PhD Thesis

Investigation of Dynamic Processes and Self-Organization by Spectroscopic Methods and Molecular Modelling

Anasztázia Hetényi

Institute of Pharmaceutical Chemistry, University of Szeged,

Szeged, Hungary

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A. Introduction and aims

In recent decades, 1,3-*X,N*-heterocycles and β-amino acid derivatives have played an important role in drug therapy and drug research. These compounds serve as good models for a better comprehension of several important phenomena in chemistry, for example the dynamic processes of ring–chain tautomerism and ring–ring epimerization and the behaviour of self-organizing foldameric systems.

The tautomeric equilibria of the 2-aryl-1,3-*X,N*-heterocycles can be described successfully by the Hammett–Brown linear free energy relation ship. The earlier results indicated that the proportions of the ring-closed forms strongly depend on the electronic character of the substituent on the aromatic ring. It is still an open question whether the substituent-dependent stability difference between the epimeric forms in the ring–ring epimerization can be explained by the stereoelectronic interactions related to the anomeric effect.

The unnatural foldamers with well-defined secondary structure preferences, especially β-peptides, are attracting increasing interest and have a wide range of potential applications because they are able to adopt specific, compact conformations. The conformationally constrained β-peptide oligomers containing cyclic side-chains are among the most thoroughly studied models in foldamer chemistry. The chain length-independence of the folding pattern of β-peptide oligomers raises the still open question of whether these oligomers exhibit a real folding process, or whether the conformational space of the monomers is too preorganized to allow partly folded or other stable secondary structures. Another major challenge in foldamer science is to prove that higher-order structural levels are available for β-peptides and that their formation can be tailored by the β-amino acid sequence.

In the field of the dynamic processes, the scope of my PhD thesis covers an analysis of the limitations of the Hammett–Brown equation in the ring–chain tautomerism of *cis*- and *trans*-1-aminomethylcyclohexane-1,2-diols and in the ring–ring epimerization of 2-aryl-1,3-*N,N*-heterocyclic derivatives. In the field of the self-organizing foldamers, the goal was to study the limits of the conformational flexibility and self-assembling

properties of the *β*-peptides with constrained side-chains for the *trans*-2-aminocyclohexanecarboxylic acid and *cis*-2-aminocyclopentanecarboxylic acid foldamers.

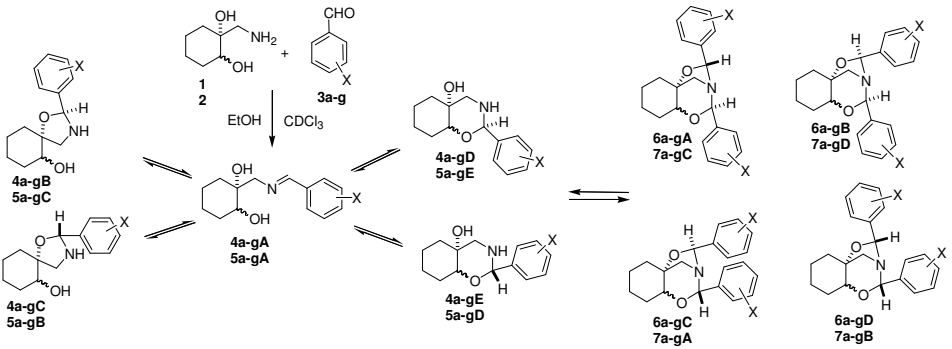
B. Methods

In order to achieve the above goals, mainly NMR spectroscopy was utilized, together with some other complementary methods, including IR, CD, DLS and TEM, besides molecular modelling.

C. Results

1. The reactions of *cis*- or *trans*-1-aminomethylcyclohexane-1,2-diols (**1** or **2**) with one equivalent of aromatic aldehydes **3a–g**, at 300 K in $CDCl_3$ resulted in a complex multicomponent equilibrium mixture of **4a–g** and **6a–g** (or **5a–g** and **7a–g**), (Scheme 1) in each case consisting of a five-component ring–chain tautomeric system **4A–E** (or **5A–E**), involving the Schiff base, two epimeric spiro-oxazolidines, two epimeric condensed 1,3-oxazines, and some of the four tricyclic compounds **6A–D** (or **7A–D**). In the complex multicomponent equilibrium mixture, the five-component, ring–chain tautomeric system **4A–E** (or **5A–E**) was found to be adequately described by the Hammett–Brown linear free energy equation.

