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Electronic Supplementary Information

Materials and methods

Materials

Ellipticine (cat# 324688) was obtained from Calbiochem. Heparin sodium salt from porcine mucosa (Sigma, cat# 51551), heparan sulfate sodium salt from porcine mucosa (Iduron, cat# GAG-HS01 BN1) and chondroitin 6-sulfate sodium salt from shark cartilage (Sigma, cat# C4384) were used as supplied. All other reagents were of analytical grade.

Preparation of ellipticine and GAG solutions

2 mM stock solution of ellipticine was made in 0.005 M HCl. GAG samples were dissolved in pH 6.3 or 7.4 Tris-HCl buffer (50 mM) containing 100 mM sodium chloride. Due to their polydisperse nature, GAG concentrations were expressed using molecular weight of the average repeating disaccharide unit: 665, 464, and 503 g/mole for heparin, heparan sulfate, and chondroitin 6-sulfate, respectively.

Circular dichroism and UV absorption spectroscopic measurements

CD and absorption spectra were recorded on a JASCO J-715 spectropolarimeter at 25 ± 0.2 °C and represent the average of three scans obtained by collecting data at a scan speed of 100 nm/min. Spectroscopic experiments were conducted in 50 mM Tris-HCl pH 6.3 buffer (100 mM NaCl) using a rectangular quartz cell of 1 cm optical path length (Hellma, USA). Temperature control was provided by a Peltier thermostat equipped with magnetic stirring. UV absorption spectra were obtained by conversion of the high tension (HT) voltage applied to the photomultiplier tube into absorbance units. CD and UV curves of ellipticine-GAG mixtures were corrected by blank buffer solution. JASCO CD spectropolarimeters record CD data as ellipticity (‘Θ’) in units of millidegrees (mdeg). The quantity of ‘Θ‘ is converted to molar circular dichroic absorption coefficient ($\Delta\varepsilon$ in M^{-1} cm^{-1}) using the equation $\Delta\varepsilon$ =

Θ/(33982cl), where, ‘c’ is the molar concentration of the ligand (mol/L), and ‘l’ is the optical pathlength expressed in cm.

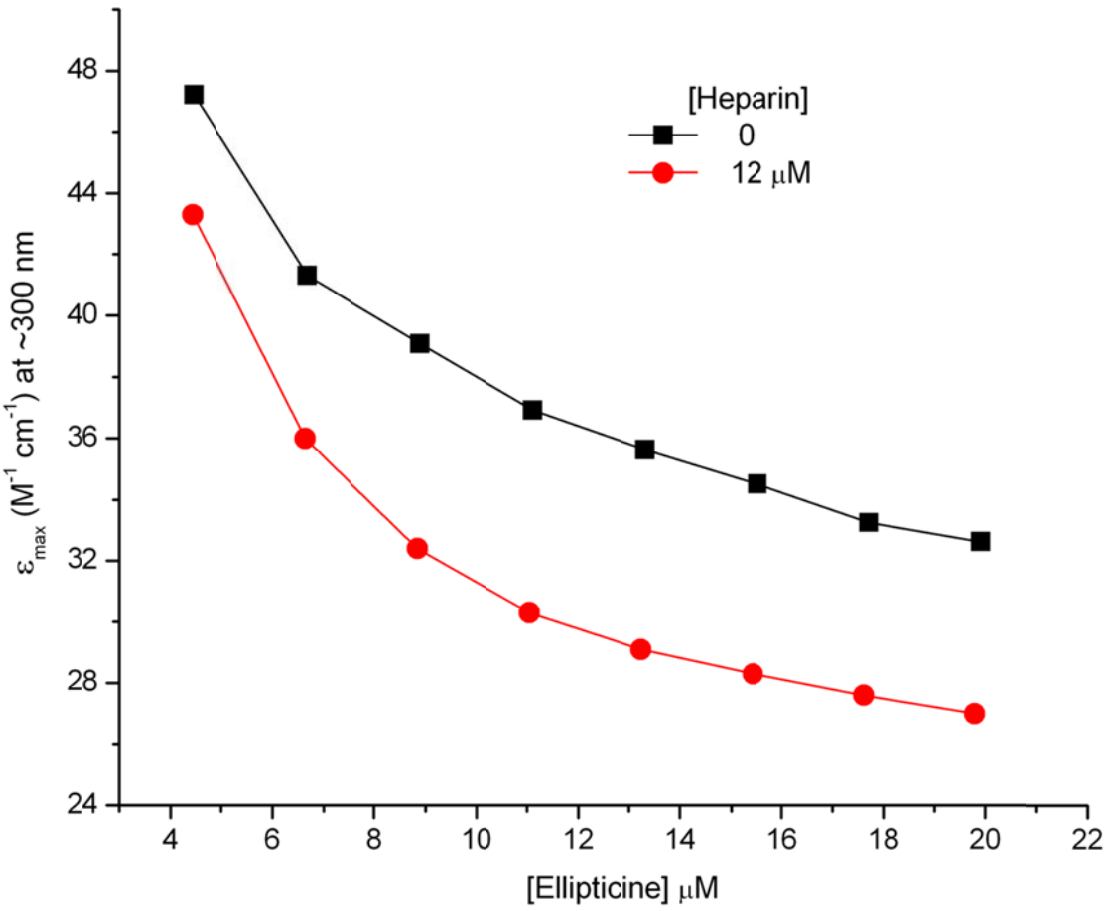
During the CD-pH titration, pH values of the sample solution was checked by using a bench-top Mettler Toledo SevenEasy pH meter (model S20).

