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Comparative ^{13}C relaxation study of *R* and *S* isomers of the 1-acetoxyethyl ester of cefuroxime. Influence of C—H bond lengths on relaxation data consistency

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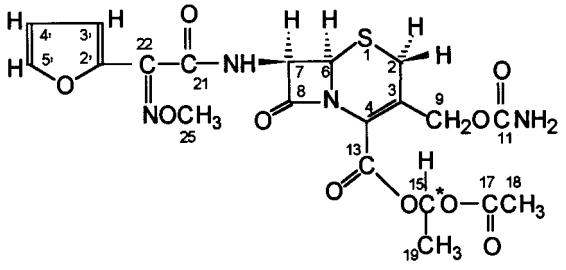
ABSTRACT: ^{13}C NMR longitudinal relaxation times and nuclear Overhauser enhancements were measured for two diastereomers of the 1-acetoxyethyl ester of cefuroxime at two magnetic fields. The relaxation parameters of ^{13}C nuclei located in the rigid core of the cefuroxime ester showed inconsistency within the frame of the relaxation model assuming axially symmetric overall reorientation and C—H bond lengths derived from the PM3 method. The consistency of relaxation data was restored allowing for the increase in C—H bond lengths reflecting the influence of vibrational corrections. The diastereomers, exhibiting differences in biological activity, differ in the ^{13}C relaxation parameters of the side ester moiety. This difference was analysed with the aid of the model-free approach.

KEYWORDS: NMR; ^{13}C relaxation; cephalosporins; cefuroxime ester; molecular motions; relaxation data consistency

INTRODUCTION

The 1-acetoxyethyl ester of cefuroxime (ACC), a prodrug of the antibiotic cefuroxime,[1–3] is shown in Scheme 1. ACC occurs in the form of two diastereomers, denoted in this paper as *R* and *S*. This kind of isomerism is caused by the presence of an asymmetric carbon atom with *R* or *S* configuration at position 15 in the ester group in the molecule containing several asymmetric centres. The diastereomers exhibit substantial differences in chemical and biological activities.[4,5]

The different activities of these diastereomers may be related to conformational differences in solution. The nuclear Overhauser effect observed in ^{1}H NMR spectra is well suited to solving structural and/or conformational problems.[6,7] In the case of the ACC diastereomers, however, no interactions among ^{1}H nuclei located in different side groups have been observed in both the laboratory and rotating frame at 9.4 and 14.1 T. Also,



Scheme 1

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diastereomeric chemical shift differences in the ^{13}C NMR spectra for the majority of carbons are smaller than 0.1 ppm. Only carbons in proximity to the chiral C-15 atom display larger differences (see Table 1). They can be, however, attributed to the inherent sensitivity of ^{13}C chemical shifts to the local structure differences.[8,9]

Other NMR parameters which depend strongly on the molecular structure and dynamics have proved to be the longitudinal relaxation times of ^{13}C nuclei, $T_1(^{13}C)$, usually supplemented by nuclear Overhauser enhancements, $\eta_{C\{H\}}$.[9,10] In this work, ^{13}C relaxation techniques were applied in the comparative study of the *R* and *S* isomers.

RESULTS AND DISCUSSION

Experimental T_1 and η values obtained for both ACC diastereomers at 9.4 and 14.1 T are given in Table 1. Since the cross peaks in NOESY spectra at both magnetic fields showed the same polarity as diagonal peaks (negative NOE regime, i.e. $\omega_H \tau_e > 1.12$),[7] it can be assumed that all experiments were carried out outside the extreme narrowing region.[11] This is confirmed by the $T_1(^{13}C)$ *vs.* B_0 dependence for carbon nuclei expected to relax exclusively via a dipolar mechanism (e.g. C-2, C-6, C-7) and η values markedly smaller than $\eta_{max} = \gamma_H/2\gamma_C = 1.988$.[12]

Relaxation of ring carbons

If one wishes to interpret the relaxation data quantitatively, a realistic model of molecular motion has to be

