

Electronic Supplementary Information to

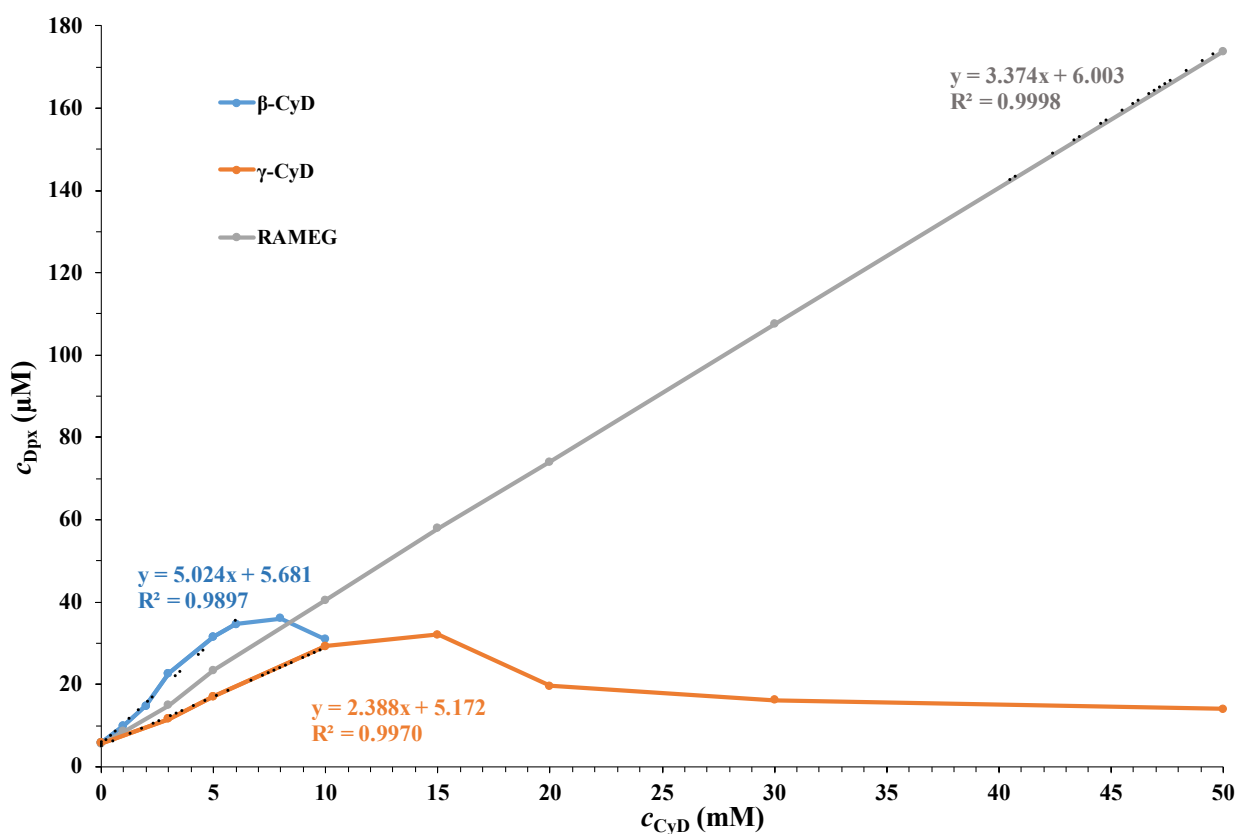
# NMR, CD and UV spectroscopic studies reveal uncommon binding modes of dapoxetine to native cyclodextrins

András Darcsi<sup>1</sup>, Zoltán Szakács<sup>2\*</sup>, Ferenc Zsila<sup>3</sup>, Gergő Tóth<sup>4</sup>, Ákos Rácz<sup>4</sup>, Szabolcs Béni<sup>1\*</sup>

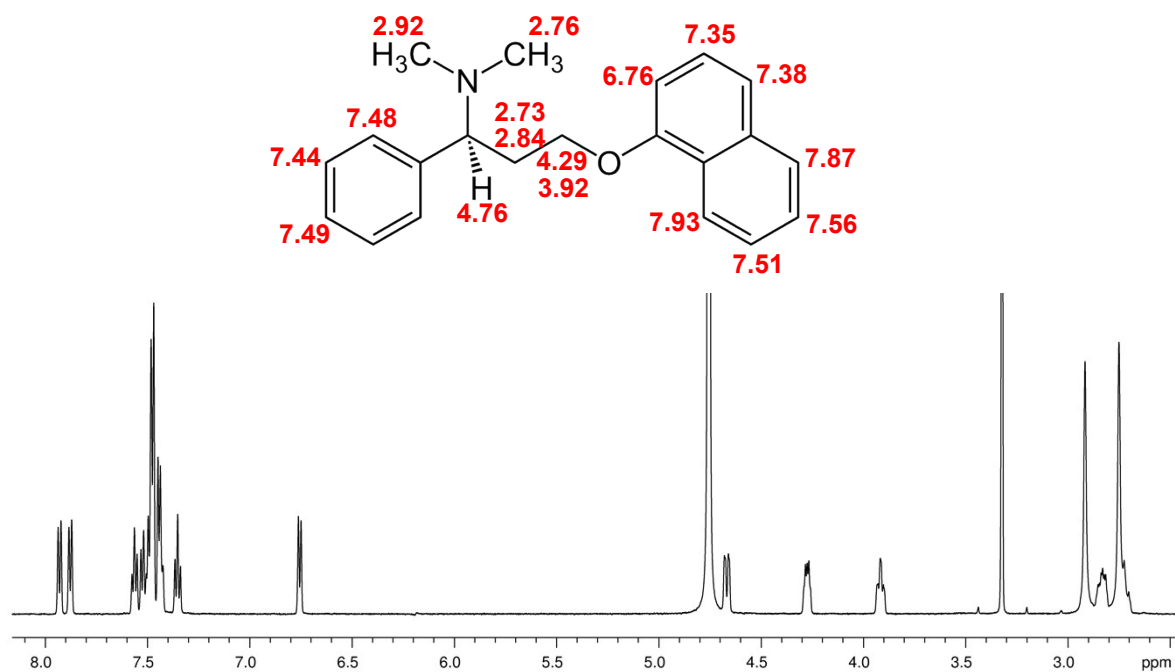
## Contents:

|                                                                                                                                                                             |           |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1. Phase solubility profile of Dpx with $\beta$ -, $\gamma$ - and random methylated $\gamma$ -CyDs at pH 7.4                                                                | Fig. S1.  |
| 2. <sup>1</sup> H NMR spectrum and assignment of Dpx (600MHz, acidified D <sub>2</sub> O)                                                                                   | Fig. S2.  |
| 3. <sup>1</sup> H NMR chemical shift titration spectra of Dpx with $\gamma$ -CyD (600MHz, acidified D <sub>2</sub> O)                                                       | Fig. S3.  |
| 4. Principal results of evaluation of the Dpx/ $\gamma$ -CyD NMR titration by the OPIUM                                                                                     | Table S1. |
| 5. Chemical shift profile of Dpx H15 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S4.  |
| 6. Chemical shift profile of Dpx H13 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S5.  |
| 7. Chemical shift profile of Dpx H14 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S6.  |
| 8. Chemical shift profile of Dpx H6 upon titration with $\gamma$ -CyD                                                                                                       | Fig. S7.  |
| 9. Chemical shift profile of Dpx H7 upon titration with $\gamma$ -CyD                                                                                                       | Fig. S8.  |
| 10. Chemical shift profile of Dpx H8 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S9.  |
| 11. Chemical shift profile of Dpx H1 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S10. |
| 12. Chemical shift profile of Dpx H3 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S11. |
| 13. Chemical shift profile of Dpx H3' upon titration with $\gamma$ -CyD                                                                                                     | Fig. S12. |
| 14. Chemical shift profile of Dpx H2 upon titration with $\gamma$ -CyD                                                                                                      | Fig. S13. |
| 15. Chemical shift profile of Dpx H2' upon titration with $\gamma$ -CyD                                                                                                     | Fig. S14. |
| 16. Chemical shift profile of $\gamma$ -CyD H3 in the titration of Dpx                                                                                                      | Fig. S15. |
| 17. Chemical shift displacement values with respect to free Dpx upon formation of the Dpx· $\gamma$ -CyD and 2Dpx· $\gamma$ -CyD complexes, results of the OPIUM evaluation | Fig. S16. |
| 18. Expanded region of the 600 MHz 2D ROESY spectrum of 0.9 mM Dpx and 2.1 mM $\gamma$ -CyD                                                                                 | Fig. S17. |
| 19. <sup>1</sup> H NMR chemical shift titration spectra of Dpx with $\beta$ -CyD (600MHz, acidified D <sub>2</sub> O)                                                       | Fig. S18. |
| 20. Principal results of evaluation of the Dpx/ $\beta$ -CyD NMR titration by the OPIUM                                                                                     | Table S2. |
| 21. Chemical shift profile of Dpx H15 upon titration with $\beta$ -CyD                                                                                                      | Fig. S19. |
| 22. Chemical shift profile of Dpx H12 upon titration with $\beta$ -CyD                                                                                                      | Fig. S20. |
| 23. Chemical shift profile of Dpx H13 upon titration with $\beta$ -CyD                                                                                                      | Fig. S21. |
| 24. Chemical shift profile of Dpx H9 upon titration with $\beta$ -CyD                                                                                                       | Fig. S22. |

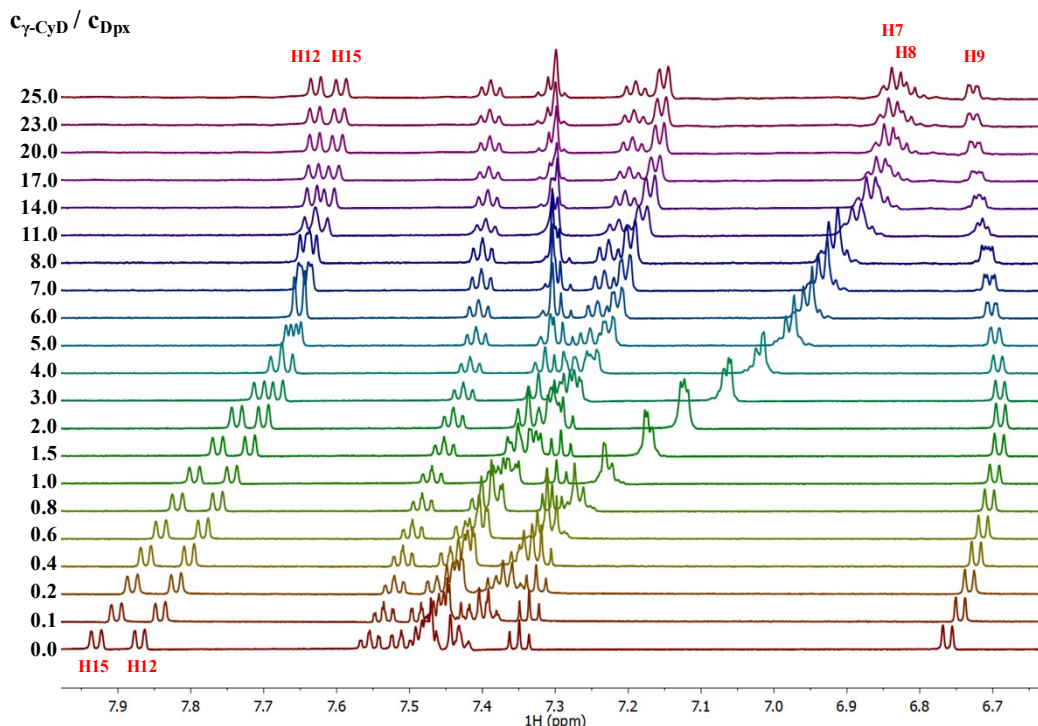
25. Chemical shift profile of Dpx H1 upon titration with  $\beta$ -CyD **Fig. S23.**
26. Chemical shift profile of Dpx H2' upon titration with  $\beta$ -CyD **Fig. S24.**
27. Chemical shift profile of Dpx H3' upon titration with  $\beta$ -CyD **Fig. S25.**
28. Chemical shift profile of Dpx H1 upon titration with  $\beta$ -CyD **Fig. S26.**
29. Chemical shift profile of Dpx H3 upon titration with  $\beta$ -CyD **Fig. S27.**
30. Chemical shift profile of Dpx H5 upon titration with  $\beta$ -CyD **Fig. S28.**
31. Chemical shift profile of Dpx H6 upon titration with  $\beta$ -CyD **Fig. S29.**
32. Chemical shift profile of Dpx H4 upon titration with  $\beta$ -CyD **Fig. S30.**
33. Species distribution plot for the NMR titration of Dpx with  $\beta$ -CyD, generated with the HysS software **Fig. S31.**
34. Chemical shift displacement values with respect to free Dpx upon formation of the Dpx· $\beta$ -CyD and 2Dpx· $\beta$ -CyD complexes, results of the OPIUM evaluation **Fig. S32.**
35. Expanded region of the 600 MHz 2D ROESY spectrum of 1.5 mM Dpx and 1.5 mM  $\beta$ -CyD **Fig. S33.**
36. Changes of the CD and UV absorption spectrum of 23  $\mu$ M Dpx measured upon sequential addition of  $\beta$ -CyD into the sample solution (pH  $\sim$  4) **Fig. S34.**
37. CD and UV absorption spectra of Dpx measured in acetonitrile and 1,4-dioxane (25 °C) **Fig. S35.**



