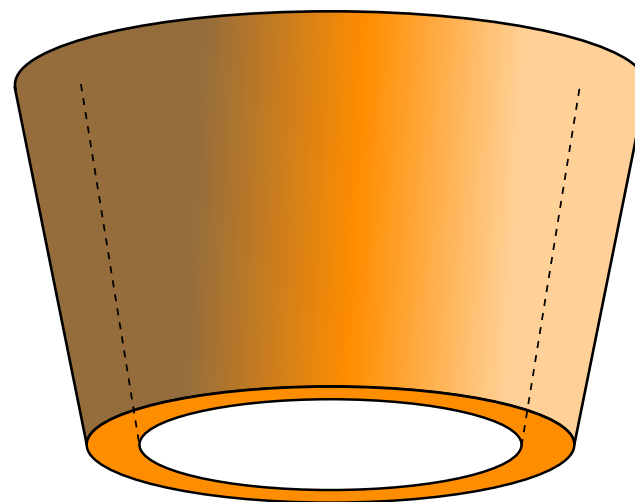
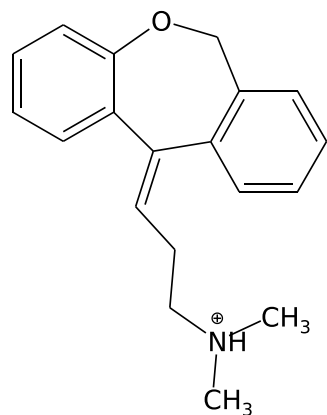
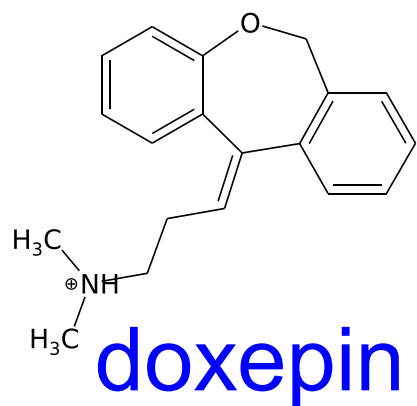


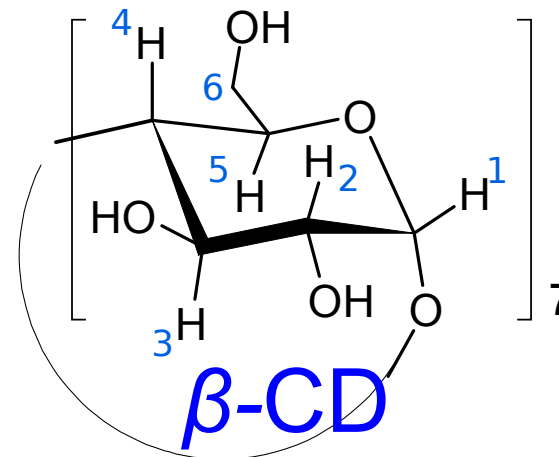
Revealing ligand-receptor interaction NMR titration

Jan Novotný

Introduction of reacting partners

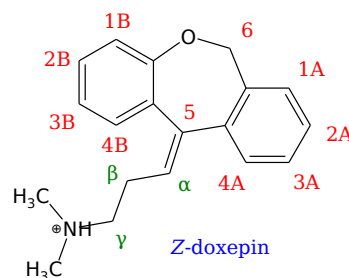
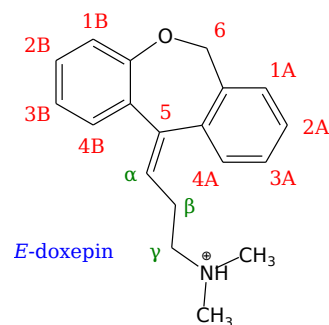


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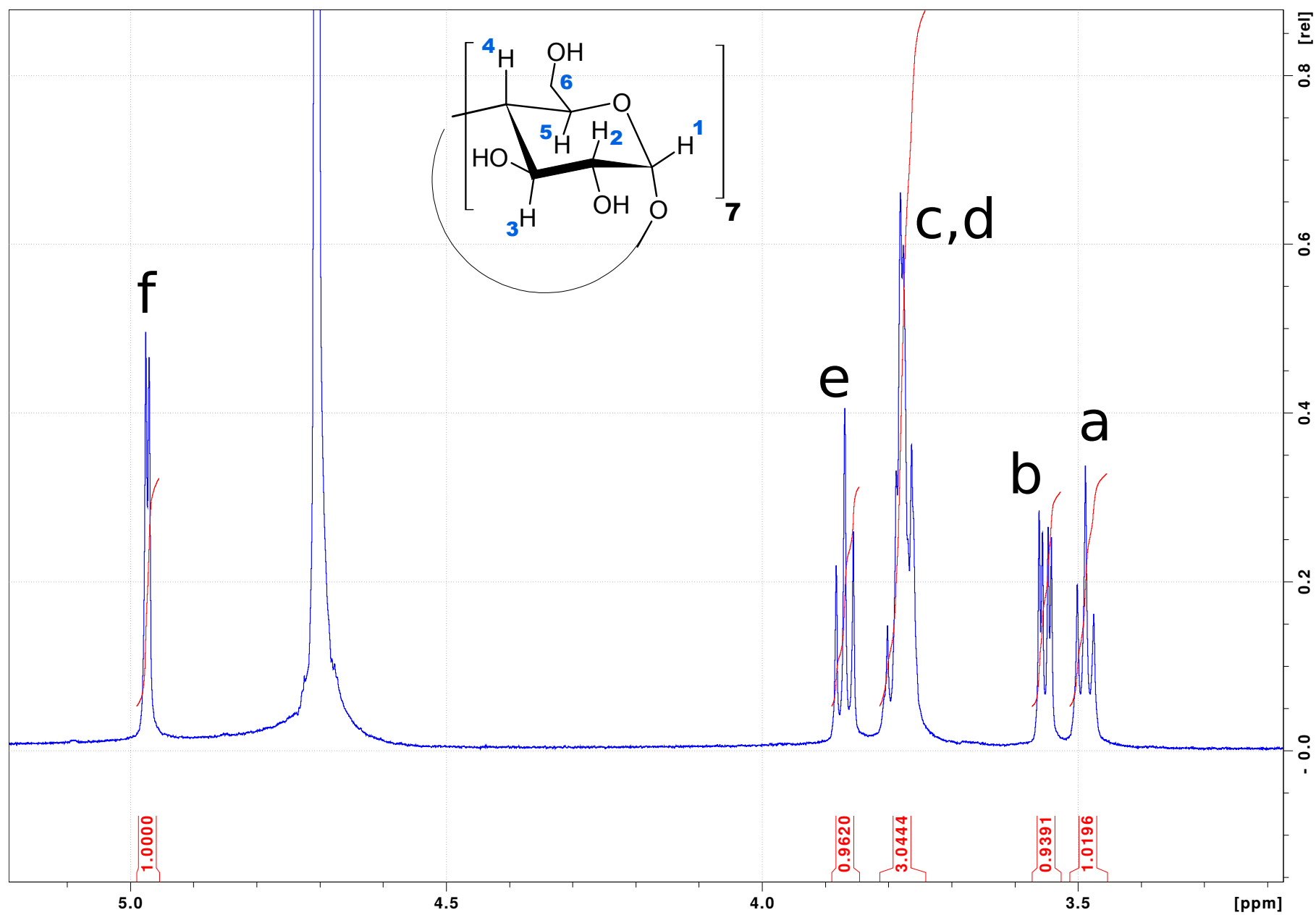


Recommended procedure

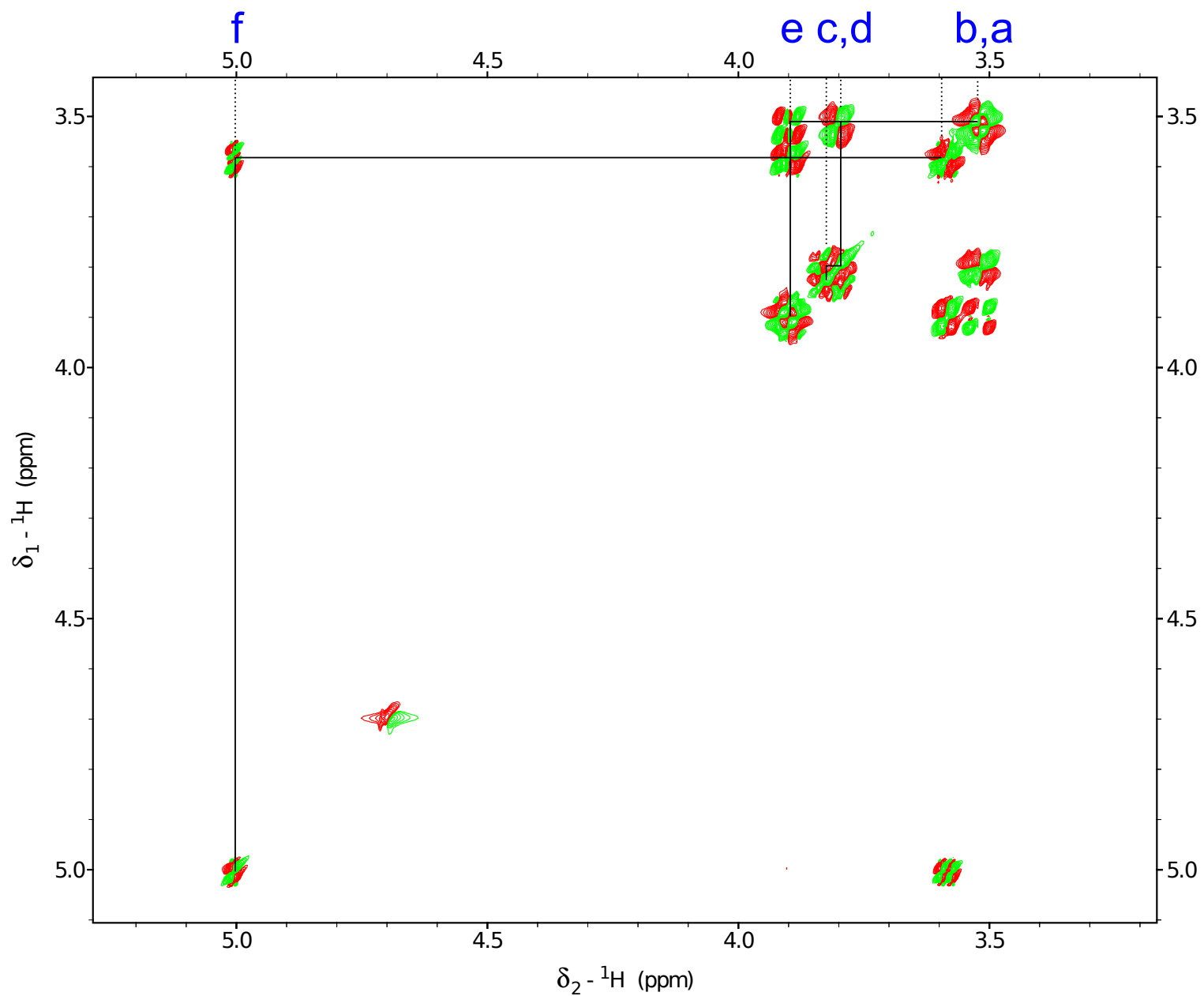
- 1 Assignment of free receptor - β -cyklodextrine (1D ^1H , DQF-COSY)
- 2 Assignment of free ligand - doxepine (1D ^1H , DQF-COSY, NOESY)
 - Identification of proton resonances of ring A and B
 - Determination of major and minor conformation of doxepin
- 3 1D NMR titration - rearrangement of β -cyklodextrine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



