Short thesis for the degree of doctor of philosophy (PhD)

Synthesis of new potentially biologically active sulfur- and selenium-containing carbohydrate derivatives

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1. Objectives of the doctoral thesis

Carbohydrates not only perform beneficial functions for the living organism, but also play a role in the recognition events related to diseases. Human galectin-3 (*h*Gal-3), a galactose-binding lectin, is implicated in numerous physiological and pathological processes. *P. aeruginosa* is an opportunistic human pathogen associated with cystic fibrosis. The tetrameric *P. aeruginosa* lectin LecA is a virulence factor and an anti-biofilm drug target.

Nowadays much attention is being paid to the development of Gal-3 and LecA inhibitors, including various glycoconjugates and glycomimetics.

Among sulfur- and selenium-containing carbohydrate derivatives, there are many examples of selective Gal-3 and LecA lectin inhibitors.

In my research work, I aimed to synthesize new types of divalent thio- and selenodigalactosides, which can be potential Gal-3 lectin inhibitors and which contain different substituents (homo- and heterocyclic, bimane derivatives) in position C-3 through oxygen or sulfur atoms. We were also focused on the synthesis of new, potential LecA lectin inhibitors, as bivalent selenogalactopyranosides, by CuAAC reaction with homo- and heteroaromatic ring systems or bimane framework as their central units.

Finally, we planned the preparation of monovalent aralkyl β-D selenoglycopyranoside derivatives which can be competitive ligands in lectin binding studies. Through the selenium atom, it is possible to examine the biological activity of selenoglycosides using ^{77}Se NMR methods.

2. Applied mehods

During our research work, we used the toolbox of preparative organic chemistry and structural analysis methods. We used thin-layer

chromatography to follow the reactions and check the purity of the compounds. The products were purified by column chromatography, preparative thick layer chromatography and crystallization. The verification of the structures of the new derivatives was carried out using modern NMR methods, as well as IR and MS methods.

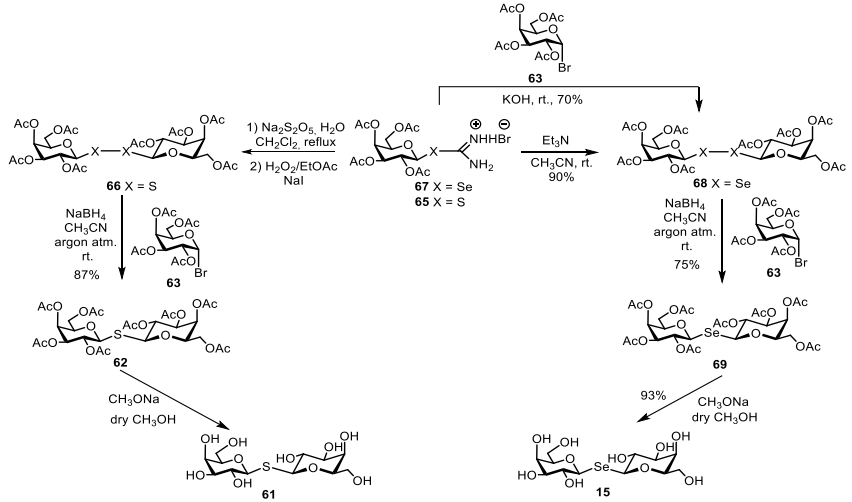
3. New scientific results

3.1. 3,3'-di-*O*-aralkyl TDG, SeDG and 3,3'-di-*S*-aryl TDG derivatives

3.1.1. 3,3'-di-*O*-aralkyl TDG derivatives

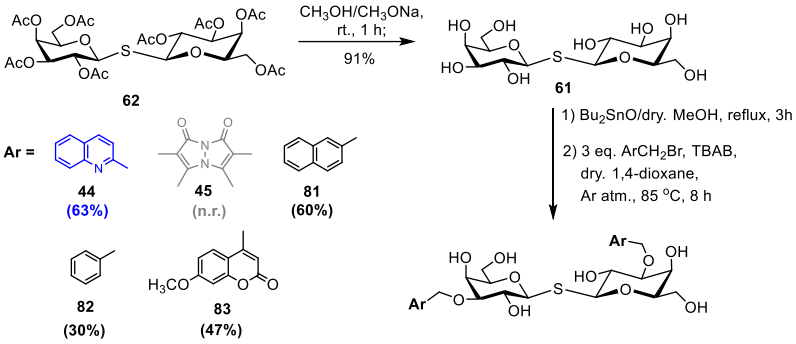
To study the interaction of TDG derivatives with hGal-3 using STD NMR spectroscopy and molecular docking simulations, new compounds were synhtesised.