**Fig. S1.** Phase solubility profile of Dpx with  $\beta$ -,  $\gamma$ - and random methylated  $\gamma$ -CyDs at pH 7.4



**Fig. S2.** 600 MHz  $^1\text{H}$  NMR spectrum and assignment (in ppm) of 3 mM Dpx in  $\text{D}_2\text{O}$  solution (acidified by 40 mM  $\text{CD}_3\text{COOD}$ ), referenced to internal  $\text{CH}_3\text{OH}$  at 3.32 ppm



**Fig. S3.** 600 MHz  $^1\text{H}$  NMR chemical shift titration spectra of Dpx with  $\gamma$ -CyD

**Table S1.** Principal results of evaluation of the Dpx/ $\gamma$ -CyD NMR titration by the OPIUM computer program: ranking of the equilibrium models according to two goodness-of-fit criteria (both indicators yield lower values for globally better fitting models)

| Equilibrium model   | The iterated equilibrium constant(s)<br>$\pm$ their standard deviation |                 | Hamilton's<br>R factor | Akaike's information<br>criterion |
|---------------------|------------------------------------------------------------------------|-----------------|------------------------|-----------------------------------|
| {Dpx·CyD, 2Dpx·CyD} | $\log K_{11}$                                                          | $2.82 \pm 0.04$ | 0.0733                 | 824.97                            |
|                     | $\log (K_{11}K_{21})$                                                  | $5.47 \pm 0.09$ |                        |                                   |
| {Dpx·CyD, Dpx·2CyD} | $\log K_{11}$                                                          | $3.63 \pm 0.09$ | 0.0871                 | 914.30                            |
|                     | $\log (K_{11}K_{12})$                                                  | $6.0 \pm 0.1$   |                        |                                   |
| {Dpx·CyD}           | $\log K_{11}$                                                          | $2.78 \pm 0.01$ | 0.2053                 | 1332.37                           |
| {2Dpx·CyD}          | $\log K_{21}$                                                          | $5.48 \pm 0.01$ | 0.2191                 | 1366.07                           |
| {Dpx·2CyD}          | $\log K_{12}$                                                          | $6.12 \pm 0.05$ | 0.5249                 | 1820.49                           |

Definition of Hamilton's R factor:

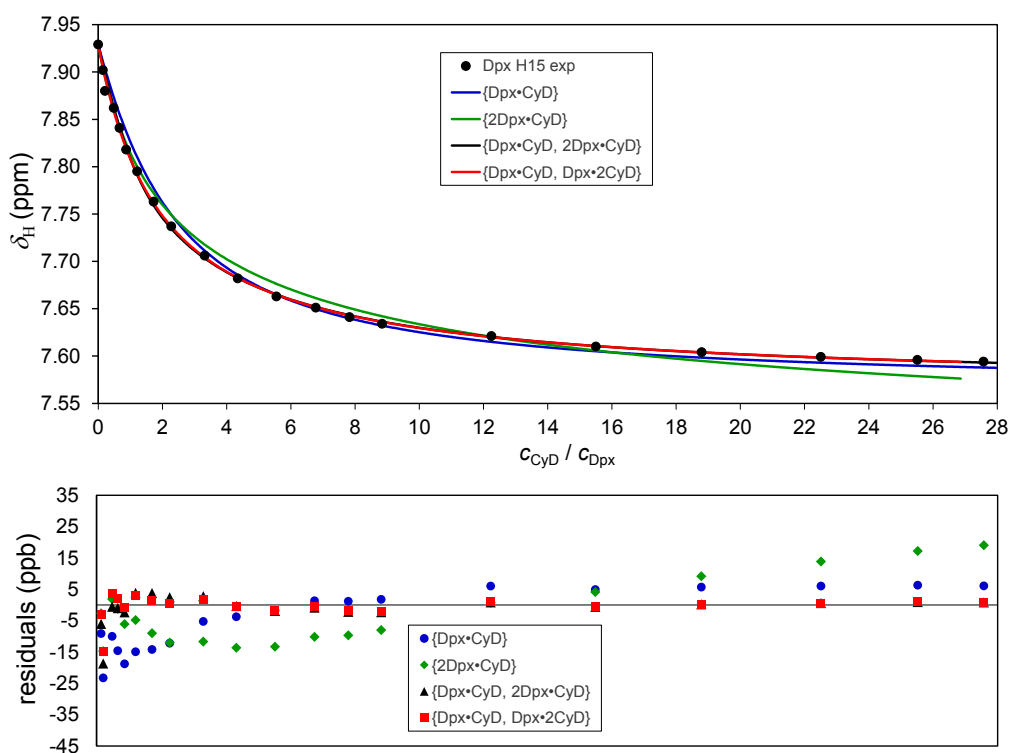
$$R = \sqrt{\frac{\sum_{k=1}^n (y_{\text{exp},k} - y_{\text{calc},k})^2}{\sum_{k=1}^n y_{\text{exp},k}^2}}$$

Definition of Akaike's information criterion:

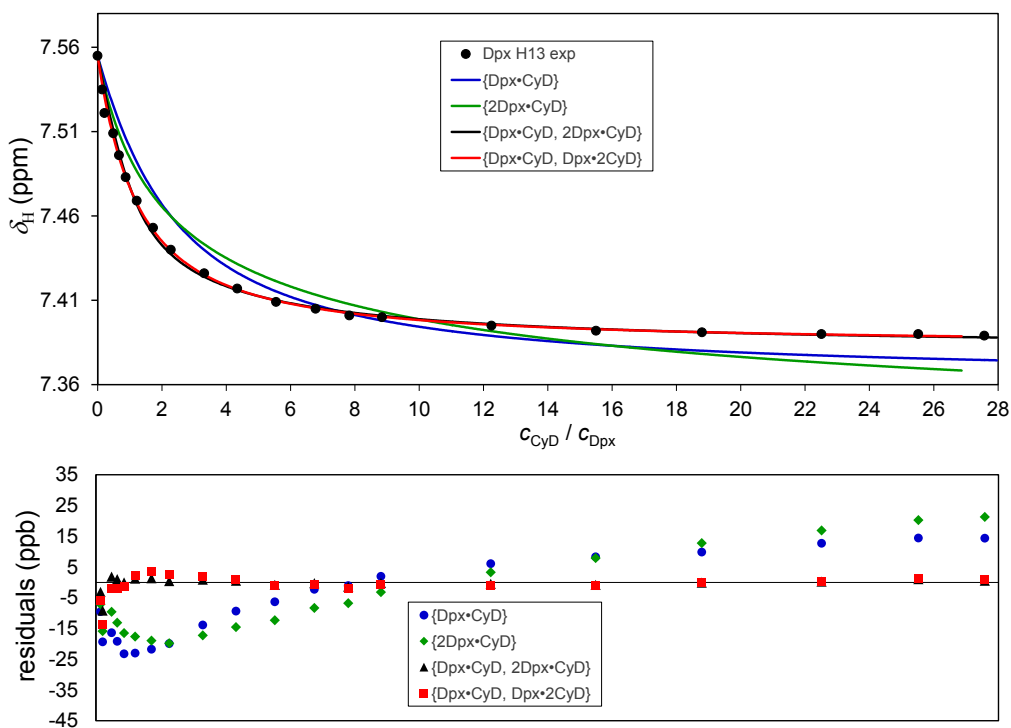
$$\text{AIC} = n \ln \left( \frac{\sum_{k=1}^n (y_{\text{exp},k} - y_{\text{calc},k})^2}{n} \right) + 2m$$

where  $y_k$  is the measured or by the model calculated chemical shift at the  $k$ th point of titration,  $n$  is the number of all data points and  $m$  is the number of estimated parameters.

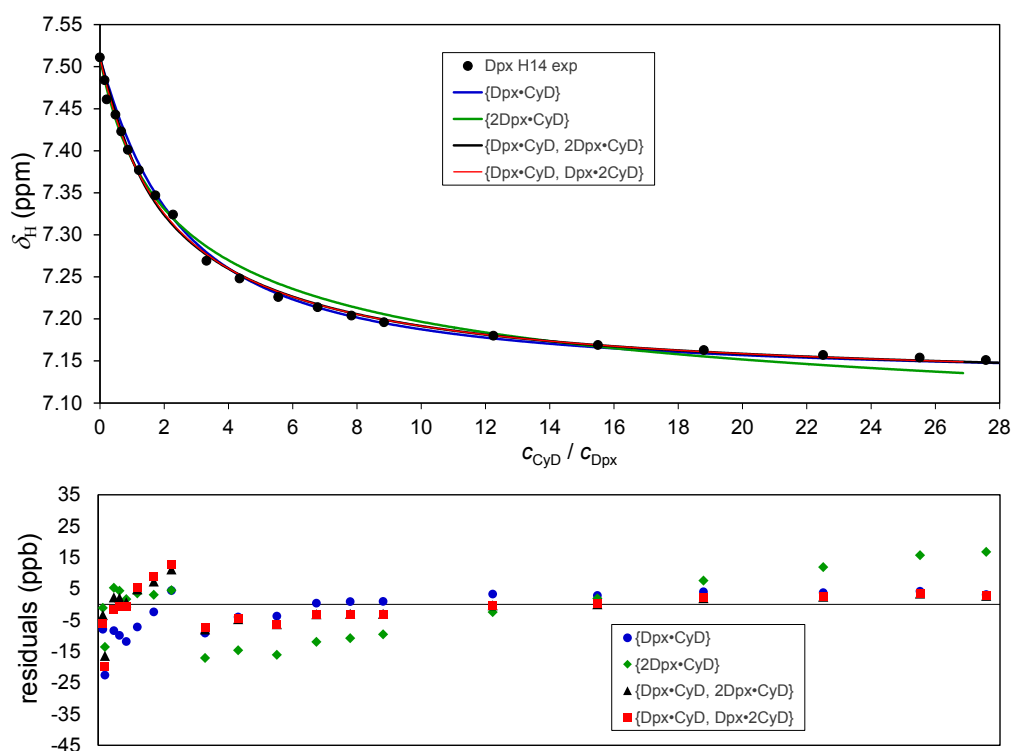
For further details, see e.g. Meloun, M., Pluharová, M. *Analytica Chimica Acta* 2000. 416: 55-68.



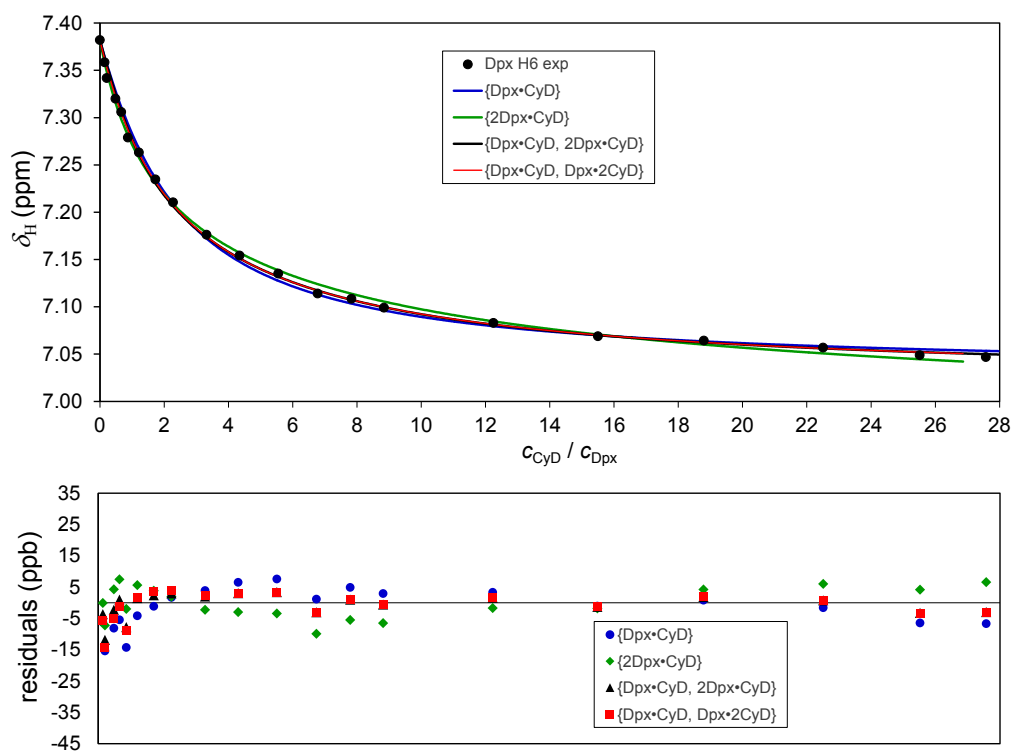
**Fig. S4.** Chemical shift profile of Dpx H15 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



