Chirality in Pharmaceutical Compounds and Absolute Configuration Determination

Crystallization: Screening, Application & Process Development

X-ray Diffraction Unit

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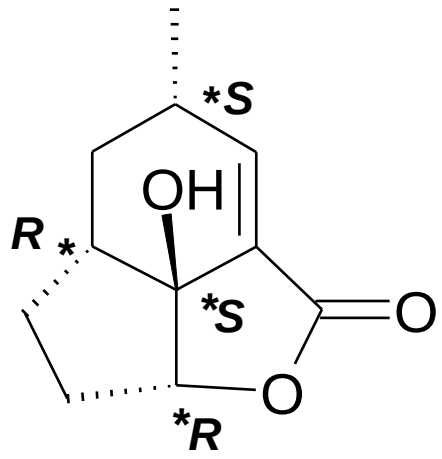
CRYSFORMA

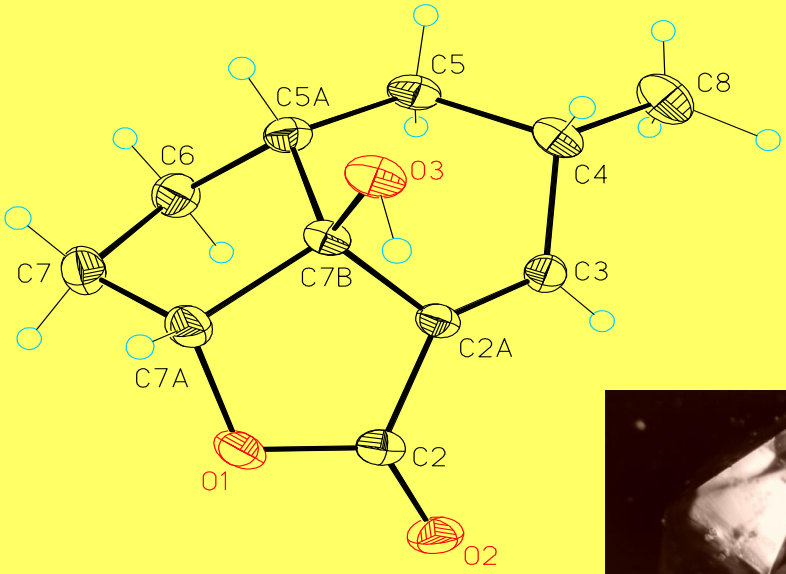
From chiral crystals to the absolute configuration of molecules: Is it an easy way?

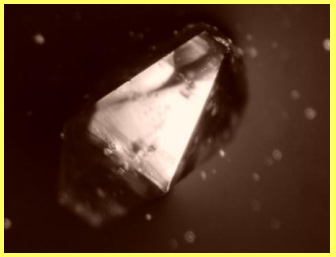
(–)-Galiellalactone



Absolute configuration:

4*S*, 5a*R*, 7a*R*, 7b*S*





Orthorhombic, $P2_12_12_1$
R_1: 3,45 %

Flack: 0.01(19)
Hooft/Parsons: -0.06(04)/-0.04(05)

• F. Nussbaum, R. Hanke, T. Fahrig, J. Benet-Buchholz; *Eur. J. Org. Chem.* ***2004****, 2783-2790.*

Determination of Absolute Configuration of APIs

- **Definitions in Chirality**
- **Precedents**
- **Methodologies**
- **Single Crystal X-ray Structure Determination:**
  - **Background**
  - **Candidate samples**
  - **Sample and Crystal selection**
  - **Validation and measurements**
  - **Examples**
- **Final schedule**

Chirality

Definitions in Chirality:

An object or a system is chiral if it is distinguishable from its mirror image; that is, it cannot be superposed onto it.

The word chirality is derived from the Greek word meaning “hand”

The most universally recognized example for chirality are the human hands.

Live is chiral

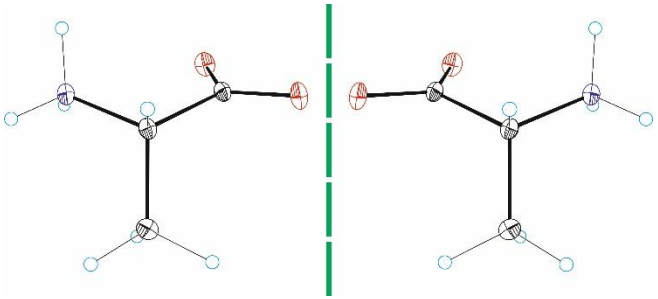
Chirality

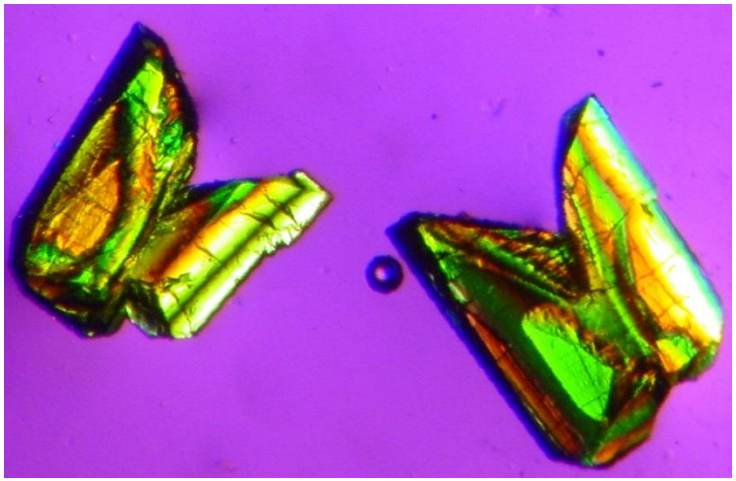
Definitions in Chirality:

- Each molecule which cannot be superposed with its mirror image is “chiral”.
- Molecules with a mirror plane are called “achiral”.

The feature that is most often the cause of chirality in molecules is the presence of an asymmetric carbon atom (four different substituents)

Two mirror images of a chiral molecule are called enantiomers or optical isomers





Conglomerate of crystals forming enantiomeric butterflies

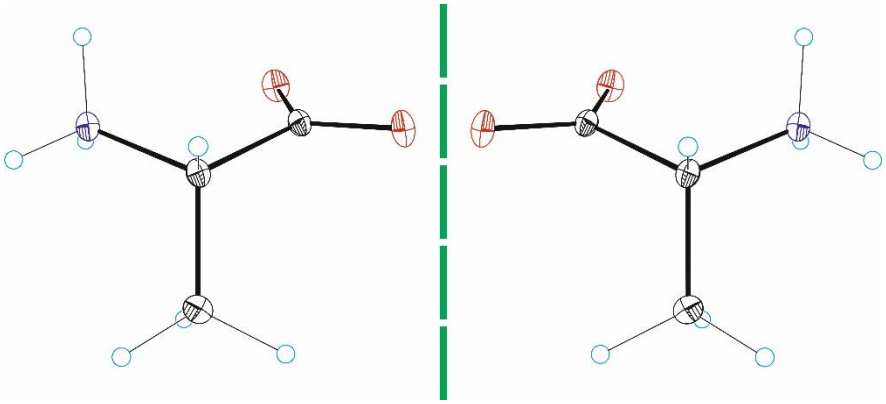
Chirality

Definitions in Chirality:

Pairs of enantiomers are often designated as "right- or left-handed".

As polarized light passes through a chiral molecule, the plane of polarization, when viewed along the axis toward the source, will be rotated in a clockwise (to the right) or counter-clockwise (to the left).

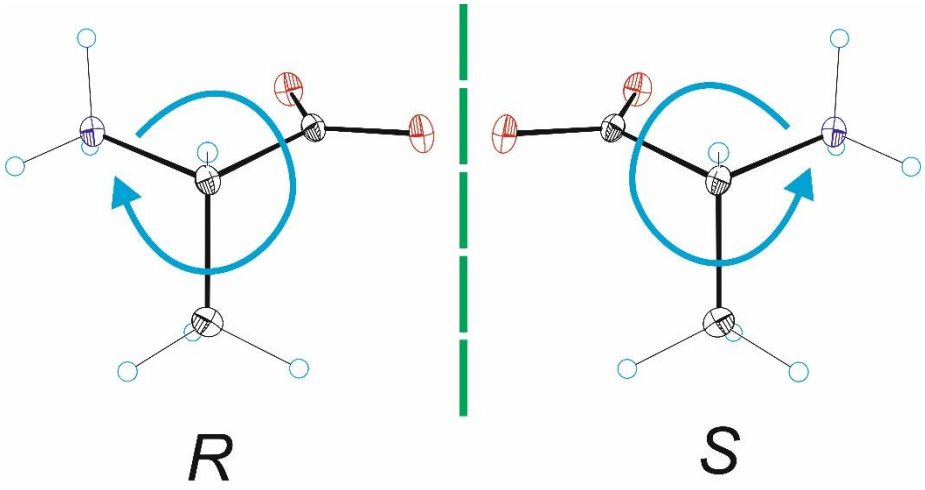
*The Enantiomers of the same compound are called **d-isomer** (dextrotatory) and **l-isomer** (levorotatory)*



Chirality

Definitions in Chirality:

Absolute configuration *in stereochemistry refers to the spatial arrangement of the atoms of a chiral molecular entity (or group) and its stereochemical description e.g.* ***R or S****, referring to Rectus, or Sinister, respectively.*



Hydrogen atom should be behind the carbon atom

The R- or S-configuration is assigned by the Cahn-Ingold-Prelog priority rules

Chirality

Definitions in Chirality:

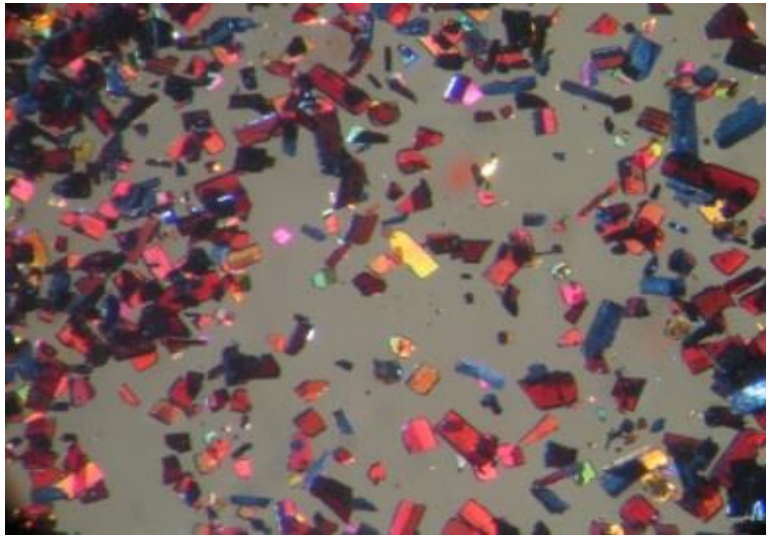
Chiral molecules can have one or more chiral centers.

With one stereo center it can crystallize forming:

- *Pure chiral crystals*
- *Racemic crystals*

} *Different powder X-ray diffraction patterns.*

- *Crystals with a non stoichiometric composition.*



Chirality

Definitions in Chirality:

Starting from the racemic mixture *and in depending of the affinity between the racemic molecules it can be distinguished between:*

Conglomerate: *Mixture of enantiomerically pure crystals*

Racemic compound: *Single crystals with both enantiomers*

} *Different PXRD patterns.*

Pseudo-racemate: *Both enantiomers coexist in an unordered manner in the crystal*

(Quasiracemate: *Mixture of two similar bust distinct compounds. (Chemically different but sterically similar)*

First successful resolution of a racemate was done by Luis Pasteur pulling away crystals of a conglomerate of Tartaric Acid.

