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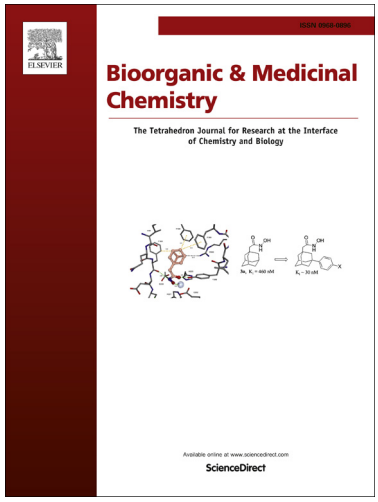

Glucopyranosylidene-spiro-iminothiazolidinone, a New Bicyclic Ring System: Synthesis, Derivatization, and Evaluation for Inhibition of Glycogen Phosphorylase by Enzyme Kinetic and Crystallographic Methods

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PII: S0968-0896(14)00452-0
DOI: http://dx.doi.org/10.1016/j.bmc.2014.05.076
Reference: BMC 11643

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 17 March 2014
Revised Date: 25 May 2014
Accepted Date: 29 May 2014

Please cite this article as: Czifrák, K., Páhi, A., Deák, S., Kiss-Szikszai, A., Kövér, K.E., Docsa, T., Gergely, P., Alexacou, K-M., Papakonstantinou, M., Leonidas, D.D., Zographos, S.E., Chrysina, E.D., Somsák, L., Glucopyranosylidene-spiro-iminothiazolidinone, a New Bicyclic Ring System: Synthesis, Derivatization, and Evaluation for Inhibition of Glycogen Phosphorylase by Enzyme Kinetic and Crystallographic Methods, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.05.076

Glucopyranosylidene-spiro-iminothiazolidinone, a New Bicyclic Ring System: Synthesis, Derivatization, and Evaluation for Inhibition of Glycogen Phosphorylase by Enzyme Kinetic and Crystallographic Methods

Katalin Czifrák,[a] András Páhi,[a] Szabina Deák,[a] Attila Kiss-Szikszai,[a] Katalin E. Kövér,[b] Tibor Docsa,[c] Pál Gergely,[c] Kyra-Melinda Alexacou,[d] Maria Papakonstantinou,[d] Demetres D. Leonidas,[d,#] Spyros E. Zographos,[d] Evangelia D. Chrysina,[d*] László Somsák[a*]

[a]*Department of Organic Chemistry, University of Debrecen, POB 20, H-4010 Debrecen, Hungary*

[b]*Department of Inorganic and Analytical Chemistry, University of Debrecen, POB 21, H-4010 Debrecen, Hungary*

[c]*Department of Medical Chemistry, Medical and Health Science Centre, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*

[d]*Institute of Biology, Medicinal Chemistry and Biotechnology, The National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, GR-116 35, Athens, Greece*

[#] Present address: Department of Biochemistry and Biotechnology, University of Thessaly, 26 Ploutonos Str., 41221 Larissa, Greece

[*] Corresponding authors – tel.: +302107273851, fax: +302107273831, e-mail: echrysina@eie.gr (EDC); tel.: +3652512900 ext 22348, fax: +3652512744, e-mail: somsak@tigris.unideb.hu (LS).

Abstract

The reaction of thiourea with *O*-perbenzoylated *C*-(1-bromo-1-deoxy-β-D-glucopyranosyl)formamide gave the new anomeric spirocycle 1*R*-1,5-anhydro-D-glucitol-spiro-[1,5]-2-imino-1,3-thiazolidin-4-one. Acylation and sulfonylation with the corresponding acyl chlorides (RCOCl or RSO_2Cl where R = *t*Bu, Ph, 4-Me-C_6H_4, 1- and 2-naphthyl) produced the corresponding 2-acylimino- and 2-sulfonylimino-thiazolidinones, respectively. Alkylation by MeI, allyl-bromide and BnBr produced mixtures of the respective *N*-alkylimino- and *N,N'*-dialkyl-imino-thiazolidinones, while reactions with 1,2-dibromoethane and 1,3-dibromopropane furnished spirocyclic 5,6-dihydro-imidazo[2,1-*b*]thiazolidin-3-one and 6,7-dihydro-5*H*-thiazolidino[3,2-*a*]pyrimidin-3-one, respectively. Removal of the *O*-benzoyl protecting groups by the Zemplén protocol led to test compounds most of which proved micromolar inhibitors of rabbit muscle glycogen phosphorylase *b* (RMGP*b*). Best inhibitors were the 2-benzoylimino- (K_i = 9 μM) and the 2-naphthoylimino-thiazolidinones (K_i = 10 μM). Crystallographic studies of the unsubstituted spiro-thiazolidinone and the above most efficient inhibitors in complex with RMGP*b* confirmed the preference and inhibitory effect that aromatic (and especially 2-naphthyl) derivatives show for the catalytic site promoting the inactive conformation of the enzyme.

Keywords

Anomeric spirocycle; thiazolidinone; type 2 diabetes; glycogen phosphorylase; inhibitor; X-ray protein crystallography; structure-based drug design

1. Introduction

A large proportion of clinically used drugs are inhibitors of enzymes and many current drug discovery and development efforts aim at the quest of such compounds.[1] Finding efficient inhibitors for a particular enzyme, and especially increasing the efficacy of such compounds, usually makes use of the three dimensional structure of the enzyme in complex with substrate analogues, inhibitors, as well as the kinetic and mechanistic behaviour of the enzyme in their presence, an approach called rational inhibitor (or drug) design.

Inhibition of glycogen phosphorylase (GP), the main regulatory enzyme of glycogen metabolism, has been connected to type 2 diabetes mellitus,[2-5] and during the last decade also to other diseased states such as myocardial[6, 7] and cerebral[8, 9] ischemia as well as tumors[10-13] as amply discussed in recent reviews and primary research articles. Liver and muscle isoforms of GP have been thoroughly characterized, the structural features of the proteins and the binding sites are well known and have been surveyed,[14, 15] therefore these enzymes are ideal targets for rational and structure-based inhibitor design.

Several structural classes of compounds for inhibition of GP have been designed and evaluated[2, 5, 16] (mostly with the prototype of GP[14] isolated from rabbit muscle (RMGP*b*)) among which the most populated group is that of the glucose analogues which bind mostly at the catalytic site.[14, 15, 17] The evolution of some glucose based compounds leading to the first low micromolar inhibitor of GP is exemplified in Chart 1 by compounds **A-C**. Thus, introduction of an acylamino type substituent in the β-anomeric position of **A** to give **B** resulted in enhanced binding by more than one order of magnitude. Formal ring closure of the substituents to yield spiro-hydantoin **C** gave an inhibitor even more efficient than **B**.

Synthesis of the essentially equipotent spiro-thiohydantoin **D** proved much simpler[18] than that of **C**, and **D** was shown to have considerable *in vivo* effects in lowering blood sugar levels.[19]

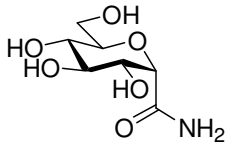
The structural features responsible for the strong binding of the spiro-hydantoins **C** and **D** to RMGP*b* were established by X-ray crystallography, and summarized as follows:[20, 21] a) a hydrogen bond exists between the β-NH group of the hydantoin and the main chain oxygen of His377; b) the rigid planar spiro-hydantoin moiety undergoes little loss of conformational entropy on binding; c) the hydrogen bonding capability of the hydantoin polar groups exploit existing water structure and recruit new waters to complete networks to protein atoms, thereby providing additional enthalpic interactions.

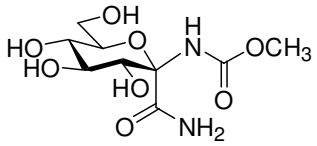
The first submicromolar glucose derived inhibitor of GP (**E** in Chart 1) was found among *N*-acyl-*N'*-β-D-glucopyranosyl ureas.[17, 22] In this series X-ray crystallography of the enzyme-inhibitor complexes[2, 23] indicated the absence of the above mentioned hydrogen bond between the β-NH group and His377 O; therefore, the stronger binding was attributed to extensive interactions with the large aromatic appendage of the inhibitor in the so-called β-channel of the protein. A variant of **E** with a 3,5-dimethyl-phenyl group in place of the 2-naphthyl moiety was shown to improve glucose tolerance and to rearrange hepatic metabolism in diabetic mice.[24]

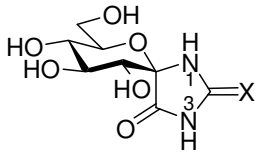
These observations shifted the focus of inhibitor design towards interactions in the β-channel and led to new principles[25] stating that efficient inhibitors advantageously have a rigid spirobicyclic scaffold which should not necessarily have an H-bond donor towards His377 (although this can be beneficial if available), but a suitably oriented, large aromatic substituent must be present to fit into the β-channel. These principles were first validated by

the synthesis and enzymatic evaluation of glucopyranosylidene-spiro-oxathiazolines[25, 26] (e. g. **F** in Chart 1) and further corroborated by spiro-isoxazolines[27] **G** among which the 2-naphthyl derivatives proved to be nanomolar inhibitors.

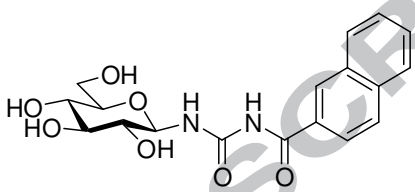
Chart 1. Inhibition of GP by selected derivatives of D-glucose (K_i [μM] against RMGP*b*)

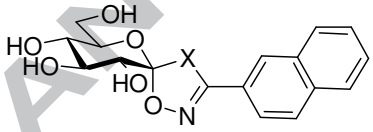
A  370[28]

B  16[30]

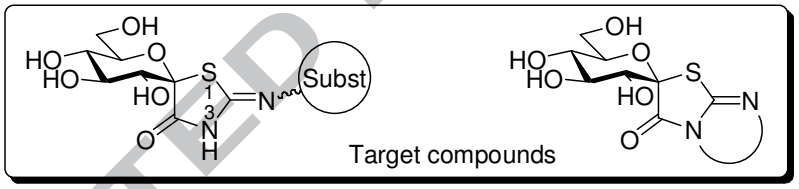
C  X = O 3.1[31] 4.2[32]

D X = S 5.1[32, 33]

E  0.35[29]

F  X = S 0.16[25, 26]

G X = CH_2 0.63[27]



Taking into account the above design principles and the importance of the hydrogen bond forming capacity of the polar groups in the inhibitor molecules as proven for the spiro-hydantoins, here we report on the synthesis of glucopyranosylidene-spiro-iminothiazolidinones (target compounds in Chart 1) which have, in comparison to **F** and **G**, additional polar moieties in the α-position of the sugar ring. These compounds have been tested for their inhibitory effects towards RMGP*b* by enzyme kinetic assays and, for selected inhibitors, crystallographic methods.

2. Results and discussion

2.1. Synthesis

Reactions of α-halogen carbonyl compounds with thioamides are generally used for the construction of the thiazole ring. As a variant of the method, α-halogen carboxylic acid derivatives (most often esters) and thiourea (or its substituted derivatives) can be reacted to give 2-imino-thiazolidin-4-ones or the tautomeric 2-amino-Δ^2-thiazolin-4-ones.[34] The latter reaction type is exemplified by some syntheses of spiro-thiazolidinones.[35-38] Related spirobicyclic systems containing a sugar ring are very scarce: to the best of our knowledge a ribofuranosylidene-spiro-thiazolidine-2,4-dione[39] and epimers of galactopyranosylidene-spiro-thiazolidine-2,4-dithione as well as galactopyranosylidene-spiro-2-amino-Δ^2-thiazolin-4-thione[40] were reported.

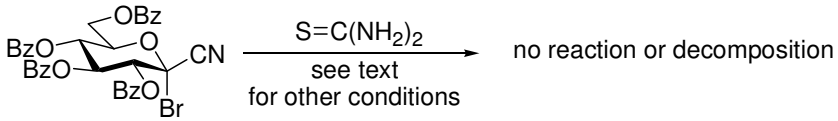
Since the above ribofuranosylidene-spiro-thiazolidine-2,4-dione[39] was prepared by reacting *O*-perbenzoylated 1-bromo-D-ribofuranosyl cyanide with thiourea, in our first attempts similar reactions of the analogous glucopyranosyl compound **1**[18] (Table 1) were tried. Unfortunately, no reaction took place under the reported conditions[39] (1.3-2 equiv of thiourea, EtOH-sulfolane solvent mixture, 100 ºC) either with conventional or microwave heating. Changing the solvent to nitrobenzene or DMF and raising the reaction temperature to 150 ºC brought about no transformation while in *N*-methylpyrrolidone or ethylene glycol only decomposition could be observed.

