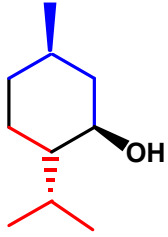


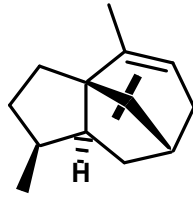


**The methylerythritol phosphate pathway
for the formation of isoprene units:
a new target for antimicrobial drugs**

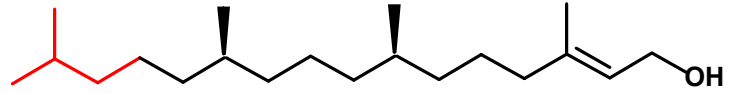
**CNRS / Université de Strasbourg
Institut de Chimie
France**



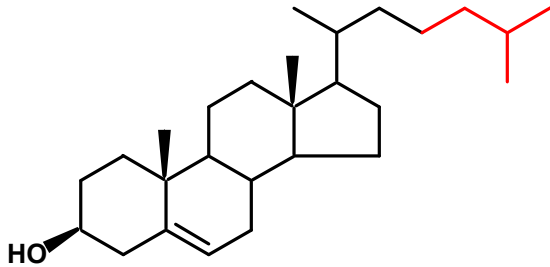
C₁₀ menthol



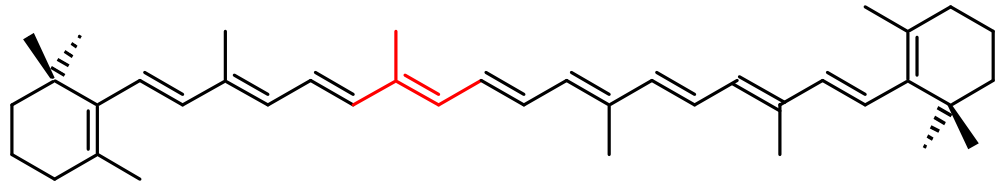
C₁₅ patchoulene



C₂₀ phytol

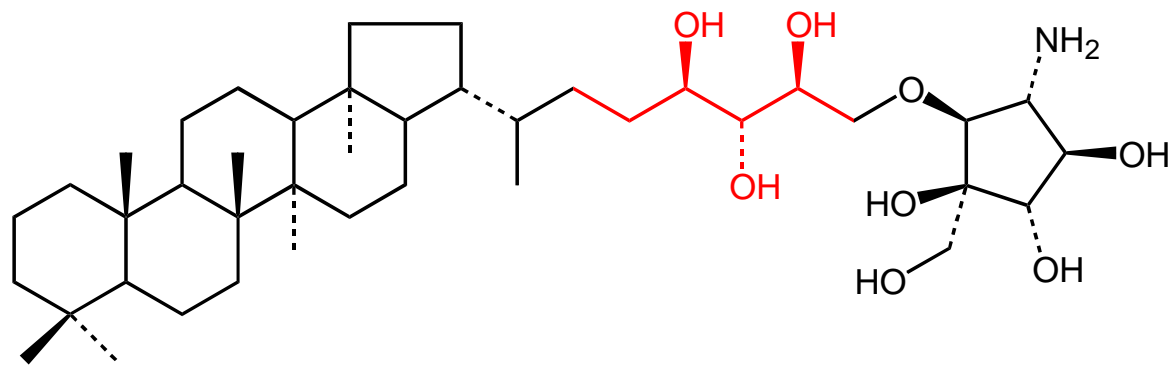
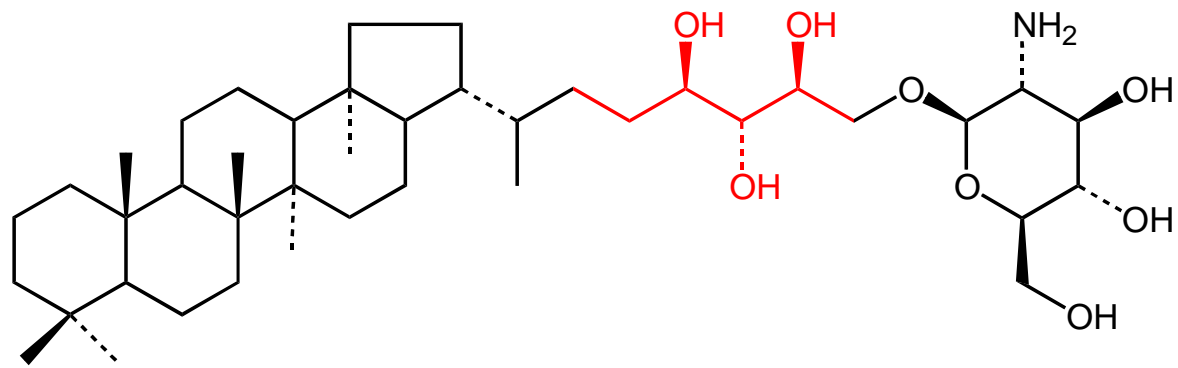


**C₃₀ derivative
cholesterol**

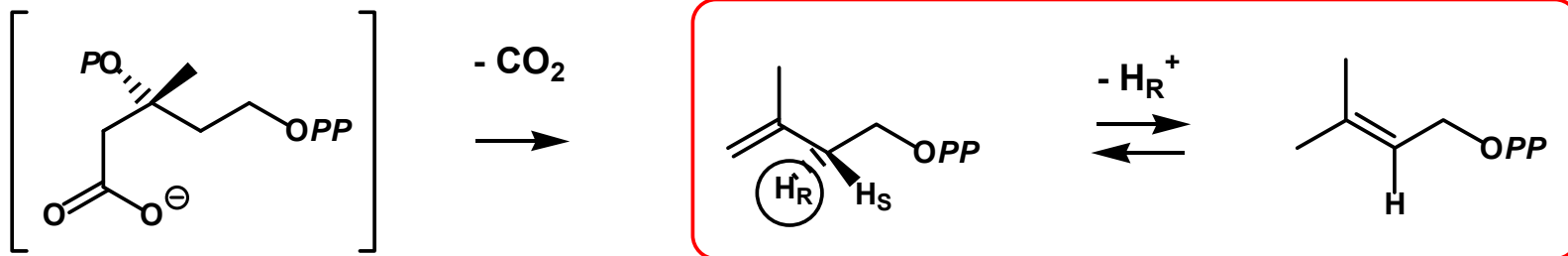
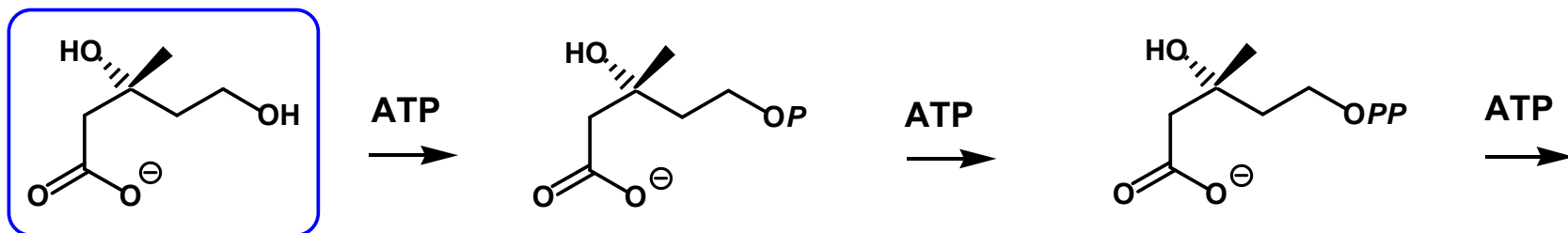
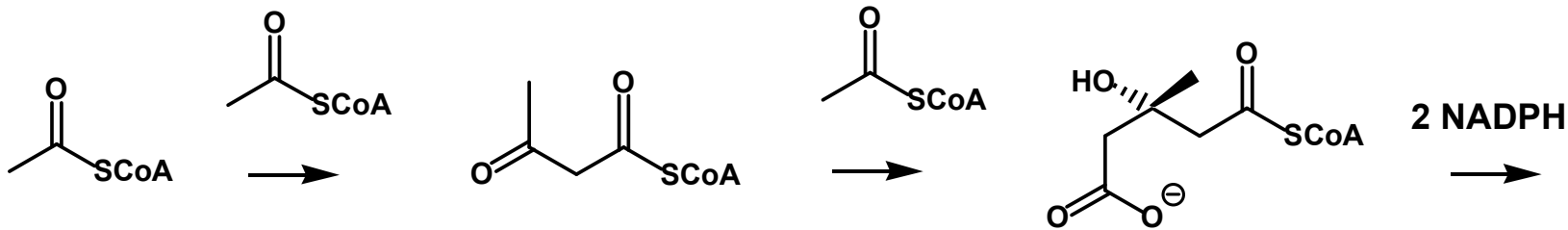


