



Synthesis of Glycogen Phosphorylase Inhibitors

theses of doctoral (PhD) dissertation

Nagy Veronika

Supervisors:

Dr. Jean-Pierre Praly

Dr. Somsák László

University of Debrecen, Faculty of Sciences,
Department of Organic Chemistry
University Claude Bernard Lyon 1, UMR-CNRS UCBL 5622

Debrecen, 2003.

1. Introduction

Glucose has a central role in the energy supply of the body. Blood glucose levels are controlled by the continuous interaction of two metabolic pathways: the glucose and the glycogen metabolism. Insufficient operation of this complex system – regulated by enzymes and hormones – results in altered, usually chronically elevated blood sugar levels. This syndrome is *diabetes mellitus*, a serious disease becoming one of the main contributors to worldwide mortality through its long term complications. The end of the 20th century has seen a dramatic increase in the number of patients diagnosed with diabetes worldwide. This disease afflicts approximately 6 % of the adult population in the Western society.

Depending on whether patients secrete insulin or not, diabetes can be divided into two basic types: type I, or insulin-dependent *diabetes mellitus* (IDDM) and type II, or non-insulin-dependent *diabetes mellitus* (NIDDM) which represents ~ 90% of all diabetic cases.

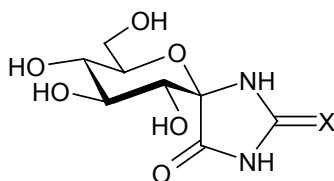
Contrary to type I diabetes most of type II diabetics can synthesize and secrete insulin although not in sufficient quantities or insulin exerts no or only a late effect on stimulating glucose uptake by cells and on stimulating glycogen synthesis. NIDDM typically afflicts people over 40 and the symptoms develop more slowly. Patients require complex treatment that involves regular physical activity and dietary regulation besides the use of oral hypoglycemic agents.

Biological/biochemical backgrounds of diabetes formation are not known. Since all of its symptoms and complications originate from the altered, elevated blood glucose levels current treatments aim to maintain a constant, approximately normal blood glucose level. For type II diabetes several sorts of oral hypoglycemic drugs (sulfonylureas, biguanides, thiazolidinediones, acarbose) are in use for symptomatic treatments but they have several adverse side effects as well as the danger of causing hypoglycemia. Therefore other therapeutic concepts (among others novel insulin secretagogues, insulin sensitizers, glucagon receptor antagonists, inhibitors of hepatic glucose output, combination therapies) are intensively investigated, and a wholly nutritional therapy has been suggested, as well.

A newly investigated approach is the inhibition of glycogen phosphorylases (GP-s) which are the main regulatory enzymes of glucose production in the liver, in the muscles and in the brain.

At the beginning of my PhD studies glucopyranosylidene-spiro-hydantoin **20** and its thio derivative **21** were the most effective glucose analogue inhibitors of glycogen

phosphorylases. However, neither of them was available in larger quantities sufficient for biological investigations.



20 X = O RMGPb $K_i = 3.1 \mu\text{M}$

21 X = S RMGPb $K_i = 5.1 \mu\text{M}$

(RMGPb: rabbit muscle glycogen phosphorylase *b*)

Taking the spiro-(thio)hydantoins as lead structures the aim of my PhD research has been the development of glucose analogue inhibitors of glycogen phosphorylases:

- elaboration of a scalable synthetic sequence for the preparation of glucopyranosylidene-spiro-thiohydantoin.
- developing new intramolecular spirocyclisations to obtain novel glucopyranosylidene-spiro-heterocycles with potential GP inhibitory activity.

