Prohapten activation and subsequent interaction with proteins: Mechanistic understanding and quantitative follow up…

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IDEA workshop on pre- & prohaptens
Brussels, June 17-18th, 2015

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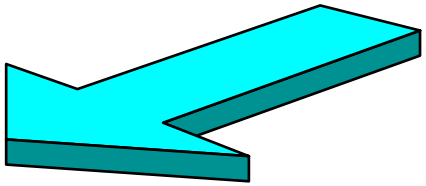
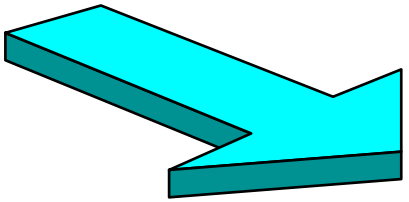
Metabolic studies

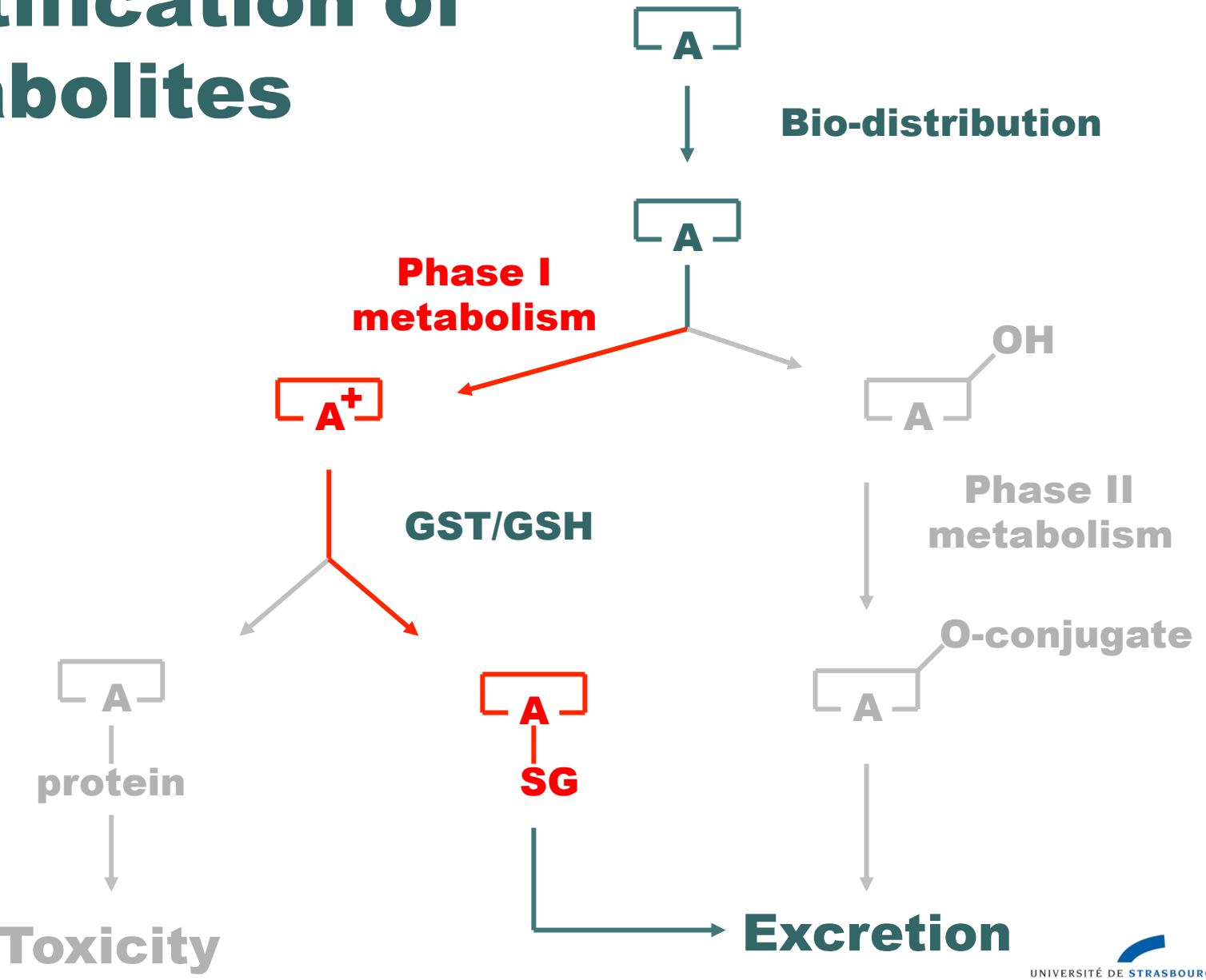


Liver metabolism…



Skin metabolism…

Identification of metabolites



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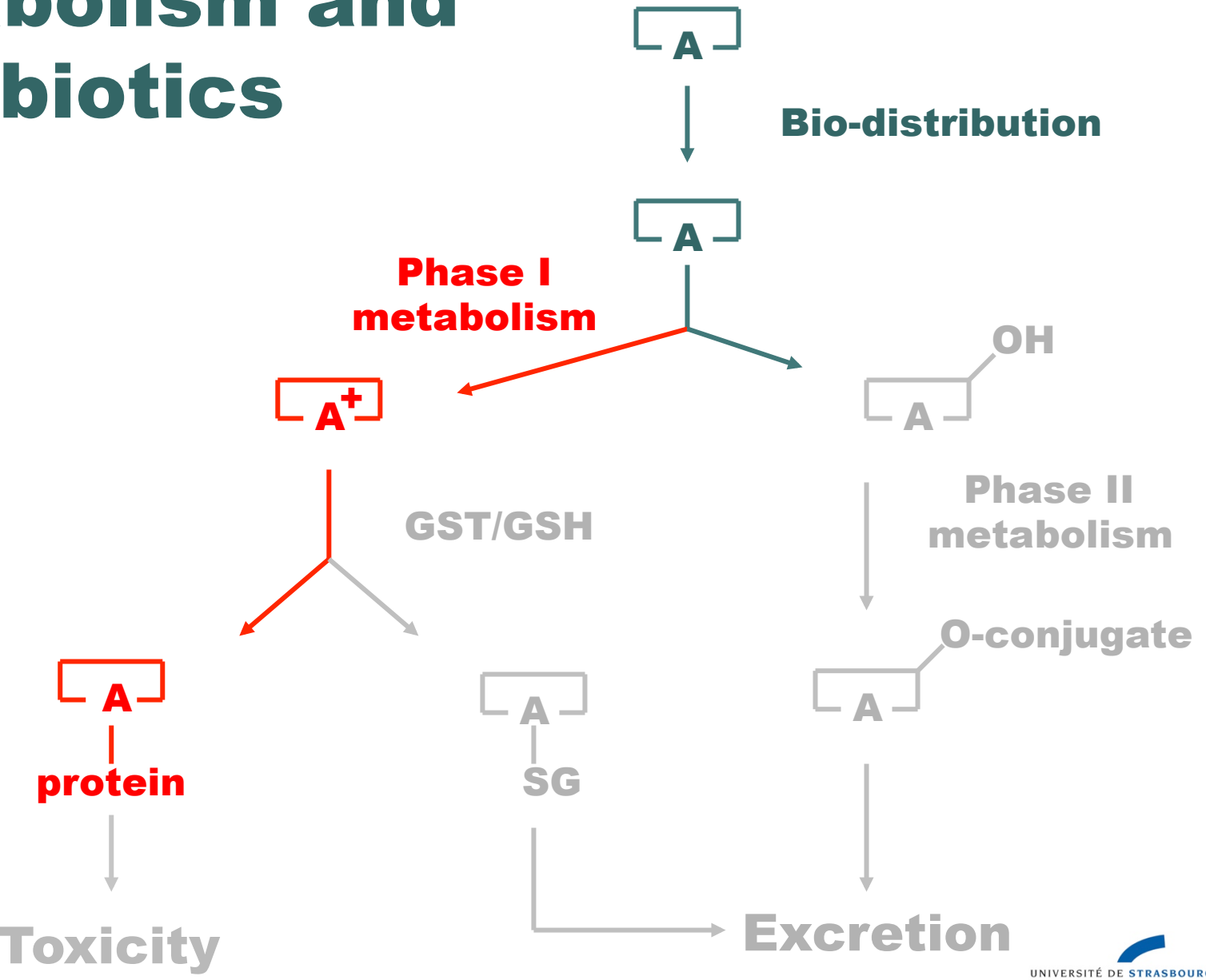
Metabolism and xenobiotics