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Table 1. ^{13}C chemical shifts, longitudinal relaxation times (accuracy <3%) and NOE factors (accuracy 10%) in *R* and *S* diastereomers of the 1-acetoxyethyl ester of cefuroxime

			T_1 (s)				η			
	δ (ppm)		9.4 T		14.1 T		9.4 T		14.1 T	
Carbon	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
C-19	19.24	19.11	0.415	0.486	0.468	0.541	1.64	1.61	1.62	1.59
C-18	20.66	20.68	1.28	1.44	1.45	1.60	0.96	1.10	0.79	0.84
C-2	26.21	26.10	0.128	0.132	0.180	0.171	0.84	0.94	0.68	0.59
C-6	57.69	57.63	0.219	0.222	0.303	0.300	0.83	0.89	0.64	0.55
C-7	58.94	59.03	0.214	0.222	0.300	0.297	0.79	0.89	0.60	0.53
C-9	62.09	62.06	0.150	0.153	0.205	0.197	1.05	1.15	0.89	0.84
C-25	62.47	62.49	0.89	0.95	0.99	0.99	0.81	0.90	0.68	0.63
C-15	88.30	89.26	0.338	0.359	0.448	0.461	1.05	1.13	0.80	0.91
C-4′	112.18	112.21	0.246	0.242	0.292	0.289	0.73	0.84	0.58	0.54
C-3′	113.03	113.07	0.232	0.233	0.290	0.288	0.65	0.72	0.47	0.44
C-4	124.37	124.27	1.55	1.58	0.98	0.95	0.06	0.09	0.03	0.03
C-3	127.94	127.51	0.894	0.910	0.606	0.613	0.26	0.24	0.07	0.01
C-22	144.99	145.02	1.32	1.34	0.80	0.79	0.08	0.05	0.00	0.00
C-2′	145.47	145.47	2.81	2.91	1.89	1.85	0.20	0.18	0.06	0.07
C-5′	145.56	145.59	0.237	0.233	0.293	0.272	0.66	0.75	0.49	0.42
C-11	156.42	156.44	2.25	2.23	1.70	1.69	0.54	0.49	0.24	0.15
C-13	159.28	159.50	1.63	1.73	1.01	1.02	0.13	0.13	0.02	0.02
C-21	161.72	161.75	1.38	1.38	0.92	0.90	0.16	0.16	0.06	0.03
C-8	163.61	163.97	1.23	1.27	0.80	0.83	0.12	0.12	0.05	0.02
C-17	168.70	168.80	2.16	2.57	1.47	1.77	0.21	0.21	0.07	0.07

assumed. It should take into account that elongated ACC molecules are expected to reorient anisotropically and the dipolar contribution to the total relaxation cannot be simply calculated with the aid of an η value away from the extreme narrowing limit without prior knowledge of the correlation time.

Despite these problems, carbons C-2, C-6 and C-7 located within the rigid core of the ACC molecule are expected to relax exclusively via a dipolar mechanism to directly attached protons ($T_1 = T_{1,\mathrm{DD}}$) with a correlation time characterizing the overall tumbling. The dipolar longitudinal relaxation times $T_{1,\mathrm{DD}}$ and nuclear Overhauser enhancements η for carbon nuclei are given by the equations[13,14]

$$T_{1,\mathrm{DD}}^{-1} = 0.1D_{\mathrm{CH}}^2 \times [3J(\omega_{\mathrm{C}}) + J(\omega_{\mathrm{H}} - \omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{C}})] \quad (1)$$

$$\eta = (\gamma_{\mathrm{H}}/\gamma_{\mathrm{C}})(0.1D_{\mathrm{CH}}^2 T_1) \times [6J(\omega_{\mathrm{H}} + \omega_{\mathrm{C}}) - J(\omega_{\mathrm{H}} - \omega_{\mathrm{C}})] \quad (2)$$

