Revealing ligand-receptor interaction NMR titration

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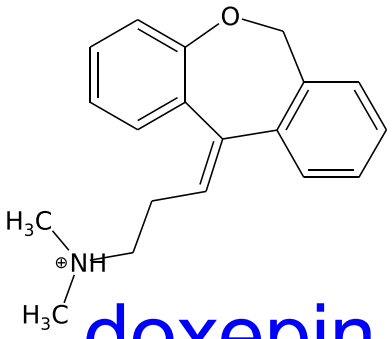
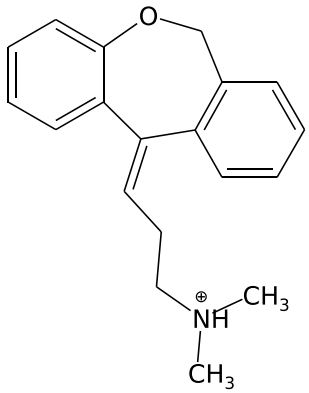
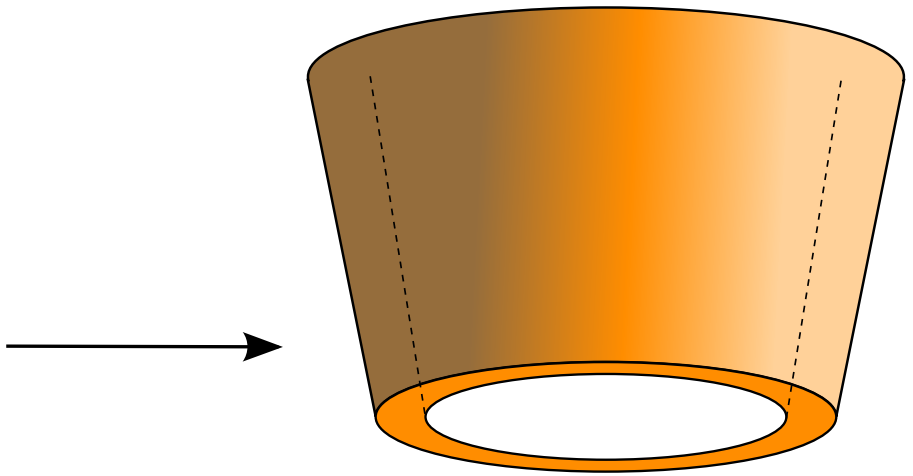
May 2, 2018

Introduction of reacting partners



doxepin ?





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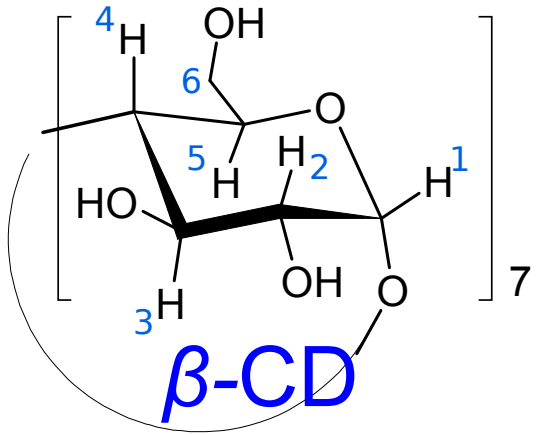
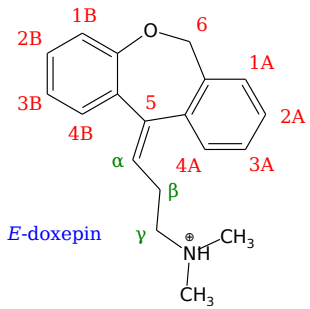
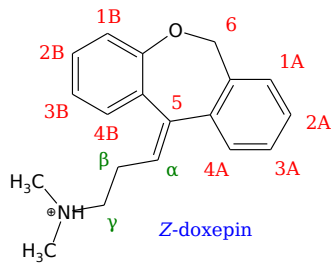


β-CD

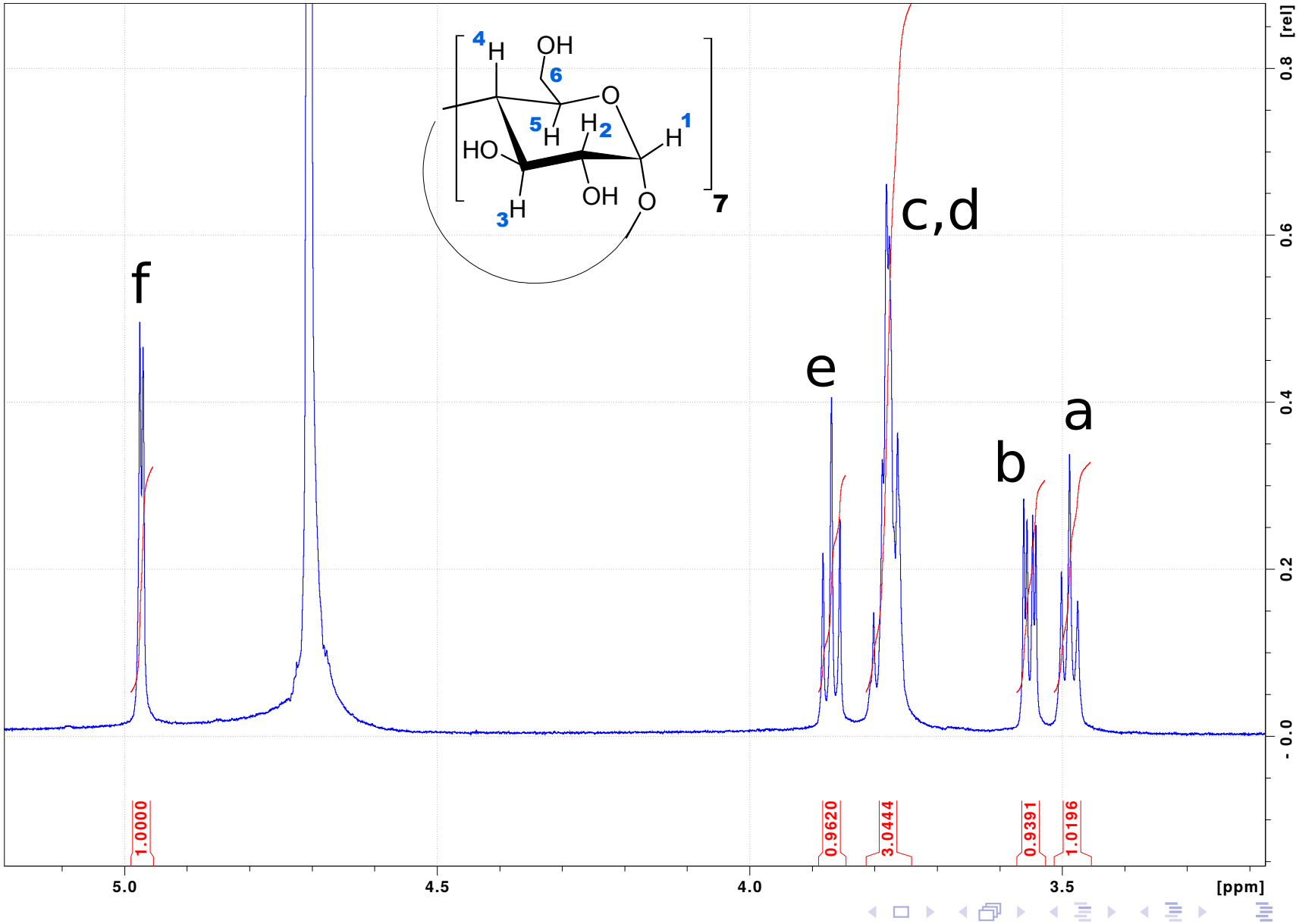
Recommended procedure

1. Assignment of free receptor: β -cyklodextrine (1D ^{1}H, DQF-COSY)
2. Assignment of free ligand: doxepine (1D ^{1}H, DQF-COSY, NOESY)
   - Identification of proton resonances of ring *A* and *B*
   - Determination of major and minor conformation of doxepin
3. 1D NMR titration: rearrangement of β -cyclodextrin resonaces upon interaction $\Rightarrow$ identification of perturbed protons
4. 1D NMR titration: rearrangement of doxepin resonaces upon complexation $\Rightarrow$ estimation of binding mode
5. ROESY spectrum of complex: ROE intermolecular contacts
6. Fitting the titration isotherm

