Synthesis and incorporation of new β-sugar amino acids into foldamers

Booklet of Thesis

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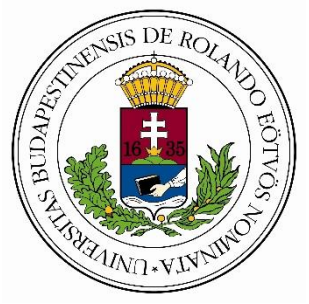

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2019

1. Introduction

Nowadays the spread of protein and peptide-base drugs is becoming ever more prominent.[1] As a result, the interest is growing in synthetic oligomers which are capable of forming structures similar to biomolecules, thereby, even replacing those. Foldamers[2] also belong to this group because they can adopt helical, β-stranded or turn motif spontaneously, similar to the secondary building blocks of proteins. Their greatest advantage is that - in contrast to natural polypeptides - the configuration of the building blocks allows us to design and predict the structure of the emergent nanosystem.[3] Hence, foldamers have already appeared in pharmaceutical research as potential lead molecules.[4]

For building blocks of peptidomimetic foldamers non-natural amino acids and sugar amino acids may be suitable.[5,6] They are grouped by their cyclic or acyclic structure, by the nature of the amide bond (α→ε) and by their ring size (from 3 to7-membered). The most studied monomers of cyclic β-amino acids are 2-aminocyclopentanecarboxylic acid (ACPC)[7] and 2-aminocyclohexanecarboxylic acid (ACHC),[8] due to their rigid structure and well-predictable properties. Various stereoisomers of these two monomers have already been used to build up numerous of homo- and heterooligomers of different lengths which proved their ability to compose helical or β-stranded structure.[7-10]

The main drawback of ACPC and ACHC derivatives and their foldamers is their apolar character which hampers their pharmaceutical application. Therefore, sugar amino acid building blocks have come into view. These compounds advantageously combine the benefits of amino acids and carbohydrates. As they are both biocompatible and hydrophilic, their foldamers also have similar properties.

Among the β-sugar amino acids, the furanoid D-ribo- and D-xilofuranuronic acids[11-13] (-RibAFU(ip)- and -XylAFU(ip)-) from D-glucose and various D-glucosamine[14] and D-galactosamine carboxylic acid (-GlcAPC- and -GalAPC-) derivatives are the most used. The monomers prepared in several steps were substituted with different protecting groups at both the amino (Fmoc or Boc), the carboxyl (methyl- or ethyl ester) and the hydroxyl groups (Bn or Bz) according to their further application.

The synthesis of some pyranoid uronic acid derivatives was described, namely the methyl 4-amino-4-deoxy-D-gluco- and methyl 4-amino-4-deoxy-D-galactopyranoside uronic acid (-GlcAPU(Me)- and -GalAPU(Me)-),[15] but they have not been used as foldamer building blocks. These compounds could be suitable monomers, since foldamers of their furanoid ring counterparts have been proven to form various types of helical structures.

2. Aims

During my PhD work my aim was to work out the large-scale synthesis of new pyranoid β-sugar amino acids which are suitable for solid phase peptide synthesis, then to use them as building blocks in the synthesis of new types of oligomers and α/β-chimera peptides.

Fmoc protected 2-amino-2-deoxy-D-mannopyranosyl-carboxylic acid (Fmoc-ManAPC-OH, **1**) was prepared based on the synthesis of well-known foldamer monomer, the C-2 epimer, H-GlcAPC-OH. The synthesis of the new C-4 epimer pair sugar amino acids, namely the Fmoc protected methyl-4-amino-4-deoxy-D-gluco- and D-galactopyranoside uronic acids (Fmoc-GlcAPU(Me)-OH, **2** and Fmoc-GalAPU(Me)-OH, **3**) was carried out in a single route *via* a common intermediate.

I planned to incorporate these sugar amino acids into model compounds containing amide bonds to find the best conditions for peptide coupling. For this reason, I examined the formation and stability of active esters formed during the couplings as a function of time. Based on the results I planned the synthesis of homooligomers and examined their structure.

