

Charge density study of *N*-acetyl-L-tyrosine ethyl ester monohydrate derived from CCD area detector data

S. DAHAOUI,^{a*} C. JELSCH,^b J. A. K. HOWARD^a AND C. LECOMTE^b

^aCrystallography Group, Department of Chemistry, University of Durham, South Road, Durham DH1 3LE, England, and ^bLaboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques (LCM³B), ESA CNRS 7036, Université Henri Poincaré, Nancy 1, Faculté des Sciences, BP 239, 54506 Vandoeuvre lès Nancy CEDEX, France. E-mail: slimane.dahaoui@durham.ac.uk

(Received 23 May 1998; accepted 22 September 1998)

Abstract

The crystal structure, thermal vibrations and electron density of the peptide *N*-acetyl-L-tyrosine ethyl ester monohydrate, C₁₃H₁₇NO₄·H₂O, have been analysed using single-crystal X-ray diffraction data collected at 110 K with Mo *K*α radiation to a resolution of $(\sin \theta/\lambda)_{\max} = 1.1 \text{ \AA}^{-1}$. A CCD area detector was used to collect 98 393 data during one week. A multipolar atom density model was fitted against the 10 189 unique data with $I > 2\sigma(I)$ [$R(F) = 0.027$, $wR(F) = 0.020$, g.o.f. = 0.65] in order to map the valence electron distribution. These deformation densities compare very well with those obtained from conventional diffractometers equipped with scintillation detectors. This work shows that area detectors permit charge density studies in a more routine way than is possible with conventional diffractometers.

1. Introduction

It is well known that charge density studies need accurate X-ray diffraction measurements and careful data reduction. This step, which requires considerable experimental expertise, is very time consuming when using a conventional diffractometer equipped with a one-point-scintillation detector. Two-dimensional (2D) detectors like CCDs (charge-coupled devices) or imaging plates are widely used in protein crystallography and are becoming more and more popular for routine crystal structure determinations (2–24 h data collection times). However, until recently very few electron density studies have been carried out using 2D-detector data. The first experiments using conventional X-ray sources and 2D detectors devoted to charge density studies were discussed in a session of the XI Sagamore conference (August 1997, Prince Albert, Canada) by Coppens (1997) and Pinkerton (1997), and at the BCA Spring Meeting (April 1998, St Andrews, Scotland) by Dahaoui *et al.* (1998). Other experiments using synchrotron radiation at HASYLAB, Hamburg, Germany, (Koritsanszky *et al.*, 1998) and at the ESRF, Grenoble, France, (Graafsma *et al.*, 1998) have estab-

lished the possibility of carrying out accurate measurements for charge density work.

In this paper we discuss the results of one of the first accurate charge density studies obtained from a CCD-based diffractometer: we show that this seven-day data set gives precise details of the charge density which would have needed at least one month of data collection time on a conventional diffractometer.

The results of the study of *N*-acetyl-L-tyrosine ethyl ester monohydrate will be archived in our charge-density data bank of peptides and related compounds (Pichon-Pesme *et al.*, 1995; Jelsch *et al.*, 1998). Indeed, the fine description of the electron density can be extended to structures of lower resolution by applying the notion of transferability of the charge and density parameters.

2. Data collection and data reduction analysis

The crystal structure at room temperature of *N*-acetyl-L-tyrosine ethyl ester monohydrate was first reported by Pieret *et al.* (1972) ($R = 0.107$) and refined (to $R = 0.052$) by Soriano-García (1993).