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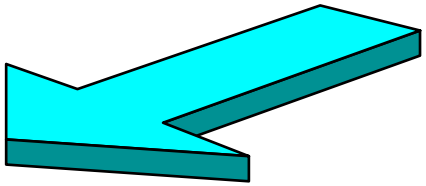
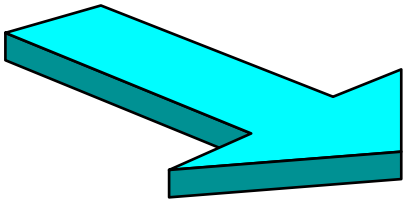
Two major pitfalls



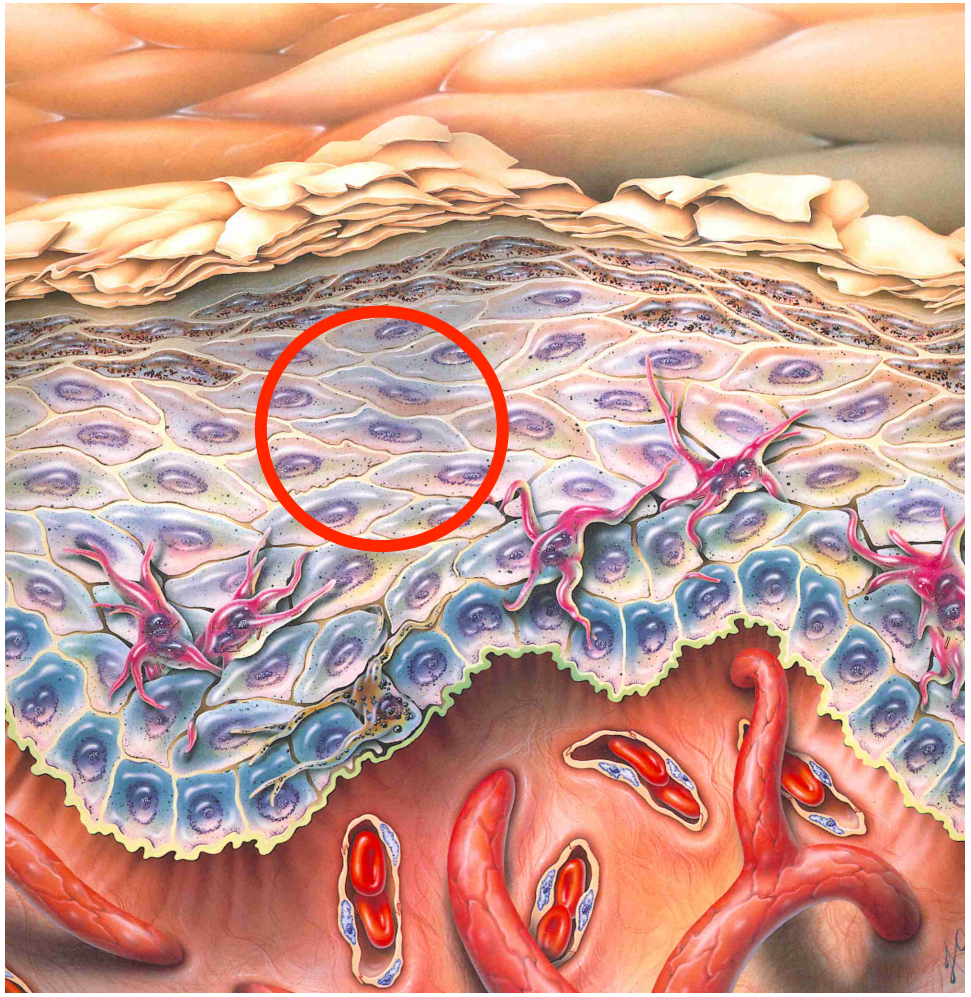
Liver vs skin…



Highly reactive intermediates…

Non invasive approach…

- Direct observation…
- Highly non homogeneous environment…



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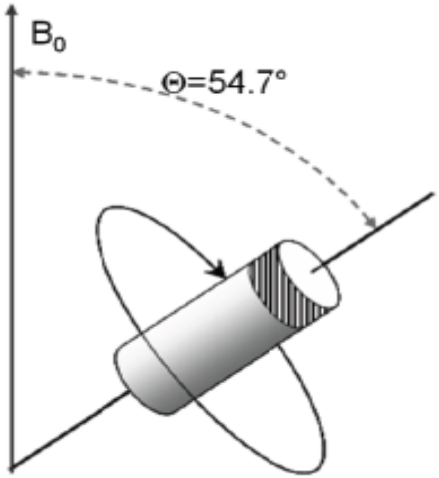
HRMAS NMR…

- High-Resolution Magic Angle Spinning “HRMAS” Nuclear Magnetic Resonance…
- Bring to zero inhomogeneity associated with the sample…



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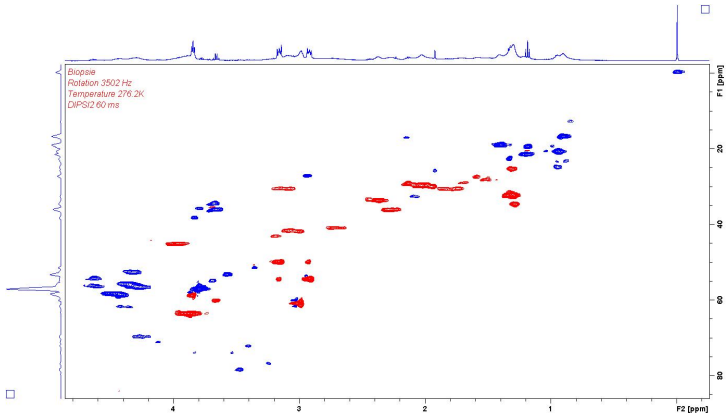
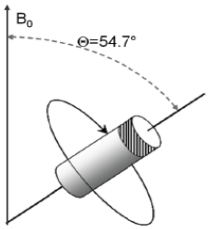
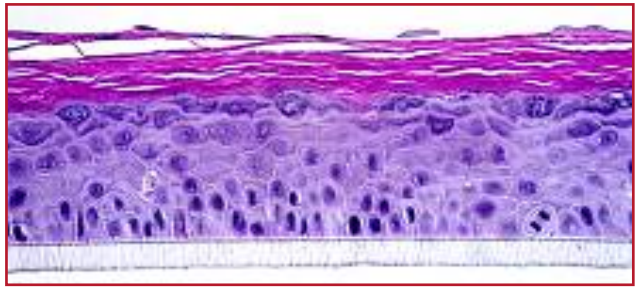
HRMAS NMR…

Test Chemical







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Is it possible to follow the metabolism of chemicals in Reconstructed Human Epidermis ?

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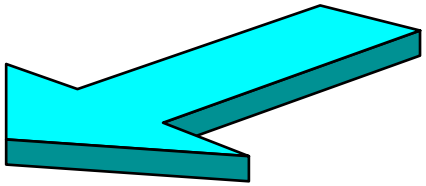
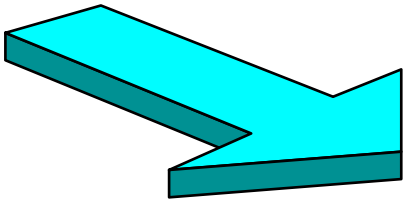
Follow-up of the RIFM project…



Activation of cinnamyl alcohol…



Hydrolysis of iso/eugenyl acetates…

The cinnamyl alcohol story…

- Cinnamyl alcohol has been considered for many years as “the” model of pro-hapten, being activated by alcohol dehydrogenase to form the reactive cinnamaldehyde…
- However, about half of the patients sensitized to cinnamyl alcohol do not react when patch-tested with cinnamaldehyde…
- This suggests that at least one alternative metabolic pathway is taking place in Human epidermis, activating cinnamyl alcohol into at least another unknown reactive intermediate…

