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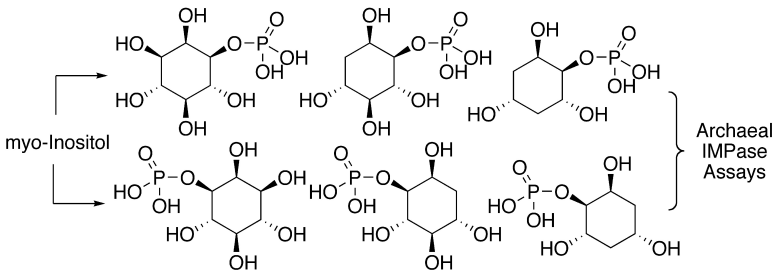

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Chemistry and Biology of Deoxy-*myo*-inositol Phosphates: Stereospecificity of Substrate Interactions within an Archaeal and a Bacterial IMPase

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The chemistry and biology of the inositol phosphates has emerged as a complex field due in part to the ubiquity of inositol phosphate-dependent cellular signaling[1] and also the challenge of site-selective organic synthesis. A significant opportunity for study of high-resolution ligand−receptor interactions between the inositol phosphates and their targets would be enhanced by highly efficient access to remodeled small-molecule analogues, including those with modified and even deleted key hydroxyls.

There are a plethora of inositol phosphatases with differing specificities. While crystal structures exist of several of these enzymes, in some cases even with ligands bound, it is not always clear what is responsible for substrate specificity. Among the best studied cases is the mammalian IMPase where structures along with select deoxy-inositol phosphate analogues have been used to test the role of key hydroxy groups in substrate binding.[2a−e] However, there is a series of proteins in the Mg^{2+}/Mn^{2+}-dependent inositol monophosphatase (IMPase) family with different functions as well as specificities. The IMPase enzymes from hyperthermophilic archaea also exhibit fructose 1,6-bisphosphatase activity.[3] Understanding how different ligands sit in the active site and how interactions with the protein contribute to overall specificity can be difficult. Figure 1 shows the active site of the IMPase/FBPase from *Archaeoglobus fulgidus* with D-I-1P bound in the presence of two Ca^{2+} ions that inhibit enzyme activity.[3b] The dotted lines indicate a hydrogen-bonding network, in particular, a specific interaction between the inositol hydroxyl group at C3 and the protein. In contrast, the C5−OH does not interact directly with any protein residues. (For comparison, neither the C3−OH nor the C5−OH appears to aid in substrate binding to the mammalian IMPase.)[2] If the inositol hydroxyl/protein interactions contribute critically to substrate binding by the archaeal IMPase leading to formation of the Michaelis complex, or to the efficiency of catalysis, then their removal should reduce k_{cat} or increase the K_m for the deoxy-substrate. In the crystal structure, there is also the problem that the inhibitory Ca^{2+} may alter the substrate interacting with the enzyme. Kinetic studies that explore the interactions of select inositol hydroxyls with their protein targets require efficient syntheses of analogues of defined stereochemistry, with and without key hydroxyl groups present.

While a select few examples of deoxygenated analogues of enantiomerically pure *myo*-inositol monophosphates have been prepared,[2] existing methods require lengthy schemes that rely on multiple protecting groups, resolutions, and/or extensive remodeling of chiral pool intermediates. As a first step toward improved access, we recently reported the syntheses of D-*myo*-inositol-1-phosphate (D-I-1P, **1**), and its enantiomer D-*myo*-inositol-3-phosphate (D-I-3P = L-I-1P, **2**), employing peptide-catalyzed asymmetric phosphorylations.[4] Notably, we discovered peptides **3** and **4** that exhibited enantiodivergent catalysis (Scheme 1).

Using the asymmetric phosphorylation chemistry as a conduit to stereoselective synthesis of the deoxygenated versions of I-1P analogues required a delineation of the relative reactivity of the 3-

