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Article

Anomeric spironucleosides of β-D-glucopyranosyl uracil as potential inhibitors of glycogen phosphorylase

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Abstract: Glycogen phosphorylase (GP) has been a target for compounds, inhibitors of GP that might prevent unwanted glycogenolysis under high glucose conditions and thus aim at the reduction of excessive glucose production from the liver, in the case of type 2 diabetes. Anomeric spironucleosides, such as hydantocidin, present a rich synthetic chemistry and possess important biological function. Herein, the Suárez radical methodology is successfully applied to synthesize the first example of a 1,6-dioxa-4-azaspiro[4.5]decane system, not been previously constructed *via* a radical pathway, starting from 6-hydroxymethyl-β-D-glucopyranosyluracil. It is shown that in the rigid pyranosyl conformation the required [1,5]-radical translocation is a minor process. The stereochemistry of the spirocycles obtained was unequivocally determined based on the chemical shifts of key sugar protons in the ^{1}H NMR spectra. The two spirocycles were found to be modest inhibitors of RMGP*b*.

Keywords: Type 2 diabetes; glycogen phosphorylase; anomeric spironucleosides; 1,6-dioxa-4-azaspiro[4.5]decane; [1,5]-radical translocation.

1. Introduction

A molecular approach that aims at the reduction of excessive glucose production from liver in the case of type 2 diabetes, involves the inhibition of glycogen phosphorylase (GP), a key enzyme in carbohydrate metabolism with great importance in the breakdown of glycogen reserves.[1] Glucose is a physiological regulator of hepatic glycogen metabolism that promotes inactivation of GP and acts synergistically with insulin, leading to reduced glycogen degradation and enhanced glycogen synthesis. Because of its central role in the regulation of glycogen metabolism, GP has been a target for compounds, inhibitors of GP that might prevent unwanted glycogenolysis under high glucose conditions.[2,3] The three dimensional structure of the T-state GPb in complex with α-D-Glucose has been used as the starting point for the design and synthesis of a series of glucose analogues, which are inhibitors that bind the catalytic site of the enzyme.[4] Design of inhibitors requires that the inhibitor stabilizes the T state of the enzyme and mimics the glucose contacts. The study of GP inhibition is an on-going challenge for researchers in physiology, synthetic and medicinal chemistry. One of the early lead inhibitors of GPb was pyranosyl spironucleosides[5] (**2a**,**b**, Figure 1), the structure of which was inspired from hydantocidin (**1**), a natural spiro compound with herbicidal and plant growth regulatory activity. A number of attempts to derivatize the initial structure in order to produce a more potent inhibitor led to other spiro-heterocycles with stronger affinity for GP.[6]

We present here a methodology, that involves a key [1,5]-radical translocation step, for the synthesis of anomeric spironucleosides **4a**,**b** (Figure 1), which were found to be modest inhibitors of rabbit muscle glycogen phosphorylase (RMGP*b*). Spirocyclic nucleosides present a rich synthetic

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chemistry and possess important biological function.[7,8] The targets in this paper contain a rare 1,6-dioxa-4-azaspiro[4.5]decane structure,[9] which has not been previously constructed *via* a radical pathway.[10] In the similar 1,6-dioxa-4-azaspiro[4.4]nonane system (**3**, Figure 1) we have reported an efficient protocol, utilizing a 6-lithiation strategy for generating a 6-hydroxymethyluridine intermediate followed by oxidative cyclization through a [1,5]-radical translocation strategy.[11,12] We were interested in applying this protocol in the related "decane" system.

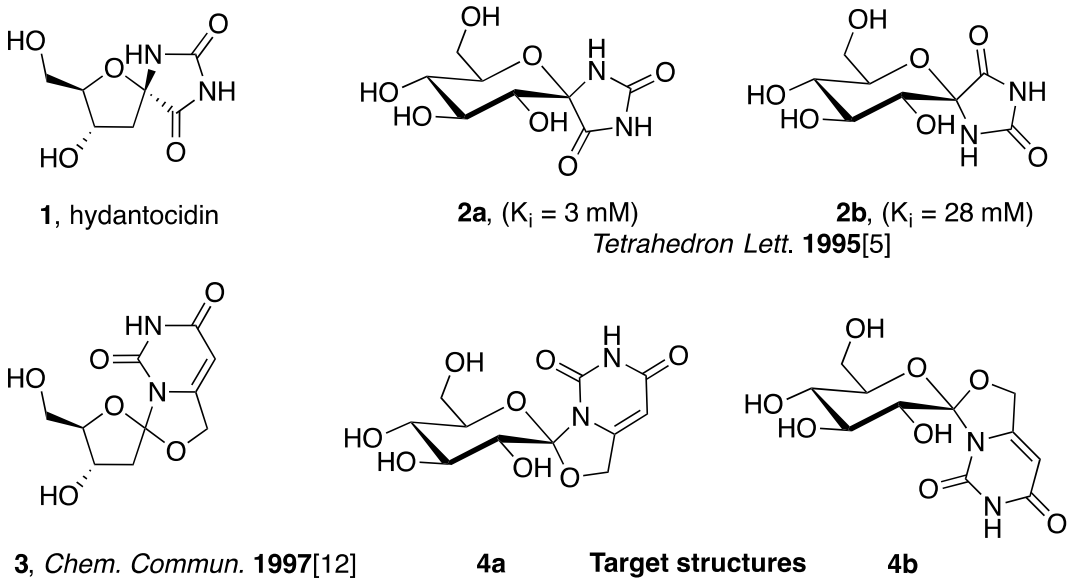

Figure 1. Structures associated with this study.

