

**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  $\alpha$ - $\beta$ - $\beta$ - $\alpha$  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<sup>a</sup>, Fabrice Camus<sup>a</sup>, Mogens Christensen<sup>b</sup>, Marc Fleurant<sup>a</sup>, Andy Fitch<sup>c</sup>, Phil Pattison<sup>a,d</sup>, Marc Schiltz<sup>a</sup>, <sup>a</sup>Laboratory of Crystallography, EPFL, Lausanne, Switzerland. <sup>b</sup>University of Aarhus, Denmark. <sup>c</sup>ESRF Grenoble, France. <sup>d</sup>Swiss-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<sup>a</sup>, Gert J. Kruger<sup>b</sup>, Gavin Blewett<sup>a</sup>, Helgard G.Raubenheimer<sup>a</sup>, <sup>a</sup>Department of Chemistry and Polymer Science, University of Stellenbosch, South Africa. <sup>b</sup>Department 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) (C<sub>36</sub>H<sub>30</sub>P<sub>2</sub>PdS<sub>2</sub>) it was found that two crystalline forms were obtained from two different solvent mixtures: one without solvent, the other containing THF (C<sub>4</sub>H<sub>8</sub>O). 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-S<sub>2</sub>P 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  $\pi$ - $\pi$  interactions between one of the phenyl rings in the diphenyldithiophosphate ligand and phenyl rings in the neighbouring molecules.

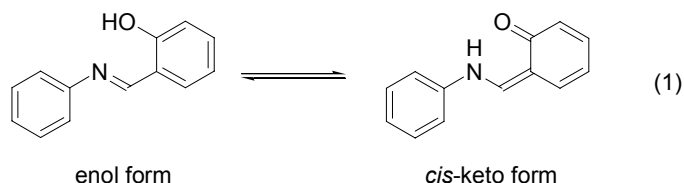
[1] Allen F.H., *Acta Cryst.*, 2002, B58, 380.**Keywords:** conformation rings, intermolecular interactions, computer simulation of structure**P.08.08.10***Acta Cryst.* (2005). A61, C330**Keto Forms in Schiff Bases of Salicylaldehydes: Structural and Theoretical Aspects**Spyros Chatziefthimiou, Yannis 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*

of Arts and Sciences, The University of Tokyo, Komaba, Meguro, Tokyo 153-8902, Japan. E-mail: ogawa@ramie.c.u-tokyo.ac.jp

Proton tautomerism is a general phenomenon in organic molecules and plays a vital role in many fields of chemistry and biochemistry. The tautomerism of salicylideneanilines [eq(1)] has attracted a considerable attention because it is closely related to thermo- and photochromism. Salicylideneanilines greatly favor the enol form over the *cis*-keto form in the gas phase. We demonstrate here that the instability of the *cis*-keto form is substantially reduced by intermolecular hydrogen bonding and also by self-association, on the basis of our variable temperature X-ray crystallographic and UV-vis absorption spectroscopic studies.[1]-[3].



[1] Ogawa K., Kasahara Y., Ohtani Y., Harada J., *J. Am. Chem. Soc.*, 1998, **120**, 7107. [2] Ogawa K., Harada J., Fujiwara T., Yoshida S., *J. Phys. Chem. A*, 2001, **105**, 3425. [3] Fujiwara T., Harada J., Ogawa K., *J. Phys. Chem. B*, 2004, **108**, 4035.

**Keywords:** proton tautomerism, self-association, salicylideneanilines

#### P.08.08.12

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#### The First Observation of All Six Elastic Constants in Tetragonal Hen Egg-white Lysozyme Crystals

Haruhiko Koizumi<sup>a</sup>, Masaru Tachibana<sup>a</sup>, Kenichi Kojima<sup>a</sup>, <sup>a</sup>Graduate School of Integrated Science, Yokohama City University. E-mail: kojima@yokohama-cu.ac.jp

It is interesting to investigate the elastic property of protein crystals. Previously, we have reported the longitudinal sound velocity of tetragonal hen egg-white (HEW) lysozyme crystals which are one of typical protein crystals using an ultrasonic pulse-echo method [1]. However, there is no measurement of all components of elastic constants,  $C_{ij}$  in tetragonal HEW lysozyme crystals since the measurements of transverse ultrasonic wave have not been carried out yet. In case of pulse-echo method, in order to generate a transverse ultrasonic wave in the crystals, a transducer must be in close contact with the crystal. However, hydrated protein crystals are fragile, and consequently, it was difficult to glue a transducer to a protein crystal. Recently, we found that the sound velocity increases with exposure to open air and approaches a constant value [2]. This suggested that the protein crystals became harder due to dehydration. Therefore, we have succeeded in the contact between a crystal and a transducer using dehydrated crystals. This led us generating a transverse ultrasonic wave through the dehydrated HEW lysozyme crystals at 42% relative humidity (RH). As a result, all components of elastic constants in dehydrated crystals were determined.

[1] a) Tachibana M., Kojima K., Ikuyama R., Kobayashi Y., Ataka M., *Chem. Phys. Lett.*, 2000, **332**, 259; b) Tachibana M., Kojima K., Ikuyama R., Kobayashi Y., Ataka M., *Chem. Phys. Lett.*, 2002, **354**, 360. [2] Tachibana M., Koizumi H., Kojima K., *Physical Review E*, 2004, **69**, 051921.