A good quality crystal (0.3 × 0.2 × 0.2 mm) was selected for the X-ray diffraction study. A preliminary short and quick data collection on a Siemens SMART CCD-based diffractometer (50 kV and 40 mA) at room temperature with Mo *K*α radiation confirmed without ambiguity that it was orthorhombic, space group $P2_12_12_1$. This room-temperature data collection was performed as follows: the data covered a sphere of reciprocal space by combining four sets of runs; each set had a different φ angle (0, 88, 180 and 268°) and each frame covered 0.3° in ω for 10 s exposure time. The crystal-to-detector distance was 4.51 cm. The data were 99% complete to 55° 2 θ [$(\sin \theta/\lambda) = 0.77 \text{ \AA}^{-1}$]. Crystal decay was monitored by repeating the 50 initial frames at the end of the data collection and analysing the duplicate reflections. Cell parameters [$a = 7.3827(2)$, $b = 13.1421(4)$, $c = 14.5150(4) \text{ \AA}$] were retrieved using SMART software (Siemens, 1995) on 411 selected reflections. Data reduction was performed using SAINT

Table 1. A summary of the 110 K X-ray data collection strategy

Run No.	2θ	ω_0	φ	χ	Frames	Time per frame (s)
0†	-29	-26	0	54.70	909	20
1†	-29	-21	88	54.70	635	20
2†	-29	-26	0	54.70	50	20
3†	-29	-26	180	54.70	909	20
4†	-29	-21	268	54.70	635	20
5†	-29	-26	0	54.70	50	20
12	-29	-26	0	54.70	909	20
13	-29	-21	45	54.70	635	20
14	-29	-26	0	54.70	50	20
15	-29	-26	135	54.70	909	20
16	-29	-21	225	54.70	635	20
17	-29	-26	0	54.70	50	20
18	+29	-26	0	54.70	200	20
19	-74	-66	0	54.70	901	40
20	-74	-66	90	54.70	901	40
21	-74	-66	180	54.70	901	40
22	-74	-66	270	54.70	901	40
23	-74	-66	45	54.70	451	40
24	-74	-66	135	54.70	451	40
25	-74	-66	225	54.70	451	40
26	-74	-66	315	54.70	451	40

† Frame collection repeated twice.

software (Siemens, 1995), which corrects for Lorentz and polarization effects, and decay. The structure was solved by direct methods using *SHELXS86* (Sheldrick, 1990) and refined by a least-squares method on F^2 using *SHELXL93* (Sheldrick, 1993). All H atoms (including the H atoms of the water molecule) were located by difference Fourier synthesis. The refined parameters included anisotropic mean-square displacements for non-H atoms, and positions and isotropic mean-square displacements for H atoms [$R(F^2) = 0.038$]. Details of the experiment, fractional coordinates, displacement parameters, bond distances and angles have been deposited as supplementary material.†

As the room-temperature crystal structure was of good quality and as intense diffraction was observed for high 2θ values, this crystal was deemed suitable for a charge density study. The crystal was cooled to 110 K with an Oxford Cryostream N₂ open-flow cryostat and the unit-cell parameters were refined. The *ASTRO* program (Siemens, 1995) was then used to check the completeness to a given resolution with the required level of redundancy. Then two batches of data were collected: the first one at low θ covered the whole sphere of reciprocal space up to 55° in 2θ and the second (high-order) batch was 93.8% complete to at least 109° in 2θ . Low-angle frames were collected for 20 s and high-angle frames for 40 s in order to increase the precision of the measurements and to compensate for the decrease of the diffracted intensity at high 2θ values. Each run had a different φ angle and each frame covered 0.2° in ω in

order to obtain accurate intensity profiles. The first low-order runs were repeated because the valence electron density information is carried by the low-angle reflections. The complete data-collection strategy is summarized in Table 1. A total of 9764 frames were collected over a period of seven days.

The unit cell was then refined using 512 reflections of the low-angle batch only, with a threshold $I/\sigma(I) > 50$, and with a minimum and maximum resolution of 0.5 and 0.8 \AA^{-1} , respectively [cell parameters obtained: $a = 7.235(2)$, $b = 13.056(4)$, $c = 14.415(4) \text{ \AA}$]. Data reduction was performed as for the room-temperature data for Lorentz and polarization effects [*SAINTE*; Siemens (1995)]. The procedure of integration of frames is described by Kabsch (1993). Each three-dimensional peak profile was placed inside a three-dimensional box of a given size. The size of the box is constant for all low-angle frames and 1.5 times larger in terms of peak width (x and y directions) for high-order frames because of the $K\alpha_1$ and $K\alpha_2$ splitting. Although absorption is negligible with Mo $K\alpha$ radiation ($\mu = 0.098 \text{ mm}^{-1}$), the intensities have been corrected for beam inhomogeneity and decay using *SADABS* (Sheldrick, 1996).