2. Results

2.1. Synthesis. Initially, we attempted to access compound **7** (Scheme 1) by direct lithiation of the known 2,3,4,6-tetra-*O*-benzyl-*β*-D-glucopyranosyluracil.[13] The protocol was based on the previous well-established 6-lithiation of protected 2-deoxy- and ribouridines followed by the reaction with dimethylformamide or ethyl formate to generate the corresponding 6-formyluridines.[11,14–16] Although a major product formed under these conditions, spectral analysis revealed that it was the product of a selective mono-debenzylation and did not contain a formyl group. Although we could not unequivocally determine the position of debenzylation, we hypothesized that this had occurred in position-3, proximal to the 6-position where the initial lithiation is expected to occur (data not shown). This reductive debenzylation could be reminiscent of a previous method employing lithium naphthalenide.[17]

The above result prompted us to change our strategy and include a previously reported[18] N^1-(β-D-glucopyranosyl)-6-methyluracil (**6a**) intermediate in our synthesis, as exemplified in Scheme 1. Under optimized conditions, the *N*-glycosylation reaction of persilylated 6-methyluracil, in the presence of an excess of TMSOTf in DCE, led to the formation of three products **6a-c**, isolated after column chromatography in 67, 30 and 3% yield, respectively. As determined by ESI-MS and NMR, the major product was the expected N^1-glycosylated 6-methyluridine **6a**, whereas the N^3-glycosylated analogue **6b** was isolated in 30 % yield, together with a small amount of N^1,N^3-bisglycosylated isomer **6c**.

The main feature that differentiated **6b** from **6a** in the ^{1}H NMR spectrum was a substantial downfield shift of H-2′ (δΔ = 0.8 ppm) in **6b**, induced by the magnetic anisotropy effect of the second vicinal amidic 4-carbonyl. A similar effect was observed in the more complex spectra of the bis-substituted analogue **6c**, and the $[M+H]^+$ peak at 786.3 amu (ESI-MS) clearly differentiated **6c** from the other two isomers ($[M+H]^+$ at 457.2 amu).

The allylic methyl group of **6a** was oxidized to the corresponding aldehyde **7** in the presence of selenium dioxide in dioxane : acetic acid,[19] in 67 % yield. Apart from the aldehyde **7**, isolated in 67% yield and recognized in ^{1}H NMR by it's characteristic aldehydic proton at 9.90 ppm, a second

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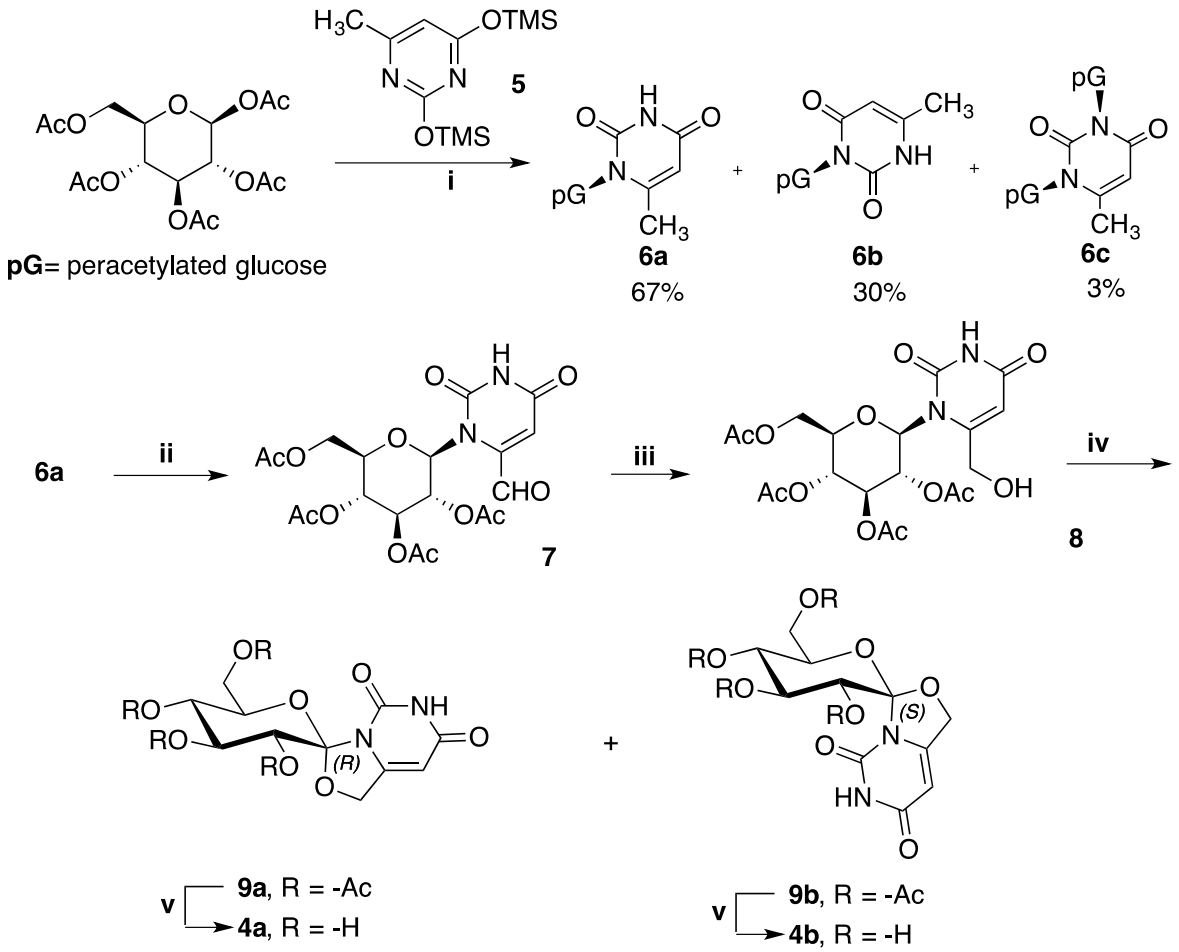
more polar product was isolated in low yield, identified as the allylic alcohol **8**. It's formation is expected by the mechanism of the reaction which follows an electrophilic allylic addition to selenium followed by a [2,3]-sigmatropic rearrangement.[20,21] Although we were unable to push the reaction to provide exclusively **8**, performing the reaction under strictly anhydrous conditions maximized the formation of aldehyde **7**. Reduction of aldehyde **7** with $NaBH_4$ in $CHCl_3$/isopropanol, in the presence of silica gel, at 0 °C[22] led to partial removal of acetate groups. By lowering the reaction temperature to -30 °C, exclusive formation of the allylic alcohol **8** was observed and the product was isolated in 90% yield.



