A simple method for the assignment of the relative stereochemistry of 2-substituted clavams

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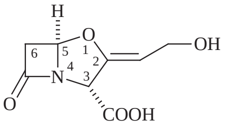
Bentley and Hunt's empirical rule for the determination of the *anti*/*syn* relationship of protons attached to C-2 and C-5 atoms in 2-substituted clavams does not predict the correct stereochemistry in a series of 2-substituted clavams synthesised by us. In this paper we propose a simple method for determination of the relative stereochemistry of 2-substituted clavams based on nuclear Overhauser effect (NOE) experiments and assignment of the diastereotopicity of protons attached to the C-3 atom. Circular dichroism (CD) measurements and X-ray crystallography provide stereochemical and spectral assignments.

Introduction

All natural clavams isolated so far[1] bear no substituent at the C-6 carbon atom. Clavulanic acid **1** and its *O*-protected derivatives are known to have (3*R*,5*R*)-configuration and both express strong β-lactamase inhibition and weak antibacterial activity.[1] Other clavams, represented here by compounds **2**–**5**, feature (*S*)-configuration at the C-5 atom, are monosubstituted at C-2, and exhibit activity against a number of species of fungi.[1] Several attempts at their synthesis have been reported,[2] which use condensation of commercially available 4-acetoxyazetidin-2-one **6** with specially prepared chiral alcohols as a crucial step. Recently, we have published a new general method of a synthesis of 2-substituted clavams, based on [2+2]cycloaddition of chlorosulfonyl isocyanate to vinyl ethers, derived from sugars[3–5] and chiral polyols obtained from readily available hydroxy acids.[6,7]



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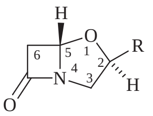
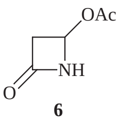


2: $R = COOH$
3: $R = CH_2CH(NH_2)COOH$
4: $R = CH_2CH_2OH$
5: $R = CH_2OH$



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The isolation[1] and the synthesis[2–7] of many clavams have raised the need of a routine and unambiguous stereochemical assignment of stereogenic sites. Bentley and Hunt[8] published NMR spectral data which allow the differentiation of diastereomers of 2-substituted clavams. They found that the difference between the chemical shifts of methylene protons at C-3 was *ca.* 1–1.4 ppm if H-2 and H-5 were in an *anti*-relationship, whereas if H-2 and H-5 atoms were *syn*, the chemical shifts of the protons at C-3 differ by only 0.4–0.5 ppm. Bentley and Hunt's rule,[8] which provided the method of determination of the relative configuration at C-2 and C-5 atoms of 2-substituted clavams, has been applied by other authors to assign the configuration of new clavams.[2]

The absolute stereochemistry at the ring junction of investigated clavams[2] has been also established on the basis of regularities drawn from CD-spectra.[9] A strong positive Cotton effect observed at around 230 nm was linked to the (5*R*)-configuration of the clavam skeleton, whereas a negative Cotton effect was assigned to the *epi*-series having (5*S*)-configuration.[9]

We have found that Bentley and Hunt's rule could not be successfully applied to compounds from our series (structures shown below)[4,6,7] since in several cases chemical shift differences of H-3 and H-3′ atoms were not in the ranges predicted by the rule. This situation has prompted us to investigate the problem in more detail in order to establish an unequivocal spectral proof of the relative configuration of synthesised clavams.

Results and discussion

In the present study we selected nine clavams **7**–**15** which have been obtained recently.[4,6,7] All compounds were investigated by ^1H NMR spectroscopy, compounds **7**, **8**, **10**–**12** were investigated by circular dichroism (CD) spectroscopy, and two crystalline clavams **7** and **10** were the subject of X-ray studies. It should be pointed out that in all cases the absolute configurations at C-2 of compounds **7**–**15** were known from their sugar[4] or hydroxy acid precursors.[6,7] The relative stereochemistry at C-2 and C-5 proved therefore the absolute stereochemistry of each investigated molecule.