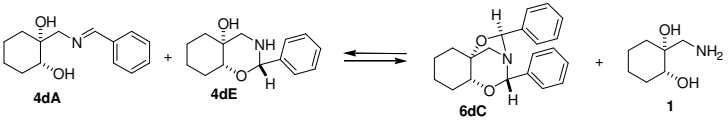
Scheme 1



a, *p*-NO_2; **b**, *m*-Cl; **c**, *p*-Cl; **d**, H; **e**, *p*-Me; **f**, *p*-OMe; **g**, *p*-NMe_2

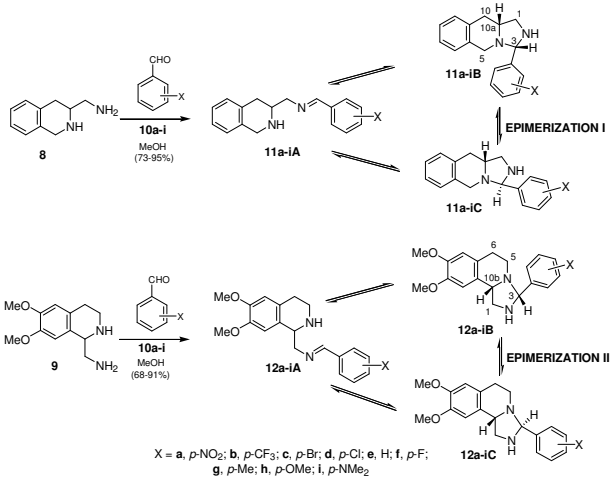
2. During a search for the possible reaction pathways of the formation of the tricyclic compounds **6A**–**D** (or **7A**–**D**) we found that they proceed via the reactions between the condensed 1,3-oxazines **4E** (or **5E**) and the Schiff base **4A** (or **5A**) by aldehyde transfer and aminodiol elimination (Scheme 2).

Scheme 2



3. An extended view of the stereoelectronic interactions affords a detailed description of the substituent-dependent stability changes exhibited by the 2aryl-1,3-*N*,*N*-heterocycles **11** (or **12**) in the corresponding ring–ring epimerization (Scheme 3). The measured reaction free energies of the epimerization reactions of conformationally inflexible 2-aryl-1,3-*N*,*N*-heterocycles **11B**, **11C** (or **12B**, **12C**) were found to correlate well with the sum of the hyperconjugative stabilization energies of all the vicinal donor-acceptor orbital overlaps around C2, obtained from *ab initio* NBO analysis, and both quantities correlated linearly with the Hammett–Brown substituent constant.

Scheme 3



4. The individual stereoelectronic interactions (n_N–$\sigma^*_{C2–N}$, n_N–$\sigma^*_{C2–Ar}$ and n_N–$\sigma^*_{C2–H}$) in **11B** ⇌ **11C** (or **12B** ⇌ **12C**) were also observed to exhibit a substituent-dependence,

despite their distance from the 2-aryl substituent and the non-periplanar arrangement of the localized molecular orbitals. The higher the electron-withdrawing effect of the 2-aryl substituent, the larger was the stabilization for n_N–$\sigma^*_{C2–Ar}$, while the overlaps n_N–$\sigma^*_{C2–N}$ and n_N–$\sigma^*_{C2–H}$ changed in the opposite sense (Figure 1). The different polarization of the acceptor σ^* orbitals caused by the 2-aryl substituent accounted for the observed propagation of the substituent effect. These results promote a detailed explanation of the useful tautomeric behaviour of the 2-aryl-1,3-*X,N*-heterocycles, and reveal the nature of the connection between the anomeric effect and the Hammett-type linear free energy relationship.

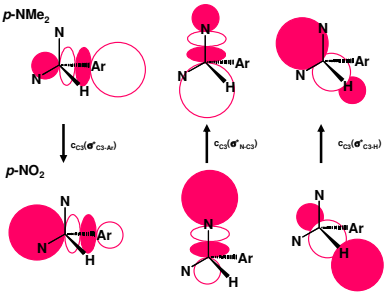


Figure 1

5. The conformationally constrained backbone of the homo-oligomers of *trans*-2-aminocyclohexanecarboxylic acid is flexible enough to afford both 10- and 14-helical motifs; in turn, this observation provides evidence of the true folding process of the *β*-petides. Homo-oligomers without protecting groups constructed by using *trans*-2-aminocyclohexanecarboxylic acid monomers **21**-**24** showed that the tetramer **22** tends to adopt a 10-helical motif, while both *ab initio* theory and NMR measurements point to the pentamer **23** and hexamer **24** forming the known 14-helix (Figure 2).

