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Evidence of the structure of 4-(4′-acetoxybenzylidene)-2-methyl-5-oxazolone and its phenylpropenoic acid derivatives

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Abstract

4-(4′-Acetoxybenzylidine)-2-methyl-5-oxazolone (*Z*-isomer) was synthesized, which upon basic hydrolysis yielded the unexpected 2-acetoxy-3-(*p*-hydroxyphenyl)-propenoic acid and treatment with acetic acid formed 2-acetoxy-3-(*p*-acetoxyphenyl)-propenoic acid. The structures of the three compounds were assigned by NMR spectroscopy. An X-ray crystallographic study of the starting azlactone confirmed its structure and supported the fact that the *Z*-configuration was retained during the synthesis of the phenylpropenoic acid derivatives.

Keywords: NMR; X-ray; Azlactones; Phenylpyruvic acids; Phenylpropenoic acids; Macrophage migration inhibitory factor

1. Introduction

This study is a continuation of our interest in the synthesis of phenylpyruvic acids and derivatives via the basic or acidic hydrolysis of their corresponding azlactones. Compounds of this type are potential substrates or inhibitors of the phenylpyruvate tautomerase activity catalyzed by the macrophage migration inhibitory factor (MIF) [1–6]. Our study on phenylpyruvic acids has so far revealed that different substituents (e.g. Cl, NO_2…) as well as their positions in the aromatic ring have an influence on their keto/enol ratio, with the enol form always predominating [7]. NMR studies have further shown that the solvent and temperature also influence the keto/enol ratio [8]. In the case of the synthesis of *p*-hydroxyphenylpyruvic acid, interesting results were observed. The basic hydrolysis of 4-(4′-acetoxybenzylidine)-2-methyl-5-oxazolone (*Z*-isomer) **2** did not yield the expected phenylpyruvic acid, but its enol acetate **3** (Synthetic scheme). Conversion of **2** to the enol acetate derivative **4** (Synthetic scheme), by treatment with acetic acid, a method developed in our laboratory for the preparation of enol acetates [9], made it possible to confirm the structure of the enol acetate **3** by NMR spectroscopy. Compound **4** not only has the enol acetate but also an aromatic acetate group. This study, supported by previous studies

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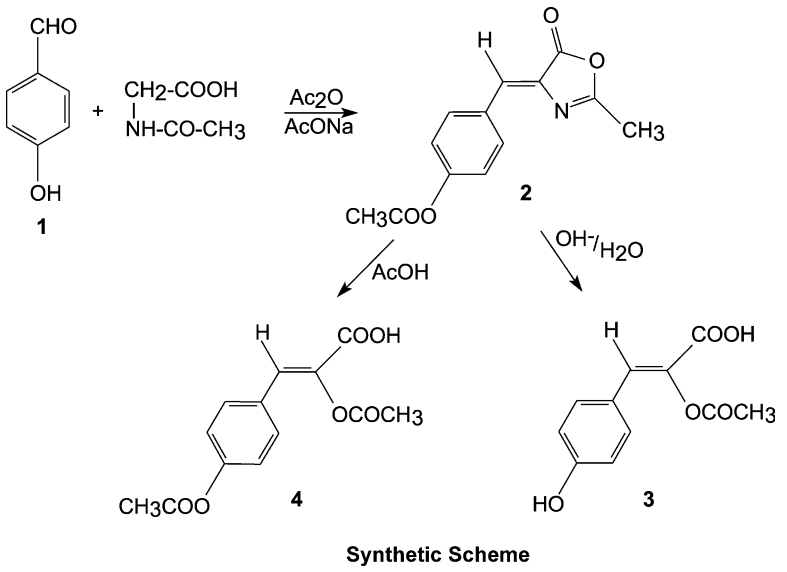

Scheme 1.

[9–11], has now shown that NMR spectroscopy can not only be used to distinguish between these two kinds of esters but also to confirm the structures of these types of phenylpyruvic acid (phenylpropenoic acid) derivatives. An X-ray crystallographic study was also performed on **2** in order to confirm the spectroscopic findings (Scheme 1).

2. Experimental

2.1. Synthesis

The synthesis of the azlactone (*Z*), **2**, involved the Erlenmeyer method [9,11–13] for the preparation of aromatic azlactones, starting from *p*-hydroxybenzaldehyde and *N*-acetylglycine. Basic hydrolysis of **2**, surprisingly yielded **3**, the enol acetate derivative instead of the enol form of the phenylpyruvic acid, which we expected to get. The diacetoxy derivative, **4**, was obtained from **2** by treatment with acetic acid.

