We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

4,400 Open access books available
117,000 International authors and editors
130M Downloads

154 Countries delivered to
TOP 1% Our authors are among the most cited scientists
12.2% Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Encapsulation of Vitamin C into β-Cyclodextrin for Advanced and Regulatory Release

Subhadeep Saha and Mahendra Nath Roy

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70035

Abstract

Host-guest inclusion complex (IC) of vitamin C with β-cyclodextrin (β-CD) in aqueous medium has been explored by spectroscopic, physicochemical and calorimetric methods as stabilizer, carrier and regulatory releaser. Job plot has been drawn by UV-visible spectroscopy to confirm the 1:1 stoichiometry of the host-guest assembly. Stereo-chemical nature of the inclusion complex has been explained by two-dimensional (2D) NMR spectroscopy. Surface tension and conductivity studies further support the inclusion process. Association constants for the vitamin C-β-CD inclusion complex have been calculated by UV-visible spectroscopy using both Benesi-Hildebrand method and non-linear programme, while the thermodynamic parameters have been estimated with the help of van't Hoff equation. Isothermal titration calorimetric study has been performed to determine the stoichiometry, association constant and thermodynamic parameters with high accuracy.

Keywords: vitamin C, β-cyclodextrin, inclusion complex

1. Introduction

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide containing seven glucopyranose units, bound by α-(1→4) linkages forming a truncated conical structure [1, 2]. Thus because of its unique structure, i.e. fairly rigid and well-defined hydrophobic cavities and hydrophilic rims having primary and secondary —OH groups (Figure 1), it is of particular interest in the modern science [3, 4]. β-CD is used for controlled delivery of organic, inorganic, biological and pharmaceutical molecules due to their ability to form inclusion complexes with diverse guest molecules by encapsulating the non-polar part of the guest into its hydrophobic cavity and stabilizing the polar part by the polar rims [5, 6]. The use of β-CD already has a long history in pharmaceuticals, pesticides, foodstuffs, etc. for the solubility, bioavailability, safety, stability and as a carrier of the guest molecules [7, 8].
β-CD has been widely employed as not only excellent receptors for molecular recognition but also excellent building blocks to construct functional materials, where they could be applied to construct stimuli-responsive supramolecular materials [9]. Series of external stimuli, e.g. enzyme activation, light, temperature, changes in pH or redox and competitive binding may be employed to operate the release of guest molecules from the inclusion composites [10, 11]. Recently cyclodextrin-modified nanoparticles are of great interest as these supramolecular macrocycles significantly combine and enhance the characteristics of the entities, such as the electronic, conductance, thermal, fluorescence and catalytic properties expanding their potential applications as nanosensors, drug-delivery vehicles and recycling extraction agents [12]. Different sophisticated probes based on semiconductor nanocrystals and other nanoparticles have been designed for this purpose because of their potential applications in the fabrication of molecular switches, molecular machines, supramolecular polymers, chemosensors, transmembrane channels, molecule-based logic gates and other interesting host-guest systems [13–15].

Figure 1. Molecular structure of vitamin C and β-cyclodextrin.
In this article vitamin C (Figure 1), is an essential human nutrient with many important functions in biological systems. Scurvy, fatigue, depression and connective tissue defects are the common syndromes caused by deficiency of vitamin C [16, 17]. Thus to protect this important bio-molecule from external effects (e.g. oxidation, structural modification, etc.) and for its regulatory release, it is crucial to investigate whether this molecule can be encapsulated into the β-CD molecule and to explore the thermodynamic aspect of the process. In this present chapter, the formation of host-guest inclusion complex (IC) of the vitamin C with β-CD (the cavity dimension of which is more appropriate than other CDs to encapsulate a great variety of molecules) has been explored particularly towards its formation, stabilization, carrying and controlled release without chemical modification by different dependable methods like two-dimensional rotating-frame nuclear overhauser effect spectroscopy (2D ROESY) NMR, UV-Vis spectroscopy, surface tension (γ), conductivity and isothermal titration calorimetric studies, which primarily focuses on the encapsulation of the bio-molecule into the cavity of β-CD. The stoichiometry, association constants and thermodynamic parameters for the inclusion complex have been determined to communicate a quantitative data regarding the encapsulation of the vitamin by β-CD.

