

Theses of PhD dissertation

**SYNTHESES OF SULFUR- AND SELENIUM CONTAINING
GLYCOMIMETICS**

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1. Introduction and objectives

Cell-surface *N*- and *O*-glycosides constitute a unique pattern of glycan structure often called as a fingerprint of the cell. The formation of these structures are strictly regulated via reactions catalyzed by cooperative actions of glycosyl transferases and glycosydases. The diversity of sequence and steric shape of glycosyl clusters are encoding an enormous amount of information giving rise to the recent emergence of the concept of „sugar code”. One approach of decoding this information is based on the interactions of glycans with carbohydrate receptor proteins. Carbohydrate-protein recognition processes are receiving increased attention in recent years. One of the most important representatives of carbohydrate-recognition proteins are the lectins which have first been isolated from plants such as the *Viscum album* agglutinin (VAA).

Animal and human lectins, on the other hand, are responsible for a wide range of physiologically relevant processes such as intracellular signalling, cell-cell or cell-intercellular matrix interactions. Galectins, an important class of carbohydrate-recognition proteins, while weakly interacting with galactose are displaying increased specificity and affinity toward lactose and *N*-acetyllactosamine. Abnormal regulations of galectins such as enhanced production levels are in the background of pathological processes such as inflammation, rheumatoid arthritis fibrosis or cancer.

Increasing insight into the nature of lectin-glycan interactions has led to the development of clinically efficient glycomimetics such as sialyl Lewis-X for inhibiting allergic reactions in ischemic tissues or Bimosiamose which was found efficient for treating respiratory tract inflammation in asthmatic patients.

Galectin-3 has recently emerged as a significant therapeutic target in glycomimetics-directed research. Digalactosyl thioglycosides substituted at C-3 or C-1 substituted multivalent lactose derivatives were found to show most promising effects. For instance, a derivative labeled as TD131_1 efficiently decreased the resistance of thyroid tumor cells especially when combined with doxorubicin or ionizing irradiation. A lactulosyl-L-leucin derivative was found to enhance cell apoptosis induced by Taxol. Galectin-1 and galectin-3 very often exert opposing physiological effects; their selective inhibition is therefore of

importance. The affinity of a C-3 substituted symmetric digalactosyl sulfide derivative toward galectin-3 was found to exceed the affinity toward galectin-1 by a factor of 200.

The main objective of my PhD work was to synthesize novel glycomimetics as potential inhibitors against plant agglutinins and/or lectins that are harmful to animal/human organisms. These derivatives were designed to contain glycosidic linkages resistant to hydrolysis by glycosidase enzymes.

The targeted molecules are characterized by the attachment of mono- or diglycosyl moieties as suitable epitopes for lectin binding to a central isocyclic aromatic core such as benzene or naphthalene as well as to the heterocyclic aglycone 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione („bimane”). For the presentation of lectin-compatible cognate sugars galactosyl-, glucosyl-, mannosyl- or lactosyl derivatives have been prepared.

2. Experimental techniques

Established methods of synthetic organic chemistry at the macro-, semimicro- or micro level have been utilized to obtain the desired compounds. The reactions were monitored by thin-layer chromatography, crystallization or column chromatography have been used for isolation/purification purposes. Identification and structural characterization of the new derivatives have been achieved via 1D/2D NMR spectroscopy, single crystal X-ray diffraction and high resolution mass spectrometry, in addition to classical analytical techniques.

3. Results

3.1. Syntheses of glycosylated benzene- and naphthalene derivatives

Reactions of **22** 1,4-bis(bromomethyl)naphthalene with **15(b,d)** per-*O*-acetyl-D-glycopyranosyl thiols furnished **26**, **27** thioglycosides, whereas **20**, **22**, **25**, **28**, **29** bis(bromomethyl)arenes reactions with **17b** 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl selenouronium bromides resulted in the formation of **30-34** seleno-glycosides (fig. 1.). The **41-45** dithio-glycosides were obtained via reacting **15(b,d)** per-*O*-acetyl- β -D-glycopyranosyl thiols with **38-40** bis-methanethiosulfonic acid esters (fig. 2.).