Of the 98 393 integrated reflections 610 were measured only once and 97 783 were multiple measurements which, on averaging using *SORTAV* (Blessing, 1987), gave 14 787 unique structure-factor amplitudes to a resolution of $(\sin \theta/\lambda)_{\max} = 1.1 \text{ \AA}^{-1}$. Internal agreement factors are $R_{\text{int}}(F^2) = 0.027$ and $wR(F^2) = 0.032$ [$w = 1/\sigma^2(F^2)$]. No problem from $\lambda/2$ contamination appeared in our data set and no systematically absent reflections were observed, in contrast to the case discussed in detail by Kirschbaum *et al.* (1997).

† Supplementary data for this paper are available from the IUCr electronic archives (Reference: AN0551). Services for accessing these data are described at the back of the journal.

Table 2. Bond-vibration analysis

Z^2 is the squared amplitude in the bond direction ($A-B$) and ΔZ^2 is the difference for the atom pair. $\langle X^2 \rangle$ is the mean-square amplitude perpendicular to the bond. The bond lengths have been corrected for rigid motion (Busing & Levy, 1964).

Atom A	Atom B	Z_A^2	Z_B^2	ΔZ^2	$\langle X_A^2 \rangle$	$\langle X_B^2 \rangle$	Corrected length (Å)
CA	N	0.0112	0.0109	-0.0004	0.0110	0.0132	1.4452
C	O	0.0129	0.0130	0.0001	0.0129	0.0191	1.2415
C	N	0.0127	0.0124	-0.0004	0.0130	0.0124	1.3473
C	C1	0.0119	0.0124	0.0005	0.0135	0.0251	1.5125
C2	O1	0.0105	0.0106	0.0002	0.0126	0.0250	1.2210
C2	O2	0.0112	0.0116	0.0004	0.0123	0.0166	1.3253
C2	CA	0.0092	0.0096	0.0004	0.0133	0.0119	1.5302
CD1	CG	0.0131	0.0127	-0.0004	0.0162	0.0125	1.3987
CD1	CE1	0.0123	0.0122	0.0000	0.0167	0.0163	1.3959
C3	O2	0.0098	0.0094	-0.0005	0.0219	0.0177	1.4530
C3	C4	0.0160	0.0163	0.0003	0.0188	0.0315	1.5051
CB	CA	0.0122	0.0121	-0.0001	0.0143	0.0106	1.5410
CB	CG	0.0112	0.0109	-0.0003	0.0148	0.0133	1.5139
CZ	CE1	0.0105	0.0106	0.0001	0.0155	0.0171	1.3971
CZ	OH	0.0109	0.0112	0.0003	0.0153	0.0222	1.3723
CE2	CZ	0.0116	0.0118	0.0002	0.0213	0.0148	1.3960
CD2	CE2	0.0119	0.0119	0.0000	0.0190	0.0212	1.3956
CD2	CG	0.0116	0.0114	-0.0002	0.0191	0.0131	1.3946

3. Refinement

The charge density model was obtained from the multipole model (*MOLLY*; Hansen & Coppens, 1978). In addition to a scale factor and the conventional atomic and displacement parameters, several atomic density deformation parameters were refined. The electron density of an atom is described by

$$\rho_{\text{atom}}(\mathbf{r}) = \rho_{\text{core}}(r) + P_v \kappa^3 \rho_v(\kappa r) + \sum_{l=0}^{l_{\text{max}}} \kappa'^3 R_l(\kappa' r) \sum_{m=0}^l P_{lm\pm} y_{lm\pm}(\theta, \varphi),$$

where ρ_{core} and ρ_v are the spherically averaged core and valence electron density, y_{lm} represents the multipolar spherical harmonic angular functions in real form, R_l represents the Slater-type radial functions and κ, κ' are the contraction/expansion coefficients of the perturbed density. The coordinates r, θ and φ refer to the local atom-centred Cartesian axes. P_v is the refined valence population parameter, which gives the charge transfer with respect to the number N_v of electrons in the valence orbitals of the atom; the net charge is $(N_v - P_v)$. P_{lm} are the multipolar population parameters.

