Synthesis of *N*-Glucopyranosidic Derivatives as Potential Inhibitors that Bind at the Catalytic Site of Glycogen Phosphorylase

T. Gimisis*

Organic Chemistry Laboratory, Department of Chemistry, School of Sciences, National and Kapodistrian University of Athens, 15771 Panepistimiopolis, Athens, Greece

Abstract: Glycogen phosphorylase (GP) is a promising molecular target for the treatment of Type 2 diabetes. The design of potential inhibitors for the catalytic site of the enzyme is based on the high affinity for β-D-glucopyranose and the presence of a β-cavity that extends from the sugar anomeric position forming a 15 x 7.5 x 10 Å available space. This review is focused on our efforts towards the design and synthesis of various families of potential inhibitors, including *N*-β-D-glucopyranosyl oxamic acid esters and oxamides, *N*-β-D-glucopyranosylaminocarbonyl L-aminoacids and peptides, as well as glucose-derived purine and pyrimidine nucleosides, spiro- and other bicyclic derivatives. Kinetic and crystallographic study of the interactions of these inhibitors with GP has increased our understanding of the importance of the various functional groups within the catalytic site and has pointed the way towards the *in silico* prediction and design of potent inhibitors, which are both synthetically viable and pharmacologically relevant.

Keywords: *N*-β-D-glucopyranosyl oxamic acid esters and oxamides, *N*-β-D-glucopyranosylaminocarbonyl-L-aminoacids, purine and pyrimidine nucleosides, glycogen phosphorylase, synthetic inhibitors.

Dedicated to the memory of Dr. Nikos G. Oikonomakos.

INTRODUCTION

Recent information on protein-carbohydrate interactions in physiological and pathological conditions substantiates the intuitive belief in carbohydrates as candidates for drug design. In the case of Type 2 diabetes (T2D), the main regulatory enzyme of glycogenolysis is glycogen phosphorylase (GP), which catalyzes the breakdown of glycogen to glucose-1-phosphate (G-1-P) to be eventually converted to glucose. GP is a promising therapeutic target for the treatment of T2D, as inhibition of hepatic GP could suppress glucose production.

The reader can find detailed information regarding the structure and function of GP, as well as the differences between hepatic and muscle GP, in the adjacent articles of this special issue of MRMC and in recent reviews that deal with the synthesis of potential inhibitors of GP [1,2]. Our interest in the chemistry of nucleosides [3-7] has drawn our attention to the synthesis of potential inhibitors of GP (GPIs) with emphasis on derivatives of β-D-glucopyranose, suitable for binding at the catalytic site of the enzyme. The catalytic site, buried in the center of the GP subunit in order to be protected from the aqueous environment and promote phosphorolysis, contains the essential cofactor pyridoxal-5'-phosphate (PLP), covalently bonded as a Schiff base to the ε-NH_2 of a lysine residue. In the less active T state, which is promoted by GPIs through stabilization of the closed

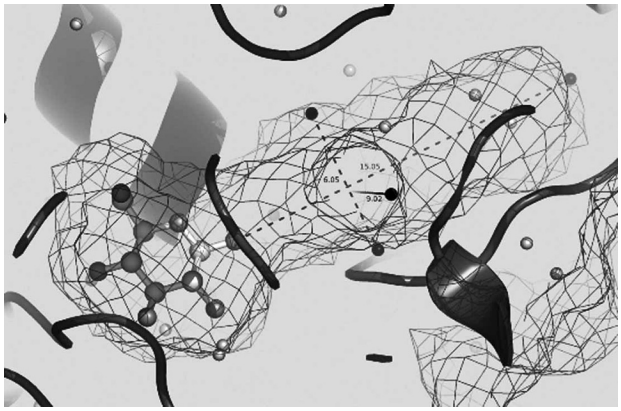

Fig. (1). Approximate dimensions of the β-cavity in the catalytic site of GPb. There are 18 crystallographic waters at r.t. (35 at 100 K) within the catalytic site [8].

position of the 280s loop, the catalytic site can accommodate, apart from glucose, β-substituents at the anomeric position of the sugar that extend towards a catalytic subsite called the β-cavity, an empty space approximately 15 Å in length, 6 Å wide and 9 Å in height (Fig. **1**). This long and narrow cavity is lined with both polar and non-polar groups. Above the anomeric carbon of glucose and in close proximity lies the main chain carbonyl of His377, which adds a constraint and a potential hydrogen bond interaction to be established with the atoms proximal to the anomeric position of the GPI. The interest of all recent work on catalytic site GPIs has been to identify the functional groups that enhance binding beyond the well-established contacts of glucose. We present herein a mini-review on the synthesis of open chain

*Address correspondence to this author at the Organic Chemistry Laboratory, Department of Chemistry, School of Sciences, National and Kapodistrian University of Athens, 15771 Panepistimiopolis, Athens, Greece; Tel: 30210 727 4928; Fax: +30210 727 4761;
E-mail: gimisis@chem.uoa.gr

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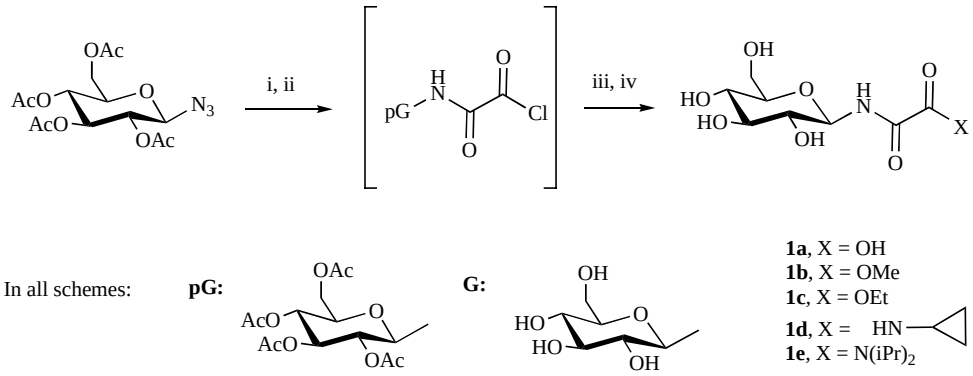


Scheme 1. (i) H_2, Pd/C, THF, hexane, (ii) filtration, excess ClCOCOCl, (iii) evaporation, NuH, (iv) ROLi, ROH. Tsipos, P.; Gimisis, T. *unpublished results.*

and cyclic β-D-glucopyranosyl derivatives that we have investigated over the last five years during our collaboration with the group of the late Dr N. Oikonomakos, as well as the understanding we have gained through the QSAR studies performed.