**Keywords:** ultrasonics, elastic properties, protein crystals

#### P.08.08.13

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#### 5,5'-Disubstituted-3,3'-Methanediyl-bis-Indoles as Potential Antitumorals

Irena Wolska<sup>a</sup>, Dorota Maciejewska<sup>b</sup>, <sup>a</sup>Department of Crystallography, Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland. <sup>b</sup>Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02 097 Warsaw, Poland. E-mail: iwolska@amu.edu.pl

Certain compounds found in the diet are a potential source of new

classes of chemotherapeutic and chemopreventive agents. Indole-3-carbinol, a major indole metabolite from cruciferous vegetables, and its natural condensation product 3,3'-diindolylmethane, have been found to inhibit the development of tumors in breast, uterus and liver [1-3]. However, both compounds may exhibit adverse tumor promoting activity in other organs. In search for new *anti* cancer agents we have decided to investigate 3,3'-diindolylmethane derivatives which may reduce the growth of human tumor cell lines by the inhibition of the binding of some transcription factors to the adequate sequences in DNA.

To determine structural parameters important for the biochemical activity we have performed the <sup>1</sup>H and <sup>13</sup>C NMR data and single crystal X-ray analysis of 5,5'-disubstituted-3,3'-methanediyl-bis-indoles. The results of these studies indicate insignificantly different molecular structures of the investigated compounds but significantly different networks of intermolecular interactions in crystals. Interesting NH... $\pi$  hydrogen bonds are observed which may have a functional role in biological features.

[1] Grubbs C.J., et al., *Anticancer Res.*, 1995, **15**, 709. [2] Cover C.M., et al., *J. Biol. Chem.*, 1998, **273**, 3838. [3] Hong C., et al., *Bioch. Pharmacol.*, 2002, **63**, 1085.

**Keywords:** anticancer compounds, 5,5'-disubstituted-3,3'-methanediyl-bis-indoles, X-ray single-crystal diffraction

#### P.08.08.14

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#### Intermolecular Interactions in 1,1'-Binaphthyl, Polymorphs and Symmetry Breaking

C. Ignacio Sainz-Díaz<sup>a</sup>, Africa P. Martín-Islán<sup>b</sup>, Julyan H. E. Cartwright<sup>a</sup>, Alfonso Hernández-Laguna<sup>b</sup>, <sup>a</sup>Instituto Andaluz de Ciencias de la Tierra, CSIC - Universidad de Granada, Av. Fuentenueva s/n, 18002-Granada (Spain). [sainz@lec.ugr.es](mailto:sainz@lec.ugr.es). <sup>b</sup>Estación Experimental del Zaidín, CSIC, C/ Profesor Albareda, 1, 18008-Granada (Spain). E-mail: [sainz@lec.ugr.es](mailto:sainz@lec.ugr.es)

A theoretical-experimental work is presented related with the chiral symmetry breaking of melting crystallization of 1,1'-binaphthyl derivatives and polymorphism. We confirm that the chiral symmetry breaking can be observed in crystallization from a melt of 1,1'-binaphthyl by a constant stirring during the crystallization. Crystallographic studies by Powder X-ray diffraction (PXRD) reveal two crystallographic forms of 1,1'-binaphthyl: one chiral form (P4<sub>2</sub>1<sub>2</sub>) with either R or S enantiomers of the trans-1,1'-binaphthyl conformer and another racemic crystal (C2/c) with both enantiomers of the cis-1,1'-binaphthyl conformer. Quantum mechanical calculations of the crystal lattice for 1,1'-binaphthyl and 2,2'-dihydroxy-1,1'-binaphthyl polymorphs were performed by Density Functional Theory approximation. Our calculations reproduce the crystal lattice parameters and PXRD pattern finding the P4<sub>2</sub>1<sub>2</sub> form with lower energy than the C2/c form for 1,1'-binaphthyl. The main intermolecular interactions in 1,1'-binaphthyl crystals are weak aromatic CH/ $\pi$  hydrogen bonds, which are responsible for enantiomeric discrimination in the molecular recognition during crystallization. The C2/c form achieves a more efficient packing than the chiral one, but intermolecular interactions in P4<sub>2</sub>1<sub>2</sub> form are stronger than in C2/c form. In 2,2'-dihydroxy-1,1'-binaphthyl the intermolecular interactions are stronger with hydrogen bonds between the hydroxyl groups and polymorphs can be predicted by Monte Carlo simulated annealing.

**Keywords:** 1,1'-binaphthyl, intermolecular interactions, chiral recognition

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#### Experimental and Theoretical Study of Weak Interactions in Simple Molecular Solids

Bartolomeo Civalieri<sup>c</sup>, Davide Viterbo<sup>a</sup>, Riccardo Bianchi<sup>b</sup>, Gianluca Croce<sup>a</sup>, Marco Milanese<sup>a</sup>, Roberto Orlando<sup>a</sup>, <sup>a</sup>DiSTA - Università del Piemonte Orientale, Alessandria. <sup>b</sup>ISTM, CNR, Milano. <sup>c</sup>Dipartimento