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Supplementary Information

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

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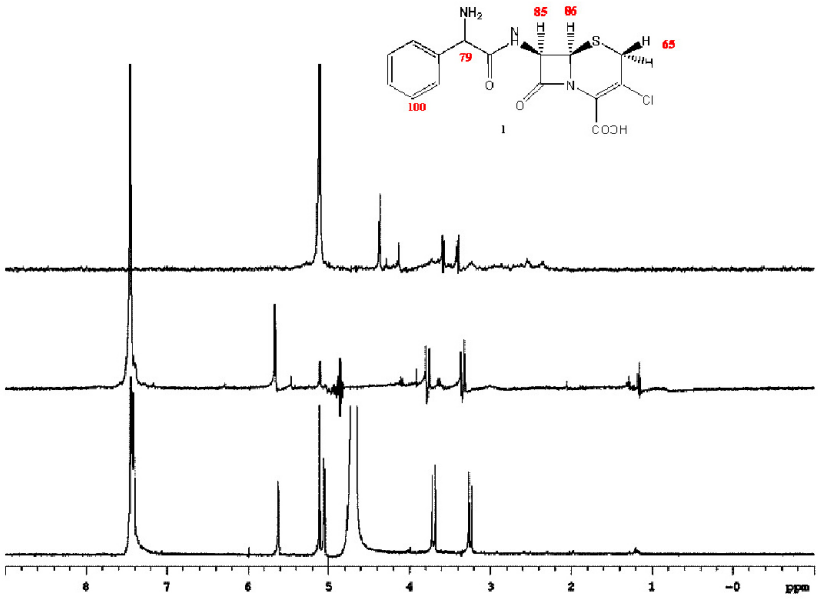

Figure S1. Epitope mapping of **1** with HAS: ^{1}H proton spectra for complex **1** + HSA (bottom), reference spectrum for the mixture HSA + **1** (middle) and corresponding STD NMR spectrum (top).

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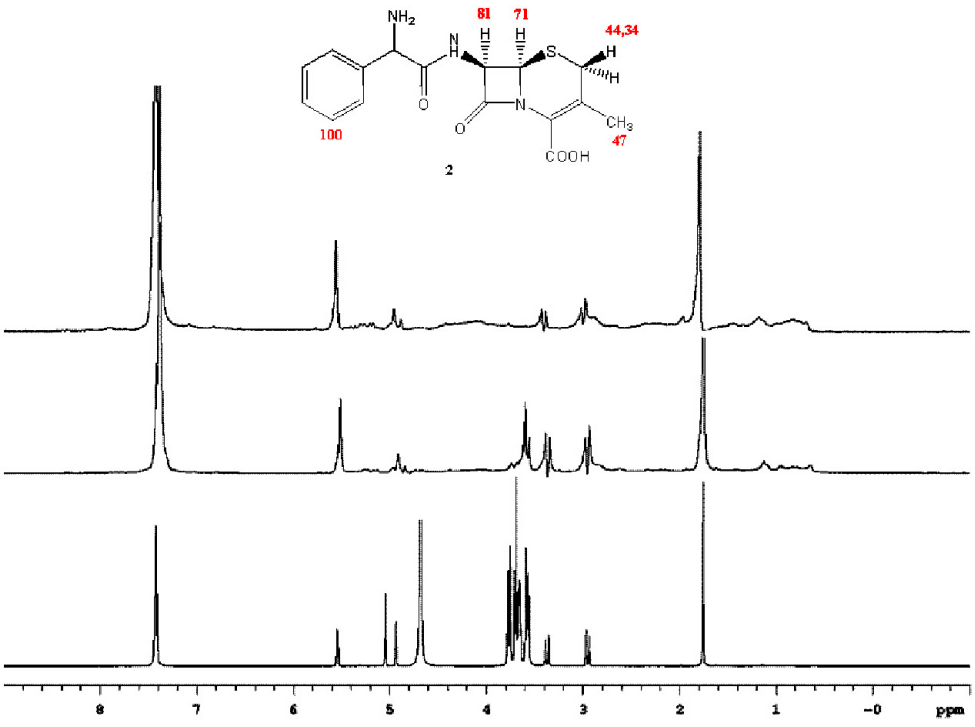

Figure S2. Epitope mapping of **2** with HAS: ^{1}H proton spectra for complex **2** + HSA (bottom), reference spectrum for the mixture HSA + **2** (middle) and corresponding STD NMR spectrum (top).

