Supporting Information for

Revisiting the IspH Catalytic System in the Deoxyxylulose Phosphate Pathway: Achieving High Activity

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15) Laupitz, R.; Hecht, S.; Amslinger, S.; Zepeck, F.; Kaiser, J.; Richter, G.; Schramek, N.; Steinbacher, S.; Huber, R.; Arigoni, D.; Bacher, A.; Eisenreich, W.; Rohdich, F.

17) Grawert, T.; Kaiser, J.; Zepeck, F.; Laupitz, R.; Hecht, S.; Amslinger, S.; Schramek, N.; Schleicher, E.; Weber, S.; Haslbeck, M.; Buchner, J.; Rieder, C.; Arigoni, D.; Bacher, A.; Eisenreich, W.; Rohdich, F.

27) Rekittke, I.; Wiesner, J.; Rohrich, R.; Demmer, U.; Warkentin, E.; Xu, W. Y.; Troschke, K.; Hintz, M.; No, J. H.; Duin, E. C.; Oldfield, E.; Jomaa, H.; Ermler, U.

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1. General information

NMR spectra were recorded on a Varian UNITY PLUS 400. High-resolution mass spectra were obtained in the Boston University Chemical Instrumentation Center using a Waters Q-TOF spectrometer. Chelation resin Chelex 100 and LB broth were bought from Bio-Rad and Amresco, respectively. All other reagents and solvents were used as supplied by Sigma-Aldrich and Pharmco. The kinetics assays were performed in an S.I. Photonics CCD-440 spectrophotometer. Mössbauer spectra were recorded in the constant acceleration mode with a Janis cryostat equipped with an electromagnet. Isomer shifts are reported relevant to iron metal at room temperature.

2. Purification of [^{57}Fe]-labeled IspH protein

BL21(DE3) cells harboring the IspH/pASK-IBA3^{+}/pDB1281 constructs were grown in LB medium supplemented with [^{57}Fe]. The cells were grown at 37^{o}C until the OD_{600} reached 0.1. D-arabinose was then added to induce *isc* operon expression. When the OD_{600} reached 0.6, the solution was cooled on ice and then induced with 400 ng/mL anhydrotetracycline. After growing at 25 ^{o}C for an additional 24 hr, cells were harvested and IspH protein was purified anaerobically following a previously reported protocol (JACS, 2008, 130, 2164 – 2165). The SDS-PAGE and the UV-Vis spectrum of purified [^{57}Fe]-enriched IspH are shown in Figures 1S-a and 1S-b.

