Bioorganic & Medicinal Chemistry 20 (2012) 1511–1520

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and evaluation of malonate-based inhibitors of phosphosugar-metabolizing enzymes: Class II fructose-1,6-bis-phosphate aldolases, type I phosphomannose isomerase, and phosphoglucose isomerase

Stéphanie Desvergnes [†], Stéphanie Courtiol-Legourd [†], Racha Daher, Maciej Dabrowski, Laurent Salmon [*], Michel Therisod [*]

Univ. Paris-Sud, Laboratoire de Chimie Bioorganique et Bioinorganique, ICMMO, UMR8182, LabEx LERMIT, Orsay F-91405, France
CNRS, Orsay F-91405, France

ARTICLE INFO

Article history:
Received 27 September 2011
Revised 20 December 2011
Accepted 22 December 2011
Available online 2 January 2012

Keywords:
Enzyme inhibitor
Hydroxamate
Malonate
Phosphate
Monosaccharide

ABSTRACT

In the design of inhibitors of phosphosugar metabolizing enzymes and receptors with therapeutic interest, malonate has been reported in a number of cases as a good and hydrolytically-stable surrogate of the phosphate group, since both functions are dianionic at physiological pH and of comparable size. We have investigated a series of malonate-based mimics of the best known phosphate inhibitors of class II (zinc) fructose-1,6-bis-phosphate aldolases (FBAs) (e.g., from *Mycobacterium tuberculosis*), type I (zinc) phosphomannose isomerase (PMI) from *Escherichia coli*, and phosphoglucose isomerase (PGI) from yeast. In the case of FBAs, replacement of one phosphate by one malonate on a bis-phosphorylated inhibitor (**1**) led to a new compound (**4**) still showing a strong inhibition (K_i in the nM range) and class II versus class I selectivity (up to 8×10^4). Replacement of the other phosphate however strongly affected binding efficiency and selectivity. In the case of PGI and PMI, 5-deoxy-5-malonate-D-arabinonohydroxamic acid (**8**) yielded a strong decrease in binding affinities when compared to its phosphorylated parent compound 5-phospho-D-arabinonohydroxamic acid (**2**). Analysis of the deposited 3D structures of the kinetically evaluated enzymes complexed to the phosphate-based inhibitors indicate that malonate could be a good phosphate surrogate only if phosphate is not tightly bound at the enzyme active site, such as in position 7 of compound **1** for FBAs. These observations are of importance for further design of inhibitors of phosphorylated-compounds metabolizing enzymes with therapeutic interest.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Number of enzymes act on phosphorylated substrates or catalyze the transfer of phosphate groups to/from various metabolic intermediates. Abnormal levels of phosphorylated biological entities in mammals have been linked to a wide range of diseases such as cancer, diabetes, atherosclerosis, immunodeficiency, cystic fibrosis (or mucoviscidosis) and inflammation. In addition, virulence and development of pathogenic microorganisms (bacteria, protozoae) have been shown to partly or entirely rely on levels of phospho-entities. Consequently, proteins and enzymes that recognize the phosphoester group have been considered as attractive therapeutic targets. Particularly, because of their implication in key metabolic pathways, phosphosugar-metabolizing enzymes such as 6-phosphofructo-2-kinase,[1] phosphoglucose isomerase,[2] phosphomannose isomerase,[3] 3-phosphoglycerate kinase,[4] glyceraldehyde-3-phosphate dehydrogenase,[5] fructose-1,6-bisphosphate aldolase (FBA),[5,6] and glutamine:fructose-6-phosphate amidotransferase[7] have been considered for the clinical development of chemotherapeutic agents, under the form of enzyme inhibitors.

Indeed, selective inhibitors of most of the fore-mentioned enzymes have been prepared, which have been proved to be efficiently active in vitro. Very disappointing results however were most often obtained when these compounds were tested for an in vivo activity. For example, we previously reported the synthesis of potent and selective inhibitors of class II (zinc dependent) FBA.[8–10] These compounds have a potential therapeutic interest as antibiotics, since class II FBA is present in many pathogenic bacteria (*Yersinia pestis, Mycobacterium tuberculosis, Clostridium difficile, Pseudomonas aeruginosa...*), yeasts (*Candida albicans...*), protozoae (*Giardia lamblia...*) and absent from human where class I FBA

Abbreviations: 5PAH, 5-phospho-D-arabinonohydroxamic acid; F6P, D-fructose 6-phosphate; FBA, fructose-1,6-bis-phosphate aldolase; FBP, D-fructose 1,6-bis-phosphate; G6P, D-glucose 6-phosphate; HEI, high energy intermediate; M6P, D-mannose-6-phosphate; β-M6P, β-D-mannopyranose 6-phosphate; PDB, Protein Data Bank; PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase.

* Corresponding authors. Tel.: +33 1 69 15 63 11; fax: +33 1 69 15 72 81.
E-mail addresses: laurent.salmon@u-psud.fr (L. Salmon), michel.therisod@u-psud.fr (M. Therisod).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.12.050

operates. They were rationally designed as transition-state analogues of the catalyzed reaction of cleavage of D-fructose 1,6-bisphosphate (FBP), and bear a zinc-chelating group to induce a selectivity for class II FBA (Fig. 1).

Compound **1** (and others reported in Refs. 8–10) presents interesting inhibitory power with K_i in the nanomolar range and selectivity up to 10^5 for microbial enzymes. Crystallographic studies also confirmed that **1** is tightly bound into the active site of class II FBA.[10] However, when **1** and its congeners were assayed in vivo against cultivated pathogens (*M. tuberculosis, Y. pestis, C. albicans*), no growth inhibition could be observed at concentrations up to 1 mM (there are strong evidence that at least in *M. tuberculosis*, class II *fba* is an essential gene). This negative result is not so surprising in view of the polarity of these phosphorylated products, unlikely to spontaneously cross (microbial) cytoplasmic membranes. Moreover, phosphatases are ubiquitously present, which can dephosphorylate **1** before it can reach its target. Similarly, 5-phospho-D-arabinonohydroxamic acid 5PAH (**2**, Fig. 2) designed as a high energy intermediate (HEI) analogue was first reported as the best inhibitor of phosphoglucose isomerase (PGI), which interconverts D-glucose 6-phosphate (G6P) and D-fructose 6-phosphate (F6P), with K_i in the low micromolar range.[11–13] The same compound turned out to behave also as the strongest known inhibitor of phosphomannose isomerase (PMI), which interconverts D-mannose 6-phosphate (M6P, epimer of G6P at carbon 2) and F6P.[14] Kinetic and molecular modeling studies on PMI from *C. albicans* allowed us to propose a detailed mechanism of the isomerization reaction catalyzed by the enzyme.[15,16] Besides its use as probe in elucidating enzyme mechanisms, compound **2** is of therapeutic interest since the multifunctional glycolytic enzyme PGI is also the cytokine autocrine motility factor involved in tumor cell migration and evolution of metastatic lesions.[17,18] PMI was reported to be essential for the survival or pathogenesis of several bacteria, parasites, and yeasts, including the fungal pathogen *C. albicans* involved in candidosis.[3] Similarly to **1**, compound **2** is unlikely to have any in vivo biological activity in view of its high polarity and the presence of a hydrolysable phosphate group.

For these reasons, we decided to build up analogues of these strong inhibitors bearing a non-hydrolyzable surrogate of the phosphate group(s). We chose to introduce a malonate group, since

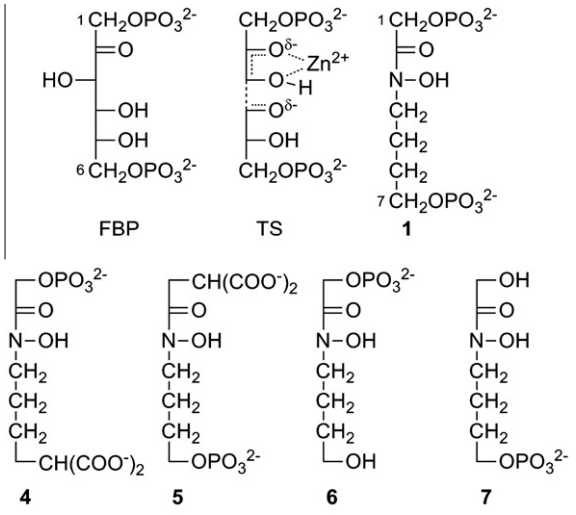


Figure 1. D-Fructose 1,6-bis-phosphate (FBP) as substrate of class II D-fructose-1,6-bis-phosphate aldolase (FBA); TS: transition-state of the reaction catalyzed by FBA; *N*-(4-phosphobutyl)-phosphoglycolohydroxamic acid (**1**) as a selective inhibitor of FBA; malonylated analogues (**4** and **5**) of **1**, and their 'naked' counterparts (**6** and **7**, respectively).