*2.1.1. Synthesis of 4-(4′-acetoxybenzylidene)-2-methyl-5-oxazolone, **2***

A mixture of *p*-hydroxybenzaldehyde (12.2 g; 0.1 mol), *N*-acetylglycine (11.7 g; 0.1 mol), anhydrous sodium acetate (16.4 g; 0.2 mol) and acetic anhydride (50 ml) was stirred for 2.5 h at 105–110 °C. The reaction mixture was poured into a mixture (120 ml) of equal volumes of ethanol and water and stirred for 30 min. The product that precipitated out was filtered off, washed with the ethanol/water mixture and dried (15.2 g; 0.062 mol). Recrystallization from methanol gave yellow crystals of **2**, m.p. 140–143 °C.

*2.1.2. Synthesis of 2-acetoxy-3-(p-hydroxyphenyl)-propenoic acid, **3***

The azlactone **2** (2.0 g; 0.0082 mol) was boiled gently under reflux in a sodium hydroxide solution (20%; 20 ml). After 2 h, the mixture was cooled and acidified with hydrochloric acid (6 M) to a pH of 1.4, stirred well and allowed to cool in a refrigerator overnight. The crystals that formed were filtered off, washed with water and dried (0.73 g; 0.0033 mol). Recrystallization from water gave off-white crystals of **3**, decomposing at 198–200 °C.

*2.1.3. Synthesis of 2-acetoxy-3-(p-acetoxyphenyl)-propenoic acid, **4***

The azlactone **2** (3.0 g; 0.012 mol) was boiled under reflux in acetic acid (90%, 30 ml) for 4 h. Water (30 ml) was added and the mixture cooled in ice. The crystals that formed were filtered off, washed with water and dried (1.87 g; 0.0071 mol). Recrystallization from a benzene/methanol (1:2) mixture gave

Table 1
^{1}H NMR data (δ, ppm) of **2**, **3** and **4**

Hydrogen atom	**2**	**3**	**4**
H-2, H-6 (Ar)[a]	8.21 (d, $J_0 = 8.7$)	7.51 (d, $J_0 = 8.7$)	7.67 (d, $J_0 = 8.5$)
H-3, H-5 (Ar)	7.27 (d, $J_0 = 8.7$)	6.81 (d, $J_0 = 8.7$)	7.17 (d, $J_0 = 8.5$)
Ar–CH=C	7.23 (s)	7.22 (s)	7.25 (s)
CH_3–C=N	2.39 (s)	–	–
CH_3–C=O (Ar)	2.30 (s)	–	2.27 (s)
CH_3–C=O (En)	–	2.00 (s)	2.01 (s)

[a] Ar: aromatic, En: enolic, d: doublet, s: singlet, J_0 in Hz.

cream-coloured crystals of **4**, m.p. 227–228 °C (decomp.)

2.2. Spectroscopic analysis

NMR analyses of compounds **2**, **3** and **4** were performed on a Bruker Avance 300 spectrometer, using DMSO-*d*6 as solvent and TMS as internal standard (Tables 1 and 2). The melting points (uncorrected) were determined on an electrothermal melting point apparatus.

2.3. X-ray analysis

Yellow crystals of **2** were obtained from methanol. The selected crystal (dimensions $0.37 \times 0.22 \times 0.07\ mm^3$) was mounted on an Enraf-Nonius CAD-4 diffractometer using graphite-monochromated CuKα radiation. Lattice parameters were determined by least-squares refinement of 25 reflections with $18° < \theta < 32°$. The intensities were collected with $\omega - 2\theta$ scan mode, up to $\theta = 65°$. The intensities of two standard reflections, measured every 90°, showed no significant deviation. The data were corrected for Lorentz and polarization effects but not for absorption ($\mu = 8.81\ cm^{-1}$). The structure was solved by direct methods using MolEN [14] and refined anisotropically (Table 3). It was determined that the structure corresponded to the *Z* azlactone. The final residuals were $R = 0.0633$ (all data) and $R = 0.0486$ [$I > 2\sigma(I)$]. The final fractional coordinates are listed in Table 4. The bond lengths and angles are given in Tables 5 and 6. Selected torsion angles are shown in Table 7. The molecular structure with the atomic numbering scheme adopted, is shown in Fig. 1.