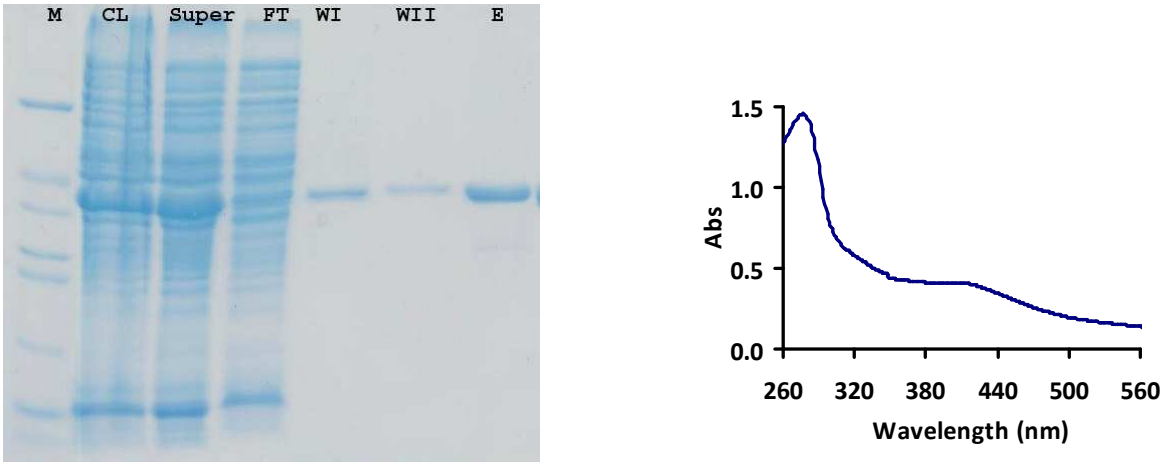


Figure 1S. a) SDS-PAGE of the anaerobically purified IspH. M, sigma low range marker; CL, cell lysate; Super, supernatant; FT, flow through from the streptavidin resin; WI, 50 mL washing of the resin after IspH binding; WII, 100 mL washing buffer after IspH binding; E, 2.5 mM desthiobiotin elution from streptavidin resin. b) The UV-vis spectrum of the anaerobically purified [^{57}Fe]-enriched IspH using Strep-Tactin resin from IBA, Inc.

3. Mössbauer spectra of IspH

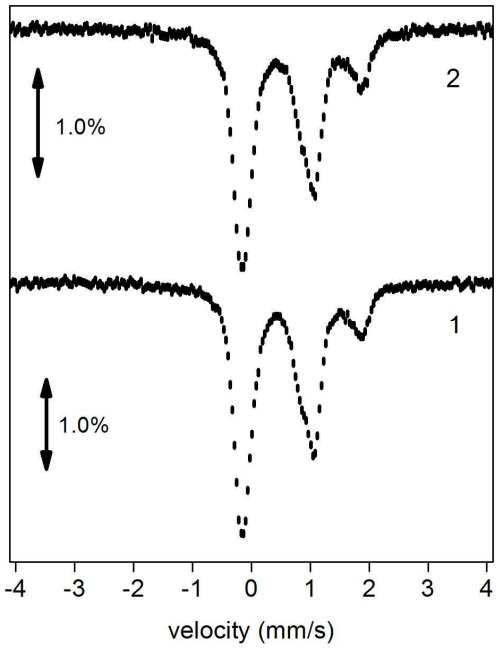


Figure 2S-A. IspH Mössbauer spectra were obtained in the absence (1) and presence (2) of NADPH-Fpr-FldA at 4.2 K with an external magnetic field of 0.1T applied perpendicularly to the γ-ray beam. For making sample (2) (0.90 mM [^{57}Fe] enriched IspH with 1.8 mM NADPH, 5 μM FldA and 2 μM Fpr): The mixture was incubated at the Coy-chamber anaerobically on ice for 30 min. The samples were then frozen in liquid nitrogen for Mössbauer spectrometry analysis.

In ref. 17, a preparation of as isolated IspH was shown to exhibit an asymmetric EPR signal at g≈2.0 which was attributed to a [3Fe-4S]$^{1+}$ cluster. The 4.2 K/0.1 T Mössbauer spectrum from our preparation (Figure 1) consists of the quadruple doublets A, B and C for at least 90% of total iron with no indication for a [3Fe-4S]$^{1+}$ cluster. In Figure 2S-B, we compare the 4.2K spectrum with a spectrum recorded at 78K. We observed that apart from the doublets A, B, and C, a fourth doublet, X is present with δ = 0.29 mm/s and ΔE_Q = 0.78 mm/s accounting for ≈10% of total iron. Such parameters are consistent with Fe(III)(S=5/2) in a tetrahedral environment comprising sulfur ligands. This doublet however is missing in the 4.2K/1.0KG spectrum. Most probably, this doublet represents a half integer spin system for which the spectrum magnetically splits at 4.2K giving rise to a broad background. Such a behavior is characteristic for [3Fe-4S]$^{1+}$ clusters [T.A. Kent, N.H. Huynh, E. Münck, Proc. Natl Acad. Science USA, 1980, 77, 6574-6576], although a monomeric Fe(III)(S=5/2) species cannot be excluded. In order to estimate the effect of a [3Fe-4S]$^{1+}$ cluster in the 4.2K spectrum, we calculated a spectrum assuming representative parameters for such a cluster [Y. Sanakis, A.L. Macedo, I. Moura, J.J.G. Moura, V. Papapefthymiou, E. E. Münck, J. Am. Chem. Soc., 2000, 122, 11855-11863 and references therein] accounting for 10% of total iron. As it is seen in Figure 2S-C, such a contribution could hardly be discernible in the 4.2 K spectrum. As a conclusion, our studies suggest that in our preparation, a [3Fe4S]$^{1+}$ cluster cannot account for more than 10% of total iron.

