Carbon–hydrogen and carbon–carbon coupling patterns in the cephalosporin series

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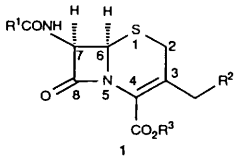
The ^{13}C NMR spectra of the skeleton of 7-amino-3-cephem-4-carboxylic acid† derivatives and the 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetyl substituent were analysed searching for the rules which might be helpful in the structure determination of new cephalosporin antibiotics and their isomers. The ^{13}C NMR decoupled spectra were fully interpreted on the basis of ^{13}C–^{1}H and ^{13}C–^{13}C coupling patterns. The method for unambiguous assignment of the *Z*/*E* geometry of 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetyl groups was established.

The synthetic studies on cephalosporin derivatives are still an important topic of applied organic chemistry. This is due to both unsatisfactory activity of known compounds and growing resistance of micro-organisms towards this class of antibiotics. The structure determination of newly obtained cephalosporin derivatives and their isomers on the basis of common NMR techniques often meets difficulties.

The signals of carbon atoms bonded to hydrogen atoms can be easily identified by means of either chemical shifts, or DEPT and CH-correlation techniques. However, the assignment of signals of numerous quaternary carbon atoms is troublesome. The identification based on chemical shifts often results in ambiguous conclusions. On the other hand, the long-range proton–quaternary carbon couplings should provide very useful structural information. Although many papers on the synthesis have been published[1] only very few works reported ^{13}C NMR data of the title compounds.[2]

The purpose of this work was the methodical re-investigation of the ^{13}C NMR data for some cephalosporins possessing various substituents at the C(7) position of **1**. Since the modern



1

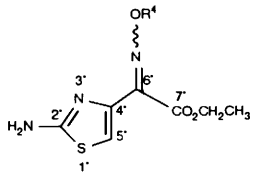
1a: $R^1 = R^2 = R^3 = H$
1b: $R^1 = R^3 = H$, $R^2 = OAc$
1c: $R^1 = H$, $R^2 = OAc$, $R^3 = CH_2OCH_3$
1d: $R^1 = PhOCH_2$, $R^2 = H$, $R^3 = CH_2OCH_3$
1e: $R^1 = PhOCH_2$, $R^2 = R^3 = H$
1f: R^1 =  , $R^2 = H$, $R^3 = Na$

β-lactam antibiotics, especially the III generation cephalosporins, usually carry the 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetyl group, we also analysed the spectra of some model substituted ethyl 2-(2-aminothiazol-4-yl)acetates **2**, **3** in order to recognize features useful for the structure determination of similar and more complicated structures.

Results and discussion

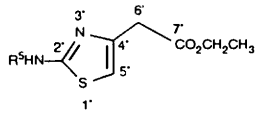
^{1}H NMR data of derivatives of ethyl 2-(2-aminothiazol-4-yl)-acetate 2a–d, 3a–b

The ^{1}H NMR data are presented in Table 1. The assignment of the signal was made on the basis of the signal multiplicity, signal integral, chemical shifts and simple comparison of the spectra.



2

2a: *Z*, $R^4 = H$
2b: *E*, $R^4 = H$
2c: *Z*, $R^4 = CH_3$
2d: *E*, $R^4 = CH_3$



3

3a: $R^5 = H$
3b: $R^5 = HCO$

^{13}C NMR data of derivatives of ethyl 2-(2-aminothiazol-4-yl)-acetate 2a–d, 3a–b

The signals of carbon atoms bonded to hydrogen atoms C(5′), CH_2 and CH_3 were easily identified by means of $^{1}J_{^{13}C-^{1}H}$ couplings, DEPT and CH correlation techniques. The high frequency signals of quaternary carbon atoms (the range above 160 ppm) were assigned to the C-2′ atom (doublet in the coupled ^{13}C NMR spectra) and to *C*=O (either triplet or multiplet in the coupled spectra). The remaining signals were assigned to C(4′) and C(6′) atoms (Table 2).

The chemical shift and proton–carbon coupling constants are not sufficient for unambiguous identification of the signals of quaternary carbon atoms of the single compounds [*e.g.* the hydrogen atom at C(5′) is coupled to all carbon atoms located in the thiazole ring and also to the carbon atom of the imino group]. Moreover, even if one can find a different pattern of C–H coupling in *Z versus E* derivatives [*e.g.* $J_{C(6)-H(5)} \approx 2$ Hz for *Z* and $J_{C(6)-H(5)} \approx 0$ for *E* isomers], the prediction of the correct structure of the imino substituent might be doubtful. The recognition of *E*/*Z* geometry constitutes an important problem appearing in the synthesis of the III generation cephalosporins.[3] For further elucidation of this topic, the $J_{^{13}C-^{13}C}$ INADEQUATE spectra were run for compounds **2a–d** and **3a–b**.

The $J_{^{13}C-^{13}C}$ coupling constants, different for each pair of the directly bonded carbon atoms, confirmed our previous assignment of chemical shifts. Also, we have obtained evidence that the C–H coupling pattern of the hydrogen atom bonded directly to the thiazole ring H(5′) is not necessarily related to atomic distances between the relevant atoms [since $^{3}J_{C(2)-H(5)} > {}^{2}J_{C(4)-H(5)}$]. Then, the chemical shift assignments

† The IUPAC recommended name is 7-amino-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid. The numbering system used throughout the text is given in structure **1**.