2. Methods

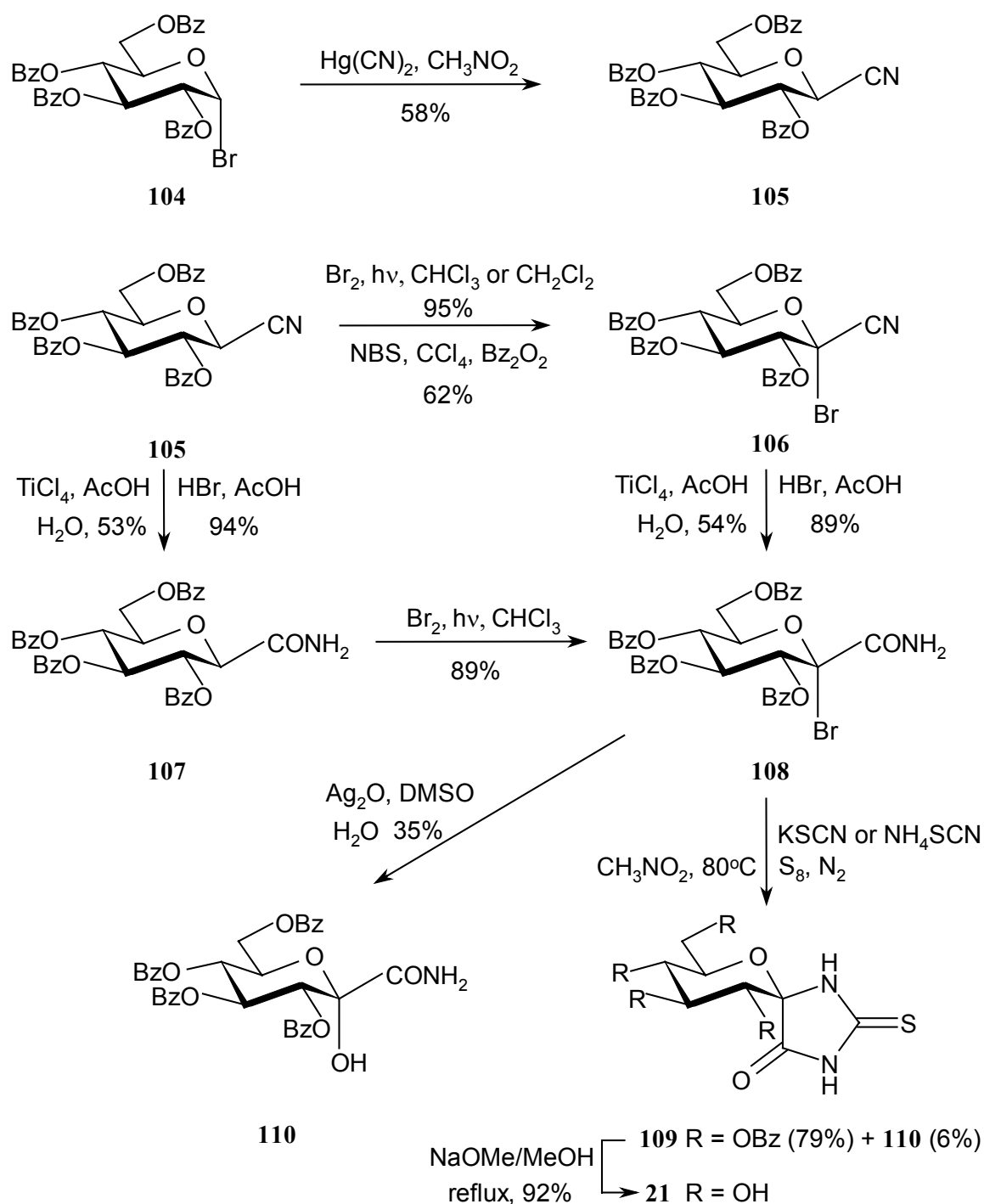
The macro- and micro methods of the modern preparative organic chemistry were used in my synthetic work.

Thin layer chromatography was applied to follow the reactions, to control the purity of compounds. Column chromatography and crystallisation were used for the purification of the products. Melting point, elemental analysis, optical rotation determination, mass- and NMR spectroscopic methods were applied for the verification of the purity and the structures of the prepared substances.