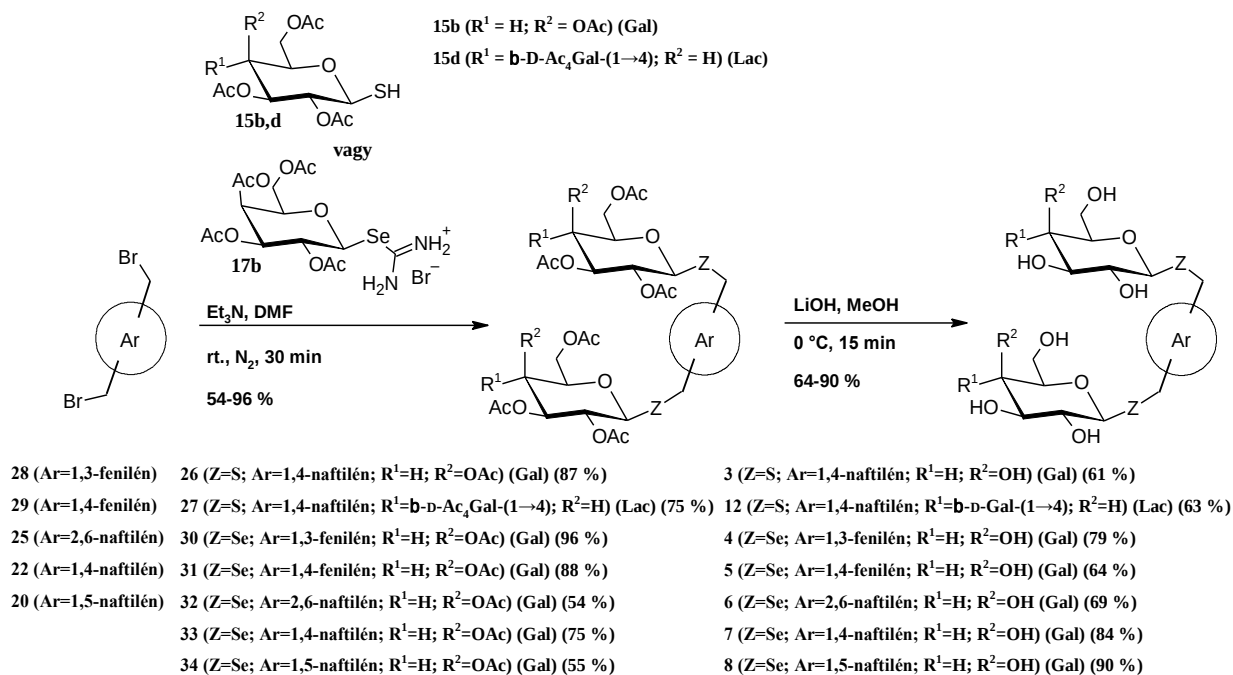


Fig. 1.

