

NMR Detection of Isomers Arising from Restricted Rotation of the C-N Amide Bond of *N*-Formyl-*o*-toluidine and *N,N'*-bis-Formyl-*o*-tolidine[†]

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[†] Dedicated to Professor *Lutz F. Tietze* on the occasion of his 60th birthday

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Abstract: Full and unambiguous assignment of all ¹H- and ¹³C-NMR resonances of the isomers due to restricted C-N amide bond rotation of *N*-formyl-*o*-toluidine and *N,N'*-bis-formyl-*o*-tolidine in DMSO-*d*₆ is reported. The *cis*-isomer predominates in the equilibrium mixture of both compounds as 1D-NOE difference experiments show.

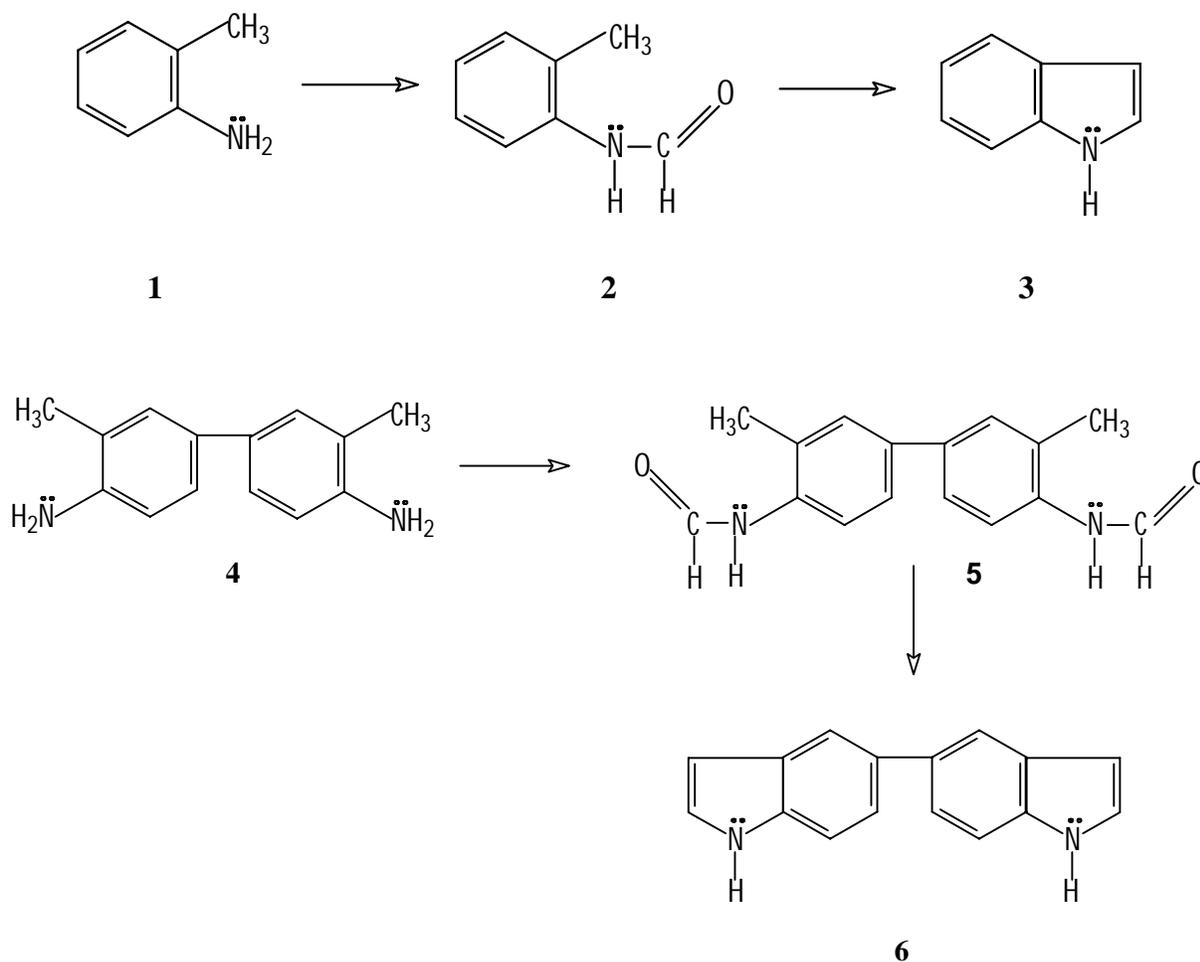
Keywords: Amide bond, restricted rotation, ¹H-NMR, ¹³C-NMR, NOE