OPEN CHAIN ANALOGUES

N-β-D-Glucopyranosyl Oxamic Acid, its Esters and Oxamides

In our earliest work, *N*-β-D-glucopyranosyl oxamic acid, its two esters and two oxamides were prepared, through a three-step, one-pot process, outlined in Scheme **1**. The method entailed hydrogenolysis of protected β-D-glucopyranosyl-azide to the corresponding amine, which was added *in situ* to an excess of oxalyl chloride. After the amine had reacted, evaporation of the excess of oxalyl chloride and quenching the intermediary chloride with *O* and *N* nucleophiles provided the protected target compounds. Deprotection with a suitable lithium alkoxide yielded compounds **1a-e** [9].

Compounds **1a-d** proved to be competitive inhibitors of rabbit muscle GPb (with respect to α-D-glucose-1-phosphate) with K_i values between 0.2–1.4 mM [10]. These substrates were extended to aromatic oxamides, in a related work, of which the 2-naphthyl derivative exhibited low micromolar activity [2].

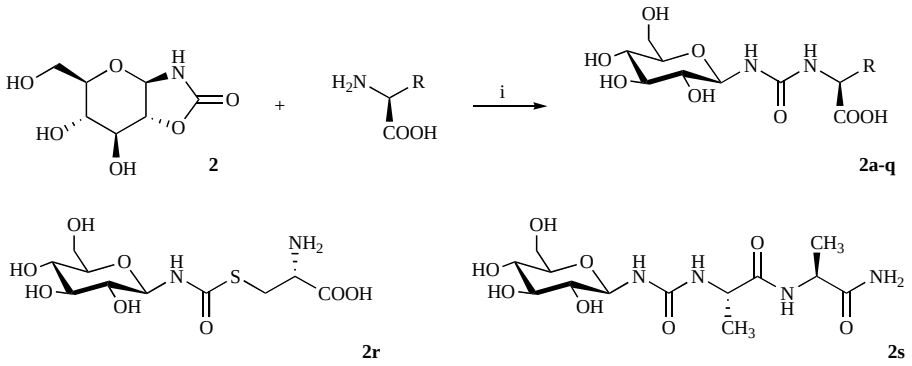
N-β-D-Glucopyranosylaminocarbonyl L-Aminoacids

There is a number of *N*-acyl-*N*'-β-D-glucopyranosyl ureas that have been synthesized as potential inhibitors of GPb. From these studies, it was concluded that aromatic groups directed within a specific area of the β-cavity could significantly increase the inhibitory effect of the compound, bringing the K_i values below the one-micromolar range [2].

The aim of a recent study [11] was the development of a simple methodology that would permit, through a key "spring-loaded" intermediate, access to a large number of *N*-(β-D-glucopyranosyl)-urea derivatives. The intermediate should be easily prepared and stable, exert a high and general reactivity and lead to a fast and quantitative isolation of the potential inhibitors. These characteristics fall under the definition of "click" reactions as given by K. B. Sharpless [12]: "an expanding set of powerful, selective, and modular 'blocks' that work reliably in both small- and large-scale applications to generate substances by joining small units together with heteroatom links (C-X-C)".

Such a "spring-loaded" intermediate is Steyermark's oxazolidinone (**2**, Scheme **2**), discovered in 1962 [13]. Its synthesis was optimized by I. Pinter in 1995 [14], and its synthetic potential was only recently revealed by Y. Ichikawa *et al.* [15]. In this last paper, the authors prepared urea-tethered neoglucoconjugates and pseudo-oligosaccharides in water by the reaction of simple amines with **2** at temperatures up to 70 ^{o}C. The *trans*-diequatorial fusion of the oxazolidinone ring in **2** is responsible for the observed reactivity and resembles a spring-loaded system, which is ready to "click". An additional important feature of this chemistry is the utilization of the unprotected, free sugar that leads directly to the water-soluble final targets in one key-step.

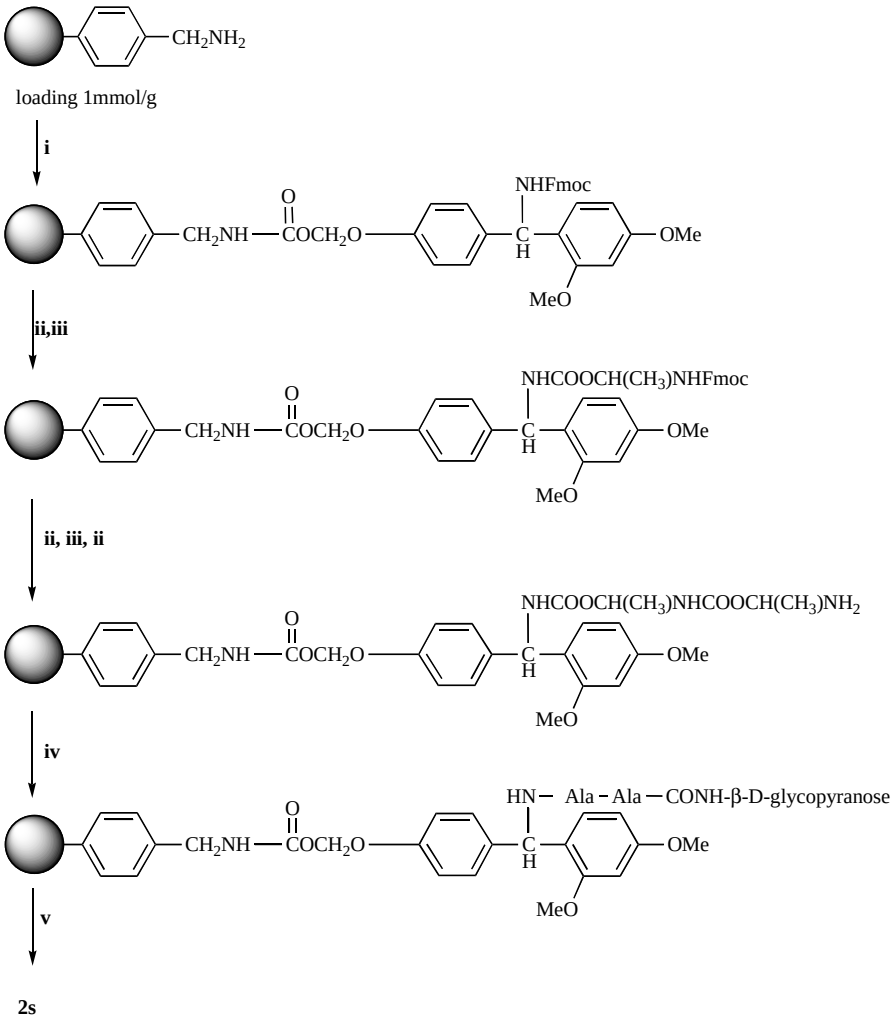
This type of "click" chemistry was applied to the synthesis of *N*-β-D-glucopyranosylaminocarbonyl L-amino acids as potential inhibitors of GPb with the aim of expanding the methodology to dipeptide and tripeptide urea-tethered neoglucoconjugates that would allow combinatorial access to libraries of potential inhibitors with members ranging from up to 20 for natural L-aminoacids, up to 400 for dipeptide- and up to 8000 possible tripeptide conjugates providing a large structural variety of targets. As a proof of concept, 18 urea-tethered *N*-β-D-glucopyranosyl L-amino acids were prepared by the above methodology in solution as well as a dipeptide (Ala-Ala) conjugate by solid-phase synthesis [11]. In this study, it was found that addition of a stoichiometric quantity of triethylamine was necessary for the reaction to occur readily at room temperature. By monitoring the reaction by NMR, it was proven that triethylamine opens the oxazolidinone ring providing a reactive cationic intermediate that reacts readily with all L-amino acids, used in this study, to provide the final products quantitatively as triethylamine salts. The products were purified by reverse phase HPLC and characterized by NMR. It was found that, with the exception of cysteine, they all corresponded to the expected *N*-(β-D-glucopyranosylaminocarbonyl)-L-amino acids **2a-q** (Scheme **2**, Table **1**). In the case of cysteine, the more nucleophilic thiol reacted chemoselectively with the oxazolidinone to provide *S*-β-D-glucopyranosylaminocarbonyl L-cysteine **2r**.