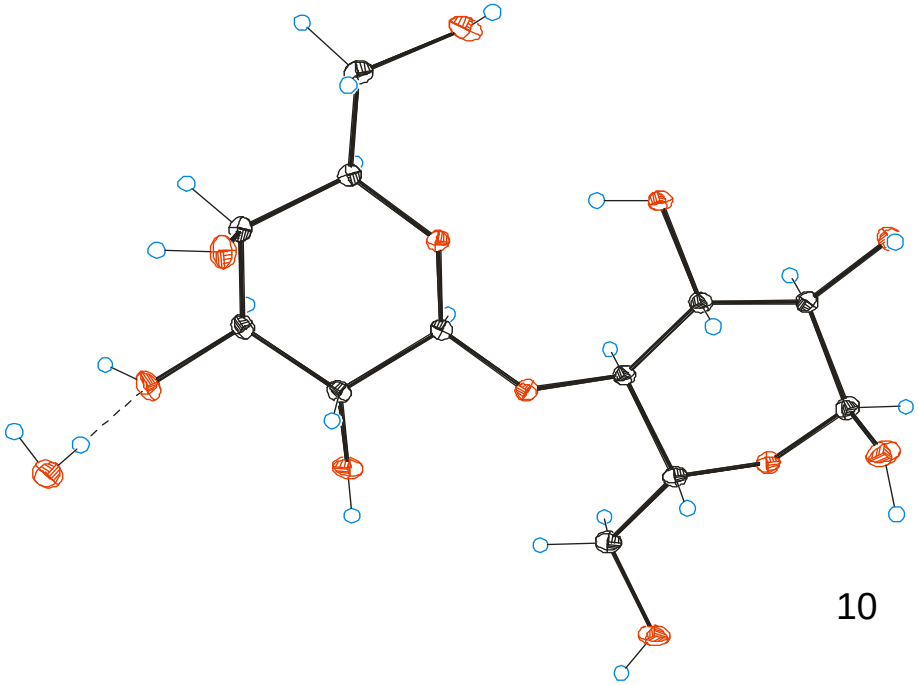
Chirality

Definitions in Chirality:

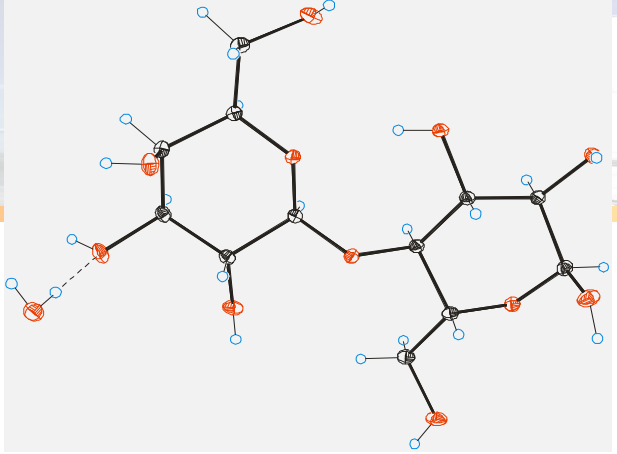
Chiral molecules *crystallized from an enantiomerically pure solution: Structure with a space group which does not contain mirror or inversion symmetry.*

There are 65 space groups in which chiral molecules can crystallize called Chiral Space Groups of Sohncke Groups.

Achiral molecules *can, in contrast, crystallize in "centrosymmetrical" or chiral space groups.*



Chirality



Definitions in Chirality:

Chiral molecule with more than one chiral center can result pairwise to:

Enantiomers: *All stereo centers are inverted, corresponds still to enantiomers.*

Have the same physical properties.

Diastereoisomers: *One or more stereocenters but not all are inverted.*

Diastereoisomers have different physical properties.

Meso isomers*: Non-optically active member of a set of stereoisomers. It contains two o more stereogenic centers but it is not chiral since it is "superposable" on its mirror image. The meso compound is bisected by an internal plane of symmetry.*

n *chiral centers result to a theoretical maximum of* 2^n *stereoisomers if there are not meso forms.*

Chirality

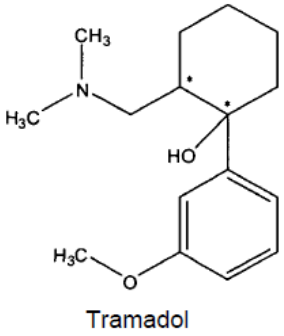
Example of chiral system: Naproxene + Tramadol

Patents: US20140350110A1 / WO2010043412A1

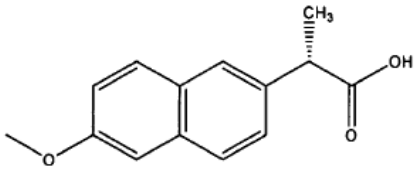
±Tramadol + *S*-Naproxene: Chiral salt of +Tramadol & *S*-Naproxen

-Tramadol + *S*-Naproxene: Chiral co-crystal of a Salt 1:2

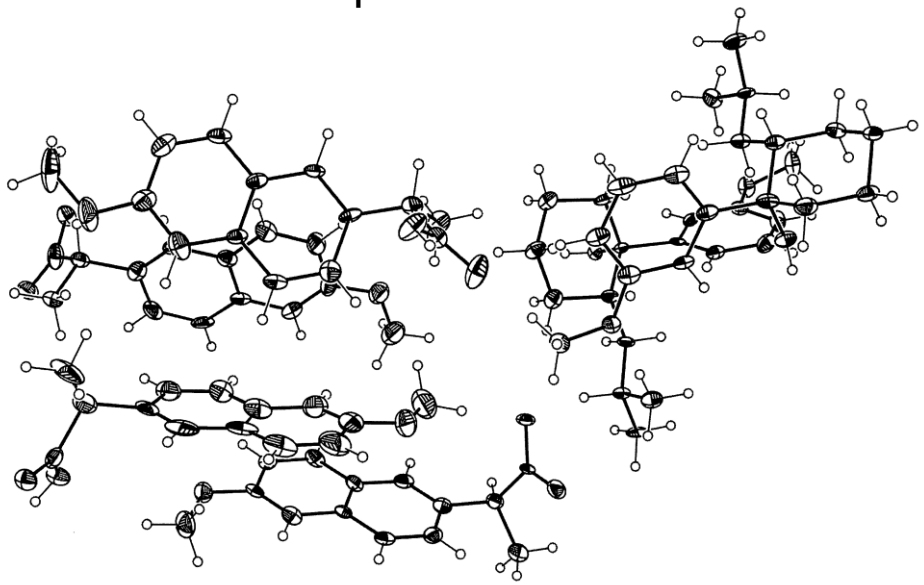
±Tramadol + *S/R*-Naproxene: Chiral crystals in form of a Conglomerate of +Tramadol & *S*-Naproxen and -Tramadol & *R*-Naproxen.



Tramadol



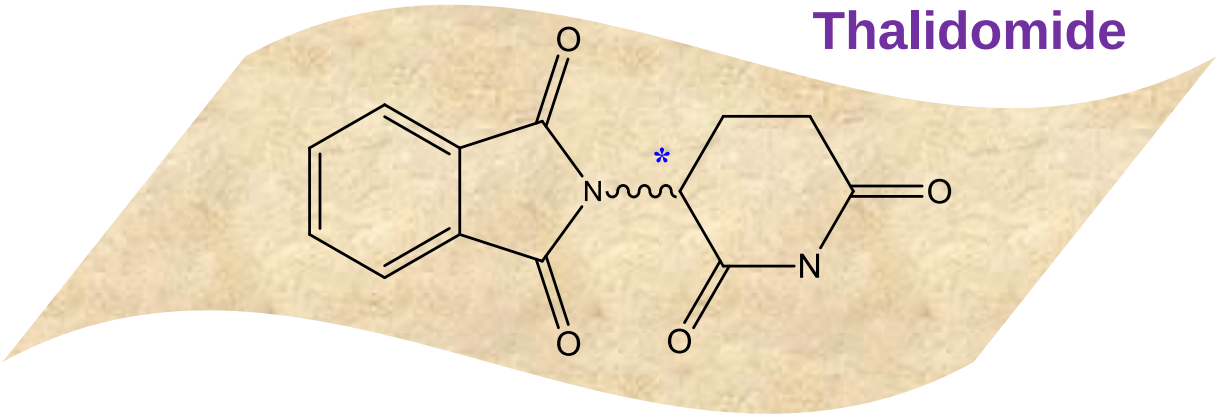
(S)-Naproxen



Chiral Active Pharmaceutical Ingredients

Thalidomide: Developed in the 1950s as a sedative, was acclaimed to be a wonder drug that provided a "save, sound sleep". It was used by pregnant women to reduce the symptoms of the morning sickness.

Thalidomide



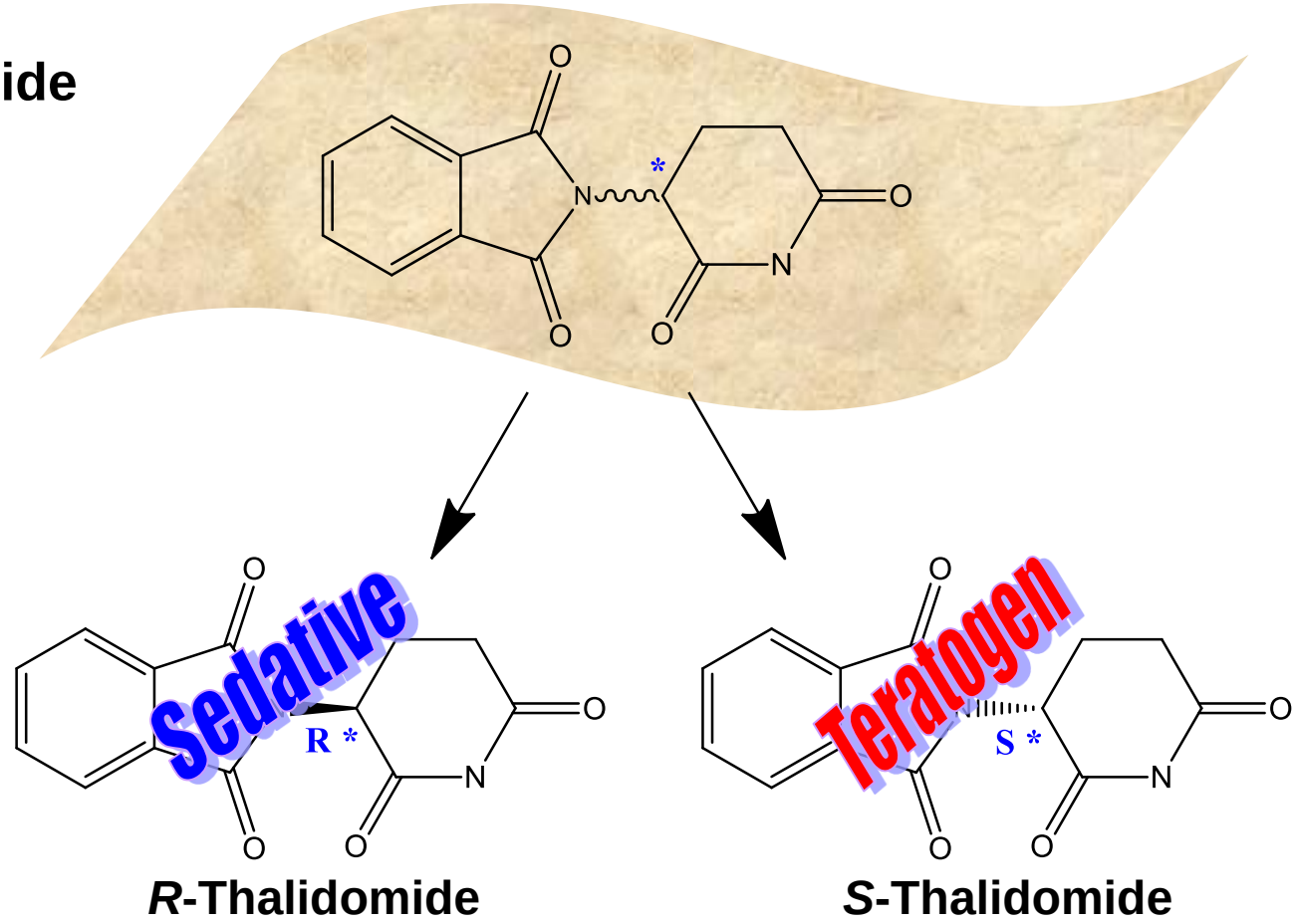
It came out to be a strong teratogen which caused dysmelia (stunted limb growth) in human babies by maternal usage.[1]

[1] *Thalidomide and congenital abnormalities*
McBride, W.G.; Lancet, Volume 2, Issue 721, Pages 1358, 1961.

