

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Tetrahedron 60 (2004) 9099–9111

Tetrahedron

# Thermodynamics of binding between $\alpha$ - and $\beta$ -cyclodextrins and some *p*-nitro-aniline derivatives: reconsidering the enthalpy–entropy compensation effect

Paolo Lo Meo,\* Francesca D'Anna, Michelangelo Gruttadauria, Serena Riela and Renato Noto\*

Dipartimento di Chimica Organica 'E. Paternò', Università degli Studi di Palermo, Viale delle Scienze, Parco d'Orleans II, Pad. 17, 90128 PALERMO, Italy

Received 23 April 2004; revised 1 July 2004; accepted 28 July 2004

Available online 26 August 2004

**Abstract**—The thermodynamics of binding between native  $\alpha$ - and  $\beta$ -cyclodextrin towards several *p*-nitro-aniline derivatives was examined, in order to gain further insights about the occurrence of different interaction modes for the two hosts. Valuable information was achieved regarding the 'expanded hydrophobic sphere' of  $\alpha$ -cyclodextrin. Furthermore, very interesting and unexpected aspects of the behavior of  $\beta$ -cyclodextrin were enlightened, such as the crucial role played by hydrogen bond interactions. Experimental data were examined under the perspective of the 'enthalpy–entropy compensation effect', and some ideas about this topic are discussed.

© 2004 Elsevier Ltd. All rights reserved.

## 1. Introduction

Binding properties of both native and chemically modified cyclodextrins towards suitably sized and shaped organic molecules have been the object of extensive studies during the last decades.<sup>1</sup> These cyclic, bucket-shaped oligosaccharides owe their popularity to their wide range of industrial, as well as research applications, ranging from pharmaceuticals,<sup>2</sup> food and cosmetics technology<sup>3</sup> to separation<sup>4</sup> and chiral discrimination,<sup>5</sup> reaction micro-environment,<sup>6</sup> catalysis,<sup>7</sup> enzyme mimics<sup>8</sup> and stereo-selective synthesis.<sup>9</sup>

A thorough understanding of the various factors affecting the host–guest inclusion phenomenon at a molecular level is needed in such a context, so considerable efforts have been devoted to this task.<sup>10–12</sup> There is now a general agreement that the binding equilibrium is the result of a fine balance between different stabilization sources,<sup>11</sup> including host desolvation and solvent reorganization, hydrophobic, dipolar and hydrogen bond interactions, conformational strain release. Their mutual interplay is classically discussed within Tabushi's scheme,<sup>12</sup> in terms of: (i) desolvation of the host cavity; (ii) desolvation of the guest; (iii) 'neat' inclusion of the guest into the host cavity; (iv)

reorganization of the solvent pool. There is not any obvious hierarchy among the previously mentioned factors, and none of them can be assumed a priori as the ultimate driving force for the overall process. Therefore, a careful systematic investigation on the thermodynamics of binding is needed,<sup>13</sup> because inclusion constants alone are not able to provide us with exhaustive information.

As observed for other classes of supramolecular ligands (such as porphyrins, crown ethers, cryptands or calixarenes),<sup>14</sup> the existence of a specific enthalpy–entropy compensation effect for inclusion in cyclodextrins has been claimed.<sup>14–19</sup> This finding is interesting both as an interpretation tool and as a topic of investigation. It relies on the simple and intuitive idea that the more strongly host and guest bind together, the more the resulting host–guest complex will suffer for the loss of conformational freedom.<sup>14,19</sup> Its actual existence and correct interpretation have been the object of intense debate and also of severe criticism.<sup>20</sup> It was first empirically proposed that the slope and the intercept of the  $T\Delta S^\circ$  versus  $\Delta H^\circ$  correlation plot might be considered respectively as a measure of the loss of degrees of freedom and of the extent of desolvation for the species involved.<sup>14</sup> However, more recently it has been suggested, on the basis of thermodynamic arguments,<sup>20b,c</sup> that the actual source of the compensation effect should be related to contributions due to solvent reorganization. Methodological objections have also been put forward, owing to the interdependence in the determination of  $\Delta H^\circ$

**Keywords:** Cyclodextrins; Thermodynamics.

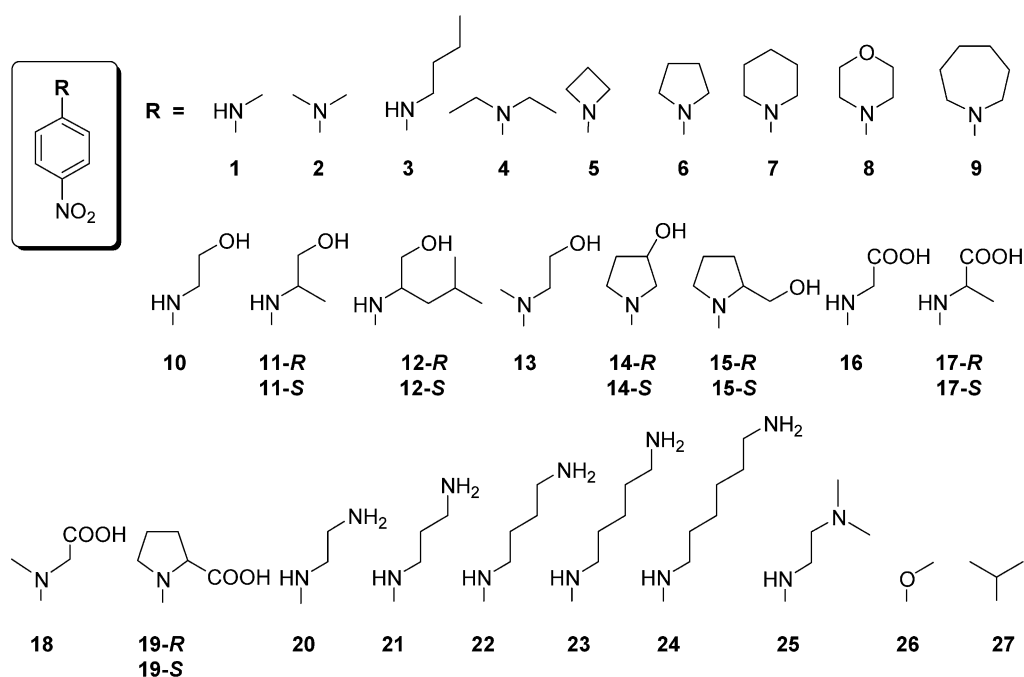
\* Corresponding authors. Tel.: +39-91-596919; fax: +39-91-596825; e-mail: [rnoto@unipa.it](mailto:rnoto@unipa.it)

and  $\Delta S^\circ$  values, which can lead to a ‘pseudo-compensation effect’.<sup>18,20a,21</sup>

Recently we have been interested in elucidating the inclusion properties of native and chemically modified cyclodextrins towards aromatic derivatives.<sup>5b,11,22</sup> In particular, we have investigated the thermodynamics of binding for  $\alpha$ -cyclodextrin ( $\alpha$ CD) and  $\beta$ -cyclodextrin ( $\beta$ CD) towards some *N*-substituted *p*-nitro-benzene derivatives (compounds **1–9**, **16**, **19-S**, **26** and **27** of Scheme 1).<sup>11</sup> These guests penetrate the cyclodextrin cavity with the nitro-group directed towards the primary rim of the host.<sup>11,22</sup> The two hosts showed quite different behaviors. Binding by  $\alpha$ CD was an enthalpy-driven process, while inclusion in  $\beta$ CD was both enthalpy- and entropy-driven. Good  $\Delta H^\circ$  versus  $T\Delta S^\circ$  correlations were found for both hosts, having very similar slopes (near to 0.96), although values for  $\alpha$ CD and  $\beta$ CD respectively were reciprocally uncorrelated. A careful data analysis<sup>11</sup> led us to the conclusion that the narrow  $\alpha$ CD cavity is able to include quite rigidly only the aromatic moiety of the guest, while its ancillary chain **R** stays exposed to the structured water molecules in the surroundings of the secondary host rim. These water molecules form a so-called ‘expanded hydrophobic sphere’,<sup>13</sup> having different properties with respect to the solvent bulk. On the other hand, the larger  $\beta$ CD cavity seems able to include the entire guest (with a certain flexibility), so it can interact with both its aromatic moiety and ancillary chain.

Although we were able to obtain convincing evidence about the occurrence of these different interaction modes, nonetheless several other questions arose. For example, the ‘expanded hydrophobic sphere’ for  $\alpha$ CD and its interaction with the ancillary chain of the guest seemed to be an ad hoc hypothesis, which needed further experimental support.

Furthermore, it seemed interesting to investigate how much the ‘expanded hydrophobic sphere’ of  $\alpha$ CD, as well as the cavity and/or the secondary rim of  $\beta$ CD, were able to discriminate particular properties of the ancillary chain, such as its chirality or the presence of charged groups. Further doubts also came from enthalpy–entropy compensation correlations, because their similarity, despite the characteristics of the two hosts which appeared to be so different, seemed suspect. Consequently, we could also ask how reliable could be the thermodynamic data directly coming from van’t Hoff plots analysis.<sup>23</sup> Therefore, we extended our study to *p*-nitro-aniline derivatives **10–25** (Scheme 1). We focused on suitable aminoalcohol, aminoacid and diamine derivatives, selected in such a way as to show appreciable differences in their properties, such as their hydrophobicity, conformational freedom, hydrogen bond ability, and the possibility to change their protonation state and charge by varying the pH value of the solvent medium, depending on their ancillary chain **R**. Among them, six enantiomeric pairs were also examined. It should be stressed that different enantiomers, as well as differently charged forms of the same guests, will presumably experience different interactions with the host, and thus have to be formally considered as different guests. Binding constants were measured by means of UV–Vis spectrophotometry at different temperatures, ranging from 288 to 313 K in a suitable phosphate buffer solution. All guests were studied at pH=6.0; aminoacid derivatives **16–19** were also studied at pH=2.5, while diamine derivatives **20–25** were also studied at pH=11.0. These pH values were chosen for consistency with our previous works,<sup>11,22</sup> in order to study the behavior of both the ionized and the neutral form of these guests. Experimental data, together with those for guests **1–9**, *p*-nitro-anisole (**26**) and *p*-nitro-isopropyl-benzene (**27**), were all subjected to a suitable statistical analysis before comparative examination.



Scheme 1. Guests **1–27**.

