

Regioisomeric Family of Novel Fluorescent Substrates for SHIP2

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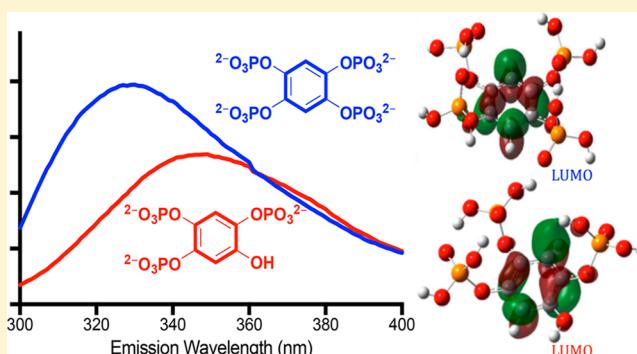
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Supporting Information

ABSTRACT: SHIP2 (SH2-domain containing inositol 5-phosphatase type 2) is a canonical 5-phosphatase, which, through its catalytic action on PtdInsP₃, regulates the PI3K/Akt pathway and metabolic action of insulin. It is a drug target, but there is limited evidence of inhibition of SHIP2 by small molecules in the literature. With the goal to investigate inhibition, we report a homologous family of synthetic, chromophoric benzene phosphate substrates of SHIP2 that display the headgroup regiochemical hallmarks of the physiological inositide substrates that have proved difficult to crystallize with 5-phosphatases. Using time-dependent density functional theory (TD-DFT), we explore the intrinsic fluorescence of these novel substrates and show how fluorescence can be used to assay enzyme activity. The TD-DFT approach promises to inform rational design of enhanced active site probes for the broadest family of inositide-binding/metabolizing proteins, while maintaining the regiochemical properties of *bona fide* inositide substrates.

KEYWORDS: Inositol phosphate, 5-phosphatase, SHIP2, TD-DFT, fluorescence, HPLC, ligand



In eukaryotic cells, many signaling pathways are regulated by levels of inositol phosphates (inositides), phosphatidylinositol phosphates (phosphoinositides), and the proteins that are their cognate binding partners. The balance of these is controlled by families of kinases and phosphatases that phosphorylate and dephosphorylate these molecules at specific positions, designated locants, of the inositol ring.¹ SH2-domain containing inositol 5-phosphatase type 2 (SHIP2) belongs to a family of phosphatases that hydrolyze the 5-phosphate of inositides and phosphoinositides. The substrates are known to include inositol pentakisphosphates (InsP₅), tetrakisphosphates (InsP₄), trisphosphates (InsP₃), phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P₃), and phosphatidylinositol 4,5-bisphosphate (PtdInsP₂).²

An understanding of how these enzymes function is important to pathologies such as diabetes and cancer.² SHIP2 reduces levels of PtdInsP₃, while increasing levels of PtdIns(3,4)P₂. Consequently, this enzyme regulates the PI3K/Akt pathway that is linked to cell proliferation and the metabolic action of insulin.^{3,4} SHIP2 is expressed in many cell types and has additionally been linked to regulation of diverse cellular processes such as calcium signaling, cytoskeletal remodeling, protein trafficking, and phagocytosis.^{2,5} While a mechanistic explanation of inhibition of PI3-kinases has

underpinned therapeutic interventions in cancer,^{6,7} therapeutic inhibition of 5-phosphatases lacks similar foundation.

Previous research for inhibitors of SHIP2 has typically assayed activity using phosphate release with D-*myo*-inositol-1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P₄) or D-*myo*-phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃).⁸ Thus, Suwa et al. identified AS1949490 as a novel, small molecule inhibitor of SHIP2. In the absence of structural data or evidence of catalytic modification of the inhibitor, the exact mode of inhibition is not clear, though from an enzymological perspective the nature of inhibition is competitive with respect to inositide substrate. It is possible that within the chemical landscape of inhibition, competitive substrate analog inhibitors will be found. However, further mechanistic analysis would require identification of the products of such reactions, something limited for *bona fide* substrates by lack of a suitable chromophore in these molecules. Alternatively, radiolabeled substrates could be used, but this would demand complex

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synthetic work and HPLC resolution of substrates and products.

