NMR Determination of Absolute Configurations in 2-Alkylthiazolidine-4-carboxylic Acids

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The condensation of L-cysteine with enantiomerically pure aldehyde **2a** and **2b** afforded a mixture of 2-substituted thiazolidine diastereomers **1a-b** and **1c-d**, respectively. The present paper deals with the use of nuclear Overhauser effect (nOe) in determining the absolute configurations of the stereocenters.

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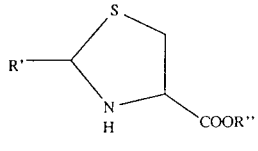
Glutathione (L-glutamyl-L-cysteinylglycine) is a natural scavenger of reactive oxygen intermediates and free radicals which are thought to be involved in many disease processes [1]. L-Cysteine prodrugs, like *N*-acetyl-L-cysteine, are known to increase intracellular glutathione levels [2]. A non-enzymatic opening of the ring of 2-methyl thiazolidine-(4R)-carboxylic acid is reported [3,4] to deliver into cells L-cysteine and acetaldehyde which is further metabolically oxidized to acetic acid. In order to check the possibility of combining the action of L-cysteine prodrugs with

Scheme 1



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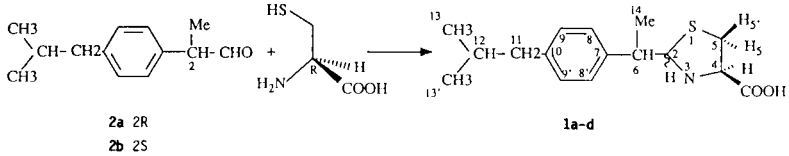


2a 2R
2b 2S

1a-d

Table 1

^{1}H, ^{13} Chemical Shifts and ^{1}H Coupling Constents of Compounds **1a,d** in DMSO-d_6

Compound	H(2)	H(4)	H(5)	H(5')	H(6)	H(11)	H(12)	Me(13/13')	Me(14)	J(4,5)	J(4,5')	J(5,5')	J(6) Me(14)	J(2,6)	J(11,12)
1a	4.62	3.64	3.07	2.57	3.05	2.4	1.8	0.83	1.34	7	9	9	6.5	9	6.4
1b	4.78	3.93	3.01	2.85	2.85	2.38	1.8	0.85	1.23	5.8	5.8	9.5	6.5	8.6	6.4
1c	4.76	4.00	2.97	2.8	2.83	2.4	1.8	0.85	1.28	6.4	6.4	0	6.4	9	6.5
1d	4.62	3.64	3.18	2.7	3.06	2.4	1.8	0.86	1.26	6.8	9.6	9.6	6.4	8.7	6.5

Compound	C(2)	C(4)	C(5)	C(6)	C(7)	C(8,8')	C(9,8')	C(10)	C(11)	C(12)	C(13,13')	C(14)	C=0
1a	77.2	65.7	37.3	44.7	14.2	127.5	129.2	139.5	44.5	29.8	22.4	21	172.8
1b	76.7	64.5	36.9	45.3	14.2	127.5	129	139.2	44.6	29.8	22.4	19.6	173.3
1c	76.3	64.8	37.3	46.2	14.2	127.5	129.1	139.2	44.5	29.8	22.4	21	173.3
1d	77.4	65.5	36.9	44.3	14.2	127.5	129.1	139.1	44.5	29.8	22.4	20.5	172.7

an antiinflammatory action [5] we synthesized a series of 2-substituted thiazolidine derivatives **1** of selected antiinflammatory acids [6]. The new compounds might have the capability to deliver, instead of acetaldehyde, an aldehyde that after oxidation affords an antiinflammatory acid.

The condensation of L-cysteine with enantiomerically pure aldehydes **2a** and/or **2b** afforded a mixture of thiazolidine diastereomers **1a-b** (4:1 ratio) and **1c-d** (4:1 ratio), respectively (see Scheme 1); the cyclization gives rise to a new chiral center at the C(2) position. The structural features of **1a-d** were determined from proton coupling constants and chemical shift data. Their ^{1}H- and ^{13}C-nmr spectra were fully assigned using 1D and 2D chemical shift correlation spectroscopy (see Table 1); in Figures 1 and 2 are presented the phase sensitive DQCOSY spectra.

