BETA-CYCLODEXTRIN MODIFICATION AND HOST-GUEST COMPLEXATION

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CHAPTER 3

STRUCTURAL EFFECTS IN COMPLEXING:
6-(4’-t-BUTYLANILINO)-NAPHTALENE-2-
SULPHONATE (BNS) MONOMER AND DIMER
WITH βCD AND LINKED βCD DIMERS
3.1. Introduction

To further investigate the cooperative complexing effect of βCD and linked βCD dimers, another dye, 6-(4'-t-butylanilino)-naphtalene-2-sulphonate (6-(4'-t-butyl)-phenyl)-naphtalene-2-sulphonate, BNS’, Fig. 3.1 and 3.2), was chosen as the guest. Although the BNS’ structure is quite similar to that of TNS’, there are only a few studies of BNS’ as discussed in a recent review.1

This guest is chosen because it has a strong UV absorption spectrum and fluoresces when complexed in a βCD annulus; thereby facilitating a study of host-guest complexation through both phenomena. It also possesses a 1H NMR spectrum readily distinguishable from those of βCD and linked βCD dimers which permits ready identification of cross-peaks in 2D ROESY structural studies. In addition, BNS’ dimerizes at sufficiently high concentrations and raises the possibility of studying both its monomer and dimer complexation.

3.1.1. Dye Dimerisation and 1:2 Host:Guest Complexation

Molecular aggregation is very important in many areas of dye chemistry and biochemistry.2 In earlier studies of dye-CD systems, the dimerisations of several organic dyes was investigated. The dimerisation constant, \( K_d \) (Eqn. 3.1) of tropaeolin 000 No.2 (TR’) (3.1, Appendix 1) is \( 9.10 \times 10^2 \) dm\(^3\) mol\(^{-1}\), the \( K_d \) of roccellin (3.2, Appendix 1) is \( 1.64 \times 10^4 \) dm\(^3\) mol\(^{-1}\), the \( K_d \) of pyronine B (PB\(^+\), 3.3, Appendix 1) is \( 1.3 \times 10^3 \) dm\(^3\) mol\(^{-1}\), and the \( K_d \) of crystal violet (3.4, Appendix 1) is \( 6.0 \times 10^2 \) dm\(^3\) mol\(^{-1}\). Several 1:2 host:guest complexes have also been examined. For example, TR’ (which is slightly larger than BNS’), is apparently unable to form a host-guest complex with αCD. However, its dimer is complexed in both γCD and βCD. The stability constants (Eqns. 3.2-3.9, where x is the linker) for the complexation of TR’ by βCD (determined from temperature-jump measurements) and 66βCD\(_2\)su and 66βCD\(_2\)ur (determined by UV titrations) are shown in Table 3.1, where \( K_{12} = K_1 \times K_{2a} \) and \( K_{2b} = K_{12} / K_d \) are calculated from the literature.3,7
\[ 2 \text{TR}' \xrightleftharpoons{K_d} (\text{TR}')_2 \]  
\[ \text{CD} + \text{TR}' \xrightleftharpoons{K_1} \text{CD.(TR')} \]  
\[ \text{CD, TR}' + \text{TR}' \xrightleftharpoons{K_{2a}} \text{CD.(TR')}_2 \]  
\[ \text{CD} + 2 \text{TR}' \xrightleftharpoons{K_{12}} \text{CD.(TR')}_2 \]  
\[ \text{CD} + (\text{TR}')_2 \xrightleftharpoons{K_{2b}} \text{CD.(TR')}_2 \]  
\[ \beta\text{CD}_{2x} + \text{TR}' \xrightleftharpoons{K_1} \beta\text{CD}_{2x}.\text{TR}' \]  
\[ \beta\text{CD}_{2x}.\text{TR}' + \text{TR}' \xrightleftharpoons{K_{2a}} \beta\text{CD}_{2x}(\text{TR}')_2 \]  
\[ \beta\text{CD}_{2x} + 2 \text{TR}' \xrightleftharpoons{K_{12}} \beta\text{CD}_{2x}(\text{TR}')_2 \]  
\[ \beta\text{CD}_{2x} + (\text{TR}')_2 \xrightleftharpoons{K_{2b}} \beta\text{CD}_{2x}(\text{TR}')_2 \]  

**Table 3.1.** Stability constants for complexation of TR' by βCD and linked βCD dimers in aqueous solution.

<table>
<thead>
<tr>
<th>Host</th>
<th>(K_1) (dm(^3) mol(^{-1}))</th>
<th>(K_{2a}) (dm(^3) mol(^{-1}))</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD</td>
<td>7.1 (± 0.7) (\times 10^2)</td>
<td>4.0 (± 0.7) (\times 10^6)</td>
<td>(^3)</td>
</tr>
<tr>
<td>66βCD(_{2su})</td>
<td>3.1 (± 0.6) (\times 10^3)</td>
<td>6.0 (± 2.0) (\times 10^3)</td>
<td>(^7)</td>
</tr>
<tr>
<td>66βCD(_{2ur})</td>
<td>5.1 (± 0.8) (\times 10^4)</td>
<td>1.6 (± 0.5) (\times 10^5)</td>
<td>(^7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(K_{2b}) † (dm(^3) mol(^{-1}))</th>
<th>(K_{12}) † (dm(^6) mol(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD</td>
<td>3.1 (\times 10^6)</td>
<td>2.8 (± 1.4) (\times 10^9)</td>
</tr>
<tr>
<td>66βCD(_{2su})</td>
<td>2.1 (\times 10^4)</td>
<td>1.9 (± 2.6) (\times 10^7)</td>
</tr>
<tr>
<td>66βCD(_{2ur})</td>
<td>9.0 (\times 10^6)</td>
<td>8.2 (± 1.3) (\times 10^9)</td>
</tr>
</tbody>
</table>

† calculated from the literature\(^3,7\)

### 3.1.2. Structure and Properties of BNS\(^-\)

The structure of BNS\(^-\) is closely related to TNS\(^-\), where the methyl group is replaced by a \(t\)-butyl group. The absorption spectrum of BNS\(^-\) possesses bands centred at 265.0 nm (\(ε = \ldots \)

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2.46 × 10^4 dm^3 mol^{-1} cm^{-1}) and 317.5 (ε = 1.98 × 10^4 dm^3 mol^{-1} cm^{-1}) (Figs. 3.16, 3.18, 3.20, 3.22, 3.24, and 3.26), and is quite similar to that of TNS\(^{-}\) (Chapter 2). In this study, excitation at 320 nm is used to produce BNS\(^{-}\) fluorescence which compares with 320 nm\(^8\) or 325 nm\(^9\) used in earlier studies.

![Figure 3.1. 6-(4'-t-Butylanilino)-naphtalene-2-sulphonate, BNS\(^{-}\).](image)

**Figure 3.1.** 6-(4’-t-Butylanilino)-naphtalene-2-sulphonate, BNS\(^{-}\).

![Figure 3.2. Molecular models (no H and lone pairs shown) of BNS\(^{-}\), constructed and energy-minimised (MM2) using Chem3D\(^10\) (C - green, O - red, N - dark blue, S - yellow). The C\(^6\)-N-C\(^{1'}\) angle is 122° and the planes of the naphthyl and phenyl groups are rotated by 60° with respect to each other.](image)

**Figure 3.2.** Molecular models (no H and lone pairs shown) of BNS\(^{-}\), constructed and energy-minimised (MM2) using Chem3D\(^10\) (C - green, O - red, N - dark blue, S - yellow). The C\(^6\)-N-C\(^{1'}\) angle is 122° and the planes of the naphthyl and phenyl groups are rotated by 60° with respect to each other.

### 3.1.3. Complexation of BNS\(^{-}\) by \(\beta\)CD and Linked \(\beta\)CD Dimers

Previous studies suggested that it is the \(t\)-butylphenyl group rather than the naphthyl group of BNS\(^{-}\) that complexes in the \(\beta\)CD annulus because of the greater hydrophobicity of the \(t\)-butylphenyl group.\(^{11,12}\) The \(K_1\) (Eqn. 3.10) value for the complexation of BNS\(^{-}\) by \(\beta\)CD (Table 3.2) is generally about 10-fold greater than that for TNS\(^{-}\) (Table 2.1, Chapter 2). Although several studies give a \(K_2\) value (Section 2.1.2, Chapter 2) for complexation of TNS\(^{-}\) by \(\beta\)CD, none have reported a \(K_2\) (Eqn. 3.11) for BNS\(^{-}\).

\[
\begin{align*}
\beta\text{CD} + \text{BNS}^- & \rightleftharpoons \text{K}_1 \beta\text{CD.BNS}^- & (3.10) \\
\beta\text{CD.BNS}^- + \beta\text{CD} & \rightleftharpoons \text{K}_2 \beta\text{CD}_2\text{.BNS}^- & (3.11)
\end{align*}
\]
Sikorski and Petter\(^9\) reported the complexation of BNS\(^-\) by \(\beta\)CD and a range of dithiol-6,6 linked \(\beta\)CD dimers (Table 3.2). The fluorimetric titrations were performed at 296 K in 0.01 mol dm\(^{-3}\) phosphate buffer at pH 7.0, with excitation at 325 nm. It was pointed out from the study that the optimum length linker for complexation with BNS\(^-\) was \(-S(CH_2)_2S-\), with \(K_1 = 8.2 \times 10^6\) dm\(^3\) mol\(^{-1}\), and that as the linker length was increased, the free energy (\(\Delta G^o\)) of complexing dropped by about 1.0 kJ mol\(^{-1}\) per methylene group added. This is said to be consistent with a model for complexation in which the two \(\beta\)CD annuli cooperate in complexing the two ends of the ligand in analogy to the chelate effect.\(^9,12\) That is, the longer the linker, the more entropy must be lost to form the highly ordered \(\beta\)CD\(_2\).BNS\(^-\) host-guest complex. This account of the advantages of ditopic complexing has been called into question by Zhang and Breslow\(^12\) who determined that enthalpy, not entropy, was responsible for the tighter complexing on the part of linked \(\beta\)CD dimer hosts with ditopic guests.\(^9\)

Other studies shown that BNS\(^-\) forms a very stable complex with linked \(\beta\)CD dimers (Eqn. 3.12, where \(x\) is the linker) such as \(N,N'\)-bis(6\(^\alpha\)-\(\beta\)-cyclodextrinyl)imidazolium\(^11\) (66\(\beta\)CD\(_2\)im.BNS\(^-\)) and \(N,N'\)-bis(3\(^\alpha\)-\(\beta\)-cyclodextrinyl) (3,5-bis(mercaptomethyl)pyridine)\(^8\) (33\(\beta\)CD\(_2\)mp.BNS\(^-\)) (Table 3.2).

\[
\beta\text{CD}_2x + \text{BNS}^- \rightleftharpoons K_1 \beta\text{CD}_2x\cdot\text{BNS}^- \quad (3.12)
\]
Table 3.2. Stability constants, $K_1$ for complexation of BNS by $\beta$CD and linked $\beta$CD dimers in aqueous solution.