C₄₀ β-carotene

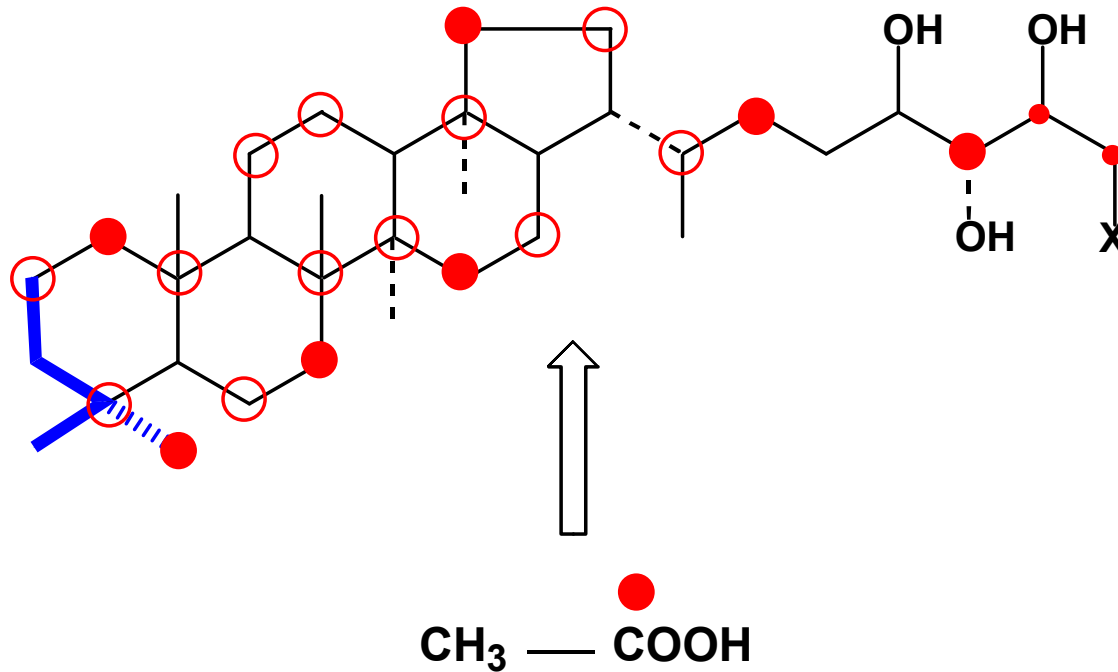
ISOPRENOIDS



Major hopanoids from *Zymomonas mobilis*



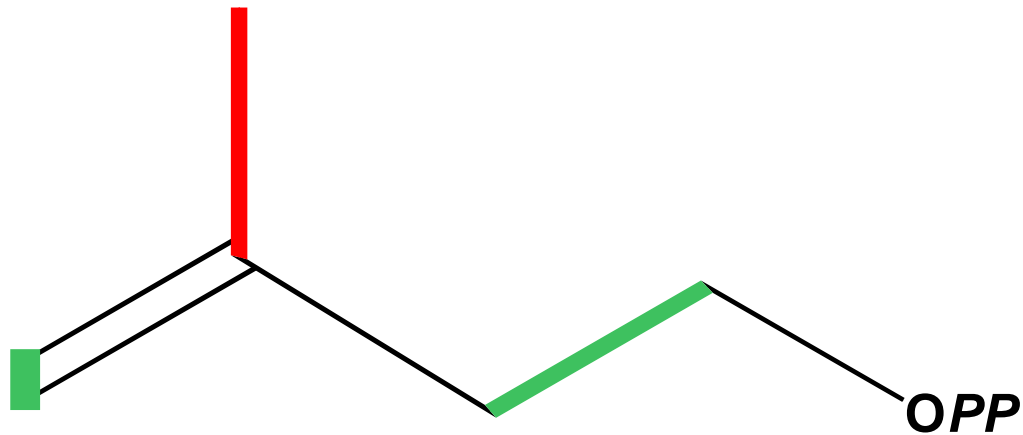
Mevalonate pathway for isoprenoid biosynthesis



Methylobacterium organophilum, *M. fujisawaense*,
Rhodopseudomonas palustris, *R. acidophila*.

Incorporation of $[1-^{13}\text{C}]$ acetate into hopanoids:

- observed labelling pattern ●
- expected labelling pattern ○



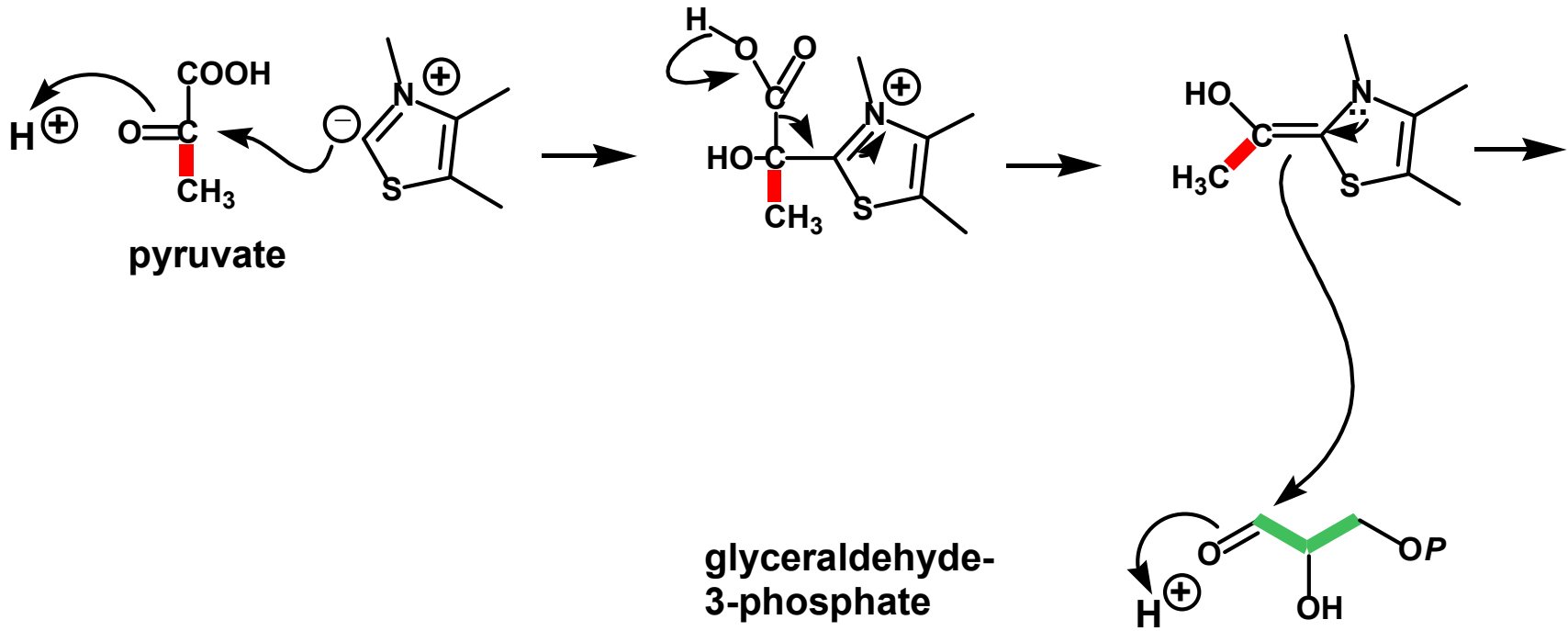
Isopentenyl diphosphate biosynthesis in eubacteria