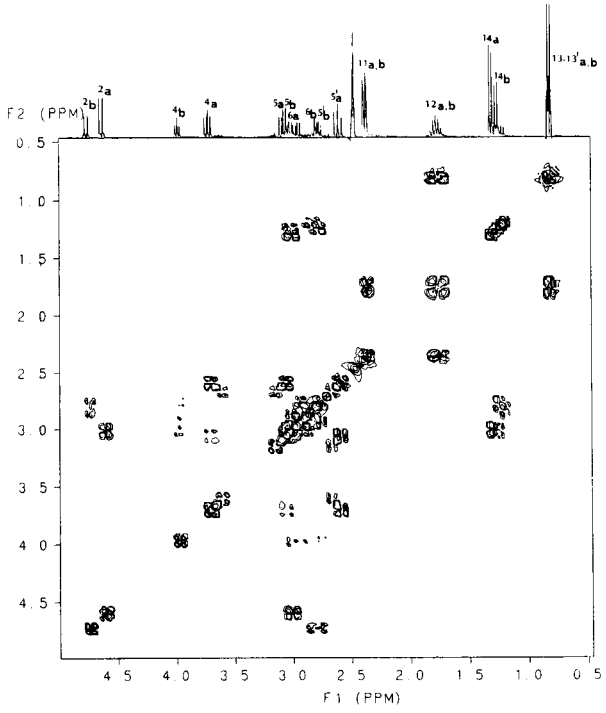


Figure 1. 2DQCOSY of **1a-b** at 300 MHz in dimethyl sulfoxide-d_6.

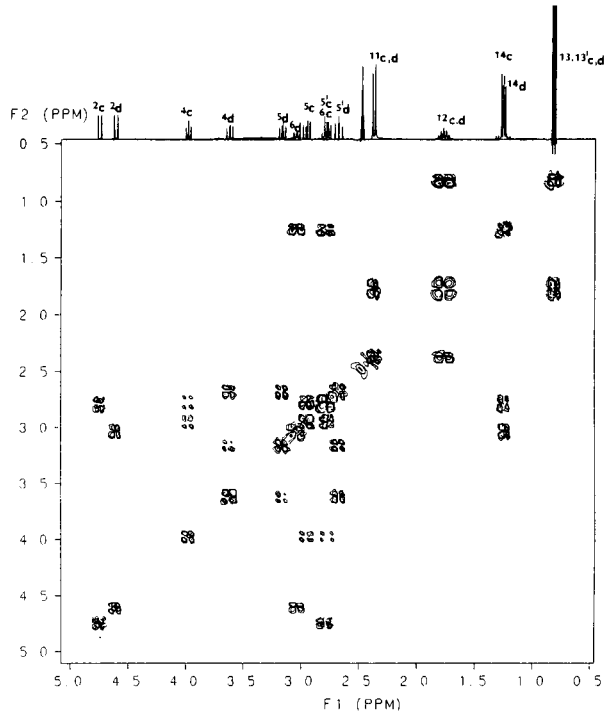
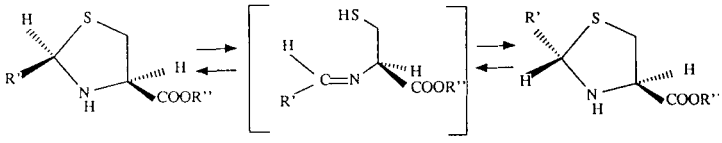


Figure 2. 2DQCOSY of **1c-d** at 300 MHz in dimethyl sulfoxide-d_6.

The investigation of the behaviour of these thiazolidines by ^{1}H-nmr showed that **1a-b** as well as **1c-d** are in equilibrium. The equilibration results from epimerization at C(2) position, as suggested by the well known solution behavior of similar thiazolidines (see Scheme 2) [3,7-10]. The equilibration time of diastereomers (**1a-d**) is between 1 and 72 hours, strongly depending upon the nature of the solvent. Solvents like deuteriochloroform, deuteriodimethyl sulfoxide, deuteriomethanol, deuteriodimethylformamide do not lead to a decomposition product so that the final mixture composition (~1:1 for **1a:1b** and **1c:1d**, see Table 2) defines the thermodynamic equilibrium ratio, while in deuterioacetone and deuteriotetrahydrofuran, since the decomposition takes place, we can't define the final diastereomer ratio as resulting from the thermodynamic equilibrium (see Table 2).