The spectral density functions, $J(\omega_i)$, depend on the assumed model of motion. Woessner[15] developed a theory to describe the case of anisotropic reorientation of a rigid molecule. For axial symmetry appropriate spectral density functions are given by Eqns (42)–(44) in Ref. 15. The dipolar coupling constant, D_{CH}, is expressed by

$$D_{\mathrm{CH}}\ (\mathrm{rad\ s^{-1}}) = -\mu_0 \gamma_{\mathrm{H}} \gamma_{\mathrm{C}} h/(8\pi^2 r_{\mathrm{CH}}^3) \quad (3)$$

where r_{CH} denotes the effective, vibrationally averaged C—H distance,[16] defined according to $r_{\mathrm{CH}} = \langle r_{\mathrm{CH}}^{-3} \rangle^{-1/3}$, and the other symbols have their usual meanings.

A detailed knowledge of the molecular geometry is necessary before performing calculations on the relaxation parameters of anisotropically reorienting molecules. The geometries used in this paper were obtained with the PM3 method (details are given in the Experimental section). Optimized conformations of both diastereomers may be well approximated by a prolate axially symmetric ellipsoid. According to the Woessner theory, molecular tumbling of the axially symmetric top is described by two rotational diffusion constants, $D_{\parallel}$ and $D_{\perp}$.[15] Two additional parameters to be determined are polar coordinates, θ and φ, which define the relative orientation of the diffusion and moment of inertia tensors. These four parameters are obtained in the minimization procedure of the target function F:

$$F = \sum \{[T_{1,\mathrm{exp}} - T_{1,\mathrm{calc}})/\Delta T_1]^2 + [(\eta_{\mathrm{exp}} - \eta_{\mathrm{calc}})/\Delta\eta]^2\} \quad (4)$$

where summation is over different carbon sites and magnetic fields. ΔT_1 and $\Delta\eta$ are the accuracies of the experimentally determined relaxation parameters. The C—H bond lengths appearing in the expression describing $T_{1,\mathrm{calc}}$ were taken from the PM3 geometry and were close to 0.111 nm (Table 2).

The minimization results were unsatisfactory. The calculated η values were systematically *ca.* 50% larger than the experimental values to an extent much exceeding the experimental accuracy [Fig. 1(B)]. Diffusion constants and effective correlation times calculated according to Eqn (5)[15,17] are given in Table 2. It is noteworthy that similar problems with the reproducibility of NOE results for ^{13}C and ^{15}N nuclei away

Table 2. Parameters for the overall tumbling of *R* and *S* diastereomers of the 1-acetoxyethyl ester of cefuroxime obtained in the optimization procedures with fixed and fitted C—H bond lengths

	r_{CH} from PM3		r_{CH} fitted	
Parameter	*R*	*S*	*R*	*S*
$D_{\|}$ (10^8 rad s^{-1})	6.9 ± 0.7	6.1 ± 0.7	3.7 ± 0.3	3.8 ± 0.3
$D_{\perp}$ (10^8 rad s^{-1})	4.6 ± 0.6	4.1 ± 0.6	2.6 ± 0.2	2.7 ± 0.2
θ, φ (°)	-65, -6	80, 81	-29, -21	-39, 36
d(C-2, H) (nm)	0.1109		0.1160	0.1157
	0.1110		0.1161	0.1159
d(C-6, H) (nm)	0.1112		0.1131	0.1130
d(C-7, H) (nm)	0.1116		0.1135	0.1134
τ_{eff} (ps)	320 ± 30	360 ± 40	570 ± 30	560 ± 30

from the extreme narrowing regime have been reported previously.[18–21]

$$\tau_{eff} = 3/(5D_{\|} + 13D_{\perp}) \quad (5)$$

Inconsistency in the longitudinal relaxation times and NOE factors for ^{13}C nuclei within the assumed relaxation model may be efficiently removed allowing for variation of the C—H bond lengths as presented schematically in Fig. 2. The dipolar relaxation mechanism depends strongly on the distance between relaxing nuclei, as follows from Eqns (1) and (3). Therefore, the dipolar relaxation of non-quaternary carbons is dominated by the interactions with directly attached protons. A smaller r_{CH} value corresponds to a shorter correlation time τ_c for a given T_1 value [horizontal line in Fig. 2(A)]. In turn, a shorter correlation time τ_c corresponds to a larger η value provided that the molecular tumbling is outside the extreme narrowing limit (vertical lines in Fig. 2).