3. Results

3.1. Spectroscopic analysis

The ^{1}H NMR data of **2**, **3** and **4** appear in Table 1. The single proton signal at $\delta = 7.3$ for **2** is the characteristic absorption of the benzylic proton (Ar–**CH**=C), confirming the *Z*-configuration of this azlactone. The chemical shift value of the benzylic proton can also be used to distinguish between the *Z* and *E* isomers (further down field at $\delta \approx 7.5$) of these

Table 2
^{13}C NMR data (δ, ppm) of **2**, **3** and **4**

Carbon atom	**2**	**3**	**4**
CH=C–*C*=O	167.2 (s)	–	–
CH_3–*C*=N	166.7 (s)	–	–
CH_3–*C*=O (Ar)[a]	168.8 (s)	–	169.0 (s)
CH_3–*C*=O (En)	–	169.40 (s)	169.3 (s)
*C*OOH	–	166.7 (s)	166.3 (s)
C-1 (Ar)	132.3 (s)	124.0 (s)	127.3 (s)
C-2, C-6 (Ar)	133.1 (d)	131.9 (d)	130.9 (d)
C-3, C-5 (Ar)	122.3 (d)	115.5 (d)	122.0 (d)
C-4 (Ar)	152.2 (s)	158.7 (s)	150.8 (s)
Ar–CH=*C*	130.6 (s)	124.6 (s)	131.3 (s)
Ar–*C*H=C	128.7 (d)	132.6 (d)	130.1 (d)
CH_3–C=O (Ar)	20.7 (q)	–	20.8 (q)
CH_3–C=O (En)	–	22.6 (q)	22.5 (q)
CH_3–C=N	15.2 (q)	–	–

[a] Ar: aromatic, En: enolic, s: singlet, d: doublet, q: quartet.

Table 3
Crystal data and structure refinement for **2**

Chemical formula	$C_{13}H_{11}NO_4$
Formula weight	245.23
Temperature	293(2) K
Wavelength	1.54180 Å
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions	$a = 26.522(2)$ Å, $\alpha = 90°$
	$b = 11.783(1)$ Å, $\beta = 90°$
	$c = 7.457(1)$ Å, $\gamma = 90°$
Volume	2330.4(4) Å^3
Z	8
Calculated density	1.398 mg m^{-3}
Absorption coefficient	0.881 mm^{-1}
$F(000)$	1024
Crystal size	$0.37 \times 0.22 \times 0.07$ mm^3
Theta range	3–65°
Index ranges	$2 \le h \le 30$, $0 \le k \le 13$, $0 \le l \le 8$
Reflections collected/unique	1844/1844
Completeness to $2\theta = 65°$	93.0%
Max. and min. transmission	0.9409 and 0.7365
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1844/0/165
Goodness-of-fit on F^2	1.050
Final R indices $[I > 2\sigma(I)]$	$R = 0.0486$, $R_w^2 = 0.1266$
R indices (all data)	$R = 0.0633$, $R_w^2 = 0.1394$
Extinction coefficient	0.0059(6)
Largest diff. peak and hole	0.299 and −0.280 eÅ^{-3}

Table 4
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å$^2 \times 10^3$) for **2**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor

	x	y	z	U(eq)
C(1)	7935(1)	3849(2)	−1111(3)	38(1)
C(2)	7759(1)	2867(2)	−273(3)	41(1)
C(3)	7259(1)	2750(2)	205(3)	41(1)
C(4)	6929(1)	3619(2)	−195(3)	40(1)
C(5)	7090(1)	4611(2)	−980(3)	44(1)
C(6)	7594(1)	4728(2)	−1414(3)	44(1)
C(7)	8453(1)	3991(2)	−1723(3)	43(1)
C(8)	8797(1)	3188(2)	−2068(3)	41(1)
C(9)	9299(1)	3437(2)	−2822(4)	52(1)
O(10)	9512(1)	2382(1)	−3155(3)	57(1)
C(11)	9162(1)	1593(2)	−2588(3)	48(1)
N(12)	8753(1)	2003(2)	−1951(3)	45(1)
O(13)	9521(1)	4298(2)	−3118(3)	79(1)
C(14)	9309(1)	399(2)	−2791(5)	67(1)
O(15)	6419(1)	3612(1)	304(2)	52(1)
C(16)	6136(1)	2660(2)	203(4)	50(1)
O(17)	6290(1)	1783(2)	−337(4)	91(1)
C(18)	5609(1)	2893(3)	815(4)	62(1)

