Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 911–923

BIOORGANIC & MEDICINAL CHEMISTRY

Inhibitors of Glycogen Phosphorylase *b*: Synthesis, Biochemical Screening, and Molecular Modeling Studies of Novel Analogues of Hydantocidin

Yankanagouda S. Agasimundin, Mary W. Mumper and Ramachandra S. Hosmane*

Laboratory for Drug Design and Synthesis, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, MD 21250, USA

Received 25 November 1997; accepted 19 January 1998

Abstract—The synthesis and biochemical screening of four novel spironucleosides **1**–**4** against rabbit liver glycogen phosphorylase *b* (Gp*b*), along with molecular modeling studies on compound **2** and its 4-hydroxy analogue **VII**, have been presented. Gp*b* is a key enzyme of glycogen metabolism, and is known to be involved in the control of diabetes mellitus. The general strategy for synthesis involved base-catalyzed condensation of diethyl 2,4-dioxoimidazolidine-5-phosphonate (**5**) with either 2-deoxy-D-ribose or D-ribose, followed by sequential reactions involving ring-closure with phenylselenenyl chloride and reduction with tri-*n*-butyltin hydride catalyzed by azobisisobutyronitrile. Compounds **2** and **4** were found to be weak competitive inhibitors of Gp*b*, whereas **1** and **3** were inactive.

Introduction

Glycogen phosphorylase (GP; α-1,4-glucan:orthophosphate glycosyl transferase; E.C. 2.4.1.1)[1] is a key enzyme in the regulation of muscle and hepatic glycogen metabolism, and catalyzes the first step in the intracellular degradation of glycogen to produce α-glucose- 1-phosphate (G-1-P) (see eq (1)).[2] Glycogen is the carbohydrate reserve of most mammalian cells, and as warranted by cellular demands for energy, glycogen is broken down by GP into G-1-P, which either undergoes glycolysis to provide energy for muscle contraction or is converted to glucose in the liver to supply food to other organ tissues.

$$(\alpha\text{-1,4-glucoside})_n + Pi \rightleftarrows (\alpha\text{-1,4-glucoside})_{n-1} \text{ (glycogen)} + \alpha\text{-glucose-1-phosphate (G-1-P)} \quad (1)$$

The reverse reaction, glycogen synthesis from G-1-P, becomes important when there is comparatively less cellular demand for energy, for example in the resting state or after a meal, and the energy needs to be stored as glycogen. Although GP is also capable of catalyzing this reverse reaction, its primary function under intracellular conditions is to catalyze the forward reaction, while another related enzyme glycogen synthase[3] (E.C. 2.4.1.11), which acts in harmony with GP in the control of glycogen metabolism, is known to be primarily responsible for carrying out the reverse reaction.

Glycogen phosphorylase is regulated both by reversible phosphorylation and allosteric interactions.[2c,d,g,h] The enzyme exists in two forms: the phosphorylated, highly active, *a* form (Gp*a*) with a predominant R-state conformation, and the nonphosphorylated, much less active, *b* form (Gp*b*) with a predominant T-state conformation.[4] However, Gp*b* can be activated by micromolar concentrations of AMP,[2d,5] one of its positive allosteric effectors,[6] and then its catalytic activity closely parallels that of Gp*a*.[2d] Thus, the allosterically activated Gp*b* can be effectively employed for in vitro biochemical investigations of catalytic properties of Gp*a*. The phosphorylation of inactive Gp*b* (T-state) to form active Gp*a* (R-state), on the other hand, is catalyzed by the

*Corresponding author. Tel.: 410 455 2520; Fax: 410 455 1148.

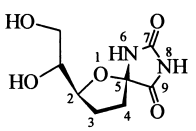
0968-0896/98/$19.00
PII: S0968-0896(98)00055-8

enzyme phosphorylase kinase[2f] upon stimulation from hormonal or neuronal signals in response to cellular demands for energy.[12] The reverse reaction, dephosphorylation of active Gp*a* to produce inactive Gp*b* at times of resting or after a meal, is catalyzed by protein phosphatase 1 (PP1),[3b,c] an important enzyme that is regulated by insulin and which promotes glycogen synthesis, while also inactivating the enzymes of glycogen break down (glycogenolysis), including GP and phosphorylase kinase.[1,2] Thus, the delicate balance between glycogen degradation and glycogen synthesis is controlled by elaborate phosphorylation and dephosphorylation events (regulation by reversible phosphorylation) as well as by modulation with several positive and negative effector metabolites such as glucose, AMP, ATP, and G-6-P (allosteric regulation).[1,2]

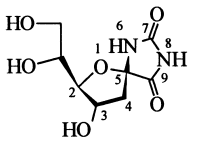
Diabetes mellitus is a disorder of chronically elevated blood glucose levels (hyperglycaemia). Over 75% of patients suffering from this disease have type II form, also known as non-insulin-dependent diabetes mellitus (NIDDM).[7–9] Hyperglycaemia is a consequence of inadequate insulin release or insulin resistance,[10] which leads to impaired glycogen synthesis (see PP1 above) in the muscle as well as impaired inactivation of both glycogenolysis and gluconeogenesis (synthesis of glucose from smaller molecular precursors) in the liver, resulting in increased glucose levels in the blood stream. Inhibition of glycogen phosphorylase[11] is believed to assist in shifting the equilibrium between glycogen degradation and glycogen synthesis in favor of glycogen synthesis in both muscle and liver, and therefore, GP inhibitors may be clinically useful for the treatment of diabetes mellitus, especially the non-insulin-dependent diabetes mellitus, NIDDM or Type II diabetes.

We report here the synthesis and inhibition studies of four spiro nucleosides **1**–**4** against rabbit muscle Gp*b*. Compounds **1**–**4** are analogues of the recently discovered powerful and non-toxic herbicide hydantocidin.[12] Also reported are the molecular modeling studies on the active compound **2** and its promising 4-hydroxy analogue **VII**.

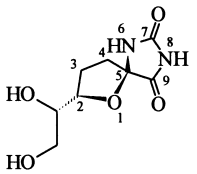
The rationale for targeting compounds **1**–**4** as potential Gp*b* inhibitors was based on structures of some of the known inhibitors of this enzyme, namely **I**–**VI**[1b,11b,11e–j] (see Chart 1), the most potent being the spironucleoside **IV** ($K_i \sim 10^{-6}\,M$). While all of the the inhibitors of Chart 1 incorporate glucose (**I**) as the common structural moiety, **I** alone is only moderately active ($K_i \sim 10^{-3}\,M$). Substitution of the anomeric hydroxyl with a carboxamide functionality as in **II** or with a six-membered spiro-heterocycle as in **III** result in incremental enhancement of activity. The best results were obtained by replacement of the anomeric hydroxyl with the spiro-fused five-membered imidazolidinedione ring, as in **IV** or **V**. Furthermore, the mode of fusion at the spiro ring junction seems to be important since the (*R*)-fused **V** was 10 times less potent than the (*S*)-fused **IV**. Regardless of the *R*/*S* fusion at the spiro junction, five-membered spiro-heterocycles, nevertheless, seem to be better than the six-membered as indicated by the respective K_i's of **IV** or **V** versus **III**. What is not clear is how important is the ring size of the sugar. Would a five-membered ribose sugar ring be better or worse? Also, how many hydroxy groups on the sugar are really necessary? It has recently been shown that the number of sugar hydroxyls present on the hydantocidin molecule plays a crucial role in its herbicidal and other biological activities.[13] In that context, a structural equivalent of **IV** or **V** with a five-membered sugar ring with retainment of all of the hydroxyls would be **VII** or **VIII** (see Chart 2). While the latter two are our ultimate synthetic targets, the anticipated facile synthesis of analogues of **VII** or **VIII** lacking one or two sugar hydroxy groups, using the readily available D-ribose or 2-deoxy-D-ribose, prompted us to set compounds **1**–**4** described



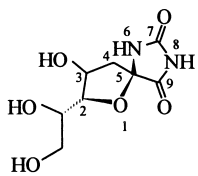
1



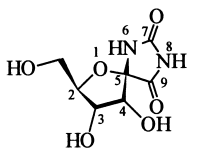
2



3



4



Hydantocidin

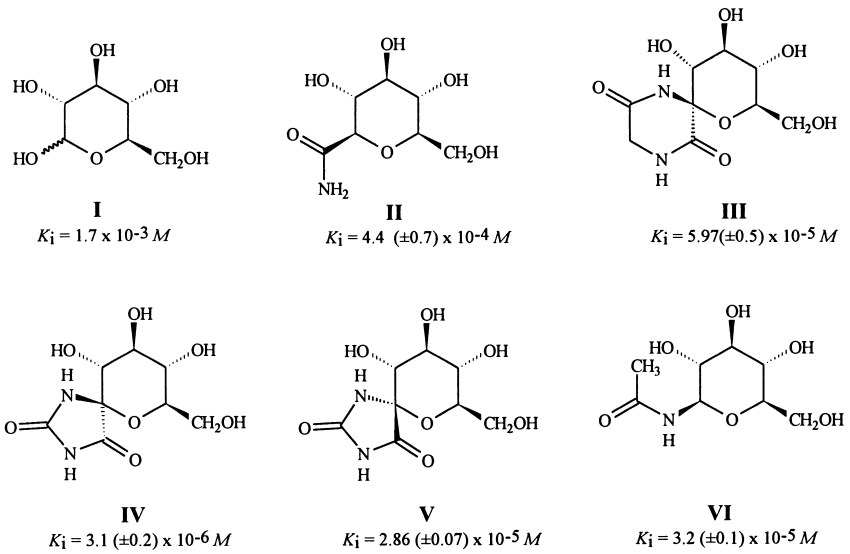


Chart 1. Inhibitors of glycogen phosphorylase.