<table>
<thead>
<tr>
<th>Host</th>
<th>$K_1$ (dm$^3$ mol$^{-1}$)</th>
<th>Method</th>
<th>Host structure (See appendix 1)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$CD</td>
<td>$5.57 (\pm 0.3) \times 10^4$</td>
<td>Calorimetry</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>$\beta$CD</td>
<td>$3.8 (\pm 0.1) \times 10^4$</td>
<td>Fluorimetry</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>66$\beta$CD$_2$im</td>
<td>$3.5 (\pm 1.7) \times 10^7$</td>
<td>Fluorimetry</td>
<td>3.5</td>
<td>11</td>
</tr>
<tr>
<td>33$\beta$CD$_2$mp</td>
<td>$3.5 (\pm 0.1) \times 10^5$</td>
<td>Fluorimetry</td>
<td>3.12</td>
<td>8</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(SS)</td>
<td>$3.67 (\pm 0.50) \times 10^6$</td>
<td>Calorimetry</td>
<td>3.6</td>
<td>12</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(SS)</td>
<td>$5 \times 10^6$</td>
<td>Fluorimetry</td>
<td>3.6</td>
<td>13</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(SS)</td>
<td>$7.9 (\pm 2.1) \times 10^4$</td>
<td>Fluorimetry</td>
<td>3.6</td>
<td>9</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(S(CH$_2$)$_2$S)</td>
<td>$8.2 (\pm 1.2) \times 10^6$</td>
<td>Fluorimetry</td>
<td>3.7</td>
<td>9</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(S(CH$_2$)$_2$S)</td>
<td>$2.5 (\pm 0.6) \times 10^6$</td>
<td>Fluorimetry</td>
<td>3.8</td>
<td>9</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(S(CH$_2$)$_4$S)</td>
<td>$6.8 (\pm 1.1) \times 10^5$</td>
<td>Fluorimetry</td>
<td>3.9</td>
<td>9</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(S(CH$_2$)$_5$S)</td>
<td>$4.9 (\pm 1.0) \times 10^5$</td>
<td>Fluorimetry</td>
<td>3.10</td>
<td>9</td>
</tr>
<tr>
<td>66$\beta$CD$_2$(S(CH$_2$)$_6$S)</td>
<td>$1.5 (\pm 0.4) \times 10^5$</td>
<td>Fluorimetry</td>
<td>3.11</td>
<td>9</td>
</tr>
</tbody>
</table>

The aim of this research is to study the dimerisation of BNS$^-$ and the complexation of BNS$^-$ monomer and dimer by $\beta$CD and the five linked $\beta$CD dimers. The linkers of the $\beta$CD dimers are substituted onto either a C$^3A$ (with inversion) or a C$^6A$ of $\beta$CD. These dimers are $\text{N,N-bis(6}^A\text{-deoxy-6}^A\text{-}\beta\text{-cyclodextrin)succinamide, 66}$\textbf{\textit{$\beta$CD$_2$su}}, $\text{N-}$(2$^A$S,3$^A$S)-3$^A$-deoxy-3$^A$-$\beta$-cyclodextrin)-N-$\prime$-(6$^A$-deoxy-6$^A$-$\beta$-cyclodextrin)urea, 36\textbf{\textit{$\beta$CD$_2$su}}, \text{N,N-bis(2$^A$S,3$^A$S)-3$^A$-deoxy-3$^A$-$\beta$-cyclodextrin)succinamide, 33}$\textbf{\textit{$\beta$CD$_2$su}}, $\text{N,N-bis(6}^A\text{-deoxy-6}^A\text{-}\beta\text{-cyclodextrin)-urea, 66}$\textbf{\textit{$\beta$CD$_2$ur}}, and $\text{N-}$(2$^A$S,3$^A$S)-3$^A$-deoxy-3$^A$-$\beta$-cyclodextrin)-N-$\prime$-(6$^A$-deoxy-6$^A$-$\beta$-cyclodextrin)urea, 36\textbf{\textit{$\beta$CD$_2$ur}}$ (Chapter 2). The aim is also to extent the work done in Chapter 2 to find how the linking of $\beta$CD in dimers and the inversion of the C$^2A$ and C$^3A$ carbons of the C$^3A$ substituted $\beta$CD glucopyranose units of 33\textbf{\textit{$\beta$CD$_2$suc}}, 36\textbf{\textit{$\beta$CD$_2$suc}}, and 36$\beta$CDur affect complex stability.
3.2. Synthesis and Dimerisation Studies of BNS⁻

3.2.1. Synthesis of BNS⁻

The sodium salt of BNS⁻ was prepared from 2-amino-6-naphtalesulfonic acid and p-t-butyl-aniline in aqueous solution of Na₂S₂O₅ by a Bucherer reaction (Scheme 3.1) in a similar manner to that reported.¹³,¹⁴

![Scheme 3.1. Preparation of BNS⁻](image)

3.2.2. UV Titration Studies

Evidence for dimerisation of BNS⁻ under low concentration conditions in aqueous phosphate buffer at pH 7.0 and ionic strength 0.10 mol dm⁻³ at 298.2 K was sought by monitoring the absorbance change of 10 sample solutions containing BNS⁻ in increasing concentration over the range 5.06 × 10⁻⁶ - 4.93 × 10⁻⁵ mol dm⁻³ (Fig. 3.3). Absorbance spectra were recorded at 0.25 nm intervals over the range 220-350 nm.

It is seen that over the concentration range 5.06 × 10⁻⁶ - 4.93 × 10⁻⁵ mol dm⁻³ BNS⁻ exhibits a linear increase in UV absorbance in the range 220-350 nm in aqueous phosphate buffer (pH 7.0, I = 0.10 mol dm⁻³, 298.2 K) in accordance with the Beer-Lambert relationship and implies that there is no significant formation of the (BNS⁻)₂ dimer (Fig. 3.4).
Figure 3.3. Increasing absorbance of the UV spectra of BNS in aqueous phosphate buffer (298.2 K, pH 7.0, I = 0.10 mol dm$^{-3}$) as [BNS]$_{\text{total}}$ increases (5.06 $\times$ 10$^{-6}$ - 4.93 $\times$ 10$^{-5}$ mol dm$^{-3}$).

Figure 3.4. Linear increase in the UV absorbance of BNS under the same conditions as for Fig. 3.3.
3.2.3. 1D $^1$H NMR Titration Studies

The 1D $^1$H NMR titration studies on dimerisation of BNS$^-$ were carried out at [BNS$^-$]$_{total}$ about 50-500 fold greater than in the UV studies, by monitoring the variation of the $^1$H (300 MHz) resonance of the $t$-butyl resonance of BNS$^-$ in D$_2$O phosphate buffer at pH 7.0, ionic strength 0.10 mol dm$^{-3}$ at 298.2 K, for 7 sample solutions containing BNS$^-$ with concentrations increasing over the range $2.00 \times 10^{-4} - 2.00 \times 10^{-2}$ mol dm$^{-3}$ (Fig. 3.5).

The data were fitted using HypNMR 2003$^{15}$ (Protonic Software)$^{16}$ The non-linear fitted plots of resonance of the $t$-butyl resonance of BNS$^-$ (ppm) versus concentration (mol dm$^{-3}$) of BNS$^-$ together with the experimental values and speciation (%) to [BNS$^-$]$_{total}$ are shown in Fig. 3.6.

It can be seen that over the concentration range $0.02-4.00 \times 10^{-2}$ mol dm$^{-3}$ BNS$^-$ in D$_2$O solution (phosphate buffer, pH 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) the $^1$H NMR resonance of the $t$-butyl group moves upfield with increasing [BNS$^-$]$_{total}$ in D$_2$O solution consistent with increasing shielding (Figs. 3.5 and 3.6). This is consistent with the formation of a (BNS$^-$)$_2$ dimer in which the $t$-butyl protons of one BNS$^-$ experience shielding as a consequence of being in close proximity to the high $\pi$ electron density of the naphthalene group of a second BNS$^-$ in a dimer structure approximating to that shown in Scheme 3.2. Fitting of an algorithm for BNS$^-$ dimerisation to the variation of $\delta$ for $t$-butyl with concentration (Figure 3.6) yielded a dimerisation constant, $K_d = 2.63 \pm 0.02 \times 10^2$ dm$^3$ mol$^{-1}$. This small $K_d$ is consistent with insignificant formation of (BNS$^-$)$_2$ under the dilute conditions of the UV (Section 3.3.3) and fluorimetric (Section 3.3.4) studies, and is also consistent with the formation of (BNS$^-$)$_2$ at the higher concentration of the $^1$H NMR titration for the complexation of BNS$^-$ by $\beta$CD and the series of linked $\beta$CD dimers described below.

From the speciation plot (Fig. 3.6), the dimerisation of BNS$^-$ at [BNS$^-$]$_{total} = 2.00 \times 10^{-2}$ mol dm$^{-3}$ reached 73.5% of (BNS$^-$)$_2$ dimer formed and 26.5% of free BNS$^-$ At the concentration ([BNS$^-$]$_{total} = 2.00 \times 10^{-3}$ mol dm$^{-3}$) that complexation of BNS$^-$ by $\beta$CD and the series of linked $\beta$CD dimers 1D $^1$H NMR titration studies were carried out (Section 3.3.5), the speciation of (BNS$^-$)$_2$ dimer and free BNS$^-$ were 39.0% and 61.0%, respectively, in the absence of $\beta$CD or a linked $\beta$CD dimer.
Figure 3.5. 1D $^1$H NMR (300 MHz) titration spectra of BNS$^-$ as [BNS$^-$]$_{\text{total}}$ increases (2.00 × 10$^{-4}$ - 2.00 × 10$^{-2}$ mol dm$^{-3}$) in D$_2$O phosphate buffer (pD 7.0, I = 0.10 mol dm$^{-3}$, 298.2 K).

