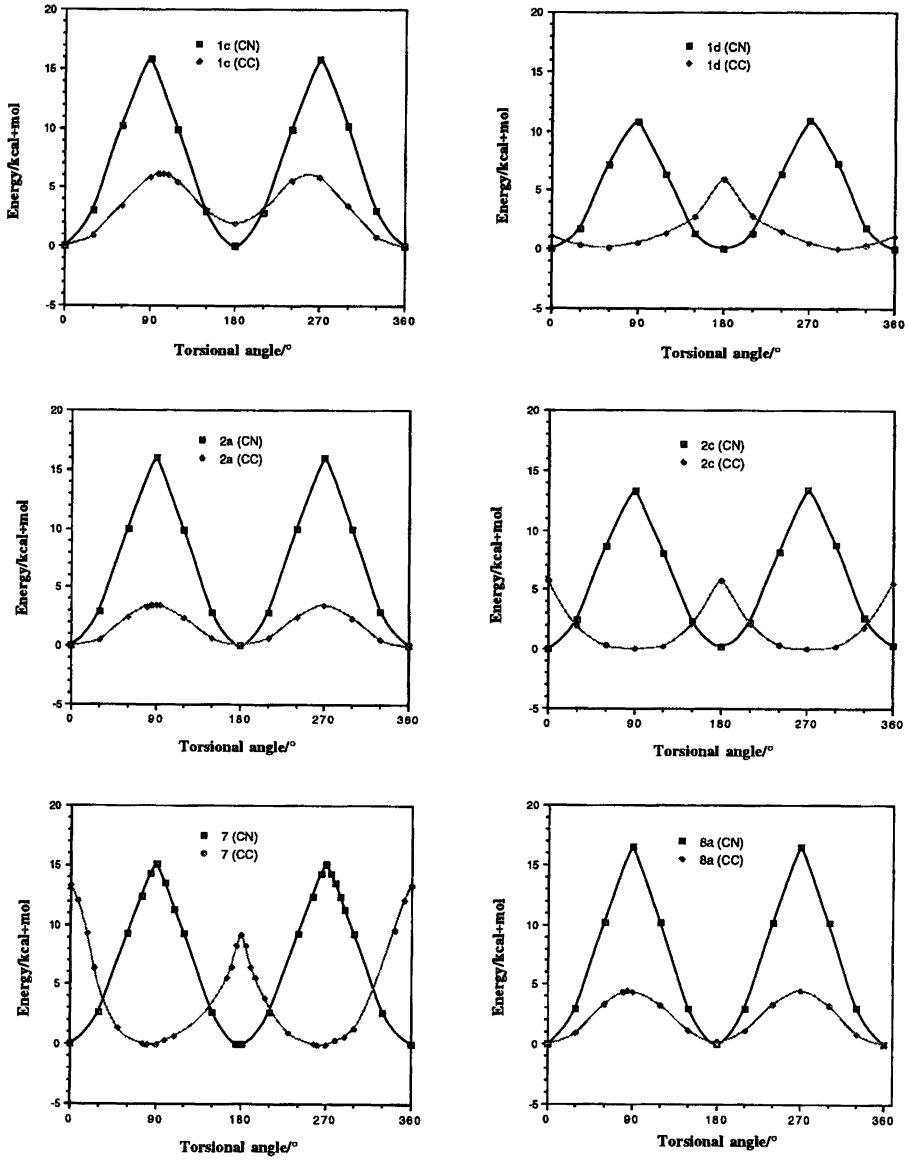


Fig. 4. MM2(91) energy profiles for rotation around the C–N and C–C bonds in some selected NADH/NAD^+ analogues. 1 kcal mol^{-1} = 4.184 kJ mol^{-1}.

the different degrees of cross conjugation. The torsional potential energy profiles in some selected examples have been calculated using the driver technique and are shown in Fig. 4. The calculated barriers are given in Table 2.

Table 2. Calculated barriers and relative conformer energies in some NADH/NAD^+ analogues.