Calculation of ellipticine-GAG binding parameters

Induced CD titration data (ellipticity in mdeg) were fitted according to the one-site specific binding with a Hill slope equation using Graphpad Prism software (ver. 6.01):

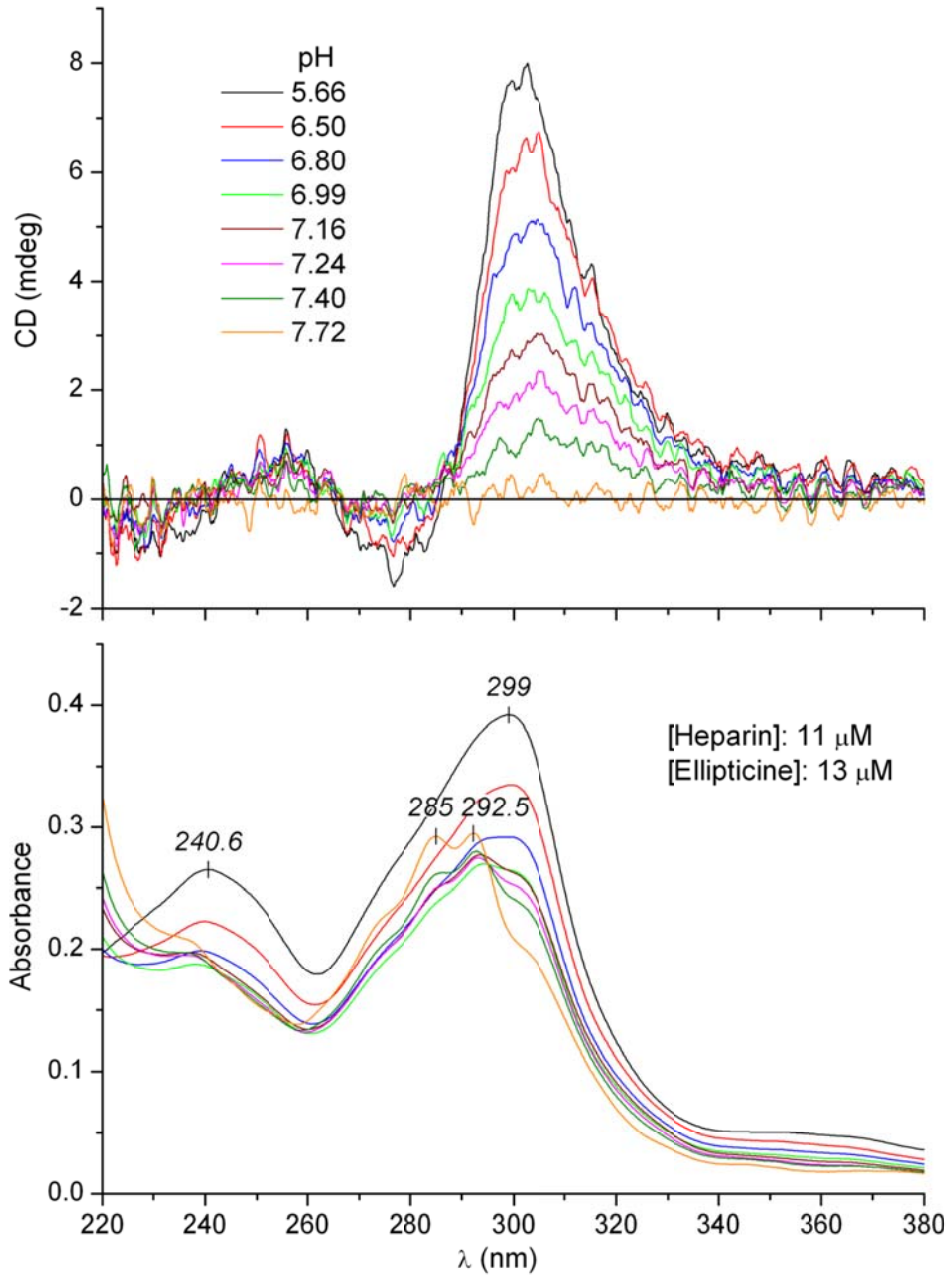
$$Y = B_{max} * X^h / (K_d^h + X^h)$$

where Y is the ICD value of ellipticine, B_{max} is the maximum extrapolated specific binding, X is the concentration of ellipticine in μM, K_d is the the equilibrium dissociation constant of ellipticine (expressed in the same units as X), and *h* is the Hill coefficient.



