Synthesis of and a Comparative Study on the Inhibition of Muscle and Liver Glycogen Phosphorylases by Epimeric Pairs of D-Gluco- and D-Xylopyranosylidene-spiro-(thio)hydantoins and *N*-(D-Glucopyranosyl) Amides

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D-Gluco- and D-xylopyranosylidene-spiro-hydantoins and -thiohydantoins were prepared from the parent sugars in a six-step, highly chemo-, regio-, and stereoselective procedure. In the key step of the syntheses *C*-(1-bromo-1-deoxy-*β*-D-glycopyranosyl)formamides were reacted with cyanate ion to give spiro-hydantoins with a retained configuration at the anomeric center as the major products. On the other hand, thiocyanate ions gave spiro-thiohydantoins with an inverted anomeric carbon as the only products. On the basis of radical inhibition studies, a mechanistic rationale was proposed to explain this unique stereoselectivity and the formation of *C*-(1-hydroxy-*β*-D-glycopyranosyl)formamides as byproducts. Enzyme assays with *a* and *b* forms of muscle and liver glycogen phosphorylases showed spiro-hydantoin **12** and spiro-thiohydantoin **14** to be the best and equipotent inhibitors with K_i values in the low micromolar range. The study of epimeric pairs of D-gluco and D-xylo configurated spiro-hydantoins and *N*-(D-glucopyranosyl)amides corroborated the role of specific hydrogen bridges in binding the inhibitors to the enzyme.

Introduction

Diabetes mellitus and its complications afflict 3% of the population of Europe and North America and 140 million people worldwide.[1] The disease is characterized by chronic elevated blood sugar levels for which several reasons like defects of insulin action in tissues or impairment of pancreatic insulin secretion may be responsible. Excessive hepatic glucose production can also significantly contribute to diabetic hyperglycemia. The non-insulin-dependent diabetes mellitus (NIDDM or type II diabetes) appears in ∼75% of the diabetic patients. Hyperglycemia in type II diabetics can be controlled by dietary regulation and exercise, which can be combined with the use of oral hypoglycemic agents. However, this treatment is not entirely satisfactory because blood glucose concentration cannot be regulated as efficiently as under normal physiological conditions. This can result in complications occurring in the later life of the diabetic patient.[2] Therefore, there is an obvious need for novel agents and/or therapeutic strategies that could act more closely to the physiological regulation of blood sugar level.

Hepatic glucose output is regulated by a complex system of enzymes that is under hormonal, neuronal, and metabolic control. The main regulatory enzyme of this system is glycogen phosphorylase, which catalyzes the first step of glycogen breakdown. The inhibition of this enzyme may help to shift the balance between glycogen degradation and synthesis in favor of the latter. On the basis of the fact that weak inhibitors of glycogen phosphorylase are weakly hypoglycemic, the concept of inhibition of this enzyme as a potential tool for controlling hyperglycemia was put forward.[3]

To find potent glucose analogue inhibitors of glycogen phosphorylase, extensive synthetic,[3−6] crystallographic,[7−11] molecular modeling,[3,8,12−14] and enzymological[3−5,7,12,14] studies have been carried out. As a result of these investigations, glucopyranosylidene-spiro-hydantoin **12** emerged as the strongest glucose analogue inhibitor of muscle glycogen phophorylase *b* known to date.[4] However, the published synthetic routes to **12** were rather lengthy on one hand or gave the much less efficient inhibitor **20** as the major product on the other.[4−6]

In this paper we disclose full experimental details of a simple, short, and highly chemo-, regio-, and stereoselective procedure for the preparation of D-glucopyranosylidene-spiro-(thio)hydantoins,[15] which was also applied to the synthesis of their D-xylo configurated analogues. Kinetic data obtained for the first time with liver glycogen phosphorylases for glucose analogue inhibitors demonstrate that such compounds may possess hypoglycemic activity. Furthermore, investigation of D-xylo configurated compounds as well as other *N*- and *C*-glycosyl amide derivatives shed light on the importance of some particular H bonds between the enzyme and the inhibitor.

