doi.org/10.1002/hlca.202200117

Perspective

HELVETICA chimica acta

X-Ray Crystallographic Studies of Quasi-Racemates for Absolute Configuration Determinations

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In memory of Professor *Jack D. Dunitz*

The determination of the absolute configuration of chiral molecular entities by means of X-ray crystallography is overall of central importance in stereochemistry. However, the growth of enantiopure single crystals often represents an unsurmountable and frustrating hurdle. Quasi-racemic crystals of biomacromolecules, for which the absolute configuration is predetermined by the chiral monomers, allowed the X-ray crystallographic analysis of systems that are difficult to crystallize as pure enantiomers, with aims other than the determination of the absolute structure. Taking advantage of the greater propensity of quasi-racemic mixtures to co-crystallize compared to growing enantiomerically pure crystals of a single compound, we herein describe the use of X-ray crystallography of quasi-racemates for the absolute configuration determination. We expect that this approach is particularly useful to establish the sense of selectivity in the development of stereoselective methods by simplified crystallizations, while confirming the consistent selectivity with a second molecular structure within the same measurement.

Keywords: absolute configuration, atropisomerism, chirality, quasi-racemates, X-ray diffraction..

Since the seminal discoveries by *Bijvoet*, X-ray crystallography evolved to a method of choice to determine the absolute configuration of chiral, enantiomerically enriched compounds.[1–6] However, growing crystals of diffraction quality and suitable size often represents a considerable challenge, particularly for certain families of compounds. Furthermore, the high tendency that a chiral molecule crystallizes with its opposite enantiomer as racemate even in enantioenriched samples is well known, rendering the growth of enantiopure crystals a constricting obstacle for numerous projects.[7,8] The challenge of growing suitable crystals for X-ray crystallography is also regularly encountered in biomacromolecules, in which difficult crystallizations are repeatedly observed when analyzing proteins or oligonucleotides.[9,10] The determination of the absolute configuration of biomacromolecules is typically unnecessary as the chirality is encoded in the structurally established building blocks. Various biomacromolecules have been synthesized with an unnatural configuration to obtain racemates or quasi-racemates which allow for more straightforward crystallization compared to single enantiomers, as predicted by *Wukovitz* and *Yeates*[11] (*Figure 1*). Quasi-racemates mimic racemic mixtures. They consist of pairs of similar, nearly mirror symmetric (and preferably isosteric), but distinct molecules,[12] as first discovered by *Pasteur* during the co-crystallization of (+)-bitartrate and (−)-bimalate salts.[13,14] *Gellman* and colleagues obtained high-resolution structures from crystals of two quasi-racemic variants of the villin headpiece subdomain (VHP) and could then assess the accommodation of noncanonical residues in nativelike protein conformations.[15] *Liu* and coworkers demon-

Supporting information for this article is available on the WWW under https://doi.org/10.1002/hlca.202200117

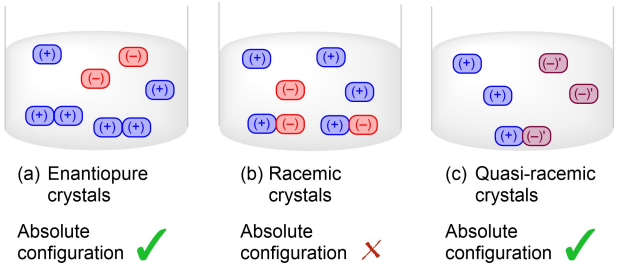


Figure 1. Schematic crystallizations.