Scheme 2. (i) Et_3N, H_2O, r.t., 1 h, quant.
Stathi, A.; Chegazi, M.; Gimisis, T.; *unpublished results.*

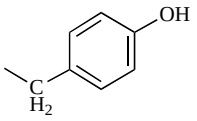
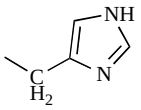
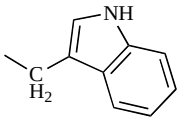
The urea-tethered Ala-Ala dipeptide conjugate **2s** was prepared by a standard solid-phase synthesis on an aminomethyl resin [16,17] with a Rink amide handled by utilizing the Fmoc strategy (Scheme **3**) [18]. The free amine of the dipeptide, bound on the solid phase, reacted readily with **2** in DMF (necessary for efficient swelling of the resin) and the final product was isolated after acid treatment and filtration of the released resin in quantitative yield and high purity.

The results from the crystallographic and kinetic studies are summarized in Table **1**. The best inhibitory activity was exhibited by the alanine and phenylalanine urea-tethered



Scheme 3. (i) Rink amide, HOBt, DIC, DMF (ii) 20% piperidine in DMF (iii) Fmoc-Ala-OH, HOBt, DIC, DMF (iv) **2**, DMF (v) $TFA/H_2O/TPS/CH_2Cl_2$.

Table 1. Crystallographic and Kinetic Results for Compounds 2a-s[a]

Amino acid/peptide in compounds 2a-s	R	Density in the catalytic site	Inhibition	K_i (mM)
Gly (2a)	–H	Yes		2.10 ±0.02
Ala (2b)	$-CH_3$	Yes		0.51 ± 0.01
Val (2c)	$-CH(CH_3)_2$	Yes		1.45 ± 0.17
Pro (2d)	$-(CH_2)_2-$	Yes		1.60 ± 0.31
Ile (2e)	$-CH(CH_3)CH_2CH_3$	Yes	11.8 % at 4 mM	
Leu (2f)	$-CH_2CH(CH_3)_2$	Partial	No inh. at 1 mM	
Thr (2g)	$-CH(CH_3)OH$	Yes		2.10 ± 0.96
Ser (2h)	$-CH_2OH$	Yes		2.30 ± 0.11
Met (2i)	$-(CH_2)_2SCH_3$	Yes		1.20 ± 0.14
Phe (2j)	$-CH_2Ph$	Partial		0.35 ± 0.04
Tyr (2k)		Partial	6.3 % at 1 mM	
Asp (2l)	$-CH_2COOH$	Partial	No inh. at 1 mM	
Asn (2m)	$-CH_2CONH_2$	Partial	No inh. at 1 mM	
Glu (2n)	$-(CH_2)_2CO_2H$	Partial	No inh. at 1 mM	
Gln (2o)	$-(CH_2)_2CONH_2$	Partial	No inh. at 1 mM	
His (2p)		No	No inh. at 1 mM	
Trp (2q)		No		1.86 ± 0.02
Cys	2r	Partial	No inh. at 1 mM	
AlaAla	2s	No	11.0 % at 1 mM	

[a]Mamais, M,; Sovantzis, D.; Gimisis, T.; Chrysina, E.; *unpublished results.*

neoglucoconjugates. Most of the other neutral-sidechain aminoacids gave mM inhibition constants, whereas most acidic- or basic-sidechain aminoacids (with the exception of tryptophane) gave no inhibition. Finally the dipeptide derivative **2s** exhibited diminished inhibition when compared with the corresponding single-alanine conjugate. It was concluded that the β-channel, that extends beyond the urea moiety, may not support well aliphatic groups and especially polar groups on saturated aliphatic chains.

