Fluoresceininated Aminohexanol Tethered Inositol Hexakisphosphate: Studies on Arabidopsis thaliana and Drosophila melanogaster and Docking with 2P1M Receptor

Sujeet Kumar Thakur, Krishnendu Goswami, Pallavi Rao, Shivam Kaushik, Bhanu Pratap Singh, Pinky Kain, Shailendra Asthana, Saikat Bhattacharjee, Prasenjit Guchhait, and Sambasivan V. Eswaran*

ABSTRACT: Inositol hexakisphosphate (InsP₆; phytic acid) is considered as the second messenger and plays a very important role in plants, animals, and human beings. It is the principal storage form of phosphorus in many plant tissues, especially in dry fruits, bran, and seeds. The resulting anion is a colorless species that plays a critical role in nutrition in many plant tissues, especially in dry fruits, bran, and seeds. The animal, and human beings. It is the principal storage form of phosphorus and is believed to cure many diseases. A fluoresceininium aminohexanol tethered inositol hexakisphosphate (III) had been synthesized earlier involving many complicated steps. We describe here a simple two-step synthesis of (III) and its characterization using different techniques such as matrix-assisted laser desorption ionization mass spectrometry, tandem mass spectrometry, and Fourier transform infrared, ultraviolet–visible, ultraviolet-fluorescence, ¹H nuclear magnetic resonance (NMR), and two-dimensional NMR spectroscopies. The effect of (III) has been investigated in the model systems, Arabidopsis thaliana and Drosophila melanogaster. Using Schrodinger software, computational studies on the binding of (III) with the protein 2P1M (Auxin-receptor TIR1-adaptor ASK1 complex) has revealed strong binding propensity with this compound. These studies on the fluoresceininium tethered phytic acid could have far reaching implications on its efficacy for human health and treatment of diseases (cancer/tumor and glioblastoma) and for understanding phosphorous recycling in the environment, especially for plant systems.

INTRODUCTION

Inositol hexakisphosphate (InsP₆; phytic acid) is found in significant amounts in dry fruits, beans, seeds, rice, wheat, and many other edible foods.¹⁻⁷ It is also referred to as the "second messenger" in cellular systems, though 1,4,5-inositol trisphosphate is considered as the "real messenger." Its potential to cure many diseases based on its powerful antioxidant property has been highlighted.² Its efficacy against cancers via inhibition of metastasis, enhancing Nk cells, increasing tumor suppressor p53 gene activity, and apoptosis has been noted.³ Now a days, there is increasing discussion about inositol pyrophosphate, InsP₇, and its biological application as a modulator of protein functions.⁴⁻⁵

InsP₆ is popular in alternative therapy and is recommended to be taken on an empty stomach. Branded versions ("Cell Forte" and "Vita Cost") are commercially available online.⁶ The ability of InsP₆ to chelate ions, especially iron, has been associated with the treatment of iron-dependent neural degeneration. It has been reported that the viability of cells of glioblastoma, a deadly brain tumor, decreases following treatment with increasing doses of InsP₆, and it has also been demonstrated that InsP₆ is taken up by tumor cells and causes inhibition of their growth.⁷

These observations substantiate the popular perception in the general public that consuming dry fruits such as almonds,⁹ and so forth rich in InsP₆ is beneficial against major diseases. The only question on their mind is how much dry fruits should one consume on a daily basis? Excess of anything is bad, and it is clear that consuming excess of InsP₆-containing dry fruits could, via chelation, sequester important cations/minerals, which could be detrimental to human health. Inositol cyclic-phosphate has been implicated in diabetes type II.¹⁰ InsP₆ is involved in plague through activation of acetyl transferase activity of AvrA-YopJ protein, which deregulates MAPK and NFkB, affecting the innate immune activity.¹¹ Studies have been carried out on Arabidopsis thaliana in relation to myo-inositol phosphate synthase 1 gene (MIPS1) in plants.¹² The important role of PP-InsP₆ in plant physiology in

http://pubs.acs.org/journal/acsofd

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ACS Omega 2020, 5, 9585−9597
https://dx.doi.org/10.1021/acsomega.0c00961
terms of plant hormones and inorganic phosphate signaling along with its emerging role in bioenergetics homeostasis have been highlighted.\textsuperscript{13,14} Overexpression of phytic acid improves the plant growth under osmotic condition via stimulation of enzymatic and non-enzymatic antioxidant systems and its regulatory role in phosphate homeostasis; phytic acid may be also involved in fine tuning osmotic stress response in plants.\textsuperscript{15}

In a recent study, biotinylated inositol hexakisphosphate\textsuperscript{16} was used to study DNA double-strand break repair and the involvement of nonhomologous end joining factor. A recent book on the subject links it to agriculture and environment.\textsuperscript{17} It is well known that monogastric animals cannot digest phytate (salt of myo-inositol hexakisphosphate), and supplementing phosphate-rich seed diet for pigs and poultry leads to environmental pollution and also caused calamities such as the Chesapeake Bay tragedy in U.S.A and in Gippsland, Australia, the areas often associated with large cattle populations. Supplementing animal diets with microbial phytase is the most successful for controlling such pollution.\textsuperscript{18}

Phytic acid is the hexa phosphoric ester of myo-inositol hexakisphosphate (InsP\textsubscript{6}).\textsuperscript{19} Previously, myo-inositol hexakisphosphate (InsP\textsubscript{6})\textsuperscript{20} was shown to bind to DNA-PK and stimulate end joining in vitro.\textsuperscript{21} InsP\textsubscript{6} also stimulates the joining of complementary DNA ends in a cell-free system. Moreover, the binding data suggested that InsP\textsubscript{6} bound to DNA-PKcs (not to Ku).\textsuperscript{22} Furthermore, the bindings of DNA ends and InsP\textsubscript{6} to Ku are independent of each other. Thus, InsP\textsubscript{6} could be useful as a marker for in vitro and in vivo plant and animal cell systems.\textsuperscript{23}

Synthesis, biochemistry, and therapeutic potential of “inositol phosphate” and its derivatives have been compiled.\textsuperscript{24–26} Phytic acid is the hexaphosphoric ester of 1,2,3,4,5,6-hexahydroxy cyclohexane. Because of ring inversion of the cyclohexane ring, it can exist in two different conformations, one having the phosphate group at position-2 axially oriented and the other five-phosphate groups oriented equatorially.\textsuperscript{27}

This conformer could coexist with the other conformer [having the five hydroxyl/phosphoric ester groups being oriented axially (ax.) and just one group being equatorially (eq.) oriented]. These authors stated that “there is no interconversion between the 1 ax/5 eq and 5 ax/1 eq conformers, except at intermediate pH of 9.0–9.5”.\textsuperscript{28}

In the current paper, an attempt has been made to answer the following questions:

- Can the title compound (III) be synthesized without any protection/deprotection steps?
- How can compound (III) be characterized? Would mass spectrometry (MS) and nuclear magnetic resonance (NMR) [\textsuperscript{1}H NMR, \textsuperscript{13}C NMR, and two-dimensional (2D)-NMR] be more useful for this purpose?
- Can the special reactivity of the axially oriented phosphoric acid at position 2 of the cyclohexane ring in InsP\textsubscript{6} be exploited? Can it be selectively esterified with the hydroxyl group of aminohexanol tethered to fluorescein? Can it happen for the complete exclusion of all other five equatorially oriented phosphoric acids in InsP\textsubscript{6}?
- Will (III) be internalized by A. thaliana?
- Will (III) be taken up by Drosophila melanogaster and would it be involved in the growth and development cycle of the fruit fly through the stages, viz. eggs, larvae, and pupae to the adult fruit fly?
- Would (III) “dock” well with the proteins PDB 2P1M and IPMQ, both of which are relevant to InsP\textsubscript{6}? Will the Schrodinger docking software tools be useful for this study?