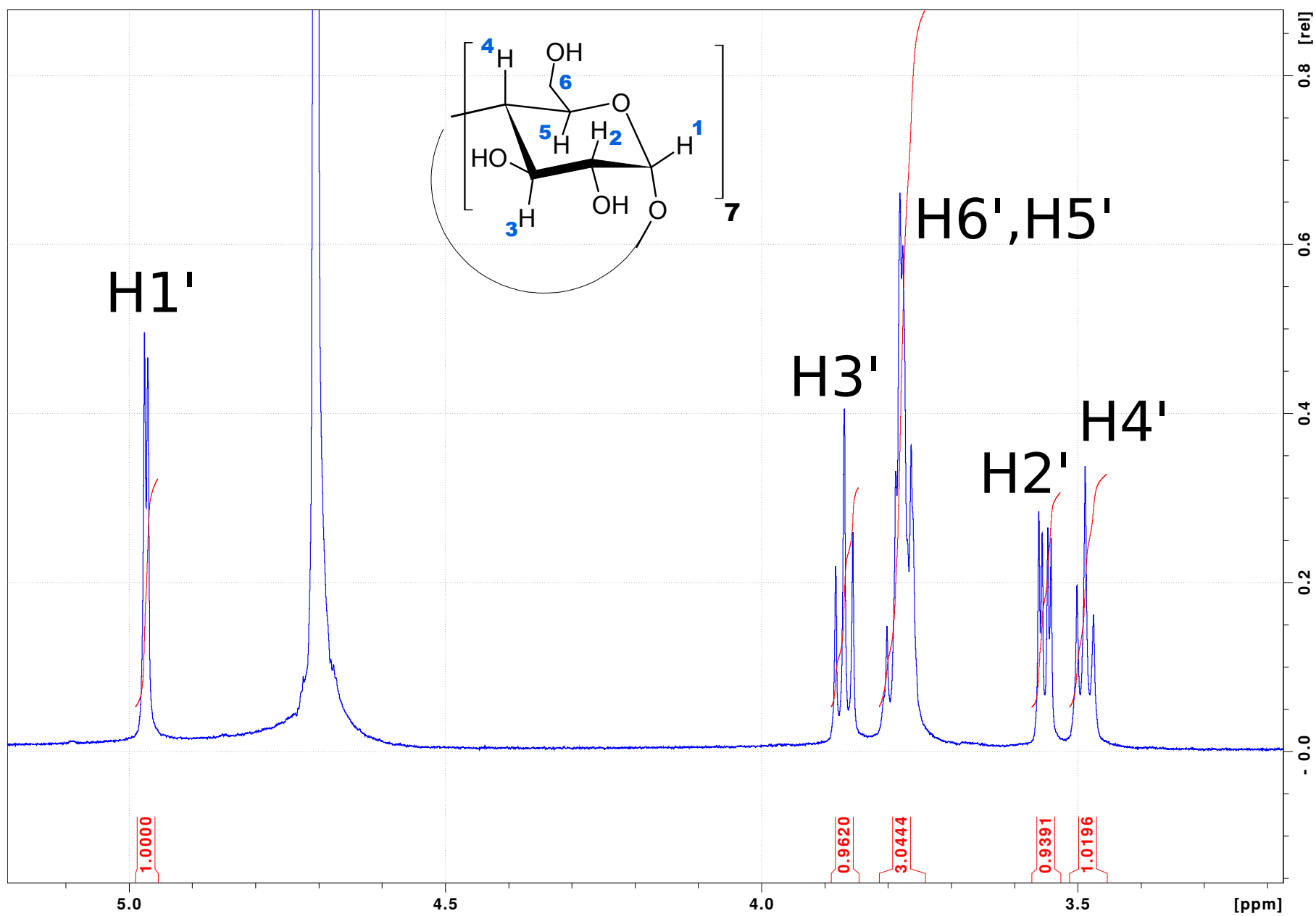
1D ^1H of β -cyklodextrine in D_2O



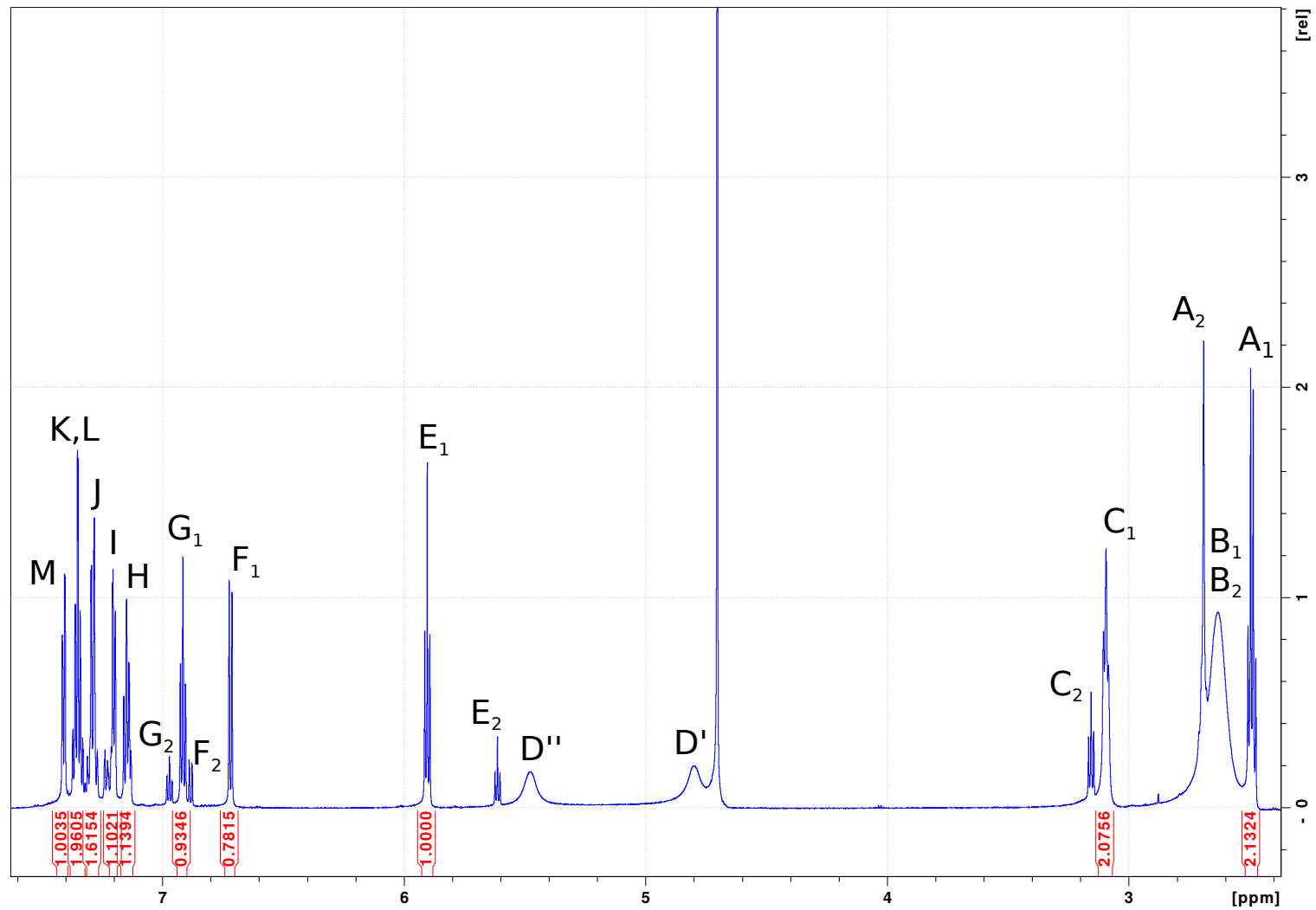
DQF-COSY of β -cyclodextrine in D_2O



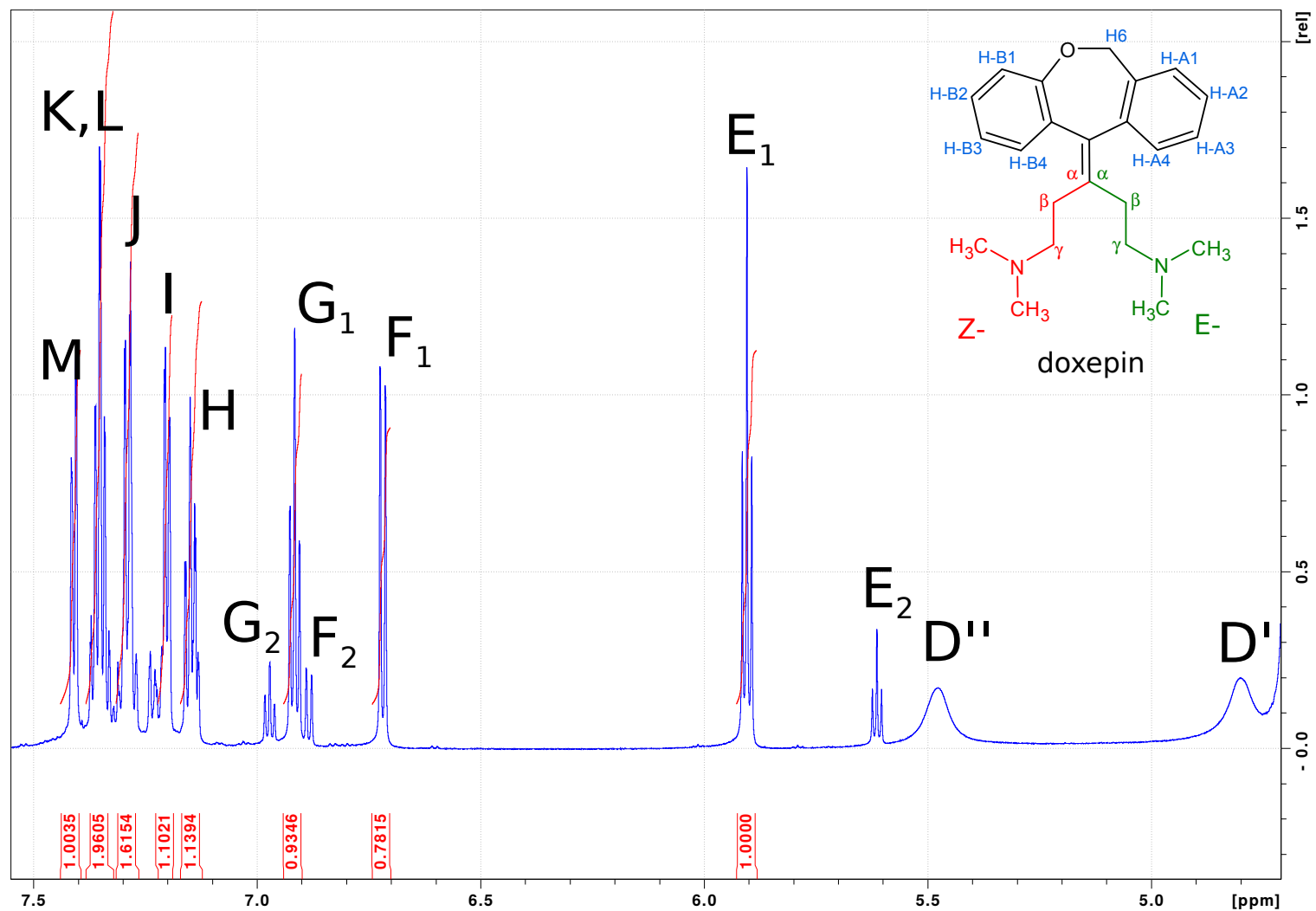
1D ^1H of β -cyklodextrine in D_2O



