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Synthesis and Biological Evaluation of Substrate-Based Inhibitors of 6-Phosphogluconate Dehydrogenase as Potential Drugs Against African Trypanosomiasis

Christophe Dardonville,^a Eliana Rinaldi,^b Stefania Hanau,^b Michael P. Barrett,^c Reto Brun^d and Ian H. Gilbert^{a,*}

^aWelsh School of Pharmacy, Redwood building, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK

^bDipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Via Luigi Borsari 46, 44100 Ferrara, Italy

^cIBLS, Division of Infection & Immunity, University of Glasgow, Glasgow G12 8QQ, UK

^dSwiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

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Abstract—The synthesis and biological evaluation of three series of 6-phosphogluconate (6PG) analogues is described. (2R)-2-Methyl-4,5-dideoxy, (2R)-2-methyl-4-deoxy and 2,4-dideoxy analogues of 6PG were tested as inhibitors of 6-phosphogluconate dehydrogenase (6PGDH) from sheep liver and also *Trypanosoma brucei* where the enzyme is a validated drug target. Among the three series of analogues, seven compounds were found to competitively inhibit 6PGDH from *T. brucei* and sheep liver enzymes at micromolar concentrations. Six inhibitors belong to the (2R)-2-methyl-4-deoxy series (**6**, **8**, **10**, **12**, **21**, **24**) and one is a (2R)-2-methyl-4,5-dideoxy analogue (**29b**). The 2,4-dideoxy analogues of 6PG did not inhibit both enzymes. The trypanocidal effect of the compounds was also evaluated in vitro against *T. brucei rhodesiense* as well as other related trypanosomatid parasites (i.e., *Trypanosoma cruzi* and *Leishmania donovani*).

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Introduction

Human African trypanosomiasis is a major health problem in sub-Saharan Africa, caused by, *Trypanosoma brucei gambiense* in West and Central Africa and *T. b. rhodesiense* in East Africa.^{1,2} The drugs currently used to treat sleeping sickness are far from satisfactory because of major side effects,³ the parenteral mode of administration, increasing resistance⁴ and the unaffordable price for African countries. Moreover, some of these drugs (suramin, pentamidine) are unable to cross the blood–brain barrier in sufficient quantity to treat late-stage cases of HAT.⁵

Bloodstream forms of *T. brucei* produce ATP exclusively through glycolysis, making the inhibition of any of the glycolytic enzymes a potential therapeutic approach.⁶ We decided to target the enzyme 6-phos-

phogluconate dehydrogenase (6PGDH), the third enzyme of the pentose phosphate pathway⁷ (PPP) which generates NADPH and ribulose-5-phosphate (Fig. 1). The PPP plays a crucial role in the host–parasite relationship because it maintains a pool of NADPH, which amongst other roles is involved in protecting the parasite against oxidative stress, and it generates carbohydrate intermediates used in nucleotide and other biosynthetic pathways.

The gene encoding the *T. brucei* 6PGDH has been cloned,⁸ and the enzyme purified⁹ and crystallized. The enzyme is remarkable for its degree of divergence with regard to other eukaryotic 6PGDHs. This can be

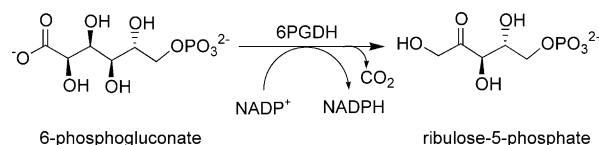


Figure 1.

*Corresponding author. Fax.: +44-(0)29-2087-4149; e-mail: gilbert-ih@cf.ac.uk

attributed to the fact that the trypanosomatid 6PGDH has been derived from a cyanobacterial precursor.¹⁰ However, despite sharing only 35% amino acid sequence identity with the sheep liver enzyme, residues which make direct contact with the substrate in the

active site appear to be conserved between the two enzymes. Similarly, most of the residues contacting the co-enzyme are conserved.¹¹

RNA interference has recently demonstrated that 6PGDH is essential for growth of bloodstream form *T. brucei*.¹² One possible mechanism by which cell death results from inhibition of 6PGDH involves an accumulation of 6PG, which in turn is a potent inhibitor of 6-phosphoglucose isomerase¹³ and thus the glycolytic pathway, which leads to increased passage of glucose 6-phosphate through the PPP and hence increased levels of 6PG, initiating a toxic positive feedback loop. This mode of action has been supported in yeast¹⁴ and *Drosophila*.¹⁵

Crystallographic studies of the sheep liver¹⁶ and the *T. brucei* enzyme have shown that, in spite of the conservation of the amino-acid residues, spatial differences at the active site exist and they may be exploited for selective drug design. Of particular note is the fact that the trypanosomal enzyme has a difference in the binding pocket in the region of the 2-OH group of the substrate. Selective inhibitors of the trypanosomal enzyme have been identified, including sugar derivatives¹⁷ and 2-

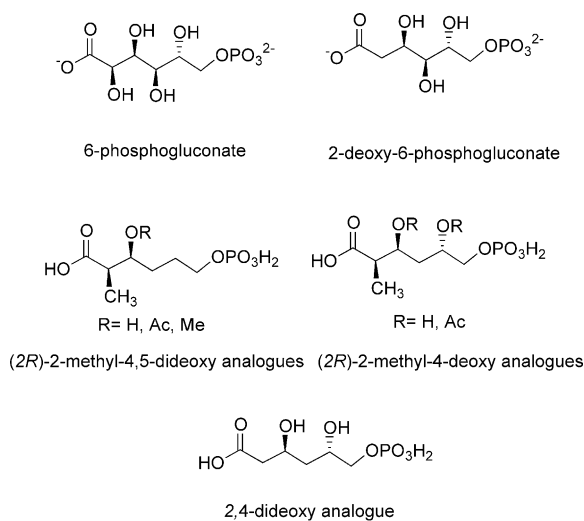


Figure 2.