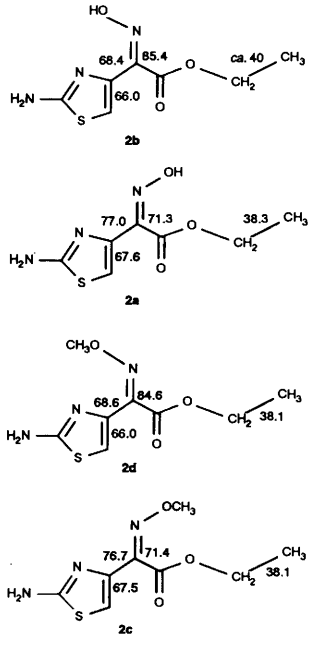
Table 1 ^{1}H NMR data of derivatives of the ethyl 2-(2-aminothiazol-4-yl)acetates **2a–d**, **3a–b**

	δ_H [a]					
Group	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**
CH(5′)	6.80	7.51	6.88	7.49	6.29	7.00
OH	11.57	12.53	—	—	—	—
OCH_3	—	—	3.86	3.97	—	—
CH_2(6′)	—	—	—	—	3.43	3.68
NH_2 [b] (NHCO)	7.13	7.10	7.24	7.11	6.85	12.2
HC=O	—	—	—	—	—	8.45
CH_2CH_3 [c]	4.25	4.21	4.27	4.23	4.05	4.07
	(7.1)	(7.1)	(7.1)	(7.1)	(7.2)	(7.1)
CH_2CH_3 [d]	1.24	1.22	1.25	1.25	1.17	1.16
	(7.1)	(7.1)	(7.1)	(7.1)	(7.2)	(7.1)

[a] All spectra were taken in [$^2H_6$]DMSO solution; the solvent peak (δ_{DMSO} = 2.49) was used as the internal reference; all signals appear as singlets, except the signals of ethyl groups. [b] Broad peaks, *ca.* 10 Hz. [c] Quartet. [d] Triplet, $^3J_{CH_2-CH_3}$ coupling constants (measured in Hz) are given in parentheses.

based only on the ^{1}H–^{13}C correlation experiments may lead to inaccurate conclusions, because the signal of the C(2′) atom of the aminothiazole ring may be interpreted as the C(4′) signal.[2a]

The $^1J_{^{13}C-^{13}C}$ coupling constants of the 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetyl fragment are typical for the conjugated double bond system (Scheme 1). Note that the results



Scheme 1 $^1J_{^{13}C-^{13}C}$ (Hz) of *Z* and *E* isomeric ethyl 2-(-2-aminothiazol-4-yl)-2-iminoacetates, ([$^2H_6$]DMSO)

obtained for the C(4′)–C=N and N=C–C=O bonds differ significantly in pairs of *E* and *Z* isomers (**2a**, **2c** *versus* **2b**, **2d**). It was found that the lone pair of the nitrogen atom increases the magnitude of the $^1J_{^{13}C-^{13}C}$ coupling constant (typically 8–11 Hz) for the C–C bond in the *syn* position in relation to the lone pair.[4] Such analogous phenomena occur for derivatives of 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetic acid and the value of $^1J_{^{13}C-^{13}C}$ coupling constants can be used for identification of the *syn–anti* isomers.

^{1}H NMR data of 7-amino-3-cephem-4-carboxylic acid derivatives 1a–e

The chemical shifts and H–H couplings in cephalosporins **1a–e** are typical and are presented in Table 3. The coupling pattern was confirmed by the homo-decoupling experiment.

^{13}C NMR data of 7-amino-3-cephem-4-carboxylic acid derivatives 1a–e

The ^{13}C signal assignment based only on chemical shifts may not lead to an accurate conclusion, particularly in the case of quaternary centres. Our aim was to assign unambiguously all ^{13}C peaks by various methods to analyse the ^{13}C–^{1}H and ^{13}C–^{13}C coupling pattern and to apply the results to more complicated molecules of the III generation cephalosporins in further work.

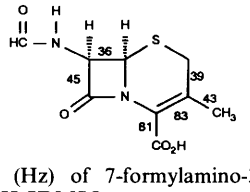
The signals of the protonated carbon atom were identified easily by the DEPT technique, the analysis of $^1J_{^{13}C-^1H}$ coupling constants and by the ^{13}C–^{1}H correlation technique (if necessary). The signals of quaternary carbon atoms are identified either by their long-range coupling patterns or by selective heterodecoupling (Table 4). In some cases analyses of ^{13}C–^{13}C couplings were necessary.

Since the cephem skeleton is a bicyclic, strained molecule with a number of heteroatoms and a relatively small amount of hydrogen atoms, a complex long-range C–H coupling pattern may be expected. In order to simplify the discussion of origin of multiplicity of ^{13}C atoms signals, the data for every atom are shown separately.

***(a)* C(2) atom.** The signal forms a triplet of multiplets. The analysis of the multiplet is usually puzzling owing to various small coupling constants with H(6) and protons of the methyl or methylene side-chain.

