Howard's giant leap for a chiral world: the Flack parameter

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CRYSFORMA

A Chiral World: Introduction

Several **biological processes** take place in a **defined stereo-chemistry** so that we can generally say that **live**, and of course human live, developed in a **chiral world**.

Determination of the stereo chemistry of molecules is of crucial importance when trying to **correct biological processes**, which generate disease, affliction and illness.

Howard Flack provided an elegant and enormously popular solution to the problem of **absolute structure determination:** He regarded crystalline chiral samples as **twins** containing x and 1-x twin fractions of two enantiomers with a refinable parameter universally know as **the Flack parameter**.

A Chiral World

In 1998, being the manager of the X-ray Diffraction Unit at the Central Research Laboratories at Bayer (Germany) I was asked to determine the stereochemistry of a chiral API (active pharmaceutical ingredient). After reading the available literature I determined the absolute structure using a single crystal of the API by “state of the art” technology: The Flack Parameter was around 0 and the standard deviation below 0.01. I was lucky, I had measured a dichloromethane solvate of the API. I went very proud to my boss showing the results after assigning the absolute configuration. My boss’s answer was: **“Everything looks great but you have to validate the methodology…….”**

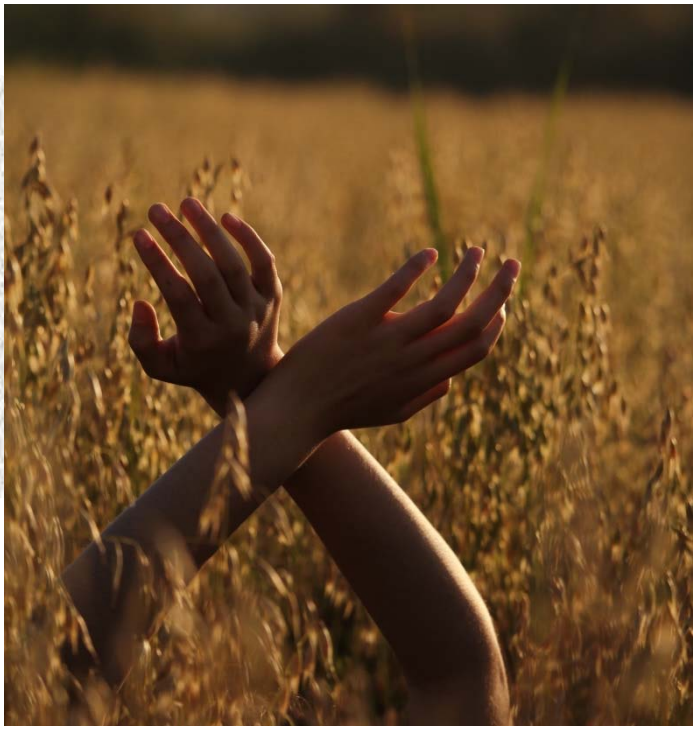
Chirality: Definitions

Chiral: 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.

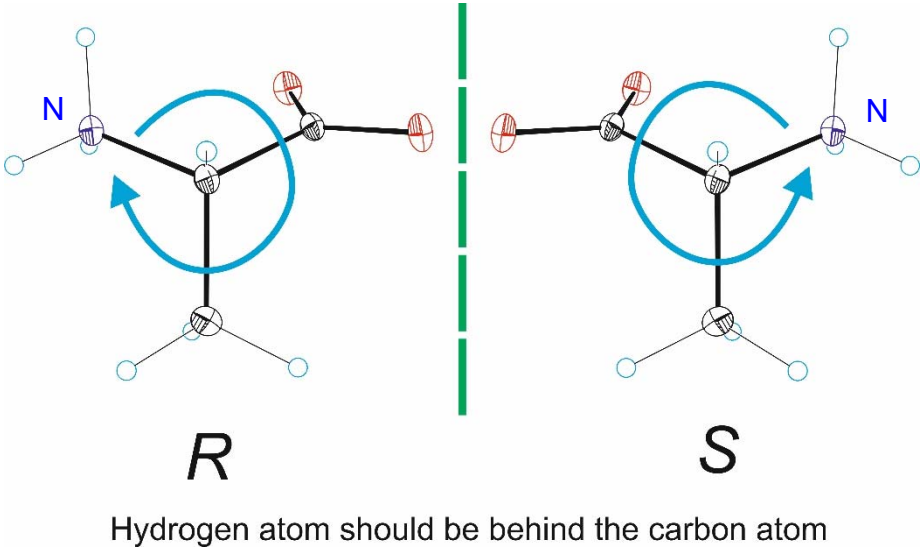
One of the first things I learned from Howard Flack is that for avoiding conceptual errors, clear definitions are needed