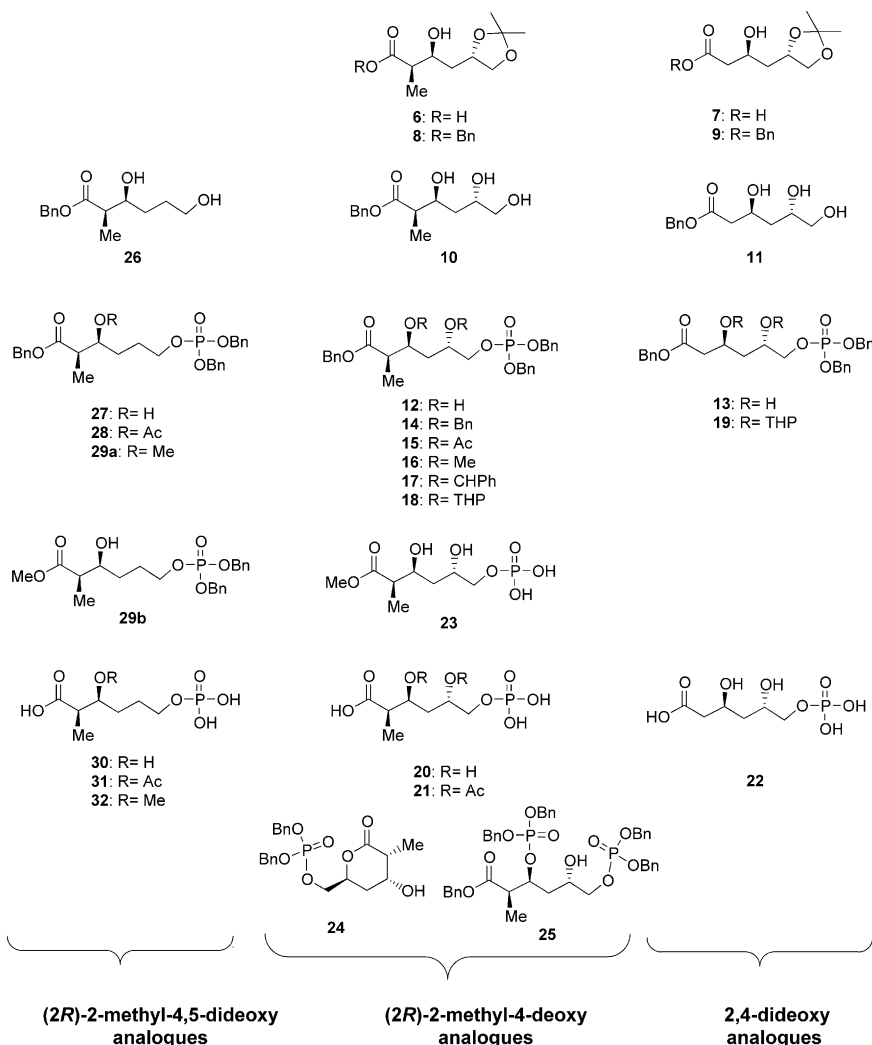


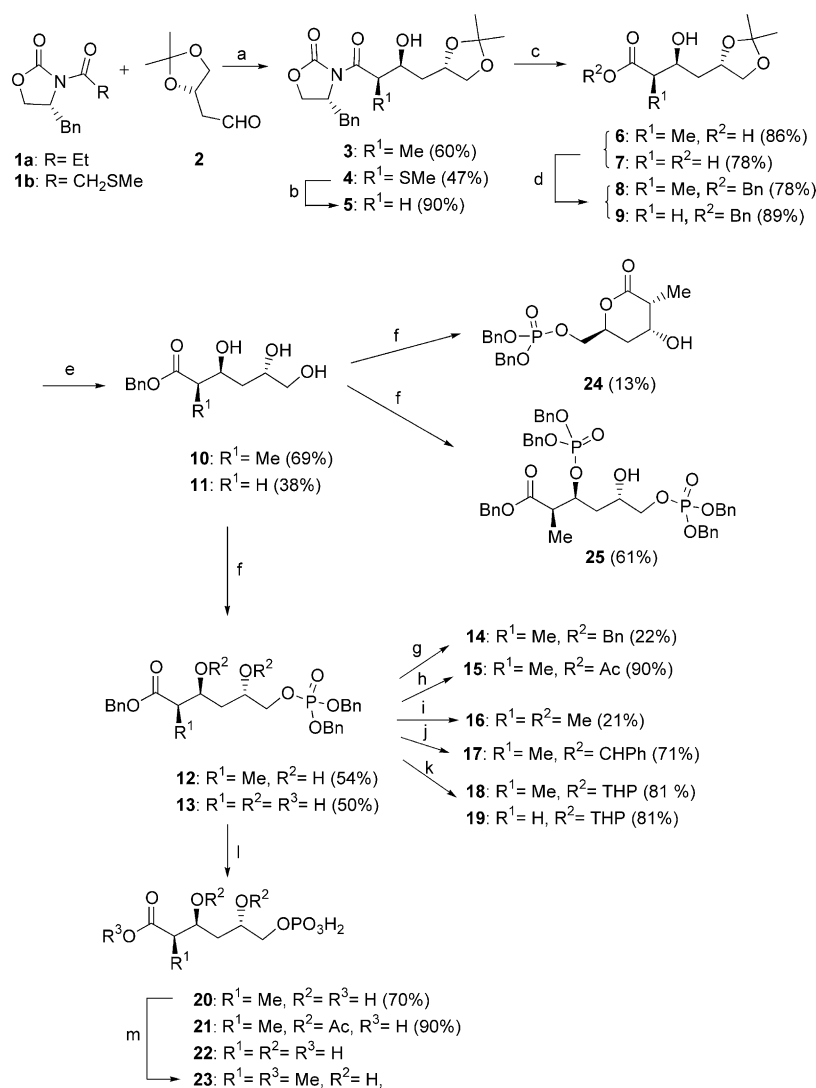
Figure 3. Compounds tested as inhibitors of 6PGDH.

deoxy-6-phosphogluconate (Fig. 2) which shows several orders of magnitude more potency in inhibition of the *T. brucei* enzyme ($K_i=4.4\ \mu\text{M}$) compared to that of sheep liver ($K_i=770\ \mu\text{M}$).^{18a} Investigation of the mechanism of action of 6PGDH from different species by Rippa et al.^{18b} suggested that the C2 hydroxyl of 6PG was important for the binding of the substrate, for communication between subunits in the 6PGDH homodimer and for the keto-enol tautomerization. However there were important differences between species as evidenced by the differences in binding of 2-deoxy-6-phosphogluconate.

Molecular modelling studies on the interaction between 6PG and the *T. brucei* enzyme revealed a number of possibly important functionalities involved in the interaction.¹⁹ Modelling indicates that there is little scope for interaction between the enzyme and the 4-OH, although studies using the *Candida* enzyme did indicate that 6-phosphogalactonate (the 4'-epimer of 6PG) was not bound by the enzyme, perhaps indicating steric hindrance if the 4-OH was in the 4*S*-conformation.²⁰

In designing inhibitors of 6PGDH that might have efficacy as anti-trypanosomal drugs, a number of considerations need to be made. Phosphorylated carbohydrates are unlikely to possess good drug-like characteristics due to metabolic instability and impermeability at biological membranes. However, deoxy and/or protected analogues of 6PG may represent inhibitors with better pharmacokinetic properties. Introduction of a methyl group in C2 was considered potentially capable of forming hydrophobic interactions with *T. brucei* enzyme due to the differences in binding pocket found juxtaposed to the 2-position in this variant of the enzyme.

Considering these facts, we designed three series of 6PG analogues: (2*R*)-2-methyl-4,5-dideoxy, (2*R*)-2-methyl-4-deoxy and 2,4-dideoxy analogues (Fig. 2) that were used to probe the active site of *T. brucei* and sheep 6PGDH around the 2-, 4- and 5-positions. To assess the importance of the individual free OH groups, we also prepared some derivatives protected on the 3-OH and 5-OH



Scheme 1. (a) Bu₂BOTf, CH₂Cl₂, Et₃N, 0°C, then **2**, -78°C; (b) Bu₃SnH, 1,1'-azobis(cyclohexanecarbonitrile), benzene, reflux, 2 h; (c) LiOH, H₂O₂, 0°C; (d) BnBr, K₂CO₃, DMF, rt; (e) CuCl₂·2H₂O, *i*PrOH; (f) (BnO)₃P, I₂, -78°C, CH₂Cl₂; (g) CCl₃NHOBn, TfOH, CH₂Cl₂, rt; (h) Ac₂O, DMAP, pyridine, rt; (i) MeO₃BF₄, DTBMP, CH₂Cl₂, rt; (j) PhCH(OMe)₂, PPTS, DMF; (k) DHP, PPTS, CH₂Cl₂; (l) H₂, Pd/C, MeOH then HCl 1 M; (m) MeOH/HCl, rt.

groups (i.e., Ac, Me, Bn, benzylidene). The three series of compounds were tested as inhibitors of 6PGDH of *T. brucei* and sheep liver and their trypanocidal effect was also evaluated in vitro against *T. brucei* sub-species as well as other related trypanosomatids parasites (i.e., *Trypanosoma cruzi* and *Leishmania donovani*).