***(b)* C(3) and C(4) atoms.** The signals form either sextet, quintet or multiplet, depending on the nature of the substitution at the carboxy group and in the side chain. Small couplings for C(2)H_2 and C(3)CH_2 or C(3)CH_3 are observed. The identification by means of chemical shifts or coupling constants may be questionable.

The unambiguous signal assignment was made for compounds **1a** and **1e** by means of analysis of $^1J_{^{13}C-^{13}C}$ coupling constants. The complete data for cephalosporin **1a** is presented in the Scheme 2. The results obtained for **1e** were not



Scheme 2 $^1J_{^{13}C-^{13}C}$ (Hz) of 7-formylamino-3-cephem-3-methyl-4-carboxylic acid **1a**, [$^2H_6$]DMSO

fully interpreted and we suggest assigning the signal δ = 123.0 to the C(4) atom (^1J = 85.3 and 82.7 Hz) and the signal δ = 130.8 to the C(3) atom ($^1J \approx$ 43 Hz found also at the methyl group connected to this carbon atom). Since in general our

Table 2 ^{13}C NMR data of derivatives of ethyl 2-(2-aminothiazol-4-yl)acetate **2a–d**, **3a–b**

	δ_C ^{13}C–^{1}H coupling constants, J/Hz					
	2a	**2b**	**2c**	**2d**	**3a**	**3b**
C(2′)	168.7, d $^3J_{C\text{-}H(5)}$ = 8.7	167.0, d $^3J_{C\text{-}H(5)}$ = 8.4	168.8, d $^3J_{C\text{-}H(5)}$ = 8.7	166.8, d $^3J_{C\text{-}H(5)}$ = 8.4	168.1, d $^3J_{C\text{-}H(5)}$ = 8.3	159.4, qq $^3J_{C\text{-}H(5)}$ = 4.5 $^3J_{C\text{-}OCH}$ = 4.0
C(4′)	142.2, d $^2J_{C\text{-}H(5)}$ = 4.5	138.3, d $^2J_{C\text{-}H(5)}$ = 4.1	141.1, d $^2J_{C\text{-}H(5)}$ = 4.5	137.6, d $^2J_{C\text{-}H(5)}$ = 4.4	144.3, td $^2J_{C\text{-}H(5)}$ = 4.9 $^2J_{C\text{-}H(6)}$ = 6.9	143.8, m $^2J_{C\text{-}H(5)}$ = 3.8 $^2J_{C\text{-}H(6)}$ = 5.5
C(5′)	106.4, d $^1J_{C\text{-}H}$ = 191.8	114.7, d $^1J_{C\text{-}H}$ = 196.9	108.5, d $^1J_{C\text{-}H}$ = 191.8	116.1, d $^1J_{C\text{-}H}$ = 197.1	103.0, dt $^1J_{C\text{-}H}$ = 189.8 $^2J_{C\text{-}H(6)}$ = 4.5	110.8, dq $^1J_{C\text{-}H}$ = 182.9 $^1J_{C\text{-}H(6)}$ = 4.3
C(6′)	146.8, dd $^3J_{C\text{-}OH}$ = 8.7 $^3J_{C\text{-}H(5)}$ = 2.1	144.5, d $^3J_{C\text{-}OH}$ = 5.3 $^3J_{C\text{-}H(5)}$ ≈ 0	146.8, d $^3J_{C\text{-}H(5)}$ = 2.0	144.9, s	36.9, td $^1J_{C\text{-}H}$ = 129.7 $^3J_{C\text{-}H(5)}$ = 1.8	36.5, m $^1J_{C\text{-}H}$ = 130.2
OCH_3	—	—	62.4, q $^1J_{C\text{-}H}$ = 144.5	63.0, q $^1J_{C\text{-}H}$ = 144.9	—	—
C(7′)	163.4, t $^3J_{C\text{-}CH_2}$ = 3.25	164.2, t $^3J_{C\text{-}CH_2}$ = 2.8	162.3, t $^3J_{C\text{-}CH_2}$ = 3.5	163.3, t $^3J_{C\text{-}CH_2}$ = 3.25	170.0, m $^3J_{C\text{-}CH_2}$ ≈ 3.6	169.9, m $^3J_{C\text{-}CH_2}$ = 3.6
CH_2CH_3	61.1, tq $^1J_{C\text{-}H}$ = 148.7 $^2J_{C\text{-}CH_3}$ = 4.5	61.2, tq $^1J_{C\text{-}H}$ = 148.3 $^2J_{C\text{-}CH_3}$ = 4.3	61.4, tq $^1J_{C\text{-}H}$ = 149.0 $^2J_{C\text{-}CH_3}$ = 4.4	61.4, tq $^1J_{C\text{-}H}$ = 148.5 $^2J_{C\text{-}CH_3}$ = 4.4	60.0, tq $^1J_{C\text{-}H}$ = 147.6 $^2J_{C\text{-}CH_3}$ = 4.5	60.3, tq $^1J_{C\text{-}H}$ = 147.7 $^2J_{C\text{-}CH_3}$ = 4.5
CH_2CH_3	14.0, qt $^1J_{C\text{-}H}$ = 127.3 $^2J_{C\text{-}CH_2}$ = 2.65	14.1, q $^1J_{C\text{-}H}$ = 127.0 $^2J_{C\text{-}CH_2}$ ≈ 0	13.9, qt $^1J_{C\text{-}H}$ = 127.3 $^2J_{C\text{-}CH_2}$ = 2.7	13.9, qt $^1J_{C\text{-}H}$ = 127.1 $^2J_{C\text{-}CH_2}$ = 2.6	14.0, qt $^1J_{C\text{-}H}$ = 126.8 $^2J_{C\text{-}CH_2}$ = 2.7	14.0, qt $^1J_{C\text{-}H}$ = 126.7 $^2J_{C\text{-}CH_2}$ = 4.5
C(O)H	—	—	—	—	—	159.4, d $^1J_{C\text{-}H}$ = 204.4

