New types and new biological applications of *C*-glycopyranosyl heterocycles

NKFI-ID: FK125067
Report period: 01-09-2017 – 31-08-2022

Closing report

Aims of this research project

The project, as formulated in the proposal, had the following general goals:

- a systematic study of the synthetic possibilities to get mono- or multiply *C*-glycopyranosylated six membered N-heterocycles;
- syntheses of *C*-glycosyl 1,2,4,5-tetrazines suitably functionalized with fluorescent conjugates for further utilization in biomolecular labeling;
- formation of platinum metal half-sandwich complexes incorporating *C*-glycosyl heterocycles as mono- or bidentate ligands for cytotoxic assays.

This report is constructed to follow the above objectives by describing i) syntheses of new 5-membered glycopyranosyl heterocycles (e.g. as ligands of platinum-group metal complexes), ii) syntheses of 6-membered *C*-glycopyranosyl heterocycles, iii) syntheses of platinum-group metal complexes, and iv) biological studies of the prepared compounds. Among the results those that have already been published will be described in a concise manner with reference to the publication, while those awaiting for publication will be presented in more details. In the reference list the publications resulted from this project are marked in bold.

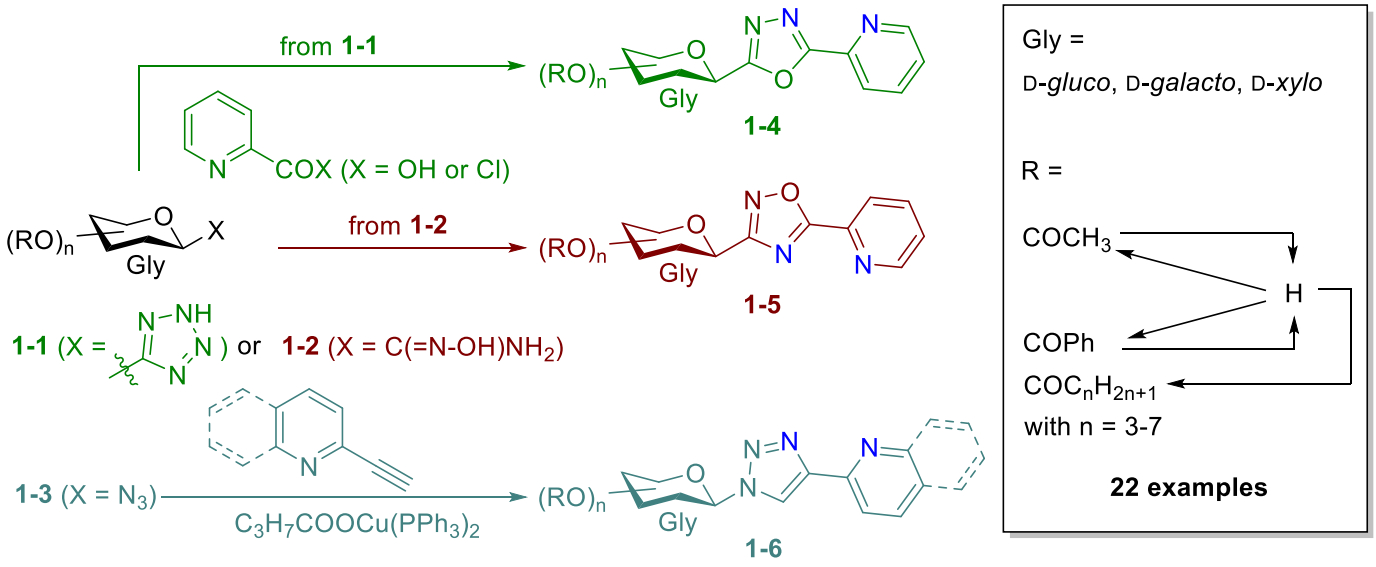
Results of this research project

1. Syntheses

1.1. Synthesis of five-membered *C*-glycopyranosyl heterocycles

The half-sandwich type complexes of platinum-group metals belong to the widely investigated classes of transition metal complexes. Their members have emerged, among others, as promising drug candidates in cancer therapy.[1-6] Although some half-sandwich type complexes containing sugar moieties were described earlier,[7-12] there was no precedent in the literature for the incorporation of real glycosyl heterocycles as ligands into the coordination sphere of such complexes. For the formation of the first representatives of these complexes, the syntheses of different glycopyranosyl heterocycles with the potential to be used as *N*,*N*-chelators were envisaged.

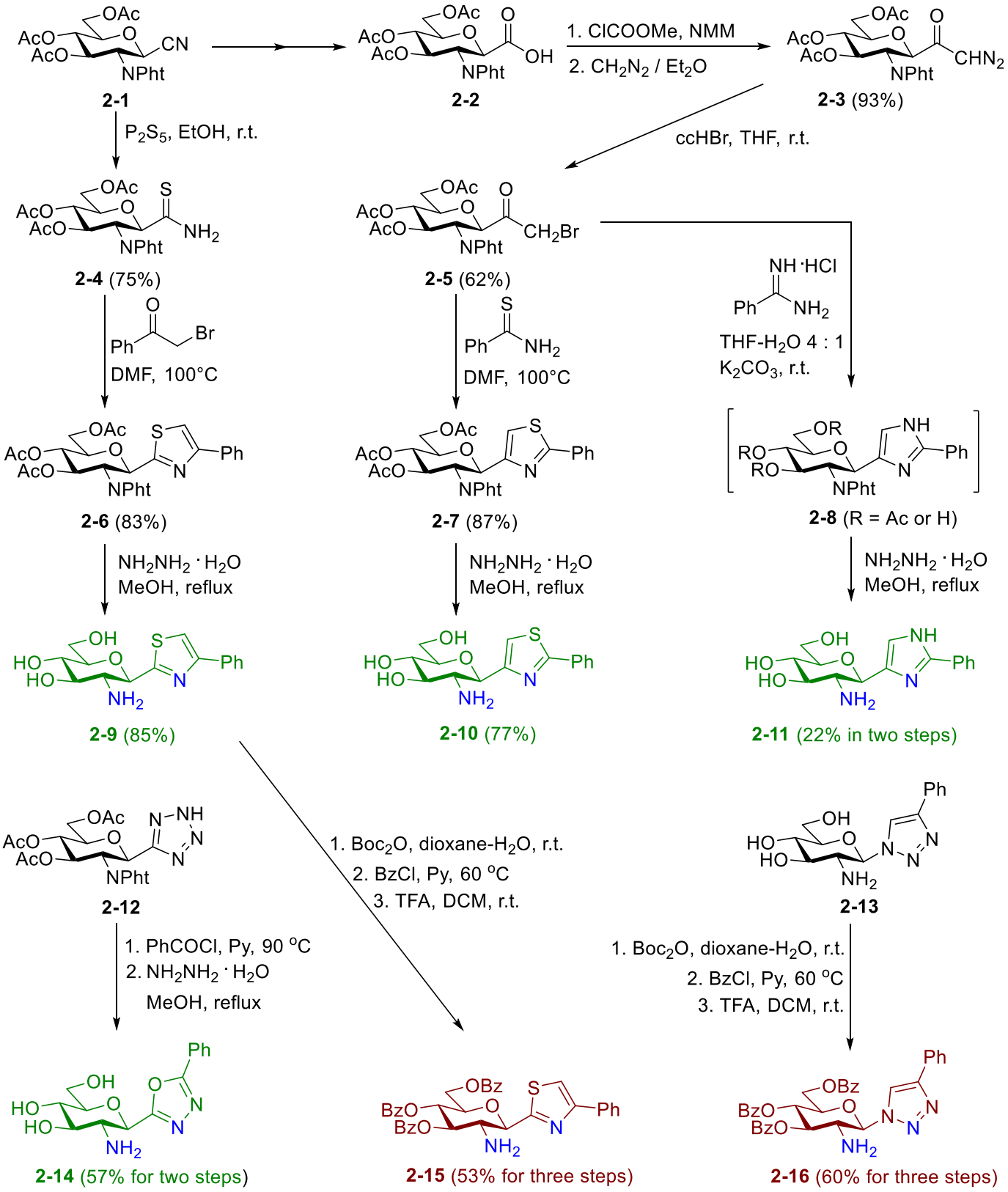
Several 2-pyridyl substituted *C*-glycopyranosyl oxadiazoles with various sugar configurations were prepared by the adaptation of earlier elaborated methods[13-15] (Scheme 1). 2-(β-D-Glycopyranosyl)-1,3,4-oxadiazoles **1-4** in *O*-peracylated form were prepared by ring-transformations of the corresponding *C*-glycopyranosyl tetrazoles **1-1** with activated picolinic acid, while an isomeric 3-(β-D-glucopyranosyl)-5-(2-pyridyl)-1,2,4-oxadiazole **1-5** was obtained by the ring-closure of *C*-glycopyranosyl formamidoxime **1-2** with picolinoyl chloride.[16,17] In addition, 1-(β-D-glucopyranosyl)-4-hetaryl-1,2,3-triazoles **1-6** (hetaryl = 2-pyridyl, 2-quinolinyl) were synthesized by Cu(I) catalyzed azide-alkyne cycloaddition[18] of *O*-peracetylated glucosyl azide **1-3** with 2-ethynylated heterocycles.[16,17] In order to increase the structural diversity of these ligands the synthesis of their *O*-deprotected and various *O*-peracylated derivatives were also carried out by performing standard *O*-deacylation and *O*-peracylation reactions.[16,17,19]



Scheme 1. Synthesis of hetaryl substituted *C*- and *N*-glycopyranosyl azoles

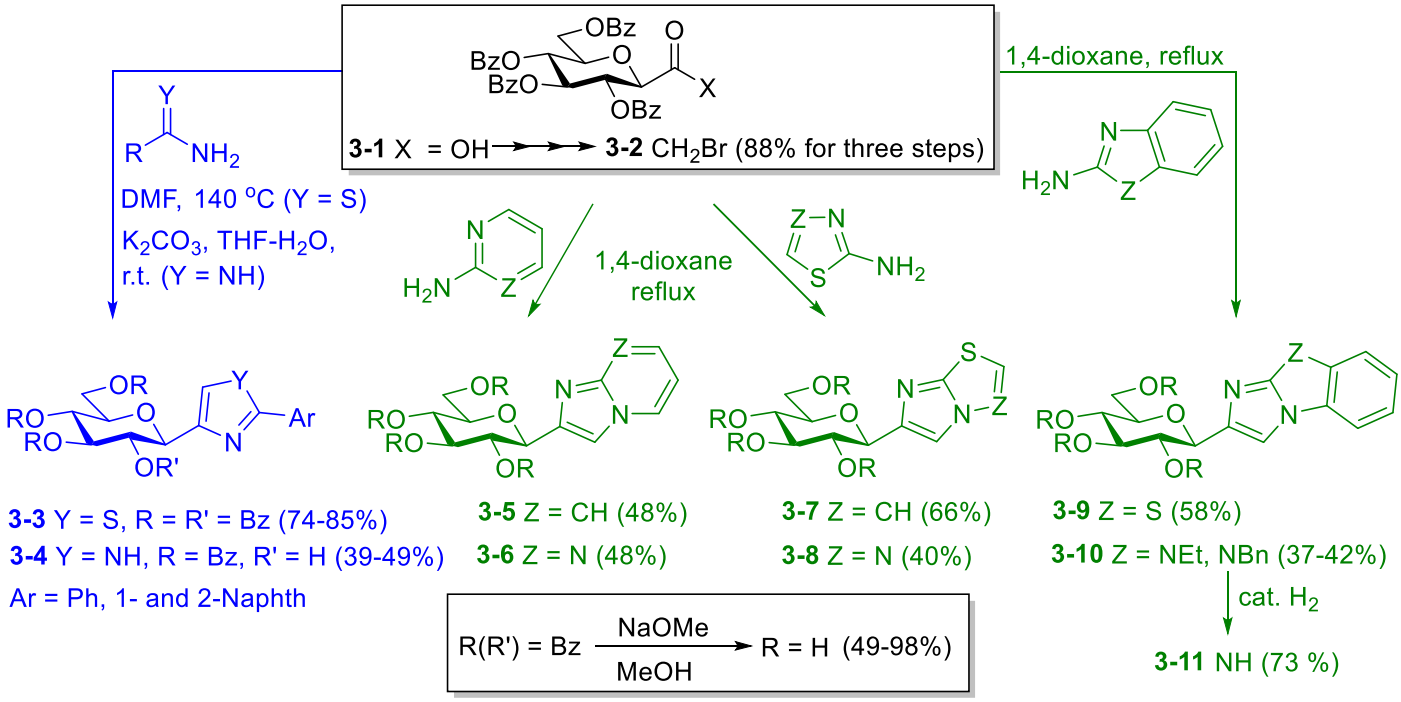
Glucosaminyl N-heterocycles were also considered to be useful *N*,*N*-bidentate ligands for the formation of the planned metal complexes. To this end, the preparation of a set of new glucosaminyl azoles were performed (Scheme 2). First, *C*-(2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)thioformamide (**2-4**) and bromomethyl-(2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-ketone (**2-5**) as precursors were synthesized starting from the corresponding cyanide[20] **2-1**. Thioamide **2-4** was prepared by the treatment of cyanide **2-1** with P_2S_5 in EtOH in high yields, while the bromomethyl ketone **2-5** was obtained by the transformation of acid **2-2** (prepared from cyanide **2-1** in two steps[21]) into diazomethyl ketone **2-3**, followed by bromination with HBr in good overall yield. Subsequent cyclisations of these precursors with thiobenzamide as well as with phenacyl bromide, followed by deacylations of the resulting **2-6** and **2-7** with hydrazine hydrate afforded the corresponding phenyl substituted 2- and 4-*C*-(2'-amino-2'-deoxy-β-D-glucopyranosyl)thiazoles **2-9** and **2-10**, respectively, in high yields. The ring-closure of **2-5** with benzamidine gave, after complete deacylation of intermediate **2-8**, the 4(5)-*C*-(2'-amino-2'-deoxy-β-D-glucopyranosyl)-2-phenyl-imidazole (**2-11**) in low yield. The synthesis of 2-(2'amino-2'-deoxy-β-D-glucopyranosyl)-5-phenyl-1,3,4-oxadiazole **2-14** was also accomplished starting from the appropriate 5-glycosyl-tetrazole[22] **2-12**. In addition, thiazole

2-9 was converted into the *O*-perbenzoylated analog **2-15** in a three-step procedure including the protection of the NH_2 group as a carbamate, *O*-perbenzoylation with benzoyl chloride and subsequent acid-mediated liberation of the NH_2 group. In the same route, *O*-perbenzoylated 1-(2'-amino-2'-deoxy-β-D-glucopyranosyl)-4-phenyl-1,2,3-triazole **2-16** was also achieved starting from the earlier prepared *O*-unprotected derivative[23] **2-13**.