3. New scientific results

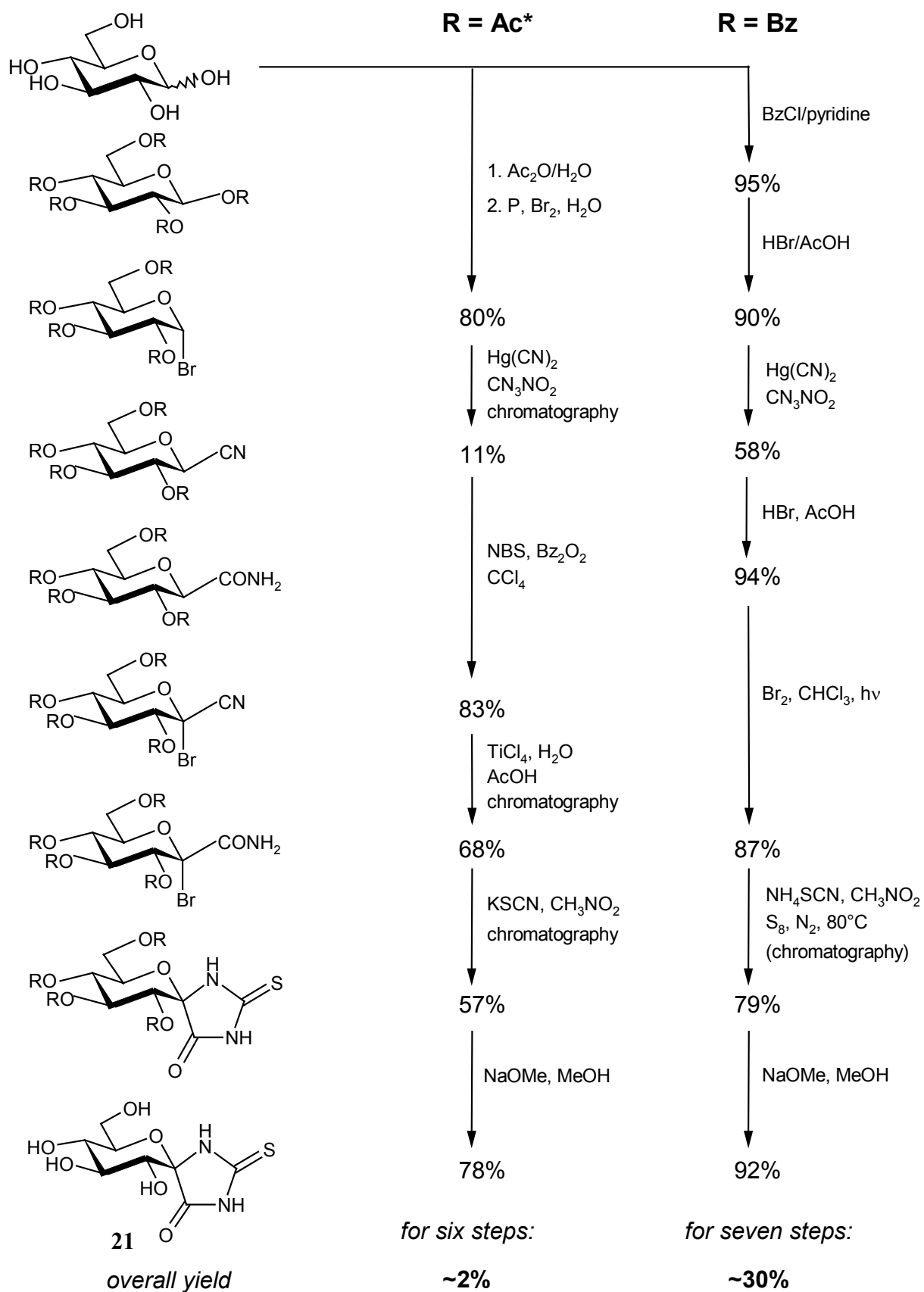
3.1. Large scale synthesis of the glucopyranosylidene-spiro-thiohydantoin

On the basis of a procedure (Ősz et al. *Bioorg. Med. Chem. Lett.* **1999**, 9, 1385-1390.) giving glucopyranosylidene-spiro-thiohydantoin **21** in a very poor yield (~2%) we have worked out a new method by which the overall yield for **21** has grown to 30%.



a.) By changing the acetyl protecting groups applied in the original synthesis to benzoyl the yield of the key intermediate glucopyranosyl-cyanide **105** increased from 11% to 58%, as well as the chromatographic separations were no longer needed. However, by the chromatographic purification of the mother liquor further 16-19% crop of **105** was obtained. This means that the formation of cyano-benzylidene derivative is not significant.

b.) NBS used earlier in the bromination reactions was changed to elemental bromine, hereby work-up of the reaction mixtures became more simple.



*Ősz et al. *Bioorg. Med. Chem. Lett.* **1999**, 9, 1385-1390.

It was shown, that carbon tetrachloride generally used in similar reactions was replaceable by the more available chloroform or dichloromethane and the radical brominations could be effected at lower temperature.