Two decades ago, Prestwich’s group\textsuperscript{29–31} carried out a very complicated multistep synthesis and purification\textsuperscript{32} of fluoresceinated aminohexanol tethered InsP\textsubscript{6} (III). A more recent synthesis of a similar fluoresceinated InsP\textsubscript{6} with a much smaller side-chain and with a more stable ether linkage, though somewhat shorter, requires the attention of a specially trained and experienced organic chemist. Based on the special high reactivity of the exposed axially oriented phosphate group at position 2 in InsP\textsubscript{6}, we hypothesized that a very simple synthesis of (III) could be undertaken, which could be handled even by an ordinary laboratory attendant. Such a simple two-step synthesis is described in this paper.

Our compound (III) described in this paper is homogenous as shown by preparative thin-layer chromatography (P-TLC); mass spectral data m/z = 1156.9. The NMR coupling constant (as shown in Table 1) for coupling in our case is 9.7 Hz.

Table 1. Composition of the Medium for Growth Studies on D. melanogaster

| s. no. | components | 250 mL | 1 L |
|-------|------------|--------|-----|
| 1     | corn flour | 20 g   | 80 g|
| 2     | D-glucose  | 5 g    | 20 g|
| 3     | sugar      | 10 g   | 40 g|
| 4     | ager       | 2 g    | 8 g |
| 5     | yeast powder| 3.75 g | 15 g|
| 6     | propionic acid | 1 mL | 4 mL|
| 7     | TEGO (dissolved in ethanol) | 0.3125 g | 1.25 g (diss in 3 mL ethanol) |
| 8     | orthophosphoric acid | 150 μL | 600 μL |

aTEGO = trade name for methyl-para-hydroxy benzoate, an antimicrobial preservative in foods.

Further, our experiments have been done in D\textsubscript{2}O at (pH = 7) and not at alkaline pH. Thus, compound (III) represents the preferred axial conformer without any interconversion to the other conformer.

## RESULTS AND DISCUSSION

### Characterization of (III)
Fluoresceinated amino hexanol tethered inositol hexakisphosphate (III) had been synthesized previously by a complex multistep synthesis. In this paper, we report a simple two-step synthesis of (III) (Scheme 1), whose three-dimensional (3D) structure is shown in Figure 1.

**Earlier Mass Spectral Studies on Phytic Acid.** The mass spectrum of phytic acid shows the molecular ion peak at m/z 658.823. In its “tandem” MS/MS spectrum it successively loses meta phosphoric acid (98 amu) and loss of (80 amu) observed at m/z (460.90) for loss of meta phosphoric acid (loc. cit.). Electrospray ionization (ESI)-MS and MS/MS of phytic acid show the [M−2H]\textsuperscript{2+} ion, and this has been used to confirm the fragmentation pattern of phytic acid. It was concluded that ESI-high-resolution mass spectrometry of inositol phosphates is unusual and highly “characteristic” and can be used for “the detection of the compound in environmental matrices” and “soils and manures.”\textsuperscript{33} The authors also state that these studies...
are "complicated by the potentially labile elimination of meta phosphoric acid" HPO$_3$. Despite the mass spectra of InsP$_6$ being complicated, these could be used for "the exploration of organic phosphorous cycling in the environment."

**MALDI-MS and MS/MS of Compound (III).** Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) studies of compound (III), purified by P-TLC, was done using methanol solution and is shown in Figure 2. In this mass spectrum, the peak of $m/z$ 1156.9 is observed for compound III (C$_{33}$H$_{41}$NO$_{30}$P$_{6}$ + potassium, K), and the calculated value is 1156.1122 so that the mass error percent is 0.78, which shows the successful conjugation between fluorescein and phytic acid using the linker molecule aminohexanol. Underivatized phytic acid shows M$^+$ at $m/z$ 658.823, the base peak, and in the MS/MS spectrum of the $m/z$ 658.823 peak, a loss of phosphate meta phosphoric acid (98 amu) is observed at $m/z$ 60.92. This is followed by another loss (80 amu) observed at $m/z$ 460.90 for loss of HPO$_3$ (loc. cit). MS/MS spectrum of the peak $m/z$ 1082.1783 results in a calculated value of 1081.9057 for C$_{33}$H$_{37}$NO$_{28}$P$_{6}$ $m/z$ (error percent is 0.02%), 1066.2053 gives calculated for C$_{33}$H$_{39}$NO$_{28}$P$_{6}$ $m/z$ value 1065.9108 + 1H equal to $m/z$ 1066.9186 (error percent is 0.06%), and 1051.8262 gives calculated for C$_{33}$H$_{38}$NO$_{28}$P$_{5}$ $m/z$ value 1051.9492 (error percent is 0.01%). M$^+$ peak 1117.9363 for calculated peak C$_{33}$H$_{41}$NO$_{30}$P$_{6}$ − 2 $\times$ H$_2$O showed the most intense peak at $m/z$ 893.0007 (loss of P$_{3}$O$_{6}$ unit $m/z$ 188.8908 amu error percent is 0.0007%). MS/MS spectra of the $m/z$ 1066.2053 and 1051.8262 peaks are all shown in (Figure S1).

![Scheme 1. Synthesis of Compound (III)](image)

![Figure 1. (a) 3D structure of (III). (b) P-TLC plate showing band for (III). (c) Fluorescence in a test tube containing a solution of (III) in methanol [seen under an ultraviolet (UV) lamp, 366 nm]. (d) Fluorescence of (III) in a Petri dish under ordinary visible light.](image)

![Figure 2. MALDI-MS spectrum of (III).](image)
Ultraviolet–Visible Studies on (III). UV–visible (UV–vis) spectrum of (III) showed peaks at 427.59 and 482.01 nm (Figure 3a). Its fluorescence spectrum shows a large shift toward higher wavelengths (Figure 3b).

Fourier Transform Infrared Studies on Compound (III). Fourier transform infrared (FT-IR) spectrum of (III) recorded in methanol at room temperature is shown in Figure S2. The FT-IR spectral analysis showed peaks at 1072.75, 1109.56, 1537.37, 1581.98, 1616.17, and 3389.64 cm\(^{-1}\).

Figure 3. (a) UV–vis spectrum of compound (III). (b) Fluorescence spectrum of (III).

Figure 4. COSY spectrum of (III) showing correlation of aromatic protons at \(\delta\) 8.566 and 8.676 with CH\(_2\)–NH peaks at \(\delta\) 3.338 and 3.374 ppm.

