Acta Crystallographica Section A
Foundations of Crystallography
ISSN 0108-7673

Received 13 January 2006
Accepted 21 March 2006

Invarioms for improved absolute structure determination of light-atom crystal structures

B. Dittrich,[a]* M. Strumpel,[b] M. Schäfer,[c] M. A. Spackman[a] and T. Koritsánszky[d]

[a]Chemistry M313, School of Biomedical, Biomolecular and Molecular Sciences, University of Western Australia, Crawley 6009, Australia, [b]Institut für Chemie/Kristallographie der Freien Universität Berlin, Berlin, D-14195 Germany, [c]Schering AG Research Laboratories, Structural Biology, Müllerstrasse 178, Berlin, D-13342 Germany, and [d]Department of Chemistry, Middle Tennessee State University, MTSU Box 0395, 1301 East-Main Street, Murfreesboro, TN 37132-0001, USA. Correspondence e-mail: birger@cyllene.uwa.edu.au

The determination of molecular absolute configuration from an X-ray analysis for structures that contain only light elements is challenging owing to the weak anomalous dispersion signal. The achievable precision of the Flack x parameter for such structures is therefore limited, especially when the independent-atom model is employed. Invariom modelling can improve this situation. Invarioms are theoretically predicted pseudoatoms within the Hansen & Coppens multipole formalism. They are transferable from one molecule to another and provide generalized aspherical atomic form factors. It is shown that, by application of the invariom approach, the precision and standard uncertainty of the Flack x parameter and therefore the reliability of deducing molecular chirality in an absolute structure determination can be improved.

1. Introduction

Absolute configuration of light-atom structures is of critical importance in the pharmaceutical industry.

Although the phenomenon of chirality was known for many years, the relevance of the knowledge of the absolute structure of a drug applied to the human organism was not recognized until the Contergan/Thalidomide scandal in the early sixties. These events made clear that the absolute structure can be of utmost importance and that it should be carefully analysed for every drug candidate before registering it as a drug, even though it has later been found that Thalidomide racemizes *in vivo*. The proportion of single-enantiomer drugs among new drugs introduced into the market is rapidly growing and reached ≈70% in 2002 (Agranat *et al.*, 2002). Therefore, stringent legal requirements to investigate absolute configuration apply for drug molecules. A related aspect is the possibility to extend or sidestep patents by invoking chirality.

Single-crystal X-ray structure analysis allows the determination of the absolute structure *via* the intensity difference between Friedel pairs due to anomalous dispersion. Usually the aim of such an analysis is to assign absolute configuration to chiral molecules in a crystal structure and considerable effort has been devoted to this area of research. To resolve ambiguities when comparing the R factor for the inverted and the correct chiral structure, Rogers (1981) introduced the η parameter. This led to significant improvements in the determination of the molecular absolute configuration (Jones, 1984) or, synonymously, absolute chirality.[1] Finally, the introduction of an improved x parameter allowed the unambiguous assignment of enantiomorph polarity (Flack, 1983) and can be considered a breakthrough. Today Flack's x parameter is widely used and implemented in many least-squares refinement programs, for example *CRYSTALS* (Betteridge *et al.*, 2003), *SHELXL* (Sheldrick, 1997) and *GFMLX* (Bernardinelli & Flack, 1985). A more recent publication (Flack & Bernardinelli, 1999) clarified possible errors that can occur in such an analysis, including a useful glossary of terms, and an overview with practical hints can be found in another article by the same authors (Flack & Bernardinelli, 2000).

An absolute structure determination requires that the anomalous dispersion signal is sufficiently pronounced. The determination of the absolute configuration of organic molecules containing only light elements (C, H, N or O) is challenging and it is in most cases impossible to reliably assign absolute structure to a crystal structure that consists of organic molecules when no heavier atom is present.

The choice of a suitable wavelength at synchrotron sources can enhance the anomalous dispersion signal, unfortunately not without reducing the attainable resolution.