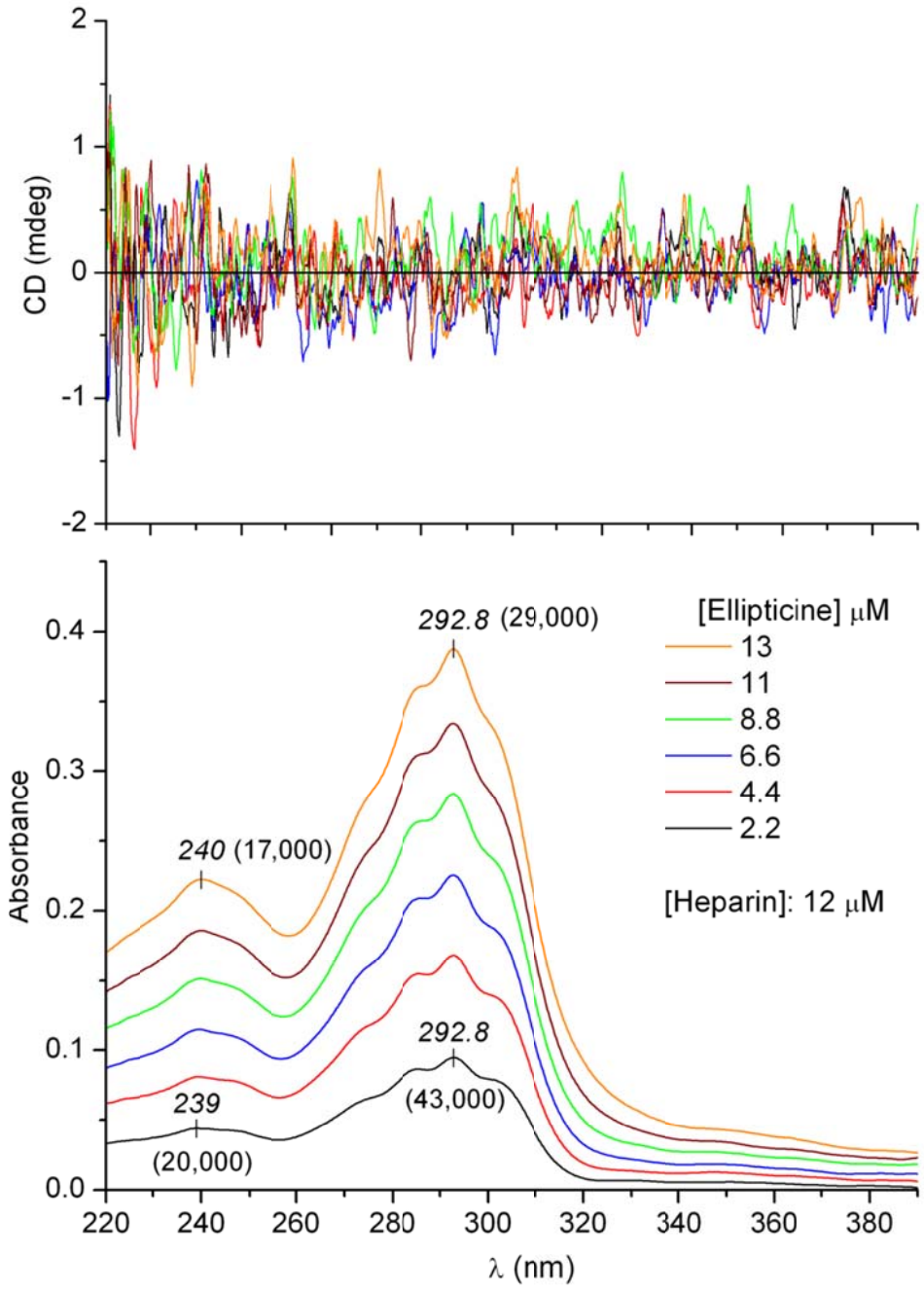
Supplementary Figure 1

Comparison of ε_{max} values of ellipticine obtained in the absence and presence of heparin in pH 6.3 Tris-HCl buffer solution (100 mM NaCl, 25 °C).