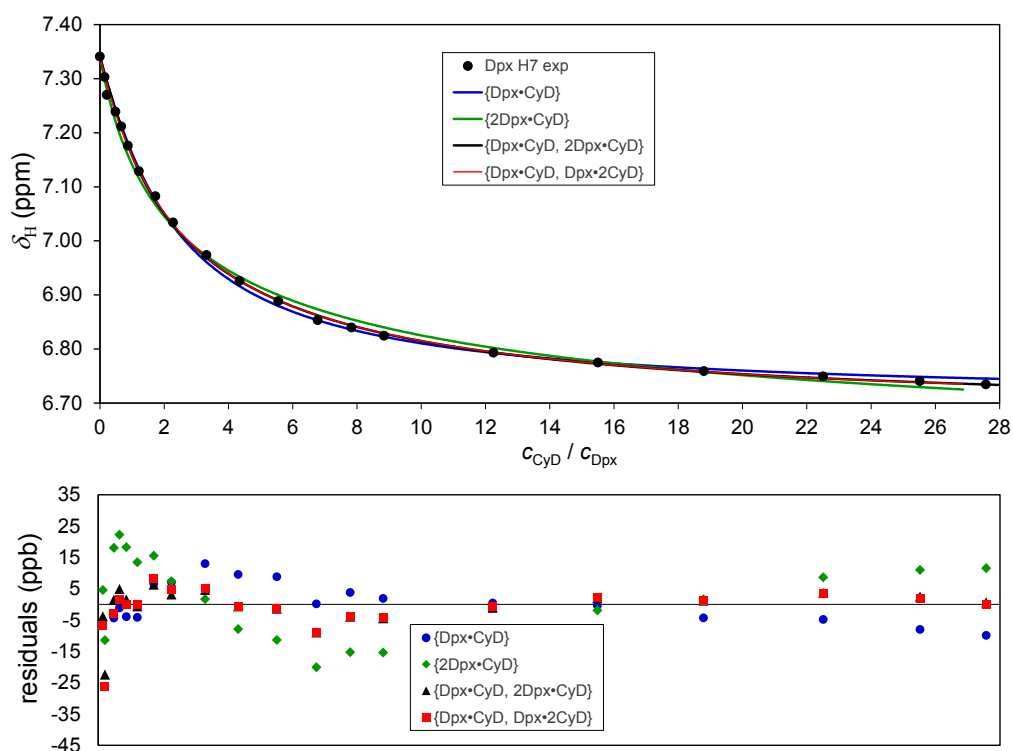
**Fig. S5.** Chemical shift profile of Dpx H13 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



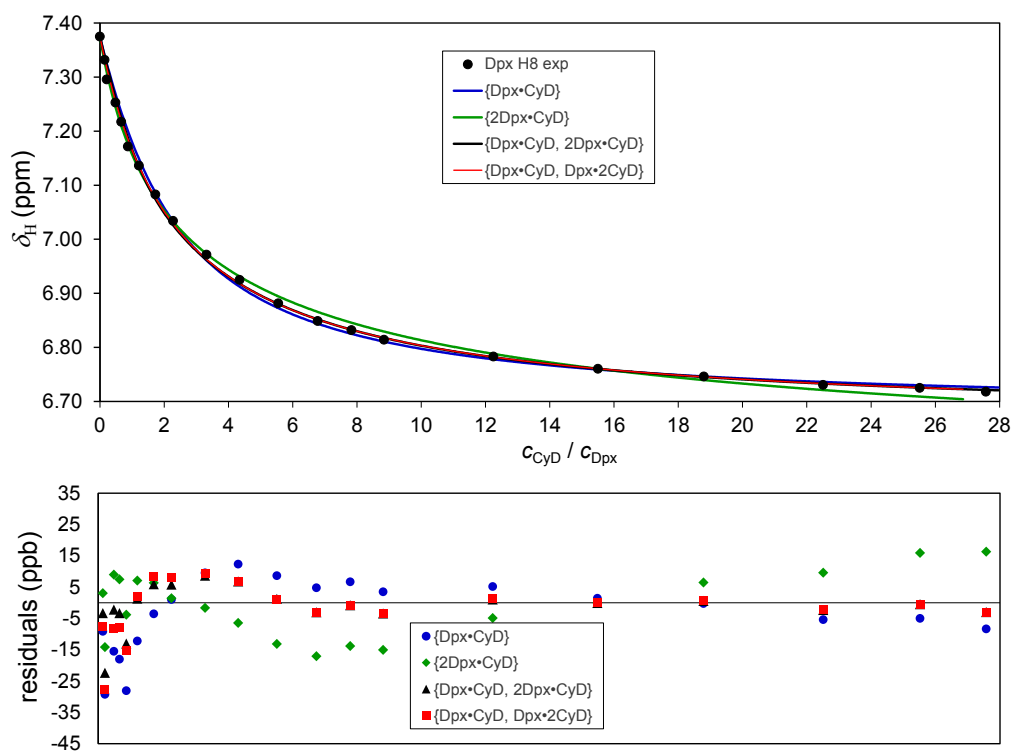
**Fig. S6.** Chemical shift profile of Dpx H14 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



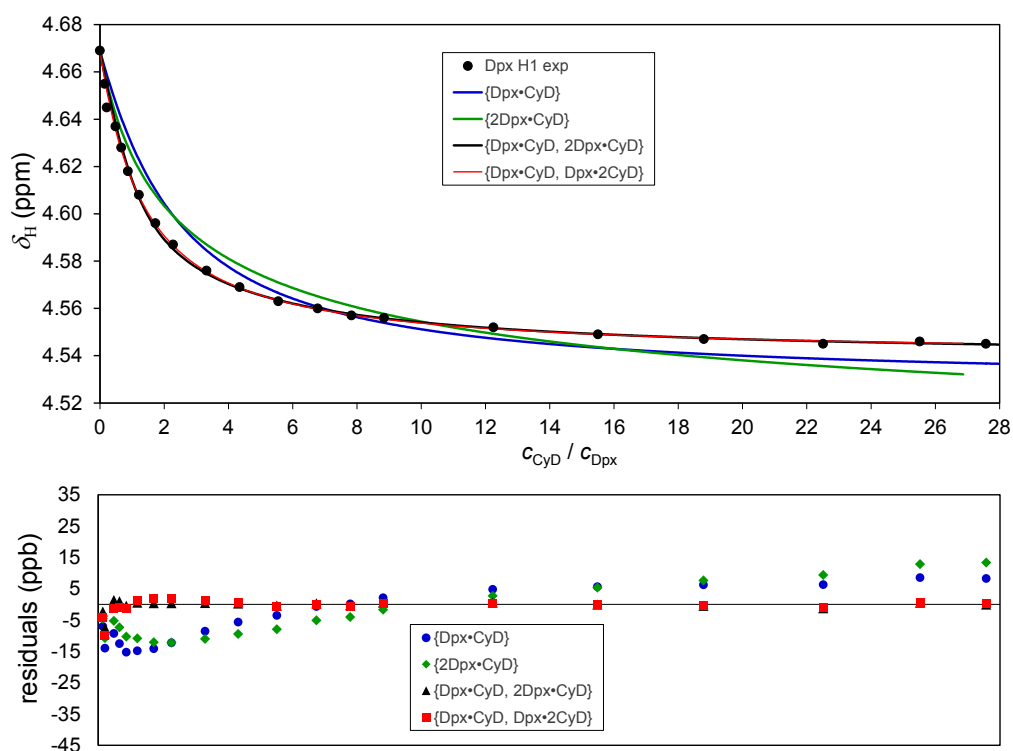
**Fig. S7.** Chemical shift profile of Dpx H6 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



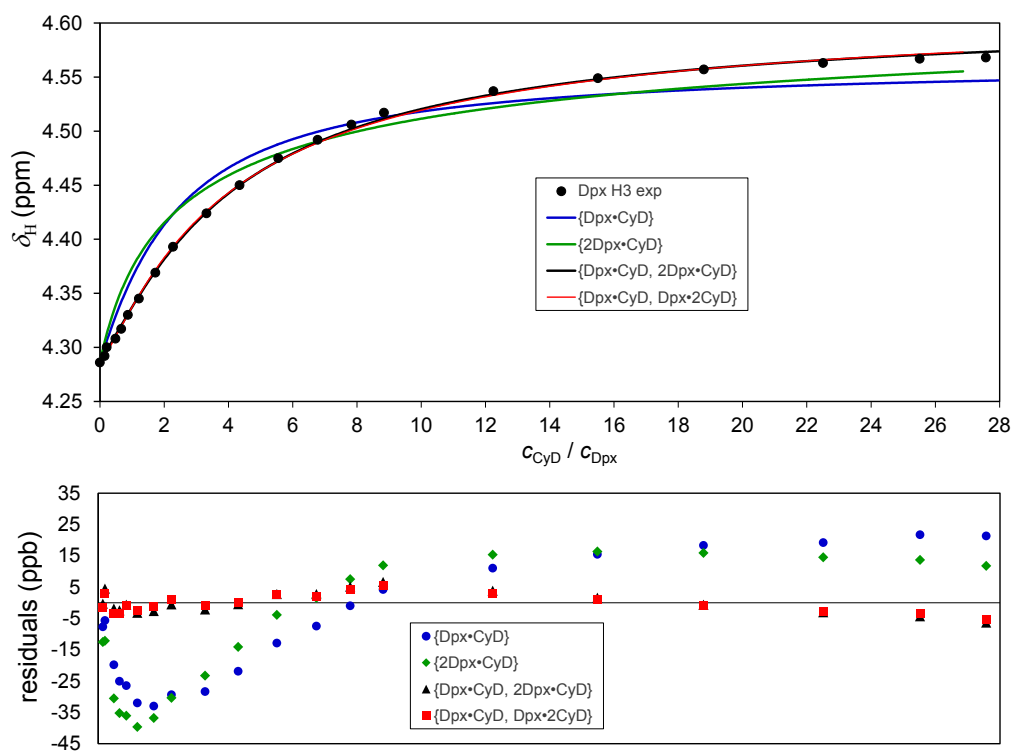
**Fig. S8.** Chemical shift profile of Dpx H7 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S9.** Chemical shift profile of Dpx H8 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