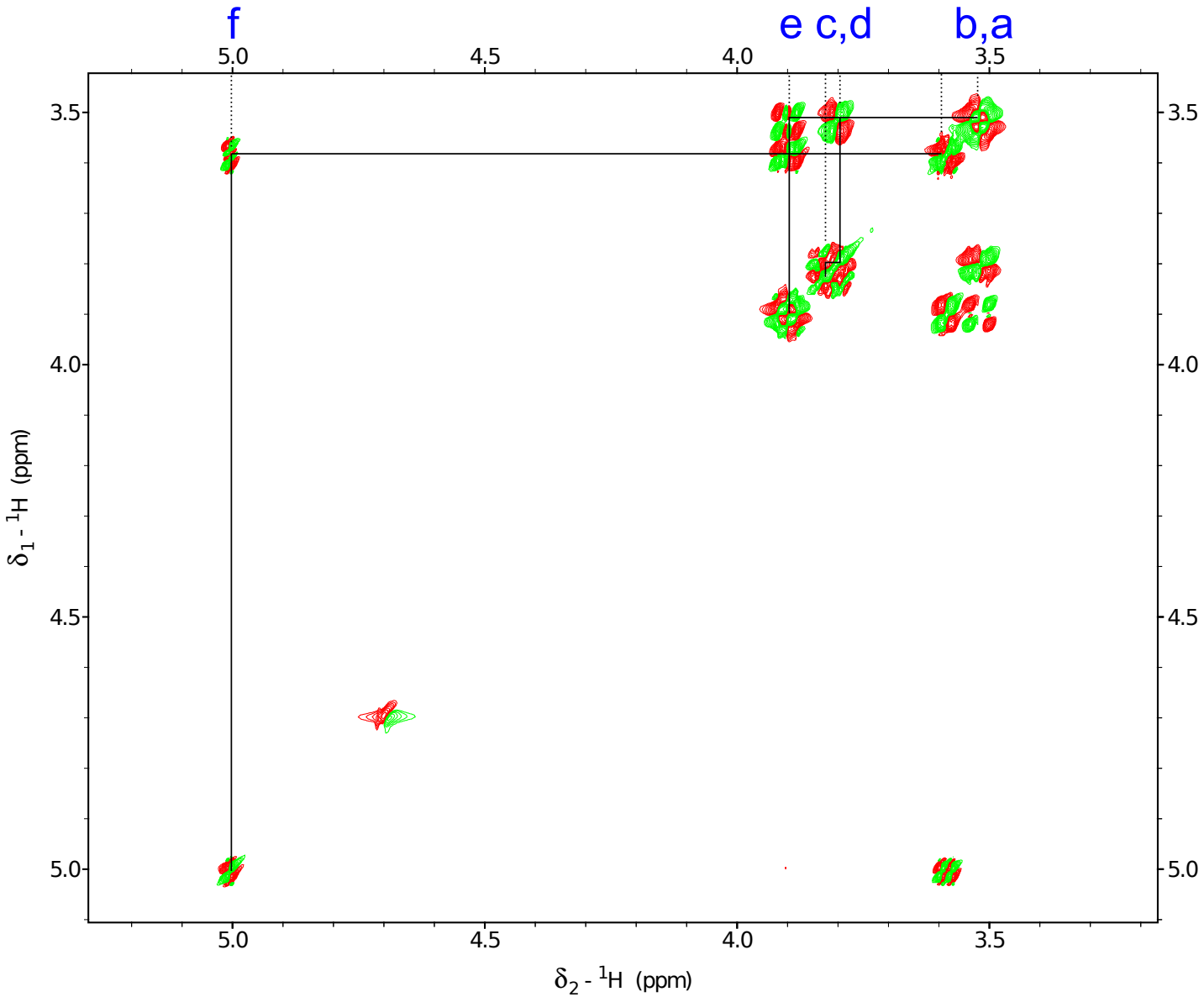




1D ^{1}H of β -cyclodextrin in D_2O



DQF-COSY of β -cyclodextrin in D_2O



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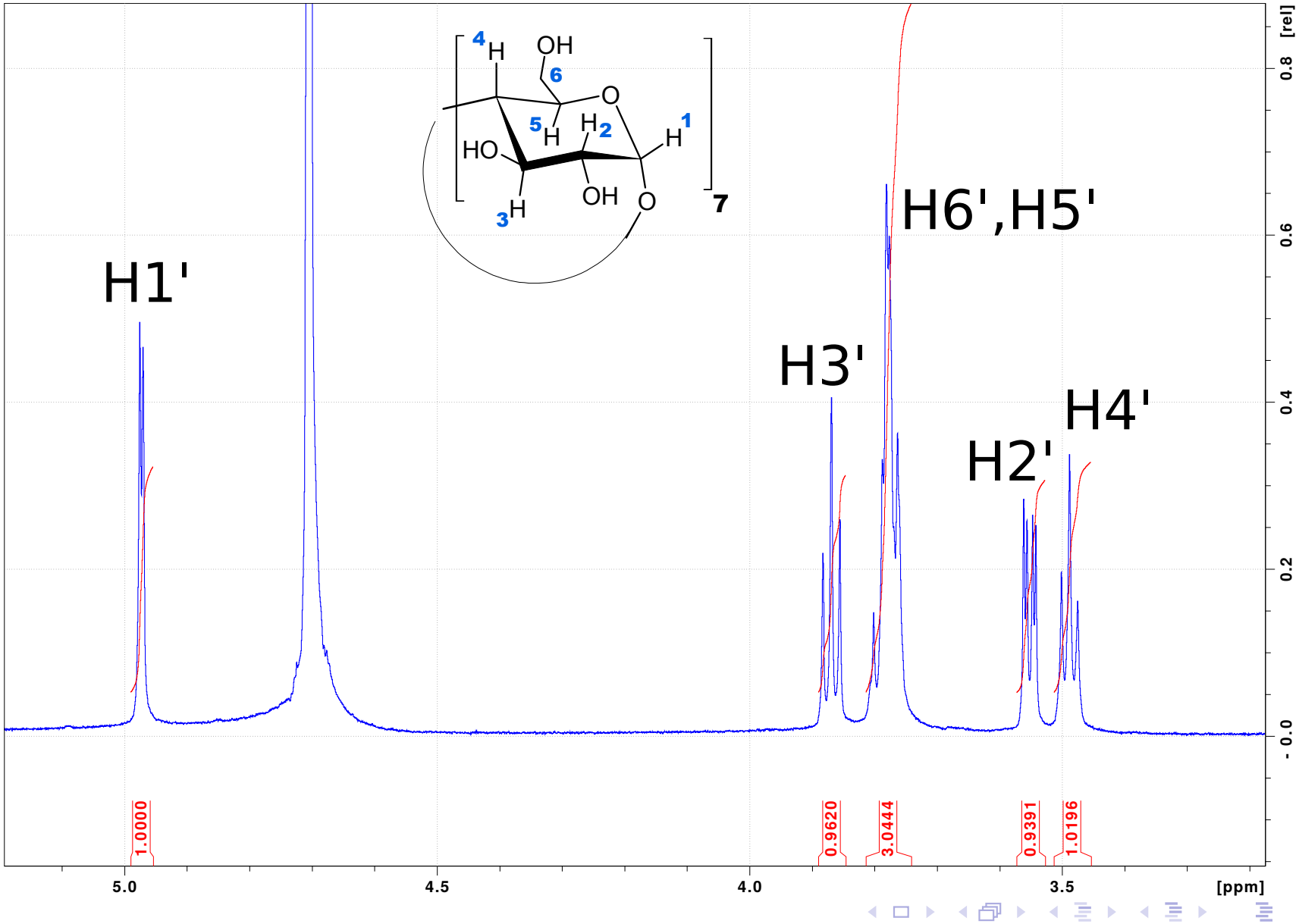
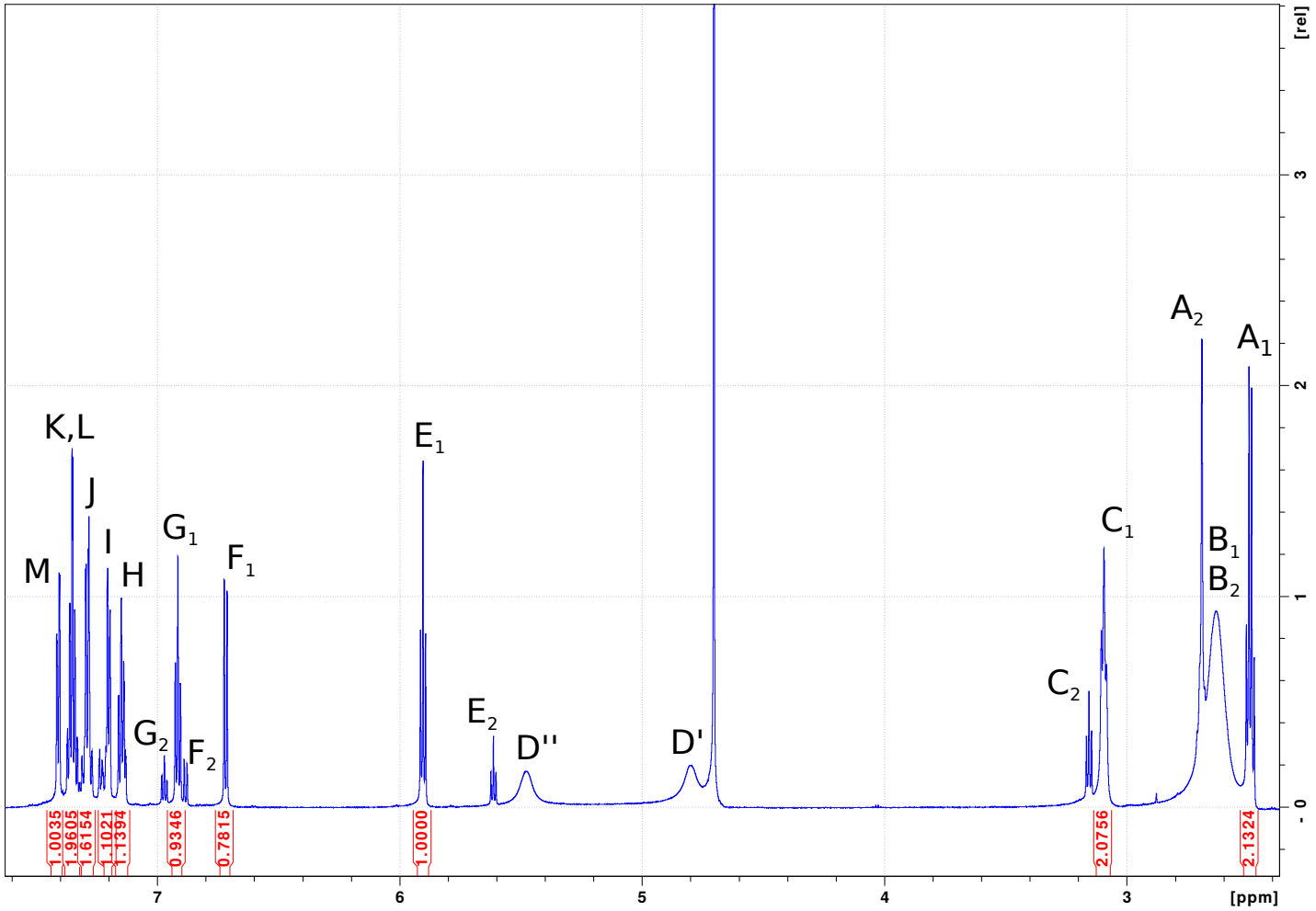
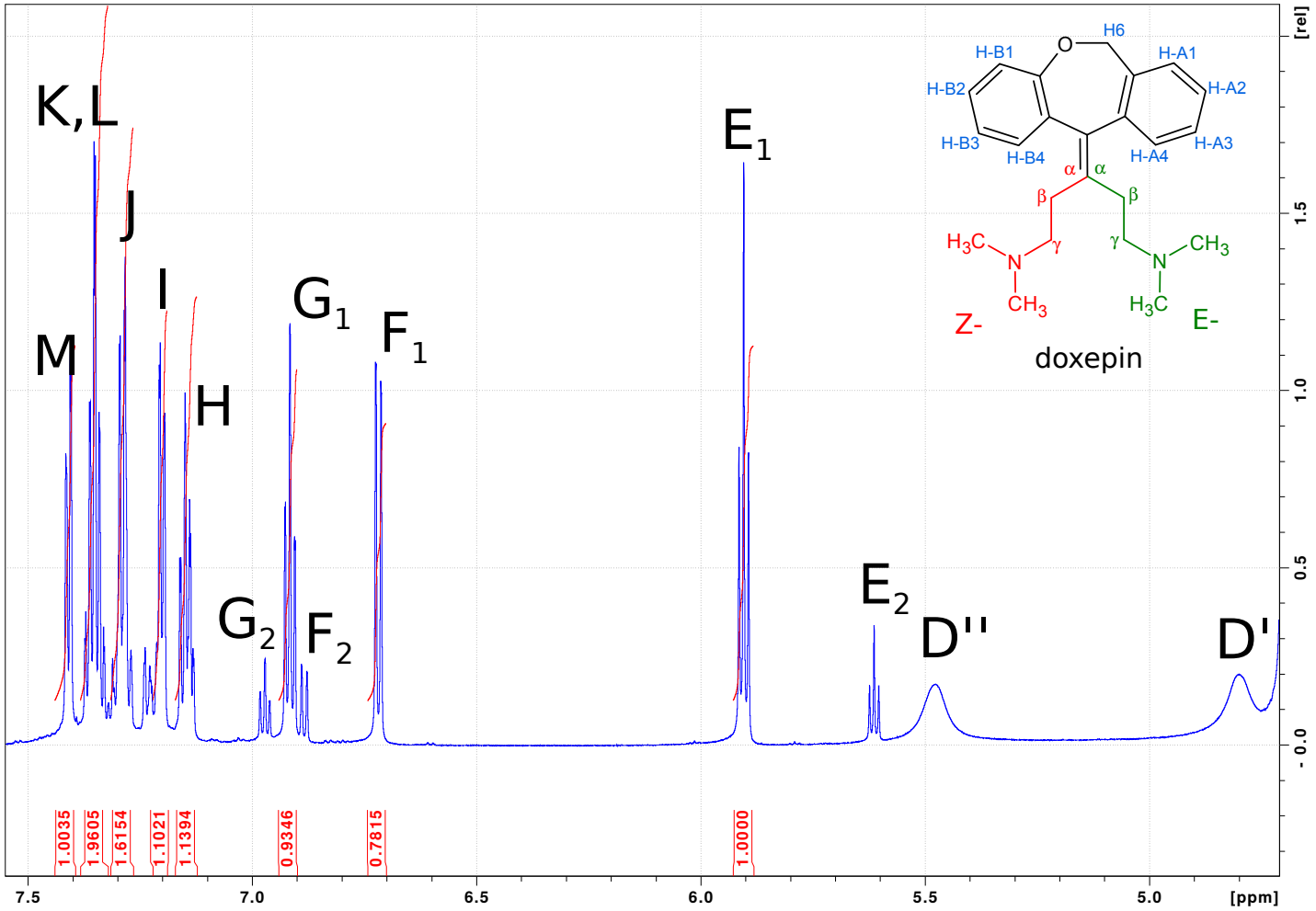