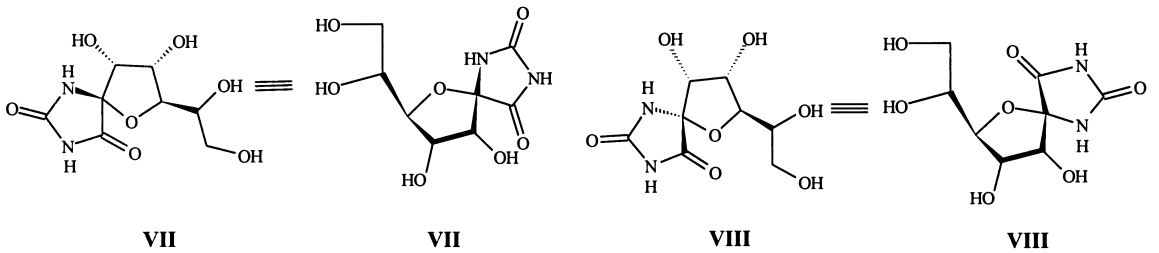


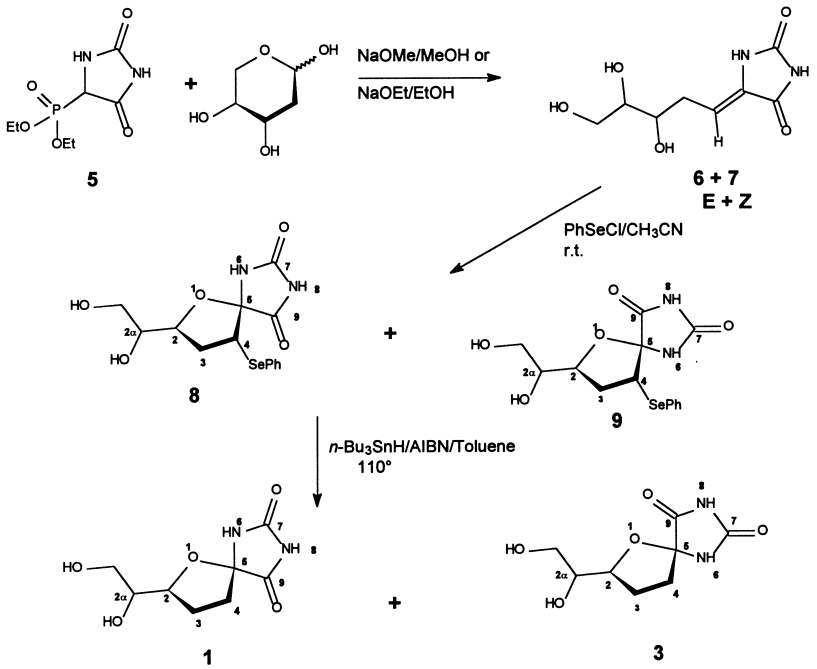
Chart 2. 5:5-Fused structural equivalents of **IV** and **V**.

herein as our initial synthetic targets. However, with judicious synthetic manipulations, our approach would also be applicable to access other novel 5:5-fused spironucleosides, including the mentioned future targets **VII** and **VIII** as well as the parent hydantocidin and its mono- and dideoxy forms.

Results and Discussion

Organic synthesis

Synthesis of [2α*R*,2*S*,5*S*]-2α,β-dihydroxyethyl-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (1) and [2α*R*,2*S*,5*R*]-2α,β-dihydroxyethyl-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (3). Key starting material for the synthesis, diethyl 2,4-dioxoimidazolidine-5-phosphonate (**5**) (Scheme 1) was readily prepared from hydantoin following the procedure of Meanwell et al.[14] The reaction of **5** with 2-deoxy-D-ribose was carried out in the presence of sodium ethoxide in ethanol at room temperature. The product *E*,*Z*-5-[3*S*,4*R*,5-trihydroxy-1-pentylidene]imidazolidine-2,4-dione (**6**+**7**), precipitated from the reaction mixture. Similar results were obtained when the reaction was carried out in the presence of sodium methoxide in anhydrous methanol. Treatment of the precipitate with hot anhydrous ethanol and filtration gave the mixture **6**+**7** in high purity. The ribose adduct is insoluble in hot ethanol, while the starting phosphonate and 2-deoxy-D-ribose are soluble. ^{1}H NMR showed the product to be a mixture of *E* and *Z* isomers in approximately a 1:1 ratio. Though easily accomplished, separation of these isomers was unnecessary, since during the subsequent step either isomer leads to the formation of an identical mixture of anomeric spironucleosides. Therefore a mixture of **6**+**7** was reacted with phenylselenenyl chloride in acetonitrile at room temperature. The product phenylselenenyl ether (**8**+**9**) precipitated from the reaction mixture after stirring overnight. While it was possible to separate the *E* and *Z* isomers, **6** and **7**, by fractional crystallization from ethanol–water, the anomeric isomers **8** and **9** were inseparable by this method even after many other solvent systems were tried. Therefore, the separation of isomers was carried out in the final step.



Scheme 1. Synthesis of α,β-(2α-hydroxymethyl)-3,4-dideoxyhydantocidin.

The first step in phenylselenenyl chloride reaction is presumed to be the reversible electrophilic addition of phenylselenonium ion ($PhSe^+$) to the double bond of **6** or **7**. The intermediate subsequently suffers intramolecular nucleophilic attack by one of the side-chain hydroxy groups at the olefinic carbon atom that can sustain the most stable carbocation. Since such an attack occurs at C-5 (hydantoin ring carbon) resulting in the formation of a spirocyclic ether, it is assumed that **6** or **7**, which can be considered both as an enamine and an α,β-unsaturated carbonyl compound, behaves more like an enamine. The alternate possibility of formation of a nonspiro nucleoside (see box C in Figure 1) was ruled out based on the ^{1}H NMR spectral data of **8** and **9**, which revealed the absence of an anomeric proton (usually seen at ~4.0–5.0 δ). Furthermore, after removal of the phenylselenenyl group to give compounds **1** and **3** (see Scheme 1), the observed proton multiplets at ~2 δ (due to four protons) can only be explained by the presence of adjacent diastereotopic sugar ring CH_2–CH_2 protons. It was, however, difficult to distinguish between a spiro [4,4] nonane (box A in Figure 1) and spiro [5,4] decane (box B) on the basis of ^{1}H and ^{13}C NMR spectral data alone. Nevertheless, after an extensive investigation of phenylselenenyl halide reactions, Nicolaou and co-workers[15] have established that such cyclization reactions lead preferentially to the formation of five- rather than six-membered rings. However, substantial spectroscopic and/or chemical evidence was necessary for a conclusive structural assignment.

The anomeric mixture of **8** and **9** was treated with tri-*n*-butyltin hydride in anhydrous toluene with a catalytic amount of azobisisobutyronitrile (AIBN) in order to reductively remove the phenylselenenyl moiety. After flash or rotating disc chromatography, the solvent was evaporated to give a light yellow oil, which solidified to form X-ray quality crystals upon standing at room temperature for about a week. The ^{1}H NMR of this solid revealed the presence of a single anomer. Single-crystal X-ray diffraction analysis (*R* factor $<3\%$) of this solid revealed it to be compound **1**, confirming the structure to be that of the (+)-hydantocidin analogue (see Figure 2). Now it was possible to assign anomeric designations to all structures of this series based upon ^{1}H NMR.

Addition of ether to the ethanol washings from the crystals of **1** caused precipitation of a light-yellow solid whose ^{1}H NMR showed it to consist primarily of the other anomer **3**. Reverse-phase HPLC separation was utilized to obtain this anomer in a pure form.