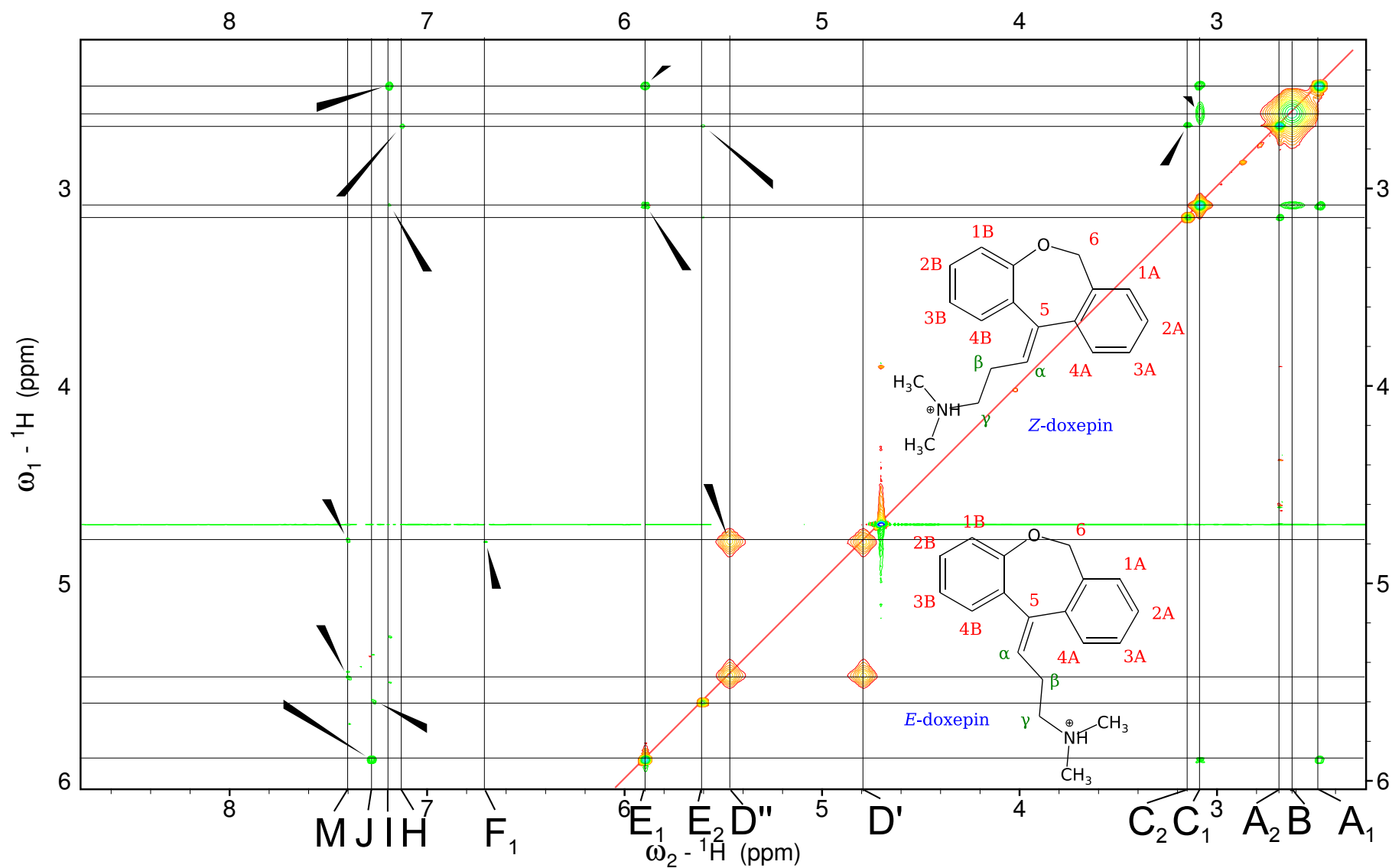
1D ^1H of doxepine in D_2O



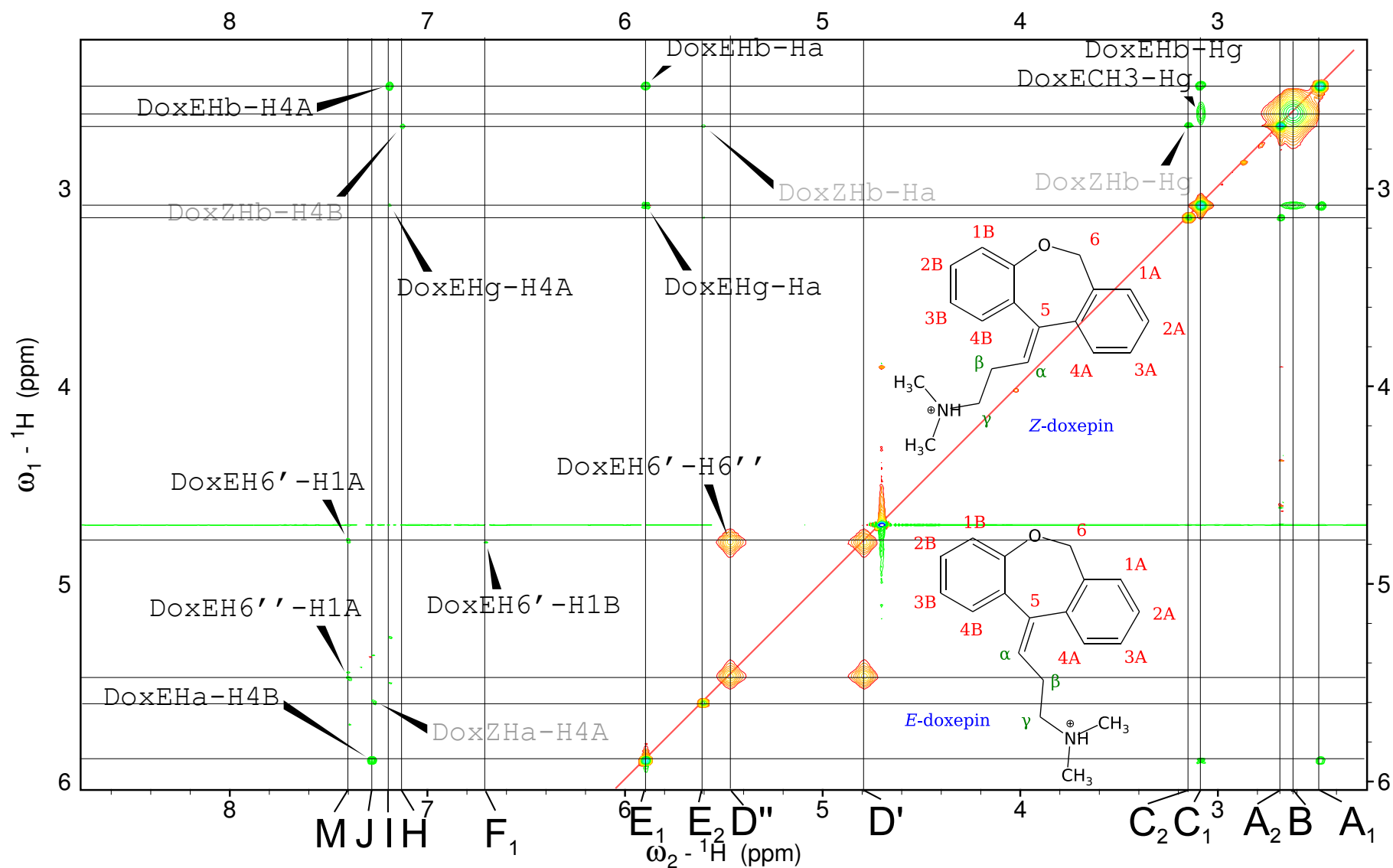
1D ^1H of doxepine in D_2O



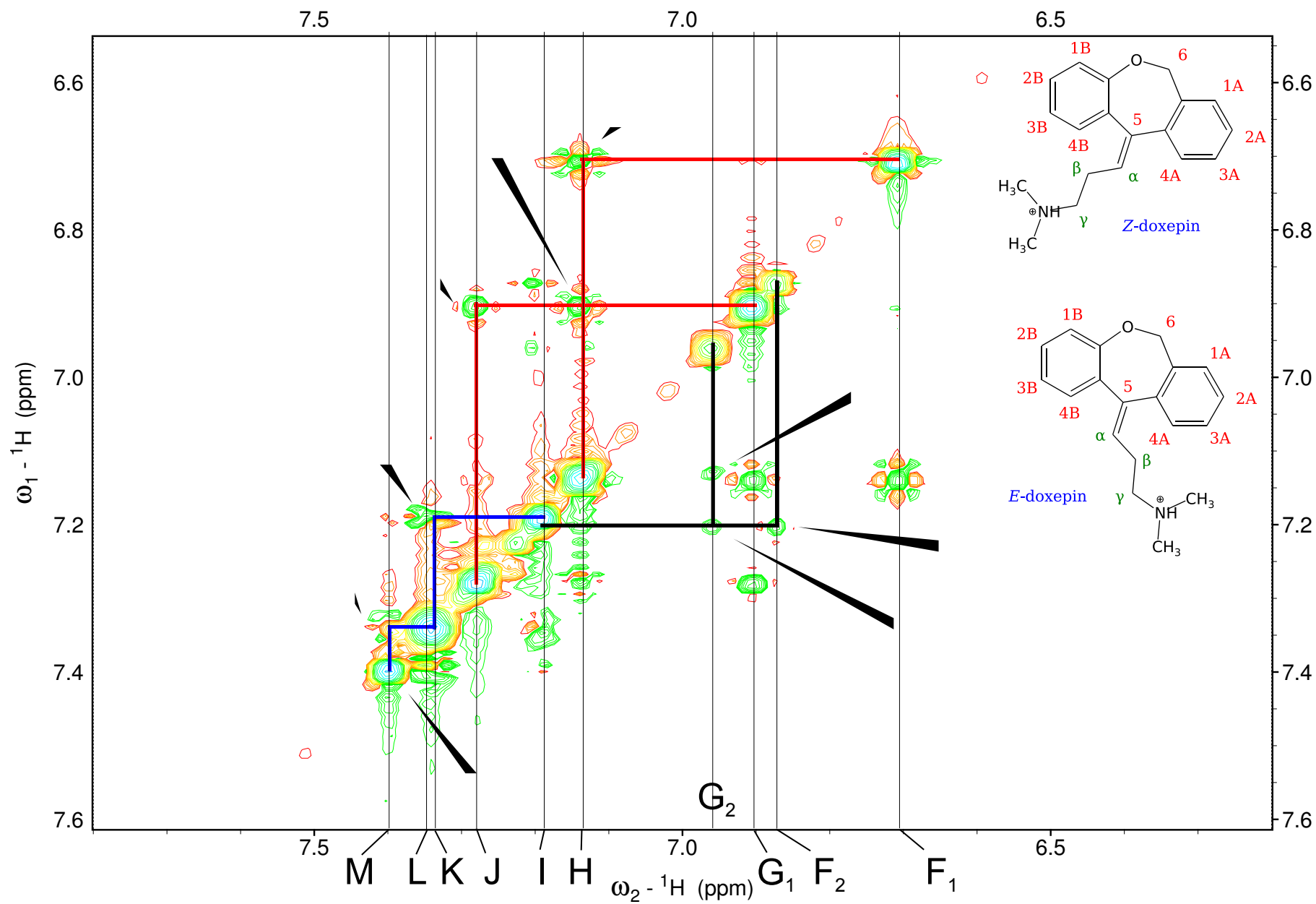
NOESY 700ms of doxepine in D₂O



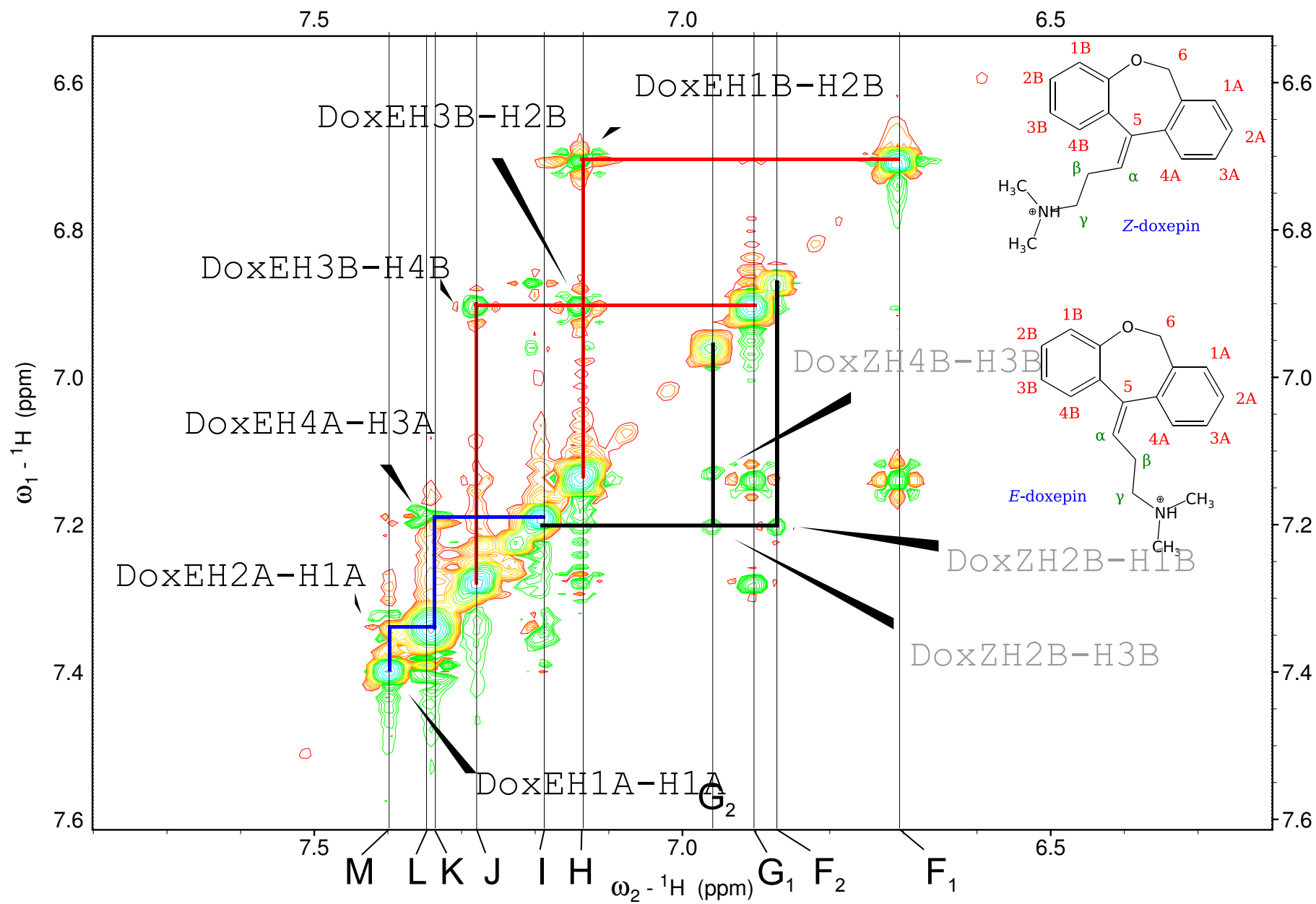
