Bioorganic & Medicinal Chemistry 17 (2009) 7100–7107

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and evaluation of non-hydrolyzable D-mannose 6-phosphate surrogates reveal 6-deoxy-6-dicarboxymethyl-D-mannose as a new strong inhibitor of phosphomannose isomerases

Johanna Foret [a,b], Benoit de Courcy [c,d,e], Nohad Gresh [e], Jean-Philip Piquemal [c,d], Laurent Salmon [a,b,*]

[a] Univ Paris-Sud, Equipe de Chimie Bioorganique et Bioinorganique, ICMMO, UMR8182, Bâtiment 420, 15 rue Georges Clemenceau, Orsay F-91405, France
[b] CNRS, Equipe de Chimie Bioorganique et Bioinorganique, ICMMO, UMR8182, Bâtiment 420, 15 rue Georges Clemenceau, Orsay F-91405, France
[c] UPMC Univ Paris 06, UMR 7616, Laboratoire de Chimie Théorique, Case Courrier 137, 4 Place Jussieu, F-75252 Paris Cedex 05, France
[d] CNRS, UMR 7616, Laboratoire de Chimie Théorique, Case Courrier 137, 4 Place Jussieu, F-75252 Paris Cedex 05, France
[e] Univ Paris Descartes, Laboratoire de Pharmacochimie Moléculaire et Cellulaire, U648 INSERM, UFR Biomédicale des Saints-Pères, 45 rue des Saint-Pères, F-75006 Paris, France

ARTICLE INFO

Article history:
Received 8 July 2009
Revised 28 August 2009
Accepted 3 September 2009
Available online 6 September 2009

Keywords:
Phosphomannose isomerase inhibitors
Sugar phosphates
Malonates
Phosphonates
Polarizable molecular mechanics
Zinc metalloenzymes

ABSTRACT

Non-hydrolyzable D-mannose 6-phosphate analogues in which the phosphate group was replaced by a phosphonomethyl, a dicarboxymethyl, or a carboxymethyl group were synthesized and kinetically evaluated as substrate analogues acting as potential inhibitors of type I phosphomannose isomerases (PMIs) from *Saccharomyces cerevisiae* and *Escherichia coli*. While 6-deoxy-6-phosphonomethyl-D-mannose and 6-deoxy-6-carboxymethyl-D-mannose did not inhibit the enzymes significantly, 6-deoxy-6-dicarboxymethyl-D-mannose appeared as a new strong competitive inhibitor of both *S. cerevisiae* and *E. coli* PMIs with K_m/K_i ratios of 28 and 8, respectively. We thus report the first malonate-based inhibitor of an aldose–ketose isomerase to date. Phosphonomethyl mimics of the 1,2-*cis*-enediolate high-energy intermediate postulated for the isomerization reaction catalyzed by PMIs were also synthesized but behave as poor inhibitors of PMIs. A polarizable molecular mechanics (SIBFA) study was performed on the complexes of D-mannose 6-phosphate and two of its analogues with PMI from *Candida albicans*, an enzyme involved in yeast infection homologous to *S. cerevisiae* and *E. coli* PMIs. It shows that effective binding to the catalytic site occurs with retention of the Zn(II)-bound water molecule. Thus the binding of the hydroxyl group on C1 of the ligand to Zn(II) should be water-mediated. The kinetic study reported here also suggests the dianionic character of the phosphate surrogate as a likely essential parameter for strong binding of the inhibitor to the enzyme active site.

1. Introduction

Phosphomannose isomerases (PMIs, E.C. 5.3.1.8) are metal-dependent aldose–ketose isomerases involved in the reversible isomerization of D-fructose 6-phosphate (F6P) to D-mannose 6-phosphate (M6P) in prokaryotic and eukaryotic cells (Chart 1).[1] This reaction is the first step of the mannose metabolism pathway resulting in the generation of GDP-D-mannose, an important precursor of many mannosylated structures such as glycoproteins, nucleotide sugars, glycolipids, fungi cell wall components, and bacterial exopolysaccharides.[2] PMI was reported to be essential for the survival or pathogenesis of bacteria from *Mycobacterium smegmatis*[3] and *Pseudomonas aeruginosa*,[4] and for the virulence of the protozoan parasite *Leishmania mexicana*.[5] PMI activity also appeared essential in the case of yeasts like *Saccharomyces cerevisiae*,[6] *Candida albicans*,[7] *Cryptococcus neoformans*,[8] and *Aspergillus nidulans*.[9] Therefore, PMI can be considered as a suitable target for the development of antibacterial, antiparasitic, and antifungal agents. Indeed, in most human tissues, its inhibition should not im-

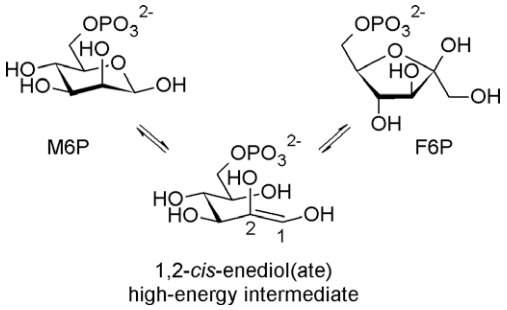


Chart 1. Mannose 6-phosphate (M6P) to fructose 6-phosphate (F6P) reversible isomerization reaction catalyzed by phosphomannose isomerases (PMIs) involving the postulated 1,2-*cis*-enediol(ate) high-energy intermediate.

* Corresponding author. Tel.: +33 1 69 15 63 11; fax: +33 1 69 15 72 81.
E-mail address: laurent.salmon@u-psud.fr (L. Salmon).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.09.005

pair human metabolism, because the bulk of the central metabolism M6P that is utilized for glycoprotein synthesis is likely not derived from F6P but originates from efficient uptake of D-mannose through a specific exogenous mannose transporter followed by phosphorylation by hexokinase.[10] In some tissues such as liver and intestine where this pathway appears less efficient, a deficiency in human PMI activity leads to the severe metabolic disorder with hepatic–intestinal presentation CDGS 1b (carbohydrate-deficient glycoprotein syndrome type 1b),[11,12] that is successfully treated by oral D-mannose.[13] Hence, a bitherapeutical approach combining the enzyme inhibitor and D-mannose should alleviate in such cases the side-effects on humans of PMI inhibition. In most microorganisms, the efficiency of such M6P formation from exogenous D-mannose remains to be evaluated. In the pathogenic yeast C. NEOFORMANS, this pathway of exogenous mannose uptake was reported to be much less efficient than in humans, and PMI was validated as an excellent therapeutic target.[8] Finally, in the case of local treatments such as against yeast infections (candidiasis) and cutaneous leishmaniasis, PMI inhibition will most likely not affect human metabolism.

The existence of PMI was first demonstrated by Slein.[14] It was then isolated by Gracy and Noltmann in brewer's yeast and shown to be a monomeric 45 kDa protein containing an essential zinc cation.[15] *S. cerevisiae*, *Escherichia coli*, *C. albicans*, and human PMIs, among others, belong to the type I class of three structurally unrelated families of proteins identified with PMI activity,[16] except for a very small conserved amino acid sequence motif, which makes up part of the active site. Type I PMIs have been shown to have very similar characteristics with a high level of sequence identity (>40%), particularly in the region of the active site.[16,17] Therefore, conclusions that may be drawn from a type I PMI can most likely be applied to other type I PMIs, including the prokaryotic and eukaryotic PMIs studied in this paper. One of the sole high-resolution X-ray crystal structures of a PMI was reported by Cleasby et al. for *C. albicans*.[18] We previously reported 5-phospho-D-arabinonohydroxamic acid as the strongest known PMI inhibitor,[19] as well as the theoretical structure of the inhibitor bound to the zinc cofactor at the active site of the enzyme from *C. albicans*.[20] However, phosphorylated compounds are likely to be of limited therapeutical interest, not only because of their ionic character which do not allow them to cross cells membranes, but also because of their high sensitivity to hydrolysis by phosphatases.

For these reasons, numerous analogues of phosphorylated molecules containing a phosphate surrogate were reported in the literature as enzyme inhibitors, ligand of protein receptors, or molecular tools for the study of nucleic acid metabolism, including phosphorothioate,[21,22] phosphonomethyl (or phosphonate),[23–35] fluoroalkyl phosphonate,[29,36] sulfonate,[37] malonyl ether,[29,38–42] malonate,[29,39,40,43,44] fluoroalkyl malonates,[45,46] and carboxymethyl (or carboxylate) analogues.[43] Numerous phosphate surrogates were notably used in the mechanistic study of 3-dehydroquinate synthase.[47,48] In the case of ribose-5-phosphate isomerase, we reported 4-deoxy-4-phosphonomethyl-D-erythronate as the only known efficient hydrolytically stable competitive inhibitor of an aldose–ketose isomerase to date.[30] In the case of phosphomannose isomerase, no such hydrolytically stable substrate or reaction intermediate analogues were evaluated to date regarding their possible enzyme inhibitory properties.