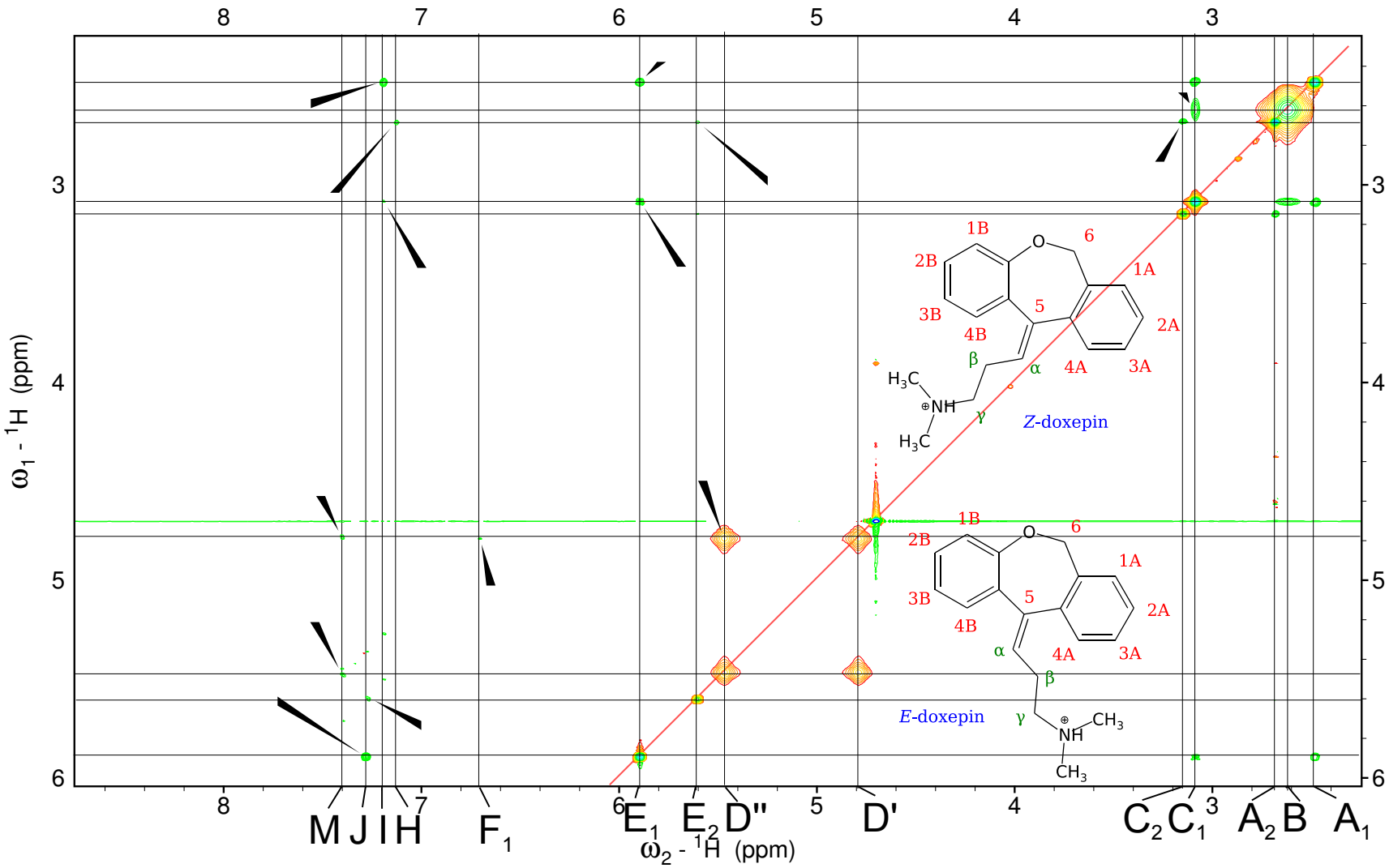
1D ^{1}H of doxepin in D_2O



1D ^{1}H of doxepin in D_2O



NOESY 700ms of doxepin in D_2O



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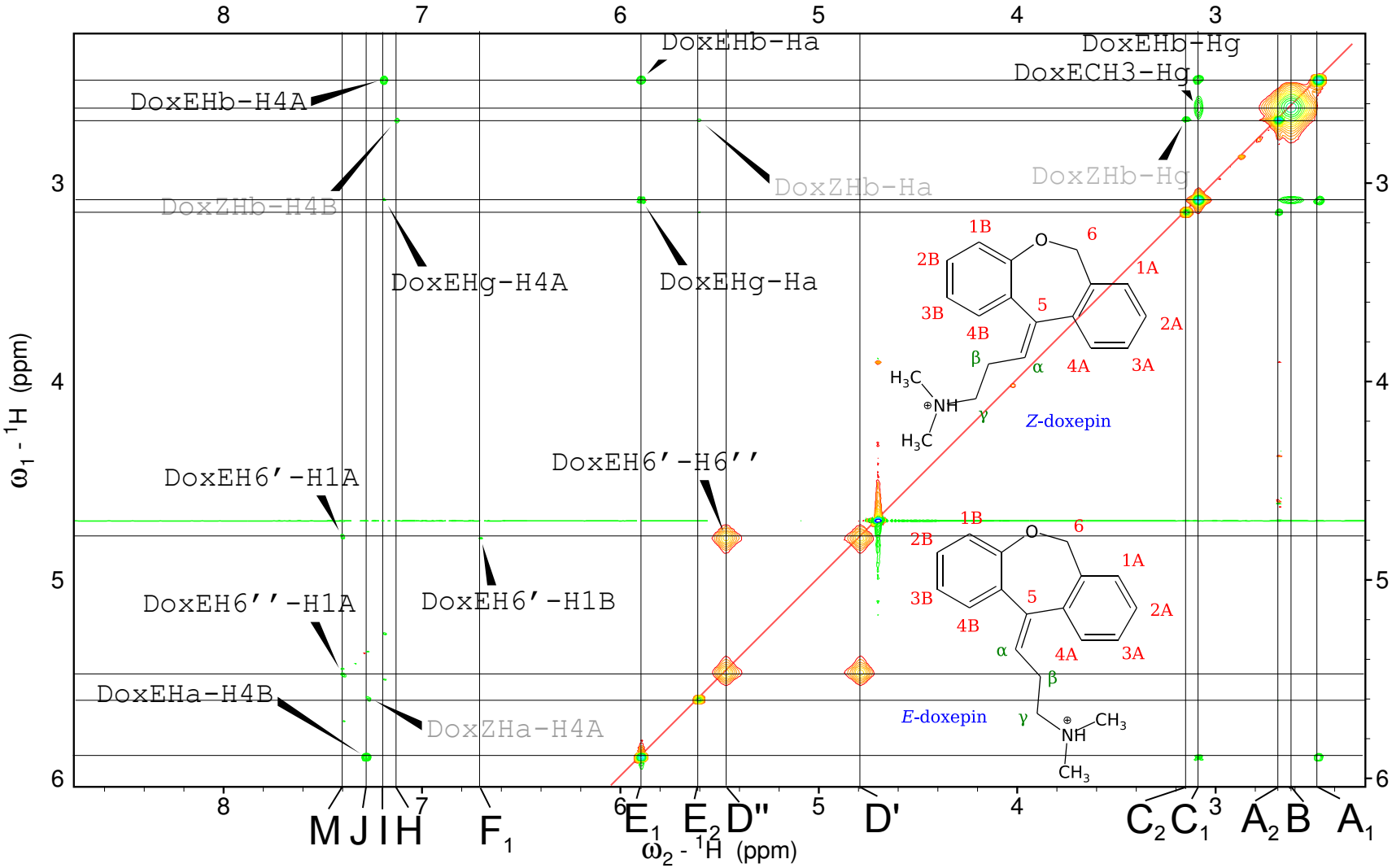

