Selective Inhibition of *Trypanosoma brucei* 6-Phosphogluconate Dehydrogenase by High-Energy Intermediate and Transition-State Analogues

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Two series of compounds were designed to mimic the transition state and high-energy intermediates (HEI) of the enzymatic reaction of 6-phosphogluconate dehydrogenase (6PGDH). Sulfoxide analogues (**7**−**11**) were designed to mimic the transition state during the oxidation of the substrate to 3-keto-6-phosphogluconate, an enzyme-bound intermediate of the enzyme. Hydroxamate and amide derivatives of D-erythronic acid were designed to mimic the 1,2-*cis*-enediol HEI of the 6PGDH reaction. These two series of compounds were assayed as competitive inhibitors of the *Trypanosoma brucei* and sheep liver enzymes, and their selectivity value (ratio sheep/parasite) was calculated. The sulfoxide transition-state analogues showed weak and selective inhibition of the *T. brucei* enzyme. The hydroxamic derivatives showed potent and selective inhibition of the *T. brucei* 6PGDH with a K_i in the nanomolar range.

Introduction

Human African trypanosomiasis is an important disease that has recently become resurgent in Africa. The disease is caused by parasitic protozoa of the *brucei* group of the genus *Trypanosoma*. In our search for new antitrypanosomal agents, we decided to target 6-phosphogluconate dehydrogenase (6PGDH), whose essential nature to growth of the bloodstream form of *T. brucei* has been recently demonstrated by RNA interference.[1] 6PGDH is the third enzyme of the pentose phosphate pathway[2] (PPP), which generates NADPH and ribulose-5-phosphate by oxidative decarboxylation of 6-phosphogluconate (6PG) (Figure 1). Hence, 6PGDH helps to maintain a pool of NADPH, which serves to protect the parasite against oxidative stress, and it generates carbohydrate intermediates used in nucleotide and other biosynthetic pathways.

We have previously reported the design and synthesis of noncarbohydrate substrate analogues of 6PGDH as potential inhibitors of this enzyme.[3,4] Such deoxy and/or protected analogues of 6PG were designed as potential inhibitors with improved pharmacokinetic properties compared to phosphorylated carbohydrates, which are unlikely to possess good druglike characteristics due to metabolic instability and impermeability at biological membranes. However, such strategy afforded only a few weak inhibitors with K_i values larger than the K_m value of 6PG.[3,4] These results prompted us to use another approach: that of transition-state analogues and analogues of high-energy intermediates formed during the reaction. It has been postulated and proved in many cases that such analogues may be good enzyme inhibitors. This is because enzymes increase reaction rates by stabilizing the transition state and/or high-energy intermediates.[5,6]

According to the mechanism of 6PGDH described in the literature,[7,8] there are two intermediate species in the enzyme reaction (Figure 1): a 3-keto-6PG entity and a putative 1,2-enediol high-energy intermediate (HEI). The recent report of some phosphate, phosphonate, and hydroxamate sugar derivatives showing selective inhibition of 6PGDH from *T. brucei* over the sheep liver enzyme,[9] and especially the powerful and selective inhibition displayed by 4-phospho-D-erythronate ($K_{i(T.brucei)} = 0.13\ \mu M$ and $K_{i(sheep)} = 10.7\ \mu M$), reinforced the idea that analogues of the enediol intermediate could give potent inhibitors of 6PGDH. We present in this paper the design and synthesis of two series of inhibitors (i.e., sulfoxide and hydroxamate derivatives) mimicking the transition-state species of 6PGDH (Figure 1).

Inhibitor Design. The "sulfoxide" analogues were designed to mimic the polarization of the C=O bond in the 3-keto-6PG species (Figure 1). Site-directed mutagenesis and crystallographic studies suggest that the carbonyl group of the 3-keto-6PG intermediate accepts a proton from the general acid of the enzymatic reaction (i.e., Lys183 and Glu190 of the sheep liver 6PGDH and Lys185 of the *T. brucei* enzyme), which facilitates the decarboxylation step.[10] Because 2-deoxy-3-keto-6PG was previously found to bind 6PGDH from *T. brucei* with high affinity and competitively inhibit the oxidation of 6PG,[11] we considered the preparation of 2,4-dideoxy sulfoxide TSA (**11**) in which the sulfoxide group would mimic the polarization of the C=O bond. Moreover, the tetrahedral geometry around the sulfur atom renders the C3-position more alike to an sp^3 hybridized carbon, such as in the proposed transition state (Figure 2). Some protected analogues and the thioether analogues (com-

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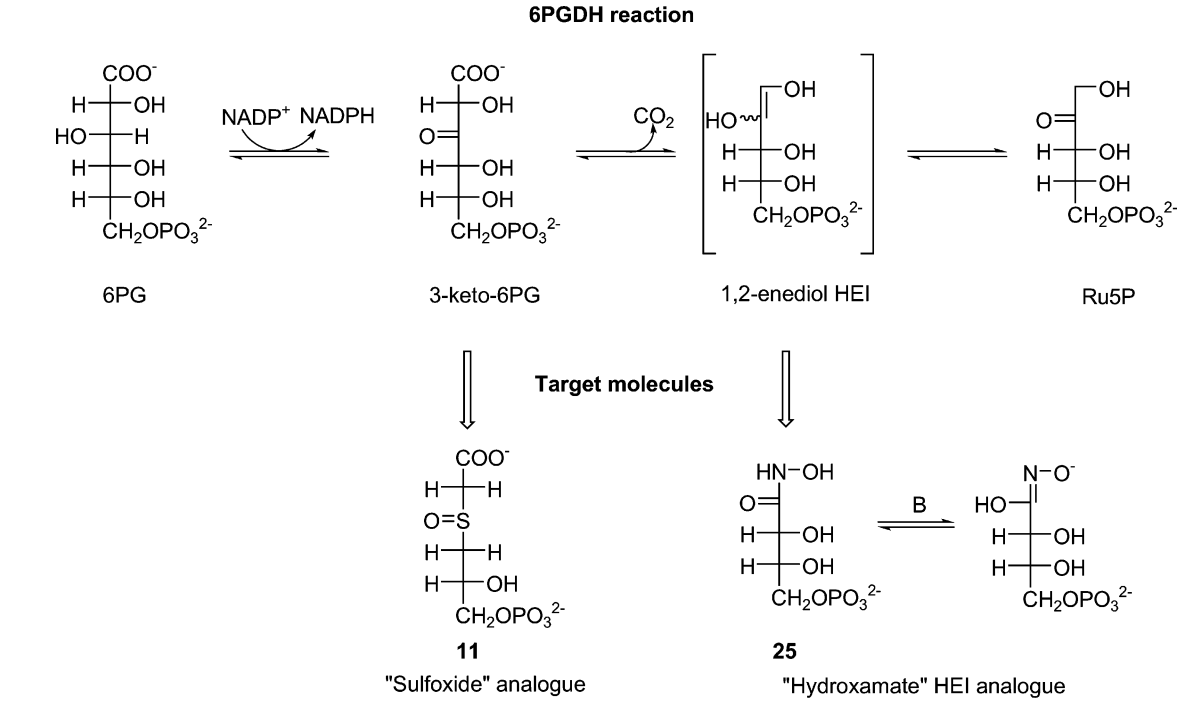


Figure 1. 6PGDH reaction and target compounds.

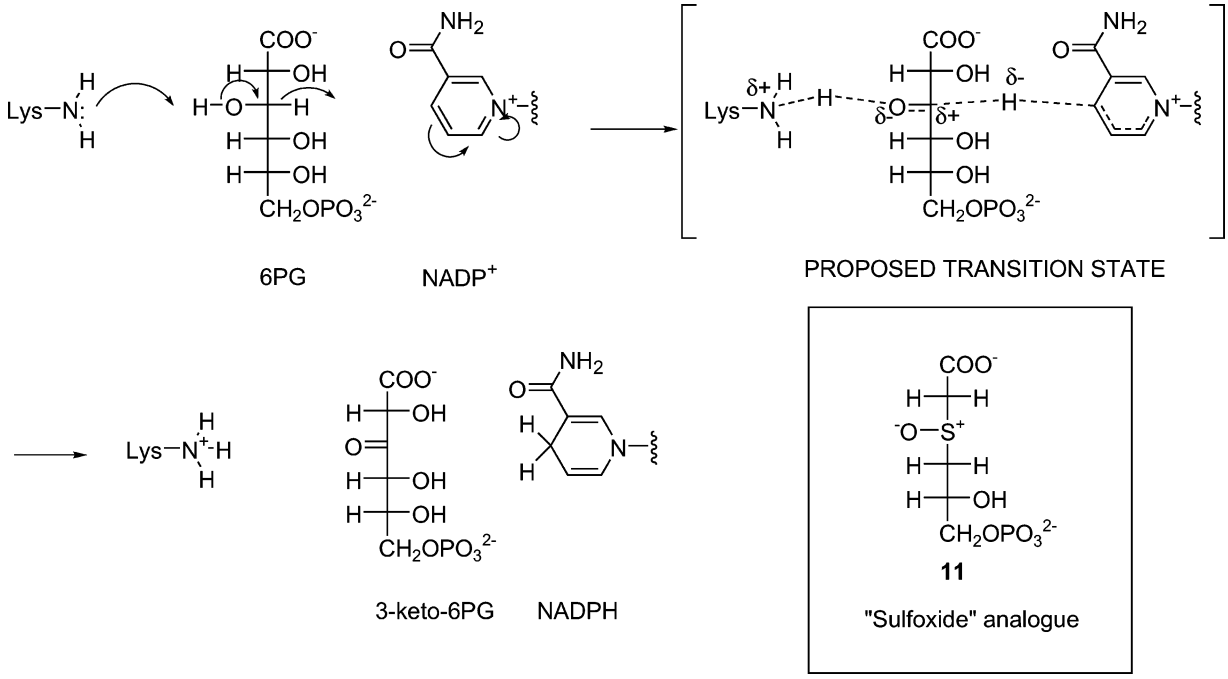


Figure 2. Proposed transition state for oxidation of 6PG and the sulfoxide mimic.

