Environmentally relevant hydrolysis of anticancer drugs explored by DFT calculations

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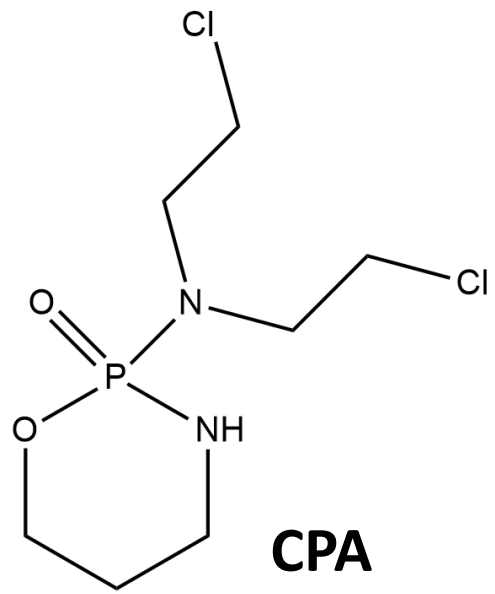
Cyclophosphamide and Ifosfamide

Alkylating cytostatics

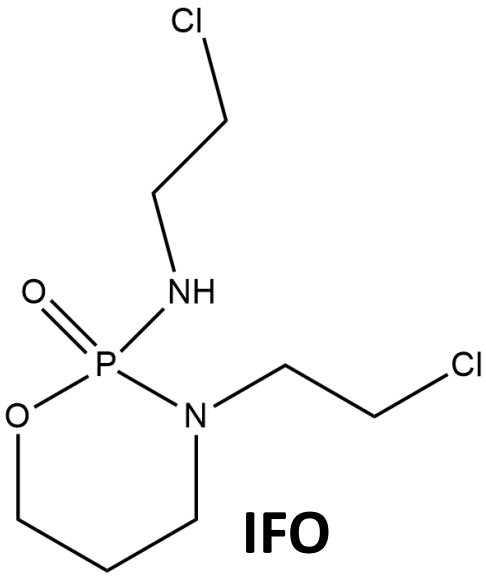
Treatment of leukaemia, lymphomas, solid tumours, and autoimmune disorders



CPA

25% of the applied dose is excreted unchanged in urine

Prodrugs → parent compounds are pharmacologically inactive, activated by CYP450



IFO

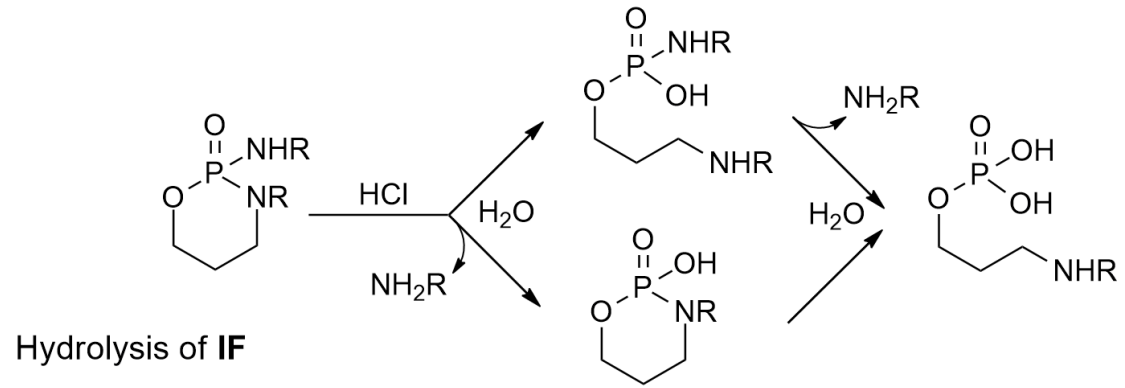
Ecotoxicity?

Hydrolysis of IFO and CPA

- Both compounds hydrolyse in neutral and acidic environments
- High proton concentrations catalyse the reaction

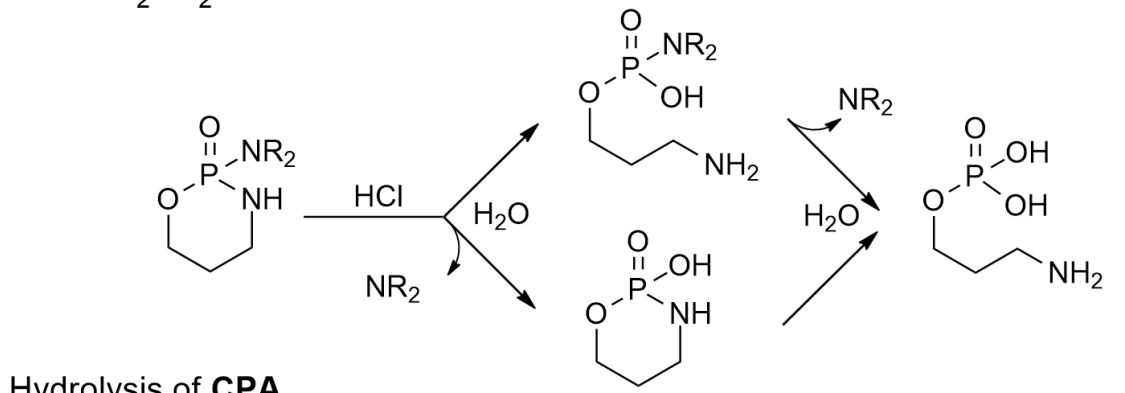
Two competing processes:

- Opening of the oxazaphosphinane ring
- Elimination of the chloroethylamine groups



