

Supplementary Information

β -Lactam Antibiotics Epitope Mapping with STD NMR Spectroscopy: a Study of Drug-Human Serum Albumin Interaction

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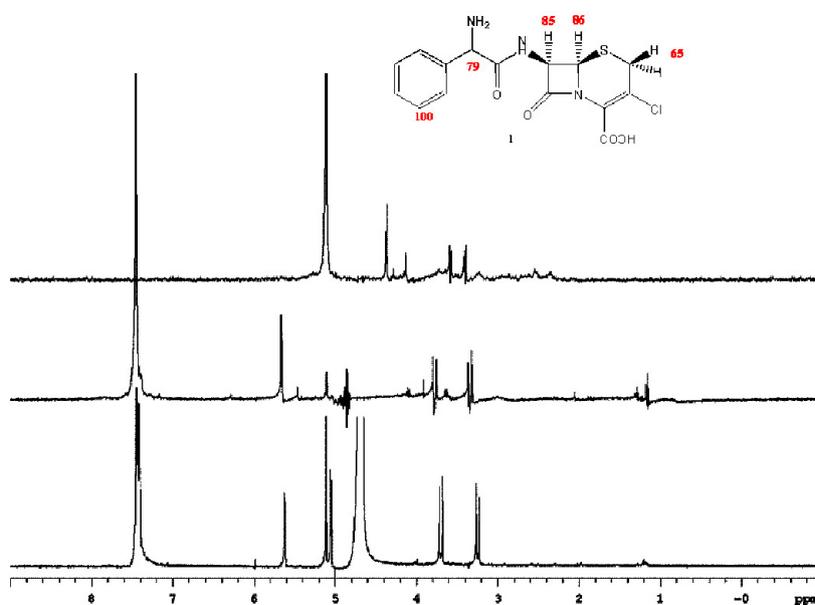


Figure S1. Epitope mapping of **1** with HAS: ¹H proton spectra for complex **1** + HSA (bottom), reference spectrum for the mixture HSA + **1** (middle) and corresponding STD NMR spectrum (top).

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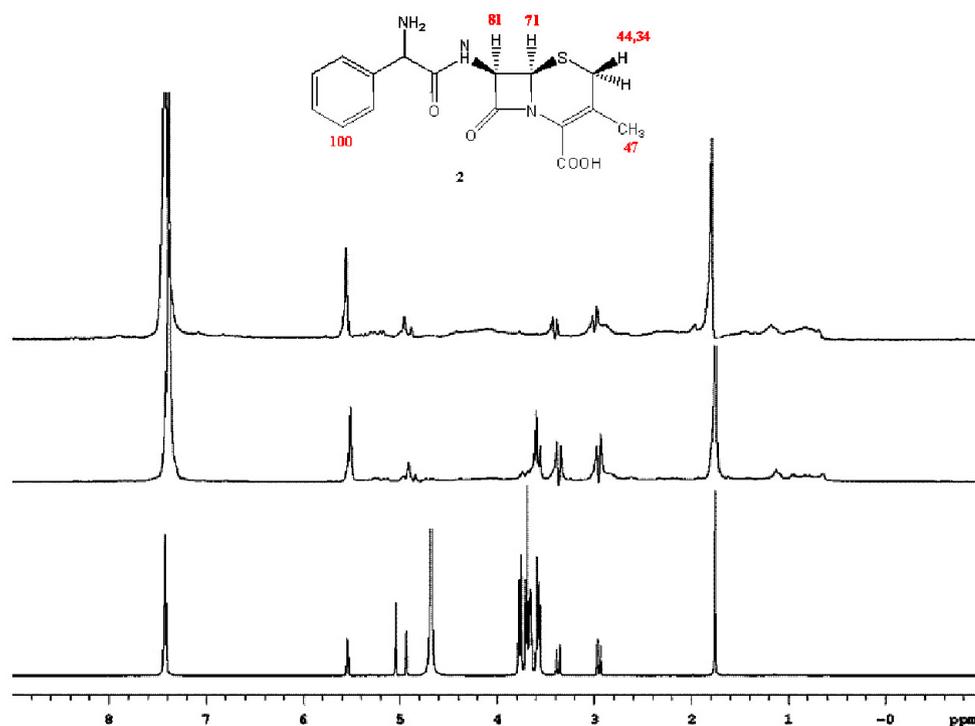


