2*C*-Methyl-D-erythritol 4-phosphate enhances and sustains cyclodiphosphate synthase IspF activity

*J. Kipchirchir Bitok and Caren Freel Meyers**

Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

*Corresponding author: cmeyers8@jhmi.edu

SUPPORTING INFORMATION

Table of Contents

Figure S1: ***Ni^{2+} affinity purification of C-His_6 E. coli IspF***. IspF was eluted 5-500 mM imidazole.

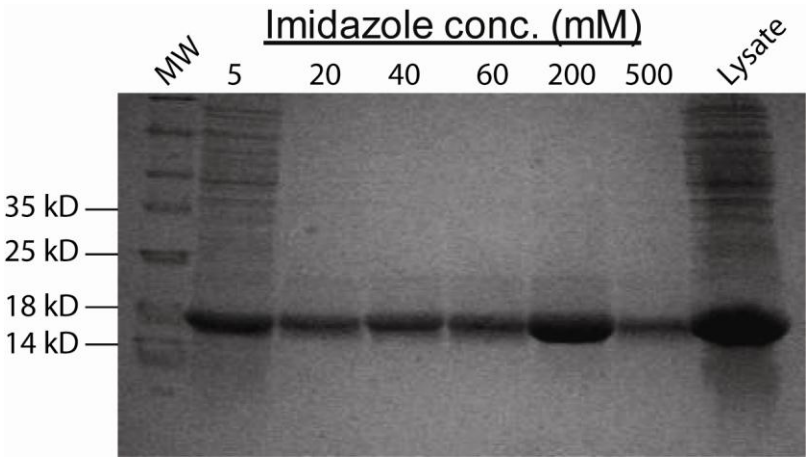


Figure S2: ***IspF-catalyzed MEcDP and CMP formation.*** Formation of MEcDP was confirmed using ^{1}H – ^{31}P two-dimensional NMR (Figure S2a,b). A reaction containing 50 mM phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 2 mM 4-diphosphocytidyl-2*C*-methyl-D-erythritol-2-phosphate, 1.5 µM IspF and 50 µg/mL BSA in a total volume of 150 µL was analyzed by H–P–NMR. NMR analysis was carried out following reported procedures.[1, 2] Magnetization transfers in NMR experiments are depicted in blue ($^{1}H \rightarrow {}^{31}P$) and red ($^{31}P \rightarrow {}^{31}P$). (**a**) Constant-time CT ^{1}H–^{31}P HSQC showing H_1:P_A, H_5:P_A, and H_4:P_B correlations. (**b**) CT ^{1}H–^{31}P–^{31}P COSY spectrum indicating magnetization transfer between P_A and P_B. This is evident in H_1:P_B, H_5:P_B, and H_4:P_A correlations. (**c**) HPLC stackplot showing IspF-catalyzed conversion of CDPME2P to CMP.