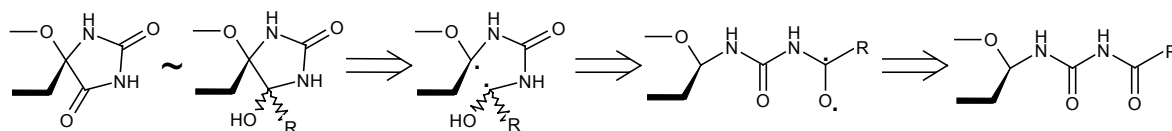
c.) The partial hydrolysis of the cyano groups in **105** and **106** was carried out with HBr in acetic acid, thus the corresponding benzoylated C-(β -D-glucopyranosyl)-formamides (**107**, **108**) were obtained as crystalline crude products in high yields.

d.) Reaction of **108** with ammonium or potassium thiocyanate in nitromethane in the presence of elemental sulfur to suppress radical-mediated pathways under nitrogen atmosphere gave spiro-thiohydantoin **109** and hydroxy-amide **110**. Compound **110** was also prepared independently from **108** by silver oxide-promoted hydrolysis in dimethylsulfoxide. Debenzoylation of **109** was accomplished by the Zemplén method in methanol to give inhibitor **21** in 92% yield.

e.) Thiohydantoin **21** was prepared in gram scale quantities by this new method allowing extended biological investigations with glycogen phosphorylases performed at the Department of Medicinal Chemistry of University of Debrecen. Compound **21** significantly lowered the enzyme activity in both *in vitro* and *in vivo* experiments and was shown to diminish blood sugar levels *in vivo*. These findings corroborate the concept of using glycogen phosphorylase inhibitors as a potential antihyperglycaemic agents.

3.2. Synthesis and photoreactions of *N*-acyl-*N'*- β -D-glucopyranosyl ureas

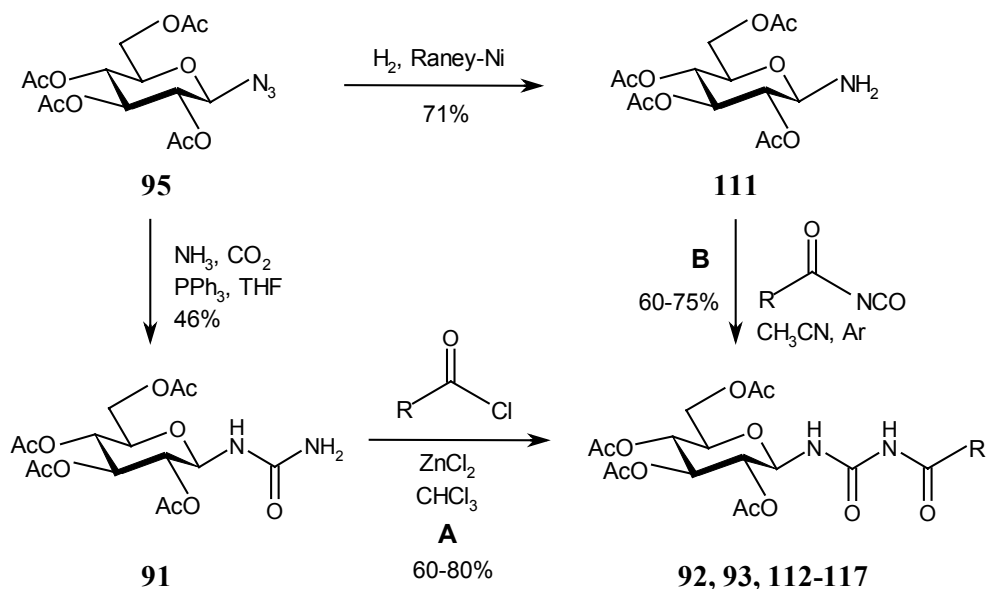
Radical reactions at the anomeric center of sugars show high regio- and stereoselectivity, that is why we have planned the synthesis of compounds similar to glucopyranosylidene-spiro-hydantion **20** in a photochemical way (Norrish type II reaction) according to the retrosynthetic scheme.



a.) Protected *N*-acyl-*N'*- β -D-glucopyranosyl ureas (**92**, **93**, **112-117**), which could be convenient precursors for this photocyclisation, were prepared from acetylated glucopyranosyl azide **95** in two ways: by the acylation of glucopyranosyl urea (Route **A**) or *via* glucopyranosyl amine with acyl isocyanates (Route **B**).

b.) Photolyses of per-*O*-acetylated *N*-acetyl- (**92**) and *N*-benzoyl-*N'*-β-D-glucopyranosyl ureas (**93**) were carried out under various conditions. These resulted in Norrish type I *N*-deacylation only, therefore, these molecules proved to be unsuitable for photocyclisation.

c.) The ureas were deprotected by classic Zemplén method or with KHSO₄ or ammonia in methanol to give **118-125**, while **126** was obtained by Raney-Ni reduction of **120**.