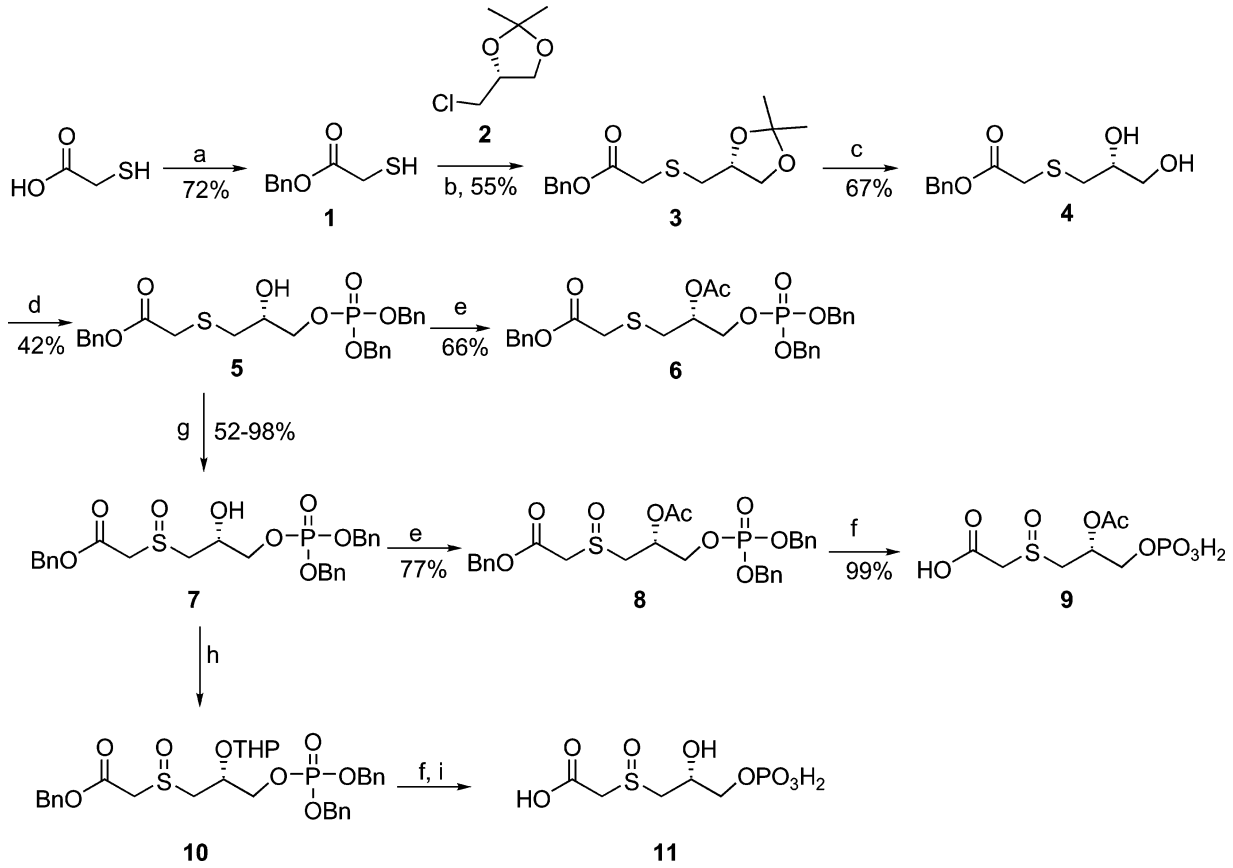
pounds **5** and **6**, Scheme 1) in which the sulfur atom is not oxidized (i.e., only H-bond acceptor) were also prepared as a means of comparison.

The use of the hydroxamate function to closely mimic the 1,2-*cis*-enediolate HEI (with the O_1, N, C_2, and O_2 coplanar) has been reported on many occasions. Especially, the synthesis and inhibition of 5-phosphate-D-arabinohydroxamic acid derivatives as potent TSA inhibitors of 6-phosphate-D-glucose isomerases was described by Salmon et al.[12] D-Arabinohydroxamic acid-5-phosphate was a potent competitive inhibitor of phosphoglucose isomerase from *T. brucei* ($K_i = 50$ nM).[13] Our hypothesis was that 4-phospho-D-erythronohydroxamic acid **25** (Figure 1) should mimic the 1,2-enediol HEI of the 6PGDH reaction (i.e., the enol of ribulose-5-phosphate). We designed an efficient synthetic route to protected 4-phospho-D-erythronohydroxamic acid derivatives (Schemes 2 and 3) that allowed the preparation of **25** (five steps, 23.1% overall)[14] and also a series of protected hydroxamate (**18**−**21** and **24**) and amide (**22** and **23**) derivatives with potentially better pharmacokinetic properties (Scheme 2).

Results and Discussion

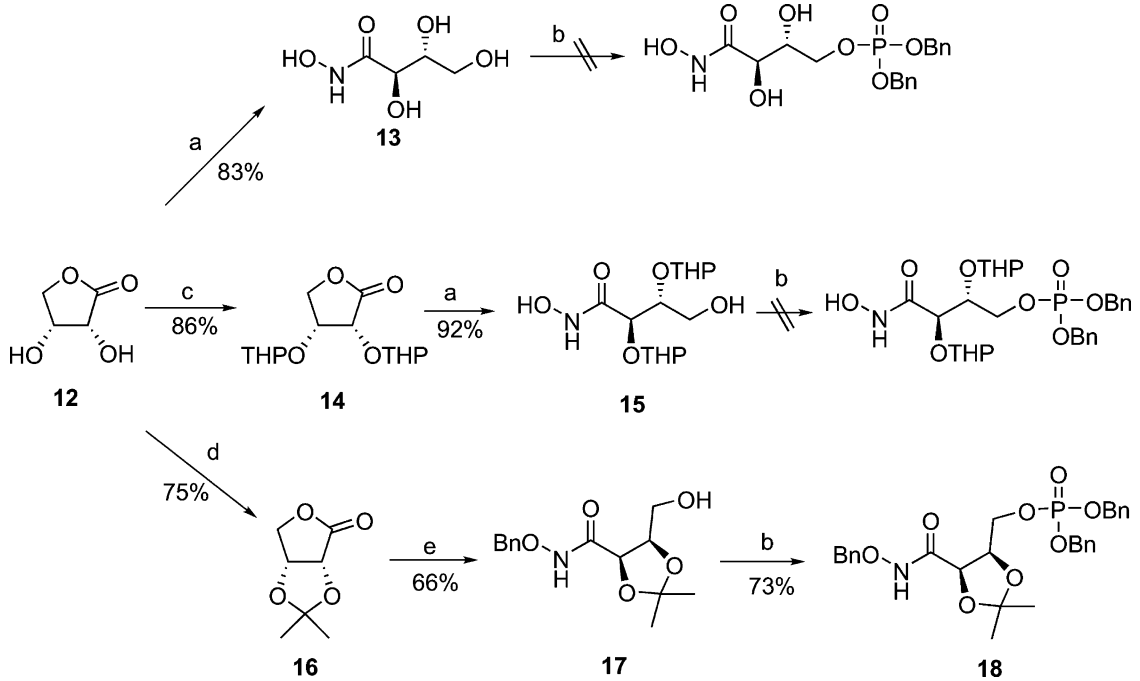
Chemistry. A. Synthesis of [(2*R*)-2-Hydroxy-3-phosphonooxypropane-1-sulfinyl]acetic Acid Analogues (Scheme 1). Mercaptoacetic acid benzyl ester **1**, obtained following a reported procedure,[15] was re-

Scheme 1[a]



[a] (a) BnOH, K_2CO_3; (b) **2**, K_2CO_3, DMF; (c) $CuCl_2 \cdot 2H_2O$, *i*-PrOH; (d) $(BnO)_3P$, I_2, CH_2Cl_2, −78 °C; (e) Ac_2O, DMAP, CH_2Cl_2, pyridine; (f) H_2, Pd−C 5%, MeOH; (g) 30% H_2O_2, AcOH, CH_3CN; (h) 3,4-DHP, PPTS, CH_2Cl_2; (i) HCl.

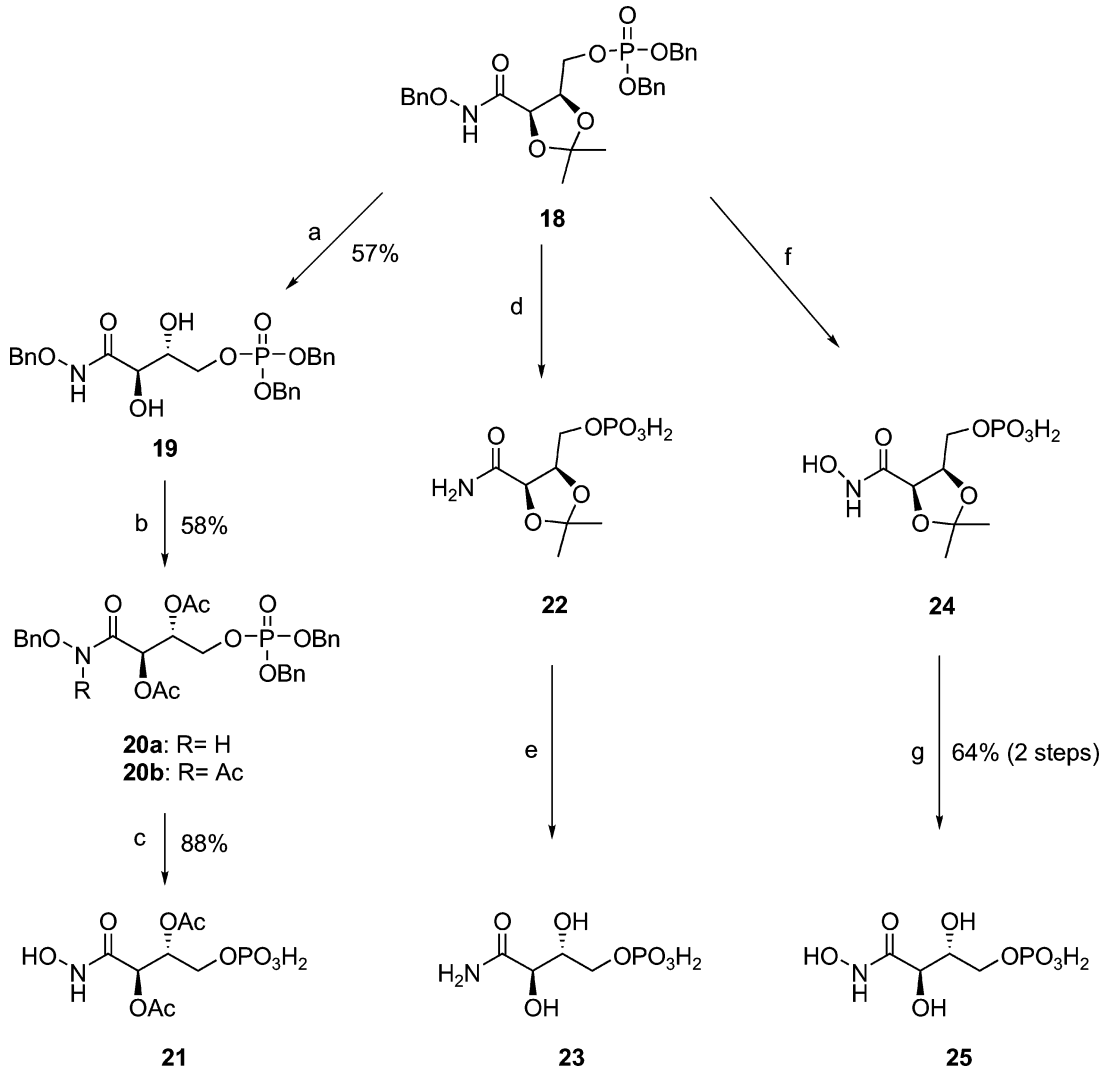
Scheme 2[a]



[a] (a) NH_2OH, 50 % H_2O; (b) $(BnO)_3P$, I_2, CH_2Cl_2, −78 °C; (c) DHP, PPTS, CH_2Cl_2; (d) acetone, $(MeO)_2CMe_2$, PPTS; (e) $AlMe_3$, $BnONH_2$, CH_2Cl_2.

acted with the enantiomerically pure chloro derivative **2** in the presence of K_2CO_3 to afford **3** in 55% yield. Thioether **3** was subsequently deprotected with copper chloride[16] to give **4** (85%). Diol **4** was phosphorylated selectively on the primary alcohol function with dibenzylphosphoiodinate (BnO_3P/I_2) working at low temperature to yield **5** (42%). Selective oxidation of the sulfur atom of **5** was achieved with 30% H_2O_2/AcOH[17] at 0 °C

Scheme 3[a]



[a] (a) Dowex 50WX8, H_2O, MeOH; (b) Ac_2O, DMAP, pyridine; (c) 10% Pd−C, H_2, MeOH, 2.5 h; (d) Raney Ni, H_2, MeOH, 20 h; (e) HCl (pH 2), CH_3CN, 24 h; (f) 20% $Pd(OH)_2$-C, H_2, MeOH, 24 h; (g) CH_3CN, H_2O, 24 h.