Figure 3.6. Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.5 where curve a is the best fit of the algorithm for dimerisation of BNS$^-$ (Eqn. 3.13) to the resonance variation, fitted by HypNMR 200315 (Protonic Software).$^{16}$ Right ordinate: speciation with [BNS$^-$]$_{\text{total}}$ = 100%, curve b = free BNS$^-$ % and curve c = (BNS$^-$)$_2$ %.

$$\text{BNS} + \text{BNS} \rightleftharpoons \text{BNS}_2 \quad K_d = 2.63 (\pm 0.02) \times 10^2 \text{ dm}^3 \text{ mol}^{-1}$$  \hfill (3.13)

Scheme 3.2. The dimerisation of BNS$.\,$
3.2.4. 2D $^1$H ROESY NMR Studies

2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) studies of dimerisation of BNS$^-$ were carried out at [BNS]$_{\text{total}} = 2.00 \times 10^{-2}$ mol dm$^{-3}$ in D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K). Under these conditions the percentage of (BNS$^-$)$_2$ is 73.5\% (Fig. 3.7).

The cross-peaks arising from dipolar interactions between $t$-butyl and aromatic BNS$^-$ protons BNS$^-$ protons arise dominantly from interactions between the adjacent $t$-butyl protons with $H^{2'-3'}$ (rectangle A) and with $H^{1,3,4,8}$ (rectangle B, Fig. 3.7). As the NOE is proportional to $r_{AB}^{-6}$ where $r_{AB}$ is the distance between interacting nuclei A and B, the cross-peak in rectangular B indicated the close through space interaction of the $t$-butyl and naphthyl group consistent with the dimerisation of (BNS$^-$)$_2$ as approximately shown in Scheme 3.2.

![Figure 3.7. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) at 2.00 $\times$ 10$^{-2}$ mol dm$^{-3}$ in BNS. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the t-butyl protons with phenyl and naphthyl protons of BNS, respectively. (N.B The t-butyl resonance is greatly truncated in this Figure).]
3.3. Studies of Complexation of BNS⁻ by βCD and Linked βCD Dimers

3.3.1. 2D ¹H ROESY NMR Studies

2D ¹H ROESY NMR (600 MHz, 0.3 s mixing time) studies of complexation of BNS⁻ were carried out at equimolar concentrations (2.00 × 10⁻³ mol dm⁻³) of BNS⁻ and either βCD or each of the series of linked βCD dimers in D₂O phosphate buffer solution (pD 7.0, I = 0.10 mol dm⁻³, 298.2 K). Under these conditions the 1:1 host-guest complex is the greatly dominant species in solution (see Section 3.3.5).

The cross-peaks arising from dipolar interactions between the BNS⁻ and βCD protons arise dominantly from interactions between the phenyl and t-butyl BNS⁻ protons and the βCD H³, H⁵ and H⁶ protons (although H⁶ cannot be clearly distinguished from H² and H⁴) with the naphthalene protons generating no cross-peaks (Fig. 3.8). This is consistent with the hydrophobic t-butylphenyl group occupying the βCD annulus whilst the more polar naphthalene-2-sulphonate group resides outside the βCD annulus. In contrast, most of the BNS⁻ protons form cross-peaks in 6βCD₂su.BNS⁻ and 6βCD₂ur.BNS⁻ are consistent with simultaneous complexation of BNS⁻ t-butylphenyl and naphthyl groups in the linked βCD annuli as shown in Fig. 3.9 and 3.12, respectively. The linked βCD dimers proton resonances consist of a broad range of overlapping multiplets because substitution at C⁶A renders the seven glucopyranose units inequivalent (as is also the case for substitution at C³A).

The spectra of 36βCD₂su.BNS⁻ (Fig. 3.10), 33βCD₂su.BNS⁻ (Fig. 3.11) and 36βCD₂ur.BNS⁻ (Fig. 3.13) also show cross-peaks arising from most of the BNS⁻ protons, but some of the aromatic resonances are substantially broadened in contrast to the t-butyl resonance which shows little broadening. This suggests some restraint to rapid rotation of BNS⁻ within the linked annuli possibly as a result of the altrose inversion of the C³A linked βCD. While the protons of the t-butyl group as a whole and its integral methyl groups are likely to experience rapid environmental averaging as a result of the relatively free rotation about bonds, such a mode of environmental averaging is less available to the aromatic protons in the naphthalene group in particular.
Figure 3.8. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) equimolar at $2.00 \times 10^{-3}$ mol dm$^{-3}$ in BNS$^{-}$ and βCD. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular H$^3$, H$^5$ and H$^6$ protons of βCD with the aromatic protons of BNS$^{-}$ and the t-butyl protons of BNS$^{-}$, respectively. Approximate complex structures are shown above.
Figure 3.9. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) equimolar at $2.00 \times 10^{-3}$ mol dm$^{-3}$ in BNS and $66\beta$CD$_{2su}$. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular H$^1$, H$^5$ and H$^6$ protons of $\beta$CD with the aromatic protons of BNS and the t-butyl protons of BNS, respectively. An approximate complex structure is shown above.
Figure 3.10. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) equimolar at $2.00 \times 10^{-3}$ mol dm$^{-3}$ in BNS- and $\beta$CD$_{2su}$. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular $H^3$, $H^5$ and $H^6$ protons of $\beta$CD with the aromatic protons of BNS and the t-butyl protons of BNS, respectively. Approximate complex structures are shown above.
Figure 3.11. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, I = 0.10 mol dm$^{-3}$, 298.2 K) equimolar at 2.00 $\times$ 10$^{-3}$ mol dm$^{-3}$ in BNS and $\beta$CD$_2$su. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular H$^5$, H$^6$ and H$^7$ protons of $\beta$CD with the aromatic protons of BNS and the t-butyl protons of BNS, respectively. An approximate complex structure is shown above.
Figure 3.12. 2D $^1$H ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a D$_2$O phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) equimolar at $2.00 \times 10^{-3}$ mol dm$^{-3}$ in BNS and $66\beta$CD$_2$ur. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular $H^1$, $H^5$ and $H^6$ protons of $\beta$CD with the aromatic protons of BNS and the t-butyl protons of BNS, respectively. An approximate complex structure is shown above.
Figure 3.13. 2D $^1H$ ROESY NMR (600 MHz, 0.3 s mixing time) spectrum of a $D_2O$ phosphate buffer solution (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K) equimolar at $2.00 \times 10^{-3}$ mol dm$^{-3}$ in BNS and $36\beta$CD$_{2}$ur. The green rectangles drawn on the spectrum, A and B, contain the cross-peaks arising from the NOE interactions of the annular $H^3$, $H^5$ and $H^6$ protons of $\beta$CD with the aromatic protons of BNS and the t-butyl protons of BNS, respectively. Approximate complex structures are shown above.
3.3.2. Molecular Modelling Studies

Molecular models of the 1:1 host:guest complexes were constructed and energy-minimised (MM2) using Cambridge Chem3D Ultra 8.0\textsuperscript{10} and are displayed in the space-filling representation with the hydrogens and lone pairs omitted to give a clearer view (Fig. 3.14). The models show that both hydrophobic regions of BNS\textsuperscript{-} fit well into both annuli of each linked βCD dimer and that the urea system fits better than the succinamide system. In principle, one βCD could rotate 180° about the linker with respect to the second βCD, but as the $K_1$ values (Table 3.3) for $\beta$CD$_2$.BNS$^-$ complexes are all larger than that for $\beta$CD.BNS$^-$ it appears that both βCD annuli of the linked dimers are involved in complexation and therefore that such a rotation is unlikely. The steric energies of complexes are also shown. Among the $\beta$CD$_2$.TNS$^-$ complexes, $66\beta$CD$_2$.su.TNS$^-$ and $66\beta$CD$_2$.ur.TNS$^-$ are more stable than the others.

![Molecular Models](image)

\begin{itemize}
  \item \textit{a.} $\beta$CD.BNS$^-$  \\
  \hspace{1cm} $E = 41.8$ kJ/mol
  \\
  \hspace{1cm} $E = 39.2$ kJ/mol
  \\
  \item \textit{b.} $66\beta$CD$_2$.su.BNS$^-$, $E = -3.2$ kJ/mol
\end{itemize}

\textbf{Figure 3.14.}
Figure 3.14. (continued) Space-filling representations (no H and lone pairs shown) of complexes BNS by βCD and linked βCD dimers (1:1), constructed and energy-minimised (MM2) using Chem3D\(^1\) (O - red, N - dark blue, S - yellow, βCD C - grey, BNS C - green).

c. 36βCD\(_{su}\).BNS
\(E = 127.7 \text{ kJ/mol}\)

d. 33βCD\(_{su}\).BNS, \(E = 98.8 \text{ kJ/mol}\)

e. 66βCD\(_{ur}\).BNS, \(E = 110.5 \text{ kJ/mol}\)

c. 36βCD\(_{su}\).BNS
\(E = 175.8 \text{ kJ/mol}\)

\[\beta CD_{su}\text{-BNS-}, E = 127.7 \text{ kJ/mol}\]
\[\beta CD_{su}\text{-BNS-}, E = 98.4 \text{ kJ/mol}\]
\[\beta CD_{ur}\text{-BNS-}, E = 110.5 \text{ kJ/mol}\]
The relevant approximate of BNS’ and the linked βCD dimers measured using Chem3D\textsuperscript{10} is shown in Fig. 3.15. The size of βCD is consistent with previous reports.\textsuperscript{19} In general, the length of BNS’ fits well in all of the linked βCD dimers studied, but fits better in the urea linked βCD dimers than in the succinamide linked βCD dimers.

**Figure 3.15.** Approximate dimensions of BNS’ and linked βCD dimers measured using Chem3D. For 66βCD\textsubscript{2su} d = 14.1 Å, 36βCD\textsubscript{2su} d = 13.5 Å, 33βCD\textsubscript{2su} d = 12.4 Å, 66βCD\textsubscript{2ur} d = 9.8 Å and 36βCD\textsubscript{2ur} d = 11.2 Å. The distance from O\textsubscript{3B} to O\textsubscript{6B} in the glucopyranose unit of βCD is 7.8 Å, the annular diameter measured from H\textsuperscript{5A} to H\textsuperscript{5D} is 7.5 Å and from H\textsuperscript{3A} to H\textsuperscript{3D} is 7.9 Å. The distance d in each linked βCD dimer is measured from the mid-point of this distance projected into the annulus centre of each βCD of each linked dimer.