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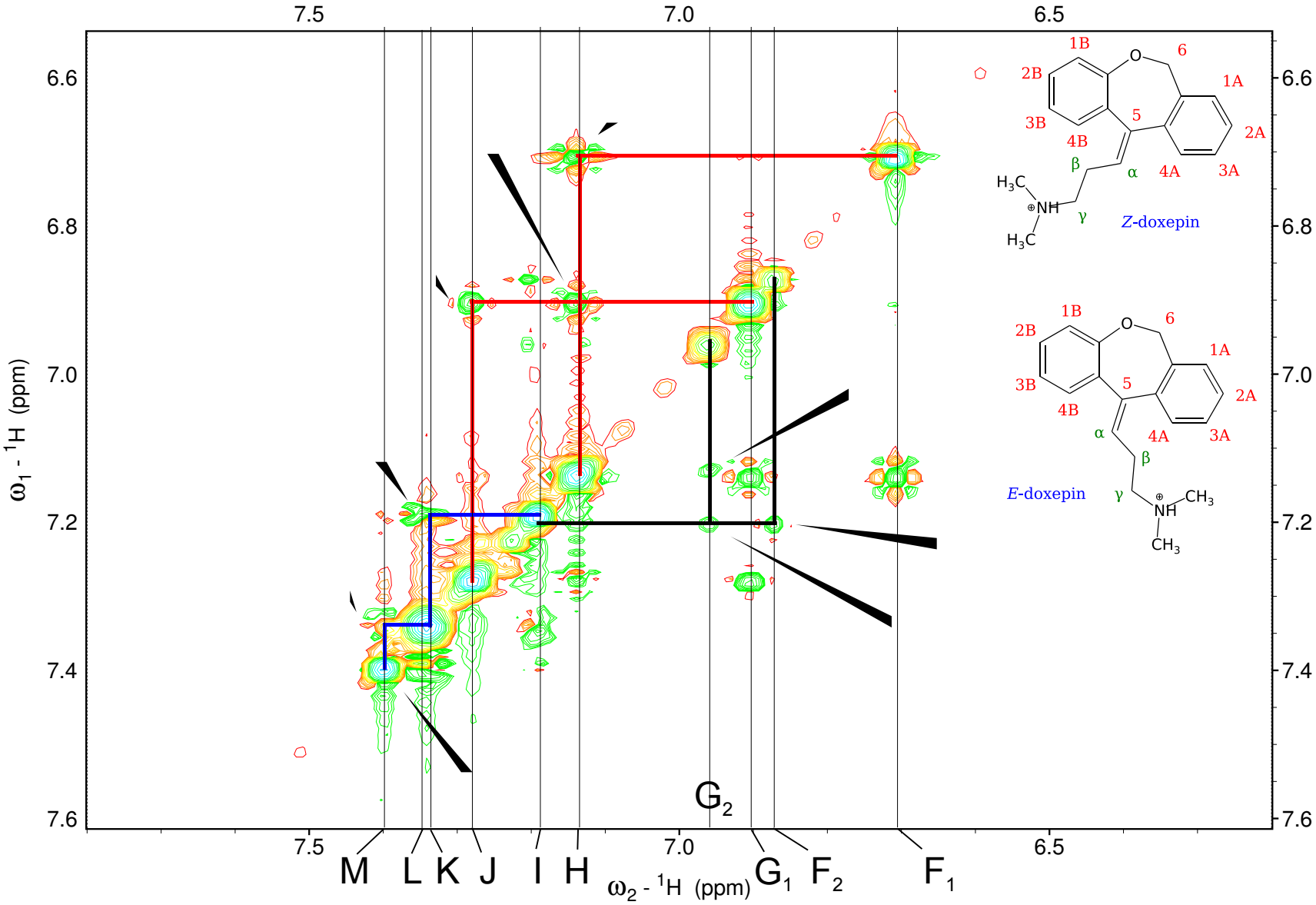

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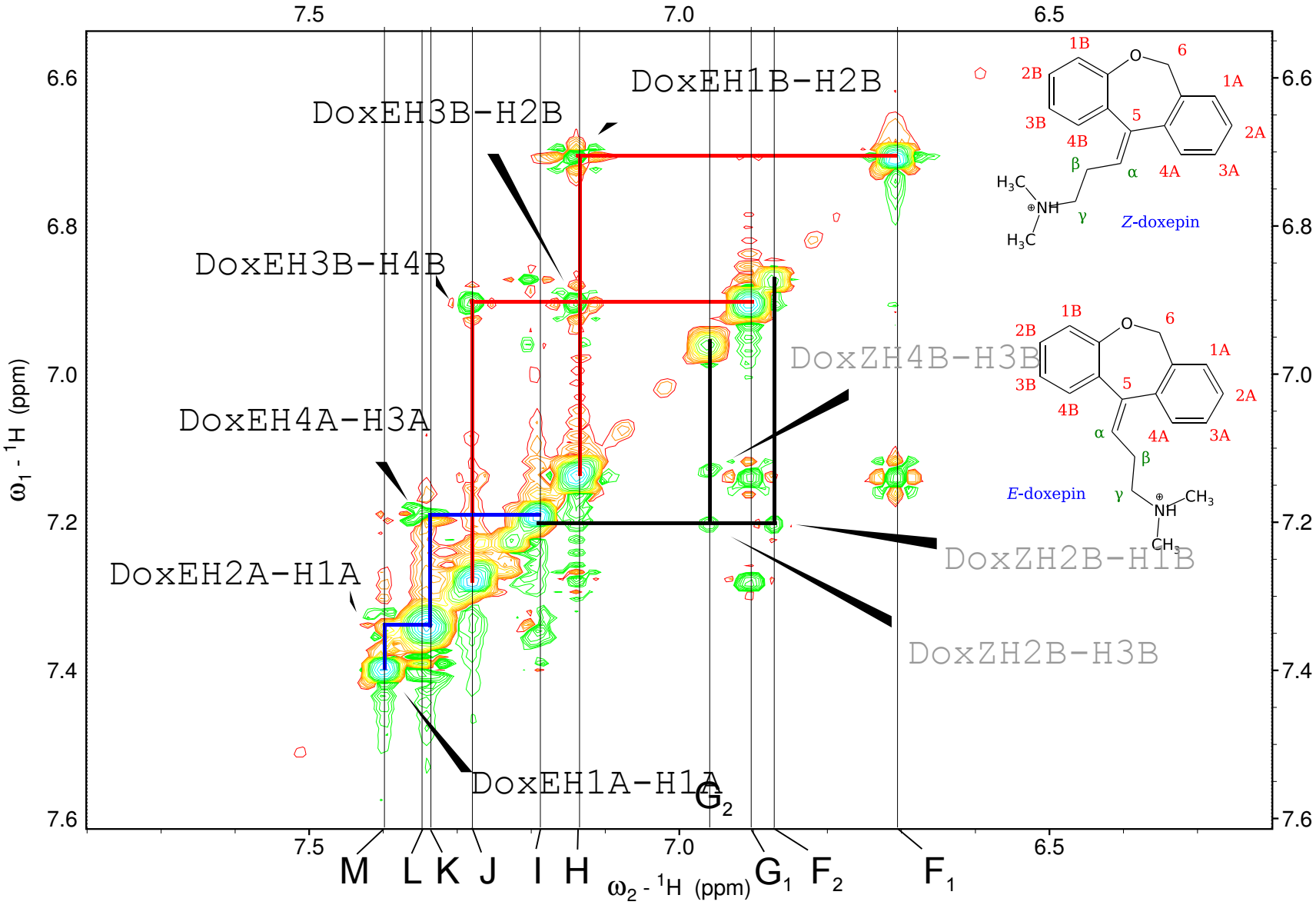
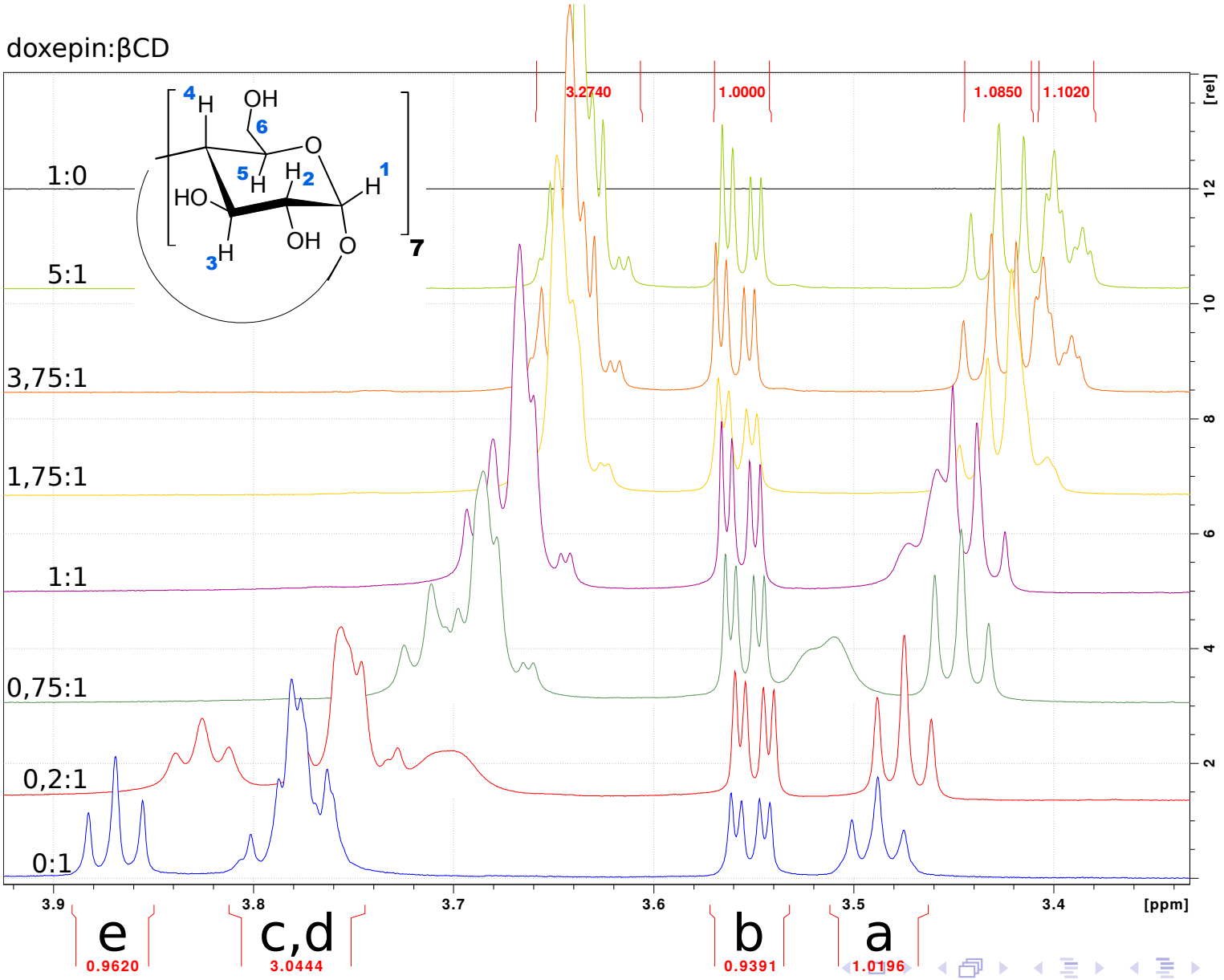