Theses:

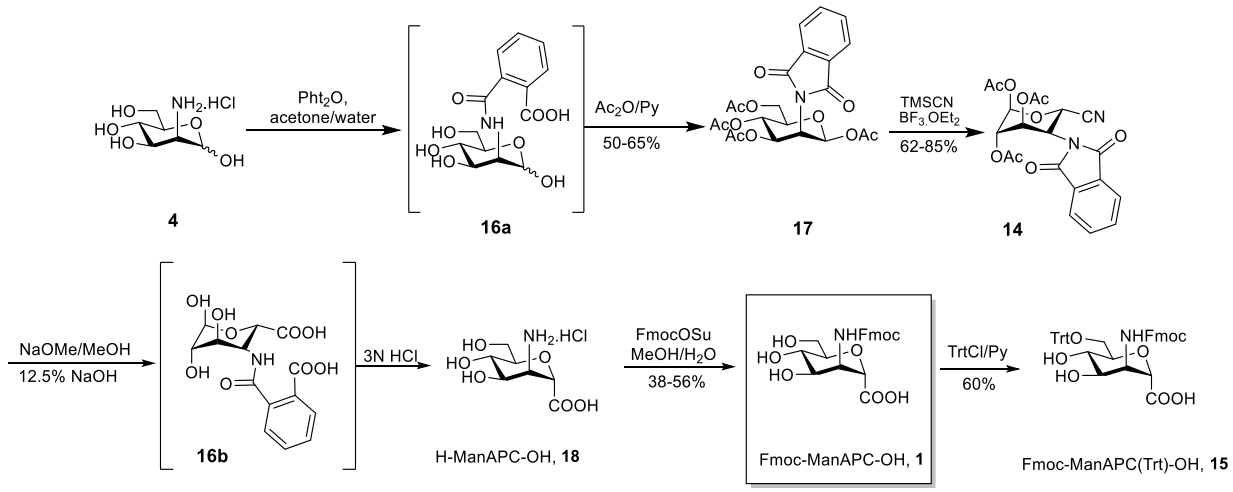
- I worked out the scalable synthesis of three new β-sugar amino acids:
  - The new Fmoc-ManAPC-OH (**1**) sugar amino acid was synthetized based on well-known analogue, H-GlcAPC-OH, in 6 steps.
  - The configuration of the new intermediate, produced in the designed synthetic pathway of D-mannosamine (**4**), was confirmed with structural investigations on different D-hexose and D-hexosamine arylhydrazones.
  - The synthesis of the 4,5-*cis*-*trans* pair Fmoc-GlcAPU(Me)-OH (**2**) and Fmoc-GalAPU(Me)-OH-t (**3**) was carried out *via* a common intermediate in 9 steps.
- I successfully applied these new β-sugar amino acids in peptide syntheses:
  - Model compounds were synthetized for the examination of amide bond formation: Ac-GlcAPU(Me)-NHMe (**5**) and Ac-GalAPU(Me)-NHMe (**6**) derivatives.
  - -GXXG- model peptides were synthetized (X: -GlcAPU(Me)- (**7**) or -GalAPU(Me)- (**8**)) with various coupling agents and the conditions of the amide bond formation were studied and optimized.
  - From Fmoc-GlcAPU(Me)-OH (**2**) two new homooligomers: the tetramer (**9**) and the hexamer (**10**) were synthetized and their structures were investigated with ECD and NMR spectroscopy.

3. Results and discussion

3.1. Pyranoid sugar amino acids I.: H-ManAPC-OH

During the production of Fmoc-ManAPC-OH (**1**) sugar amino acid the synthetic path designed for C-2 epimer D-glucosamine derivatives was applied.[14] First, to form D-mannosamine (**2**) from D-mannose *via* per-*O*-acetyl-D-mannose 4-nitrophenylhydrazone (**12**), the benzylamine moiety was built in at C-2 position. The reaction proceeded through an acyclic intermediate, therefore to determine the configuration of the new 2-benzylamino derivative (**13**), the structures of different D-hexose and D-hexosamine arylhydrazones were examined with ^{1}H-NMR-, IR-, and mass-spectrometry and with molecular dynamic simulations. According to the results, the amino group at C-2 position, and the equatorial hydroxyl groups on the pyranoid ring as well as the electron withdrawing NO_2 group of the arylhydrazone moiety are necessary to ensure the formation of the complete hydrogen bond network stabilizing the cyclic form of hexose and hexosamine 4-nitrophenylhydrazones. Based on this finding and on the crystal structure of the new (*N*-acetyl-2-benzylamino)-2-deoxy-D-hexose 4-nitrophenylhydrazone (**13**) we unequivocally confirmed the D-*gluco* configuration of this new intermediate.

