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Glucose-based spiro-heterocycles as potent inhibitors of glycogen phosphorylase

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ABSTRACT

Glucopyranosylidene-spiro-1,4,2-oxathiazoles were prepared in high yields by NBS-mediated spiro-cyclization of the corresponding glucosyl-hydroximothioates. In an effort to synthesize analogous glucopyranosylidene-spiro-1,2,4-oxadiazolines, with a nitrogen atom instead of the sulphur, attempted cyclizations resulted in aromatization of the heterocycle with opening of the pyranosyl ring. Enzymatic measurements showed that some of the glucose-based inhibitors were active in the micromolar range. The 2-naphthyl-substituted 1,4,2-oxathiazole displayed the best inhibition against RMGPb (K_i = 160 nM), among glucose-based inhibitors known to date.

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1. Introduction

Type 2 diabetes mellitus[1] is currently estimated to affect more than 5% of the adult population in Western societies, and its incidence is expected to increase considerably in the future.[2,3] This is in particular due to the dramatic increase in obesity even among young adults and children.[4–6] Striking enhancements in the prevalence of the disease is predicted for the developing countries of Africa, Asia, and South America.[2] Current preventive and therapeutic strategies do not achieve adequate control of blood glucose to diminish chronic morbidity, and there is a need to develop novel healthcare interventions to address this substantial biomedical challenge.[7]

Hepatic glucose output is elevated in type 2 diabetic patients and current evidence indicates that glycogenolysis (release of monomeric glucose from the glycogen polymer storage form) is an important contributor to the abnormally high production of glucose by the liver. Glycogen phosphorylase (GP) is the rate limiting enzyme in the liver responsible for glycogen breakdown to produce glucose and related metabolites for energy supply.[8] Due to its key role in modulation of glycogen metabolism, pharmacological inhibition of GP has been regarded as an effective therapeutic approach to treating type 2 diabetes.[9–11]

Several types of compounds for inhibition of GP have been reported,[10–13] and among them derivatives of D-glucose represent, as ligands of the catalytic site, the most populated class (Chart 1).[12–15] There are three mammalian GP isoenzymes termed as 'brain', 'muscle', or 'liver' GP depending on the tissue in which they are preferentially expressed. All isoenzymes can be converted from the inactive form (GP*b*) into the active GP*a* form through the phos-

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	R			X	
A	CH_3	32[16]	**G**	O	3.1[28,29]
B	CF_3	75[24]			4.2[26,30]
C	CH_2N_3	49[25]	**H**	S	5.1[26,30]
D	C_6H_5	81[16], 144[26]			
E	$C_{10}H_7$ (2-naphthyl)	10[27]			
F	CH=CH-$C_{10}H_7$ (2-naphthyl)	3.5[27]			

H-bridges between His-377 and *N*-acyl-β-D-glucopyranosylamine type inhibitors in the catalytic site of GP

	R	
I	CH_3	305[31]
J	C_6H_5	4.6[31]
K	4-*t*Bu-C_6H_4	0.7[20]
L	$C_{10}H_7$ (1-naphthyl)	15[20]
M	$C_{10}H_7$ (2-naphthyl)	0.35[20]

N 26

O

Chart 1. Inhibitory constants (K_i [μM]) for some representative inhibitors of rabbit muscle glycogen phosphorylase.[24,25,27]

phorylation of Ser-14. Isoforms of GP can be obtained from rabbit squeletal muscles (RMGP*a*/RMGP*b*) or human liver (HLGP*a*/HLGP*b*). Because of a ~80% homology between the liver and muscle isoforms of GP, it is a general practice to use the more readily available RMGP (most frequently in the unphosphorylated *b* form) for preliminary kinetic studies (Chart 1), even though, for in vivo experiments, the existence of isoforms raises the question of the selectivity of inhibition.[13] The functional form of GP is a homodimer having a C-2 symmetry. The catalytic site of GP is buried at the centre of the monomers, and is accessible to the bulk solvent through a 15 Å long channel. The site has been extensively investigated with glucose analogue inhibitors which promote the less active T (tensed) state through stabilisation of the closed position of the 280s loop. Upon transition from the T state to the R (relaxed) state, the 280s loop becomes disordered and displaced, resulting in the activation of the enzyme. Crystallographic investigations have shown the existence of an empty pocket in the direction of the β-anomeric substituent of D-glucopyranose, lined by both polar and non-polar group, and named the β-channel.[10]

N-Acetyl-β-D-glucopyranosylamine[16] **A** was one of the first efficient glucose analogue inhibitors of GP. A large array of compounds with the *N*-acyl-β-D-glucopyranosylamine structure (e.g., **B**–**F**) was synthesized, tested, and also investigated by X-ray crystallography.[10] These studies revealed that a H-bridge between the amide NH and main chain CO of His377 (Chart 1) significantly contributed to the binding. This H-bridge was also observed in GP crystal complexes of the low micromolar inhibitor spiro-hydantoins **G**[17] and **H**,[18] and was, thereby, considered to be an essential feature for strong inhibition. The rigidity of the spiro-bicyclic structure of the latter compounds with a pyranoid sugar and a five-membered heterocyclic ring was also considered to be a decisive factor for the efficiency.[17] On the other hand, *N*-β-D-glucopyranosyl-*N*′-acyl ureas[12,13,19] (e.g., **I**–**M**) with large aromatic acyl moieties (**K**, **M**) properly oriented so as to interact with the side chains of the so-called β-channel next to the catalytic site, exhibit nanomolar inhibition[12,13,20] despite the lack of the H-bond mentioned above. Therefore, interactions in the so called β-channel of the enzyme must play a more important role than that particular H-bond.

Based on the binding peculiarities of these inhibitors, we envisaged to prepare compounds which might unify the structural features of the best inhibitors, that is, spiro junction between the anomeric carbon of D-glucopyranose and a five-membered hetero-

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cycle, as well as a large aromatic substituent capable of interactions in the β-channel. This design was supported by the finding that spiro-oxathiazole **N**, available from earlier synthetic studies,[21,22] proved to be a micromolar inhibitor. Therefore, synthesis of further compounds of this series was carried out[23] and, in order to facilitate the formation of an additional H-bond, preparation of spiro-oxadiazolines of type **O** was also attempted.

2. Results and discussion

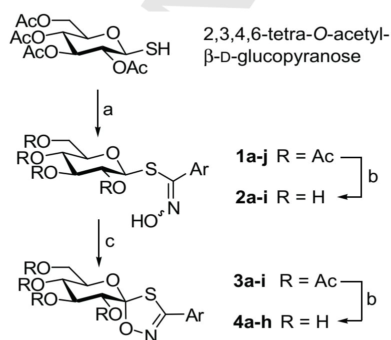
2.1. Preparation of glucosyl-hydroximothioates

The D-glucosyl hydroximothioates **1a–j** were prepared from per-*O*-acetylated 1-thio-β-D-glucopyranose and in situ formed nitrile oxides used in slight excess (1.2 equiv), following a procedure reported previously by Rollin[32] (Scheme 1, Table 1). The synthesis of **1i** (Ar = 4-pyridyl) was optimized by increasing the amount of 4-pyridyl-hydroximoyl chloride used for producing the corresponding nitrile oxide with yields growing as follows: 6% (1.4 equiv), 48% (3.8 equiv), 78% (5 equiv). The prepared hydroximothioates which appeared to be stable under normal conditions were deacetylated by standard procedures (NaOMe/MeOH or MeOH/H_2O/Et_3N) to afford the corresponding unprotected products in high yields (Scheme 1).

In an attempt to prepare molecules susceptible to bind more tightly at the active site of GP through non covalent interactions involving a polar group near the anomeric center of the ligand, we tried to oxidize compound **1i** (Ar = 4-pyridyl) with *m*-CPBA, as applied earlier for the synthesis of glucosyl-phenyl-sulfoxides.[33] The oxidation was not selective, since the reaction mixture was shown by TLC to contain a mobile compound, and two major polar products. One of them was shown to be the *N*-oxide **1j** (Ar = 4-pyridyl-*N*-oxide), isolated in ca 30% yield.

2.2. NBS-mediated cyclization of hydroximothioates

The spiro-cyclization of phenyl hydroximothioate **1a** proceeded in $CHCl_3$ with the same selectivity as already observed in CCl_4,[22,33] affording a 5:1 mixture of the (1*S*)/(1*R*)-epimers of **2a** (Scheme 1). The reaction of hydroximothioates **1b,c,e,g,h** with *N*-bromosuccinimide (NBS) in freshly distilled CCl_4 or $CHCl_3$ was stopped when TLC monitoring indicated complete conversion of the substrates, ca 45 min at reflux under irradiation with a tungsten lamp (60 W). This afforded the spiro-bicycles **3b,c,e,g,h** as a mixture of epimers, with the predominance of the (1*S*)-configured compounds. Separation by flash column chromatography afforded first the pure (1*S*)-epimers. The fractions collected next contained epimeric mixtures whose composition was estimated by ^1H NMR to permit calculation of the yield for each epimer (Table 1). This



Scheme 1. Synthesis of D-glucopyranosylidene-spiro-oxathiazoles. Reagents and conditions: (a) ArC(Cl) = NOH, Et_3N; (b) NaOMe, MeOH, or MeOH, Et_3N, H_2O, rt; (c) NBS, *hν*, 45 min, in refluxing CCl_4 or $CHCl_3$.

spiro-cyclization is believed to proceed through a 1,5-hydrogen transfer to alkoxyl radicals to create predominantly α oriented C–O bonds.[34,35] Spiro-cyclization of **1f** was not selective under the applied conditions (heating for 1 h with a lamp), yielding a multicomponent mixture. Among the four fractions obtained by column chromatography, only the third one was pure enough to get exploitable NMR data corresponding probably to the (1*R*)-**3f** spiro compound, because of the observed pattern of the proton signals resonating at δ = 5.59, 5.10, 5.27 and 4.10 ppm for protons H-2, H-3, H-4 and H-5, respectively (in $CDCl_3$).

Treatment of **1i** with NBS (2 equiv) in boiling chloroform for 30 min resulted in the formation of two major and two minor compounds (TLC). Column chromatography afforded only one homogeneous fraction identified as (1*R*)-**3i** (30%), based on NMR data and the fact that (1*R*)-epimers were found to be significantly more dextrorotatory than the (1*S*)-epimers.[22,33] Since spiro-cyclization afforded in this case a higher proportion of the (1*R*)-epimer, we wondered whether this was due to the basic properties of pyridine. Therefore, the spiro-cyclization of **1a** (Ar = phenyl) was carried out in the presence of DBU (1 equiv) affording **2a** (34% yield) in a 1:1 (1*S*)/(1*R*) ratio after repeated column chromatographies. As compared to the selectivity normally observed (60% yield, 5:1 (1*S*)/(1*R*) ratio), this change suggested a variation along the possible reaction pathways.

The Zemplén deacetylation was achieved in high yields to obtain the desired spiro-oxathiazoles **4b,c,e,g,h**. The final products had a very poor solubility in H_2O, MeOH, EtOH (less than 0.1 mg/mL) but were soluble in DMF or DMSO.