Table 5
Bond lengths (Å) for **2**

C(1)–C(6)	1.393(3)
C(1)–C(2)	1.396(3)
C(1)–C(7)	1.458(3)
C(2)–C(3)	1.379(3)
C(3)–C(4)	1.380(3)
C(4)–C(5)	1.375(3)
C(4)–O(15)	1.404(2)
C(5)–C(6)	1.382(3)
C(7)–C(8)	1.340(3)
C(8)–N(12)	1.404(3)
C(8)–C(9)	1.474(3)
C(9)–O(13)	1.193(3)
C(9)–O(10)	1.387(3)
O(10)–C(11)	1.381(3)
C(11)–N(12)	1.279(3)
C(11)–C(14)	1.468(3)
O(15)–C(16)	1.351(3)
C(16)–O(17)	1.181(3)
C(16)–C(18)	1.496(3)

Table 6
Bond angles (°) for **2**

C(6)–C(1)–C(2)	118.2(2)
C(6)–C(1)–C(7)	118.4(2)
C(2)–C(1)–C(7)	123.4(2)
C(3)–C(2)–C(1)	121.3(2)
C(2)–C(3)–C(4)	118.7(2)
C(5)–C(4)–C(3)	121.7(2)
C(5)–C(4)–O(15)	114.7(2)
C(3)–C(4)–O(15)	123.3(2)
C(4)–C(5)–C(6)	119.0(2)
C(5)–C(6)–C(1)	121.0(2)
C(8)–C(7)–C(1)	128.4(2)
C(7)–C(8)–N(12)	129.3(2)
C(7)–C(8)–C(9)	123.2(2)
N(12)–C(8)–C(9)	107.3(2)
O(13)–C(9)–O(10)	121.9(2)
O(13)–C(9)–C(8)	133.2(2)
O(10)–C(9)–C(8)	104.9(2)
C(11)–O(10)–C(9)	105.9(2)
N(12)–C(11)–O(10)	115.4(2)
N(12)–C(11)–C(14)	128.8(2)
O(10)–C(11)–C(14)	115.7(2)
C(11)–N(12)–C(8)	106.4(2)
C(16)–O(15)–C(4)	121.7(2)
O(17)–C(16)–O(15)	123.7(2)
O(17)–C(16)–C(18)	125.9(2)
O(15)–C(16)–C(18)	110.4(2)

Table 7
Selected torsion angles (°) for **2**

C(6)–C(1)–C(7)–C(8)	− 158.3(2)
C(1)–C(7)–C(8)–C(9)	174.9(2)
C(5)–C(4)–O(15)–C(16)	145.2(2)
C(4)–O(15)–C(16)–C(18)	180.0(2)

kind of 2-methyl azlactones [15,16]. The absorption of this proton can be easily observed in the methyl azlactones than in the phenyl azlactones, as the absorption of the extra aromatic protons in the latter compounds tend to mask the absorption of the benzylic proton.

With compound **3**, where we expected the enol form of 4-hydroxyphenylpyruvic acid to form, its ^{1}H NMR spectrum clearly showed that, instead, the enol acetate was formed. The three-proton singlet at $\delta = 2.00$ is characteristic of the methyl protons of the enol acetate. The benzylic proton of the enol acetate absorbs at $\delta = 7.22$ which is similar to values found for similar derivatives ($\delta = 7.1–7.6$) [9–11]. The benzylic proton of phenylpyruvic acids (enol form) absorbs higher upfield ($\delta = 6.37–6.8$). This is further confirmed by the ^{13}C NMR spectrum of **3** which shows the absorption of 11 carbon atoms and not nine as it would have been the case for the phenylpyruvic acid. The benzylic carbon of the enol acetate absorbs at $\delta = 132.6$ (chemical shift values of $\delta = 120–133$ were reported for similar enol acetates) while the same proton of phenylpyruvic acids (enol form) absorbs higher upfield (104–112 ppm) [8]. These values are also in agreement with values obtained for the enol acetate of the 4-hydroxy-3-methoxy derivative [10].