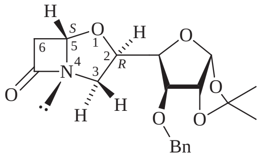
Circular dichroism measurement

The UV and CD spectra of compounds **7**, **8**, **10**–**12** are presented in Table 1 and Fig. 1. Compounds **7**, **8**, **10**, **12** show the same pattern of sign sequence in CD spectra, *i.e.* the negative long wavelength CD band observed at *ca.* 230 nm is followed by a positive short wavelength CD band at approximately 195 nm. The CD spectrum of **11** shows the opposite pattern in the same spectral range, and in contrast to compounds **7**, **8**, **10**, **12**, exhibits a positive Cotton effect at 232 nm and a negative one at 195 nm. A third Cotton effect at approximately 185 nm, well separated only for **10** and **12** is also present in CD spectra of investigated compounds. This band, assigned to a $\pi\rightarrow\pi^*$ transition is positive for all compounds studied in accordance with the literature data.[2c,9] The presence of a benzyl substituent in **7** provided the additional band at 204 nm visible as a shoulder in

Table 1 CD and UV data of the clavams **7**, **8**, **10**–**12** measured in acetonitrile

Compound	UV ε/dm^3 cm^{-1} mol^{-1} (λ_{max}/nm)		CD $\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)		
7	17 014 (195) 12 840 (204)	690 (235)	+[a]	+9.07 (197.5)	−19.68 (233.9)
8	5 790 (198)	730 (232)	+[a]	+13.68 (194.2)	−24.06 (233.3)
10	6 990 (200)	950 (233)	+21.73 (183)	+15.80 (190.8)	−22.75 (232.8)
11	5 434 (196)	714 (228)		−7.79 (195.0)	+12.03 (231.8)
12	9 300 (199)	1 700 (230)	+11.11 (185)	+6.23 (197.9)	−16.26 (234.2)

[a] Maximum not reached above 178 nm.



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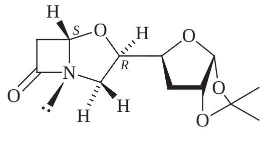

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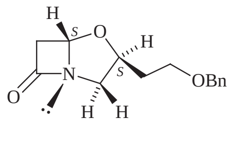

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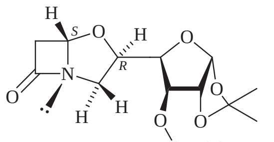

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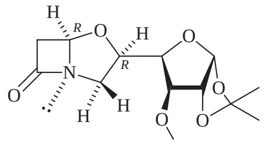

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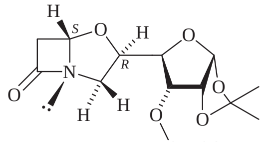

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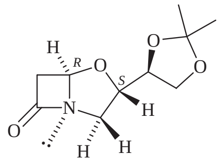

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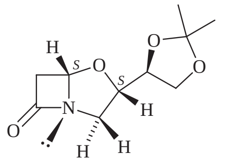

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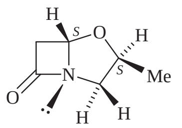

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the UV spectrum. Superposition of this band increases intensity of the band at 195 nm. All other clavams **8**, **10**–**12** do not display such a band.

The enantiomorphic shape of CD curves in the range 260–190 nm is caused by the reversal of the absolute stereochemistry at the ring junction of bicyclic β-lactams. Following the literature data[2c,9] the positive sign of the CD band at 232 nm in the spectrum of **11** points to an (*R*)-configuration of the ring junction (C-5), whereas the negative sign of this band for compounds **7**, **8**, **10**, **12** proves the (*S*)-configuration at C-5. The stereochemistry deduced from CD measurements was confirmed by X-ray crystallography in two cases.

X-Ray crystallography

X-Ray structure analysis of compounds **7** and **10** provided valuable information on geometry of the 2-substituted clavam skeleton (Figs. 2 and 3). The molecules of compounds **7** and **10** have long N-4–C-7 and short C-7–O-8 bond lengths, characteristic for β-lactam rings in biologically active penams and cephems. As a result of the ring fusion the β-lactam nitrogen atom is pyramidal. This is evidenced by the sum of three valence angles of the nitrogen atom equal to 327.2(2)° and 326.7(3)° or by the distance 0.474(2) and 0.477(3) Å of the nitrogen atom from the least-squares plane containing C-3, C-5 and C-7, respectively for compounds **7** and **10**.[10] On the basis of the observed pyramidality of the β-lactam nitrogen atom in compounds **7** and **10**, a lone pair has been generated at a distance 0.6 Å from the parent atom. Distances between protons located at C-5 and C-3 and the generated lone pair for both compounds are shown in Table 2.