As discussed in Section 2.4, Chapter 2, the succinamide linker is more flexible than the urea linker and, joining the linker to βCD through a primary C\textsuperscript{6A} carbon, appears to allow more flexibility than joining through a secondary inverted C\textsuperscript{3A}. While hydrogen bonding between the βCD hydroxy groups and water occurs, the structural constraints discussed above probably maintain the relative sizes of the interannular distances. The size of the interannular distance is not the only determinant of complex stability. While 66βCD\textsubscript{2ur} and 36βCD\textsubscript{2ur} with the shortest and second shortest distances, respectively, form the most and third most stable complexes with BNS’, the second most stable complex is 66βCD\textsubscript{2su} which has the longest distance (Table 3.5). In contrast to the two urea linked βCD dimer complexes, the stability variation 66βCD\textsubscript{2su}.BNS’ > 36βCD\textsubscript{2su}.BNS’ > 33βCD\textsubscript{2su}.BNS’ is the inverse of the interannular distances in the succinamide linked βCD dimers. The angles
between the planes of the βCD annuli and the presence of an altrose inversion probably also influence the relative complex stabilities.

3.3.3. UV Titration Studies

The complexation behaviour of BNS− in the βCD and linked βCD dimer annuli in aqueous phosphate buffer at pH 7.0, ionic strength 0.10 mol dm−3 and 298.2 K was studied by monitoring the absorbance change of 24 sample solutions containing BNS− (1.0 × 10−5 mol dm−3) with increasing concentrations of either βCD (0-2 × 10−4 mol dm−3); 66βCD2su (0-1 × 10−4 mol dm−3); 36βCD2su (0-2 × 10−5 mol dm−3); 33βCD2su (0-6 × 10−5 mol dm−3); 66βCD2ur (0-2 × 10−5 mol dm−3) or 36βCD2ur (0-6 × 10−5 mol dm−3) (Figs. 3.16, 3.18, 3.20, 3.22, 3.24 and 3.26, respectively). Twenty-four reference solutions for each system contained the same concentrations of βCD or linked βCD dimers as in the corresponding sample solutions. Absorbance spectra were recorded at 0.25 nm intervals over the range 220-350 nm. Some data points were discarded as outliers.

The data were fitted using the Specfit20 program through the Matlab21 protocol for the range of wavelengths where significant spectral change occurred. The models for fitting the complexation of BNS− by βCD and linked βCD dimers were 1:1 complexes. The algorithms arising from the equilibria shown in Eqns. 3.10 and 3.12 were fitted to the experimental data. Algorithms for the formation of 2:1 and 1:2 complexes as additional species did not fit the experimental data.

The non-linear fitted plots of molar absorbance (dm−3 mol−1 cm−1) versus concentration for the complexation of BNS− with βCD, 66βCD2su, 36βCD2su, 33βCD2su, 66βCD2ur and 36βCD2ur at a selected wavelength, together with the experimental values are shown in Fig. 3.17, 3.19, 3.21, 3.23, 3.25 and 3.27, respectively. The values obtained for the stability constants (K1) of the complexes are given in Table 3.3 and shown in Fig. 3.28.
Figure 3.16. Absorption spectrum of BNS \((1.0 \times 10^{-5} \text{ mol dm}^{-3})\) alone (blue curve) and in the presence of increasing concentration of \(\beta\text{CD} \ (0-2 \times 10^{-4} \text{ mol dm}^{-3})\) in aqueous solution at pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) and 298.2 K. The arrows show the direction of intensity change upon increasing \(\beta\text{CD}\) concentration. The \(\lambda_{\text{max}}\) values of complex \(\beta\text{CD}.BNS\) are 267.0 and 317.5 nm. An isosbestic point occurs at 241.0 nm.

Figure 3.17. Absorption change of BNS \((1.0 \times 10^{-5} \text{ mol dm}^{-3})\) in the presence of increasing concentration of \(\beta\text{CD} \ (0-2 \times 10^{-4} \text{ mol dm}^{-3})\) at 265.0 nm, fitted by the Specfit program from data shown in Fig. 3.16, in the regions 245-275 and 300-335 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.10.
Figure 3.18. Absorption spectrum of BNS (1.0 $\times$ 10$^{-5}$ mol dm$^{-3}$) alone (blue curve) and in the presence of increasing concentration of 66[\(\beta\)CD$_2$su] (0-1 $\times$ 10$^{-4}$ mol dm$^{-3}$) in aqueous solution at pH 7.0 (I = 0.10 mol dm$^{-3}$) and 298.2 K. The arrows show the direction of intensity change upon increasing 66[\(\beta\)CD$_2$su] concentration. The $\lambda_{\text{max}}$ values of complex 66[\(\beta\)CD$_2$su].BNS$^-$ are 278.0 and 324.0 nm. Isosbestic points occur at 235.0, 272.5, 287.5 and 302.0 nm.

Figure 3.19. Absorption change of BNS (1.0 $\times$ 10$^{-5}$ mol dm$^{-3}$) in the presence of increasing concentration of 66[\(\beta\)CD$_2$su] (0-1 $\times$ 10$^{-4}$ mol dm$^{-3}$) at 324.0 nm, fitted by the Specfit$^\text{20}$ program from data shown in Fig. 3.18, in the regions 275-285 and 305-345 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.20. Absorption spectrum of BNS $\left(1.0 \times 10^{-5} \text{ mol dm}^{-3}\right)$ alone (blue curve) and in the presence of increasing concentration of $36\beta\text{CD}_{2}\text{su}$ $\left(0-2 \times 10^{-5} \text{ mol dm}^{-3}\right)$ in aqueous solution at pH 7.0 ($I = 0.10 \text{ mol dm}^{-3}$) and 298.2 K. The arrows show the direction of intensity change upon increasing $36\beta\text{CD}_{2}\text{su}$ concentration. The $\lambda_{\text{max}}$ values of complex $36\beta\text{CD}_{2}\text{su}.\text{BNS}^-$ are 272.5 and 317.5. Isosbestic points occur at 235.0, 275.0, 286.5 and 306.0 nm.

Figure 3.21. Absorption change of BNS $\left(1.0 \times 10^{-5} \text{ mol dm}^{-3}\right)$ in the presence of increasing concentration of $36\beta\text{CD}_{2}\text{su}$ $\left(0-2 \times 10^{-5} \text{ mol dm}^{-3}\right)$ at 265.0 nm, fitted by the Specfit20 program from data shown in Fig. 3.20, in the region 250-275 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.22. Absorption spectrum of BNS (1.0 × 10⁻⁵ mol dm⁻³) alone (blue curve) and in the presence of increasing concentration of 33βCD₂su (0-6 × 10⁻⁵ mol dm⁻³) in aqueous solution at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K. The arrows show the direction of intensity change upon increasing 33βCD₂su concentration. The λ_{max} values of complex 33βCD₂su.BNS⁻ are 270.0 and 320.0 nm. Isosbestic points occur at 238.0 and 273.0 nm.

Figure 3.23. Absorption change of BNS⁻ (1.0 × 10⁻⁵ mol dm⁻³) in the presence of increasing concentration of 33βCD₂su (0-6 × 10⁻⁵ mol dm⁻³) at 260.0 nm, fitted by the Specfit²⁰ program from data shown in Fig. 3.22, in the region 245-275 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.24. Absorption spectrum of BNS (1.0 × 10^{-5} mol dm^{-3}) alone (blue curve) and in the presence of increasing concentration of 66βCD_{2}ur (0-2 × 10^{-5} mol dm^{-3}) in aqueous solution at pH 7.0 (I = 0.10 mol dm^{-3}) and 298.2 K. The arrows show the direction of intensity change upon increasing 66βCD_{2}ur concentration. The λ_{max} values of complex 66βCD_{2}ur.BNS are 278.0 and 323.0 nm. Isosbestic points occur at 234.5, 273.0, 287.0 and 303.0 nm.

Figure 3.25. Absorption change of BNS (1.0 × 10^{-5} mol dm^{-3}) in the presence of increasing concentration of 66βCD_{2}ur (0-2 × 10^{-5} mol dm^{-3}) at 320.0 nm, fitted by the Specfit20 program from data shown in Fig. 3.24, in the regions 250-270 and 315-345 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.26. Absorption spectrum of BNS \((1.0 \times 10^{-5} \text{ mol dm}^{-3})\) alone (blue curve) and in the presence of increasing concentration of \(36\beta\text{CD}_{2}\text{ur} (0-6 \times 10^{-5} \text{ mol dm}^{-3})\) in aqueous solution at pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) and 298.2 K. The arrows show the direction of intensity change upon increasing \(36\beta\text{CD}_{2}\text{ur}\) concentration. The \(\lambda_{\text{max}}\) values of complex \(36\beta\text{CD}_{2}\text{ur}.\text{BNS}\) are 267.0 and 316.5 nm. Isosbestic points occur at 244.0, 277.0 and 289.0 nm.

Figure 3.27. Absorption change of BNS \((1.0 \times 10^{-5} \text{ mol dm}^{-3})\) in the presence of increasing concentration of \(36\beta\text{CD}_{2}\text{ur} (0-6 \times 10^{-5} \text{ mol dm}^{-3})\) at 265.0 nm, fitted by the Specfit\textsuperscript{20} program from data shown in Fig. 3.26, in the regions 250-275 and 305-330 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
**Table 3.3.** Stability constants for complexation of BNS \((1.0 \times 10^{-5} \text{ mol dm}^{-3})\) with βCD and linked βCD dimers in aqueous phosphate buffer at pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) and 298.2 K.

<table>
<thead>
<tr>
<th>Host Conc. ranges (mol dm(^{-3}))</th>
<th>(K_1^a) (dm(^3) mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD 0-2.00 × 10(^{-4})</td>
<td>5.54 (±0.02) × 10(^4)</td>
</tr>
<tr>
<td>66βCD(_{2su}) 0-1.00 × 10(^{-4})</td>
<td>1.25 (±0.01) × 10(^6)</td>
</tr>
<tr>
<td>36βCD(_{2su}) 0-2.00 × 10(^{-5})</td>
<td>7.39 (±0.06) × 10(^5)</td>
</tr>
<tr>
<td>33βCD(_{2su}) 0-6.00 × 10(^{-5})</td>
<td>1.86 (±0.02) × 10(^5)</td>
</tr>
<tr>
<td>66βCD(_{2ur}) 0-2.00 × 10(^{-5})</td>
<td>3.64 (±0.03) × 10(^6)</td>
</tr>
<tr>
<td>36βCD(_{2ur}) 0-6.00 × 10(^{-5})</td>
<td>1.61 (±0.01) × 10(^5)</td>
</tr>
</tbody>
</table>

\(a\) The errors shown are the fitting errors, but when experimental error is taken into account the overall error is ± 3%.