Introduction

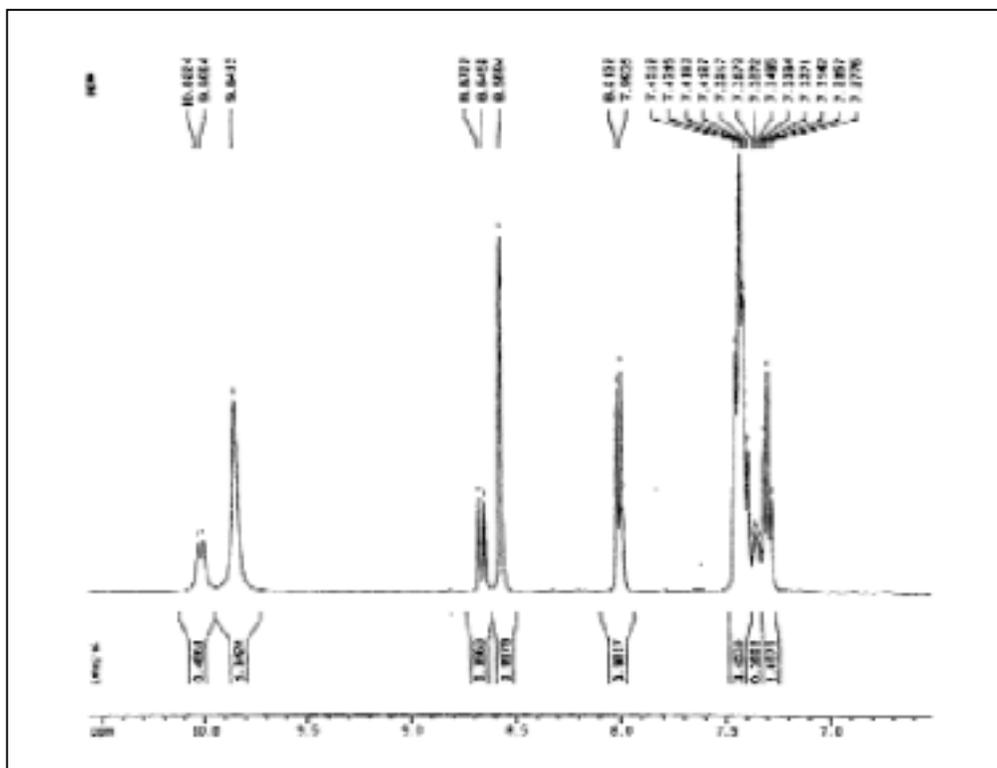
The Madelung indole synthesis is a method for producing these heterocycles via a base-catalyzed thermal cyclization of *N*-acyl-*o*-toluidides [1,2]. At the same time, it is one of the few known reactions by which an unsubstituted indole nucleus can be obtained [3-5]. Consequently, we decided to use this

reaction in order to obtain 5,5'-biindole as a starting material for the synthesis of indoloquinolizines with potential antitumor activity [6]. For our investigation we needed to synthesize *N*-formyl-*o*-toluidine (**2**), starting from *o*-toluidine (**1**), as well as *N,N'*-bis-formyl-*o*-toluidine (**5**), starting from *o*-toluidine (**4**), in order to transform them into indole (**3**) and to 5,5'-biindole (**6**), respectively (Scheme 1).

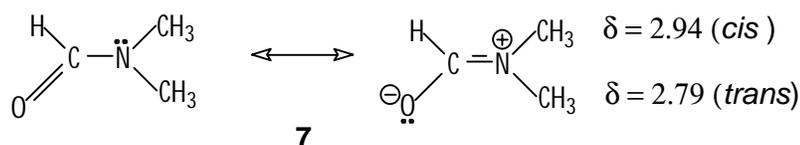
Scheme 1



During the spectroscopic characterization of the amides **2** and **5** we noted the presence of two isomers in solution (DMSO- d_6), observing double signals for the protons of the amine group and of the formyl group in the $^1\text{H-NMR}$ spectra, as well as double signals for all carbons of both compounds in the $^{13}\text{C-NMR}$ spectra. The ratio of the isomers, as deduced from the integration of the formyl proton signals, is approximately 3:1 in both compounds (Figures 1-2). It is known that in a few cases single bond rotation is so slow that *cis* and *trans* isomers can be isolated even where no double bond exists [7], e.g. in thioamides [8, 9] and in certain amides [10-16], because resonance gives the single bond some double bond character and slows rotation [17].

