Clemens Mügge

Molecular Structures, Dynamics and Interactions

High resolution NMR spectroscopy is one of the most important methods for molecular structure elucidation on atomic level. By means of different measurement techniques NMR can provide information about the molecular structure as well as dynamics and intra- and intermolecular interactions. The applications of NMR spectroscopy embrace the whole range of molecules starting from low weight and inorganic compounds up to high molecular biopolymers (900 kDa proteins).

Natural products

Natural products, preferably those of ethnobotanically organisms are promising starting points for drug development. Their isolation and structural elucidation are the first steps in the identification of new lead-structures. A major part of today's pharmaceuticals is directly or indirectly related to natural products, that means compounds of herbal, animal, microbial or endogenous origin.

From the large variety of natural products we have so far dealt with

- the contents of medicinal plants, in particular, the alkaloids from *Amaryllidaceae*,
- the steroid oligoglycosides from maritime sources,
- the antibiotics from microorganisms (*Actinomyceten*)
- the peptide and non peptide contents of fungi

with the aim of finding new active compounds.

The identification and structure elucidation of biological compounds can be carried out successfully by using a combination of separation and structure elucidation techniques. Thus, the direct coupling of HPLC with MS or NMR became an indispensable tool for rapid and efficient investigation in this field. The often complementary information of the spectroscopic methods UV/VIS, MS and NMR is of special importance. Using them simultaneously as hyphenated techniques provides considerable advantages for the rapid identification of natural compounds in extracts. During investigation of the metabolism of already known strains of *Streptomyces olivacues* by means of the modern *on-line* techniques more than 30% of the iden-