**Figure 3.28.** Stability constants \((K_1)\) for complexation of BNS by a) βCD, b) 66βCD\(_{2su}\), c) 36βCD\(_{2su}\), d) 33βCD\(_{2su}\), e) 66βCD\(_{2ur}\) and f) 36βCD\(_{2ur}\) in aqueous phosphate buffer at pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) and 298.2 K.

UV titration studies for the complexation of BNS by βCD and linked βCD dimer show that while the change induced in the spectrum of BNS by 66βCD\(_{2su}\) \((\lambda_{\text{max}} = 278.0 \text{ and } 325.0 \text{ nm}, \epsilon = 28000 \text{ and } 28500 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})\) differs markedly from that induced by βCD, this difference is less for 36βCD\(_{2su}\) \((\lambda_{\text{max}} = 273.0 \text{ and } 318.0 \text{ nm}, \epsilon = 22100 \text{ and } 19700 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})\) and 33βCD\(_{2su}\) \((\lambda_{\text{max}} = 270.0 \text{ and } 320.0 \text{ nm}, \epsilon = 22900 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})\) and 33βCD\(_{2su}\) \((\lambda_{\text{max}} = 270.0 \text{ and } 320.0 \text{ nm}, \epsilon = 22900 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})\)
and 18800 dm$^3$ mol$^{-1}$ cm$^{-1}$). A similar relationship holds for 66βCD2ur ($\lambda_{\text{max}} = 278.0$ and 324.0 nm, $\varepsilon = 24600$ and 25100 dm$^3$ mol$^{-1}$ cm$^{-1}$) and 36βCD2ur ($\lambda_{\text{max}} = 267.0$ and 318.0 nm, $\varepsilon = 22200$ and 17400 dm$^3$ mol$^{-1}$ cm$^{-1}$).

The equivalent C$^{6A}$ substituted βCD of 66βCD2su and 66βCD2ur limits the orientations of the ends of BNS$^-$ simultaneously complexed in both annuli to a single orientation with respect to the primary and secondary ends of the annuli. However, BNS$^-$ complexing in 33βCD2su reverses this orientation, whereas complexing in 36βCD2su and 36βCD2ur potentially offers a choice. As the spectra of BNS$^-$ in 66βCD2su.BNS$^-$ and 66βCD2ur.BNS$^-$ differ most from of BNS$^-$ in βCD.BNS$^-$ it may be inferred that the orientation of BNS$^-$ in the latter complex has the t-butyl group adjacent to the primary hydroxyl end of the βCD annulus as is necessarily the case in 33βCD2su.BNS$^-$. An extension of this reasoning indicates that while BNS$^-$ has the choice of two opposite orientations when simultaneously bound in the C$^{3A}$ and C$^{6A}$ substituted βCD of 36βCD2su.BNS$^-$ and 36βCD2ur.BNS$^-$, that with the t-butylphenyl group in the C$^{3A}$ substituted βCD is probably favoured as judged on the basis of the similarity of the UV spectra of BNS$^-$ in these complexes and in βCD.BNS$^-$. Generally, the changes in the absorbance spectrum of complexed BNS$^-$ reflect changes in its hydration in the complexed environment and possibly some change in the relative orientation of the planes of the t-butylphenyl and naphthyl groups as twisting about the -NH- group occurs to optimize complexation in the linked βCD dimers.

The dominant host-guest complexes detected in the UV studies were 1:1 species, and resonance isosbestic points were observed in all cases. The 33βCD2su.BNS$^-$ and 66βCD2ur.BNS$^-$ complexes encompass the range of stabilities of the linked βCD dimer complexes and are three and 66 times more stable than the βCD.BNS$^-$ complex, respectively. The $K_1$ data for the 1:1 linked βCD dimer complexes are consistent with C$^{6A}$-C$^{6A}$ linking of βCD maximizing stability, while the altrose inversion in the C$^{3A}$-C$^{6A}$ and C$^{3A}$-C$^{3A}$ linked βCD annuli decreases their ability to accommodate BNS$^-$ and optimize the host-guest hydrophobic interactions. The nature of the linker also causes some variation in the magnitude of $K_1$ and, in combination with the C$^{3A}$ and C$^{6A}$ substitutions in the linked βCD dimers, produces the observed $K_1$ variation. The >10-fold increase in $K_1$ observed for the 66βCD2su.BNS$^-$, 36βCD2su.BNS$^-$ and 66βCD2ur.BNS$^-$ systems by comparison with $K_1$
for $\beta$CD.BNS$^-$ indicates a cooperativity in simultaneous complexing of BNS$^-$ in their linked $\beta$CD annuli.

By comparison with previous studies under similar condition (Table 3.2), the $K_1$ of $\beta$CD.BNS$^-$ $(5.54 \pm 0.02) \times 10^4 \text{ dm}^3 \text{ mol}^{-1}$ is consistent with the value of Zhang and Breslow $(5.57 \pm 0.3) \times 10^4 \text{ dm}^3 \text{ mol}^{-1}$ measured by a calorimetric method.$^{12}$

### 3.3.4. Fluorescence Titration Studies

The complexation behaviour of BNS$^-$ in $\beta$CD and linked $\beta$CD dimer annuli in aqueous phosphate buffer at pH 7.0, ionic strength 0.10 mol dm$^{-3}$ and 298.2 K was studied by monitoring the fluorescence intensity change of 30 sample solutions containing BNS$^-$ ($1.0 \times 10^{-6}$ mol dm$^{-3}$) with increasing concentrations of either $\beta$CD ($0-1 \times 10^{-4}$ mol dm$^{-3}$); $\beta$CD$_2$su ($0-1 \times 10^{-5}$ mol dm$^{-3}$); $\beta$CD$_2$su ($0-1 \times 10^{-5}$ mol dm$^{-3}$); $\beta$CD$_2$su ($0-5 \times 10^{-5}$ mol dm$^{-3}$); $\beta$CD$_2$ur ($0-5 \times 10^{-6}$ mol dm$^{-3}$) or $\beta$CD$_2$ur ($0-5 \times 10^{-5}$ mol dm$^{-3}$) (Figs. 3.29, 3.31, 3.33, 3.35, 3.37 and 3.39, respectively). Emission spectra were recorded at 0.5 nm intervals over the range 350-550 nm. All emission spectra were recorded at an excitation wavelength of 320.0 nm. Some data points were discarded as outliers.

The data were fitted using the Specfit$^{20}$ program through the Matlab$^{21}$ protocol for the range of wavelengths where significant spectral change occurred. The models for fitting the complexation of BNS$^-$ by $\beta$CD and linked $\beta$CD dimers were 1:1 complexes. The algorithm arising from the equilibria shown in Eqns. 3.10 and 3.12 were fitted to the experimental data. Algorithms for the formation of 2:1 and 1:2 complexes as additional species did not fit the experimental data.

The non-linear fitted plots of relative fluorescence intensity versus concentration for the complexation of BNS$^-$ with $\beta$CD, $\beta$CD$_2$su, $\beta$CD$_2$su, $\beta$CD$_2$su, $\beta$CD$_2$ur and $\beta$CD$_2$ur at selected wavelengths, together with the experimental values are shown in Figs. 3.30, 3.32, 3.34, 3.36, 3.38 and 3.40, respectively. The obtained stability constants, $K_1$, for the complexes formed are given in Table 3.4 and shown in Fig. 3.41.
Figure 3.29. Emission of BNS (1.0 × 10^{-6} mol dm^{-3}) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of βCD (0-1 × 10^{-4} mol dm^{-3}) in aqueous solution at pH 7.0 (I = 0.10 mol dm^{-3}) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 5 and 10 nm, respectively. The λ_{max} value of complex βCD.BNS is 464.5 nm.

Figure 3.30. Emission of BNS (1.0 × 10^{-6} mol dm^{-3}) in the presence of increasing concentration of βCD (0-1 × 10^{-4} mol dm^{-3}) at 464.0 nm, fitted by the Specfit^{20} program from data shown in Fig. 2.29, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.10.
Figure 3.31. Emission of BNS (1.0 \times 10^{-6} \text{ mol dm}^{-3}) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of \(66\beta\text{CD}_{2}\text{su}\) (0-1 \times 10^{-4} \text{ mol dm}^{-3}) in aqueous solution at pH 7.0 (I = 0.10 \text{ mol dm}^{-3}) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 2.5 and 5 nm, respectively. The \(\lambda_{\text{max}}\) value of complex \(66\beta\text{CD}_{2}\text{su}.\text{BNS}\) is 433.0 nm.

Figure 3.32. Emission of BNS (1.0 \times 10^{-6} \text{ mol dm}^{-3}) in the presence of increasing concentration of \(66\beta\text{CD}_{2}\text{su}\) (0-1 \times 10^{-5} \text{ mol dm}^{-3}) at 430.0 nm, fitted by the Specfit program from data shown in Fig. 2.31, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.33. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of \(36\beta CD2su\) (0-1 × 10⁻⁵ mol dm⁻³) in aqueous solution at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 2.5 and 5 nm, respectively. The \(\lambda_{max}\) value of complex \(36\beta CD2su.BNS\) is 432.0 nm.

Figure 3.34. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) in the presence of increasing concentration of \(36\beta CD2su\) (0-1 × 10⁻⁵ mol dm⁻³) at 433.0 nm, fitted by the Specfit²⁰ program from data shown in Fig. 2.33, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.35. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of \(33\beta CD_{2su}\) (0-5 × 10⁻⁵ mol dm⁻³) in aqueous solution at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 2.5 and 5 nm, respectively. The \(\lambda_{\text{max}}\) value of complex \(33\beta CD_{2su}.BNS^-\) is 436.5 nm.

Figure 3.36. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) in the presence of increasing concentration of \(33\beta CD_{2su}\) (0-5 × 10⁻⁵ mol dm⁻³) at 435.0 nm, fitted by the Specfit²⁰ program from data shown in Fig. 2.35, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.37. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of 66βCD₂ur (0-6 × 10⁻⁶ mol dm⁻³) in aqueous solution at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 2.5 and 5 nm, respectively. The λ_max value of complex 66βCD₂ur.BNS is 431.0 nm.

