Supporting Information
for

Sequential Formation of a Ternary Complex among Dihexylammonium, Cucurbit[6]uril and Cyclodextrin with Positive Cooperativity

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Contents

1. ESI-MS Spectral Data for the Ternary Complex Formation of Dihexylamine (DHA) with Cucurbituril (CB[6]) and β-Cyclodextrin (CD)

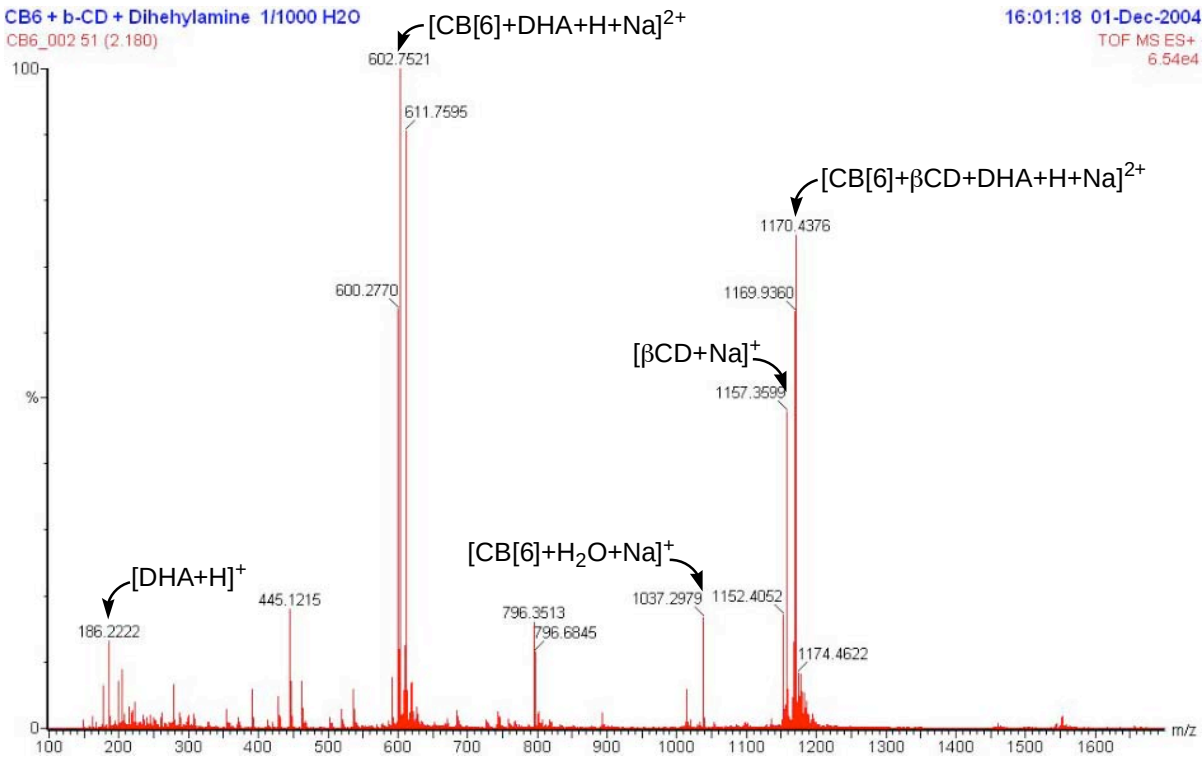


Figure S1-1. ESI-MS Spectra of a Mixture of DHA, CB[6], and CD (5 nM DHA•2HCl + 5 nM CB[6] + 5 nM α-CD in H_2O containing 20 nM NaCl)

The CB-CD-DHA ternary supramolecular complex was clearly detected as dicationic $[CB[6]+\beta CD+DHA+H+Na]^{2+}$ species, while the other component species as mono- and dicationic fragment peaks, as indicated above.

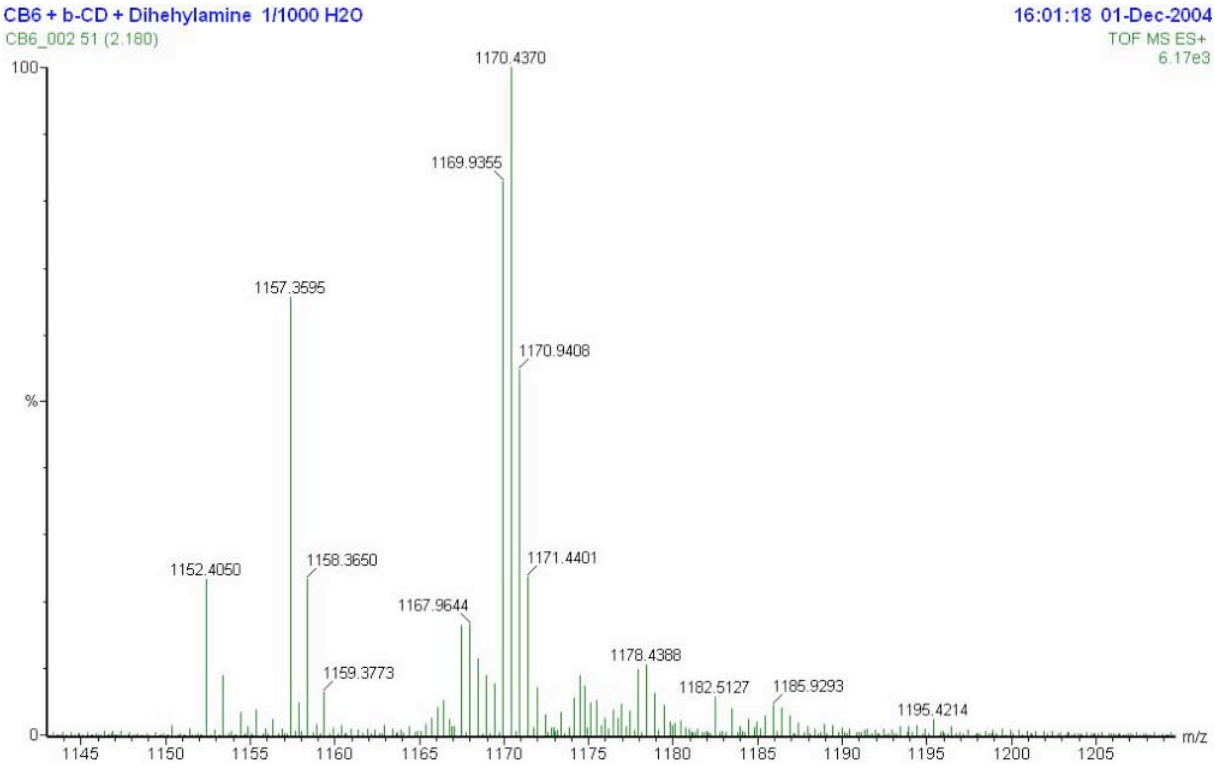


Figure S1-2. ESI-MS Spectra of [CB[6]•DHA•β-CD•Na]$^{2+}$ Complex (Enlargement 1)

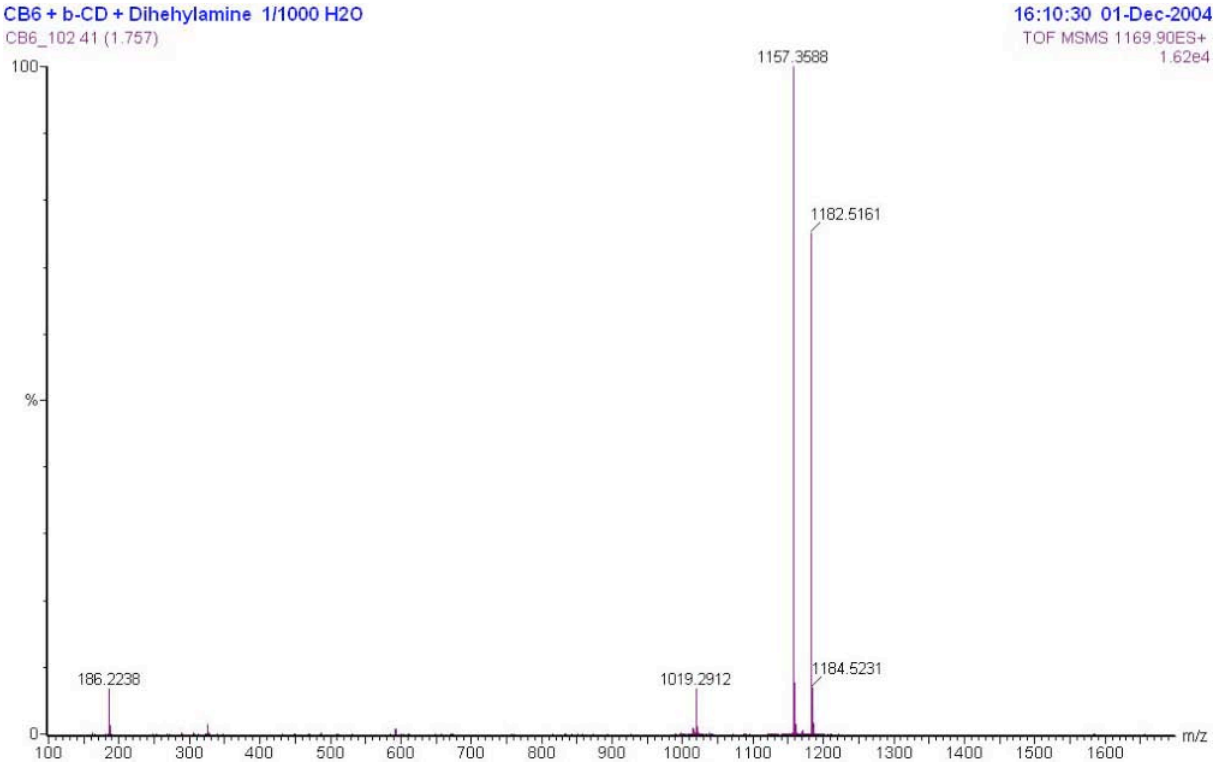


Figure S1-3. ESI-MS Spectra of $[CB[6]\bullet DHA\bullet \beta\text{-}CD\bullet Na]^{2+}$ Complex