With a view to finding alternative substrates of 5-phosphatases that have analytically useful chromophoric properties, we investigated the benzene polyphosphate compounds shown in Figure 1. Originally, the benzene

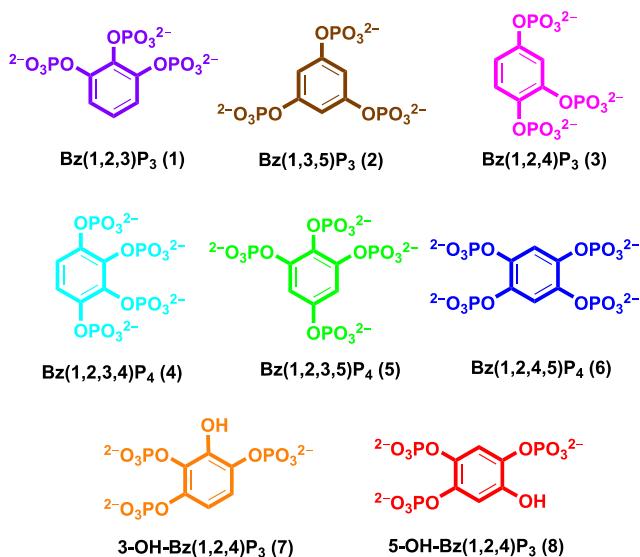


Figure 1. Structures of benzene phosphates used in this study.

phosphate compounds **1** to **6** were designed as substrate analogs to substitute as ligands to inositol phosphate binding proteins/enzymes, enabling crystallographic resolution of protein–ligand complexes and insight into enzyme inhibition.^{9,10} While simple benzene phosphates appear not to be substrates of 5-phosphatases,^{9–11} compound **7**, 3-OH-Bz(1,2,4)P₃, was synthesized and has been shown by assay of release of phosphate to be a substrate for type I *myo*-inositol 1,4,5-triphosphate-5-phosphatase (INPPSA).¹¹ Benzene phosphate analogues were also used to uncover mechanistic information in cocrystallization studies with INPPSB.¹²

We now show that the chromophoric properties of benzene phosphates vary depending on the regiochemistry of substituents around the benzene ring and that several members of this family have an unanticipated, useful, intrinsic fluorescence. Alongside conventional spectroscopic measurements, we use time-dependent density functional theory (TD-DFT) to examine how the regiochemistry modifies the spectroscopic properties. Compound **6**, Bz(1,2,4,5)P₄, proved to be intensely fluorescent, a property potentially explainable by TD-DFT. It is a tantalizing observation that the four phosphate groups of this compound are stereochemically homotopic, remaining indistinguishable even in the chiral environment of the enzyme active site. Since 5-phosphatases yield only a single product from Ins(1,3,4,5)P₄, should benzene phosphates be substrates, we expect a single, identifiable product **8** 5-OH-Bz(1,2,4)P₃ of the dephosphorylation of compound **6** Bz(1,2,4,5)P₄. Compound **8** 5-OH-Bz(1,2,4)P₃ was, however, not synthesized, so in the absence of reference material, we sought to use TD-DFT to predict its fluorescence with the expectation of being able to use this prediction to identify whether compound **6** Bz(1,2,4,5)P₄ could be a substrate of SHIP2. We therefore investigated the possibility that benzene phosphates themselves are substrates for

dephosphorylation by SHIP2 and the chromophoric properties of this class of molecule might allow construction of assays complementary to those that employ the measurement of released inorganic phosphate.

Previously, benzene phosphates have been shown to be inhibitors of INPPSA,⁷ INPPSB,¹² and SHIP2,⁸ with compound **6** Bz(1,2,4,5)P₄ yielding IC₅₀ values in single figures and tens of micromolar range against Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ substrates, tested at 1 and 100 μM concentrations, respectively. We also recently described the use of a fluorescent conjugate of Ins(1,3,4,5,6)P₅ (2-FAM-InsP₅, Figure 2) as an active site probe of inositol pentakisphosphate 2-

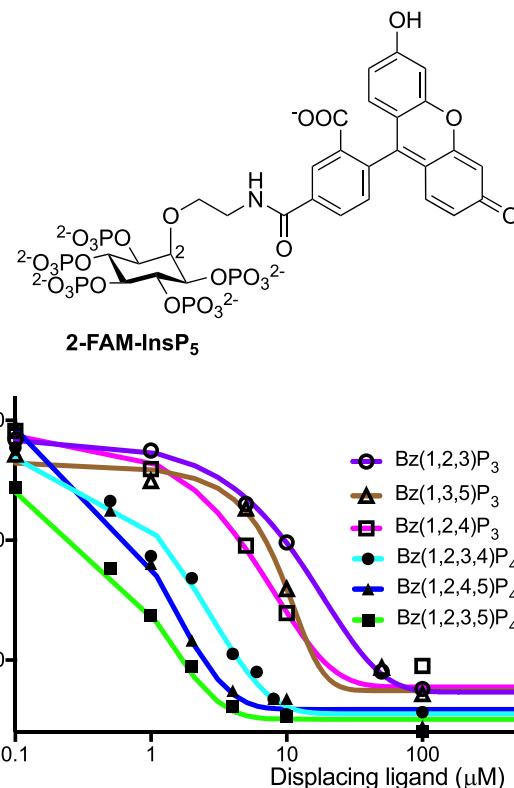


Figure 2. Displacement of 2-FAM-InsP₅ from SHIP2 by benzene phosphates. The experiments used 2 nM 2-FAM-InsP₅ and 1 μM SHIP2. Results shown as mean of triplicate experiments (results with standard deviations given in Table 1).

kinase¹³ and as an intracellular probe.¹⁴ Here, combining the use of two classes of ligand, we show that displacement of 2-FAM-InsP₅ affords an assay of benzene phosphate binding to SHIP2 (Figure 2 and Table 1).