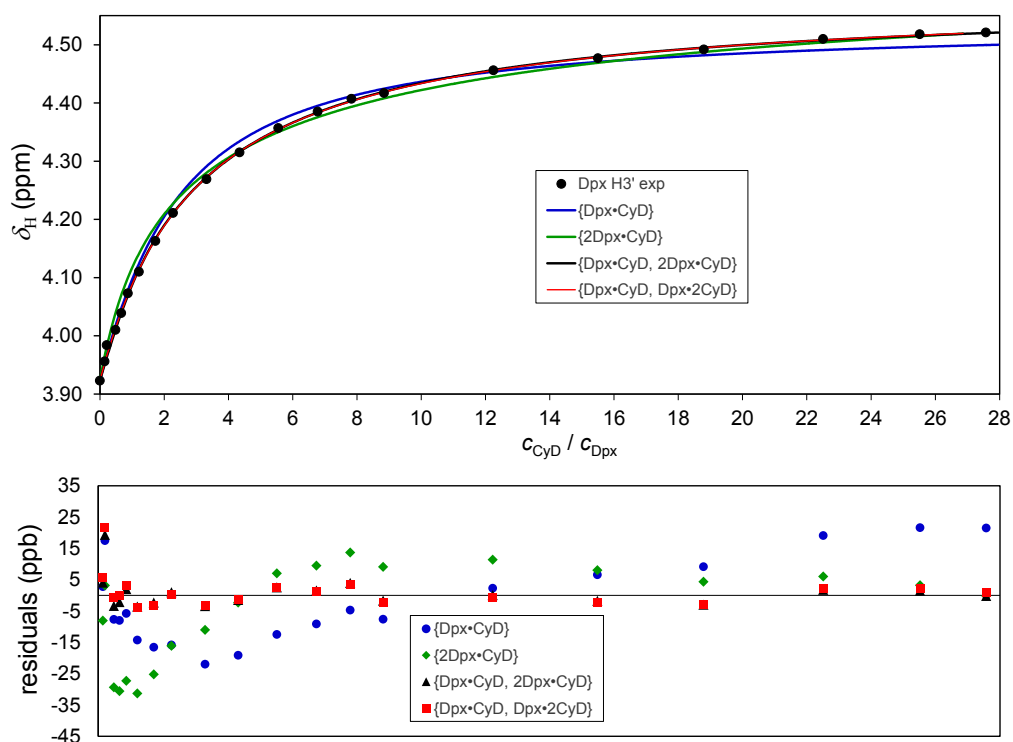


**Fig. S10.** Chemical shift profile of Dpx H1 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

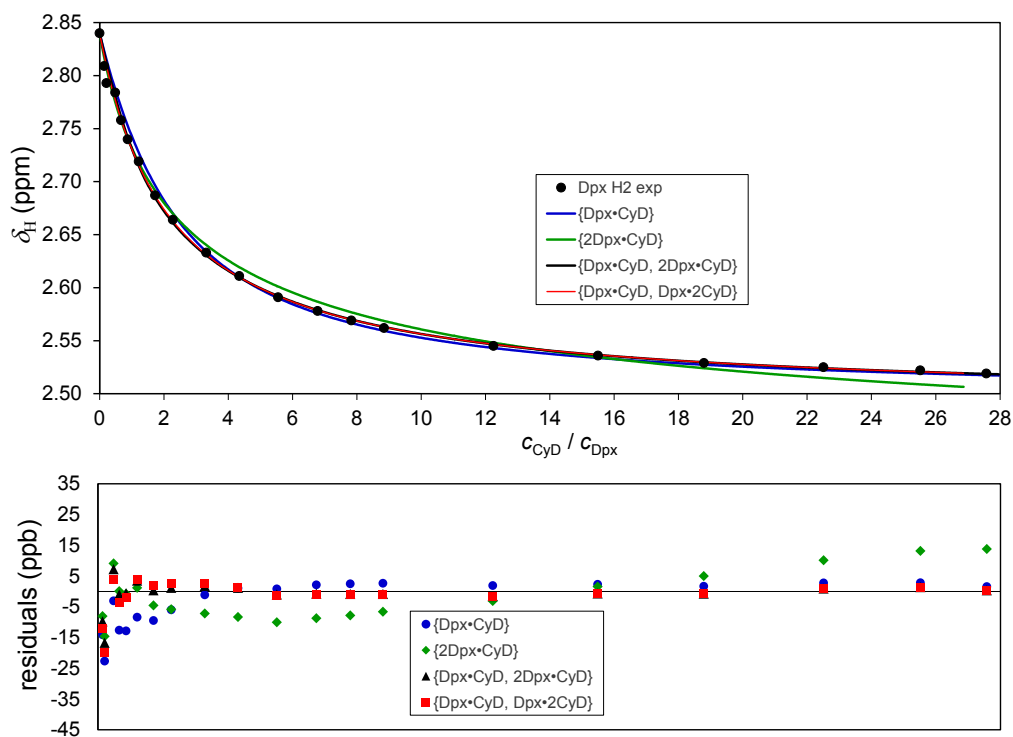


**Fig. S11.** Chemical shift profile of Dpx H3 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

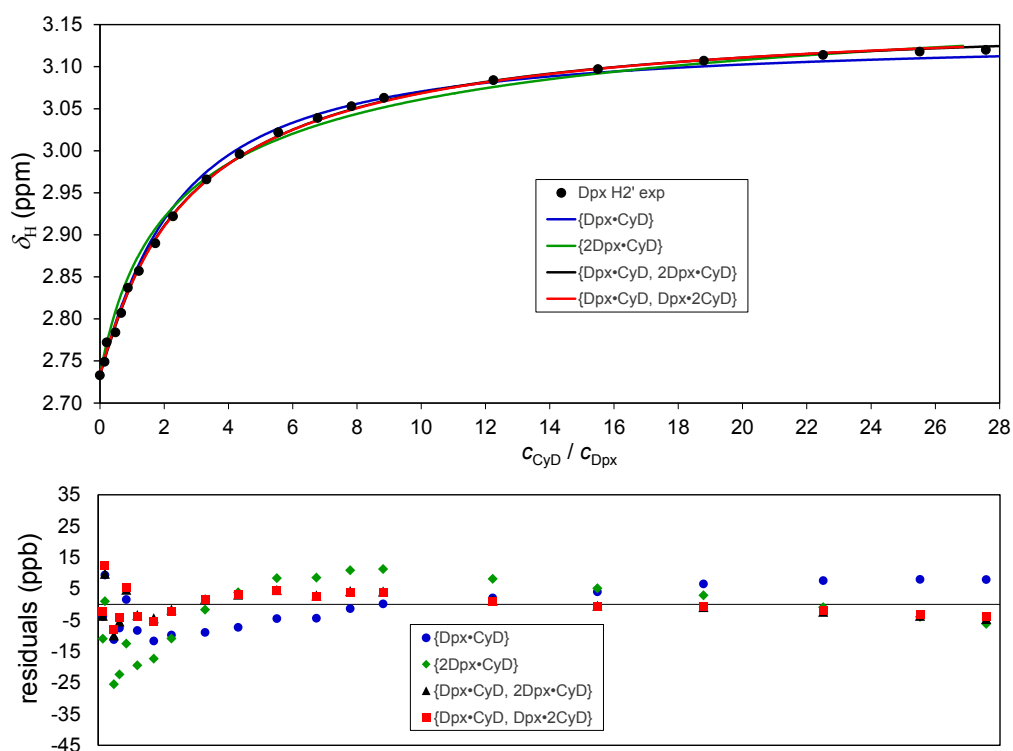




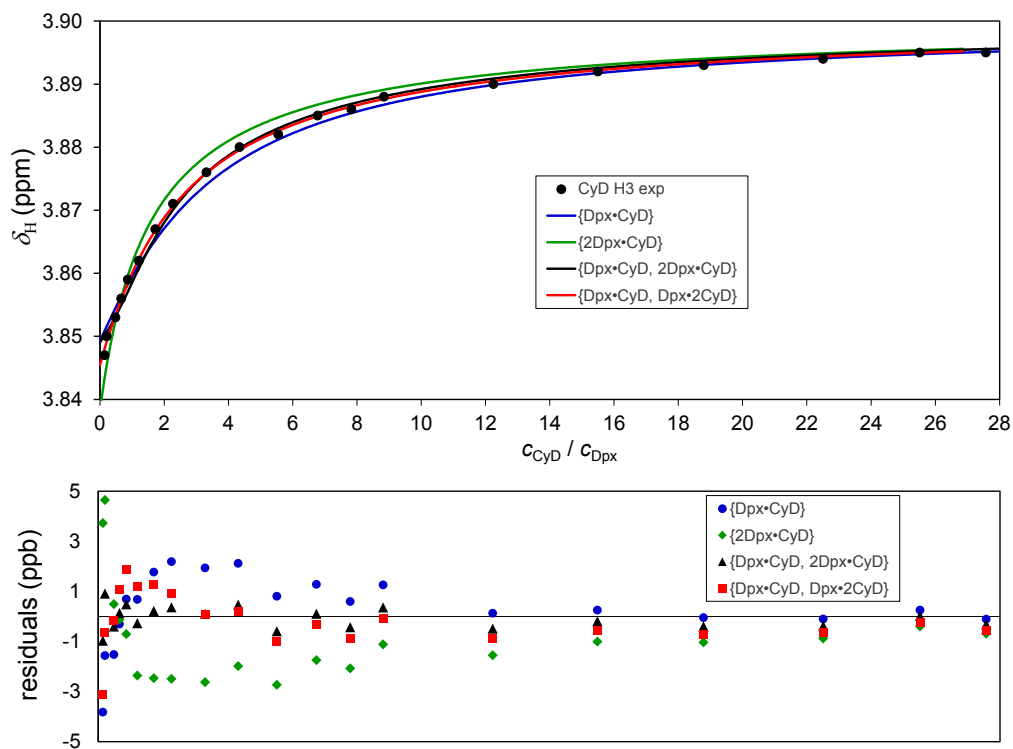
**Fig. S12.** Chemical shift profile of Dpx H3' upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S13.** Chemical shift profile of Dpx H2 upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S14.** Chemical shift profile of Dpx H2' upon titration with  $\gamma$ -CyD. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



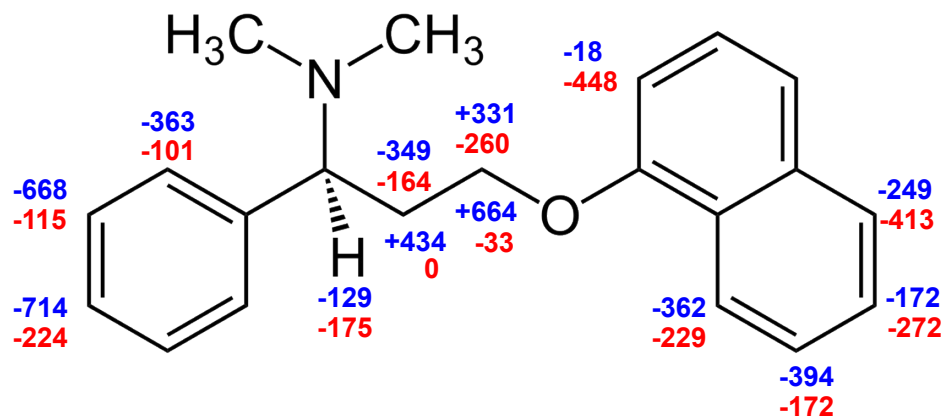
**Fig. S15.** Chemical shift profile of  $\gamma$ -CyD H3 in the titration of Dpx. Four equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

blue values in ppm:

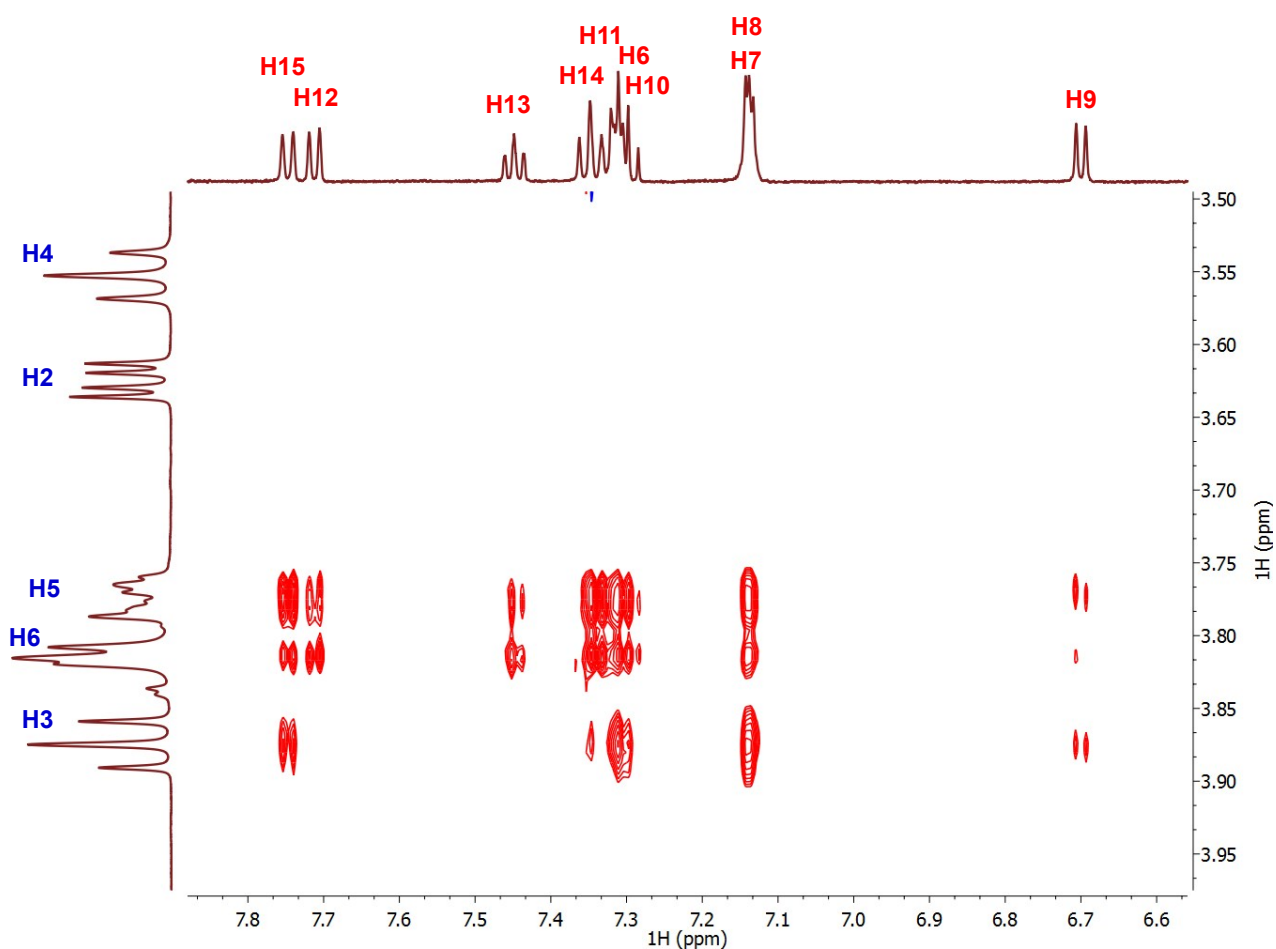
$$\Delta\delta_{\text{Dpx}\cdot\text{CyD}}^i = \delta_{\text{Dpx}\cdot\text{CyD}}^i - \delta_{\text{Dpx}}^i$$

red values in ppb:

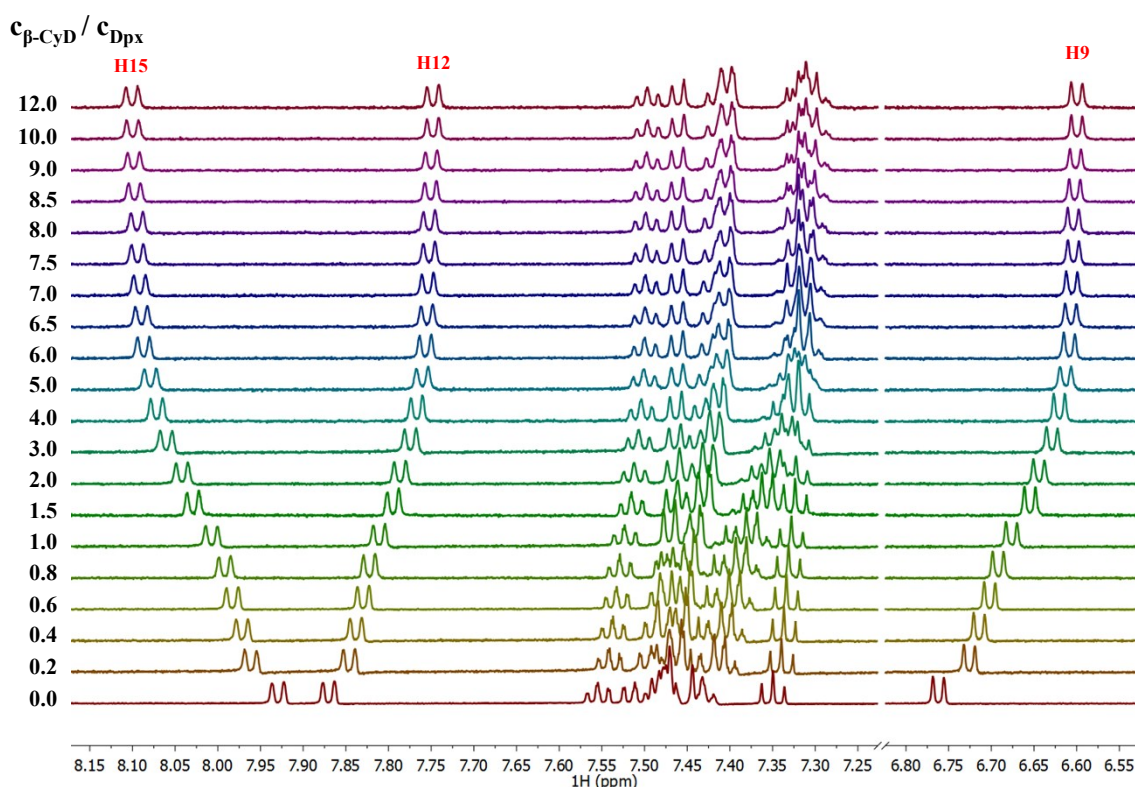
$$\Delta\delta_{2\text{Dpx}\cdot\text{CyD}}^i = \delta_{2\text{Dpx}\cdot\text{CyD}}^i - \delta_{\text{Dpx}}^i$$



