Synthesis of Glycogen Phosphorylase Inhibitors

doctoral (PhD) dissertation

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Debrecen, 2003. október 10.

Nagy Veronika

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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.[1]

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.[1, 2]

Both are believed to have genetic origins but other (environmental/ immunologic) factors can also induce formation of *diabetes*. In case of type I, which is an autoimmune disease, the β-cells of Langerhans islets of pancreas do not secrete any insuline and in the absence of this hormone the tissues are unable to take up glucose. Therefore glucose accumulates in blood resulting in hyperglycemia. As the concentration of blood-glucose augments osmotic forces come into play that tend to increase the blood volume and urine output (polyuria). When the concentration of blood-sugar exceeds a certain limit, glucose appears in the urine (glycosuria). Because of the increased liquid loss of the body the patients feel strong thirst (polydipsia). Since glucose cannot enter into the cells they are forced to find another source of energy for instance ketone bodies secreted by liver. Thus the concentration of ketone bodies in blood increases (ketonemia) and they also appear in urine (ketonuria). Therewith muscular weekness and weight loss can

be observed. These symptomes appear relatively rapidly, but diabetes has other serious long-term complications involving disorders in the cardiovascular, renal, neural and visual systems, which are the main reasons of the death of patients. Blood sugar levels in type I diabetes are controlled by insulin therapy with comparative success.[3]

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. Contrary to type I diabetes NIDDM typically afflicts people over 40 and the symptomes develop more slowly. Patients require complex treatment that involves regular physical activity and dietary regulation besides the use of oral hypoglycemic agents.[3]

Efficient causes and biological/biochemical backgrounds of diabetes formation are not known. Since all of its symptomes 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.[4-7] Therefore other therapeutic concepts (among others novel insulin secretagogues, insulin sensitizers, glucagon receptor antagonists, inhibitors of hepatic glucose output, combination therapies) are intensively investigated,[1, 6, 8-12] and a wholly nutritional therapy has been suggested, as well.[13]

A newly investigated approach is the inhibition of glycogen phosphorylases which are the main regulatory enzymes of glucose production in the liver, in the muscles and in the brain. We set ourselves the aim to develop new glucose analogue inhibitors of glycogen phosphorylases.

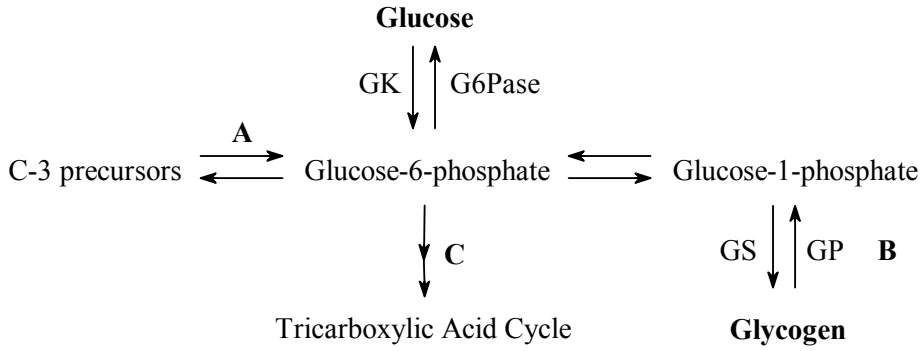
2. Literature survey

2.1. Biological background

2.1.1. Metabolism of glycogen and enzymes involved

The liver is the major regulator of plasma glucose levels in the post-absorptive state. Several studies have shown that in type II diabetics hepatic glucose production (HGP) is significantly elevated in comparison to nondiabetics and this is directly correlated to fasting hyperglycemia. Therefore HGP can be a therapeutic target in type II diabetes.[1, 9, 11, 14, 15]

The liver produces glucose by two pathways: gluconeogenesis (*de novo* synthesis of glucose), and glycogenolysis (break-down of glycogen) (**Scheme 1.**).



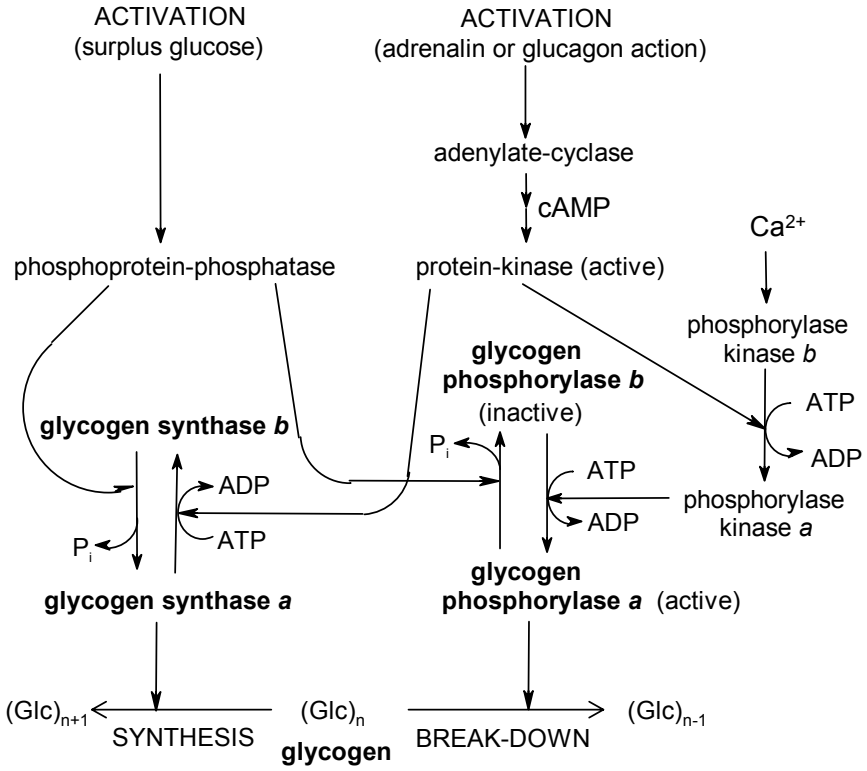
A: Gluconeogenesis
B: Glycogenolysis
C: Glycolysis

Enzymes: G6Pase: Glucose-6-phosphatase
GK: Glucokinase
GP: Glycogen phosphorylase
GS: Glycogen synthase

Scheme 1. Overview of glycogenolysis and gluconeogenesis

The relative contribution of glycogenolysis may well account 70 % to the net hepatic glucose production, furthermore a substantial portion of glucose formed by gluconeogenesis is cycled through the glycogen pool prior to be effluxed from the liver cells.[9, 15, 16]

The main regulatory enzymes of glycogen metabolism are glycogen phosphorylase (GP) and glycogen synthase (GS) (**Scheme 2.**). The change-over of glycogen synthesis and degradation depends on the blood-sugar levels. These two enzymes are controlled by phosphorylation and by a number of allosteric effectors including glucose, glucose-6-phosphate, cAMP and calcium as well as hormonal regulation.[17]



Scheme 2. Schematic representation of the glycogen metabolism

The glycogen synthase exists in two major forms *in vivo*. Synthase *a* is the more active, least phosphorylated form and synthase *b* is the less active, fully phosphorylated form. In contrast, glycogen phosphorylase is most active in its phosphorylated form (GP*a*) and less active in the dephosphorylated form (GP*b*). Accordingly, phosphorylation is associated with an inactivation of GS and activation of GP. The dephosphorylation of GP and GS is interrelated and

catalyzed by the glycogen associated protein phosphatase. Both GP and GS are acted upon by kinases and phosphatases that can be controlled by second messengers generated when hormones, such as insulin, glucagon and epinephrine interact with their receptors.[9, 18]

Gluconeogenesis, *de novo* synthesis of glucose from lactate, amino acids, and other precursor molecules, can also contribute to the elevated blood glucose levels, however, it has been clearly demonstrated that glucose arising from gluconeogenesis also passes through the glycogen cycle. That is why the inhibition of hepatic GP could suppress glucose production originated from both glycogenolysis and gluconeogenesis.[9, 18, 19]

2.1.2. Glycogen phosphorylases

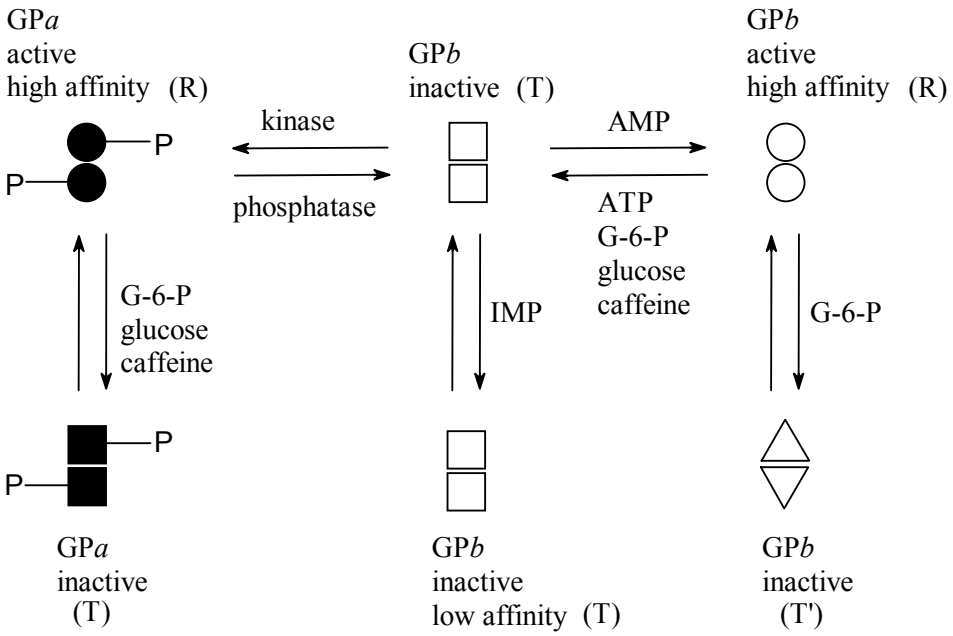
Glycogen phosphorylase (R-1,4-glucan:orthophosphate glucosyltransferase) catalyzes the breakdown of the storage polysaccharide glycogen to produce glucose-1-phosphate (G-1-P).

Mammalian GP has three isoforms according to the tissue where they can be found: muscle, liver and brain GP. Their structures and functions are very similar, therefore various species and tissue isoforms of GP, either in the phosphorylated or unphosphorylated state, have been used to assess the inhibitory activity of putative inhibitors. For accessibility reasons generally the phosphorylated or unphosphorylated rabbit muscle glycogen phosphorylase (RMGP*a* or RMGP*b*, respectively) or the phosphorylated recombinant human liver enzyme (rHLGP*a*) are used for investigations.

The functional form of GP is a homodimer (M_r about 2 x 97500 Da) consisting of two identical subunits and having a C-2 symmetry. This quaternary structure allows allosteric regulation since there are two binding sites for each effector per enzyme (**Figure I.** Please see Appendix). Beside the catalytic centers, where G-1-P and glycogen bind as substrates and glucose acts as an inhibitor, other binding sites have been discovered, as well. At the so-called allosteric (or AMP or

nucleotide) site e. g. AMP activates, while ATP and G-6-P inhibit the enzyme. The inhibitor (or purine or nucleoside) site binds purine compounds, like caffeine, nucleosides, nucleotides and flavopiridol. The new allosteric (or indole) sites, located inside the large central cavity formed on association of the two subunits, have been reported recently to accommodate indole-carboxamide type inhibitors.[19]

The allosteric binding of different effectors produces different conformations of GP, either active or inactive, which are very important for its regulation and activity. These conformations can be considered within the Monod-Wyman-Changeux model for allosteric enzymes[20] (**Scheme 3.**). In this model the "T state" of an allosteric enzyme has low activity and low affinity for substrates and effectors while the "R state" has high activity and high affinity for substrates and effectors.



Scheme 3. Allosteric transition of glycogen phosphorylase (adapted from Ref. [21])

Crystallographic studies have shown that in native T state GP there is no access from the buried catalytic site to the surface because access is blocked by the

so-called 280s loop.[19] The key transition between inactive T-state and active R-state GP involves movement and disruption of the 280s loop, which allows a crucial arginine to enter the catalytic site, and which also opens up access for glycogen to the catalytic site. The shift and disruption of the 280s loop is associated with subsequent destruction of the inhibitor site and changes at the intersubunit contacts of the dimer which give rise to allosteric effects. In the R-state GP, the 280s loop is not well ordered and its position has not been established definitively. A certain effector promotes or stabilizes either the R or the T state which can be seen by its effect on the activity of the enzyme.

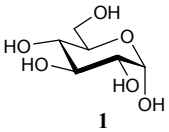
2.1.3. Inhibitors of glycogen phosphorylases

2.1.3.1. Derivatives of D-glucose and other monosaccharides

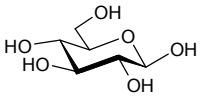
D-Glucose (**1**, **2**) is a physiological allosteric inhibitor of GP (for K_i values see **Scheme 4**.).[22] Its analogues are competitive inhibitors and bind at the catalytic site of the enzyme. The binding of α-D-glucose is affected by hydrogen bonds between each of the hydroxyl groups and the corresponding protein atoms.

Glucose, on binding at the catalytic site, promotes the less active T state through stabilization of the closed position of the 280s loop which blocks access for the substrate to the catalytic site.[19]

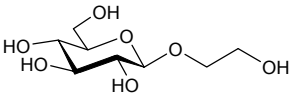
Crystallographic studies of the enzyme-glucose complex have shown an empty pocket in β direction for the sugar ring lined by both polar and nonpolar groups.[19] The 2-hydroxyethyl β-D-glucopyranoside (**3**) aimed to fill this β-pocket binds so that the aglycon partially fills the empty place and allows a hydrogen bond from terminal O to Asp339 at the end of the pocket.[19, 22] The compound exhibits a poorer inhibition constant compared to α-D-glucose, which is due to the loss of entropy on binding of the flexible aglycon. Among other *O*- and *C*- glucosides examined as GP inhibitors the most effective compounds (**4**-**7**) are shown in **Scheme 4**.[22, 23]



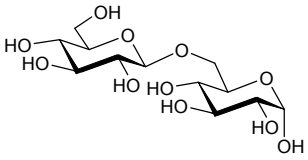
1
K_i = 1700 μM



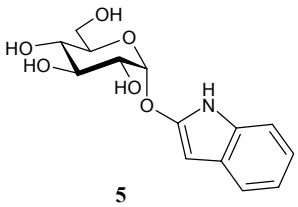
2
K_i = 7400 μM



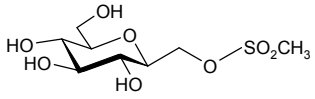
3
K_i = 25300 μM



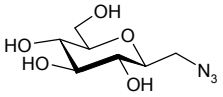
4
K_i = 16300 μM



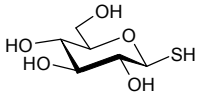
5
K_i = 2600 μM



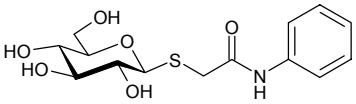
6
K_i = 4800 μM



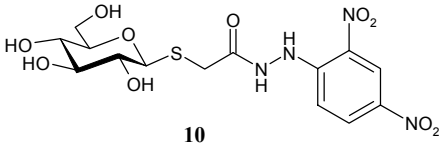
7
K_i = 15200 μM



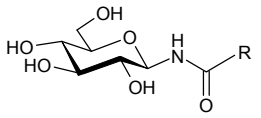
8
K_i = 1000 μM



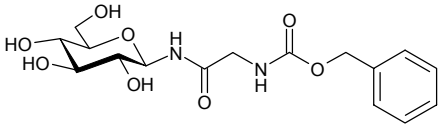
9
K_i = 3600 μM



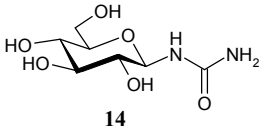
10
K_i = 650 μM



11 R = CH_3 K_i = 32 μM
12 R = C_6H_5 K_i = 81 μM[23]
144 μM[25]



13
K_i = 21 μM



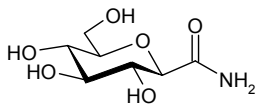
14
K_i = 140 μM

Scheme 4. Glucose analogue inhibitors of GP-s **I**. K_i values measured with RMGP*b*

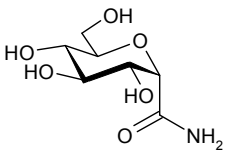
Since the β-pocket has a mixed polar/non-polar nature a nonpolar but easily polarizable group may be suitable to fill it. The 1-deoxy-1-thio-β-D-glucopyranose (**8**) was found a better inhibitor than either α- or β-D-glucose. In the crystal complex the thiol group is in van der Waals interaction with the enzyme. However, attempts to extend the thioglucoside derivatives with additional groups (e.g. **9**) did not lead to further promising compounds. The hydrazide **10** do not bind at the catalytic site but the dinitrophenyl group are sandwiched between aromatic groups of GP at the inhibitor binding site.[19]

The amide nitrogen of *N*-acyl-β-D-glucopyranosylamines (**11**, **12**, **13**) makes a strong hydrogen bond to the peptide main chain carbonyl O of His377. Thus the *N*-acetyl-β-D-glucopyranosylamine (1-GlcNAc, **11**) is ~60 times better inhibitor than glucose.[23] Replacement of the methyl group by an NH_2-group (**14**) results in a lower inhibitory activity.[23]

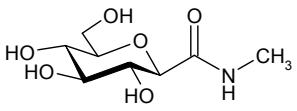
Several 2,6-anhydro-heptonamides (**15-18, Scheme 5.**) were also examined *vis a vis* GP-s.[24]



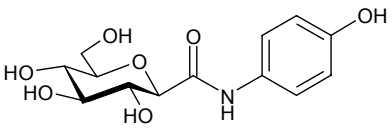
15
K_i = 370 μM



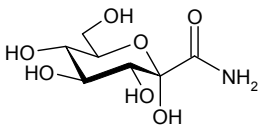
16 K_i = 440 μM



17
K_i = 160 μM



18
K_i =4400 μM



19
K_i = 3100 μM

Scheme 5. Glucose analogue inhibitors of GP-s **II**. K_i values measured with RMGP*b*

Both β- (**15**) and α-heptonamide (**16**) exhibited similar binding affinities which are greater than that of D-glucose itself. The α-heptonamide (**16**) forms water-mediated hydrogen bonds with its CO and NH groups to the enzyme. In the case of β-heptonamide **15** a strong hydrogen bond is formed between the NH_2 group and the peptide main chain carbonyl of His377 and another weak one between the carbonyl group of heptonamide and Asn284. The methyl amide **17** was the best inhibitor among 2,6-anhydro-heptonamides. The amide nitrogen forms one hydrogen bond directly with the carbonyl O of His377 and the methyl group is in a secondary interaction with non-polar groups of the enzyme displacing a water molecule. In the complex with **18** the aromatic group fits in the β-pocket but there are small adjustments in the protein structure, therefore binding of **18** is less favourable.[19]

Hept-2-uloson-amide **19** also proved rather ineffective in spite of the presence of the carboxamide group.[25]

Glucopyranosylidene-spiro-heterocycles (**20**-**31**, **Scheme 6**.) were also taken into consideration as potential GP inhibitors. Glucopyranosylidene-spiro-hydantoin **20** was found to be the most effective glucose analogue inhibitor.[26] Crystallographic studies of RMGP*b*-**20** complex have shown that **20** binds at the active site of GP. The hydrogen bond between the N7-H and the CO of His377 is the same as in the case of 1-GlcNAc (**11**). O8 binds to Asp339 through a water molecule and O10 also takes part in a hydrogen bond to Asp283 through another water molecule. The rigid nature of the hydantoin ring results in little conformational energy change on binding, which is another favourable factor. The hydrogen binding capability of the hydantoin polar groups exploits existing water structure and creates additional water network to the protein, thereby providing additional enthalpic contributions.[27] The 8-thio derivative (**21**) has similar binding properties determined by protein crystallography of the RMGP*b*-thiohidantoin complex (**Figure 1**. and **Figure II**., please see also Appendix). Thiohydantoin **21** binds also at the active site and the hydantoin ring installs in the same position as in

the event of **20**. The same hydrogen bonds are formed with N7 and O10 and the thiohydantoin derivative also stabilizes the closed position of the 280s loop in the same manner as **20** does.[28]

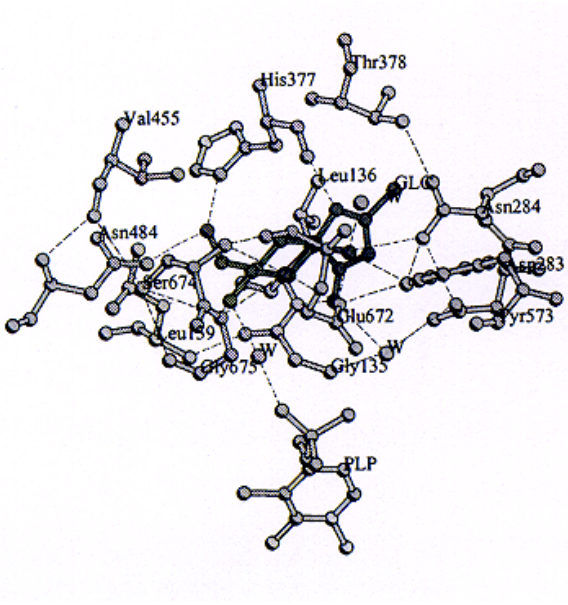
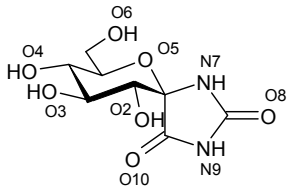


Figure 1. Stucture of GP*b*-**21** complex determined by X-ray crystallography at 273 K.[27]

Kinetic studies with muscle and liver GP*b* and GP*a* enzyme render thiohydantoin **21** and hydantoin **20** to be equipotent inhibitors in the low micromolar range.[25]

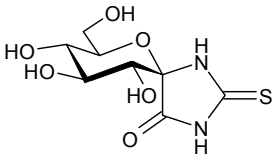
The epimeric spiro-hydantoin **22** was found to be significantly weaker inhibitor than **20**.[26, 29] This can be originated from the lack of the hydrogen bonding groups in suitable positions.

The xylopyranosylidene-spiro-hydantoin **23** and its thio derivative **24** are practically ineffective which indicate the importance of the hydrogen bond between the CH_2OH group of the sugar ring and the protein and confirm the high specificity of the catalytic site.[25]



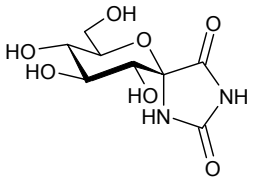
20

K_i = 3.1 μM[26]
4.2 μM[25]
RMGP*a* K_i = 26 μM[25]
RLGP*b* K_i = 12.8 μM[25]
RLGP*a* K_i = 16.5 μM[25]



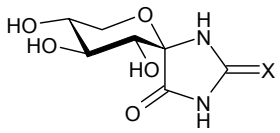
21

K_i = 5.1 μM
RMGP*a* K_i = 10.9 μM
RLGP*b* K_i = 7 μM
RLGP*a* K_i = 28.9 μM

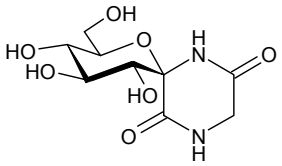


22

K_i = 105 μM[29]
320 μM[31]
28.6 μM[26]
RLGP*a* K_i = 2050 μM[29]

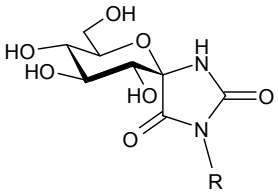


23 X = O K_i > 11500 μM
24 X = S K_i > 10000 μM

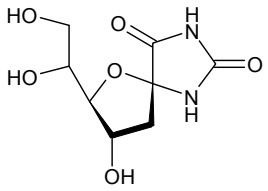


25

K_i = 59.7 μM

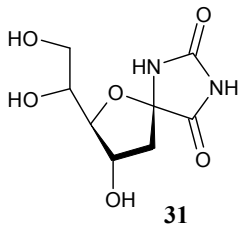


26 R = CH_3 K_i = 1200 μM
27 R = OH K_i = 39 μM
28 R = NH_2 K_i = 146 μM
29 R = $NHCOCH_3$ K_i = 550 μM



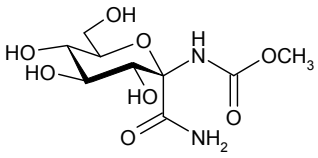
30

K_i = 220000 μM



31

K_i = 8200 μM



32

K_i = 16 μM

Scheme 6. Glucose analogue inhibitors of GP-s **III**. K_i values measured with RMGP*b* unless otherwise stated (Numbering used in protein crystallographic experiments are also shown for **20**)

The glucopyranosylidene-spiro-diketopiperazine **25** binds in a similar manner to the enzyme as hydantoin **20** but the piperazine ring is more flexible. This additional conformational flexibility leads to an unfavourable entropic loss in binding energy and results in weaker inhibition.[30]

Various *N*(9)-substituted derivatives (**26**-**29**) of **20** were also synthesized and tested as GP inhibitors and only the *N*-hydroxy derivative **27** proved to be fairly effective.[31]

Glucofuranose isomers **30** and **31** showed no inhibition of GP*b* suggesting that the enzyme does not bind the glucofuranose moiety even if the hydantoin is present in the compound.[32]

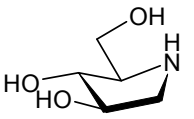
Compound **32** an „open chain" hydantoin derivative is also a powerful inhibitor showing that both the α-carboxamido group and the *N*-acyl-glucopyranosylamine part can be useful for the binding.[31]

2.1.3.2. Iminosugars

The GPs catalyzed phosphorylation of glycogen was postulated to proceed through a glycosyl cation like transition state,[33] therefore mimics of glycosylium ions could be able to inhibit GPs. Thus the best glycosidase inhibitor iminosugars were examined as GP inhibitors.

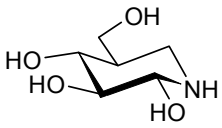
The five-membered iminosugar 1,4-dideoxy-1,4-imino-arabinitol (DAB, **33**, **Scheme 7**.) proved to be the most effective[34], while the six-membered glycosidase inhibitors were less active. Noeuromycin[35] **34** has a poorer effect on GP than isofagomine **35** in spite of the presence of OH in position 2.

The *N*-substituted derivatives of isofagomine (**36**-**42**) did not exhibit a better inhibitory effect on GP than isofagomine itself.[36] Similar observations were made with azafagomine **43** and its substituted derivatives. While **44** is effective β-glucosidase inhibitors, its IC_{50} value with GP is one order of magnitude worse than that of azafagomine.[37] Among some iminosugar-annelated heterocycles nojiritetrazole **46** was the most effective GP inhibitor, while on reducing the number of cyclic nitrogen atoms or introducing some substituents the inhibitory activities decreased dramatically (e. g. **45**).[38, 39]



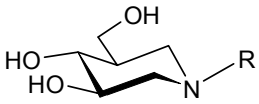
33

RMGP*a* K_i = 0,392 μM
RMGP*b* K_i = 0,342 μM
RLGP*a* K_i = 0,374 μM
HLGP*a* K_i = 0,530 μM

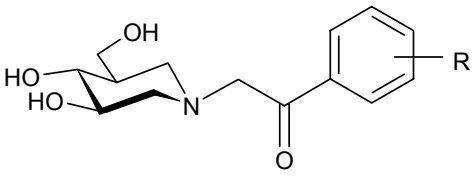


34

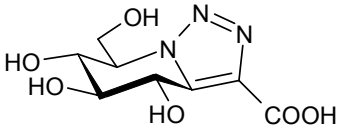
IC_{50} = 4,0 μM



	R	IC_{50}(μM)
35	H	0,7
36	CH_2=$CHCH_2$-	4,0
37	Ph-$CH_2CH_2CH_2$-	1,0
38	Ch-$CH_2CH_2CH_2$-	2,0

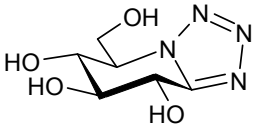


	R	IC_{50}(μM)
39	H	7,0
40	3,4-di-F	8,0
41	3-OCH_3	8,0
42	3-F	6,0



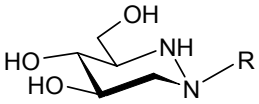
45

K_i = 7400 μM

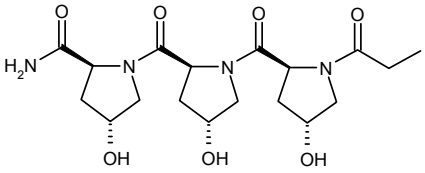


46

K_i = 53 μM



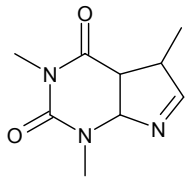
43 R = H IC_{50}= 13,5 μM

44 R =  IC_{50}= 200 μM

Scheme 7. Iminosugar derivative GP inhibitors tested with RLGP*a* unless otherwise stated

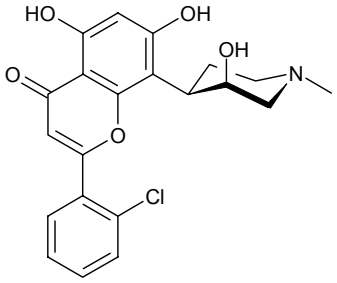
2.1.3.3. Other inhibitors

Beside the glucose analogue inhibitors binding in general at the catalytic site of the enzyme several other effectors can act upon the activity of GP.