We have recently introduced the concept of invarioms (Dittrich *et al.*, 2004). By defining intermolecular transferable pseudoatoms (invarioms) within the Hansen & Coppens

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[1] For the use of the term 'absolute' in crystallography, see Glazer & Stadnicka (1989).

doi:10.1107/S0108767306010336

'aspherical atom' or 'multipole' formalism (Hansen & Coppens, 1978), the independent-atom model (IAM) can be replaced by the generalized invariom scattering model (Dittrich *et al.*, 2005). The invariom approach is based on the nearest/next-nearest-neighbour approximation that is also used in recent work by Koritsanszky *et al.* (2002). Thus, for each element, only a limited number of invarioms exists. A database has been created for invariom modelling and, although the invariom concept is in principle applicable to all elements, our database presently contains invarioms only for the elements H, C, N, O, P and S. Multipole populations of the invarioms were determined by first-principles calculations *via* theoretical structure factors from geometry-optimized model compounds. A detailed description of database construction and citations of preceding and related work can be found in our earlier paper (Dittrich *et al.*, 2005). In essence, invariom modelling provides an improved scattering-factor model. Therefore, the least-squares fit of aspherical scatterers to experimental structure factors leads to an improvement of the *R* factor and other figures of merit. Additionally, enhanced inversion-distinguishing power can result when the IAM model is replaced with invarioms.

This has an impact on the absolute structure determination because precision and standard uncertainty of a Flack-parameter determination can be significantly improved. After some theoretical remarks, we present results for four absolute structure determinations on light-atom structures measured with copper and molybdenum radiation, using the invariom database.

2. Theory

2.1. Least-squares refinement

For conventional least-squares refinement based on F^2, the following residual is minimized:

$$\varepsilon = \sum_{\mathbf{H}} w_{\mathbf{H}}[|F_o(\mathbf{H})|^2 - |F_c(\mathbf{H})|^2]^2. \tag{1}$$

This involves taking the partial derivatives of the calculated structure factors with respect to the refinable parameters, while taking into account their weighting scheme w (Rollett, 1970). Upon incorporation of a Flack x parameter defined in the following way (Flack & Bernardinelli, 1999),

$$\begin{aligned} |G(\mathbf{H},x)|^2 &= (1-x)|F_c(\mathbf{H})|^2 + x|F_c(\bar{\mathbf{H}})|^2 \\ &= |F_c(\mathbf{H})|^2 + x[|F_c(\bar{\mathbf{H}})|^2 - |F_c(\mathbf{H})|^2] \\ &= |F_c(\mathbf{H})|^2 - x\Delta_{\mathbf{H}}, \end{aligned} \tag{2}$$

where $\Delta_{\mathbf{H}}$ is $|F_c(\mathbf{H})|^2 - |F_c(\bar{\mathbf{H}})|^2$, the residual changes to

$$\begin{aligned} \varepsilon' &= \sum_{\mathbf{H}} w_{\mathbf{H}}[|F_o(\mathbf{H})|^2 - |G(\mathbf{H},x)|^2]^2 \\ &= \sum_{\mathbf{H}} w_{\mathbf{H}}[|F_o(\mathbf{H})|^2 - |F_c(\mathbf{H})|^2 + x\Delta_{\mathbf{H}}]^2 \\ &= \varepsilon + 2x\sum_{\mathbf{H}} w_{\mathbf{H}}[|F_o(\mathbf{H})|^2 - |F_c(\mathbf{H})|^2]\Delta_{\mathbf{H}} + x^2\sum_{\mathbf{H}} w_{\mathbf{H}}\Delta_{\mathbf{H}}^2. \end{aligned} \tag{3}$$

From equations (2) and (3), the role of $\Delta_{\mathbf{H}}$ is quite obvious and the smaller the values of $\Delta_{\mathbf{H}}$ are, the closer ε' is to ε, and the smaller the difference between $F_c(\mathbf{H})$ and $F_c(\bar{\mathbf{H}})$. Because of this, $\Delta_{\mathbf{H}}$ provides a measure of the inversion distinguishing power of the model, and it plays an important role in the discussion of the following sections. For determination of x, additional derivatives have to be added to the least-squares procedure; for example,

$$\frac{\partial\varepsilon'}{\partial x} = 2\sum_{\mathbf{H}} w_{\mathbf{H}}[|F_o(\mathbf{H})|^2 - |F_c(\mathbf{H})|^2]\Delta_{\mathbf{H}} + 2x\sum_{\mathbf{H}} w_{\mathbf{H}}\Delta_{\mathbf{H}}^2. \tag{4}$$

Although x is a linear parameter, refining it together with all other parameters is necessary to ascertain the standard uncertainty of x (Flack & Bernardinelli, 2000), and we discuss this further below.