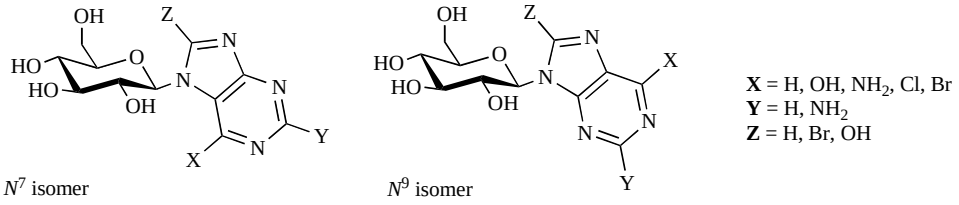
CYCLIC DERIVATIVES

β-D-Glucopyranosyl Purine Nucleosides and Derivatives

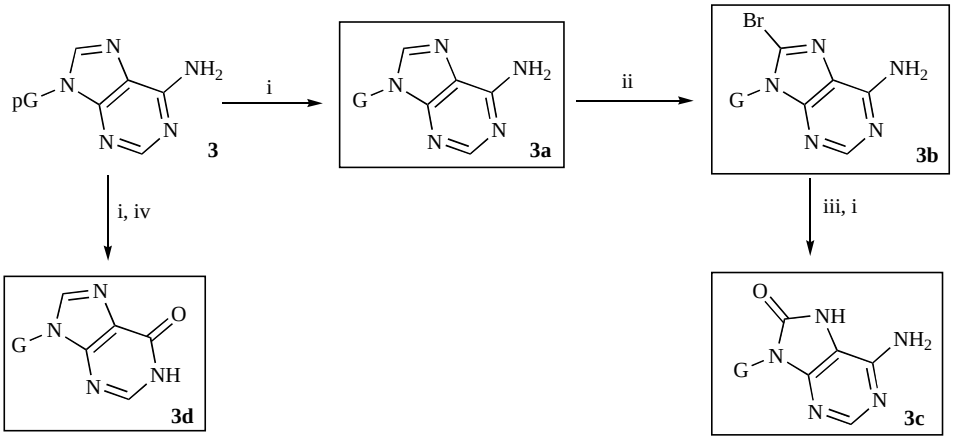
Purines are known to bind in the inhibitor or caffeine-binding site, located on the surface of the enzyme, forming π-π stacks between the aromatic side chains of Tyr613 and Phe285 [1,2]. We were interested in studying the effect of adding a β-D-glucopyranose at the 7- or 9-position of substituted purines and specifically monitoring their ability to enter into the catalytic site as a function of their substitution pattern and the effect that this has on their inhibition constants. Therefore, several 7- or 9-(β-D-glucopyranosyl)-purines have been prepared with the purpose of testing them as potential inhibitors of GPb. They can be described by the general formulae in Scheme **4** [19].

I. Adenine (3a, X = NH_2, Y = Z = H), 8-bromoadenine (3b, X = NH_2, Y = H, Z = Br), 8-oxoadenine (3c, X = NH_2, Y = H, Z = OH), and inosine (3d, X = OH, Y = Z = H)

The regiospecific synthesis of the known 9-(β-D-glucopyranosyl)-adenine **3** [20,21] was effected by applying the Saneyoshi conditions [22] on 1,2,3,4,6-penta-*O*-acetyl-β-



Scheme 4. General structures of 7- or 9-(β-D-glucopyranosyl)-purines synthesized.



Scheme 5. (i) 7 N NH_3 in MeOH, r.t., 92 – 97 % (ii) Br_2, aq. AcONa, pH = 5, 50 °C, 3 h, 29 % (iii) Ac_2O, AcOH, 80 °C, 4h, 95 %, (iv) $NaNO_2$, AcOH, r.t., 2.5 h, 55 %.

Pantzou, A.; Cismas, C.; Gimisis, T.; *unpublished results.*

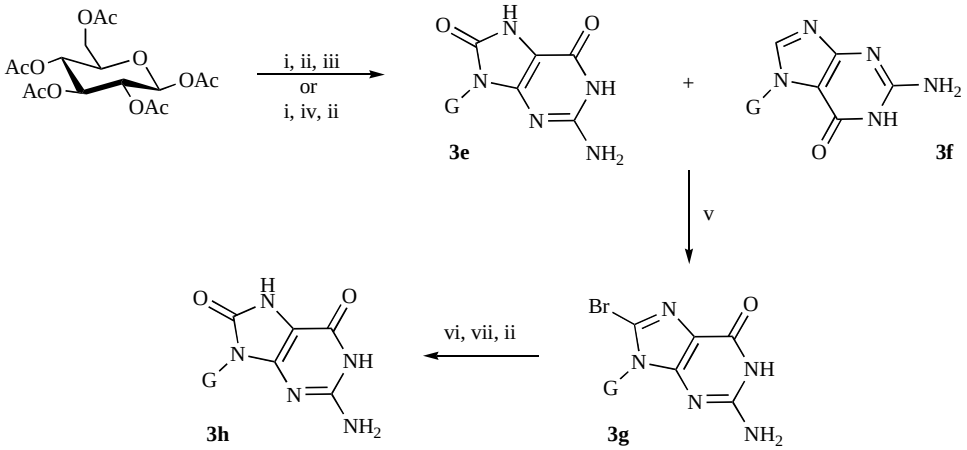
D-glucose. Under these conditions, the free adenine base provided protected 9-(β-D-glucopyranosyl)-adenine, with complete regio-selectivity, in high yield. Standard deprotection provided the free nucleoside **3a**, which was brominated [23,24] to the new 8-bromoderivative **3b**. Acetolysis [25] converted the latter intermediate to the fully protected 8-oxoderivative, which gave the new 8-oxoadenine-nucleoside **3c**, upon methanolysis (Scheme **5**). In a parallel route, oxidative deamination of protected 9-(β-D-glucopyranosyl)-adenine was effected through the acetolysis of a diazonium intermediate to provide the known [24] 9-(β-D-glucopyranosyl)-hypoxanthine (**3d**).