2. Result and discussion

2.1. Job plot reveals the stoichiometry of the host-guest inclusion complex

One of the best methods used to recognize the stoichiometry of the host-guest inclusion complexes is the Job’s method, known as the continuous variation method, which has been applied here by using UV-visible spectroscopy [18]. A set of solutions for the vitamin and β-CD was prepared varying the mole fraction of the guest in the range 0–1. Job plot was generated by plotting ΔA × R against R, where ΔA is the difference in absorbance of the vitamin without and with β-CD and R = [Vit]/([Vit] + [β-CD]) [19, 20]. Absorbance values were measured at 298.15 K.

![Figure 2](http://dx.doi.org/10.5772/intechopen.70035)

Figure 2. Job plot of vitamin C-β-CD system at 298.15 K. R = [Vit]/([Vit] + [β-CD]), ΔA = absorbance difference of vitamin C without and with β-CD.
$\lambda_{\text{max}}$ for each solution at 298.15 K. The value of $R$ at the maximum deviation gives the stoichiometry of the inclusion complex (IC), i.e. ratio of guest and host is 1:2 if $R = 0.33$; 1:1 if $R = 0.5$; 2:1 if $R = 0.66$, etc. In the present work maxima of the plot was found at $R = 0.5$, which suggest 1:1 stoichiometry of the host-guest inclusion complex (Figure 2).

2.2. 2D NMR spectra analysis

Two-dimensional (2D) NMR spectroscopy gives most powerful evidence about the spatial proximity between the host and the guest atoms by observations of the intermolecular dipolar cross-correlations [21, 22]. Any two protons that are located within 0.4 nm in space can

![Figure 3](image-url)  
(a) Stereo-chemical configuration of $\beta$-cyclodextrin, (b) truncated conical structure of $\beta$-cyclodextrin with interior and exterior protons.

Vitamin C
produce a nuclear overhauser effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or rotating-frame NOE spectroscopy (ROESY) [23, 24]. In the structure of β-CD the H3 and H5 protons are situated inside the conical cavity, particularly, the H3 are placed near the wider rim while H5 are placed near the narrower rim, the other H1, H2 and H4 protons are located at the exterior of the β-CD molecule (Figure 3) [25, 26]. Thus the inclusion phenomenon within the cyclodextrin cavity may be confirmed by the appearance of NOE cross-peaks between the H3 or H5 protons of the host and the protons of the guest identifying their spatial contacts [27, 28]. For this purpose, 2D ROESY has been obtained of the 1:1 molar mixture of vitamin C with β-CD. The ROESY spectra in D$_2$O shows significant correlations between the H-3, H-5 protons of β-CD and the CH$_2$, CHOD, CH protons of vitamin C (Figure 4). This result confirms the encapsulation of the vitamin molecule within the cavity.
of β-CD. Here in addition, the H6 protons of β-CD were not affected by the inclusion process, which tell that the guest vitamin molecule was included into the β-CD cavity via the wider rim (Figure 5) [29].

2.3. Surface tension study elucidates the inclusion as well as stoichiometric ratio of the host and guest

Surface tension (γ) study gives important clue about the formation and the stoichiometry of the host-guest IC [30-32]. Due to ionic interactions there was significant increase in γ of the aqueous solution of vitamin. β-CD, in contrast, because of having hydrophobic outer surface and hydrophilic rims, hardly show any change in γ while dissolved in aqueous medium for a wide range of concentration [32]. In the present study γ of aqueous vitamin has been measured with increasing concentration of β-CD at 298.15 K. The vitamin showed progressively falling trend of γ with increasing concentration of β-CD, may be due to encapsulation of the vitamin molecule from the surface of the solution into the hydrophobic cavity of β-CD forming host-guest inclusion complex (Figure 6) [33]. The plot also shows that there is a single discernible break in the curve, which not only points out the formation of IC but also indicates the 1:1 stoichiometric ratio for the IC formed (Figure 7) [34, 35]. The value of γ and corresponding concentrations of vitamin and β-CD at break have been listed in Table 1, which also indicate that at break point the concentration ratio of host and guest is about 1:1, establishing the formation of 1:1 IC between the studied vitamin and β-CD [8, 36].