Synthesis of [2α*R*,2*S*,3*S*,5*S*]-2α,β-dihydroxyethyl-3-hydroxy-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (2) and [2α*R*,2*S*,3*S*,5*R*]-2α,β-dihydroxyethyl-3-hydroxy-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (4). Reaction of diethyl-2,4-dioxoimidazolidine-5-phosphonate (**5**)[14] with D-ribose was carried out (Scheme 2) in the presence of sodium ethoxide in ethanol in a manner analogous to the synthesis of **1** and **3** described above. The product,

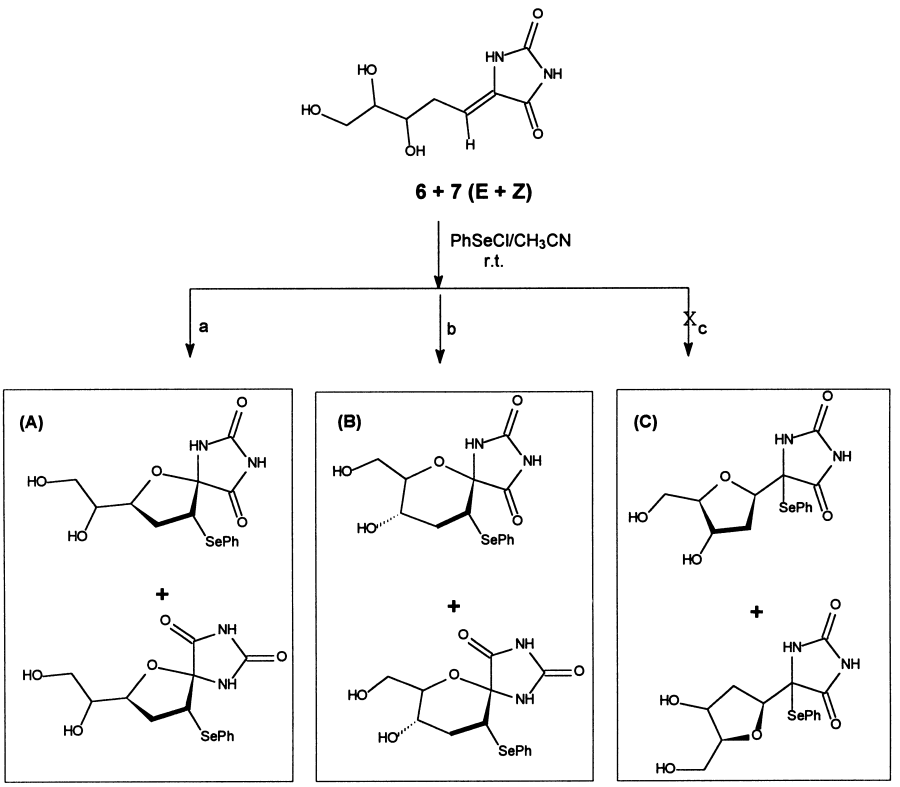


Figure 1. Three possible pathways for ring-closure of the $E+Z$ isomer (**6**+**7**) upon reaction with phenylsenenyl chloride.

5-[2*S*,3*S*,4*R*,5-tetrahydroxy-1-pentylidine]imidazolidine-2,4-dione (**10**+**11**), was again a mixture of *E* and *Z* isomers. The yields for this series of reactions were generally lower in comparison to the previous series. The reaction of **10**+**11** with phenylselenenyl chloride in anhydrous acetonitrile produced the spiro compounds **12** and **13**. Separation of the anomers by silica gel flash chromatography or rotating disk chromatography was unsuccessful, but the isomers could be separated by HPLC in the final step.

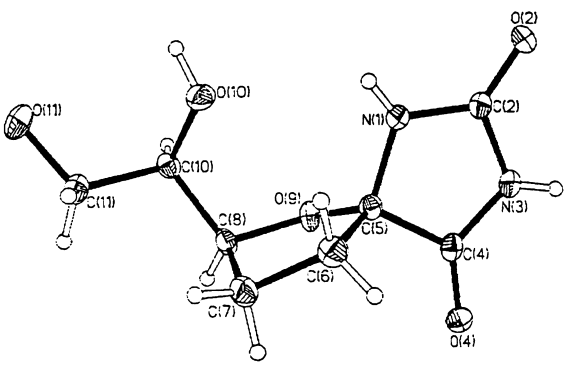
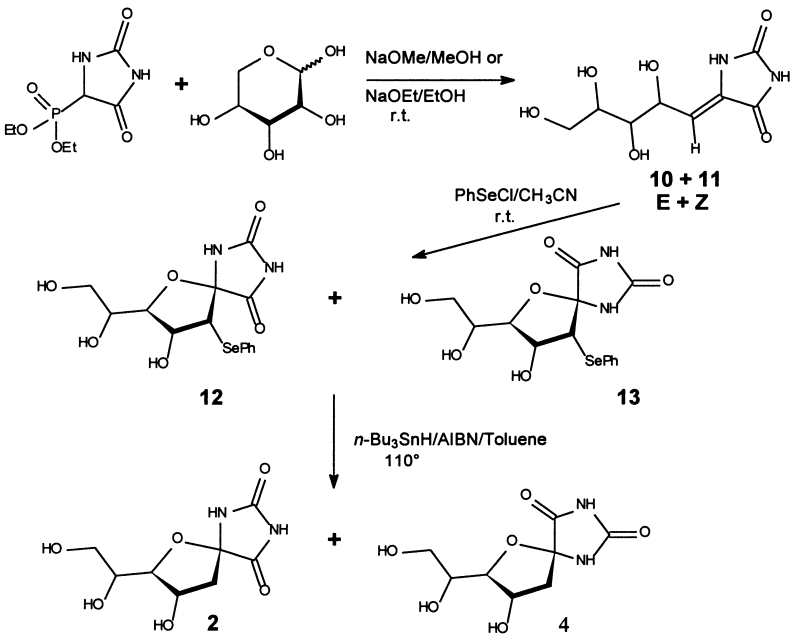


Figure 2. The ORTEP view of compound **1** showing the atom numbering scheme.

Reductive removal of the phenylselenenyl group to give spiro-nucleosides **2** and **4** was completed by reacting **12**+**13** with tributyltin hydride in anhydrous toluene at 110 °C in the presence of the free radical initiator AIBN. This gave the product spironucleoside as a mixture of diastereomers. Separation of the anomers by reverse phase HPLC gave **2** and **4** in 2.4:1 ratio.

Biochemical studies

Inhibition of glycogen phosphorylase *b*. Glycogen phosphorylase *b* was assayed, using the procedure of Martin et al.[11b] in the direction of glycogen synthesis at pH 7.0 and 25 °C by measuring the release of inorganic phosphate (refer to eq (1) above). The enzyme (20 μg/mL) was assayed in 40 mM Tris–acetate buffer (pH 7.0), 2 mM EDTA, 1% glycogen and 1.6 mM AMP with concentrations of glucose-1-phosphate (G-1-P) between 5 and 20 mM and concentrations of inhibitors between 5 and 20 mM. The enzyme was assayed as purchased from Sigma. Enzyme preparations exhibited K_M of 1.2 (± 0.3)$\times 10^{-2}$ M at saturating AMP (1.6 mM) and glycogen (1%). Kinetic data were evaluated using Lineweaver–Burk plots. Regression analysis was performed using Quatro-Pro™ 5.0 spreadsheet.



Scheme 2. Synthesis of α,β-2α-hydroxymethyl-4-deoxyhydantocidin.

The graph in Figure 3 shows the plot of data for compound **2**. As can be seen, spironucleoside **2** is a competitive inhibitor of glycogen phosphorylase *b* with a K_i of $8.2\ (\pm 0.4)\times 10^{-3}$ M. Compound **4** is a weak competitive inhibitor with a K_i of $2.2\ (\pm 0.3)\times 10^{-1}$ M. Compounds **1** and **3** show neither inhibition nor activation in these conditions.

