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Sugar Derivatives as New 6-Phosphogluconate Dehydrogenase Inhibitors Selective for the Parasite *Trypanosoma brucei*

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Abstract—Sugar derivatives mimicking compounds which take part in the catalysed reaction have been assayed as alternative substrates and/or competitive inhibitors of 6-phosphogluconate dehydrogenase from *Trypanosoma brucei* and sheep liver. Phosphonate analogues have been synthesised and the new compound 5-deoxy-5-phosphono-D-arabinonate shows good selectivity towards the parasite enzyme. A number of 4-carbon and 5-carbon aldonates are strong inhibitors of the parasite enzyme with K_i values below the substrate K_m and some acyl derivatives are also potent inhibitors. At least five of the compounds showing a significant selectivity for the parasite enzyme represent leads for trypanocidal drugs against this recently validated target.

Introduction

The pentose phosphate pathway enzyme, 6-phosphogluconate dehydrogenase (decarboxylating, 6PGDH, EC 1.1.1.44), is one of the chosen targets for drug design against African trypanosomiasis.[1,2] This type of infectious disease, affecting both humans and animals in Sub-Saharan Africa and caused by different subspecies of the protozoan parasite *Trypanosoma brucei*, is re-emerging, and chemotherapy, which is the only practical means of control, needs an increase in both commercial and scientific investment.[3,4] 6PGDH catalyses the $NADP^+$-dependent oxidative decarboxylation of 6-phospho-D-gluconate (6PG) to D-ribulose 5-phosphate (Ru5P), via 3-keto 6PG and a probable 1,2-enediol as intermediates (Scheme 1).[5,6] The importance of this enzyme is highlighted by the fact that in eukaryotes *Drosophila* and yeast, deletion of the correspondent gene is lethal.[7,8] RNA interference technology has also shown the gene to be essential in bloodstream forms *T. brucei* (M. P. Barrett, personal communication). At the same time, the substrate 6PG inhibits the key glycolytic enzyme phosphoglucose isomerase[9,10] and thus glycolysis, which is the only source of ATP for bloodstream form *T. brucei*. Significant differences have been found between the *T. brucei* and the mammalian 6PGDHs, both in the sequences[11] and in the kinetics[12,13] and inhibitors have been found that show significant selectivity for the parasite enzyme, such as some triphenylmethane derivatives.[1] Crystallographic structures[2] and molecular modelling[1] show critical differences between the two above-mentioned 6PGDHs, which can explain the inhibitors' selectivity and which lead us to continue looking for inhibitors and studying drug design. Until now, 2-deoxy-6PG (**1**, Fig. 1) has been shown to be the most selective inhibitor for the parasite 6PGDH with indications of an affinity for the *T. brucei* enzyme which is more than 170-fold increased compared to that of sheep liver.[12,13] Thus sugar derivatives which mimic the substrate, the product and the intermediates of the catalysed reaction are worthy of study as *T. brucei* 6PGDH inhibitors. We have found good selectivity values with some phosphate sugar derivatives and some which are phosphonate analogues.

Results

6-Carbon sugar derivatives and bio-isosteric analogues

Several analogues of 6PG have been tested as alternative substrates or inhibitors of 6PGDH (**2**–**4**, Fig. 1).

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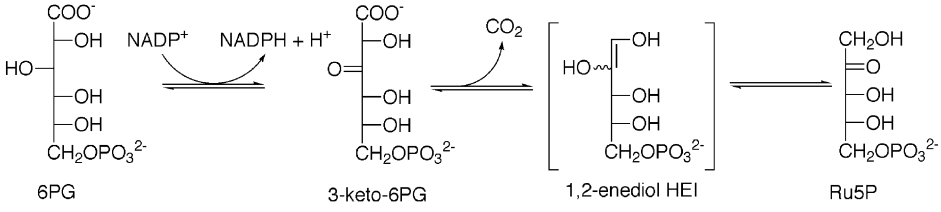
This group includes 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate (**2**), an analogue of the substrate 6PG in which the C–O–P moiety is replaced by C–CH_2–P;[14] this resulting in a bio-isosteric replacement. The two enzymes clearly differ from each other when using 6PG analogues which act as alternative substrates. As already reported,[12,13] compared to 6PG, 2-deoxy-6-phosphogluconate (**1**) shows a 100-fold increase in the K_m value with the sheep liver enzyme, against a 2-fold increase with the *T. brucei* enzyme. 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate (**2**) is a substrate for both the *T. brucei* and the sheep liver 6PGDHs, and the K_m value is similar to that of 6PG for both the 6PGDHs, while the catalytic efficiency (k_{cat}/K_m) is 20-fold less for the *T. brucei* 6PGDH, and only 6-fold less for the sheep enzyme (Table 1, where K_m is represented as K_s).