2.3. Attempted preparations of glucosylidene-spiro-1,2,4-oxadiazolines

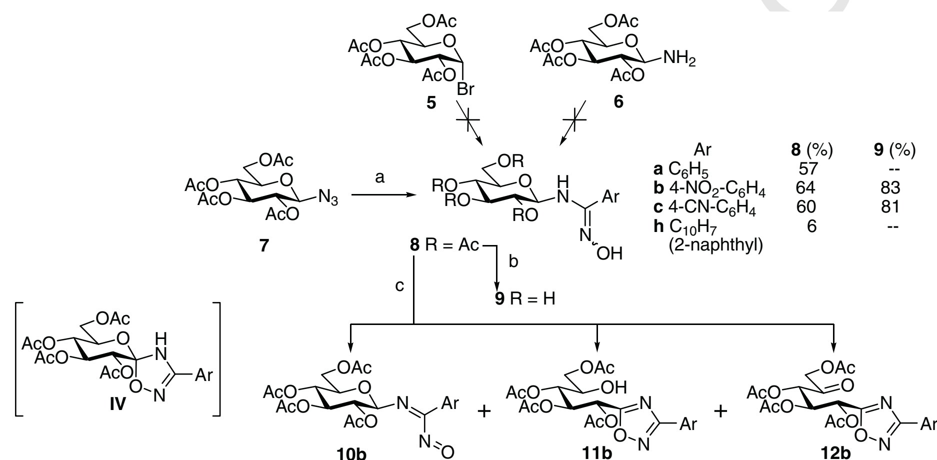
The synthesis of glucosylidene-spiro-1,2,4-oxadiazolines was planned as nitrogen-containing spiro analogues by applying again a NBS-mediated cyclization reaction to *N*-β-D-glucosyl-amidoximes **8** (Scheme 2). A detailed retrosynthetic analysis and the preparation of the amidoximes[36] are presented in the Supporting Information. Preparation of the required amidoxime **8** from per-*O*-acetylated glucopyranosyl bromide **5** or glucopyranosylamine **6** failed. Fortunately, in the presence of trimethylphosphine, acetylated β-D-glucopyranosyl azide **7** was transformed into the corresponding phosphinimine derivative[37] which reacted readily with hydroximoyl chlorides[38–40] and gave the target molecules **8a–c,h**. The NMR spectra of the crude products indicated the presence of two species (~1:10 ratio) assumed to be the *E* and *Z*-configured isomers. Due to the poor stability of some of these compounds, only **8b** and **8c** could be deacetylated by the Zemplén method to give **9b** and **9c**.

The oxidative photocyclization of 4-nitrobenzamidoxime **8b** was carried out under conditions similar to those applied in the synthesis of spiro-oxathiazoles **3**. A chloroform solution of **8b** and NBS was irradiated with a heating lamp (60 or 375 W). NBS (4 equiv, twice as much as for the oxathiazoles) had to be added at once or portion wise to completely transform the starting material. Three experiments afforded mixtures which did not contain the expected spiro-molecule **IV** (Scheme 2) but separation by column chromatography afforded **10b**, **11b**, and **12b** in variable proportions (Table 2). Nitroso compound **10b** should result from **8b** by oxidation without cyclization. However, formation of **11b** and **12b** clearly indicated that spiro-cyclization must have occurred followed by ring opening of the pyranosyl ring to allow aromatization of the oxadiazole ring. Compound **12b** displayed in addition a carbonyl due to oxidation of the liberated hydroxyl at C-5. The product distribution was highly dependent on the applied conditions which were not optimized, although it was possible to obtain **12b** as a single product.

Table 1
Preparation of hydroximothioates **1–2** and glucosylidene-spiro-oxathiazoles **3–4**

Ar=	Compound/yield[a] (%)				
Ph	**2a**/9432	**1a**/9032	(1*S*)-**3a**/46	(1*R*)-**3a**/11	(1*S*)-**4a**/90
p-NO2Ph	**2b**/9132	**1b**/6032	(1*S*)-**3b**/*33*	(1*R*)-**3b**/*16*	(1*S*)-**4b**/71
p-CNPh			(1*S*)-**3c**/*15*	(1*R*)-**3c**/*7*	(1*S*)-**4c**/84
p-FPh	**2d**/9532	**1d**/6532	(1*S*)-**3d**/3022	(1*R*)-**3d**/1622	(1*S*)-**4d**/79
p-MeOPh	**2e**/9732	**1e**/7132	(1*S*)-**3e**/*69*	(1*R*)-**3e**/*17*	(1*S*)-**4e**/79
3,4,5-triMeOPh		**1f**/85	Unselective reaction		
p-PhPh			(1*S*)-**3g**/*61*	(1*R*)-**3g**/*18*	(1*S*)-**4g**/97
2-Naphthyl			(1*S*)-**3h**/*36*	(1*R*)-**3h**/*16*	(1*S*)-**4h**/94
4-Pyridyl	**2i**/90	**1i**/78	(1*S*)-**3i**/0	(1*R*)-**3i**/30	(1*R*)-**4i**/95
4-Pyridyl-*N*-oxide		**1j**/30			

[a] Calculated yields given in italics take into account the ratio of anomers estimated by ^{1}H NMR in mixed fractions after purification.



Scheme 2. Attempted preparations of glucosylidene-spiro-oxadiazolines **IV**. Reagents and conditions: (a) i) PMe_3, ii) ArC(Cl) = NOH, Et_3N; (b) NaOMe, MeOH; (c) see conditions in Table 2.

Table 2
Photolysis of **8b** under various conditions

Lamp (W)	NBS (equiv)	Yield of **10b** (%)	Yield of **11b** (%)	Yield of **12b** (%)
60	4	31	0	~3
60	4 × 1	0	0	45
375	4	0	20	10

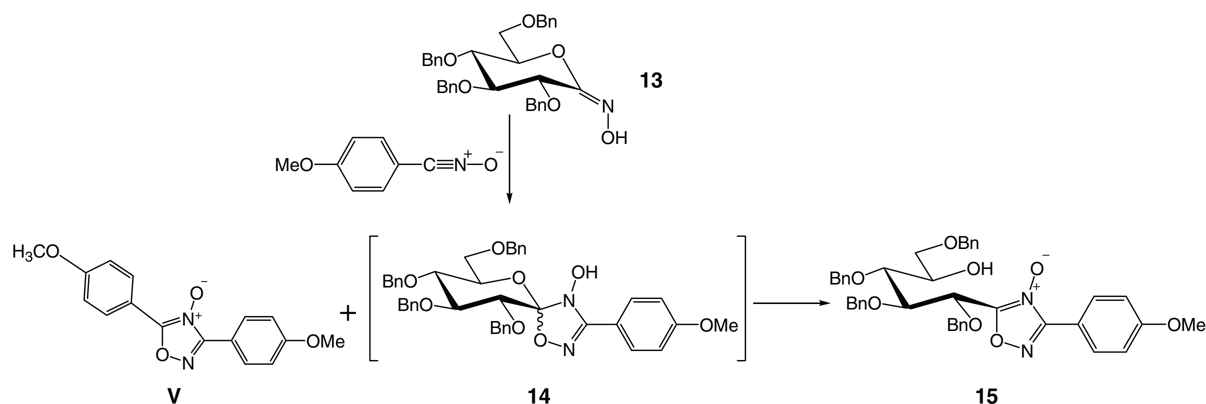
1,3-Dipolar cycloaddition of nitrile oxides to benzyl-protected glucosyl-hydroximolactone **13**[41,42] was envisaged as another approach to analogous glycopyranosylidene-spiro-1,2,4-oxadiazolines. Cycloadditions with aryl nitrile oxides derived from α-chloro-benzaldoxime, α-chloro-4-methoxybenzaldoxime, and α-chloro-4-nitrobenzaldoxime proved to be disappointing since **13** underwent partial conversion after 24 h in boiling toluene in the presence of 8 equiv of 4-methoxybenzonitrile oxide. Among the isolated products (Scheme 3), we could identify the *N*-oxide dimer **V**[43] deriving from the nitrile oxide used and **15** (13% isolated yield). Based on the conversion of **13**, the calculated yield for **15** was 22%. The low reactivity of **13** was confirmed by attempted reactions with either trimethylsilyldiazomethane in refluxing dioxane, or with bromonitrile oxide, which resulted only in slow and unselective transformations. Isolation of **V** (m/z = 299.1 $[M+H]^+$) proved the existence, in the reaction mixture, of the 4-methoxybenzonitrile oxide which reacted slowly with **13**. Mass spectrometry (ESI) confirmed that cycloaddition took place but it was not appropriate to discriminate between structures **14** or **15** which both might correspond to the observed ions (m/z = 703 $[M+H]^+$). ^{13}C NMR spectroscopy indicated chemical shifts at 140.8 ppm (N_2CAr) and 154.2 ppm (NCO) characteristic of the oxadiazole *N*-oxide ring.[44] Formation of an aromatic oxadiazole ring with opening of the pyrane ring was in analogy with our previous observations from attempted spiro-cyclization of benzamidoximes.

2.4. Inhibition of glycogen phosphorylase

Enzymatic tests (Table 3) with deprotected hydroximothioates **2**, benzamidoximes **9b,c**, and the (1*R*)-configured spiro-oxathiazole (1*R*)-**4i** showed no significant inhibition of rabbit muscle glycogen phosphorylase (RMGP) *b*. In contrast, several (1*S*)-configured spiro-compounds (1*S*)-**4** showed inhibition in the low micromolar range, except for compounds (1*S*)-**4b,c** having polar substituents (4-nitro and 4-cyano, respectively). The most efficient was the 2-naphthyl derivative (1*S*)-**4h** with a nanomolar K_i which appears to be the best glucose-based inhibitor known at the moment. This shows conclusively that a rigid spiro-bicyclic structure, attached to a properly oriented large apolar aromatic group as the 2-naphthyl are favorable structural features for strong binding at the catalytic site of GP. Further exploitation of these findings is in progress for the design of more potent inhibitors.

3. Conclusion

The recent identification of glycogen phosphorylase as a biological target for the treatment of hyperglycemia led to the design of a large collection of glucose-based inhibitors displaying inhibitory activities in the micromolar range.[10,12–15] Among them, the glucopyranosylidene-spiro-hydantoins[28–30] and *N*-acyl-*N*′-β-D-glucopyranosyl urea[20,31] derivatives were shown to be the best inhibitors.



Scheme 3. Attempted cyclization of glucosyl-hydroximolactone **13**.

Table 3
Inhibition of rabbit muscle glycogen phosphorylase (RMGP) *b*

Compound	Inhibition (μM)	Compound	Inhibition (μM)
2b	IC50 > 10 000	(1*S*)-**4a**	K_i = 26
2c	No inhibition at 625	(1*S*)-**4b**	IC50 = 250
2e	No inhibition at 312.5	(1*S*)-**4c**	IC50 = 700
2g	Insoluble in DMSO	(1*S*)-**4d**	K_i = 48
2h	Insoluble in DMSO	(1*S*)-**4e**	K_i = 8.2
2i	No inhibition at 625	(1*S*)-**4g**	IC50 = 250
9b	No inhibition at 625	(1*S*)-**4h**	K_i = 0.16
9c	K_i = 1 800	(1*R*)-**4i**	No inhibition at 625

Based on the binding peculiarities of these types of inhibitors as revealed by X-ray crystallography, we set up a new design principle towards better inhibitors.[23] To validate this approach, we designed a synthesis of properly substituted glucopyranosylidene-spiro-oxathiazoles by NBS-mediated spiro-cyclization at the anomeric center of glucopyranosyl-hydroximothioate precursors. Attempted preparations of the corresponding aza-analogs, in which the sulphur atom is replaced by a nitrogen, failed and led to heteroaromatic structures with an acyclic carbohydrate moiety. The 2-naphthyl substituted glucopyranosylidene-spiro-oxathiazole displayed the strongest inhibition with a K_i value of 160 nM. The present study provides the best inhibitor of GP in the glucose-based family and strengthens the need for additional investigations towards inhibition of this enzyme.