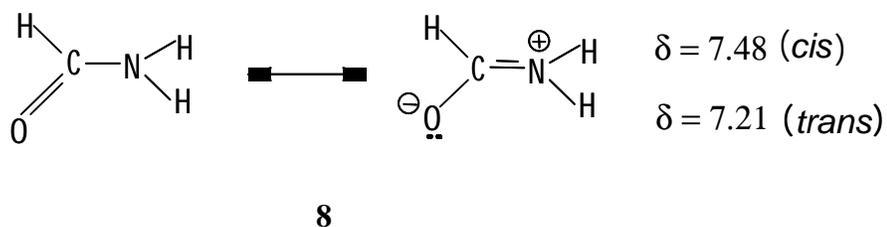
Figure 1. Aromatic part of the $^1\text{H-NMR}$ spectrum of **2** (in $\text{DMSO-}d_6$ solution)

Scheme 2



For example, in dimethylformamide (**7**, Scheme 2) the two methyl groups are non-equivalent due to the mentioned hindered rotation about the C-N bond. In the corresponding $^1\text{H-NMR}$ spectrum two methyl signals are found at $\delta = 2.79$ and 2.94 , together with a singlet at $\delta = 8.0$ for the formyl proton. If one saturates the methyl signal at $\delta = 2.94$, the intensity of the formyl proton signal increases by 18 %. When instead the other methyl signal is saturated, a decrease of 2 % is observed [18a]. In DMF the NOE experiments led to a correct assignment of the methyl signals. Since the signal of the isolated formyl proton is only enhanced when a particular one of the two methyl signals is saturated (the downfield one), this must correspond to the *cis* methyl group (*trans* to carbonyl group). Likewise, two signals for the methyl carbons are observed in the spectrum of $^{13}\text{C-NMR}$, at $\delta = 30$ and 36 , whereby the downfield signal corresponds to the methyl group, *trans* to the carbonyl group [19a].

Scheme 3



In the case of formamide (**8**, Scheme 3) the two protons on the nitrogen atom are also non-equivalent [19b]. Irradiating in the absorption frequency of ^{14}N , 3 quartets centered at $\delta = 8.21$, 7.48 and 7.21 are observed in the $^1\text{H-NMR}$ spectrum [20a, 21]. The downfield signal corresponds to the formyl proton, which presents two couplings of $J = 2.18$ and 13.49 Hz. These couplings correspond to those we expected for the *cis* N-H ($\delta = 7.48$, $J = 2.18$ and 2.60 Hz) with the formyl proton as well as for the *trans* N-H ($\delta = 7.21$, $J = 2.6$ and 13.49 Hz) with the formyl proton. Thus, the two protons on the nitrogen atom show a geminal coupling constant $J = 2.6$ Hz.

It is interesting to observe that the coupling constant of the *cis* protons in formamide is of smaller magnitude (2.18 Hz) compared to that of the *trans* protons (13.49 Hz), similar to what is seen in a typical double bond C=C [18b].

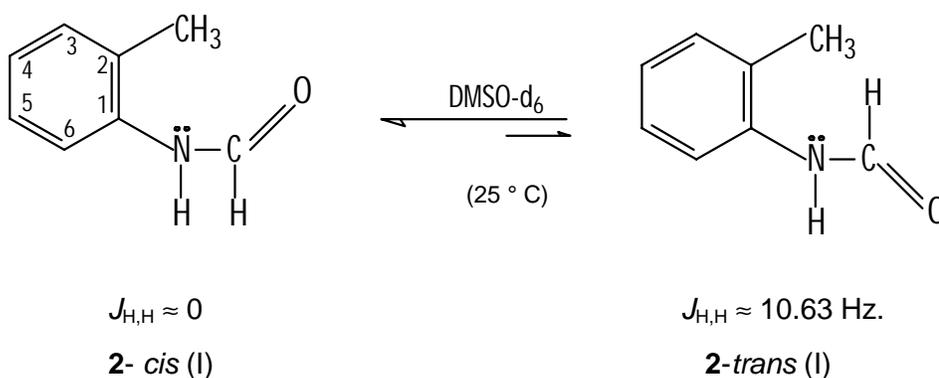
Based on the reasoning described, it is expected that for monosubstituted formamides, as is the case of the amides **2** and **5**, *cis* and *trans* isomers are present in solution which can be detected by way of NMR techniques. Thus, the purpose of our investigation was to assign in detail the configurations of