Table 2 Distances (Å) between generated lone pair (lp) and protons located at the C-3 and C-5 atoms in clavams **7** and **10**

	lp–H3β	lp–H3α	lp–H5β	H3β–H5β	H3α–H5β
7	2.07(4)	2.31(5)	2.08(4)	3.32(3)	3.82(3)
10	2.04(2)	2.35(3)	2.11(2)	3.21(2)	3.86(6)

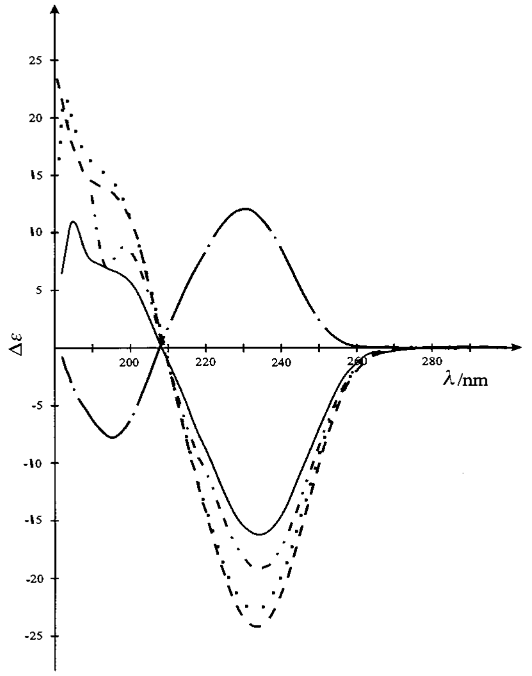

Fig. 1 CD spectra of **7** (——·——), **8** (————), **10** (·····), **11** (—·—·—·—) and **12** (———) in acetonitrile

According to our crystallographic data,[10] twist $^{C5}T_{O1}$ and envelope E_{C3} conformations are assigned to the oxazolidine ring in compounds **7** and **10**, respectively (Fig. 4). This shows that the distances between protons located at C-3 and C-5 are not sensitive to conformational changes at the oxazolidine ring, thus it can be used for diagnostic purposes.

^{1}H NMR studies

Vicinal coupling constants between protons of the 2-substituted clavam skeleton do not show clear dependence on the relative configuration at C-2 and C-5 carbon atoms,[4,6,7] therefore they cannot be used as an unequivocal probe of configuration of clavams **7**–**15**.

The relative configurations of all studied clavams were established unambiguously on the basis of steady-state ^{1}H nuclear Overhauser effect (NOE) experiments. For compounds **7**, **10**

Table 3 Experimental and theoretical (in parentheses) steady-state NOEs for clavams **7** and **10**

Irradiated proton		Observed proton					
		H5β	H2α	H3α	H3β	H6β	H6α
H5β	**7**		0.6 (0.9)	0.0 (−0.1)	1.1 (1.0)	6.4 (6.1)	−0.2 (−0.8)
	10		1.3 (0.5)	−0.2 (0.0)	0.5 (0.5)	6.7 (4.1)	−0.2 (−0.2)
H2α	**7**	1.6 (1.6)		9.1 (8.5)	−0.6 (0.2)	0.4 (−0.2)	1.5 (0.5)
	10	1.8 (0.7)		6.8 (4.4)	−3.3 (−0.5)	−0.2 (−0.1)	1.0 (0.0)
H3α	**7**	0.3 (−0.5)	14.8 (19.3)		36.8 (35.5)	0.0 (0.1)	0.6 (0.2)
	10	−1.2 (−0.1)	14.9 (9.8)		34.4 (36.0)	−0.9 (0.0)	−0.3 (0.0)
H3β	**7**	3.2 (3.4)	−1.6 (0.5)	35.1 (31.4)		0.0 (−0.1)	0.6 (0.0)
	10	2.8 (1.5)	−0.5 (−1.1)	34.7 (36.1)		0.4 (0.0)	0.8 (0.0)
H6β	**7**	15.4 (14.7)	−0.7 (−0.2)	0.1 (0.1)	0.1 (−0.1)		36.4 (39.6)
	10	15.9 (10.8)	−0.1 (−0.2)	0.1 (0.0)	−1.0 (0.0)		38.0 (37.4)
H6α	**7**	−0.4 (−1.8)	1.5 (1.5)	0.0 (0.1)	0.3 (0.0)	34.4 (36.8)	
	10	−1.2 (−0.5)	2.0 (0.9)	−0.4 (0.0)	−0.3 (0.0)	37.3 (36.1)	

