PD 121406

Synthesis and spirocyclization of bis-*C*,*C*-glycopyranosyl compounds

Closing report

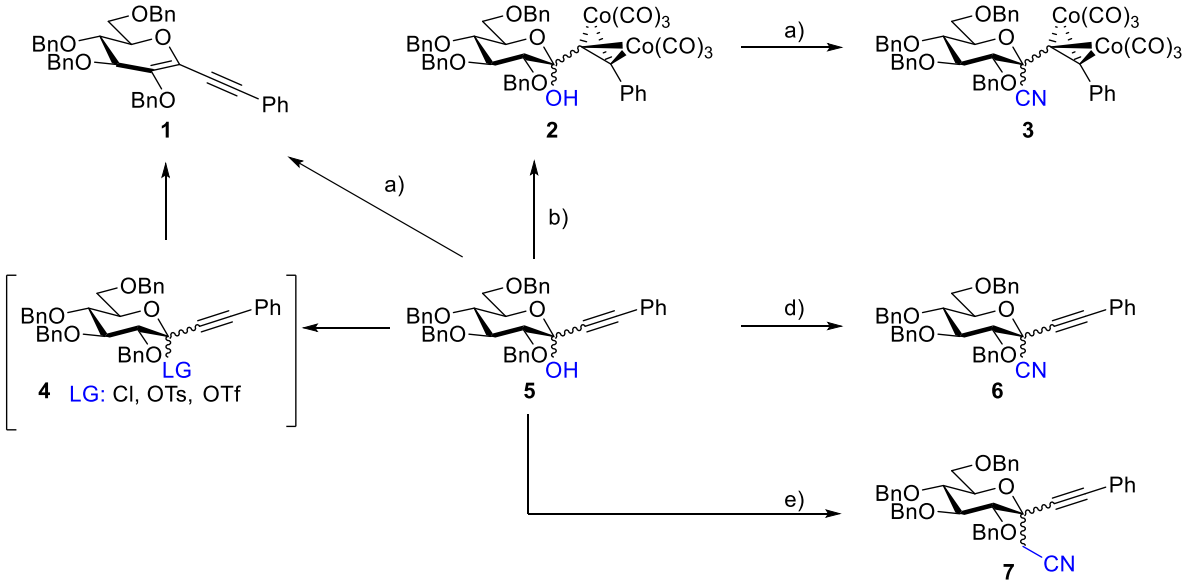
Among glycopyranosylidene spirocycles we can find many compound with considerable biological activity. A special class within these compounds is the group of bis-*C*,*C*-glycopyranosylidene-spirocycles, where two carbon substituents are attached to the sugar C1 (anomeric) carbon.

C-Glycosyl derivatives of ulosonic acids are scarcely known in the literature, such type of structures are limited only to the 3-deoxy type sialic acid derivatives. The main focus of this research was on a systematic study on the synthesis of bis-*C*,*C*-glycopyranosyl compounds from fully substituted heptulosonic acid derivatives, followed by cyclization to novel spirocycles.

In this report only the unpublished results will be discussed in detail, the published ones will be presented only in a concise form.

Ionic reactions toward bis-*C*,*C*-glycopyranosyl compounds

Substitution reactions of 1,2-dideoxy-1-phenyl-4,5,6,8-tetra-*O*-benzyl-β-D-gluco-oct-1-yn-3-ulopyranose (**5**) was investigated with *C*-nucleophiles (Scheme 1.). Direct activation of **5** by Lewis acids in the presence of Me_3SiCN or attempts to transform the anomeric OH to a better leaving group (**4**) resulted in immediate formation of glycal **1**. Replacement of the OH was possible on a dicobalt hexacarbonyl protected derivative **2** to give the bis-*C*,*C*-glycosyl compound **3**. Substitution reactions with Me_3SiCN and Me_3SiCH_2CN under Mitsunobu conditions gave the anomeric mixtures of the expected *C*-glycosyl compounds **6** and **7**, respectively, in moderate yields. To obtain precursors for spirocyclization various transformations of the nitrile group (to $CONH_2$, $CSNH_2$, CH_2NH_2) of **3**, **6** and **7** were attempted, however, these experiments typically resulted in the decomposition of the starting material under the applied conditions. Investigations were discontinued in this area.

