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Synthesis and Structural Characterisation of Two Novel Diastereoisomeric Naproxen Appended β-Cyclodextrin Derivatives

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Abstract

The condensation reaction of 6-deoxy-6-amino(2-aminoethyl)-cyclomaltoheptaose (β-CyDen) and racemic [(*R*,*S*)-2-(6-methoxy-2-naphthyl) propanoic acid] (*R*,*S*-naproxen) affords two new diastereoisomeric naproxen-appended β-cyclodextrin derivatives. The structural analysis of these two β-CyD derivatives, undertaken by combined use of circular dichroism (CD) and 1D or 2D NMR techniques, shows that the naphthalene ring of the naproxen is included in the CyD cavity in both derivatives. The CD spectra are consistent with an axial complexation model, moreover, the self-inclusion mode is further corroborated by ^{1}H NMR ROESY spectra which also suggest the orientation of the naphthyl moiety in the cavity of both the diastereoisomers.

Introduction

α, β, and γ cyclodextrins (α-, β-, and γ-CyDs) are cyclic oligosaccharides consisting of six, seven or eight D-glucopyranose residues, respectively, linked by α-1,4 glycosidic bonds. Due to their macrocyclic structure, CyDs possess a hydrophobic cavity which allows them to form inclusion complexes in aqueous solution with suitable guest molecules [1, 2]. CyDs have been successfully used in a wide variety of applications including the development of sensing molecules [3], the catalysis of regio- and stereo-selective chemical reactions [4], enzyme models [5], chiral recognition [6] and the transport of pharmacologically active compounds [7].

The potential use of CyDs for drug protection and delivery has led to a large number of papers dealing with the interaction of CyDs with many pharmaceutically important compounds including 2-arylpropionic acids (APAs), which represent a large class of non-steroidal anti-inflammatory drugs [7, 8]. Naproxen [2-(6-methoxy-2-naphthyl)propanoic acid], ketoprofen [2-(3-benzoylphenyl)propanoic acid] and ibuprofen [2-(4-isobutylphenyl)propanoic acid] are well-known examples of widely used APAs.

APAs have been shown to be ideal complex-forming partners for cyclodextrins. Indeed, their polarity, molecular mass, and structure make them good candidates for the inclusion into the CyD cavity. In this regard, it is worth mentioning that inclusion of these drugs in CyD reduce the ulcerogenic activity observed after oral administration [9]. On the other hand, linking covalently APAs or other non-steroidal anti-inflammatory compounds to a β-CyD moiety affords products displaying physico- and bio-pharmaceutical properties which may differ greatly from those of the inclusion complex [7]. Moreover, the easy biodegradation of CyDs, render these CyD conjugates particularly useful as a source of site-specific delivery of drugs to the colon intestine [10–16]. This aspect could be of particular interest in view of the chemopreventive action in colon carcinogenesis explicated by several non-steroidal anti-inflammatory drugs [17, 18].

In this context, two new β-CyD derivatives, obtained by linking the two enantiomers of naproxen with the 6-deoxy-6-amino(2-aminoethyl)-cyclomaltoheptaose (β-CyDen), have been synthesized [19]. The conjugation of the racemic naproxen with the β-CyDen allows these new derivatives to be separated and characterised as diastereoisomeric compounds.

This paper reports the synthesis and the spectroscopic (NMR and CD) characterisation of two new diastereoisomers, i.e. 6-deoxy-6-{[(2*S*) 2-(6-methoxy-2-naphthyl) propanamido]ethylamino} cyclomaltoheptaose **(1S)** and 6-deoxy-6-{[(2*R*) 2-(6-methoxy-2-naphthyl) propanamido]ethylamino} cyclomaltoheptaose **(1R).**

The present study demonstrates that in both **1S** and **1R**, the naphthalene ring of the appended-naproxen moiety is deeply inserted into the β-CyD cavity.

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Experimental

Materials

6-deoxy-6-(2-aminoethylamino)-β-cyclodextrin (β-CyDen) was prepared from the parent β-cyclodextrin as described elsewhere [19]. (*S*)-(+)-naproxen or (*S*)-2-(6-methoxy-2-naphthyl)propionic acid was purchased from Sigma-Aldrich. The *S*-isomer of naproxen was racemised by following the procedure described by Tsai *et al.* [20] Complete racemisation was checked by optical rotation measurements. Synthesis grade peptide *N*,*N* dimethylformamide (DMF) (Applied biosystem) was used for the synthesis of the derivatives. 2-(l-*H*-Benzotriazole-l-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and *N*-hydroxybenzotriazole (HOBT) were purchased from Novabiochem.

*Synthesis of **1S** and **1R***

The diastereoisomers were synthesised according to Scheme 1: *(S,R)* (±) naproxen (41 mg, 0.18 mmol), HOBT (24 mg, 0.18 mmol), TBTU (57 mg, 0.18 mmol) were added to a solution of β-CyDen (191 mg, 0.16 mmol), dissolved in anhydrous DMF (3.6 ml). The reaction mixture was stirred vigorously at room temperature for one hour under a nitrogen stream. Progress of the reaction was followed by TLC (eluent: *n*PrOH/H_2O/EtAc/NH_3 5:4:4:1); two products with $R_f = 0.50$ and $R_f = 0.60$ were obtained. The DMF was evaporated to dryness under vacuum; the residue was dissolved in methanol and precipitated with ethyl ether. The precipitate, dissolved in a minimum amount of water, was loaded into a CM Sephadex C-25 column (3 × 60 cm, NH_4^+ form) and eluted initially with water (400 ml) and then with a linear gradient of aqueous NH_4HCO_3 (0–0.3 M, 3000 ml). The products were spotted by TLC using an anysaldehyde reagent [21]. The first eluted fractions, containing the product with R_f = 0.50, were combined and concentrated to dryness under vacuum at 40 °C. The solid residue was dissolved in a minimum amount of water and poured into acetone. The resulting precipitate was filtered out and dried under reduced pressure. The purity of the product was tested by HPLC using a 4 × 125 mm Lichrospher 100 RP-18 chromatographic column (particle size: 5 μm; pores: 100 Å; eluent: 88%H_2O/12%CH_3CN both containing 0.1% trifluoroacetic acid; flux: 1 ml/min; λ = 254 nm; retention time: 8.0 min). The product obtained (100 mg, 0.07 mmol, 86% yield) as a white solid had the following physical constants: m.p. 242–244 °C (dec.). FAB-MS: *m*/*z* 1389 $[M + H]^+$. $[\alpha]^{25}_D$ + 136.7 (*c* 0.1 in H_2O). Elementary analysis for $C_{58}H_{88}O_{36}N_2 \cdot 5H_2O$. Calculated: C 47.07, H 6.68, N 1.89; found: C 47.00, H 6.73, N 1.94.