With the stereoisomers of 6PG, 6-phospho-D-mannonate (**3**) and 6-phospho-D-galactonate (**4**), the enzymes from sheep liver and *T. brucei* show the same behaviour. Compound **3** inhibits both the 6PGDHs in a competitive way, even if for both it seems to have a low affinity: the K_i values are 0.8 and 0.13 mM for the *T. brucei* and the sheep liver 6PGDHs, respectively. Catalytic activity on **3** is not significant from both parasite and sheep liver 6PGDHs since it is, respectively, 4 and 5 orders of magnitude less than with 6PG. Compound **4** inhibits both enzymes but again the affinity is low since the K_i values are 0.19 mM for the *T. brucei* 6PGDH and 0.56 mM for the sheep liver 6PGDH. Catalytic activity on **4** is also not significant from both 6PGDHs since it is 3 orders of magnitude less than with 6PG.



Scheme 1. 6-Phosphogluconate dehydrogenase reaction. 6-Phospho-D-gluconate (6PG) is oxidised to 3-keto 6-phospho-D-gluconate (3-keto-6PG) which is then decarboxylated to a probable 1,2-enediol high-energy intermediate (HEI) (between brackets) of the product D-ribulose 5-phosphate (Ru5P).

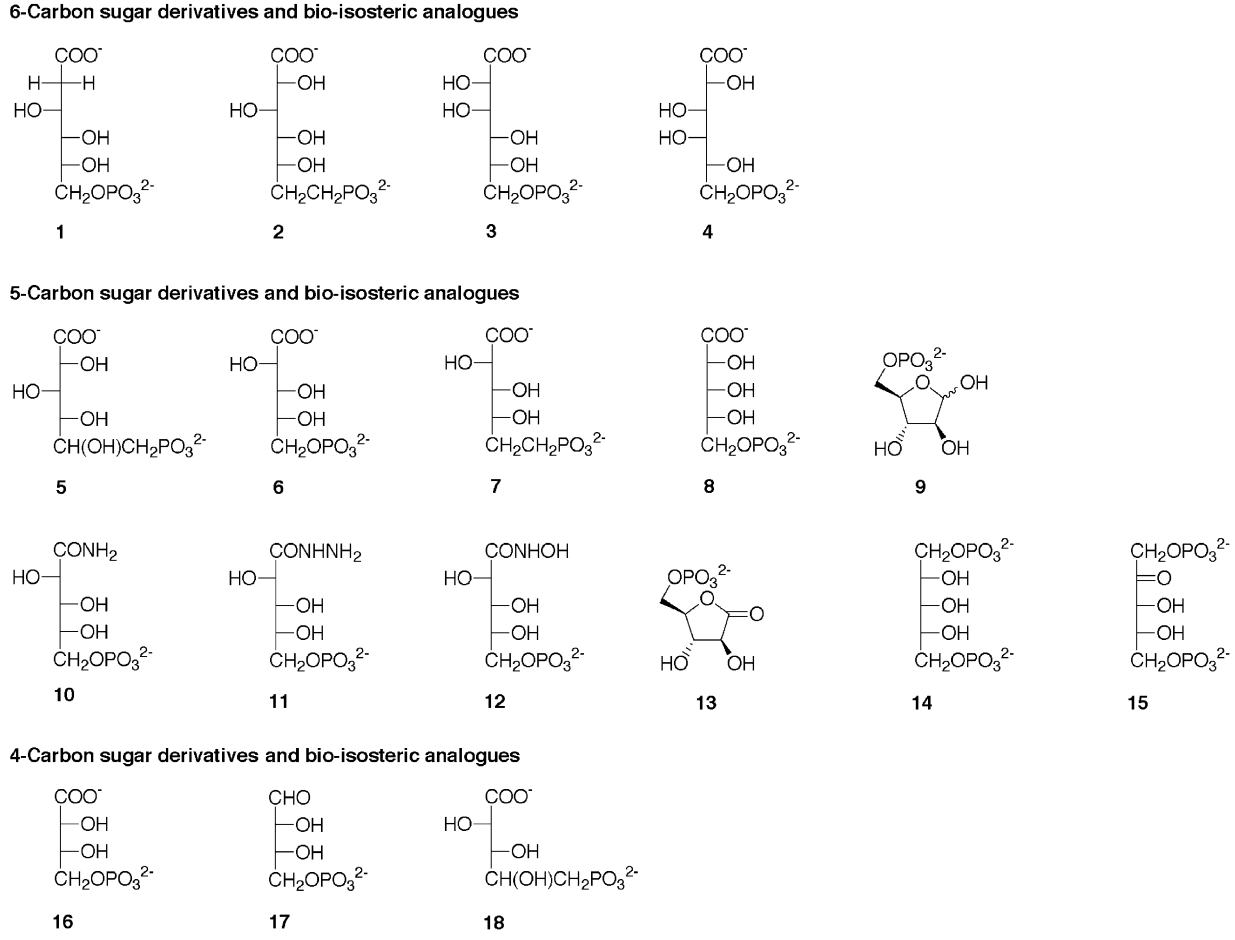


Figure 1. Structures and numbering of the compounds used in the present study.

