Microcoil NMR Study of the Interactions between Doxepin, β-Cyclodextrin, and Acetate during Capillary Isotachophoresis

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Supporting information table of contents:

EXPERIMENTAL SECTION

Normal Phase HPLC Isolation of the Doxepin Z-Isomer. The separation was performed on a 4.6 x 150 mm Kromasil silica column (Sigma-Aldrich, Inc., St. Louis, MO) using an Agilent 1100 series HPLC. An isocratic solvent system of 25% chloroform and 75% acetonitrile containing 0.1% triethylamine was used for elution. The separation was run at a constant flow rate of 1.0 mL/min and was monitored using UV detection at 254 nm. The Z-isomer fraction was collected in chilled centrifuge tubes that were covered with aluminum foil to prevent light from entering the tube.

Structure Confirmation of the Doxepin Z-Isomer by NMR. All ^{1}H NMR spectra were measured using a Bruker Avance NMR spectrometer operating at a frequency of 599.84 MHz equipped with a Protasis/MRM TXI microcoil probe. The lyophilized sample isolated by HPLC was reconstituted in 4 μL of D_2O. The sample was sandwiched between layers of chloroform and injected into capillary of the probe until the sample was positioned completely in the active volume of the microcoil, as monitored by the spectrometer lock level. The ^{1}H NMR survey spectrum (Figure S1) was acquired by averaging 1024 transients with 16 dummy scans. A relaxation delay of 3 s was used, and FIDs were acquired into 26,452 data points following the

application of the 90° pulse. FIDs were apodized by multiplication by an exponential function equivalent to 1.0 Hz line broadening prior to Fourier transform and zero-filled to 131072 points.

The COSY spectrum (Figure S2) was acquired using the standard cosyphpr Bruker pulse sequence with presaturation during the 1.5 s relaxation delay. A total of 32 transients were acquired in F2 with 64 dummy scans for each of the 256 increments measured in the F1 dimension. The spectra were zero-filled to 4096 points in the F2 dimension and 2048 points in the F1 dimension. All processing was performed using Bruker Topspin software (version 1.3).

The TOCSY spectrum (Figure S3) was acquired using the standard mlevphpr Bruker pulse sequence with presaturation during the 1.5 s relaxation delay. A total of 32 transients were acquired in F2 with 64 dummy scans for each of the 512 increments measured in the F1 dimension. A 200 ms mixing time was used. The spectra were zero-filled to 4096 points in the F2 dimension and 1024 points in the F1 dimension. All processing was performed using Bruker Topspin software (version 1.3).

The NOESY spectrum (Figure S4) was acquired using the standard noesyphpr Bruker pulse sequence with presaturation during the 1.5 s relaxation delay and during the NOESY mixing time. A total of 40 transients were acquired in F2 with 64 dummy scans and 448 increments measured in the F1 dimension. A 400 ms mixing time was used. The spectra were zero-filled to 4096 points in the F2 dimension and 2048 points in the F1 dimension. All processing was performed using Bruker Topspin software (version 1.3).

RESULTS

NMR Structure Confirmation of the Doxepin Z-isomer. To confirm the identity of the Z isomer and complete the resonance assignments for this compound approximately 0.5 mg was isolated through a normal phase HPLC separation of the commercial preparation. The isolated

material was subjected to one- and two-dimensional ^{1}H-NMR experiments using a commercial TXI-microcoil probe from Protasis. Figure S1 shows the ^{1}H-NMR survey spectrum of the isolated material. Assignments of the doxepin proton resonances as well as those of the triethylamine (TEA) impurity were made using the COSY and TOCSY spectra shown in Figures S2 and S3 and by comparison to reference chemical shift data for the E-isomer.[29] To confirm the stereochemistry of the isolated doxepin molecule, Figure S4 shows an expansion of the aromatic region of the NOESY spectrum. The NOESY cross-peaks between the α proton near the double bond to a proton on the "A" ring (most likely the A_4 proton), and between the β proton to "B" ring (most likely the B_4 proton) confirm that the isolated compound was the doxepin Z-isomer.