Figure 5. Feasible and restricted inclusion of the guest into the host molecule.

Figure 6. Formation of inclusion complex of vitamin C with β-CD.
2.4. Conductivity study demonstrates inclusion process and its stoichiometric ratio

Conductivity ($\kappa$) measurement is an important tool to elucidate the inclusion phenomenon in solution phase [30, 32]. It indicates the formation as well as the stoichiometry of the IC formed [37, 38]. In this study, the conductivity of the solution decreases gradually as the vitamin molecules are encapsulated into the cavity of $\beta$-CD, i.e. the conductivity of the solution is markedly affected by the inclusion phenomenon. At a certain concentration of $\beta$-CD and the vitamin a single break was found in conductivity curve signifying the formation of 1:1 IC (Figure 8) [30]. The values of $\kappa$ and corresponding concentration of the vitamin and $\beta$-CD at the break point have been listed in Table 2, which reveal that the ratio of the concentrations of vitamin C and $\beta$-CD at the break point is approximately 1:1, suggesting that vitamin C-cyclodextrin inclusion complex is equimolar, i.e. the host-guest ratio is 1:1 (Figure 6).

2.5. Ultraviolet spectroscopy: association constants and thermodynamic parameters

Association constants ($K_a$) have been calculated for the vitamin-$\beta$-CD IC by UV-visible spectroscopy. As the vitamin molecules go from the polar aqueous environment to the apolar

![Figure 7. Variation of surface tension of aqueous vitamin C solution with increasing concentration of $\beta$-cyclodextrin at 298.15 K.](image)

<table>
<thead>
<tr>
<th>Concentration of $\beta$-CD (mM)</th>
<th>Concentration of vitamin (mM)</th>
<th>$\gamma^a$ (mN m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.94</td>
<td>5.06</td>
<td>72.98</td>
</tr>
</tbody>
</table>

* Standard uncertainties ($u$): temperature: $u(T) = \pm 0.01$ K, surface tension: $u(\gamma) = \pm 0.1$ mN m$^{-1}$.

Table 1. Values of surface tension ($\gamma$) at the break point with corresponding concentrations of $\beta$-CD and vitamin C at 298.15 K$^a$.

cavity of β-CD making the IC, there is a change in molar extinction coefficient ($\Delta \varepsilon$) of the chromophore of the vitamin [39]. The changes in absorbance ($\Delta A$) of vitamin C (261–265 nm) were studied against the concentration of β-CD at different temperatures to determine the association constants ($K_a$). On the basis of reliable Benesi-Hildebrand method for 1:1 host-guest complex the double reciprocal plots have been drawn using Eq. (1) [20, 40].

$$
\frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon[V]K_a} \times \frac{1}{[\beta-CD]} + \frac{1}{\Delta \varepsilon[V]}
$$

(1)

The values of $K_a$ for the system were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot (Table 3) [41, 42].

The thermodynamic parameters can easily be derived basing upon the association constants found at various temperatures by the above method with the help of van’t Hoff equation Eq. (2).

$$
\ln K_a = \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}
$$

(2)
There is a linear relationship between $\ln K_a$ and $1/T$ in the above equation, on the basis of which the thermodynamic parameters $\Delta H/C_{14}$ and $\Delta S/C_{14}$ for the formation of IC may be obtained [32, 37, 43].