Scheme 2. Synthesis of new *C*- and *N*-glucosaminyl azoles

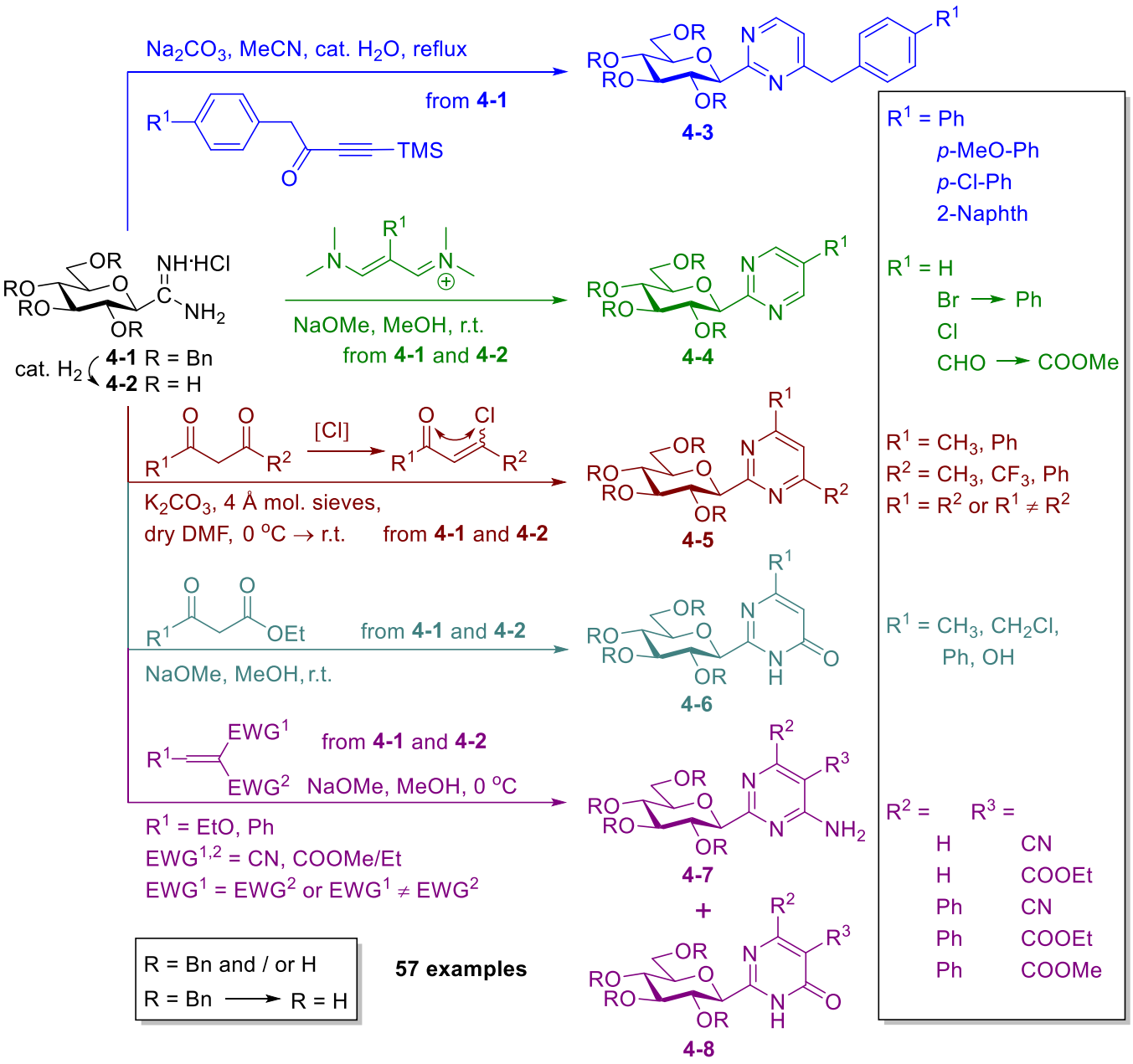
Motivated by the promising computational studies for predicting new potential inhibitors of the glycogen phosphorylase enzyme made by the Joseph M. Hayes's group, the preparation of 2-aryl-4-(β-D-glucopyranosyl)imidazoles and thiazoles was accomplished (Scheme 3). First, bromomethyl-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-ketone[24] **3-2** was synthesized starting from the corresponding anhydro-aldonic acid[25] **3-1** in a similar way to that described for compound **2-5**. Its cyclocondensation with the corresponding aromatic thioamide and carboxamidines, followed by cleavage of the *O*-benzoyl protecting groups furnished the test compounds **3-3** and **3-4**, respectively, in good to excellent yields.[26] In addition, the ring-closure of **3-2** with several 2-aminoheterocyles was also performed (Scheme 3) to get a set of annulated *C*-(β-D-glucopyranosyl)imidazoles (**3-5**–**3-11**) for the inhibition of the same enzyme.[24]



Scheme 3. Synthesis of new *C*-(β-D-glucopyranosyl)imidazoles and thiazoles

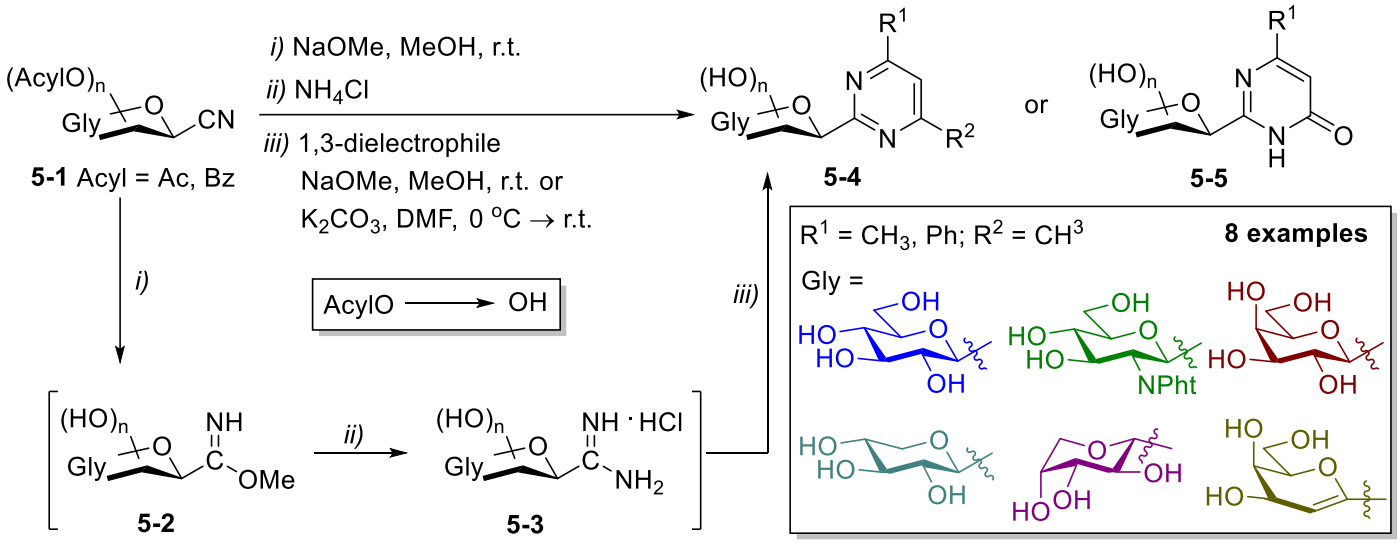
1.2. Synthesis of six-membered *C*-glycopyranosyl heterocycles

An extensive synthetic work was carried out on the synthesis of 2-*C*-glycopyranosyl pyrimidines which were practically completely unknown (only one representative obtained as a minor component of a regioisomeric mixture could be found in the chemical literature[27]) before. A large set of diversely substituted 2-(β-D-glucopyranosyl)pyrimidines (Scheme 4, **4-3**–**4-8**) was obtained by Pinner type cyclocondensations of the *O*-perbenzylated glucosyl formamidine[28] **4-1** with different 1,3-dielectrophiles (trimetylsilylated alkynyl ketones, vinamidinium salts, α,β-unsaturated β-chloroketones obtained from β-diketones, β-ketoesters, dimethyl malonate and methylenemalonic acid derivatives), followed by removal of the *O*-benzyl protecting groups.[29-31] Since the *O*-debenzylation of the sugar part of the heterocycles proved to be problematic in several cases, the ring-closing reactions were also performed by using the *O*-deprotected glucosyl formamidine[29] **4-2** obtained easily from **4-1** providing a more efficient route to get the *O*-unprotected **4-3**–**4-8**, among others, in terms of the yields.[29,30]



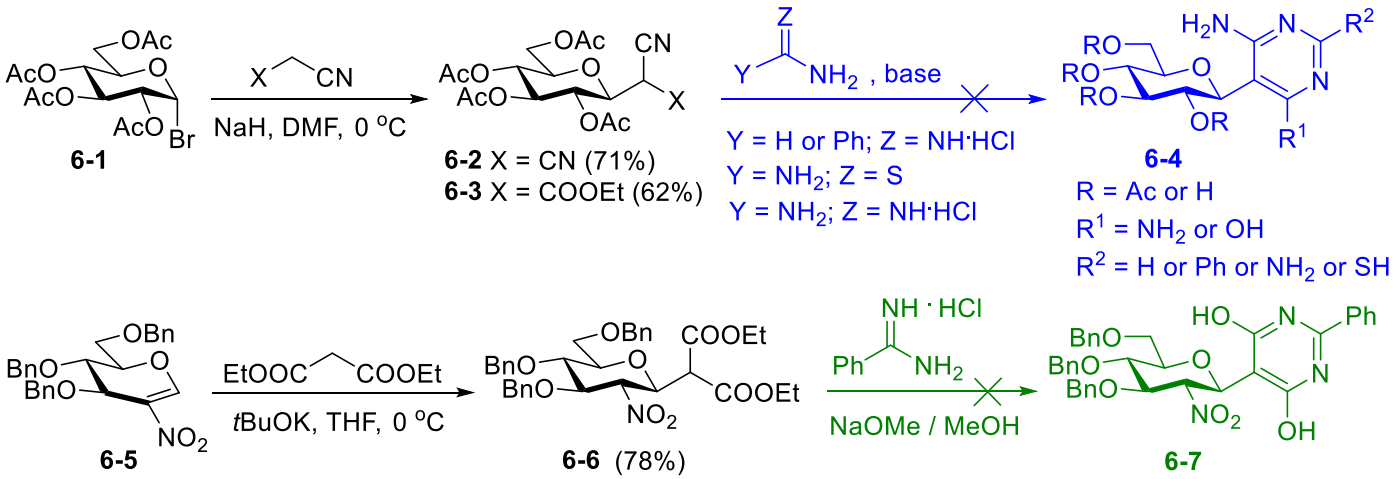
Scheme 4. Synthesis of variously substituted 2-*C*-(β-D-glucopyranosyl)pyrimidines

For the preparation of 2-*C*-glycopyranosyl pyrimidines a continuous three-step procedure starting from easily accessible *O*-peracylated D-glycopyranosyl cyanides was also worked out (Scheme 5).[29] Treatment of the corresponding glycosyl cyanide **5-1** with NaOMe, followed by the reaction of the resulting iminoester **5-2** with NH_4Cl and subsequent cyclisation of the *in situ* obtained amidine **5-3** with the 1,3-dielectrophilic reagent gave the desired pyrimidines **5-4** or **5-5** with different sugar configurations in moderate to high overall yields (25-94 %).[29]



Scheme 5. One-pot three-step preparation of 2-*C*-glycopyranosyl pyrimidines from *O*-peracylated glycopyranosyl cyanides

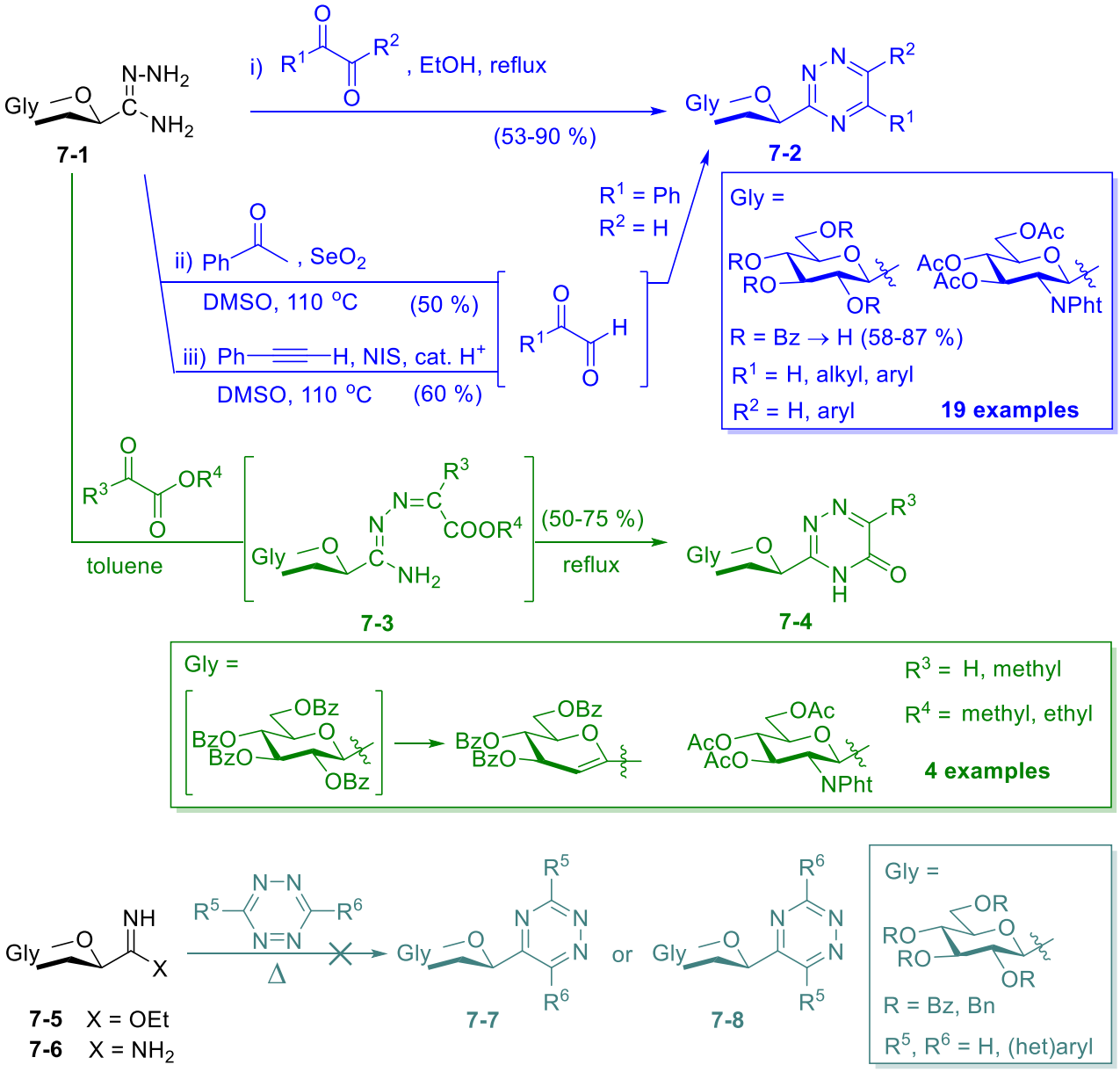
The preparation of 5-*C*-(β-D-glycopyranosyl)pyrimidines was also investigated (Scheme 6). By applying literature methods,[32-34] 2-*C*-glycopyranosylated malonic acid derivatives **6-2**, **6-3** and **6-6** were synthesized first from *O*-peracetylated α-D-glucopyranosyl bromide **6-1** and *O*-perbenzylated 2-nitroglucal **6-5**, respectively. The cyclocondensations of these precursors were then examined with carboxamidines, thiourea and guanidine under different basic conditions (e.g. NaOMe/MeOH, K_2CO_3/THF-H_2O, NaH/DMF, Et_3N/1,4-dioxane), however, the expected heterocyles **6-4** and **6-7** could be obtained in neither cases.