92 R = CH₃ 56% (A)

93 R = C₆H₅ 30% (A)

112 R = 4-NO₂-C₆H₄ 60% (A)
65% (B)

113 R = 3-Cl-C₆H₄ 74% (B)

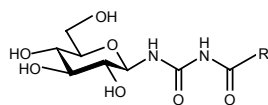
114 R = 4-OAc-C₆H₄ 72% (A)
20% (B)

115 R = 1-naphthyl 73% (A)

116 R = 2-naphthyl 29% (A)

117 R = 2-indolyl 41% (A)

K_i (μM), RMGPb



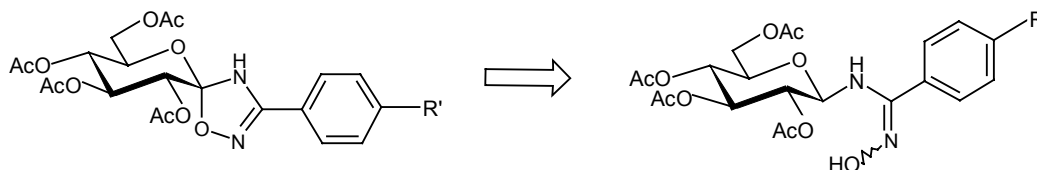
118	R = CH ₃	370.5
119	R = C ₆ H ₄	4.6
120	R = 4-NO ₂ -C ₆ H ₄	3.0
121	R = 3-Cl-C ₆ H ₄	113.6
122	R = 4-OH-C ₆ H ₄	3.6
123	R = 1-naphthyl	7.8
124	R = 2-naphthyl	0.4
125	R = 2-indolyl	8.0
126	R = 4-NH ₂ -C ₆ H ₄	IC ₅₀ = 14

d.) These compounds were found to inhibit GP, and among them *N*-2-naphthoyl-*N'*- β -D-glucopyranosyl urea (**124**) has been the most effective with its nanomolar K_i value, and is at present the best glucose analogue inhibitor of GP.

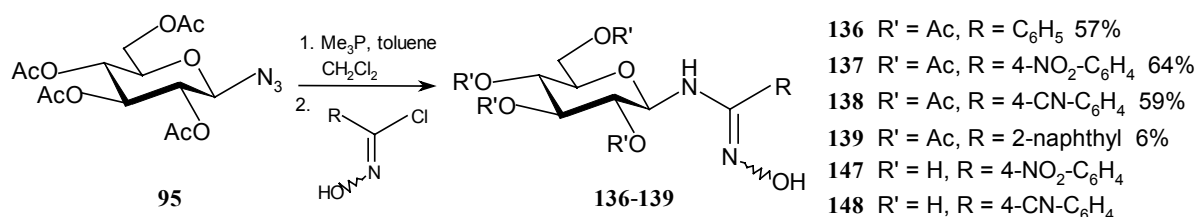
e.) Crystallographic studies have shown that some of these compounds bind also at the so-called new allosteric site of GP besides the catalytic site, which is a unique property among glucose derivatives. With the preparation of *N*-acyl-*N'*- β -D-glucopyranosyl ureas new leads have been discovered for the inhibition of GP.