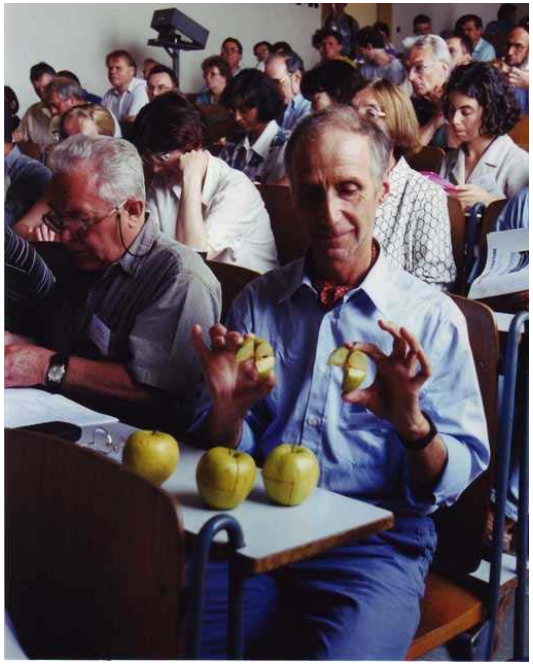
Paula Benet

Chirality: Definitions

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



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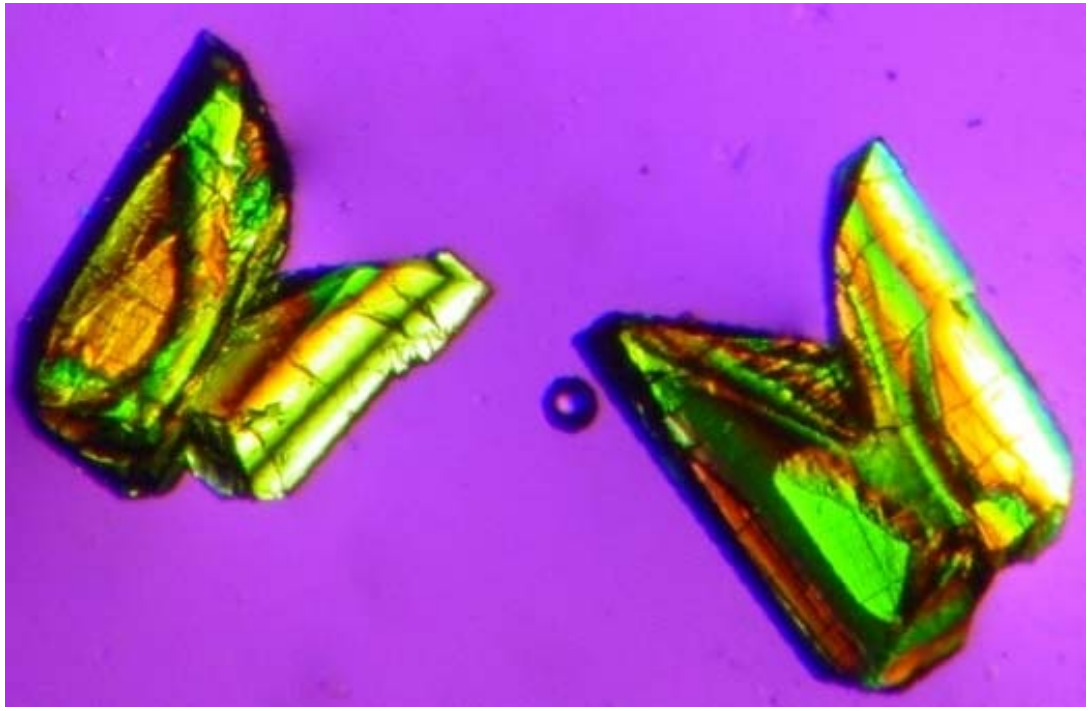
La coupe du roi

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

Chirality: Definitions

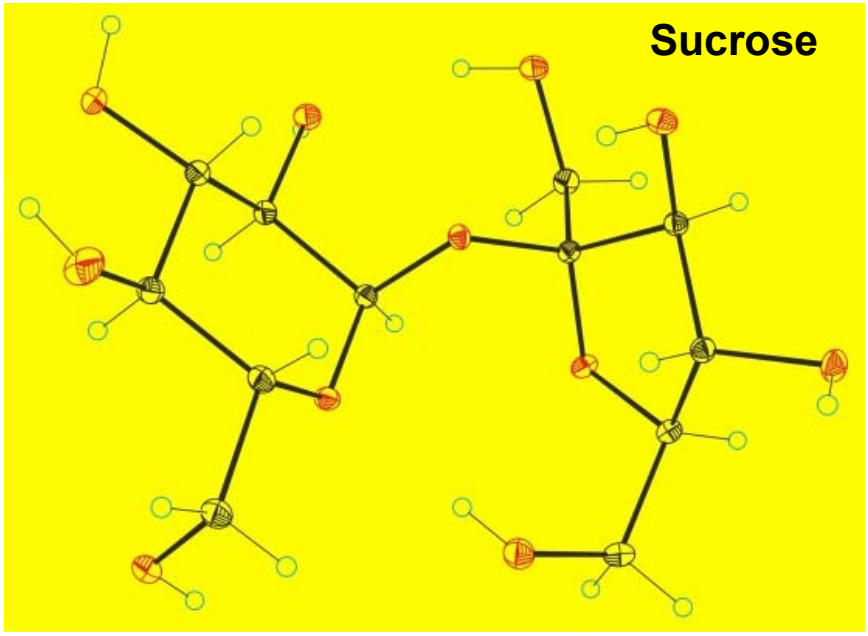
Absolute structure: *The spatial arrangement of the atoms of a physically identified non-centrosymmetric crystal and its description by way of unit-cell dimensions, space group, and representative coordinates of all atoms.*



Conglomerate of crystals forming enantiomeric butterflies

Chirality: Definitions

Chiral molecules can have <u>one or more chiral centers</u>.



*In case or having only one stereo center wird R- and S- configuration a compound can crystallize forming a **pure chiral crystal (conglomerate)**, a **racemic crystal** or a crystal with a **non stoichiometric composition**.*

Chirality: Definitions

Chiral space groups: *If chiral molecules are crystallized from an enantiomerically pure solution they must have a structure with a space group which does not contain mirror or inversion symmetry. There are 65 space groups in which chiral molecules can crystallize. They are called Chiral Space Groups of Sohncke Groups.*

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

Chirality: Definitions

If the chiral molecule has more than one chiral center the number of different combinations results pairwise to:

Enantiomers: *All stereo centers are inverted, corresponds still to enantiomers. Have the same physical properties.*

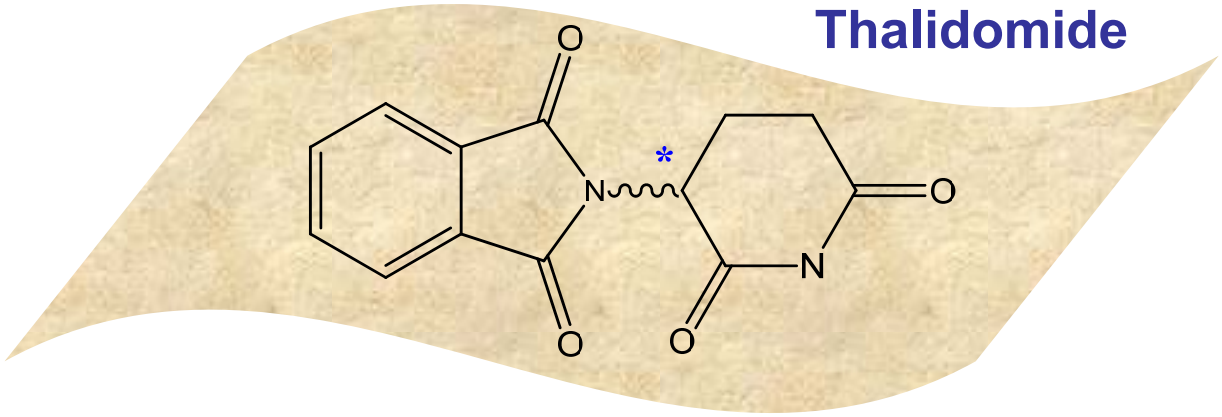
Diastereoisomers: *One or more stereocenters but not all are inverted. Diastereoisomers have different physical properties.*

Meso isomer*: 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* $\boldsymbol{2^n}$ *stereoisomers if there are not meso forms.*

Chiral Active Pharmaceutical Ingredients: Precedents

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.