Scheme 6. Attempts for the synthesis of 5-*C*-(β-D-glycopyranosyl)pyrimidines

Simple synthetic methods were elaborated for the preparation of hitherto unknown 3-*C*-(β-D-glycopyranosyl)-1,2,4-triazines and -1,2,4-triazin-5(4*H*)-ones (Scheme 7).[35] The ring-closing reactions of *O*-peracylated *C*-glycosyl formamidrazones[35,36] **7-1** with pre-formed (route *i*) or *in situ* generated 1,2-dicarbonyl derivatives (obtained from methyl ketones or alkynes under oxidative conditions in routes *ii* and *iii*, respectively) gave a series of variously substituted 3-glycosyl-1,2,4-triazines **7-2** (substituent: H, 5-alkyl, 5-aryl, 5,6-diaryl) in good to excellent yields. Among the direct (*i*) and the *one-pot* reactions (*ii* and *iii*) the former one proved to be more efficient in terms of the yields. In case of the glucose derivatives the *O*-benzoyl protecting groups of the new compounds were cleaved by the Zemplén method to get test compounds for further biological studies.[35] In addition, cyclisation of the same amidrazones **7-1** with α-oxocarboxylic esters via N^1-alkoxycarbonylalkylidene amidrazones **7-3** were also effected resulting in 1,2,4-triazin-5(4*H*)-ones **7-4** in moderate to good yields.[35]

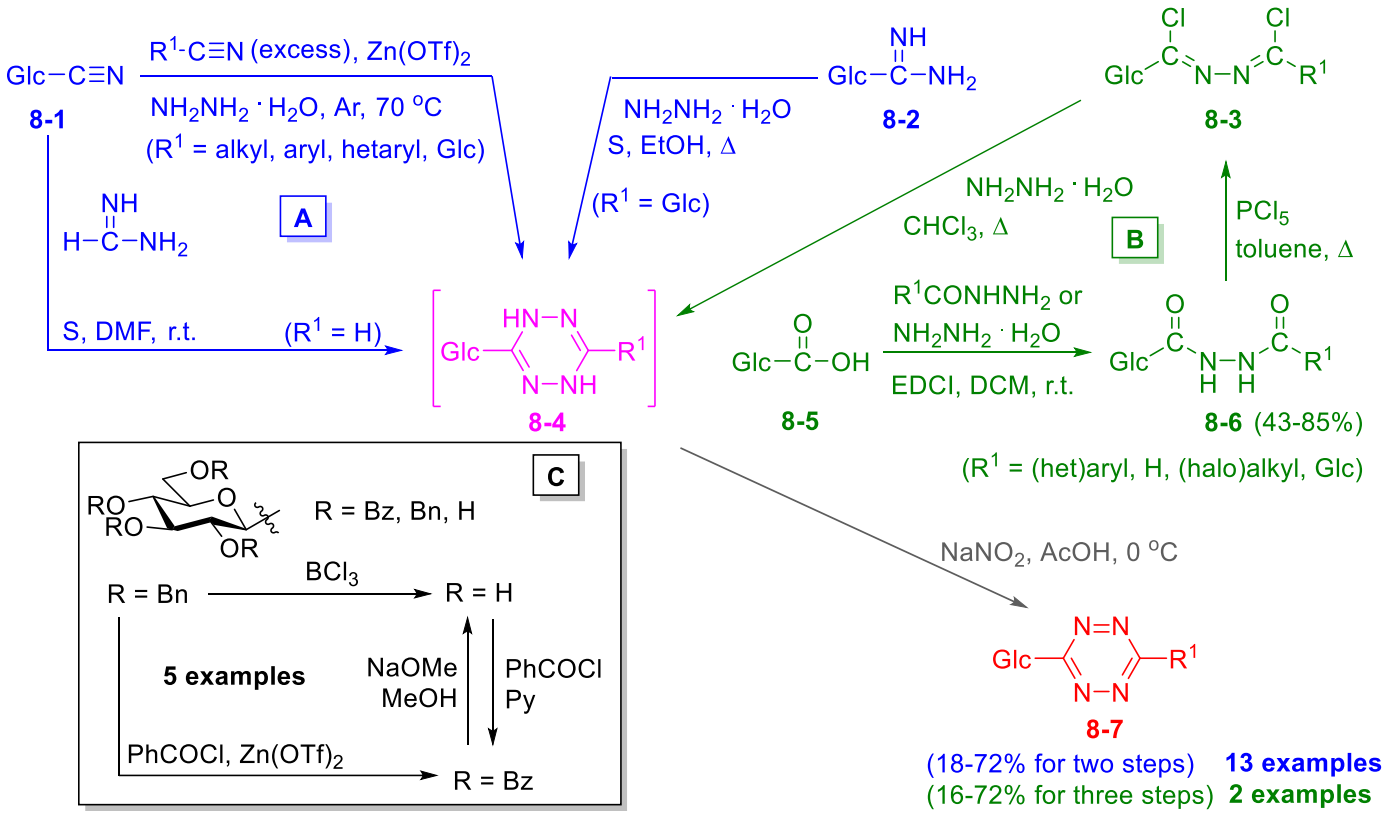
To get further constitutional isomers of *C*-glucopyranosyl 1,2,4-triazines, inverse electron demand Diels-Alder (IEDDA) reactions of phenyl- and (2-pyridyl)-substituted 1,2,4,5-tetrazines with *O*-protected *C*-glucosyl formimidate[37] **7-5** and formamidines[28,36] **7-6** were investigated in different solvents (DCM, EtOH, toluene, *m*-xylene) under heating, however, the formation of none of the expected 5-(β-D-glucopyranosyl)-1,2,4-triazines **7-7** or **7-8** was observed (Scheme 7).



Scheme 7. Experiments towards *C*-(β-D-glycopyranosyl)-1,2,4-triazines and -1,2,4-triazin-5(4*H*)-ones

1,2,4,5-Tetrazine having an azadiene functionality within the ring is a versatile heterocycle with broad synthetic potential to access further N-heterocycles (e.g. pyridazine, 1,2,4-triazines, pyrazoles)[38-41] and to develop very fast chemical transformations for bioorthogonal applications.[42-46] Since *C*-glycosyl 1,2,4,5-tetrazines were completely unknown in the chemical literature, an extensive study was carried out to find feasible methods for the syntheses of representatives of this compound class. One of the general synthetic strategies to get a 1,2,4,5-tetrazine is based on the formation of a 1,4-dihydrotetrazine intermediate constructed by ring forming reactions of suitable electrophilic carbon precursors with hydrazine, followed by an oxidative aromatization.[47,48] For the formation of *C*-glucopyranosyl 1,2,4,5-tetrazines a great number of experiments based on ring-closure reactions of anhydro-aldonic acid derivatives were performed, among them, the most important ones were compiled in Scheme 8 (routes A and B).[49] Several unsymmetrical 3-(β-D-glucopyranosyl)-6-susbtituted 1,2,4,5-tetrazines **8-7** (R^1 = alkyl, aryl, hetaryl) were obtained by Lewis acid catalyzed Pinner type cyclocondensations of *O*-perbenzylated glucopyranosyl cyanide[28] **8-1** with liquid or low melting

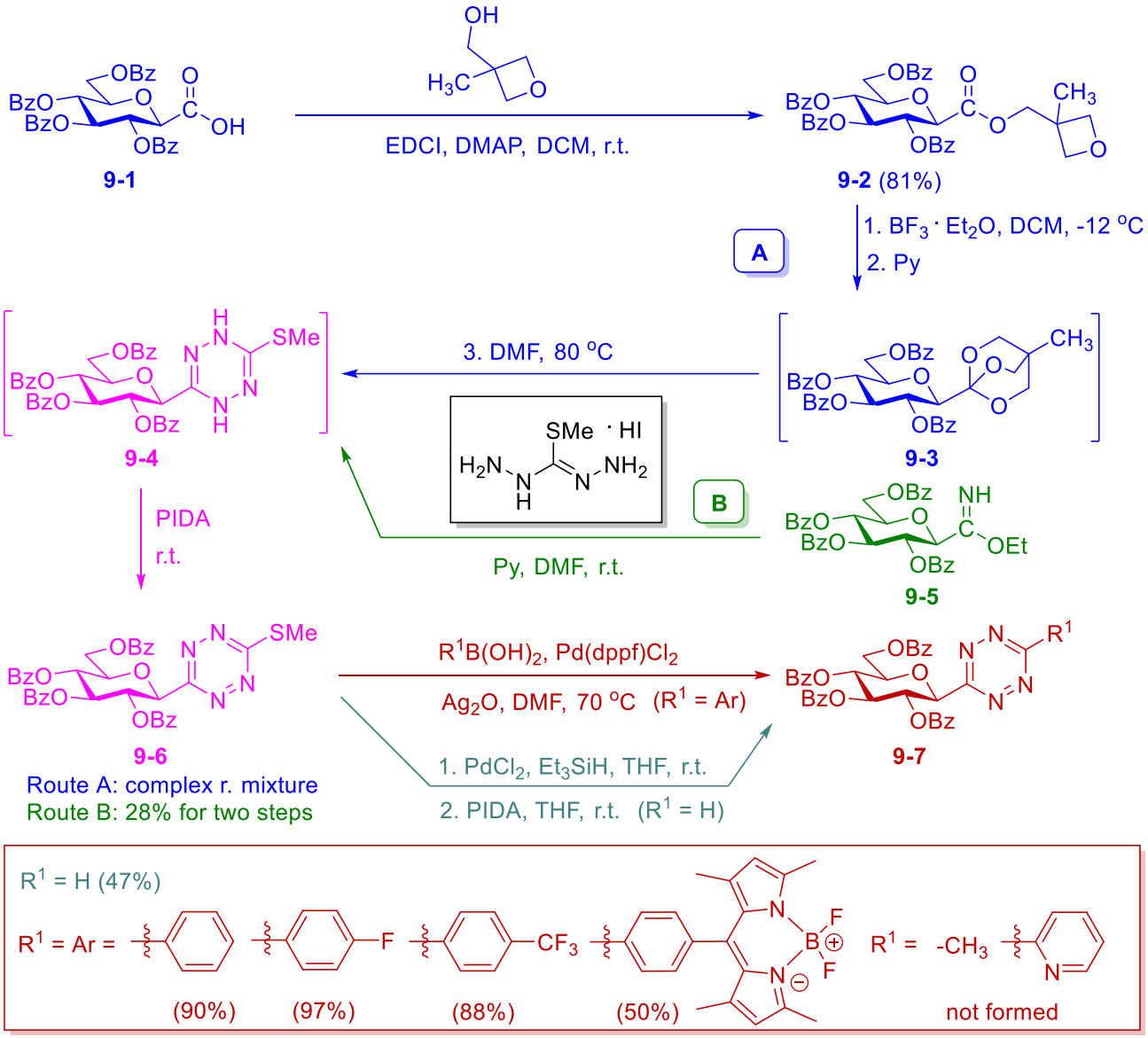
nitriles and hydrazine hydrate, followed by the oxidation of the resulting 1,4-dihydrotetrazine intermediates **8-4**.[49] 3-Mono- and 3,6-bis-glucopyranosylated 1,2,4,5-tetrazines **8-7** (R^1 = H and Glc, respectively) were synthesized by Pinner type cyclisation and subsequent oxidation steps starting from the same cyanide[28] **8-1** or *O*-unprotected *C*-glucopyranosyl formamidine[29] **8-2**.[49] A series of *N*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosylcarbonyl)-*N'*-acyl hydrazines **8-6**, as an additional set of precursors, was prepared from the corresponding anhydro aldonic acid[25] **8-5** with carboxylic acid hydrazides or with hydrazine hydrate. Compounds **8-6** were transformed into 1,2-bis-chloroalkylidene-hydrazines **8-3** by chlorination, which were then treated with hydrazine monohydrate and subjected in the final step to oxidation. This chlorination-cyclisation-oxidation sequence was succesfully applied for a high-yielding preparation of *O*-perbenzoylated 3-(β-D-glucopyranosyl)-6-phenyl-1,2,4,5-tetrazine **8-7** (R^1 = Ph, 72 % combined yield for three steps). Unfortunately, the same three-step procedures carried out with other members of **8-6** failed by leading to undesirable 1,3,4-oxadiazole type by-products or inseparable product mixtures.[49] The basic protecting group compatibility of the prepared *C*-glucopyranosyl tetrazines was also investigated, and verified by a set of *O*-debenzylation, *O*-debenzoylation and *O*-benzoylation reactions (Scheme 8, C).[49]



Scheme 8. Syntheses of 3-(β-D-glucopyranosyl)-6-substituted-1,2,4,5-tetrazines by ring-formation reactions