Figure S2. Epitope mapping of 2 with HAS: ^1H proton spectra for complex 2 + HSA (bottom), reference spectrum for the mixture HSA + 2 (middle) and corresponding STD NMR spectrum (top).

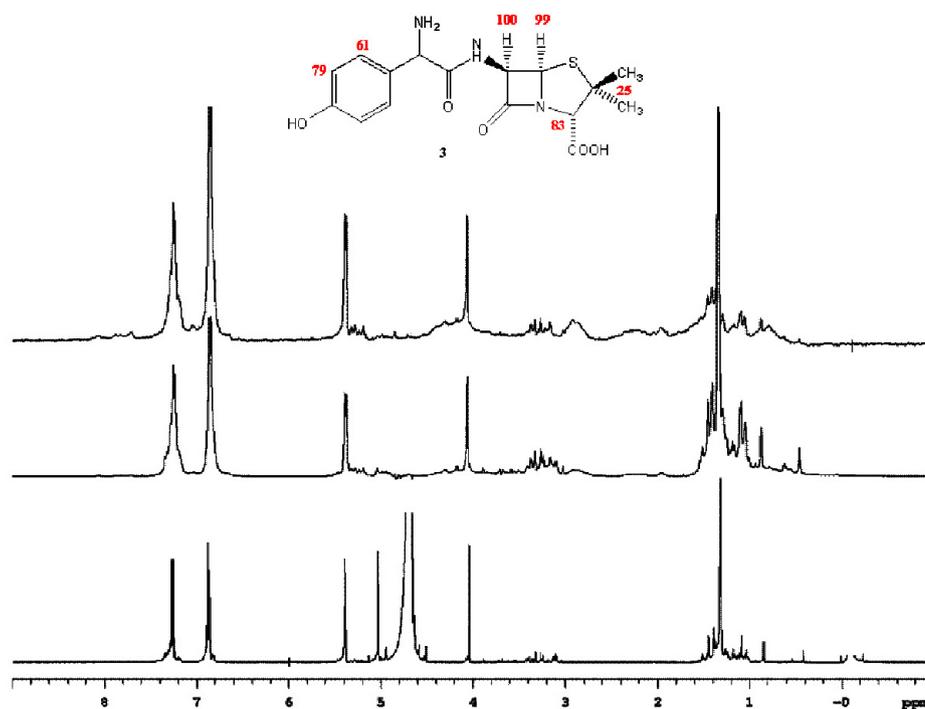


Figure S3. Epitope mapping of 3 with HAS: ^1H proton spectra for complex 3 + HSA (bottom), reference spectrum for the mixture HSA + 3 (middle) and corresponding STD NMR spectrum (top).