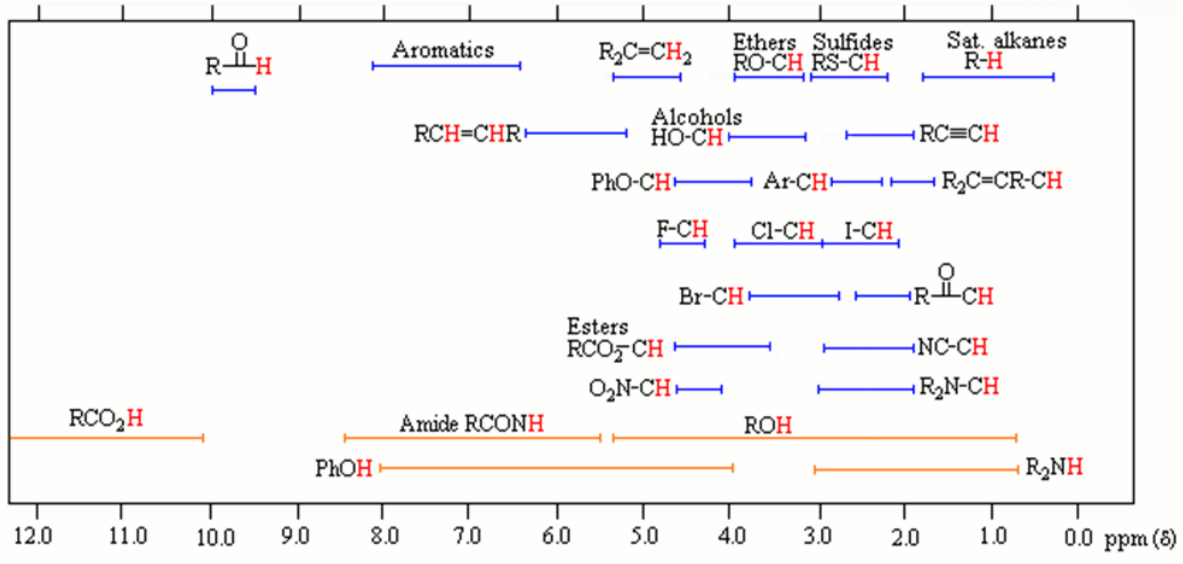
Hydrolysis of **IF**

R = -CH_2CH_2Cl



Hydrolysis of **CPA**

R = -CH_2CH_2Cl



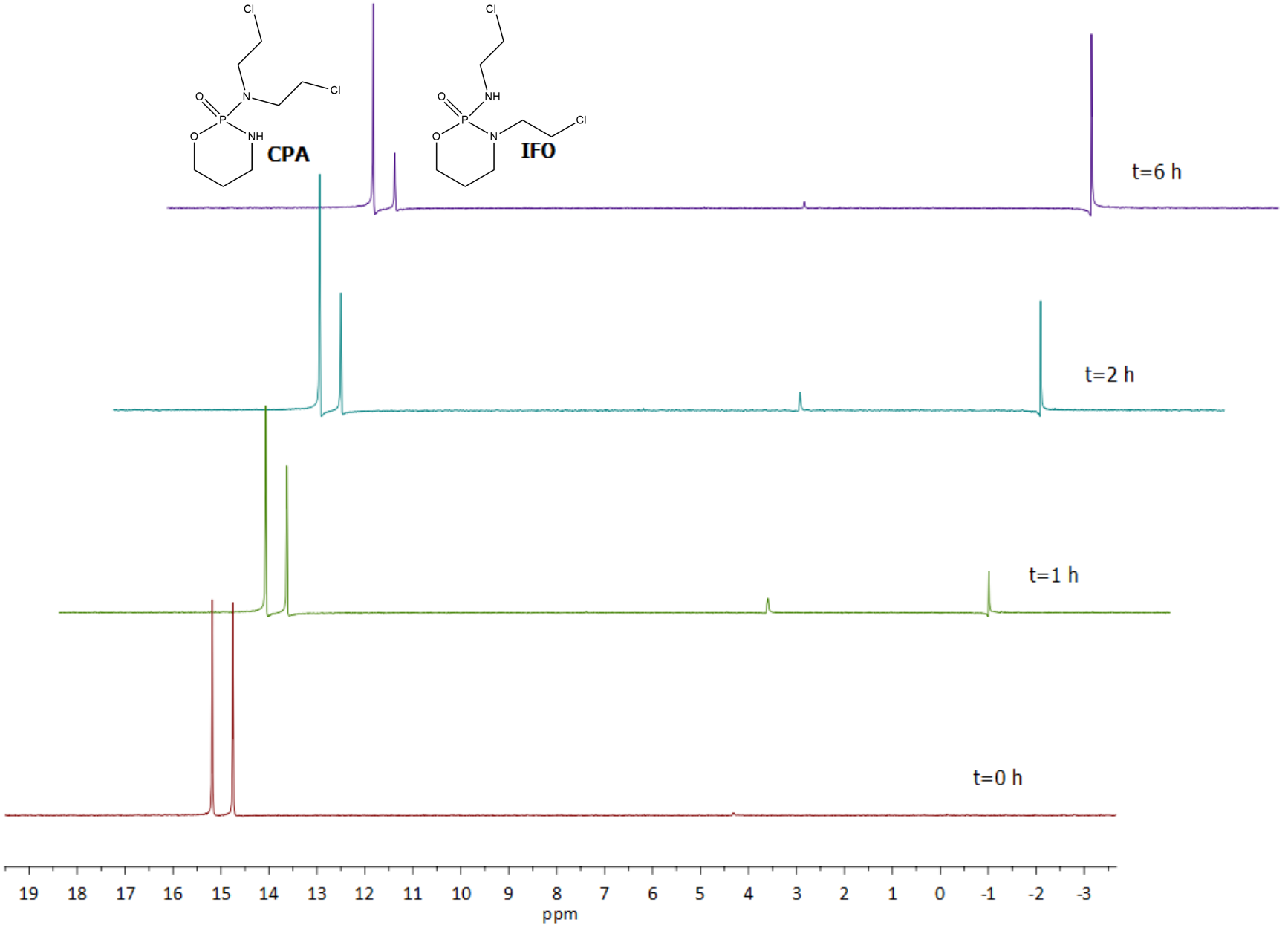
NMR analysis

Varian INOVA 400 NMR

- Hydrolysis of an IFO and CPA solution observed by phosphorous NMR

- IFO reacts faster than CPA
  - the IFO intermediate products signal is seen