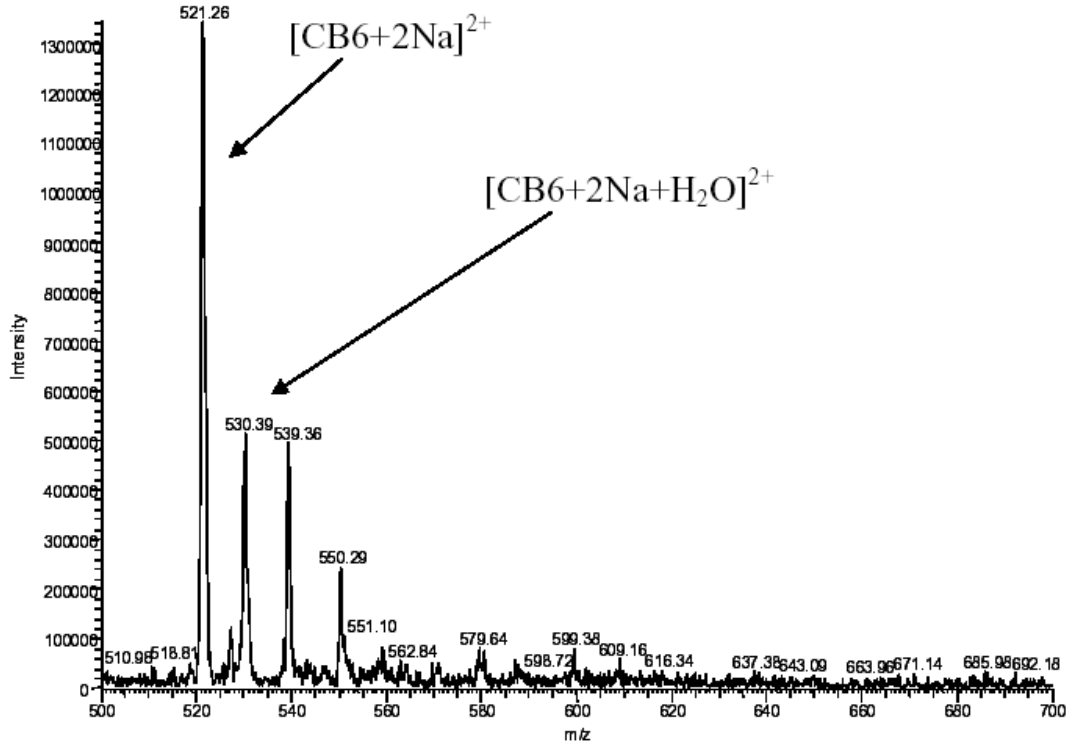


Figure S1-4. ESI-MS Spectra of $[CB[6]\bullet 2Na]^{2+}$ Complex

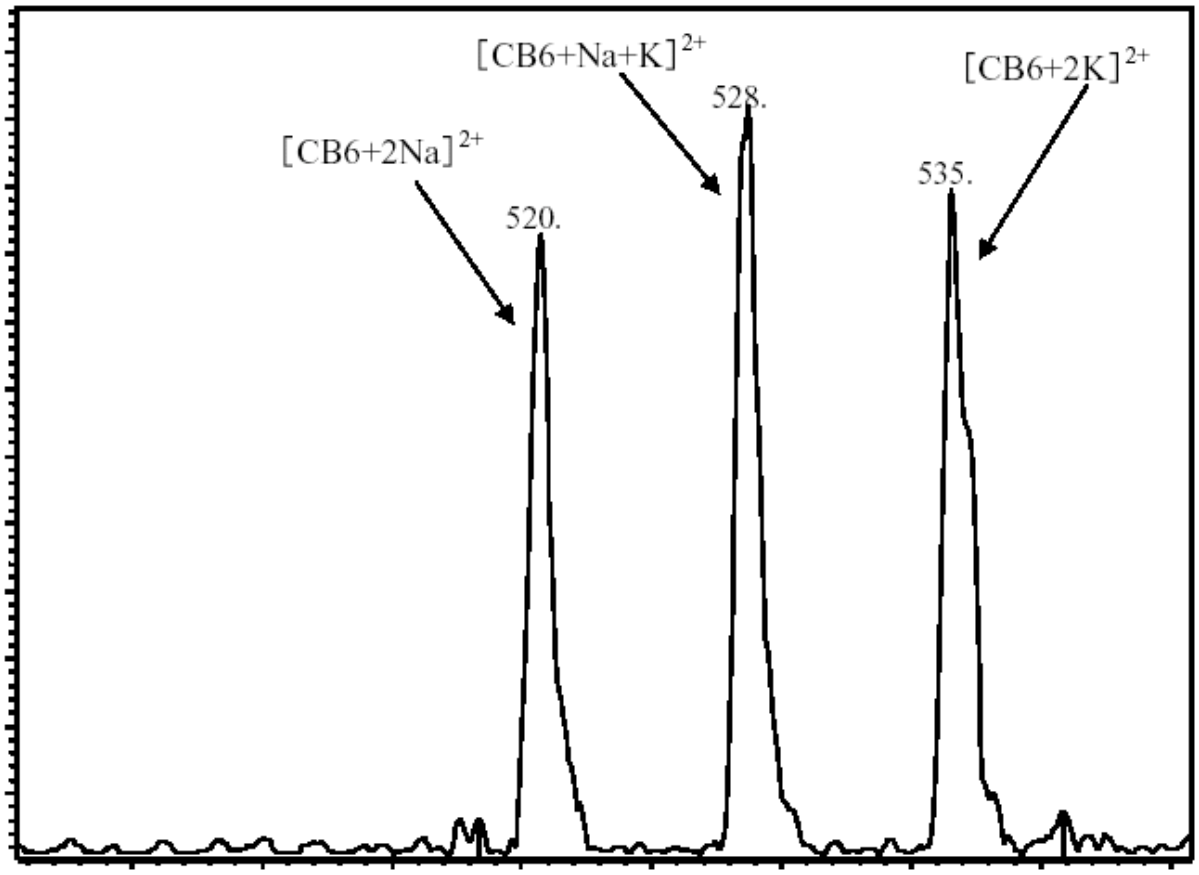


Figure S1-5. Results of ESI-MS study to illustrate that CB[6] always exists in aqueous solution as di-cationic species even if different cations are in the solution.

2. NMR Spectral Data for the Stepwise Ternary Complex Formation of Dihexylamine (DHA) with Cucurbituril (CB[6]) and Cyclodextrins (CD)

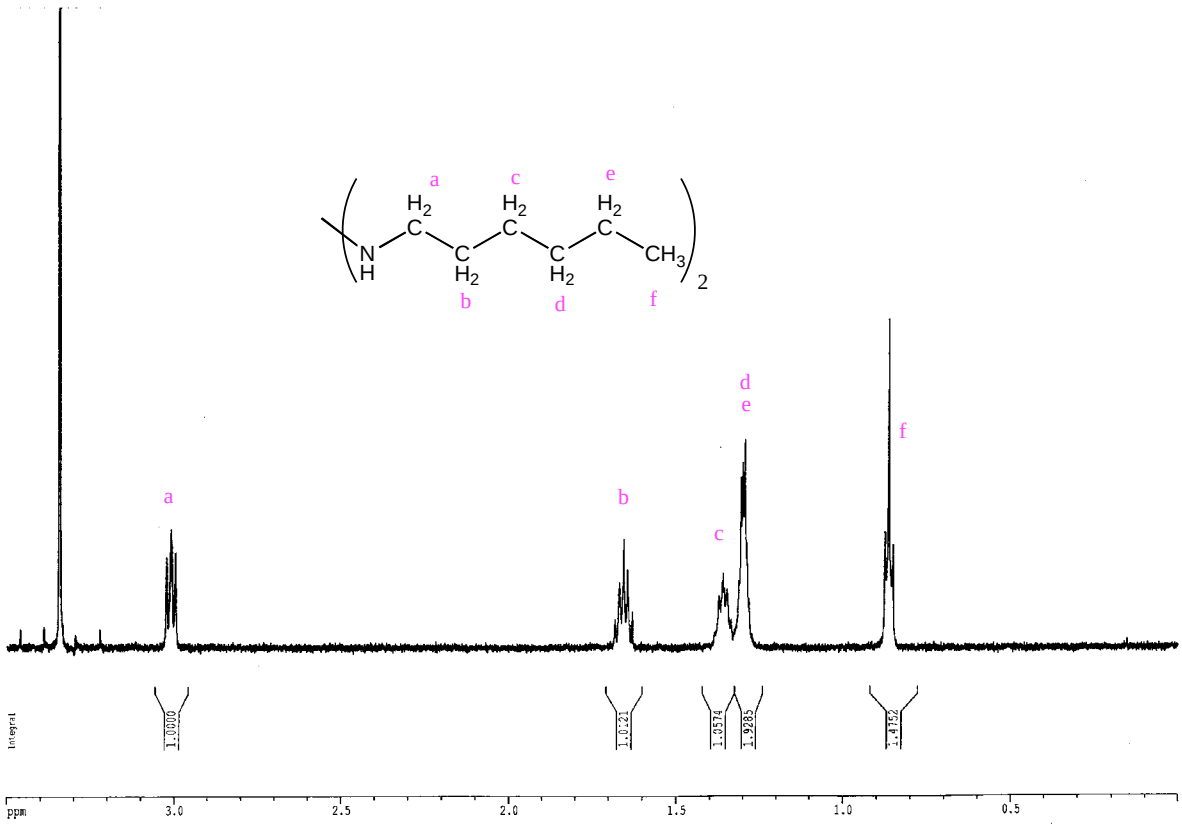


Figure S2-1. 1D ^{1}H NMR spectrum of DHA (1 mM DHA in D_2O containing 4 mM HCl and 0.2 M NaCl)