3.3. Experiments towards the synthesis of glucopyranosylidene-spiro-1,2,4-oxadiazolines

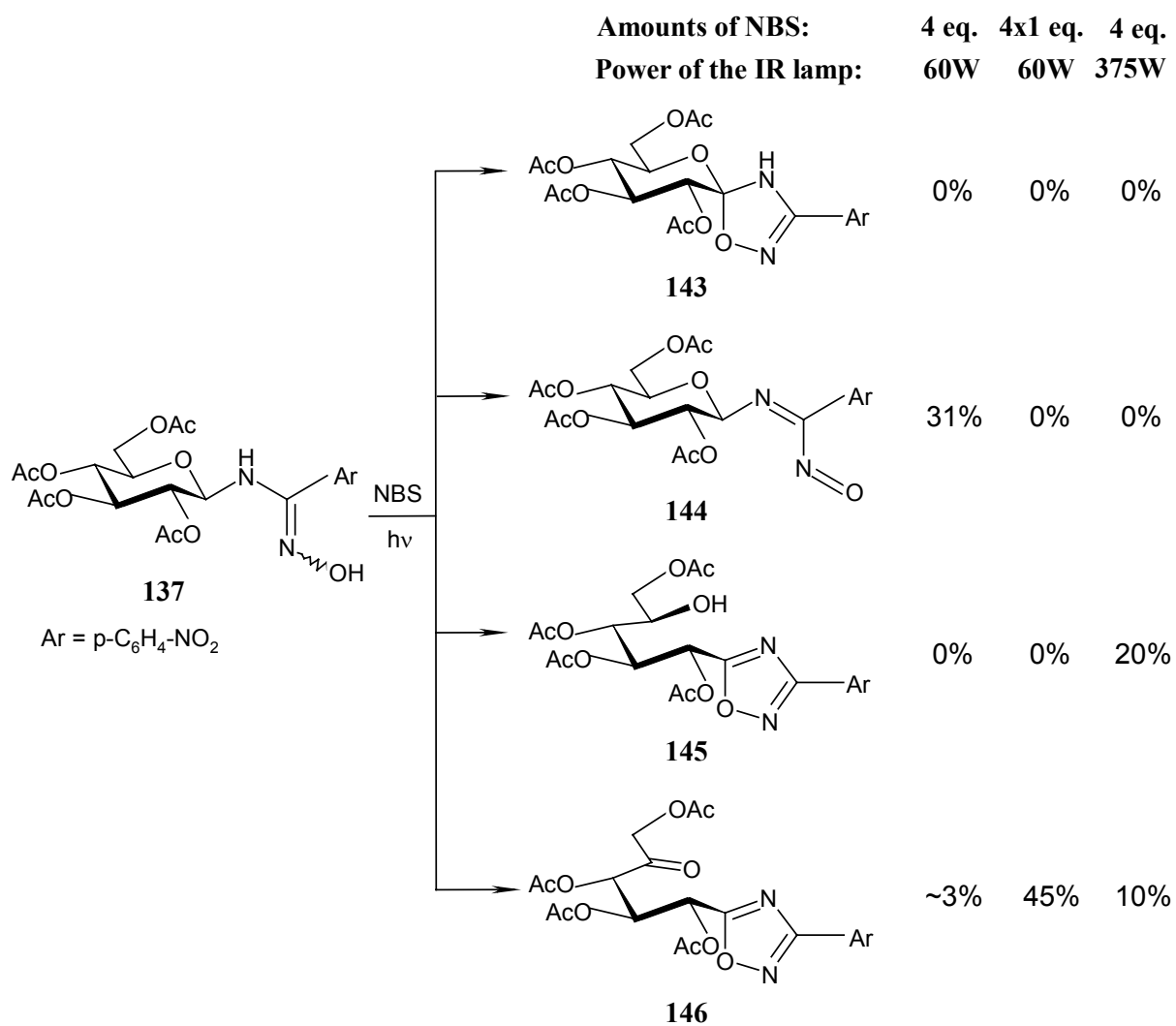
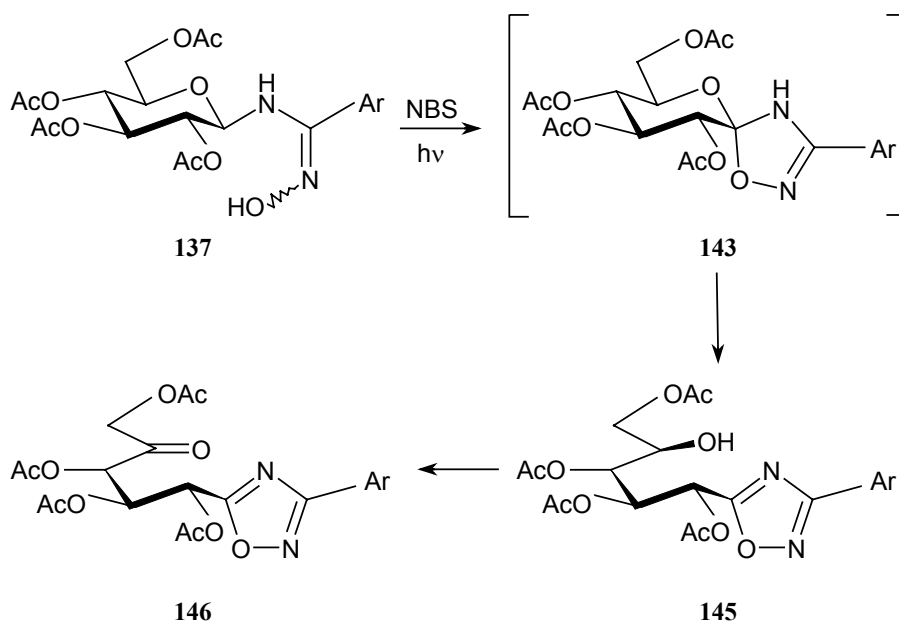
New glucopyranosylidene-spiro-oxadiazolines were also targeted on the basis of kinetic and crystallographic results with the above molecules as well as some glucopyranosylidene-spiro-oxathiazoles which were found to be micromolar inhibitors of GP. This synthesis was planned by the photolysis of per-*O*-acetylated *N*- β -D-glucopyranosyl amidoximes under oxidative circumstances.