Table 4 Experimental steady-state NOEs for clavam **11**

Irradiated proton	Observed proton					
	H5α	H2α	H3β	H6α	H3α	H6β
H5α		4.5	0.2	4.8	0.4	−0.2
H2α	7.2		−0.5	−0.1	10.5	−0.1
H3β	0.4	−0.4		0.5	43.5	0.15
H6α	12.8	2.7	5.4		−2.6[a]	37.7
H3α	3.4	15.3	33.9	−11.9[a]		3.8
H6β	−0.5	1.3	0.9	45.3	−1.4	

[a] Direct saturation due to unselective irradiation.

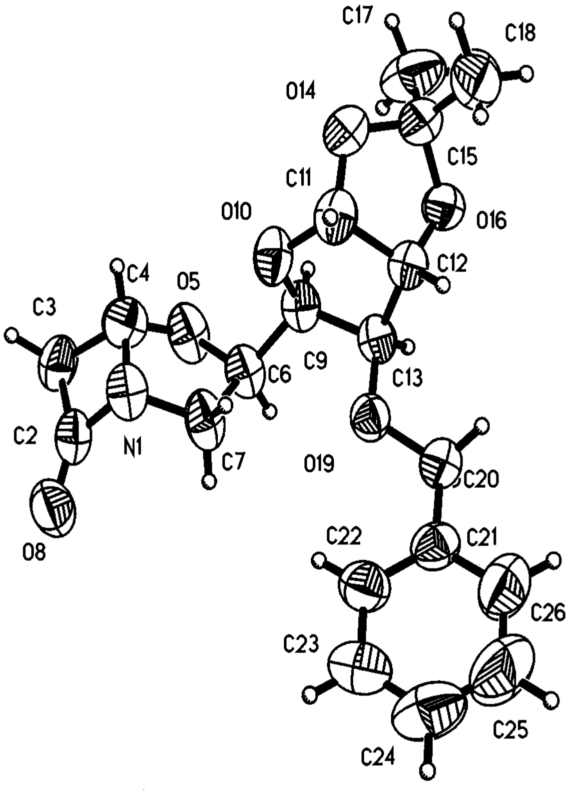

Fig. 2 ORTEP diagram of the molecule of compound **7**. Thermal ellipsoids shown at 50% probability level.

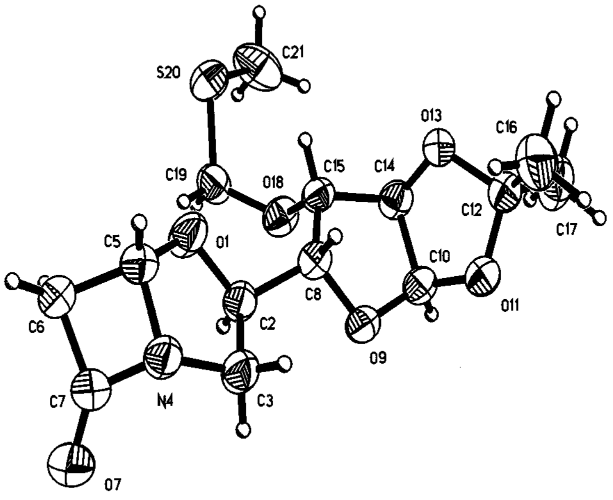

Fig. 3 ORTEP diagram of the molecule of compound **10**. Thermal ellipsoids shown at 50% probability level.

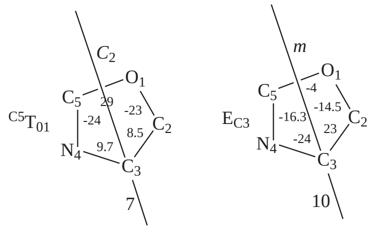

Fig. 4 Values of the torsion angles (in °) observed in five-membered rings

and **11** NOEs were quantitatively determined using our previously published procedure (Tables 3 and 4).[11] For other clavams **8**, **9** and **12**–**15** enhancements were estimated from standard NOE difference experiments using free-induction decay (FID) subtraction.[4,6,7] The stereospecific assignment of diastereotopic protons in both methylene groups was established by means of NOEs (Tables 3, 4 and 5).