Syntheses of thio- and seleno-glycosides based on benzene/naphthalene core

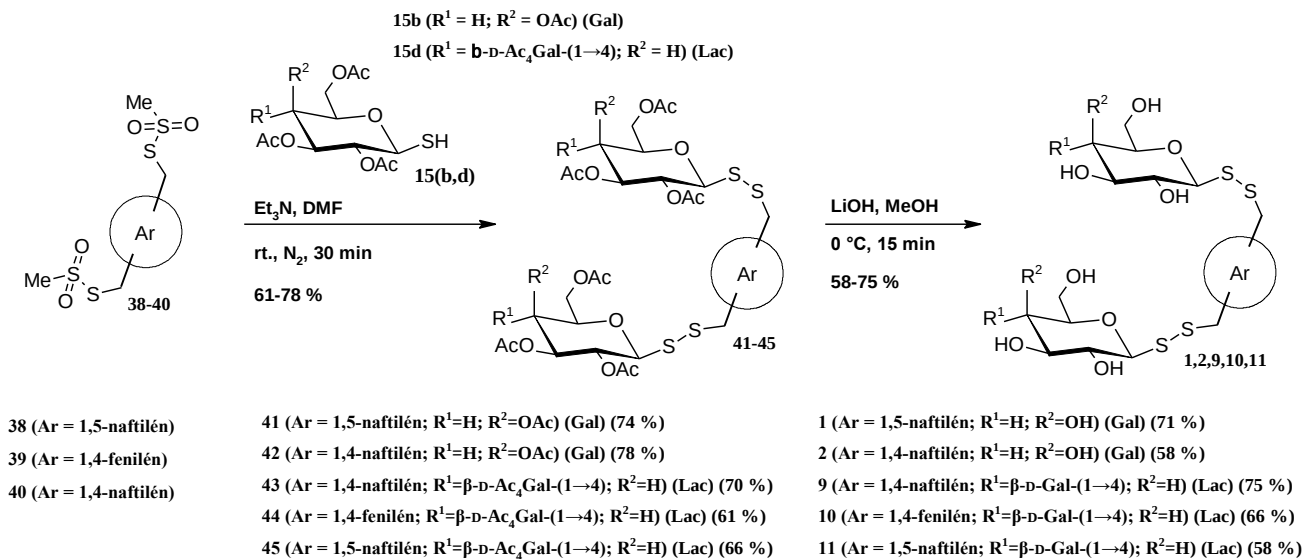


Fig. 2.

Syntheses of dithioglycosides based on benzene/naphthalene core

The acetyl protecting groups from the per-O-acetylated derivatives **41-45**, **26**, **27**, **30-34** have been removed by treatment with stoichiometric amounts of lithium hydroxide to furnish aromatic glycoconjugates **1-12** with free OH-groups in good yields.

3.2. Structural characterization and biological activity of glycosylated benzene- and naphthalene derivatives

The conformational properties of selected examples from among molecules **1-12** were explored by Dr. Krisztina Fehér (University of Gent) using molecular dynamics calculations. Inter-headgroup orientations were characterized by distances between anomeric (C-1) atoms of the two glycosyl units attached to the aromatic cores. These distances varied in the range between 4-11 Å for selenogalactosides **4** and **5** or disulfido lactosides **9** and **11** indicating accessibility to nearly the full conformational space allowed by rotations around the glycosidic bonds.

The biological activities of **1-12** were investigated by Prof. Hans-Joachim Gabius and his group (Ludwig Maximilians University, Munich). Inhibitory activities against lectin binding to glycans were tested in three different systems. Solid phase assays using a surface-presented glycoprotein (asialofetuin) indicated enhancements of binding up to 10fold with respect of the cognate sugar with the disulfide **2** and selenoglycoside **6** as the most efficient inhibitors against the plant toxin VAA. On the other hand, disulfide **9** emerged as the most active molecule to inhibit binding of three different human galectins to glycans on the cell-surface of human tumor cell lines as demonstrated by flow cytofluorometric analysis. Taking a step further to test the inhibitory potencies of our naphthalene-based glycoconjugates on more complex systems murine tissue sections were used in a histochemical approach. In these tests compounds **9** and **12** displayed outstanding efficiency in preventing the binding of human lectins to the tissues investigated; for instance, the lactose-presented **9** produced a ca. 100fold increase with respect to free lactose to inhibit binding of galectin-8 to fixed murine jejunum tissue.

3.3. Syntheses of glycosylated bimane derivatives

Reactions of **62** *syn*- and **63** *anti*-(CH₂Br;CH₃)B with **15(a-d)** per-*O*-acetylated-D-glycopyranosyl thiols furnished thioglycosides **64(a-d)**, **65(a-d)** thio-glycosides whereas the same dibromobimanes provided **66(a,b)**, **67(a-c)** selenoglycosides (fig. 3.) when reacted with **17(a-c)** 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl selenouronium bromides.

