m13.p26

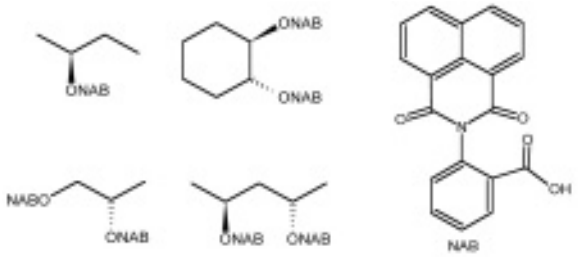
Factors controlling the helicity and stacking of N-(1,8-naphthaloyl)-2-aminobenzoic acid esters: interplay between weak hydrogen bonds, π-π stacking and dipolar interactions

A. Plutecka, U. Rychlewska, M. Hoffmann

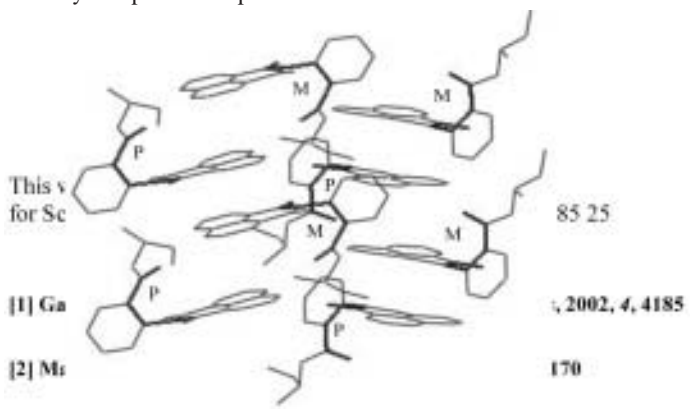
Dept. of Chemistry, Adam Mickiewicz University, Poznań, Poland

Keywords: weak interactions, twinning, multiple asymmetric units

Several esters of chiral mono- and dialcohols and *N*-(1,8-naphthaloyl)-2-amino benzoic acid (NAB) have been investigated by X-ray crystallography and quantum-chemical methods. The investigated molecules can be considered as consisting of two parts: the aliphatic core (either linear or cyclic) and the aromatic NAB moiety(ies).



Mutual arrangement of the two parts is such that either the aliphatic skeleton is put away from the naphtalimide unit (conformer A, extended) or it is situated above the unit (conformer B, folded) [1]. Quantum chemical calculations (ONIOM method [2]) indicate that the A conformer is energetically preferred over the B one, in both mono- and diesters, and that the two aromatic rings constituting the NAB chromophore are inclined to each other at exactly right angles. In crystals the situation is more complex, as we observe both the A and B conformers, and deviations from orthogonality of the aromatic fragments. The latter effect combined with severe twisting around the C_{aryl}-C_{ester} bond induces plus (P) or minus (M) helicity into the NAB moiety. The investigated crystals, being chiral, display a tendency to form multiple asymmetric units and non-merohedral twins. These structural peculiarities may be connected with the fact that the face stacking interactions involve molecules of opposite helicity and that both the molecular helicity and the crystal packing are mostly stabilized by antiparallel dipolar interactions.



This work was supported by the Polish State Committee for Scientific Research as scientific project no. 4 T09A 185 25

[1] Gawroński, J.; Kwit, M.; Gawrońska, K. *Organic Letters*, 2002, *4*, 4185

[2] Maseras, F.; Morokuma, K. J. *Comp. Chem.,* 1995, *16*, 1170

m13.p27

Structure of non-structural protein 9 of human coronavirus 229E

Rajesh Ponnusamy, Jeroen R. Mesters, Ralf Moll, Rolf Hilgenfeld

Institute of Biochemistry, Center for Structural and Cell Biology in Medicine University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Keywords: viral replication, common cold, RNA interaction

Coronaviruses are a group of positive-stranded RNA viruses which carry the largest single-stranded RNA viral genome known to date (~30 Kb). They can replicate in the cytoplasm of the infected host cells and cause a variety of respiratory and gastrointestinal illnesses. The coronavirus replicase gene consists of two large ORFs (ORF 1a and 1b) and codes for two polyproteins, pp1a and pp1ab. The polyproteins are subsequently processed by viral proteinases to yield the functional non-structural proteins. They mediate all the functions necessary for viral replication and transcription. The crystal structure of the HCoV 229E main proteinase has been determined earlier by our group [1]. The 3' region of ORF1a codes for a number of relatively small polypeptides (Nsp6 to Nsp11) and the products assemble into a membrane-associated viral replication/transcription complexes [2]. Nsp9 is a single stranded oligonucleotide binding protein with different topology from the OB fold family known from SARS-CoV Nsp9 structure [3], [4]. The crystal structure shows HCoV-229E Nsp9 as a symmetric dimer with a different mode of dimer formation compared to what was observed in SARS-CoV Nsp9. The dimer is mediated by hydrophobic interaction, four hydrogen bonds and a disulfide bridge. To investigate further the dimer formation, Nsp9 Cys67 was mutated to alanine. The crystal structure of the Nsp9 mutant shows a similar dimer conformation observed as in SARS-CoV Nsp9. Reasons for the structural differences will be discussed. Using zone-interference gel electrophoresis [5] and fluorescence spectroscopy, we demonstrated that HCoV 229E Nsp9 binds non-specifically to nucleic acids. Co-crystallization of Nsp9 and nucleic acids is in progress.

This project is being supported by the Sino-European Project on SARS Diagnostics and Antivirals (SEPSDA; contract no. SP22-CT-2004-003831; www.sepsda.info) and by VIZIER (contract no. LSHG-CT-2004-511960; www.vizier-europe.org), both of which are funded by the European Commission.

[1] Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R. & Hilgenfeld, R. (2003). Coronavirus main protease ($3CL^{pro}$) structure: basis for design of anti-SARS drugs. *Science* 300, 1763-1767.

[2] Herold, J., Raabe, T., Schelle-Prinz, B. & Siddell, S.G. (1993). Nucleotide sequence of the human coronavirus 229E RNA polymerase locus. *Virology* 195, 680-691.

[3] Egloff, M.P., Ferron, F., Campanacci, V., Longhi, S., Rancurel, C., Duartre, H., Snijder, E. J., Gorbalenya, A.E., Cambillau, C. & Canard, B. (2004) The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. *Proc. Natl. Acad. Sci. USA* 101, 3792-3796.

[4] Sutton, G., Fry, E., Carter, L., Sainsbury, S., Walter, T., Nettleship, J., Barrow, N., Owens, R., Gilbert, R., Davidson, A., Siddell, S., Poon, L.L.M., Diprose, J., Alserton, D., Walsh, M., Grimes, J. M. & Stuart, D.I. (2004). The nsp9 replicase protein of SARS-coronavirus, structure and functional insights. *Structure* 12, 341-353.

[5] Abrahams, J.P., Kraal, B. & Bosch, L. (1988). Zone-interference gel electrophoresis: a new method for studying weak protein-nucleic acid complexes under native equilibrium conditions. *Nucl. Acids Res.* 16, 10099-10108.