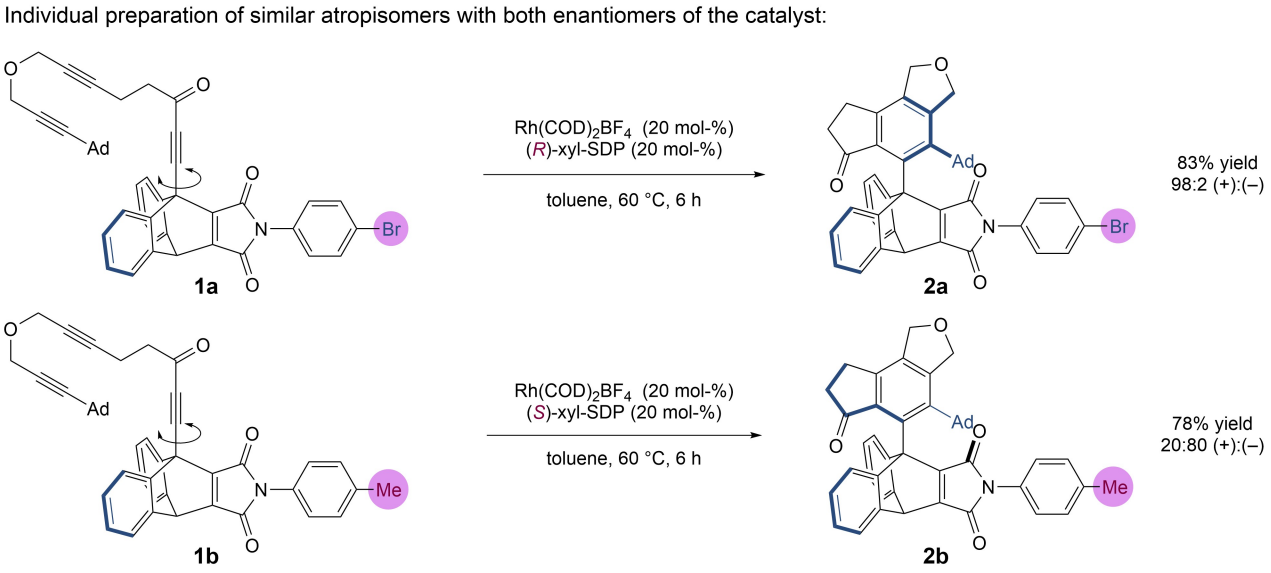
strated that monomeric D-ubiquitin (D-Ub) cocrystallizes with different dimers, trimers or tetramers of L-Ub.[16] A strong propensity of racemic DNA mixtures to form crystals was also observed by the group of *Huc*.[17]

Among chiral small molecules, quasi-racemate crystallography has been explored mostly to study molecular behavior and molecular recognition ever since the first discovery in 1848.[13,14,18–24] However, this strategy could also be immensely useful for chemists developing methods for stereoselective synthesis when determining the absolute configuration of small molecules, particularly when faced with the challenge of getting suitable crystalline material for X-ray crystallographic analysis.

We encountered this challenge of obtaining enantiopure crystals during our investigation of C(sp^2)–C(sp^3) atropisomers (*Scheme 1*)[25] and hence first investigated the Electronic Circular Dichroism (ECD) spectra of (+)-**2a** and (−)-**2b** with the aim to assign the absolute configuration by comparison of measured and calculated ECD spectra.

For (+)-**2a**, signs of *Cotton* bands at 400, 345, 280, 250, 235 and 212 nm match the signs of calculated values at 419, 340, 275, 240, 227 and 202 nm respectively (*Figure 2*). Meanwhile, signs of *Cotton* bands for (−)-**2b** at 395, 342, 280, 248, 237 and 212 nm aligned with the signs of calculated values at 421, 340, 275, 238, 225 and 202 nm, respectively. However, the signals were overall too weak for a fully reliable determination of the absolute configuration and the assignment was therefore only tentative.

After numerous attempts to confirm the absolute configuration by growing a suitable enantiopure crystal for X-ray crystallography, we thus resorted to the preparation of a quasi-racemic mixture. Herein, we describe in detail how the use of a quasi-racemate for X-ray crystallography provides an efficient approach for the determination of the absolute configuration of small molecules. This strategy is simple, general, and not limited to the crystallization of atropisomeric compounds.[25] It may take advantage of co-crystallizing related stereoisomers prepared with opposite enantiomers of a catalyst for any chiral product. Furthermore, the successful measurement establishes not only the absolute configuration and validates the connectivity, but also confirms the sense of selectivity of the catalytic method by the two product molecules in the same crystal.



Scheme 1. Preparation of similar (Br *vs.* Me) enantioenriched atropisomers with opposite enantiomers of the ligand xyl-SDP.

