



Application Note SC-XRD 512

Absolute Structure Determination of Light Atom Compounds

- D8 QUEST & D8 VENTURE – Pushing the limits

The absolute configuration is a crucial detail in the description of chiral compounds. Although the structural differences between such molecules and their mirror images may not be apparent to the inexperienced observer, the chirality can have very pronounced effects on the chemistry of the compounds. The terpenoids (*R*)-Carvone, which smells like spearmint, and (*S*)-Carvone with a caraway

smell (Figure 1), illustrate just one example that can have consequences if confused. Other pairs of compounds have much more extreme differences. Therefore, in addition to the verification of the synthetic success, the unambiguous determination of chiral centers is of vital importance in fields such as organic chemistry, food and flavors, and pharmaceutical research.

Small intensity differences are important

Single crystal X-ray diffraction (SC-XRD, SCD) is the standard method for determining the absolute configuration of molecules since only a few experimentally challenging or indirect alternatives, such as optical rotatory dispersion (ORD) exist.^[1]

The determination of the absolute configuration by X-ray diffraction makes use of small intensity differences between symmetry-equivalent reflections (Bijvoet pairs) caused by anomalous dispersion. The challenge is to measure these differences with the required accuracy.

A number of requirements must be fulfilled in order to assign the “handedness” of the stereocenter(s) in the compound from single crystal X-ray data:

- at least two different elements must be present in the crystal,
- the sample must crystallize in a chiral space group,
- all molecules in the asymmetric unit must have the same configuration (i.e., the crystal must be enantiopure), and
- an absolute structure distinguishing parameter (P_{abs}) must be sufficiently determined with a low standard uncertainty to exclude inversion twinning or the assignment of the incorrect “handedness”.

More reliable parameters recently established

For many years, the absolute structure distinguishing parameter P_{abs} was the Flack^[2] x parameter. Recently, modern discriminators^[3,4] like the Parsons' Q and the Hooft γ became more popular, which made the estimation of the standard uncertainties more reliable compared to the original method.

All three distinguishing parameters confirm the correct determination of the absolute configurations and consequently the absolute structure if they adopt a value close to zero with a small standard uncertainty. Commonly accepted criteria for a successful absolute structure determination are $P_{\text{abs}} < |0.1|$ with $\sigma(P_{\text{abs}}) < 0.1$.

This is normally an easy task if the crystal contains elements from period 3 or later of the periodic table (Na to Ar), leading to a relatively strong anomalous signal. The signal is most pronounced if an X-ray wavelength close to (but smaller than) the absorption edge of an atom in the crystal is used. Generally, softer radiation increases the anomalous dispersion.