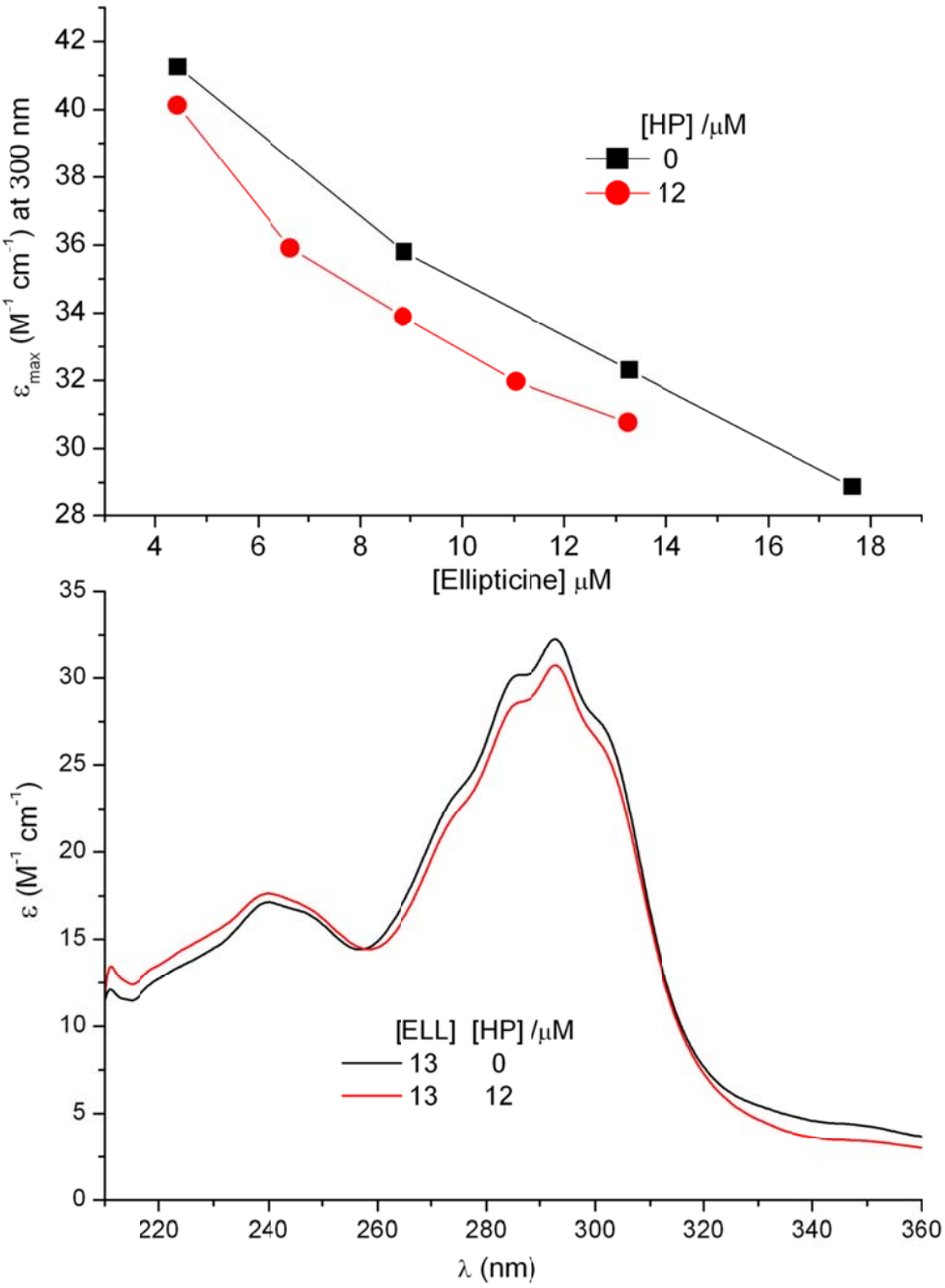
Supplementary Figure 2

The pH dependence of CD and absorption spectra of ellipticine-heparin mixture (50 mM Tris-HCl buffer, 100 mM NaCl, 25 °C).