4. Experimental

4.1. General methods

Dichloromethane, chloroform and carbon tetrachloride were washed with water (3 times), then dried ($CaCl_2$) prior distillation over CaH_2, and kept away from light. Triethylamine and water were distilled twice. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with Silica Gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/H_2O (1/1 v/v) followed by heating. Brominated compounds were visualized on TLC plates by spraying first a 0.1% w/v fluorescein solution in absolute MeOH, then a 1:1 mixture of H_2O_2 (30% in water) and AcOH. Upon gentle heating, bromo-compounds gave pink-colored spots. Silica gel column chromatography was performed with Geduran® Silica Gel Si 60 (40–63 μm) purchased from Merck (Darmstadt, Germany). Preparative reversed phase chromatography (RP-18) was performed using a 15 × 150 mm column of fully endcapped Silica Gel 100 C_{18} (>400 mesh, Fluka). ^1H and ^{13}C NMR spectra were recorded at 23 °C using Bruker DRX300 or DRX500 spectrometers with the residual solvent as the internal standard. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; dd, doublet of doublet; ddd, doublet of doublet of doublet; t, triplet; td, triplet of doublet; q, quadruplet; m, multiplet; br, broad; p, pseudo. Structure elucidation was deduced from 1D and 2D NMR spectroscopy which allowed, in most cases, complete signal assignments based on COSY, HSQC, and HMBC correlations. Atom numbering follows that of the parent carbohydrate for the description of the sugar signals in the NMR spectra. Uncertain assignments are indicated by an asterisk. NMR solvents were purchased from Euriso-Top (Saint Aubin, France). HRMS (LSIMS) mass spectra were recorded in the positive mode using a Thermo Finnigan Mat 95 XL spectrometer. MS (ESI) mass spectra were recorded in the positive mode (unless stated otherwise) using a Thermo Finnigan LCQ spectrometer. Optical rotations were measured using a Perkin Elmer polarimeter. A collection of compounds **1** and **2** have been previously described except for their ^{13}C NMR data.[32] 1-Thio-β-D-glucopyranosyl hydroximothioates **2a,b,d,e** were previously reported.[32] Compound (1*S*)-**3d** was previously reported.[22]

4.1.1. General procedure A for the preparation of O-peracetylated hydroximothioates 1

Preparation adapted from previously reported procedures.[21,32]

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1 mmol) and hydroximoyl chloride (1.2 mmol, 1.2 equiv) were dissolved in CH_2Cl_2 (5 mL). After adding triethylamine (3 mmol, 3 equiv), the mixture was stirred at room temperature and TLC monitoring showed completion of the reaction. The solvent was evaporated and the residue purified by flash silica gel column chromatography (PE/EtOAc 1:1, then 2:3) to afford the desired hydroximothioates **1**.

4.1.2. General procedure B for the preparation of deacetylated compounds 2i and (1R)-4i

The acetylated compound (0.25 mmol) was dissolved in a mixture of water (5 mL), MeOH (5 mL) and triethylamine (1 mL). After stirring overnight at room temperature, TLC showed deacetylation was complete. Evaporation of the volatiles under reduced pressure afforded a residue corresponding to pure deacetylated product.

4.1.3. General procedure C for the preparation of spiro-oxathiazoles 3

A solution of hydroximothioate **1** (1 mmol) and *N*-bromosuccinimide (2 mmol) in CCl_4 (20 mL, for **3b–e,g,h**) or $CHCl_3$ (20 mL for **3a,f,i**) was boiled and illuminated by a 60 W heat lamp for 45 min. The reaction was diluted with EtOAc (150 mL) and the organic layer was washed with 5% aqueous Na_2SO_3 (2 × 100 mL) and water (2 × 100 mL), dried (Na_2SO_4), filtered and evaporated under

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reduced pressure. The residue was purified by flash column chromatography (PE then PE/EtOAc 65:35) to afford the desired acetylated spiro-oxathiazole **3**.

4.1.4. General procedure D for the Zemplén deacetylation

A solution of an acetylated compound **1** or **3** (150 mg) and NaOMe (5 mg) in MeOH (15 mL) was stirred at rt for 3 h. The reaction was neutralized to pH 5–6 with Amberlite IR-120 resin (H^+ form) and the resin washed with MeOH (2 × 10 mL). The filtrate was evaporated under reduced pressure to afford the desired hydroximothioates **2** and spiro-oxathiazoles **4**, respectively.

4.1.5. General procedure E for the synthesis of *N*-glycosyl-amidoximes 8

Trimethyl phosphine (2.68 mL, 1 M in toluene) was added with stirring to a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide **7** (1 g, 2.68 mmol) in dry dichloromethane (10 mL). After gas evolution had ceased and TLC showed complete transformation of the starting azide **7** (10–15 min), a hydroximoyl chloride (1 equiv) was added. After 20–30 min, the reaction mixture was poured into water, and the organic layer was separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic layers were combined, washed with 10% aq sodium carbonate and water (5 × 20 mL), dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash column chromatography (EtOAc/hexane, 1:3) or crystallization. An optimized synthesis of **8c** is also presented below.

4.2. Synthesis of O-peracetylated hydroximothioates 1

4.2.1. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-4-nitrobenzhydroximoyl-1-thio-β-D-glucopyranose 1b

From tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1.8 g, 5.3 mmol) according to general procedure A yielding **1b** (1.38 g, 2.61 mmol, 53%) as a yellow crystalline product.

R_f = 0.48 (PE/EtOAc, 1:1); $[\alpha]_D^{25}$ +7.5 (*c* 0.57, MeOH), lit.[32] $[\alpha]_D$ +8.0 (*c* 1, $CHCl_3$); mp = 156–157 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.40 (s, 1H, OH), 8.30 (d, 2H, *J* = 7.9 Hz, H-ar), 7.62 (d, 2H, *J* = 7.9 Hz, H-ar), 5.11–5.02 (m, 3H, H-2 H-3 H-4), 4.63 (d, 1H, *J* = 9.2 Hz, H-1), 4.12 (dd, 1H, *J* = 3.8 and 11.9 Hz, H-6), 4.01 (dd, 1H, *J* = 2.6 Hz, H-6′), 3.24 (ddd, 1H, H-5), 2.11, 2.07 (2s, 6H, CH_3CO), 1.99 (s, 6H, CH_3CO); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.6, 170.2 (2s, CH_3CO), 169.3 (s, 2C, CH_3CO), 149.4 (C=N), 148.6, 139.2 (2s, CH-ar), 129.8 (s, 2C, CH-ar), 123.5 (s, 2C, CH-ar), 81.3 (C-1), 75.8, 73.4, 70.1, 67.8 (4s, C-2 C-3 C-4 C-5), 61.8 (C-6), 20.7, 20.6 (2s, 2C, CH_3CO), 20.4 (s, 2C, CH_3CO); Anal. Calcd for $C_{21}H_{24}N_2O_{12}S$ (528.50): C, 47.73; H, 4.58; N, 5.30; S, 6.07. Found: C, 47.51; H, 4.76; N, 5.65; S, 5.99.

4.2.2. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-4-cyanobenzhydroximoyl-1-thio-β-D-glucopyranose 1c

From tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1.5 g, 4.1 mmol) according to general procedure **A** yielding **1c** (900 mg, 1.77 mmol, 48%) as a white crystalline product.

R_f = 0.19 (PE/EtOAc, 2:1); $[\alpha]_D^{25}$ +23 (*c* 0.28, MeOH); mp = 165–167 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 10.10 (s, 1H, OH), 7.80–7.70 (m, 4H, H-ar), 5.10–5.00 (m, 3H, H-2 H-3 H-4), 4.62 (d, 1H, *J* = 9.2 Hz, H-1), 4.12 (dd, 1H, *J* = 3.8 and 11.9 Hz, H-6), 3.98 (dd, 1H, *J* = 2.6 Hz, H-6′), 3.22 (ddd, 1H, H-5), 2.09, 2.05 (2s, 6H, CH_3CO), 1.99 (s, 6H, CH_3CO); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.6, 170.1, (2s, CH_3CO), 169.3 (s, 2C, CH_3CO), 149.0 (C=N), 137.5, 132.1 (2s, CH-ar), 129.4 (s, 2C, CH-ar), 117.9 (s, 2C, CH-ar), 113.4 (CN), 81.1 (C-1), 75.6, 73.4, 70.0, 67.7 (4s, C-2 C-3 C-4 C5), 61.7 (C-6), 20.6, 20.4 (2s, CH_3CO), 20.3 (s, 2C, CH_3CO); Anal. Calcd for $C_{21}H_{24}N_2O_{10}S$ (508.11): C, 51.96; H, 4.76; N, 5.51; S, 6.31. Found: C, 51.71; H, 4.88; N, 5.32; S, 6.11.

4.2.3. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-4-methoxybenzhydroximoyl-1-thio-β-D-glucopyranose 1e

From tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1.63 g, 4.47 mmol) according to general procedure **A** yielding **1e** (1.17 g, 2.28 mmol, 51%) as a white crystalline product.

R_f = 0.27 (PE/EtOAc, 1:1); $[\alpha]_D^{25}$ +29 (*c* 0.44, CH_2Cl_2); lit.[32] $[\alpha]_D$ +13 (*c* 1, $CHCl_3$); mp = 157–161 °C lit.[32] mp = 150 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.46 (s, 1H, OH), 7.46 (d, 2H, *J* = 7.9 Hz, H-ar), 6.93 (d, 2H, *J* = 7.9 Hz, H-ar), 5.10–4.99 (m, 3H, H-2 H-3 H-4), 4.50 (d, 1H, *J* = 10.5 Hz, H-1), 4.12 (dd, 1H, *J* = 3.6 and 11.9 Hz, H-6), 3.98 (dd, 1H, *J* = 1.2 Hz, H-6′), 3.86 (s, 3H, OCH_3), 3.13 (ddd, 1H, H-5), 2.10, 2.04 (2s, 6H, CH_3CO), 1.96 (s, 6H, CH_3CO); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.6, 170.2 (2s, CH_3CO), 169.2 (s, 2C, CH_3CO), 160.8 (C=N), 152.2, 130.3, 124.5 (2s, CH-ar), 113.7 (2s, CH-ar), 81.4 (C-1), 75.3, 73.7, 69.4, 67.8 (4s, C-2 C-3 C-4 C5), 61.8 (C-6), 55.3 (OCH_3), 20.6, 20.5, 20.4, 20.3 (4s, CH_3CO); Anal. Calcd for $C_{22}H_{27}NO_{11}S$ (513.52): C, 51.46; H, 5.30; N, 5.51; S, 6.24. Found: C, 51.62; H, 5.46; N, 5.50; S, 6.01.

4.2.4. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-3,4,5-trimethoxybenzhydroximoyl-1-thio-β-D-glucopyranose 1f

Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (553 mg, 1.57 mmol) dissolved in CH_2Cl_2 (30 mL) was reacted with 3,4,5-trimethoxybenzhydroximoyl chloride (4.92 mmol, 3 equiv) upon stirring at room temperature in the presence of NEt_3 (3 mL, 22 mmol, 14 equiv) and under argon. After the reaction was over (~30 min), aqueous washings and concentration afforded a crude oil which was purified by column chromatography (PE/EtOAc 1:1, then 2:3), yielding **1f** (770 mg, 1.35 mmol, 85% yield) as a colorless foam.

R_f = 0.23 (PE/EtOAc, 1:1); $[\alpha]_D^{20}$ +27.8 (*c* 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (s, 1H, OH), 6.75 (s, 2H, H-ar), 5.03 (m, 3H, H-2 H-3 H-4), 4.68 (d, 1H, *J* = 9.5 Hz, H-1), 4.06 (dd, 1H, *J* = 3.8 and 12.5 Hz, H-6), 3.93 (dd, 1H, *J* = 2.3 Hz, H-6′), 3.84 (s, 9H, OMe), 3.19 (ddd, 1H, H-5), 2.00, 1.99, 1.94, 1.92 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.7, 170.2, 169.7 (4s, CH_3CO), 153.6 (s, 2C), 151.8 (C=N), 140.0, 128.4, 106.8 (s, 2C, CH-ar), 82.1 (C-1), 76.2 (C-5), 74.1, 70.8, 68.1 (3s, C-2 C-3 C-4), 61.8 (C-6), 21.0, 20.92, 20.91, 20.8 (4s, CH_3CO); Anal. Calcd for $C_{24}H_{31}NO_{13}S$ (573.57): H, 5.45; N, 2.44; O, 36.26. Found: H, 5.20; N, 2.25; O, 35.46.