Association constants ($K_a^\psi$) have also been calculated for the vitamin C-β-CD IC by UV-visible spectroscopy with the help of non-linear programme basing upon the changes in absorbance as a result of encapsulation of the vitamin molecule inside into the apolar cavity of β-CD [44]. The following equilibrium is supposed to exist between the host and the guest for 1:1 IC [1].

$$V_f + CD_f K_a^\psi \rightleftharpoons IC$$ (3)

The association constant ($K_a^\psi$) for the formation of IC may be expressed as

$$K_a^\psi = \frac{[IC]}{[V_f][CD_f]}$$ (4)

Here, [IC], [V], and [CD] represent the equilibrium concentration of IC, free vitamin molecule and free CD respectively. According to the binding isotherm, the association constant ($K_a^\psi$) for the formation of IC may be expressed as [45]

$$K_a^\psi = \frac{[IC]}{[V_f][CD_f]} = \frac{(A_{obs} - A_o)}{(A - A_{obs})[CD_f]}$$ (5)

where

$$[CD_f] = [CD]_{ad} - \frac{[V]_{ad}(A_{obs} - A_o)}{(A - A_o)}$$ (6)

Here, $A_o$, $A_{obs}$, and $A$ are the absorbance of vitamin molecules at initial state, during addition of CD and final state, respectively. $[V]_{ad}$ and [CD]$_{ad}$ are the concentration of vitamin molecule and the added CD, respectively. Thus, the values of $K_a^\psi$ for both the systems were evaluated from the binding isotherm by applying non-linear programme (Table 4) [7, 46]. The corresponding

<table>
<thead>
<tr>
<th>Temp ($K$)</th>
<th>$K_a \times 10^{-3}$ (M$^{-1}$)</th>
<th>$\Delta H$ (kJ mol$^{-1}$)</th>
<th>$\Delta S$ (J mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>288.15</td>
<td>4.19</td>
<td>$-21.67$</td>
<td>$-5.87$</td>
</tr>
<tr>
<td>293.15</td>
<td>3.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>298.15</td>
<td>3.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303.15</td>
<td>2.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>308.15</td>
<td>2.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>313.15</td>
<td>2.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard uncertainties in temperature $u$ are: $u(T) = \pm 0.01$ K.

* Mean errors in $K_a = \pm 0.02 \times 10^{-3}$ M$^{-1}$; $\Delta H = \pm 0.01$ kJ mol$^{-1}$; $\Delta S = \pm 0.01$ J mol$^{-1}$ K$^{-1}$.

Table 3. Association constant ($K_a$) and thermodynamic parameters $\Delta H^\psi$ and $\Delta S^\psi$ of vitamin C-β-cyclodextrin inclusion complex.
thermodynamic parameters have been derived basing upon the association constants found from various isotherms by the above method with the help of van’t Hoff equation (Eq. (2)) (Table 4) [37, 43].

The values of $\Delta H^\circ$ and $\Delta S^\circ$ for the formation of IC were found negative suggesting that the inclusion process is exothermic and entropy controlled but not entropy driven (Table 3) [37]. These results may be explained on the basis of molecular association that was taking place while the IC was being formed between $\beta$-CD and the vitamin. Because of this, there is a drop of entropy, which is unfavourable for the spontaneity of the IC formation. This effect is conquered by higher negative value of $\Delta H^\circ$, making the overall inclusion process thermodynamically favourable.

### Table 4. Association constants ($K_a^\psi$) obtained from non-linear programme and the corresponding thermodynamic parameters $\Delta H^\circ$ and $\Delta S^\circ$ of vitamin C-$\beta$-cyclodextrin inclusion complex.

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>$K_a^\psi \times 10^{-3}$ (M$^{-3}$)</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (J mol$^{-1}$ K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>288.15</td>
<td>4.21</td>
<td>$-21.83$</td>
<td>$-6.40$</td>
</tr>
<tr>
<td>293.15</td>
<td>3.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>298.15</td>
<td>3.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303.15</td>
<td>2.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>308.15</td>
<td>2.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>313.15</td>
<td>2.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard uncertainties in temperature $u$ are: $u(T) = \pm 0.01$ K.