5-Carbon sugar derivatives and bio-isosteric analogues

Several 5-carbon sugar derivatives have been tested as 6PGDH inhibitors (**5**–**15**, Fig. 1). This group includes 6-deoxy-6-phosphono-D-gluconate (**5**), where the $CH(OH)CH_2$ moiety replaces the CH_2O part of the phosphate ester in 5-phospho-D-xylonate, and 5,6-dideoxy-6-phosphono-D-*arabino*-hexonate (**7**), with the methylene group replacing the oxygen of the phosphate ester in **6**. The K_i values for both the *T. brucei* and the sheep liver 6PGDHs are reported in Table 2.

With the sheep liver enzyme, the K_i values of all the inhibitors are higher than the K_m values of 6PG, indicating that the shortening of the carbon chain negatively affects the binding. A different result is observed with the *T. brucei* enzyme; indeed one half of the inhibitors have K_i values equal to, or lower than the K_m value of the substrate. This different behaviour of the two enzymes brings out a marked selectivity of these inhibitors (Table 2, last column).

Some findings must be pointed out. With both enzymes, even if with different affinity, 5-phospho-D-ribonate (**8**) is a better inhibitor than 5-phospho-D-arabinonate (**6**), despite the fact that this latter compound retains the same configuration of the substrate 6PG (Table 2). In fact **8** has the inverted configuration at C2, which corresponds to the C3 of 6PG if we suppose these analogues bind to the enzyme by preserving the position of the phosphate group. The result is quite surprising, the C3 of 6PG being the site of the enzymatic oxidation, and it might be expected to have more stringent steric requirements.

Another unexpected result is the low affinity of 5,6-dideoxy-6-phosphono-D-*arabino*-hexonate (**7**) compared to the bio-isosteric phosphate ester, 5-phospho-D-arabinonate (**6**) (Table 2). The corresponding 6-carbon sugar derivatives, 6PG and 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate (**2**), have a nearly identical K_m (Table 1). This means that a general rule regarding the effect on the binding cannot be drawn from the replacement of the oxygen atom with a methylene group.

Another difference between the sheep liver and the *T. brucei* enzymes is the sensitivity to inhibition by derivatives of 5-phospho-D-arabinose (**9**, Fig. 1), namely 5-phospho-D-arabinonate, 5-phospho-D-arabinonamide, 5-phospho-D-arabinonhydrazide, 5-phospho-D-arabinonohydroxamic acid, and 5-phospho-D-arabinono-1,4-lactone (respectively **6**, **10**, **11**, **12**, and **13**, Fig. 1). As reported in Table 2, for the sheep enzyme, the efficiency of inhibition is in the order amide (**10**) > carboxylate (**6**) > hydrazide (**11**) > lactone (**13**) > hydroxamic acid (**12**), while for the *T. brucei* enzyme the order is carboxylate (**6**) > amide (**10**) = hydroxamic acid (**12**) > lactone (**13**) > hydrazide (**11**).

The most surprising result is the strong inhibition by 6-deoxy-6-phosphono-D-gluconate (**5**). According to a schematic simplification, this compound could bind to the enzyme either by preserving the correct position of the carbohydrate chain, or by preserving the position of the phosphate/phosphonate group. In the first case a low affinity is expected, due to the improper location of the phosphonate moiety in the phosphate binding site. In the second case two carbon atoms (C2 and C3 corresponding to the C3 and C4 of 6PG) would have an incorrect configuration, and a bulky CHOH group would replace the methylene bound to the phosphate, generating steric hindrance.

Finally, both 1,5-diphospho-ribitol and 1,5-diphospho-D-ribulose (respectively **14** and **15**, Fig. 1), weakly inhibit 6PGDHs in a competitive manner and display only a slight selectivity for the *T. brucei* over the sheep liver enzyme (Table 2).

Table 1. Kinetic parameters for *T. brucei* and sheep liver 6PGDHs with 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate (**2**) and 6-phospho-D-gluconate (6PG) as substrates

Parameter	*T. brucei*		Sheep liver	
	2	6PG	**2**	6PG
k_{cat} (s^{-1})	1.4	31.8	0.53	9
K_S (μM)	4.8	5.3	10.5	29.6
k_{cat}/K_S (M^{-1} s^{-1})	292	6000	50.5	304

Table 2. Inhibitory effect of compounds shown in Figure 1 on 6PGDH from *T. brucei* and sheep liver

6PGDH Inhibition inhibitor	K_i versus substrate (μM)		Selectivity (ratio sheep/parasite)
	T. brucei	Sheep	
1	4.4[a]	770[a]	175
3	800±10	135±2	0.17
4	190±7	565±5	3
5	1.30±0.15	35.80±0.06	27.5
6	3.4±0.3	109±3	32
7	21.2±0.8	1000±5	47
8	0.95±0.05	67±3	70
9	25.0±0.5	710±3	28
10	5.60±0.08	80.30±0.08	14.3
11	26.70±0.0015	128.00±0.08	4.8
12	5.8±0.3	307±5	53
13	11.00±0.83	147±1	13
14	122.0±0.9	500±2	4
15	274±4	1000±5	3.6
16	0.13±0.01	10.7±0.8	83
17	175.0±0.5	1590±10	9.1
18	7.0±0.4	433±3	62

[a]From ref 13.