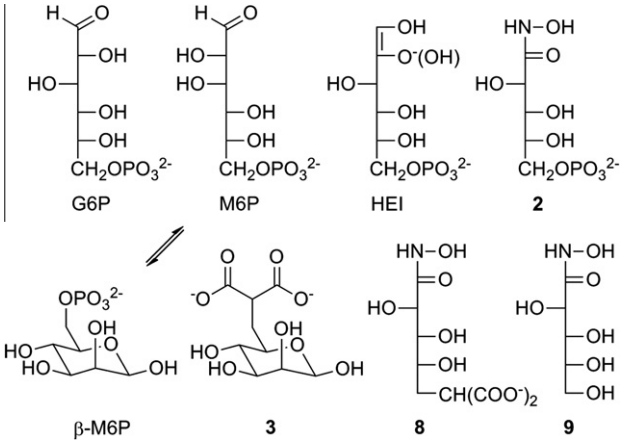


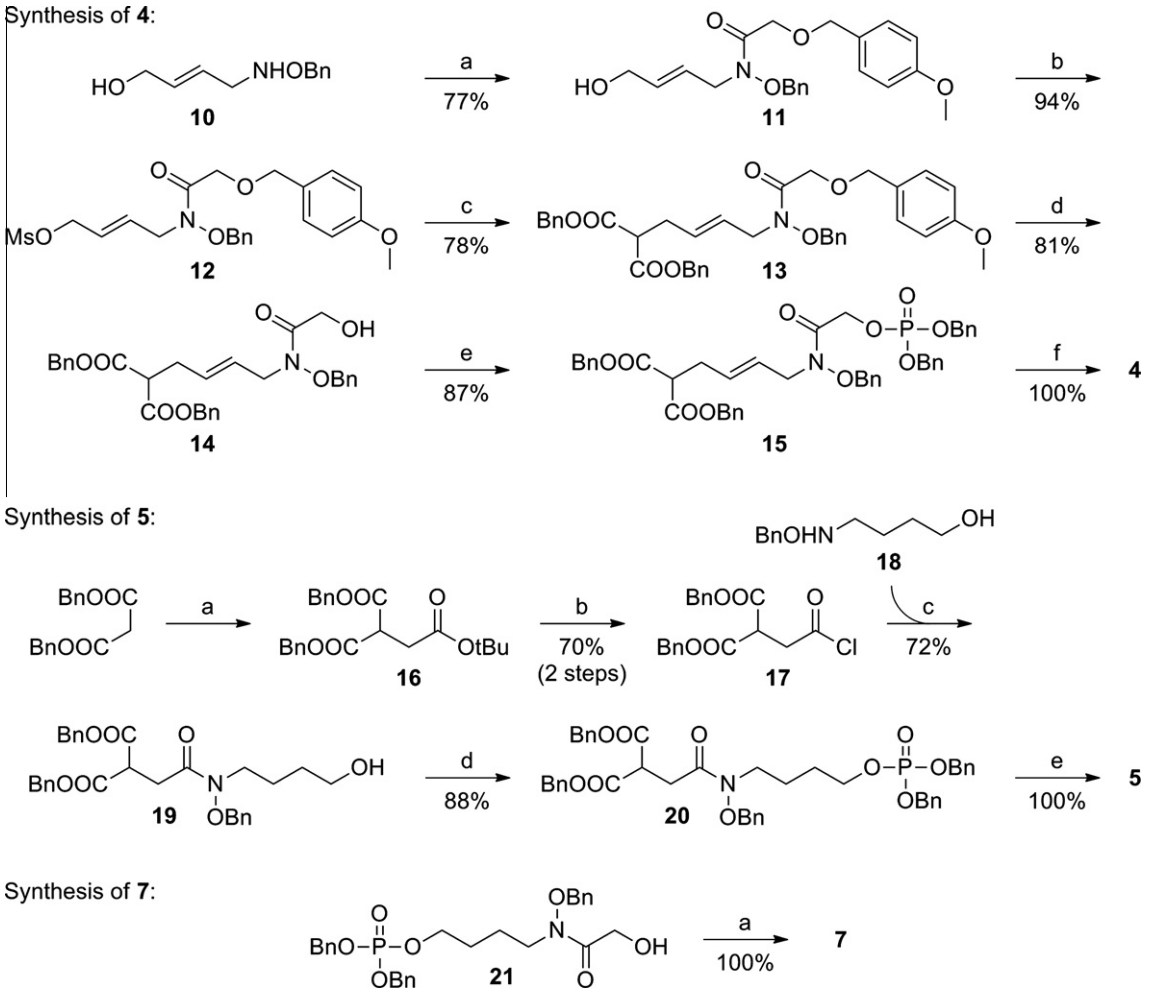
Figure 2. D-Glucose 6-phosphate (G6P) and D-mannose 6-phosphate (M6P) as substrates of phosphoglucose isomerase (PGI) and phosphomannose isomerase (PMI), respectively; HEI: high energy intermediate of the reactions catalyzed by the two enzymes; 5-phospho-D-arabinonohydroxamic acid (**2**) as a HEI analogue inhibitor of both PGI and PMI; 6-deoxy-6-malonate-β-D-mannopyranose (**3**) as the malonylated analogue inhibitor of the PMI cyclic substrate β -D-mannopyranose 6-phosphate (β -M6P); malonylated analogue (**8**) of **2**, and its 'naked' counterpart (**9**).

this function is also easy to derivatize into fatty-acid esters. Inhibitors bearing such a malonic ester could be considered as lipophilic prodrugs, able to cross biological membranes, and readily hydrolyzed in the cell by various non-specific lipases or esterases. Malonate has been used more or less successfully as surrogate of phosphate in synthesis of inhibitors of various enzymes.[19–28] We recently reported the preparation and successful evaluation of the malonylated analogue of β-D-mannopyranose 6-phosphate (β-M6P), namely 6-deoxy-6-malonate-D-mannopyranose (**3**, Fig. 2) as a hydrolytically resistant inhibitor of PMI.[29] This product was characterized as a purely competitive substrate analogue inhibitor of PMI, with inhibition constants K_i in the micromolar range, and K_m/K_i ratio values of 10 to 30, depending on the enzyme source. We therefore undertook the synthesis and evaluation on class II FBA of compounds **4** and **5** as mono-malonylated analogues of inhibitor **1**. For a useful comparison, we also prepared and tested the 'naked' compounds **6** and **7** (Fig. 1). Similarly, compound **8**, the malonylated analogue of 5PAH **2**, as well as its 'naked' analogue **9**, were synthesized and evaluated on PMI and PGI (Fig. 2).

2. Results and discussion

2.1. Chemistry

The chemical syntheses of new class II FBA inhibitors **4**, **5**, and **7** are depicted in Scheme 1 (the syntheses of **1** and **6** were reported previously[10]). Indeed, compound **10**[9] was acylated in methanol / triethylamine with the *para*-methoxybenzyl (PMB) ether of glycolyl chloride,[30,31] chosen for having a protection orthogonal to the benzyl group, to afford **11**, which was subsequently mesylated to give compound **12**. The dibenzylmalonate group was introduced through displacement of the mesylate to afford **13**, then **14** after removal of the PMB ether with DDQ. **4** was finally obtained after a classical phosphorylation with dibenzyl-*N*-diisopropylphosphoramidite and oxidation with ^{t}butylhydroperoxide which gave **15**, followed by one-step removal of the five benzyl groups (40% overall yield from **10**). Very similarly, the dibenzylmalonyl glycolyl chloride **17** was condensed in methanol/triethylamine with the *N*-alkylhydroxylamine **18**[10] to afford **19**, which was then phosphorylated as described above to give compound **20**. The five benzyl groups were finally removed by hydrogenolysis to lead to the



Scheme 1. Synthesis of class II D-fructose-1,6-bis-phosphate aldolase inhibitors **4**, **5**, and **7**. Reagents and conditions: Synthesis of **4**—(a) Cl-CO-CH_2-O-PMB, MeOH, 0 °C; (b) MsCl, CH_2Cl_2, DIPEA, 0 °C; (c) $CH_2(COOBn)_2$, NaH, DMF, rt; (d) DDQ, CH_2Cl_2/H_2O, rt; (e) $(iPr)_2NP(OBn)_2$, triazole/imidazole, CH_2Cl_2, rt then ^tBuOOH; (f) H_2, Pd-C 10%, 2 equiv $NaHCO_3$, EtOH/H_2O. Synthesis of **5**—(a) NaH, $BrCH_2COO^tBu$, DMF, 0 °C to rt; (b) TFA, CH_2Cl_2, rt then $(COCl)_2$, DMF cat., CH_2Cl_2, rt; (c) MeOH, NEt_3, 0 °C; (d) $(iPr)_2NP(OBn)_2$, triazole/imidazole, CH_2Cl_2, rt then ^tBuOOH; (e) H_2, Pd-C 10%, 2 equiv $NaHCO_3$, THF/H_2O. Synthesis of **7**—(a) H_2, Pd-C 10%, 1 equiv $NaHCO_3$, THF/H_2O, on a previously reported synthetic intermediate.[10]

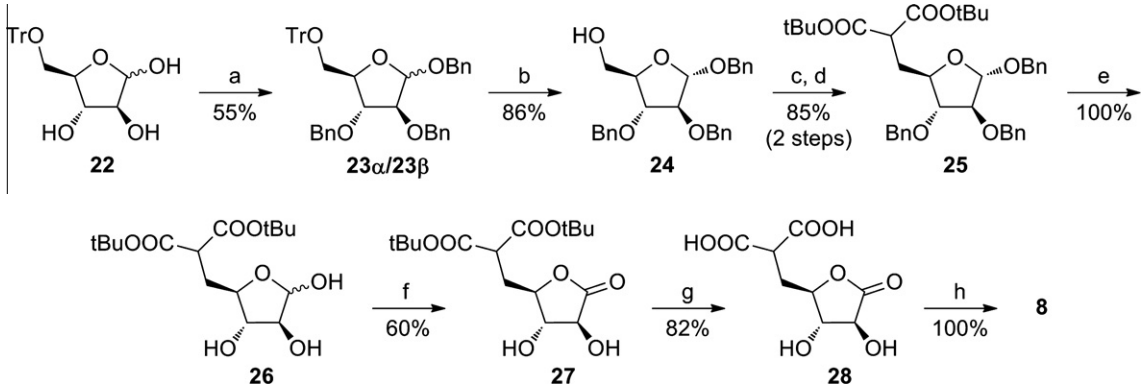
free compound **5** (44% overall yield from ^{t}butylbromoacetate and dibenzylmalonate). Compound **7** was simply obtained after deprotection of **21**[10] whose synthesis was previously described. As proven by ^{13}C NMR, throughout the syntheses as well as on the final deprotected compounds, one single conformer of the hydroxamate function was formed. We cannot however decide if it is the s-*Z* or s-*E* conformer. Moreover, there is no evidence that the conformer present in solution is the same as the one (s-*Z*) present in the active site of the enzymes.[10]

The chemical synthesis of the new PGI/PMI inhibitor 5-deoxy-5-dicarboxymethyl-D-arabinonohydroxamic acid **8** is depicted in Scheme 2 (the syntheses of **2**,[11,12] **3**,[29] and **9**[32,33] were reported previously). Benzylation of 5-*O*-trityl-D-arabinofuranose **22**[34,35] was achieved using the conditions reported by Cabaret et al. for the synthesis of the L enantiomers.[36] The two anomers were separated by flash-chromatography to afford **23**α (δ(C-1) = 105 ppm) and **23** β (δ(C-1) = 99 ppm) in 39% and 16%, respectively (overall yield: 55%; lit.[35] <40%). Following removal of the trityl group of **23**α in AcOH/H_2O as reported, **24** (α-anomer: δ(C-1) = 105 ppm; lit.[35] 105 ppm) was obtained in 86% yield (lit.[35] 72%). Reaction with trifluoromethanesulfonic anhydride and diisopropylethylamine afforded benzyl 2,3-di-*O*-benzyl-5-*O*-trifluoromethanesulfonyl-α-D-arabinofuranoside which was not purified and used immediately in the next reaction. Addition to a dry THF solution of ditertiobutylmalonate and sodium hydride gave compound **25** (α–anomer: δ(C-1) = 105 ppm) in 85% yield (two steps). Following deprotection of the hydroxyl groups by hydrogenolysis on $Pd(OH)_2/C$ to give compound **26** quantitatively, oxidation of the anomeric hydroxyl group led to 5-deoxy-5-ditertiobutylmalonate-D-arabinono-1,4-lactone **27** in 60% yield. Deprotection of the malonate group was successfully achieved with trifluoroacetic acid in dry dichloromethane to afford the lactone **28**, which, upon reaction with 50% aqueous hydroxylamine gave the final product 5-deoxy-5-malonate-D-arabinonohydroxamic acid (hydroxylammonium salt) **8** in 82% yield (two steps).