Scheme 2. (i) TMSOTf, DCE, reflux, 1 h, 67%, (ii) SeO_2, dioxane, AcOH, reflux, 5 h, 67%, (iii) $NaBH_4$, silica gel, $CHCl_3$, propanol, -30 °C, 1 h, 90%, (iv) DIB, I_2, CH_2Cl_2, hv, r.t., 2.5 h, 18% (**9a**:**9b** = 1.25:1), and 50% (**7**), (v) NH_3 (7N in MeOH), r.t., 16 h, 100%.

The key step photolysis of **8**, under the standard Suárez conditions,[23] in the presence of DIB and I_2, in DCM and under visible light (150 W) irradiation, led to the isolation of three products in 50%, 10% and 8% yield. The major product was, surprisingly, the aldehyde **7**, whereas the two minor products corresponded to the expected isomeric spironucleosides **9a,b**. The reaction is expected to proceed through the generation of an alkoxyl radical intermediate, followed by a [1,5]-radical translocation[24] to generate a C-1′ radical intermediate which in turn after oxidation and anionic cyclization provides the spirocycles **9a,b**.[11,12] The main formation of aldehyde **7** can be explained by a possible disproportionation reaction of the above alkoxy radical intermediate to aldehyde **7** and alcohol **8**, with the latter entering back in the reaction cycle (see discussion below). This indicates that the rigidity of the β-D-glucopyranosyl ring makes the [1,5]-hydrogen atom transfer less favorable in this system than in the previously observed flexible ribosyl system.[11,12] Final removal

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of the acetate protection of **9a,b** with ammonia in methanol led to the isolation of the target spirocycles **4a,b** in quantitative yield.

2.2 Kinetic experiments. RMGP*b* was isolated, purified and recrystallized according to previously established protocols.[25] Compounds **4a,b** were assayed in the direction of glycogen synthesis for their inhibitory effect on RMGP*b* as described before.[25,26] They both exhibited competitive inhibition with respect to the substrate glucose-1-phosphate, at constant concentrations of glycogen (0.2% w/v) and AMP (1 mM). Compound **4b** was found to be a stronger inhibitor of RMGP*b* (35% inhibition at 1 mM) than **4a** (26% inhibition at 1 mM).

3. Discussion

The stereochemistry of the two spirocyclic products could be inferred unequivocally, from the ^{1}H NMR spectra, as can be seen in Figure 2. Both spectra contain features that can be explained by the magnetic anisotropy induced by the 2-C=O group onto the sugar α- or β- hydrogens depending on the stereochemistry of the new spiro-center. As the new spirocycle locks the configuration of the pyrimidine ring in respect to the sugar ring, the 2-C=O is spaced in the vicinity of H-2′ in the ⍲-anomer **9a** and on the other hand, in the vicinity of H-3′ and H-5′, in the *S*-anomer **9b**. This results in significant downfield shifts of the corresponding sugar Hs in the ^{1}H NMR spectra. Specifically, there is a 0.55 ppm shift of H-2′ going from the *S*- to ⍲-anomer (5.62 to 6.17 ppm), whereas there is a 0.48 and 0.84 ppm shift for H-5′ (4.18 to 4.65 ppm) and H-3′ (5.47 to 6.31 ppm), respectively, going from the ⍲- to the *S*-anomer (Figure 2). The remaining Hs (H-4′, H-6′, H-5 and H-7) have similar chemical shifts in the two spectra, with the difference that the pairs of H-6′ and H-7 protons appear as AB quartets in the case of the more congested *S*-anomer **9b**, whereas they collapse to singlets in the case of the *R*-anomer **9a**.