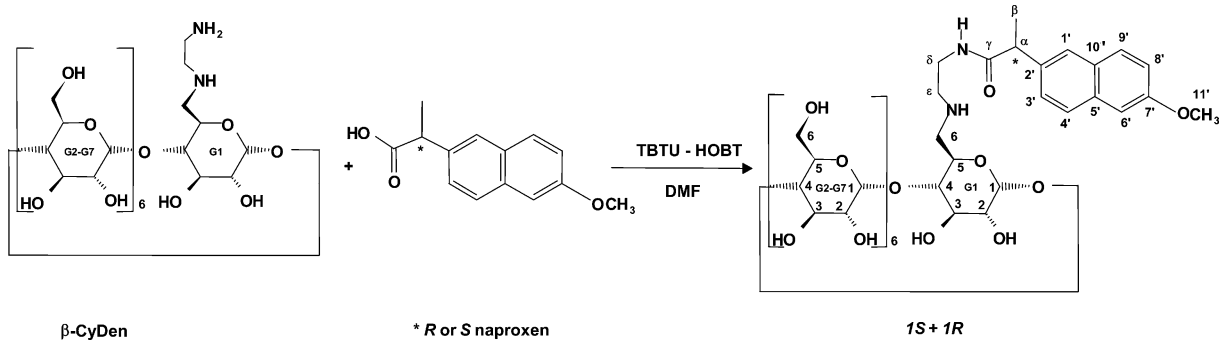
The fractions containing the product with $R_f = 0.60$, eluted later from the CM Sephadex C-25, were combined and processed as above described for the derivative with $R_f = 0.50$. The HPLC analysis of the $R_f = 0.60$ compound gave a single peak with a retention time of 5.4 min by using the same gradient as above. The product thus obtained (107 mg, 0.07 mmol, 87% yield) as a white solid had the following physical constants: m.p. 248–250 °C (dec.). FAB-MS: *m*/*z* 1389 $[M + H]^+$. $[\alpha]^{25}_D$ + 159.5 (*c* 0.1 in H_2O). Elementary analysis for $C_{58}H_{88}O_{36}N_2 \cdot 5H_2O$. Calculated: C 47.07, H 6.68, N 1.89; found: C 46.95, H 6.75, N 1.93.

*Synthesis of **1S***

In order to assign the stereochemistry of the diastereoisomers, the synthesis was repeated by starting off from the optically pure (*S*)-(+)-naproxen and the β-CyDen and following the same procedure described above. This afforded a compound showing identical optical, chromatographical and mass weight constants as the product with $R_f = 0.60$.

Chromatography

Product separation was achieved by ion exchange chromatography performed on a CM-Sephadex C-25 (Dry bead size: 40–120 μm; Pharmacia) of 3 × 60 cm, in the NH_4^+ form column. The product purity was tested by HPLC with a HP 1050 series instrument on a Lichrospher 100 RP-18 column (particle size 5 μm; pores: 100 Å; 4 × 125 mm). TLC was performed by using glass plates pre-coated with 0.2 mm silica gel 60 F_{254} (Merck).



Scheme 1.

Optical rotation and pH measurements

The optical rotation was recorded on a Jasco DIP-370 digital polarimeter and is expressed in units of 10^{-1} deg cm^2 g^{-1}. pH (pD) values were measured with an Orion Model SA 520 pH meter equipped with a MI410 combined pH electrode (Microelectrodes, Inc., USA). The potentiometric system was routinely calibrated by using standard buffer solutions of the appropriate pH value. The potentiometric measurements, used to have an estimate of p*K* values, were carried out by means of a home assembled fully automated apparatus that makes use of Metrohm equipment (meter, E 654; combined glass electrode, EA 125; burette, E 665). E^o and slope of the electrode system were determined by titrating 2.5 ml of HNO_3 with carbon dioxide-free NaOH. p*K* values were estimated by titrating 2.5 ml of a solution containing the ligand (3.5×10^{-3} M) and HNO_3 (4.0×10^{-3} M) with NaOH (0.1 M) The titration cell was thermostated at 25 ± 0.1°C. Solutions to be titrated were kept under nitrogen and were magnetically stirred. The potentiometric data were processed by using HYPERQUAD that measures the error square sum based on electrode potential [22].

NMR spectroscopy

High field NMR experiments were carried out on 5.0 mM sample solutions at 30 °C on a Varian Unity-plus spectrometer operating at 499.87 MHz. 1D ^{1}H NMR spectra were collected using 16 K data points over a spectral width of 4400 Hz. Homonuclear 2D experiments (DQF-COSY [23], TOCSY [24] and T-ROESY [25]) were obtained in the phase sensitive mode according to Ruben–States–Haberkorn [26] procedure. 2D experiments were typically acquired with 2 K data points in the t_2 dimension and 512 t_1 increments. T-ROESY spectra were acquired using two different mixing times (150 ms and 300 ms). TOCSY spectra were recorded using a MLEV-17 [24] sequence with 7.0 kHz spin locking field and 80 or 100 ms mixing times, whereas lD TOCSY [27] spectra were performed using a 80 ms Gaussian soft pulse and an array of mixing times ranging from 5 ms to 105 ms. Inverse detection proton-carbon correlation experiments, HSQC [28] and HMBC [29] were carried out with GARP-1 [30] modulated decoupling, acquiring 2 K data points for 256 (HSQC) or 128 (HMBC) increments of t_1 (zero filled to 512 points) with 16 (HSQC) or 140 (HMBC) transients for each increment. All spectra were acquired in D_2O, the HDO signal was suppressed by selective irradiation during the repetition delays. The experiments were performed at pH = 7.5 (pH-meter reading uncorrected for the deuterium effect). The pH of the solutions was adjusted by adding the appropriate amount of DCl. All the experiments were processed with the software supplied by the manufacturers.