It is widely accepted in relaxation calculations that one can assume a C—H bond length for aliphatic carbons of 0.109 nm, as obtained from diffraction data. Emsley and Lindon[22] noted, however, that each method of determining molecular structure gave a different average of the internuclear distances. Moreover, Diehl and Niederberger[23] proved that for direct C—H bonds the vibrational corrections are extremely important. The average $\langle r_{CH}^{-3} \rangle^{-1/3}$ may be a few per cent greater than r_{CH} values based on rotational spectra or electron

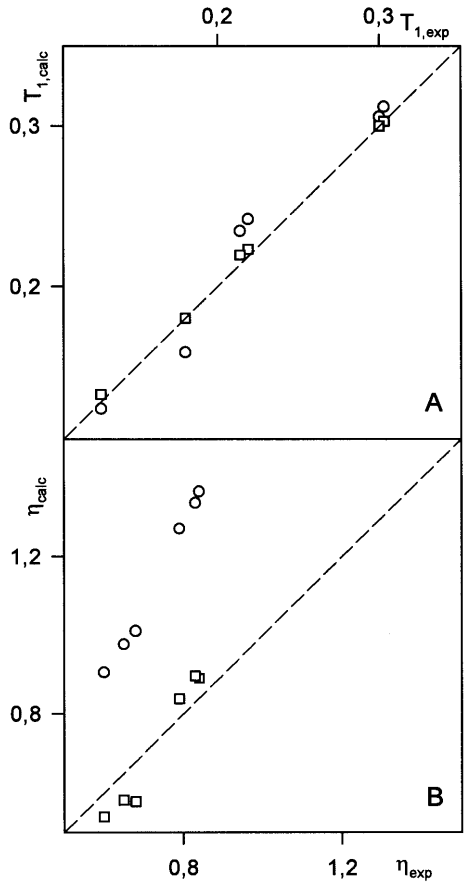

Figure 1. Comparison of experimental and calculated values of (A) ^{13}C longitudinal relaxation times and (B) NOEs for the *R* diastereomer of 1-acetoxyethyl ester of cefuroxime. Calculations were performed using appropriate parameters given in Table 2. ○, Fixed C—H bond lengths; □, fitted C—H bond lengths.

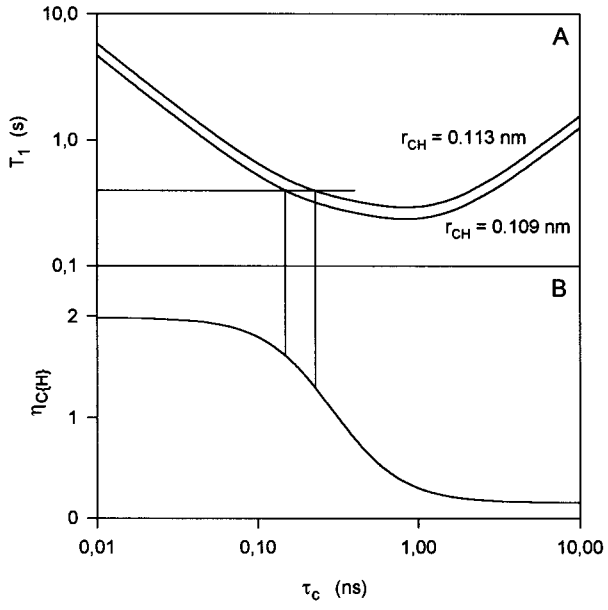

Figure 2. Dipolar longitudinal relaxation times T_1(^{13}C) and nuclear Overhauser enhancements $\eta_{C\{H\}}$ calculated for a C—H pair at B_0 = 14.1 T. Isotropic reorientation of the C—H vector was assumed.

diffraction data.[24] Therefore, it is well justified to vary a C—H bond length in order to restore consistency of T_1 and NOE data. One should stress that this effect remains unnoticed in the extreme narrowing limit, often resulting in the determination of false values of correlation times.