Molecular modeling studies

Molecular modeling of spironucleoside 2 and its 4-hydroxy analogue VII. Molecular modeling was performed using BIOSYM/INSIGHT/DISCOVER (see Experimental for details). The crystal coordinates of rabbit muscle glycogen phosphorylase *b* (Gp*b*, T-State) were imported from the Brookhaven National Protein Data Bank. Spironucleosides **2** and **VII** were built, energy-minimized to convergence, and then docked into Gp*b*, followed by energy minimization of the Gp*b*-**2** and Gp*b*-**VII** complexes. The stereoviews of the respective energy-minimized structures are shown in Figure 4(a) and (b), while the close-up monoviews of the same are depicted in Figure 5(a) and (b). As shown in Figure 4(a), compound **2** exhibits two hydrogen bonds with the protein: **2**-N^8H....N^1-HIS377 (1.77 Å) and **2**-$O^{2\alpha}$(H)....HO-THR676 (2.36 Å). On the other hand, compound **VII** (see Figure 4(b), which contains only an additional OH group at position 4 as compared with **2**), reveals a total of seven hydrogen bonds with Gp*b*: **VII**-$O^{2\alpha}$(H)....HO-THR676 (2.36 Å), **VII**-$O^{2\alpha}$(H)....HN-THR676 (1.94 Å), **VII**-$O^{2\beta}$H....$N^{\varepsilon}(H_2)$-LYS568 (2.34 Å), **VII**-O^3(H)....$N^{\varepsilon}(H_2)$-LYS574 (2.18 Å), **VII**-O^9(=C)....H_2N-C(O)-ASN284 (2.05Å), **VII**-N^8H....N^1-HIS377 (2.07Å), and **VII**-O^7(=C)....H_2N-C(O)-ASN484 (2.13 Å). This dramatic difference in hydrogen bonding array of **VII** as contrasted with **2**, upon addition of a single hydroxy group at position 4 of **2** is especially intriguing since this added OH group does not itself show any participation in hydrogen bonding with Gp*b*. As expected from the enhanced protein–ligand interactions, the complex Gp*b*-**VII** showed considerably lower energy (Insight Energy = −144 kcals/mol) as compared with the complex Gp*b*-**2** (I.E. = −13 kcals/mol).

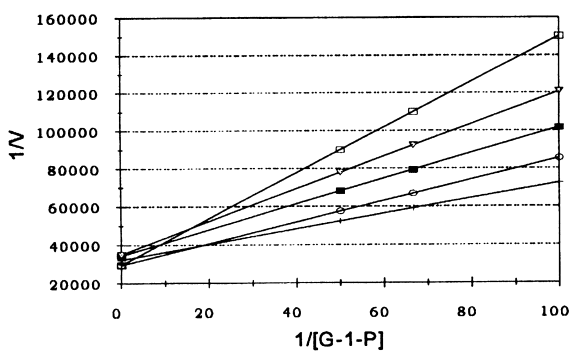


Figure 3. Inhibition of glycogen phosphorylase *b* by **2**. Inhibitor concentrations were 0 (+), 5 (O), 10 (■), 15 (▽), and 20 (□) mM. Y intercept values were calculated by linear regression.

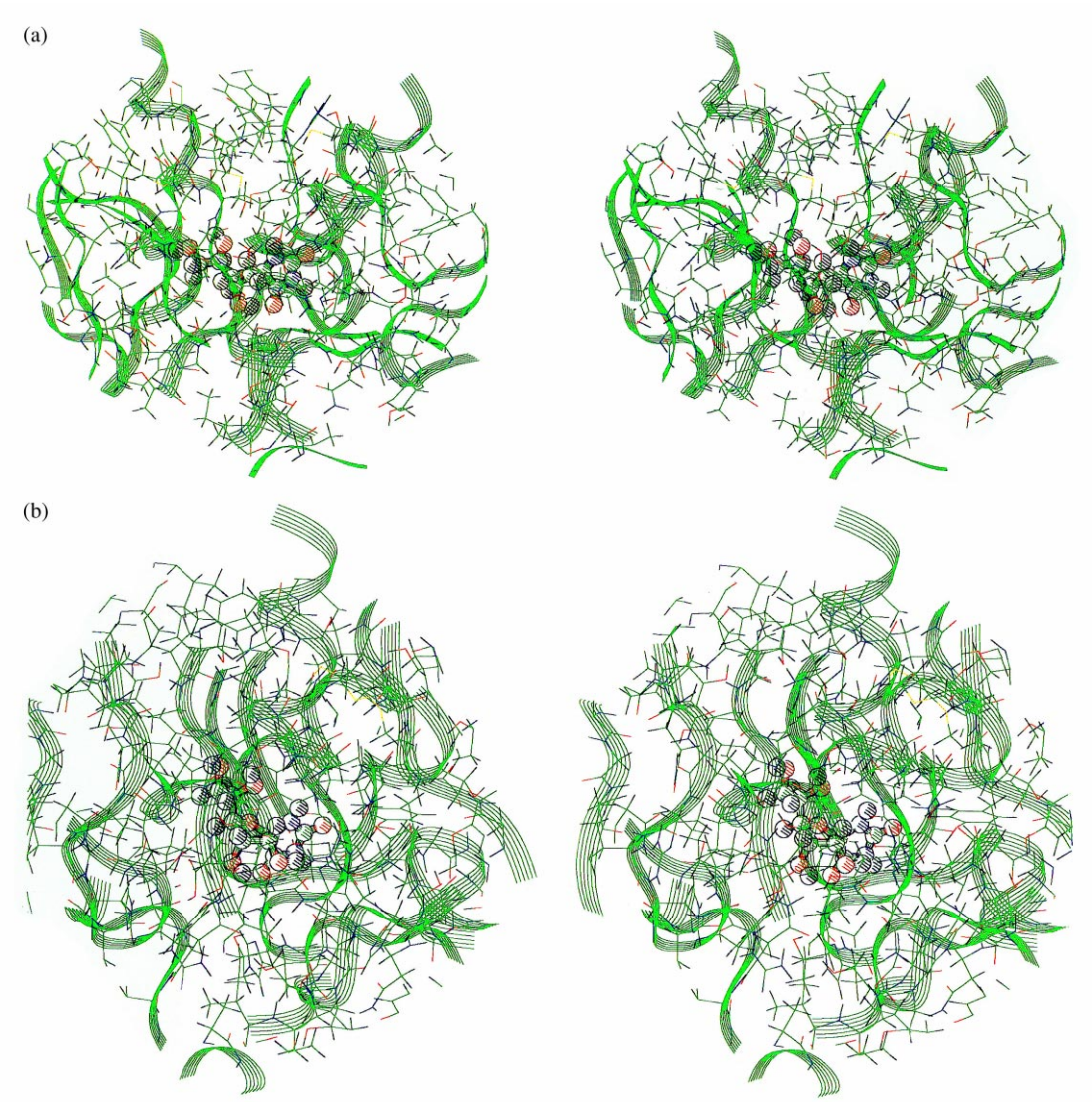


Figure 4. Stereoviews of the energy-minimized protein–ligand complexes: (a) GP*b*–**2** and (b) GP*b*–**VII**. Only those residues of GP*b* that are located within a 14 Å radius from the ligand are shown.

In order to simulate the natural environment even further, each of the above energy-minimized complexes was soaked in a layer of water, 5 Å thick, all around the unconstrained residues in the active site, stretching in a radius of 15Å from the site of ligand. While the energy-minimization to convergence of each solvated complex revealed, as expected, substantially lower energy (I.E. in kcals/mol for Gp*b*-**2** = −1894; Gp*b*-**VII** = −1915) than the unsolvated species, ligand in neither complex picked up any additional hydrogen bond with the added water. The femto-second molecular dynamics simulation (300 K, 1000 iterations) was performed on each solvated complex. As revealed from graphs computed from the dynamics trajectories, the potential energy of each complex, after an initial surge during the equilibration period of about 100 iterations, remained essentially constant, ranging from −712 to −685 kcals.

Conclusion

We have successfully synthesized the spironucleoside analogues **1**–**4**. The overall synthetic strategy involves attachment of the sugar ring to the target base-analogue, which is different from other known methods of synthesis of such spironucleosides,[11a] including hydantocidins,[16] wherein the synthesis normally commences