4-Carbon sugar derivatives and bio-isosteric analogues

In this group (Fig. 1), compounds tested as inhibitors were 4-phospho-D-erythronate (**16**), 4-phospho-D-erythrose (**17**) and 5-deoxy-5-phosphono-D-arabinonate (**18**) where the $CH(OH)CH_2$ moiety replaces the CH_2O part of the phosphate ester in 4-phospho-D-threonate.

4-Phospho-D-erythronate (**16**) is the most powerful inhibitor among all the compounds assayed (Fig. 2). It also displays the highest selectivity value for the parasite enzyme compared to the sheep liver one (K_i ratio = 83, Table 2). Instead, 4-phospho-D-erythrose (**17**), although it inhibits 6PGDH competitively, has a low affinity for

both the parasite and the sheep enzymes (Table 2). 5-Deoxy-5-phosphono-D-arabinonate (**18**) shows a high affinity for the *T. brucei* 6PGDH and, similarly to **16**, a high selectivity value (K_i ratio = 62, Table 2).

Discussion

Bearing a rational drug design in mind, targeting the *T. brucei* 6PGDH in a selective way with respect to the mammalian enzyme, we started from the significant differences between the two 6PGDHs: in the kinetics and affinity for the substrate analogue 2-deoxy-6PG (**1**, Fig. 1).[12] In the screening of a large number of sugar derivatives (Fig. 1) mimicking the compounds which take part in the catalysed reaction (Scheme 1), the strongest *T. brucei* 6PGDH inhibitor has been shown to be the 4-carbon aldonate 4-phospho-D-erythronate (**16**). Its structural analogue 5-deoxy-5-phosphono-D-arabinonate (**18**) is one of the most potent inhibitors, too. If we assume that all the inhibitors assayed bind to the active site mainly by the phosphate group, the C1 of the 4-carbon compounds should correspond to the C3 of the substrate 6PG and the intermediate 3-keto-6PG, and to the C2 of the postulated 1,2-enediol high-energy intermediate (HEI) and the product of the reaction Ru5P (Scheme 1). The K_i value of **16** for the *T. brucei* 6PGDH being an order of magnitude lower than the respective K_m for 6PG (Table 2) is in agreement with the fact that the inhibitor mimics the supposed 1,2-enediol (ate) intermediate of the 6PGDH catalysed reaction (Scheme 1): the double bond between C1 and C2 of the HEI would match exactly the position of one of the C1–O bond of the carboxylate moiety in **16**. Also, we found previously that the intermediate analogue 2-deoxy-3-keto-6PG has a K_i value 18-fold lower for the *T. brucei* 6PGDH compared to that of the sheep liver one.[12] Now we see that the selectivity of 4-phospho-D-erythronate (**16**) for the parasite enzyme compared to the sheep liver one is 83. Thus analogues of the enediol intermediate appear as lead compounds in the search for selective drugs targeting the *T. brucei* 6PGDH. Structural reasons for this large selectivity may be elucidated by crystallographic studies, up-to-date data show that the active site is at the interface between the two equal subunits of the enzyme[16] (Fig. 3, PDB entry: 1PGP) and that in the *T. brucei* enzyme there are major inter-subunit interactions.[2] Figure 3 shows the residues directly binding 6PG in the sheep liver 6PGDH:[17] Arg-287, Tyr-191, Lys-260, Thr-262, Arg-446 (belonging to the second

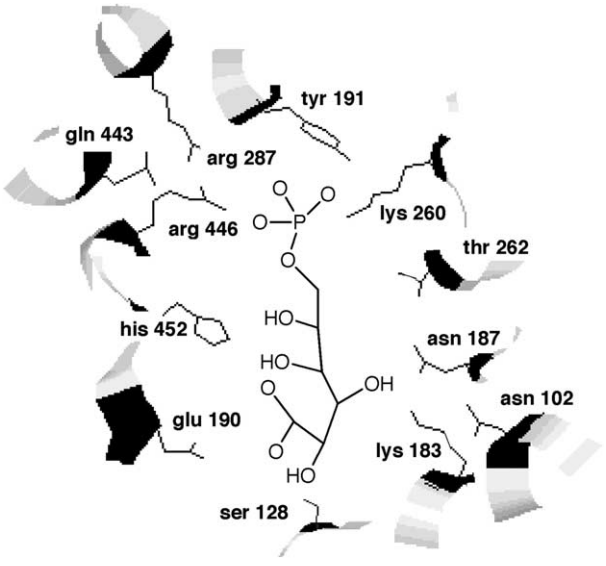


Figure 3. The sheep liver 6PGDH substrate binding site. Gln-443, Arg-446 and His-452 belong to the second subunit. In the *T. brucei* enzyme, Ser-450 replaces Gln-443.