**Table 1.**  $pK_a$  values for aminoacid derivatives **16–19**

Guest	$pK_a$	pH	% <sub>HA</sub>	% <sub>A<sup>-</sup></sub>
<b>16</b>	$3.52 \pm 0.08$	2.5	91.3	8.7
		6.0	0.3	99.7
<b>17</b>	$3.54 \pm 0.03$	2.5	91.6	8.4
		6.0	0.3	99.7
<b>18</b>	$3.18 \pm 0.01$	2.5	82.7	17.3
		6.0	0.2	99.8
<b>19</b>	$3.34 \pm 0.01$	2.5	87.4	12.6
		6.0	0.2	99.8

**Table 2.**  $pK_{BH^+}$  values for diamine derivatives **20–25**

Guest	$pK_{BH^+}$	pH	% <sub>BH<sup>+</sup></sub>	% <sub>B</sub>
<b>20</b>	$9.02 \pm 0.01$	6.0	99.8	0.2
		11.0	0.1	99.9
<b>21</b>	$10.05 \pm 0.05$	6.0	100.0	—
		11.0	10.1	89.9
<b>22</b>	$10.19 \pm 0.01$	6.0	100.0	—
		11.0	13.4	86.6
<b>23</b>	$10.45 \pm 0.01$	6.0	100.0	—
		11.0	22.0	78.0
<b>24</b>	$10.08 \pm 0.01$	6.0	100.0	—
		11.0	10.7	89.3
<b>25</b>	$8.43 \pm 0.01$	6.0	99.6	0.4
		11.0	0.3	99.7

## 2. Results and discussion

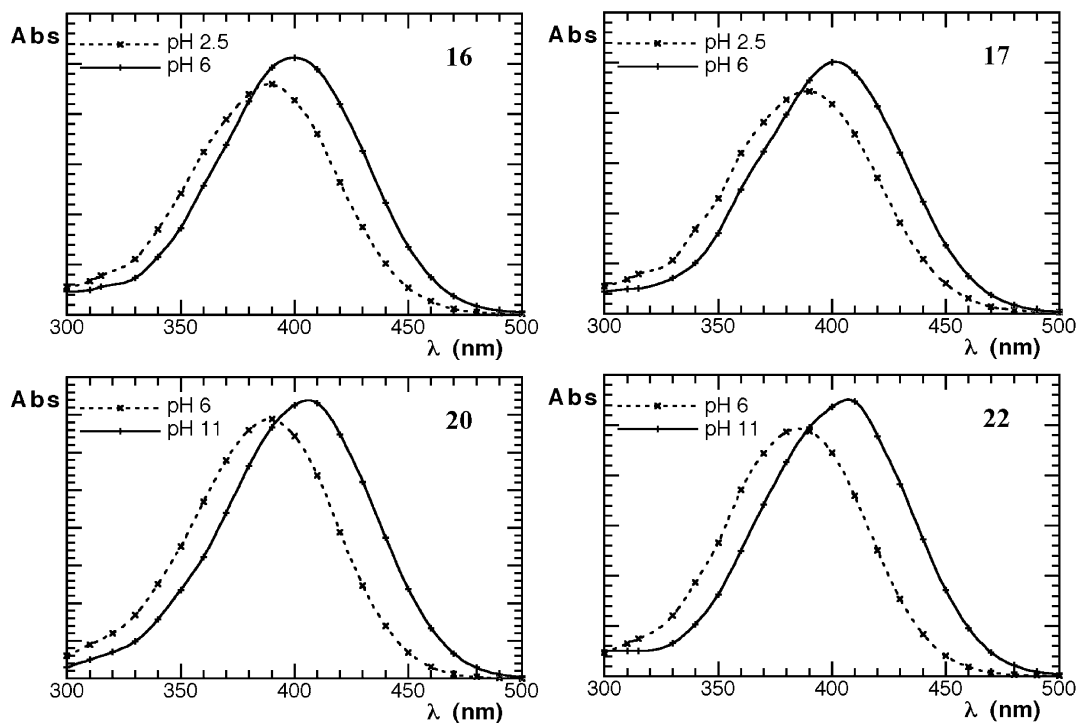
### 2.1. Behavior of guests **16–25** in solution

As a preliminary work, the ionization equilibria of aminoacid and diamine derivatives **16–25** in buffer solution at different pH values had to be examined. In Table 1 the  $pK_a$  values of *N*-(*p*-nitro-phenyl)-aminoacid derivatives **16–19** are reported. These guests are, in general, weaker acids than the corresponding free aminoacids<sup>24</sup> (relative to

their first dissociation constant. The mean difference in  $pK_a$  values is ca. 1.2). This finding can probably be attributed to an unfavorable effect of the hydrophobic *p*-nitro-phenyl moiety on the solvation of the ionized carboxyl group. From these data we can immediately deduce that compounds **16–19** are fully ionized at pH=6.0. At pH=2.5 the undissociated acid form predominates, but an amount of ionized form, ranging up to 17%, is still present. Clearly, this is not strictly negligible; however, at a first approximation level we can assume guests **16–19** to be not ionized at this pH value.<sup>25</sup>

Data related to diamine derivatives **20–25** are reported in Table 2. In general, their  $pK_{BH^+}$  values are comparable to those of the corresponding diamines<sup>26</sup> with the exception of ethylenediamine derivatives **20** and **25**, for which higher dissociation constants are found. The latter behavior can also be attributed to the effect of the *p*-nitro-phenyl moiety on the solvation of the charged ammonium group, the effect becoming weaker on increasing the diamine chain length. Also for these compounds data indicate complete ionization at pH=6.0. At pH=11.0 the free base forms fully predominate for compounds **20** and **25**. For compounds **21–24** an amount of ionized form (ranging up to 22%) is still present; however also in this case we can assume at a first approximation that its presence is not relevant.

The spectroscopic behavior of short chain derivatives **16**, **17**, **20** and **25** is quite interesting. Significant shifts of the absorption maximum in the UV–Vis spectra are observed on passing from their neutral to their ionized forms (Fig. 1). We can explain this observation considering that for all these compounds the aniline N atom is a hydrogen bond donor, which is able to interact with the functional group at the chain end. This interaction, influencing the conformational

**Figure 1.** UV–Vis spectra of guests **16**, **17**, **20** and **22**.

**Table 3.** Binding constants at 298.15 K and thermodynamic parameters for inclusion of guests **1–27** in  $\alpha$ CD

Entry	Guest	pH	$K$ ( $M^{-1}$ , 298.15 K)	$\Delta G^\circ$ ( $kJ\ mol^{-1}$ )	From van't Hoff plots		Corrected (Eq. 3)	
					$\Delta H^\circ$ ( $kJ\ mol^{-1}$ )	$T\Delta S^\circ$ ( $kJ\ mol^{-1}$ )	$\Delta H^\circ$ ( $kJ\ mol^{-1}$ )	$T\Delta S^\circ$ ( $kJ\ mol^{-1}$ )
1	<b>1</b>	6.0	990 ± 24 <sup>a</sup>	−17.09 ± 0.06 <sup>a</sup>	−37.8 ± 0.6 <sup>a</sup>	−20.7 ± 0.6 <sup>a</sup>	−34.7 ± 0.5	−17.6 ± 0.5
2	<b>2</b>	6.0	1178 ± 38 <sup>a</sup>	−17.52 ± 0.08 <sup>a</sup>	−38.8 ± 1.0 <sup>a</sup>	−21.3 ± 1.0 <sup>a</sup>	−35.9 ± 0.4	−18.4 ± 0.4
3	<b>3</b>	6.0	1453 ± 35 <sup>a</sup>	−18.04 ± 0.06 <sup>a</sup>	−32.6 ± 0.6 <sup>a</sup>	−14.6 ± 0.6 <sup>a</sup>		
4	<b>4</b>	6.0	1118 ± 23 <sup>a</sup>	−17.39 ± 0.05 <sup>a</sup>	−34.8 ± 1.4 <sup>a</sup>	−17.4 ± 1.4 <sup>a</sup>	−35.3 ± 0.5	−17.9 ± 0.5
5	<b>5</b>	6.0	1197 ± 29 <sup>a</sup>	−17.56 ± 0.06 <sup>a</sup>	−38.0 ± 0.3 <sup>a</sup>	−20.5 ± 0.3 <sup>a</sup>	−36.0 ± 0.4	−18.4 ± 0.4
6	<b>6</b>	6.0	2123 ± 120 <sup>a</sup>	−18.98 ± 0.14 <sup>a</sup>	−36.4 ± 0.5 <sup>a</sup>	−17.5 ± 0.5 <sup>a</sup>	−39.5 ± 0.7	−20.6 ± 0.7
7	<b>7</b>	6.0	1346 ± 22 <sup>a</sup>	−17.85 ± 0.04 <sup>a</sup>	−38.9 ± 1.0 <sup>a</sup>	−20.9 ± 1.0 <sup>a</sup>	−36.9 ± 0.4	−19.0 ± 0.4
8	<b>8</b>	6.0	932 ± 11 <sup>a</sup>	−16.94 ± 0.03 <sup>a</sup>	−34.0 ± 1.3 <sup>a</sup>	−17.1 ± 1.3 <sup>a</sup>	−34.2 ± 0.6	−17.3 ± 0.6
9	<b>9</b>	6.0	3602 ± 87 <sup>a</sup>	−20.29 ± 0.06 <sup>a</sup>	−42.4 ± 0.2 <sup>a</sup>	−22.1 ± 0.2 <sup>a</sup>	−43.1 ± 1.1	−22.9 ± 1.1
10	<b>10</b>	6.0	1043 ± 38	−17.22 ± 0.09	−34.5 ± 0.7	−17.3 ± 0.7	−34.9 ± 0.5	−17.7 ± 0.5
11	<b>11-R</b>	6.0	1221 ± 39	−17.61 ± 0.08	−37.3 ± 0.5	−19.7 ± 0.5	−36.0 ± 0.4	−18.4 ± 0.5
12	<b>11-S</b>	6.0	1251 ± 51	−17.67 ± 0.10	−36.1 ± 0.7	−18.5 ± 0.7	−36.1 ± 0.4	−18.5 ± 0.5
13	<b>13</b>	6.0	1329 ± 48	−17.82 ± 0.09	−37.5 ± 0.3	−19.7 ± 0.4	−36.6 ± 0.4	−18.7 ± 0.5
14	<b>14-R</b>	6.0	1282 ± 41	−17.73 ± 0.08	−39.2 ± 0.9	−21.5 ± 0.9	−36.5 ± 0.4	−18.7 ± 0.4
15	<b>14-S</b>	6.0	1362 ± 82	−17.88 ± 0.15	−39.4 ± 0.9	−21.5 ± 0.9	−36.9 ± 0.4	−19.0 ± 0.4
16	<b>15-R</b>	6.0	1792 ± 43	−18.56 ± 0.06	−39.8 ± 0.6	−21.2 ± 0.6	−38.5 ± 0.6	−19.9 ± 0.6
17	<b>15-S</b>	6.0	1778 ± 29	−18.54 ± 0.04	−39.6 ± 0.4	−21.1 ± 0.4	−38.4 ± 0.6	−19.9 ± 0.6
18	<b>16</b>	2.5	1010 ± 61 <sup>a</sup>	−17.14 ± 0.15 <sup>a</sup>	−20.4 ± 0.8 <sup>a</sup>	−3.3 ± 0.8 <sup>a</sup>		
19	<b>16</b>	6.0	1010 ± 37 <sup>a</sup>	−17.14 ± 0.09 <sup>a</sup>	−30.6 ± 0.9 <sup>a</sup>	−13.5 ± 0.9 <sup>a</sup>	−34.7 ± 0.5	−17.6 ± 0.5
20	<b>17-R</b>	2.5	723 ± 44	−16.31 ± 0.15	−36.6 ± 0.6	−20.3 ± 0.6	−32.8 ± 0.7	−16.5 ± 0.7
21	<b>17-R</b>	6.0	1118 ± 45	−17.39 ± 0.10	−30.6 ± 0.5	−13.2 ± 0.6	−35.4 ± 0.4	−18.0 ± 0.4
22	<b>17-S</b>	2.5	891 ± 32	−16.83 ± 0.09	−35.2 ± 1.0	−18.4 ± 1.0	−33.9 ± 0.6	−17.0 ± 0.6
23	<b>17-S</b>	6.0	1006 ± 24	−17.13 ± 0.06	−32.7 ± 0.5	−15.5 ± 0.5	−34.8 ± 0.5	−17.6 ± 0.5
24	<b>18</b>	2.5	856 ± 59	−16.73 ± 0.17	−39.3 ± 0.6	−22.5 ± 0.7		
25	<b>18</b>	6.0	819 ± 33	−16.62 ± 0.10	−31.9 ± 0.9	−15.3 ± 0.9	−33.5 ± 0.6	−16.8 ± 0.6
26	<b>19-R</b>	2.5	943 ± 23	−16.97 ± 0.06	−34.5 ± 0.3	−17.5 ± 0.3	−34.3 ± 0.6	−17.4 ± 0.6
27	<b>19-R</b>	6.0	1226 ± 30	−17.62 ± 0.06	−34.6 ± 0.4	−17.0 ± 0.4	−36.0 ± 0.5	−18.4 ± 0.5
28	<b>19-S</b>	2.5	1039 ± 25 <sup>a</sup>	−17.21 ± 0.06 <sup>a</sup>	−30.8 ± 0.8 <sup>a</sup>	−17.2 ± 0.8 <sup>a</sup>	−34.8 ± 0.5	−17.6 ± 0.5
29	<b>19-S</b>	6.0	1187 ± 14 <sup>a</sup>	−17.54 ± 0.03 <sup>a</sup>	−35.7 ± 1.1 <sup>a</sup>	−17.6 ± 1.1 <sup>a</sup>	−35.8 ± 0.5	−18.2 ± 0.5
30	<b>20</b>	11.0	1197 ± 24	−17.56 ± 0.05	−36.4 ± 1.0	−18.9 ± 1.0	−35.9 ± 0.5	−18.3 ± 0.5
31	<b>20</b>	6.0	849 ± 31	−16.71 ± 0.09	−35.1 ± 0.8	−18.4 ± 0.8		
32	<b>21</b>	11.0	1843 ± 60	−18.63 ± 0.08	−39.5 ± 0.4	−20.8 ± 0.4	−38.8 ± 0.6	−20.2 ± 0.6
33	<b>21</b>	6.0	1756 ± 43	−18.51 ± 0.06	−37.2 ± 0.6	−18.7 ± 0.6	−38.5 ± 0.5	−19.9 ± 0.5
34	<b>22</b>	11.0	1507 ± 55	−18.13 ± 0.09	−37.6 ± 0.9	−19.4 ± 0.9	−37.4 ± 0.5	−19.3 ± 0.5
35	<b>22</b>	6.0	1770 ± 36	−18.53 ± 0.05	−37.4 ± 0.6	−18.9 ± 0.6	−38.5 ± 0.5	−19.9 ± 0.5
36	<b>23</b>	11.0	1390 ± 79	−17.93 ± 0.14	−35.8 ± 0.9	−17.8 ± 0.9	−37.0 ± 0.4	−19.1 ± 0.5
37	<b>23</b>	6.0	1620 ± 65	−18.31 ± 0.10	−37.8 ± 0.6	−19.5 ± 0.6	−37.8 ± 0.5	−19.5 ± 0.5
38	<b>24</b>	11.0	1356 ± 66	−17.87 ± 0.12	−36.9 ± 0.6	−19.1 ± 0.6	−36.7 ± 0.4	−18.9 ± 0.5
39	<b>24</b>	6.0	1513 ± 37	−18.14 ± 0.06	−34.8 ± 0.8	−16.7 ± 0.8	−37.3 ± 0.5	−19.2 ± 0.5
40	<b>25</b>	11.0	1236 ± 35	−17.64 ± 0.07	−36.5 ± 0.8	−18.9 ± 0.8	−36.1 ± 0.4	−18.5 ± 0.4
41	<b>25</b>	6.0	1065 ± 26	−17.27 ± 0.06	−34.6 ± 0.5	−17.3 ± 0.5	−35.2 ± 0.5	−17.9 ± 0.5
42	<b>26</b>	6.0	315 ± 50 <sup>a</sup>	−14.25 ± 0.39 <sup>a</sup>	−35.9 ± 0.4 <sup>a</sup>	−21.7 ± 0.6 <sup>a</sup>		
43	<b>27</b>	6.0	505 ± 79 <sup>a</sup>	−15.42 ± 0.39 <sup>a</sup>	−28.5 ± 2.3 <sup>a</sup>	−12.9 ± 2.3 <sup>a</sup>	−30.7 ± 1.0	−15.2 ± 1.1