The real and imaginary dispersion corrections to the form factors (Creagh & McAuley, 1992) were used in the structure-factor calculations. First a high-order (HO) refinement was performed, using 3060 reflections with $0.9 < \sin \theta/\lambda < 1.1 \text{ \AA}^{-1}$ and $I > 2\sigma(I)$, to obtain the best estimate of the atomic positions and displacement parameters of the non-H atoms. All xyz and U^{ij} were refined. Convergence was achieved at $R(F) = 0.064$, $wR(F) = 0.067$ and g.o.f. = 0.68. At the end of the HO refinement, the rigid-bond test (Hirshfeld, 1976) was

applied for non-H atoms: the test gives hopeful results with a maximum discrepancy $\Delta Z^2 = 7 \times 10^{-4} \text{ \AA}^2$ for the C2—O1 and C3—O2 bonds. The positional and isotropic displacement parameters of the H atoms were refined at low angle ($\sin \theta/\lambda < 0.5 \text{ \AA}^{-1}$), then the H atoms were shifted along the Csp^3-H , Csp^2-H , $OW-H$ and $N-H$ bond vectors to average bond-distance values determined from neutron-diffraction studies (Allen, 1986) of 1.085, 1.076, 0.96 and 1.032 Å, respectively. These distances were kept fixed during all further refinements. The multipolar refinement was carried out using all 10 189 reflections with $I > 2\sigma(I)$ with the following strategy: scale factor, P_v then κ, P_v and κ, P_{lm} then κ', xyz and U^{ij} for the non-H atoms. Then all the parameters were refined together with all multipolar parameters except κ' of the H atoms. At each step the refinement was cycled until convergence. The H-atom coordinates were also shifted to neutron-diffraction values at each step and H-atom isotropic displacement parameters were adjusted ($\sin \theta/\lambda < 0.5 \text{ \AA}^{-1}$). During the refinement no extinction correction was deemed necessary. In the multipolar refinements, the multipole expansion was truncated at the octopole level for all non-H atoms and one single dipole centered on the H atom along the C(O,N)—H bond was calculated. Besides the constraint of electroneutrality of the unit cell, the H atoms Hsp^2 linked to C atoms (CD1, CD2, CE1 and CE2) of the tyrosine group were assumed to have identical electron density deformations. At the end of the refinement, statistical factors were $R(F) = 0.027$, $wR(F) = 0.020$ [$w = 1/\sigma^2(F)$] and g.o.f. = 0.65 for 10 189 observed reflections [$I > 2\sigma(I)$] and $N_{\text{par}} = 544$; six reflections only had $(|F_{\text{obs}}| - |F_{\text{calc}}|/\sigma) > 3$. The results of a rigid-bond test are excellent, as shown in Table 2. It is