Origin of the carbon atoms from ^{13}C labelling experiments

- **C₂ unit** from pyruvate decarboxylation
- **Glyceraldehyde 3-phosphate**

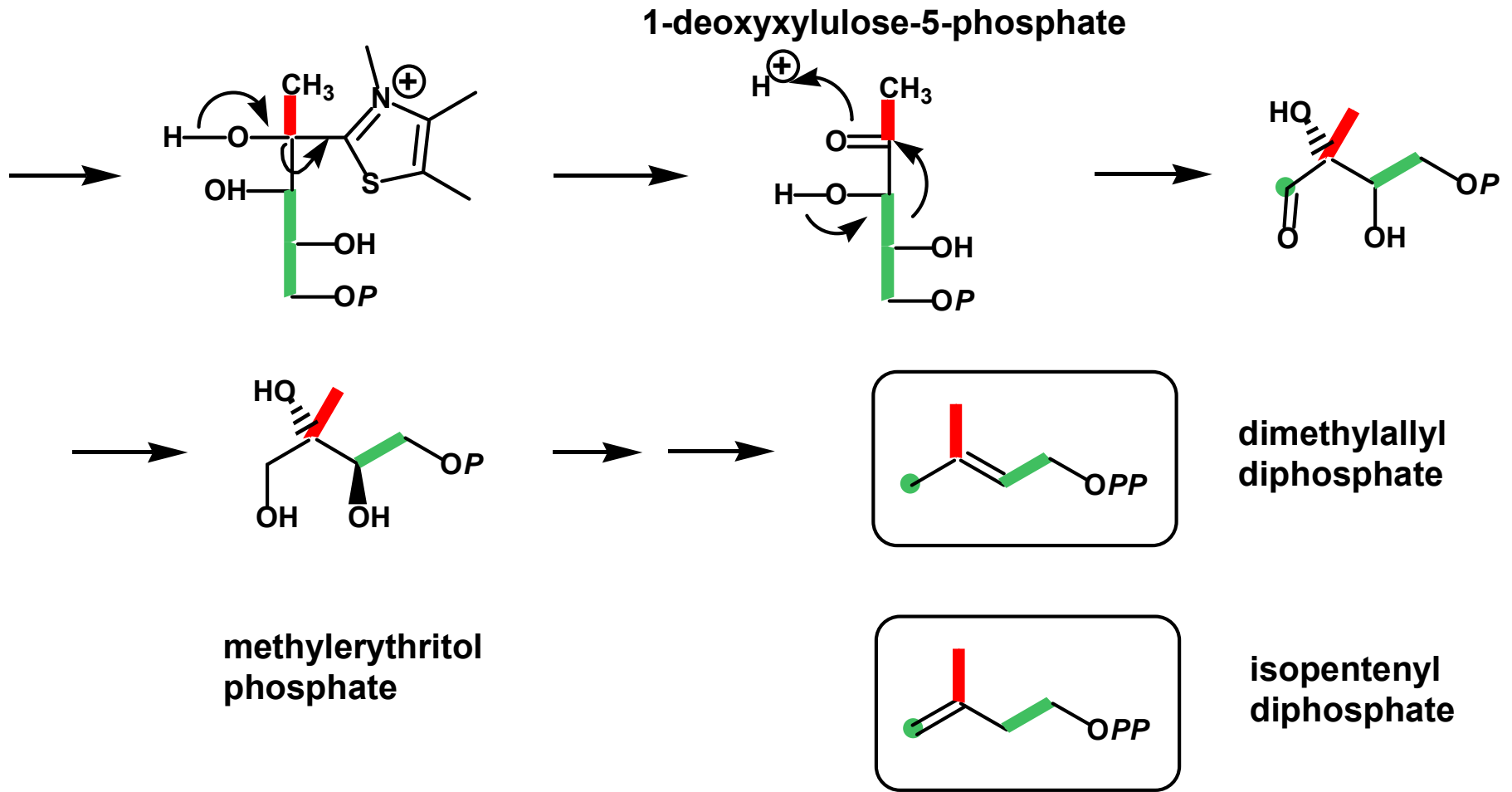
Rohmer *et al.*, *Biochem. J.* **295**, 517, 1993; *J. Am. Chem Soc.* **118**, 2564, 1996

Pyruvate decarboxylase

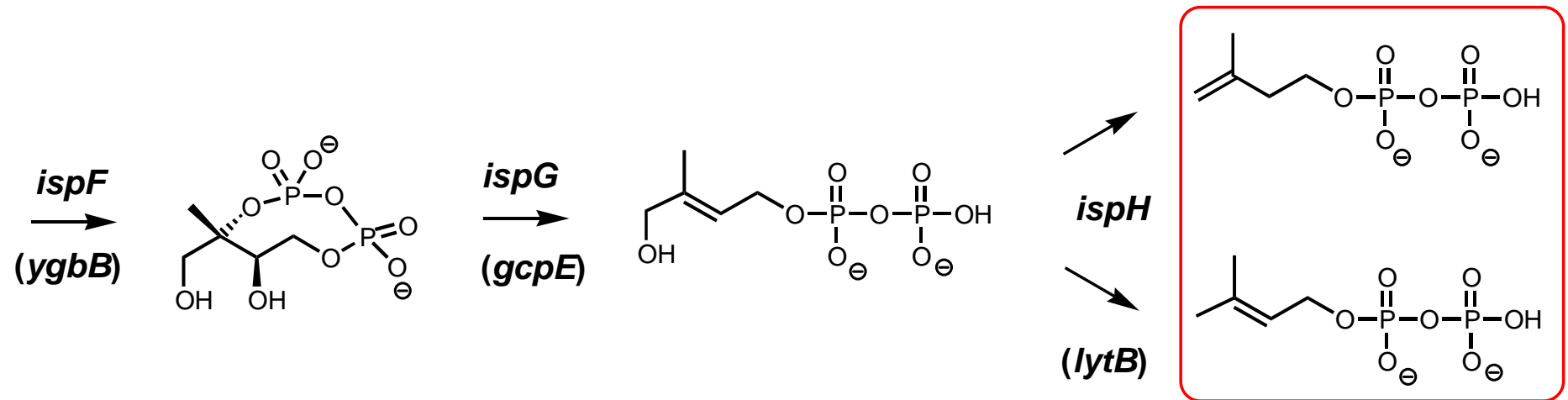
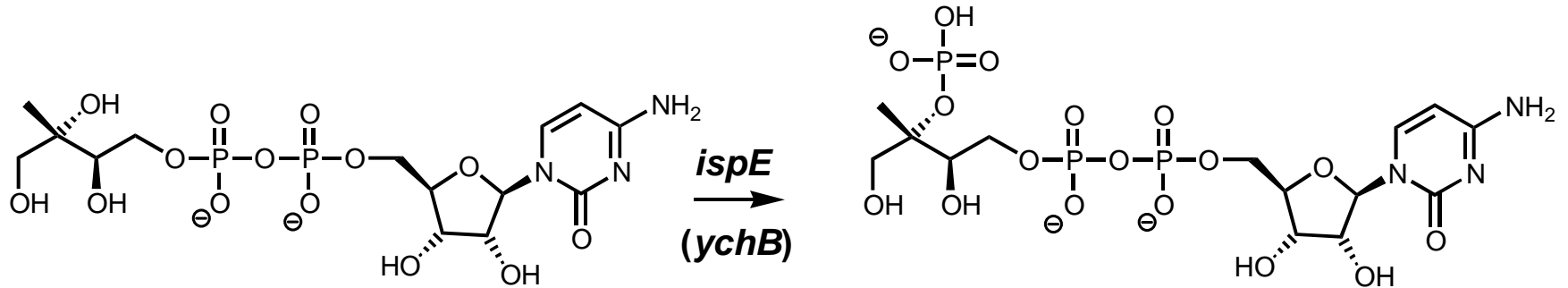
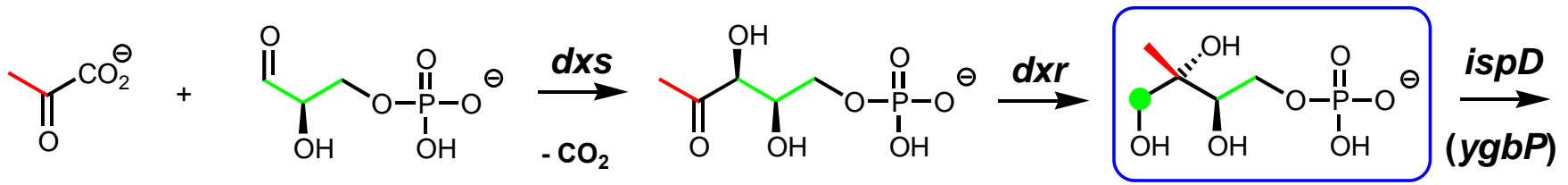