In our general search for isosteric and isoelectronic non-hydrolyzable inhibitors of aldose–ketose isomerases of potential therapeutical interest, we report here the synthesis and kinetic evaluation of three mimics of the PMI substrate M6P in which the phosphate group has been replaced by a phosphonomethyl, a dicarboxymethyl (or 2-malonyl), and a carboxymethyl group, to give, respectively, 6-deoxy-6-phosphonomethyl-D-mannose[24] (6PMM, **1**), 6-deoxy-6-dicarboxymethyl-D-mannose (6DCM, **2**), and 6-deoxy-6-carboxymethyl-D-mannose (6CM, **3**) as depicted in Chart 2. The methylated analogues of compounds **1**–**3** in which the anomeric hydroxyl group is replaced by a methoxy group in the α configuration, respectively, methyl 6-deoxy-6-phosphonomethyl-α-D-mannopyranoside, methyl 6-deoxy-6-dicarboxymethyl -α-D-mannopyranoside, and methyl 6-deoxy-6-carboxymethyl-α-D-mannopyranoside, were previously synthesized and evaluated for binding affinity to a cation-independent M6P receptor, namely the M6P/insulin-like growth factor II, by the Berkowitz et al.[29] and Montero/Morère groups.[43] We do not expect the above methylated analogues to bind tightly to PMI, notably because the α-anomer of M6P, in contrast to the β-anomer, has no activity as a substrate and is, at best, a poor inhibitor of the enzyme.[49] Because high-energy intermediate (or transition state) analogues acting as inhibitors are expected to bind more tightly to the enzyme active site, we also report the synthesis and kinetic evaluation of phosphonomethyl analogues of the reaction intermediate postulated for the isomerization reaction catalyzed by PMIs (Chart 1). Modeling of the complexes of the M6P substrate and of two of its analogues, 6PMM (**1**) and 6DCM (**2**), with the active site of *C. albicans* PMI was performed by the SIBFA polarizable molecular mechanics procedure developed by some of us.[50] It sheds light on the essential interactions taking place between PMI and these ligands at both the Zn-binding site and the cavity entrance which has two cationic residues, Arg304 and Lys310.

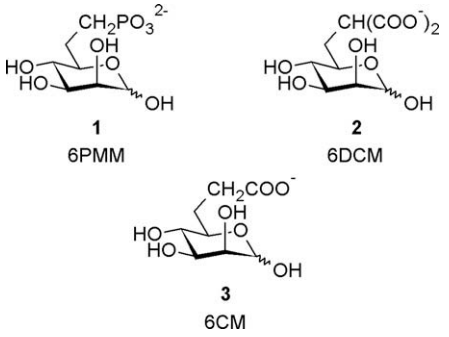


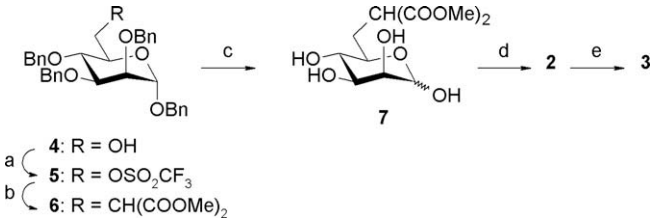
Chart 2. Structure of the mannose 6-phosphate (M6P) analogues synthesized and evaluated in this study: 6-deoxy-6-phosphonomethyl-D-mannopyranose (6PMM, **1**), 6-deoxy-6-dicarboxymethyl-D-mannopyranose (6DCM, **2**), and 6-deoxy-6-carboxymethyl-D-mannopyranose (6CM, **3**).

2. Results and discussion

2.1. Synthesis of enzyme inhibitors

The dipotassium salt of 6-deoxy-6-phosphonomethyl-D-mannopyranose[24] (6PMM, **1**, Chart 2) was obtained from the commercially available methyl α-D-mannopyranoside in 30% yield (seven steps) using reported procedures.[23,24,51]

Synthesis of the disodium salt of 6-deoxy-6-dicarboxymethyl-D-mannopyranose (6DCM, **2**) was achieved in four steps from the known benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **4**[52] by the route shown in Scheme 1. The synthetic strategy, similar to that reported for the synthesis of the methyl mannopyranoside analogue of **2**,[29,43] involves the displacement of sugar triflate by dimethylmalonate as the key step to accessing the title compound. Indeed, triflate compounds are known to be very versatile electrophiles in making sugar derivatives with a whole range of functionality in place of phosphorous.[53,54] A benzyl instead of a methyl group was chosen as the protecting group of the hydroxyl function on C1 in **4** in order to achieve its deprotection at room temperature without hydrolysis and decarboxylation of the dimethylmalonate function. Trifluoromethanesulfonic anhydride was added to the



Scheme 1. Synthesis of the M6P analogues 6-deoxy-6-dicarboxymethyl-D-mannopyranose (6DCM, **2**) and 6-deoxy-6-carboxymethyl-D-mannopyranose (6CM, **3**) from benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **4**. Reagents and conditions: (a) $(CF_3SO_2)_2O$, $(iPr)_2EtN$, CH_2Cl_2, −40 °C, 3 h; (b) $H_2C(COOMe)_2$, THF, NaH, reflux, 15 h, 64% (two steps); (c) H_2 (15 bar), $Pd(OH)_2/C$, MeOH, rt, 2 d, 94%; (d) 1—NaOH 1 M, rt, 15 h, 2—Dowex®-50WX8 (H^+), 3—Dowex®-50WX8 (Na^+), 99%; (e) 1—Dowex®-50WX8 (H^+), H_2O, reflux, 18 h, 2—Dowex®-50WX8 (Na^+), 100%.

primary alcohol **4** in the presence of diisopropylethylamine in dichloromethane to form benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside **5**. Following work-up, compound **5** was used immediately for the nucleophilic substitution with dimethylmalonic acid in the presence of sodium hydride in THF to give benzyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-dimethylmalonate-α-D-mannopyranoside **6** in 64% yield (two steps). Hydrogenolysis over $Pd(OH)_2/C$ afforded 6-deoxy-6-dimethylmalonate-D-mannopyranose **7** in 94% yield, which, upon hydrolysis in 1 M sodium hydroxide aqueous solution, cation-exchange chromatography on a Dowex® resin column (H^+ form, then Na^+ form) and lyophilization gave the malonate M6P analogue **2** in 99% yield.

Compound **2** also served as the starting compound for the synthesis of the sodium salt of 6-deoxy-6-carboxymethyl-D-mannopyranose (6CM, **3**, Scheme 1). The carboxylate M6P analogue **3** was obtained in one step in quantitative yield through simple decarboxylation of **2** in aqueous medium under reflux in the presence of Dowex® resin (H^+ form).

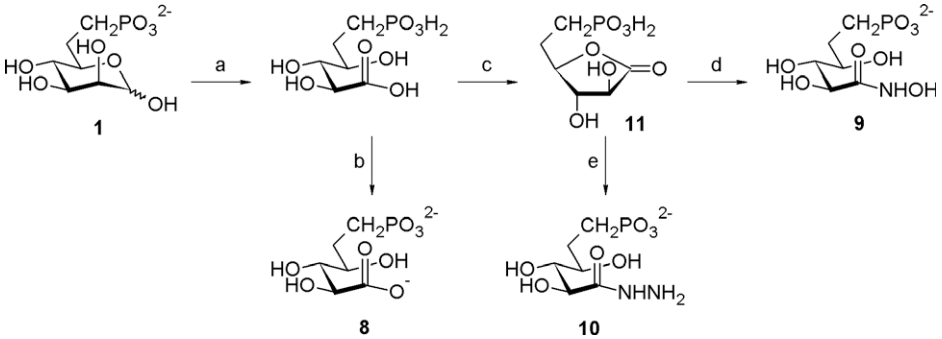
As depicted in Scheme 2, **1** was also used as a starting compound for the synthesis of 5-deoxy-5-phosphonomethyl-D-arabinonate (5PMAA, **8**[25]), 5-deoxy-5-phosphonomethyl-D-arabinonohydroxamic acid (5PMAH, **9**[55]), and 5-deoxy-5-phosphonomethyl-D-arabinonohydrazide (5PMAHz, **10**). As reported for the synthesis of 5-phospho-D-arabinonate from 6-phosphate D-fructose[56,57] or 6-phosphate D-glucose,[57] oxidative cleavage of **1** by molecular oxygen in 0.5 M sodium hydroxide aqueous solution (Spengler–Pfannenstiel conditions) followed by cation exchange on a Dowex® resin (H^+ form) yielded 5-deoxy-5-(dihydrogenophosphono)-D-arabinonic acid (not isolated). Following cation exchange on a Dowex® resin (Na^+ form), the trisodium salt of 5-deoxy-5-phosphonomethyl-D-arabinonate (5PMAA, **8**[25]) was obtained in 50% yield from **1** (no yield nor data were reported in the literature[25] for its synthesis from 6-deoxy-6-phosphonomethyl-D-glucose[58]). Alternatively, evaporation under high vacuum and lyophilization yielded the synthon 5-deoxy-5-(dihydrogenophosphonomethyl)-D-arabinono-1,4-lactone **11**[55] in 50% yield from **1** (lit.[55] 71% yield from 6-deoxy-6-phosphonomethyl-D-glucose.[58] ^1H, ^{13}C, and ^{31}P NMR spectral data matched that reported[55]). Subsequent nucleophilic addition of aqueous hydroxylamine or aqueous hydrazine followed by lyophilization, gave, respectively, the dihydroxylammonium salt of **9**[55] (yield 100%, lit.[55] 72% for the disodium salt of **9**) or the dihydrazinium salt of **10** (yield 100%).

