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

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1. Introduction

Type 2 diabetes mellitus 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. This is in particular due to the dramatic increase in obesity even among young adults and children. Striking enhancements in the prevalence of the disease is predicted for the developing countries of Africa, Asia, and South America. 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.

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. 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.

Several types of compounds for inhibition of GP have been reported, and among them derivatives of D-glucose represent, as ligands of the catalytic site, the most populated class. All isoenzymes can be converted from the inactive form (GPb) into the active GPa form through the phosphorylation by the glucokinase.

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<sub>i</sub> = 160 nM), among glucose-based inhibitors known to date.

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phorylation of Ser-14. Isoforms of GP can be obtained from rabbit skeletal muscles (RMGP\textsubscript{a}/RMGP\textsubscript{b}) or human liver (HLGP\textsubscript{a}/HLGP\textsubscript{b}). Because of a \textasciitilde 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.\textsuperscript{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 \( \beta \)-anomeric substituent of D-glucopyranose, lined by both polar and non-polar group, and named the \( \beta \)-channel.\textsuperscript{16} 

\( \beta \)-Acetyl-\( \beta \)-D-glucopyranosylamine\textsuperscript{16} A was one of the first efficient glucose analogue inhibitors of GP. A large array of compounds with the \( \beta \)-acyl-\( \beta \)-D-glucopyranosylamine structure (e.g., B–F) was synthesized, tested, and also investigated by X-ray crystallography.\textsuperscript{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 \textsuperscript{G,} and \textsuperscript{H,} 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.\textsuperscript{17} On the other hand, \( \beta \)-acetyl-\( \beta \)-D-glucopyranosyl-N\textsubscript{\textsuperscript{0}}-acyl ureas\textsuperscript{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 \( \beta \)-channel next to the catalytic site, exhibit nanomolar inhibition\textsuperscript{12,13,20} despite the lack of the H-bond mentioned above. Therefore, interactions in the so called \( \beta \)-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 \( \beta \)-glucopyranose and a five-membered hetero-
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, proved to be a micromolar inhibitor. Therefore, synthesis of further compounds of this series was carried out 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 α-glucosyl hydroximothioates 1a–j were prepared from per-O-acetylated α-thio-β-α-glucopyranose and in situ formed nitrile oxides used in slight excess (1.2 equiv), following a procedure reported previously by Rollin (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/H2O/Et3N) 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 anomerice 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-sulfonoxides. 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-pyriddyloxime), isolated in ca 60% yield.

2.2. NBS-mediated cyclization of hydroximothioates

The spiro-cyclization of phenyl hydroximothioate 1a proceeded in CHCl₃ with the same selectivity as already observed in CCl₄, affording a 5:1 mixture of the (1S)/(1R)-epimers of 2a (Scheme 1). The reaction of hydroximothioates 1b,c,e,g,h,i with N-bromosuccinimide (NBS) in freshly distilled CCl₄ or CHCl₃ was stopped when 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/H2O/Et3N) to afford the corresponding unprotected products in high yields (Scheme 1).

The oxidative photocyclization of 4-nitrobenzamidoxime proceeded in CHCl₃ with the same selectivity as already observed in CCl₄, affording a 5:1 mixture of the (1S)/(1R)-epimers of 2a (Scheme 1).

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 H₂O, 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-α-glucosyl-amidoximes 8 (Scheme 2). A detailed retrosynthetic analysis and the preparation of the amidoximes 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-α-glucopyranosyl azide 7 was transformed into the corresponding phosphinimine derivative which reacted readily with hydroxymyl chlorides and gave the target molecules 8a–c, which were isolated in ca 30% yield.