1D ^{1}H NMR titration: β -cyclodextrin



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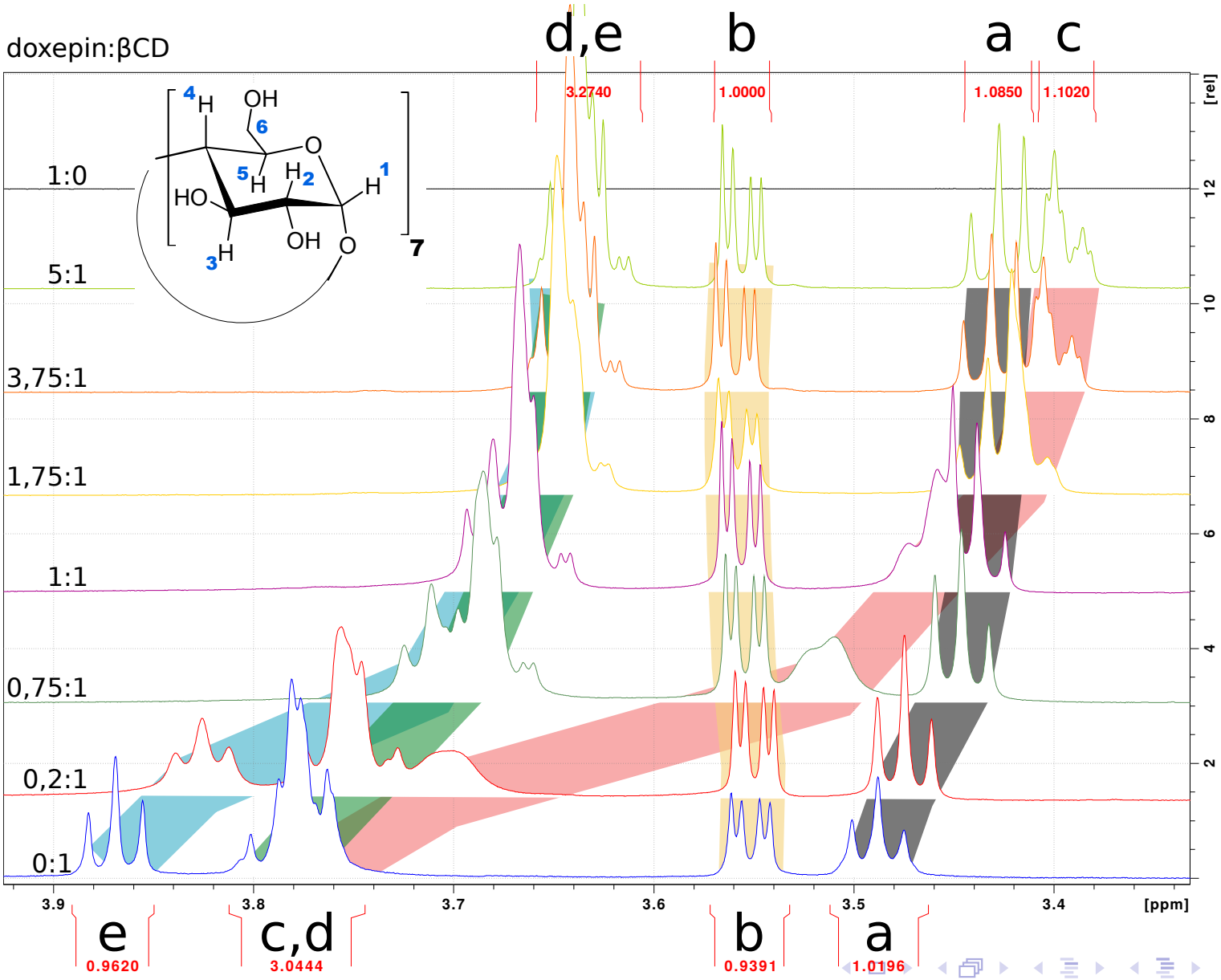

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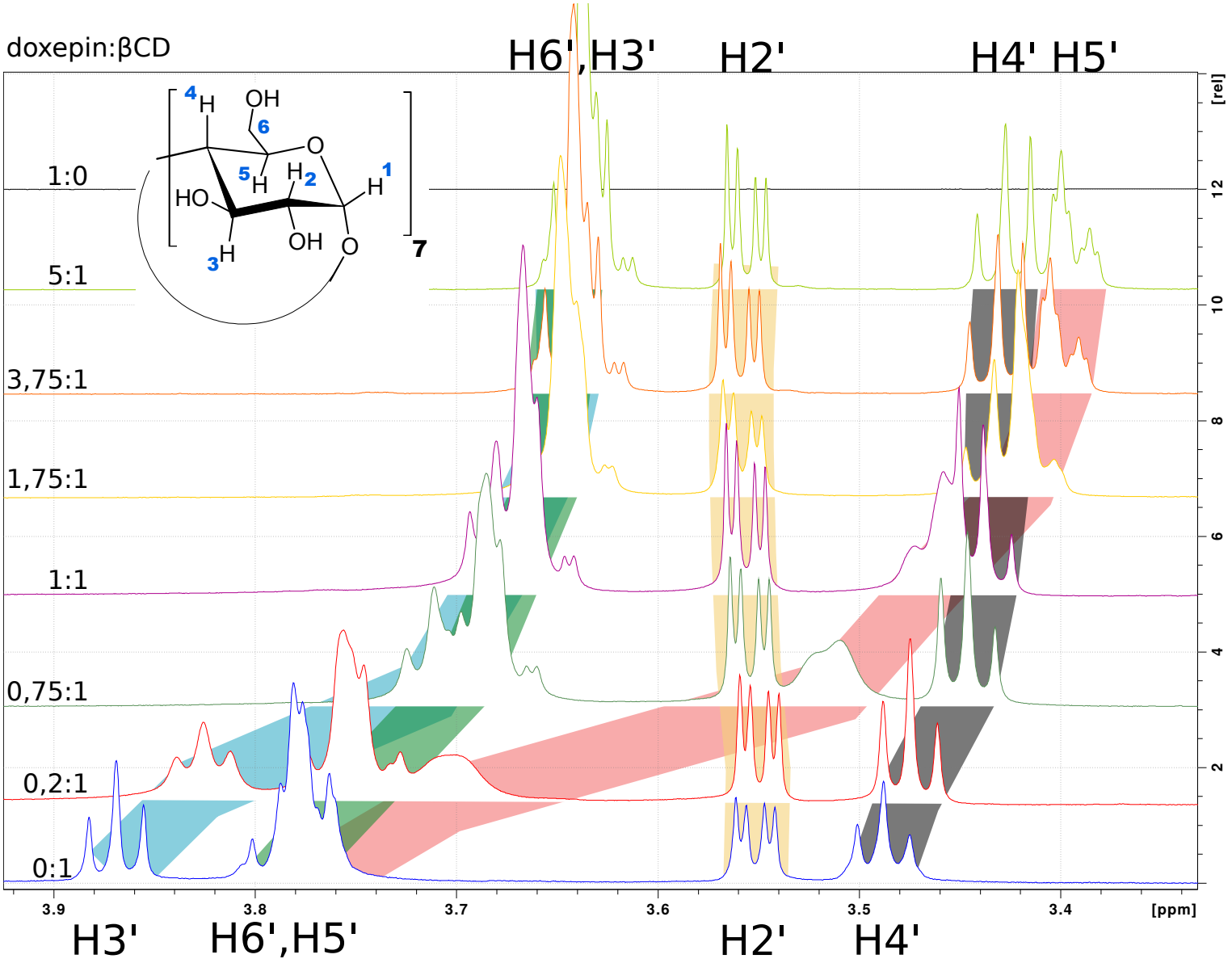
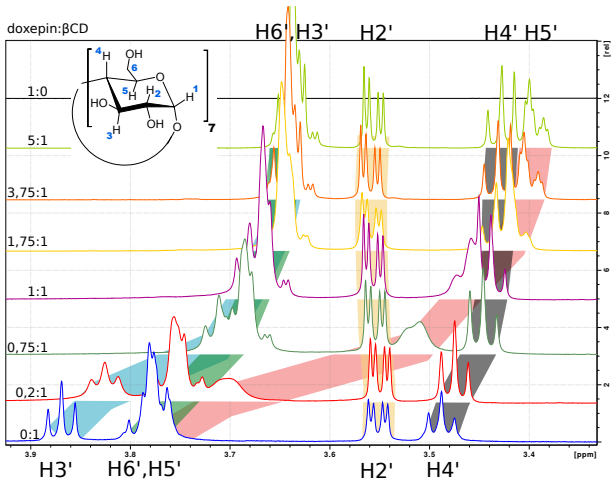
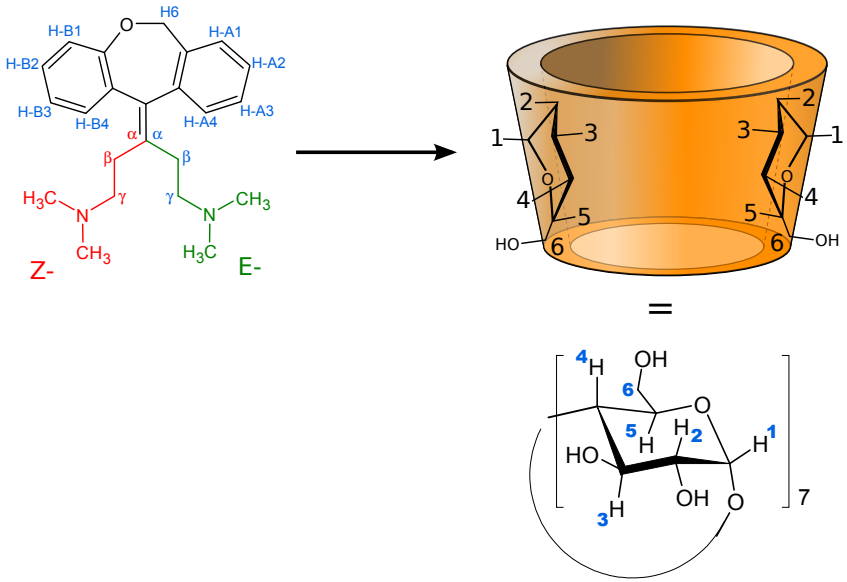


