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SUMMARY OF SCIENTIFIC ACCOMPLISHMENTS (ATTACHED TO HABILITATION PROCEDURE APPLICATION FORM)

"Helical oligourea foldamers as structural and functional mimetics of peptides and proteins"

Warsaw, 28.01.2019

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1. Name: **Karolina Pułka-Ziach (neé Pułka)**

2. Education – diplomas, academic titles and degrees:

2007 – PhD in chemistry, with honors, Faculty of Chemistry, University of Warsaw, doctoral dissertation titled "*Synthesis and application of cyclic derivatives of tryptophan for the preparation of analogs of biologically active peptides*", written under the supervision of Prof. Aleksandra Misicka-Kęsik.

2002 - MSc in chemistry, with honors, the College of Inter-faculty Individual Studies in Mathematics and Natural Sciences at the University of Warsaw, master's thesis titled "Research on the aggregation of fragment 109-126 of prion protein", written under the supervision of Prof. Aleksandra Misicka-Kęsik.

3. Employment at academic institutions:

09.2007 to present – adjunct (polish *adiunkt*), Faculty of Chemistry, University of Warsaw (including: May 2011 to September 2013 - unpaid leave related to a postdoctoral internship; November 2014 to July 2016 – sick leave and maternity leave)

04.2007 to 09.2007 - independent scientific and technical staff member, Faculty of Chemistry, University of Warsaw

4. Indication of "scientific achievement" as defined in Art. 16 paragraph 2 of the Act of 14 March 2003 on academic degrees and titles and on titles and degrees in art (Dz. U. 2016 no. 882 as amended, Dz. U. 2016 no. 1311):

a) title of the scientific achievement:
"Helical oligourea foldamers as structural and functional mimetics of peptides and proteins"

b) list of publications constituting the scientific achievement:

[H1] C. Douat-Casassus, **K. Pulka**, P. Claudon, G. Guichard "Microwave-enhanced solid-phase synthesis of *N,N'*-linked aliphatic oligoureas and related hybrids."
***Organic Letters,* 2012**, 14, 3130-3133. IF = 6.142

[H2] G. W. Collie, **K. Pulka-Ziach**, C. M. Lombardo, J. Fremaux, F. Rosu, M. Decossas, L. Mauran, O. Lambert, V. Gabelica, C. D. Mackereth, G. Guichard "Shaping quaternary assemblies of watersoluble nonpeptide helical foldamers by sequence manipulation."
***Nature Chemistry,* 2015**, 7, 871-878. IF = 27.893

[H3] G. Collie, **K. Pulka-Ziach**, G. Guichard "Surfactant-facilitated crystallisation of water soluble foldamers."
***Chemical Science,* 2016**, 7, 3377-3383. IF = 8.668

[H4] G. Collie, **K. Pulka-Ziach**, G. Guichard "In situ iodination and Xray crystal structure of a foldamer helix bundle."
***Chemical Communications,* 2016**, 52, 1202-1205. IF = 6.319

[H5] G. W. Collie, R. Bailly, **K. Pulka-Ziach**, C. M. Lombardo, L. Mauran, N. Taib-Maamar, J. Dessolin, C. D. Mackereth, G. Guichard "Molecular recognition within the cavity of a foldamer helix bundle: encapsulation of primary alcohols in aqueous conditions."
***Journal of the American Chemical Society,* 2017**, 139, 6128−6137. IF = 13.858

[H6] K. Pulka-Ziach, S. Sęk "α-Helicomimetic foldamers as electron transfer mediators."
Nanoscale, **2017**, **9**, 14913-14920. IF = 7.367

[H7] K.Pulka-Ziach, S. Antunes, C. Perdriau, B. Kauffmann, M. Pasco, C. Douat, G. Guichard "Postelongation strategy for the introduction of guanidinium units in the main chain of helical oligourea foldamers"
***Journal of Organic Chemistry,* 2018**, 83, 2530-2541. IF = 4.849

[H8] K. Pulka-Ziach "Influence of reaction conditions on the oxidation of thiol groups in model peptidomimetic oligoureas."
Journal of Peptide Science, **2018**, 24, e3096. DOI: 10.1002/psc.3096 IF = 1.972

[H9] K. Pulka-Ziach, A. K. Puszko, J. Juhaniewicz-Debinska, S. Sek „Electron transport and a rectifying effect of oligourea foldamer films entrapped within nanoscale junctions"
The Journal of Physical Chemistry C, **2019**, 123, 1136-1141. IF = 4.484

Total IF (according to the year of publication) = 81.552
Average IF per publication: 9.061

c) description of the scientific objective of the listed publications and their potential use

The process of folding of natural oligomers (proteins, nucleic acids) plays an extremely important role in nature, because the spatial structure of biomolecules determines their functions, and any abnormalities may lead to the loss of biological activity or serious dysfunction. Globally, the structure of biomolecules comprises several levels and the primary structure, i.e. the sequence of individual residues (amino acids or nucleotides), determines the secondary structure, from which tertiary and quaternary structures further assemble, as the next level of organization. Therefore, a thorough understanding of the factors affecting the process of folding and self-assembly is a fundamental issue that straddles the divide between chemistry and biology. The study of foldamers forms part of this crucial research field. The term "foldamer" was introduced into the scientific literature 20 years ago by S. H. Gellman[1] to systematize the terminology regarding non-natural, synthetic oligomers inspired by nature. The group of foldamers includes oligomers that adopt well-defined, stable and predictable secondary structures, the most commonly used of which is the helix.[2,5] The main chain of foldamers can be formed by β- and γ-peptides, aromatic polyamides, aza-aromatic oligomers, oligoureas and other building blocks.[4]

Oligoureas with the general formula $[\text{-CH(R)-CH}_2\text{-NH-CO-NH}]_n$ (**Figure 1a**) are a class of peptidomimetics, because it is possible to introduce the side chains of all natural amino acids. However, what distinguishes oligourea foldamers from peptides is the stability against proteolytic enzymes, as well as the ability to adopt a stable, secondary structure.

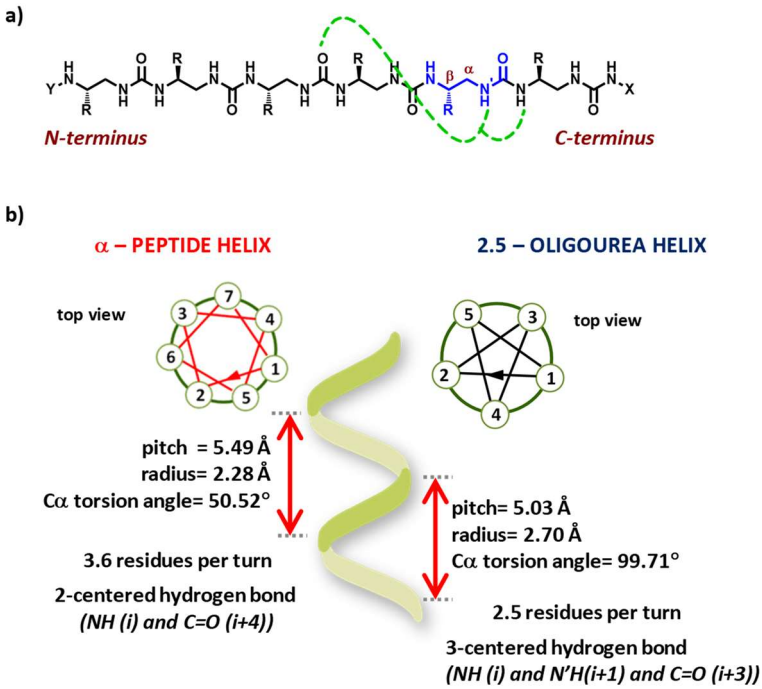


Figure 1. Oligourea foldamer: a) general pattern of the oligourea oligomer (the urea residue is marked in blue, while the green color shows the hydrogen bonds stabilizing the helix); b) comparison of the basic parameters of α- and 2.5-helices.

The oligoureas fold into 2.5-helices , which means that one helix turn is formed with 2.5 residues.[6] This helix is stabilized by 3-centered hydrogen bonds, closing the 12- and 14-membered pseudo-ring between N***H***(*i*) and N'***H***(*i+1*) and C=***O*** (*i+3*). The presence of an additional hydrogen bond (by comparison to peptides), stabilizing the helix, makes even short oligomers (from four urea residues)

fold into 2.5-helix, stable in solid state and in solution.[7] In addition, the folding capability is a feature of the main chain and does not depend on the type of side chains of urea residues, which distinguishes oligoureas from peptides, where the side chains of individual amino acid residues strongly determine the secondary structure adopted by peptides. The basic parameters of α- and 2.5-helix are similar, which makes oligourea foldamers excellent structural mimetics of peptides (**Figure 1b**).

Investigating the synthesis and application of oligourea foldamers and their derivatives is the main scientific objective of my habilitation project. I was interested in optimizing the synthesis of new building blocks that can be successfully used in the synthesis of urea oligomers on solid support under the microwave irradiation. In addition, I also studied the synthesis of oligourea derivatives in which one or several urea groups were replaced by γ-amide bonds or guanidinium groups. Another goal was to use oligoureas to study phenomena and physicochemical processes previously reserved for peptides and their derivatives. Among that processes are: self-assembly into higher-order spatial structures, self-assembly to form monolayers on the surface, study of the electron transport process.

Synthesis of oligourea foldamers and their derivatives

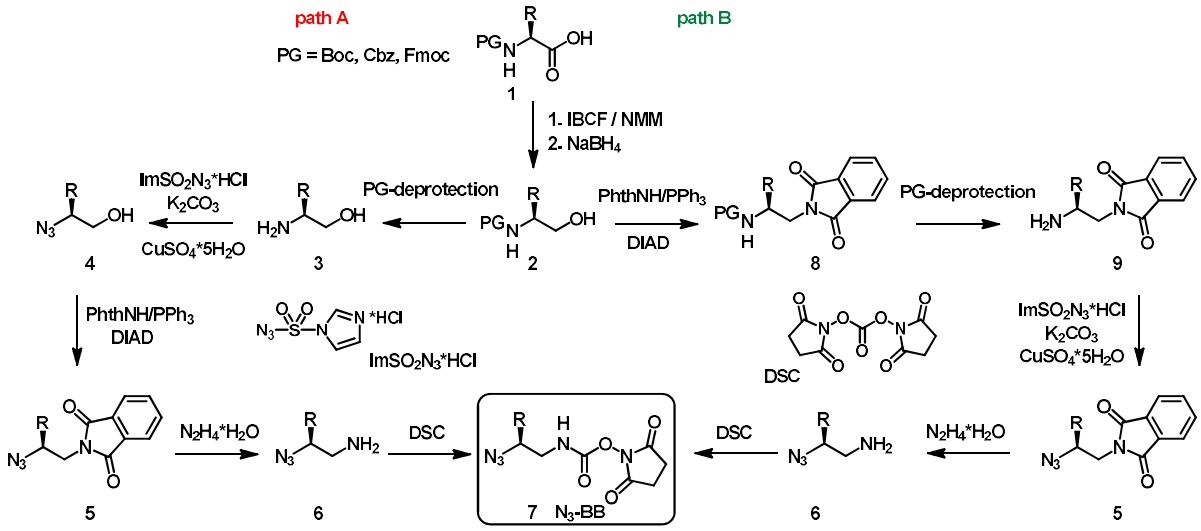
The synthesis of *N,N'*-disubstituted aliphatic oligoureas uses activated building blocks based on a monoblocked ethylenediamine derivatives. The oligomer synthesis may be carried out in solution or on a solid support (SPoUS-Solid Phase oligoUrea Synthesis). Intensive research has been underway for a number of years to optimize the synthesis on a solid support, because it allows the time of obtaining long oligoureas to be significantly reduced, and often also leads to final compounds being obtained with higher yield than in solution.