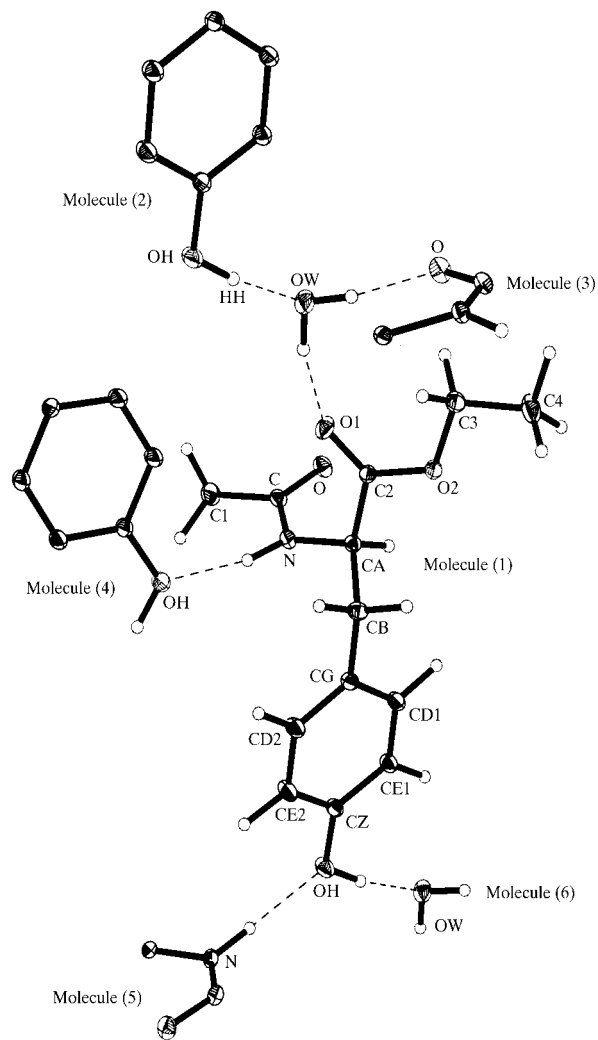
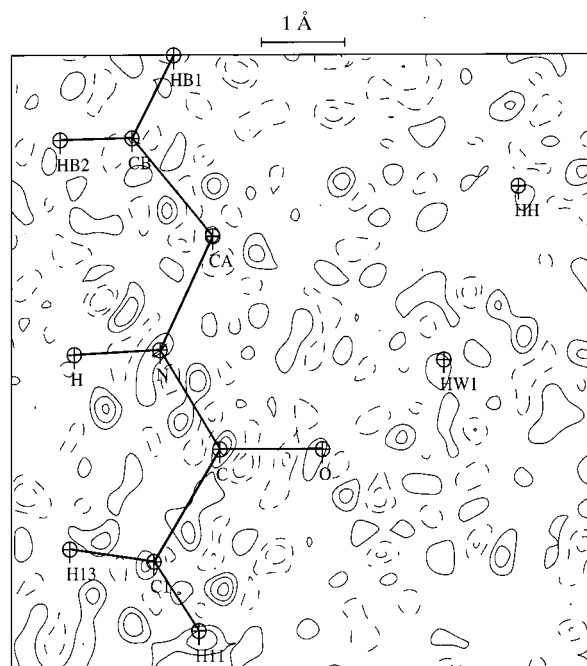


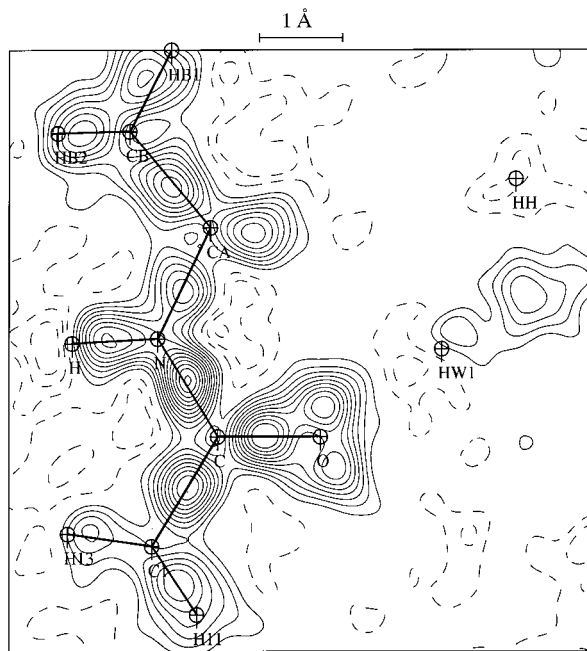
Fig. 1. ORTEP (Johnson, 1976) view of the packing of $C_{13}H_{17}NO_4 \cdot H_2O$ with 50% probability displacement ellipsoids for non-H atoms. Symmetry codes: molecule (1) x, y, z ; molecule (2) $x, 1 + y, z$; molecule (3) $-\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$; molecule (4) $-x, \frac{1}{2} + y, \frac{1}{2} - z$; molecule (5) $-x, -\frac{1}{2} + y, \frac{1}{2} - z$; molecule (6) $x, -1 + y, z$. Hydrogen bonds are indicated by dotted lines.