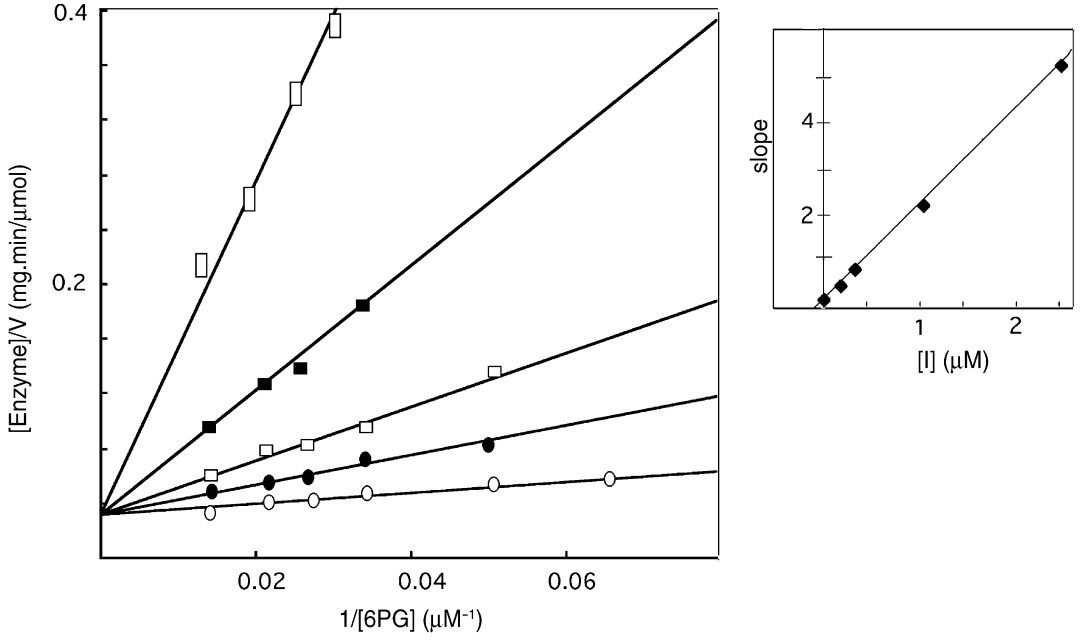


Figure 2. Inhibition of *T. brucei* 6PGDH by D-erythronate 4-phosphate (**16**). Left: double reciprocal plot of initial reaction velocity versus 6PG concentration obtained at various inhibitor concentrations (Lineweaver–Burk graphical representation): ○, no inhibitor; ●, 0.15 μM; □, 0.3 μM; ■, 1 μM; ▯, 2.5 μM. Right: secondary plot of slopes of lines from the Lineweaver–Burk plot, from which the K_i is determined.

subunit) are ligands of the phosphate of the substrate, His-452 (belonging to the second subunit) makes at least a hydrogen bond with a hydroxyl of the central part of the substrate, Lys-183 and Asn-187 bind to the 3-hydroxyl while Ser-128 and Glu-190 interact with the carboxyl group. Asn-102 is within 4 Å of the 6PG site too, while Gln-443 is a second neighbour to the substrate which can make a hydrogen bond with Arg-446. Replacement of Gln with a Ser (Ser-450) in the *T. brucei* 6PGDH does not allow the hydrogen bond with the corresponding Arg (Arg-453).[2] Thus, even if all the residues directly binding 6PG are conserved in the parasite enzyme, critical differences in the distances between charged residues are present in the active site,[1] also due to substitutions in residues second neighbours to 6PG.[2] 5-Deoxy-5-phosphono-D-arabinonate (**18**) also has a high selectivity for the parasite 6PGDH. 4-Phospho-D-erythrose, although it is not a high-affinity inhibitor, shows a certain selectivity too (Table 2).

The good inhibition properties of trypanosomal 6PGDH by the 5-carbon aldonates and compounds with a similar structure (Table 2) are a little more surprising, even more so if we consider that the highest affinity for the *T. brucei* 6PGDH is shown by compounds which have at the C2 (correspondent to the C3 of 6PG) a stereochemistry which is opposite to that of 6PG (Fig. 1). In fact, 6-deoxy-6-phosphono-D-gluconate (**5**) and 5-phospho-D-ribonate (**8**), which both have the *R* configuration at C2, in contrast to the *S* configuration at the C3 of 6PG (Scheme 1), have K_i values 5-fold lower than the substrate K_m value (Table 1). Their affinities are higher than those of 5-phospho-D-arabinonate (**6**) and its derivatives (**10**–**13**), which all have the *S* configuration at the C2 as 6PG at the C3, and have K_i values for the *T. brucei* 6PGDH of the same order of magnitude as the substrate K_m. It seems that compounds **5** and **8** bind to the active site in a way different from the arabino derivatives. In this last family 5-phospho-D-arabinonohydroxamic acid (**12**) has a good parasite 6PGDH selectivity and only 5-phospho-D-arabinonhydrazide (**11**) and 5-phospho-D-arabinose (**9**) have no high affinity for the parasite enzyme, even if the latter compound shows a significant selectivity for the *T. brucei* 6PGDH. Another cyclic compound, 5-phospho-D-arabinono-1,4-lactone (**13**), shows a moderate affinity for the *T. brucei* 6PGDH and selectivity, in contrast to 5-phospho-D-ribose and 2-deoxy-5-phospho-D-ribose which do not inhibit the enzyme (data not shown).

