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Glucose derived inhibitors of glycogen phosphorylase☆

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ABSTRACT

Design, synthesis, and structure–activity relationships of glucose analogue inhibitors of glycogen phosphorylase are surveyed.

1. Introduction

One of the major aims of chemical biology [1], the young and developing scientific field between chemistry and biology, is to find matches between the biological and chemical space [2]. The chemical space comprises (small) molecules, some of which show complementary features to certain points of the biological space constituted by the structure of binding sites of biomacromolecules (mainly but not only proteins). Good matches may result in efficient agonists/antagonists of receptors or activators/inhibitors of enzymes. Such interactions contribute to the basic understanding of the way of biological action of the macromolecule, and may ultimately be utilised in drug design and discovery.

In the context of this survey, the biological space is represented by glycogen phosphorylase (GP), the main regulatory enzyme of glycogen metabolism. GP, catalysing the rate-determining step of glycogen degradation in the liver by phosphorolysis, is directly responsible for the regulation of blood glucose levels. Thus, the enzyme has become a validated target in combatting non-insulin-dependent, or type 2, *diabetes mellitus* (NIDDM or T2DM), and its inhibitors are considered as potential antidiabetic agents. The biochemical and pharmacological background of this research has been amply summarized in several reviews of the past decade, therefore, the reader is kindly referred to those papers [3–8].

Diverse classes of compounds [4,9–12] can be found among inhibitors of GP binding to one (or in specific cases more) of the so far discovered binding sites of the enzyme (Fig. 1). The most populated class of compounds is that of glucose derivatives, first proposed and investigated [4,13,14] by Fleet, Johnson, and Oikonomakos,[1] which bind primarily to the active site of GP. This article highlights the most important "historical" moments of GP inhibitor design among glucose analogues, and the main emphasis is put on developments of the past couple of years, not, or not fully, included in the last comprehensive reviews [11,12]. Although the design of compounds was heavily based on and supported by results of crystallographic investigations of enzyme–inhibitor complexes and molecular dockings, the syntheses and structure–activity relationships of the inhibitors are pointed out in this overview.

2. Early glucose analogue inhibitors of glycogen phosphorylase

The weak binding of D-glucose anomers **1** and **2** to the catalytic site of GP to act as the physiological regulator of

☆ Dedicated to Professor András Lipták on the occasion of his 75th birthday.

Adresse e-mail : somsak@tigris.unideb.hu.

[1] Passed away on Aug 31, 2008.

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doi:10.1016/j.crci.2010.09.004

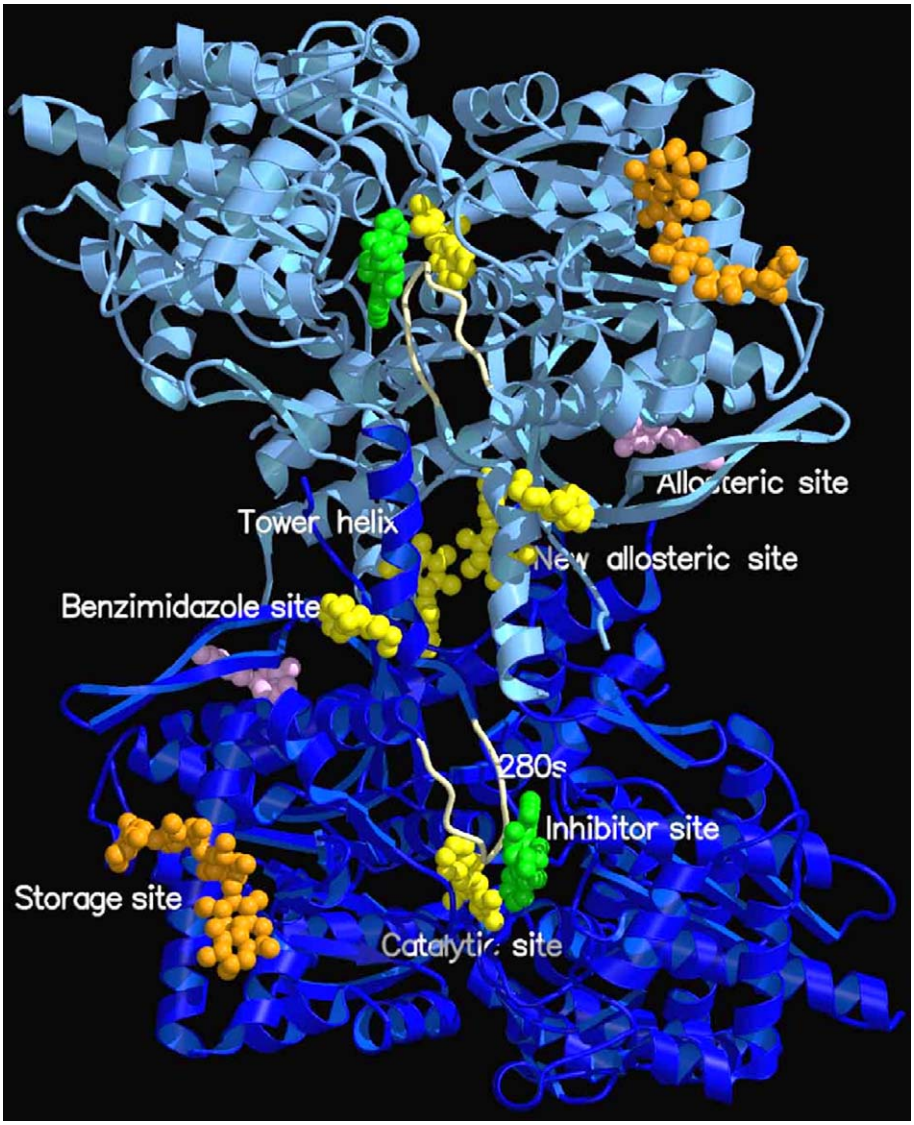


Fig. 1. A schematic diagram of the muscle GPb dimeric molecule viewed down the molecular dyad. The positions are shown for the catalytic, allosteric, glycogen storage, the caffeine, the indole site, and the novel binding site for benzimidazole. The catalytic site, marked by 2-β-D-glucopyranosyl benzimidazole, is buried at the centre of the subunit and is accessible to the bulk solvent through a 15 Å-long channel. Binding of the competitive inhibitor benzimidazole promotes the less active T state through stabilization of the closed position of the 280 s loop (shown in white). The allosteric site, which binds the activator AMP (indicated in the figure), is situated at the subunit–subunit interface some 30 Å from the catalytic site. The inhibitor site or caffeine binding site, which binds purine compounds, such as caffeine and flavopiridol (indicated), is located on the surface of the enzyme some 12 Å from the catalytic site and, in the T state, obstructs the entrance to the catalytic site tunnel. The glycogen storage site (with bound maltopentaose) is on the surface of the molecule some 30 Å from the catalytic site, 40 Å from the allosteric site and 50 Å from the new allosteric inhibitor site. The new allosteric or indole binding site, located inside the central cavity, formed an association of the two subunits, bound indole-2 carboxamide analogues, *N*-benzoyl-*N*'-β-D-glucopyranosyl urea, and benzimidazole (indicated). The novel binding site with bound benzimidazole, also located on the surface of the molecule, is some 31 Å from the catalytic site, 32 Å from the allosteric site, and 32 Å from the indole site (figure by courtesy of N.-G. Oikonomakos and E.-D. Chrysina).