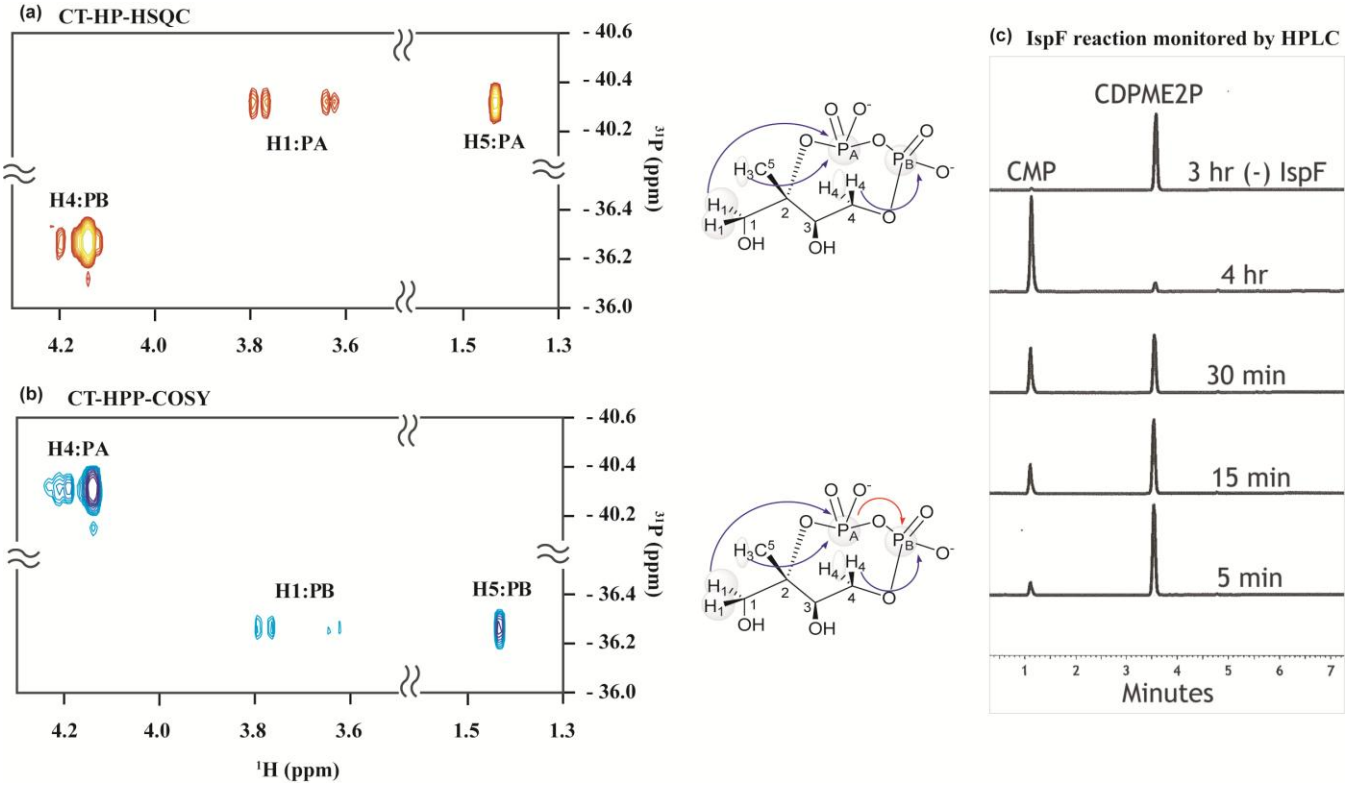


Figure S3: ***Dependence of IspF rate on pH.*** The dependence of IspF initial rate on pH was determined following the described general IspF assay. Each reaction contained 200 μM 4-diphosphocytidyl-2*C*-methyl-D-erythritol-2-phosphate at pH 6.0, 7.0, 7.4, 8.0, 9.0 and 10.0. HPLC sample preparation and analyses were carried out as described. IspF showed apparent maximal activity at pH 7.4.