Results and Discussion

Chemistry

Synthesis of (2*R*)-2-methyl-4-deoxy and 2,4-dideoxy analogues (Scheme 1). Our synthetic strategy²¹ for the synthesis of the (2*R*)-2-methyl-deoxy analogues of 6PG relied on the Evans aldol reaction for the installation of the chiral centres in the 2- and 3-positions.²² We have previously reported the synthesis of compounds **26–28**, **29a,b**, **30–32** (Fig. 3) and now this approach was extended to the synthesis of the 2,4-dideoxy analogues (**4**, **5**, **7**, **9**, **11**, **13**, **19**, **22**) as described below.

Diastereoselective boron aldol reaction of aldehyde (3*S*)-**2**²³ with the chiral oxazolidinones **1a** and **1b** afforded (2*R*,3*S*)-**3** (60%) and (2*R*,3*S*)-**4** (47%), respectively. Reductive desulfurization^{23,24} of **4** with Bu₃SnH in refluxing toluene afforded **5** in high yield (90%).

The benzyl esters **8** and **9** were prepared in two steps from **4** and **5**, respectively: the chiral auxiliary was first removed with LiOH/H₂O₂,²⁵ affording the pure acids **6** and **7** in good yield (86 and 78%, respectively) by acid-base workup. The acids were subsequently esterified with benzyl bromide and K₂CO₃ in DMF affording **8** and **9** (78 and 89%, respectively).

Following the removal of the isopropylidene protecting group with CuCl₂/iPrOH, triol **10** and **11** were selectively phosphorylated with dibenzylphosphoiodinate [prepared from (BnO)₃P and I₂ in CH₂Cl₂]²⁶ working at –78 °C. It is noteworthy that when the reaction was carried out at higher temperature (i.e., –10 °C to +15 °C over 2 h), the lactone product **24** was obtained instead (13%). Besides, when 2.5 equivalents of phos-

phorylating agent were added at once, the diphosphorylated compound **25** was obtained as major product (61%). The second phosphorylation in **25** was thought to occur on the 3-OH, based on the downfield chemical shift observed in the ¹³C NMR spectra for the CH (+0.1 ppm) and CH₃ (+0.5 ppm) in β-position and the up-field chemical shift for the carbonyl of the ester group (–1 ppm) in the γ-position.

The free hydroxyl groups of phosphates **12** and **13** were protected with acetyl (**15**), methyl (**16**), benzyl (**14**), benzylidene (**17**) and THP groups (**18**). Phosphate **20** was obtained by hydrogenolysis of **15** with 5% Pd/C. Compounds **20** and **22** were obtained from **18** and **19**, respectively, by hydrogenolysis followed by mild acidic hydrolysis of the THP protecting groups.²⁷

Biological results

All compounds were tested for their potency as competitive inhibitors with respect to substrate (6PG) against 6-phosphogluconate dehydrogenases of *T. brucei* and sheep liver (Table 1 and Fig. 3). The compounds were also assayed for their in vitro toxicity against blood-stream form trypomastigotes (the clinically relevant stage) of *T. brucei rhodesiense*. The compounds were also assayed against two related parasites, *T. cruzi* (intracellular amastigotes) and *L. donovani* (intracellular amastigotes), responsible for Chagas' disease and visceral Leishmaniasis, respectively (Table 2).

Table 2. In vitro antiparasitic activity

Compd	<i>T. brucei</i> IC ₅₀ (μM) ^a	<i>T. cruzi</i> IC ₅₀ (μM) ^b	<i>L. donovani</i> IC ₅₀ (μM) ^c	Cytotoxicity L6-cells IC ₅₀ (μM)
3	55.4	> 239	> 79.5	
6	415	> 413	> 138	
7	96.6	179.4	> 147	
8	254	153.2	> 97	
9	> 306	> 306	> 102	
10	51.1	> 333	97.8	
11	354	240	72	
12	146.4	89.6	31.4	
13	91.4	49.2	47.1	
14	11.6	98.3	> 42	
15	8.2	25.4	35.9	
16	156	22.3	> 54	
17	80	24.4	dnp	
19	81.2	9.5	dnp	24.9
20	336	> 349	> 116	
21	> 263	> 263	> 88	
22	> 369	> 369	> 123	
23	> 331	> 331	> 110	
24	40.5	> 214	> 71	
25	37.8	8.5	dnp	38 ^e
26	20.2	> 357	dnp ^d	204.8
27	7.46	43.8	31.4	65
28	59.1	19	dnp	
29a	45	91.6	> 57	
29b	31.9	14	dnp	39.4
30	> 372	> 372	> 124	324.8
31	> 317	> 317	> 106	
32	196	234	> 117	

Table 1. 6PGDH inhibition^a

Compd	Sheep 6PGDH K _i (μM)	<i>T. brucei</i> 6PGDH K _i (μM)
6PG ^b	16	3.5
2-Deoxy-6PG ^c	770	4.4
29b	65	> 1000
6	650	800
8	650	800
10	> 1000	500
12	100	174
21	400	> 1000
24	600	> 1000

^aAll compounds were assayed for inhibition of 6PGDH. Only compounds displaying inhibition are shown; Lineweaver–Burk plots where the standard errors of linear regressions were below 0.05 are an estimate of the accuracy of the enzyme inhibition assays.

^bK_m values from ref 18b.

^cK_i values from ref 18a.

^aControl: melarsoprol, IC₅₀ = 0.0055 μM.

^bControl: benznidazole, IC₅₀ = 1.076 μM.

^cControl: pentamidine, IC₅₀ = 9.3 μM.

^dDetermination not possible due to cytotoxicity on the host cell.

^eMinimum inhibitory concentration (μg/mL).

Inhibition of 6PGDH of *T. brucei* and sheep liver enzymes

Among all the compounds assayed against sheep liver and *T. brucei* 6PGDH enzymes, seven had a competitive inhibitory activity, with a K_i in the micromolar range. These results are reported in Table 1. Four compounds selectively inhibited either the sheep 6PGDH (**21**, **24** and **29b**) or the *T. brucei* enzyme (**10**). Moreover, three compounds (**6**, **8** and **12**) inhibited both enzymes with a low selectivity index in favour of the sheep enzyme (1.2–1.5). Three molecules (**20**, **22** and **30**), which are the most structurally related to 6PG, were not inhibitors of the enzyme. Therefore, they were also tested as substrates of the enzyme, but none of them displayed any activity.

Discussion

Comparison of data allows several important conclusions to be drawn. However care has to be taken when trying to draw structure–activity relationships, as inhibitors may be binding in different orientations.

C4-Position

The lack of inhibitory activity of the fully deprotected compounds **20**, **22** and **30**, which are particularly close analogues of 6PG is informative. The lack of inhibitory activity of **22** is particularly interesting when considering that 2-deoxy-6-phosphogluconate is an excellent inhibitor of the *T. brucei* enzyme [K_i (*T. brucei*) = 4.4 μM] and a moderate inhibitor of the sheep one [K_i (sheep) = 770 μM].^{18a} The presence of a hydroxyl group at C4 appears to be essential for the observed inhibitory activity of 2-deoxy-6-phosphogluconate in the case of the *T. brucei* enzyme. However the 4-OH may not be strictly essential in the sheep enzyme as seen by the inhibition given by the 3,5-acetate protected compound **21** (K_i = 400 μM) in the sheep enzyme. The presence of acetate protecting groups (H-bond acceptor) in C3 and C5 instead of OH groups (H-bond acceptor/donor) could account for the affinity and selectivity of this molecule for the sheep 6PGDH active site.