Figure 3.38. Emission of BNS (1.0 × 10⁻⁶ mol dm⁻³) in the presence of increasing concentration of 66βCD₂ur (0-5 × 10⁻⁶ mol dm⁻³) at 430.0 nm, fitted by the Specfit²⁰ program from data shown in Fig. 2.37, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Figure 3.39. Emission of BNS (1.0 × 10^{-6} mol dm^{-3}) alone (lowest intensity (blue) curve in the montage) and in the presence of increasing concentration of 36\(\beta\)CD2ur (0-5 × 10^{-5} mol dm^{-3}) in aqueous solution at pH 7.0 (I = 0.10 mol dm^{-3}) and 298.2 K, as a function of wavelength when excited at 320.0 nm with excitation and emission slit widths of 2.5 and 5 nm, respectively. The \(\lambda_{max}\) value of complex 36\(\beta\)CD2ur.BNS is 436.5 nm.

Figure 3.40. Emission of BNS (1.0 × 10^{6} mol dm^{-3}) in the presence of increasing concentration of 36\(\beta\)CD2ur (0-5 × 10^{-5} mol dm^{-3}) at 435.0 nm, fitted by the Specfit program from data shown in Fig. 2.39, in the region 400-500 nm. The purple circles represent data points and the blue solid line represents the best fit to the algorithm arising from the equilibrium shown in Eqn. 3.12.
Table 3.4. Stability constants, $K_1$, for complexation of BNS ($1.0 \times 10^6$ mol dm$^{-3}$) by $\beta$CD and the series of linked $\beta$CD dimers in aqueous phosphate buffer at pH 7 ($I = 0.10$ mol dm$^{-3}$) and 298.2 K.

<table>
<thead>
<tr>
<th>Host</th>
<th>Conc. ranges (mol dm$^{-3}$)</th>
<th>$K_1^a$ (dm$^3$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$CD</td>
<td>0-1.00 $\times$ 10$^{-4}$</td>
<td>4.67 ($\pm$0.01) $\times$ 10$^4$</td>
</tr>
<tr>
<td>66$\beta$CD$_{2su}$</td>
<td>0-1.00 $\times$ 10$^{-5}$</td>
<td>3.30 ($\pm$0.01) $\times$ 10$^6$</td>
</tr>
<tr>
<td>36$\beta$CD$_{2su}$</td>
<td>0-1.00 $\times$ 10$^{-5}$</td>
<td>1.01 ($\pm$0.01) $\times$ 10$^6$</td>
</tr>
<tr>
<td>33$\beta$CD$_{2su}$</td>
<td>0-5.00 $\times$ 10$^{-5}$</td>
<td>1.10 ($\pm$0.01) $\times$ 10$^5$</td>
</tr>
<tr>
<td>66$\beta$CD$_{2ur}$</td>
<td>0-5.00 $\times$ 10$^{-6}$</td>
<td>4.35 ($\pm$0.02) $\times$ 10$^6$</td>
</tr>
<tr>
<td>36$\beta$CD$_{2ur}$</td>
<td>0-5.00 $\times$ 10$^{-5}$</td>
<td>2.96 ($\pm$0.01) $\times$ 10$^5$</td>
</tr>
</tbody>
</table>

$^a$ The errors shown are the fitting errors, but when experimental error is taken into account the overall error is $\pm$ 3%.

Figure 3.41. Stability constants, $K_1$, for complexation of BNS by a) $\beta$CD, b) 66$\beta$CD$_{2su}$, c) 36$\beta$CD$_{2su}$, d) 33$\beta$CD$_{2su}$, e) 66$\beta$CD$_{2ur}$ and f) 36$\beta$CD$_{2ur}$ in aqueous phosphate buffer at pH 7 ($I = 0.10$ mol dm$^{-3}$) and 298.2 K.

It can be seen that the BNS$^-$ relative fluorescence increases on complexation by $\beta$CD and the series of linked $\beta$CD dimers consistent with decreased quenching by water dipoles through complexation in the $\beta$CD and linked $\beta$CD annuli. By comparison with the complex stability of $\beta$CD.BNS$^-$, the highest stability of linked $\beta$CD dimer complexes with BNS$^-$ is shown by 66$\beta$CD$_{2ur}$ (93 times that of $\beta$CD.BNS$^-$); while those of 66$\beta$CD$_{2su}$,
36βCD₂su, 36βCD₂ur, and 33βCD₂su (70, 21, 6 and 2 times that of βCD.BNS⁻, respectively) are lower. Thus, cooperative complexing occurs for the first four linked βCD dimers studied, while the 33βCD₂su.BNS⁻ complex shown only a statistical enhancement in stability.

The BNS⁻ fluorescence maximum occur at 359.0 nm and 481.5 nm with relative fluorescences of 1.1 a.u. and 0.5 a.u. (arbitrary fluorescence units), respectively, while those for βCD.BNS⁻ are 464.5 nm and 134 a.u. Substantially greater changes are observed for 66βCD₂su.BNS⁻ (433.0 nm, 209 a.u.), 36βCD₂su.BNS⁻ (432.0 nm, 154 a.u.), 33βCD₂su.BNS⁻ (436.5 nm, 137 a.u.), 66βCD₂ur.BNS⁻ (431.0 nm, 245 a.u.), and 63βCD₂su.BNS⁻ (436.5 nm, 142 a.u.). These changes are consistent with complexation changing the BNS⁻ environment and enhancing fluorescence as a consequence of complexation inside the hydrophobic βCD annulus.

By analogy to closely related TNS⁻, the changes in the observed $\lambda_{\text{max}}$ are consistent with the existence of three excited states of BNS⁻ whose relative populations are environment dependent.²² Excitation ($\pi \rightarrow \pi^*$) from the BNS⁻ $S_0$ ground state in which the planes of the naphthyl and phenyl groups are rotated with respect to each other results in three BNS⁻ excited states (Fig. 3.42). The first is $S_{1,\text{np}}$ (excitation $\lambda_{\text{max}} = 320.0$ nm, emission $\lambda_{\text{max}} = 482.0$ nm) in which the nonplanarity is retained. Electron transfer produces two BNS⁻ charge transfer excited states: $S_{1,\text{ct, np}}$ (excitation $\lambda_{\text{max}} = 320-330$ nm, emission $\lambda_{\text{max}} \sim 400$ nm) in which the naphthyl and phenyl planes approach co-planarity, and $S_{1,\text{ct,perp}}$ (excitation $\lambda_{\text{max}} = 290.0$ nm, emission $\lambda_{\text{max}} = 406.0$ nm) in which the naphthyl and phenyl planes are perpendicular to each other. In water the $S_{1,\text{ct,perp}}$ excited state dominates as a result of water hydrogen bonding to the amine nitrogen of BNS⁻ while $S_{1,\text{ct, np}}$ is much less populated and $S_{1,\text{np}}$ is even less populated. However, in non-polar and viscous solvents $S_{1,\text{np}}$ becomes dominantly populated. The latter solvent conditions resemble the hydrophobic and motion restricting environment which BNS⁻ experiences in the βCD annulus. Accordingly, the decrease of the observed emission $\lambda_{\text{max}}$ for BNS⁻ in the linked βCD dimer complexes is consistent with $S_{1,\text{np}}$ becoming the dominant excited state, as shown for 66βCD₂ur.BNS⁻ in Fig. 3.43, whereas the observed $\lambda_{\text{max}} = 406.0$ nm for BNS⁻ in water is a consequence of emission from the dominant $S_{1,\text{ct,perp}}$ excited state.
The increased BNS\(^-\) fluorescence with complexation probably results from a combination of three factors. The first is the decrease in the relative populations of the charge transfer S\(_{1,ct,np}\) and S\(_{1,ct,perp}\) excited states, which are likely to decay more rapidly than the S\(_{1,np}\) excited state. The second is the isolation of BNS\(^-\) from the quenching pathway provided by water oscillators, and the third is the decrease in the number of rotational degrees of freedom for BNS\(^-\) which also decreases the effectiveness of quenching.\(^{23}\)

By comparison with previous studies under similar condition (Table 3.2), the stability constant of $\beta$CD.BNS\(^-\) (4.67 (±0.01) \times 10^4 \text{ dm}^3 \text{ mol}^{-1})$ is consistent with the value of Breslow and Halfon (3.8 (±0.1) \times 10^4 \text{ dm}^3 \text{ mol}^{-1}).\(^{11}\)
Figure 3.42. Three dimensional plot of the fluorescence of BNS (1.0 × 10⁻⁵ mol dm⁻³) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K as a function of excitation and emission wavelength at 2 nm intervals.

Figure 3.43. Three dimensional plot of the fluorescence of BNS (1.0 × 10⁻⁶ mol dm⁻³) and 66βCD₂ur (5.0 × 10⁻⁶) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm⁻³) and 298.2 K as a function of excitation and emission wavelength at 2 nm intervals. Under these conditions [66βCD₂ur:BNS] = 0.95 × 10⁻⁶ mol dm⁻³.
3.3.5. 1D $^1$H NMR Titration Studies

The complexation of dimerised BNS$^-$ in the $\beta$CD and the linked $\beta$CD dimers was carried out by monitoring the variation of the $^1$H (300 MHz) resonance of the $t$-butyl resonance of BNS$^-$ in D$_2$O phosphate buffer at pH 7.0, ionic strength 0.10 mol dm$^{-3}$ and 298.2 K. Fifteen sample solutions containing BNS$^-$ with increasing concentrations of either $\beta$CD, 66$\beta$CD$_{2su}$, 36$\beta$CD$_{2su}$, 33$\beta$CD$_{2su}$, 66$\beta$CD$_{2ur}$ or 36$\beta$CD$_{2ur}$ over the range $0.5 \times 10^{-3}$ (mol dm$^{-3}$) were studied (Figs. 3.44, 3.46, 3.48, 3.50, 3.52 or 3.54, respectively).