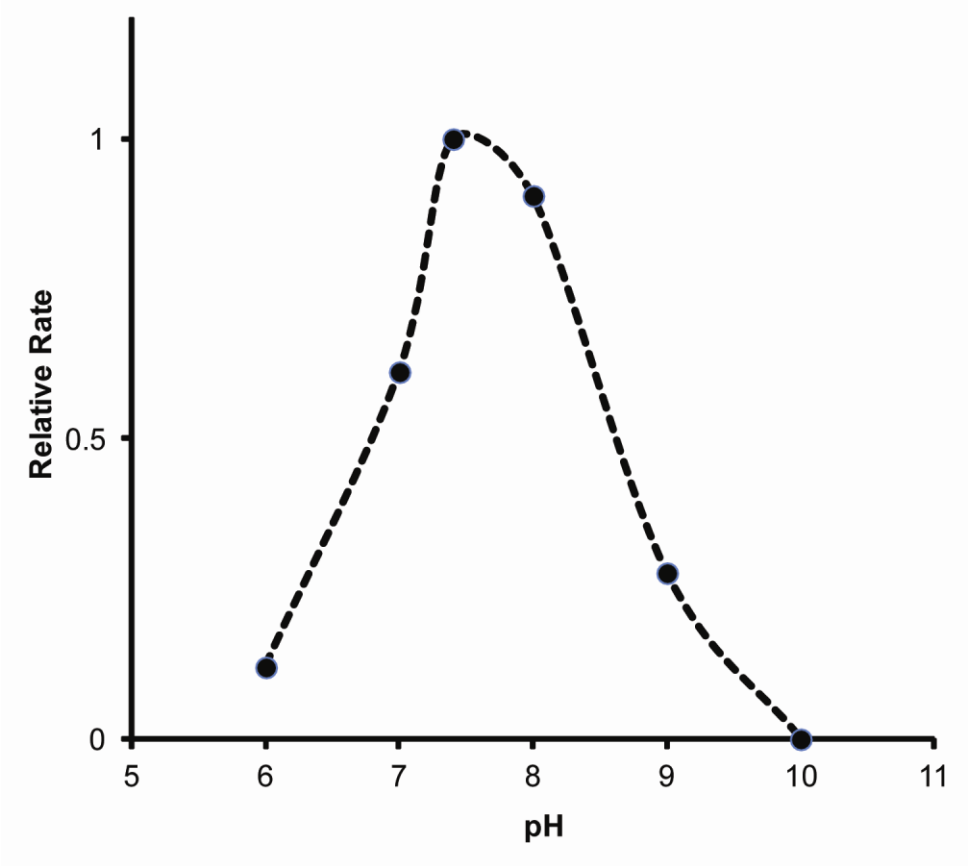


Figure S4: ***Michaelis-Menten kinetic analyses***. IspF-catalyzed formation of CMP was kinetically characterized in the absence or presence of additives at 20°C. (**a**) Kinetic parameters of IspF in the absence of additives; (**b**) Kinetic parameters of IspF in the presence of 500 μM MEP; (**c**) Kinetic parameters of IspF in the presence of 500 μM ME.

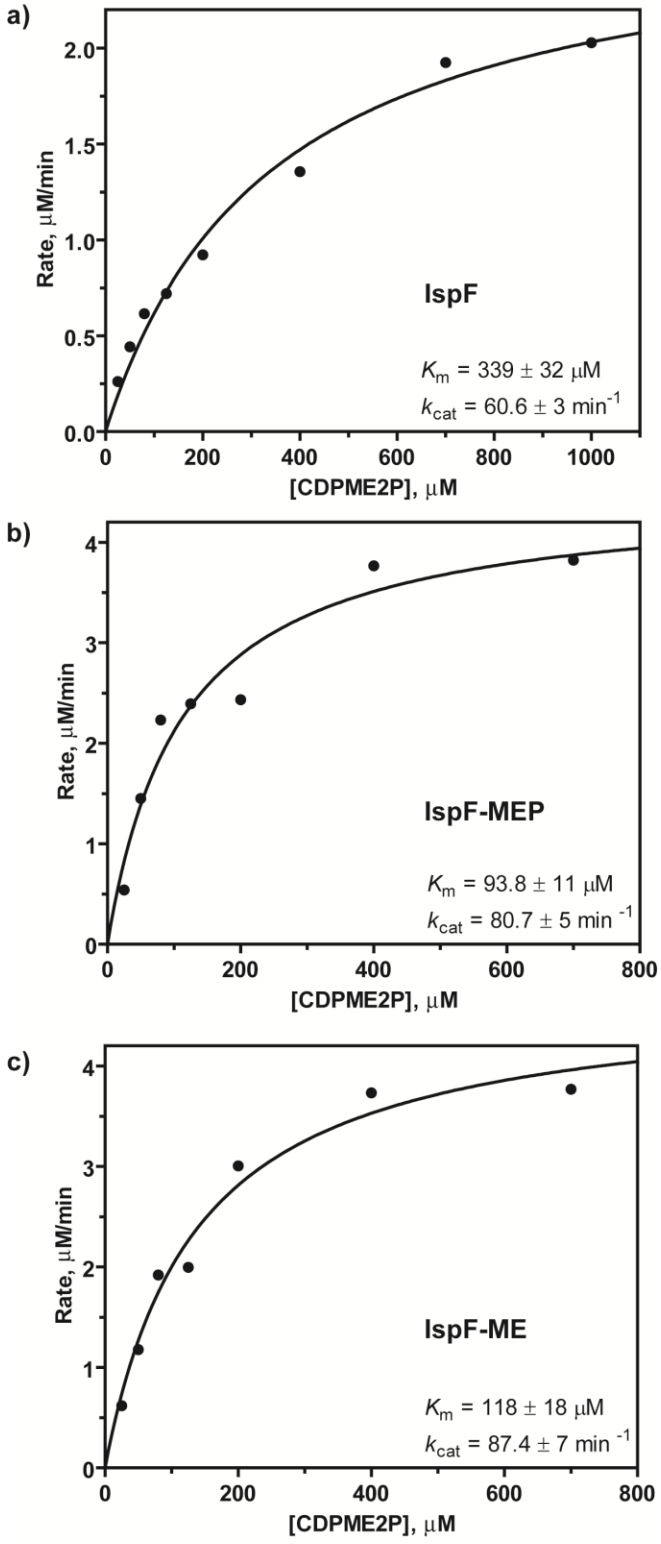


Figure S5: ***IspF inhibition by CDP.*** Concentrations of CDP used were in the range 0.1-1000 μM. The IC_{50} was calculated using GraFit software version 7 (Figure S5).[3]