In the literature, there are several known ways of introducing urea bonds, by using activated building blocks, such as isocyanate derivatives[8], *p*-nitrophenyl carbamates[9-11] or succinimidyl carbamates.[6,12]

Research focused on obtaining active building blocks, and using them subsequently for the synthesis on a solid support under microwave irradiation (what significantly reduces the time of obtaining desired compounds), is described in the article **H1.** As building blocks for the introduction of urea units, I decided to use ethylenediamine derivatives in which one amine group was masked as an azide group $-N_3$, while the other amino group was transformed into the active succinimidyl carbamate. I used two synthetic paths, **A** and **B** – shown in **Scheme 1**. These routes differ in the sequence of individual steps. In the case of both synthetic routes, the starting compounds were α-amino acids with a suitably protected α-amino group. I used derivatives with Boc and Cbz protecting groups; in the article **H2** also Fmoc.

The first step in both cases was to reduce the carboxyl group to the hydroxyl group in a two-stage reaction: in the first stage, in the presence of isobutyl chloroformate (IBCF), a mixed anhydride was formed, which was next reduced with $NaBH_4$ to the desired amino alcohol **2**. The obtained amino alcohol **2** was then subjected to subsequent reactions. Having obtained a building block via path **A**, first I deprotected the amine group (applying TFA, when the Boc protecting group was used, or in the hydrogenation reaction when the Cbz group was used), and then I subjected it to the so-called *diazotransfer reaction*, resulting in the formation of azido alcohol **4**. For this reaction I used $ImSO_2N_3$*HCl,[13] reagent, which is an imidazole derivative. This compound is crystalline and has a much higher stability than commonly used trifluoromethanesulfonic azide. The next step is the Mitsunobu reaction using phthalimide as a nucleophile. As a result of this reaction, the hydroxyl group in compound **4** was substituted with an amino group protected with a phthaloyl group and compound **5** was formed. The next two steps were the removing of the phthaloyl protecting group with N_2H_4*H_2O

and the reaction with disuccinimidyl carbonate (DSC), which results in obtaining the desired building block **7** (**N_3-BB**).



Scheme 1. The synthesis of carbamate building blocks with -N_3 group

Publication **H1** describes the synthesis of several building blocks. Phe, Tyr and Orn derivatives were obtained via path **A** with good overall yields (>30% after 6 steps). However, when I tried to synthetize the Leu building block **7** in the same way, I obtained a very low overall yield (<10%). This was due to the high volatility of the two intermediate compounds, namely azidoalcohol **4** and azidoamine **6**. Therefore I changed the sequence of steps (path **B**) and so I first carried out the Mitsunobu reaction, obtaining compound **8**, which subsequently underwent deprotection and reaction with $ImSO_2N_3$*HCl, resulting in compound **5**. The next two steps were carried out in the same manner as via path **A**, with one exception, after work-up the reaction mixture consisting of azido-amine **6**, the solvent was not fully evaporated to avoid loss of compound **6**. This allowed Ala and Leu, as well as Trp derivative building blocks to be obtained, with good overall yields (27-43% after 6 steps).

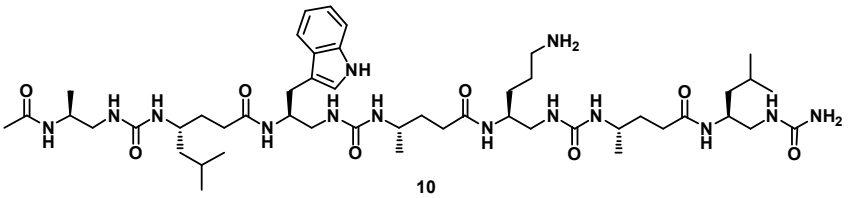


Figure 2. Urea-γ- amide hybrid obtained on solid support under microwave irradiation

The next stage of work was to optimize the conditions for the microwave assisted synthesis of oligomers on solid support. For the reduction of the -N_3 group to the -NH_2 group we chose the Staudinger reaction with PMe_3 in aqueous media. The use of water as a reagent (and solvent) narrowed the choice of resin to a hydrophilic polymer, with good swelling properties in a wide range of solvents, including water. NovaPEG Rink amide resin was used, from which oligomers are cleaved with trifluoroacetic acid.

The conditions for the synthesis of oligourea foldamers and their derivatives were optimized taking into account the synthesis of oligomers whose main chain is made only of urea units as well as urea-γ-amide hybrids, in which every second urea unit was replaced with the isostructural γ-amino acid residue (**Figure 2**). We achieved the best yields and purity of the planned oligomers using the following reaction conditions:

a) *coupling of carbamate building blocks with N_3 (N_3-BB) group*: 1.5 eq. N_3-BB, 2.5 eq. DIPEA, DMF, 70°C, 25W, 2 x 15 min.
b) *reduction of N_3 group to NH_2 group*: 10 eq. PMe_3 in THF (1M), 7:3 1,4-dioxane : water, 70°C, 25W, 2 x 30 min.
c) *coupling of Fmoc-γ-amino acids:* 1.5 eq. Fmoc-γ-AA, 1.5 eq. HBTU, 1.5 eq. HOBt, 5 eq. DIPEA, DMF, 50°C, 50W, 2 x 12 min.
d) *removal of Fmoc protecting group*: 20% piperidine solution in DMF, 50°C, 50W, 1 x 4 min., 1 x 8 min.

Moreover, to show the efficiency of the synthetic method using MW irradiation, I synthetized a urea-γ-amide hybrid **10** using carbamate building blocks with Fmoc protecting group (Fmoc-BB) at room temperature, without microwave assistance. The synthesis lasted much longer, and the purity of the obtained compound was more than 3 times smaller than using N_3-BB and microwave irradiation. Except for the desired compound, shorter oligomers were also formed, in which one or two urea units were missing (**Figure 3**).

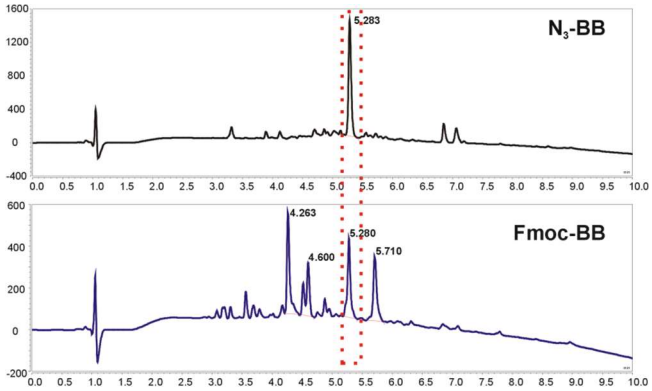


Figure 3. Comparison of the efficiency of the synthesis of the urea-γ-amide hybrid **10** (λ=200 nm) using azide building blocks (N_3-BB, black chromatogram) with Fmoc-protected building blocks (blue chromatogram). The peak from the appropriate compound is marked.

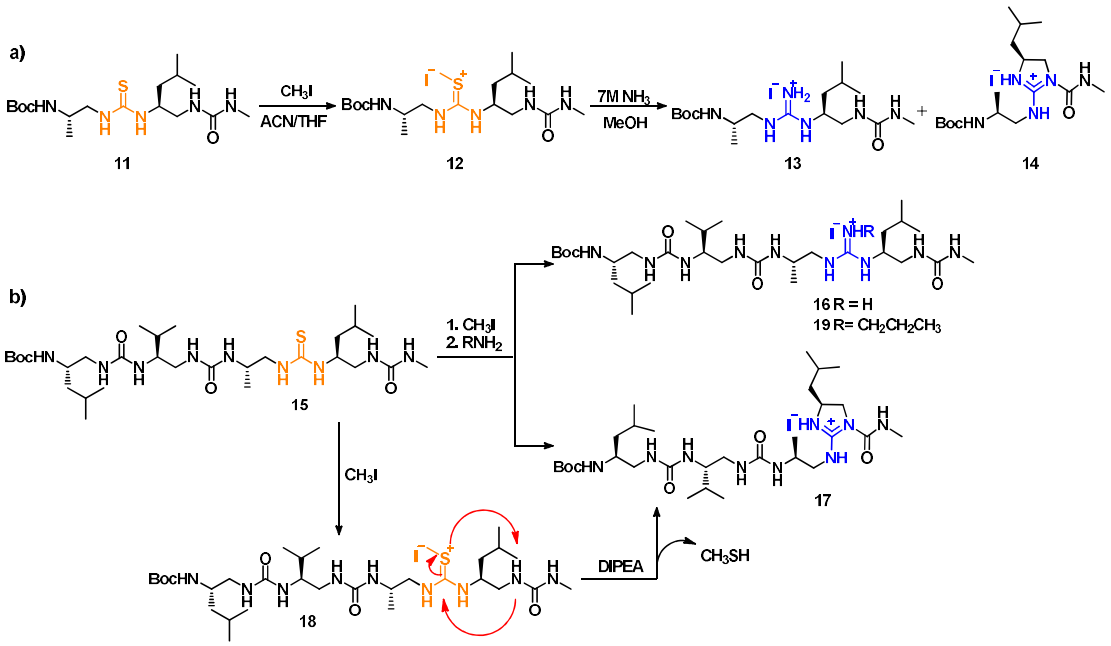
The method of the synthesis of carbamate building blocks and oligourea foldamers and their derivatives on solid support under microwave irradiation, which I devised and described in **H1**, was used to obtain building blocks (I obtained 18 building blocks in total) and oligomers, described in publications **H2-H5** and in other projects in which I have participated and which I currently lead.

As I mentioned above, oligourea foldamers can be treated as peptidomimetics. A strategy frequently used in the search for new peptidomimetics involves substitutions within a peptide bond. A similar approach was used for oligoureas, where individual urea groups were replaced by γ-amide bonds,[14,15] carbamate bonds **Błąd! Nie zdefiniowano zakładki.**,[16] or thio- and selenium urea bonds.[17] Next, for these new hybrids, the effect of substitutions on the secondary structure was determined. Analysis of the literature showed that there are no reports on hybrid derivatives in which one of urea groups is replaced by a guanidinium moiety. Guanidine derivatives exhibit strongly basic properties and are protonated over a wide range of pH. Therefore, it was interesting to check what impact the introduction of the guanidinium residue would have on the helicity of the oligourea chain, because the donor-acceptor properties change in consequence of such substitution.

The preparation and conformational studies of oligourea mimetics with a guanidinium residue are the subject of publication **H7**. I performed the optimization of preparation of the new compounds using the solution methodology, as it offers more control over the course of the reaction. I decided to use

the method of obtaining guanidine derivatives known from the literature, which uses thioureas as starting compounds.[18-21]

I tested dimer **11** (**Scheme 2a**) under various reaction conditions known from the literature[18-21] and controlled the progress of the reaction by RP-HPLC method. Of all the conditions tested, the two-step method proved to be the most efficient. In the first step thiourea is S-methylated with CH_3I and the S-methylthiouronium iodide **12** thus obtained, undergoes nucleophilic substitution with the appropriate amine at elevated temperature (40-45°C). In my case the amine was NH_3 used as a 7M solution in CH_3OH (**Scheme 2a**). As a result of this reaction two products were obtained: the linear **13**, consistent with the assumption, and the cyclic **14** (N,N',N''- trisubstituted guanidinium derivative) resulting from nucleophilic intramolecular attack (**Table 1**). It is also worth noting that in each case guanidinium derivatives (both cyclic and linear) were obtained in the form of iodide salts.



Scheme 2. Scheme of a two-step synthesis of guanidinium derivatives: a) from dimer **11;** b) from tetramer **15**

Subsequently, I conducted a reaction on a longer oligomer, tetramer **15**, under the same conditions (**Scheme 2b**). As the main product I obtained the cyclic product **17** and – in a smaller quantity – the linear product **16** (**Table 1**).