NOESY 700ms of doxepine in D₂O



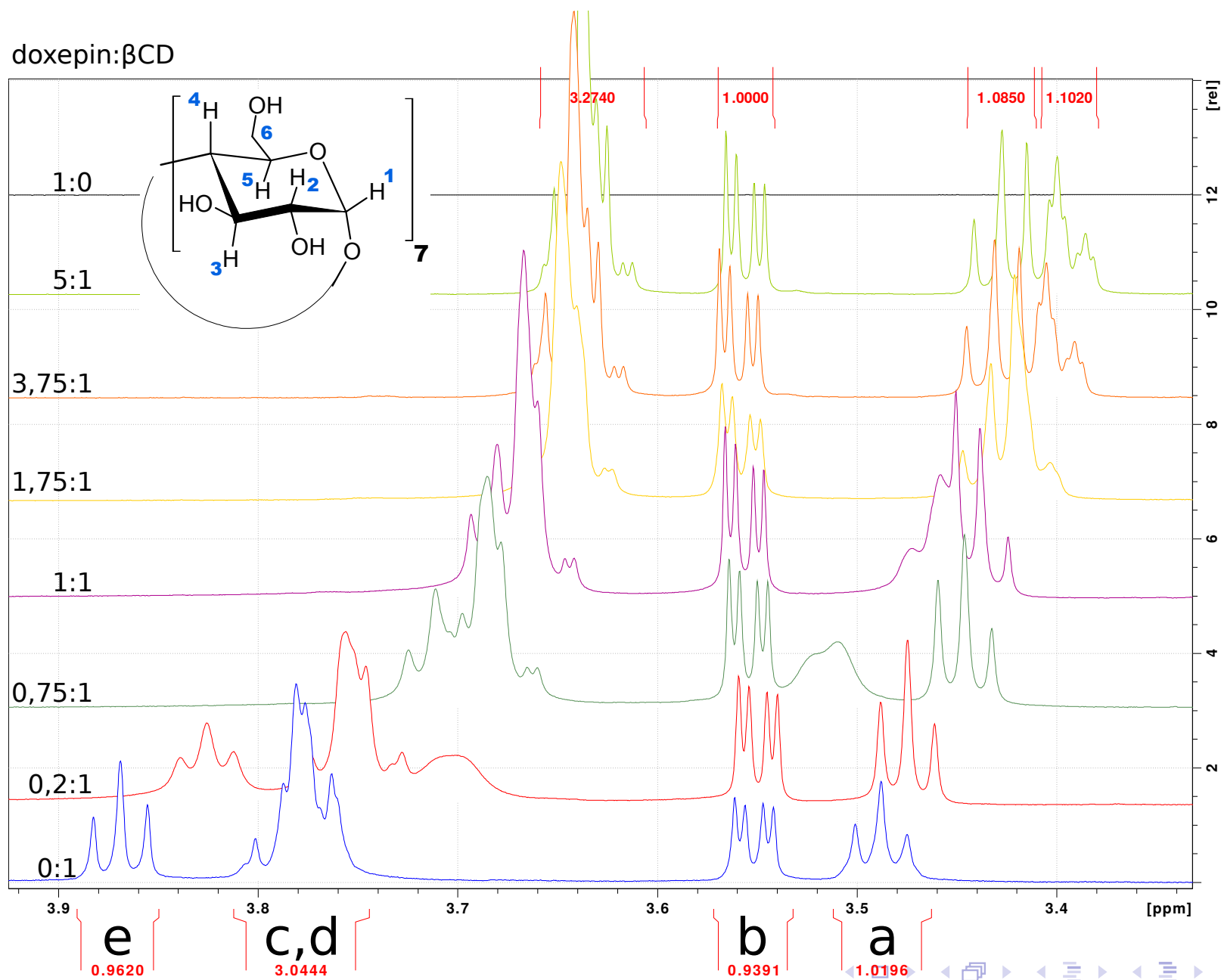
NOESY 700ms of doxepine in D₂O



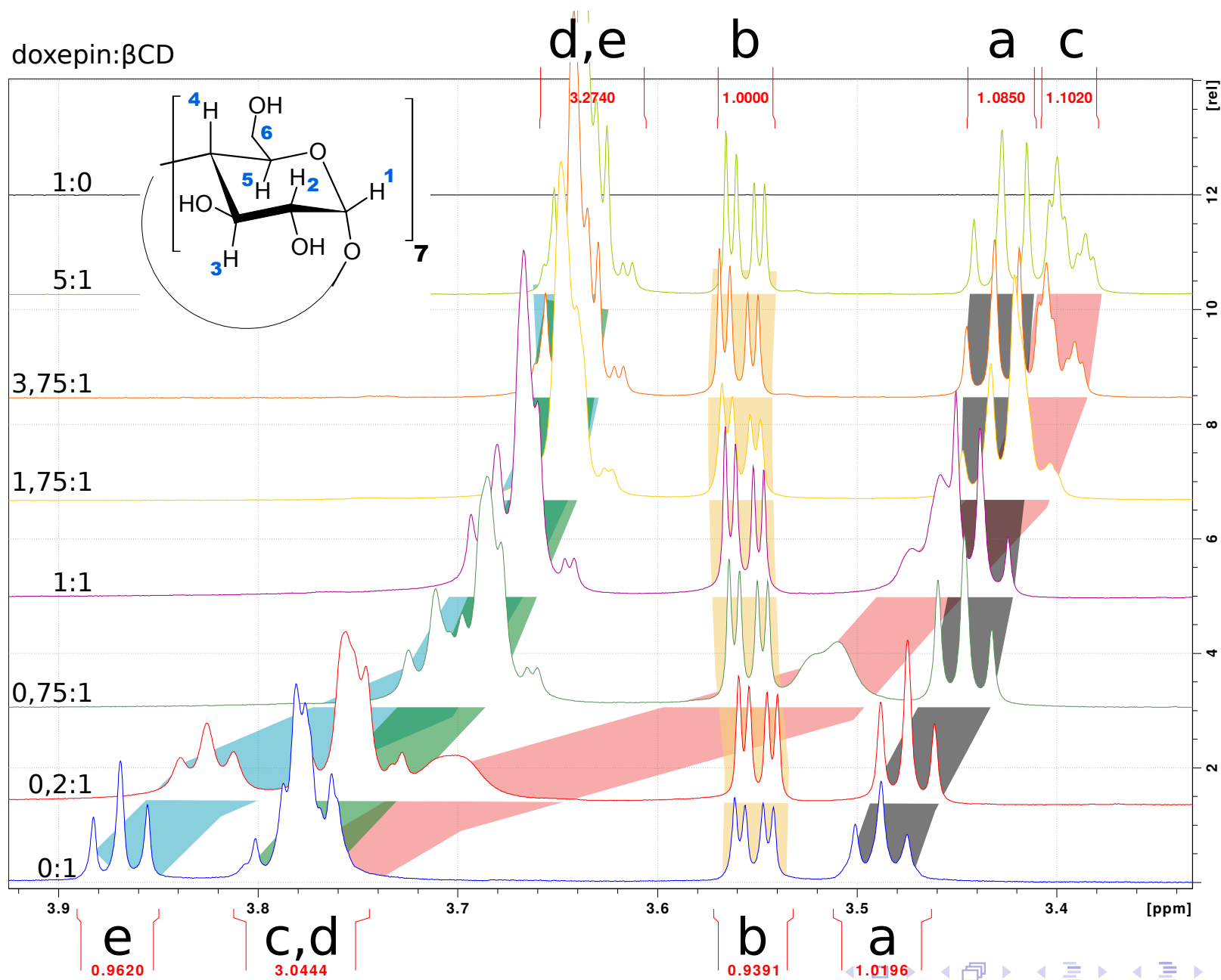
NOESY 700ms of doxepine in D₂O



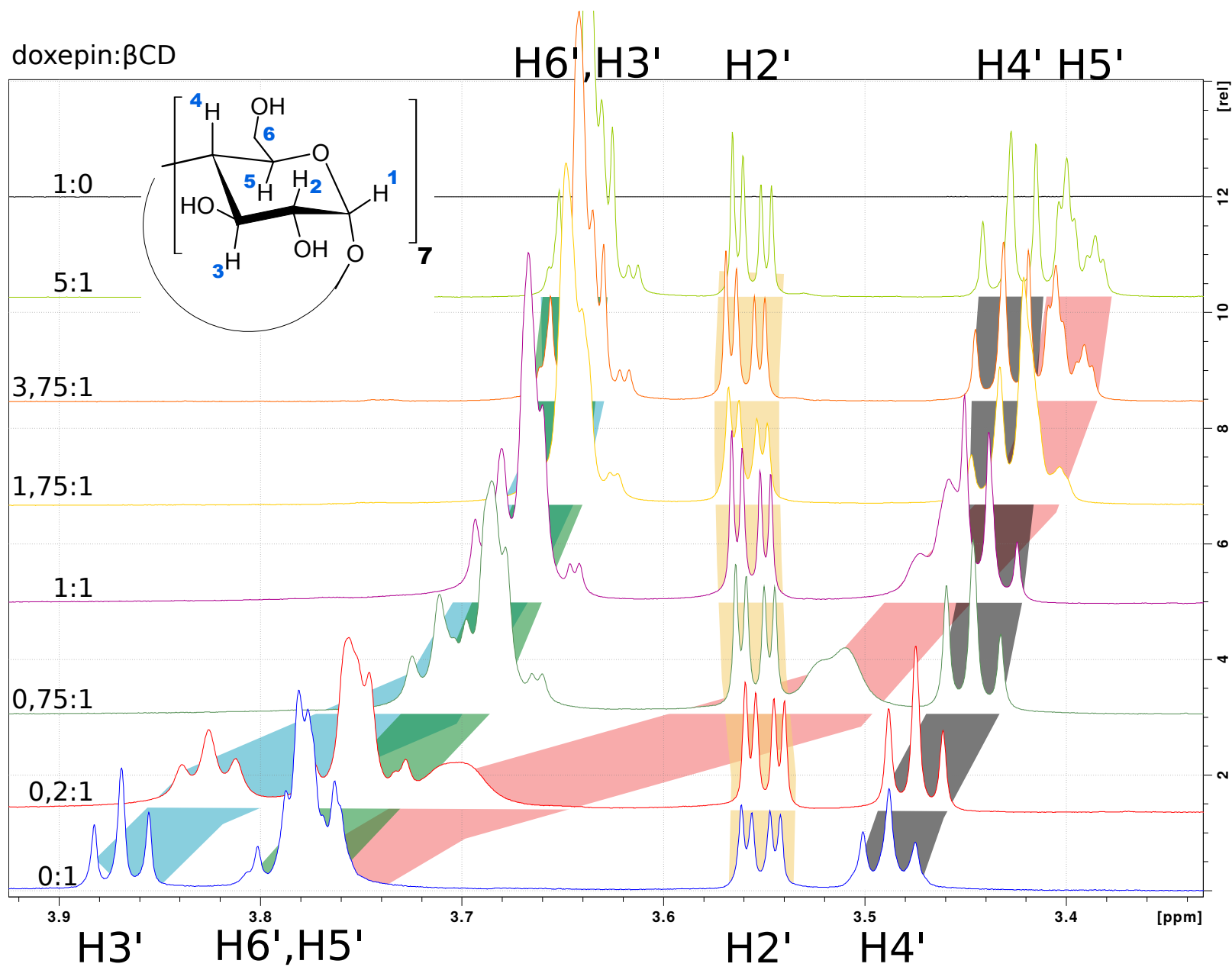
1D ^1H NMR titration: β -cyclodextrine