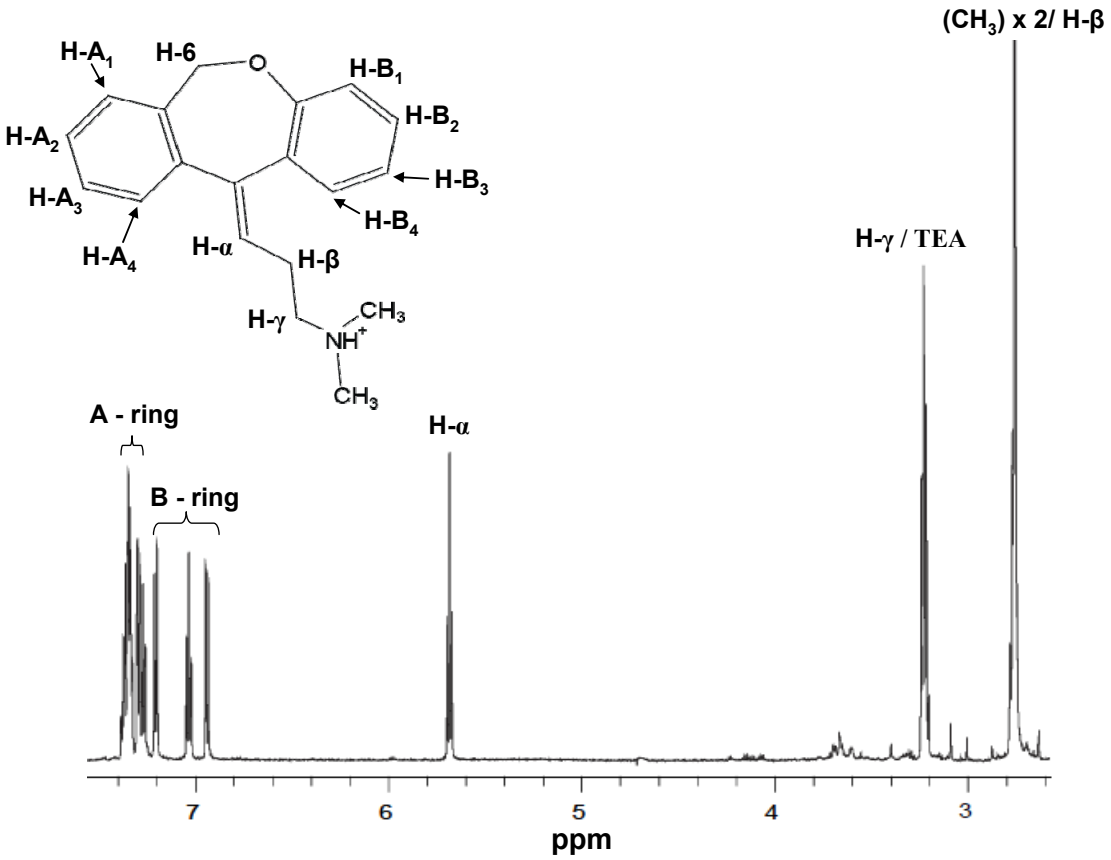

Figure S1. ^{1}H NMR survey spectrum of approximately 0.5 mg of the doxepin Z-isomer isolated by normal phase HPLC and reconstituted in 4 μL of D_2O.