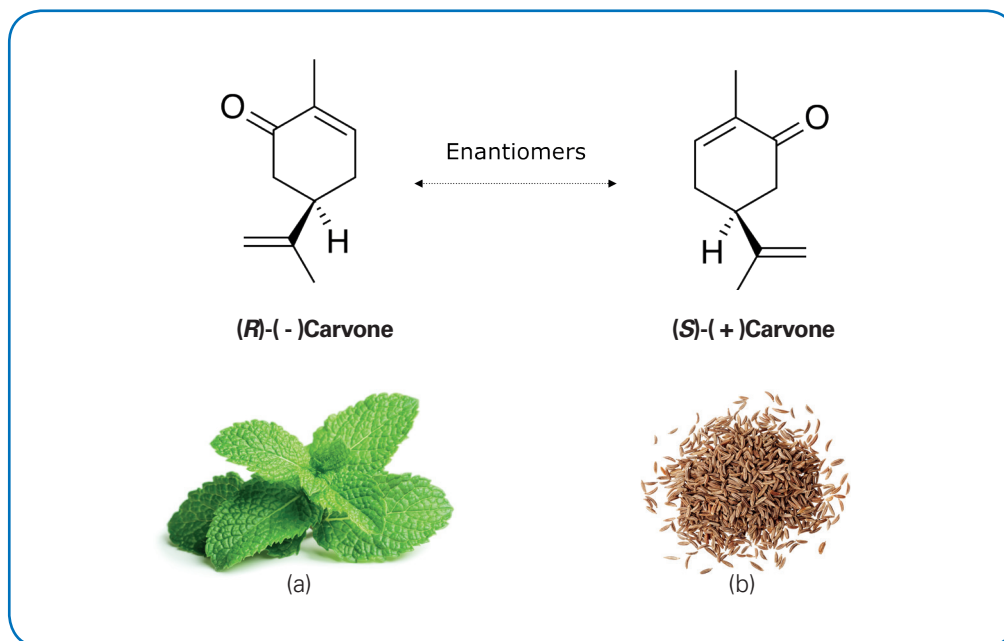


Figure 1: Carvone enantiomers smell like (a) spearmint or (b) caraway.

Period 2 element compounds now accessible

Unfortunately, the majority of pharmaceuticals consist of period 2 elements (Li to Ne) only. In many cases, they are “light-atom compounds” consisting of only carbon, nitrogen, or oxygen. For many years, these were thought to be inappropriate for the direct determination of the absolute structure as their anomalous signal was considered too weak, even with Cu-K α radiation. Light-atom compounds were traditionally derivatized or transferred into their salts in order to introduce heavier atoms into the compounds. At that time, H. Flack established a suitability factor to quantify the magnitude of the anomalous dispersion called “Friedif”^[5]. Friedif values above 80 qualified compounds for absolute structure determination from good quality crystals. Within the last five years, the situation has changed significantly.

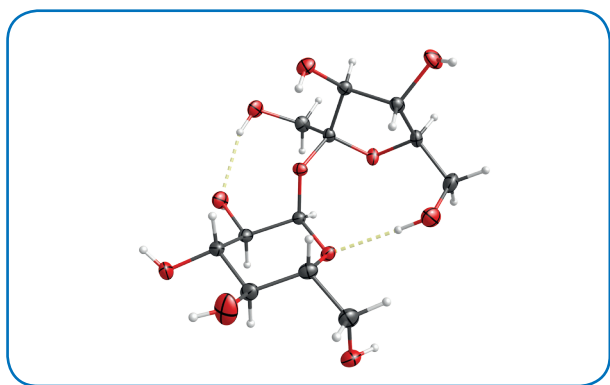


Figure 2: Sucrose

Best determination of the anomalous signal

Today, the absolute structure of organic light atom structures with Friedif values far below 80 can often be successfully determined. Recent advances in technology, in both hardware and software, are driving a paradigm shift. In particular, the new I μ S 3.0 microfocus source technology, combined with large active-area, state-of-the-art CPAD detectors, such as the PHOTON II, have removed all ambiguity in the direct determination of absolute structure. Instruments such as the D8 QUEST or the D8 VENTURE, which feature these components, are greatly facilitating the accurate measurement of the Bijvoet pair differences. In addition, the slim components and open goniometer design of these instruments support this sophisticated application by increasing the

accessible resolution for Cu-K α radiation beyond 0.80 Å. As a consequence, more Bijvoet pairs are measured, the anomalous signal is better determined, and the standard uncertainty of P_{abs} is minimized. This standard deviation is further lowered with an increased data multiplicity^[6] and data collection at low temperature. The former is easily achieved thanks to the large PHOTON II detector (active area 10 × 14 cm²), while modern low-temperature devices are essential for the latter.

Pushing the limits

Three compounds with impressively low Friedif values were chosen as test samples for the latest generation of D8 QUEST and D8 VENTURE instruments. Both systems feature the I μ S 3.0 and the PHOTON II CPAD detector. Sucrose (Friedif = 36), threonine (Friedif = 35) and alanine (Friedif = 34) were investigated.