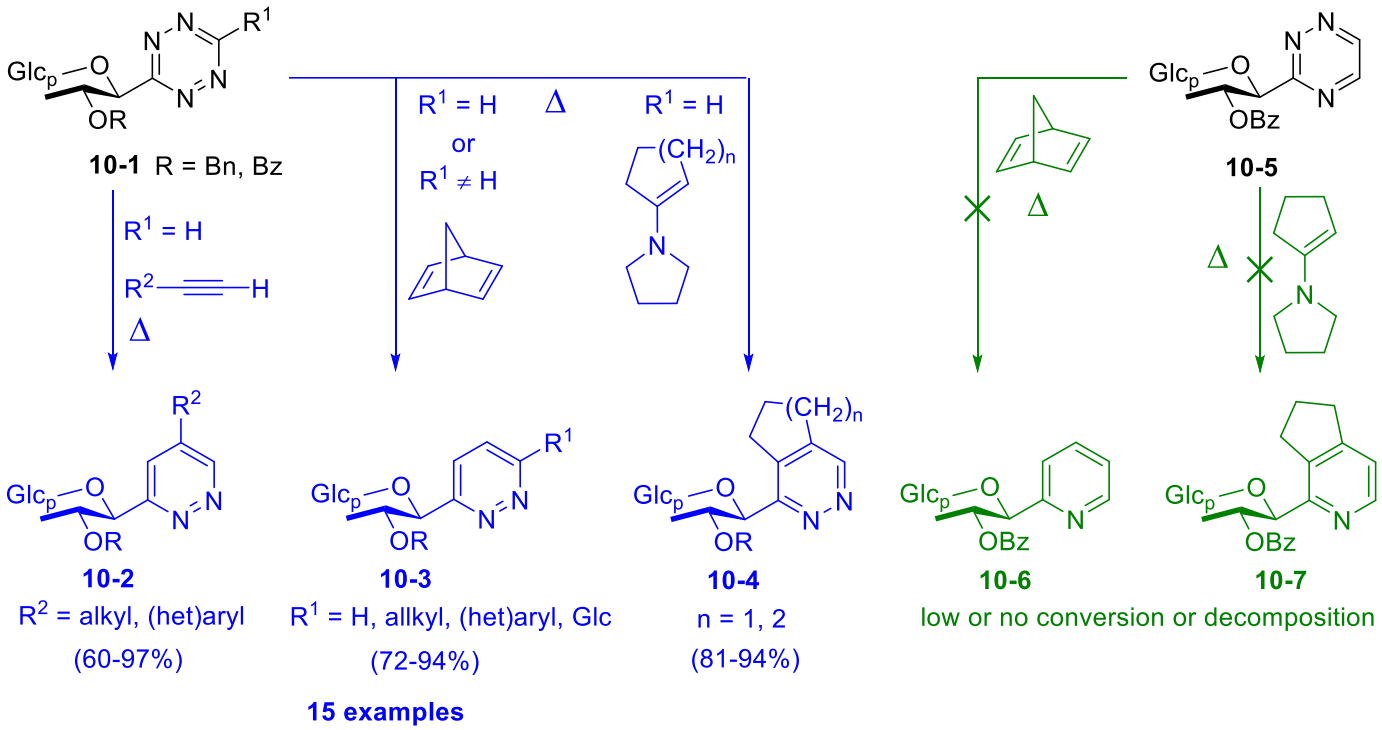
For the formation of unsymmetrical 3,6-disubstituted 1,2,4,5-tetrazines cross-couplings of pre-formed 1,2,4,5-tetrazines substituted with an exchangeable group (e.g. halide, alkylsulfanyl) provides an another synthetic possibility.[47,48] In order to study such reactions, the preparation of *O*-perbenzoylated 3-(β-D-glucopyranosyl)-6-(methylthio)-1,2,4,5-tetrazine **9-6** was investigated first (Scheme 9). The adaptation of a literature procedure[50] shown as route A in Scheme 9 was attempted first. Thus, **9-1** was converted to an activated (3-methyloxetan-3-yl)methyl carboxylic ester **9-2** in high yield. However, its treatment with $BF_3{\cdot}Et_2O$, followed by condensation of the resulting oxabicyclo[2.2.2]octyl orthoester **9-3** with *S*-methyisothiocarbonohydrazidium iodide and subsequent oxidation by nitrosation or by PIDA led to a complex reaction mixture. Therefore, another possible route[51] (B) was tried, wherein the *O*-perbenzoylated ethyl *C*-(β-D-glucopyranosyl)formimidate[37] **9-5** was cyclised with the above hydrazidinium salt, followed by the aromatization of the resulting dihydrotetrazine intermediate **9-4** by PIDA. In this way, the target **9-6** could be obtained in acceptable overall yield. The cross-coupling reactions of **9-6** were then studied with boronic acids in the presence of $Pd(dppf)Cl_2$ and Ag_2O. Its reactions with arylboronic acids were smoothly accomplished affording the expected 6-aryl-1,2,4,5-tetrazines **9-7** in high yields. By applying this method, a fluorescent 6-BODIPY-substituted tetrazine could also be prepared in moderate yield. However, our attempts to couple **9-7** with methylboronic and pyridine-2-boronic acid failed, multicomponent

mixtures were obtained. The transformation of **9-7** into the corresponding unsubstituted 3-glycosyl-1,2,4,5-tetrazine **9-7** (R^1 = H) was also performed by reductive cleavage of the methylthio group with Et_3SiH followed by an oxidative re-aromatization. To extend the scope of the *C*-glycosyl 1,2,4,5-tetrazines, the synthesis of further derivatives with sugar configuration other than glucopyranose (e.g. galactopyranose, ribofuranose) according to route B has begun, and the results of Scheme 9 will be published in the near future.



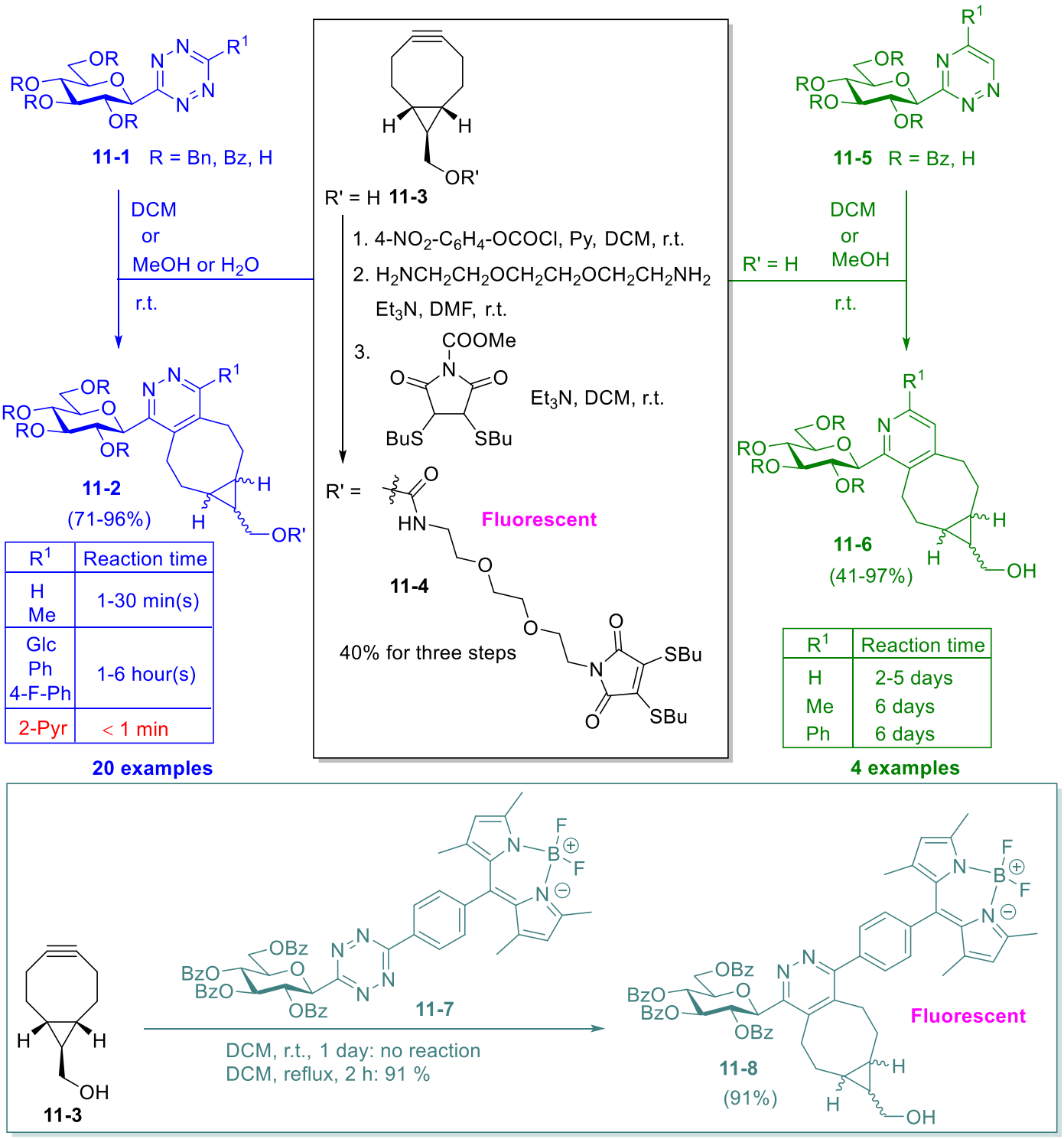
Scheme 9. Synthesis of 3-(2',3',4',6'-tetra-*O*-benzoyl-β-D-glucopyranosyl)-6-(methylthio)-1,2,4,5-tetrazine and its cross-coupling reactions

To assess the synthetic applicability of the prepared *C*-glucosyl 1,2,4,5-tetrazines towards further *C*-glucosyl azines, their inverse electron demand Diels-Alder (IEDDA) reactions with various dienophiles were studied (Schemes 10 and 11 and Table 1). In parallel reactions, similar transformations of the *C*-glucosyl 1,2,4-triazines were also investigated. Thermal reactions of the tetrazines **10-1** with terminal alkynes, norbornadiene, and 1-pyrrolidino-1-cycloalkenes were smoothly effected providing an easy access to 3-(β-D-glucopyranosyl)-5- and 6-substituted and -4,5-alkylene pyridazines **10-2**, **10-3** and **10-4**, respectively (Scheme 10).[49] It is to be noted that prior to our work, 3-glycopyranosyl pyridazines were essentially unknown, only a sole glucopyranosyl derivative obtained from gluconolactone in a multistep reaction sequence was described in the literature.[52] For the preparation of 2-glucopyranosyl pyridines the IEDDA reactions of a 3-glucopyranosyl-1,2,4-triazine **10-5** with the same dienophiles were also attempted, however, these ring-transformations into **10-6** and **10-7** could not be triggered even at elevated temperature (e.g. in boiling *m*-xylene): no or negligible conversion and slow decomposition of **10-5** was observed.[35]



Scheme 10. Experiments towards 3-(β-D-glucopyranosyl)pyridazines and 2-(β-D-glucopyranosyl)pyridines by IEDDA reactions

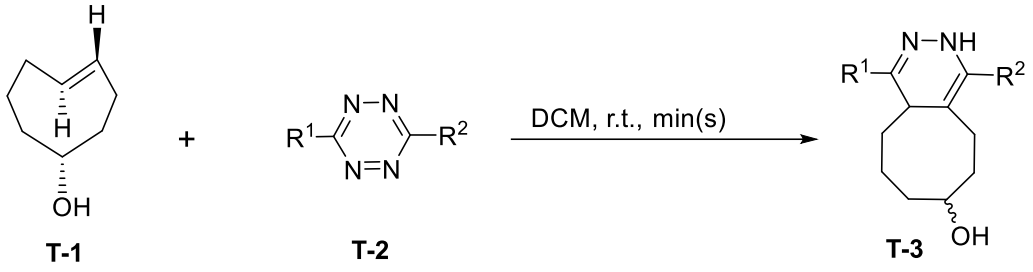
For future bioorthoganal applications, the reactivity of the *C*-glucosyl 1,2,4,5-tetrazines and 1,2,4-triazines was tested in strain-promoted IEDDA reactions (Scheme 11 and Table 1). First, a bicyclononyne ((1*R*,8*S*,9*R*)-bicyclo[6.1.0]non-4-yn-9-yl)methanol,[53] BCN, **11-3**) known as a reactive dienophile was prepared, which was transformed in three steps into a dithiomaleimide containing fluorescent derivative **11-4** (Scheme 11). The IEDDA reactions of **11-3** and **11-4** with the 1,2,4,5-tetrazines **11-1** were easily accomplished at room temperature in most of the cases resulting in the expected annulated pyridazines **11-2** as mixtures of two diastereoismers in high yields. Among the tetrazines tested, the sterically least crowded (R^1 = H or CH$_3$) and the most electron deficient (R^1 = 2-pyridyl) derivatives proved to be the most reactive. Unfortunately, the BODIPY-containing fluorescent tetrazine **11-7** was the least reactive compound of the series, gentle heating had to be applied for its conversion into pyridazine **11-8**. Analogous cycloadditions of the same BCN reagents with 1,2,4-triazines **11-5** also proceeded at room temperature, however, due to the decreased reactivity of the 1,2,4-triazines compared to that of tetrazines the formation of the desired annulated pyridines **11-6** required prolonged reaction time.[35]

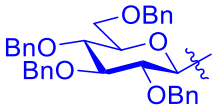
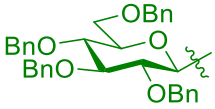
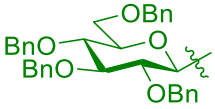
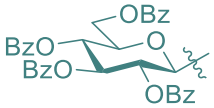
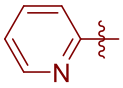
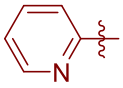
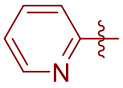


Scheme 11. Strain-promoted IEDDA reactions of *C*-glucosyl 1,2,4,5-tetrazines and 1,2,4-triazines

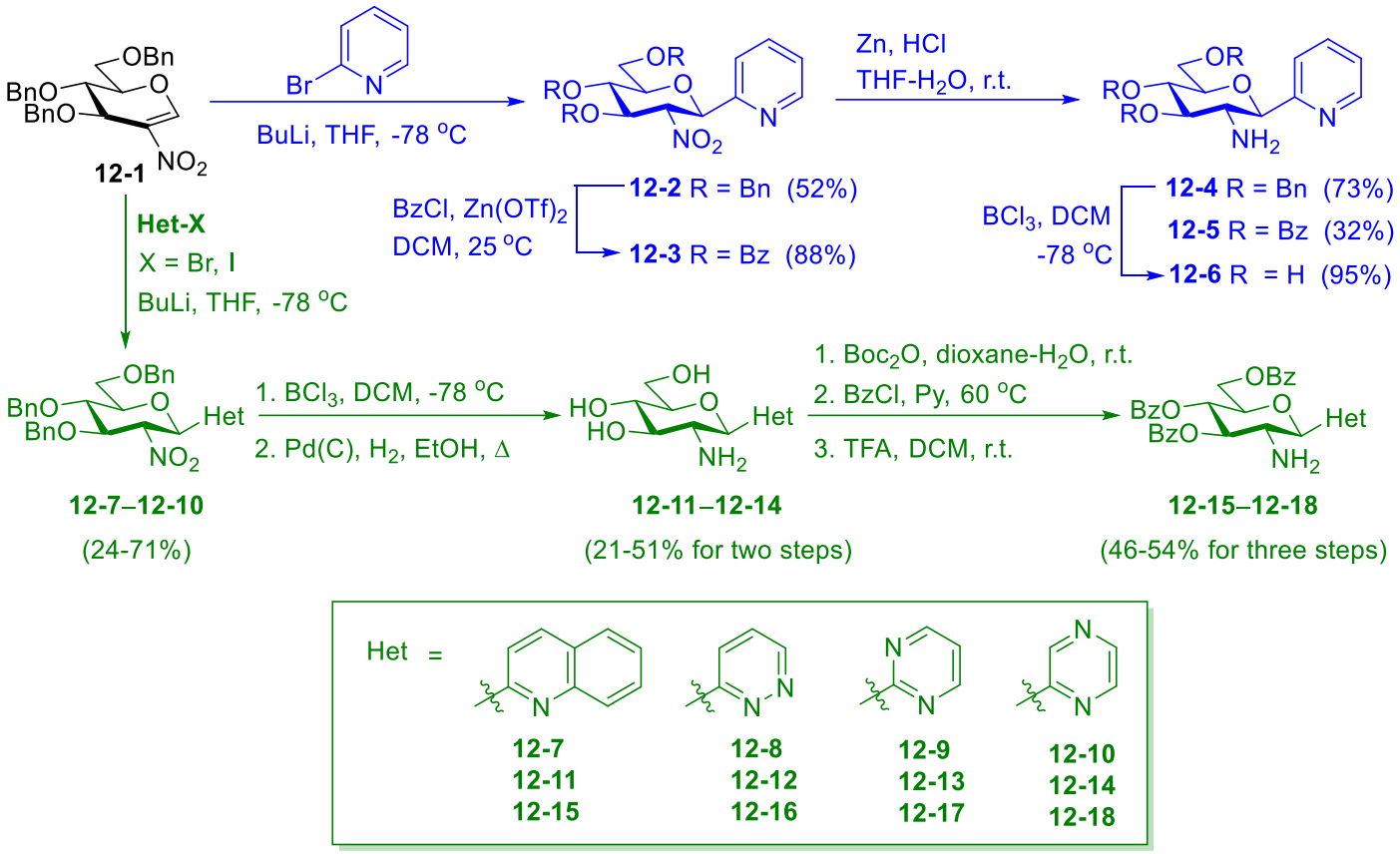
Based on a literature method, axial *trans*-4-cycloocten-1-ol[54] (TCO, **T-1** in Table 1) was prepared in order to study its applicability in the direct synthesis of fluorescent 1,4-dihydropyridazine-based sugar conjugates. Test reactions of TCO with the *O*-perbenzylated 3-glucosyl-, 3,6-bis-glucosyl- and the *O*-perbenzoylated 3-glucosyl-6-phenyl-1,2,4,5-tetrazines (entries 1-3) as well as with the control 3-mono- and 3,6-dipyridyl-1,2,4,5-tetrazines (entries 4 and 5) took place at very high rates. The visualization of the TLC plates under UV lamp indicated that at least one (hetero)aromatic substituent attached to the **T-2** tetrazine ring was required to induce fluorescent property in the resulting cycloadducts **T-3** (entries 1 and 2 *cf.* entries 3-5).