the isomers observed in DMSO- d_6 solutions of *N*-formyl-*o*-toluidine (**2**) and *N,N'*-bis-formyl-*o*-toluidine (**5**), by analyzing the spectra of ^1H - and ^{13}C -NMR, by running additional NOE experiments and using bidimensional spectra (COSY, HMQC, HMBC) for the full assignment of the signals.

Results and Discussion

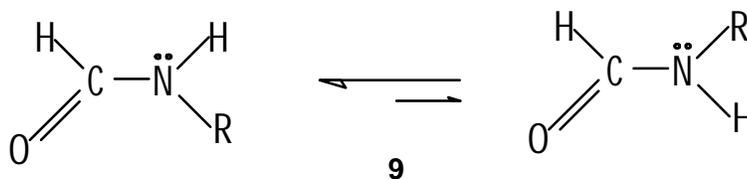
The preferred conformation solution of *N*-formyl-*o*-toluidine (**2**) in DMSO- d_6 is the *cis* one, based on the fact that the ^1H -NMR signals for the N-H and the formyl proton of the major isomer present appear as singlets at $\delta = 9.84$ and 8.57 respectively, i.e. with a coupling constant $J \approx 0$, whereas the signals corresponding to the minor isomer are present as doublets at $\delta = 10.01$ and 8.66 respectively, showing a coupling constant $J \approx 10.63$ Hz, i.e. in the latter isomer a coupling constant of greater magnitude is observed, which is in accordance with the observation made for formamide (minor coupling constant for the *cis* NH-CH protons and major coupling constant for the corresponding *trans* protons) [20a, 21].

Scheme 4



The aforementioned observation is congruent with the one made in a series of *N*-monoalkyl substituted formamides **9** (Scheme 5) in which in their NMR spectra (mainly ^{13}C) the *cis* isomer predominates up to 80% over the *trans* isomer [20b].