All six benzene phosphate derivatives chosen displaced 2 nM 2-FAM-InsP₅ from SHIP2 with IC₅₀ values in the low micromolar range, the benzene tetrakisphosphates being more effective than benzene trisphosphates, with IC₅₀ values approximately 1 order of magnitude smaller than the trisphosphates.

Most commonly, 5-phosphatase activity is assayed as the release of inorganic phosphate from Ins(1,3,4,5)P₄ or PtdIns(3,4,5)P₃. We assayed the ability of SHIP2 to release phosphate from inositol phosphates and also benzene phosphates. We tested whether, by virtue of the lower pK_a of the hydroxyl of a theoretical dephosphorylated benzene phosphate (leaving group),^{15,16} benzene phosphates might be

Table 1. Comparison of Published IC₅₀ Values for Inhibition of 5-Phosphatases by Benzene Phosphates to Experimentally Determined IC₅₀ Values Found for Displacement of 2-FAM-InsP₅ from SHIP2

compound	IC ₅₀ (μ M) 2-FAM-InsP ₅ SHIP2, this study	IC ₅₀ (μ M) Ins(1,3,4,5)P ₄ SHIP2 ^{10,12}	IC ₅₀ (μ M) Ins(1,4,5)P ₃ INPPSA ⁹	IC ₅₀ (μ M) Ins(1,4,5)P ₃ INPPSB ¹²
Bz(1,2,3)P ₃	14.23 \pm 0.1	>1000	86 \pm 28	33.5 \pm 6.8
Bz(1,3,5)P ₃	11.25 \pm 2.9		16 \pm 9	
Bz(1,2,4)P ₃	7.02 \pm 0.05		14 \pm 9	
Bz(1,2,3,4)P ₄	2.78 \pm 0.59		98 \pm 16	
Bz(1,2,3,5)P ₄	1.01 \pm 0.43		78 \pm 50	
Bz(1,2,4,5)P ₄	1.02 \pm 0.1	69.3 \pm 15.4 ¹⁰ 108.3 \pm 20.3 ¹²	4 \pm 2	6.3 \pm 0.8

better substrates than simple inositol phosphate substrate (Figure 3).

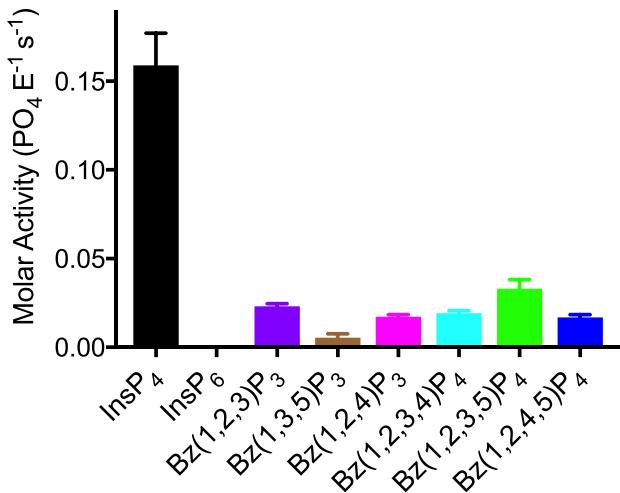


Figure 3. Benzene phosphates as substrates of SHIP2. Compounds tested at 100 μ M. For Ins(1,3,4,5)P₄ and InsP₆, the concentration of SHIP2 was 1 μ M and for the benzene phosphates, 4 μ M, with triplicate reactions run for 10 min at 30 °C.