II. Guanine (3e, 3f, X = OH, Y = NH_2, Z = H), 8-bromoguanine (3g, X = OH, Y = NH_2, Z = Br) and 8-oxoguanine (3h, X = OH, Y = NH_2, Z = OH)

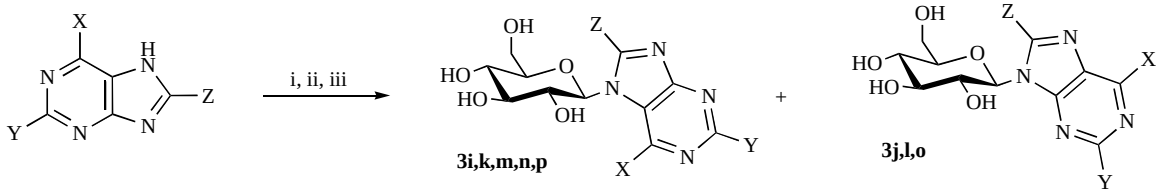
The synthesis of the known 9- and 7-(β-D-glucopyranosyl)-guanine **3e** and **3f** [26,27], respectively, is described in Scheme **6**. We have employed a Vorbrüggen *N*-glycosylation of pentaacetylated glucose, followed by deprotection of the inseparable 3:1 mixture of 9- and 7-isomers. Fractional crystallization from water following Vorbrüggen's work on the ribo-analogues [28], separated the least soluble N^7- from the N^9-isomer. When 1-acetyl-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranose was utilized following the work of Garner [26], we failed to obtain a better than 3:1 N^9:N^7 regioisomeric ratio. On the other hand employment of the Robin reagent, N^2-acetyl-O^6-diphenylcarbamoylguanine [27], provided exclusively the N^9-isomer **3e**, albeit in low yield. Nevertheless, the diphenylcarbamoyl protection of O^6 rendered the mixture of regioisomers separable and this method was used to obtain the protected glucoguanines in pure form (Scheme **6**). Bromination of **3e** in water provided the new 8-bromo derivative **3g** in good yield. It was found that the N^7-isomer was unreactive under these conditions and thus we employed the conditions directly on the mixture of regioisomers, to obtain, after precipitation, **3g** in pure form. Finally, acetyl protection, followed by acetolysis of the bromide and removal of the acetates, yielded the new 8-oxoderivative **3h**.

III. Purine (3i, 3j, X = Y = Z = H), 6-chloropurine (3k, 3l, X = Cl, Y = Z = H), 6-bromopurine (3m, X = Br, Y = Z = H), 2-amino-6-chloropurine (3n, 3o, X = Cl, Y = NH_2, Z = H) and 2,6-diaminopurine (3p, X = Y = NH_2, Z = H)

The synthesis of these substituted purines was based on the same three-step sequence, starting with trimethylsilylation of the base, *N*-glycosylation in the presence of TMSOTf, separation of the regioisomers formed and deprotection of the acetyl groups (Scheme **7**). Acetylation of 2,6-diaminopurine was necessary before silylation, but 2-amino-6-chloropurine was directly silylated so that the sugar acetyl protection could be removed at low temperature, in order to preserve the 6-chloro substituent. Similarly, low temperature was also used for the formation of **3k-m**. The *N*-glycosylation of purine gave a mixture of N^9:N^7 regioisomers in a 3:1 ratio, whereas the analogous reaction of 6-chloropurine gave a 4:1 ratio and that of 2-amino-6-chloropurine a 2.5:1 ratio, respectively. In the reaction of 6-bromopurine, or 2,6-diacetamidopurine, the N^9 regioisomer is obtained exclusively. The spectral characterization and identification of the two regioisomers is based on the empiri-



Scheme 6. (i) Trimethylsilylated N^2-acetylguanine [29], TMSOTf, DCE, 80 °C, 2.5 h, 55 % (**3e**:**3f** 3:1). (ii) 7 N NH_3, MeOH, overnight, quant. (iii) fractional crystallization from H_2O. (iv) diphenylcarbamoyl chloride, DIEA, DMAP, pyridine, r.t., 2.5 h, **3e**, 66 %, **3f**, 22 %. (v) Br_2, H_2O, r.t., 85 %. (vi) Ac_2O, Et_3N, DMAP, MeCN, 45 °C, 6 h, 74 %. (vii) AcOH, AcONa, reflux, 24 h, 60 %.
Cismas, C.; Pantzou, A.; Stathis, D.; Gimisis, T.; *unpublished results.*



Scheme 7. (i) BSA, DCE, 15 min, 80 °C. (ii) pG-OAc, TMSOTf, DCE, 70 °C. (iii) 7 N NH_3, MeOH, -5 °C, overnight.
Pantzou, A.; Gimisis, T.; *unpublished results.*

cal rules developed by J. Kjeillberg and N.G. Johansson [30].

A summary of the results from the crystallographic and kinetic studies is presented in Table **2**. Density for each inhibitor **3a-p** was found in the inhibitor site of the enzyme. Nevertheless, only a small number of them appeared to bind within the catalytic site. Those inhibitors exhibited a stronger binding affinity, with the best K_i being that of 7-(β-D-glucopranosyl)-guanine (K_i = 170 μM). It is noteworthy that only the 7-isomer of glucoguanine could enter the catalytic site, whereas no density was found in the catalytic site for the 9-isomer, which at the same time exhibited very low inhibition (13 % at 1 mM). We could conclude that of the 9-isomers, only those with substitution at the 6-position could enter the catalytic site, whereas substitution at position-2 and position-8 prevented the substituted purines from entering the catalytic site.

β-D-Glucopyranosyl Pyrimidine Nucleosides and Derivatives

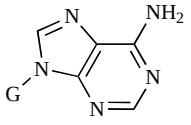
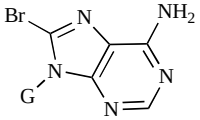
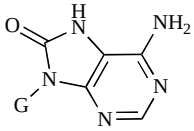
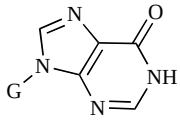
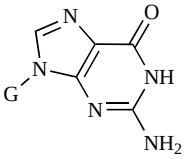
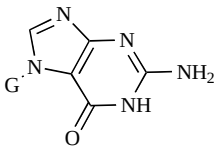
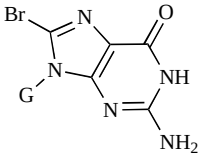
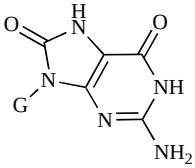
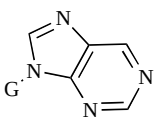
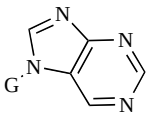
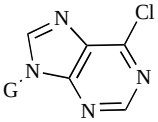
I. Simple Pyrimidines

The class of β-D-glucopyranosyl pyrimidine nucleosides (**4a-f**, Scheme **8**) was recently identified as competitive inhibitors of GPb [31]. They possess the motif of *N*-acyl-*N'*-β-D-glucopyranosyl urea, but in this case the urea is part of a pyrimidine ring.