Results and Discussion

Synthesis of D-Glycopyranosylidene-spiro-(thio)-hydantoins and *N*-D-Glucopyranosylamides. The spiro heterocycles were synthesized by adapting and improving our recently published protocol for the preparation of D-galacto- and D-arabinopyranosylidene-spiro-

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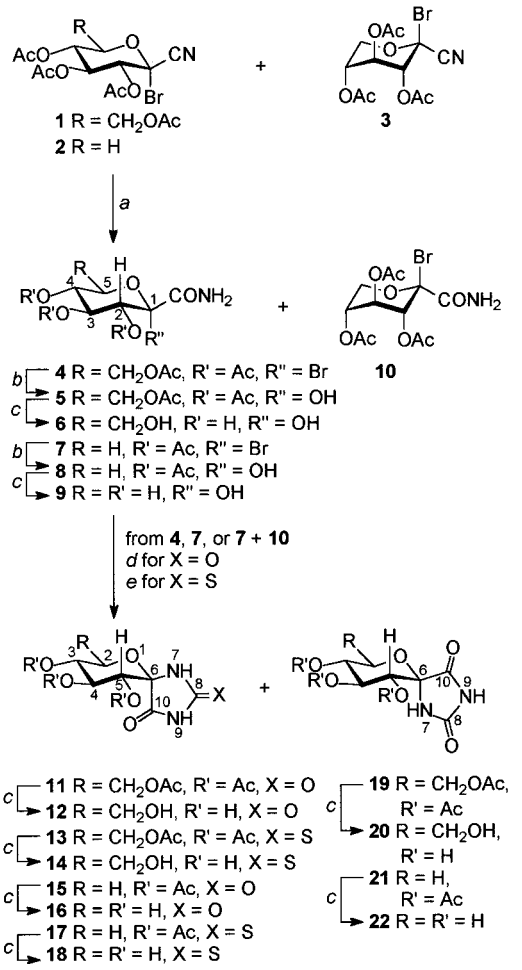
Scheme 1. Preparation of Glycopyranosylidene-spiro-(thio)hydantoins[a]



[a] (a) $TiCl_4$, H_2O (1 equiv), AcOH, 0 °C → room temp; (b) Ag_2O, H_2O (1 equiv), DMSO, room temp; (c) NaOMe (cat.), MeOH, room temp; (d) AgOCN (4 equiv), CH_3NO_2, 80 °C; (e) KSCN (4 equiv), S_8, CH_3NO_2, 80 °C, N_2 atm.

(thio)hydantoins.[16] Thus, acetylated 1-bromo-1-deoxy-β-D-glycopyranosyl cyanides (**1** or **2**, Scheme 1) readily obtained by radical-mediated bromination[17] from per-*O*-acetylated β-D-glycopyranosyl cyanides were treated with $TiCl_4$ in AcOH in the presence of 1 equiv of H_2O to give, after chromatographic purification, *C*-(1-bromo-1-deoxy-β-D-glycopyranosyl)formamides **4** (68%) and **7** (66%). Reaction of **4** with AgOCN in CH_3NO_2 at 80 °C gave spiro-hydantoins **11** (4%) and **19** (25%) together with hydroxyamide **5** (53%). Similar reaction of **7** gave spiro-hydantoins **15** and **21** and hydroxyamide **8** in a 1:1:2 ratio (^{1}H NMR). Under the same conditions as those for a mixture of **7** and **10** obtained by partial hydrolysis of **2** and **3**, the product mixture of the photobromination **15**, **21**, and **8** were isolated in 7%, 15%, and 27% yield, respectively. Compounds **5** (85%) and **8** (83%) were also prepared from **4** and **7**, respectively, by Ag_2O-promoted hydrolysis in DMSO in the presence of 1 equiv of H_2O. When **4** or **7** was reacted with AgSCN or KSCN in CH_3NO_2 at 80 °C, spiro-thiohydantoins **13** (57%) and **17** were the only cyclized products accompanied by **5** (19%) and **8**, respectively. Carrying out these reactions in the presence of a small amount of elemental sulfur under nitrogen atmosphere in order to suppress radical-mediated pathways raised the yield of **13** to 79%, and only 4% of **5** was obtained; the respective yields for **17** and **8** were 64% and 15%. See the mechanistic proposal below to rationalize the use of the above conditions.