Hypothetical biogenetic scheme

for the biosynthesis of isoprenic units in eubacteria, Part 1.



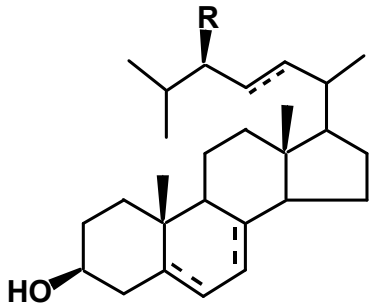
Hypothetical biogenetic scheme for the biosynthesis of isoprenic units in eubacteria, Part 2.



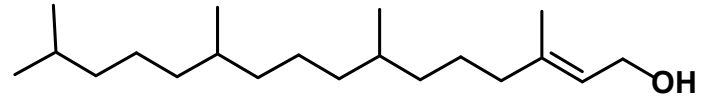
**Methylerythritol phosphate pathway (MEP) pathway:
 from pyruvate and glyceraldehyde phosphate to IPP and DMAPP**

CYTOPLASM

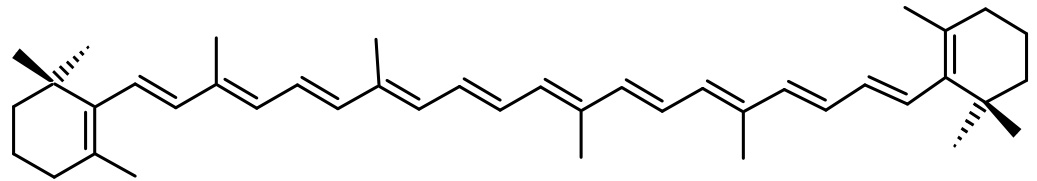
CHLOROPLASTS



sterols

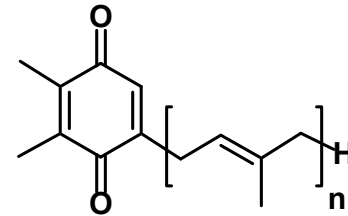


phytol



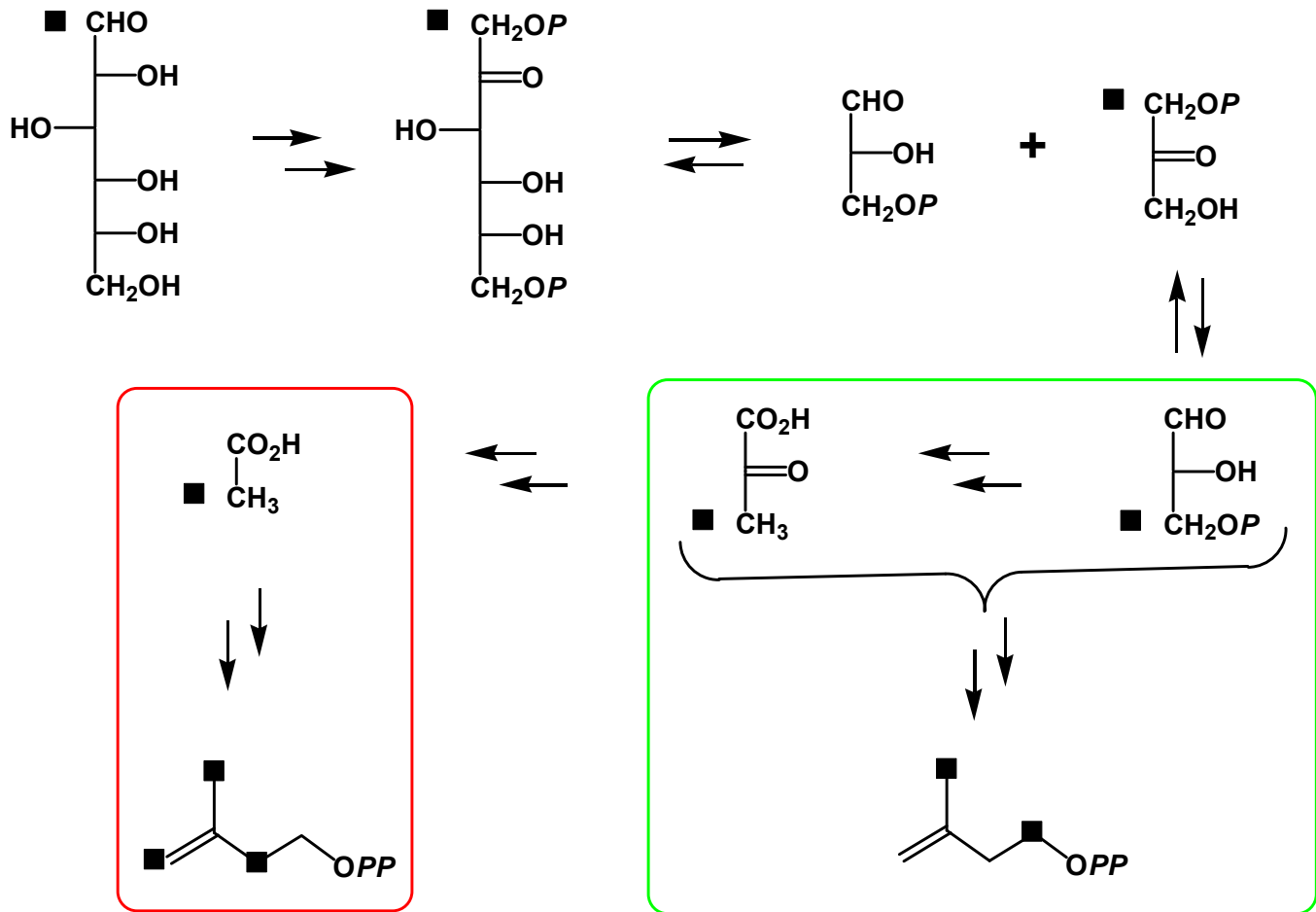
carotenoids

ISOPRENOIDS



plastoquinone

FROM PLANTS AND ALGAE



higher plants

cytoplasm (MVA)