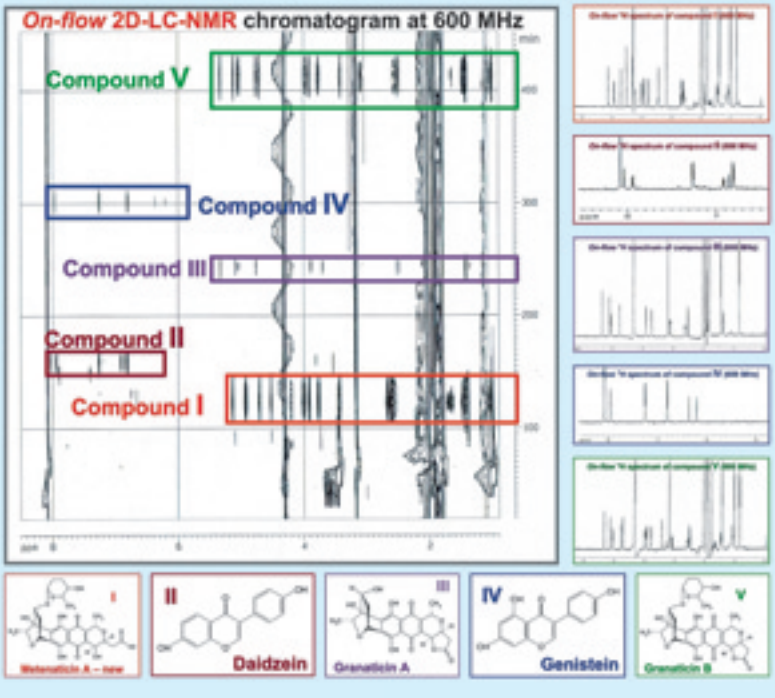


Fig. 1
On-flow LC-NMR extract of Streptomyces olivaceus *(TÜ22)*

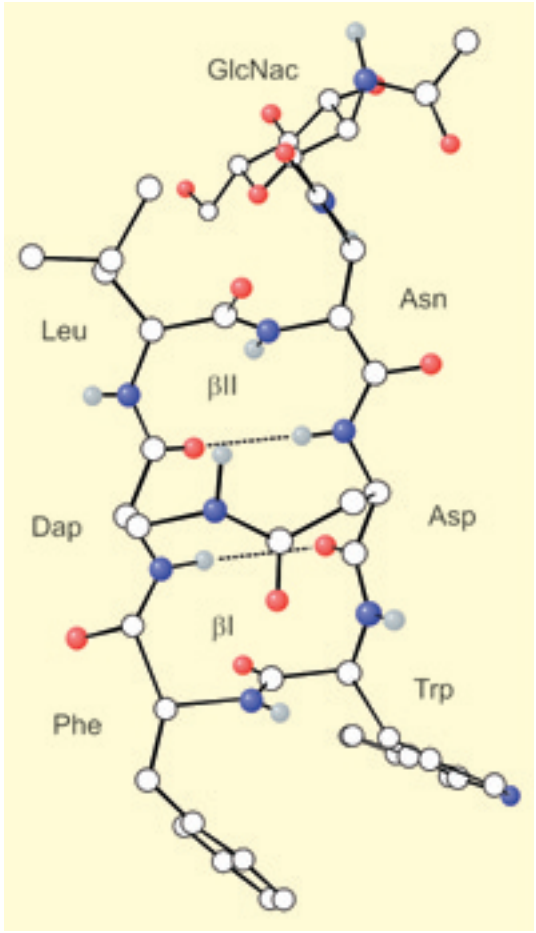


Fig. 2
A bioactive, bicyclic hexapeptide. The active conformation on the receptor is forming a ß-turn conformation.

Molekulare Strukturforschung

Die molekulare Strukturforschung im ursprünglichen Sinn als Bestimmung der dreidimensionalen Struktur isolierter Moleküle geht gegenwärtig in einer wesentlich erweiterten Form in Studien zum Verständnis komplexer Wechselwirkungen zwischen den Molekülen auf. Hauptziel unserer Arbeiten ist es, mit Hilfe der hochauflösenden kernmagnetischen Resonanzspektroskopie (NMR) die in ausgewählten molekularen Modellsystemen auftretenden Wechselwirkungen auf struktureller Basis möglichst umfassend aufzuklären, um hieraus induktiv generelle Aspekte der Spezifität molekularer Erkennung und Funktion abzuleiten.

tified metabolites turned out to be hitherto unknown. By using the hyphenated techniques LC-MS and LC-NMR a full structure elucidation is possible for a wide range of substances from biological samples at the ng level. Moreover the methods established by us allow the quantification of individual components from the LC-NMR chromatograms. (Fig. 1)

Peptides

The ultimate goal of molecular biology is to understand biological processes in terms of the chemistry and physics of the participating macromolecules. One of the essential differences between the chemistry of living systems and that of nonliving ones is the overwhelming structural complexity of biological macromolecules. It is impossible to unravel the chemistry of life in molecular detail without knowing at atomic or close to atomic resolution the structure of biological macromolecules, especially the proteins. On this background we focus on the conformational analysis of cyclic penta- and hexapeptides, which are excellent models mimicking protein structures.

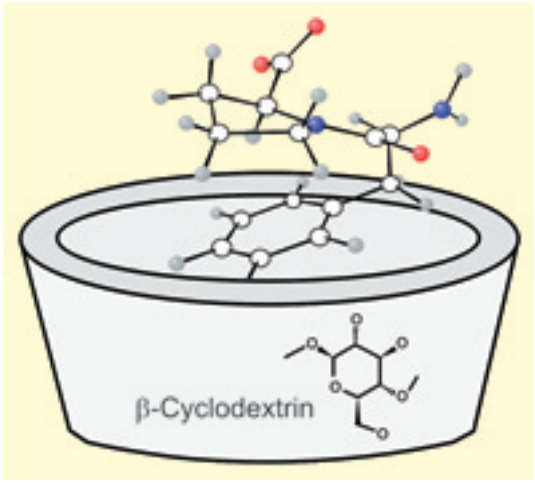
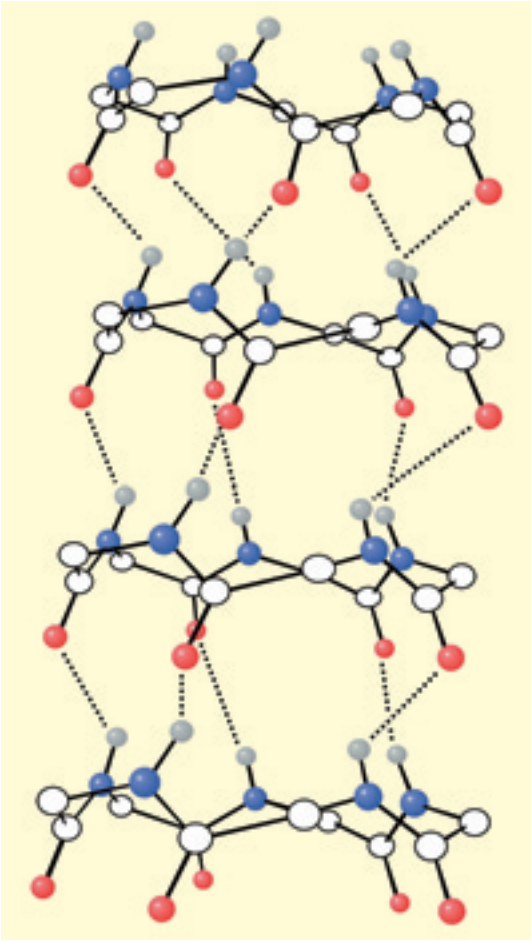