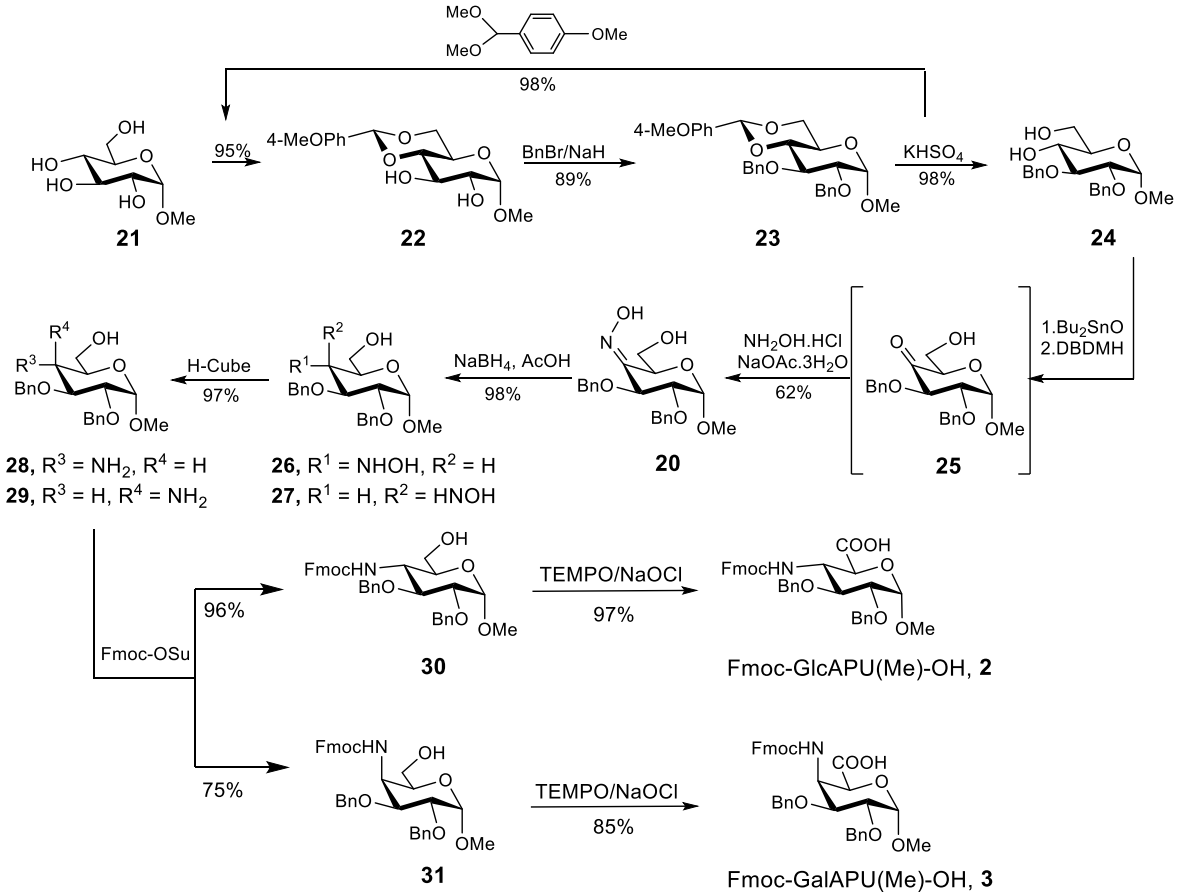
Sugar amino acid **1** was synthetized from D-mannosamine (**4**) in 6 steps. The key step of the pathway, the chain elongation at C-1 resulted in the 1,2-*trans N*-phthaloyl-per-*O*-acetyl-D-mannosamine cyanide (**14**). Both the configuration and the unexpected $^1C_4$ pyranoid ring conformation of the compound was demonstrated by determining its crystal structure. Transformation of this new compound led to Fmoc protected derivatives suitable for peptide synthesis, the Fmoc-ManAPC-OH (**1**) and Fmoc-ManAPC(Trt)-OH (**15**) sugar amino acids in good overall yields.