4.2.5. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-(4-phenyl)-benzhydroximoyl-1-thio-β-D-glucopyranose 1g

From tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1.94 g, 5.32 mmol) according to general procedure A yielding **1g** (1.20 g, 2.14 mmol, 40%) as a white crystalline product.

R_f = 0.28 (PE/EtOAc, 1:1); $[\alpha]_D^{25}$ +5.7 (*c* 0.17, MeOH); mp = 101–103 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.53 (s, 1H, OH), 7.72–7.63 (m, 6H, H-ar), 7.55–7.41 (m, 3H, H-ar), 5.16–5.00 (m, 3H, H-2 H-3 H-4), 4.55 (d, 1H, *J* = 9.2 Hz, H-1), 4.25 (dd, 1H, *J* = 3.6 and 11.9 Hz, H-6), 4.15 (dd, 1H, *J* = 2.6 Hz, H-6′), 3.15 (ddd, 1H, H-5), 2.12, 2.09, 2.01, 1.98 (4s, 12H, CH_3CO); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.6, 170.2, 169.2 (4s, CH_3CO), 151.9 (C=N), 142.7, 139.8, 131.9, 129.3, 128.9 (s, 2C), 127.8, 127.6, 127.3, 127.0 (s, 2C), 126.9 (CH-ar), 81.3 (C-1), 75.8, 73.7, 69.9, 67.8 (4s, C-2 C-3 C-4 C-5), 61.2 (C-6), 20.6, 20.5, 20.4, 20.3 (4s, CH_3CO); Anal. Calcd for $C_{27}H_{29}NO_{10}S$ (559.60): C, 57.95; H, 5.22; N, 2.50; S, 5.73. Found: C, 57.71; H, 4.99; N, 2.66; S, 5.52.

4.2.6. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-2-naphthoyl-hydroximoyl-1-thio-β-D-glucopyranose 1h[23]

From tetra-*O*-acetyl-1-thio-β-D-glucopyranose (875 mg, 2.40 mmol) according to general procedure A yielding **1h** (999 mg, 1.87 mmol, 78%) as a white crystalline product.

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4.2.7. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-isonicotinoyl-hydroximoyl-1-thio-β-D-glucopyranose 1i

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (107 mg, 0.29 mmol) and 4-pyridyl-hydroximoyl chloride (250 mg, 1.59 mmol, 5.6 equiv) were introduced in a 25 mL flask. After the flask was flushed with argon, anhydrous dichloromethane (6 mL) and triethylamine (0.53 mL, 3.77 mmol, 13 equiv) were added with stirring. After the dark brown solution was stirred for 30 min at room temperature, the reaction was finished (TLC). The mixture, diluted with dichloromethane (25 mL), was washed with a satd aq $NaHCO_3$ solution (25 mL), then with water (25 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to afford a crude yellow oil (245 mg) which was subjected to column chromatography (PE/EtOAc 2:3 gradually changed to 0:1, with 0.1% Et_3N) to afford an orange-colored solid. After treatment of a dichloromethane solution with charcoal, **1i** was obtained a white solid upon filtration and evaporation (112 mg, 0.23 mmol, 78% yield).

R_f = 0.12 (PE/EtOAc, 2:3); $[\alpha]_D^{20}$ +9.3 (*c* 1, CH_2Cl_2); mp = 139–140 °C; ^{1}H NMR (300 MHz, CD_3COCD_3) δ 11.61 (s, 1H, OH), 8.69 (dd, 2H, *J* = 1.6 and 4.4 Hz, H-ar), 7.56 (dd, 2H, H-ar), 5.23 (t, 1H, *J* = 9.0 Hz H-3), 5.01 (t, 1H, H-2), 4.98 (t, 1H, H-4), 4.95 (d, 1H, *J* = 10.0 Hz, H-1), 4.12 (dd, 1H, *J* = 5.5 and 12.5 Hz, H-6), 3.98 (dd, 1H, *J* = 2.3 Hz, H-6′), 3.56 (ddd, 1H, H-5), 2.06, 2.02, 1.94, 1.92 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 171.6, 171.1, 170.9, 170.8 (4s, CH_3CO), 152.0 (s, 2C, CH-ar), 149.9 (C=N), 143.0, 124.9 (s, 2C, CH-ar), 82.5 (C-1), 77.3 (C-5), 75.0 (C-3), 72.1, 70.0 (2s, C-2 C-4),* 63.8 (C-6), 21.6, 21.5, 21.4, 21.3 (4s, CH_3CO); HRMS (FAB > 0) $C_{20}H_{25}N_2O_{10}S$ $[M+H]^+$ Calcd 485.1230, found 485.1233; Anal. Calcd for $C_{20}H_{24}N_2O_{10}S$ (484.28): C, 49.58; H, 4.99; N, 5.78; O, 33.02; S, 6.62. Found: C, 49.51; H, 4.96; N, 5.70; O, 32.95.

4.2.8. 2,3,4,6-Tetra-*O*-acetyl-1-*S*-(*Z*)-isonicotinoyl-hydroximoyl-1-thio-β-D-glucopyranose-*N*-oxide 1j

A solution of **1i** (195 mg, 0.4 mmol) in chloroform (10 mL) was cooled down to 0 °C before the addition of *m*-chloroperbenzoic acid (139 mg, 0.8 mmol, 2 equiv). After 8 h, TLC monitoring showed two main polar products. After concentration under reduced pressure, the crude mixture was purified by flash column chromatography (EtOAc containing 0.1% Et_3N gradually enriched in MeOH from 0% to 6%) affording **1j** (62 mg, 0.12 mmol, 30% yield) as an orange solid. When the reaction was carried out with a lower amount of *m*-CPBA (1 equiv), the yield was 25%.

R_f = 0.20 (EtOAc/MeOH, 9:1); $[\alpha]_D^{20}$ – 17.7 (*c* 0.53, MeOH); mp = 152 °C darkening, 182 °C decomposition; ^{1}H NMR (300 MHz, CD_3COCD_3) δ 11.49 (s, 1H, OH), 8.07 (d, 2H, *J* = 7.2 Hz, H-ar), 7.50 (d, 2H, H-ar), 5.14 (t, 1H, *J* = 9.2 Hz, H-3), 4.98 (d, 1H, *J* = 10.1 Hz, H-1), 4.89 (dd, 1H, *J* = 9.0 Hz, H-2), 4.88 (t, 1H, *J* = 9.7 Hz, H-4), 4.04 (dd, 1H, *J* = 5.5 and 12.4 Hz, H-6), 3.93 (dd, 1H, *J* = 2.3 Hz, H-6′), 3.68 (ddd, 1H, H-5), 1.92, 1.89, 1.83, 1.80 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, CD_3COCD_3) d 170.2, 169.7, 169.4, 169.3 (4s, CH_3CO), 147.2 (C=N), 139.3 (s, 2C, CH-ar), (C^{IV} not seen), 126.3 (s, 2C, CH-ar), 81.3 (C-1), 75.7 (C-5), 73.5 (C-3), 70.6 (C-2), 68.5 (C-4), 62.5 (C-6), 20.0 (m, 4C, CH_3CO).

4.3. Deacetylation of *O*-peracetylated hydroximothioates

4.3.1. 1-*S*-(*Z*)-4-Nitrobenzhydroximoyl-1-thio-β-D-glucopyranose 2b

From compound **1b** (200 mg, 0.38 mmol) according to general procedure D yielding **2b** (111 mg, 0.3 mmol, 82%) as a white crystalline product.

R_f = 0.12 ($CHCl_3$/MeOH, 9:1); $[\alpha]_D^{25}$ +7.5 (*c* 0.56, MeOH), lit.[32] $[\alpha]_D^{25}$ +5 (*c* 1, MeOH); mp = 184–187 °C; ^{1}H NMR (360 MHz, D_2O) δ 8.27 (d, 2H, *J* = 7.9 Hz, H-ar), 7.72 (d, 2H, *J* = 7.9 Hz, H-ar), 4.24 (d, 1H, *J* = 9.2 Hz, H-1), 3.58 (dd, 1H, *J* = 1.2 and 11.9 Hz, H-6), 3.51 (dd, 1H, *J* = 3.9 Hz, H-6′), 3.36, 3.28, 3.20 (3t, 3H, *J* = 9.2 Hz, H-2 H-3 H-4), 2.66 (ddd, 1H, H-5); ^{13}C NMR (90 MHz, D_2O) δ 154.5 (C=N), 149.9, 140.1, 131.6 (s, 2C, CH-ar), 125.0 (s, 2C, CH-ar), 84.4 (C-1), 81.5, 78.5, 73.6, 70.3 (4s, C-2 C-3 C-4 C-5), 61.7 (C-6); Anal. Calcd for $C_{13}H_{16}N_2O_8S$ (360.34): C, 43.33; H, 4.48; N, 7.77; S, 8.90. Found: C, 43.44; H, 4.26; N, 7.16; S, 8.70.

4.3.2. 1-*S*-(*Z*)-4-Cyanobenzhydroximoyl-1-thio-β-D-glucopyranose 2c

From compound **1c** (200 mg, 0.39 mmol) according to general procedure D yielding (122 mg, 0.36 mmol, 86%) as a slightly colored crystalline product.

R_f = 0.74 ($CHCl_3$/MeOH, 7:3); $[\alpha]_D^{20}$ +23 (*c* 0.27, MeOH); mp = 156–160 °C; ^{1}H NMR (360 MHz, D_2O) δ 7.80 (d, 2H, *J* = 7.9 Hz, H-ar), 7.72 (d, 2H, *J* = 7.9 Hz, H-ar), 4.24 (d, 1H, *J* = 9.2 Hz, H-1), 3.62 (dd, 1H, *J* = 5.3 and 13.2 Hz, H-6), 3.52 (dd, 1H, *J* = 1.3 Hz, H-6′), 3.40–3.10 (m, 3H, H-2 H-3 H-4), 2.75 (ddd, 1H, H-5); ^{13}C NMR (90 MHz, D_2O) δ 148.3 (C=N) 139.7, 132.8 (2s, CH-ar), 132.6 (2s, CH-ar), 119.5, 111.0 (CN), 83.3 (C-1), 80.0, 77.2, 72.6, 69.2 (4s, C-2 C-3 C-4 C-5), 60.4 (C-6); Anal. Calcd for $C_{14}H_{16}N_2O_6S$ (340.36): C, 49.41; H, 4.74; N, 8.23; S, 9.42. Found: C, 49.24; H, 4.66; N, 8.42; S, 9.12.

4.3.3. 1-*S*-(*Z*)-4-Methoxybenzhydroximoyl-1-thio-β-D-glucopyranose 2e

From compound **1e** (300 mg, 0.58 mmol) according to general procedure D yielding **2e** (172 mg, 0.50 mmol, 86%) as a white crystalline product.