sterols

triterpenes

sesquiterpenes

ubiquinone

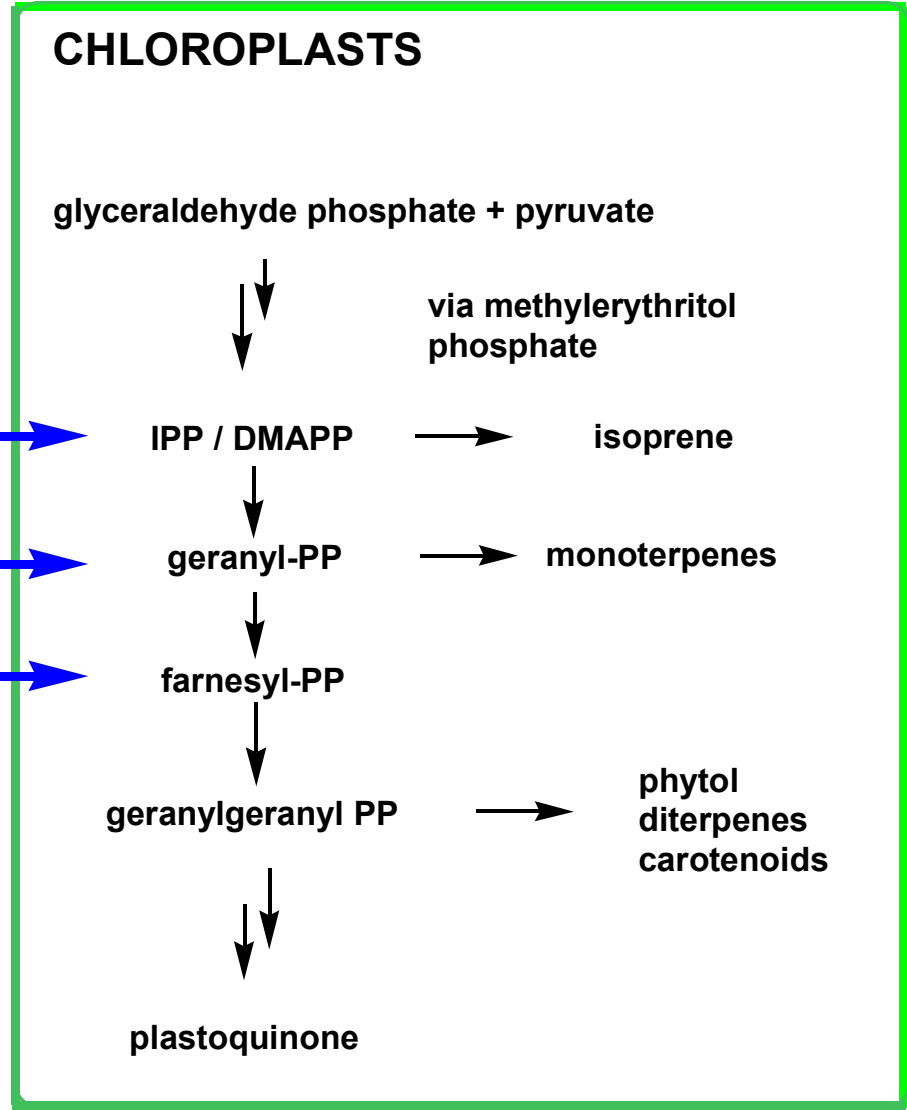
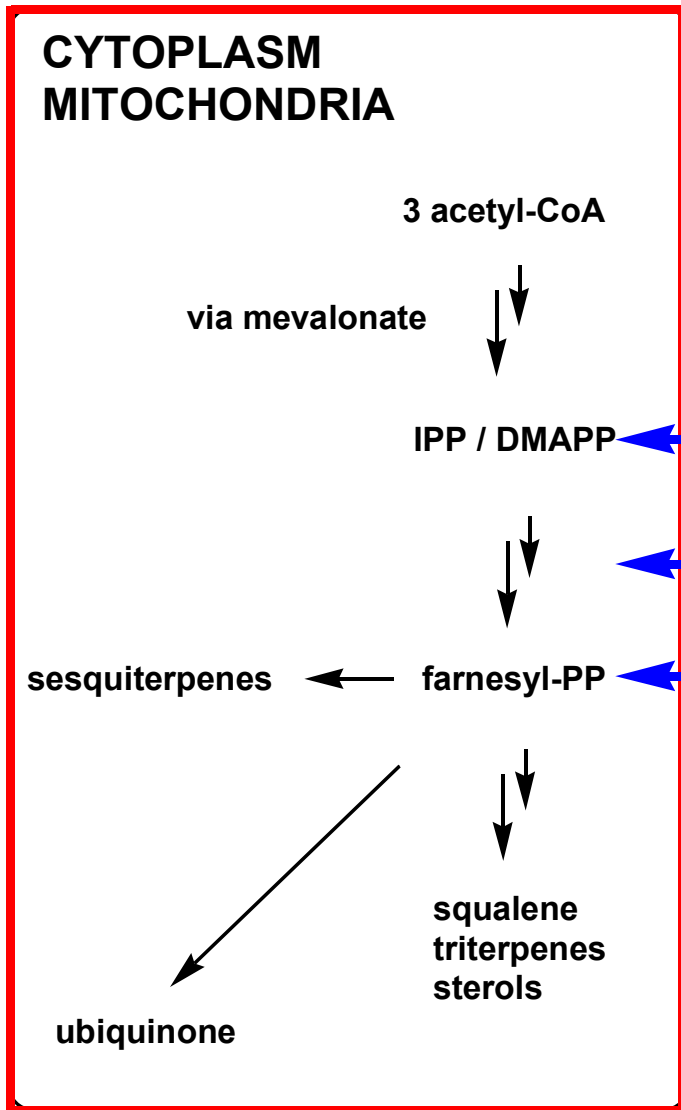
plastids (MEP)

phytol, carotenoids, plastoquinone

hemi-, mono- and diterpenes

sesquiterpenes

Isoprenoid biosynthesis in photosynthesizing eukaryotes



C₅

C₁₀

C₁₅

C₂₀

C₄₀

C₃₀

C_{5n}

Hypothetical compartmentation of isoprenoid biosynthesis in higher plants

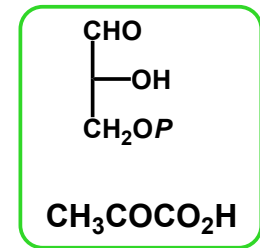
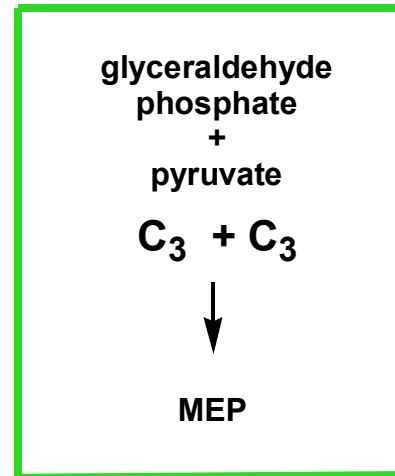
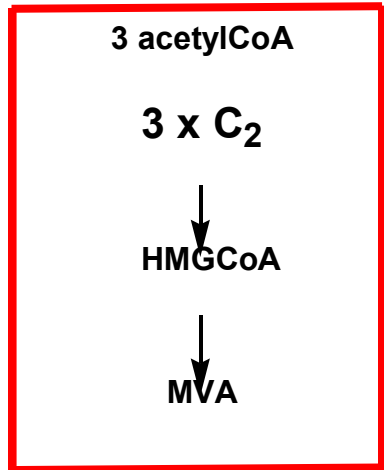
Carbon and energy sources