Molecular models were generated using the HYPERCHEM software package (HYPERCHEM, Hypercube release 5.01 license no. 500-10002406, Hypercube Inc., Waterloo, Ontario, 1996) and geometry optimisation was accomplished by using the MM^+ force field.

Circular dichroism (CD) spectroscopy

CD spectra were obtained at 25 °C under a constant flow of nitrogen on a Jasco model J-810 spectropolarimeter which had been calibrated with an aqueous solution of ammonium *d*-camphorsulphate [31]. The measurements were carried out in water at pH = 7.4 using 1 mm or 5 mm cuvettes. The pH of the solutions was adjusted by adding the appropriate amount of H_2SO_4. All experiments were performed in the UV region (190–400 nm). The spectra represent the average of 2–10 scans: CD intensities are expressed in $\Delta\varepsilon$ (M^{-1} cm^{-1}).

Result and discussion

*Structure determination of **1S** and **1R***

As indicated in the experimental section the FAB-MS spectra of both the compounds gave identical molecular peaks at *m/z* corresponding to the correct molecular weight. p*K* values, determined by running two independent titrations for each ligand, turned out to be 6.8 ± 0.1 and 6.6 ± 0.1 for **1S** and **1R**, respectively.

The structure of the two products was confirmed through the combined analysis of homonuclear (DQF-COSY, TOCSY, T-ROESY) as well as heteronuclear (HSQC, HMBC) 2D NMR techniques. Furthermore a selective excitation experiment lD TOCSY was also employed to aid the specific assignment of each glucopyranosyl unit. All the NMR experiments were run by adjusting the pH-meter reading to 7.5. This value actually corresponds to a pH value of 7.1 if measured in H_2O according to the well known equation p*D* = pH + 0.4 [32]. At this pH value, the signals result from both the protonated and unprotonated species. However, the percentage of protonated and unprotonated form does not differ significantly for both **1S**, and **1R**. The choice of this pH was dictated by: (i) the need to work in the physiological pH range; (ii) the need to work at a pH value as close as possible to the one employed to run the CD experiments; (iii) the broadening of the NMR signal with pH increases.

The 500 MHz ^{1}H NMR spectra of **1S** and **1R** in deuterium oxide are reported in Figures la and b.

Due to the unsymmetrical modification, the CyD moiety shows a complex NMR profile caused by a multitude of magnetically unequivalent protons overlapping within a few ppm. In our previous work on unsymmetrically modified β-CyD, a NMR study was carried out through an integrated use of high field homonuclear and heteronuclear 2D NMR experiments [33, 34]. This allowed a detailed structural and conformational picture of the studied derivatives to be

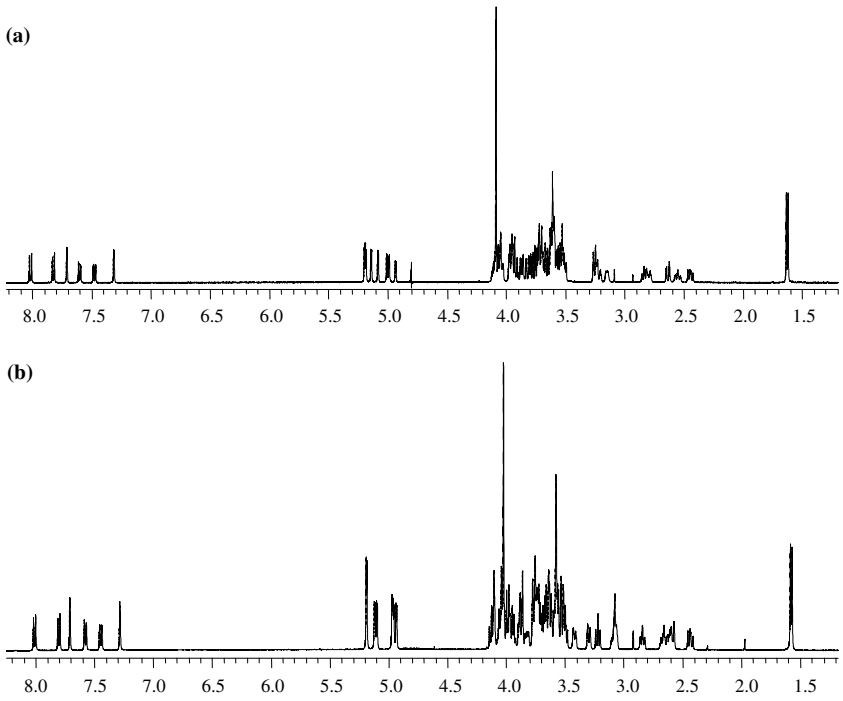


Figure 1. 500 MHz ^{1}H NMR spectra of **1S** [5.0×10^{-3} M] (a) and **IR** [5.0×10^{-3} M] (b) in D_2O at pH 7.5.

obtained. Thus, a similar approach was employed in the present study. In the first step, one-dimensional ^{1}H and ^{13}C (proton decoupled) NMR spectra were obtained for each compound. Subsequently, a heteronuclear 2D HSQC experiment was used to correlate ^{1}H NMR signals with those of protonated carbons. A careful analysis of the two-dimensional DQF-COSY and TOCSY spectra aided in the assignments of proton signals; long-range proton-carbon connectivities were analysed through the heteronuclear 2D HMBC experiments to complete the assignments of carbon signals.