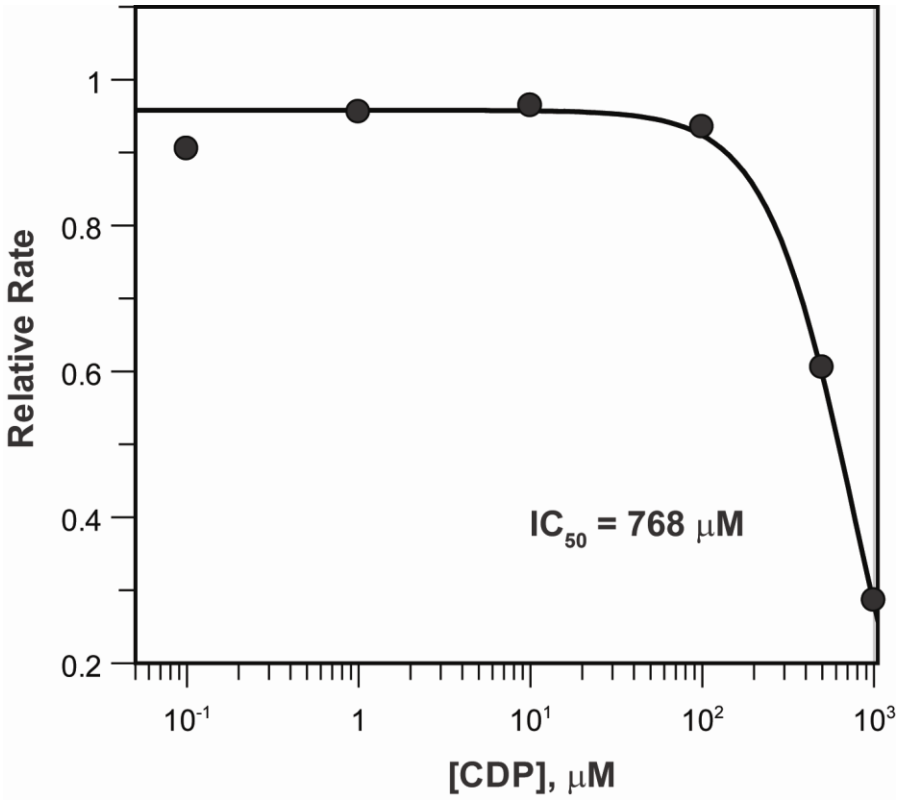
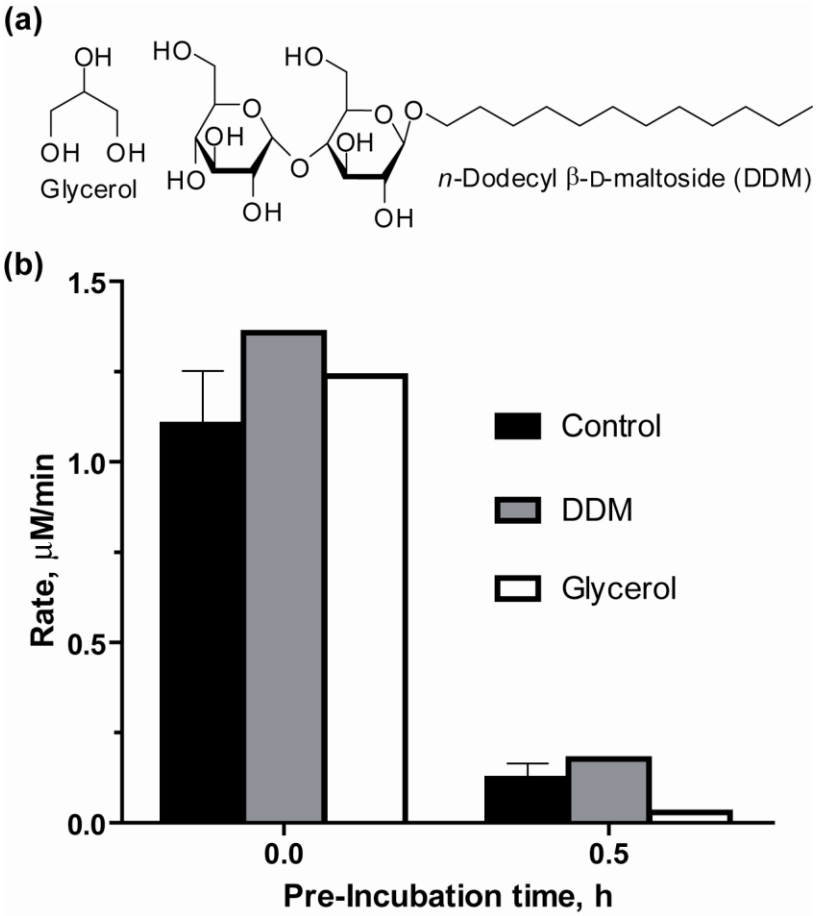