Table 1. Results of guanidinylation reactions of compounds **11** and **15**.

substrate	nucleophile	solvent[a]	time [h]	product ratio linear:cyclic[b]
11	7M NH_3	CH_3OH	48	33:67 (**13:14**)
15	7M NH_3	CH_3OH	24	26:74 (**16:17**)
15	*n*-$PrNH_2$	CH_3OH	24	15:85 (**19:17**)
15	*n*-$PrNH_2$	CH_3CN	120	73:27 (**19:17**)
15	0.5M NH_3 in 1,4-dioxane	CH_3CN	120	85:15 (**16:17**)
15	0.5M NH_3 in 1,4-dioxane	THF	38	97:3 (**16:17**)

[a] reactions were carried out at a temperature of 40-45°C

[b] determined by RP-HPLC; product numbers are given in parentheses

I also demonstrated that the cyclic product is obtained from the S-methylthiuronium derivative **18** due to the basic reaction conditions. Compound **18**, dissolved in CH_3OH in the presence of DIPEA, was converted to product **17** in 24 hours (**Scheme 2b**). In addition, I tested the influence of the solvent on the course of the reaction. I used *n*-$PrNH_2$ ($CH_3(CH_2)_2NH_2$) as a nucleophile. It turned out that the solvent has a considerable influence on the reaction, and so in CH_3OH a cyclic product **17** was predominantly formed, whereas in CH_3CN the main product was the desired linear compound **19**, but the reaction was slower. The effect of the solvent was tested again when the nucleophile was NH_3 (used as 0.5M solution in 1,4-dioxane). It turned out that both in CH_3CN and in THF the linear product **19** was formed as the main product (**Table 1**). In the next stage of work I focused on selecting such substrate structure that only the desired linear product is obtained. The formation of a cyclic product was associated with the presence of a urea group preceding the S-methylthiouronium residue, so it seemed reasonable to replace this urea residue with γ- amino acid (compounds **20-22**, **Figure 4a**).

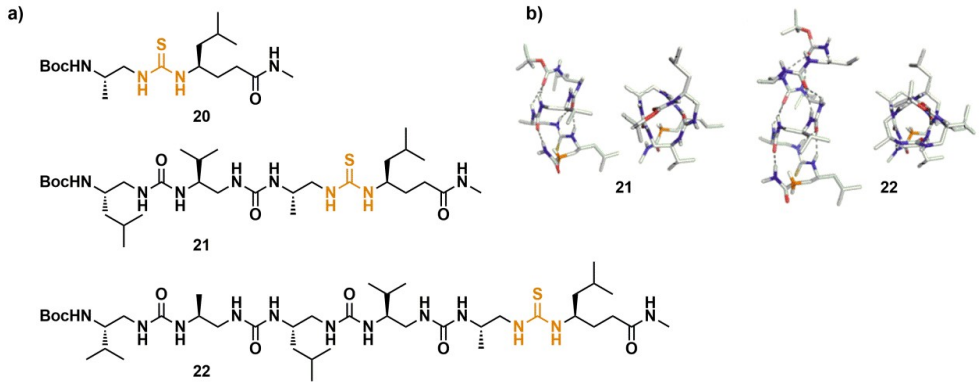
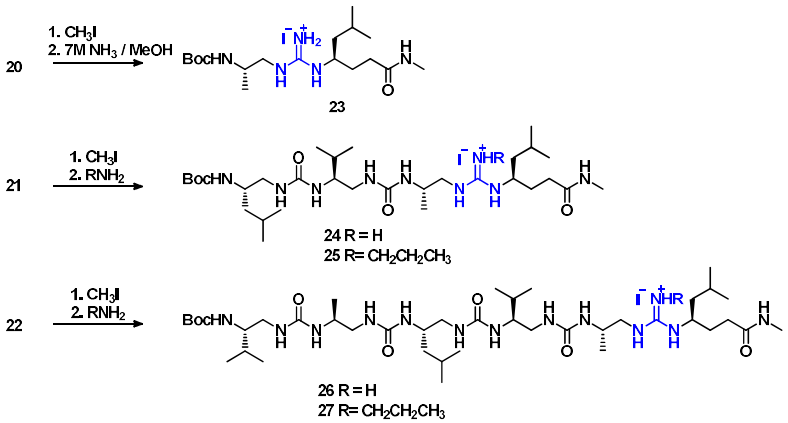


Figure 4. Thiourea derivatives with γ- amino acid residue: a) substrates for the guanidinylation reaction; b) crystal structures of compounds **21** and **22**

Both compound **21** and **22** adopted 2.5-helical structure in the solid state, as confirmed by X-Ray analysis (**Figure 4b**).



Scheme 3. The guanidinylation reaction of foldamers with γ-amino acid at C-terminus

Guanidinylation reaction of appropriate S-methylthiouronium derivatives with NH_3 as well as *n*-$PrNH_2$ led, with a good or very good yield, to the formation of guanidinium derivatives (**Scheme 3**) containing 2 (compound **23**), 4 (compounds **24** and **25**) or 6 (compounds **26** and **27**) residues in the main chain (**Table 2**).

Table 2. Results of guanidinylation reactions of compounds **20-22**

substrate	nucleohile	solvent[a]	time [h]	reaction yield[b] [%]
20	7M NH_3	CH_3OH	48	20 (**23**)
21	0.5M NH_3 in 1,4-dioxane	CH_3CN	90	55 (**24**)
21	0.5M NH_3 in 1,4-dioxane	THF	38	74 (**24**)
21	*n*-$PrNH_2$	CH_3CN	90	46 (**25**)
22	0.5M NH_3 in 1,4-dioxane	CH_3CN	24	92 (**26**)
22	*n*-$PrNH_2$	CH_3CN	60	60 (**27**)

[a] reactions were carried out at temperature of 40-45°C
[b] isolated yield; product numbers are given in parentheses

The obtained foldamers with one guanidinium residue were then tested for the effect of the guanidinium residue on the conformation of the oligomer. The position of the guanidinium residue in the main chain was purposefully chosen near the so-called C-terminus of the oligomer, to have the least destabilizing effect on the conformation of the entire foldamer.

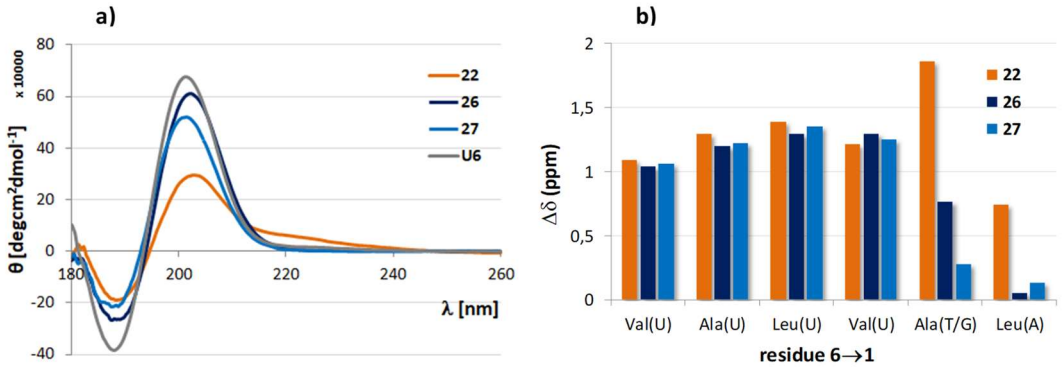


Figure 5. Conformational studies of hexamers containing a guanidinium group: a) CD spectra of thiourea **22** and corresponding guanidinium derivatives **26, 27** as compared to the spectrum of homooligourea (**U6**, gray); b) comparison of the differences in chemical shifts (Δδ) of protons of α-CH_2 groups

A comparison of the circular dichroism spectra (CD) of urea-guanidinium hybrids (oligomers **26** and **27**) with the spectrum of homooligurea (**U6**) shows that these hybrids tend to fold into a helix (**Figure 5a**). Analysis of NMR spectra, in particular the comparison of chemical shifts of protons of urea groups, as well as the differences in chemical shifts of diastereotopic protons of α-CH_2 (**Figure 5b**) and the proton/deuterium exchange rate (H/D) of the protons of urea groups allows to conclude on the stability of the secondary structure. Even though the first four residues (counting from the N-terminus) assume to adopt a stable helical conformation, the last two residues (including guanidinium) are characterized by much lower conformational stability.
The results presented in article **H7** (optimization of synthesis and proving the conformational stability of guanidinium oligourea derivatives) allowed me to design new compounds that are the subject of my Sonata Bis project.

Foldamers as structural mimetics of peptides and proteins

The tertiary and quaternary structure of proteins determines their function, and any abnormalities may lead to the loss of their biological activity or serious dysfunction. One of the frequently occurring

motifs of higher-order protein structures are the so-called coiled coils,[22] also referred to as helix bundles. They are composed of two to four (but also more) amphiphilic α-helices and are clearly distinguished in protein molecules.[23] The interfaces between helices are predominantly composed of hydrophobic amino acid residues (usually Leu, Ile, Val). The hydrophobic amino acid residues usually occupy positions ***a*** and ***d*** in a 7-amino acid repeating motif, referred to as *heptad repeat abcdefg* (**Figure 6a**).[24] Positions ***e*** and ***g*** are occupied by charged amino acids. The combination of hydrophobic and ionic interactions determines the orientation of helices in relation to one another, and thus the topology of entire structures (**Figure 6a**). As these structural elements are found in natural proteins, they therefore refer to water-soluble compounds. Therefore, for several years, intensive research on water soluble foldamers, capable of creating higher-order structures (tertiary and quaternary) has been carried out.[25-30]

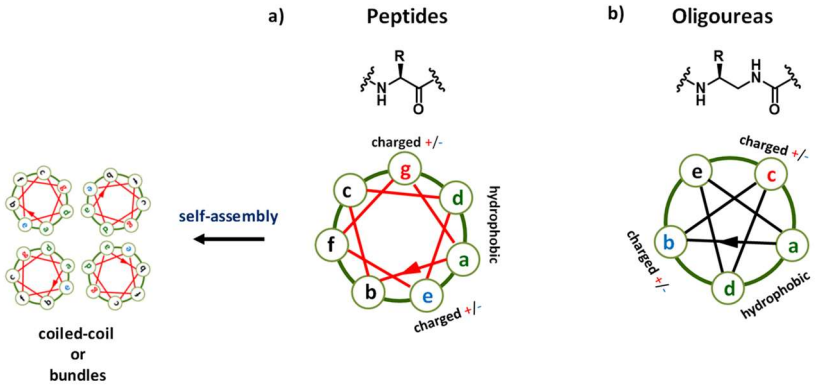
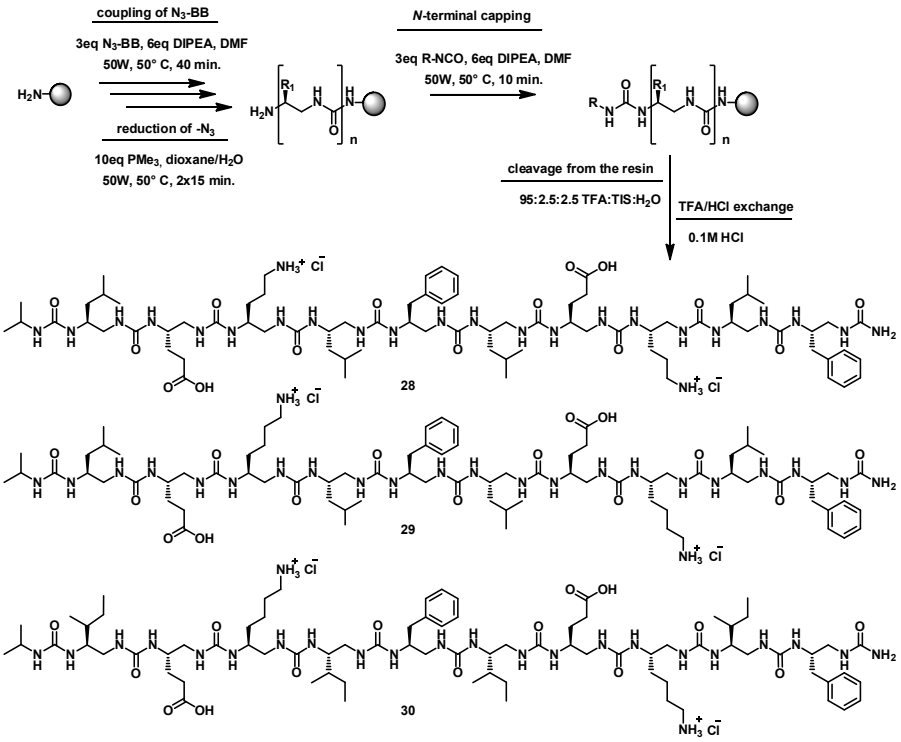


Figure 6. Schematic representation of helices: a) peptide helix, showing the so-called *heptad repeat* and principles of self-assemble in quaternary structures; b) oligourea helix

Peptide foldamers capable of forming bundles, such as β-peptides[25-27] and α/β-peptide hybrids,[28-30] are known in the literature; oligourea foldamers dovetail excellently with this trend of research. As I mentioned above, it is possible to introduce the side chains of all natural amino acids into the oligourea chain, which makes these compounds ideal candidates in the search for water-soluble sequences that are able to self-assemble into quaternary structures.