2.2. Inhibition studies on FBAs

The inhibitory properties of compounds **1**, **4**–**7** were next evaluated on FBAs from various sources following standard procedures.[8] The quantitative results are given in Table 1.

All the tested compounds gave purely competitive-inhibition kinetics patterns on the *C. albicans* enzyme, considered as representative of class II FBAs. As reported previously, compound **1**, in which the two phosphate groups at positions 1 (p1) and 7 (p7, Fig. 1) mimic the phosphates present at p1 and p6 of the substrate



Scheme 2. Synthesis of PGI/PMI inhibitor **8**. Reagents and conditions: (a) KOH, BnBr, Bu_4NBr; (b) $AcOH/H_2O$, 65 °C; (c) Tf_2O, DIPEA, CH_2Cl_2, −40 °C; (d) $(^tBuOOC)_2CHNa$, THF reflux; (e) H_2, $Pd(OH)_2/C$, rt; (f) Br_2, $BaCO_3$, H_2O/dioxane, rt; (g) TFA, CH_2Cl_2, rt; (h) 50% aq NH_2OH.

Table 1
In vitro biochemical evaluation of inhibitor **1**, **4**–**7** on D-fructose-1,6-bis-phosphate aldolases (FBA) from various sources[a]

FBA source (class)	K_M (μM)	K_i (μM)				
	FBP	**1**[b]	**4**	**5**	**6**[b]	**7**
Rabbit muscle (I)[c]	17	57.5	257	500	400	>1000[d]
C. albicans (II)	350	0.003	0.05	2300	0.3	0.76
H. pylori (II)[c]	37	0.07	0.04	250	5.5	1.2
M. tuberculosis (II)[c]	21[b]	0.0016	0.004	500	0.19	0.175
Y. pestis (II)[c]	55[b]	0.018	0.008	1500	0.2	0.08

[a] Estimated standard error value = 5%.
[b] Values from Ref. 10.
[c] K_i estimated from IC_{50}, measured at [S] = K_M.
[d] No inhibition at the given concentration.

D-fructose 1,6-bis-phosphate (FBP), respectively, strongly and selectively inhibits class II versus class I FBAs.[10] Compound **4**, in which a malonate mimics the p7 phosphate group of compound **1**, is still a strong and selective inhibitor of class II FBAs, with, depending on the FBA source, a limited loss (2.5 to 16), or an even better inhibitory power (case of *Helicobacter pylori*) and selectivity as compared to **1**. The comparison between **4** and **6** (on which a simple hydroxyl group is present at p7 while maintaining the phosphate group at p1 also confirms the efficiency of a malonate as a surrogate of phosphate at this position. On the other hand, the replacement of the phosphate group at p1 of **1** by a malonate in **5** gave K_i values in the millimolar range and a complete loss of Class II versus Class I selectivity. The comparison between **5** and **7** (on which a simple hydroxyl group is present at p1 while maintaining the phosphate group at p6) also indicates that a malonate at this position not only does not mimic the phosphate at p1 of FBP, but has a negative effect on binding of the inhibitor. Interestingly, compound **7** lacking the phosphate group at p1 is ignored by class I FBA and efficiently recognized by class II FBAs, with low K_i values and high selectivities. The fact that compounds **6** (phosphorylated at p1) and **7** (phosphorylated at p7) gave similar inhibition constants may suggest that either phosphate group in compound **1** is important for binding at the active site of FBA. However, **6** and **7** are 'naked' compounds, which lack a phosphate or a malonate group at p7 or p1, respectively, so that these two compounds are only good but not strong inhibitors. Our discussion about the relative importance of either phosphate in compound **1** is based on the effect of its replacement by a malonate group. Clearly, comparison of the inhibition constants of compounds **4** and **5** shows that such a replacement of the phosphate group at p1 or p7 by a malonate leads to very different conclusions. In the case of compound **4**, the importance of the phosphate group at p1 is further highlighted by the fact that its non-phosphorylated analogue (unpublished result) proved to be inactive against FBAs. The above kinetic results confirm that the two phosphate groups at p1 and p7 are not equally important for an analogue of FBP to be recognized by FBA. Indeed, analysis of crystallographic data obtained with **1** in the active site of *H. pylori*[10] (Fig. 3, PDB ID code = 3N9S) and *M. tuberculosis*[37] (PDB ID code = 4A22) FBAs indicates that the phosphate group at p7 is bound to only two active site residues (Arg280 and Ser49) through three hydrogen bonds, and to seven water molecules. The case of the phosphate group at p1 is rather different: the binding network implies only one water molecule, but six hydrogen bonds to five active site residues (Gly181, Lys184, Ser213, Asp255, and Thr256), with four over six H-bonds coming from NH groups of the C α chain. In addition, two of these residues are consecutive in the sequence (Asp255 and Thr256). Consequently, the phosphate group at p1 appears much more tightly bound to the protein than the one at p7. Although the structure of a malonate is chemically different from its phosphate bio-isoster, both functions are most likely dianionic at pH >7 used for the kinetics.[29] However, molecular dynamics revealed that the malonate group occupies about 13% more volume than the phosphate.[20] This small difference is of little importance in replacement of a loosely bound phosphate (p7 of **1**) by a malonate (case of **4**). In the case of a tight-binding interaction, like for the phosphate at p1 of **1**, the malonate substitution could induce steric clashes (case of **5**). Moreover, a malonate function has three rotatable bonds, while a phosphate ester group has only one. This implies a more negative binding entropy for a malonylated than a phosphorylated analogue to the enzyme active site, and still a more negative binding entropy of a malonate at p1, where the phosphate group is very ordered, than at p6, where it is less. Our results clearly show that in this series of FBA inhibitors, malonate can effectively replace phosphate only if this group is not essential for binding of the FBP analogue to the enzyme active site, that is, the phosphate group at p6 of FBP (or p7 in analogues **1**, **4**–**7**). The phosphate group in p1 position being crucial to enzyme binding of substrate and analogues is likely to be difficult to mimic. Our hypothesis is also in accordance with dihydroxyacetone phosphate being the unique entity that binds to FBA with the phosphate group in p1, while a large spectrum of aldehydes are accepted by the enzyme for binding at the p6 domain.

2.3. Inhibition studies on PGI/PMI

The inhibitory properties of compounds **2**, **8**, and **9** were evaluated on D-glucose-6-phosphate isomerase (PGI) from yeast and D-mannose-6-phosphate isomerase (PMI) from *Escherichia coli* following standard procedures.[12,16] The quantitative results are

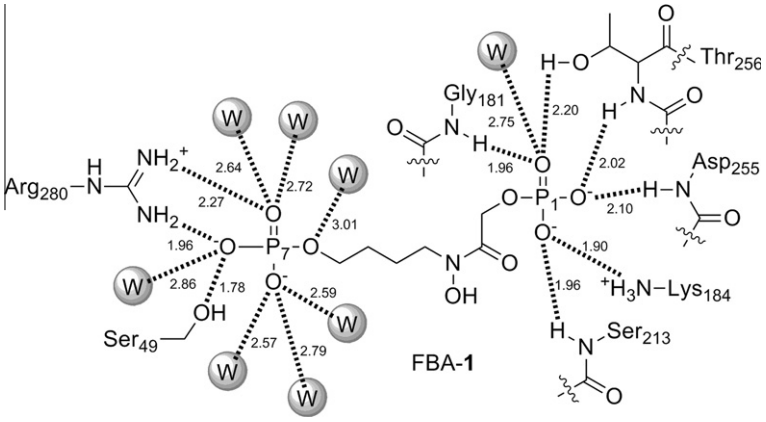


Figure 3. Schematic representation of the active site of FBA from *H. pylori* complexed to *N*-(4-phosphobutyl)-phosphoglycolohydroxamic acid (**1**) (PDB ID code = 3N9S[10]) showing the bonding patterns of the two phosphate groups in positions 1 (p1) and 7 (p7). Crystallographic water molecules (W) are highlighted as spheres and hydrogen bonds by dashed lines (H-bonds distances are given in angstrom). Distances involving water molecules are given between oxygen atoms.

Table 2
In vitro biochemical evaluation of inhibitors **2**, **8**, and **9** on D-glucose-6-phosphate isomerase (PGI) and D-mannose-6-phosphate isomerase (PMI)[a]

Enzyme	K_M (μM)		K_i (μM)		
	F6P	**M6P**	**2**	**8**	**9**
Yeast PGI	84	—	0.23[b]	2290[c]	5540[c]
E. coli PMI	—	354	0.08[d]	162	10000[c,e]

[a] Estimated standard error value = 5%.
[b] Ref. 11.
[c] IC_{50} value.
[d] Ref. 16.
[e] Ref. 38.

given in Table 2. The phosphate analogue inhibitor **2** is known as the most powerful competitive inhibitor of all PGI and PMI evaluated so far,[11,12,14,16] with K_i values in the sub-micro-molar range and K_M/K_i ratios from 400 to 4500 for yeast PGI and *E. coli* PMI, respectively. Considering now compound **8**, the malonylated analogue of **2**, a much higher K_i value was determined for *E. coli* PMI with a 2000-fold decrease in binding affinity. A similar tendency is observed for yeast PGI with an IC_{50} value above 2 mM for **8** as compared to a K_i value of 0.23 μM for **2**. In the case of these transition state analogue inhibitors, malonate does definitely not appear as a good mimic of phosphate. Interestingly, compound **3** (Fig. 2), the weak malonylated analogue inhibitor of the PMI substrate β-M6P, gave a K_i value in the same range of magnitude (115 μM[29] vs 162 μM for **8**). This comparison confirms that phosphate replacement of compound **2** by malonate in compound **8** is likely responsible of the dramatic loss in enzyme binding affinity. However, the situation is worse in the case of compound **9** (the non-phosphorylated/-non-malonylated transition state analogue of **2**/**8**), which does not inhibit neither yeast PGI nor *E. coli* PMI. These results confirm that the phosphate group in substrates and analogues accounts for most of the binding affinity to either enzyme, is thus definitely essential, but can be very partly replaced by a malonate group. Examination of the previously reported X-ray crystal structure (rabbit muscle PGI-**2** complex,[13] Fig. 4A) and model structure (*Candida albicans* PMI-**2** complex,[16] Fig. 4B) gives interesting information that could explain the above kinetic results. Active site residues of PGI from yeast and rabbit muscle are highly conserved, so that kinetic results determined with yeast PGI can be corroborated by conclusions drawn from the rabbit muscle PGI-**2** complex structure. A similar comment can be formulated for both *E. coli* and *C. albicans* PMI, for which no X-ray crystal structures of the enzyme–inhibitor complexes are available. In the