The assignment of signals due to the APA moiety was a relatively easy task, and was accomplished as first, starting with the diastereomer ***1S***, bearing the S-naproxen pendant. On the basis of previously reported NMR data on naproxen [35], assignments, reported in Table 1 for ^{1}**H** aromatic signals ($\Delta\delta$ 7.2–8.2) and 7′-OMe (δ 4.08) in IS, were straightforward and clearly corroborated by DQF-COSY and TOCSY data. A similar analysis of ^{13}C NMR spectrum, supported also by HSQC and HMBC data, allowed ^{13}C signals of the naproxen moiety to be assigned (Table 1). Assignment of ^{1}H and ^{13}C NMR signals due to the β-CyDen portion (namely at α, β, δ and ε positions) was somewhat less obvious, because of their overlapping with the entanglement of glucopyranose signals, and was accomplished thanks to the 2D NMR spectra. A detailed and careful analysis of the above cited two-dimensional NMR experiments, together with 1D TOCSY selective excitation experiments, allowed not only the assignment of ^{1}H signals due to the functionalised glupyranose G1 unit, but also a complete assignment of the remaining six glucopyranose G2–G7 units (Table 2). The sharp separation of all anomeric signals indicates the reduction of the sevenfold symmetry of the modified β-cyclodextrin macrocycle and suggests a strong interaction of the aromatic naphthalene nucleus with the β-CyD cavity [3a, 36, 37]. This peculiarity offered a convenient starting point for this step of the analysis: in particular, through the 1D TOCSY experiment, the spin system from H(1) to H(6) was analysed following the correlations through the glucopyranose ring, thus circumventing the difficulty of extensive overlapping in the region between δ 3.5 and δ 4.2. For example, the selective excitation of the signal at δ

Table 1. ^{1}H and ^{13}C NMR assignments for the pendant naproxen moiety in **1S** and **1R** [values in brackets][a]

Position	δ_H	δ_C
α	3.92 [3.96]	49.1 [48.4]
β	1.61 [1.58]	19.2 [19.2]
γ		179.9 [179.4]
δ	3.19, 3.59 [3.05, 3.60]	41.7 [41.2]
ε	2.52, 2.78 [2.67, 2.62]	51.4 [49.6]
1′	7.69 [7.71]	126.8 [126.8]
2′		139.9 [140.0]
3′	7.58 [7.59]	128.9 [127.8]
4′	7.81[7.80]	129.7 [129.2]
5′		135.9 [135.5]
6′	7.28 [7.28]	108.7 [108.4]
7′		160.4 [160.0]
8′	7.44 [7.44]	121.3 [120.9]
9′	8.01 [8.00]	131.8 [131.4]
10′		131.3 [130.9]
11′	4.08 [4.02]	58.8 [58.5]

[a]Run at 500 MHz in D_2O. Chemical shifts are in ppm and refer to the residual water peak assigned at 4.8 ppm. Scheme 1 shows the numbering system used for the assignments. Values obtained from DQF-COSY and HSQC.

Table 2. [1]H NMR data for **1S** and **1R** [values in brackets][a]

Position	H(1)	H(2)	H(3)	H(4)	H(5)	H(6)
G1	4.91 [4.93]	3.54[3.56]	3.50[3.48]	3.22[3.22]	2.81[2.84]	2.42,2.62 [2.44,2.58]
G2	4.99 [4.95]	3.65[3.64]	3.92[3.87]	3.66[3.66]	3.53[3.42]	3.84,3.84 [3.76,3.86]
G3	5.18 [5.18]	3.76[3.75]	4.07[4.12]	3.70[3.75]	4.06[4.04]	3.94,4.02 [3.96,4.04]
G4	5.06 [5.10]	3.61[3.62]	3.63[3.68]	3.50[3.52]	3.24[3.29]	3.52,3.52 [3.52,3.57]
G5	4.97 [4.97]	3.55[3.57]	3.59[3.56]	3.58[3.56]	3.16[3.07]	3.62,3.70 [3.62,3.72]
G6	5.12 [5.12]	3.72[3.72]	4.06[4.03]	3.75[3.71]	3.94[4.07]	4.03,4.09 [3.98,4.09]
G7	5.16 [5.18]	3.70[3.70]	3.93[3.98]	3.52[3.50]	3.73[3.82]	3.59,3.78 [3.64,3.64]

[a]Conditions as specified in Table 1.

4.91 H(1)–G1, with gradual increase of the mixing time, allowed the sequential correlations with H(2)–G1 (δ 3.54), H(3)–G1 (δ 3.50), H(4)–G1 (δ 3.22), H(5)–G1 (δ 2.81), H(6)-G1 (δ 2.42 and δ 2.62) (see Figure 2). These assignments were corroborated by 2D NMR data: in particular, HSQC showed a single carbon signal at δ 52.1, C(6)–G1, which is typical of a carbon linked to a nitrogen atom [33, 34, 38], correlating with both distinct ^{1}H signals of the diastereotopicH(6)–G1 protons; DQF-COSY clearly confirmed the geminal coupling of these latter signals as well as their vicinal correlation with H(5)–G1. This allowed the unambiguous identification of the modified glucopyranosyl unit G1. All the other signals of the cyclodextrin moiety of **1S** were assigned in the same manner (Table 2). The partial overlapping of H(1)–G3 (δ 5.18) with H(1)–G7 (δ 5.16) complicated the interpretation of 1D TOCSY experiments, however unambiguous assignments within glucopyranosyl units G3 and G7 was made possible by the dispersion of ^{13}C signals.

At this point, the assignments within each glucopyranose units were established, but the correct sequence from G1 to G7, that is, the sequence by which the glucose units are connected, still had to be defined. This was accomplished through the analysis of two-dimensional T-ROESY experiments, which showed clear cross-correlations between H(1) and H(4) of contiguous glucopyranosyl units (not reported).

A similar methodology was applied to assign signals of the ^{1}H NMR spectrum of the diastereoisomer, bearing R-naproxen, **1R**. This was facilitated by the complete analysis of the ^{1}H NMR of isomer **1S**,