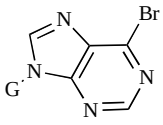
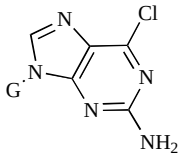
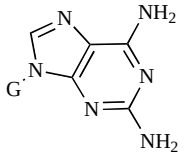
The syntheses of derivatives **4a** [32], **4b** [33,34], **4c** [35-37], **4d** [33] and **4e** [38,39] were relatively straightforward (Scheme **8**) and have been already reported. We have utilized slight modifications [40-42] of the original TMSOTf-catalyzed, Vorbrüggen *N*-glycosylation [28,43,44]. Compound **4f**, for which only *N*-methylated derivatives have been reported [45], was prepared by a modification [46] of the reported procedure. Specifically, in order to avoid the formation of a bis-substituted product the reaction was performed at ambient temperature, with $SnCl_4$ as catalyst and using an increased excess of silylated base. The silylated bases were synthesized by an original procedure that allows monitoring of the number of introduced silyl groups. Deprotection in the presence of methanolic ammonia at room temperature afforded the corresponding deprotected derivatives **4a-f** in quantitative yields [31]. The structural assignment of the compounds was based on ^1H and ^{13}C NMR, as well as ESI-MS, comparison with published data and finally, on the crystallographic data obtained from the complexes of these inhibitors in the catalytic site of GPb.

As shown in Table **3** [47], compounds **4a-d** exhibited low micromolar inhibition constants, with the best inhibitors being **4b** and **4d**. Substitution at C-5 (H → CH_3, **4a**) or C-4 (O → NH_2, **4c**) did not affect the activity, whereas substitution of C-6 (C → N, **4e**) or at C-6 (H → O, **4f**) led to inhibitors from 16, to more than 200 times weaker, respectively. This fact was attributed to the close proximity of the carbonyl of His377 to the C-6 center, an association that has been shown in many cases to be critical. It is interesting to compare the above results with those of a recently reported related family of compounds [48], where the 3'-hydroxyl

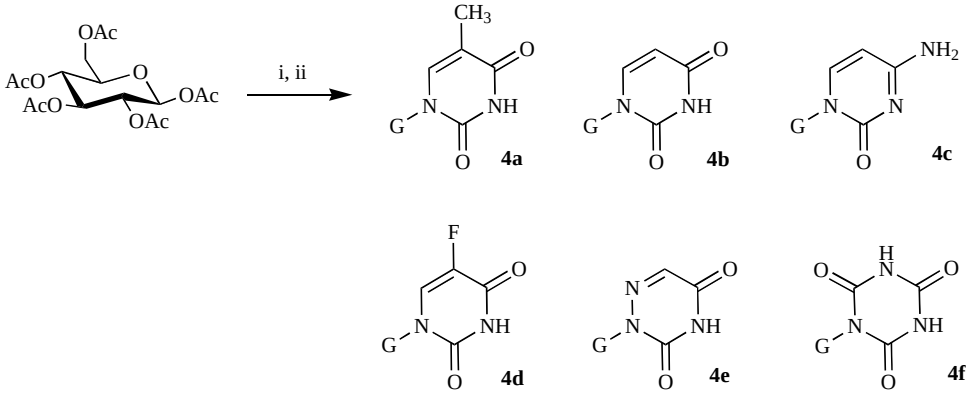
Table 2. Crystallographic and Kinetic Results for Compounds 3a-p[a]

Compound	Structure	Density in the catalytic site	Density in the inhibitor site	K_i (mM)
3a		Yes	Yes	0.31 ± 0.02
3b		No	Yes	N.D.[1]
3c		No	Yes	N.D.[1]
3d		Yes	Yes	N.D.[1]
3e		No	Yes	13 % at 1
3f		Yes	Yes	0.17 ± 0.05
3g		No	Yes	2.7 % at 1
3h		Partial	Yes	0.73 ± 0.04
3i		Yes	Yes	1.87 ± 0.09
3j		Yes	Yes	1.87 ± 0.11
3k		Partial	Yes	0.76 ± 0.05

(Table 2). Contd…..

Compound	Structure	Density in the catalytic site	Density in the inhibitor site	K_i (mM)
3m		N.D.		0.52 ± 0.05
3n		Partial	Yes	0.85 ± 0.09
3p		No	Partial	13 % at 2

[1]Not determined.
[a]Chegazi, M.; Sovantzis, D.; Hadjiloi, D.; Pantzou, A.; Cismas, C.; Gimisis, T. Chrysina, E.; *unpublished results.*



Scheme 8. (i) trimethylsilylated base, 2.3 eq. TMSOTf, DCE, reflux, 2.5 - 3.5 h, 60 - 97 % (for **4f**: 1.2 eq. $SnCl_4$, DCE, *r.t.,* 2.5 h, 56 %). (ii) 7 N NH_3 in CH_3OH, r.t., quantitative.

group has been replaced by a fluorine atom. This substitution caused a dramatic decrease by three orders of magnitude in the corresponding inhibition with 3'-F-**4b**, 3'-F-**4c** and 3'-F-**4d**, exhibiting a K_i of 3.46, 4.01 and 3.67 mM, respectively.

II. Glucopyranosylidene-Spiro-Oxazolo-Pyrimidines

One of the best, known inhibitors for the catalytic site of GPb with a K_i = 3.1 μM, is D-glucopyranosylidene-spiro-hydantoin [8]. Apart from the favorable contacts that this inhibitor makes within the catalytic site, it is believed that the rigidity of the spiro structure is also responsible for the strong binding. We, therefore, reasoned that conversion of the strong inhibitor **4b** to a spiro structure would further enhance its binding affinity. Based on our previous work on constructing anomeric spironucleosides [3,49-51], we designed a synthesis leading to the new orthoamide spironucleosides **5a,b** (Scheme **9**). Specifically, Vorbrüggen-type *N*-glycosylation of trimethylsilylated 6-methyluracil furnished a 67:30:3 separable mixture of **4g**:**4h**:**4i** with the desired *N*-1 monoglycosylated base as the major product. Oxidation of the allylic 6-methyl group with selenium dioxide and reduction of the produced aldehyde furnished the 6-hydroxymethyl-derivative **4j**. In the key step, photolysis of an *in situ* generated hypoiodite produces an alkoxyl radical that, after a cascade of reactions [3], leads to, after deprotection, an anomeric mixture of spironucleosides **5a**:**5b**, in a 5:4 ratio. The yield of spirocyclization is low and the major product (50 %) arises from the autooxidation of the intermediate alkoxyl radical to the 6-aldehyde that can be recycled. This indicates that the produced alkoxyl radical conformation is not the one necessary for the [1,5]-radical translocation step, when compared with the related ribofuranosyl-system [50]. The anomeric configuration of each spironucleoside was established based on the anisotropy effect induced by the 2-carbonyl group either to H-2' and H-4' for the β-isomer **5a**, or to the H-3' for the α-anomer **5b**. Unfortunately, both spironucleosides exhibited millimolar inhibition constants (K_i = 2.1 and 1.07 mM for **5a** and **5b**, respectively) indicating that the conformations forced by the rigid spiro structure were not well accommodated within the catalytic site.