The structure of the diacetoxy derivative, **4**, as enol acetate, was confirmed by its ^{1}H and ^{13}C NMR spectra, which further supports the structure of **3**. ^{1}H NMR spectral values of the methyl protons of the aromatic acetate group ($\delta \approx 2.30$) and the enolic acetate group ($\delta \approx 2.00$) can be used to confirm the presence of, or distinguish between these two groups. The ^{13}C NMR chemical shift values of these methyl carbons can also be effectively used to distinguish between these two esters, as the aromatic ester gives a quartet at $\delta \approx 20.7$ and the enolic ester, a quartet at $\delta \approx 22.6$. The chemical shift values of the carbonyl carbons of these esters are very close and could not be used to distinguish between them.

The similar absorption values for the benzylic proton at $\delta = 7.23$, 7.22 and 7.25 for **2**, **3** and **4**, respectively, can therefore be used to confirm the

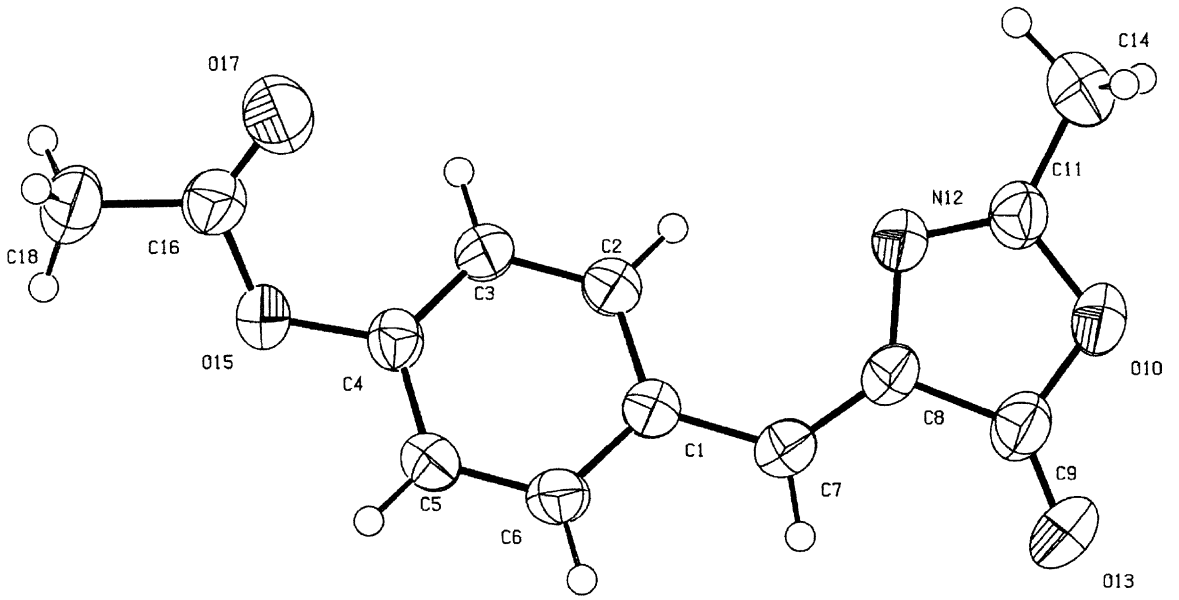

Fig. 1. ORTEP view of **2**.

existence of the *Z*-configuration of such compounds. The ^{13}C chemical shift values for the same benzylic carbon at $\delta = 128.7$, 132.6 and 130.1 for **2**, **3** and **4**, respectively, also seem to be good indicators for the confirmation of the *Z*-configurations.

3.2. X-ray analysis

Bond lengths and angles of the azlactone **2** have the suspected values. The *Z*-configuration was confirmed by this single crystal X-ray analysis. The N(12) nitrogen atom and the aromatic ring are on the same side of the C(7)–C(8) double bond. The relative orientation of the ester group versus the aromatic ring is defined by the two torsion angles C(5)–C(4)–O(15)–C(16) = 145.2(2)° and C(4)–O(15)–C(16)–C(18) = 180.0(2)°.

4. Discussion

The basic hydrolysis of the azlactone (*Z*) **2**, yields the enol acetate derivative **3**. The same phenomenon is observed with the 4-hydroxy-3-methoxy derivative [9]. The enolic hydroxyl group seems to react readily with the acetate group that becomes available during hydrolysis of the aromatic acetate group and the azlactone ring. Whereas the aromatic acetate is easily hydrolyzed, the enol acetate must be quite stable to form under these conditions and to resist hydrolysis. However, we found that the 3-hydroxy-4-methoxy substituted derivative does form the enol form of the phenylpyruvic acid when hydrolyzed under similar conditions [8]. It appears that the enol acetate is less stable when the phenolic hydroxyl is in the 3-position and therefore hydrolyzes under these conditions. This was confirmed using an AMPAC energy calculation on both enol acetates, of 4-hydroxy and 3-hydroxy compounds, the former appearing to be the most stable one.

