Development & Applications of ^{31}P-NMR Spin Trapping; Toward a Detailed Understanding of Radical Mechanism of Action of Oxidative Enzymes

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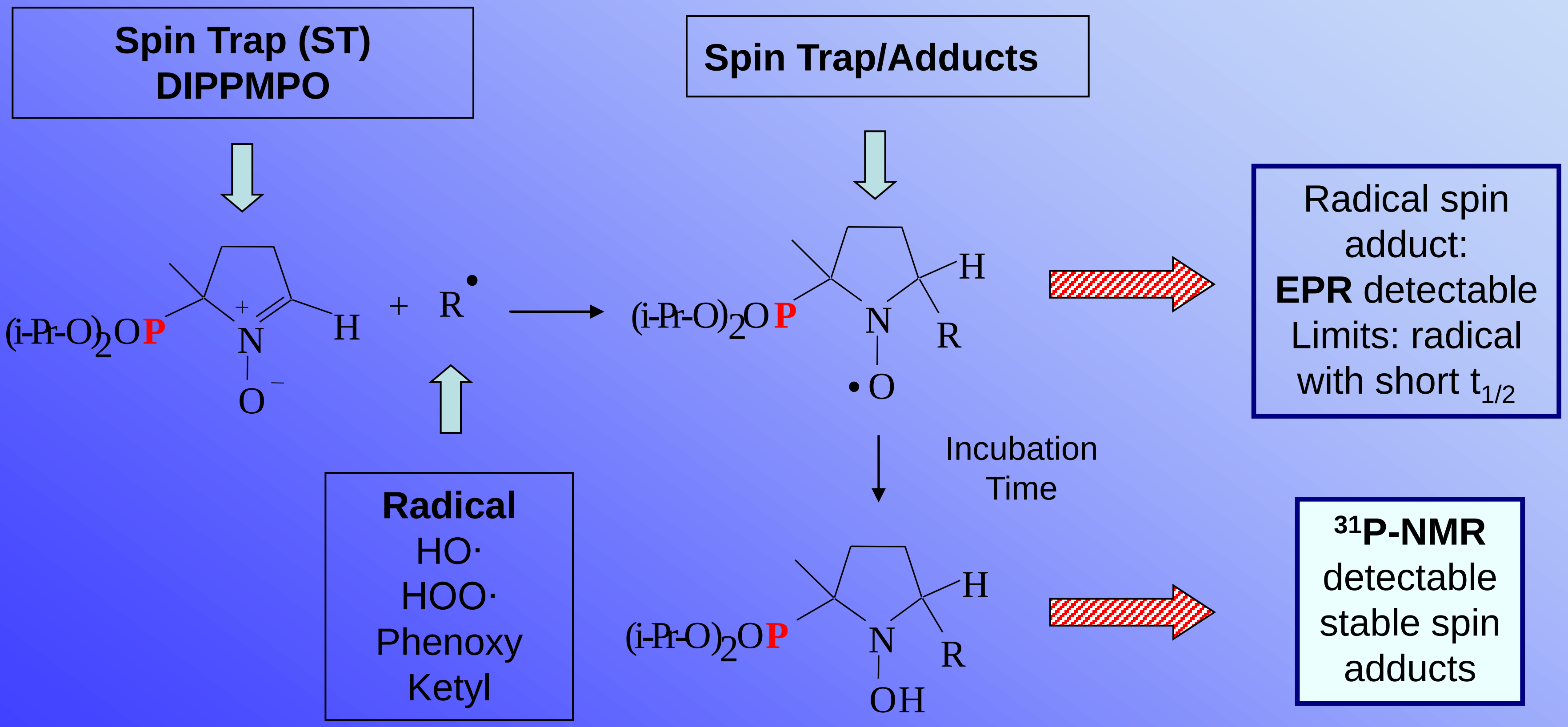
Spin Traps and Spin Adducts

- ✓ ST are molecules that form stable radical adducts;
- ✓ Radicals adducts are suitably detected using EPR;
- ✓ Using ST such as 5-diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO), adducts can be detected and quantified accurately by ^{31}P-NMR;



Objectives

✓ Correlate the ^{31}P-NMR chemical shifts to the nature of the radicals being trapped.

✓ Study the mechanism of oxidative enzymes

HRP (Horse Radish Peroxydase)

- H - Atom Abstraction from benzylic alcohols → Ketyl radical
- Single Electron Oxidation of phenols → Phenoxy radical

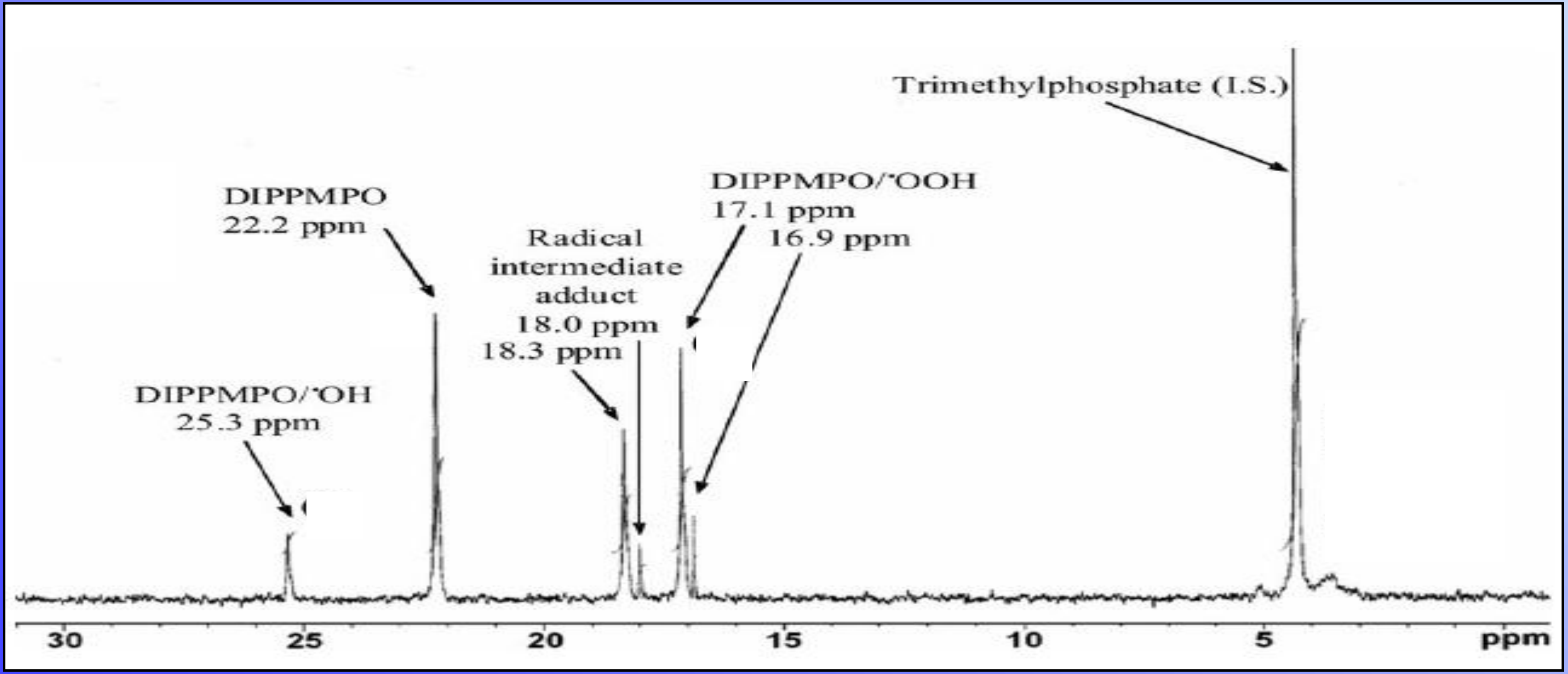
Develop quantitative ^{31}P-NMR techniques aimed at unraveling complex radical pathways in organic, chemo-enzymatic & eventually reactions within living cells

Spin Trapping of Oxygen-Centered Radicals by DIPPMPO

Argyropoulos D. S. et al. *Bioorganic & Medicinal Chemistry* 2006, 15: 4017-4028

Oxygen-Centered Radicals

Species	Chemical shift (ppm)
DIPPMPO	22.2
DIPPMPO/·OH	25.3
DIPPMPO/·OOH	16.9, 17.1
Intermediate radical species	18.0, 18.3



Argyropoulos D. S. et al. *Bioorganic & Medicinal Chemistry* 2006, 15: 4017-4028

Spin Trapping of Carbon-Centered Radicals by DIPPMPO

Argyropoulos D. S. et al. *Bioorganic & Medicinal Chemistry* 2006, 15: 4017-4028

Carbon-Centered Radicals

Species	Chemical shift (ppm)
DIPPMPO	22.2
DIPPMPO/·CH_3	23.1
DIPPMPO/·CH_2OH	22.6
DIPPMPO/·$CH(OH)CH_3$	27.3
DIPPMPO/·$C(O)CH_3$	30.2
DIPPMPO/·$C(OH)(CH_3)_2$	29.0
DIPPMPO/·$C(OH)(CH_3)Ph$	28.0