[a] All spectra were taken in [$^2H_6$]DMSO solution; the solvent peak (δ_{DMSO} 39.5) was used as the reference; abbreviations: d doublet, t triplet, q quartet, m multiplet, dd and dq doublets of doublets and quartets, td and tq triplets of doublets and quartets, qt quartet of triplets.

Table 3 ^1H NMR data of 7-amino-3-cephem-4-carboxylic acid derivatives **1a–f**

	δ_H [a] $J_{^1H\text{-}^1H}$/Hz [b]					
	1a	**1b**	**1c**	**1d**	**1e**	**1f**
C(2)H_2 [c]	3.33, d 3.55, d (18.2) [d]	3.49, d 3.62, d (18.2) [d]	3.56, d 3.68, d (18.2) [d]	3.44, d 3.59, d (18.3) [d]	3.38, d 3.53, d (18.1) [d]	3.11, d 3.41, d (18.1) [d]
C(6)*H*	5.04, d (4.7) [e]	5.11, d (4.9) [e]	5.16, d (4.9) [e]	5.12, d (4.6) [e]	5.06, d (4.6) [e]	4.96, d (4.6) [e]
C(7)H	5.67, q (8.7) [f] (4.7) [e]	5.78, q (8.8) [f] (4.9) [e]	5.84, q (8.8) [f] (4.9) [e]	5.70, q (8.3) [f] (4.6) [e]	5.61, q (8.3) [f] (4.6) [e]	5.53, q (8.1) [f] (4.6) [e]
C(3)CH_3	2.00, s	—	—	2.06, s	2.02, s	1.95, s
C(3)CH_2OAc [c]	—	4.68, d 4.99, d (12.9) [d]	4.71, d 4.95, d (13.1) [d]	—	—	—
OAc	—	2.01, s	2.02, s	—	—	—
CH_2OCH_3 [c]	—	—	5.33, d 5.38, d (6.0) [d]	5.29, d 5.39, d (6.1) [d]	—	—
CH_2OCH_3	—	—	3.42, s	3.42, s	—	—
CONH	8.96, d (8.8) [f]	9.02, d (8.8) [f]	9.05, d (8.8) [f]	9.10, d (8.3) [f]	9.06, d (8.3) [f]	9.49, d (8.1) [f]
R[1]	*H*CONH: 8.12, s	*H*CONH: 8.13, s	*H*CONH 8.14, s	PhOCH_2: [c] 4.61, d 4.66, d (15.0) [d] Ph: 6.95, m 7.29, m 6.97, m	PhOCH_2: [c] 4.60, d 4.64, d (15.0) [d] Ph: 6.9, m 7.27, m 6.9, m	C(5′)H: 6.71, s CH_3O: 3.83, s NH_2: 7.34, s (br)

[a] All spectra were taken in [$^2H_6$]DMSO solution, using the solvent peak (δ_{DMSO} 2.49) as the reference; abbreviations: s singlet, d doublet, q quartet, m multiplet. [b] The magnitudes of coupling constants are given in parentheses. [c] AB system. [d] $^2J_{gem}$. [e] $^3J_{H(6)\text{-}H(7)}$. [f] $^3J_{H(7)\text{-}NH}$.

results show that $^2J_{C(3)\text{-}H(2)} > {}^3J_{C(4)\text{-}H(2)}$, the signal assignment for compounds **1b** and **1d** is simple. However, the case of **1c** is not so obvious.

(*c*) **C(6) atom.** The signal usually forms a doublet of quartets or doublet of multiplets, depending on the coupling constant magnitudes. Typically, long-range couplings with the H(7) proton (*ca.* 2.5 Hz) and with only one C(2)H proton (*ca.* 5.5 Hz) are observed.