The data were fitted by HypNMR 2003$^{15}$ (Protonic Software).$^{16}$ The non-linear fitted plots of resonance of the $t$-butyl resonance of BNS$^-$ (ppm) versus concentration (mol dm$^{-3}$) of $\beta$CD, 66$\beta$CD$_{2su}$, 36$\beta$CD$_{2su}$, 33$\beta$CD$_{2su}$, 66$\beta$CD$_{2ur}$ or 36$\beta$CD$_{2ur}$, together with the experimental values and speciation ($\%$) to [BNS$^-$]$_{total}$ are shown in Figs. 3.45, 3.47, 3.49, 3.51, 3.53 and 3.55, respectively. The derived $K_{12}$ (accumulated constant, Eqn. 3.14 and 3.15) appear in Table 3.5 together with calculated values of $K_{2a}$ (stepwise constant, $K_{2a} = K_{12}/K_1$, where $K_1$ are values determined by UV titrations) for complexation of BNS$^-$ in $\beta$CD.BNS$^-$ and $\beta$CD$_{2x}$.BNS$^-$, and $K_{2b}$ (stepwise constant, $K_{2b} = K_{12}/K_d$) for complexation of (BNS$^-$)$_2$ by $\beta$CD and the linked $\beta$CD dimers to form 1:2 host:guest complexes (Scheme 3.3).

\begin{align*}
2 \text{BNS}^- &+ \text{B} & \xrightleftharpoons{K_{12}} & \text{B} \text{(BNS)}_2 & (3.14) \\
2 \text{BNS}^- &+ \text{B} \text{CD}_{2x} & \xrightleftharpoons{K_{12}} & \text{B} \text{CD}_{2x} \text{(BNS)}_2 & (3.15)
\end{align*}

The complexation of BNS$^-$ by $\beta$CD and the series of linked $\beta$CD dimers to form 1:2 host:guest complexes under these conditions involves a sequence of sterically orienting interactions and solvation changes which are not separately identifiable from experiment. It is, nevertheless, informative to consider the formation of the various complexes in a reaction with two stages.$^{3-5}$ The two possible mechanisms and accompanying equilibrium constants are shown in Scheme 3.3.
Figure 3.44. 1D $^1$H NMR (300 MHz) titration spectrum of BNS (2.00 × 10^{-3} mol dm^{-3}) (red curve) and in the presence of increasing concentration of $\beta$CD (0-5 × 10^{-3} mol dm^{-3}) in $D_2O$ phosphate buffer (pD 7.0, I = 0.10 mol dm^{-3}, 298.2 K).

Figure 3.45. Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS under the same conditions as in Fig. 3.44 where curve a is the best fit of the algorithm for complexation of BNS to the resonance variation, fitted by HypNMR 200315 (Protonic Software). Right ordinate: speciation with [BNS]_{total} = 100%, curve b = free BNS %, curve c = (BNS)$_2$ %, d = $\beta$CD.BNS %, and e = $\beta$CD.(BNS)$_2$ %.
**Figure 3.46.** 1D $^1$H NMR (300 MHz) titration spectra of BNS$^-$ (2.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) (red curve) and in the presence of increasing concentration of 66$\beta$CD$_{2su}$ (0-5 $\times$ 10$^{-3}$ mol dm$^{-3}$) in D$_2$O phosphate buffer (pD 7.0, I = 0.10 mol dm$^{-3}$, 298.2 K).

**Figure 3.47.** Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.46 where curve a is the best fit of the algorithm for complexation of BNS$^-$ to the resonance variation, fitted by HypNMR 2003$^{15}$ (Protonic Software).$^{16}$ Right ordinate: speciation with [BNS$^-$]$_{total}$ $\equiv$ 100%, curve b = free BNS %, curve c = (BNS)$^2$ %, d = 66$\beta$CD$_{2su}$.BNS %, and e = 66$\beta$CD$_{2su}$. (BNS)$^2$ %.
**Figure 3.48.** 1D $^1H$ NMR (300 MHz) titration spectra of BNS$^-$ (2.00 × 10$^{-3}$ mol dm$^{-3}$) (red curve) and in the presence of increasing concentration of 36βCD$_{2su}$ (0-5 × 10$^{-3}$ mol dm$^{-3}$) in D$_2$O phosphate buffer (pD 7.0, I = 0.10 mol dm$^{-3}$, 298.2 K).

**Figure 3.49.** Left ordinate: variation of the $^1H$ (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.48 where curve a is the best fit of the algorithm for complexation of BNS$^-$ to the resonance variation, fitted by HypNMR 200315 (Protonic Software).16 Right ordinate: speciation with $[\text{BNS}^-]_{\text{total}} = 100\%$, curve b = free BNS$^-$ %, curve c = (BNS$^-$)$_2$ %, d = 36βCD$_{2su}.\text{BNS}^-$ %, and e = 36βCD$_{2su}.(\text{BNS}^-)_2$ %.
Figure 3.50. 1D $^1$H NMR (300 MHz) titration spectra of BNS$^-$(2.00 × 10$^{-3}$ mol dm$^{-3}$) (red curve) and in the presence of increasing concentration of $^{33}\beta$CD$_{2su}$ (0-5 × 10$^{-3}$ mol dm$^{-3}$) in D$_2$O phosphate buffer (pD 7.0, I = 0.10 mol dm$^{-3}$, 298.2 K).

Figure 3.51. Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.50 where curve a is the best fit of the algorithm for complexation of BNS$^-$ to the resonance variation, fitted by HypNMR 2003$^{15}$ (Protonic Software).$^{16}$ Right ordinate: speciation with $[\text{BNS}]_{\text{total}} = 100\%$, curve b = free BNS %, curve c = (BNS)$_2$ %, d = $^{33}\beta$CD$_{2su}$.BNS %, and e = $^{33}\beta$CD$_{2su}$.BNS$_2$ %.
Figure 3.52. 1D $^1$H NMR (300 MHz) titration spectra of BNS$^-$ ($2.00 \times 10^{-3}$ mol dm$^{-3}$) (red curve) and in the presence of increasing concentration of 66βCD$_{2}$ur (0-5 $\times 10^{-3}$ mol dm$^{-3}$) in D$_2$O phosphate buffer (pD 7.0, $I = 0.10$ mol dm$^{-3}$, 298.2 K).

Figure 3.53. Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.52 where curve a is the best fit of the algorithm for complexation of BNS$^-$ to the resonance variation, fitted by HypNMR 2003$^{15}$ (Protonic Software).$^{16}$ Right ordinate: speciation with [BNS$^-$]$_{\text{total}} = 100\%$, curve b = free BNS $\%$, curve c = (BNS)$^2$ $\%$, d = 66βCD$_{2}$ur.BNS $\%$, and e = 66βCD$_{2}$ur.(BNS)$^2$ $\%$. 

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Figure 3.54. 1D $^1$H NMR (300 MHz) titration spectra of BNS$^-$ (2.00 \times 10^{-3} \text{ mol dm}^{-3}) (red curve) and in the presence of increasing concentration of 36$\beta$CD$_2$ur (0-5 \times 10^{-3} \text{ mol dm}^{-3}) in D$_2$O phosphate buffer (pD 7.0, $I = 0.10 \text{ mol dm}^{-3}$, 298.2 K).

Figure 3.55. Left ordinate: variation of the $^1$H (300 MHz) resonance of the t-butyl resonance of BNS$^-$ under the same conditions as in Fig. 3.54 where curve a is the best fit of the algorithm for complexation of BNS$^-$ to the resonance variation, fitted by HypNMR 2003\textsuperscript{15} (Protonic Software).\textsuperscript{16} Right ordinate: speciation with [BNS$^-$]$_{total} \equiv 100\%$, curve b = free BNS $\%$, curve c = (BNS)$^2$$\%$, d = 36$\beta$CD$_2$ur.BNS $\%$, and e = 36$\beta$CD$_2$ur.(BNS)$^2$ $\%$. 
Table 3.5. Complexation constants for 1:2 host-guest complexes of BNS \((K_{2a}, K_{2b}\) (Scheme 3.3)) determined by \(^1\)H NMR spectroscopy in \(D_2O\) phosphate buffer (pH 7.0, \(I = 0.10 \text{ mol dm}^{-3}\)) at 298.2 K.

<table>
<thead>
<tr>
<th>Host</th>
<th>(K_1^a) (\text{dm}^3\text{mol}^{-1})</th>
<th>(K_{2a}^a) (\text{dm}^3\text{mol}^{-1})</th>
<th>(K_{2b}^a) (\text{dm}^3\text{mol}^{-1})</th>
<th>(K_{12}^a) (\text{dm}^6\text{mol}^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta)CD</td>
<td>(5.54 (\pm 0.02) \times 10^4)</td>
<td>(2.29 (\pm 0.01) \times 10^2)</td>
<td>(4.83 (\pm 0.02) \times 10^4)</td>
<td>(1.27 (\pm 0.01) \times 10^7)</td>
</tr>
<tr>
<td>66(\beta)CD(_{2su})</td>
<td>(1.25 (\pm 0.01) \times 10^6)</td>
<td>(2.57 (\pm 0.02) \times 10^2)</td>
<td>(1.22 (\pm 0.03) \times 10^6)</td>
<td>(3.21 (\pm 0.02) \times 10^8)</td>
</tr>
<tr>
<td>36(\beta)CD(_{2su})</td>
<td>(7.39 (\pm 0.06) \times 10^5)</td>
<td>(2.30 (\pm 0.01) \times 10^2)</td>
<td>(6.46 (\pm 0.03) \times 10^5)</td>
<td>(1.70 (\pm 0.01) \times 10^8)</td>
</tr>
<tr>
<td>33(\beta)CD(_{2su})</td>
<td>(1.86 (\pm 0.02) \times 10^5)</td>
<td>(2.57 (\pm 0.02) \times 10^2)</td>
<td>(1.82 (\pm 0.03) \times 10^5)</td>
<td>(4.78 (\pm 0.02) \times 10^7)</td>
</tr>
<tr>
<td>66(\beta)CD(_{2ur})</td>
<td>(3.64 (\pm 0.03) \times 10^6)</td>
<td>(1.72 (\pm 0.02) \times 10^2)</td>
<td>(2.38 (\pm 0.04) \times 10^6)</td>
<td>(6.27 (\pm 0.03) \times 10^8)</td>
</tr>
<tr>
<td>36(\beta)CD(_{2ur})</td>
<td>(1.61 (\pm 0.01) \times 10^5)</td>
<td>(1.80 (\pm 0.02) \times 10^3)</td>
<td>(1.10 (\pm 0.03) \times 10^6)</td>
<td>(2.89 (\pm 0.02) \times 10^8)</td>
</tr>
</tbody>
</table>

* The errors shown are the fitting errors, but when experimental error is taken into account the overall error is \(\pm 3\%\).