<sup>a</sup> From Ref. 11.

equilibria of the molecule, is favored because it involves the formation of a five-membered pseudo-cycle. A change in the protonation state of the latter group will heavily affect the occurrence of this intramolecular hydrogen bond. Its formation induces a variation in the local dipole moment of the aryl chromophore moiety, causing a bathochromic shift of the absorption maximum.

Aminoalcohol derivatives **11** and **12** presumably share the same behavior, although it is not possible to point it out in this way. The ease of forming the intramolecular hydrogen bond rapidly decreases as the chain length increases. In particular, we observed that along the series of the diamino derivatives **20–22**, a bathochromic shift of 17 nm is found for **20**, which decreases to 8 nm for **21** and to only 2 nm for **22**. Therefore, we can presume that the former two-carbon-chain molecule can be mostly found in its pseudo-cyclic conformation; whereas the latter four-carbon-chain derivative is almost completely in a free-chain conformation. For the intermediate three-carbon-chain compound the two

conformational states are probably populated in comparable amounts.

## 2.2. Complexation behavior of $\alpha$ CD and $\beta$ CD towards guests **1–27**. A first overview of inclusion constants, van't Hoff parameters and chiral selection properties

Binding constants at 298.15 K and van't Hoff parameters for complexation of  $\alpha$ CD and  $\beta$ CD towards substrates **1–27** are reported in Tables 3 and 4 respectively. The data indicate remarkable differences in behavior between the two examined hosts, in agreement with our previous observations.<sup>11</sup> Complexation with  $\alpha$ CD is an essentially enthalpy-driven process, with  $\Delta H^\circ$  values ranging from  $-20.4$  to  $-42.4$   $kJ\ mol^{-1}$ , while  $T\Delta S^\circ$  values range from  $-3.3$  to  $-22.5$   $kJ\ mol^{-1}$ . However, most of the  $\Delta G^\circ$  values are restricted in a narrow range of ca.  $2.7$   $kJ\ mol^{-1}$ , which corresponds to only modest variations in binding constants. Interestingly, this indicates that we cannot gain significant information simply by consideration of the binding

**Table 4.** Binding constants at 298.15 K and thermodynamic parameters for inclusion of guests **1–27** in  $\beta$ CD

Entry	Guest	pH	$K$ ( $M^{-1}$ , 298.15 K)	$\Delta G^\circ$ ( $kJ\ mol^{-1}$ )	From van't Hoff plots		Corrected (Eq. 3)		Group
					$\Delta H^\circ$ ( $kJ\ mol^{-1}$ )	$T\Delta S^\circ$ ( $kJ\ mol^{-1}$ )	$\Delta H^\circ$ ( $kJ\ mol^{-1}$ )	$T\Delta S^\circ$ ( $kJ\ mol^{-1}$ )	
44	<b>1</b>	6.0	1080±48 <sup>a</sup>	-17.31±0.11 <sup>a</sup>	-12.9±0.7 <sup>a</sup>	4.3±0.7 <sup>a</sup>	-13.8±0.7	3.5±0.7	A
45	<b>2</b>	6.0	710±26 <sup>a</sup>	-16.27±0.09 <sup>a</sup>	-10.9±0.6 <sup>a</sup>	5.3±0.6 <sup>a</sup>	-13.0±0.7	3.3±0.7	C
46	<b>3</b>	6.0	917±52 <sup>a</sup>	-16.90±0.14 <sup>a</sup>	-17.9±0.8 <sup>a</sup>	-1.1±0.8 <sup>a</sup>	-17.0±0.3	-0.1±0.4	B
47	<b>4</b>	6.0	588±28 <sup>a</sup>	-15.80±0.12 <sup>a</sup>	-14.1±0.9 <sup>a</sup>	1.7±0.9 <sup>a</sup>	-14.3±0.4	1.5±0.4	B
48	<b>5</b>	6.0	764±28 <sup>a</sup>	-16.45±0.09 <sup>a</sup>	-11.9±0.4 <sup>a</sup>	4.5±0.4 <sup>a</sup>	-13.0±0.7	3.5±0.7	C
49	<b>6</b>	6.0	1297±42 <sup>a</sup>	-17.76±0.08 <sup>a</sup>	-12.7±0.6 <sup>a</sup>	5.0±0.6 <sup>a</sup>	-13.0±0.7	4.7±0.7	C
50	<b>7</b>	6.0	2640±64 <sup>a</sup>	-19.52±0.06 <sup>a</sup>	-13.1±1.2 <sup>a</sup>	6.4±1.2 <sup>a</sup>	-13.0±0.7	6.5±0.7	C
51	<b>8</b>	6.0	647±26 <sup>a</sup>	-16.04±0.10 <sup>a</sup>	-14.7±0.6 <sup>a</sup>	1.3±0.6 <sup>a</sup>	-13.0±0.7	3.1±0.7	C
52	<b>9</b>	6.0	17297±349 <sup>a</sup>	-24.18±0.05 <sup>a</sup>	-23.2±0.4 <sup>a</sup>	0.0±0.4 <sup>a</sup>			
53	<b>10</b>	6.0	610±25	-15.89±0.10	-20.6±0.6	-4.7±0.6	-18.8±0.4	-2.9±0.5	A
54	<b>11-R</b>	6.0	641±26	-16.01±0.10	-17.6±0.4	-1.6±0.5	-18.3±0.4	-2.2±0.4	A
55	<b>11-S</b>	6.0	566±25	-15.70±0.11	-15.7±0.6	0.0±0.6	-19.2±0.5	-3.5±0.5	A
56	<b>12-R</b>	6.0	1177±57	-17.52±0.12	-14.4±0.9	3.1±0.9	-13.0±0.8	4.5±0.8	A
57	<b>12-S</b>	6.0	1204±44	-17.57±0.09	-11.6±0.8	6.0±0.8	-12.8±0.8	4.8±0.8	A
58	<b>13</b>	6.0	734±27	-16.35±0.09	-14.8±0.4	1.5±0.4	-15.6±0.4	0.8±0.4	B
59	<b>14-R</b>	6.0	954±27	-17.00±0.07	-13.3±0.6	3.6±0.6	-13.0±0.7	4.0±0.7	C
60	<b>14-S</b>	6.0	957±19	-17.01±0.05	-13.2±0.7	3.8±0.7	-13.0±0.7	4.0±0.7	C
61	<b>15-R</b>	6.0	1293±31	-17.75±0.06	-16.2±0.3	1.5±0.3	-19.2±0.5	-1.4±0.5	B
62	<b>15-S</b>	6.0	1182±43	-17.53±0.09	-15.4±0.2	2.1±0.3	-18.6±0.4	-1.1±0.4	B
63	<b>16</b>	6.0	348±25 <sup>a</sup>	-14.50±0.18 <sup>a</sup>	-22.4±1.2 <sup>a</sup>	-8.0±1.2 <sup>a</sup>	-24.0±1.1	-9.5±1.2	A
64	<b>17-R</b>	2.5	377±38	-14.70±0.25	-27.4±3.1	-12.5±3.1	-22.4±0.9	-7.7±1.0	A
65	<b>17-R</b>	6.0	396±27	-14.82±0.17	-25.4±0.7	-10.6±0.8	-22.2±0.9	-7.4±0.9	A
66	<b>17-S</b>	2.5	544±44	-15.61±0.20	-20.5±1.5	-4.8±1.5	-19.3±0.5	-3.7±0.6	A
67	<b>17-S</b>	6.0	540±50	-15.59±0.23	-18.4±1.8	-2.7±1.8	-19.4±0.5	-3.9±0.6	A
68	<b>18</b>	6.0	423±51	-14.98±0.30	-12.7±1.1	2.3±1.2	-12.4±0.5	2.6±0.6	B
69	<b>19-R</b>	2.5	858±42	-16.74±0.12	-20.4±0.4	-3.7±0.5	-16.5±0.3	0.2±0.4	B
70	<b>19-R</b>	6.0	594±34	-15.82±0.14	-13.1±0.4	2.7±0.6	-14.3±0.4	1.5±0.4	B
71	<b>19-S</b>	2.5	1018±49	-17.16±0.12	-20.8±0.2	-3.6±0.3	-17.7±0.4	-0.5±0.4	B
72	<b>19-S</b>	6.0	665±59 <sup>a</sup>	-16.10±0.22 <sup>a</sup>	-13.1±0.4 <sup>a</sup>	3.0±0.5 <sup>a</sup>	-15.0±0.4	1.1±0.4	B
73	<b>20</b>	11.0	684±39	-16.17±0.14	-18.4±1.0	-2.2±1.0	-17.7±0.4	-1.5±0.4	A
74	<b>20</b>	6.0	303±35	-14.16±0.29	-10.2±0.6	3.9±0.7	-9.8±0.8	4.3±0.8	B
75	<b>22</b>	11.0	1149±70	-17.46±0.15	-20.4±0.5	-3.0±0.6	-18.5±0.4	-1.0±0.5	B
76	<b>22</b>	6.0	865±35	-16.76±0.10	-17.9±0.3	-1.1±0.5	-16.7±0.3	0.1±0.4	B
77	<b>25</b>	11.0	689±28	-16.19±0.10	-16.6±1.1	-0.4±1.1	-17.5±0.4	-1.3±0.4	A
78	<b>25</b>	6.0	369±49	-14.64±0.33	-8.7±0.9	5.9±0.9	-11.2±0.6	3.5±0.7	B
79	<b>26</b>	6.0	175±25 <sup>a</sup>	-12.80±0.35 <sup>a</sup>	-5.8±0.2 <sup>a</sup>	7.0±0.4 <sup>a</sup>	-6.7±1.2	6.0±1.2	B
80	<b>27</b>	6.0	1450±88 <sup>a</sup>	-18.04±0.15 <sup>a</sup>	-7.9±0.3 <sup>a</sup>	10.2±0.4 <sup>a</sup>			