Our results indicate that while the 4-OH is important for the binding of the phosphate analogues of 6PG, it is not absolutely essential in the case of protected analogues since high micromolar level inhibition is seen with several inhibitors of the 2-methyl-4-deoxy series (**6**, **8**, **10**, **12**, **24** and **29b**), which either have no phosphate or a protected phosphate. This latter result may reflect a different binding mode of the compounds in the enzyme active site.

C2-Position

No 2,4-dideoxy analogues (**7**, **9**, **11**, **13**, **19**, **22**) inhibited the enzyme, whilst the inhibitors have a (2*R*)-methyl group at the 2-position (compare compounds **6** and **7**; **8** and **9**; **10** and **11**; **12** and **13**). These results may confirm the hypothesis that there is a binding pocket in the

enzyme active site that is filled by substituents in the (2*R*)-position, and substituents at this position should increase the affinity of inhibitors [e.g., this is exemplified in the *T. brucei* enzyme by comparison of the K_m values for 6PG (K_m = 3.5 μM) and 2-deoxy-6PG (K_m = 9.6 μM)].^{18b}

C3-Position

All the compounds tested in this study had the 3*S* stereochemistry as in 6PG. The 3*S*-configuration is presumably preferred as previously reported for the *Candida* enzyme in which the K_i for 6-phosphoallionate (in which the stereochemistry at the C3 position is inverted) is about 20-fold higher than K_m for 6PG.²⁰ More recently, Pasti et al. concluded that an oxygen at C3 of 6PG, independent of the stereochemistry, was fundamental for binding to the active site.¹⁷ Also the presence of blocking groups on the 3-OH seemed to reduce activity, implying the H-bonding interaction of the 3-OH with the enzyme active site is important (compare **12** with **14**–**18**). However, the presence of an acetate blocking group (H-bond acceptor) did not always reduce activity (compare **15** and **12**; **21** and **20**).

C5- and C6-Positions

Either a hydroxyl group or a large lipophilic group seems to give activity at the 5-position. Compare the activity of the (2*R*)-methyl-4-deoxy analogues **10**, **12** and **21** with their non-active (2*R*)-methyl-4,5-dideoxy counterparts **26**, **27** and **31**). Similarly, there is an improvement of inhibition when adding the benzyl-protected phosphate moiety (compound **12**, K_i (*T. brucei*) = 174 μM) to the triol **10** (K_i = 500 μM). These results may reflect different binding modes compared to 6PG.

C1-Position

There is no clear data on SAR of our compounds at position C1. Thus compounds **6** and **8** show similar inhibition, despite one being a carboxylic acid (**6**) and the other a benzyl ester (**8**). Compound **29b** which carries a methyl ester protecting group is highly selective towards the sheep enzyme (K_i (sheep) = 65 μM). Compound **27**, however, which is similar to compound **29b** but carries a bulky benzyl ester rather than a methyl ester, has no inhibitory effect indicating that the binding pocket is not large enough to accommodate the bulkier substituents. Intriguingly, the presence of the 5-OH in compound **12** allows this compound to participate in some important interactions in both enzymes in spite of the bulky benzyl ester at position C1. It is possible that analogues do not bind in the manner predicted based on their homology to 6PG.

In vitro toxicity. The results of the in vitro toxicity on three related trypanosomatid parasites (*T. brucei*, *T. cruzi* and *L. donovani*) are reported in Table 2. Among the 27 compounds that were assayed, three of them (**27**, **14** and **15**) showed a moderate in vitro antiparasitic activity against *T.b. rhodesiense* trypomastigotes (<12 μM), two compounds (**19**, **25**) had a moderate

toxicity against *T. cruzi* amastigotes ($< 10 \mu\text{M}$) but none had significant anti-leishmanial activity.

The most active compounds against *T.b. rhodesiense* were the (2*R*)-2-methyl protected analogues of 6PG. Benzyl and especially acetate protection of the hydroxyl groups in positions 3 and 5 (compounds **14** and **15**, respectively) appeared to be the most effective, with a 12- and 18-fold increase in activity, respectively, if compared with the methyl protection (compound **16**). The benzylidene protection (compound **17**) gave only a moderate improvement of activity ($\text{IC}_{50} = 80 \mu\text{M}$) compared to the unprotected **12** ($\text{IC}_{50} = 146.4 \mu\text{M}$). The fact that the corresponding deprotected (2*R*)-2-methyl analogues of 6PG (**20** and **30**) were totally inactive in vitro probably reflects the highly charged character of these molecules (i.e., phosphate and carboxylate moieties) at physiological pH that would prevent them crossing the cell membranes of the parasite. As a whole, all compounds bearing unprotected phosphates (**20–23**, and **30–32**) and free carboxylic acids (**6**, **7**) showed poor in vitro cytotoxicity.

An interesting feature is the increase in activity of the (2*R*)-2-methyl-4,5-dideoxy analogue **27** ($\text{IC}_{50} = 7.5 \mu\text{M}$) compared with its corresponding acetylated analogue **28** ($\text{IC}_{50} = 59 \mu\text{M}$). On the contrary, the (2*R*)-2-methyl-4-deoxy analogue **12** ($146 \mu\text{M}$) is less potent than its acetylated counterpart **15** ($\text{IC}_{50} = 8.2 \mu\text{M}$); the only difference between the compounds **27**, **28**, **12**, and **15** is the presence of the 5-hydroxyl group. Variability in lipophilicity, facilitating uptake at the plasma membrane may explain this difference.

All the trypanosomatids have structurally similar 6PGDH enzymes and it is likely that they would respond similarly to most inhibitors, although this remains to be verified experimentally. Although there is little correlation between anti-parasitic activity and enzyme inhibition, the toxicity of the compounds could be at least in part due to inhibition of 6PGDH, although this needs to be verified experimentally. In fact, the two most potent compounds against *T. brucei* (**14** and **15**) are both protected analogues of **12** which is the most potent 6PGDH inhibitor of the series. In order to show anti-parasitic activity, the compounds must cross the *T. brucei* plasma membrane and fully protected analogues of **12** could meet the pharmacokinetic criteria to cross the membrane. Hence, metabolic conversion of different products within the cell (for example removal of protecting groups) could yield products with differing inhibitory activity than the parents, thus lack of potent 6PGDH inhibitory activity by a given compound might not necessarily mean that the cytotoxic effect is unrelated to inhibition of 6PGDH by a metabolite of the parent compound.

For *T. cruzi* amastigotes, compounds must cross the host cell membrane and then that of the parasites. In the case of *Leishmania* the compounds must cross three membranes, with that of the parasitophorous vacuole joining those of the host and parasite surfaces. The variant biology of the different kinetoplasts

has made development of a single entity that treats diseases caused by all three groups difficult, and also makes interpretation on modes of action of toxicity difficult.

Conclusion

Based on the results of inhibition of this series of compounds several conclusions regarding interactions crucial for binding could be made. First of all, there may be a possibility of esters binding to the active site. Secondly, the 2-position appears more important to the sheep enzyme than the *T. brucei*. Regarding the C3 position, blocking the hydroxyl groups probably reduces activity. Regarding the 4-OH and 5-OH, they appear important in binding to the *T. brucei* enzyme although the 4-OH is probably less important in the case of the sheep 6PGDH. Finally, blocked phosphate appeared to show good activity compared to the unblocked analogues. This could be due to a different mode of binding, increased lipophilicity or a change in conformation of the active site.