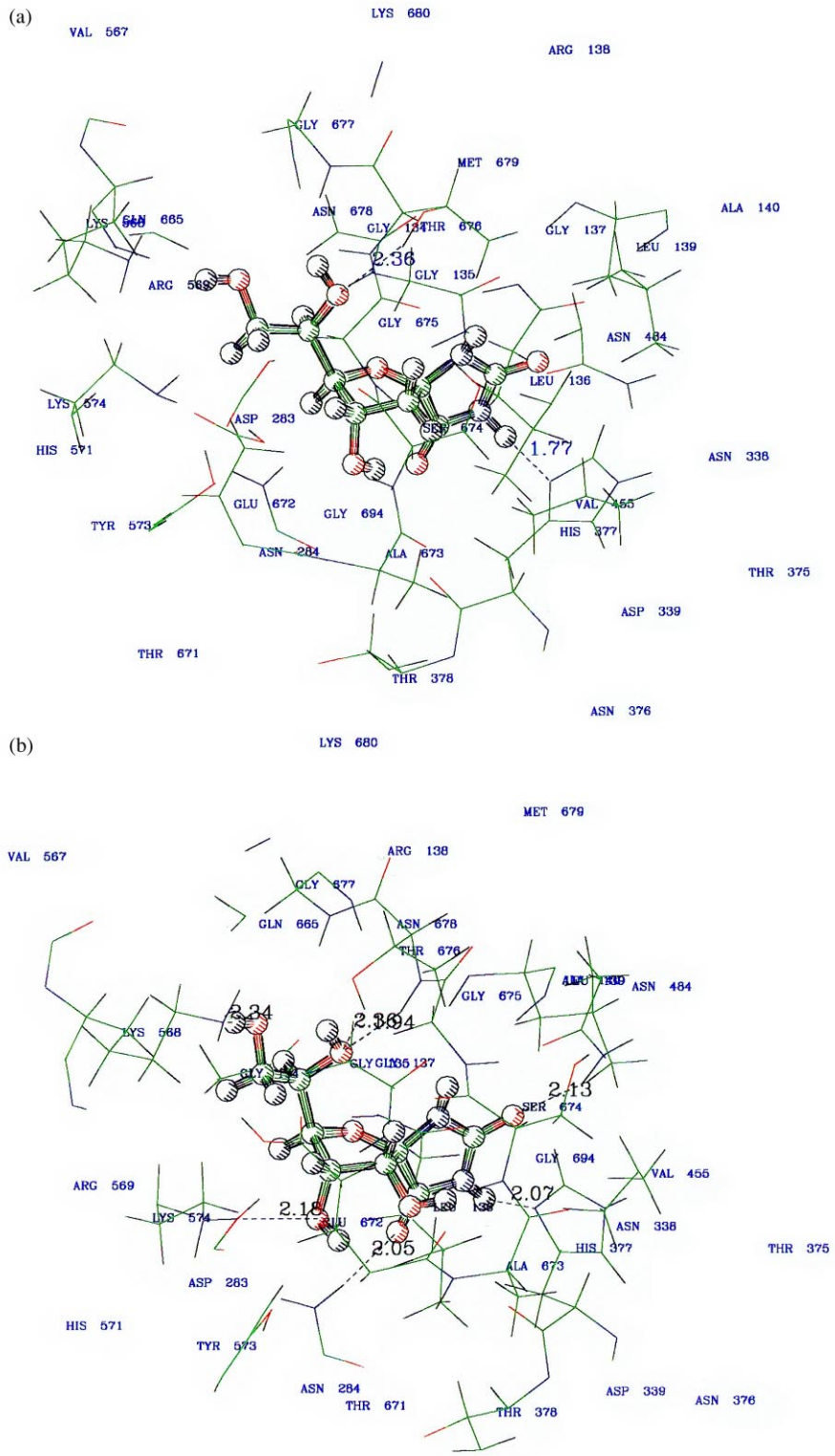


Figure 5. A close-up view of the energy-minimized protein–ligand complexes shown in Figure 4: (a) GP*b*–**2** and (b) GP*b*–**VII**. Only those residues of GP*b* that are located within a 6 Å radius from the ligand are shown.

from sugar onto which the target heterocycle is built. Most of these reported syntheses are long, tedious, and poor-yielding. Our synthesis, by contrast, is simple, convenient, short, as well as versatile for both deoxy and dideoxy sugar analogues. With further manipulations, the approach is conceivably applicable for the synthesis of not only the contemplated target dihydroxy sugar analogues (e.g. **VII**) but also the parent hydantocidin as well as its several analogues and derivatives of potential therapeutic and biological interest. The four compounds were screened in vitro against rabbit muscle glycogen phosphorylase *b*. Compounds **2** and **4** were found to be weak competitive inhibitors of the enzyme, while the remaining two were inactive. Our molecular modeling studies with glycogen phosphorylase *b* suggest that addition of an hydroxyl group at the four position of **2**, as in **VII**, could further strengthen the binding of inhibitor to the enzyme, and hence, strengthen the inhibition. Such an endeavor is currently in progress.

Experimental

General

^{1}H NMR spectra were recorded at 80 or 300 MHz in DMSO-d_6, unless otherwise stated. The data are reported in the following format: chemical shift (all relative to Me_4Si), multiplicity (s = singlet, d = doublet, dt = double triplet, dd = double doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constants, integration and assignment. X-Ray crystal structure analysis was performed at the Department of Chemistry, Southern Methodist University, Dallas, TX, USA. Chemical ionization (CI) mass spectra were performed using isobutylene as the carrier gas. The high-resolution FAB mass spectral analyses were performed by the Mass Spectral Facility, Department of Biochemistry, Michigan State University. Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA. Evaporations were done under reduced pressure on a rotary evaporator. Column chromatography was performed as described by Still et al.[17] Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} (0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

(*E*,*Z*)-5-(3*S*,4*R*,5-Trihydroxy-1-pentylidene)imidazolidine-2,4-dione (6 + 7). To a magnetically stirred solution of sodium methoxide prepared from freshly cut sodium (0.220 g, 9.6 mg. atom) and anhydrous methanol (25 mL) was added diethyl 2,4-dioxoimidazolidine-5-phosphonate (2.25 g, 9.6 mmol) and 2-deoxy-D-ribose (1.37 g, 10 mmol). The mixture was stirred overnight and a white compound precipitated. Reaction mixture was diluted with methanol to redissolve and adsorbed onto silica gel (10 g). Flash chromatography on an 80 g silica gel column eluted with $CHCl_3$:MeOH (9:1) gave 1.24 g (60%) of a clear glass which was dissolved in hot ethanol. Upon cooling, 620 mg of a white crystalline compound (mp 152–154 °C) precipitated. Assignment Z-isomer: ^{1}H NMR (DMSO-d_6) δ 10.89 (s, ex. w. D_2O, 1H, O=CNHC=O), 10.01 (s, ex. w. D_2O, 1H, C=CNHC=O), 5.65 (t, J = 8 Hz, 1H, =CH), 4.70, 4.60, 4.38 (3 bs, ex. w. D_2O, 3H, 3 OH′s), 3.53–3.28 (m, 4H, CH_2OH + 2 C*H*OH′s), 2.44 and 2.26 (2 m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 165.0 (C=O), 154.3 (C=O), 131.3 (imidazole C-4), 115.8 (vinyl CH) 75.2, 71.2, 63.6 (CH_2OH + 2 CHOH′s), 29.9 (CH_2). Dropwise addition of petroleum ether (bp 40–60 °C) to the mother liquor until turbid and left standing overnight yielded 550 mg of white powder, mp 178–180 °C, assigned as *E*-isomer; ^{1}H NMR (DMSO-d_6) δ 10.87 (s, ex. w. D_2O, 1H, O=CNHC=O), 9.91 (s, ex. w. D_2O, 1H, C=CNHC=O), 5.567 (t, J = 7.5, 1H, =CH), 4.62, 4.54, 4.37 (3 bs, ex. w. D_2O, 3H, 3 OH′s), 3.50–3.26 (m, 4H, CH_2OH + 2 C*H*OH′s), 2.92 and 2.59 (2 m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 165.1 (C=O), 154.2 (C=O), 129.8 (imidazole C-4), 110.5 (vinyl CH), 75.1, 71.4, 63.3 (CH_2OH + 2 CHOH′s), 30.0 (CH_2); MS m/z (MH^+) 217; Anal. calcd for $C_8H_{12}N_2O_5$: C, 44.45; H, 5.55; N, 12.56. Found: C,44.47; H, 5.60; N, 12.95.

[2α*R*,2*S*,4,5*S*]-2α,β-Dihydroxyethyl-4-selenophenyl-1-oxa-6,8-diazaspiro[4,4]nonane-7,9-dione (8) and [2α*R*,2*S*,4,5*R*]-2α-hydroxy-2α,β-dihydroxyethyl-4-selenophenyl-1-oxa-6,8-diazaspiro[4,4]nonane-7,9-dione (9). To a stirred suspension of **6** + **7** (0.864 g, 4 mmol) in anhydrous acetonitrile, phenylselenenyl chloride (0.77 g, 4 mmol) was added and the reaction mixture was stirred at room temp for 20 h. The solution changed from bright orange-red to a very pale yellow. The reaction mixture was adsorbed onto 2 g of flash silica gel (particle size 40–63 μm). Flash chromatography of this material ($CHCl_3$:MeOH, 6:1–4:1) and evaporation of UV absorbing fractions with faster moving R_f than the starting material gave a colorless foam (1.01 g, 68%). When the foam was dissolved in a minimum of acetonitrile and allowed to stand, clear crystals precipitated. ^{1}H NMR of the crystals (mp, 92 °C) shows two isomers (**8** and **9**) in virtually 1:1 ratio: ^{1}H NMR (DMSO-d_6) δ 11.10 and 11.00 (2 s, ex. w. D_2O, 1H, O=CNHC=O), 9.38 and 8.74 (2s, ex. w. D_2O, 1H, qCNHC=O), 7.48–7.31 (m, 5H, Ar CH′s), 5.17 and 4.83 (2 bs, ex. w. D_2O, 1H, 2α OH), 4.54 (m, ex. w. D_2O, 1H, 2α CH_2O*H*), 3.94 and 3.87 (2 m. 1H, SeCH), 3.43 (m, 4H, 2α CH_2OH, 2α C*H*OH, C2H), 2.60–1.96 (4 m, 2H, C3H_2); MS (CI w/ CH_3OH) m/z 373 (MH^+); Anal. calcd for $C_{14}H_{16}N_2O_5Se$: C, 45.29; H, 4.34; N, 7.55. Found: C, 45.37; H, 4.42; N, 7.47.