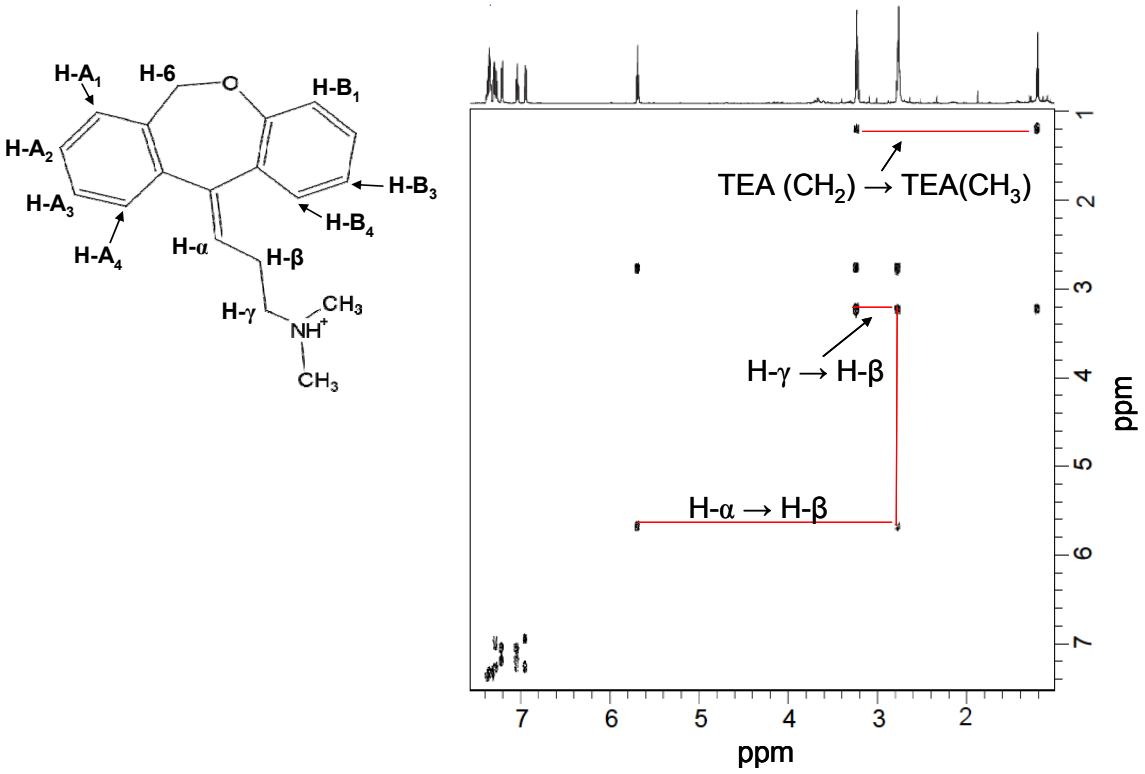

Figure S2. COSY spectrum measured for approximately 0.5 mg of the doxepin Z-isomer isolated by normal phase HPLC and reconstituted in 4 μL of D_2O. The red lines indicate cross peaks that correspond to the through bond coupling of the H-β proton to both the H-α and H-γ protons of doxepin. The cross peaks near 1 ppm represent through bond coupling of the methyl and methylene protons of TEA, an impurity introduced in the HPLC separation.