Despite the long distance involved,

nature of carbon affects ^{31}P-NMR chemical shift

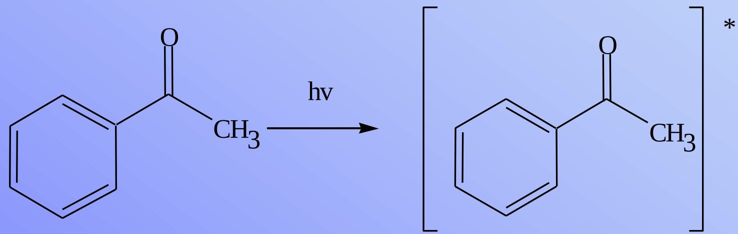
$3^0 > 2^0 > 1^0$

Argyropoulos D. S. et al. *Bioorganic & Medicinal Chemistry* 2006, 15: 4017-4028

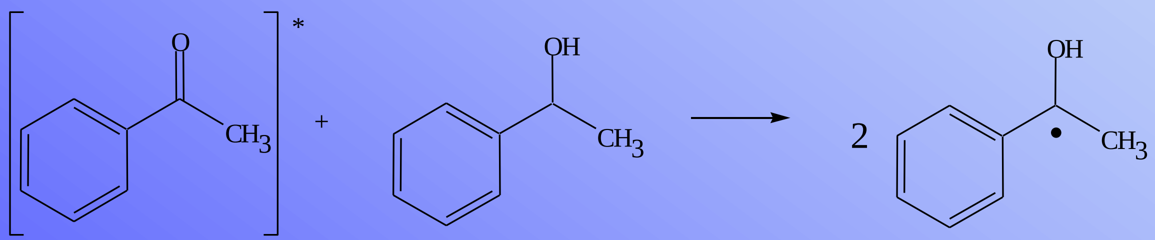
Spin Trapping of Ketyl Radical by DIPPMPO

L. Zoia, Argyropoulos., D. S.,"Ketyl Radical Detection Using Quantitative ^{31}P NMR Spin Trapping", Chemitry ; J. Phys. Org. Chem. 2009, DOI:10.1002/poc. 1561.

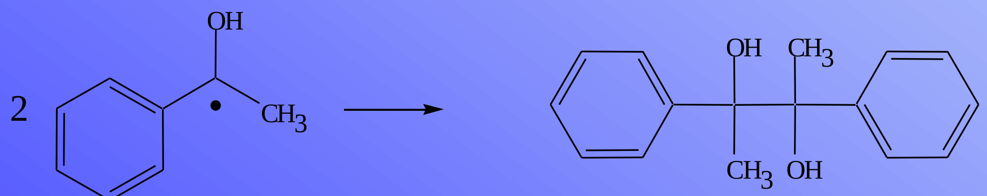
1-Phenylethanol-1-yl (Ketyl) Radical



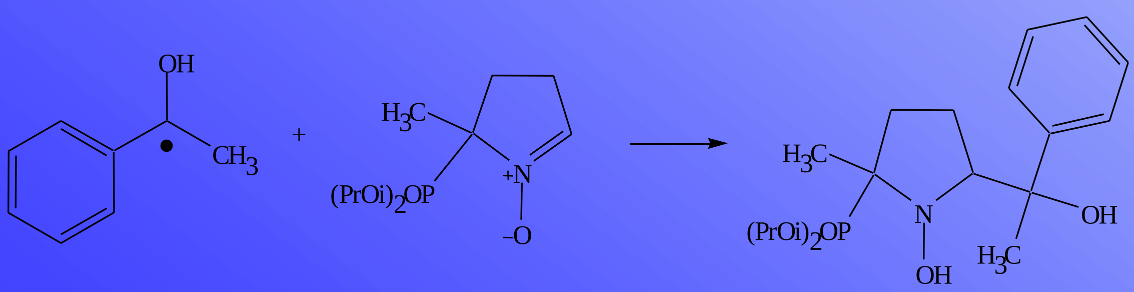
Generation of triplet state (n-π)* of acetophenone with UV irradiation



H-abstraction from H-donor



Pinacol coupling self termination reaction (*meso*, *d* and *l* products)

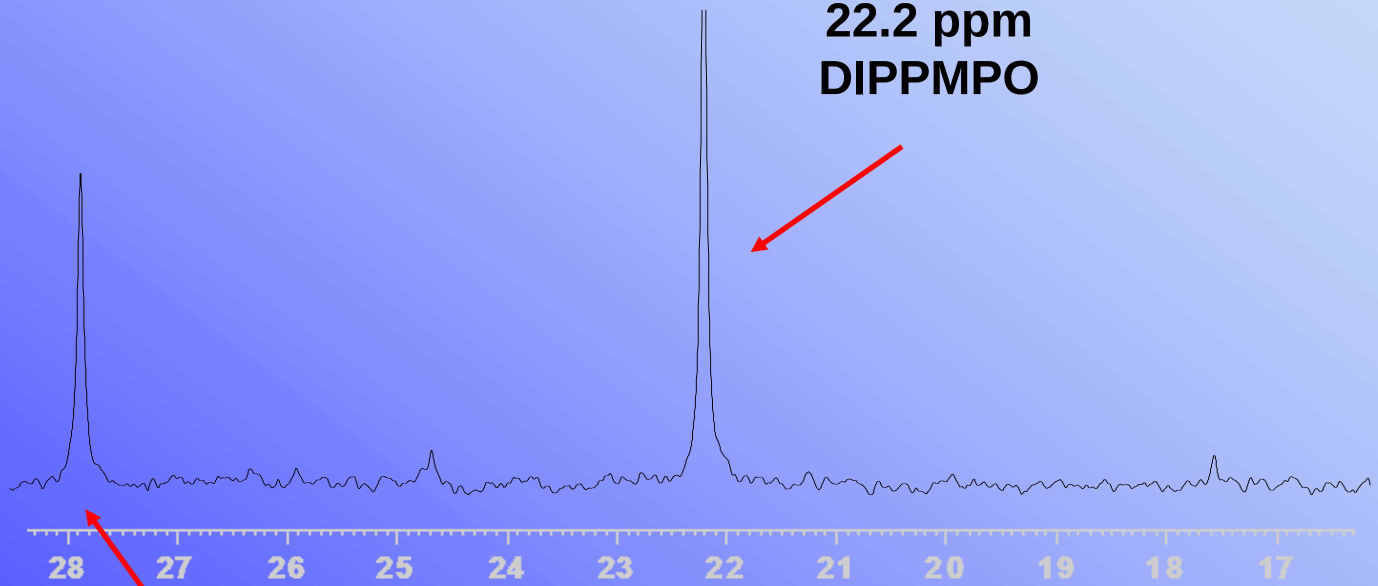


Trapping of Ketyl radicals with DIPPMPO (28.0 ppm)