Since 2-deoxy-5-phospho-D-ribonate shows a K_i higher than 1 mM (data not shown) we conclude that an oxygen at this position (C3 of 6PG), independent of the stereochemistry, is fundamental for binding to the active site. At the same time, the double bond at the C1 of these compounds (C2 position of 6PG) is also important for the binding to the parasite enzyme. In fact the compounds 1,5 diphospho-ribitol (**14**) and 1,5 diphospho-D-ribulose (**15**) have a lower affinity for the parasite 6PGDH. On the contrary, the low affinity for both 6PGDHs and the lack of selectivity shown by the 6-carbon substrate analogues, 6-phospho-D-mannonate (**3**) and 6-phospho-D-galactonate (**4**), indicate that two vicinal hydroxyls with the same *S* configuration at either C2 and C3 or C3 and C4, are an obstacle for tight binding to 6PGDH. The high affinity shown by 6-deoxy-6-phosphono-D-gluconate (**5**) for the parasite 6PGDH indicates, on the other hand, that an opposite stereochemistry at the hydroxyl in C4 (with contemporary stereochemistry change at C3: there are not two vicinal hydroxyls with the same configuration) is not an obstacle to tight binding to the active site. A different conclusion has been reported for the yeast 6PGDH.[6]

5,6-Dideoxy-6-phosphono-D-*arabino*-hexonate (**7**), bio-isosteric to 5-phospho-D-arabinonate (**6**), shows a good selectivity for the parasite enzyme. Generally all the phosphonate compounds assayed showed a good selectivity for the parasite 6PGDH, which indicates that this enzyme binds the phosphonate group better than the sheep liver enzyme. This result is in agreement with the crystallographic structures which show that a hydrogen bond which defines the orientation of the substrate phosphate is lacking in the parasite enzyme (between Gln-443 and Arg-446 in Figure 3, see above in Discussion).[2] The crystal of the enzyme in ammonium sulfate binds two sulfate ions tightly at the 6PG binding site, in equivalent positions to the phosphate and the carboxyl of the substrate respectively.[2,15] Analysing the crystallographic structures of both the *T. brucei* and the sheep liver 6PGDHs (respective PDB entries: 1PGJ and 2PGD), we realised that the distance between the two sulfates is shorter in the parasite enzyme than in the mammalian one: 6.46 Å between the two sulfur atoms in the *T. brucei* enzyme against 6.78 Å in the sheep liver one. This might explain why the first binds the 5-carbon derivatives studied better. Phosphonates inhibitors may be promising compounds in view of therapeutic applications: in constrast to phosphates derivatives, they are not subject to hydrolysis by kinases. Furthermore, they might be more easily delivered inside the cell because of their slightly lower polarity.

We finally report that the bio-isosteric phosphonate analogue of 6PG, 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate (**2**), is substrate for both the *T. brucei* and the sheep liver 6PGDHs, in agreement with previous reports in which **2** was shown as a substrate of the yeast 6PGDH.[14] Since for the *T. brucei* 6PGDH the catalytic efficiency with this alternative substrate is 20-fold less than with 6PG, while for the sheep liver enzyme it is only 6-fold less, the final effect of this compound might be that of inhibiting the parasite 6PGDH, while it is not too toxic for the mammalian enzyme. Hence a therapeutic potential of this last compound may be also considered.

Conclusion

Summing up, half of the 18 compounds shown have selectivity values over 25, and thus they are good leads for potential trypanocide drugs. We shall now assay them as trypanocidals and try to obtain crystals of complexes of the 6PGDH with some of them, which will enable us to perform X-ray diffraction studies.