The NMR spectra of the crude products indicated the presence of two species (~1:1 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 deacylated 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. 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 14 (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

<table>
<thead>
<tr>
<th>Ar=</th>
<th>Compound/yield (%)</th>
<th>(15)-3a/46</th>
<th>(15)-3b/33</th>
<th>(15)-3c/15</th>
<th>(15)-3d/3022</th>
<th>(15)-3e/17</th>
<th>(15)-4a/90</th>
<th>(15)-4b/71</th>
<th>(15)-4c/4</th>
<th>(15)-4d/79</th>
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<td>1a/9032</td>
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<td></td>
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<td>(15)-4e/4</td>
<td></td>
<td>(15)-4f/97</td>
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<tr>
<td>p-NO2Ph</td>
<td>2b/9132</td>
<td>1b/6032</td>
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<td>(15)-3c/7</td>
<td>(15)-3d/1622</td>
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<td>1e/7132</td>
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<td>(15)-3d/122</td>
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<td>(15)-4e/79</td>
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<td>4-Pyridyl</td>
<td>3g/90</td>
<td>1j/30</td>
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<tr>
<td>4-Pyridyl-N-oxide</td>
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* Calculated yields given in italics take into account the ratio of anomers estimated by 1H NMR in mixed fractions after purification.

Scheme 2. Attempted preparations of glucosylidene-spiro-oxadiazolines 4. Reagents and conditions: (a) i) PMe3, ii) ArC(Cl) = NOH, Et3N; (b) NaOMe, MeOH; (c) see conditions in Table 2.

Table 2
Photolysis of 8b under various conditions

<table>
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<tr>
<th>Lamp (W)</th>
<th>NBS (equiv)</th>
<th>Yield of 10b (%)</th>
<th>Yield of 11b (%)</th>
<th>Yield of 12b (%)</th>
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<tr>
<td>60</td>
<td>4</td>
<td>31</td>
<td>0</td>
<td>~3</td>
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<td>60</td>
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<td>45</td>
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<td>375</td>
<td>4</td>
<td>20</td>
<td>20</td>
<td>10</td>
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1,3-Dipolar cycloaddition of nitrile oxides to benzyl-protected glucosyl-hydroximolactone 13 was envisaged as another approach to analogous glycopyranosylidene-spiro-1,2,4-oxadiazoles. 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 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]+). 13C NMR spectroscopy indicated chemical shifts at 140.8 ppm (N_{2}C_{ar}) 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 (1R)-configured spiro-oxadiazole (1R)-4i showed no significant inhibition of rabbit muscle glycogen phosphorylase (RMPG) b. In contrast, several (1S)-configured spiro-compounds (1S)-4 showed inhibition in the low micromolar range, except for compounds (1S)-4b,c having polar substituents (4-nitro and 4-cyano, respectively). The most efficient was the 2-naphthyl derivative (1S)-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 is 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 glycopyranosylidene-spiro-hydantoins25–28 and N-acyl-N'-β-D-glucopyranosyl ureas29–31 derivatives were shown to be the best inhibitors.
umn chromatography was performed with Geduran washed with water (3 times), then dried (CaCl2) prior distillation. The 2-phur atom is replaced by a nitrogen, failed and led to heteroaromatic structures with an acyclic carbohydrate moiety. The 2-naphthyl substituted glucopyranosylidene-spiro-oxathiazole dis-somatic structures with an acyclic carbohydrate moiety. The 2-naphthyl substituted glucopyranosylidene-spiro-oxathiazole dis-somatic structures with an acyclic carbohydrate moiety. The 2-naphthyl substituted glucopyranosylidene-spiro-oxathiazole dis-

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 glucopyranosylidene-hydroximothioate precursors. Attempted preparations of the correspondingaza-analogs, in which the sulfur 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 Ki 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 (CaCl2) prior distillation over CaH2 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 F254 (Merck). TLC plates were inspected by UV light (λ = 254 nm) and developed by treatment with a mixture of 10% H2SO4 in EtOH/H2O (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 H2O2 (30% in water) and AcOH. Upon gentle heating, bromo-compounds gave pink-colored spots. Silica gel column chromatography was performed with Geduran Silica Gel 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 C18 (>400 mesh, Fluka). 1H and 13C 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 Eu riso-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 13C NMR data.22 1-Thio-b-n-glucopyranosyl hydroximothioates 2a,b,d,e were previously reported.22 Compound (15)-3d was previously reported.