R_f = 0.70 ($CHCl_3$/MeOH, 7:3); $[\alpha]_D^{25}$ – 85 (*c* 0.31, MeOH) lit.[32] $[\alpha]_D^{25}$ –7 (*c* 1, MeOH); mp = 146–148 °C; ^{1}H NMR (360 MHz, D_2O) δ 7.45 (d, 2H, *J* = 7.9 Hz, H-ar), 7.04 (d, 2H, *J* = 7.9 Hz, H-ar), 4.22 (d, 1H, *J* = 9.2 Hz, H-1), 3.85 (s, 3H, OCH_3), 3.20–3.44 (m, 3H, H-2 H-3 H-4), 3.68 (dd, 1H, *J* = 3.9 and 13.2 Hz, H-6), 3.56 (dd, 1H, *J* = 2.6 Hz, H-6′), 2.75 (ddd, 1H, H-5); ^{13}C NMR (90 MHz, D_2O) δ 159.5 (C=N), 151.0, 130.9 (2s, CH-ar), 126.7 (2s, CH-ar), 113.8, 83.2 (C-1), 79.9, 77.3, 72.3, 69.1 (4s, C-2 C-3 C-4 C-5), 60.4 (C-6), 55.6 (OCH_3); Anal. Calcd for $C_{14}H_{19}NO_7S$ (345.37): C, 48.69; H, 5.55; N, 4.06; S, 9.28. Found: C, 48.44; H, 5.36; N, 4.26; S, 9.02.

4.3.4. 1-*S*-(*Z*)-(4-Phenyl)-benzhydroximoyl-1-thio-β-D-glucopyranose 2g

From compound **1g** (300 mg, 0.53 mmol) according to general procedure D yielding (190 mg, 0.48 mmol, 90%) as a yellow oil.

R_f = 0.58 ($CHCl_3$/MeOH, 7:3); $[\alpha]_D^{25}$ +5.7 (*c* 0.17, MeOH); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.40 (s, 1H, OH), 7.52–7.20 (m, 9H, H-ar), 4.98–4.51 (m, 4H, OH), 4.15 (d, 1H, *J* = 9.2 Hz, H-1), 4.24 (dd, 1H, *J* = 5.8 and 13.2 Hz, H-6), 4.17 (dd, 1H, *J* = 2.6 Hz, H-6′) 4.16–3.72 (m, 3H, H-2 H-3 H-4), 3.24 (ddd, 1H, H-5); ^{13}C NMR (90 MHz, DMSO-d_6) δ 151.1 (C=N), 140.9, 140.1, 129.9, 129.7, 129.6, 129.5, 129.3, 128.2, 128.1, 127.1, 126.9, 126.7, 84.4 (C-1), 80.4, 79.5, 73.8, 70.2 (4s, C-2 C-3 C-4 C-5), 60.9 (C-6); Anal. Calcd for $C_{19}H_{21}NO_6S$ (391.45): C, 58.30; H, 5.41; N, 3.58; S, 8.19. Found: C, 58.14; H, 5.16; N, 3.12; S, 8.02.

4.3.5. 1-*S*-(*Z*)-2-Naphthoyl-hydroximoyl-1-thio-β-D-glucopyranose 2h

From compound **1h** (343 mg, 0.64 mmol) according to general procedure D yielding (200 mg, 0.55 mmol, 85%) as a yellow oil.

R_f = 0.68 ($CHCl_3$/MeOH, 7:3); $[\alpha]_D^{25}$ +4.4 (*c* 0.3, MeOH); mp = 150–152 °C; ^{1}H NMR (360 MHz, D_2O) δ 8.04–7.54 (m, 7H, H-ar), 4.24 (d, 1H, *J* = 10.6 Hz, H-1), 3.56 (dd, 1H, *J* = 3.9 and 13.2 Hz, H-6), 3.44 (dd, 1H, *J* = 2.6 Hz, H-6′) 3.36, 3.28, 3.12 (3t, 3H, *J* = 9.2 Hz, H-2 H-3 H-4), 2.52 (ddd, 1H, H-5); ^{13}C NMR (90 MHz, D_2O) δ 148.3 (C=N), 139.7, 132.8, 132.7, 132.6 (s, 2C), 129.8 (s, 2C), 129.6, 119.6, 111.5, 83.3 (C-1), 80.1, 77.2, 72.6, 69.2 (4s, C-2 C-3 C-4 C-5), 60.5 (C-6); Anal. Calcd for $C_{17}H_{19}NO_6S$ (365.41): C, 55.88; H, 5.24; N, 3.83; S, 8.77. Found: C, 55.74; H, 5.06; N, 3.52; S, 8.55.

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4.3.6. 1-S-(Z)-Isonicotinoyl-hydroximoyl-1-thio-β-D-glucopyranose 2i

600 A solution composed of **1i** (90 mg, 0.18 mmol) in a mixture of MeOH (2 mL), water (2 mL) and NEt_3 (0.4 mL) was treated according to general procedure B. The residue was purified (RP-18: water) to afford **2i** (53 mg, 0.16 mmol, 90% yield) as a white solid.

R_f = 0.48 (EtOAc/MeOH, 4:1); $[\alpha]_D^{20}$ – 1.6 (c 1, H_2O); mp = 140 °C darkening, 175 °C decomposition; ^{1}H NMR (300 MHz, D_2O) δ 8.62 (dd, 2H, J = 1.5 and 5.5 Hz, H-ar), 7.57 (dd, 2H, H-ar), 4.33 (d, 1H, J = 9.8 Hz, H-1), 3.60 (dd, 1H, J = 2.4 and 12.7 Hz, H-6), 3.52 (dd, 1H, J = 4.9 Hz, H-6′), 3.40 (t, 2H, J = 9.2 Hz, H-2), 3.31 (t, 1H, J = 9.0 Hz, H-4), 3.25 (t, 1H, J = 8.7 Hz, H-3), 2.75 (ddd, 1H, H-5); 610 ^{13}C NMR (75 MHz, D_2O) δ 152.7 (C=N), 149.6 (s, 2C, CH-ar), 141.6, 124.3 (s, 2C, CH-ar), 83.1 (C-1), 80.3 (C-5), 77.3 (C-3), 72.5, 69.2 (2s, C-2, C-4),* 60.5 (C-6); Anal. Calcd for $C_{12}H_{16}N_2O_6S$, 0.5 H_2O: C, 44.30; H, 5.27; N, 8.61; O; 31.97; S, 9.86. Found: C, 44.34; H, 5.26; N, 8.62; O, 32.45.

4.4. Spiro-cyclization

4.4.1. (1S)- and (1R)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(phenyl)-1,4,2-oxathiazole (1S)-3a and (1R)-3a

When the spiro-cyclization was carried out with **1a** (278 mg) in 620 $CHCl_3$ in the presence of NBS (2 equiv) and DBU (1 equiv) according to the general procedure C, TLC suggested a ~1:1 ratio of the (1S)/(1R)-epimers. Separation by repeated column chromatographies with a gradient of PE/EtOAc as the mobile phase afforded (1S)-**3a** (44 mg, 16%) and (1R)-**3a** (51 mg, 18%).

4.4.2. (1S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(4-nitrophenyl)-1,4,2-oxathiazole (1S)-3b

A solution composed of **1b** (827 mg, 1.56 mmol) and NBS (557 mg, 3.13 mmol) was treated according to the general procedure C to afford a first crop of pure (1S)-**3b** (259 mg) as a white foam and 630 an additional crop (144 mg) of a mixture of (1S)-**3b** and (1R)-**3b** in a 7:93 ratio, respectively, (^{1}H NMR). The calculated yields were, respectively, 33% and 16% for the (1S)- and (1R)-epimers.

R_f = 0.62 (PE/EtOAc, 3:2); $[\alpha]_D^{20}$ +33 (c 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, 2H, J = 9.0 Hz, H-ar), 7.87 (d, 2H, J = 9.0 Hz, H-ar), 5.64 (m, 2H, H-2 H-3), 5.29 (m, 1H, H-4), 4.44 (ddd, 1H, J = 2.1, 3.6 and 10.3 Hz, H-5), 4.35 (dd, 1H, J = 3.6 and 12.7 Hz, H-6′), 4.10 (dd, 1H, J = 2.1 and 12.7 Hz, H-6), 2.05, 2.07, 2.09, 2.10 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 169.5, 169.3, 169.2, (4s, CH_3CO), 154.4 (C=N), 149.3, 132.8, 128.8 640 (s, 2C, CH-ar), 124.1 (s, 2C, CH-ar), 123.4 (C-1), 70.9 (C-2 or C-3), 70.8 (C-5), 67.8 (C-2 or C-3), 67.2 (C-4), 60.9 (C-6), 20.6 (s, CH_3CO), 20.4 (s, 3C, CH_3CO); MS (ESI >0) m/z = 549.0 [M+Na]$^+$, 1074.5 [2M+Na]$^+$; HRMS (ESI >0) m/z = $C_{21}H_{22}N_2NaO_{12}S$ [M+Na]$^+$ calcd 549.0791, found 549.0792.

4.4.3. (1S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(4-cyanophenyl)-1,4,2-oxathiazole (1S)-3c

A solution composed of **1c** (680 mg, 1.34 mmol) and NBS (476 mg, 2.67 mmol) was treated according to the general procedure C to afford a first crop of pure (1S)-**3c** (103 mg, 15%) as a white 650 foam and an additional crop (48 mg) of a mixture of (1S)-**3c** and (1R)-**3c** in a 5:95 ratio, respectively, (^{1}H NMR). The calculated yields were, respectively, 15% and 7% for the (1S)- and (1R)-epimers.

R_f = 0.49 (PE/EtOAc, 3:2); $[\alpha]_D^{20}$ +35 (c 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, 2H, J = 8.7 Hz, CH-ar), 7.74 (d, 2H, J = 8.7 Hz, CH-ar), 5.28 (m, 1H, H-4), 5.64 (m, 2H, H-2 H-3), 4.44 (ddd, 1H, J = 2.1, 3.6 and 10.3 Hz, H-5), 4.33 (dd, 1H, J = 3.6 and 12.7 Hz, H-6′), 4.10 (dd, 1H, J = 2.1 and 12.7 Hz, H-6), 2.09 (s, 6H, CH_3CO), 2.04, 2.06 (2s, 6H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 660 170.4, 169.6, 169.29, 169.26, (4s, CH_3CO), 154.5 (C=N), 132.6 (s, 2C, CH-ar), 131.2, 128.4 (s, 2C, CH-ar), 123.3 (C-1), 117.7, 115.1 (C≡N), 70.84 (C-2 or C-3), 70.80 (C-5), 67.9 (C-2 or C-3), 67.3 (C-4), 60.9 (C-6), 20.6 (CH_3CO), 20.4 (s, 3C, CH_3CO); MS (ESI >0) m/z = 529.0 [M+Na]$^+$, 1034.5 [2M+Na]$^+$; HRMS (ESI > 0) m/z = $C_{22}H_{22}N_2NaO_{10}S$ [M+Na]$^+$ calcd 529.0893, found 529.0898.

4.4.4. (1S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(4-methoxyphenyl)-1,4,2-oxathiazole (1S)-3e

A solution composed of **1d** (690 mg, 1.34 mmol) and NBS (478 mg, 2.68 mmol) was treated according to the general procedure C to afford a first crop of pure (1S)-**3e** (372 mg) as a white foam and 670 an additional crop (218 mg) of a mixture of (1S)-**3e** and (1R)-**3e** in a 45:55 ratio, respectively, (^{1}H NMR). The calculated yields were, respectively, 69% and 17% for the (1S)- and (1R)-epimers.