Chiral Active Pharmaceutical Ingredients

After a detailed investigation of the effects of this drug, the *R*-enantiomer resulted to be sedative and the *S*-enantiomer teratogen.

Thalidomide

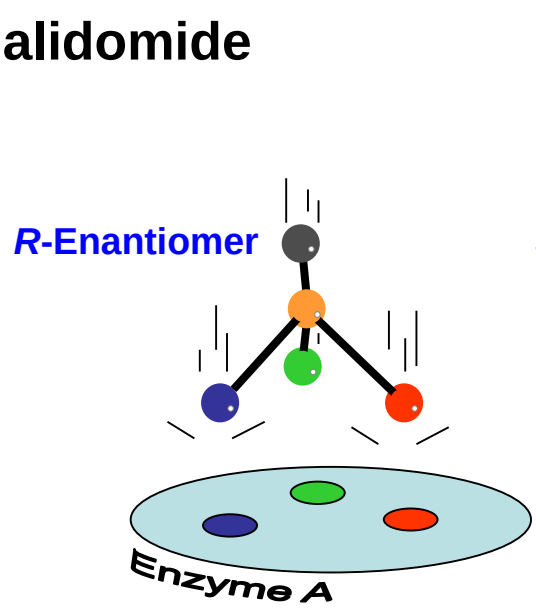


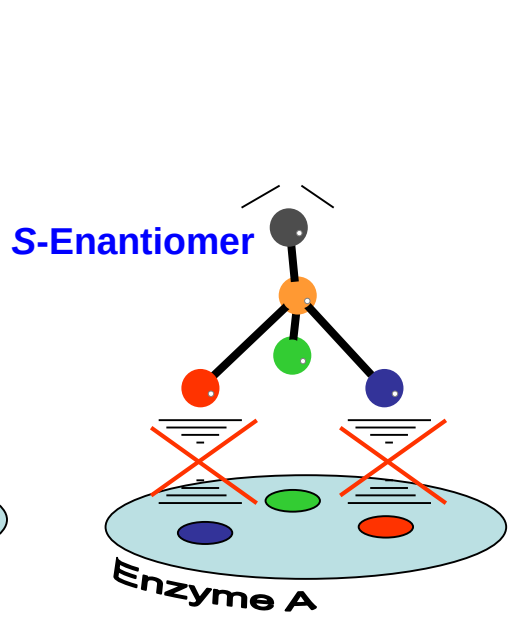
***R*-Thalidomide** ***S*-Thalidomide**

Chiral Active Pharmaceutical Ingredients

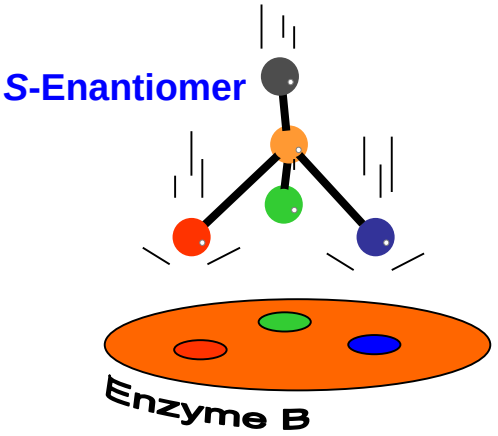
Chiral biological environment

Thalidomide





Sedative effect

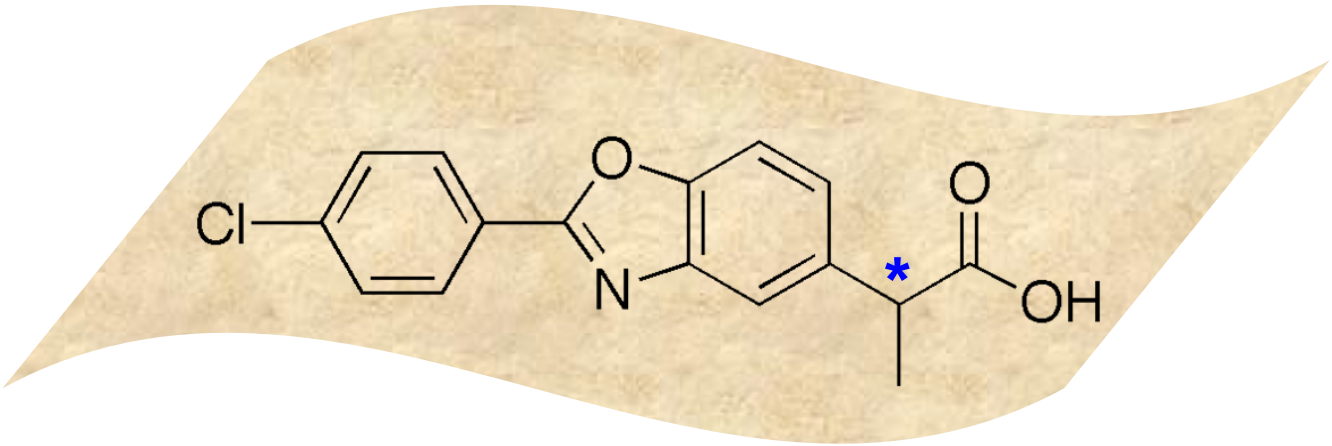


Teratogen effect

Chiral Active Pharmaceutical Ingredients

Benoxaprofen

In a similar case, another new "wonder drug" of the 80s, the anti-inflammator Benoxaprofen (Oraflex), was forced to be withdraw from the marked because of liver damage caused by the "inactive" *R*-isomer.[2,3]



[2] *Liver and kidney pathology in severe adverse reactions associated with the non-steroidal anti-inflammatory drug Benoxaprofen (Opren).*
Macsween, RNM. and Dische, Fe; Journal of Pathology, Volume 140, Issue 2, Pages 123-124, 1983.
[3] *A retrospective study of the molecular toxicology of Benoxaprofen.*
Lewis, DFV. Ioannides, C. and Parke D.V.; Toxicology, Volume 65, Issue 1-2, Pages 33-47, 1990.

Chiral Active Pharmaceutical Ingredients

Actually, there is no doubt that knowing the pharmacology and the pharmacokinetics of enantiomers is an important fact in the development of new APIs (Active Pharmaceutical Ingredients) which are chiral.

Regulatory entities as the FDA (U.S Food and Drug Administration) require sensitive analytical methods to ensure the safety and efficacy of chiral Drugs.

For the approval of either a racemate or a pure enantiomer clinical investigations are necessary to compare the safety and efficacy of the racemate and its enantiomers.[4]

[4] *The FDA perspective on the development of stereoisomers: The pharmacological, biological, and chemical consequences of molecular asymmetry*
Chirality, Volume 1, Issue 1, Pages 2-6, 1989.

Determination of Absolute Configuration

Methods for the determination of absolute configuration:

Direct methods:

- X-ray diffraction analysis
- Chiroptical Methods (supported by ab initio calculations):
  - Electronic Circular Dichroism (ECD)
  - Optical Rotatory Dispersion (ORD in UV-VIS)
  - Vibrational Circular Dichroism (VCD, ROA)

Indirect methods (relative configuration):

- Assignment based on NMR
- Assignment based on enzymatic transformations

Conditioned by the availability of single crystals, the direct method based on X-ray diffraction analysis, is probably the most reliable.

Of course it is recommended to use more that one methodology.

Determination of Absolute Configuration

Ways to determine the absolute configuration by Single Crystal X-ray Diffraction:

Direct methods (absolute structure):

- Molecules with heavy atoms (heavy than Si)

  Straight forward method.
  A heavy atom can be added to the molecule by synthesis or by co-crystallization (for example using CH_3Cl as crystallization solvent).

- Molecules with only light atoms (CHNO)

  Not easy due to the “weak distinguishing power”.

Indirect methods (relative configuration):

- Molecules with one known chiral center.
- Co-crystallization with a molecule with a known chiral center.

Determination of Absolute Configuration

Determination of the absolute configuration by X-ray diffraction:

Based on the **anomalous scattering effects** produced on the atoms contained in the structure. Is only effective if the differences between Friedel-related reflections collected from a single crystal are large enough to be detected.

Traditionally: Based on the single crystal X-ray structure determination performed using $Cu_{K\alpha}$ or $Mo_{K\alpha}$ radiation on compounds containing atoms **heavier than Si**.

Anomalous scattering effects: Much weaker (specially using $Mo_{K\alpha}$ radiation) at **organic molecules** containing only "*light atoms*"*.

Basic problem: Weak inversion distinguishing capacity on the determination of the absolute configuration of APIs (Active Pharmaceutical Ingredients) and natural products.

* CHNO Molecules

Absolute Configuration of CHNO Molecules

Basic problem: "Distinguishing power"

Low differences in the anomalous dispersion for C,N,O-atoms

The most difficult case to determine the absolute configuration applies when the analysed molecule contains **only** "**light atoms**" like carbon, nitrogen and oxygen and **no known chiral centres** are available.

In this case, traditionally, Copper Kα radiation is taken as the wavelength of choice and Molybdenum Kα radiation was considered traditionally as a "taboo", since its anomalous dispersion effects are expected to weak to be suitable.

Absolute Configuration of CHNO Molecules

We postulated: The determination of the absolute structure by using **$Mo_{K\alpha}$ radiation** is possible based on

a) The number of reflections that can be collected using $Mo_{K\alpha}$ radiation is ten times larger than using $Cu_{K\alpha}$ radiation for the same 2θ range without massive increment on data acquisition time. Consequently, the limitation of the weaker resonant scattering of $Mo_{K\alpha}$ radiation can be compensated by a **substantial increase in the number of reflections**.

b) Because of that the effect of the **resonant scattering is angle independent**, the **relative contribution** to the intensity differences **increases** at very high resolution accessible with $Mo_{K\alpha}$ radiation (specially measuring at low temperature).

1) E.C. Escudero-Adán*, J. Benet-Buchholz*, P. Ballester; Acta Cryst. (2014) B70, 660-668.

Absolute Configuration of CHNO Molecules

Erroneous Interpretations or Determinations

Determination not possible in several structures of chiral organic molecules published in the Cambridge Data Base in which the measurement was performed using Molybdenum radiation and not enough data were collected.

Erroneous determinations in some appeared publications in which was affirmed that the absolute structure of an organic compound was determined properly. These affirmations were wrong since the standard uncertainties were (far) below the edge of trueness.

Absolute Configuration of CHNO Molecules

Methods to determine the absolute configuration from enantiopure crystals up to the nineties:

1951 (first method): Bijvoet J.M., Peerdeman A.F., van Bommel A.J., Nature 168 (1951) 271.

