Final report K119509: Synthesis and antibaterial evaluation of semisynthetic glycopeptides and multivalent carbohydrates

Results in the field of multivalent carbohydrates:

Sevaral potential ligands of carbohydrate-specific bacterial lectins were synthesized and investigated as potenial candidates for anti-adhesion therapy.

Four multivalent mannoside derivatives were prepared as potential inhibitors of lectin BC2L-A, one of the virulence factors deployed by *B. cenocepacia* in the infection process. An (α1→2)-thio-linked mannobioside mimic bearing an azide functionalized aglycon was conjugated to different multivalent scaffolds such as propargylated calix[4]arenes, methyl gallate and pentaerythritol by azide-alkyne 1,3-dipolar cycloaddition. The interaction between the glycoclusters and the mannose binding BC2L-A lectin from *B. cenocepacia* was examined by isothermal microcalorimetry, surface plasmon resonance, inhibition of yeast agglutination and analytical ultracentrifugation. We proved that these multivalent glycodendrons can cross-link and aggregate BC2L-A lectin and this process resulted in interference with biofilm formation and bacteria-host cell interaction. The results were published in *Carbohydrate Research.*

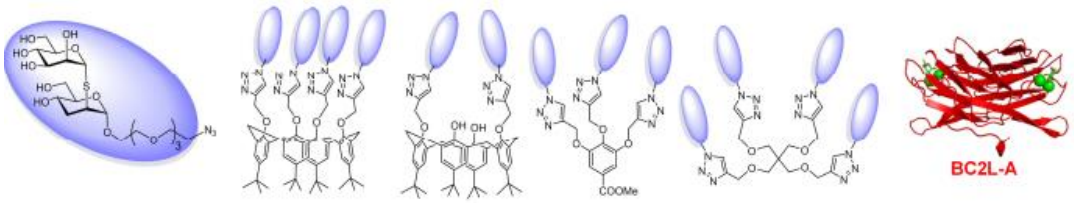


Figure 1. Structures of the (α1→2)-thio-linked mannobioside-containing glycoconjugates and the target lectin BC2L-A

Series of self–assembling mannosides were also synthesized and the characterization of the binding properties of glycoconjugates towards mannose-specific lectin BC2L-A from *Burkholderia cenocepacia* were investigated. We have demonstrated an approach how to achieve multivalency in inhibitors of lectins via self-assembling 1,2-thiomannobioside glycoconjugates. Lipophilic carriers containing amphiphilic compounds (hexadecyl- and pyrrolidinofullerene-chain) were prepared and investigations of their ability to interact with and inhibit mannose-specific lectin BC2L-A were carried out. The results confirmed that both compounds are able to inhibit binding activity of the lectin. High inhibitory potencies were achieved in the current research, with the hexadecyl chain containing self-assembled 1,2-thiomannobioside glycoconjugate being eight times better inhibitor than simple D-mannose as determined by yeast agglutination inhibition assay. Significantly increased cross-linking activity was observed, too. This cross-linking activity even made SPR measurements impossible and resulted in aggregates visible by naked eye during yeast agglutination assay when higher concentration of lectin BC2L-A was used. Considering pyrrolidinofullerene self-assembling mannoside-containing compound, such behaviour was not detected. The shape and the size of the cluster were characterized by DLS and AFM and were found out to be heterogeneous and irregular, however stable. The clusters were not disrupted even in 25% DMSO which could be therefore used as a solubilizing agent for achievement of high

concentration. The ability of self-assembling mannocluster to interact with native lectin BC2L-A on the bacterial surface and cross-link and aggregate *Burkholderia cenocepacia* was also tested with positive result. The preparation of the manuscript are in progress.