Fig. 3
Complex of ß-cyclodextrine and a Phe-cisPro-sandwich.

Fig. 4
Molecules of a cyclic all-L pentapeptide forming a peptide nanotube.



Conformational investigations of cyclic peptides are a general tool for the study of regular turn structures. Regular turns are a third type of structural motifs besides the better known α-helices and β-sheets. On average, one quarter of the amino acid residues of a peptide are located in regular turns. The study of cyclic peptides provides insight into backbone angles, intramolecular distances and side chain orientations of turn structures and, moreover, into the relationship between amino acid sequence and secondary structure. (Fig. 2)

Cyclic peptides are also suited for the investigation of other structural elements which occur in proteins. For example, the close contact of the amino acid side chains of cis-proline and phenylalanine is detectable in the cysteine brigded peptide cyclo(Cys-Phe-Pro-Ala-Cys). Such a sandwich-like arrangement and other hydrophobic interactions could be reasons for the existence of rare cis-peptide bonds in proteins. Hydrophobic interactions are responsible, too, for the formation of molecular complexes of peptides (as, e.g. Phe-cisPro) and cyclodextrines. Cyclodextrines are bucket-like molecules, featering a hydrophilic exterior and a hydrophobic inside. These molecules were found to have widespread applications, ranging from medicine & cosmetics to analytical chemistry. (Fig. 3)

Cyclic pentapeptides which contain L-amino acids exclusively, can self-assemble to nanotubes stabilised by intermolecular hydrogen bonds. This behaviour is often typical for natural antibiotics consisting of D,L-amino acid subunits. (Fig. 4)

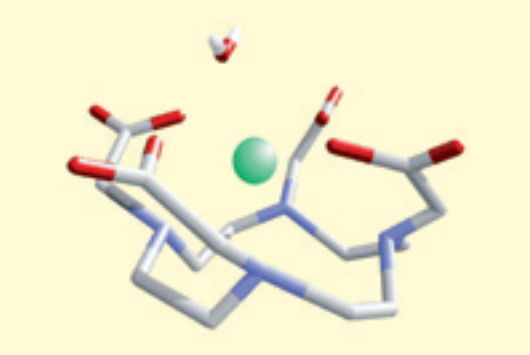


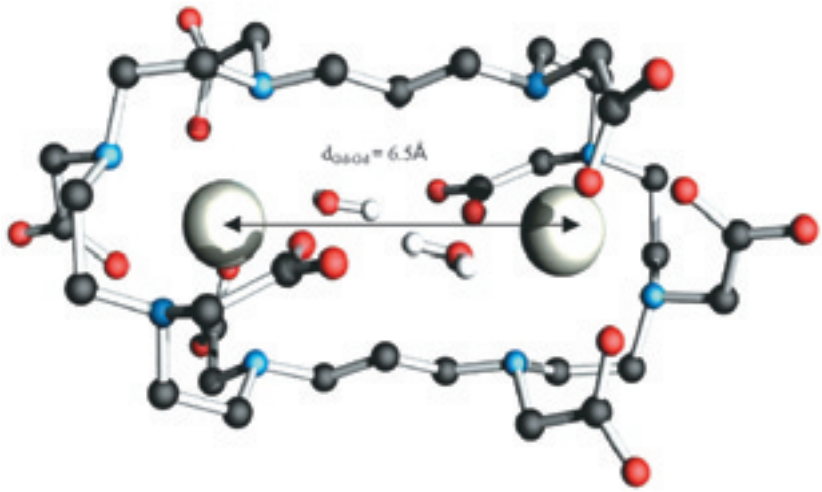
Fig.5
Solid state structure of $[Gd(DOTA)(H_2O)]^-$.