1965 (R-factor ratio test): Hamilton W.C., Acta Cryst. 18 (1965) 502-510

1981 (first refinable parameter): Rogers, D., Acta Cryst. A37 (1981) 734–741.

1983 (established traditional method): Flack H.D., Acta Cryst. A39 (1983) 876.

1990 (probabilistic method): Le Page, Y., Gabe, E. J. & Gainsford, G. J. J. Appl. Cryst., 23, (1990) 406–411.

Absolute Configuration of CHNO Molecules

Flack Parameter: x(u)

The **Flack parameter x** is a value obtained after refinement of the structure which should be zero if the absolute configuration has been determined properly and one in the inverted structure has been refined. The Flack x parameter encodes the relative abundance of the two components in an inversion twin.

$$|F(h,x)|^2 = (1-x)\,|F(h)|^2 + x\,|F(-h)|^2$$

The correctness of the Flack parameter is determined by its standard uncertainty **u**.

Strong inversion-distinguishing value: **u: 0.04**
Enantiopure-sufficient inversion-distinguishing power: **u: 0.08**

Flack H.D., *Acta Cryst.*, 1983, *A39*, 876-881.
Flack H.D., Bernardinelli G., *Acta Cryst.*, 1999, *A55*, 908-915.
Flack H.D., Bernardinelli G., *J. Appl. Cryst.*, 2000, *33*, 1143-1148.

Absolute Configuration of CHNO Molecules

State of the Art
Advances for the determination of Absolute configuration of CHNO molecules

Using the Flack Parameter:

"The **standard uncertainty values** obtained seemed to be **too high** so that they did not reflect the reality.

Are the applied limits of trueness to strength?"

Since in "light atom" molecules, the weak distinguishing power in the determination of the absolute configuration leaded to standard uncertainties at the edge of trueness, several new methodologies were developed trying to get a stronger inversion-distinguishing power.

Absolute Configuration of CHNO Molecules

New methodologies:

A) Hooft et al. described a post-refinement Bayesian statistical procedure which defines the probability that a refined absolute structure is correct.
a) Hooft R. W. W., Straver L. H. & Spek A. L. J. Appl. Cryst. 41, (2008) 96–103. b) Hooft R. W. W., Straver L. H. & Spek A. L. J. Appl. Cryst. 43, (2010) 665–668.

B1) Parsons & Flack reported an alternative technique based on quotient restraint (using differences free from systematic errors).
Parsons S., Flack H., Acta Cryst. A39 (2004) S61.

B2) Parsons, Wagner et al. described a methodology in which refinement weights are modified for data in proportion to their sensitivity to the Flack parameter.
Parsons S., Wagner T., Presly O., Wood P. A. & Cooper R. IJ. Appl. Cryst. 45, (2012) 417–429.

B3) Parsons et al. described the estimation of the uncertainty by using differences and quotients refining with TOPAS-Academic.
(Parsons S., Flack H.D., Wagner T., Acta Cryst. B69 (2013) 249-259.)

Absolute Configuration of CHNO Molecules

New methodologies:

Hooft-Parameter

Parsons-Parameter

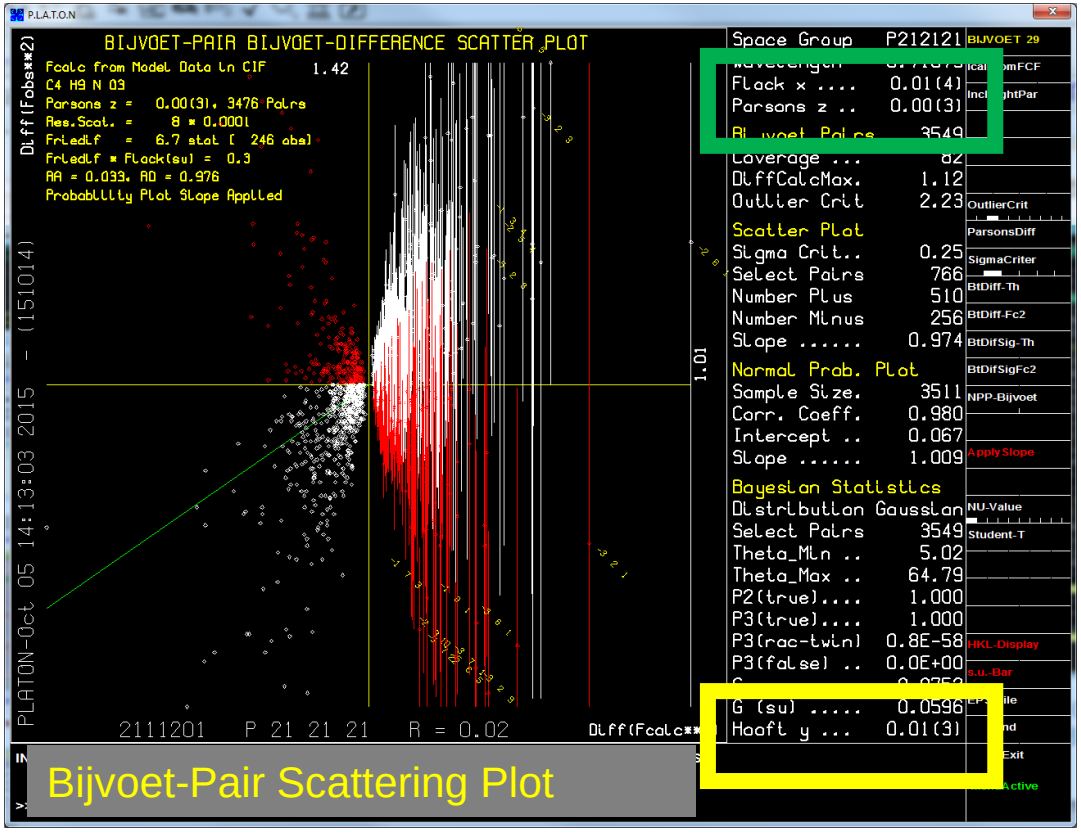
Technique: Measuring Strategy & Background

Determination of absolute structure using Bayesian statistics on Bijvoet differences

New probabilistic approach for the determination of the absolute structure of a compound which is known to be enantiopure based on Bijvoet-pair differences.

Hooft Parameter: y(u)

The standard uncertainty is generally improved by factor three.



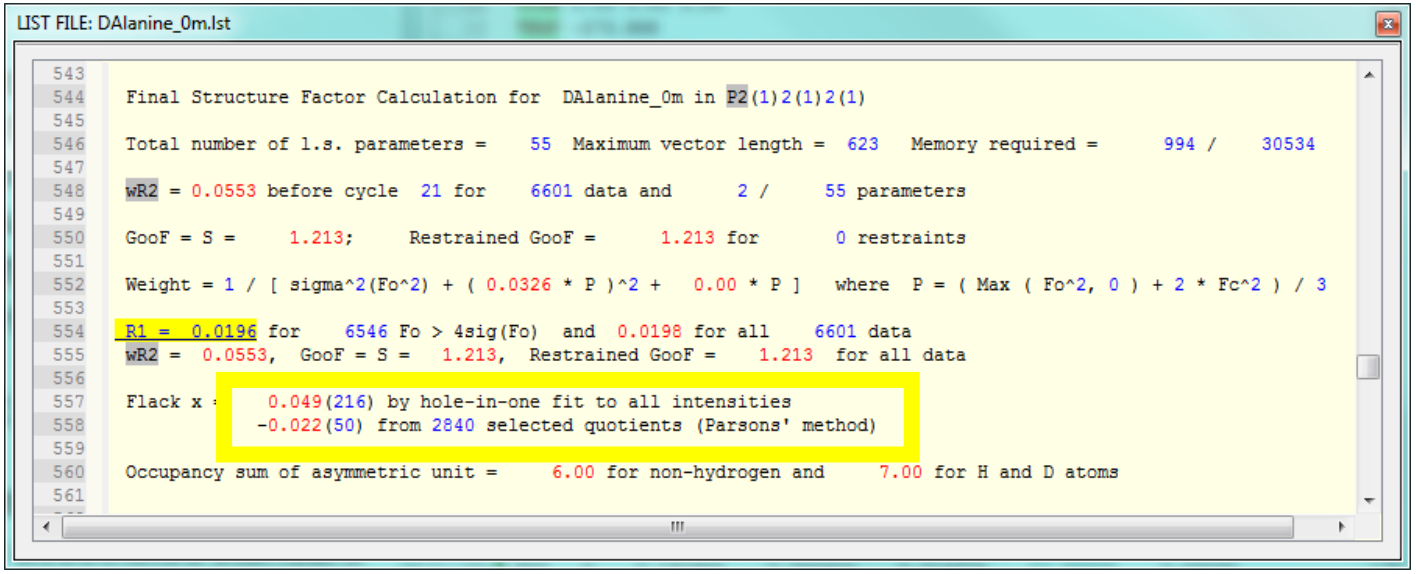
Hooft, R. W. W., Straver, L. H. & Spek, A. L. J. Appl. Cryst. 41, (2008) 96–103.
Hooft, R. W. W., Straver, L. H. & Spek, A. L. J. Appl. Cryst. 43, (2010) 665–668.
Platon: Speck, A.L., Acta Cryst. 2009, D65, 148-155.

Technique: Measuring Strategy & Background

Parsons Method z(u) and traditional revised Flack Parameter x(u)

In the new SHELX2014 version the refinement of a chiral structure gives:

- A traditional (but revised) Flack parameter. The standard uncertainty is higher than in the classical Flack parameter implemented in SHELX93
- A new “Parsons” z parameter calculated with selected quotients based on the Parsons’ method. The standard uncertainty is generally improved by factor three.



Parsons S., Flack H., Acta Cryst. A39 (2004) S61.
SHELXL 2014; George M. Sheldrick 1993-2013.

Absolute Configuration of CHNO Molecules

Goal: ***Determination of the absolute configuration of chiral active pharmaceutical ingredients (APIs).***

Approach:

Validated method: It should make the determination of the absolute configuration from the point of view of a pharmaceutical company credible.

Should take advantage and make profitable the state of the art of the evaluating and measuring techniques.

Absolute Configuration of CHNO Molecules

Standard Industry Samples:

- The analyzed standards and APIs are enantiopure compounds which are synthetically prepared or are natural products.

- Most of the APIs are well characterized and pure compounds.

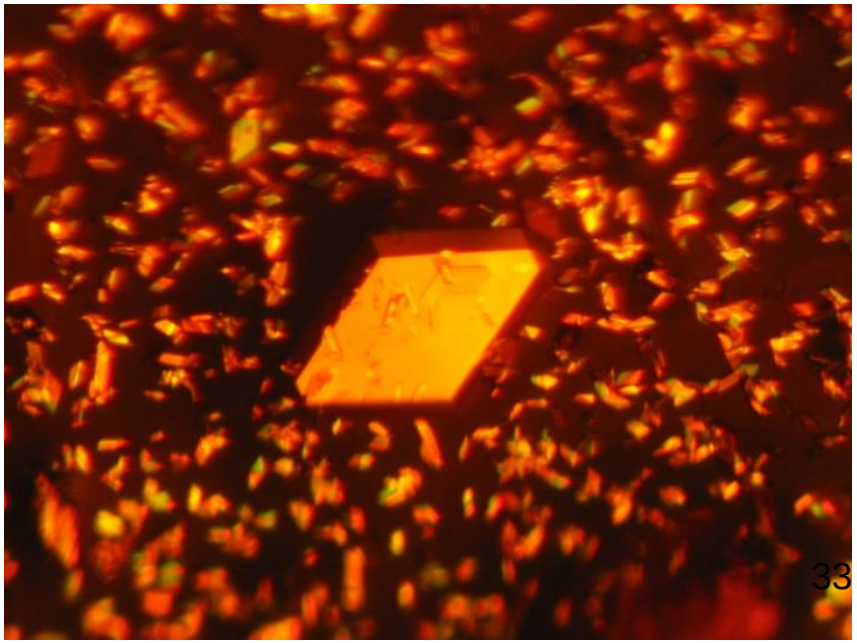
- Normally they are available in enough quantity for crystallization.