Ins(1,3,4,5)P₄ and InsP₆ were included as positive and negative controls, respectively. Phosphate release was determined with molybdenum blue, using ferrous sulfate as a reducing agent.¹⁷ While slower than the reaction with Ins(1,3,4,5)P₄, phosphatase activity was noted with all compounds except InsP₆, a substrate for phytases¹⁸ but not for inositol polyphosphate 5-phosphatases. As the benzene phosphates are poorer substrates than Ins(1,3,4,5)P₄, the rate of dephosphorylation must be influenced by factors other than just the pK_a of the leaving group. In an earlier study,¹¹ we postulated that OH groups in the substrate are likely to have a mechanistic role, showing that during dephosphorylation of compound 7 3-OH-Bz(1,2,4)P₃ by INPPSA, stabilization of the phenolic OH proton accompanied deprotonation of the phosphate groups. In order to test this theory, we compared phosphate release from compound 7 3-OH-Bz(1,2,4)P₃ and compound 4 Bz(1,2,3,4)P₄ (Figure S1). The rate determined for compound 7 3-OH-Bz(1,2,4)P₃ was almost twice that observed for compound 4 Bz(1,2,3,4)P₄. However, this rate is comparable to that observed for compound 5 Bz(1,2,3,5)P₄, suggesting that the regiochemistry of the ring substitution may also have an influence.

Having gained evidence for binding of benzene phosphates to SHIP2 and their catalytic processing by SHIP2, we

speculated that the spectroscopic properties of benzene phosphates might be exploited to follow catalysis. Excitation and emission scans of acetonitrile solutions of compound 4 Bz(1,2,3,4)P₄, compound 5 Bz(1,2,3,5)P₄, compound 6 Bz(1,2,4,5)P₄, and tryptophan were measured, and the spectral details compared to those predicted by TD-DFT (Figure 4 and

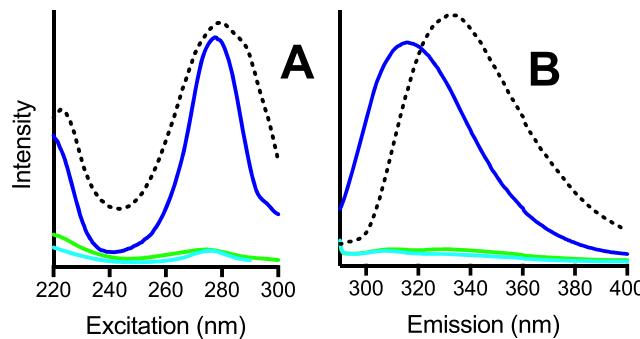


Figure 4. Fluorescence spectra of 500 μ M Bz(1,2,3,4)P₄ (cyan line), 500 μ M Bz(1,2,3,5)P₄ (green line), 100 μ M Bz(1,2,4,5)P₄ (blue line), and 10 μ M tryptophan (black dotted line) in acetonitrile. (A) Excitation scans determined at emission maxima and (B) emission scans excited at 280 nm.

Tables S1 and S2). Spectral predictions for compound 8 5-OH-Bz(1,2,4)P₃ are included in the table, but the pure compound was not available to test. The theoretical methods employ approaches used in TD-DFT studies of tryptophan.^{19,20} A detailed discussion of the TD-DFT methods and results is included in Supporting Information.

Significantly, on excitation at 280 nm, the measured fluorescence of compound 6 Bz(1,2,4,5)P₄ was 30 times more intense than that of the next most fluorescent benzene tetrakisphosphate, compound 5 Bz(1,2,3,5)P₄, and approximately 10% of that of tryptophan (Figure 4). These observations match the trends predicted by TD-DFT (Tables S1 and S2). The calculations for compound 8 5-OH-Bz(1,2,4)P₃ predict that the excitation maximum of 5-OH-Bz(1,2,4)P₃ is 6 nm greater than that of compound 6 Bz(1,2,4,5)P₄, a prediction confirmed by empirical measurement (Figure 5 and Table S1). Similarly, TD-DFT predicts the emission maximum for compound 8 5-OH-Bz(1,2,4)P₃ is 25 nm greater than that of compound 6 Bz(1,2,4,5)P₄ (Figure 5 and Table S2).

As TD-DFT predicts that compound 6 Bz(1,2,4,5)P₄ and its hydrolysis product compound 8 5-OH-Bz(1,2,4)P₃ show significant fluorescence intensity, we devised an HPLC separation that could be used with fluorescence detection for