1D ^1H NMR titration: β -cyclodextrine

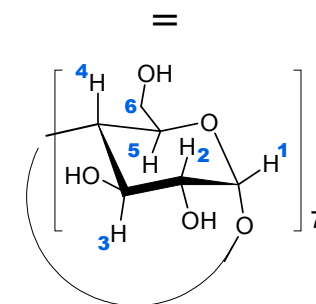
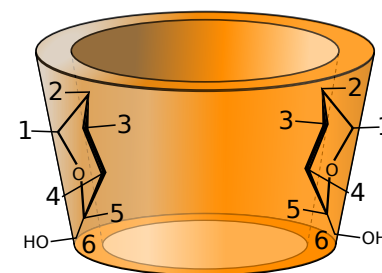
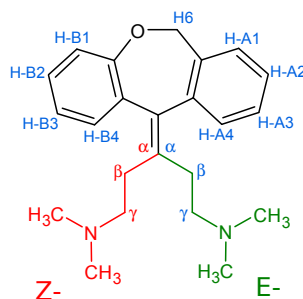
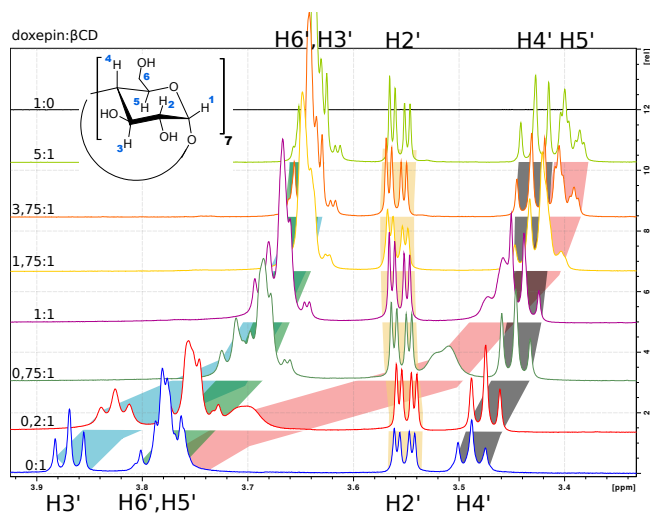


1D ^1H NMR titration: β -cyclodextrine