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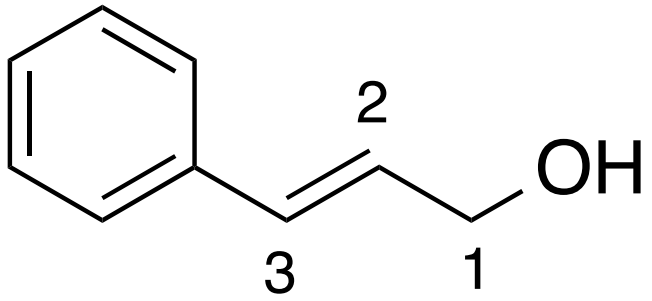
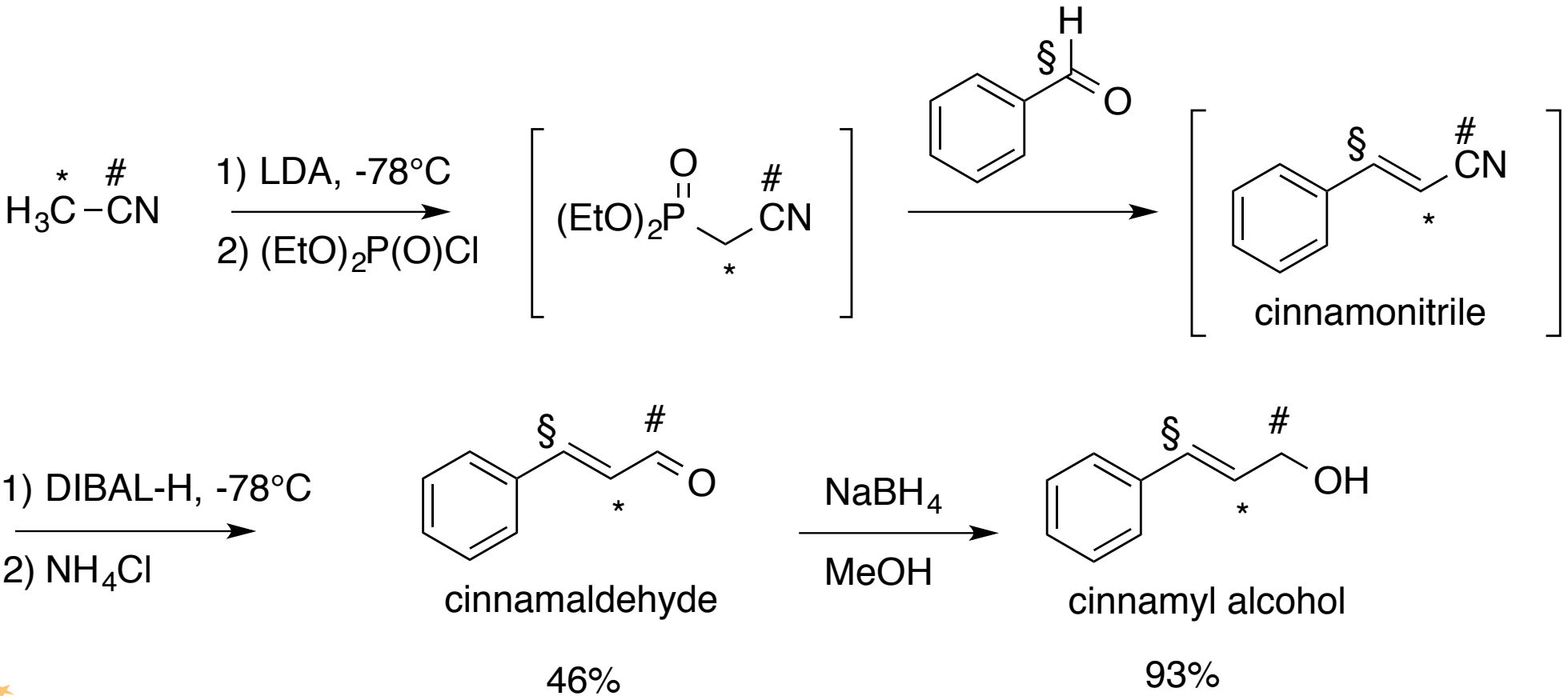
The cinnamyl alcohol story…

- The aim of this initial phase was to assess the potential of the HRMAS NMR / RHE model to investigate the metabolism of cinnamyl alcohol in a living 3D tissue…
- Cinnamyl alcohol was synthesized carbon-13 substituted either at position 1, 2 or 3…

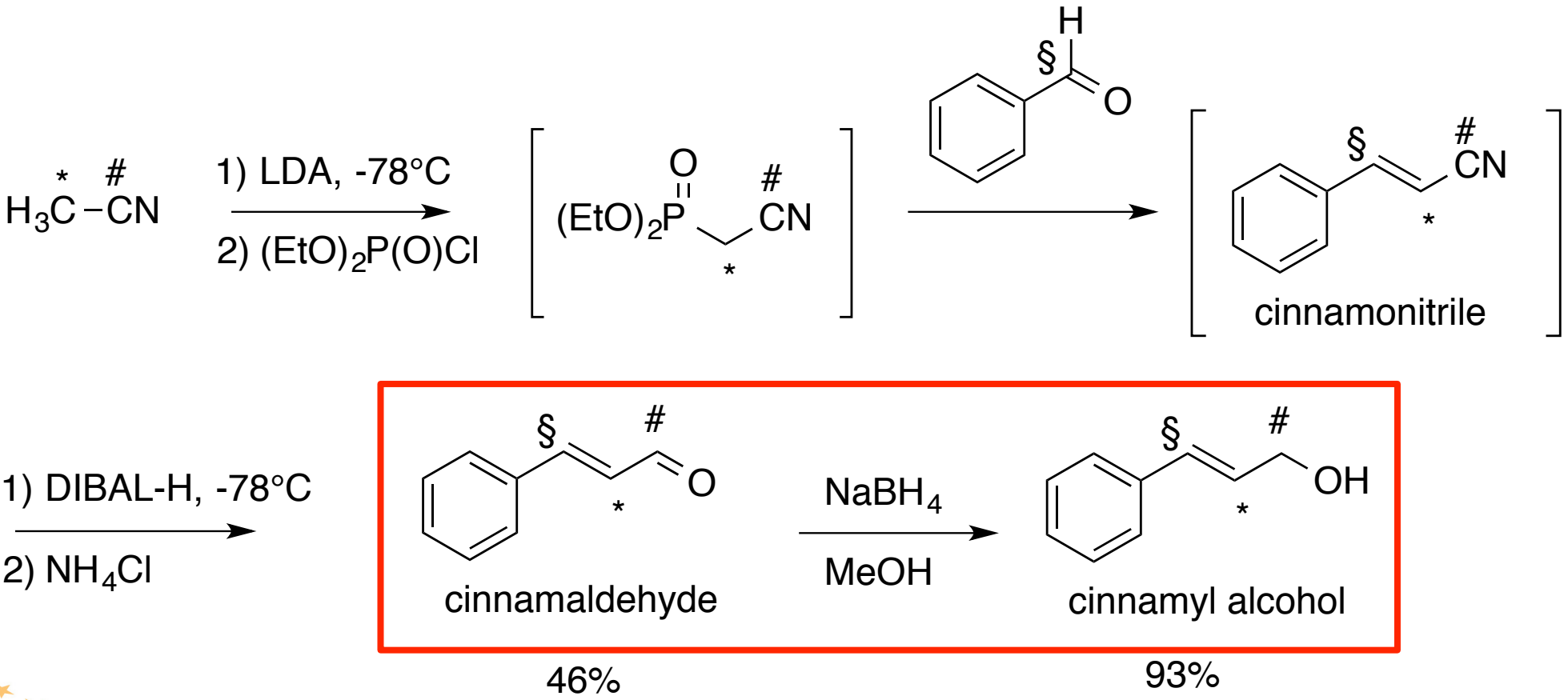


1-(^{13}C)
2-(^{13}C)
3-(^{13}C)

The cinnamyl alcohol story...



The cinnamyl alcohol story...