**Fig. S16.** Chemical shift displacement values with respect to free Dpx upon formation of the Dpx·γ-CyD and 2Dpx·γ-CyD complexes, results of the OPIUM evaluation



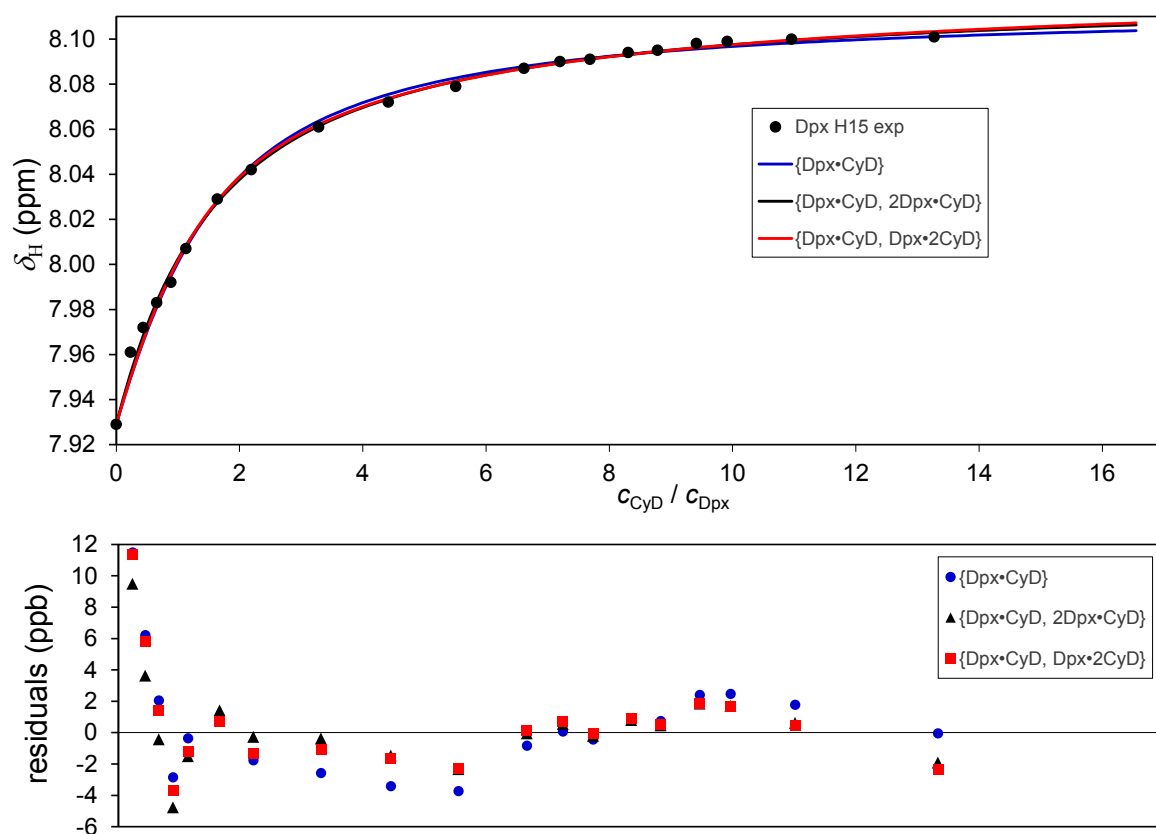
**Fig. S17.** Expanded region of the 600 MHz 2D ROESY spectrum of 0.9 mM Dpx (horizontal trace with assignment) and 2.1 mM γ-CyD (vertical trace with assignment)



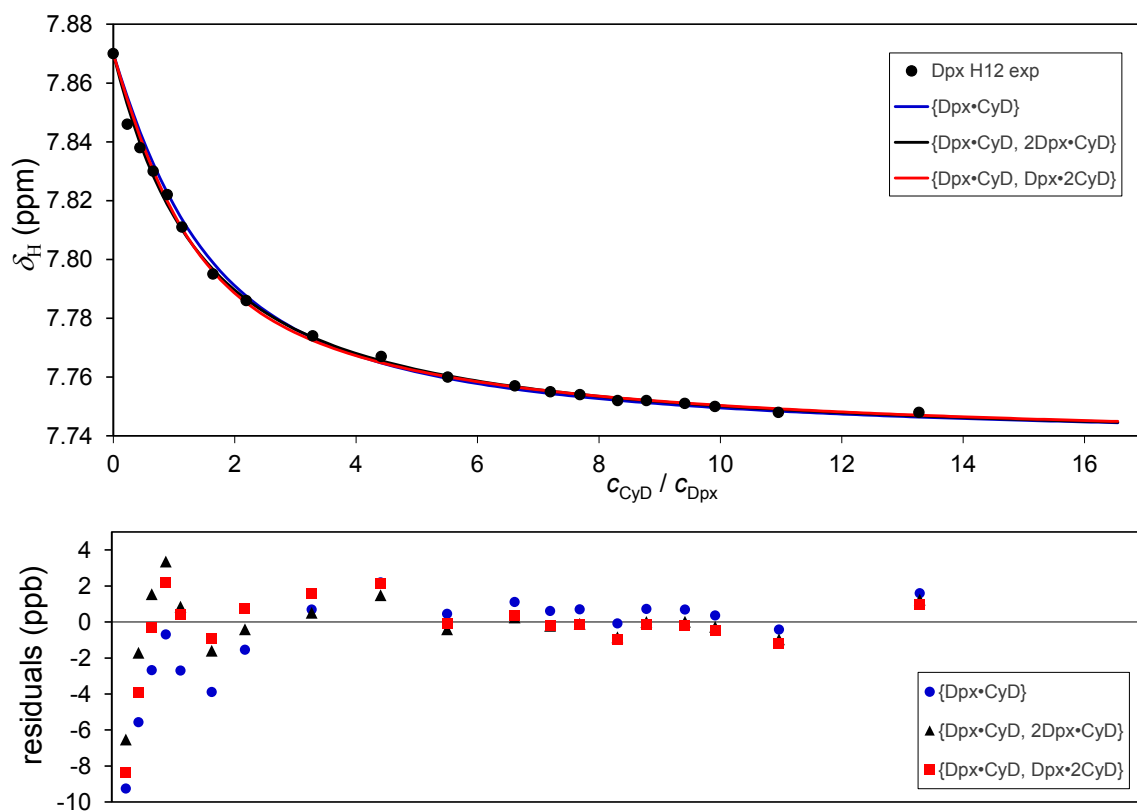
**Fig. S18.** 600 MHz  $^1\text{H}$  NMR chemical shift titration spectra of Dpx with  $\beta\text{-CyD}$

**Table S2.** Principal results of evaluation of the Dpx/ $\beta\text{-CyD}$  NMR titration by the OPIUM computer program: ranking of the equilibrium models according to two goodness-of-fit criteria (both indicators yield lower values for globally better fitting models; for their definitions, see Table S1)

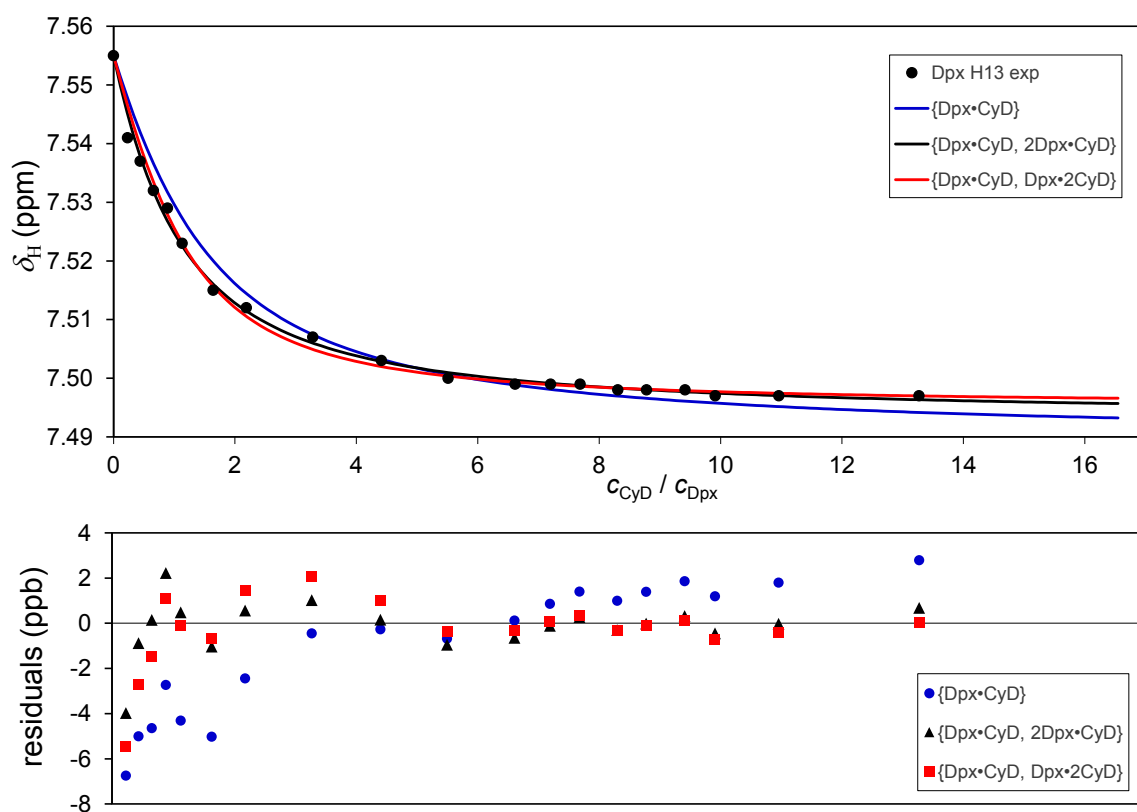
| Equilibrium model   | The iterated equilibrium constant(s)<br>$\pm$ their standard deviation |                 | Hamilton's<br>R factor | Akaike's information<br>criterion |
|---------------------|------------------------------------------------------------------------|-----------------|------------------------|-----------------------------------|
| {Dpx·CyD, 2Dpx·CyD} | $\log K_{11}$                                                          | $2.96 \pm 0.09$ | 0.0473                 | 482.08                            |
|                     | $\log (K_{11}K_{21})$                                                  | $5.40 \pm 0.25$ |                        |                                   |
| {Dpx·CyD, Dpx·2CyD} | $\log K_{11}$                                                          | $3.4 \pm 0.1$   | 0.0516                 | 521.24                            |
|                     | $\log (K_{11}K_{12})$                                                  | $5.7 \pm 0.3$   |                        |                                   |
| {Dpx·CyD}           | $\log K_{11}$                                                          | $3.06 \pm 0.01$ | 0.0668                 | 613.15                            |
| {2Dpx·CyD}          | $\log K_{21}$                                                          | $5.62 \pm 0.02$ | 0.0893                 | 745.75                            |
| {Dpx·2CyD}          | $\log K_{12}$                                                          | $7.31 \pm 0.09$ | 0.1846                 | 1076.7                            |