R_f = 0.43 (PE/EtOAc, 3:2); $[\alpha]_D^{20}$ +52 (c 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, 2H, J = 8.9 Hz, CH-ar), 6.94 (d, 2H, J = 8.9 Hz, CH-ar), 5.62 (m, 2H, H-2 H-3), 5.26 (m, 1H, H-4), 4.41 (ddd, 1H, J = 2.0, 3.6 and 10.3 Hz, H-5), 4.34 (dd, 1H, J = 3.6 and 12.6 Hz, H-6′), 4.07 (dd, 1H, J = 2.0 and 12.6 Hz, H-6), 3.85 (s, 3H, OMe), 2.09, 2.08, 2.05, 2.03, (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 169.6, 169.5, 169.4 (4s, CH_3CO), 162.2, 155.9 680 (C=N), 129.6 (s, 2C, CH-ar), 122.1 (C-1), 119.3, 114.3 (s, 2C, CH-ar), 71.1 (C-2 or C-3), 70.5 (C-5), 67.9 (C-2 or C-3), 67.5 (C-4), 61.1 (C-6), 55.4 (OMe), 20.7 (CH_3CO), 20.5 (s, 3C, CH_3CO); MS (ESI >0) m/z = 511.8 [M+H]$^+$, 534.0 [M+Na]$^+$, 1022.4 [2M+H]$^+$, 1044.6 [2M+Na]$^+$; HRMS (ESI >0) m/z = $C_{22}H_{25}NNaO_{11}S$ [M+Na]$^+$ calcd 534.1046, found 534.1049.

4.4.5. (1R)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(3,4,5-trimethoxyphenyl)-1,4,2-oxathiazole (1R)-3f

Spiro-cyclization of **1f** (580 mg) was attempted as before (NBS: 690 360 mg, 2 equiv; $CHCl_3$, 25 mL) upon heating with a 60 W tungsten lamp for 1 h. TLC showed that the transformation was not selective, yielding a multicomponent mixture. Workup and flash column chromatography (PE then PE/EtOAc 1:1) of the crude product (827 mg) afforded four fractions (90, 33, 21, 41 mg). Only the third one was pure enough and led to exploitable NMR spectra (COSY, HSQC), concluding to the presence of (1R)-**3f.**

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 2H, CH-ar), 5.59 (d, 1H, J = 10.2 Hz, H-2), 5.27 (t, 1H, H-4), 5.10 (dd, 1H, J = 9.5 and 10.2 Hz, H-3), 4.35 (dd, 1H, J = 3.3 and 12.3 Hz, H-6), 4.10 (m, 2H, 700 H-5 H-6′), 3.90 (s, 9H, OMe), 2.08, 2.07 (2s, 6H, CH_3CO), 2.05 (s, 6H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.3, 169.6, 168.9, (4s, CH_3CO), 155.6 (C=N), 153.8 (s, 2C), 141.4, 127.4 (C-1), 122.8, 105.7 (s, 2C, CH-ar), 73.7 (C-3), 71.0 (C-5), 68.7 (C-2), 67.5 (C-4), 61.42 (C-6), 61.40 (OMe), 56.7 (s, 2C, OMe), 20.7 (CH_3CO), 20.6 (s, 2C, CH_3CO), 20.5 (CH_3CO).

4.4.6. (1S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-[(4-phenyl)-phenyl]-1,4,2-oxathiazole (1S)-3g

A solution composed of **1g** (200 mg, 357 μmol) and NBS (127 mg, 715 μmol) was treated according to the general proce- 710 dure C to afford a first crop of pure (1S)-**3g** (65 mg) as a white foam and an additional crop (90 mg) of a mixture of (1S)-**3g** and (1R)-**3g** in a 3:2 ratio, respectively. The calculated yields were, respectively, 61% and 18% for the (1S)- and (1R)-epimers.

R_f = 0.52 (PE/EtOAc, 3:2); $[\alpha]_D^{20}$ +45 (c 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, 2H, J = 8.5 Hz, CH-ar), 7.65 (d, 2H, J = 8.5 Hz, CH-ar), 7.57–7.63 (m, 2H, CH-ar), 7.37–7.50 (m, 3H, CH-ar), 5.64 (m, 2H, H-2 H-3), 5.28 (m, 1H, H-4), 4.44 (ddd, 1H, J = 2.0, 3.7 and 10.3 Hz, H-5), 4.36 (dd, 1H, J = 3.7 and 12.6 Hz, H-6′), 4.09 (dd, 1H, J = 2.0 and 12.6 Hz, H-6), 2.09, 2.06 (2s, 6H, CH_3CO), 2.04 (2s, 6H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 720 169.6, 169.5, 169.4 (4s, CH_3CO), 156.1 (C=N), 144.5, 139.6, 128.9 (s, 2C, CH-ar), 128.4 (s, 2C, CH-ar), 128.1 (CH-ar), 127.5 (s, 2C,

CH-ar), 127.1 (s, 2C, CH-ar), 125.8, 122.4 (C-1), 71.0 (C-2 or C-3), 70.6 (C-5), 68.0 (C-2 or C-3), 67.5 (C-4), 61.1 (C-6), 20.7 (CH_3CO), 20.5 (s, 3C, CH_3CO); MS (ESI >0) m/z = 557.8 $[M+H]^+$, 580.0 $[M+Na]^+$, 1114.4 $[2M+H]^+$, 1136.7 $[2M+Na]^+$; HRMS (ESI >0) m/z = $C_{27}H_{27}NNaO_{10}S$ $[M+Na]^+$ calcd 580.1253, found 580.1252.

4.4.7. (1*S*)-2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(2-naphthyl)-1,4,2-oxathiazole (1*S*)-3h[23]

730 A solution composed of **1h** (655 mg, 1.23 mmol) and NBS (437 mg, 2.46 mmol) was treated according to the general procedure C to afford a first crop of pure (1*S*)-**3h** (181 mg) as a white foam and an additional crop (160 mg) of a mixture of (1*S*)-**3h** and (1*R*)-**3h** in a 1:2 ratio, respectively. The calculated yields were, respectively, 36% and 16% for the (1*S*)- and (1*R*)-epimers.

4.4.8. (1*R*)-2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-glucitol-spiro[1.5]-3-(4-pyridyl)-1,4,2-oxathiazole (1*R*)-3i

A solution composed of **1i** (636 mg, 1.31 mmol) and NBS (467 mg, 2.62 mmol) in anhydrous chloroform (30 mL) was treated 740 with for 30 min according to the general procedure C. TLC monitoring showed the appearance of two main products (more mobile than **1i**) and two minor products. Workup and flash chromatography with petroleum ether EtOAc (containing 0.1% Et_3N) 7:3 then 2:3 afforded a mixture (107 mg) with 3 components (NMR) some being brominated (as shown by TLC using a specific staining reagent with fluorescein), and (1*R*)-**3i** (190 mg, 0.39 mmol, 30% yield) as a transparent solid which appeared labile at room temperature and was kept at 0 °C.

R_f = 0.63 (EtOAc); mp = 60–61 °C; $[\alpha]_D^{20}$ +181.7 (*c* 0.9, CH_2Cl_2); ^1H 750 NMR (300 MHz, $CDCl_3$) δ 8.75 (dd, 2H, *J* = 1.6 and 4.4 Hz, H-ar), 7.57 (dd, 2H, H-ar), 5.63 (d, 1H, *J* = 10.2 Hz, H-2), 5.28 (t, 1H, *J* = 9.7 Hz, H-4), 5.11 (dd, 1H, *J* = 9.5 Hz, H-3), 4.34 (dd, 1H, *J* = 4.2 and 12.9 Hz, H-6), 4.12 (m, 2H, H-5 H-6′), 2.08, 2.07, 2.06, 2.05 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 170.3, 169.5, 168.9 (4s, CH_3CO), 154.2 (C=N), 151.0 (s, 2C, CH-ar), 135.2, 128.3 (C-1), 122.0 (s, 2C, CH-ar), 73.5 (C-3), 71.1 (C-5), 68.6 (C-2), 67.3 (C-4), 61.3 (C-6), 21.1, 21.0, 20.90, 20.92 (4s, CH_3CO); Anal. Calcd for $C_{20}H_{22}N_2O_{10}S$ (482.46): C, 49.79; H, 4.60; N, 5.81; O, 33.16. Found: C, 50.07; H, 4.68; N, 5.55; O, 33.41.

760 4.5. Deacetylation of spiro-oxathiazoles

4.5.1. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(4-nitrophenyl)-1,4,2-oxathiazole (1*S*)-4b

A solution composed of (1*S*)-**3b** (255 mg) was treated according to general procedure D to afford (1*S*)-**4b** (124 mg, 71%) as a pale yellow solid.

R_f = 0.43 (EtOAc/MeOH, 85:15); $[\alpha]_D^{20}$ +58.8 (*c* 1, DMSO); mp = 182–183 °C; ^1H NMR (300 MHz, CD_3OD, 50 °C) δ 8.30 (d, 2H, *J* = 8.9 Hz, CH-ar), 7.90 (d, 2H, *J* = 8.9 Hz, CH-ar), 3.92 (ddd, 1H, *J* = 2.3, 4.7 and 9.5 Hz, H-5), 3.90 (d, 1H, *J* = 9.5 Hz, H-2), 3.82 770 (dd, 1H, *J* = 2.3 and 12.2 Hz, H-6′), 3.78 (t, 1H, *J* = 9.5 Hz, H-3), 3.76 (dd, 1H, *J* = 4.7 and 12.2 Hz, H-6), 3.53 (t, 1H, *J* = 9.5 Hz, H-4); ^{13}C NMR (75 MHz, CD_3OD, 50 °C) δ 154.5 (C=N), 150.7, 135.3, 129.8 (s, 2C, CH-ar), 129.0, 125.2 (s, 2C, CH-ar), 77.7 (C-5), 76.2 (C-3), 73.0 (C-2), 70.8 (C-4), 62.2 (C-6); MS (ESI <0) m/z = 392.9 $[M+Cl]^-$, 750.5 $[2M+Cl]^-$; HRMS (ESI <0) m/z = $C_{13}H_{14}ClN_2O_8S$ $[M+Cl]^-$ calcd 393.0159, found 393.0158.

4.5.2. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(4-cyanophenyl)-1,4,2-oxathiazole (1*S*)-4c

A solution composed of (1*S*)-**3c** (100 mg) was treated according 780 to general procedure D to afford (1*S*)-**4c** (56 mg, 84%) as a pale yellow foam.

R_f = 0.35 (EtOAc/MeOH, 85:15); $[\alpha]_D^{20}$ +36.3 (*c* 1, DMSO); ^1H NMR (300 MHz, CD_3OD) δ 7.85 (m, 4H, CH-ar), 3.86–3.93 (m, 1H, H-5), 3.89 (d, 1H, *J* = 9.6 Hz, H-2), 3.81 (dd, 1H, *J* = 2.3 and 12.3 Hz, H-6′), 3.70–3.78 (m, 2H, H-3 H-6), 3.51 (t, 1H, *J* = 9.0 Hz, H-4); ^{13}C NMR (75 MHz, CD_3OD) δ 155.8 (C=N), 134.0 (s, 2C, CH-ar), 133.7, 129.4 (s, 2C, CH-ar), 128.8 (C-1), 119.0, 115.6 (CN), 77.6 (C-5), 76.1 (C-3), 72.7 (C-2), 70.6 (C-4), 61.9 (C-6); MS (ESI <0) m/z = 372.9 $[M+Cl]^-$; HRMS (ESI <0) m/z = $C_{14}H_{14}ClN_2O_6S$ $[M+Cl]^-$ calcd 373.0261, found 373.0264. 790

4.5.3. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(4-fluorophenyl)-1,4,2-oxathiazole (1*S*)-4d

Prepared by general procedure D from (1*S*)-**3d** (500 mg) to afford (1*S*)-**4d** (392 mg, 79%) as a pale yellow solid.