Our next goal was to create potential ligands of the newly discovered L-fucose specific PHL lectin isolated from *Photorhabdus asymbiotica*. PHL is a homo-dimer with two sets of binding sites and contains up two seven L-fucose specific binding sites per monomer. α-L-fucoside-containing mono-, di-, tri- and tetravalent glycoclusters were synthesized as potential ligand of PHL lectin. Methyl gallate and pentaerythritol were chosen as multivalent scaffolds and the fucoclusters were built from the above mentioned cores by coupling with different oligoethylene bridges and propargyl α-L-fucosides using 1,3-dipolar azide-alkyne cycloaddition. The interaction between fucoside derivates and PHL lectin was investigated by several biophysical and biological methods, ITC and SPR measurements, hemagglutination inhibition assay and investigation of bacterial aggregation properties were carried out. The aggregation behaviour of the PHL lectin upon titration with the five α-L-fucoside-containing derivatives has been investigated and compared by ^{1}H NMR spectroscopy, monitoring the change (decrease) in protein signal intensity as a function of the actual ligand concentration. To monitor the binding of the high-affinity multivalent ligands and to identify their probable binding sites, competition STD experiments were also carried out. We performed a series of *in vitro* aggregation assays to reveal the aggregation properties of fucosylated compounds towards *P. asymbiotica subsp. australis*. X-ray crystallography was also used to investigate the PHL/monovalent fucoside complex. Moreover, the NMR and X-ray data revealed the details of interaction between α-L-fucoside monomer unit and PHL. The newly synthesized multivalent glycoclusters proved to be up to several orders of magnitude better ligands than L-fucose. We have found potential ligands of the newly discovered L-fucose specific PHL lectin isolated from *Photorhabdus asymbiotica*. The newly synthesized multivalent glycoclusters proved to be up to several orders of magnitude better ligands than the natural ligand, L-fucose. These results were published in a D1-ranked journal (Synthesis of alpha-L-fucopyranoside-presenting glycoclusters and investigation of their interaction with *Photorhabdus asymbiotica* lectin (PHL), *Chemistry - A European Journal*, 2018, 24, 4055 – 4068. doi: 10.1002/chem.201705853).

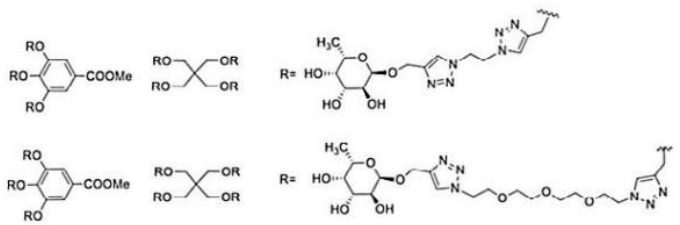


Figure 2. Structure of the tri- and tetravalent fucoclusters as potential ligand of PHL lectin

Series of mono- and multivalent α-L-fucosides with different scaffolds and valency were prepared and their interaction with six fucolectins (AOL, AAL, RSL, AFL, PA-IIL, BC2L-C) were tested. We have published these results in a Q1-ranked, open-access journal (Son Le Thai, Lenka Malinovska, Michaela Vašková, Erika Mező, Viktor Kelemen, Anikó Borbás, Petr

Hodek, Michaela Wimmerová and Magdolna Csávás: Investigation of binding affinity of a broad array of L-fucosides with six fucose-specific lectins from bacterial and fungal origin. Molecules 2019, 24 (12), 2262). Series of multivalent α-L-fucoside containing glycoclusters and variously decorated L-fucosides were synthesized to find potential inhibitors of fucose-specific lectins and study the structure-binding affinity relationships. Tri- and tetravalent fucoclusters were built up using copper-mediated azide-alkyne click chemistry. Series of fucoside monomers and dimers were synthesized using various methods, namely glycosylation, azide-alkyne click reaction, photoinduced thiol-en addition and sulfation. The interactions of compounds with six fucolectins of bacterial or fungal origin were tested using hemagglutination inhibition assay. As a result, a tetravalent, α-L-fucose presenting glycocluster showed to be orders of magnitude better ligand than a simple monosaccharide for tested lectins in most cases, which can nominate it as a universal ligand for studied lectins. This compound was also able to inhibit adhesion of *Pseudomonas aeruginosa* cells to human epithelial bronchial cells. A trivalent fucocluster with protected amino functional group seems also to be a promising candidate to design glycoconjugates and chimeras.