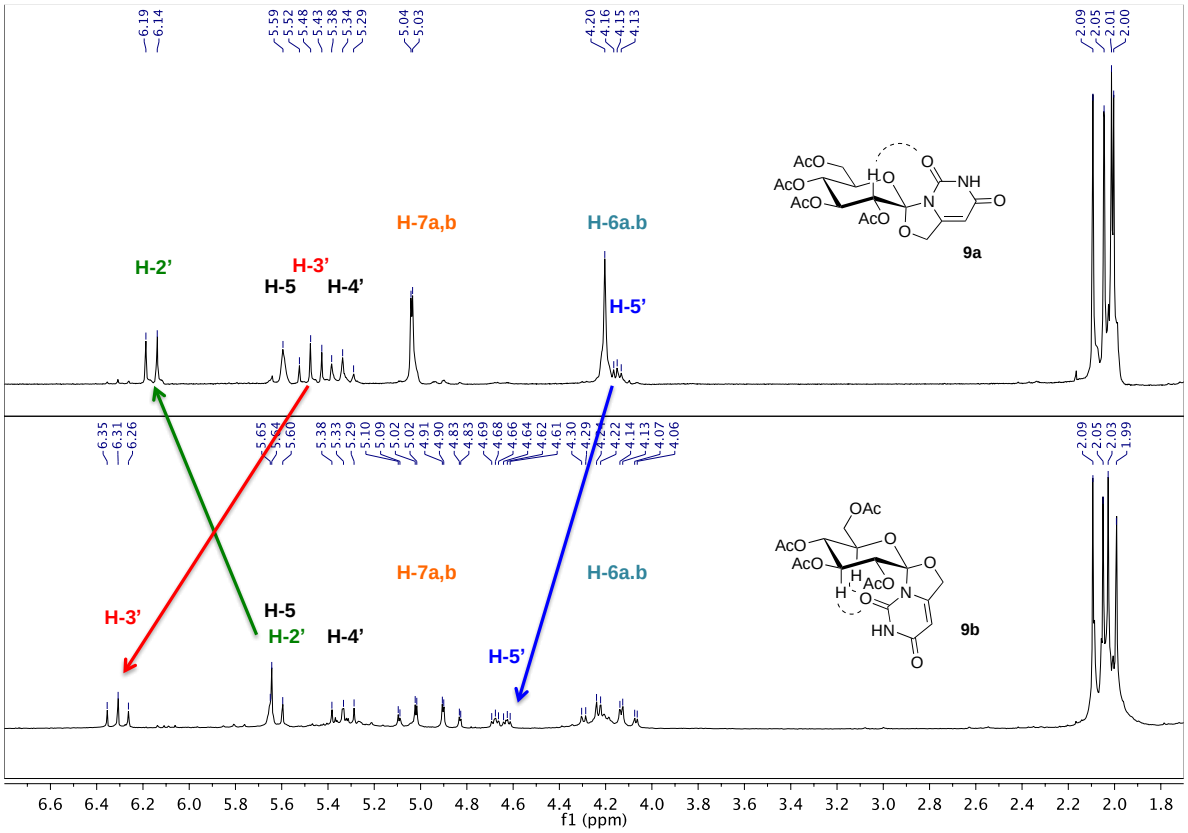

Figure 2. Comparison of ^{1}H NMR spectral shifts in compounds **9a**,**b**.

The same trends reported above for the protected derivatives were observed also in the case of the final compounds **4a**,**b**. Specifically, there was a 0.61 ppm shift of H-2′ going from the *S*- (**4b**) to *R*- (**4a**) anomer (3.95 to 4.56 ppm), whereas there is a 0.37 and 0.79 ppm shift for H-5′ (3.90 to 4.27 ppm) and H-3′ (3.83 to 4.62 ppm), respectively, going from the *R*- (**4a**) to the *S*- (**4b**) anomer. The remaining

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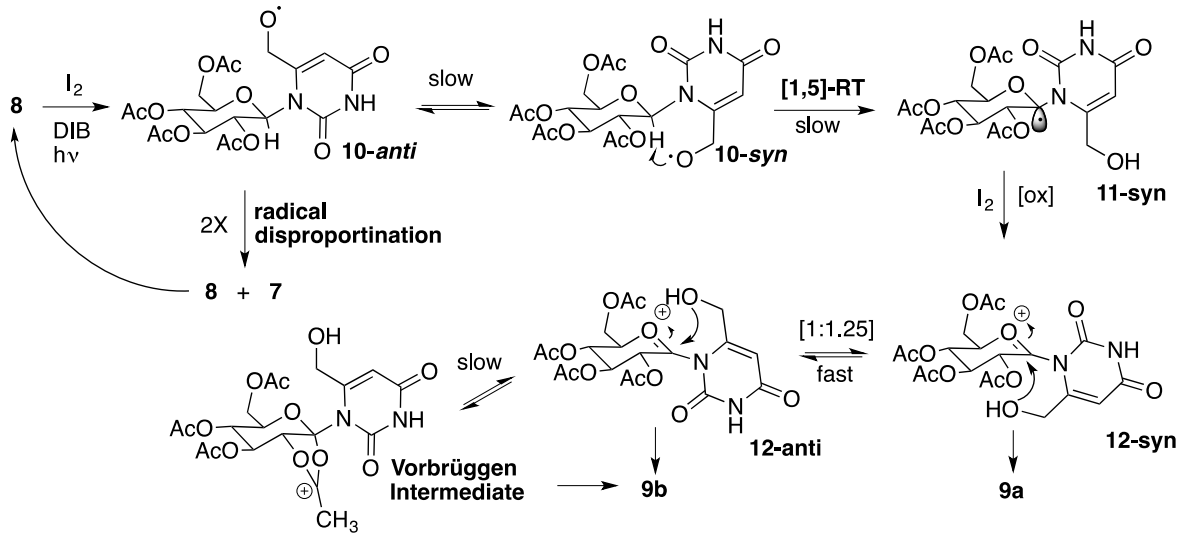
Hs (H-4′, H-6′, H-5 and H-7) had similar chemical shifts in the two spectra, with the difference, again in this case, that the pairs of H-6′ and H-7 protons appear as AB quartets in the case of the more congested *S*-anomer **4b**, whereas they collapse to singlets in the case of the *R*-anomer **4a**.