Based on the structural requirements described above for self-assembly observed in proteins, we proceeded to design the sequence of oligourea foldamers (**Figure 6b**), so that they contain hydrophobic side chains (Leu) and side chains of charged amino acids (Glu, Lys or Orn) at appropriate positions. We intended to conduct self-assembly studies in solid state (crystallization) and in solution. The first designed sequences contained *p*-bromophenyl or *p*-bromobenzyl residue at the N-terminus. Despite the presence of side chains containing carboxyl and amino groups, these compounds did not dissolve in the aqueous media. We obtained water solubility for compounds containing an isopropyl residue at the N-terminus. The first designed water-soluble oligourea foldamers are the subject of publication **H3**. I synthesized all oligomers using the solid phase method (SPoUS) with the carbamate building blocks with -N_3 group (article **H1**). The solid phase synthesis was performed under the microwave irradiation, and the conditions of the individual steps were slightly modified in relation to those described in article **H1** (**Scheme 4**). This was caused by the presence of protected functional groups in the side chains of individual building blocks. The compounds **28-30** were cleaved from the resin with trifluoroacetic acid, and the purification was carried out by semi-preparative RP-HPLC. After lyophilization, the pure compounds were obtained as trifluoroacetates. A number of attempts were made to crystallize such derivatives, but none of them was successful. Therefore, we decided to exchange the counterion with a chloride anion (**Scheme 4**).



Scheme 4. Synthesis of water-soluble oligourea foldamers **28-30**

Several hundred crystallization conditions were tested (commercially available kits), but none of them yielded satisfactory results. Only the use of surfactants (detergents) such as CTAB (hexadecyltrimethylammonium bromide) and SDS (sodium dodecyl sulfate) as components of the crystallization mixture (0.5M NaCl, 10mM $MgCl_2$, 100mM HEPES buffer and 10mM CTAB or SDS) allowed to grow crystals of compounds **28-30** suitable for X-Ray analysis.

It turned out that in the crystal structure the individual helices form a superhelix (**Figure 7c**).

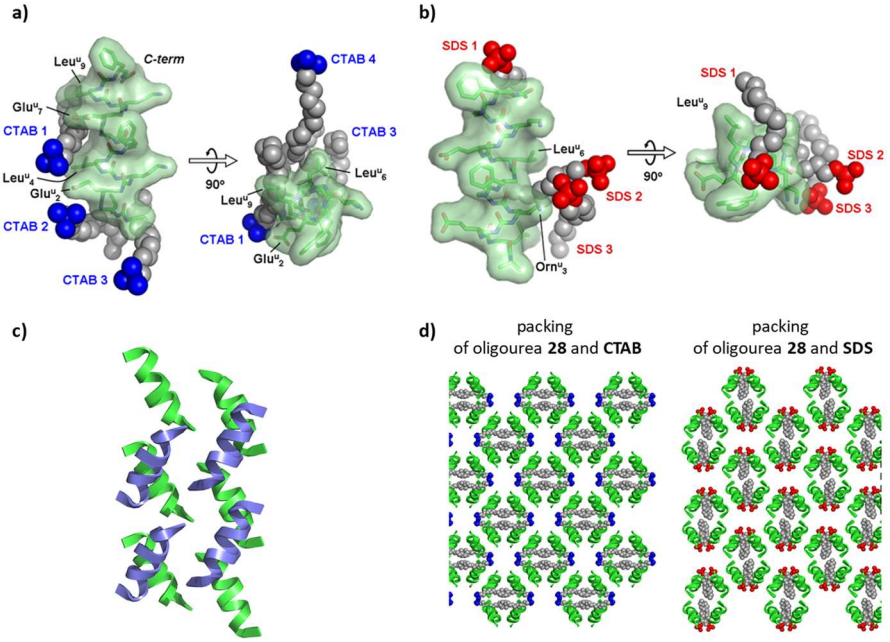


Figure 7. Crystal structures obtained for oligoureas in the presence of surfactants: a) oligourea **28** in the presence of CTAB; b) oligourea **28** in the presence SDS; c) coiled coil formed from individual helices (for clarity, detergent molecules are not shown); d) packing of oligoureas and detergents in the crystal lattice

In addition, it turned out that the surfactants, both positively and negatively charged, acted as binders, the "molecular glue" of foldamer molecules in the crystal lattice. In the case of CTAB, the positively charged trimethylammonium "head" was located near the carboxyl groups of the Glu^u residues (superscript *u* depicts the urea residue), while the aliphatic CTAB "tail" hydrophobically interacted with the side chains of the Leu^u residues (**Figure 7a,d**). In the case of SDS, the negatively charged sulfate group was located near the positively charged amino groups in the side chains of the oligourea molecule (**Figure 7b,d**). The comparison of the parameters of the helix obtained as a result of crystallization from the aqueous environment in the presence of surfactants, with the parameters of the helix obtained as a result of crystallization from organic solvents, shows that the conditions of crystallization have a negligible effect on the geometry of the helix.

In parallel, I strove to optimize the primary structure to obtain compounds capable of self-assembly into quaternary structures called bundles. My research on this subject is described in articles **H2, H4** and **H5**. The starting compound for introducing structural modifications was oligomer **29**. As I show at **Figure 8**, we decided to extend the main chain with an additional Leu^u residue unit leading to an increase in the number of hydrophobic interactions, while in the crystal structures described above such a role was played by a detergent hydrophobic chain. Further, we replaced one of the Phe^u to Tyr^u to improve solubility of the compound in water and the other one with Ala^u with a short side chain. The new oligomer **31** was obtained by solid phase synthesis, with N_3-BB strategy under the conditions given in **Scheme 4**, and the trifluoroacetate ion was substituted by chloride anion.

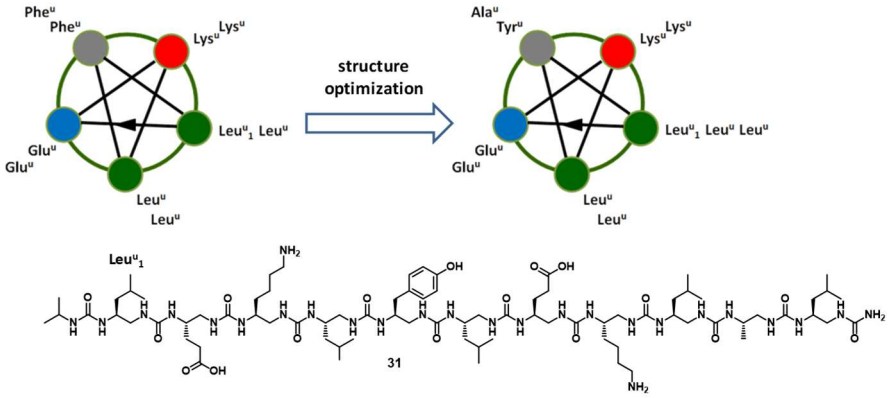


Figure 8. Optimization of the primary structure in order to achieve self-assembly to higher-order structures

It turned out that relatively small changes in the primary structure had considerable consequences in terms of self-assembly to higher-order structures, as described in **H2** . It is worth noting here that the addition of detergent was not required to crystallize compound **31** or the compounds described below. The crystallization conditions were as follows: 20% isopropanol, 200mM $CaCl_2$ and 100mM buffer CH_3COONa with pH 4.6. The crystal structure of foldamer **31** showed the formation of a capsule, a helix bundle composed of 6 independent oligourea helices, forming three dimers, each of which consists of helices arranged antiparallel to each other. This structure resembles a capsule with a hydrophobic core, formed by Leu^u side chains and charged side chains of Lys^u and Glu^u outside of the capsule (**Figure 9**). The measured interior volume of the capsule ("cavity" in **Figure 9**) is 495$Å^3$. In addition to the studies in the solid state, the formation of a well-organized quaternary structure was also confirmed in solution with a number of methods, such as native mass spectrometry (native ESI-MS), circular dichroism or high-field nuclear magnetic resonance spectroscopy (700 and 800MHz).

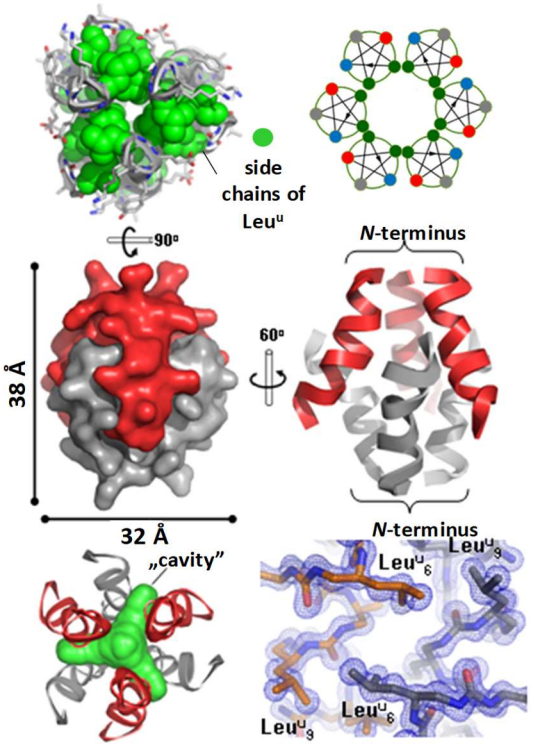


Figure 9. Self-assembly and crystal structure of oligourea **31**

Native ESI-MS shows that the peak corresponding to the hexameric structure of compound **31**, has the highest intensity among all peaks observed for multimer structures (**Figure 10a**). To confirm that the data obtained with ESI-MS result from the formation of a quarternary structure, not just cluster ions, compound **32** was obtained (negative control), in which the central Leu^u residue was substituted with Asn^u (**Figure 10c**).

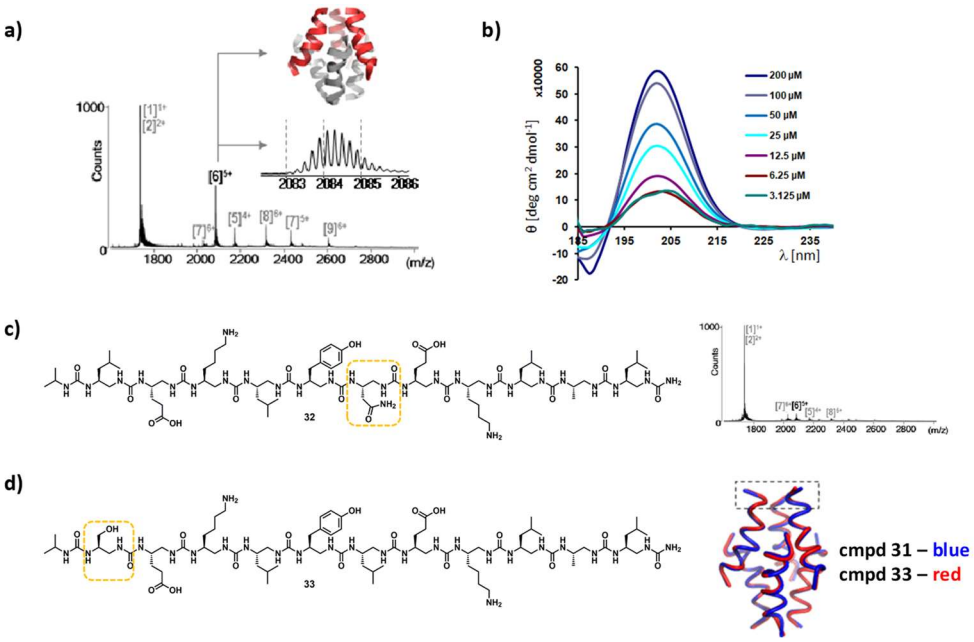


Figure 10. The research in the self-assembly processes in aqueous solution: a) spectrum of the native ESI-MS of foldamer **31**; b) CD measurements as a function of concentration for compound **31**; c) the sequence of foldamer **32** (with the modification site marked) and the spectrum of the native ESI-MS; d) the sequence of foldamer **33** (with the modification site selected) and the overlay of the crystal structure of compounds **31** and **33**

The assumption was that compound **32** should not assemble into a quaternary structure, due to the absence of a hydrophobic side chain of the central Leu^{u} unit necessary to stabilize the capsule. Indeed, with ESI-MS, almost no peaks could be observed except for the peak corresponding to a single helix (**Figure 10c**). In addition, no crystals of compound **32** were obtained, either.