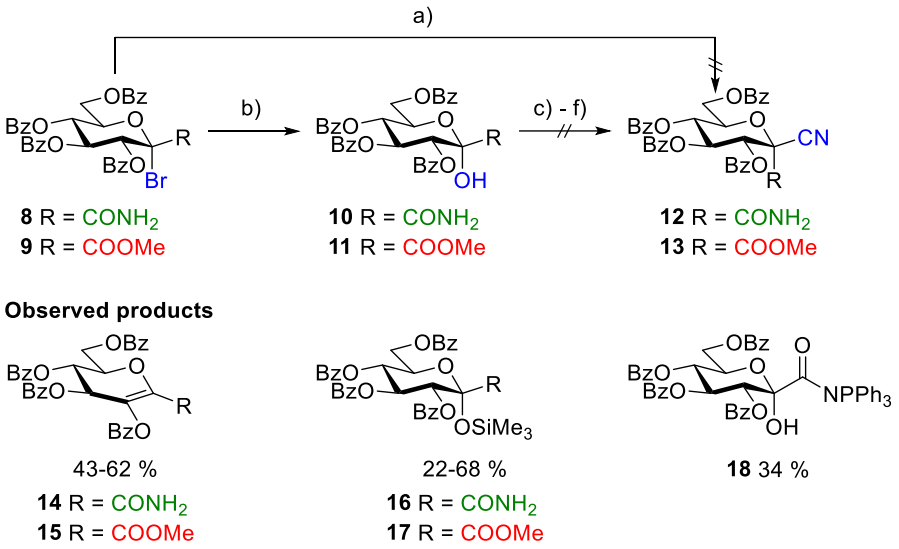


Scheme 1. Reaction conditions: a) Me_3SiCN, Me_3SiOTf, DCM, 61 %; b) $Co_2(CO)_8$, DCM, 81 %; Me_3SiCN, DEAD, PPh_3, THF, 42 %; d) Me_3SiCH_2CN, DEAD, PPh_3, THF, 39 %

Substitution of bromide in (ulosylbromide)onic acid derivatives by *O*-, *N*-, and *S*-nuclepohiles is a relatively easy task, however, their reactions with *C*-nucleophiles are unexplored so far.

Our research group has decades of experience in the synthetic use of glycosyl cyanides. Previously, we have developed several methods for the transformation of the nitrile group into various functions, so one of our main goals was to introduce this group to the anomeric center of ulosonic acids to yield precursors for spirocycle synthesis.

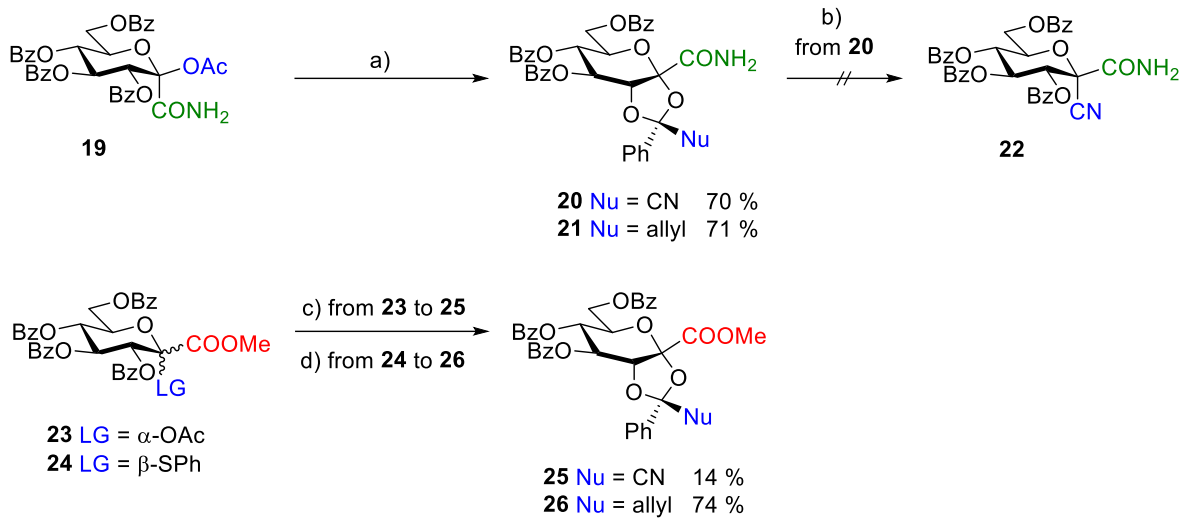
The replacement of bromide in (ulosylbromide)onic acid derivatives **8** and **9** with cyanide was investigated to obtain bis-*C*,*C*-glycosyl compounds **12** and **13**, respectively (Scheme 2.). Reactions were performed using various combinations of cyanide sources (NaCN, KCN, $Hg(CN)_2$) and solvents (DMF, DMSO, MeCN, $MeNO_2$) at different temperatures but the only observable products were glycals **14** and **15** formed by HBr elimination.