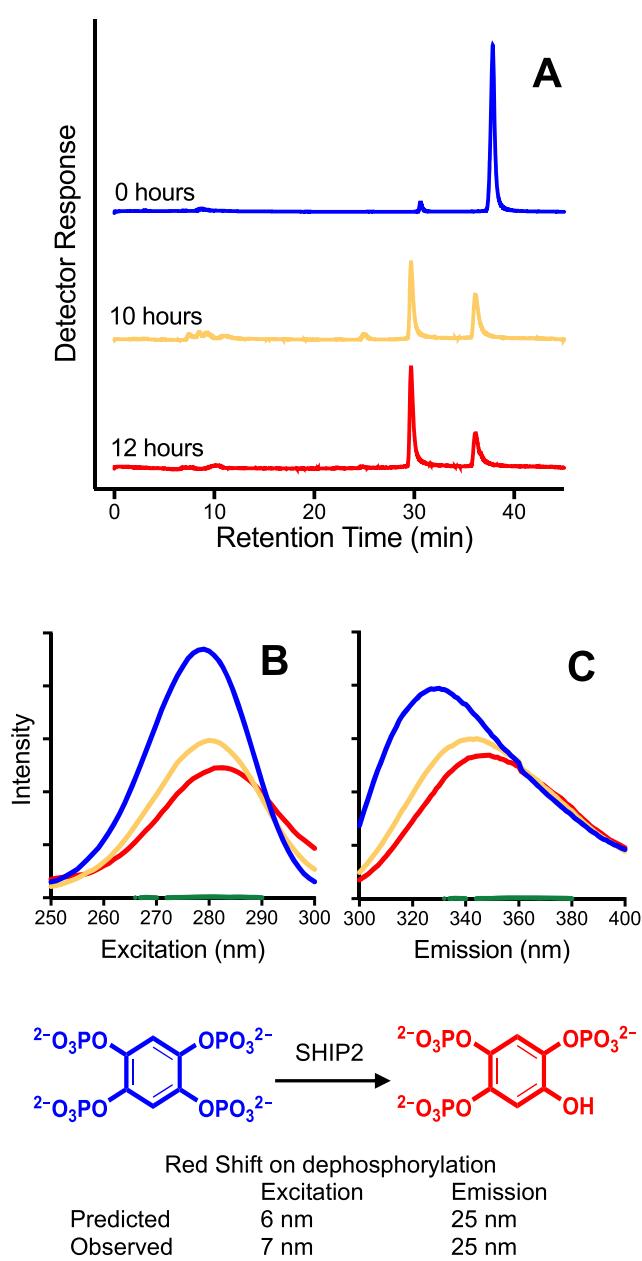


Figure 5. (A) HPLC analysis of reaction of Bz(1,2,4,5)P₄ (100 μ M) incubated with SHIP2 (100 nM) at 16 °C for 0 h (blue line), 10 h (orange line), and 12 h (red line). Substrate and products were detected by fluorescence (excitation at 280 nm, emission at 330 nm). (B,C) Fluorescence of the same samples diluted 100-fold in acetonitrile. SHIP2 (100 nM) alone shown as a dark green line on the baseline: (B) excitation scans with emission at 330 nm and (C) emission scans excited at 280 nm.

benzene phosphates. First, we established separations following UV absorbance (Figure S2). HPLC resolved all the discrete benzene trisphosphates and tetrakisphosphates used in this study. Compound 6 Bz(1,2,4,5)P₄ that eluted at approximately 37 min could also be detected by fluorescence with excitation at 280 nm and emission at 330 nm. In the first instance, we wanted to use the HPLC method to investigate whether SHIP2 could convert compound 6 Bz(1,2,4,5)P₄ into compound 8 5-OH-Bz(1,2,4)P₃. Therefore, we selected

conditions that would limit the extent of reaction to a predominant single dephosphorylation. Extended incubation of 100 μ M of compound 6 Bz(1,2,4,5)P₄ with 100 nM SHIP2 allowed us to monitor the progression of the reaction without interference from other fluorescent products as judged by HPLC (Figure 5A). Over a period of 12 h, the fluorescence intensity of the parent peak at 37 min diminished correlating with the appearance of an earlier-eluting peak at 30 min (Figure 5A). As we did not have the assumed product, compound 8 5-OH-Bz(1,2,4)P₃, to confirm the identity of the peak at 30 min (Figure 5A), we sought confirmation by recording fluorescence excitation and emission spectra of the accumulated products (Figures 5B,C).

Samples analyzed in Figure 5A were diluted 100-fold into acetonitrile, and fluorescence spectra were recorded (Figure 5B,C). As the proportion of the product increased, the red shifts in the fluorescence spectra increased correspondingly. After 12 h incubation, the excitation maximum of the assumed product, compound 8 5-OH-Bz(1,2,4)P₃, was 7 nm greater than that of the starting material (Figure 5B). This compares favorably to the shift of +6 nm predicted by TD-DFT for compound 6 Bz(1,2,4,5)P₄ converting to compound 8 5-OH-Bz(1,2,4)P₃ (Table S1). The emission maximum of the product was 25 nm greater than that of the starting material (Figure 5C). This also compares favorably to the predicted shift of +25 nm for compound 6 Bz(1,2,4,5)P₄ converting to compound 8 5-OH-Bz(1,2,4)P₃ (Table S2). As discussed in Supporting Information, the predicted transition energies for excitation and emission differ systematically from the experimentally determined values so that variations between compounds can be defined in terms of wavelength shifts. A similar observation has previously been noted for this type of TD-DFT analysis.^{19,20} From the close match between the predicted and observed wavelength shifts, we can conclude that compound 8 5-OH-Bz(1,2,4)P₃ is formed when compound 6 Bz(1,2,4,5)P₄ is incubated with SHIP2 under these conditions.