Reactions with non-sugar thiols resulted in the formation of novel sulfur containing bimanes such as **68** *syn*-(CH₂SAc;CH₃)B, **69** *syn*-(CH₂SH;CH₃)B, **71** *anti*-(CH₂SAc;CH₃)B (3. ábra), including **70** *syn*-(CH₂S-;CH₃)B featuring an intramolecular disulfide bridge.

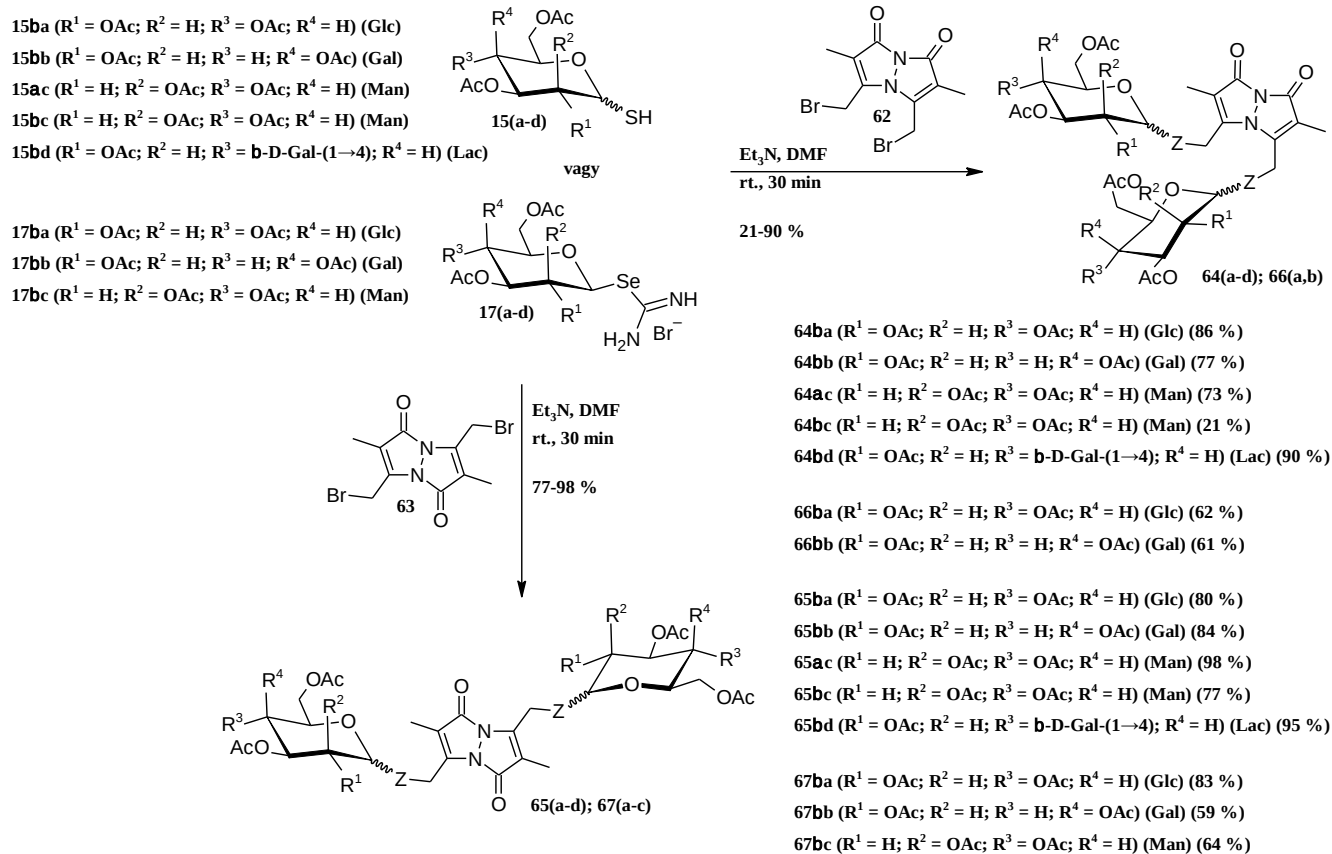


Fig. 3.
Syntheses of bimane thio- and selenoglycosides

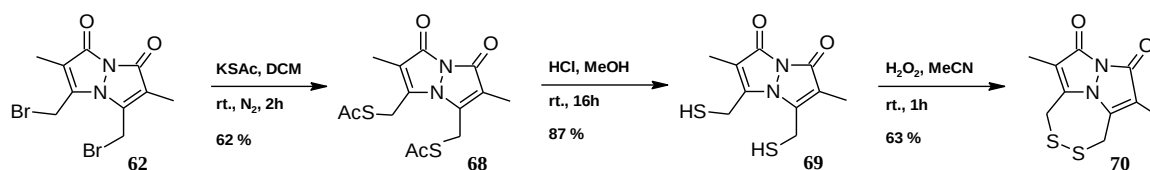


Fig. 4.
Syntheses of non glycosylated bimanane derivatives

A further interglycosidic linkage motif, the disulfide bond, has been introduced to obtain novel glycosylated bimananes **74(a-c)** *syn*-(CH₂S₂GlyAc₄;CH₃)B via reacting **73(a-c)** *N*-phthaloyl-*S*-(2,3,4,6-tetra-*O*-acetyl- β -D-glycopyranosyl)sulfenamides with the bimanane dithiol **69** *syn*-(CH₂SH;CH₃)B.