The next trials were performed with the conventionally used α-halogen ester type compounds **2** and **3**[41] (Table 1, Entries 1-4). Under microwave heating conditions the desired spiro-

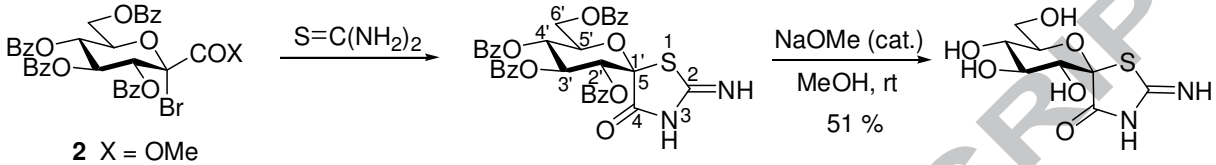
thiazolidinone **5** was obtained albeit the conversion of the starting compounds was incomplete. The active ester **3** showed somewhat higher reactivity (Entry 3). In a parallel experiment (Entry 5) the α-bromo carboxamide type **4**[18] was also shown to be converted to **5**. Since the preparation of **4** is shorter than those of **2** and **3** further optimizations (Entries 5-10) were carried out with this compound (as far as we know this type of cyclization with α-halogen carboxamides is unknown in the literature). Although the conversion of the starting material remained incomplete in these experiments, under the best conditions (Entries 7 and 10) approx. 75-80 % of **4** could be transformed into **5** by using large excess of thiourea. Removal of the sugar protecting groups was effected by the Zemplén protocol to give the unprotected **6** in acceptable yield.

Structural elucidation of **5** was based on NMR methods. Complete assignments of protons and carbons were made by COSY and HSQC correlations. The $^4C_1$ conformation of the glucopyranose ring followed from the vicinal proton-proton coupling constants. The ^{1}H NMR spectrum showed the presence of a broad singlet at 10.8 ppm for N(3)H, while the signal for C(2)=NH was hidden among the aromatic CH resonances (for comparison the NH signals for $CONH_2$ of **4** appeared as 2 broad singlets between 5-7 ppm). The ^{13}C NMR spectrum exhibited a resonance at 183.0 ppm for C(4), and another one at 179.7 ppm for C(2). Carbonyls of the protecting groups appeared as expected. The configuration of the spiro carbon C(1') was established on the basis of three-bond heteronuclear coupling between H(2') and C(4). This coupling was measured by a sensitivity enhanced gradient long-range ^{13}C-^{1}H correlation experiment (G-HSQMBC) and the obtained ~5.9 ± 0.3 Hz value indicated *trans* arrangement of the relevant atoms in the $^4C_1$ conformation. Cross peaks were observed also between N(3)H and C(1'), C(4) and C(2), but C(2)=NH gave a cross peak only with C(2), thereby corroborating ring formation.

Table 1. Preparation of glucopyranosylidene-spiro-iminothiazolidinone



1



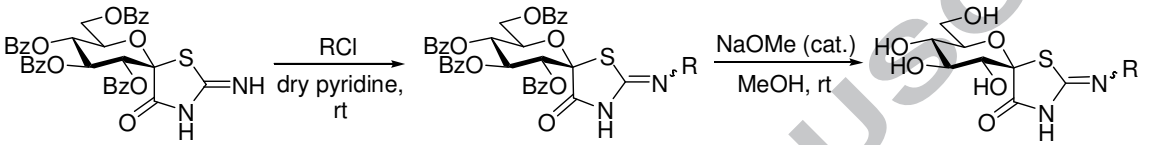
2 X = OMe
3 X = OC_6Cl_5
4 X = NH_2

5 **6**

Entry	Starting compound	Solvent	Thiourea (equiv.)	Temp. (ºC)	Heating	Reaction time	Conversion of starting compd. (%)	Yield of **5** (%)
1.	**2**	acetone	1.5	95	MW/150W	30 min	41	99
2.	**2**	EtOH	1.5	95	MW/100W	30 min	42	99
3.	**3**	acetone	1.5	95	MW/150W	30 min	80	55
4.	**3**	EtOH	1.5	95	MW/100W	30 min	50	99
5.	**4**	acetone	1.5	reflux	oil bath	12 h	54	76
6.	**4**	acetone	2.5	reflux	oil bath	7 days	72	82
7.	**4**	acetone	12	rt	-	11 days	89	86
8.	**4**	EtOH	12	rt	-	11 days	80	81
9.	**4**	EtOH	1.5	95	MW/100W	30 min	43	61
10.	**4**	EtOH	10	120	MW/100W	1 h	82	97

In order to introduce a substituent to approach the β-channel of the enzyme acylation was investigated first (Scheme 1). A series of aliphatic and aromatic carboxylic acid chlorides and arenesulfonylchlorides were used to get carboxamides **7-13** and sulfonamides **14-17**, respectively, in medium to good yields. In these reactions only the the C(2)=NH group was substituted as revealed by broad singlets between 9-12 ppm for N(3)H of the iminothiazolidinone ring. Configuration of the C=N double bond was not investigated at this stage, but the single series of signals in the NMR spectra indicated formation of one isomer

only. This structural feature was later deduced from the crystallographic investigation of the enzyme-inhibitor complexes. *O*-Debenzoylation under Zemplén conditions resulted in a loss of the *N*-acyl moiety from **7** and **8** furnishing thiazolidinone **6**. Deprotection of **9-13** gave the corresponding **18-22** in modest yields, probably because of the sensitivity of the carboxamides to the slightly basic conditions, while sulfonamides **14-17** gave the *O*-unprotected **23-26** in almost quantitative yields.



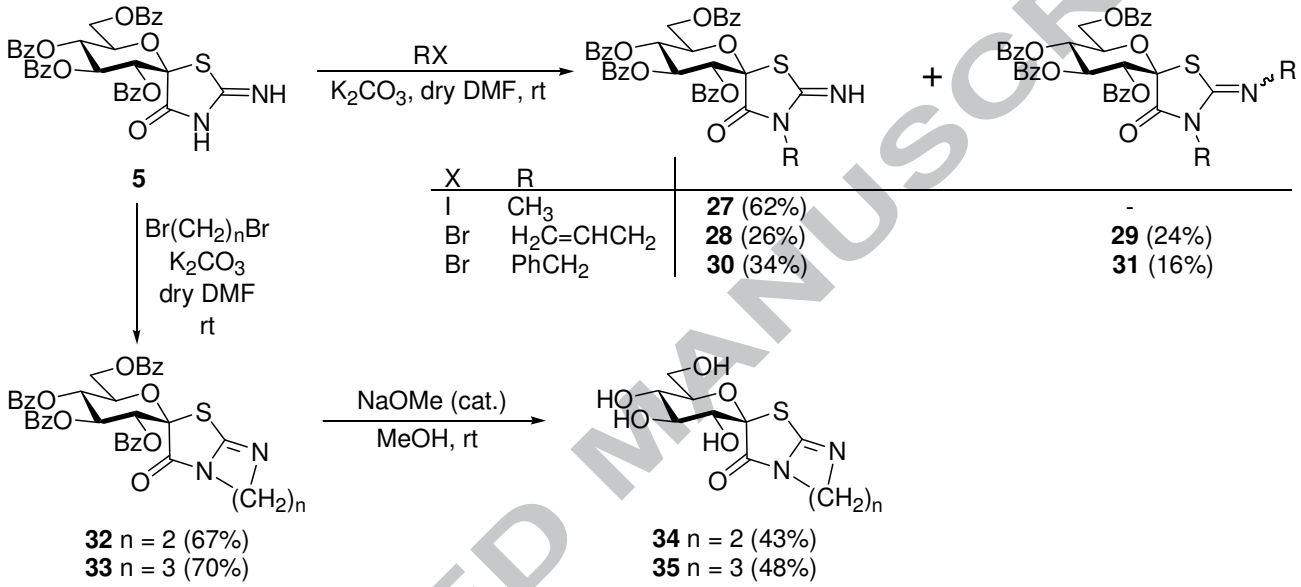
5 **7-17** **18-26**

R		Yield (%)		Yield (%)
Me-CO	**7**	76	thiazolidinone **6** (R = H)	20
*i*Pr-CO	**8**	81	thiazolidinone **6** (R = H)	61
*t*Bu-CO	**9**	82	**18**	26
Ph-CO	**10**	84	**19**	58
4-Me-C_6H_4-CO	**11**	53	**20**	43
1-naphthyl-CO	**12**	73	**21**	56
2-naphthyl-CO	**13**	89	**22**	50
Ph-SO_2	**14**	55	**23**	98
4-Me-C_6H_4-SO_2	**15**	70	**24**	97
1-naphthyl-SO_2	**16**	45	**25**	96
2-naphthyl-SO_2	**17**	62	**26**	98

Scheme 1. Acylation of glucopyranosylidene-spiro-iminothiazolidinone **5**

Alkylation of **5** was studied with various alkyl halides in the presence of K_2CO_3 in DMF (Scheme 2). Methyl iodide gave only a single product **27** for which the presence of the methyl group attached to N(3) was indicated by the absence of the broad singlet of the corresponding proton around 10 ppm. Reactions with allyl or benzyl bromides resulted in mixtures of monoalkylated products **28** and **30** as well as dialkyl derivatives **29** and **31**, respectively, which could be separated by column chromatography. α,ω-Dibromoalkanes furnished good

yields of the ring-annelated compounds **32** and **33** which were deprotected by the Zemplén method to give **34** and **35** in acceptable yields. The position of the alkyl group in compounds **27**, **28**, and **30** followed from the absence of the broad singlet of N(3)H. In the proton spectra of the dialkylated compounds **29** and **31-33** no NH signals could be observed. Aliphatic and additional aromatic resonances for the introduced groups appeared as expected.



Scheme 2. Alkylation of glucopyranosylidene-spiro-iminothiazolidinone **5**

2.2. Enzyme kinetic studies and structure-activity relationships

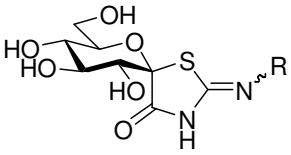
The new compounds were evaluated for their inhibitory potency against RMGP*b*. The kinetic studies were performed in the direction of glycogen synthesis and the results obtained are summarized in Table 2. Thiazolidinone derivative **6** proved to be a low micromolar inhibitor, however, a comparison to spiro-hydantoins **C** and **D** in Chart 1 shows that **6** is 5-8 times less efficient than the hydantoins. This may be due to the loss of the direct H-bridge to His377 carbonyl oxygen shown to be crucial in the binding of the hydantoins to the enzyme.

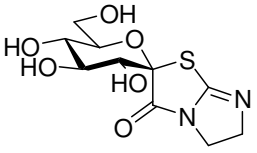
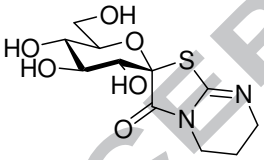
Acylation of **6** at the exocyclic NH gave inhibitors of varying efficiency depending on the structure of the acyl group. While the large pivaloyl moiety of **18** was not beneficial for the inhibition resulting in a more than 5-fold weakening of the binding compared to that of the parent **6**, the aromatic groups in **19**, **21**, and **22** made the inhibition ~2 times stronger. Somewhat unexpectedly, however, these aroyl derivatives had rather similar inhibition in contrast to observations with inhibitor types **E-G** in Chart 1, where the size and orientation of the aromatic part had a strong bearing on the inhibition generally rendering the 2-naphthyl compounds to be the best inhibitors.[17] Introduction of a methyl group in the 4-position of the phenyl ring as in **20** resulted in a weaker inhibition than that of **19**, and this is again different from compounds **E-G** in which this modification (or 4-OMe substitution) gave more effective inhibitors.[17, 22] This behaviour may reveal a rather unfavourable orientation of the aromatic part of these compounds in the active site β-pocket of the enzyme.

Sulfonylation of **6** as in compounds **23-26** gave inhibitors of still micromolar efficiency, however, these proved weaker than **6** and were pairwise less efficient than their acylated counterparts **19-22**, respectively. This may be due to the presence of the tetrahedral sulfonyl linker which can force the aromatic ring in more unfavourable positions than in the acylated derivatives. Similar phenomena were observed on introduction of CH_2 or SO_2 groups[17, 42] in place of planar moieties of inhibitors of type **E** in Chart 1 as well as in analogous compounds.

Extension of **6** by annelating a further ring to the thiazolidinone gave practically inactive compounds **34** and **35** showing that increasing the size of the molecules in this particular direction, being obviously out of the direction of the β-channel, is highly detrimental for the binding.

Table 2. Inhibition of RMGP*b* by the new spirocyclic compounds



	R	K_i [μM]
6	H	24 ± 1.7
18	*t*Bu-CO	137 ± 15*
19	Ph-CO	9 ± 0.6*
20	4-Me-C_6H_4-CO	35 ± 3.1
21	1-naphthyl-CO	15 ± 1.1*
22	2-naphthyl-CO	10 ± 1*
23	Ph-SO_2	143 ± 21*
24	4-Me-C_6H_4-SO_2	63 ± 5
25	1-naphthyl-SO_2	29 ± 3.7
26	2-naphthyl-SO_2	153 ± 23
34		48 % inhibition at 1 mM
35		No inhibition up to 1 mM

*Calculated from the IC_{50} values by the Cheng-Prusoff equation:[43] $K_i = IC_{50}/(1 + [S]/K_m)$.

