PYRIDAZINES 47.[1] THE CONFIGURATION OF NOVEL THIOSEMICARBAZONE DERIVATIVES OF PYRIDAZINECARBALDEHYDES AND ALKYL PYRIDAZINYL KETONES

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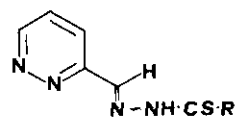
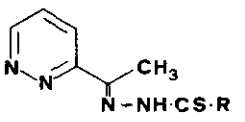
Abstract - Structure and configuration of thiosemicarbazone derivatives **1-6**, containing a 3-pyridazinyl (**1,2,3**), 4-pyridazinyl (**4,5**) or 2-pyridyl moiety (**6**) were determined by means of ^{1}H and ^{13}C nmr spectroscopy.

INTRODUCTION

Thiosemicarbazones (TSCs) derived from various N-heteroaromatic carbaldehydes and ketones, in particular pyridine derived TSCs, represent an interesting class of bioactive compounds due to antiviral,[2] antibacterial,[3] antimalarial,[4] antileucemic[5] and antineoplastic[6] activities observed in this series. Recently, we reported on the synthesis of a variety of related pyridazine-derived TSCs of types **1-5** and on investigations of their antiviral activity.[7] The present article is devoted to spectroscopic studies which were undertaken in order to confirm the structure and to elucidate the configuration of these compounds and of some of their 2-pyridine analogues.



1a-e



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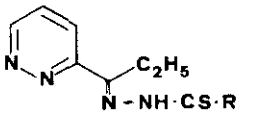

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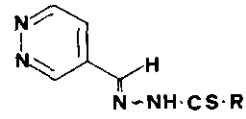

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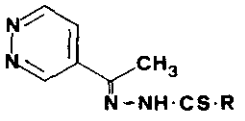

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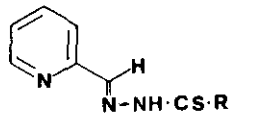
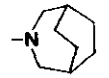
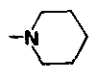


6a,b

1-5	a	b	c	d	e
R	$-SCH_3$				

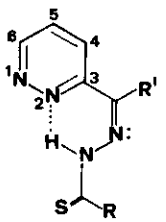
6	a	b
R		

Scheme 1

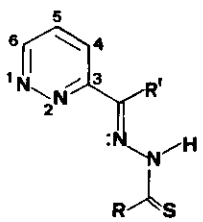
RESULTS AND DISCUSSION

Thiosemicarbazones derived from 3-pyridazinecarbaldehyde and alkyl 3-pyridazinyl ketones (1,2,3)

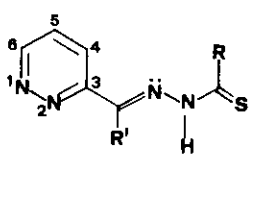
According to the ^1H nmr spectra (Table 1), several of the TSCs containing the 3-pyridazinyl moiety, namely compounds **1d**, **2c**, **2d**, **3a**, **3b**, **3c**, **3d** are mixtures of E- and Z-isomers. The most remarkable differences regarding chemical shifts of corresponding protons in the two isomeric forms were observed for the resonance signals attributable to the N-H protons: one species with δ(NH)=14-15 ppm, the other with δ(NH)=9.5-12.9 ppm, respectively. Previously it has been shown by Grifantini and co-workers[8] in the 2-pyridine and 1-isoquinoline series that in the spectra of Z-TSCs, there is an extreme downfield shift of the N-H signal (δ~15 ppm) due to intramolecular hydrogen bonding. Considering these findings, we assign E-configuration to the predominant isomers with the N-H resonance at higher field. From the integration of the peak areas, an E/Z-ratio of ≥ 9:1 can be concluded. By contrast, the spectra of TSCs **1b**, **1c**, **1e**, **2b**, **2e**, and **3e** exhibit signals of only one isomeric form. Since the signal of the N-H proton in these spectra appears in the range of 9.5-11.5 ppm, E-configuration is assigned to these compounds.