We tested several methods known in the literature for the production of thiodigalactoside (TDG, **61)** and selenodigalactoside (SeDG, **15**) as starting materials, but none of them proved to have a good yield and could be scaled up. Finally we have developed a new synthetic method to obtain the compounds in several grams (**Sceme 1.).**



Scheme 1: The gram-scale of TDG (**61**) and SeDG (**15)**

We planned the synthesis of novel *N*-heterocycled derivatives. The synthesis of 3,3'-(quinoline-2-yl)methyl-di-*O*-disubtituted TDG derivative (**44**, Scheme 2) was succesful by Sn-acetal mediated regioselective substitution of TDG with excess of the reagent (2-(bromomethyl)quinoline, 3 eq.) in 1,4-dry dioxane, at 85 °C. Unfortunately, the bimane substrate (potencial flourescent probe) was not reactive under the conditions used. To compare the binding properties of the novel derivative to *h*Gal-3, three known 3,3'-aralkyl-disubstitued thiodigalactosides described previously, namely naphthalene-2-yl)methyl (**81**), benzyl (**82**) and (7-methoxy-2H-1-benzopyran-2-on-4-yl)methyl (**83**) derivatives at **Scheme 2**, were also synthesised using modified strategies for their preparation.

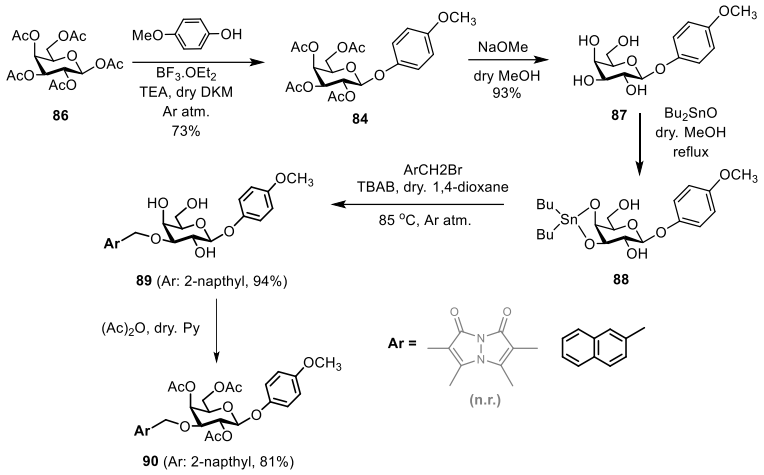


Scheme 2: Synthetic route of 3,3'-*O*-aralkyl disubstituted symmetrical thiodigalactosides

3.1.2. Synthesis strategies for the preparation of 3,3'-di-*O*-aralkyl-SeDG derivatives

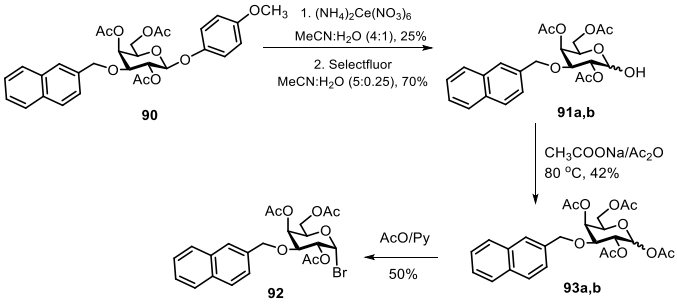
The preparation of 3,3'-di-*O*-aralkyl-SeDG derivatives was planned to be carried out by analogy with the synthesis of 3,3'-di-*O*-aralkyl-TDG derivatives. Sn acetal-mediated regioselective substitution of SeDG with excess of the reagents (*syn*-bimane, quinolone, naphthalene derivatives, 3 eq.) in 1,4-dry dioxane, at 85 °C were unsuccessful. Based

on literature methods, we prepared the SeDG derivatives starting from monosaccharide derivatives (**87**, **Scheme 3**.)



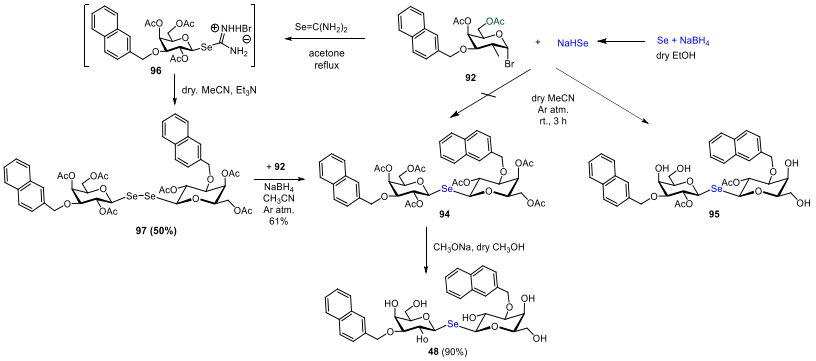
Scheme 3: Synthesis of PMP 3,3'-di-*O*-naphthalene-2-yl-β-D-galactopyranoside derivative