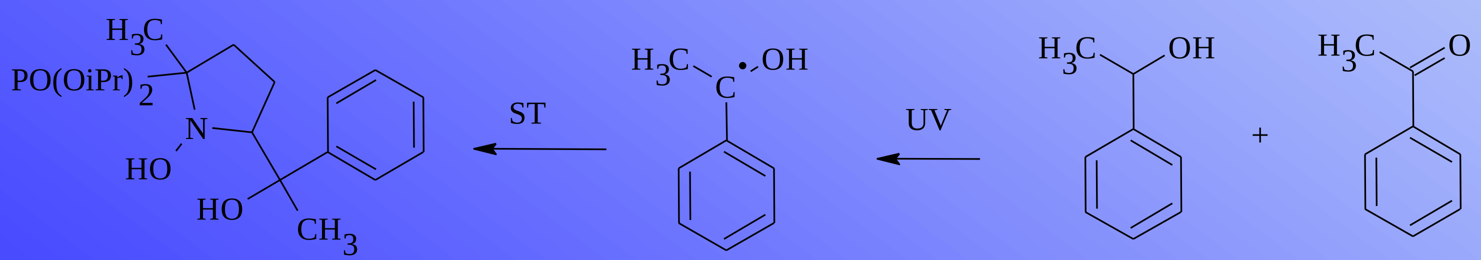
^{31}P-NMR Spectra Interpretation

Photochemical generation of ketyl radical

22.2 ppm
DIPPMPO

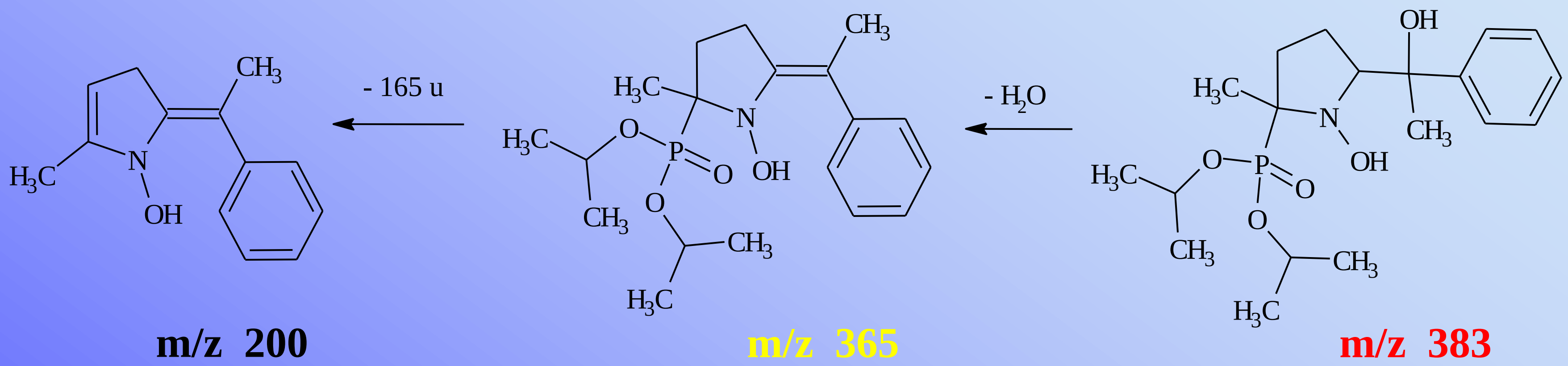


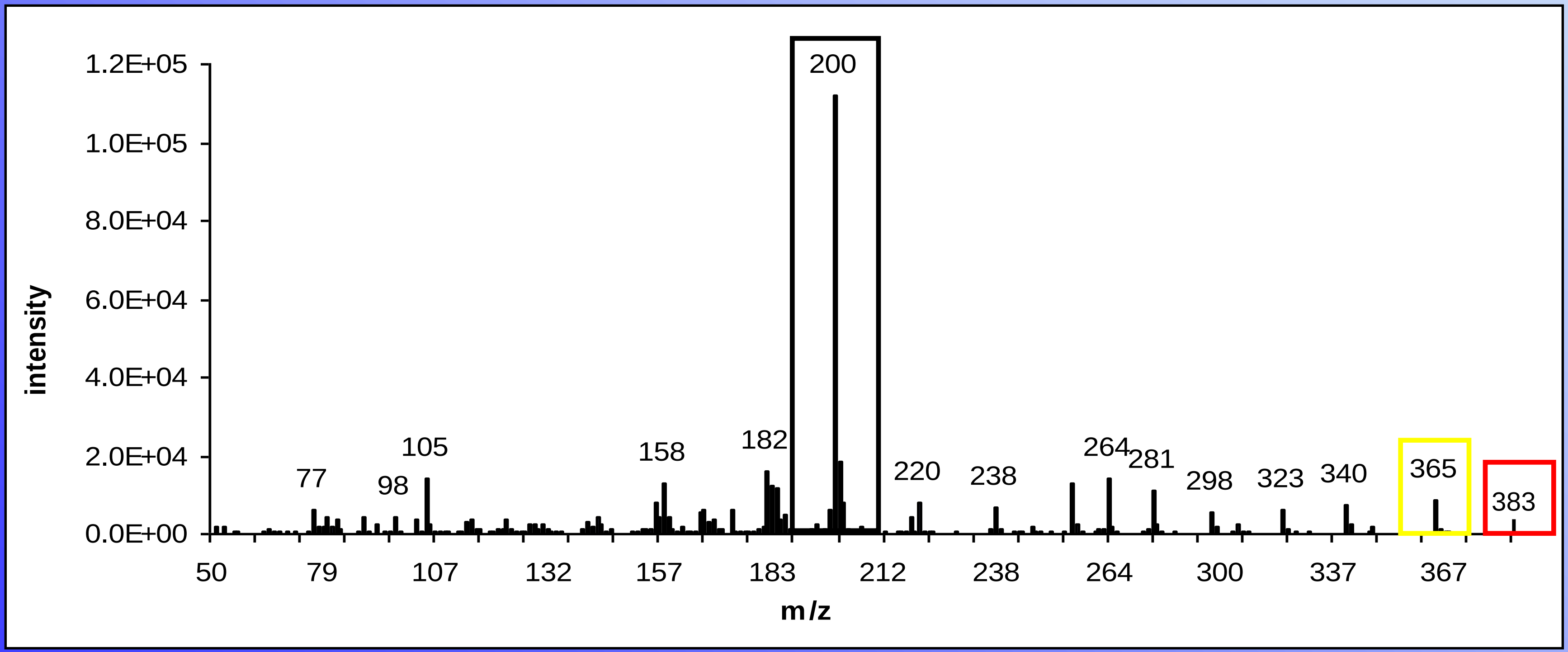
- Acetophenone
- Phenethylalcohol
- ST



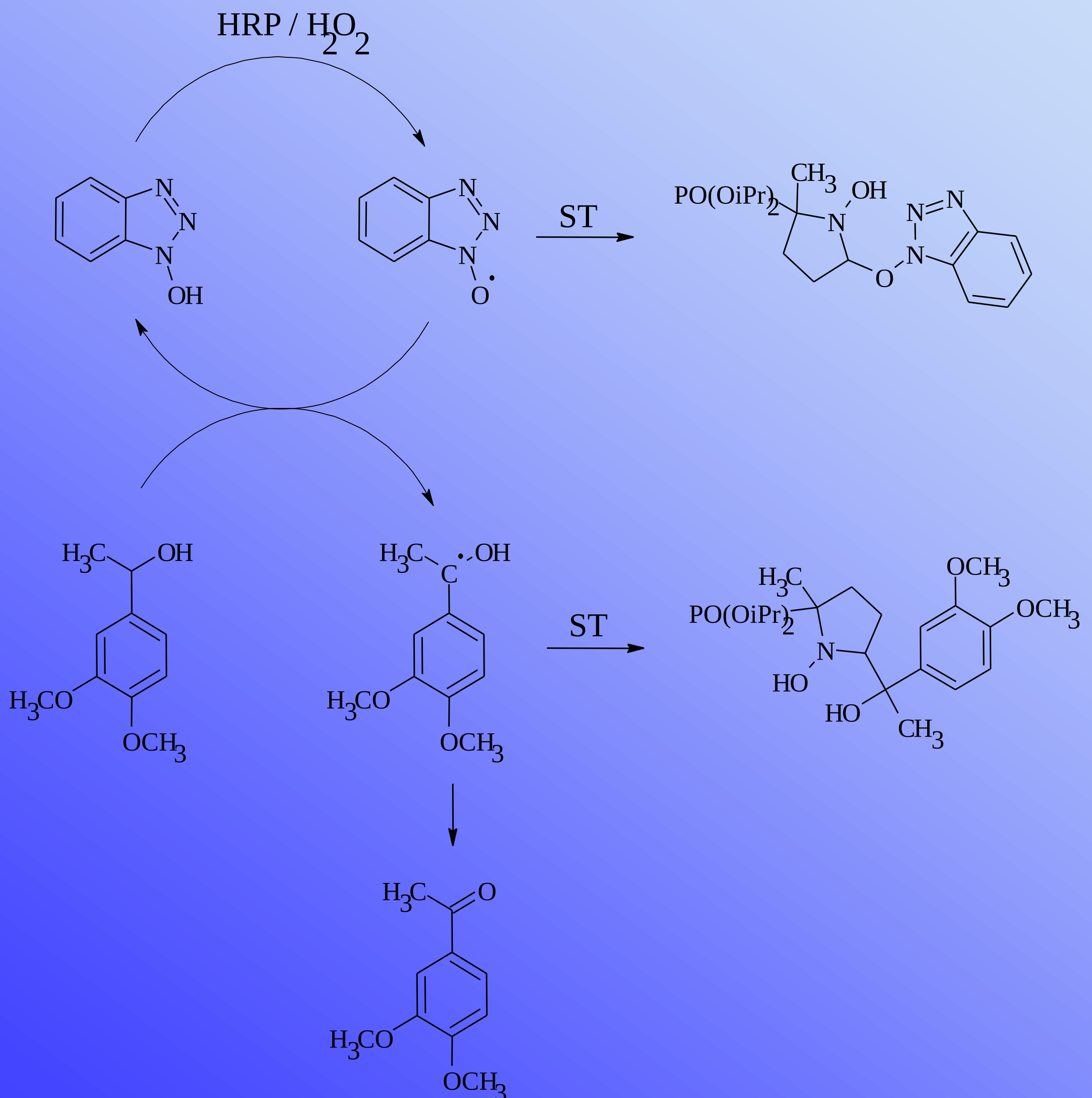
28.0 ppm

MS of DIPPMPO/Ketyl





HRP–HBT System with DIPPMPO



HRP/HBT cycle

Trapping of Ketyl Radical

Decay of Ketyl radical by disproportionation