<sup>a</sup> From Ref. 11.

constants alone. On the other hand, complexation with  $\beta$ CD shows less negative  $\Delta H^\circ$  values, ranging from  $-5.8$  to  $-24.2\ kJ\ mol^{-1}$ , and  $T\Delta S^\circ$  values correspondingly ranging from  $-12.5$  to  $10.2\ kJ\ mol^{-1}$ ; so only in some cases is the process both enthalpy and entropy driven. Furthermore,  $\Delta G^\circ$  values show a moderate variability, spreading over a  $11.4\ kJ\ mol^{-1}$  range. Lack of correlation between  $\Delta G^\circ$ ,  $\Delta H^\circ$  or  $T\Delta S^\circ$  values for  $\alpha$ CD versus  $\beta$ CD is fully confirmed. Compensation  $T\Delta S^\circ$  versus  $\Delta H^\circ$  plots show fairly good linear correlations, according to the following relationships:

for  $\alpha$ CD:  $T\Delta S^\circ = (14.8 \pm 1.9) + (0.92 \pm 0.03)\ \Delta H^\circ$  ( $n=40$ ,  $r=0.985$ );

for  $\beta$ CD:  $T\Delta S^\circ = (16.7 \pm 1.8) + (1.02 \pm 0.05)\ \Delta H^\circ$  ( $n=36$ ,  $r=0.966$ ).

Because both slopes are close to the critical value of 0.96 (vide infra), suspicion of a pseudo-compensation effect cannot be ruled out. In particular, the slope value for  $\beta$ CD should account for an almost perfect compensation between enthalpy and entropy at 298.15 K, in striking contrast with the fact that  $\Delta G^\circ$  values actually show appreciable variations.

Data regarding chiral selection properties are summarized in Table 5.  $\alpha$ CD does not act as a chiral selector. Modest selections are indeed observed only for guest **17**. However, fair selectivities are found for  $\beta$ CD, in particular with alaninol and alanine derivatives **11** and **17**. It is also very interesting to notice that inversion of chiral selection is found on comparing the two aminoalcohol derivatives **11**

**Table 5.** Chiral selection data

Host	Guest	PH	$K_R/K_S$	
$\alpha$ CD	<b>11</b>	6.0	0.98±0.05	
	<b>14</b>	6.0	0.94±0.06	
	<b>15</b>	6.0	1.01±0.03	
	<b>17</b>	2.5	0.81±0.06	
	<b>17</b>	6.0	1.11±0.05	
	<b>19</b>	2.5	0.91±0.03	
	<b>19</b>	6.0	1.03±0.03	
	$\beta$ CD	<b>11</b>	6.0	1.13±0.07
		<b>12</b>	6.0	0.98±0.06
<b>14</b>		6.0	1.00±0.03	
<b>15</b>		6.0	1.09±0.05	
<b>17</b>		2.5	0.69±0.09	
<b>17</b>		6.0	0.73±0.08	
<b>19</b>		2.5	0.84±0.06	
<b>19</b>	6.0	0.89±0.09		

and **15** with the related aminoacid derivatives **17** and **19**. No selectivity is found for the pyrrolidinol derivatives **14**, indicating that chiral recognition depends on the distance between the chiral center and the aromatic moiety of the guest. Strangely, no selection is found for the leucinol derivatives **12**. The chiral selectivities reported here are comparable, or even better than selections reported<sup>16,27</sup> for aminoacids or their simple derivatives with natural or chemically modified  $\beta$ CDs (much better results have only been obtained in more structured systems, such as ternary complexes<sup>5b</sup>). These results may be easily explained on the basis of the different interaction models illustrated previously. The possibility to observe any chiral discrimination is indeed linked to the occurrence of an effective interaction between the stereogenic center and the host cavity, as happens in  $\beta$ CD, while the ‘expanded hydrophobic sphere’ of  $\alpha$ CD is not structured enough to accomplish any recognition.

### 2.3. Formation of 2:1 complexes with $\alpha$ CD

We already observed<sup>11</sup> that  $\alpha$ CD is able to form 2:1 host-guest complexes, along with the usual 1:1 complexes, with some of our guests. In particular, we had been able to measure the second partial association constant  $K_{\alpha,2}$ , and the related thermodynamic parameters, for guests **1**, **3**, **5**, **6** and **16**, and we were able to detect qualitatively the incipient formation of the 2:1 complexes at high (> 10 mM)  $\alpha$ CD concentration for guests **2**, **4**, **7** and **8**.<sup>28</sup>

We were able to qualitatively detect the incipient presence of a 2:1 complex also with ethanolamine derivative **10**. Interestingly, among substrates **11–15** and **17–19** we never had any evidence about the presence of detectable amounts of 2:1 complexes, even at high (50 mM)  $\alpha$ CD concentration. Comparing the latter substrates with guests **6**, **10** and **16**, we can deduce that the formation of the 2:1 complex is hampered by placing either a sterically demanding or a strongly hydrophilic group on the pyrrolidine, ethanolamine or glycine frameworks of **6**, **10** or **16** respectively. For diamine derivatives **20–25** incipient formation of a 2:1 complex was always qualitatively detected at pH=11.0 (guests in their neutral form). In particular, we found indicative values of  $K_{\alpha,2}$  at 298.15 K of  $20 \pm 10 \text{ M}^{-1}$  and  $40 \pm 20 \text{ M}^{-1}$  for **23** and **24** respectively, although the data did not allow us to get a reliable evaluation of the related thermodynamic parameters. However, at pH=6.0, the incipient formation of the 2:1 complex was qualitatively detected only for **23** and **24**. This finding further confirms that the presence of a hydrophilic group on the ancillary chain has a negative effect on the formation of the 2:1 complex.

### 2.4. Statistical analysis of binding constants and determination of corrected thermodynamic parameters

Before proceeding with a careful comparative examination of experimental data, we had to establish whether the compensation effect was real, and thus how reliable van't Hoff parameters were. As we already mentioned, regardless of the experimental procedure adopted to obtain enthalpy and entropy variations for a generic processes series (microcalorimetry or van't Hoff plot analysis) their values

are determined simultaneously. Consequently, their best estimates and indeterminations are correlated.<sup>20a</sup> In fact, in a  $\Delta H^\circ$  versus  $T\Delta S^\circ$  plot the confidence region for each experimental datum should actually be represented by a thin ellipse<sup>29</sup> (whose major diameter has a slope equal to 1). Under these circumstances, it can be rigorously demonstrated that the covariance between  $\Delta H^\circ$  and  $T\Delta S^\circ$  is non-zero. As a consequence, good linear relationships are anyway found, having slopes depending on the mean value of the operational temperature, and irrespective of the existence of a real compensation. In particular, for a series of van't Hoff experiments carried out between 283 and 343 K, a slope of ca. 0.96 should be expected.<sup>30</sup> The latter result is dangerously similar to most of the slope values reported in literature for studies on cyclodextrins.<sup>13–16</sup>

In order to solve this problem, different approaches have been tried and reported in literature.<sup>17,20a</sup> In particular, Alper and Gelb developed a suitable statistical analysis method of equilibrium constant data,<sup>18,21</sup> which could be considered as an ‘extended van't Hoff’ treatment. Their method is based on a simultaneous regression analysis of all the various van't Hoff correlations for a set of experiments with  $n$  different guests, by means of Eq. 1:

$$R \text{Ln } K_{ij} = -\Delta H^\circ_i/T_{ij} + \Delta S^\circ_i \quad (1)$$

where index  $i$  refers to the generic  $i$ -th experiment, and the index  $j$  individuates the generic  $j$ -th datum within the  $i$ -th experiment. If a real enthalpy–entropy compensation effect exists, according to the Eq. 2:

$$\Delta H^\circ_i = \Delta H^\circ_0 + \Theta \Delta S^\circ_i \quad (2)$$

then Eq. 1 may be re-written as:

$$R \text{Ln } K_{ij} = (\Delta H^\circ_0 + \Theta \Delta S^\circ_i)/T_{ij} + \Delta S^\circ_i \quad (3)$$

In Eqs. 2 and 3  $\Theta$  is defined as the ‘compensation’ or ‘isoequilibrium’ temperature, while  $\Delta H^\circ_0$  represents the enthalpic gain on inclusion in absence of any entropic variation. Eq. 3 corresponds to a non-linear regression problem with  $n+2$  parameters, namely  $\Delta H^\circ_0$ ,  $\Theta$  and the  $n$  different  $\Delta S^\circ_i$  values; these parameters have in turn to be determined through the usual  $\chi^2$  minimizing condition, with  $\chi^2$  defined as:

$$\chi^2 = \sum_i \sum_j (y_{ij} - Y_{ij})^2 / \sigma_{ij}^2 \quad (4)$$

where  $y_{ij}$  is the calculated value of  $R \text{Ln } K_{ij}$ ,  $Y_{ij}$  is the corresponding experimental value, and  $\sigma_{ij}^2$  is the variance of  $Y_{ij}$ . If the minimum value of  $\chi^2$  is similar to the number of degrees of freedom for the data set, then the compensation effect is real and we directly obtain the best estimates for  $\Theta$ ,  $\Delta H^\circ_0$  and the  $\Delta S^\circ_i$  values.<sup>31</sup> Uncertainties on these values can be subsequently obtained by means of a suitable Monte-Carlo procedure.<sup>32</sup> Differently, if the  $\chi^2$  value is much higher than the number of degrees of freedom, or if  $\Theta$  is negative, or if the uncertainty on  $\Theta$  is larger than its own value, then the compensation effect on the entire data set is false, and a deeper analysis is needed. Noticeably, if a null  $\Theta$  value is found, this indicates a set of isoenthalpic reactions, for which a simpler fitting equation can be used:

$$R \text{Ln } K_{ij} = -\Delta H^\circ_i/T_{ij} + \Delta S^\circ_i \quad (5)$$

Differently, if  $\Theta$  tends to infinity, we have a set of isoentropic reactions, which can be treated according to Eq. 6:

$$R \ln K_{ij} = -\Delta H^\circ_i/T_{ij} + \Delta S^\circ_0 \quad (6)$$

In performing this kind of statistical data analysis, a crucial role is played by  $\sigma_{ij}^2$  values.<sup>18</sup> After the original Alper and Gelb's work,<sup>21</sup>  $\sigma_{ij}$  has been referred to as the 'experimental uncertainty' for  $Y_{ij}$ .<sup>18,20a</sup> In particular, analyzing a series of data from inhomogeneous sources concerning cyclodextrin complexation equilibria, the same authors chose to fix a minimum value of  $0.15 R$  to this 'uncertainty', in order to account for the possibility of unknown systematic errors.<sup>18</sup> This corresponds to a minimum 15% indetermination on  $K_{ij}$  values. In our opinion, this choice involves a 'goodness-of-fit' evaluation criterion which is not sufficiently restrictive. Our data set is indeed homogeneous in origin, and refers to strictly homogeneous substrates. Furthermore, in our case we chose to give  $2\sigma$ -wide confidence intervals for our association constants, and we have found that the mean relative indetermination on our  $K_{ij}$  values is 5%. There is the general agreement that a 5–6% indetermination appears reasonable for thermodynamic as well as for kinetic constant values. Therefore, everything considered, in our opinion a minimum value for  $\sigma_{ij}$  of  $0.03 R$  ( $0.25 \text{ J mol}^{-1} \text{ K}^{-1}$ , accounting for a 6% indetermination) seemed a more suitable choice. In other words, on the grounds of Alper's symbolism we can set:

$$\sigma_{ij} = \text{MAX}\{\varepsilon_{R \ln K_{ij}}/2, 0.03R\} \quad (7)$$

that is, we set  $\sigma_{ij}$  as half the value of the 'experimental'  $R \ln K_{ij}$  error ( $\varepsilon_{R \ln K_{ij}}$ ) if this is larger than  $0.03 R$ , otherwise as  $0.03 R$ . It should be noticed that this value is less than 1% of the entire range of  $R \ln K_{ij}$  examined. Submitting our data to this kind of analysis, we obtained very interesting results.

All data for  $\alpha$ CD were first treated together as belonging to a single compensation model, leading to a poor result ( $\Theta = 523 \text{ K}$ ,  $\Delta H^\circ_0 = -3.8 \text{ kJ mol}^{-1}$ ;  $\chi^2 = 268.96$ , 43 entries, 199 data points, 154 degrees of freedom). On the grounds of the deviations between calculated and experimental  $R \ln K_{ij}$  values, a careful inspection of the entire data set led us to the conclusion that a group of five data subsets did not fit with the model and had to be excluded, namely those for guests **3**, **16** at pH=2.5, **18** at pH 2.5, **20** at pH 6.0 and **26**. After their elimination, results were much more satisfying:  $\Theta = 479 \pm 45 \text{ K}$ ,  $\Delta H^\circ_0 = -6.4 \pm 2.8 \text{ kJ mol}^{-1}$ ;  $\chi^2 = 134.28$ , 38 entries, 177 data points, 137 degrees of freedom.

Corrected thermodynamic parameters obtained in this way are also reported in Table 3 and are illustrated in Figure 2. These results undoubtedly account for a real compensation effect. The slope for the  $T\Delta S^\circ$  versus  $\Delta H^\circ$  correlation is  $\beta = 0.62 \pm 0.06$ . The calculated  $\Theta$  value is comparable with those reported by Alper<sup>18</sup> and by Linert<sup>17</sup> in their works for similar cases. The relatively high uncertainty on both  $\Theta$  and  $\Delta S^\circ_0$  (which are interdependent) may be due to the fact that  $\Theta$  is actually quite far from the operational temperature range.<sup>20a</sup>

Analysis of data for  $\beta$ CD similarly shows that a single

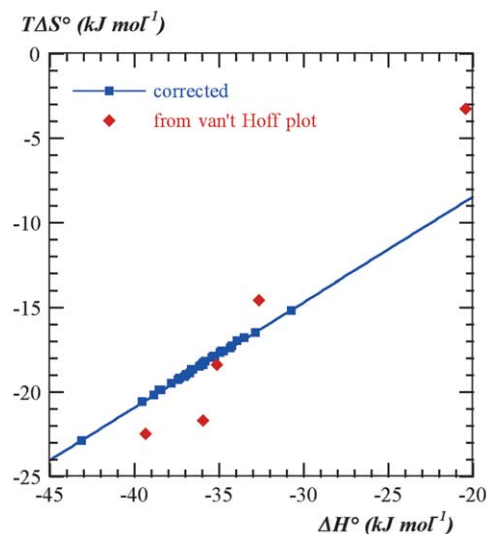


Figure 2. Corrected  $T\Delta S^\circ$  versus  $\Delta H^\circ$  plot for  $\alpha$ CD.

compensation model is absolutely unsuitable ( $\Theta = -213 \text{ K}$  (!),  $\Delta H^\circ_0 = -15.3 \text{ kJ mol}^{-1}$ ;  $\chi^2 = 428.12$ , 37 entries, 173 data points, 134 degrees of freedom). So, also in this case we had to carefully inspect the data on the grounds of the deviations between calculated and experimental  $R \ln K_{ij}$  values, as well as of the differences between van't Hoff and calculated thermodynamic parameters. This analysis led us to the unexpected conclusion, with very satisfactory results, that three different groups of guests may be reasonably defined:

Group A, guests **1**, **10–12**, **16** at pH=6.0, **17** (both at pH=2.5 and 6.0) **20** at pH=11.0, **25** at pH=11.0:  $\Theta = 235 \pm 8 \text{ K}$ ,  $\Delta H^\circ_0 = -16.5 \pm 0.1 \text{ kJ mol}^{-1}$ ;  $\chi^2 = 42.12$ , 13 entries, 59 data points, 44 degrees of freedom;

Group B, guests **3**, **4**, **13**, **15**, **18** at pH=6.0, **19** (both at pH=2.5 and 6.0), **20** at pH=6.0, **22** (both at pH=2.5 and

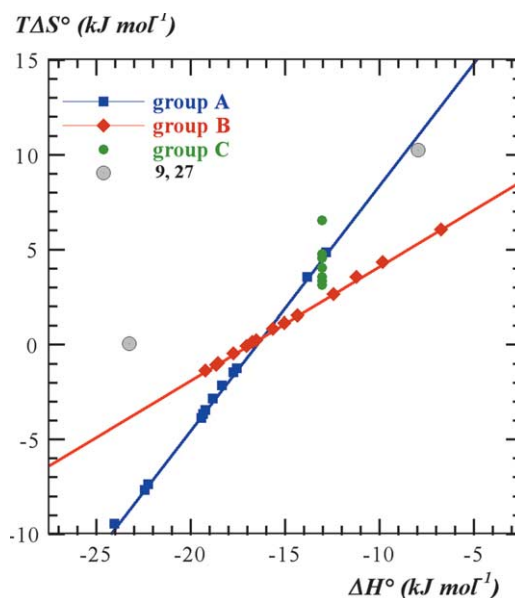


Figure 3. Corrected  $T\Delta S^\circ$  versus  $\Delta H^\circ$  plot for  $\beta$ CD.

6.0), **25** at pH=6.0, **26**:  $\Theta=494\pm 38$  K,  $\Delta H^\circ_0=-16.2\pm 0.2$  kJ mol<sup>-1</sup>;  $\chi^2=53.48$ , 15 entries, 71 data points, 54 degrees of freedom;

Group C, guests **2**, **5–8**, **14**. Isoenthalpic ( $\Theta=0$  K),  $\Delta H^\circ_0=-13.0\pm 0.7$  kJ mol<sup>-1</sup>;  $\chi^2=12.80$ , 7 entries, 33 data points, 24 degrees of freedom.

Guests **9** and **27** cannot be inserted in any of the preceding groups. Corrected thermodynamic parameters for  $\beta$ CD, also reported in Table 4, and are illustrated in Figure 3. The slopes of the  $T\Delta S^\circ$  versus  $\Delta H^\circ$  correlations for groups A and B are  $\beta_A=1.27\pm 0.04$  and  $\beta_B=0.60\pm 0.05$  respectively.

A fair agreement is generally found between the corrected  $\Delta H^\circ_i$  or  $T\Delta S^\circ_i$  values and those directly coming from van't Hoff plots. Differences show a standard deviation of 2.0 kJ mol<sup>-1</sup>, but they are significantly large, indeed, only for guests **16** and **17** at pH=6.0 with  $\alpha$ CD, and for guests **15** and **19** with  $\beta$ CD. A careful analysis of the data presented above offers us a confirmation of the two different interaction models already proposed for  $\alpha$ CD and  $\beta$ CD respectively.<sup>11</sup> Nonetheless, data presented here provide us also with further interesting and unexpected insights.

## 2.5. Comparative analysis of thermodynamic data for $\alpha$ CD

Binding constants and  $-\Delta H^\circ$  values with  $\alpha$ CD for aminoalcohol guests regularly increase along the series **10**<**11**<**13**≈**14**<**15**, on increasing the hydrophobicity of the ancillary chain. The same behavior may be deduced on comparing these guests with the related aminoacid derivatives **16–19** (excepting **18** at pH=2.5). However, comparison between the neutral (at pH=2.5) and the ionized (at pH=6.0) forms of the latter guests is not easy to perform for several reasons. As a matter of fact, **16** and **18** go beyond the general fitting model, and further difficulties derive from the discrepancy of the calculated  $\Delta H^\circ$  values with respect to van't Hoff values for **16** and **17** at pH=6.0. Unfortunately, we do not have for the moment a satisfactory rationale for the fact that some entries have to be excluded from the general fitting model. Probably for **20** at pH=6.0 this could be related to the high solvation demand of the cationic ammonium tail group, while for **26** the problem could be the high indetermination of the association constants. Anyway, we notice that binding constants at pH=6.0 are comparable (**16**, **18**) or even higher (**17**, **19**) than at pH=2.5. Presumably, this behavior is the overall result of a balance between several contrasting factors, including the different hydrophobicity and possible conformational changes for the guest, induced on changing its protonation state. It should also be remembered that, as shown by the comparison between guests **3**, **4** and **6**, the interaction of the 'expanded hydrophobic sphere' with the ancillary chain seems to be disfavored on increasing the conformational freedom of the chain itself.

Under the latter perspective, the behavior of diamino derivatives **20–25** appears very interesting. For the short two-carbon-chain guests **20** and **25**, higher  $K$  and  $-\Delta H^\circ$  values are found at pH=11.0 than at pH=6.0. This finding is in striking contrast with the usual rule that binding

constants are expected to decrease on passing from a nearly neutral (pH=6.0) to an alkaline (pH=11.0) buffer,<sup>22</sup> due to the partial deprotonation of the host, and consequently its more difficult desolvation.<sup>33</sup> Also for the three-carbon-chain guest **21** the neutral form is a little more favorably included than the ionized one. Differently, longer chain derivatives **22–24** show higher binding constants at pH=6.0, according to the usual rule, and thus irrespective of the presence of a charged group at the end of the long ancillary chain. At both pH values, neither  $K$  nor  $-\Delta H^\circ$  values vary monotonically along the series **20–24**, but pass through a maximum for **21** at pH=11.0, while at pH=6.0 the highest values are found for **21** and **22**. It is interesting to notice that the usual effects of  $K$  and  $-\Delta H^\circ$  increase on increasing the number of methylene units, observed for different classes of linear alkyl compounds,<sup>13</sup> is completely overruled in this case. Furthermore, comparison between **21** and **3** shows that the replacement of the methyl group with the more hydrophilic amino or ammonium groups at the end of the ancillary chain improves the binding affinity of the guest. The binding constant for **3** may be rather compared with values found for the longest chain guest **24**.