The above analysis allowed us to better interpret the ^{1}H NMR spectrum of compound **8** (in dmso-d6) which appeared as a mixture of tautomers, indicating a slow, in the NMR time scale, rotation around the glycosidic C$^{1'}$-N^1 bond, due to the new 6-hydroxymethyl substituent. The existence of tautomers was indicated by the presence of two amidic hydrogens at 11.48 and 11.32 ppm in a ~1:2 ratio. Two more characteristic low field signals were a doublet at 6.43 ppm and a triplet at 6.08 ppm exhibiting the same ~1:2 ratio. By applying the analysis above, performed for the final spirocyclic products, one can assign the doublet to the H-2′ of the conformer with the 2-C=O group in β-position and the triplet to the H-3′ of the conformer with the 2-C=O group in α-position. The observed ratio, at equilibrium, of the two conformers, becomes important in the next key step, as explained below.

Regarding the mechanism of the key step, it is expected that, under the Suárez conditions, an alkoxy radical intermediate is produced that may exist in two possible conformations (**10-syn**, **10-anti**, Scheme 2). These two conformers are similar to the ones observed above for alcohol **8** and are expected to be formed in a similar ~1:2 ratio (**10-syn**:**10-anti**). The rigidity of the pyranosyl chair conformation may not allow a fast interconversion between the conformers for steric reasons and the product distribution may be affected directly by these two conformer populations and their corresponding reactivity. Specifically, **10-anti** cannot undergo a [1,5]-hydrogen shift and an alternative [1,6]-hydrogen atom transfer from the 2-position of the sugar is known to be disfavored in the presence of acetyl protection.[23] The only available pathway for conformer **10-anti** is a radical disproportionation reaction leading to **7** and **8**, of which the latter enters back in the reaction cycle, while the former accumulates (Scheme 2).



Scheme 2. Proposed mechanism for the formation of **9a**,**b**.

On the other hand, conformer **10-syn** possesses a suitable conformation for a [1,5]-radical translocation leading to **11-syn** intermediate. After oxidation of **11-syn**, in the presence of I_2, the **12-syn** oxonium ion may exist in equilibrium with conformer **12-anti**, through rotation of the C$^{1'}$-N^1 bond, and also with a possible Vorbrüggen-type intermediate, formed through the anchimeric assistance of the 2′-acetyl group. The Vorbrüggen intermediate is the main species that determines the stereochemistry of the final product in *N*-glycosylation reactions,[27] and if the same was true in our system, exclusive formation of the *S*-anomer **9b** would be expected. Nevertheless, in our system a 1:1.25 *S*:*R* mixture of anomers **9b**:**9a** is obtained. This result allows to draw two major conclusions regarding the mechanism. First, rotation around the C$^{1'}$-N^1 bond in the oxonium ion **12-syn** to produce **12-anti** has to be faster than cyclization in order to allow the formation of the second, prior

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to cyclization, and there must be no major thermodynamic difference between the two conformers. Second, the formation of the Vorbrüggen intermediate must not be favored in this system, for steric reasons, and even if it is formed, through conformer **12-anti**, the process rate is comparable to that of cyclization of **12-syn** conformer to the observed *S*-anomer **9a** (Scheme 2).

Kinetics determined that the *S* anomeric spirocycle **4b** exhibited 1.25 times higher inhibition than the *R* anomer **4a**. The difference could be associated with the locked *syn* conformation of the pyrimidine ring with regard to the *β*-D-glucose moiety and possible unfavorable interactions of **4a** within the catalytic site between the backbone CO of His377 with the uracil 2-C=O, as has been observed previously with protein crystallography (unpublished results). Although we attempted to obtain X-ray crystallographic data by soaking crystals of RMGP*b* with either **4a** or **4b**, the rather low affinity of both spirocycles did not allow to collect sufficient data for establishing their binding in the catalytic site and studying their interactions. Both spironucleosides are stronger binders than the physiological inhibitors of GP, *β*- and *α*-D-glucose.[28] For example, **4b** is about 7 and 1.5 times stronger than *β*- and *α*-D-glucose, respectively. The new compounds, nevertheless, exhibit a rather low inhibition profile compared with the known spirohydantoin derivative of glucopyranose[5] and other known strong catalytic site inhibitors of RMGP*b*.[29,30] We have established previously that *anti* is the desirable conformation of the pyrimidine ring at the anomeric position of β-D-glucose leading to strong inhibition,[30] and the current results confirm this finding. Anomeric spironucleosides are rigid structures and given that they possess the correct conformation, they are expected to bind strongly to the catalytic site of GP. Our current studies are therefore directed towards anomeric spironucleosides with locked *anti* conformations and these results will be reported in due course.

4. Materials and Methods

All reagents and solvents were purchased from commercial sources and used without further purification, unless otherwise stated. All reactions were carried out under an argon atmosphere on a magnetic stirrer and monitored by thin-layer chromatography. Compounds were purified by flash chromatography on silica gel 40 – 60 μm, 60Å. NMR measurements were performed with a Varian Mercury 200 Nuclear Magnetic Resonance Spectrometer (at 200 MHz for ^{1}H and at 50 MHz for ^{13}C, respectively). The deuterated solvents used for NMR spectroscopy were $CDCl_3$ and D_2O. Chemical shifts are given in ppm and were referenced on residual solvent peaks for $CDCl_3$ (δ 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR), whereas for D_2O an external reference of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt was used. Coupling constants were measured in Hz. Hydrogen atom assignments, when given, are based on COSY spectra. Melting points were obtained by using a Gallenkamp Sanyo apparatus and are uncorrected. Mass Spectrometry experiments were carried out in a Thermo Finnigan Surveyor MSQ plus Mass Spectrometer, using the Electron Spray Ionization technique (ESI-MS). High-Resolution Mass Spectrometry experiments were carried out in a Q-TOF Bruker MaXis Impact HR-Mass Spectrometer. 1,2,3,5,6-penta-O-acetyl-*β*-glucopyranose was synthesized using standard synthetic protocols.[31] AMP, Glc-1-Phosphate (dipotassium salt) and oyster glycogen were obtained from Sigma-Aldrich and used without further purification. Oyster glycogen was freed of AMP according to Helmreich and Cori.[32]