CD experiments carried out for compound **31** also confirmed the formation of capsules. It is known from the literature that the higher the concentration of a compound that potentially can be organized into higher-order structures, the higher the molar ellipticity should be observed.[31] We were able to measure such dependence for compound **31** (**Figure 10b**). Another oligomer obtained was the derivative **33** (**Figure 10d**), in which Leu^{u} residue at the *N*-terminus was substituted by Ser^{u} residue. It turned out that this foldamer, like compound **31**, self-assembled into a capsule-like structure with a similar morphology; however, the stability of this structure, studied by CD, was slightly lower than for **31**.

In addition to modification of the hydrophobic fragment of the helix (compounds **32** and **33**) we also introduced modifications in the charged, hydrophilic fragment. Compound **34** was obtained and it differed from the compounds described above in terms of the distribution of charged and hydrophobic residues (**Figure 11**). It turned out that such modifications of the primary structure led to a completely different way of self-assembly.

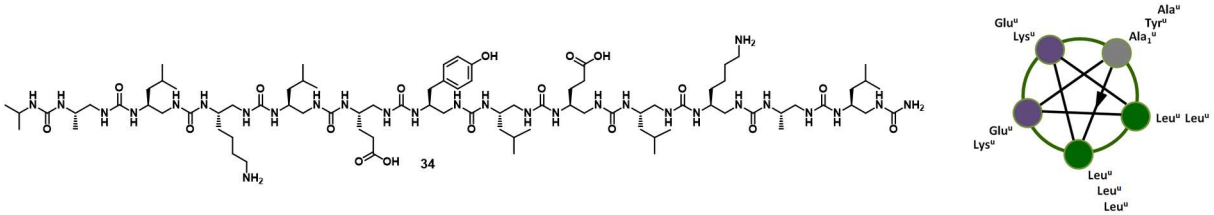


Figure 11. Sequence of compounds **34**

The crystal structure of **34** showed that superhelix channels (composed of 6 coiled coils) were formed, with charged residue units inside and hydrophobic residues on the outside. The interior of the channels was filled with water molecules.

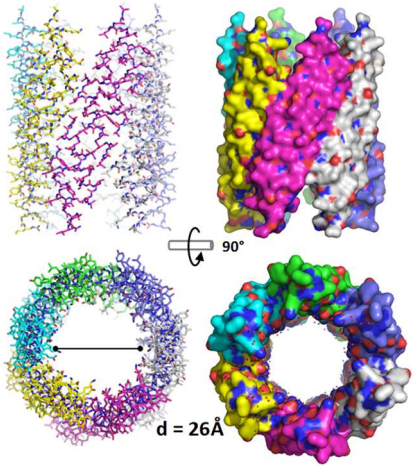


Figure 12. Superhelical channels formed by compound **34**

Thus, it can be seen that by properly choosing the primary structure, we can control the individual molecules' mode of self-assembly. In addition, minor changes in the sequence, substitution or shift of individual residues lead either to the loss of self-assembly capacity or to the formation of completely different higher-order structures (capsules or channels, **Figure 13**).

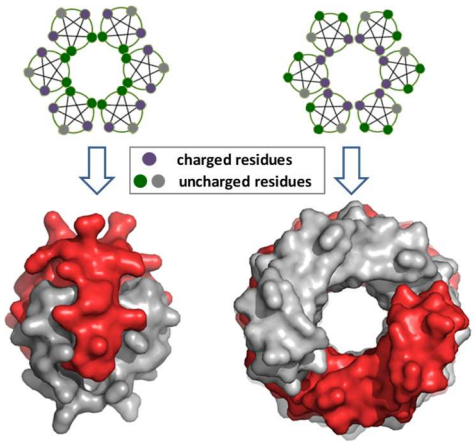


Figure 13. Dependence of the quaternary structure on the primary structure

Taking advantage of the fact that compound **31** forms capsules (**Figure 9**) we proceeded to the next stage of the research, which consisted in an attempt to close a guest molecule inside the capsule. This is discussed in **H5**, and also – indirectly – in **H4**. As the interior of the capsule is hydrophobic (side chains of Leu^{u} residues), initially we selected I_2 as the guest molecule (**H4** article). We started the experiments, adding the iodide solution to the crystallization mixture (the concentration of compound **31** in the solution was 10 mg/ml, 15% isopropanol, 100mM buffer CH_3COONa with pH 4.6, 200mM $CaCl_2$ and 0.75mM I_2).

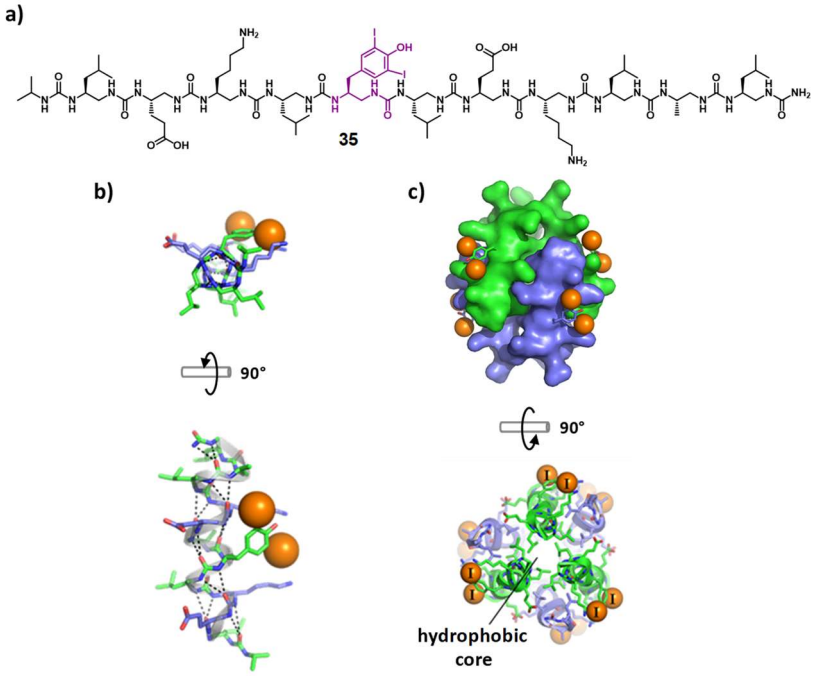


Figure 14. Foldamer **35:** a) sequence of compound, di-iodo Tyr^{u} residue is marked in purple; b) single helix, iodine atoms are marked in orange; c) a capsule formed by 6 helices of compound **35**

It turned out that I_2 was not encapsulated, but rather it reacted chemically with compound **31**. The aromatic ring of Tyr^{u} urea residue underwent, in the crystallization conditions, double electrophilic substitution at the *ortho* positions relative to the hydroxyl group. As a result, compound **35** was obtained (**Figure 14a**). Subsequently, it turned out that substitution in Tyr^{u} aromatic ring had no effect upon the secondary structure and the compound **35** folded into 2.5-helix (**Figure 14b**). What is more, these helices were organized in capsules, almost identical to those obtained for oligourea **31** (**Figure**

14c). The Tyr^{u} ring is located on the outside of the capsule, so chemical interference in this place has no negative effects on the self-assembly process to the quaternary structure.

In article **H5** we were able to prove that it is possible to introduce a guest molecule to the oligourea capsule. The literature provides examples of foldamer capsules that bind guest molecules, but most often they are single helices that "wrap" on a guest, that act as a template.[32-35]

For the complexation studies, we used compound **31.** Thorough analysis of the interior of the obtained capsule showed that it consists of an almost spherical chamber and three "tunnels" arranged at 120° angles (**Figure 15a**). The crystallization mixture contained isopropanol. We managed to obtain crystals with molecules of this alcohol as a guest inside the capsule. This interior, as I mentioned above, is hydrophobic, but in each "tunnel" there are two carbonyl groups of Leu_6^{u} residues, potentially being acceptors of hydrogen bonds (**Figure 15b**).

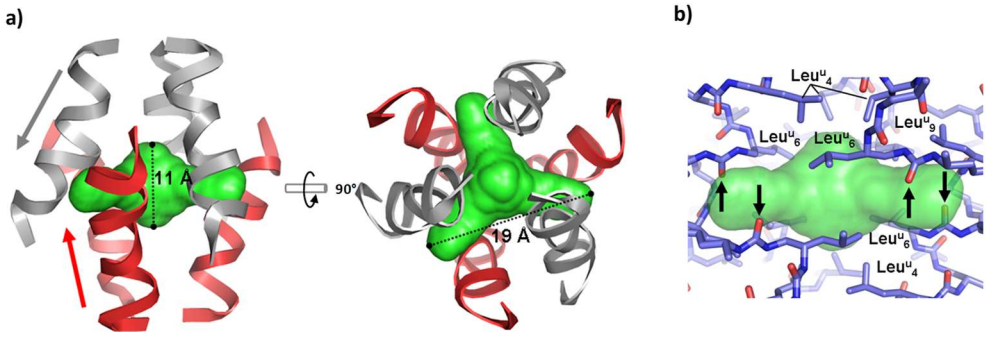


Figure 15. Capsule consisting of 6 helices of compound **31**: a) with an empty space in the center; b) Leu_6^{u} residues (arrows indicate potential acceptor sites for hydrogen bonds)

In the crystals of compound **31** with isopropanol, the -OH groups of the alcohol molecule form hydrogen bonds with the oxygen of the carbonyl group of Leu_6^{u}, and the aliphatic chain occupies the "tunnel". The ratio of oligourea to alcohol was 2: 1. These preliminary studies with isopropanol have encouraged us to check how alcohols with longer, unbranched aliphatic chains will behave like guests. We conducted the experiments in the presence of the corresponding alcohols in solution using CD method and in solid state using X-Ray crystallography. With the help of CD, we have measured the so-called *melting points*, representing the thermal stability of a given spatial structure (**Table 3**). The higher the melting point, the more stable the structure is.

Table 3. The influence of the guest molecule on the stability and volume of the capsule

guest	Tm (°C)[a]	ΔTm (°C)	inside volume of the capsule (Å^3)[b]
without guest	41.5	-	495
isopropanol	46.4	+4.9	513
1-butanol	50.8	+9.3	517.4
1-pentanol	54.9	+13.4	526.4
1-hexanol	53	+11.5	543.0
2-ethoxyethanol	44.5	+3.0	502.9
2-propoxyethanol	47.1	+5.6	484.8
1,4-butanediol	43.6	+2.1	500.5

[a] determined using CD

[b] determined on the basis of crystal structures

Furthermore, we were able to obtain crystals of compound **31** in the presence of all tested alcohols (**Figure 16**). It turned out that the volume of the capsule varied depending on the guest's size and was the largest for 1-hexanol (**Table 3**). In addition to simple aliphatic alcohols, we also studied the binding of alcohols with polar groups in the chain. The molecules of these alcohols intercalated into the interior of the capsules, and also in the case of 1,4-butanediol, hydrogen bonds among the individual guest molecules were observed (**Figure 16**).