Scheme 2. Reaction conditions: a) NaCN or KCN or $Hg(CN)_2$, DMF or DMSO or MeCN or $MeNO_2$; b) Ag_2O, DMSO, H_2O; c) Me_3SiCN, DEAD, PPh_3, THF; d) Me_3SiCN, Me_3SiOTf, DCM; e) Me_3SiCN, I_2, DCM; f) acetone cyanohydrin, DEAD, PPh_3, THF

Activation of the anomeric hydroxyl group in the presence of Me_3SiCN is a well-known method for the preparation of glycosyl cyanides. Treatment of the ulopyranosonamide **10** with Me_3SiCN in the presence of TMSOTf or iodine gave silyl ether **16** in good yield. Reaction of methyl ulopyranosonate **11** and ulosonamide **10** under Mitsunobu conditions resulted in silyl ether **17** and *N*-acylphosphinimine **18**, respectively.

Since the activation of OH group was not successful in the above experiments, glycosyl donors with better leaving groups were prepared (Scheme 3.). Irrespectively of the initial anomeric configuration and concentration of the *C*-nucleophile Me_3SiOTf promoted reactions of acetate donors **19** and **23** gave dioxolane type products **20**, **21** and **25** instead of the expected bis-*C*,*C*-glycosyl compounds. Rearrangement of **20** to the target **22** was not successful under the applied conditions. Activation of thioglycoside **24** in the presence of allyl-$SnBu_3$ resulted in **26**.



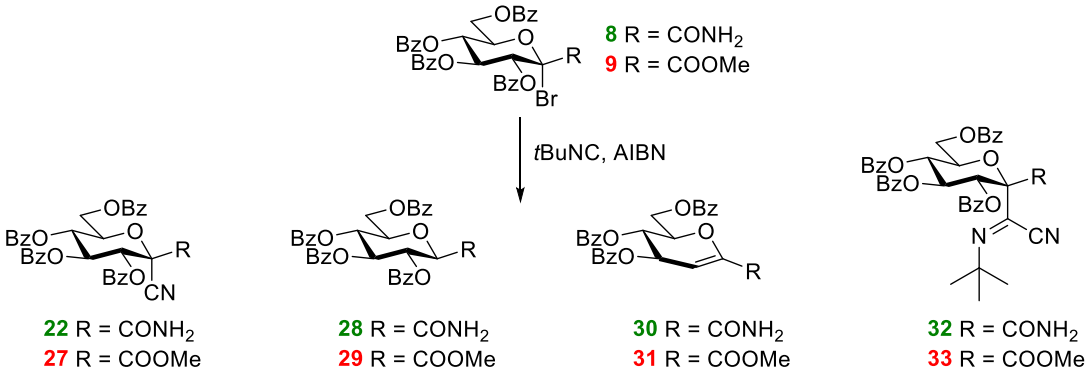
Scheme 3. Reagents and conditions: a) Me_3SiNu, Me_3SiOTf, DCM; b) $BF_3 \cdot OEt_2$, $MeNO_2$, Δ or Me_3SiCN, Me_3SiOTf, DCE Δ or Me_3SiCN, $HgBr_2$, $MeNO_2$; c) Me_3SiCN (as a solvent), Me_3SiOTf, DCM; c) Bu_3Sn-allyl, Ph_2SO, Tf_2O, TTBP, DCM.

The above results in the field of ionic reactions led to the following conclusions: a) SN type substitution of bromine in (ulosylbromide)onic acid derivatives seems to be highly unfavored. Low reactivity of the tertiary bromide and high basicity of the ^-CN leads to HBr elimination. b) the destabilizing effect of the electron withdrawing group at the anomeric position and the participating neighboring group leads to stable 1,2-dioxolenium ion type intermediate. The resulting products seem to be resistant to rearrangement.