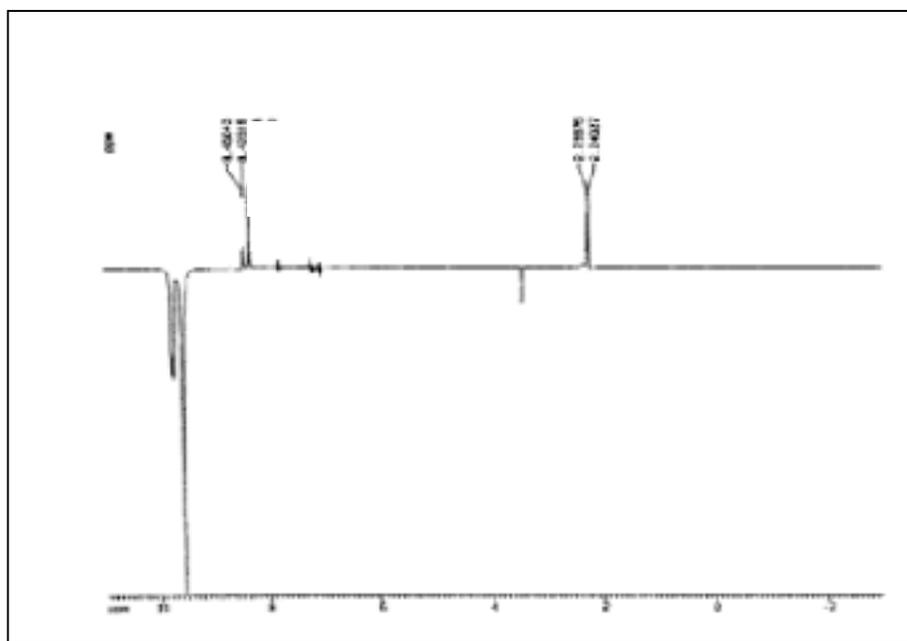
Scheme 5



R = methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl

In the case of the *N*-methyl, *N*-ethyl- and *N*-*tert*-butylformamide signals for the formyl protons of the *cis* isomer ($d, J = 0.3\text{--}2\text{ Hz}$) as well as of *trans* isomer ($d, J = 12\text{ Hz}$) could be clearly observed in the $^1\text{H-NMR}$ spectra (CDCl_3) which coincides again with the observation made for formamide. When analyzing the $^{13}\text{C-NMR}$ spectra of these ten monoalkylamides it can be seen that the signals of the carbonyl carbon and those of the first two carbons of the alkyl substituent of the *trans* isomer always appear downfield compared to the corresponding signals of the *cis* isomer. Additional information about the configuration of the isomers of **2** (and also of **5**) can be obtained with help of NOE experiments. Figures 3-4 show the NOE experiments, in which the N-H signals of both isomers of **2** and **5** are saturated.

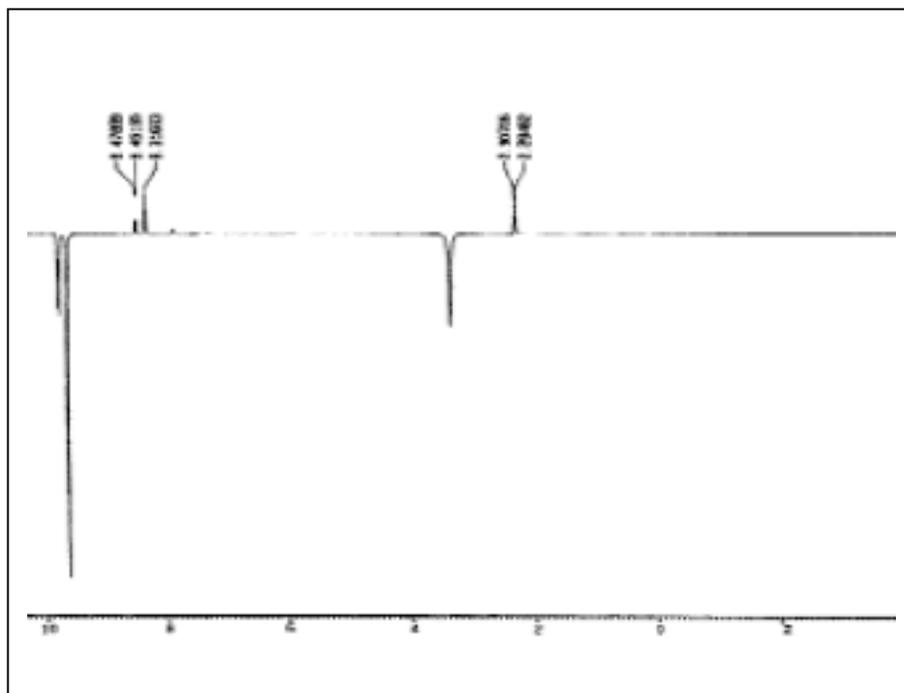
Figure 3. NOE Experiment on **2**.



In the signals of the formyl protons we observe an increase by approx. 11% for the singlet at $\delta = 8.57$ and by 8 % for the doublet at $\delta = 8.66$, which indicates precisely that the signals of N-H and the formyl proton of the isomer in a greater porportion represent protons closer to each other and therefore correspond to the *cis* isomer. Additional analysis of the $^1\text{H-NMR}$ spectra allows one to distinguish for *cis*-**2** a doublet at $\delta = 8.0$ ($J = 7.88\text{ Hz}$) for the proton in position 6 and a triplet at $\delta = 7.29$ ($J = 7.34\text{ Hz}$) for the proton in position 4. The two remaining aromatic protons of *cis*-**2** as well as all the aromatic protons of *trans*-**2** appear as a multiplet in the 7.40 to 7.35 ppm region.

The signal of the aromatic protons of **2** are found at lower field compared to those observed for *o*-toluidine ($\delta = 6.59\text{--}7.01$, CDCl_3 , 400 MHz) [20c]. This is due to the inductive effect (-I) of the amido group, which withdraws charge from the aromatic ring, in opposition to the amine group, which in the *o*-toluidine introduces charge into the aromatic ring by means of resonance.