A second optimization was performed, fitting four diffusion parameters and two factors representing elongation of r_{CH} in methylene (C-2) and methine (C-6, C-7) groups. In order to obtain a very good fit for all relaxation parameters, it was sufficient to increase r_{CH} by 2–4%. At the same time, diffusion constants and τ_{eff} changed by more than 60% (Table 2). The quality of this fit in comparison with the fit obtained without C—H bond elongation is presented in Fig. 1.

No meaningful difference in the overall tumbling of ACC diastereomers was observed, but a difference in their biological activities was expected to be manifested in motions of side groups, particularly the 1-acetoxyethyl ester substituent.

Internal rotation of side groups

Qualitative interpretation of the diastereomeric differences in T_1s and NOEs for carbons C-15, C-17, C-18 and C-19 in the ester moieties is fairly straightforward. The smaller T_1 and NOE (only for C-15 and C-18) values observed for *R* diastereomer indicate longer effective correlation times at a given site as compared with the *S* diastereomer. Since the correlation times for tumbling of the rigid central part of the ACC molecule in both diastereomers are equal, one can conclude that internal motions of the ester moiety in the *R* diastereomer are slower and/or more restricted than those in the *S* diastereomer, presumably owing to the intramolecular steric interactions.

A quantitative description of these relaxation data calls for a model of motion in which multiple internal rotations are superposed on the anisotropic overall molecular tumbling. Theoretically available,[25,26] this approach requires far too many parameters to be practically feasible. Alternatively, the model-free approach (MFA) of Lipari and Szabo,[27] not so demanding in the number of parameters, may be applied. Formally, the MFA accounting for anisotropic overall motion should be used. Nevertheless, it was shown recently[28] that the MFA formalism for isotropic motion well described the cases of moderate anisotropy up to $r = D_{\|}/D_{\perp} = 2.0$. Since the r values for the *R* and *S* diastereomers are both equal to 1.4, it is assumed that isotropic overall tumbling well represents the overall motion of ACC molecules in the MFA.

In the MFA formalism, the spectral density function is

$$J(\omega) = S^2\tau_c/(1 + \omega^2\tau_c^2) + (1 - S^2)\tau/(1 + \omega^2\tau^2) \quad (6)$$

with $\tau^{-1} = \tau_c^{-1} + \tau_{int}^{-1}$. The overall motion is described by a correlation time τ_c and internal motion(s) by a generalized order parameter S which is a measure of the degree of spatial restriction of the motion and an effective correlation time τ_{int} corresponding to the rate of these motions.[27]

In the optimization procedure, which gives the MFA parameters, the τ_c values characterizing the overall motion were taken from the results of the motional analysis of ring carbons (τ_{eff} in Table 2) and the two remaining parameters, S^2 and τ_{int}, were fitted. The S^2 value for C-15 in the *R* diastereomer is slightly larger than that in the *S* diastereomer (Table 3), possibly indicating a smaller motional freedom of the C-15—H-15 vector. The inaccuracies of τ_{int} values for C-15 are relatively large and drawing conclusions from their difference does not seem to be well justified. In contrast, the MFA parameters for the C-19 methyl carbon differ in τ_{int} rather than in S^2, suggesting slower internal motions in the *R* diastereomer. The MFA-derived results for C-15 and C-19 might indicate that internal motions of the central part of the ester moiety in the *R* diastereomer are slower and more restricted.