Compound	$\Delta E^{\ddagger}$(C–C)/kcal mol^{-1} [a]	$\Delta E^{\ddagger}$(C–N)/kcal mol^{-1}	ΔE^{o}/kcal mol^{-1}	Comment
1c	6.1	15.8	1.9	*cis* more stable
1d	1.1	10.8	(0)	Enantiomeric conformers
2a	3.4	16.0	0.1	
2c	5.6	13.2	(0)	Enantiomeric conformers
7	9.2	15.1	(0)	Enantiomeric conformers
8a	4.4	16.5	0.2	
8b	1.8	12.9	0	Enantiomeric conformers

[a] Barriers refer to major→minor transition.

Discussion

Rotational barriers around C–N bonds in simple amides and thioamides have been extensively studied.[11] Substituent effects, solvent effects, acid catalysis, *etc.* have been examined by experimental as well as computational methods.[11] Thioamide barriers are correlated with corresponding amide barriers by eqn. (1).[21]

$$\Delta G^{\ddagger}(\text{thioamide}) = 1.11\Delta G^{\ddagger}(\text{amide}) + 1.13 \quad (1)$$

This relation reflects the larger electron demand of the thiocarbonyl group due mainly to less effective π-overlap between first- and second-row elements. We here show how one can make use of this property in two respects: (i) the π-barrier between the ring and the thioamide group is higher than in the corresponding amide and (ii) the greater rigidity of the thioamide group and, to some extent, the larger size of the sulfur atom lead to more severe steric interactions in the planar states.

Unfortunately, solubility problems and the large temperature range inevitably led to the use of several different solvents. This hampers a straightforward comparison of the barriers. An analysis of published rotational amide barriers in different solvents, both systematic studies, as for *N,N*-dimethylbenzamide,[22] and from various sources[11] shows that the barrier for tertiary amides increases with the polarity of the solvent roughly by 0.025ε (ε = dielectric constant). Hydrogen bonding is also known to influence the barrier, which should be kept in mind when the data shown in Table 1 are discussed.

Throughout the series it is obvious that the barrier to rotation around the (thio)amide C–N bond is much higher than the barrier to rotation around the aryl–(thio)amide bond, no matter whether the latter barrier reflects rotation over a 90° twisted or over a planar transition state. The tertiary amides are *twisted* in the ground state and have steric barriers ($\Delta G^{\ddagger}$ = 6–10 kcal mol^{-1}) over the planar state. The C–N barriers are considerably larger, ($\Delta G^{\ddagger}$ = 11–17 kcal mol^{-1} for amides, and 12–21 kcal mol^{-1} for thioamides). The primary amides and thioamides are *planar or nearly planar* and possess ring–amide barriers ($\Delta G^{\ddagger} \approx$ 4–7 kcal mol^{-1}) over the perpendicular aryl–amide state. The *cis*-**I** conformation indicated in Scheme 1 is strongly preferred for **1**, but in **6b** the two conformations differ by only ca. 0.3 kcal mol^{-1}. The barrier heights are governed by the efficiency of the cross-conjugation in the ground and transition states, and by the steric interactions in the planar conformations.

The results from the MM2 calculations are in very good agreement with the experimental results. Molecule **1c** is planar and the *cis* conformation 1.9 kcal mol^{-1} more stable than *trans*. The calculated gas-phase C–N and C–C barriers are 15.8 and 6.1 kcal mol^{-1}, respectively. This indicates that the experimental barrier, 11.4 kcal mol^{-1}, is probably affected by the proton exchange mechanism as well as rotation. Tropp and Redfield measured 13.3 kcal mol^{-1} for NADH in water–methanol solution.[13]

In the nicotinamide analogue, **2a**, the calculated *cis–trans* energy difference and the C–C barrier are smaller. The corresponding tertiary amide, **2c**, is twisted in the ground state, and an *o*-methyl substituent as in **7** causes the amide group to twist 80° and doubles the magnitude of the steric barrier over the planar transition state. Even so, the C–N barrier is ca. 5 kcal mol^{-1} higher than the C–C barrier.

Conclusion. The exchange process observed by NMR spectroscopy for NADH[13,14] is due to C–N rotation (and possible proton exchange) and not to aryl–amide rotation. In primary NADH analogues 2–4 kcal mol^{-1} are needed to twist the amide group into the conformation proposed for the transition state for hydride transfer. Aromatic tertiary amides, on the other hand, are twisted in the ground state, hence assuming the required conformation for hydride transfer, which could be used to test further the 'twisted hypothesis' and to explore in model studies.

