

P.08.08.7*Acta Cryst.* (2005). A61, C330**Increasing Thermostability of N-carbamoyl-D-amino Acid Amidohydrolase by Introducing Additional Intermolecular Disulfide Bridges**Wen-Ching Wang, Wei-Chun Chiu, Cheng-Yu Chen, Ji-Yu You, *Institute of Molecular and Cellular Biology and Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan.* E-mail: wewang@life.nthu.edu.tw

N-carbamoyl-D-amino-acid amidohydrolase is an industrial biocatalyst to hydrolyze *N*-carbamoyl D-amino acids for producing valuable D-amino acids. The crystal structure of *N*-carbamoyl-D-amino-acid amidohydrolase in the unliganded and liganded forms demonstrate a tetramer with α - β - β - α fold and a C172-E47-K127 catalytic triad. Crucial binding residues N173, R175, and R176 are also identified. Four mutants were further generated to engineer enzymes with additional intermolecular disulfide bridges: P178C at helix 6, A222C at helix 8, P295C/F304C and A302C from the C-terminal segment near a 2-fold axis. A302C and P295C/F304C showed an increase of 8.8°C and 3.7°C respectively in apparent melting temperature than that of the wild-type enzyme, while there was hardly any change for P178C and A222C. Crystal structures of A222C and A302C were determined and showed limited conformational change. An intermolecular disulfide bridge was observed in A302C but not in A222C. Enzymatic kinetic analysis of A302C revealed a 1.5-fold enhancement in k_{cat}/K_m at 55°C and 4.2-fold increase at 65°C. Our results suggest that introducing an intermolecular disulfide bridge at the C-terminal segment of *N*-carbamoyl-D-amino-acid amidohydrolase near a dyad axis is a useful approach for enhanced thermostability.

Keywords: N-carbamoyl-D-amino acid amidohydrolase, disulfide linkages, thermostability**P.08.08.8***Acta Cryst.* (2005). A61, C330**How Close Can Halogen Atoms Get in a Crystal – Triphenylmethylbromide**Céline Besnard^a, Fabrice Camus^a, Mogens Christensen^b, Marc Fleurant^a, Andy Fitch^c, Phil Pattison^{a,d}, Marc Schiltz^a, ^aLaboratory of Crystallography, EPFL, Lausanne, Switzerland. ^bUniversity of Aarhus, Denmark. ^cESRF Grenoble, France. ^dSwiss-Norwegian BL, ESRF, Grenoble, France. E-mail: celine.besnard@epfl.ch

The crystal structure of triphenylmethylbromide (TPMB) displays unusually close halogen...halogen contacts between neighbouring molecules. The shortest Br...Br distance is 3.203 Å at room temperature, which is about 0.5 Å smaller than the sum of the van-der-Waals radii [1]. An investigation of the crystal structure of TPMB as a function of temperature was performed, to check if the lattice contraction would allow a further compression of the Br...Br contacts.

We found that a reversible phase transition occurs at about 160K, where the single crystal splits up into different domains. The so-formed twinned crystal recovers back to a single domain on passing through the phase transition back to the high temperature form.

Analysis of the twinned data allowed us to solve the structure of the low temperature phase which turns out to be isomorphous to one of the three known polymorphs of triphenylmethylchlorid (TPMC) [2]. Inspection of the low and high temperature structures reveals the mechanism of the phase transition: upon lowering the temperature, the Br...Br distances decrease down to a limiting value at 160K. Beyond that point, the molecules undergo a rearrangement whereby the C-Br bonds are tilted away from the 3 fold-axis, thus leading to a lowering of the crystal symmetry from P-3 to P-1.