2,4-di-(trimethylsilyloxy)-(6-methylpyrimidine) (5). A suspension of 6-methyluracil (1 g, 7.93 mmol), well grinded ammonium sulphate (80 mg, 0.60 mmol 0.076 eq) in HMDS (8.4 ml, 39.7 mmol, 5 eq) was heated to 120 °C under anhydrous conditions until full dissolution occurred. Upon completion, the excess of HMDS was removed through distillation, toluene was added twice (5 mL) followed by distillation to remove all traces of excess HMDS to yield 2.1 g (7.8 mmol, 97%) of the title compound which was characterized without further purification. **^{1}H NMR** (200 MHz, $CDCl_3$): δ = 0.34 (s, 18H), 1.95 (3H, s), 5.81 ppm (1H, s). **^{13}C NMR** (50 MHz, $CDCl_3$): δ = 0.00 (3C), 0.03 (3C), 23.4, 102.6, 162.6, 169.8, 170.0 ppm.

***N*-glycosylation of 6-methyuridine.** In a solution of **5** (1.5 g, 5.6 mmol, 1.5 eq) in dry 1,2-dichloroethane (7 ml) at r.t., a solution of TMSOTf (1.61 ml, 8.33 mmol, 2.25 eq) and 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose[31] (1.44 g, 3.7 mmol) in dry DCE (3.5 ml) was added. The reaction mixture

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was heated at reflux until full consumption of the sugar (~1 h). Then, the mixture was cooled, diluted with DCM and washed successively twice with saturated aq. $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, the solvents evaporated and the crude product was purified by column chromatography (30-70% Et_2O in EtOAc) to give, in order of elution, **6c** as a white solid (86 mg, 0.11 mmol, 3 %), **6a** as a white solid (1.13 g, 2.48 mmol, 67 %) and **6b** as a white solid (0.51 g, 1.11 mmol, 30 %).

1-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-methyluracil (6a): R_f = 0.40 (70:30 Et_2O:EtOAc). **^{1}H NMR (200 MHz, $CDCl_3$):** δ = 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.55 (s, 3H), 3.90 (bd, *J* = 9.5 Hz, 1H), 4.16 (dd, 1H, *J* = 12.2, 1.7 Hz), 4.27 (dd, 1H, *J* = 12.6, 4.2 Hz), 5.14 (t, 1H, *J* = 9.5 Hz), 5.35 (t, 1H, *J* = 9.5 Hz), 5.46 (t, 1H, *J* = 9.4 Hz), 5.57 (bs, 2H), 6.27 (d, 1H, *J* = 9.1 Hz), 8.62 (bs, 1H) ppm. **^{13}C NMR (50 MHz, $CDCl_3$):** δ = 170.7, 170.0, 169.8, 169.7, 162.7, 150.5, 139.3, 104.1, 80.5, 75.1, 72.8, 69.5, 67.9, 61.8, 20.97, 20.80 (2C), 20.78, 20.60 ppm. **HRMS (ESI):** calcd for $C_{19}H_{25}N_2O_{11}^+$ $[M+H]^+$ 457.1458 found 457.1465.

3-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-methyluracil (6b): R_f = 0.35 (70:30 Et_2O:EtOAc). **^{1}H NMR (200 MHz, $CDCl_3$):** δ = 9.70 (s, 1H), 6.18 (dd, *J* = 9.4, 8.4 Hz, 1H), 6.08 (d, *J* = 9.4 Hz, 1H), 5.52 (s, 1H), 5.30 (dd, *J* = 9.4, 8.6 Hz, 1H), 5.15 (t, *J* = 9.7 Hz, 1H), 4.30 – 4.10 (m, 2H), 3.84 (ddd, *J* = 10.1, 5.3, 2.5 Hz, 1H), 2.17 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.95 ppm (s, 3H). **^{13}C NMR (50 MHz, $CDCl_3$):** δ 170.5, 169.9, 169.6, 169.4, 162.5, 152.1, 151.5, 98.9, 78.3, 74.5, 73.7, 68.0, 67.9, 62.0, 20.6, 20.5 (2C), 20.4, 18.6 ppm. **ESI-MS:** calcd for $C_{19}H_{25}N_2O_{11}^+$ $[M+H]^+$ 457.1 found 457.2.

1,3-Bis-(tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-methyluracil (6c): R_f = 0.50 (70:30 Et_2O:EtOAc)**. ^{1}H NMR (200 MHz, $CDCl_3$):** δ = 6.34 (d, *J* = 9.9 Hz, 1H), 6.10 (d, *J* = 9.3 Hz, 1H), 6.02 (dd, *J* = 9.3, 9.3 Hz, 1H), 5.55 (s, 1H), 5.52 – 5.02 (m, 5H), 4.37 – 4.10 (m, 4H), 3.96 – 3.74 (m, 2H), 2.57 (s, 1H), 2.06 (s, 6H), 2.04 (s, 6H), 2.02 (s, 6H), 2.01 (s, 6H) ppm. **^{13}C NMR (50 MHz $CDCl_3$):** δ = 170.78, 170.60, 170.40, 170.20, 169.96, 169.62, 169.49, 169.40, 160.8, 152.9, 150.5, 103.4, 81.3, 79.2, 75.0, 74.7, 73.5, 73.3, 69.2, 68.1, 67.8, 67.5, 61.9, 61.4, 20.7 (3C), 20.6 (4C), 20.3, 20.0 ppm. **ESI-MS:** calcd for $C_{33}H_{43}N_2O_{20}^+$ $[M+H]^+$ 786.3 found 786.3.