The acetylated compounds **5**, **8**, **11**, **13**, **15**, **17**, **19**, and **21** were deprotected by the Zemplén procedure to give **6**, **9**, **12**, **14**, **16**, **18**, **20**, and **22**, respectively, in high yields.

N-Acyl-D-glucopyranosylamines **27**,[18] **28**,[19] **29**, and **30** were obtained from the corresponding per-*O*-acetylated compounds[20] by the Zemplén protocol.

Structure Elucidation of the New Compounds. The structure of the new compounds was unequivocally established from NMR data. The presence of the carboxamido group in **4**−**9** was indicated by two broad singlets in the proton spectra and a carbon resonance around 167−170 ppm. An additional broad singlet was characteristic for the free hydroxyl group in **5** and **8**.

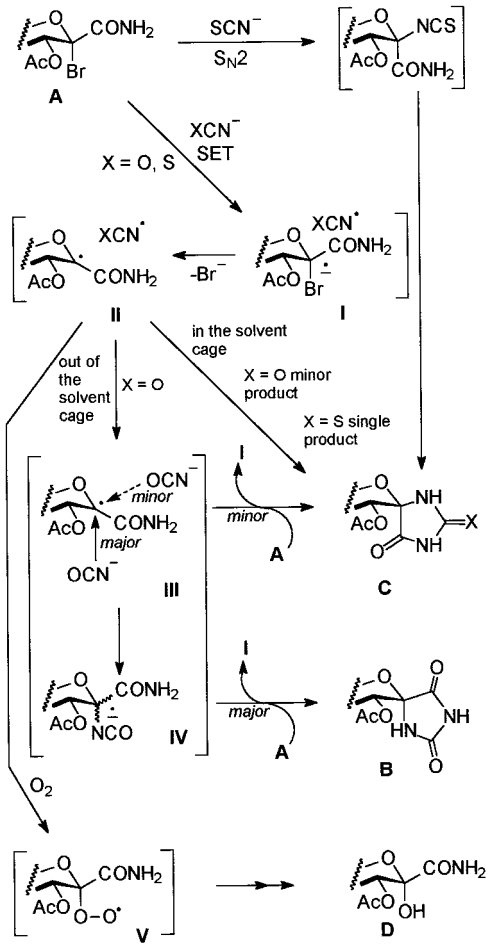
For each hydantoin the ^{13}C NMR spectra exhibited two resonances at chemical shifts characteristic[21−23] of the "amide" [δ_{C-10} 167−175 ppm], the "urea" [δ_{C-8} 155−160 ppm], or the "thiourea" [δ_{C-8} 182−186 ppm] type of (thio)carbonyl carbons. The ^{1}H chemical shifts of the NH protons are similar to those found in simple (thio)-hydantoins,[24,25] the H-9 resonances appearing at lower field (10.4−10.8 ppm in hydantoins; 11.4−11.9 ppm in thiohydantoins) than those for H-7 (7.7−8.4 ppm in hydantoins; 9.8−10.5 ppm in thiohydantoins). Long-range ^{13}C−^{1}H or ^{15}N−^{1}H connectivities detected in the respective HMBC spectra have provided further confirmation of the spiro-(thio)hydantoin structures as follow: ^{13}C−^{1}H correlations through two bonds, C-6/N**H**-7, C-8/N**H**-7, C-8/N**H**-9, C-10/N**H**-9; ^{13}C−^{1}H correlations through three bonds: C-6/N**H**-9, C-10/H-5, C-10/N**H**-7; and ^{15}N−^{1}H correlations through three bonds, N-7/H-5, N-7/N**H**-9, and N-9/N**H**-7.