the enzyme [15] raised the possibility to design glucose derivatives with much higher affinity to the active site. Enzymatic tests of a large series of α- and β-D-glucopyranosides, 1-thio-D-glucopyranosides, *N*-acyl-β-D-glucopyranosylamines and related compounds [13] revealed 1-deoxy-D-*gluco*-heptulopyranose 2-phosphate (**3**) and *N*-acetyl-β-D-glucopyranosylamine (**4**) as the first glucose derivatives with an inhibitor constant (K_i) in the low micromolar range. Anhydro-heptonamides **5** and **6** were less effective, however, a formal combination of **6** with an anomeric substituent similar to that of **4** gave again a low micromolar inhibitor **7**. Ring closure of **7** to glucopyranosylidene-spiro-hydantoin **8** strengthened the binding by a factor of ~5. The spiro-epimeric hydantoin **9** proved much less efficient, indicating that the presence of a β-D-anomeric NH was very important to make a good inhibitor. This was rationalized by crystallographic investigation of the enzyme–inhibitor complex [16] to show the presence of a specific H-bridge between NH and His377 next to the catalytic site also present in *N*-acyl-β-D-glucopyranosyla-

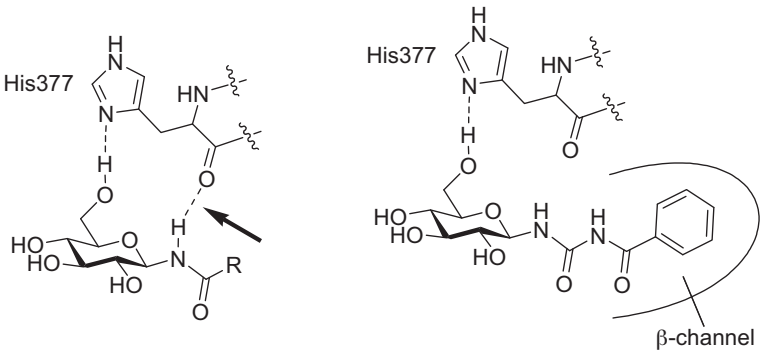


Fig. 2. Outline of binding of glucose analogues at the active site of glycogen phosphorylase (GP) highlighting (a) important H-bonds between *N*-acyl-β-D-glucopyranosylamine type inhibitors and His377 and (b) binding modes of *N*-acyl-*N*′-β-D-glucopyranosyl ureas as observed by X-ray crystallography.

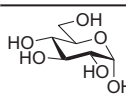
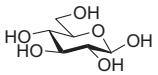
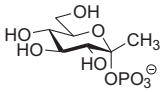
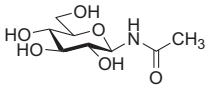
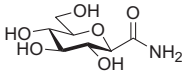
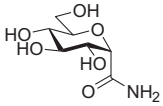
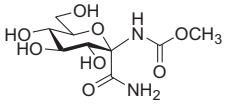
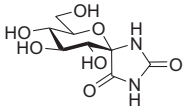
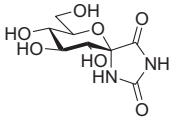
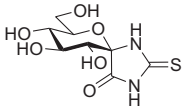
1		1700[21]
2		7400[21]
3		14[22]
4		32[23]
5		440[24]
6		370[24]
7		16[18]
8		3.1[17] 4.2[25]
9		320[18] 105[25]
10		5.1[25,26]

Fig. 3. Inhibition of glycogen phosphorylase (GP) by D-glucose and the most efficient inhibitors of early glucose analogue derivatives (K_i [μM] against RMGP*b*) [21–26].

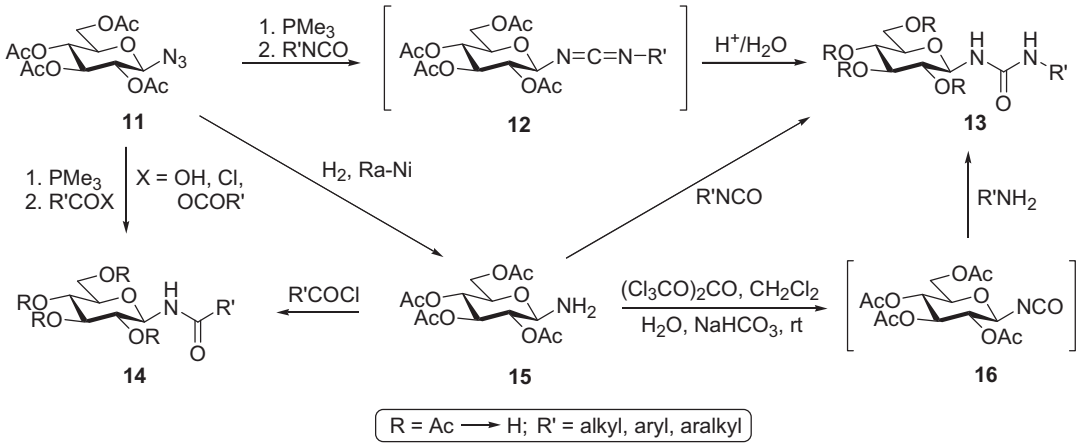
mine type inhibitors (Fig. 2a for an illustration). The synthetic problems with the stereoselective preparation of the properly configured spiro-hydantion **8** [17–19] were essentially overcome by the highly stereoselective synthesis of spiro-thiohydantoin **10** [20] which proved equipotent with **8** (Fig. 3).

3. Glucose derivatives tested recently as inhibitors of glycogen phosphorylase

3.1. N-Acyl-β-D-glucopyranosylamines and related compounds

Following the success of the first *N*-acyl-β-D-glucopyranosylamine type inhibitors like **4**, several modifications of the acyl group were carried out. A widely applied general method for the preparation of such compounds starts with the reaction of per-*O*-acetylated β-D-glucopyranosyl azide **11** with triaryl- or trialkyl phosphanes (PMe_3 proved the most advantageous [27]) and the intermediate phosphinimine is then reacted with a carboxylic acid or acid chloride or anhydride to get protected amides **14** (Scheme 1; for an exhaustive review see [28]). Reduction of **11** to **15** followed by acylation can be an alternative synthetic route. Subsequent deprotection yields test compounds of type **14** (R = H), and several recent examples as inhibitors of rabbit muscle GP *b* (RMGP*b*) are shown in Fig. 4.