Sucrose

A sucrose sample measuring 0.14 × 0.12 × 0.10 mm³ was mounted, and in an initial fast scan experiment lasting only three minutes, the unit cell was determined. In addition to the correct monoclinic unit cell, this short experiment also revealed the correct space group and delivered the interatomic connectivity. The APEX3 software suite’s integrated strategy optimizer suggested twenty-two runs and a frame width of 2° to obtain a complete dataset for point group 2 with a moderately high multiplicity. A resolution limit of **0.80 Å** ($2\theta = 149^\circ$) was chosen to facilitate the absolute structure determination. An exposure time of only 2 seconds per frame was fully sufficient for the room-temperature experiment.

High quality data - excellent model agreement

A total of 1540 frames were collected in just 50 minutes. Integration of the data yielded 16209 reflections, of which 2890 were independent (multiplicity 5.6). The 100% complete data, after scaling and absorption correction, boast a **R_{int} of 2.76%** and a **R_{sig} of 2.00%**. The structure solution was fully automated with APEX3’s new AUTOSTRUCTURE plug-in. All elements were correctly assigned, including the hydrogen atoms. Due to the excellent quality of the data set, the hydrogen atom positions could be freely refined. The refinement resulted in an excellent model agreement with **$R1 = 2.45\%$** , and **$wR2 = 5.96\%$** and low residuals below $\pm 0.2 \text{ e}/\text{Å}^{-3}$.

Both absolute structure distinguishing parameters Parsons' Q , **-0.04(4)**, and Hooft γ , **-0.04(4)**, unambiguously confirm the configuration at the stereocenters of sucrose – or in IUPAC notation, $(2R,3R,4S,5S,6R)$ -2-[($2S,3S,4S,5R$)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol, even at room temperature (Figure 2).

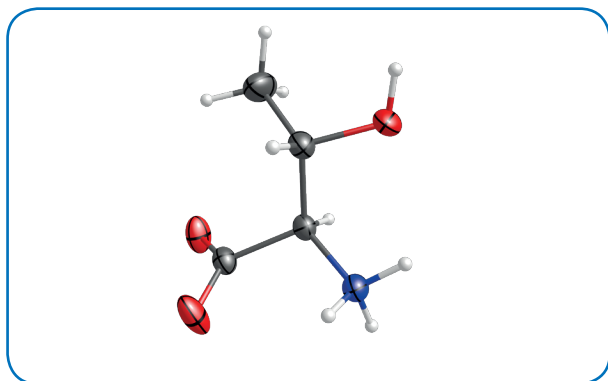


Figure 3: Threonine

Threonine

The second test crystal, threonine, was of similar size ($0.14 \times 0.12 \times 0.11 \text{ mm}^3$), but with less diffraction power than the sucrose sample, even at a measurement temperature of 100 K.

After the fast scan, APEX3's strategy optimizer recommended nine runs for the orthorhombic crystal system, again with a frame width of 2° . Exposure times of 4 seconds (for low 2θ) and 20 seconds (for high 2θ) were chosen to establish a 3.2-hour, 790-frame experiment. Normal data processing in APEX3 yielded 5464 reflections up to **0.84 Å** resolution, of which 963 were independent (100% completeness, multiplicity 5.7). For this data set, the minimum *Acta Cryst* resolution was chosen to determine whether the new-generation instrument can provide a sufficiently low $\sigma(P_{\text{abs}})$. The R_{int} (**2.61%**) and R_{sig} (**1.64%**) were promisingly low, and the model agreement criteria highlight the data quality: $R1 = \mathbf{2.31\%}$; $wR2 = \mathbf{6.28\%}$; residual density $< \pm 0.13 \text{ e}/\text{Å}^{-3}$.

Non-natural configuration revealed

In nature, the L configuration of threonine is an essential amino acid and therefore more likely. In our case, however, inspection of the chirality at the two stereocenters reveal R at the 2 position and S in the 3 position, and the L configuration can be ruled out. The visual inspection is verified by a Parsons' Q value of $-0.05(8)$. So we are clearly dealing with a synthetic compound, which can be used as a strong test case for absolute structure determination. The D configuration was later confirmed by the supplier (Figure 3).