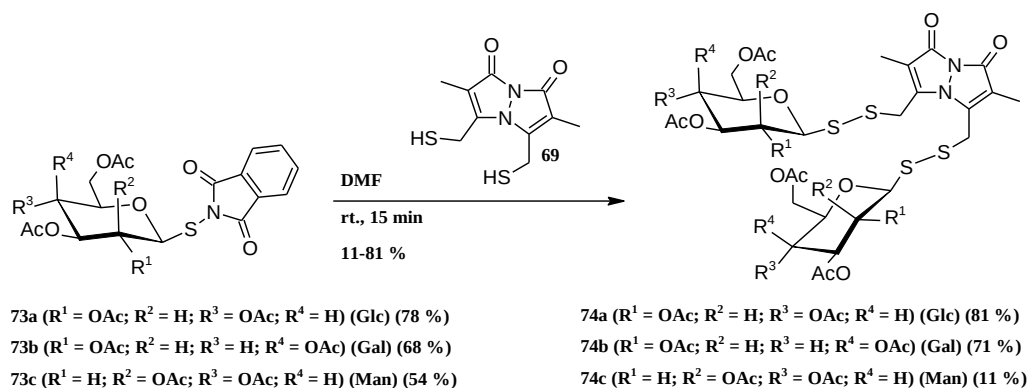


Fig. 5.
Syntheses of bimanane dithio-glycosides

Exploring further possibilities for the attachment of carbohydrate moieties to bimananes exploitation of the CuAAC („click”) reaction appeared to be an attractive option in view of the convenient availability of glycosyl azides. Reactions of per-*O*-acetyl- β -D-glycopyranosyl azides **79(a-d)** with the *syn*-(CH₃;C $\equiv$ CTMS)B **78** resulted in the formation of 1,2,3-triazolo glycosides **80(a-d)** in good yields. The trimethylsilyl groups of alkyne **78** were cleaved in a tandem reaction during the coupling with the azides. The acetyl groups of **80(a-d)** could be removed by treatment with LiOH to furnish triazolo derivatives **81(a-d)** with free OH-groups.