We were also interested in the synthesis and kinetic evaluation of the respective 2-malonyl analogues of the reaction intermediate postulated for the isomerization reaction catalyzed by PMIs. However, oxidative cleavage of **2** according to the Spengler–Pfannenstiel conditions described above followed by cation exchange on a Dowex® resin (H^+ form) did not yield the expected 5-deoxy-5-dicarboxymethyl-D-arabinonic acid in pure form. Furthermore, subsequent lyophilization yielded several lactone derivatives as a consequence of the three carboxylic acid groups present in the molecule.

2.2. Kinetic evaluation of enzyme inhibitors

Kinetic studies were performed on *E. coli* and *S. cerevisiae* PMIs as model targets for the development of PMI-inhibiting antibacterial and antifungal agents, respectively. Inhibition parameters of M6P analogues 6PMM (**1**), 6DCM (**2**) and 6CM (**3**) for the M6P to F6P isomerization reaction catalyzed by these two type I PMIs (Table 1) were determined using the phosphoglucose isomerase/D-glucose-6-phosphate dehydrogenase (PGI/G6PDH) coupled enzymes method (50 mM Hepes buffer, pH 7.1, 25 °C) in which PMI activity was spectrophotometrically assayed at 340 nm (NADPH formation) as previously described.[19] Following isomerization of M6P to F6P by PMI, then to D-glucose 6-phosphate by PGI, D-gluconolactone 6-phosphate and NADPH were formed by G6PDH with $NADP^+$ as the cofactor. Both auxiliary enzymes were added in excess so that the rate-limiting reaction was the PMI-catalyzed isomerization of M6P to F6P.

Both phosphonomethyl (6PMM, **1**) and carboxymethyl (6CM, **3**) substrate analogues gave high IC_{50} values, respectively, 4.7 and 4.6 mM against EcPMI, and 1.6 and 0.9 mM against ScPMI. Both compounds therefore behave as quite weak inhibitors of the enzymes, thus indicating that the phosphonomethyl and carboxymethyl functions are not good phosphate surrogates. In the case of the carboxymethyl analogue (6CM, **3**), the monoanionic state



Scheme 2. Synthesis of 5-deoxy-5-phosphonomethyl-D-arabinonate (5PMAA, **8**), 5-deoxy-5-phosphonomethyl-D-arabinonohydroxamic acid (5PMAH, **9**), and 5-deoxy-5-phosphonomethyl-D-arabinonohydrazide (5PMAHz, **10**) from the synthon 5-deoxy-5-(dihydrogenophosphono)-D-arabinono-1,4-lactone (5PMAL, **11**). Reagents and conditions: (a) 1—O_2, NaOH 0.5 M, rt, 7 d, 2—concd HCl, 3—satd $Ba(OH)_2$, 4—Dowex®-50WX8 (H^+); (b) Dowex®-50WX8 (Na^+), 50% (two steps); (c) lyophilization, 50% (two steps); (d) NH_2OH/H_2O, rt, 20 min, 100%; (e) H_2NNH_2/H_2O, rt, 20 min, 100%.

Table 1
Inhibition parameters of M6P analogues **1**–**3** for the M6P to F6P isomerization catalyzed by *E. coli* and yeast PMIs[a]

Inhibitor	Name	*E. coli* PMI[b] IC_{50} (K_i) (μM)	*S. cerevisiae* PMI[c] IC_{50} (K_i) (μM)
1	6PMM	4700 ± 800	1610 ± 90
2	6DCM	199 ± 5 (115 ± 5)	18.1 ± 0.6 (10.5 ± 0.5)
3	6CM	4600 ± 400	900 ± 100
8	5PMAA	>3000	nd[d]
9	5PMAH	370 ± 20	2200 ± 200
10	5PMAHz	>3000	nd[d]

[a] PMI activity assays were achieved using the PGI/G6PDH coupled enzymes method (50 mM Hepes buffer, pH 7.1, 25 °C) as previously described.[19]
[b] K_m (M6P) = 910 ± 80 μM (lit.[68] 1.21 mM).
[c] K_m (M6P) = 290 ± 30 μM (lit.[19] 121 μM; lit.[17] 650 μM; lit.[69] 1350 μM).
[d] nd: not determined.

of this phosphate surrogate would likely explain its low binding affinity to the PMI active site. With a generally accepted pK_{a2} of about 7.5–8 for phosphonate compounds as compared to 6.5 for phosphate compounds,[55,59] 6PMM (**1**) could be monoprotonated at the pH used for the kinetics (7.1), hence a lowered affinity to the PMI active site.

In the opposite, the 2-malonyl substrate analogue (6DCM, **2**) displayed low IC_{50} values on EcPMI, and particularly on ScPMI, respectively, 199 and 18.1 μM (Table 1). Double reciprocal plot of the initial reaction velocity V_o versus M6P concentration at different inhibitor concentrations revealed that 6DCM (**2**) is a competitive inhibitor of the isomerization reaction catalyzed by EcPMI and ScPMI as depicted in Figure 1A (for ScPMI). Secondary plot of the K_m/V_{max} ratio obtained at different inhibitor concentrations gave the respective K_i values of 115 and 10.5 μM (Fig. 1B), from which the respective K_m/K_i ratio values of 8 and 28 could be calculated. The K_i values appear to be about an order of magnitude in favor of the fungal enzyme as compared to the bacterial enzyme. However the ratio of the respective K_m/K_i values is only 3.5. It is not significant in terms of specificity, which confirms that the two type I PMIs have comparable active sites. Although these K_i values are only in the micromolar range, 6DCM appears as the second best inhibitor of a PMI reported to date, 5-phospho-D-arabinonohydroxamic acid being in the submicromolar range.[19] Furthermore, 6DCM represents the first 2-malonyl-based phosphate surrogate inhibitor of an aldose–ketose isomerase ever reported in the literature. Our results regarding PMI inhibition confirm earlier findings on other targets,[29,39,40,43,47] namely that the hydrolase-resistant 2-malonyl function is an efficient mimic of the phosphate group. Although the structure of a malonate as a phosphate bioisoster is chemically different, molecular dynamic simulations previously performed by one of us revealed that the malonate group occupies only about 13% more volume than the phosphate.[39] The crucial point thus appears to be the dianionic ionization state of the 2-malonyl function (ethyl malonic acid has pK_{a1} = 3.0 and pK_{a2} = 5.8),[60] as likely is the phosphate function at pH 7.1 used for the kinetics.

Phosphonomethyl mimics of the 1,2-*cis*-enediolate high-energy intermediate postulated for the isomerization reaction catalyzed by PMIs, namely 5PMAA (**8**), 5PMAH(**9**), and 5PMAHz (**10**), were also evaluated against PMIs from *E. coli* and *S. cerevisiae* (**9** only) using the same conditions as reported above (Table 1). With IC_{50} values above 3 mM against *E. coli* PMI, both 5PMAA (**8**) and 5PMAHz (**10**) behave as quite weak inhibitors. In the case of 5PMAA (**8**), the IC_{50} value obtained is sixfold higher than the value of 0.5 mM we determined for its phosphate analogue, namely 5-phospho-D-arabinonate, a bad PMI inhibitor.[19] However, the IC_{50} value obtained for 5PMAHz (**10**) is much higher than the value of 2 μM obtained for its phosphate analogue, namely 5-phospho-D-arabinonohydrazide.[61] 5PMAH (**9**) is also a quite weak inhibitor of *S. cerevisiae* PMI with an IC_{50} value of 2.2 mM, but a reasonably good inhibitor of *E. coli* PMI with an IC_{50} value of 0.37 mM. However, the loss of binding affinity is of several orders of magnitude when compared to the K_i value of 0.084 μM obtained for its phosphate analogue 5-phospho-D-arabinonohydroxamic acid.[19] Overall, replacement of the phosphate group by a phosphonomethyl group in known 1,2-*cis*-enediolate high-energy intermediate PMI analogue inhibitors strongly impairs their binding affinity to the enzyme active site.