Removal of the PMP group was performed using two methods: Removal of the PMP group with CAN was performed with low yield (25%), and after studying the literature, we finally removed the PMP group with Selectfluor® reagent in good yield (70%, **Scheme 4.)**



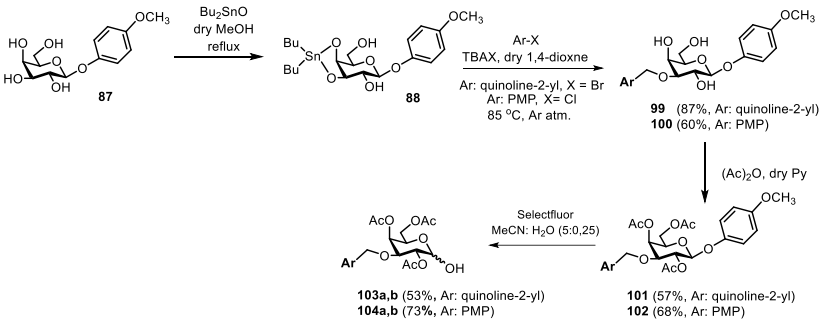
Scheme 4: Removal of the PMP group and the formation of **92** bromide

Acetilation of **91a,b** with CH_3COONa/Ac_2O followed by bromination with HBr/CH_3COOH afforded bromide derivative **92** in medium yield. The SeDG derivative **95** was prepared in the reaction of elemental selenium and derivative **92** under reductive conditions ($NaBH_4$ for reagent synthesis) in low yield. The isoselenuronium salt **96** was formed from the bromide **92** in good yield and it was reacted with triethylamine to obtain the symmetrical diselenide **97** in medium yield. Under the reductive conditions used in the production of TDG peracetate, reaction of bromide **92** with diselenide **97** afforded SeDG derivative **94** in good yield (61%, **Scheme 5.**). Deacetylation of acetate **94** with CH_3ONa/CH_3OH afforded **48** in high yield.



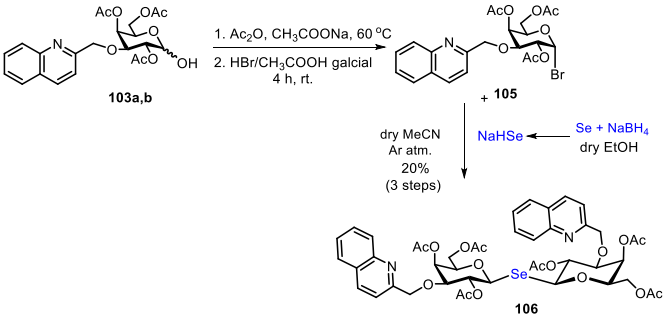
Scheme 5: The synthesis of SeDG derivative **48**

Further PMP 3,3'-di-*O*-aralkyl-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside derivatives were prepared for the synthesis of new SeDG derivatives.



Scheme 6. Synthesis of derivatives **103a,b** and **104a,b**

The protected quinoline derivative **106** was prepared in 20% overall yield using Se powder, started from anomeric mixture (**103a,b, Scheme 7.**).



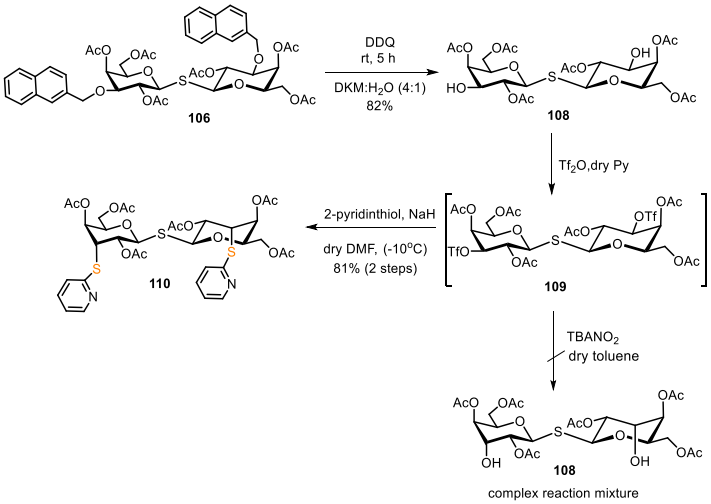
Scheme 7: The synthesis of SeDG derivative **106**

Under similar reaction conditions, 3,3'-di-*O*-PMBn-SeDG acetate could not be prepared. During the removal with the Selectfluor® reagent, the formation of fluorinated by-products in the aromatic ring were detected in the case of naphthalene and PMBn derivatives.