2.2. Role of anomalous dispersion in the IAM structure factor

Within the kinematic theory of scattering, the complex structure factor $F(\mathbf{H})$ of a sum of density units k is given by

$$\begin{aligned} F(\mathbf{H}) &= \sum_k f_k(H)t_k(H)\exp(2\pi i\mathbf{H}\cdot\mathbf{r}_k) \\ &= \sum_k f_k(H)t_k(H)[\cos(2\pi\mathbf{H}\cdot\mathbf{r}_k) + i\sin(2\pi\mathbf{H}\cdot\mathbf{r}_k)] \\ &= \sum_k f_k(H)t_k(H)[\mathrm{c}_k + i\mathrm{s}_k], \end{aligned} \tag{5}$$

where t_k is the temperature factor, which can become complex when higher-order temperature-factor models are used to describe anharmonic thermal vibrations (Coppens, 1997). In this work, we limit ourselves to the harmonic treatment of thermal motion and, because the temperature factor is real in this approximation, it is omitted from the following discussion. For absolute structure determination, anomalous dispersion has to be considered in the expression for the atomic scattering factor f_k, the Fourier transform of the atomic electron density $\rho_k(\mathbf{r})$. When a structure contains one or more density units k that are anomalous scatterers, there is an imaginary component to the scattering factor. For the anomalous scatterers, f_k becomes

$$f_k = (f_k^{\mathrm{IAM}} + f_k') + if_k'' = a_k + ib_k. \tag{6}$$

The IAM structure factor including anomalous dispersion is given by

$$\begin{aligned} F(\mathbf{H}) &= \sum_k [a_k + ib_k][\mathrm{c}_k + i\mathrm{s}_k] \\ &= \sum_k a_k\mathrm{c}_k - \sum_k b_k\mathrm{s}_k + i\sum_k b_k\mathrm{c}_k + i\sum_k a_k\mathrm{s}_k \\ &= (A_1 - A_2) + i(B_1 + B_2). \end{aligned} \tag{7}$$

In an X-ray diffraction experiment, intensities are measured and, for refinement against F^2, the difference $\Delta_{\mathbf{H}}^{\mathrm{IAM}}$ between $|F(\mathbf{H})|^2$ and $|F(\bar{\mathbf{H}})|^2$, which is due to anomalous dispersion, becomes

$$\Delta_{\mathbf{H}}^{\mathrm{IAM}} = -4A_1A_2 + 4B_1B_2. \tag{8}$$

$\Delta_{\mathbf{H}}^{\mathrm{IAM}}$ is small in the case of a weak anomalous dispersion signal, which is typical for light-atom structures or when using Mo $K\alpha$ or shorter wavelengths.

2.3. Role of anomalous dispersion and odd-order multipoles

The description of the electron density of a pseudoatom in the Hansen & Coppens multipole model (Hansen & Coppens, 1978) is given by the following formula, and has been described in detail by Coppens (1997):

$$\begin{aligned}\rho_{\rm atom}(\mathbf{r}) &= \rho_{\rm core}(r) + P_{\rm val}\kappa^3\rho_{\rm val}(\kappa r)\\ &\quad + \sum_{l=1}^{l_{\max}} \kappa'^3 R_l(\kappa' r) \sum_{m=0}^{l} P_{lm\pm}\, y_{lm\pm}(\theta,\phi)\\ &= \rho_{\rm IAM}(r) + \delta\rho(r). \qquad (9)\end{aligned}$$

In this model, the total electron density is the sum of a spherical core, a spherical valence and an aspherical valence density. $y_{lm\pm}$ are the real spherical-harmonic functions and $l_{\max}$ is the order of the multipole expansion. The P's are populations and κ, κ' are expansion/contraction parameters. To facilitate the comparison when including anomalous dispersion, the electron-density expression can also be subdivided into an IAM and a deformation part, the latter being the difference between IAM and multipole models (*i.e.* the spherical and aspherical valence deformations). In an experimental charge-density study, the multipole parameters are determined by least-squares refinement with a suitably extensive data set. When using invarioms, they are transferred from the database and kept fixed. The scattering factor for a pseudoatom k of the Hansen & Coppens multipole model [equation (9)] is given by the Fourier transform of the pseudoatomic density,

$$\begin{aligned}f_k(\mathbf{H}) &= P_{k,\rm core} f_{k,\rm core}(H) + P_{k,\rm val} f_{k,\rm val}(H/\kappa)\\ &\quad + 4\pi \sum_{l=1}^{l_{\max}} i^l \langle j_l(H/\kappa')\rangle \sum_{m=0}^{l} P_{k,lm\pm}\, y_{k,lm\pm}(\mathbf{H}/H), \qquad (10)\end{aligned}$$