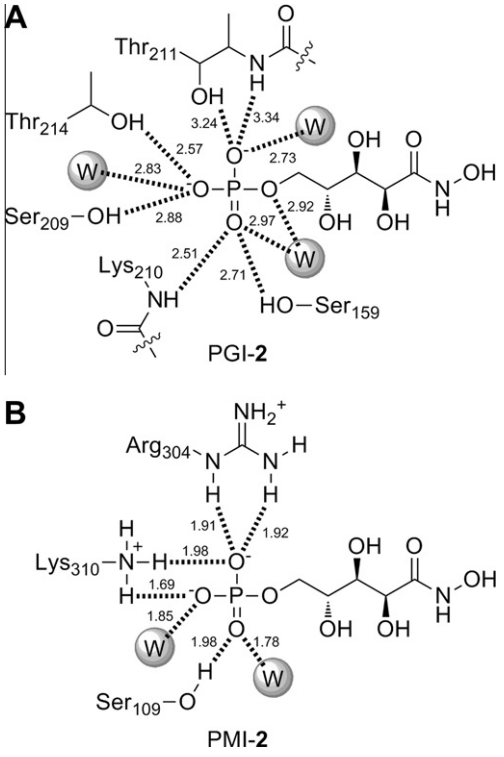


Figure 4. Schematic representations of the active site of (A) rabbit muscle PGI and (B) *Candida albicans* PMI complexed to 5-phosphate-D-arabinonohydroxamic acid **2**, depicted from the deposited crystal structure (PDB ID code = 1KOJ)[13] and from the reported polarizable molecular mechanics study,[16] respectively, showing the bonding pattern of the phosphate group in both complexes. Crystallographic (A) and modeled (B) water molecules (W) are highlighted as spheres and hydrogen bonds by dashed lines (distances are given in angstrom). In Figure 4A, all distances are given between heteroatoms, while in Figure 4B, H-bond distances are provided.

case of the PGI-**2** complex (Fig. 4A), binding pattern of the phosphate group to the enzyme involves three water molecules (four H-bonds) and five residues (six H-bonds). Interestingly, three of these residues (Ser209, Lys210, and Thr211) are consecutive (almost four with Thr214), which likely gives a rather rigid structure of the phosphate subsite. In addition, two of these residues (Lys210 and Thr211) interact with the phosphate through the NH group of the C α chain, enhancing binding and ordering of the phosphate group. Consequently, and as described above for FBA, binding of a malonate analogue to the PGI active site is likely to strongly

affect the binding energy cost both in terms of enthalpy and entropy (more negative for a malonate than for a phosphate), and thus appears unfavorable. In PMI-**2** complex (Fig. 4B), two water molecules and three active site residues are involved in the binding of the phosphate group of inhibitor **2**, five over seven H-bonds coming from the three residues. Arg304 and Lys310 appear to strongly interact with the phosphate group through two H-bonds each, with H-bond distances even shorter than most of those observed in the p1 phosphate subsite of FBA which has been described as quite well ordered (Fig. 3). The strong bonding pattern at the PMI phosphate subsite may well explain why phosphate replacement by a malonate is not energetically favorable. However, why binding of the malonylated substrate analogue **3** and transition state analogue **8** are in the same order of magnitude cannot be clearly explained from the model depicted in Figure 4B. Polarizable molecular mechanics studies of the PMI-**2** and PMI-**8** complexes involving discrete structural water molecules will be performed and published in due course.

2.4. Biological studies

Compounds **1**, **4**, and **5** were assayed for growth inhibition of cultivated *M. tuberculosis* and *Y. pestis*. No effect was observed at concentrations up to 1 mM.

3. Conclusion

With a dianionic ionization state at physiological pH as for phosphate and a steric hindrance only slightly larger, malonate has been widely considered as a good phosphate surrogate in several examples reported in the literature. Actually, in our design of an hydrolytically stable phosphosugar analogue as potential inhibitor of class II FBA, phosphate replacement by a malonate group gave an effectively strong inhibitor (compound **4**). The same success was obtained in inhibition of PMI by 6-deoxy-6-malonate-D-mannopyranose **3**, the malonylated analogue of the substrate β-M6P. In other cases, such as for PMI or PGI inhibition by **8**, the malonylated analogue of the strong inhibitor **2**, or in inhibition of FBA by **5**, such a replacement yielded inhibitors with poor or no significant binding properties. Analysis of our previously reported tridimensional crystal (FBA, PGI) and theoretical model (PMI) structures of enzyme–inhibitor complexes gives informations in accordance with the kinetic results. Somehow logically, malonate appears as a good phosphate surrogate only if phosphate binding is not crucial for the whole binding of the phosphorylated entity at the enzyme active site. We propose that binding enthalpy and entropy could be determining parameters in the overall binding energy of the malonylated analogue at the enzyme active site. In conclusion, malonate is not a versatile phosphate surrogate: such a replacement appears rather dependent on the enzyme studied, and in the case of bis-phosphorylated substrates or inhibitors, further dependent on the substitution position. These observations are of importance for further design of enzyme inhibitors with therapeutic interest.

4. Experimental protocols

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. 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 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 at 25 °C 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 Bruker DRX 400 (^1H at 400.13 MHz and ^{13}C at 100.62 MHz), Avance 360 (^1H at 360.13 MHz and ^{13}C at 90.56 MHz), DRX 300 (^1H at 300.13 MHz and ^{13}C at 75.47 MHz) or DPX 250 (^1H at 250.13 MHz, ^{13}C at 62.90 MHz, and ^{31}P at 101.26 MHz) spectrometer using NMR Notebook 2.0 WinNMR software. Chemical shift 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 4.78) for solutions in $CDCl_3$, CD_3OD and D_2O, 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. 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 electrospray with positive (ESI^+) or negative (ESI^-) ionization mode.

4.2. Synthesis of compound 4

4.2.1. (*E*)-*N*-(Benzyloxy)-*N*-(4-hydroxybut-2-enyl)-2-(4-methoxybenzyloxy)acetamide (11)

(*E*)-4-(Benzyloxyamino)but-2-en-1-ol (**10**)[9] (1.21 g, 6.23 mmol) and triethylamine (1.05 mL, 7.5 mmol) were dissolved in anhydrous methanol (15 mL) at 0 °C. 4-Methoxy-benzyloxyacetyl chloride[30,31] dissolved in dichloromethane was then added dropwise. The mixture was further stirred during 2 h, diluted with 20 mL of dichloromethane and washed three times with water. The organic phase was dried over sodium sulfate and evaporated. The product was purified by flash-chromatography (pentane/ethyl acetate 6:4) to afford the title compound (1.78 g, 77%). R_f = 0.34 (pentane/AcOEt 2:8); ^1H NMR ($CDCl_3$, 250 MHz) δ 3.79 (s, 3H, CH_3), 4.10 (d, 2H, NCH_2, *J* 6.8 Hz), 4.16 (s, 2H, OCH_2CO), 4.24 (d, 2H, *J* 8.8 Hz, CH_2OH), 4.50 (s, 2H, OCH_2PhOMe), 4.76 (s, 2H, CH_2Ph), 5.67–5.89 (m, 2H, C*H*=C*H*), 6.88 (d, 2H, *J* 12.4 Hz, o-H_{ar}-PhOMe), 7.28–7.37 (m, 7H, *H*-Ph, m-H_{ar}-PhOMe); ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 48.2 (NCH_2), 55.3 (CH_3), 62.6 (CH_2OH), 67.2 (OCH_2CO), 72.9 (OCH_2PhOMe), 77.0 (CH_2Ph), 113.8 (o-C_{ar}-PhOMe), 124.2 (*C*H=CH–CH_2OH), 128.7, 129.1, 129.4, 129.8, 134.2 (*C*-Ph, CH=*C*H-CH_2OH, m-C_{ar}-PhOMe), 159.4 (*C*=O).

4.2.2. (*E*)-4-(*N*-(benzyloxy)-2-(4-methoxybenzyloxy)acetamido) but-2-enyl methanesulfonate (12)

Compound **11** (1.88 g, 5.1 mmol), a catalytic amount of DMAP and DIPEA (1.3 mL, 7.5 mmol) were dissolved in anhydrous dichloromethane (20 mL) at 0 °C. To this stirred solution was added dropwise mesyl chloride (0.47 mL, 6.12 mmol), and the mixture was further stirred during 1 h. The mixture was diluted with more dichloromethane, washed with water, 1 M HCl, and saturated $NaHCO_3$. The organic phase was dried and evaporated to afford the title compound (2.125 g, 94%). ^1H NMR ($CDCl_3$, 250 MHz) δ 2.98 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 4.20 (s, 2H, OCH_2CO), 4.28 (d, 2H, *J* 6.8 Hz, NCH_2), 4.54 (s, 2H, CH_2PhOMe), 4.70 (d, 2H, *J* 8.8 Hz, CH_2OMs), 4.80 (s, 2H, NOCH_2Ph), 5.80–5.90 (m, 2H, C*H*=C*H*), 6.90 (d, 2H, *J* 13.6 Hz, o-H_{ar}-PhOMe), 7.29–7.40 (m, 7H, *H*-Ph, m-H_{ar}-PhOMe); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 38.2 (SCH_3), 47.8 (NCH_2), 55.3 (OCH_3), 67.2 (CH_2OMs), 69.4 (OCH_2CO), 72.9 (CH_2PhOMe), 77.1 (NOCH_2Ph),

113.9 (o-C_{ar}-PhOMe), 126.3 (NCH_2CH=), 128.8, 129.1, 129.4, 129.8, 134.2 (*C*-Ph, NCH_2CH=*C*H, m-C_{ar}-PhOMe), 159.4 (C=O).