Some compounds had trypanocidal activity although correlations between anti-parasitic activity and enzyme inhibition are not evident. Criteria involving drug-uptake and other pharmacological events such as possible metabolic conversion limit interpretations that can be made on cytotoxic effect until further studies are performed.

To conclude, this work described the rational design of substrate-based inhibitors of 6PGDH. The results reported here open the route to the design of more efficient inhibitors.

Experimental

Chemistry

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

The synthesis of compounds **2**, **3**, **6**, **8**, **10**, **12**, **14–18**, **20**, **21**, **24** and **26–32** has been described elsewhere.²¹

(4*R*)-4-Benzyl-3-(2-methylsulfonyl-acetyl)-oxazolidin-2-one (1b). Thionyl chloride (2 mL, 27 mmol) was added drop wise to a solution of (methylthio)acetic acid (2.38 g, 22.4 mmol) in Et_2O (10 mL) and the reaction was heated at ca. 40°C for 75 min. The volatiles were removed in vacuo affording the (methylthio)acetic acid chloride as a greenish oil that was used without further

purification. A 2.5 M solution of *n*BuLi in hexanes (9 mL, 22.4 mmol) was added at -65°C to a solution of (*R*)-4-benzyl-2-oxazolidinone (3.17 g, 22.4 mmol) in THF (50 mL). After 5 min, (methylthio)acetic acid chloride was added. The reaction mixture was stirred 30 min at the same temperature and 90 min at room temperature. The reaction was quenched with 5% NaHCO_3 and the solvent was removed in vacuo. The crude product was partitioned between CH_2Cl_2 and water. The aqueous phase was extracted with CH_2Cl_2 (2 $\times$) and the organic extracts were dried (MgSO_4) and concentrated. Chromatography (isolute 50 g SI) with hexane/EtOAc: 0 $\rightarrow$ 30% afforded **1b** as a yellow oil (3.1 g, 65% for two steps). R_f : 0.6 (50% EtOAc/hexane); ^1H NMR (CDCl_3) δ 7.45–7.25 (m, 5H); 4.77 (m, 1H); 4.35–4.2 (m, 2H); 3.9 (dd, 2H, $J=14.2$ Hz, $J=22.8$ Hz); 3.38 (dd, 1H, $J=3.4$ Hz, $J=13.4$ Hz); 2.85 (dd, 1H, $J=9.6$ Hz, $J=13.4$ Hz); 2.27 (s, 3H). Anal. calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_3\text{S}$: C, 58.85; H, 5.70; N, 5.28; S, 12.08; found: C, 58.72; H, 5.54; N, 4.95; S, 11.90.

(4R)-4-Benzyl-3-(2R,3S)-4-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-hydroxy-(2R)-2-methylsulfanylbutoanoyl]-1,3-oxazolan-2-one (4). A solution of **1b** (2.33 g, 8.8 mmol) in dry CH_2Cl_2 (15 mL) cooled to -10°C was treated sequentially with dibutylboron triflate (2.4 mL, 9.6 mmol) and DIPEA (2.1 mL, 12 mmol). The yellow reaction mixture was stirred for 2 h at ca. -5°C . The clear orange reaction mixture was cooled to -78°C and a solution of aldehyde **12** (1.53 g, 8.6 mmol) in CH_2Cl_2 (10 mL) was added drop wise over a 5-min period. The reaction was allowed to gradually warm up to room temperature overnight and was quenched with phosphate buffer (pH 7.2, 30 mL). The resulting mixture was stirred 4 h at room temperature. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 $\times$). The combined organic phases were dried (MgSO_4) and concentrated to give a crude orange oil. Chromatography with hexane/EtOAc: 25 $\rightarrow$ 30% afforded **4** as a yellow oil (1.54 g, 47%) and some recovered oxazolidinone **1b** (1 g); ^1H NMR (CDCl_3) δ 7.4–7.2 (m, 5H); 4.72 (m, 2H); 4.4–4.0 (m, 5H); 3.55 (dd, 1H, $J=7$ Hz, $J=8.2$ Hz); 3.4 (s, 1H); 3.2 (dd, 1H, $J=3.4$ Hz, $J=13.4$ Hz); 2.8 (dd, 1H, $J=9.4$ Hz, $J=13.4$ Hz); 2.17 (s, 3H); 1.8 (t, 2H); 1.37 (s, 3H); 1.3 (s, 3H); ^{13}C NMR (CDCl_3) δ 171.4 (C_q); 170.4 (C_q); 153.2 (C_q); 135.5 (C_q); 129.9 (CH); 129.3 (CH); 127.8 (CH); 109.1 (C_q); 73.9 (CH); 70.1 (CH_2); 66.7 (CH); 66.3 (CH_2); 55.2 (CH); 50.4 (CH); 38.9 (CH_2); 37.9 (CH_2); 27.5 (CH_3); 26.0 (CH_3); 13.5 (CH_3); $[\alpha]_D^{19} = -44.8$ (*c* 0.58, CH_2Cl_2); MS (ES^+) m/z 427 [$\text{M} + \text{NH}_4$]; ESHRMS m/z 410.1634 [$\text{M} + \text{H}$] ($\text{C}_{20}\text{H}_{28}\text{NO}_6\text{S}$ requires: 410.1637). Anal. calcd for $\text{C}_{20}\text{H}_{27}\text{NO}_6\text{S}$: C, 58.66; H, 6.65; N, 3.42; S, 7.83; found: C, 58.43; H, 6.80; N, 3.32; S, 7.45.

(4R)-4-Benzyl-3-(3S)-4-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-hydroxybutanoyl-1,3-oxazolan-2-one (5). A solution of **4** (380 mg, 0.9 mmol), tributyltin hydride (0.37 mL, 1.4 mmol) and 1,1'-azobis(cyclohexanecarbonitrile) (93 mg, 0.4 mmol) in benzene (18 mL) was refluxed (bath temperature 85°C) for 3 h. The solvent was removed in vacuo and the residue was purified by flash chromatography (Isolute 5 g SI): mobile impurities

were eluted first with 100% hexane and the product **5** was eluted with hexane/EtOAc 25%. Colourless oil (285 mg, 84%). ^1H NMR (CDCl_3) δ 7.42–7.5 (m, 5H); 4.75 (m, 1H); 4.4 (m, 2H); 4.35–4.1 (m, 3H); 3.65 (t, 1H); 3.32 (m, 2H); 3.17 (m, 2H); 2.84 (dd, 1H, $J=10.4$ Hz, $J=13.5$ Hz); 1.82 (t, 2H); 1.47 (s, 3H); 1.41 (s, 3H); ^{13}C NMR (CDCl_3) δ 172.8 (C_q); 153.8 (C_q); 135.4 (C_q); 129.9 (CH); 129.4 (CH); 127.9 (CH); 109.2 (C_q); 73.7 (CH); 70.1 (CH_2); 66.8 (CH_2); 65.8 (CH); 55.4 (CH); 43.5 (CH_2); 40.5 (CH_2); 38.2 (CH_2); 27.4 (CH_3); 26.2 (CH_3); $[\alpha]_D^{22} = -13.5$ (*c* 0.74, CH_2Cl_2); MS (ES^+) m/z 381 [$\text{M} + \text{NH}_4$]; ESHRMS m/z 364.1756 [$\text{M} + \text{H}$] ($\text{C}_{19}\text{H}_{26}\text{NO}_6$ requires: 364.1760).