Experimental

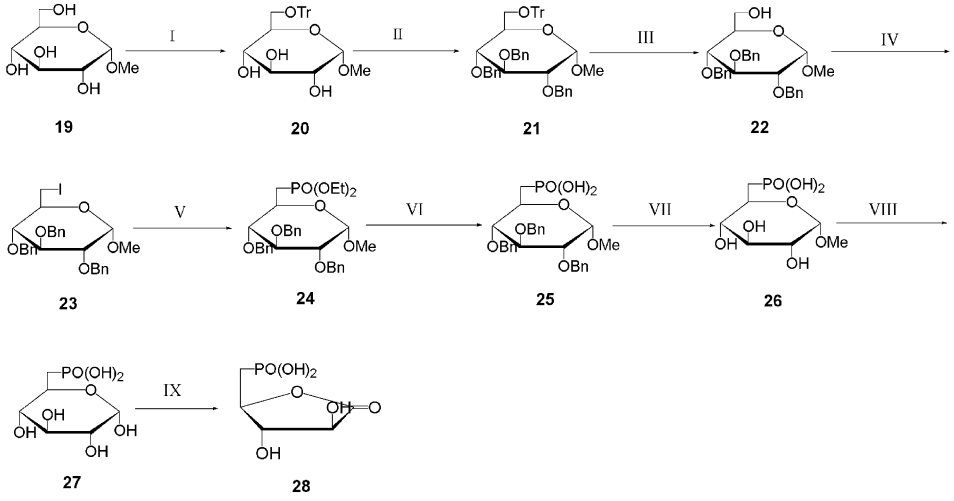
Enzyme assays

The recombinant *T. brucei* 6PGDH, overexpressed in *Escherichia coli*, was purified by a technique which was slightly modified compared to the original of Barrett.[16] Cells lysed by freeze–thaw cycles and then resuspended in 50 mM triethanolamine/HCl containing 1 mM EDTA, pH 7.5 (TEA buffer) were 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 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 0.5 mM $NADP^+$. The whole purification lasted less than 1 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. Kinetic parameters were obtained in TEA buffer, pH 8 varying either 6PG or 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate concentration in the range of 8–40 μM whilst keeping $NADP^+$ concentration fixed to 0.5 mM. Reactions with 6PG as substrate were initiated by addition of either 0.2 μg *T. brucei* 6PGDH or 0.5 μg sheep liver 6PGDH per mL reaction mixture while 10 times more enzyme was used in reactions with 6,7-dideoxy-7-phosphono-D-*gluco*-heptonate as substrate. Inhibition type and degree was evaluated by performing the assays in TEA buffer, pH 7.5 at variable 6PG concentration, whilst keeping $NADP^+$ concentration fixed to 0.26 mM. As shown in Figure 2, a number of inhibitor concentrations were tested and K_i values calculated by Lineweaver–Burk plots[1] or secondary plots;[19] experiments were repeated several times. The selectivity degree is valued as the ratio between the mammal and parasite enzymes K_i values.

Chemistry

The aldonates were produced by oxidation with bromine of the corresponding commercial sugars.[20] 5-Phospho-D-arabinonate (**6**), 5-phospho-D-arabinonamide (**10**), 5-phospho-D-arabinonhydrazide (**11**), 5-phospho-D-arabinonohydroxamic acid (**12**), and 5-phospho-D-arabino-1,4-lactone (**13**) were synthesised as already reported.[19,21,22] 1,5-Diphospho-ribitol (**14**) was produced by reduction of 1,5-diphospho-D-ribulose (**15**) with $NaBH_4$.[23] 6,7-Dideoxy-7-phosphono-D-*gluco*-heptonate (**2**) and 6-deoxy-6-phosphono-D-gluconate (**5**) were obtained by bromine oxidation of 6,7-dideoxy-7-phosphono-D-*gluco*-heptose[24] and 6-deoxy-6-phosphono-D-glucose,[24] respectively.

5-Deoxy-5-phosphono-D-arabinonate (18). The title compound **18** is a new compound which was obtained from 6-deoxy-6-phosphono-D-glucose[24] by a synthesis procedure analogous to that used to obtain 5-phospho-D-arabinonate (**6**) from 6-phospho-D-glucose.[19,21] The complete synthesis (shown in Scheme 2) started from the commercially available 1-methyl-α-D-*gluco*-pyranoside (**19**). To a solution of **19** (10 g, 51.5 mmol) in dry pyridine (200 mL), chlorotriphenylmethane (28 g, 100 mmol) was added and the reaction mixture was kept at room temperature overnight to give **20**. After concentration in vacuum, the residue was dissolved in *N,N*-dimethylformamide (DMF) and NaH (7.2 g, 300 mmol) was added to the cooled (0 °C) solution. After 20 min at room temperature, benzyl bromide (21 mL, 175 mmol) was added cautiously to the cooled solution. After 2 h, methanol (15 mL) was added and the mixture was poured into 200 mL of water. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated. Flash column chromatography of the



Scheme 2. (I) Ph_3CCl, pyridine; (II) DMF, 0 °C, NaH, 20 min, BnBr, 2 h; (III) 15 °C, HBr, acetic acid; (IV) Ph_3P, I_2, imidazole, toluene, reflux, 2 h; (V) $P(OEt)_3$, 140 °C, 24 h; (VI) 0 °C, CH_2Cl_2, Me_3SiBr, 24 h; (VII) methanol, H_2, Pd/C, 3 h; (VIII) 6 N HCl, 90 °C; (IX) O_2/1 N NaOH, 1 week.