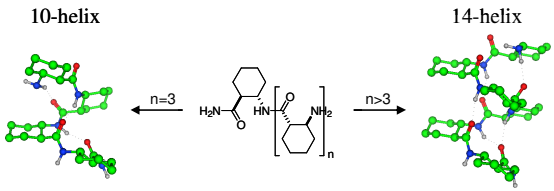


Figure 2

6. Such a conformational polymorphism is an important feature of any folded system that is designed to have a complex dynamic function in general; it is also observed for the natural α-peptides: the interplay between the α-helix and the 3_{10}-helix motif can be a crucial factor during the folding process. The revealed similar instrinsic properties of β-peptides suggest that the 10-helix may be a potential conformational intermediate in the folding process towards the thermodynamically stable 14-helix.

7. For the first time, direct evidence is presented on the tertiary structure of β-peptide foldamers, which proves that natural biopolymers are not unique in their highly structured conformational behaviour.

8. Stereochemically controlled secondary structure units of β-peptide strands and helices intrinsically self-assemble into sandwiches of pleated sheets and helix bundles, respectively (Figure 3). By residue control, the self-organization can be directed toward nano-sized fibrils or multilamellar vesicles. The results can have implications for new nanostructured materials and for a deeper understanding of protein folding and misfolding processes.

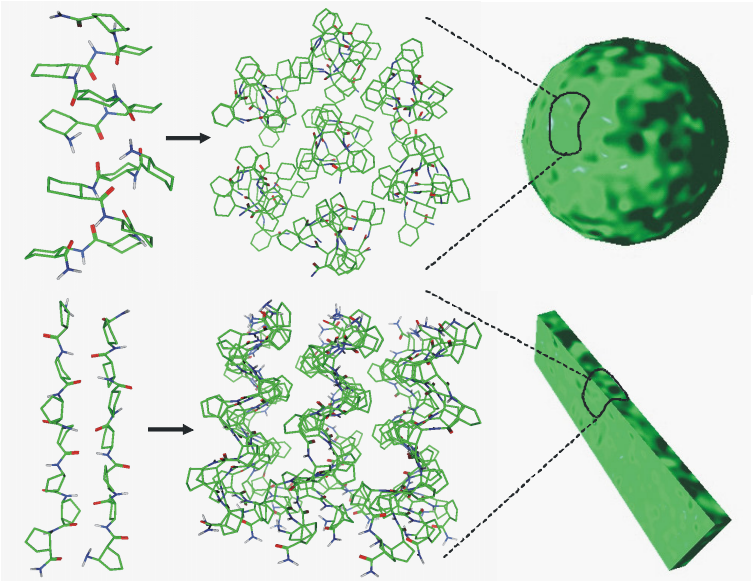


Figure 3

D. Publications

Related to the thesis

I. **Anasztázia Hetényi**, Zsolt Szakonyi, Karel D. Klika, Kalevi Pihlaja, Ferenc Fülöp:
Formation and characterisation of a multicomponent equilibrium system derived from *cis*- and *trans*-1-aminomethylcyclohexane-1,2-diol.
J. Org. Chem. **2003**, *68*, 2175-2182. i.f.: 3.462

II. **Anasztázia Hetényi**, Tamás A. Martinek, László Lázár, Zita Zalán, Ferenc Fülöp:
Substituent-dependent negative hyperconjugation in 2-aryl-1,3-*N,N*-heterocycles. Fine-tuned anomeric effect?
J. Org. Chem. **2003**, *68*, 5705-5712. i.f.: 3.462

III. **Anasztázia Hetényi**, István M. Mándity, Tamás A. Martinek, Gábor K. Tóth, Ferenc Fülöp:
Chain-length-dependent helical motifs and self-association of β-peptides with constrained side chains.
J. Am. Chem. Soc. **2005**, *127*, 547-553. i.f.: 6.903

IV. Tamás A. Martinek, **Anasztázia Hetényi**, Lívia Fülöp, István M. Mándity, Gábor K. Tóth, Imre Dékány, Ferenc Fülöp:
Biomimicking tertiary structures of β-peptides form nano-sized fibrils and membranes.
Angew. Chem. Int. Ed. submitted

Other papers

V. Zsolt Szakonyi, Tamás Martinek, **Anasztázia Hetényi**, Ferenc Fülöp:
Synthesis and transformations of enantiomeric 1,2-disubstituted monoterpene derivatives.
Tetrahedron: Asymmetry **2000**, *11*, 4571-4579. i.f.: 2.386

VI. Márta Palkó, **Anasztázia Hetényi**, Ferenc Fülöp:
Synthesis and stereochemistry of indano[1,2-*d*][1,3]oxazines and thiazines, new ring systems.
J. Heterocyclic Chem. **2004**, *41*, 69-75. i.f.: 0.814

VII. Ferenc Csende, **Anasztázia Hetényi**, Géza Stájer, Ferenc Fülöp:
Synthesis and structure of cycloalkane- and norbornane-condensed 6-aryl-1,2,4,5-tetrahydropyridazinones.
J. Heterocyclic Chem. **2004**, *41*, 259-261. i.f.: 0.814