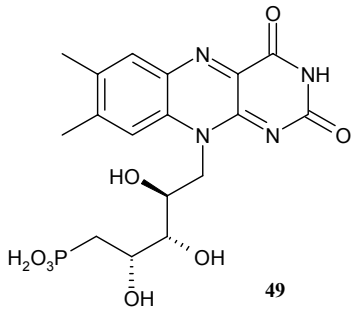
47

K_i =100-200 μM



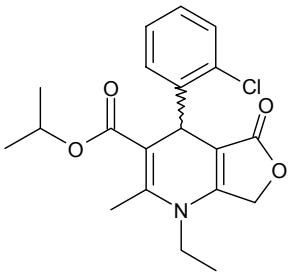
48

K_i = 0,3 μM



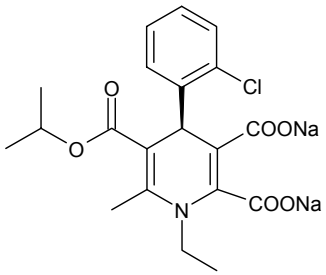
49

K_i = 10 μM



50

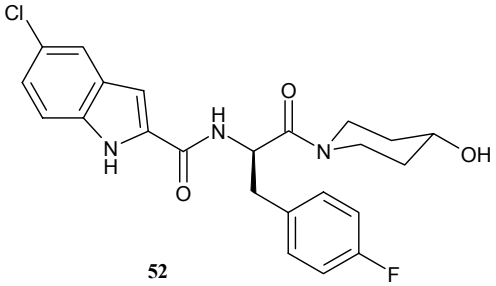
(prodrug)



51

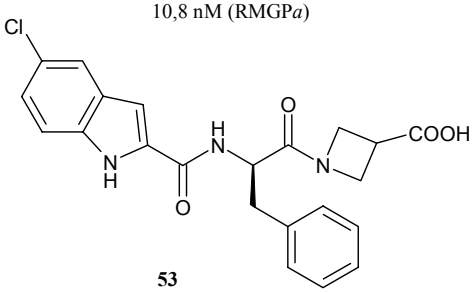
K_i = 1,6 nM (RMGP*b*)

10,8 nM (RMGP*a*)



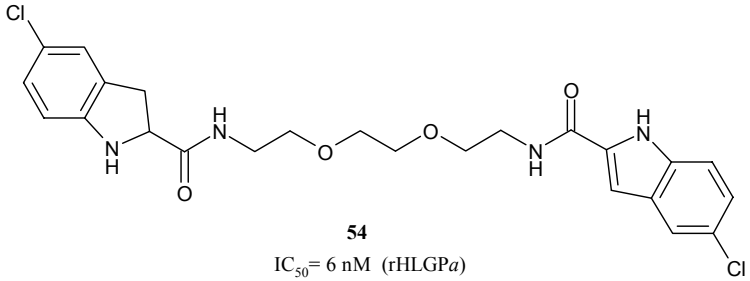
52

IC_{50}= 240 nM (RMGP*b*)



53

IC_{50}= 45 nM (rHLGP*a*)



54

IC_{50}= 6 nM (rHLGP*a*)

Scheme 8. Other inhibitors of GP

Some physiological inhibitors bind at the inhibitor binding site e. g. caffeine (**47**), flavopiridol (**48**) and flavin mononucleotid (FMN, **49**),[9, 19] these compounds exhibit synergistic inhibition with glucose (**Scheme 8**.)

Compound **50** (BAY R3401) inhibits hepatic glycogenolysis *in vivo* after its metabolic conversion to **51** (BAY W1807). Compound **51** binds at the allosteric site of the enzyme, and is to date one of the best known inhibitors of GP among all investigated molecules with its low nanomolar value of K_i.[40]

The new allosteric binding site was explored by crystallograpic studies with indole-2-carboxamide derivatives (**52-54**), which were found on the basis of high-throughput screening studies against rHLGPa and further developed by structure assisted design. They were found to act in synergism with glucose, which can be an important physiological feature: the decrease in inhibitor potency - as glucose concentrations decrease *in vivo* - should minimize the risk of hypoglycaemia.[41, 42]

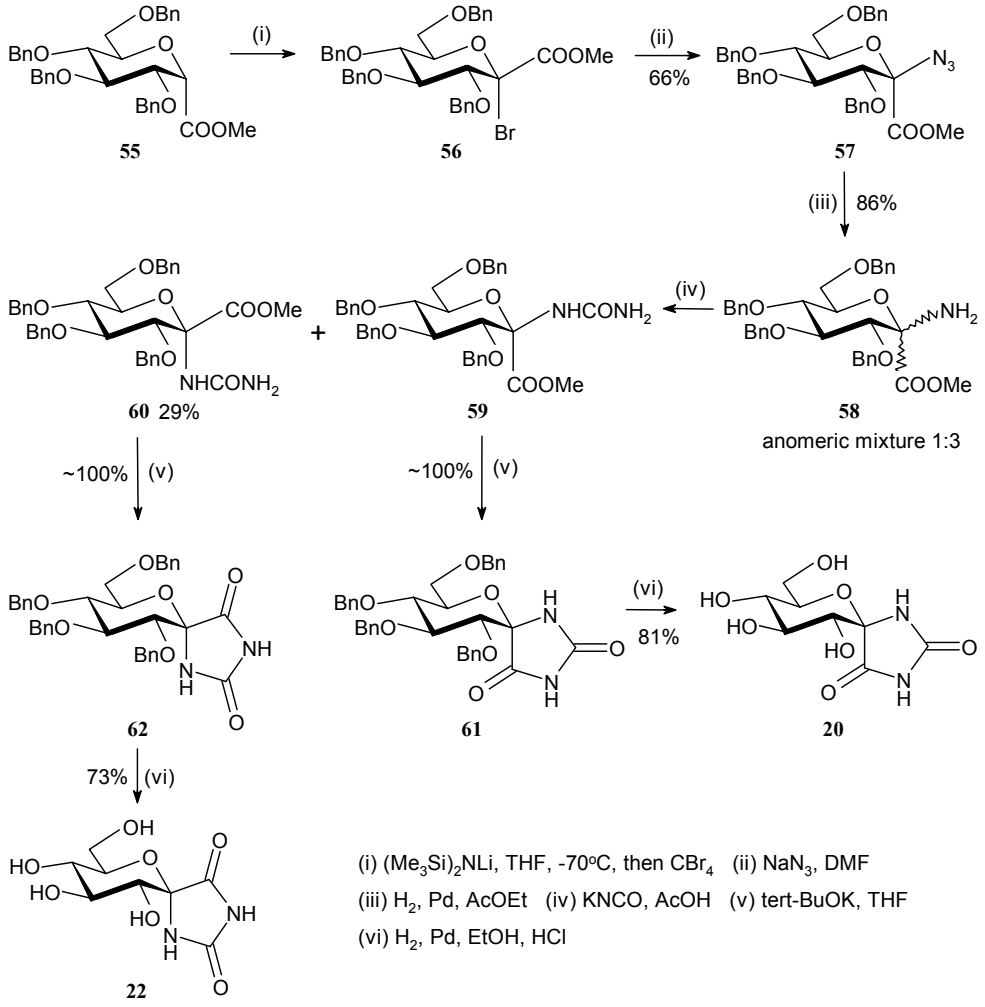
Crystallographic studies have shown that CP-320,626 (**52**) and CP-403,700 (**53**) bind at the large symmetric cavity formed by the association of the two subunits where no previous ligand interactions have been reported. One inhibitor binds to each subunit, accordingly two per homodimer.[41] Compound **54** (CP-526,423) has one of the highest reported inhibitory activity of all compounds.[42] This molecule contains two 5-chloroindole-2-carboxamide moieties joint by a diether linker, thus one inhibitor molecule per enzyme binds to the new allosteric site of GP.

2.2. Synthetic studies

2.2.1. Syntheses of glucopyranosylidene-spiro-(thio)hydantoins

For its excellent enzyme inhibitor activity various methods were developed for the preparation of glucopyranosylidene-spiro-hydantoin **20**.

The first synthesis of this compound was worked out by Bichard and her co-workers (**Scheme 9**.).[26]

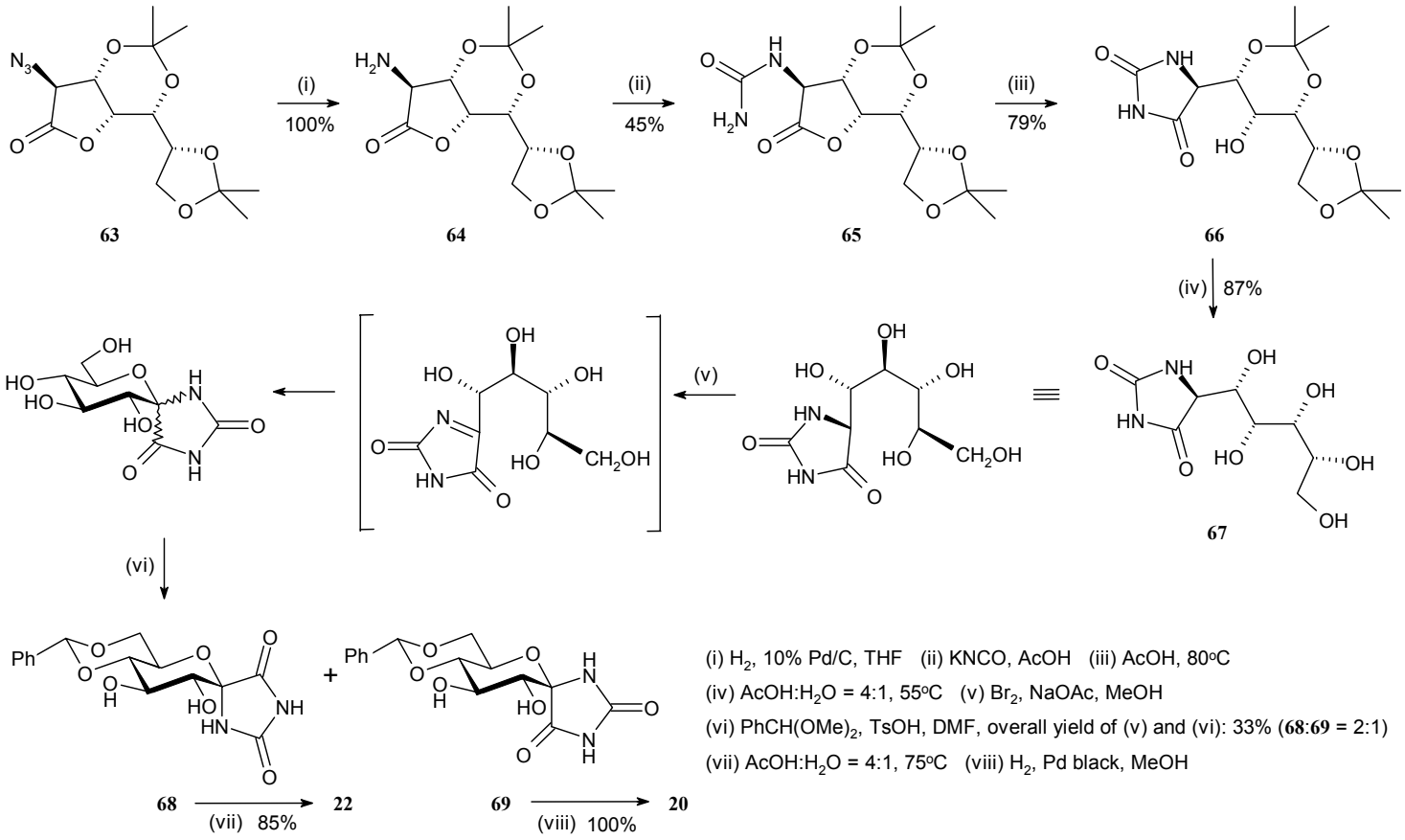


Scheme 9.

They reported the preparation of the epimeric spirohydantoins (**20**, **22**) in six steps starting from the benzylated 2,6-anhydro heptonic acid methyl ester **55**. It was first brominated (**56**) and converted to the azido derivative **57**. Hydrogenation of azido methylester **57** gave a mixture of two epimeric amines **58**, which could be separated but they readily interconverted in solution.

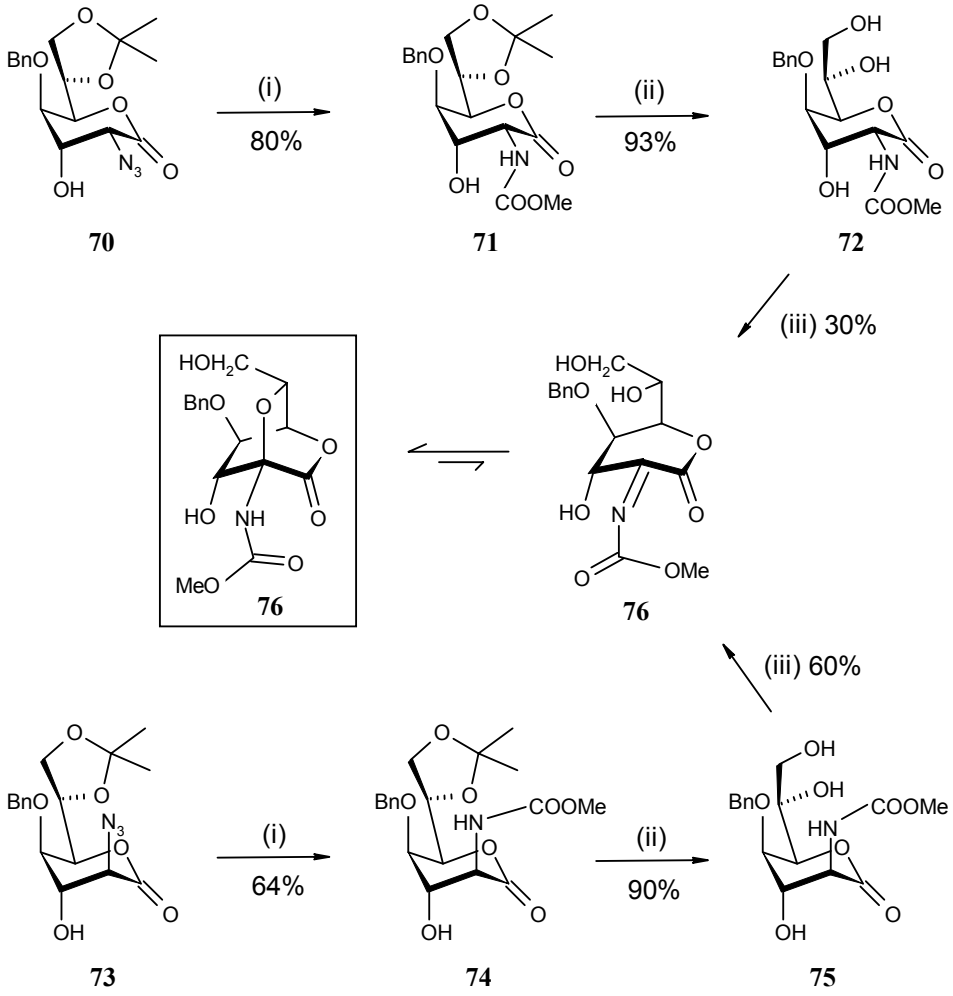
Thus a mixture of amines **58** was transformed to urea derivatives (**59**, **60**) which are separable, relatively stable, and equilibration of the anomers does not take place. The protected hydantoins (**61**, **62**) formed in quantitative yield from the corresponding urea derivatives. Although the yields of each reaction and the precise structure of several products were not published, because of the difficult separations this procedure was not suitable for the preparation of larger amounts of the target product.

De la Fuente and her co-workers have worked out two syntheses of hydantion **20**. One of them is a short, very simple method[43] giving a mixture of anomeric hydantoins together with a procedure for their separation (**Scheme 10**.). Since in practice the conversion of amine **64** to the unprotected open chain hydantoin **67** might be accomplished in one pot without isolation of **65** and **66**, the transformation of the cheap and readily avaible starting material azido heptonolactone **63** to a mixture of epimeric hydantoins requires only three steps. However, because of problems in the separation of epimers together with the fact that the major product is the much less active **22,** this synthesis is not suitable for the large scale preparation of **20**.

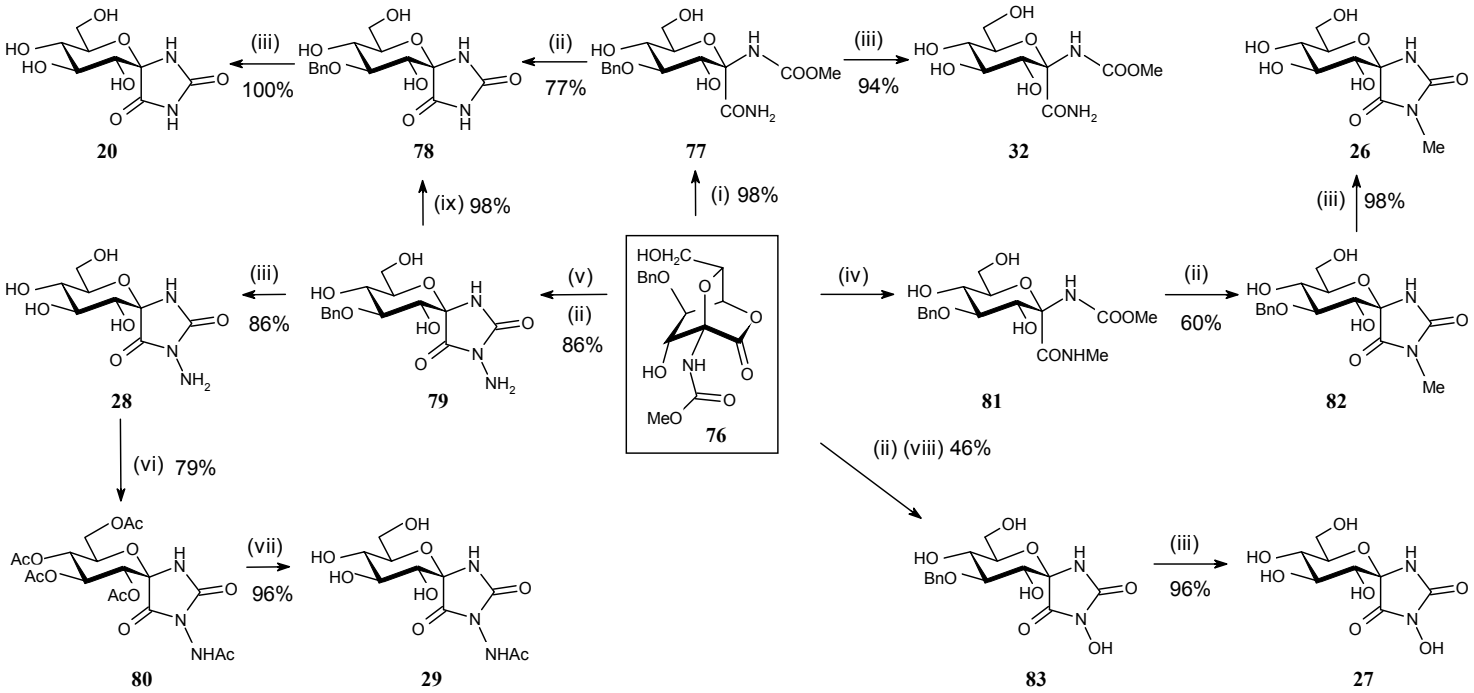

(i) 100%
(ii) 45%
(iii) 79%
(iv) 87%
(v)
(vi)
(vii) 85%
(viii) 100%
63
64
65
66
67
68
69
20
22
(i) H_2, 10% Pd/C, THF (ii) KNCO, AcOH (iii) AcOH, 80°C
(iv) AcOH:H_2O = 4:1, 55°C (v) Br_2, NaOAc, MeOH
(vi) $PhCH(OMe)_2$, TsOH, DMF, overall yield of (v) and (vi): 33% (**68**:**69** = 2:1)
(vii) AcOH:H_2O = 4:1, 75°C (viii) H_2, Pd black, MeOH

Scheme 10.

The other procedure (**Scheme 11**.)[31] gave the target compound **20** and a series of analogues (**26**-**29**) from the bicyclic lactone **76** which bears a carbamate at the bridgehead position.



Scheme 11/a.

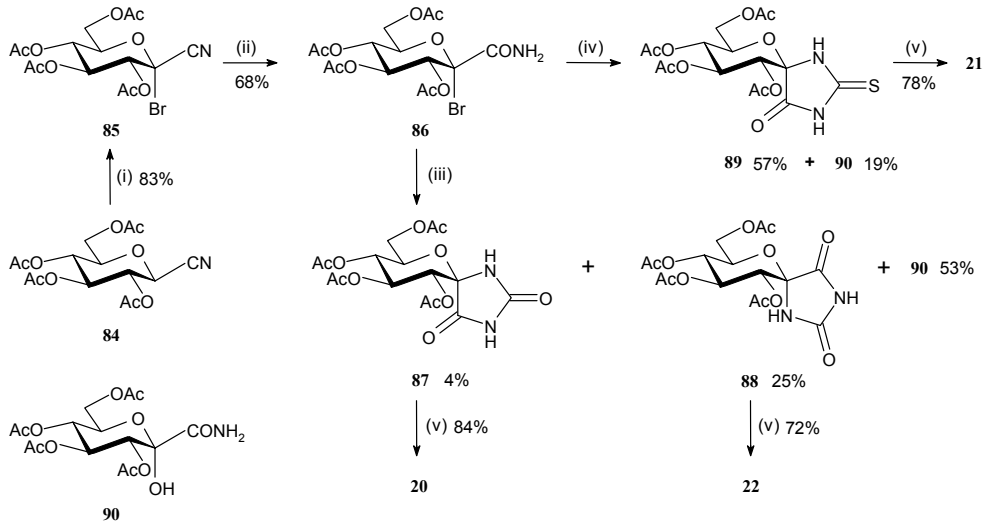


(i) NH_3, H_2O, THF (ii) tert-BuOK, DMF (iii) Pd, cyclohexene, MeOH, reflux (iv) $MeNH_2$, MeOH, THF (v) NH_2NH_2, H_2O, THF (vi) Ac_2O, pyridine
(vii) NaOMe, MeOH (viii) $NH_2OH{\cdot}HCl$, DBU, CH_2Cl_2 (ix) iso-amylnitrite, AcOH, $CHCl_3$

Scheme 11/b.

The rigid stucture of **76**, which already possesses the pyranose ring, guarantees that the anomeric nitrogen will be in the β position on ring opening of the lactone by a nucleophilic attack. This compound can be readily converted to **20**. Accordingly, the target hydantoin was prepared in six steps from the starting material azidolactone **70** in 26% yield.[44]

The procedure developed earlier in our laboratory gave hydantoin **20** from the acetylated bromo amide precursor **86** with silver cyanate (**Scheme 12**).[29]



(i) NBS, CCl_4, reflux (ii) $TiCl_4$, H_2O, Ac_2O, 0°C-r.t. (iii) AgOCN (4 eq), dry CH_3NO_2, 80°C
(iv) AgSCN (4 eq) or KSCN (2 eq), dry CH_3NO_2, 80°C (v) NaOMe, MeOH

Scheme 12.

Unfortunately, both epimers of the protected hydantoin (**87** and **88**) formed and the less useful one **88** arose in higher amount together with the hept-2-uloson amide derivative **90** which was the major product in 53% yield. On the contrary, using potassium thiocyanate in place of silver cyanate the protected derivative **89** of the biologically active thiohydantoin **21** formed in good yield (57%) as a single epimer together with a moderate amount (19%) of hydroxy amide **90**. Carrying out this reactions in the presence of a small amount of elemental sulfur under nitrogen

atmosphere in order to suppress radical-mediated pathways raised the yield of **89** to 79%, and only 4% of **90** was obtained. These findings propose that the formation of thiohydantoin **89** follows an ionic pathway while hydroxyamide **90** forms by radical reactions.[25]

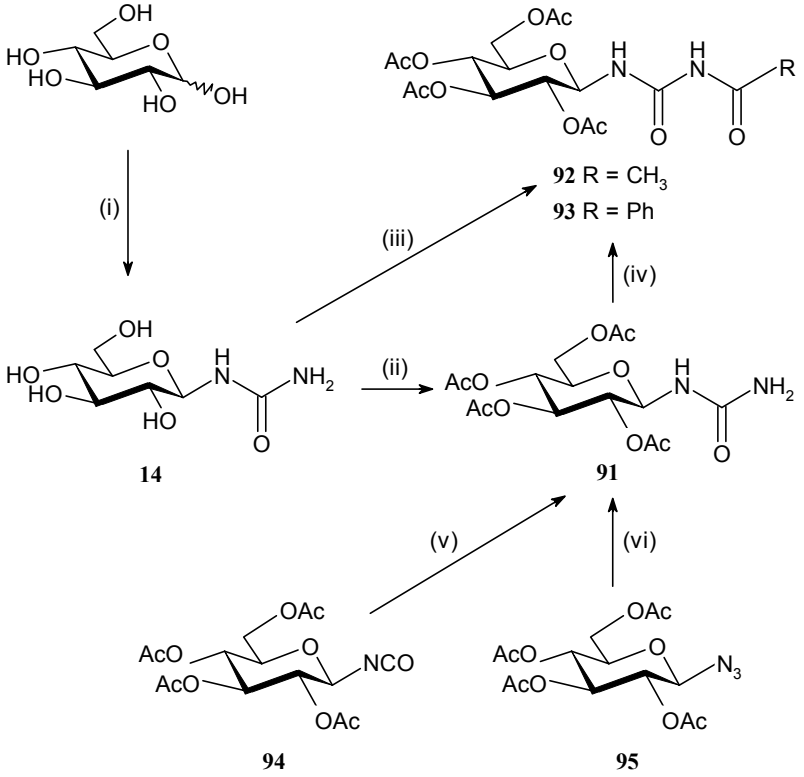
Starting from D-glucose this method involves only six steps but the overall yield of the syntheses was very low (~1-2%) because of the extremely poor production (11%) of the key acetylated glucopyranosyl cyanide **84**. In addition, several chromatographic separations were needed. This was the first and only reported synthesis of glucopyranosilidene-spiro-thiohydantoin **21** which is an equipotent GP inibitor to hydantoin **20**. Although this is a very simple and short procedure giving the desired isomer of thiohydantoin it seemed to be worth to optimise it and make it suitable for the preparation of higher amounts of inhibitor **21**.

2.2.2. *N*-Glucopyranosyl ureas

We also planned the synthesis of glucopyranosylidene-spiro-hydantoin like compounds from protected *N*-acyl-*N'*-glycopyranosyl ureas (see Results and discussion 3.2.). In the literature only a few processes are known for the synthesis of *N*-β-D-glucopyranosyl urea **14** (**Scheme 13**.).

The first method reported in 1903 by Schoorl[45] was based on the acid catalysed condensation of crude hydrolysed starch and urea. Its poor yield was later improved by others.[46,47] These methods gave the glucopyranosyl urea contaminated by a considerable amount of urea and a smaller quantity of *N*, *N'*-diglucopyranosyl urea which was removed by subsequent recrystallisations and chromathography. Protected derivatives **91-93** were synthesized by Helferich and Kosche from **14**.[46] Acetylated glucopyranosyl urea **91** was also prepared by Fischer[48] from acetylated glucopyranosyl isocyanate **94** with aqueous ammonia, this method has been optimised recently.[49]

For the preparation of **91** a more convenient method was published by Pintér and his co-workers.[50] They transformed acetylated glucopyranosyl azide **95** to the corresponding phosphinimine which in the presence of carbon dioxide behaves like a masked sugar isocyanate and readily reacts with ammonia to give **91** in 75% yield.



(i) H_2NCONH_2, H_2O, H^+ (ii) Ac_2O, dry pyridine (iii) Ac_2O, $ZnCl_2$

(iv) BzCl, $ZnCl_2$, $CHCl_3$ (v) NH_3, H_2O (vi) NH_3, CO_2, THF then PPh_3, THF

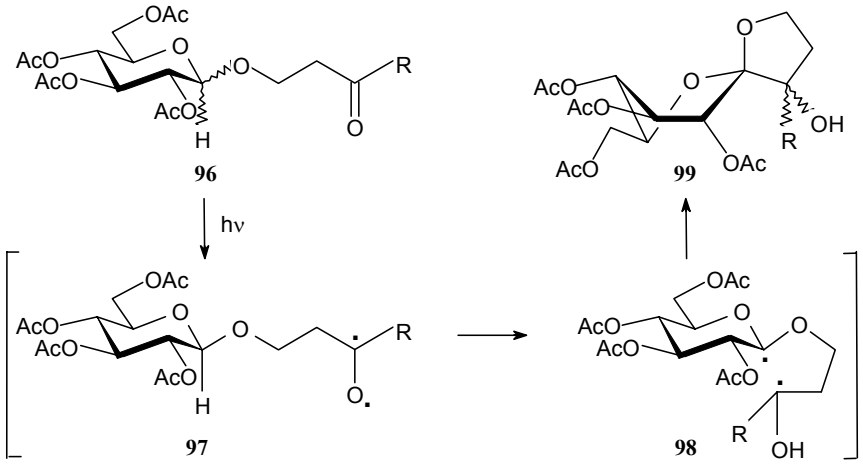
Scheme 13.

2.2.3. Photochemical syntheses of glucopyranosylidene-spiro heterocycles

It is well known that radical reactions can be carried out under very mild conditions and can be suitable for the transformations of carbohydrates. Numerous

examples were reported for the synthesis of anomeric spiro-heterocycles by photochemical reactions.