(3S)-4-[(4S)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-hydroxybutanoic acid (7). A 0.05 M solution of aldol **5** (1.06 g, 2.9 mmol) in a 3/1 THF/ H_2O mixture (45 mL/15 mL) was treated at 0°C with a 30% H_2O_2 (1.5 mL, 14.6 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (185 mg, 4.4 mmol). After 2 h at 0°C , the reaction was quenched with 1.5 M Na_2SO_3 aqueous solution (11.5 mL, 17 mmol) and buffered with a 5% NaHCO_3 aqueous solution (5 mL). THF was removed in vacuo and the aqueous solution was diluted with water and extracted with CH_2Cl_2 (2 $\times$ 50 mL). The aqueous phase was acidified to pH $\sim$ 2 with 2% HCl and extracted with EtOAc (3 $\times$ 50 mL). Combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated to yield the pure acid **7** as a colourless oil (466 mg, 78%). ^1H NMR (CDCl_3) δ 7.0 (br, 2H); 4.3–4.15 (m, 2H); 4.02 (dd, 1H, $J=6.2$ Hz, $J=8.4$ Hz); 3.5 (t, 1H, $J=7.7$ Hz); 2.5 (m, 2H); 1.65 (t, 2H); 1.32 (s, 3H); 1.29 (s, 3H); ^{13}C NMR (CDCl_3) δ 176.9 (C_q); 109.5 (C_q); 73.6 (CH); 69.9 (CH_2); 65.9 (CH); 41.9 (CH_2); 40.2 (CH_2); 27.3 (CH_3); 26.0 (CH_3); $[\alpha]_D^{22} = -7.97$ (*c* 1.38, MeOH); MS (ES^-) m/z 203 [$\text{M} - \text{H}$]; ESHRMS m/z 222.1340 [$\text{M} + \text{NH}_4$] ($\text{C}_9\text{H}_{20}\text{NO}_5$ requires: 222.1341).

Benzyl (3S)-4-[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-hydroxybutanoate (9). A solution of acid **7** (390 mg, 1.9 mmol), benzyl bromide (0.34 mL, 2.8 mmol) and K_2CO_3 (340 mg, 2.3 mmol) in DMF (12 mL) was stirred 23 h at room temperature. The reaction was diluted with CH_2Cl_2 and filtered on a path of Celite. The filtrate was concentrated in vacuo and the crude residue was purified by flash chromatography (Isolute 10 g SI) with hexane/EtOAc: 0 $\rightarrow$ 100%, affording the ester **9** as a colourless oil (500 mg, 89%). R_f : 0.45 (50% EtOAc in hexane); ^1H NMR (CDCl_3) δ 7.4 (s, 5H); 5.2 (s, 2H); 4.45–4.25 (m, 2H); 4.13 (dd, 1H, $J=6$ Hz, $J=8.1$ Hz); 3.61 (t, 1H, $J=7.2$ Hz); 3.3 (m, 1H); 2.75–2.55 (m, 2H); 1.77 (t, 1H, $J=6.5$ Hz); 1.45 (s, 3H); 1.41 (s, 3H); ^{13}C NMR (CDCl_3) δ 172.8 (C_q); 135.9 (C_q); 129.1 (CH); 128.9 (CH); 128.8 (CH); 109.3 (C_q); 73.7 (CH); 70.0 (CH_2); 67.0 (CH_2); 66.0 (CH); 42.1 (CH_2); 40.3 (CH_2); 27.4 (CH_3); 26.1 (CH_3); $[\alpha]_D^{23} = -4.58$ (*c* 2.4, MeOH); MS (ES^+) m/z 295 [$\text{M} + \text{H}$]; ESHRMS m/z 295.1545 [$\text{M} + \text{H}$] ($\text{C}_{16}\text{H}_{23}\text{O}_5$ requires: 295.1545).

Benzyl (3S,5S)-3,5,6-trihydroxyhexanoate (11). A 0.1 M solution of **9** (400 mg, 1.36 mmol) in i PrOH (14 mL) was treated with $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ (1.15 g, 6.8 mmol). The resulting green reaction mixture was stirred at room temperature for 2 h 45 min. The reaction was quenched with

1 M Na₂CO₃ (7.5 mL). After 10 min stirring, *i*PrOH was added and the reaction mixture was filtered on a path of Celite. The filter cake was rinsed with *i*PrOH. The filtrate was concentrated in vacuo and the resulting residue was dissolved in EtOAc. Insoluble inorganic salts were filtered off. The filtrate was dried (MgSO₄) and concentrated. Chromatography (Isolute 5 g SI) with hexane/EtOAc: 0→100% yielded **11** as a colourless oil (130 mg, 38%); *R*_f: 0.23 (100% EtOAc); ¹H NMR (CDCl₃) δ 7.4 (br s, 5H); 5.16 (s, 2H); 4.5–4.3 (m, 3H); 4.15 (m, 1H); 3.61 (m, 1H); 3.49 (m, 1H); 2.7–2.47 (m, 2H); 1.56 (t, 2H); ¹³C NMR (CDCl₃) δ 172.9; 136.0; 129.0; 128.8; 128.7; 69.4; 67.3; 67.0; 65.3; 42.4; 39.5; [α]_D²² = –6.67 (*c* 0.9, MeOH); MS (ES⁺) *m/z* 272 [M + NH₄]; ESHRMS *m/z* 255.1234 [M + H] (C₁₃H₁₉O₅ requires: 255.1232). Anal. calcd for C₁₃H₁₈O₅/0.6H₂O: C, 58.90; H, 7.30; found: C, 59.01; H, 7.31.

6-(Bis-benzyloxy-phosphoryloxy)-(3*S*,5*S*)-3,5-dihydroxy-hexanoic acid benzyl ester (13). A solution of tribenzylphosphite (360 mg, 1 mmol) and iodine (236 mg, 0.9 mmol) in CH₂Cl₂ (2 mL) was stirred 10 min at 0 °C and 15 min at room temperature. A part of this yellow solution (1.2 mL) was added dropwise at –78 °C to a stirred solution of **11** (125 mg, 0.49 mmol) and pyridine (0.12 mL, 1.5 mmol) in CH₂Cl₂ (2 mL). The reaction was stirred 2 h and another 0.2 mL of the dibenzylphosphoriodinate solution was added. After 1 h at the same temperature, the reaction was filtered and partitioned between CH₂Cl₂ and dilute HCl solution. The organic phase was dried (MgSO₄) and concentrated to give a crude yellow oil. Chromatography (Isolute 5 g SI) with hexane/EtOAc: 0→100% afforded **13** as a colourless oil (125 mg, 50%). ¹H NMR (CDCl₃) δ 7.4 (br s, 15H); 5.2 (s, 2H); 5.1 (m, 2H); 5.06 (m, 2H); 4.44 (m, 1H); 4.2–3.9 (m, 3H); 3.8–3.5 (br, 2H); 2.57 (m, 2H); 1.6 (t, 2H); ¹³C NMR (CDCl₃) δ 172.8 (C_q); 136.1 (C_q); 136.0 (C_q); 129.1 (2CH); 128.8 (CH); 128.7 (CH); 128.5 (CH); 72.2 (d, CH₂, *J* = 7.5 Hz); 70.1 (d, CH₂, *J* = 7.5 Hz); 67.8 (CH); 67.0 (CH₂); 65.4 (CH); 42.0 (CH₂); 38.7 (CH₂); ³¹P NMR (CDCl₃) δ +0.92; [α]_D²³ = –3.7 (*c* 0.54, MeOH); MS (ES⁺) *m/z* 532 [M + NH₄], 515 [M + H]; ESHRMS *m/z* 515.1839 [M + H] (C₂₇H₃₂O₈P requires: 515.1835). Anal. calcd for C₂₇H₃₁O₈P/0.9H₂O: C, 61.10; H, 6.23; P, 5.84; found: C, 61.06; H, 5.69; P, 5.66.