The spin rotation mechanism[11] may play a role in the total relaxation of the two methyl carbons C-18 and C-19. Therefore, the equation describing T_1 requires modification:

$$T_1^{-1} = T_{1,DD}^{-1} + T_{1,SR}^{-1} \quad (7)$$

Appropriate changes should appear also in Eqns (2) and (4).

Table 3. Model-free approach parameters for carbon nuclei located in side groups of *R* and *S* diastereomers of the 1-acetoxyethyl ester of cefuroxime

Carbon	Diastereomer	τ_{int} (ps)	S^2	$T_{1,SR}^{-1}$ (s^{-1})
C-15	*R*	48 ± 12	0.54 ± 0.03	
	S	64 ± 10	0.47 ± 0.02	
C-19	*R*	29 ± 2	0.05 ± 0.01	
	S	23 ± 2	0.05 ± 0.01	
C-18	*R*	3.4 ± 0.6	0.02 ± 0.01	0.22 ± 0.06
	S	3.7 ± 0.5	0.02 ± 0.01	0.17 ± 0.05
C-9	*R*	68 ± 11	0.54 ± 0.03	
	S	79 ± 10	0.53 ± 0.03	
C-25	*R*	4.3 ± 0.8	0.03 ± 0.01	0.44 ± 0.09
	S	5.1 ± 0.7	0.02 ± 0.01	0.50 ± 0.07

It has been found that $T_{1,SR}$ contributes significantly (*ca.* 30%) to the total relaxation of the terminal C-18 carbon but is negligible in the total relaxation of the C-19 nucleus. Although diastereomeric differences in experimental relaxation times for C-18 are meaningful, their MFA parameters do not differ. In our opinion, this is an example of the low sensitivity of the MFA formalism to certain sets of experimental relaxation parameters. Similar findings have already been reported.[29,30]

Experimental relaxation parameters for C-9 and C-25, carbon nuclei situated in two remaining side groups, exhibit no diastereomeric differences. Therefore, it is obvious that their MFA parameters are the same within the accuracy limits (Table 3). It is noteworthy that the methylene carbon C-9 shows a generalized order parameter value close to that of the C-15 nucleus and no measurable spin rotation, whereas this mechanism constitutes more than 40% of the total relaxation of the terminal methyl carbon C-25.

CONCLUSIONS

For heteronuclei, whose relaxation is dominated by dipolar interactions with directly attached proton(s), the appropriate vibrationally averaged bond lengths, usually a few per cent greater than widely used values, should be applied in order to remove discrepancies between relaxation times and NOEs appearing away from the extreme narrowing region.

Our results show that ^{13}C relaxation measurements differentiate *R* and *S* diastereomers of the 1-acetoxyethyl ester of cefuroxime in which ^{1}H nuclear Overhauser effect experiments failed and chemical shift differences were difficult to interpret. It is known that these esters differ in their in biological activity, i.e. the rate of the enzymatic hydrolysis and bioavailability;[4,5] however, these facts cannot be directly correlated. The MFA seems not to be sensitive enough to interpret fully all experimentally observed diastereomeric differences in T_1s and NOEs resulting from multiple internal motions superposed on the overall reorientation of the molecule.

EXPERIMENTAL

The synthesis and separation of the 1-acetoxyethyl ester of cefuroxime isomers has been described elsewhere.[31] Two separate samples of *R* and *S* diastereomers were prepared in Me_2SO-d_6 (euriso-top); concentrations were 200 mM. All NMR measurements were carried out on Bruker AMX 400 (B_0 = 9.4 T) and AMX 600 (B_0 = 14.1 T) spectrometers and ^{1}H and ^{13}C chemical shifts were referenced to TMS. The temperature was measured before and after each measurement with an ethylene glycol sample and was 292.9 K.