Remy and his co-workers examined photolytic ring closure of γ-oxoalkyl glycosides **96**[51] (**Scheme 14.**). They have found that both anomers lead to the same spirocycle **99**. The mechanism of the reaction is supposed to involve the formation of the carbon centered biradical **98** from **97** by abstraction of the anomeric hydrogen. Biradical **98** is liable to ring closure from the α direction.

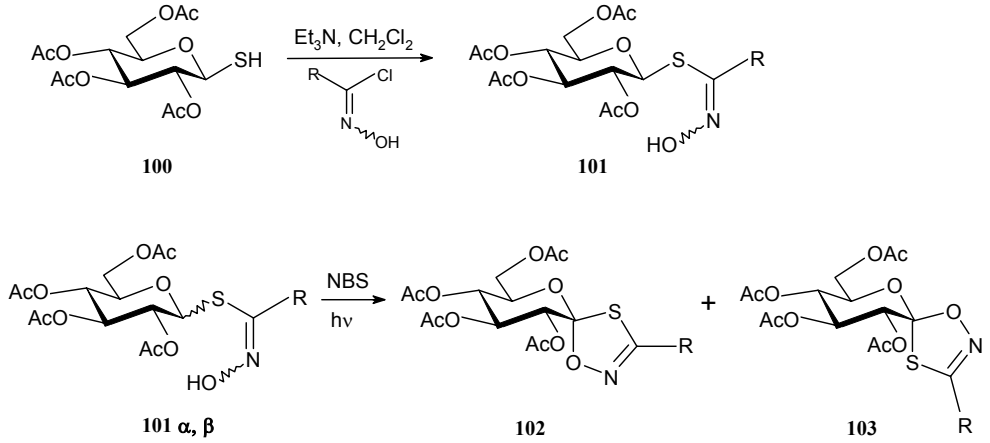


Scheme 14.

The β anomer of **96** was found more reactive than the α one which can be explained by stereoelectronic effects, that is the hydrogen atom in axial position at the anomeric carbon is easier to abstract than the equatorial one. This reaction is regioselective, only the hydrogen atom in δ position to the radical is abstracted, thus the process takes place through an energetically favourable seven membered transition state (Norrish type II reaction). Similar reactions were published by others.[52, 53]

Praly and his co-workers have worked out the photochemical synthesis of glucopyranosylidene-spiro-1,2,4-oxathiazoles **102** and **103**[54] from the corresponding thiohydroximate **101**[55] under oxidative conditions (**Scheme 15**.).

Intramolecular spirocyclisation of **101** bearing an aryl ring attached to the thiohydroximate moiety led to two epimeric spiro-oxathiazole derivatives **102** and **103** in ~45-60% total yield. This occurred with good stereoselectivity as judged from the yields and the ratio of epimers **102**:**103** was ca. 4:1. A comparison of the results obtained for both anomers of **101** showed that neither the rate nor the selectivity of the transformation were significantly affected by the anomeric configuration in **101**.

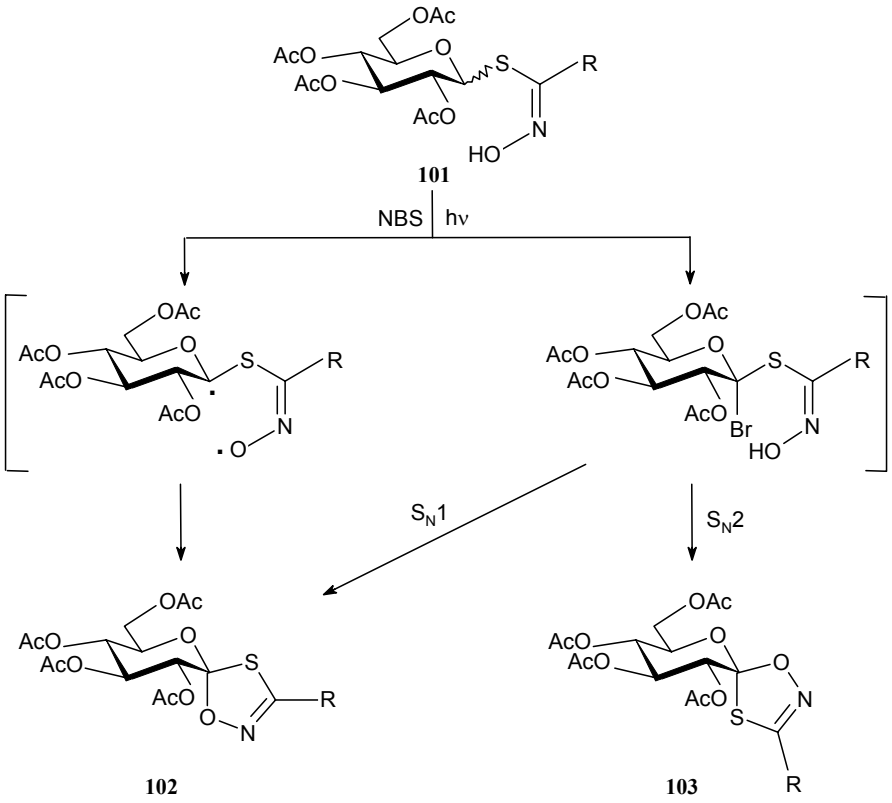


R = C_6H_5, p-C_6H_4Br, p-C_6H_4Cl, p-C_6H_4F, $C_{10}H_7$, COOEt, CN, CH_3

Scheme 15.

Spiro-oxathiazoles were also obtained in lower yields when the aryl ring in **101** was replaced by ethoxycarbonyl or cyano groups. In contrast no cyclisation occurred when **101** contained a methyl group but a simple bromination took place instead. According to the proposed mechanism the reaction can follow two pathways (**Scheme 16**.): on one hand oxidation of the hydroxyl group may lead to an oxygen-centered oximidoyl radical which abstracts the anomeric hydrogen, then in a similar way the restored hydroxymate group produces again the oxygen-centered radical and the formed biradical is liable to cyclise. On the other hand the radical bromination of the anomeric center occurs first facilitated by the presence

of the sulfur atom. This is followed by the heterolysis of the C-Br bond forming an anomeric carbocation.[56] There are several examples in the literature showing that nucleophiles attack stabilised glucopyranosyl cations stereoselectively from the α side in either an inter-[57-59] or intramolecular[60] process.



Scheme 16.

Thus the formation of the major stereoisomer **102** can be explained by an S_N1 process while the minor **103** can be generated by an S_N2 displacement of the bromine atom by the hydroximate group. Both proposed pathways give **102** as the major product. In the case of R = C_6H_5, p-C_6H_4Cl, p-C_6H_4Br and p-C_6H_4F the deprotected derivatives of **102** proved to be weak inhibitors of β-D-glycosidase from sweet almond[54] and GP (see Section 3.3.)

3. Results and discussion

As indicated in the Introduction the general aim of my PhD research has been the development of glucose analogue inhibitors of glycogen phosphorylases. Based on the literature survey this goal can be detailed as follows:

– elaboration of a scalable synthetic sequence for the preparation of glucopyranosylidene-spiro-thiohydantoin **21**.

– developing new intramolecular spirocyclisations to obtain novel glucopyranosylidene-spiro-heterocycles with potential GP inhibitory activity.

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

Glucopyranosylidene-spiro-thiohydantoin **21** was found to be one of the best glucose analog inhibitors of GP but the reported syntheses (see section 2.2.1.) were not suitable for the preparation of large amounts required for more extended biological investigations. Although the simplest procedure[29] starting from D-glucose and producing **21** as a sole isomer in six steps was attractive, the overall yield was rather low (~2). The main reasons of that are the following: a.) the key starting material, the acetylated glucopyranosyl cyanide **84** (**Scheme 12**.) could be obtained in an extremely poor yield (~11%); b.) several chromatographic separations were needed; c.) formation of a considerable amount of hydroxy amide by-product **90** was unavoidable. However, to have a really practical synthesis it seemed to be worth improving this procedure.

The preparation of acetylated glucopyranosyl cyanides was studied by Myers and Lee.[61, 62] The best procedure proposed by them for 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl cyanide, i.e. fusion of acetobromo-glucose with mercury(II) cyanide[61] gave in our hands generally 10-12% of the target compound isolated by several consecutive crystallizations and/or chromatographic purification. There is no information in the literature to explain why the cyanation of acetobromo-

glucose is so unselective under various conditions. The most important by-products are 1,2-*O*-(1-cyano-ethylidene) derivatives formed by an attack of the cyanide on the probable 1,2-acetoxonium intermediate and penta-*O*-acetyl-D-glucopyranoses.[61, 62] Another possibility for the preparation of the acetylated β-D-glucopyranosyl cyanide could have been the procedure of Köll and Förtsch starting from the corresponding acetylated *C*-glycosyl nitromethane.[63] However, several chromatographic separations would have been required even in this case.

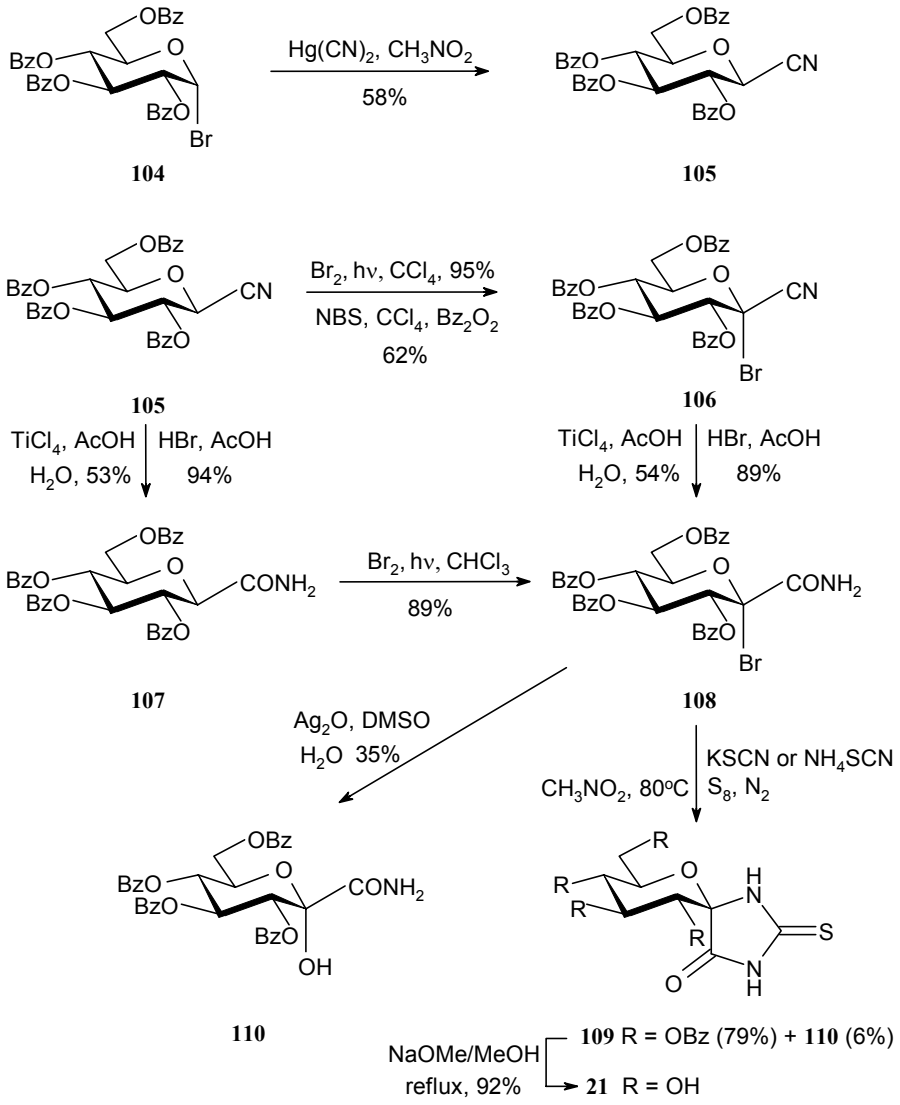
Therefore we decided to use benzoyl protecting groups with the expectation of a lower tendency for the formation of 1,2-orthoester derivatives from the 1,2-acyloxonium ion intermediate as has been also reported in certain cases for 2-*O*-benzoylated sugars relative to their 2-*O*-acetylated counterparts.[64] Another expected advantage was a higher tendency for crystallization of the benzoylated sugar derivatives.

Benzobromo-glucose **104** (**Scheme 17**.) obtained by a known procedure from penta-*O*-benzoyl-D-glucopyranoses with HBr in acetic acid[65] was treated with mercury(II) cyanide in dry nitromethane at room temperature. Crystallization of the worked-up mixture gave regularly 54-58% yields of 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl cyanide **105**. Chromatographic purification of the mother liquor gave a further 16-19% crop of **105** and the presence of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl cyanide[66] as well as 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose[67] as minor by-products could also be detected by NMR spectroscopy.

For the partial hydrolysis of the nitrile moiety in **105** $TiCl_4$ in acetic acid[68] (used in the acetylated series) or HBr in acetic acid[69] were applied. The $TiCl_4$ mediated procedure required long reaction time (5 h) and gave **107** in 53% yield while by using HBr **107** was obtained after 2 h reaction time in 94% yield as an almost analytically pure crystalline product.

Radical mediated bromination[70] of **105** could be performed with NBS in refluxing carbon tetrachloride in the presence of catalytic benzoyl-peroxide as in the acetylated series. The acetylated 1-bromoglucopyranosyl-cyanide dissolves in

hot carbon tetrachloride while the succinimide does not and it was easily separeted from the product by filtration. However, the insolubility of the benzoylated 1-bromoglucopyranosyl-cyanide **106** in this solvent made the work-up of the reaction mixture difficult (see Experimental) and this decreased the yield of **106** to 62%.



Scheme 17.

Photobromination of **105** with bromine in refluxing carbon tetrachloride[71] gave **106** as an essentially pure product in an almost quantitative yield. The above conditions could not be used for the bromination of **107** because of its very poor solubility in carbon tetrachloride even at reflux. Replacement of this solvent partially or fully with chloroform in the photobromination reaction gave excellent yields of **108** (**Table 1**). The product formed quantitatively in the chloroform reaction was again sufficiently pure for further transformations. Dichloromethane was also tried as a solvent for the photobrominations and gave similar yields for both **106** and **108**.

Table 1. Isolated yield (%) of the brominated products obtained by photobromination

Solvent	106	108
CCl_4	80 (95*)	-
CCl_4-$CHCl_3$ 1:2	-	87
$CHCl_3$	67 (quant.*)	89 (quant.*)
CH_2Cl_2	70 (quant.*)	60 (97*)

*Yield of the crude product if sufficiently pure for further transformations

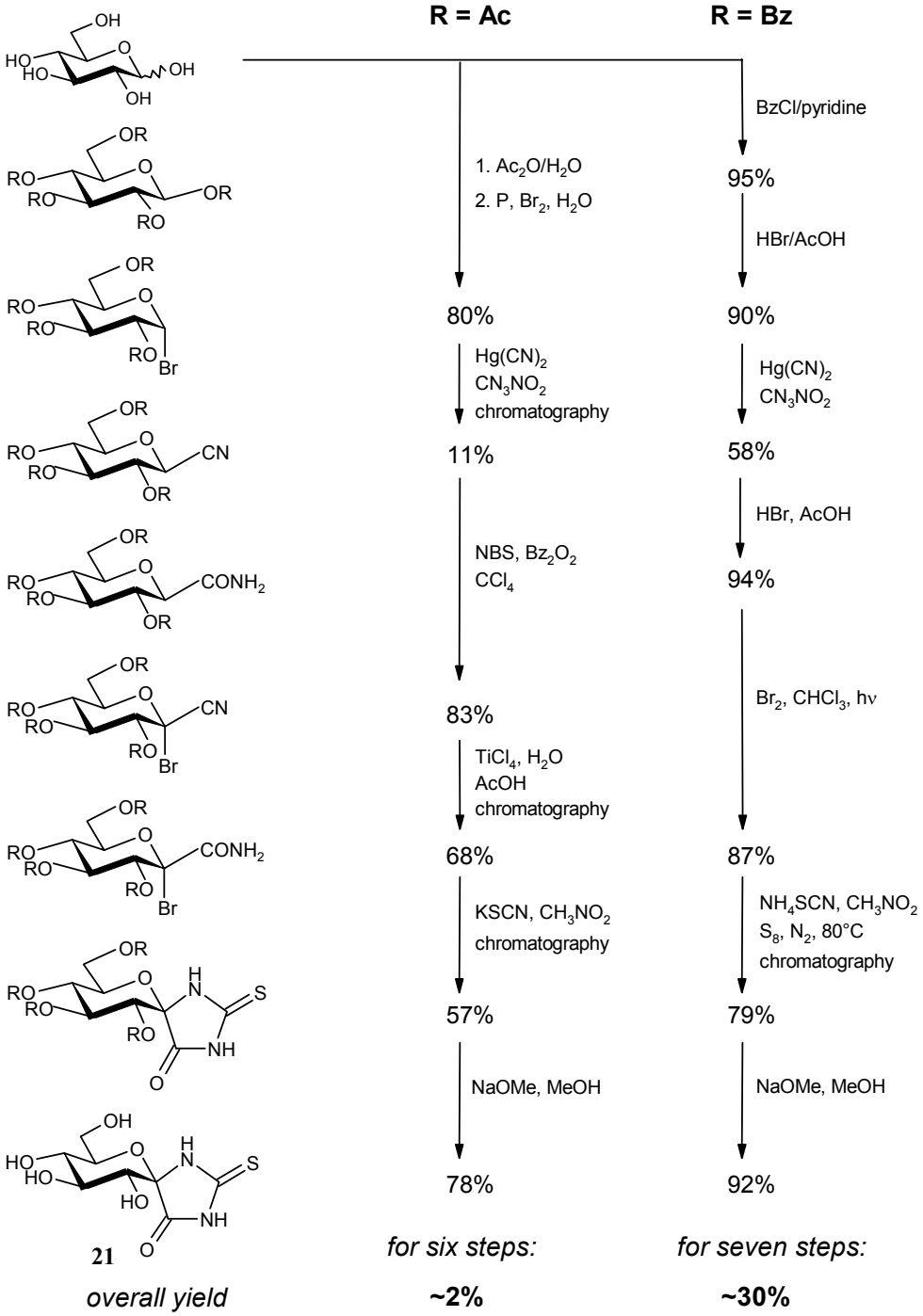
These experiments show that the use of solvents other than carbon tetrachloride is possible in photobrominations of carbohydrate derivatives. Note that recently 1,1,1-trichloroethane was suggested as a replacement for carbon tetrachloride in similar radical-mediated halogenations of *C*-glycosyl formates[72] as well as $KBrO_3$-$Na_2S_2O_4$ reagent in dichloromethane-water or benzotrifluorid ($PhCF_3$)-water biphasic solvent systems were applied to the bromination of several monosaccharide derivatives.[73] Compound **106** was also prepared by this latter method in CH_2Cl_2-H_2O biphasic solvent in 97% yield after 168 h reaction time.[73]

Reaction of **108** with ammonium or potassium thiocyanate in nitromethane in the presence of elemental sulfur to suppress radical-mediated pathways[72] under nitrogen atmosphere gave spiro-thiohydantoin **109** (79%) and hydroxy-amide **110**

(6%). Compound **110** was also prepared independently from **108** by silver oxide-promoted hydrolysis in dimethylsulfoxide in 65% yield. Debenzoylation of **109** was accomplished by the Zemplén method in methanol at reflux to give inhibitor **21** in 92% yield.

Structure elucidation of the new compounds was straightforward using NMR methods. Conformation the sugar rings was established as $^4C_1$ from the vicinal proton-proton couplings. The anomeric configuration of compounds **105** and **107** has been found to be β according to the measured coupling constants between H-1 and H-2 ($J_{1,2}$~9 Hz). In case of compounds **106**, **108** and **110** configuration of the anomeric carbons were deduced from the three bond heteronuclear couplings between H-2 and the *C*-substituent of the anomeric center.[74] Thus, the observed couplings (**106** $^3J_{\text{H-2,CN}}$ = 2.1 Hz; **108** $^3J_{\text{H-2,CONH2}}$<1 Hz; **110** $^3J_{\text{H-2,CONH2}} \approx$ 1 Hz) prove the depicted structures for these compounds. In the same manner the configuration of the spiro carbon (C-6) in **109** was assigned as *S* based on the $^3J_{\text{H-5,C-10}}$ = 6.6 Hz value. Compound **21** proved identical with a sample prepared by the previously published route.[29]

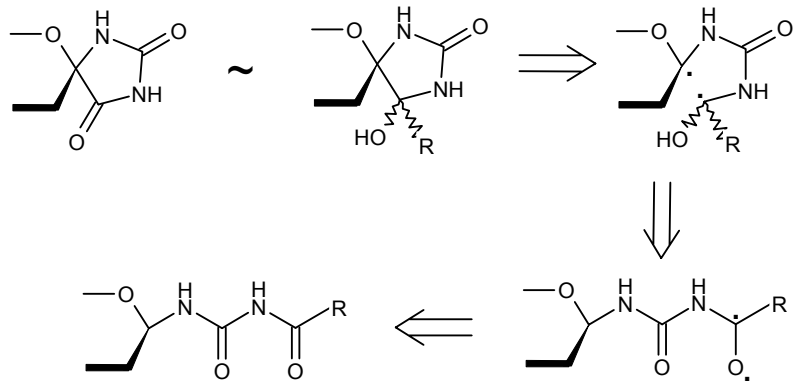
As benzobromo-glucose **104** can be made from D-glucose via the pentabenzoate in 86% yield, for the seven steps the overall yield for **21** is 30% i. e. ~15 times better than in the original synthesis (**Scheme 18**.). Most reactions were clean and the crude products could be used without any purification. Even if further treatment was necessary it was only simple crystallization. The number of chromatographic separations was reduced to one, the only chromatography required was the separation of **109** from minor by-products. However, carrying out the reaction in larger amounts **109** crystallized from the worked up reaction mixture in 56% yield, thus this chromathograpy was not essential. This highly practicable synthetic sequence allowed **21** to be prepared in gram quantities.[75] Thus, *in vitro* and *in vivo* studies of hepatic glycogen metabolism with **21** have become possible, and these results are detailed in Section 4.[76]


R = Ac
R = Bz
1. Ac2O/H2O
2. P, Br2, H2O
80%
Hg(CN)2
CN3NO2
chromatography
11%
NBS, Bz2O2
CCl4
83%
TiCl4, H2O
AcOH
chromatography
68%
KSCN, CH3NO2
chromatography
57%
NaOMe, MeOH
78%
BzCl/pyridine
95%
HBr/AcOH
90%
Hg(CN)2
CN3NO2
58%
HBr, AcOH
94%
Br2, CHCl3, hν
87%
NH4SCN, CH3NO2
S8, N2, 80°C
chromatography
79%
NaOMe, MeOH
92%
21
for six steps:
~2%
for seven steps:
~30%
overall yield

Scheme 18.

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 (see 2.2.3.), that is why we have planned the synthesis of compounds similar to glucopyranosylidene-spiro-hydantion **20** in a photochemical way according to the retrosynthetic **Scheme 19**.

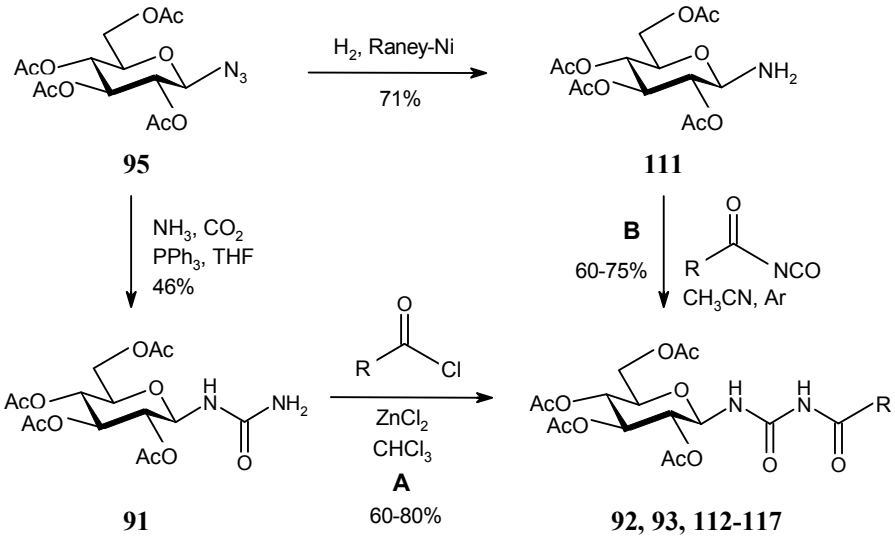


Scheme 19.

Suitable precursors are expected to take part in a Norrish type II reaction on irradiation (UV light).

Protected *N*-acyl-*N*'-glucopyranosyl ureas, which could be convenient precursors for this photocyclisation, were prepared from acetylated glucopyranosyl azide[77] **95** in two ways (**Scheme 20**). According to the method of the Pintér group[50] acetylated β-D-glucopyranosyl urea was synthesized via the corresponding phosphinimine derivative, then it was acylated in chloroform in the presence of zinc chloride (Route **A**).[46] Catalytic hydrogenation of **95** gave amine **111** which reacted with various acyl isocyanates in dry acetonitrile under argon atmosphere[78] and gave also the target molecules **93**, **112-117** in good yields (Route **B**). The structure of the new compounds was determined by NMR methods. The conformation of the sugar rings was found to be $^4C_1$ from the vicinal proton-proton couplings. The anomeric configurations were established from the measured

coupling constants between H-1 and H-2 ($J_{1,2}$~9 Hz). In the ^{13}C NMR spectra signals between 150 and 160 ppm are characteristic for the carbonyl C of the urea moiety.



92 R = CH_3 56% (**A**)
93 R = Ph 30% (**A**)
112 R = p-NO_2-C_6H_4 60% (**A**)
65% (**B**)
113 R = m-Cl-C_6H_4 74% (**B**)
114 R = p-OAc-C_6H_4 72% (**A**)
20% (**B**)
115 R = 1-naphthyl 73% (**A**)
116 R = 2-naphthyl 29% (**A**)
117 R = 2-indolyl 41% (**A**)

Scheme 20.

For the photocyclisation assays per-*O*-acetylated protected *N*-acetyl- (**92**) and *N*-benzoyl-*N*'-β-D-glucopyranosyl ureas (**93**) were chosen (**Table 2.**). We tested various reaction conditions: different dry solvents (benzene, acetonitrile, methanol) were examined as reaction media. The UV irradiations were effected by a high-efficiency medium pressure mercury lamp with and without Vicor filter but according to our experiments utilisation of the filter was indifferent. In the event of *N*-acetyl urea **92** some transformations could be carried out only in methanolic solutions but after chromatographic work up of the reaction mixtures the products were identified as 2,3,4,6-tetra-*O*-acetyl-*N*-glucopyranosyl urea **91** in each case.

Photolysis of a benzene solution of *N*-benzoyl derivative **93** gave similar results. The transformations were very slow (several days) that is why the reactions were not allowed to reach the complete conversion but were stopped and the product was isolated. In these cases instead of the expected transformation Norrish type I deacylation reactions took place. In other cases the solutions became dark brown on irradiation but no transformation of the starting material was observed. In summary photolysis of protected *N*-acyl-*N*'-glucopyranosyl ureas was found to be unsuitable for spirocyclisation because of the very long reaction times, the incomplete conversion and prevalance of the Norrish type I cleavage.