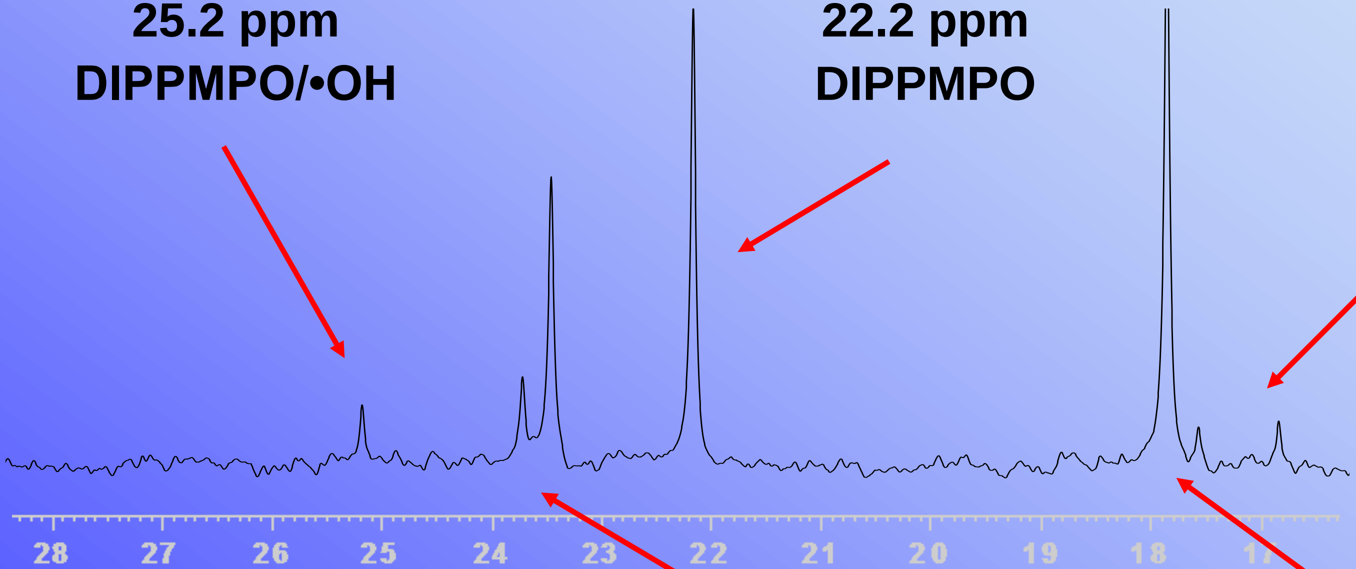
^{31}P-NMR Spectra Interpretation

HBT-HRP system w/o substrate

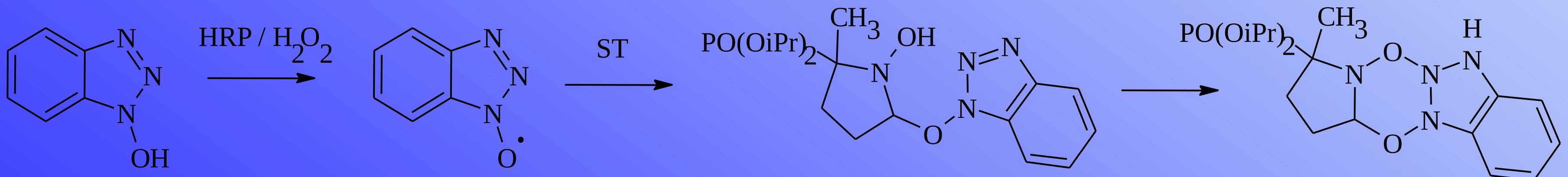
25.2 ppm
DIPPMPO/•OH

22.2 ppm
DIPPMPO

16.8/17.5 ppm
DIPPMPO/•OOH



- HBT
- ST
- HRP
- H_2O_2

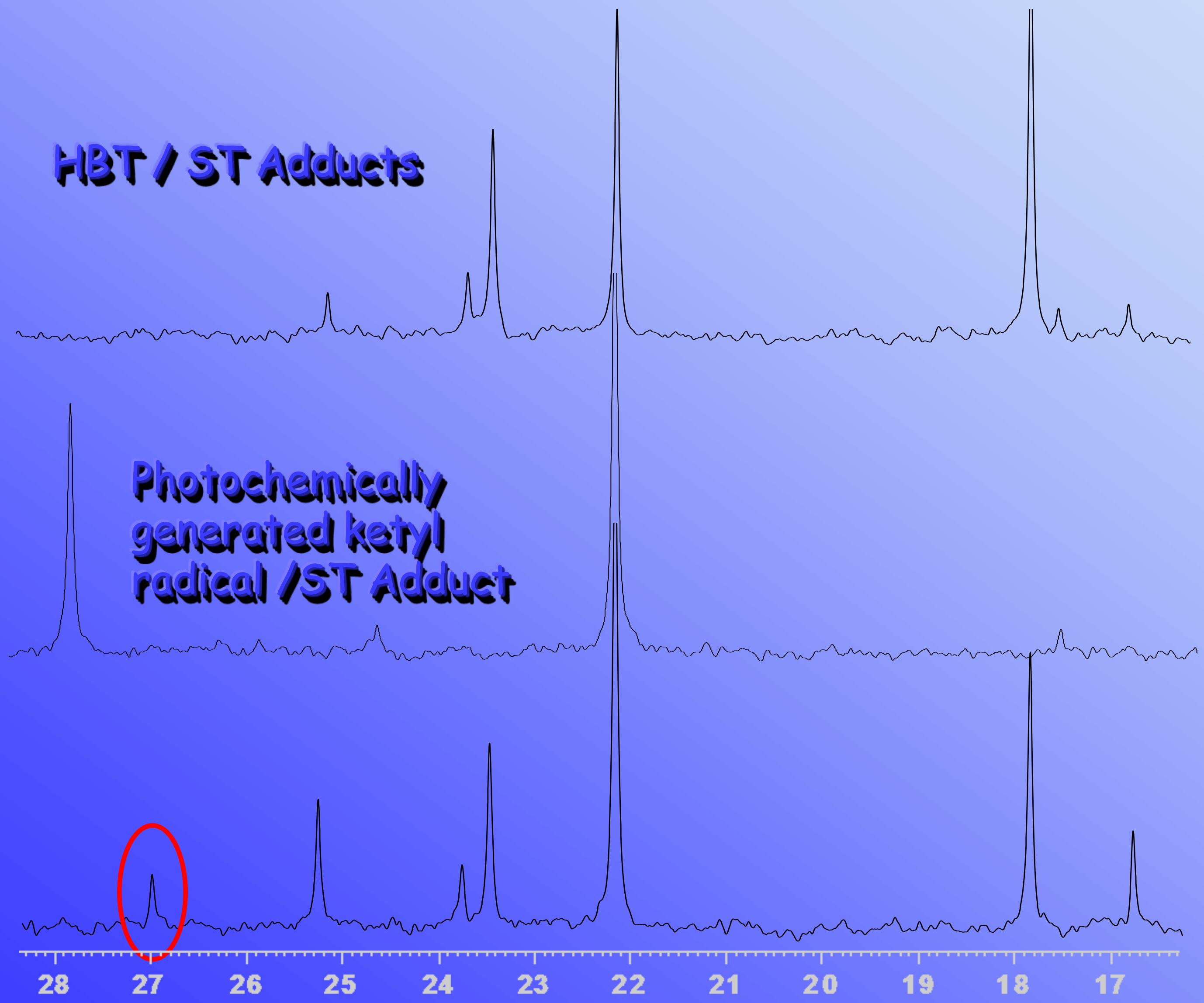


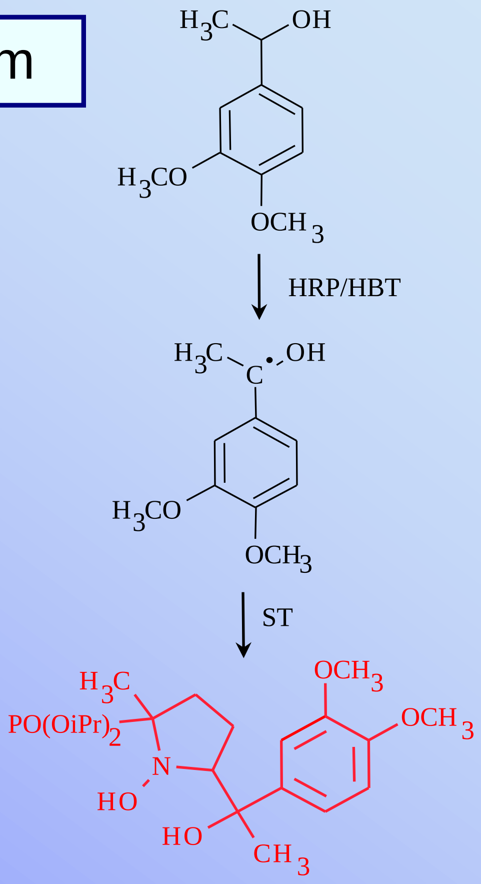
23.5 / 23.8 ppm

17.9 ppm

^{31}P-NMR Spectra Interpretation

Oxidation of Apocinol by HRP-HBT system





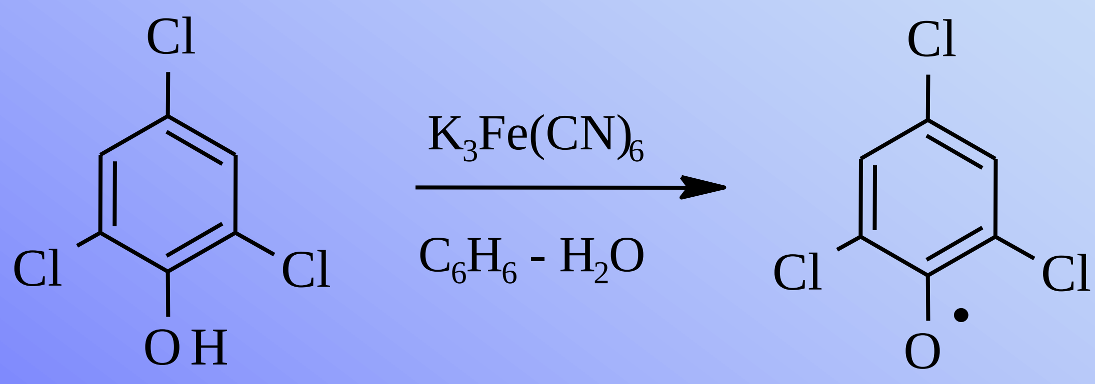
