Preparation and NMR Structure of the Cyclo-β-tripeptide [β^3-HGlu]$_3$ in Aqueous Solution: A New Class of Enterobactin-Type C_3-Symmetrical Ligands?

Preliminary Communication

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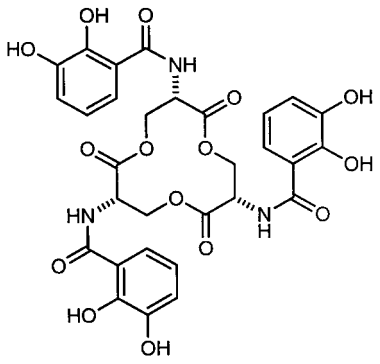
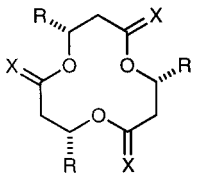
Dedicated to Professor *Armin de Meijere*, Universität Göttingen, on the occasion of his 60th birthday

To date, cyclo-β-tripeptides (twelve-membered-ring trilactams, **C**) have resisted structural investigations because of their extreme insolubility. Modelling and comparison with the corresponding tetramers indicate that the rings stack to form tube-like hydrogen-bonded aggregates (**D**). By exploiting the solubilizing effect of LiCl on peptides in THF, we were able to prepare the water-soluble title compound and determine its structure (**E**) by NMR spectroscopy. Structural similarities and differences between the trilactam ring of [β^3-HGlu]$_3$ and the corresponding trilactone ring, such as that of enterobactin (**A**), are discussed, and structures ('elongated'; **F**, **G**) are proposed that should be able to serve as tridentate or trivalent ligands for metal centers.

Introduction. – C_3-Symmetrical cyclic oligomers such as *enterobactin* (**A**) play an important role in bacterial iron uptake and storage (*siderophores*; for a review article, see [1]); these compounds bind Fe^{III} highly selectively and with extraordinary binding constants of up to 10^{49} [2]. The X-ray structure of **A** with V^{IV} revealed an all-up arrangement of the carbonyl groups with s-*trans*-conformation of the ester bonds and complexation of the metal ion by the catecholate units under the 'flat' macrocyclic ring [3].

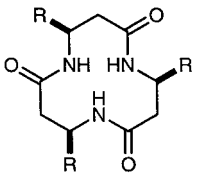


A



B

X = O, S; R = Me, Et



C

[1]) Part of the projected dissertation of *K.G.*, ETH-Zürich

In the course of our work on the mechanism of the Ca^{2+} transport through phospholipid bilayers mediated by 3-hydroxybutanoic acid (HB) oligomers [4][5], we also prepared cyclic HB oligomers and found that the twelve-membered ring in the crystal structure of the trilactones **B** is almost superimposable with that of enterobactin [6–8]. The dipole moment of **B** (X = O, R = Me) was determined to be 4.6 D in CCl_4 [7], consistent with the parallel arrangement of three C=O groups.

In contrast to the structures of the trilactones those of the corresponding highly insoluble cyclo-β-tripeptides, such as **C**, are unknown[2]). In the case of cyclo-β-tetrapeptides consisting of β^3-HAla, we were able to determine (by powder X-ray diffraction) the structures of three diastereoisomers: they adopt stacked tubular structures with a tight net of H-bonds, so-called *peptide nanotubes* [10][3]). Another cyclo-β-tetrapeptide has recently been shown to display biological activity mimicking that of a natural α-peptide hormone [12].

Modelling. – In previous work on cyclo-β-peptides with alkyl and with functionalized side chains [13–15], we had noticed the extremely poor solubility, and IR spectra indicated that these compounds also form a network of intermolecular H-bonds, maybe nanotube-type stacking in the solid state. Modelling of the parent compound (**C**; R = H), with the MacroModel program [16], by placing seven trilactam units symmetrically on top of each other and allowing for energy minimization, indeed predicts the tube stacking as shown in the model **D** (*Scheme*). As can be seen, each of the rings is turned a few degrees against its neighbor (the angle N–H···O deviates from 180°). On each tetrahedral C-atom of the rings, there is an equatorial or lateral position available for substituents and an axial one occupied by H-atoms.

The infinite pleated-sheet-like stacking suggested by this model is, of course, compatible with the insolubility of such compounds in all common solvents except for CF_3COOH, and with their very high melting points of over 200° [14][15]. Four ways occured to us to overcome such insolubility, *i.e.*, to break the intermolecular H-bonds and stabilize non-aggregated solubilized species: *i*) introduction of solubilizing side chains (bearing hydrophilic NH_3^+ or CO_2^- groups), *ii*) complexation with 'hard' metal ions such as Li^+, which complex amide O-atoms very strongly [17][4]), *iii*) prohibition of stacking by switching to *N*-methyl-amide derivatives [17][19], and *iv*) prevention of intermolecular H-bonding by incorporating geminally disubstituted ($\beta^{2,2}$ or $\beta^{3,3}$) or, alternatively, *unlike* $\beta^{2,3}$-disubstituted β-amino-acid residues (these insure that a substituent is in a sterically forbidden position on a tetrahedral C-atom of the ring).