The structure of **4** was clearly established using NMR spectroscopy. However, the mechanism for its formation remains unclear. Further investigation is currently in progress to establish the origin of the enol acetate group, i.e. whether its source is from the acetic acid or from the azlactone ring. The data from the X-ray crystal study of the azlactone **2** confirmed the existence of the *Z* isomer and that the ester group is in the same plane as the aromatic ring.

Finally, the macrophage MIF, a cytokine implicated in a number of immune and inflammatory processes, is known for interconverting the enol and keto isomers of both phenylpyruvate and *p*-hydroxyphenylpyruvate. Crystal structures of MIF complexed with competitive inhibitors have shown that the substrates exist as enols (*E*-isomers) [17,18]. Following a molecular modeling process, work is in progress to synthesize phenylpyruvic acid derivatives and parent compounds with the (*E*)-configuration as potential inhibitors of the phenylpyruvic tautomerase activity of MIF.

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References

[1] T. Calandra, R. Bucula, Crit. Rev. Immunol. 17 (1997) 77.
[2] S.L. Stamps, A.B. Taylor, S.C. Wang, M.L. Hackert, C.P. Whitman, Biochemistry 39 (2000) 9671.
[3] M. Orita, S. Yamamoto, N. Katayama, M. Aoki, K. Takayama, Y. Yamagiwa, N. Seki, H. Suzuki, H. Kurihara, H. Sakashita, M. Takeuchi, S. Fujita, T. Yamada, A. Tanaka, J. Med. Chem. 44 (2001) 540.
[4] A. Dios, R.A. Mitchell, B. Aljabari, J. Lubetsky, K. O'Connor, H. Liao, P.D. Senter, K.R. Manogue, E. Lolis, C. Metz, R. Bucala, D.J.E. Callaway, Y. Al-Abed, J. Med. Chem. 45 (2002) 2410.
[5] P.D. Senter, Y. Al-Abed, C.N. Metz, F. Benigni, R.A. Mitchell, J. Chesney, J. Han, C.G. Gartner, S.D. Nelson, G.J. Todaro, R. Bucala, Proc. Natl. Acad. Sci. USA 99 (2002) 144.
[6] J.B. Lubetsky, A. Dios, J. Han, B. Aljabari, B. Ruzsicska, R. Mitchell, E. Lolis, Y. Al-Abed, J. Biol. Chem. 277 (2002) 24976.
[7] A.J.M. Carpy, P.P. Haasbroek, J. Ouhabi, D.W. Oliver, J. Mol. Struct. 520 (2000) 191.
[8] P.P. Haasbroek, PhD Thesis, Potchefstroom University for C.H.E., Potchefstroom, South Africa (1997).
[9] P.P. Haasbroek, D.W. Oliver, A.J.M. Carpy, J. Chem. Crystallogr. 28 (1998) 139.
[10] P.P. Haasbroek, D.W. Oliver, A.J.M. Carpy, J. Chem. Crystallogr. 28 (1998) 193.

[11] D.W. Oliver, P.P. Haasbroek, J.M. Léger, A.J.M. Carpy, J. Chem. Crystallogr. 24 (1994) 665.
[12] H.E. Carter, Org. React. 3 (1946) 199.
[13] P.P. Haasbroek, D.W. Oliver, A.J. Carpy, J. Chem. Crystallogr. 28 (1998) 811.
[14] MolEN, An Interactive Structure Solution Procedure, Enraf-Nonius, Delft, The Netherlands, 1990.
[15] R. Glaser, M. Twaik, Tetrahedron Lett. 15 (1976) 1219.
[16] A.P. Morgenstern, C. Schutij, W.T. Nauta, Chem. Commun. (1969) 321.
[17] J.B. Lubetsky, M. Swope, C. Dealwis, P. Blake, E. Lolis, Biochemistry 38 (1999) 7346.
[18] A.B. Taylor, W.H. Johnson Jr., R.M. Czerwinski, H.S. Li, M.L. Hackert, C.P. Whitman, Biochemistry 38 (1999) 7444.