Scheme 1: The synthesis of Fmoc-ManAPC-OH (**1**) sugar amino acid in 6 steps *via* 2-phthalimido-2-deoxy-per-*O*-acetyl-D-mannopyranosyl cyanide (**14**)

3.2. Pyranoid ring sugar amino acids II.: H-GlcAPU(Me)-OH and H-GalAPU(Me)-OH

The syntheses of various methyl ester derivatives of pyranoid 4-amino-4-deoxy uronic acids were already described in the literature, so their production started using these methods. Attempting the use of the azido group as the amino group precursor,[15] I got unexpected result: in the presence of the carboxyl group at C-5 the sulfonate→azide replacement failed and an elimination reaction took place giving a 4,5-unsaturated derivative (**19**), which terminated this pathway. By changing the precursor of the amino group to the oxazine ring,[16] and by opening it the Fmoc-GalAPU(Me)-OH (**3**) with D-*galacto* configuration was obtained in full stereoselectivity.



Scheme 2. The synthetic steps of the two pyranoid ring uronic acids (**2** and **3**) *via* the non-selective reduction of the common oxime intermediate (**20**)

The oxime (**20**) precursor was used for target compound **2** which made possible to produce both C-4 epimer sugar amino acids (**2** and **3**) in one economical and effective route. In the first 3 steps the *O*-benzyl protected **24** compound was formed from methyl α-D-glucopyranoside (**21**), then the key intermediate oxime (**20**) was synthetized *via* the 4-oxo compound (**25**). Based on the complete structural investigation (NMR and X-ray) of this new oxime **20** a non-selective

two stepped reduction was worked out to form the methyl 2,3-di-*O*-benzyl-4-amino-4-deoxy-α-D-gluco- and D-galactopyranoside (**28** and **29**) derivatives in 1:1 ratio. This two epimers were separated after subsequent Fmoc protection by crystallizing one of them. In the last step, selective oxidation was used to synthetize the two new Fmoc-GlcAPU(Me)-OH (**3**) and Fmoc-GalAPU(Me)-OH (**3**) monomers suitable for solid phase peptide synthesis.

3.3. Peptide coupling on solid phase

First, the new sugar amino acids were used to synthesize model compounds. The formation of amide bond was examined by the production of *N*-acetyl- and *C*-methyl amide derivatives with D-*gluco* and D-*galacto* configuration of the pyranoid uronic acids, namely Ac-GlcAPU(Me)-NHMe (**5**) and Ac-GalAPU(Me)-NHMe (**6**) diamide models. There was no considerable difference in the yield of the *cis* and *trans* compound, due to the flexibility of the pyranose ring. Hence, no significant difference is expected in the application of the two monomers.

To determine the conditions of the peptide coupling, *e.g.* coupling time, coupling agents, *etc.* the Fmoc-GlcAPU(Me)-OH (**2**) sugar amino acid was used. On RAM-Tentagel® resin, the -GXXG- model peptide (**7**) was synthetized with four common coupling reagents, HATU/DIEA, PyBOP/DIEA, HOBt/DIC and HOBt/EDCI/DIEA. With this short sequence the formation of α/β, β/β and β/α amide bonds were modeled and the efficacy of the coupling was determined. To state the optimal coupling time, the formation and stability of the active esters were examined by ^{1}H NMR spectroscopy. The results could be correlated with the efficacy of couplings. We found the best coupling agent to be PyBOP/DIEA. This reagent pair was used to synthetize α/β chimera peptides -VXVG- (**32**) and -GXXG- containing Fmoc-GalAPU(Me)-OH (**3**) with similar good coupling efficacy (84%→<99%).