6-(Bis-benzyloxy-phosphoryloxy)-(3*S*,5*S*)-3,5-bis-(tetrahydro-pyran-2-yloxy)-hexanoic acid benzyl ester (19). A solution of **13** (65 mg, 0.13 mmol), 3,4-dihydro-2*H*-pyran (0.2 mL, 2.5 mmol) and PPTS (12 mg, 0.05 mmol) in CH₂Cl₂ (2 mL) was stirred at room temperature for 48 h. The volatiles were removed in vacuo and the residue was chromatographed (Isolute 1 g SI) with hexane/EtOAc: 0→70% to yield **19** as an oil (72 mg, 81%). ¹H NMR (acetone-*d*₆) δ 7.5–7.3 (m, 15H); 5.2–5.0 (m, 6H); 4.8 (m, 1H); 4.67 (m, 1H); 4.4–3.75 (m, 6.5H); 3.4 (m, 2H); 2.7–2.35 (m, 1.5H); 1.9–1.3 (m, 14H); ³¹P NMR δ +0.574; +0.501; +0.379; +0.342; MS (ES⁺) *m/z* 705 [M + Na]; ESHRMS *m/z* 705.2789 [M + Na] (C₃₇H₄₇O₁₀NaP requires: 705.2805).

(3*S*,5*S*)-3,5-Dihydroxy-6-phosphonooxy-hexanoic acid (22). A suspension of **19** (55 mg) and 5% Pd/C²⁸

(20 mg) in MeOH (5 mL) was hydrogenated 30 min at rt. The catalyst was filtered off and the solvent was removed in vacuo. The crude residue was dissolved in THF (2 mL) and 1 M aqueous HCl (2 mL) was added. The reaction was stirred 2 h at room temperature and the solvent was evaporated to give a brownish oil. ¹H NMR (D₂O) δ 4.3–3.5 (m, 4H); 2.7–2.2 (m, 2H); 1.7–1.2 (m, 2H); ¹³C NMR (D₂O) δ 176.2 (C_q); 70.4 (CH₂); 66.95 (d, CH, *J* = 7.5 Hz); 64.8 (CH); 42.5 (CH₂); 39.0 (CH₂); ³¹P NMR (D₂O) δ +1.39; MS (ES⁺) *m/z* 267 [M + Na]; ESHRMS *m/z* 267.0257 [M + Na] (C₆H₁₃O₈NaP requires: 267.0246).

(3*S*,5*S*)-3,5-Dihydroxy-(2*R*)-2-methyl-6-phosphonooxy-hexanoic acid methyl ester (23). A suspension of **18** (50 mg) and 5% Pd/C (10 mg) in MeOH (5 mL) was hydrogenated 30 min at rt. The catalyst was filtered off and the solvent was removed in vacuo. The crude residue was dissolved in MeOH/HCl (5 mL) and the reaction was stirred 2 h at rt. The solvent was evaporated and the residue was dried under high-vacuum overnight. The residue was dissolved in MeOH and the white solid that precipitated was filtered off. Concentration of the filtrate gave the methyl ester **23** as a colourless oil (24 mg); ¹H NMR (CD₃OD) δ 4.2–3.8 (m, 4H); 3.7 (s, 3H); 2.52 (quint, 1H); 1.55 (m, 2H); 1.18 (d, 3H, *J* = 6.8 Hz); ¹³C NMR (CD₃OD) δ 177.4 (C_q); 72.2 (CH₂); 70.2 (CH); 68.6 (d, CH, *J* = 7.5 Hz); 52.6 (CH₃); 47.8 (CH); 39.4 (CH₂); 12.7 (CH₃); ³¹P NMR (CD₃OD) δ –1.89 (br); [α]_D¹⁸ = –18.7 (*c* 0.1, MeOH); MS (ES[–]) *m/z* 271 [M – H]; HRMS (ES[–]) *m/z* 271.0579 (C₈H₁₇O₈P requires: 271.0583).

(3*S*)-3,6-Bis-(bis-benzyloxy - phosphoryloxy) - (5*S*) - 5 - hydroxy-(2*R*)-2-methyl-hexanoic acid benzyl ester (25). Iodine (908 mg/3.6 mmol) was added at 0 °C to a solution of tribenzylphosphite (1.3 g, 3.7 mmol) in CH₂Cl₂ (10 mL). After 10 min at 0 °C and 10 min at room temperature, the red solution was added drop wise at –78 °C to a solution of **10** (400 mg, 1.49 mmol) and pyridine (0.6 mL, 7.5 mmol) in CH₂Cl₂ (6 mL). The reaction was stirred 2 h 15 min at –78 °C, diluted with CH₂Cl₂, filtered, washed with water, dried (MgSO₄) and concentrated in vacuo. The crude oil was chromatographed (Isolute 20 g SI) with hexane/EtOAc: 25→100% to give the diphosphate as a light yellow oil. The product dissolved in CH₂Cl₂ was treated with activated charcoal and filtered. Removal of the solvent in vacuo yielded **25** as a colourless oil (715 mg, 61%); ¹H NMR (CDCl₃) δ 7.35 (br s, 25H); 5.18 (s, 2H); 5.12–4.98 (m, 8H); 4.75 (m, 1H); 4.1 (m, 2H); 3.95 (m, 2H); 2.55 (m, 1H); 1.75–1.55 (m, 2H); 1.25 (d, 3H, *J* = 7.2 Hz); ¹³C NMR (CDCl₃) δ 175.2 (C_q); 136.3 (C_q); 136.1 (C_q); 136.0 (C_q); 129.0 (CH); 128.7 (CH); 128.6 (CH); 128.4 (CH); 74.9 (CH); 70.2 (d, CH₂); 69.85 (d, CH₂); 69.6 (CH₂); 67.4 (CH); 66.8 (CH₂); 45.6 (CH); 36.8 (CH₂); 12.6 (CH₃); ³¹P NMR (CDCl₃) δ +1.13; +0.24; ESHRMS *m/z* 806.2860 [M + NH₄] (C₄₂H₅₀NO₁₁P₂ requires 806.2859). Anal. calcd for C₄₂H₄₆O₁₁P₂: C, 63.96; H, 5.88; P, 7.85; found: C, 63.93; H, 6.00; P, 7.81.

Biological tests

6PGDH enzyme assay. The recombinant *T. brucei* 6PGDH, overexpressed in *Escherichia coli*, was purified by a technique which was slightly modified compared to the original of Barrett.⁹ 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 39,000 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 flowthrough 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 one day and was monitored both by SDS-PAGE and activity assays. Enzyme was stored in the presence of 50% glycerol at –20 °C. The sheep liver 6PGDH was purified as reported.¹⁸ Activity of the enzymes at 20 °C is followed spectrophotometrically (Kontron Uvikon 930 spectrophotometer) at 340 nm, measuring the production of NADPH in absence or presence of inhibitor.