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-b-d-glucopyranose (1 mmol) and hydroximoyl chloride (1.2 mmol, 1.2 equiv) were dissolved in CH3Cl2 (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 CCl4 (20 mL, for 3b–e,g,h) or CHCl3 (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 Na2SO4 (2 × 100 mL) and water (2 × 100 mL), dried (Na2SO4), filtered and evaporated under 

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Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (μM)</th>
<th>Compound</th>
<th>Inhibition (μM)</th>
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<tr>
<td>2b</td>
<td>IC50 &gt; 10 000</td>
<td>(15)-4a</td>
<td>Kᵢ = 26</td>
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<tr>
<td>2c</td>
<td>No inhibition at 625</td>
<td>(15)-4b</td>
<td>IC50 = 250</td>
</tr>
<tr>
<td>2e</td>
<td>No inhibition at 312.5</td>
<td>(15)-4c</td>
<td>IC50 = 700</td>
</tr>
<tr>
<td>2g</td>
<td>Insoluble in DMSO</td>
<td>(15)-4d</td>
<td>Kᵢ = 48</td>
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<td>2h</td>
<td>Insoluble in DMSO</td>
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<td>Kᵢ = 8.2</td>
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<tr>
<td>2i</td>
<td>No inhibition at 625</td>
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<td>Kᵢ = 1 800</td>
<td>(1R)-4i</td>
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4.1.4. General procedure D for the Zemplén decacylation

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 left to pH 5–6 with Amberlite IR-120 resin (H+ form) and the resin was washed with MeOH (2 × 10 mL). The filtrate was evaporated under reduced pressure to afford the desired hydroximothioates 2 and spiro-oxazolione 4, respectively.

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

Phosphine (2.68 mL, 1 M in toluene) was added with stirring to a solution of 2,3,4,6-tetra-O-acetyl-1-thio-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 (Na2SO4), 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-peracylated hydroximothioates 1

4.2.1. 2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosyl-1-thio-β-D-glucopyranosyl azide 1b

To a mixture of the azide 7 (1.94 g, 5.32 mmol) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl azide 1f (0.35 g, 0.65 mmol) in dry dichloromethane (30 mL) was added a solution of 1,2-diacetylhydrazine (0.32 g, 0.65 mmol) in dry dichloromethane (10 mL). After the reaction was over (2–3 h), the mixture was poured into water, and the oil 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 (Na2SO4), 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.

4.2.2. 2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosyl-1-thio-β-D-glucopyranosyl azide 1c

To a mixture of the azide 7 (1.94 g, 5.32 mmol) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosyl azide 1f (0.35 g, 0.65 mmol) in dry dichloromethane (30 mL) was added a solution of 1,2-diacetylhydrazine (0.32 g, 0.65 mmol) in dry dichloromethane (10 mL). After the reaction was over (2–3 h), the mixture was poured into water, and the oil 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 (Na2SO4), 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.
4.2.7. 2,3,4,6-Tetra-O-acetyl-1-S(–)-inosicotinoyl-hydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 1\1

2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranosylglycoporanosyl (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 saturated aqueous NaHCO3 solution (25 mL), then with water (25 mL). The organic layer was dried (MgSO4) 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% acetic acid) to give a crude yellow oil (212 mg, 0.36 mmol, 86%) as a white solid upon filtration and evaporation (112 mg, 0.23 mmol, 78% yield).

4.3. Deacetylation of O-pentaacylated hydroximiothiases

4.3.1. 1-S(–)-4-Nitrobenzhydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 2b

From compound 1b (200 mg, 0.38 mmol) according to general procedure D yielding 2b (111 mg, 0.03 mmol, 82%) as a white crystalline solid.

4.3.2. 1-S(–)-4-Cyanobenzhydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 2c

From compound 1c (200 mg, 0.39 mmol) according to general procedure D yielding 2c (122 mg, 0.36 mmol, 86%) as a white crystalline solid.

4.3.3. 1-S(–)-4-Methoxybenzhydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 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 solid.

4.3.4. 1-S(–)-4-(Phenyl)-benzhydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 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.