Radical reactions toward bis-*C,C*-glycopyranosyl compounds

After the disappointing results of ionic reactions we investigated radical pathways to synthesize bis-*C,C*-glycosyl compounds.

Reaction of (ulosylbromide)onic acid derivatives **8** and **9** with *tert*-butyl isocyanide under various radical conditions resulted in complex mixtures of anhydro-aldonic acid derivartives **28** and **29**, glycals **30** and **31**, and iminonitrile derivatives **32** and **33** (Scheme 3.). The target cyanide **22** could be detected only in one reaction by LC-MS.

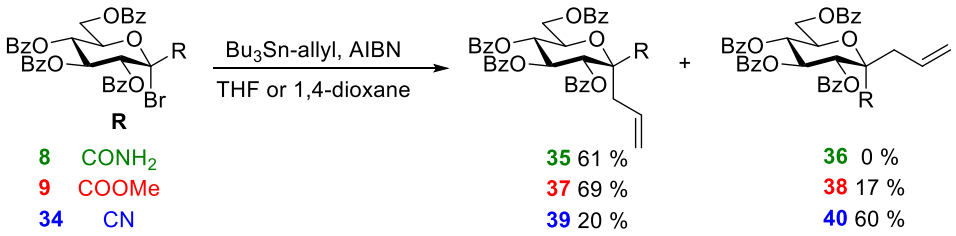


Conditions	Products (Yield)			
	22 or **27**	**28** or **29**	**30** or **31**	**32** or **33**
8, $(Me_3Si)_3SiH$, THF	**22** traces (LC-MS)	**28** 20 %	**30** 10 %	**32** 17 %
8, $(Me_3Si)_3SiH$, benzene	-	**28** 13 %	-	**32** 26 %
9, $NaBH_3CN$, Bu_3SnCl, *t*BuOH, 1,4-dioxane	-	**28** 49 %	**31** 17 %	-
9, $(Me_3Si)_3SiH$, $CHCl_3$	-	-	**31** 34 %	**33** 10 %

Scheme 3.

The outlined research plan was largely based on the syntheses, transformations and spirocyclizations of the desired ulosyl cyanides. Since such a compound could not be prepared despite all our efforts, henceforth, the synthesis and reactions of allyl derivatives were investigated.

Allylation of (ulosylbromide)onic acid derivatives (**8**, **9**, **34**) with an excess of allyltributylstannane in the presence of AIBN furnished the expected *C*-allylated products in good yields (Scheme 4.). Allylation of bromo amide **8** gave **35** in a highly α selective reaction. Methyl ester **9** and nitrile **34** gave separable mixtures of allylated products (**37**-**40**) in different ratios. Conditions of this radical allylation were optimized to reduce the amount of the costly and toxic allyltributylstannane.



Scheme 4.

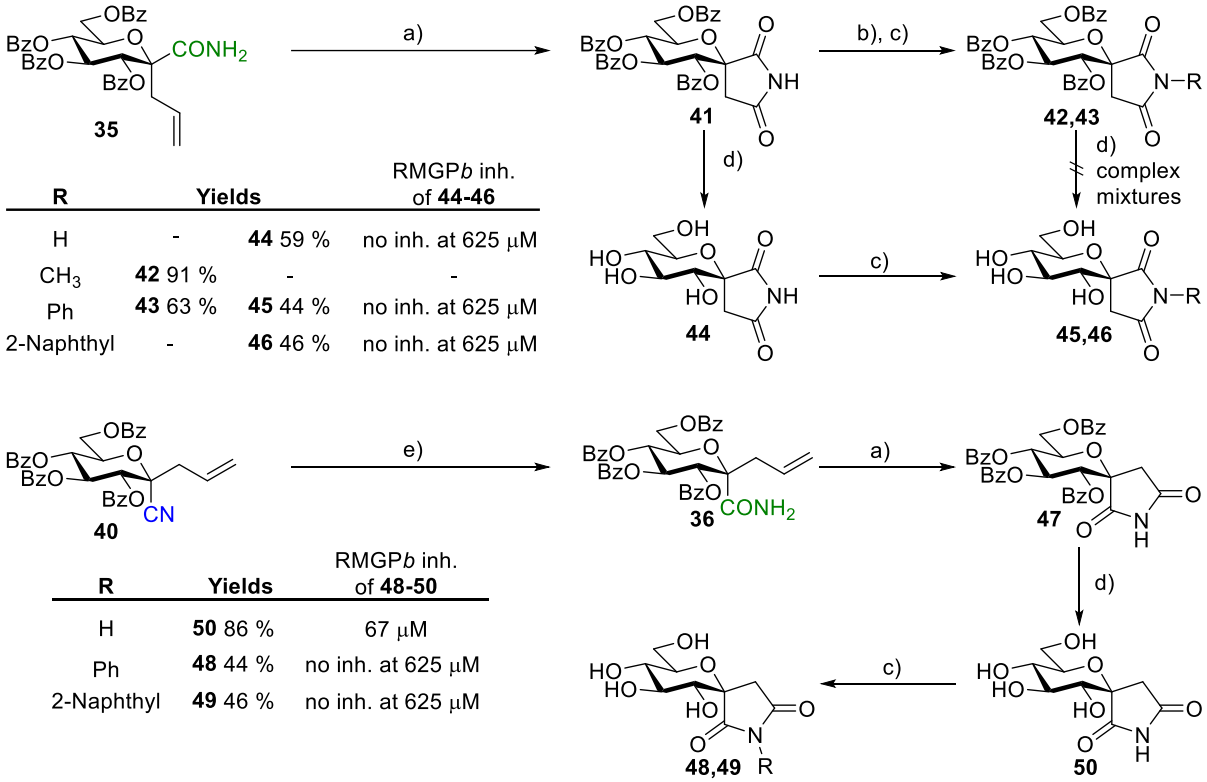
Experiments toward new bis-*C*,*C*-glycopyranosylidene spirocycles