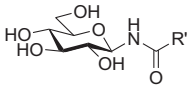
Substitution in the methyl group of *N*-acetyl-β-D-glucopyranosylamine makes the inhibition weaker (Fig. 4, compare **4** and **14a,b**). The α-anomeric trifluoroacetamide **17** proved configurationally stable (for a discussion on the stability of *N*-acyl-glycosylamine anomers, see ref. [27]) but showed no inhibition. From a larger collection of monoamides of dicarboxylic acids, **14c** showed similar inhibition to that of **4**, while its methylester **14d** proved significantly weaker. In the series of oxamic acid derivatives, the efficiencies of acid **14e** and ester **14f** were reversed, both being much less effective than **4**. Introduction of a large side chain as in **14g** made a weak inhibitor. Among aromatic amides, the 2-naphthoyl derivative **14h** proved the most efficient, and in this series, the position occupied by the aromatic moiety becomes also important (Fig. 5 also). Necessity of the intact homoaro-



Scheme 1.

matic system is indicated by 1,4-benzodioxane carboxamide **14i**. Changing the acyl part to a dimethoxyphosphoryl residue (**18**) resulted in a practical loss of inhibition.

Syntheses of analogues **19** of spiro-hydantoins **8-10** were envisaged by photocyclization of acyl urea derivatives **20** outlined in Scheme 2a. To this end, reported cyclizations of 3-oxoalkyl glycosides [38,39] **23** resulting in stereoselective formation of spiro-acetals **24** (Scheme 2b) served as analogies. Thus, a photoexcitation of **20**



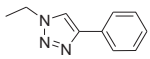
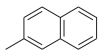
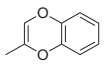
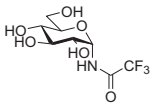
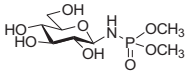
	R'	K_i [μM]
4	$-CH_3$	32[23]
14a	$-CF_3$	75[29]
14b	$-CH_2N_3$	49[30]
14c	$-CH_2CH_2COOH$	20[31]
14d	$-CH_2CH_2COOCH_3$	170[31]
14e	$-COOH$	710[32]
14f	$-COOCH_3$	210[32]
14g		180[33]
14h		10[34] 13[35]
14i		85[36]
17		No inh.[26]
18		5900[37]

Fig. 4. Inhibition of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) by *N*-acyl-β-D-glucopyranosylamines and related compounds. Illustrative examples of the most efficient members of larger series of compounds detailed in the referred papers [29–37].

might have resulted in intermediate **22** which, upon intramolecular hydrogen abstraction to give **21** and subsequent radical combination could have given the target compounds **19**.

To test this hypothesis, *N*-acyl-*N'*-β-D-glucopyranosyl ureas of type **27** (Scheme 3) were needed. Only two examples of this class of compounds were known in the literature [40] which were obtained by a modification of the original synthesis. Azide **11** was transformed to urea **26** by Pintér et al.'s method [41] and then acylation was carried out to give **27** (R = Ac, R' = Me or Ph). Irradiation of **27** under various conditions brought about a Norrish I type cleavage of the R'CO moieities leading back to **26** instead of the expected Norrish II type cyclization [42]. Quite unexpectedly, the deprotected compounds **27** (R = H, R' = Me K_i = 305 μM; R' = Ph K_i = 4.6 μM) proved efficient inhibitors of GP [43] and the benzoyl derivative had similar potency to those of spiro-hydantoins **8** and **10**. Initiated by this serendipitous finding, synthetic and enzymatic studies were started to get insight in structure–activity relationships of β-D-glucopyranosyl derivatives attached to aromatic rings by linkers of 3-6 atoms analogous to amide groups.

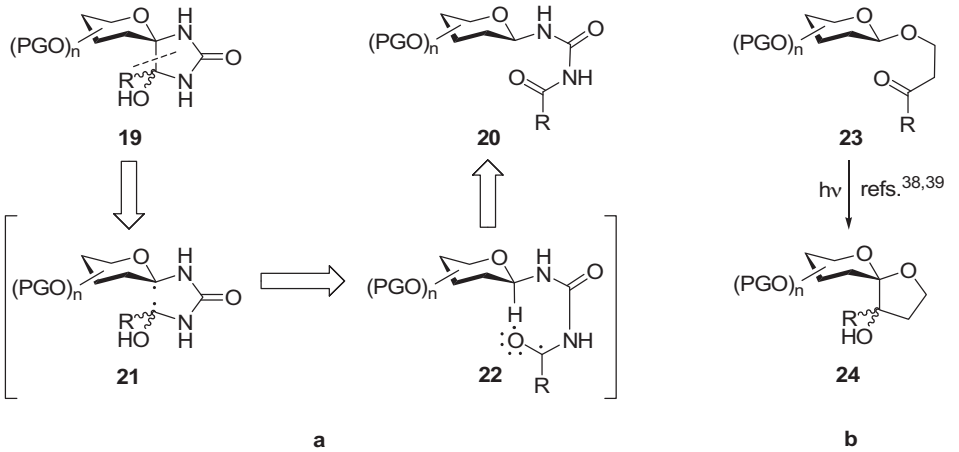
N-Aryl-*N'*-β-D-glucopyranosyl ureas **13** were obtained (Scheme 1) either via acid catalysed hydration of carbodiimide **12** obtained from azide **11** by a Staudinger type transformation, or by reacting glucosylamine **15** with isocyanates, or by *in situ* conversion of **15** into glucosylisocyanate **16** [44] followed by amine addition. Removal of the protecting groups was straightforward under Zemplén conditions. Further compounds of the protected *N*-acyl-*N'*-β-D-glucopyranosyl urea series **27** (Scheme 3) were obtained in reactions of glucosylamine **15** with acylisocyanates or from glucosylisocyanate **16** upon treatment with arenecarboxamides. During these syntheses, anomerization was observed in almost every cases thereby diminishing the yield of the target compounds [45]. Furthermore, deprotection of acyl ureas **27** was always accompanied by the cleavage of the R'CO group, both under base or acid catalysed transesterification conditions. These side reactions could be circumvented by the addition of unprotected β-D-glucopyranosylamine obtained *in situ* from β-D-glucopyranosylammonium carbamate [46] (**25**)