Table 2. Photoreactions of acetylated *N*-acetyl- and *N*-benzoyl-*N*'-β-D-glucopyranosyl ureas

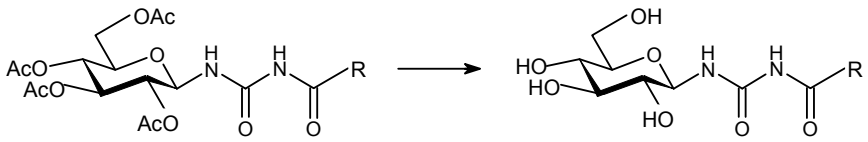
Starting material	Solvent	Reaction conditions	Reaction time	Conversion	Product (%)
92	benzene	Vicor*	2.5 h	-	-
92	benzene	*	3 h	-	-
92	acetonitrile	Vicor*	2.5 h	-	-
92	acetonitrile	*	3 h	-	-
92	methanol	Vicor*	33 h	12%	**91** (6%)
92	methanol	*	25 h	28%	**91** (<1%)
93	benzene	Vicor*	20 h	57%	**91** (6%)
93	benzene	*	50 h	+	**91****

*Irradiations were effected by a 450 W Hanovia type mercury lamp at reflux temperature

**Product was not isolated and was identified by TLC

The structure of *N*-acyl-*N*'-glucopyranosyl ureas can be regarded as "open chain" analogues of hydantoin **20**. After deprotection they proved to be very efficient inhibitors of GP (see Section 4.), even the most active glucose analogue inhibitor known to date, the 2-naphthoyl derivative (**124**, **Table 3**.), has been found among them with its nanomolar K_i value.[79]

Table 3. Synthesis of *N*-acyl-*N*'-β-D-glucopyranosyl ureas



92, **93**, **112-117** **118-126**

Substrate	R	Product	Reaction conditions*	Yield
92	CH_3	**118**	NaOMe, MeOH, 1h	82%
93	C_6H_5	**119**	NaOMe, MeOH, 2 h	90%
112	4-NO_2-C_6H_4	**120**	$KHSO_4$, MeOH, 5 days	99%
113	3-Cl-C_6H_4	**121**	NaOMe, MeOH, 0.5 h	98%
114	4-OAc-C_6H_4	**122** (R=4-OH-C_6H_4)	NaOMe, MeOH, 0.5 h	87%
115	1-naphthyl	**123**	NaOMe, MeOH, 1 h	94%
116	2-naphthyl	**124**	NaOMe, MeOH, 0.5 h	83%
117	2-indolyl	**125**	NH_3, MeOH, 1 day	70%
120	4-NO_2-C_6H_4	**126** (R=4-NH_2-C_6H_4)	Raney-Ni, MeOH, 1 h	99%

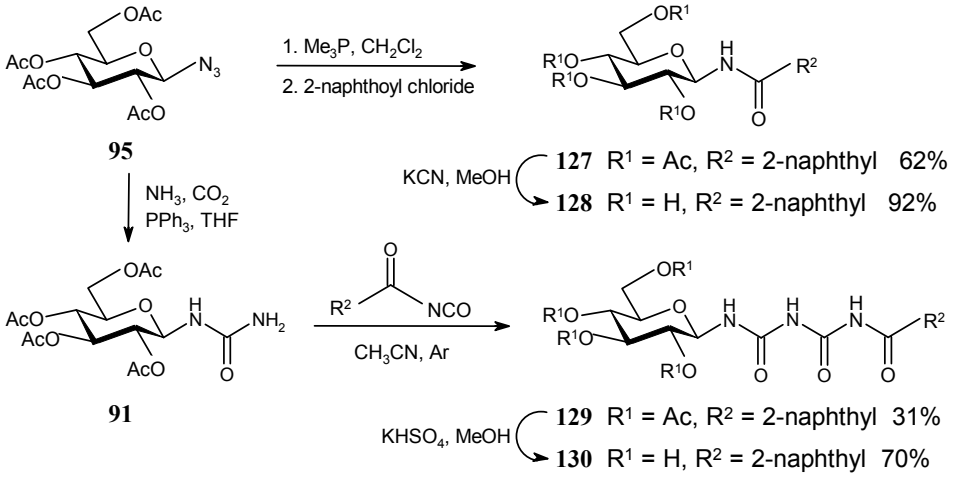
*The reactions were carried out at room temperature

Unfortunately, the character of the R group has an important influence on the deprotection, that is why it succeeded only under various conditions.

The 4-NO_2-benzoyl urea **120** was hydrogenated to form *N*-4-amino-benzoyl-*N*'-glucopyranosyl urea **126** which also proved to be efficient inhibitor of GP (see Section 4.).

Crystallographic studies of glucopyranosyl urea-GP complexes have shown that the new compounds bind to the active site of the enzyme and some of them binds also to the new allosteric site which is a unique property among the glucose analogue inhibitors. The nature of binding of urea derivatives containing an aromatic group are different from that of the spirohydantoin, they take an extended conformation at the active site of the enzyme so that the aromatic moieties fit well into the β-pocket (see Section 4.)[80]

To explore the β-pocket of the enzyme two other 2-naphthoyl derivatives were prepared: *N*-naphthoyl-β-D-glucopyranosylamine **128**[81] and *N*-2-naphthoyl-*N*''-β-D-glucopyranosyl biuret **130** (**Scheme 21.**). Compound **127** was obtained from acetylated glucopyranosyl azide **95** via a phosphinimine intermediate[82] and was deprotected with KCN in dry methanol to form **128**, while **129** formed in the reaction of 2-naphthoyl-isocyanate and acetylated glucopyranosyl urea **91** and was deacetylated with potassium hydrogensulfate in methanol.



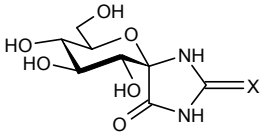
Scheme 21.

For the results of the kinetic and protein crystallographic investigations, please, see Section 4.

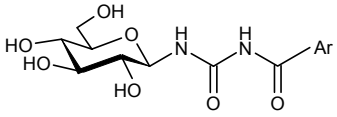
3.3. Experiments towards the synthesis of glucopyranosylidene-spiro-1,2,4-oxadiazolines

On the basis of kinetic and crystallographic results obtained with the above inhibitors and spiro-(thio)hydantoins it seemed to be worth to prepare similar glucopyranosylidene-spiro-heterocycles that contain an NH group in anomeric β position of the D-sugar in a rigid five-membered spiro ring similar to the

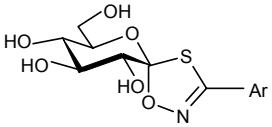
hydantoins, as well as an aromatic group in an appropriate position to fit into the β-pocket.



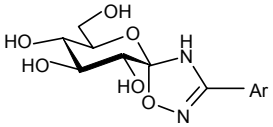
20 X = O K_i = 3.1 μM
21 X = S K_i = 5.1 μM



119 Ar = C_6H_5 K_i = 4.6 μM
124 Ar = 2-naphthyl K_i = 0.4 μM



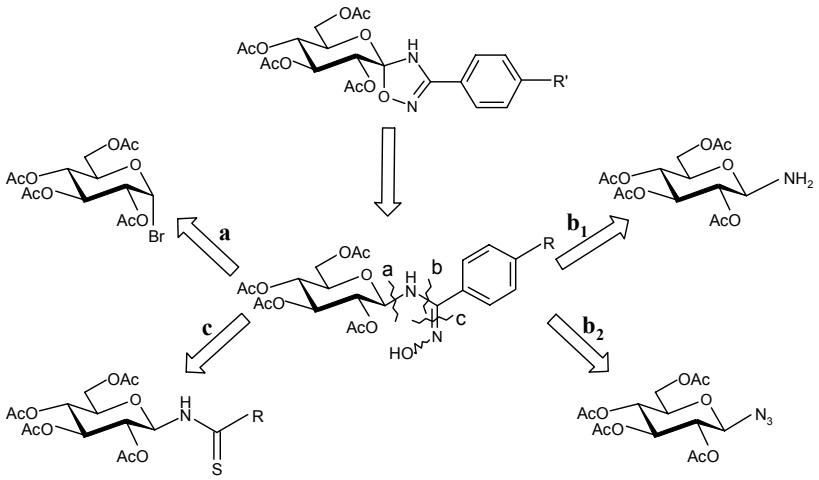
131 Ar = C_6H_5 K_i = 26.0 μM
132 Ar = 4-F-C_6H_5 K_i = 48.0 μM



Ar = phenyl, 2-naphthyl

Scheme 22.

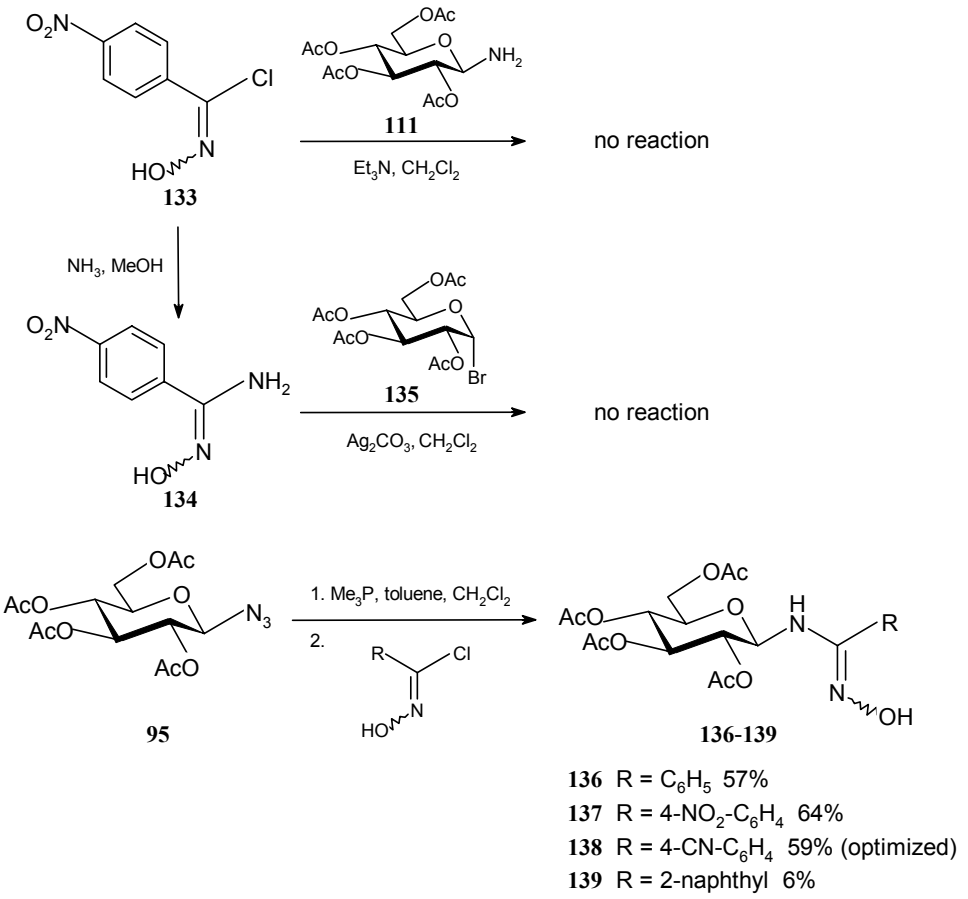
Suchlike molecules containing a sulfur atom in anomeric β position[54] have been synthesized earlier in Praly's group (see Section 2.2.3.). Among the deprotected oxathiazoles in the case of Ar = C_6H_5 (**131**, **Scheme 22**.) and Ar = p-F-C_6H_4 (**132**) the compounds proved to be efficient inhibitors of GP.[83] We planned to extend their methodology for the preparation of glucopyranosyliden-spiro-1,2,4-oxadiazolines according to the retrosynthetic **Scheme 23**.



Scheme 23.

To this end *N*-β-D-glucopyranosyl amidoxime derivatives were needed as percurors. For the preparation of these unknown compounds four synthetic pathways were considered according to disconnections a, b and c.

Route $\mathbf{b_1}$, which is analogous with the synthesis of thiohidroximates[55], seemed to be obvious,[84] and was tried first. Thus hydroximinoyl chloride **133** was prepared[85] and reacted with acetylated β-D-glucopyranosyl amine **111** in the presence of triethylamine but no transformation of the sugar was observed on the TLC plates (**Scheme 24**.).



Scheme 24.

Hydroximinoyl chloride **133** treated with methanolic ammonia gave amidoxime **134**[86, 87] which was expected to react with acetobromoglucose **135** according to route **a** but no reaction occured even in the presence of silver carbonate.

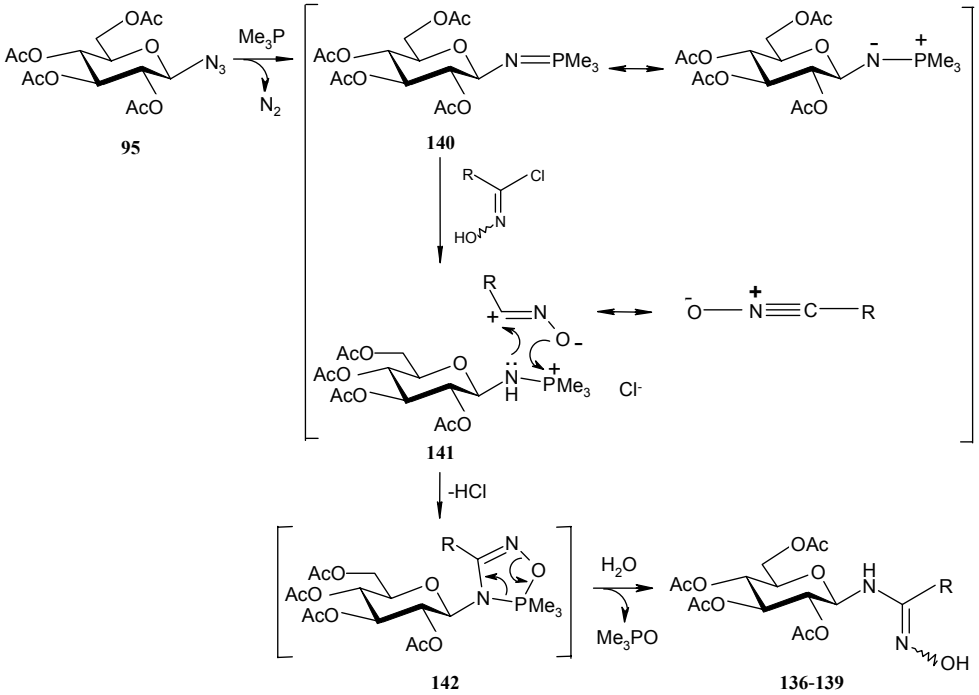
Aromatic thioamides were published to react with hydroxylamine to form the corresponding amidoximes[84] according to route **c**. We did not try to obtain glucopyranosyl amidoximes in this way because the starting material would have been preparable in three steps from acetobromoglucose and a more simple procedure detailed below was found to be appropriate.

Finally, acetylated glucopyranosyl azide **95** was transformed to the corresponding phosphinimine derivative[82] which reacted readily with hydroximinoyl chlorides and gave the target molecules **136-139** according to route $\mathbf{b_2}$. The *syn*/*anti* ratio were ~1/10 according to the NMR spectra of the crude prducts. While in the thio hydroximates various R groups could be introduced (**Scheme 15**.), our amidoximes **136-139** proved to be reasonably stable only when an electron withdrawing group was attached to position 4 of the aromatic ring.

In the case of phenyl **136** and 2-naphthyl **139** derivatives the products decomposed during the flash chromatographic purification, they were stable only at –20°C, so they were not suitable for further transformations such as deprotection or photocyclisation. In contrast, the 4-nitro-phenyl compound **137** was obtained in good yield and could be purified by simple chromatography or even recrystallised from hot ethanol. The 4-cyano benzamidoxyme **138** formed in 18% yield and proved also to be rather stable at room temperature.

During the preparation of benzamidoximes formation of unidentified polar compounds was observed. These remained on the starting point of the TLC plates even in methanolic eluents and disappeared after the aqueous work-up. In the case of 4-nitrobenzamidoxime **137** only a small amount of such by-products were obtained, but the yield of p-cyanobenzamidoxyme **138** was significantly lower (18%) because the side reactions produced an important quantity of these polar

compounds. **Scheme 25** shows a possible mechanism for the formation of the target molecules: from the starting azide **95** the corresponding phosphinimine **140** forms first which has a strongly basic character; thus it may form a salt **141** with hydroximinoyl chlorides;[82] **141** has a lower reactivity as compared to **140** but activated nitriloxides like the 4-nitrophenyl derivative can attack it. Target compounds **136-139** may form via intermediate **142** during the aqueous work-up. Presumably, if the hydroximinoyl chloride bears substituents with less electron-withdrawing character the nitriloxide is not active enough and the salt **141** is transformed to the corresponding cyclic intermediate **142** and thus products **136** and **139** only to a very low extent or not at all.



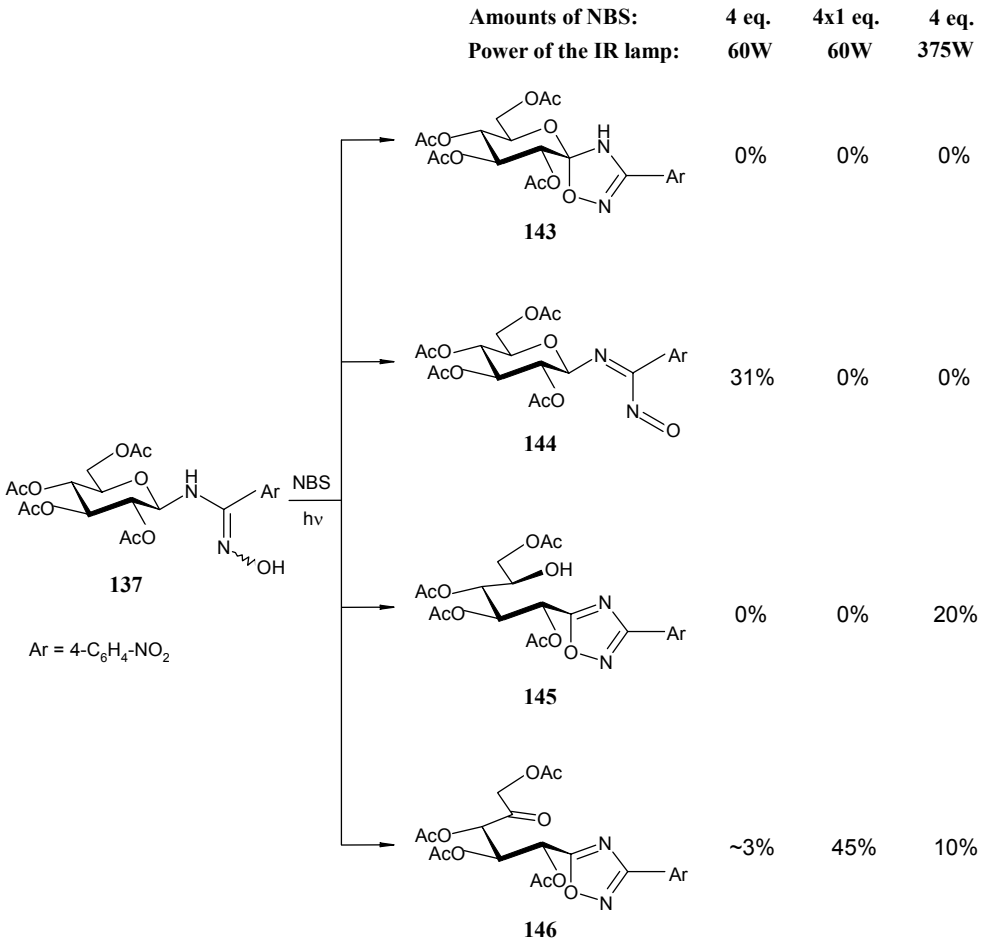
Scheme 25.

Since the 4-cyanobenzamidoxime **138** was rather stable it was worth to optimize its synthesis. To avoid the formation of the hypothetical salt **141** triethyl amine was added to a solution of the 4-cyanobenzhydroximinoyl chloride in

dichloromethane to form the corresponding nitriloxide which was reacted with a solution of phosphinimine **140** prepared meanwhile. This way, the formation of the start spot on TLC was not observed and the yield of **138** increased up to ~60%.

Experiments towards the oxidative photocyclisation were carried out under the same conditions as in the synthesis of oxathiazoles **102** (**Scheme 15.**). The results of our experiments are summarized in **Table 4**.

Table 4. Photolysis of **137** under various conditions

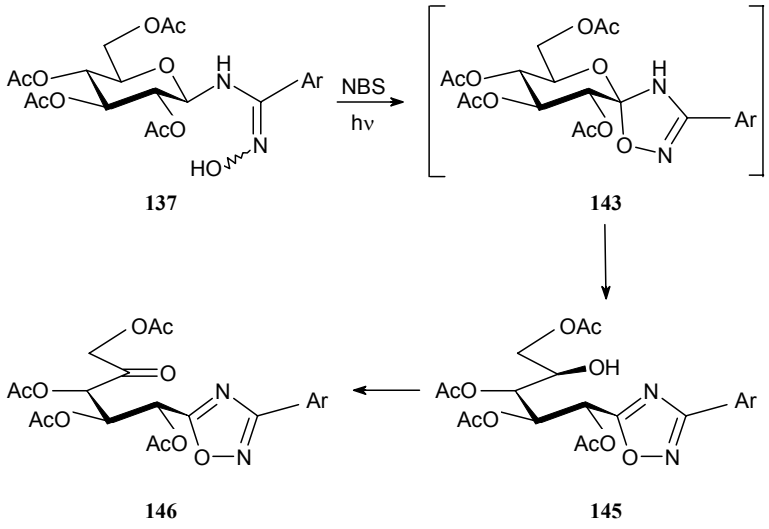


Chloroform solutions of the 4-nitrobenzamidoxime **137** with different quantities of NBS were irradiated with a heating lamp followed by separation with

coloumn chromatography. The original synthesis employed two equivalents of NBS but in our assays four equivalents led to complete transformations of the starting material.

By using a 60 W heating lamp for the irradiation and four equivalents of NBS added in one portion the formation of the desired spirocycle **143** was not observed but an oxidized compound **144** formed in moderate yield and traces of an open chain sugar derivative **146** were detected by NMR spectroscopy. Addition of NBS in four portions resulted in the exlusive formation of compound **146** in 45% yield. Carrying out the photolyses with a 375 W IR lamp the way of the addition of NBS was indifferent for the outcome of the reaction. The desired product **143** did not form in these cases, too, but another open chain compound **145** was observed together with **146**.

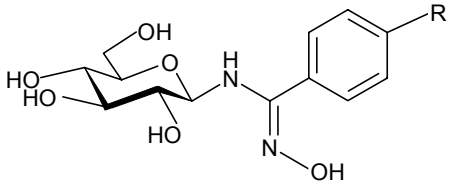
These findings can be explained by the formation of spiro-bicycle **143** which has been the target compound of these experiments. However, opening of the sugar ring in **143** along the *O,N*-acetal moiety is favoured by the formation of the aromatic 1,2,4-oxadiazole **145**, in which the secondary alcohol can be oxidized to **146** in the presence of NBS (**Scheme 26**.).



Scheme 26.

Structure of the new compounds was established by using NMR methods. The conformation of the sugar rings in amidoximes **136-139** were found to be $^4C_1$ from the vicinal proton-proton couplings. The anomeric configuration was deduced to be β-D from the couplings between H-1 and H-2 ($J_{1,2}$~9 Hz). The configuration of the C=N double bonds was not identified but it is indifferent for the cyclisations. The vicinal proton-proton couplings in the ^{1}H spectra of **145** and **146** were ~6 Hz indicating open chain sugars. In the ^{13}C spectra the chemical shift of C-5 is 30.20 ppm in the case of **145** and 197.01 ppm for **146**; these values correspond to the secondary alcohol and carbonyl group, respectively. The chemical shifts of C-1 of **145** and **146** are at 174.35 ppm and 174.30 ppm, respectively, as well as the chemical shifts of carbons N_2C-Ar are at 131.67 ppm (**145**) and 131.77 ppm (**146**) which also comfirm the aromatic structures. Compound **146** contains only five protons and from the splitting pattern of signals the lack of H-5 can be derived. The molecular weights of compounds **145** and **146** were determined by mass spectrometry: MALDI-TOF measurement was used for **145** and ESI for **146**. The matrix was dihydroxy benzoic acid (M=154.12), compound **145** had a molecular ion at 663.72 which corresponds to a **145**-martix complex and a molacular weight of 509.6 for **145** in accordance with the calculated value. In the case of **146** the molecular ion was observed at 545.9 which also contains a potassium ion, thus the molar weight of **146** was found to be 506.9 which is also identical with calculated value.

The deprotected amidoximes **147** and **148** were prepared by Zemplén's method and were examined as GP inhibitors (see Section 4.).



147 R = NO_2
148 R = CN

4. Biological and crystallographic investigations

Kinetic studies of the prepared compounds *vis a vis* GPs were carried out at the Department of Medical Chemistry of the University of Debrecen. The binding properties of the new molecules to the enzymes were determined by X-ray crystallograpy at the Institute of Biological Research and Biotechnology of the National Hellenic Research Foundation in Athens, Greece.

Muscle GP*b* was isolated from rabbit skeletal muscle[29] (see references therein) and were converted to GP*a* by phosphorylase kinase. For the crystallographic studies before data collection native T-state tetragonal RMGP*b* crystals were mounted in thin-walled glass capillaries and soaked with the inhibitors in a buffered solution.

The measured inhibitor constants (K_i) are summarized in **Table 5.** together with some reference compounds. The prepared compounds proved to be inhibitors of GP-s with low micromolar K_i values.

N-Acyl-*N*'-β-D-glucopyranosyl urea derivatives **118**-**126** efficiently inhibit GP-s. The *N*-acetylated compound **118** proved a weaker inhibitor than urea **14**. Electron density map of GP*b*-**118** complex defined the position of the inhibitor at the catalytic site (**Figure III.** see Appendix).[80]

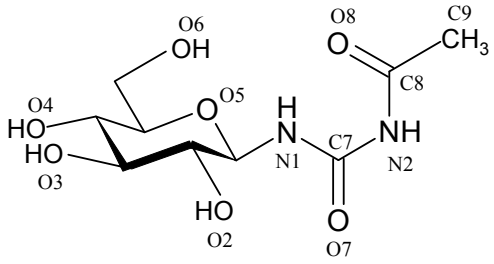
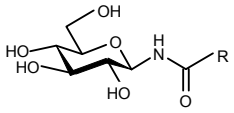
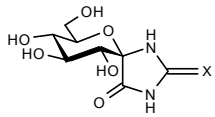
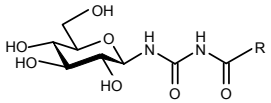
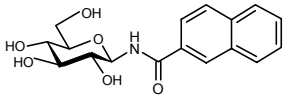
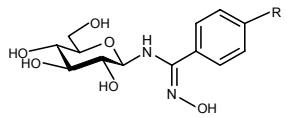


Figure 3. Numbering of **118** in the protein crystallographic results

As expected, the mode of binding of **118** to GP*b* is similar to that of **11** (1-GlcNAc) and its benzoylated derivative **12** (**Scheme 4.**) but not identical.

Table 5. K_i values of GP inhibitors

Entry	Structure	Ki (μM) RMGP*b*	RMGP*a*	Ref.
				
11	R = CH_3	32.0		22
12	R = C_6H_5	81.0		22
		144.0		24
14	R = NH_2	140.0		22
				
		3,1		25
20	X = O	4.2	26.0	24
21	X = S	5.1	10.9	24
				
118	R = CH_3	370.5		79, 80
119	R = C_6H_4	4.6	4.24	79, 80
120	R = 4-NO_2-C_6H_4	3.0	12.6	
121	R = 3-Cl-C_6H_4	113.6	~10% inhibition*	
122	R = 4-OH-C_6H_4	3.62	18.41	
123	R = 1-naphthyl	7.89	119	79
124	R = 2-naphthyl	0.47	2.64	79
125	R = 2-indolyl	8.02		
126	R = 4-NH_2-C_6H_4	IC_{50} = 14		
130	R = NHCO-2-naphthyl	~45% inhibition*		79
				
128		9.7	13.2	79
				
147	R = NO_2	no inhibition		
148	R = CN	1.8 mM		

* in 625 μM concentration

In complexes of latter analogues there is a hydrogen bond between the amide N atom and the main chain His377. In the GP*b*-**118** complex this hydrogen bond is not maintained because N1 is too far away from the main chain O of His377 (3.9 Å). However, both the carbonyl O7 and N2 contact Asp283 through a water molecule as well as O7 and carbonyl O8 also form other hydrogen bonds to the enzyme. There is also a possible intramolecular hydrogen bond from N1 to O8 (2.5 Å). One water molecule was displaced by the methyl group (C9), in addition to those displaced by the glucopyranose. The structural results show that **118** can be accommodated at the catalytic site with no significant disturbance of the structure (**Figure IV.**) and by hydrogen bonds **118** stabilizes the geometry of the 280s loop.

There are no changes at the allosteric effector site, the new allosteric site and the inhibitor site. The bound **118** adopts a rather unfavourable conformation, formed by the intramolecular contact between its N1 and O8. Computer aided calculations for the conformation energy minimum of **118** resulted in an extended conformation, different from the bound structure. In the bound acetyl urea structure, the torsion angle O7-C7-N2-C8 is 178.5°, so that the conformation about the C7-N2 bond is significantly different from that in the computed structure (0.2°) of **118**.

N-Benzoyl-*N*'-β-D-glucopyranosyl urea **119** binds also at the catalytic site with the urea and benzoyl moieties filling the empty space of the β-pocket, a side channel from the catalytic site that leads toward residue His341 (**Figure V.**, **VI.**).

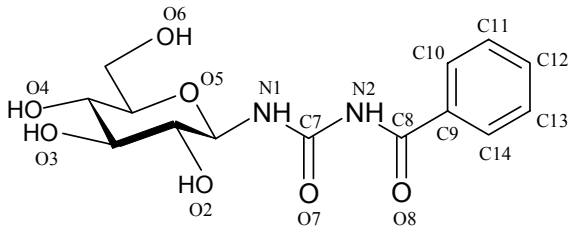


Figure 4. Numbering of benzoyl urea **119** in the crystallographic results

There are altogether 21 hydrogen bonds and 96 van der Waals interactions in the GP*b*-**119** complex. There is no hydrogen bond between the amide N1 and His377 in the **119** complex as in the case of the **118** complex. N1 and N2 are hydrogen bonded to Asp339 through two water molecules which is also hydrogen bonded to His341 of the β-pocket. The carbonyl O7 also take part in several hydrogen bonds.

There are negligible changes in the overall conformation on binding **119** to GP*b* but there is a dramatic shift in the 280s loop and some differences in the water structure at the catalytic site. The superimposition of the structures of the native T state GP*b* and the GP*b*-**119** complex are shown in **Figure VII.** The benzoyl moiety exploits a number of van der Waals contacts in the β-pocket, these contacts are dominated by polar-polar and polar-nonpolar contacts with Asn282 and Asp283. The strong affinity of **119** for GP*b* can be interpreted in terms of its extensive interactions with the protein.[80]

In **Figure VIII.** the binding of 1-GlcNAc **11**, *N*-benzoyl-β-D-glucopyranosylamine **12**, acetyl urea **118** and benzoyl urea **119** are compared within the catalytic site of GP*b*. The positions of the glucosyl components of each of the structures are similar indicating that the glucosyl recognition site does not change substantially. The largest difference is in their N1 positions, a distance that reflects the presence (2.9 Å in **11**, 3.2 Å in **12**) or absence (3.9 Å in **118** and 4.2 Å in **119**) of a hydrogen bond between N1 and the main chain O of His377 in these derivatives.