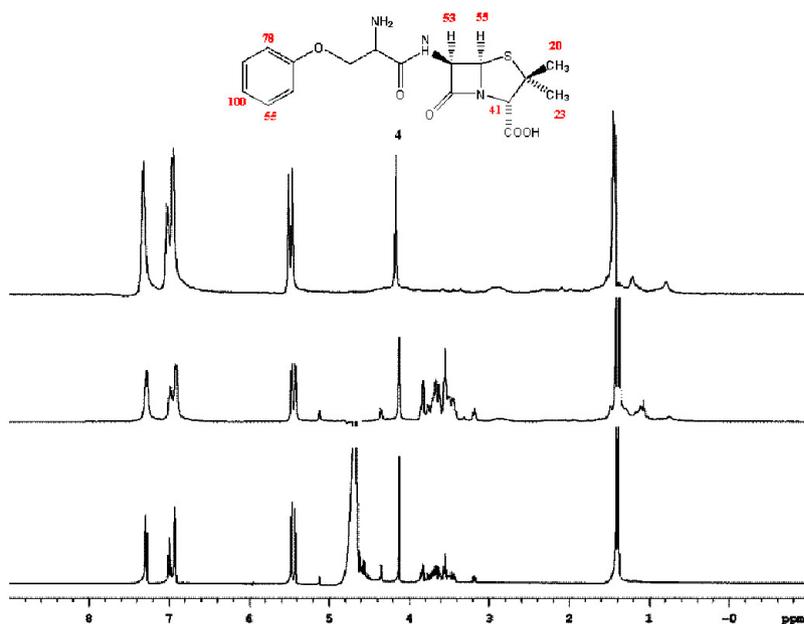


Figure S4. Epitope mapping of **4** with HSA: ^1H proton spectra for complex **4** + HSA (bottom), reference spectrum for the mixture HSA + **4** (middle) and corresponding STD NMR spectrum (top).

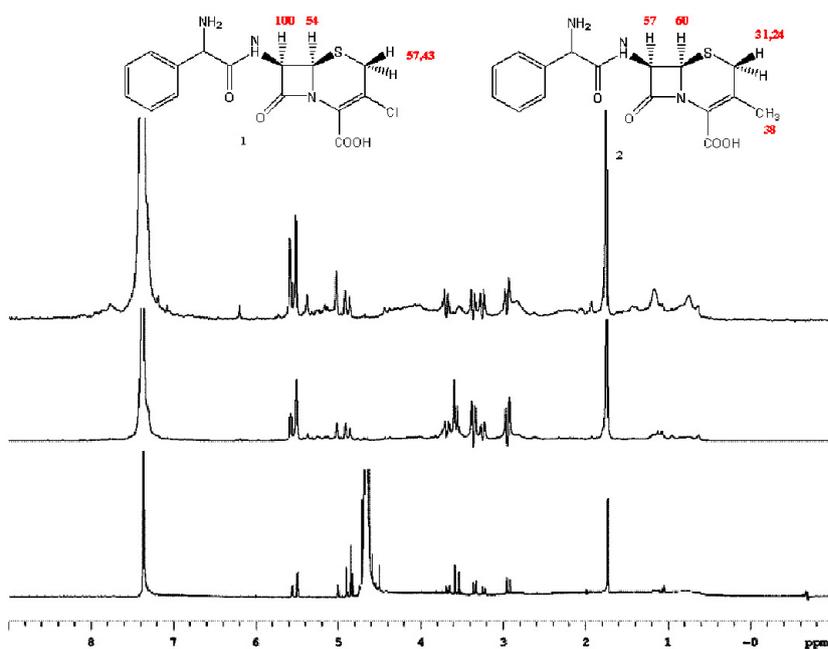


Figure S5. Epitope mapping of **1 + 2** with HSA: ^1H proton spectra for complex **1 + 2** + HSA (bottom), reference spectrum for the mixture HSA + **1 + 2** (middle) and corresponding STD NMR spectrum (top).

