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A Chiral Phosphoramidite Reagent for the Synthesis of Inositol Phosphates

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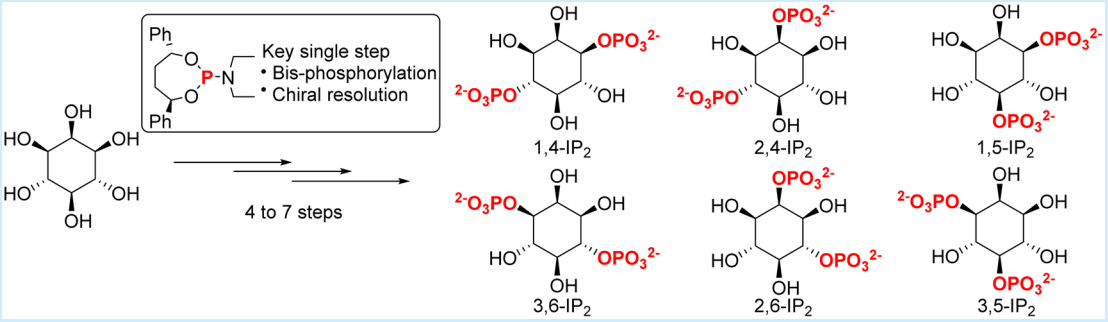
Supporting Information



ABSTRACT: There is a paucity of chiral phosphoramidite reagents or chiral catalysis methods for the synthesis of biologically relevant inositol phosphates. A new C_2-symmetrical chiral phosphoramidite has been developed and successfully applied to the synthesis of a set of chiral inositol bisphosphates. The reagent allowed bis-phosphorylation and chiral resolution, resulting in a concise synthetic route, thus expanding the toolbox available for the preparation of biologically relevant inositol phosphates in high optical purity.

Inositol phosphates and their lipidated phosphatidyl inositol congeners are important signaling molecules with crucial roles in cellular processes such as calcium release, gene regulation, and immune response.[1,2] A network of phosphatases and kinases metabolically relates the various inositol phosphates by adding or removing phosphate groups on a central *myo*-inositol core.[1] For instance, the second messenger inositol 1,4,5-trisphosphate is released by the hydrolysis of phosphatidyl inositol 4,5-bisphosphate from the plasma membrane by phospholipase C. 1,4,5-IP_3 can then be further metabolized to 1,4-IP_2 by a 5-phosphatase which regulates calcium release from intracellular stores.[3] The synthesis of well-defined structures and derivatives greatly benefited the study of the inositol phosphates, but functions have still not been attributed to all members of this large family yet.[2] This is well illustrated with inositol bisphosphates (IP_2s) that are one on the most abundant members of this family.[4] They are generally considered as downstream metabolites. However, studies have shown their potential involvement in biological functions such as a DNA polymerase activator[5] and cytoskeletal actin inducer[6] with other functions yet to be revealed. Numerous inositol phosphates and all phosphatidyl inositols are chiral molecules, which complicates their preparation in enantiomerically pure form. Many efforts to address this challenge have been made offering strategies based on chiral precursors, desymmetrization, or chiral resolution of *myo*-inositol.[7−9] However, these routes suffer from multiple protecting group manipulations and tedious purification of the charged inositol phosphates. Two paradigms have emerged for the chiral synthesis of inositol phosphates using a unique phosphorylation reaction.[10] The first employs peptide-based chiral nucleophilic catalysts. Histidine- and tetrazolylalanine-containing peptides have been used to catalyze the asymmetric phosphorylation[11,12] and phosphitylation,[13] respectively, of inositol derivatives. This method has allowed the synthesis of numerous chiral inositol phosphates, phosphatidylinositols, and analogues. The second is the chiral phosphoramidite reagent recently introduced by Jessen and coworkers that allows chiral resolution after phosphorylation and was used in the synthesis of highly phosphorylated inositol pyrophosphate molecules.[14−16] The chiral auxiliary is based on the β-cyanoethyl moiety which is accessible in a few synthetic steps from mandelic acid and is a base-labile phosphate protecting group.

Motivated by the paucity of chiral phosphoramidite reagents available for synthesis, we developed a new cyclic C_2-symmetrical phosphoramidite reagent and utilized it for bis-phosphorylation and chiral resolution in a single step. This methodology enabled rapid access to various chiral IP_2 in high optical purity (Figure 1).