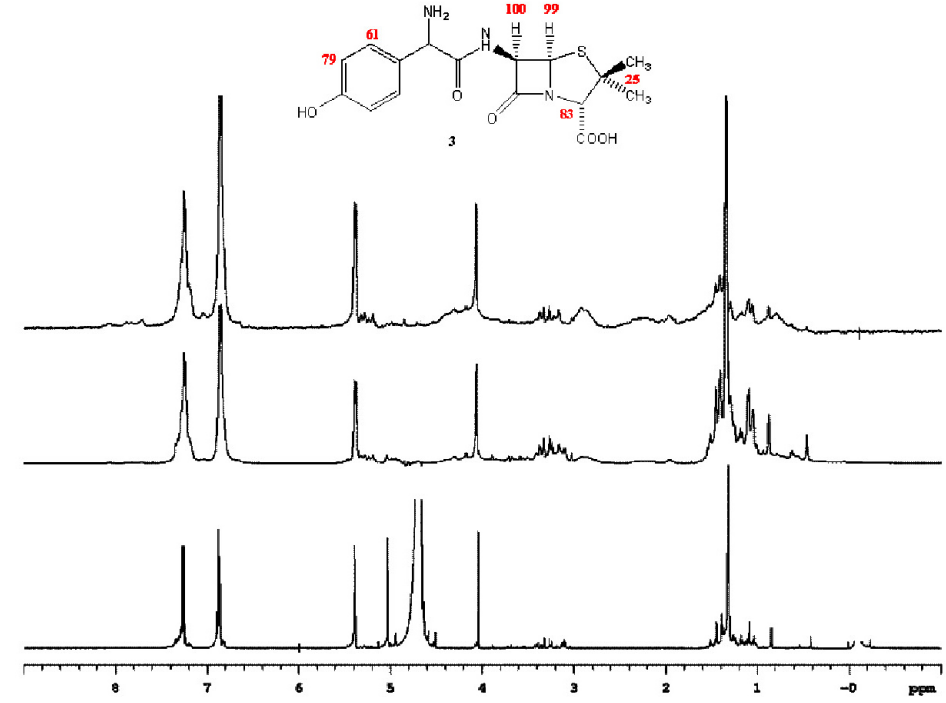

Figure S3. Epitope mapping of **3** with HAS: ^{1}H proton spectra for complex **3** + HSA (bottom), reference spectrum for the mixture HSA + **3** (middle) and corresponding STD NMR spectrum (top).

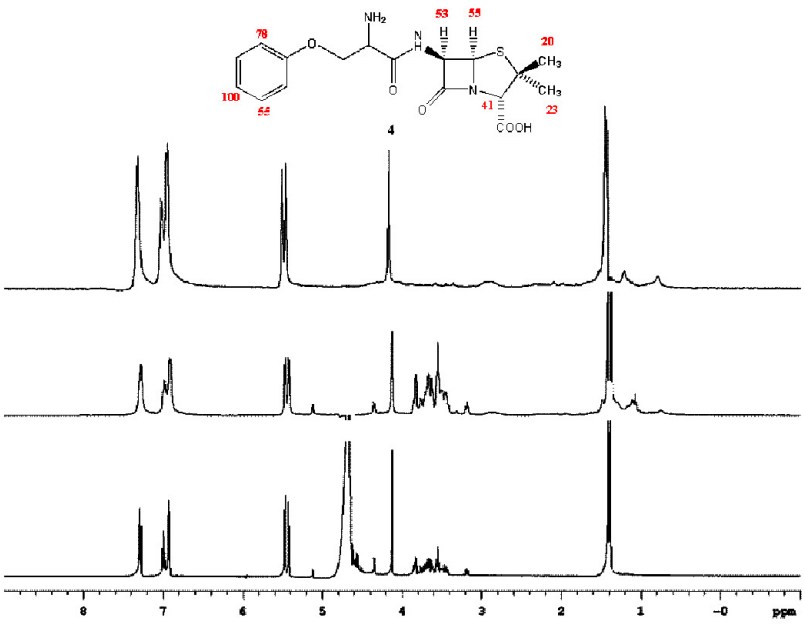

Figure S4. Epitope mapping of **4** with HAS: ^{1}H proton spectra for complex **4** + HSA (bottom), reference spectrum for the mixture HSA + **4** (middle) and corresponding STD NMR spectrum (top).