a.) The unknown precursors were obtained by a modified Staudinger reaction from per-*O*-acetylated β -D-glucopyranosyl azide reacted first with trimethylphosphine and then with aryl hydroximinoyl chlorides.



b.) Photoreactions of acetylated *N*- β -D-glucopyranosyl 4-nitro-benzamidoxime **137** in the presence of NBS suggest the formation of the target spiro-oxadiazoline **143** in which the sugar ring is liable to open to form an aromatic 1,2,4-oxadiazol **145**. The secondary alcohol can be oxidized to **146** under the reaction conditions. In conclusion the examined reaction is unsuitable for the preparation of the desired, likely unstable glucopyranosylidene-spiro-oxadiazoline. The deprotected derivatives **147** and **148** showed no inhibition of GP.



4. Possible applications of the results

A new, high yielding, simple synthesis of glucopyranosylidene-spiro-thiohydantoin **21** was developed starting from D-glucose to produce gram quantities of **21**, thus *in vitro* and *in vivo* studies of this efficient GP inhibitor on hepatic glycogen metabolism have become possible.

Various *N*-acyl-*N'*- β -D-glucopyranosyl ureas (**118-126**), which can be regarded as “open chain” analogues of hydantoin **20**, were also prepared. They proved to be very efficient inhibitors of GP, even the most active glucose analogue inhibitor known to date, the 2-naphthoyl derivative **124** has been found among them ($K_i = 0.4 \mu\text{M}$). Thus they can be the new “lead structures” of further investigations.

Possibilities of a photochemical synthesis of new glucopyranosylidene-spiro-oxadiazolines from per-*O*-acetylated *N*- β -D-glucopyranosyl amidoximes were investigated. According to our findings the target oxadiazolines are likely unstable and cannot be prepared by the method examined.

5. List of publications – Közlemények jegyzéke

5.1. Publications – Közlemények

1. László Somsák, Veronika Nagy, Tibor Docsa, Béla Tóth, Pál Gergely

Gram-scale synthesis of a glucopyranosylidene-spiro-thiohydantoin and its effect on hepatic glycogen metabolism studied *in vitro* and *in vivo*

Tetrahedron: Asymmetry **2000**, *11*, 405-408.

2. László Somsák, Veronika Nagy

A new, scalable preparation of a glucopyranosylidene-spiro-thiohydantoin: one of the best inhibitors of glycogen phosphorylases

Tetrahedron: Asymmetry **2000**, *11*, 1719-1728.

3. Nikos G. Oikonomakos, Magda Kosmopoulou, Spyros E. Zographos, Demetres D. Leonidas, László Somsák, Veronika Nagy, Jean-Pierre Praly, Tibor Docsa, Béla Tóth, Pál Gergely

Binding of *N*-acetyl-*N'*- β -D-glucopyranosyl urea and *N*-benzoyl-*N'*- β -D-glucopyranosyl urea to glycogen phosphorylase *b*

European Journal of Biochemistry **2002**, 269, 1684-1696.