Figure 5. Expanded signals in the COSY spectrum of (III) at \(\delta\) 3.338 and 3.374 correlating with signals at \(\delta\) 8.566 and 8.676 ppm.
Earlier NMR Studies on Phytic Acid. Experimental data based on earlier NMR studies of phytic acid (loc. cit) have shown that for 1 ax/5 eq conformation, \( J_{1,2} = J_{2,3} = 2.0 \pm 0.2 \text{ Hz, expt.} \) (1.7 Hz, predicted); \( J_{3,4} = J_{4,5} = 9.6 \pm 0.2 \text{ Hz, expt.} \) (9.6 Hz predicted). For the 5 ax/1 eq conformation (Figure S3), \( J_{1,2} = J_{3,4} = 2.2 \pm 0.2 \text{ Hz, expt.} \) (2.4 Hz predicted); \( J_{4,5} = 1.7 \pm 0.2 \text{ Hz, expt.} \) (0.7 Hz predicted).

\( ^1H \) NMR Spectral Studies on Compound (III). \( ^1H \) NMR of compound (III) was recorded in D_2O (and CD_3OD), and the expanded spectra of aliphatic and aromatic regions are shown in (Figures S3–S5). The \( ^1H \) NMR spectrum of (III) can be divided into three distinct regions; the aliphatic CH\(_2\) region, the cyclohexane hydrogen region, and the aromatic region of the fluorescein moiety. The four aliphatic methylene groups are observed at \( \delta 1.35 \) and 1.67 ppm.

The other two methylene groups are observed at \( \delta 4.23 \) (b, 2H, CH\(_2\)–O– unit; Figure 8) and \( \delta 3.338–3.374 \) (t, 2H, CH\(_3\)–NH unit; based on the correlation in the correlation spectroscopy (COSY) spectrum). Figures 4 and 5 show the correlation of aromatic protons at \( \delta 8.566 \) and 8.676 with CH\(_3\)–NH peaks at \( \delta 3.338 \) and 3.374 ppm.

The cyclohexane ring protons are observed at \( \delta 3.422 \) (s, 1H, 2-H) and 3.338 to 3.378 (5H, H-1, H-3, H-4, H-5, H-6). The nine aromatic protons of the fluorescein ring are observed at \( \delta 3.338 \) and 3.374 ppm.

Total correlation spectroscopy (TOCSY) spectrum of (III) shows correlation of the \( \delta 4.23 \) ppm signal of the CH\(_2\)–O hydrogens with the protons of the cyclohexane ring, as shown in (Figure S6).

2D-NMR COSY spectrum of (III) in the fluorescein unit; the region \( \delta 6.4 \) to 8.8 is shown in Figure 6. On this basis, assignments have been made which are discussed below.

It is seen that the signals at \( \delta 6.787 \) and 7.220 are correlated and hence coupled to each other, showing that they are ortho-placed with respect to each other and thus assigned to H-7', and H-6', respectively. Similarly, the two signals at \( \delta 7.427 \) and 7.977 are correlated and hence coupled to each other, showing that they are also ortho-placed with respect to each other and thus assigned to H-4' and H-5', respectively. It can also be seen that the two signals at \( \delta 8.566 \) and 8.676 are correlated and hence coupled to each other, showing that they are ortho-placed with respect to each other and thus assigned to H-5' and H-4', respectively. The three signals at \( \delta 6.874, 7.609, \) and 8.239 are observed as singlets and thus assigned to H-9', H-2', and H-2', respectively. Our NMR data are compared with values from the literature in the Supporting Information (Table S1).

Thus, P-TLC shows that compound (III) is homogenous; the mass spectral data, other spectroscopic data (FT-IR, UV–vis, and UV fluorescence), and more particularly the NMR/2D NMR data confirm the structure of (III).

Our NMR spectral studies have been done in neutral pH and not alkaline pH, and as quoted earlier, no cyclohexane ring inversion is expected to be observed. The coupling constants and more particularly the value of \( J_{1,6} \) of 7 Hz (lit. value of \( J = 9.6 \text{ Hz} \)) clearly establish the structure of (III) unequivocally.

Studies on the Model Plant A. thaliana Using (III). Compound (III) is Internalized by Root and Leaf Cell of A. thaliana Seedlings. Recently, internalization of InsP_6 by H1299 has been demonstrated. \(^{34a,b}\) InsP_6 is a highly charged molecule and therefore cannot freely pass the cell membrane because neither plants nor animals have a plasma membrane transporter for InsP_6. The authors demonstrated that extracellular InsP_6 enters the cell by a micro-endocitosis like mechanism. Once inside the lysosome (by fusion of the endocitic vesicle), InsP_6 could be dephosphorylated by a specific phosphatase MINPP1 to myo-inositol, which can then be transported inside the cell cytosol. Synthesis of membrane permeable diphosphate-containing inositol polyphosphate or inositol pyrophosphates and their cellular uptake has already been reported. To check whether compound (III) would be similarly taken up by A. thaliana, its seeds were grown with compound (III) for 7 days. As the control, seeds were also grown with fluorescein. Uptake of fluoresceinated InsP_6 or fluorescein was analyzed using a Zeiss SPS confocal micro-

Figure 6. Double quantum filtered-COSY spectrum of (III).
The characteristics of the channel used were as follows: argon laser; excitation is at 490 nm; detector gain 320. Cellular uptake by a cell is defined by the detection of the fluorescence signal in root and leaf mesenchymal cells. Fluorescence signal was not detected from fluorescein-treated seedlings (Figure S), but seedlings treated with compound (III) show a clear accumulation of fluoresceinated InsP₆ (Figure 7a,b).

**Chlorophyll Content of A. thaliana Seedlings Were Induced under Fluoresceinated InsP₆ Containing Media.** Extensive role of inositol triphosphate (InsP₃) on chloroplast development has been reported earlier. To find out, whether fluoresceinated InsP₆ plays any such role, we analyzed A. thaliana seedlings grown under either fluoresceinated InsP₆ or fluorescein. Interestingly we could see that seedlings grown with fluoresceinated InsP₆ are more greenish and contain a significant increase in the amount of chlorophyll compared to seedlings grown with fluorescein alone (Figure 8). Considering that seedling development requires inositol hexakisphosphate, our observations suggest the stimulating role of fluoresceinated InsP₆ on chlorophyll synthesis.

Expression of ICS1-transcripts in the seedling assay is shown in Figure 9.

**Salicylic Acid Biosynthesis Gene ICS1 (Isochorismate Sythase 1) Expression Is Upregulated with Fluoresceinated InsP₆.** Inducing defense responses upon phosphate supplementation was shown earlier. The rice phosphate transporter protein OsPT8 regulates disease resistance and plant growth. We discovered that the transcript level of ICS1 is upregulated in seedlings grown with fluoresceinated InsP₆ compared to fluorescein control (Figure 14). Indeed, ICS1 was found to be involved in disease resistance in A. thaliana and other plants. Salicylic acid (SA) is a potent immune signaling molecule. These results suggest that fluoresceinated InsP₆ helps in inducing defense responses in A. thaliana.