Technique: Measuring Strategy & Background

Crystals: Of good quality and representative for the sample.

This is a crucial point for the determination. The crystals obtained should be examined carefully and "singular" crystals should be rejected. If the sample is pure, the main bulk of crystals should belong to the expected compound. In case of doubts more than one crystal should be analyzed.

Be **"very" suspicious** about a large and perfect single crystal surrounded only by small and different looking crystals!!!



Technique: Measuring Strategy & Background

Crystals: Of good quality and representative for the sample

Example: Analyzed sample with **two chiral centers** with possible small amounts of the **diastereoisomer** (<95%) ⇒ Optical rotation and HPLC experiments should be performed on the crystal measured.

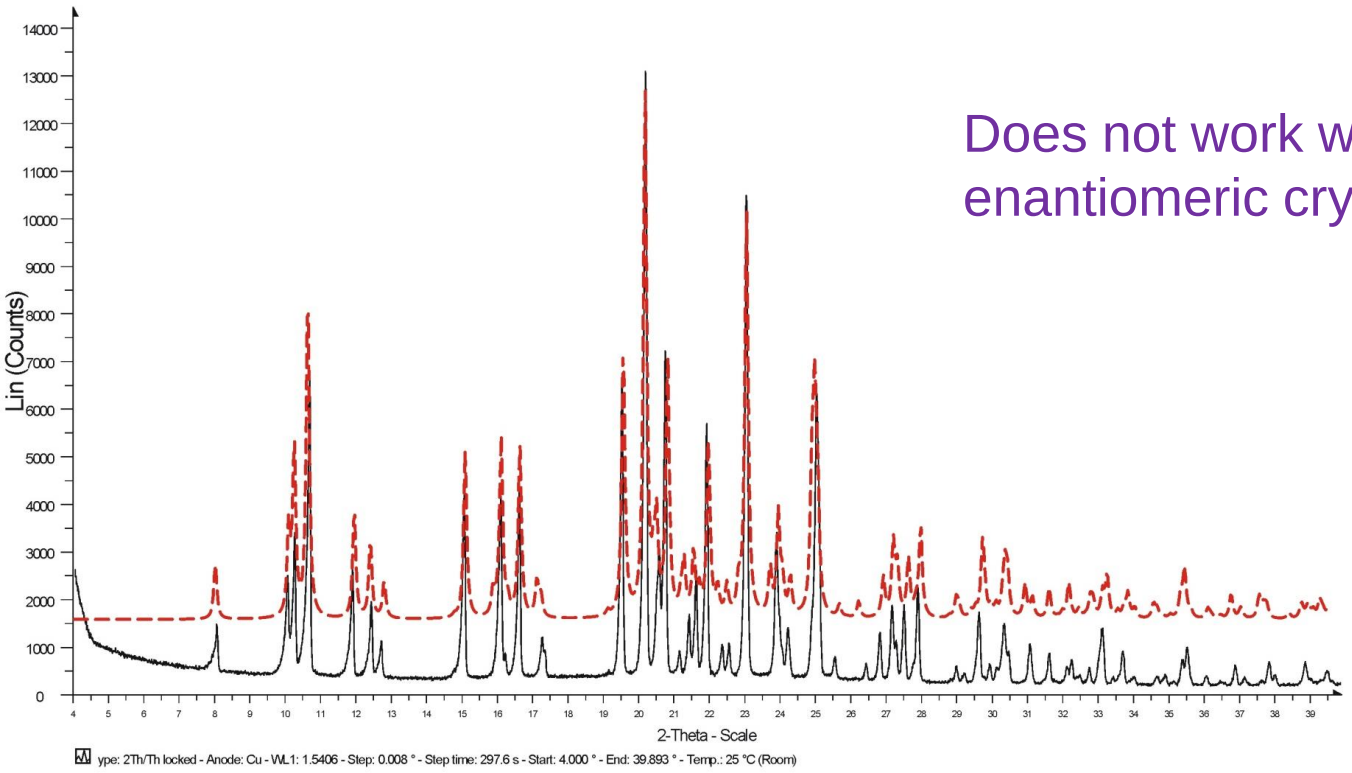
The presence of few crystals could indicate that the "possible more insoluble" wrong diastereoisomer has crystallized ⇒ The right one is still in solution or is amorphous.

This is a real case which happened to us. After the optical rotation and HPLC experiments we could clear that we were crystallizing the minor present diastereoisomer of the API. We got only few crystals and the major component was still in solution. In an second fractioned crystallization we got the right form.

Technique: Measuring Strategy & Background

Crystals: Of good quality and representative for the sample

In case of doubts ⇒ Check identity of the measured crystal by comparison of the **powder X-ray diffraction** (PXRD) patterns of the bulk and from single crystal X-ray structure determination.



Does not work with enantiomeric crystals!

Technique: Measuring Strategy & Background

Measurements:

- Measurements with full datasets and high “Friedel pair coverage”
- High redundancy (4-5)
- Maximum resolution of the device uses (2θ ~140^{o})
- Low temperature, 100 K or lower
- Good Absorption Correction

Absolute Configuration Determinations based on:

- Flack (SHELX93)
- Parsons method (SHELXL2014)
- Hooft method (Bijvoet-Pairs, Platon).

Absolute Configuration of CHNO Molecules with $Cu_{K\alpha}$

56 measurements:

$Cu_{K\alpha}$

Alanine

Carbohydrates

Macromolecules

VALIDATION

SUBSTANCE	File	R_1	wR2	Flack	Hooft	Parsons	Friedif	Reflections Total	Reflections Obsv.	Temp.	S. Group
L-Alanine	1101 LAla1RT	0,0301	0,0752	0,25(30)	0,37(10)	0,31(12)	34	730	730	RT	$P2_12_12_1$
	1102 LAla2RT	0,0339	0,0793	-0,23(38)	-0,10(20)	0,01(28)	34	701	700	RT	
	1103 LAla3RT	0,0280	0,0713	0,13(28)	0,11(12)	0,13(14)	34	751	740	RT	
	1104 LAla4RT	0,0351	0,0833	0,01(32)	0,03(15)	0,06(14)	34	733	725	RT	
	1105 LAla5RT	0,0274	0,0695	0,12(32)	0,10(13)	0,11(15)	34	725	717	RT	
	1106 LAla6RU	0,0305	0,0791	-0,06(34)	-0,18(13)	-0,16(17)	34	682	681	RT	
	1107 LAla90K	0,0274	0,0679	-0,23(31)	-0,18(14)	-0,25(15)	34	719	676	90K	
	1108 LAla9RT	0,0357	0,0852	-0,15(34)	-0,11(15)	-0,10(19)	34	700	697	RT	
	1109 LAlanRT	0,0316	0,0810	-0,24(35)	0,00(14)	0,08(18)	34	739	7[illegible]	RT	
	1110 LAl2RT	0,0349	0,0885	0,08(35)	0,07(11)	0,11(13)	34	719	711	RT	
	1111 SAlanRT	0,0471	0,1139	0,15(41)	0,11(16)	0,10(20)	34	[illegible]16	702	RT	
L-Asparigine	1201 LAspaRT	0,0301	0,0[illegible]	0,24(27)	0,[illegible]	[illegible]	33	1120	1083	RT	$P2_12_12_1$
	1202 LAsprRU	0,0316	0,0812	0,22(35)	0,35(12)	0,36(14)	33	1096	1019	RT	
L-Aspartate	1301 DilphRT	0,0279	0,0722	0,21(26)	0,38(15)	0,33(18)	34	800	769	RT	$P2_1$
L-Glutamate	1401 LGlutRU	0,0300	0,0756	0,10(27)	0,07(16)	0,12(18)	35	1078	1018	RT	$P2_12_12_1$
L-Glutamine	1501 LGlutRT	0,0383	0,0961	-0,11(35)	-0,01(15)	0,01(18)	33	1029	994	RT	$P2_12_12_1$
L-Histidine	1601 LHis90K	0,0315	0,0782	0,13(29)	0,03(13)	0,06(15)	30	1092	1058	90K	$P2_12_12_1$
	1602 LHistRT	0,0319	0,0822	0,29(33)	0,27(15)	0,22(18)	30	1111	1041	RT	
L-Serine	1701 LSer90K	0,0274	0,0683	-0,03(30)	0,15(15)	0,15(17)	34	733	712	90K	$P2_12_12_1$
	1702 LSeriRT	0,0314	0,0781	-0,14(38)	0,04(12)	0,10(14)	34	743	717	Rt	
L-Threo	1801 LThreRT	0,0266	0,0635	-0,08(28)	0,05(10)	0,03(12)	35	901	853	RT	$P2_12_12_1$
	1802 LThr90K	0,0263	0,0630	-0,07(27)	0,00(13)	-0,02(15)	35	896	846	90K	
	1803 2S3RTre	0,0371	0,0883	-0,01(30)	0,13(11)	0,10(13)	35	909	885	RT	
D-Threo-ine	1804 DTHreo_1b	0,0337	0,0875	0,05(32)	0,01(09)	0,03(09)	35	820	819	100K	$P2_12_12_1$
	1805 ThreoninUB	0,0371	0,0883	-0,01(30)	0,09(03)	0,13(03)	35	864	863	100K	
L-Valine	1901 Lvalirtg	0,0658	0,1794	-0,24(42)	0,00(20)	-0,07(0,24)	35	1439	1356	RT	$P2_1$
L-Hydroxyproline	2001 LhProRT	0,0371	0,0902	0,04(33)	0,05(09)	0,02(11)	35	1002	987	RT	$P2_12_12_1$
L-Ascorbic acid	2101 LAscoART	0,0513	0,1[illegible]	0,01 (28)	0,26(18)	[illegible]	35	1854	1740	RT	$P2_1$
	2102 LAscoRtg	0,0[illegible]33	0,1041	0,08(28)	0,30(20)	0,31(30)	35	2085	1758	RT	
ß-D-Ribofuranose 1,2,3,5-tetraacetate	3001 BDRiRT	0,0336	0,0887	0,12(18)	0,14(08)	0,13(09)	37	2[illegible]	2611	RT	$P2_12_12_1$
Methyl-a-D-Mannopyranoside	3101 aDMaRTe	0,0270	0,0690	-0,02(18)	0,08(08)	0,05(10)	37	1505	1442	RT	$P2_12_12_1$
	3102 MaLM90K	0,0326	0,0816	-0,04(21)	0,15(09)	0,17(10)	37	1431	140[illegible]	90K	
Methyl-a-L-Rhamnopyranoside	3201 MaLR90K	0,0276	0,0712	0,01(17)	0,02(05)	0,02(06)	37	1387	1372	90K	$P2_12_12_1$
	3202 MaLRhRT	0,0331	0,0863	0,17(21)	0,12(08)	0,12(09)	37	1431	1383	RT	
Methyl-ß-D-Galactopyranoside	3301 MbDG90K	0,0379	0,1371	0,27(24)	0,28(11)	0,30(13)	37	1432	1371	90K	$P2_12_12_1$
	3302 MbDGaRT	0,0451	0,1072	0,19(26)	0,22(12)	0,17(14)	37	1461	1398	RT	
	3303 BDGa90K	0,0276	0,0696	0,03(18)	0,02(07)	0,02(08)	37	1474	1452	90K	
	3304 BDGalRT	0,0318	0,0806	-0,05(22)	-0,12(09)	-0,12(10)	37	1505	1472	RT	
a-D-Heptagluconsäure-ß-Lacton	3401 aDHGlRT	0,0333	0,0818	0,13(20)	0,11(09)	0,14(11)	37	1498	1445	RT	$P2_12_12_1$
Methyl-ß-D-Glucopyranoside hemihydrate	3501 MBDGlRT	0,0309	0,0855	0,04(22)	0,06(08)	0,07(09)	37	1678	1614	RT	$P4_12_12$
1,3,4,6-Tetra-O-Acetyl-ß-D-Ribopyranoside	3601 toaR90K	0,0294	0,0753	-0,07(19)	0,35(10)	0,10(12)	37	3873	3849	90K	$P4_1$
	3602 toaRtRT	0,0487	0,1257	-0,13(36)	0,35(19)	0,11(27)	37	3855	3838	RT	
1,6-Anhydro-ß-D-Glucopyranose	3701 AnBGluc	0,03[illegible]	0,0828	-0.02(21)	0,18(11)	0,11(10)	35	1113	1105	RT	$P2_12_12_1$
(-)-(1R,2S)-2-Amino-4-methylen-cyclopentanecarbonsäure	4001 b108888	0,0300	0,0761	-0,06(22)	0,[illegible]	-0,04(13)	33	1175	1184	90K	$P2_1$
	4002 B10890K	0,0297	0,0732	0,12(25)	0,09(12)	0,11(14)	33	964	958	90K	
	4003 B1088RT	0,0320	0,0806	0,08(29)	0,10(13)	0,13(16)	33	1008	985	RT	
Cyclodepsipeptid	4101 PF1022pro	0,0397	0,0[illegible]	0,[illegible]	0,14(08)	[illegible]	33	8936	8253	90K	$P2_1$
Cyclohexadepsipeptid	4201 JES18[illegible]	0,0364	0,0981	-0,01(12)	-0,02(03)	-0,02(04)	33	[illegible]	6806	90K	$P2_12_12_1$
(-)-Galielalacton	4301 NUF300g	0,0308	0,0844	0,05(18)	-0,06(04)	-0,04(05)	34	1760	1[illegible]	90K	$P2_12_12_1$
Bisdehydratogaliellalacton	4302 NUF320g	0,0280	0,0735	-0,04(12)	-0,01(04)	0,01(04)	31	3246	3222	90K	$P2_12_12_1$
Acyldepsipeptide	4401 B646309	0,0310	0,0805	0,04(08)	0,04(03)	0,05(03)	40	7570	7420	90K	$P2_12_12_1$
	4402 B666666	0,0397	0,1025	0,03(11)	0,00(04)	0,03(05)	40	7599	7433		$P2_12_12_1$
Longicatenamycin A precursor	4501 NUF895g	0,0358	0,0889	-0,06(10)	0,05(06)	0,04(06)	34	7527	7161	90K	$P2_12_12_1$
	4602 CAM2702g	0,0396	0,0966	-0,04(13)	0,03(07)	0,02(07)	35	3052	[illegible]028	90K	$P2_1$
Lysobactin (Katanosin B)	4701 Lysobacting	0,0779	0,[illegible]	0,06(28)	-0,01(08)	[illegible]	42	10733	8349	90K	$P2_1$
Cinnabaramide A	4801 js36010ag	0,0774	0,1865	0,09(19)	0,12(05)	0,13(05)	32	26995	21014	90K	$P2_12_12_1$