(*d*) **C(7) atom.** Two long-range coupling constants are observed for compounds possessing the formyl group (**1a–c**): with a formyl proton (*ca.* 5.6 Hz) and NH proton (*ca.* 2.5 Hz); certainly only the latter (*ca.* 2.5 Hz) is observed for compounds

Table 4 ^{13}C NMR data of 7-amino-3-cephem-4-carboxylic acid derivatives **1a–f**

	δ_C ^{13}C–^{1}H coupling constants, J/Hz[a]					
	1a	**1b**	**1c**	**1d**	**1e**	**1f**
C(2)	29.2, tm $^{1}J_{C\text{-}H} \approx 143$	25.7, tm $^{1}J_{C\text{-}H} \approx 143$ $^{3}J_{C\text{-}CH_2OAc} \approx 5$	26.0, tm $^{1}J_{C\text{-}H} \approx 145$ $^{3}J_{C\text{-}CH_2OAc} \approx 4.8$	29.3, tm $^{1}J_{C\text{-}H} \approx 142$	29.2, tm $^{1}J_{C\text{-}H} \approx 139$	29.0, tm $^{1}J_{C\text{-}H} \approx 141$ $^{n}J_{C\text{-}CH} \approx 5.2$
C(3)	130.4, sext. $^{2}J_{C\text{-}H(2)} \approx 6.5$ $^{2}J_{C\text{-}CH_3} \approx 6.5$	123.8, quin. $^{2}J_{C\text{-}CH} = 5.2$	125.0, quin.[b] $^{2}J_{C\text{-}CH} = 4.8$	133.3, sext. $^{2}J_{C\text{-}H(2)} \approx 6.0$ $^{2}J_{C\text{-}CH_3} \approx 6.5$	130.8, sext. $^{2}J_{C\text{-}H(2)} \approx 6.5$ $^{2}J_{C\text{-}CH_3} \approx 6.5$	119.1, sext. $^{2}J_{C\text{-}CH} = 6.2$
C(4)	122.9, sext. $^{3}J_{C\text{-}H(2)} \approx 5.1$ $^{3}J_{C\text{-}CH_3} \approx 5.1$	126.3, quin. $^{3}J_{C\text{-}CH} = 4.6$	126.1, m[b] $^{3}J_{C\text{-}CH} \approx 6.5$ and 4.2	121.7, sext. $^{3}J_{C\text{-}H(2)} = 4.5$ $^{3}J_{C\text{-}CH_3} \approx 5.0$	123.0, sext. $^{3}J_{C\text{-}H(2)} \approx 5.2$ $^{3}J_{C\text{-}CH_3} \approx 5.2$	129.9, sext. $^{3}J_{C\text{-}CH} = 5.2$
C(6)	56.9, dm $^{1}J_{C\text{-}H} = 175.3$ $^{2}J_{C\text{-}H(7)} = 2.5$ $^{3}J_{C\text{-}H(2)} = 5.4$ and < 1	57.0, dq $^{1}J_{C\text{-}H} = 176.7$ $^{2}J_{C\text{-}H(7)} = 2.6$ $^{3}J_{C\text{-}H(2)} = 5.6$	57.2, dq $^{1}J_{C\text{-}H} = 177.3$ $^{2}J_{C\text{-}H(7)} = 2.9$ $^{3}J_{C\text{-}H(2)} = 5.6$	57.3, dm $^{1}J_{C\text{-}H} \approx 176$	57.2, dm $^{1}J_{C\text{-}H} = 175.2$ $^{2}J_{C\text{-}H(7)} = 2.6$ $^{3}J_{C\text{-}H(2)} = 6.2$ and 2	57.2, dd (br) $^{1}J_{C\text{-}H} = 172.6$ $^{n}J = 4.8$
C(7)	57.4, dq $^{1}J_{C\text{-}H} = 152.6$ $^{2}J_{C\text{-}NH} = 2.2$ $^{3}J_{C\text{-}OCH} = 5.6$	57.5, dq $^{1}J_{C\text{-}H} = 153.3$ $^{2}J_{C\text{-}NH} = 2.4$ $^{3}J_{C\text{-}OCH} = 5.6$	57.7, dq $^{1}J_{C\text{-}H} \approx 153$ $^{2}J_{C\text{-}NH} = 2.7$ $^{3}J_{C\text{-}OCH} = 5.6$	58.8, dm $^{1}J_{C\text{-}H} \approx 153$	58.7, dd $^{1}J_{C\text{-}H} = 153.1$ $^{2}J_{C\text{-}NH_n} = 2.3$	57.9, d $^{1}J_{C\text{-}H} = 151.8$