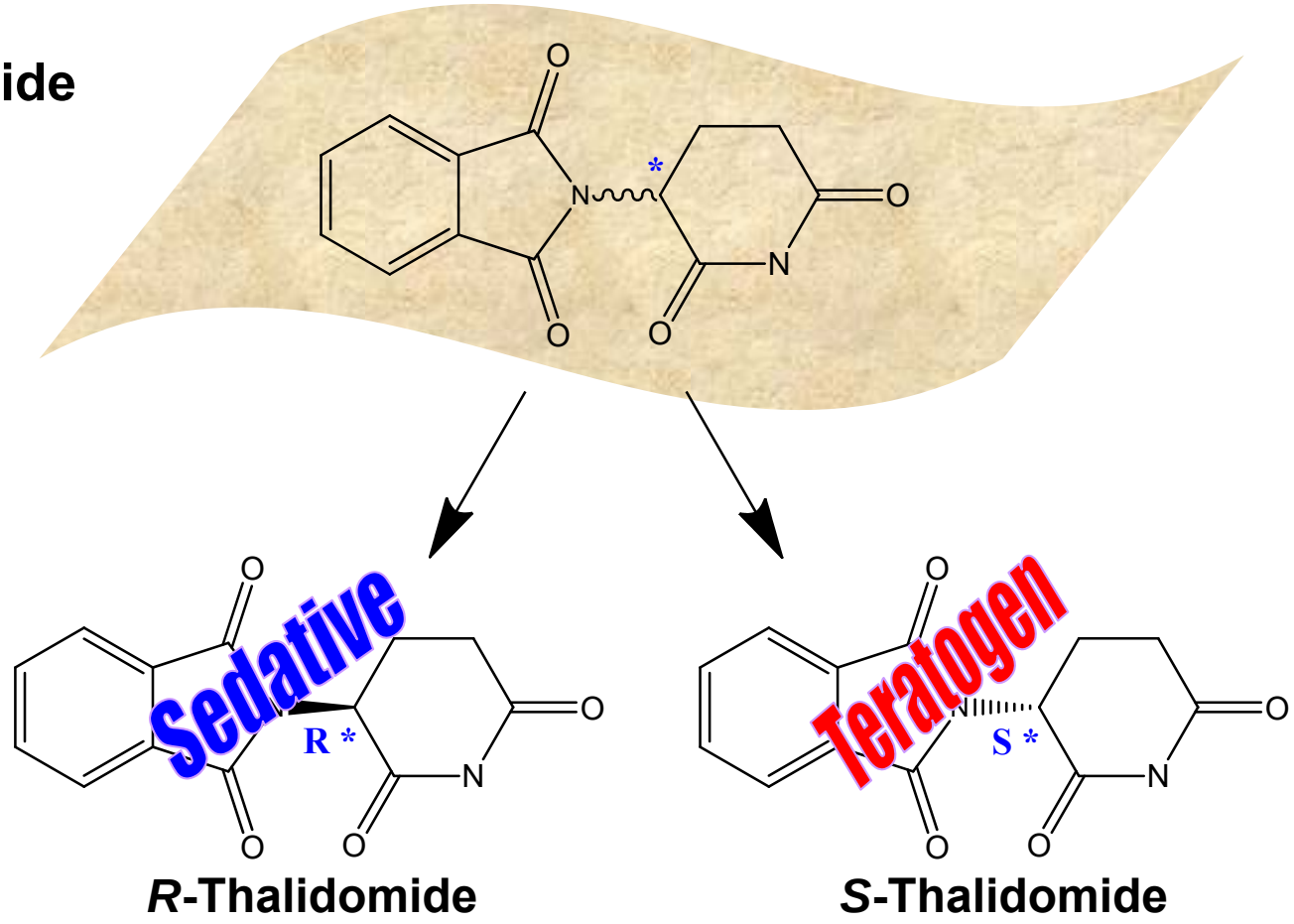
It came out to be a strong teratogen which caused dysmelia (stunted limb growth) in human babies by maternal usage.[1]

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

Chiral Active Pharmaceutical Ingredients: Precedents

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

Thalidomide

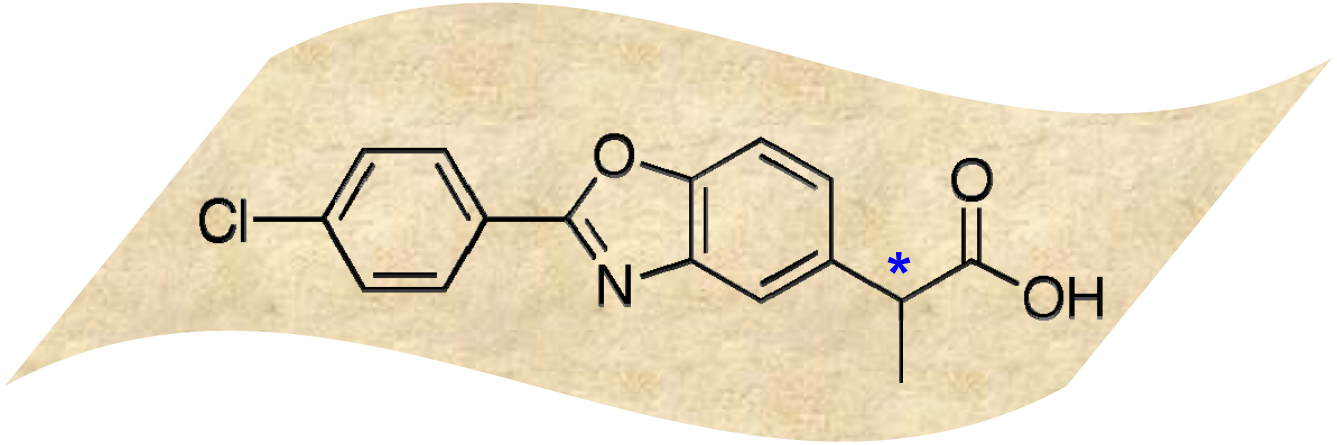


***R*-Thalidomide** ***S*-Thalidomide**

Chiral Active Pharmaceutical Ingredients: Precedents

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 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:

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

Traditionally absolute configuration determination was based on the single crystal X-ray structure determination performed using $Cu_{K\alpha}$ or $Mo_{K\alpha}$ radiation and compounds containing atom types **heavier than Si**.