The cinnamyl alcohol story…

- The behavior of cinnamyl alcohol and cinnamaldehyde in RHE was first assessed…
- Cinnamyl alcohol and cinnamaldehyde, substituted at position 1, were thus applied on RHE and post-incubated for 1, 8 and 24 h, respectively…
- Samples were processed and analyzed by HRMAS NMR to assess potential oxidation or reduction taking place at position 1…

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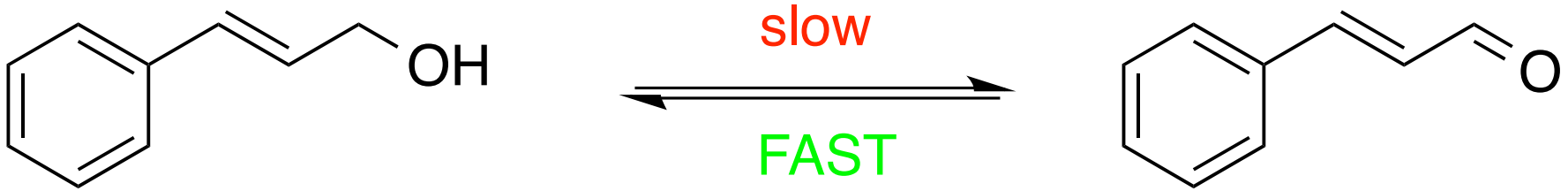
The cinnamyl alcohol story…

- The behavior of cinnamyl alcohol and cinnamaldehyde in RHE is not symmetrical…
- A rapid (already after 1 h of incubation) and significant reduction of cinnamaldehyde into cinnamyl alcohol was observed…
- The opposite was not observed (no signal of cinnamaldehyde in cinnamyl alcohol treated RHE) even after 24 h…



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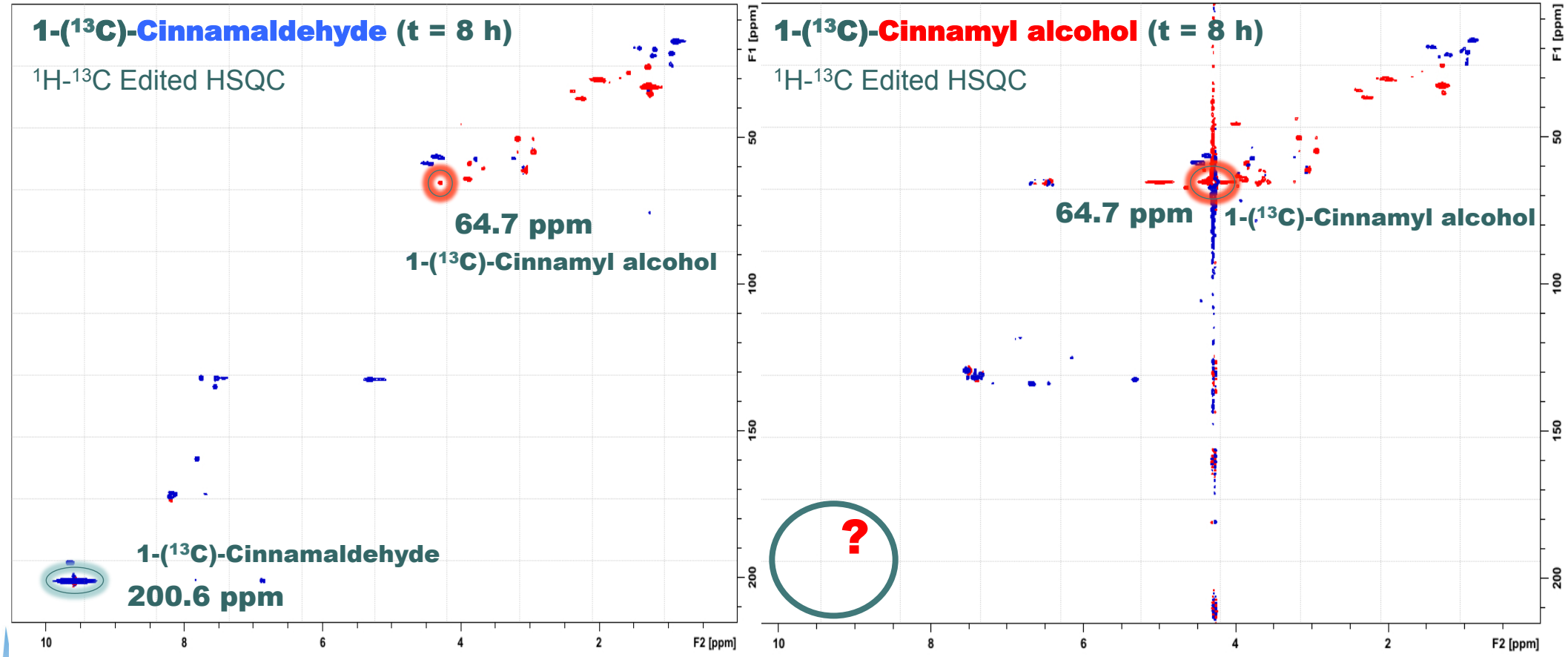
The cinnamyl alcohol story…



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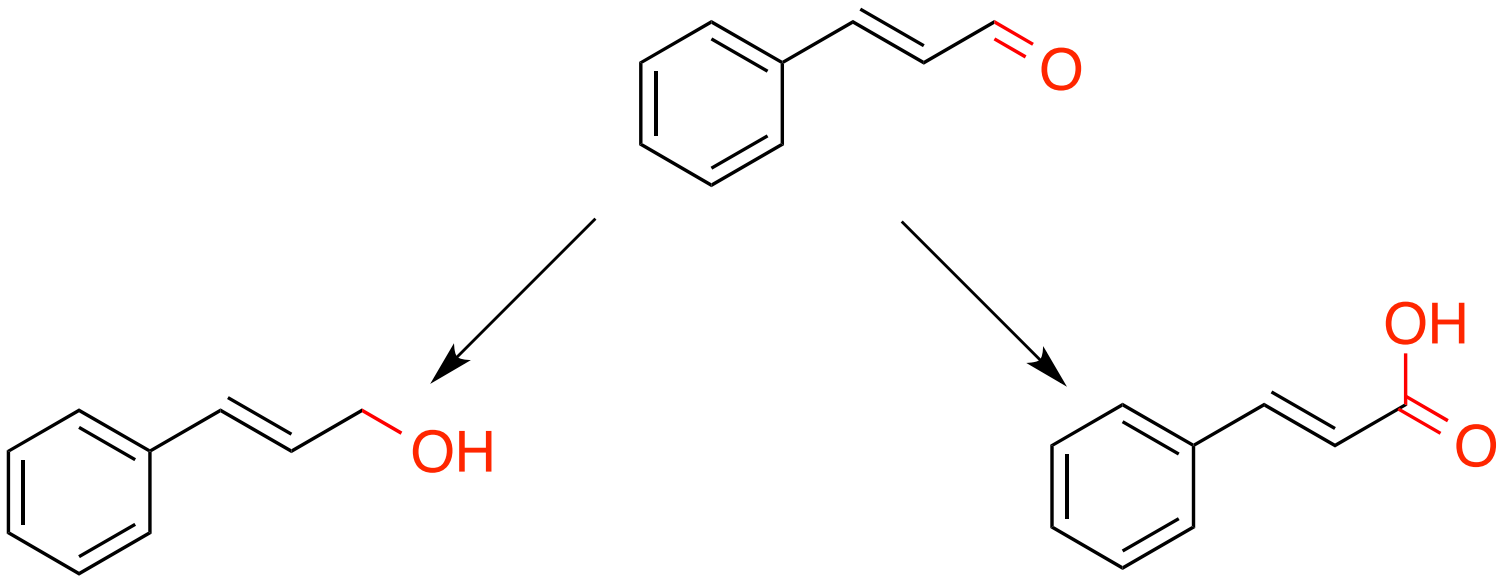
The cinnamyl alcohol story…

- Samples treated with cinnamyl alcohol and cinnamaldehyde, respectively, were also analyzed for the formation of cinnamic acid…
- A significant amount of cinnamic acid was observed in cinnamaldehyde treated samples