C(8)	164.4, t (br) $^{2}J_{C\text{-}H(7)} \approx 6.5$ $^{3}J_{C\text{-}H(6)} \approx 6.5$ $^{3}J_{C\text{-}NH} < 1$	164.7, t (br) $^{2}J_{C\text{-}H(7)} = 6.7$ $^{3}J_{C\text{-}H(6)} = 6.7$ $^{3}J_{C\text{-}NH} < 1$	165.1, tm $^{2}J_{C\text{-}H(7)} \approx 7$ $^{3}J_{C\text{-}H(6)} \approx 7$ $^{3}J_{C\text{-}NH} \approx 1.7$	164.3, t (br) $^{2}J_{C\text{-}H(7)} \approx 6.7$ $^{3}J_{C\text{-}H(6)} \approx 6.7$ $^{3}J_{C\text{-}NH} < 1$	163.9, tm $^{2}J_{C\text{-}H(7)} \approx 6$ $^{3}J_{C\text{-}H(6)} \approx 6$ $^{3}J_{C\text{-}NH} = 2.5$	162.2, t (br) $^{2}J_{C\text{-}H(7)} \approx 6.4$ $^{3}J_{C\text{-}H(6)} \approx 6.4$
C(3)CH_2R^2	19.6, qd $^{1}J_{C\text{-}H} = 129.1$ $^{3}J_{C\text{-}H(2)} = 3.3$	62.8, tm $^{1}J_{C\text{-}H} \approx 152$ $^{3}J_{C\text{-}H(2)} \approx 2.4$	62.6, td $^{1}J_{C\text{-}H} = 151.6$ $^{3}J_{C\text{-}H(2)} = 3.3$	19.5, tm $^{1}J_{C\text{-}H} = 129.5$ $^{3}J_{C\text{-}H(2)} \approx 2.7$	19.5, tm $^{1}J_{C\text{-}H} = 128.4$ $^{3}J_{C\text{-}H(2)} \approx 3.3$ and 1	19.4, qm $^{1}J_{C\text{-}H} \approx 127.5$ $^{3}J_{C\text{-}H(2)} \approx 3.5$
$CH_2O_2CCH_3$	—	170.3, m $^{2}J_{C\text{-}CH_3} = 6.5$ $^{3}J_{C\text{-}CH_2} = 3.0$	170.3, m $^{2}J_{C\text{-}CH_3} = 6.7$ $^{3}J_{C\text{-}CH_2} = 3.0$	—	—	—
$CH_2O_2CCH_3$	—	20.6, q $^{1}J_{C\text{-}H} = 129.6$	20.5, q $^{1}J_{C\text{-}H} = 129.7$	—	—	—
C(4)CO_2R^3	163.7, s (br) $^{n}J \ll 1$	162.9, s	161.1, t $^{3}J_{C\text{-}CH_2} = 3.7$	161.6, t (br) $^{3}J_{C\text{-}CH_2} \approx 2$	163.6, s	166.1, s (br)
CH_2OCH_3	—	—	92.0, tq $^{1}J_{C\text{-}H} = 174.1$ $^{3}J_{C\text{-}CH_3} = 4.9$	91.1, tq $^{1}J_{C\text{-}H} = 170.5$ $^{3}J_{C\text{-}CH_3} = 5.0$	—	—
CH_2OCH_3	—	—	57.6, qt $^{1}J_{C\text{-}H} = 143.3$ $^{3}J_{C\text{-}CH_2} = 7.3$	57.4, qt $^{1}J_{C\text{-}H} = 143.3$ $^{3}J_{C\text{-}CH_2} = 7.2$	—	—
CONH	161.8, dt $^{1}J_{C\text{-}H} = 196.7$ $^{2}J_{C\text{-}NH} \approx 3.6$ $^{3}J_{C\text{-}H(7)} \approx 3.6$	161.7, dt $^{1}J_{C\text{-}H} = 196.9$ $^{2}J_{C\text{-}NH} = 3.6$ $^{3}J_{C\text{-}H(7)} = 3.6$	161.8, dt $^{1}J_{C\text{-}H} = 197.2$ $^{2}J_{C\text{-}NH} = 3.7$ $^{3}J_{C\text{-}H(7)} = 3.7$	168.7, quin. $^{n}J_{C\text{-}H} \approx 3.8$	168.6, quin. $^{n}J_{C\text{-}H} = 3.7$	163.0, t (br) $^{2}J_{C\text{-}NH} = 3.6$ $^{3}J_{C\text{-}H(7)} = 3.6$
R^1				PhOCH_2: 66.2, t $^{1}J_{C\text{-}H} = 146.3$ Ph: 157.8, m 114.6, dq $^{1}J = 159.6$, $^{3}J = 7.8$, $^{3}J = 5.3$ 129.5, dd $^{1}J = 159.5$, $^{3}J = 8.6$ 121.2, dt $^{1}J = 160.8$, $^{3}J = 7.3$	PhOCH_2: 66.2, t $^{1}J_{C\text{-}H} = 146.2$ Ph: 157.8, m 114.6, dq $^{1}J = 161.6$, $^{3}J = 7.9$, $^{3}J = 4.6$ 129.5, dq $^{1}J = 159.5$, $^{3}J = 8.6$ $^{n}J = 1.5$ 121.2, dt $^{1}J = 160.8$, $^{3}J = 7.5$	C(6′): 149.1, d $^{3}J_{C\text{-}H(5)} = 2.4$ C(5′): 109.3, d $^{1}J_{C\text{-}H} = 190.0$ C(4′): 142.6, d $^{2}J_{C\text{-}H(5)} = 4.3$ C(2′): 168.7, d $^{3}J_{C\text{-}H(5)} = 8.7$ OCH_3: 62.0, q $^{1}J_{C\text{-}H} = 143.6$