Entry	linker	Ar A	Ar B	Ar C
1.	NHCO	81[23] 144 [26]	191[35] 444[34]	10[34] 13[35]
2.	NHCONH	18[48]	350[48] (IC_{50})	5.2[48]
3.	$NHCOCH_2$	1100 (IC_{50}) [34]	-	-
4.	NHCONHCO	4.6 [43]	10[42]	0.35[42]
5.	$NHCONHCH_2$	42 % (1 mM) [48]	-	-
6.	$NHCOCH_2CH_2$	85[34]	-	-
7.	NHCOCH=CH	18[34]	-	3.5[34]
8.	CH_2COCH=CH	-	-	52 % (100 μM)[49]*
9.	NHCOC≡C	62[34]	-	-
10.	NHCOCONH	100[50]	144[50]	56[50]
11.	CONHCONH	No inh.[48]	-	-
12.	CONHNHCO	22 % (3.75 mM)[50]	-	-
13.	NHCONHCONH	21[47]	-	-
14.	NHCONHCONHCO	-	-	45 % (625 μM)[42]
15.		151[35] 162[51]	136[35] 625[51] (IC_{50})	16[35] 36[51]

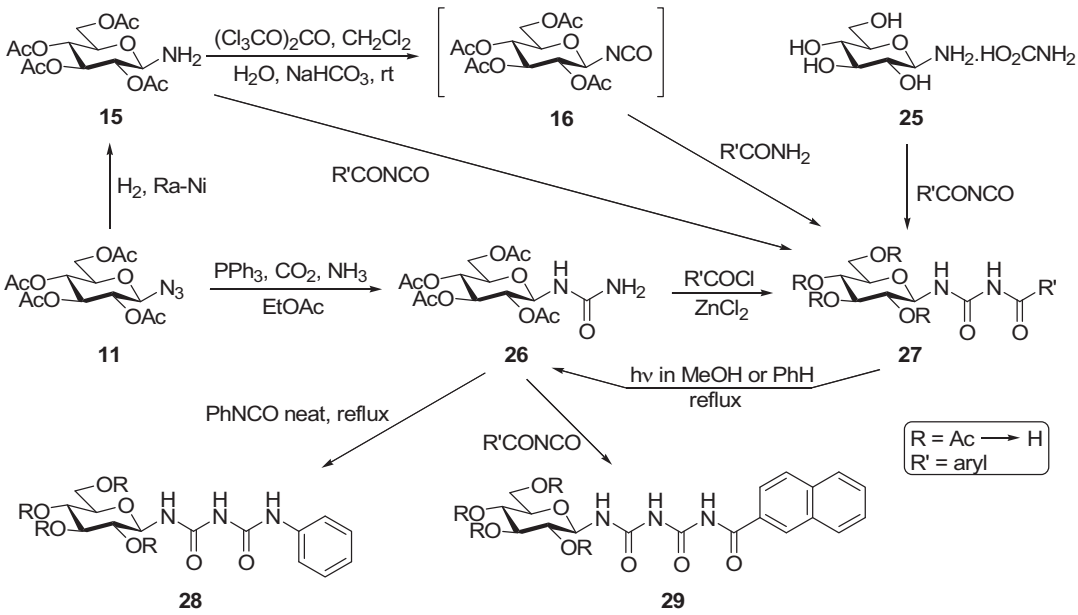
Fig. 5. Comparison of inhibition of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) (K_i [μM]) by *N*-acyl-β-D-glucopyranosylamines, *N*-substituted-*N*′-β-D-glucopyranosyl ureas and related compounds. Against rat liver glycogen phosphorylase (GP) [48–51].

to various acyl-isocyanates to give directly the unprotected **27** ureas [45]. Biurets **28** [47] and **29** [42] were prepared in reactions of urea **26** with phenyl and 2-naphthoyl isocyanates, respectively.

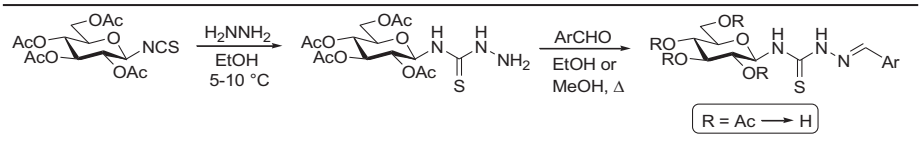
Most important results of the enzyme kinetic studies are collected in Fig. 5. Comparison of entries 1, 2, 4, 13, and 14 shows that the inhibition is strongest for the acyl urea type compounds (entry 4). Introduction of a tetrahedral element into the linker makes weaker inhibitors (compare entries 2–3, 4–6). Replacement of one NHCO by a more rigid bond (entries 4, 7, 9) seems less detrimental, although the inhibition is weakened, showing the necessity of a polar part



Scheme 2.



Scheme 3.



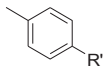
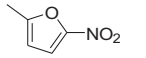
Ar	R'	K_i [μM]	Ar	Inhibition
	H	33[54]		K_i 105 μM[55]
	Cl	26[54]		
	Br	49[55]		8 % (at 625 μM)[54]
	Me	101[55]		

Fig. 6. Synthesis of aldehyde 4-(β-D-glucopyranosyl)thiosemicarbazones and their enzymatic evaluation against rabbit muscle glycogen phosphorylase *b* (RMGP*b*) [54,55].

capable for participation in H-bonds as well. Entries 7 and 8 indicate again that higher flexibility due to a rotatable element of the linker is not advantageous (of course, the absence of the H-bond donor amide moiety from the anomeric carbon must also contribute to the weaker binding). Constitutional isomers of the NHCONHCO moiety (entries 10–12) also make significantly less efficient inhibitors. Comparison of columns A–C demonstrate the importance of the size and orientation of the aromatic appendage the 2-naphthyl derivatives exhibiting the strongest binding. Accordingly, *N*-2-naphthoyl-*N'*-β-D-glucopyranosyl urea (entry 4C) was the first nanomolar glucose analogue inhibitor of GP. Protein crystallography showed acyl ureas of entries 4A and 4C to bind also to the new allosteric site of the enzyme [43].