where $\langle j_l\rangle$ is the lth-order Fourier–Bessel transform of R_l (the spherical-harmonic functions are Fourier-transform invariant):

$$\langle j_l\rangle = \textstyle\int j_l(2\pi H r) R_l(r) r^2\, {\rm d}r \qquad (11)$$

with j_l being the lth-order spherical Bessel function. The scattering factor f_k, equation (10), can be rewritten as follows:

$$\begin{aligned}f_k(\mathbf{H}) &= P_{k,\rm core} f_{k,\rm core}(H) + P_{k,\rm val} f_{k,\rm val}(H/\kappa)\\ &\quad + 4\pi \sum_{l,\rm even} i^l \langle j_l(H/\kappa')\rangle \sum_{m=0}^{l} P_{k,lm\pm}\, y_{k,lm\pm}(\mathbf{H}/H)\\ &\quad + 4\pi \sum_{l,\rm odd} i^l \langle j_l(H/\kappa')\rangle \sum_{m=0}^{l} P_{k,lm\pm}\, y_{k,lm\pm}(\mathbf{H}/H), \qquad (12)\end{aligned}$$

where the first three terms are real and the last term is imaginary. For anomalous scatterers, the multipole model scattering factor has the contribution of the deformation density δf_k added to that for the IAM.

$$f_k = f_k^{\rm IAM} + \delta f_k + f_k' + i f_k''. \qquad (13)$$

Writing all (real) even-order contributions ($l = 0, 2, 4$) as $\delta f_k^{\rm e}$ and all (imaginary) odd ones ($l = 1, 3$) as $\delta f_k^{\rm o}$, we can write the multipole model scattering factor for anomalous scatterers as

$$f_k = (f_k^{\rm IAM} + f_k' + i f_k'') + (\delta f_k^{\rm e} + i\delta f_k^{\rm o}). \qquad (14)$$

The first term in this expression can be identified as $a_k + ib_k$ [equation (6)], and labelling the even and odd deformation terms e_k and o_k, respectively, we can write

$$f_k = [a_k + ib_k] + [e_k + io_k]. \qquad (15)$$

The pseudoatom structure factor then becomes

$$\begin{aligned}F(\mathbf{H}) &= \sum_k [a_k + ib_k][{\rm c}_k + i{\rm s}_k] + \sum_k [e_k + io_k][{\rm c}_k + i{\rm s}_k]\\ &= \sum_k a_k{\rm c}_k - \sum_k b_k{\rm s}_k + i\sum_k b_k{\rm c}_k + i\sum_k a_k{\rm s}_k\\ &\quad + \sum_k e_k{\rm c}_k - \sum_k o_k{\rm s}_k + i\sum_k o_k{\rm c}_k + i\sum_k e_k{\rm s}_k\\ &= (A_1 - A_2) + i(B_1 + B_2) + (\alpha_1 - \alpha_2) + i(\beta_1 + \beta_2).\end{aligned} \qquad (16)$$

The difference $\Delta_{\mathbf{H}}^{\rm Mul}$ in the multipole model between $|F(\mathbf{H})|^2$ and $|F(\bar{\mathbf{H}})|^2$ is

$$\Delta_{\mathbf{H}}^{\rm Mul} = -4A_1A_2 + 4B_1B_2 - 4A_2(\alpha_1 - \alpha_2) + 4B_1(\beta_1 + \beta_2) \qquad (17)$$

so that we can conclude that the additional information available to determine the Flack parameter is

$$\Delta_{\mathbf{H}}^{\rm Mul} - \Delta_{\mathbf{H}}^{\rm IAM} = 4B_1(\beta_1 + \beta_2) - 4A_2(\alpha_1 - \alpha_2). \qquad (18)$$

This difference may be positive, negative or zero. This expression for the enhancement on inversion distinguishing power (*i.e.* multipoles plus anomalous dispersion, compared with anomalous dispersion only) is, strictly speaking, valid only if the same structural and thermal parameters are used in both independent-atom and multipole structural models.