We focused our efforts on the preparation of C_2-symmetric diphenyl seven-membered ring phosphoramidite **2**, which is analogous to the widely used xylylene phosphoramidite for several reasons (Scheme 1). First, the C_2 symmetry was desired to avoid any complicated isomeric mixture. Second, the benzyl ether phosphate bond is sensitive to acid and also allows

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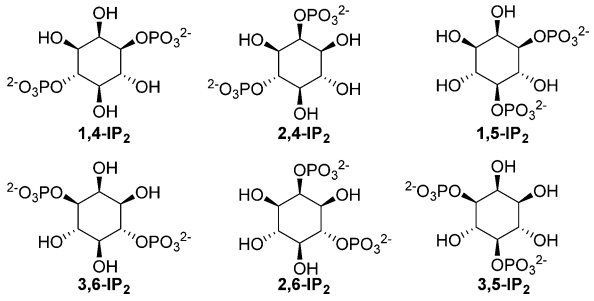
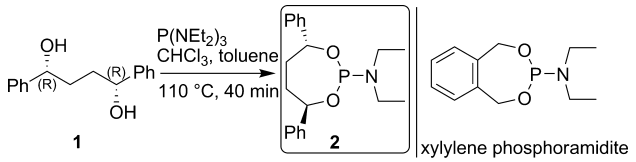


Figure 1. Chiral inositol bisphosphates (IP_2).

Scheme 1. Synthesis of C_2 Chiral Phosphoramidite



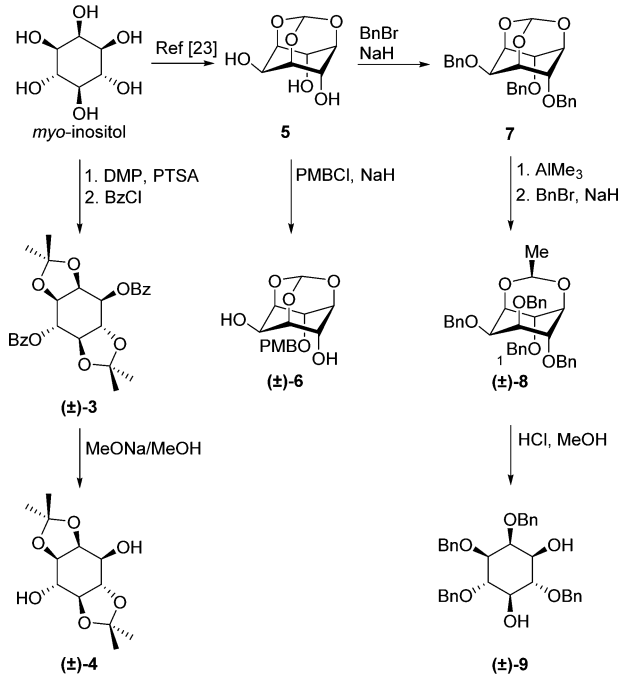
C_2-symmetric
Acid labile benzyl ether phosphate bond
Easier preparation of cyclic phosphoramidite

hydrogenolysis as an alternative deprotection strategy. Finally, we surmised that a cyclic phosphoramidite would ease the reagent preparation.[17] The chiral diol auxiliary **1** was rapidly obtained in two steps. Homocoupling of bromoacetophenone provided diphenylbutanedione,[18] which was subsequently reduced using the Corey–Bakshi–Shibata catalyst.[19] Then, following Arbuzov's procedure,[20] diol **1** reacted cleanly with phosphorus triamide affording the phosphoramidite **2**. The isolation of the phosphoramidite reagent was complicated by oxidation and a high boiling point, but the cleanliness of the reaction allowed for a one-pot *in situ* preparation of the reagent (*vide infra*).