[a] All spectra were taken in [$^2H_6$]DMSO solution; the solvent peak (δ_{DMSO} 39.5) was used as a reference; abbreviations: d doublet, t triplet, q quartet, quin. quintet, sext. sextet, m multiplet, dd, dm, dq and dt doublets of doublets, multiplets, quartets and triplets, qd and qm quartets of doublets and multiplets, td, tm and tq triplets of doublets, multiplets and quartets. [b] Assignment of C(3) and C(4) may be opposite.

1d and **1e**. The small magnitude of $^2J_{C(7)-H(6)}$ (less than 1 Hz) is interesting.

In general the comparison of the coupling constants $^1J_{C(6)-H} \approx 176$ and $^1J_{C(7)-H} \approx 153$ Hz provides a method (other than C–H correlation) for C(6) and C(7) signal assignment by means of a coupled ^{13}C NMR spectrum only.

***(e)* C(8) atom.** The signal forms a broad triplet. Three couplings are observed: $^2J_{C(8)-H(7)} \approx {}^3J_{C(8)-H(6)} \approx 7$ Hz and a small coupling with the NH group $0 < {}^3J_{C(8)-NH} < 2.5$ Hz.

***(f)* C(3) substituent.** The carbon atoms of the CH_2OAc or CH_3 groups located at the 3-position exhibit two different couplings with C(2)H_2 protons: $2.4 < {}^3J_{C-H(2)} < 3.3$ and $0 < {}^3J_{C-H(2)} < 1$ Hz. The carbonyl atom of the acetyl group ($CH_2O_2CCH_3$) displays two couplings: $^2J = 6.5$ Hz for the methyl and $^3J = 3$ Hz for the methylene protons.

***(g)* C(4) substituent.** The signal of the CO_2H carbon atom of free acids **1a**, **1b**, **1e** appears as a singlet; however, the long-range ^{13}C–^1H correlation experiment exhibits small couplings (less than 1 Hz) for protons of the 3-methyl group. In the methoxymethyl esters **1c** and **1d**, these signals form triplets owing to coupling with protons of the methylene group. One long-range coupling is observed for the methyl and methylene carbon atoms of the methoxymethyl substituent: $^3J_{CH_2-CH_3} \approx 5$ Hz and $^3J_{CH_3-CH_2} \approx 7$ Hz, respectively.

***(h)* C(7) substituent.** The signal of the carbon atom of the carbonylamino group **1a**, **1b**, **1c** appears as a doublet of triplets with two equal long-range coupling constants, $^2J_{CO-NH} \approx {}^3J_{CO-H(7)} \approx 3.7$ Hz. The signal forms a quintet for phenoxyacetyl derivatives **1d** and **1e** owing to additional couplings with hydrogen atoms of the methylene group.

Only one-bond couplings are detected for the CH_2 carbon atom of the phenoxyacetyl substituent. The coupling pattern of the phenyl group is quite typical.

Finally, we tried to apply the collected data to analyse ^{13}C NMR spectra of the III generation cephalosporin, CEFETAMET sodium salt **1f**, a compound possessing eight quaternary carbon atoms. The remaining six carbon atoms bonded to hydrogen atoms were identified easily, only signals of C(6) and C(7) very close to each other were assigned on the basis of different ^1J coupling constants (172 *versus* 152.4 Hz). The characteristic C–H long-range coupling pattern of the 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl group explained the shape of signals shifted to 168.9, 149.1 and 142.6 ppm (Table 4). Additionally, irradiation of signal H(5′) (heterodecoupling experiment) confirmed the corresponding assignments. The carbonyl carbon atoms were specified on the basis of previously found long-range coupling constants. Also the result of the heterodecoupling experiment [irradiation of H(7)] supported the initial assignment of the signals.

The simple assignment of signals of the unsaturated carbon atoms of the dihydrothiazine ring [C(3) and C(4)] based on the chemical shifts can lead to a false conclusion, since, in comparison with previous examples, the signal of C(3) was significantly shifted upfield. This statement was supported by the INADEQUATE experiment: the signal at δ 119.1 expressed two ^{13}C–^{13}C coupling constants ($^1J = 43$ and 83 Hz) and was identified as the C(3) carbon atom ($^1J = 43$ Hz was found also at the side-chain methyl group), whereas the signal at δ 129.9 was assigned to the C(4) atom ($^1J = 76$ Hz corresponding to the coupling with the carboxy group).

In general, the collected data let us formulate some conclusions which can be helpful in further work. (*i*) The signals of protonated carbon atoms can be easily assigned, either by their chemical shifts, or by DEPT and C–H correlation techniques and comparison of the magnitude of C–H coupling constants; however, the assignment of the signals based only on the ^{13}C NMR chemical shifts leads to numerous errors, especially for the quaternary carbon atoms. (*ii*) The analysis of the long-range C–H coupling pattern and selective heterodecouplings provides the unambiguous assignments of the signals; the knowledge of correct coupling constants will be applied to analyses of spectra of new cephalosporins and in further work to arrange new NMR correlation experiments. (*iii*) The chemical shifts and the magnitude of C(6′)–H(5′) and C(5′)–H(5′) coupling constants in the 2-(2-aminothiazol-4-yl)-2-alkoxyimino substituent differ slightly in the *Z* and *E* isomers whose spectra we have measured; however the difference may not be used to diagnose the geometry of the alkoxyimino group in the case of other derivatives. (*iv*) The INADEQUATE experiment and analysis of ^{13}C–^{13}C coupling constants leads to an unambiguous confirmation of signal assignments; additionally, the *Z* or *E* geometry of the imino substituent can be recognized, even if only one of these isomeric compounds is available.

Experimental

Solutions of 1.0–1.5 mol dm^{-3} concentration in [$^2H_6$]DMSO were used for the NMR studies. All measurements were recorded on a Bruker AM500 instrument.