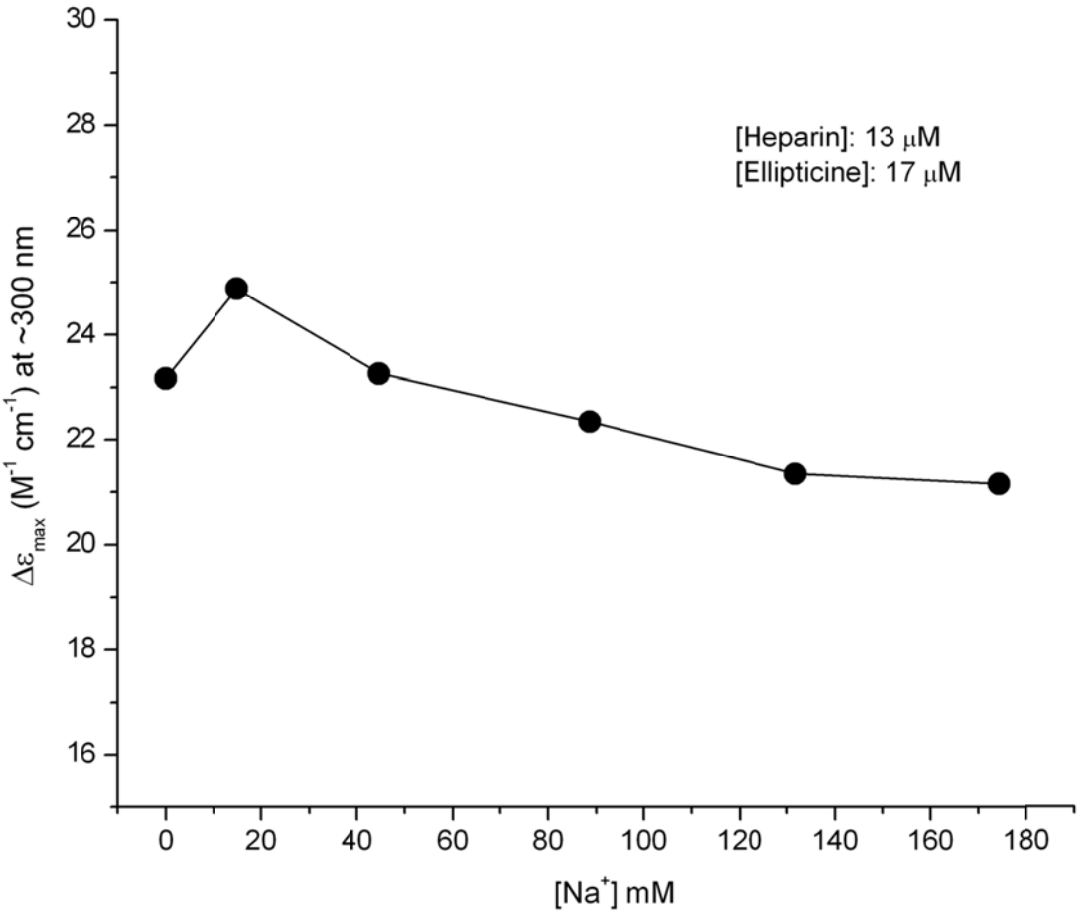
Supplementary Figure 3

CD and absorption spectra measured at increasing concentrations of ellipticine in pH 7.4 heparin solution (50 mM Tris-HCl buffer, 100 mM NaCl, 25 °C). Molar absorption coefficients (ε) calculated by using the alkaloid concentration are shown in parentheses.