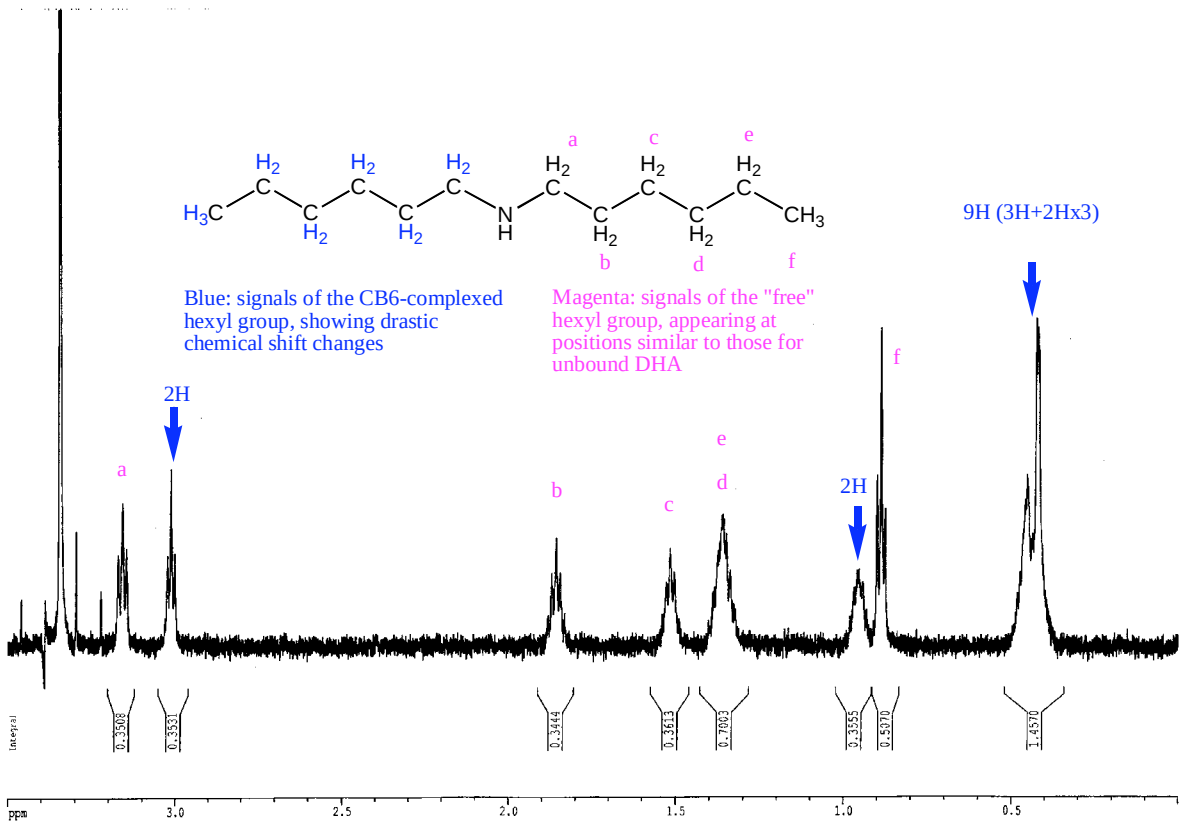


Figure S2-2. Dihexylamine signals in 1D ^{1}H NMR spectrum of a mixture of DHA and CB[6] (1 mM DHA + 1.4 mM CB[6] in D_2O containing 4 mM HCl and 0.2 M NaCl)

Compared to the chemical shifts of “free” DHA (Figure S1), each set of the aliphatic protons in the two hexyl chains are split (due to the slow guest exchange); halves of the signals (blue) are drastically upfield-shifted, while the other halves (magenta) show only small downfield shifts (particularly for Ha, Hb, and Hc, shown in magenta). These changes clearly indicate that only one of the two hexyl groups is exclusively included by CB[6], leaving the other outside the CB[6] cavity.

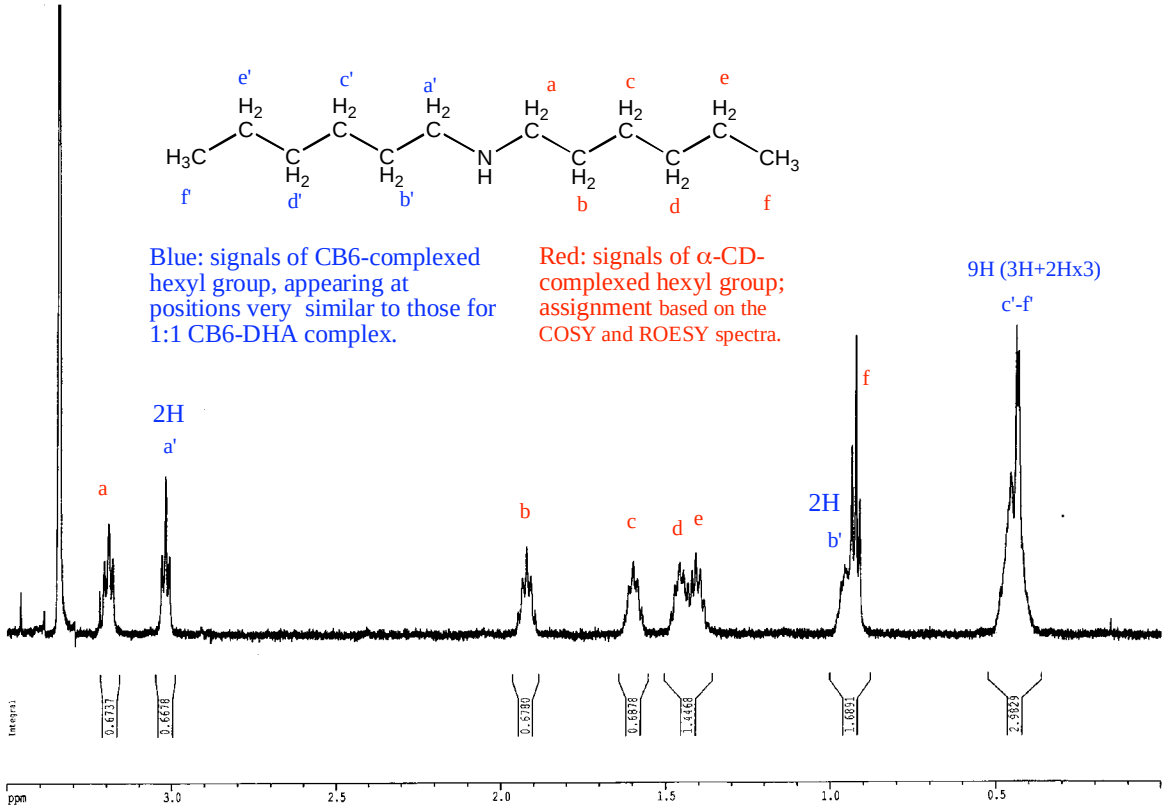


Figure S2-3. Dihexylamine signals in the 1D ^{1}H NMR spectrum of a mixture of DHA, CB[6], and α-CD (1 mM DHA + 1.5 mM CB[6] +1.5 mM α-CD in D_2O containing 4 mM HCl and 0.2 M NaCl)

The signals of CB[6]-complexed hexyl group do not show any further shift changes upon addition of α-CD, indicating very strong complexation of the first hexyl with CB[6]. No competitive inclusion between CB[6] and α-CD seems to occur.
The protons of the other hexyl group included in α-CD show moderate downfield shifts and Hd and He are separated.

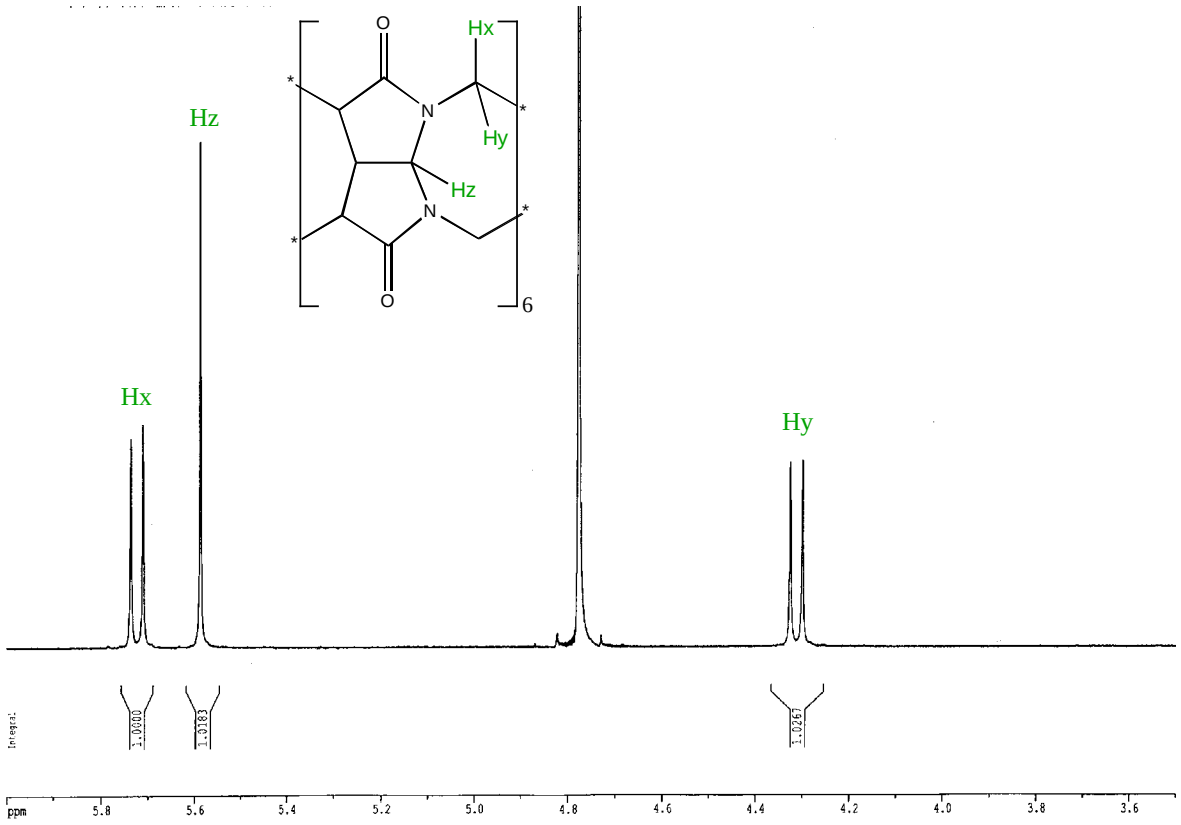


Figure S2-4. 1D ^{1}H NMR of CB[6] (2.4 mM CB[6] in D_2O containing 0.2 M NaCl)