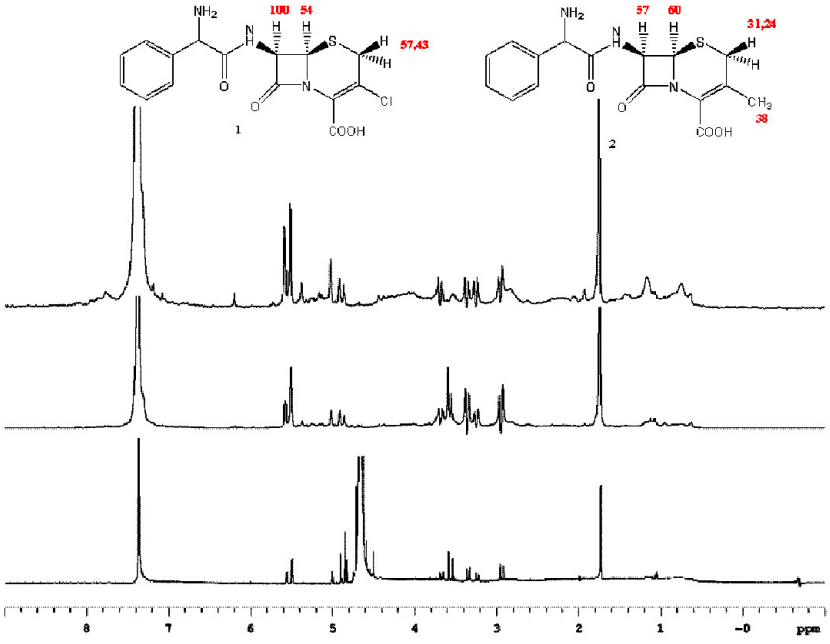

Figure S5. Epitope mapping of **1** + **2** with HAS: ^{1}H proton spectra for complex **1** + **2** + HSA (bottom), reference spectrum for the mixture HSA + **1** + **2** (middle) and corresponding STD NMR spectrum (top).

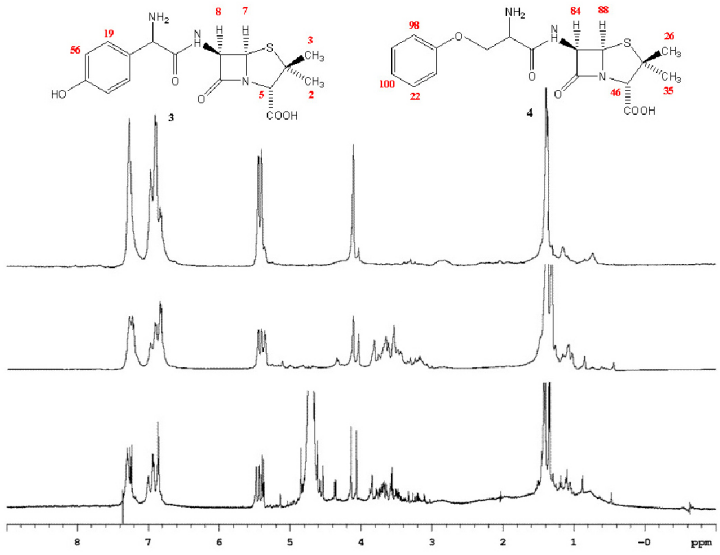

Figure S6. Epitope mapping of **3** + **4** with HAS: ^{1}H proton spectra for complex **3** + **4** + HSA (bottom), reference spectrum of the mixture HSA + **3** + **4** (middle) and corresponding STD NMR spectrum (top).

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 $A = I_{STD}/I_{off}$ and normalized setting the STD signal with the highest intensity to 100%, and the other percentages are accordingly calculated.

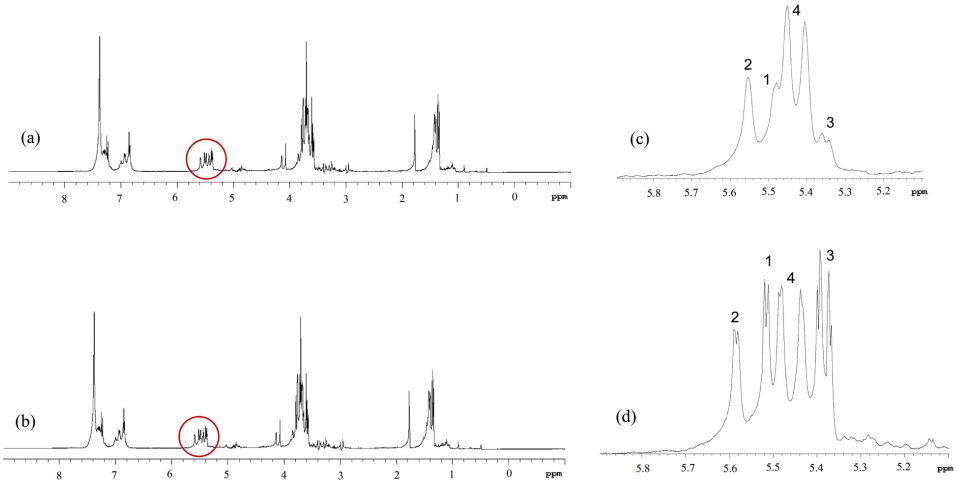

Figure S7. (a) STD NMR spectrum of HSA and a mixture of **1** + **2** + **3** + **4**, (b) STD NMR control spectrum (off resonance) of the mixture cited before. Spectra (c) and (d) correspond to amplification of circled regions in spectra (a) and (b), respectively, and refers to H-7 (**1**), H-7 (**2**), 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 $A = (I_{STD}/I_o)$ and were normalized using the largest STD effect as reference.