Interestingly, Frost and coworkers reported a study of 3-dehydroquinate (DHQ) synthase active site in which a large number of substrate analogue carbocyclic inhibitors bearing a phosphate mimic were evaluated.[47] They clearly demonstrated that access to a dianionic ionization state of the phosphate surrogate yielded strong inhibitors, as in the case of their dianionic carbamalonate for which an apparent pK_a of 4.40 was measured, as compared to the monoanionic carbacarboxylate or carbaacetate analogues which appeared as much weaker inhibitors. These results are in perfect agreement with the kinetic results we report here for 6DCM (**2**) as compared to 6CM (**3**). On the other hand, in contrast to our results, their carbaphosphonate analogues were reported to

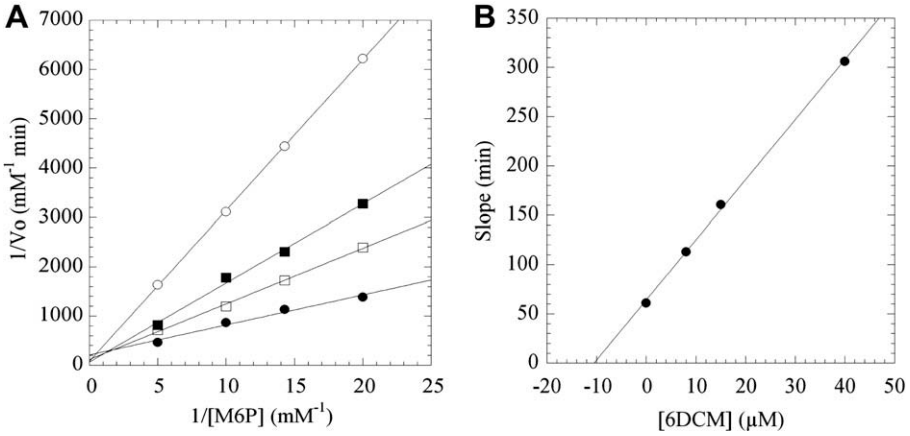


Figure 1. (A) Inhibition of *S. cerevisiae* PMI (50 mM Hepes buffer, 25 °C, pH 7.1) by 6-deoxy-6-dicarboxymethyl-D-mannopyranose (6DCM, **2**); double reciprocal plot of initial reaction velocity versus M6P concentration obtained at various concentrations of inhibitor: ●, no inhibitor; □, [I] = 8 μM; ■, [I] = 15 μM; ○, [I] = 40 μM. Lines drawn were obtained from a linear regression fit of the observed data using Michaelis–Menten equation for competitive inhibition. (B) Secondary plot of apparent K_m/V_{max} values (slopes of the straight lines in the primary graph A) versus inhibitor concentration. The *x* intercept, which is equal to $-K_i$, was determined by linear regression.

be strong inhibitors of DHQ synthase, implying that they might bind as dianions rather than monoanions. This might originate from the slightly higher pH of 7.7 used in their study as compared to 7.1 for our kinetics on PMIs, or from possible modulations of the pK_a of phosphonate as a function of the enzyme active site.

2.3. Polarizable molecular mechanics study of enzyme substrate and inhibitors

Because we did not succeed into getting high-resolution crystal structures of PMI complexes, the polarizable molecular mechanics procedure SIBFA (Sum of Interactions Between Fragments computed ab initio)[50] was used to study the PMI-M6P and PMI-inhibitor interactions. This procedure was previously applied successfully to study several inhibitor–metalloprotein complexes.[20,62–67] We used the three-dimensional structure of the type I *C. albicans* PMI[18] as a starting point to generate a theoretical model of the enzyme, and thereafter the different complexes of the model with the substrate or the inhibitors as depicted in Figure 2. Energy-minimized representations of the active site of *C. albicans* PMI complexed with β-D-mannopyranose 6-phosphate (βM6P), 6DCM (**2**), dianionic 6PMM (**1**), and monoanionic 6PMM(H) (**1**) are depicted, respectively, in Figure 2A–D. An important finding from the present study is the need to retain the Zn-bound water molecule in the optimized complex. Alternative energy-minimizations in its absence showed that none of the ligand hydroxyl groups was unable to bind Zn(II) at distances closer than 2.5 Å, while the C–O–Zn angle was non-optimal. This constitutes an uncommon instance in which ligand binding to the catalytic Zn(II) occurs through an interposed water molecule. Figure 2 clearly shows that both the phosphate group of βM6P (Fig. 2A) and the 2-malonyl group of 6DCM (Fig. 2B) can fit snugly between with Arg304, and Lys310, with additional stabilization involving Ser109. In fact, as previously observed with 5-phospho-D-arabinonohydroxamic acid,[20] this array of interactions propagates to the entire recognition site. Thus Ser109 in turn behaves as an H-bond acceptor to Gln111, which donates a second proton to the ligand ring oxygen, as well as acting as one of the Zn-ligands. Zn(II) is coordinated to Glu138, which also accepts a proton from the hydroxyl group on C2 of the ligand, while as mentioned above its water ligand donates a proton to the hydroxyl group on C1 of the ligand.

The case of the phosphonomethyl substrate analogue 6PMM (**1**) is interesting. In its dianionic form (Fig. 2C), the binding pattern of 6PMM (**1**) is quite similar to that observed for βM6P (and so for 6DCM) as shown by the superposition of the two complexes

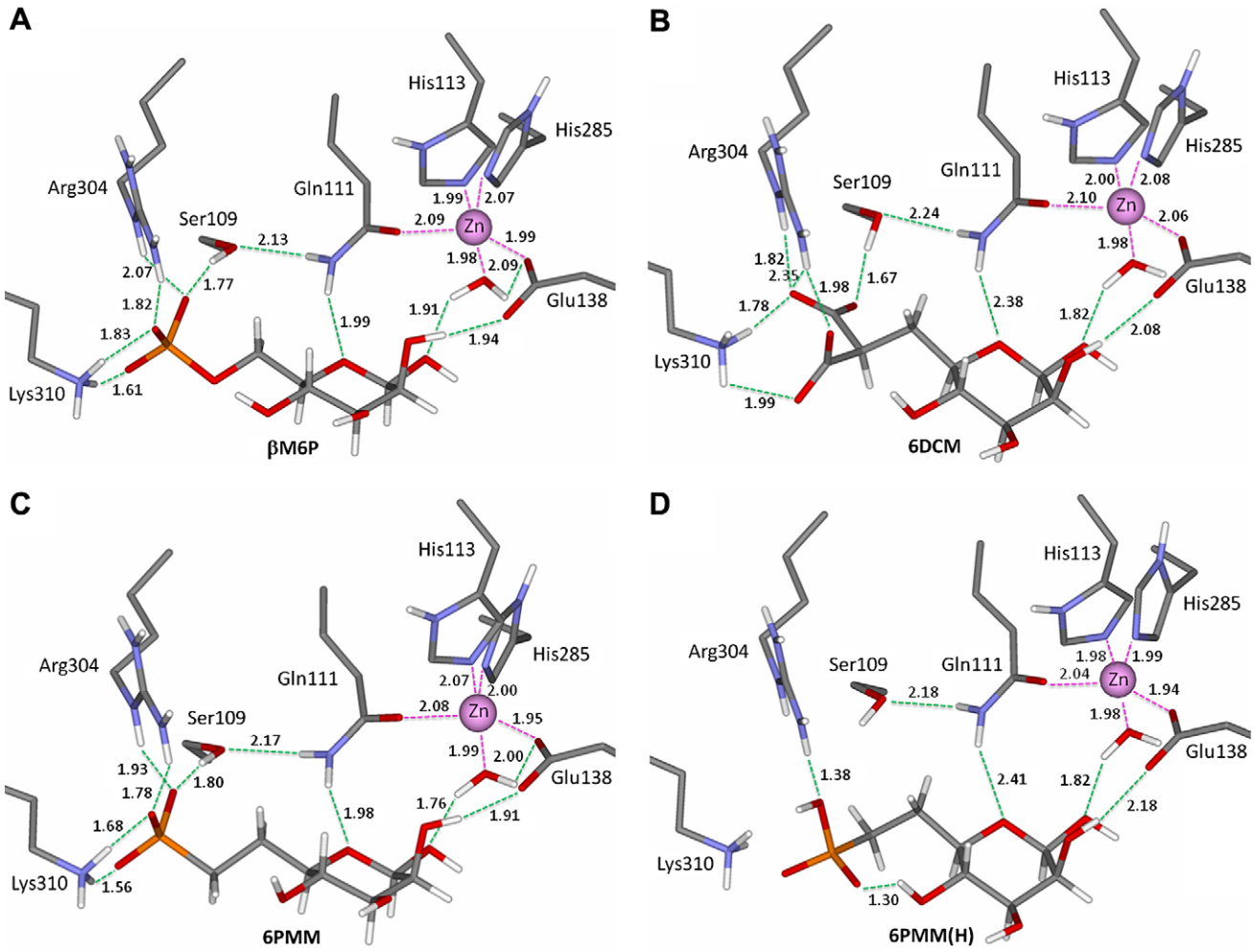


Figure 2. SIBFA representations of the active site of *C. albicans* PMI complexed to: (A) β-D-mannopyranoside 6-phosphate (βM6P); (B) 6-deoxy-6-dicarboxymethyl-D-mannopyranose (6DCM, **2**); (C) 6-deoxy-6-phosphonomethyl-D-mannopyranose (6PMM, **1**); (D) 6-deoxy-6-hydrogeno-phosphonomethyl-D-mannopyranose [6PMM(H)]. H-bonds are depicted as green dashed lines and H-bonds distances are given in angstrom. Ligand interactions at the zinc binding site are depicted as pink dashed lines. Some hydrogen atoms, active site residues, water molecules, and H-bonds were omitted for clarity of the figure. DS Visualizer 2.0 and Microsoft PowerPoint software's were used to prepare the figure.