Figure 3.56. Accumulated stability constants \((K_{12})\) for 1:2 host:guest complexation of BNS by a) \(\beta\)CD, b) 66\(\beta\)CD\(_{2su}\), c) 36\(\beta\)CD\(_{2su}\), d) 33\(\beta\)CD\(_{2su}\), e) 66\(\beta\)CD\(_{2ur}\) and f) 36\(\beta\)CD\(_{2ur}\) in aqueous solution pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) and 298.2 K.
Scheme 3.3. Equilibria for the complexation of BNS by 66βCD$_{2su}$. Analogous schemes apply for βCD and other linked βCD dimers studied.

The 1D $^1$H NMR titration studies were carried out at [BNS$^-$]$_{total}$ 50- and 500-fold greater than the UV spectrophotometric and fluorimetric studies. Consequently, formation of the (BNS$^-$)$_2$ dimer occurs simultaneously with BNS$^-$ complexation by βCD and the linked βCD dimers. The variation of $\delta$ for the t-butyl group of BNS$^-$ with $[\beta CD]_{total}$ is shown in Fig. 3.44. An algorithm incorporating (BNS$^-$)$_2$ and βCD.BNS$^-$ only could not be fitted to the variation of $\delta$. However, incorporation of the formation of βCD.(BNS$^-$)$_2$ provided an excellent fit as seen in Fig. 3.45 which also shows the speciation of the system. The increasing downfield shift of $\delta$ for the t-butyl group is broadly a consequence of the complexation of BNS$^-$ in βCD.BNS$^-$ inducing a downfield shift approximately twice the upfield shift caused by the dimerisation of BNS$^-$. The analogous data and fitting for the other linked βCD dimer systems appear in Figs. 3.46 - 3.55.

The speciation plots indicate that up to about 15% BNS$^-$ is complexed in 1:2 host:guest complexes (βCD.(BNS$^-$)$_2$ or βCD$_{2x}$.BNS$^-$)$_2$) formed when the concentration of βCD or linked βCD dimers is $1.00 \times 10^{-3}$ mol dm$^{-3}$ (that is half of [BNS$^-$]$_{total}$ $2.00 \times 10^{-3}$ mol dm$^{-3}$). When the concentration of βCD or linked βCD dimers reaches $2.00 \times 10^{-3}$ mol dm$^{-3}$, the only species observed in solution are the 1:1 host:guest complexes.
It is seen from Table 3.5 and Fig. 3.56 that the accumulated stability constants \((K_{12})\) for 1:2 host:guest complexation of \(\beta CD\) and the series of linked \(\beta CD\) dimers with BNS\(^-\) decrease in the order \(66\beta CD_{2su}.BNS^- > 36\beta CD_{2su}.BNS^- > 33\beta CD_{2su}.BNS^-\) and a similar trend applies for the urea linked \(\beta CD\) dimers. The lowest \(K_{12}\) is shown by \(33\beta CD_{2su}\) which is four times greater than \(K_{12}\) for \(\beta CD\), and the largest \(K_{12}\) is shown by \(66\beta CD_{2ur}\) which is 49 times greater than \(K_{12}\) for \(\beta CD\).

By comparison with earlier studies of the 1:2 host:guest complexation of tropaeoline (TR\(^-\)) by \(\beta CD\), \(66\beta CD_{2su}\) and \(66\beta CD_{2ur}\) (Table 3.1), it is seen that the ratios of \(K_{12}\) for BNS\(^-\)/TR\(^-\) are 0.0045 for \(\beta CD\), 16.89 for \(66\beta CD_{2su}\) and 0.076 for \(66\beta CD_{2ur}\) which indicates the critical nature of the stereochemistries of the host and the guest in determining \(K_{12}\) and complex stability.

### 3.4. Conclusion

It is seen from the variation of the magnitude of \(K_1\) (Fig. 3.57) that the \(33\beta CD_{2su}.BNS^-\) and \(66\beta CD_{2ur}.BNS^-\) complexes encompass the range of stabilities of the linked \(\beta CD\) dimer complexes and are two and 66 times more stable than the \(\beta CD.BNS^-\) complex, respectively. The \(K_1\) for the 1:1 linked \(\beta CD\) dimer complexes are consistent with C\(^{6A}\)-C\(^{6A}\) linking of \(\beta CD\) optimizing host-guest attractive interactions and complex stability, while the altrose inversions in the C\(^{3A}\)-C\(^{6A}\) and C\(^{3A}\)-C\(^{3A}\) linked \(\beta CD\) annuli decrease complex stability. Consequently, \(K_1\) for \(33\beta CD_{2su}.BNS\) and \(36\beta CD_{2ur}.BNS\) are increased in magnitude over those for \(\beta CD.BNS\) by the approximate statistical expectation only. The change in nature of the linker also causes some variation in the magnitude of \(K_1\) and, in combination with the C\(^{3A}\) and C\(^{6A}\) substitutions in the linked \(\beta CD\) dimers, produces the observed \(K_1\) variation. The \(>10.5\)-fold increase in \(K_1\) observed for the \(66\beta CD_{2su}.BNS^-\), \(36\beta CD_{2su}.BNS^-\) and \(66\beta CD_{2ur}.BNS^-\) systems by comparison with \(K_1\) for \(\beta CD.BNS^-\) indicates a cooperativity in complexation of BNS\(^-\) in their linked \(\beta CD\) annuli. (Because of the greater spectral change occurring as complexation the fluorimetrically determined \(K_1\) are probably more reliable than those determined by UV spectroscopy).
Figure 3.57. Stability constants ($K_1$) for complexation of BNS by a) βCD, b) 66βCD$_{2su}$, c) 36βCD$_{2su}$, d) 33βCD$_{2su}$, e) 66βCD$_{2ur}$ and f) 36βCD$_{2ur}$ in aqueous solution pH 7.0 (I = 0.10 mol dm$^{-3}$) and 298.2 K determined by the UV and fluorimetric methods.

The magnitudes of $K_{2a}$ varies 1.5-fold for the first five complexes listed in Table 3.5 and compare with $K_d$ ($2.63 \times 10^2$ dm$^3$ mol$^{-1}$) for the formation of (BNS$^-$)$_2$ in the absence of a host species. This could infer that the process for BNS$^-$ dimerization is similar in these five host-guest complexes and that it occurs with complexation in a single βCD annulus in each case. However, $K_{2b}$ ($=K_1 K_{2a}/K_d = [66βCD_{2su}(BNS-)_2]/([66βCD_{2su}BNS][BNS])$ and analogous concentrations for the other systems) for 66βCD$_{2su}$(BNS$^-$)$_2$ and 36βCD$_{2su}$(BNS$^-$)$_2$ are 25.3 and 13.4 times greater than that for βCD.(BNS$^-$)$_2$ because of the higher $K_1$ of the first two complexes consistent with involvement of two βCD annuli in complexing (BNS$^-$)$_2$ in the linked βCD dimer complexes. As discussed above, the angles between the planes of the adjacent faces of the linked βCD in 33βCD$_{2su}$ and 36βCD$_{2su}$ are 0° whereas those of 66βCD$_{2su}$, 36βCD$_{2ur}$, and 66βCD$_{2ur}$ are 30°, 60°, and 40°, respectively. It appears that the complexation of (BNS$^-$)$_2$ is enhanced when the angle between the planes of the adjacent βCD departs from 0°, possibly for steric reasons. The greater angle for 36βCD$_{2ur}$(BNS$^-$)$_2$ may explain $K_2$ for being 7.9-fold greater than for βCD.(BNS$^-$)$_2$. 

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Table 3.6. Complexation constants for 1:1 host-guest complexes of TNS − and BNS − (K₁) in D₂O phosphate buffer (pD 7.0, I = 0.10 mol dm⁻³) at 298.2 K.

<table>
<thead>
<tr>
<th>Host</th>
<th>K₁ (dm³ mol⁻¹) (UV)</th>
<th>K₁ (dm³ mol⁻¹) (Fluorimetry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNS −</td>
<td>BNS −</td>
</tr>
<tr>
<td>βCD</td>
<td>3.02 (±0.03) × 10³</td>
<td>5.54 (±0.02) × 10⁴</td>
</tr>
<tr>
<td>66βCD₂Su</td>
<td>1.61 (±0.01) × 10⁴</td>
<td>1.25 (±0.01) × 10⁶</td>
</tr>
<tr>
<td>36βCD₂Su</td>
<td>1.09 (±0.02) × 10⁴</td>
<td>7.39 (±0.06) × 10⁵</td>
</tr>
<tr>
<td>33βCD₂Su</td>
<td>1.07 (±0.05) × 10⁴</td>
<td>1.86 (±0.02) × 10⁵</td>
</tr>
<tr>
<td>66βCD₂Ur</td>
<td>5.51 (±0.02) × 10⁴</td>
<td>3.64 (±0.03) × 10⁶</td>
</tr>
<tr>
<td>36βCD₂Ur</td>
<td>1.83 (±0.04) × 10⁴</td>
<td>1.61 (±0.01) × 10⁵</td>
</tr>
</tbody>
</table>

⁴ The errors shown are the fitting errors, but when experimental error is taken into account the overall error is ± 3%.

The K₁ for complexation of TNS − by βCD (Table 3.6) are an order of magnitude less than those for BNS −, and K₁ for complexation of TNS − by the linked βCD dimers are one to two orders of magnitude less than those for BNS −. The K₁ variations over the five linked βCD dimer BNS − complexes are 22.7 and 39.5 while the analogous values for TNS − are 5.1 and 4.4. As the only structural difference between TNS − and BNS − is the substitution of a methyl group by a t-butyl group it is likely that the increased hydrophobicity and size of the latter is the major cause of greater stability of the BNS − complexes. This is probably also the cause of the formation of βCD₂.TNS − whereas the analogous βCD₂.BNS − was not detected. It appears that the t-butylphenyl group is sufficiently strongly complexed by βCD to inhibit the complexing of a second βCD by the naphthyl group and the formation of βCD₂.BNS − whereas the less strongly complexed methylphenyl group of TNS − does allow formation of the analogous complex.
3.5. References


10. CambridgeSoft Corporation, 100 CambridgePark Drive, Cambridge, MA 02140, USA.


16 Protonic Software, 2, Templegate Avenue, Leeds LS15 0HD, UK.