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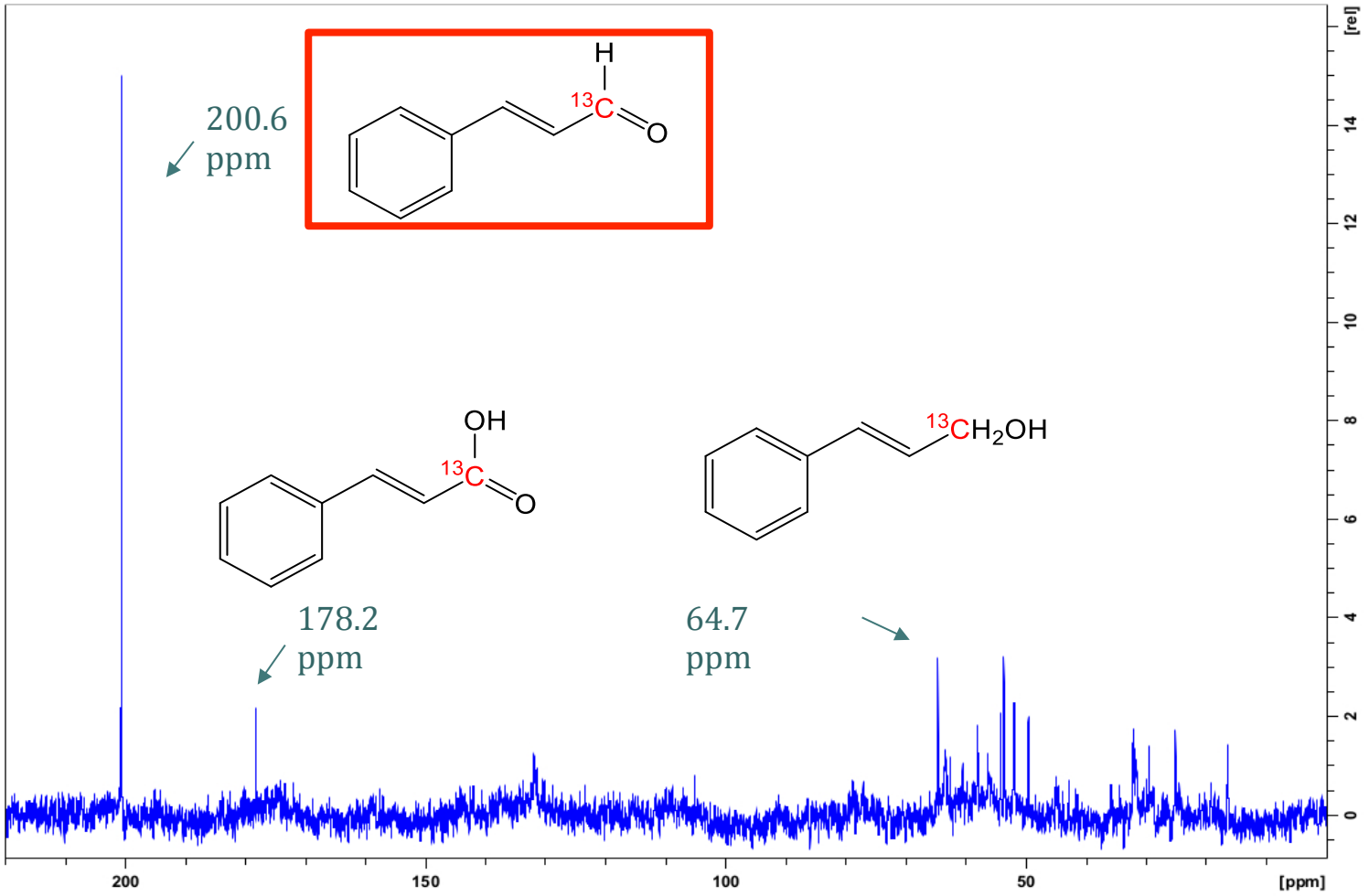
The cinnamyl alcohol story…



1-(^{13}C)-Cinnamaldehyde (t = 24 h)

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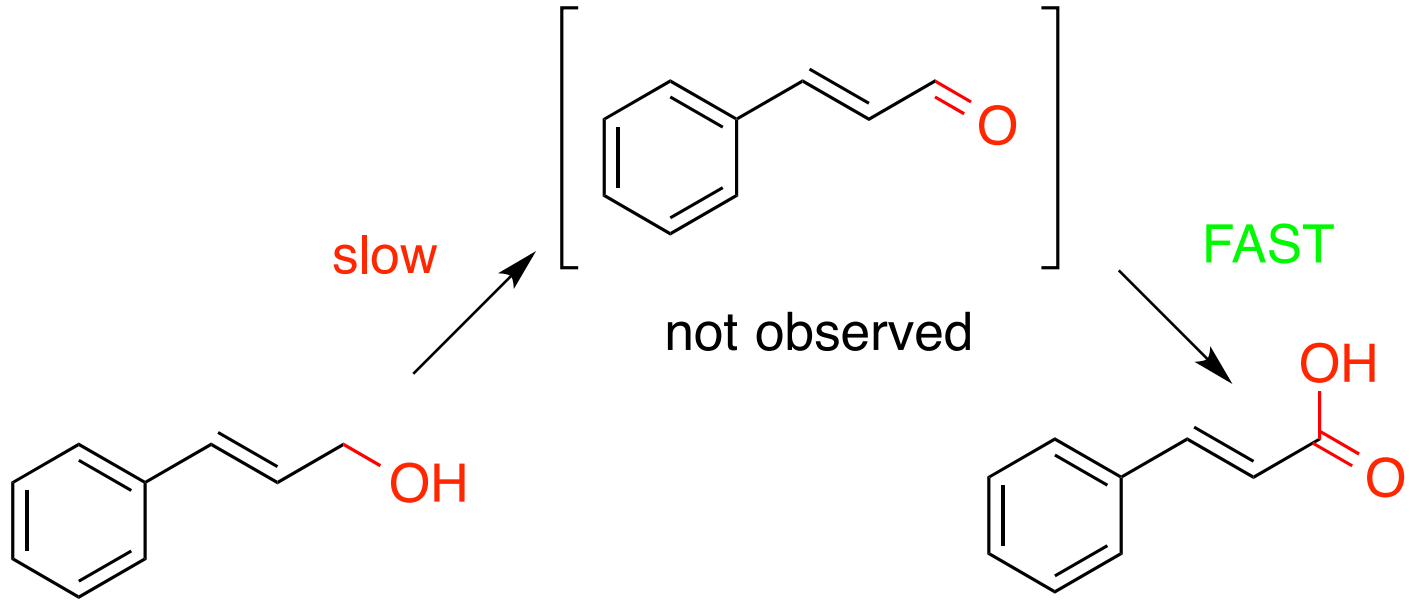
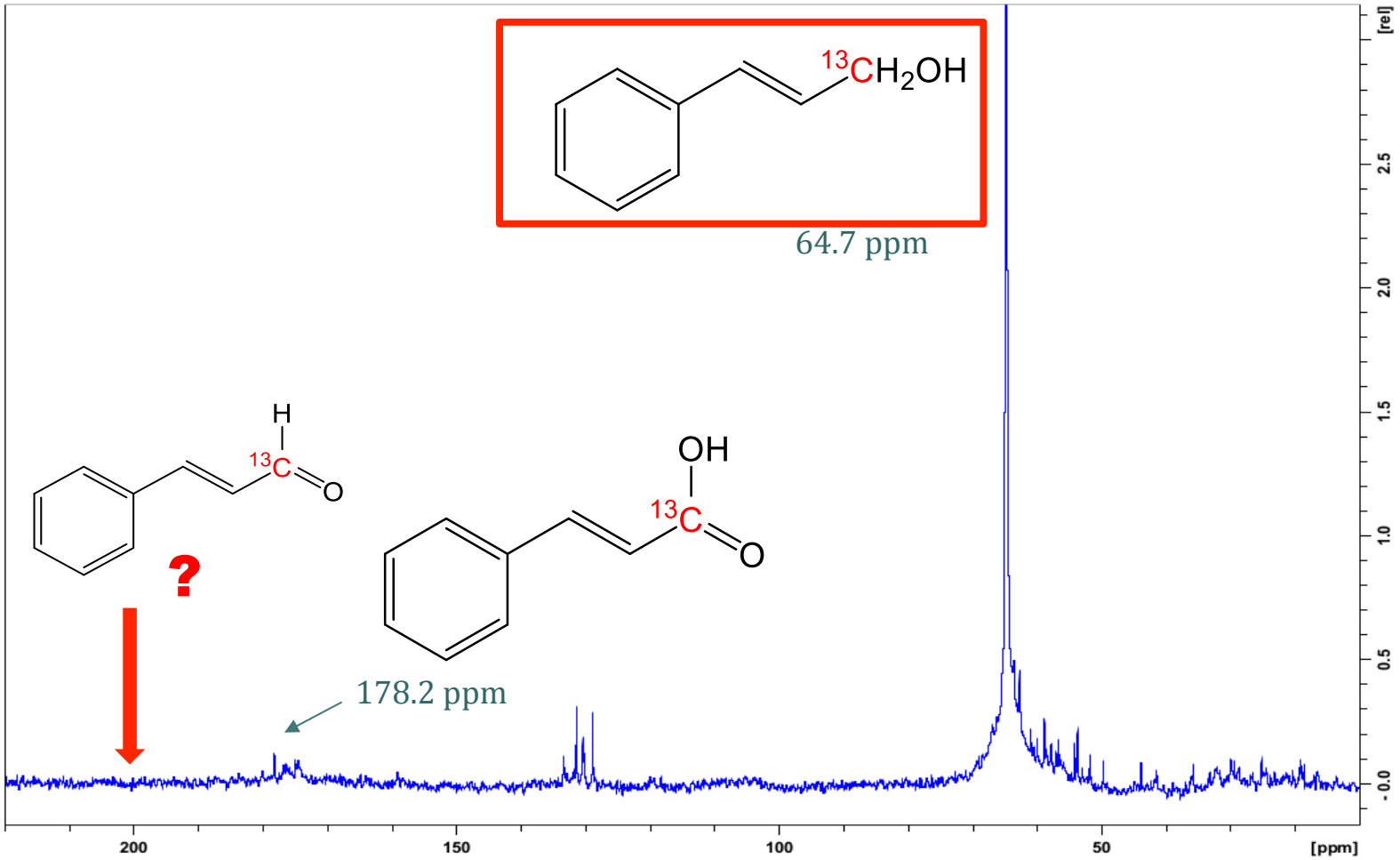
The cinnamyl alcohol story…