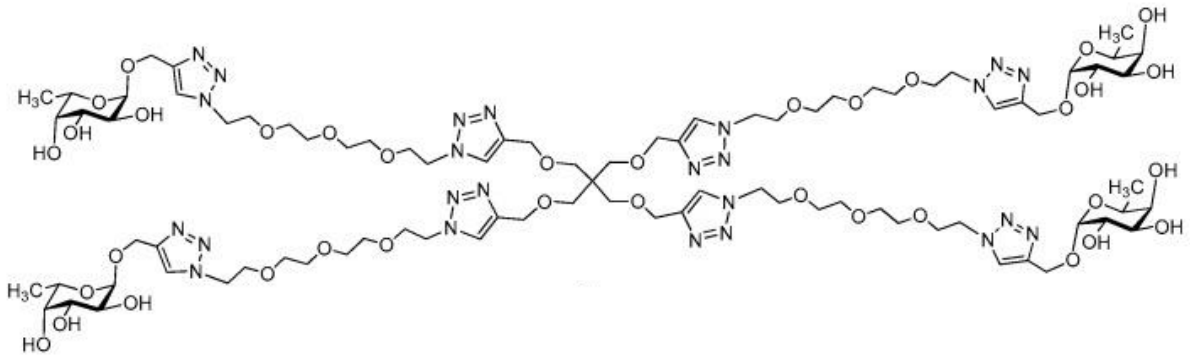


Figure 3. Tetravalent, α-L-fucose presenting glycocluster as universal ligand of focuphilic lectins

Tri- and tetravalent D-galactosides with methyl gallate and pentaerythritol scaffolds were also prepared using click chemisty as ligand of galactose-specific PA-IL lectin from *Pseudomonas aeruginosa*. *P. aeruginosa* is an opportunistic human pathogen associated with cystic fibrosis. This bacterium produces, among other virulence factors, a soluble D-galactose-specific lectin PA-IL (LecA). PA-IL plays an important role in the adhesion to the host cells and is also cytotoxic. Therefore, this protein is an interesting therapeutic target, suitable for inhibition by carbohydrate-based compounds. Methyl gallate and pentaerythritol equipped with propargyl groups were chosen as multivalent scaffolds and the galactoclusters were built from the above-mentioned cores by coupling ethylene or tetraethylene glycol-bridges and peracetylated propargyl β-D-galactosides using 1,3-dipolar azide-alkyne cycloaddition. The interaction between galactoside derivatives and PA-IL was investigated by several biophysical methods, including hemagglutination inhibition assay, isothermal titration calorimetry, analytical ultracentrifugation, and surface plasmon resonance. Their ability to inhibit adhesion of P. aeruginosa to bronchial cells was determined by ex vivo assay. The newly synthesized multivalent galactoclusters proved to be significantly better ligands than simple D-galactose for lectin PA-IL and as a result, two representatives of the dendrimers were able to decrease adhesion of *P. aeruginosa* to bronchial cells to approx. 32 % and 42 %, respectively. The

manuscript was published in *Biomolecules*, to the special issue: New Molecules: Towards the Drugs of the Future - Selected Papers from JMMC 2019.

We have some preliminary result in the synthesis of mono- and hepta-fuco- and -mannosylated ß-cyclodextrins. The mono- and heptakis-azido ß-CD was coupled with propargyl glycosides. Our further aim is to investigate the clusters as potential ligands of lectins and test the complexation effect on the aqueous solubility and bioavailability of drugs. A diploma thesis were written based on this topic (Petra Rozsos, Chemical Engineer, MSc).

The results were also published on conferences and presented as oral lectures at Annual Meeting of Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics (Mátraháza, 2018), CTB9 (Chemistry Towards Biology, 2018, Budapest) SAL (Synthesis and Analysis of Drugs, 2018, Brno), International Chemistry Conference (Romania, 2018), Joint Meeting on Medicinal Chemistry (Prague, 2019) and ELTE (Bruckner-lectures, 2019).