The anomalous scattering effects, specially using $Mo_{K\alpha}$ radiation, are much weaker at **organic molecules** containing only "*light atoms*"* so that the basic problem of the determination of the absolute configuration of APIs (Active Pharmaceutical Ingredients) and natural products is the weak inversion distinguishing capacity.

* CHNO Molecules

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.

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.
Reporting and evaluating absolute-structure and absolute-configuration determinations

Absolute Configuration of CHNO Molecules

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

Using the traditional 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.

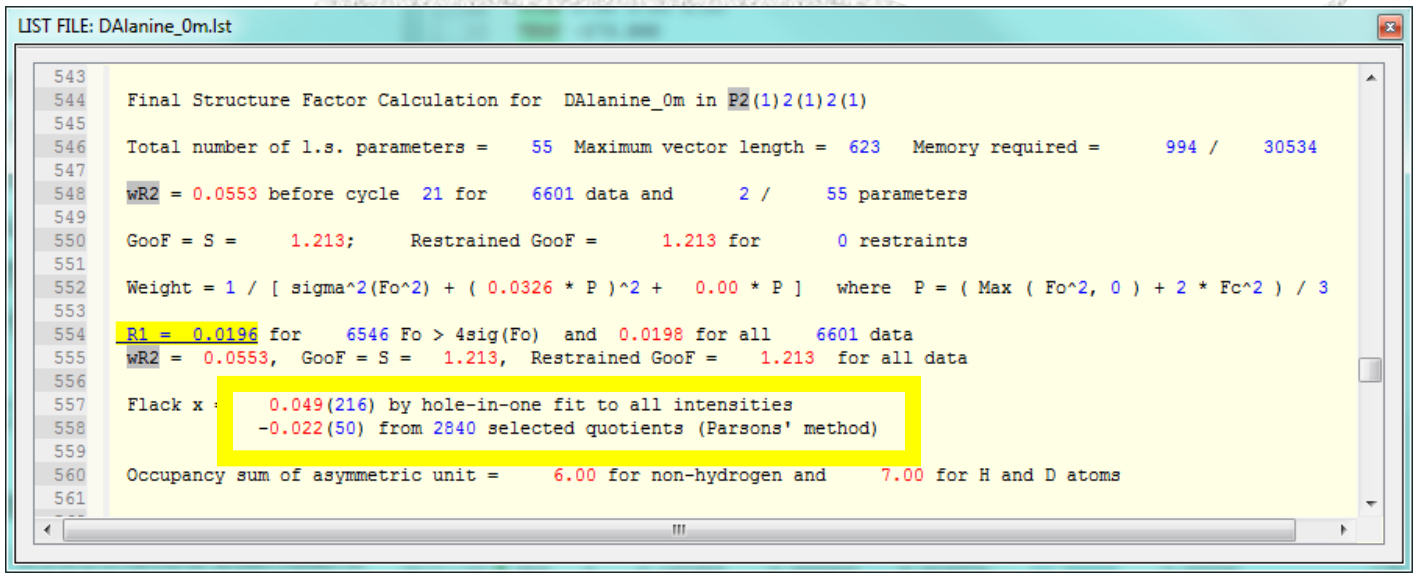
B3) Parsons et al. described the estimation of the uncertainty by using differences and quotients refining with TOPAS-Academic.
(Parsons S., Flack H.D., Wagner T., Acta Cryst. B69 (2013) 249-259.)

Absolute Configuration of CHNO Molecules

Parsons Quotients 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 Flack parameter based on selected quotients “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

Since his contribution in 1983 Howard Flack continuously revised the processes related to “absolute structure determination reporting” and “evaluating absolute configuration determinations”.

Flack H.D., Bernardinelli G., *J. Appl. Cryst.*, 2000, *33*, 1143-1148.
Reporting and evaluating absolute-structure and absolute-configuration determinations
Flack H.D., *Helvetica Chimica Acta*, 2003, *86*, 905-921.
Flack H.D., Bernardinelli G., *Crystal Engineering*, 2003, *6*, 213-223.
Flack H.D., Bernardinelli G., Clemente D.A., Linden A., and Spek A.L., *Acta Cryst.* 2006, *B62*, 695-701.
Flack H.D., Bernardinelli G., *Acta Cryst.*, 2008, *A64*, 484-493.
Shmueli U., Schiltz M., Flack H.D., *Acta Cryst.,* 2008, *A64*, 476-483.
Flack H.D. *Acta Cryst.*, 2013, *C69*, 803-807.
Parsons S., Flack H.D., Wagner T., *Acta Cryst.* 2013, *B69*, 249-259.
Flack H.D. *Chimia.*, 2014, *68*, 26-30.
Cooper, R.I., Watkin, D.J., Flack H.D., *Acta Cryst.* 2016, *C72*, 261-267.

Absolute Configuration of CHNO Molecules

Basic problem: "Distinguishing power"

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

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

Approach:

Validated method, which, specially from the point of view of a pharmaceutical company, makes the determination of the absolute configuration credible.

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

Absolute Configuration of CHNO Molecules

Pre-conditions:

- The measured crystal should be representative for the sample. In case of doubts HPLC and Optical rotation should be measured.

Approach: Candidate 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.