[2αR,2S,5S]-2α,β-Dihydroxyethyl-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (1) and [2αR,2S,5R]-2α,β-dihydroxyethyl-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (3). A mixture of **8** and **9** (0.742 g, 2 mmol), tributyltinhydride (1.165 g, 4 mmol) and azobis-isobutyronitrile (AIBN) (cat. amount) in freshly distilled anhydrous toluene was heated at 110 °C for 18 h under nitrogen. TLC (silica gel, $CHCl_3$:MeOH, 4:1) indicated the completion of reaction. Removal of solvent under diminished pressure gave the crude product **1** + **3**, which was further purified by flash chromatography ($CHCl_3$: MeOH, 4:1) to give **1** + **3**, as a colorless oil which was permitted to stand for several days when small crystals were seen to form in the bottom of the flask. After 1 week these clear crystals include approximately half of the material in the flask. The contents of the flask were carefully washed with ethanol and filtered to give 90 mg (20%) clear crystals, mp 175–176 °C. X-Ray analysis of these crystals showed them to be compound **1**. ^{1}H NMR (DMSO-d_6) δ 10.77 (s, 1H, ex. w D_2O, NH), 8.48 (s, 1H, ex. w D_2O, NH), 4.75 (d, J = 4.8 Hz, 1H, ex. w D_2O, 2α OH), 4.50 (t, J = 4.9 Hz, 1H, ex. w D_2O, 2α CH_2OH), 3.94 (m, 1H, 1H, C2H), 3.47 (m, 3H, 2α C*H*OH + 2α CH_2OH), 2.17–1.94 (m, 4H, C3H_2 + C4H_2); MS (CI w/i-C_4H_{10}) m/z 217 (MH^+); Anal. calcd for $C_8H_{12}N_2O_5$: C, 44.45; H, 5.55; N, 12.56. Found: C, 44.53; H, 5.58; N, 12.82.

Evaporation of the ethanol washings gave a light-yellow solid (0.10 g, 24%); ^{1}H NMR showed this solid to be 90% of the α anomer. This solid was purified to a single anomer using HPLC with water as eluent. The solid was dissolved in water and injected in several portions onto tandem columns (ISCO C_{18}, 21.2×250 mm + Waters Bondapak C_{18} 25×150 mm) eluted at flow rate 7 mL/min. Fractions with retention time 17.5 min were combined and the solvent evaporated to yield **3** as an off-white solid, mp 162–163 °C; ^{1}H NMR (DMSO-d_6) δ 10.77 (s, 1H, ex. w. D_2O, NH), 8.60 (s, 1H, ex. w. D_2O, NH), 4.75 (d, J = 5.1 Hz, 1H, ex. w. D_2O, 2α OH), 4.45 (t, J = 5.4 Hz, 1H, ex. w. D_2O, 2α CH_2OH), 4.00 (m, 1H, C2H), 3.37 (m, 3H, 2α C*H*OH and 2α CH_2OH), 2.23–1.89 (m, 4H, C3H_2 + C4H_2); Anal. calcd for $C_8H_{12}N_2O_5$: C, 44.45; H, 5.55; N, 12.56. Found: C, 44.59; H, 5.55; N, 12.87.

***E,Z*-5[2S,3S,4R,5-Tetrahydroxypentylidene]-2,4-imidazolidine-2,4-dione (10 + 11).** To a stirred solution of sodium methoxide prepared from anhydrous methanol (30 mL) and freshly cut sodium (0.276 g, 12 mg atom), diethyl-2,3-dioxo-imidazolidime-5-phosphonate (2.84 g, 12 mmol) and D-ribose (1.80 g, 12 mmol) were added. The reaction mixture was stirred at room temp. for 18 h. The clear solution was mixed with flash silica gel (4–5 g) and evaporated to dryness. Flash chromatography of this material ($CHCl_3$:MeOH, 4:1) gave a UV absorbing product, which was still associated with some sticky substance. It was dissolved in methanol, acetonitrile added until turbid and left overnight. The pure product separated as a fine colorless solid (1.39 g, 50%), mp 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 10.97 (s, 1H, ex. w. D_2O, NH), 9.76 (s, 1H, ex. w. D_2O, NH), 5.58 (d, 1H, vinyl CH), 5.04 (d, 1H, ex. w. D_2O, OH), 4.67 (d, 1H, ex. w. D_2O, OH), 4.65 (d, 1H, ex. w. D_2O, OH), 4.34 (s, 1H, ex. w. D_2O, OH), 4.44 (bs, 1H, C*H*OH), 3.44 (m, 4H, CH_2OH, 2 C*H*OH); ^{13}C NMR (DMSO-d_6) δ 164.30 (C = O), 154.28 (C = O), 130.2 (imidazole C-4), 111.32 and 110.76 (vinyl CH, *E*, *Z* isomers), 74.19, 72.0, 67.54, 62,75 (CH_2OH + 2 CHOH + CH_2); Anal. calcd for $C_8H_{12}N_2O_6$: C, 41.38; H, 5.21; N, 12.06. Found: C, 41.40; H, 5.20; N, 12.02.

[2αR,2S,3S,4,5S]-2α,β-Dihydroxyethyl-3-hydroxy-4-selenophenyl-1-oxa-6,8-diazaspiro[4,4]nonane-7,9-dione and [2αR,2S,3S,4,5R]-2α,β-dihydroxyethyl-3-hydroxy-4-selenophenyl-1-oxa-6,8-diazaspiro[4,4]nonane-7,9-dione (12 + 13). A stirred suspension of **10**, **11** (1.83 g, 8.0 mmol) in acetonitrile (60 mL) was treated with phenylselenenyl chloride (1.54 g, 8 mmol) in one portion at room temperature. It was stirred for 48 h, while following by TLC (silica gel, $CHCl_3$:MeOH, 4:1). Unreacted starting compound which remained insoluble was collected by filtration (0.48 g). The filtrate was evaporated with flash silica gel and chromatographed ($CHCl_3$:MeOH, 5:1) to give **12**, **13** as a colorless solid (1.70 g, 73% on the basis of unrecovered starting material); ^{1}H NMR (DMSO-d_6) δ 11.09 (bs, 1H, ex. w. D_2O, NH), 8.6, 8.2, 7.7 (3 s, 1H, ex. w. D_2O, NH), 7.56–7.28 (m, 5H, Ar-H), 5.77 (m, 1H, ex. w. D_2O, OH), 4.01 (m, 1H, ex. w. D_2O, OH), 4.0 (m, 1H, ex. w. D_2O, OH), 4.5 (m, 2H, 2 C*H*OH), 3.44–3.72 (m, 4H, CH_2OH + C*H*-SePh + C2H); MS m/z (CI w/i-C_4H_{10}) 388 (MH^+); Anal. calcd for $C_{14}H_{16}N_2O_6Se$: C, 43.42; H, 4.16; N, 7.23. Found: C, 43.98; H, 4.50; N, 6.90.

[2αR,2S,3S,5S]-2α,β-Dihydroxyethyl-3-hydroxy-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (2) and [2αR,2S,3S,5R]-2α,β-dihydroxyethyl-3-hydroxy-1-oxa-6,8-diazaspiro-[4,4]-nonane-7,9-dione (4). A mixture of **12**,**13** (0.774 g, 2 mmol), tributyltinhydride (1.5 g, 5 mmol) and azobisisobutyro-nitrile (AIBN, 75 mg) in freshly distilled anhydrous toluene (30 mL) was heated under gentle reflux for 6 h. The reaction was followed by TLC (silica gel, $CHCl_3$:MeOH, 4:1). The reaction mixture was evaporated under reduced pressure, redissolved in methanol, and adsorbed onto flash silica gel (3–4 g). Flash chromatography of this material ($CHCl_3$:MeOH, 5:1) gave the product **2**,**4** as a colorless solid (0.35 g, 76.5%), mp 219–220 °C. Analytical sample was obtained by crystallization from $CHCl_3$–MeOH (0.125 g, 40%), mp 219–220 °C; MS (CI w/i-C_4H_{10}) m/z 233 (MH^+); Anal. calcd for $C_8H_{12}N_2O_6$: C, 41.38; H, 5.21; N; 12.06. Found: C, 41.48; H, 5.18; N, 12.12.