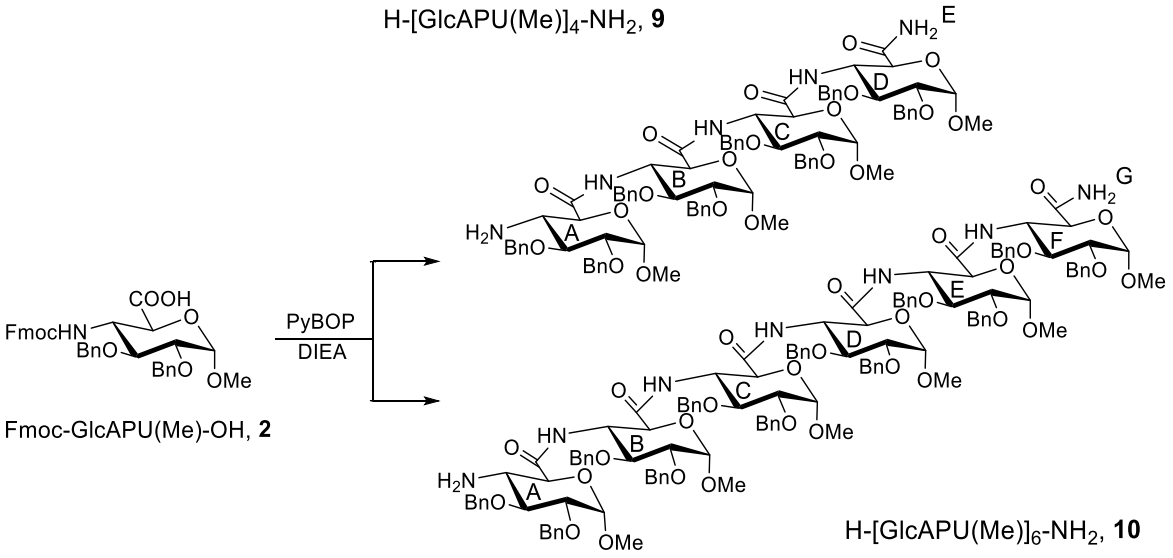
The active esters of selected α-amino acids, aliphatic and cyclic β-amino acids as well as β-sugar amino acids were investigated also with PyBOP/DIEA coupling agent. Their different behavior, the hydrolysis of the active esters and their stability was found to depend on the topology of the given amino acid resides. Based on these, we could propose new modified coupling protocols.

In case of the diamide model (**5**) and the -GXXG- model peptide (**7**) the *O*-benzyl protection was removed to study its effect on the structure.

Based on my experience with model peptides, two new homooligomers of different lengths were synthetized by using RAM-Tentagel® resin with PyBOP/DIEA reagents from Fmoc-GlcAPU(Me)-OH monomer. Both the tetramer H-[GlcAPU(Me)]$_4$-NH$_2$ (**9**) and the hexamer

H-[GlcAPU(Me)]$_6$-NH$_2$ (**10**) was produced in good overall yields and could be purified with RP-HPLC.

The purity of diamide models (**5**, **6**), α/β chimera peptides (**7**, **8**) and homooligomers (**9**, **10**) was examined by NMR, MS and HPLC measurements and their structure was investigated with ECD and NMR spectroscopy. The ECD spectra of both tetramer and hexamer oligomers may indicate helical structure, as the increasing number of building blocks caused increasing signal intensity. However, this could not be clearly confirmed by 2D NMR measurements, because only the sequential cross peaks between (*i*) and (*i+1*) or (*i*-1) were identified in their ROESY spectra. This was probably caused by the presence of *O*-benzyl protecting groups which can be removed to provide a more compact structure as a proven foldamer.



Scheme 3. Building of the two new homooligomer (**9** and **10**) from Fmoc-GlcAPU(Me)-OH (**2**) sugar amino acid with PyBOP/DIEA coupling agents

4. Summary, new results

During my PhD work I successfully synthesized three new β-sugar amino acids based on literature analogies as well as by working out new synthetic routes. In case of Fmoc-ManAPC-OH (**1**), the one step C-1 elongation resulted in 1,2-*trans* sugar amino acid in 6 steps. The 4,5-*cis*-*trans* pair Fmoc-GlcAPU(Me)-OH (**2**) and Fmoc-GalAPU(Me)-OH (**3**) was synthetized *via* the common oxime (**20**) intermediate with non-selective reduction in 9 steps.

Fmoc-GlcAPU(Me)-OH (**2**) and Fmoc-GalAPU(Me)-OH (**3**) were used to examine the peptide coupling conditions by preparing suitable model compounds containing amide bonds (**5** and **6**) and α/β chimera peptides (**7** and **8**). From the four applied coupling reagents the PyBOP/DIEA proved to be the best in the synthesis of further peptide sequence, too. Two new

homooligomers, namely the tetramer **9** and hexamer **10** were synthetized from Fmoc-GlcAPU(Me)-OH (**2**) and their structure was investigated with 2D NMR spectroscopy.