therefore clear that electron density parameters are deconvoluted from the displacement parameters. The low values of the g.o.f. at high order and at the end of the multipolar refinement show that all low- or high-resolution frames are integrated in the same way and the experimental s.u.'s are uniformly overestimated by the data reduction program *SAINT*. This underestimated weighting scheme may have some effect on tiny details of the deformation density. P_v, κ, P_{lm} and κ' parameters of the converged model have been deposited as supplementary material.†

† See deposition footnote on p. 227.



(a)



(b)

Fig. 2. (a) Residual density of the peptide bond. Non-zero contours are shown at intervals of $0.05 \text{ e } \text{Å}^{-3}$, negative contours are dashed. The residual density map is calculated by Fourier synthesis according to $\Delta\rho_{\text{res}}(\mathbf{r}) = (1/V) \sum_{\mathbf{H}} [|F_{\text{obs}}(\mathbf{H})| - |F_{\text{mul}}(\mathbf{H})|] \exp[i(\varphi_{\text{mul}} - 2\pi\mathbf{H}\cdot\mathbf{r})]$. (b) Experimental deformation density of the peptide bond calculated by Fourier synthesis according to $\Delta\rho_{\text{exp}}(\mathbf{r}) = (1/V) \sum_{\mathbf{H}} [|F_{\text{obs}}(\mathbf{H})| \exp(i\varphi_{\text{mul}}) - |F_{\text{sph}}(\mathbf{H})| \exp(i\varphi_{\text{sph}})] \exp(-2\pi i\mathbf{H}\cdot\mathbf{r})$. Contours are shown at intervals of $0.05 \text{ e } \text{Å}^{-3}$.

4. Discussion

Fig. 1 gives an *ORTEP* (Johnson, 1976) view of the local molecular packing. A critical examination of the residual density maps calculated for all data with $I > 2\sigma(I)$ permits discussion of the quality of the X-ray measurements and refinements. For example, Fig. 2(a) gives the residual experimental deformation density calculated in the peptide-link plane. Fig. 2(a) does not show any contour greater than $0.1 \text{ e } \text{Å}^{-3}$ [*i.e.* typically twice the estimated deformation electron density standard uncertainty in the unit cell: internal and external estimates of the average error in the experimental deformation maps (Cruickshank, 1949) are 0.08 and $0.05 \text{ e } \text{Å}^{-3}$, respectively], indicating that the noise level in the experimental data is low and confirming the high quality of the data.

The experimental deformation density in the same peptide-link plane as in Fig. 2(a) is given in Fig. 2(b). It is calculated using 7129 observed reflections with $\sin \theta/\lambda < 0.9 \text{ Å}^{-1}$ and $I > 2\sigma(I)$. The bonding density in the C—N, C—C1 and C—O bonds is 0.60 (5), 0.50 (5) and 0.55 (5) $\text{e } \text{Å}^{-3}$, respectively, and the O-atom lone pair is clearly observed. The average bonding density for the C—C bonds of the tyrosine group is $0.60 (5) \text{ e } \text{Å}^{-3}$. These results agree quantitatively with previous X-ray diffraction studies and with *ab initio* SCF deformation density maps (Stevens *et al.*, 1978; Swaminathan *et al.*, 1984; Lecomte *et al.*, 1992; Souhassou *et al.*, 1991, 1992). The experimental deformation density of the ester group and water molecule is also very well resolved and resembles those obtained for other peptides.