Table 3. Crystallographic and Kinetic Results for Compounds 4-9

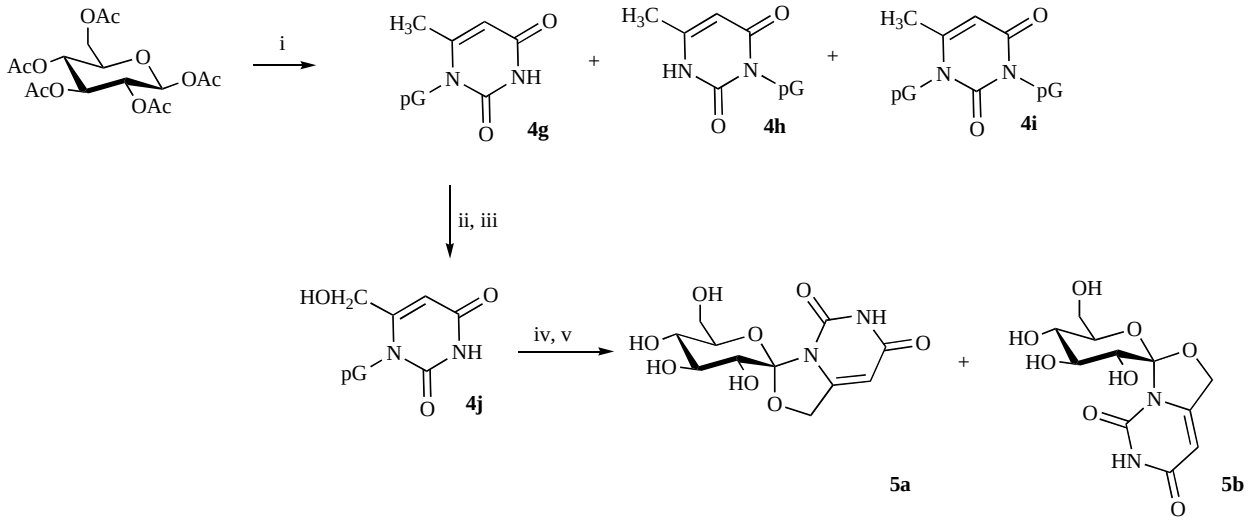
Compound	Density in the catalytic site	K_i (μM)
4a	Yes	6.6 ± 0.5
4b	Yes	6.10 ± 0.01
4c	Yes	7.7 ± 0.4
4d	Yes	5.5 ± 0.4
4e	Yes	96.0 ± 4.0
4f	Yes	1260 ± 60
5a	Partial	~2100
5b	No	~1700
6a	Yes	N.D.[1]
6b	Yes	196.9 ± 3.6
7b	Yes[2]	76.2 ± 0.4
8	Yes	1856
9a	Yes	N.I.[3]
9b	Yes	54.6 ± 1,8

[1]Not determined. [2]Two conformations of the 7-membered ring were detected with protein crystallography. [3]No inhibition

III. Tetrazolopyrimidines, Photolysis Products and Other Bicyclic Nucleosides

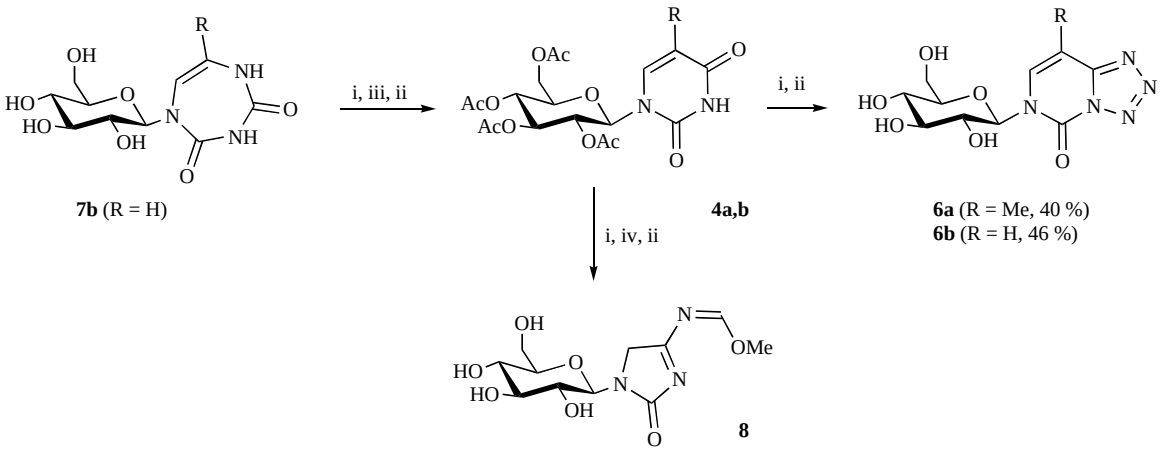
The known conditions for the conversion of thymidine to bicyclic tetrazole derivatives [52,53] were applied to the protected 9-(β-D-glucopyranosyl)-thymine and uracil (protected **4a**,**b**). The products were deprotected at low temperature to provide the photosensitive tetrazolo-derivatives **6a**,**b** in moderate yields. Tetrazolo-pyrimidines are known to exist in equilibrium with the corresponding azido-form, a valence isomerization that in this system lies completely to the side of the tetrazolo-form [54-56], as was determined from the product's spectroscopic data, as well as, from the crystallographic results of the GPb-inhibitor complexes. Photolysis of the protected tetrazolo-pyrimidine **6b** with pyrex-filtered light, under controlled conditions and in aqueous acetonitrile gave, after deprotection, the 7-membered triazepane **7b** in good yield. Alternatively, photolysis of protected **6b** in anhydrous methanol gave a complex mixture of products from which we isolated and characterized (Z)-1-(β-D-glucopyranosyl)-4-methoxyimidoformyl-2-oxo-2,5-dihydro-1*H*-imidazole (**8**, Scheme **10**) [57]. Again, in addition to ^{1}H and ^{13}C NMR spectroscopy as well as ESI-MS and SIM-MS, we were aided in the structure analysis of **8** by the crystallographic data obtained from the complex of this inhibitor in the catalytic site of GPb. As shown in Table **3**, among compounds **6b**, **7b** and **8**, **7b** proved to be the best inhibitor, but with a K_i 12 times weaker than **4b** that could be attributed to the increased flexibility of the 7-membered ring [31].