R_f = 0.69 ($CHCl_3$/MeOH, 7:3); $[\alpha]_D^{20}$ +51 (*c* 1.1, DMSO); mp = 192–193 °C; ^1H NMR (360 MHz, DMSO-d_6) δ 7.73–7.69 (m, 2H, H-ar), 7.38–7.33 (m, 2H, H-ar), 3.72–3.62 (m, 3H, H-6 H-6′ H-1), 3.53–3.23 (m, 3H, H-2 H-3 H-5); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.2, 154.7 (C=N), 130.8, 130.7, 128.3, 117.5, 117.2, 108.8 (C-1), 77.7, 75.3, 72.1, 70.1 (C-2 C-3 C-4 C-5), 61.2 (C-6); Anal. Calcd for 800 $C_{13}H_{14}NO_6S$ (331.32): C, 47.13; H, 4.26; N, 4.23; S, 9.68. Found: C, 47.33; H, 4.09; N, 4.52; S, 9.45.

4.5.4. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(4-methoxyphenyl)-1,4,2-oxathiazole (1*S*)-4e

A solution composed of (1*S*)-**3e** (370 mg) was treated according to general procedure D to afford (1*S*)-**4e** (196 mg, 79%) as a pale yellow solid.

R_f = 0.33 (EtOAc/MeOH, 85:15); $[\alpha]_D^{20}$ +43.4 (*c* 1, DMSO); mp = 192–194 °C; ^1H NMR (300 MHz, DMSO-d_6+εD_2O) δ 7.57 (d, 2H, *J* = 8.7 Hz, H-ar), 7.04 (d, 2H, *J* = 8.7 Hz, H-ar), 3.80 (s, 3H, 810 OMe), 3.43–3.71 (m, 5H), 3.24 (t, 1H, *J* = 9.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6+εD_2O) δ 161.6, 154.6, 129.2 (s, 2C, CH-ar), 126.8, 119.8, 114.8 (s, 2C, CH-ar), 76.6, 74.3, 71.0, 69.1, 60.3 (C-6), 55.6 (OMe); MS (ESI <0) m/z = 377.9 $[M+Cl]^-$; MS (ESI >0) m/z = 365.9 $[M+Na]^+$, 708.8 $[2M+Na]^+$; HRMS (ESI >0) m/z = $C_{14}H_{17}NNaO_7S$ $[M+Na]^+$ calcd 366.0623, found 366.0621.

4.5.5. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-[(4-phenyl)-phenyl]-1,4,2-oxathiazole (1*S*)-4g

A solution composed of (1*S*)-**3g** (65 mg) was treated according 820 to general procedure D to afford (1*S*)-**4g** (44 mg, 97%) as a pale yellow foam.

R_f = 0.32 (EtOAc/MeOH, 85:15); $[\alpha]_D^{20}$ +39.4 (*c* 0.6, DMSO); ^1H NMR (500 MHz, CD_3OD) δ 7.79 (d, 2H, *J* = 8.3 Hz, CH-ar), 7.73 (d, 2H, *J* = 8.3 Hz, H-ar), 7.65–7.69 (m, 2H, H-ar), 7.36–7.52 (m, 3H, CH-ar), 3.92 (ddd, 1H, *J* = 2.3, 4.6 and 9.9 Hz, H-5), 3.88 (d, 1H, *J* = 9.9 Hz, H-2), 3.82 (dd, 1H, *J* = 2.3 and 12.3 Hz, H-6′), 3.73–3.79 (m, 2H, H-3 H-6), 3.52 (t, 1H, *J* = 9.9 Hz, H-4), ^{13}C NMR (125 MHz, CD_3OD) δ 62.0 (C-6), 70.8 (C-4), 72.8 (C-2), 76.2 (C-3), 77.5 (C-5), 127.8 (C-1), 128.1 (s, 2C, CH-ar), 128.5 (s, 2C, CH-ar), 129.2 (s, 2C, CH-ar), 129.3 (s, 2C, CH-ar), 130.1 (s, 2C, CH-ar), 133.8, 141.1, 145.4, 157.2 830 (C=N); MS (ESI <0) m/z = 423.9 $[M+Cl]^-$; HRMS (ESI <0) m/z = $C_{19}H_{19}ClNO_6S$ $[M+Cl]^-$ calcd 424.0622, found 424.0623.

4.5.6. (1*S*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(2-naphthyl)-1,4,2-oxathiazole (1*S*)-4h[23]

A solution composed of (1*S*)-**3h** (180 mg) was treated according to general procedure D to afford (1*S*)-**4h** (116 mg, 94%) as a pale yellow solid.

4.5.7. (1*R*)-1,5-Anhydro-D-glucitol-spiro[1.5]-3-(4-pyridyl)-1,4,2-oxathiazole (1*R*)-4i

(1*R*)-**3i** (115 mg, 0.24 mmol) was treated according to general 840 procedure B (NEt_3, MeOH, H_2O) to afford (1*R*)-**4i** (71 mg, 0.22 mmol, 95% yield) as a white solid.

R_f = 0.40 (EtOAc/MeOH, 4:1); $[\alpha]_D^{20}$ −4 (*c* 0.3, DMF); mp = 174–175 °C; ^1H NMR (300 MHz, D_2O) δ 8.63 (dd, 2H, *J* = 1.8 and

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4.5 Hz, H-ar), 7.70 (dd, 2H, H-ar), 3.94 (d, 1H, J = 10.2 Hz, H-2), 3.81 (m, 3H, H-5 H-6 H-6′), 3.60 (t, 1H, J = 9.1 Hz, H-4), 3.40 (dd, 2H, J = 9.0 Hz, H-3); ^{13}C NMR (75 MHz, D_2O) δ 156.2 (C=N), 150.0 (s, 2C, CH-ar), 135.9, 130.8 (C-1), 122.6 (s, 2C, CH-ar), 76.7 (C-3), 75.5 (C-5), 71.6 (C-2), 68.5 (C-4), 60.2 (C-6); HRMS (FAB > 0) m/z = $C_{12}H_{15}N_2O_6S$ $[M+H]^+$ calcd 315.0651, found 315.0655.

4.6. Synthesis of *N*-glycosyl-amidoximes

4.6.1. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-benzamidoxime 8a

Prepared according to general procedure E to afford **8a** in 57% yield as a syrup. The product decomposed rapidly during the NMR spectroscopic measurement.

4.6.2. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-nitrobenzamidoxime 8b

Prepared according to general procedure E to afford **8b** (880 mg) in 64% yield.

$[\alpha]_D$ +20 (c 1.2, $CHCl_3$); mp = 183–185 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.36 (s, 1H, OH), 8.25 (d, 2H, J = 9.1 Hz, H-ar), 7.72 (d, 2H, J = 8.4 Hz, H-ar), 6.20 (d, 1H, NH), 5.12 (t, 1H, J = 9.6 Hz, H-2), 5.03 (t, 1H, J = 9.4 Hz, H-3), 4.97 (t, 1H, J = 9.2 Hz, H-4), 4.31 (t, 1H, J = 9.9 Hz, H-1), 4.12 (dd, 1H, J = 5.1 Hz, H-6), 4.02 (dd, 1H, J = 11.8 Hz, H-6′), 3.38 (ddd, 1H, J = 2.8 Hz, H-5), 2.09, 2.05, 1.97, 1.95 (4s, 12H, CH_3CO); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.4, 170.1, 170.0, 169.4 (4s, CH_3CO), 151.9 (NHC(=NOH)Ar), 148.7 (CNO_2), 136.5 (N_2C–C), 129.5, 123.6, 82.5 (C-1), 72.9, 72.5, 70.6, 68.3 (4s, C-2 C-3 C-4 C-5), 62.1 (C-6), 20.5, 20.6, 20.4 (3s, 4C, CH_3CO); Anal. Calcd for $C_{21}H_{25}N_3O_{12}$ (511.45): C, 49.32; H, 4.93; N, 8.22. Found: C, 50.02; H, 5.03; N, 8.16.

4.6.3. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-cyanobenzamidoxime 8c

Prepared according to general procedure E to afford **8c** (245 mg) in 18% yield.

Optimised synthesis of **8c**: 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl azide **7** (100 mg, 0.26 mmol) was dissolved in dry dichloromethane (1 mL) and trimethyl phosphine (0.268 mL, 1 M in toluene) was added with stirring. During gas evolution, the reagent solution was freshly prepared by adding triethylamine to a solution of p-cyanophenyl hydroximinoyl chloride (153 mg, 0.26 mmol) in dry dichloromethane (1 mL) causing the formation of triethylamine hydrochloride precipitate. When TLC showed complete transformation of the starting material **7** (~15 min), the reagent solution was added to the mixture. After 10 min, the reaction mixture was poured into water and was worked up as described in general procedure E to afford **8c** (78 mg, 60% yield).

$[\alpha]_D$ +9.8 (c 0.2, $CDCl_3$); mp = 120–123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H, OH), 7.71 (d, 2H, J = 8.6 Hz, H-ar), 7.65 (d, 2H, J = 8.3 Hz, H-ar), 6.16 (d, 1H, J = 10.4 Hz, NH), 5.14 (t, 1H, J = 9.4 Hz, H-3 or H-4), 5.03 (t, 1H, J = 8.8 Hz, H-2), 4.99 (t, 1H, J = 9.4 Hz, H-3 or H-4), 4.31 (dd, 1H, J = 8.8 Hz, H-1), 4.10 (d, 1H, J = 6.6 Hz, H-6), 4.02 (dd, 1H, J = 12.5 Hz, H-6′), 3.39 (ddd, 1H, J = 2.7 Hz, H-5), 2.09, 2.06, 2.02, 1.99 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.2, 170.0 (3s, 4C, CH_3CO), 152.3 (NHC(=NOH)Ar), 134.7 (N_2C–C), 132.3, 129.2, 128.9 (C–CN), 113.9 (CN), 82.5 (C-1), 72.9 (C-5), 72.4, 70.7, 68.3 (C-2 C-3 C-4), 62.1 (C-6), 21.0, 20.7, 20.6, 20.5 (4s, CH_3CO); Anal. Calcd for $C_{22}H_{25}N_3O_{10}$ (491.46): C, 53.77; H, 5.13; N, 8.55. Found: C, 53.43; H, 5.09; N, 8.62.

4.6.4. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-C-(2-naphthyl)-amidoxime 8h

Prepared according to the general procedure E to afford **8h** (92 mg) in 6% yield as a brownish foam which decomposed rapidly.

4.7. Deacetylation of *N*-glycosyl-amidoximes

4.7.1. *N*-(β-D-Glucopyranosyl)-4-nitrobenzamidoxime 9b

A few drops of NaOMe (2.5 M in MeOH) were added to a solution of **8b** (100 mg, 0.19 mmol) in dry methanol (7 mL). The reaction mixture was stirred at rt for 5 min before the addition of cation exchange resin Amberlyst 15 (H^+ form). The resin was filtered off and the solvent was removed. The residue was purified by crystallization to give 56 mg (83%) of **9b**.

$[\alpha]_D$ −56 (c 1.3, DMSO); mp = 165–168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 1H, OH), 8.22 (d, 2H, J = 8.4 Hz, H-ar), 7.91 (s, 2H, J = 8.2 Hz, H-ar), 6.40 (d, 1H, J = 9.84 Hz, NH), 5.1–4.65 (m, 4H, OH), 4.00–2.80 (m, 7H, H-1 H-2 H-3 H-4 H-5 H-6 H-6′); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.0 (CNO_2), 147.6 (N = CN), 138.5 (N_2C–C), 129.6, 123.1, 83.8 (C-1), 78.1, 77.3, 72.8, 70.1 (4s, C-2 C-3 C-4 C-5), 61.0 (C-6); Anal. Calcd for $C_{13}H_{17}N_3O_8$ (343.30): C, 45.48; H, 4.99; N, 12.24. Found: C, 46.02; H, 5.25; N, 11.87.

4.7.2. *N*-(β-D-Glucopyranosyl)-4-cyanobenzamidoxime 9c

A few drops of NaOMe (2.5 M in MeOH) were added to a solution of **8c** (90 mg, 0.18 mmol) in dry methanol (9 mL). The reaction mixture was stirred at rt for 25 min before the addition of cation exchange resin Amberlyst 15 (H^+ form). The resin was filtered off and the solvent was removed to give 48 mg (81%) of **9c** as a syrup.