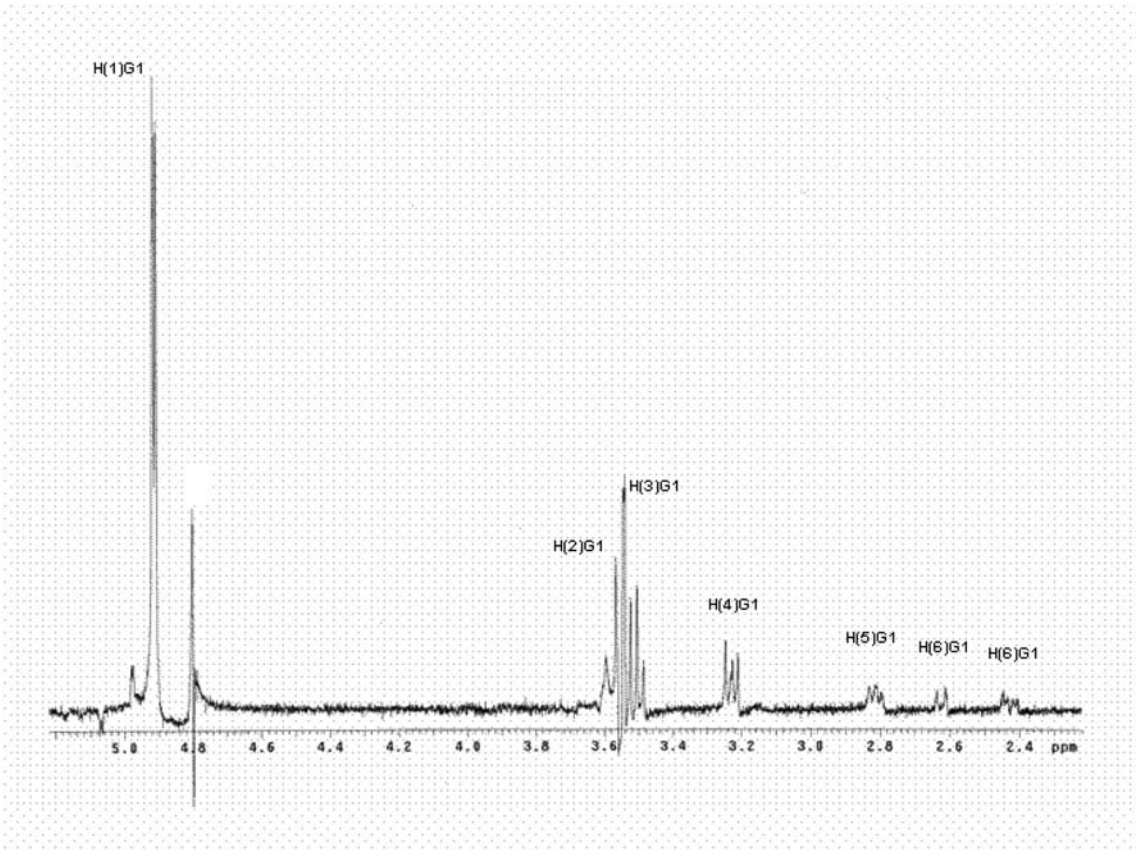


Figure 2. 500 MHz ID TOCSY spectrum (mixing time 100 ms) of **1S** [5.0×10^{-3} M] at pH 7.5. Selective excitation at δ 4.91.

although in the presence of some specific difficulties as for example the complete overlapping of H(1)–G3 with H(1)–G7 (δ 5.18) as well as partial overlapping of H(1)–G2 (δ 4.95) with H(1)–G5 (δ 4.97).

*Solution conformation of **1S** and **1R***

In order to establish whether the naproxen moiety is self-included in the β-CD cavity or not, the preferred conformation of **1S** and **1R** in aqueous solution was first investigated by means of CD spectroscopy.

The CD spectra of **1S** and **1R** at pH = 7.4 are reported in Figure 3. This pH value was a good compromise between the need to maximize the CD signal and the necessity to work at pH values as close as possible to those employed for the NMR experiments. It has to be stressed that the CD spectrum levels off at pH > 7.2; CD spectra obtained at pH 8 are virtually superimposable with those obtained at pH 7.4. Naphthalene bands can be clearly identified in these spectra. In particular the CD positive band at 234 nm and the negative signals centred around 278 nm and 323 nm, can be assigned to the ^1B_b, ^1L_a and ^1L_b transitions respectively [39]. The n–π^* transition of the amide chromophore can be found in the wavelength range of 200–225 nm; therefore the shorter wavelength positive CD band at 223 nm for **1S** and at 208 nm for **1R** might originate from the interaction of the amide n–π^* dichroich transition with the chiral environment determined by the β-CyD cavity.

Guest molecules included into the chiral cyclodextrin cavity have been reported to exhibit an induced circular dichroism (ICD) in their absorption regions [40]. For simple chromophores, such as substituted benzene and naphthalene rings, the sign of the observed ICD has been correlated, on a theoretical basis, with the orientation of the dipole transition moment of a given absorption band relative to the β-cyclodextrin seven fold axis [40].

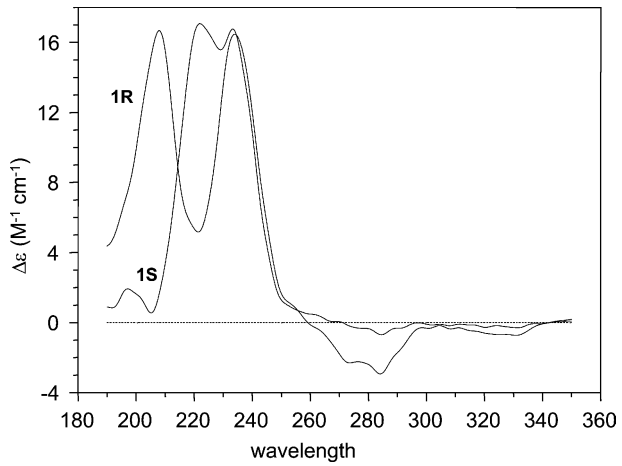


Figure 3. CD spectra of *1S* [2.05×10^{-4} M] and *1R* [2.0×10^{-4} M] in aqueous solution at pH 7.4.

The naproxen moiety can be considered as a 2-substituted naphthalene and in the case of axial complexation (i.e. with the long axis of naphthalene parallel to the β-CyD sevenfold axis) both the ^1L_a and ^1L_b transitions are expected to give negative CD curves, while the ^1B_b transition to have a positive sign [41]. The CD curves shown in Figure 3 are consistent with the axial inclusion mode of the naphthalene.

CD measurements recorded in the presence of a competitive guest (l-adamatanecarboxylic acid, ACA) revealed that the positive ellipticities decrease as ACA concentration increases (Figure 4). ACA-induced reduction of the CD signal intensities suggests that in **1S** and **1R** the pendant naphthyl moiety moves out of β-CyD cavity following guest (ACA) binding.

Furthermore, variable concentration CD experiments were also carried out at 25 °C and at pH 7.4 for both compounds. The intensity of the CD signals was not affected in the 8–200 μM concentration range, thus indicating the intramolecular nature of both the inclusion complexes (data not reported).