* Mean errors in $K_a^\psi = \pm 0.01 \times 10^{-3}$ M$^{-3}$; $\Delta H^\circ = \pm 0.01$ kJ mol$^{-1}$; $\Delta S^\circ = \pm 0.01$ J mol$^{-1}$ K$^{-1}$.

2.6. Isothermal titration calorimetry: characterization of the complexation

Isothermal titration calorimetry (ITC) is the most sensitive and accurate analytical technique for determination of binding constant and various thermodynamic parameters in host-guest complexation with precise accuracy [47]. It has become an efficient method for direct determination of the thermodynamic parameters rather than using the earlier van’t Hoff equation technique [48]. Top of Figure 9 shows the data obtained from the ITC titration of vitamin C with $\beta$-CD in water at 298 K, which describes production of exothermic heat after each injection and the magnitude of the released heat decreases progressively with each injection until complete complexation is achieved. Bottom of Figure 9 shows the experimental data and the calculated best fit binding curve of vitamin C with $\beta$-CD, that provides the stoichiometry ($N^C$), association constant ($K_a^C$), standard enthalpy ($\Delta H^\circ^C$) and standard entropy ($\Delta S^\circ^C$) (Table 5). The outcomes of calorimetric study are consistent with those obtained from the analysis of the UV-visible spectroscopic data, however, these values are little different than those obtained by the earlier spectroscopic method studied at a range of temperature, which may be partly illustrated by the fact that the association constants of CD complexes decrease with increasing temperature, on the basis of which the thermodynamic parameters $\Delta H^\circ$ and $\Delta S^\circ$ were calculated using van’t Hoff method. But, in calorimetric study these parameters were determined only at 298 K, thus, the variation of the values of association constants is not
Figure 9. ITC isotherms for the interaction of vitamin C with β-cyclodextrin at 298 K. For each titration, β-cyclodextrin concentration in sample cell was taken as 50 μM and vitamin C concentration in syringe was 500 μM. The top panel represents the raw heats of binding obtained upon titration of vitamin C to β-cyclodextrin. The lower panel is the binding isotherm fitted to the raw data using one site model.
considered here. The other fact is that in spectroscopic determination, thermodynamic parameters were estimated from association constants, which again were found out on the basis of $\Delta \epsilon$ of the vitamin, that was due to the changes in the environment around the chromophore, when this goes from the polar aqueous environment to the apolar cavity of $\beta$-CD; hence, the changes in enthalpy and entropy described there were exclusively for the formation of IC, not for the other solvent interactions taking place in the medium. But, in calorimetric determination various types of non-covalent forces, like, electrostatic, hydrophobic, van der Waals and H-bonding are involved in the host-guest interaction, thus, thermodynamic parameters represent the overall heat changes resulting from the above interactions [10, 49]. Several mechanisms have been proposed for the complexation, where the most important forces involved are van der Waals and hydrophobic interactions [50]. The binding of vitamin C with $\beta$-CD is enthalpy driven as the entropy value of the interaction is not favourable. This indicates electrostatic and hydrophobic interactions play major role in the complexation in this case.

The stoichiometry (N) of the association further suggest that only 1:1 complexation has occurred in the formation of complex of vitamin C with $\beta$-CD which is in agreement with the 1:1 complexation revealed from the Job’s method.

Formation of the host-guest IC is the dimensional suitability between the two species, which is favoured by the unique cyclodextrin molecule that provides an appropriate condition by encapsulating the apolar part of the guest molecule inside the cavity, as well as stabilizing the polar part by the polar rims [36]. The other driving force for the formation of IC is the release of the water molecules from the hydrophobic cavity into the bulk thereby increasing the entropy of the system [1, 51]. The inclusion of the guest molecule is likely from the wider rim of the $\beta$-CD molecule to make maximum contact with the cavity (Figure 5), which is also supported by ROESY spectrum. The polar $–\text{OH}$ group of the vitamin can also make H-bonds with the $–\text{OH}$ groups at both the rims of the $\beta$-CD molecule, thereby stabilizing the IC.