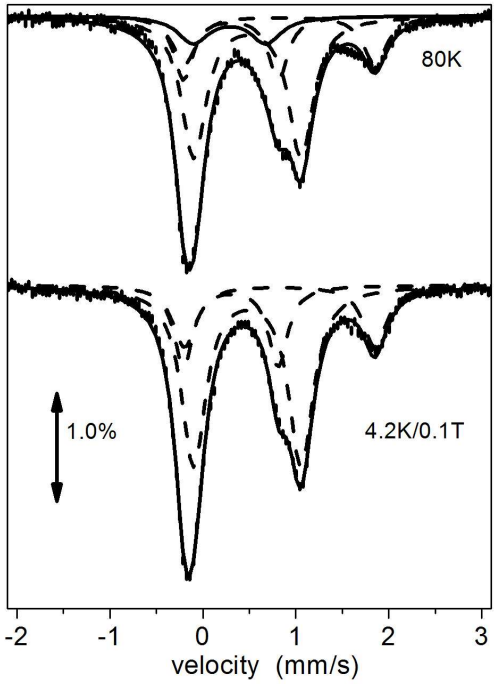


Figure 2S-B. Mössbauer spectra from isolated IspH at 4.2 K with an external magnetic field of 0.1 T applied perpendicularly to the γ-ray beam and 80 K. Dashed lines represent doublets A, B, and C. The solid line in the 78 K spectrum represents doublet X.

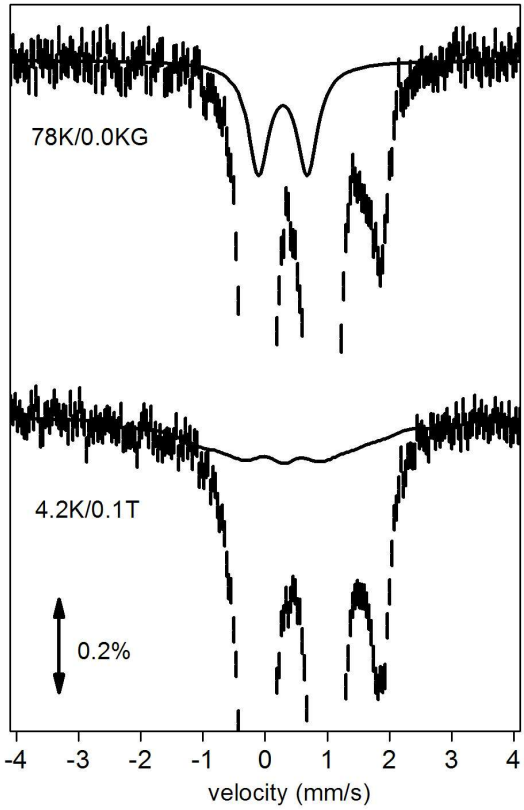


Figure 2S-C. The contribution (solid line) of a $[3Fe\text{-}4S]^{1+}$ cluster to the 4.2 K/0.1 T and 78 K spectra.

4. Syntheses of redox dyes.

4.1 Synthesis of **4a**

A solution of 5-chloro-1,10-phenanthroline (107 mg, 0.05 mmol) and 2.0 mL of 1,3-dibromopropane was refluxed for 12 hours, during which a precipitate was accumulated. The reaction mixture was cooled to room temperature, the solid was filtered off and washed with acetone, hexane, and then dried in vacuum to afford 180 mg of yellow powder identified as **4a** (86% yield). ^{1}H-NMR (D_2O, 400 MHz): 9.62 (d, *J* = 6.8 Hz, 2 H), 9.53 (d, *J* = 5.6 Hz, 1 H), 9.24 (d, *J* = 8.2 Hz, 1 H), 8.64 (s, 1 H), 8.51-8.54 (m, 1 H), 8.40-8.44 (m, 1 H), 4.97-5.04 (m, 4 H), 3.27-3.30 (m, 2 H).