In this period, we achieved the synthesis of multivalent glycoclusters which contain seleno-glycosides. Our ealier studies suggested a tetravalent lead-compound as universal ligand of lectins, (Molecules, 2019) so we have synthesized it's analogues containing seleno-fucosides, -mannosides and galactosides. The synthetic strategy was the azido-alkyne click chemistry, the seleno-glycosides were prepared from bromo-sugars, reacted with selenourea, then seleno-propargylation was carried out. The tetravalent thio- and selenogalactoside clusters and together with disulfides and diselenosides were examined by hemagglutination assay for lectin-carbohydrate interaction studies and seleno-sugars were used for ^{77}Se-NMR studies and STD-NMR experiments, even in the presence of the appropriate bacterial lectin. Part of these results was published in *Molecules*, Special IssueTargeting Carbohydrate–Protein Interactions.

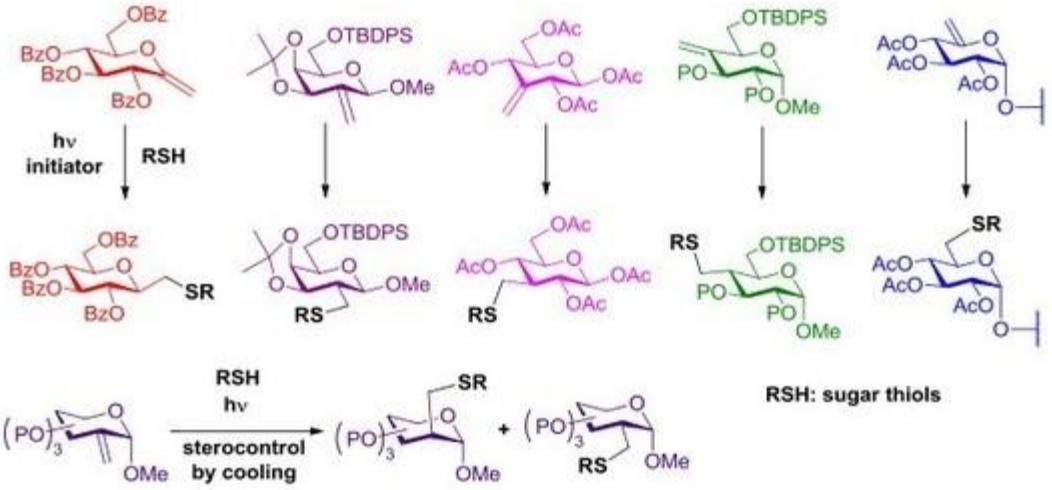
Finally, 3-fucosyl and 3'-fucosyl lactose-containing tetravalent glycoclusters were synthesized, as mixed ligands of galactophilic and fucophilic lectins. Pentaerythritol equipped with ethylene glycol-bridges and azide groups were chosen as multivalent scaffolds and the glycoclusters were built by coupling with propargyl 3-fucosyl lactoside and propargyl 3'-fucosyl lactoside using 1,3-dipolar azide-alkyne cycloaddition. The interaction between glycoclusters and PA-IL and PA-IIL were investigated by hemagglutination inhibition assay, isothermal microcalorimetry and saturation transfer difference NMR technique. The results may provide an opportunity to develop anti-adhesion therapy for the treatment of *P. aeruginosa* infection. Novel STD-NMR method development were also used to determine the protein-ligand interactions. These results are under publication to *Molecules.*