Absolute Configuration of CHNO Molecules with $Mo_{K\alpha}$

45 measurements:

Alanine

$Mo_{K\alpha}$

Threonine

VALIDATION

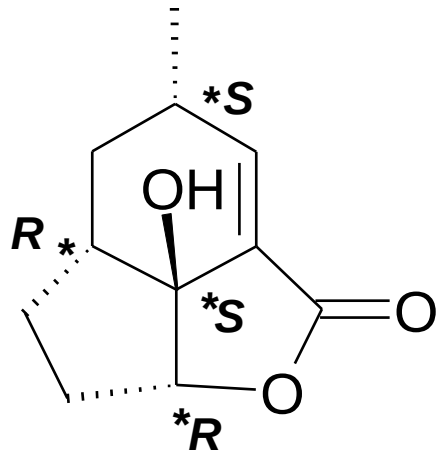
1) E.C. Escudero-Adán, J. Benet-Buchholz, P. Ballester; Acta Cryst. (2014) B70, 660-668.

Carbohydrates

Compound	Sample	$Friedif_{stat}$	$N_{ind}(I>4\sigma)$	Redundancy	Space Group	$R_1(I>4\sigma)$	x (Flack)	y (Hooft)	z (Parsons)
L-Alanine	5001 [a]		6209	4.5	$P2_12_12_1$	0.0254			0.06(8)
	5002 [a]	6.5	6393	5.6	$P2_12_12_1$	0.0299	-0.07(29)	- 0.09(9)	
	5006 [a]	6.5	6549	5.1	$P2_12_12_1$	0.0196	0.05(22)	-0.02(5)	
	5008		6172	4.8	$P2_12_12_1$	0.0238			-0.01(4)
L-Serin	5101 [a]	6.5	7308	5.2	$P2_12_12_1$	0.0270	0.03(28)	0.05(5)	0.07(5)
D-Threonine	5201 [a]	6.7	8832			0.0210	0.12(22)	0.04(5)	0.05(5)
	5202 [a]	6.7	8389	5.2	$P2_12_12_1$	0.0202		-0.03(6)	-0.03(5)
	5203 [a]	6.7	8124	5.8	$P2_12_12_1$	0.0219	0.01(24)		0.07(6)
	5204 [a]	6.7	8324	11.6	$P2_12_12_1$	0.0221	0.03(24)	0.02(6)	0.02(6)
	5205 [I,a]	6.7	7711	3.7	$P2_12_12_1$	0.0266	0.02(29)	0.00(9)	
	5206 [I,a]	6.7	6401	3.2	$P2_12_12_1$	0.0237	0.29(32)	0.10(7)	0.1
	5212 [b]	6.7	8758	5.7	$P2_12_12_1$	0.0241	0.08(26)	0.01(9)	-0.01(
	5213 [a]	6.7	8952	5.9	$P2_12_12_1$	0.0196	-0.12(19)	-0.03(5)	-0.03(
	5214 [b]	6.7	8883	5.9	$P2_12_12_1$	0.0231	0.09(24)	0.06(7)	0.0
	5215 [c]	6.7	9543	8.2	$P2_12_12_1$	0.0223	0.00(21)	-0.02(5)	-0.03(5)
	5216 [d]	6.7	8257	5.2	$P2_12_12_1$	0.0190	0.01(22)	0.00(3)	-0.01(3)
L-Threonine	5210	6.7	8473	5.6	$P2_12_12_1$	0.0223	0.11(23)	0.06(4)	0.09(3)
	5211 [a]	6.7	8060	2.6	$P2_12_12_1$	0.0235	0.07(3)	0.05(8)	0.05(8)
L-As	5301 [a]	6.5	8234				-0.06(23)	0.02(9)	-0.01(8)
L-Hy	5401 [a]	6.8	9535	5.2	$P2_12_12_1$	0.0230	0.06(26)	0.03(3)	0.03(3)
	5402 [a]	6.8	9311	11.5	$P2_12_12_1$	0.0239	0.07(26)	0.05(3)	0.06(3)
L-Glutamine	5501 [a]	6.7	9418	5.2	$P2_12_12_1$	0.0205	0.15(20)	0.07(5)	0.08(5)
L-Histidine	5602 [a]	5.7	10624	5.1	$P2_12_12_1$	0.0299	0.14(40)	0.07(7)	0.09(6)
	5603 [a]	5.7	10721	5.0	$P2_12_12_1$	0.0360	-0.10(45)	0.08(8)	0.08(11)
	5604 [a]	5.7	11241	5.1	$P2_12_12_1$	0.0314	0.09(42)	0.01(7)	0.00(7)
L-Valin	5701 [a]	6.6	18138	2.5	$P2_1$	0.0295	0.11(19)	0.10(9)	0.12(8)
L-Isoleucine	5801 [a]	6.4	19689	2.6	$P2_1$	0.0315	-0.20(20)	-0.05(9)	-0.04(8)
Methyl-α-L-rhamnopyranoside	6001 [a]	7.1	13396	5.3	$P2_12_12_1$	0.0217	0.08(16)	-0.05(5)	-0.04(4)
	6002 [a]	7.1	12004	5.3	$P2_12_12_1$	0.0233	0.03(17)	0.07(4)	0.07(4)
	6003 [a]	7.1	11628	5.5	$P2_12_12_1$	0.0238	0.14(18)	0.11(4)	0.11(4)
Methyl-α-D-mannopyranoside	6101 [a]	7	13431	4.9	$P2_12_12_1$	0.0208	-0.08(17)	-0.06(4)	-0.07(3)
Methyl-α-D-glucopyranoside	6201 [a]	7	13732	5.2	$P2_12_12_1$	0.0239	0.03(18)	0.02(4)	0.02(4)
	6202 [a]	7	13666	5.2	$P2_12_12_1$	0.0273	0.07(21)	0.02(6)	0.00(6)
ß-D-Galactose pentaacetate	6301 [a]	7	24645	5.2	$P2_12_12_1$	0.0367	-0.04(21)	0.01(5)	0.03(6)
ß-D-Lactose monohydrate	6401 [a]	6.9	23466	2.6	$P2_1$	0.0347	-0.02(22)	-0.02(5)	0.01(5)
α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside	6501 [a]	6.9	20204	2.4	$P2_1$	0.0198	-0.06(12)	0.03(5)	- 0.01(5)
	6502 [a]	6.9	20110	2.9	$P2_1$	0.0197	0.04(11)	0.05(4)	0.06(3)
	6503 [a]	6.9	21902	2.6	$P2_1$	0.0207	-0.09(12)	-0.04(4)	-0.04(5)
	6504 [a]	6.9	20599	4.7	$P2_1$	0.0190	0.04(11)	0.04(3)	0.05(3)
	6507 [I,a]	6.9	9994	1.2	$P2_1$	0.0239	-0.20(20)	0.07(6)	0.04(8)
ß-D-Maltose octaacetate	6601 [a]	7	40462	4.8	$P2_12_12_1$	0.0629	0.02(33)	0.03(8)	0.04(10)
β-D-Allose	6802 [a]	6.9	9523	6.6	$P2_12_12_1$	0.0267	0.17(25)	-0.08(8)	-0.04(8)
L-Tartaric acid	7001 [a]	6.3	8734	2.5	$P2_1$	0.0225	0.04(20)	0.02(8)	0.04(10)
$C_{15}H_{26}O_2$ (Carreras et. al. 2014)	7701 [a]	5.6	26783	3.1	$P2_12_12$	0.0393	-0.01(32)	-0.02 (10)	-0.04(11)
$C_{11}H_{14}O_6$ (Nieto et. al. 2005)	7801[a]	7	14435	3.8	$P2_12_12_1$	0.0364	0.09(31)	-0.02(8)	-0.04(7)