All these considerations suggest that the dimensions of the 'expanded hydrophobic sphere' should not exceed the length of a straight three-carbon chain. The 'sphere' seems to feel unfavorably the effects of the presence of either a conformationally free or a short and charged (or even strongly solvated) ancillary chain. Strangely, a charged group just at the edge of the 'sphere' seems to have a favorable effect on its structuring (as accounted for by **21** and **22** at pH=6.0). We may hypothesize that in this case, the need to keep the charged group out of the 'sphere' has a somewhat blocking effect on the conformational freedom of the chain. On the other hand, the highly hydrophobic chains of **3**, **23** and **24** need to avoid any contact with the bulk water, and try to penetrate the 'sphere' adopting a partly folded and flexible conformation, which makes the interaction with the 'sphere' less effective.

## 2.6. Comparative analysis of thermodynamic data for $\beta$ CD

Thermodynamic data for  $\beta$ CD are really interesting, because the unexpected existence of three well-defined guest groups seems to indicate that quite different situations may occur even within the same interaction model. As a matter of fact, group A collects those guests whose ancillary chain is able to give rise simultaneously to two or more hydrogen bonds, including **1** as a suitable 'anchor point', and excludes those guests whose ancillary chain either ends with a highly hydrophilic group (**20**, **22** and **25** at pH=6.0) or is so long (**22**) that the simultaneous formation of two hydrogen bonds may be entropically disfavored. On the other hand, group C collects guests whose cyclic ancillary chain has poor conformational freedom, including **2** as a suitable 'anchor point' (it should be remarked that their aniline-like N atom is unable to act as a hydrogen bond donor). All other guests are collected in group B, excluding **9** and **27**. The latter two guests go beyond the fitting models probably because their ancillary chains make them so highly hydrophobic that competition between different inclusion modes<sup>7,11</sup> into the  $\beta$ CD cavity (i.e., with the nitro group



directed towards either the primary or the secondary host rim) may occur.

Starting with group C, the occurrence of a set of isoenthalpic processes seems to suggest that the interaction between the host cavity and the ancillary chain actually involves only the two methylene groups directly linked to the aniline-like N atom, while the remaining chain is held far from the cavity and fairly exposed to the solvent shell. This hypothesis explains the lack of chiral selection for pyrrolidinol derivatives **14**. Furthermore, it agrees with the idea that an effective interaction of the ancillary chain with the host cavity may occur either if the chain is conformationally flexible (as for **3** and **4**, as compared to **6**) or if a suitable interacting group is held relatively ‘near’ the aniline-like N atom (as for **15** and **19**). Noticeably, inclusion entropies for the cyclic guests **5–9** and **14** follow the same order as their hydrophobicity. The latter finding suggests that solvent reorganization should be particularly involved in entropic contributions to the overall process, at least for these guests.

On the other hand, the behavior of the guests belonging to group A accounts for a situation in which the inclusion complex is particularly rigid, owing to the simultaneous occurrence of at least two hydrogen bonds between the guest and the host cavity or rim. Therefore, according to the low compensation temperature  $\Theta$  (and the high  $\beta_A$  slope), variations in the entropic contributions along the group are so large that they strikingly overwhelm enthalpic variations at ordinary temperature. In fact, this leads to the uncommon situation that lower binding constants at 298.15 K are observed for those guests whose inclusion enthalpy is more favorable. By inspection of data reported in Table 4, we notice that higher  $-\Delta H^\circ$  values (and lower  $K$  values) are found for **10** with respect to **12** and for **16** with respect to **17**, indicating that the presence of bulky groups on the structure of the guest causes unfavorable enthalpic contributions. These contributions may be responsible of the fairly good chiral recognitions observed for **11** and **17**, but they may also disfavor recognition if they become too heavy (as for **12**). We were not able to get a reliable estimation of the inclusion constant for **16** at pH=2.5. This may be due, in our opinion, to the fact that easier desolvation of the carboxyl group of **16** in its neutral form, rather than in its ionized form, may have the consequence of increasing the  $-\Delta H^\circ$  value so much as to make the overall inclusion process too disfavored. It should also be noticed that the simultaneous formation of two (or more) hydrogen bonds requires the disruption of the guest intramolecular hydrogen bond discussed above, which is obviously an enthalpy-demanding process.

The behavior of group B guests is quite different. As  $\Theta$  and  $\beta_B$  values account for, this time the host–guest complex does not appear to be too rigid, so enthalpic contribution variations are not completely erased by entropic variations. Inspection of thermodynamic data seems to indicate that inclusion enthalpy increases on increasing the hydrophobicity of the guest, as we can deduce on comparing aminoalcohol derivatives **13** and **15** with the corresponding aminoacid derivatives **18** and **19** in both their neutral (pH=2.5) and ionized (pH=6) forms. In agreement with this

trend, inclusion enthalpy for the diamino derivative **22** decreases on passing from pH=11 to pH=6. Noticeably, also diamino derivatives **20** and **25** at pH=6 are included in this group, showing quite low  $-\Delta H^\circ$  values. This confirms that their highly solvated cationic tail group is unable to interact effectively with the host cavity or rim.

## 2.7. Further remarks about the enthalpy–entropy compensation effect

The ability to individuate different classes of guests, unambiguously related to their structural features, is a particularly intriguing aspect of our data analysis. In fact, it allows us to achieve valuable information about the occurrence of different behaviors within the same interaction model (namely with  $\beta$ CD). This, in turn, can be easily related to the microscopical characteristics of the host–guest complex. Therefore, we were induced to reconsider some current ideas about the interpretation of the compensation effect.

As a matter of fact, during the last years, different attempts have been made<sup>20b,c,e</sup> in order to rigorously lead back compensation for a generic binding phenomenon to thermodynamics principles. Thus, theory ‘demonstrated’ that, at least under certain conditions, enthalpic and entropic contributions related to the reorganization of the solvent molecules, among the solvent bulk and the solvation spheres of the interacting species, ‘must’ exactly compensate. On the assumption that in aqueous medium, owing to the high structuring of water, solvation effects should prevail on the effects related to the so-called ‘nominal process’ (i.e., the neat equilibrium between solvated host, guest and complex), it has thus been inferred that solvation effects should be the actual source of the compensation effect. As a remarkable consequence, a compensation temperature  $\Theta$  near to the operational temperature (and  $\beta$  slope value close to 1) should be always expected.<sup>34</sup>

Unfortunately, our data, as well as those found by other authors,<sup>17–19</sup> strikingly contradict these conclusions. In our opinion, such a disagreement between predictions and experimental results should indicate that, at least in our situation, solvation effects are not able to entirely conceal the ‘nominal process’. In other words, our findings seem to suggest that, along a series of strictly homogeneous guests, solvation effects may be nearly constant (and thus their variations negligible). Under these conditions the occurrence of a compensation effect will actually account for information related to the ‘nominal process’ only. Linert<sup>17</sup> first warned about the opportunity to correlate only data related to series of homogeneous guests; nonetheless during the last years comprehensive correlations for very large sets of thermodynamic data have become much more popular.<sup>13,16</sup> However, under the perspective of our considerations, the latter choice clearly appears unsuitable. As a matter of fact, for a very large guests set the assumption of a nearly identical solvent reorganization is likely to be incorrect. Therefore, in this case the piece of information related to the actual host–guest interaction would simply be lost, being ‘drowned’ by solvation effects.

### 3. Conclusions

In conclusion, our study confirmed the occurrence different models of interaction between our hosts and guests, as previously proposed,<sup>11</sup> and also provided further useful insights. In particular, valuable information was deduced about the characteristics of the ‘expanded hydrophobic sphere’ for  $\alpha$ CD. Furthermore, the unexpected occurrence of different compensation effects for  $\beta$ CD towards structurally well defined classes of guests, pointed out the mutual interplay between hydrophobic, polar and hydrogen bond interactions in determining the overall thermodynamics of the process. Nonetheless, a careful statistical data analysis is needed before proceeding with an exhaustive examination of thermodynamic parameters, because direct van’t Hoff parameters are not completely reliable. Finally, some interesting critical considerations about the actual meaning of the compensation effect were presented.

### 4. Experimental

#### 4.1. Materials

Commercial  $\alpha$ CD and  $\beta$ CD (Fluka) were dried in a desiccator in vacuo over phosphorus pentoxide at 90 °C for at least 24 h and stored in the same apparatus at 40 °C; they were then used as such. All other commercial materials and reagents (Fluka, Aldrich) were used as such without further purification.

Compounds **1–9**, **16**, **19-S**, **26** and **27** were prepared according to literature references.<sup>11</sup>

Aminoalcohol derivatives **10–15** were prepared according to the following procedure: the appropriate aminoalcohol (10 mmol) and a slight excess of *p*-fluoro-nitrobenzene (11 mmol) were dissolved in DMSO (20 mL); after addition of anhydrous K<sub>2</sub>CO<sub>3</sub> (15 mmol), the mixture was allowed to react under gentle warming (45 °C) and stirring till completion (TLC). The mixture was then poured into water (200 mL); part of the product precipitated and was filtered off. The mother liquors were extracted with ethyl acetate; the organic extracts were dried on anhydrous Na<sub>2</sub>SO<sub>4</sub> and distilled in vacuo. The crude products were joined and purified by flash chromatography on silica gel, using suitable ethyl acetate–light petrol mixtures as eluents (yield 70–85%). The purified product was finally crystallized from methanol or from ethanol–light petrol prior to use.

**4.1.1. 2-*N*-(*p*-Nitro-phenyl)-amino-ethanol (10).** Yellow-orange crystals, mp 108 °C. IR (nujol)  $\nu_{\max}$  3439, 3271, 1599, 1549, 1502 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.29 (q, *J*=5.6 Hz, 2H, –NHCH<sub>2</sub>–), 3.63 (q, *J*=5.6 Hz, 2H, –CH<sub>2</sub>OH), 4.90 (t, *J*=5.6 Hz, 1H, –OH), 6.71, 8.03 (2d, *J*=9.3 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH–), 7.37 (t, *J*=5.4 Hz, 1H, –NH–). Anal. Calcd for C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.47; H, 5.35; N, 15.77.

**4.1.2. (*R*)-2-*N*-(*p*-Nitro-phenyl)-amino-propanol (11-*R*) and (*S*)-2-*N*-(*p*-nitro-phenyl)-amino-propanol (11-*S*).** Yellow crystals, mp 120–121 °C. IR (nujol)  $\nu_{\max}$  3430,

3290, 1600, 1540, 1495 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.19 (d, *J*=6.5 Hz, 3H, –CH<sub>3</sub>), 3.35–3.51 (m, 2H, –CH<sub>2</sub>OH), 3.60–3.71 (m, 1H, –NH–CH(CH<sub>3</sub>)–), 4.89 (1H, t, *J*=5.6 Hz, –OH), 6.72, 8.02 (2d, *J*=9.4 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH–), 7.16 (d, *J*=7.9 Hz, 1H, –NH–). Anal. Calcd for C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 55.09; H, 6.16; N, 14.28. Found: C, 54.98; H, 6.27; N, 14.11.

**4.1.3. (*R*)-2-*N*-(*p*-Nitro-phenyl)-amino-4-methyl-pentanol (12-*R*) and (*S*)-2-*N*-(*p*-nitro-phenyl)-amino-4-methyl-pentanol (12-*S*).** Yellow crystals, mp 98–99 °C. IR (nujol)  $\nu_{\max}$  3467, 3304, 1603, 1545, 1506 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  0.89, 0.97 (2d, *J*=6.5 Hz, 3H+3H, –CH(CH<sub>3</sub>)<sub>2</sub>), 1.40–1.55 (m, 1H, –NH–CH<), 3.36–3.50, 3.54–3.66 (2m 1H+1H, –CH<sub>2</sub>OH), 4.84 (t, *J*=5.4 Hz, 1H, –OH), 6.65, 8.09 (2d, *J*=9.4 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH–), 7.12 (d, *J*=8.5 Hz, 1H, –NH–). Anal. Calcd for C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>: C, 60.49; H, 7.61; N, 11.76. Found: C, 60.28; H, 7.88; N, 11.40.