4.2.3. (*E*)-Dibenzyl 2-(4-(*N*-(benzyloxy)-2-(4-methoxybenzyloxy) acetamido)but-2-enyl)malonate (13)

A solution of dibenzylmalonate (2.15 mL, 8.8 mmol) in anhydrous DMF (2 mL) was added dropwise to a suspension of 60% NaH (0.126 g, 5.26 mmol) in the same solvent (6 mL) at 0 °C. After 30 min, compound **12** (2.12 g, 4.38 mmol) dissolved in DMF (2 mL) was added dropwise, and the mixture was stirred 2 h at rt. Water (20 mL) was then added and the mixture was extracted with diethyl ether (3 × 50 mL). The organic phase was washed with water, brine, then dried and evaporated. Flash-chromatography (pentane/ethyl acetate 7:3) afforded the title product (2.17 g, 78%). R_f = 0.44 (pentane/AcOEt 7:3); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.72 (t, 2H, *J* 7.0 Hz, $CH_2CH(CO_2Bn)_2$), 3.70 (t, 1H, *J* 7.0 Hz, $CH(CO_2Bn)_2$), 3.84 (s, 2H, OCH_3), 4.18 (m, 4H, NCH_2, OCH_2CO), 4.56 (s, 2H, CH_2PhOMe), 4.74 (s, 2H, $NOCH_2Ph$), 5.15, 5.16 (2s, 4H, CO_2CH_2Ph), 5.55–5.70 (m, 2H, CH=CH), 6.90 (d, 2H, *J* 9.3 Hz, o-H_{ar}-PhOMe), 7.29–7.40 (m, 17H, *H*-Ph, m-H_{ar}-PhOMe); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 31.4 ($CH_2CH(CO_2Bn)_2$), 41.6 (NCH_2), 51.6 ($CH(CO_2Bn)_2$), 55.3 (OCH_3), 67.2 (OCH_2CO), 67.3 (CO_2CH_2Ph), 72.9 (CH_2PhOMe), 77.0 ($NOCH_2Ph$), 113.8 (o-C_{ar}-PhOMe), 126.3 (NCH_2CH=), 128.8, 129.1, 129.4, 129.8, 134.2 (C-Ph, NCH_2CH=CH, m-C_{ar}-PhOMe), 159.4 (*C*q-Ph), 166.3 (NC=O), 168.5 (CO_2Bn).

4.2.4. (*E*)-Dibenzyl 2-(4-(*N*-(benzyloxy)-2-hydroxyacetamido) but-2-enyl)malonate (14)

DDQ (0.93 g, 4.0 mmol) was added at once to an heterogeneous mixture of **13** (2.16 g, 3.4 mmol) in water/dichloromethane 7:1 (25 mL). The mixture was stirred overnight at rt, then washed with sodium thiosulfate until no color persisted, and brine. After drying and evaporation, the product was purified by flash-chromatography (pentane/ethyl acetate 7:3) to afford the title compound (1.4 g, 81%). R_f 0.56 (pentane/AcOEt 7:3); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.71 (t, 2H, *J* 5.5 Hz, $CH_2CH(CO_2Bn)_2$), 3.56 (t, 1H, *J* 5.6 Hz, $CH(CO_2Bn)_2$), 4.22–4.41 (m, 4H, NCH_2, CH_2OH), 4.77 (s, 2H, $NOCH_2Ph$), 5.15, 5.16 (2s, 4H, CO_2CH_2Ph), 5.57–5.70 (m, 2H, CH=CH), 7.28–7.41 (m, 15H, *H*-Ph); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 31.3 ($CH_2CH(CO_2Bn)_2$), 48.9 (NCH_2), 51.6 ($CH(CO_2Bn)_2$), 60.3 (CH_2OH), 67.2 (CO_2CH_2Ph), 76.8 ($NOCH_2Ph$), 126.6 (NCH_2CH=), 128.8, 129.1, 129.4, 129.8, 135.3 (*C*-Ph, NCH_2CH=*C*H), 166.3 (NC=O), 168.3 (CO_2Bn).

4.2.5. (*E*)-Dibenzyl 2-(4-(*N*-(benzyloxy)-2-dibenzylphosphoaceta mido)but-2-enyl)malonate (15)

A mixture of compound **14** (1.42 g, 2.75 mmol) and dibenzyldiisopropylphosphoramidite (1.9 g, 5.51 mmol) was kept under vacuum until no more bubbles formed. Triazole (0.57 g, 8.25 mmol), imidazole (0.375 g, 5.51 mmol) and anhydrous dichloromethane were added and the mixture was stirred overnight at rt under argon. ^{t}Butylhydroperoxide (aqueous solution, 0.65 mL, 5.51 mmol) was then added, and the mixture was stirred for additional 3 h. Dichloromethane (25 mL) and aqueous thiosulfate (25 mL) were added. The organic phase was washed with saturated sodium hydrogenocarbonate, dried, and evaporated. The crude product was purified by flash-chromatography (pentane/ethyl acetate 6:4) to afford the title compound (1.86 g, 87%). R_f 0.38 (pentane/AcOEt 4:6); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.70 (t, 2H, *J* 5.7 Hz, $CH_2CH(CO_2Bn)_2$), 3.55 (t, 1H, *J* 5.5 Hz, $CH(CO_2Bn)_2$), 4.15 (d, 2H, *J* 4.5 Hz, NCH_2), 4.40 (d, 2H, *J* 9.2 Hz, $NCOCH_2O$), 4.74 (s, 2H, $NOCH_2Ph$), 5.14 (d, 4H, *J* 7.2 Hz, $P(OCH_2Ph)_2$), 5.15, 5.16 (2s, 4H, CO_2CH_2Ph), 5.57–5.70 (m, 2H, CH=CH), 7.28–7.38 (m, 25H, *H*-Ph); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 31.4 ($CH_2CH(CO_2Bn)_2$), 48.6 (NCH_2), 51.6 ($CH(CO_2Bn)_2$), 64.4 (d, J_{PC} 5.4 Hz, $NCOCH_2O$), 67.2 (CO_2CH_2Ph), 69.5 (d, J_{PC} 6.3 Hz $P(OCH_2Ph)_2$), 76.7 ($NOCH_2Ph$), 126.7 (NCH_2CH=), 128.8, 129.1, 129.4, 129.8, 135.3 (*C*-Ph, NCH_2CH=*C*H), 168.4 (CO_2Bn), 169.0 (NC=O).

4.2.6. Sodium 2-carboxy-6-(2-(hydrogenphosphonatooxy)-*N*-hydroxyacetamido)hexanoate (4)

Compound **15** (0.31 g, 0.4 mmol) was dissolved in a 15:1 mixture of ethanol and water, together with sodium hydrogenocarbonate (0.068 g, 0.8 mmol). Pd/C (35 mg) was added and the mixture was vigorously stirred overnight under hydrogen (4 bars). After filtration and evaporation, compound **4** was obtained as a white powder (0.15 g, 100%). ^{1}H NMR (D_2O, 360 MHz) δ 1.25–1.26 (m, 2H, CH_2CH_2CH), 1.60 (m, 2H, NCH_2CH_2), 1.78 (m, 2H, CH_2CH), 3.18 (t, 1H, *J* 5.0 Hz, C*H*), 3.58 (t, 2H, *J* 5.5 Hz, NCH_2), 4.63 (d, 2H, *J* 5.3 Hz, $COCH_2O$); ^{13}C NMR (D_2O, 90.5 MHz) δ 23.8 (CH_2CH_2CH), 25.4 (NCH_2CH_2), 29.2 (CH_2CH), 48.1 (NCH_2), 53.7 (*C*H), 62.2 (d, J_{PC} 3.2 Hz, $COCH_2O$), 170.4 (NC=O), 177.0, 177.1 (*C*OO); HRMS (ESI^-) calcd for $C_9H_{15}NO_{10}P$: 328.0434 $[M-2Na+H]^-$, found *m/z* 328.0420.

4.3. Synthesis of compound 5

4.3.1. Dibenzyl 2-(2-*tert*-butyloxy-2-oxoethyl)malonate (16)

A solution of dibenzylmalonate (4 g, 14.08 mmol) in anhydrous DMF (17.5 mL) was added dropwise to a suspension of 60% NaH (338 mg, 8.45 mmol) in anhydrous DMF (18.7 mL) at 0 °C. After 30 min, a solution of *tert*-butyl-bromoacetate (1 mL, 6.77 mmol) in DMF (6.6 mL) was added dropwise at 0 °C, and the mixture was stirred for 2 h at rt. The reaction was quenched by addition of water (50 mL) and the mixture was extracted with diethyl ether (3 × 100 mL). The organic layers were washed with water, brine, then dried over $MgSO_4$, filtered and concentrated under vacuum to give compound **16** used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.37 (s, 9H, *t*-Bu), 2.85 (d, 2H, *J* 7.5 Hz, CH_2CH), 3.88 (t, 1H, *J* 7.5 Hz, C*H*), 5.13 (s, 4H, 2×CH_2Ph), 7.18–7.46 (m, 10H, CH_{ar}).

4.3.2. Dibenzyl 2-(2-chloro-2-oxoethyl)malonate (17)

A solution of compound **16** (6.77 mmol) in CH_2Cl_2/TFA 1:1 (5.4 mL) was stirred at room temperature for 2 h. The mixture was concentrated under vacuum. Following flash chromatography on silica gel (petroleum ether/AcOEt 10:0 to 4:6), the deprotected carboxylic acid was obtained (1.62 g, 70% from *tert*-butyl bromoacetate). ^{1}H NMR ($CDCl_3$, 360 MHz) δ 3.00 (d, 2H, *J* 7.3 Hz, CH_2CH), 3.91 (t, 1H, *J* 7.3 Hz, C*H*), 5.15 (s, 4H, 2×CH_2Ph), 7.17–7.44 (m, 10H, CH_{ar}). Oxalyl chloride (755 μL, 8.79 mmol) was added dropwise to a solution of this product (1.00 g, 2.92 mmol) in CH_2Cl_2 (2 mL) containing 2 drops of anhydrous DMF. The mixture was stirred for 2 h at room temperature and then was concentrated under vacuum to give compound **17** used in the next step without further purification.

