Discovery of a Catalytic Asymmetric Phosphorylation through Selection of a Minimal Kinase Mimic: A Concise Total Synthesis of D-*myo*-Inositol-1-Phosphate

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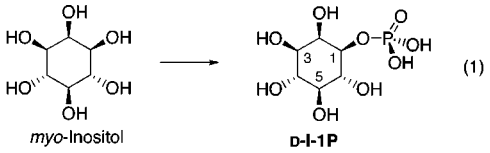
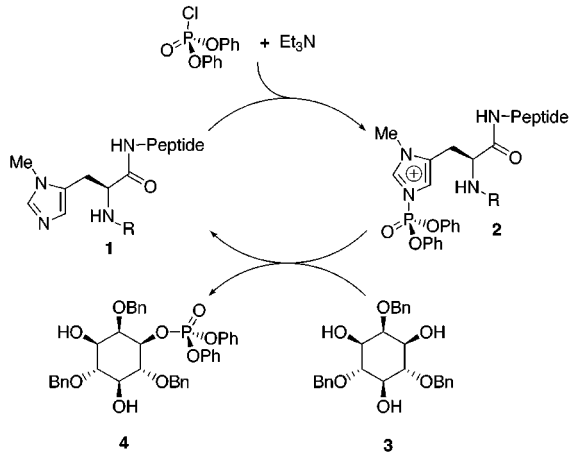
Selectivity in laboratory syntheses of polyfunctional molecules often pales in comparison to the remarkable control that nature exerts through its biosynthetic apparatus. Whereas enzymes that functionalize unique reactive sites among many within such molecules are commonplace, laboratory analogues of such catalysts are rare. Phosphorylated natural products, such as D-*myo*-inositol-1-phosphate (**D-I-1P**, eq 1),[1] provide an example. Nature often selectively installs phosphates at key sites within polyfunctional molecules as part of regulatory pathways. Signal transduction pathways depend critically on the action of enzymes that both phosphorylate (kinases) and dephosphorylate (phosphatases) their substrates. Laboratory mimics of these enzymes could prove useful tools in studying signaling processes from both a mechanistic point of view, and from the standpoint of improving synthetic access to selectively phosphorylated substances that are otherwise difficult to obtain. Herein, we report a "kinase mimic" that enables a catalytic, asymmetric synthesis of **D-I-1P**.



Because *myo*-inositol is readily available, one can imagine that a straightforward synthesis could rely on a catalyst that would effect regio- and enantioselective phosphorylation (eq 1).[2] Yet, because such a catalyst must distinguish among six stereochemically unique sites, development of such a process presents a significant challenge.[3] Nonenzymatic laboratory syntheses to date address the challenge with multistep protective group strategies. Enantioselective preparations have relied on classical resolutions, selective crystallization of *myo*-inositol derivatives that are functionalized with chiral reagents and the use of auxiliaries to form diastereomers.[4] Overall yields tend to be low. Presented below is a partial solution to the proposed selective, monofunctionalization of free *myo*-inositol.

We recently began investigating the possibility of regio- and enantioselective phosphorylation of such polyfunctional substrates

Scheme 1



using small peptide-based catalysts. Along these lines, we have found several octapeptides that catalyze asymmetric acylation reactions with high selectivity factors ($k_{ent1}/k_{ent2} > 50$), despite the fact that such catalysts are orders of magnitude smaller (in terms of molecular weight) than lipases and acylases.[5] These observations, coupled with the growing literature on the mechanistic basis for kinase action,[6] prompted us to investigate low-molecular weight peptide-based catalysts (and catalytic cycles) as illustrated by Scheme 1. It is now well documented that the first step in a number of signal transduction cascades is phosphorylation of histidine, initiating a nucleophilic catalytic mechanism.[7] We therefore speculated that small peptides based on modified His residues (e.g., **1**) might function in analogy to His-dependent kinases to form intermediates such as the phosphorylated catalyst **2**. On the basis of the pendant peptide sequence, the high-energy phospho-imidazolium ion would be generated in a chiral environment, rich with functionality that could plausibly interact with multifunctional substrates in a site-specific fashion. As a result, phosphate transfer (to substrates such as **3**) might occur with both regio- and enantioselectivity to produce enantio- and regiopure products such as **4**, regenerating catalyst **1** for further rounds of the catalytic cycle.