5. Conclusion

Precise electron density in molecular compounds can now be obtained with excellent accuracy in a much shorter time by using high-resolution low-temperature X-ray diffraction data from a CCD area detector. This will allow us to build a complete data bank of transferable experimental electron density parameters (*i.e.* aspherical scattering factors) for structures of lower resolution in the very near future.

References

- Allen, F. H. (1986). *Acta Cryst.* **B42**, 515–522.
 Blessing, R. H. (1987). *Crystallogr. Rev.* **1**, 3–58.

- Busing, W. R. & Levy, H. A. (1964). *Acta Cryst.* **17**, 142–146.
 Coppens, P. (1997). XI Sagamore Conference, Prince Albert, Canada, Abstract RA52, 65.
 Creagh, D. C. & McAuley, W. J. (1992). *International Tables for Crystallography*, Vol. C, edited by A. J. C. Wilson, pp. 206–222. Dordrecht: Kluwer Academic Publishers.
 Cruickshank, D. W. J. (1949). *Acta Cryst.* **2**, 65–82.
 Dahaoui, S., Jelsch, C., Howard, J. A. K. & Lecomte, C. (1998). BCA Spring Meeting, St Andrews, Scotland, 5–8 April. Abstract CP-7.
 Graafsma, H., Souhassou, M., Puig-Molina, A., Harkema, S., Kvik, Å. & Lecomte, C. (1998). *Acta Cryst.* **B54**, 193–195.
 Hansen, N. K. & Coppens, P. (1978). *Acta Cryst.* **A34**, 909–921.
 Hirshfeld, F. L. (1976). *Acta Cryst.* **A32**, 239–244.
 Jelsch, C., Pichon-Pesme, V., Lecomte, C. & Aubry, A. (1998). *Acta Cryst.* **D54**, 1306–1318.
 Johnson, C. K. (1976). *ORTEP*II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.
 Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
 Kirschbaum, K., Martin, A. & Pinkerton, A. A. (1997). *J. Appl. Cryst.* **30**, 514–516.
 Koritsanszky, T., Flaig, R., Zobel, D., Krane, H.-G., Morgenroth, W. & Luger, P. (1998). *Science*, **279**, 356–358.
 Lecomte, C., Ghermani, N., Pichon-Pesme, V. & Souhassou, M. (1992). *J. Mol. Struct.* **255**, 241–260.
 Pichon-Pesme, V., Lecomte, C. & Lachekar, H. (1995). *J. Phys. Chem.* **99**, 6246–6250.
 Pieret, A. F., Durant, F., Germain, G. & Koch, M. (1972). *Cryst. Struct. Commun.* **1**, 75–77.
 Pinkerton, A. A. (1997). XI Sagamore Conference, Prince Albert, Canada, Abstract RM50, 63.
 Sheldrick, G. M. (1990). *Acta Cryst.* **A46**, 467–473.
 Sheldrick, G. M. (1993). *SHELXL93. Program for the Refinement of Crystal Structures*. University of Göttingen, Germany.
 Sheldrick, G. M. (1996). *SADABS. Siemens Area Detector Absorption Correction Program*. University of Göttingen, Germany.
 Siemens (1995). *ASTRO and SAINT. Data Collection and Processing Software for the SMART System*. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA.
 Soriano-García, M. (1993). *Acta Cryst.* **C49**, 96–97.
 Souhassou, M., Lecomte, C., Blessing, R. H., Aubry, A., Rohmer, M. M., Wiest, R., Bénard, M. & Marraud, M. (1991). *Acta Cryst.* **B47**, 253–266.
 Souhassou, M., Lecomte, C., Ghermani, N., Rohmer, M. M., Wiest, R., Bénard, M. & Blessing, R. H. (1992). *J. Am. Chem. Soc.* **114**, 2371–2382.
 Stevens, E. D., Rys, J. & Coppens, P. (1978). *J. Am. Chem. Soc.* **100**, 2324–2329.
 Swaminathan, S., Craven, B. M., Spackman, M. A. & Stewart, R. F. (1984). *Acta Cryst.* **B40**, 398–404.