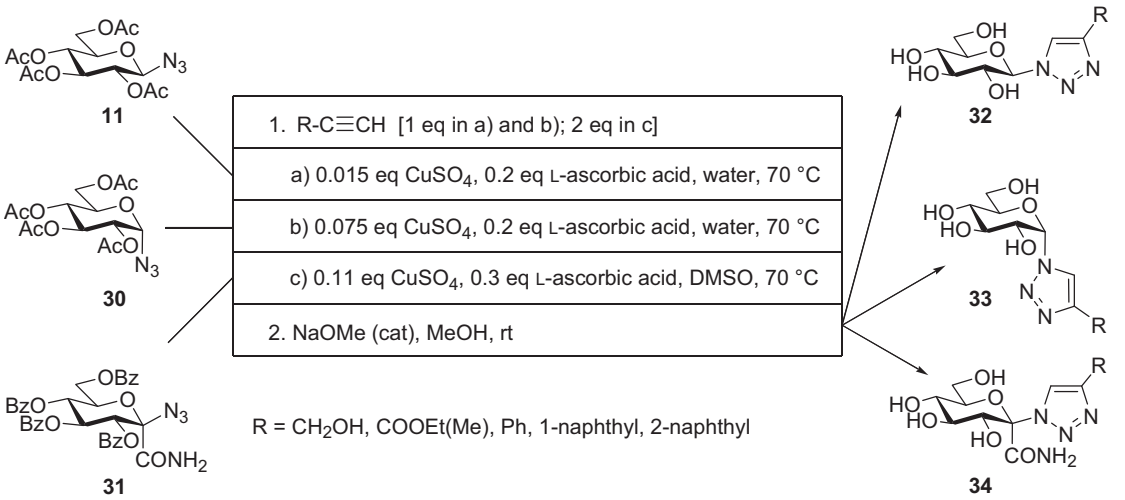
X-Ray crystallographic studies of GP–*N*-acyl-*N'*-β-D-glucopyranosyl urea complexes revealed that, contrary to the *N*-acyl-β-D-glucopyranosylamines, there is no H-bond between the β-anomeric NH and His377 (Fig. 2b) [43]. As the acyl ureas are much more inhibitory than the corresponding glucosylamines (Fig. 5, entries 1 and 4), the stronger binding must be due to extended interactions of the urea and especially the aromatic parts of the molecules in the β-channel[2] of the enzyme. This observation was utilized in further inhibitor design discussed in Section 3.4.

Very recently, a new series of aldehyde 4-(β-D-glucopyranosyl)-thiosemicarbazones [52,53] was prepared from per-*O*-acetylated β-D-glucopyranosylisothiocyanate (Fig. 6) and several of them showed micromolar inhibition.

3.2. *N*-β-D-glucopyranosyl heterocycles

The problems encountered in the synthesis of *N*-acyl-β-D-glucopyranosyl ureas necessitated a quest for more stable compounds. To this end, bioisosteric replacement of

[2] The β-channel or β-pocket is an empty space next to the catalytic site of GP in the direction of the β-anomeric substituent of bound D-glucose surrounded by amino acid side chains of mixed character.



Scheme 4.

NHCO moieties in acyl ureas and related compounds was envisaged. As the first example of such studies, the NHCO unit of *N*-acyl-β-D-glucopyranosylamines was changed to 1,2,3-triazole because some literature examples indicated similarities [56] of these two moieties. Three series of 1-D-glucopyranosyl-4-substituted-1,2,3-triazoles [51] were prepared by copper(I) catalysed azide-alkyne cycloaddition (CuAAC) [57] outlined in Scheme 4. From β-D-glucopyranosyl azide **11** conditions 1a, frequently applied in the literature, proved to be a straightforward way to the per-*O*-acetylated 1-β-D-glucopyranosyl-4-substituted-1,2,3-triazoles in 58–96% yields. Transformations of the α-azide **30** required higher catalyst loads (conditions 1b) and the yields for the corresponding per-*O*-acetylated 1-α-D-glucopyranosyl-4-substituted-1,2,3-triazoles were lower (36–72%). The aqueous conditions were unsatisfactory for the reactions of (hept-2-ulopyranosylazide)onamide **31** for which conditions 1c were found the best to give 75–87% of the corresponding *O*-protected glucosyl triazoles with 51–73% conversion of the starting **31** in one day. Removal of the protecting groups was effected by the Zemplén protocol to give triazoles **32-34** in generally very good yields.

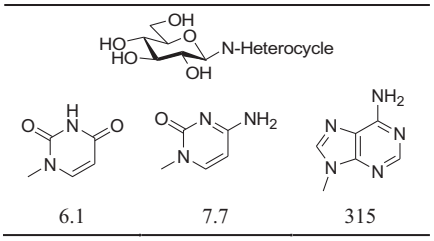
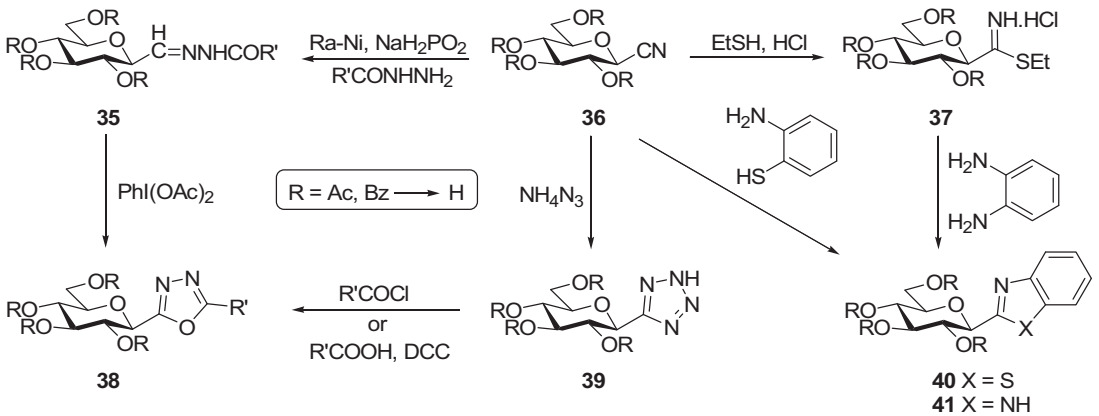
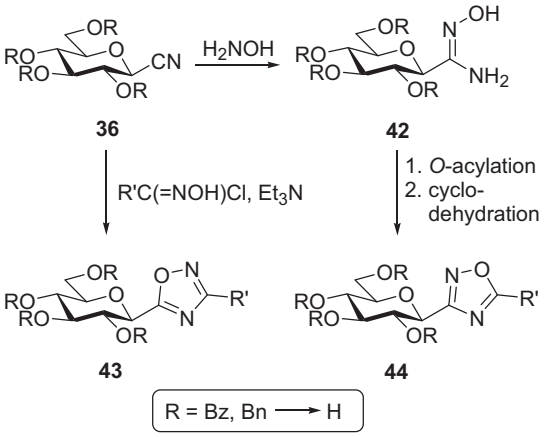


Fig. 7. Inhibitory effect of β-D- glucopyranosyl nucleosides against rabbit muscle glycogen phosphorylase *b* (RMGP*b*) [58] (K_i [μM]) [58].

From these 1,2,3-triazoles, only compounds **32** showed significant inhibition (e.g. R = CH_2OH K_i = 26 μM [51] or 14 μM; [35]). Inhibitor constants for other members of this series can be found in Fig. 5, entry 15 to show acceptable similarity with those of glucosyl amides in entry 1. Comparative crystallographic studies of the amide and triazole series revealed that pairs of the compounds with the same aglycon bound to the enzyme in essentially the



Scheme 5.



Scheme 6.