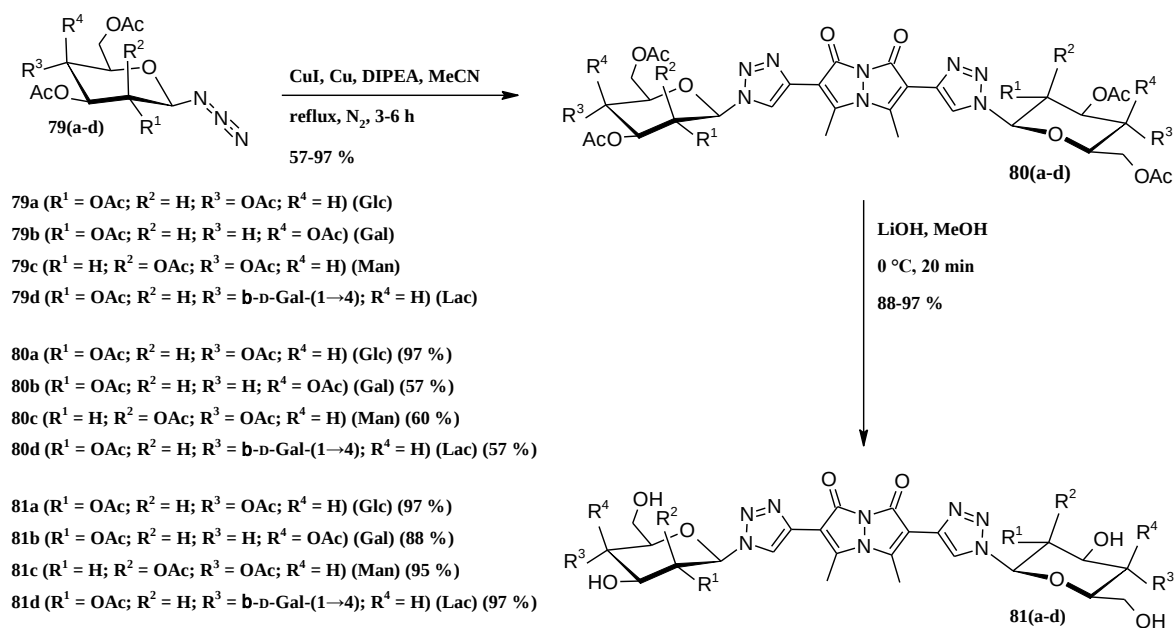


Fig. 6.
Syntheses of bimane dithio-glycosides

3.4. Structural characterisation, spectroscopic data and bioactivity of glycosylated bimane derivatives

To investigate potential influence of appended glycosyl moieties on the known fluorescent properties of the bimane skeleton UV-Vis absorption and fluorescence spectra of the novel glycosylated bimanies have been measured. *Syn* isomers were found to be fluorescent as expected with one notable exception: missing fluorescence in *Se*-glycoside **66** is likely to be attributed to a photoinduced electron-transfer (PET) process between *Se* and the bimane fluorophore. Configuration changes in the glycosyl units did not, however, have any noticeable influence on the fluorescent properties.

Single-crystal X-ray diffraction measurements have been performed by Dr. Attila Bényei to determine the solid state structures of **64** *syn*-(CH₂SGlcAc₄;CH₃)B, **65** *anti*-(CH₂SGlcAc₄;CH₃)B, **68** *syn*-(CH₂SAC;CH₃)B, **70** *syn*-(CH₂S-;CH₃)B, **80** *syn*-(CH₃;TAGlcAc₄)B. Two independent molecules have often been observed in the asymmetric units. Crystallographic analysis in several cases revealed nonplanarity of the bimane skeleton ie., the angle subtended by the mean planes of the two pyrazolone rings

varying between 0-50 deg. This angle was found to be 13/22 deg. in **68** *syn*-(CH₂SAc;CH₃)B, 5/19 deg. in **64** *syn*-(CH₂SGlcAc₄;CH₃)B, and 4/19 deg. in **70** *syn*-(CH₂S-;CH₃)B for the corresponding two molecules in the asymmetric units. Distances between the glycosyl units in different molecules, characterized by the separation of anomeric C-atoms, are varying within an extended range as exemplified by 5.9 Å for **65** *anti*-(CH₂SGlcAc₄;CH₃)B to 14.0 Å for **80** *syn*-(CH₃;TAGlcAc₄)B.