**Defense Signaling Regulator EDS1 (Enhanced Disease Susceptibility 1) Transcripts Are Upregulated by Fluoresceinated InsP₆.** Recent understanding of plant immune signaling pathways genetically place SA and enhanced disease susceptibility 1 (EDS1) downstream of resistant protein activation. Previously, our assays demonstrated upregulated ICS1 upon fluoresceinated InsP₆ treatment. To determine if EDS1 played a role in the same context, we checked the EDS1 protein levels upon each treatment. We identified significant accumulation of EDS1 protein in fluoresceinated InsP₆-treated seedlings compared to fluorescein controls (Figure 10). Taken together, these results suggest that, indeed, fluoresceinated InsP₆ augments defense responses in A. thaliana.

**Studies on the Model System Adult D. melanogaster Using (III).** Feeding response of D. melanogaster on new

![Figure 7](image-url)  
**Figure 7.** Uptake of fluoresceinated InsP₆ through the root system (a) and leaf cell of A. thaliana seedlings (b). Shown are the fluorescence and bright-field representative images at 400-fold magnification. The scale bar is 50 μm.

![Figure 8](image-url)  
**Figure 8.** (a) A. thaliana seedlings were grown with fluorescein or fluoresceinated InsP₆ containing nutrient media. (b) Chlorophyll content was measured on fluorescein- and fluoresceinated InsP₆-treated seedlings. * represents the statistical significant difference (P < 0.0001) in the expression level between treated and untreated samples.

![Figure 9](image-url)  
**Figure 9.** Expression of ICS1-transcripts. Whole seedlings of each treatment were collected for total RNA extraction followed by qRT-PCR analysis (n = 3). * represents the statistical significant difference (P < 0.0001) in the expression level between treated and untreated samples.
fluoresceinated aminohexanol tethered inositol hexakis phosphate (III) and its effect on ingestion.

To study the effect of this new fluoresceinated aminohexanol tethered inositol hexakisphosphate on fruit flies, adult *D. melanogaster* were fed on this compound. Fluorescence was observed in the abdominal region of flies that ingested the compound. The flies which did not ingest the compound did not show fluorescence in any region. As most of the flies ingested the compound, it shows that the flies can easily feed on it and ingest it and might have a taste for it as shown in Figure 11. Flies feeding on the compound in the presence of 100 mM sucrose compared to just the fluoresceinated aminohexanol tethered inositol hexakis phosphate feeding alone concentration is shown in Figure 11. Since not much difference is seen in the intensity and feeding behavior between 0.25 and 0.5 mg/mL concentration, we later used 0.25 mg/mL concentration only for all assays.

The graphical representation of the feeding preferences of adult *D. melanogaster* on different concentrations of fluoresceinated aminohexanol tethered inositol hexakis phosphate tested is shown in Figure 12 at different concentrations.

Significance of the flies feeding preference is more toward lower concentration of 0.25–0.5 mg/mL of the compound. A small percentage of the flies did not feed on the compound at any concentration. The T test values of 0.5 and 0.25 clearly show that there is a difference in the absence of sucrose.

**Effect of (III) on the Drosophila Larval Feeding.** The larvae of *D. melanogaster* also showed a feeding preference on the fluoresceinated aminohexanol tethered inositol hexakis phosphate (F) mixed with agar (A). The fluorescence intensity in larvae after ingestion of fluoresceinated aminohexanol tethered inositol hexakis phosphate (F) mixed with 1% agar compared to the compound mixed with agar (A) and sucrose (S) were more or less similar, indicating that larvae feeding on the fluoresceinated aminohexanol tethered inositol hexakis phosphate (F) can feed on the compound even in the absence of sucrose (Figure 13a–c).

Larval molting was also not affected in general in the presence of fluoresceinated aminohexanol tethered inositol hexakis phosphate as seen on 1% agar media with and without sucrose.

In the “larval feeding assay” on the basis of T test analysis, we observe that high feeding is observed in the absence of sucrose.

**Effect of Compound (III) on the Development of *D. melanogaster***. The effect of fluoresceinated aminohexanol tethered inositol hexakisphosphate in nutrient media (nutrient media 1 and nutrient media 2) and without the fluorescent label (F + nutrient media 1 and F + nutrient media 2) was studied. No differences in the development of larvae or pupae are found in the presence or absence of the compound.

We also collected and counted eggs on 1% agar with and without the compound, as shown in Figure S7. We have observed that in the presence of the fluoresceinated compound, egg laying has increased.

**Effect of Compound (III) on the Pupae of *D. melanogaster***. The fluorescence was absent in pupae from normal media when compared to pupae with normal media mixed with fluoresceinated aminohexanol tethered inositol hexakisphosphate (F). These results indicate that the compound does not degrade and stays even in the pupal stages (Figure 14a,b).

Thus, the above-mentioned result confirms the interaction and retention of compound (III) in pupae and the positive response of the life cycle of the flies.

**Effect of Compound (III) on the Development of Flies of *D. melanogaster***. During our experiments, we observed that flies can easily lay eggs in the presence of fluoresceinated aminohexanol tethered inositol hexakis phosphate and larvae can easily feed on it. We also observed fluorescence in the pupae and then later in the adult flies. Compared to...
Fluoresceinated aminohexanol tethered inositol hexakis phosphate (F) mixed with normal media, only minimal background fluorescence was observed in normal media flies. Later, we counted the number of pupae that eclosed as adult flies, as shown in the graphical representation of the number of pupae, and the number of pupae that did not evolve as flies (Figure 15 a−e). Our data suggest that fluoresceinated aminohexanol tethered inositol hexakis phosphate has a significant effect on the pupal development and eclosion.

Thus, in vivo and significance effect were captured by the different microscopic techniques and different concentrations and at different stages such as eggs, larvae, pupae, and at the adult state. This also shows that the interaction with compound (III) provides better effect on the flies and the enhancement of their life cycle.

In the future work, we plan to confirm these claims by coupling with biochemical confirmation, for example, by MS analyses with root extracts treated with the InsP$_6$-fluorescent conjugate or by analyzing the control and treated extracts by polyacrylamide gel electrophoresis (PAGE) coupled with TiO$_2$ enrichment. A green chemistry method for carrying out the CuO nanoparticle-catalyzed click reaction with very high yields has been described. Similar experiments could also be taken up using *D. melanogaster*. InsP$_6$ is a direct precursor for inositol pyrophosphates, such as InsP$_7$. These pyrophosphates are fundamental to a large number of biological processes. Therefore, in the future work, it would be interesting to see whether the IP$_6$-conjugate can be used as a substrate by InsP$_6$K-type proteins to generate fluoresceinated-IP$_7$.

*D. melanogaster* is a widely used model organism for research purposes. It only has four chromosomes which makes the study easier. Its life cycle is of around 10−12 days. Differentiating male fruit flies from female flies is easy. Because of their small size, *Drosophila* can easily be stored in a laboratory. The food, present in the abdominal area, can be detected by viewing under a microscope.

All above-mentioned studies indicate that after feeding of the phytic acid derivative, the fluorescence can be easily assessed in the gut. May be fluoresceinated aminohexanol tethered inositol hexakis phosphate (III) has the potential to be used as a tag for studies to mark the neurons or for looking at the effect of various compounds that are hazardous for human health.