ANIMALS, FUNGI
PLANTS (cytoplasm)
ARCHAEA
EUBACTERIA

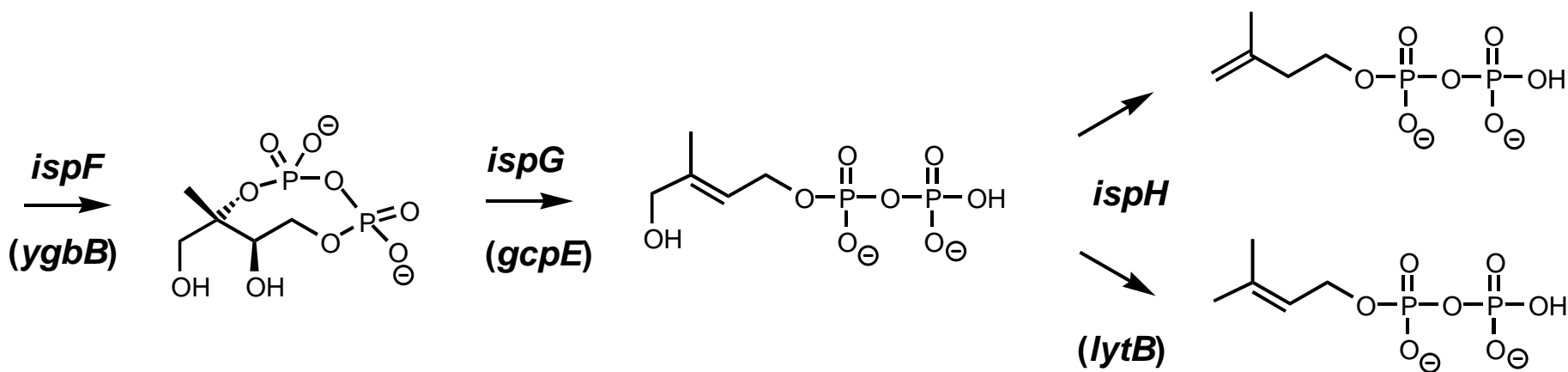
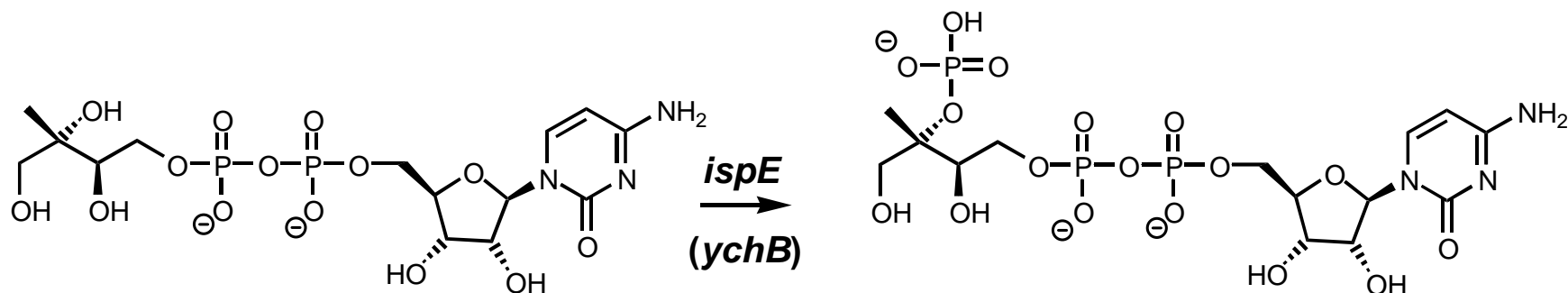
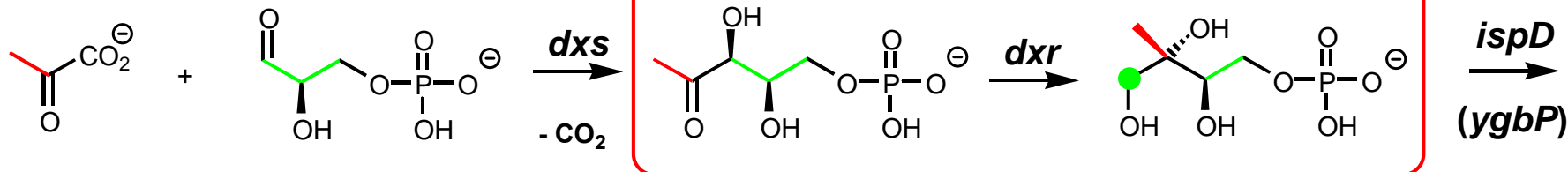
CHLOROPLASTS (plants, charophyta,
rhodophyta, chrysophyta)
GREEN ALGAE, *Plasmodium*, *Prototheca*
EUBACTERIA



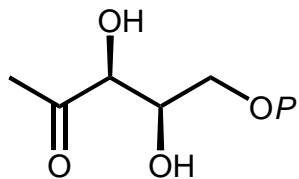
3 x CH₃CO₂H



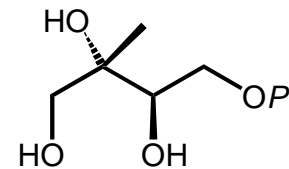
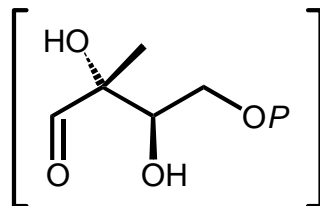
C₅: Isopentenyl-PP \rightleftharpoons Dimethylallyl-PP \Rightarrow Polyterpenoids



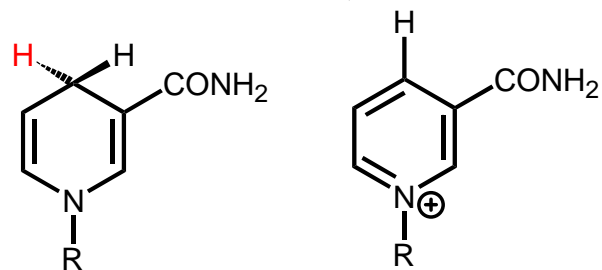
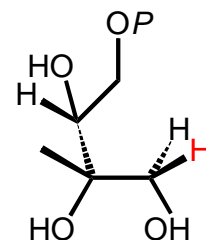
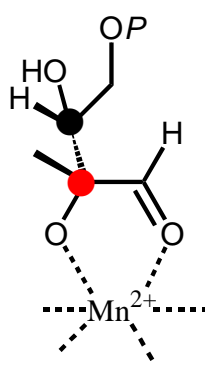
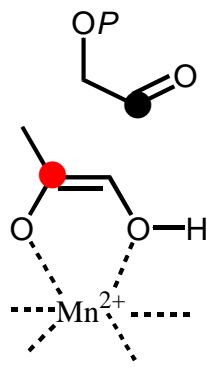
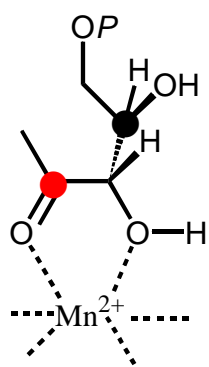
**Methylerythritol phosphate pathway (MEP) pathway:
 deoxyxylulose phosphate synthase (DXS) and reductoisomerase (DXR)**



1-deoxyxylulose 5-phosphate



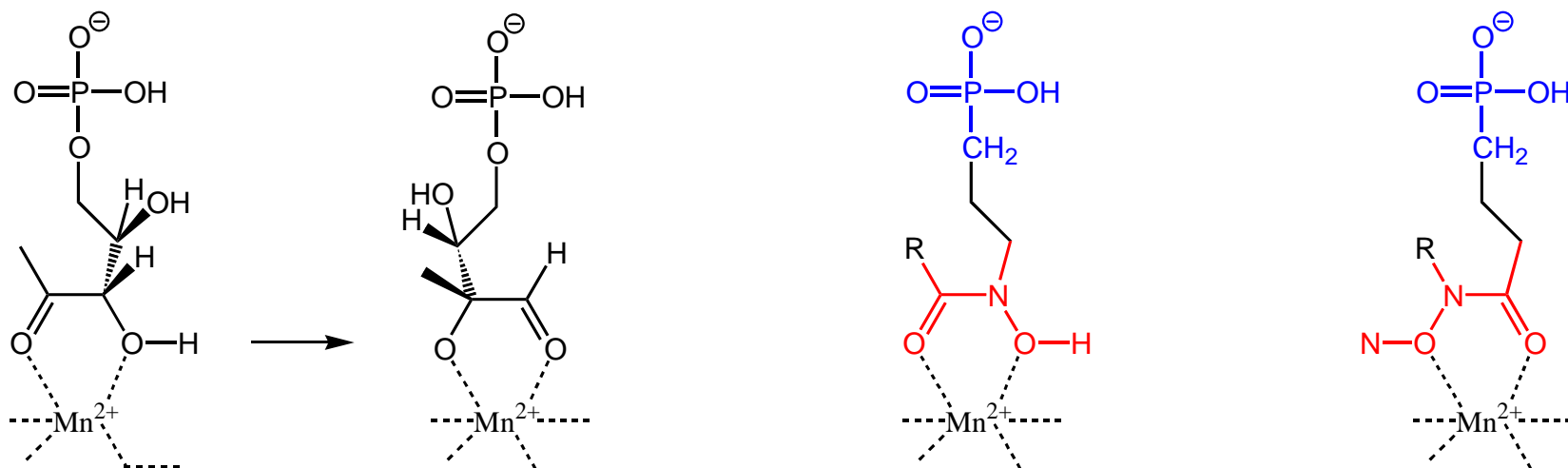
2-C-methylerythritol 4-phosphate



1-Deoxyxylulose 5-phosphate reducto-isomerase

Kuzuyama et al., 1998; Takahashi et al., 1998; Arigoni et al., 1999; Radykewicz et al., 1999;