- Samples treated with cinnamyl alcohol and cinnamaldehyde, respectively, were analyzed for the formation of cinnamic acid…
- Only a minute amount of cinnamic acid was detected in cinnamyl alcohol treated samples…



The cinnamyl alcohol story…



1-(^{13}C)-Cinnamic alcohol (t = 1 h)

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The cinnamyl alcohol story…

- Samples treated with cinnamyl alcohol and cinnamaldehyde, respectively, were also qualitatively analyzed for the formation of metabolites/adducts…
- A very clear difference was observed with the formation of new correlation signals but associated to very different patterns (no common signals associated to metabolites/adducts)…
- Signals associated to the reactivity of cinnamaldehyde were not observed in RHE treated with cinnamyl alcohol…

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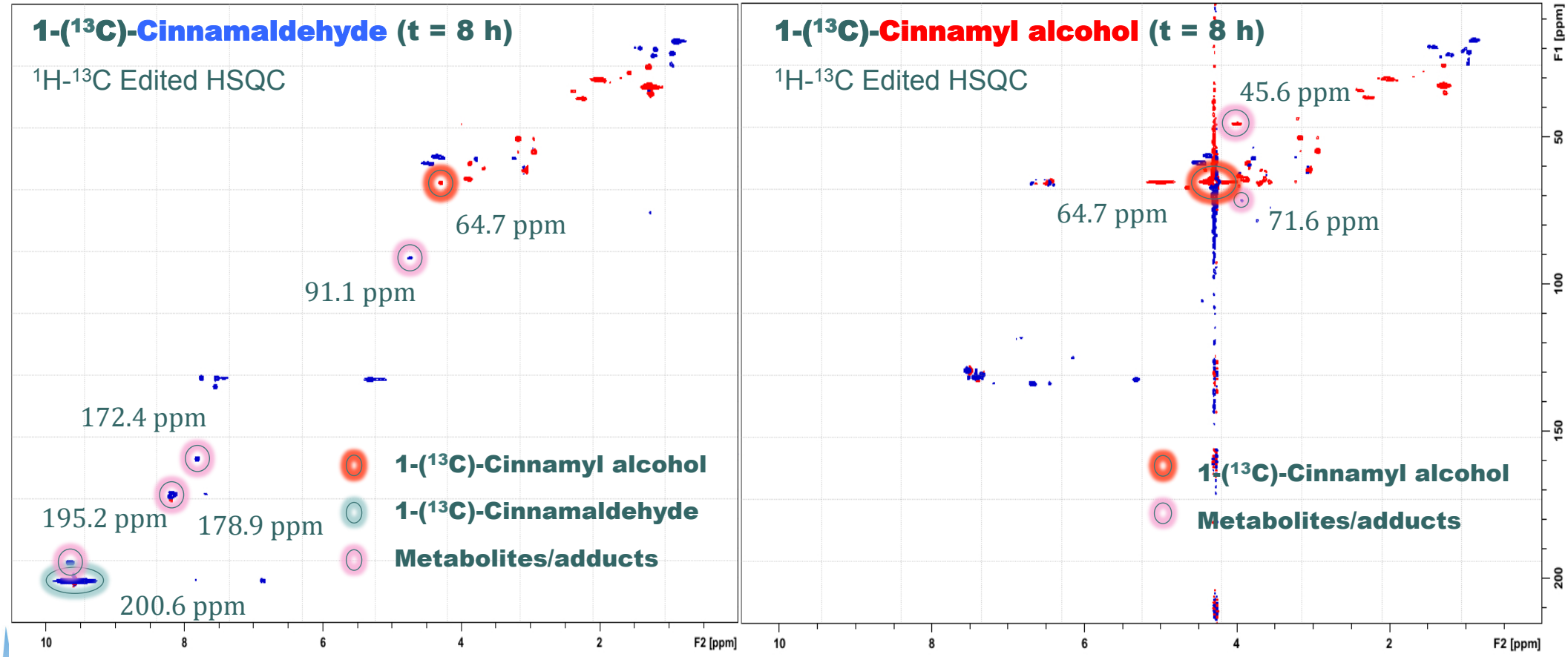
The cinnamyl alcohol story…



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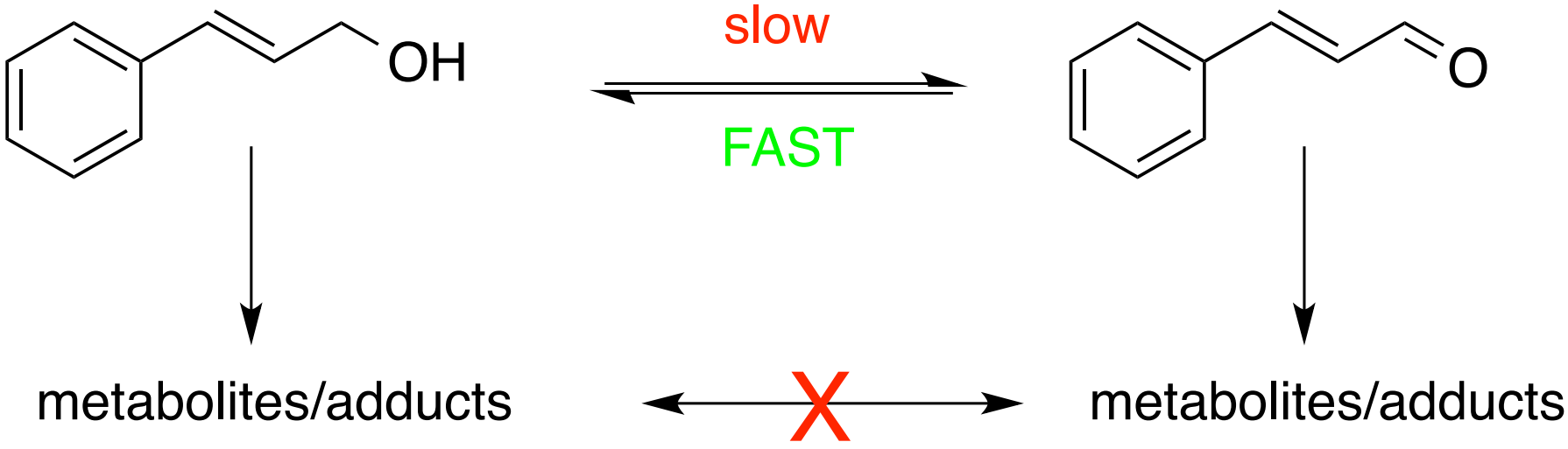
The cinnamyl alcohol story…

- **The “dogma” of cinnamyl alcohol being a sensitizer through its oxidation into cinnamaldehyde can therefore be seriously questioned…**



The iso/eugenyl acetates story…

- Eugenol and isoeugenol are used as pure material but also as eugenyl and isoeugenyl esters (acetates or phenyl acetates)…
- According to LLNA data, acetate derivatives of eugenol and isoeugenol are classified as non-sensitizers while their parent materials are classified as weak and moderate skin sensitizers, respectively…
- However, clinical studies have shown that some individuals sensitized to isoeugenol also reacted when patch-tested to isoeugenyl acetate…

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The iso/eugenyl acetates story…