It is readily shown that not only is $\Delta_{\mathbf{H}}$ a measure of the inversion distinguishing power of the model but it is also influential in determining the standard uncertainty in x, $\sigma(x)$. From equation (3), the second derivative is given by

$$\frac{\partial^2\varepsilon'}{\partial x^2} = 2\sum_{\mathbf{H}} w_{\mathbf{H}}\Delta_{\mathbf{H}}^2 \qquad (19)$$

and, hence, to a good approximation, we can equate the inverse of this curvature to the variance in x,

$$\sigma^2(x) \approx \frac{1}{2\sum_{\mathbf{H}} w_{\mathbf{H}}\Delta_{\mathbf{H}}^2} \qquad (20)$$

and hence

$$\sigma(x) \approx \left(\frac{1}{2\sum_{\mathbf{H}} w_{\mathbf{H}}\Delta_{\mathbf{H}}^2}\right)^{1/2}. \qquad (21)$$

From this result, we see that $\sigma(x)$ is largely determined by the magnitudes of $\sigma^2(F_o^2)$ and by $\Delta_{\mathbf{H}}^2$; a smaller $\sigma(x)$ will result from either smaller $\sigma^2(F_o^2)$ (*i.e.* more precise X-ray data) or larger $\Delta_{\mathbf{H}}^2$ (*i.e.* greater discriminating power). These approximate expressions are illuminating, but all results reported below for $\sigma(x)$ have been obtained from the full inverse least-squares matrix.

Table 1
Refinement data for (Z)-17-α-propenyl-3,3-trimethylenedioxyestr-5[10]-ene-17-ol (Tcx).

Empirical formula	$C_{24}H_{36}O_3$
Formula weight [g mol^{-1}]	372.53
Cell setting, space group	Orthorhombic, $P2_12_12_1$ (No. 19)
Z	4
Temperature [K]	100 (2)
Unit-cell dimensions:	
a [Å]	7.7228 (4)
b [Å]	14.2576 (8)
c [Å]	18.7153 (10)
V [Å^3]	2060.72 (19)
Calculated density [g cm^{-3}]	1.201
F(000)	816.0
Crystal size [mm]	0.25 × 0.10 × 0.10
Wavelength λ [Å]	1.5418
Absorption coefficient μ [mm^{-1}]	0.60
Absorption correction	None
θ range [°]	3.90 to 69.97
$(\sin\theta/\lambda)_{\max}$ [Å^{-1}]	0.609
No. of measured, independent and observed reflections	12736, 3755, 3198
Criterion for observed reflections	$I > 2\sigma(I)$, refined against F^2
Overall completeness [%]	96.5
Weighting scheme	Based on measured s.u.'s†
$R_{\rm int}(F^2)$‡ [%]	7.48

† $w = 1/\sigma^2$. ‡ $R_{\rm int}(F^2) = \sum |F_o^2 - F_o^2(\text{mean})| / \sum F_o^2$.

3. Experimental

The structure of the steroid derivative (Z)-17-α-propenyl-3,3-trimethylenedioxyestr-5[10]-ene-17-ol (Tcx) is reported here.[2] Fig. 1(a) shows an *ORTEP* representation (Burnett & Johnson, 1996) of the molecule after invariom refinement and the atomic numbering scheme. The molecule has five chiral C atoms and crystallizes in space group $P2_12_12_1$. Data were measured with Cu $K\alpha$ radiation and area detection on a Bruker CCD diffractometer (Table 1). A second light-atom structure, (+)-α-methyl-4-carboxyphenylglycine [MCPG, T = 150 K, Fig. 1(b)], measured with Cu $K\alpha$ to a similar resolution (≈0.6 Å^{-1} in $\sin\theta/\lambda$), was taken from the literature. X-ray data and starting coordinates were taken from Wilson *et al.* (1997). Data were remeasured for two further examples. For the structure of vitamin C, the third example (Wagner, 2005), Cu $K\alpha$ radiation was used [T = 100 K, Fig. 1(c), only one of the two conformationally different molecules in the asymmetric unit shown]. The structure was originally reported by Hvoslef (1968). The fourth example is the dipeptide L-phenylalanyl-L-proline monohydrate [FP, T = 90 K, Fig. 1(d)], where the structure has been reported (Panneerselvam & Chacko, 1989). For this example, charge-density-quality Mo $K\alpha$ data have been measured up to high resolution and a detailed study of these data will be reported elsewhere.

4. Invariom modelling and refinement

Experimental data were modelled using the Hansen & Coppens multipole formalism (Hansen & Coppens, 1978) as implemented in the program package *XD* (Koritsánszky *et al.*, 2003). The program incorporates a pseudoatom representation of deformations of the electron density $\rho(\mathbf{r})$, for example due to chemical bonding, using the electron-density expression given in equation (9). An appropriate invariom was assigned to each atom in the crystal structures. The procedure for the building of the database of theoretically derived multipole populations has been described in detail (Dittrich *et al.*, 2005).