2.3. Crystallographic studies

Structural studies were performed for the parent spirocycle **6** and the two best inhibitors of the series (**19**, **22**). The crystal structures of RMGP*b*:**6**, RMGP*b*:**19**, and RMGP*b*:**22** were determined at high resolution 1.95 Å, 1.90 Å, and 2.1 Å, respectively using mainly synchrotron radiation at Daresbury Laboratory, UK. The data collection and processing statistics along with those from refinement and model quality are summarized in Table 3S. Crystallographic numbering for **6**, **19**, and **22** is shown in Tables 4S, 7S, and 10S respectively.

The results showed that all three compounds bound at the catalytic site of the enzyme as indicated by the difference electron density maps (Figure 1).

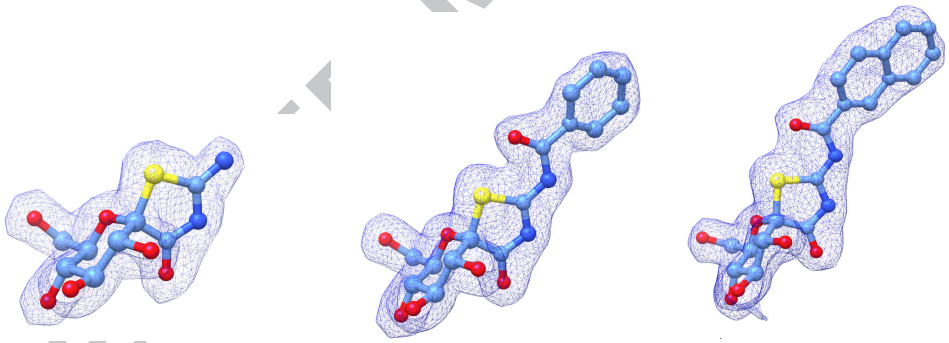


Figure 1. Schematic representation of the $2F_o$ - F_c electron density maps contoured at 1.0 σ level of the refined complex structures of RMGP*b*:**6** (left), RMGP*b*:**19** (middle), and RMGP*b*:**22** (right) of the enzyme. The figure was prepared with *UCSF Chimera*.[44]

More specifically, **6** forms a total of 16 hydrogen bond and 78 van der Waals interactions upon binding (Figure 2) most of which come from the sugar peripheral hydroxyl groups. Comparison with the RMGP*b*-glucopyranosylidene-spiro-hydantoin (**C** in Chart 1) complex structure determined previously[20, 45] showed that replacement of N-1 (cf formula **C** in Chart 1) with sulfur resulted in less favourable binding compared to spirohydantoin **C**. In agreement

with the kinetic results obtained, the ligand affinity was reduced. As discussed in the introduction, an H-bond from an NH group to the carbonyl oxygen of His377 could be considered added value for improved affinity for a number of glucose derivatives; however, it was not a prerequisite for strong binding. Therefore, the weaker binding could not be attributed solely to the loss of the hydrogen bond formation. Careful inspection of the residues in the ligand environment after superposition of the three dimensional complex structure of RMGP*b*:**6** on RMGP*b*:spirohydantoin showed that the atoms of the spiro heterocycle have shifted by ~0.5 Å (an incline by ~16°) while the carbonyl oxygen of His377 has moved away by ~0.4 Å from its original position. The repulsive forces which might develop between the sulfur atom inserted in the spiro ring and the backbone oxygen of His377 (at a distance of 3.3 Å) could partially explain the worse inhibition of enzyme activity despite the increased number of interactions formed (two additional hydrogen bonds and eight van der Waals interactions). In addition, it was anticipated that substitution of the carbonyl oxygen at position (2) of the spiro ring (cf **C** in Chart 1 and **5** in Table 1) with an NH group would have improved the affinity. However, it seems that this NH forms water-mediated interactions with the 280s loop residues equivalent to those of the carbonyl oxygen in the RMGP*b*:spirohydantoin complex structure (Tables 4S-6S, Figure 2).[45] To achieve this, the solvent structure is slightly perturbed (shifts by ~0.4 Å are observed) and the most profound change is that of Wat53 O, that forms a direct H-bond with N1 of **6** and shifts by ~0.9 Å to optimize its contact with Asp283 OD1. The atoms of both residues Asp283 and Asp284 also move by ~ 0.4 Å.

The three dimensional structure of **19** in complex with RMGP*b* provided further evidence on the preference of the so-called 'β-pocket' of the catalytic site for aromatic groups, as long as they are oriented across the β-channel. Compound **19** binds tightly at the catalytic site

forming a favourable network of interactions (19 hydrogen bonds and 109 van der Waals interactions (Tables 7S-9S, Figure 3)). These interactions seem to outweigh the alterations of the solvent structure induced upon binding which is also supported by the entropic effect introduced by the release of water molecule. The slightly worse inhibition potency that it exhibits compared to the lead compound spirohydantoin of glucopyranose (Table 2), might be both due to the sulfur atom introduced in the spiro ring, similar to **6**, but also due to the perturbation of the solvent structure to accommodate the phenyl ring. Water 60 O of RMGP*b*:**6** is not present in the new complex structure, destabilizing the side chain of Asp339 (dihedral χ2 rotates by ~40 °), and Wat55 O shifts by ~1.0 Å to move away from the phenyl ring. Minor changes are observed in the side chain of His341 (imidazole ring atoms shift by ~0.3 Å to ~0.5 Å (Figure 4). The increased number of van der Waals interactions formed with residues in the vicinity seems to outweigh the energy loss due to these alterations.

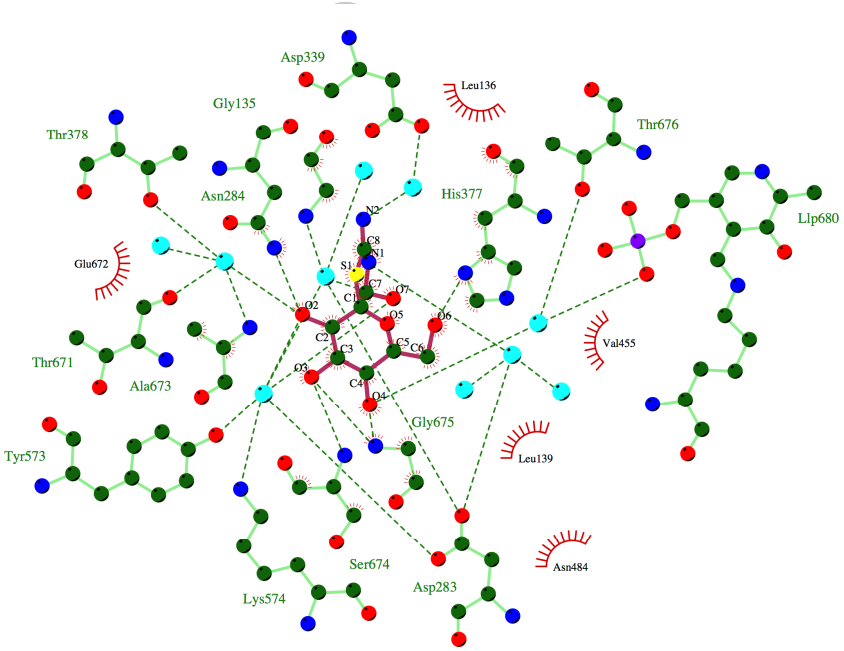


Figure 2. LIGPLOT diagram of **6** interacting with the active-site residues lying in the vicinity. Hydrogen bonds are shown as dark green dashed lines. Additional residues forming van der Waals interactions with the ligand are represented by red semicircles with radiating spokes.[46] Water molecules are depicted in cyan as round spheres.

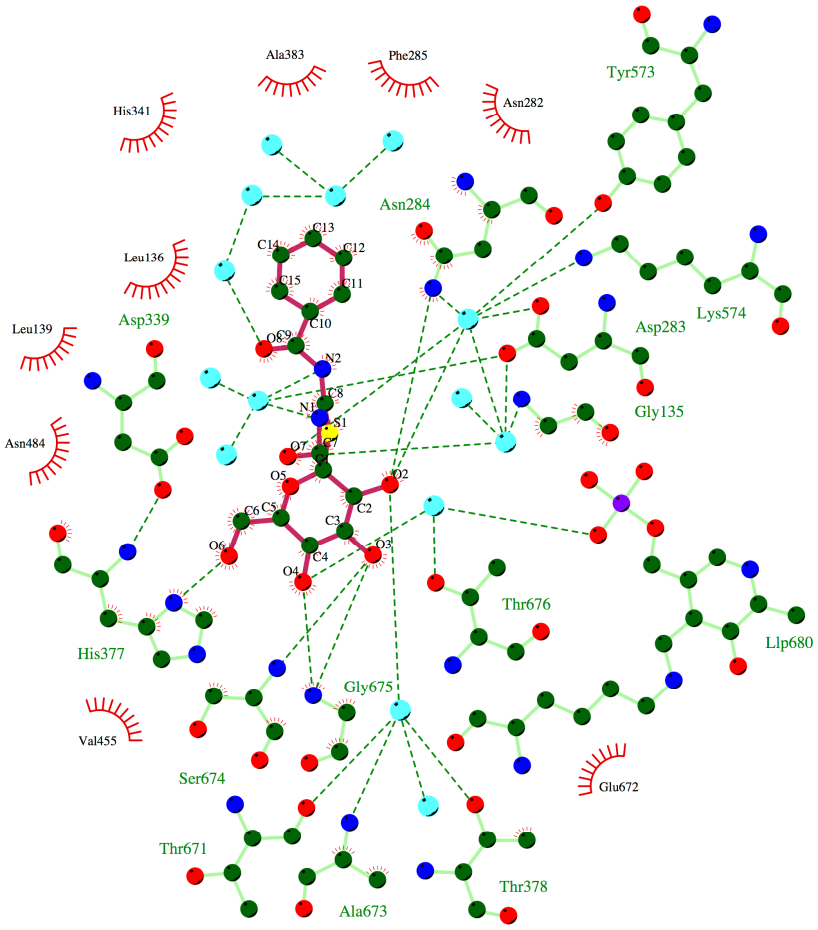


Figure 3. LIGPLOT diagram of **19** interacting with the active-site residues lying in the vicinity. Hydrogen bonds are shown as dark green dashed lines. Additional residues forming van der Waals interactions with the ligand are represented by red semicircles with radiating spokes.[46] Water molecules are depicted in cyan as round spheres.

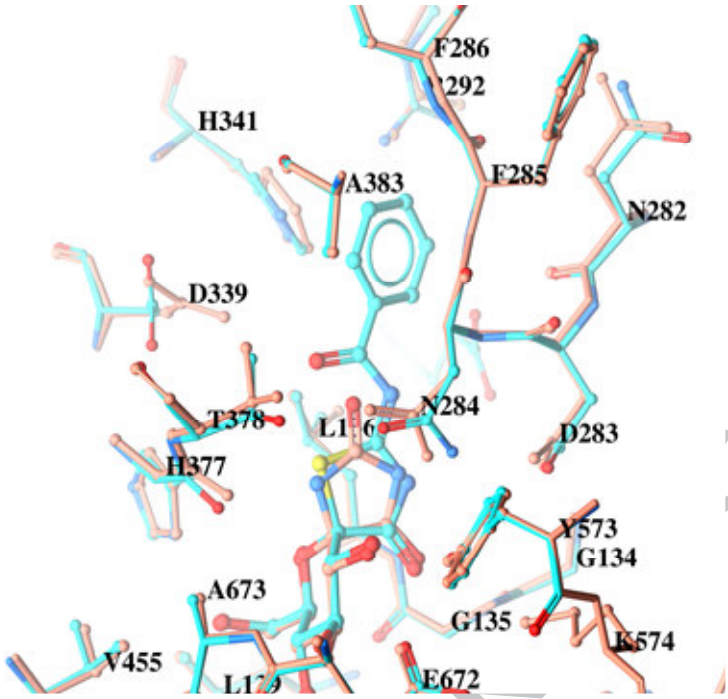


Figure 4. Superposition of the crystal structures of RMGP*b* in complex with spirohydantoin (**C** in Chart 1, shown in orange), with RMGP*b*:**19** (shown in cyan) bound at the catalytic site of the enzyme. The figure was prepared with program *MolSoft*.[47]

Replacement of the phenyl ring of **19** with a 2-naphthyl group resulted in compound **22** that was also studied in complex with RMGP*b*. X-ray diffraction data collected at 2.1 Å resolution slightly lower compared with the previous two complex structures. As it was anticipated, the difference electron density maps (Figure 1, right) confirmed that compound **22** also binds at the catalytic site of the enzyme and the 2-naphthyl group is accommodated in the so-called β-subsite, in accordance with previous compounds with such a substituent.[2]

A portion of additional electron density was observed at the new allosteric site of the enzyme that could be attributed to the 2-naphthyl group only. Previous studies have shown that glucose analogues bearing this group bound at both the catalytic and the new allosteric site.