Alanine

Alanine is the smallest amino acid with an asymmetric carbon atom. A plate-like $0.14 \times 0.13 \times 0.03 \text{ mm}^3$ crystal was selected and measured at 100 K. A strategy was calculated for 100% completeness with a multiplicity above 5. Exposure times between 1 second and 4 seconds were used, which led to more than 99% observed reflections from the 1585 frames of an initial set of runs collected in only 1.3 hours.

The impact of higher data multiplicity

Then, extra runs were added by extending the strategy in the optimizer to achieve a much higher multiplicity beyond 23. This additional data will be used below to analyze the effects of higher multiplicity on the absolute structure determination's quality factors.

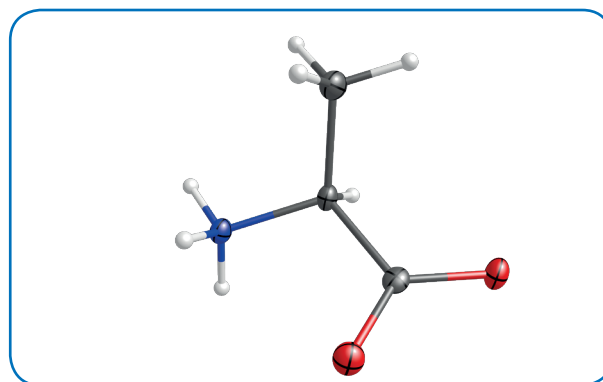


Figure 4: Alanine

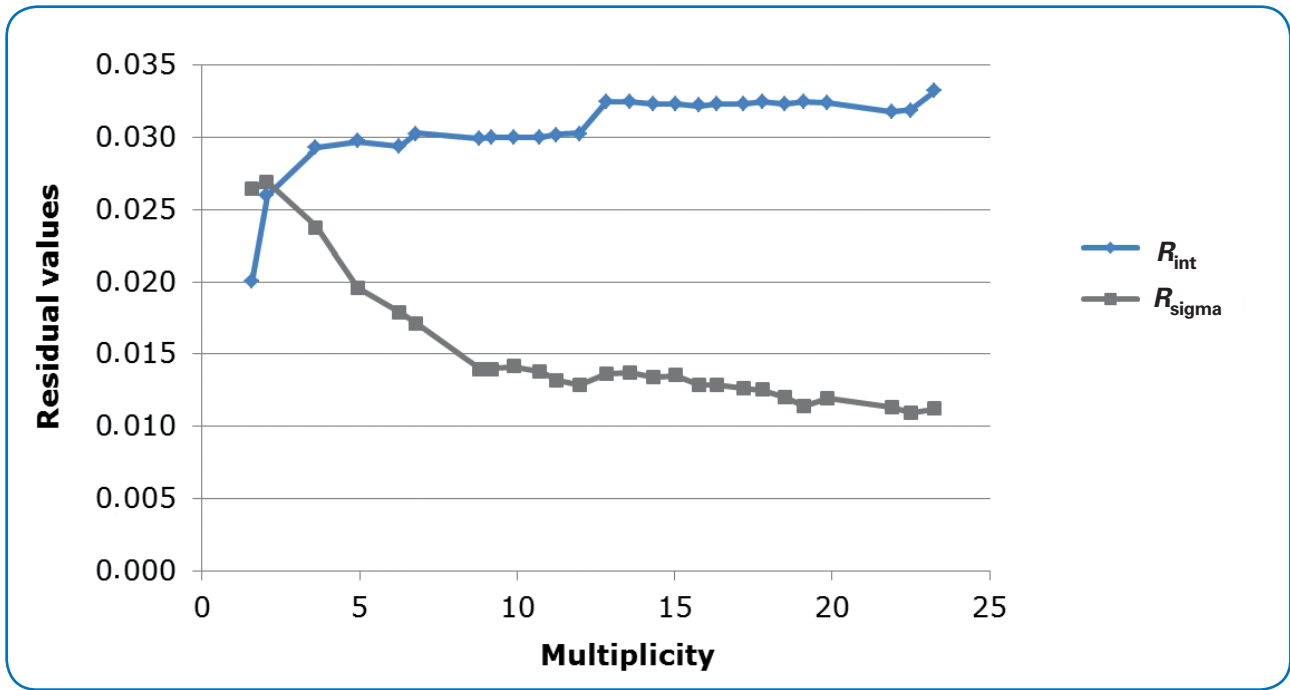


Figure 5: Residual values versus increasing multiplicity for the L-alanine data set.

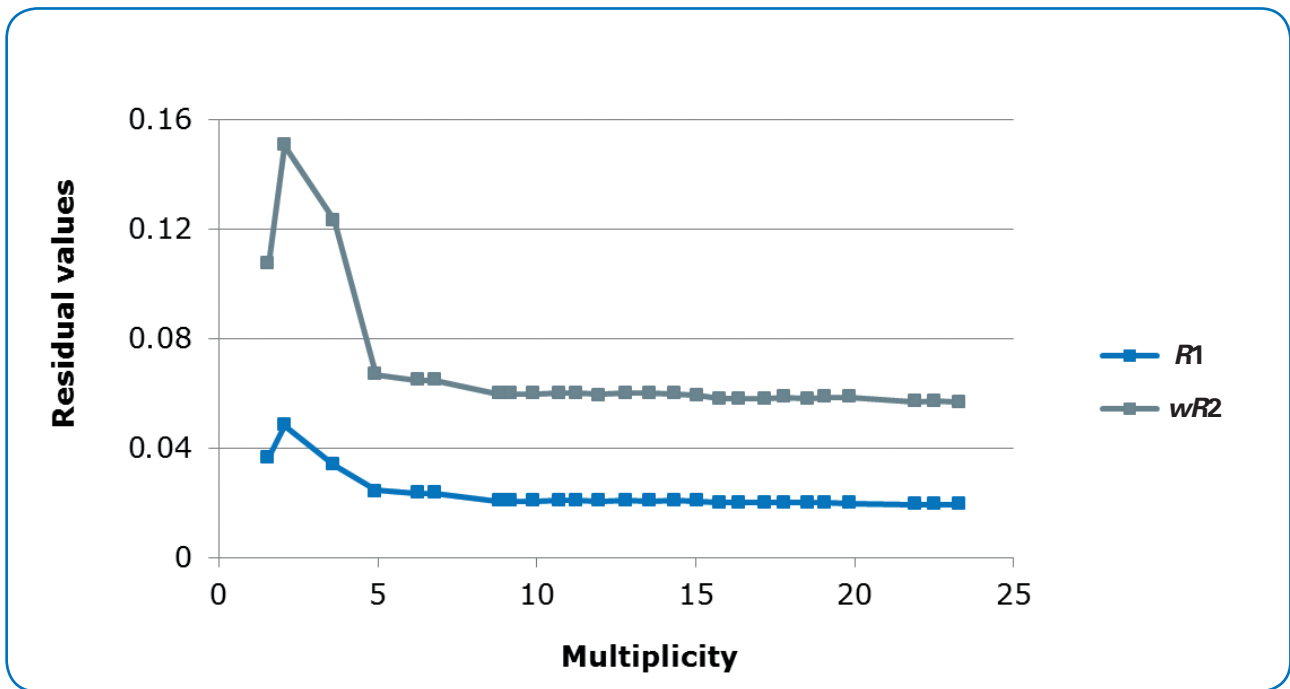


Figure 6: $R1/wR2$ versus increasing multiplicity for the L-alanine data set.