Results in the field of carbohydrate chemistry

For the preparation of glycoconjugates, we develop new methods for the synthesis of enzymatically stable O-, C-, S- and N-glycosides. The photoinitiated thiol−ene addition reaction is ideally suited for the synthesis of carbohydrate mimetics, moreover it is a highly stereo- and regioselective, and environmentally friendly reaction. A comprehensive study on UV-light-induced reactions of 2,3-unsaturated O-, C-, S- and N-glycosides with various thiols was performed and published in *Chem. Asian. J.* The effect of experimental parameters and structural variations of the alkenes and thiols on the efficacy and regio- and stereoselectivity of the reactions was systematically studied and optimized. The type of anomeric heteroatom was

found to profoundly affect the reactivity of 2,3-unsaturated sugars in the thiol−ene couplings. Hydrothiolation of 2,3-dideoxy O-glycosyl enosides efficiently produced the axially C2-S-substituted addition products with high to complete regioselectivity. Moderate efficacy and varying regio- and stereoselectivity were observed with 2,3-unsaturated N-glycosides and no addition occurred onto the endocyclic double bond of C-glycosides. Upon hydrothiolation of 2,3-unsaturated S-glycosides, the addition of thiyl radicals was followed by elimination of the thiyl aglycone resulting in 3-S-substituted glycals.

Moreover, in 2020, we reported on stereoselective synthesis of carbon-sulfur-bridged disaccharide mimetics by the free radical addition of carbohydrate thiols onto the exo-cyclic double bond of unsaturated sugars. A systematic study on UV-light initiated radical mediated hydrothiolation reactions of enoses bearing an exocyclic double bond at C1, C2, C3, C4, C5, and C6 positions of the pyranosyl ring with various sugar thiols was performed. The effect of temperature and structural variations of the alkenes and thiols on the efficacy and stereoselectivity of the reactions was systematically studied and optimized. The reactions proceeded with high efficacy and, in most cases, with complete diastereoselectivity producing a broad array of disaccharide mimetics coupling through an equatorially oriented methylensulfide bridge. These results were published in *Int. J. Mol. Sci.*



Novel results (Eszenyi et al., *Chem. Eur. J.*, 2018) was also published about promoting thiol-ene click reactions, which does not connect to the grant strictly, although it is a powerfool technique to create glycoclusters and in the future it is going to be used in the synthesis of multivalent structures.

Book chapter:

The Comprehensive Glycoscience was published in 2004, and chapter „Protecting Group Manipulations in Carbohydrate Synthesis" was refreshed during this year. I have updated two subchapters, namely „Protection of Hydroxyl Groups" and „Esters", in a 34 page-long version. It was published as Protecting Group Manipulations in Carbohydrate Synthesis. In: Reference Module in Chemistry, Molecular Sciences and Chemical Engineering.

Results in the field of carbohydrate-antibiotic chimeras

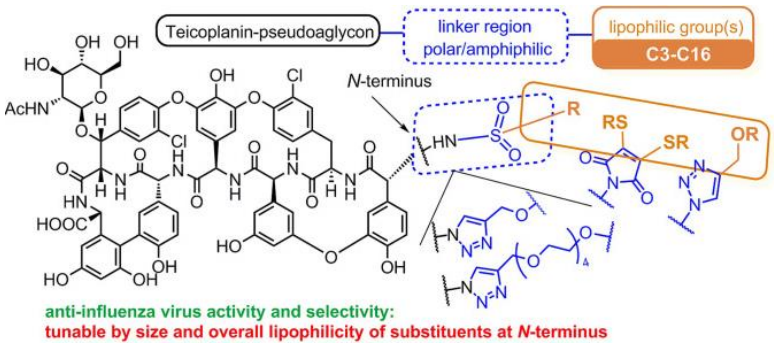
Preliminary experiments were carried out in the field of combination of anti-adhesion therapy with antibiotics. PA lectins of *P. aeruginosa* has got specificity to L-fucose and D-galactose, and it is well-known that ciprofloxacin is a widely used antibiotic against this infection. In order to create chimeric carbohydrate-antibiotic conjugate, L-fucose and D-galactose containing trivalent glycodendron was synthesized with an additional azido function. During our work, 6 chimeric fluoroquinolone antibiotics were synthesized including 4 fucoside- and 2 galactoside-containing chimeras. Although the antibacterial activity of these chimeric compounds was weaker than the effect of their fluoroquinolone parents, this result boded well for the future application of chimeric antibiotics. Despite the fact that there are many hurdles to overcome before chimeric antibiotics could make a success in antibiotic chemistry, it is worth considering this as a new tool to fight against bacterial infection and resistance. Additionally, CuAAC and photoinduced thiol-ene radical addition of click reaction have a great of advantages in synthetic chemistry. They are widely applied to conjugate molecules with suitable functional groups into one single compound in just one step-reaction with high yield and little by-product. There is no doubt that their application will continue to hold important postition in the future, particularly in the field of drug design and development.