- The mechanism underlying this observation is still not clear…
- It can be hypothesized that isoeugenyl esters are hydrolyzed either enzymatically (epidermal esterase) or chemically (hydrolysis)…
- The aim of this initial phase was to assess the potential of the “HRMAS NMR / RHE model” to investigate and characterize the behavior of eugenyl- and isoeugenyl acetates in a living 3D tissue…

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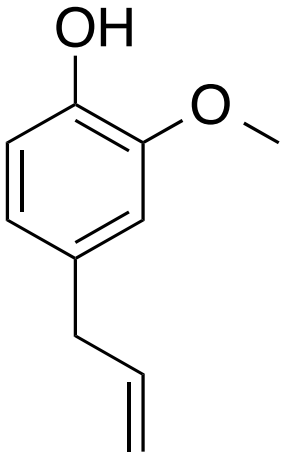
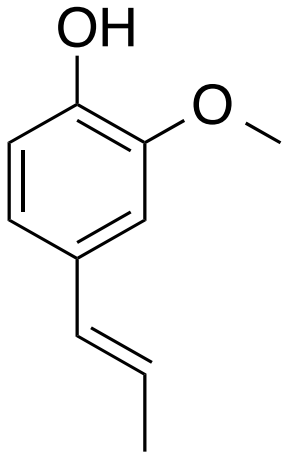
The iso/eugenyl acetates story…

- Carbon-13 substituted iso/eugenyl acetates were synthesized
  - To increase the sensitivity…
  - To discriminate between acetates released by iso/eugenyl derivatives and other acetates…



1



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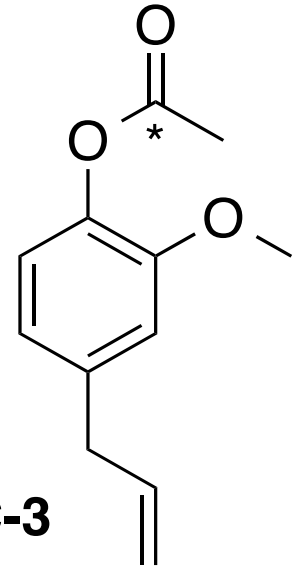
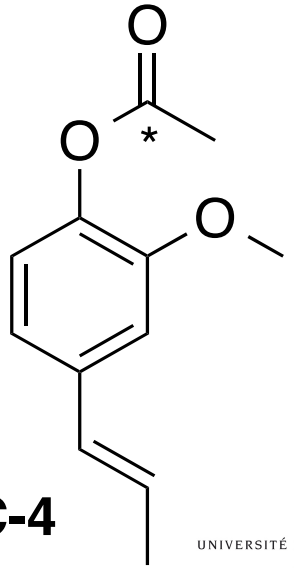


^{13}C-3



^{13}C-4

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The iso/eugenyl acetates story…

- The stability of eugenyl and isoeugenyl acetates was first assessed in a 1:3 mixture of acetonitrile and phosphate buffer (PBS pH 7.4)…
- Reactions were followed by ^{13}C NMR over a period of one month…
- Both eugenyl and isoeugenyl acetates were found to be rather stable toward chemical hydrolysis with only a slow release of free acetate over time…

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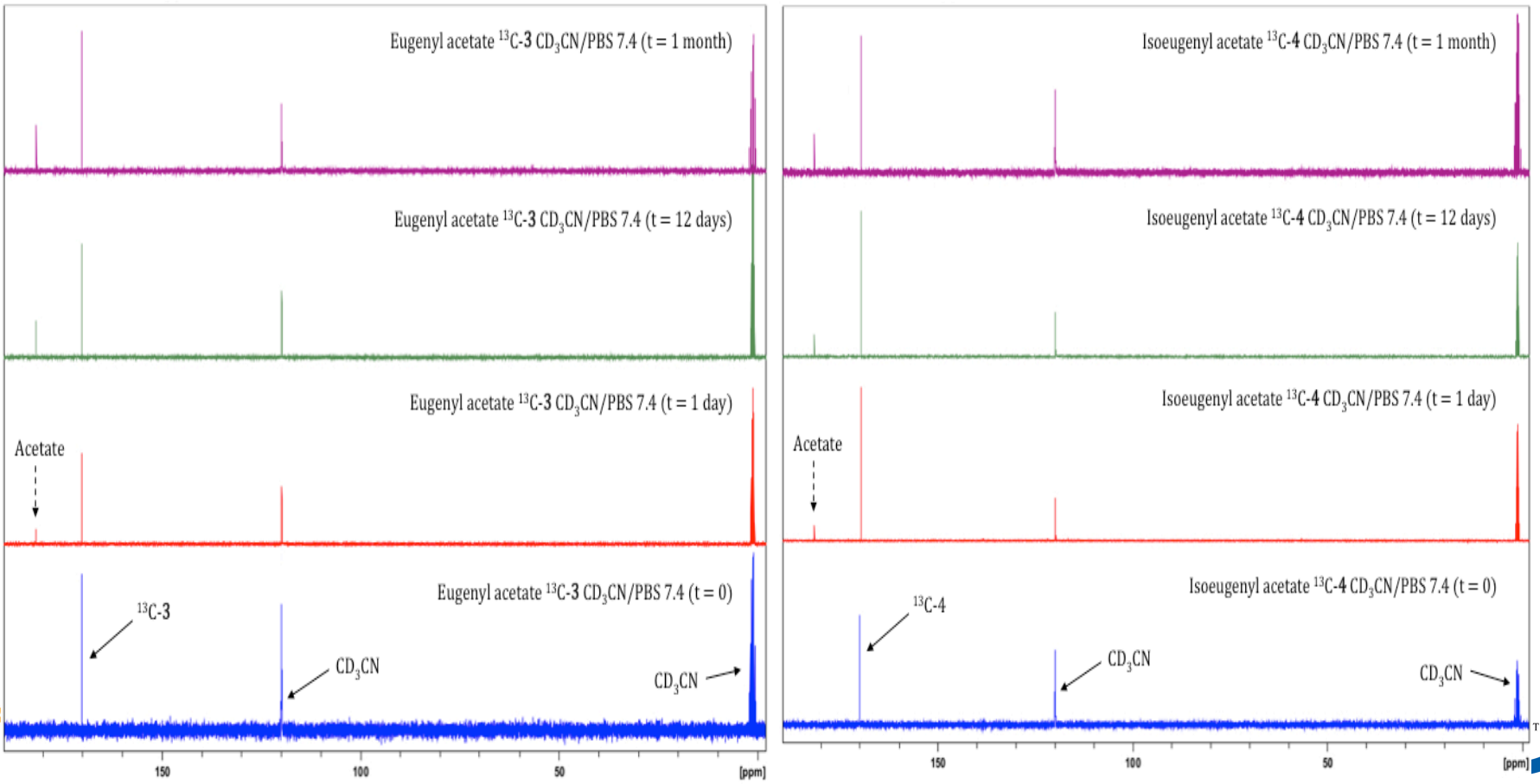
The iso/eugenyl acetates story…

- **Stability of iso/eugenyl acetates in a 1:3 mixture of acetonitrile and PBS pH 7.4**



The iso/eugenyl acetates story…

- The stability of iso/eugenyl acetates was then tested on RHE…
- Eugenyl and isoeugenyl acetates, ^{13}C-3 and ^{13}C-4 were applied on three samples of RHE and incubated for 1, 8 and 24 h, respectively…
- Samples were then processed and analyzed by HRMAS NMR using a 1D-^{13}C sequence…
- With both eugenyl- and isoeugenyl acetates, only one signal at 183.4 ppm, corresponding to a free acetate (CH_3-COO^-), was detected…