4. László Somsák, Veronika Nagy, Zsuzsa Hadady, Tibor Docsa, Pál Gergely

Glucose analog inhibitors of glycogen phosphorylases as potential Antidiabetic agents: recent developments

Current Pharmaceutical Design **2003**, 9, 1177-1189. invited review

5.2. Lectures and posters in the field of the dissertation – A dolgozat témakörében bemutatott előadások és poszterek

1. Marietta Tóth, Veronika Nagy, László Somsák

Synthesis of D-gluco- and D-xylopyranosylidene-spiro-(thio)hydantoins and their effect on muscle and liver glycogen phosphorylases

MTA Szénhidrátkémiai Munkabizottságának Nemzetközi Előadóülése, May 26-27. 1999. Mátrafüred

2. Veronika Nagy, Zsuzsa Hadady, László Somsák, Nikos G. Oikonomakos, Tibor Docsa, Béla Tóth, Pál Gergely

New glucose analogue inhibitors of glycogen phosphorylases which can also bind at the new allosteric site

MTA Szénhidrátkémiai Munkabizottságának Nemzetközi Előadóülése, May 15-16. 2001. Mátrafüred

3. BélaTóth, Tibor Docsa, Veronika Nagy, László Somsák, Pál Gergely

A glikogén foszforiláz glükózanalóg inhibitorai, mint lehetséges hipoglikémiás szerek

A Magyar Fiziológiai Társaság LXVI. Kongresszusa, June 6-8. 2001. Szeged

4. Veronika Nagy, Zsuzsa Hadady, László Somsák, Nikos G. Oikonomakos, Tibor Docsa, Béla Tóth, Pál Gergely

Glikogén foszforiláz enzimek új glükózanalóg inhibitorainak előállítása

Magyar Kémikusok Egyesülete, Vegyészkonferencia, June 27-29. 2001. Hajdúszoboszló

5. László Somsák, Veronika Nagy, Zsuzsa Hadady, Nikos G. Oikonomakos, Tibor Docsa, Béla Tóth, Pál Gergely

New Glucose Analogue Inhibitors of Glycogen Phosphorylase

11th European Carbohydrate Symposium, September 2-7. 2001. Lisboa, Portugal

6. Veronika Nagy, Zsuzsa Hadady, László Somsák, Jean-Pierre Praly, Nikos G. Oikonomakos, Tibor Docsa, Béla Tóth, Pál Gergely

New glucose analogue inhibitors of glycogen phosphorylase

VI^{ème} Journée du Groupe Lyonnais des Glycosciences, November 29. 2001. Lyon, France

7. Veronika Nagy, Zsuzsa Hadady, László Somsák, Jean-Pierre Praly, Nikos G. Oikonomakos, Tibor Docsa, Béla Tóth, Pál Gergely

Nouveaux dérivés du D-glucose inhibiteurs de la glycogène phosphorylase

XIX^{ème} Journées de Chimie et Biochimie des Glucides, May 20-24. 2002. Albé, France

8. Evangelia D. Chrysina, Magda N. Kosmopoulou, C. Tiraidis, G. Loupasakis-Loupasis, Demetres D. Leonidas, Nikos G. Oikonomakos, Veronika Nagy, László Somsák, Pál Gergely, Jean-Pierre Praly

Structural studies of N'-β-D-glucopyranosyl urea analogues in complex with glycogen phosphorylase b

Congress of the Hellenic Society of Biochemistry & Molecular Biology, October 25-27. 2002. Ioannina, Greece

9. Veronika Nagy, Tibor Docsa, Pál Gergely, Nikos G. Oikonomakos, Jean-Pierre Praly, László Somsák

N-acyl-N'-β-D-glucopyranosyl-ureas as glycogen phosphorylase inhibitors

12th European Carbohydrate Symposium, July 6-11. 2003. Grenoble, France

10. Veronika Nagy, László Somsák, Jean-Pierre Praly

Experiments towards new glucopyranosylidene-spiro-heterocycles

1st Hungarian-Austrian Carbohydrate Conference, September 24-26. 2003. Burg Schlaining, Austria

11. László Somsák, Veronika Nagy, Zsuzsa Hadady, Attila Krakomperger, Matietta Tóth

New glucose analogue inhibitors of glycogen phosphorylase

1st Hungarian-Austrian Carbohydrate Conference, September 24-26. 2003. Burg
Schlaining, Austria

5.3. Lecture in other field– V. 3. Egyéb témakörben bemutatott előadás

Veronika Nagy, László Somsák

Preparation of 1-carboxamido-glycosides as potential glycosidase inhibitors/ inactivators

MTA Szénhidrátkémiai Munkabizottságának Nemzetközi Előadóülése, May 15-16. 2001.
Mátrafüred