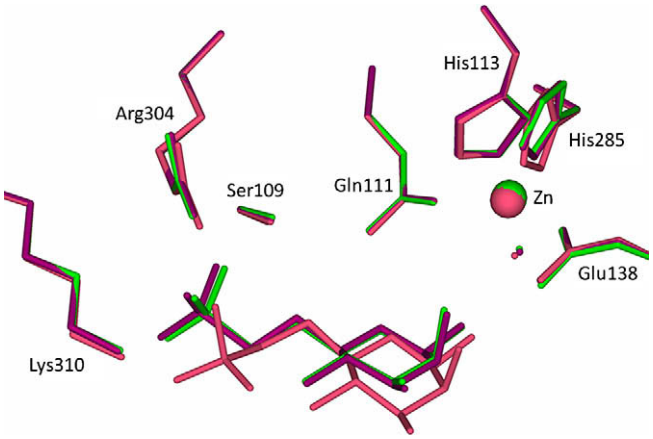


Figure 3. Superposition of the active sites of *C. albicans* PMI complexed to (i) β-D-mannopyranose 6-phosphate (βM6P) in green, (ii) 6-deoxy-6-phosphonomethyl-D-mannopyranose (6PMM, **1**) in purple, and (iii) 6-deoxy-6-hydrogenophosphonomethyl-D-mannopyranose [6PMM(H)] in pink, obtained by structural alignment (DS Visualizer 2.0). Hydrogen atoms, H-bonds, and some active site residues and water molecules were omitted for clarity of the figure. DS Visualizer 2.0 and Microsoft PowerPoint software's were used to prepare the figure.

depicted in Figure 3. Replacement of the oxygen atom on carbon C-6 of the substrate M6P by a methylene group does not impair binding of the inhibitor to the PMI active site. However, this observation does not agree with the kinetic results which indicate that the phosphonomethyl analogue is not a PMI inhibitor. Representing the inhibitor in its likely monoanionic form, 6PMM(H), shows that only one attractive interaction of the phosphonomethyl group takes place with Arg304 (Fig. 2D). Superimposition with the two previous PMI complexes (βM6P and dianionic 6PMM) reveals a distinct and most likely weaker binding pattern (Fig. 3).

In order to unravel the origin of the experimentally observed preferential binding of 6DCM (**2**) than 6PMM (**1**) to PMI, energy balances are underway. As in our previous studies,[19,62–65] they take into account the ligand–PMI intermolecular interaction and solvation energy of the complex on the one hand, and the conformational energy cost and desolvation energies of the ligand and the protein on the other hand. These results will be detailed and published separately in due course. They indicate that a sole continuum representation of solvation cannot account for such a preferential binding. On the other hand, including a limited array (nine) of highly polarized discrete water molecules in the binding site can exert a decisive effect in favor of 6DCM. The so-augmented interaction site is shown as Supplementary data for the 6DCM complex. It is seen to extend to three anionic residues of PMI.

3. Conclusion

In conclusion, we report 6-deoxy-6-(dicarboxymethyl)-D-mannopyranose, a non-hydrolyzable D-mannose 6-phosphate analogue in which the phosphate group was replaced by a dicarboxymethyl (or 2-malonyl) group, as a strong inhibitor of PMIs from *E. coli* and *S. cerevisiae*. This compound also represents the first malonate-based inhibitor of an aldose–ketose isomerase reported to date. By contrast, phosphonomethyl and carboxymethyl mimics of M6P do not inhibit significantly the enzymes, most likely because of their monoanionic state. SIBFA molecular modeling showed the binding to the catalytic zone to occur with retention of the Zn-bound water molecule, while both dianionic phosphate and malonate can snugly bind to both Arg304 and Lys310 residues at the entrance of the binding site. Malonate-based inhibitors offer promising therapeutic outcomes considering that, in addition to be known as hydrolase-resistant, they could be vectorized by hydrophobic labile chains in order to increase their intracellular uptake, and thereby their potential efficiency toward the targeted enzyme. The demonstration in the present work that the malonate derivative has a one order of magnitude better binding property than the parent phosphate is the incentive for related comparisons in the 5-phospho-D-arabinonohydroxamic acid series of derivatives. Synthesis of the 2-malonyl analogue of the reaction intermediate postulated for the isomerization reaction catalyzed by PMIs, namely 5-deoxy-5-(dicarboxymethyl)-D-arabinonohydroxamic acid, will thus be undertaken as an extension of the present work.

4. Experimental

4.1. General materials and methods

Unless otherwise stated, all chemical reagents were of analytical grade, obtained from Acros, Alfa Aesar, or Aldrich, and used without further purification. Solvents were obtained from SDS or VWR-Prolabo. MeOH, CH_2Cl_2, and THF were dried by refluxing with, respectively, Mg/I_2, CaH_2, and Na/benzophenone, then distilled and used immediately. Flash chromatography was performed using silica gel (35–70 μm, E. Merck) under N_2 pressure. Ion-exchange chromatography was performed on Dowex®-50WX8 cation-exchange resin (H^+ or Na^+ form, 50–100 or 100–200 mesh, Acros), eluting with purified water (Millipore, 18.2 MΩ). Unless otherwise stated, all organic extracts were dried over Na_2SO_4 and filtered. Concentration of solutions was performed under diminished pressure at temperature <30 °C using a rotary evaporator. All air- and moisture-sensitive reactions were performed under an atmosphere of argon. Analytical TLC was performed using Silica Gel 60 F_{254} pre-coated aluminum plates (E. Merck). Spots were visualized by treatment with 5% ethanolic H_2SO_4 followed by heating and/or by absorbance of UV light at 254 nm. Optical rotations were measured on a Jasco DIP-370 digital polarimeter at 589 nm. NMR spectra were recorded at 297 K in $CDCl_3$, CD_3OD, and D_2O with a Bruker DPX 250 (^1H at 250.13 MHz, ^{13}C at 62.90 MHz, and ^{31}P at 101.26 MHz), DRX 300 (^1H at 300.13 MHz, ^{13}C at 75.47 MHz, and ^{31}P at 121.50 MHz), or Avance 360 (^1H at 360.13 MHz and ^{13}C at 90.56 MHz) spectrometer using NMR Notebook 2.0 WinNMR software. Chemical shifts are reported in ppm (δ) and coupling constants in Hz (J_{ij}). ^1H NMR spectra were referenced to internal residual chloroform (δ 7.26), CD_2HOD (δ 3.31), and HOD (δ 4.80) for solutions in $CDCl_3$, CD_3OD, and D_2O, respectively. For CH_2 groups, H-5′, H-6′, and H-7′ resonances appear arbitrarily at lower field than H-5, H-6, and H-7 resonances, respectively. ^{13}C NMR spectra were referenced to solvent for solutions in $CDCl_3$ (δ 77.0) and CD_3OD (δ 49.1), and to dioxane (δ 67.4) for solutions in D_2O. ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). In most cases, COSY, HSQC, and/or DEPT135 NMR spectra were recorded for assigning resonances. Infrared spectra were recorded with a FTIR Bruker IFS-66 spectrometer. Low-resolution mass spectrometry (MS) and high-resolution mass spectrometry (HRMS) analyses were performed by Imagif (ICSN-CNRS, Gif-sur-Yvette) by electrospray with positive (ESI^+) or negative (ESI^-) ionization mode.