Z



E



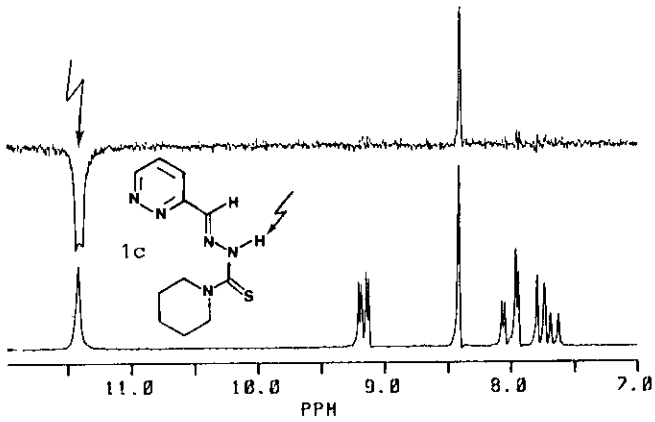
E

1 R'=H 2 R'=CH_3 3 R'=CH_2CH_3

Scheme 2

These results are further supported by NOE-difference experiments performed with some typical model substances. Thus, for instance, irradiating the methyl resonance of the main isomer of **2d** or the formyl-H transition of **1c**, respectively, enhanced the corresponding N-H signals. In reverse experiments a positive NOE on the lines mentioned above was detected when the N-H transitions were irradiated

(Figure 1). This through-space connection between the N-H proton and the methyl or formyl protons is only possible in the E-configurated species, where the involved protons are spatially close (see Scheme 2). In accordance, on irradiation of the pyridazine H-4 resonance in compound **1c** or **2d** the corresponding N-H signals remained unaffected. Employing such NOE-difference series, it was also possible to prove that compounds **1a** and **2a**, (the ^{1}H nmr spectra of which show signals of only one isomeric form) as well as the major isomer of **3a** are present in the E-configuration. In the case of these compounds the chemical shift of the N-H signal (12.8-13.6 ppm) does not permit the unequivocal assignment of configuration.



NOE-difference spectrum of **1c** obtained on irradiation of the N-H proton

80 MHz ^{1}H nmr spectrum of **1c** (range: 7 - 12 ppm)

Figure 1

Comparison of the ^{1}H nmr data (Table 1) of all E/Z- pairs shows a downfield shift for the pyridazine protons H-5 and H-6 in the Z-form compared to the signals of the E-isomer, whereas the pyridazine proton in *o*-position to the side-chain (H-4) is more shielded in the Z-form. These findings are in good agreement with observations in the 2-pyridine series[8] and can be explained as follows: The relative deshielding of H-5 and H-6 in the Z-isomers is consistent with a withdrawal of electron density from the pyridazine ring *via* the ring nitrogen atom N-2, which is involved in hydrogen bonding. On the other hand, the relative upfield shift of pyridazine H-4 in the Z-isomers may be interpreted in terms of anisotropy of the sp^2-hybridized exocyclic nitrogen atom, which comes close to the pyridazine H-4 in one of the possible conformations of the E-isomer (Scheme 2). In the Z-form the stereochemistry prevents such an interaction and consequently in this isomer the pyridazine H-4 is relatively more shielded.

Table 1: ^{1}H Nmr chemical shifts (ppm, DMSO-d_6) for thiosemicarbazones containing the 3-pyridazinyl moiety (1,2,3)