**4.1.4. 2-*N*-Methyl-*N*-(*p*-nitro-phenyl)-amino-ethanol (13).** Yellow crystals, mp 104–105 °C. IR (nujol)  $\nu_{\max}$  3437, 1593, 1578, 1517 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.14 (s, 3H, >NCH<sub>3</sub>), 3.58–3.69 (m, 4H, –CH<sub>2</sub>–CH<sub>2</sub>OH), 4.88 (br s, 1H, –OH), 6.71, 8.03 (2d, *J*=9.3 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>N<). Anal. Calcd for C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 55.09; H, 6.16; N, 14.28. Found: C, 55.37; H, 6.00; N, 14.25

**4.1.5. (*R*)-*N*-(*p*-Nitro-phenyl)-3-hydroxy-pyrrolidine (14-*R*) and (*S*)-*N*-(*p*-nitro-phenyl)-3-hydroxy-pyrrolidine (14-*S*).** Red-orange crystals, mp 177–178 °C. IR (nujol)  $\nu_{\max}$  3468, 1599, 1556, 1518 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.96–2.18 (m, 2H, >NCH<sub>2</sub>CH<sub>2</sub>–), 3.25–3.45 (m, 4H, –CH<sub>2</sub>–N–CH<sub>2</sub>–), 4.45–4.51 (m, 1H, >CH–OH), 5.16 (d, *J*=3.6 Hz, 1H, –OH), 6.63, 8.08 (2d, *J*=9.3 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>N<). Anal. Calcd for C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 57.69; H, 5.81; N, 13.45. Found: C, 57.82; H, 5.88; N, 13.20.

**4.1.6. *N*-(*p*-Nitro-phenyl)-(*D*)-prolinol (15-*R*) and *N*-(*p*-nitro-phenyl)-(*L*)-prolinol (15-*S*).** Yellow crystals, mp 114 °C. IR (nujol)  $\nu_{\max}$  3456 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.93–2.14 (m, 4H, –CH<sub>2</sub>CH<sub>2</sub>–), 3.22–3.41 (m, 2H, >N–CH<sub>2</sub>–), 3.48–3.57 (m, 2H, –CH<sub>2</sub>–OH), 3.92–4.00 (m, 1H, >NCH–), 4.97 (dd, *J*=6.0, 5.6 Hz, 1H, –OH), 6.73, 8.09 (2d, *J*=9.4 Hz, 2H+2H, *p*NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>N<). Anal. Calcd for C<sub>12</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.28; H, 6.59; N, 12.40.

Aminoacid derivatives **17**, **18** and **19-*R*** were prepared according to the following procedure: the appropriate aminoacid (10 mmol) was treated with an equimolar amount of 1 M tetrabutylammonium hydroxide in methanol; the solvent was removed in vacuo, and the residue was dissolved in DMSO (20 mL). A slight excess of *p*-fluoro-nitrobenzene (11 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (11 mmol) was added and the mixture was allowed to react under gentle warming (45 °C) and stirring till completion (TLC). The mixture was then poured into cold water (200 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The aqueous phase was collected, acidified with HCl up to pH=2.0 and extracted with ethyl acetate; the latter organic extract was dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo, and the residue finally

purified by chromatography on silica gel with light petrol–ethyl acetate mixtures as eluents (yield 60–80%). In some cases it was convenient to isolate the product as the sodium salt: the product was dissolved in a minimum amount of methanol and an equimolar amount of a methanol concentrated NaOH solution was carefully added; the resulting solution was then dropped in a ten-fold volume of Et<sub>2</sub>O, and the finely precipitated product was filtered off.

**4.1.7. *N*-(*p*-Nitro-phenyl)-(D)-alanine sodium salt (17-R·Na) and *N*-(*p*-Nitro-phenyl)-(L)-alanine sodium salt (17-S·Na).** Red-orange powder, 82–84 °C. IR (nujol)  $\nu_{\max}$  3269, 1611, 1580, 1547, 1501 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.43 (d, *J*=6.9 Hz, 3H, -CH<sub>3</sub>), 4.03–4.12 (m, 1H, -NHCH(CH<sub>3</sub>)-), 6.69, 8.02 (2d, *J*=8.8 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.42 (d, *J*=7.2 Hz, 1H, -NH-). Anal. Calcd for C<sub>9</sub>H<sub>9</sub>N<sub>2</sub>NaO<sub>4</sub>: C, 46.56; H, 3.91; N, 12.07. Found: C, 46.42; H, 3.88; N, 11.91.

**4.1.8. *N*-(*p*-Nitro-phenyl)-sarcosine (18).** Yellow solid, mp 120–140 °C (decomp.), IR (nujol)  $\nu_{\max}$  1740, 1599, 1583, 1518 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.16 (s, 3H, >N-CH<sub>3</sub>), 4.35 (s, 2H, -CH<sub>2</sub>-), 6.82, 8.11 (2d, *J*=9.4 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>N<), 12.95 (br s, 1H, -COOH). Anal. Calcd for C<sub>9</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>: C, 51.43; H, 4.80; N, 13.33. Found: C, 51.72; H, 4.68; N, 13.41.

**4.1.9. *N*-(*p*-Nitro-phenyl)-(D)-proline (19-R).** Orange-brown solid; mp >250 °C (decomp.). IR (nujol)  $\nu_{\max}$  1720 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO)  $\delta$  1.96–2.40 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>CH<sub>2</sub>-CH<), 3.43–3.64 (m, 2H, >N-CH<sub>2</sub>-), 4.47 (dd, *J*=8.6, 2.4 Hz, 1H, >CH-COOH), 6.63, 8.12 (d, *J*=9.3 Hz, 2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>N<). Anal. Calcd for C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.77; H, 6.45; N, 11.40.

Diamine derivatives **20–25** were prepared as follows: *p*-fluoro-nitrobenzene (10 mmol) was dissolved in DMSO (20 mL) and 5 equiv of the appropriate diamine were added. The mixture was allowed to react at 80 °C under stirring till completion (TLC). The mixture was poured into water (200 mL), acidified with HCl up to pH=4 and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The aqueous phase was then treated with a concentrated NaOH solution up to pH=12 and extracted with ethyl acetate. The organic extracts were dried on anhydrous Na<sub>2</sub>SO<sub>4</sub> and distilled in vacuo. The residue was finally purified by crystallization from methanol (yield 75–90%). Sometimes it was convenient to isolate the low-melting product as hydrochloride: the product was dissolved in a minimum amount of methanol and a slight excess of 12 M HCl was slowly added; the resulting solution was then dropped in a ten-fold amount of Et<sub>2</sub>O and the product was finally recovered by filtration.

**4.1.10. *N*-(*p*-Nitro-phenyl)-1,2-diamino-ethane (20).** Yellow solid, mp 138–141 °C. IR (nujol)  $\nu_{\max}$  3360, 3359, 3224, 3171, 1600, 1550, 1501 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.68 (br s, 2H, NH<sub>2</sub>), 2.78 (t, *J*=6.3 Hz, 2H, -CH<sub>2</sub>NH<sub>2</sub>), 3.13–3.23 (m, 2H, -NHCH<sub>2</sub>-), 6.70, 8.04 (2d, *J*=9.3 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.36 (br t, 1H, -NH-). Anal. Calcd for C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>: C, 53.03; H, 6.12; N, 23.19. Found: C, 52.81; H, 5.99; N, 23.31.

**4.1.11. *N*-(*p*-Nitro-phenyl)-1,3-diamino-propane (21).** Yellow crystals, mp 108–110 °C. IR (nujol)  $\nu_{\max}$  3358, 3298, 3275, 3229, 1603, 1549, 1504 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.48 (br s, 2H, -NH<sub>2</sub>), 1.68 (quint., *J*=6.7 Hz, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 2.58 (t, *J*=6.7 Hz, 2H, -CH<sub>2</sub>-NH<sub>2</sub>), 3.25 (br t, *J*=6.7 Hz, 2H, -NHCH<sub>2</sub>-), 6.68, 8.05 (2d, *J*=9.2 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.39 (br s, 1H, -NH-). Anal. Calcd for C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.40; H, 6.90; N, 21.29.

**4.1.12. *N*-(*p*-Nitro-phenyl)-1,4-diamino-butane (22).** Yellow-orange crystals, mp 100 °C. IR (nujol)  $\nu_{\max}$  3352, 3294, 3216, 3167, 1600, 1550, 1500 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (br s, 2H, -NH<sub>2</sub>), 1.40–1.52, 1.57–1.69 (2m, 2H+2H, -CH<sub>2</sub>-CH<sub>2</sub>-), 2.55–2.64 (m, 2H, -CH<sub>2</sub>NH<sub>2</sub>), 3.15–3.22 (m, 2H, -NHCH<sub>2</sub>-), 6.68, 8.03 (2d, *J*=9.2 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.39 (br t, 1H, -NH-). Anal. Calcd for C<sub>10</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>: C, 57.40; H, 7.23; N, 20.08. Found: C, 57.70; H, 7.49; N, 20.21.

**4.1.13. *N*-(*p*-Nitro-phenyl)-1,5-diamino-pentane hydrochloride (23·HCl).** Yellow-orange powder, mp 161–163 °C. IR (nujol)  $\nu_{\max}$  3321, 1603, 1528, 1501 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.40–1.52 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 1.57–1.73 (m, 2H+2H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 2.77–2.86 (quint., *J*=6.4 Hz, 2H, -NHCH<sub>2</sub>-), 3.14 (br t, *J*=6.4 Hz, 2H, -CH<sub>2</sub>NH<sub>3</sub><sup>+</sup>), 3.50 (br s, 3H, -NH<sub>3</sub><sup>+</sup>), 6.71, 8.03 (2d, *J*=9.3 Hz, 2H+2H, pNO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>NH-), 7.53 (br s, 1H, -NH-). Anal. Calcd for C<sub>11</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 50.87; H, 6.99; N, 16.18. Found: C, 50.69; H, 7.08; N, 15.98.

**4.1.14. *N*-(*p*-Nitro-phenyl)-1,6-diamino-exane (24).** Yellow-orange crystals, mp 89–90 °C. IR (nujol)  $\nu_{\max}$  3225, 3178 1607, 1551, 1504 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.36–1.45, 1.55–1.64 (2m, 6H+2H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 1.87 (br s, 2H, -NH<sub>2</sub>), 2.54–2.57 (m, 2H, -CH<sub>2</sub>NH<sub>2</sub>), 3.15–3.22 (m, 2H, -NHCH<sub>2</sub>-), 6.68, 8.03 (2d, *J*=9.3 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.36 (br t, 1H, -NH-). Anal. Calcd for C<sub>12</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>: C, 60.74; H, 8.07; N, 17.71. Found: C, 60.57; H, 7.94; N, 17.81.

**4.1.15. *N,N*-Dimethyl-*N'*-(*p*-nitro-phenyl)-1,2-diamino-ethane hydrochloride (25·HCl).** Yellow powder, mp 178–180 °C. IR (nujol)  $\nu_{\max}$  3244, 2611, 2469, 1594, 1539, 11498 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO)  $\delta$  2.85 (s, 6H, -NH(CH<sub>3</sub>)<sub>2</sub><sup>+</sup>), 3.31 (2H, t, *J*=6.6 Hz, -CH<sub>2</sub>-NH<sup>+</sup><), 3.62–3.70 (m, 2H, -NHCH<sub>2</sub>-), 6.81, 8.08 (2d, *J*=9.2 Hz, 2H+2H, pNO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NH-), 7.39 (br t, 1H, -NH-), 10.86 (br s, 1H, -NH(CH<sub>3</sub>)<sub>2</sub><sup>+</sup>). Anal. Calcd for C<sub>10</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>2</sub>: C, 48.88; H, 6.56; N, 17.10. Found: C, 48.69; H, 6.67; N, 16.95.