Table S1. ***Rates of IspF-catalyzed CMP formation in the presence or absence of additives***: isopentenyl diphosphate (IDP),dimethylallyl diphosphate (DMADP), geranyl diphosphate (GDP), farnesyl diphosphate (FDP), 1-deoxy-D-xylulose 5-phosphate (DXP),4-diphosphocytidyl-2*C*-methyl-D-erythritol (CDPME), (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBDP), 2*C*-methyl-D-erythritol 4-phosphate (MEP), Deoxyxylulose (DX), D-erythritol 4-phosphate (EP), or 2*C*-methyl-D-erythritol (ME).

	[a]Time, (min)	Rate, μM • min^{-1}	Relative Rate
Control		1.12 ± 0.22	1.00
[b]1:4:2		1.35 ± 0.13	1.21
[b]10:40:20		1.22 ± 0.16	1.09
[b]100:400:200		1.57 ± 0.04	1.40
[b]200:800:400		1.42 ± 0.21	1.27
Control	0	1.10 ± 0.15	1.00
	30	0.12 ± 0.04	0.11
	24 h	0	0.00
[c]IDP	0	1.74 ± 0.23	1.58
	30	0.33 ± 0.11	0.30
[c]DMADP	0	1.88 ± 0.41	1.70
	30	0.34 ± 0.02	0.31
[c]GDP	0	1.97 ± 0.18	1.79
	30	0.66 ± 0.04	0.59
[c]FDP	0	1.16 ± 0.07	1.05
	30	0.41 ± 0.08	0.37
[c]DXP	0	1.07 ± 0.22	0.96
	30	0.17 ± 0.05	0.16
[c]CDPME	0	1.24 ± 0.24	1.12
	30	0.19 ± 0.03	0.17
[c]HMBDP	0	1.57 ± 0.18	1.43
	30	0.28 ± 0.01	0.25
[c]MEP	0	2.25 ± 0.13	2.03
	30	1.99 ± 0.11	1.80
	24 h	2.19 ± 0.21	1.99
[c]DX	0	1.15 ± 0.19	1.04
	30	0.25 ± 0.03	0.23
[c]EP	0	1.46 ± 0.09	1.33
	30	0.31 ± 0.03	0.28
[c]ME	0	2.17 ± 0.38	1.97
	30	2.12 ± 0.05	1.92
	24 h	1.91 ± 0.09	1.73

[a]Pre-incubation time, before the reaction was initiated with the substrate CDPME2P.
[b]Ratio of IDP:GDP:FDP concentrations in μM. [c]Used at a final concentration of 500 μM.

Figure S6: ***Evaluation of activity-enhancing or activity-stabilizing properties of glycerol and n-dodecyl β-D-maltoside (DDM)***. The IspF reaction was carried out in the presence of known enzyme stabilizers, glycerol (500 μM) or DDM (0.005%). (**a**) Structures of glycerol and DDM; (**b**) Rate of CMP formation in the presence of 500 μM glycerol, or 0.005% DDM.). **(c)** Summary of the effects of the enzyme stabilizers in (b).



(c)

Entry	Pre-incubation Time, min	Rate, μM/min	Relative Rate
Control	0	1.10 ± 0.15	1.00
	30	0.12 ± 0.04	0.11
DDM	0	1.36	1.23
	30	0.18	0.16
Glycerol	0	1.24	1.12
	30	0.03	0.03

Figure S7: ***AC_{50} determinations of MEP and ME***. Representative AC_{50} determinations for MEP (**a**) or ME (**b**) in the presence of 100 μM CDPME2P at 20 °C.