4.3.5. 1-S(–)-2-Naphthhydroximoyl-1-thio-β-D-glucopyranosylglycoporanosyl 2h

From compound 1h (343 mg, 0.64 mmol) according to general procedure D yielding 2h (200 mg, 0.38 mmol, 82%) as a white crystalline solid.
4.3.6. 1-S-(2)-Isonicotinoyl-hydroxymethyl-1-thio-β-o-glucopyranose 2i
A solution composed of 11 (90 mg, 0.18 mmol) in a mixture of MeOH (2 mL), water (2 mL) and NEt₃ (0.4 mL) was treated according to general procedure B. The residue was purified by RP-18: water to afford 2i (53 mg, 0.16 mmol, 90% yield) as a white solid.

4.4. Spiro-cyclization

4.4.1. (15S)- and (1R)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-α-glucitol-spiro[1.5]-3-(4-phenyl)-1,4,2-oxathiazole (15S)-3a and (1R)-3a

When the spiro-cyclization was carried out with 1a (278 mg) in CHCl₃ 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 (15S)- and (1R)-epimers. Separation by repeated column chromatography with a gradient of PE/EtOAc as the mobile phase afforded (15S)-3a (44 mg, 16%) and (1R)-3a (51 mg, 18%).

4.4.2. (15S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-α-glucitol-spiro[1.5]-3-(4-nitrophene)-1,4,2-oxathiazole (15S)-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 (15S)-3b (259 mg) as a white foam and an additional crop (144 mg) of a mixture of (15S)-3b and (1R)-3b in a 7:93 ratio, respectively. (1H NMR). The calculated yields were, respectively, 33% and 16% for the (15S)- and (1R)-epimers.

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

Spiro-cyclization of 1f (580 mg) was attempted as before: NBS (360 mg, 2 equiv) with CHCl₃ (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).

1H NMR (300 MHz, CDCl₃) δ 6.93 (d, 2H, J = 9.0 Hz, H-2, 1H, J = 9.0 Hz, H-3, 8.25 (m, 1H, H-4), 3.59 (m, 2H, H-5, H-6), 4.10, 2.07 (4s, 12H, CH₃CO); 13C NMR (75 MHz, CDCl₃) δ 170.6, 169.4, 169.3, 169.3. 30.7 (OMe), 155.4 (C=N), 193.4, 132.8, 128.8 (s, 2C, CH₃CO), 124.1 (s, 2C, CH₂), 123.4 (3C, 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), 206 (s, CH₃CO), 20.4 (s, 3C, CH₂CO); MS (ESI>0) m/z +511.8 [M+H]+, 534.0 [M+Na]+, 1022.4 [2M+H]+, 1404.6 [2M+Na]+; HRMS (ESI>0) m/z +c2H₂H₂N₂NaO₁₂S [M+Na]+ calcd 534.1046, found 534.1049.

4.4.6. (15S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-α-glucitol-spiro[1.5]-3-[3-(4-phenyl)-phenyl]-1,4,2-oxathiazole (15S)-3g

A solution composed of 1g (200 mg, 357 µmol) and NBS (127 mg, 715 µmol) was treated according to the general procedure C to afford a first crop of pure (15S)-3g (65 mg) as a white foam and an additional crop (90 mg) of a mixture of (15S)-3g and (1R)-3g in a 3:2 ratio, respectively. The calculated yields were, respectively, 61% and 18% for the (15S)- and (1R)-epimers.