Experimental

General. Flash chromatography was performed according to Taber.[23] Light petroleum (b.p. 60–70°C) and ethyl acetate for chromatography, were distilled before use. Melting points are uncorrected. The high resolution mass spectra [MS(hr)] were recorded on a Jeol SX 102 mass spectrometer using the direct inlet technique. ^{1}H NMR spectra were recorded on a Varian XL300 spectrometer: δ in ppm downfield from Me_4Si as an internal standard. Infrared (IR) spectra were measured as thin films on NaCl plates, or as KBr tablets. The purity of the products was assessed by TLC and ^{1}H NMR spectroscopy.

[$^2H_6$]Dimethyl ether, for low temperature NMR, was prepared from [$^2H_4$]methanol.[23] The samples containing [$^2H_6$]dimethyl ether or dichlorofluoromethane (Freon 21) as solvents were degassed by repeated cycles of freeze–pump–thawing before being sealed off under high vacuum.[24] The populations and rate constants were evaluated by visual fitting of the experimental spectra to spectra calculated by the McConnell formalism for uncoupled two-site exchange systems.[25–27] The evaluations of T_2 and δν values for bandshape calculations were performed as described previously.[28] Temperature calibration of the NMR spectrometer was performed with methanol and ethylene glycol according to the method described by van Geet.[29] Errors in $\Delta G^{\ddagger}$, ± 0.1 kcal mol^{-1}, are given with the assumption that the temperature could be determined to an accuracy of ± 1 K.[30]

Molecular mechanics calculations were performed using the MM2(91) force field implemented in the MacMimic program package.[31]

General procedure for the benzylation of pyridine derivatives. The pyridine amide and benzyl chloride or bromide were dissolved in acetone–DMF. When the reaction was complete, the crystals were filtered off and washed with acetone.

*1-Benzyl-3-carbamoylpyridinium chloride (***3a***).* Nicotinamide (2.04 g, 16.7 mmol) and benzyl chloride (2.0 ml, 17 mmol) were refluxed in 20 ml of acetone and 6 ml of DMF for 20 h. White crystals (1.56 g, 38% yield) were obtained: m.p. 228–230°C (lit.[32] 236°C); IR: ν 3382, 3784, 3142, 2933, 2842, 1694; 1643, 1583, 1509, 1492, 1437 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.73 (1 H, s), 9.32 (1 H, d), 9.00 (1 H, d), 8.72 (1 H, br s), 8.26–8.32 (1 H, m), 8.19 (1 H, br s), 7.44–7.61 (5 H, m), 5.93 (2 H, s).

*1-Benzyl-3-thiocarbamoylpyridinium chloride (***3b***).* Thionicotinamide (0.50 g, 3.6 mmol) and benzyl chloride (0.41 ml, 3.6 mmol) were dissolved in 5 ml of acetone and 2 ml of DMF. The reaction mixture was stirred at room temperature for 5 days. Yellow crystals (0.06 g, 6% yield) were obtained: m.p. 195–197°C; IR: ν 3247, 3050, 2935, 2850, 1646, 1630, 1583, 1510, 1494, 1457, 1438, 1405 cm^{-1}; ^1H NMR (DMSO-d_6): δ 10.63 (1 H, br s), 10.37 (1 H, br s), 9.71 (1 H, s), 9.24 (1 H, d), 8.88 (1 H, d), 8.17–8.22 (1 H, m), 7.44–7.61 (5 H, m), 5.92 (2 H, s).

*1-Benzyl-3-dimethylcarbamoylpyridinium bromide (***3c***).* *N,N*-Dimethylnicotinamide (2.02 g, 13.5 mmol) and benzyl bromide (1.6 ml, 14 mmol) were dissolved in 6 ml of acetone. The reaction mixture was stirred at room temperature for 19 h to give 2.33 g (54% yield) of white crystals: m.p. 147–154°C; IR: ν 3040, 3000, 2978, 2908, 1640, 1490, 1450, 1440, 1404 cm^{-1};^1H NMR (DMSO-d_6): δ 9.50 (1 H, s), 9.31 (1 H, d), 8.71 (1 H, d), 8.23–8.27 (1 H, m), 7.44–7.62 (5 H, m), 5.92 (2 H, s), 3.05 (3 H, s), 2.97 (3 H, s).