DQF-COSY,[32] NOESY[33] and ROESY[34,35] time-domain data (4096 × 512) were obtained with use of the TPPI method[36] to provide frequency sign discrimination in the F_1 dimension. Sixteen scans for each FID and a relaxation delay of 4 s were used. Mixing times for NOESY and ROESY spectra at both magnetic fields were 400 ms; the field strength for spin lock applied within the ROESY mixing time was 2.6 kHz.

The complementary data from COSY and NOESY spectra were used to assign all ^{1}H resonances. The HMBC[37] sequence rather than HMQC or HSQC was used to correlate ^{1}H and ^{13}C resonances owing to the large number of quaternary carbon atoms in the ACC molecule. C-22 and C-2′ resonances were discriminated with their T_1 and η values. The HMBC spectrum was measured at 9.4 T in the magnitude mode using a time-domain data size of 2048 × 512, a relaxation delay of 2.2 s and 64 transients per FID.

^{13}C longitudinal relaxation times T_1 were measured with the fast inversion–recovery method[38] (relaxation delays 6.5 and 3.6 s at 9.4 and 14.1 T, respectively). Ten different evolution times from 0.001 to 5.5 s (9.4 T) or 4 s (14.1 T) were employed and T_1 values were calculated using a three-parameter non-linear least-squares fit.[39] The nuclear Overhauser enhancements $\eta_{C\{H\}}$ were measured by a standard method.[40] Three or more independent measurements were carried out for all relaxation parameters.

The geometries of the *R* and *S* isomers used in this paper were obtained with the semi-empirical PM3 method.[41,42] After energy minimization of the standard PM3 geometry, the ester group was rotated around the COO—R bond in steps of 20°. No significant difference was observed in the rotation barriers on comparing the *R* and *S* isomers the values being 35 kJ mol^{-1} for both. In general, the calculated bond lengths were consistent with the literature x-ray data for cephalosporins.[43–45]

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REFERENCES

1. A. M. Emmerson, *J. Antimicrob. Chemother.* **22**, 101 (1988).
2. S. M. Harding, P. E. O. Williams and J. Ayrton, *Antimicrob. Agents Chemother.* **25**, 78 (1984).
3. E. Ridgway, K. Stewart, G. Rai, M. C. Kalsey, C. C. Bielawska, *J. Antimicrob. Chemother.* **27**, 663 (1991).
4. I. Oszczapowicz, E. Małafiej, A. Horoszewicz-Małafiej, M. Szelachowska, C. Kuklewicz and E. Sierańska, *Acta Pol. Pharm.* **52**, 471 (1995).
5. B. Tejchman, M. Horodecka, M. Jaromińska and I. Oszczapowicz, *Acta Pol. Pharm.* **52**, 477 (1995).
6. J. H. Noggle and R. E. Schirmer, The Nuclear Overhauser Effect. Chemical Applications. Academic Press, New York (1971).
7. D. Neuhaus and M. P. Williamson, The Nuclear Overhauser Effect in Structural and Conformational Analysis. VCH, New York (1989).
8. N. K. Wilson and J. B. Stothers, *Top. Stereochem.* **8**, 1 (1974).
9. H. O. Kalinowski, S. Berger and S. Braun, *Carbon-13 NMR Spectroscopy*. Wiley, New York (1988).
10. F. W. Wehrli and T. Wirthlin, Interpretation of Carbon-13 NMR Spectra. Heyden, London (1976).