All the above presented CD results strongly suggest the inclusion of the naphthalene pendant within the β-CyD cavity.

Such a conclusion is supported by the NMR results. In particular, the T-ROESY spectra showed a series of NOE correlations between the protons of the aromatic

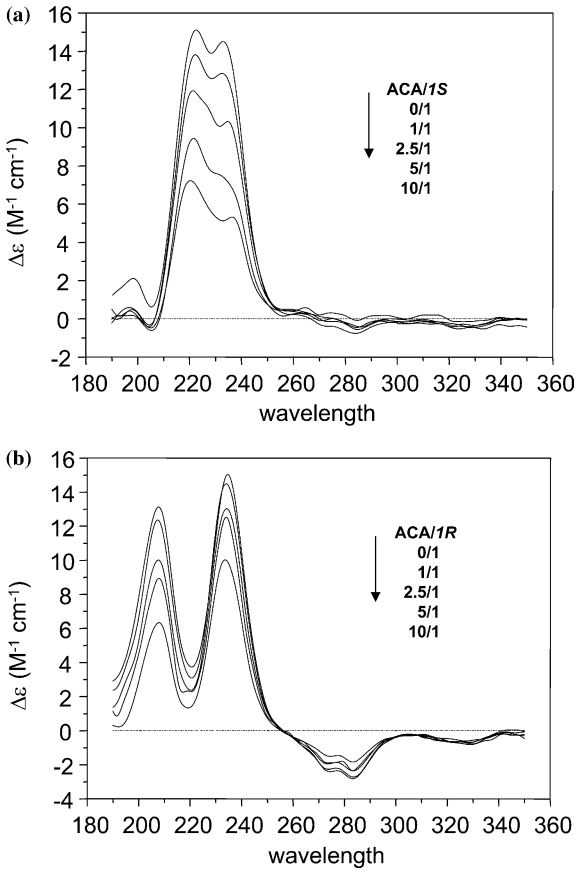


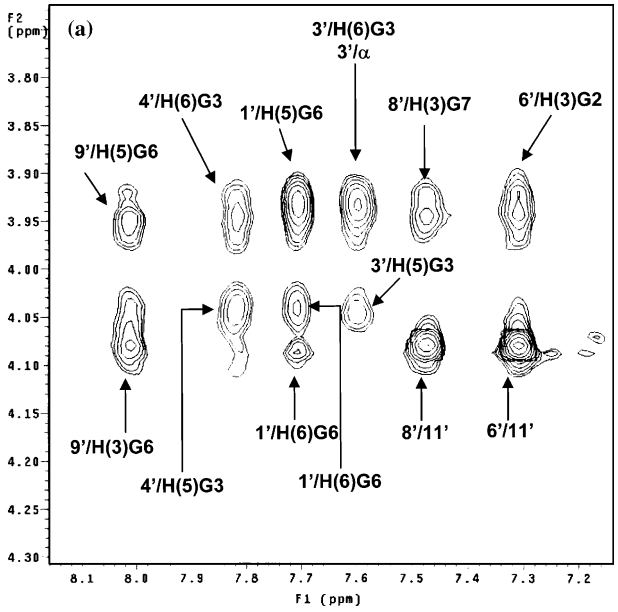
Figure 4. CD spectra of *1S* [2.05×10^{-4} M] (a) and *1R* [2.0×10^{-4} M] (b) in aqueous solution at pH 7.4 at different *ACA*/*IS* and *ACA*/*IR* ratios (values indicated in the Figures).

naphthalene condensed rings and several protons of the cyclodextrin moiety (Figure 5). The analysis of the observed NOE connectivities, revealed the close proximity of the naphthalene protons and the H(3) and H(5) protons of the G3 and G6 glucopiranosyl units (see Figure 5). Furthermore the presence of NOE correlations between aromatic protons 1′, 3′, and 4′, and some protons in position 6 of the β-CyD is in agreement with the self-inclusion of the aromatic pendant into the β-CyD cavity.

The comparison of the proton chemical shift values reported in Table 2 for each diastereoisomer, allows some considerations about the effect of the inclusion of the naphthalene ring into the β-CyD cavity. In both compounds a considerable dispersion of the chemical shift values of H(3) ($\Delta\delta = 0.57$ for **1S**; $\Delta\delta = 0.64$ for **1R**), H(5) ($\Delta\delta = 1.25$ for **1S**; $\Delta\delta = 1.23$ for **1R**) and the diastereotipic protons H(6) ($\Delta\delta = 0.48$; 0.57 for **1S**; $\Delta\delta = 0.46$; 0.52 for **1R**) is detected. By contrast H(2) ($\Delta\delta = 0.22$ for **1S**; $\Delta\delta = 0.19$ for **1R** and H(4) ($\Delta\delta = 0.25$ for **1S** and **1R**; with the exception of the H(4)–G1 protons of both compounds which resonate at $\delta = 3.22$) protons, resonate in a narrower spectral region, thus appearing less affected. In particular, the internal H(3) and H(5) protons of the G1, G4, and G5 units, generally have low chemical shift while those of the G3 and G6 units show higher chemical shift values.

This phenomenon, that is clearly related to the shielding and deshielding effects of the aromatic nucleus [3a, 34, 41], together with the assigned NOE correlations, allowed the disposition of the aromatic moiety within the cyclodextrin cavity to be estimated.

Figure 6 proposes the possible aqueous solution structures of **1S** and **1R**, based on NMR results. It has to be underlined that these structures have been obtained by introducing the interproton distance constraints determined through the ROESY data, into the minimization procedure. Finally, it should be mentioned that the deduced conformations represent the mean of the protonated and unprotonated forms existing in slightly different proportion for the two diastereoisomers.