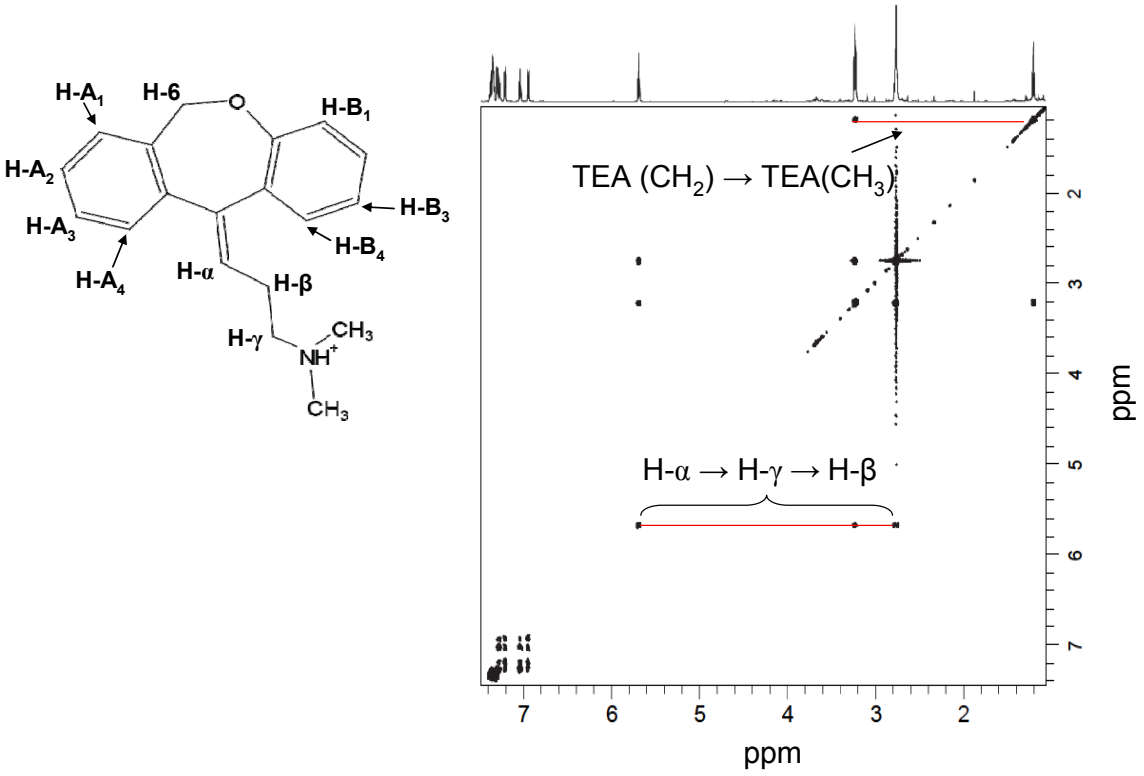

Figure S3. TOCSY spectrum measured for approximately 0.5 mg of the doxepin Z-isomer isolated by normal phase HPLC and reconstituted in 4 μL of D_2O. The red lines indicate cross peaks that correspond to the coupling through multiple bonds in the same spin system. The cross peaks around 5.7 ppm in the F1 dimension indicate that the H-α, H-β, and H-γ protons are all in the same spin system, as expected. The cross peaks near 1 ppm in the F1 dimension again represent through bond coupling of the methyl and methylene protons of TEA, an impurity introduced in the HPLC separation.

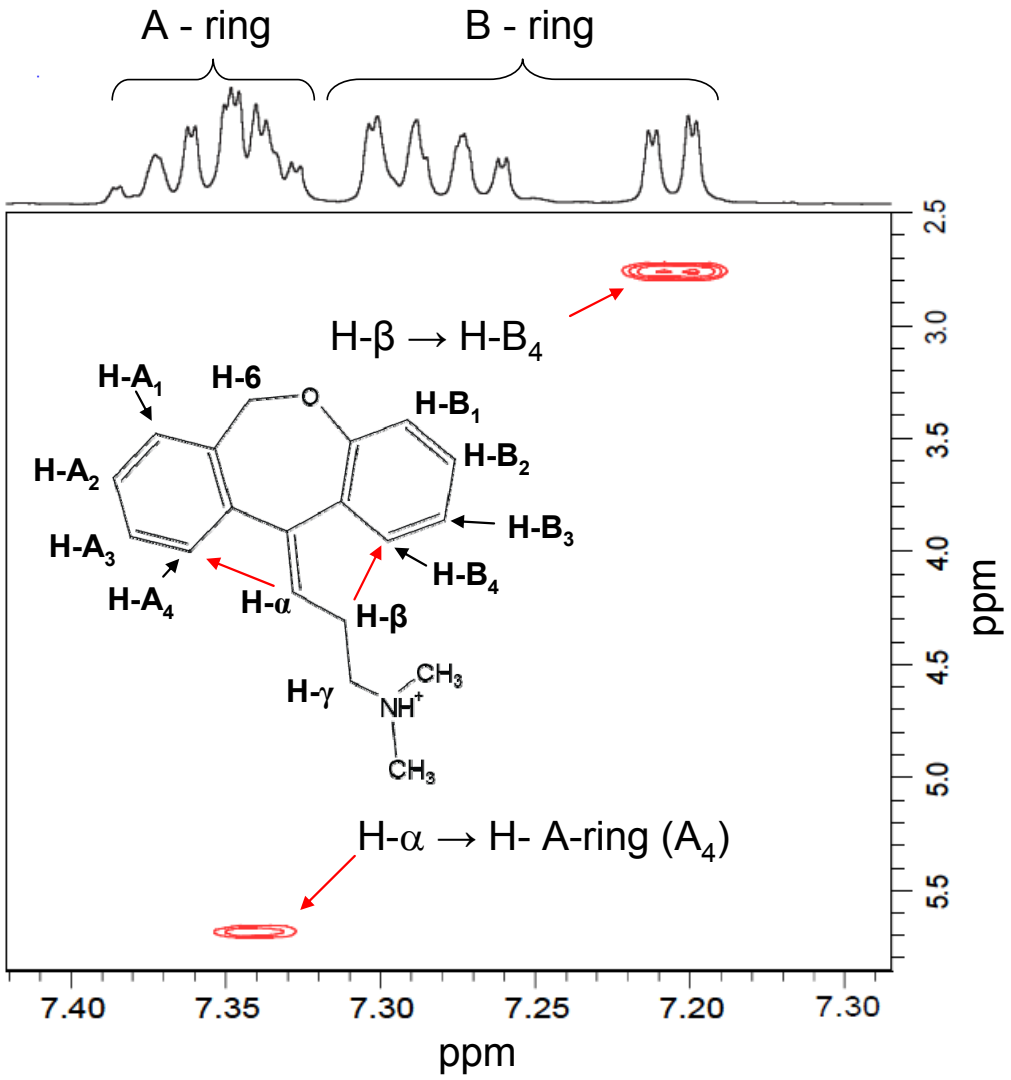

Figure S4. NOESY spectrum measured for approximately 0.5 mg of the doxepin Z-isomer isolated by normal phase HPLC and reconstituted in 4 μL of D_2O. The cross-peaks (shown in red) between the α proton to a proton on the doxepin A ring (most likely the A_4 proton), and between the β proton to B ring (most likely the B_4 proton) confirm that the isolated compound is the doxepin Z-isomer.