NMR contrast agents

One of the fastest growing diagnostic techniques used in medicine is magnetic resonance imaging (MRI). As in X-Ray imaging, it is often necessary to enhance contrast between different parts of tissue. In particular, gadolinium(III) was found to be an excellent contrast agent that relaxes the water protons in vivo, thus allowing enhanced discrimination of the tissues in a MRI experiment. However, the toxicity of uncoordinated gadolinium is a considerable drawback, hence the majority of commercial MRI contrast agents are derivatives of the Gd-DOTA or Gd-DTPA chelates, i.e. mononuclear paramagnetic complexes formed with polyaminocarboxylate ligands.

The best investigated compounds of this class are the mononuclear lanthanide(III) chelates of the macrocyclic ligand DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate), which show the ninefold coordinated metal ions in a mono-capped square-antiprismatic coordination sphere. It is further characterized by one water molecule in the first coordination sphere. (Fig. 5)

Fig. 6
Solid state structure of $[Na_2Gd_2(OHEC)(H_2O)_2]$.



Whereas it is understandable that the relaxivity increases with the rate of water exchange in the complex, other factors influencing the relaxivity are less obvious. In fact, in large molecular systems, besides intramolecular motions the electronic relaxation can also be affected and this effect is not yet understood. One reason could be an interaction between the paramagnetic metal ions occuring when they are close to each other. We will try to answer the question if there is any electronic interaction between the two paramagnetic ions. For this purpose, we synthesized a series of bilanthanide chelates of polyaminocarboxylate ligands, e.g. H_8OHEC. (Fig. 6)

The binuclear chelates are useful models since the two lanthanide cations are close to each other (r_{Gd-Gd} = 6.5 Å). Further advantages of this type of contrast agents are the increase of molecular rotational correlation time and the doubling of the number of water molecules in the inner coordination sphere while the kinetic inertness and thermodynamic stability is maintained.

With the aim of increasing both their efficiency and their specifics towards particular organs or tissues, new macromolecular agents such as dendrimers and micelles have been developed during this last decade. The delivery of contrast agents into tissues *in vivo* is restricted by transport across the capillary microvascular endothelial barrier. Our strategy for mediating the uptake of contrast agents in cells is the covalent linkage of contrast agents to membrane-permeable peptides. Peptides that have been used for the conjugation with DOTA-like contrast agents are cationic at physiological ph values, such as polylysine and arginine-rich sequences.

Protein-ligand interactions

Knowledge about chemical bonding between ligands and receptors is the key for understanding of many biological processes. Of special interest are the protein-ligand interactions which play a key role at etiogenesis of serious diseases such as AIDS or cancer.

By using new methods of NMR spectroscopy now it is possible to investigate and understand protein-protein and protein-ligand interactions on an atomic level.

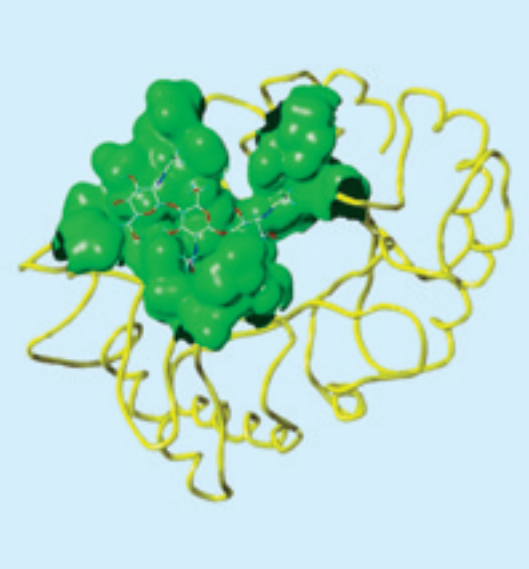


Fig. 7
Hevamine chitotriose complex

Nowadays, NMR provides a broad range of specialized pulse sequences, thereby facilitating not only the in-depth study of the structure and dynamics but also of the interaction between molecules. Specifically, these experiments allow, for instance

- the detection of intermolecular interactions,
- the identification of areas of molecules, that actively participate in the bonding (binding epitopes) and
- the deduction of bioactive conformations of interacting molecules.

The aim of our future work is the characterization of individual interactions of selected biomolecules by combining known procedures with new techniques. By this means, we want to deduce general aspects of the specifics of molecular recognition.

Recently, we have started our research in the field of receptor-ligand and enzym-inhibitor interactions in hevamin-chitotriose complexes. (Fig. 7)

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