Rf = 0.52 (PE/ EtOAc, 3:2); [α]D 45 +45 (c 1, CH₂Cl₂); 1H NMR (300 MHz, CDCl₃) δ 7.74 (d, 2H, J = 8.5 Hz, CH₂), 7.65 (2H, J = 8.5 Hz, CH₂), 7.57–7.63 (3H, 2C, CH₂), 7.37–7.50 (3H, 2C, CH₂), 6.84 (m, 4H, 6H, CH₂CO); 13C NMR (75 MHz, CDCl₃) δ 169.6, 169.5, 169.4 (4s, CH₃CO), 156.1 (C=N), 144.5, 139.6, 128.9 (s, 2C, CH-2), 128.4 (s, 2C, CH-3), 128.1 (CH-4, CH-5), 127.5 (s, 2C, CH₂CO), 170.4, 169.6, 169.29, 169.26, (4s, CH₃CO), 154.5 (C=N), 132.6 (2C, CH-5), 131.2, 128.4 (s, 2C, CH-2, 123.3 (C-5), 117.7, 115.1 (C=N), 70.84 (C-2 or C-3), 70.50 (C-2 or C-3), 67.3 (C-4), 60.9 (C-6), 20.6 (CH₃CO), 20.4 (3C, CH₃CO); MS (ESI>0) m/z +529.0 [M+Na]+, 1034.5 [2M+Na]+; HRMS (ESI>0) m/z c2H₂H₂N₂NaO₁₂S [M+Na]+ calcd 529.0893, found 529.0898.

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 (CH3CO), 20.5 (s, 3C, CH2O); 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 = Z = C27H27NNaO10S [M+Na]+ calc 580.1253, found 580.1252.

4.4.7. (1S,3S,5S)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-o-glucitol-spiro[1.5]-3-(2-naphthyl)-1,4,2-oxathiazole (15)-3h

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 (15)-3h (181 mg) as a white foam and an additional crop of (15)-3h (200 mg) of a mixture of (15)-3h and (1R)-3h in a 1:2 ratio, respectively. The calculated yields were, respectively, 36% and 16% for the (1S)- and (1R)-enipimers.

4.4.8. (1R)-2,3,4,6-Tetra-O-acetyl-1,5-anhydro-o-glucitol-spiro[1.5]-3-(4-pyridyl)-1,4,2-oxathiazole (1R)-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 with 30 min according to the general procedure C. TLC monitoring showed the appearance of two main products (more mobile than 11) and two minor products. Workup and flash chromatography with petroleum ether EtOAc containing 0.1% Et3N 7:3 then 2:3 afforded a mixture (107 mg) with 3 components (NMR) some being bismolated (as shown by TLC using a specific staining reagent with fluorescent dye, and (1R)-3i (190 mg, 0.39 mmol, 30% yield) as a transparent solid which appeared labile at room temperature and was kept at 0 °C.

131.8 (s, 2C, CH-ar), 135.2, 128.3 (C-1), 122.0 (s, 2C, CH-ar), 84.6 (s, C2H5O), 83.6 (s, 2C), 79.7 (C-5), 73.5 (C-3), 71.1 (C-6), 68.6 (C-2), 67.3 (C-4), 61.3 (C-6), 21.1, 21.0, 20.90, 20.92 (4s, CH3CO); Anal. Calcd for C29H23NO10S: C, 49.79; H, 4.60; N, 5.81; O, 33.16. Found: C, 49.07; H, 4.68; N, 5.55; O, 33.41.

4.5. Decacylation of spiro-oxathiazoles

4.5.1. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(4-nitrophenyl)-1,4,2-oxathiazole (15)-4b

A solution composed of (15)-3b (255 mg) was treated according to general procedure D (15)-4b (254 mg, 71%) as a pale yellow solid.

131.9 (s, 2C, CH-ar), 134.8, 133.7, 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 (s, CH2O); MS [ESI < 0] m/z = 392.8 [M+Cl]-, 750.5 [2M+Cl]+, HRMS [ESI < 0] m/z = C21H14Cl2NO10S [M+Cl]+ calc 744.0622, found 744.0623.

4.5.2. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(4-cyanophenyl)-1,4,2-oxathiazole (15)-4c

A solution composed of (15)-3c (100 mg) was treated according to general procedure D to afford (15)-4c (56 mg, 84%) as a pale yellow foam.

4.5.3. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(4-fluorophenyl)-1,4,2-oxathiazole (15)-4d

Prepared by general procedure D from (15)-3d (500 mg) to afford (15)-4d (392 mg, 79%) as a pale yellow solid.

4.5.4. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(4-methoxyphenyl)-1,4,2-oxathiazole (15)-4e

A solution composed of (15)-3e (370 mg) was treated according to general procedure D to afford (15)-4e (196 mg, 79%) as a pale yellow solid.