The compound was incorporated in the model of the complex structure and was subjected to refinement. The resulting density was not satisfactory, suggesting that the compound binds, very weakly though, at the new allosteric site so **22** was not included as a ligand at this site. A portion of continuous electron density observed in the vicinity of the active site, above the β-subsite were the 2-naphthyl group was located, was attributed to dithiothreitol (DTT) that was used during the enzyme isolation and purification process (for details see Supporting information and Figure 5).

Detailed investigation of the binding mode of **22** and comparison with **19** revealed that there are structural changes at the catalytic site of the enzyme induced by changes that might also facilitate the binding of DTT. More specifically, **22** forms a total of 17 hydrogen bonds and 126 Van der Waals interactions which are summarized in Tables 10S-12S and graphically presented in Figure 5.

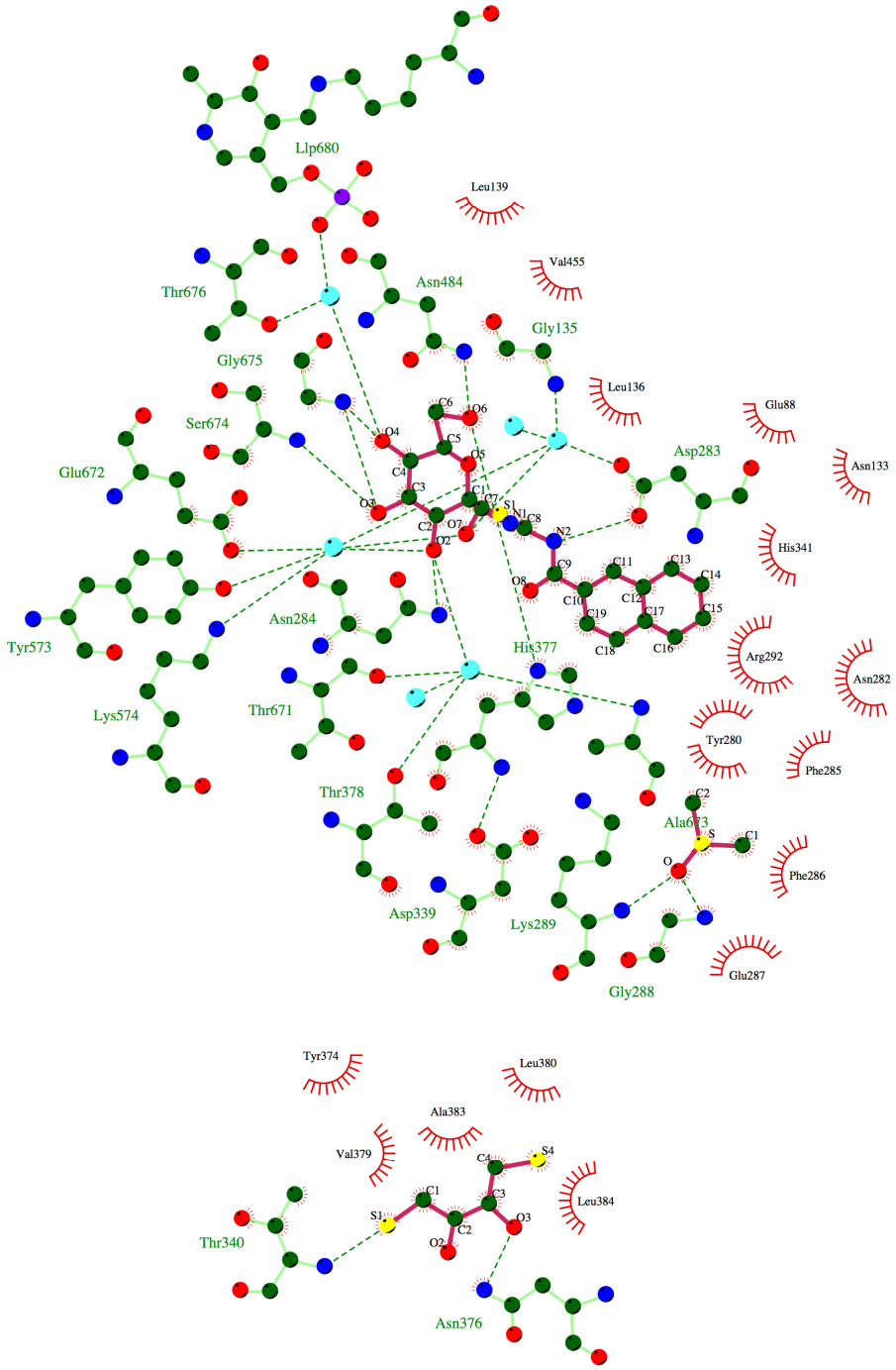


Figure 5. LIGPLOT diagram of **22** (top) interacting with the active-site residues lying in the vicinity. The interactions formed by DTT at the same site are also shown (bottom) Hydrogen bonds are shown as dark green dashed lines. Additional residues forming van der Waals interactions with the ligand are represented by red semicircles with radiating spokes.[46] Water molecules are depicted in cyan as round spheres.

Superposition of the crystal structures of RMGP*b*:**22** with RMGP*b*:**19** bound at the catalytic site of the enzyme (Figure 6) showed that replacement of the phenyl group by the 2-naphthyl one led to significant changes at the catalytic site, especially in the residues lining the 280s loop (282-287). More specifically, three water molecules Wat159 O, Wat160 O, Wat161 O and Wat162 O are displaced to avoid steric interaction with the ligand 2-naphthyl group. This re-arrangement is accompanied by flipping of the peptide bond between Asn282 and Asn283 ~180° and rotation of their dihedral angles; Asn282 (χ1, χ2) rotate by (~98°, 24°), respectively and Asp283 dihedral angle χ2 to rotates by 116°. The perturbation of the 280s loop residues includes Asn284, the backbone atoms of which shift by 0.7 to 0.9 Å and its side chain changes also changes conformation (dihedral angles (χ1, χ2) rotate by (~28°, 42°)). The phenylalanine residues Phe285 and Phe286 also participate in the consecutive changes induced by shifts of their backbone atoms (ranging from ~0.5 to 0.9 Å) and similarly, Glu287 that alters its conformation substantially (dihedrals (χ1, χ3) rotate by (~70°, 114°)) and the orientation of the peptide bond it forms with Gly288. The latter is correlated with the presence of a DMSO molecule that binds in the vicinity of Glu287 at a position previously occupied by a water molecule (Wat162 O in RMGP*b*:**19** complex structure) at the β-subsite of the catalytic channel. The presence of the DMSO molecule appears to further stabilize the perturbed 280s loop residues in their new position. The more hydrophobic part of **22** is lying now closer to the 280s loop increasing the number of van der Waals interactions (Table 12S) and creating more space at the opposite side of the catalytic site. This allowed the binding of DTT that displaced two water molecules Wat165 O and Wat166 O and induced changes in the Asp339 side chain (dihedrals (χ1, χ3) rotate by (~38°, 84°)) and main chain atoms (shifts by ~0.6 Å). Similar amendments take place in the environment of Asp339 starting from Asn388 (backbone atoms shift by ~0.5 A and dihedral angle χ1 rotates by ~20°) affecting Leu136 side chain (rotation of dihedral angle χ2 by ~96°). In addition, all atoms of the 380s loop (378-

380) are subjected to minor shifts by 0.4-0.5 Å. Finally, His341 ring inclines by ~35° so as to optimize the stacking interactions between the aromatic moiety of the ligand and the imidazole ring. Overall, despite the slightly reduced hydrogen bond interactions formed in the RMGPb:**22** complex upon binding of the ligand, and the re-organization of the β-pocket at the active site of the enzyme, it appears that compound **22** is tightly bound at the catalytic site of the enzyme in agreement with the kinetic results. The presence of DMSO and DTT seem also to have facilitated this binding.

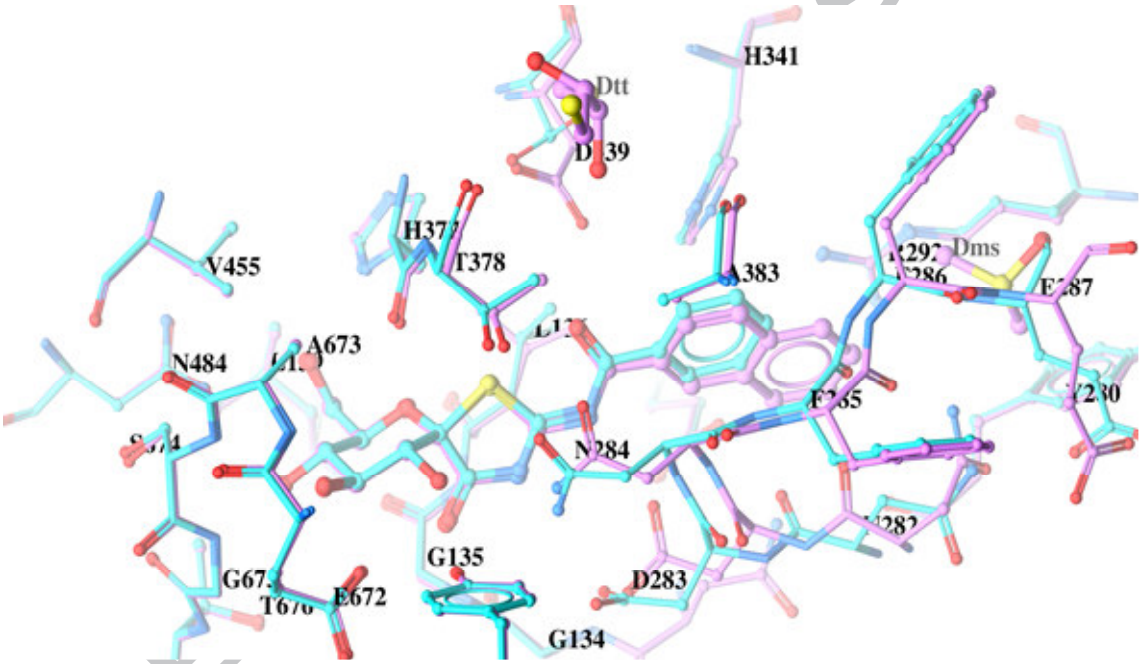


Figure 6. Superposition of the crystal structures of RMGP*b*:**19** (shown in cyan) with RMGP*b*:**22** (shown in mauve) bound at the catalytic site of the enzyme. The figure was prepared with program *MolSoft*.[47]

Comparison of all three ligands showed that their location and orientation at the active site of the enzyme is very similar (Figure 7). In particular, the small incline in the spiro ring observed when one of its nitrogen atoms was replaced by a sulphur atom allowed the ligands to bind along the β-channel by orienting their most hydrophobic groups to optimally interact

with residues lining this subsite. This is clearly shown for all ligands including compound **22**, in which the position of almost all atoms is also maintained except for the naphthyl group that needs to optimise its interaction with the 280s loop residues.

Figure 7. Superposition of spirohydantoin (**C** in Chart 1, shown in orange) and the ligands **6** (shown in green), **19** (shown in cyan) and **22** (shown in mauve) bound at the corresponding RMGP*b* complex structures. The figure was prepared with program *MolSoft*.[47]

3. Conclusion

A new spirobicyclic ring system 1*R*-1,5-anhydro-D-glucitol-spiro-[1,5]-2-imino-1,3-thiazolidin-4-one (**6**) was obtained in the reaction of *C*-(2,3,4,6-tetra-*O*-benzoyl-1-bromo-1-deoxy-β-D-glucopyranosyl)formamide and thiourea. Alkylation, acylation, and sulfonylation gave further functionalized derivatives of the anomeric spirocycle. *O*-Deprotected compounds prepared by the Zemplén method and tested against rabbit muscle glycogen phosphorylase *b* proved to be micromolar inhibitors of the enzyme. Best inhibitors were 1*R*-1,5-anhydro-D-glucitol-spiro-[1,5]-2-benzoylimino- (**19**) and -2-naphthoylimino-1,3-thiazolidin-4-ones (**22**) (K_i 9 and 10 μM, respectively). The corresponding sulfonylimino derivatives (formal replacement of the CO group by an SO_2 moiety) were much less efficient inhibitors probably

due to the tetrahedral geometry of the sulfonyl group. Further extension of the ring system by the formation of an annelated cycle gave inactive compounds.

Structural studies of RMGP*b* in complex with **6**, **19**, and **22** allowed detailed investigation of the binding mode of these compounds at the active site of the enzyme providing interpretation of their inhibitory potency assessed by the kinetic experiments. The atoms introduced at the spirocycle determine the geometrical features and chemical properties of the inhibitor and act as a probe for the adjustments induced in ligand environment upon binding. Taking into account previous studies it can be concluded that aromatic moieties such as the 2-naphthyl group show a preference for the active site and compounds like **19** and **22** remain attractive lead compounds for further optimization using the structure-based drug design approach. Further investigation is required for shedding light on the cavity formed above the β-subsite and its potential role in promoting in the inactive conformation of the enzyme.