The *conformations* of the pyranose rings were shown to be $^4C_1$ by vicinal ^{1}H−^{1}H coupling constants (see Supporting Information) in all cases. This ensures that H-2 or H-5 atoms on the pyranose rings are in the *axial* position. Therefore, the *configurations* of the anomeric carbons (C-1 or C-6) could be deduced from the values of heteronuclear three-bond coupling constants[16,26] $^3J_{H-2,CONH2}$ and $^3J_{H-5,C-10}$. The configurational assignments were supported by ^{1}H chemical shift rules recently established for spiro-hydantoins.[16]

A Mechanistic Proposal. The main peculiarities of the (thio)hydantoin forming reactions in the D-gluco and D-xylo configurations described in this work, as well as in the D-galacto and D-arabino ones,[16] can be summarized as follows: (1) the stereoselectivity of the ring-closing step at the anomeric center depends on the nucleophile, while with silver cyanate the highly selective formation of a retention product (like **19** and **21**) was observed; ring closure with both silver and potassium thiocyanate gave thiohydantoins (like **13** and **17**) with inversion of the anomeric configuration exclusively. (2) The formation of *C*-(1-hydroxy-D-glycosyl)formamides (like **5** and **8**) was unavoidable in each of these transformations.

To rationalize these observations and to improve the selectivity of the reactions, numerous modifications and inhibition experiments were carried out (see Supporting Information) and we propose the following mechanism

Scheme 2. Proposed Mechanism of Glycopyranosylidene-spiro-(thio)hydantoin Formation



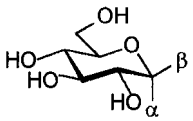
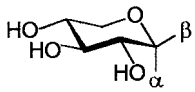
(Scheme 2). A highly probable general reaction that may proceed independent of solvent and cation can be started by a single-electron transfer (SET) from (thio)cyanate to bromoamide **A** to give radical anion **I**. From **I** a bromide can split off to give radical **II**, which may combine with the $SCN^{\bullet}$ radical in the solvent cage,[27] followed by a tautomeric ring closure furnishing **C**. This route is indistinguishable from a bimolecular substitution, and **C** (X = S) may indeed be formed by the classical S_N2 route (**A** → **C**) with complete inversion. If radical **II** escapes from the solvent cage, it may react with a cyanate ion to give **IV**. Because of well-known stereoelectronic reasons,[28] axial attack of the OCN^- ion may be favored as shown in **III** (major) leading to **B** after ring closure. Isomers **C** can also be formed in these reactions via **III** (minor) as byproducts. Oxidation of radical anion **IV** may proceed by the starting material **A**, thereby giving rise to a chain reaction. This is in keeping with the finding that significantly lower than stoichiometric amounts of radical traps or radical anion traps could completely inhibit the reaction. A similar mechanism was proposed for the formation 1-azido-1-deoxy-D-glycopyranosyl cyanides.[26]

Out of the solvent cage radical **II** may also be trapped by triplet oxygen to give the hydroperoxyl radical **V**. This can then abstract a hydrogen from the solvent and undergo known thermal decomposition[29] to **D**. This possibility is corroborated by several reports on the formation of alcohols via radicals trapped by molecular oxygen.[30−32] Formation of **D** on this pathway is not a chain reaction; therefore, small amounts of radical traps do not exert very efficient inhibition.[30]

As a result of these studies, we have obtained tools for suppressing hydroxyamide formation, as was demonstrated by the enhanced yield for the D-glycopyranosylidene-spiro-thiohydantoins **13** and **17** (see above).