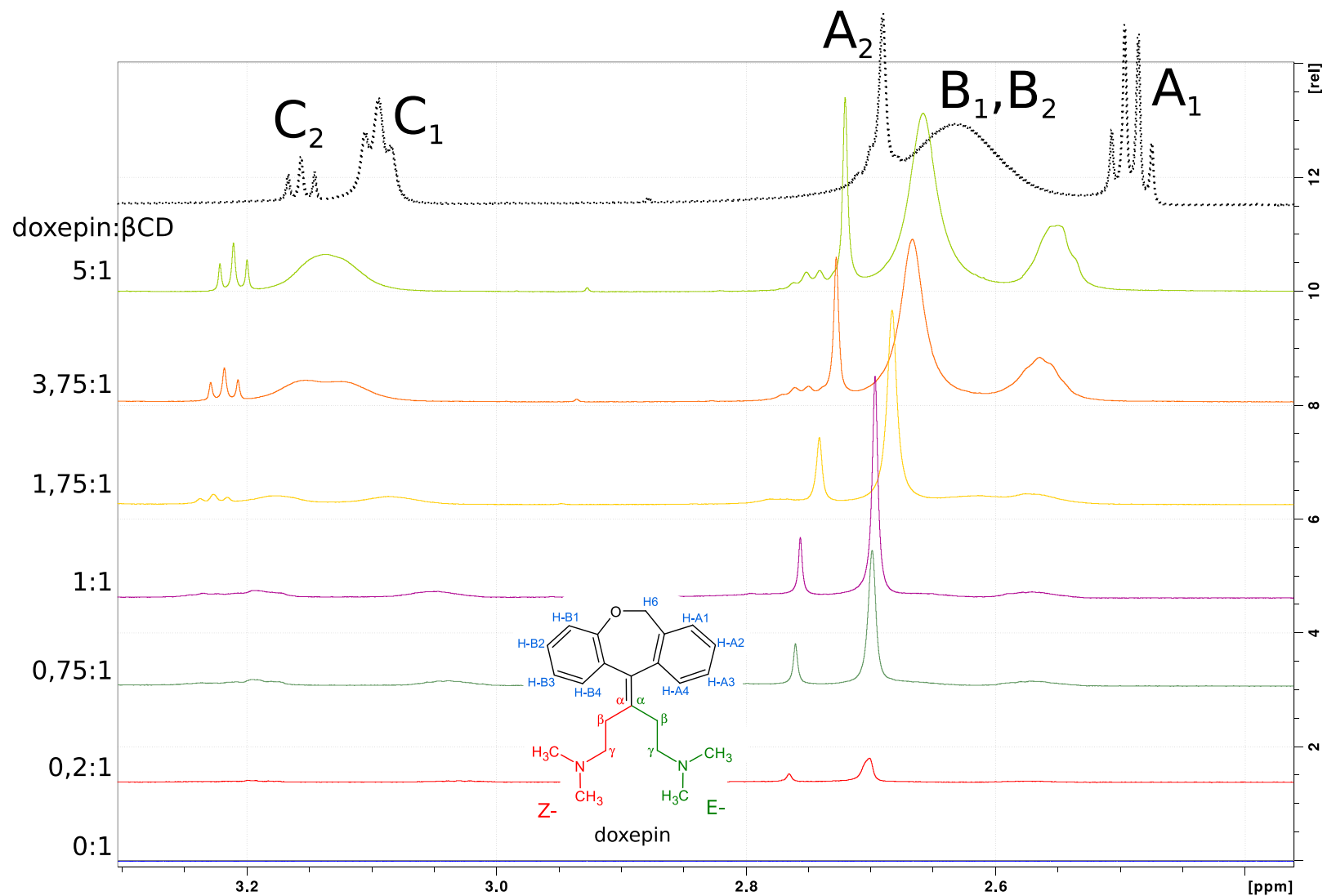


1D ^1H NMR titration: β -cyklodextrine

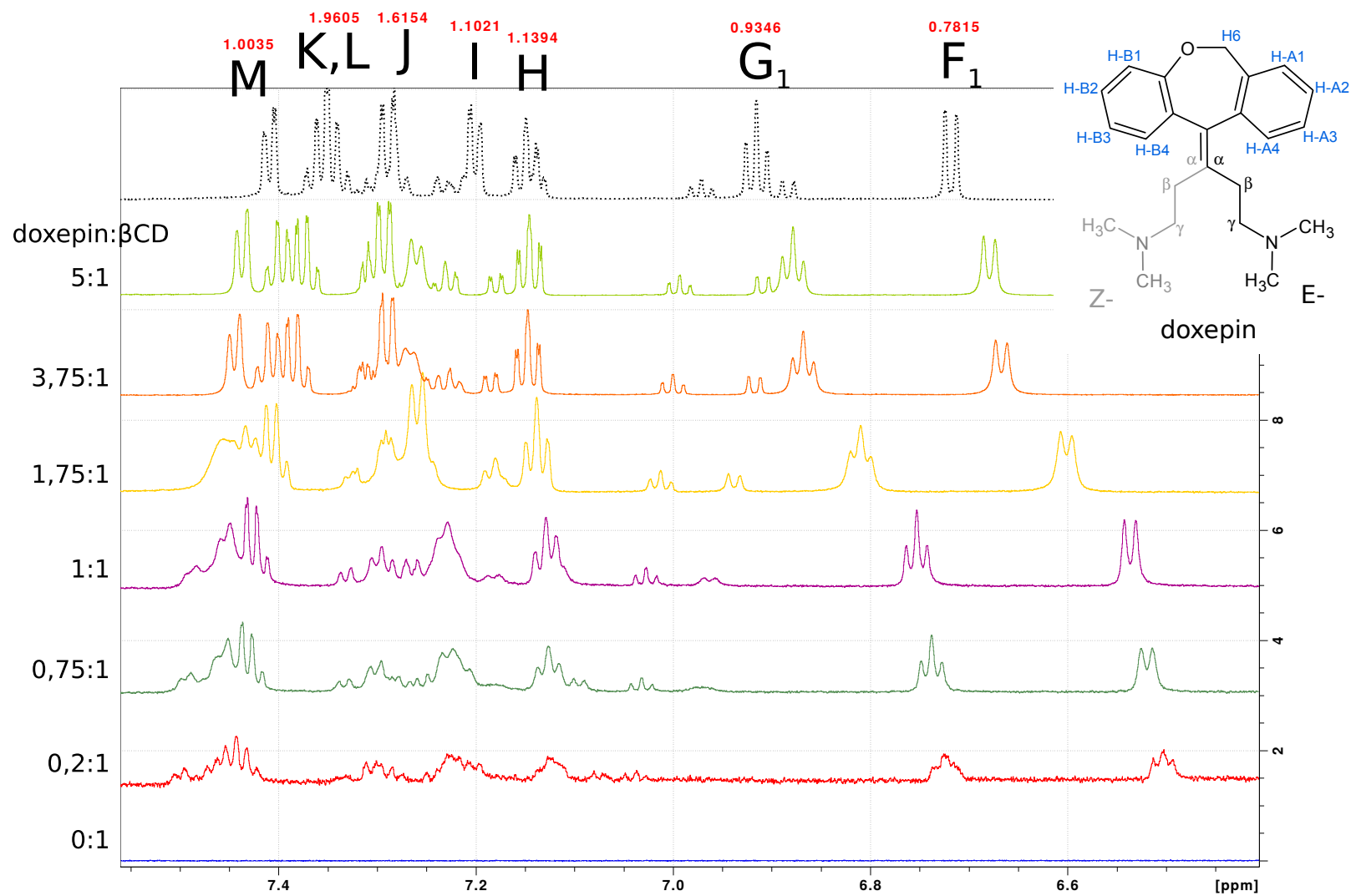
Inner β -CD protons **H3'** and **H5'** are dominantly exposed to shielding induced by ring current of aromatic ring.