Based on my results the three new sugar amino acids are useful for solid phase peptide synthesis, hence, we can apply those, routinely. Furthermore, they can be incorporated into foldamers and various peptide-based drugs to modify their pharmacokinetic properties advantageously.

5. References

1. Lau, J. L.; Dunn, M. K. *Bioorg. Med. Chem.* **2018,** *26*, 2700-2707.
2. Gellman, S. H. *Acc. Chem. Res.* **1998,** *31*, 173-180.
3. Mándity, I. M.; Wéber, E.; Martinek, T. A.; Olajos, G.; Tóth, G. K.; Vass, E.; Fülöp, F. *Angew. Chem., Int. Ed.* **2009,** *48*, 2171-2175.
4. Gopalakrishnan, R.; Frolov, A. I.; Knerr, L.; Drury, W. J.; Valeur, E. *J. Med. Chem.* **2016,** *59*, 9599-9621.
5. Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001,** *101*, 3893-4011.
6. Risseeuw, M.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. *Amino Acids* **2013,** *45*, 613-689.
7. Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J., Jr.; Gellman, S. H. *Nature* **1997,** *387*, 381-384.
8. Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996,** *118*, 13071-13072.
9. Martinek, T. A.; Tóth, G. K.; Vass, E.; Hollósi, M.; Fülöp, F. *Angew. Chem., Int. Ed.* **2002,** *41*, 1718-1721.
10. Mándity, I. M.; Fülöp, L.; Vass, E.; Tóth, G. K.; Martinek, T. A.; Fülöp, F. *Org. Lett.* **2010,** *12*, 5584-5587.
11. Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stockle, M.; Kessler, H. *Chem.- Eur. J.* **2002,** *8*, 4366-4376.
12. Chandrasekhar, S.; Reddy, M. S.; Jagadeesh, B.; Prabhakar, A.; Rao, M.; Jagannadh, B. *J. Am. Chem. Soc.* **2004,** *126*, 13586-13587.
13. Nagy, A.; Csordás, B.; Zsoldos-Mády, V.; Pintér, I.; Farkas, V.; Perczel, A. *Amino Acids* **2017,** *49*, 223-240.
14. Suhara, Y.; Kurihara, M.; Kittaka, A.; Ichikawa, Y. *Tetrahedron* **2006,** *62*, 8207-8217.
15. Naka, T.; Hasizume, T. *Agric. Biol. Chem.* **1970,** *34*, 1855-1858.
16. van den Bos, L. J.; Codee, J. D. C.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Biomol. Chem.* **2003,** *1*, 4160-4165.

6. List of publications

6.1. The thesis based on the following publications

V. Goldschmidt Gőz, I. Pintér, A. Csámpai, I. Jákli, V. Zsoldos-Mády, A. Perczel
Hydrogen-Bonding Network Anchors the Cyclic Form of Sugar Arylhydrazones
European Journal of Organic Chemistry (**2016**), *20*, 3419-3426.

V. Goldschmidt Gőz, I. Pintér, V. Harmat, A. Perczel,
Approaches to Pyranuronic β-Sugar Amino Acid Building Blocks of Peptidosaccharide Foldamers
European Journal of Organic Chemistry (**2018**), *3*, 355-361.

A. Nagy, **V. Goldschmidt Gőz,** I. Pintér, V. Farkas, A. Perczel,
α/β-Chimera peptide synthesis with cyclic β-sugar amino acids: the efficient coupling protocol
Amino Acids (**2019**), *4*, 669-678.

V. Goldschmidt Gőz, A. Nagy, V. Farkas, E. Keszei, A. Perczel,
Unwanted hydrolysis or α/β-peptide bond formation: How long the rate-limiting coupling step should take? *RSC: Advances* (**2019**), Accepted

6.2. Related posters and presentations on international or national conferences

<u>Presentations in English:</u>

V. Goldschmidt Gőz, A. Nagy, V. Farkas, I. Pintér, A. Perczel: *Puzzle pieces: C-4 and C-3epimer β-SAAs as new building blocks of foldamers*; 9th Conference Chemistry towards Biology; Budapest, 24-27.09.2018.