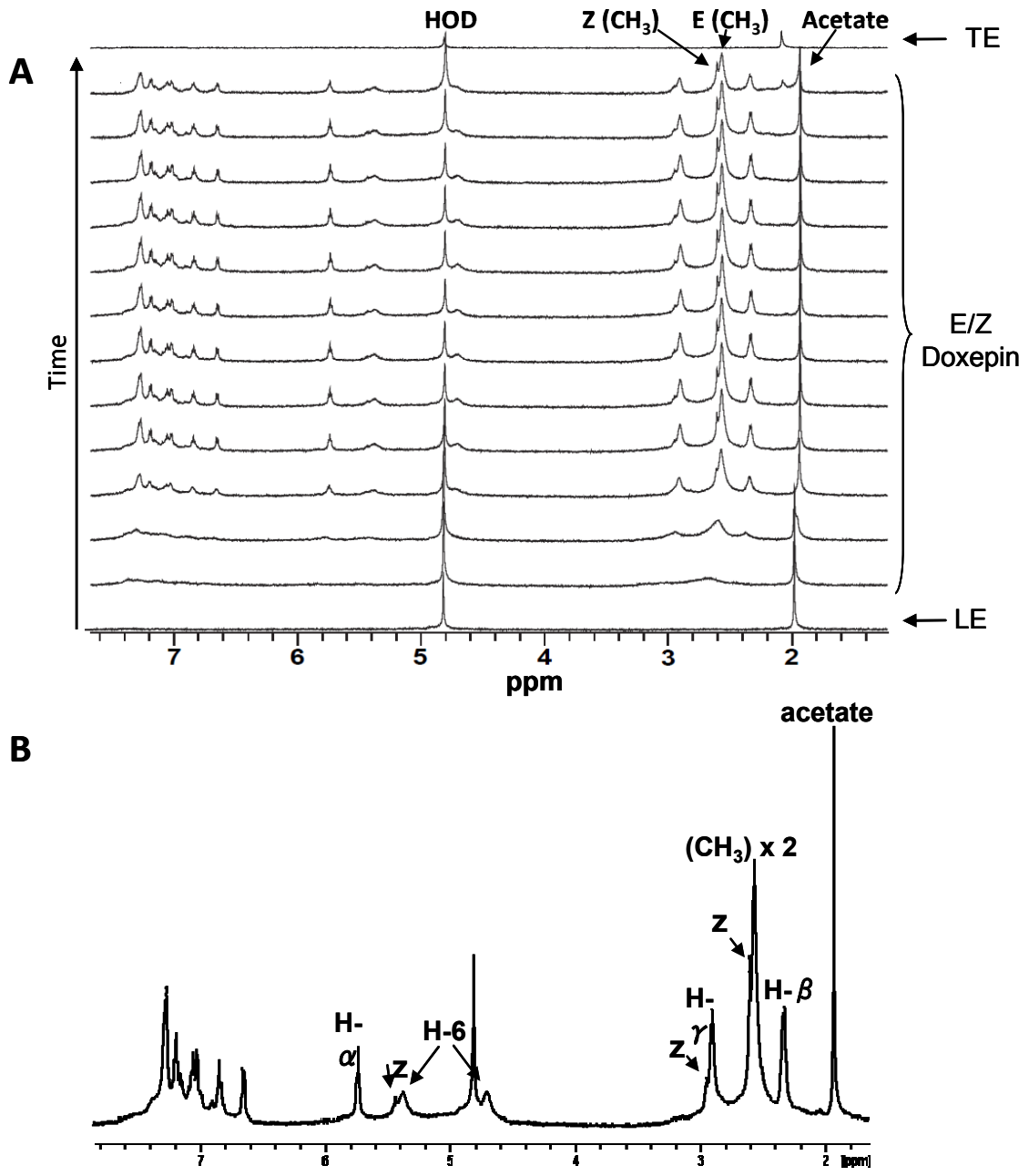

Figure S5. A) Results of the online cationic cITP-NMR analysis of 9 nanomoles of doxepin with a LE of 160 mM NaOAc at pD 5.0 and a TE of 160 mM acetic acid-d_4. B) Spectrum resulting from coaddition of all of the cITP-NMR spectra in (A) containing resonances of doxepin.