(CH_3CN was used as a co-solvent to avoid freezing the reaction mixture), affording the sulfoxide **7** as a racemic mixture of two diastereoisomers (as shown by ^1H NMR). Acetylation of the alcohols **5** and **7** with Ac_2O/DMAP/pyridine afforded **6** and **8**, respectively. Hydrogenolysis of **8** with 5% Pd-C afforded **9** quantitatively. Sulfoxide **11** was obtained by reductive deprotection followed by mild acidic hydrolysis of its precursor **10**.

B. Synthesis of Hydroxamic Acid Analogues (Schemes 2 and 3). This synthetic strategy allowed the preparation of various analogues (e.g., acetate-protected, amide derivative) starting from the commercially available D-erythronic-γ-lactone (**12**) as chiral synthon.

Following the method of Salmon et al.,[18] the treatment of D-erythronic-γ-lactone **12** with aqueous hydroxylamine afforded cleanly the hydroxamic acid **13** (83%; Scheme 2). Using the same conditions, the THP-protected lactone **14** was transformed into the hydroxamate **15** in excellent yield (92%). Various attempts of direct phosphorylation of the unprotected hydroxamates **13** and **15** with dibenzylphosphoiodinate were unsuccessful and led to untreatable mixtures. Thus, an alternative strategy consisting in the use of benzyl-protected hydroxylamine was used. Treatment of D-erythronic-γ-lactone **16**[19] with $BnONH_2$ in CH_2Cl_2 for 30 min, followed by addition of Me_3Al at room temperature,[20] afforded the corresponding γ-hydroxybenzylhydroxamate **17** (66%). Alcohol **17** was successfully phosphorylated with an excess of dibenzylphosphoiodinate in CH_2Cl_2, affording the fully protected intermediate **18** in good yield (73%).

Removal of the acetylidene protecting group of **18** (Scheme 3) was achieved by treatment with acidic resin (either Dowex 50WX8-200 or Amberlite IR-120) in MeOH−H_2O to give the diol **19** in moderate yield (∼50%). It should be noted that such deprotection was rather slow (2−3 days) and did not occur with the copper chloride method. Diol **19** was acetylated with Ac_2O/DMAP/pyridine, affording the diacetylated compound **20a**. When 4 equiv of Ac_2O was used, the triacetylated compound **20b** was also isolated as a side product (22%). The acetate **20a** was cleanly hydrogenated with 10% Pd−C/MeOH to afford **21** in good yield (88%).

The amide analogue **23** was obtained by hydrogenolysis of the protected hydroxamate **18** with Raney nickel followed by acidic hydrolysis of the benzylidene moiety. The pure product was isolated by crystallization from EtOH.

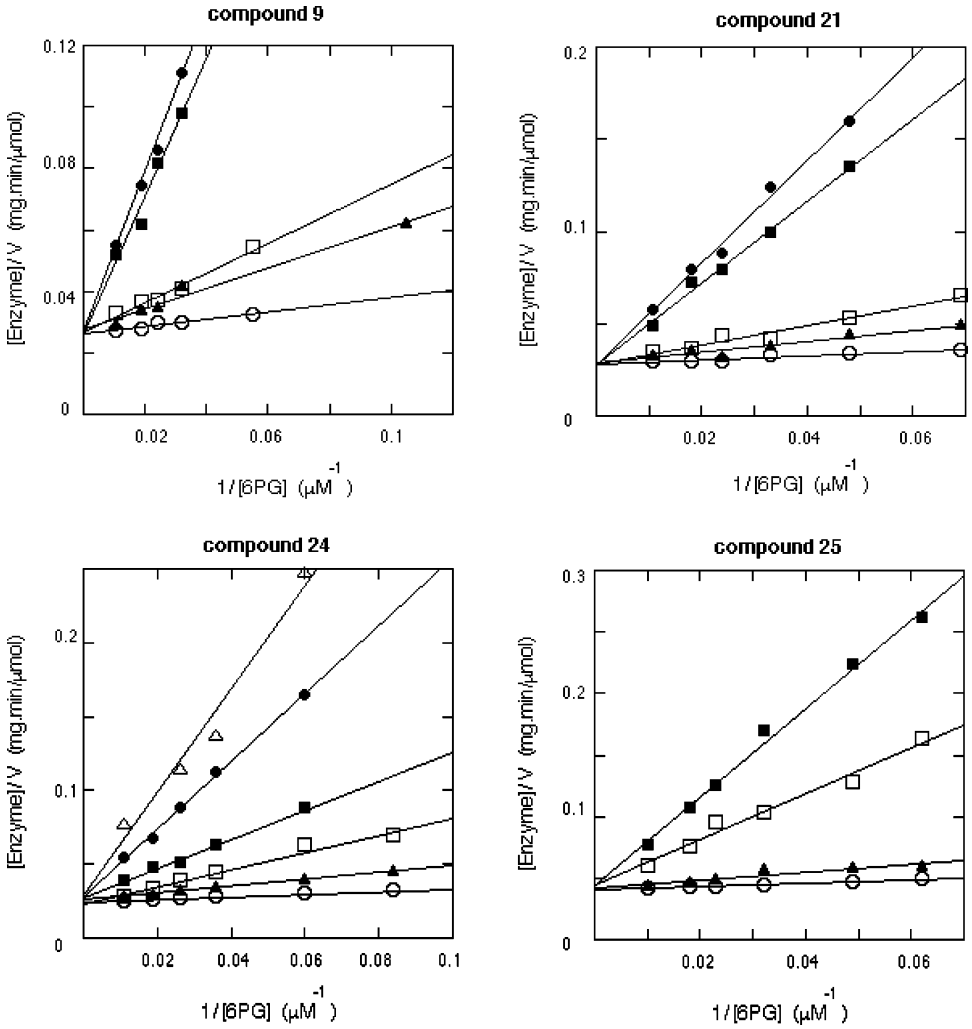


Figure 3. Inhibition of *T. brucei* 6PGDH as represented by a double-reciprocal plot of initial reaction velocity versus 6PG concentration obtained at various inhibitor concentrations (Lineweaver−Burk graphical representation): ○, no inhibitor in all of the plots; compound **9**, (▲) 10 μM, (□) 20 μM, (■) 100 μM, (●) 200 μM; compound **21**, (▲) 0.2 μM, (□) 0.5 μM, (■) 1 μM, (●) 2 μM; compound **24**, (▲) 0.05 μM, (□) 0.1 μM, (■) 0.2 μM, (●) 0.5 μM, (△) 1 μM; compound **25**, (▲) 0.1 μM, (□) 0.2 μM, (■) 0.5 μM.

The hydroxamate analogue **25** was obtained by reductive deprotection of the benzyl protecting group with a palladium catalyst followed by hydrolysis of the benzylidene protecting group (Scheme 3). Because the formation of the amide derivative (i.e., **22**) was a potential side reaction,[21] various catalysts (e.g., 5% Pd−C, 10% Pd−C, 5% Pd−$BaSO_4$) and reaction times were tried. Interestingly, the use of 20% palladium hydroxide gave the best results (no amide side product was observed), and the hydroxamate **24** was obtained quantitatively. The hydrolysis of the isopropylidene moiety of **24** occurred naturally in water without adding any inorganic acid. This behavior is possibly due to the acidity of the phosphate (e.g., the pH of an aqueous solution of **24** is ∼1−2).

Biological Results. A. Inhibition of *T. brucei* and Sheep Liver 6PGDH. Compounds were assayed against the *T. brucei* and sheep enzymes, and their selectivity ratio was calculated (Table 1). Examples of inhibition plots are shown in Figure 3.

B. Sulfoxide Derivatives. Of the sulfoxide inhibitors, the fully deprotected transition-state analogue **11** was a weak inhibitor of 6PGDH ($K_{i(T.brucei)}$ = 198 μM; $K_{i(sheep)}$ = 784 μM). The compound was selective for the *T. brucei* enzyme. The 5-OH acetate analogue **9** was a more potent inhibitor and showed greater selectivity. The reason for this greater activity is not clear, but is presumably due to an additional interaction with the acetate in the *T. brucei* active site. The inhibitory activities of these compounds show that analogues with protected phosphates (**7**, **8**, and **10**) were not active.

C. Hydroxamic Acid and Amide Derivatives. The hydroxamic derivatives **21**, **24**, and **25** were the most potent competitive inhibitors of 6PGDH with K_i values in the nanomolar range and selectivity ratios (*T. brucei*/sheep) of 4.5, 31, and 254, respectively (Table 1). The lack of inhibition of compound **13** shows the importance of the phosphate group for the interaction with the active site of the enzyme. Interestingly, in compounds **21** and **24** the 2- and 3-hydroxyl groups were protected. This may indicate that the hydroxyl groups are not essential for binding. The amide analogues **22** and **23** also inhibited both enzymes, with **23** being very potent and selective for *T. brucei* (26-fold). The amide **22** (acetylidene-protected analogue of **23**) was 300 times less effective as an inhibitor than **23**, with a lower selectivity for the *T. brucei* enzyme.

Only one of the fully protected hydroxamic acid analogues (i.e., **20b**) displayed some inhibition (of mixed type). Compound **20b**, fully protected with acetates and

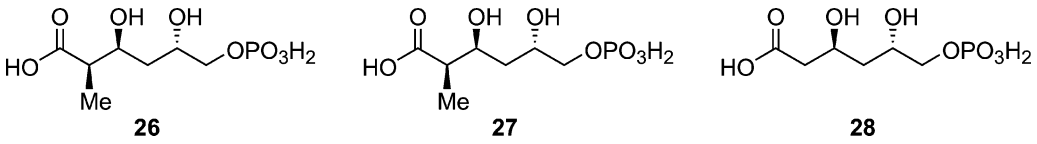


Figure 4. Analogues showing no activity.