Kinetic Studies. The kinetic studies with glycogen phosphorylases were performed as described previously.[15] Inhibitor constants (K_i) determined with muscle glycogen phosphorylase *b* for compounds with D-glucose or D-xylose skeletons are given in Table 1. The glucopyranosylidene-spiro-hydantoin **12** and its 8-thio analogue **14** have been identified as the highest affinity inhibitors of muscle GP*b*[4,15] (entries 1 and 2). These findings are supported by X-ray studies: analysis of the structure of muscle GP*b*−**12** complex pointed out the significance in binding of a H bond between N-7-H of the hydantoin ring and the peptide backbone oxygen of His377;[9] this important H bridge has been shown to be present in the muscle GP*b*−**14** complex as well.[33] The epimeric **20** is obviously devoid of this interaction with the enzyme, and in agreement with published data,[5] it proved to be a less efficient inhibitor (entry 3). The importance of this H bond to the protein is further corroborated by the loss of inhibition in the α-configurated **26** compared to its β-anomer **25** (entries 6 and 7). Such a comparison was not possible earlier because of the lack of a suitable synthetic method[20] to produce *N*-(α-D-glycosyl) amides. The presence of the trifluoromethyl group in **25** caused a significant weakening in the inhibition compared to that in **23** (entry 4). The reason for this is not obvious: CH_3 and CF_3 groups are similar in size; the H-bond donating capacity of the NH moiety in the trifluoroacetamido group can be higher than that of the acetamido group, and this might predict a stronger binding; the hydrophobicity is much higher for the CF_3 moiety[34] with respect to the CH_3 group; however, the presence of the much more hydrophobic phenyl group in **24** (entry 5) led to a smaller decrease of the inhibitory activity. Further studies to clear this point are in progress. Hydroxyamide **6** proved to be a weak inhibitor (entry 8). This seems to be surprising when compared to α-D-glucose (**28**) and β-carboxamide **29**, which show stronger inhibition and have the hydroxyl and the carboxamido groups in the same position as in **6** (entries 10 and 11, respectively). The joint presence of these moieties at the anomeric center is clearly highly detrimental for the binding. A possible reason for this may be an intramolecular H bond between the anomeric OH and $CONH_2$ groups in **6**. A similar intramolecular H bond has recently been observed in *C*-(1-azido-1-deoxy-D-glycopyranosyl)formamides in solution[35] and in the solid phase.[36] Such a H bond may alter the conformational position of the groups involved, thereby preventing formation of favorable interactions between these groups and the protein, which are present in the complexes GP*b*−**28**[3] or GP*b*−**29**.[7] This observation also needs further investigations. Changing the configuration of the sugar ring from D-gluco to D-xylo by removal of the hydroxymethyl side chain brought about a decrease of 4 orders of magnitude in the inhibition (entries 13−15). This is in keeping with earlier data obtained with 6-deoxy-D-glucose[37] and clearly indicates the importance

Table 1. Inhibitor Constants (K_i) Determined for Muscle Glycogen Phosphorylase *b*

entry	compound sugar skeleton	no.	anomeric substituent α	anomeric substituent β	K_i [μM]	ref
1		**12**		CONH−CONH	3.1	4
					4.2	this work
2		**14**		CONH−CSNH	5.1	this work
3		**20**		NHCO−NHCO	320	5
					105	this work
4		**23**	H	$NHCOCH_3$	32	8
					31	this work
5		**24**	H	$NHCOC_6H_5$	81	8
					144	this work
6		**25**	H	$NHCOCF_3$	710	this work
7		**26**	$NHCOCF_3$	H	no inhibition	this work
8		**6**	OH	$CONH_2$	3100	this work
9		**27**	H	OH	7400	3
10		**28**	OH	H	1700	3
11		**29**	H	$CONH_2$	440	7
12		**30**	$CONH_2$	H	370	7
13		**16**		CONH−CONH	11500	this work
14		**18**		CONH−CSNH	>10000[a]	this work
15		**22**		NHCO−NHCO	>10000[a]	this work
16		**9**	OH	$CONH_2$	no inhibition	this work

[a] Because of insufficient sample quantity, no precise value could be obtained.

in binding of an optimal hydrogen bond between the side chain of His377 in the enzyme and the oxygen of the hydroxymethyl side chain in the inhibitor revealed by X-ray studies.[9,33]

Potential antidiabetic compounds should exert their inhibitory action on the active *a* form of glycogen phosphorylase, and especially important is their effect on the hepatic enzymes. For the best inhibitors K_i values [μM] were also determined with liver glycogen phosphorylases *b* (**12**, 12.8 ± 1.1; **14**, 7.0 ± 1.0) and *a* (**12**, 16.5 ± 1.3; **14**, 29.8 ± 2.9) as well as with muscle phosphorylase *a* (**12**, 26.0 ± 2.4; **14**, 10.9 ± 1.0) to show similar potency for both compounds.[15]

Conclusion

The synthetic procedure described in this paper allows the stereoselective preparation of glycopyranosylidene-spiro-(thio)hydantoins in six steps from the corresponding free sugar. Although the peculiar stereoselectivity of the hydantoin-forming reaction disfavored obtention of the efficient glycogen phosphorylase inhibitor **12**, the high-yielding preparation of its thio analogue **14** provides an easy access to an equipotent inhibitor of both *b* and *a* forms of muscle and liver glycogen phosphorylases. Further improvements[38] of the synthetic sequence have facilitated acquisition of **14** in gram quantities. This has allowed us to demonstrate that **14** is effective in inhibiting glycogen phosphorylase *a* activity in hepatic cells both in vitro and in vivo,[39] indicating that this compound and its analogues can be useful biochemical tools for the study of the importance of glycogen phosphorylases in the regulation of hyperglycemia.