The ^1H and ^{13}C spectra were obtained by standard instrumental procedures. The solvent signal was used as internal reference: 2.49 ppm for ^1H NMR and 39.5 ppm for ^{13}C NMR, respectively.

The frequency of 125.76 MHz was used for ^{13}C NMR measurements. Typically, 32 scans were acquired for decoupled ^{13}C spectra as well as for DEPT experiments. About 100–150 scans were collected for coupled carbon spectra. The resolution enhancement (Bruker parameters LB = −1.2 and GB = 0.2) was applied during processing. The measurements were carried out with the spectral resolution of 0.898 Hz per point.

The standard Bruker programs: XHCORRD (adjusted for coupling constant 140 Hz) and COLOC (adjusted for coupling constant 6 Hz) were used for ^{13}C–^1H 2D correlation experiments; a 4096 × 128 (4096 × 256) points data matrix was collected. The continuous wave decoupling and decoupler power of 0.2–0.4 mW were applied for heterodecoupling experiments.

The INADEQUATE experiment was adjusted either for a coupling constant of 80 Hz or for constant 30/90 Hz; *ca.* 12 000 scans were acquired. Typically, the 2 mol dm^{-3} solutions were used, measurements were run at 30 °C to increase the solubility of the compound. In some cases just good quality ^{13}C decoupled spectra (12 000 scans) were used for determination of $^1J_{^{13}C-^{13}C}$ coupling constants.

The compounds of which spectra were recorded, are known and were prepared according to the published methods: 7-formylamino-3-methyl-3-cephem-4-carboxylic acid **1a** and 7-formylamino-3-acetoxymethyl-3-cephem-4-carboxylic acid **1b**,[5] methoxymethyl 7-formylamino-3-acetoxymethyl-3-cephem-4-carboxylate **1c** and methoxymethyl 7-phenoxyacetamino-3-methyl-3-cephem-4-carboxylate **1d**,[6] 7-phenoxyacetamino-3-methyl-3-cephem-4-carboxylic acid **1e**,[7] (*Z*)- and (*E*)-ethyl 2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate and (*Z*)- and (*E*)-ethyl 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate,[8] ethyl 2-(2-aminothiazol-4-yl)acetate[9] and ethyl 2-(2-formylaminothiazolyl-4-yl)acetate.[10]

References

1 (*a*) H. Wild, *Protective Groups in β-Lactam Chemistry and Introduction and Transformation of Functional Groups in β-Lactam Chemistry* in *The Organic Chemistry of β-Lactams*, ed. G. I. Georg, VCH, New York, 1993, pp. 1–120; (*b*) S. Kukolja and R. R. Chauvette, *Cephalosporin Antibiotics Prepared by Modification at the C-3 Position* in *Chemistry and Biology of β-Lactam Antibiotics*, ed. R. B. Morin and M. Gorman, Academic Press, New York, 1982, vol. 1, pp. 93–198.

2 (*a*) M. Tashiro, E. Magome, S. Miki and K. Sakagami, *Chem. Pharm. Bull.*, 1992, **40**, 1623; (*b*) A. I. Chernyshev, S. V. Shorshnev, I. B. Vtorov and A. A. Zhelaev, *Khim. Farm. Zh.*, 1991, 81; (*c*)

Y. Hamashima, *Synthesis, Biological Properties, and Pharmacokinetics of 7432-S, a New Oral Cephem* in *Recent Advances in the Chemistry of β-Lactam Antibiotics*, eds. P. H. Bentley and R. Southgate, The Royal Society of Chemistry, London, 1989, pp. 77–90; (*d*) K. A. Alvi, C. D. Reeves, J. Peterson, J. Lein, *J. Antibiotics*, 1995, **48**, 338.
3 M. Ochiai, A. Morimoto, Y. Matsushita, T. Okada, *J. Antibiotics*, 1981, **34**, 160.
4 (*a*) V. V. Shcherbakov, L. B. Krivdin, G. A. Kalabin and B. A. Trofimov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1986, 1210; (*b*) C. V. Zinchenko, R. Yu. Kiselev and L. B. Krivdin, *Zh. Org. Khim.*, 1991, **27**, 1233; (*c*) K. Kamieńska-Trela, *^{13}C-^{13}C Spin-Spin Coupling Constants* in *Annual Reports on NMR Spectroscopy*, ed. G. A. Webb, Academic Press, London, 1995, vol. **30**, pp. 131–230.
5 Ger. Offen. 1,955,475/1970 (*Chem. Abstr.*, 1970, **73**, 25 453).
6 J. Pankowski and J. Winiarski, *Org. Prep. Proc. Int.*, 1994, **26**, 327.
7 R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper, F. L. José, I. G. Wright, E. M. Van Heyningen and G. W. Huffman, *J. Org. Chem.*, 1971, **36**, 1259.
8 R. Bucourt, R. Heymes, A. Lutz, L. Penaste and J. Perronnet, *Tetrahedron*, 1978, **34**, 2233.
9 M. Steude, *Liebigs Ann. Chem.*, 1891, **261**, 30.
10 H. Takasugi, H. Kochi, T. Masugi, H. Nakano and T. Takaya, *J. Antibiotics*, 1983, **36**, 846.

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