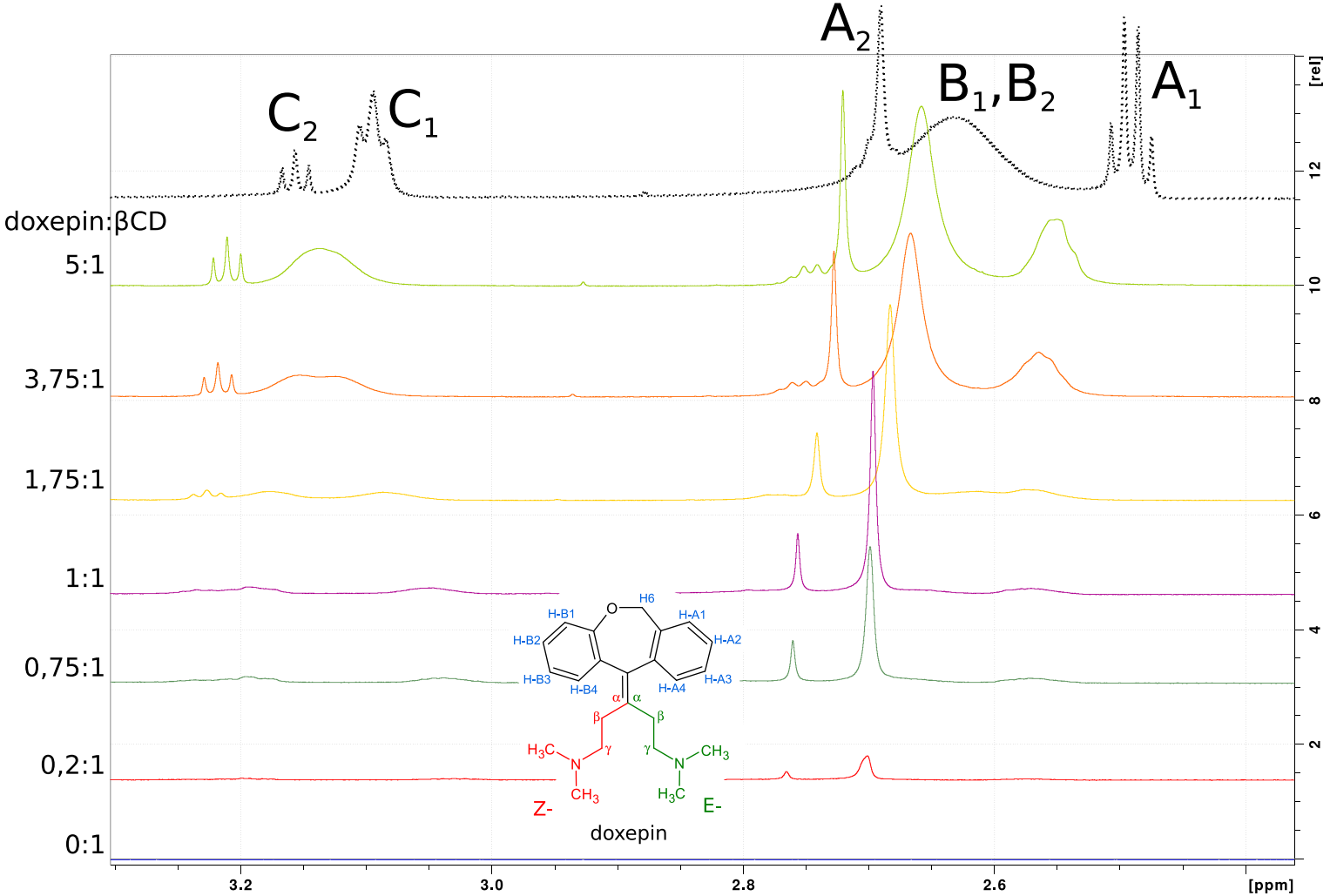
1D ^{1}H NMR titration: β -cyclodextrin

Inner β-CD protons **H3'** and **H5'** are dominantly exposed to shielding induced by ring current of aromatic ring.