Table 1. Test reactions of TCO with 1,2,4,5-tetrazine derivatives



Entry	R^1	R^2	Observation
1		-H	Formation of isomeric mixtures of ***non-fluorescent*** cycloadducts
2			Formation of a ***non-fluorescent*** cycloadduct
3			Formation of isomeric mixtures of ***fluorescent*** cycloadducts
4		-H	Formation of a ***fluorescent*** cycloadducts
5			Formation of isomeric mixtures of ***fluorescent*** cycloadducts

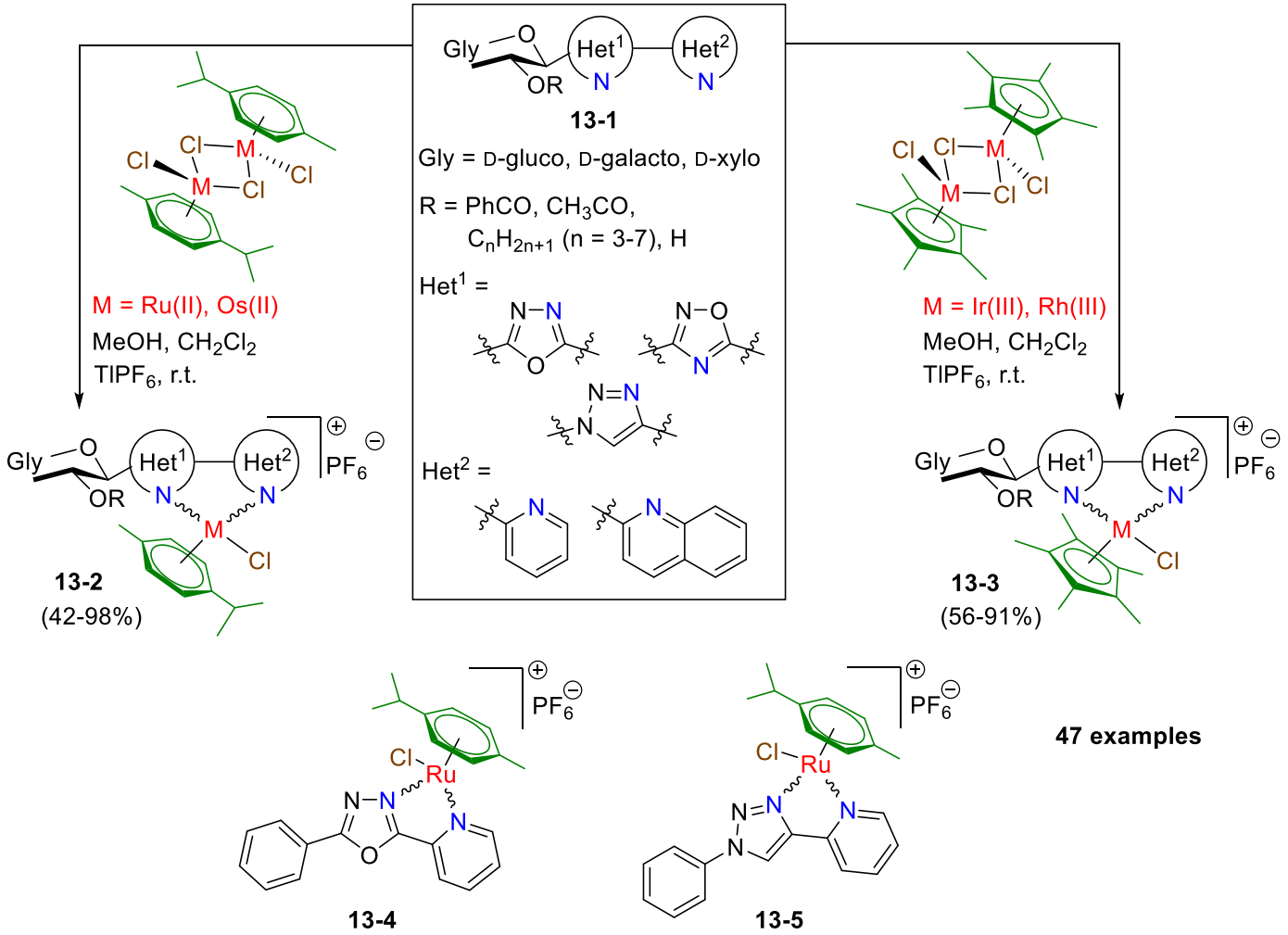
In order to obtain further *C*-glucosaminyl type ligands with 6-membered heterocycles for complex formation reactions, the synthesis of a set of *C*-glucosaminyl azines was carried out based on nitro-Michael addition of lithiated heterocycles to *O*-perbenzylated 2-nitroglucal[55] **12-1** (Scheme 12). Coupling of the *in situ* formed 2-lithiated pyridine with **12-1** resulted in the *O*-perbenzylated 2-*C*-(2'-deoxy-2'-nitro-β-D-glucopyranosyl)pyridine **12-2** in good yield. The exchange of the *O*-benzyl protecting groups to benzoyl groups by a Lewis acid mediated reaction[56] of **12-2** with benzoyl chloride furnished the *O*-perbenzoylated analog **12-3** in excellent yield. Reduction of the nitro group of **12-2** and **12-3** by Zn-HCl gave the *O*-perbenzylated and -perbenzoylated glucosamine derivatives **12-4** and **12-5**, respectively, in moderate to high yields. *O*-Debenzylation of **12-4** was also effected to give the *O*-unprotected pyridine **12-6** in excellent yield. The addition reactions were also performed with lithiated quinoline, pyridazine, pyrimidine and pyrazine to get further representatives of *C*-(2'-deoxy-2'-nitro-β-D-glucopyranosyl)azines (Scheme 12). Surprisingly, similar transformations of **12-7–12-10** as described for the pyridine derivatives (the direct Bn→Bz exchange and the reduction of the nitro group of the *O*-protected glycosyl heterocycles) proved to be problematic in several cases. Therefore another route to obtain the desired *O*-unprotected and *O*-perbenzoylated *C*-glucosaminyl azines had to be worked out. Since simultaneous cleavage of the *O*-benzyl protecting groups and reduction of the nitro group of **12-7–12-10** could not be achieved by applying catalytic hydrogenation conditions, a two-step procedure was performed to get the *O*-unprotected glucosamine derivatives **12-11–12-14**. A Lewis acid mediated *O*-debenzylation of **12-7–12-10**, followed by the $NO_2 \rightarrow NH_2$ transformation upon catalytic hydrogenation furnished **12-11–12-14** in acceptable overall yields. After that, the same protecting group manipulation was applied as described earlier for compounds **2-9→2-15** and **2-13→2-16** in Scheme 2, resulting in the *O*-perbenzoylated derivatives **12-15–12-18** in good overal yields.



Scheme 12. Synthesis of *C*-glucosaminyl azines from 2-nitroglucal

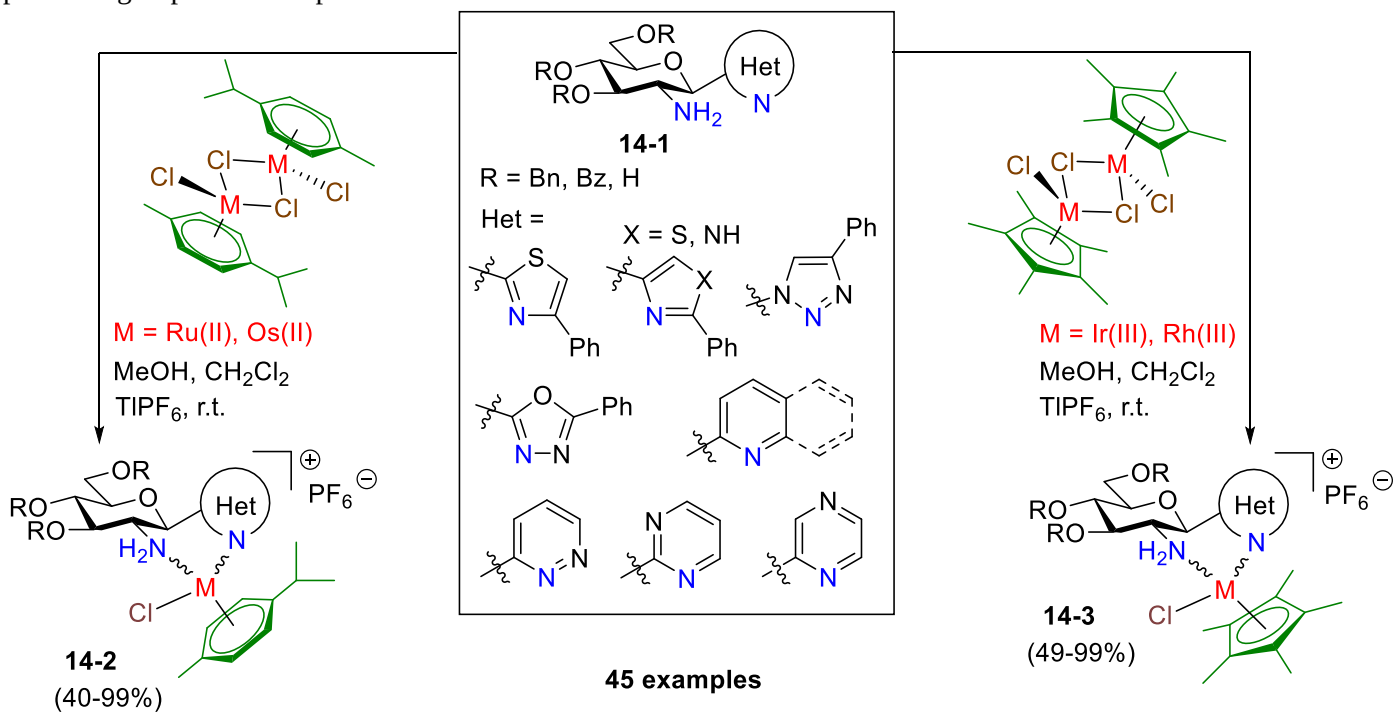
1.3. Synthesis of half-sandwich type platinum-group metal complexes with glycopyranosyl heterocyclic *N,N*-bidentate ligands

C- and *N*-Glycopyranosyl heterocyles shown in Schemes 1, 2 and 12 were used as *N,N*-bidentate ligands in the formation of platinum-group metal half-sandwich complexes. Treatment of the corresponding hetaryl-substituted *C*-glycopyranosyl oxadiazoles and *N*-glucopyranosyl-1,2,3-triazoles (**13-1** in Scheme 13) with dichloro(η^6-*p*-cymene)ruthenium(II) and osmium(II) dimers in the presence of the halide abstractor $TlPF_6$ resulted in the desired cationic complexes **13-2** with five-membered chelate rings.[16,17,19,57] The synthesis of analogous complexes **13-3** constructed from **13-1** with dichloro(pentamethylcyclopentadienyl)iridium(III) and rhodium(III) dimers were also carried out.[17,19,57] Each complex was obtained as a mixture of two diastereoisomers. For comparative biological studies, two further complexes with non-sugar based azole ligands **13-4** and **13-5** were also synthesized.[16]



Scheme 13. Synthesis of half-sandwich complexes with glycopyranosyl azole ligands

Another large set of half-sandwich complexes (Scheme 14, **14-2** and **14-3**), with six-membered chelate rings, was produced by the reaction of the glucosaminyl heterocycles **14-1** with the aforementioned dimeric chloro-bridged platinum-group metal complexes.



Scheme 14. Synthesis of half-sandwich complexes with glucosaminyl heterocyclic ligands

It is to be noted that the use of glucopyranosyl cyanide[58] and pre-formed *O*-perbenzoylated and *O*-deprotected 2-(β-D-glucopyranosyl)-5-phenyl-1,3,4-oxadiazoles[13] as monodentate ligands were also investigated, however their attempted complexations with dichloro(η^6-*p*-cymene)ruthenium(II) dimer did not result in the formation of the expected half-sandwich complexes. Therefore, further experiments in this direction with similar *C*-glucopyranosyl azoles, e.g. with the newly prepared imidazoles and thiazoles shown in Scheme 3, were discontinued.

The distribution coefficient of each complex shown in Schemes **13** and **14** was determined in an *n*-octanol-aqueous PBS (phosphate buffered saline, pH = 7.4) system. Based on these data, it can be seen that the cationic complexes having sugar-based ligands *O*-protected with large hydrophobic acyl (e.g. Bz, COC_nH_{2n+1} with $n \geq 3$) or benzyl groups have lipophilic character (logD = +1.5 – +3.3), while the complexes constructed with *O*-unprotected or *O*-peracetylated derivatives remain hydrophilic (logD = -0.75 – -2.1).[16,17,19]

During preliminary solution equilibrium studies performed by Péter Buglyó's group the proton dissociation processes and metal ion binding capabilities of selected *C*-glycopyranosyl heterocyclic *N*,*N*-chelators have also been explored. Routine titrations revealed slow decomposition processes in the basic pH range. Interestingly, narrowing the used pH range, low basicity of the N donor atoms of the coordinating part of the ligands was found. It could also be seen that the presence of the ligands prevent the studied organoruthenium cation from hydrolysis thus suggesting strong metal ion – ligand interaction.