In order to investigate further the effects of SHIP2 on compound 6 Bz(1,2,4,5)P₄, 100 μ M of the substrate was incubated for 2 h with increasing amounts of SHIP2. This demonstrated that the conversion of compound 6 Bz(1,2,4,5)P₄ into its first hydrolysis product, compound 8 5-OH-Bz(1,2,4)P₃, is dependent on the SHIP2 concentration and that compound 6 Bz(1,2,4,5)P₄ alone is stable under these conditions (Figure S3). Longer incubation and use of tandem UV-fluorescence detection resulted in a more complicated product profile with peaks of increased absorbance: fluorescence ratio at 30, 25, 23, and 13 min (Figure S4). This is consistent with successive dephosphorylation generating a sequence of different hydroxybenzene phosphate compounds with different elution profiles as observed for inositol phosphates.²¹

The change in fluorescence that accompanies the conversion of substrate to product can be used to develop a real-time fluorescence-based assay to follow dephosphorylation of compound 6 Bz(1,2,4,5)P₄ by the exemplar 5-phosphatase SHIP2 (Figure 6). In the absence of competing additives, the fluorescence intensity at 325 nm reduces, and the maximum shifts to a longer wavelength as the reaction between SHIP2 and compound 6 Bz(1,2,4,5)P₄ progresses (Figure 6A). Figure 6B traces how the emission intensity at 325 nm (the maximum for compound 6 Bz(1,2,4,5)P₄) decreases with time. It also shows how this decrease is affected by additives that are

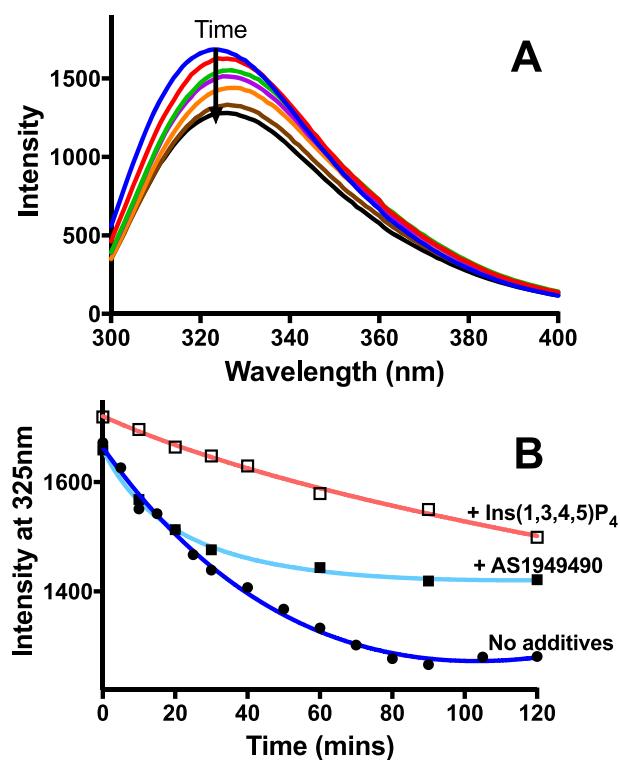


Figure 6. (A) Change in fluorescence of compound 6 $\text{Bz}(1,2,4,5)\text{P}_4$ ($100 \mu\text{M}$) incubated with SHIP2 ($1 \mu\text{M}$) at 23°C recording emission scans excited at 280 nm over a period of 2 h . (B) Change in emission intensity at 325 nm with time for compound 6 $\text{Bz}(1,2,4,5)\text{P}_4$ incubated with SHIP2; with no other additives (dark blue line), with $\text{Ins}(1,3,4,5)\text{P}_4$ ($50 \mu\text{M}$) (peach line), and with SHIP2 inhibitor AS1949490 ($100 \mu\text{M}$) (light blue line).

reported to be substrates or inhibitors of SHIP2 activity. The rate of the intensity decrease slows down considerably in the presence of the natural substrate $\text{Ins}(1,3,4,5)\text{P}_4$, which, as shown in Figure 3, is 10 times more active in terms of phosphate release than any of the benzene phosphates. In the presence of the SHIP2 inhibitor AS1949490, there is an initial decrease in fluorescence intensity that matches that of the reaction with no additives indicating that, in the initial stages under these conditions, we are not seeing inhibition of SHIP2 activity with compound 6 $\text{Bz}(1,2,4,5)\text{P}_4$. This experiment is not an exhaustive analysis of the substrates competing for SHIP2 activity but serves to demonstrate the utility of benzene phosphate fluorescence in this context.