We have now made use of the first two methods, which allowed us to prepare and study the structure of a cyclo-β-tripeptide in aqueous solution.

Preparation of [β^3-HGlu]$_3$. – The trilactam **1** derived from glutamic acid with three benzyl-ester groups in the side chains, which could previously not be debenzylated for reasons of poor solubility [15], was dissolved in high concentrations in THF containing

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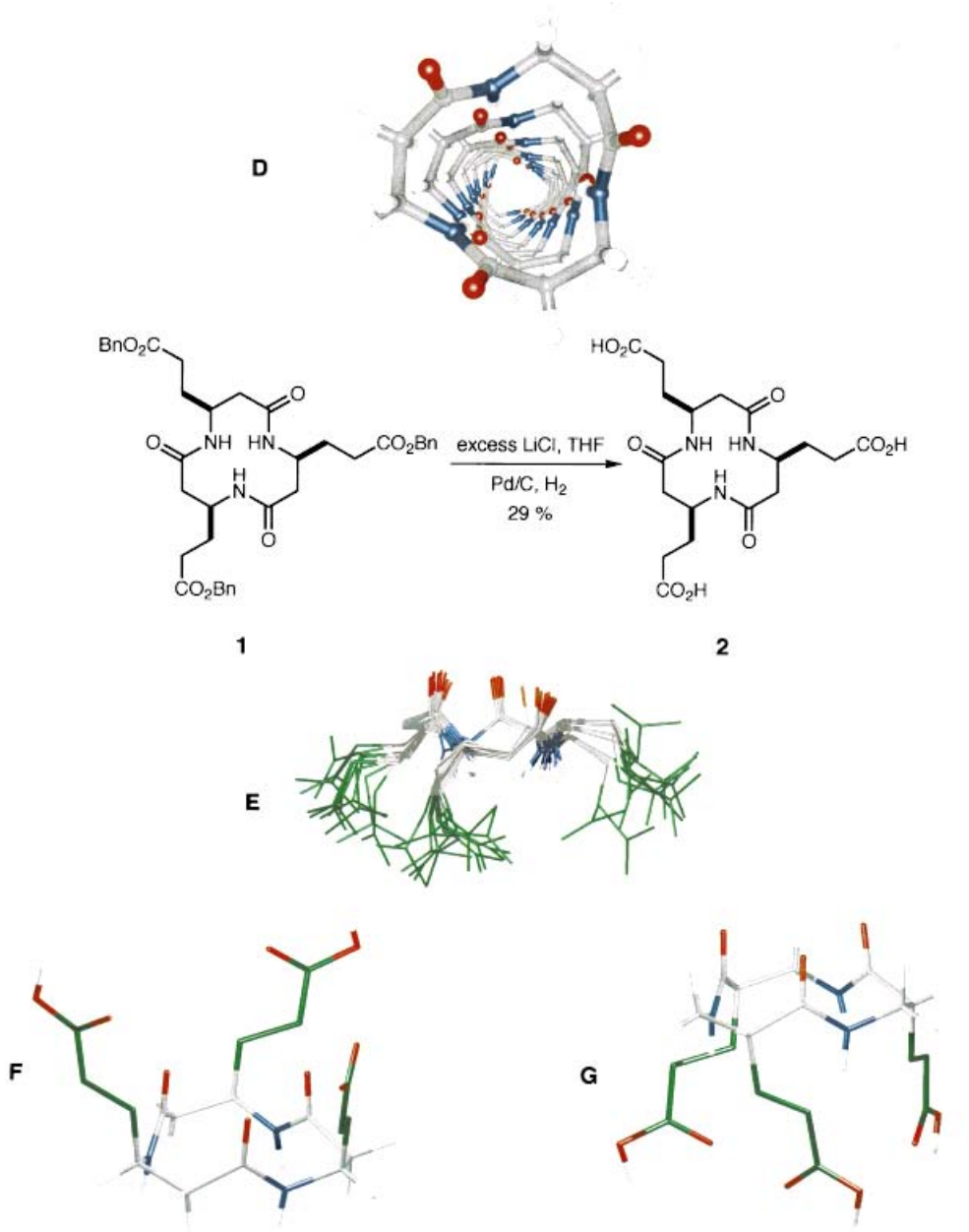
[2]) Early molecular mechanics calculations by *Drey* and co-workers did not provide conclusive structural information about the achiral cyclo-β-tripeptide **C** (R = H) [9].

[3]) Incorporation of a cyclo-β-tetrapeptide in phospholipid bilayers induces ion conductivity [11].

[4]) ΔH^0 values of up to –37 kcal/mol have been measured [18].