1-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-formyluracil (7). In a solution of **6a** (2.1 g, 4.63 mmol) in dry dioxane (40 ml), selenium oxide was added (1.5 g, 13.9 mmol, 3 eq) and acetic acid (1.32 ml, 23.1 mmol). The reaction mixture was heated at reflux until full consumption of the starting material (~5 h). Then, the mixture was cooled, diluted with ethyl acetate and washed successively with saturated aq. $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, the solvents evaporated and the crude product was purified by column chromatography (97:3, EtOAC:Et_2O) to yield the title compound as a white solid (1.5 g, 3.1 mmol, 67%). R_f = 0.50 (100% EtOAc). **^{1}H NMR** (200 MHz, $CDCl_3$): δ = 9.90 (s, 1H), 8.53 (s, 1H), 6.28 (d, *J* = 2.3 Hz, 1H), 6.11 (d, *J* = 9.4 Hz, 1H), 5.61 (t, *J* = 9.3 Hz, 1H), 5.41 (t, *J* = 9.4 Hz, 1H), 5.24 (t, *J* = 9.7 Hz, 1H), 4.19 (m, 2H), 3.92 (ddd, *J* = 9.9, 3.3, 3.0 Hz, 1H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H) ppm. **^{13}C NMR (50 MHz, $CDCl_3$):** δ = 20.2, 20.4 (2C), 20.5, 61.0, 67.2, 70.3, 72.2, 74.9, 81.3, 110.0, 147.0, 150.2, 161.6, 169.4, 169.7, 170.0, 170.4, 183.4 ppm. **HRMS (ESI):** calcd for $C_{19}H_{23}N_2O_{12}^+$ $[M+H]^+$ 471.1251 found 471.1255.

1-(Tetra-*O*-acetyl-*β*-D-glucopyranosyl)-6-hydroxymethyluracil (8). In a solution of **7** (0.2 g, 0.425 mmol) in 2-propanol (2.5 ml) and chloroform (0.6 ml), dry silica gel was added (43 mg) and the suspension was cooled to -30 °C. Then, $NaBH_4$ (0.161 g, 4.25 mmol, 10 eq) was added and the reaction mixture was stirred until full consumption of the starting material (~1 h). Then, the mixture was diluted with DCM, filtered through Celite® and the filtrate washed successively with saturated aq. $NaHCO_3$ solution, water and saturated sodium chloride solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, the solvents were evaporated and the crude product was purified by column chromatography (EtOAc) to give the title compound as a white solid (146 mg, 0,38 mmol, 90%). R_f = 0.40 (70:30, Et_2O:EtOAc). **^{1}H NMR (200 MHz, DMSO-d6):** (mixture of tautomers) δ = 1.92 (s, 3H), 1.96 (s, 3H), 2.01 (s, 6H), 4.30 (m, 2H), 4.92 (t, *J* = 9.6 Hz, 1H), 5.26-5.62 (m, 2H), 5.69 (s, 1H), 5.84 (s, 2H), 6.08 (t, *J* = 9.0 Hz, 1H), 6.42 (d, *J* = 9.4 Hz, 1H), 11.32 ppm (s, 1H), 11.48 ppm (s, 1H). **^{13}C NMR (50 MHz, $CDCl_3$):** δ = 20.4, 20.5 (2C), 20.7, 60.1, 61.2, 67.5, 69.8, 72.6, 75.2, 81.5, 102.9, 151.3, 157.2, 162.8, 169.5, 169.8, 170.4, 170.7 ppm. **HRMS (ESI):** calcd for $C_{19}H_{25}N_2O_{12}^+$ $[M+H]^+$ 473.1407 found 473.1410.

Spirocyclization of 8. A solution of **8** (150 mg, 0.32 mmol) in dichloromethane (16 ml) was degassed by argon gas bubbling for 10 min. Then, diacetoxyiodobenzene (155 mg, 0.48 mmol, 1.5 eq)

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and iodine (91 mg, 0.32 mmol, 1 eq) were added. Photolysis was carried out at r.t., with two 75 W Philips Standard 230 V visible light lamps, for 2.5 h. Afterwards, the reaction was quenched by 10% aq. $Na_2S_2O_3$ solution and then extracted with dichloromethane. The organic layer was collected, dried over anhydrous Na_2SO_4, and then filtered, the solvent was evaporated and the crude product was purified by column chromatography (EtOAc: Et_2O gradient) to give, in order of elution, compound **7** (75 mg, 0.16 mmol, 50%), **9a** as a white solid, (12 mg, 0.026 mmol, 8%) and **9b** as a white solid (10 mg, 0.032 mmol, 10%).

(3*R*,3'*R*,4'*S*,5'*R*,6'*R*)-6'-(acetoxymethyl)-5,7-dioxo-1,3',4',5,5',6,6',7-octahydrospiro[oxazolo[3,4-*c*]pyrimidine-3,2'-pyran]-3',4',5'-triyl triacetate (9a): R_f = 0.60 (70:30 Et_2O:EtOAc). **^{1}H NMR (200 MHz, $CDCl_3$):** δ = 6.16 (d, *J* = 9.8 Hz, 1H), 5.59 (s, 1H), 5.48 (t, *J* = 10.0 Hz, 1H), 5.34 (t, *J* = 9.5 Hz, 1H), 5.04 (m, 2H), 4.20 (s, 2H), 4.17 – 4.10 (m, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H) ppm. **^{13}C NMR (50 MHz, $CDCl_3$):** δ = 170.7, 170.1, 169.2, 168.7, 163.1, 151.3, 145.7, 112.1, 93.8, 71.4, 71.3, 68.6, 68.2, 67.4, 61.2, 20.72, 20.62, 20.60 20.52 ppm. **HRMS (ESI):** calcd for $C_{19}H_{23}N_2O_{12}^+$ $[M+H]^+$ 471.1246 found 471.1239.