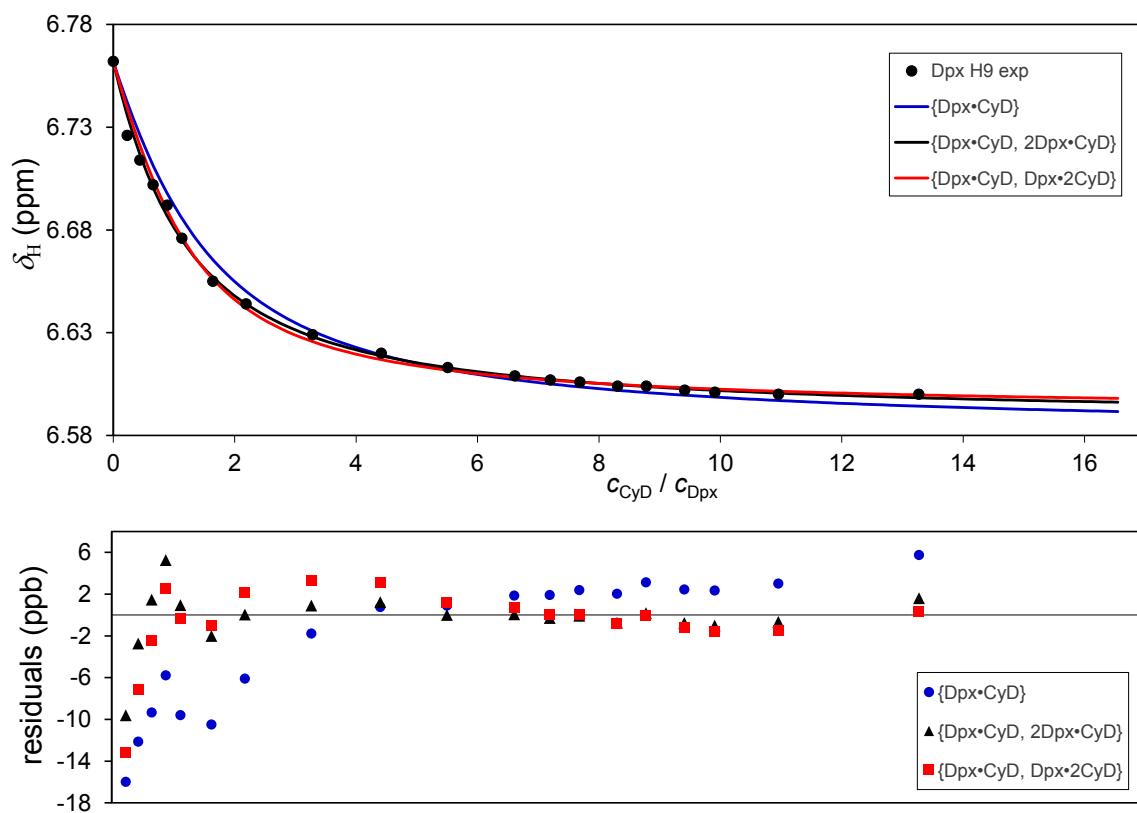
**Fig. S19.** Chemical shift profile of Dpx H15 upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



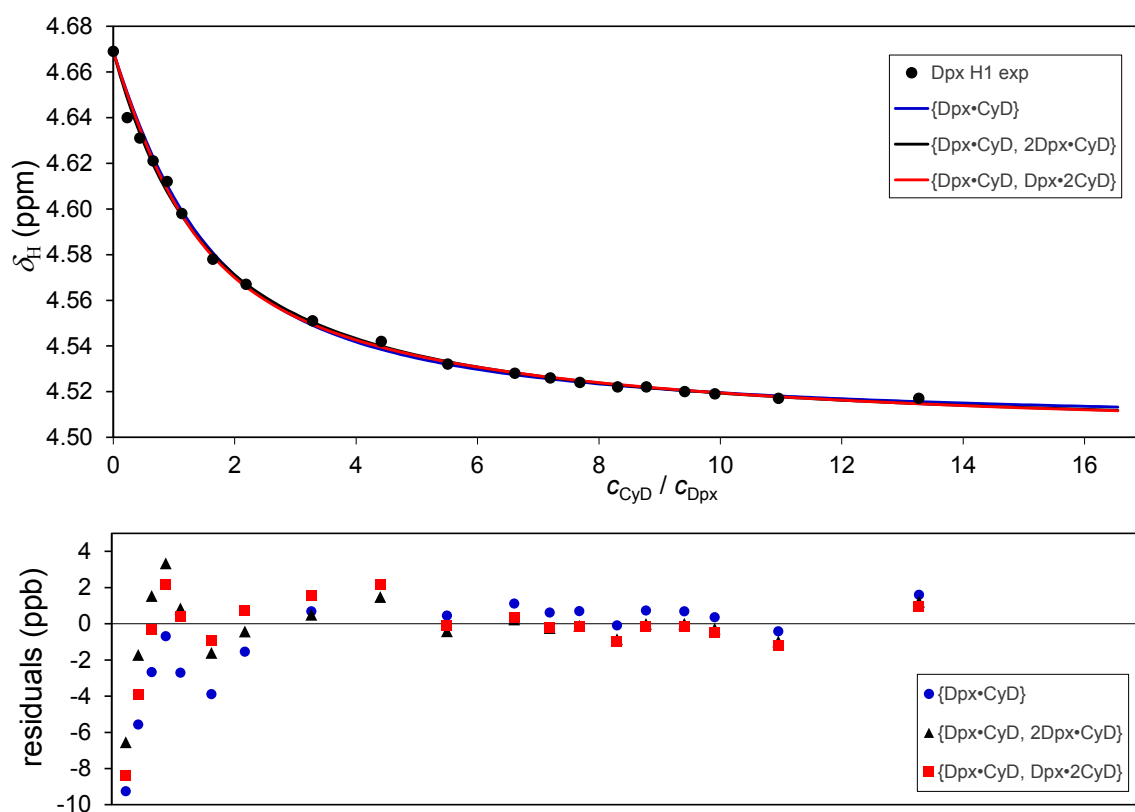
**Fig. S20.** Chemical shift profile of Dpx H12 upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



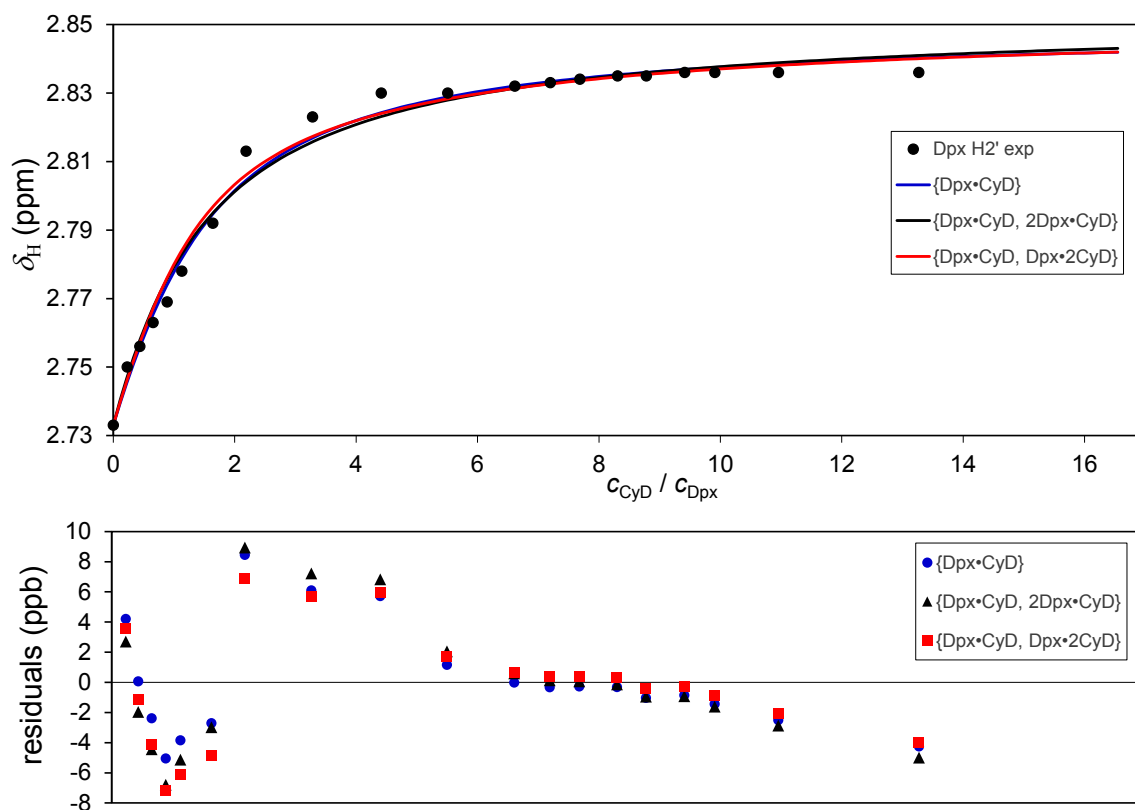
**Fig. S21.** Chemical shift profile of Dpx H13 upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



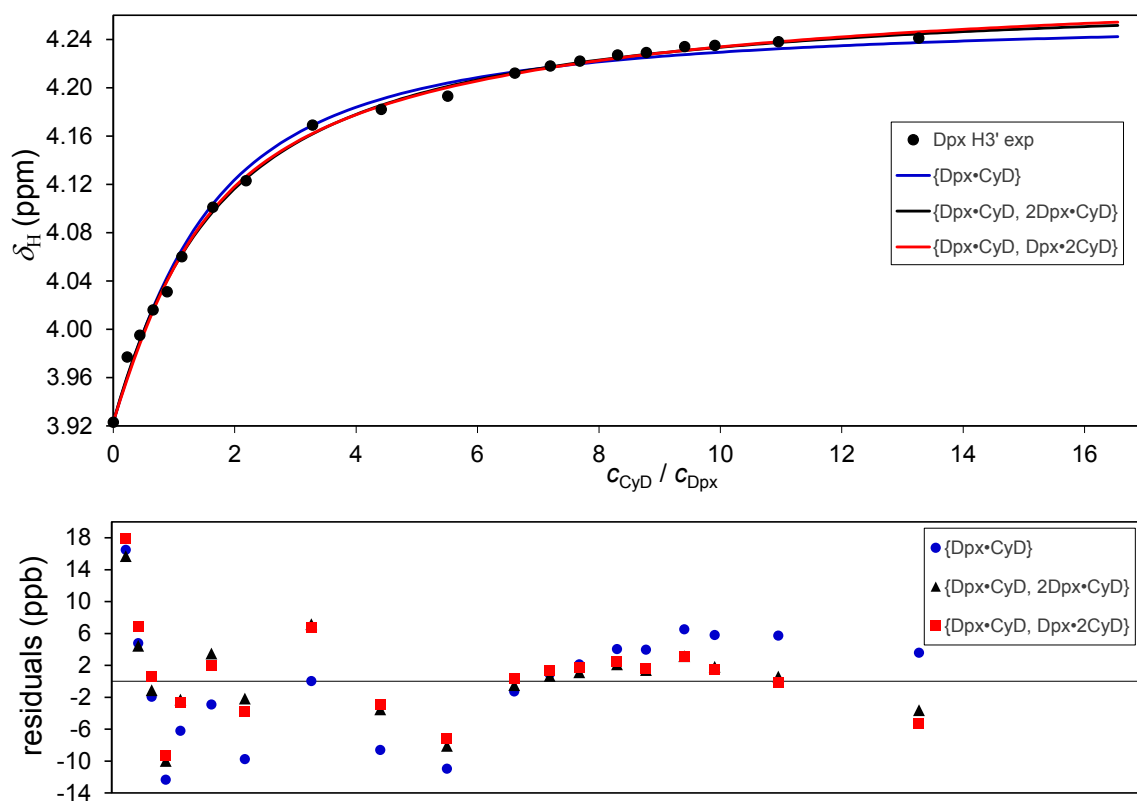
**Fig. S22.** Chemical shift profile of Dpx H9 upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



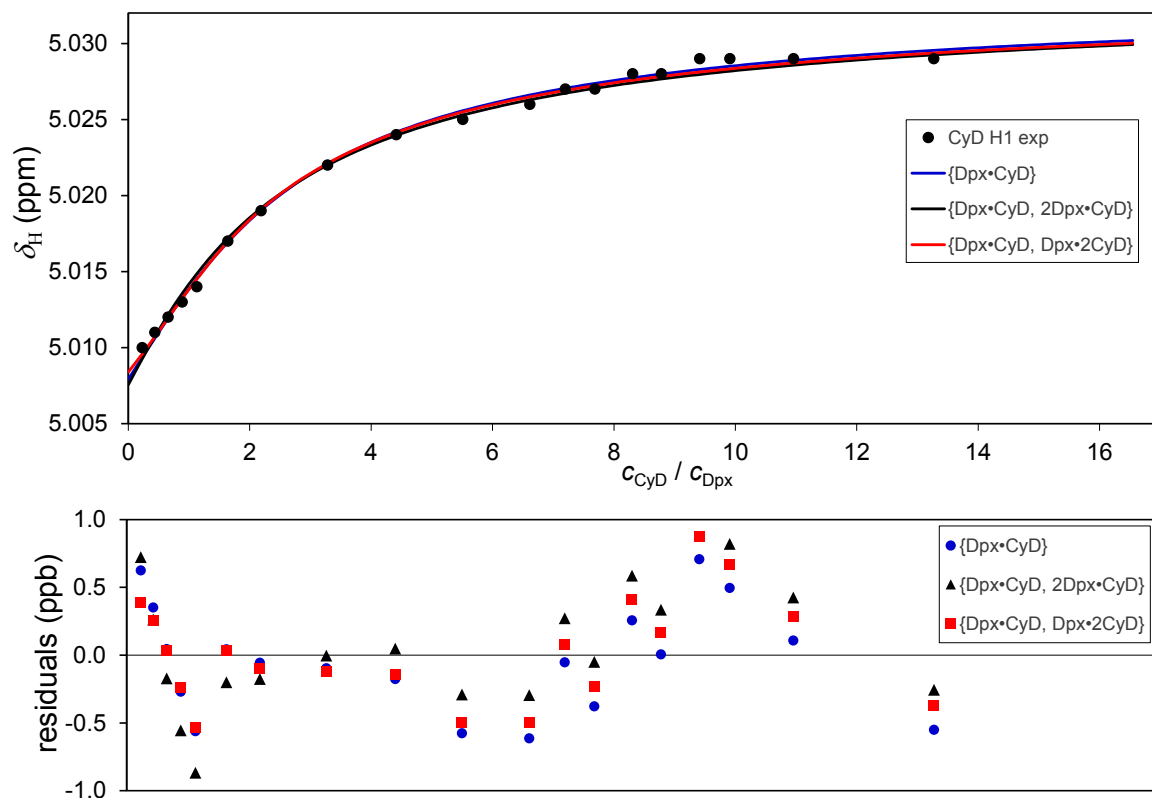
**Fig. S23.** Chemical shift profile of Dpx H1 upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S24.** Chemical shift profile of Dpx H2' upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