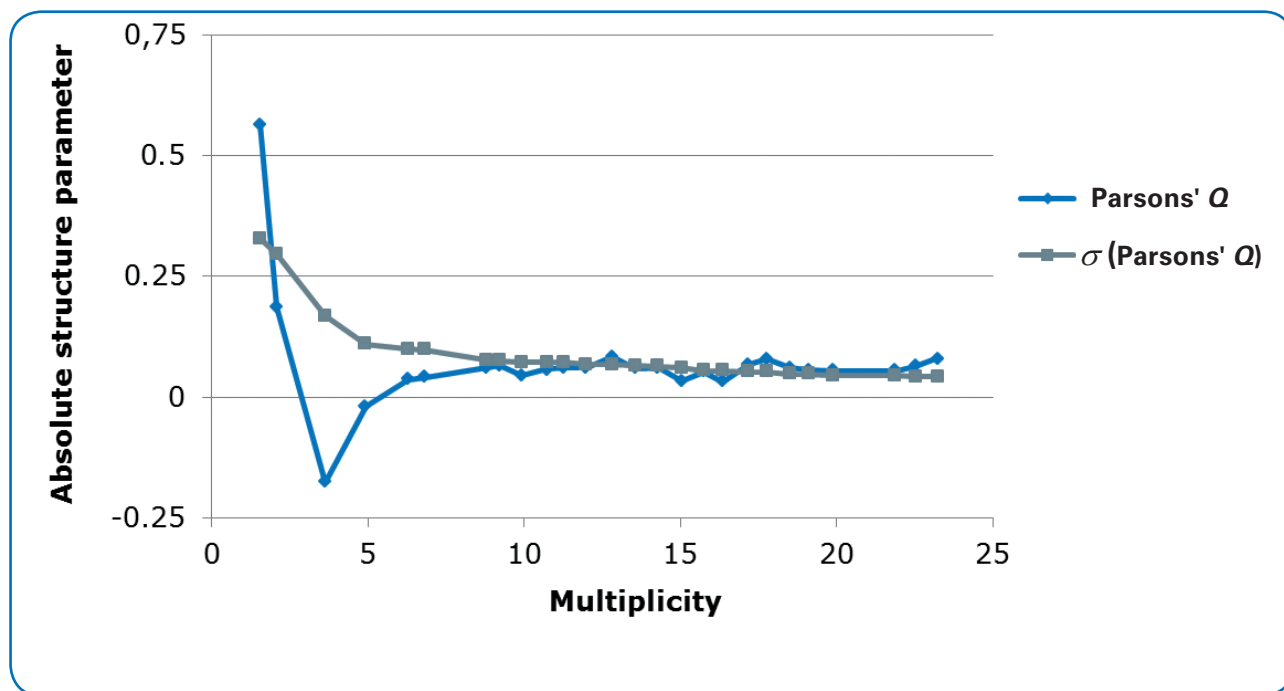


Figure 7: Absolute structure parameter (Parsons' Q) and $\sigma(\text{Parsons' } Q)$ versus increasing multiplicity for the L-alanine dataset.

There are multiple known cases of CCD detector data sets for which absolute structure determination (i.e., $P_{\text{abs}} < 0.1$) could only be achieved with a data multiplicity higher than 10.^[3,7] The PHOTON II detector's large active area greatly accelerates data collection compared to traditional CCDs or smaller CMOS detectors. But how much multiplicity is necessary to assign one enantiomer over the other? We used the high-multiplicity data set of L-alanine to investigate how much multiplicity is necessary to reliably establish the absolute structure.

The data processing yielded 4710 reflections up to 0.83 Å resolution, of which 770 were independent (average multiplicity 6.1; $R_{\text{int}} = 2.53\%$, $R_{\text{sig}} = 1.56\%$). The data delivered an excellent model of alanine (Figure 4) with **$R1$ of 1.99%**, **$wR2$ of 5.88%**, and insignificant residuals ($< \pm 0.15 \text{ e}/\text{Å}^3$). The Parsons' Q parameter of 0.02(7) clearly identifies this component as L-alanine, the second most common amino acid in the human body.