4.2. Synthesis

4.2.1. 6-Deoxy-6-dicarboxymethyl-D-mannopyranose, disodium salt (2)

Compound **7** (0.92 g, 3.12 mmol) was dissolved in 1 M aq NaOH (22 mL) and vigorously stirred at rt for 15 h. Following concentration of the reaction mixture, the residue was successively eluted with water on two Dowex®-50WX8 ion-exchange columns (1-H^+

form, 2-Na^+ form) and lyophilized to afford **2** (0.96 g, 99%) as a white powder: $[\alpha]_D^{25}$ −159 (*c* 1, H_2O); FTIR (KBr): ν_{max} 3402, 2930, 1596, 1387, 1061 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 4.98 (s, 0.6H, H-1α), 4.84 (s, 0.4H, H-1β), 3.79 (m, 1H, H-2), 3.67 (dd, 0.6H, J_{3-4} 9.7, J_{3-2} 3.0 Hz, H-3α), 3.63 (t, 0.6H, J_{5-6} 9.4, J_{5-4} 9.4 Hz, H-5α), 3.47 (dd, 0.4H, J_{3-4} 9.5, J_{3-2} 2.8 Hz, H-3β), 3.37 (m, 2H, H-4 and H-7), 3.10 (t, 0.4 H, J_{5-6} 9.4, J_{5-4} 9.4 Hz, H-5β), 2.28 (br d, 1H, $J_{6-6'}$ 14.0 Hz, H-6′), 1.80 (dd, J_{6-5} 9.4, $J_{6-6'}$ 14 Hz, 1H, H-6); ^{13}C NMR (75 MHz, D_2O): δ 177.9 and 177.6 (2COONa), 93.8 (C-1α), 93.7 (C-1β), 74.0 (C-5β), 72.8 (C-3β), 71.1 (C-4β), 70.5 (C-2β), 70.8, 70.6, 70.1, and 70.0 (C-2α, C-3α, C-4α, and C-5α), 52.6 (C-7β), 52.4 (C-7α), 31.6 C-6); ESI^+MS: *m/z* 289.0 (100) $[M-Na+2H]^+$, 311.0 (38) $[M+H]^+$; $HRESI^+MS$: calcd for $C_9H_{13}O_9Na_2$: 311.0355 $[M+H]^+$, found *m/z* 311.0356.

4.2.2. 6-Deoxy-6-carboxymethyl-D-mannopyranose, sodium salt (3)

Compound **2** (100 mg, 0.32 mmol) was dissolved in water (5 mL). Dowex®-50WX8 ion-exchange resin (H^+ form, 2 g) was added to the soln and the reaction mixture was stirred under reflux for 18 h. The soln was then filtered and directly eluted on a Dowex®-50WX8 ion-exchange column (Na^+ form). Following concentration and lyophilization, the title compound (80 mg, 100%) was obtained as a white solid: $[\alpha]_D^{25}$ +19 (*c* 0.95, H_2O); FTIR (KBr): ν_{max} 3427, 2924, 1582, 1413, 1063 cm^{-1}; ^1H NMR (250 MHz, D_2O): δ 5.04 (s, 0.4H, H-1α), 4.76 (s, 0.6H, H-1β), 3.84 (m, 1H, H-2), 3.72 (dd, 0.6H, J_{3-4} 9.6, J_{3-2} 2.3 Hz, H-3β), 3.65 (m, 0.6H, H-5β), 3.52 (dd, 0.4H, J_{3-4} 9.6, J_{3-2} 1.9 Hz, H-3α), 3.41 (t, 0.6H, J_{4-3} 9.6, J_{4-5} 9.6 Hz, H-4β), 3.32 (t, 0.4H, J_{4-3} 9.6, J_{4-5} 9.6 Hz, H-4α), 3.15 (t, 0.4H, J_{5-6} 9.6, J_{5-4} 9.6 Hz, H-5α), 2.33–2.11 (2H, m, H-7 and H-7′), 2.03 (m, 1H, H-6′), 1.59 (m, 1H, H-6); ^{13}C NMR (62.5 MHz, D_2O): δ 183.0 (COONa), 93.9 (C-1β), 93.7 (C-1α), 75.2 (C-5α), 72.9 (C-3α), 71.3 (C-5β), 71.1 (C-2α), 70.6 (C-2β), 70.4 (C-3β), 70.3 (C-4), 33.4 (C-7, 27.4 C6); ESI^+MS: *m/z* 229.1 (100) $[M-OH+H]^+$, 245.1 (75) $[M+H]^+$, 267.1 (6) $[M+Na]^+$; $HRESI^+MS$: calcd for $C_8H_{14}O_7Na$: 245.0637 $[M+H]^+$, found *m/z* 245.0641.

4.2.3. Benzyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-dimethylmalonate-α-D-mannopyranoside (6)

Benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **4**[52] (6 g, 11.1 mmol) was dissolved in anhyd CH_2Cl_2 (50 mL) under argon. Diisopropylethylamine (4.9 mL, 28.3 mmol) was added and the soln was stirred and cooled to −40 °C. Following dropwise addition of trifluoromethanesulfonic anhydride (2.8 mL, 16.6 mmol), the reaction mixture was stirred for 3 h. Thereafter, the soln was allowed to warm to rt and ethyl acetate (50 mL) was added. The resulting soln was washed with satd aq NH_4Cl (50 mL), then neutralized with satd aq $NaHCO_3$ (50 mL). The organic phase was dried and concentrated under diminished pressure to afford benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside **5** (7.8 g) as an intense red oil which was not further purified and used immediately for the next reaction: R_f 0.71 (4:1 petroleum ether/ethyl acetate).

In a two-neck round bottom flask placed under argon and containing anhyd THF (85 mL) was dissolved dimethyl malonate (1.7 mL, 28.8 mmol). NaH (60% in oil, 574 mg, 14.4 mmol) was then slowly added to the solution under vigorous stirring. The reaction mixture was further stirred at rt for 1 h. A soln of the benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside **5** previously synthesized in anhyd THF (17 mL) was then added dropwise to the sodium dimethyl malonate THF soln. The reaction mixture was stirred under reflux for 15 h, then cooled to rt, and neutralized by addition of MeOH (5 mL) and satd aq NH_4Cl (100 mL). The aq phase was extracted with diethyl ether (3 × 100 mL). The combined organic phases were washed with satd aq NaCl (100 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash-chromatography (2:1 petroleum ether/ethyl acetate) to give **6** (4.65 g, 64% for two steps) as a pale yellow oil: R_f 0.31 (2:1 petroleum ether/ethyl acetate); ^1H NMR (300 MHz, D_2O): δ 7.44–7.38 (m, 20H, Ph), 5.06 (d, 1H, *J* 10.8 Hz, OCH_2Ph), 4.97 (s, 1H, H-1), 4.86–4.66 (m, 6H, OCH_2Ph), 4.48 (d, 1H, *J* 11.9 Hz, OCH_2Ph), 4.05 (m, 1H, H-3), 3.90 (m, 2H, H-2 and H-7), 3.82 (m, 2H, H-4 and H-5), 3.81 (s, 3H, CH_3'), 3.72 (s, 3H, CH_3), 2.79–2.71 (ddd, 1H, $J_{6'-6}$ 14.2, $J_{6'-5(7)}$ 9.8, $J_{6'-7(5)}$ 4.7 Hz, H-6′), 2.32–2.22 (ddd, 1H, $J_{6-5(7)}$ 10.2, $J_{6-7(5)}$ 2.0 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.6 and 169.4 (2*C*OOMe), 138.2, 138.1, 138.0, and 137.0 (4C_q Ph), 128.2–127.4 (20 *C*H Ph), 96.7 (C-1), 79.9 (C-3), 78.5 (C-4), 75.1 (*C*H_2Ph), 74.5 (C-2), 72.5 (*C*H_2Ph), 72.1 (*C*H_2Ph), 69.2 (C-5), 68.4 (*C*H_2Ph), 52.3 (2CH_3), 48.2 (C-7), 31.2 (C-6); ESI^+MS: *m/z* 677.3 (100) $[M+Na]^+$, 678.3 (43), 679.3 (11); $HRESI^+MS$: calcd for $C_{39}H_{42}O_9Na$: 677.2721 $[M+Na]^+$, found *m/z* 677.2722.