- The final products signals overlap



$^{31}P\{^{1}H\}$ NMR spectra stack of CPA/IFO hydrolysis

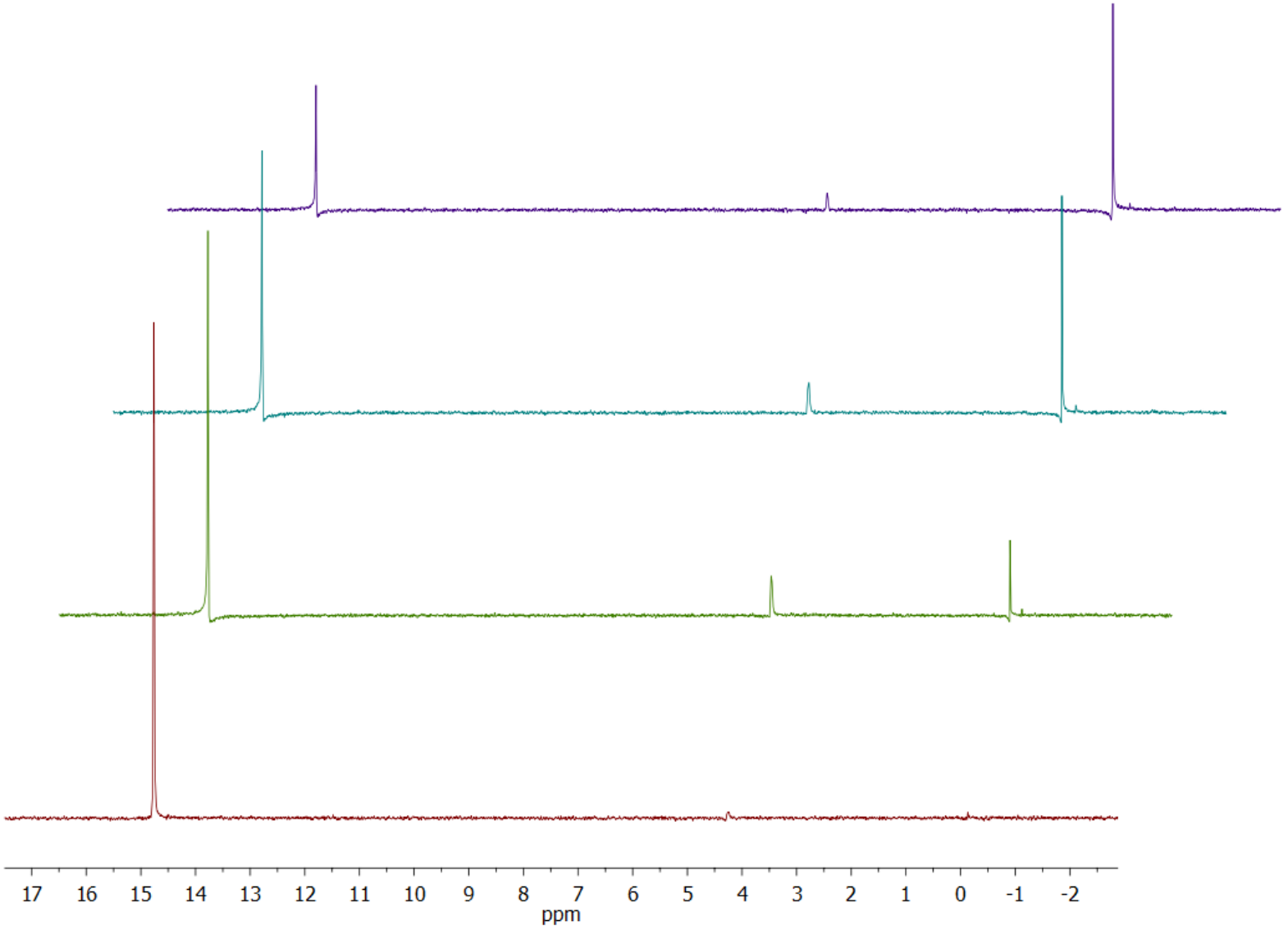
IFO gives a single sharp ^{31}P signal (14.8 ppm)

Good method of following the reaction

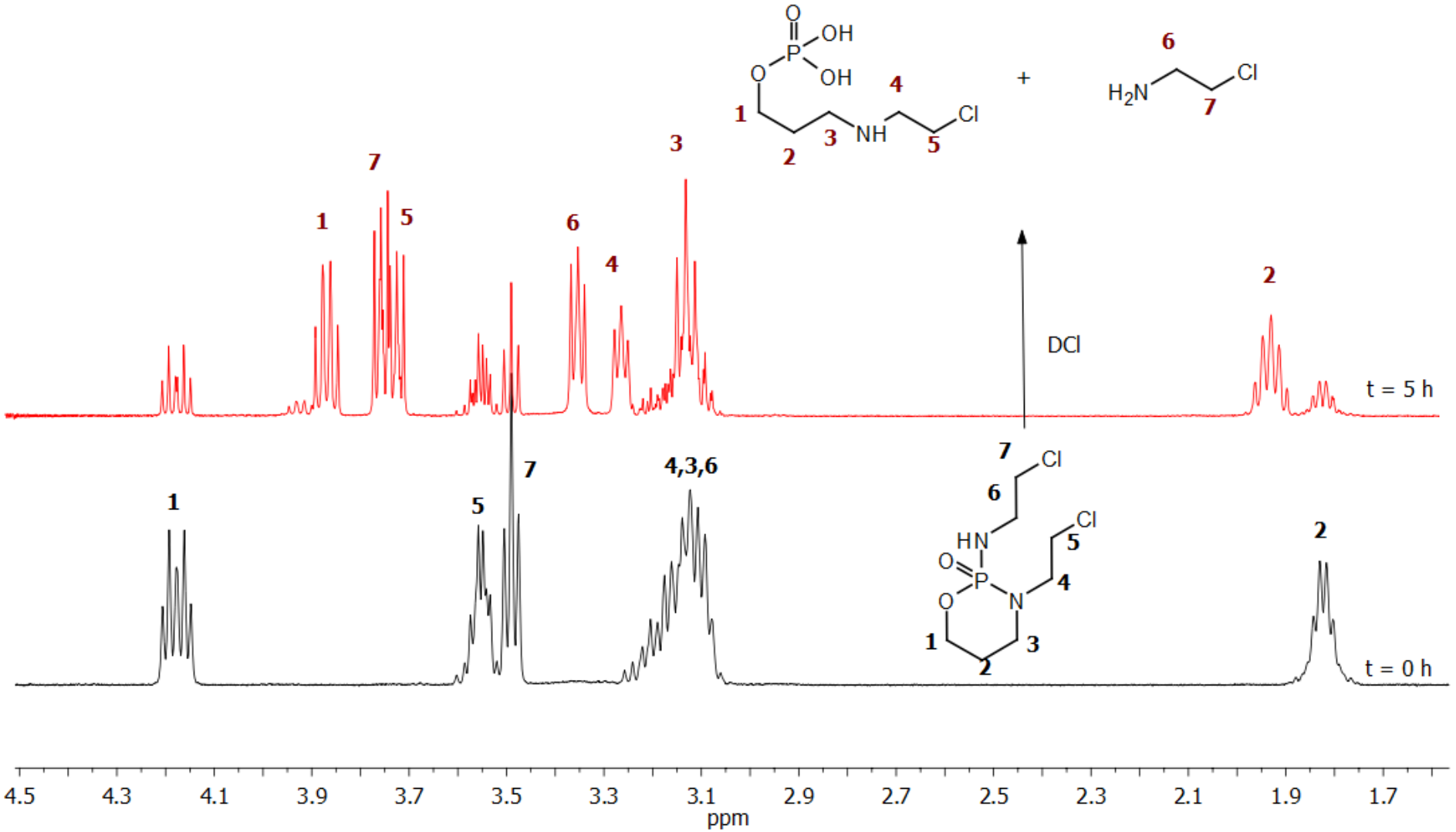
Signal integrals can be used to calculate reaction kinetics