Table 1. 6PGDH Inhibition[a]

compd	K_i versus substrate (μM)		selectivity (ratio sheep/ *T. brucei*)
	T. brucei	sheep	
6PG	3.5[b]	16[b]	
2-deoxy-6PG	4.4[c]	770[c]	
4-phospho-D-erythronate	0.13[d]	10.7[d]	83
7	NI[e]	NI	
8	NI	NI	
9	40	625	15.6
10	NI	NI	
11	198	784	4.0
20a	NI	NI	
20b	109	192	1.76
21	0.08	0.36	4.5
22	588	714	1.21
23	1.52	39.16	25.7
24	0.035	1.1	31.4
25	0.01	2.54	254

[a] All compounds were assayed for inhibition of 6PGDH. Only compounds displaying inhibition are shown; Lineweaver−Burk plots for which the standard errors of linear regressions were below 0.05 are an estimate of the accuracy of the enzyme inhibition assays. [b] K_M values from ref 11. [c] K_i values from ref 9. [d] K_i values from ref 9. [e] NI = no inhibition.

Table 2. In Vitro Antiparasitic Activity

compd	*T. brucei* IC_{50}[a] (μM)	*T. cruzi* IC_{50}[b] (μM)	*L. donovani* IC_{50}[c] (μM)	*P. falciparum* IC_{50} (μM)	cytotoxicity L6 cells IC_{50} (μM)
3	>304	215	>101	>169	na[d]
4	75	212	>117	>195	na
5	47	124	23	65	na
6	137	>161	28	62	na
7	82	>169	>56	>94	na
8	36	>157	27	18	60
9	113	>296	>99	>164	na
10	73	82	na	7	91
11	244	>343	na	>191	343
12	na	na	na	na	na
13	116	290	>199	18	na
14	113	>315	>105	78	na
15	61	>282	>94	~157	na
16	262	>569	>190	>316	na
17	3.5	>32	30	53	37
18	126	>166	>55	>92	na
19	130	>179	33	>100	na
20a	60	>154	>17	>85	na
20b	49	>143	>48	>80	na
21	229	>285	na	>159	>285
22	212	131	na	>196	>220
23	277	>418	>139	>232	>418
24	>332	>332	>111	>184	>332
25	>389	>389	>130	>216	269

[a] Control: melarsoprol, IC_{50} = 0.0055 μM. [b] Control: benznidazole, IC_{50} = 1.076 μM. [c] Control: pentamidine, IC_{50} =9.3 μM. [d] Data not available.

benzyl protecting groups, was a weak inhibitor of both 6PGDH enzymes with a low selectivity (1.76-fold) for the parasite enzyme.

In Vitro Toxicity. The results of the in vitro toxicity against three related trypanosomatid parasites (*T. brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*) and against the malaria parasite *Plasmodium falciparum* are reported in Table 2. Four compounds (**5**, **8**, **17**, and **20b**) showed significant toxicity (IC_{50} < 50 μM) against *T. brucei*; five compounds (**5**, **6**, **8**, **17**, and **19**) were also significantly active against *L. donovani* (IC_{50} < 35 μM), and three compounds (**8**, **10**, and **13**) had interesting antiplasmodium activity (IC_{50} < 20 μM). None of the tested compounds had a significant toxicity toward *T. cruzi*.

Only one 6PGDH inhibitor (**20b**) had trypanocidal activity against *T. brucei*. This result could possibly be explained by the highly hydrophilic character of the hydroxamate and sulfoxide analogues studied that would prevent their penetration inside the cells. The higher lipophilicity of **20b** may allow this molecule to cross the membranes and reach its intracellular target. However, more experimental data are needed to check that the in vitro toxicity of this compound is the result of 6PGDH inhibition.

Discussion

The sulfoxides (**7**−**11**) were designed to be transition-state analogues. They have proved to be weak inhibitors of 6PGDH. In previous studies,[4] we have shown that the substrate analogues (**26**−**28**, Figure 4) are not inhibitors of the enzyme at concentrations of up to 1 mM nor are they substrates of the enzyme. This is presumably due to the necessity of the 2- and/or 4-OH groups for interaction with the enzyme. In the analogue **11**, these hydroxyl groups (2- and 4-OH) are not present, yet the compound causes significant inhibition of the enzyme. This implies that the presence of the sulfoxide group may somehow compensate for the lack of interaction at the 2- and 4-positions.

The hydroxamic acid analogues **21**, **24**, and **25** were very potent inhibitors of *T. brucei* 6PGDH with K_i values of 0.08, 0.035, and 0.01 μM, respectively, lower than the K_m of the substrate 6PG (K_m = 3.5 μM).[11] These results are in agreement with the fact that these compounds probably mimic the 1,2-enediol transition state postulated for the 6PGDH reaction. As expected, the order of potency for these inhibitors is hydroxamate > acid > amide. The amide analogue **23** was a 150-fold weaker inhibitor than the hydroxamic acid **25** and a 12-fold weaker inhibitor than the acid 4-phospho-D-erythronate (Table 1).

Protection of the hydroxyls of **25** with acetate groups or with an isopropylidene group reduced the potency of inhibition by 8-fold in compound **21** and reduced selectivity in favor of the *T. brucei* enzyme. In the case of the isopropylidene-protected **24**, the observed inhibition on *T. brucei* and sheep 6PGDH (0.035 and 1.1 μM, respectively) is very similar to the inhibition observed for the unprotected **25** (0.01 and 2.54 μM). Because we observed that the hydrolysis of the acetylidene protecting group of **24** occurred very easily in aqueous media (vide infra), it is possible that **24** was partially hydrolyzed during the assays so the measured inhibition would in reality be that of the hydrolyzed compound (i.e., **25**). However, the diacetate (**21**), which is more stable, did not suffer from these problems.

When protected in compounds **21** and **24**, the oxygen atoms at the 2- and 3-positions can act as hydrogen bond

acceptors, but not hydrogen bond donors. The relatively small reduction in activity compared to compound **25** may imply that the 2- and 3-hydroxyls act as hydrogen bond acceptors. However, care has to be taken in this interpretation as there is a large reduction in activity for the amide compound (**23**) when the hydroxyls are blocked with an isopropylidene group (**22**). Another possible explanation would be the enhanced conformational constraints imposed by the protecting groups that would prevent the compound from adopting the optimum binding conformation in the active site.

The lack of significant in vitro activity against the parasites and the lack of correlation between enzyme inhibition and antiparasitic toxicity are likely to be due to the charged nature of the compounds preventing or reducing cellular permeation. This is a common feature of phosphorylated or other charged molecules.

Nevertheless, these represent good lead compounds for the development of 6PGDH inhibitors as potential anti-parasitics. They will require modification to give cellular uptake and metabolic stability.

Conclusion

Transition-state and high-energy intermediate analogues of intermediates in the 6-phosphogluconate dehydrogenase reaction are potent inhibitors of this enzyme. The analogues **9**, **21**, and **23**−**25** appear to have selectivity toward the trypanosomal enzyme, which is known to be essential to these parasites. They therefore represent excellent leads to develop as potential drugs, although the compounds need to be adapted in a way that increases cellular permeability if they are to reach their target in situ.

Experimental Section

Chemistry. All reaction solvents were purchased anhydrous from Aldrich Chemical Co. and used as received. Reactions were monitored by TLC using precoated silica gel 60 F254 plates. Chromatography was performed with silica gel (220−240 mesh) or with Isolute prepacked columns. All reactions requiring anhydrous conditions or an inert atmosphere were performed under a positive pressure of N_2. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded at 300, 75, and 121 MHz, respectively.

Synthesis of "Sulfoxide" Analogues. A. Mercaptoacetic Acid Benzyl Ester (1).[15] A flask fitted with a Dean−Stark apparatus containing a solution of mercaptoacetic acid (8 mL, 65.6 mmol), benzyl alcohol (6.8 mL, 65.8 mmol), and concentrated sulfuric acid (3 drops) in toluene (200 mL) was heated to reflux for 2 h. The solvent was removed under reduced pressure, and the product was purified by distillation under reduced pressure (high-vacuum pump). The fraction with bp 78−88 °C (bp 86 °C) contained the pure benzyl ester (8.6 g, 72%): colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.4 (s, 5H), 5.22 (s, 2H), 3.35 (d, 2H), 2.1 (t, 1H).

B. [(4*R*)-2,2-Dimethyl-[1,3]dioxolan-4-ylmethylsulfanyl]acetic Acid Benzyl Ester (3). A mixture of **1** (1.34 g, 7.36 mmol), (4*R*)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane (1 mL, 7.36 mmol), and K_2CO_3 (1.5 g, 11 mmol) in DMF (12 mL) was stirred at room temperature for 2 days. The reaction mixture was diluted with CH_2Cl_2 and filtered on Celite. The filtrate was concentrated under reduced pressure, and the product was purified by chromatography (Isolute 20 g SI) with hexane/EtOAc, 0 → 15%, colorless oil (1.2 g, 55%); ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 5H), 5.22 (s, 2H), 4.32 (quint, 1H, J = 6.2 Hz), 4.1 (dd, 1H, J = 6.1 Hz, J = 8.3 Hz), 3.73 (dd, 1H, J = 6.3 Hz, J = 8.3 Hz), 3.4 (dd, 2H, J = 14.9 Hz, J = 23.4 Hz), 2.9 (dd,1H, J = 6.2 Hz, J = 13.6 Hz), 2.78 (dd, 1H, J = 6.2 Hz, J = 13.6 Hz), 1.46 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6 (s), 135.9 (s), 129.1 (d), 128.9 (d), 128.8 (d), 110.1 (s), 75.7 (d), 69.1 (t), 67.6 (t), 35.9 (t), 34.5 (t), 27.3 (q), 26.0 (q); $[\alpha]^{23}_D$ = +12.17 (*c* 2.3, MeOH); MS (ES^+) *m/z* 319 [M + Na]; Anal. ($C_{15}H_{20}O_4S$) C, H.