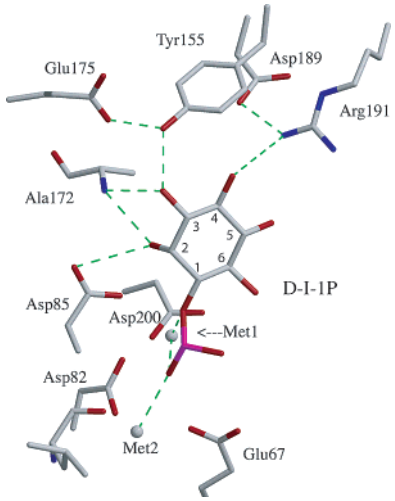
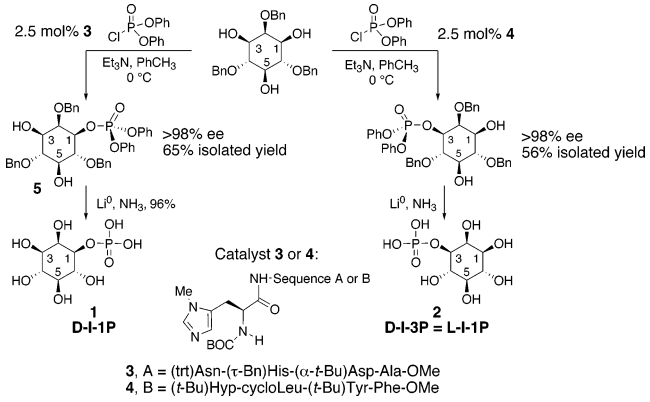
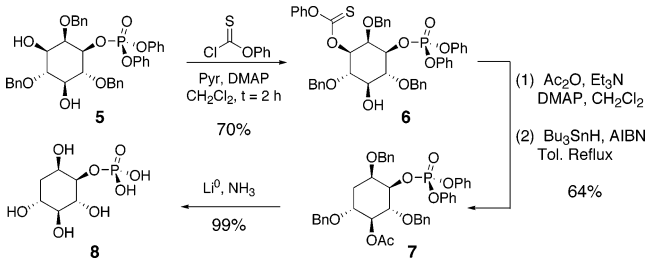


Figure 1. Interactions of bound D-I-1P with *A. fulgidus* IMPase side chains.

Scheme 1



Scheme 2



and 5-positions of intermediate **5**. Consistent with earlier reports,[5] we found that selective monothiocarbonylation delivered monophosphate **6** (70% yield, Scheme 2). Acetate protection of the 5-hydroxyl followed by standard free radical deoxygenation afforded **7** (64% yield). Global deprotection under dissolving metal conditions then yielded 3-deoxy-D-*myo*-inositol-1-phosphate **8** in

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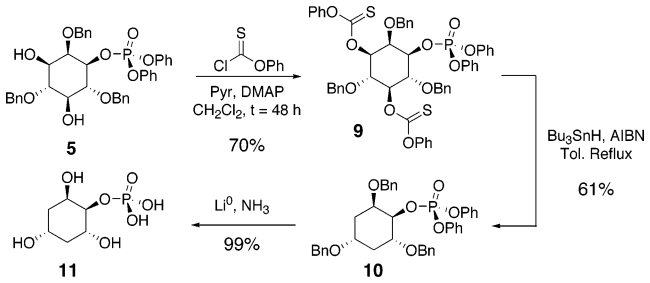
Scheme 3



stereochemically pure form (99% yield). With enantiodivergent catalysts **3** and **4** in hand for the asymmetric phosphorylation, we also carried out the identical sequence in the D-*myo*-inositol-3-phosphate series to give *ent*-**8** (1-deoxy-D-*myo*-inositol-3-phosphate[6]).

The preparation of the 3,5-dideoxy-compounds **11** and *ent*-**11** followed an analogous plan (Scheme 3). In this case, both the 3- and 5-positions may be subjected to thiocarbonylation for prolonged reaction time (due to the lower reactivity of the 5-hydroxyl group. Thus, bis(thiocarbonate)-**9** could be obtained in 70% yield. Simultaneous radical deoxygenation, followed by dissolving metal reduction to cleave the protecting groups, affords **11** (61% yield).

The D-I-1P series (**1**, **8**, **11**) and the L-I-1P series (D-I-3P series: *ent*-**1**, *ent*-**8**, *ent*-**11**) with 3-deoxy and 3,5-dideoxy alterations were examined as substrates for *A. fulgidus* IMPase (assayed at 85 °C) and for *E. coli* SuhB (assayed at 37 °C). The latter enzyme has many kinetics characteristics similar to those of eukaryotic IMPase enzymes.[7] The *A. fulgidus* IMPase crystal structure with D-I-1P bound shows hydrogen-bond interactions between the inositol C3-hydroxyl and Tyr155 (polarized by Glu175) and the amide nitrogen of Ala172. Consistent with this, the K_m for **8** is increased compared to **1**. The $\Delta\Delta G$ extracted from this increase in K_m reflects a change of only ~2.2 kJ/mol. Removal of the C-5 hydroxyl group did not significantly increase K_m further. More interesting was the very pronounced increase in K_m for all the D-I-3P or (L-series) substrates for the archaeal IMPase (Table 1). The averaged $\Delta\Delta G$ reflected in the increased K_m of *A. fulgidus* IMPase for a specific L- versus D-substrate was 8.4 ± 1.0 kJ/mol. A comparison of K_m within the L-I-1P (D-I-3P) series, showed much smaller increases: the difference in K_m for *ent*-**8** versus *ent*-**1** reflects only about 1 kJ/mol, while removing both 3- and 5-hydroxyl groups (*ent*-**11**) in this series costs about 3 kJ/mol for formation of the Michaelis complex. The results suggest that when L-I-1P is bound in the active site, the C5-hydroxyl group is likely to interact with the protein, while the C3-hydroxyl group has a considerably weaker interaction. More critically, the large difference in K_m between the L- and D- series is equivalent to removing a hydrogen-bonding interaction of the L-I-1P ligand with the protein. [For comparison, the mammalian IMPase binds D- and L-I-1P somewhat differently (60° change in orientation), but without significantly affecting kinetic parameters.[2c]] Removal of hydroxyl groups also affected the k_{cat} of the *A. fulgidus* IMPase differently for the D-I-1P versus L-I-1P series. Removal of the two hydroxyl groups decreased k_{cat} with the D-I-1P series but increased k_{cat} for the L-I-1P series.