**Docking of (III) with the PM1K Receptor Using the Schrodinger Software Suite.** Bioinformatics studies on (III) has been performed using the Schrodinger software suite.
In these studies, the docking cavity and its corresponding energy were taken into account. The study involved the Auxin receptor TIR1 in a complex with the adaptor ASK1 (PDB code 2P1M). Three different protein crystal structures 2P1M, 1N4K, and 4ZAI were taken for molecular docking studies. First, axial and equatorial ligands were docked to the 2P1M protein. For the axial conformer, the docking energy is $-5.01$ kcal/mol, and for the equatorial conformer, the docking energy is $-7.24$ kcal/mol. The binding site residues for axial and equatorial are mostly common though their binding orientation is different. The common amino acid residues at the binding site are R111, S138, K113, R114, R164, R344, R403, R401, R436, R435, K485, R484, K74, E72, F49, K47, K25, D24, and L23. In the case of the axial conformer, some residues are distinct E109, R509, V48, V71, and S70. Because of different orientations of the axial and equatorial conformers, the equatorial mode of the ligand is shown apart from many common residues such as W320, R318, E165, E342, L378, S37, H78, G51, R560, R509, M460, and E459 and other set of residues as well. Hydrogen bond, van der Waal, and pi–pi interactions are responsible for the high binding affinity. The residues involved in hydrogen bonding are R436, R114, K113, K485, R484, and R509, whereas for the equatorial conformer, the residues are K113, R344, R403, R401, R436, R435, K485, K74, E72, K25, and E459, and pi–pi stacking is observed with F49 shown in Figure S8. From the residue-wise analysis, the docking data shows that the binding of the equatorial conformer is much better than that of the axial conformer. The binding site derived from docking studies using Schrodinger software is shown in Figure 16, which confirms that fluoresceinated aminohexanol tethered inositol hexakis phosphate (III) binds well to protein 2P1M.

**CONCLUSIONS**

Fluoresceinated aminohexanol tethered inositol hexakisphosphate compound (III) has been prepared by a facile and convenient two-step synthesis unlike previous synthetic methods, which involved multistep synthesis and many protection/deprotection steps. This beautiful, green fluorescent compound has been characterized by modern spectroscopic techniques (FT-IR, UV–vis, and NMR studies). 2D NMR spectral studies have been used to make assignments for individual hydrogen atoms in the molecule. Its mass spectrum (M+, m/z = 1156.9) helped nail the molecule. In the UV–fluorescence spectrum ($\lambda_{\text{max}}$ 457.28–487.01 nm), a bathochromic shift of 29.93 nm was observed.

We detected internalization of fluoresceinated aminohexanol tethered inositol hexakisphosphate compound (III) into the root and leaf cellular systems and its subsequent enhancement of the chlorophyll content in whole seedlings. Therefore, compound (III) has been successfully demonstrated by us as a growth enhancer on the model plant A. thaliana. The insect ingested the compound, and fluorescence was carried over to the eggs, larvae, and pupae, showing that it survives these stages in the fly’s development cycle. The data generated in this paper could be useful for studying the role of InsP$_6$ in intact cells. InsP$_6$ is a direct precursor for inositol pyrophosphates, such as InsP$_7$. These pyrophosphates are fundamental to a large number of biological processes. The importance of InsP$_6$ in environmental studies, soils, and phosphorous recycling is also foreseen.
EXPERIMENTAL SECTION

Materials and Methods. Synthesis of (III). N-Hydroxy succinimidyl fluorescein (40 mg, 0.931 mmol) and 1-amino-6-hexanol (10 mg, 0.854 mmol) were taken in a flask with 20 mL of dichloromethane. For increasing the solubility, 10–15 drops of methanol was also added into the vessel, and the solution was stirred using a magnetic needle on a magnetic stirrer overnight at room temperature.

After overnight stirring, the solvent was evaporated using a Heidolph rotary evaporator. Phytic acid (56 mg, 0.848 mmol) along with dicyclohexyl carbodiimide (DCC) (16 mg, 0.776 mmol) were then added followed by dichloromethane (50 mL) and again stirred overnight at room temperature using a magnetic needle.

Any solid (DCC urea) obtained at this stage was filtered away; the solvent was removed by evaporation, when a yellowish orange colored solid was obtained, which was dried over anhydrous phosphorous pentoxide in a vacuum desiccator overnight. The yield was 72 mg (Scheme 1). Mass spectrum of (III) showed the molecular ion peak, M+ at m/z 1156.9, which represents its molecular weight.

Experimental Methods for Studies on A. thaliana Using (III). Plant growth conditions: Seeds of A. thaliana Columbia (Col-0) were stratified in the dark at 4 °C for 3 days. The seeds were then surface sterilized with 30% bleach solution and rinsed three times with sterile water. Murashige and Skoog agar media-containing Petidishes (0.5X Murashige and Skoog, 0.5% sucrose, 1% phytoagar, pH 5.7) were prepared either with 0.5% sucrose or with 10 μM fluorescein or with 10 μM fluoresceinated amino-hexanol tethered inositol hexakisphosphate (fluorescein InsP6).

After plating the seeds, the plates were sealed with a parafilm and placed in a growth room for a short day (12 h dark:12 h light) photoperiod cycle with a constant temperature of 22 °C for 7 days.

Analysis of Cellular Uptake of Fluoresceinated InsP6 in A. thaliana Seedlings. Uptake of fluoresceinated InsP6 or fluorescein was analyzed using a Zeiss SPS confocal microscope. The characteristics of the channel used were as follows: argon laser; excitation 490 nm; detector gain 320. Seven day old seedlings grown with fluoresceinated tethered inositol hexakisphosphate (fluorescein InsP6).

Measurement of Chlorophyll Content. Chlorophyll extraction using dimethyl sulfoxide. One hundred milligram of seedlings was taken into a vial containing 7 mL of dimethyl sulfoxide (DMSO) (Sisco research Lab Pvt. Ltd.) and incubated for 6 h at 65 °C. After a short spin, the supernatants were transferred to another tube, and the volume was made up to 10 mL with DMSO.

Determination of chlorophyll content. From the prepared DMSO extraction, 3.0 mL of extract was transferred to a cuvette, and the optical density values were measured at 645, 663, and 652 nm using a spectrophotometer with DMSO as the blank. Chlorophyll content was then calculated according to the Arnon equation.45

RNA isolation and qPCR analysis. Seven day old seedlings were grown in liquid nitrogen, and total RNA was extracted with RNA iso Plus (Takara) according to the manufacturer’s instruction. Extracted RNA was treated with TURBO DNA-free Kit (Invitrogen) to inactivate the DNase and was quantified using a nano-spectrophotometer (Thermo Fisher Scientific). Two microgram of RNA was then reverse transcribed to cDNA using iScript cDNA synthesis kit as instructed by the manufacturer (Bio-Rad). Quantitative real-time polymerase chain reaction (PCR) was then performed using the HOT FIREPol EvaGreen qPCR Supermix in a ABI system (Thermo Fisher Scientific, Waltham, MA, USA). Mean PCR efficiencies per ampiclon was then calculated through LinReg PCR. SAND (At2g28190) was used to normalize the efficiency corrected relative expression primers. Primers used in quantitative reverse transcription qPCR (qRT)-PCR are listed below.