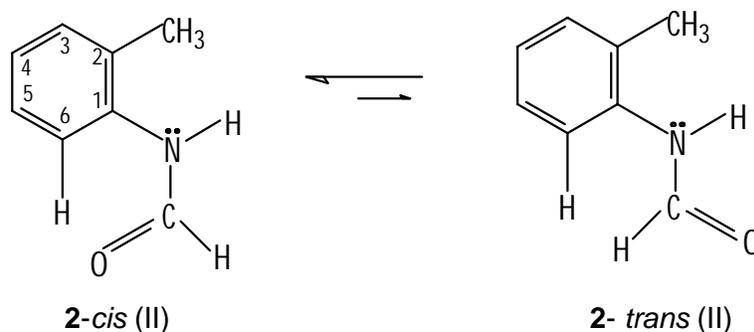
Figure 4. NOE Experiment on 5.



The effects of the substituents in the aromatic rings become normally apparent in the order $o > p > m$ and therefore it is expected that the proton in the o -position to the amido group in **2** (position 6) is at lower field compared to the remaining aromatic protons. In fact, in *cis*-**2**, this proton, 6-H, indeed appears at lower field compared to the others ($\delta = 8.00$), however, it is strange that the corresponding proton in *trans*-**2** is present at a higher field, in the multiplet at $\delta = 7.40-7.35$. The foregoing observation can be explained, if we assume that the isomers of **2** are really present in the conformations (II) indicated below (Scheme 6):

In these conformations (II) a steric hindrance between the formyl group and the methyl group of position 2 is avoided. However, a certain steric interaction between 6-H and the carbonyl group in *cis*-**2** (and which is not present in *trans*-**2**) can be the cause for the additional downfield displacement of this proton.

Scheme 6



The presence of the conformations II of **2** seems to be confirmed by the NOE experiment shown in Figure 3. When irradiating in the signals of the N-H of the two isomers of **2** an increase in intensity of the signals of the methyl groups is observed, which confirms a spacial proximity of the protons under discussion. In the ^{13}C -NMR spectrum it is possible to differentiate perfectly the signals of all carbons of both isomers of **2**. In accordance with the expectations, the signals of the isomer in lower proportion (*trans*-**2**) are present at lower field compared to those corresponding to the isomer in higher proportion (*cis*-**2**).

The definite assignment of the chemical shifts of protons and carbons of both isomers of **2** can be achieved by taking into account the bidimensional experiments H,H-COSY, HMQC, HMBC as well as DEPT 135. These assignments are shown in Tables 1 and 2.

Table 1. ^1H (400 MHz) and ^{13}C NMR (100MHz) Spectral Data for *cis*-**2** in DMSO- d_6

Position	δ_{H} , mult., J in Hz	δ_{C}	$^{\text{a}}$ DEPT 135	COSY ^1H - ^1H correlations	$^{\text{b}}$ HMQC $^1J_{\text{CH}}$	$^{\text{b}}$ HMBC (12Hz) $^3J_{\text{CH}}$
H-C=O	8.57, s	160.18	(+) CH	NH, 6-H	Formyl-H	-----
NH	9.84, s	NH	NH	Formyl-H	-----	-----
1	-----	135.73	(0) C_{q}	-----	-----	Formyl-H
2	-----	129.60	(0) C_{q}	-----	-----	3-H 5-H 4-H 6-H
2- CH_3	2.47, s	17.96	(+) CH_3	3-H	CH_3	
3	(7.45-7.33), m	130.62	(+) CH	4-H	3-H	5-H
4	7.30, t, 7.34	124.98	(+) CH	3-H	4-H	6-H
5	(7.45-7.33), m	126.33	(+) CH	6-H	5-H	3-H
6	8.00, d, 7.88	123.14	(+) CH	5-H, Formyl-H	6-H	4-H

a) DEPT shows CH, CH_2 , CH_3 , C_{q}

b) Correlation from C to the indicated hydrogens

Table 2. ^1H (400 MHz) and ^{13}C -NMR (100MHz) Spectral Data for *trans*-**2** in DMSO- d_6