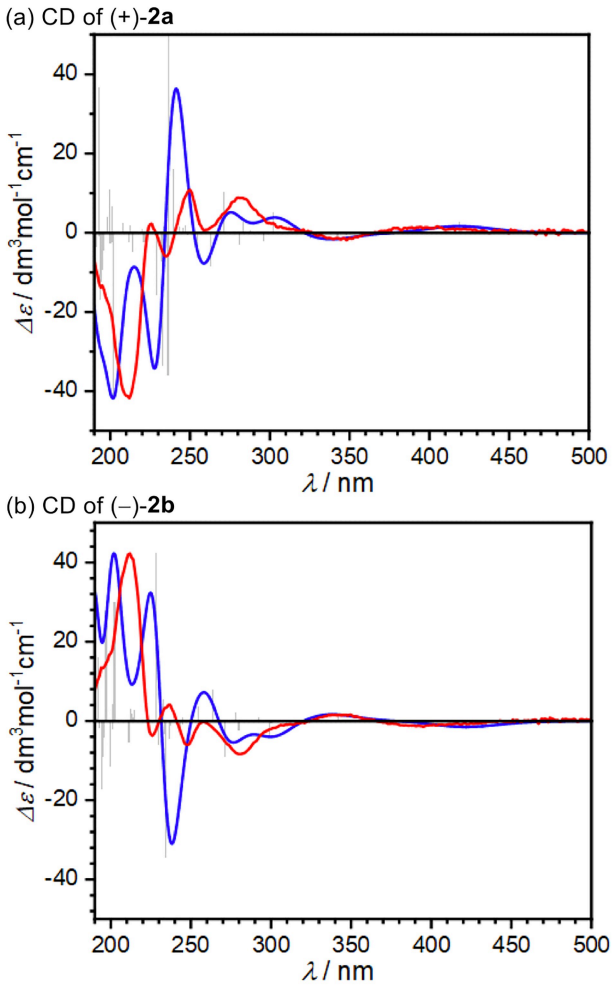


Figure 2. Experimental (red) and calculated (blue) ECD spectra with calculated transitions (grey lines) in acetonitrile of (a) (+)-**2a** and (b) (−)-**2b**.

The enantioenriched C(sp^2)–C(sp^3) atropisomers were prepared according to a method recently developed in our group (*Scheme 1*).[25] Specifically, out of six possible atropisomers, a chiral rhodium complex derived from $Rh(cod)_2BF_4$ and (*R*)-xyl-SDP in toluene at 60 °C controls the selective formation of the particular stereoisomer (+)-**2a** which arises from the restricted rotation around the C(sp^2)–C(sp^3) stereogenic axis. Instead of the *para*-bromo substitution on the aryl group of the maleimide moiety, (−)-**2b** with a *para*-methylphenyl unit was introduced as the corresponding quasi-enantiomer of (+)-**2a**, that could be prepared by the general conditions employing the opposite (S_a)-configured xyl-SDP ligand. Notably, the enantiomeric purity for both chiral compounds (+)-**2a** and (−)-**2b** were readily enhanced to 99% ee in the mother liquor after simple recrystallization by partial evaporation of a CH_2Cl_2/propan-2-ol 1:6 (*v/v*) solution (*Figure 3,a*).

With these highly enantioenriched compounds in hand, we once more attempted to individually grow crystals by changing the solvent systems and other recrystallization conditions. However, all attempts were unsuccessful until we turned to a quasi-racemic sample. An equimolar mixture of compounds (+)-**2a** and (−)-**2b** (1:1) was thereby dissolved in MeOH, CH_2Cl_2 and nitromethane and by slow evaporation of the resulting solution, we were able to obtain transparent colorless crystals consisting of (+)-**2a** and (−)-**2b**. The several crystals in the sample appeared to be of the same form, the co-crystals of (+)-**2a** and (−)-**2b**, as confirmed by randomly chosen crystals that were dissolved and measured by HPLC analysis (*Figure 3,b*). A crystal in the form of a colorless plate (0.17×0.13×0.05 mm^3) was selected and the crystallographic study performed.[25] In this specific case, due to the degradation of the sample during longer measurements presumably through the damage by the beam, data collection upon synchrotron radiation was used in order to reach the required resolution and completeness of the data. In the molecular structure (*Figure 4*), the pair of quasi-racemic molecules arranged around a pseudo-inversion center of symmetry, nearly forming a

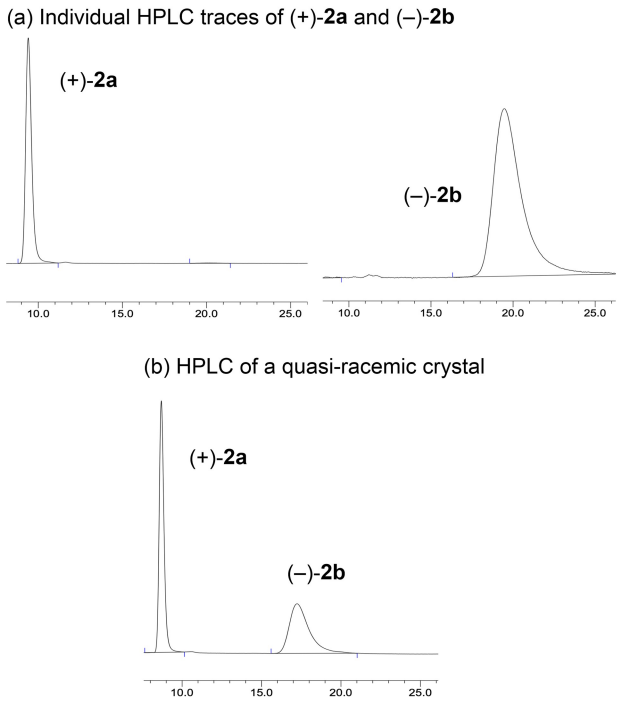


Figure 3. HPLC traces of enantioenriched samples and a dissolved quasi-racemic crystal.