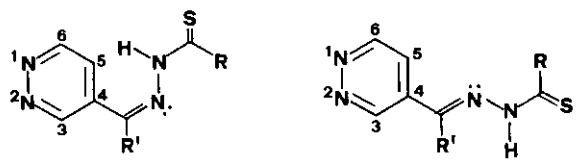
Comp.	pyridazine-H** H-4	H-5	H-6	formyl-,methyl- or ethyl-H	H of R	N-H
E-1a*	8.04	7.75	9.21	8.47	2.53	13.56
E-1b#	8.05	7.72	9.18	8.47	3.77(2',5'), 1.91(3',4')	11.44
E-1c*	7.99	7.72	9.16	8.41	3.87(2',6'), 1.62(3',4',5')	11.43
E-1d#	8.02	7.73	9.17	8.53	3.91(2',7'), 1.77(3',6'), 1.65(4',5')	11.27
Z-1d#	-+	-+	9.28	7.66	-+	14.84
E-1e#	8.00	7.71	9.16	8.43	4.03(2',4'), 2.09(1',5'), 1.67(6',7', 8',9')	11.20
E-2a*	8.21	7.77	9.26	2.53	2.57	12.75
E-2b#	8.19	7.72	9.21	2.50	3.78(2',5'), 1.91(3',4')	9.80
E-2c*	8.13	7.70	9.19	2.48	3.84(2',6'), 1.61(3',4',5')	9.89
Z-2c*	8.01	7.97	9.30	2.40	-+	14.51
E-2d*	8.16	7.72	9.19	2.50	3.90(2',7'), 1.77(3',6'), 1.51(4',5')	9.64
Z-2d*	8.03	7.98	9.30	2.42	-+	15.01
E-2e#	8.15	7.71	9.21	2.51	4.06(2',4'), 2.08(1',5'), 1.69(6',7', 8',9')	9.84
E-3a*	8.16	7.73	9.23	3.18(CH_2) 1.07(CH_3)	2.51	12.86
Z-3a*	8.07	7.94	9.32	2.80(CH_2) 1.16(CH_3)	2.49	14.86
E-3b*	8.16	7.70	9.19	3.12(CH_2) 1.09(CH_3)	3.75(2',5'), 1.89(3',4')	9.85
Z-3b*	8.09	7.97	9.30	2.82(CH_2) 1.16(CH_3)	-+	14.49
E-3c*	8.10	7.69	9.17	3.10(CH_2) 1.07(CH_3)	3.83(2',6'), 1.59(3',4',5')	9.95
Z-3c*	8.05	7.95	9.28	2.78(CH_2) 1.13(CH_3)	-+	14.11
E-3d#	8.16	7.79	9.21	3.13(CH_2) 1.10(CH_3)	3.93(2',7'), 1.78(3',6'), 1.56(4',5')	9.70
Z-3d#	-+	-+	9.32	2.85(CH_2) 1.19(CH_3)	-+	14.81
E-3e#	8.13	7.73	9.21	3.13(CH_2) 1.12(CH_3)	4.07(2',4'), 2.09(1',5'), 1.69(6',7', 8',9')	10.30

* 400 MHz spectrum
\# 80 MHz spectrum
\+ overlap with signals of the predominant E-isomer
** typical coupling constants: E-isomers: ^3J(H-4, H-5): 8.7 Hz; ^4J(H-4, H-6): 1.7 Hz; ^3J(H-5, H-6): 4.9 Hz
Z-isomers: ^3J(H-4, H-5): 8.8 Hz; ^4J(H-4, H-6): 1.5 Hz; ^3J(H-5, H-6): 5.0 Hz

The ^{13}C nmr data for compounds **1-3** are summarized in Table 4. Assignments were made by several methods. The signals of the quaternary C-atoms could be easily identified using the J-modulated spin echo technique[9] (decoupler switch-off delay τ = 7 ms for an average $^{1}J(^{13}C,^{1}H)$ coupling constant of 143 Hz) and NOE considerations. In addition to information obtained from the ^{1}H coupled spectra, the assignment of the pyridazine C-resonances was achieved by comparison with the chemical shift values of model pyridazine compounds.[10] The appearance of C=S resonances between 179-202 ppm unequivocally excludes that in DMSO-d_6 solution the TSCs under consideration are present in the S-H tautomeric form. Comparing the chemical shifts of the isomeric pairs of compounds **2c**, **2d** and **3c**, the most characteristic differences between E- and Z-forms concern the resonances of pyridazine C-4 and of C=N as well as the signals of the aliphatic carbon atoms in α-position to the C=N double bond (CH_3 or CH_2, respectively). The observed effects (downfield shift for pyridazine C-4 and for the CH_3 or CH_2 resonances, upfield shift for C=N in the Z-form relative to the E-form) can be attributed to steric and electronic reasons. These interpretations are in good agreement with results obtained with comparable compounds like E- and Z-arylhydrazone derivatives of 2-pyridinecarbaldehyde.[11]

Thiosemicarbazones derived from 4-pyridazinecarbaldehyde and methyl 4-pyridazinyl ketone (**4,5**)

In the ^{1}H nmr spectra of all TSCs of type **4** and **5** (Table 2) only one isomeric form could be detected. In contrast to TSCs **1-3**, neither for the E- nor for the Z-form of TSCs incorporating a 4-pyridazinyl core (Scheme 3) the formation of an intramolecular hydrogen bond is possible. Thus, in this series the chemical shift