Transformations of the allyl groups were investigated to build new functional groups for further cyclizations. Ozonolysis of amide **35** followed by oxidation resulted in a spirocyclic

pyrrolidine-2,5-dione **41** (Scheme 5.). Alkylation and arylation of **41** afforded the *N*-substituted succinimides **42** and **43** which gave complex mixtures under the attempted debenzoylation conditions. A reversed sequence, debenzoylation to **44** followed by *N*-arylation resulted in the target compounds **45** and **46**.

Compounds with the opposite configuration at the spiro carbon were also prepared. First nitrile **40** was transformed into amide **36**, then according to the above strategy the deprotected succinimide **50** was prepared via **47**. *N*-arylation of **50** yielded **48** and **49**.

Rabbit muscle glycogen phosphorylase b (RMGP*b*) inhibition of the deprotected spirocycles was tested, only compound **50** showed low micromolar inhibition.

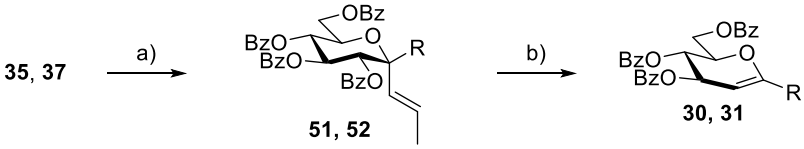


R	Yields		RMGP*b* inh. of **44-46**
H	-	**44** 59 %	no inh. at 625 μM
CH_3	**42** 91 %	-	-
Ph	**43** 63 %	**45** 44 %	no inh. at 625 μM
2-Naphthyl	-	**46** 46 %	no inh. at 625 μM

R	Yields	RMGP*b* inh. of **48-50**
H	**50** 86 %	67 μM
Ph	**48** 44 %	no inh. at 625 μM
2-Naphthyl	**49** 46 %	no inh. at 625 μM

Scheme 5. Reagents and conditions: a) 1. O_3, DCM, 2. Et_3N, 3. CrO_3·pyr, 86 %; b) CH_2N_2, DCM; c) $ArB(OH)_2$, $Cu(OAc)_2$, MeOH; d) NaOMe, MeOH; e) Et_2NHOH, 1,4-dioxane, 83 %.

Synthesis of malonic acid derivatives was attempted, as they can be valuable precursors for the synthesis of new spirocycles (Scheme 6.). Allylated compounds **35** and **37** were rearranged into the corresponding propenyl derivatives **51** and **52**, respectively. Ozonolysis followed by oxidative workup resulted in decarboxylation and benzoate elimination to give glycal derivatives **30** and **31** instead of the desired malonic acid derivatives.

