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5-Amino-2,4,6-tribromoisophthalic acid: the MAD triangle for experimental phasing

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The title compound, $C_8H_4Br_3NO_4$, shows an extensive hydrogen-bond network. In the crystal structure, molecules are linked into chains by COO—H···O bonds, and pairs of chains are connected by additional COO—H···O bonds. This chain bundle shows stacking interactions and weak N—H···O hydrogen bonds with adjacent chain bundles. The three Br atoms present in the molecule form an equilateral triangle. This can be easily identified in the heavy-atom substructure when this compound is used as a heavy-atom derivative for experimental phasing of macromolecules. The title compound crystallizes as a nonmerohedral twin.

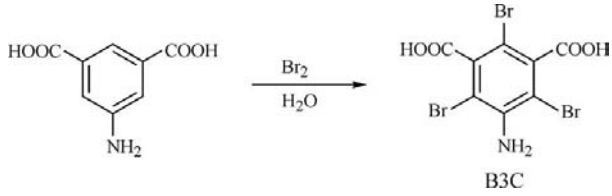
Comment

Heavy atoms are utilized for the derivatization of biological macromolecules to enable experimental phase determination. Nowadays, techniques are commonly used that exploit the anomalous scattering of certain atoms. SAD (single-wavelength anomalous dispersion) is suitable for in-house phasing if the atoms concerned have an appreciable anomalous signal at the Cu *Kα* wavelength. At a synchrotron, a series of experiments may be carried out close to the absorption edge of the particular heavy atom, maximizing the sum of the anomalous signals and the difference of the dispersive signals (multi-wavelength anomalous dispersion, MAD).

Incorporation of heavy atoms is required if the biomolecule does not possess sufficient intrinsic anomalous scatterers. Expression of a selenomethionine derivative enables structure solution by MAD or SAD methods. Incorporation of other anomalous scatterers such as halides (Dauter *et al.*, 2000) or the tantalum bromide cluster (Schneider & Lindqvist, 1994) is achieved using less demanding approaches, *e.g.* soaking or cocrystallization of the native protein with the heavy-atom salt. However, most heavy-atom derivatives show low occupancies of the heavy-atom sites due to nonspecific binding.

We have recently reported the synthesis and characterization of 5-amino-2,4,6-triiodoisophthalic acid, hereinafter I3C (Beck & Sheldrick, 2008). This is a representative of a new class of compounds that can be used for heavy-atom derivatization. It combines functional groups for hydrogen bonding to the protein with an easily recognizable arrangement of heavy atoms. It has been applied for SAD phasing (Beck *et al.*, 2008). There is also a report of a novel structure that was solved using I3C (Sippel *et al.*, 2008). Unfortunately, I3C cannot be used for multi-wavelength experiments since the energy of the iodine absorption edge is not accessible at a typical macromolecular crystallography synchrotron beamline.

Since MAD experiments yield better phase information, we were also interested in the analogous bromine compound (the bromine absorption edge falls within the normal energy range of a synchrotron beamline). This report characterizes the new phasing tool 5-amino-2,4,6-tribromoisophthalic acid, B3C. Two carboxyl groups and one amino group for hydrogen bonding, combined with three Br atoms arranged in an equilateral triangle, render B3C a suitable phasing tool for MAD experiments. B3C has been used for MAD phasing with synchrotron data (Beck *et al.*, 2009) and also for SAD phasing using data collected only at Cu *Kα* (Beck *et al.*, unpublished results).



B3C

In the crystal environment, B3C has no internal symmetry and crystallizes in the space group $P2_1/c$ with four molecules in the asymmetric unit. The bond lengths and angles fall within normal ranges. A displacement ellipsoid plot is shown in Fig. 1. In the crystal structure, molecules are linked to chains *via* COO—H···O bonds (Fig. 2). The first carboxyl group of each molecule forms cyclic dimers with a neighboring carboxyl

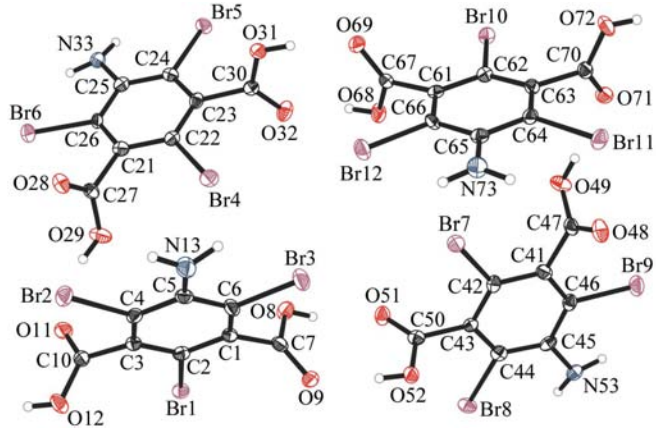


Figure 1
The asymmetric unit of B3C, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