4.2.4. 6-Deoxy-6-dimethylmalonate-D-mannopyranose (7)

Compound **6** (2.4 g, 3.7 mmol) dissolved in anhyd MeOH and $Pd(OH)_2/C$ (420 mg) were stirred under H_2 (15 bar) for 2 d. The mixture was then filtered and concentrated to give the intermediate compound benzyl 6-deoxy-6-dimethylmalonate-D-mannopyranoside. The latter dissolved in anhyd MeOH (50 mL) and $Pd(OH)_2/C$ (420 mg) were stirred again under H_2 (15 bar) for 3 d, filtered and concentrated to give **7** (1.017 g, 94%) as a colorless oil: R_f 0.47 (9:1 ethyl acetate/MeOH); ^1H NMR (360 MHz, CD_3OD): δ 5.03 (s, 0.7H, H-1α), 4.65 (s, 0.3H, H-1β), 3.83–3.65 (m, 2H, H-5 and H-7), 3.73 and 3.72 (2s, 6H, 2OCH_3), 3.48–3.37 (m, 3H, H-2, H-3, and H-4), 2.50 (ddd, 1H, $J_{6'-6}$ 14.2, $J_{6'-5(7)}$ 10.1, $J_{6'-7(5)}$ 5.2 Hz, H-6′), 1.98 (ddd, 1H, $J_{6-5(7)}$ 9.5, $J_{6-7(5)}$ 2.5 Hz, H-6); ^{13}C NMR (90.5 MHz, CD_3OD): δ 171.8, 171.7, 171.3, and 171.2 (4*C*OOMe: α- and β-anomers), 95.4 and 95.3 (C-1α and C-1β), 74.8, 74.5, 72.9, 72.6, 72.4, 71.9, 70.4 (C-2, C-3, C-4, and C-5: α- and β-anomers), 53.1 and 53.0 (2CH_3), 49.3 (C-7), 32.0 (C-6); ESI^+MS: *m/z* 316.9 (100) $[M+Na]^+$, 317.9 (12); $HRESI^+MS$: calcd for $C_{11}H_{18}O_9Na$: 317.0843 $[M+Na]^+$, found *m/z* 317.0836.

4.2.5. Preparation of 5-deoxy-5-phosphonomethyl-D-arabinonate, trisodium salt (8)[25]

The dipotassium salt of 6-deoxy-6-phosphonomethyl-D-mannopyranose[24] (460 mg, 1.38 mmol) was dissolved in 1 M NaOH (6.8 mL, 3.4 mmol). The reaction mixture was vigorously stirred at rt under O_2 for 7 d, using a volumetric device which allowed measurement of dioxygen consumption. The barium salt of **8** was obtained using the reported procedure,[56] dispersed in a few mL of water, and eluted with water on a Dowex®-50WX8 ion-exchange columns (H^+ form). The aq solution was then directly eluted on a Dowex®-50WX8 ion-exchange columns (Na^+ form), concentrated under vacuum and lyophilized to afford the trisodium salt of **8** (215 mg, 50%) as a white solid: ^1H NMR (300 MHz, D_2O): δ 4.15 (s, 1H, H-2), 3.60 (d, 1H, J_{3-4} 8.4 Hz, H-3), 3.53 (td, 1H, J_{4-5} 8.4, $J_{4-5'}$ 2.4 Hz, H-4), 1.94–1.41 (m, 4H, H-5′, H-5, H-6′, H-6); ^{13}C NMR (75 MHz, D_2O): δ 179.7 (C-1), 74.8 (C-3), 71.6 (C-2), 71.2 (d, J_{4-P} 16.7 Hz, C-4), 27.2 (C-5), 23.9 (d, J_{6-P} 134 Hz, C-6); ^{31}P NMR (121.5 MHz, D_2O): δ 26.1; ESI^-MS: *m/z* 264.9 (48) $[M-2Na+H]^-$, 246.9 (23) $[M-2Na+H-H_2O]^-$, 243.0 (88) $[M-3Na+2H]^-$, 224.9 (100) $[M-3Na+2H-H_2O]^-$; $HRESI^-MS$: calcd for $C_6H_{12}O_8P$: 243.0270 $[M-3Na+2H]^-$, found *m/z* 243.0278.

4.2.6. Preparation of 5-deoxy-5-phosphonomethyl-D-arabinonohydroxamic acid, dihydroxylammonium salt (9)[55]

6-Deoxy-6-dihydrogenophosphono-D-arabinono-1,4-lactone **11**[55] (110 mg, 0.486 mmol) was dissolved in a 50% aq NH_2OH soln (2.4 mL, 40 mmol). The reaction mixture was stirred at rt for 20 min, concentrated under high vacuum at rt, and lyophilized to afford the title compound as a white solid (160 mg, 100%): $[\alpha]_D^{25}$

−21 (*c* 0.95, H_2O); FTIR (KBr): ν_{max} 3194, 1651, 1042; ^{1}H NMR (300 MHz, D_2O): δ 4.05 (s, 1H, H-2), 3.27 (d, 1H, J_{3-4} 8.4 Hz, H-3), 3.22 (td, 1H, J_{4-5} 8.4, $J_{4-5'}$ 2.4 Hz, H-4), 1.36–1.04 (m, 4H, H-5′, H-5, H-6′, H-6), not in good agreement with the literature[55] for the disodium salt; ^{13}C NMR (75 MHz, D_2O): δ 171.3 (C-1), 73.6 (C-3), 70.4 (d, J_{4-P} 14.8 Hz, C-4), 70.1 (C2), 27.0 (C-5), 23.6 (d, J_{6-P} 133 Hz, C-6)), not in good agreement with the literature[55] for the disodium salt; ^{31}P NMR (121.5 MHz, D_2O): δ 25.3, lit.[55] 26.18 and 26.16 (2s) for the disodium salt; ESI$^-$MS: *m/z* 258.1 (100) $[M-2NH_3OH+H]^-$, lit.[55] 258 $[M-2Na+H]^-$; ESI$^-$MS: *m/z* calcd for $C_6H_{13}NO_8P$: 258.0379 $[M-2NH_3OH+H]^-$, found 258.0383.

4.2.7. 5-Deoxy-5-phosphonomethyl-D-arabinonohydrazide, dihydrazinium salt (10)

6-Deoxy-6-dihydrogenophosphono-D-arabinono-1,4-lactone **11**[55] (70 mg, 0.309 mmol) was dissolved in a 64% aq hydrazine soln. The reaction mixture was stirred at rt for 20 min, concentrated under high vacuum at rt, and lyophilized to afford the title compound (100 mg, 100%) as a white solid: ^{1}H NMR (360 MHz, D_2O): δ 4.28 (s, 1H, H-2), 3.53 (d, 1H, J_{3-4} 8.4 Hz, H-3), 3.46 (td, 1H, J_{4-5} 8.4, $J_{4-5'}$ 2.4 Hz, H-4), 1.82–1.19 (m, 4H, H-5′, H-5, H-6′, H-6); ^{13}C NMR (90.5 MHz, D_2O): δ 173.8 (C-1), 74.0 (C-3), 70.8 (d, J_{4-P} 14.8 Hz, C-4), 70.6 (C-2), 27.6 (C-5), 24.4 (d, J_{6-P} 131 Hz, C-6); ^{31}P NMR (101 MHz, D_2O): δ 23.2; ESI$^-$MS: *m/z* 257.0 (45) $[M-2NH_2NH_3+H]^-$, 224.9 (100) $[M-2NH_2NH_3-NH_2NH_2+H]^-$; HRESI$^-$MS: calcd for $C_6H_{14}N_2O_7P$ 257.0539 $[M-2NH_2NH_3+H]^-$, found 257.0552.

Acknowledgements

The ab initio computations were performed using resources from GENCI (CINES/IDRIS), Grant 2009-075009, and the Centre de Ressources Informatiques de Haute Normandie (CRIHAN, Rouen, France), Grant 1998053. We wish to thank Nicole Audiffren (CINES) and Guy Moebs (CRIHAN) for their help. We also wish to thank the Ligue Nationale contre le Cancer (Comité de Paris) for financial support.

Supplementary data

Supplementary data (structure of the active site of CaPMI complexed to 6DCM including additional discrete water molecules and ^{13}C NMR spectra of new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.09.005.