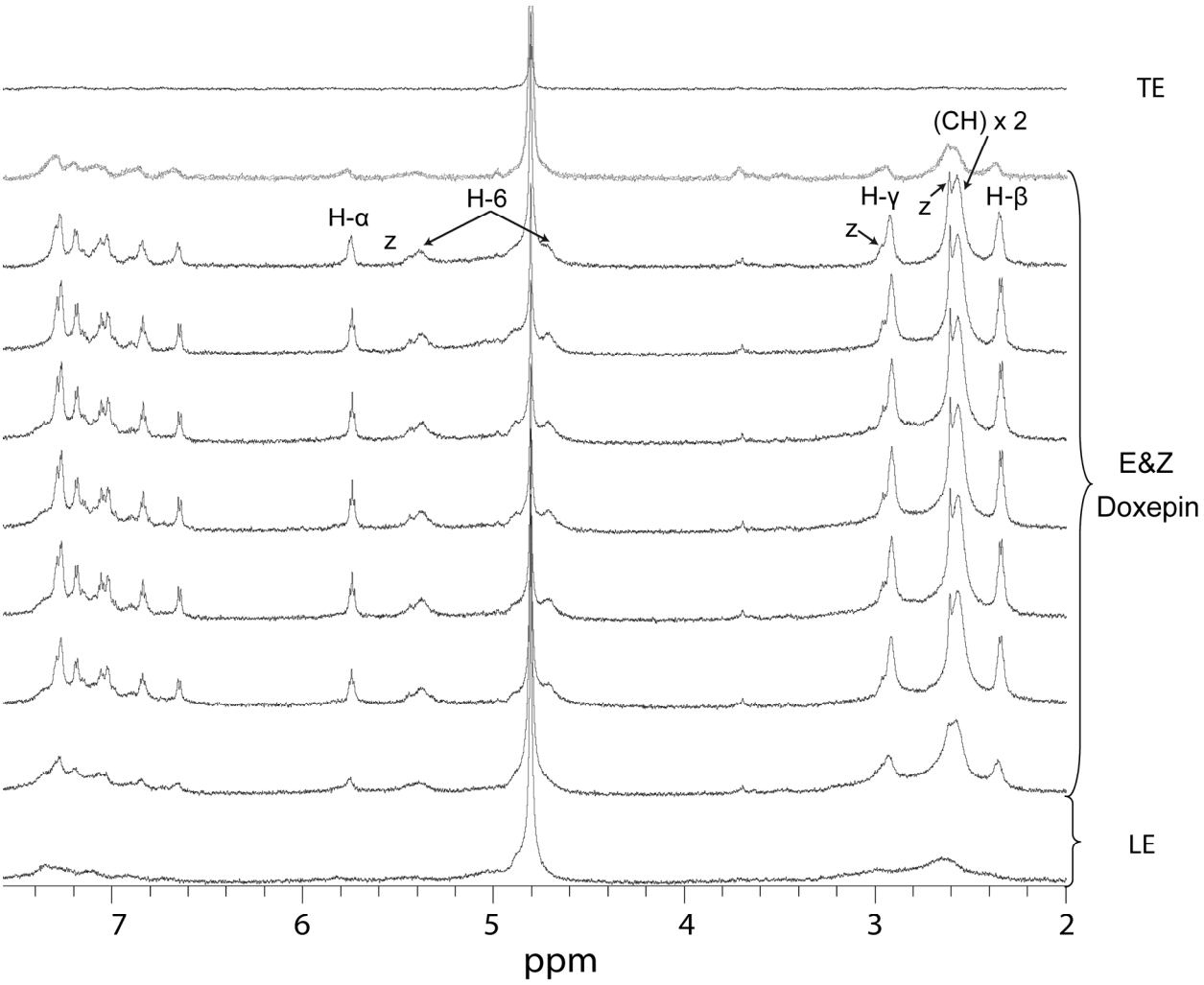

Figure S6. Results of the online cationic cITP-NMR analysis of 9 nanomoles of doxepin with a LE of 160 mM NaOAc at pD 5.0 and a TE of 160 mM acetic acid-d_4. The LE and TE both contain 0.8 mM β-CD.