The mixture was dissolved in water and injected in several portions onto HPLC tandem columns (ISCO C_{18}, 21.2×250 mm + Waters Bondapak C_{18} 25×150 mm) eluted at flow rate 7 mL/min. Fractions with retention time of 27 min were collected and solvent evaporated. After trituration with ether and filtration, an off-white solid was collected (0.060 g, mp 235 °C, dec) This compound was assigned to be **2** on the basis of NMR data, using **1** as a model compound: ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H, ex. w. D_2O, NH), 8.56 (s, 1H, ex. w. D_2O, NH), 5.03 (d, J = 5.7 Hz, 1H, ex. w. D_2O, OH), 4.65 (t, J = 3.9 Hz, 1H, ex. w D_2O, CH_2OH), 4.43 (d, J = 3.9 Hz, 1H ex. w D_2O, OH), 4.13 (m, 1H, C2H) 3.79–3.35 (m, 4H, 2α C*H*OH, 2α CH_2OH), 2.42–2.19 (m, 2H, C4H_2).

The fractions eluted at 17 min were pooled and solvent evaporated. Again, ether trituration was required to yield an off-white solid (25 mg), mp 180 °C. On the basis of NMR data, this compound was assigned to be **4**: ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H, ex. w. D_2O, NH), 7.68 (s, 1H, ex. w. D_2O, NH), 5.12 (d, J = 5.3 Hz, 1H, ex. w. D_2O, OH), 4.88 (t, J = 4.0 Hz, 1H, ex. w D_2O, 2α CH_2OH), 4.56 (d, J = 4.2 Hz, 1H ex. w D_2O, OH), 4.01 (m, 1H, C2H), 3.76–3.42 (m, 4H, 2α C*H*OH, 2α CH_2OH), 2.04–1.85 (m, 2H, C4H_2).

Enzyme studies. Rabbit muscle glycogen phosphorylase *b* was utilized. AMP disodium salt, oyster glycogen, G-1-P were used as purchased from Sigma. The inhibitors were freshly dissolved just before use. Phosphorylase activity in the direction of glycogen synthesis was determined at pH 7.0 and 25 °C by measuring the inorganic phosphate released in the reaction using the method of Fiske and Subbarow.[18] The enzyme (20 μg/mL) was assayed in 40 mM Tris–acetate buffer (pH 7.0), 2 mM EDTA, 1% glycogen, and 1.6 mM AMP with concentrations of glucose-1-phosphate (G-1-P) between 5 and 20 mM. Inhibitor concentrations were between 5 and 20 mM. Assay volume was 0.5 mL in every instance. Enzyme and glycogen were preincubated for 15 min at 25 °C before the reaction was initiated with the substrate mixtures containing AMP and G-1-P with or without inhibitor. Aliquots were removed every 15 s and the reaction was assayed for a total of 75 s. Kinetic data were analyzed by Lineweaver–Burk double reciprocal plots.

Molecular modeling studies. These studies were performed on a Silicon Graphics, Inc. Indigo R4000 workstation, using the molecular modeling software Biosym/Insight/Discover, available from Molecular Simulations, Inc., San Diego, California. The X-ray coordinates of rabbit muscle glycogen phosphorylase *b* (Gp*b*, T-state) were imported from the Brookhaven National Laboratory Protein Data Bank. The ligands were energy-minimized to convergence, and then docked into Gp*b*, followed by energy-minimization of the ligand-Gp*b* complexes. During minimization of the complexes, all atoms that were 15 Å or further from the ligand were fixed with a temperature constant of 300 K. No constraints were applied to the remaining residues in and around the ligand site. There were no morse or cross terms. Each complex was minimized to convergence using consecutive Steepest Descent, Conjugate Gradient, and Newton–Raphson energy minimization protocols. The final computational parameters for each of the energy-minimized complexes are as follows: Gp*b*–**2** complex: Total Insight Energy-13.811129 kcal., Average Absolute Derivative 0.0001895, Stand. Dev. of Abs. Deriv. 0.0001518, and RMS Deriv. 0.0002428; Gp*b*–**VII** Complex: Total Insight Energy-144.167913 kcal, Average Absolute Derivative 0.0176970, Stand. Dev. of Abs. Deriv. 0.0212888, and RMS Deriv. 0.0276839.

To mimic the natural environment, each of the above energy-minimized complexes was soaked in a layer of water, 5 Å thick, all around the unconstrained residues, stretching in a radius of 15 Å from the site of ligand. Energy minimizations to convergence of the aquated protein–ligand complexes were carried out as described before for the unhydrated species.

The femto-second molecular dynamics simulations were carried out on each of the above aquated complexes at 300 K (1000 iterations). The graphs of energy versus frame were generated from the respective dynamics trajectories.

Single-crystal X-ray diffraction analyses. A colorless crystal of **2** was mounted on a Nicolet *R3m/V* diffractometer. Final unit cell parameters were obtained by least-squares fit of the angles of 24 accurately centered reflections ($16° < 2\theta < 26°$). Intensity data were collected in the range of $3.0° \leq 2\theta \leq 50.0°$ at −43 °C using graphite monochromated $M_o{\cdot}K_\alpha$ ($\lambda = 0.71073$ Å) radiation. The scan was θ/2θ, 1696 reflections were collected with 1497 unique with $R_{int} = 0.0150$. Three standard reflections monitored after every 150 reflections did not show any significant change in intensity during the data collection. Data were collected for Lorentz and polarization effects, but not for absorption. The structure was solved by direct-methods with the *SHELXTL*-Plus package.[19] Full-matrix least-squares refinement was performed. Scattering factors were taken from the *International Tables for X-ray Crystallography*.[20] Hydrogen atoms were located on DF maps, and refined at fixed isotropic temperature factors (U = 0.08 Å^2). The weight had the form $\omega = [\sigma^2(F_o) + g(F_o)^2]^{-1}$ where g = 0.00065. The final cycles of refinement converged at $R = \Sigma||F_o|-|F_c||/\Sigma|F_o| = 0.0299$, $\omega R = [\Sigma\omega(|F_o|-|F_c|)^2/\Sigma\omega(F_o)^2]^{1/2} = 0.0636$, GOF = 1.067 for observed reflections [$I > 3.0\sigma(I)$]. Maximum and minimum residuals of 0.142 and −0.183 e/Å^3 were shown on the final difference Fourier maps.

Crystallographic data for 2. $C_8H_{12}N_2O_5$, $M_r = 216.20$, $a = 4.8680$ (10) Å, $\alpha = 90°$, $b = 18.039$ (4) Å, $\beta = 93.21°$ (3), $c = 10.481$ (2) Å, $\gamma = 90°$, $V = 918.9$ (3) Å^3, $Z = 4$, $\rho = 1.563$ g·cm^{-3}, $(M_o \cdot K_\alpha) = 0.71073$ Å. Final $R = 0.0254$ for 1470 reflections [$I > 3.0\sigma(I)$].

Acknowledgements

This paper is dedicated to Professor Nelson J. Leonard on the occasion of his 80th birthday. The research was supported by grants (#RO1 GM49249 and #RO1 CA 71079) from the National Institutes of Health. We thank Dr Hongming Zhang and Prof. Narayan S. Hosmane of the Department of Chemistry, Southern Methodist University, Dallas, Texas, for single-crystal X-ray diffraction analyses of Compound **1**. The Michigan State University Mass Spectrometry Facility was supported in part from a grant (# P41RR00480-0053) from the National Institutes of Health.

Supporting information available: X-ray crystallographic data, including tables of positional parameters, bond distances, bond angles, torsional angles, and anisotropic displacement parameters (9 pages). This information can be obtained directly from the senior author marked by an asterisk on the title page.

References and Notes

1. (a) For a recent review on glycogen phosphorylase *b*, see Livanova, N. B.; Kornilaev, B. A. *Biochemistry* (Moscow) **1996**, *61*, 1432. (b) For a minireview on glycogen phosphorylase, see Johnson, L. N.; Barford, D. *J. Biol. Chem.* **1990**, *265*, 2409.

2. (a) Ortmeyer, H. K.; Bodkin, N. L.; Hansen, B. C. *Am. J. Physiol.* **1997**, *272*, E133. (b) Barford, D.; Johnson, L. N. *Nature* (London) **1989**, *340*, 609. (c) Johnson, L. N.; Barford, D. *FASEB J.* **1992**, *6*, 2274. (d) Barford, D.; Hu, S.-H.; Johnson, L. N. *J. Mol. Biol.* **1991**, *218*, 233. (e) Childress, C. C.; Sacktor, B. *J. Biol. Chem.* **1970**, *245*, 2927. (f) Andreeva, I. E.; Makeeva, V. F.; Livanova, N. B.; Kurganov, B. I. *Biochemistry* (Moscow) **1995**, *60*, 1009. (g) Johnson, L. N.; O'Reilly, M. *Curr. Opin. Struct. Biol.* **1996**, *6*, 762. (h) Lin, K.; Hwang, P. K.; Fletterick, R. J. *J. Biol. Chem.* **1995**, *270*, 26833.