An important synthetic carbohydrate chemical result is that the PMP group can be selectively removed with Selectfluor® from the ^{2}NAP, quinolin-2-ylmethyl and PMBn protecting groups.

3.1.3. Synthesis of 3,3'-di-*S*-pyridin-2-yl-thiodiguloside derivative

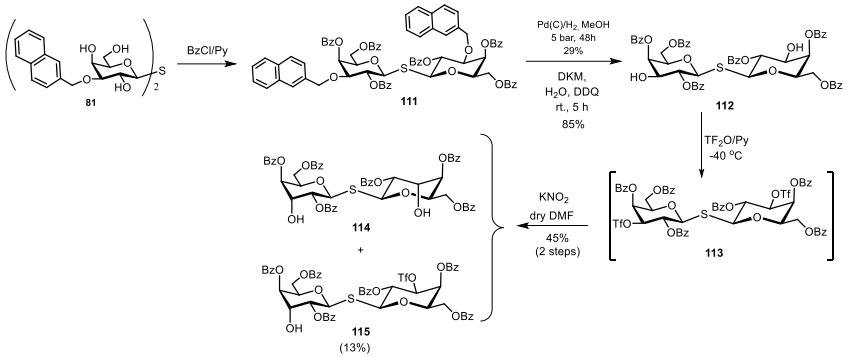
Based on *in silico* methods, it was established that the replacement of oxygen atoms in 3,3' positions with sulfur atoms and their substitution with aryl groups can result promising TDG derivatives in *h*Gal-3 lectin binding studies. Starting from 3,3'-di-*O*-naphthyl-2-yl-methyl-TDG derivative, an attempt was made to further transform the 3,3'-di-OH derivative with triflate formation and D-L inversion. Unfortunately, this reaction led to a complex mixture (**Scheme 8**.). The derivative **108** offers new possibilities for the synthesis of a dithio-thiodiguloside derivative, which can be a potential inhibitor of relevant galactophilic galectins.



Scheme 8: Synthesis of 3,3'-di-*S*-pyridin-2-yl-thiodiguloside derivative **110**

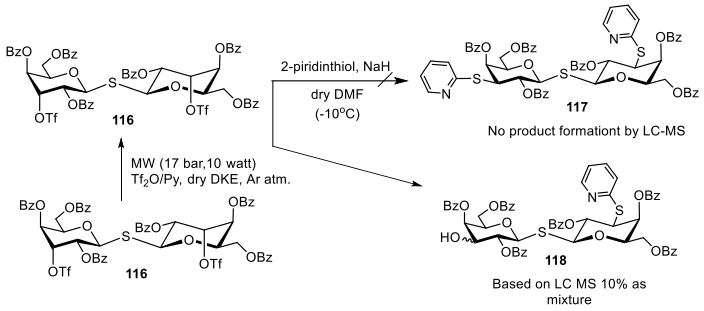
By reacting ditriflate derivative **109** with pyridine-2-thiol in the presence of NaH, thiodiguloside **110** was synthesized in good yield (**Scheme 8**.)

Since further transformations of the acetyl protected derivatives did not lead to aryl 3,3'-dithio galactose derivative, new synthesis was carried out with benzoyl protecting groups. Benzoylated **112** derivative was synthesized in two steps from **81** in good yield. In the hydrolytic step involving inversion following the transformation of the OH groups into triflate, we get a two-component mixture (**114** and **115**, **Sheme 9**.).



Scheme 9: Preparation of compound **114**

The syntheis and further transformation of 3,3'-*di*-O-triflyl gulo derivative **113** was carried out in different ways. Reaction of **116** with pyridine-2-thiol in the presence of NaH afforded derivative **118** as a mixture (10%) by LC-MS mesurements.