residue on silica gel 60 (230–400 mesh) with cyclohexane/ethyl acetate (5/1) gave **21** (15 g, 41% yield from **19**). 45% Hydrogen bromide in glacial acetic acid (12 mL) was added to a cooled (15 °C) solution of **21** (15 g, 21.2 mmol) in glacial acetic acid (200 mL). The mixture was shaken for 90 s, quickly filtered into ice-water, and the precipitate was washed with glacial acetic acid (10 mL). The filtrate and washings were extracted three times with dichloromethane, the combined extracts were washed three times with water and evaporated in vacuum. Flash chromatography of the residue on silica gel 60 (cyclohexane/ethyl acetate, 3/1) gave **22** (2 g, 20% yield). A mixture of **22** (2 g, 4.3 mmol), triphenyl phosphine (1.35 g, 5.1 mmol), imidazole (0.7 g, 10.3 mmol), iodine (1.3 g, 5.1 mmol) and anhydrous toluene (40 mL) was boiled under reflux for 2 h, then cooled to room temperature, diluted with ether (50 mL), washed with 5% aqueous $Na_2S_2O_3$ (2×20 mL) and concentrated. The resulting brown solid was triturated with ether (50 mL) and filtered through Celite to remove most of crystalline triphenylphosphine oxide. The solution was concentrated and the residue was eluted from a column of silica gel with cyclohexane/ethyl acetate (7/1) to give **23** (2 g, 81% yield) as a syrup. When heated for 24 h at 140 °C with triethylphosphite, compound **23** underwent the Arbuzov reaction to give the substituted phosphonate analogue **24** of 1-methyl-6-phospho-α-D-glucopyranoside, which was purified by flash chromatography (cyclohexane/ethyl acetate, 3/1) in 57% yield. A solution of **24** (660 mg, 1.12 mmol) in dichloromethane (15 mL) was treated at 0 °C under dry nitrogen with bromotrimethylsilane (830 μL, 6.3 mmol). After 24 h, the solvent was evaporated and the residue (**25**) was dissolved in methanol (90% v/v, 20 mL) and hydrogenolyzed in the presence of 10% Pd/C for 3 h. The catalyst was removed by filtration and the solvent evaporated in vacuum. Hydrolysis of **26** to give **27** was carried out by dissolution in 6 N HCl at 90 °C, overnight (67% yield). Compound **27** (200 mg, 0.75 mmol) was then dissolved in 10 mL 1 N NaOH and vigorously stirred at room temperature under 1 atm of oxygen for 1 week. The reaction mixture was adjusted with concentrated HCl to pH 1.5 and bubbled with N_2 to remove dissolved CO_2. This procedure was followed by adjustment to pH 5 with saturated aqueous barium hydroxide. The resulting solution was filtered and the acid barium salt precipitated by the addition of 2 volumes of absolute ethanol. The isolated barium salt was suspended in 50 mL of water, brought into solution with the minimum amount of concentrated HCl, and reprecipitated as before. A third precipitation was performed in the same manner but with only a single volume of ethanol. The material, dried in vacuum and then dissolved in a minimum volume of water, was passed through a column of Sephadex gel LH20 (38×3 cm) with methanol/water (1/1) from which 5-deoxy-5-(dihydrogeno-phosphono)-D-arabinono-1,4-lactone (**28**, 35 mg, 0.148 mmol) was recovered (19.7 yield). **28**: ^{1}H NMR (300 MHz, D_2O) δ 4.42 (d, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 4.37–4.27 (m, 1H, H-4), 3.99 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 2.25–1.98 (m, 2H, 2H-5); ^{13}C NMR (75 MHz, D_2O) δ 176.0 (C-1), 78.0 (d, $J_{c,p}$ = 12.2 Hz, C-4), 77 (d, $J_{c,p}$ = 4.0 Hz, C-3), 73.3 (C-2), 30.7 (d, $J_{c,p}$ = 35.5 Hz, C-5); ^{31}P NMR (121 MHz, D_2O) δ 22.3. The neutralised aqueous solution of the 5-deoxy-5-(dihydrogeno-phosphono)-D-arabinono-1,4-lactone, giving 5-deoxy-5-phosphono-D-arabinonate (**18**), was used in the inhibition assays.

5,6-Dideoxy-6-phosphono-D-*arabino*-hexonate (7). The title compound **7** was synthesised in a different way from that known as starting from D-arabinose,[25] starting from **22** (Scheme 2). A mixture of **22** (100 mg, 0.215 mmol), activated 4 Å powdered molecular sieves (1 g), and dry CH_2Cl_2 (4 mL) were stirred at room temperature for 15 min, and then pyridinium chlorochromate (230 mg, 1.07 mmol) was added. The suspension was stirred for 45 min and then 30 mL of cyclohexane and 60 mL of ether were added. The mixture was filtered through silica gel (30 g) and concentrated to give a compound which has an aldehydic group in place of the CH_2I group in compound **23**. This last compound was then condensed with tetraethylmethylenediphosphonate (Wittig–Horner) to give the corresponding alkene product. Following the same procedure as described in Scheme 2 for the preparation of **27** from **24**, 6,7-dideoxy-7-phosphono-D-*gluco*-heptose was obtained (the analogue of **27** with an additional methylene group in position 7). The title compound **7** was obtained from 6,7-dideoxy-7-phosphono-D-*gluco*-heptose according to the same procedure as described for the preparation of **28** from **27** in Scheme 2. All the analytical data were consistent with the reported values.[25]

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