[1] Dunand A., Gerdl R., *Acta Cryst.*, 1984, B40, 59. [2] Kahr B., Carter R.L., *Mol. Cryst. Liq. Cryst.*, 1992, 219, 79.**Keywords:** halogen contacts, phase transition, polymorphism**P.08.08.9***Acta Cryst.* (2005). A61, C330**4-membered Metallothiophosphate Rings – Flat or Puckered?**Catharine Esterhuysen^a, Gert J. Kruger^b, Gavin Blewett^a, Helgard G.Raubenheimer^a, ^aDepartment of Chemistry and Polymer Science, University of Stellenbosch, South Africa. ^bDepartment of Chemistry and Biochemistry, University of Johannesburg, South Africa. E-mail: ce@sun.ac.za

During the preparation of the complex (diphenylphosphinodithiolato)(phenyl)(triphenylphosphine)-palladium(II) (C₃₆H₃₀P₂PdS₂) it was found that two crystalline forms were obtained from two different solvent mixtures: one without solvent, the other containing THF (C₄H₈O). Single crystal X-ray diffraction analysis showed that the molecular structures differed in the planarity of the 4-membered palladium dithiophosphate rings, with the complex in the crystal containing solvent taking on a flat conformation, whereas in the crystal without solvent the ring is planar. In order to explain these results the experimental conformations were compared to the conformations of 4-membered metal-S₂P rings reported in the Cambridge Structural Database (CSD) [1], where it was shown that a flat conformation is more common than a puckered one. DFT calculations at the B3LYP level of theory indicate that the flat conformation of a model metallothiophosphate ring is very slightly lower in energy (1.2 kcal/mol) than the puckered conformation, thus supporting the CSD analysis. Closer investigation of the crystal packing shows that the puckering can be traced back to weak off-set face-to-edge π - π interactions between one of the phenyl rings in the diphenyldithiophosphate ligand and phenyl rings in the neighbouring molecules.

[1] Allen F.H., *Acta Cryst.*, 2002, B58, 380.**Keywords:** conformation rings, intermolecular interactions, computer simulation of structure**P.08.08.10***Acta Cryst.* (2005). A61, C330**Keto Forms in Schiff Bases of Salicylaldehydes: Structural and Theoretical Aspects**Spyros Chatziefthimiou, Yannis Lazarou, Eugene Hadjoudis, Irene M. Mavridis, *Institute of Physical Chemistry, National Center for Scientific Research "Demokritos", P.O. Box 60228, Ag. Paraskevi 15310, Greece.* E-mail: spchatzi@chem.demokritos.gr

Schiff Bases of Salicylaldehydes undergo enol-keto tautomerism involving proton transfer from the hydroxylic oxygen to the imino nitrogen atom [1]. The difference in molecular conformation (planar and non-planar for thermochromic and photochromic compounds respectively) and the resulting different crystal packing has been considered crucial for the chromobehaviour of N-salicylideneanilines. In contrast, studies of N-salicylideneacylamines suggested that the electron density on the imine N-atom is what is crucial to thermochromism, rather than molecular planarity, which on the other hand is detrimental to photochromism. It is natural therefore, to suppose that by influencing the electron density on the N-atom by substitution in the salicylaldehyde or/and the amine moiety of the molecule, keto or enol forms of the compounds may be observed. However, no stable keto form had been observed in the crystalline state so far among the substituted N-salicylideneamines and therefore it has not been characterised structurally. In the present study we report the first structural characterisation in the crystalline state of the *cis*-keto form of a number of N-salicylideneamines, which derive from methoxy substituted salicylaldehydes and aliphatic amines. Moreover, we compare it to the theoretical results derived from DFT quantum mechanical calculations in an attempt to understand the important characteristics that render the *cis*-keto form more stable for certain derivatives in this class of N-salicylideneamines.

[1] Hadjoudis E., Mavridis I. M., *Chem. Soc. Rev.*, 2004, 33, 579.**Keywords:** density functional theory, photochromism, tautomerism**P.08.08.11***Acta Cryst.* (2005). A61, C330-C331**Role of Self-Association in Proton Tautomerism of Salicylideneanilines**Keiichiro Ogawa, Toshikatsu Fujiwara, Jun Harada, *Graduate School*