ICS1 forward primer: 5’TACCTACACGTCCGAAA-GACG3’ ICS1 reverse primer: 5’GAGGCTTTGACAA-CACCTCTGT3’

Protein extraction and analysis. Seven day old seedlings were ground with 6 M urea. The homogenates were mixed with a loading buffer containing 4%(w/v) sodium dodecyl sulphate (SDS), 125 mM Tris–HCl, 2% (v/v) 2-mercaptoethanol, 0.05% (w/v) bromophenol blue, and 20% (v/v) glycerol and boiled for 10 min at 100 °C, followed by loading on an 6% SDS-PAGE gel for Western blot analysis. The monoclonal anti-α-EDS1 antibody produced in mouse (customized from Bio Bharati, 1:10,000 dilutions) was used to detect the endogenous EDS1 levels. The goat anti-rabbit-lgG polyclonal antibody horseradish peroxidase-conjugated (Sigma, 1:5000 dilutions) was used as the secondary antibody.

Experimental Methods on Studies on D. melanogaster Using (III). Materials for studies on D. melanogaster

1. Wild-type CsBz flies
2. 1% agar and 1% agar + 100 mM sucrose media.
3. Fluoresceinated tethered compound.
4. Fly media composition for raising the flies

The composition of the medium for growth studies on D. melanogaster is shown in Table 1.

Steps involved:

1. Two vials each of 1% agar and two of fluoresceinated tethered compound plus 1% agar were taken. Each vial had 3 mL of media present in it. Fluoresceinated tethered compound (0.25 mg/mL) was present, which means each vial had 0.75 mg/mL fluoresceinated tethered compound.
2. Twenty female flies and 10 male flies were put in each vial for egg laying for 24 h.

After 24 h, the flies were removed and the following were done:

1. The eggs were observed under the fluorescence microscope.
2. The number of eggs in both 1% agar vials and vials which also contain the fluoresceinated tethered compound (III) were counted.
3. The eggs were then allowed to hatch into larvae and were shifted to normal media; later, they were again checked under a microscope for fluorescence. Both, first and second instar stages of larvae were checked.
4. The larvae were then allowed to grow into pupae. The pupae that eclosed as adult flies were also checked under a fluorescence microscope.

Steps Involved in Computational Studies Using (III). Protein Structure Preparations. Crystals of 2P1M, 1N4K, and 4ZAI were obtained from the Protein Data Bank (www.pdb.org). The crystals were in APO forms.

The protein structure was optimized and then minimized using the protein
optimization was done using the OPLS3 force field.

**Ligand Preparation in Axial and Equatorial Arrangements.** The bioactive molecules in their axial and equatorial forms were prepared using Schrödinger’s module LIGPREP (version 2017-1)49,50 which generates tautomers, and the possible ionization states at the pH range 7 ± 251 also generates all stereoisomers of the compound if necessary. The optimization was done using the OPLS3 force field.52

**Binding Site Identification.** Two ligand-independent (Site Map) and ligand-dependent (molecular dockings) methods were used to identify the most likely binding site of the compounds. The optimized structures of 2P1M, 1N4K, and 4ZAI by molecular dynamics simulation were used for binding site identification.

**Site Map Analysis.** Site Map53 program of Schrodinger Suite was used for calculating the binding site of compounds. The different parameters such as site score, size, exposure score, enclosure, hydrophobic/hydrophilic character, contact, and donor/acceptor character were used for the calculation of the potential binding site. Drug ability of the site is denoted by D score. These scores were derived by Halgren4 by executing the Site Map Program on a number of proteins that have inhibitors bound with potencies in the submicromolar range was used, and statistical analyses were performed to produce optimized scores. The OPLS-2003 force field55 was employed, and a standard grid was used with 15 site points per reported site and cropped at 4.0 Å from the nearest site point.

**Molecular Docking.** Molecular docking was used further to assess the robustness of results provided by Site Map. The docking studies were performed using both AUTODOCK4.2 (loc.cit) and GLIDE. The two tools were required to identify the putative binding site as no cocrystal (with inhibitor) is reported for these molecules.56,57 The docking with AUTODOCK was performed using three different protocols: (1) blind docking was performed to explore the possible binding sites at the surface of the whole protein to rule out any bias; (2) focused docking was performed on the most populated cluster obtained from blind docking by entering the docking grid on the center of mass of cluster representative conformation of compounds; and (3) guided docking was performed using the coordinates of the binding site58 to observe the expansion of compounds from the center of the pocket, taking the center of mass of compounds as a grid. Interestingly, the different dockings were carried out at the most likely site identified by Site Map, followed by the most populated cluster obtained through blind docking, followed by focused and guided docking that coincide well. In all these protocols, 200 conformers were generated separately to observe their convergence at the catalytic site.

The grid coordinates, energy evaluations, and generations of all three protocols are provided here. GLIDE and GLIDE XP docking methods (a module of MAESTRO) were also implemented to obtain the consensus result as well as to remove the chances of errors.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00961.

MS/MS spectrum for m/z value 115,6,9, FT-IR spectrum of compound (III), 1H NMR spectra, assignment tables for NMR analysis, TOCSY spectrum of (III) showing the correlation of the 4.23 ppm signal of the CH—O hydrogen with the protons of the cyclohexane ring, graphical representation of the number of eggs in 1% agar media and 1% agar with F, and protein docking interaction with the 2P1M diagram (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
Sambasivan V. Eswaran — Teri Deakin Nano Biotechnology Centre (TDNBC), Gurugram 122002, Haryana, India; orcid.org/0000-0002-2097-4377; Email: sv.eswaran@gmail.com, C_sv.eswaran@teri.res.in, eswaran.s@deakin.edu

**Authors**
Sujeet Kumar Thakur — TERI School of Advanced Studies, New Delhi 110070, India
Krishnendu Goswami — Regional Centre for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Pallavi Rao — Amity University, Noida 201313, Uttar Pradesh, India
Shivam Kaushik — Regional Centre for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Bhanu Pratap Singh — Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Pinky Kain — Regional Centre for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Shailendra Asthana — Translational Health Science and Technology Institute, NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Saikat Bhattacharjee — Regional Centre for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad 121001, Haryana, India
Prasenjit Guchhait — Regional Centre for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad 121001, Haryana, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c00961

**Author Contributions**
S.K.T. helped in synthesis, NMR, MS studies, and drafting the manuscript; S.B. proposed the problem and supervised the A. thaliana work; K.G. carried out the plant work; P.K. supervised the work on D. melanogaster; P.R. and S.K. did the experiments on D. melanogaster; S.A. supervised the computational work using Schrodinger software; B.P.S. did the computational work; P.G. gave material support and strategic inputs and helped in deciding the collaborations; S.V.E. planned the synthesis, NMR, and MS studies, set up the collaborations, drafted the manuscript, and coordinated with the collaborators)

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
The corresponding author thanks Dr Alok Adholeya, Director, TDNBC for providing facilities. We thank the Executive
Director, Regional Centre for Biotechnology (RCB), Faridabad, Haryana for the facilities. We thank Dr. Dinesh Mahajan, Translational Health Science and Technology Institute (THSTI), and Dr. Nirpender Singh, Advanced Technology Platform Center (ATPC), Regional Center for Biotechnology (RCB), NCR Biotech Science Cluster, Faridabad, Haryana, India for mass spectral studies. We thank Dr. K. V. Ramanathan, Sophisticated Instruments Facility, Department of NMR, Indian Institute of Science (IISc), Bangalore, India and Dr. Néel Sarovar Bhawesh, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India for NMR spectral studies. We are thankful to THSTI intramural core grant (S.A.) funding. We are thankful to Prof. S. Uma Devi for checking and improving the English language in the final manuscript.