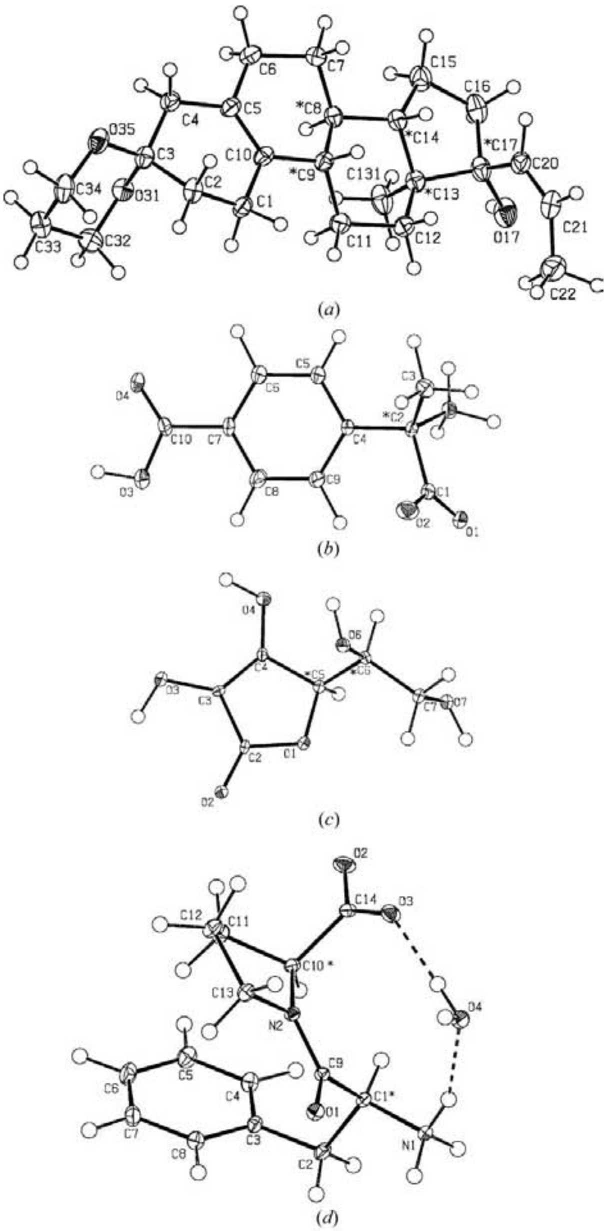

Figure 1
ORTEP representations of the crystal structures used in the present work. Compound names are given in the text; thermal ellipsoids with 50% probability. Chiral C atoms are marked with a star.

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[2] Supplementary data for this paper are available from the IUCr electronic archives (Reference: CN5008). Services for accessing these data are described at the back of the journal.

Table 2
Invarioms, site symmetry and model compounds used for aspherical refinement of the Tcx structure.

Atom name	Invariom	Model compound	Invariom site symmetry
O(31), O(35)	O1c1c	$O(CH_3)_2$	$mm2$
O(17)	O1c1h	$OH(CH_3)$	m
C(1), C(2), C(4), C(6), C(7), C(11), C(12), C(15), C(16), C(33)	C1c1c1h1h	$CH_2(CH_3)_2$	$mm2$
C(3)	C1o1o1c1c	$C(OH)_2(CH_3)_2$	m
C(5), C(10)	C2c1c1c	$H_2C{=}C(CH_3)_2$	m
C(8), C(9), C(14)	C1c1c1c1h	$CH(CH_3)_3$	$3m$
C(13)	C1c1c1c1c	$C(CH_3)_4$	$3m$
C(17)	C1o1c1c1c	$COH(CH_3)_3$	$3m$
C(20), C(21)	C2c1c1h	$H_2C{=}CH(CH_3)$	m
C(22), C(131)	C1c1h1h1h	$CH_3(CH_3)$	3
C(32), C(34)	C1o1c1h1h	$COH(CH_3)_2H$	m
H(1*A*)–H(7*B*), H(11*A*)–H(12*B*), H(15*A*)–H(16*B*), H(33*A*, *B*)	H1c[1c1c1h]	$CH_2(CH_3)_2$	6
H(8), H(9), H(14)	H1c[1c1c1c]	$CH(CH_3)_3$	6
H(13*A*)–H(13*C*), H(22*A*)–H(22*C*)	H1c[1c1h1h]	$CH_3(CH_3)$	6
H(20), H(21)	H1c[2c1c]	$H_2C{=}CH(CH_3)$	6
H(32*A*, *B*), H(34*A*, *B*)	H1c[1o1c1h]	$CH_2(OH)(CH_3)$	6
H(17)	H1o[1c]	CH_3OH	6