2. Biological results

The new 2-aryl-4-(β-D-glucopyranosyl)thiazoles and imidazoles (e.g. **I** and **II** in Fig. 1), designed and synthesized based on promising *in silico* screening experiments,[26] were tested for the inhibition of the isoforms of glycogen phosphorylase to get new low micromolar inhibitors of the biologically relevant hlGPa enzyme.[59] These compounds were also tested in *ex vivo* studies and the best inhibitor of the series **II** displayed significant GP inhibitory activity (60 %) in human hepatocarcinoma HepG2 cells.[59] X-Ray crystallographic studies of the enzyme-inhibitor complexes showed the binding peculiarities of these molecules and revealed a new sulfur σ-hole phenomenon for the thiazole derivatives.[59] The inhibitory effect of the annulated *C*-glycopyranosyl imidazoles was also determined against rmGPb, however these compounds exhibited no or very week inhibition (e.g. **III** and **IV**).[24]

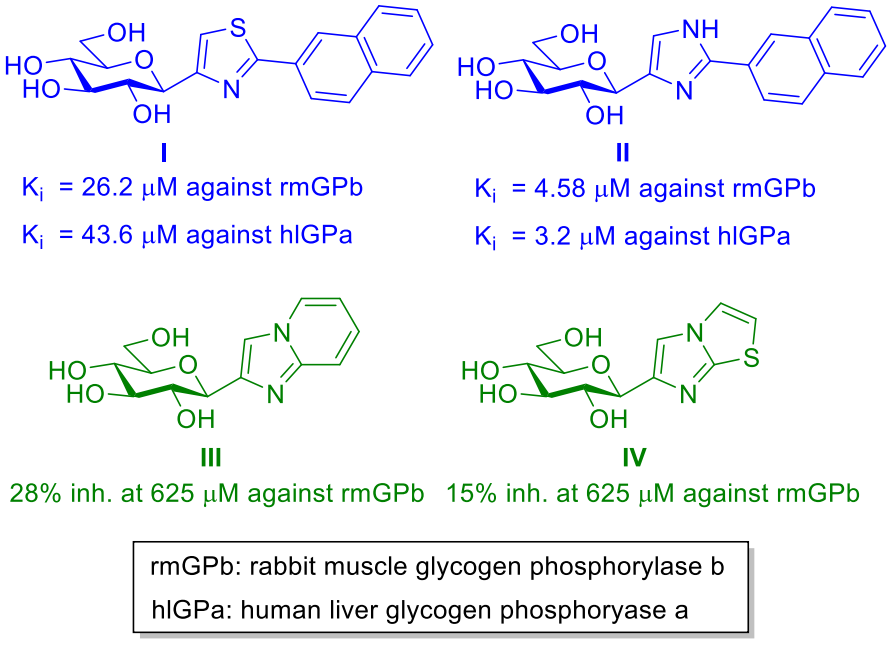


Figure 1. Inhibitory effect of the newly prepared *C*-glucopyranosyl azoles against glycogen phosphorlyase

The human *O*-(*N*-acetyl-β-D-glucosaminyl)-L-serine/threonine *N*-acetylglucosaminyl hydrolase enzyme (OGA) is in the forefront of glycomimetic design due to its multiple effects on various proteins thereby modulating a large number of physiological and pathological events.[60] Since *C*-glucosaminyl heterocycles have not yet been tried as inhibitors of OGA, some compounds available by modifications of the syntheses of 5-membered *C*-glucosaminyl heterocycles (Scheme 2) such as **V** and **VI** were tested against this enzyme, as well. Unfortunately, these compounds showed no

significant inhibitory effect (Fig. 2), therefore, these investigations were halted. On the other hand, lactone hydrazone type compounds **VII** and **VIII** proved low nanomolar inhibitors of OGA and, as a completion of previously started investigations, their structure-activity relationship has been studied and published in two papers.[61,62]

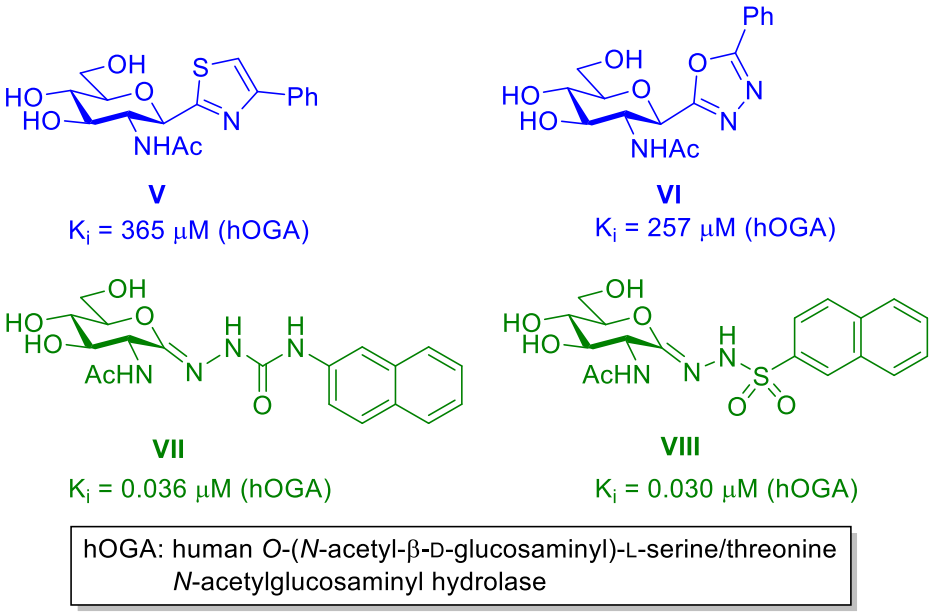


Figure 2. Inhibition of hOGA by *C*-glucosaminyl azoles and other glucosamine derivatives

The inhibitory potential of the 2-*C*-glycopyranosyl pyrimidines was tested against some glycoenzymes. None of the compounds displayed inhibitory activity against glycogen phosphorylase, however, some members of the series (**IX-XI** in Fig. 3) proved to be submillimolar inhibitors of α-glucosidase and β-galactosidase enzymes.[29,30]

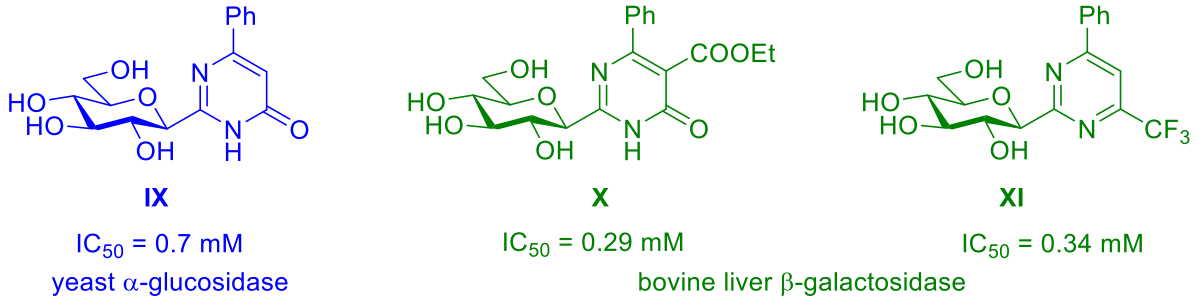


Figure 3. Inhibitory activity of the prepared 2-*C*-glycopyranosyl pyrimidines against glycosidase enzymes

4-Arylmethyl-2-(β-D-glucopyranosyl)-pyrimidines were evaluated as inhibitors of sodium dependent glucose contransporters SGLT1 and SGLT2 to reveal compounds, e.g. **XII** in Fig. 4, exhibiting low micromolar inhibition against the SGLT2 protein.[31] Despite the structural resemblance of **XII** to dapagliflozin **XIII** (an active ingredient as SGLT2 inhibitor of an oral antidiabetic drug), this compound proved to be three order of magnitude weaker inhibitor than the reference compound, remained, however, still selective for SGLT2 over SGLT1. The (*C*-β-D-glucopyranosyl(het)aryl)arene type **XII** and **XIII** were tested against glycogen phosphorylase, but none of them displayed inhibitory potential.[31] To identify new SGLT inhibitors, this study was also extended to the investigation of (*C*-β-D-glucopyranosylhetaryl)arene type GP inhibitors prepared earlier by us. Among these compounds, the 1,2,4-triazole **XIV** and the imidazole **XV**, known as the best nanomolar glucose analog inhibitors of GP,[63-66] also showed significant SGLT inhibition. Thus, the first SGLT-GP dual acting inhibitors were developed providing a novel concept and the potential of application for future antidiabetic therapy.[31]

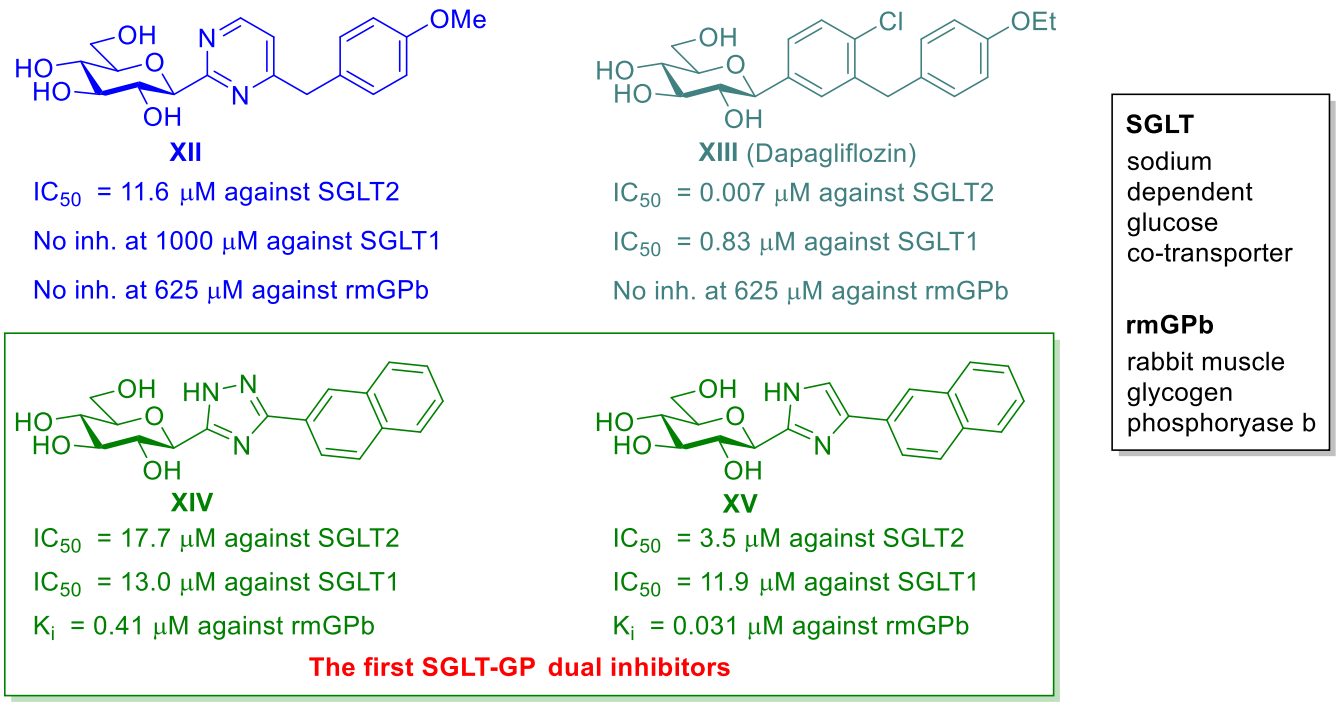


Figure 4. Inhibitory activity of *C*-glucopyranosyl heterocycles against sodium dependent glucose co-transporters

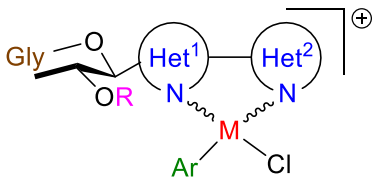
The prepared half-sandwich type platinum-group metal complexes containing glycopyranosyl azole ligands were studied for inhibiting proliferation of different cancer cells (Fig. 5, **XVI**), and several of them showed low micromolar (e.g. **XVII**) or in certain cases submicromolar (e.g. **XVIII**) cytostatic activities.[16,17,19,57] The inhibitory effects of most of the complexes proved to be selective for cancer cell lines, their toxic and cytostatic properties were absent or stongly reduced when probed on untransformed (noncancerous) human primary fibroblasts. In most of the cases the Hill coefficient of the complexes was in the range of 2-3, suggesting cooperative binding properties during their at present not fully understood way of action. Based on preliminary biological investigations, the antiproliferative effect of these complexes was associated with reactive oxygen species production,[16,17,19,57] nevertheless, further studies are needed to reveal the mechanism of the strong antineoplastic effects of the compounds.

By means of the large number of test compounds **XVI** displaying structural diversity and their biological results structure-activity relationships were also extensively examined, and the main general findings were highlighted in Fig. 5 and explained in details in our published papers.[16,17,19]

One of the most important structural requirements for biological efficiency is that of the presence of the sugar moiety *O*-protected with large hydrophobic acyl, preferably with benzoyl groups. These results are in accordance with the favorably increased lipophilic character (logD) of these complexes. Another crucial point is the metal ion with the coordinating arene or arenyl type ligand, revealing preference of the Ru(II) and Os(II) complexes with *p*-cymene (*p*-cym) ligand.[16,17,19] While the organoruthenium and -osmium cations have a formal +2 oxidation state and bear a neutral hexahapto bound arene ligand (*p*-cym), in organorhodium and -iridium cations with the +3 oxidation state of the metal ions pentahapto coordinated negatively charged arenyl (pentamethylcyclopentadienyl = Cp*) ligand can be found. This difference provides different electron density along the metal ions, different hydrolytic behaviour of the aqua species together with different steric hindrance of the aromatic units. The different kinetic behaviour of these metal ions may also significantly influence their likely biological effects providing optimal lifetime in various biological media. In this context the following inertness order can be given: Rh < Ru << Os ~ Ir.

In addition, the complexes displaying antineoplastic activities proved to be effective against Gram positive multirestistant bacteria. The best compound of the series was compound **XVIII** exhibiting low micromolar antimicrobial effects on *Staphylococcus aureus* and *Enterococcus* species (Fig. 5).[57,67]

Structure-activity relationships showing increasing biological activities:



XVI

R: H/$COCH_3$ inactive; R = COC_nH_{2n+1} (n = 3-7) < COPh

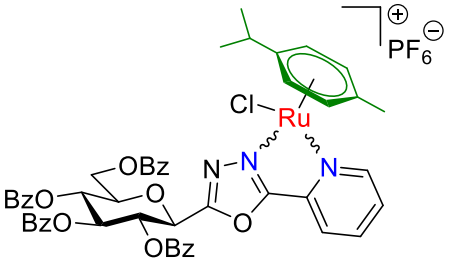
Gly: D-xylo < D-gluco ~ D-galacto

Het^1: 1,2,4-oxadiazole < 1,3,4-oxadiazole < 1,2,3-triazole

Het^2: 2-pyridyl < 2-quinolinyl

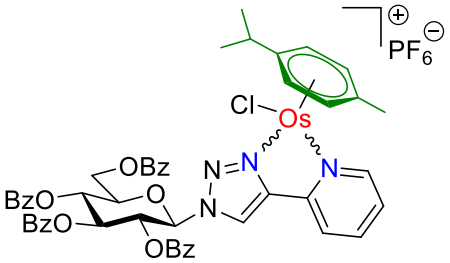
M: Ir(III)/Rh(III) with Cp* < Ru(II)/Os(II) with *p*-cym

The sugar based ligands and the non-sugar based complexes had no or negligible biological effects.