4.2 Synthesis of **4b**

Following a procedure similar to that described for compound **4a** using 1,10-phenanthroline monohydrate and 1,3-dibromopropane as the starting material, 380 mg of yellow powder identified as **4b** was obtained in 99% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.52 (d, *J* = 5.6 Hz, 2 H), 9.32 (d, *J* = 7.6 Hz, 2 H), 8.45 (s, 2 H), 8.38-8.44 (m, 2 H), 5.00 (t, *J* = 6.4 Hz, 4 H), 3.26-3.30 (m, 2 H).

4.3 Synthesis of **4c**

Following a procedure similar to that described for compound **4a** using 4-methyl-1,10-phenanthroline and 1,3-dibromopropane as the starting material, 250 mg of gray powder identified as **4c** was obtained in 63% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.48 (d, *J* = 5.6 Hz, 1 H), 9.28-9.31 (m, 2 H), 8.51 (dd, *J* = 47.6, 8.8 Hz, 2 H), 8.36-8.39 (m, 1 H), 8.24 (d, *J* = 6.0 Hz, 1 H), 4.97 (t, *J* = 6.4 Hz, 2 H), 4.91 (t, *J* = 7.2 Hz, 2 H), 3.19-3.24 (m, 2 H), 3.04 (s, 3 H).

4.4 Synthesis of **4d**

Following a procedure similar to that described for compound **4a** using 1,10-phenanthroline monohydrate and 1,2-dibromoethane as the starting material, 330 mg of yellow powder identified as **4d** was obtained in 90% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.50 (d, *J* = 5.6 Hz, 2 H), 9.39 (d, *J* = 8.4 Hz, 2 H), 8.56 (s, 2 H), 8.45-8.49 (m, 2 H), 5.56 (s, 4 H).

4.5 Synthesis of **5b**

A mixture of 4,4′−dipyridyl (312 mg, 2.0 mmol) and 1.5 mL of bromoethane in 3.0 mL of acetonitrile was refluxed for 6 hours, during which a precipitate was formed. After cooling to room temperature, the precipitate was filtered off and washed with hot chloroform, acetone, hexane, and then dried in vacuum to yield 710 mg of yellow powder identified as **5b** (95% yield). ^{1}H-NMR (D_2O, 400 MHz): 8.98 (d, *J* = 5.6 Hz, 4 H), 8.39 (d, *J* = 5.6 Hz, 4 H), 4.59-4.63 (m, 4 H), 1.55 (t, *J* = 7.2 Hz, 6 H).

4.6 Synthesis of **5c**

Following a procedure similar to that described for compound **5b**, 202 mg of yellow powder identified as **5c** was obtained in 21% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.04 (d, *J* = 6.2 Hz, 4 H), 8.39 (d, *J* = 6.4 Hz, 4 H), 4.94-5.01 (m, 2 H), 1.60 (d, *J* = 6.8 Hz, 12 H).

4.7 Synthesis of **5e**

A mixture of 4,4′−dipyridyl (784 mg, 5.0 mmol) and iodomethane (0.41 mL, 6.5 mmol) in 10.0 mL of CH_2Cl_2 was refluxed for 2 hours, during which a precipitate was formed. After cooling to room temperature, the precipitate was filtered off and washed with ethyl acetate. The solid was recrystallized in MeOH to yield 1.32 gram of yellow product **5b** (88% yield). ^{1}H-NMR (D_2O, 400 MHz): 8.74 (d, *J* = 6.4 Hz, 2 H), 8.62 (d, *J* = 5.6 Hz, 2 H), 8.23 (d, *J* = 6.4 Hz, 2 H), 7.76 (d, *J* = 5.6 Hz, 2 H), 4.28 (s, 3 H).