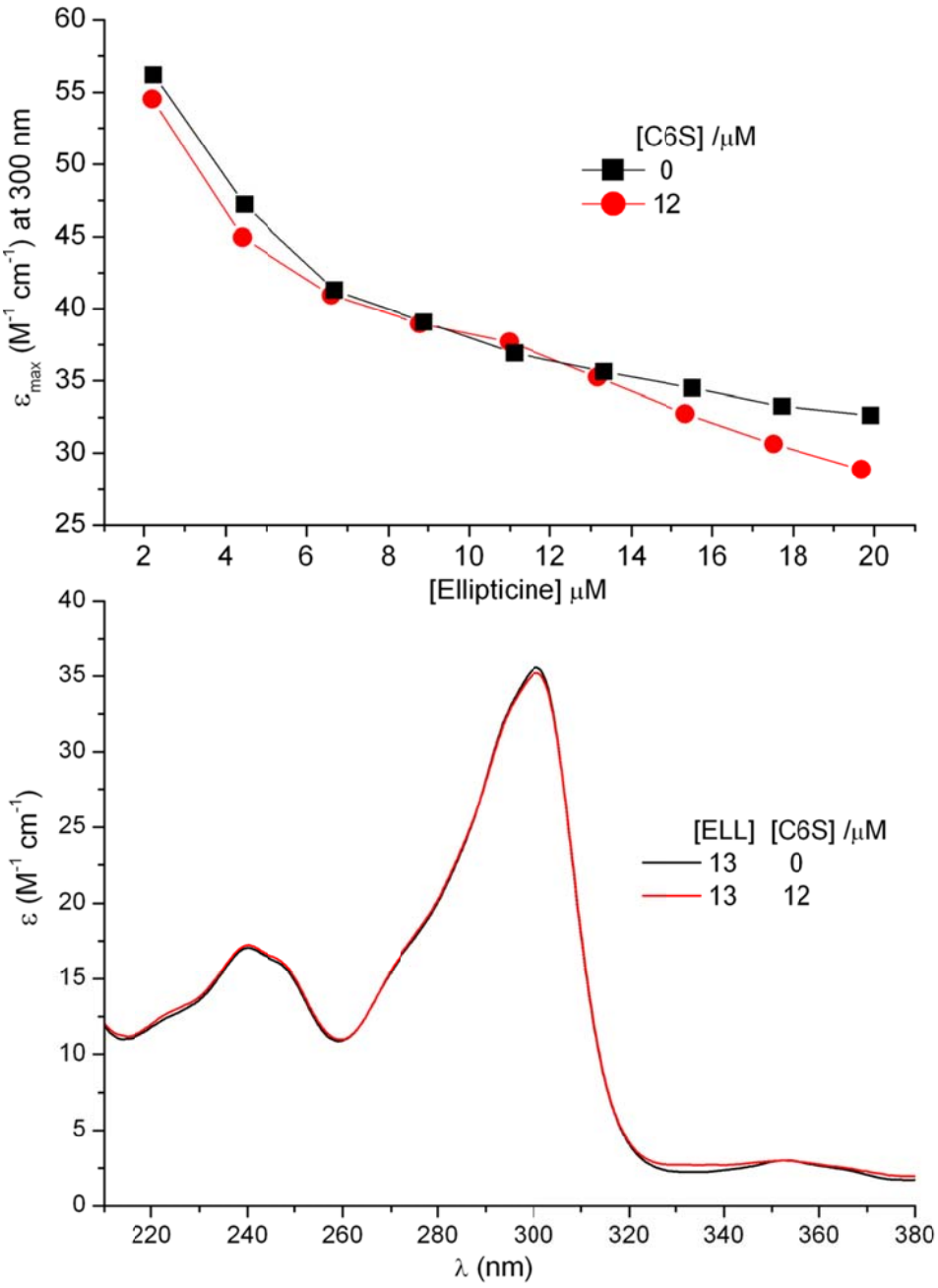
Supplementary Figure 4

Top panel: ε_{max} values of ellipticine (ELL) obtained in the absence and presence of heparin (HP) in pH 7.4 Tris-HCl buffer solution (100 mM NaCl, 25 °C). Bottom panel: UV absorption spectra of ellipticine measured in heparin and heparin-free pH 7.4 Tris-HCl buffer solution.



Supplementary Figure 5

Effect of increasing sodium ion concentration on the molar circular dichroic absorption coefficient ($\Delta\varepsilon_{max}$) of ellipticine bound to heparin (deionized water, 25 °C).



Supplementary Figure 6

Top panel: ε_{max} values of ellipticine obtained in the absence and presence of C6S in pH 6.3 Tris-HCl buffer solution (100 mM NaCl, 25 °C). Bottom panel: UV absorption spectra of ellipticine measured with C6S and in C6S-free solution (pH 6.3 Tris-HCl buffer).