4. Outlook: development potentials

In order to gain insight into the recognition process of cell-surface glycans by lectins novel glycoconjugates were synthesized by introducing S/Se atoms and disulfide linkages instead of glycosidic oxygen in derivatives containing benzene- or naphthalene-based central aromatic skeletons. The targeted interactions are responsible of various aspects of pathophysiology in a wide range of diseases such as inflammation, rheumatoid arthritis fibrosis or cancer.

The novel bivalent thio-, disulfido- and selenoglycosides attached to benzene/naphthalene cores display galactose, for blocking the plant toxin VAA, or lactose, the canonical ligand of adhesion/growth-regulatory human galectins.

The anticipated lectin inhibitory activities could indeed be confirmed by using a range of biochemical/physical techniques such as solid-phase assays using a surface-presented glycoprotein, follow cytofluorimetric analysis to demonstrate prevention of lectin binding to surfaces of human carcinoma cells and histochemical assays on murine organ tissue sections as models. Activity enhancements per sugar unit were detected to an extent of up to nearly 100fold relative to free cognate sugar.

The results open perspectives for extended applicability of the studied glycoclusters in testing physiologically relevant interactions of biomedically relevant lectins.

In a further study syntheses and characterization of a set of novel glycoconjugates derivatives with appended mono- and disaccharide moieties, based on the 1,5-diazabicyclo[3.3.0]octa-3,6- diene-2,8-dione (bimane) heteroaromatic ring system have been described. No carbohydrate derivatives of bimanans have been described before.

Mono- and disaccharide residues were attached to *syn*- or *anti*-bimane central cores via thio-, dithio- or selenoglycosidic linkages to obtain novel fluorescent or nonfluorescent glycoconjugates. Cu(I)-catalyzed cycloaddition of glycosyl azides to a bimane diethynyl derivative furnished further bivalent glycoconjugates with sugar residues linked to the central bimane core via 1,2,3-triazole rings. Crystal and molecular structures of several glycosylated and non-glycosylated bimanies have been determined together with fluorescence data for the new compounds. Potential applications with carbohydrate-binding proteins, such as lectins, or more complex biological systems are foreseen particularly in view of the fluorescent properties of these molecules.

Preliminary assays have indicated moderate inhibition activity on the growth of *Trypanosoma brucei* parasite, the causing agent of African sleeping disorder for some of our novel derivatives such as **69** (CH₂SH;CH₃)B, **70** (CH₂S-;CH₃)B, **68** (CH₂SAc;CH₃)B and **64βc** (CH₂S-β-ManAc₄,CH₃)B.



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Candidate: Tamás Szabó
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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Szabó, T.**, Bényei, A., Szilágyi, L.: Bivalent glycoconjugates based on 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione ("bimane") as a central scaffold.
Carbohydr. Res. 473, 88-98, 2019. ISSN: 0008-6215.
DOI: <http://dx.doi.org/10.1016/j.carres.2019.01.002>
IF: 2.074 (2017)
2. Kaltner, H., **Szabó, T.**, Fehér, K., André, S., Balla, S., Manning, J. C., Szilágyi, L., Gabius, H. J.:
Bivalent O-glycoside mimetics with S/disulfide/Se substitutions and aromatic core: Synthesis, molecular modeling and inhibitory activity on biomedically relevant lectins in assays of increasing physiological relevance.
Bioorg. Med. Chem. 25 (12), 3158-3170, 2017. ISSN: 0968-0896.
DOI: <http://dx.doi.org/10.1016/j.bmc.2017.04.011>
IF: 2.881





List of other publications

Foreign language scientific articles in international journals (1)

3. Illyés, T. Z., **Szabó, T.**, Szilágyi, L.: Glycosylation via mixed disulfide formation using glycosylthio-phtalimides and -succinimides as glycosylsulfenyl-transfer reagents.
Carbohydr. Res. 346 (12), 1622-1627, 2011. ISSN: 0008-6215.
DOI: <http://dx.doi.org/10.1016/j.carres.2011.04.020>
IF: 2.332

Total IF of journals (all publications): 7,287

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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.