VIII. Ferenc Miklós, **Anasztázia Hetényi**, Pál Sohár, Géza Stájer:
Preparation and structure of di-*exo*-condensed norbornane heterocycles.
Monatsh. Chem. **2004**, *135*, 839-847. i.f.: 0.904

IX. István Szatmári, **Anasztázia Hetényi**, László Lázár, Ferenc Fülöp:
Transformation reactions of the Betti base analog aminonaphthols.
J. Heterocyclic Chem. **2004**, *41*, 367-373. i.f.: 0.814

X. Zita Zalán, **Anasztázia Hetényi**, László Lázár, Ferenc Fülöp:
Substituent effects in the ring-chain tautomerism of 4-aryl-1,3,4,6,7,11b-hexahydro-2H-pyrimido[6,1-*a*]isoquinolines.
Tetrahedron **2005**, *61*, 5287-5295. i.f.: 2.643

XI. Zsolt Szakonyi, Szilvia Gyónfalvi, Enikő Forró, **Anasztázia Hetényi**, Norbert De Kimpe, Ferenc Fülöp:
Synthesis of 3- and 4-hydroxy-2-aminocyclohexanecarboxylic acids by iodocyclization.
Eur. J. Org. Chem. **2005**, in press. i.f.: 2.426

E. Conference lectures related to the thesis

XII. **Anasztázia Hetényi**, Karel D. Klika, Kalevi Pihlaja, Zsolt Szakonyi, Ferenc Fülöp:
A study of the conformation and ring-chain tautomerism of aminodiol derivatives by NMR spectroscopy.
2001 Nordic NMR Symposium, 23rd Finnish NMR Symposium
Helsinki, Finland, 26-29 August, 2001, Abstr.: 33 old.

XIII. Zsolt Szakonyi, **Anasztázia Hetényi**, Ferenc Fülöp, Karel D. Klika, Kalevi Pihlaja:
Stereoselective synthesis and ring enclosure of carbocyclic aminodiols.
85th CSC Conference & Exhibition
Vancouver, Canada, 1-5 June, 2002, Abstr.: 1136 OR PS.

XIV. Martinek A. Tamás, **Hetényi Anasztázia**, Zalán Zita, Lázár László, Fülöp Ferenc:
Sztereoelektronikus stabilizáció detektálása nem periplanáris geometriák esetén. Finomhangolható anomer-effektus?
Elméleti Szerveskémiai Munkabizottsági ülés
Budapest, 2003. január 30.

XV. **Hetényi Anasztázia**, Martinek Tamás, Lázár László, Zalán Zita, Fülöp Ferenc:
Sztereoelektronikus hatások szerepe a 2-arilszubsztituált 1,3-X,N-heterociklusos epimerek szubsztituensfüggő stabilitáskülönbségében.
Vegyészkonferencia 2003
Hajdúszoboszló, 2003. június 26-28., Abst.: P-39.

XVI. **Hetényi Anasztázia**:
Ciklusos β-aminosav oligomerek térszerkezetének változása a lánchosszal.
A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány és a SZAB Szerves és Gyógyszerkémiai Munkabizottság 4. tudományos előadásülése
Szeged, 2004. január 14.

XVII. **Hetényi Anasztázia**, Mándity M. István, Martinek A. Tamás, Tóth K. Gábor, Fülöp Ferenc:
Konformációsan gátolt β-peptidek lánchosszfüggő helikális szerkezete és önrendeződése.
MTA-Peptidkémiai Munkabizottság ülése
Balatonszemes, 2004. május 26-28.

XVIII. **Anasztázia Hetényi**, István Mándity, Tamás A. Martinek, Gábor K. Tóth, Ferenc Fülöp:
True folding of conformationally constrained β-peptides: chain length-dependent secondary structure.
3rd International and 28th European Peptide Symposium
Prague, Czech Republic, 5-10 September, 2004, Abstr.: P-590.

XIX. Martinek Tamás, **Hetényi Anasztázia**, Mándity István, Fülöp Lívia, Tóth Gábor, Fülöp Ferenc:
Béta-peptidek kiralitással szabályozott harmadlagos szerkezetei.
MTA-Peptidkémiai Munkabizottság ülése
Balatonszemes, 2005. május 30-június 1.

XX. Martinek Tamás, Mándity M. István, **Hetényi Anasztázia**, Tóth K. Gábor, Forró Enikő, Fülöp Ferenc:
További lépések a β-peptidek harmadlagos szerkezete felé.
Vegyészkonferencia 2005
Hajdúszoboszló, 2005. június 28-30., Abstr.: P-62.