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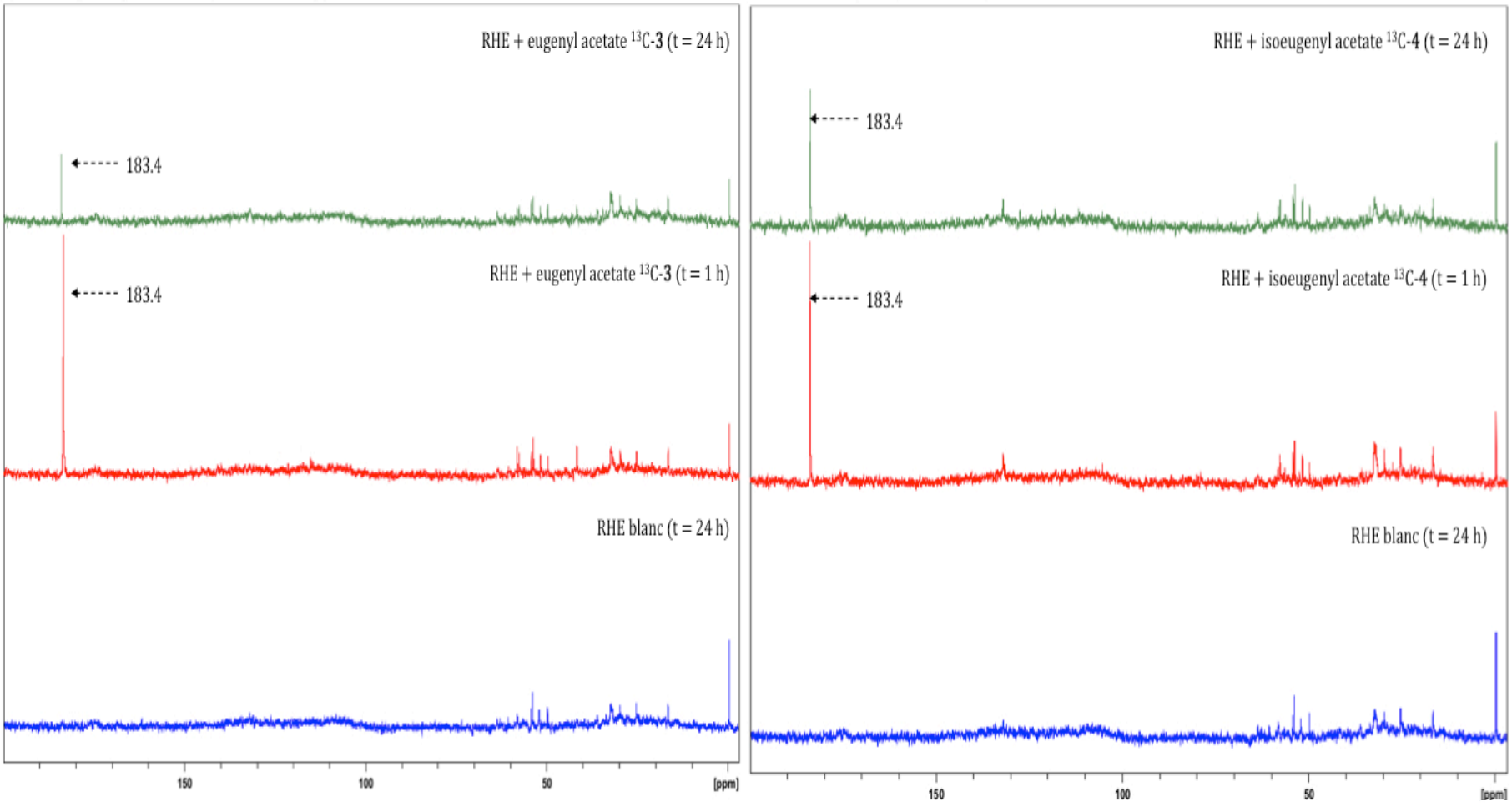
The iso/eugenyl acetates story…

- **Stability of eugenyl and isoeugenyl acetate on RHE**



The iso/eugenyl acetates story…

- A second set of experiments was carried out with isoeugenyl acetate ^{13}C-4 following incubation times of 5, 10, 15, 30 and 45 minutes, respectively…
- Spectra obtained indicate a very fast hydrolysis of isoeugenyl acetate ^{13}C-4…
- Even after 5 min, the residual signal of isoeugenyl acetate (δ 170.8 ppm) was very small with a major signal at δ 183.4 ppm corresponding to the hydrolyzed acetate …

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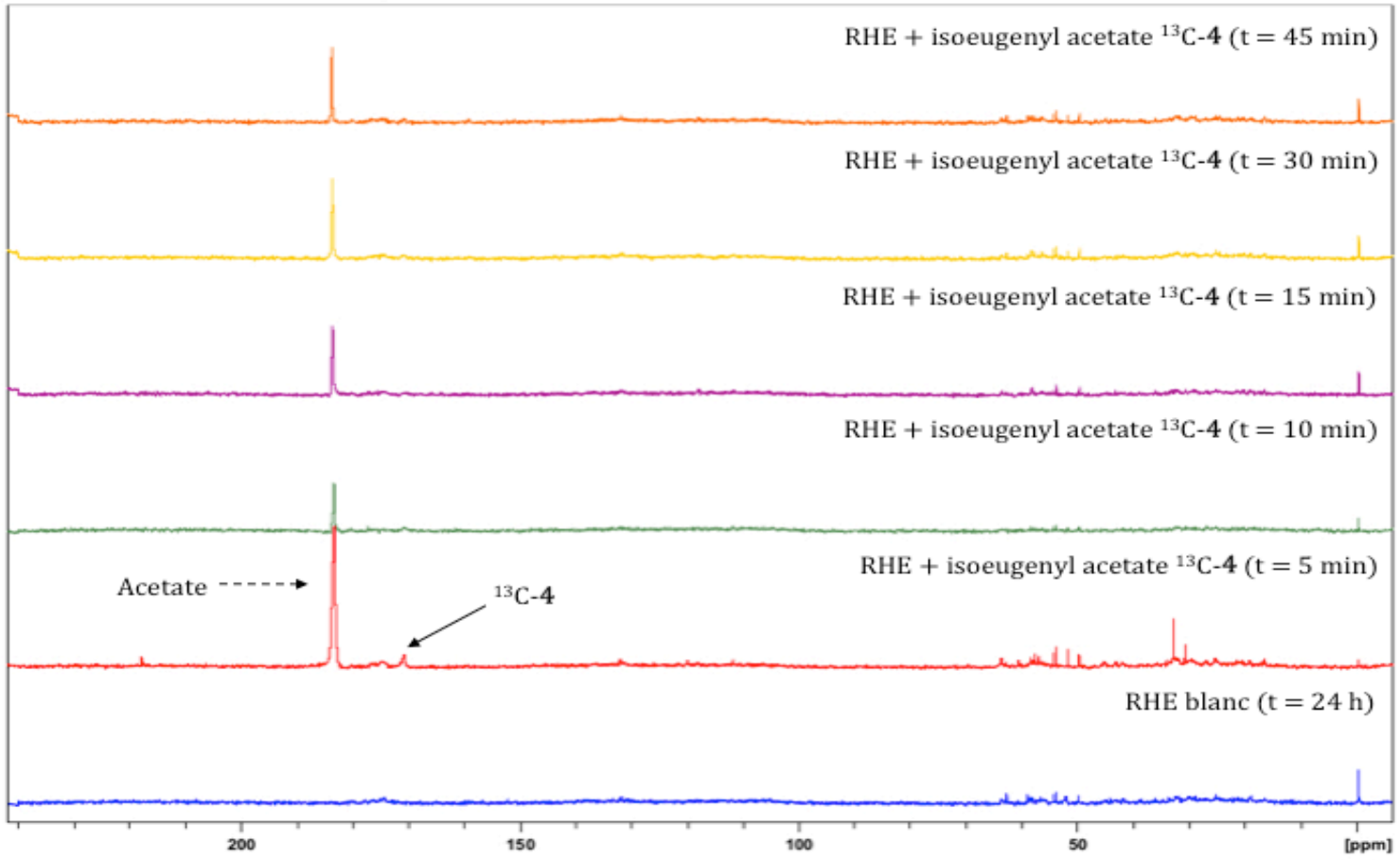
The iso/eugenyl acetates story…

- **Stability of isoeugenyl acetate on RHE**



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The iso/eugenyl acetates story…

- Pre-treatment of RHE with inhibitors of esterase…
- Use of “fixed” RHE to confirm the role of enzymes in the hydrolysis of eugenyl and isoeugenyl acetates…
- Model the hydrolysis reaction of eugenyl and isoeugenyl acetates with porcine liver esterase…
- **Perform the reaction at a lower temperature to slow down the enzymatic kinetic rate…**

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Conclusions/Perspectives…

- Results obtained so far clearly confirmed the high potential of HRMAS NMR, in association with RHE, to investigate activation mechanisms of prohaptens…
- Iso/eugenyl acetates appeared to be highly sensitive to hydrolysis…
- The formation of cinnamaldehyde by epidermal oxidation of cinnamyl alcohol, as a source of sensitization, appeared to be highly questionable…
- Further studies are needed…

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