Benzoyl urea **119** is the first reported glucose analogue molecule which occupies not only the active site but also the new allosteric inhibitor site of GP.[80] The conformation of the **119** at this site is not identical with that described above for the active site. In the benzoyl urea structure the torsion angle O7-C7-N2-C8 (for numbering see **Figure 4.**) is -179.3°, so that the conformation about the C7-N2 bond is in *trans* geometry, significantly different from that in the catalytic site

(0.2°) of **119** (**Figure IX.** Appendix). In addition, the phenyl ring is coplanar to the urea moiety, whereas at the catalytic site the plane of the phenyl ring is inclined ~30° to the plane of the urea moiety. **119**, on binding at the new allosteric inhibitor site of GP*b,* makes a total of nine hydrogen bonds and exploits 80 van der Waals interactions and there are 21 contacts with the symmetry related subunit (**Figure X.**). The urea moiety is located between the side chains of Arg60 and Lys191 and exploits 14 van der Waals contacts with the enzyme. The O2 and O3 of the glucopyranose moiety are in hydrogen bonds with their symmetry related pairs in the central cavity of the enzyme.

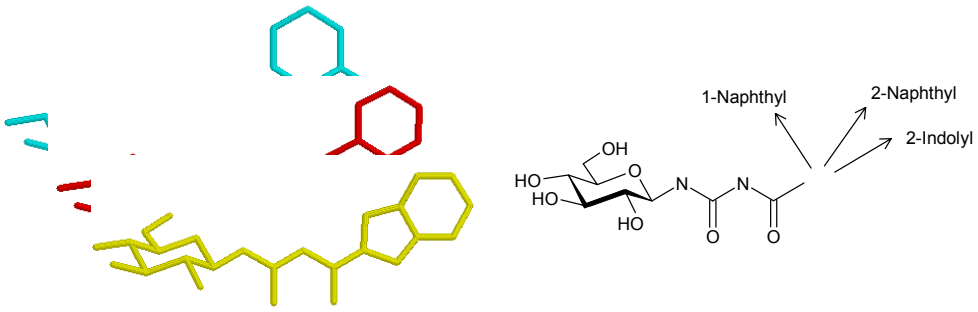
Substituting the aromatic ring of the benzoyl urea moiety with polar groups in para position (**120**, **122**, **126**) brings about no significant change in the K_i values. Their binding properties are similar to the benzoyl urea **119**, they were observed at the active site of GP*b* and the 4-nitro derivative **120** binds also to the new allosteric site. **Figure XI.** shows the comparison of benzoyl- **119**, 4-aminobenzoyl- **126** and 2-naphthoyl urea **124** derivatives at the active site.

The *N*-2-naphthoyl-*N*'-glucopyranosyl urea **124** is to date the best glucose analogue inhibitor of GP*b* with its nanomolar K_i value, and it also binds to the new allosteric site of GP*b* in addition to the active centre. **124** binds to the active site in a similar way as benzoyl urea **119** does (**Figure XII.**) but at the new allosteric site it takes an other conformation (**Figure XIII**.). The O5 and O7 are in hydrogen bonds through a water molecule, O8 also hydrogen bonds through a water with the enzyme. In addition there is a hydrogen bond from O8 to the O6 of the symmetry related inhibitor molecule but no contact between the two sugar moieties.

We prepared with one NHCO moiety "shorter" and "longer" analogues of **124** to examine the effect of the position of the naphthyl group on the binding properties. The *N*-2-naphthoyl-glucopyranosylamine **128** proved also to be a rather active inhibitor (**Table 5.**) but placing the naphthyl group closer to the sugar makes the binding weaker by one order of magnitude. This distance might have an optimum which was confirmed by the practical inactivity of the longer 2-naphthoyl

biuret derivative **130**. Similar tendency could be obtained in the case of the benzoylated series: *N*-glucopyranosyl-benzamide **12** was also a weaker inhibitor than the urea derivative **137**. Structures of the bound **119**, **124** and **128** at the catalytic site of GP*b* are shown in **Figure XIV**. Molecules **119** and **124** superimpose well, there is a very slight deflection in the torsion angles around the bond C8-C9 while the naphthylamide **128** occupies a slightly different position. **128** binds also at the new allosteric site and its binding is similar to that of the benzoyl urea **119**. **Figure XV.** demonstrates that both the aromatic and the sugar moieties fit well but their conformations differ significantly from that of naphthoyl urea **124**.

Comparing the positions of the aromatic groups of urea derivatives **123-125** bound at the catalytic site of GP, each of them determines a direction with respect to the glucopyranosyl urea moiety (**Scheme 27.**). From the kinetic and crystallographic results it seems that the 2-naphthyl moiety fits in the best way into the β-pocket of the enzyme, consequently further modifications of the basic structure are worthy to be considered into the direction determined by **124**.



Scheme 27. *N*-1-naphthoyl- **123** (cyan), *N*-2-naphthoyl- **124** (red) and *N*-indol-2-carbonyl-*N*'-glucopyranosyl urea **125** (yellow) bound at the active site of GP.

More extended investigations for the details on binding of the new inhibitor molecules to the protein are in progress. In summary, with the preparation of *N*-

acyl-*N*'-β-D-glucopyranosyl ureas new lead structures have been discovered for the inhibition of GP-s.

The gram scale availability of glucopyranosylidene-spiro-thiohydantoin facilitated extended *in vitro* and *in vivo* investigations with **21**. Results of preliminary experiments are shown in **Figure 5.**[76] **Figure 5/A** illustrates the effect of D-glucose and spiro-thiohydantoin **21** on the dephosphorylation of GP*a* in gel-filtered extracts from rat liver catalyzed by phosphorylase phosphatase. The filtrates were supplemented with 5 mM ammonium hydrogensulphate and 1 mM magnesium acetate (•) in the presence of 5 mM D-glucose (■) or 100 μM **21** (▲). The filtrates were incubated at 30°C and samples were withdrawn at the indicated times. The results are mean values ± S.D. of four independent experiments. Apparently both ligands can enhance the dephosphorylation (inactivation) of hepatic Gp*a*; however, **21** applied in a much lower concentration seems to be more effective.

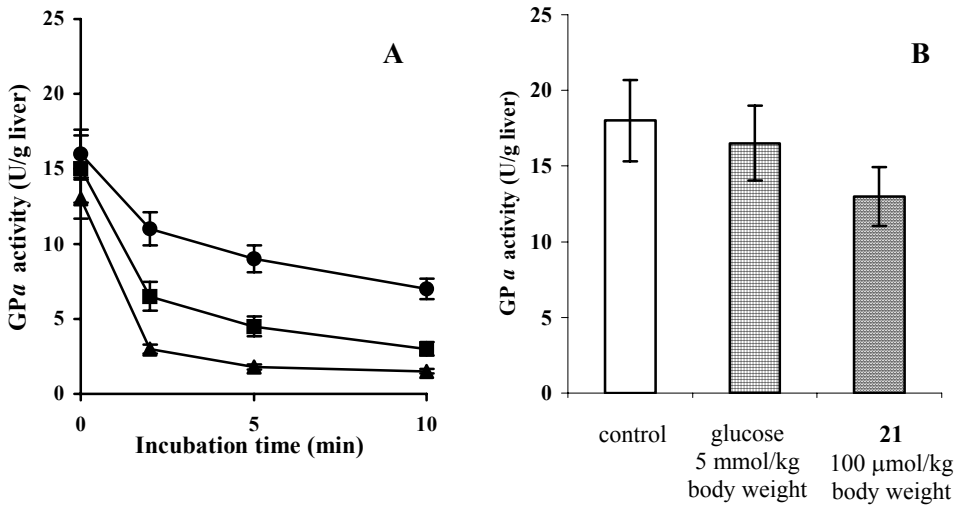


Figure 5. Effect of D-glucose and **21** on the activity of hepatic GP*a in vitro* (**A**) and *in vivo* (**B**)

Figure 5/B demonstrates the effect of intravenous administration of either D-glucose or **21** on the activity of GP*a* in rat liver. D-Glucose or **21** in a dose of 5 mmol or 100 μmol per body weight kg, respectively, was injected into the portal vein of Wistar rats. Liver samples were taken before (control) and 5 min after the administration of D-glucose or **21**. Results are means ± S.D. for four independent experiments. It can be seen that **21** significantly decreases the active form of GP*a* in liver. These results strongly support the expectations that glucose analogue inhibitors of GP-s can be useful as therapeutic agents for the treatment of type II diabetes.

5. Experimental

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter at room temperature. NMR spectra were recorded with Bruker WP 200 SY (200/50 MHz for $^{1}H/^{13}C$), Bruker AM 360 (360/90 MHz for $^{1}H/^{13}C$) or Bruker AM 400 (400/100 MHz for $^{1}H/^{13}C$) spectrometers. Chemical shifts are referenced to Me_4Si (^{1}H), or to the residual solvent signals (^{13}C). In some cases the protons of the sugar ring were not assigned. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (Merck) (eluent EtOAc:hexane 1:2, unless stated otherwise), and the spots were visualized under UV light and by gentle heating. For column chromatography Kieselgel 60 (Merck, particle size 0.063-0.200 mm) was used. Organic solutions were dried over anhydrous $MgSO_4$ and concentrated in vacuo at 40-50ºC (bath temperature). Nitromethane, acetonitrile and carbon tetrachloride were distilled from P_4O_{10} and stored over molecular sieves (3 Å). Dry methanol was distilled from magnesium methylate. Other solvents were of commercial analytical grade quality and have been used without further purification.

5.1. Preparation of glucopyranosylidene-spiro-thiohydantoin **21**

*2,3,4,6-Tetra-O-benzoyl-β- D-glucopyranosyl cyanide **105***

Benzobromo-glucose[65] **104** (9.89 g, 15 mmol) was dissolved in dry nitromethane (40 ml) and mercury(II) cyanide (3.79 g, 15 mmol, dried at 100ºC in vacuo for 20 h) was added. The mixture was stirred at rt for 2 days. The solids were then filtered off, washed with nitromethane, and the solvent was removed from the combined filtrate and washings. The residue was dissolved in chloroform, the solution filtered if necessary and washed with 1 M aq. KBr solution (2x). After drying the solvent was removed and the remaining syrup was crystallized from diethyl ether to give 5.09 g (56%) of **105**. Chromatographic purification (eluent EtOAc:hexane 1:5) of a 0.41 g crop of the material (4.06 g) obtained after evaporating the solvent from the mother liquor gave 0.18 g (corresponding to another 19%) of **105**. Mp 114-116ºC; $[\alpha]_D$ +52 (c=1.06, $CHCl_3$); ^{1}H NMR (CDCl3) δ (ppm) 8.1-7.7, 7.6-7.2 (m, 20H, 4xC_6H_5), 5.9-5.8, 5.73-5.67 (strongly coupled signals, 3H, H-2,3,4), 4.67 (d, 1H, H-1, $J_{1,2}$=9 Hz), 4.65 (dd, 1H, H-6, $J_{6,6'}$=12.4 Hz), 4.47 (dd, 1H, H-6', $J_{5,6'}$=5.4 Hz), 4.20 (ddd, 1H, H-5, $J_{4,5}$=10.1 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 166.25, 165.98, 165.22, 164.81 (*C*=O), 114.52 (*C*N), 77.45, 73.25, 69.83, 68.72, 67.11 (C-1–C-5), 62.76 (C-6). Anal. calcd for $C_{35}H_{27}NO_9$ (605.60): C 69.41, H 4.49, N 2.31. Found: C 69.05, H 4.47, N 2.11. Two other minor components of the mother liquor were identified on the basis of their ^{1}H NMR spectra as 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl cyanide[66] and 2,3,4,6-tetra-*O*-benzoyl-α-D-gluco-pyranose.[67]

*2,3,4,6-Tetra-O-benzoyl-1-bromo-1-deoxy-β-D-glucopyranosyl cyanide **106***

(a) *With bromine:* Glucosyl cyanide **105** (400 mg, 0.66 mmol) was dissolved in carbon tetrachloride (10 ml) and bromine (0.14 ml, 2.64 mmol) and some $BaCO_3$ were added. The mixture was placed in an Erlenmeyer flask above a heat lamp (375 W, white, distance from the lamp ~1±2 cm, height of the solution 1-1.5 cm)

and refluxed until TLC showed complete transformation (~2.5 h). It was then filtered, and the filtrate washed with 5% aq. $NaHSO_3$ and satd aq. $NaHCO_3$ solutions. Drying and evaporation of the solvent gave 431 mg (95%) of **106** as white crystals from which an analytical sample was obtained by recrystallization from ethanol. Mp 152-154ºC; $[\alpha]_D$ +119 (c=0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 8.13-7.23 (m, 20H, 4xC_6H_5), 6.11 (t, 1H, H-3, $J_{3,4}$=9.6 Hz), 5.84 (t, 1H, H-4, $J_{4,5}$=9.6 Hz), 5.82 (d, 1H, H-2, $J_{2,3}$=9.6 Hz), 4.72 (ddd, 1H, H-5, $J_{5,6}$=2.2 Hz), 4.69 (dd, 1H, H-6, $J_{6,6'}$=12.7 Hz), 4.56 (dd, 1H, H-6', $J_{5,6'}$=4.7 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 167.20, 165.80, 165.33, 165.10 (*C*=O), 113.90 (*C*N, $J_{H\text{-}2,CN}$=2.1 Hz), 81.26 (C-1), 75.16, 72.22, 70.59, 67.99 (C-2–C-5), 61.82 (C-6). Anal. calcd for $C_{35}H_{26}BrNO_9$ (684.49): C 61.41, H 3.82, N 2.04. Found: C 61.42, H 3.62, N 1.97.

The reactions in $CHCl_3$ and CH_2Cl_2 were performed similarly except that instead of $BaCO_3$ K_2CO_3 was added as the acid scavenger and at the biginning of the work up filtration was no longer needed. In the $CHCl_3$ reaction additional portions of Br_2 had to be added to the mixture (see also the procedure for the preparation of **108**). Work-up was effected by adding 5% aq. $NaHSO_3$ solution to the cooled reaction mixture. After shaking and separating the phases the organic solution was washed with water, dried, and the solvent removed to give the crystalline crude product which was recrystallized from ethanol. Yields are indicated in **Table 1**.

(b) *With N-bromosuccinimide:* Glucosyl cyanide **105** (6.75 g, 11.15 mmol) was dissolved in carbon tetrachloride (140 ml); bromotrichloromethane (60 ml), *N*-bromosuccinimide (4.96 g, 27.86 mmol) and some benzoyl-peroxide were added. The mixture was heated under reflux for 2 h, during which time the product precipitated as a white solid. After cooling to rt dichloromethane was added to the mixture until dissolution of all of the solids, and the solution was washed with 5% aq. Na_2SO_3 (2x), satd aq. $NaHCO_3$, and water. Drying and evaporation of the solvents left a solid which was suspended in a small amount of ethanol, boiled

under reflux for several minutes and the warm suspension was filtered to give a crystalline substance. Recrystallization from ethyl acetate gave **106** (4.78 g, 62%).

*C-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)formamide **107***

(a) *With HBr/AcOH:* Glucosyl cyanide **105** (13.1 g, 20 mmol) was suspended in a solution of HBr in acetic acid (20 ml, 20% m/m) and the mixture was stirred at rt for 3 h. The resulting solution was poured into ice-water (200 ml), which was then extracted with chloroform (2x200 ml). The unified $CHCl_3$ phases were washed with satd aq. $NaHCO_3$ (2x100 ml), then with water (100 ml), dried, and the solvent removed. The crystalline residue **107** (11.7 g, 94%) was sufficiently pure for the further step. An analytical sample was obtained by dissolving the crude material in chloroform and precipitating it with diethyl ether. Mp 226-228ºC; $[\alpha]_D$ +26 (c=0.98, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.15-7.8, 7.6-7.2 (m, 20H, 4xC_6H_5), 6.52 (br s, 1H, CONH_2), 5.96 (pseudo t, 1H, H-3, $J_{2,3}$~$J_{3,4}$~9.2 Hz), 5.8-5.6 (m, 3H, H-2,4, CONH_2), 4.71 (dd, 1H, H-6, $J_{5,6}$=2.2 Hz), 4.51 (dd, 1H, H-6', $J_{6,6'}$=12.8 Hz), 4.26 (d, 1H, H-1, $J_{1,2}$=10.1 Hz), 4.20 (ddd, 1H, H-5, $J_{5,6'}$=4.8 Hz); ^{13}C NMR ($CDCl_3$) δ 169.40 (*C*ONH_2), 166.04, 165.80, 165.57, 165.50 (*C*=O), 76.54, 76.43, 73.80, 70.32, 69.31 (C-1–C-5), 63.02 (C-6). Anal. calcd for $C_{35}H_{29}NO_{10}$ (623.62): C 67.41, H 4.69, N 2.25. Found: C 67.14, H 4.69, N 2.49.

(b) *With $TiCl_4$:* Glucosyl cyanide **105** (9.94 g, 16 mmol) was suspended in acetic acid (16 ml), the suspension was cooled to 0ºC in an ice-bath, and $TiCl_4$ (3.58 g, 32 mmol) followed by water (0.0295 ml, 16 mmol) was added. The mixture turned yellow and was stirred at rt until TLC had indicated disappearance of the starting material (3-4 days). It was then diluted with ice-water (100 ml) and extracted with chloroform (3x80 ml). The combined chloroform phases were washed with ice-cold satd aq. $NaHCO_3$ until neutral, then with water, dried and the solvent evaporated. The residue was crystallized from diethyl ether to give 6.2 g (53%) of **107**.

*C-(2,3,4,6-Tetra-O-benzoyl-1-bromo-1-deoxy-β-D-glucopyranosyl) formamide **108***

(a) By photobromination of **107**: *C*-Glucosyl formamide **107** (200 mg, 0.32 mmol) was dissolved in chloroform (6 ml), bromine (0.07 ml, 1.28 mmol) and some $BaCO_3$ were added, and the mixture was irradiated and refluxed by a heat lamp as described in procedure (a) for **105**. After 1 h the mixture decolorized and 0.1 ml Br_2 was added again. This was repeated after another 0.5 h. After TLC had shown complete transformation (~2 h from the start) the mixture was filtered, washed with 5% aq. $NaHSO_3$ and satd aq. $NaHCO_3$ solutions, dried, and the solvent removed. The residual syrup (264 mg) crystallized on addition of diethyl ether to give 201 mg (89%) of **108**. Mp 170-173ºC; $[\alpha]_D$ +101 (c=1.02, CH_3OH); ^1H NMR ($CDCl_3$) δ (ppm) 8.15-7.74, 7.68-7.21 (m, 20 H, $4xC_6H_5$), 6.63 (br s, 1H, CONH_2), 6.14 (t, 1H, H-3, $J_{3,4}$=9.5 Hz), 5.81 (t, 1H, H-4, $J_{4,5}$=9.5 Hz), 5.76 (d, 1H, H-2, $J_{2,3}$=9.5 Hz), 5.44 (br s, 1H, CONH_2), 4.85 (dd, 1H, H-6, $J_{5,6}$=2.2 Hz), 4.72 (ddd, 1H, H-5, $J_{5,6'}$=4.2 Hz), 4.52 (dd, 1H, H-60, $J_{6,6'}$=12.4 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 167.31 (CONH_2, $J_{H\text{-}2,CONH2}$ <1 Hz), 166.30, 165.41, 164.89, 164.70 (C=O), 93.19 (C-1), 74.74, 71.99, 70.21, 67.62 (C-2–C-5), 61.80 (C-6). Anal. calcd for $C_{35}H_{28}BrNO_{10}$ (702.52): C 59.84, H 4.02, N 1.99. Found: C 60.15, H 4.08, N 2.23. The photobrominations in other solvents (**Table 1**.) were performed similarly. In the CH_2Cl_2 reaction no further addition of Br_2 was necessary.

(b) *By partial hydrolysis of **106** with $TiCl_4$:* Prepared from bromo-cyanide **106** (7.7 g, 11.31 mmol) as described for the preparation of amide **107** to give 4.28 g (54%) of **108**.

(c) *By partial hydrolysis of **106** with HBr/AcOH:* Prepared from bromo-cyanide **106** (0.5 g, 0.73 mmol) as described for the preparation of amide **107** to give 425 mg (83%) of **108** after crystallization from diethyl ether.

*(2R,3R,4S,5R,6S)3,4,5-Tribenzoyloxy-2-benzoyloxymethyl-7,9-diaza-1-oxa-spiro [4,5]decane-10-one-8-thione **109***

C-(1-Bromoglucosyl)formamide **108** (4 g, 5.69 mmol) was dissolved in dry nitromethane (23 ml). Molecular sieves (3 Å), ammonium thiocyanate (1.732 g, 22.79 mmol) and elemental sulfur (18 mg, 0.56 mmol) were added, and the mixture was stirred in an 80ºC bath under N_2 atmosphere for 7 h. The syrupy residue obtained after filtration and solvent removal was dissolved in dichloromethane, the solution filtered, washed with satd aq. NH_4Cl solution, dried, and concentrated. The remaining syrup was separated by silica gel column chromatography with EtOAc:hexane 2:5 eluent. The first fraction which crystallized from methanol gave 3.09 g (79%) of thiohydantoin **109**. Mp 199-202ºC; $[\alpha]_D$ +26 (c=1.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 9.08 (br s, 1H, N*H*), 8.09-7.13 (m, 21H, 4xC_6H_5, N*H*), 6.67 (t, 1H, H-4, $J_{3,4}$=10 Hz), 5.95 (d, 1H, H-5, $J_{4,5}$=9.7 Hz), 5.91 (pseudo t, 1H, H-3, $J_{2,3}$=10 Hz), 5.21 (ddd, 1H, H-2, $J_{2,CH2a}$=2.5 Hz), 4.75 (dd, 1H, C*H*2a, J_{CH2}=12.6 Hz), 4.44 (dd, 1 H, C*H*2b, $J_{2,CH2b}$=4.0 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 181.93 (C-8), 169.90 (C-10, $J_{H\text{-}5,C\text{-}10}$=6.6 Hz), 165.73, 165.60, 165.51 (*C*=O), 87.75 (C-6), 71.69, 70.90, 70.41, 68.91 (C-2–C-5), 62.84 (CH_2). Anal. calcd for $C_{36}H_{28}N_2O_{10}S$ (680.68): C 63.52, H 4.15, N 4.12. Found: C 63.50, H 4.16, N 4.24. The second fraction (227 mg) contained three substances (monitored in diethyl ether:hexane 1:1) which were not characterized. The third fraction gave 231 mg (6%) of hydroxy-amide **110**.

*C-(2,3,4,6-Tetra-O-benzoyl-1-hydroxy-β-D-glucopyranosyl)formamide **110***

Bromo-amide **108** (200 mg, 0.285 mmol) was dissolved in DMSO (12 ml); silver oxide (66 mg, 0.285 mmol) and water (5.12 ml, 0.285 mmol) were added. The mixture was stirred at rt in the dark for 3 h. It was than filtered and the filtrate diluted with water. The precipitate was filtered off, dissolved in dichloromethane, and the solution was washed with 10% aq. NH_4SCN solution. After drying and

solvent removal the residue was recrystallized from ethanol to give 119 mg (65%) of **110**. Mp 255-258ºC; $[\alpha]_D$ +48 (c=0.89, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 8.15-7.15 (m, 20H, 4xC_6H_5), 6.51 (br s, 1H, CONH_2), 6.22 (pseudo t, 1H, H-3, $J_{3,4}$=9.6 Hz), 5.81 (pseudo t, 1H, H-4, $J_{4,5}$=9.8 Hz), 5.67 (d, 1H, H-2, $J_{2,3}$=9.9 Hz), 5.63 (br s, 1H, CONH_2), 5.23 (br s, 1H, O*H*), 4.70 (ddd, 1H, H-5, $J_{5,6}$=2.8 Hz), 4.65 (dd, 1H, H-6, $J_{6,6'}$=12.5 Hz), 4.48 (dd, 1H, H-6', $J_{5,6'}$=4.8 Hz); ^{13}C NMR (DMSO-d_6) δ (ppm) 168.88 *C*ONH_2, ($J_{H\text{-}2,CONH2} \leq 1$ Hz), 165.49, 165.16, 164.81, 164.65 (*C*=O), 94.47 (C-1), 72.15, 71.64, 69.13, 68.70 (C-2–C-5), 62.96 (C-6). Anal. calcd for $C_{35}H_{29}NO_{11}$ (639.62): C 65.72, H 4.57, N 2.19. Found: C 65.53, H 4.71, N 2.31.

(2R,3S,4S,5R,6S)3,4,5-Trihydroxy-2-hydroxymethyl-7,9-diaza-1-oxa-spiro[4,5] decane-10-one-8-thione ***21***

Thiohydantoin **109** (3.09 g, 4.53 mmol) was dissolved in absolute methanol (20 ml) and 1 M methanolic sodium methoxide solution (1.5 ml) was added. The reaction mixture was refluxed for 2 h, cooled to rt and then neutralized with a cation exchange resin Amberlyst 15 (H^+ form). Filtration and solvent removal left a syrup which solidified to an amorphous product on addition of diethyl ether. This material was filtered off and washed several times with hexane to remove traces of methyl-benzoate. The product was crystallised from water to give 1.1 g (92%) of **21** which proved identical to the reported compound. Mp 144-146ºC $(\alpha)_D$ +19 (c=1.007, CH_3OH) (lit.[29] $[\alpha]_D$ +19 (c=2.34, CH_3OH)); ^{1}H NMR (D_2O) δ (ppm) 4.23 (m unres., 1H, H-2), 4.13 (t, 1H, H-4, $J_{3,4}$~$J_{4,5}$~9.5 Hz), 3.87-3.62 (m, 3H, H-5, CH_2), 3.49 (t, 1H, H-3, $J_{2,3}$~9.5 Hz); ^{13}C NMR (D_2O) δ (ppm) 187.16 (C-8), 175.97 (C-10), 91.81 (C-6), 77.63, 74.83, 74.68, 71.37 (C-2–C-5), 63.14 (CH_2).

5.2. Preparation of *N*-acyl-*N*'-β-D-glucopyranosyl ureas

General methods for the preparation of N-*acyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-glucopyranosyl) ureas*

***Method A**:* To a solution of an acyl chloride (18 mmol) in 20 ml of dry chloroform anhydrous zinc chloride (80 mg, 0.59 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl urea **91** (1 g, 2.56 mmol) were added with stirring. The reaction mixture was refluxed until TLC showed the complete transformation of **91**. Then the reaction mixture was poured into ice-water and was extracted with chloroform (2x). The organic phases were collected and washed with satd. aq. $NaHCO_3$ solution and water. After drying the solvent was evaporated under vacuo and the residue was purified by column chromatography (eluent: EtOAc: hexane = 1:2).

Method B: To a suspension of NaOCN (1.21 g, 18.7 mmol) in dry acetonitrile an acyl chloride (14.4 mmol) in acetonitrile (30 ml) and $SnCl_4$ (84 µl, 0.72 mmol) were added under argon with stirring. The mixture was stirred under reflux for 8 h and after cooling to rt 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl amine **111** (1 g, 2.88 mmol) was added under argon. After strirring for 30 min some drops of water were added and the mixture was filtered. The solvent was evaporated and the residue purified by column chromatography (eluent: EtOAc:hexane = 1:1).

N-*4-nitrobenzoyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea **112***

(a) *With **Method A:*** Reaction time: 6h. Starting from 1 g of urea **91** the yield was 840 mg (60%), mp 113-116ºC $[\alpha]_D$ +51.3 (c=1.100, $CHCl_3$); ^{1}H NMR (DMSO, 360 MHz) δ (ppm) 11.93 (s, 1H, N*H*), 9.59 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=9.2 Hz), 5.67 (t, 1H, *J*=9.2 Hz), 5.44 (t, 1H, *J*=9.2 Hz), 5.08 (t, 1H, *J*=9.2 Hz), 4.94 (t, 1H, *J*=9.6 Hz), 4.2-4.1 (m unres., 2H, H-6, H-5), 4.01 (d, 1H, H-6', *J*=10.5 Hz), 2.00, 1.99, 1.95, 1.90 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm) 169.87, 169.43, 169.22, 169.04 (4x acetyl *C*O), 166.47 (NH*C*OAr), 149.55 (NH*C*ONH), 139.20, 129.95, 128.99, 123.50 (Ar), 77.58 (C-1), 72.71, 72.22, 70.68, 67.67 (C-2–C-5), 61.58 (C-6), 20.40, 20.27, 20.21, 20.16 (4x acetyl CH_3). Anal. calcd for $C_{22}H_{25}N_3O_{13}$ (539.46): C 48.98, H 4.67, N 7.79. Found: C 49.03, H 4.71, N 7.31.

(b) *With* ***Method B:*** Starting from 250 mg **111** yield: 255 mg (65%).