In order to evaluate the methodology, we prepared racemic partially protected inositol analogues using established methodology (Scheme 2). Once phosphorylated and resolved, these intermediates would give rise to a series of biologically relevant inositol bisphosphates. The widely used intermediate (±)-**4** with free 1 and 4 hydroxyl groups was prepared following the procedure described by Khersonsky and Chang.[21] While treatment of *myo*-inositol with acetone ketal afforded a mixture of regioisomers, a subsequent benzoylation enabled the separation of the desired isomer (±)-**3** and basic deprotection generated the racemic compound (±)-**4**. Intermediates (±)-**6** and (±)-**9** were prepared from *myo*-inositol orthoformate **5**.[22,23] Regioselective alkylation of the orthoformate with PMBCl in the presence of NaH afforded the racemic (±)-**6** in 75% yield. As for intermediate (±)-**9**, it was accessed in four high yielding steps. After benzylation, orthoformate **7** was selectively reduced with $AlMe_3$ to release the enantiotopic hydroxyl group on position 3/1 which was subsequently benzylated to obtain (±)-**8**.[24] Finally, the remaining acetal was hydrolyzed to liberate hydroxyl groups on position 1/3 and 5 affording intermediate (±)-**9** with an overall yield of 64%.

Phosphitylation with phosphoramidite **2** was performed on these racemic inositol intermediates, to simultaneously introduce the two phosphate groups and resolve the diastereomeric mixture in order to obtain optically pure diphosphate inositol compounds (Table 1). The phosphoramidite **2**, generated *in situ* in degassed solvent, was used directly in the presence of tetrazole in the phosphitylation reaction followed by oxidation with *m*-CPBA. Tetrazole was used in excess to compensate for the diethylamine released during phosphoramidite formation.

Scheme 2. Preparation of the Racemic Diols Inositol Intermediates[a]



[a]Bn: benzyl. Bz: benzoyl. DMP: 2,2-dimethoxypropane. PMB: *p*-methoxybenzyl. PTSA: *p*-toluenesulfonic acid.

The phosphorylated diastereomeric mixtures were obtained with full conversion and were purified by normal SiO_2 flash column chromatography. Diastereomers with phosphate on positions 1,4 and 3,6 were separated by crystallization affording diastereomer 1,4-P*$_2$ **10a** in an excellent diastereomeric ratio (>99:1, Table 1, entry 1). The absolute configuration was assigned by X-ray analysis revealing the phosphate groups in positions 1 and 4 (SI Figure S1). Recrystallization of the mother liquor furnished its diastereomer 3,6-P*$_2$ **10b** also with excellent optical purity (dr >99:1) (Table 1, entry 2). We were delighted to see that diastereomers **11a** and **11b**, with phosphate on positions 2,4 and 2,6, respectively, could be separated by normal SiO_2 flash column chromatography and they were both obtained with exquisite optical purity (dr >99:1) (Table 1, entries 3 and 4). In this case, the absolute configuration was determined in the next step by optical rotation measurement of the deprotected **2,6-IP$_2$**. Finally, the 1,5- and 3,5-bisphosphate diastereomers were also separated by crystallization. The 1,5-P*$_2$ **12a** could be obtained with high purity (dr >99:1) (Table 1, entry 5), and its absolute configuration was determined by X-ray analysis displaying the phosphate groups in positions 1 and 5 (SI Figure S2). However, diastereomer 3,5-P*$_2$ **12b** was only accessed with a moderate diastereomeric ratio of 85:15 (Table 1, entry 6).

Deprotection of the bisphosphate diastereomers to access the inositol bisphosphates was achieved in a single step for each intermediate (Scheme 3). This straightforward deprotection was a considerable advantage, as inositol phosphate compounds are sensitive and difficult to purify. Hence the phosphorylated

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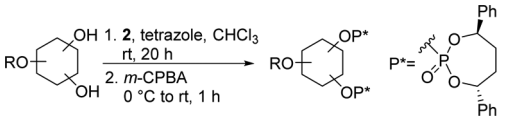
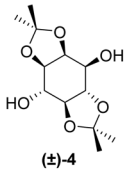
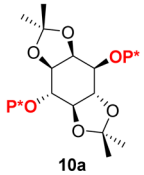
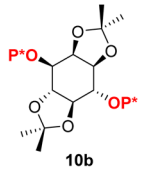
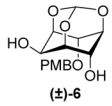
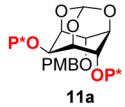
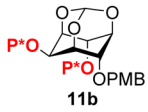
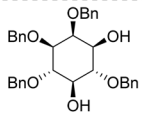
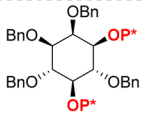
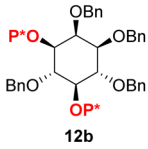
Table 1. Chiral Resolution via Phosphitylation[a]