As the reaction begins 3 additional signals emerge

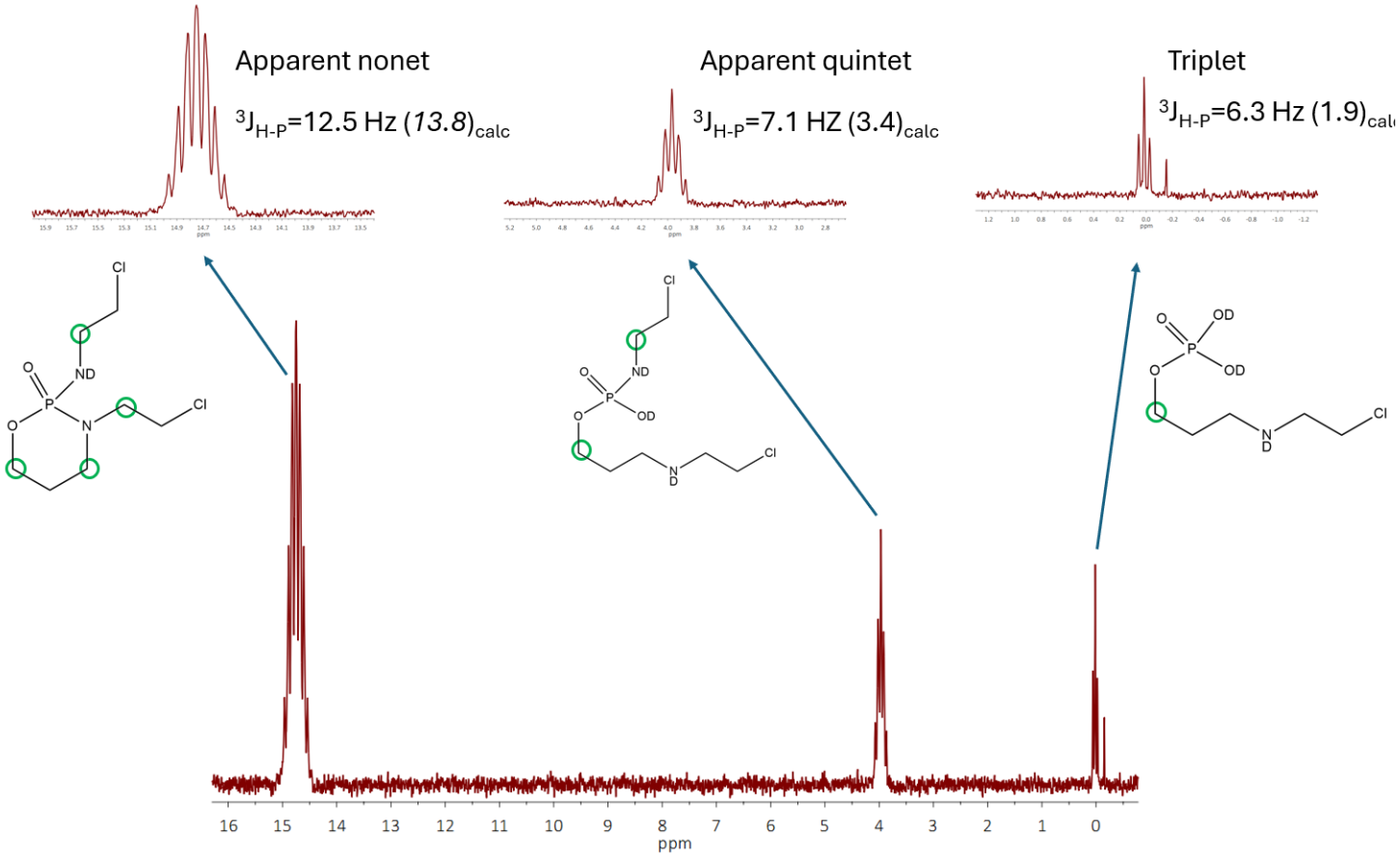
- intermediate product (5.1 ppm)
- final product (0 ppm)
- phosphate (-0.1 ppm)



$^{31}P\{^{1}H\}$ NMR spectra stack of IFO hydrolysis



$^{1}H\{^{13}C\}$ NMR spectra of IFO hydrolysis

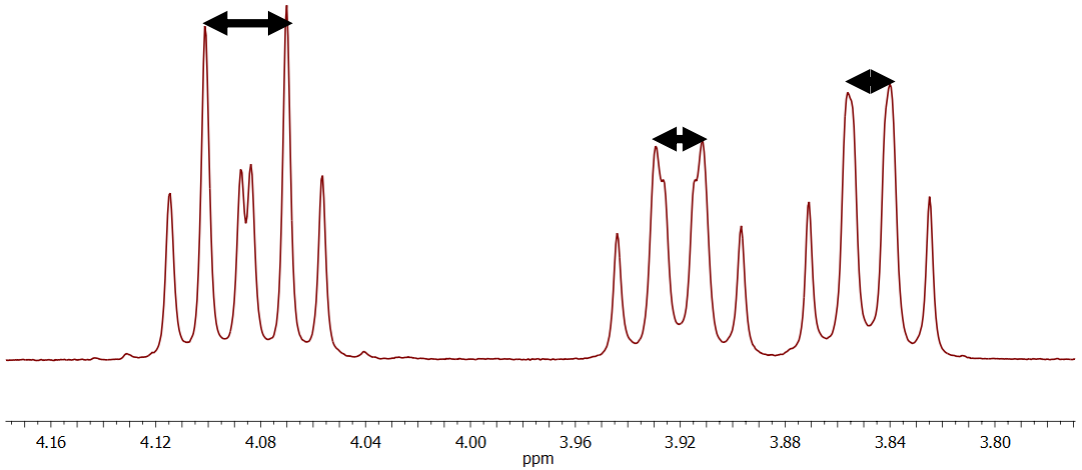


^{31}P NMR spectra of IFO hydrolysis

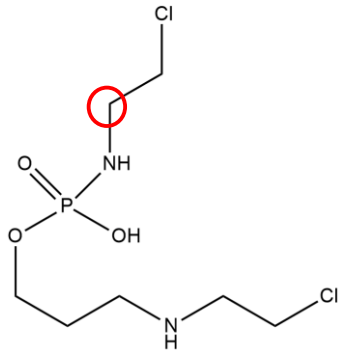
$^3J_{H-P}$ couplings decrease during the reaction