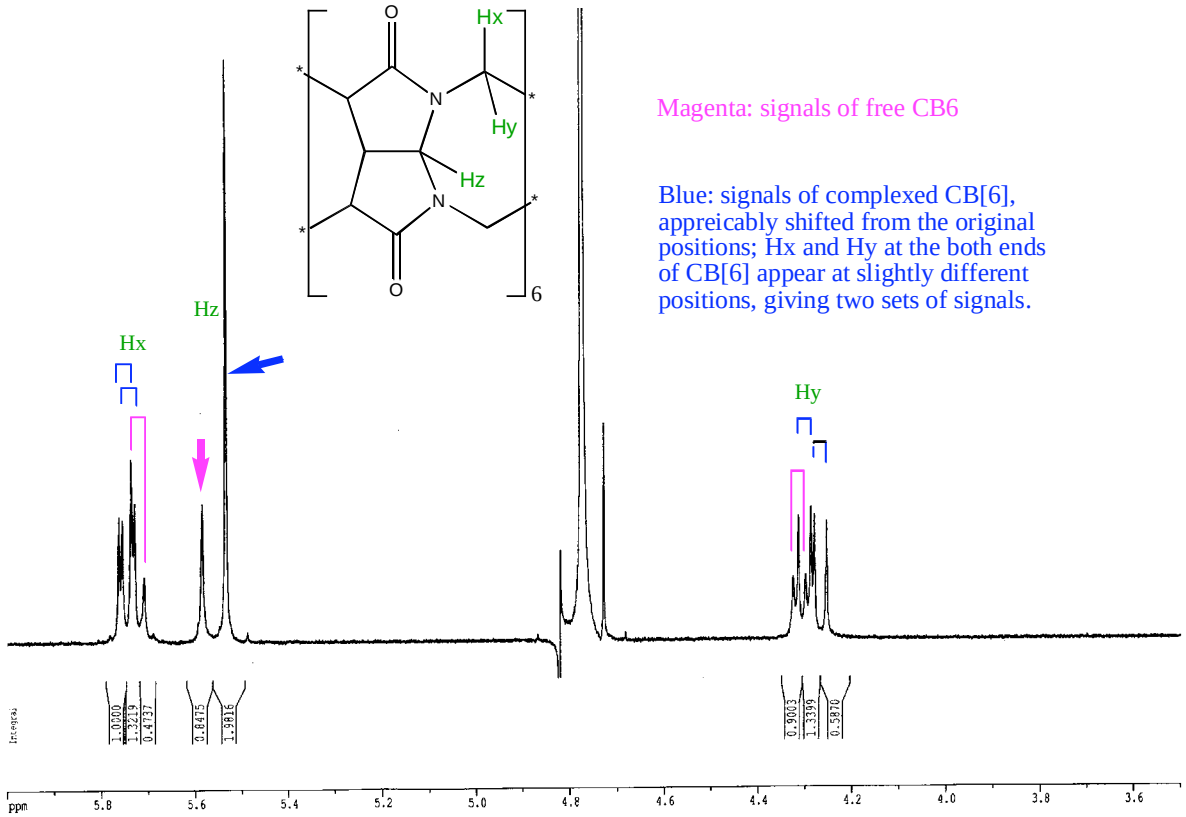


Figure S2-5. CB[6] signals in 1D ^{1}H NMR spectrum of a mixture of DHA and CB[6] (1 mM DHA + 1.4 mM CB[6] in D_2O containing 4 mM HCl and 0.2 M NaCl)

Because of the use of an excess amount of CB[6] over DHA, free (magenta) and complexed (blue) CB[6] give separate signals, which are clearly assigned from their chemical shifts. The separate signals indicate a slow exchange of DHA included in CB[6].
Compared to the relevant protons of free CB[6], Hx is shifted to the downfield, whilst Hy and Hz show upfield shifts upon inclusion of DHA.
Interestingly, Hx and Hy of complexed CB[6] (blue) are split to two sets of signals, while Hz gives one singlet signal, indicating that the two portal protons are differentiated upon complexation of DHA.

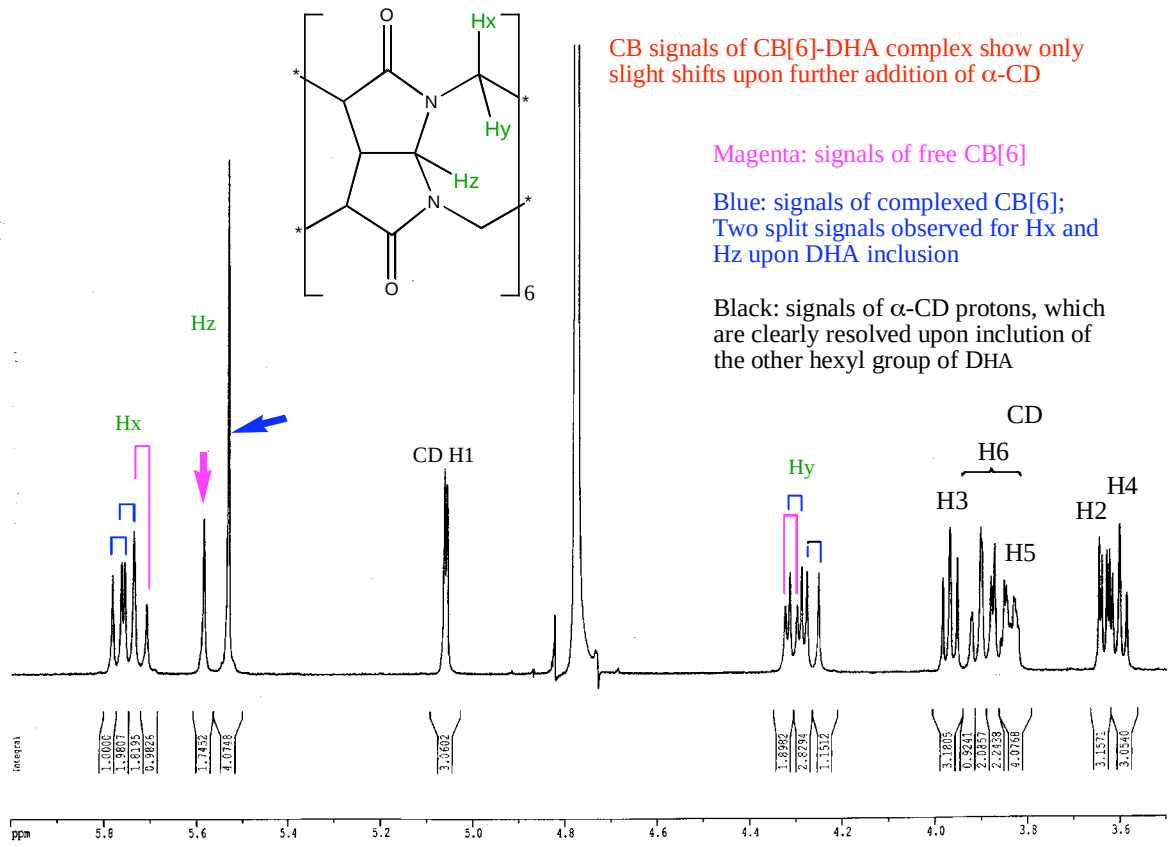
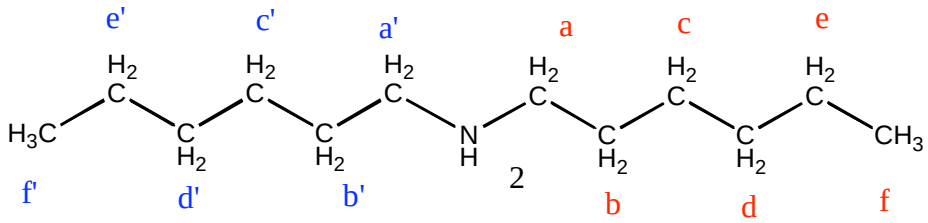


Figure S2-6. CB[6] signals in 1D ^{1}H NMR spectrum of a mixture of DHA, CB[6], and α-CD (1 mM DHA + 1.5 mM CB[6] + 1.5 mM α-CD in D_2O containing 4 mM HCl and 0.2 M NaCl)

Further addition of α-CD to the solution of DHA + CB[6] (Spectrum 5) causes slight but appreciable changes in DHA's Hx and Hy, due to the inclusion complexation of the other hexyl group by α-CD, giving a ternary complex.