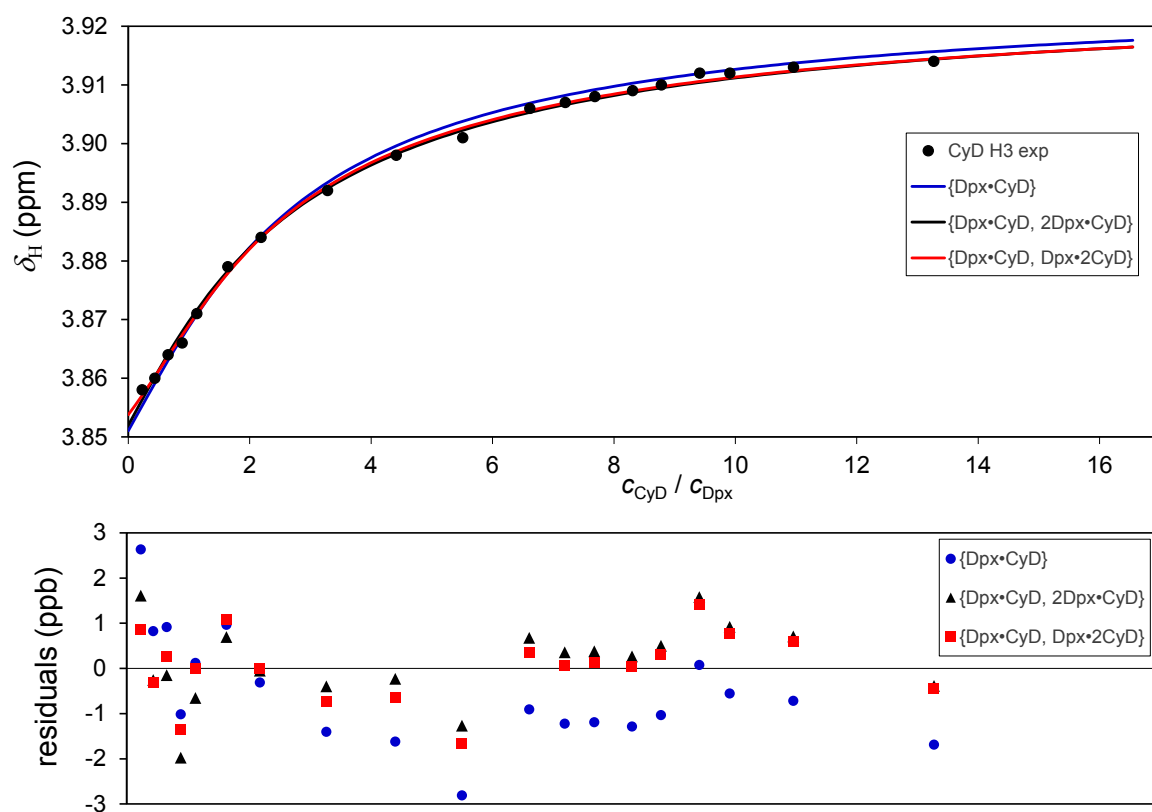


**Fig. S25.** Chemical shift profile of Dpx H3' upon titration with  $\beta$ -CyD. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

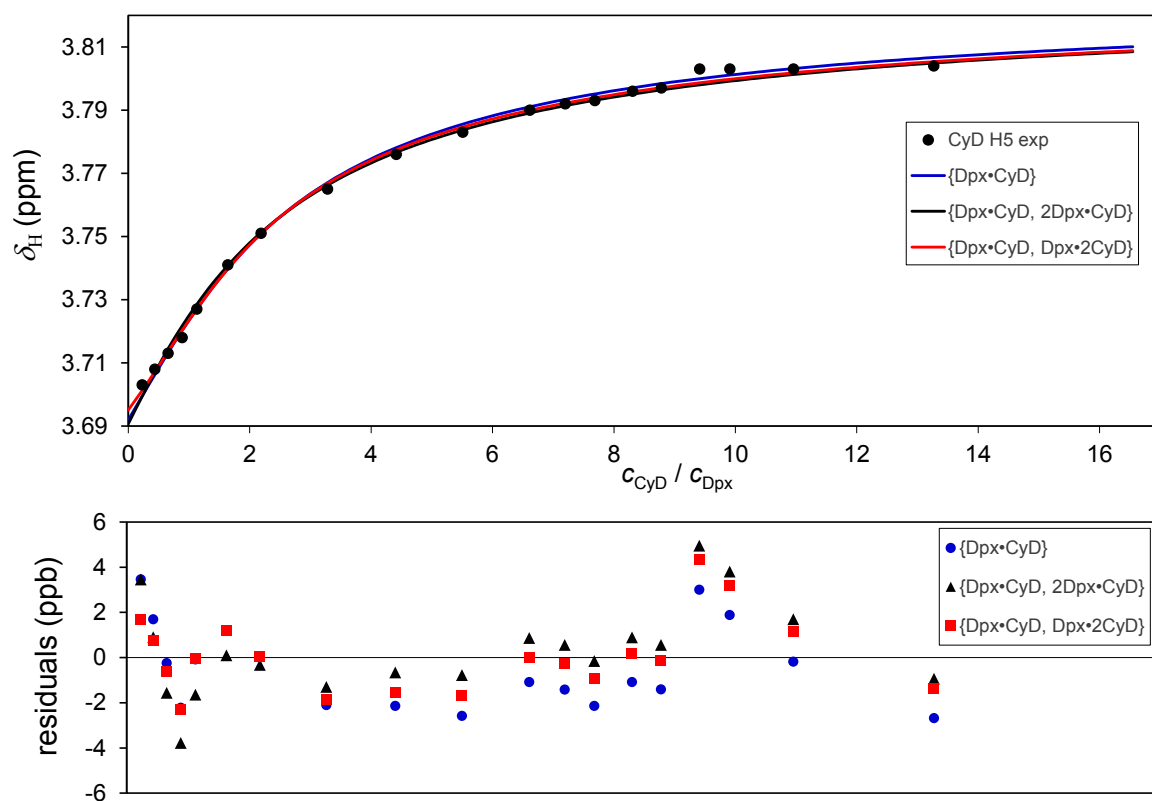


**Fig. S26.** Chemical shift profile of  $\beta$ -CyD H1 in the titration of Dpx. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below

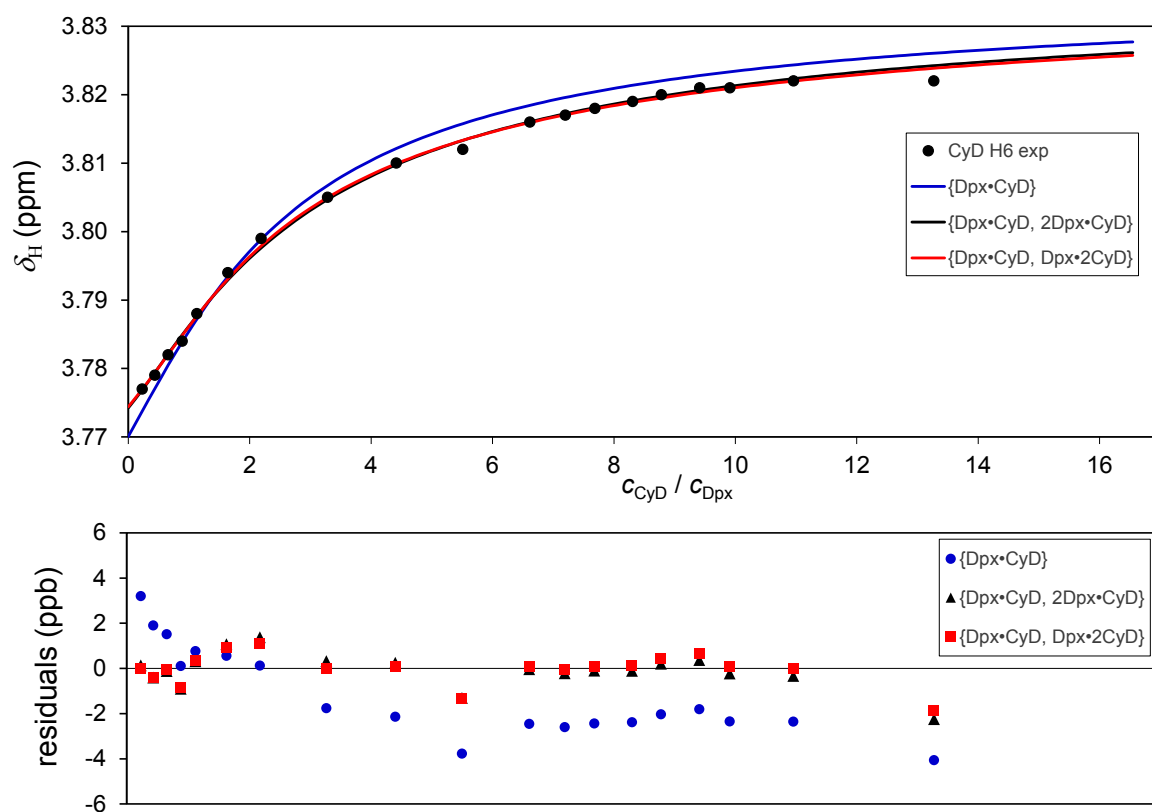




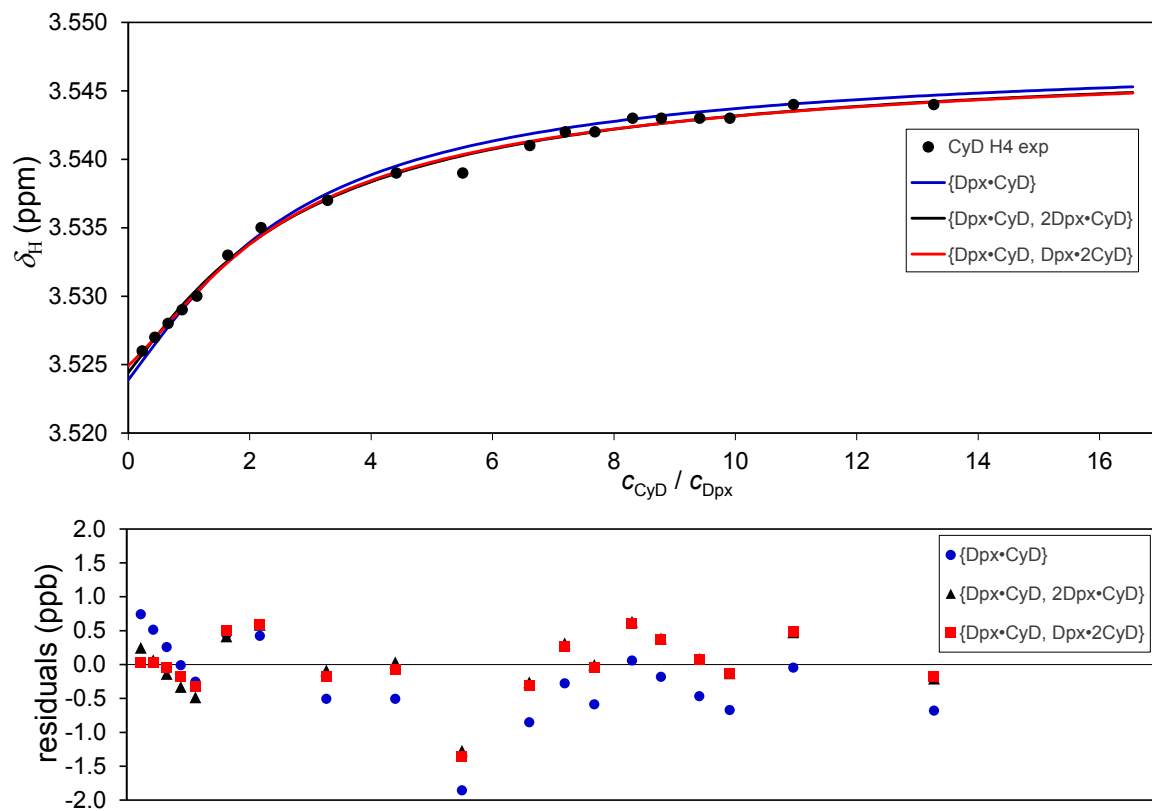
**Fig. S27.** Chemical shift profile of  $\beta$ -CyD H3 in the titration of Dpx. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