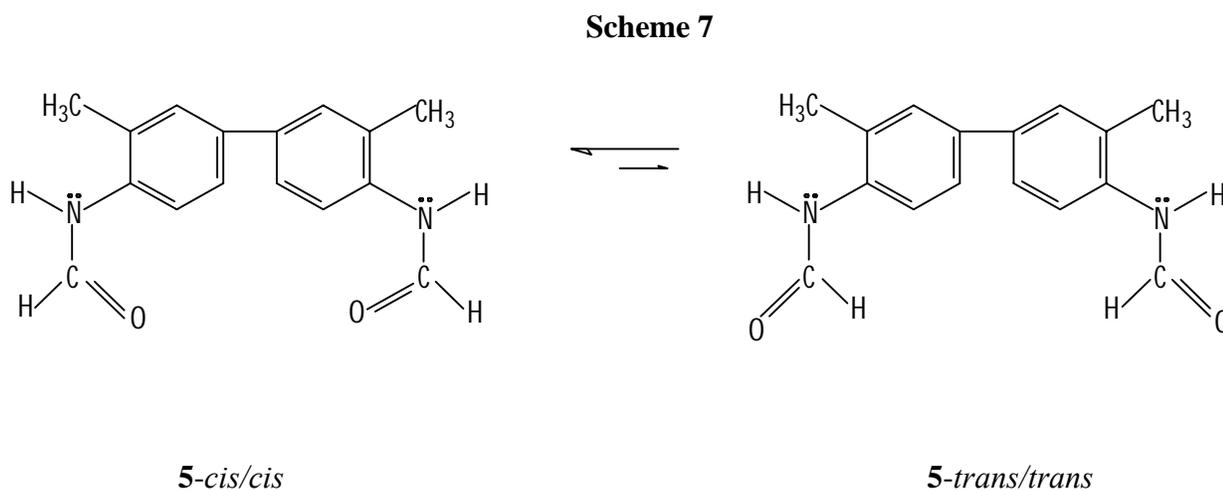
Position	δ_{H} , mult., J in Hz	δ_{C}	$^{\text{a}}$ DEPT 135	COSY ^1H - ^1H correlations
H-C=O	8.66, d, 10.63	163.95	(+) CH	NH
NH	10.01, d, 10.63	NH	NH	Formyl-H
1	-----	136.03	(0) C_{q}	-----
2	-----	129.60	(0) C_{q}	-----

Position	δ_H , mult., J in Hz	δ_C	^a DEPT 135	COSY ¹ H- ¹ H correlations
2-CH ₃	2.49, s	17.82	(+) CH ₃	-----
3	(7.45-7.33), m	131.02	(+) CH	-----
4	(7.45-7.33), m	125.63	(+) CH	-----
5	(7.45-7.33), m	126.99	(+) CH	-----
6	(7.45-7.33), m	122.09	(+) CH	-----

a) DEPT shows CH, CH₂, CH₃, C_q

b) Correlation from C to the indicated hydrogens

In a similar way we found that in a solution of *N,N'*-bis-formyl-*o*-tolidine in DMSO-*d*₆ the isomer with the *cis/cis* conformation (Scheme 7) predominates as NOE experiments are showing (see above, Figure 4).



The assignment of the chemical shifts of protons and carbons of the two isomers of **5** are shown in Tables 3 and 4 (due to the symmetry of **5**, we refer only to positions 1 to 6, like **2**).

Table 3. ¹H(400 MHz) and ¹³C NMR (100MHz) Spectral Data for **5-cis** in DMSO-*d*₆

Position	δ_H , mult., J in Hz	δ_C	^a DEPT 135	COSY ¹ H- ¹ H correlations	^b HMQC ¹ J _{CH}	^b HMBC (12Hz) ³ J _{CH}
H-C=O	8.31, s	159.84	(+) CH	NH	Formyl-H	NH
NH	9.62, s	NH	NH	Formyl-H	-----	-----
1	-----	134.87	(0) C _q	-----	-----	Formyl-H 3-H, 5-H
2	-----	129.40	(0) C _q	-----	-----	6-H
2-CH ₃	2.29, s	17.93	(+) CH ₃	3-H	CH ₃	3-H

Position	δ_H , mult., J in Hz	δ_C	^a DEPT 135	COSY ¹ H- ¹ H correlations	^b HMQC ¹ J_{CH}	^b HMBC (12Hz) ³ J_{CH}
3	7.52, s	128.22	(+) CH	2-CH ₃	3-H	5-H
4	-----	135.69	(0) C _q	-----	-----	6-H
5	7.45, d, 8.06	124.00	(+) CH	6-H	5-H	3-H
6	7.85, d, 8.30	122.86	(+) CH	5-H	6-H	NH

a) DEPT shows CH, CH₂, CH₃, C_q

b) Correlation from C to the indicated hydrogens

Table 4. ¹H(400 MHz) and ¹³C NMR (100MHz) Spectral Data for **5-trans** in DMSO-*d*₆