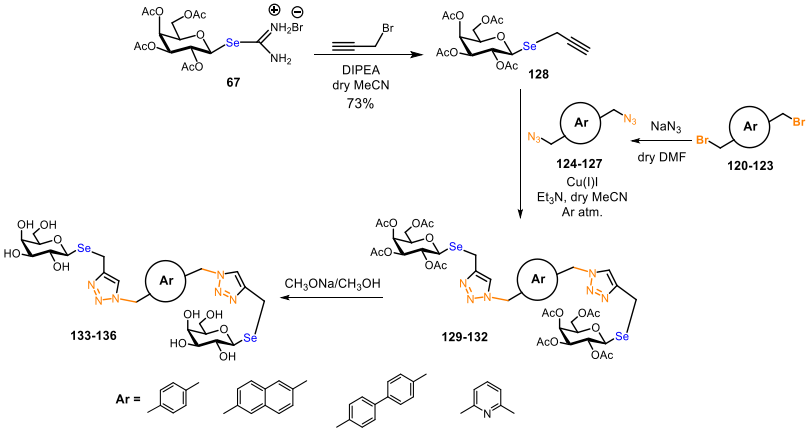


Scheme 10: The synthesis of 3,3'-*di*-*O*-triflyl gulo derivative

3.2. The synthesis of bivalent aralkyl 1,2,3-triazol-4-ylmethyl-seleno-β-D-galactopyranosides

Selenogalactosides were prepared by the CuAAC reaction, utilizing homo- and heteroaromatic rings or bimane scaffolds as the central "spacer" units in the derivatives. Homo- and heteroaromatic dimethyl diazides were synthesized from the corresponding bromides in dry DMF at room temperature in good yields. Conversion of fluorescent dibromobimane to diazide with NaN_3 and TMSA was unsuccessful.

By reacting the salt **67** with propargyl bromide in dry CH_3CN, prop-2-yn-1-yl 2,3,4,6-tetra-*O*-acetyl-β-D-selenogalactopyranoside **128** was obtained in good yield. (**Scheme 11**.)



Scheme 11: CuAAC reaction of **128** with aromatic diazidomethyl derivatives

The aromatic diazidomethyl derivatives (**124-127**) were reacted with **128** in the presence of, Cu(I) catalyst, in dry CH_3CN under an inert atmosphere; the new bivalent compounds **129-132** were obtained in good yields (**Table 1.**).

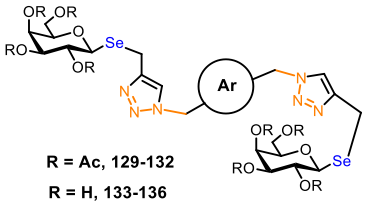
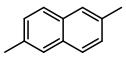
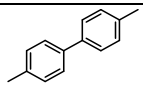
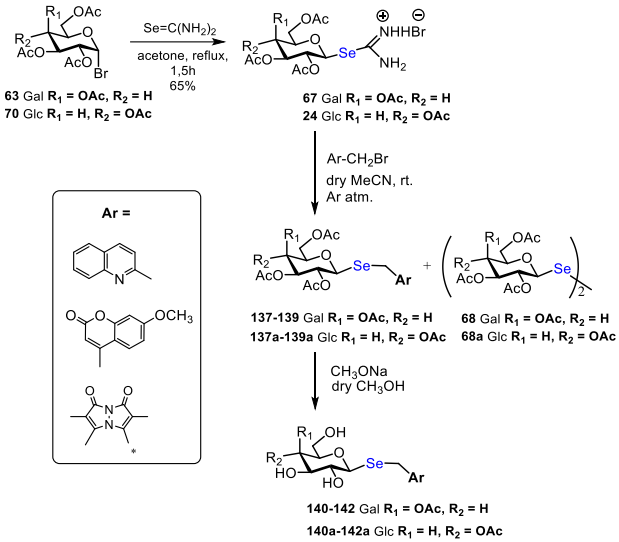
Aralkyl 1,2,3-triazolil-4-ylmethyl-seleno-β-D-galactopyranosides	Ar	Isolated yields (%)	R
		56 82	**129** (Ac) **133** (H)
		71 78	**130** (Ac) **134** (H)
		60 80	**131** (Ac) **135** (H)
		50 82	**132** (Ac) **136** (H)

Table 1: Isolated yields of aralkyl 1,2,3-triazolil-4-ylmethyl-seleno-β-D-galactopyranosides

The removal of the protecting groups was carried out under Zemplén conditions (**Scheme 11**.).

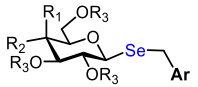
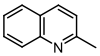
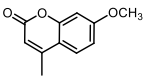
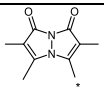
3.3. The synthesis of new monovalent selenoglycosides

The use of the bivalent selenodigalactoside for the investigation of carbohydrate-protein interactions is known, thanks to the ^{77}Se NMR active nucleus. We synthesized selenoglycosides in which the aglycones occur as substituents among the 3,3'-di-*O*-aralkyl TDG derivatives, which were proven to bind to *h*Gal-3 galectin. The new derivatives were prepared from selenuronium salts **67**, **24** by reactions with 2-(bromomethyl)quinoline and 4-bromomethyl-7-methoxycoumarin, at room temperature, in dry acetonitrile, in the presence of Et_3N base, in an argon atmosphere.