No better model with extra data

Figures 5 through 7 illustrate the evolution of key data quality criteria of L-alanine with increasing multiplicity.

The R_{sig} decreases with increasing multiplicity (Figure 5) while the R_{int} steadily increases, a well-known feature of this parameter. This is why it should be replaced by the R_{pim} , which is more independent from the multiplicity. As expected, the data accuracy rises with more redundant data. The $R1/wR2$ parameters (Figure 6) show high fluctuations when the data is not yet complete, and then steadily drop until a plateau is reached. Additional data only marginally improve the quality—this is why incomplete data sets should not be published. It is impressive that the Bijvoet pair differences are already sufficiently well determined with a multiplicity of about 4.5 (Figure 7). More data stabilizes the absolute value and steadily improves the standard uncertainty of the Parsons' Q . While there is still improvement between 5- and 8-fold multiplicity, there are only insignificant changes with extra data beyond. At that point, reflection intensities are already determined to such high accuracy that additional reflections do not result in a change of the averaged reflection intensity. The accuracy limit for the particular crystal is reached, and stochastic errors have been minimized.

Summary

The latest-generation D8 QUEST and D8 VENTURE diffractometers enabled us to unambiguously determine the absolute structure of pure organic light-atom compounds, thanks to the high-brilliance microfocus μS 3.0 source and the PHOTON II CPAD detector. Until today, routine absolute structure determination of pure light-atom structures with weak anomalous signals (and low Friedif values) posed a challenge to the crystallographer. We investigated three typical organic samples and assigned the correct absolute structure in very short experiment times—direct, quick, and unequivocal. One example was taken to demonstrate the D8 QUEST's and D8 VENTURE's ability to provide the required information with data multiplicity significantly lower than that of ancient CCD systems.

D8 QUEST & D8 VENTURE: Essential for your research

This makes the D8 QUEST or D8 VENTURE an essential research instrument for the daily work of organic chemists involved in the development and characterization of pharmaceuticals, natural products, or functionalized materials. The next paradigm shift in crystallography is in sight.

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Element	Sucrose	D-Thr	L-Ala
Friedif	36	35	34
Total time [min]	52	190	75
Exposure/frame [s]	2	4/20	1/3/4
Frame width [deg]	2	2	1.2
Multiplicity	5.9	5.7	6.1
$\ \sigma$	57.7	54.7	70.0
R_{int} [%]	2.8	2.6	2.5
R_1 [%]	2.3	2.3	2.0
wR_2 [%]	5.6	6.3	5.9
Parsons' Q	0.04(5)	-0.05(8)	0.02(7)
Residual density [$e/\text{\AA}^3$]	0.2/-0.1	0.1/-0.1	0.1/-0.1

Table 1: Summary of refinement parameters

Literature

- 1 M. Pitzer et al. (2013). *Science* **V341**, 1096.
- 2 H. D. Flack (1983). *Acta Cryst.* **A39**, 879.
- 3 S. Parsons' et al. (2013). *Acta Cryst.* **B69**, 249.
- 4 R. W. W. Hooft et al. (2008). *J. Appl. Cryst.* **41**, 96.
- 5 H. D. Flack et al. (2008). *Acta Cryst.* **A64**, 484.
- 6 P. Müller (2009). *Crystallography Reviews* **15**, 57.
- 7 E. C. Escudero-Adán, et al. (2014). *Acta Cryst.* **B70**, 660.

Key features of Bruker single crystal diffractometers for absolute structure determination on light-atom structures

- Ability to measure subtle intensity differences in the Bijvoet mates most accurately due to the strong μS 3.0 X-ray source and sensitive PHOTON II detector
- Large accessible 2θ range of the FIXED-CHI and KAPPA goniometer



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