Blue: signals of the hexyl group inlcuded in CB[6]

Red: signals of the hexyl group included by α-CD (assignment based on ROESY)

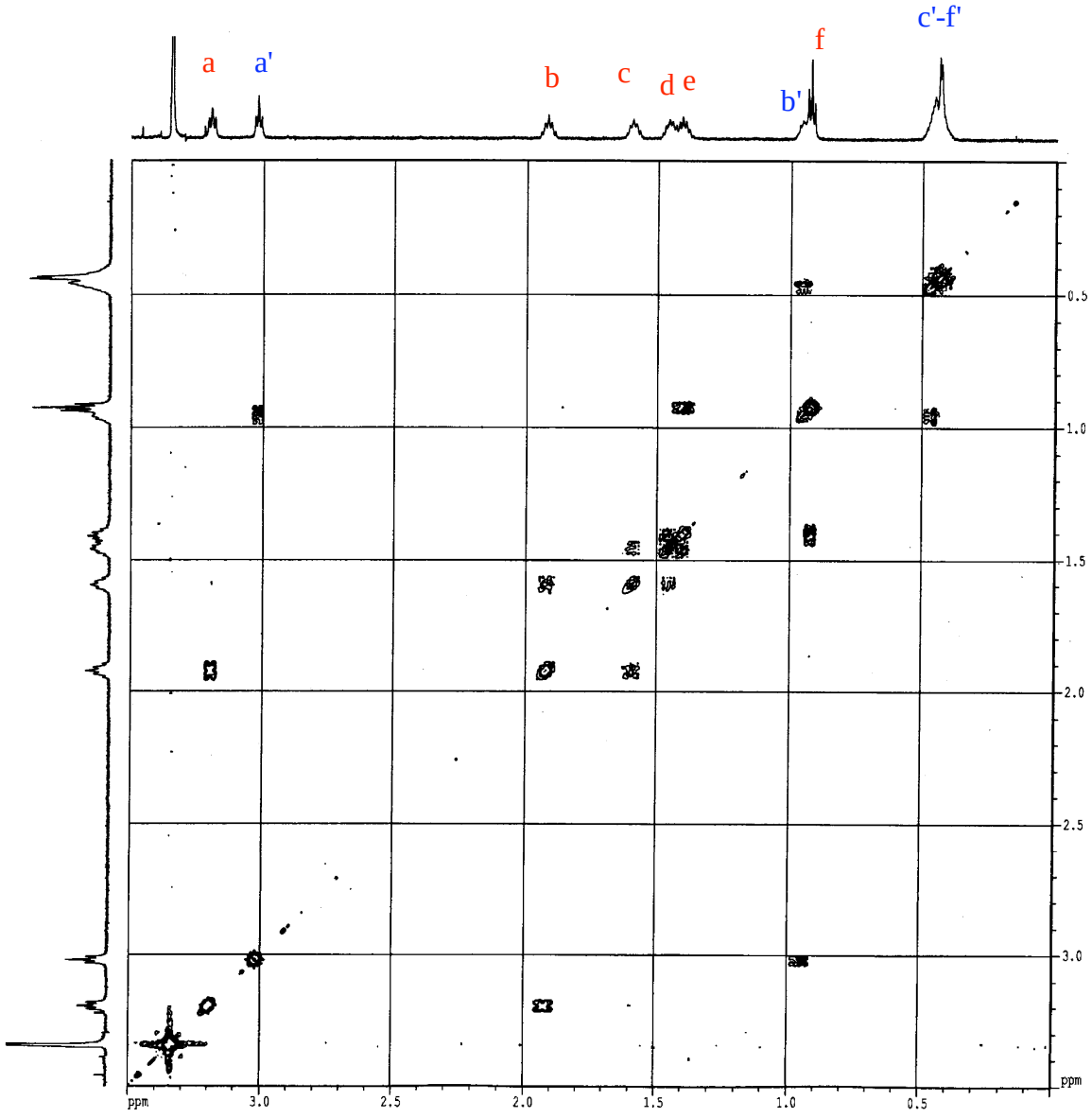


Figure S2-7. COSY spectrum of a mixture of DHA, CB[6], and α-CD (2 mM DHA•2HCl + 2.4 mM CB[6] + 2.4 mM α-CD in D_2O containing 0.2 M NaCl)

The two hexyl groups of DHA are clearly discriminated from each other (shown in red and blue).

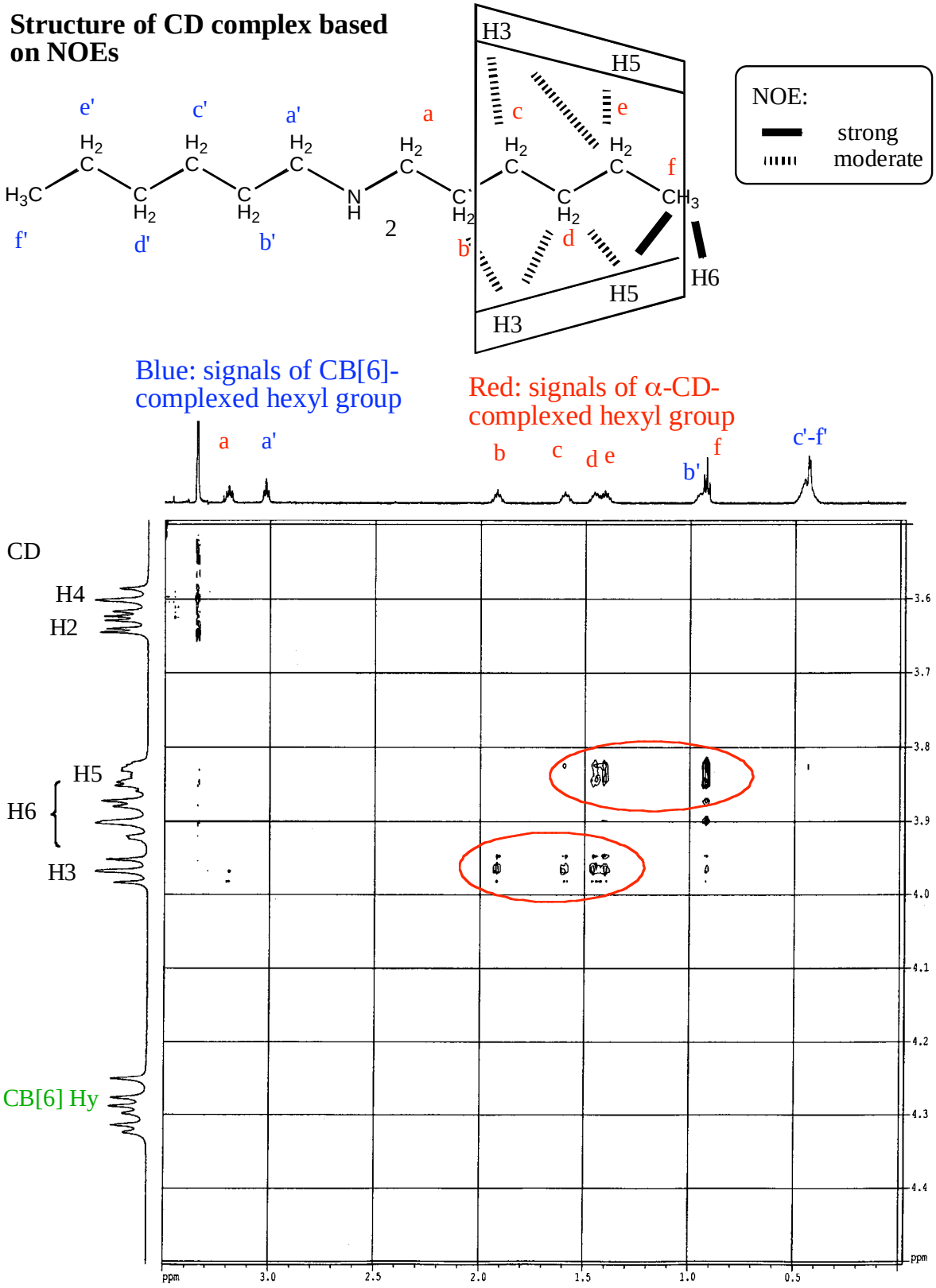


Figure S2-8. ROESY spectrum of a mixture of DHA, CB[6], and α-CD (2 mM DHA•2HCl + 2.4 mM CB[6] + 2.4 mM α-CD in D_2O containing 0.2 M NaCl)

One of the two hexyl groups that is included by CB[6] does not show any NOEs with α-CD

protons, whilst the other hexyl group shows very clear crosspeaks with α-CD protons as listed below.

DHA protons	α-CD protons		
	H3	H5	H6
Ha	no	no	no
Hb	medium	no	no
Hc	medium	weak	no
Hd	medium	medium	no
He	medium	medium	no
Hf	weak	strong	strong

These NOEs indicate that the hexyl group penetrates into the CD cavity from the secondary side.