4.3.3. Dibenzyl 2-(2-(benzyloxy(4-hydroxybutyl)amino)-2-oxoethyl)malonate (19)

A solution of compound **17** (2.92 mmol) in CH_2Cl_2 (6.3 mL) was added dropwise to a solution of *N*-(4-hydroxybutyl)-*O*-benzylhydroxylamine **18**[10] (3.65 mmol) and triethylamine (611 μL, 4.39 mmol) in anhydrous methanol (7.9 mL) at 0 °C. The reaction mixture was stirred for 30 min at rt, and then diluted with CH_2Cl_2 (20 mL) and washed with water (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. After purification by flash chromatography on silica gel (petroleum ether/AcOEt 10:0 to 4:6), compound **19** was obtained (1.10 g, 72% from compound **17**). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.43–1.58 (m, 2H, $HOCH_2CH_2$), 1.60–1.77 (m, 2H, CH_2CH_2N), 3.06 (d, 2H, *J* 7.5 Hz, CH_2CH), 3.52–3.73 (m, 4H, $HOCH_2$ and CH_2N), 4.02 (t, 1H, *J* 7.5 Hz, C*H*), 4.81 (s, 2H, $NOCH_2Ph$), 5.10 (d, 2H, *J* 12.5 Hz, 2×CH_2Ph), 5.17 (d, 2H, *J* 12.5 Hz, 2×CH_2Ph), 7.17–7.44 (m, 15H,

CH_{ar}); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 23.3 (CH_2CH_2N), 29.5 ($HOCH_2CH_2CH_2$), 32.0 (CH_2CH), 45.5 (CH_2N), 47.7 (CH_2CH), 62.1 ($HOCH_2CH_2$), 67.4 (2×CO_2CH_2Ph), 76.5 ($NOCH_2Ph$), 128.2–129.2 (15×CH_{ar}), 134.4 (C_{ar}, NOBn), 135.4 (2×C_{ar}, CO_2Bn), 168.7 (2×CO_2Bn), 171.5 (CO); MS (ESI^+) *m/z* 542 (100%) $[M+Na]^+$.

4.3.4. Dibenzyl 2-(2-(benzyloxy(4-dibenzylphosphobutyl)amino) -2-oxoethyl)malonate (20)

1,2,4-Triazole (239 mg, 3.46 mmol) and imidazole (353 mg, 5.19 mmol) were added to a solution of compound **19** (900 mg, 1.73 mmol) and dibenzyl-diisopropylphosphoramidite (1.20 g, 3.46 mmol) in anhydrous CH_2Cl_2 (24 mL). The reaction mixture was stirred overnight at rt under argon. A 70% aqueous solution of *tert*-butylhydroperoxide (494 μL, 3.46 mmol) was then added at 0 °C, and the mixture was stirred another 2 h at rt. A 1 M solution of sodium thiosulfate (25 mL) was added and the aqueous layer was washed with CH_2Cl_2 (3 × 25 mL). The collected organic layers were washed with a saturated solution of $NaHCO_3$ (25 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. After purification by flash chromatography on silica gel (petroleum ether/AcOEt 10:0 to 6:4), compound **20** was obtained (1.19 g, 88%). ^1H NMR ($CDCl_3$, 250 MHz) δ 1.54–1.80 (m, 4H, $POCH_2CH_2CH_2$), 3.09 (d, 2H, *J* 7.5 Hz, CH_2CH), 3.55–3.70 (m, 2H, CH_2N), 3.98 (q, 2H, *J* 6.5 Hz, $POCH_2$), 4.05 (t, 1H, *J* 7.5 Hz, CH), 4.81 (s, 2H, $NOCH_2Ph$), 5.03 (d, 2H, *J* 7.5 Hz, $POCH_2Ph$), 5.06 (d, 2H, *J* 7.5 Hz, $POCH_2Ph$), 5.13 (s, 2H, $COOCH_2Ph$), 5.21 (s, 2H, $COOCH_2Ph$), 7.19–7.53 (m, 25H, CH_{ar}); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 23.0 (CH_2CH_2N), 27.4 (d, J_{PC} 6.5 Hz, $POCH_2CH_2CH_2$), 32.0 (CH_2CH), 45.2 (CH_2N), 47.7 (CH_2CH), 67.4 (d, J_{PC} 6.0 Hz, $POCH_2CH_2$), 67.4 (2×CO_2CH_2Ph), 69.3 (d, *J* 5.5 Hz, 2×$POCH_2Ph$), 76.6 ($NOCH_2Ph$), 128.0–129.4 (25×CH_{ar}), 134.4 (C_{ar}, NOBn), 135.4 (2×C_{ar}, CO_2Bn), 136.0 (2×C_{ar}, POBn), 171.5 (CO), 179.2 (2×CO_2Bn); ^{31}P NMR ($CDCl_3$, 101.2 MHz) δ −0.91; MS (ESI^+) *m/z* 802 (22%) $[M+Na]^+$.

4.3.5. Sodium 2-(2-(hydroxy(4-(hydrogenphosphonatooxy)butyl) amino)-2-oxoethyl)malonate (5)

To a solution of compound **20** (156 mg, 0.20 mmol) and sodium hydrogenocarbonate (34 mg, 0.40 mmol) in a 5:1 mixture of THF and H_2O (15 mL) 10% Pd/C (35 mg) was added. After to be purged first with nitrogen and then with hydrogen, the reaction mixture was stirred vigorously at room temperature for 5 h under hydrogen. The mixture was filtered through Celite, and the filtrate was concentrated under vacuum to give compound **5** (75 mg, 100%). ^1H NMR (D_2O, 360 MHz): δ 1.56–1.67 (m, 2H, $POCH_2CH_2CH_2$), 1.67–1.79 (m, 2H, $CH_2CH_2CH_2N$), 2.97 (d, 2H, *J* 7.0 Hz, CH_2CH), 3.51 (t, 1H, *J* 7.5 Hz, CH), 3.68 (t, 2H, *J* 6.5 Hz, CH_2N), 3.80 (m, $POCH_2$); ^{13}C NMR (D_2O, 90.5 MHz): δ 22.6 (CH_2CH_2N), 27.5 (d, J_{PC} 7.0 Hz, $POCH_2CH_2CH_2$), 33.0 (CH_2CH), 47.9 (CH_2N), 54.3 (CH_2CH), 64.0 (d, J_{PC} 4.5 Hz, $POCH_2CH_2$), 173.6 (CO), 178.5 (2×CO_2H); ^{31}P NMR (D_2O, 101.2 MHz): δ 3.76; HRMS (ESI^-) calcd for $C_9H_{15}NO_{10}P$: 328.0434 $[M-2Na+H]^-$, found *m/z* 328.0306.

4.4. Synthesis of compound 7

4.4.1. 4-(*N*,2-Dihydroxyacetamido)butyl dihydrogen phosphate (7)

A reaction mixture containing compound **21**[10] (103 mg, 0.20 mmol), sodium hydrogenocarbonate (34 mg, 0.40 mmol) in THF/H_2O 5:1 (15 mL), and 10% Pd/C (35 mg) was stirred vigorously at rt for 2 h under hydrogen. The mixture was filtered through Celite, and the filtrate was concentrated under vacuum to give compound **7** (49 mg, 100%). ^1H NMR (D_2O, 250 MHz) δ 1.45–1.76 (m, 4H, $CH_2CH_2CH_2N$), 3.58 (t, 2H, *J* 6.5 Hz, CH_2N), 3.69 (q, 2H, *J* 6.5 Hz, $POCH_2$), 4.32 (s, 2H, CH_2OH); ^{13}C NMR (D_2O, 90.5 MHz) δ 22.4 (CH_2CH_2N), 27.5 (d, J_{PC} 7.5 Hz, $POCH_2CH_2CH_2$), 48.5 (CH_2N), 59.3 (CH_2OH), 64.5 (d, J_{PC} 4.5 Hz, $POCH_2CH_2$), 171.8 (CO); ^{31}P NMR (D_2O, 101.2 MHz) δ 3.65; HRMS (ESI^-) calcd for $C_6H_{13}NO_7P$: 242.0430 $[M-H]^-$, found *m/z* 242.0439.

4.5. Synthesis of compound 8

4.5.1. Preparation of benzyl 2,3-di-*O*-benzyl-5-*O*-trityl-D-arabinof uranoside (23α/23β[35]

To a solution of trityl arabinose **22**[34,35] (5 g, 12.74 mmol) in benzyl bromide (15 mL) was added crushed KOH (2.5 g). After addition of tetrabutylammonium bromide (250 mg) as catalyst, the mixture was stirred overnight at rt. Diethyl ether was added (50 mL) and the solution was decanted, washed with water (2 × 20 mL), dried over Na_2SO_4, and evaporated. The residue was purified by chromatography with petroleum ether/ethyl acetate (9:1) as eluent to give **23α** as a colorless oil (3.29 g) and **23β** as a colorless oil (1.35 g) (overall yield = 55%). **23α**: R_f 0.53 (petroleum ether/ethyl acetate: 9:1); $[\alpha]_{589}$ +32.47° (*c* 0.85, dichloromethane); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 64.0 (C-5α), 69.0 (C-1 α –OCH_2), 72.1, 72.2 (C-2 α –OCH_2, C-3 α –OCH_2), 83.3 (C-2α), 84.1 (C-3α), 86.9 (C-4α), 88.5 (CPh_3), 105.3 (C-1α), 129.0–127.1 (CH_{ar} Ph), 137.8, 138.2 (Cq_{ar} Bn), 144.2 (Cq_{ar} CPh_3); MS (ESI^+) *m/z* 685.52 (100%) $[M+Na]^+$, 701.47 (10%) $[M+K]^+$; **23β**: R_f 0.36 (petroleum ether/ethyl acetate: 9:1); $[\alpha]_{589}$ −37.40° (*c* 1, dichloromethane); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 66.2 (C-5β), 68.9 (C-1 β –OCH_2), 72.5, 72.6 (C-2 β –OCH_2, C-3 β –OCH_2), 77.4 (C-2β), 80.7 (C-3β), 83.1 (C-4β), 84.4 (CPh_3), 99.0 (C-1β), 127.2–129.4 (CH_{ar} Ph), 137.8, 138.1 (Cq_{ar} Bn), 144.1 (Cq_{ar} CPh_3); MS (ESI^+) *m/z* 685.52 (100%) $[M+Na]^+$, 701.47 (10%) $[M+K]^+$.