N-*3-chlorobenzoyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea* ***113***

Prepared from glucopyranosylamine **111** (50 mg, 0.144 mmol) as described in ***Method B*** to give 56.7 mg (74%) of **113**. Mp 155-158ºC $[\alpha]_D$ -1.1 (c=1.000, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 10.05 (s, 1H, N*H*), 8.64 (s, 1H, N*H*), 8.1-7.3 (m, 4H, Ar), 5.42 (d, 1H, H-1, $J_{1,2}$=9.3 Hz), 5.40 (t, 1H, *J*=9.4 Hz) 5.11 (t, 1H, *J*=9.9 Hz) 5.07 (t, 1H, *J*=9.6 Hz) (H-2–H-4), 4.34 (dd, 1H, H-6, $J_{5,6}$=4.3 Hz), 4.10 (dd, 1H, H-6', $J_{6,6'}$=12.5 Hz), 3.92 (ddd, 1H, H-5, $J_{5,6'}$=2.1 Hz), 2.07, 2.05 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 170.69, 169.94, 169.67, 166.67 (4x acetyl *C*O), 165.99 (NH*C*OAr), 156.10 (NH*C*ONH), 135.09, 134.62, 130.22, 130.08, 128.24, 127.90 (Ar), 78.96 (C-1), 73.72, 72.64, 70.92, 68.25 (C-2–C-5), 61.69 (C-6), 20.75, 20.63 (4x acetyl CH_3). Anal. calcd for $C_{22}H_{25}N_2O_{11}Cl$ (528.90): C 49.96, H 4.76, N 5.30. Found: C 49.58, H 4.86, N 4.35.

N-*4-acetoxybenzoyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea* ***114***

(a) *With* ***Method A:*** Reaction time: 1 day. From 500 mg of **91** the yield was 510 mg (72%) mp 184-186ºC $[\alpha]_D$ -16.6 (c=1.090, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 10.34 (s, 1H, N*H*), 9.97 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=7.8 Hz), 8.13 (d, 2H, Ar, *J*=8.9 Hz), 7.28 (d, 2H, Ar, *J*=8.9 Hz), 6.00 (dd, 1H, H-1, $J_{1,2}$=5.2 Hz), 5.40 (t, 1H, H-3, $J_{3,4}$=10.1 Hz), 5.19 (dd, 1H, H-2, $J_{2,3}$=10.4 Hz), 5.15 (t, 1H, H-4, $J_{4,5}$=9.7 Hz), 4.32 (dd, 1H, H-6, $J_{5,6}$=4.1 Hz), 4.04 (dd, 1H, H-6', $J_{6,6'}$=12.4 Hz), 3.97 (ddd, 1H, H-5, $J_{5,6'}$=3.2 Hz), 2.34, 2.05, 2.04 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 169.79, 169.52, 169.45, 168.56 (4x acetyl *C*O), 167.80 (NH*C*OAr), 154.77 (NH*C*ONH), 129.81, 128.71, 122.16 (Ar), 75.17 (C-1), 70.17, 68.73, 68.43, 68.04 (C-2–C-5), 60.30 (C-6), 21.07, 20.57, 20.51 (4x acetyl CH_3). Anal. calcd for $C_{24}H_{28}N_2O_{13}$ (552.50): C 52.18, H 5.11, N 5.07. Found: C 51.90, H 5.26, N 4.72.

(b) *With* ***Method B:*** Starting from 50 mg glucopyranosylamine **111** the yield: 61 mg (20%).

N-*1-naphthoyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea* **115**

Prepared from glucopyranosyl urea **91** (200 mg, 0.512 mmol) by ***Method A***. Reaction time: 1.5 h. Yield: 312 mg (73%) mp 186-188ºC $[\alpha]_D$ -32.6 (c=1.150, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.38 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=8.26 Hz), 8.96 (s, 1H, N*H*), 8.35 (d, 1H, Ar, *J*=8.0 Hz), 8.02 (d, 1H, Ar, *J*=8.0 Hz), 7.89 (d, 1H, Ar, *J*=7.5 Hz), 7.74 (d, 1H, Ar, *J*=6.0 Hz), 7.60-7.46 (m, unres., 3H, Ar), 5.31 (t, 1H, H-1, $J_{1,2}$=9.3 Hz), 5.23 (t, 1H, *J*=9.5 Hz) 5.15 (t, 1H, *J*=9.5 Hz) 5.13 (t, 1H, *J*=9.3 Hz) (H-2–H-4), 4.26 (t, 1H, H-6, $J_{5,6}$=3.3 Hz), 4.06 (d, 1H, H-6', $J_{6,6'}$=12.3 Hz), 3.76 (m unres., 1H, H-5), 2.06, 2.04, 2.02 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 170.62, 170.06, 169.74, 169.60 (4x acetyl *C*O), 169.38 (NH*C*OAr), 153.72 (NH*C*ONH), 133.68, 132.90, 130.68, 129.89, 128.52, 127.93, 126.84, 126.49, 124.96, 124.24 (Ar), 79.04 (C-1), 73.52, 72.97, 70.10, 68.00 (C-2–C-5), 61.55 (C-6), 20.67, 20.56 (4x acetyl CH_3). Anal. calcd for $C_{26}H_{28}N_2O_{11}$ (544.52): C 57.35, H 5.18, N 5.14. Found: C 57.37, H 5.34, N 5.05.

N-*2-naphthoyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea* **116**

(a) *With* ***Method A:*** Starting from 800 mg of urea **91**. Reaction time: 1 day. Yield: 334 mg (29%). Mp 184-185ºC $[\alpha]_D$ -64.4 (c=1.200, $CHCl_3$); ^1H NMR ($CDCl_3$+DMSO-d_6, 360 MHz) δ (ppm) 10.23 (s, 1H, N*H*), 9.65 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=8.5 Hz), 8.57 (s, 1H, Ar), 8.10-7.6 (m unres., 6H, Ar), 5.34 (t, 1H, H-1, $J_{1,2}$=9.1 Hz), 5.33 (t, 1H, *J*=9.6 Hz) 5.16 (t, 1H, *J*=9.7 Hz) 5.09 (t, 1H, *J*=9.5 Hz) (H-2–H-4), 4.17 (dd, 1H, H-6, $J_{5,6}$=3.7 Hz), 3.95 (d, 1H, H-6', $J_{6,6'}$=12.6 Hz), 3.64 (m unres., 1H, H-5), 2.03, 2.00 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 90 MHz) δ (ppm) 170.54, 170.03, 169.74, 169.31 (4x acetyl *C*O), 168.11 (NH*C*OAr), 155.04 (NH*C*ONH), 135.47, 132.24, 129.47, 129.25, 128.83, 128.69, 128.56, 127.76, 127.04, 123.79 (Ar), 78.94 (C-1), 73.46, 72.95, 70.14, 67.99 (C-2–C-5), 61.47 (C-6), 20.53, 20.45 (4x acetyl CH_3). Anal. calcd for $C_{26}H_{28}N_2O_{11}$ (544.52): C 57.35, H 5.18, N 5.14. Found: C 57.38, H 5.32, N 5.17.

N-*(indol-2-carbonyl)*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)urea **117**

Prepared according to ***Method A***. Reaction time: 3 days. Starting from 500 mg of urea **91** the yield was 284 mg (41%). Mp 135-138ºC, $[\alpha]_D$ –56.2 (c=1.050, MeOH) ^{1}H NMR ($CDCl_3$+DMSO-d_6, 360 MHz) δ (ppm) 11.73 (s, 1H, N*H*), 10.89 (s, 1H, N*H*), 9.28 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=9.3 Hz), 7.58 (d, 2H, Ar, *J*=5.4 Hz), 7.14 (d, 1H, Ar, *J*=7.9 Hz), 7.19 (t, 1 H, Ar, *J*=7.5 Hz), 7.01 (t, 1H, Ar, *J*=7.5 Hz), 5.41 (t, 1H, *J*=9.6 Hz) 5.38 (t, 1H, *J*=9.4 Hz) 5.00 (t, 1H, *J*=9.3 Hz) 4.98 (t, 1H, *J*=10.0 Hz) (C-1–C-4), 4.19 (dd, 1H, H-6*,* $J_{5,6}$=4.0 Hz), 4.03 (m unres., 1H, H-5), 3.99 (d, 1H, H-6', $J_{6,6'}$=12.6 Hz), 1.99, 1.97, 1.94 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 90 MHz) δ (ppm) 169.56, 168.98, 168.95, 168.78 (4x acetyl *C*O), 161.98 (NH*C*OAr), 153.25 (NH*C*ONH), 137.43, 128.48, 126.59, 124.54, 121.99, 119.87, 112.24, 106.97 (Ar), 77.77 (C-1), 72.22, 72.15, 69.86, 67.89 (C-2–C-5), 61.49 (C-6), 20.23, 20.11, 20.05 (4x acetyl CH_3). Anal. calcd for $C_{24}H_{27}N_3O_{11}$ (533.50): C 54.03, H 5.10, N 7.88. Found: C 53.47, H 5.18, N 7.64.

General method for the deprotection of N-*acyl*-N*'-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)ureas*

Method C: A solution of an *N*-acyl-*N*'-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)urea **91** in dry methanol was treated with a catalytic amount of a methanolic solution of NaOMe at rt. After TLC had shown disappearance of the starting material the reaction mixture was neutralized with a cation exchange resin Amberlyst 15 (H^+ form). After filtration the solvent was removed and the residue was purified by crystallisation.

N-*acetyl*-N*'-β-D-glucopyranosyl urea **118***

Prepared by ***Method C*** from 376 mg of **92**. Yield: 239 mg (82%). Mp 143-146ºC (decomp.), $[\alpha]_D$ –26.20 (c=0.580, H_2O) ^{1}H NMR (D_2O, 200 MHz) δ (ppm) 4.99 (d, 1H, H-1, $J_{H\text{-}1,\ NH}$=8,1 Hz), 3.90 (d, 1H, H-6, $J_{6,6'}$=11.7 Hz), 3.73 (dd, 1 H,

H-6', $J_{5,6'}$=4.8 Hz), 3.6-3.3 (m unres., 4H, H-2–H-5) 2.17 (s, 3H, CH_3); ^{13}C NMR (D_2O, 50 MHz) δ (ppm) 175.60 (NH*C*OCH_3), 155.63 (NH*C*ONH), 80.65 (C-1), 78.00, 76.82, 72.38, 69.77 (C-2–C-5), 61.09 (C-6), 23.81 (CH_3). Anal. calcd for $C_9H_{16}N_2O_7$ (264.23): C 40.91, H 6.10, N 10.60, O 42.38. Calcd for $C_9H_{16}N_2O_7$ x 1.5 H_2O (273.25): C 39.56, H 6.27, N 10.25, O 43.92. Found: C 39.88, H 6.52, N 9.86, O 43.82.

N-*benzoyl*-N*'-β-D-glucopyranosyl urea* ***119***

Prepared by ***Method C*** from 430 mg of **93** and the crude product was purified by column chromatography (eluent: chloroform:methanol: ethylacetate=7:2:1). Yield: 257 mg (90%). Mp 196-199ºC (decomp.), $[\alpha]_D$ –11.57 (c=0.950, MeOH) ^{1}H NMR (CD_3OD, 360 MHz) δ (ppm) 7.83 (d, 2H, Ar, *J*=7.2 Hz), 7.54 (t, 1H, Ar, *J*=7.4 Hz), 7.43 (t, 2H, Ar, *J*=7.9 Hz), 4.92 (d, 1H, H-1, $J_{H\text{-}1,NH}$=8,9 Hz), 3.78 (dd, 1H, H-6, $J_{5,6}$=1.8 Hz), 3.60 (dd, 1 H, H-6', $J_{5,6'}$=4.6 Hz, $J_{6,6'}$=12.0 Hz), 3.4-3.2 (m unres., 4H, H-2–H-5); ^{13}C NMR (CD_3OH, 90 MHz) δ (ppm) 171.40 (NH*C*OAr), 157.10 (NH*C*ONH), 135.00, 134.92, 130.64, 129.93 (Ar) 82.91 (C-1), 80.57, 79.73, 75.28, 72.18 (C-2–C-5), 63.53 (C-6). Anal. calcd for $C_{14}H_{18}N_2O_7$ (326.31): C 51.53, H 5.56, N 8.59. Found: C 50.96, H 5.65, N 8.37.

N-*4-nitrobenzoyl*-N*'-β-D-glucopyranosyl urea* ***120***

Acetylated *N*-4-nitrobenzoyl-*N'*-β-D-glucopyranosyl urea **112** (155 mg, 0.28 mmol) was dissolved in dry methanol and some crystals of $KHSO_4$ were added. The mixture was filtered after five days and the solvent was evaporated under vacuo. The crystalline crude product was recrystallised from methanol to give 105 mg (90%) of **120**. Mp 214-215ºC (decomp.) $[\alpha]_D$ +2.17 (c=0.460, DMSO) ^{1}H NMR (DMSO, 200 MHz) δ (ppm) 11.20 (s, 1H, N*H*), 8.95 (s, 1H, N*H*), 8.33 (d, 2H, Ar, *J*=8.7 Hz), 8.13 (d, 2H, Ar, *J*=8.7 Hz), 4.81 (d, 1H, H-1, $J_{1,2}$=8.9 Hz), 3.65 (m, unres., 1H, H-6), 3.44 (dd, 1H, H-6', $J_{5,6'}$=4.8 Hz, $J_{6,6'}$=11.8 Hz), 3.3-3.0 (m

unres., 4H, H-2–H-5); ^{13}C NMR (DMSO, 50 MHz) δ (ppm) 167.42 (NH*C*OAr), 153.21 (NH*C*ONH), 150.04, 138.31, 129.95, 123.79 (Ar) 80.46 (C-1), 78.74, 77.19, 73.05, 69.88 (C-2–C-5), 60.96 (C-6). Anal. calcd for $C_{14}H_{17}N_3O_9$ (371.31): C 45.29, H 4.61, N 11.32. Found: C 50.01, H 4.32, N 11.25.

N-*3-chlorobenzoyl*-N*'-β-D-glucopyranosyl urea* ***121***

Prepared by ***Method C*** from 513 mg of **113**. Yield: 343 mg (98%). Mp 201-203ºC (decomp.) $[\alpha]_D$ +28.70 (c=1.160, MeOH) ^{1}H NMR (CD_3OD, 200 MHz) δ (ppm) 8.54 (s, 1H, N*H*), 7.9-7.4 (m, unres., 4H, Ar), 5.72 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=4.8 Hz), 5.12 (dd, 1H, H-1, $J_{1,2}$=8.6 Hz), 3.8-3.6 (m. unres., 3H), 3.46 (dd, 1H, H-6, $J_{5,6}$=1.4 Hz, $J_{6,6'}$=8.14 Hz), 3.42-3.3 (m., 2H, H-6', H-5); ^{13}C NMR (CD_3OD, 50 MHz) δ (ppm) 170.68 (NH*C*OAr), 169.73 (NH*C*ONH), 137.56, 135.86, 133.20, 131.45, 129.23, 127.56 (Ar) 82.02 (C-1), 80.09, 79.27, 73.99, 71.65 (C-2–C-5), 62.92 (C-6). Anal. calcd for $C_{14}H_{17}N_2O_7Cl$ (360.75): C 46.61, H 4.75, N 7.77. Found: C 46.71, H 5.07, N 4.20.

N-*4-hydroxybenzoyl*-N*'-β-D-glucopyranosyl urea* ***122***

Prepared by ***Method C*** from 50 mg of **114**. Yield: 27 mg (87%). Mp 203-205ºC (decomp.) $[\alpha]_D$ +2.52 (c=1.070, DMSO) ^{1}H NMR (DMSO+CD_3OD, 300 MHz) δ (ppm) 9.59 (s, 1H, N*H*), 8.56 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=8.5 Hz), 7.10 (d, 2H, Ar, *J*=8.6 Hz), 6.09 (d, 2H, Ar, *J*=8.6 Hz), 4.14 (d, 1H, H-1, $J_{1,2}$=9.11 Hz), 2.98 (d, 1H, H-6, $J_{6,6'}$=11.6 Hz), 2.80 (dd, 1H, H-6', $J_{5,6'}$=4.36 Hz), 2.6-2.37 (m., 4H, C-2–C-5); ^{13}C NMR (DMSO+CD_3OD, 75 MHz) δ (ppm) 162.70 (NH*C*OAr), 155.02 (NH*C*ONH), 131.11, 123.74, 115.86 (Ar) 81.23 (C-1), 79.24, 78.14, 73.83, 70.63 (C-2–C-5), 61.82 (C-6). Anal. calcd for $C_{14}H_{18}N_2O_8$ (342.31): C 49.12, H 5.30, N 8.18. Found: C 46.85, H 5.35, N 7.48.

N-*1-naphthoyl*-N*'-β-D-glucopyranosyl urea* ***123***

Prepared by ***Method C*** from 55 mg of **115**. Yield: 35 mg (94%). Mp 218-221ºC $[\alpha]_D$ +11.4 (c=1.100, DMSO) ^{1}H NMR (DMSO+CD_3OD, 300 MHz) δ (ppm) 12.66 (s, 1H, NH), 10.76 (d, 1H, NH, $J_{H\text{-}1,\ NH}$=9.4 Hz), 9.83 (d, 1H, Ar, *J*=7.4 Hz), 9.72 (d, 1H, Ar, *J*=7.96 Hz), 9.63 (dd, 1H, Ar, *J*=7.1 Hz, *J*=9.3 Hz), 9.38 (d, 1H, Ar, *J*=6.4 Hz), 9.25-9.15 (m., unres., 3H, Ar), 6.56 (t, 1H, H-1, $J_{1,2}$=9.0 Hz), 5.38 (dd, 1H, H-6, $J_{5,6}$=1.4 Hz, $J_{6,6'}$=11.73 Hz), 5.18 (dd, 1H, H-6', $J_{5,6'}$=4.8 Hz), 5.0-4.1 (m., 4H, C-2–C-5); ^{13}C NMR (DMSO+CD_3OD, 75 MHz) δ (ppm) 171.1 (NHCOAr), 153.9 (NHCONH), 133.71, 132.24, 131.86, 129.90, 128.88, 127.76, 126.95, 125.24, 125.13 (Ar) 80.93 (C-1), 79.05, 77.70, 73.56, 70.28 (C-2–C-5), 61.41 (C-6). Anal. calcd for $C_{18}H_{20}N_2O_7$ (376.37): C 57.44, H 5.36, N 7.44. Found: C 57.15, H 5.35, N 7.48.

N-*2-naphthoyl*-N*'-β-D-glucopyranosyl urea* ***124***

Prepared by ***Method C*** from 100 mg of **116**. Yield: 58 mg (83%). Mp 212-213ºC $[\alpha]_D$ +6.51 (c=0.180, DMSO), ^{1}H NMR (DMSO, 360 MHz) δ (ppm) 11.02 (s, 1H, NH), 9.15 (d, 1H, NH, $J_{H\text{-}1,\ NH}$=9.5 Hz), 8.2-7.5 (m., unres., 7H, Ar), 4.84 (t, 1H, H-1, $J_{1,2}$=8.60 Hz), 4.01-3.08 (m., 10H, 4xO*H*, H-1–H-6'); ^{13}C NMR (DMSO, 90 MHz) δ. (ppm) 168.50 (NHCOAr), 153.50 (NHCONH), 134.72, 134.65, 132.15, 130.01, 129.59, 129.38, 129.16, 127.85, 127.64, 126.53 (Ar) 80.33 (C-1), 78.6, 77.27, 73.16, 69.70 (C-2–C-5), 60.81 (C-6). Anal. calcd for $C_{18}H_{20}N_2O_7$ (376.37): C 57.44, H 5.36, N 7.44. Found: C 57.35, H 5.28, N 7.36.

N-*(indol-2-carbonyl)*-N*'-β-D-glucopyranosyl urea* ***125***

Prepared by ***Method C*** from 70 mg of **117** and the syrupy crude product was purified by column chromatography (eluent: chloroform:methanol=9:1). Yield: 34 mg (71%). Mp 187-190˚C $[\alpha]_D$ +13.00 (1.410, MeOH) ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) 7.64-7.05 (indole), 5.58 (d, 1H, NH, *J*=5.2 Hz), 4.99 (d, 1H, NH, $J_{H\text{-}}$

$_{1, NH}$=8.9 Hz), 3.86 (dd, 1H, H-6, $J_{5,6}$=1.8 Hz), 3.78 (dd, 1H, H-6', $J_{6,6'}$=11.9 Hz), 3.7 (t, 1H, *J*=9.5 Hz) 3.68 (t, 1H, *J*=9.5 Hz) 3.54 (t, 1H, *J*=9.8 Hz) (C-2–C-4), 3.42 (ddd, 1H, H-5, $J_{5,6'}$=9.8 Hz), 3.38 (t, 1H, H-1, $J_{1,2}$=8.8 Hz), 3.37 (pd, 1H, indole NH, *J*<1 Hz); ^{13}C NMR (CD_3OD, 90 MHz) δ (ppm) 164.20 (NHCOAr), 156.49 (NHCONH), 139.43, 130.12, 128.78, 126.47, 123.52, 121.68, 113.32, 107.83, 107.75 (Ar) 82.14 (C-1), 75.73, 74.62, 74.51, 71.44 (C-2–C-5), 62.75 (C-6). Anal. calcd for $C_{16}H_{19}N_3O_7$ (365.35): C 52.60, H 5.24, N 11.50. Anal. calcd for $C_{16}H_{19}N_3O_7$ x 2 H_2O (383.39): C 50.73, H 5.45, N 11.90. Found: C 48.93, H 5.13, N 10.38.

N-*4-aminobenzoyl*-N*'-β-D-glucopyranosyl urea* ***126***

N-4-nitrobenzoyl-*N*'-β-D-glucopyranosyl urea **120** (37 mg, 1.1 mmol) was dissolved in dry methanol (7 ml) and some Raney-Ni catalyst (Type BLM112W) was added. Hydrogen was bubbled into the stirred mixture for 1 h when TLC showed the disappearance of the starting material. The catalyst was filtered off on a Celite pad and the solvent was evaporated under vacuo. The crystalline residue was recrystallised from methanol to give 34 mg (99%) of **126**. Mp 220-221ºC (decomp.) $[\alpha]_D$ 0.0 (c=1.070, DMSO) ^{1}H NMR (DMSO, 300 MHz) δ (ppm) 10.26 (s, 1H, N*H*), 9.28 (d, 1H, N*H*, $J_{H\text{-}1, NH}$=8.8 Hz), 7.73 (d, 2H, Ar, *J*=9.0 Hz), 6.54 (d, 2H, Ar, *J*=8.5 Hz), 5.99 (s, 2H, NH_2), 5.23 (d, 1H, OH, *J*=5.3 Hz), 5.03 (d, 1H, OH, *J*=4.4 Hz), 4.96 (d, 1H, OH, *J*=4.8 Hz), 4.78 (t, 1H, H-1, $J_{1,2}$=9.1 Hz), 4.55 (t, 1H, OH, *J*=5.6 Hz), 3.64 (dd, 1H, H-6', $J_{5,6'}$=4.3 Hz, $J_{6,6'}$=11.0 Hz), 3.23-2.9 (m, unres., 5H, C-2–C-6); ^{13}C NMR (DMSO, 50 MHz) δ (ppm) 167.80 (NH*C*OAr), 154.07 (NH*C*ONH), 153.46, 130.20, 117.93, 112.18 (Ar) 80.25 (C-1), 78.53, 77.29, 73.16, 69.81 (C-2–C-5), 60.86 (C-6). Anal. calcd for $C_{14}H_{19}N_3O_7$ (341.32): C 49.27, H 5.61, N 12.31. Found: C 50.03, H 5.54, N 12.26.

5.3. Photolysis of N-acyl-N'-β-D-glucopyranosyl ureas

Photolysis of N-*acetyl*-N*'-β-D-glucopyranosyl urea* ***92***

N-acetyl-N'-β-D-glucopyranosyl urea **92** (43 mg, 0.1 mmol) was dissolved in dry benzene (2 ml) under argon athmosphere in a quartz tube (diameter 25 mm) which was placed in 1 cm distance from a medium pressure mercury lamp (Hanovia, 450 W) covered with Vicor filter. On irradiation the solution got warm to reflux temperature and became dark brown but after 2.5 h no transformation of **92** was observed on TLC plates. Removing the light filter the irradiation was continued for further 3 h without any reaction of **92**.

The same experiment was carried out using dry acetonitrile as solvent, the solution was irradiated through Vicor filter for 2.5 h and for 3 h without filter but starting **92** was not transformed.

Dry methanolic solution of **92** (150 mg, 0.34 mmol) was irradiated under the same conditions using Vicor filter and after 30 min a new spot appeared on the TLC plate. The irradiation was continued for 33 h by which time the product seemed to appear in higher amount. The reaction mixture was separated by coloumn chromatography (eluent: ethylacetate), the starting **92** was recovered in 88% and the product proved to be **91** N-β-D-glucopyranosyl urea formed in 6% yield (8 mg).

Without Vicor filter similar result was observed (for yields see **Table 2**.)

Photolysis of N-*benzoyl*-N*'-β-D-glucopyranosyl urea* ***93***

N-benzoyl-*N*'-β-D-glucopyranosyl urea **93** (183 mg, 0.37 mmol) was dissolved in dry benzene (7 ml) under argon athmosphere in a quartz tube (diameter 25 mm) which was placed in 1 cm distance from a medium pressure mercury lamp (Hanovia, 450 W) surrounded by a Vicor filter. After 30 min irradiation a new product appeared on TLC plate with the same R_f value than that of **91**. After 20 h of irradiation the filter was removed and the mixture was

irradiated for further 50 h and was sepatated by coloumn chromatography (eluent: ethylacetate). The starting **93** was recovered in 43% (80 mg) and 12 mg (6%) of **91** was isolated as product.

5.4. Synthesis and photolysis of *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amidoximes

General method for the preparation of N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl) amidoximes*

Method D: 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide **95** (1 g, 2.68 mmol) was dissolved in dry dichloromethane (10 ml) and a toluene solution of trimethyl phosphine (2.68 ml, 1M) was added with stirring. After cessation of gas evolution when TLC showed complete transformation of the starting material (10-15 min) hydroximinoyl chloride (1 eq) was added. After 20-30 min the reaction mixture was poured into water, and the organic layer was separated. The aqueous solution was extracted with dichloromethane (2x20 ml), the organic phases were collected and washed with 10 % aq. sodium carbonate soln. and water (5x). After drying the solvent was removed under vacuo and the residue was purified by flash coloumn chromatography (eluent: ethylacetate:hexane=1:3) or crystallisation.

N-(*2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosy)benzamidoxime **136***

Prepared by ***Method D***. Yield: 57%, syrup. The product decomposed rapidly during the NMR spectroscopic measurement.

N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-4-nitrobenzamidoxime **137***

Prepared by ***Method D***. Yield: 880 mg (64%) mp 183-185ºC $[\alpha]_D$ +20.0 (c=1.220, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm) 9.36 (s, 1H, O*H*), 8.25 (d, 2H, Ar, *J*=9.1 Hz), 7.72 (d, 2H, Ar, *J*=8.4 Hz), 6.20 (d, 1H, N*H*), 5.12 (t, 1H, H-2, $J_{2,3}$=9.6 Hz), 5.03 (t, 1H, H-3, $J_{3,4}$=9.4 Hz), 4.97 (t, 1H, H-4, $J_{4,5}$=9.2 Hz), 4.31 (t,

1H, H-1, $J_{1,2}$=9.9 Hz), 4.12 (dd, 1H, H-6, $J_{5,6}$=5.1), 4.02 (dd, 1H, H-6', $J_{6,6'}$=11.8 Hz), 3.38 (ddd, 1H, H-5, $J_{5,6'}$=2.8 Hz), 2.09, 2.05, 1.97, 1.95 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm) 170.44, 170.15, 169.96, 169.36 (4x acetyl *C*O), 151.91 (NH*C*(=NOH)Ar), 148.71 (*C*NO_2), 136.55 (N_2C-*C*), 129.53, 123.62 (Ar), 82.49 (C-1), 72.93, 72.47, 70.64, 68.28 (C-2–C-5), 62.08 (C-6), 20.52, 20.62, 20.40 (4x acetyl CH_3). Anal. calcd for $C_{21}H_{25}N_3O_{12}$ (511.45): C 49.32, H 4.93, N 8.22. Found: C 50.02, H 5.03, N 8.16.

N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-4-cyanobenzamidoxime* ***138***

Prepared by ***Method D***: Yield: 245 mg (18%), mp 120-123ºC $[\alpha]_D$ +9.81 (c=0.200, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.50 (s, 1H, O*H*), 7.71 (d, 2H, Ar, *J*=8.6 Hz), 7.65 (d, 2H, Ar, *J*=8.3 Hz), 6.16 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=10.4 Hz), 5.14 (t, 1H, H-3/4, $J_{3,4}$=9.4 Hz), 5.03 (t, 1H, H-2, $J_{2,3}$=8.8 Hz), 4.99 (t, 1H, H-3/4, $J_{4,5}$=9.4 Hz), 4.31 (dd, 1H, H-1, $J_{1,2}$=8.8 Hz), 4.10 (d, 1H, H-6, $J_{5,6}$=6.6 Hz), 4.02 (dd, 1H, H-6', $J_{6,6'}$=12.5 Hz), 3.39 (ddd, 1H, H-5, $J_{5,6'}$=2.7 Hz), 2.09, 2.06, 2.02, 1.99 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 170.40, 170.19, 169.95, (4x acetyl *C*O), 152.29 (NH*C*(=NOH)Ar), 134.73 (N_2C-*C*), 132.29, 129.19 (Ar), 128.94 (*C*-CN), 113.94 (CN), 82.50 (C-1), 72.93 (C-5), 72.45, 70.66, 68.30 (C-2–C-4), 62.07 (C-6), 20.98, 20.68, 20.59, 20.47 (4x acetyl CH_3). Anal. calcd for $C_{22}H_{25}N_3O_{10}$ (491.46): C 53.77, H 5.13, N 8.55. Found: C 53.43, H 5.09, N 8.62.