1D ^1H NMR titration: doxepin

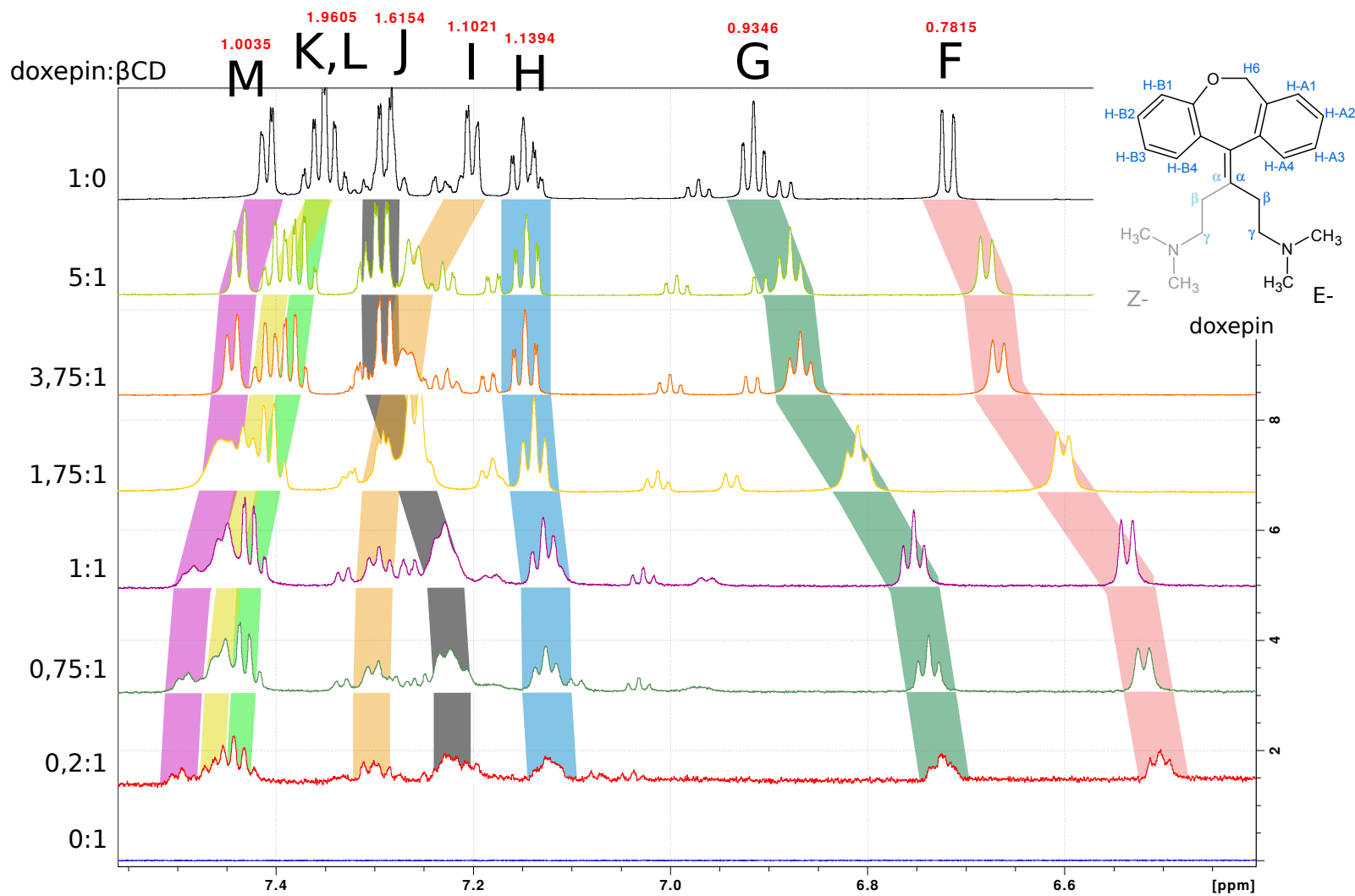


1D ^1H NMR titration: doxepin



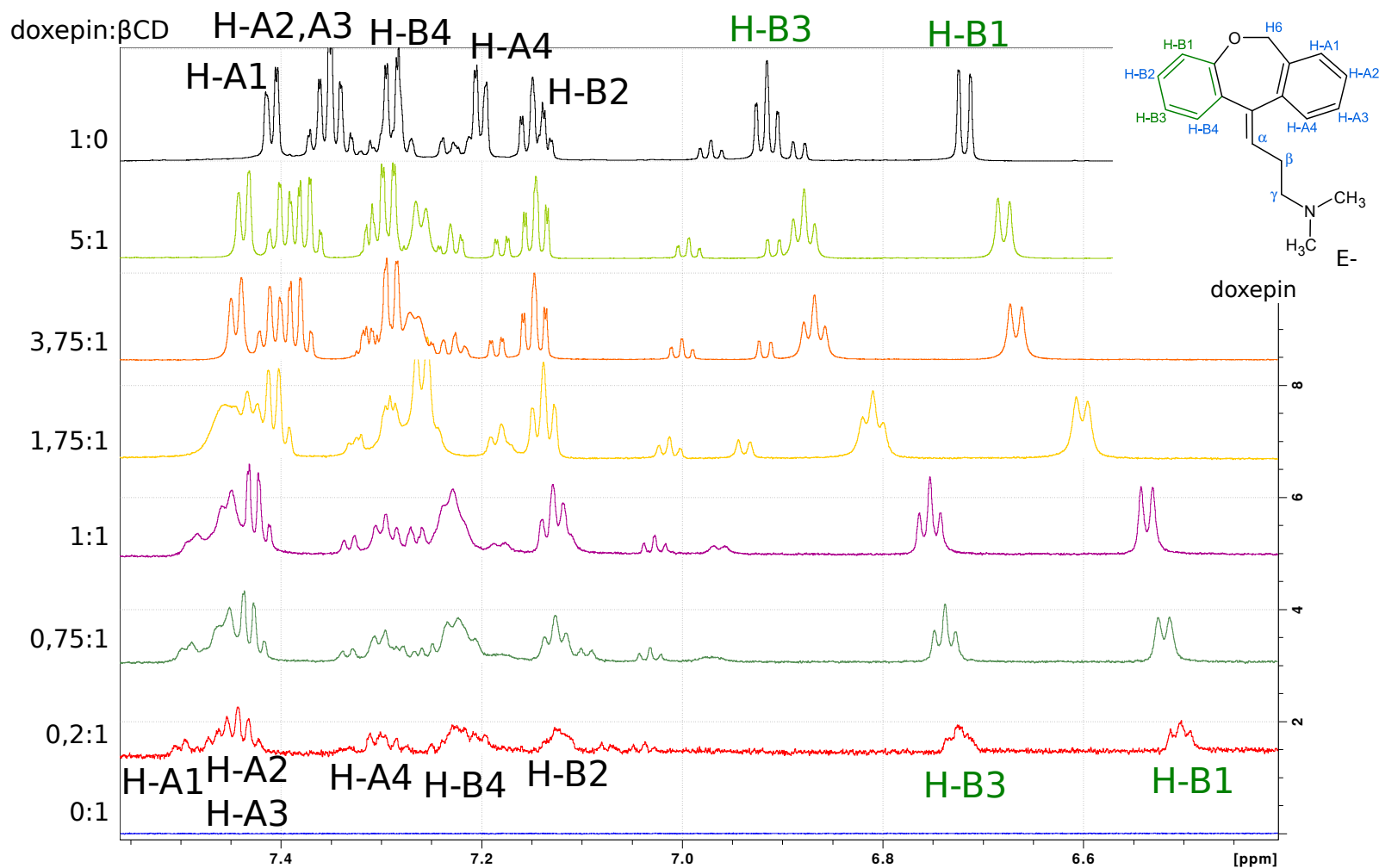
Find the most perturbed resonances and estimate the time regime of interaction:

1D ^1H NMR titration: doxepin



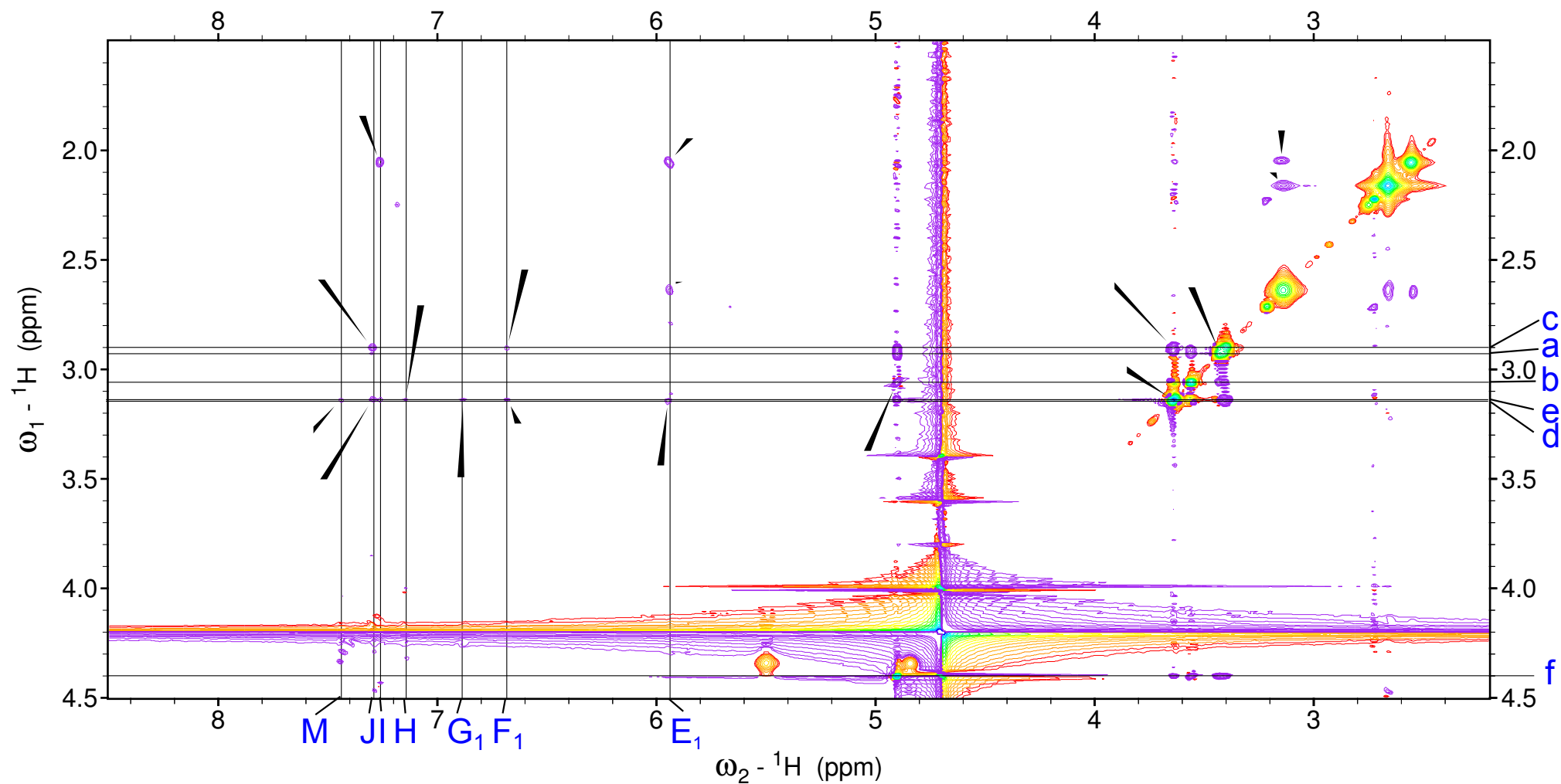
Find the most perturbed resonances and estimate the time regime of interaction:

1D ^1H NMR titration: doxepin

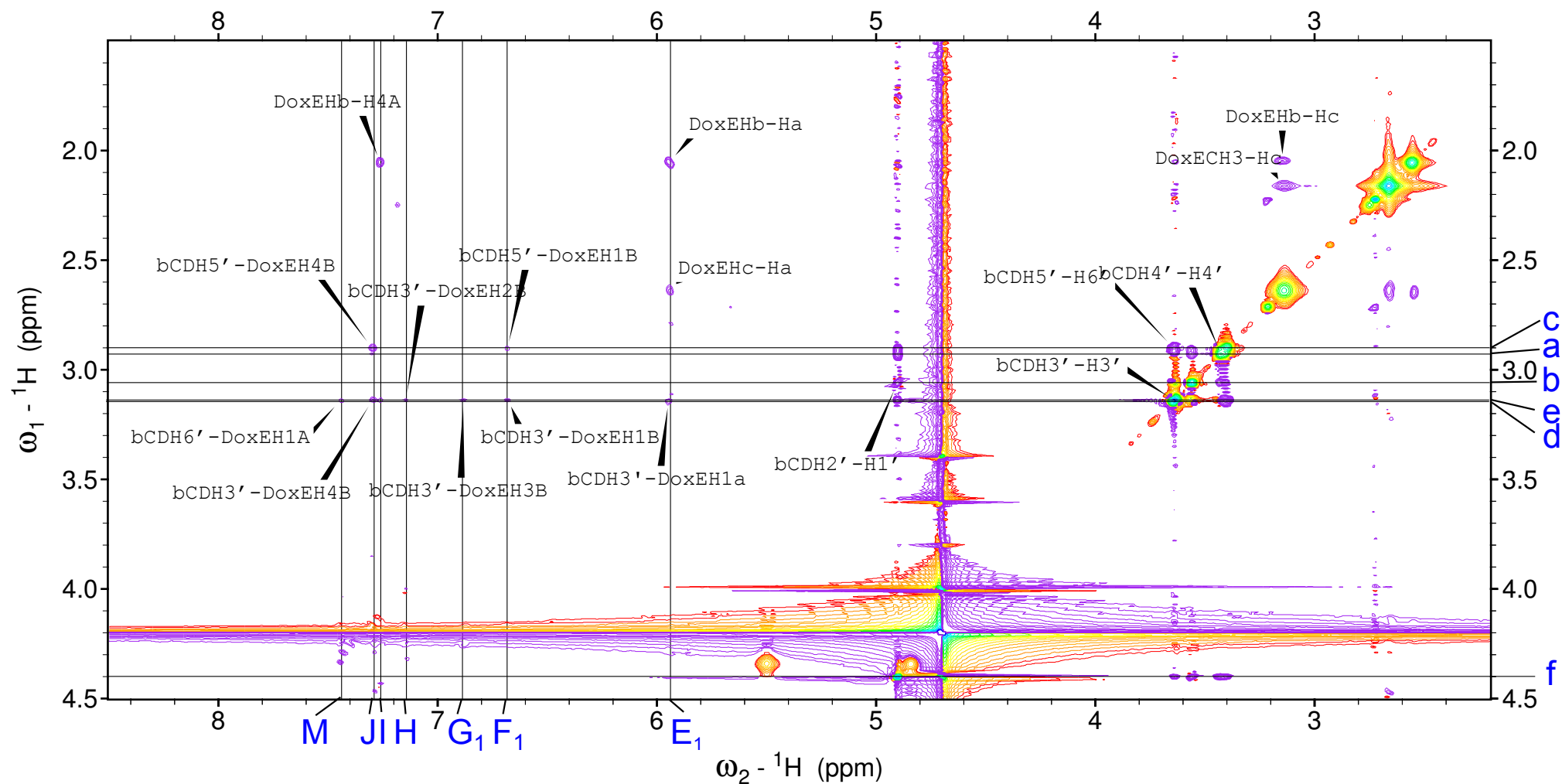


One set of broaden signals of doxepin \Rightarrow fast/intermediate rate of exchange.

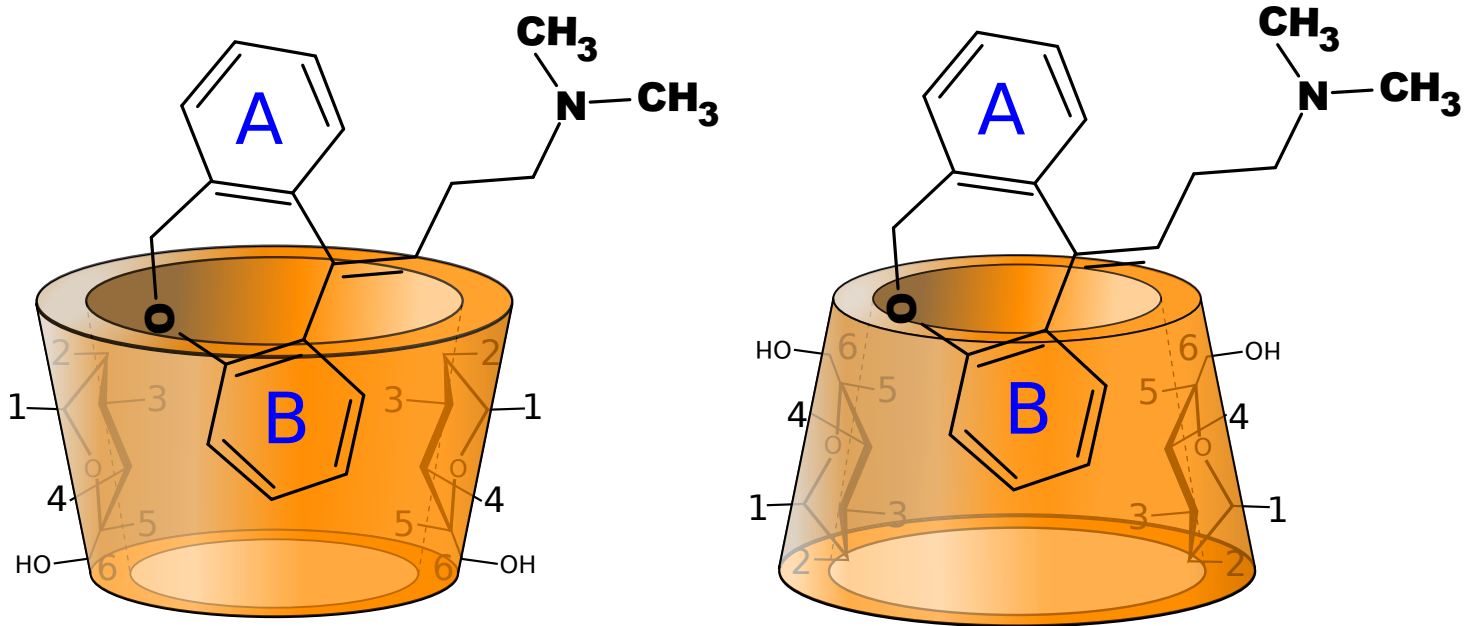
ROESY 300ms - doxepin: β -cyklodextrine=5:1



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

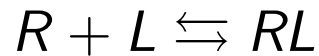


Proposed orientation of doxepine in β -cyklodextrine



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

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



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

Upon titration we are gradually changing L_0 concentration:

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

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