C. [(2*R*)-2,3-Dihydroxypropylsulfanyl]acetic Acid Benzyl Ester (4). A mixture of **3** (617 mg, 2.1 mmol) and $CuCl_2 \cdot 2H_2O$ (1.7 g, 10.4 mmol) in ^{i}PrOH (20 mL) was stirred at room temperature for 3.5 h. The reaction was quenched with 1 M Na_2CO_3 (10.5 mL). When the evolution of CO_2 stopped, the mixture was diluted with ^{i}PrOH and filtered on a pad of Celite. The filtered cake was rinsed with ^{i}PrOH. The solvent was removed under reduced pressure, and the resulting residue was partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc (3 × 20 mL). Organic extracts were dried ($MgSO_4$) and concentrated. Chromatography (Isolute 5 g SI) with hexane/EtOAc, 0 → 100%, afforded the product as a colorless oil (360 mg, 67%): ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 5H), 5.22 (s, 2H), 3.83 (m, 1H), 3.72 (dd, 1H, J = 3.4 Hz, J = 11.4 Hz), 3.55 (dd, 2H, J = 6.1 Hz, J = 11.4 Hz), 3.38 (d, 2H, J = 1.6 Hz), 2.83 (dd, 2H, J = 4.5 Hz, J = 14 Hz), 2.73 (dd, 1H, J = 8.1 Hz, J = 14 Hz); ^{13}C NMR ($CDCl_3$) δ 171.4 (s), 135.7 (s), 129.1 (d), 129.0 (d), 128.9 (d), 70.9 (d), 67.9 (t), 65.7 (t), 36.8 (t), 34.6 (t); $[\alpha]^{24}_D$ = −12.16 (*c* 3.7, MeOH); MS (ES^+), *m/z* 279 [M + Na]; Anal. ($C_{12}H_{16}O_4S$/0.5 H_2O) C, H.

D. [3-(Bis(benzyloxy)phosphoryloxy)-(2*R*)-2-hydroxypropane-1-sulfinyl]acetic Acid Benzyl Ester (5). A 0.31 M solution of dibenzylphosphoiodinate[3] (1.5 mL, 0.46 mmol) in CH_2Cl_2 was added dropwise at −78 °C to a solution of **4** (100 mg, 0.39 mmol) and pyridine (0.1 mL, 1.2 mmol) in CH_2Cl_2 (2 mL). After 1 h, another 0.17 mmol of dibenzylphosphoiodinate was added. The reaction was stirred for 1 h at −78 °C and poured into water. The organic phase was separated, dried ($MgSO_4$), and concentrated. Chromatography (Isolute 5 g SI) with hexane/EtOAc, 0 → 50%, afforded the product as a colorless oil (43 mg, 21%): ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 15 H), 5.15 (s, 2H), 5.07 (m, 2H), 5.05 (m, 2H), 4.0 (dd, 2H, J = 5 Hz, J = 8 Hz), 3.88 (m, 1H), 3.31 (s, 2H), 2.74 (dd, 1H, J = 4.9 Hz, J = 14.1 Hz), 2.64 (dd, 1H, J = 7.3 Hz, J = 14.1 Hz); ^{13}C NMR ($CDCl_3$) δ 171 (s), 136.1 (s), 136.0 (s), 135.7 (s), 129.1 (2 × d), 129.0 (d), 128.9 (d), 128.5 (d), 70.4 (d, J_{C-P} = 6 Hz), 70.11 (d, J_{C-P} = 1.5 Hz), 70.03 (d, J_{C-P} = 1.5 Hz), 69.8 (d, J_{C-P} = 6.7 Hz), 67.8 (t), 36.1 (t), 34.7 (t); ^{31}P NMR ($CDCl_3$) δ +1.05; MS (ES^+), *m/z* 539 [M + Na]; $[\alpha]^{21}_D$ = −3.49 (*c* 0.86, CH_2Cl_2); Anal. ($C_{26}H_{29}O_7PS$/ 2.9 H_2O) C, H.

E. [(2*R*)-2-Acetoxy-3-(bis(benzyloxy)phosphoryloxy)-propylsulfanyl]acetic Acid Benzyl Ester (6). A solution of **5** (70 mg, 0.136 mmol), acetic anhydride (45 μL, 0.5 mmol), and DMAP (5 mg) in pyridine (1 mL) was stirred for 18 h at room temperature. The solvent was removed in vacuo, and the crude oil was chromatographed (Isolute 2 g SI) with hexane/EtOAc, 0 → 50%, to afford **6** as an oil (50 mg, 66%): R_f 0.46 (50% EtOAc in hexane); ^{1}H NMR ($CDCl_3$) δ 7.4 (br s, 15H), 5.21 (s, 2H), 5.17−5.05 (m, 5H), 4.2−4.1 (m, 2H), 3.36 ($^1/_2$ ABX, J = 14.9 Hz, 1H), 3.3 ($^1/_2$ ABX, J = 14.9 Hz, 1H), 2.82 (ABX, 2H, J = 6.2, 6.7, 14.3 Hz), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6 (s), 170.3 (s), 136.0 (s), 135.99 (s), 135.8 (s), 129.1 (d), 128.9 (d), 128.8 (d), 128.4 (d), 70.8 (d, J_{C-P} = 7.5 Hz), 69.93 (d, J_{C-P} = 5.2 Hz), 67.7 (t), 67.0 (d, J_{C-P} = 5.2 Hz), 34.1 (t), 32.4 (t), 21.3 (q); ^{31}P NMR ($CDCl_3$) δ +0.24; MS (ES^+), *m/z* 581 [M + Na]; $[\alpha]^{21}_D$ = +5.77 (*c* 0.52, CH_2Cl_2); ESHRMS, *m/z* 576.1813 [M + NH_4] ($C_{28}H_{35}NO_8PS$ requires 576.1821).

F. [3-(Bis(benzyloxy)phosphoryloxy)-(2*R*)-2-hydroxypropane-1-sulfinyl]acetic Acid Benzyl Ester (7). To a solution of **5** (110 mg, 0.21 mmol) in AcOH/CH_3CN (1.5 mL/ 0.5 mL) cooled to 0 °C was added a solution of 30% H_2O_2 in water (0.1 mL, 1 mmol). The reaction was stirred for 4.5 h at 0 °C and poured into an aqueous 5% $NaHCO_3$ solution. The reaction was partitioned between water and CH_2Cl_2, and the aqueous phase was extracted (2 × 15 mL) with the same solvent. The combined organic extracts were dried ($MgSO_4$) and concentrated to give a crude oil that was pure enough for use in the next synthetic step (110 mg, 98%). For analytic purpose, the product was purified by chromatography (Isolute 2 g SI) with hexane/EtOAc, 0→ 100%: colorless oil; R_f 0.41 (100% EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.4 (br s, 15H), 5.25 (s, 2H),

5.18−5.02 (m, 4H), 4.55−4.3 (m, 1H), 4.15−3.95 (m, 2H), 3.95−3.7 (m, 2H), 3.1−2.85 (m, 2H), 2.15 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 165.6 (s), 165.3 (s), 136.0 (s), 135.9 (s), 135.2 (s), 129.2 (CH), 129.1 (CH), 129.0 (2 × CH), 128.6 (CH), 128.5 (CH), 71.1 (d, CH_2, J = 6 Hz), 70.95 (d, CH_2, J = 6 Hz), 70.2 (m, CH_2), 68.26 (CH_2), 68.24 (CH_2), 65.54 (d, CH, J = 6 Hz), 65.2 (d, CH, J = 6 Hz), 56.6 (CH_2), 56.1 (CH_2), 55.8 (CH_2), 53.5 (CH_2); ^{31}P NMR ($CDCl_3$) δ +0.8; MS (ES^+), *m/z* 555 [M + Na]; Anal. ($C_{26}H_{29}O_8PS/1H_2O$) C; Calcd H, 5.13.; S, 5.82; P, 5.62. Found: H, 4.63; S, 5.08; P, 6.36.

G. [(2*R*)-2-Acetoxy-3-(bis(benzyloxy)phosphoryloxy)-propane-1-sulfinyl]acetic Acid Benzyl Ester (8). A solution of **7** (45 mg, 0.084 mmol), acetic anhydride (20 μl, 0.2 mmol), and DMAP (3 mg, 0.24 mmol) in pyridine (1 mL) was stirred for 19 h at room temperature. The solvent was removed in vacuo, and the crude oil was partitioned between CH_2Cl_2 and saturated NH_4Cl aqueous solution. The aqueous phase was extracted with CH_2Cl_2 (two times). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography (Isolute 1 g SI) with hexane/EtOAc, 0 → 50%, afforded **8** as a yellowish oil (37 mg, 77%): R_f 0.55 (100% EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.4 (br s, 15H), 5.38 (m, 1H), 5.24 (m, 2H), 5.18−5.0 (m, 4.5H), 4.4−4.0 (m, 2.5H), 3.79 (ABX, J = 14.1 and 31.6 Hz, 1H), 3.73 (ABX, J = 14 and 17.7 Hz, 1H), 3.1 (m, 2H), 2.04 (d, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4 (s), 170.2 (s), 165.1 (s), 165.05 (s), 136.0 (s), 135.9 (s), 135.1 (s), 129.19 (d), 129.16 (d), 129.13 (d), 129.0 (d), 128.5 (d), 70.05 (d, J_{C-P} = 5.6 Hz), 68.38 (t), 68.35 (t), 67.7 (d, J_{C-P} = 5.4 Hz), 67.45 (d, J_{C-P} = 5.6 Hz), 67.4 (d, J_{C-P} = 7.4 Hz), 66.95 (d, J_{C-P} = 7.3 Hz), 56.8 (t), 55.8 (t), 54.3 (t), 52.7 (t), 21.23 (q), 21.15 (q); ^{31}P NMR ($CDCl_3$) δ +0.33, +0.23; MS (ES^+), *m/z* 597 [M + Na]; Anal. ($C_{28}H_{31}O_9PS/0.5\ H_2O$) C, H; Calcd: S, 5.49; P, 5.31. Found: S, 4.87; P, 4.03.

H. [(2*R*)-2-Acetoxy-3-phosphonooxypropane-1-sulfinyl]acetic acid (9). Hydrogenolysis of a solution of **8** (25 mg/0.043 mmol) in MeOH with 5% Pd−C (15 mg) for 20 min afforded **9** as a colorless oil (13 mg, 99%): ^{1}H NMR (D_2O) δ 5.4−5.2 (m, 1H), 4.1−3.85 (m, 3H), 3.8 (d, 1H), 3.35−3.1 (m, 2H), 1.98 (d, 3H); ^{13}C NMR (D_2O) δ 173.4, 173.34, 169.1, 168.9, 68.8, 68.7, 67.5, 67.4, 65.91 (J_{C-P} = 5.2 Hz), 65.8 (J_{C-P} = 5.2 Hz), 52.5, 51.8, 20.7, 20.5; ^{31}P NMR (D_2O) δ +1.13; MS (ES^-), *m/z* 303 [M − H]; ESHRMS, *m/z* 302.9941 ($C_7H_{12}O_9PS$ requires 302.9940).