References and notes

1. Jensen, S.; Reeves, P. *Biochem. Biophys. Acta* **1998**, *1382*, 5.
2. Wu, B.; Zhang, Y.; Zheng, R.; Guo, C.; Wang, P. *FEBS Lett.* **2002**, *519*, 87.
3. Patterson, J.; Waller, R.; Jeevarajah, D.; Billman-Jacobe, H.; McConville, M. *Biochem. J.* **2003**, *372*, 77.
4. Shinabarger, D.; Berry, A.; May, T.; Rothmel, R.; Fialho, A.; Chakrabarty, A. *J. Biol. Chem.* **1991**, *266*, 2080.
5. Garami, A.; Ilg, T. *J. Biol. Chem.* **2001**, *276*, 6566.
6. Payton, M.; Rheinnecker, M.; Klig, L.; DeTiani, M.; Bowden, E. *J. Bacteriol.* **1991**, *173*, 2006.
7. Smith, D.; Proudfoot, A.; De Tiani, M.; Wells, T.; Payton, M. *Yeast* **1995**, *11*, 301.
8. Wills, E.; Roberts, I.; Del Poeta, M.; Rivera, J.; Casadevall, A.; Cox, G.; Perfect, J. *Mol. Microbiol.* **2001**, *40*, 610.
9. Smith, D.; Payton, M. *Mol. Cell. Biol.* **1994**, *14*, 6030.
10. Panneerselvam, K.; Etchison, J.; Freeze, H. *J. Biol. Chem.* **1997**, *272*, 23123.
11. De Koning, T.; Dorland, L.; Van Diggelen, O.; Boonman, A.; De Jong, G.; Van Noort, W.; De Schryver, J.; Duran, M.; Van den Berg, I.; Gerwig, G.; Berger, R.; Poll-The, B. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 38.
12. Jaeken, J.; Matthijs, G.; Saudubray, J.; Dionisi-Vici, C.; Bertini, E.; De Lonlay, P.; Henri, H.; Carchon, H.; Schollen, E.; Van Schaftingen, E. *Am. J. Hum. Genet.* **1998**, *62*, 1535.
13. Hendriksz, C.; Mc Clean, P.; Henderson, M.; Keir, D.; Worthington, V.; Imtiaz, F.; Schollen, E.; Matthijs, G.; Winchester, B. *Arch. Dis. Child.* **2001**, *85*, 339.
14. Slein, B. *J. Biol. Chem.* **1950**, *186*, 753.
15. Gracy, R.; Noltman, E. *J. Biol. Chem.* **1968**, *243*, 3161.
16. Proudfoot, A.; Turcatti, G.; Wells, T.; Payton, M.; Smith, D. *Eur. J. Biochem.* **1994**, *219*, 415.
17. Proudfoot, A.; Payton, M.; Wells, T. *J. Protein Chem.* **1994**, *13*, 619.
18. Cleasby, A.; Wonacott, A.; Skarzynski, T.; Hubbard, R.; Davies, G.; Proudfoot, A.; Bernard, A.; Payton, M.; Wells, T. *Nat. Struct. Biol.* **1996**, *3*, 470.
19. Roux, C.; Lee, J.; Jeffery, C.; Salmon, L. *Biochemistry* **2004**, *43*, 2926.
20. Roux, C.; Gresh, N.; Perera, L.; Piquemal, J.; Salmon, L. *J. Comput. Chem.* **2007**, *28*, 938.
21. Liu, X.; Moody, E. C.; Hecht, S. S.; Sturla, S. J. *Bioorg. Med. Chem.* **2008**, *16*, 3419.
22. Kowalska, J.; Lewdorowicz, M.; Darzynkiewicz, E.; Jemielity, J. *Tetrahedron Lett.* **2007**, *48*, 5475.
23. Vidil, C.; Morère, A.; Garcia, M.; Barragan, V.; Hamdaoui, B.; Rochefort, H.; Montero, J. *Eur. J. Org. Chem.* **1999**, 447.
24. Belakhov, V.; Dovgolevsky, E.; Rabkin, E.; Shulami, S.; Shoham, Y.; Baasov, T. *Carbohydr. Res.* **2004**, *339*, 385.
25. Pasti, C.; Rinaldi, E.; Cervellati, C.; Dallocchio, F.; Hardré, R.; Salmon, L.; Hanau, S. *Bioorg. Med. Chem.* **2003**, *11*, 1207.
26. Hajduch, J.; Nam, G.; Kim, E. J.; Fröhlich, R.; Hanover, J. A.; Kirk, K. L. *Carbohydr. Res.* **2008**, *343*, 189.
27. Devreux, V.; Wiesner, J.; Jomaa, H.; Van der Eycken, J.; Van Calenbergh, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4920.
28. Hirsch, G.; Grosdemange-Billiard, C.; Tritsch, D.; Rohmer, M. *Tetrahedron Lett.* **2004**, *45*, 519.
29. Berkowitz, D. B.; Maiti, G.; Charette, B. D.; Dreis, C. D.; MacDonald, R. G. *Org. Lett.* **2004**, *6*, 4921.
30. Burgos, E.; Salmon, L. *Tetrahedron Lett.* **2004**, *45*, 3465.
31. Khanjin, N.; Montero, J. *Tetrahedron Lett.* **2002**, *43*, 4017.
32. Le Camus, C.; Badet-Denisot, M.; Badet, B. *Tetrahedron Lett.* **1998**, *39*, 2571.
33. Arth, H.; Fessner, W. *Carbohydr. Res.* **1997**, *305*, 313.
34. Wiemer, D. F. *Tetrahedron* **1997**, *53*, 16609.
35. Engel, R. *Chem. Rev.* **1977**, *77*, 349.
36. Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. *J. Org. Chem.* **2000**, *65*, 4498.
37. Jeanjean, A.; Gary-Bobo, M.; Nirdé, P.; Leiris, S.; Garcia, M.; Morère, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6240.
38. Dourlat, J.; Valentin, B.; Liu, W.; Garbay, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3943.
39. Chen, H.; Luzy, J.; Gresh, N.; Garbay, C. *Eur. J. Org. Chem.* **2006**, 2329.
40. Gao, Y.; Luo, J.; Yao, Z.; Guo, R.; Zou, H.; Kelley, J.; Voigt, J. H.; Yang, D.; Burke, T. R. *J. Med. Chem.* **2000**, *43*, 911.
41. Miller, M. J.; Cleary, D. G.; Ream, J. E.; Snyder, K. R.; Sikorski, J. A. *Bioorg. Med. Chem.* **1995**, *3*, 1685.
42. Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R. *J. Med. Chem.* **1995**, *38*, 4270.
43. Jeanjean, A.; Garcia, M.; Leydet, A.; Montero, J.; Morère, A. *Bioorg. Med. Chem.* **2006**, *14*, 3575.
44. Miller, M. J.; Anderson, K. S.; Braccolino, D. S.; Cleary, D. G.; Gruys, K. J.; Han, C. Y.; Lin, K.; Pansegrau, P. D.; Ream, J. E.; Douglas Sammons, R.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1435.
45. Clavel, C.; Barragan-Montero, V.; Montero, J. *Tetrahedron Lett.* **2004**, *45*, 7465.
46. Gurgui-Ionescu, C.; Toupet, L.; Uttaro, L.; Fruchier, A.; Barragan-Montero, V. *Tetrahedron* **2007**, *63*, 9345.
47. Tian, F.; Montchamp, J.; Frost, J. W. *J. Org. Chem.* **1996**, *61*, 7373.
48. Bender, S. L.; Widlanski, T.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7560.
49. Rose, I.; O'Connell, E.; Schray, K. *J. Biol. Chem.* **1973**, *248*, 2232.
50. Gresh, N.; Cisneros, G. A.; Darden, T. A.; Piquemal, J. *J. Chem. Theory Comput.* **2007**, *3*, 1960.
51. Borén, H.; Eklind, K.; Garegg, P.; Lindberg, B.; Pilotti, Å. *Acta Chem. Scand.* **1972**, *26*, 4143.
52. Alais, J.; Veyrières, A. *Carbohydr. Res.* **1981**, *92*, 310.
53. Berkowitz, D. B.; Eggen, M.; Shen, Q.; Sloss, D. G. *J. Org. Chem.* **1993**, *58*, 6174.
54. Shen, Q.; Sloss, D. G.; Berkowitz, D. B. *Synth. Commun.* **1994**, *24*, 1519.
55. Sengmany, S. PhD thesis, Université Paris-Sud, Orsay, France, 2003.
56. Chirgwin, J.; Noltmann, E. *J. Biol. Chem.* **1975**, *250*, 7272.
57. Hardré, R.; Bonnette, C.; Salmon, L.; Gaudemer, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3435.
58. Chambers, R. D.; O'Hagan, D.; Brian Lamont, R.; Jaina, S. C. *J. Chem. Soc., Chem. Commun.* **1990**, 1053.
59. Crofts, P. C.; Kosolapoff, G. M. *J. Am. Chem. Soc.* **1953**, *75*, 3379.
60. Kortum, G.; Vogel, W.; Andrussow, K. *Dissociation Constants of Organic Acids in Aqueous Solution*; Butterworths: London, 1961.
61. Roux, C. PhD thesis, Université Paris-Sud, Orsay, France, 2004.
62. Gresh, N.; Roques, B. *Biopolymers* **1997**, *41*, 145.
63. Antony, J.; Gresh, N.; Olsen, L.; Hemmingsen, L.; Schofield, C.; Bauer, R. *J. Comput. Chem.* **2002**, *23*, 1281.
64. Gresh, N.; Derreumaux, P. *J. Phys. Chem. B* **2003**, *107*, 4862.
65. Antony, J.; Piquemal, J.; Gresh, N. *J. Comput. Chem.* **2005**, *26*, 1131.
66. Garmer, D.; Gresh, N.; Roques, B. *Proteins* **1998**, *31*, 42.
67. Jenkins Miller, L. M.; Hara, T.; Durell, S. R.; Hayashi, R.; Inman, J. K.; Piquemal, J.; Gresh, N.; Appella, E. *J. Am. Chem. Soc.* **2007**, *129*, 11067.
68. Gao, H.; Yu, Y.; Leary, J. *Anal. Chem.* **2005**, *77*, 5596.
69. Gracy, R.; Noltman, E. *J. Biol. Chem.* **1968**, *243*, 5410.