The reduction trend is also seen using DFT calculations

- NMR=(SpinSpin) option
- B3LYP/6-311++G(3df,2pd) level of theory

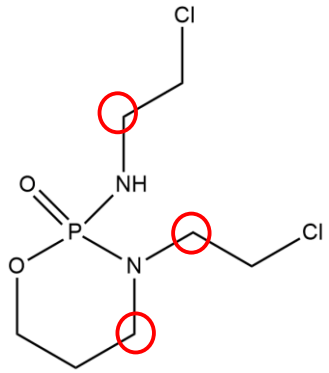


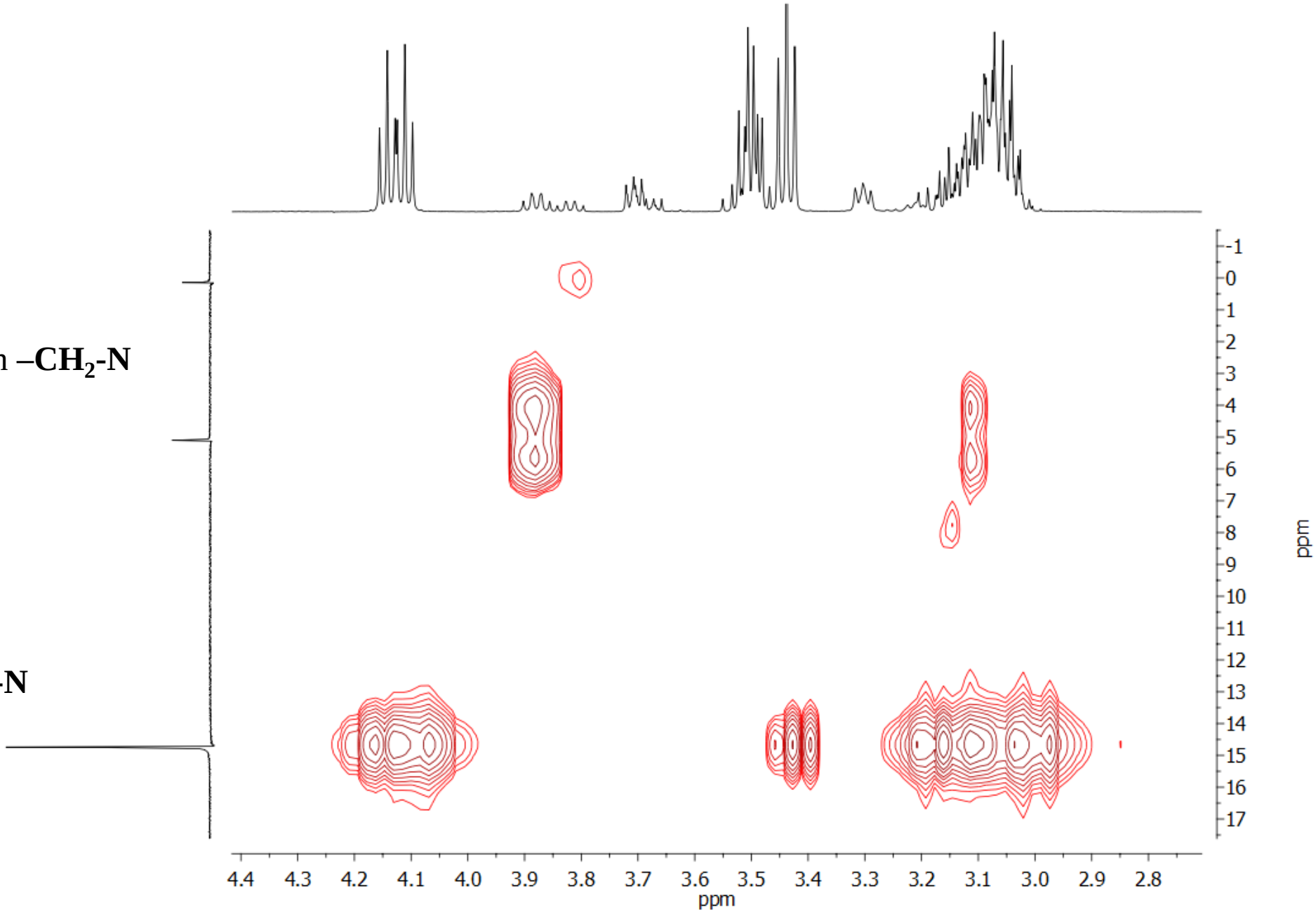
^{1}H{^{13}C} NMR spectra of IFO showing the multiplets of the parent compound, intermediate product, and final product



Intermediate → 1 interaction with **–CH_2-N**

IFO → 3 interactions with **–CH_2-N**





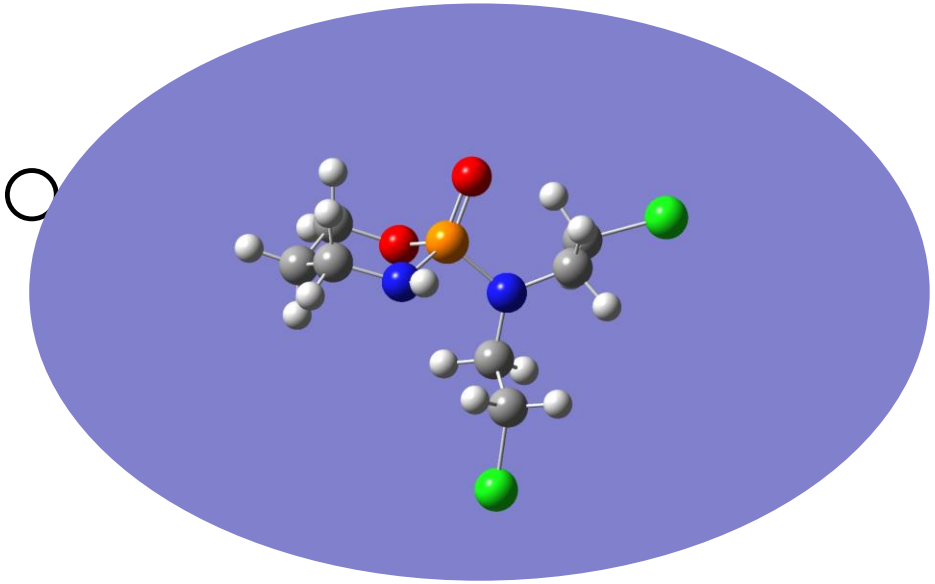
$^{1}H-^{31}P$ HMBC of IFO hydrolysis

DFT calculations

- Geometry optimization
- Frequency calculation
- Transition state optimization
- NMR spin-spin coupling

- Using **Gaussian16** software installed on “Supek” supercomputer at the University computer center “SRCE” in Zagreb