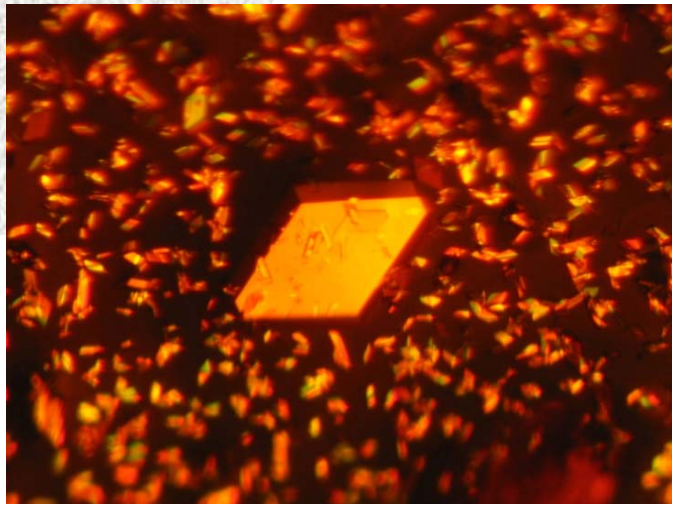
Absolute Configuration of CHNO Molecules

Pre-conditions:

Crystals: Should be of good quality, representative for the sample and with a relatively large medium size according to the features of the device used (typically around 0.05 to 0.3 mm).

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!!!



Absolute Configuration of CHNO Molecules

Pre-conditions:

Crystals: Should be of good quality, representative for the sample and with a relatively large medium size according to the features of the device used (typically around 0.05 to 0.3 mm).

If the sample analyzed, for example, has **two chiral centers** and small amounts of the **diastereoisomer** can be present (<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 and 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.

Absolute Configuration of CHNO Molecules

Pre-conditions:

Crystals: Should be of good quality, representative for the sample and with a relatively large medium size according to the features of the device used (typically around 0.05 to 0.3 mm).

All measurements were performed trying to collect full data sets with high Friedel pair coverage. The redundancy should be around 4-5 (allowing a good absorption correction). The measurements should reach the maximum resolution of the devices used (Copper 0.82 Å, max. 2θ ~140º and Molybdenum 0.39 Å, max. 2θ ~130º). The measurement time in all devices should the from 12 to 48 hours. Low temperature (100 K) should be used.

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

56 measurements:

Alanine

Carbohydrates

Macromolecules

VALIDATION

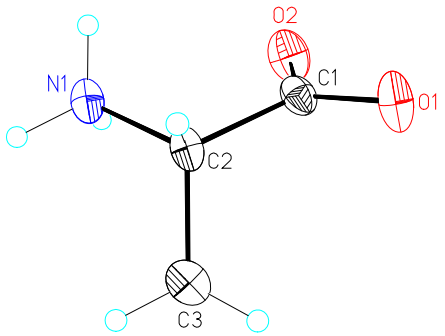
Internal validation report BAYER AG, Leverkusen (Germany) 2000.

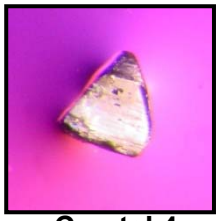
SUBSTANCE	File	R_1	wR2	Flack	Hooft	Parsons	Friedif	Reflections Total	Reflections Obsv.	Temp.	S. Group
L-Alanine	1101 LAlaRT	0,0301	0,0752	0,25(30)	0,37(10)	0,31(12)	34	730	730	RT	$P2_12_12_1$
	1102 LAla2RT	0,0339	0,0793	-0,23(38)	-0,10(20)	0,01(28)	34	701		RT	
	1103 LAla3RT	0,0280	0,0713	0,13(28)	0,11(12)	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 SAlaRT	0,0471	0,1139	0,15(41)	0,11(16)	0,10(20)	34	[illegible]16	702	RT	
L-Asparigine	1201 LAspaRT	0,0301	[illegible]	[illegible]	[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[illegible]	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[illegible]e	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	0,1256	0,01(28)	0,26(15)	0,20(21)	35	1854	1740	RT	$P2_1$
	2102 LAscoRtg	0,0383	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	2760	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	1400	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 toaRmRT	0,0487	0,1257	-0,13(36)	0,35(19)	0,11(27)	37	3865	3838	RT	
1,6-Anhydro-ß-D-Glucopyranose	3701 AnBGluc	0,0316	0,0828	-0,02(21)	0,18(11)	0,11(10)	33	1113	1105	RT	$P2_12_12_1$
(-)-(1R,2S)-2-Amino-4-methylen-cyclopentanecarbonsäure	4001 b108888	0,0300	0,0761	[illegible]	[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	0,0993	[illegible]	[illegible]	[illegible]	33	8936	8253	90K	$P2_1$
Cyclohexadepsipeptid	4201 JES184[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	1760	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	$P2_1$
	4602 CAM[illegible]702g	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	[illegible]	[illegible]	[illegible]	[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 $Cu_{K\alpha}$

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

Smallest molecule with only 6 atoms





Crystal 4

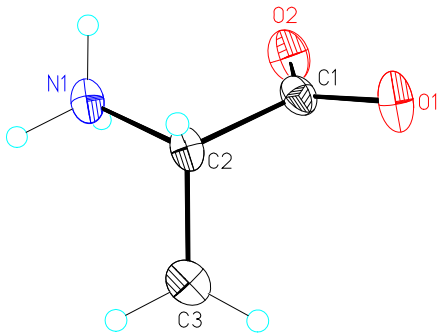
File	R_1	wR2	Flack(U)	Hooft(U)	Parsons	Friedif	Reflections		Temp.	S. Group	Chiralität
							Total	Obsv.			
1102 LAla2RT	0,0339	0,0793	**-0,23(38)**	**-0,10(20)**	**0,01(28)**	34	701	669	RT	$P2_12_12_1$	S2
1103 LAla3RT	0,0280	0,0713	**0,13(28)**	**0,11(12)**	**0,13(14)**	34	751	740	RT		S2
1104 LAla4RT	0,0351	0,0833	**0,01(32)**	**0,03(15)**	**0,06(14)**	34	733	725	RT		S2
1105 LAla5RT	0,0274	0,0695	**0,12(32)**	**0,10(13)**	**0,11(15)**	34	725	717	RT		S2
1106 LAla6RU	0,0305	0,0791	**-0,06(34)**	**-0,18(13)**	**-0,16(17)**	34	682	681	RT		S2
1107 LAla90K	0,0274	0,0679	**-0,23(31)**	**-0,18(14)**	**-0,25(15)**	34	719	676	90 K		S2
1108 LAla9RT	0,0357	0,0852	**-0,15(34)**	**-0,11(15)**	**-0,10(19)**	34	700	697	RT		S2
1109 LAlanRT	0,0316	0,0810	**-0,24(35)**	**0,00(14)**	**0,08(18)**	34	739	706	RT		S2
1110 SAl2RT	0,0349	0,0885	**0,08(35)**	**0,07(11)**	**0,11(13)**	34	719	711	RT		S2
1111 SAlanRT	0,0471	0,1139	**0.15(41)**	**0,11(16)**	**0,10(20)**	34	716	702	RT		S2

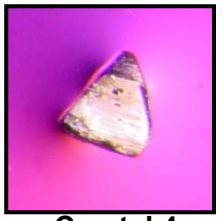
All the samples measured are corresponding to the correct inversion twin. The standard uncertainties for the classical Flack parameter are around 0.3, far away from the limit (0.08) set for the Enantiopure-sufficient inversion-distinguishing power. In the case of Hooft and Parsons the standard uncertainties are around 0.15.