Scheme 2

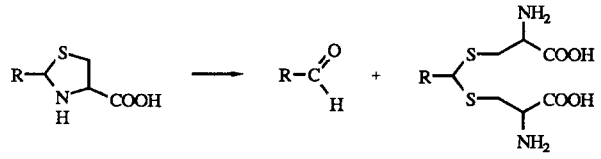


No change in the configuration at C(4) has been observed under the above reaction conditions [8]. Moreover, the ^{1}H- and ^{13}C-nmr spectra of thiazolidines **1a** and/or **1b** do not show any detectable amount of thiazolidines **1c** or **1d**, thus indicating that no epimerization occurs at C(6). Since we know the configuration at C(4) and C(6) we focused our attention to define the absolute configuration at stereocenter C(2). To this end, we perform 2D nOe (NOESY) studies in deuteriodimethyl sulfoxide after reaching the equilibrium ratio of **1a,b** and **1c,d** (see Table 2). It is worth noting that in DMSO the 5% convertion of one diastereomer into the other is about 3 minutes showing that the equilibration rate is very slow with respect to the NOESY data acquisition.

Table 2
Epimerization Rate of Thiazolidine **1a**, **1b** and **1c**, **1d** in Several Solvents

Compound	Solvent	Ratio [a] 1a/1b	Ratio [a] 1c/1d	Time hours
1a,b	$CDCl_3$	insoluable		1 15'
1c,d	$CDCl_3$		58/42	1
1a,b	DMSO	56/44		1
1c,d	DMSO		50/50	1
1a,b	CD_3OD	64/36		1
1c,d	CD_3OD		45/55	2 10'
1a,b	DMF	53/47		48
1c,d	DMF		53/47	3
1a,b	Acetone	55/45		72 [b]
1c,d	Acetone		50/50	24 [b]
1a,b	THF	80/20		1 [b]
1c,d	THF		48/52	4 [b]

[a] Ratios are given for **1a-b** and/or **1c-d** starting from a mixture ca 4:1 (see text). [b] We see a decompsition product deriving from the reaction:



Determination of Absolute Configuration at C(2) by nOe Measurements.

The ^1H-^1H nuclear Overhauser effect has been widely used to establish internuclear distances and thus to determine molecular structures in solution [11]. Nuclear Overhauser measurements have been proven useful for the study of small molecules in which magnetic dipolar relaxation of the nuclei is in extreme narrowing limit [12]. In our case the nOe measurements to H_4 are adequate for a clear assignment of *cis*/*trans* configuration for isomers **1a,d**. Moreover the NOESY data allow us to determine the absolute configuration at C(2). In the present application the 2D nOe phase sensitive pulse sequence [13] 90° - $t_{1/2}$ - 90° - τ_m - 90° - t_2 (FID) was used; the mixing time, τ_m, allowed for magnetization exchange due to dipole-dipole relaxation between two spins. It has been shown theoretically [14a,b] and experimentally [15a,b] that the intensities of the off-diagonal peaks are dependent on the length of the mixing time and are of appreciable intensity in a 2D nOe spectrum when the mixing time is in order of magnitude of spin-lattice relaxation time.

Figure 3 which is the 2D nOe spectrum (recorded with τ_m = 0.4s) of **1a** and **1b** (as a mixture *ca.* 3:2.4) shows interactions between H(2)-H(4) and H(4)-H(5) (downfield resonance) for the major isomer **1a**; while the H(4) proton reveals nOe with both H(5) and H(6) (weaker) in the minor isomer **1b**. In Figure 4 is reported the 2D nOe spectrum of **1c** and **1d** (as a mixture *ca.* 1:1), here the situation is reversed: the minor isomer **1d** shows interactions between H(2)-H(4) and H(4)-H(5) while the major isomer **1c** shows nOe between H(2) and H(5) (upfield resonance) and between H(4) and both H(5) and H(6) (weaker).