Scheme. *Cyclo-β-tripeptides.* The model **D** calculated for the structure of cyclo-β-tripeptide **C** (R = H). This model is in agreement with experimentel facts such as very high melting points and IR spectra of cyclo-β-tripeptides. The cyclo-β-tripeptide **1** is deprotected using the Li-salt effect in THF, yielding the water-soluble triacid **2**. The bundle of structures **E** is representative of the *solution structure* of **2** *in water*, as determined by NMR spectroscopy. The substituents occupy lateral positions on the trilactam ring, a conformer with axial dispositions of the side chains (obtained by ring inversion) as in **F** is destabilized by $A^{1,3}$ strain. For the corresponding β^2-peptide (see model **G**), axial substituents should be tolerated, thus metal complexation underneath the trilactam ring may be possible with this isomer.



a large excess of LiCl (up to 23 equiv.)[5]). The solution thus obtained was used for the hydrogenolytic ester cleavage over Pd on charcoal (see *Scheme*). The reaction was rather slow, and the yields in the heterogeneous mixture varied between 20 and 35%[6]). Nevertheless, we were able to isolate and purify by reversed phase HPLC (eluent 1% CF_3CO_2H in H_2O/MeCN) the triacid **2** in pure form. The compound is extremely water-soluble.

NMR Analysis of Triacid 2. – The NMR spectrum in D_2O clearly demonstrates that the predominant conformation possesses a three-fold symmetry (by the magnetic equivalence of the NH, C(β)–H, C(α)–H, and C(α)–H′ protons of all three residues). By symmetry, it is, therefore, sufficient to determine the structure of the β-tripeptide **2** by assigning only three torsion angles of one of the component β-amino acids. Scalar ^3J-coupling constants lead directly to the torsion angles *via* the *Karplus* equation [23–26]: The distinct J values observed in D_2O (11.7 and 2.9 Hz for C(β)H–C(α)H^{ax} and C(β)H–C(α)H^{lat}) clearly indicate an antiperiplanar arrangement of the C(β)H^{ax}–C(α)H^{ax} protons. Furthermore, the large NH,C(β)H coupling constant (9.9 Hz)[7]) establishes the antiperiplanar disposition of the corresponding H-atoms. These values were used as torsion-angle restraints for the structure determination by the program X-PLOR [27]. The resulting bundle **E** of conformations, which is representative of the structure in aqueous solution, is shown in the *Scheme*.

Discussion and Conclusions. – It is obvious that the experimentally determined structure **E** of the twelve-membered ring is essentially identical with the trilactam components in model **D**. The structure is characterized by an all-up arrangement of the C=O groups. The laterally disposed side chains bent downwards so that the carboxylic-acid groups populate the half-space underneath the peptidic macrocycle. This solution structure is very similar to the solid-state structures observed for the triolides **B**. Furthermore, the J values measured are nearly identical to those observed for the corresponding H-atoms in the 3_1-helix of β-peptides [28][29]. Therefore, the structure may be referred to as a 'helix without a pitch' or, conversely, the 3_1-helix may be considered to be composed of trilactam units cut open and bent to form a left-handed single helix pitch[8]).

There is both a resemblence and a fundamental difference between the structures of **2** and of enterobactin. The twelwe-membered ring of enterobactin (**A**) is essentially superimposable with that of **2**, with the NH groups in **2** replaced by the O-atom in **A**. The NH–CO–Aryl substituents on the enterobactin trilactone occupy axial positions, which, in the case of the neighboring lactone O-atoms, do not lead to any $A^{1,3}$ repulsion. The side chains in **2** occupy lateral positions, so that the acid groups can approach each other only when bent down, which was actually observed in aqueous solution. A complexation of a trivalent ion by the three carboxylate groups of **2** would probably

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5) This effect was originally discovered with α-peptides [20–22] and is now used frequently in peptide synthesis (for a review, see [17]).

6) *Cf.* the debenzylation of a cyclo-β-tetrapeptide [12].

7) The NH resonance dissapears after several min due to exchange with D_2O.

8) *Cf.* the X-ray crystal structure of a linear β-tripeptide containing a nascent helix, Fig. 3 in [30].

not be possible on steric grounds; one or two additional CH_2 groups in the side chains, should, however, allow for a geometry in which the trilactam derivative becomes a C_3-symmetrical ligand for such an ion. Inversion of the trilactam ring conformation to put the side chains in axial positions (see model **F**) is impossible because of six massive 1,5-repulsive interactions between CH_2 groups and carbonyl O-atoms! An axial substitution on a trilactam structure is conceivable with β^2- instead of β^3-amino-acid building blocks (model **G**), when only the NH H-atoms of the three amide bonds are in a 1,5 coplanar arrangement with the side chain CH_2 groups.

We have started work to synthesize analogues of **2** with both possible C_3-symmetrical types of substitution patterns, the one bearing elongated side chains, and the corresponding β^2-amino-acid-derived isomers (*cf.* **G**).

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