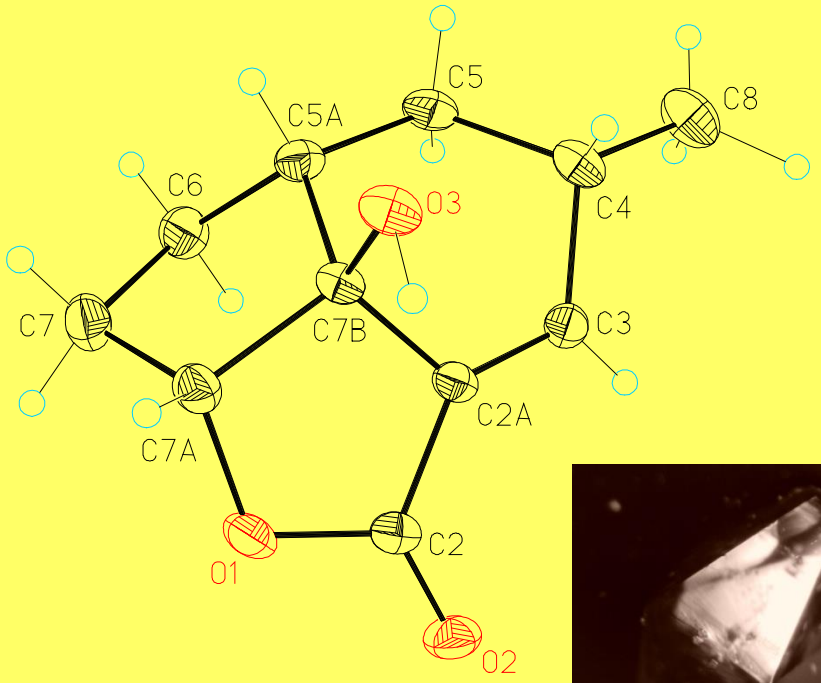
Quirality with $Cu_{K\alpha}$ radiation

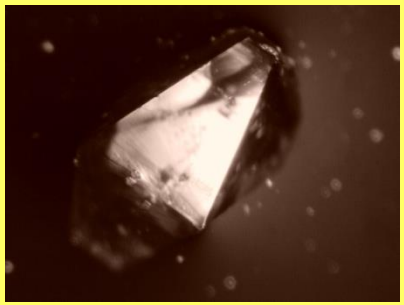
(–)-Galiellalactone



Absolute configuration:

4*S*, 5a*R*, 7a*R*, 7b*S*



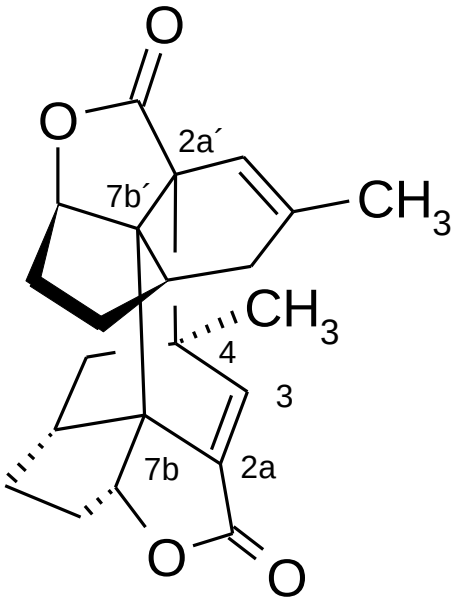


Orthorhombic, $P2_12_12_1$
R_1: 3,45 %
Flack/Hooft/Parsons: 0.01(19)/-0.06(04)/-0.04(05)

- F. Nussbaum, R. Hanke, T. Fahrig, J. Benet-Buchholz; *Eur. J. Org. Chem.* ***2004****, 2783-2790.*

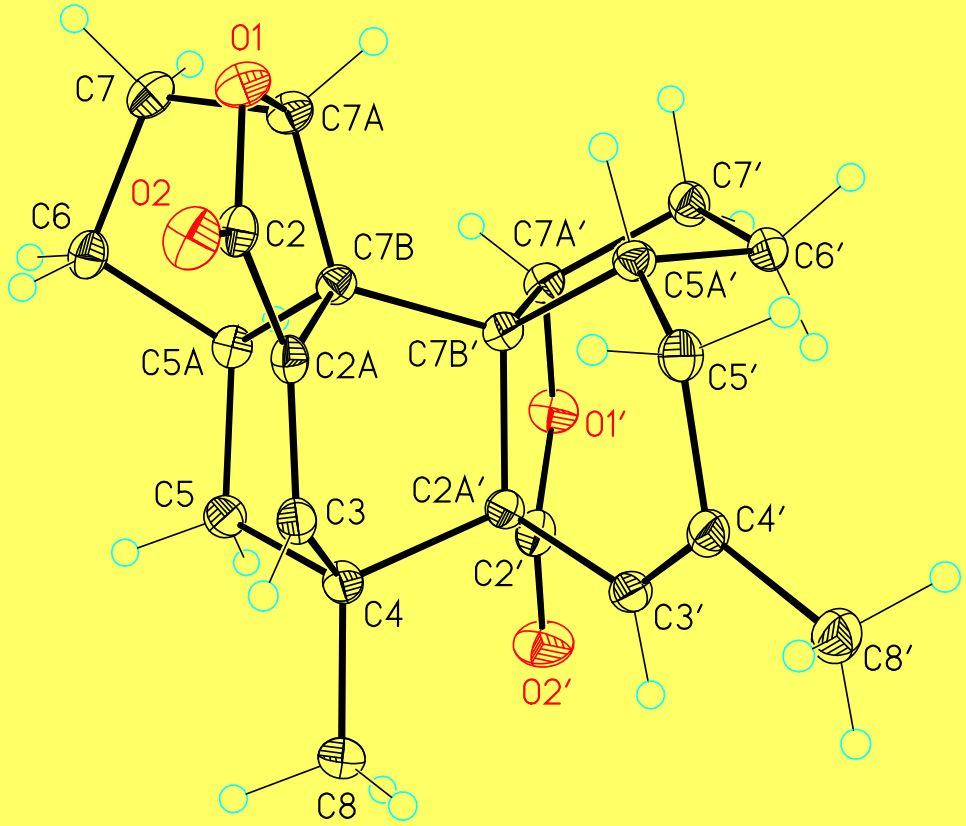
Quirality with $Cu_{K\alpha}$ radiation

Bisdehydrogaliellalactone



Absolute configuration:

2a`*S*, 5a`*R*, 7a`*R*, 4*R*, 7b`*S*,
5a*R*, 7a*R*, 7b*S*

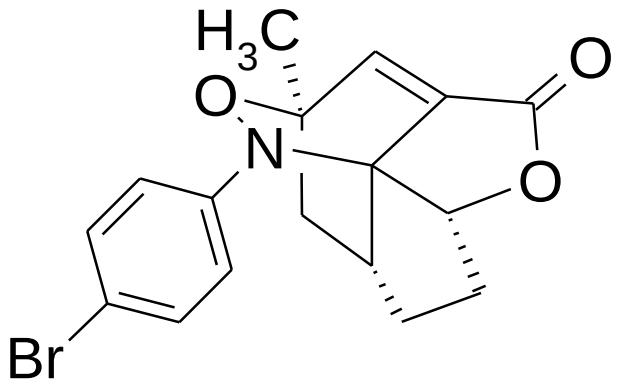


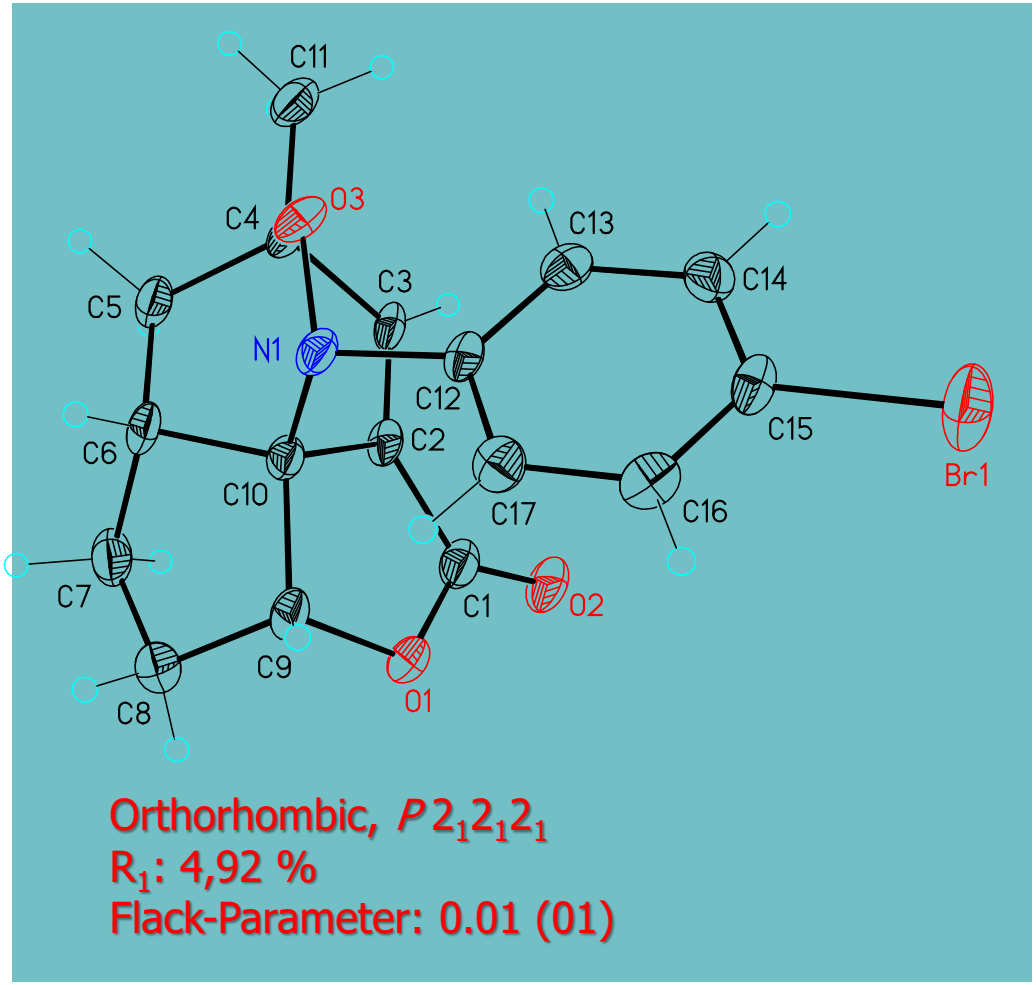
Orthorhombic, $P2_12_12_1$
R_1: 3,45 %
Flack/Hooft/Parsons: -0.04(13)/-0.01(04)/0.01(04)

• F. Nussbaum, R. Hanke, T. Fahrig, J. Benet-Buchholz; *Eur. J. Org. Chem.* ***2004***, *2783-2790.*

Quirality with $Mo_{K\alpha}$ radiation

Bisdehydrogaliellalactone



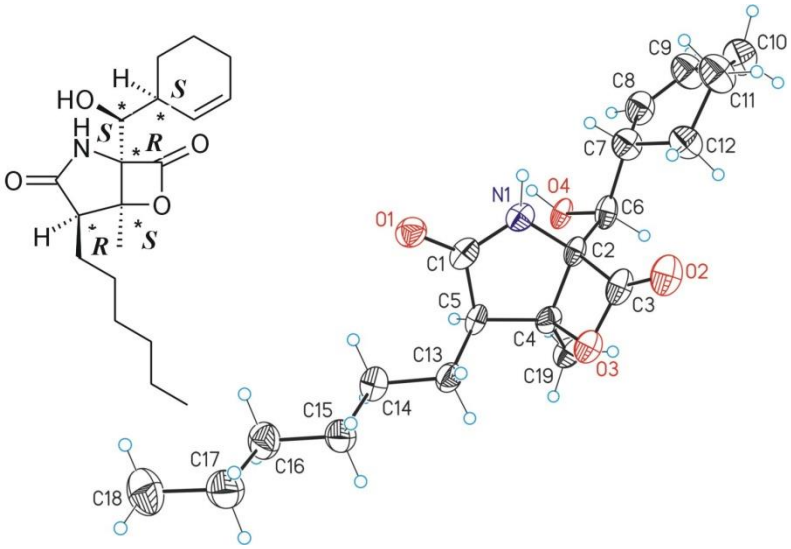


• F. Nussbaum, R. Hanke, T. Fahrig, J. Benet-Buchholz; *Eur. J. Org. Chem.* ***2004****, 2783-2790.*