While there is much to take account of when designing such an experiment, we have proved the utility of TD-DFT to select from a regiosomeric family of benzene tris- and tetrakisphosphates for the compound with the best fluorescent properties to monitor 5-phosphatase, specifically SHIP2, activity. Interestingly, benzene phosphates appear to undergo successive dephosphorylations with SHIP2, in contrast to the single 5-dephosphorylation of inositol phosphate substrates. By judicious choice of a symmetrical tetrakisphosphate and careful titration of enzyme, we were able to limit the extent of reaction to a predominant single dephosphorylation. Interestingly, toward the end of the experiment shown in Figure 6A there is a slight shift to shorter wavelength again, most likely

reflecting further dephosphorylation of the $5\text{-OH-}\text{Bz}(1,2,4)\text{P}_3$ product.

To explore further the catalytic flexibility engendered in phosphate-substituted benzenes, we also sought to define the products of successive dephosphorylation of the more weakly fluorescent compound 4, $\text{Bz}(1,2,3,4)\text{P}_4$ (Figure S5), for which a number of potential dephosphorylation products are available. Accordingly, we included compound 7 3-OH-Bz(1,2,4) P_3 and the newly synthesized compounds 9 1,2-Di-OH-Bz(3,4) P_2 and 10 1,3-Di-OH-Bz(2,4) P_2 in our analysis (full details and structures of these compounds are given in Supporting Information). We did not detect the accumulation of compound 7, 3-OH-Bz(1,2,4) P_3 from compound 4 $\text{Bz}(1,2,3,4)\text{P}_4$. This does not rule out its production or that of 1-OH-Bz(2,3,4) P_3 as there is the possibility that the first product of hydrolysis is rapidly dephosphorylated to Di-OH-Bz P_2 products. Indeed, compound 9 1,2-Di-OH-Bz(3,4) P_2 = 3,4-Di-OH-Bz(1,2) P_2 was generated by SHIP2 from compound 4 $\text{Bz}(1,2,3,4)\text{P}_4$ (Figure S4) and, from Figure S1, we can see that compound 7 3-OH-Bz(1,2,4) P_3 is a better phosphate-releasing substrate than compound 4 $\text{Bz}(1,2,3,4)\text{P}_4$. Consistently, SHIP2 action on compound 7 3-OH-Bz(1,2,4) P_3 yielded multiple products including compound 9 1,2-Di-OH-Bz(3,4) P_2 = 3,4-Di-OH-Bz(1,2) P_2 , but not compound 10 1,3-Di-OH-Bz(2,4) P_2 = 2,4-Di-OH-Bz(1,3) P_2 (Figure S4). [2,3-Di-OH-Bz(1,4) P_2 was not available to test.] The intricacies of this analysis demonstrate further the advantages of a planned approach to selecting substrates with the most suitable properties for probing selected mechanisms of protein activity.

To summarize, with interest in the utility of benzene phosphates as ligands of diverse inositol/phosphoinositide-binding proteins/enzymes, we have shown that, uniquely among characterized ligands of 5-phosphatases, benzene phosphates have the properties of *bona fide* substrate analogs. Indeed, benzene tris- and tetrakisphosphates are inhibitors of Type I and Type II inositol polyphosphate 5-phosphatases,^{9,12} while compound 4 $\text{Bz}(1,2,3,4)\text{P}_4$ is a tight binding ligand of the PH domain of PKB/Akt.²² Compound 6 $\text{Bz}(1,2,4,5)\text{P}_4$ is an inhibitor of SHIP2¹⁰ and a structure was solved for a binary complex with INPPS2B.¹² Here we show that benzene phosphates are surrogate substrates of the canonical 5-phosphatase SHIP2 by virtue of their regiochemistry. This mimics well the stereoisomerism but not enantiomerism of inosides and phosphoinosides. Clearly these compounds could find utility with a range of inositol phosphate phosphatases beyond 5-phosphatases, e.g., 3-phosphatases typified by PTEN or 4-phosphatases such as SopB. The demonstration that they are substrates and can be used for real-time assays affords great opportunity for the study of allosteric regulation of catalytic activity by ligand binding to distal domains of the full-length protein.