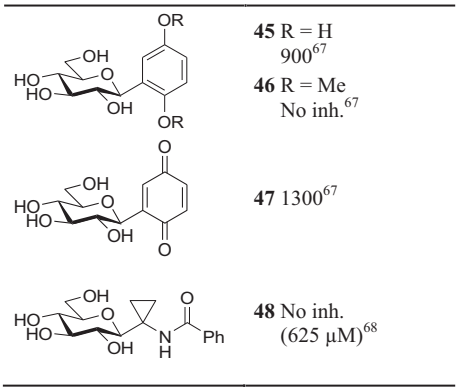


Fig. 9. β-D-Glucopyranosyl carbocycles as inhibitors of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) (K_i [μM]).

same way in most cases [35]. Thereby, the bioisosteric relationship for NHCO-1,2,3-triazole was proven for the GP case as well.

Investigations of some *N*-β-D-glucopyranosyl derivatives of pyrimidine and purine heterocycles ("glucosyl nucleosides") showed these compounds to have inhibitory effect towards GP, and the best inhibitors are collected in Fig. 7.

3.3. *C*-β-D-glucopyranosyl derivatives

The first *C*-β-D-glucopyranosyl heterocycles tested as inhibitors of GP were methyl-1,3,4-oxadiazole **38**, tetrazole **39**, benzothiazole **40**, and benzimidazole **41** (Scheme 5, R = H in each) [59]. Common starting material for the syntheses of these compounds was the per-*O*-acetylated or -benzoylated 2,6-anhydro-aldononitrile (β-D-glucopyranosyl cyanide) **36**. 1,3-Dipolar cycloaddition of protected **36** with azide ion gave 5-β-D-glucopyranosyl tetrazole **39** which was transformed into 2-β-D-glucopyranosyl-5-substituted-1,3,4-oxadiazoles **38** via an *N*-acyl-nitrilimine intermediate obtained by acylation of **39** [59,60]. Oxadiazoles **38** could also be prepared by oxidation [60] of 2,6-anhydro-aldose acylhydrazones [61] **35**, and the two pathways proved comparable with respect to yields and operational difficulties. Nitrile **36** was ring-closed to benzothiazole **40** with 2-aminothiophenol. The analogous reaction with 1,2-diaminobenzene was unsuccessful, therefore, benzimidazole **41** was obtained via thioimidate **37**. Deprotection was carried out by the Zemplén method.

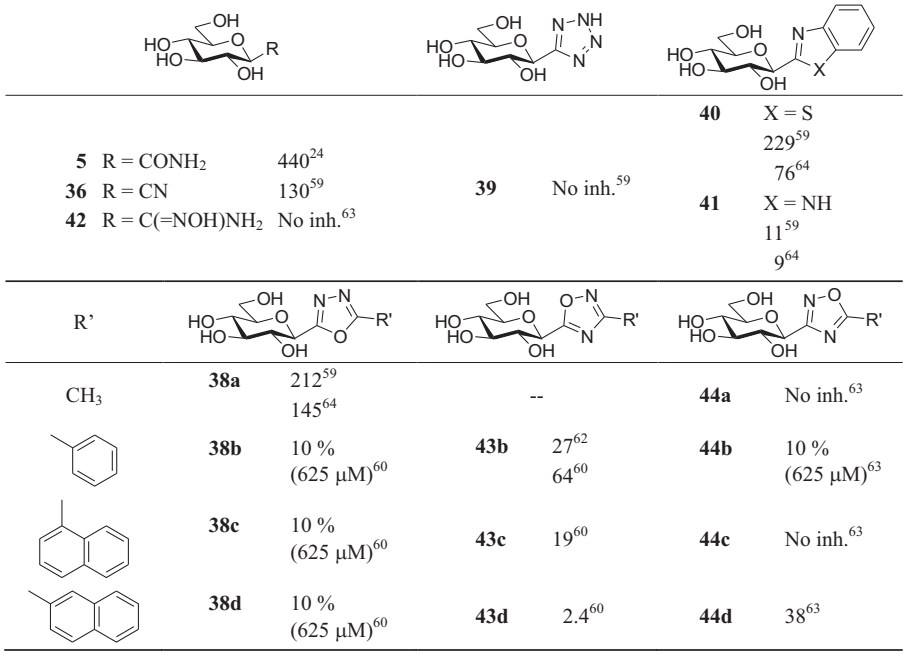
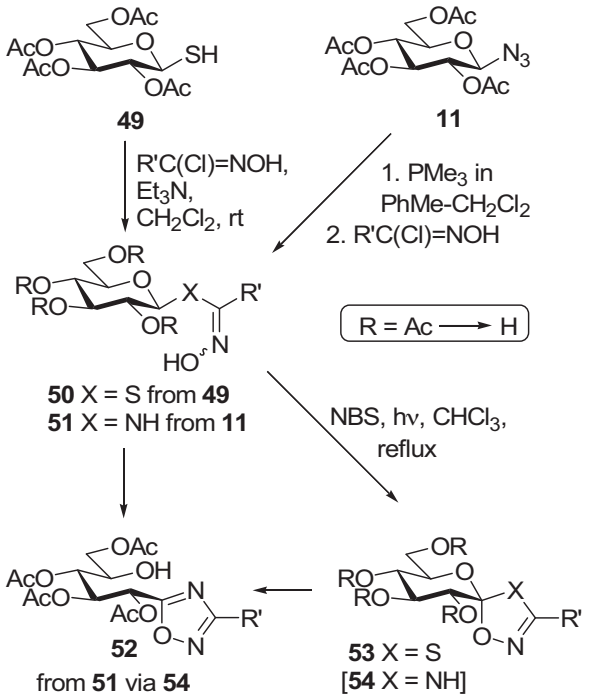


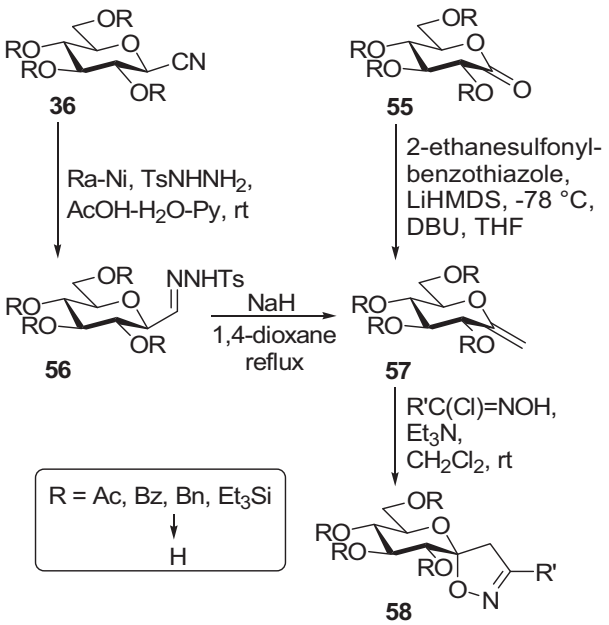
Fig. 8. *C*-β-D-glucopyranosyl heterocycles and their precursors as inhibitors of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) (K_i [μM]).