Two more bicyclic nucleosides, analogues of tetrazolopyrimidine **6b**, have also been synthesized, as shown in Scheme **11** [58]. They both contain a phthalazine-fused bicycle derivative, namely a 2,3-dihydrophthalazine-1,4-dione (**9a**) and phthalazin-1(2*H*)-one (**9b**). They were both constructed using the standard TMSOTf-catalyzed, Vorbrüggen-type, *N*-glycosylation of the corresponding trimethylsilylated base with peracetylated glucose. It is interesting to note that phthalazinone **9b** is a mesoionic structure, as established by the protein crystallographic data of the complex of **9b** with GPb. In this compound, the *N*-glycosidic bond is formed with the imidic nitrogen atom at position-3 and this nitrogen, being quartenary, contains a formal positive charge. For neutrality, compound **9b** must contain the amidic nitrogen deprotonated, with a formal negative charge. This type of mesoionic nucleosides has been previously reported to arise under kinetically controlled *N*-glycosylation reaction conditions [59]. The conditions we have utilized favor the thermo-

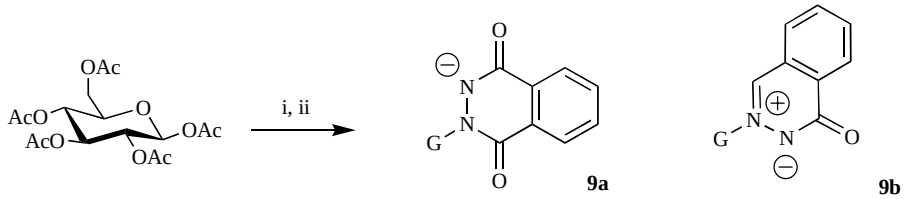


Scheme 9. (i) trimethylsilylated base, TMSOTf, DCE, reflux, 1 h, 67 %. (ii) SeO_2, AcOH, dioxane, r.t., 5 h, 67 %. (iii) $NaBH_4$, $CHCl_3$/2-propanol (4:1), SiO_2, -40 ^{0}C, 1 h, 90 %. (iv) DIB, I_2, DCM, hν, 2.5 h, 18 %. (v) 7 N NH_3 in MeOH, r.t., overnight, quant.

Stathi, A.; Gimisis, T.; *unpublished results.*



Scheme 10. (i) $POCl_3$, $TMSN_3$, CH_3CN, 70 °C, 17 h. (ii) 7 N NH_3 in MeOH, -5 °C, overnight. (iii) hν (Mercury 400 W lamp in pyrex filtered glass), H_2O/MeCN (3:2), 30 h, 65 %. (iv) hν (as in iii), dry MeOH, 44 h, 7 %.
Stathis, D.; Gimisis, T.; *unpublished results.*



Scheme 11. (i) trimethylsilylated base, TMSOTf, DCE, reflux, 2-3 h. (ii) 7 N NH_3 in MeOH, r.t., overnight, quant..
Grammatopoulos, P.; Gimisis, T.; *unpublished results.*

dynamic product arising from reaction of the amidic nitrogen at position-2. A comparison with the related 6-azauracil glucoconucleoside **4e**, in which the *N*-glycosidic bond is formed at the "thermodynamic" *N*-1 position, indicates a particular stability of the mesoionic structure in **9b**. As an inhibitor, **9b** was the strongest fused bicyclic inhibitor among all purines and tetrazolopyrimidines studied to date (K_i = 54.6 μM). Its activity is in stark contrast with that of phthalazine-1,4-dione **9a**, which exhibited no inhibition up to 1 mM. A possible explanation of this total lack for inhibition for **9a** could be ascribed to the fact that the free amidic nitrogen at position-3 of **9a** has a formal negative charge in physiological pH. From these two systems (**9a,b**) it is suggested that the interaction of the main chain carbonyl group of His-377 with these inhibitors within the catalytic site is critical and can be seen as a positive interaction when a positive charge exists in the vicinity (e.g., **9b**) and a negative interaction when a there is a negative charge present (e.g., **9a**). This important interaction should be taken into account in any inhibitor design for the catalytic site.

CONCLUSIONS

The strongest inhibitors revealed from the above studies proved to be 1-(β-D-glucopyranosyl)-pyrimidines, such as **4b** and **4d**. These compounds are our leads towards developing stronger inhibitors. A recent [31] theoretical *in silico* study that takes into account possible tautomeric forms as well as partial charges based on the pKa values of the basic or acidic groups present in the potential inhibitor is described in the same special issue in the mini-review by Hayes and Leonidas. With the aid of these theoretical studies we have been designing and synthesizing second-generation glucopyrimidine inhibitors substituted in the 4-position with aryl groups in order to enhance their activity. Having a reliable prediction tool at our disposal, we will be able to design simple synthetic procedures for the fast access to highly potent inhibitors that can be tested for their activity *in vivo* and to determine their bioavailability based on their functional characteristics. Based on this work and the work of other researchers in the field, a large number of suitable inhibitors with variable functionality and favorable *in vivo* properties may be available in the near future.

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ABBREVIATIONS

T2D = Type 2 diabetes

GP = glycogen phosphorylase

GPIs = glycogen phosphorylase inhibitors

PLP = pyridoxal-5'-phosphate

HOBT = hydroxybenzotriazole

DIC = diisopropyl carbodiimide

DMF = dimethylformamide

Fmoc = fluorenylmethyloxycarbonyl

TFA = trifluoroacetic acid

TPS = triisopropyl silane

DCE = dichloroethane

DIEA = diisopropylethylamine

DMAP = dimethylaminopyridine

BSA = bistrimethylsilylacetamide

TMSOTf = trimethylsilyl trifluoromethanesulfonate

DCM = dichloromethane

DIB. = diacetoxyiodosobenzene

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