4.5.2. Preparation of benzyl 2,3-di-*O*-benzyl-α-D-arabinofuranosi de (24)[35]

Compound **23α** (3.35 g, 5.05 mmol) was dissolved in a mixture of acetic acid (44 mL) and water (11 mL). The reaction was heated to 65 °C. After 3 h, TLC (petroleum ether/ethyl acetate: 1:1) indicated completed consumption of starting material. The solvent was removed in vacuo (azeotrope 3× with toluene) and the crude product was purified by wet-flash chromatography (the column was initially eluted with petroleum ether/ethyl acetate (9:1) to wash out TrOH and the product was obtained on elution with petroleum ether/ethyl acetate (1:1) to afford **24** as a colorless oil (2.1 g, 99%). R_f 0.27 (petroleum ether/ethyl acetate: 1:1); $[\alpha]_{589}$ +41.6° (*c* 1, dichloromethane); ^1H NMR ($CDCl_3$, 360 MHz) δ 3.65 (dd, 1H, *H*-3, $J_{5'5}$ 12.3, $J_{5'4}$ 4.1 Hz), 3.83 (dd, 1H, *H*-5, $J_{55'}$ 12.3, J_{54} 2.7 Hz), 4.00 (dd, 1H, *H*-3, J_{34} 6.8, J_{32} 2.7 Hz), 4.10 (dd, 1H, *H*-2, J_{23} 2.7, J_{21} 0.9 Hz), 4.38–4.40 (m, 1H, *H*-4, J_{43} 6.8, $J_{45'}$ 4.1, J_{45} 2.7 Hz), 4.45 (d, 1H, C-3,2-OCH_AH_B, J_{BA} 12.3 Hz), 4.49 (d, 1H, C-3,2-OCH_AH_B, J_{AB} 12.3 Hz), 4.50 (d, 1H, C-2,3-OCH_AH_B, J_{BA} 12.3 Hz), 4.51 (d, 1H, C-2,3-OCH_AH_B, J_{AB} 12.3 Hz), 4.59 (d, 1H, C-1-OCH_AH_B, J_{BA} 12.3 Hz), 4.77 (d, 1H, C-1-OCH_AH_B, J_{AB} 12.3 Hz), 5.10 (s, 1H, *H*-1), 7.26–7.36 (m, 15H, Ph); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 65.2 (C-5), 69.0 (C-1-CH_2), 72.1, 72.4 (C-2-CH_2, C-3-CH_2), 82.4 (C-4), 83.0 (C-3), 88.2 (C-2), 105.5 (C-1), 127.1, 127.8, 127.9, 128.1, 128.6, 128.7 (CH_{ar}), 137.5, 137.9, 138.0 (Cq_{ar}); MS (ESI^+) *m/z* 443.33 (100%) $[M+Na]^+$.

4.5.3. Benzyl 2,3-di-*O*-benzyl-5-deoxy-5-ditertiobutylmalonate-α-D-arabinofuranoside (25)

Compound **24** (600 mg, 1.43 mmol) was dissolved in dry CH_2Cl_2 (10 mL) under argon. Diisopropylethylamine (0.61 mL, 3.71 mmol) was added and the solution was stirred and cooled to −40 °C. Following dropwise addition of trifluoromethane-sulfonic anhydride (0.36 mL, 2.15 mmol), the reaction mixture was stirred for 3 h. Thereafter, the solution was allowed to warm to rt and ethyl acetate (5 mL) was added. The resulting solution was washed with satd NH_4Cl (5 mL), then neutralized with satd $NaHCO_3$ (5 mL). The organic phase was dried and concentrated in vacuo to afford benzyl 2,3-di-*O*-benzyl-5-trifluoromethane-sulfonyl-α-D-arabinofuranoside as an

intense red oil which was not further purified and used immediately for the next reaction: R_f 0.89 (petroleum ether/ethyl acetate: 8:2). In a two-neck round bottom flask placed under argon and containing dry THF (100 mL) was dissolved ditertiobutyl malonate (0.64 mg, 2.86 mmol). NaH (60% in oil, 74 mg) was then slowly added to the solution under vigorous stirring. The reaction mixture was further stirred at rt for 30 min. A solution of benzyl 2,3-di-*O*-benzyl-5-*O*-trifluoromethanesulfonyl-α-D-arabinofuranoside previously synthesized in dry THF (25 mL) was then added dropwise to the sodium ditertiobutyl malonate THF solution. The reaction mixture was stirred under reflux for 1 h, then cooled to rt, and neutralized by addition of methanol (0.5 mL) and satd NH_4Cl (10 mL). The aq phase was extracted with diethyl ether (3 × 10 mL). The combined organic phases were washed with satd NaCl (10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by wet-flash chromatography (petroleum ether/ethyl acetate: 95:5) to give **25** as colorless oil (500 mg, 85% for two steps). R_f 0.81 (petroleum ether/ethyl acetate: 8:2); $[\alpha]_{589}$ +51.6° (*c* 0.9, dichloromethane); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.38 (s, 18H, CH_3), 3.38 (dd, 1H, *H*-6, J_{65} 8.6, $J_{65'}$ 6.4 Hz), 3.48 (ddd, 1H, *H*-5′, $J_{5'5}$ 12.3, $J_{5'4}$ 8.6, $J_{5'6}$ 6.4 Hz), 3.68 (dd, 1H, *H*-3, J_{32} 3.6, J_{34} 0.9 Hz), 3.78 (ddd, 1H, *H*-5, $J_{55'}$ 12.3, J_{56} 8.6, J_{54} 4.1 Hz), 4.13 (dd, 1H, *H*-2, J_{23} 3.6, J_{21} 0.9 Hz), 4.15–4.23 (m, 1H, *H*-4), 4.51 (d, 1H, C-3,2-OCH$_A$*H*$_B$, J_{BA} 12.3 Hz), 4.52 (d, 1H, C-3,2-OC*H*$_A$H$_B$, J_{AB} 12.3 Hz), 4.58 (d, 1H, C-2,3-OCH$_A$*H*$_B$, J_{BA} 12.3 Hz), 4.60 (d, 1H, C-2,3-OC*H*$_A$H$_B$, J_{AB} 12.3 Hz), 4.65 (d, 1H, C-1-OCH$_A$*H*$_B$, J_{AB} 12.3 Hz), 4.82 (d, 1H, C-1-OC*H*$_A$H$_B$, J_{AB} 12.3 Hz), 5.10 (s, 1H, *H*-1), 7.35–7.43 (m, 15H, Ph); ^{13}C NMR ($CDCl_3$, 90.5 MHz) δ 28.1 (*C*H$_3$), 32.5 (*C*-5), 51.4 (*C*-6), 68.7 (C-1-OCH$_2$), 72.3 (C-2-OCH$_2$, C-3-OCH$_2$), 78.7 (*C*-4), 81.5, 81.6 (*C*Me$_3$), 87.8 (*C*-3), 89.0 (*C*-2), 104.7 (*C*-1), 127.8, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6 (*C*H$_{ar}$), 137.7, 137.9, 138.1 (*C*q$_{ar}$), 168.6, 168.7 (*C*=O); MS (ESI$^+$) *m/z* 641.42 (100%) [M+Na]$^+$; HRMS (ESI$^+$) calcd for $C_{37}H_{46}O_8$: 641.3090 [M+Na]$^+$, found *m/z* 641.3095.

4.5.4. 5-Deoxy-5-ditertiobutylmalonate-D-arabinofuranose (26)

Compound **25** (500 mg, 0.8 mmol) dissolved in ethyl acetate and $Pd(OH)_2$/C (92 mg) were stirred under H_2 for 3 days. The mixture was then filtered and concentrated to give **26** (α/β = 6:4) as a colorless oil (360 mg, 100%). ^{1}H NMR (CD_3OD, 360 MHz) δ 1.49 (s, 18H, CH_3), 2.10–2.20 (m, 1H, *H*-5′), 2.22–2.29 (m, 1H, *H*-5), 3.46–3.52 (m, 0.6H, *H*-6α), 3.67–3.74 (m, 0.4H, *H*-6β), 3.79 (d, 0.4H, *H*-2β, $J_{2\beta3\beta}$ 2.3 Hz), 3.84 (dd, 0.6H, *H*-2α, $J_{2\alpha3\alpha}$ 4.8, $J_{2\alpha1\alpha}$ 4.5 Hz), 3.94–4.07 (m, 2H, *H*-4, *H*-3), 5.19 (d, 0.6H, *H*-1α, $J_{1\alpha2\alpha}$ 2.7 Hz), 5.25 (d, 0.4H, *H*-1β, $J_{1\beta2\beta}$ 4.5 Hz); ^{13}C NMR (CD_3OD, 90.5 MHz) δ 33.8, 35.6 (*C*-5α, *C*-5β), 45.1 (*C*H$_3$), 52.2 (*C*-6), 78.9, 80.5, 80.7, 81.6, 82.4, 84.4 (*C*-2, *C*-3, *C*-4*C*Me$_3$), 97.3 (*C*-1β), 103.4 (*C*-1α), 170.1, 170.4 (*C*=O); ν_{max} 3442 (br s, OH), 2979, 2934 (C$_{sp3}$-H), 1727 (C=O ester) cm^{-1}.

4.5.5. 5-Deoxy-5-ditertiobutylmalonate-D-arabinono-1,4-lactone (27)

Compound **26** (100 mg, 0.3 mmol) was dissolved in water (1 mL) and 1,4-dioxane (2 mL). $BaCO_3$ (178 mg, 0.9 mmol) was added and the solution was stirred and cooled to 0 °C. Following dropwise addition of bromine (0.05 mL, 0.9 mmol), the reaction mixture was stirred for 2 days at rt. The solution was neutralized with 1 M sodium thiosulfate (1 mL). The mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with satd aq NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified on preparative plate (petroleum ether/ethyl acetate: 95:5) and lyophilized to afford the lactone **27** as a colorless oil (62 mg, 60%). $[\alpha]_{589}$ +28.5° (*c* 1, water); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 18H, CH_3), 2.15 (ddd, 1H, *H*-5′, $J_{5'5}$ 12.3, $J_{5'6}$ 8.5, $J_{5'4}$ 6.0 Hz), 2.44 (ddd, 1H, *H*-5, $J_{55'}$ 12.3, J_{52} 8.5, J_{54} 3.8 Hz), 3.45 (dd, 1H, *H*-6, J_{65} 8.5, $J_{65'}$ 6.0 Hz), 4.11 (dd, 1H, *H*-3, J_{34} 8.5, J_{32} 8.5 Hz), 4.22 (td, 1H, *H*-4, J_{43} 8.5, J_{45} 3.8 Hz), 4.51 (d, 1H, *H*-2, J_{23} 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.1 (*C*H$_3$), 31.7 (*C*-5), 50.5 (*C*-6), 74.8 (*C*-2), 78.1 (*C*-3), 78.7 (*C*-4), 82.6, 82.7 (*C*Me$_3$), 168.5, 168.6 (*C*O*O*^{t}Bu), 174.2 (*C*-1); ν_{max} 3364 (br s, OH), 2980, 2934 (C$_{sp3}$-H), 1799 (C=O lactone), 1725 (C=O ester) cm^{-1}; MS (ESI$^+$) *m/z* 369.31 (100%) [M+Na]$^+$; HRMS (ESI$^+$) calcd for $C_{16}H_{26}O_8Na$: 369.1525 [M+Na]$^+$, found *m/z* 369.1513.