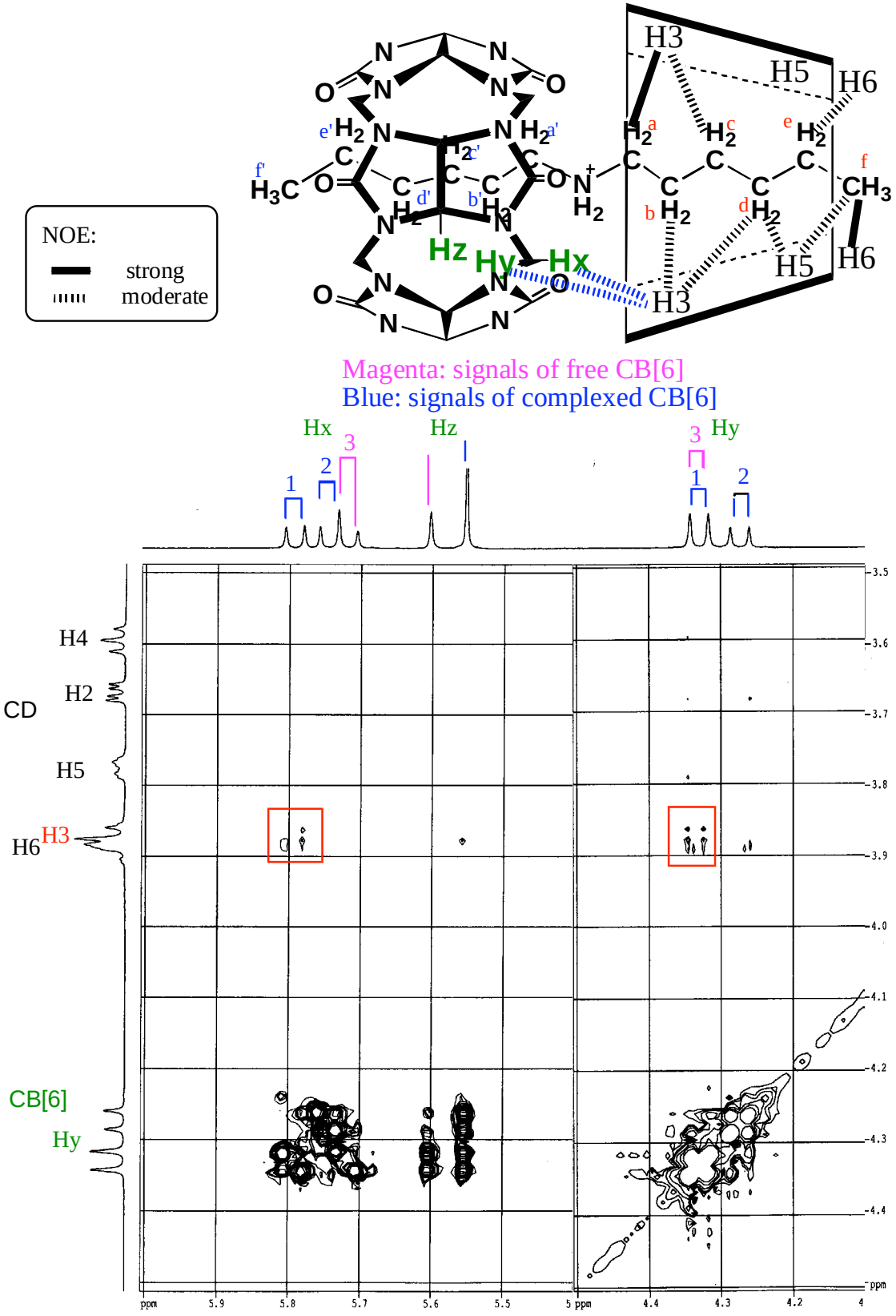


Figure S2-9. ROESY spectrum (mixing time 0.2 s) of a mixture of 1 mM DHA + 1.5 mM CB[6] +1.1 mM β-CD in D_2O containing 4 mM HCl and 0.2 M NaCl (CB[6] part)

Moderate crosspeaks between CD's H3 and CB[6]'s Hx1 and Hy1 are seen, indicating the capping complexation of CD to the DHA-CB[6] complex.

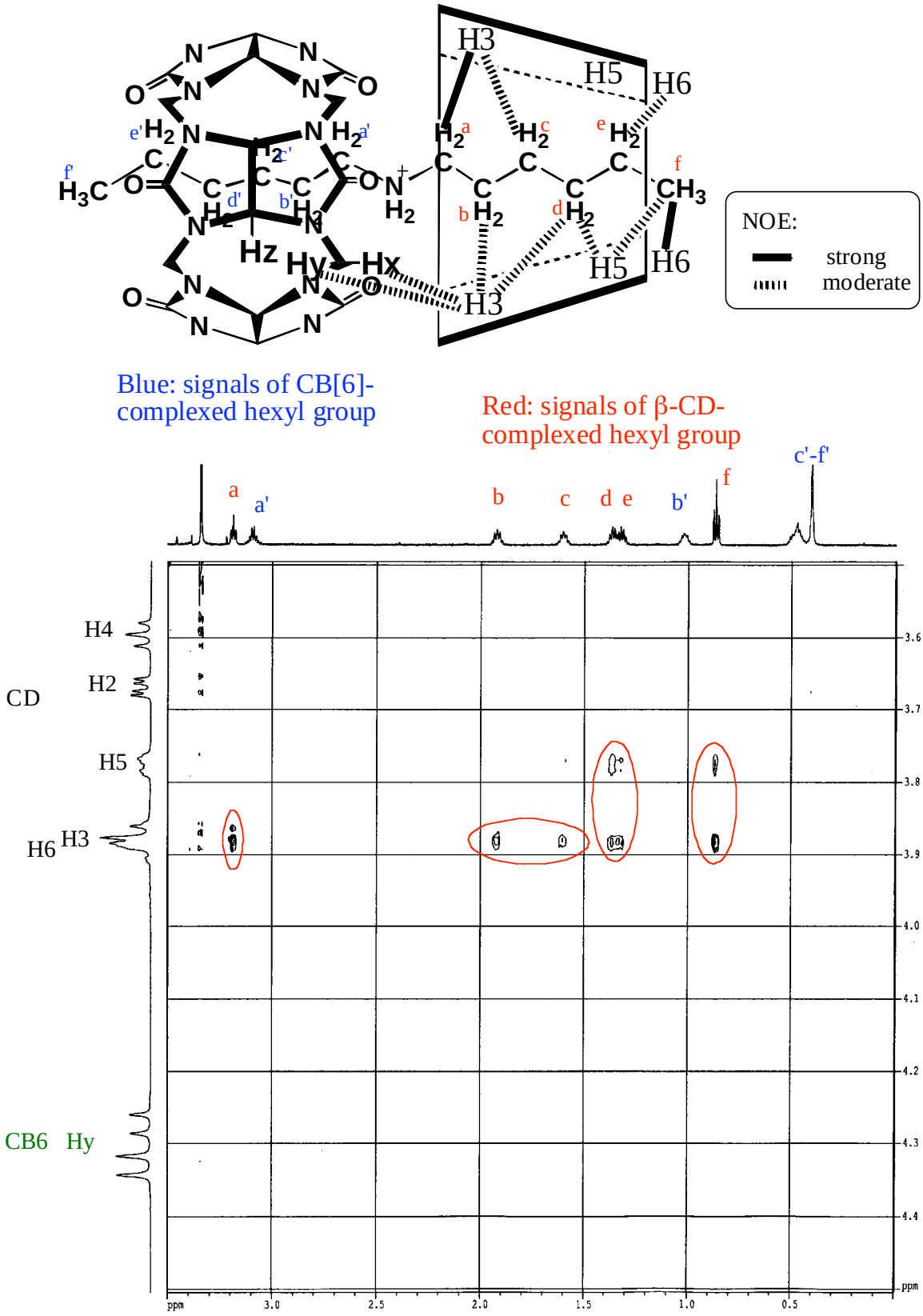


Figure S2-10. ROESY spectrum (mixing time 0.2 s) of a mixture of 1 mM DHA + 1.5 mM CB[6] +1.1 mM β-CD in D_2O containing 4 mM HCl and 0.2 M NaCl (β-CD part)

One of the two hexyl groups that is included by CB[6] does not show any NOEs with β-CD protons, whilst the other hexyl group shows very clear crosspeaks with β-CD protons as listed

below.

DHA protons	β-CD protons		
	H3	H5	H6
Ha	strong	no	no
Hb	medium	no	no
Hc	medium	no	no
Hd	medium	medium	no
He	no	no	medium
Hf	no	medium	strong

These NOEs indicate that the hexyl group penetrates into the CD cavity from the secondary side.

Summary of the NMR Studies

One of the two hexyl groups of dihexylamine (DHA) accommodated in the CB[6] cavity showed drastic chemical shift changes, while the other hexyl group included in CD gave clear NOE crosspeaks with CD's H3 protons in the ROESY spectra. These NMR spectral examinations confirmed the formation of a ternary complex of DHA with CB[6] and CD, as indicated by microcalorimetric titrations.

3. ITC Data for the Complexation of α-, β-, and γ-Cyclodextrin toward Dihexylamine (DHA) - Cucurbituril (CB[6]) 1:1 Complex.

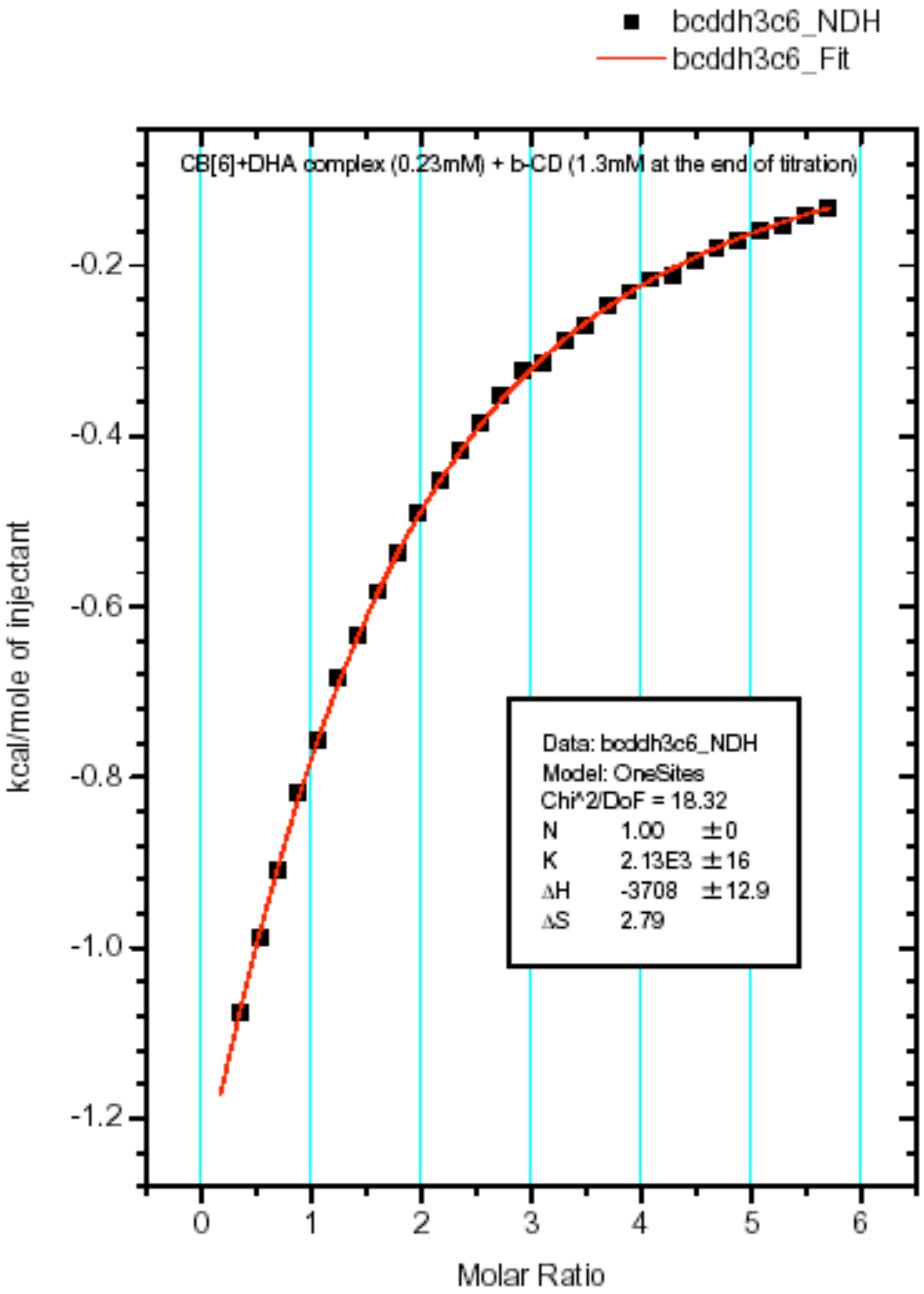


Figure S3-1. Computer simulation of ITC titration curve in the case of complexation of β-CD toward 1:1 DHA-CB[6] complex.