4. Experimental

4.1. General methods

Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Microwave assisted reactions were carried out in a CEM-Discover Focused Microwave Synthesis System (2450 MHz) with a built-in infrared temperature sensor. Optical rotations were determined with a Perkin-Elmer 241 polarimeter at room temperature (21-24 °C). NMR spectra were recorded with Bruker 360 (360/90 MHz for $^{1}H/^{13}C$) or Avance DRX 500 (500/125 MHz for $^{1}H/^{13}C$) spectrometers. Chemical shifts are referenced to Me_4Si (^{1}H),

or to the residual solvent signals (^{13}C). LCMS spectra were recorded by a Thermo Accela HPLC & LTQXL mass spectrometer system. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (Merck), and the plates were visualised 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 under diminished pressure at 40-50 ºC (water bath). Acetone was distilled from $KMnO_4$. DMF, thiourea, acid chlorides, and alkyl halides were purchased from Sigma-Aldrich.

4.2. General procedure I for the acylation/sulfonylation of 5 (preparation of 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-acyl(or sulfonyl)imino-1,3-thiazolidin-4-ones **7-17**)

To a solution of **5** (0.20 g, 0.29 mmol) in dry pyridine (3 mL) an acid chloride (3-5 equiv) was added. The mixture was then stirred at rt until disappearance of the starting material (TLC, 1:1 EtOAc-hexane). The solvent was evaporated and the obtained oil was coevaporated with toluene (2 x 5 mL), then purified by column chromatography as specified with the particular compounds.

4.3. General procedure II for Zemplén-deacylation

To a solution of an *O*-peracylated compound in dry MeOH 1-2 drops of a ~1 M methanolic NaOMe solution were added, and the reaction mixture was kept at rt until completion of the transformation (TLC 1:1 $CHCl_3$-MeOH). Amberlyst 15 (H^+ form) was then added to remove sodium ions, the resin was filtered off, and the solvent was removed. If the residue was chromatographically not uniform it was purified by column chromatography or crystallisation.

4.4. General procedure III for the alkylation of 5 (preparation of 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-imino-3-alkyl-1,3-thiazolidin-4-ones **27, 28, 30** and 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-alkylimino-3-alkyl-1,3-thiazolidin-4-ones **29, 31-33**)

To the solution of **5** (0.20 g, 0.29 mmol) in dry DMF (3 mL) containing dry K_2CO_3 (0.04 g, 0.29 mmol) a mono- or dihalogen alkane (0.58 mmol) was added. The mixture was stirred at rt until the disappearance of the starting material (TLC, 1:1 EtOAc-hexane). It was then diluted with water (10 mL) and extracted with EtOAc (5 x 5 mL). The organic layer was washed with water (10 mL) and dried. The solvent was removed and the obtained oil was purified by column chromatography.

4.5. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-imino-1,3-thiazolidin-4-one (5)

C-(2,3,4,6-Tetra-*O*-benzoyl-1-bromo-1-deoxy-β-D-glucopyranosyl)formamide (**4**, 1.00 g, 1.42 mmol) was dissolved in dry acetone (10 mL) and thiourea (1.02 g, 17.04 mmol) was added in one portion. The mixture was stirred at rt for 11 days, it was then filtered and the solvent evaporated. The obtained crude product was purified by column chromatography (eluent, 1:1 EtOAc-hexane) to give 0.11 g recovered **4** (conv.: 89 %) and 0.74 g (86 %) of **5** as an amorphous solid material. R_f=0.21 (1:1 EtOAc-hexane); $[\alpha]_D$ +132 (c 0.70, $CHCl_3$); In the NMR spectra complete signal assignments are based on COSY, HSQC, and HSQMBC correlations. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 10.8 (s, 1H, NH), 8.18-7.17 (m, 21H, ArH, NH), 6.99 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.10 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.92 (pseudo t, 1H, $J_{4,5}$ 10.6

Hz, H-4), 5.56 (ddd, 1H, $J_{5,6}$ 3.8 Hz, H-5), 5.20 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.41 (dd, 1H, $J_{5,6'}$ 1.2 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 125 MHz HSQC): δ (ppm) 183.0 (*C*ONH, $^3J_{H\text{-}2,CONH}$= ~5.9 Hz, HSQMBC), 179.7 (*C*=N), 166.8, 165.5, 165.3 165.0 (*C*O), 97.0 (C-1), 73.0 (C-5), 71.9 (C-3), 70.2 (C-2), 68.8 (C-4), 62.2 (C-6); Anal. Calcd for $C_{36}H_{28}N_2O_{10}S$ (680.68): C, 63.52; H, 4.15; N, 4.12. Found: C, 63.58; H, 4.05; N, 4.01.

Alternatively, a solution of **4** (0.10 g, 0.14 mmol) and thiourea (0.84 g, 1.4 mmol) in dry EtOH (5 mL) was irradiated by microwaves (100W, 120 °C) for 60 min. The solvent was then removed and the residue purified as above to give 18 mg recovered **4** (conv. 82 %) and 0.08 g (97 %) of **5**.

4.6. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-imino-1,3-thiazolidin-4-one (6)

Prepared from **5** (0.18 g, 0.26 mmol) according to General procedure **II** and purified by column chromatography (eluent, 1:1 $CHCl_3$-MeOH) to give 0.04 g (51%) of **6** as a colourless oil. R_f=0.45 (1:1 $CHCl_3$-MeOH); $[\alpha]_D$ +16 (c 0.25, H_2O); ^{1}H NMR (D_2O, 360 MHz): δ (ppm) 4.32 (ddd, 1H, $J_{5,6}$ 4.0 Hz, H-5), 4.24 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.90 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.75 (dd, 1H, $J_{6,6'}$ 11.9 Hz, Hz, H-6), 3.68 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'), 3.43 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4); ^{13}C NMR (D_2O, 90 MHz): δ (ppm) 187.9 (*C*ONH), 181.9 (*C*=N), 100.5 (C-1), 77.5, 73.1, 72.9, 69.4 (C-2 - C-5), 61.2 (C-6); Anal. Calcd for $C_8H_{12}N_2O_6S$ (264.26): C, 36.36; H, 4.58 N, 10.60. Found: C, 36.26; H, 4.68; N, 10.48.

4.7. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-acetylimino-1,3-thiazolidin-4-one (7)

Prepared from **5** (0.15 g, 0.22 mmol) and AcCl (0.05 mL, 1.10 mmol) according to General procedure **I**. Column chromatography (eluent, 1:1 EtOAc-hexane) gave 0.12 g (76 %) of **7** as a yellow powder. Mp: 129-131°C; $[\alpha]_D$ +89 (c 0.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 12.60 (s, 1H, NH), 8.05-7.25 (m, 20H, ArH), 6.72 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.14 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.83 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.19 (ddd, 1H, $J_{5,6}$ 1.1 Hz, H-5), 4.64 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.49 (dd, 1H, $J_{5,6'}$ 2.1 Hz, H-6'), 2.47 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 183.9, 182.1, 172.0, 166.0, 165.4, 165.1, 164.5 (*C*O, *C*=N), 133.7-128.2 (Ar*C*), 89.9 (C-1), 71.6, 70.5, 70.1, 68.5 (C-2 - C-5), 62.5 (C-6), 29.1 (*C*H_3); Anal. Calcd for $C_{38}H_{30}N_2O_{11}S$ (722.72): C: 63.15; H: 4.18; N: 3.88; Found: C, 63.19; H, 4.03; N, 3.75.

4.8. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1.5]-2-(2-methyl-propanoyl)imino-1,3-thiazolidin-4-one (8)

Prepared from **5** (0.15 g, 0.22 mmol) and $(CH_3)_2CHCOCl$ (0.12 mL, 1.10 mmol) according to General procedure **I**. Column chromatography (eluent, 1:1 EtOAc-hexane) gave 0.13 g (81 %) of **8** as a white powder. Mp: 128-129°C; $[\alpha]_D$ +92 (c 0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 12.22 (s, 1H, NH), 8.06-7.24 (m, 20H, ArH), 6.74 (pseudo t, $J_{3,4}$ 9.2 Hz, H-3), 6.15 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.85 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.23 (ddd, 1H, $J_{5,6}$ 1.2 Hz, H-5), 4.64 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.49 (dd, 1H, $J_{5,6'}$ 2.1 Hz, H-6'), 3.11 (m, 1H, CH), 1.22 (d, 3H, *J* 7.9 Hz, CH_3), 1.16 (d, 3H, *J* 7.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 183.6, 182.0, 179.0, 165.9, 165.4, 165.1 164.5 (*C*O, *C*=N), 133.6-128.2 (Ar*C*), 89.8

(C-1), 71.6, 70.6, 70.2, 68.5 (C-2 - C-5), 62.4 (C-6), 35.6 (*C*H), 18.8, 18.5 (2 CH_3); Anal. Calcd for $C_{40}H_{34}N_2O_{11}S$ (750.77) : C: 63.99; H: 4.56; N: 3.73; Found: C, 63.89; H, 4.43; N, 3.65.

4.9. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-pivaloylimino-1,3-thiazolidin-4-one (9)

Prepared from **5** (0.15 g, 0.22 mmol) and $(CH_3)_3CCOCl$ (0.14 mL, 1.10 mmol) according to General procedure **I**. Column chromatography (eluent, 1:1 EtOAc-hexane) gave 0.14 g (82 %) of **9** as a white powder. Mp: 133-134°C; $[\alpha]_D$ +72 (c 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 10.67 (s, 1H, NH), 8.10-7.22 (m, 20H, ArH), 6.78 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.12 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.92 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 5.23 (ddd, 1H, $J_{5,6}$ 1.1 Hz, H-5), 4.84 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.42 (dd, 1H, $J_{5,6'}$ 2.2 Hz, H-6'), 1.18 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 181.9, 181.6, 177.8, 166.2, 165.2, 165.2, 164.5 (*C*O, *C*=N), 133.6-128.2 (Ar*C*), 95.5 (*C*$(CH_3)_3$), 89.9 (C-1), 71.9, 70.6, 70.5, 68.4 (C-2 - C-5), 62.3 (C-6), 26.2 (3*C*H_3); Anal. Calcd for $C_{41}H_{36}N_2O_{11}S$ (764.80): C: 64.39; H: 4.74; N: 3.66; Found: C, 64.45; H, 4.68; N, 3.55.

4.10. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-benzoylimino-1,3-thiazolidin-4-one (10)

Prepared from **5** (0.20 g, 0.29 mmol) and PhCOCl (0.05 m, 0.87 mmol) according to General procedure **I**. Column chromatography (eluent, 1:2 EtOAc-hexane) gave 0.19 g (84 %) of **10** as a white powder. Mp: 269-271°C; $[\alpha]_D$ +129 (c 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 9.88 (s, 1H, NH), 8.14-7.21 (m, 25H, ArH), 6.80 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.17 (d, 1H,

$J_{2,3}$ 9.2 Hz, H-2), 5.93 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.33 (ddd, 1H, $J_{5,6}$ 1.2 Hz, H-5), 4.79 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.49 (dd, 1H, $J_{5,6'}$ 3.3 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 178.1, 171.8,171.3, 166.3, 165.4, 165.1, 164.5 (*C*O, *C*=N), 133.9-128.2 (Ar*C*), 88.9 (C-1), 72.4, 70.8, 70.6, 68.5 (C-2 - C-5), 62.4 (C-6); Anal. Calcd for $C_{43}H_{32}N_2O_{11}S$ (784.79): C, 65.81; H, 4.11; N, 3.57. Found: C, 65.69; H, 4.03; N, 3.45.

4.11. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-4-methylbenzoylimino-1,3-thiazolidin-4-one (11)

Prepared from **5** (0.30 g, 0.44 mmol) and 4-Me-C_6H_4COCl (0.30 mL, 2.20 mmol) according to General procedure **I**. Column chromatography (eluent, 1:2 Acetone-hexane) gave 0.19 g (53 %) of **11**as a yellow oil. R_f=0.88 (2:1 EtOAc-hexane); $[\alpha]_D$ +40 (c 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.62 (s, 1H, NH), 8.02-7.04 (m, 25H, ArH), 6.83 (pseudo t, 1H, $J_{3,4}$ 8.0 Hz, H-3), 6.15 (d, 1H, $J_{2,3}$ 9.7 Hz, H-2), 5.93 (t, 1H, $J_{4,5}$ 9.7 Hz, H-4), 5.36 (ddd, 1H, $J_{5,6}$ 2.3 Hz, H-5), 4.64 (dd, 1H, $J_{6,6'}$ 11.7 Hz, H-6), 4.45 (dd, 1H, $J_{5,6'}$ 3.6 Hz, H-6'), 3.40 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 181.6, 176.8, 175.3, 166.2, 165.8, 165.1 164.6 (*C*O, *C*=N), 149.0-123.9 (Ar*C*), 89.7 (C-1), 71.9, 71.1, 70.8, 69.9 (C-2 - C-5), 62.6 (C-6), 50.2 (*C*H_3); Anal. Calcd for $C_{44}H_{34}N_2O_{11}S$ (Mol. Wt.: 798.81, Ex. Mass.: 798.19): C, 66.16; H, 4.29; N, 3.51. Found: C, 65.99; H, 4.13; N, 3.55; ESI-MS (positive mode) m/z: 821.182 $[M+Na]^+$.