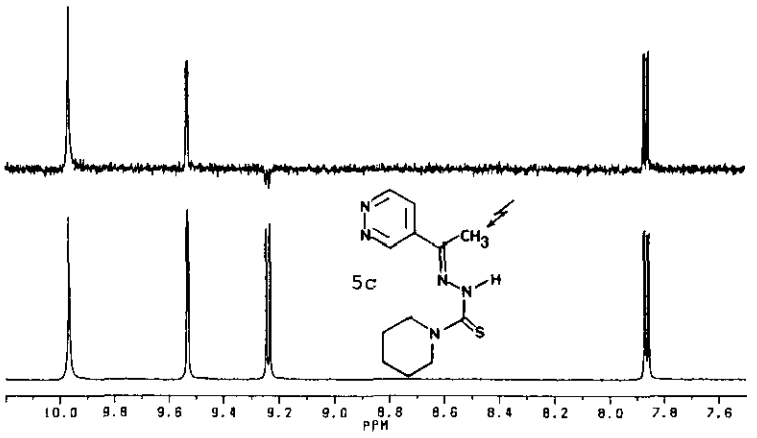


Z **E**

4 R'=H **5** R'=CH_3

Scheme 3

of the N-H proton cannot be considered as a suitable probe for the determination of the configuration. An unequivocal assignment, however, could be achieved by NOE-difference experiments similar to those described for compounds **1c** and **2d**. Irradiation of the formyl-H resonances in compounds **4a** and **4b** or of the methyl-H transition in compounds **5a** and **5c**, respectively, enhanced the corresponding N-H signal, as well as the signals of pyridazine H-3 and H-5 (Figure 2), whereas a perturbation of the N-H resonances led to a positive NOE on the corresponding formyl or methyl signals. On the other hand, no NOE could be registered on the N-H signals when irradiating the pyridazine H-3 resonances. Thus, E-configuration has to be assigned to compounds **4** and 5. An interesting detail is the negative indirect NOE on H-6 *via* H-5 obtained on irradiation of the methyl protons in **5a** and **5c**. This phenomenon indicates a linear arrangement of the methyl-H, H-5 and H-6.



NOE-difference spectrum of **5c** obtained on irradiation of the methyl protons

400 MHz ^{1}H nmr spectrum of **5c** (range: 7.8-10 ppm)

Figure 2

Table 2: ^{1}H Nmr chemical shifts (ppm, DMSO-d_6) for thiosemicarbazones containing the 4-pyridazinyl moiety (**4,5**)

Comp.	pyridazine-H* H-3	H-5	H-6	formyl- or methyl-H	H of R	N-H
E-4a	9.46	7.88	9.30	8.22	2.55	13.68
E-4b	9.40	7.78	9.21	8.11	3.75(2',5'), 1.89(3',4')	11.50
E-4c	9.37	7.76	9.22	8.08	3.86(2',6'), 1.62(3',4',5')	11.45
E-4d	9.39	7.77	9.23	8.20	3.91(2',7'), 1.75(3',6'), 1.56(4',5')	11.28
E-4e	9.38	7.76	9.23	8.10	4.03(2',4'), 2.08(1',5'), 1.67(6',7', 8',9')	11.45
E-5a	9.58	7.94	9.30	2.40	2.54	12.72
E-5b	9.56	7.88	9.23	2.30	3.76(2',5'), 1.90(3',4')	9.81
E-5c	9.53	7.86	9.23	2.30	3.84(2',6'), 1.61(3',4',5')	9.97
E-5d	9.55	7.88	9.24	2.30	3.91(2',7'), 1.78(3',6'), 1.56(4',5')	9.68
E-5e	9.52	7.86	9.24	2.30	4.02(2',4'), 2.06(1',5'), 1.66(6',7', 8',9')	9.90

*typical coupling constants: ^{4}J(H-3,H-5): 2.4 Hz; ^{5}J(H-3,H-6); 1.2 Hz, ^{3}J(H-5,H-6): 5.5 Hz