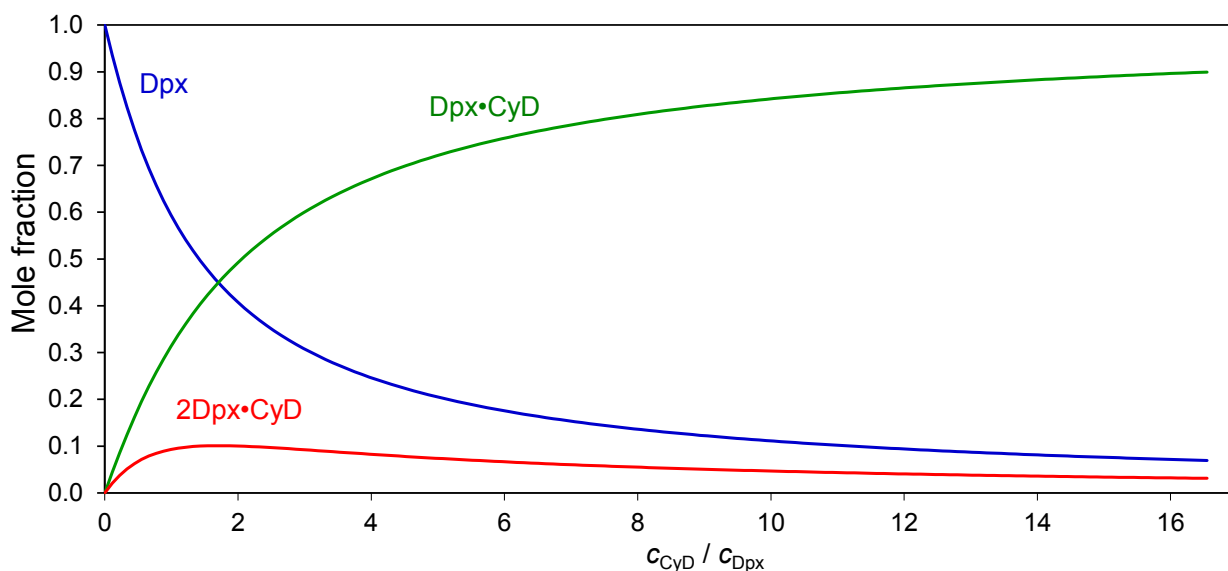
**Fig. S28.** Chemical shift profile of  $\beta$ -CyD H5 in the titration of Dpx. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S29.** Chemical shift profile of  $\beta$ -CyD H6 in the titration of Dpx. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



**Fig. S30.** Chemical shift profile of  $\beta$ -CyD H4 in the titration of Dpx. Three equilibrium models fitted with the OPIUM program are shown in solid curves and the corresponding residuals are shown below



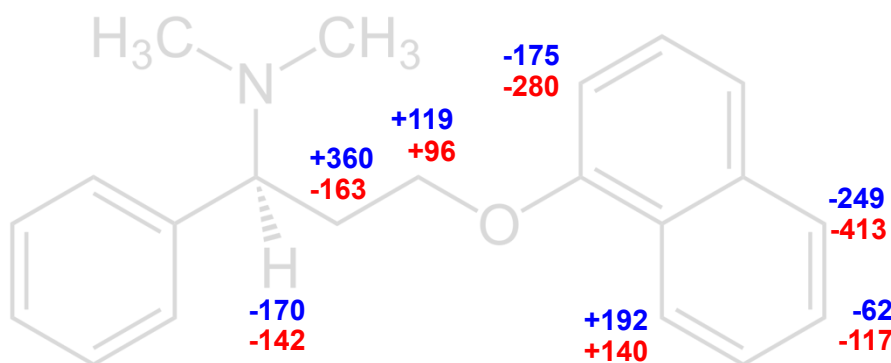
**Fig. S31.** Species distribution plot for the NMR titration of Dpx with  $\beta$ -CyD, generated with the HysS software, using the stability constants iterated by the OPIUM program

**blue values in ppm:**

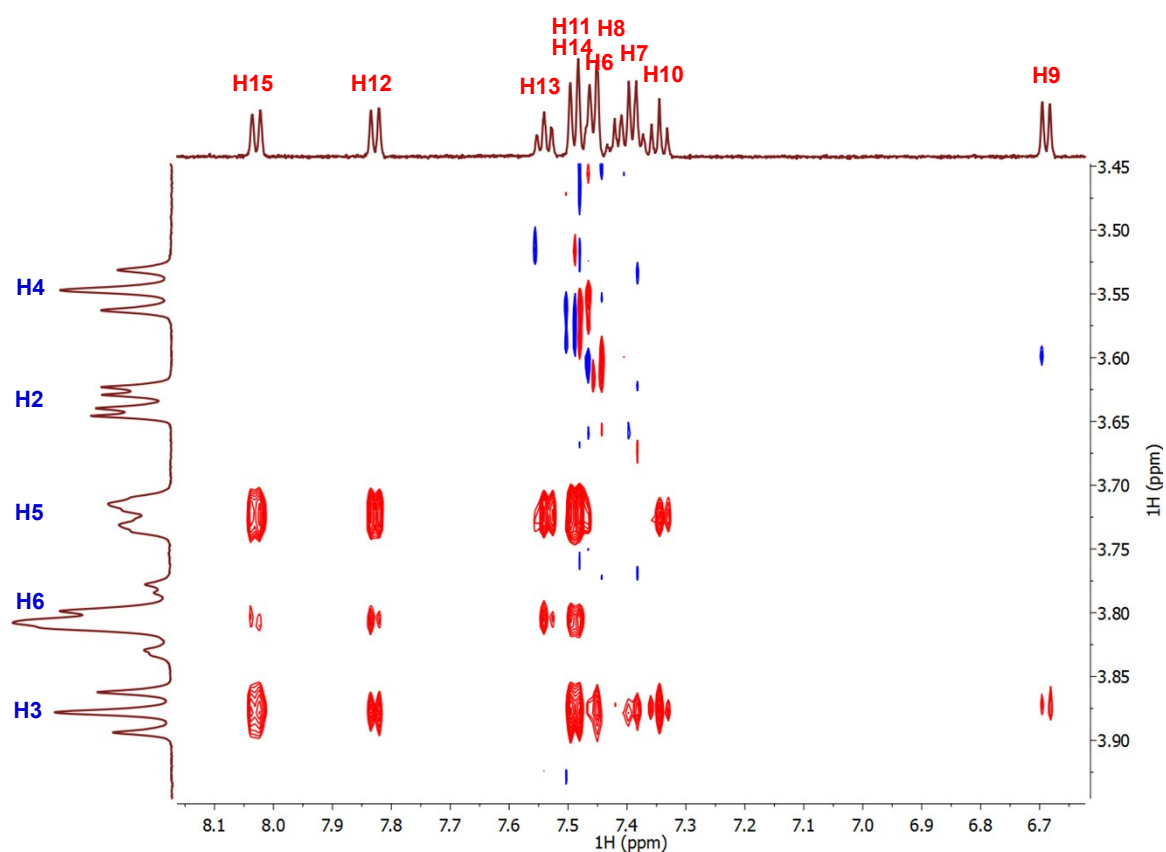
$$\Delta\delta_{\text{Dpx}\cdot\text{CyD}}^i = \delta_{\text{Dpx}\cdot\text{CyD}}^i - \delta_{\text{Dpx}}^i$$

**red values in ppb:**

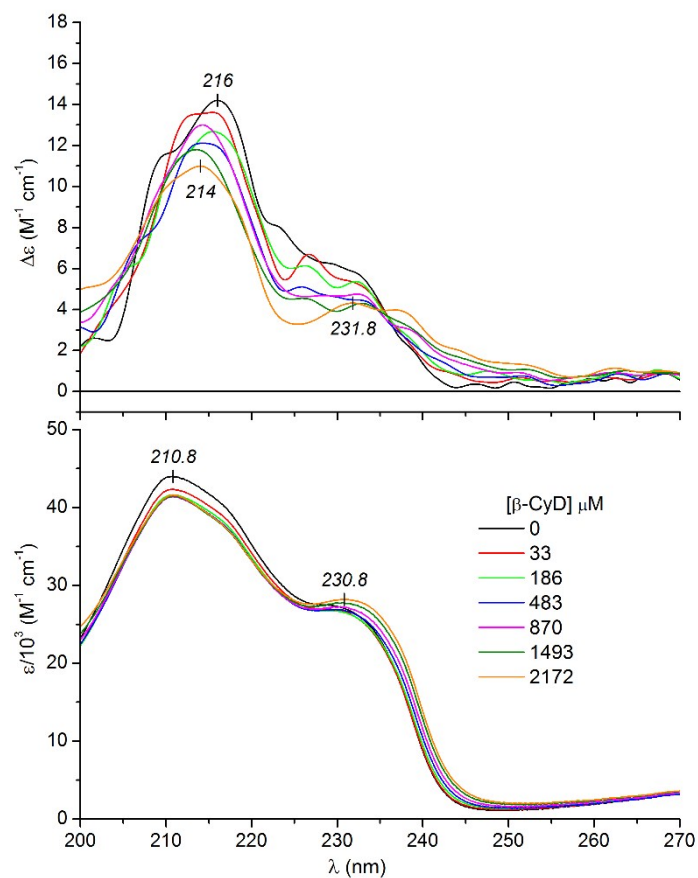
$$\Delta\delta_{2\text{Dpx}\cdot\text{CyD}}^i = \delta_{2\text{Dpx}\cdot\text{CyD}}^i - \delta_{\text{Dpx}}^i$$



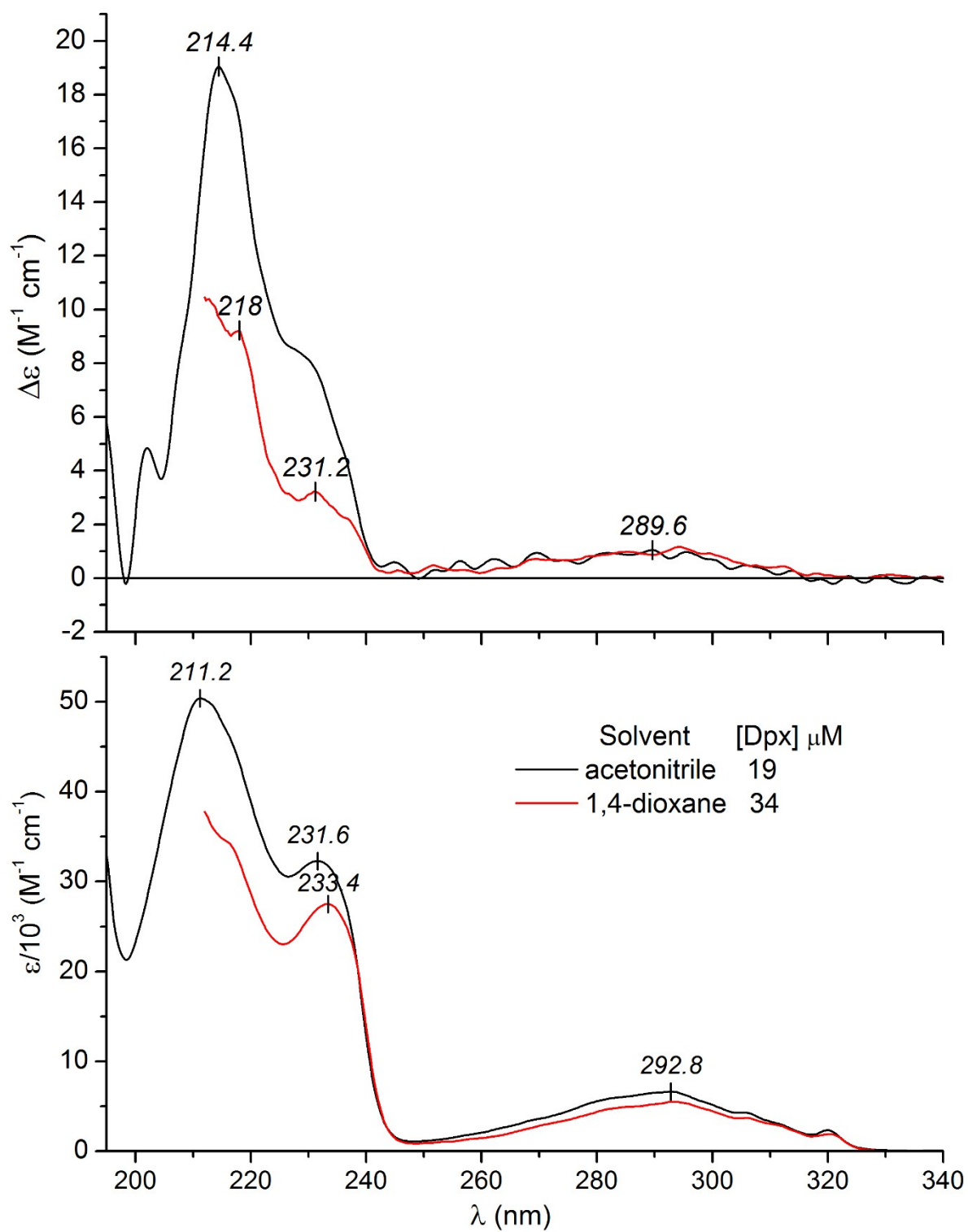
**Fig. S32.** Chemical shift displacement values with respect to free Dpx upon formation of the Dpx· $\beta$ -CyD and 2Dpx· $\beta$ -CyD complexes, results of the OPIUM evaluation



**Fig. S33.** Expanded region of the 600 MHz 2D ROESY spectrum of 1.5 mM Dpx (horizontal trace with assignment) and 1.5 mM  $\beta$ -CyD (vertical trace with assignment)



**Fig. S34.** Changes of the CD and UV absorption spectrum of 23  $\mu$ M Dpx measured upon sequential addition of  $\beta$ -CyD into the sample solution (pH  $\sim$  4)



**Fig. S35.** CD and UV absorption spectra of Dpx measured in acetonitrile and 1,4-dioxane (25 °C)