When invariom modelling was originally introduced (Dittrich *et al.*, 2004), H atoms were modelled by taking into account the nearest-neighbour atoms only. Recently, it has been found that a better fit to the experimental structure factors, and a smaller deviation from electroneutrality for the sum of the monopole populations, can be achieved when next-nearest neighbours are also taken into account for all H atoms. In the latest version of our database, two further details differ from the procedure described previously (Dittrich *et al.*, 2005): In order to avoid density overlap, the size of the cubic cell for the calculation of theoretical structure factors has been increased to 30 Å following earlier work (Volkov *et al.*, 2004). Furthermore, the double-zeta basis set of Dunning/Huzinaga D95++(3df, 3pd) available in *GAUSSIAN* (Frisch *et al.*, 1998) was used instead of the 6–311++G(3df, 3pd) basis set for geometry optimizations of the model compounds.

The electron density of the molecules in the crystal was obtained by superposition of invariom electron densities. In the least-squares refinement, multipole parameters taken from the invariom database were not refined. Thus, compared to an IAM refinement, the number of refinable parameters does not increase and invariom refinement – using fixed multipole parameters – of the low-resolution Cu $K\alpha$ data sets was feasible. Only a scale factor, the Flack x parameter, positional and thermal parameters were refined. We emphasize that, when a refinement of the Flack parameter is the aim of a study employing data of limited resolution, simultaneous refinement of Flack and multipole parameters has to be avoided. As an example, Table 2 lists all invarioms, their local atomic site symmetry (Kurki-Suonio, 1977) and the model compounds for Tcx. *XD* input files were generated with the preprocessor program *INVARIOMTOOL* (Hübschle & Dittrich, 2004).

5. Results and discussion

For the present work, we included a Flack parameter in a locally modified version of the full-matrix least-squares refinement program *XDLSM* of the *XD* suite (Koritsánszky *et al.*, 2003). We tried to obtain more information from the diffraction data in challenging cases of organic molecules, where the anomalous dispersion signal is very weak and only due to light atoms, mostly oxygen. For the examples given below, the Flack parameter was refined simultaneously with positional and thermal parameters in order to obtain the correct standard uncertainty.

In the latest issues of *Acta Crystallographica* Sections C and E, a large number of studies cite the original publication, where Flack's x parameter was first introduced (Flack, 1983). When heavier atoms or third-row elements are present in a structure, the inversion-distinguishing power is good and an absolute structure determination is usually attempted and successful, although most authors do not take special care to optimize their measurement strategy in order to measure all Friedel pairs. Because most current studies use Mo $K\alpha$ radiation, the Flack parameter is generally not reported, or labelled inconclusive, for light-atom structures. This also holds when Cu $K\alpha$ is used. As the information on absolute configuration is of critical importance for the pharmaceutical industry, it is disappointing that for a large fraction of chiral organic molecules the absolute structure cannot be determined reliably as part of a routine structure determination.

Aspherical atom modelling with invarioms leads to an improvement of molecular geometry and standard uncertainties, thermal-motion description and the figures of merit (*e.g.* the R factor). The reduction of the R factor on account of the invariom model is often only of the order of 0.5% for Cu $K\alpha$ data, where the resolution is inherently limited, but we observe a greater reduction of up to or in excess of 1% for Mo $K\alpha$ data sets.

In Table 3, the Flack parameter is listed with selected figures of merit for the IAM and the invariom-model refinements for the four example structures. When the Flack x parameter is refined for both structural models, its precision (*i.e.* standard uncertainty) improves when the invariom model is used and in

Table 3
R factor and Flack parameter for invariom and independent-atom models for four light-atom absolute structure determinations.