These results were published in conferences as oral lectures (International Workshop on Chemistry and Chemical Biology of Carbohydrates, Nucleic Acids and Antibiotics and Antibiotics and Chemistry Towards Biology) and the publication is under construction.

Results in the field of antibiotics

A selection of some derivatives of teicoplanin pseudoaglycon were tested in vitro against clinical vancomycin-resistant Enterococcus strains possessing vanA, vanB or both genes. The tested compounds contain different carbohydrates and aryl groups as lipophilic moieties. About one-third of the teicoplanin-resistant strains were shown to be susceptible to one or more of the glycopeptide derivatives. The results were published in *The Journal of Antibiotics.*

Six series of semisynthetic lipophilic glycopeptide antibiotic derivatives were evaluated for in vitro activity against influenza A and B viruses. The new teicoplanin pseudoaglycon-derived lipoglycopeptides were prepared by coupling one or two side chains to the N-terminus of the glycopeptide core, using various conjugation methods. Three series of derivatives bearing two lipophilic groups were synthesized by attaching bis-alkylthio maleimides directly or through linkers of different lengths to the glycopeptide. Access to the fourth and fifth series of compounds was achieved by click chemistry, introducing single alkyl/aryl chains directly or through a tetraethylene glycol linker to the same position. A sixth group of semisynthetic derivatives was obtained by sulfonylation of the N-terminus. Of the 42 lipophilic teicoplanin pseudoaglycon derivatives were tested, about half showed broad activity against influenza A and B viruses, with some of them having reasonable or no cytotoxicity. Minor differences in the side chain length as well as lipophilicity appeared to have significant impact on antiviral activity and cytotoxicity. Several lipoglycopeptides were also found to be active against human coronavirus. The results were published in a D1-ranked journal (Zsolt Szűcs, Viktor Kelemen, Son Le Thai, Magdolna Csávás, Erzsébet Rőth, Gyula Batta, Annelies Stevaert, Evelien Vanderlinden, Lieve Naesens, Pál Herczegh, Anikó Borbás: Structure-activity relationship studies of lipophilic teicoplanin pseudoaglycon derivatives as new anti-influenza virus agents. *European Journal of Medicinal Chemistry,* 157, pp 1017-1030 (2018)).