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

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

Smallest molecule with only 6 atoms





Crystal 4

File	R_1	wR2	Flack(U)	Hooft(U)	Parsons	Friedif	Reflections		Temp.	S. Group	Chiralität
							Total	Obsv.			
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1104 LAla4RT	0,0351	0,0833	**0,01(32)**	**0,03(15)**	**0,06(14)**	34	733	725	RT		S2
1105 LAla5RT	0,0274	0,0695	**0,12(32)**	**0,10(13)**	**0,11(15)**	34	725	717	RT		S2
1106 LAla6RU	0,0305	0,0791	**-0,06(34)**	**-0,18(13)**	**-0,16(17)**	34	682	681	RT		S2
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1108 LAla9RT	0,0357	0,0852	**-0,15(34)**	**-0,11(15)**	**-0,10(19)**	34	700	697	RT		S2
1109 LAlanRT	0,0316	0,0810	**-0,24(35)**	**0,00(14)**	**0,08(18)**	34	739	706	RT		S2
1110 SAl2RT	0,0349	0,0885	**0,08(35)**	**0,07(11)**	**0,11(13)**	34	719	711	RT		S2
1111 SAlanRT	0,0471	0,1139	**0.15(41)**	**0,11(16)**	**0,10(20)**	34	716	702	RT		S2

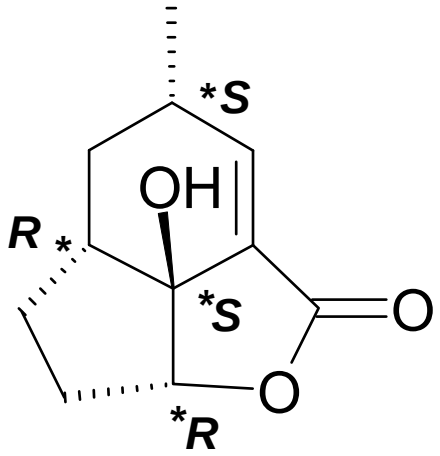
One option to improve the absolute structure determination is to measure more than one crystal and calculate the mean value of the x,y,z parameters and its standard uncertainties.

Mean values for Alanine

Flack: 0.02(12)
Hooft: 0.00(0.06)
Parsons: 0.04(0.07)

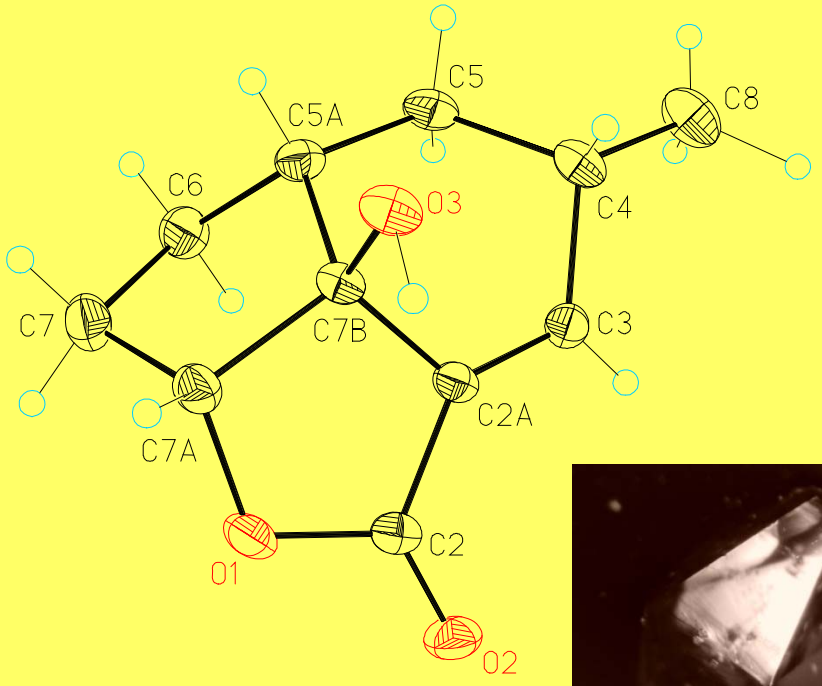
Quirality with $Cu_{K\alpha}$ radiation

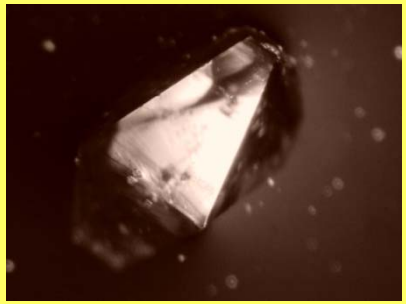
(–)-Galiellalactone



Absolute configuration:

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



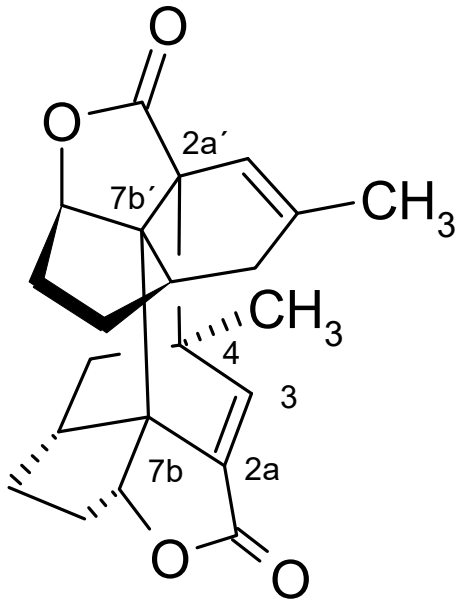


Orthorhombic, $P\,2_12_12_1$
R_1: 3,45 %
Flack/Hooft/Parsons: 0.01(19)/-0.06(04)/-0.04(05)