$[\alpha]_D$ −132 (c 0.6, MeOH); ^{1}H NMR (360 MHz, CD_3OD) δ 7.65 (d, 2H, J = 9.2 Hz, H-ar), 7.39 (d, 2H, J = 7.9 Hz, H-ar), 3.55 (t, 1H, J = 11.8 Hz), 3.40–3.1 0(m, 5H); ^{13}C NMR (90 MHz, CD_3OD) δ 156.4 (N–C=N), 134.6 (N_2C–C), 124.9, 119.9 (NC–C), 108.1 (CN), 84.6 (C-1), 79.6, 78.6, 74.2, 71.5 (4s, C-2 C-3 C-4 C-5), 62.9 (C-6); Anal. Calcd for $C_{14}H_{17}N_3O_6$ (323.31): C, 52.01; H, 5.30; N, 13.00. Found: C, 52.52; H, 5.23; N, 12.65.

4.8. *N*-(Phenyl-nitrosomethylene)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl amine 10b

Photolysis of **8b** *with a 60 W heating lamp using 4 equiv of NBS in one portion*: A solution of **8b** (200 mg, 0.39 mmol) in dry chloroform (8 mL) was placed in a 100 mL erlenmeyer flask equipped with a reflux condenser and NBS (278 mg, 1.56 mmol) was added in one portion. The mixture was irradiated with a 60 W white heating lamp from a distance of 1 cm. After 15 min, chloroform was added (30 mL), the mixture was washed with 5% aq Na_2SO_3, satd aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered and the solvent was removed under vacuo. The residue was purified by column chromatography (EtOAc/hexane, 1:4) affording **10b** as syrup in 30% yield (58 mg). Traces of **12b** (~3%) were also detected in the crude product by NMR spectroscopy.

Compound **10b**: $[\alpha]_D$ +22 (c 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, 2H, J = 8.3 Hz, H-ar), 8.05 (d, 2H, J = 8.9 Hz, H-ar), 5.32 (d, 1H, J = 9.8 Hz, H-1), 5.23 (t, 1H, J = 9.5 Hz, H-3), 4.83 (t, 2H, J = 9.4 Hz, H-2 H-4), 4.44 (dd, 1H, J = 12.9 Hz, H-6), 4.13 (dd, 1H, J = 6.0 Hz, H-6′), 3.90 (ddd, 1H, J = 1.9 Hz, H-5), 2.16, 2.01, 1.99, 1.93 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 169.5, 169.4, 169.2 (4s, CH_3CO), 157.3 (N_2C–C), 156.6 (NC=N), 150.1 (CNO_2), 131.5, 123.7, 82.0 (C-1), 75.3, 72.2, 68.7, 66.8 (C-2 C-3 C-4 C-5), 61.0 (C-6), 20.7, 20.4, 20.3, 20.2 (4s, CH_3CO); Anal. Calcd for $C_{21}H_{23}N_3O_{12}$ (509.43): C, 49.51; H, 4.55; N, 8.25. Found: C, 49.48; H, 4.76; N 8.22.

4.9. 3-(4-Nitrophenyl)-5-(D-*gluco*-1,2,3,5-tetraacetoxy-4-hydroxypentyl)-1,2,4-oxadiazole 11b

Photolysis of **8b** *with a 375 W heating lamp using 4 equiv of NBS in one portion*: A solution of **8b** (200 mg, 0.39 mmol) in dry chloroform (12 mL) was placed in a 100 mL erlenmeyer flask equipped with a reflux condenser and NBS (278 mg, 1.56 mmol, 4 equiv)

was added in one portion. The mixture was irradiated with a 375 W white heating lamp from a distance of 1 cm. After 2 h, the mixture was diluted with chloroform (30 mL), washed with 5% aq Na_2SO_3, satd aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered and the solvent was removed under vacuo. The residue was purified by column chromatography (EtOAc/hexane, 1:4) affording **12b** (13 mg, 13%) and **11b** (20 mg, 20%) as a syrup.

Compound **11b**: $[\alpha]_D$ +26.2 (*c* 0.26, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 2H, *J* = 8.9 Hz, H-ar), 8.24 (d, 2H, *J* = 8.8 Hz, H-ar), 6.16 (d, 1H, *J* = 6.7 Hz, H-2), 5.73 (dd, 1H, *J* = 3.1 and 7.3 Hz, H-4), 5.20–5.17 (m, 1H, H-5), 3.53 (dd, 1H, *J* = 3.6 and 11.9 Hz, H-6), 3.39 (dd, 1H, *J* = 6.4 Hz, H-6′), 2.18, 2.11, 2.10, 2.08 (4s, 12H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3 (C-1), 169.5, 169.4, 169.3, 169.0 (4s, CH_3CO), 166.9 (NC=N), 149.6 (CNO_2), 131.8 (C–CN_2), 128.5, 124.1, 69.6, 69.2, 68.6 (C-2 C-3 C-4), 65.7 (C-6), 30.2 (C-5), 20.6, 20.5, 20.3, 20.2 (4s, CH_3CO); MS (FAB > 0) *m*/*z* = 663.7 [M+dihydroxybenzoic acid]$^+$; Anal. Calcd for $C_{21}H_{23}N_3O_{12}$ (509.43): C, 49.51; H, 4.55; N, 8.25. Found: C, 49.76, H 4.58, N 8.17.

4.10. 3-(4-Nitrophenyl)-5-(D-*xylo*-1,2,3,5-tetraacetoxy-4-oxopentyl)-1,2,4-oxadiazole 12b

Photolysis of **8b** *with a 60 W heating lamp using 4 equiv of NBS in 4 portions*: A solution of **8b** (1 g, 1.96 mmol) in dry chloroform (40 mL) was placed in a 250 mL erlenmeyer flask equipped with a reflux condenser and NBS (348 mg, 1.96 mmol, 1 equiv) was added. The mixture was irradiated with a 60 W white heating lamp from a distance of 1 cm. After 30 min, NBS (348 mg, 1.96 mmol, 1 equiv) was added to the reaction mixture. An additional 2 equiv of NBS were added in two portions after 105 min and 135 min, respectively. After 2 h 45 min total reaction time, the mixture was diluted with chloroform (120 mL), washed with 5% aq Na_2SO_3, satd aq $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered and the solvent was removed under vacuo. The residue was purified by column chromatography (EtOAc/hexane, 1:4) affording **12b** as a sole syrupy product in 45% yield (447 mg).

Compound **12b**: $[\alpha]_D$ +19.4 (*c* 0.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, 2H, *J* = 10.1 Hz, H-ar), 8.22 (d, 2H, *J* = 9.1 Hz, H-ar), 6.27 (d, 1H, *J* = 5.9 Hz, H-2), 5.90 (dd, 1H, *J* = 3.7 Hz, H-3), 5.54 (d, 1H, H-4), 4.87 (d, 1H, *J* = 17.3 Hz, H-6), 4.72 (d, 1H, H-6′), 2.19, 2.13, 2.12, 2.01 (4s, 12H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.1 (C-5), 174.3 (C-1), 169.7, 169.4, 169.2, 168.9 (4s, CH_3CO), 167.0 (N_2CAr), 149.6 (CNO_2), 131.7 (N_2C–C), 128.5, 124.1, 73.7 (C-3), 69.5 (C-4), 66.4 (C-6), 65.4 (C-2), 23.1, 20.2, 20.1 (3s, 4C, CH_3CO); MS (FAB > 0) *m*/*z* = 508 [M+H]$^+$; Anal. Calcd for $C_{21}H_{21}N_3O_{12}$ (507.41): C, 49.71; H, 4.17; N, 8.28. Found: C, 49.58; H, 4.23; N, 8.24.

4.11. (1*R*,2*S*,3*R*,4*R*)-1,2,3,5-Tetra-*O*-benzyl-1-*C*-[3-(4-methoxyphenyl)-4*N*-oxido-1,2,4-oxadiazole-5-yl]pentitol 15

A solution of Et_3N (240 µL, 1.72 mmol, 12 equiv) in toluene (2 mL) was added dropwise over 8 h (syringe pump) to a solution of hydroximolactone **13** (80 mg, 0.14 mmol) and α-chloro-*p*-methoxybenzaldoxime (210 mg, 1.15 mmol, 8 equiv) in refluxing toluene (15 mL). After 24 h reflux, TLC showed a partial conversion of **13** into several products. After concentration, the residue was purified by column chromatography (PE/EtOAc, 7:3) to afford unreacted **13** (33 mg) then **15** (13 mg, 13% isolated yield). Based on the converted starting material, the calculated yield was 22%.

Compound **15**: R_f = 0.55 (EP/EtOAc, 7:3); ^1H NMR (500 MHz, $CDCl_3$) δ 7.15–7.38 (m, 22H, H-ar), 6.88 (d, 2H, *J* = 8.7 Hz, H-ar), 4.75 (d, 1H, *J* = 12.2 Hz, CH_2Ph), 4.66 (m, 2H, H-5 CH_2Ph), 4.59 (d, 1H, *J* = 12.4 Hz, CH_2Ph), 4.55 (m, 3H, CH_2Ph), 4.47 (br s, 1H, CH_2Ph), 4.41 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 4.18 (br s, 1H, H-2), 3.96 (m, 1H, H-3), 3.84–3.88 (m, 2H, H-4 H-6), 3.79 (s, 4H, H-6′ OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.3, 154.2 (NCO), 140.8 (NC=N), 137.8, 137.4, 137.0, 136.6, 130.1, 127.0–128.6 (m, 16C, CH-ar), 121.4 (s, 2C, CH-ar), 114.2 (s, 2C, CH-ar), 80.8 (C-3), 77.2 (C-4), 77.1 (C-5), 73.4 (CH_2Ph), 73.0 (CH_2Ph), 72.9 (C-2), 71.7 (CH_2Ph), 71.0 (CH_2Ph), 67.7 (C-6), 55.5 (OCH_3); MS (ESI >0) *m*/*z* = 703.0 [M+H]$^+$, 725.0 [M+Na]$^+$, 1404.9 [2M+H]$^+$, 1426.9 [2M+Na]$^+$; HRMS (ESI >0) *m*/*z* = $C_{42}H_{43}N_2O_8$ [M+H]$^+$ calcd 703.3019; found 703.3022.

4.12. Enzymatic methods

Glycogen phosphorylase *b* was prepared from rabbit skeletal muscle according to the method of Fischer and Krebs,[45] using dithiothreitol instead of L-cysteine, and recrystallized at least three times before use. Kinetic experiments were performed in the direction of glycogen synthesis as described previously.[18] Kinetic data for the inhibition of rabbit skeletal muscle glycogen phosphorylase were collected using different concentrations of α-D-glucose-1-phosphate (2–20 mM), constant concentrations of glycogen (1% w/v) and AMP (1 mM), and various concentrations of inhibitors. Inhibitors were dissolved in dimethyl sulfoxide (DMSO) and diluted in the assay buffer (50 mM triethanolamine, 1 mM EDTA and 1 mM dithiothreitol) so that the DMSO concentration in the assay should be lower than 1%. The enzymatic activities were presented in the form of double-reciprocal plots (Lineweaver–Burk) applying a nonlinear data analysis program. The inhibitor constants (K_i) were determined by Dixon plots, by replotting the slopes from the Lineweaver-Burk plots against the inhibitor concentrations.[26,30] The means of standard errors for all calculated kinetic parameters averaged to less than 10%.

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Supplementary data

Supplementary data (attempted preparations of glucopyranosylidene-spiro-oxadiazolines, separation of amidoximes, structural elucidation of amidoximes and oxidation products) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.05.080.

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