**References**

(1) (a) Raboy, V. Seeds for a better future: ‘low phytate’ grains help to overcome malnutrition and reduce pollution. *Trends Plant Sci.* 2001, 6, 458–462. (b) Freed, C.; Adepoju, O.; Gillaspy, G. Can Inositol Pyrophosphates Inform Strategies for Developing Low Phytate Crops? *Plants* 2020, 9, 115. (c) Joy, A.; Balaji, S. Drug-like-ness of Phytic Acid and Its Analogues. *Open Microbiol. J.* 2015, 9, 41–149. (2) Graf, E.; Empson, K.L.; Eaton, JW Phytic acid. A natural antioxidant. *J. Biol. Chem.* 1987, 262, 11647–11650. (3) Powell, E.; Piwnica-Worms, D.; Piwnica-Worms, H. Contribution of p53 to metastasis. *Cancer Discovery* 2014, 4, 405–414. (4) Rao, F.; Xu, J.; Fu, C.; Cha, J. Y.; Gadalla, M. M.; Xu, R.; Barrow, J. C.; Snyder, S. H. Inositol pyrophosphates promote tumor growth and metastasis by antagonizing liver kinase B1. *Proc. Natl. Acad. Sci. U.S.A.* 2015, 112, 1773–1778. (5) Bhandari, R.; Saiardi, A.; Ahmadibeni, Y.; Snowman, A. M.; Resnick, A. C.; Kristiansen, T. Z.; Molina, H.; Pandey, A.; Werner, J. K.; Jurlin, K. R.; Xu, Y.; Prestwich, G. D.; Parang, K.; Snyder, S. H. Protein pyrophosphorylation by inositol pyrophosphates is a posttranslational event. *Proc. Natl. Acad. Sci. U.S.A.* 2007, 104, 15305–15310. (6) 5400 ken Sound Blvd Northwest Suite 500 Boca, Phone-1-561-959-7240, www.vitacost.com. (7) Karmakar, S.; Banik, N. L.; Ray, S. K. Molecular mechanism of inositol hexaphosphate-mediated apoptosis in human malignant glioblastoma T98G cells. *Neurochem. Res.* 2007, 32, 2094–2102. (8) Shamsuddin, A. M.; Yang, G. Y.; Vucenik, I. Novel anti-cancer functions of IP6: growth inhibition and differentiation of human mammary cancer cell lines in vitro. *Anticaner Res.* 1996, 16, 3287–3292. (9) Brian, Q. Inositol phosphates in foods. *Advances in Food and Nutrition Research; Elsevier, 2003; pp 1–60. (10) Foster, S. R.; Dilworth, L. L.; Sparks, J.; Alexander-Lindo, R. L.; Omoruyi, F. O. Combined Inositol Hexakisphosphate and Inositol Supplement Consumption Improves Serum Alpha-Amylase Activity and Hematological Parameters in Streptozotocin-Induced Type 2 Diabetic Rats. *Adv. Pharmacol. Sci.* 2019, 2019, 4143137. (11) Mittal, R.; Peak-Chew, S. Y.; Sade, R. S.; Vallis, Y.; McMahon, H. T. The Acetyltansferase Activity of the Bacterial Toxin YopJ ofYersinial Activated by Eukaryotic Host Cell Inositol Hexakisphosphate. *J. Biol. Chem.*, 2010, 285, 19927–19934. (12) Donahue, J. L.; Alford, S. R.; Torabinjed, J.; Kerwin, R. E.; Nourbakhsh, A.; Ray, W. K.; Herrick, M.; Huang, X.; Lyons, B. M.; Hein, P. P.; Gillaspy, G. E. The Arabidopsis thaliana Myo-Inositol 1-Phosphate Synthase Gene Is Required for Myo-inositol Synthesis and Suppression of Cell Death. *Plant Cell* 2010, 22, 888–903. (13) Azevedo, C.; Saiardi, A. Eukaryotic Phosphate Homeostasis: The Inositol Pyrophosphate Perspective. *Trends Biochem. Sci.* 2016, 42, 219. (14) Desfougères, Y.; Wilson, M. S. C.; Laha, D.; Miller, G. J.; Saiardi, A. ITPK1 mediates the lipid-independent synthesis of inositol phosphates controlled by metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 2019, 116, 24551–24561. (15) Belgaroui, N.; Lacombe, B.; Rouached, H.; Hanin, M. Phytase overexpression in Arabidopsis improves plant growth under osmotic stress and in combination with phosphate deficiency. *Sci. Rep.* 2018, 8, 1137. (16) Jiao, C.; Summerlin, M.; Bruzik, K. S.; Hanakahi, L. Synthesis of biotinylated inositol hexakisphosphate to study DNA double-strand break repair and affinity capture of IP6-binding proteins. *Biochemistry* 2015, 54, 6312–6322. (17) Reitz, A. B. Inositol Phosphates and Derivatives Synthesis, Biochemistry, and Therapeutic Potential; Oxford University Press, 1991. (18) Inositol Phosphates: Linking Agriculture and the Environment; Benjamin Turner, L., Richardson, A. E., Mullaney, E. J., Eds.; CABI, 2007; p 288. (19) Sarmah, B.; Latimer, A. J.; Appel, B.; Wente, S. R. Inositol polyphosphates regulate zebrlish left-right asymmetry. *Dev. Cell* 2005, 9, 133–145. (20) Raboy, V. myo-Inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* 2003, 64, 1033–1043. (21) Hanakahi, L. A.; West, S. C. Specific interaction of IP6 with human Ku70/80, the DNA-binding subunit of DNA-PK. *EMBO J.* 2002, 21, 2038–2044. (22) Ma, Y.; Lieber, M. R. Binding of inositol hexakisphosphate (IP6) to Ku but not to DNA-PKcs. *J. Biol. Chem.* 2002, 277, 10756–10759. (23) Yin, M.-x.; Catimel, B.; Gregory, M.; Condon, M.; Kapp, E.; Holmes, A. B.; Burgess, A. W. Synthesis of an inositol hexakisphosphate (IP6) affinity probe to study the interactome from a colon cancer cell line. *Integr. Biol.* 2016, 8, 309–318. (24) Wang, H.; Godage, H. Y.; Riley, A. M.; Weaver, J. D.; Shears, S. B.; Potter, B. V. L. Synthetic inositol phosphate analogs reveal that PP1PSK2 has a surface-mounted substrate capture site that is a target for drug discovery. *Chem. Biol.* 2014, 21, 689–699. (25) Shears, S. B. Inositol Pentakis- and Hexakisphosphate Metabolism Adds Versatility to the Actions of Inositol Polyphosphates Novel Effects on Ion Channels and Protein Traffic. *Subcell. Biochem.* 1996, 26, 187–226. (26) Sasakawa, N.; Sharif, M.; Hanley, M. R. Metabolism and biological activities of inositol pentakisphosphate and inositol hexakisphosphate. *Biochem. Pharmacol.* 1995, 50, 137–146. (27) Bauman, A. T.; Chateauneuf, G. M.; Boyd, B. R.; Brown, R. E.; Murthy, P. N. P. Conformational inversion processes in phytic acid: NMR spectroscopic and molecular modeling studies. *Tetrahedron Lett.* 1999, 40, 4489–4492. (28) Barrientos, L.; Scott, J. J.; Murthy, P. P. N. Specificity of Hydrolysis of Phytic acid by alkaline phytase from lilly pollen. *Plant Physiol.* 1994, 106, 1489–1495. (29) Marecek, J. F.; Prestwick, G. D. Synthesis of Tethered Phytic Acid. *Tetrahedron Lett.* 1991, 32, 1863–1866. (30) Best, M. D.; Zhang, H.; Prestwick, G. D. Inositol polyphosphates, diphosphoinositol polyphosphates and phosphatidylinositol polyphosphate lipids: structure, synthesis, and development of probes for studying biological activity. *Nat. Prod. Rep.* 2010, 27, 1403–1430. (31) Prestwick, G. D. Touching all the bases: Synthesis of inositol polyphosphate and phosphonostidea affinity probes from glucose. *Acc. Chem. Res.* 1996, 29, 503–513. (32) Chaudhary, A.; Mehrotra, B.; Prestwick, G. D. Rapid purification of reporter group-tagged inositol hexakisphosphate on ion-exchange membrane adsorbers. *BioresTechniques* 1997, 23, 427–430. (33) McIntyre, C. A.; Arthur, C. J.; Evershed, R. P. High-resolution mass spectrometric analysis of myo-inositol hexakisphosphate using electrospray ionisation Orbitrap. *Rapid Commun. Mass Spectrom.* 2017, 31, 1681–1689. (34) (a) Windhorst, S.; Lin, H.; Blechner, C.; Fanick, W.; Brandt, L.; Brehm, M. A.; Mayr, G. W. Tumour cells can employ extracellular...
Ins(1,2,3,4,5,6)P6 and multiple inositol-polyphosphate phosphatase 1 (MINPP1) dephosphorylation to improve their proliferation. Biochem. J. 2013, 450, 115–125. (b) Pavlovic, I.; Thakor, D. T.; Bigler, L.; Wilson, M. S. C.; Lah, D.; Schaf, G.; Sairadi, A.; Jessen, H. J. Prometabolites of 5-Dipospho-myo-inositol Pentakisphosphate. Angew. Chem. 2015, 54, 9622–9626.