The ^{13}C nmr data for compounds **4** and **5** are summarized in Table 4. Assignments were made as described for compounds **1-3**. The results of the NOE-difference experiments, indicating E-configuration for compounds **4** and **5** as described above, are supported by the ^{13}C nmr data. Thus, for instance, the chemical shifts of the non-aromatic carbons in the "side-chain" of compounds **5c** and **5d** are in better agreement with the data of the E-isomers of the 3-pyridazinyl analogoues **2c** and **2d** than with those of **Z-2c** and **Z-2d**. The similar magnitude of the ^{1}J (^{13}C,^{1}H) spin coupling constant of the formyl-C atom in **4c** (169.1 Hz) and in **E-1c** (169.8 Hz) points to E-configuration of **4c**. Such types of coupling constants are known to be very sensitive to stereochemical changes due to the fact that the influence of the sp^2-hybridized nitrogen's lone pair in the E-form differs markedly compared to that in the Z-form.[12] This has been demonstrated for comparable E/Z-diastereomeric hydrazones, oximes and imines,[12,13] which show differences of 10-15 Hz in the above mentioned ^{1}J (^{13}C,^{1}H) coupling constant between E- and Z-isomers. Thus, for **Z-4c** a markedly larger ^{1}J (^{13}C,^{1}H) coupling constant has to be anticipated compared to that observed with **E-1c** due to the now cis-position of the coupled formyl-H and the nitrogen lone-pair orbital.

Table 3: ^{1}H Nmr chemical shifts (ppm, DMSO-d_6) of thiosemicarbazones containing the 2-pyridyl moiety (**6**)

	pyridine-H						
Comp.	H-3	H-4	H-5	H-6	formyl-H	H of R	N-H
E-6a	7.87	7.78	7.33	8.55	8.19	3.75(2',5'), 1.88(3',4')	11.68
Z-6a	7.70	8.01	7.50	8.75	7.52	-[+]	14.90
E-6b	7.83	7.78	7.32	8.55	8.16	3.86(2',6'), 1.61(3',4',5')	11.21
Z-6b	7.71	8.04	7.49	8.70	7.52	3.93(2',6'), -[+] (3',4',5')	14.88

[+] overlap with signals of the predominant E-isomer

Table 4: ^{13}C Nmr chemical shifts (ppm, DMSO-d_6) of compounds **1-6**

	aromatic C						
Comp.	C-3	C-4	C-5	C-6	C=N-	C=S	other C, notes
E-1a	155.85	123.15	127.42	151.85	143.51	200.01	16.87(SCH_3); J(N=C-H): 171.7 Hz
E-1c	156.64	122.72	127.23	151.29	140.45	180.13	cycloaliphatic C: 51.11(2',6'), 25.66(3',5'), 23.76(4'); ^{1}J(N=C-H): 169.8 Hz
E-2a	157.02	123.75	127.01	151.80	149.07	201.24	17.95(SCH_3); 12.84(CH_3)
E-2c	157.54	123.46	126.93	151.41	146.99	181.98	cycloaliphatic C: 51.34(2',6'), 25.66(3',5'), 23.75(4'); 12.04(CH_3)
Z-2c	155.21	128.67	127.46	150.99	137.13	180.93	cycloaliphatic C: 50.33(2',6'), 25.38(3',5'), 23.75(4'); 21.47(CH_3)
E-2d	157.50	123.76	126.97	151.38	147.83	181.35	cycloaliphatic C: 51.82(2',7'), 26.98(3',6'), 26.25(4',5'); 11.96(CH_3)
Z-2d	156.30	128.78	127.42	150.95	137.67	179.39	cycloaliphatic C: -[+]; 21.31(CH_3)
E-3a	156.40	124.08	127.11	151.74	152.90	201.50	17.03(SCH_3); 18.70(CH_2); 10.87(CH_3)
Z-3a	155.96	128.57	127.30	151.33	144.30	201.03	16.71(SCH_3); 27.47(CH_2); 10.58(CH_3)
E-3c	156.90	123.70	126.98	151.31	150.22	182.08	cycloaliphatic C: 51.47(2',6'), 25.65(3',5'), 23.74(4'); 17.70(CH_2); 10.14(CH_3)
Z-3c	156.04	128.58	126.86	150.95	140.82	180.08	cycloaliphatic C: 50.72(2',6'), 25.51(3',5'), 23.74(4'); 27.23(CH_2); 11.39(CH_3)
E-4c	148.25	132.60	122.61	151.71	137.43	180.06	cycloaliphatic C: 51.21(2',6'), 25.68(3',5'), 23.75(4'); ^{1}J(N=C-H): 169.1 Hz
E-5a	147.82	134.85	122.94	151.69	146.55	201.59	17.07(SCH_3); 13.69(CH_3)
E-5c	147.77	135.41	122.38	151.54	143.82	181.97	cycloaliphatic C: 51.45(2',6'), 25.71(3',5'), 23.76(4'); 12.92(CH_3)
E-5d	147.81	135.34	122.32	151.46	144.69	181.23	cycloaliphatic C: 52.01(2',7'), 26.94(3',6'), 26.20(4',5'); 12.71(CH_3)
E-6b	119.19	136.56	123.70	149.30	143.29	180.22	cycloaliphatic C: 51.18(2',6'), 25.66(3',5'), 23.78(4'); pyridine C-2: 153.44
Z-6b	124.16	138.39	125.76	147.84	135.25	179.50	cycloaliphatic C: 50.06(2',6'), 25.31(3',5'), 23.78(4'); pyridine C-2: 151.60