- ST
- HRP
- HBT
- H_2O_2
- APOCINOL

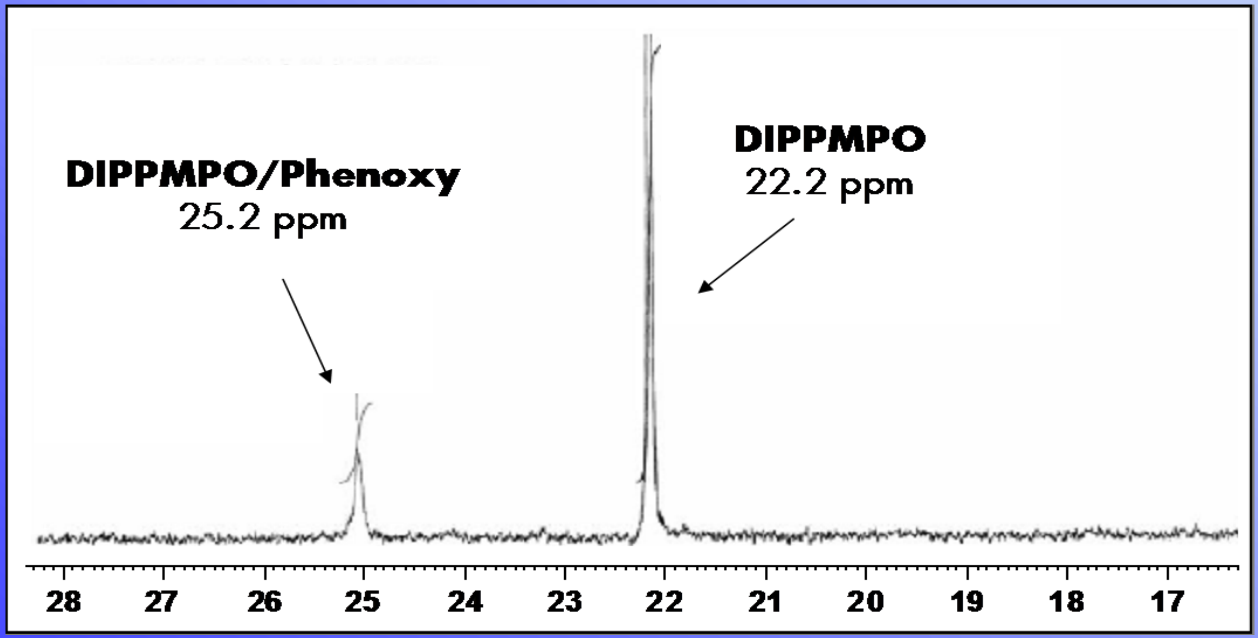
Spin Trapping of Phenoxy Radical by DIPPMPO

L. Zoia, Argyropoulos., D. S., "Phenoxy Radical Detection Using Quantitative ^{31}P NMR Spin Trapping", J. of Physical Organic Chemistry; 22 1070–1077, (2009); www.interscience.wiley.com) DOI 10.1002/poc.1561, 2009.

Generation of phenoxy radical

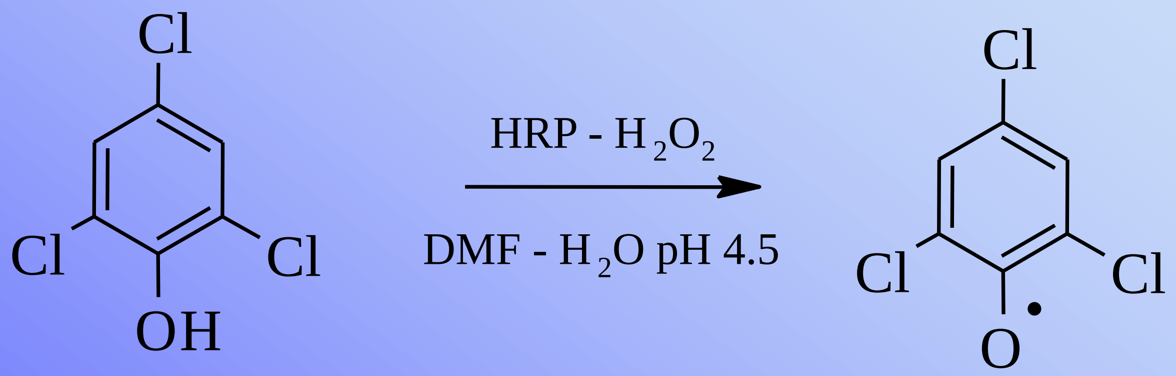
Generation of phenoxy radical with Iron Cyanide