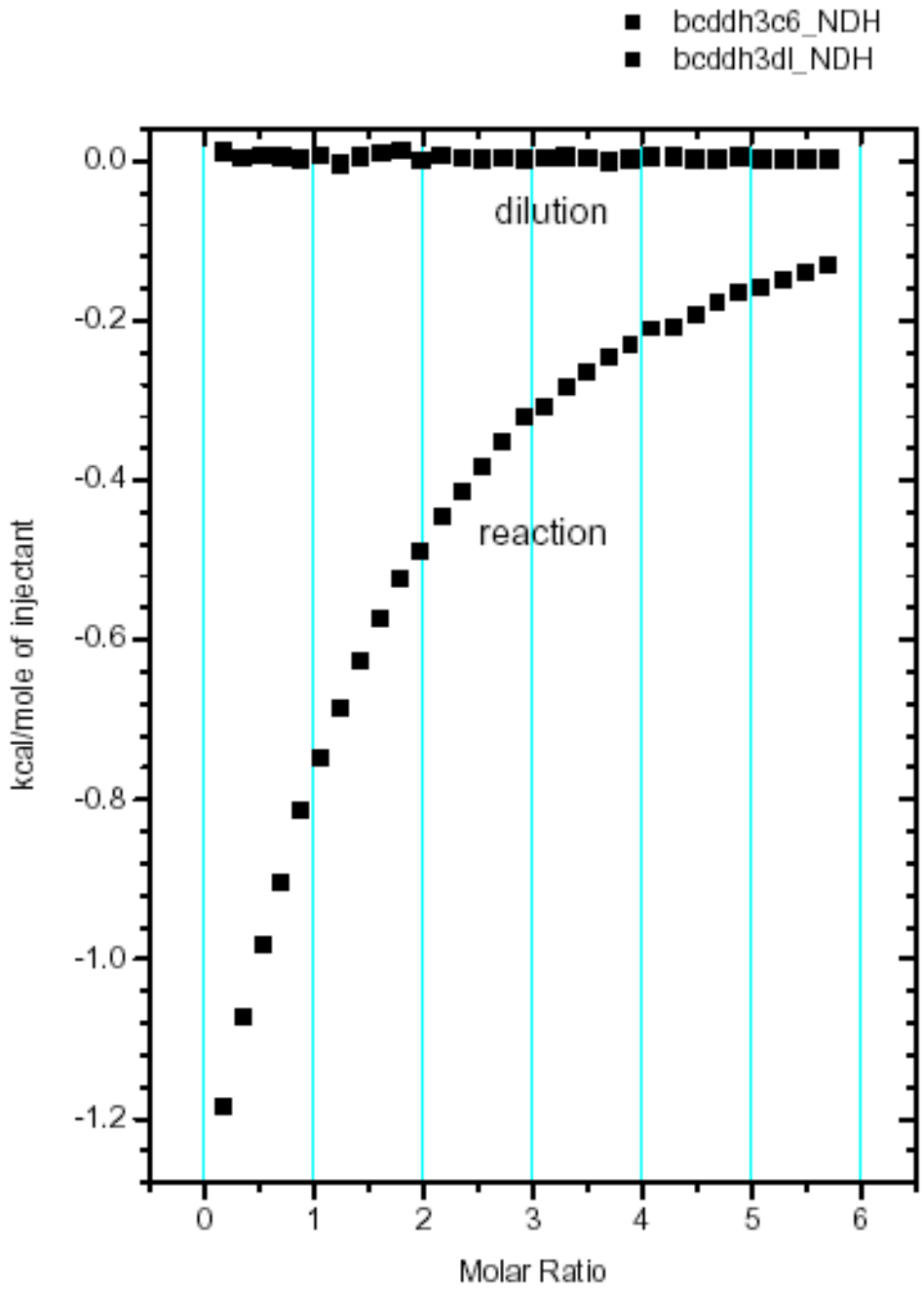


Figure S3-2. Heat effects observed upon titration of β-CD solution into solution of DHA-CB[6] complex (reaction) and upon dilution of initial β-CD solution (dilution); concentration of reactants shown at Figure S3-1.

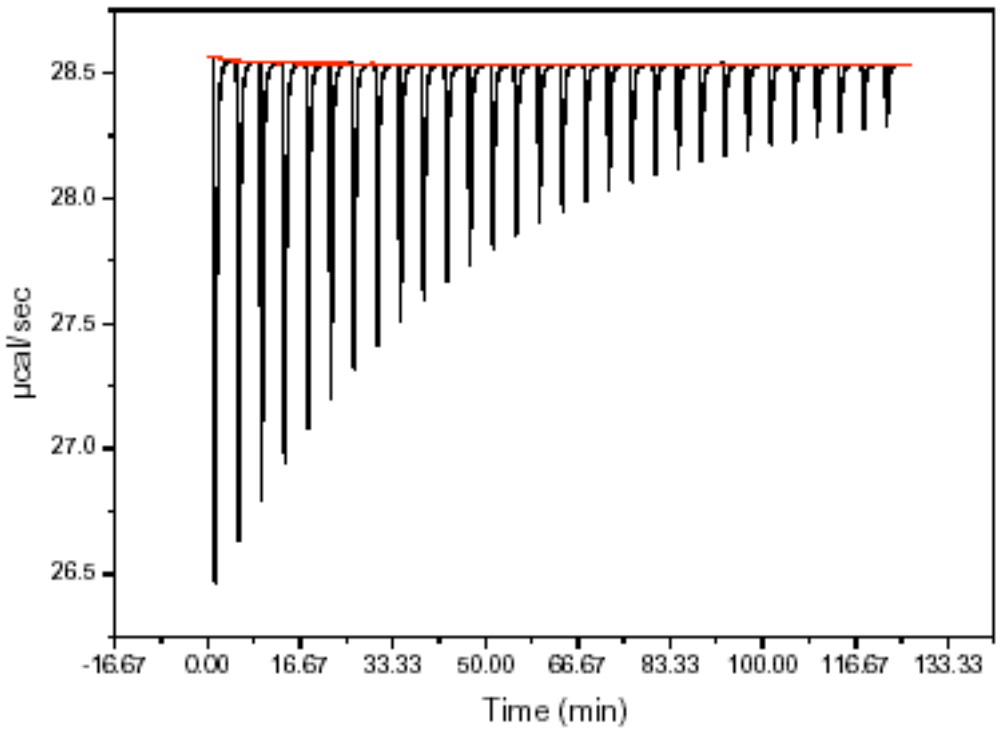


Figure S3-3. Raw ITC data for titration of β-CD solution into solution of DHA-CB[6] complex.

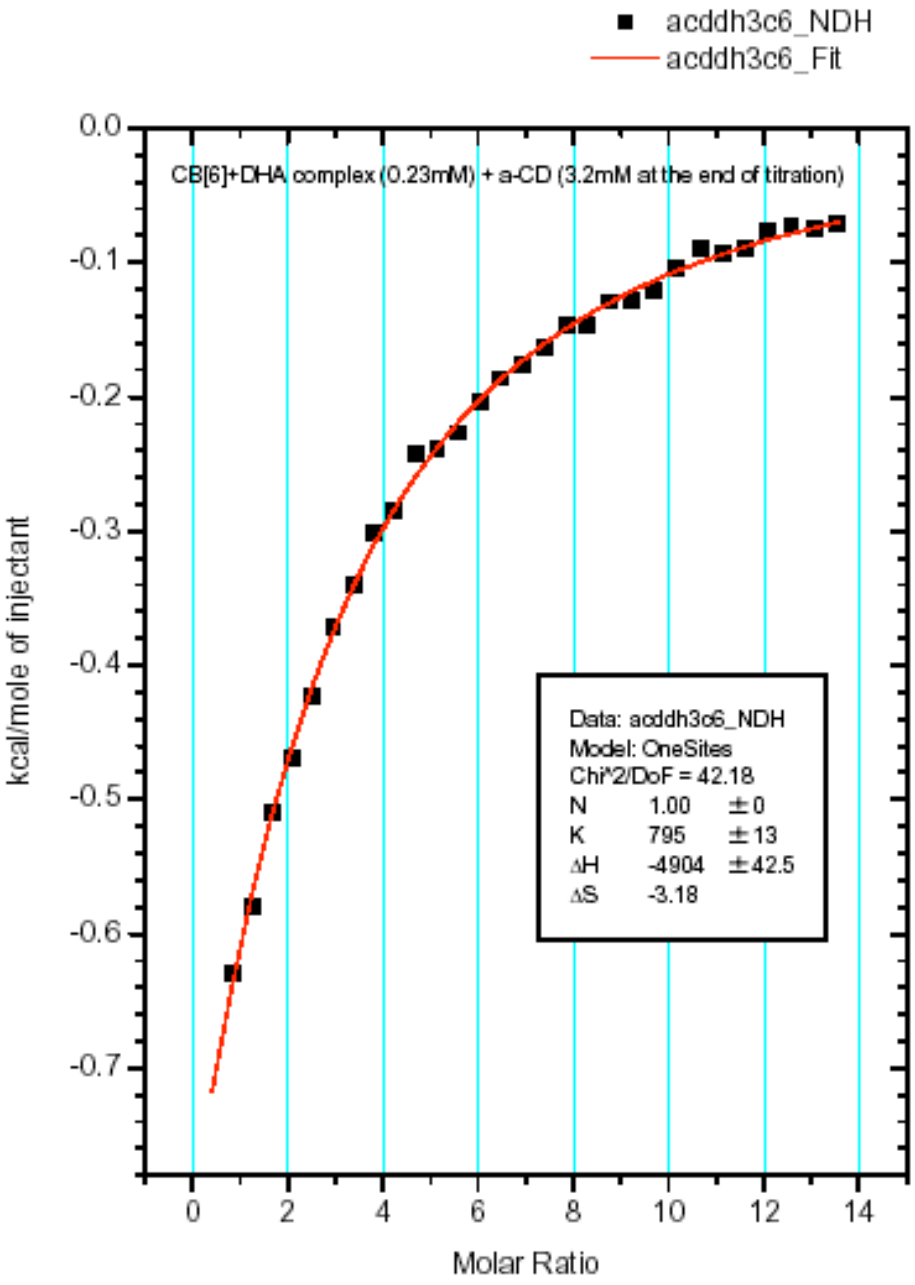


Figure S3-4. Computer simulation of ITC titration curve in the case of complexation of α-CD toward 1:1 DHA-CB[6] complex.