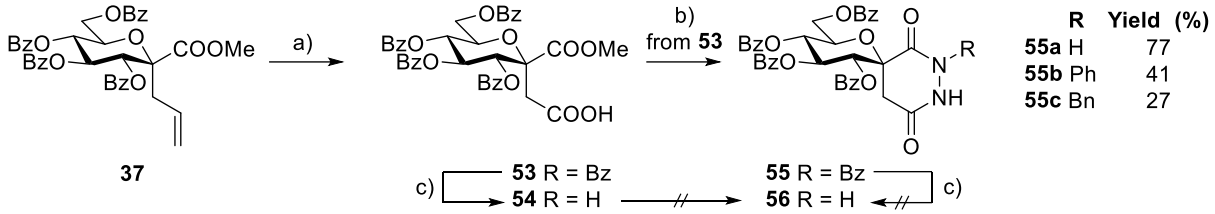


R	Product (Yield)
$CONH_2$	**51** (64 %) **30** (49 %)
COOMe	**52** (37 %) **31** (33 %)

Scheme 6. Reagents and conditions: a) $PdCl_2$, toluene; b) 1. O_3, MeCN, H_2O, 2. $NaClO_2$

Monomethyl succinate derivative **53** was obtained from **37** by ozonolysis (Scheme 7.). Spirocyclization of **53** with hydrazines was successful to give tetrahydropyridazine-3,6-diones

55 in moderate yields. Standard deprotection of **55** to the expected **56** gave complex mixtures. Cyclization of the deprotected **54** into **56** failed under the studied conditions.



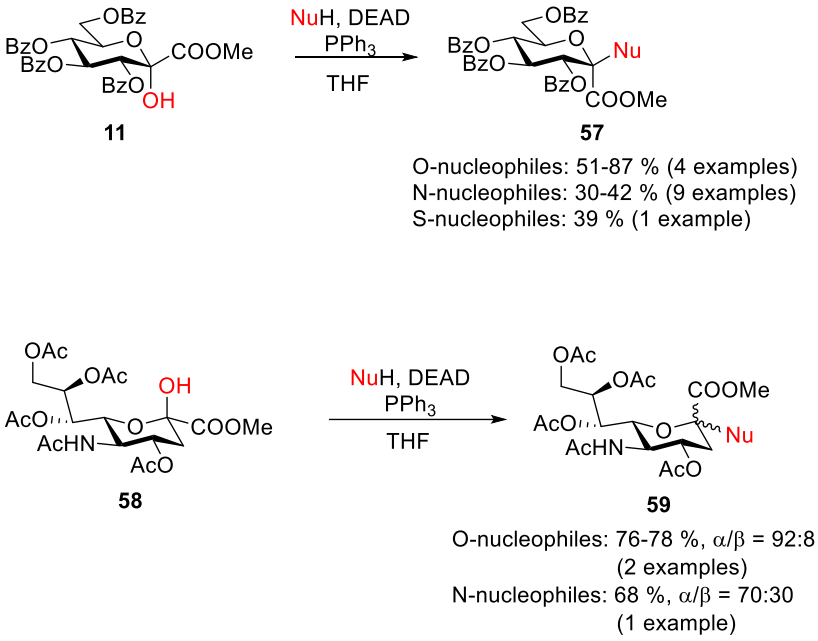
Scheme 7. Reagents and conditions: a) 1. O_3, MeCN, H_2O, 2. $NaClO_2$, 80 %; b) 1. CDI, DCM or DMF, 2. hydrazine derivative; c) NaOMe, MeOH

The above detailed results have not yet published; some additional results are needed to reach the publishable level.

Other results

Glucopyranosylidene-spiro-benzo[b][1,4]oxazinones, a cyclic hydroxamic acid derivative spiro-benzo[b][1,4]-4-hydroxyoxazinone and the corresponding analogous thio compounds (spiro-benzo[b][1,4]thiazinones) were prepared from ulosonic acid derivatives. The above compounds shows similarities to effective GP inhibitors, furthermore, they are spirocyclic analogues of the allelophatic natural products HBOA and DIBOA glucosides. The enzymekinetic studies revealed very weak RMGP*b* inhibition (max 28% at 625 μM) and plant growth tests of the hydroxamic acid showed modest growth inhibition of garden cress. Published in Journal of Agricultural and Food Chemistry.[1]