Exploring the reactio

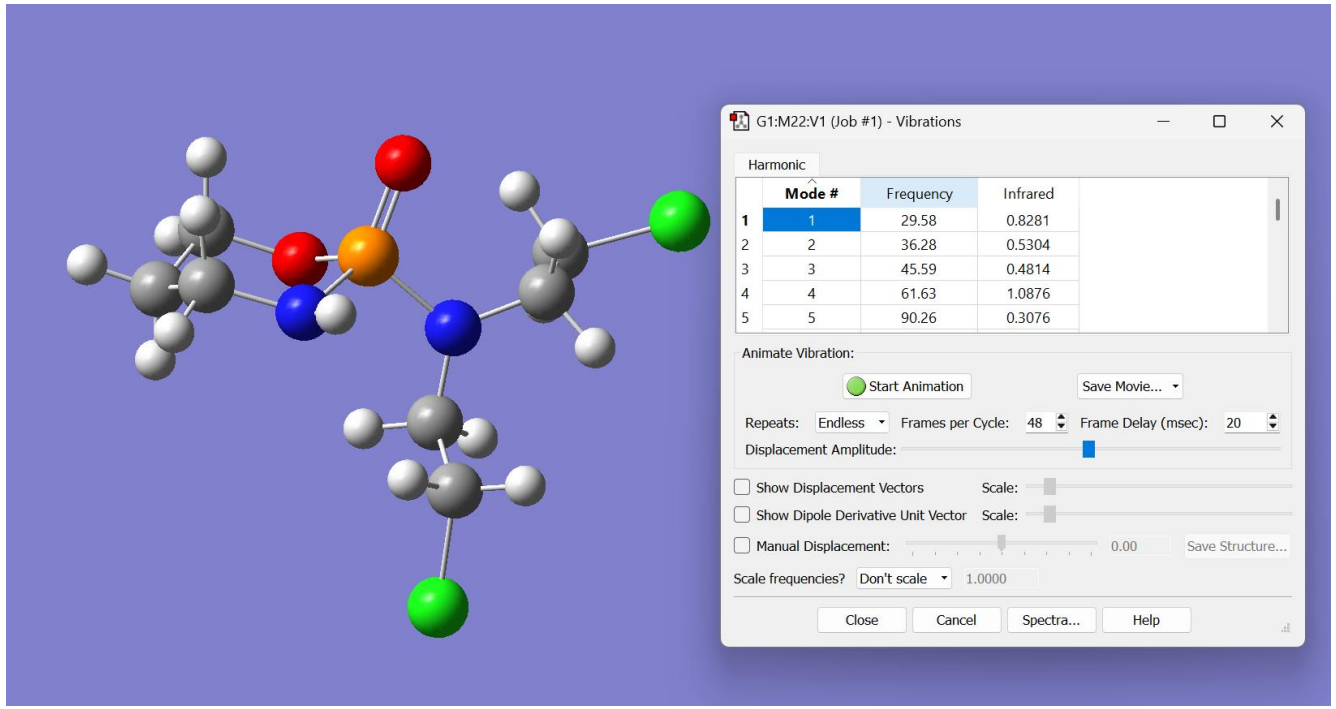


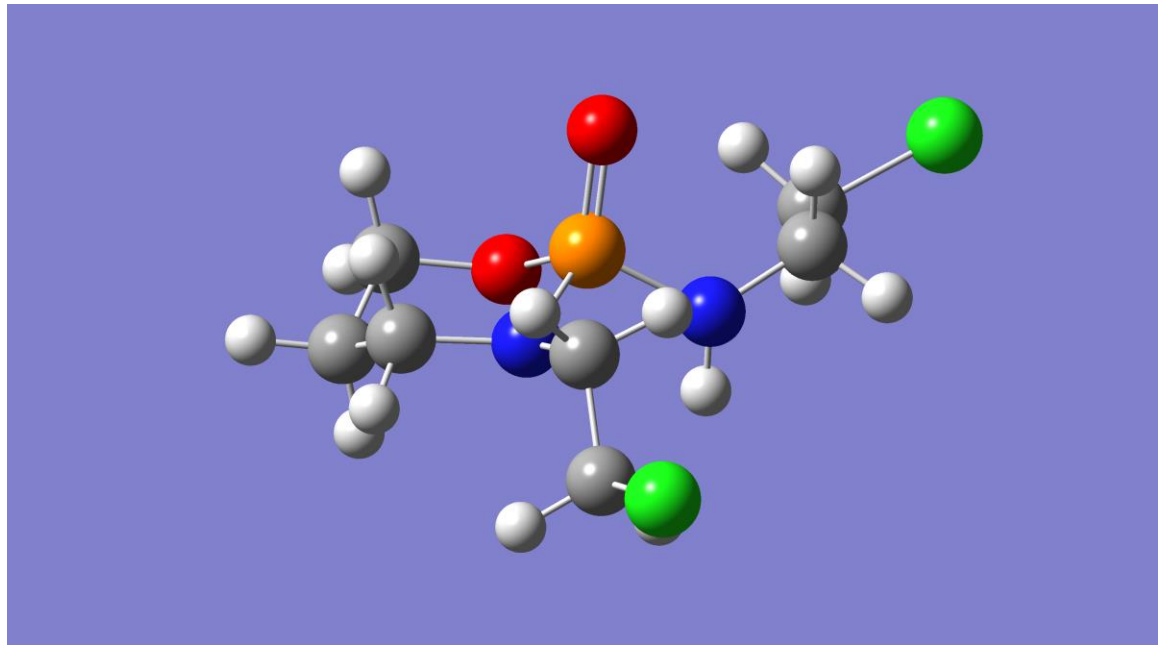
- PES – potential energy surface
- Conformational analysis of the parent compounds
- Geometry optimization of the compounds and transition states
- Frequency calculation to determine minima and stationary points
- SMD solvation in water

Most stable conformations of CPA and IFO in water

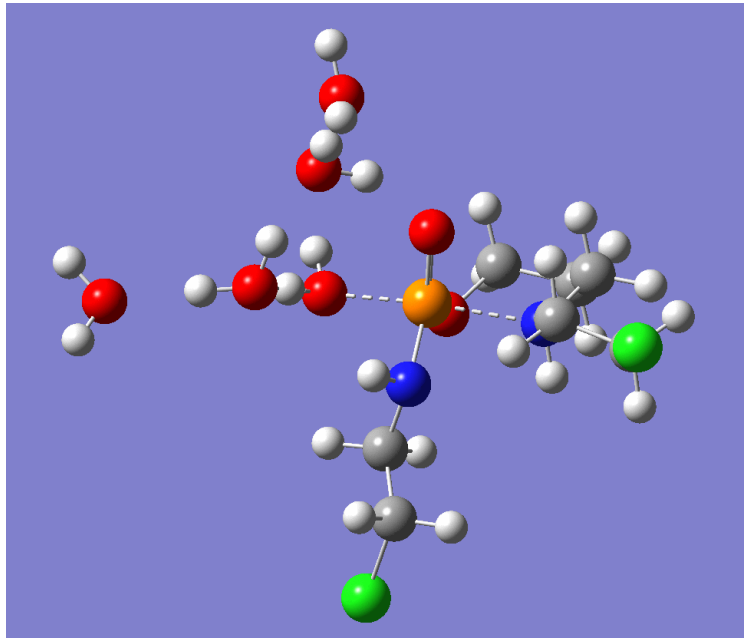
#scrf=(smd,solvent=water,externaliteration,dovacuum)