4.8 Synthesis of **6a**

Following a procedure similar to that described for compound **4a** using 2,2′-dipyridyl and 1,2-dibromoethane as the starting material, 320 mg of powder identified as **6a** was obtained in 93% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.07 (d, *J* = 6.0 Hz, 2 H), 8.22-8.26 (m, 2 H), 5.20 (s, 4 H).

4.9 Synthesis of **6b**

Following a procedure similar to that described for compound **4a** using 5,5′-dimethyl-2,2′-dipyridyl and 1,2-dibromoethane as the starting material, 320 mg of powder identified as **6b** was obtained in 86% yield. ^{1}H-NMR (D_2O, 400 MHz): 8.81 (d, *J* = 6.4 Hz, 2 H), 8.62 (s, 2 H), 8.00 (d, *J* = 6.0 Hz, 2 H), 5.06 (s, 4 H), 2.66 (s, 6 H).

4.10 Synthesis of **6c**

Following a procedure similar to that described for compound **4a** using 4,4′-dimethyl-2,2′-dipyridyl and 1,2-dibromoethane as the starting material, 714 mg of gray powder identified as **6b** was obtained in 96% yield. ^{1}H-NMR (D_2O, 400 MHz): 8.89 (s, 2 H), 8.53-8.65 (m, 4 H), 5.11 (s, 4 H), 2.53 (s, 6 H).

4.11 Synthesis of **6d**

Following a procedure similar to that described for compound **4a** using 2,2′-dipyridyl and 1,3-dibromopropane as the starting material, 320 mg of powder identified as **6d** was obtained in 90% yield. ^{1}H-NMR (D_2O, 400 MHz): 9.11 (d, *J* = 5.6 Hz, 2 H), 8.78 (t, *J* = 8.0 Hz, 2 H), 8.37 (d, *J* =7.2 Hz, 2 H), 8.27 (t, *J* = 7.2 Hz, 2 H), 4.89-4.85 (m, 2 H), 4.37-4.46 (m, 2 H), 2.81-2.83 (m, 2 H).

4.12 Synthesis of **6e**

Following a procedure similar to that described for compound **4a** using 4,4′-dimethyl-2,2′-dipyridyl and 1,3-dibromopropane as the starting material, 390 mg of white powder identified as **6e** was obtained in 99% yield. ^{1}H-NMR (D_2O, 400 MHz): 8.95 (s, 2 H), 8.56 (d, *J* = 8.0 Hz, 2 H), 8.19 (d, *J* = 8.0 Hz, 2 H), 4.79-4.83 (m, 2 H), 4.31-4.39 (m, 2 H), 2.75-2.82 (m, 2 H), 2.54 (s, 6 H).

4.13 Synthesis of **6f**

Following a procedure similar to that described for compound **4a** using 5,5′-dimethyl-2,2′-dipyridyl and 1,3-dibromopropane as the starting material, 320 mg of powder identified as **6f** was obtained in 83% yield. ^{1}H-NMR (D_2O, 400 MHz): 8.42 (d, *J* = 5.6 Hz, 2 H), 8.14 (s, 2 H), 8.02-8.04 (m, 2 H), 4.77-4.83 (m, 2 H), 4.24-4.33 (m, 2 H), 2.70-2.75 (m, 2 H), 2.64 (s, 6 H).

5. Steady-state kinetic IspH analysis using dithionite and methyl viologen or MDQ as mediators

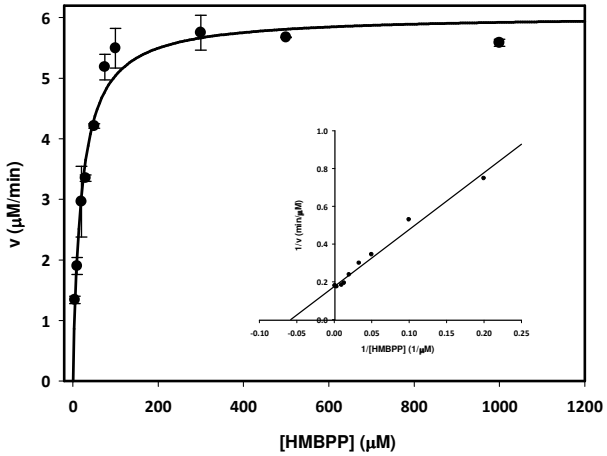


Figure 3S-1. IspH steady-state kinetic analysis with dithionite/MV at 37 °C. Assay mixtures contained 10 nM IspH, *ca* 1.0 mM dithionite reduced methylviologen in 100 mM Tris-HCl, pH 8.0 and various amount of HMBPP in a total volume of 300 μL. The reaction was monitored photometrically at 734 nm. The data was fitted by SigmaPlot.