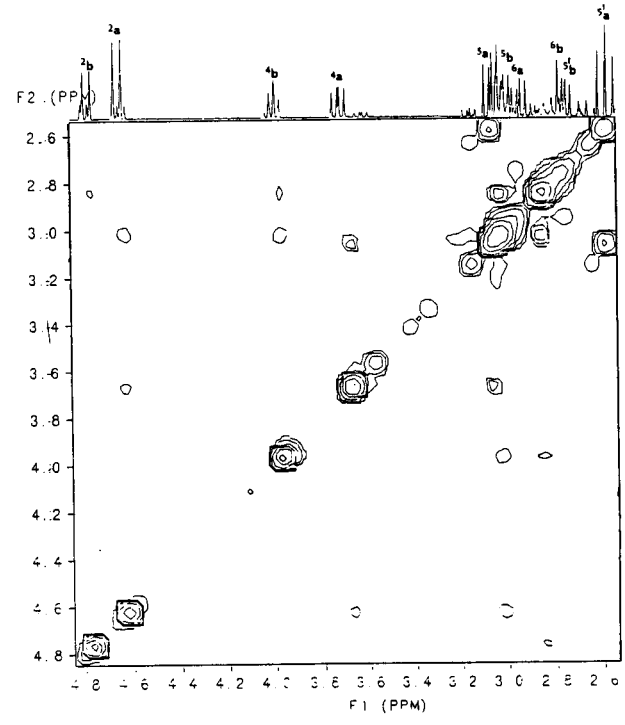


Figure 3. 2D nOe spectrum of **1a-b** at 300 MHz in dimethyl sulfoxide-d_6.

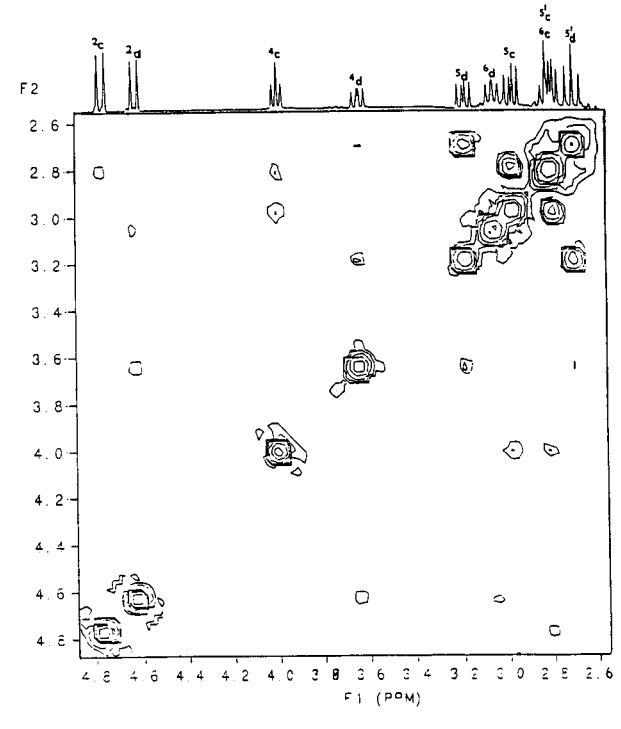


Figure 4. 2D nOe spectrum of **1c-d** 300 MHz in dimethyl sulfoxide-d_6.