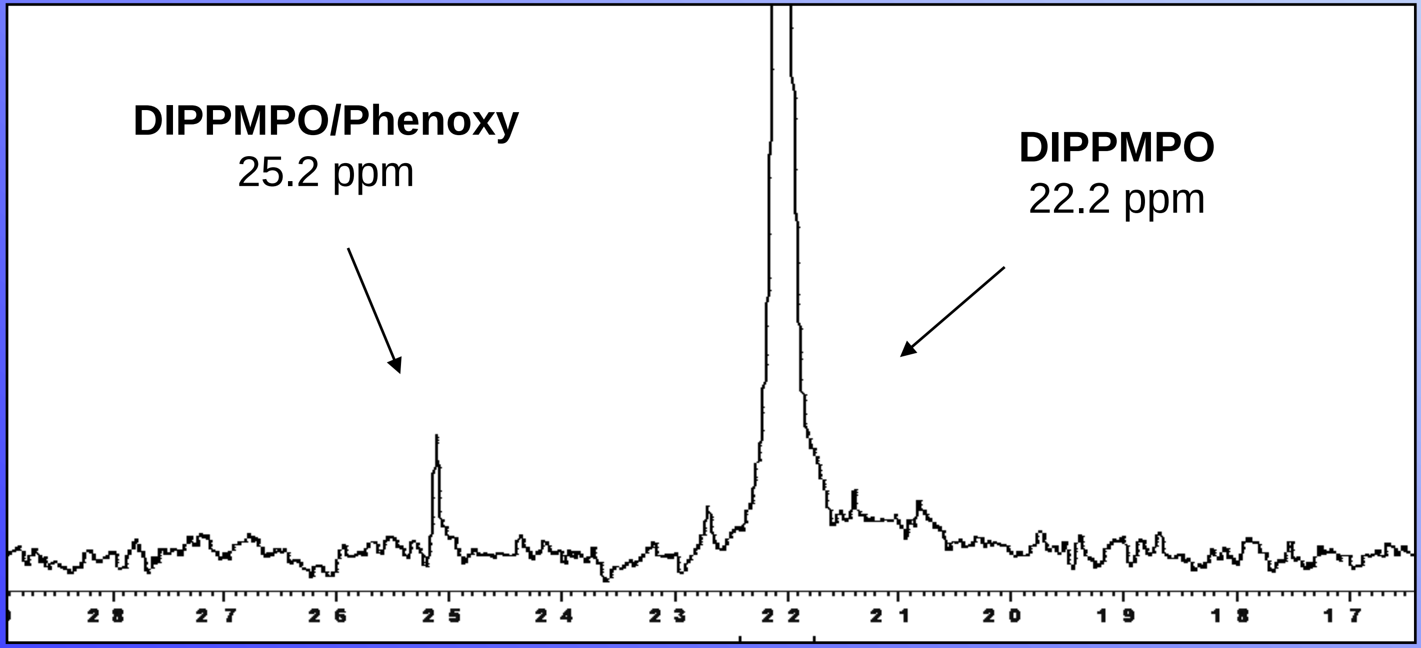




Generation of phenoxy radical

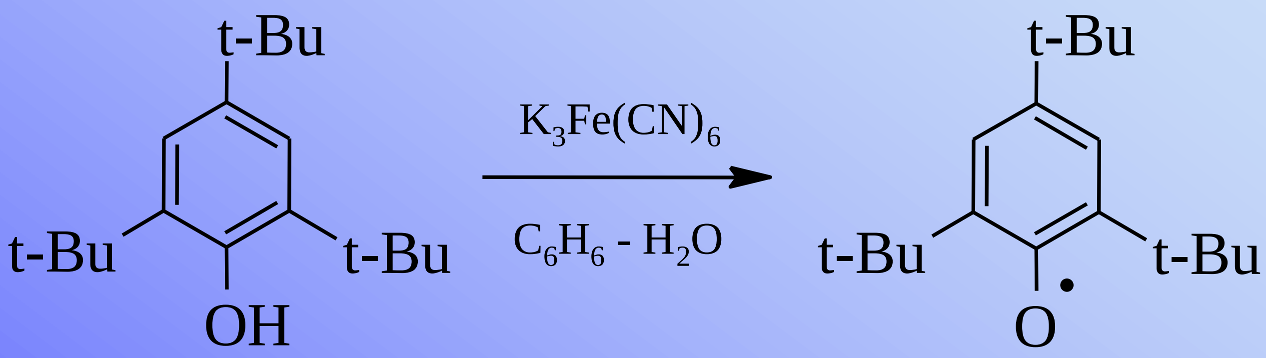
Generation of phenoxy radical with HRP

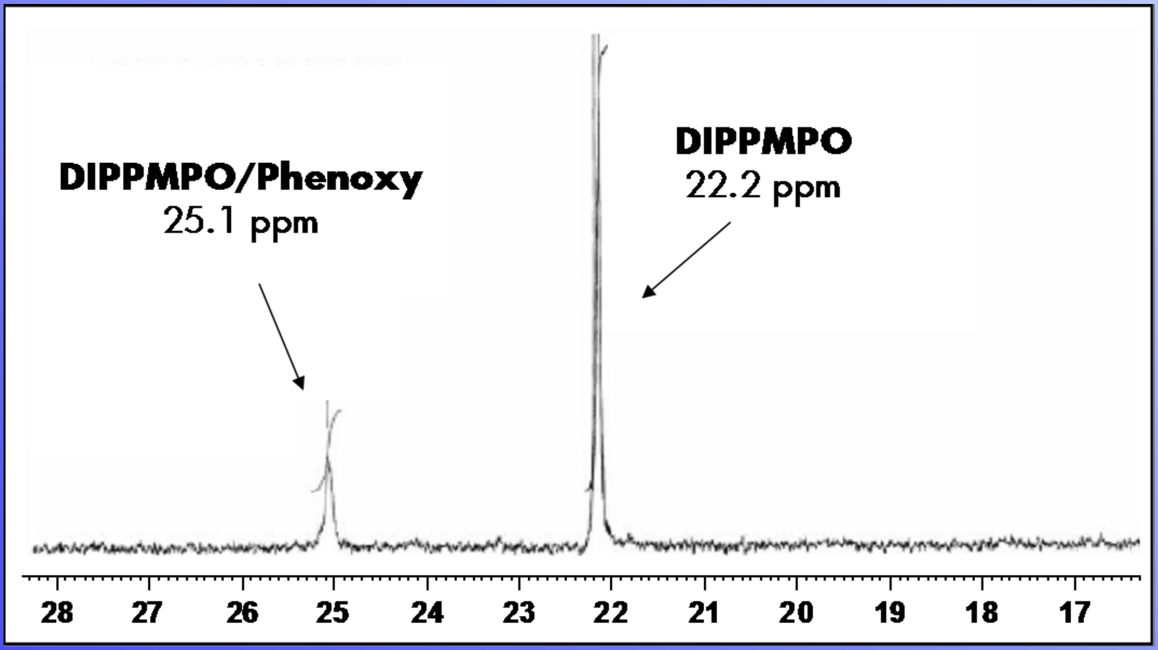




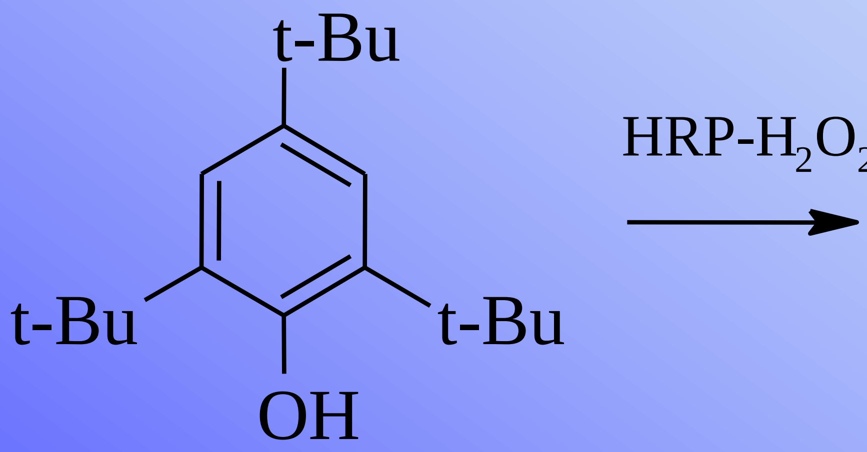
Generation of phenoxy radical

Generation of phenoxy radical with Iron Ferrocyanide





2,4,6 Tri-tert-Butylphenol

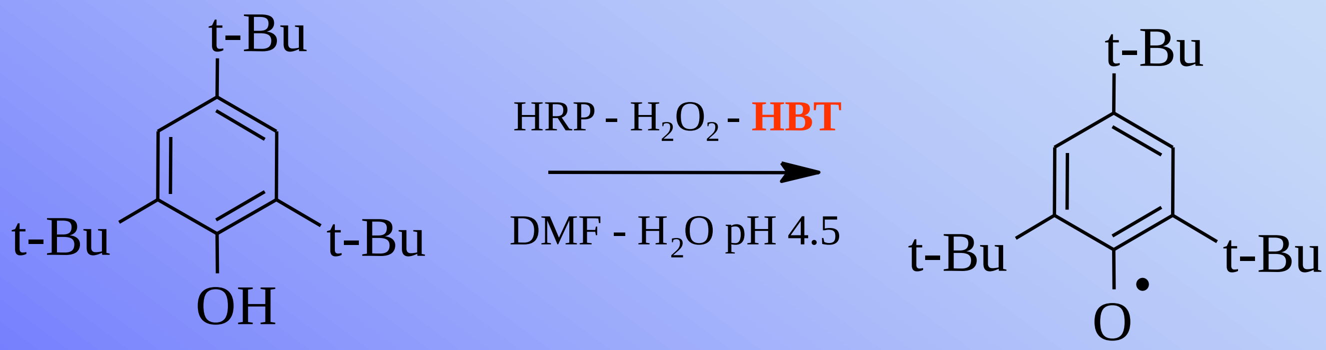


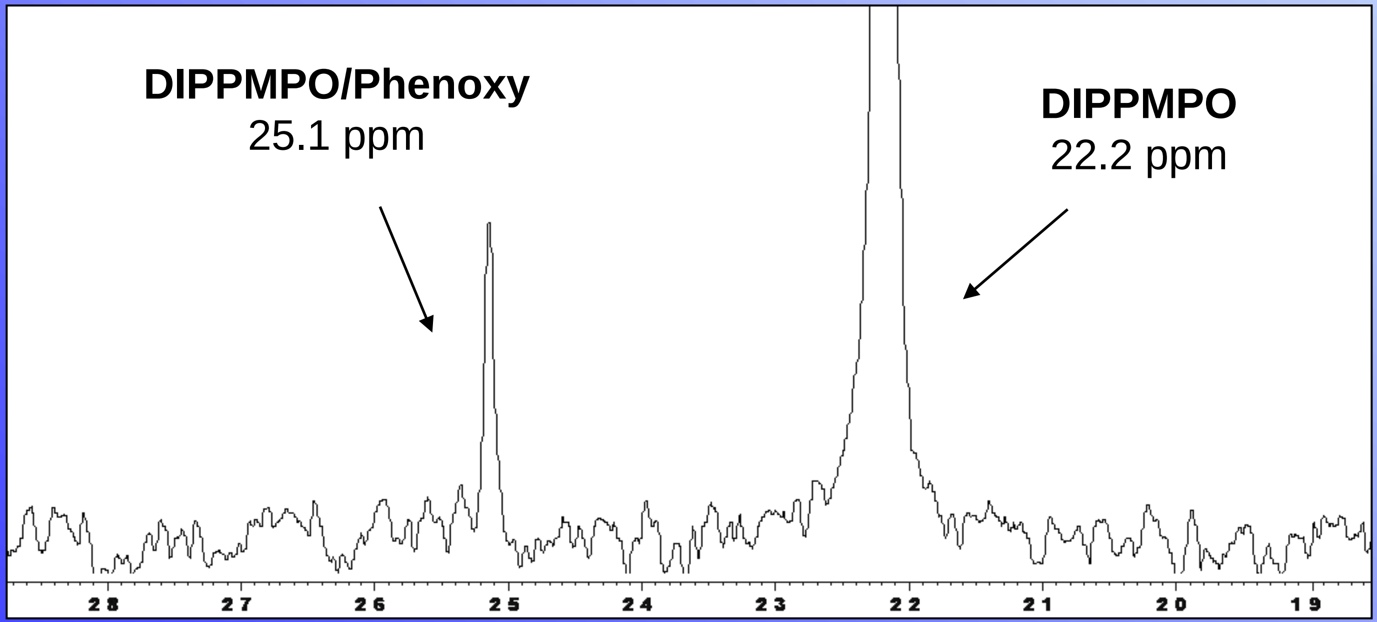
NO REACTION
GC-MS data and ^{31}P-NMR indicates that no reaction occurs.

The phenolic group is hindered by *tert*-butyl groups
The enzyme cannot approach to the phenolic group