Theoretical NOEs were calculated (scaled using an external relaxation parameter[12]) for clavams **7** and **10** using interproton distances taken from X-ray data. They are in good agreement with experimental values. Goodness of fit of the calculated to the experimental NOEs was assessed by the use of a root-mean-square deviation (RMS_{NOE}) factor in a manner similar to its use in crystallography.[13] Experimental NOE patterns obtained for compounds of the same configuration **7** and **10** are very similar while they are clearly different for **11**, which is epimeric to **10** at C-5 (Tables 3 and 4). Low RMS_{NOE} factors (0.105 for **7** and 0.124 for **10**) indicate that the clavam skeletons of both compounds adopt similar conformations in the crystal state and in solution. Furthermore, the fact that experimental NOEs can

Table 5 ^{1}H NMR Chemical shifts (δ_H) of clavam protons in compounds **7**–**15** recorded in $CDCl_3$

	H-2α	H-2β	H-3α	H-3β	Δδ(3α–3β)	H-5β	H-5α	H-6β	H-6α	H-2/H-5
7[a]	4.44		4.03	3.12	+0.91	4.89		2.55	2.41	*anti*
7	4.54		3.97	3.12	+0.85	5.32		3.26	2.80	*anti*
8	4.28		3.98	2.89	+1.09	5.34		3.27	2.84	*anti*
9	4.38		3.98	2.65	+1.33	5.29		3.27	2.83	*anti*
10	4.49		3.98	3.08	+0.90	5.33		3.27	2.79	*anti*
11	4.56		3.14	3.93	−0.79		5.19	2.89	3.24	*syn*
12	4.52		3.94	3.06	+0.88	5.41		3.28	2.85	*anti*
13		4.37	2.93	3.95	−1.02[b]		5.38	3.27	2.84	*anti*
14		4.38	3.61	3.12	+0.49[b]	5.24		3.25	2.91	*syn*
15	4.36		3.96	2.53	+1.43	5.33		3.28	2.83	*anti*

[a] Spectrum recorded in C_6D_6. [b] The change in the sign of Δδ(3α–3β) is due to change of configuration on C-2.

Table 6 Crystal data and structure refinement for **7** and **10**

Identification code	**7**	**10**
Empirical formula	$C_{19}H_{23}NO_6$	$C_{14}H_{21}NO_6S$
Formula weight	361.38	331.38
Temperature/K	293(2)	
Wavelength/Å	1.541 78	
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
Unit cell dimensions		
a/Å	6.4811(8)	8.6840(5)
b/Å	14.8269(9)	11.0653(4)
c/Å	19.6435(10)	9.3006(6)
β/°	—	116.461(5)
Volume/Å^3	1887.6(3)	800.08(7)
Z	4	2
Density (calculated)/mg m^{-3}	1.272	1.376
Absorption coefficient/mm^{-1}	0.788	2.060
F(000)	768	352
Crystal size/mm	0.27 × 0.14 × 0.14	0.27 × 0.14 × 0.07
θ-range for data collection/°	3.73 to 74.93	5.31 to 72.62
Index ranges	$-8 \leqslant h \leqslant 0, 0 \leqslant k \leqslant 18, 0 \leqslant l \leqslant 24$	$-9 \leqslant h \leqslant 9, 0 \leqslant k \leqslant 13, -10 \leqslant l \leqslant 0$
Reflections collected	2115	1432
Independent reflections	2115 [$R_{int} = 0.00$]	1348 [$R_{int} = 0.048$]
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	2112/0/236	1348/1/200
Goodness-of-fit on F^2	0.609	1.012
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0357$, $wR_2 = 0.1145$	$R_1 = 0.0341$, $wR_2 = 0.1063$
R indices (all data)	$R_1 = 0.0361$, $wR_2 = 0.1195$	$R_1 = 0.0342$, $wR_2 = 0.1065$
Absolute structure parameter	0.3(3)	0.00(2)
Extinction coefficient	0.0026(11)	0.006(2)
Largest diff. peak and hole (e Å^{-3})	0.142 and −0.104	0.278 and −0.146

be sufficiently well reproduced by calculations increases our confidence in stereochemical assignments based on NOEs.