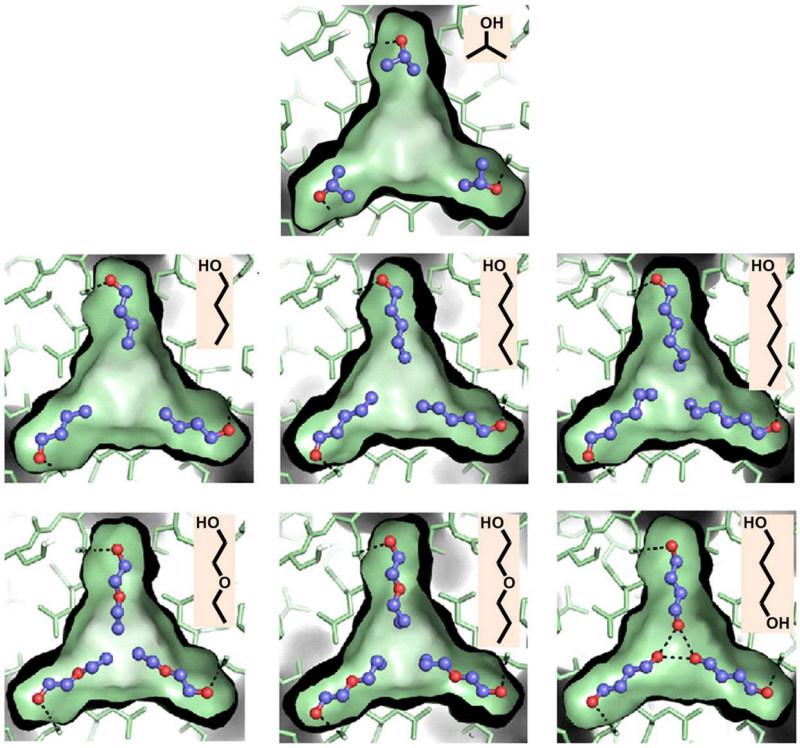


Figure 16. Crystal structures of capsule-guest complexes (fragments of a capsule with a binded guest)

The studies described in publication **H5** show that it is possible to "fill" the capsule formed by oligourea helices with guest molecules and this leads to only minor changes in the quaternary structure. In addition, these results may contribute in the future to the design of new drug delivery systems or nanoreactors to carry out chemical reactions.

Foldamers as functional mimetics of peptides and proteins

As I mentioned at the beginning of this presentation, aliphatic *N,N'*-disubstituted oligoureas fold into 2.5-helix. This makes them attractive models for the study of physicochemical properties related to the secondary structure of oligomers. One of such properties is the phenomenon of electron transport. This process is one of the fundamental reactions in nature.[36] It plays a key role in photosynthesis, cellular respiration, enzymatic reactions, drug activation and many others. The mechanism of this process is still not fully understood for native proteins, which is why simpler model systems are being searched for. Peptides have proven to be excellent mediators of electron transport.[37-42] It also turns out that the efficiency of this process can be modulated by changes in the secondary structure of the mediator and α-helix is one of the most effective structural elements involved in the electron transport process. Currently, it is believed that the electron transport process in peptides takes place via two mechanisms.[43] One of them is tunneling, in other words a one-step "jump" of an electron, while the

other is called hopping, a multi-step "jump" of an electron between neighboring units (**Figure 17** on the example of studies using AFM).

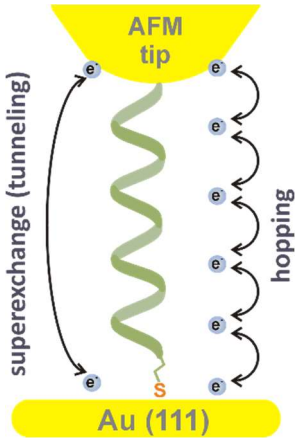


Figure 17. Postulated mechanisms of electron transport through helical mediators as exemplified by AFM examination

In addition, theoretical predictions suggest[44] that for helical molecules with increasing chain lengths, the mechanism changes from tunneling to hopping. This theory was very difficult to verify for α-peptides, due to the high conformational lability of compounds made up of less than 10 amino acid residues. For this reason, I thought that oligourea foldamers would serve as excellent model compounds to prove (or disprove) the theory. Research into this issue is described in the publications **H6** and **H9**, and is also the subject of my Opus Project.

The electron transport process can be investigated by various methods, however, the first stage usually involves the preparation of a self-assembled monolayer on the conductive surface, most commonly gold. The molecule that forms the monolayer must therefore have an anchoring group, most often the –SH thiol group. In the case of foldamers I studied, I chose the cysteamine urea unit to incorporate -SH function. The synthesis and structure of actual compounds tested for the transport of electrons in the **H6** article will be presented further in the text.

During the synthesis of these compounds, I noticed that the thiol group is oxidized to disulfide bridges, and the rate of this reaction considerably depends on the environment. I decided to explore this issue in depth to avoid unwanted dimerization during the synthesis and analytical characterization of the foldamers, especially while collecting NMR spectra.

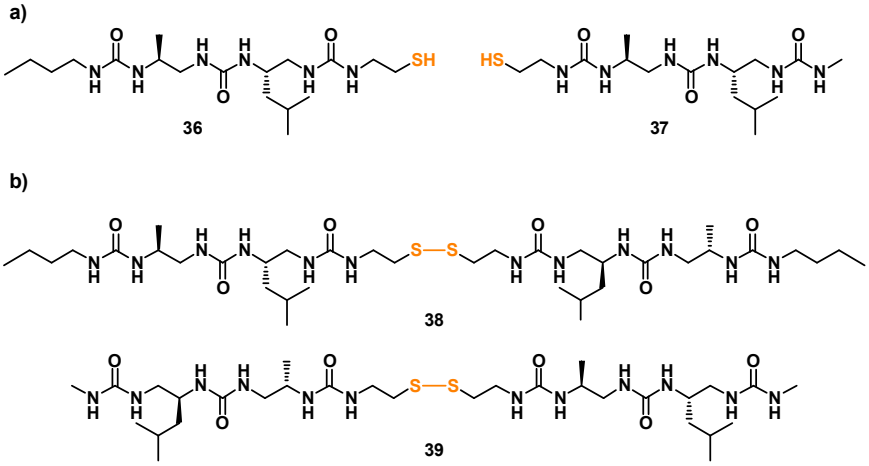


Figure 18. a) Oligoureas bearing a thiol group studied for the oxidation; b) Corresponding dimers with a disulfide bond

The results of studies on dimerization are presented in article **H8**. I chose short oligoureas containing a thiol group at the C- or N-terminus (**Figure 18a**, compounds **36** and **37**, respectively). It turned out that the rate of oxidation reaction essentially does not depend on the position of –SH group in the main chain (N- or C-terminus), but it is considerably influenced by the reaction temperature and the solvent. The progress of the oxidation reaction was investigated by RP-HPLC method. I chose two solvents for the tests: CH_3OH and DMSO (as these are often used in the NMR experiments). I tested the oxidation reaction at the same concentration (2mM), at room temperature and at 40°C and 60°C (**Figure 19**). It turned out that the reaction in CH_3OH was very slow regardless of the temperature. Similar reaction rate was observed in DMSO at room temperature. After 72 hours less than 5% of dimers **38** and **39** were present in the respective samples (**Figure 18**).

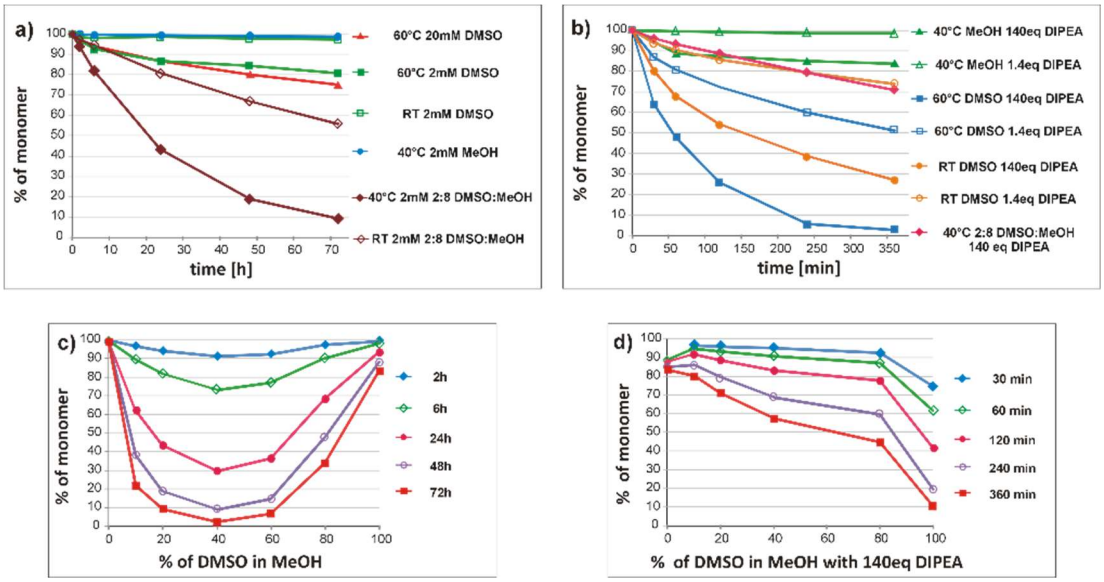
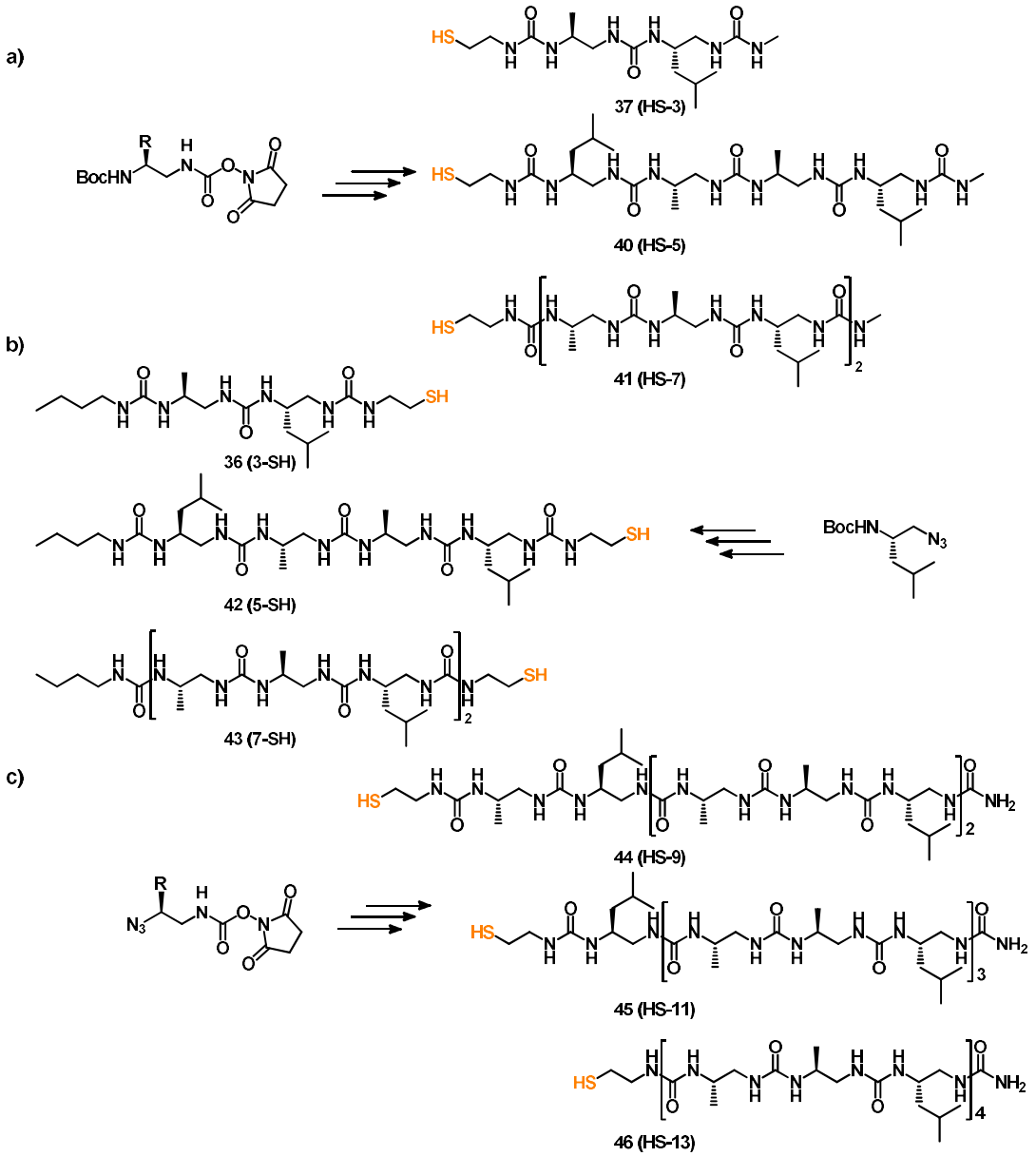