*1-Benzyl-3-dimethylthiocarbamoylpyridinium chloride (***3d***).* *N,N*-Dimethylthionicotinamide (0.48 g, 2.88 mmol) and benzyl chloride (0.34 ml, 3.0 mmol) were dissolved in 1.5 ml of acetone and refluxed for 16 h. The product separated as an oil, which crystallized after having been rigorously stirred with some additional acetone at room temperature. Yellow crystals (0.58 g, 69% yield) were obtained: m.p. 130–132°C; IR: ν 3050, 2995, 2930, 1626, 1581, 1532, 1493, 1451 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.42 (1 H, s), 9.20 (1 H, d), 8.56 (1 H, d), 8.14–8.19 (1 H, m), 7.44–7.60 (5 H, m), 5.88 (2 H, s), 3.54 (3 H, s), 3.24 (3 H, s).

*1-Benzyl-3-carbamoyl-1,4-dihydropyridine (***1a***)* was prepared according to Mauzerall and Westheimer:[33] m.p. 104–109°C (lit.[33] 120–122°C); IR: ν 3350, 3168, 2813, 1679, 1640, 1560, 1490, 1449, 1428 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.23–7.38 (5 H, m), 7.16 (1 H, d), 5.72–5.76 (1 H, m), 5.15 (2 H, br s), 4.75 (1 H, dt), 4.29 (2 H, s), 3.17–3.19 (2 H, m).

*1-Benzyl-3-thiocarbamoyl-1,4-dihydropyridine (***1b***)* was prepared according to Mauzerall and Westheimer[33] with the following modifications. The solution of sodium carbonate and sodium dithionite in water (10 ml) was flushed with gaseous nitrogen before the 1-benzyl-3-thiocarbamoylpyridinium chloride (0.12 g, 0.45 mmol) was added. The reaction was run in room temperature for 2 h, the yellow solid collected by filtration and washed with water to give 0.07 g (70% yield) of product: m.p. 88–92°C with decomposition; IR: ν 3395, 3273, 3162, 2800, 1669, 1617, 1576, 1491, 1442 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.03 (1 H, d), 7.24–7.43 (5 H, m), 6.54 (2 H, br s), 5.75–5.79 (1 H, m), 4.96–5.01 (1 H, m), 4.43 (2 H, s), 3.22–3.24 (2 H, m); MS(hr): 230.0878; calc. for $C_{13}H_{14}N_2S$: 230.0878.

*1-Isopropyl-3-dimethylthiocarbamoylpyridinium iodide (***3e***).* *N,N*-Dimethylthionicotinamide (0.33 g, 2.0 mmol), absolute ethanol (0.4 ml) and freshly distilled isopropyl iodide (0.25 ml, 2.5 mmol) were refluxed for 22 h. Light petroleum was added with stirring and an oil separated. The solvent was decanted off and the procedure was repeated for three times. Acetone was added with stirring and after a while crystals precipitated. The light yellow crystals were filtered off to give 0.13 g (19% yield) of product: m.p. 197–201°C (*n*-butanol), IR: ν 3080, 3045, 2994, 2925, 1621, 1582, 1534, 1483, 1449, 1404, 1391 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.25 (1 H, s), 9.13 (1 H, d), 8.53 (1 H, d), 8.13–8.18 (1 H, m), 5.03 (1 H,m), 3.55 (3 H, s), 3.25 (3 H, s), 1.62 (6 H, d).

3-Bromobenzophenone was prepared according to Koopal[34] with the following modifications. After 3 h the reaction was quenched with water. The mixture was extracted with chloroform. The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc). White crystals were obtained (94% yield): m.p. 75–77°C (lit.[34] 77°C); ^1H NMR ($CDCl_3$): δ 7.34–7.94 (9 H, m).

3-Bromodiphenylmethane was prepared from 3-bromobenzophenone (6.35 g, 24.3 mmol) according to Vogel.[35] The crude product was purified by column chromatography (light petroleum). A colorless liquid was obtained (2.93 g, 49% yield): ^1H NMR ($CDCl_3$): δ 7.09–7.34 (9 H, m), 3.92 (2 H, s).