Stock phosphate buffer solutions were prepared according to literature reports and used within a few days, after checking the actual pH value. Freshly double-distilled water was used for the preparation of the buffers, which were in turn used as solvents for the preparation of the measurement solutions.

## 4.2. Measurement of pK<sub>a</sub> or pK<sub>BH</sub><sup>+</sup> of 17–18 and 20–25

(i) A weighed amount (ca. 40 μmol) of the sodium salts of

**17** or **18** was introduced in a water-jacketed vessel thermostated at  $298.1 \pm 0.3$  K and was dissolved with double-distilled water (20 mL) under magnetic stirring. A stream of fine Argon bubbles was passed for 15 min through the solution, which was then titrated with a 0.1 M standardized HCl solution introduced into the vessel by a microsyringe. (ii) A weighed amount (ca. 40  $\mu$ mol) of the diamine derivatives **20**, **21**, **22** or **24** was introduced in a water-jacketed vessel thermostated at  $298.1 \pm 0.3$  K and was dissolved with a 0.0025 M standardized HCl solution (20 mL) under magnetic stirring. A stream of fine Argon bubbles was passed for 15 min through the solution, which was then titrated with a 0.1 M standardized NaOH solution introduced into the vessel by a microsyringe. (iii) A weighed amount (about 40  $\mu$ mol) of the hydrochlorides of derivatives **23** or **25** was introduced in a water-jacketed vessel thermostated at  $298.1 \pm 0.3$  K and was dissolved with double-distilled water (20 mL) under magnetic stirring. A stream of fine Argon bubbles was passed for 15 min through the solution, which was then titrated with a 0.1 M standardized NaOH solution introduced into the vessel by a microsyringe.

In each case, the titration experiment was performed by following the pH value variations. Data were finally processed fitting the pH versus added base curve by means of the proper equation obtained analytically.

#### 4.3. UV–Vis spectra and binding constants measurement

Solutions for UV–Vis spectra and binding constants measurements were prepared at a fixed concentration of guest (usually about 30 mM) and at a concentration of host ranging up to 0.05 M for  $\alpha$ -CD, or up to 0.008 M for  $\beta$ -CD (according to the maximum solubility of the two cyclodextrins). UV–Vis spectra were recorded at different temperatures ranging from 288.15 to 318.15 K on a Beckmann DU-7 spectrophotometer equipped with a Peltier temperature controller, able to keep the temperature within a  $\pm 0.1$  K indetermination. Suitable work wavelengths for each guest were chosen after recording some ‘difference spectra’ by comparison of the samples without cyclodextrin and in the presence of given amounts of cyclodextrin. The absorbances of the different solutions at the work wavelength were processed by direct non-linear regression analysis.<sup>11,35</sup>

#### Acknowledgements

Financial support from the University of Palermo (funds for selected research topics) and Italian MIUR within the National Research Project ‘Non-aromatic heterocycles in stereo-controlled processes’ is gratefully acknowledged.

#### Supplementary Data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2004.07.079.

#### References and notes

- (a) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743–1753 and references therein. (b) Easton, C. J.; Lincoln, S. F. *Chem. Soc. Rev.* **1996**, *25*, 163–170. (c) Szejtli, J.; Osa, T. *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Elsevier: Oxford, Vol. 3, 1996. (d) Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325–1357. (e) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803–822.
- Ukeama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045–2076.
- Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035–2044.
- (a) Li, S.; Purdy, W. C.; Schneiderman, E. *Chem. Rev.* **1992**, *92*, 1457–1470. (b) Stalcup, A. M. *J. Chromatogr. B* **2000**, *745*, 83–102.
- (a) Lipkowitz, K. B.; Coner, R.; Peterson, M. A.; Morreale, A.; Shakelford, J. *J. Org. Chem.* **1998**, *63*, 732–745, and references therein. (b) D’Anna, F.; Riela, R.; Lo Meo, P.; Gruttadauria, M.; Noto, R. *Tetrahedron: Asymmetry* **2002**, *13*, 1755–1760.
- Guernelli, S.; Laganà, M. F.; Spinelli, D.; Lo Meo, P.; Noto, R.; Riela, S. *J. Org. Chem.* **2002**, *67*, 2948.
- (a) Gadosy, T. A.; Boyd, M. J.; Tee, O. S. *J. Org. Chem.* **2000**, *65*, 6879–6889. (b) Tee, O. S.; Giorgi, J. B. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1013–1018.
- (a) Breslow, R.; Czarnik, A. W.; Lauer, .; Leppkes, R.; Winkler, J.; Zimmermann, S. *J. Am. Chem. Soc.* **1986**, *108*, 1969–1979. (b) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011. (c) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146–153.
- Takahashi, K. *Chem. Rev.* **1998**, *98*, 2013–2033.
- (a) Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn* **1979**, *52*, 2808–2814. (b) Rekarisky, M. V.; Mayhew, M. P.; Goldberg, R. N.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Phys. Chem. B* **1997**, *101*, 87–100. (c) Wedig, M.; Laug, S.; Christians, T.; Thunhorst, M.; Holzgrabe, U. *J. Pharm. Biomed. Anal.* **2000**, *27*, 531–540.
- Lo Meo, P.; D’Anna, F.; Riela, S.; Gruttadauria, M.; Noto, R. *Org. Biomol. Chem.* **2003**, *1*, 1584–1590, and references cited therein.
- Tabushi, I.; Kiyosuke, Y.; Sugimoto, T.; Yamamura, K. *J. Am. Chem. Soc.* **1978**, *100*, 916–919.
- Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917, and references cited therein.
- (a) Inoue, Y.; Hakushi, T. *J. Chem. Soc., Perkin Trans. 2* **1985**, 935–946. (b) Inoue, Y.; Liu, Y.; Tong, L.-H.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 10637–10644.
- (a) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475–481. (b) Liu, Y.; Han, B.-H.; Li, B.; Zhang, Y.-M.; Zhao, P.; Chen, Y.-T.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1998**, *63*, 1444–1454.
- (a) Rekharsky, M. V.; Inoue, Y. *J. Am. Chem. Soc.* **2000**, *122*, 4418–4435. (b) Rekharsky, M. V.; Inoue, Y. *J. Am. Chem. Soc.* **2001**, *123*, 813–826.
- Linert, W.; Han, L.-F.; Lukovits, I. *Chem. Phys.* **1989**, *139*, 441–455.
- Gelb, R. I.; Alper, J. S. *J. Phys. Org. Chem.* **1995**, *8*, 825–832.
- Yi, Z.-P.; Chen, H.-L.; Huang, Z.-Z.; Huang, Q.; Yu, J.-S. *J. Chem. Soc., Perkin Trans. 2* **2000**, 121–127.
- (a) Liu, L.; Guo, Q.-X. *Chem. Rev.* **2001**, *101*, 673–695 and references therein. (b) Grunwald, E.; Steel, C. *J. Am. Chem. Soc.* **1995**, *117*, 5687–5692. (c) Sharp, K. *Protein Sci.* **2001**, *10*, 661–667. (d) Ranatunga, R.; Vitha, M. F.; Carr, P. W.

- J. Chromatogr. A* **2002**, *946*, 47–49. (e) Vitha, M. F.; Carr, P. W. *Ind. Eng. Chem. Res.* **2003**, *42*, 6290–6293.
21. Alper, J. S.; Gelb, R. I. *J. Phys. Org. Chem.* **1993**, *6*, 273–280.
  22. (a) D'Anna, F.; Lo Meo, P.; Riela, S.; Gruttadauria, M.; Noto, R. *Tetrahedron* **2001**, *57*, 6823–6827. (b) Lo Meo, P.; D'Anna, F.; Riela, S.; Gruttadauria, M.; Noto, R. *Tetrahedron* **2002**, *58*, 6039–6045.
  23. Weber, G. *J. Phys. Chem.* **1995**, *99*, 1052–1059.
  24. *CRC Handbook of Chemistry and Physics*, 80th ed.; Lide, D. R., Ed.; CRC: Boca Raton, 1999, pp 8–42 and following.
  25. Data of Table 1 also allow us to rule out the occurrence of a zwitterionic form for aminoacid derivatives **16–19** at pH 2.5. As a matter of fact, the behaviour of a hypothetical zwitterionic form should be comparable to that of a *p*-nitro-anilinium ion, for which a  $pK_a$  value of 1 or lower should be expected.
  26. Lide, D. R., Ed.; *CRC Handbook of Chemistry and Physics* 80th ed.; CRC: Boca Raton, 1999, 8–42 and following.
  27. Liu, Y.; Zhang, Y.-M.; Qi, A.-D.; Chen, R.-T.; Yamamoto, K.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1997**, *62*, 1826–1830. (b) Rekarisky, M.; Yamamura, H.; Kawai, M.; Inoue, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5360–5361.
  28. The incipient formation of a 2:1 complex is easily deduced by the deviations, in the absorbance versus  $\alpha$ CD concentration plots, from the trend expected under the hypothesis of the existence of a 1:1 complex only.
  29. Kita, F.; Adam, W.; Jordan, P.; Nau, W. M.; Wirz, J. *J. Am. Chem. Soc.* **1999**, *121*, 9265–9275.
  30. More precisely, a 'compensation temperature' of 310 K is expected, see Ref. 21
  31. More often the compensation relationship is written as:

$$T\Delta S_i^\circ = T\Delta S_0^\circ + \beta\Delta H_i^\circ \quad (8)$$

with  $\beta = T/\Theta$ . By comparison of Eqs. 2 and 8 we easily obtain that  $\Delta H_0^\circ = -\Theta\Delta S_0^\circ$ . Inserting Eq. 8 in Eq. 3, two further relationships can be easily deduced:

$$R \ln K_{ij} = \Theta\Delta S_0^\circ/T_{ij} + (1 - \Theta/T_{ij})\Delta S_i^\circ \quad (9)$$

$$R \ln K_{ij} = (1/1 - \Theta/T_{ij})\Delta H_i^\circ + \Delta S_0^\circ \quad (10)$$

Eq. 9 is equivalent to the original Eq. 3, differently, Eq. 10 allows us to obtain directly  $\Delta H_i^\circ$  rather than  $\Delta S_i^\circ$  values. The actual use of either relationship may somewhat appear as a matter of taste. We tried to use both, obtaining the same results; however in our opinion Eq. 10 is the most convenient to handle.

32. Alper, J. S.; Gelb, R. I. *J. Phys. Chem.* **1990**, *94*, 4747–4751.
33. A similar effect could also be caused by partial protonation in the presence of an acidic buffer. This fact should be taken into account, when discussing the behaviour of aminoacid guests **16–19**.
34. It is strange to observe that in some occasions (see Ref. 20b) this thesis has been supported with experimental data for which there is the concrete danger of dealing with a pseudo-compensation effect. In fact, it should be considered that, under these circumstances, a real compensation would be indistinguishable from a false one.
35. Inoue, Y.; Yamamoto, K.; Wada, T.; Everitt, S.; Gao, X.-M.; Hou, Z.-J.; Tong, L.-H.; Jiang, S.-K.; Wu, H.-M. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1253–1257. (b) Liu, Y.; Li, B.; You, C.-C.; Wada, T.; Inoue, Y. *J. Org. Chem.* **2001**, *66*, 225–232.