Initial studies verified that an alkylimidazole, such as that resident in **1**, functions efficiently as a catalyst for alcohol phosphorylation in substoichiometric quantity [2.5 mol % *N*-methylimidazole (NMI); diphenylchlorophosphate (DPCP) as the phosphorylating agent. Table 1].[8] In particular, conversions of 66−95% are achieved during the catalytic phosphorylation of several cyclic secondary alcohols (entries 1−3). The key to catalytic turnover is the use of a stoichiometric amount of Et_3N as an additive during the reaction. Under these conditions, the background rate is negligible (<5%).

We then turned our attention to the question of enantio- and regioselective phosphorylation of an appropriate *myo*-inositol derivative. Our previous studies of asymmetric acyl transfer employing low-molecular weight peptide-based catalysts suggested that these catalysts afford their highest enantioselectivities in non-

(1) (a) Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197−205. (b) Potter, B. V. L. *Nat. Prod. Rep.* **1990**, *7*, 1−24. (c) Agranoff, B. W.; Fisher, S. K. In *Inositol Phosphates and Derivatives: Synthesis, Biochemistry and Therapeutic Potential*; Reitz, A. B., Ed.; ACS Symposium Series 463: American Chemical Society: Washington, DC, 1991; pp 20−32.

(2) Such a process constitutes a desymmetrization reaction. For reviews of enantioselective desymmetrization, see: (a) Poss, C. S.; Schreiber, S. L. *Acc. Chem. Res.* **1994**, *27*, 9−17. (b) Willis, M. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1765−1784.

(3) For a discussion of the complex sequence of reactions in the biosyntheses of the enantiomeric I-1P molecules, see: Tian, F.; Migaud, M. E.; Frost, J. W. *J. Am. Chem. Soc.* **1999**, *121*, 5795−5796 and references therein.

(4) For a review, see: Billington, D. C. *The Inositol Phosphates: Chemical Synthesis and Biological Significance*; VCH: New York, 1993.

(5) (a) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 6496−6502. (b) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J.; Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638−11643.

(6) Pirrung, M. C. *Chem. Biol.* **1999**, *6*, R167−R175.

(7) For example, see: (a) Bilwes, A. M.; Alex, L. A.; Crane, B. R.; Simon, M. I. *Cell* **1999**, *96*, 131−141. (b) Mizuguchi, H.; Cook, P. F.; Tai, C. H.; Hasemann, C. A.; Uyeda, K. *J. Biol. Chem.* **1999**, *274*, 2166−2175. (c) Fraser, M. E.; James, M. N.; Bridger, W. A.; Wolodko, W. T. *J. Mol. Biol.* **1999**, *285*, 1633−1653.

(8) The NMI-DPCP adduct has been observed spectroscopically. See: Mal'tseva, T. V.; Ivanova, E. M.; Korobeinicheva, I. K. *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk.* **1985**, 112−116.

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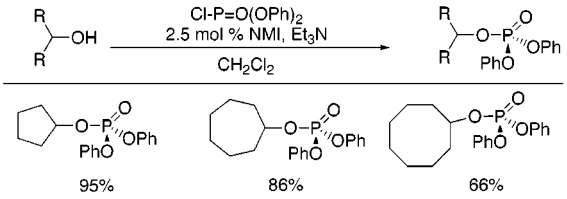
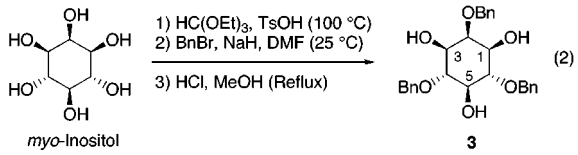
Table 1. Amine-Based Catalysis of Alcohol Phosphorylation[a]



[a] Analysis by ^{1}H NMR (400 MHz). Reactions were quenched after 12 h.

polar solvents.[9,10] We therefore prepared derivative **3** (eq 2), where the benzyl ethers (i) confer solubility under these conditions and (ii) reduce the site-selectivity problem to three unique hydroxyl groups.[11]



A small peptide library (39 members)[12] was then screened for regio- and enantioselectivity for monophosphorylation of **3**. The library was examined in parallel, with 39 independent reaction vessels, under a uniform set of small-scale conditions (25 °C, 2.5 mol % unpurified peptide, $PhCH_3$ solvent). A two-stage achiral/chiral HPLC assay allowed for a determination of the overall product distribution; the enantioselectivity for the phosphorylation of the 1- versus 3-position of **3** is shown in Figure 1a.[13] Notable from the data is that each catalyst indeed affords a different level of enantioselectivity during the catalytic phosphorylation, underscoring the ability of the peptide secondary structure to influence the stereochemical course of the reaction. Most significant is the performance of *unpurified* pentapeptide **6**, which affords **(−)-4** with good enantioselectivity (90% ee) under the parallel screen conditions.