Compound	Formula	% oxygen	Cell setting	Space group	$(\sin\theta/\lambda)_{\max}$ (Å^{-1})	Cell dimensions (Å, °)	$R_1(F)$†‡	$R_w(F)$‡	x‡
Tcx	$C_{24}H_{36}O_3$	12.9	Orthorhombic	$P2_12_12_1$	0.61	a = 7.7228 (4) b = 14.2576 (8) c = 18.7153 (10	3.81 4.04	3.63 3.82	0.03 (25) −0.05 (26)
MCPG	$C_{10}H_{11}NO_4$	30.6	Monoclinic	$P2_1$	0.63	a = 8.698 (3) b = 5.819 (3) c = 10.032 (3) β = 107.48 (3)	2.66 3.44	3.15 3.87	−0.03 (23) −0.14 (28)
Vitamin C	$C_6H_8O_6$	54.5	Monoclinic	$P2_1$	0.61	a = 6.395 (2) b = 6.240 (2) c = 17.095 (3) β = 99.341 (9)	1.95 2.48	1.86 2.55	−0.01 (12) −0.03 (16)
FP	$C_{14}H_{20}N_2O_4$	22.8	Tetragonal	$P4_32_12$	1.20	a, b = 8.197 (1) c = 41.226 (1)	2.27 3.36	2.10 3.78	0.08 (17) 0.11 (30)

† $R_1(F) = \sum ||F_o| - |F_c|| / \sum |F_o|$, $R_w(F) = \sum w(|F_o| - |F_c|) / \sum w|F_o|$, ‡ The top line is for the invariom model and bottom line for the IAM.

all cases the invariom result is closer to the ideal value of 0.0 for a correct structure. In the structures investigated here, the inversion-distinguishing power is considered weak and these are cases where the standard uncertainty of the Flack parameter multiplied by three falls in the range $0.1 \leq 3\sigma \leq 0.3$, as pointed out by Flack & Bernardinelli (2000). For Cu *Kα* data, invariom modelling improves the standard uncertainty by only a moderate amount. Still the fact that the standard uncertainty of the Flack parameter is reduced encourages the use of the invariom scattering model, especially when one takes into account that the substances studied here are a challenge for absolute structure determination. The third example of vitamin C, where the oxygen content is more favourable for the purpose of a light-atom absolute structure determination, also shows that small improvements are important. When the IAM is used, the inversion-distinguishing power is weak (3σ = 0.48), while for the invariom refinement 3σ is reduced to 0.36, an inversion-distinguishing power close to what is considered enantiopure sufficient. The fourth example, a charge-density-quality data set of a dipeptide, highlights the importance of incorporating more realistic atomic scattering factors (*i.e.* invarioms in this case). Although the anomalous dispersion signal is especially weak for Mo *Kα* data, the standard uncertainty of the Flack parameter is significantly reduced even though the oxygen content is lower than for vitamin C. High resolution is important; when the FP data are truncated at $(\sin\theta/\lambda)_{\max}$ = 0.7 Å^{-1}, the Flack parameter and its standard uncertainty is 0.14 (32), whereas it is 0.08 (17) at full resolution. As most modern diffractometers are equipped with Mo *Kα* X-ray sources, this is an important outcome of this study. It has been emphasized above that simultaneous refinement of the Flack and multipole parameters has to be avoided. This also holds for data of charge-density quality, where multipole parameters could be refined, as ambiguities in phases can occur in non-centrosymmetric space groups, for which absolute structure determination may be required.

6. Conclusions

The aim of this study was to explore if the use of an improved scattering-factor model (invarioms) provides significant additional information on the Flack parameter. Results for Tcx, MCPG and vitamin C data collected with Cu *Kα* radiation show that the situation improves when the invariom scattering model is used. For the dipeptide FP, where high-resolution Mo *Kα* data were collected, the most significant improvement was achieved. The main sources of improvement are a better scattering-factor model, which is expected to yield more accurate phases, and the additional inversion-distinguishing power of the aspherical scattering model. Therefore, invariom modelling is recommended, especially when an absolute structure determination is the goal of a single-crystal X-ray structure analysis. In addition, for standard structural work, the advantages of invariom modelling, such as more accurate molecular geometry, reduced standard uncertainties and more physically meaningful thermal parameters (Dittrich *et al.*, 2005), are worth considering. The preprocessor program used in this work allows a rapid application of the modelling process, starting from a conventional IAM refinement. Although we believe that invariom modelling is a useful contribution to absolute structure determination, whether or not light-atom structural data really provide enough information should remain subject to skepticism in each individual case.

We thank Professor Howard Flack for providing the source code of his implementation of an enantiomorph polarity parameter (program *GFMLX*) and for helpful discussions. Furthermore we thank Dr T. Wagner for providing Cu *Kα* data for vitamin C, and Dr B. Menzenbach (Jenapharm) for synthesis of Tcx. Dr P. Lienau's support in initial stages of this work and Linda Nielsen's help in preparing the manuscript are also acknowledged.

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