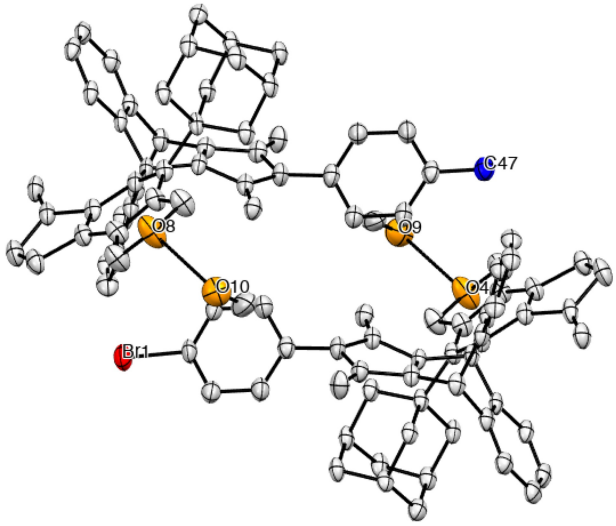


Figure 4. Crystal structure of the quasi-racemate of (+)-**2a** and (−)-**2b** illustrating approximate inversion symmetry (with highlighted proximity of the dihydroisobenzofuran oxygen atoms with the two MeOH O(9) and O(10)).

mirror image. MeOH co-crystallized in the cell, as highlighted by the proximity of the oxygen atoms 9 and 10 with the dihydroisobenzofuran rings (oxygen atoms 4 and 8). The absolute configuration of both (+)-**2a** and (−)-**2b** was finally established.

Conclusions

In conclusion, we describe that X-ray crystallography of a quasi-racemic mixture is an efficient approach for the determination of the absolute configuration after a facile crystallization of similar $C(sp^2)$–$C(sp^3)$ atropisomeric compounds individually obtained with both enantiomers of the catalyst. The tendency to form the racemate or quasi-racemate crystals was confirmed after attempting to grow enantiopure crystals. This strategy may be useful even in the case with an inconclusive *Flack* parameter to confirm the consistent sense of stereoinduction of a catalytic method, for instance when studying molecules lacking atoms displaying sufficiently strong anomalous dispersion. In this scenario, a full assignment is however only possible if the configuration of one of the molecules of the quasi-racemates is already established. Overall, it is anticipated that the practicality and ease of implementation of this approach will facilitate the determination of the absolute configuration by X-ray crystallography and that quasi-racemates will be generally useful to assign the sense of stereoselectivity of novel catalytic methods.

Experimental Section

Preparation of the Quasi-Racemate[25]

(+)-**2a** and (−)-**2b** were individually prepared from **1a** and **1b** using the enantiomeric ligands (*R*)-xyl-SDP and (*S*)-xyl-SDP, respectively. An equimolar mixture of (+)-**2a** and (−)-**2b** was dissolved in a mixture of MeOH, CH_2Cl_2 and nitromethane and allowed to crystallize by slow evaporation at ambient temperature to give the quasi-racemate as crystalline colorless plates. The composition of the quasi-racemate was qualitatively confirmed by HPLC with one of the crystals as shown in *Figure 3,b* using a *Chiralcel IA* analytical column (1.0 mL min^{-1}, 2-PrOH/*n*-heptane, 30:70): $t_{(+)\text{-}\mathbf{2a}} = 8.7$ min and $t_{(-)\text{-}\mathbf{2b}} = 17.2$ min, before the X-ray crystallography was performed as described in Ref. [25].

Acknowledgements

We gratefully acknowledge the *Swiss National Science Foundation* (155902 and 175746), the University of Basel, the NCCR Molecular Systems Engineering (182895) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 101002471). We thank Prof. *Marcel Mayor* for the usage of equipment, Dr. *Johannes Georg Rebelein* for the remote operation of Swiss Synchrotron at the Paul Scherrer Institute (PSI) and Dr. *Kirill Nikitin* of the University College Dublin for helpful discussions.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Author Contribution Statement

X. W. and *C. S.* conceived the study, designed the experiments and analyzed the data. *X. W.* performed the experiments and *J. M.* the CD measurements and calculations. *A. P.* carried out the X-ray crystallographic analysis. *X. W.*, *J. M.*, *A. P.* and *C. S.* wrote the manuscript.

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Received September 9, 2022
Accepted September 13, 2022