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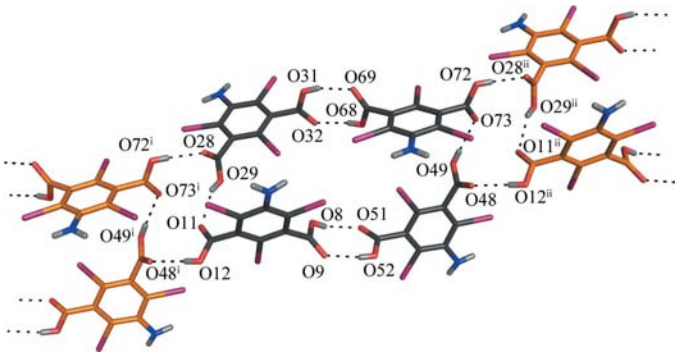

Figure 2
The hydrogen bonding of B3C. Molecules form chains that are connected pairwise to each other. Symmetry-equivalent C atoms are depicted in a lighter shade. For symmetry operators, please refer to Table 2.

group (*e.g.* O8—H8···O51 and O52—H52···O9; see Table 2 for details of the hydrogen-bond geometry), as observed frequently for carboxylic acids in the solid state (Leiserowitz, 1976). The second carboxyl group is also involved in hydrogen bonding, forming a one-dimensional chain (*e.g.* O12—H12···O48^{i}; see Table 2 for symmetry code). Instead of a dimeric interaction, one hydrogen bond is also formed to an adjacent chain (*e.g.* O29—H29···O11; Table 2). Therefore, a one-dimensional hydrogen-bond network is found in the crystal structure of B3C. In trimesic acid, with three carboxyl groups, a two-dimensional network is found, not limited to chains but extending within a plane (Duchamp & Marsh, 1969).

A bundle of chains of B3C shows weak hydrogen bonding *via* one amino group (N33) to adjacent chain bundles (Table 2). In addition, stacking of benzene rings [ring C41–C46 with symmetry-equivalent ring C21^{iv}–C26^{iv}; symmetry code: (iv) $-x+1, \frac{1}{2}+y, \frac{3}{2}-z$] is also observed as a contact between the chain bundles. No direct face-to-face contact is present, but rather an offset geometry, favoured by decreased π–π repulsion and increased σ–π attraction (Hunter & Sanders, 1990). Close C···C contacts are observed between atoms C42 and C25^{iv} [3.690 (5) Å], C42 and C26^{iv} [3.889 (5) Å], C43 and C25^{iv} [3.803 (5) Å], and C43 and C26^{iv} [3.843 (5) Å]. In addition, a close C···Br distance is also observed [C46···Br6^{iv} = 3.620 (4) Å]. The angle between the two benzene ring planes is 7.9 (2)°.

The molecular arrangement in the crystal lattice differs from the packing found in crystals of the iodine derivative I3C (Beck & Sheldrick, 2008). In B3C, no solvent water molecule is present. Increased halogen–carbon bond lengths and van der Waals radii (2.15 Å for I *versus* 1.95 Å for Br; Weast, 1984) explain why the cyclic dimeric hydrogen bonds of two neighbouring carboxyl groups found in B3C are not favoured for I3C: the I atoms of two molecules would come in close proximity and repel each other. Hydrogen bonds to the solvent water molecule are observed for I3C. These are also present in unsubstituted 5-aminoisophthalic acid (Dobson & Gerkin, 1998), where, since the substituents are missing, the packing is different from that observed in I3C and B3C. The unsubstituted acid crystallizes as a zwitterion, with one carboxylate and an $-NH_3^+$ group. The carboxyl groups are in plane with the aromatic ring. The negative charge of the carboxylate group can therefore be delocalized across the π system, leading to an increase in acid strength compared with B3C and I3C. Here, clear indications for protonated carboxyl groups can be found (Table 1). In all carboxyl groups, one C—O bond is significantly shorter than the other, indicating one double bond and one single bond. Interestingly, the bond lengths for C30—O31/C30—O32 and C67—O68/C67—O69 do not show such large differences, but still indicate protonation of O31 and O68, respectively (Table 1).