Scheme 12: Aralkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosides

In the reaction with 4-bromomethyl-*syn*-bimane derivative, no selenoglycoside formation was observed.

Aralkyl β-D-selenoglycopyranosides	**Ar**	**Isolated yields**	**R_1**	**R_2**	**R_3**
 **137-139 R_1 = OAc, R_2 = H** **137a-139a R_1 = H, R_2 = OAc** **140-142 R_1 = OAc, R_2 = H** **140a-142a R_1 = H, R_2 = OAc**		**137** (78%) **140** (83%)	OAc OH	H H	Ac H
		**138** (76%) **141** (82%)	OAc OH	H H	Ac H
		**139** (not formed)	OAc	H	Ac
		**137a** (75%)	H H	OAc OH	Ac H

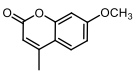
		140a (70%)			
		**138a** (71%) **141a** (80%)	H H	OAc OH	Ac H

Table 2: Yields of the selenoglycosides

The structure verification of the new derivatives was carried out by evaluating NMR measurements.

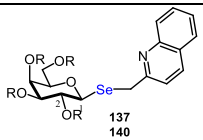
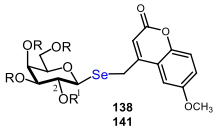
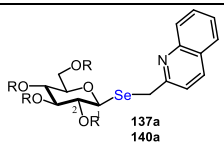
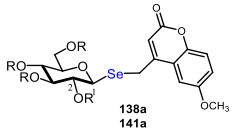
Aralkil β-D-selenoglycopyranosides	**δ ^{1}H (ppm, 500 MHz)** *Se*-CH_2	**$^{2}J_{H,H}$ (Hz)** *Se*-CH_2
 **137 140**	4.26- 4.08 (**137**, R= Ac, $CDCl_3$) 4.61- 4.36 (**140**, R = H, CD_3OD)	12.0 13.1
 **138 141**	4.09- 3.92 (**138**, R= Ac, $CDCl_3$) 4.21- 4.05 (**141**, R = H, CD_3OD)	overlapping signals 12.1
 **137a 140a**	4.14- 4.02 (**137a**, R= Ac, $CDCl_3$) 4.39- 4.22 (**140a,** R = H, CD_3OD)	11.9 12.0
 **138a 141a**	4.01- 3.90 (**138a**, R= Ac, $CDCl_3$) 4.12- 4.03 (**141a**, R = H, DMSO-d_6)	11.8 11.8

Table 3: Characteristic NMR spectral data of $SeCH_2$ protons in selenoglycosides

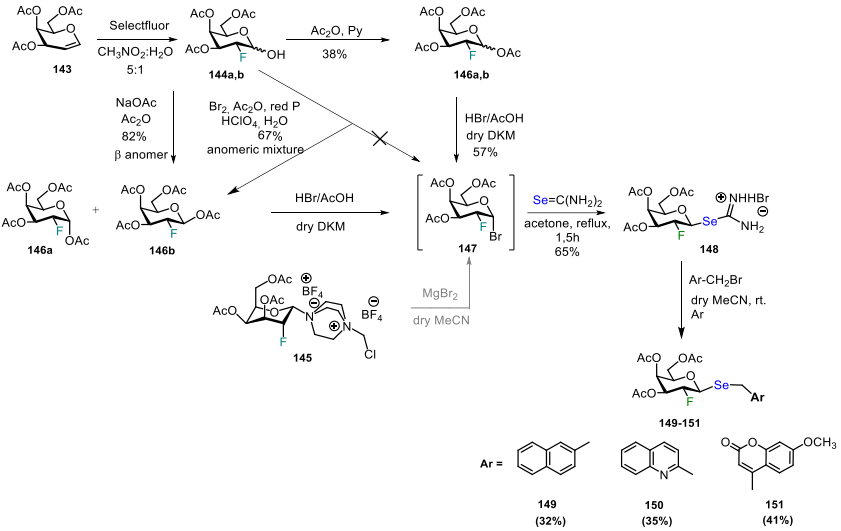
The β-D-galactopyranoside derivatives are potential Gal-3 and LecA monovalent inhibitors in interaction studies using ^{77}Se NMR methods; glucose analogs can be tested as competitive non-binding ligands in binding assays. At the same time the new selenoglycosides

137-139 and **137a-139a** were sent for parasitological studies together with other selenium-containing carbohydrate derivatives. Dr. Marcelo Comini, professor at the Pasteur Institute in Montevideo, Uruguay and his group confirmed that the new derivatives showed activity against *T. brucei* parasite.