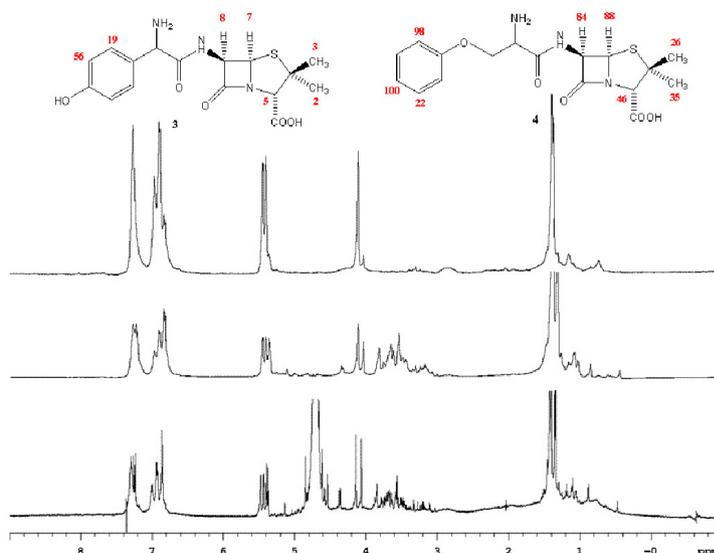


Figure S6. Epitope mapping of **3 + 4** with HAS: ^1H proton spectra for complex **3 + 4** + HSA (bottom), reference spectrum of the mixture HSA + **3 + 4** (middle) and corresponding STD NMR spectrum (top).

Epitope Mapping

The saturation degrees were calculated by determining individual signal intensities in the STD spectrum (I_{STD}) and in the reference STD spectrum (I_{off}). The relative amount of transferred saturation is measured by $A = I_{\text{STD}}/I_{\text{off}}$ and normalized setting the STD signal with the highest intensity to 100%, and the other percentages are accordingly calculated.

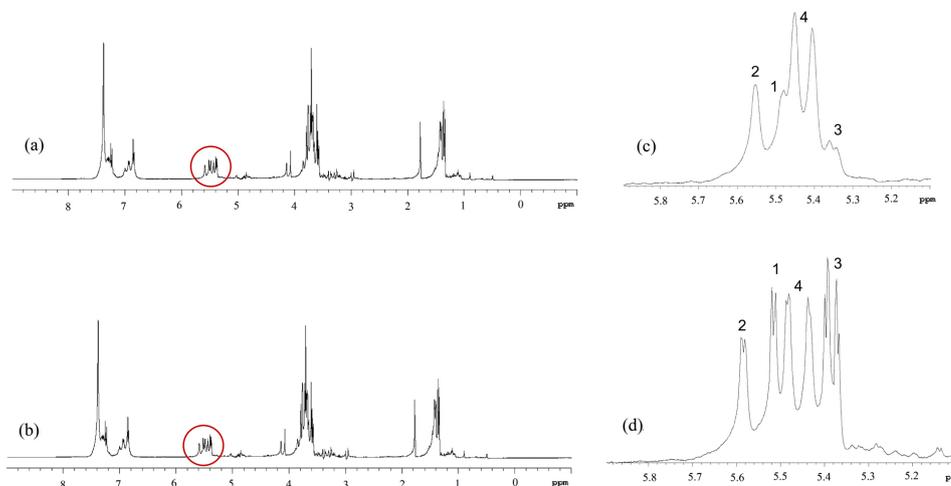


Figure S7. (a) STD NMR spectrum of HSA and a mixture of **1 + 2 + 3 + 4**. (b) STD NMR control spectrum (off resonance) of the mixture cited before. Spectra (c) and (d) correspond to amplification of circled regions in spectra (a) and (b), respectively, and refers to H-7 (**1**), H-7 (**2**), H-6 and H-5 (**3**), and H-6 and H-5 (**4**).

Basis of the above calculation

(i) Assignments of the signals

(ii) Individual signal intensities in the STD spectrum on resonance (I_{on}) and in the reference NMR spectrum off resonance (I_{off}) were calculated. An important observation is that for compounds **1** and **2**, the signal on spectra

corresponds to one hydrogen atom (H-7), while for compounds **3** and **4** it corresponds to two hydrogen atoms (H-5 and H-6). This should be taken into account when calculating individual intensities.

(iii) The relative degrees of saturation were measured by $A = (I_{\text{STD}}/I_{\text{off}})$ and were normalized using the largest STD effect as reference.