[+] overlap with signals of the predominant E-isomer

Thiosemicarbazones derived from 2-pyridinecarbaldehyde (6)

The ^{1}H and ^{13}C nmr data of the pyridine-derived TSCs **6a,b** (included in these investigations as comparison materials) are collected in Tables 3 and 4. Compounds **6a,b** turned out to represent mixtures of E/Z isomers (E/Z ratio: **6a** 8:1, **6b** 6:1).

Ir spectroscopic characteristics of compounds **1-6**

The most characteristic bands in the ir spectra of compounds **1-6** are the N-H stretching frequencies in the range of 3350-3050 cm^{-1}, the C=S stretching frequencies between 1270-1210 cm^{-1}, and for the methyl dithioates **1a, 2a, 3a, 4a** and **5a** also a strong band at about 680 cm^{-1} which is typical for S-CH_3 stretching vibrations. Together with the absence of S-H absorption bands at 2600-2550 cm^{-1}, this clearly indicates the described TSC derivatives **1-6** to exist also in the solid state in the C=S tautomeric form.

CONCLUSION

Based on ^{1}H nmr spectroscopy including NOE-difference experiments, the configuration of a series of novel TSCs recently synthesized as potential antiviral agents could be determined unequivocally. Compounds **1-3**, containing a 3-pyridazinyl moiety exist either in the E-form or as E/Z-isomeric mixtures with the Z-portions below 10% in DMSO-d_6 solution. The 4-pyridazinyl derived TSCs **4** and **5** exclusively occur in the E-form. Compounds **6a,b**, bearing a 2-pyridyl system turned out to form E/Z-isomeric mixtures with higher amounts of the Z-isomer (up to 20%). ^{13}C Nmr spectra of compounds **1-6** support the results obtained from the ^{1}H nmr spectra. According to the ir spectra all TSCs investigated exist as thioxo tautomers also in the solid state.

EXPERIMENTAL

The nmr spectra were recorded from DMSO-d_6 solutions in 5 mm sample tubes on a Bruker AC-80 or a Bruker AM-400 Fourier-transform spectrometer equipped with an Aspect 3000 computer (operating frequencies for ^{1}H: 80.13 MHz or 400.14 MHz, ^{13}C: 20.15 MHz or 100.61 MHz). The probe temperature was 30°C. The centre of the

solvent multiplet was used as internal standard, which was related to TMS with δ 2.49 ppm for ^{1}H and δ 39.50 ppm for ^{13}C. According to the spectral parameters used, the digital resolution in ^{1}H nmr spectra was 0.5 Hz/point (for the determination of pyridazine-H coupling constants 0.2 Hz/point), in broad-band decoupled or J-modulated spin echo ^{13}C nmr spectra 0.9-1.2 Hz/point. ^{1}H-Coupled ^{13}C nmr spectra were obtained using the gated decoupling technique (digital resolution 0.2 Hz/point). Ir spectra (KBr) were recorded on a Jasco IRA-1 spectrophotometer.

For the preparation of compounds **1-6** see ref.[7]

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