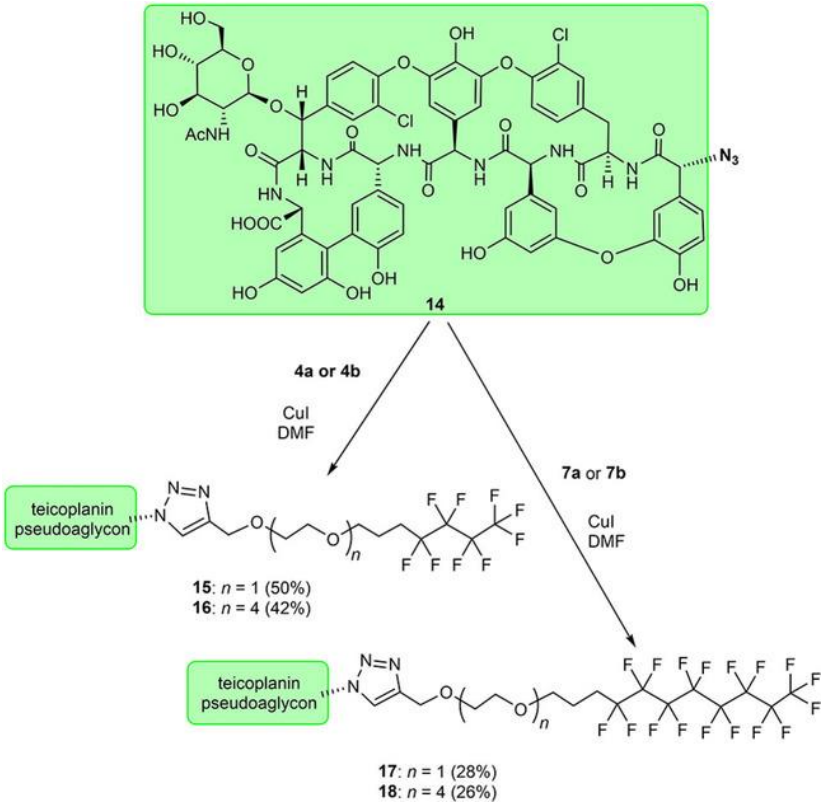


Ten analogues of a teicoplanin pseudoaglycon derivative have been synthesized with the aim of optimizing the in vitro activity of the compound against VanA type vancomycin resistant enterococci (VRE) isolated from hospitalized patients. Teicoplanin, vancomycin, and oritavancin were used as reference antibiotics for the antibacterial evaluations. One of the new derivatives exhibited far superior activity than the original compound. The in vitro MICs measured were comparable to that of oritavancin against the investigated VRE strains. The results were published in *The Journal of Antibiotics*, 2019, Zsolt Szűcs, Eszter Ostorházi, Máté Kicsák, Lajos Nagy, Anikó Borbás, Pál Herczegh: New semisynthetic teicoplanin derivatives have comparable in vitro activity to that of oritavancin against clinical isolates of VRE.

In the field of carbohydrate-antibiotic chimeras, we have synthesized eight hybrid compounds. Fuco- and galactocluster-fluorquinolone hybrids were prepared, as PA-IIL lectin of P. aeruginosa has got specificity to L-fucose and PA-IL lectin has got specificity to D-galactose, moreover it is well-known that ciprofloxacin is a widely used antibiotic against this infection.

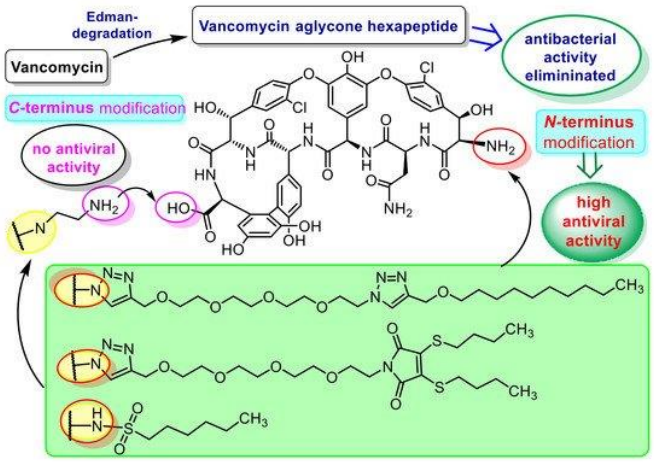
During the third year, new results (Szőke K, Czompa A, Lekli I, Szabados-Fürjesi P, Herczeg M, Csávás M, Borbás A, Herczegh P, Tósaki Á: A new, vasoactive hybrid aspirin containing nitrogen monoxide-releasing molsidomine moiety. *Eur. J. Pharm. Sci.*, 2019, 131 (2019) 159–166.) were also published about the synthesis of molsidomine-aspirin hybrid compound, which does not connect to the grant strictly, although it is also worth mentioning in the field of synthesis of chimeras as new candidates in the treatment of cardiovascular diseases.