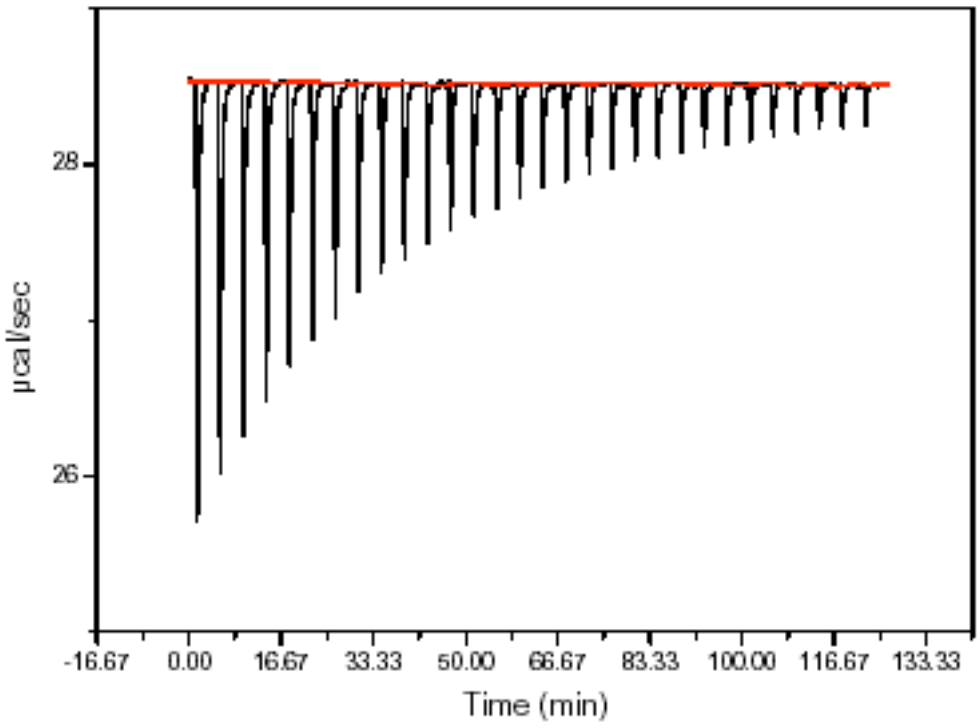


Figure S3-5. Raw ITC data for titration of α-CD solution into solution of DHA-CB[6] complex; concentration of reactants shown at Figure S3-4.

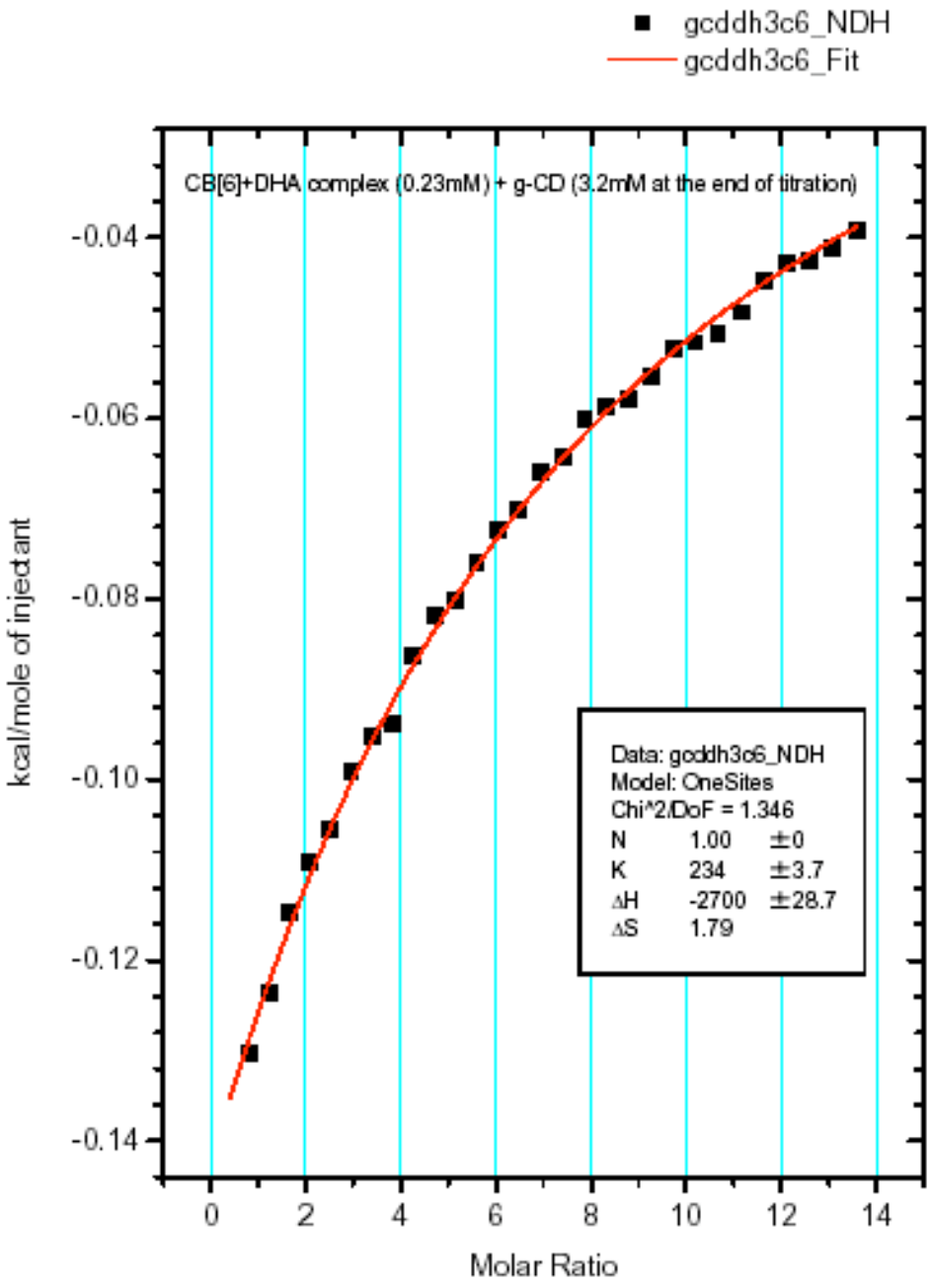


Figure S3-6. Computer simulation of ITC titration curve in the case of complexation of γ-CD toward 1:1 DHA-CB[6] complex

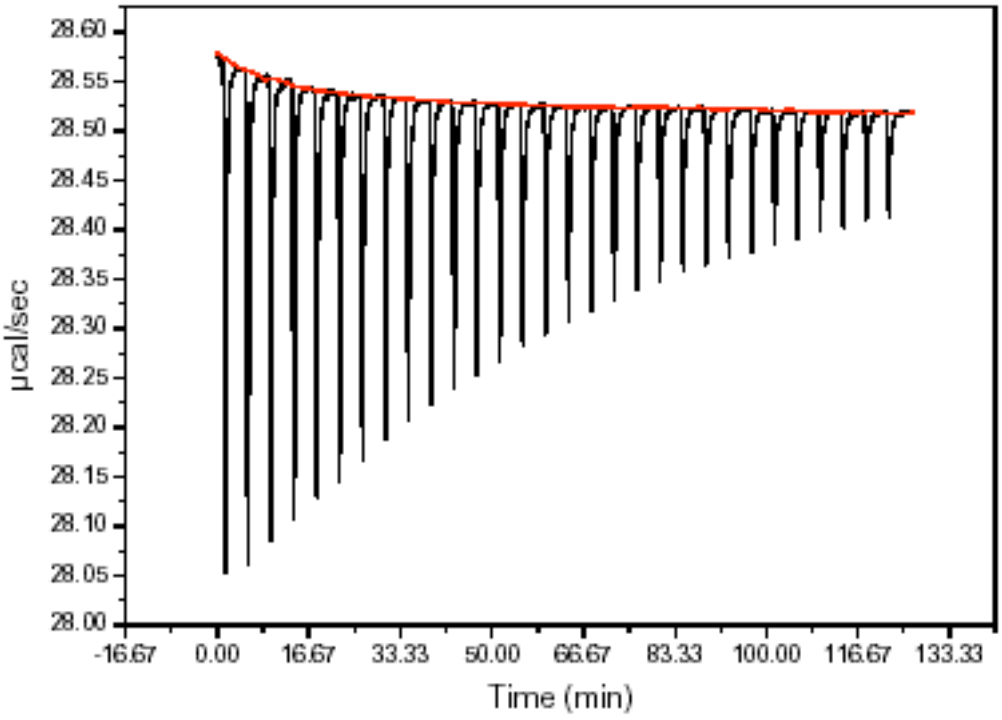


Figure S3-7. Raw ITC data for titration of α-CD solution into solution of DHA-CB[6] complex; concentration of reactants shown at Figure S3-6.