The distance between Br atoms in B3C varies from 5.652 (1) to 5.695 (1) Å. For each B3C molecule, the Br atoms form an equilateral triangle that can be easily identified in the heavy-atom substructure when this compound is used as a heavy-atom derivative for experimental phasing of macromolecules. The geometry of B3C as determined by this crystal structure has been used to generate restraints for the refinement of B3C in macromolecules (Beck *et al.*, 2009). These restraints are available by email request from author TB.

The crystal is nonmerohedrally twinned. The first and second domains are related by a twofold axis about the real axis 1 0 0. Integration using both orientation matrices simultaneously was carried out using *SAINT* (Bruker, 2007). The fractional contribution of the second domain was refined to 0.3468 (6).

Experimental

B3C was prepared according to the method shown in the scheme (see *Comment*). 5-Aminoisophthalic acid (3.32 g, 18 mmol) was added to water (100 ml). Bromine (9.6 g, 3.0 ml, 60 mmol) was added dropwise with stirring. After stirring for 24 h, the resulting precipitate was filtered off, washed with small amounts of water and dried *in vacuo*. B3C was obtained as a pink–white solid (yield 4.98 g, 66%) and was recrystallized from a water–acetonitrile solution (1:1 *v*/*v*) by slow evaporation of the solvents to obtain crystals suitable for single-crystal X-ray diffraction.

Crystal data

$C_8H_4Br_3NO_4$
M_r = 417.85
Monoclinic, $P2_1/c$
a = 16.728 (2) Å
b = 11.449 (2) Å
c = 23.649 (3) Å
β = 99.69 (3)°
V = 4464.6 (11) Å^3
Z = 16
Cu $K\alpha$ radiation
μ = 13.44 mm^{-1}
T = 100 K
0.10 × 0.10 × 0.05 mm

Data collection

Bruker SMART 6000 diffractometer
Absorption correction: multi-scan (*TWINABS*; Sheldrick, 2008*a*)
T_{min} = 0.347, T_{max} = 0.553
97602 measured reflections
8717 independent reflections
8413 reflections with $I > 2\sigma(I)$
R_{int} = 0.048

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.026
$wR(F^2)$ = 0.064
S = 1.13
8717 reflections
626 parameters
106 restraints
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.64 e Å^{-3}
$\Delta\rho_{min}$ = −0.55 e Å^{-3}

Table 1
Selected bond lengths (Å).

C7—O9	1.212 (5)	C47—O48	1.207 (5)
C7—O8	1.312 (5)	C47—O49	1.321 (5)
C10—O11	1.219 (5)	C50—O51	1.212 (5)
C10—O12	1.303 (5)	C50—O52	1.312 (5)
C27—O28	1.213 (5)	C67—O69	1.236 (5)
C27—O29	1.309 (5)	C67—O68	1.289 (5)
C30—O32	1.240 (5)	C70—O71	1.215 (5)
C30—O31	1.288 (5)	C70—O72	1.300 (5)

Table 2
Hydrogen-bond geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O8—H8···O51	0.75 (3)	1.97 (3)	2.713 (4)	167 (6)
O12—H12···O48^{i}	0.76 (3)	1.96 (3)	2.719 (4)	177 (6)
O52—H52···O9	0.76 (3)	1.92 (3)	2.667 (4)	170 (6)
O49—H49···O71	0.75 (3)	1.93 (3)	2.664 (4)	165 (7)
O31—H31···O69	0.76 (3)	1.91 (3)	2.649 (4)	165 (6)
O68—H68···O32	0.75 (3)	1.91 (3)	2.659 (4)	171 (6)
O72—H72···O28^{ii}	0.76 (3)	1.92 (3)	2.673 (4)	171 (6)
O29—H29···O11	0.76 (3)	1.99 (4)	2.662 (4)	147 (6)
N33—H33*A*···O68^{iii}	0.88 (2)	2.39 (3)	3.237 (5)	164 (5)

Symmetry codes: (i) $x+1, y, z$; (ii) $x-1, y, z$; (iii) $-x+1, y-\frac{1}{2}, -z+\frac{3}{2}$.

H atoms were located in a difference Fourier map. The N—H and O—H bond lengths for amino and carboxyl H atoms were restrained to be equal, respectively. Benzene rings and carboxylate groups were restrained to planarity. For all H atoms, U_{iso}(H) = 1.5U_{eq}(parent).

Data collection: *APEX2* (Bruker 2007); cell refinement: *SAINT* (Bruker 2007); data reduction: *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008*b*); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008*b*); molecular graphics: *SHELXTL* (Sheldrick, 2008*b*) and *pyMOL* (DeLano, 2008); software used to prepare material for publication: *SHELXL97* and *SHELXTL*.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: EG3012). Services for accessing these data are described at the back of the journal.

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