4.12. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-(1-naphthoylimino)-1,3-thiazolidin-4-one (12)

Prepared from **5** (0.14 g, 0.20 mmol) and 1-naphthoyl chloride (0.10 mL, 0.60 mmol) according to General procedure **I**. Column chromatography (eluent, 1:3 EtOAc-hexane) gave 0.13 g (73 %) of **13** as a colourless oil. R_f=0.52 (1:1 EtOAc-hexane); $[\alpha]_D$ +119 (c 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 11.9 (s, 1H, NH), 8.85-7.12 (m, 27H, ArH), 6.81 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.20 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.94 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.34 (ddd, 1H, $J_{5,6}$ 1.1 Hz, H-5), 4.76 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 4.49 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 178.7, 175.9, 172.3, 166.3, 165.3, 165.1, 164.6 (*C*O, *C*=N), 134.3-124.9 (Ar*C*), 89.2 (C-1), 72.3, 70.7 (2), 68.5 (C-2 - C-5), 62.4 (C-6); Anal. Calcd for $C_{47}H_{34}N_2O_{11}S$ (834.84): C, 67.62; H, 4.10; N, 3.36. Found: C, 67.68; H, 4.16; N, 3.28.

4.13. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-(2-naphthoylimino)-1,3-thiazolidin-4-one (13)

Prepared from **5** (0.15 g, 0.22 mmol) and 2-naphthoyl chloride (0.13 g, 0.66 mmol) according to General procedure **I**. Column chromatography (eluent, 1:3 EtOAc-hexane) gave 0.16 g (89 %) of **12** as a white powder. Mp: 235-236 °C; $[\alpha]_D$ +121 (c 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 10.80 (s, 1H, NH), 8.50-7.33 (m, 27H, ArH), 6.79 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.16 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.93 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.30 (ddd, 1H, $J_{5,6}$ 1.1 Hz, H-5), 4.80 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.46 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 176.3, 176.1, 173.5, 166.2, 165.4, 165.1, 164.5 (*C*O, *C*=N), 135.8-

124.5 (Ar*C*), 88.7 (C-1), 72.4, 70.8, 70.6, 68.5 (C-2 - C-5), 62.4 (C-6); Anal. Calcd for $C_{47}H_{34}N_2O_{11}S$ (834.84): C, 67.62; H, 4.10; N, 3.36. Found: C, 67.57; H, 4.23; N, 3.30.

4.14. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-phenylsulfonylimino-1,3-thiazolidin-4-one (14)

Prepared from **5** (0.25 g, 0.37 mmol) and $PhSO_2Cl$ (0.28 mL, 1.85 mmol) according to General procedure **I**. Column chromatography (eluent, 1:1 Acetone-hexane) gave 0.14 g (55 %) of **14** as a yellowish oil. R_f=0.21 (2:1 EtOAc-hexane); $[\alpha]_D$ +48 (c 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 9.18 (s, 1H, NH), 8.04-7.14 (m, 25H, ArH), 6.72 (pseudo t, 1H, $J_{3,4}$ 9.3 Hz, H-3), 6.06 (d, 1H, $J_{2,3}$ 9.7 Hz, H-2), 5.86 (t, 1H, $J_{4,5}$ 9.7 Hz, H-4), 5.32 (ddd, 1H, $J_{5,6}$ 1.2 Hz, H-5), 4.59 (dd, 1H, $J_{6,6'}$ 11.7 Hz, H-6), 4.43 (dd, 1H, $J_{5,6'}$ 3.1 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 180.6, 169.6, 166.1, 165.5, 165.0, 164.5 (*C*O, *C*=N), 138.8-124.7 (Ar*C*), 88.5 (C-1), 72.6, 71.1, 70.8, 68.5 (C-2 - C-5), 62.3 (C-6); Anal. Calcd for $C_{42}H_{32}N_2O_{12}S_2$ (Mol. Wt.: 820.84, Ex. Mass.: 820.14): C, 61.46; H, 3.93; N, 3.41. Found: C, 61.56; H, 4.03; N, 3.21; ESI-MS (positive mode) m/z: 843.131 $[M+Na]^+$.

4.15. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-(4-methylphenylsulfonylimino)-1,3-thiazolidin-4-one (15)

Prepared from **5** (0.15 g, 0.23 mmol) and 4-Me-$C_6H_4SO_2Cl$ (0.17 g, 1.04 mmol) according to General procedure **I**. Column chromatography (eluent, 5:1 EtOAc-hexane) gave 0.14 g (70 %) of **15** as a yellowish crystalline product from hexane. Mp: 161-164 °C; $[\alpha]_D$ +88 (c 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 7.99-7.10 (m, 24H, ArH), 6.80 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.00 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.77 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.30 (ddd, 1H,

$J_{5,6'}$ 2.1 Hz, $J_{5,6}$ 1.1 Hz, H-5), 4.38-4.32 (m, 2H, H-6, H-6'), 2.10 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 178.3, 170.6, 166.0, 165.9, 165.3, 164.7 (*C*O, *C*=N), 139.2-126.6 (Ar*C*), 88.7 (C-1), 72.3, 71.0 (2), 68.6 (C-2 - C-5), 62.3 (C-6), 21.4 (*C*H_3); Anal. Calcd for $C_{43}H_{34}N_2O_{12}S_2$ (Mol. Wt.: 834.86, Ex. Mass.: 834.16): C, 61.86; H, 4.10; N, 3.36. Found: C, 61.76; H, 4.13; N, 3.31; ESI-MS (positive mode) m/z: 857.142 $[M+Na]^+$.

4.16. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-(1-naphthylsulfonylimino)-1,3-thiazolidin-4-one (16)

Prepared from **5** (0.24 g, 0.35 mmol) and 1-naphthylsulfonyl chloride (0.40 g, 1.75 mmol) according to General procedure **I**. Column chromatography (eluent, 2:1 EtOAc-hexane) gave 0.14 g (45 %) of **16** as a yellowish oil. R_f=0.30 (2:1 EtOAc-hexane); $[\alpha]_D$ +65 (c 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 9.94 (s, 1H, NH), 8.48-7.12 (m, 27H, ArH), 6.57 (t, 1H, $J_{3,4}$ 9.7 Hz, H-3), 6.02 (d, 1H, $J_{2,3}$ 9.7 Hz, H-2), 5.87 (t, 1H, $J_{4,5}$ 9.7 Hz, H-4), 5.15 (ddd, 1H, $J_{5,6}$ 1.5 Hz, H-5), 4.62 (dd, 1H, $J_{6,6'}$ 12.3 Hz, H-6), 4.42 (dd, 1H, $J_{5,6'}$ 3.1 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 183.6, 169.9, 166.1, 165.2, 165.0, 164.3 (*C*O, *C*=N), 134.7-123.9 (Ar*C*), 88.4 (C-1), 73.3, 70.9, 70.6, 68.1 (C-2 - C-5), 62.2 (C-6); Anal. Calcd for $C_{46}H_{34}N_2O_{12}S_2$ (Mol. Wt.: 870.90, Ex. Mass.: 870.16): C, 63.44; H, 3.94; N, 3.22. Found: C, 63.59; H, 4.01; N, 3.38; ESI-MS (positive mode) m/z: 893.141 $[M+Na]^+$.

4.17. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-(2-naphthylsulfonylimino)-1,3-thiazolidin-4-one (17)

Prepared from **5** (0.24 g, 0.35 mmol) and 2-naphthylsulfonyl chloride (0.40 g, 1.75 mmol) according to General procedure **I**. Column chromatography (eluent, 2:1 EtOAc-hexane) gave

0.19 g (62 %) of **17** as a yellowish oil. R_f=0.17 (2:1 EtOAc-hexane); $[\alpha]_D$ +40 (c 0.43, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.36-7.28 (m, 27H, ArH), 6.57 (t, 1H, $J_{3,4}$ 9.7 Hz, H-3), 6.03 (d, 1H, $J_{2,3}$ 9.7 Hz, H-2), 5.88 (t, 1H, $J_{4,5}$ 9.7 Hz, H-4), 5.22 (ddd, 1H, $J_{5,6}$ 2.5 Hz, H-5), 4.65 (dd, 1H, $J_{6,6'}$ 12.3 Hz, H-6), 4.46 (dd, 1H, $J_{5,6'}$ 3.7 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 180.2, 170.3, 166.1, 165.3, 165.0, 164.3 (*C*O, *C*=N), 135.7-121.8 (Ar*C*), 88.4 (C-1), 73.4, 71.2, 70.5, 68.2 (C-2 - C-5), 62.3 (C-6); Anal. Calcd for $C_{46}H_{34}N_2O_{12}S_2$ (Mol. Wt.: 870.90, Ex. Mass.: 870.16): C, 63.44; H, 3.94; N, 3.22. Found: C, 63.56; H, 3.84; N, 3.33; ESI-MS (positive mode) m/z: 893.141 $[M+Na]^+$.

4.18. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-pivaloylimino-1,3-thiazolidin-4-one (18)

Prepared from **9** (0.13 g, 0.17 mmol) according to General procedure **II** and purified by column chromatography (eluent, 9:1 $CHCl_3$-MeOH) to give 0.02 g (26%) of **18** as a yellow oil. R_f=0.17 (9:1 $CHCl_3$-MeOH); $[\alpha]_D$ -69 (c 0.05, MeOH); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 4.36 (ddd, 1H, $J_{5,6}$ 4.0 Hz, H-5), 4.23 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.84 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'), 3.72 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.69 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 3.40 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 185.6, 178.9, 165.9 (*C*O, *C*=N), 94.9 (C-1), 86.0 (*C*$(CH_3)_3$) 78.4, 75.0, 74.2, 70.8 (C-2 - C-5), 62.7 (C-6), 27.1 (*C*H_3); Anal. Calcd for $C_{13}H_{20}N_2O_7S$ (348.38): C, 44.82; H, 5.79; N, 8.04. Found: C, 44.75; H, 5.72; N, 8.08.

4.19. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-benzoylimino-1,3-thiazolidin-4-one (19)

Prepared from **10** (0.13 g, 0.16 mmol) according to General procedure **II** and purified by column chromatography (eluent, 4:1 $CHCl_3$-MeOH) and then crystallised from water to give 0.04 g (55 %) of **19** as a white solid. Mp: 242-244°C; $[\alpha]_D$ +27 (c 0.20, H_2O); ^{1}H NMR (D_2O,

360 MHz): δ (ppm) 7.94-7.89 (m, 2H, ArH), 7.50-7.43 (m, 3H, ArH), 4.35 (ddd, 1H, $J_{5,6}$ 2.1 Hz, H-5), 4.25 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.94 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.85 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 3.74 (dd, 1H, $J_{5,6'}$ 4.0 Hz, H-6') 3.52 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4); ^{13}C NMR (D_2O, 90 MHz): δ (ppm) 177.2, 175.4, 170.5 (*C*O, *C*=N), 132.7-129.0 (Ar*C*), 92.3 (C-1), 76.9, 73.0, 72.8, 69.2 (C-2 - C-5), 61.1 (C-6); Anal. Calcd for $C_{15}H_{16}N_2O_7S$ (368.36): C, 48.91; H, 4.38; N, 7.60. Found: C, 48.87; H, 4.43; N, 7.55.

4.20. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(4-methylbenzoylimino)-1,3-thiazolidin-4-one (20)

Prepared from **11** (0.19 g, 0.23 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:3 $CHCl_3$-MeOH) to give 0.04 g (43 %) of **20** as a white crystalline product. Mp: 149-152°C; $[\alpha]_D$ +52 (c 0.37, H_2O); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 8.03 (d, 2H, *J* 8.0 Hz, ArH), 7.23 (d, 1H, *J* 8.0 Hz, ArH), 4.34 (ddd, 1H, $J_{5,6}$ 1.1 Hz, H-5), 4.20 (t, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.80 (dd, 1H, $J_{6,6'}$ 12.3 Hz, H-6), 3.66 (d, 1H, $J_{2,3}$ 9.3 Hz, H-2), 3.63 (dd, 1H, $J_{5,6'}$ 4.9 Hz, H-6') 3.35 (t, 1H, $J_{4,5}$ 9.3 Hz, H-4), 2.35 (s, 1H, CH_3); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 181.0, 179.1, 176.5 (*C*O, *C*=N), 145.2-130.1 (Ar*C*), 92.9 (C-1), 78.3, 75.2, 74.3, 70.9 (C-2 - C-5), 62.8 (C-6), 21.7 (*C*H_3); Anal. Calcd for $C_{16}H_{18}N_2O_7S$ (Mol. Wt.: 382.39, Ex. Mass.: 382.08): C, 50.26; H, 4.74; N, 7.33. Found: C, 50.37; H, 4.63; N, 7.45; ESI-MS (positive mode) m/z: 405.075 $[M+Na]^+$.