3-Benzylbenzoic acid was prepared from 3-bromodiphenylmethane (2.93 g, 11.9 mmol) according to *Organic Syntheses*[36] with the following modifications. The halide solution was added over a period of 10 min and refluxing was maintained for 3 h. The crude product (1.40 g, 55% yield) was used in the next step without further purification: m.p. 102–104°C (lit.[37] 107–108°C); ^1H NMR ($CDCl_3$): δ 7.94–7.98 (2 H, m), 7.17–7.45 (7 H, m), 4.04 (2 H, s).

4-Methyl-4-phenyl-2-pentanone was prepared according to Hoffman[38] with the following modifications. Mesityl oxide (5.8 ml, 51 mmol) was added over a period of 10 min and the reaction was allowed to continue for 4 h. The reaction mixture was poured onto ice and extracted with diethyl ether. The combined organic phases were extracted with water and saturated $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The crude product was distilled *in vacuo* (b.p. 119–122°C/13 mmHg) to give 5.4 g (60% yield) of product: ^{1}H NM ($CDCl_3$): δ 7.17–7.39 (5 H, m), 2.74 (2 H, s), 1.79 (3 H, s), 1.42 (6 H, s).

2,4-Dimethyl-4-phenyl-2-pentanol was prepared from 4-methyl-4-phenyl-2-pentanone (4.28 g, 24.3 mmol).[39] The crude product was purified by column chromatography (light petroleum–EtOAc 4:1) to give 4.01 g (86% yield) of product: ^{1}H NMR ($CDCl_3$): δ 7.14–7.43 (5 H, m), 2.00 (2 H, s), 1.42 (3 H, s), 1.00 (3 H, s).

1,1,3,3-Tetramethylindane was prepared from 2,4-dimethyl-4-phenyl-2-pentanol (3.89 g, 20.2 mmol) according to Bogert and Davidson[40] with the following modifications. When the reaction was complete, it was poured into water and extracted with diethyl ether. The combined organic phases were extracted with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum) to give 2.72 g (77% yield) of product: ^{1}H NMR ($CDCl_3$): δ 7.10–7.22 (5 H, m), 1.91 (2 H, s), 1.30 (12 H, s).

5-Bromo-1,1,3,3-tetramethylindane was prepared from 1,1,3,3-tetramethylindane (1.02 g, 5.85 mmol) according to *Organic Syntheses*[41] with the following modifications. The mixture was cooled with an ice–water bath and the bromine was added over a period of 10 min. The reaction was allowed to stand for 6 h with cooling and for 18 h at room temperature. The reaction mixture was poured into water and extracted with ether. The combined organic phases were extracted twice with 3% NaOH, once with water, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum) to give 1.21 g (82% yield) of product: ^{1}H NMR ($CDCl_3$): δ 7.21–7.33 (2 H, m), 6.96–6.99 (1 H, m), 1.90 (2 H, s), 1.29 (6 H, s), 1.28 (6 H, s).

5-Carboxy-1,1,3,3-tetramethylindane was prepared from 5-bromo-1,1,3,3-tetramethylindane (1.19 g, 4.70 mmol) according to *Organic Syntheses*[37] with the following modifications. The halide solution was added over a period of 15 min and reflux was maintained for 22 h. The crude product (0.59 g, 58% yield) was used in the next step without further purification: ^{1}H NMR ($CDCl_3$): δ 7.87–7.99 (2 H, m), 7.19–7.22 (1 H, m), 1.96 (2 H, s), 1.55 (6 H, s), 1.53 (6 H, s).

1-Benzylindole-3-carbaldehyde was prepared from indole-3-carbaldehyde (4.86 g, 32.1 mmol) according to Kalir and Szara.[42] The crude product was recrystallized from methanol to give 5.70 g (75% yield) of product: m.p. 104–105°C (lit.[42] 113–114°C); ^{1}H NMR ($CDCl_3$): δ 10.01 (1 H, s), 8.31–8.35 (1 H, m), 7.72 (1 H, s), 7.16–7.39 (8 H, m), 5.36 (2 H, s).