3. (a) Carabaza, A.; Ciudad, C. J.; Baque, S.; Guinovart, J. J. *FEBS Lett.* **1992**, *296*, 211. (b) Pugazhenthi, S.; Yu, B.; Gali, R. R.; Khandelwal, R. L. *Biochim. Biophys. Acta* **1993**, *1179*, 271. (c) Johnson, D. F.; Moorhead, G.; Caudwell, F. B.; Cohen, P.; Chen, Y. H.; Chen, M. X.; Cohen, P. T. W. *Eur. J. Biochem.* **1996**, *239*, 317.

4. (a) Maddaiah, V. T.; Madsen, N. B. *J. Biol. Chem.* **1966**, *241*, 3873. (b) Oikonomakos, N. G.; Zographos, S. E.; Johnson, L. N.; Papageorgiou, A. C.; Acharya, K. R. *J. Mol. Biol.* **1995**, *254*, 900. (c) Brickey, D. A.; Naranan, V.; Sucic, J. F.; Rutherford, C. L. *Mol. Cell. Biochem.* **1990**, *97*, 17. (d) Johnson, L.N.; Snape, P.; Martin, J. L.; Acharya, K. R.; Barford, D.; Oikonomakos, N. G. *J. Mol. Biol.* **1993**, *232*, 253. (e) Juan-Serrano, F. S.; Fernandez-Gonzalez, M.; Sanchez-Lopez, J. L.; Garcia-Martin, L. O. *Comp. Biochem. Physiol.* **1993**, *106B*, 925. (f) Helmreich, E. J. M. *BioFactors* **1992**, *3*, 159. (g) Perutz, M. F. *Quarterly Reviews of Biophysics* **1989**, *22*, 172. (h) Johnson, L. N. *Carlsberg Res. Commun.* **1989**, *54*, 203. (i) Oikonomakos, N. G.; Johnson, L. N.; Acharya, K. R.; Stuart, D. I.; Barford, D.; Hajdu, J.; Varvill, K. M.; Melpidou, A. E.; Papageorgiou, A. C.; Graves, D. J.; Palm, D. *Biochemistry* **1987**, *26*, 8381. (j) Sprang, S. R.; Goldsmith, E. J.; Fletterick, R. J.; Withers, S. G.; Madsen, N. B. *Biochemistry* **1982**, *21*, 5364.

5. (a) Helmreich, E.; Cori, C. F. *Proc. Nat. Acad. Sci.* **1964**, *51*, 131. (b) Carabaza, A.; Ricart, M. D.; Mor, A.; Guinovart, J. J.; Ciudad, C. J. *J. Biol. Chem.* **1990**, *265*, 2724. (c) Sprang, S. R.; Withers, S. G.; Goldsmith, E. J.; Fletterick, R. J.; Madsen, N. B. *Science* **1991**, *254*, 1367.

6. Browner, M. F.; Hackos, D.; Fletterick, R. *Struct. Biol.* **1994**, *1*, 327.

7. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* **1979**, *28*, 1039.

8. WHO Expert Committee. Second report on diabetes mellitus. WHO Technical Report Series. No. 646, Geneva, World Health Organization, 1980.

9. (a) Reaven, G. M.; Moore, J.; Greenfield, M. *Diabetes* **1983**, *32*, 601. (b) Ginsberg, H. et al . *J. Clin. Invest.* **1975**, *55*, 454. (c) DeFronzo, R. A.; Ferrannini, E.; Koivisto, V. *Am. J. Med.* **1983**, *74(supplement 1A)*, 52. (d) Kelterman, O. G. et al. *J. Clin. Invest.* **1981**, *68*, 957. (e) Bogardus, C. et al. *J. Clin. Invest.* **1984**, *74*, 1238. (f) DeFronzo, R. A. Lilly Lecture 1987. The triumvirate β cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* **1988**, *37*, 667.

10. Osei, K. In *Diabetes, Clinical Science in Practice*; Leslie, R. D. G.; Robbins, D. C., Eds; Cambridge University: 1995; Chapter 12.

11. (a) Bichard, C. J. F.; Mitchell, E. P.; Wormald, M. R.; Watson, K. A.; Johnson, L. N.; Zographos, S. E.; Koutra, D. D.; Oikonomakos, N. G.; Fleet, G. W. J. *Tetrahedron Lett.* **1995**, *36*, 2145. (b) Martin, J. L.; Veluraja, K.; Ross, K.; Johnson, L. N.; Fleet, G. W. J.; Ramsden, N. G.; Bruce, I.; Orchard, M. G.; Oikonomakos, N. G.; Papageorgiou, A. C.; Leonidas, D. D.; Tsitoura, H. S. *Biochemistry* **1991**, *30*, 10101. (c) Watson, K. A.; Mitchell, E. P.; Johnson, L. N.; Son, J. C.; Bichard, C. J. F.; Orchard, M. G.; Fleet, G. W. J.; Oikonomakos, N. G.; Leonidas, D. D.; Kontou, M.; Papageorgiou, A. C. *Biochemistry* **1994**, *33*, 5745. (d) Papageorgiou, A. C.; Oikonomakos, N. G.; Leonidas, D. D. *Arch. Biochem. Biophys.* **1989**, *27*, 376. (e) Board, M.; Hadwen, M.; Johnson, L. N. *Eur. J. Biochem.* **1995**, *228*, 753. (f) Board, M.; Bollen, M.; Stalmans, W.; Kim, Yl; Fleet, G. W. J.; Johnson, L. N. *Biochem. J.* **1995**, *311*, 845. (g) Martin, J. L.; Johnson, L. N.; Withers, S. G. *Biochemsitry* **1990**, *29*, 10745. (h) Martin, J. L.; Veluraja, K.; Ross, K.; Johnson, L. N.; et al. *Biochemistry* **1991**, *30*, 10101. (i) Bichard, C. J. F.; Mitchell, E. P.; Wormald, M. R.; Watson, K. A.; Johnson, L. N. et al. *Tetrahedron Lett.* **1995**, *36*, 2145. (j) Krülle, T. M.; Watson, K. A.; Gregoriou, M.; Johnson, L. N. et al. *Tetrahedron Lett.* **1995**, *36*, 8291.

12. (a) Nakajma, M.; Itoi, K.; Takamatsu, Y.; Kinashita, T.; Okasaki, T.; Kawakubo, K.; Shindou, M.; Honma, T.;

Tohigamori, M.; Haneishi, T. *J. Antibiot*. **1991**, *44*, 293. (b) Takahashi, S.; Nakajima, M.; Kinoshita, T; Haruyama, H.; Sugai, S.; Honma, T.; Sato, S.; Haneishi, T. In *Natural and Engineered Pest Management Agents*; American Chemical Society, 1994; Chapter 6. (c) Hanessian, S.; Sancéau, J.-Y.; Chemla, P. *Tetrahedron* **1995**, *51*, 6669. (d) Fairbanks, A. J.; Fleet, G. W. *Tetrahedron* **1995**, *51*, 3881. (e) Nakajima, N.; Kirihara, M.; Matsumoto, M.; Hashimoto, M.; Katoh, T.; Terashima, S. *Heterocycles* **1996**, *42*, 503. (f) Haruyama, H,; Takayama, T.; Kinoshita, T.; Kondo, M.; Nakajima, M.; Haneishi, T. *J. Chem. Soc. Perkin Trans*. **1991**, *1*, 1637.

13. Mio, S.; Sano, H.; Shindou, M.; Honma, T.; Sugai, S. *Agric. Biol. Chem*. **1991**, *55*, 1105.

14. Meanwell, N. A.; Roth, H. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. *J. Org. Chem*. **1991**, *56*, 6897.

15. (a) Nicolaou, K. C.; Magolda, R. L.; Sipio, W. J.; Barnette, W. E.; Lysenka, Z.; Jowlie, M. M. *J. Am. Chem. Soc*. **1980**, *102*, 3784. (b) Nicolaou, K. C. *Tetrahedron* **1981**, *37*, 4097.

16. (a) Mio, S.; Ichinose, R.; Goto, K.; Sugai, S. *Tetrahedron* **1991**, *47*, 2111. (b) Mio, S.; Kumagawa, Y.; Sugai, S. *Tetrahedron* **1991**, *47*, 2133. (c) Chemla, P. *Tetrahedron Lett*. **1993**, *34*, 7391.

17. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem*. **1978**, *43*, 2923.

18. Fiske, E.H.; Subbarow, Y. *J. Biol. Chem*. **1925**, *66*, 375.

19. Sheldrick, G. M., *SHELXTL-Plus*, **1990**, Siemans Analytical X-ray Instruments, Inc., USA.

20. *International Tables for X-ray Crystallography*, **1974**, *Vol. IV*, Birmingham, Kynock Press.