11. A. Abragam, Principles of Nuclear Magnetism. Clarendon Press, Oxford (1989).
12. D. Doddrell, V. Glushko and A. Allerhand, *J. Chem. Phys.* **56**, 3683 (1972).
13. I. Solomon, *Phys. Rev.* **99**, 559 (1955).
14. K. F. Kuhlman, D. M. Grant and R. K. Harris, *J. Chem. Phys.* **52**, 3439 (1970).
15. D. E. Woessner, *J. Chem. Phys.* **37**, 647 (1962).
16. N. N. Szeverenyi, R. R. Vold and R. L. Vold, *Chem. Phys.* **18**, 23 (1976).
17. H. Beierbeck, R. Martino and J. K. Saunders, *Can. J. Chem.* **57**, 1224 (1979).
18. D. J. Wilbur, R. S. Norton, A. O. Clouse, R. Addleman and A. Allerhand, *J. Am. Chem. Soc.* **98**, 8250 (1976).
19. G. M. Clore, A. Szabo, A. Bax, L. E. Kay, P. C. Driscoll and A. M. Gronenborn, *J. Am. Chem. Soc.* **112**, 4989 (1990).
20. T. Bremi, M. Ernst and R. R. Ernst, *J. Phys. Chem.* **98**, 9322 (1994).
21. M. L. Remerowski, H. A. M. Pepermans, C. W. Hilbers and F. J. M. van de Ven, *Eur. J. Biochem.* **235**, 629 (1996).
22. J. W. Emsley and J. C. Lindon, NMR Spectroscopy Using Liquid Crystal Solvents. Pergamon Press, Oxford (1975).
23. P. Diehl and W. Niederberger, *J. Magn. Reson.* **9**, 495 (1973).
24. K. Dill and A. Allerhand, *J. Am. Chem. Soc.* **101**, 4376 (1979).
25. D. Wallach, *J. Chem. Phys.* **47**, 5258 (1967).
26. Y. K. Levine, N. J. M. Birdsale, A. G. Lee, J. C. Metcalfe, P. Partington and K. C. G. Roberts, *J. Chem. Phys.* **60**, 2890 (1974).
27. G. Lipari and A. Szabo, *J. Am. Chem. Soc.* **104**, 4546, 4559 (1982).
28. J. M. Schurr, H. P. Babcock and B. S. Fujimoto, *J. Magn. Reson. B* **105**, 211 (1994).
29. M. J. Dellwo and A. J. Wand, *J. Magn. Reson.* **91**, 505 (1991).
30. A. Ejchart, paper presented at the 5th Chianti Workshop on Magnetic Resonance Nuclear and Electron Relaxation, San Miniato, Italy (1993).
31. M. Sasinowska-Motyl, I. Wiśniewska, W. Gumułka, I. Oszczapowicz, M. Szelachowska and B. Interewicz, *Acta Pol. Pharm.* **52**, 391 (1995).
32. M. Rance, O. W. Sorensen, G. Bodenhausen, G. Wagner, R. R. Ernst and K. Wuethrich, *Biochem. Biophys. Res. Commun.* **117**, 479 (1983).
33. J. Jeener, B. H. Meier, P. Bachmann and R. R. Ernst, *J. Chem. Phys.* **71**, 4546 (1971).
34. A. A. Bothner-By, R. L. Stevens, J. Lee, C. D. Warren and R. W. Jeanloz, *J. Am. Chem. Soc.* **106**, 811 (1984).
35. A. Bax and D. G. Davis, *J. Magn. Reson.* **63**, 207 (1985).
36. D. Marion and K. Wuethrich, *Biochem. Biophys. Res. Commun.* **113**, 967 (1983).
37. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **108**, 2093 (1986).
38. D. Canet, G. C. Levy and I. R. Peat, *J. Magn. Reson.* **18**, 199 (1975).
39. M. Sass and D. Ziessow, *J. Magn. Reson.* **25**, 263 (1977).
40. D. Canet, *J. Magn. Reson.* **23**, 361 (1976).
41. M. J. S. Dewar, *Int. J. Quantum Chem.* **44**, 427 (1992).
42. P. Kollman, *Chem. Rev.* **93**, 2395 (1993).
43. M. van Meerssche, G. Germain, J. P. Declercq, B. Coene and C. Moreaux, *Cryst. Struct. Commun.* **8**, 287 (1979).
44. R. M. Sweet and F. Deahl, *J. Am. Chem. Soc.* **92**, 5489 (1970).
45. J. P. Declercq, G. Germain, C. Moreaux and M. van Meerssche, *Acta Crystallogr., Sect. B* **33**, 3868 (1977).