V. Goldschmidt Gőz, A. Nagy, V. Farkas, I. Pintér, A. Perczel: *Puzzle pieces: C-4 and C-3epimer β-SAAs as new building blocks of foldamers*; Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences, Mátrafüred, 23-25.05.2018.

V. Goldschmidt Gőz, I. Pintér, A. Perczel: *Pyranuronic β-sugar amino acids as foldamer building blocks***,** Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences, Mátraháza, 31.05.-02.06.2017.

V. Goldschmidt Gőz, I. Pintér, V. Farkas, A. Perczel: *New synthetic approaches to pyranoid β-SugarAminoAcids,*8th Conference Chemistry towards Biology; Brno, 28.08.-09.01.2016.

V. Gőz, V. Zsoldos-Mády, I. Pintér, A. Perczel: *Pyranuronic sugar amino acids (SAA): new ways for the synthesis of the precursors*, Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences; Mátraháza, 27-29.05.2015.

V. Gőz, V. Zsoldos-Mády, I. Pintér, A. Csámpai, A. Perczel: *Cyclic or acyclic? Studies on the structure of aldose arylhydrazones*, Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences; Mátraháza, 21-23.05.2014.

V. Gőz, V. Zsoldos-Mády, I. Pintér, A. Perczel: *A novel approach toward 2-amino-2-dezoxyaldose derivatives: benzylamine as proper nucleophile,* Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences; Mátrafüred, 22-24.05.2013.

Presentations in Hungarian:

Goldschmidtné Gőz V., Nagy A., Farkas V., Keszei E., Perczel A.: *A peptidkötés kialakulásának vizsgálata: aktívészterek, mennyi ideig kapcsoljunk?*, Peptidkémiai Munkabizottság ülése; Balatonszemes, 2019.05.27-29.

Goldschmidtné Gőz V., Pintér, I., Perczel A.: *Piranóz gyűrűs β-cukoraminosavak, mint alkalmas foldamer építőelemek*, Peptidkémiai Munkabizottság ülése; Balatonszemes, 2017.05.29-31.

Goldschmidtné Gőz V., Pintér, I., Farkas, V., Perczel A: *Új szintézisutak piranóz β-cukoraminosavamid származékok előállítására*, Peptidkémiai Munkabizottság ülése; Balatonszemes, 2016.05.30-07.01.

Kapros A., **Gőz V.**, Zsoldosné Mády V., Pintér I., Perczel A.: *Piranóz-gyűrűs cukoraminosavak, mint peptidoglikán építőelemek,* Peptidkémiai Munkabizottság ülése; Balatonszemes, 2014. 05.28-30.

Gőz V., Zsoldosné Mády V., Pintér I., Perczel A.: *Új szintetikus megközelítés 2-amino-2-dezoxi-aldohexózok előállítására: Benzilamin, mint alkalmas nukleofil,* XIX. Nemzetközi Vegyészkonferencia; Nagybánya, 2013.11.21-24.

Posters:

V. Goldschmidt Gőz, I. Pintér, A. Perczel: *New synthetic approaches to C-2 epimer β-Sugar Amino Acids, building blocks of foldamers,* 9th Conference Chemistry towards Biology; PhD-Day, Budapest, 24-27.09.2018.

Farkas V., **Goldschmidt Gőz, V**., Nagy, A: *Új módszer a láthatáron: peptid alapú hatóanyagok, szubsztrátok gazdaságos és gyors szintézise áramlásos rendszerű peptidszintézis készülékkel*, MedInProt 6. Konferencia; Budapest, 04.26.2018.

V. Goldschmidt Gőz, I. Pintér, V. Farkas, A. Perczel*: Single pathway to 4-epimer pyranoid β-sugar amino acids, building blocks of new β-foldamers*, 19th European Carbohydrate Symposium, EUROCARB 2017; Barcelona, 02-07.07.2017.

V. Goldschmidt Gőz, I. Pintér, V. Zsoldos-Mády, A. Csámpai, A. Perczel: *A circular H-bond network anchors the pyranosyl form of common monosaccharide arylhydrazones;* Debrecen Colloquium on Carbohydrates, DEBCARB 2015; András Lipták Memorial Conference; Debrecen, 06-08.11.2015.