I. (2*R*)-[3-(Bis(benzyloxy)phosphoryloxy)-2-(tetrahydropyran-2-yloxy)propane-1-sulfinyl]acetic Acid (10). A solution of **7** (110 mg, 0.21 mmol), 3,4-dihydropyran (1 mL, 9 mmol), and PPTS (10 mg, 0.04 mmol) in CH_2Cl_2 (1.5 mL) was stirred at room temperature for 30 h. The reaction was quenched with a few drops of Et_3N, and the solvent was removed under reduced pressure. Chromatography (Isolute 2 g SI column) with hexane/EtOAc, 0 → 100%, afforded the product as a colorless oil (103 mg, 79%): R_f 0.12 (hexane/EtOAc, 1/2); ^{1}H NMR ($CDCl_3$) δ 7.4 (br s, 15H), 5.22 (m, 2H), 5.2−5.0 (m, 4H), 4.82−4.65 (m, 1H), 4.42−4.18 (m, 2H), 4.1−3.7 (m, 4H), 3.5 (m, 1H), 3.25−2.9 (m, 2H), 1.85−1.4 (m, 6H); ^{13}C NMR (D_2O) δ 165.6 (s), 165.5 (2 × s), 165.4 (s), 136.2 (s), 136.1 (2 × s), 136.0 (s), 135.3 (s), 135.2 (s), 129.1 (2 × d), 129.0 (d), 128.9 (d), 128.5 (d), 101.8 (d), 99.9 (d), 99.2 (d), 96.5 (d), 72.1 (d, J = 7 Hz), 70.9 (d, J = 7 Hz), 69.9 (m, CH_2), 69.6 (d, J = 6 Hz), 69.4 (t, J = 6 Hz), 69.0 (t, J = 6 Hz), 68.2 (t, J = 6 Hz), 68.15 (t, J = 6 Hz), 67.6 (t, J = 6.7 Hz), 67.05 (t, J = 6 Hz), 64.2 (t), 63.5 (t), 63.3 (t), 63.1 (t), 57.2 (t), 56.8 (t), 56.3 (t), 56.2 (t), 55.6 (t), 54.5 (t), 52.9 (t), 31.1 (t), 31.0 (2 × t), 29.5 (t), 25.5 (t), 20.4 (t), 20.1 (t), 19.9 (t), 19.6 (t); ^{31}P NMR (D_2O) δ +0.47, +0.34, +0.18; MS (ES^+), *m/z* 640 [M + Na].

J. (2*R*)-(2-Hydroxy-3-phosphonooxypropane-1-sulfinyl)acetic Acid (11). Hydrogenolysis of a solution of **10** (80 mg, 0.13 mmol) in MeOH (5 mL) with 5% Pd−C (50 mg) for 20 min afforded a colorless oil. The oil was dissolved in H_2O and stirred at room temperature overnight. The solvent was removed in vacuo. Ether-mediated precipitation of the crude product dissolved in a little MeOH afforded **11** as a white powder (15 mg, 44%): ^{1}H NMR (CD_3OD) δ 4.25 (m, 1H), 4.15−3.70 (br m, 4H), 3.1 (m, 2H); ^{13}C NMR (CD_3OD) δ 169.9 (s), 168.9 (s), 71.1 (m, t), 70.8 (m, t), 67.2 (m, d), 66.6 (m, d), 58.0 (t), 56.3 (t); ^{31}P NMR (D_2O) δ +1.16 (br); MS (ES^-), *m/z* 261 [M − H]; ESHRMS, *m/z* 260.9836 ($C_5H_{10}O_8PS$ requires 260.9839).

Synthesis of Hydroxamic Acid Analogues. A. (2*R*,3*R*)-2,3,4,*N*-Tetrahydroxybutyramide (13). Fifty percent aqueous hydroxylamine (0.3 mL, 5 mmol) was added to a solution of D-erythronic acid-γ-lactone (126 mg, 1.07 mmol) in water (1.7 mL). After 20 min at room temperature, the solvent was evaporated under reduced pressure to dryness (bath temperature of 25 °C). To remove the excess of hydroxylamine, water was added to the residue and evaporated again under high vacuum. The oily residue was triturated with cold EtOH to give a white gum. The product was crystallized with EtOH and rinsed with Et_2O: white solid (126 mg, 83%); $[\alpha]^{20}_D$ = +3.75 (*c* 0.8, H_2O); ^{1}H NMR (D_2O) δ 4.03 (br s, 1H), 3.75 (br s, 1H), 3.5 (br s, 2H); ^{13}C NMR (D_2O) δ 170.6 (s), 72.6 (d), 71.5 (d), 61.8 (t); Anal. ($C_4H_9NO_5$) C, H, N.

B. (3*R*,4*R*)-3,4-Di(tetrahydro-2*H*-2-pyranyloxy)tetrahydro-2-furanone (14). A solution of D-erythronic acid-γ-lactone (565 mg, 4.8 mmol), 3,4-dihydropyran (4.3 mL, 48 mmol), and PPTS (360 mg, 1.4 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h. Et_3N was added to the reaction mixture (0.2 mL, 1.4 mmol), and the solvent was removed under reduced pressure. Chromatography (Isolute 25 g SI column prewashed with CH_2Cl_2/Et_3N and sequentially rinsed with CH_2Cl_2 and hexane) with hexane/EtOAc, 0 → 40%, afforded the product as a colorless oil that solidified on standing (1.18 g, 86%): ^{1}H NMR ($CDCl_3$) δ 5.05 (t, 1H), 4.92 (t, 1H), 4.5 (m, 2H), 4.32 (m, 2H), 3.9−3.7 (m, 2H), 3.55−3.4 (m, 2H), 1.9−1.35 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 174.5 (s), 99.7 (d), 98.8 (d), 73.1 (d), 72.4 (d), 71.6 (t), 62.95 (t), 62.86 (t), 30.46 (t), 30.43 (t), 25.7 (t), 25.65 (t), 19.43 (t), 19.37 (t); MS (ES^+), *m/z* 309 [M + Na].

C. 4,*N*-Dihydroxy-(2*R*,3*R*)-2,3-bis(tetrahydropyran-2-yloxy)butyramide (15). Fifty percent aqueous hydroxylamine (0.6 mL, 10 mmol) was added to a solution of **14** (290 mg, 1 mmol) in THF (7 mL). After 18 h at room temperature, the solvent was evaporated under reduced pressure. To remove the excess of hydroxylamine, water was added and evaporated again. The product was dried under high vacuum: ^{1}H NMR ($CDCl_3$) δ 4.9−4.65 (m, 2H), 4.55−4.4 (m, 1H), 4.2−3.5 (m, 7H), 2.0−1.5 (br m, 12H); ^{13}C NMR ($CDCl_3$) δ 167.8 (s), 167.4 (s), 167.0 (s), 166.8 (s), 99.4 (d), 99.1 (d), 96.5 (d), 96.0 (d), 79.3 (d), 78.7 (d), 75.3 (d), 75.2 (d), 74.4 (d), 73.3 (d), 63.0 (d), 62.4 (d), 61.3 (d), 61.1 (d), 61.0 (d), 60.5 (d), 58.9 (d), 30.0 (t), 29.8 (t), 29.7 (t), 29.5 (t), 29.3 (t), 29.1 (t), 29.0 (t), 24.3 (t), 24.2 (t), 20.0 (d), 19.3 (t), 18.8 (t), 18.5 (t), 18.2 (t), 18.0 (t), 17.7 (t), 17.5 (t); MS(ES^-), *m/z* 318 [M − H]; ESHRMS, *m/z* 320.1703 ($C_{14}H_{26}O_7N$ requires 320.1709).

D. (3a*R*,6a*R*)-2,2-Dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4-one (16). A solution of D-erythronic acid-γ-lactone (3.015 g, 25.5 mmol), 2,2-dimethoxypropane (10 mL, 81 mmol), and PPTS (1 g, 4 mmol) was stirred for 20 h at room temperature. The reaction was quenched with Et_3N (0.6 mL), and the volatiles were removed under reduced pressure. Chromatography (Isolute 50 g SI) with hexane/EtOAc, 0 → 50%, afforded the product as a white solid (2.85 g, 71%): ^{1}H NMR ($CDCl_3$) δ 4.94 (dd, 1H, J = 3.6 and 5.6 Hz), 4.8 (d, 1H, J = 5.6 Hz), 4.53 (d, 1H, J = 10.8 Hz), 4.46 (dd, 1H, J = 11 and 3.6 Hz), 1.55 (s, 3H), 1.46 (s, 3H); $[\alpha]^{24}_D$ = −113.3 (*c* 0.3, H_2O) [Lit.[22] −103.4 (*c* 1, H_2O)].

E. (4*R*,5*R*)-5-Hydroxymethyl-2,2-dimethyl-[1,3]dioxolane-4-carboxylic Acid Benzyloxyamide (17). Regeneration of $BnONH_2$ Base. A solution of *O*-benzylhydroxylamine hydrochloride (2.18 g, 13.6 mmol) in MeOH (10 mL) was treated at 0 °C with a MeONa methanolic solution (700 mg, 13 mmol, 10 mL MeOH). After 1 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and filtered. The mother liquor was concentrated under reduced pressure, and the residue was treated with hot EtOAc. The solution was filtered, and the mother liquor was concentrated to give *O*-benzylhydroxylamine as a free base (1.6 g, 13 mmol). A mixture of lactone **16** (1.43 g, 9 mmol) and $BnONH_2$ (1.6 g, 13 mmol) in CH_2Cl_2

was stirred for 30 min at room temperature. The reaction was cooled to 0 °C, and a 2 M solution of Me_3Al in hexane (5 mL, 9.9 mmol) was added dropwise. The reaction was stirred for 1.5 h at room temperature and poured into a 5% aqueous $NaHCO_3$ solution. The thick aluminum salt precipitate was washed several times with CH_2Cl_2 and MeOH. The washings were concentrated under reduced pressure, and the crude residue was dissolved in EtOAc and dried with $MgSO_4$. Chromatography (Isolute 25 g SI) with hexane/EtOAc, 0 → 50%, afforded the product as a colorless solid (1.4 g, 56%): mp 111−112 °C; ^{1}H NMR ($CDCl_3$) δ 9.0 (br s, 1H), 7.45 (m, 5H), 4.97 (2H, ABX, J = 11 and 17.8 Hz), 4.62 (d, 1H, J = 7.6 Hz), 4.55 (m, 1H), 3.85 (br, 1H), 3.70 (dd, 1H, J = 7.1 and 11.9 Hz), 3.28 (br, 1H), 1.39 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.9 (s), 135.1 (s), 129.7 (d), 129.5 (d), 129.1 (d), 110.6 (s), 79.0 (t), 78.0 (t), 76.7 (d), 61.6 (t), 26.9 (q), 24.7 (q); $[\alpha]^{21}_D$ = −50 (*c* 0.48, $CHCl_3$); MS(ES$^+$), *m/z* 304 [M + Na]; Anal. ($C_{14}H_{19}NO_5$) C, H, N.