3. Experimental

3.1. Materials

Vitamin C and $\beta$-cyclodextrin of puriss grade were bought from Sigma-Aldrich, Germany and used as purchased. The mass fraction purity of vitamin C and $\beta$-cyclodextrin was $\geq0.99$ and $\geq0.98$, respectively.
3.2. Apparatus and procedure

Prior to the start of the experimental work, solubility of β-cyclodextrin and the vitamin has been precisely checked in triply distilled and degassed water (with a specific conductance of $1 \times 10^{-6}$ S cm$^{-1}$) and observed that the selected vitamin was freely soluble in all proportion of aqueous β-cyclodextrin. All the stock solutions of the vitamin were prepared by mass (weighed by Mettler Toledo AG-285 with uncertainty 0.0003 g), and then the working solutions were obtained by mass dilution at 298.15 K. Adequate precautions were made to reduce evaporation loss during mixing.

UV-visible spectra were recorded by JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The measuring temperature was held constant by an automated digital thermostat.

Two-dimensional (2D) ROESY spectra were recorded in D$_2$O at 300 MHz using Bruker Avance 300 MHz instrument at 298 K.

The surface tension experiments were done by platinum ring detachment method using a Tensiometer (K9, KRÜSS; Germany) at the experimental temperature. The accuracy of the measurement was within ±0.1 mN m$^{-1}$. Temperature of the system has been maintained by circulating auto-thermostat water through a double-wall glass vessel containing the solution.

Specific conductance values of the experimental solutions were measured by Mettler Toledo Seven Multi conductivity meter with uncertainty of ±1.0 µS m$^{-1}$. The measurements were made in an auto-thermostated water bath maintaining the temperature at 298.15 K and using the HPLC grade water with specific conductance of 6.0 µS m$^{-1}$. The cell was calibrated using a 0.01 M aqueous KCl solution. The uncertainty in temperature was ±0.01 K.

Isothermal titration calorimetry was used to obtain association constant at 298 K using a MicroCal VP-ITC (MicroCal, Inc., Northampton, MA, USA). The thermal equilibration step at 298 K was followed by an initial 120 s delay step and the subsequent 25 injections of each vitamin to β-CD (injection duration of 10 s and spacing of 180 s). Each injection generated a heat-burst curve between micro cal s$^{-1}$ versus time (min). The saturation curve between kcal/mol of injectant versus molar ratio was determined by integration, using Origin 7.0 software (Microcal, Inc.) to give the measure of the heat associated with the injection. The binding affinity and thermodynamic parameters of the binding process were obtained by fitting the integrated heats of binding the isotherm to the one site binding model to give the association constant ($K_a$), stoichiometry ($N^E$), binding enthalpy ($\Delta H^E$) and the entropy ($\Delta S^E$).

4. Conclusion

The present study explains that vitamin C forms IC with β-CD in aqueous medium, which can be used as regulatory releaser of the vitamin. Two-dimensional (2D) ROESY NMR study confirms the inclusion phenomenon and its mechanism. Surface tension and conductivity studies also show that the ICs have been formed, the stoichiometry of which were confirmed as 1:1 by Job
plots. The association constants and thermodynamic parameters have been estimated for both the ICs by reliable spectroscopic and calorimetric techniques with high accuracy. Thus, this work communicates both qualitative and quantitative idea about the formation of IC of β-CD with vitamin C suggesting its potential applications in pharmaceutical industries and medical sciences.

Author details

Subhadeep Saha and Mahendra Nath Roy*

*Address all correspondence to: mahendraroy2002@yahoo.co.in

Department of Chemistry, University of North Bengal, India

References


[45] Spies MA, Schowen RL. The trapping of a spontaneously “flipped-out” base from double helical nucleic acids by host-guest complexation with α-cyclodextrin: The intrinsic base-flipping rate constant for DNA and RNA. Journal of the American Chemical Society. 2002;124:14049-14053