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

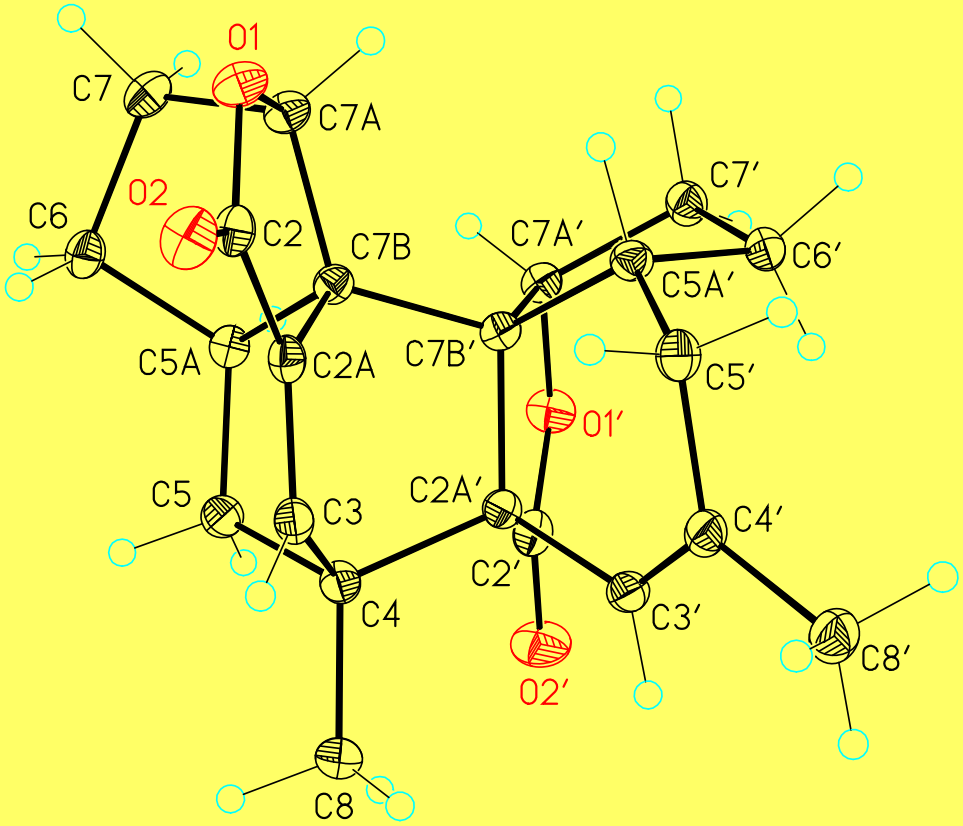
Quirality with $Cu_{K\alpha}$ radiation

Bisdehydrogaliellalactone



Absolute configuration:

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

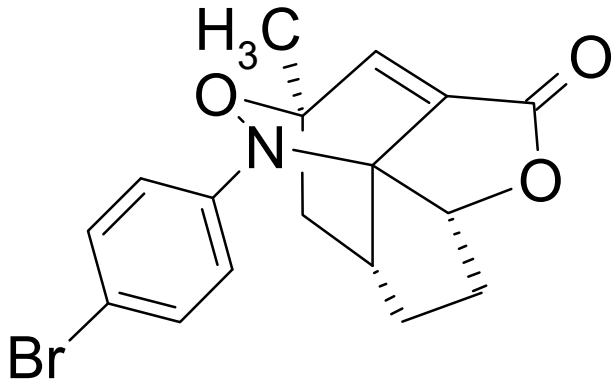


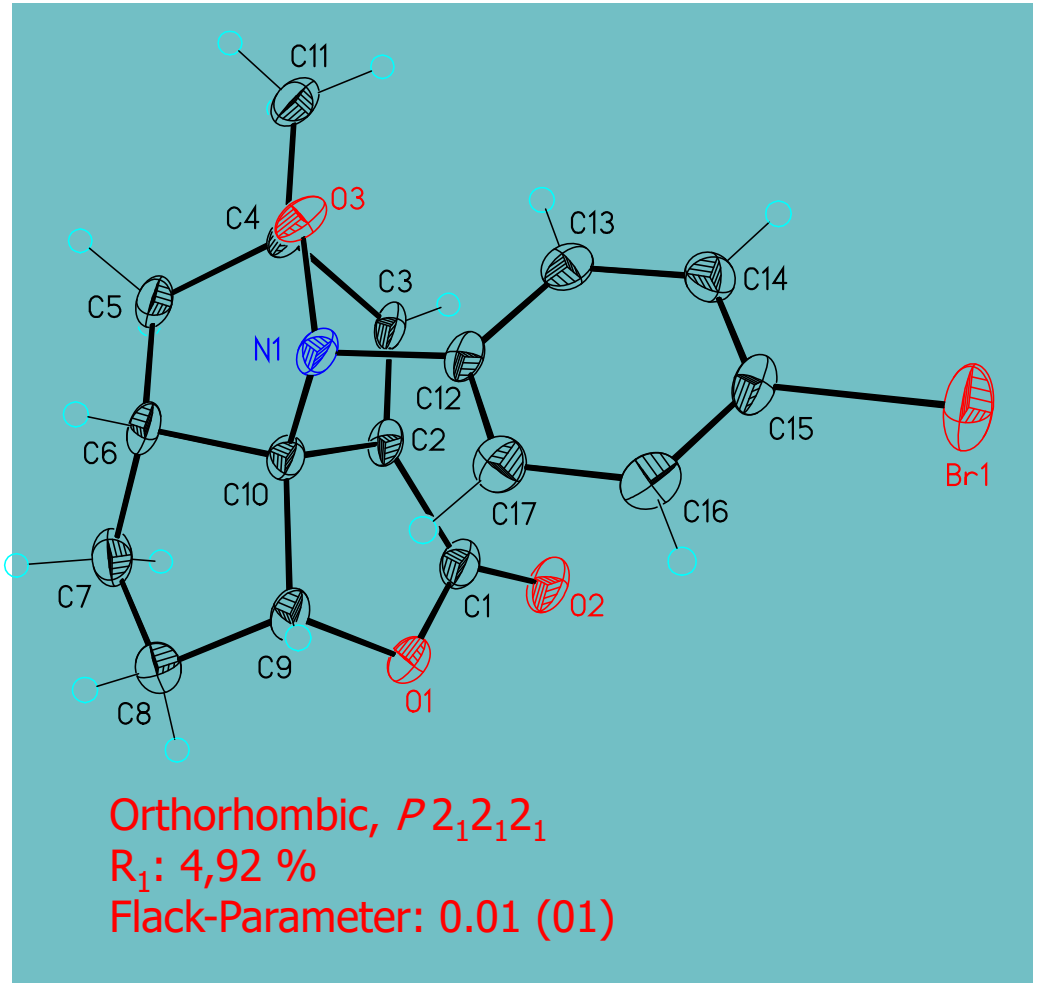
Orthorhombic, $P\,2_12_12_1$
R_1: 3,45 %
Flack/Hooft/Parsons: -0.04(13)/-0.01(04)/0.01(04)