F. Phosphoric Acid Dibenzyl Ester (4*R*,5*R*)-5-Benzyloxycarbamoyl-2,2-dimethyl-[1,3]dioxolan-4-ylmethyl Ester (18). A solution of dibenzylphosphoiodinate[3] (1.8 mmol, 1.4 equiv) in CH_2Cl_2 (2.5 mL) was added at −78 °C to a solution of alcohol **17** (343 mg, 1.2 mmol) and pyridine (0.29 mL, 3.7 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 1.5 h at −78 °C and diluted with CH_2Cl_2. The precipitate was filtered off and the solution concentrated under reduced pressure. Chromatography (Isolute 20 g SI) with hexane/EtOAc, 0 → 70%, afforded the phosphate **18** as a colorless oil (480 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 8.88 (s, 1H, NH), 7.5−7.3 (m, 15H), 5.08 (d, 2H, J = 2.1 Hz), 5.05 (d, 2H, J = 2.1 Hz), 4.9 (ABX, 2H, J = 11 and 18.8 Hz), 4.65−4.5 (m, 2H), 4.35 (m, 1H), 4.2−4.0 (m, 1H), 1.41 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.2 (s), 136.3 (s), 136.2 (s), 135.2 (s), 129.7 (d), 129.3 (d), 129.1 (d), 129.0 (d), 128.9 (d), 128.4 (2 × d), 111.1 (s), 79.0 (t), 76.35 (d, J_{C-P} = 7.5 Hz), 75.4 (d), 69.75 (t, J_{C-P} = 7.5 Hz), 69.7 (t), 66.35 (t, J_{C-P} = 7.5 Hz), 27.0 (q), 24.9 (q); ^{31}P NMR ($CDCl_3$) δ −0.08; $[\alpha]^{24}_D$ = +20 (*c* 0.2, CH_2Cl_2); MS(ES$^+$), *m/z* 564 [M + Na]; Anal. ($C_{28}H_{32}NO_8P$/0.3 H_2O) C, H, N, P.

G. Phosphoric Acid Dibenzyl Ester (2*R*,3*R*)-3-Benzyloxycarbamoyl-2,3-dihydroxypropyl Ester (19). A solution of **18** (48 mg, 0.09 mmol) in MeOH (5 mL) was stirred with Dowex 50WX8-200 (1.5 mL in H_2O) at room temperature for 48 h. The resin was filtered off, and the solvent was removed under reduced pressure. The residue dissolved in EtOAc was dried ($MgSO_4$) and concentrated. Chromatography (Isolute 1 g SI) with hexane/EtOAc, 0 → 100%, gave the diol **19** as a colorless oil (25 mg, 57%): R_f 0.13 (25% EtOAc in hexane); ^{1}H NMR ($CDCl_3$) δ 9.55 (br, 1H), 7.6−7.3 (m, 15H), 5.2−5.05 (m, 4H), 5.02 (s, 2H), 4.35 (td, 1H, J = 3.5 and 11.5 Hz), 4.2 (td, 1H, J = 2.1 and 11.5 Hz), 4.07 (d, 1H, J = 8.9 Hz), 3.87 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 170.9 (s), 135.6 (s), 135.2 (s), 129.6 (d), 129.4 (d), 129.3 (d), 129.2 (d), 129.1 (2 × d), 128.5 (2 × d), 78.9 (t), 72.4 (CH, d), 70.6 (t, J_{C-P} = 5.2 Hz), 70.5 (t, J_{C-P} = 5.2 Hz), 68.8 (d), 68.3 (t); ^{31}P NMR ($CDCl_3$) δ +2.47; $[\alpha]^{22}_D$ = +8 (*c* 0.5, CH_2Cl_2); MS(ES$^+$), *m/z* 524 [M + Na]; ESHRMS, *m/z* 524.1453 ($C_{25}H_{28}NO_8NaP$ requires 524.1450).

H. Acetic Acid (1*R*,2*R*)-2-Acetoxy-1-benzyloxycarbamoyl-3-(bis(benzyloxy)phosphoryloxy)propyl Ester (20a). A solution of **19** (66 mg, 0.132 mmol), acetic anhydride (27 μL, 0.3 mmol), and DMAP (3 mg) in pyridine (1.5 mL) was stirred for 15 h at room temperature. The solvent was removed under reduced pressure, and the crude oil was chromatographed (Isolute 2 g SI) with hexane/EtOAc, 0 → 80%, to afford **20a** as an oil (45 mg, 58%): ^{1}H NMR ($CDCl_3$) δ 8.8 (br, 1H), 7.5−7.25 (m, 15H), 5.4 (m, 1H), 5.2 (m, 1H), 5.15−5.0 (m, 4H), 4.95 (s, 2H), 4.22 (m, 2H), 2.1 (s, 3H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.0, 169.7, 164.1, 135.9, 135.2, 129.8, 129.4, 129.1, 129.0, 128.5, 128.45, 78.8, 77.7, 70.83 (J_{C-P} = 7.5 Hz), 70.06 (J_{C-P} = 5.7 Hz), 64.7 (J_{C-P} = 7.5 Hz), 21.1, 20.9; ^{31}P NMR ($CDCl_3$) δ +0.32; $[\alpha]^{20}_D$ = +6.7 (*c* 0.3, CH_2Cl_2); MS(ES$^+$), *m/z* 608 [M + Na]; ESHRMS, *m/z* 603.2107 ($C_{29}H_{36}PN_2O_{10}$ requires 603.2108).

I. Acetic Acid (1*R*,2*R*)-2-Acetoxy-1-(acetylbenzyloxyaminocarbonyl)-3-(bis(benzyloxy)phosphoryloxy)propyl Ester (20b). The same procedure as for **20a** using 4 equiv of Ac_2O was followed. Purification by chromatography afforded **20b**, which was eluted first (11.5 mg, 22%) followed by **20a** (21 mg, 40%): ^{1}H NMR ($CDCl_3$) δ 7.6−7.3 (m, 15H), 5.9 (br d, 1H), 5.52 (m, 1H), 5.2−4.95 (m, 6H), 4.28 (m, 2H), 2.4 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H); MS (ES$^+$), *m/z* 650 [M + Na]; ESHRMS, *m/z* 645.2217 ($C_{31}H_{38}PN_2O_{11}$ requires 645.2213).

J. Acetic Acid (2*R*,1*R*)-2-Acetoxy-1-hydroxycarbamoyl-3-phosphonooxypropyl Ester (21). Hydrogenolysis of a solution of **20a** (40 mg) in MeOH (5 mL) with 5% Pd−C (45 mg) for 2.5 h afforded **21** as a brownish oil (19 mg, 88%): ^{1}H NMR (CD_3OD) δ 5.3 (br, 1H), 5.13 (br, 1H), 4.1 (br, 1H), 2.03 (s, 3H, Ac), 1.98 (s, 3H, Ac); ^{13}C NMR (CD_3OD) δ 169.4 (s), 168.98 (s), 164.1 (br, s), 70.27 (d, J_{C-P} = 7.5 Hz), 69.1 (br, d), 62.8 (t), 18.7 (q), 18.4 (q); ^{31}P NMR (CD_3OD) δ +0.67 (br); MS (ES$^-$), *m/z* 314 [M − H]; ESHRMS, *m/z* 314.0287 ($C_8H_{13}NO_{10}P$ requires 314.0283).

K. Phosphoric Acid Mono-[(4*R*,5*R*)-5-carbamoyl-2,2-dimethyl-[1,3]dioxolan-4-ylmethyl] Ester (22). A solution of **18** (100 mg, 0.185 mmol) in MeOH (5 mL) was hydrogenated with Raney nickel (50% in water, ∼50 mg) for 22 h. The product was crystallized with EtOAc and rinsed with Et_2O, affording **22** as an off-white solid (23 mg, 49%): ^{1}H NMR (CD_3OD) δ 4.65 (br, 2H), 4.2 (br, 1H), 3.9 (br, 1H), 1.62 (s, 3H, Me), 1.42 (s, 3H, Me); ^{13}C NMR (CD_3OD) δ 175.9 (s), 113.1 (s), 79.6 (d), 78.7 (d), 66.9 (t), 28.9 (q), 26.7 (q); ^{31}P NMR (CD_3OD) δ +2.16 (br); MS(ES$^-$), *m/z* 254 [M − H]; $[\alpha]^{24}_D$ = +32.6 (*c* 0.46, MeOH); ESHRMS, *m/z* 254.0429 ($C_7H_{13}PNO_7$ requires 254.0435).

L. 4-Phospho-D-erythronamide (23).[14] Compound **22** was dissolved in a mixture of CH_3CN (1 mL) and H_2O (2 mL). The pH of the solution was adjusted to 1−2 with 1 M HCl. The deprotection reaction was followed by mass spectrometry. After 24 h at room temperature, the solvent was removed under reduced pressure, and the product was crystallized with EtOH and washed with Et_2O to yield an off-white solid (31% from **22**): ^{1}H NMR (D_2O) δ 4.05 (br, 1H), 4.0−3.6 (br m, 3H); ^{13}C NMR (D_2O) δ 177.8, 72.6 (2 × d), 66.2 (t); ^{31}P NMR (D_2O) δ +1.63 (br); $[\alpha]^{24}_D$ = +7.1 (*c* 0.28, H_2O); MS (ES$^-$), *m/z* 214 [M − H]; ESHRMS, *m/z* 214.0125 ($C_4H_9PNO_7$ requires 214.0117).

M. Phosphoric Acid Mono-[(5*R*,4*R*)-5-hydroxycarbamoyl-2,2-dimethyl[1,3]dioxolan-4-ylmethyl] Ester (24). Hydrogenolysis of a solution of **18** (70 mg) in MeOH (5 mL) with 20% $Pd(OH)_2$ (45 mg) for 24 h afforded **24** as a brownish oil (35 mg, 100%): ^{1}H NMR (CD_3OD) δ 4.65 (m, 2H), 4.2 (br m, 1H), 3.9 (br m, 1H), 1.55 (s, 3H, Me), 1.35 (s, 3H, Me); ^{13}C NMR (CD_3OD) δ 167.6 (s), 111.7 (s), 77.45 (d, J_{C-P} = 7.5 Hz), 76.0 (d, J_{C-P} = 12.4 Hz), 66.1 (t, J_{C-P} = 4.5 Hz), 27.0 (q), 24.8 (q); ^{31}P NMR (CD_3OD) δ +1.17 (br); $[\alpha]^{20}_D$ = +15 (*c* 0.2, MeOH); MS(ES$^-$), *m/z* 270 [M − H]; ESHRMS, *m/z* 270.0384 ($C_7H_{13}NO_8P$ requires 270.0384).

N. 4-Phospho-D-erythronohydroxamic Acid (25). A solution of **24** (45 mg, 0.17 mmol) in a mixture of CH_3CN (2 mL) and water (1.5 mL) was stirred for 23 h at room temperature. Ether-mediated precipitation of the crude product dissolved in MeOH afforded **25** as a brownish hygroscopic solid (25 mg, 64%): ^{1}H NMR (D_2O) δ 4.02 (br, 1H), 3.85 (br m, 3H); ^{13}C NMR (D_2O) δ 170.4 (s), 71.1 (d, J_{C-P} = 7.5 Hz), 70.6 (d), 65.8 (t, J_{C-P} = 7.5 Hz); ^{31}P NMR (D_2O) δ +1.39 (br); $[\alpha]^{22}_D$ = −7 (*c* 0.5, H_2O) [Lit.[14] $[\alpha]^{28}_D$ = −14.1 (*c* 1.17, H_2O)]; MS(ES$^-$), *m/z* 230 [M − H]; ESHRMS, *m/z* 230.0074 ($C_4H_9NO_8P$ requires 230.0071).