4.21. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(1-naphthoylimino)-1,3-thiazolidin-4-one (21)

Prepared from **12** (0.10 g, 0.12 mmol) according to General procedure **II** and purified by column chromatography (eluent, 4:1 $CHCl_3$-MeOH) to give 0.03 g (56 %) of **21** as a colourless oil. R_f=0.59 (7:3 $CHCl_3$-MeOH); $[\alpha]_D$ +38 (c 0.20, DMSO); ^{1}H NMR (D_2O, 360 MHz): δ (ppm) 8.31-7.27 (m, 7H ArH), 4.31 (ddd, 1H, $J_{5,6}$ 1.0 Hz, H-5), 4.22 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.88 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.77 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 3.67 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6') 3.45 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4); ^{13}C NMR (D_2O, 90 MHz): δ (ppm) 182.3, 177.9, 175.4 (*C*O, *C*=N), 134.6-125.1 (Ar*C*), 92.9 (C-1), 77.0, 73.3, 73.1, 69.5 (C-2 - C-5), 61.4 (C-6); Anal. Calcd for $C_{19}H_{18}N_2O_7S$ (418.42): C, 54.54; H, 4.34; N, 6.70. Found: C, 54.48; H, 4.39; N, 6.67.

4.22. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(2-naphthoylimino)-1,3-thiazolidin-4-one (22)

Prepared from **13** (0.14 g, 0.16 mmol) according to General procedure **II** and purified by column chromatography (eluent, 4:1 $CHCl_3$-MeOH) to give 0.04 g (50 %) of **22** as an amorphous white material. R_f=0.45 (4:1 $CHCl_3$-MeOH); $[\alpha]_D$ +35 (c 1.06, DMSO); ^{1}H NMR (DMSO-d_6, 360 MHz): δ (ppm) 8.78 (s, 1H, NH), 8.20-7.60 (m, 7H, ArH), 6.05, 5.14, 4.56 (3s, 3H, OH), 4.18 (dd, 1H, *J* 5.3 Hz, *J* 5.2 Hz, OH) , 4.15 (ddd, 1H, $J_{5,6}$ 1.0 Hz, H-5), 3.95 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.65 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 3.54 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.42 (dd, 1H, $J_{5,6'}$ 4.0 Hz, H-6') 3.16 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4); ^{13}C NMR (DMSO-d_6, 90 MHz): δ (ppm) 186.9, 176.3 (2) (*C*O, *C*=N), 135.0-125.1 (Ar*C*), 90.9 (C-1), 77.5, 73.6, 72.4, 69.1 (C-2

- C-5), 61.1 (C-6); Anal. Calcd for $C_{19}H_{18}N_2O_7S$ (418.42): C, 54.54; H, 4.34; N, 6.70. Found: C, 54.44; H, 4.43; N, 6.75.

4.23. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-phenylsulfonylimino-1,3-thiazolidin-4-one (23)

Prepared from **14** (0.16 g, 0.19 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:1 $CHCl_3$-MeOH) to give 0.08 g (98 %) of **23** as a white crystalline product. Mp: 156-159 °C; $[\alpha]_D$ +82 (c 0.41, H_2O); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 7.93-7.90 (m, 2H, ArH), 7.56-7.47 (m, 3H, ArH), 4.35 (ddd, 1H, $J_{5,6}$ 1.4 Hz, H-5), 4.28 (t, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.75 (dd, 1H, $J_{6,6'}$ 12.1 Hz, H-6), 3.70 (d, 1H, $J_{2,3}$ 9.3 Hz, H-2), 3.62 (dd, 1H, $J_{5,6'}$ 4.9 Hz, H-6'), 3.34 (t, 1H, $J_{4,5}$ 9.3 Hz, H-4); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 182.0, 173.8 (*C*O, *C*=N), 143.8-127.8 (Ar*C*), 98.1 (C-1), 77.8, 74.9, 74.3, 71.0 (C-2 - C-5), 62.8 (C-6); Anal. Calcd for $C_{14}H_{16}N_2O_8S_2$ (Mol. Wt.: 404.42, Ex. Mass.: 404.03): C, 41.58; H, 3.99; N, 6.93. Found: C, 41.65; H, 3.89; N, 7.05; ESI-MS (positive mode) m/z: 427.026 $[M+Na]^+$.

4.24. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(4-methylphenylsulfonylimino)-1,3-thiazolidin-4-one (24)

Prepared from **15** (0.16 g, 0.19 mmol) according to General procedure **II** and purified by column chromatography (eluent, 4:1 $CHCl_3$-MeOH) to give 0.08 g (97 %) of **24** as a colourless oil. R_f=0.32 (7:3 $CHCl_3$-MeOH); $[\alpha]_D$ +39 (c 0.20, MeOH); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 7.78 (d, 2H, *J* 7.9 Hz, ArH), 7.35 (d, 2H, *J* 7.9 Hz, ArH), 4.29 (ddd, 1H, $J_{5,6}$ 2.1 Hz, H-5), 4.18 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.79 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.69 (dd, 1H,

$J_{6,6'}$ 11.9 Hz, H-6), 3.66 (dd, 1H, $J_{5,6'}$ 4.0 Hz, H-6'), 3.37 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 2.42 (s, 3H, CH_3); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 179.4, 173.7 (*C*O, *C*=N), 145.2-127.9 (Ar*C*), 94.9 (C-1), 78.9, 75.0, 74.4, 70.6 (C-2 - C-5), 62.6 (C-6), 21.5 (*C*H_3); Anal. Calcd for $C_{15}H_{18}N_2O_8S_2$ (Mol. Wt.: 418.44, Ex. Mass: 418.05): C, 43.36; H, 4.34; N, 6.69. Found: C, 43.26; H, 4.43; N, 6.55; ESI-MS (positive mode) m/z: 441.042 $[M+Na]^+$.

4.25. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(1-naphthylsulfonylimino)-1,3-thiazolidin-4-one (25)

Prepared from **16** (0.13 g, 0.14 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:3 $CHCl_3$-MeOH) to give 0.06 g (96 %) of **25** as a white crystalline product. Mp: 169-172 °C; $[\alpha]_D$ +71 (c 0.49, H_2O); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 8.69-7.56 (m, 7H, ArH), 4.26 (ddd, 1H, $J_{5,6}$ 1.8 Hz, H-5), 4.16 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.80 (dd, 1H, $J_{6,6'}$ 11.7 Hz, H-6), 3.71 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.64 (dd, 1H, $J_{5,6'}$ 5.5 Hz, H-6'), 3.37 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 178.4, 171.2 (CO, *C*=N), 137.2-125.3 (Ar*C*), 94.2 (C-1), 79.1, 75.1, 74.4, 70.5 (C-2 - C-5), 62.6 (C-6); Anal. Calcd for $C_{18}H_{18}N_2O_8S_2$ (Mol. Wt.: 454.47, Ex. Mass: 454.05): C, 47.57; H, 3.99; N, 6.16. Found: C, 47.53; H, 4.11; N, 6.32; ESI-MS (positive mode) m/z: 477.043 $[M+Na]^+$.

4.26. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,5]-2-(2-naphthylsulfonylimino)-1,3-thiazolidin-4-one (26)

Prepared from **17** (0.19 g, 0.21 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:3 $CHCl_3$-MeOH) to give 0.10 g (98 %) of **26** as a white crystalline product. Mp: 168-171 °C; $[\alpha]_D$ +68 (c 0.35, H_2O); ^{1}H NMR (CD_3OD, 360 MHz):

δ (ppm) 8.47-7.58 (m, 7H, ArH), 4.34 (ddd, 1H, $J_{5,6}$ 2.1 Hz, H-5), 4.26 (pseudo t, 1H, $J_{3,4}$ 9.1 Hz, H-3), 3.74 (dd, 1H, $J_{6,6'}$ 11.7 Hz, H-6), 3.69 (d, 1H, $J_{2,3}$ 9.3 Hz, H-2), 3.60 (dd, 1H, $J_{5,6'}$ 4.9 Hz, H-6'), 3.42 (pseudo t, 1H, $J_{4,5}$ 9.3 Hz, H-4); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 182.1, 174.6 (*C*O, *C*=N), 140.7-123.9 (Ar*C*), 98.2 (C-1), 77.8, 74.9, 74.3, 71.0 (C-2 - C-5), 62.8 (C-6); Anal. Calcd for $C_{18}H_{18}N_2O_8S_2$ (Mol. Wt.: 454.47, Ex. Mass: 454.05): C, 47.57; H, 3.99; N, 6.16. Found: C, 47.66; H, 3.87; N, 6.25; ESI-MS (positive mode) m/z: 477.043 $[M+Na]^+$.

4.27. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-imino-3-methyl-1,3-thiazolidin-4-one (27)

Prepared from **5** (0.30 g, 0.44 mmol) and CH_3I (0.01 mL, 0.88 mmol) according to General procedure **III**. Column chromatography (eluent, 1:2 EtOAc-hexane) gave 0.1 g (62 %) of **27** as a colourless oil. R_f=0.62 (1:2 EtOAc-hexane); $[\alpha]_D$ +97 (c 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.07-7.25 (m, 21H, ArH, NH), 6.69 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 5.96 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.85 (pseudo t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 5.20 (ddd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 4.65 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.35 (dd, 1H, $J_{5,6'}$ 3.9 Hz, H-6'), 3.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 169.8, 166.0, 165.3, 165.0, 164.7, 155.8 (*C*O, *C*=N), 133.8-128.1 (Ar*C*), 90.8 (C-1), 73.7, 71.5, 71.1, 68.5 (C-2 - C-5), 62.4 (C-6), 28.4 (*C*H_3); Anal. Calcd for $C_{37}H_{30}N_2O_{10}S$ (694.71) Számított: C: 63.97, H: 4.35, N: 4.03. Found: C, 63.88; H, 4.41; N, 4.00.

4.28. Allylation of 5 to 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-imino-3-allyl-1,3-thiazolidin-4-one (28) and 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-allylimino-3-allyl-1,3-thiazolidin-4-one (29)

Prepared from **5** (0.10 g, 0.15 mmol) and CH_2CHCH_2Br (0.03 mL, 0.30 mmol) according to General procedure **III**. Column chromatography (eluent, 1:2 EtOAc-hexane) gave as the first fraction 0.025 g (25 %) of **28** as a colourless oil. R_f=0.37 (1:2 EtOAc-hexane); $[\alpha]_D$ +119 (c 0.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.06-7.26 (m, 21H, ArH, NH), 6.70 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 5.96 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.85-5.80 (m, 2H, CH, H-4), 5.25 (dd, 2H, *J* 15 Hz, *J* 9.2 Hz, CH_2), 5.16 (ddd, 1H, $J_{4,5}$ 9.2 Hz, $J_{5,6'}$ 2.1 Hz, $J_{5,6}$ 1.1 Hz, H-5), 4.64 (dd, 1H, $J_{6,6'}$13.2 Hz, H-6), 4.44-4.42 (m, 3H, H-6', CH_2); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 169.1, 166.0, 165.2, 165.1, 164.9, 155.2 (*C*O,*C*=N), 133.4-128.2 (Ar*C*, CH), 111.8 (CH_2), 90.6 (C-1), 73.6, 71.2, 71.1, 68.5 (C-2 - C-5), 62.5 (C-6), 43.9 (*C*H_2); Anal. Calcd for $C_{39}H_{32}N_2O_{10}S$ (720.74) C: 64.99, H: 4.48, N: 3.89. Found: C, 64.89; H, 4.43; N, 3.67.

The second fraction contained 0.30 g (26 %) of **29** as a colourless oil, R_f=0.17 (1:2 EtOAc-hexane); $[\alpha]_D$ +33 (c 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.08-7.25 (m, 20H, ArH), 6.79 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.10 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.85 (pseudo t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 5.62 (m, 1H, CH), 5.54-5.43 (m, 2H, CH, H-5), 5.10 (dd, 2H, *J* 15 Hz, *J* 6.6 Hz, CH_2), 4.98 (dd, 2H, *J* 15 Hz, *J* 6.6 Hz, CH_2), 4.60 (dd, 1H, $J_{6,6'}$13.2 Hz, $J_{5,6}$ 4.0 Hz, H-6), 4.43 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'), 3.88-3.76 (m, 4H, 2 x CH_2); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 183.9, 177.8, 165.1, 165.0 (2), 164.9 (*C*O, C=N), 133.6-128.2 (Ar*C*, 2 x *C*H), 119.7, 119.5 (2 x CH_2), 97.9 (C-1), 72.1, 71.3, 70.9, 68.7 (C-2 - C-5), 62.7 (C-6), 52.6, 52.2 (2 x *C*H_2); Anal. Calcd for $C_{42}H_{36}N_2O_{10}S$ (760.81) C: 66.33, H: 4.77, N: 3.68. Found: C, 66.29; H, 4.63; N, 3.61.