A systematic study was performed by using Mitsunobu conditions for glycosylation with ulopyranosonate **11** and **58** (Scheme 8.). These substrates with a tertiary OH group are very scarcely applied under these conditions due to the steric hindrance of the alcoholic functional group. From a set of 47 *O*-, *N*-, *S*- and *C*-nucleophiles, phenols and *N*-hydroxy compounds with a pK_a of 5-8, phthalimide, benzotriazole, 6-chloropurine, an oxazolidinedione and several tetrazoles with a pK_a of 4-8, and thiophenol gave the corresponding products **57** and **59** in moderate to very good yields, while *C*-nucleophiles were unreactive. Reactions with **11** were highly stereoselective at room temperature while reactions of sialic acid derivative **58** showed selectivity only at -30 °C. These series of experiments allowed to establish the scope and limitations of this transformation. A manuscript has been submitted to New Journal of Chemistry.[2]



Scheme 8.

Synthesis and of *O*-peracetylated glucopyranosylidene-spiro-thiazolinones was carried out from (ulopyranosyl bromide)onamide. *O*-Deacylation was studied under various conditions. Together with *O*-deprotection the reversible addition of the solvent to the heterocycle was also observed. Alcohol and water additions were examined and the absolute configuration of the new stereogenic center was determined by CD and TDDFT-ECD computations. These results were published in Molecules.[3]

An *in silico* screening revealed a library of promising glycogen phosoporylase inhibitor 3-(β-D-glucopyranosyl)-5-substituted-1,2,4-triazoles. A small group of these compounds have been selected and synthesized. Three new 1,2,4-triazoles displayed sub-micromolar K_i values. The enzyme-inhibitor interactions were studied by X-ray crystallography. These results were published in European Journal of Medicinal Chemistry.[4]

New, potent *C*-glucosyl azole type GP inhibitors have been designed and synthesized, motivated by *in silico* predictions. The most potent inhibitors showed K_i values in a low micromolar range against RMGP*b*. The synthesized compounds were effective at inhibiting glycogenolysis at low micromolar concentrations in hepatocytes.[5]

An another paper describes the synthesis of new *C*- and *N*-β-D-glucopyranosyl imidazoles, 1,2,3-triazoles and tetrazoles for glycogen phosphorylase inhibition. The study demonstrates that the structure of heterocycle has a major effect on the binding. Interchange of the substituents on the heterocycle can result in the complete loss of inhibition. Published in Molecules.[6]

Structure-activity relationships of *C*- and *N*-glucopyranosyl azoles were studied against isoforms of GP. X-ray crystallography of the enzyme inhibitor complexes explained the importance of a hydrogen bond interaction between the aglycon heterocycle and the enzyme.[7]

A paper has been published in Pure and Applied Chemistry which summarizes the transformations of glycosyl cyanides and other precursors to glycomimetics.[8]

Summary

Our efforts to synthesize bis-*C*,*C*-glycopyranosylidene spirocycles have been moderately successful. During this pathfinder research we encountered a large number of failures, and ran into several dead ends. Investigations will be continued in this area since it is a rather secluded spot in carbohydrate chemistry with a promise of unique results. The experience gained during this research will be utilized for the synthesis of new spirocyclic glycomimetics within the framework of FK132222 project.

Diploma theses related to this research project

Magos Nóra: Spirociklusos bisz-*C*,*C*-glikozil vegyületek előállítása ulozonamid származékokból (BSc diploma thesis, 2018)

Nagy Attila: Glükopiranozilidén-spiro-szukcinimidek előállítása (BSc diploma thesis, 2019)

Csák Szabina: Új típusú bisz-*C*,*C*-glükopiranozilidén-spiro-heterociklusok előállítása (BSc diploma thesis, 2020)