The results for SuhB provide an intriguing contrast. The D-I-1P series showed no significant change in K_m as hydroxyl groups were removed from the substrate. Furthermore, the K_m values for the L-I-1P series were very similar to those for the D-I-1P series, although a small (but statistically significant) increase was observed with *ent*-**8**; this was reduced when the C5-hydroxyl was subsequently removed. The only significant difference in k_{cat} occurred for *E. coli* SuhB hydrolysis of L-I-1P (*ent*-**1**). Removal of the hydroxyl at C3 of this isomer enhanced k_{cat} 3- to 4-fold (Table 1).

Table 1. Kinetic Parameters Extracted for IMPase Enzymes

	A. fulgidus IMPase (85 °C)		*E. coli* SuhB (37 °C)	
substrate[a]	K_m (mM)	k_{cat} (s^{-1})	K_m (mM)	k_{cat}(s^{-1})
D-I-1P (**1**)	0.11 ± 0.02	3.3 ± 0.2	0.068 ± 0.01	4.4 ± 0.3
3-deoxy (**8**)	0.23 ± 0.05	3.9 ± 0.3	0.047 ± 0.005	4.4 ± 0.1
3,5-dideoxy (**11**)	0.16 ± 0.01	2.5 ± 0.1	0.051 ± 0.008	4.0 ± 0.2
L-I-1P (D-I-3P, *ent*-**1**)	1.7 ± 0.4	3.6 ± 0.2	0.061 ± 0.03	1.4 ± 0.2
3-deoxy (*ent*-**8**)	2.3 ± 0.6	3.2 ± 0.3	0.20 ± 0.03	5.6 ± 0.4
3,5-dideoxy (*ent*-**11**)	4.9 ± 0.8	5.8 ± 0.4	0.10 ± 0.02	4.4 ± 0.3

[a] Assays were carried out in 50 mM Tris HCl, pH 8.0, with 4 and 8 mM free Mg^{2+} for the archaeal and *E. coli* enzymes, respectively.

Whatever the rate-limiting step, the activation energy for *ent*-**1** hydrolysis must be reduced when the C1-hydroxyl group of *ent*-**1** is removed. Since no crystal structure exists of the *E. coli* IMPase, we can predict that in terms of substrate binding, the interactions of the enzyme with the C3−OH group of L-I-1P may be destabilizing. Thus, the substrate kinetics with SuhB are reminiscent of those observed with the mammalian IMPase,[2b] whereas the archaeal system exhibits very different behavior.

Can the kinetics with these defined inositol phosphate isomers contribute to our understanding of the biology of the archaeal enzyme? In *A. fulgidus*, the IMPase is involved in synthesizing an unusual solute (L,L′-di-*myo*-inositol-1,1′-phosphate, DIP),[8] which is used for osmotic balance but also produced in response to heat stress.[9] Biosynthesis of DIP requires both L-I-1P (converted to CDP-inositol) and *myo*-inositol (produced from L-I-1P by the IMPase). To ensure that there is adequate L-I-1P for DIP synthesis, the IMPase needs to be regulated. The dramatically increased K_m for L-I-1P could well be part of this control since little *myo*-inositol would be generated until a significant amount of L-I-1P accumulated.

In summary, rapid synthesis of point-by-point "mutated" IPs can shed light on their interactions with protein targets, and uncover differences among proteins. Expanded studies of these compounds, and their phosphatidyl analogues, should increase our understanding of their roles in biology.

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Supporting Information Available: Experimental details and characterization for the synthetic chemistry and the enzyme assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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