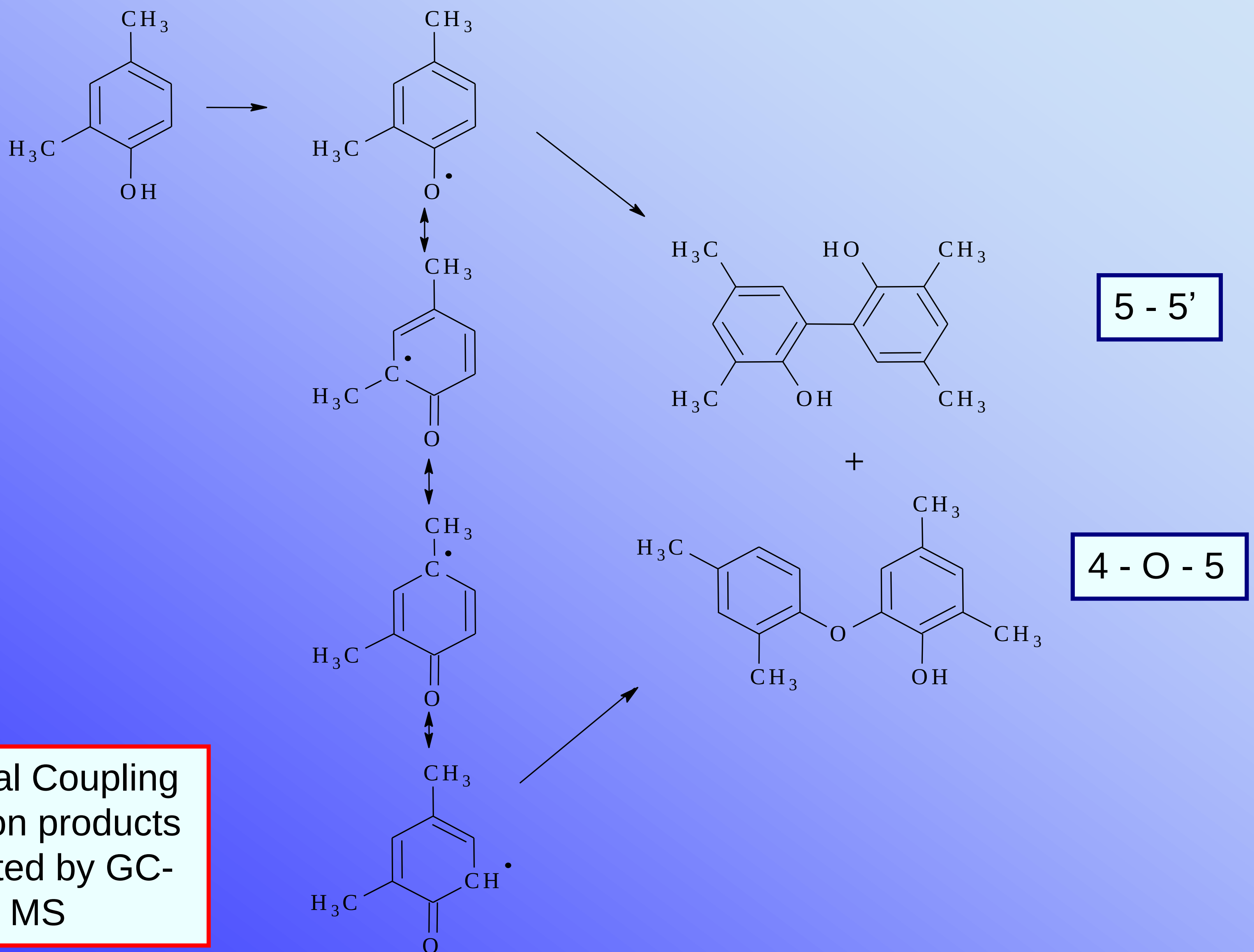
Generation of phenoxy radical

Generation of phenoxy radical with HRP/HBT



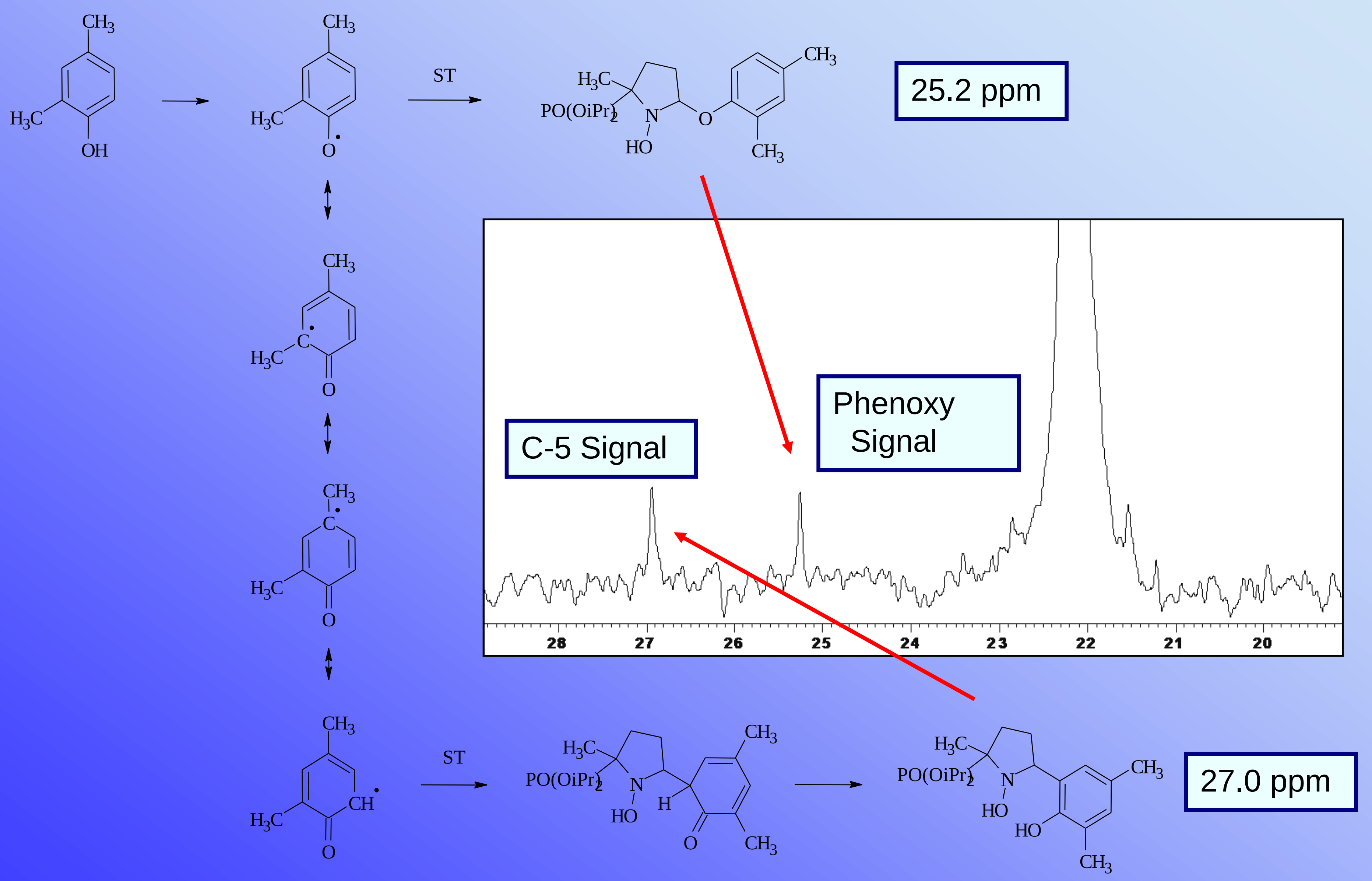


2,4 Dimethyl phenol

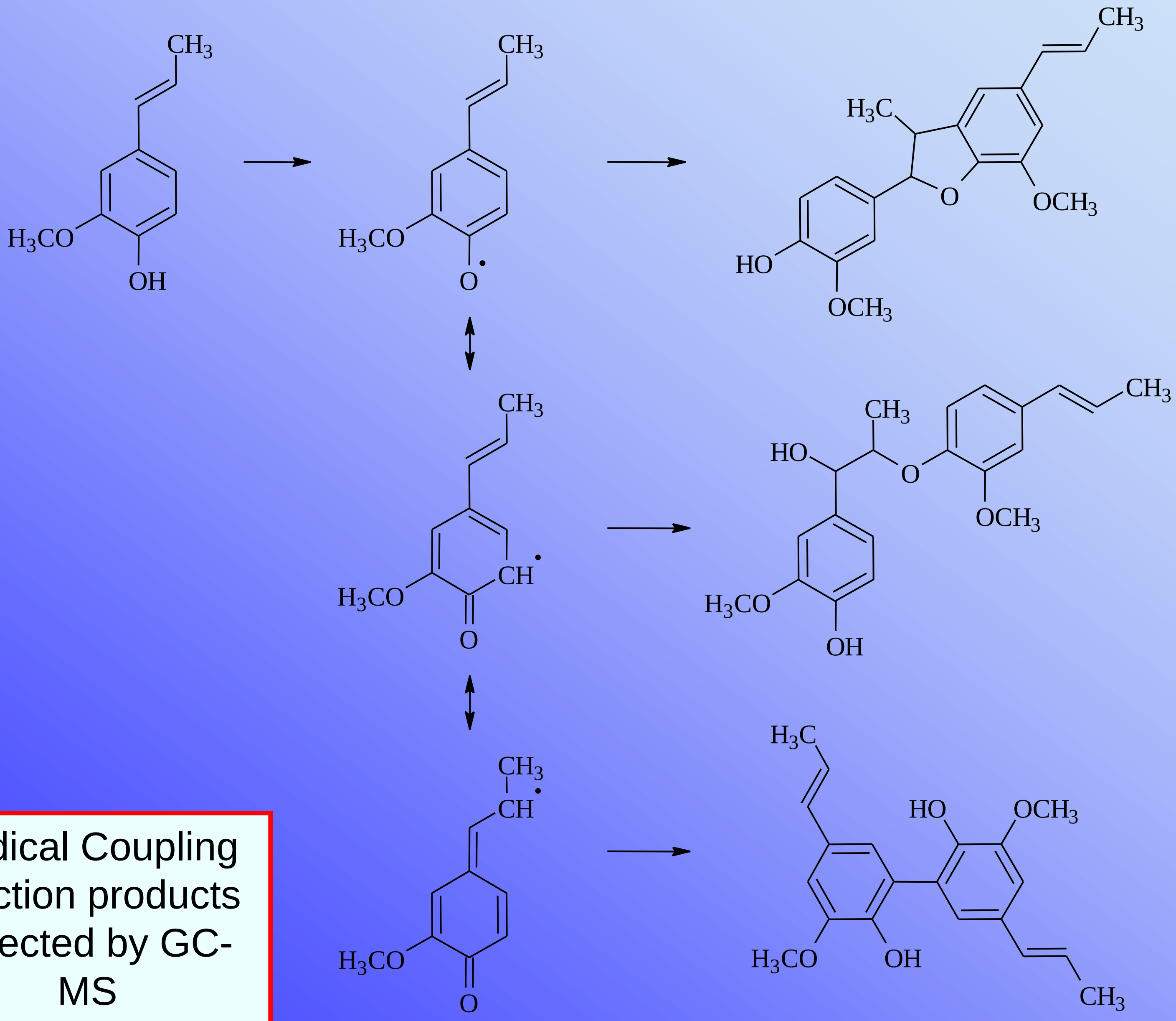


Radical Coupling reaction products detected by GC-MS

2,4 Dimethyl phenol/ST Zutropf



Isoeugenol



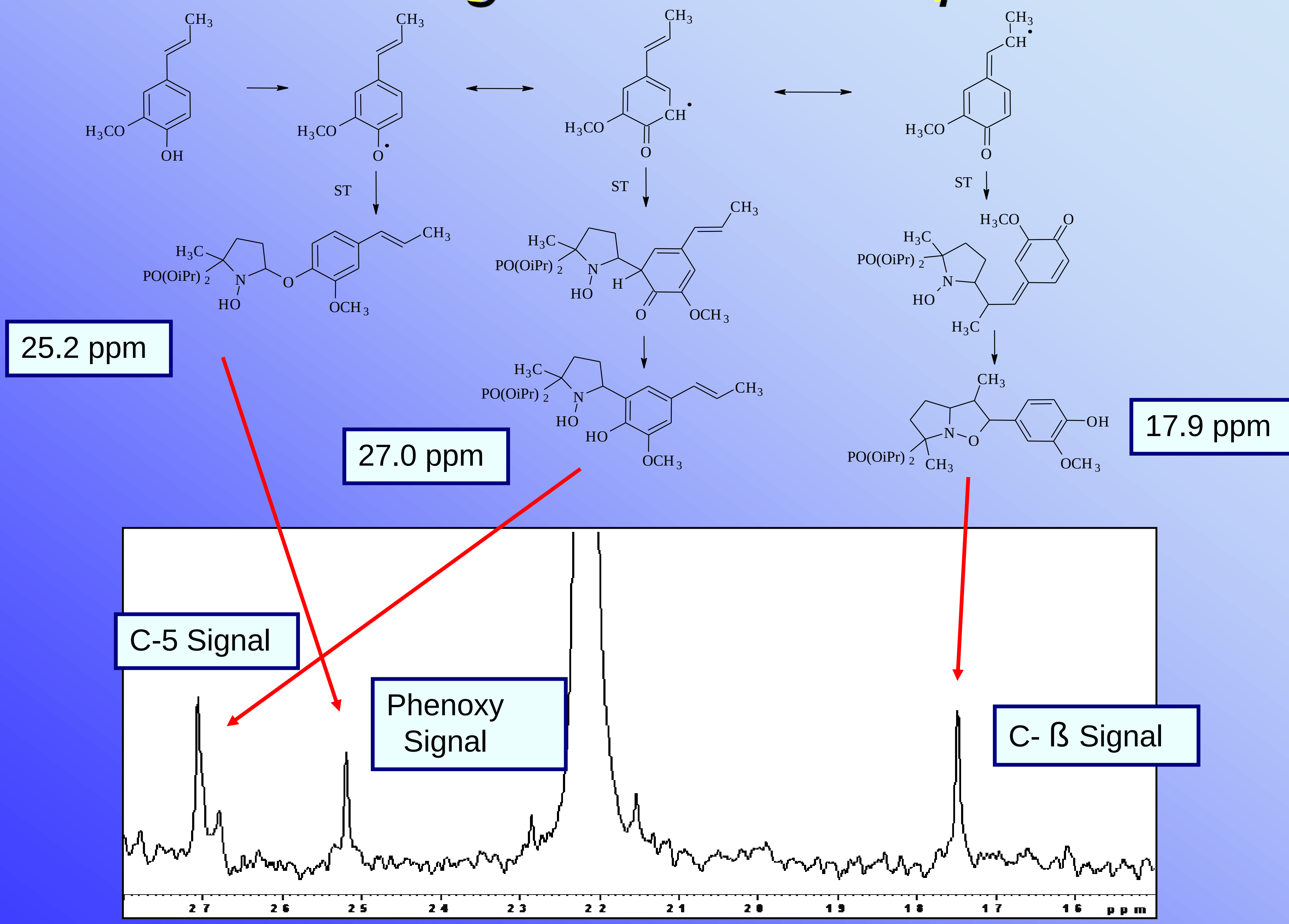
ß - 5

ß - O - 4

5 - 5'

Radical Coupling reaction products detected by GC-MS

Isoeugenol/ST Zutropf



Conclusions

- ✓ DIPPMPO spin trapping detected by ^{31}P-NMR is an effective tool for the identification and quantification of oxygen- and carbon-centered free radical species, such as:
  - Hydroxyl radicals
  - Superoxide radicals
  - Phenoxy radicals
  - Ketyl radicals
- ✓ These techniques could be used to understand mechanisms of radical activity in a variety of biomolecular processes.