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Other publication

1. **Szabó Tamás**, Neu József, Garadnay Sándor, (R,S)-N,N-diizopropil-3-(2-hidroxi-5-(hidroximetil)fenil)-3-fenilpropilamin rezolválása (R)-N-acetil-fenilglicinnel, Bilingual lecturebook on spectroscopy: on the 80th birthday of professor Dr. Ferenc Billes, by Ziegler Ildikó and Fejes István, Esztergom **2014**. p. 101-106. ISBN 978-963-08-9474-6

Patents

1. **Szabó Tamás**, Neu József, Garadnay Sándor; Rezolválás N-acil-fenilglicin származékokkal, Richter Gedeon Nyrt. (P1200360)
2. Neu József, **Szabó Tamás**, Garadnay Sándor; Ipari eljárás fesoterodin előállítására, Richter Gedeon Nyrt. (P1200361)
3. Garadnay Sándor, Neu József, **Szabó Tamás**; Eljárás benzazepin származékok előállítására, Richter Gedeon Nyrt. (P1400545, EP3221297)
4. **Szabó Tamás**, Neu József, Garadnay Sándor; Eljárás fíngolimod hidroklorid előállítására, Richter Gedeon Nyrt. (P1500617, HU230806)
5. Neu József, **Szabó Tamás**, Garadnay Sándor; Process for the preparation of high-purity Prasugrel, Richter Gedeon Nyrt. (P1600389, WO2017221187)
6. Neu József, Garadnay Sándor, **Szabó Tamás**; Industrial process for the preparation of cariprazine, Richter Gedeon Nyrt. (P1600420, WO2018007986)
7. **Szabó Tamás**, Neu József, Garadnay Sándor; Process for the preparation of 4-(4-aminophenyl)morpholin-3-one, Richter Gedeon Nyrt. (P1800007)

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1. **Szabó Tamás**, Szilágyi László, Glikozilezett bimán származékok szintézise Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése Balatonszemes, 2014. május 21-23.
2. **Szabó Tamás**, Szilágyi László, Glikozilezett bimán származékok szintézise Szénhidrát, Nukleinsav és Antibiotikum Munkabizottság Ülése, Mátraháza 2014. május 27-29.

3. **Szabó Tamás**, Herbert Kaltner, Fehér Krisztina, Sabine André, Balla Sára, Joachim C. Manning, Szilágyi László, Hans-Joachim Gabius, Aromás magra épített bivalens S/Se-glikozid-mimetikumok: szintézis, lektin-kötődés, hisztokémia, MKE Vegyészkonferencia, Hajdúszoboszló 2017. június 19-21.

Posters

1. **Szabó Tamás**, Szilágyi László, Glikozilezett Bimán származékok szintézise, Vegyészkonferencia, Hajdúszoboszló, 2013. június 26-28.
2. **Szabó Tamás**, Szilágyi László, Syntheses of Glycosylated Bimane Derivatives, 15th Blue Danube Symposium on Heterocyclic Chemistry, Olomouc, 2013. szeptember 1-5.
3. **Szabó Tamás**, Szilágyi László, Thio- and selenoglycosyl derivatives of bimanies as potential antitrypanosomal agents. Syntheses and fluorescent studies, XVI International Conference on Heterocycles in Bioorganic Chemistry, Metz, 2015. június 8-11.
4. **Szabó Tamás** és Szilágyi László, Bimánok tio- és szeleno-glikozid származékai mint potenciális anti-trypanosoma ágensek. Szintézisek és fluoreszcens mérések, MKE 2. Nemzeti Konferencia, Hajdúszoboszló, 2015. augusztus 31. - szeptember 2.
5. Herbert Kaltner, **Szabó Tamás**, Fehér Krisztina, Sabine André, Balla Sára, Joachim C. Manning, Szilágyi László, Hans-Joachim Gabius, Bivalent O-glycoside mimetics with S/disulfide/Se substitutions and aromatic core: synthesis, lectin binding, histochemistry, 17th Blue Danube Symposium on Heterocyclic Chemistry, Linz, 2017. augusztus 30. – szeptember 2.