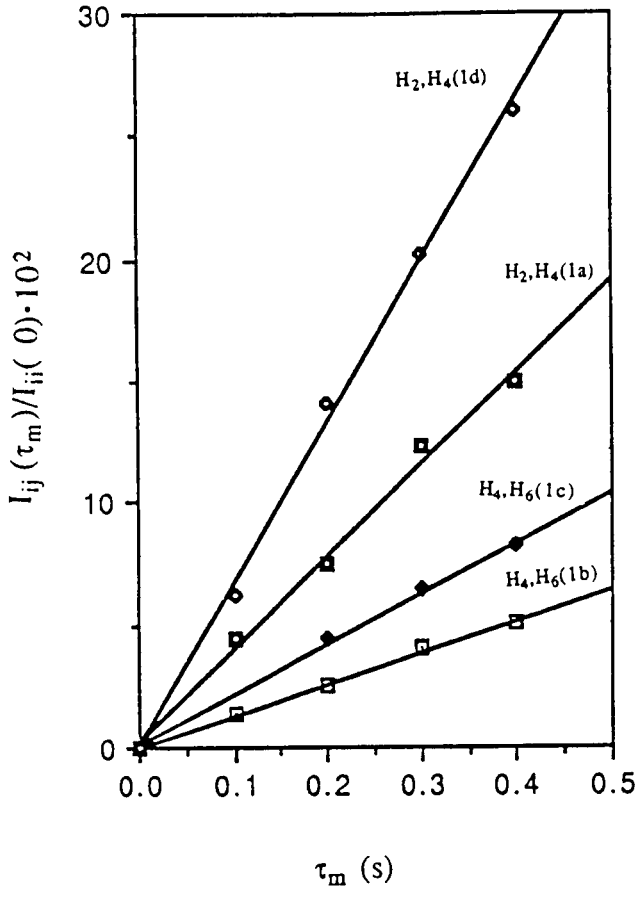


Figure 5. Dependence of the cross peaks intensities on the mixing time in the 2D nOe spectra.

2D nOe spectra, similar to those shown in Figure 3 and 4, were recorded with different mixing times; as expected the build-up rate (as function of τ_m) of nOe between the pair H(2)-H(4) for **1a**, **1d** and H(4)-H(6) for **1b**, **1c** is linear. Values of σ_{ij} [16a,c-17] (cross-relaxation due to dipole-dipole interaction between spins i and j) obtained according to the approximate equation

$$I_{ij}(\tau_m) / I_{ii}(0) = \sigma_{ij}\,\tau_m \qquad (1)$$

are found to be 0.44 $\pm$0.02s^{-1} and 0.6 $\pm$0.02s^{-1} for H(2)-H(4) of **1a** and **1d**, respectively, and 0.125 $\pm$0.02s^{-1}, 0.225 $\pm$0.02s^{-1} for interaction H(4)-H(6) of **1b**, **1c** respectively.

Interproton distances are calculated from equation (2) [18] which holds for isotropic overall motion under the exteme narrowing conditions:

$$\sigma_{ij} = \frac{\gamma_i^2\,\gamma_j^2\,\hbar^2}{2\,r_{ij}^6}\,\tau_c(ij) \qquad (2)$$

where γ_i and γ_j are the gyromagnetic ratios of spins i and j separated by a distance r_{ij}, and τ_c(ij) is the rotational correlation time. The data obtained from ^{13}C spin-lattice relaxation (T_1) measurement indicate that **1a,d** reorient isotropically as a whole (see Table 3); and the maximum nOe values of 3.00 $\pm$0.1 for the protonated carbons (obtained from spectra with S/N = 200/1) shows that these molecules relax predominantly *via* the dipole-dipole mechanism under the extreme narrowing condition. As a consequence we calculate an average value of τ_c (11.7 x 10^{-11}s) for compounds **1a,d** from ^{13}C T_1 of Table 3, and the interproton distances, from equation (3) are 2.9 Å, 2.8 Å for H(2)-H(4) of **1a** and **1d**, respectively, and 3.6 Å, 3.2 Å for H(4)-H(6) of compounds **1b** and **1c** respectively; these values have uncertainties of $\pm$0.3 Å.

Table 3

^{13}C Spin-lattice Relaxation Rate (S) of **1a,d** Dimethylsulfoxide-d_6

T1 (C^{13})	1a	1b	1c	1d
C(2)	0.39	0.37	0.39	0.4
C(4)	0.35	0.34	0.36	0.35
C(5)	0.19	0.19	0.19	0.2
C(6)	0.39	0.39	0.4	0.41
C(7)	3.5	3.7	3.5	3.8
C(8)	0.5	0.5	0.5	0.5
C(9)	0.5	0.5	0.5	0.5
C(10)	3.6	3.6	3.5	3.5
C(11)	0.39	0.39	0.4	0.41
C(12)	0.8	0.8	0.9	0.9
C(13)	0.8	0.8	0.9	0.9
C(14)	0.5	0.51	0.61	0.68

Comparable results are obtained using the equation (3) [19]:

$$r_{ij} / r_{kl} = (\sigma_{kl} / \sigma_{ij})^{1/6} \qquad (3)$$

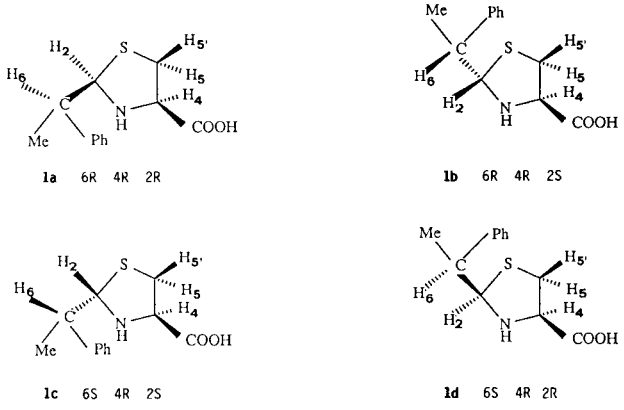
In fact, under conditions of dominant dipole-dipole interactions and isotropic overall molecular motion, the proton-proton dipolar contribution to the relaxation process are pairwise additive and decrease as a sixth power of the interproton distances. In equation (3) r_{ij} can be calculated if the cross-relaxation, σ_{kl}, is measured for protons k and l whose distance, r_{kl}, is known.

In our case the proton-proton H(2)-H(4) distances of **1a**, **1d** and H(4)-H(6) of **1b**, **1c** can be obtained from the σ_{kl} value between H(5)-H(5'), separated by a known distance of 1.75 Å [16a,b]. Analysis of these interproton distances serves as an indicator of the consistency of nOe approach to determination of the conformation in solution of compounds **1a-d**.

The proximity of H(2) and H(4) protons in **1a** and **1d** gives rise to a strong nOe which is an evidence for a *cis* conformation for these thiazolidine rings, this is possible only when the absolute configuration of C(2) is (R) taking into account that the configuration at C(4) is (R) (and does not change in solution). Moreover the interaction between H(4) and H(6) protons, shown in the case of **1b** and **1c** derivatives, is a test for a *trans* conformation of thiazolidine rings, this conformation being allowed only when the absolute configuration at C(2) is (S) (see Scheme 3). These

results are confirmed by the interactions of the geminal protons, indeed there is nOe between H(4)-H(5) in **1c** and **1d** and only in **1c** is there nOe between H(2) and H(5').

Scheme 3



Analysis of 1 D-NMR Data of **1a-d**.

Several features of the conformations of **1a-d** are evident if we analyze the spin-spin coupling and chemical shift data in Table 1. The H(2) proton exhibits the following chemical shifts: δ 4.62 in **1a** and **1d**, δ 4.78 and 4.76 in **1b** and **1c**, respectively. The sum of coupling constants between geminal protons H(5),H(5') with H(4) {J[H(5), H(4)] + J[H(5), H(4)]} is 16, 16.4 Hz for **1a** and **1d**, respectively and 11.6, 12.8 Hz for **1b** and **1c**, respectively. The proton at C(2) position is at lower field in *trans* derivatives **1b**, **1d** and that the sum of coupling constants J[H(5), H(4)] + J[H(5'), H(4)] is <13 Hz for *trans* and >16 Hz for *cis* derivatives.

These results and the literature reports [20] seem to suggest a general rule: coupling constants and chemical shifts of 2-substituted-4-thiazolidine carboxylic acid derivatives can be taken as features for the *cis,trans* arrangement of their substituents.

EXPERIMENTAL

2-Substituted-4-thiazolidine carboxylic acid derivatives **1a,d** utilized were prepared as previously described [6]. Approximately 7 mg of thiazolidine was dissolved in 0.5 ml of deuteriodimethyl sulphoxide. The solution was transferred to a previously constricted 5 mm nmr tube and degassed by several freeze-pump-thaw cycles on a high-vacuum line to remove dissolved oxygen. All spectra were recorded with a Varian XL 300 spectrometer operating at 299.997 MHz; 2D DQCOSY homocorrelated experiments were carried out using a spectral width of 1600 Hz in each domain, a 2048 x 2048 data matrix, 256 time increments and 32 transients for each FID. The 90° pulse was 10 μs and a relaxation delay of 5s was used. The 2D-NOESY experiments were carried out with several mixing times from 0.1 to 1.5s in phase sensitive mode and relaxation delays of 6s, 5 times greater than the longest T_1, to insure quantitative peak intensities. The data matrix were 1024 x 1024, 256 increments and 256 transient for each FID. The temperature was controlled at 300°K for all 2-D nOe experiments.

^{13}C spin-lattice relaxation times were measured by the inversion-recovery method. A three parameter fitting procedure was used for the calculation of ^{13}C T_1 values. The statistical error and reproducibility were 5% and 10% for ^{13}C-nmr relaxation measurements, respectively.

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