Figure 19. The influence of reaction conditions on the dimerization rate: a) in CH_3OH or/and in DMSO at different temperatures; b) in CH_3OH or in DMSO at different temperatures with the addition of DIPEA; c) in DMSO and CH_3OH mixtures; d) in DMSO and CH_3OH mixtures with the addition of DIPEA

At the same time, at 60°C, the amount of dimer increased to 20%. For samples dissolved in DMSO, I also examined the effect of concentration by comparing samples in which the initial monomer concentration was 2 and 20mM. I conducted concentration experiments at 60°C. It turned out that the concentration has little effect on the reaction rate. Next, I examined the effect of the presence of a base (a tertiary amine, DIPEA) on the rate of the disulfide bond formation reaction. It is known from the literature that the presence of a base accelerates the rate of dimerization due to the formation of highly nucleophilic thiolates.[45] I conducted the reaction in the presence of a small amount of the base (1.4 eq.) and an excessive amount (140 eq.). The greater the amount of amine in the reaction mixture, the faster the oxidation (**Figure 19b**). In addition, in DMSO the reaction proceeded much faster than in CH_3OH. Because the reaction rates in CH_3OH and in DMSO varied significantly, I decided to check the course of oxidation in a mixture of these two solvents, increasing the amount of DMSO in CH_3OH. The results I obtained were in a way surprising. It turned out that the rate of dimerization strongly depends on the amount of DMSO and the reaction was the fastest when the DMSO:CH_3OH volume ratio was 2:3 (**Figure 19c**). However, when I added 140 eq. of DIPEA to the reaction mixture, the dimer content in the mixture grew gradually with increasing DMSO content (**Figure 19d**). The results obtained in the publication **H8**, allow for example to select proper conditions for NMR measurements of thiols to avoid

unwanted dimerization. On the other hand, if the synthetic goal is to obtain a dimer, on the basis of these results suitable conditions can be chosen to facilitate dimerization.

The research carried out during the preparation of article **H8** allowed me to avoid unwanted dimerization in the synthesis of the compounds being the subject of article **H6**. The main objective of the work reported in **H6** was to check whether oligourea foldamers can be used as electron transport mediators and what the mechanism of this process is. Therefore I synthesized two series of oligomers with varied position of the cysteamine residue (at the C- or N-terminus of the foldamer).



Scheme 5. Oligoureas for studying the electron transport and starting substrates: a) with a thiol group at the N-terminus (synthesis in solution); b) with a thiol group at the C-terminus (synthesis in solution); c) with a thiol group at the N-terminus (microwave-enhanced solid-phase synthesis).

The synthesis of oligomers was carried out in solution, with the use of carbamate building blocks with Boc-protected amino group. In each series I obtained three oligoureas (**Scheme 5a,b**; compound symbols show the location of the thiol group and the total number of urea bonds) differing in the number of residues: dimer (**37 HS-3** and **36 3-HS**), tetramer (**40 HS-5** and **42 5-HS**) and hexamer (**41 HS-7** and **43 7-HS**).

All compounds were analyzed with a number of physicochemical methods in terms of the conformation they adopt (**Figure 20a-d**). Studies by NMR spectroscopy (comparison of differences in chemical shifts of diastereotopic protons of α-CH_2 groups, **Figure 20a**), and especially by circular dichroism, allowed the determination of the conformation of compounds in solution. Tetramers and hexamers displayed the characteristic profile of the CD spectrum, with the strong Cotton effect at λ=202-203 nm, while for dimers the CD band had very low intensity (**Figure 20b**). This indicates a large conformational lability of the shortest oligoureas. In addition, comparison of the CD spectra for tetramer and hexamer confirms the dependence of the stability of the secondary structure on the length of the oligomer.

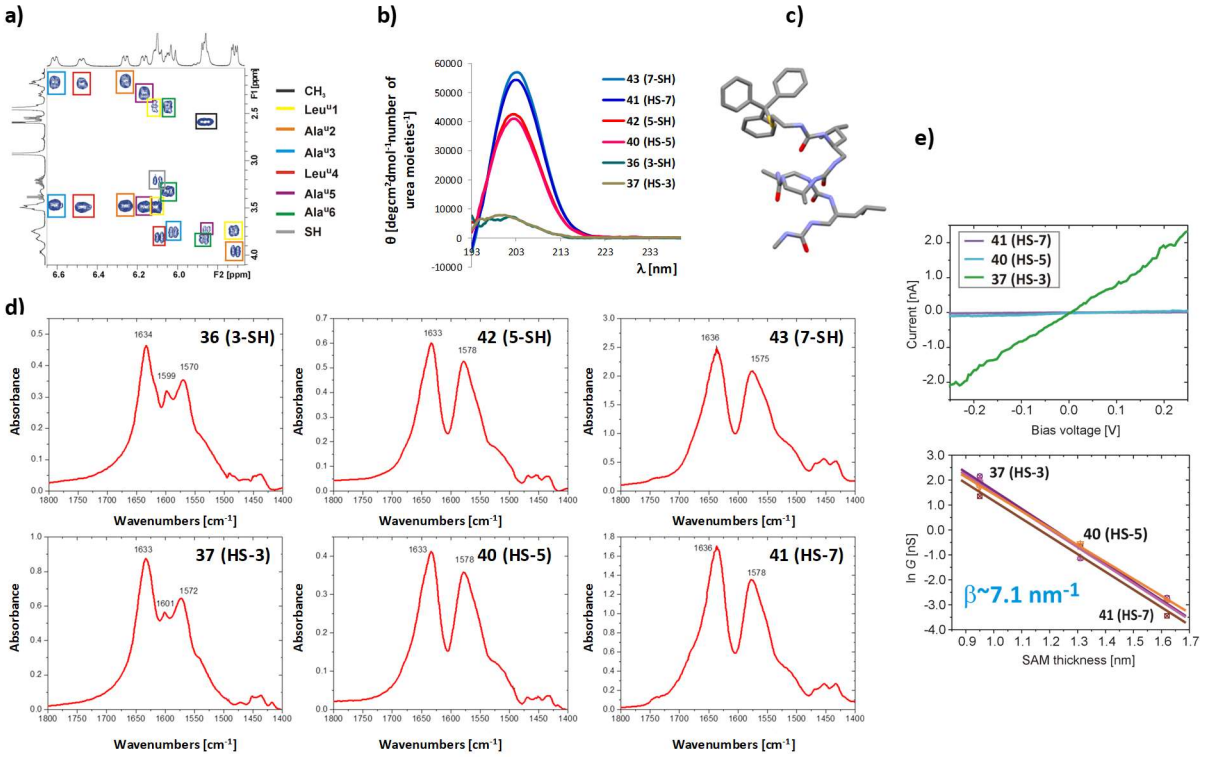


Figure 20. Studies of a conformation and electron transport of short oligoureas: a) fragment of COSY spectrum of compound **41**, showing differences in the chemical shift of α-CH_2 protons; b) CD spectra; c) the X-Ray structure of derivative of compound **40** with the Trt group; d) FTIR spectra; e) exemplary current-voltage relationships and the dependence of conductivity on the thickness of the monolayer

The structure in the solid state was examined with the FTIR method (**Figure 20d**). Helical oligoureas, similarly to peptides, display a characteristic IR spectrum.[46,47] Two strong bands appear at 1630-1638 cm^{-1} (so called *urea I*) and 1571-1578 cm^{-1} (called *urea II*). The presence of absorption bands in this region indicates that oligoureas adopt 2.5-helix. As can be seen, the FTIR spectra of tetramers and hexamers are almost identical, while in the spectra of the shortest oligomers an additional band is present at approx. 1600 cm^{-1}, which may indicate a higher conformational lability and *cis-trans* isomerization of individual urea bonds (**Figure 20d**). For **Trt-S-5** tetramer (derivative of the compound **40** with Trt protection) I obtained monocrystal, suitable for X-Ray studies, which additionally confirmed the helical structure in the solid state (**Figure 20c**). The results of the conformational studies show that the location of the cysteamine residue (N- or C-terminus of the oligomer) does not affect the helicity of the oligoureas. Having confirmed the secondary structure, we subjected all oligourea foldamers to the self-assembly process on the gold surface, and then examined them for electron transport by Atomic Force Microscopy (AFM). We found that electron transport through oligourea foldamers

consisting of up to 6 urea residues occurs in accordance with the tunneling mechanism. This is evidenced by the value of the tunneling coefficient β ~7 nm^{-1} (**Figure 20e**). AFM measurements were typically performed at a constant force of 0.5 nN applied to the probe. In order to check the elasticity of the monolayer (as well as the stability of the secondary structure), we gradually increased the force and examined the conductivity of this monolayer. It turned out that at force to about 3 nN, the conductivity grew, which was in line with expectations, because the distance between the AFM tip and metal contact decreased (the helix compresses like a spring or/and the tilt angle is changed); at the range of 4-5 nN the conductivity remained at constant level, but when the force applied to the probe was higher than 5nN, the changes of the conductance was irregular. This was most probably caused by changes in the structure of the monolayer and the distortion in the helical structure of the oligoureas.

Studies on the conductivity of oligoureas with a longer main chain (up to 12 urea residues) are the subject of article **H9**. Three compounds **44-46** (octamer **HS-9**, decamer **HS-11** and dodecamer **HS-13**) were obtained on a solid support under the microwave irradiation. The cysteamine residue was attached to the N-terminus (δ+ pole of dipole) of the oligomer (**Scheme 5c**).

Table 4. Comparison of the theoretical length of oligourea helices with the thickness of the monolayers formed by them

Compound	Length of oligourea helix [nm][a]	Thickness of the monolayer under 0,5 nN applied load [nm][b]
44 (HS-9)	2,02	1,82±0,2
45 (HS-11)	2,43	2,09±0.33
46 (HS-13)	2,84	2,58±0,35

[a] the length of the helix was estimated based on the assumption that the compounds fold into 2.5-helix and that 2.48 residues formed one turn, corresponding to 5.03Å per turn.[16] The length of 4Å was assumed for the cysteamine spacer 4 Å.

[b] the thickness of monolayers was determined with the AFM method

As in the case of the compounds being the subject of the article **H6**, the secondary structure (2.5-helix) of foldamers **44-46** was confirmed by circular dichroism and FTIR spectra. In addition, PM IRRAS spectra (Polarization Modulation-Infrared Reflection-Adsorption Spectroscopy, **Figure 21a**) were also recorded. By this experiments we showed that the secondary structure of the tested compounds did not change after the formation of the monolayer and still remained helical. Moreover, the comparison of the ratio of the surface area of the *urea I* / *urea II* band suggests that oligourea helices are almost vertical in relation to the gold surface (the greater the difference in the intensity of the two bands, the more vertical orientation of the compounds in relation to the surface).[48-50] This is additionally confirmed by the comparison of the monolayer thickness with the theoretical length of helices of individual compounds (**Table 4**). Compounds **44-46** were then studied for electron transport with the AFM method and the coefficient β = 0.92-0.95 nm^{-1} was determined (**Figure 21b**). A comparison of β values for short (2-6 urea residues, article **H6**) and long (8-12 urea residues, article **H9**) oligoureas allowed us to state that the electron transport mechanism changes with the increase of the main chain length, and the change from tunneling to hopping occurs when the thickness of the monolayer is approx. 1.8-2.0 nm. However, the most interesting feature of oligoureas **44-46** related to the electrical properties of the monolayers obtained from them was observed at high voltages (±1.0V, **Figure 21c**).