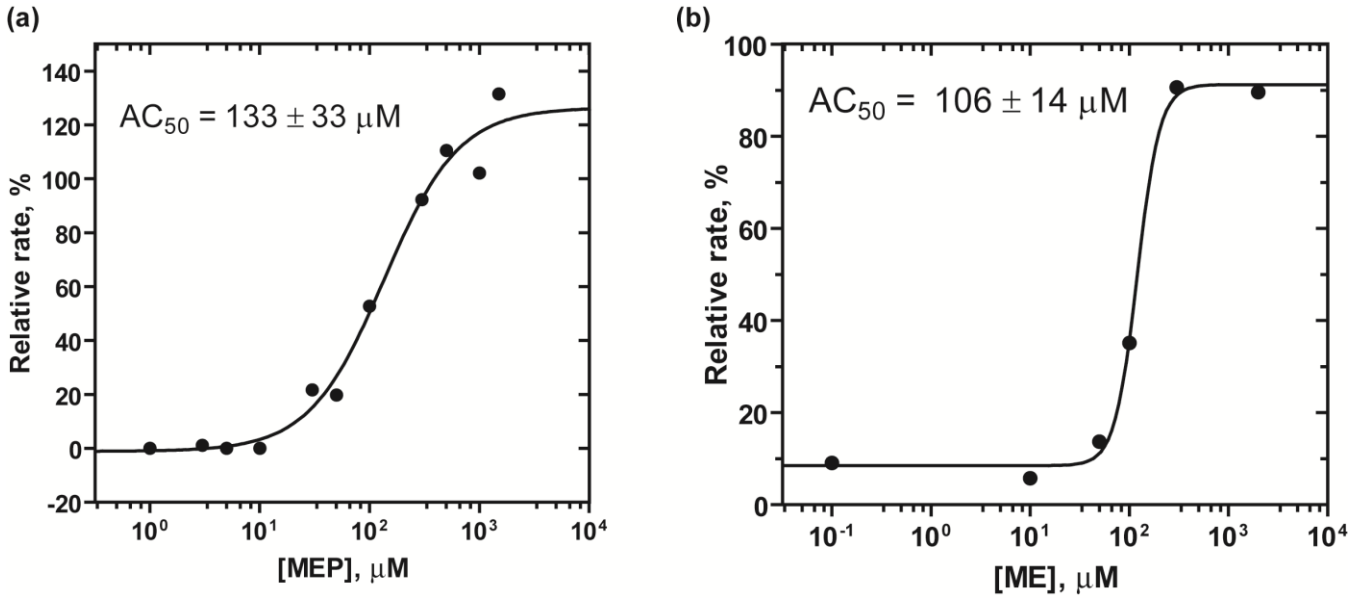


Table S2. ***Effects of CDP and isoprenoid diphosphates on the activity of the IspF-MEP complex.***

Conditions	Rate, μM • min^{-1}
(-) MEP control	1.10 ± 0.15
(-) MEP (+) CDP	0.45 ± 0.02
(+) MEP control	2.06 ± 0.07
(+) MEP (+) CDP	2.28 ± 0.29
(+) MEP (+) HMBDP	2.20 ± 0.31
(+) MEP (+) IDP	2.10 ± 0.37
(+) MEP (+) DMADP	2.34 ± 0.28
(+) MEP (+) GDP	2.03 ± 0.24
(+) MEP (+) FDP	1.17 ± 0.21

REFERENCES

1. Majumdar, A.; Shah, M. H.; Bitok, J. K.; Hassis-LeBeau, M.; Freel Meyers, C. L. Probing phosphorylation by non-mammalian isoprenoid biosynthetic enzymes using ^{1}H-^{31}P-^{31}P correlation NMR spectroscopy. *Mol. BioSyst.* **2009**, *5,* 935–944.

2. Majumdar, A.; Sun, Y.; Shah, M.; Freel Meyers, C. L. Versatile ^{1}H-^{31}P-^{31}P COSY 2D NMR Techniques for the Characterization of Polyphosphorylated Small Molecules. *J. Org. Chem.* **2010**, *75,* 3214–3223.

3. Leatherbarrow, R. J. *GraFit Version 7*, Erithacus Software Ltd., Horley, U.K. **2010**, .