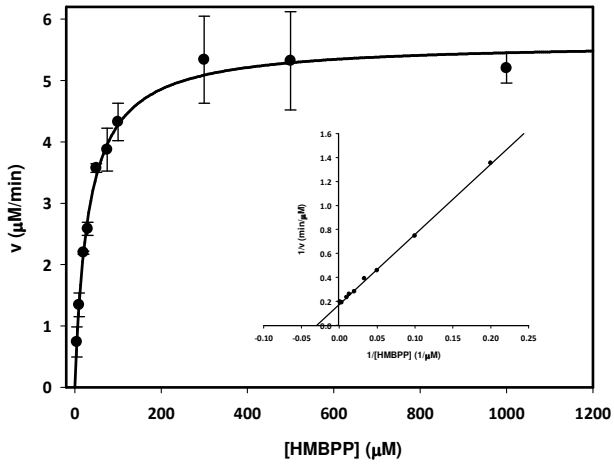
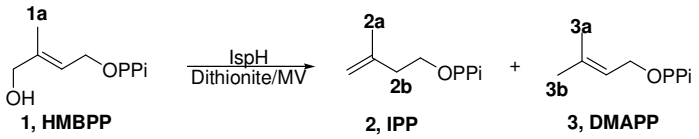


Figure 3S-2. IspH steady-state kinetic analysis with dithionite/MDQ (**6c**) at 37 °C. Assay mixtures contained 5.0 nM IspH, *ca* 1.0 mM dithionite reduced MDQ (**6c**) in 100 mM Tris-HCl, pH 8.0 and various amount of HMBPP in a total volume of 300 μL. The reaction was monitored photometrically at 760 nm. The data was fitted by SigmaPlot.

6. IspH activities under different conditions

[a]Assayed with NADPH, Fpr and FldA as electron transfer system. [b]IspH was purified aerobically as maltose binding fusion. [c]Analyzed products formation by ion-pair HPLC and liquid scintillation counting. [d]Assayed with photoreduced deazaflavin (DAF). [e]IspH was purified as the histindine-tagged form aerobically. [f]Measured products' radioactivity by liquid scintillation counting after TLC separation. [g]IspH was reconstituted with Na_2S, ferric chloride and dithiothreitol anaerobically. [h]The assay was in presence of 30 mM NaF. [i]IspH was purified from an *isc* operon as the his-tagged form anaerobically. [j]Determined by ^{13}C NMR with radiolabled HMBPP. [k]Monitored NADPH consumption by UV. [l]Assayed with NADH-Fpr-FldA. [m]Assayed with NADPH-FldA and ferredoxin reductase (Fdr) from spinach. [n]Assayed with NADPH, ferredoxin (FdX) and Fdr from spinach. [o]Using dithionite (DT) as reducing agent. [p]IspH was purified from an *isc* operon as Strep-tagged form anaerobically. [q]Assayed with reduced form of methyl viologen (MV) and DT as reductant. [r]The reaction was performed at 60°C. [s]Determined by monitoring the oxidation of DT reduced methyl viologen (MV) by UV. [t]Assayed with NADPH, *P. falciparum* ferredoxin (Fd), and ferredoxin-$NADP^+$ reductase (FNR).