1-Benzylindole-3-carboxylic acid was prepared from 1-benzylindole-3-carbaldehyde (5.94 g, 52.2 mmol) according to Papayan and Galstyan[43] with the following modifications. The crude product was treated with charcoal and recrystallised from methanol to give 3.34 g (53% yield) of product: m.p. 193–195°C (decomp.) (lit.[43] 193–194°C); ^{1}H NMR ($CDCl_3$): δ 8.24–8.27 (1 H, m), 7.95 (1 H, s), 7.16–7.37 (8 H, m), 5.36 (2 H, s).

Synthesis of carboxamides from carboxylic acids was performed according to *Organikum*[44] with the following modifications. Thionyl chloride was used in an amount sufficient to dissolve the acid. In the case of pyridine and indolecarboxylic acid chlorides at least 5 equivalents of the amine were used. The reaction mixture was poured into neutral ice–water.

N,N-*Dimethylnicotinamide* (**2c**). Nicotinic acid (5.01 g, 40.7 mmol) was treated according to the procedure above. The ice–water mixture was continuously extracted with chloroform until no more product could be detected by TLC in the water phase. The organic phase was dried with $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography (EtOAc–MeOH 4:1) to give 5.66 g (92% yield) of product: m.p. 35–39°C; ^{1}H NMR ($CDCl_3$): δ 8.65–8.69 (2 H, m), 7.75–7.79 (1 H, m), 7.34–7.38 (1 H, m), 3.14 (3 H, s), 3.02 (3 H, s).

3-Benzyl-N,N-*dimethylbenzamide* (**4a**). 3-Benzylbenzoic acid (0.68 g, 3.2 mmol) was treated according to the procedure above. The ice–water mixture was extracted with ether and the combined organic phases were dried with Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum–EtOAc 1:3) to give 0.66 g (86% yield) of a colorless oil: IR: ν 3055, 3023, 2922, 1715, 1625, 1580, 1492, 1477, 1448, 1389 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.16–7.33 (9 H, m), 4.00 (2 H, s), 3.08 (3 H, br s), 2.93 (3 H, br s); MS(hr): 239.1311; calc. for $C_{16}H_{17}NO$: 239.1310.

5-Dimethylcarbamoyl-1,1,3,3-tetramethylindane (**5a**). 1,1,3,3-Tetramethylindane-5-carboxylic acid (0.50 g, 2.3 mmol) was treated according to the procedure above. The ice–water mixture was extracted with ether and the combined organic phases were dried with Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum–EtOAc 1:3). White crystals (0.48 g, 85% yield) were obtained: m.p. 98–99°C; IR: ν 3039, 2946, 2857, 1617, 1604, 1571, 1501, 1482, 1443, 1395 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ

7.10–7.25 (3 H, m), 3.10 (3 H, br s), 3.01 (3 H, br s), 1.92 (2 H, s), 1.30 (12 H, s); MS(hr): 245.1774; calc. for $C_{16}H_{23}NO$: 245.1780.

1-Benzyl-3-carbamoylindole (**6a**). 1-Benzylindole-3-carboxylic acid (0.41 g, 1.6 mmol) was treated according to the procedure above. The ice–water mixture was extracted with chloroform and the combined organic phases were dried with Na_2SO_4, filtered and evaporated. The crude product was recrystallized with methanol–water to give 0.28 g (69% yield) of product: m.p. 168–169°C; IR: ν 3388, 3330, 3265, 3183, 3100, 1635, 1605, 1528, 1463, 1438, 1412, 1389 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.98–8.01 (1 H, m), 7.75 (1 H, s), 7.14–7.36 (8 H, m), 5.70 (2 H, br s), 5.33 (2 H, s); MS(hr): 250.1107; calc. for $C_{16}H_{14}N_2O$: 250.1106.

3-Dimethylcarbamoyl-2-methylquinoline (**7**) was made from 2-methyl-3-quinolinic acid hydrochloride salt[45] (0.38 g, 1.7 mmol) according to Miyano[46] with the following modifications. When preparing the potassium salt, 1 M potassium hydroxide was added until all crystals were dissolved. The potassium salt was covered with toluene, oxalyl chloride (1.2 ml, 14 mmol) was added and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo*. The acid chloride was treated according to the procedure above. The ice–water mixture was extracted with chloroform and the combined organic phases were dried with Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc–MeOH 5:1) to give 32 mg (9% yield) of product: ^1H NMR ($CDCl_3$): δ 8.01 (1 H, d), 7.96 (1 H, s), 7.68–7.78 (2 H, m), 7.48–7.53 (1 H, m), 3.18 (3 H, s), 2.87 (3 H, s), 2.69 (3 H, s); MS(hr): 214.1104; calc. for $C_{13}H_{14}N_2O$: 214.1106.