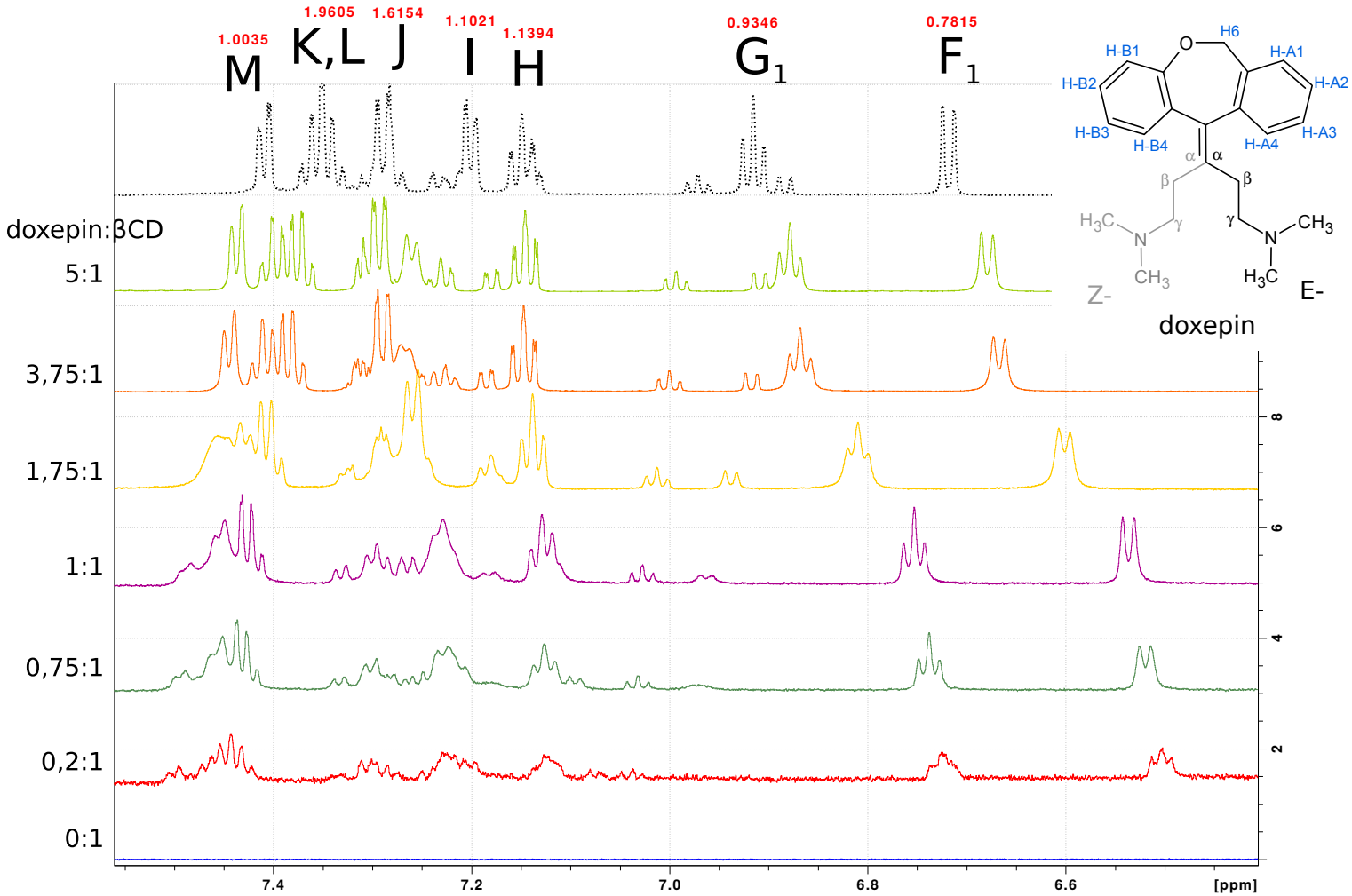




1D ^{1}H NMR titration: doxepin

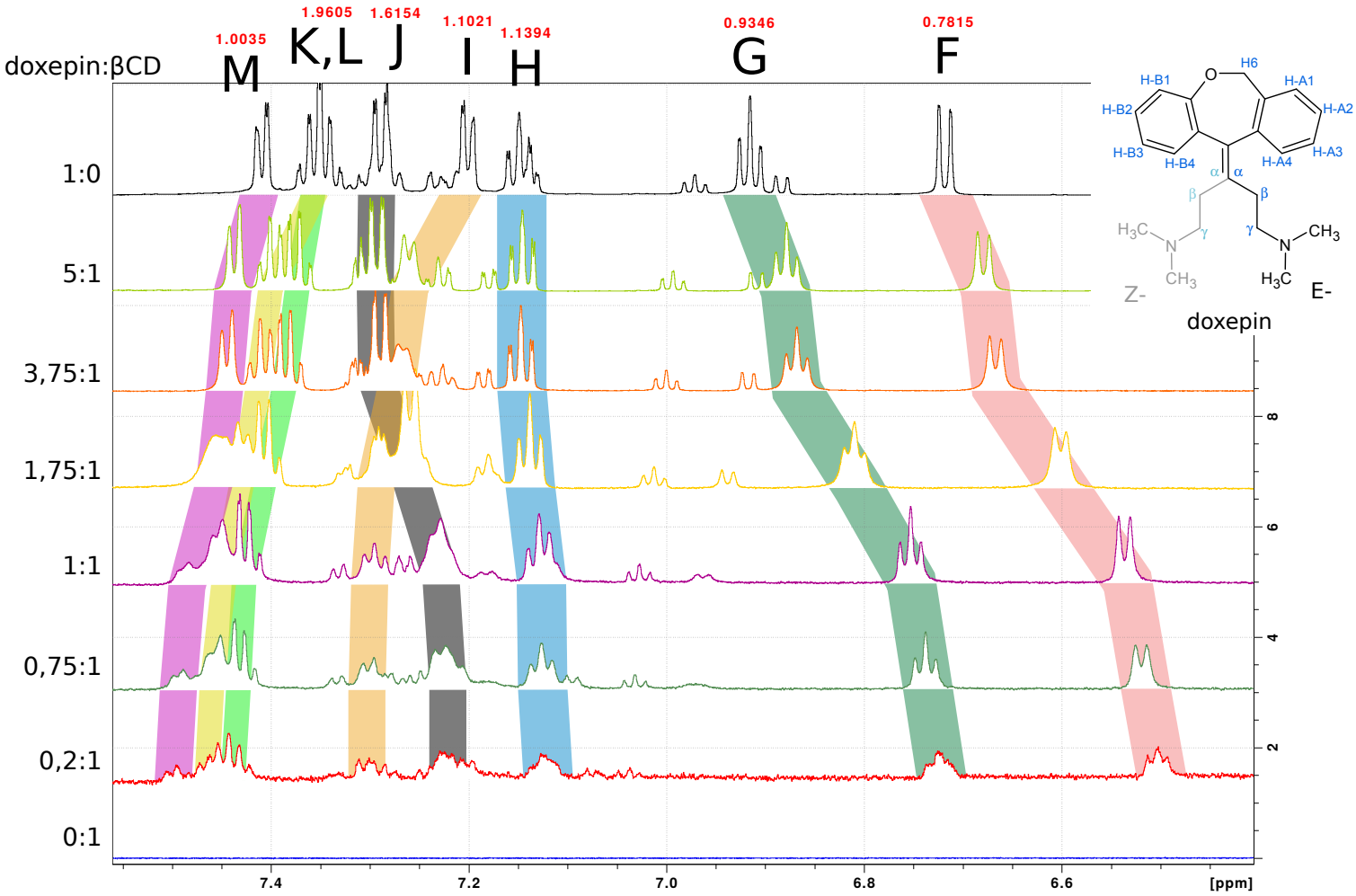


1D ^{1}H NMR titration: doxepin



Find the most perturbed resonances and estimate the time regime of interaction:

1D ^{1}H NMR titration: doxepin



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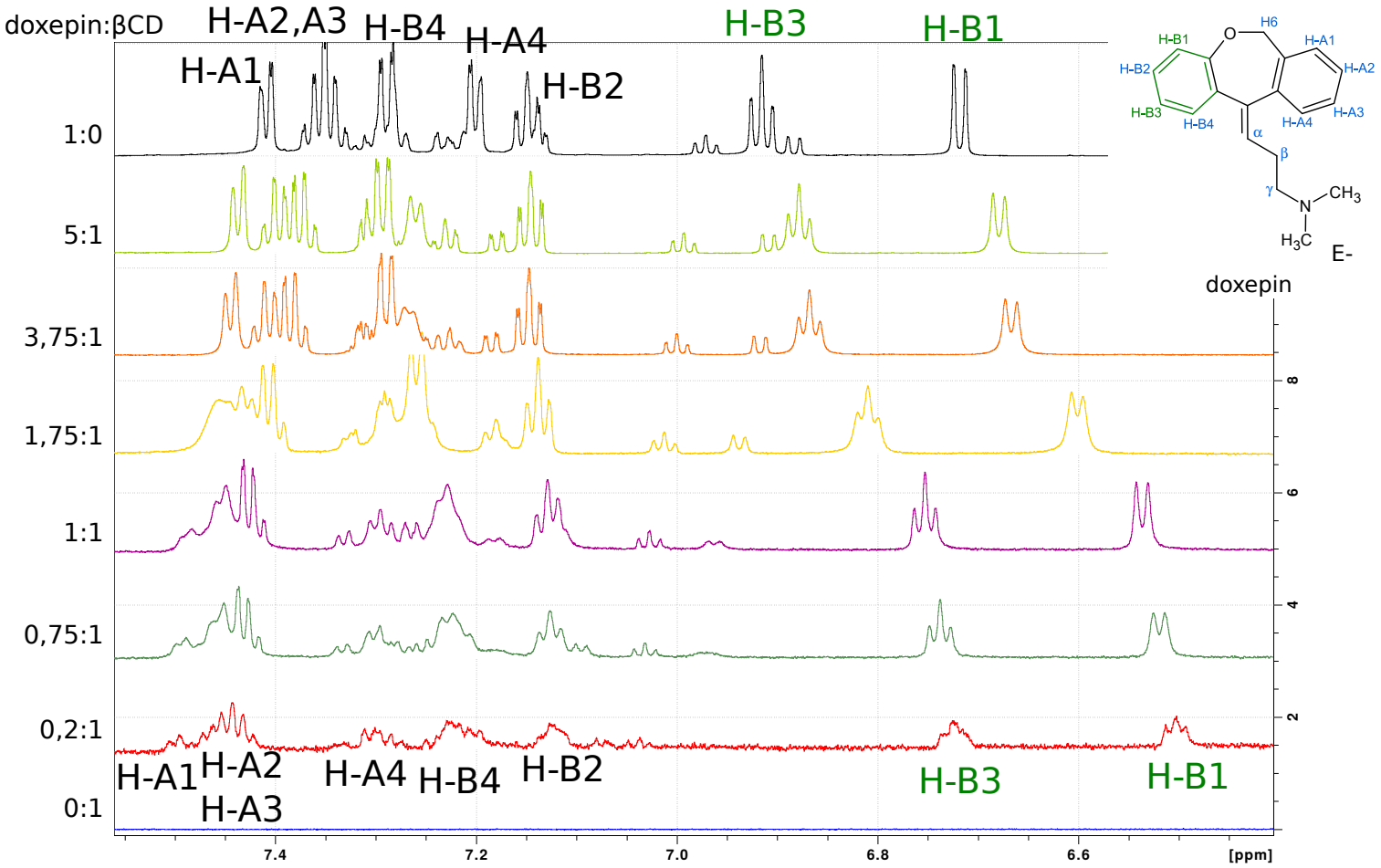
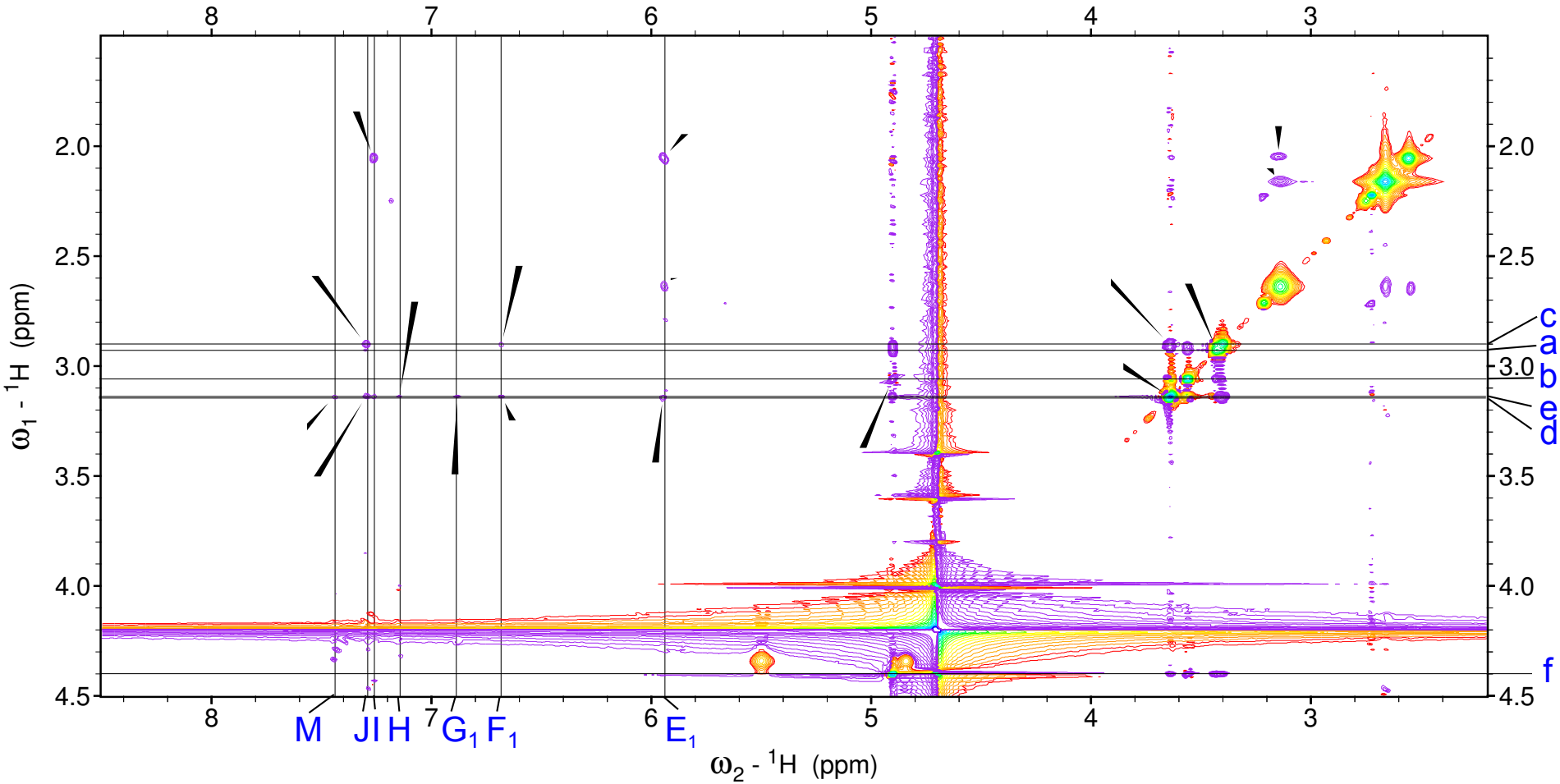