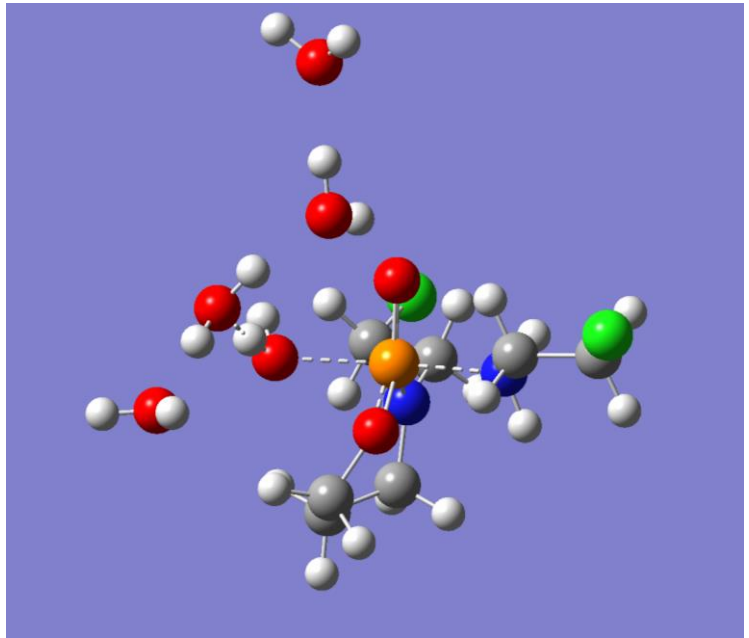
```
# opt freq b3lyp/6-31+g(d,p)

conformer 1

0 1
 C                -2.57975300    1.71769400   -1.34656300
 C                -3.40426700    0.63027500    0.78117200
 C                -3.62851700    0.80165700   -0.72333000
 P                -0.73133600    0.98928700    0.39634700
 O                -0.31077500    2.23784800    1.09386200
 N                 0.51752900   -0.11215800    0.26947000
 C                 1.88746100    0.30516400    0.60834200
 C                 2.56859500    1.02117100   -0.56010500
 Cl                4.31131200    1.35323000   -0.16499100
 N                -2.03619600    0.12031000    1.04617800
 O                -1.24997600    1.17711900   -1.13664900
 C                 0.34933900   -1.39528800   -0.42429700
 C                 0.20518700   -2.54095200    0.57810500
 Cl                0.08900500   -4.13359400   -0.29057600
 H                -1.89197200   -0.05845700    2.03690500
 H                -2.62472300    2.72918700   -0.92400600
 H                -2.69558400    1.78853800   -2.43011500
 H                -4.11321800   -0.09982300    1.18344200
 H                -3.58308300    1.58683900    1.29499000
```

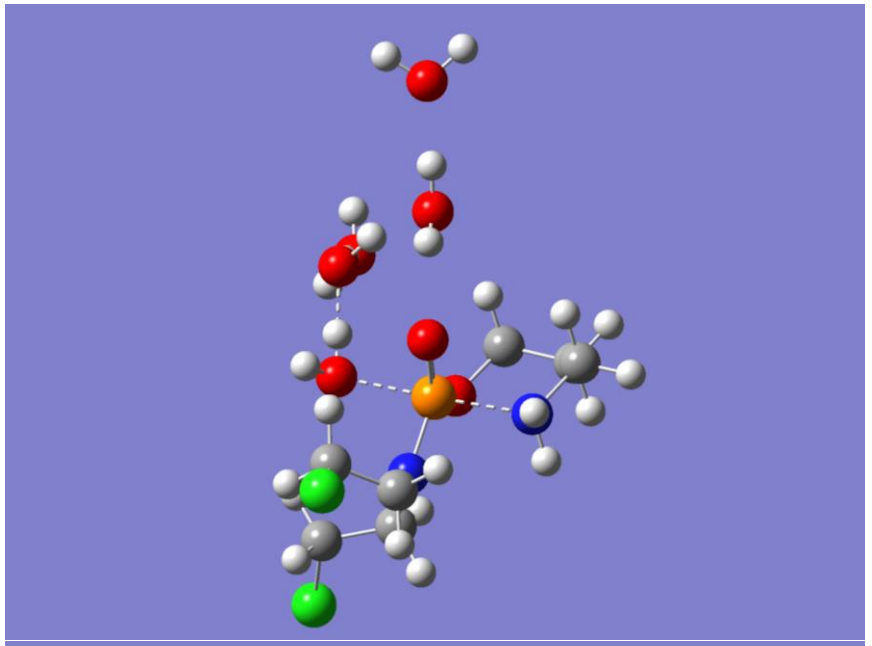


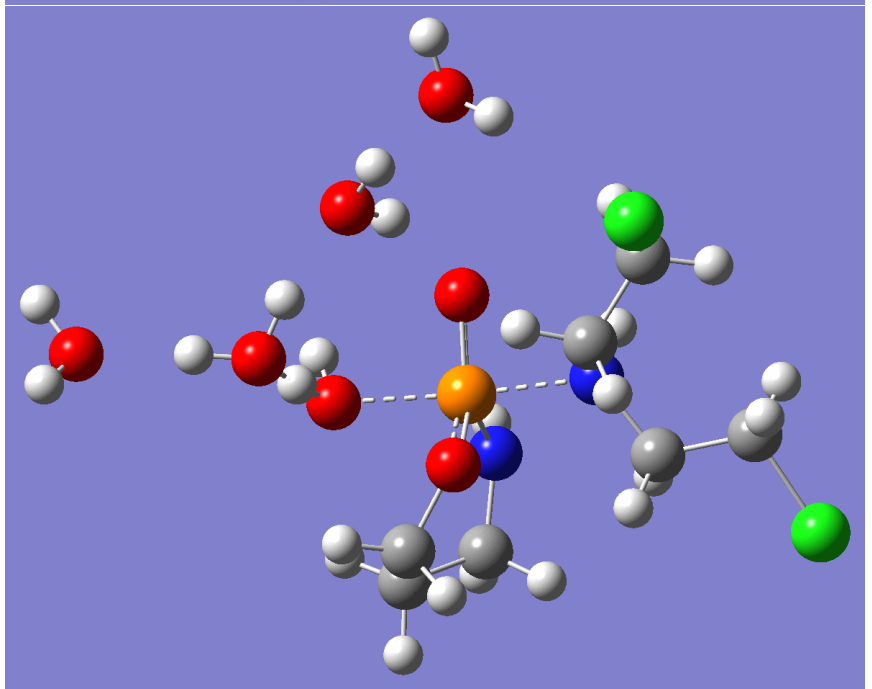


opt=(ts,calcfc,noeigentest) freq B3LYP/6-31+G(d,p)