4.29. Benzylation of 5 to 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-imino-3-benzyl-1,3-thiazolidin-4-one (30) and 1*R*-1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,5]-2-benzylimino-3-benzyl-1,3-thiazolidin-4-one (31)

Prepared from **5** (0.30 g, 0.44 mmol) and $PhCH_2Br$ (0.16 mL, 1.32 mmol) according to General procedure **III**. Column chromatography (eluent, 1:3 EtOAc-hexane) gave as the first fraction 0.12 g (34 %) of **30** as a colourless oil. R_f=0.12 (1:3 EtOAc-hexane); $[\alpha]_D$ +104 (c 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.10-7.23 (m, 26H, ArH, NH), 6.74 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 5.99 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.85 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.18 (ddd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 5.03-5.01 (m, 2H, CH_2), 4.63 (dd, 1H, $J_{6,6'}$13.2 Hz, H-6), 4.66 (dd, 1H, $J_{5,6'}$ 4.0 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 169.5, 166.0, 165.2, 165.0, 164.7, 154.8 (*C*O, *C*=N), 135.2-127.8 (Ar*C*), 90.5 (C-1), 73.5, 71.2, 71.0, 68.5 (C-2 - C-5), 62.5 (C-6), 45.2 (*C*H_2); Anal. Calcd for $C_{43}H_{34}N_2O_{10}S$ (770.80): C, 67.00; H, 4.45; N, 3.63. Found: C, 66.95; H, 4.37; N, 3.68.

The second fraction gave 0.06 g (16 %) of **31** as a colourless oil R_f=0.07 (1:3 EtOAc-hexane); $[\alpha]_D$ +139 (c 0.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.10-6.90 (m, 30H, ArH), 6.85 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.18 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.89 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.54 (ddd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 4.93 (d, 1H, *J* 14.5 Hz,CH_2), 4.69-4.63 (m, 2H, CH_2, H-6), 4.45 (dd, 1H, $J_{6,6'}$13.2 Hz, $J_{5,6'}$ 4.0 Hz, H-6'), 4.30-4.28 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 184.2, 178.7, 166.1, 165.2 (2), 164.8 (*C*O, *C*=N), 135.2-127.8 (Ar*C*), 98.1 (C-1), 72.2, 71.2, 71.0, 68.7(C-2 - C-5), 62.6 (C-6), 52.9, 52.5 (2 x *C*H_2); Anal. Calcd for $C_{50}H_{40}N_2O_{10}S$ (860.92): C, 69.75; H, 4.68; N, 3.25. Found: C, 69.71; H, 4.62; N, 3.30.

4.30. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,2]-(5,6-dihydro-imidazo[2,1-*b*]thiazolidin-3-one) (32)

Prepared from **5** (0.20 g, 0.29 mmol) and $Br(CH_2)_2Br$ (0.075 mL, 0.87 mmol) according to General procedure **III**. Column chromatography (eluent, 1:1 EtOAc-hexane) gave 0.14 g (67 %) of **32** as white crystals. Mp: 202-205 °C; $[\alpha]_D$ +195 (c 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.07-7.23 (m, 20H, ArH), 6.67 (pseudo t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 5.98 (d, 1H, $J_{2,3}$ 10.6 Hz, H-2), 5.85 (pseudo t, 1H, $J_{4,5}$ 10.6 Hz, H-4), 5.20 (ddd, 1H, $J_{5,6}$ 4.0 Hz, H-5), 4.66 (dd, 1H, $J_{5,6'}$ 2.6 Hz, H-6'), 4.66 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.32-4.24 (m, 1H, CH_2), 4.21-4.13 (m, 1H, CH_2), 3.88-3.74 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 166.0, 165.2, 165.0, 164.5 163.3, 156.1 (*C*O, *C*=N), 133.7-128.5 (Ar*C*), 97.6 (C-1), 73.8, 72.0, 70.9, 68.4 (C-2 - C-5), 62.4 (C-6), 60.5, 41.7 (2 x *C*H_2); Anal. Calcd for $C_{38}H_{30}N_2O_{10}S$ (706.72): C, 64.58; H, 4.28; N, 3.96. Found: C, 64.49; H, 4.33; N, 3.88.

4.31. 1*R*-1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-glucitol-spiro-[1,2]-(6,7-dihydro-5*H*-thiazolidino[3,2-*a*]pyrimidin-3-one) (33)

Prepared from **5** (0.20 g, 0.29 mmol) and $Br(CH_2)_3Br$ (0.09 mL, 0.87 mmol) according to General procedure **III**. Column chromatography (eluent, 1:1 EtOAc-hexane) gave 0.15 g (70 %) of **33** as a white crystals. Mp: 234-236 °C; $[\alpha]_D$ +181 (c 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ (ppm) 8.05-7.24 (m, 20H, ArH), 6.69 (t, 1H, $J_{3,4}$ 9.2 Hz, H-3), 6.00 (d, 1H, $J_{2,3}$ 9.2 Hz, H-2), 5.87 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 5.20 (ddd, 1H, $J_{5,6}$ 2.3 Hz, H-5), 4.66 (dd, 1H, $J_{6,6'}$ 13.2 Hz, H-6), 4.66 (dd, 1H, $J_{5,6'}$ 1.1 Hz, H-6'), 3.83-3.81 (m, 1H, CH_2), 3.72-3.68 (m, 1H, CH_2), 3.48-3.42 (m, 2H, CH_2), 1.90-1.87 (m, 1H, CH_2), 1.73-1.70 (m, 1H, CH_2); ^{13}C NMR ($CDCl_3$, 90 MHz): δ (ppm) 166.0, 165.9, 165.3, 164.9, 164.3, 147.5 (*C*O, *C*=N), 133.6-

128.3 (Ar*C*), 88.3 (C-1), 73.4, 71.5, 70.0, 68.5 (C-2 - C-5), 62.4 (C-6), 46.2, 40.6, 19.2 (3 x *C*H_2); Anal. Calcd for $C_{39}H_{32}N_2O_{10}S$ (720.74): C, 64.99; H, 4.48; N, 3.89. Found: C, 64.89; H, 4.38; N, 3.80.

4.32. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,2]-(5,6-dihydro-imidazo[2,1-*b*]thiazolidin-3-one) (34)

Prepared from **32** 0.14 g (0.20 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:3 $CHCl_3$-MeOH) to give 0.025 g (43 %) of **34** as a pale yellowish oil. R_f=0.36 (7:3 $CHCl_3$-MeOH); $[\alpha]_D$ +103 (c 0.20, MeOH); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 4.36-4.24 (m, 4H, H-5, H-6, CH_2), 4.17 (t, 1H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz, H-3), 3.86-3.74 (m, 2H, H-2, H-6'), 3.69-3.63 (m, 2H, CH_2), 3.38 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4); ^{13}C NMR (CD_3OD, 90 MHz): δ (ppm) 166.9, 160.9 (*C*O, *C*=N), 102.7 (C-1), 80.0, 76.4, 74.6, 70.6 (C-2 - C-5), 62.6 (C-6), 60.8, 42.5 (2 x *C*H_2); Anal. Calcd for $C_{10}H_{14}N_2O_6S$ (290.29): C, 41.37 H, 4.86; N, 9.65. Found: C, 41.28; H, 4.77; N, 9.60.

4.33. 1*R*-1,5-Anhydro-D-glucitol-spiro-[1,2]-(6,7-dihydro-5*H*-thiazolidino[3,2-*a*]pyrimidin-3-one) (35)

Prepared from **33** (0.15 g, 0.20 mmol) according to General procedure **II** and purified by column chromatography (eluent, 7:3 $CHCl_3$-MeOH) to give 0.03 g (48 %) of **35** as a colourless oil. R_f=0.40 (7:3 $CHCl_3$-MeOH); $[\alpha]_D$ +106 (c 0.20, MeOH); ^{1}H NMR (CD_3OD, 360 MHz): δ (ppm) 4.26 (ddd, 1H, $J_{5,6}$ 4.0 Hz, $J_{5,6'}$ 2.1 Hz, H-5), 4.18 (t, 1H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz, H-3), 3.80 (dd, 1H, $J_{6,6'}$ 11.9 Hz, H-6), 3.69-3.61 (m, 4H, H-2, H-6', CH_2), 3.54-3.47 (m, 2H, CH_2), 3.36 (t, 1H, $J_{4,5}$ 9.2 Hz, H-4), 3.34-3.30 (m, 2H, CH_2); ^{13}C NMR (CD_3OD, 90

MHz): δ (ppm) 171.3, 153.6 (*C*O, *C*=N), 92.7 (C-1), 79.6, 75.7, 74.7, 70.7 (C-2 - C-5), 62.7 (C-6), 47.0, 41.5, 20.4 (3 x CH_2); Anal. Calcd for $C_{11}H_{16}N_2O_6S$ (304.32): C, 43.41 H, 5.30; N, 9.21. Found: C, 43.33; H, 5.37; N, 9.29.

4.34. Enzyme preparation

RMGPb was isolated from rabbit skeletal muscle and purified as described previously.[48] Kinetic studies were performed in the direction of glycogen synthesis in the presence of various concentrations of the inhibitors. Enzyme activity was measured at pH 6.8 by the release of inorganic phosphate as described.[49]

4.35. Crystal complex formation and X-ray crystallographic data collection and processing

Native T-state RMGPb crystals were grown in the tetragonal lattice, space group $P4_32_12$ and prior to data collection were soaked in a buffered solution (10 mM Bes, pH 6.7) with 20 mM of **6** (for 21 h) and 20 mM **19** (for 3.9 h). Diffraction data for both complexes were collected from single crystals at room temperature, using the synchrotron radiation source at SRS-Daresbury Laboratory UK, beamline PX10.1 (λ = 0. 97976 Å). Integration of the reflections and data reduction was performed using the programs DENZO and SCALEPACK from HKL-package.[50]

4.36. Crystal structure determination

The structures of RMGP*b* in complex with the ligands **6**, **19**, and **22** were determined by applying standard protocols for molecular replacement and refinement as implemented in

CCP4 suite[51] using the programs PHASER and REFMAC. Ligand models were fitted to $2F_o$-F_c and F_o-F_c electron density maps after adjustment of their torsion angles using the program for molecular graphics COOT.[52] The quality of the model was improved by alternate cycles of manual rebuilding with COOT where required and refinement with REFMAC. The data collection and refinement statistics along with the model quality of the complex structures are summarized in Table 3S.

The stereochemistry of the protein residues was validated by PROCHECK and the analyses of the complex structures involving mapping of hydrogen bond and van der Waals interactions of the ligands with the residues lining the catalytic site was performed with CONTACT.[51] A distance cut off at 3.3 Å between the electronegative atoms for H-bonds and 4.0 Å for van der Waals interactions was applied. The programme calculates the angle O…H…N (where the hydrogen position is unambiguous) and the angle source… oxygen-bonded carbon atom. Suitable values are 120° and 90°. The network of interactions is described in Tables 4S-12S. Structural comparisons were performed with the program LSQKAB[51] by superposition of the atomic coordinates of the new complexes with RMGP*b*:spirohydantoin. The root mean square deviation in Cα positions were determined for residues (24-249), (261-281), (289-313), (326-549), (558-830). The figures were prepared using the programs Chimera[44] and Molsoft.[47] The coordinates of the new structures in complex with RMGP*b* were deposited with the protein data bank PDBe (www.pdbe.org) with codes: 4ctm for **6**, 4cto for **19**, and 4ctn for **22**.

Acknowledgements

This work was supported in Hungary by the Hungarian Scientific Research Fund (OTKA 46081, 109450), as well as by the projects BAROSS REG_EA_09-1-2009-0028

(LCMS_TAN), TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2.A-11/1/KONV-2012-0025, co-financed by the European Union and the European Social Fund. TD thanks for a Bolyai János Research Fellowship from the Hungarian Academy of Sciences, and research support from the University of Debrecen (5N5X 1IJ0 KUDT 320).

This work was supported in Greece by the EU Marie Curie Early Stage Training (EST) contract no MEST-CT-020575; a Marie Curie Host Fellowships for the Transfer of Knowledge (ToK) contract no MTKD-CT-2006-042776 under FP6; the FP7 Capacities coordination and support actions REGPOT-2009-1-No 245866 'ARCADE'; The research leading to these results has received funding from the European Community's Sixth (RII3/CT/2004/5060008, IHPP HPRI-CT-1999-00012) and Seventh Framework Programme (FP7/2007-2013) under BioStruct-X (grant agreement N°283570) awarded to both SRS-Daresbury Laboratory and EMBL-Hamburg. We would also like to acknowledge the assistance of the staff at SRS-Daresbury beamline 9.6 and EMBL PETRA III beamline P14 for providing excellent facilities for X-ray data collection.

Supplementary material

X-Ray data collection and processing statistics; crystallographic numbering and hydrogen bond network (direct and water mediated) as well as van der Waals interactions of compounds **6**, **19**, and **22** at the catalytic site of RMGP*b* (Tables 3S-12S); LCMS records for compounds **19**, **21**, **22**, and **24**; identification of DTT bound to the protein.

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Glucopyranosylidene-spiro-iminothiazolidinone, a New Bicyclic Ring System: Synthesis, Derivatization, and Evaluation for Inhibition of Glycogen Phosphorylase by Enzyme Kinetic and Crystallographic Methods

K. Czifrák, A. Páhi, Sz. Deák, A. Kiss-Szikszai, K. E. Kövér, T. Docsa, P. Gergely, K.-M. Alexacou, M. Papakonstantinou, D. D. Leonidas, S. E. Zographos, E. D. Chrysina,* L. Somsák*