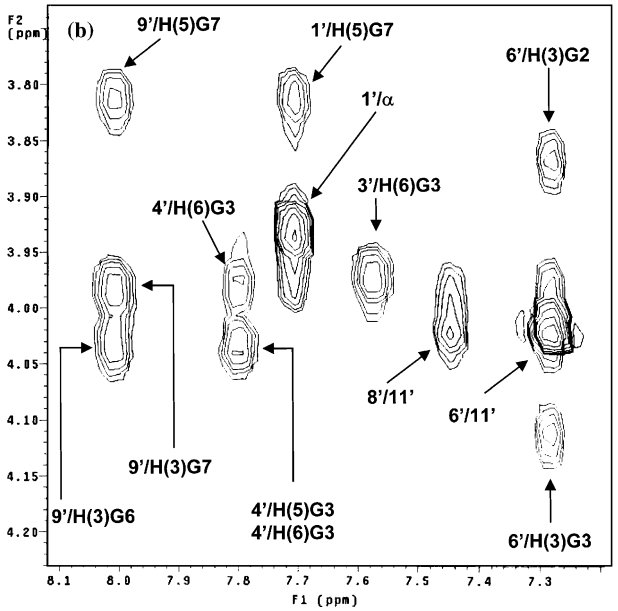


Figure 5. Portions of the 500 MHz T-ROESY spectra of **1S** [5.0×10^{-3} M] (a) and **1R** [5.0×10^{-3} M] (b) in D_2O at pH 7.5.

Concluding remarks

Two novel diastereoisomeric β-CyD derivatives bearing *S*- or *R*-naproxen moieties, were synthesised as a new class of compounds with potential therapeutic activity. Their structure was determined by means of NMR; the CD and NMR results suggest that both compounds adopt a folded conformation in aqueous solution with the naphthalene ring self-included in the β-CyD cavity. The

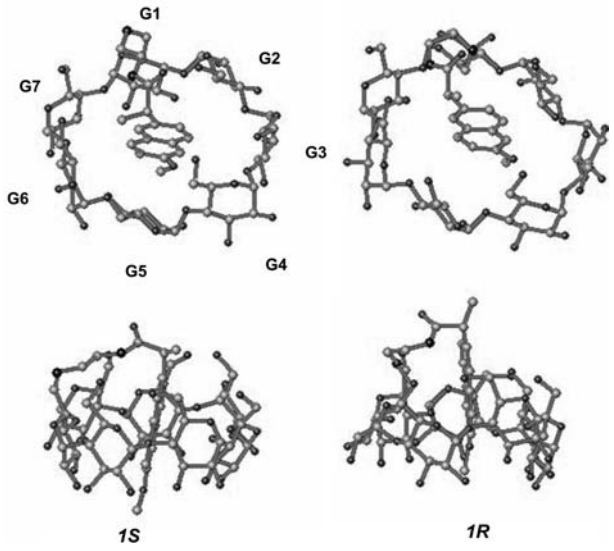


Figure 6. Top (upper panel) and side (lower panel) views of the energy-minimised structures of **1S** and **1R** as estimated from the NMR data. Hydrogen atoms are not shown for clarity. G1–7 refer to the glucose units.

presence of the ethylendiamino spacer between the CyD torus and the naproxen residue provides both the length and flexibility that allow the almost complete encapsulation of the naphthalene ring. The water solubility of **1S** and **1R** compounds was found to be of the order of 3.50×10^{-3} M at pH = 7.4 and $t = 25$ °C. It is of particular relevance that, this value while still being lower than that of the unmodified β-CyD (1.63×10^{-2} M) [43], is of a few order of magnitude larger for those reported in the literature for similar β-CyD derivatives conjugated with pharmaceutically active compounds at the primary hydroxyl position, that generally fall in the range of 10^{-5}–10^{-6} M. [10, 11, 16]. In this regard, it should be mentioned that the naproxen itself shows an intrinsic water solubility of 6.16×10^{-5} M [44]. Finally, the good solubility of **1S** and **1R** is also in accordance with the formation of the self-inclusion complexes.