(3*S*,3'*R*,4'*S*,5'*R*,6'*R*)-6'-(acetoxymethyl)-5,7-dioxo-1,3',4',5,5',6,6',7-octahydrospiro[oxazolo[3,4-*c*]pyrimidine-3,2'-pyran]-3',4',5'-triyl triacetate (9b): R_f = 0.40 (70:30 Et_2O:EtOAc). **^{1}H NMR (200 MHz, $CDCl_3$):** δ 8.61 (s, 1H), 6.31 (t, *J* = 9.2 Hz, 1H), 5.65 (s, 2H), 5.62 (d, *J* = 9.3 Hz, 1H), 5.33 (dd, *J* = 10.1, 8.9 Hz, 1H), 5.06 (dd, *J* = 14.5, 1.6 Hz, 1H), 4.87 (dd, *J* = 14.5, 1.0 Hz, 1H), 4.65 (ddd, *J* = 9.6, 3.6, 2.5 Hz, 1H), 4.26 (dd, *J* = 12.7, 3.6 Hz, 1H), 4.10 (dd, *J* = 12.7, 2.5 Hz, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H) ppm. **^{13}C NMR (50 MHz, $CDCl_3$):** δ = 170.6, 170.0, 169.6, 169.1, 163.3, 152.7, 148.3, 114.5, 94.2, 72.6, 72.4, 71.0, 67.3, 67.2, 61.4, 20.7, 20.52 (2C), 20.4 ppm. **HRMS (ESI):** calcd for $C_{19}H_{23}N_2O_{12}^+$ $[M+H]^+$ 471.1246 found 471.1241.

(3*R*,3'*R*,4'*S*,5'*S*,6'*R*)-3',4',5'-trihydroxy-6'-(hydroxymethyl)-3',4',5',6'-tetrahydrospiro[oxazolo[3,4-c]pyrimidine-3,2'-pyran]-5,7(1H,6H)-dione (4a). A solution of **9a** (20 mg, 0.043 mmol) in methanolic ammonia (7 N, 0.35 ml) was stirred for 12 h at room temperature, until full conversion to a single compound. Then, the solvent was evaporated and the compound dried under high vacuum, to yield the title compound as a white solid (13 mg, 0.043 mmol, 100%). **^{1}H NMR (200 MHz, D_2O):** δ 5.88 (s, 1H), 5.20 (s, 2H), 4.56 (d, *J* = 9.7 Hz, 1H), 3.97 – 3.75 (m, 4H), 3.64 (t, *J* = 9.1 Hz, 1H) ppm. **^{13}C NMR (50 MHz, D_2O):** δ = 169.6, 157.1, 142.5, 116.8, 96.7, 78.3, 76.5, 72.5, 71.6, 70.8, 63.0 ppm. **HRMS (ESI):** calcd for $C_{11}H_{15}N_2O_8^+$ $[M+H]^+$ 303.0823 found 303.0830.

(3*S*,3'*R*,4'*S*,5'*S*,6'*R*)-3',4',5'-trihydroxy-6'-(hydroxymethyl)-3',4',5',6'-tetrahydrospiro[oxazolo[3,4-c]pyrimidine-3,2'-pyran]-5,7(1H,6H)-dione (4b). A solution of **9b** (29 mg, 0.062 mmol) in methanolic ammonia (7 N, 0.51 ml) was stirred for 12 h at room temperature, until full conversion to a single compound. Then, the solvent was evaporated and the compound was dried under high vaccuum to yield the title compound as a white solid (19 mg, 0.062 mmol, 100%). **^{1}H NMR (200 MHz, D_2O):** δ = 5.81 (s, 1H), 5.18 (d, *J* = 15.0 Hz, 1H), 5.08 (d, *J* = 14.9 Hz, 1H), 4.62 (t, *J* = 9.2 Hz, 2H), 4.26 (ddd, *J* = 10.1, 5.3, 2.2 Hz, 1H), 3.95 (d, *J* = 9.5 Hz, 2H), 3.85 (dd, *J* = 12.4, 2.1 Hz, 1H), 3.70 (dd, *J* = 12.5, 5.5 Hz, 2H), 3.58 (t, *J* = 9.6 Hz, 1H) ppm. **^{13}C NMR (50 MHz, D_2O)** δ = 169.5, 158.2, 149.4, 119.6, 96.4, 79.5 , 77.3, 76.2, 71.6, 70.2, 63.4 ppm. **HRMS (ESI):** calcd for $C_{11}H_{15}N_2O_8^+$ $[M+H]^+$ 303.0823 found 303.0828.

5. Conclusions

In conclusion, we have successfully applied the Suárez radical methodology to synthesize the first example of a 1,6-dioxa-4-azaspiro[4.5]decane system starting from 6-hydroxymethyl-β-D-glucopyranosyluracil. We have shown that in the rigid pyranosyl conformation the required [1,5]-radical translocation is a minor process. The stereochemistry of the spirocycles obtained was unequivocally determined by the chemical shifts of key sugar protons in the ^{1}H NMR spectra. Finally, the two spirocycles were found to be modest inhibitors of RMGP*b*, corroborating the finding that *anti* should be the desirable conformation of the pyrimidine ring of future anomeric spironucleosides that may lead to strong inhibition of GP.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, ^{1}H and ^{13}C NMR spectra of new compounds (Figures S1-S10). Tables of kinetic measurements (Tables S1-S2).

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