Publications

1. Kun, S.; Kánya, N.; Galó, N.; Páhi, A.; Mándi, A.; Kurtán, T.; Makleit, P.; Veres, S.; Sipos, Á.; Docsa, T.; Somsák, L. Glucopyranosylidene-spiro-benzo[b][1,4]oxazinones and -benzo[b][1,4]thiazinones: Synthesis and Investigation of Their Effects on Glycogen Phosphorylase and Plant Growth Inhibition. *Journal of Agricultural and Food Chemistry* **2019**, 6884–6891.
2. Kánya, N.; Kun, S.; Batta, G.; Somsák, L. Glycosylation with Ulosonates under Mitsunobu Conditions: Scope and Limitations. *New Journal of Chemistry* **2020,** submitted.
3. Szabó, E. K.; Kun, S.; Mándi, A.; Kurtán, T.; Somsák, L. Glucopyranosylidene-spiro-thiazolinones: synthetic studies and determination of absolute configuration by TDDFT-ECD calculations. *Molecules* **2017,** 22, 1760/1-1760/15.
4. Kun, S.; Begum, J.; Kyriakis, E.; Stamati, E. C. V.; Barkas, T. A.; Szennyes, E.; Bokor, É.; Szabó, E. K.; Stravodimos, G. A.; Sipos, Á.; Docsa, T.; Gergely, P.; Moffatt, C.; Patraskaki, M. S.; Kokolaki, M. S.; Gkerdi, A.; Skamnaki, V. T.; Leonidas, D. D.; Somsák, L.; Hayes, J. M. A multidisciplinary study of 3-(β-D-glucopyranosyl)-5-substituted-1,2,4-triazole derivatives as glycogen phosphorylase inhibitors: computation, synthesis, crystallography and kinetics reveal new potent inhibitors. *European Journal of Medicinal Chemistry* **2018,** 147, 266-278.
5. 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 Chemical Biology* **2018**, 1460-1470.
6. Kun, S.; Bokor, É.; Sipos, Á.; Docsa, T.; Somsák, L. Synthesis of new *C*- and *N*-β-D-glucopyranosyl derivatives of imidazole, 1,2,3-triazole and tetrazole, and their evaluation as inhibitors of glycogen phosphorylase. *Molecules* **2018,** 23, 666.
7. Kyriakis E.; Karra, A. G., Papaioannou, O.; Solovou, T.; Skamnaki, V. T.; Liggrib, P. G. V.; Zographos, S. E.; Szennyes, E.; Bokor, É.; Kun, S.; Psarr, 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 and offer new insights to structure-based design. *Bioorganic & Medicinal Chemistry* **2020**, 28, 115196.
8. Somsák, L.; Bokor, É.; Juhász, L.; Kun, S.; Lázár, L.; Tóth, É.; Tóth, M. New Syntheses towards *C*-Glycosyl Type Glycomimetics. *Pure and Applied Chemistry* **2019,** 91, 1159-1175.

Besides the above publications 2 oral lectures and 4 posters were presented in domestic and international conferences.

1. Kun, S.; Szabó, E. Katalin; Kánya, N.; Galó, N.; Páhi, A.; Mándi, A.; Kurtán, T.; Somsák, L. Glucopyranosylidene-spirocycles with five and six membered heterorings: Synthesis, CD studies and inhibition of glycogen phosphorylase. Eurocarb 19, Barcelona, Spain, 2nd – 6th of July, 2017, P32, (Poster)
2. Kun, S.; Szabó, E. Katalin; Kánya, N.; Galó, N.; Páhi, A.; Mándi, A.; Kurtán, T.; Somsák, L. Glükopiranozilidén spirociklusok öt- és hattagú heterogyűrűvel: szintézis, CD vizsgálatok és glikogén foszforiláz gátlás. Magyar, Kémikusok Egyesülete Vegyészkonferencia 2017: Program és előadásösszefoglalók pp. 67-67. (Poster)
3. Kun, S.; Kánya, N.; Magos, N.; Somsák L. Studies towards the synthesis of new bis-*C*,*C*-glycosylic compounds. Annual meeting of the Working Committee for Carbohydrates,

Nucleic Acids and Antibiotics of the Hungarian Academy of Sciences, Mátrafüred, Hungary, 23rd - 25th of May, 2018, (oral lecture)

4. Kánya, N.; Kun, S.; Somsák L. A study for the application of the Mitsunobu reaction on a heptulopyranosonic ester. International Workshop on Chemistry and Chemical Biology of Carbohydrates, Nucleic Acids and Antibiotics; Mátrafüred, Hungary, 22nd - 24th of May, 2019, (oral lecture)
5. Kun, S.; Kánya, N.; Magos, N.; Somsák L. Novel bis-*C*,*C*-glycosyl derivatives: *C*-glycosides of heptulosonic acids and their spirocyclization. Eurocarb 20, Leiden, Netherlands, 30th of June - 4th of July, 2019, P164, (Poster)
6. Kánya, N.; Kun, S.; Somsák L. Modifications of heptulopyranosonic acid esters using the Mitsunobu-reaction. Eurocarb 20, Leiden, Netherlands, 30th of June - 4th of July, 2019, P164, (Poster)