Scheme 7.

Per-*O*-benzoylated or -benzylated nitriles **36** were also transformed into two other series of 1,2,4-oxadiazoles (Scheme 6). 1,3-dipolar cycloaddition with nitrile-oxides generated *in situ* furnished 5-β-D-Glucopyranosyl-3-substituted-1,2,4-oxadiazoles **43** [60,62]. Addition of hydroxylamine to **36** produced amidoxime **42** which upon *O*-acylation with either carboxylic acids or acid chlorides followed by cyclodehydration gave 3-β-D-glucopyranosyl-5-substituted-1,2,4-oxadiazoles **44** [63]. The protecting groups were removed by standard methods.

Results of enzyme kinetic studies are presented in Fig. 8. β-D-Glucopyranosyl cyanide **36** is a somewhat better inhibitor than anhydro-aldonamide **5**, while tetrazole **39** and amidoxime **42** are inactive. Benzimidazole **41** binds



Scheme 8.

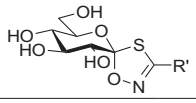
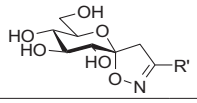
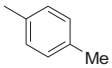
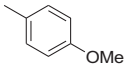
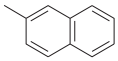
R'					
	**53a**	26		**58a**	19.6
		-		**58b**	7.9
			Refs.[69,70]		Ref.[74]
	**53c**	8.2		**58c**	6.6
	**53d**	0.16		**58d**	0.63

Fig. 10. Inhibition of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) (K_i [μM]) by glucopyranosylidene-spiro-heterocycles [74].

stronger than benzothiazole **40**, and this can be attributed to the H-bond between the NH of the heterocycle and His377 which is necessarily absent for **40**. X-ray crystallography has shown **41** also to be present at the new allosteric site and the new "benzimidazole site" has been discovered by investigating this compound (Fig. 1) [64]. From the three oxadiazole series (**38**, **43**, **44**), compounds **43** are the most active. The tendency of strengthening the inhibition by a large and properly oriented aromatic substituent can be observed in the oxadiazoles, too: compounds with a 2-naphthyl appendage (**43d**, **44d**) are the best inhibitors. Although all three oxadiazoles could be considered as bioisosteric replacements [65,66] of NHCO, these results suggest that in the case of GP, 5-β-D-glucopyranosyl-3-substituted-1,2,4-oxadiazoles **43** are the best choice.

β-D-Glucopyranosyl hydroquinone derivative **46** in its *O*-acetyl protected form was prepared by aromatic electrophilic substitution in 1,4-dimethoxybenze using penta-*O*-acetyl-β-D-glucopyranose as a source of glucosylium ion. Subsequent oxidation gave protected benzoquinone **47** which was reduced to **45** [67]. The deprotected

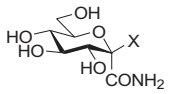
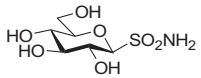
	 X	K_i [μM]
6	H	370[24]
7	NHCOOMe	16[18]
59	OEt	21 % (at 625 μM)[75]
60	SPh	No inh. (at 625 μM)[75]
61	NHPh	No inh. (at 625 μM)[75]
62	N_3	1800[78]
63	NHCOMe	310[78]
64		No inh. (at 625 μM)[76]

Fig. 11. Inhibition of rabbit muscle glycogen phosphorylase *b* (RMGP*b*) by various monosaccharide derivatives [78].

compounds were moderately inhibitory against GP (Fig. 9). Cyclopropane **48** was obtained from per-*O*-benzoylated nitrile **36** by EtMgBr–Ti(O*i*Pr)$_4$ followed by Zemplén deprotection [68]. This compound had no inhibition of GP.

3.4. Glucopyranosylidene-spiro-heterocycles

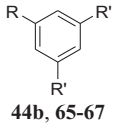
Studies on *N*-acyl-β-D-glucopyranosylamines and *N*-acyl-*N*'-β-D-glucopyranosyl ureas allowed one to conclude that it is possible to make very efficient inhibitors even in the absence of a H-bond to His377, provided that interactions in the β-channel are strong enough. Combining these facts with the spirobicyclic structure of hydantoins, a novel design principle for efficient glucose-based inhibitors of GP could be set up [69,70]:

- such molecules should have a rigid spirobicyclic scaffold in which a (preferably five-membered hetero) cycle is attached to the anomeric carbon of D-glucopyranose;
- this cycle, although it may, should not necessarily be a H-bond donor towards His 377;
- a suitably oriented, large aromatic appendage must be present on this cycle to fit into the β-channel.

This principle was first verified by spiro-oxathiazolines **53**, the synthesis of which followed well elaborated pathways [71] (Scheme 7): per-*O*-acetylated 1-thio-β-D-glucopyranose **49** was reacted with *in situ* generated nitrile-oxides to give hydroximothioates **50** which underwent a ring-closure upon oxidation by NBS to yield the target compounds **53** after Zemplén deprotection. Synthesis of the analogous spiro-oxadiazoline **54** was also attempted. To this end, glucosyl azide **11** was transformed in a Staudinger type reaction into *N*-β-D-glucopyranosyl amidoxime **51**. Oxidative treatment of **51** gave oxadiazole **52** probably via **54**. The driving force for the tautomeric ring opening must be the aromatization of the heterocycle.

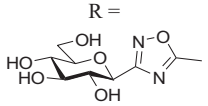
A series of glucopyranosylidene-spiro-isoxazolines **58** was prepared by 1,3-dipolar cycloaddition of nitrile-oxides to *exo*-glycals **57** (Scheme 8) [62]. The exomethylene sugars were made by Julia olefination of per-*O*-benzylated or -silylated lactone **55**. Protecting group exchange to get the per-*O*-acetylated **57** was necessary because upon hydrogenolytic debenzylation of **58**, the isoxazoline ring

Homotrivalent glucose derivatives[79]

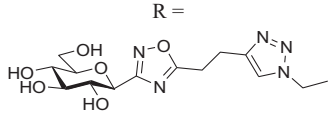


44b, **65-67**

R =

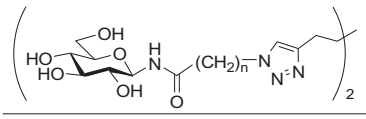


R =



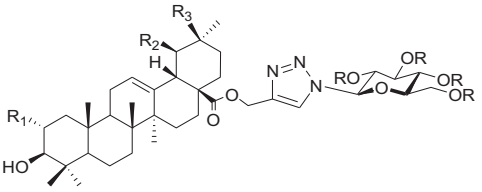
Inhibition of RMGP*b* at 625 μM	**44b** R' = H 10 %	**65** R' = R 35 %	**66** R' = H No inh.	**67** R' = R 30 %