When the specific assignment of all protons in the clavam skeletons of **6**–**15** had been established, we observed that proton H-3 located *syn* to the lone pair of the nitrogen atom is always shielded in the ^{1}H NMR spectrum with respect to its geminal partner. We suggest that this regularity may be used for the assignment of the relative configuration of 2-substituted clavams as follows.

Crystallographic studies of clavams **7** and **10** showed that the β-lactam nitrogen atom is highly pyramidal. This known fact, which helps to determine the biological activity of β-lactam antibiotics,[14] is a consequence of the *cis*-fusion of the four-membered β-lactam ring and five-membered oxazolidine ring. Therefore, the H-5 proton and the nitrogen lone electron pair must always lie on the same face of both rings, which means that the relative configurations of the C-5 carbon atom and the N-4 nitrogen atom are correlated. The difference in chemical shifts of methylene protons at C-3 can be rationalised in terms of magnetic anisotropy of the nitrogen lone electron pair which causes shielding of the *syn* proton and deshielding of the *anti* proton. Similar effects of the nitrogen lone pair have already been reported in the literature for azacyclohexanes.[15] In addition, the more shielded proton, on C-3 the atom *syn* to H-5 and to the lone pair, exhibits a small long range coupling constant to the H-6 proton located on the same face of the rings. This kind of coupling can be treated as a homoallylic coupling analogue.

A simple NOE experiment, *i.e.* saturation of the well separated H-2 proton resonance and observation of through-space interactions to both H-3 protons, gives unambiguous information on the relative position of these protons. The differences in NOEs from H-2 to H-3β and H-3α (Tables 3 and 4) are large enough that even poorly performed NOE experiments provide a clear and reliable stereochemical assignment.

In conclusion, stereochemical assignment of protons on the C-3 atom with respect to the lone electron pair (on the basis of chemical shift) and an NOE experiment provide sufficient information to assign the relative position of H-2 and H-5 protons.

Experimental

The syntheses of compounds **7**, **8**, **10**–**12**,[4] **9**, **15**,[7] **13**, **14**[6] have been published recently. CD measurements were run in acetonitrile on an AVIV 62D spectrophotometer. UV spectra were performed on a Cary 1E spectrophotometer in acetonitrile.

^{1}H NMR spectra were run on an AM-500 Bruker spectrometer in $CDCl_3$ solution at room temperature. Steady-state NOE measurements for **7**, **10** and **11** were taken on the same spectrometer on samples degassed by the freeze–thaw technique using a routine program for multiplet irradiation. The timing of the experiments was as follows: 15 s irradiation time, 5 s acquisition time, and 1 s relaxation time. The irradiating power was low, and good selectivity was achieved in almost all cases. The saturation factor for multiplets was of the order of 0.5–0.8. Limits of integration for each signal were kept constant for all irradiation lists. Fractional NOEs were calculated using absorption spectra scaled against the reference spectrum and were corrected for different saturation factors. Usually eight averaging cycles, each of eight scans preceded by two dummy scans, were performed.

The theoretical NOEs for **7** and **10** were calculated on the basis of X-ray geometry using the computer program NOE,[11] which is available on request from the authors. The program NOE is based on steady-state equations for multi-spin NOEs in the presence of external relaxation.[12]

The estimated precision of all experimental NOEs is ±1%. Root-mean-square deviations, RMS_{NOE}, of experimental and theoretical NOEs calculated for **7** and **10** are equal to 0.105 and 0.124 respectively.

X-Ray structure determination of compounds **7** and **10**. Single crystals suitable for diffractometric measurements were obtained from CH_2Cl_2 solution. Reflections were collected on the Nonius MACH3 diffractometer using the ω–2θ scan technique. Intensities were corrected for Lorentz and polarisation factors. The structures were solved by direct methods using SHELXS86[16] and refined using SHELXL93[17] programs. Hydrogen atoms attached to the carbon atoms were generated geometrically and refined in riding mode. Lone pairs located at the β-lactam nitrogen atoms were calculated using an ancillary program. Information regarding crystallographic data and structure solution and refinement is collected in Table 6.

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