Hoeffler et al., 2002; Steinbacher et al., 2003; Wong & Cox, 2007



R = H

fosmidomycin $K_i = 38$ nM

$K_i = 170$ nM

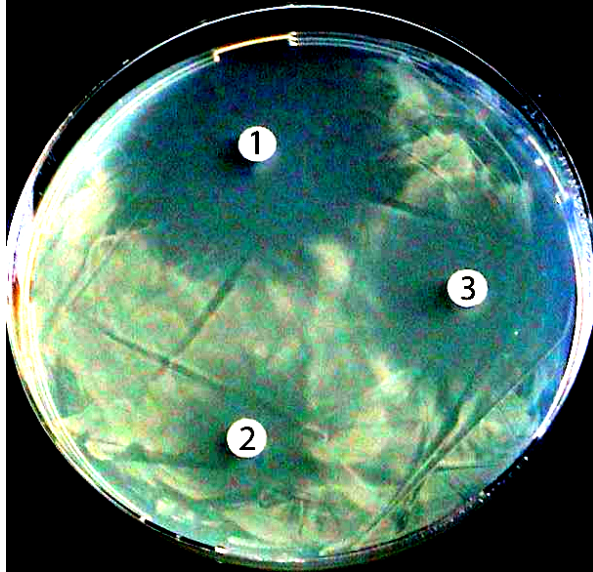
R = CH₃

FR900098

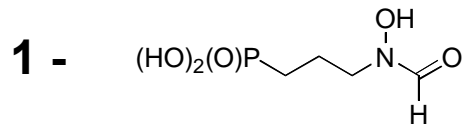
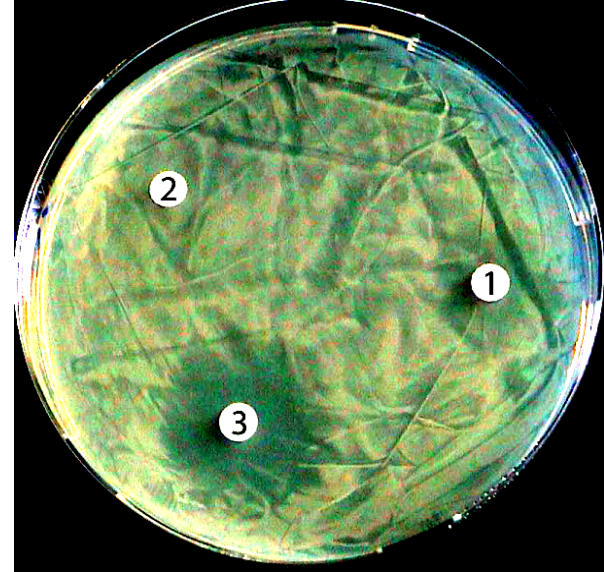
$K_i = 54$ nM

Inhibitors of 1-deoxyxylulose 5-phosphate reducto-isomerase

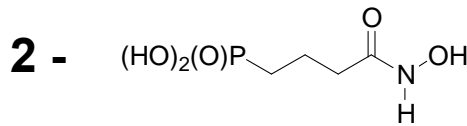
Wild type



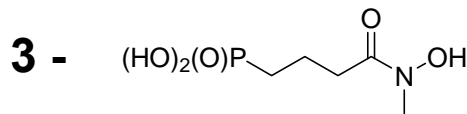
Fosmidomycin resistant strain



fosmidomycin 50 µg/disk

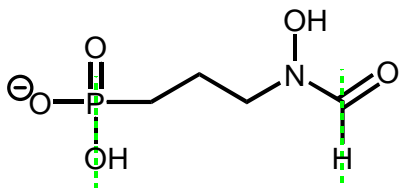
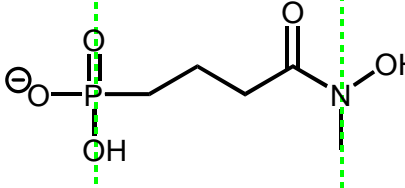
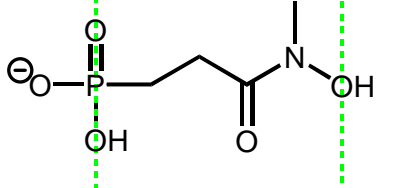
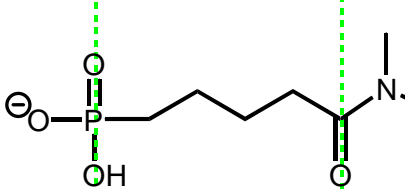
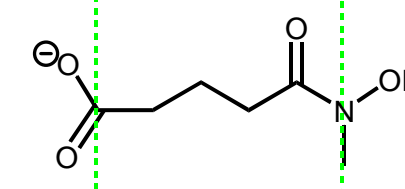
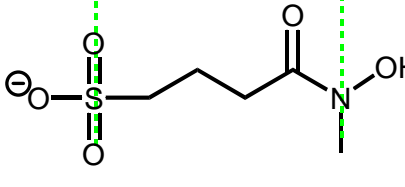


50 µg/disk



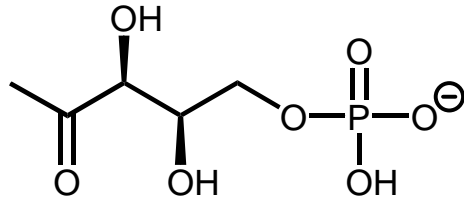
50 µg/disk

Growth inhibition of *Escherichia coli* by DXR inhibitors

	without	with	preincubation
	0.25	0.032	
	0.50	0.049	
	77	19	
	0.90	0.11	
	270	25	
	564	48	
			IC₅₀ μM

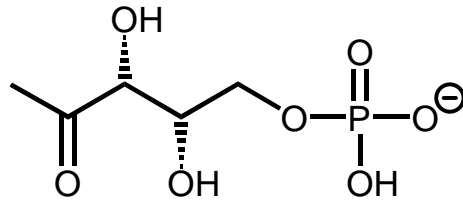
Inhibition of deoxyxylulose phosphate reducto-isomerase by fosmidomycin analogues