Experimental Section

General Considerations. Melting points were measured in open capillary tubes or on a Kofler hot stage and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter at room temperature. NMR spectra were recorded with Bruker WP 200 SY (200/50 MHz for $^1H/^{13}C$) or Avance DRX 500 (500/125/50 MHz for $^1H/^{13}C/^{15}N$) spectrometers. Chemical shifts are referenced to Me_4Si (^1H), to the residual solvent signals (^{13}C), or to NH_4Cl as external standard (^{15}N). Fast-atom bombardment (FAB) mass spectra were obtained using a VG-7070MS mass spectrometer. TLC was performed on DC-Alurolle Kieselgel 60 F_{254} (Merck), and the plates were visualized by gentle heating. For column chromatography Kieselgel 60 (Merck, particle size 0.063−0.200 mm) was used. Organic solutions were dried over anhydrous $MgSO_4$ and concentrated in vacuo at 40−50 °C (water bath). Nitromethane was distilled from P_4O_{10} directly in the reaction flask or was stored over 3 Å molecular sieves.

General Procedure I for the Preparation of *C*-(Per-*O*-acetyl-1-bromo-1-deoxy-D-glycopyranosyl)formamides (4 or 7). To a suspension of a glycosyl cyanide (**1** or **2**) in AcOH (1−1.5 mL/mmol), cooled in an ice bath, were added with stirring $TiCl_4$ (2−4 equiv) and H_2O (1 equiv). After 30 min the ice bath was removed and the mixture was stirred for 6 days at room temperature. Then the solution was poured with continuous stirring into a mixture of ice and water and the crude product was extracted with $CHCl_3$ (3×). The combined extracts were washed with cold saturated $NaHCO_3$ solution and H_2O and dried, and the solvent was evaporated. The crude product was sufficiently pure for further transformations.

General Procedure II for the Preparation of *C*-(Per-*O*-acetyl-1-hydroxy-D-glycopyranosyl)formamides (5 or 8). To a solution of **4** or **7** in dry DMSO (18 mL/mmol) were added with stirring Ag_2O (1 equiv) and H_2O (1 equiv). The reaction mixture was stirred at room temperature until disappearance of the starting material (TLC, eluent: EtOAc/hexane 1:1). After filtration the solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc and passed through a 3 cm layer of silica gel. Evaporation of the solvent left a syrupy crude product processed as given for the particular compounds.

General Procedure III for the Preparation of Per-*O*-acetylated d-glycopyranosylidene-spiro-(thio)hydantoins 11, 13, 15, 17, 19, 21. To a solution of **4** or **7** in dry CH_3NO_2 (15 mL/mmol) were added molecular sieves (3 Å) and an

MXCN salt (4 equiv of KSCN or freshly prepared dry AgSCN or AgOCN). The reaction mixture was stirred at 80 °C under nitrogen atmosphere for 3−4 h. After filtration and evaporation of the solvent the crude product was purified by column chromatography using ethyl acetate/hexane (1:2 to 1:1) as eluent.

General Procedure IV for the Removal of Acetyl Protecting Groups. To the solution of an acetylated compound in dry MeOH (10−15 mL/mmol) were added a few drops of a 1 M methanolic NaOMe solution, and the mixture was stirred at room temperature until disappearance of the starting material (TLC, eluent: chloroform/methanol 1:1). After neutralization with a cation-exchange resin (H^+ form) and filtration, the solvent was removed to furnish the product, which was further purified if necessary.

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Supporting Information Available: ^{1}H NMR chemical shift rules for the determination of configuration in the spirohydantoins, modifications of the reaction conditions and inhibition experiments supporting the mechanistic proposal, experimental data (compound characterization, details of inhibition experiments, and enzyme assays), and additional references. This material is available free of charge via the Internet at http://pubs.acs.org.

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