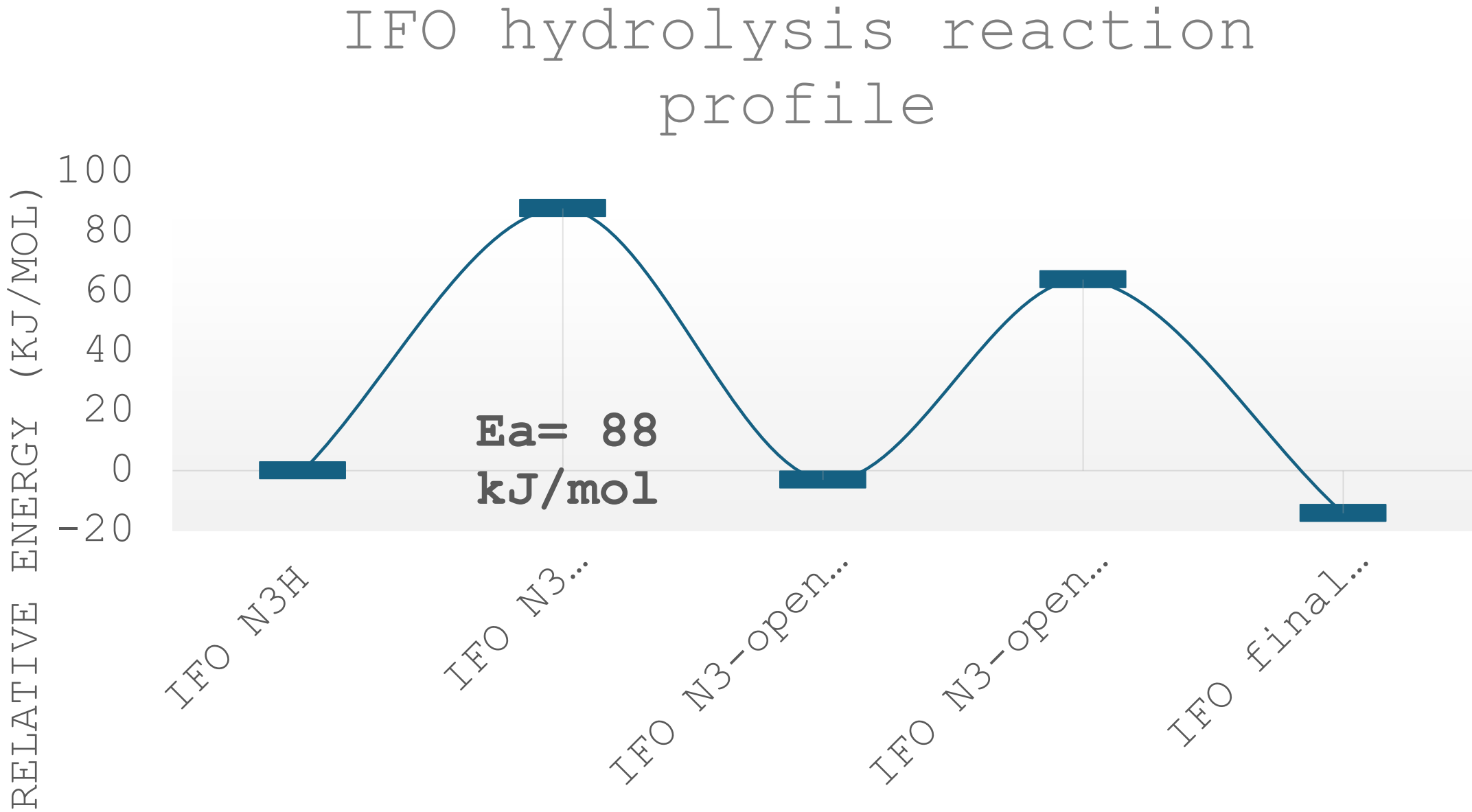




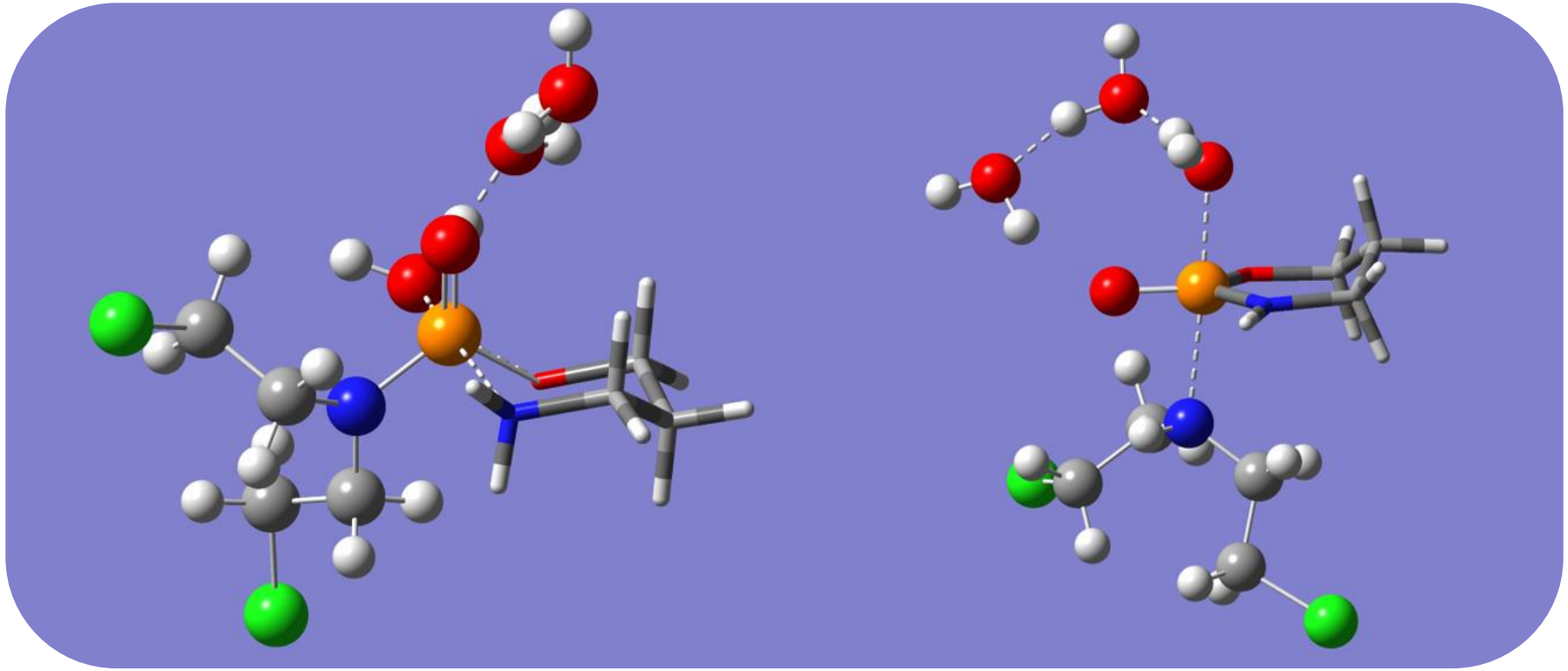
Name	Activation energy (Ea) [kJ/mol]
CPA N3 hydrolysis	91
CPA N7 hydrolysis	115
IFO N3 hydrolysis	88
IFO N7 hydrolysis	97

Activation energies of different hydrolysis pathways. N3 corresponds to the opening of the ring, while N7 corresponds to the elimination of 2-chloroethylamine group

- Opening of the ring is faster for both molecules
- IFO has a lower Ea and thus hydrolyses faster than CPA



Energy profile of the faster process in IFO hydrolysis (opening of the ring in IFO)



Different ring conformations during the hydrolysis of the P-N3 and the P-N7 bonds

This could explain the difference in energy

Currently investigating analogues with different ring sizes to test the hypothesis

Thank you for your attention!

PHARMA-ECO

HRZZ
Hrvatska zaklada za znanost

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