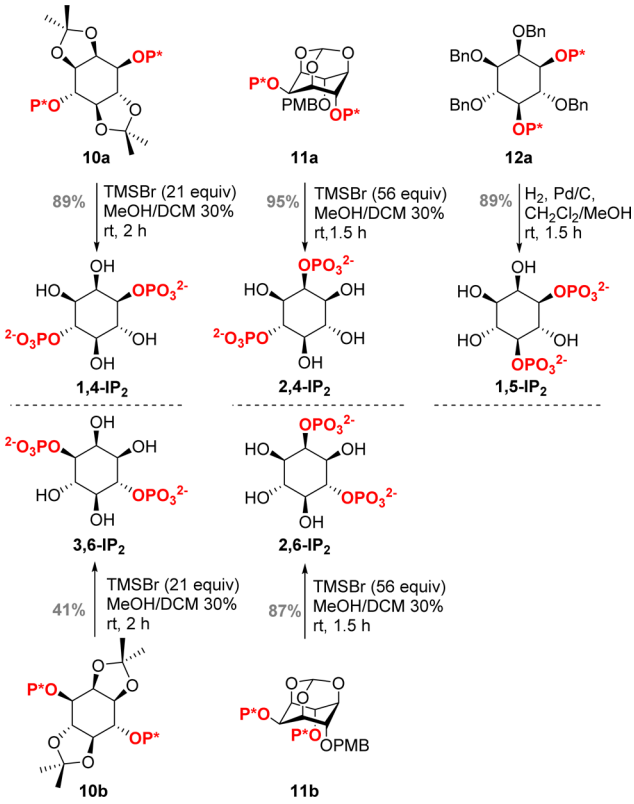
entry	substrate	product	yield[b] (%)	dr[c]
1	 (±)-4	 10a	16	>99:1
2		 10b	13	>99:1
3	 (±)-6	 11a	24	>99:1
4		 11b	16	>99:1
5	 (±)-9	 12a	52	>99:1
6		 12b	11	85:15

[a]Reaction conditions: 1. **2** (4 equiv), 1*H*-tetrazole (23 equiv), $CHCl_3$, rt, 20 h; 2. *m*-CPBA (10 equiv). [b]Recovered yield based on the diastereomer itself which represents 50% of the racemic starting material. [c]Diastereomeric ratio determined by ^{31}P and ^{1}H NMR spectroscopy.

acetal intermediates **10a** and **10b**, as well as the orthoformates **11a** and **11b**, were fully deprotected by treatment with TMSBr and were purified by precipitation to isolate pure compounds in good yields. Benzylated intermediate **12a** was deprotected by hydrogenolysis and like its counterparts was purified by precipitation to give **1,5-IP$_2$**.

Overall, the development of the new cyclic C_2-symmetrical phosphoramidite reagent offers new methodology to rapidly access various chiral IP_2 in high optical purity. Phosphorylation with the cyclic C_2-symmetric phosphoramidite reagent allowed the introduction of the two phosphate groups on racemic inositol derivatives and their chiral resolution in a single step. Although the optically pure IP_2 derivatives could only be obtained in low to moderate yields, this method avoids the additional protection/deprotection steps necessary for classic chiral resolution. Moreover, the auxiliary lability allowed access

Scheme 3. Single Step Deprotection



to the final inositol phosphates in a single high yielding step, as all protecting groups could be removed simultaneously with acidic conditions or hydrogenation. Although this methodology is not meant for a process scale-up application, it proved to be efficient in the preparation of a chiral inositol bisphosphates library. Their preparation starting from the commercially available *myo*-inositol was achieved in a record number of four steps and a maximum of seven steps, while synthesis using classic chiral resolution or chiral substrate starting material suffers from multiple protection/deprotection steps.[2S]

This cyclic C_2-symmetric phosphoramidite comes to expand the toolbox for general chiral inositol phosphates synthesis. The reactivity, similar to the widely used xylylene phosphoramidite, should also give the possibility to rapidly access numerous chiral inositol phosphates as well as their metabolically stable analogues thiophosphates. The synthesis of optically pure inositol phosphates and their analogues remains an invaluable tool to help decipher their biological role.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01374.

Crystallographic information on compounds **10a** and **12a** (CIF)

Experimental procedures, analytical data of all new compounds, and X-ray crystallographic data (PDF)

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Notes

The authors declare no competing financial interest.

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