Position	δ_H , mult., J in Hz	δ_C	^a DEPT 135	COSY ¹ H- ¹ H correlations	^b HMBC (12Hz) ³ J_{CH}
H-C=O	8.46, d, 10.77	163.55	(+) CH	NH	-----
NH	9.78, d, 10.77	NH	NH	Formyl-H	-----
1	-----	136.51	(0) C _q	-----	5-H
2	-----	130.61	(0) C _q	-----	-----
2-CH ₃	2.29, s	17.81	(+) CH ₃	-----	-----
3	7.52, s	128.68	(+) CH	-----	-----
4	-----	134.96	(0) C _q	-----	-----
5	7.27, d, 8.08	124.56	(+) CH	6-H	-----
6	7.45, d, 8.06	122.03	(+) CH	5-H	-----

a) DEPT shows CH, CH₂, CH₃, C_q

b) Correlation from C to the indicated hydrogens

Conclusions

We have presented the complete ¹H- and ¹³C-NMR chemical shifts of the *cis* and *trans* isomers of *N*-formyl-*o*-toluidine (**2**) and *N,N'*-bis-formyl-*o*-toluidine (**5**) in DMSO-*d*₆. The two isomers are formed in solution due to restricted C-N-amide bond rotation and the *cis*-isomer predominates in the equilibrium mixture.

Acknowledgments

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Experimental

General

NMR spectra were recorded in DMSO- d_6 at 25°C on a Bruker DPX400 spectrometer operating at 400.13 MHz for ^1H , and 100.61 MHz for ^{13}C , using the solvent signals as internal references. Thin layer chromatography (TLC) was performed on precoated plates (Aldrich TLC aluminium backed sheets coated with silica 60F₂₅₄) with detection by UV light. FTIR spectra were taken on a Perkin-Elmer spectrometer using potassium bromide pellets. Mass spectrometry was determined on a GC Perkin-Elmer Autosystem and a Perkin-Elmer Turbomass mass spectrometer.

N-formyl-*o*-toluidine (**2**) [4]. *o*-Toluidine (43 g, 0.4 mol) and 90% (w/w) formic acid (15 mL, 0.4 mol) were mixed together in a 100 mL round-bottomed flask fitted with a reflux condenser, and the mixture was heated on a boiling water bath (96-98 °C) for 3 hours. The reflux condenser was replaced by a Claisen still-head and an air condenser arranged for distillation under reduced pressure, and the product was distilled using a water pump. At 40 mm Hg and 42 °C a first fraction consisting of a mixture of water and formic acid was collected, then unreacted *o*-toluidine (130 °C) and finally (199 °C) the *N*-formyl-*o*-toluidine was collected as a pale yellow oil, which solidified on cooling. The yield was 45.5 g (84 %). M.p. 53 °C. TLC: $R_f = 0.61$ (Kieselgel; benzene-chloroform, 2:1). IR ν_{max} : 3256, 3208, 3045, 2880, 1667 (C=O), 1591, 1553, 752 (aromatic ring) cm^{-1} ; GC-MS: ($R_t = 5.37$ min) m/z 135 $[\text{M}]^+$, 106 (100%) $[\text{M}-29]^+$, 77 (calcd. for $\text{C}_8\text{H}_9\text{NO}$, 135.16).

N,N'-bis-formyl-*o*-toluidine (**5**). *o*-Toluidine (5 g, 0.024 mol) and 90% (w/w) formic acid (25 mL, 0.65 mol) were mixed together in a 50 mL round-bottomed flask fitted with a reflux condenser and the mixture was heated on a boiling water bath (96-98 °C) for 1 hour. After cooling the precipitate formed was filtered off, washed with cold water and dried in a vacuum desiccator over CaCl_2 . The yield was 4.87 g (77 %) of a white solid. M.p. 265-270 °C; TLC: $R_f = 0.44$ (Kieselgel, benzene-acetone, 3:1); IR ν_{max} : 3241, 3030, 2883, 1668 (C=O), 1595, 1533, 765 (aromatic ring) cm^{-1} ; GC-MS: ($R_t = 5.19$ min) m/z 253 $[\text{M}-15]^+$, 135, 106 (100%), 77 (calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2$, 268.31).

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Sample Availability: samples of compounds **2** and **5** are available from MDPI