Optimised synthesis of ***138***: 2,3,4,6-tetra-*O*-acetyl-glucopyranosyl azide **95** (100 mg, 0.26 mmol) was dissolved in dry dichloromethane (1 ml) and a toluene solution of trimethyl phosphine (0.268 ml, 1M) was added with stirring. During gas evolution the reagent solution was prepared freshly by the following way: hydroximinoyl chloride (153 mg, 0,26 mmol) was dissolved in dry dichloromethane (1 ml) and triethylamine was added (formation of triethylamine hydrochloride precipitate). When TLC showed complete transformation of the starting material **95** (~15 min) the reagent solution was added to the mixture. After 10 min the reaction mixture was poured into water and was worked up as described

in ***Method D***. Yield: 78 mg (60%), the product was identical with that prepared by ***Metode D***.

N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)*-C-*2-naphthyl amidoxime **139***

Prepared by ***Method D***: Yield: 92 mg (6%) brownish foam, rapidly decomposes.

Photolysis of N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-4-nitrobenzamid-oxime **137***

With a 60 W heating lamp using 4 eq of NBS in one portion: *N*-β-D-glucopyranosyl-p-nitrobenzamidoxime **137** (200 mg, 0.39 mmol) was dissolved in dry chloroform (8 ml) in a 100 ml Erlenmeyer flask equipped with a reflux condenser and 4 equivalent of NBS (278 mg, 1,56 mmol) were added in one portion. The mixture was irradiated with a 60 W white heating lamp from 1 cm. After 15 min chloroform was added (30 ml), the mixture was washed with 5% aq. Na_2SO_3 soln., satd. aq. $NaHCO_3$ soln. and water. After drying the solvent was removed under vacuo and the residue was purified by column chromatography (eluent:ethylacetate:hexane=1:4) giving **144** as syrupy product in 30% yield (58 mg). $[\alpha]_D$ +21.77 (c=0.400, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.39 (d, 2H, Ar, *J*=8.3 Hz), 8.05 (d, 2H, Ar, *J*=8.9 Hz), 5.32 (d, 1H, H-1, $J_{1,2}$=9.8 Hz), 5.23 (t, 1H, H-3, $J_{3,4}$=9.5 Hz), 4.83 (t, 2H, H-2, H-4, $J_{4,5}$=9.4 Hz), 4.44 (dd, 1H, H-6, $J_{6,6'}$=12.9 Hz), 4.13 (dd, 1H, H-6', $J_{5,6'}$=6.0 Hz), 3.90 (ddd, 1H, H-5, $J_{5,6}$=1.9 Hz), 2.16, 2.01, 1.99, 1.93 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 170.17, 169.53, 169.38, 169.16 (4x acetyl *C*O), 157.35 (N_2C-*C*), 156.60 (NC=N), 150.13 (*C*NO_2), 131.47, 123.71 (Ar), 82.02 (C-1), 75.26, 72.19, 68.72, 66.80 (C-2–C-5), 61.03 (C-6), 20.72, 20.40, 20.35, 20.17 (4x acetyl CH_3). Anal. calcd for $C_{21}H_{23}N_3O_{12}$ (509.43): C 49.51, H 4.55, N 8.25. Found: C 49.48, H 4.76, N 8.22. Traces of **146** (~3%) were also detected in the crude product by NMR spectroscopy.

With a 60 W heating lamp using 4 eqs of NBS in 4 portion: *N*-β-D-glucopyranosyl-p-nitrobenzamidoxime **137** (1 g, 1.96 mmol) was dissolved in dry chloroform (40 ml) in a 250 ml Erlenmeyer flask equipped with a reflux condenser and one equivalent of NBS (348 mg, 1,96 mmol) was added. The mixture was irradiated with a 60 W white heating lamp from 1 cm. After 30 min 348 mg of NBS was added to the reaction mixture. Addition of further 1-1 eq. of NBS was effected after 105 min, and 135 min, respectively. After 2 h 45 min total reaction time the mixture was diluted with chloroform (120 ml), washed with 5% aq. Na_2SO_3 soln., satd. aq. $NaHCO_3$ soln. and water. After drying the solvent was removed under vacuo and the residue was purified by column chromatography (eluent:ethylacetate:hexane=1:4) giving **146** as a sole, syrupy product in 45% yield (447 mg). $[\alpha]_D$ +19.38 (c=0.280, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.32 (d, 2H, Ar, *J*=10.1 Hz), 8.22 (d, 2H, Ar, *J*=9.1 Hz), 6.27 (d, 1H, H-2, $J_{2,3}$=5.9 Hz), 5.90 (dd, 1H, H-3, $J_{3,4}$=3.7 Hz), 5.54 (d, 1H, H-4), 4.87 (d, 1H, H-6, $J_{6,6'}$=17.3 Hz), 4.72 (d, 1H, H-6'), 2.19, 2.13, 2.12, 2.01 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 197.09 (C-5), 174.35 (C-1), 169.69, 169.37, 169.17, 168.94 (4x acetyl *C*O), 166.95 (N_2*C*Ar), 149.63 (*C*NO_2), 131.67 (N_2C-*C*), 128.47, 124.14 (Ar), 73.66 (C-3), 69.55 (C-4), 66.44 (C-6), 65.45 (C-2), 231, 20.20, 20.14 (4x acetyl CH_3). Anal. calcd for $C_{21}H_{21}N_3O_{12}$ (507.41): C 49.71, H 4.17, N 8.28. Found: C 49.58, H 4.23, N 8.24. MS: 508 (M+1).

With a 375 W heating lamp using 4 eq of NBS in one portion: *N*-β-D-glucopyranosyl-p-nitrobenzamidoxime **137** (200 mg, 0.39 mmol) was dissolved in dry chloroform (12 ml) in a 100 ml Erlenmeyer flask equipped with a reflux condenser and 4 equivalent of NBS (278 mg, 1,56 mmol) were added in one portion. The mixture was irradiated with a 375 W white heating lamp from 1 cm. After 2 h chloroform was added (30 ml), the mixture was washed with 5% aq. Na_2SO_3 soln., satd. aq. $NaHCO_3$ soln. and water. After drying the solvent was removed under vacuo and the residue was purified by column chromatography (eluent:ethylacetate:hexane=1:4) giving **146** in 13% (13 mg) and **145** as syrupy

product in 20% yield (20 mg). $[\alpha]_D$ +26.2 (c=0.260, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.33 (d, 2H, Ar, *J*=8.9 Hz), 8.24 (d, 2H, Ar, *J*=8.8 Hz), 6.16 (d, 1H, H-2, $J_{2,3}$=6.7 Hz), 5.73 (dd, 1H, H-4, *J*=3.06 Hz, *J*=7.3 Hz), 5.20-5.17 (m unres., 1H, H-5), 3.53 (dd, 1H, H-6, $J_{5,6}$=3.6 Hz, $J_{6,6'}$=11.9 Hz), 3.39 (dd, 1H, H-6', $J_{5,6'}$=6.4 Hz), 2.18, 2.11, 2.10, 2.08 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 174.30 (C-1), 169.49, 169.44, 169.34, 169.01 (4x acetyl *C*O), 166.89 (N*C*=N), 149.56 (*C*NO_2), 131.77 (*C*-CN_2), 128.48, 124.07 (Ar), 69.62, 69.19, 68.57 (C-2–C-4), 65.73 (C-6), 30.20 (C-5), 20.62, 20.55, 20.35, 20.24 (4x acetyl CH_3). Anal. calcd for $C_{21}H_{23}N_3O_{12}$ (509.43): C 49.51, H 4.55, N 8.25. Found: C 49.76, H 4.58, N 8.17. MS: 663,72 (M+matrix, matrix: 154 g/mol), M=509.

5.5. Miscellaneous

N-*2-naphthoyl*-N*''-(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl) biuret* ***129***

To a suspension of NaOCN (121 mg, 1.87 mmol) in dry acetonitrile naphthoyl chloride (1.44 mmol) in acetonitrile (30 ml) and $SnCl_4$ (8.4 μl, 0.072 mmol) were added under argon with stirring. The mixture was stirred under reflux for 8 h and after cooling to rt 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl urea **91** (500 mg, 1.28 mmol) was added under argon. After strirring for overnight some drops of water were added and the mixture was filtered. The solvent was evaporated and the residue purified by column chromatography (eluent: EtOAc:hexane = 1:1) to give **129** in 31% (235 mg) yield. Mp 165-167ºC $[\alpha]_D$ – 32.75 (c=1.160, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz) δ (ppm) 10.81 (s, 1H, N'*H*), 8,82 (s, 1H, Ar), 8.82 (d, 1H, N*H*, $J_{H\text{-}1,\ NH}$=6.7 Hz), 8.42 (s, 1H, N''*H*), 8.00-7.60 (m, unres., 6H, Ar), 5.27 (t, 2H, H-1, H-2, $J_{1,2}$=9.3 Hz), 5.08 (t, 1H, H-3, *J*=9.3 Hz) 4.99 (t, 1H, H-4, *J*=9.4 Hz) 4.27 (dd, 1H, H-6, $J_{5,6}$=4.7 Hz, $J_{6,6'}$=12.7 Hz), 4.1-4.0 (m, unres., 1H, H-6'), 3.83 (ddd, 1H, H-5, $J_{5,6'}$=10.0 Hz), 2.08, 2.04, 2.00 (s, 4x3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ (ppm) 170.59, 169.94, 169.84 (4x acetyl *C*O), 169.45 (N'''H*C*OAr), 153.62 (N'H*C*ON''H), 146.73 (N''H*C*ON'''H), 135.62,

129.63, 129.37, 129.14, 128.93, 127.84, 127.35, 123.23 (Ar), 78.90 (C-1), 73.36, 72.80, 70.17, 68.16 (C-2–C-5), 61.89 (C-6), 20.40 (4x acetyl CH_3). Anal. calcd for $C_{27}H_{29}N_3O_{12}$ (587.54): C 55.20, H 4.98, N 7.15. Found: C 56.32, H 5.00, N 6.95.

N-*2-naphthoyl*-N*''-β-D-glucopyranosyl-biuret* ***130***

N-2-naphthoyl-*N*''-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) biuret **129** (165 mg, 0.28 mmol) was dissolved in the mixture of dry methanol (3 ml) and chloroform (1 ml). $KHSO_4$ (3 mg) was added and the reaction mixture was stirred at rt. After 3 weeks the salt was filtered off, the solvent was evaporated and the residue was purified by coloumn chromatography (eluent: ethylacetate:methanol = 7:3). Yield: 81 mg (70%). Mp 188-191ºC $[\alpha]_D$ +5.32 (c=0.180, DMSO); ^{1}H NMR (DMSO-d_6, 360 MHz) δ (ppm) 10.69 (s, 1H, N''*H*), 9,68 (s, 1H, N'''*H*), 7.81 (s, 1H, Ar), 7.57 (pd, 1H, N'*H*), 7.23-6.8 (m, unres., 6 H, Ar), 4.53 (s, 1H, O*H*), 4.41 (s, 1H, O*H*), 4.36 (s, 1H, O*H*), 4.20 (pt, 1H, H-1) 4.11 (s, 1H, O*H*) 3.90-2.20 (m, unres., 6H, H-2–H-6'); ^{13}C NMR (DMSO-d_6, 90 MHz) δ (ppm) 168.06 (N'''H*C*OAr), 152.29 (N'H*C*ON''H), 151.95 (N''H*C*ON'''H), 134.87, 131.68, 129.60, 129.25, 128.73, 128.28, 127.61, 127.09, 123.96 (Ar), 80.35 (C-1), 78.57, 77.11, 72.93, 69.67 (C-2–C-5), 60.73 (C-6). Anal. calcd for $C_{19}H_{21}N_3O_8$ (419.39): C 54.41, H 5.05, N 10.02. Found: C 53.98, H 5.16, N 9.77.

N-*(β-D-glucopyranosyl)-4-nitrobenzamidoxime* ***147***

N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-4-nitrobenzamidoxime* ***137*** (100 mg, 0.19 mmol) was dissolved in dry methanol (7 ml) and some drops of methanolic NaOMe solution was added. The reaction mixture was stirred at rt. After 5 min cation exchange resin Amberlyst 15 (H^+ form) was added. The miture was filtered off, the solvent was removed and the residue was purified by crystallisation to give 56 mg (83%) of **147**. Mp 165-168ºC $[\alpha]_D$ –56,30 (c=1.280, DMSO); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 10.39 (s, 1H, NO*H*), 8.22 (d,

2H, Ar, *J*=8.4 Hz), 7.91 (s, 2H, Ar, *J*=8.2 Hz), 6.40 (d, 1H, N'*H*, *J*=9.84 Hz), 5.1-4.65 (m, unres., 4H, 4xOH), 4.0-2.8 (m., unres., 7H, H-1–H-6'); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 152.04 (*C*NO_2), 147.59 (N=*C*N), 138.47 (N_2C-*C*), 129.61, 123.14 (Ar), 83.84 (C-1), 78.15, 77.34, 72.85, 70.15 (C-2–C-5), 61.00 (C-6). Anal. calcd for $C_{13}H_{17}N_3O_8$ (343.30): C 45.48, H 4.99, N 12.24. Found: C 46.02, H 5.25, N 11.87.

N-*(β-D-glucopyranosyl)-4-cyanobenzamidoxime* ***148***

N-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-4-cyanobenzamidoxime* ***138*** (90 mg, 0.18 mmol) was dissolved in dry methanol (9 ml) and some drops of methanolic NaOMe solution was added. The reaction mixture was stirred at rt. After 25 min cation exchange resin Amberlyst 15 (H^+ form) was added. The miture was filtered off, the solvent was removed to give **148** in 81% (48 mg) yield as a syrup. $[\alpha]_D$ –132.07 (c=0.640, MeOH); ^{1}H NMR (CD_3OD, 360 MHz) δ (ppm) 7.65 (d, 2H, Ar, *J*=9.2 Hz), 7.39 (d, 2H, Ar, *J*=7.9 Hz), 3.55 (t, 1H, *J*=11.8 Hz), 3.4-3.1 (m, unres., 5H); ^{13}C NMR (CD_3OD, 90 MHz) δ 156.38 (N-C=N), 134.64 (Ar, N_2C–*C*), 124.87 (Ar), 119.92 (NC-*C*), 108.10 (*C*N), 84.65 (C-1), 79.59, 78.56, 74.18, 71.49 (C-2–C-5), 62.89 (C-6). Anal. calcd for $C_{14}H_{17}N_3O_6$ (323.31): C 52.01, H 5.30, N 13.00. Found: C 52.52, H 5.23, N 12.65.

6. Summary

Diabetes is one of the most dangerous disease worldwide which afflicts approximately 6 % of the adult population in the Western society. Type II or non-insulin-dependent *diabetes mellitus* (NIDDM) represents ~90% of all diabetic cases. A newly investigated approach for the potential treatment of NIDDM is the inhibition of liver glycogen phosphorylase (GP) which is the main regulatory enzyme of blood sugar levels.

Glucopyranosylidene-spiro-hydantoin **20** and its thio derivative **21** are among the most effective glucose analogue inhibitors of glycogen phosphorylases. On the basis of a procedure 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%. By changing the acetyl protecting groups to benzoyl the yield of the key intermedier glucopyranosyl cyanide **105** (**Scheme 17.**) increased from 11% to 58%, as well as the chromatographic separations were no longer needed. NBS used earlier in the bromination reactions was changed to elemental bromine, hereby work-up of the reaction mixtures became more simple. It was shown, that carbon tetrachloride generally used in similar reactions was replaceable by chloroform or dichloromethane and the radical brominations could be effected at lower temperature. 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. Thiohydantoin **21** was prepared in gram scale quantities by this new method allowing extended biological investigations with glycogen phosphorylases. 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.

For the synthesis of compounds analogous to spiro-(thio)hydantions **20** and **21** a photochemical approach based on the known Norrish type II cyclisation of γ-oxoalkyl glycosides was examined. 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. The deprotected ureas (**92→118**, K_i = 370.5 μM; **93→119**, K_i = 4.6 μM) were found to be inhibitors of glycogen phosphorylases. Following this finding different aromatic acyl groups (ArCO) were attached to the β-D-glucopyranosyl urea basic structure (Ar = 4-NO_2-phenyl, **120**; 3-Cl-phenyl, **121**; 4-OH-phenyl, **122**; 1-naphthyl, **123**; 2-naphthyl, **124**; 2-indolyl, **125**) and their binding to GP was studied by kinetic and protein crystallographic methods. Among these inhibitors *N*-2-naphthoyl-*N*'-β-D-glucopyranosyl urea **124** (K_i = 0.4 μM) has been the most effective, and is at present the best glucose analogue inhibitor of GP. Crystallographic studies have shown that some of these compounds bind also at the 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.

New glucopyranosylidene-spiro-oxadiazolines were also targeted (**Scheme 22.**) 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. The precursors were obtained from per-*O*-acetylated β-D-glucopyranosyl azide reacted first with trimethylphosphine and then with aryl hydroximinoyl chlorides (Ar = phenyl **136**, 4-NO_2-phenyl **137**, 4-CN-phenyl **138**, 2-naphthyl **139**). Photo-reactions of acetylated *N*-β-D-glucopyranosyl 4-nitro-benzamid oxime **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 amid-oximes (**147**, **148**) showhed no significant inhibition of GP.

7. Összefoglalás

A cukorbetegség (*diabetes mellitus*) a világ egyik legsúlyosabb betegsége mely a fejlett tárasadalmak lakosságának ~6%-át érinti.[1] A cukorbetegséget két fő típusra oszhatjuk attól függően, hogy a beteg szervezete termel-e inzulint vagy sem. Ez alapján megkülönböztetünk ún. inzulinfüggő, vagy más néven I típusú, valamint nem-inzulinfüggő, vagy II. típusú *diabetes*t. A betegek mintegy 90%-a a II típusú *diabetes*ben szenved, melyre jellemző, hogy a szervezet képes ugyan inzulin termelésére, de nem megfelelő mennyiségben, ill. az inzulin nem, vagy csak késve fejti ki hatását: a sejtek glükózfelvételének serkentését és a glikogénszintézis előmozdítását.[1, 2] Szemben az I. típussal, a tünetek főként idős korban jelentkeznek és sokkal lassabban fejlődnek ki. A betegség közvetlen kiváltó okait, kialakulásának biológiai, biokémiai hátterét nem ismerjük. Mivel minden tünete és szövődménye a kórosan magas ill. változó vércukorszintre vezethető vissza, a kezelés a normálist megközelítő, állandó vércukorszint biztosítását jelenti. A kezelés jelenleg tüneti szinten lehetséges változó sikerrel.

A II típusú diabetes kezelésére egy újonnan vizsgált megközelítés a vércukorszint beállításáért felelős máj glikogén-foszforiláz (GP) enzim gátlása. Az enzimnek számos glükóz-analóg inhibítora ismert, melyek közül a leghatékonyabbak a **20** glükopiranozilidén-spiro-hidantoin illetve **21** tiohidantoin (**Scheme 6**.).[25] Az e vegyületek szintézisére korábban alkalmazott eljárások[26, 31, 43] egyike sem volt alkalmas a kiterjedtebb biológiai vizsgálatokhoz szükséges, nagyobb mennyiségű inhibítor előállítására. Ezeket a módszereket a sok reakciólépés és/vagy alacsony hozamok mellett az jellemezte, hogy a termék spiro-hidantoin mindkét izomerje (**20**, **22**) keletkezett, ám csak az egyikhez rendelhető számottevő biológiai aktivitás. Az irodalomban csupán egy eljárás[25] talalható, melynek során az aktív tiohidantoin származék **21**, bár igen alacsony összkitermeléssel (~2%), de egyedüli izomerként keletkezik. Ezen eljárás alapján kidolgoztunk egy új módszert, mellyel **21** hozama

~15-szörösére növekedett (**Scheme 17.**). A védőcsoportokat acetilről benzoilra cserélve a szintézis kulcsvegyületének, a **105** glükopiranozil-cianidnak a hozama 11%-ról 58%-ra nőtt, valamint a kromatográfiás elválasztások is elhagyhatóvá váltak. A gyökös brómozási reakciókban korábban használt *N*-bróm-szukcinimidet elemi brómmal váltottuk ki, ezáltal leegyszerűsödött a reakcióelegyek feldolgozásának módja. Megmutattuk, hogy az ilyen reakciókban általánosan alkalmazott széntetraklorid oldószer helyettesíthető kloroformmal ill. dilklórmetánnal, amelyek mellett a reakciók alacsonyabb hőmérsékleten is kivitelezhetővé válnak. A cianocsoport részleges hidrolízisét **105** és **106** esetén jégecetes HBr-dal végeztük, így tiszta, kristályos nyerstermékként, jó hozammal nyertük a megfelelő benziolezett *C*-(β-D-glükopiranozil)-formamidokat (**107**, **108**). Az új módszerrel grammos tételben állítottuk elő a tiohidantoint, melynek megvizsgálták a máj glikogén-foszforiláz enzim aktivitására gyakorolt hatását. Ezek szerint a tiohidantoin **21** mind *in vitro*, mind *in vivo* körülmények között igen jelentősen csökkenti az enzimaktivitást, illetve *in vivo* kísérletekben a vércukorszintet, így egy olyan anyag nagy léptékű szintézisét dolgoztuk ki, mely potenciálisan vércukorszint-csökkentő terápiás szer lehet.[75, 76]

Az irodalomból ismert γ-oxoalkil-glikozidok Norrish II típusú gyűrűzárási reakciói[51] alapján megkíséreltünk fotokémiai módszerrel előállítani a **20** és **21** spiro-(tio)hidantoinokhoz hasonló szerkezetű molekulákat (**Scheme 19**). Különböző körülmények között végeztük el a per-*O*-acetilezett *N*-acetil (**92**) és *N*-benzoil-*N*'-β-D-glükopiranozil karbamid (**93**) fotolízisét, ám a várt átalakulás helyett Norrish I típusú *N*-dezacileződés történt. Ezek alapján azt mondhatjuk, hogy a vizsgált karbamidok nem alkalmasak fotolitikus gyűrűzűrásra. A védőcsoportok eltávolítása utan azonban ezek a karbamidszármazékok (**92→118**, K_i = 370.5 μM; **93→119**, K_i = 4.6 μM) a glikogén foszforiláz inhibítorainak bizonyultak.[80] Ezt követően különböző aromás acilcsoportokat (ArCO) kapcsoltunk a karbamid alapszerkezethez (Ar = 4-NO_2-fenil, **120**; 3-Cl-fenil, **121**; 4-OH-fenil, **122**; 1-naftil, **123**; 2-naftil, **124**; 2-indolil, **125**) melyek enzimhez való

kötődését enzimkinetikai és fehérjekrisztallográfiai módszerekkel vizsgálták. Ezen inhibítorok közül a **124** *N*-2-naftoil-*N*'-β-D-glükopiranozil karbamidot találták a leghatékonyabbnak (K_i = 0.4 μM) mely a jelenleg ismert legjobb glükózanalóg GP inhibítor.[79] A krisztallográfiai vizsgálatok szerint az általunk előállított karbamidszármazékok közül egyesek nemcsak a GP enzim katalitikus helyén kötődnek, hanem az ún. új allosztérikus helyen is, mely eddig nem tapasztalt, egyedülálló tulajdonság a glükózanalóg inhibítorok között. Az *N*-acil-*N*'-β-D-glükopiranozil karbamidok előállításával új vezérmolekulákat fedeztünk fel a GP enzim gátlására.

A fenti molekulákkal valamint egyes, hatékony GP inhibítornak talált glükopiranozilidén-spiro-oxatiazolokkal[54] végzett kinetikai és röntgenkrisztallográfiai vizsgátatok eredményei alapján új glükopiranozilidén-spiro-oxadiazolok előállítását terveztük per-*O*-acetilezett *N*-β-D-glükopiranozil-amidoximok oxidatív körülmények között végzett fotolízisével. E prekurzorokat per-*O*-acetilezett-β-D-glükopiranozil azidból készítetük trimetilfoszfin majd aril-hidroximinoil-kloridok hozzáadásával (**Scheme 24**.). A **137** acetilezett *N*-β-D-glükopiranozil-4-nitrobenzamidoxim fotolízisét NBS jelenlétében végeztük el különböző körülmények között (**Table 4.**). A képződött termékek szerkezete alapján feltételezzük (**Scheme 26.**) a kívánt **143** spiro-oxadiazolin kialakulását, melyben azonban a cukorgyűrű felnyílik és egy aromás 1,2,4,-oxadiazol (**145**) jön létre. A keletkezett szekunder alkohol a reakciókörülmények között **146** oxovegyületté alakulhat át. Az elvégzett kísérletek alapján a célvegyület *N*-β-D-glükopiranozilidén-spiro-oxadiazolin feltehetően instabil és az általunk vizsgált módszerrel nem állítható elő.

8. Résumé

Les diabètes sont des pathologies sévères qui affectent ~3% de la population mondiale.[1] Les pathologies diabétiques (*diabetes mellitus)* correspondent à deux types distincts, de type I ou *diabetes mellitus* insulino-dépendante et de type II ou *diabetes mellitus* non-insulino dépendante. Le type II représent environ 90% de tous les cas diabètiques. Le plupart des patients diabètiques peuvent produire l'insuline, souvent en quantité insuffisante. D'autre part, l'insuline peut exercer son effet partiellement ou pas du tout sur la stimulation de l'absorption du D-glucose par les cellules et la stimulation de la synthèse du glycogène.[1, 2]

Contrairement au diabète du type I, celui de type II affecte les personnes de plus de 40 ans et les symptômes se développent plus lentement.

Dans le cas du diabète de type I, le niveau de D-glucose dans le sang est contrôlable par une thérapie à l'insuline, tandis qu'en cas du diabète de type II, on utilise une régulation diététique et des médicaments hypoglycémiques.

Toutes ces thérapies sont des traitements symptomatiques lourds. Aussi beaucoup d'efforts sont déployés pour développer des méthodes pour maintenir le niveau physiologiquement normal de D-glucose dans le sang.

L'une des approches envisagées dans le contexte du *diabetes mellitus* non-insulino dépendante est l'inhibition de la glycogène phosphorylase (GP) qui est l'enzyme régulateur principal du niveau du D-glucose dans le sang.

On a trouvé parmi les meilleurs inhibiteurs de la GP les glucopyranosylidène-spiro-(thio)hydantoïnes[25] (**20, 21**, **Scheme 6**). Les procédures[26,31,43] appliquées précédemment pour la prépatarion de ces produits ne sont pas convenables pour la synthèse des inhibiteurs en quantité plus grande qui permet les investigations biologiques plus étendues. Ces méthodes contiennent beaucoup d'étapes réactionelles et/ou elles ont des rendements faibles, en plus elles ont donné les deux isomères de produit (**20**, **22**); toutefois seulement l'une a de

l'activité biologique importante. Dans la littérature une seule procédure[25] se trouve qui rend le dérivé thiohydantoïne actif comme une seule isomère mais avec un rendement bas (~2%).

Sur la base de ce procédure nous avons développé une nouvelle série de réaction avec laquelle le rendement brut de la thiohydantoïne **21** est multiplié par ~15 fois (**Scheme 17.**). En changeant les groupements protecteurs d'acétyle à benzoyle, le rendement du produit clef (cyanure de glucopyranosyl) a augmenté de 11% à 58%, ainsi que les étapes de chromatographie ont devenus inutiles. On a remplacé par brome l'*N*-bromure de succinimide employé précédemment dans les réactions de bromination radicalaires; de cette façon la méthode de traitement du mélange réactionnel s'est bien simplifié.

D'après de nos expériences le solvant tétrachlorure de carbone utilisé ordinairement dans les réactions pareilles peut être changé par du chloroforme ou du dichlorométhane, avec lesquels ces réactions peuvent s'effectuer en température plus ambiante.

L'hydrolyse partielle du groupement cyanide en cas **105** et **106** a été exécutée par du bromure d'hydrogène dans acide acétique, ainsi on a récupéré les *C*-carboxamides des β-D-glucopyranoses (**107**, **108**) avec des rendements excellents.

Nous avons préparé la dérivé thiohydantoïne **21** en quantité de gramme et son effet sur l'activité de l'enzyme glycogène phosphorylase du foie a été examiné. Selon ces investigations la thiohydantoïne diminue significativement l'activité de l'enzyme GP *in vitro* et *in vivo*, en plus le niveau du D-glucose dans le sang dans les experiments *in vivo*. En conséquence on a développé la synthèse à grande échelle d'un produit qui peut être une remède hypoglycémique potentionnelle.[75, 76]

On a investigué les possibilité de la synthèses des analogues de la glucopy-ranosylidène-spiro-hydantoïne par une voie nouvelle photochimique selon la **Schéma 19** rétrosynthétique.

A la base des réactions similaires de littérature[51] on a essayé les photolyses de *N*-acétyle- (**92**) et de *N*-benzoyle-*N*'-(tétra-*O*-acétyle-β-D-glucopyranosyl)-urée (**93**) sous les conditions diverses.

Bien que la photocyclisation n'ait pas réussit dans norte mains, parce qu'à la place de la transformation attendue une N-déacylation de type Norrish II a été lieu, après la déprotection les acyl-glucosyl-urées se sont révélées des inhibiteirs de la GP (**92→118**, K_i = 370.5 μM; **93→119**, K_i = 4.6 μM).[80] De ce fait on a lié des groupements acyl aromatiques divers (ArCO) à la structure de base d'urée (Ar = 4-NO_2-phényle, **120**; 3-Cl-phényle, **121**; 4-OH-phényle, **122**; 1-naphthyle, **123**; 2-naphthyle, **124**; 2-indolyle, **125**). Leur fixation à l'enzyme GP a été examinée par des méthodes cinétiques et cristallographiques. Parmi ces molécules le *N*-2-naphthoyl-*N*'-β-D-glucopyranosyl urée **124** inhibite l'enzyme par un K_i nanomolaire (K_i = 0.4 μM). De cette manière ce produit est le meilleur inhibiteur analogue du D-glucose de la glycogène phosphorylase.[79]

Une propriété bien intéressante des quelques-uns de ces molécules est leurs aptitudes à s'attacher au prétendu nouveau site allostérique de la GP aussi en dehors du site actif; c'est une propriété unique parmi les inhibiteur analogue du D-glucose. Par conséquence, les *N*'-acyl-*N*-(β-D-glucopyranosyl)-urées peuvent être de nouveaux « lead structures » pour l'inhibition de la GP.