Further synthetic modifications of teicoplanin pseudoaglycon were also carried out. Glycopeptide antibiotics and their lipophilic derivatives have emerged as relevant inhibitors of diverse viruses. We published a new strategy for the synthesis of dual hydrophobic and lipophobic derivatives of glycopeptides to produce selective antiviral agents without membrane-disrupting activity. Perfluorobutyl and perfluorooctyl moieties were attached through linkers of different length to azido derivatives of vancomycin aglycone and teicoplanin pseudoaglycone, and the new derivatives were evaluated against a diverse panel of viruses. The teicoplanin derivatives displayed strong anti-influenza virus activity at nontoxic concentrations. Some of the perfluoroalkylated glycopeptides were also active against a few other viruses such as herpes simplex virus or coronavirus. These data encourage further exploration of glycopeptide analogues for broad antiviral application. These results were published in *ChemMedChem.*



N-Terminal guanidine derivatives of teicoplanin antibiotics strongly active against glycopeptide resistant *Enterococcus faecium* were synthesized and published in *The Journal of Antibiotics* during this period. We designed and prepared guanidine and lipophilic guanidine derivatives of the glycopeptide antibiotic teicoplanin to armed them with activity against the most threatening nosocomial bacteria, multiresistant enterococci. From teicoplanin and its pseudoaglycone, a series of N-terminal guanidine derivatives have been prepared with free and amide C-terminal parts. Six aliphatic and aromatic lipophilic carbodiimides were prepared and used for the synthesis of lipophilic guanidine teicoplanin conjugates. All new N-terminal guanidine antibiotics showed high activity against a standard panel of Gram-positive bacteria. Four selected derivatives displayed excellent antibacterial activity against a series of nosocomial VanA *Enterococcus faecium* strains.

Glycopeptide vancomycin were modified to result potent antiviral agents devoid of antibacterial activity. Glycopeptide derivatives have emerged as a promising new class of antiviral agents. To avoid potential antibiotic resistance, these antiviral glycopeptides are preferably devoid of antibiotic activity. We prepared six vancomycin aglycone hexapeptide derivatives with the aim of obtaining compounds having anti-influenza virus but no antibacterial activity. Two of them exerted strong and selective inhibition of influenza A and B virus replication, while antibacterial activity was successfully eliminated by removing the critical N-terminal moiety. In addition, these two molecules offered protection against several other viruses, such as herpes simplex virus, yellow fever virus, Zika virus, and human coronavirus, classifying these glycopeptides as broad antiviral molecules with a favorable therapeutic index. These results were published in *Pharmaceuticals*, Special Issue „Glycopeptide Antibiotic 2020”.



Finally, a pioneer work was started in 2020, the very first synthetic modifications of pleuromutilin and lefamulin at alkene position C19-C20 were achieved using photoinitiated thiol-addition with wide-range of thiols, moreover radical addition reactions with perfluoroalkyl iodides. The antibacterial properties of the novel semisynthetic pleuromutilin derivatives were investigated on a panel of bacterial strains, containing susceptible and multiresistant pathogens and normal flora members. We have discovered several novel semisynthetic pleuromutilin derivatives with excellent antimicrobial properties. The publication is under process.

SUMMARY

As a summary, several multivalent glycoconjugates, carbohydrate-antibiotic chimeras and semisynthetic antibiotic were prepared with high biological relevance. The project resulted in 15 high-quality, peer-reviewed publication in international journals strongly connecting to the research plan, a book-chapter, eight conference presentations, and further five articles in the field of carbohydrate chemistry and four manuscripts is still under submission. The PI would like to say grateful thanks for the financial support.

Further academic achievements: Habilitation, 2020. september

Supervision: Le Thai Son, thesis under construction (Synthesis of glycomimetics, carbohydrate-containing chimeras and antibiotics with potential biological relevance)

There are some manuscripts under construction:

1. Evaluation of self-assembling 1,2-thiomannobioside glycoconjugates as potential multivalent ligands of mannose-binding lectin from *Burkholderia cenocepacia*

2. Synthesis of fucosylated lactose-presenting glycoclusters, investigation of their interactions with *Pseudomonas aeruginosa* lectin A (PA-IL) and B (PA-IIL)

3. Synthesis of multivalent α-L-fucopyranoside and fluoroquinolone carboxylic acid presenting chimeras and investigation of their antibacterial effect

4. The very first modification of pleuromutilin and lefamulin by photoinitiated radical addition reactions –Synthesis and antibacterial studies