General procedure for the synthesis of thioamides from carboxamides. In a typical experiment, the amide (41 mmol) and Lawesson's reagent[47] (24 mmol) were dissolved in 50–100 ml of dry 1,2-dimethoxyethane and stirred at room temperature under a nitrogen atmosphere.

Thionicotinamide **2b** *from nicotinamide.* After 6 days, the reaction mixture was poured into ice–water. The mixture was made basic with concentrated NaOH and continuously extracted with chloroform for 24 h. The chloroform was removed *in vacuo* and the crude product recrystallized from water. Yellow needles were obtained (4.2 g, 74% yield): m.p. 186–188°C (decomp.) (lit.[48] 191.5–192.5°C); ^1H NMR (DMSO-d_6): δ 10.07 (1 H, br s), 9.71 (1 H, br s), 9.00 (1 H, d), 8.65–8.67 (1 H, m), 8.18–8.22 (1 H, m), 7.43–7.47 (1 H, m).

N,N-*Dimethylthionicotinamide* (**2d**). *N,N*-Dimethylnicotinamide (2.53 g, 16.8 mmol) was treated according to the procedure above. After 6 days, the reaction mixture was poured into ice–water. The mixture was made basic with concentrated NaOH and extracted with chloroform. The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc–MeOH 19:1). A yellow oil was obtained (2.46 g, 88% yield): ^1H NMR ($CDCl_3$): δ 8.56–8.58 (2 H, m), 7.66–7.70 (1 H, m), 7.28–7.33 (1 H, m), 3.61 (3 H, s), 3.21 (3 H, s).

3-Benzyl-N,N-*dimethylthiobenzamide* (**4b**). **4a** (0.63 g, 2.6 mmol) was used according to the procedure above. After two days, the reaction mixture was poured into ice–water and extracted with ether. The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum–EtOAc 3:2). A yellow oil was obtained (0.56 g, 83% yield): IR: ν 3079, 3054, 3010, 2923, 1596, 1579, 1512, 1492, 1477, 1449, 1424, 1403, 1388 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.32 (9 H, m), 3.98 (2 H, s), 3.58 (3 H, s), 3.12 (3 H, s); MS(hr): 255.1083; calc. for $C_{16}H_{17}NS$: 255.1082.

5-Dimethylthiocarbamoyl-1,1,3,3-tetramethylindane (**5b**). **5a** (0.22 g, 0.90 mmol) was treated according to the procedure above. After 20 h, the reaction mixture was poured into ice–water and extracted with ether. The organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum–EtOAc 3:1) and recrystallised from MeOH–water 7:3. Light yellow needles were obtained (0.15 g, 64% yield): m.p. 129–133°C; IR: ν 3025, 2949, 2920, 2859, 1570, 1517, 1482, 1460, 1447, 1394 cm^{-1}; ^{1}NMR ($CDCl_3$): δ 7.03–7.18 (3 H, m), 3.61 (3 H, s), 3.19 (3 H, s), 1.91 (2 H, s), 1.29 (12 H, s); MS(hr): 261.1563; calc. for $C_{16}H_{23}NS$: 261.1551.

1-Benzyl-3-thiocarbamoylindole (**6b**). **6a** (0.13 g, 0.52 mmol) and 10 ml of solvent were treated according to the procedure above. After 5 days, the reaction mixture was poured into ice–water. The mixture was made basic with concentrated NaOH and extracted with chloroform. The combined organic phases were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (light petroleum–EtOAc 1:1) and recrystallised from MeOH–water. Yellow crystals were obtained (0.09 g, 65%): m.p. 148–158°C; IR: ν 3457, 3290, 3162, 3094, 1592, 1519, 1466, 1450, 1437 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.14 (1 H, s), 7.93–7.35 (1 H, m), 7.16–7.38 (9 H, m), 5.35 (2 H, s), MS(hr): 266.0873; calc. for $C_{16}H_{14}N_2S$: 266.0878.

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