3.4. Aralkyl 2-deoxy-2-fluoro-3,4,6-tri-O-acetyl-β-D-selenogalactopyranosides

Based on the results of the parasitological tests, the question arose as to whether the bioactivity of the selenoglycosides produced by us can be increased if an acetyl group in the carbohydrate derivatives is replaced by a fluorine atom. In order to answer the question, we planned to produce a new type of aralkyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-β-D-selenogalactopyranosides, with the aim of detecting potential antiparasitic activity against *T. brucei.* 3,4,6-tri-*O*-acetylgalactal **143** was obtained from acetobromogalactose by an elimination reaction in CH_3CN in good yield.

The fluorination reaction of **143**, which was carried out with Selectfluor® in a 5:1 mixture of CH_3NO_2: H_2O; the expected anomeric mixture **144a,b** was formed with a low yield (30%), while the salt **145** was isolated from the reaction mixture with a 60% yield (**Scheme 13.**)



Scheme 13: The synthesis of aralkyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-β-D-selenogalactopyranosides

Acetylation of **144a,b** with CH_3COONa/Ac_2O followed by bromination reaction afforded 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide **147** in good yield. Selenuronium salt formation was carried out in similar manner to **24** or **67** formation (**Scheme 12**.), in reaction of **147** bromide with selenourea in acetone at reflux temperature.

The isoselenuronium salt **148** was reacted with various aryl methyl bromides, in dry CH_3CN, at room temperature, under an argon atmosphere, in the presence of Et_3N according to the method described for aralkyl 2,3,4,6-tetra-*O*-acetyl-β-D-selenogalactopyranosides.

The presence of a fluorine atom in a molecule is confirmed by the multiplets appearing as a result of $^{19}F–^{1}H$ and $^{19}F–^{13}C$ couplings in the ^{1}H and ^{13}C spectra of the given compounds. **Table 4** summarizes the characteristic NMR spectral characteristics of aralkyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-β-D-selenogalactopyranosides.

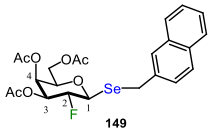
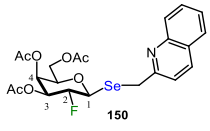
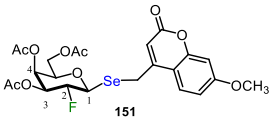
2-dezoxy-2-fluoro-3,4,6-tri-*O*-acetyl-β-D-selenogalactopyranosides	δ ^{13}C (ppm, 125 MHz)	$^{n}J_{C,F}$ (Hz)
 **149**	C-1 75.8 C-2 87.4 *Se*-CH_2 27.1	$^{1}J_{C2,F}$ = 187.1 $^{2}J_{C1,F}$ = 26.0 $^{2}J_{C3,F}$ = 19.9 $^{3}J_{C4,F}$ = 8.34
 **150**	C-1 76.7 C-2 87.3 *Se*-CH_2 29.2	$^{1}J_{C2,F}$ =187.5 $^{2}J_{C1,F}$ = 25.6 $^{2}J_{C3,F}$ = 19.8 $^{3}J_{C4,F}$ = 8.14
 **151**	C-1 75.9 C-2 87.1 *Se*-CH_2 21.2	$^{1}J_{C2,F}$ = 187.0 $^{2}J_{C1,F}$ = 26.0 $^{2}J_{C1,F}$ = 19.3 $^{3}J_{C1,F}$ = 8.17

Table 4: Characteristic ^{13}C (ppm) and ^{n}JC,F (Hz) values of derivatives **149-151**

4. Biological studies, results

The **44** quinoline derivative binds to *h*Gal-3, which binding was confirmed by Dr. István Timári and Bence László Farkas with ^{1}H STD NMR measurements.

Selenoglycosides have been tested in parasitological studies: coumarin derivative **141** (EC_{50} 4.1 μM) and quinoline derivative **137a** (EC_{50} 1.2 μM) showed activity against *T. brucei*.

2-F-selenogalactoside derivatives were tested in SARS-CoV-19 antiviral tests in South Korea. Based on the results, quinoline and coumarin derivatives have antiviral effects. The EC_{50} value determined for the quinoline **150** derivative is 21.3 μM, while the EC_{50} for the **151** coumarin derivative is 29.5 μM.

5. Possible application of the results

The optimized method for the synthesis of TDG and SeDG is suitable for synthesis of several non symmetrical thio- and selenglycosides.

The new synthesis of 3,3’-dithio-(pyridine-2-yl)-thiodiguloside derivative provides new perspectives for the preparation of new dithiodigulosides with diverse structures.

Based on the anti-parasitic and anti-viral effects of selenoglycosides, the synthesis of new selenoglycosides containing natural heterocycles may open up interesting synthetic possibilities.