XVII

Cell line	IC_{50} (μM)*	Hill slope
A2780	6.19	2.62
ID8	5.66	3.04
Capan2	1.81	1.81
U251	4.17	2.46
MCF7	3.74	2.01
Fibroblast	Not effect	



XVIII

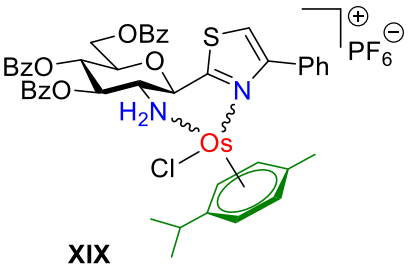
Cell line	IC_{50} (μM)*	Hill slope
A2780	0.73	3.01
ID8	1.96	3.14
Capan2	1.71	1.33
Saos	4.17	-
Fibroblast	Not effect	

***Cytostatic activity** in different cell models:
-ovarian cancer (A2780, ID8),
-pancreatic adenocarcinoma (Capan2),
-gliobastoma (U251),
-breast cancer (MCF7)
-sarcoma (Saos)
-primary human dermal fibroblast

Antimicrobial activity of XVIII
MIC = 0.3-5.0 μM
on Gram-positive
multiresistant bacteria
-*Staphylococcus aureus*
-*Enterococcus species*

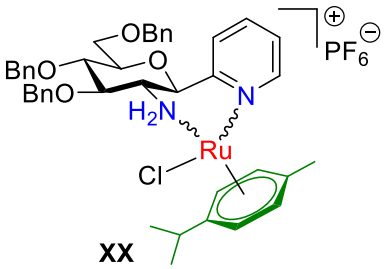
Figure 5. Antineoplastic and antimicrobial activities of the prepared half-sandwich complexes of the glycopyranosyl azoles

Similar biological studies are in progress for complexes derived from the glucosaminyl heterocycles. The preliminary antiproliferative studies in different cancer cell models show that the complexes with five-membered heterocyclic glucosamine derivatives are inactive or only slightly effective (e.g. **XIX** in Fig. 6). Some members of the six-membered heterocyclic complex series have low micromolar antineoplastic activity (e.g. **XX**), but show significant inhibition on non-transformed human dermal fibroblasts, are thus not selective. A manuscript containing the syntheses of the latter type of complexes is in preparation and will be published soon when completed with the biological results.[68]



XIX

30% inhibition at 100 μM



XX

IC_{50} = 1.86 μM

A2780 ovarian cancer cell line

Figure 6. Antineoplastic activity of selected half-sandwich complexes of the glucosaminyl heterocycles

Collaborations

The aforementioned biological and computational studies were performed in the frame of domestic and international collaborations. The principal scientists of these research teams with their contributions are listed below:

Joseph M. Hayes (School of Pharmacy & Biomedical Sciences, University of Central Lancashire, United Kingdom):
Computational studies related to the inhibitors of GP and hOGA.

Demetres D. Leonidas (Department of Biochemistry and Biotechnology, University of Thessaly, Greece):
Enzyme kinetic, ex vivo and protein crystallography studies related to the inhibitors of GP.

Tibor Docsa (Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Hungary):
Kinetic studies carried out in vitro or in cell modells for the determination of GP and SGLT inhibitory effects of the compounds.

Gyöngyi Gyémánt (Department of Inorganic & Analytical Chemistry, University of Debrecen, Hungary):
Enzyme kinetic studies of the compounds for glycosidase inhibition.

Péter Bay (Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Hungary*)*:
Studies of the antineoplastic effects of the compounds.

Gábor Kardos (Department of Metagenomics, University of Debrecen, Hungary):
Studies of the antimicrobial effects of the compounds.

Significance of the results

Synthesized and fully characterized were close to 400 compounds from which ~170 new chemical entities were used as test compounds in various biological studies.

Synthetic methods were elaborated for completely unknown or barely reported 6-membered *C*-glycopyranosyl heterocycles (such as pyridines, quinolines, pyridazines, pyrimidines, pyrazines, 1,2,4-triazines and 1,2,4,5-tetrazines) which are now available for various applications.

Among the otherwise unknown *C*-glucopyranosyl 1,2,4,5-tetrazines the first fluorescent derivatives were synthesized that may pave the way to a new type of bioorthogonal labeling of biomolecules.

Novel types of platinum group metal half sandwich complexes were designed and synthesized several of which proved to be superior to the clinically used platinum complexes as antineoplastic agents, and are also potential antibiotics against multiresistant Gram-positive bacteria.

As a new direction of antidiabetic therapy, a novel concept of dual-target SGLT2-GP inhibitors was laid down and demonstrated experimentally.

Publications and other scientific reports

Related to the scientific results of this work 16 publications (marked in bold in the reference list) have been reported altogether. Among them, there are 13 full papers (refs. 16, 17, 24, 26, 29, 30, 31, 35, 49, 59, 61, 62, 67) and a review article (ref. 66), published in international journals, and an additional overview published as a book chapter (ref. 65). In addition, a patent application has also been filed (ref. 57). One of the papers received the publication prize of the Lajos Kisfaludy Foundation (ref. 29). An additional paper, including our synthetic results and biological studies of our domestic collaborators has been submitted (ref. 19, underlined) and another manuscript is in preparation (ref. 68, dashed lined). Based on further results of this project presented in this report at least three further publications are foreseen. In the course of this research project 22 oral lectures and posters were presented in domestic and international conferences. Two PhD dissertations and 11 diploma theses have been completed and an additional PhD dissertation is in preparation.

References

(1) Melchart, M.; Sadler, P. J. Ruthenium Arene Anticancer Complexes. In *Bioorganometallics*, Jaouen, G. Ed.; Wiley-VCH Verlag GmbH & Co. KGaA, 2006; pp 39-64. DOI: 10.1002/3527607692.ch2

(2) Hanif, M.; Babak, M. V.; Hartinger, C. G. Development of anticancer agents: wizardry with osmium. *Drug Discov. Today* **2014**, *19*, 1640-1648. DOI: 10.1016/j.drudis.2014.06.016.

(3) Leung, C. H.; Zhong, H. J.; Chan, D. S. H.; Ma, D. L. Bioactive iridium and rhodium complexes as therapeutic agents. *Coord. Chem. Rev.* **2013**, *257*, 1764-1776. DOI: 10.1016/j.ccr.2013.01.034.

(4) Kenny, R. G.; Marmion, C. J. Toward Multi-Targeted Platinum and Ruthenium Drugs-A New Paradigm in Cancer Drug Treatment Regimens? *Chem. Rev.* **2019**, *119*, 1058-1137. DOI: 10.1021/acs.chemrev.8b00271.

(5) Štarha, P.; Trávníček, Z. Non-platinum complexes containing releasable biologically active ligands. *Coord. Chem. Rev.* **2019**, *395*, 130-145. DOI: 10.1016/j.ccr.2019.06.001.

(6) Máliková, K.; Masaryk, L.; Štarha, P. Anticancer Half-Sandwich Rhodium(III) Complexes. *Inorganics* **2021**, *9*, 26. DOI: 10.3390/inorganics9040026.

(7) Böge, M.; Fowelin, C.; Bednarski, P.; Heck, J. Diaminohexopyranosides as Ligands in Half-Sandwich Ruthenium(II), Rhodium(III), and Iridium(III) Complexes. *Organometallics* **2015**, *34*, 1507-1521. DOI: 10.1021/om5013117.

(8) Florindo, P.; Marques, I. J.; Nunes, C. D.; Fernandes, A. C. Synthesis, characterization and cytotoxicity of cyclopentadienyl ruthenium(II) complexes containing carbohydrate-derived ligands. *J. Organomet. Chem.* **2014**, *760*, 240-247. DOI: 10.1016/j.jorganchem.2013.09.004.

(9) Florindo, P. R.; Pereira, D. M.; Borralho, P. M.; Rodrigues, C. M. P.; Piedade, M. F. M.; Fernandes, A. C. Cyclopentadienyl-Ruthenium(II) and Iron(II) Organometallic Compounds with Carbohydrate Derivative Ligands as Good Colorectal Anticancer Agents. *J. Med. Chem.* **2015**, *58*, 4339-4347. DOI: 10.1021/acs.jmedchem.5b00403.

(10) Florindo, P. R.; Pereira, D. M.; Borralho, P. M.; Costa, P. J.; Piedade, M. F. M.; Rodrigues, C. M. P.; Fernandes, A. C. New $[(\eta^5\text{-}C_5H_5)Ru(N\text{-}N)(PPh_3)][PF_6]$ compounds: colon anticancer activity and GLUT-mediated cellular uptake of carbohydrate-appended complexes. *Dalton Trans.* **2016**, *45*, 11926-11930. DOI: 10.1039/c6dt01571a.

(11) Fernandes, A. C. Synthesis, Biological Activity and Medicinal Applications of Ruthenium Complexes Containing Carbohydrate Ligands. *Curr. Med. Chem.* **2019**, *26*, 6412-6437. DOI: 10.2174/0929867326666190124124350.

(12) Hamala, V.; Martišová, A.; Šťastná, L. C.; Karban, J.; Dančo, A.; Šimarek, A.; Lamač, M.; Horáček, M.; Kolářová, T.; Hrstka, R.; Gyepes, R.; Pinkas, J. Ruthenium tetrazene complexes bearing glucose moieties on their periphery: Synthesis, characterization, and *in vitro* cytotoxicity. *Appl. Organomet. Chem.* **2020**, *34*, e5896. DOI: 10.1002/aoc.5896.

(13) Tóth, M.; Kun, S.; Bokor, É.; Benltifa, M.; Tallec, G.; Vidal, S.; Docsa, T.; Gergely, P.; Somsák, L.; Praly, J.-P. Synthesis and structure-activity relationships of *C*-glycosylated oxadiazoles as inhibitors of glycogen phosphorylase. *Bioorg. Med. Chem.* **2009**, *17*, 4773-4785. DOI: 10.1016/j.bmc.2009.04.036.

(14) Benltifa, M.; Vidal, S.; Fenet, B.; Msaddek, M.; Goekjian, P. G.; Praly, J.-P.; Brunyánszki, A.; Docsa, T.; Gergely, P. In the Search of Glycogen Phosphorylase Inhibitors: 5-Substituted 3-*C*-Glucopyranosyl-1,2,4-Oxadiazoles from β-D-Glucopyranosyl Cyanides upon Cyclization of *O*-Acyl-amidoxime Intermediates. *Eur. J. Org. Chem.* **2006**, 4242-4256. DOI: 10.1002/ejoc.200600073.

(15) Cecioni, S.; Argintaru, O.-A.; Docsa, T.; Gergely, P.; Praly, J.-P.; Vidal, S. Probing multivalency for the inhibition of an enzyme: glycogen phosphorylase as a case study. *New J. Chem.* **2009**, *33*, 148-156. DOI: 10.1039/B812540F.

(16) Kacsir, I.; Sipos, A.; Ujlaki, G.; Buglyó, P.; Somsák, L.; Bai, P.; Bokor, É. Ruthenium half-sandwich type complexes with bidentate monosaccharide ligands show antineoplastic activity in ovarian cancer cell models through reactive oxygen species production. *Int. J. Mol. Sci.* 2021, *22*, 454. DOI: 10.3390/ijms221910454.

(17) Kacsir, I.; Sipos, A.; Bényei, A.; Janka, E.; Buglyó, P.; Somsák, L.; Bai, P.; Bokor, É. Reactive oxygen species production is responsible for antineoplastic activity of osmium, ruthenium, iridium and rhodium half-sandwich type complexes with bidentate glycosyl heterocyclic ligands in various cancer cell models. *Int. J. Mol. Sci.* 2022, *23*, 813. DOI: 10.3390/ijms23020813.

(18) Bokor, É.; Koppány, C.; Gonda, Z.; Novák, Z.; Somsák, L. Evaluation of bis-triphenylphosphano-copper(I)-butyrate ($C_3H_7COOCu(PPh_3)_2$) as catalyst for the synthesis of 1-(D-glycopyranosyl)-4-substituted-1,2,3-triazoles. *Carbohydr. Res.* **2012**, *351*, 42-48. DOI: 10.1016/j.carres.2012.01.004.

(19) Kacsir, I.; Sipos, A.; Kiss, T.; Major, E.; Bajusz, N.; Tóth, E.; Buglyó, P.; Somsák, L.; Kardos, G.; Bai, P.; Bokor, É. Half Sandwich-Type Osmium, Ruthenium, Iridium and Rhodium Complexes with Bidentate Glycosyl Heterocyclic Ligands Induce Cytostasis in Platinum-Resistant Ovarian Cancer Cells and Bacteriostasis in Gram-Positive Multiresistant Bacteria. *Front. Chem.* **2022**, submitted.

(20) Myers, R. W.; Lee, Y. C. Improved preparations of some per-*O*-acetylated aldohexopyranosyl cyanides. *Carbohydr. Res.* **1986**, *154*, 145-163. DOI: 10.1016/s0008-6215(00)90029-6.

(21) Áts, S. C.; Lausberg, E.; Lehmann, J.; Sandhoff, K. Synthesis of 4-acetamido-3,7-anhydro-2-azi-1,2,4-trideoxy-D-glycero-D-gulooctitol, a good competitive inhibitor and potential photoaffinity reagent for human β-hexosaminidase. *Liebigs Ann. Chem.* **1990**, 1261-1264. DOI: 10.1002/jlac.1990199001226.

(22) Koppány, C. D. Új *C*-(β-D-glikopiranozil)-azolok előállításának vizsgálata a glikogén foszforiláz enzim gátlására. Diploma thesis, University of Debrecen, Debrecen, **2014**.

(23) Bokor, É.; Kyriakis, E.; Solovou, T. G. A.; Koppány, C.; Kantsadi, A. L.; Szabó, K. E.; Szakács, A.; Stravodimos, G. A.; Docsa, T.; Skamnaki, V. T.; Zographos, S. E.; Gergely, P.; Leonidas, D. D.; Somsák, L. Nanomolar inhibitors of glycogen phosphorylase based on β-D-glucosaminyl heterocycles: a combined synthetic, enzyme kinetic and protein crystallography study. *J. Med. Chem.* **2017**, *60*, 9251-9262. DOI: 10.1021/acs.jmedchem.7b01056.

(24) Szennyes, E.; Bokor, É.; Docsa, T.; Sipos, Á.; Somsák, L. Synthesis of *C*-β-D-glucopyranosyl derivatives of some fused azoles for the inhibition of glycogen phosphorylase. *Carbohydr. Res.* 2019, *472*, 33-41. DOI: 10.1016/j.carres.2018.11.003.

(25) Czifrák, K.; Szilágyi, P.; Somsák, L. Anomeric α-azido acid (2-azido-2-deoxy-hept-2-ulopyranosonic acid) derivatives en route to peptides incorporating sugar amino acids. *Tetrahedron: Asymm.* **2005**, *16*, 127-141. DOI: 10.1016/j.tetasy.2004.11.064.