La synthèse des nouveaux glucopyranosylidène-spiro-oxadiazolines a été aussi visée (**Scheme 22.**) a la base les investigations cinétiques et cristallographiques avec les produit ci-dessus et quelques glucopyranosylidène-spiro-oxathiazoles[54] qui sont trouvés des inhibiteurs bien efficaces de la GP. On a planifié cette syntèse par la photolyse des amidoximes acétylés (**Scheme 22.**) parmis les conditions oxidatives. On a obtenu ces précurseurs a partir d'azoture de glucopyranosyle per-*O*-acetylée par la réaction avec Me_3P et puis chlorures d'hydroximoyl (**Scheme 24.**). Photolyse de *N*-β-D-glucopyranosyl-4-nitro-benzamidoxime **137** a été effectuée en présence de NBS parmi les conditions diverses (**Table 4.**). A la base de la structure des produits formés on suppose la

formation de spiro-oxadiazoline **143** désiré, dans lequel le cycle de sucre s'ouvre et un 1,2,4-oxadiazol (**145**) aromatique se produit. Cet alcool secondaire peut se transformer au oxo-composé **146** parmi les conditions reactionelles. En considération nos expériments le produit de but spiro-oxadiazoline est probablement instable et n'est pas préparable par la méthode examinée.

9. References

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Abbreviations

1-GlcNAc	*N*-acetyl-β-D-glucopyranosylamine
Ac	acetyl
ADP	adenosine diphosphate
AMP	adenosine monophosphate
aq	aqueous
Ar	aromatic ring
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphosphate
Bn	benzyl
Bu	butyl
Bz	benzoyl
cAMP	cyclic adenosine monophosphate
Ch	cyclohexyl
d	doublet
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
dd	double doublet
ddd	double double doublet
δ	chemical shift
DMF	*N*,*N*-dimethyl formamide
DMSO	dimethyl sulfoxide
eq	equivalent
ESI	electrospray ionization
FMN	flavin mononucleotide
G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate
G6Pase	glucose-6-phosphatase
GK	glucokinase
GP	glycogen phosphorylase
GS	glycogen synthase
h	hour
HGP	hepatic glucose production

His	histidine
IC_{50}	50% inhibitory concentration
IDDM	insulin dependent *diabetes mellitus*
IR	infrared
J	coupling constant
K_i	inhibitor constant
Lys	lysine
m	multiplet
MALDI-TOF	matrix assisted laser desorption ionisation-time of fligth
Me	methyl
min	minute
mp	melting point
MS	mass spectrometry
NBS	*N*-bromosuccinimide
NIDDM	non insulin dependent *diabetes mellitus*
NMR	nuclear magnetic resonance
Ph	phenyl
P_i	inorganic phosphate
R_f	retention factor
rHLGP	recombinant human liver glycogen phosphorylase
RLGP	rat liver glycogen phosphorylase
RMGP	rabbit muscle glycogen phosphorylase
rt	room temperature
s	singlet
SD	standard deviation
S_N1	monomolecular nucleophilic substitution
S_N2	bimolecular nucleophilic substitution
t	triplet
THF	tetrahydrofurane
TLC	thin layer chromatography
Ts	tosyl, 4-toluenesulphonyl
unres	unresolved
UV	ultra violet

Synthesis of Glycogen Phosphorylase Inhibitors

Glikogén-foszforiláz inhibítorok előállítása

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében a kémia tudományágban

Írta: Nagy Veronika, okleveles vegyész, kémia tanár.

Készült a Debreceni Egyetem TTK Kémia Doktori Iskolája
(*Szénhidráttartalmú természetes anyagok kémiája* c. (K/5) alprogramja) keretében

Témavezetők: Dr. Somsák László és Dr. Jean-Pierre Praly

A doktori szigorlati bizottság:

elnök: Dr. Antus Sándor ……

tagok: Dr. Kövér Katalin ……

Dr. Kuszmann János ……

A doktori szigorlat időpontja: 2003. …… … .

Az értekezés bírálói:

Dr. Bozó Éva ……

Dr. Patrick Rollin ……

A bírálóbizottság:

elnök: Dr. Lipták András ……

tagok: Dr. Szurmai Zoltán ……

Dr. Jean-Pierre Praly ……

Dr. Jean-Marc Lancelin ……

Az értekezés védésének időpontja: 2003. december

Appendix

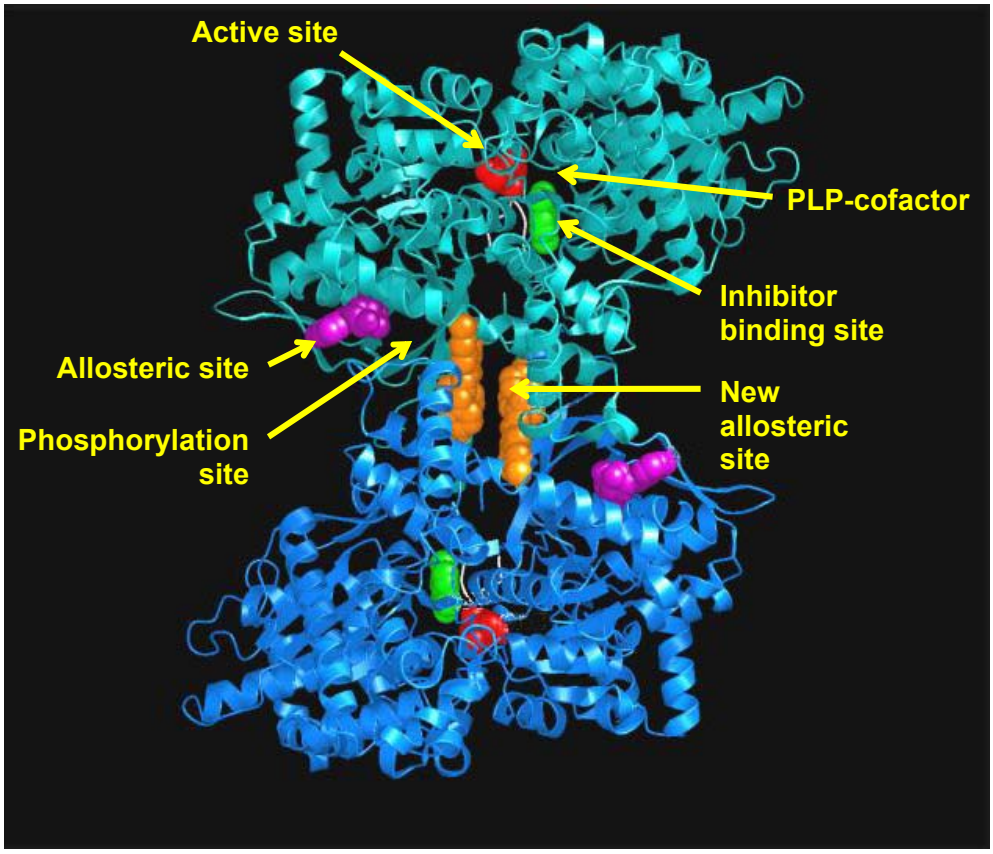

Figure I. X-Ray structure of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) showing the binding sites of effectors.[21]

Glucose (shown in red) binds at the catalytic site, which includes the essential cofactor pyridoxal 5'-phosphate (PLP). The allosteric site binds the activator AMP (shown in magenta) and other phosphorylated compounds. The inhibitor site, which binds purine compounds, nucleosides, nucleotides and flavopiridol (shown in green) is located on the surface of the enzyme. The new allosteric binding site, located inside the central cavity formed on association of the two subunits, binds e. g. indole derivatives (shown in orange).

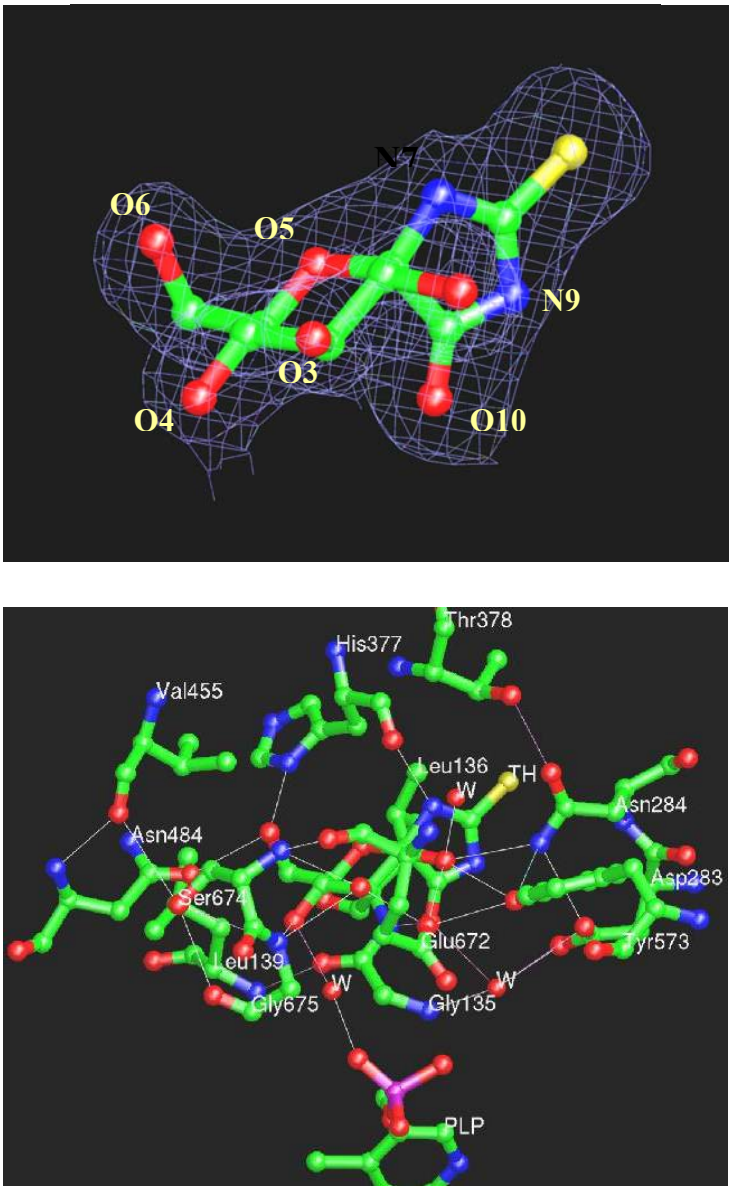

Figure II. Structure of GP-glucopyranosylidene-spiro-thiohydantoin 21 complex determined by X-ray crystallography

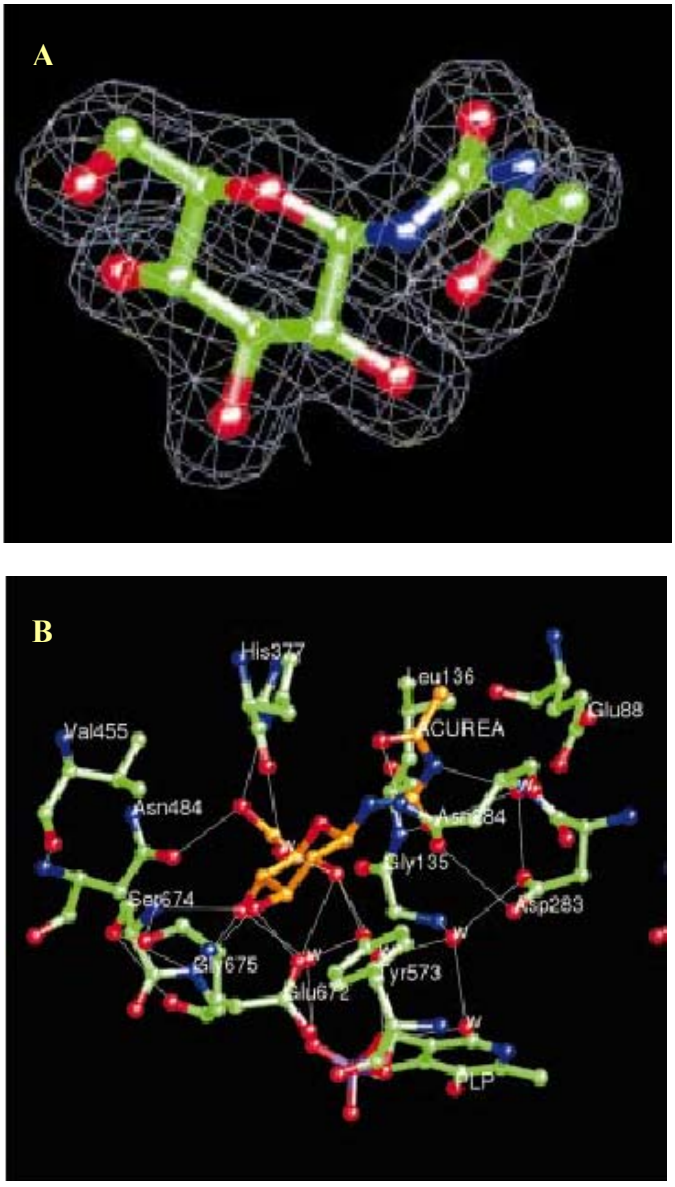

Figure III. Electron density (A) and interactions (B) of *N*-acetyl-*N*'-β-D-glucopyranosyl urea 136 at the catalitic site og RMGP*b*

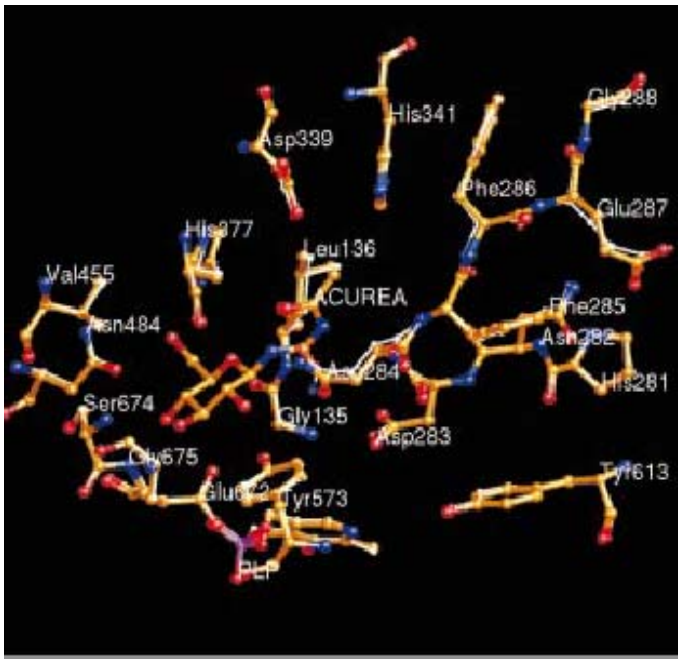

Figure IV. Comparison of 136 bound to GP*b* (orange) with native GP*b* (white) in the vicinity of the catalytic site

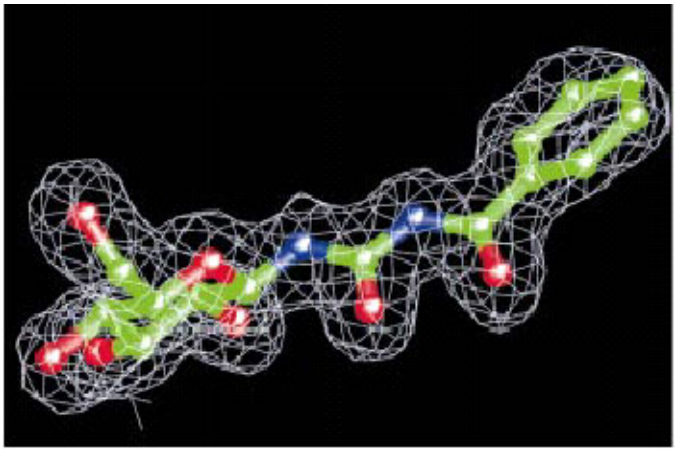

Figure V. Electron-density of *N*-benzoyl-*N*'-β-D-glucopyranosyl urea 137 at the catalytic site

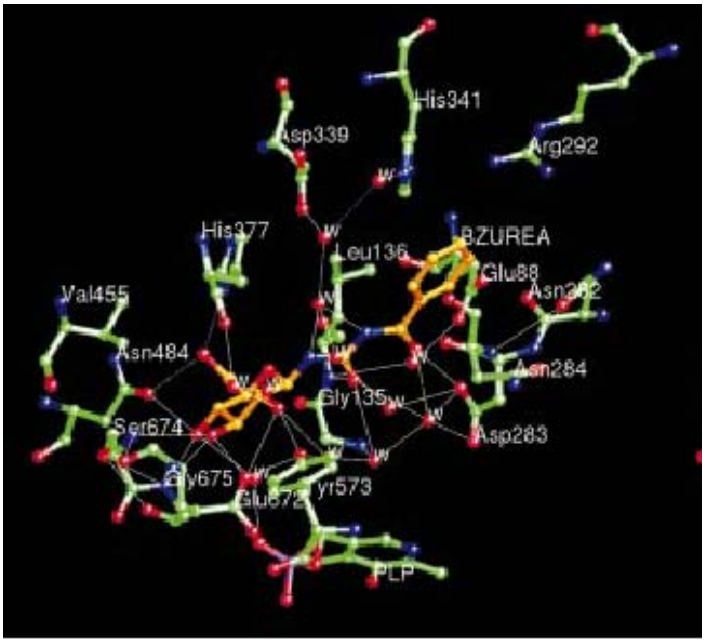

Figure VI. Interactions between *N*-bezoyl-*N*'-β-D-glucopyranosyl urea 137 and GP*b* in the vicinity of the catalytic site.

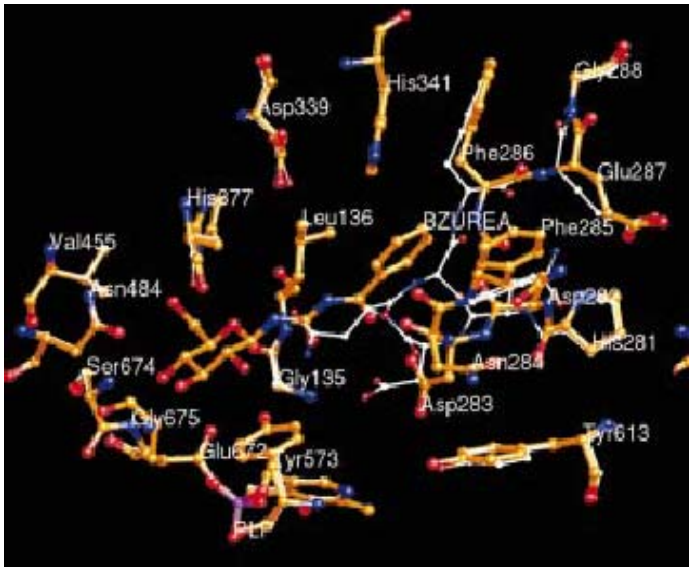

Figure VII. Comparison of *N*-bezoyl-*N*'-β-D-glucopyranosyl urea 137 bound to GP*b* (orange) with native GP*b* (white) in the vicinity of the catalytic site.

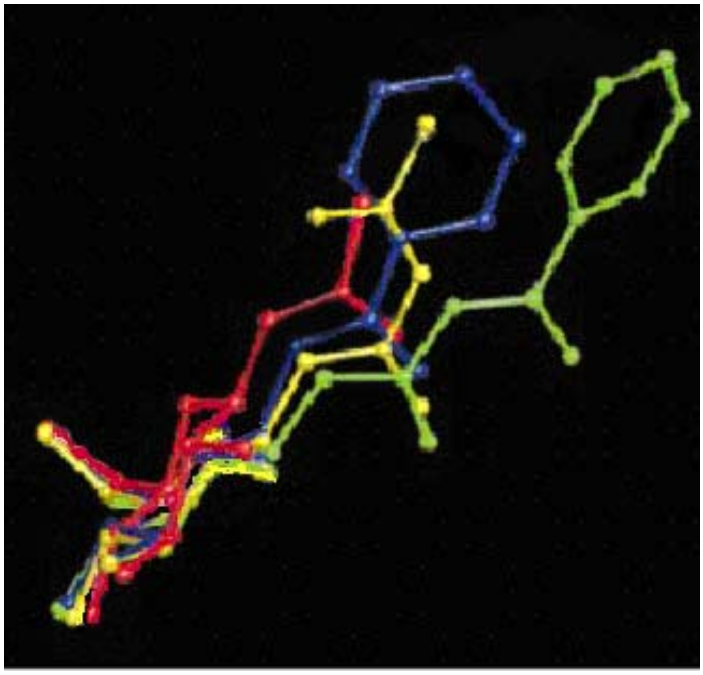

Figure VIII. Superimposition of the positions of the inhibitors 1-GlcNAc 11 (red), *N*-benzoyl-glucopyranosylamine 12 (blue), acetyl urea 136 (yellow), and benzoyl urea 137 (green) bound at the catalytic site of GPb.

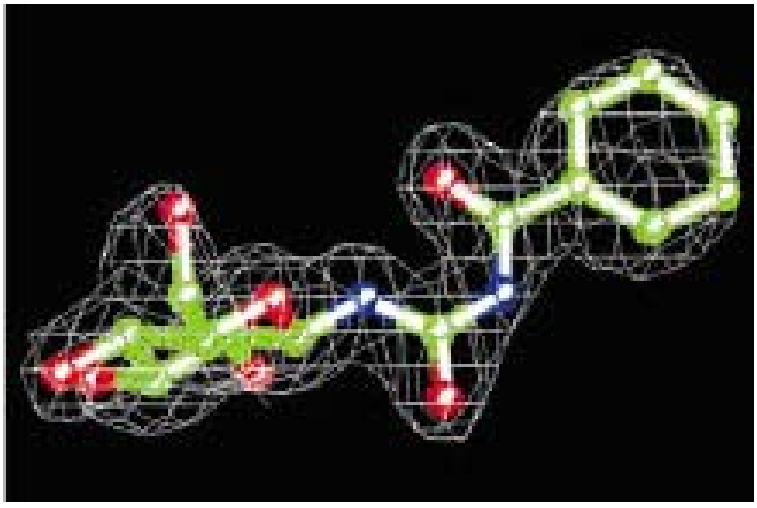

Figure IX. Electron-density map of benzoyl urea 137 at the new allosteric site.

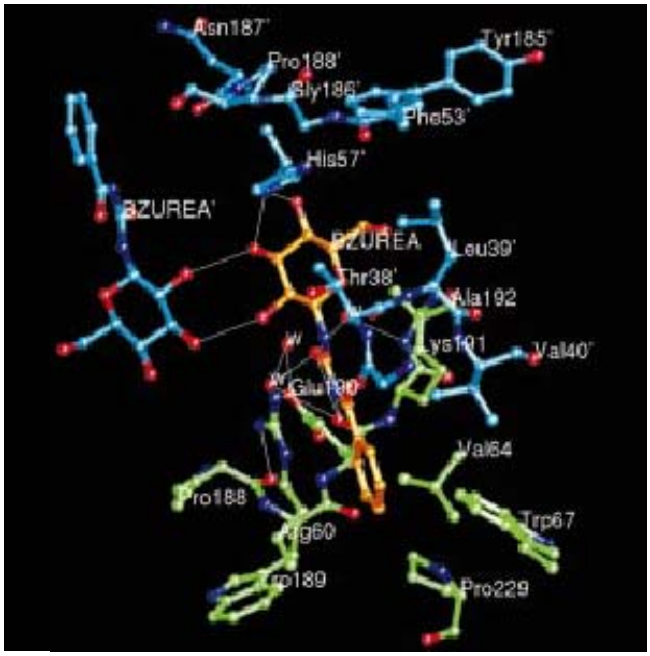

Figure X. Interactions between benzyol urea 137 and GP*b* in the vicinity of the new allosteric site.

Residues from one subunit are shown in green, and their symmetry related equivalents from the other subunit are shown in cyan.

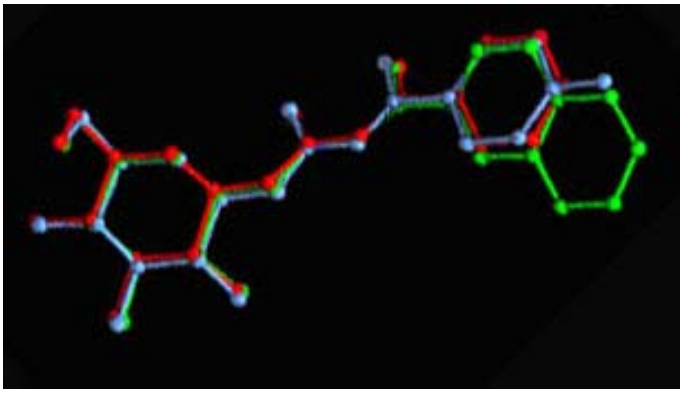

Figure XI. Superimposition of benzoyl urea 137 (red), 4-aminobenzoyl urea (blue) and 2-naphthoyl urea (green) at the active site of GP*b*.

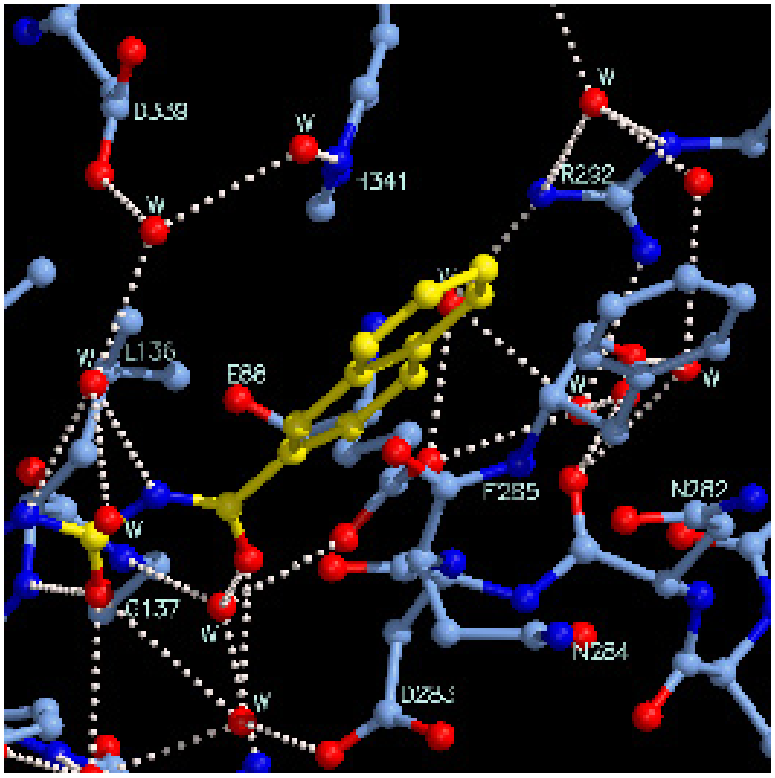

Figure XII. 2-naphthoyl moiety of 142 at the vicinity of the active site of GP

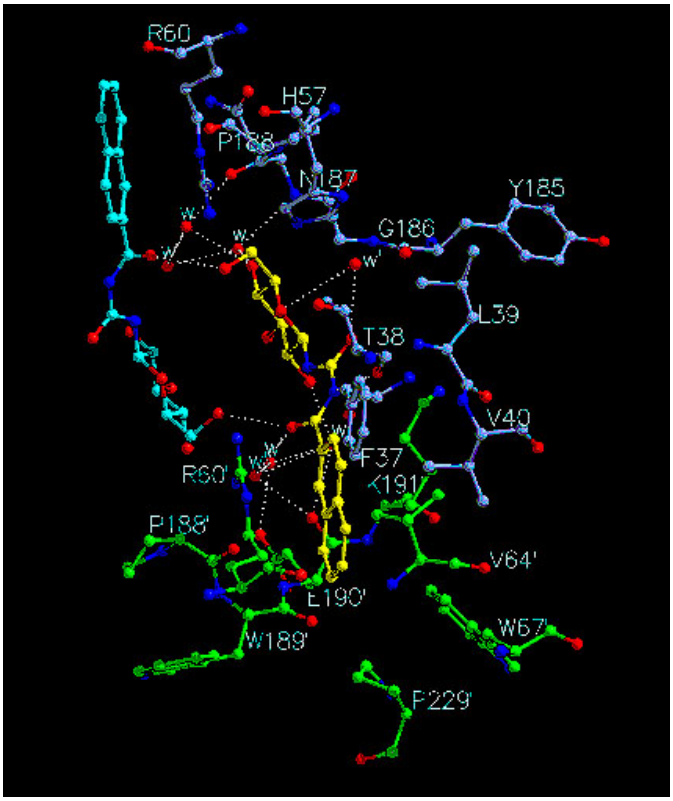

Figure XIII. Interactions between 2-naphthoyl urea 142 and GP*b* in the vicinity of the new allosteric site.

Residues from one subunit are shown in green, and their symmetry related equivalents from the other subunit are shown in cyan.