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Registry number: DEENK/527/2024.PL
Subject: PhD Publication List

Candidate: Fanni Hőgye
Doctoral School: Doctoral School of Chemistry
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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Hőgye, F.**, Farkas, L. B., Balogh, Á. K., Szilágyi, L., Samar, A., Bajza, I., Borbás, A., Fehér, K., Illyés, T. Z., Timári, I.: Saturation Transfer Difference NMR and Molecular DockingInteraction Study of Aralkyl-Thiodigalactosides as Potential Inhibitors of the Human-Galectin-3 Protein. *Int. J. Mol. Sci. 25* (3), 1-18, 2024. EISSN: 1422-0067.
   DOI: http://dx.doi.org/10.3390/ijms25031742
   IF: 4.9 (2023)
2. Dibello, E., Oddone, N., Franco, J., Illyés, T. Z., Medeiros, A., Kiss-Szikszai, A., **Hőgye, F.**, Kövér, K. E., Szilágyi, L., Comini, M. A.: Selenosugars targeting the infective stage of Trypanosoma brucei with high selectivity.
   Int. J. Parasitol.-Drugs Drug Resist. 24, 1-6, 2024. ISSN: 2211-3207.
   DOI: http://dx.doi.org/10.1016/j.ijpddr.2024.100529
   IF: 4.1 (2023)

Total IF of journals (all publications): 9
Total IF of journals (publications related to the dissertation): 9

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

24 October, 2024

Conference participations

Oral presentations

1. Hőgye Fanni, Illyés Tünde Zita, Szilágyi László

Bimán tartalmú, potenciális lektingátló hatású szénhidrátszármazékok szintézise

MTA Szénhidrát-, Nukleotid- és Antibiotikumkémiai Munkabizottság Előadóülés, Online; 2021. június.

2. Hőgye Fanni, Illyés Tünde Zita, Szilágyi László

Tio-diglikozidok szintézise és funkcionalizálása

Erdélyi Magyar Műszaki Tudományos Társaság, XXVII. Nemzetközi Vegyészkonferencia, Online; 2021. október.

3. Hőgye Fanni, Illyés Tünde Zita, Szilágyi László

Kísérletek 3,3'-ditio-tio-digalaktozid előállítására és aralkil halogenidekkel történő továbbalakítására

Tavaszi Szél Konferencia (III. helyezés), Miskolc, 2023. május.

4. Hőgye Fanni, Tóth Viktória, Illyés Tünde Zita, Szilágyi László

Homo- és heteroaromás szubsztituenseket tartalmazó szelenoglikozidok szintézise

MTA Szénhidrát-, Nukleotid- és Antibiotikumkémiai Munkabizottság Előadóülése, Mátrafüred, 2023. június.

5. Hőgye Fanni, Farkas László Bence, Timári István, Illyés Tünde Zita, Szilágyi László, Bajza István

Tio-digalaktozid 3,3'-helyzeteiben történő módosításai

Erdélyi Magyar Műszaki Tudományos Társaság, XXIX. Nemzetközi Vegyészkonferencia, Marosvásárhely, 2023. november.

Poster presentations

1. Fanni Hőgye, Tünde Zita Illyés, László Szilágyi

Syntheses of 2-fluoro-selenogalactoside derivatives

Debrecen Colloquium on Carbohydrates 2020 in 2022 Rezső Bognár Memorial Conference on Glycomimetics, Debrecen.

2. Fanni Hőgye, Viktória Tóth, Tünde Zita Illyés, László Szilágyi

Aralkil szelenoglikozidok és módosított tiodigalaktozidok előállítása MKE 4. Nemzeti Konferencia, Eger, 2023. július.

Other posters not related to the PhD thesis

1. László Bence Farkas, Álex Kálmán Balogh, Fanni Hőgye, László Szilágyi, Tünde Zita Illyés, Krisztina Fehér, István Timári
Investigating Galactoside-Human Galectin-3 Interactions with Advanced NMR and Computational Methods
Experimental Nuclear Magnetic Resonance (ENC), april 6-10, 2024, Pacific Grove, CA, USA.
2. László Bence Farkas, Fanni Hőgye, Álex Kálmán Balogh, Tamás Gáti, László Szilágyi, Samar Alnukari, Krisztina Fehér, Tünde Zita Illyés and István Timári
Interaction of Aralkyl-Thiodigalactosides as Potential Inhibitors of Human-Galectin-3 Investigated by Saturation Transfer Difference NMR and Molecular Docking Simulation
Final iNEXT-Discovery Consortium Meeting & 4th Symposium on Recent Advances in Cryo-EM, Brno, june 10-13, 2024.