D-DXP



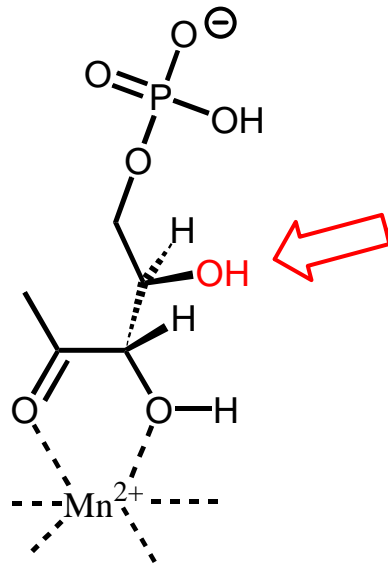
$$K_M = 30 \mu\text{M}$$

L-DXP



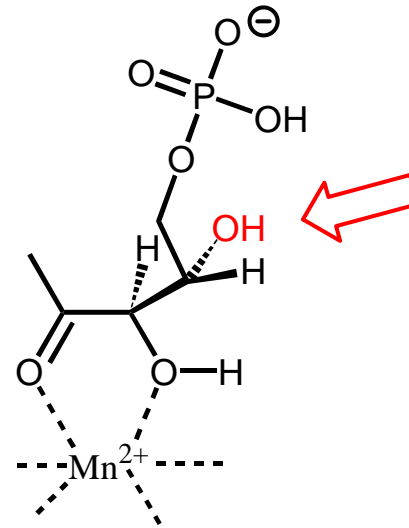
$$K_I = 147 \mu\text{M}$$

**1-Deoxyxylulose 5-phosphate reducto-isomerase.
L-DXP as competitive inhibitor**



D-DXP

substrate

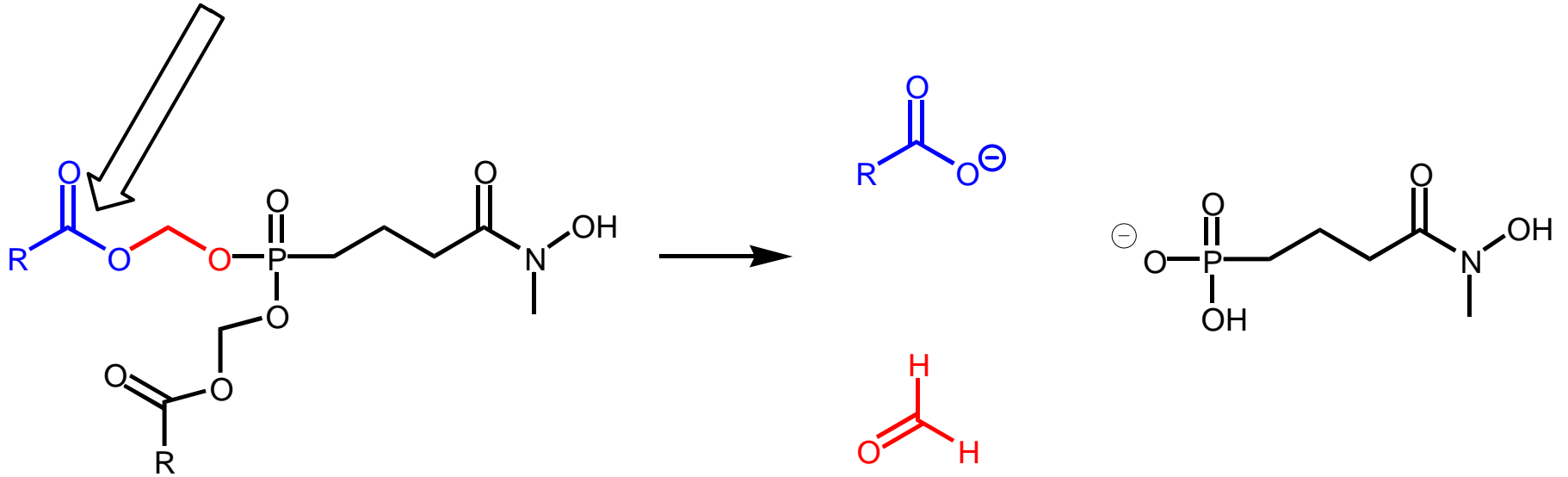


L-DXP

competitive inhibitor

**1-Deoxyxylulose 5-phosphate reducto-isomerase.
L-DXP as competitive inhibitor**

hydrolysis by
esterases,
lipases...

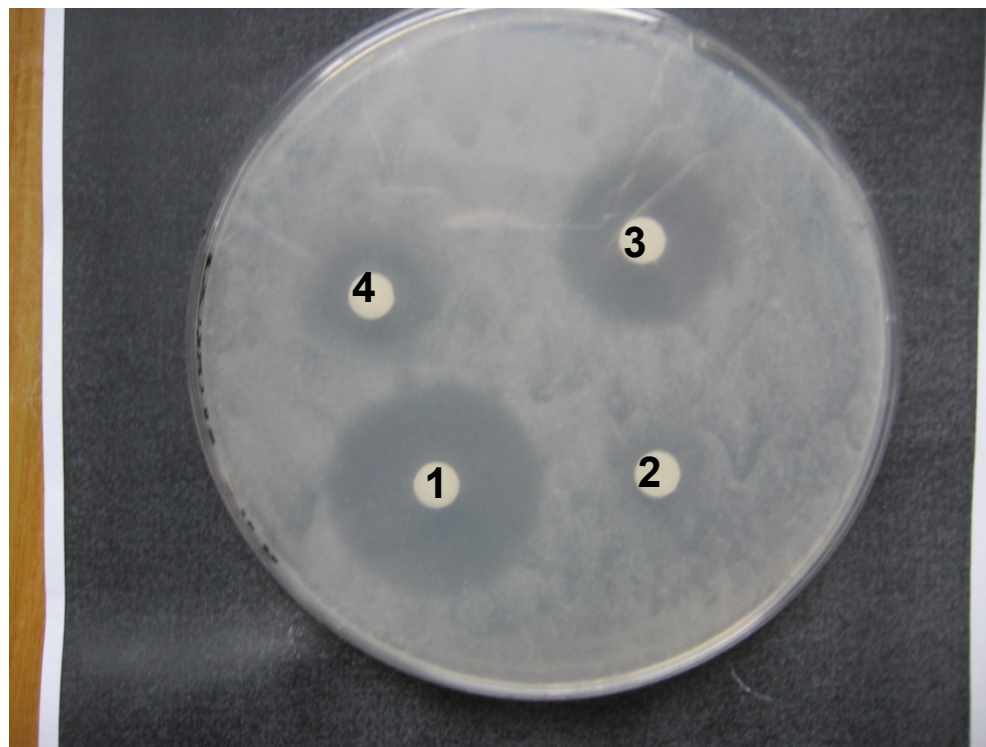


prodrug

drug

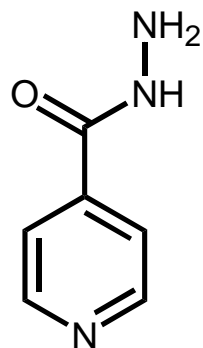
Lipophilic prodrugs of fosmidomycin analogues

4 R = -Ph

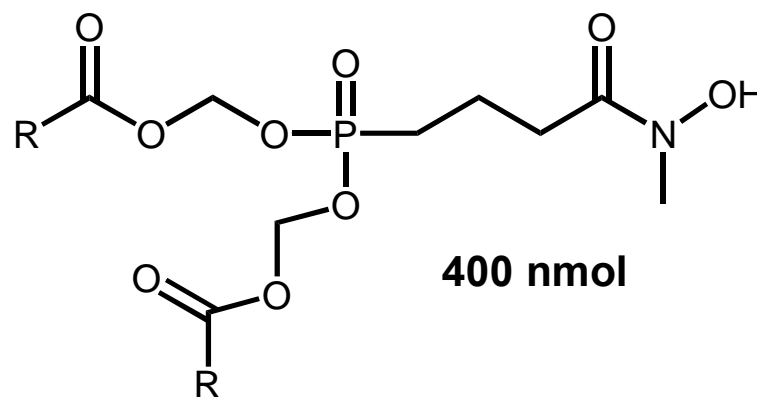


3 R = *n*-Pr

2 R = *t*-Bu



1 30 nmol



400 nmol

Growth inhibition of *Mycobacterium smegmatis* by isoniazide (1) and fosmidomycin analogue prodrugs (2 - 4)

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Ecole Nationale Supérieure de Chimie de Mulhouse**

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T. DUVOLD
G. FLESCH**

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L. KUNTZ
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**S. PONAIRE
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D. TRITSCH
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Prof. Dr. H. LICHTENTHALER, J. SCHWENDER**

**Forschungszentrum Jülich, FRG, Institut für Biotechnologie
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**Universität Lübeck / Technische Universität Kaiserslautern, FRG
Prof. Dr. A.X. TRAUTWEIN / Prof. Dr. V. SCHÜNEMANN**