One set of broaden signals of doxepin $\Rightarrow$ fast/intermediate rate of exchange.

ROESY 300ms - doxepin:β -cyclodextrin=5:1



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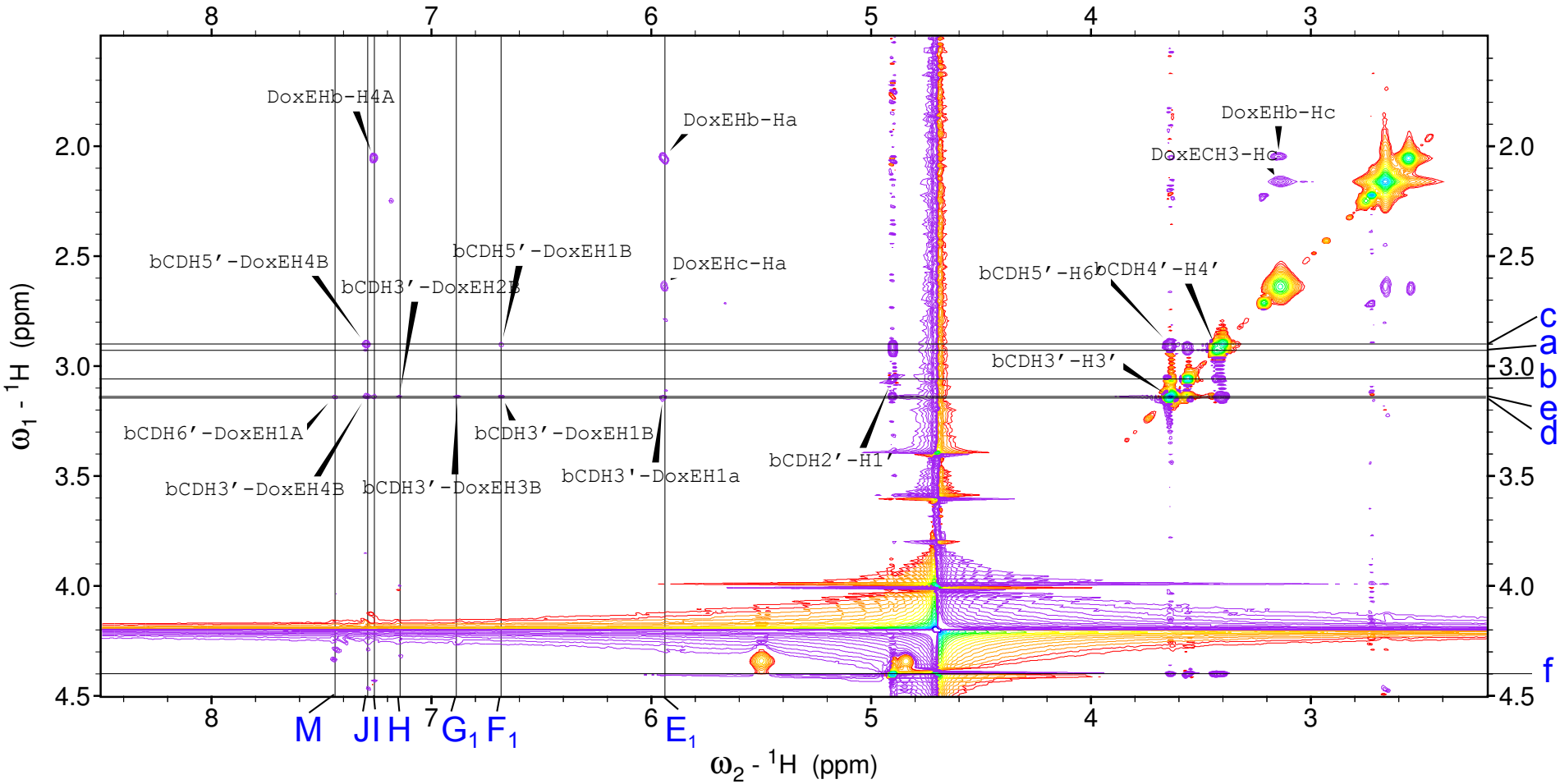
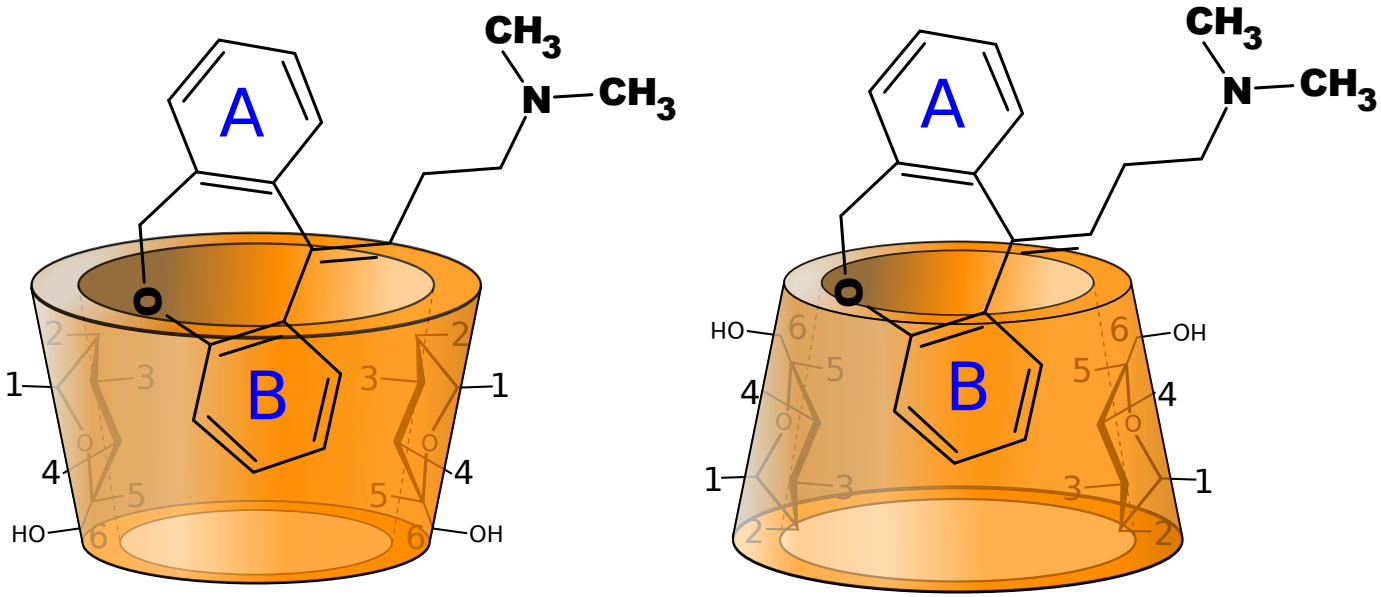


Proposed orientation of doxepine in β -cyclodextrin



1D ^{1}H NMR titration: determination of stoichiometry/K_a

$$R + L \leftrightarrows RL$$

$$K_a = \frac{[RL]}{[R][L]} = \frac{[RL]}{(R_0 - [RL])(L_0 - [RL])}$$

Upon titration we are gradually changing L_0 concentration:

$$\delta_R = [RL]\delta_{R_{bound}} + (R_0 - [RL])\delta_{R_{free}}$$

$$\Delta\delta_R = \delta_R - \delta_{R_{free}}$$

Titration curve of $\Delta\delta$(H1') as a function of increasing concentration of doxepine.