(26) Barr, D.; Szennyes, E.; Bokor, É.; Al-Oanzi, Z. H.; Moffatt, C.; Kun, S.; Docsa, T.; Sipos, Á.; Davies, M. P.; Mathomes, R.; Snape, T. J.; Agius, L.; Somsák, L.; Hayes, J. M. Identification of *C*-β-D-glucopyranosyl azole type inhibitors of glycogen phosphorylase that reduce glycogenolysis in hepatocytes: *in silico* design, synthesis, *in vitro* kinetics and *ex-vivo* studies. *ACS Chem. Biol.* 2019, *14*, 1460-1470. DOI: 10.1021/acschembio.9b00172.

(27) Togo, H.; Ishigami, S.; Fujii, M.; Ikuma, T.; Yokoyama, M. Synthesis of *C*-nucleosides via radical coupling reaction. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2931-2942. DOI: 10.1039/P19940002931.

(28) Szennyes, E.; Bokor, É.; Batta, G.; Docsa, T.; Gergely, P.; Somsák, L. Improved preparation of 4(5)-aryl-2-(β-D-glucopyranosyl)-imidazoles, the most efficient glucose analogue inhibitors of glycogen phosphorylase. *RSC Adv.* **2016**, *6*, 94787-94794. DOI: 10.1039/C6RA21839C.

(29) Szennyes, E.; Bokor, É.; Langer, P.; Gyémánt, G.; Docsa, T.; Sipos, Á.; Somsák, L. The first general synthesis of 2-*C*-(β-D-glycopyranosyl)pyrimidines and their evaluation as inhibitors of some glycoenzymes *New J. Chem.* 2018, *42*, 17439-17446. DOI: 10.1039/C8NJ04035D.

(30) Szennyes, E.; Gyémánt, G.; Somsák, L.; Bokor, É. Synthesis of new series of 2-*C*-(β-D-glucopyranosyl)pyrimidines and their evaluation as inhibitors of some glycoenzymes. *Molecules* 2020, *25*, 701. DOI: 10.3390/molecules25030701.

(31) Sipos, Á.; Szennyes, E.; Hajnal, N. É.; Kun, S.; Szabó, K. E.; Uray, K.; Somsák, L.; Docsa, T.; Bokor, É. Dual-Target Compounds against Type 2 Diabetes Mellitus: Proof of Concept for Sodium Dependent Glucose Transporter (SGLT) and Glycogen Phosphorylase (GP) Inhibitors. *Pharmaceuticals* 2021, *14*, 364. DOI: 10.3390/ph14040364.

(32) Feuillastre, S.; Chajistamatiou, A. S.; Potamitis, C.; Zervou, M.; Zoumpoulakis, P.; Chrysina, E. D.; Praly, J. P.; Vidal, S. *C*-Glucosylated malonitrile as a key intermediate towards carbohydrate-based glycogen phosphorylase inhibitors. *Bioorg. Med. Chem.* **2012**, *20*, 5592-5599. DOI: 10.1016/j.bmc.2012.07.033.

(33) Inaba, Y.; Yano, S.; Mikata, Y. Preparation of *C*-glycoside Pendant β^2- and $\beta^{2,2}$-Amino Acids. *Bull. Chem. Soc. Jpn.* **2008**, *81*, 606-616. DOI: 10.1246/bcsj.81.606.

(34) Pachamuthu, K.; Gupta, A.; Das, J.; Schmidt, Richard R.; Vankar, Yashwant D. An Easy Route to 2-Amino-β-*C*-Glycosides by Conjugate Addition to 2-Nitroglycals. *Eur. J. Org. Chem.* **2002**, 1479-1483. DOI: 10.1002/1099-0690(200205)2002:9<1479::AID-EJOC1479>3.0.CO;2-P.

(35) Bokor, É.; Ferenczi, A.; Hashimov, M.; Juhász-Tóth, É.; Götz, Z.; Zaki, A. I.; Somsák, L. First synthesis of 3-glycopyranosyl-1,2,4-triazines and some cycloadditions thereof. *Molecules* 2022, 7801. DOI: 10.3390/molecules27227801.

(36) Bokor, É.; Fekete, A.; Varga, G.; Szőcs, B.; Czifrák, K.; Komáromi, I.; Somsák, L. *C*-(β-D-Glucopyranosyl)formamidrazones, formic acid hydrazides and their transformations into 3-(β-D-glucopyranosyl)-5-substituted-1,2,4-triazoles: a synthetic and computational study. *Tetrahedron* **2013**, *69*, 10391-10404. DOI: 10.1016/j.tet.2013.09.099.

(37) Bokor, É.; Szilágyi, E.; Docsa, T.; Gergely, P.; Somsák, L. Synthesis of substituted 2-(β-D-glucopyranosyl)-benzimidazoles and their evaluation as inhibitors of glycogen phosphorylase. *Carbohydr. Res.* **2013**, *381*, 179-186. DOI: 10.1016/j.carres.2013.01.011.

(38) Boger, D. L. Diels-Alder Reactions of Azadienes. *Tetrahedron* **1983**, *39*, 2869-2939. DOI: 10.1016/s0040-4020(01)92154-4.

(39) Boger, D. L. Diels-Alder Reactions of Heterocyclic Azadienes - Scope and Applications. *Chem. Rev.* **1986**, *86*, 781-793. DOI: 10.1021/cr00075a004.

(40) Neunhoeffer, H. Tetrazines and Pentazines. In *Comprehensive Heterocyclic Chemistry*, Katritzky, A. R., Rees, C. V. Eds.; Vol. 3; Pergamon, 1984; pp 531-572.

(41) Neunhoeffer, H. 1,2,4,5-Tetrazines. In *Methoden der Organischen Chemie (Houben-Weyl), Hetarenes III, Part 3*, 4th ed.; Vol. E9; Georg Thieme Verlag, 1997; pp 870-916.

(42) Shih, H. W.; Kamber, D. N.; Prescher, J. A. Building better bioorthogonal reactions. *Curr. Opin. Chem. Biol.* **2014**, *21*, 103-111. DOI: 10.1016/j.cbpa.2014.07.002.

(43) King, M.; Wagner, A. Developments in the Field of Bioorthogonal Bond Forming Reactions-Past and Present Trends. *Bioconjugate Chem.* **2014**, *25*, 825-839. DOI: 10.1021/bc500028d.

(44) Zheng, M. M.; Zheng, L.; Zhang, P. Y.; Li, J. B.; Zhang, Y. Development of Bioorthogonal Reactions and Their Applications in Bioconjugation. *Molecules* **2015**, *20*, 3190-3205. DOI: 10.3390/molecules20023190.

(45) Oliveira, B. L.; Guo, Z.; Bernardes, G. J. L. Inverse electron demand Diels-Alder reactions in chemical biology. *Chem. Soc. Rev.* **2017**, *46*, 4895-4950. DOI: 10.1039/c7cs00184c.

(46) Liu, F.; Liang, Y.; Houk, K. N. Bioorthogonal Cycloadditions: Computational Analysis with the Distortion/Interaction Model and Predictions of Reactivities. *Acc. Chem. Res.* **2017**, *50*, 2297-2308. DOI: 10.1021/acs.accounts.7b00265.

(47) Choi, S. K.; Kim, J.; Kim, E. Overview of Syntheses and Molecular-Design Strategies for Tetrazine-Based Fluorogenic Probes. *Molecules* **2021**, *26*, 1868. DOI: 10.3390/molecules26071868.

(48) Wang, L.; Zhang, J. W.; Zhao, J. Z.; Yu, P.; Wang, S.; Hu, H. M.; Wang, R. Recent synthesis of functionalized *s*-tetrazines and their application in ligation reactions under physiological conditions: a concise overview. *Catal. Rev.-Sci. Eng.* **2020**, *62*, 524-565. DOI: 10.1080/01614940.2020.1726009.

(49) Bokor, É.; Kecskés, D. T.; Gombás, F.; Fehér, A.; Kardos, E.; Dabian, A.; Vonza, Z.; Szennyes, E.; Somsák, L. First representatives of *C*-glycosyl 1,2,4,5-tetrazines: Synthesis of 3-β-D-glucopyranosyl 1,2,4,5-tetrazines and their transformation into 3-β-D-glucopyranosyl pyridazines. *New J. Chem.* 2022, Advance article. DOI: 10.1039/D2NJ03920F.

(50) Xie, Y.; Fang, Y.; Huang, Z.; Tallon, A. M.; am Ende, C. W.; Fox, J. M. Divergent Synthesis of Monosubstituted and Unsymmetrical 3,6-Disubstituted Tetrazines from Carboxylic Ester Precursors. *Angew. Chem. Int. Ed.* **2020**, *59*, 16967-16973. DOI: 10.1002/anie.202005569.

(51) Neumann, K.; Gambardella, A.; Lilienkampf, A.; Bradley, M. Tetrazine-mediated bioorthogonal prodrug–prodrug activation. *Chem. Science* **2018**, *9*, 7198-7203. DOI: 10.1039/C8SC02610F.

(52) Kang, S. Y.; Song, K. S.; Lee, J.; Lee, S. H.; Lee, J. Synthesis of pyridazine and thiazole analogs as SGLT2 inhibitors. *Bioorg. Med. Chem.* **2010**, *18*, 6069-6079. DOI: 10.1016/j.bmc.2010.06.076.

(53) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. Readily Accessible Bicyclononynes for Bioorthogonal Labeling and Three-Dimensional Imaging of Living Cells. *Angew. Chem. Int. Edit.* **2010**, *49*, 9422-9425. DOI: 10.1002/anie.201003761.

(54) Vázquez, A.; Dzijak, R.; Dračínský, M.; Rampmaier, R.; Siegl, S. J.; Vrabel, M. Mechanism-Based Fluorogenic *trans*-Cyclooctene–Tetrazine Cycloaddition. *Angew. Chem. Int. Ed.* **2017**, *56*, 1334-1337. DOI: 10.1002/anie.201610491.

(55) Delaunay, T.; Poisson, T.; Jubault, P.; Pannecoucke, X. Stereoselective Access to β-*C*-Glycosamines by Nitro-Michael Addition of Organolithium Reagents. *Eur. J. Org. Chem.* **2014**, 3341-3345. DOI: 10.1002/ejoc.201402001.

(56) Polat, T.; Linhardt, R. J. Zinc triflate-benzoyl bromide: a versatile reagent for the conversion of ether into benzoate protecting groups and ether glycosides into glycosyl bromides. *Carbohydr. Res.* **2003**, *338*, 447-449. DOI: 10.1016/s0008-6215(02)00481-0.

(57) Bai, P.; Balázs, B.; Bokor, É.; Buglyó, P.; Kacsir, I.; Kardos, G.; Kiss, G. A.; Sipos, A.; Somsák, L.; Tóth, Z. Half sandvich transition metal complexes and uses thereof. Hungary 2022. Hungarian patent application P2100326.

(58) Somsák, L.; Nagy, V. A new, scalable preparation of a glucopyranosylidene-spiro-thiohydantoin: one of the best inhibitors of glycogen phosphorylases. *Tetrahedron: Asymm.* **2000**, *11*, 1719-1727. Corrigendum 2247. DOI: 10.1016/S0957-4166(00)00107-5.

(59) Kyriakis, E.; Karra, A. G.; Papaioannou, O.; Solovou, T.; Skamnaki, V. T.; Liggri, P. G. V.; Zographos, S. E.; Szennyes, E.; Bokor, É.; Kun, S.; Psarra, A.-M. G.; Somsák, L.; Leonidas, D. D. The architecture of hydrogen and sulfur σ-hole interactions explain differences in the inhibitory potency of *C*-β-D-glucopyranosyl thiazoles, imidazoles and an *N*-β-D-glucopyranosyl tetrazole for human liver glycogen phosphorylase. *Bioorg. Med. Chem.* 2020*, 28,* 115196. DOI: 10.1016/j.bmc.2019.115196.

(60) Ma, J.; Wu, C.; Hart, G. W. Analytical and Biochemical Perspectives of Protein *O*-GlcNAcylation. *Chem. Rev.* **2021**, *121*, 1513-1581. DOI: 10.1021/acs.chemrev.0c00884.

(61) Kiss, M.; Szabó, E.; Bocska, B.; Sinh, L. T.; Fernandes, C. P.; Timári, I.; Hayes, J. M.; Somsák, L.; Barna, T. Nanomolar inhibition of human OGA by 2-acetamido-2-deoxy-D-glucono-1,5-lactone semicarbazone derivatives. *Eur. J. Med. Chem.* 2021*, 223,* 113649. DOI: 10.1016/j.ejmech.2021.113649.

(62) Kiss, M.; Timári, I.; Barna, T.; Mészáros, Z.; Slámová, K.; Bojarová, P.; Křen, V.; Hayes, J. M.; Somsák, L. 2-Acetamido-2-deoxy-D-glucono-1,5-lactone Sulfonylhydrazones: Synthesis and Evaluation as Inhibitors of Human OGA and HexB Enzymes. *Int. J. Mol. Sci.* 2022*, 23,* 1037. DOI: 10.3390/ijms23031037.

(63) Bokor, É.; Docsa, T.; Gergely, P.; Somsák, L. *C*-Glucopyranosyl-1,2,4-triazoles as new potent inhibitors of glycogen phosphorylase. *ACS Med. Chem. Lett.* **2013**, *4*, 612-615. DOI: 10.1021/ml4001529.

(64) Bokor, É.; Kun, S.; Docsa, T.; Gergely, P.; Somsák, L. 4(5)-Aryl-2-*C*-glucopyranosyl-imidazoles as new nanomolar glucose analog inhibitors of glycogen phosphorylase. *ACS Med. Chem. Lett.* **2015**, *6*, 1215-1219. DOI: 10.1021/acsmedchemlett.5b00361.

(65) Bokor, É. *N*- and *C*-Glycopyranosyl heterocycles as glycogen phosphorylase inhibitors. In *Recent Trends in Carbohydrate Chemistry - Synthesis, Structure and Function of Carbohydrates*, Rauter, A. P., Christensen, B. E., Somsák, L., Kosma, P., Adamo, R. Eds.; Vol. 1; Elsevier, 2020; pp 253-300.

(66) Somsák, L.; Bokor, É.; Juhász, L.; Kun, S.; Lázár, L.; Juhász-Tóth, É.; Tóth, M. New Syntheses towards *C*-Glycosyl Type Glycomimetics. *Pure Appl. Chem.* 2019*, 91,* 1159-1175. DOI: 10.1515/pac-2019-0208.

(67) Balázs, B.; Tóth, Z.; Kacsir, I.; Sipos, A.; Buglyó, P.; Somsák, L.; Bokor, É.; Kardos, G.; Bai, P. Targeting multiresistant Gram-positive bacteria by ruthenium, osmium, iridium and rhodium half-sandwich type complexes with bidentate monosaccharide ligands. *Front. Chem.* 2022*, 10,* 868234. DOI: 10.3389/fchem.2022.868234.

(68) Kacsir, I.; Sipos, A.; Buglyó, P.; Kardos, G.; Somsák, L.; Bai, P.; Bokor, É. Half-sandwich type complexes of *C*-glucosaminyl azines: syntheses, antineoplastic and antimicrobial activities. **2022**, in preparation.

Debrecen, November 21, 2022.

Éva Bokor
principal investigator