7. IspH reaction product characterization.



1, HMBPP **2, IPP** **3, DMAPP**

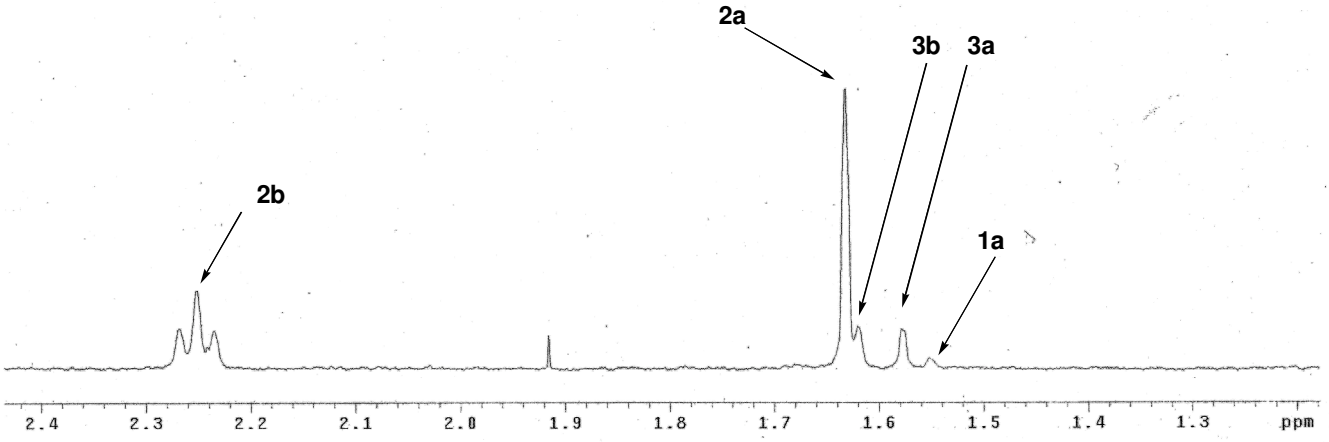


Figure 4S-a. The ^{1}H-NMR of IspH reaction mixture. IPP : DMAPP is 4.7 : 1.0

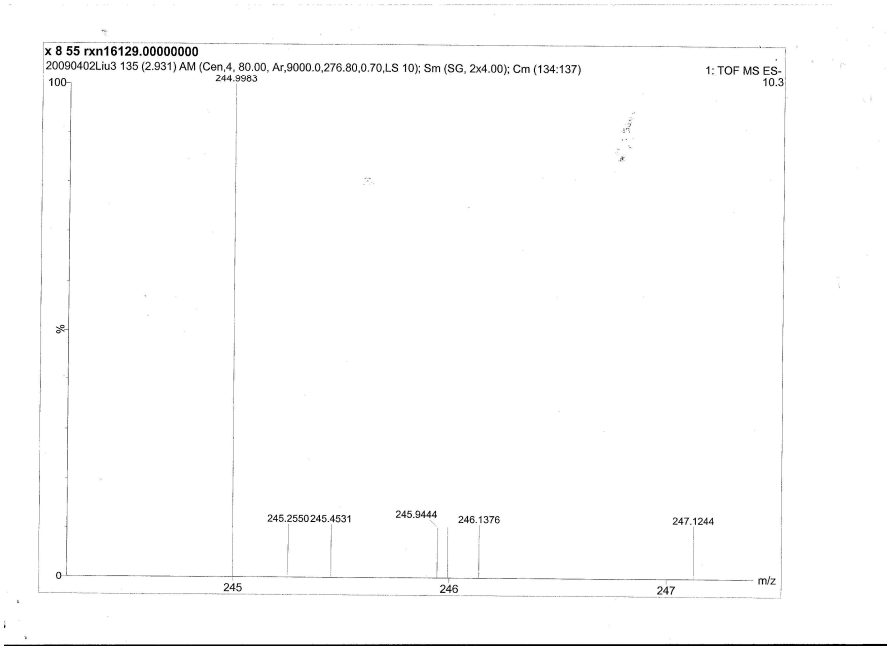


Figure 4S-b. The high resolution ESI-MS of IspH reaction mixture (calculated molecular weight for IPP and DMAPP as $[M-H]^-$ form was 244.9980, and found 244.9983).