Because of its relatively strong absorption and fluorescence emission, compound 6 $\text{Bz}(1,2,4,5)\text{P}_4$ is shown to be a promising spectroscopic probe for inositol 5-phosphatase(s). It may also be of use for proteins lacking catalytic activity since, as demonstrated, it is a good ligand of inositol phosphate-binding sites. Beyond this, the combination of TD-DFT and enzymological approaches could direct the synthesis of new probes for the study of specific aspects of protein structure and function. For real time assays, designed to screen potential inhibitors of protein activity, there is opportunity to design chromophores with enhanced, perhaps further red-shifted, fluorescence. Spectral separation from tryptophan, either in

excitation and/or emission, might enable polarization-based approaches to inhibitor screening of inositol phosphate-metabolizing enzymes as we have described with 2-FAM-InsP₅ for IPK1,¹³ but without the potential constraints of bulky fluorophore substituents. Probes might be synthesized to retain the ligand coordination of *bona fide* substrates and/or might afford a range of binding constants tailored for particular experimental scenarios, cf. ion-sensing fluorescent probes. Thus, we envisage our methods will enable prediction and testing of poly-substituted benzenes that retain the regiochemistry of a favored ligand while building in the spectroscopic opportunity of substitution with other functionalities. The diversification of simple aryl ligands to include other functionalities may further find use as structure-stabilizing ligands enabling crystallization of recalcitrant inositol-related protein targets.¹² In summary, there is wide-ranging potential in this field for the application of TD-DFT to aid decisions over probe synthesis and experiment design.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.9b00368](https://doi.org/10.1021/acsmedchemlett.9b00368).

Experimental Procedures including chemical syntheses, protein purification, analytical methods, and TD-DFT calculations. Figure S1: Assay of benzene phosphates and hydroxybenzene phosphates as substrates of SHIP2. Figure S2: HPLC separation of benzene phosphates. Figure S3: HPLC of SHIP2 action on compound 6 Bz(1,2,4,5)P₄. Figure S4: Tandem UV-fluorescence HPLC assay of SHIP2 action on compound 6 Bz(1,2,4,5)P₄. Figure S5: HPLC separation of compound 4 Bz(1,2,3,4)P₄, compound 7 3-OH-Bz(1,2,3)P₃, compound 9 1,2-Di-OH-Bz(3,4)P₂, and compound 10 1,3-Di-OH-Bz(2,4)P₂. TD-DFT: Results, Tables, and Figures ([PDF](#))

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Author Contributions

These authors contributed equally to this work. G.W., K.B., H.W., C.P., S.J.M. and C.A.B. performed experiments. G.W., V.O., S.J.M., A.M.R., B.V.L.P., and C.A.B. designed the study and wrote the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

2-FAM-InsP₅, 2-O-(2-(5-fluoresceinylcarboxy)-aminoethyl)-*myo*-inositol 1,3,4,5,6-pentakisphosphate (triethylammonium salt); 3-OH-Bz(1,2,4)P₃, 3-hydroxybenzene 1,2,4-trisphosphate; 5-OH-Bz(1,2,4)P₃, 5-hydroxybenzene 1,2,4-trisphosphate; 1,2-Di-OH-Bz(3,4)P₂, 1,2-dihydroxybenzene-3,4-bisphosphate; 1,3-Di-OH-Bz(2,4)P₂, 1,3-dihydroxybenzene-2,4-bisphosphate; 2,3-Di-OH-Bz(1,4)P₂, 2,3-dihydroxybenzene-1,4-bisphosphate; BzP, benzene phosphate; Bz(1,2,3)P₃, benzene 1,2,3-trisphosphate; Bz(1,2,4)P₃, benzene 1,2,4-trisphosphate; Bz(1,3,5)P₃, benzene 1,3,5-trisphosphate; Bz(1,2,3,4)P₄, benzene 1,2,3,4-tetrakisphosphate; Bz(1,2,3,5)P₄, benzene 1,2,3,5-tetrakisphosphate; Bz(1,2,4,5)P₄, benzene 1,2,4,5-tetrakisphosphate; EDTA, ethylenediamine tetra-acetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; His, histidine; HOMO, highest occupied molecular orbital; HPLC, high-pressure liquid chromatography; IC₅₀, half-maximal inhibitory concentration; INPP5A, type I inositol 5-phosphatase; INPP5B, type II inositol 5-phosphatase; INPPSE, inositol polyphosphate 5-phosphatase E; Ins(1,4,5)-P₃, inositol 1,4,5-trisphosphate; Ins(1,3,4,5)P₄, inositol 1,3,4,5-tetrakisphosphate; LUMO, lowest unoccupied molecular orbital; OCRL-1, Lowe oculocerebrorenal syndrome protein (INPPSF); PKB, Protein Kinase B; PtdIns(3,4)P₂, phosphatidylinositol 3,4-bisphosphate; PtdIns(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; PtdIns(3,4,5)P₃, phosphatidylinositol 3,4,5-trisphosphate; SYNJ1, synaptjanin-1; SYNJ2, synaptjanin-2; SHIP1, SH2-domain containing inositol 5-phosphatase type 1; SHIP2, SH2-domain containing inositol 5-phosphatase type 2; TCEP, tris(2-carboxyethyl) phosphine; TD-DFT, time-domain density functional theory; TEV, Tobacco Etch Virus

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