Biological Tests. A. 6PGDH Enzyme Assay. The recombinant *T. brucei* 6PGDH, overexpressed in *Escherichia coli*, was purified according to a technique that was slightly modified compared to the original of Barrett.[9,23] Cells were lysed by freeze−thaw cycling using liquid nitrogen and then resuspended in 50 mM triethanolamine/HCl containing 1 mM EDTA, pH 7.5 (TEA buffer). Cells were then centrifuged at 39000 rpm for 30 min in a Beckman XL-70 ultracentrifuge using a Ti70 rotor. The supernatant was applied to a 15 mL DEAE-Sepharose column equilibrated with TEA buffer, then washed with the same buffer, and the flow-through material absorbing at 280 nm was loaded directly onto a 5 mL 2′,5′-ADP−Sepharose column, equilibrated with TEA buffer. After

washing, the enzyme was eluted by a TEA buffer containing 0.5 M NaCl and the specific activity assayed in the presence of 1.5 mM 6PG and 0.25 mM $NADP^+$. The whole purification lasted less than 1 day and was monitored both by SDS−PAGE and by activity assays. Enzyme was stored in the presence of 50% glycerol at −20 °C. The sheep liver 6PGDH was purified as reported.[8] Activity of the enzymes at 20 °C is followed spectrophotometrically (Kontron Uvikon 930 spectrophotometer) at 340 nm, measuring the production of NADPH in the absence or presence of inhibitor.

Compounds to assay as inhibitors were dissolved in methanol, DMSO, or water. Inhibition type and degree were evaluated by performing the assays in TEA buffer, pH 7.5, at variable 6PG concentration (8−40 μM) while keeping the $NADP^+$ concentration fixed at 0.26 mM (which is a saturating concentration, being 260-fold and 40-fold the K_m for $NADP^+$ in the *T. brucei* and sheep liver 6PGDH, respectively). When lack of solubility of the compounds did not allow assaying a high inhibitor concentration in the conditions described above, the enzyme activity was assayed in TEA buffer in the presence of either 20% methanol or 10 or 20% DMSO. (This was necessary for only a few compounds, and these solvents did not affect enzyme activity.) The reaction was initiated by the addition of either 0.2 μg of *T. brucei* 6PGDH or 0.5 μg of sheep liver 6PGDH per milliliter of reaction mixture. A number of inhibitor concentrations were tested, and inhibition type was established by Lineweaver−Burk plots, where the standard errors of linear regressions were below 0.05 and after experiments were repeated several times. The K_i values were then calculated by linear regression from the secondary plots where the slopes of the lines from the Lineweaver−Burk plot are plotted against inhibitor concentration.[9] The selectivity degree is valued as the ratio between the mammal and parasite enzymes K_i values.

B. In Vitro Pharmacology, ***Trypanosoma brucei rhodesiense*.** Minimum essential medium (50 μL) supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μL of trypanosome suspension (*T. b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL) was then added to each well and incubation continued for a further 2−4 h. The plate was then read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Fluorescence development was expressed as percentage of the control, and IC_{50} values were determined.

C. In Vitro Pharmacology, *Trypanosoma cruzi*. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μL in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the α-galactosidase (Lac Z gene) were added in 100 μL per well with 2× of a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. After 96 h, the minimum inhibitory concentration (MIC) was determined microscopically. For measurement of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The color reaction that developed during the following 2−4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated. Cytotoxicity was assessed in the same assay using noninfected L-6 cells and the same serial drug dilution. The MIC was determined microscopically after 4 days.

D. In Vitro Pharmacology, *Leishmania donovani*. Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat-inactivated FBS into Lab-tek 16-chamber slides. After 24 h, *L. donovani* amastigotes were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. The next day the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37 °C under a 5% CO_2 atmosphere for 96 h. Then the medium was removed, and the slides were fixed with methanol and stained with Giemsa. The ratio of infected to noninfected macrophages was determined microscopically, expressed as a percentage of the control and the IC_{50} value calculated by linear regression.

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References

(1) Barrett, M. P.; Gilbert, I. H. Perspectives for new drugs against Trypanosomiasis and Leishmaniasis. *Curr. Topics Med. Chem.* **2002**, *2*, 471−482.
(2) Barrett, M. P. The pentose phosphate pathway and parasitic protozoa. *Parasitol. Today* **1997**, *13*, 11−16.
(3) Dardonville, C.; Gilbert, I. H. Synthesis of (*R*)-2-methyl-4-deoxy and (*R*)-2-methyl-4,5-dideoxy analogues of 6-phosphogluconate as potential inhibitors of 6-phosphogluconate dehydrogenase. *Org. Biomol. Chem.* **2003**, *1*, 552−559.
(4) Dardonville, C.; Rinaldi, E.; Hanau, S.; Barrett, M. P.; Brun, R.; Gilbert, I. H. Synthesis and biological evaluation of substrate-based inhibitors of 6-phosphogluconate dehydrogenase as potential drugs against African Trypanosomiasis. *Bioorg. Med. Chem.* **2003**, *11*, 3205−3214.
(5) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: London, U.K., 1992; pp 172−173.
(6) Schramm, V. L. Development of transition state analogues of purine nucleoside phosphorylase as anti-T-cell agents. *Biochim. Biophys. Acta* **2002**, *1587*, 107−117.
(7) Berdis, A. J.; Cook, P. F. Chemical mechanism of 6-phosphogluconate dehydrogenase from *Candida utilis* from pH studies. *Biochemistry* **1993**, *32*, 2041−2046.
(8) Hanau, S.; Dallocchio, F.; Rippa, M. NADPH activates a decarboxylation reaction catalysed by lamb liver 6-phosphogluconate dehydrogenase. *Biochim. Biophys. Acta* **1992**, *1122*, 273−277.
(9) Pasti, C.; Rinaldi, E.; Cervellati, C.; Dallocchio, F.; Hardré, R.; Salmon, L.; Hanau, S. Sugar derivatives as new 6-phosphogluconate dehydrogenase inhibitors selective for the parasite *Trypanosoma brucei*. *Bioorg. Med. Chem.* **2003**, *11*, 1207−1214.
(10) Hanau, S.; Rinaldi, E.; Dallocchio, F.; Gilbert, I. H.; Dardonville, C.; Adams, M. J.; Gover, S.; Barrett, M. P. 6-phosphogluconate dehydrogenase: a target for chemotherapy in African trypanosomiasis. *Curr. Med. Chem.* **2004**, in press.
(11) Rippa, M.; Giovannini, P. P.; Barrett, M. P.; Dallocchio, F.; Hanau, S. 6-Phosphogluconate dehydrogenase: the mechanism of action investigated by a comparison of the enzyme from different species. *Biochim. Biophys. Acta* **1998**, *1429*, 83−92.
(12) (a) Hardré, R.; Bonnette, C.; Salmon, L.; Gaudemer, A. Synthesis and evaluation of a new inhibitor of phosphoglucose isomerases: the enediolate analogue 5-phospho-D-arabinohydroxamate. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3435−3438. (b) Bonnette, C.; Salmon, L.; Gaudemer, A. Synthesis of 5-phosphate-D-arabinohydroxamic acid, a potent transition state analogue inhibitor of 6-phosphate-D-glucose isomerases. *Tetrahedron Lett.* **1996**, *37*, 1221−1224. (c) Hardré, R.; Salmon, L. Competitive inhibitors of yeast phosphoglucose isomerase: synthesis and evaluation of new types of phosphorylated sugars from the synthon D-arabinolactone-5-phosphate. *Carbohydr. Res.* **1999**, *318*, 110−115.
(13) Hardré, R.; Salmon, L.; Opperdoes, F. Competitive inhibition of *Trypanosoma brucei* phosphoglucose isomerase by D-arabino-5-phosphate derivatives. *J. Enzyme Inhib.* **2000**, *15*, 509−515.
(14) A synthesis of compounds **23** and **25** starting from potassium D-erythronate (7 steps, 12.3% overall) was published following the submission of this paper. Burgos, E.; Salmon, L. Synthesis and evaluation of new 4-phospho-D-erythronic acid derivatives as competitive inhibitors of spinach ribose-5-phosphate isomerase. *Tetrahedron Lett.* **2004**, *45*, 753−756.
(15) Woulfe, S. R.; Miller, M. J. The synthesis of substituted [[3(*S*)-(acylamino)-2-oxo-1-azetidinyl]thio]acetic acids. *J. Org. Chem.* **1986**, *51*, 3133−3139.
(16) Iwata, M.; Ohrui, H. A simple regioselective partial hydrolysis of di-*O*-isopropylidene monosaccharides with copper(II) ion. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2837−2838.
(17) Ohta, H.; Kato, Y.; Tsuchihashi, G. Studies on enzymic oxidation of sulfides. V. Switching of the direction of enzyme-mediated oxidation and reduction of sulfur-substituted 2-propanols and 2-propanones. *J. Org. Chem.* **1987**, *52*, 2735−2739.

(18) Salmon, L.; Prost, E.; Merienne, C.; Hardré, R.; Morgant, G. A convenient preparation of aldonohydroxamic acids in water and crystal structure of L-erythronohydroxamic acid. *Carbohydr. Res.* **2001**, *335*, 195−204.
(19) Gypser, A.; Peterek, M.; Hans-Dieter, S. D-Erythronolactone as a C_4 building unit. Part 2. A short and efficient synthesis of both enantiomers of *epi*-muricatacin, a diastereoisomer of the native acetogenin from *Annona muricata*. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1013−1016.
(20) Takahashi, H.; Iwai, Y.; Hitomi, Y.; Ikegami, S. Novel synthesis of L-ribose from D-mannose-1,4-lactone. *Org. Lett.* **2002**, *4*, 2401−2403.
(21) Nikam, S. S.; Komberg, B. E.; Johnson, D. R.; Doherty, A. M. Synthesis of hydroxamic acids: Pd/$BaSO_4$ as a new catalyst for the deprotection of *O*-benzyl hydroxamates. *Tetrathedron Lett.* **1995**, *36*, 197−200.
(22) Cohen, N.; Banner, B. L.; Lopresti, R. J.; Wong, F.; Rosenberger, M.; Liu, Y. Y.; Thom, E.; Liebman, A. A. Enantiospecific syntheses of leukotrienes C4, D4, and E4, and [14,15-$^3H_2$]-leukotriene E4 dimethyl ester. *J. Am. Chem. Soc*. **1983**, *105*, 3661−3672.
(23) Barrett, M. P.; Phillips, C.; Adams, M. J.; Le Page, R. W. Overexpression in *Escherichia coli* and purification of the 6-phosphogluconate dehydrogenase of *Trypanosoma brucei*. *Protein Expr. Purif.* **1994**, *5*, 44−49.

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