4.5.6. 5-Deoxy-5-dihydrogenomalonate-D-arabinono-1,4-lactone (28)

Compound **27** (90 mg, 0.26 mmol) was dissolved in dry dichloromethane (1.5 mL) under argon. The solution was cooled to 0 °C and TFA was added (1.5 mL). The reaction mixture was stirred at 0 °C for 35 min then 2.5 h at rt. The solution was concentrated in vacuo (azeotrope 3 × 20 mL with toluene) at rt and lyophilized to afford the title compound **28** as a colorless oil (50 mg, 82%). ^{1}H NMR (D_2O, 360 MHz) δ 2.30 (dd, 1H, *H*-5′, $J_{5'5}$ 14.5, $J_{5'4}$ 9.1 Hz), 2.52 (dd, 1H, *H*-5, $J_{55'}$ 14.5, J_{54} 2.7 Hz), 3.61–3.80 (m, 1H, *H*-6), 4.16 (dd, 1H, *H*-3, J_{32} 9.1, J_{34} 9.1 Hz), 4.37 (ddd, 1H, *H*-4, J_{43} 9.1, $J_{45'}$ 9.1, J_{45} 2.7 Hz), 4.58 (d, 1H, *H*-2, J_{23} 9.1 Hz); ^{13}C NMR (D_2O, 90.5 MHz) δ 31.0 (*C*-5), 73.7 (*C*-2), 77.0 (*C*-4), 78.9 (*C*-3), 172.8, 173.1 (*C*OOH), 175.9 (*C*-1); MS (ESI$^-$) *m/z* 233.05 (100%) [M−H]$^-$; HRMS (ESI$^-$) calcd for $C_8H_9O_8$: 233.0297 [M−H]$^-$, found *m/z* 233.0293.

4.5.7. 5-Deoxy-5-hydrogenomalonate-D-arabinonohydroxamic acid, hydroxylammonium salt (8)

Compound **28** (50 mg, 0.21 mmol) was dissolved in a 50% aq NH_2OH solution (1.5 mL, 20.3 mmol). The reaction mixture was stirred at rt for 25 min, concentrated under high vacuum at rt and lyophilized to afford the title compound **8** as a white solid (70 mg, 100%); $[\alpha]_{589}$ +17.6° (*c* 0.9, water); ^{1}H NMR (D_2O, 400 MHz) δ 1.51 (dd, 1H, *H*-5′, $J_{5'5}$ 14.1, $J_{5'4}$ 9.8 Hz), 1.98 (d, 1H, *H*-5, $J_{55'}$ 14.1 Hz), 3.37 (dd, 1H, *H*-4, $J_{45'}$ 9.8, J_{43} 8.2 Hz), 3.45 (d, 1H, *H*-3, J_{34} 8.2 Hz), 4.23 (s, 1H, *H*-2); ^{13}C NMR (D_2O, 100.6 MHz) δ 33.7 (*C*-5), 54.9 (*C*-6), 68.9 (*C*-3), 70.2 (*C*-2), 74.3 (*C*-4), 171.1 (*C*-1), 179.1, 179.4 (*C*OO$^-$); ν_{max} 3087 (br s, OH), 2290 (C$_{sp3}$-H), 1636 (C=O), 1553 (COO$^-$) cm^{-1}; HRMS (ESI$^-$) calcd for $C_8H_{12}NO_9$: 266.0512 [M−NH$_3$OH]$^-$, found *m/z* 266.0515.

Acknowledgments

We are very grateful to Professor Jurgen Sygusch (Université de Montréal, Canada) for the kind supply of *H. pylori* FBA, to Dr. Mary Jackson (Colorado State University, U.S.A.) for the kind supply of *M. tuberculosis* and *Y. pestis* FBAs, and to Dr. Jean-Michel Bruneau (Novexel, Romainville, France) for the kind supply of *C. albicans* FBA. The 'Région Ile-de-France' (SD and RD) and the 'Ministère de l'Enseignement Supérieur et de la Recherche' (SCL) are gratefully acknowledged for financial support, as well as Delphine Arquier (ICMMO) for mass spectrometry data. Our laboratory is a member of the Laboratory of Excellence LERMIT supported by a grant from ANR (ANR-10-LABX-33).

Supplementary data

Supplementary data (NMR spectra of new compounds evaluated on enzymes) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.050.

References and notes

1. Clem, B.; Telang, S.; Clem, A.; Yalcin, A.; Meier, J.; Simmons, A.; Rasku, M. A.; Arumugam, S.; Dean, W. L.; Eaton, J.; Lane, A.; Trent, J. O.; Chesney, J. *Mol. Cancer Ther.* **2008**, *7*, 110.
2. Haga, A.; Tanaka, N.; Funasaka, T.; Hashimoto, K.; Nakamura, K. T.; Watanabe, H.; Raz, A.; Nagase, H. *J. Mol. Biol.* **2006**, *358*, 741.
3. Smith, D. J.; Proudfoot, A. E. I.; De Tiani, M.; Wells, T. N. C.; Payton, M. A. *Yeast* **1995**, *11*, 301.
4. Kotsikorou, E.; Sahota, G.; Oldfield, E. *J. Med. Chem.* **2006**, *49*, 6692.
5. Cáceres, A. J.; Michels, P. A. M.; Hannaert, V. *Mol. Biochem. Parasitol.* **2010**, *169*, 50.

6. Azéma, L.; Lherbet, C.; Baudoin, C.; Blonski, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3440.
7. Buse, M. G. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *290*, E1.
8. Fonvielle, M.; Coinçon, M.; Daher, R.; Desbenoit, N.; Kosieradzka, K.; Barilone, N.; Gicquel, B.; Sygusch, J.; Jackson, M.; Therisod, M. *Chem. Eur. J.* **2008**, *14*, 8521.
9. Daher, R.; Therisod, M. *ACS Med. Chem. Lett.* **2010**, *1*, 101.
10. Daher, R.; Coinçon, M.; Fonvielle, M.; Gest, P. M.; Guerin, M. E.; Jackson, M.; Sygusch, J.; Therisod, M. *J. Med. Chem.* **2010**, *53*, 7836.
11. Hardré, R.; Bonnette, C.; Salmon, L.; Gaudemer, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3435.
12. Hardré, R.; Salmon, L. *Carbohydr. Res.* **1999**, *318*, 110.
13. Arsenieva, D.; Hardré, R.; Salmon, L.; Jeffery, C. J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5872.
14. Roux, C.; Lee, J. H.; Jeffery, C. J.; Salmon, L. *Biochemistry* **2004**, *43*, 2926.
15. Roux, C.; Gresh, N.; Perera, L. E.; Piquemal, J.-P.; Salmon, L. *J. Comput. Chem.* **2007**, *28*, 938.
16. Roux, C.; Bhatt, F.; Foret, J.; de Courcy, B.; Gresh, N.; Piquemal, J.-P.; Jeffery, C. J.; Salmon, L. *Proteins* **2011**, *79*, 203.
17. Liotta, L. A.; Mandler, R.; Murano, G.; Katz, D. A.; Gordon, R. K.; Chiang, P. K.; Schiffmann, E. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 3302.
18. Watanabe, H.; Takehana, K.; Date, M.; Shinozaki, T.; Raz, A. *Cancer Res.* **1996**, *56*, 2960.
19. Berkowitz, D. B.; Maiti, G.; Charette, B. D.; Dreis, C. D.; MacDonald, R. G. *Org. Lett.* **2004**, *6*, 4921.
20. Chen, H.; Luzy, J.-P.; Gresh, N.; Garbay, C. *Eur. J. Org. Chem.* **2006**, 2329.
21. Gao, Y.; Luo, J.; Yao, Z.-J.; Guo, R.; Zou, H.; Kelley, J.; Voigt, J. H.; Yang, D.; Burke, T. R. *J. Med. Chem.* **2000**, *43*, 911.
22. Dourlat, J.; Valentin, B.; Liu, W.-Q.; Garbay, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3943.
23. Miller, M. J.; Cleary, D. G.; Ream, J. E.; Snyder, K. R.; Sikorski, J. A. *Bioorg. Med. Chem.* **1995**, *3*, 1685.
24. 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.
25. Clavel, C.; Barragan-Montero, V.; Montero, J.-L. *Tetrahedron Lett.* **2004**, *45*, 7465.
26. Gurgui-Ionescu, C.; Toupet, L.; Uttaro, L.; Fruchier, A.; Barragan-Montero, V. *Tetrahedron* **2007**, *63*, 9345.
27. Jeanjean, A.; Garcia, M.; Leydet, A.; Montero, J.-L.; Morère, A. *Bioorg. Med. Chem.* **2006**, *14*, 3575.
28. Miller, M. J.; Anderson, K. S.; Braccolino, D. S.; Cleary, D. G.; Gruys, K. J.; Han, C. Y.; Lin, K.-C.; Pansegrau, P. D.; Ream, J. E.; Douglas Sammons, R.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1435.
29. Foret, J.; de Courcy, B.; Gresh, N.; Piquemal, J.-P.; Salmon, L. *Bioorg. Med. Chem.* **2009**, *17*, 7100.
30. Piers, E.; Lemieux, R. *Organometallics* **1995**, *14*, 5011.
31. Lal, B.; Gangopadhyay, A. K.; Rajagopalan, R.; Vasantrao Ghate, A. *Bioorg. Med. Chem.* **1998**, *6*, 2061.
32. Kobashi, K.; Kumashi, K.; Hase, J. *Biochim. Biophys. Acta* **1971**, *227*, 429.
33. Salmon, L.; Prost, E.; Merienne, C.; Hardré, R.; Morgant, G. *Carbohydr. Res.* **2001**, *335*, 195.
34. Izumi, M.; Tsuruta, O.; Hashimoto, H. *Carbohydr. Res.* **1996**, *280*, 287.
35. Winzar, R.; Philips, J.; Kiefel, M. *Synlett* **2010**, 583.
36. Cabaret, D.; Wakselman, M. *Can. J. Chem.* **1990**, *68*, 2253.
37. de la Paz Santangelo, M.; Gest, P. M.; Guerin, M. E.; Coinçon, M.; Pham, H.; Ryan, G.; Puckett, S. E.; Spencer, J. S.; Gonzalez-Juarrero, M.; Daher, R.; Lenaerts, A. J.; Schnappinger, D.; Therisod, M.; Ehrt, S.; Sygusch, J.; Jackson, M. *J. Biol. Chem.* **2011**, *286*, 40219.
38. Foret, J. Ph.D Thesis, Université Paris-Sud (Orsay, France), **2009**.