(35) Garcia, C.; Lohmann, A.; Lamodière, E.; Catinot, J.; Buchala, A.; Doermann, P.; Métraux, J. P. Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of Arabidopsis. Plant Physiol. 2008, 147, 1279–1287.

(36) Zhu, J.; Hong, D. D.; Wakisaka, M. Phytic Acid Extracted from Rice Bran as a Growth Promoter for Euglena gracilis. Open Chem. 2019, 17, 57–63.

(37) Khan, G. A.; Vogtjatzki, E.; Glausser, G.; Poirier, Y. Phosphate Deficiency Induces the Jasmonate Pathway and Enhances Resistance to Insect Herbivory. Plant Physiol. 2016, 171, 632–644.

(38) Dong, Z.; Li, W.; Liu, J.; Li, P.; Pan, S.; Liu, S.; Gao, J.; Liu, L.; Liu, X.; Wang, G. L.; Dai, L. The rice phosphate transporter protein OsPT8 regulates disease resistance and plant growth. Sci. Rep. 2019, 9, 5408.

(39) An, C.; Mou, Z. Salicylic Acid and its Function in Plant Immunity. J. Integr. Plant Biol. 2011, 53, 412–428.

(40) Venugopal, S. C.; Jeong, R. D.; Mandal, M. K.; Zhu, S.; Chandra-Shekara, A. C.; Xia, Y.; Hersh, M.; Stromberg, A. J.; Navarre, D.; Kachroo, A.; Kachroo, P. Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. PLoS Genet. 2009, 5, e1000545.

(41) Joglekar, S.; Suliman, M.; Bartsch, M.; Halder, V.; Maintz, J.; Bautor, J.; Zeier, J.; Parker, J. E.; Kombrik, E. Chemical Activation of EDS1/PAD4 Signaling Leading to Pathogen Resistance in Arabidopsis. Plant Cell Physiol. 2018, 59, 1592–1607.

(42) Wilson, M. S. C.; Bulley, S. J.; Pisan, F.; Irvine, R. F.; Saiardi, A. A novel method for the purification of inositol phosphates from biological samples reveals that no phytate is present in human plasma or urine. Open Biol. 2015, 5, 150014.

(43) Vadivelu, M.; Sugirbha, S.; Dheen Kumar, P.; Arun, Y.; Karthikeyan, K.; Praveen, C. Solvent-free implementation of two dissimilar reactions using recyclable CuO nanoparticles under ball-milling conditions: synthesis of new oxindole-triazole pharmaco-phores. Green Chem. 2017, 19, 3601–3610.

(44) Wilson, M. S. C.; Livermore, T. M.; Saiardi, A. Inositol pyrophosphates: between signalling and metabolism. Biochem. J. 2013, 452, 369–379.

(45) Thota, S. G.; Bhandari, R. The emerging roles of inositol pyrophosphates in eukaryotic cell physiology. J. Biosci. 2015, 40, 593–605.

(46) Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 1949, 4, 1.

(47) Esa, N.; Narayanaswamy, R.; Rjai, L. K. Molecular Docking Analysis of Phytic Acid and 4-hydroxysolucine as Cyclooxygenase-2, Microsomal Prostaglandin E Synthase-2, Tyrosinase, Human Neutrophil Elastase, Matrix Metalloproteinase-2 and -9, Xanthine Oxidase, Squalene Synthase, Nitric Oxide Synthase, Human Aldose Reductase, and Lipoxigenase Inhibitors. Pharmacogn. Mag. 2017, 13, 521.

(48) Asthana, S.; Shukla, S.; Vargiu, A. V.; Ceccarelli, M.; Ruggerone, P.; Paglietti, G.; Marongiu, M. E.; Blois, S.; Giliberti, G.; La Colla, P. Different molecular mechanisms of inhibition of bovine viral diarrhea virus and hepatitis C virus RNA-dependent RNA polymerases by a novel benzimidazole. Biochemistry 2013, 52, 3752–3764.

(49) Kant, S.; Asthana, S.; Missiakas, D.; Pancholi, V. A novel STK1-targeted small-molecule as an “antibiotic resistance breaker” against multidrug-resistant Staphylococcus aureus. Sci. Rep. 2017, 7, 5067.

(50) Mattapally, S.; Singh, M.; Murthy, K. S.; Asthana, S.; Banerjee, S. K. Computational modeling suggests impaired interactions between NKX2.5 and GATA4 in individuals carrying a novel pathogenic D16N NKX2.5 mutation. Oncotarget 2018, 9, 13713.