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References

1. G. Wenz: *Angew. Chem.* **106**, 851 (1994); *Angew. Chem. Int. Ed. Engl.* **33**, 803 (1994).
2. J. Szejtli: *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.
3. See for example: (a) H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda, and A. Uena: *J Am. Chem. Soc.* **118**, 10980 (1996); (b) A. Uena: ACS Symposium-538. In A.W. Czarnik (ed.), *Fluorescent Chemosensor for Ion and Molecule Recognition,* American Chemical Society, Washington, DC, 1993, pp. 74–84; (c) F. Hamada, Y. Kondo, K. Ishikawa, H. Ito, I. Suzuki, T. Osa and A. Ueno: *J Incl. Phenom.* **17**, 267 (1994); (d) F. Hamada, Y. Kondo, R. Ito, I. Suzuki, T. Osa and A. Ueno: *J. Incl. Phenom.* **15**, 273 (1993); (e) A. Uena, T. Kuwabara, A. Nakamura and F. Toda: *Nature* **356**, 136 (1992); (f) A. Ueno, S. Minato and T. Osa: *Anal. Chem.* **64**, 2562 (1992).
4. (a) K. Takahashi: *Chem. Rev.* **98**, 2013 (1998); (b) M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag, Berlin, Heidelberg, New York, 1978, p. 61.
5. R. Breslow and S. Dong: *Chem. Rev.* **98**, 1997 (1998).
6. (a) S. Li and W.C. Purdy: *Chem. Rev.* **92**, 1457 (1992); (b) J. Snopek, E. Smolková Keulemansová, T. Cserháti, K.H. Gahm and A. Stalcup: *Comprehensive Supramolecular Chemistry*, Pergamon, Oxford, 1996, Vol. 3, p. 515.
7. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
8. (a) W. Sanger: *Angew. Chem., Int. Ed. Engl.* **19**, 344 (1980); (b) J. Szejtli: *Cyclodextrin and their Inclusion Complexes*, Akademio Kaido, Budapest, 1982, p. 296.
9. (a) B. Bole-Vundik, K. Verhujak, and J. Zmitek: *J. Pharm. Pharmacol.* **48**, 1153 (1996); (b) J. Szejtli and L. Szente: *Pharmazie* **36**, 694 (1985).
10. (a) J.H. Coates, C.E. Easton, S.J. van Eyk, B.L. May, P. Singh, and S.F. Lincoln: *J Chem. Soc.Chem. Commun.* 759 (1991); (b) F. Hirayama, K. Minami and K. Uekama: *J. Pharm. Pharmacol.* **48**, 27 (1996).
11. K. Uekama, K. Minami, and F. Hirayama: *J. Med. Chem.* **40**, 2755 (1997).
12. K. Minami, F. Hirayama, and K. Uekama: *J. Pharm. Sci.* **87**, 715 (1998).
13. F. Hirayama, T. Ogata, H. Yano, H. Arina, K. Udo, M. Takano, and K. Uekama: *J. Pharm. Sci.* **89**, 1486 (2000).
14. H. Yano, F. Hirayama, H. Arima and K. Uekama: *J. Pharm. Sci.* **90**, 493 (2001).
15. H. Yano, F. Hirayama, H. Arima, and K. Uekama: *J. Pharm.* Sci. **90**, 2103 (2001).
16. M. Kamada, F. Hirayama, K. Udo, H. Yano, H. Arima, and K. Uekama: *J Control. Release* **82**, 407 (2002).
17. Z. Zhang and R.N. DuBois: *Gastroenterology* **48**, 1012 (2000).
18. R. Masunaga, H. Kohno, D. Kuna Dhar, T. Katoh, H. Tabara, M. Tachibana, H. Kubata, and N. Nagasue: *Dis. Colon Rectum* **44**, 1008 (2001) and references cited therein.
19. R.P. Bonomo, V. Cucinotta, F. D'Alessandro, G. Impellizzeri, G. Maccarrone, E. Rizzarelli, and G. Vecchio: *J. Inclusion Phenom.* **15**, 717 (1993).
20. S.W. Tsai and H.J. Wie: *Enzyme and Microbial Technology* **16**, 328 (1994).
21. J.C. Touchstone and M.F. Dobbins: *Practice of Thin Layer Chromatography*, Wiley-Interscience, New-York, 1983, p. 180.
22. P. Gans, A. Sabatini and A.Vacca: *Talanta* **43**, 1739 (1996).
23. M. Rance, O.W. Sørensen, G. Bodenhausen, G. Wagner, R.R. Ernst, and K. Wüthrich: *Biochem. Biophys. Res. Commun.* **117**, 479 (1983).
24. A. Bax and D.G. Davis: *J Magn. Reson.* **65**, 355 (1985).
25. T.L. Hwang and A.J. Shaka: *J. Magn. Reson.* Ser. B **102**, 155 (1993), and references cited therein.
26. (a) D.J. States, R.A. Haberkorn, and D.J. Ruben: *J. Magn. Reson.* **48**, 286 (1982); (b) M. Ohuchi, M. Hosono, K. Furihata and H. Seto: *J. Magn. Reson.* **72**, 279 (1987).
27. (a) D.G. Davis and A. Bax: *J. Am. Chem. Soc.* **107**, 7197 (1985); (b) S. Subramanian and A. Bax: *J. Magn. Reson.* **71**, 325 (1987); (c) H. Kessler, H. Oschkinat and C. Griesinger: *J. Magn. Reson.* **70**, 106 (1986).
28. (a) A.L. Davis, J. Keeler, E.D. Laue, and D. Moskav: *J. Magn. Reson.* **98**, 207 (1992); (b) G. Bodenhausen and D. Ruben: *Chem. Phys. Lett.* **69**, 185 (1980).
29. (a) A. Bax and M.F. Summers: *J. Am. Che. Soc.* **108**, 2093 (1996); (b) M.F. Summers, L.G. Marzilli and A. Bax: *J. Am. Chem. Soc.* **108**, 4285 (1986).
30. A.J. Shaka, P.B. Barker, and R. Freeman: *J. Magn. Reson.* **64**, 547 (1985).
31. G.C. Chen and J.T. Yang: *Anal. Lett.* **10**, 1195 (1977).
32. P.K. Glasoe and F.A. Long. *J. Phys. Chem.* **64**, 188 (1960).
33. G. Impellizzeri, G. Pappalardo, E. Rizzarelli and C. Tringali: *J. Chem. Soc., Perkin Trans.* **2**, 1435 (1996).
34. G. Impellizzeri, G. Pappalardo, F. D' Alessandro, E. Rizzarelli, M. Saviano, R. Iacovino, E. Benedetti, and C. Pedone: *Eur. J. Org. Chem.* 1065 (2000).
35. A. Ganza-Gonzalez, J.L Vila-Jato, S. Anguiano-Igea, F.J. Otero-Espinar and J. Blanco-Méndez: *Int. J. Pharm.* **106**, 179 (1994).
36. R. Corradini, A. Dossena, R. Marchelli, A. Panagia, G. Sartor, M. Saviano, A. Lombardi, and V. Pavone: *Chem. Eur. J.* **2**, 373 (1996).
37. (a) H. Parrot-Lopez, H. Galons, A.W. Coleman, F. Djedaïni, N. Keller, and B. Perly: *Tetrahedron Asymm.* **1**, 367 (1990); (b) F. Djedaïni-Pilard, N. Azaroual-Bellanger, M. Gosnat, D. Vernet and B. Perly: *J. Chem. Soc., Perkin Trans.* **1** 723 (1995); (c) S. Hanessian, A. Benalil and M.T. Phan Viet: *Tetrahedron* **51**, 10131 (1995); (d) P.R. Ashton, E.Y. Hartwell, D. Philp, N. Spencer and J.F. Stoddart: *J. Chem. Soc, Perkin Trans.* **2**, 1263 (1995).
38. E. Breitmaier and W. Voelter: *Carbon-13 NMR Spectroscopy*, VCH, Weinheim, New York, 1990, p. 237.
39. M.M. Velazquez, M. Valero, L.J. Rodriguez, S.M.B. Costa, and M.A. Santos: *J. Photochem. Photobiol. B. Biology* **29**, 23 (1995).
40. (a) M. Kodaka: *J. Am. Chem. Soc.* **115**, 3702 (1993); (b) M. Kodaka: *J. Phys. Chem.* **95**, 2110 (1991).
41. (a) J.N. Murrel: *The Theory of Electronic Spectra of Organic Molecules*, Wiley, New York, 1963; (b) K. Harata, H. Uedaira: *Bull. Chem. Soc. Jpn.* **48**, 375 (1975).
42. H.-J. Schneider, F. Hacket, V. Rüdiger, and H. Ikeda: *Chem. Rev.* **98**, 1755 (1998).
43. J. Szejtli: *Chem. Rev.* **98**, 1743 (1998).
44. A. Avdeef, C.M. Berger, and C. Brownell: *Pharm. Res.* **17**, 85 (2000).