4.5.5. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(4-phenoxyphenyl)-1,4,2-oxathiazole (15)-4f

A solution composed of (15)-3g (65 mg) was treated according to general procedure D to afford (15)-4f (44 mg, 97%) as a pale yellow foam.

4.5.6. (1S,15)-1,5-Anhydro-o-glucitol-spiro[1.5]-3-(2-naphthyl)-1,4,2-oxathiazole (15)-4h

A solution composed of (15)-3h (180 mg) was treated according to general procedure D to afford (15)-4h (116 mg, 94%) as a pale yellow solid.

4.5 Hz, H-ar), 7.70 (d, 2H, H-ar), 3.94 (d, 1H, J = 9.1 Hz, H-2), 3.81 (m, 3H, H-5 H-6 H-7); 13C NMR (75 MHz, CDCl3) δ 85.0 (s, 1H, OH), 7.71 (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), 4.93 (t, 1H, J = 8.8 Hz, H-2), 4.99 (t, 1H, J = 9.4 Hz 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 (t, 1H, J = 12.5 Hz, H-6), 3.39 (dd, 1H, J = 2.7 Hz, H-5), 2.09, 2.06, 2.02, 1.99 (4s, 12H, CH3CO); 1H NMR (360 MHz, CDCl3) δ 8.98 (s, 1H, OH), 8.22 (d, 2H, J = 8.9 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, CH3CO); 13C NMR (75 MHz, CDCl3) δ 170.4, 169.2, 169.1, 169.0 (4s, CH3CO), 157.3 (N=C=O), 151.0 (CO2), 147.6 (NH), 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. Calc'd for C13H17N3O8 (343.30): C, 45.48; H, 4.99; N, 12.24. Found: C, 45.48; H, 4.99; N, 12.25. 4.8. (N-Phenyl-nitrosomethylene)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine amine 10b 4.8.1. Solution of 8b with a 60 W heating lamp using 4 equiv of NBS 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 exchanged by using 4 equiv of NBS 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 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. The residue was purified by crystallization to give 56 mg (83%) of 9b.

4.8.2. Deacetylation of 10b with a 60 W heating lamp using 4 equiv of NBS 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 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. The residue was purified by crystallization to give 56 mg (83%) of 9b.

4.7. Deacetylation of N-glycosyl-amidoximes

4.7.1. N2(p-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.

4.7.2. N2(p-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 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 9c as a syrup.
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 Na₂SO₃, satd aq NaHCO₃ and water. The organic layer was dried (Na₂SO₄), 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:** $\delta_{1H}$ 2.62 (c 0.26, CH₂Cl₂); $^1H$ NMR (400 MHz, CDCl₃) $\delta$ 3.33 (d, 2H, $J = 8.9$ 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, CH₃CO); $^{13}C$ NMR (75 MHz, CDCl₃) $\delta$ 197.1 (C-5), 174.3 (C-1), 169.4, 169.2, 168.9 (4s, CH₂CO), 167.0 (N=C=Nil), 149.6 (CNO₂), 131.7 (N=C=C), 128.5, 124.1, 69.6, 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, CH₃).

**Compound 12b:** $\delta_{1H}$ 19.4 (c 0.3, CHCl₃); $^1H$ NMR (300 MHz, CDCl₃) $\delta$ 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, CH₃CO); $^{13}C$ NMR (75 MHz, CDCl₃) $\delta$ 197.1 (C-5), 174.3 (C-1), 169.4, 169.2, 168.9 (4s, CH₂CO), 167.0 (N=N=C=Nil), 149.6 (CNO₂), 131.7 (N=C=C), 128.5, 124.1, 69.6, 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, CH₃).

**4.10. 2-(4-Nitrophenyl)-5-[(3-oxoethyl)-1,2,3,5-tetraacetoxy-4-oxopentyl]-1,2,4-oxadiazol-1-ylidine-spiro-oxadiazolines, separation of amidoximes, structural elucidation of amidoxydine and oxadiazolines**

**Supplementary data**

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

**References and notes**

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