The efficiency of electron transport in oligourea foldamers was found to depend on the direction of electron flow. The magnitude of the current flowing through the mediator at positive bias voltage (i. e. aligned with the dipole moment) was much higher than at negative voltage (**Figure 21d**). This difference is expressed by the rectification ratio coefficient (RR), which is the current ratio at positive and negative bias voltages.

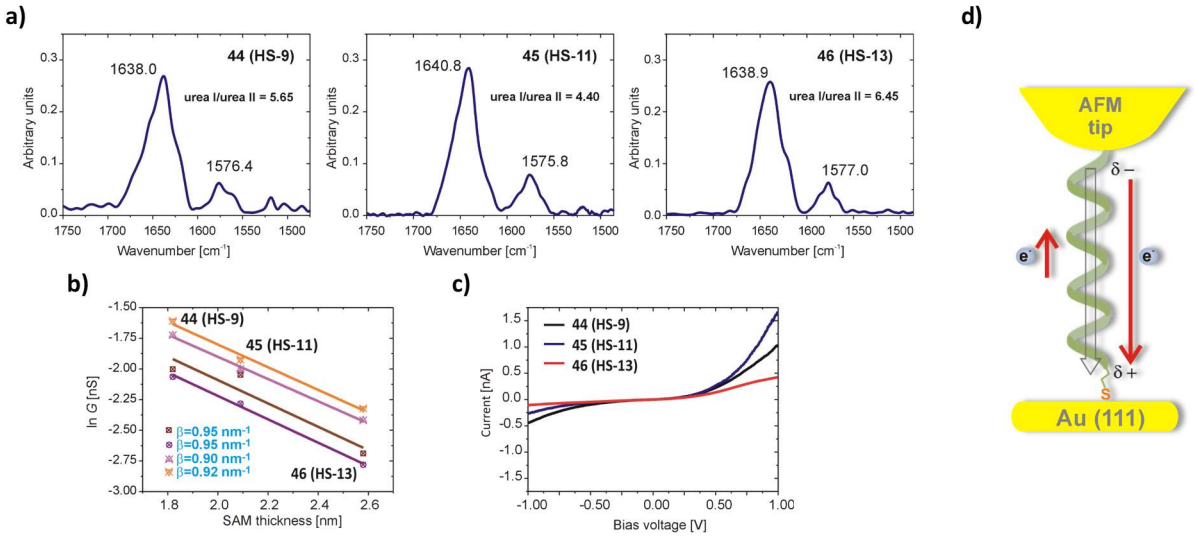


Figure 21. a) PM IRRAS spectra of oligoureas **44-46** (region of *urea I* and *urea II* bands); b) Dependence of conductivity on the thickness of the monolayer; c) Exemplary current-voltage relationships for high bias voltages; d) A graphical representation of the dependence of the efficiency of electron transport on the direction of the dipole

The determined rectification ratios are much higher (3-4 times) than for peptides with the same chain length. This proves that helical oligoureas may in the future be excellent candidates for the construction of new molecular diodes, and may be used to design new materials for nanoelectronics.

It is my believe that the above description clearly indicates that oligourea foldamers and their derivatives represent scientifically very attractive structural and functional mimetics of peptides and proteins. I am sure that interest in these compounds will grow, and oligoureas will be used, for example, as drug delivery systems or materials with new properties.

I consider my main achievements to be as follows:

- developing an efficient method for preparation of carbamate building blocks with -N_3 group as a masked amine group (N_3-BB). This method is particularly applicable to obtain urea building blocks with aliphatic side chains
- developing and optimizing a method of introducing the guanidinium group to the main chain of a foldamer. The starting compound for the guanidinylation reaction is a foldamer with a thiourea group and this reaction occurs with very good yields, also for oligomers already folded into a helix
- designing and obtaining the first water-soluble oligourea foldamers capable of self-assembly into higher-order structures. Small changes in the primary structure of the studied compounds

(mutual "shifts" of hydrophobic and hydrophilic residues) led to completely different quaternary structures (closed capsules vs. open channels)

- designing and obtaining oligourea foldamers as electron transport mediators. Being capable of self-assembly on the surface in the form of monolayers, oligoureas show a number of very interesting properties in terms of stability and conductivity of these monolayers.

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5. Description of other research (artistic) achievements

Total number of publications: **21**

Number of publications after obtaining the doctoral degree: **18**

Number of citations (based on the Web of Science [WoS] database) = **250**, as of 28 January 2019

Number of citations without self-citations = **217**

H-index **= 9**, as of 28 January 2019

Total impact factor according to the Journal Citation Reports (JCR) list, according to the year of publication: **IF_{total} = 135.607**

Before obtaining the doctoral degree - publications **M1-M3**

After obtaining the doctoral degree (publications that are not presented as a part of the monothematic cycle) - publications **D1-D9**

[M1] L. Frankiewicz, **K. Pulka**, A. W. Lipkowski, A. Misicka "Cross interaction of beta-amyloid peptide and prion protein fragments."
***Letters in Peptide Science*, 2002**, 9, 77-81. IF = 0.904

[M2] M. Karbarz, **K. Pulka**, A. Misicka, Z. Stojek "pH and Temperature-Sensitive N-Isopropylacrylamide Ampholytic Networks Incorporating L-Lysine."
***Langmuir*, 2006**, 22, 7843-7847. IF = 3.902

[M3] K. Pulka, D. Feytens, I. Van den Eynde, R. De Wachter, P. Kosson, A. Misicka, A. Lipkowski, N.N. Chung, P.W. Schiller, D. Tourwé "Synthesis of 4-amino-3-oxo-tetrahydroazepino[3,4-b]indoles: new conformationally constrained Trp analogs."
***Tetrahedron*, 2007**, 63, 1459-1466. IF = 2.869

[D1] K. Pulka, P. Kulis, D. Tymecka, L. Frankiewicz, W. Kozminski, M. Wilczek, A. Misicka "Diastereoselective Pictet-Spengler condensation of tryptophan with α-amino aldehydes as chiral carbonyl components."
***Tetrahedron*, 2008**, 64, 1506-1514. IF = 2.897

[D2] K. Pulka, D. Feytens, A. Misicka, D. Tourwé "New tetracyclic tetrahydro-beta-carbolines as tryptophan-derived peptidomimetics."
***Molecular Diversity*, 2010**, 14, 97-108. IF = 3.721

[D3] K. Pulka "Pictet-Spengler reactions for the synthesis of pharmaceutically relevant heterocycles."
***Current Opinion in Drug Discovery and Development*, 2010**, 13, 669-684. IF = 4.507, **artykuł na zaproszenie**

[D4] K. Pulka, A Misicka "Influence of reaction conditions on products of the Pictet–Spengler condensation."
***Tetrahedron*, 2011**, 67, 1955-1959. IF = 3.025

[D5] K. Pulka, M. Slupska, A. Puszko, M. Misiak, M. Wilczek, W. Kozminski, A. Misicka *"Peptides and peptidoaldehydes as substrates for the Pictet-Spengler reaction."*
Journal of Peptide Science, **2013**, 19, 433-440. IF=1.862

[D6] M. Slupska, **K. Pulka-Ziach**, E. Deluga, P. Sosnowski, B. Wilenska, W. Kozminski, A. Misicka "Synthesis of rigid tryptophan mimetics by diastereoselective Pictet-Spengler reaction of β3-homo-tryptophan derivatives with chiral α-aminoaldehydes."
Journal of Peptide Science, **2015**, 21, 893-904. IF=1.951

[D7] K. Pulka-Ziach, V. Pavet, N. Chekkat, K. Estieu-Gionnet, R. Rohac, M.-Ch. Lechner,C.R. Smulski, G. Zeder-Lutz, D. Altschuh, H. Gronemeyer, S. Fournel, B. Odaert, G. Guichard "Thioether analogues of disulfide-bridged cyclic peptides targeting death receptor 5: conformational analysis, dimerization a and consequences for receptor activation."
ChemBioChem, 2015, 16, 293-301. IF = 2.850

[D8] J. Fremaux, L. Mauran, **K. Pulka-Ziach**, B. Kauffmann, B. Odaert, G. Guichard "α-Peptide–oligourea chimeras: stabilization of short α-helices by non-peptide helical foldamers."
Angewandte Chemie-International Edition, 2015, 54, 9816-9820 IF = 11.709

[D9] C. M. Lombardo, G. W. Collie, **K. Pulka-Ziach**, F. Rosu, V. Gabelica, C. D. Mackereth, G. Guichard "Anatomy of an oligourea six-helix bundle."
Journal of the American Chemical Society, **2016**, 138, 10522-10530. IF = 13.858

During the preparation of my master's thesis, and then during the doctoral studies and after obtaining the doctoral degree, I was involved in various projects, which is reflected in the publications **M1-M3** and **D1-D9**. These various projects concerned the study of aggregation of β-amyloid fragments and the prion protein (publication **M1**) and the synthesis of amino acid derivatives that can be incorporated into a polymer network (publication **M2**). The main topic of my PhD thesis was the synthesis of cyclic tryptophan derivatives, in which the additional cycle consists of 7- or 6-atoms. I optimized the synthesis of 7-membered derivatives (related to the structure of indoloazepinone) during my internship at the laboratory of Prof. Dirk Tourwé (VUB in Brussels, two 3-months stays). The main synthetic problem consisted in introducing the formyl group at the 2' position of the indole ring of Trp. I tested direct methods known from the literature, but none of them led to the desired compound. Indirect method – synthesis of a cyclic Trp derivative with a 6-membered ring (tetrahydro-β-carboline derivative) in the Pictet-Spengler reaction, followed by oxidation with SeO_2 of the resulting intermediate, led to obtaining the proper compound. These issues are the subject of publication **M3**. The Pictet-Spengler reaction in its chiral variant, i.e. using optically active aldehydes, being amino acid and peptide derivatives, was the subject of my doctorate, as well as the scientific work before leaving for my post-doctoral internship. In the series of articles on this topic (**D1-D6**), I thoroughly studied Pictet-Spengler reaction. I explained the influence of the aminoaldehyde structure on the stereochemical result of the reaction (publication **D1**). I showed that by properly controlling the reaction conditions (temperature, solvent, DMSO additive, pH) different products may be obtained (publication **D4**), and that it is possible to further transform tetrahydro-β-carboline derivatives into compounds containing 4 conjugated rings, i.e. with very limited freedom of rotation (publication **D2**). In subsequent works, instead of aldehydes I used peptides with a formyl group at the C-terminus (publication **D5**) or instead of natural Trp the unnatural β-Trp was used (publication **D6**). Additionally, due to my interest in the Pictet-Spengler

reaction and its application, I was asked to write a review article on this topic (publication **D3**). During the post-doctoral internship I was involved in numerous projects. Some of the publications written at that time, concerning the subject of oligourea foldamers and their derivatives, have been included in the series of publications being the subject of my habilitation procedure. In addition, I was also involved in the synthesis of urea-peptide chimeras (publication **D8**) and preparation of building blocks that were used to obtain derivatives to thoroughly explain the effect of the primary structure on the self-assembly process of oligourea foldamers to the above-described capsules (publication **D9**). I was also involved in a project on the synthesis of cyclic peptides interacting with the DR-5 receptor. I obtained a cyclic peptide with a disulfide bridge, which was next transformed into a thioether bridge under basic conditions. Then I examined the stereochemical consequences of this transformation by synthesizing a lanthionine derivative (publication **D7**).

Since returning from my postdoctoral internship, I have continued my scientific cooperation with the laboratory of Prof. Gilles Guichard (Institut Européen de Chimie et Biologie in France). In addition, I have undertaken new scientific cooperation with the group of Prof. Sławomir Sęk (Faculty of Chemistry, University of Warsaw) and Dr. Yulia Moskalenko (the group of Prof. Christina Thiele, (Technische Universität Darmstadt in Germany). The results of that cooperations have already been published or are being prepared for submission.

Karolina Pulka-Ziach