Peptide **6** was then resynthesized, purified to chromatographic homogeneity and applied to an asymmetric total synthesis of **D-I-1P** (Scheme 2). Treatment of **3** with DPCP (1.6 equiv) and Et_3N (1.7 equiv), 2.0 mol % purified **6** in $PhCH_3$ (0 °C, 8 h) affords monophosphate **4** as a single enantiomer (>98% ee by chiral HPLC, 65% isolated yield). The only other detectable products of the reaction are a small amount of the 1,3-bis-(phosphate) derived from **3** (∼10%) and recovered starting material (**3**, ∼10%). Optically pure **4** was then converted to **D-I-1P** in a single step by dissolving metal deprotection (96% yield; synthetic: $[\alpha]_D$ +3.5 *c* 1.0, pH = 9; natural: $[\alpha]_D$ +3.5 *c* 1.0, pH = 9).[14] The synthesis reliably delivers >0.5 g of the natural product salt in convenient laboratory-scale glassware, starting from ∼ 1 g of *myo*-inositol in one week's time.

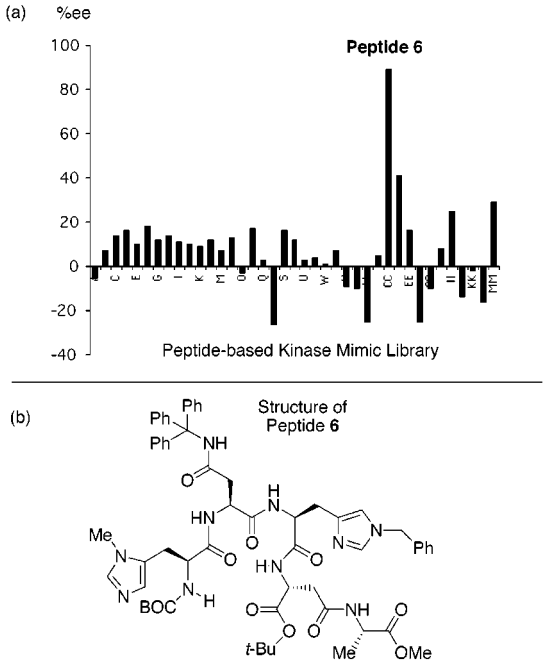
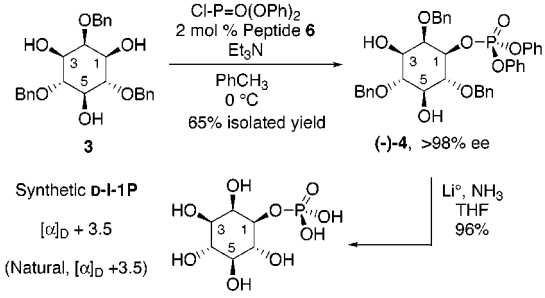


Figure 1. (a) Results of enantioselectivity screen for monophosphorylation of triol **3**. The identity of the 39 catalysts that were evaluated may be found in the Supporting Information. (b) The structure of the most selective catalyst, peptide **6**.

Scheme 2



From the standpoint of the selective synthesis of phosphorylated natural products, it appears likely from this study that other unique catalysts exist that will effect other site-selective reactions such that greatly simplified synthetic schemes may be achieved. Studies along these lines, as well as experiments to probe the mechanisms by which these catalysts work, are ongoing in our laboratory.

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Supporting Information Available: Experimental details for all aspects of this study (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Miller, S. J.; Copeland, G. T.; Papaioannou, N.; Horstmann, T. E.; Ruel, E. M. *J. Am. Chem. Soc.* **1998**, *120*, 1629−1630.

(10) There are often advantages to employing actual enzymes in organic solvents also. See: Klibanov, A. M. *Nature* **2001**, *409*, 241−246.

(11) Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1423−1429.

(12) Library members were synthesized on solid support and used following cleavage *without* purification. Members ranged in size from tetrapeptides to octapeptides. Please see the Supporting Information.

(13) When reactions are conducted in toluene solution, the only observed products were the **D-I-1P**, **L-I-1P**, **I-1,3-bisP**, and recovered starting material (**3**). The 5-position undergoes minimal phosphorylation (<5%) with these catalysts (including NMI) under these conditions.

(14) **D-I-1P** was isolated and characterized as the bis(cyclohexylamine) salt in accord with convention.