Compounds to assay as inhibitors were dissolved in either methanol, DMSO or water. Inhibition type and degree was evaluated by performing the assays in TEA buffer, pH 7.5 at variable 6PG concentration (8–40 μ M), whilst keeping NADP⁺ concentration fixed to 0.26 mM. When lack of solubility of the compound did not allow to assay a high inhibitor concentration in the conditions described above, enzyme activity was assayed in TEA buffer in the presence of either 20% methanol or 10 or 20% DMSO. The concentration of inhibitors was fixed and the reaction was initiated by the addition of either 0.2 μ g *T. brucei* 6PGDH or 0.5 μ g sheep liver 6PGDH per mL reaction mixture. The mean K_i values were calculated by Lineweaver–Burk plots where the standard errors of linear regressions were below 0.05 and after experiments were repeated several times. The selectivity degree is valued as the ratio between the mammal and parasite enzymes K_i values.

Compounds **20** and **22** were assayed as substrates in the presence of 0.25 mM NADP⁺ in TEA buffer, pH 7.5, at concentrations of 2.6 and 3.4 mM, respectively, against both sheep and *T. brucei* enzymes.

In vitro pharmacology, *T.b. rhodesiense*. Minimum Essential Medium (50 μ L) 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 μ L of trypanosome suspension (*T.b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5% CO₂ atmosphere for 72 h. Alamar Blue (10 μ L) 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 emission wavelength of 590 nm. Fluorescence development was expressed as percentage of the control, and IC₅₀ values determined.

In vitro pharmacology, *T. cruzi*. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μ L 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 galactosidase (Lac Z gene) were added in 100 μ L per well with 2 $\times$ of a serial drug dilution. The plates were incubated at 37 °C in 5% CO₂ for 4 days. After 96 h, the minimum inhibitory concentration (MIC) was determined microscopically. For measurement of the IC₅₀ the substrate CPRG/Nonidet was added to the wells. The colour reaction which developed during the following 2–4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve IC₅₀ values were calculated. Cytotoxicity was assessed in the same assay using non-infected L-6 cells and the same serial drug dilution. The MIC was determined microscopically after 4 days.

In vitro pharmacology, *L. 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* amastigote were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. Next day, the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37 °C under a 5% CO₂ atmosphere for 96 h. Then the medium was removed, the slides fixed with methanol and stained with Giemsa. The ratio of infected to non-infected macrophages was determined microscopically, expressed as percentage of the control and the IC₅₀ value calculated by linear regression.

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References and Notes

1. WHO/CDS/CSR/ISR/2000.1, <http://www.who.int/emc>.
2. <http://www.who.int/tdr/diseases/trypan/diseaseinfo.htm>.
3. World Health Organ. Tech. Rep. Ser. **1998**, 881, 1.
4. (a) De Koning, H. P. *Int. J. Parasitol.* **2001**, *31*, 512. (b) Denise, H.; Barrett, M. P. *Biochem. Pharmacol.* **2001**, *61*, 1.
5. Keiser, J.; Stich, A.; Burri, C. *TRENDS Parasitol.* **2001**, *17*, 42.
6. (a) Oppendoes, F. R. *Biochem. Soc. Trans.* **1990**, *18*, 729. (b) Oppendoes, F. R.; Michels, P. A. M. *Int. J. Parasitol.* **2001**, *31*, 482.
7. Barrett, M. P. *Parasitol. Today* **1997**, *13*, 11.
8. Barrett, M. P.; Le Page, R. W. F. *Mol. Biochem. Parasitol.* **1993**, *57*, 89.

9. Barrett, M. P.; Phillips, C.; Adams, M. J.; LePage, R. W. F. *Protein Expres. Purif.* **1994**, *5*, 44.
10. Krepinsky, K.; Plaumann, M.; Martin, W.; Schnarrenberger, C. *Eur. J. Biochem.* **2001**, *268*, 2678.
11. Phillips, C.; Dohnalek, J.; Gover, S.; Barrett, M. P.; Adams, M. J. *J. Mol. Biol.* **1998**, *282*, 667.
12. Barrett, M. P.; Gilbert, I. H. *Curr. Topics Med. Chem.* **2002**, *2*, 471.
13. Marchand, M.; Kooystra, U.; Wierenga, R. K.; Lambeir, A.-M.; Beeumen, J. V.; Oppendoes, F. R.; Michels, P. A. *Eur. J. Biochem.* **1989**, *184*, 455.
14. Lobo, Z.; Maitra, P. K. *Mol. Gen. Genet.* **1982**, *185*, 367.
15. Gvozdev, V. A.; Gerasimova, T. I.; Kogan, G. L.; Braslavskaya, O. Yu. *FEBS Lett.* **1976**, *64*, 85.
16. (a) Phillips, C.; Gover, S.; Adams, M. J. *Acta Cryst.* **1995**, *D51*, 290. (b) Adams, M. J.; Ellis, G. H.; Gover, S.; Naylor, C. E.; Phillips, C. *Structure* **1994**, *2*, 651.
17. Pasti, C.; Rinaldi, E.; Cervellati, C.; Dallochio, F.; Hardré, R.; Salmon, L.; Hanau, S. *Bioorg. Med. Chem.* **2003**, *11*, 1207.
18. (a) Hanau, S.; Rippa, M.; Bertelli, M.; Dallochio, F.; Barrett, M. P. *Eur. J. Biochem.* **1996**, *240*, 592. (b) Rippa, M.; Giovannini, P. P.; Barrett, M. P.; Dallochio, F.; Hanau, S. *Biochim. Biophys. Acta* **1998**, *1429*, 83.
19. Bertelli, M.; El-Bastawissy, E.; Knaggs, M. H.; Barrett, M. P.; Hanau, S.; Gilbert, I. H. *J. Comput. Aid. Mol. Des.* **2001**, *15*, 465.
20. Berdis, A. J.; Cook, P. F. *Biochemistry* **1993**, *32*, 2041.
21. Details of the development of our synthetic approach for the preparation of the (2*R*)-2-methyl-4-deoxy analogues (compounds **2**, **3**, **6**, **8**, **10**, **12**, **14–18**, **20**, **21**) and (2*R*)-2-methyl-4,5-dideoxy analogues (compounds **26–32**) have been described elsewhere: Dardonville, C.; Gilbert, I. H. *Org. Biomol. Chem.* **2003**, *1*, 552.
22. Evans, D. A. *Aldrichim. Acta* **1982**, *15*, 23.
23. (a) Meyers, A. I.; Lawson, J. P.; Walker, D. G.; Linderman, R. J. *J. Org. Chem.* **1986**, *51*, 5111. (b) Hanessian, S.; Ugolini, A.; Therien, M. *J. Org. Chem.* **1983**, *48*, 4427. (c) Boger, D. L.; Curran, T. T. *J. Org. Chem.* **1992**, *57*, 2235.
24. (a) Ueno, Y.; Miyamo, T.; Okawara, M. *Tetrahedron Lett.* **1982**, *23*, 443. (b) Vedejs, E.; Powell, D. W. *J. Am. Chem. Soc.* **1982**, *104*, 2046.
25. Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141.
26. (a) Gefflaut, T.; Lemaire, M. *Valentin, M-L., Bolte, J. J. Org. Chem.* **1997**, *62*, 5920. (b) Kis, K.; Wungsintaweeikul, J.; Eisenreich, W.; Zenk, M. H.; Bacher, A. *J. Org. Chem.* **2000**, *65*, 587.
27. It should be noted that compounds **20** and **22** could not be obtained in a pure form directly by hydrogenolysis of **12** and **13**, respectively. Phosphate migration ability of this kind of molecules has been discussed in another article. See [ref 21](#).
28. We used 5% Pd/C from Fluka Chemical company to avoid early deprotection of the THP. Kaisalo, L. H.; Hase, T. A. *Tetrahedron Lett.* **2001**, *42*, 7699.