Quirality with $Cu_{K\alpha}$ radiation

Cinnabaramide

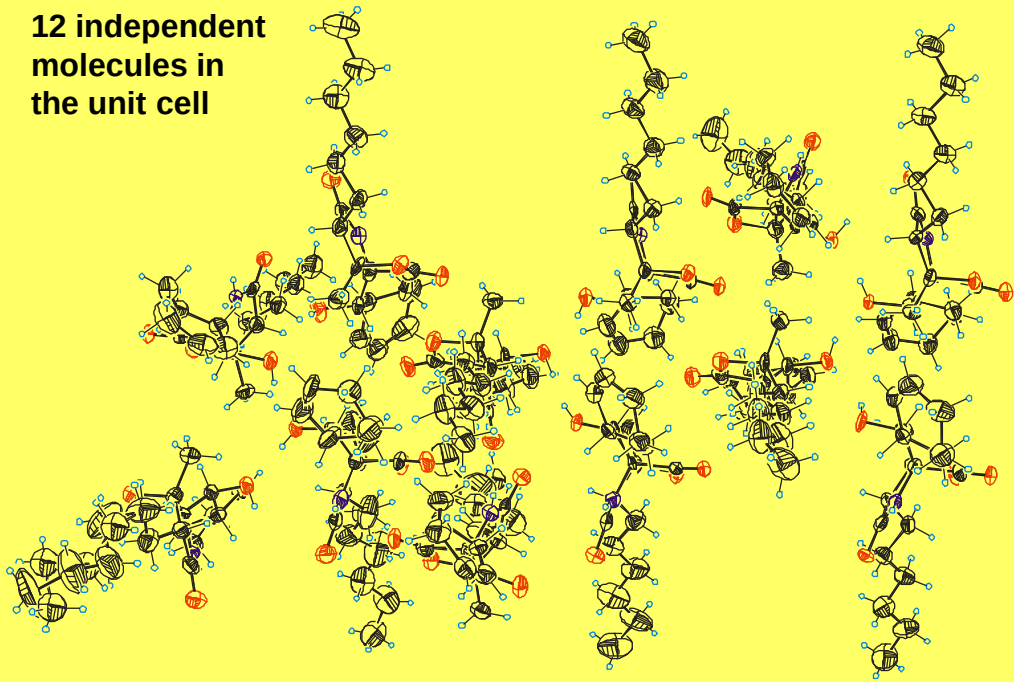
The largest cell



Absolute configuration:

R(C2);S(C4);R(C5);S(C6);S(C7)

12 independent molecules in the unit cell

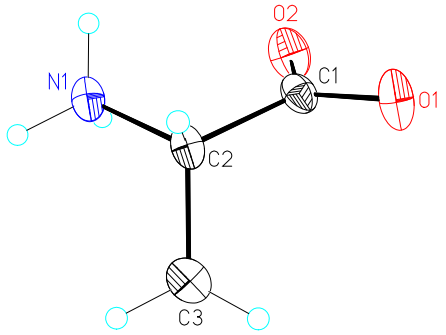


Orthorhombic, $P2_12_12_1$
Volume: 22178.2(7)
Reflections Fo>4σ(Fo): 21119
Parameters 2676
R_1: 7,81 %
Flack/Hooft/Parsons: 0.02(19)/0.12(05)/0.13(05)

• M. Stadler, J. Bitzer, A. Mayer-Bartschmid, H. Müller, J. Benet-Buchholz, F. Gantner, H.-V. Tichy, P. Reinemer, K. Bacon; *J. Nat. Prod.* ***2007****, 246-252.*

Absolute Configuration of CHNO Molecules with $Mo_{K\alpha}$

Measurements of L-Alanine with $Mo_{K\alpha}$-Radiation



Smallest molecule with only 6 atoms

Compound	Sample	$Friedif_{stat}$	$N_{ind}(I>4\sigma)$	Redundancy	Space Group	$R_1(I>4\sigma)$	x (Flack)	y (Hooft)	z (Parsons)
L-Alanine	5001 [a]	6.5	6209	4.5	$P2_12_12_1$	0.0254	-0.08(28)	0.05(8)	0.06(8)
	5002 [a]	6.5	6393	5.6	$P2_12_12_1$	0.0299	-0.07(29)	- 0.09(9)	-0.06(10)
	5006 [a]	6.5	6549	5.1	$P2_12_12_1$	0.0196	0.05(22)	-0.02(5)	0.02(5)
	5008 [b]	6.5	6172	4.8	$P2_12_12_1$	0.0238	0.02(23)	-0.01(5)	-0.01(4)

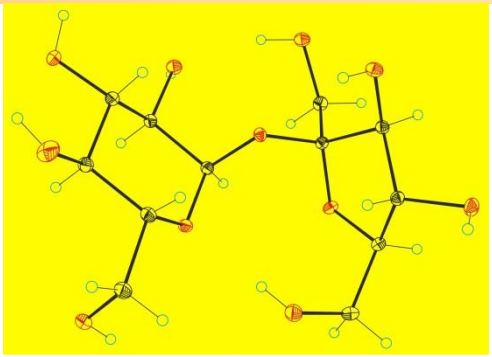
Flack Parameter: Standard uncertainties around 0.25.

Hooft / Parsons parameter: the standard uncertainties in range 0.04 to 0.10.

The mean value of the Flack parameter for all crystals is: -0.02(0.13)

Absolute Configuration of CHNO Molecules with $Mo_{K\alpha}$

Carbohydrates: Sucrose



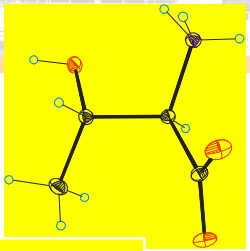
Compound	Sample	$Friedif_{stat}$	$N_{ind}(I>4\sigma)$	Redundancy	Space Group	$R_1(I>4\sigma)$	x (Flack)	y (Hooft)	z (Parsons)
α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside (Sucrose)	6501 [a]	6.9	20204	2.4	$P2_1$	0.0198	-0.06(12)	0.03(5)	- 0.01(5)
	6502 [a]	6.9	20110	2.9	$P2_1$	0.0197	0.04(11)	0.05(4)	0.06(3)
	6503 [a]	6.9	21902	2.6	$P2_1$	0.0207	-0.09(12)	-0.04(4)	-0.04(5)
	6504 [a]	6.9	20599	4.7	$P2_1$	0.0190	0.04(11)	0.04(3)	0.05(3)

Flack parameter: Standard uncertainties around 0.11.

Hooft / Parsons parameter: Standard uncertainties in the range 0.03-0.05.
(clearly below the limit of 0.08 set for the Enantiopure-sufficient inversion-distinguishing power)

Standard uncertainties are improving with higher number of independent reflections.

Comparison of data with $Mo_{K\alpha}$ and $Cu_{K\alpha}$ radiation



Measurements on Threonine

Compound	Sample	Temp	$Friedif_{stat}$	$N_{ind}(I>4\sigma)$	Redundancy	Space Group	$R_1(I>4\sigma)$	x (Flack)	y (Hooft)	z (Parsons)
	$Mo_{K\alpha}$ Radiation									
D-Threonine	5201	100 K	6.7	8832	5.2	$P2_12_12_1$	0.0210	0.12(22)	0.04(5)	0.05(5)
	5202	100 K	6.7	8389	5.2	$P2_12_12_1$	0.0202	-0.01(21)	-0.03(6)	-0.03(5)
	5203	100 K	6.7	8124	5.8	$P2_12_12_1$	0.0219	0.01(24)	0.06(6)	0.07(6)
	5204	100 K	6.7	8324	11.6	$P2_12_12_1$	0.0221	0.03(24)	0.02(6)	0.02(6)
	5205 [I]	100 K	6.7	7711	3.7	$P2_12_12_1$	0.0266	0.02(29)	0.00(9)	-0.01(10)
	5206 [I]	100 K	6.7	6401	3.2	$P2_12_12_1$	0.0237	0.29(32)	0.10(7)	0.10(8)
	5212	100 K	6.7	8758	5.7	$P2_12_12_1$	0.0241	0.08(26)	0.01(9)	-0.01(10)
	5213	100 K	6.7	8952	5.9	$P2_12_12_1$	0.0196	-0.12(19)	-0.03(5)	-0.03(4)
	5214	100 K	6.7	8883	5.9	$P2_12_12_1$	0.0231	0.09(24)	0.06(7)	0.07(7)
	5215	100 K	6.7	9543	8.2	$P2_12_12_1$	0.0223	0.00(21)	-0.02(5)	-0.03(5)
	5216	100 K	6.7	8257	5.2	$P2_12_12_1$	0.0190	0.01(22)	0.00(3)	0.01(3)
L-Threonine	5210	100 K	6.7	8473	5.6	$P2_12_12_1$	0.0223	0.11(23)	0.09(4)	0.09(3)
	5211 [I]	100 K	6.7	8060	2.6	$P2_12_12_1$	0.0235	0.07(25)	0.05(8)	0.05(8)
	$Cu_{K\alpha}$ Radiation									
	1801	297 K	35	901	5.9	$P2_12_12_1$	0.0266	-0.08(28)	0.05(10)	0.03(12)
	1802	90 K	35	896	5.7	$P2_12_12_1$	0.0263	-0.07(27)	0.00(13)	-0.02(15)
	1803	297 K	35	909	4.7	$P2_12_12_1$	0,0371	-0.01(30)	0.13(11)	0.10(13)
	1804	100 K	35	820	4.3	$P2_12_12_1$	0.0337	0.05(32)	0.01(9)	0.03(9)
	1805	100 K	35	901	5.9	$P2_12_12_1$	0.0371	-0.01(30)	0.09(3)	0.13(3)

Similar or Better U(x) values for the same compound with Mo!!

Absolute Configuration of CHNO Molecules with $Mo_{K\alpha}$

Refinements at low-resolution ranges

Explicar millor

% of Theoretical reflections	Requirement Cut-off ($Å^{-1}$)	$\|y\| \leq 0.1$	$u_y \leq 0.1$	$\|z\| \leq 0.1$	$u_z \leq 0.1$	$\|y\|,u_y \leq 0.1$	$\|z\|,u_z \leq 0.1$
100%	No cut-off	44(100%)	44(100%)	43(98%)	43(98%)	44(100%)	42(95%)
48%	1.0	40(91%)	43(98%)	39(89%)	39(89%)	39(89%)	33(75%)
34%	0.9	38(86%)	39(89%)	39(89%)	33(75%)	34(77%)	31(70%)
25%	0.8	35(80%)	33(75%)	35(80%)	32(73%)	29(66%)	34(36%)
15%	0.7	23(52%)	17(39%)	23(52%)	16(37%)	13(30%)	11(25%)
10%	0.6	22(50%)	7(16%)	21(48%)	5(11%)	7(16%)	3(7%)

The use of the lowest resolutions cuts caused a dramatic increase in the standard uncertainties and in some cases also the wrong absolute structures were implied.

Absolute Configuration of CHNO Molecules with $Mo_{K\alpha}$

Determination of absolute configuration: Final schedule

- **Enantiopure Samples:** Make all possible previous analysis to be sure that sample is enantiopure.
- **Right crystal:** Select the right crystal from the bulk avoiding singular samples.
- **Complete Data:** Completeness, maximum resolution and redundancy.
- **Precise evaluation of the refined data**: Limits of standard uncertainty.
- **Additional crystals:** Measure more than one crystal in case of doubts.
- **Use of additional techniques:** Make use of HPLC and optical rotation on the measured crystals compared to the main bulk.
- **Previous measurement of some standard chiral crystals:** Information about device limits.

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