Homobivalent glucose derivatives[80]

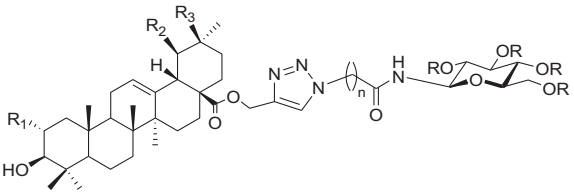


68 n = 1, 5, 10, 15 — No inhibition of RMGP*b* at 625 μM

Heterobivalent triterpene–glucose derivatives[80]

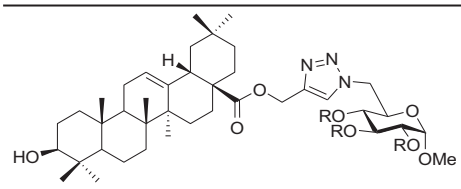


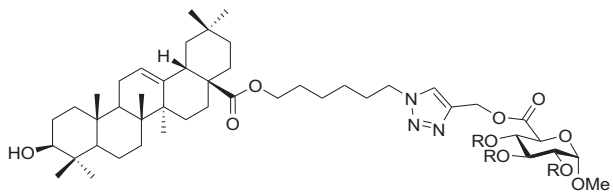
Oleanolic acid (OA) R_1 = H, R_2 = H, R_3 = CH_3
Ursolic acid (UA) R_1 = H, R_2 = CH_3, R_3 = H
Maslinic acid (MA) R_1 = OH, R_2 = H, R_3 = CH_3



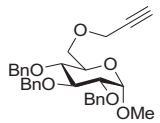
		Acid	R	n	Inhibition of RMGP*a* (IC_{50} [μM])
69	**a**	OA	Ac	-	337
	b	OA	H	-	26
	c	UA	Ac	-	51
	d	UA	H	-	45
	e	MA	Ac	-	no inh.
	f	MA	H	-	779
70	**a**	OA	Ac	1	68
	b	UA	Ac	10	65
	c	MA	Ac	1	78

Fig. 12. Probing multivalency for the inhibition of rabbit muscle glycogen phosphorylase (RMGP).

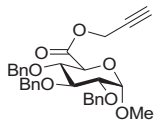




		R	
71	**a**	Ac	no inh.
	b	H	1.14
72	**a**	Bn	11.6
	b	H	no inh.



73 289



74 37

Fig. 13. Triterpene–glucose conjugates and protected monosaccharide derivatives [81] as inhibitors of rabbit muscle glycogen phosphorylase *a* (RMGP*a*) (IC_{50} [μM]).

also opened up due to a cleavage of the N–O bond. *O*-deacetylation of **58** could be achieved by the Zemplén protocol. Another way to **57** was reported by transforming per-*O*-acylated nitriles **36** to 2,6-anhydro-aldose tosylhydrazones followed by a Bamford-Stevens type carbene generation to yield the target *exo*-glycals [72,73].

Enzyme kinetic investigation of these spirocycles (Fig. 10) indicated low micromolar inhibition of GP by the phenyl substituted derivatives **53a** and **58a**. Substitution in the para-position of the aromatic ring gave somewhat better inhibitors (**53c**, **58b**,**c**). The 2-naphthyl derivatives (**53d**, **58d**) were nanomolar inhibitors, thereby fully validating the design principles.

3.5. Miscellaneous compounds

Several *O*-, *S*-, and *N*-glucosides (Fig. 11, **59**-**63**) of β-D-*gluco*-hept-2-ulopyranosonamide were prepared by nucleophilic substitutions of the corresponding glycosyl bromide [75]. These compounds can be regarded as anomerically extended variants of amide **6** for which a β-anomeric carbamate moiety (**7**) significantly improved the inhibitory efficiency. On the other hand, the new substitution patterns of **59**-**63** weakened the inhibition.

Sulfonamide **64** prepared recently by two different methods [76,77] had no inhibition against RMGP*b*.

Very recently, multivalent molecules have been designed and proposed for inhibition of GP [79]. Compound **65** (Fig. 12) was prepared by acylation of amidoxime **42** with trimesic acid chloride. To get compound **67** containing a spacer, **42** was acylated with 4-pentynoic acid followed by CuAAC with 1,3,5-tris(azidomethyl)benzene. These compounds have three glucose units, each potentially capable to bind to an active site of GP. It was found that the homotrivalent derivatives **65** and **67** had slightly better inhibitory activity than the corresponding monovalent compounds **44b** and **66**, respectively. Homobivalent compounds **68** were made by CuAAC from *N*-ω-azidoalkanoyl-β-D-glucopyranosylamines and 1,7-octadiyne, but had no effect on the enzyme [80].

Potentially heterobivalent compounds were designed by tethering pentacyclic triterpenes and D-glucose derivatives [80]: C-28 propargyl esters of oleanolic, ursolic, or maslinic acids were coupled by CuAAC with β-D-glucopyranosyl azide and *N*-ω-azidoalkanoyl-β-D-glucopyranosylamines to give compounds **69** and **70**, respectively. Derivatives with both per-*O*-acetylated and unprotected sugar parts were tested against GP and the best inhibitors are shown in Fig. 12. Micromolar inhibitors could be identified among both protected and unprotected glucose derivatives, and also the triterpene part and, in some cases, the linker length had a bearing on the efficiency of the compounds.

Oleanolic acid and D-glucose were also conjugated via C-6 ethers and glucuronic esters in several ways [81]. Most efficient compounds are **71b** and **72a** (Fig. 13) interestingly with an unprotected and a protected sugar unit, respectively. Based on molecular docking, **71b** was proposed to bind at the allosteric site of GP. Per-*O*-benzylated precursor sugars **73** and **74** containing a propargyl group also exhibited inhibition of GP, the latter in the low micromolar range.

4. Conclusion

Extensive synthetic efforts supported by crystallographic studies on enzyme–inhibitor complexes have resulted in several new types of glucose analogue inhibitors of GP. Among them, *N*-acyl-*N*′-β-D-glucopyranosyl ureas, glucopyranosylidene-spiro-oxathiazolines and -isoxazolines represent novel scaffolds which, in the presence of suitable substituents, exhibit nanomolar efficiency. Further increase in the binding strength of glucose analogues may be expected from a better exploitation of interactions of the molecules in the β-channel of the enzyme. This will need a strong collaboration between synthetic and computational chemists, as well as crystallographers and biochemists. Nevertheless, due to the extremely high flexibility of the catalytic site of GP, synthesis and enzyme kinetic study of a large number of compounds will be inevitable.

5. Note added in proof

While this manuscript was under review, an interesting paper appeared on enzyme kinetic and crystallographic investigations of a series of 3-deoxy-3-fluoro-β-D-glucopyranosyl pyrimidine derivatives [82].

Acknowledgement

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