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

Quirality with $Mo_{K\alpha}$ radiation

Bisdehydrogaliellalactone





Orthorhombic, $P2_12_12_1$
R_1: 4,92 %
Flack-Parameter: 0.01 (01)

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

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

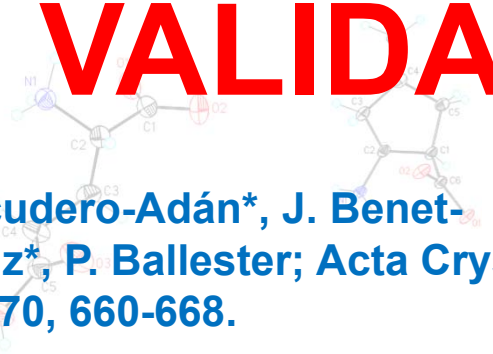
45 measurements:

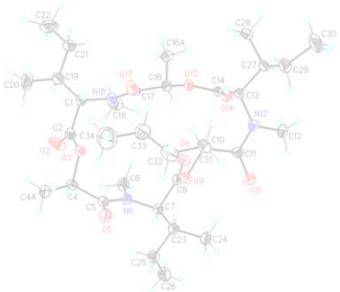
Alanine

Threonine

VALIDATION

Carbohydrates





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

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	5.2		0.0210	0.12(22)	0.04(5)	0.05(5)
	5202 [a]	6.7	8589	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 [l,a]	6.7	7711	3.7	$P2_12_12_1$	0.0266	0.02(29)	0.00(9)	
	5206 [l,a]	6.7	6401	3.2	$P2_12_12_1$	0.0237	0.29(32)	0.10(7)	
	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)	0.07(
	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)		0.09(3)
	5211 [a]	6.7	8060	2.6	$P2_12_12_1$	0.0235		0.05(8)	0.05(8)
L-Aspartic	5301 [a]	6.5	8234				-0.06(23)	0.02(9)	-0.01(8)
L-Hydroxyprolin	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	13309	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 [l,a]	6.9	9994	1.2	$P2_1$	0.0239	-0.20(20)	0.07(5)	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	9520	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)

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

In contrast to the general opinion, we postulated that the determination of the absolute structure by using **$Mo_{K\alpha}$ radiation** is possible since:

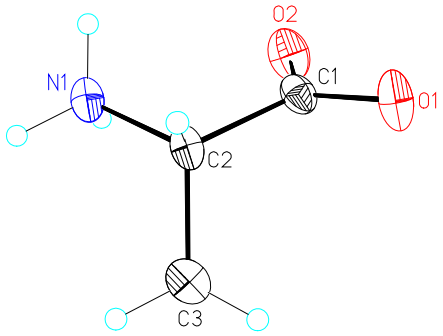
a) The number of reflections that can be collected using $Mo_{K\alpha}$ radiation is ten times larger than using $Cu_{K\alpha}$ radiation for the same 2θ range without massive increment on data acquisition time. Consequently, the limitation of the weaker resonant scattering of $Mo_{K\alpha}$ radiation 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 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)
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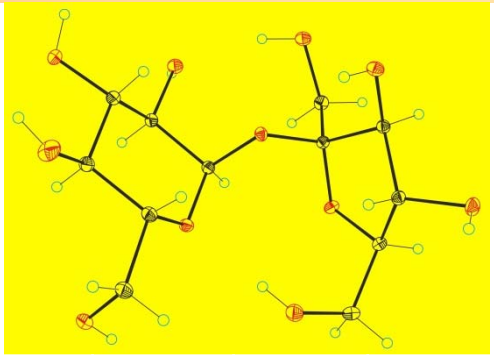
	5002 [a]	6.5	6393	5.6	$P2_12_12_1$	0.0299	-0.07(29)	- 0.09(9)	-0.06(10)
	5006 [a]	6.5	6549	5.1	$P2_12_12_1$	0.0196	0.05(22)	-0.02(5)	0.02(5)
	5008 [b]	6.5	6172	4.8	$P2_12_12_1$	0.0238	0.02(23)	-0.01(5)	-0.01(4)

All the samples measured are corresponding to the correct inversion twin. The standard uncertainties for the classical Flack parameter are around 0.25. In the case of Hooft and Parsons the standard uncertainties are in the 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)

The standard uncertainties for the Flack parameter are vey low with values around 0.11

All the standard uncertainties for the Hooft and the Parsons parameter are, with values in the range 0.3-0.5, clearly below the limit (0.08) set for the Enantiopure-sufficient inversion-distinguishing power.

As by the measurement with Copper radiation the standard uncertainties are improving with higher number of independent reflections.

Conclusions:

If the **pre-conditions** are fulfilled the determination of the absolute structure / absolute configuration using the new technologies should be unambiguously possible for any type of molecule containing “let’s say” at least as heaviest atom a Oxygen atom.

Howard Flack was on of the main actors which, with all his life contribution, made this conclusion possible.

In Memoriam of Prof. Howard D. Flack

The Chiral World

Message sent by Howard before ChemKrist 2013 in Mühlheim:
I have already taken Daniel and Danielette off the shelf so that I do not forget them (as I did for the 2013 Zurich school of crystallography and had to have them sent by express post). I have also taken out my sharp kitchen knife (and emergency plaster) so that I can cut up an apple to amuse the participants. I hope to find a suitable apple in Muelheim.