Fluorescent Chemosensor for Organic Guests and Copper(II) Ion Based on Dansyldiethylenetriamine-Modified β-Cyclodextrin

Roberto Corradini,*,† Arnaldo Dossena,† Gianni Galaverna,† Rosangela Marchelli,† Anna Panagia,† and Giorgio Sartor‡

Dipartimento di Chimica Organica e Industriale, University of Parma, Viale delle Scienze, I-43100 Parma, Italy, and Istituto di Chimica Biologica, University of Parma Via Gramsci, I-43100 Parma, Italy

Received February 25, 1997

A modified cyclodextrin containing a dansyldiethylenetriamine metal-binding group (6-deoxy-6-*N*-(*N*″-dansyldiethylenetriamino)-β-cyclodextrin, CD-dien-DNS) was synthesized. The conformation of CD-dien-DNS was studied by 2D NMR (ROESY spectra) in D_2O, by circular dichroism, and by fluorescence. The results were compared with those previously obtained with the analogous 6-deoxy-6-*N*-(*N*′-dansylethylenediamino)-β-cyclodextrin (CD-en-DNS) and were consistent with the self-inclusion of the dansyl group within the macrocycle cavity. However, the orientation of the dansyl group for CD-dien-DNS was found to be equatorial, whereas for CD-en-DNS it was axial, suggesting a dependence of the orientation of the dansyl group upon the length of the linker. In the presence of lipophilic organic molecules, CD-dien-DNS showed sensing properties similar to those observed for CD-en-DNS, suggesting a similar "in-out" movement of the dansyl group, due to competitive inclusion of the guest. Unlike CD-en-DNS, CD-dien-DNS was found to be a fluorescent chemosensor for copper(II) ion, with a linear response up to a 1:1 molar ratio, suggesting that a more flexible conformation of the linker and the presence of additional binding sites allow binding of the metal ion by the amino and sulfonamidate groups. Good selectivity for Cu(II), when compared with Fe(II), Co(II), Ni(II), and Zn(II), was observed. The CD-dien-DNS copper(II) complex was shown to behave as a chemosensor for bifunctional molecules, such as amino acids. In fact, upon addition of alanine, tryptophan, and thyroxine, the negligible fluorescence intensity of Cu(CD-dien-DNS) complex was "switched on", with a response dependent on the amino acid side chain.

Introduction

Cyclodextrins (CDs)[1] have recently received great attention for their host−guest properties[2] and for their potential use as building blocks for supramolecular structures.[3] They have been extensively used in molecular recognition of neutral molecules, being able to discriminate between guests of different shapes and dimensions.

In order to achieve a more efficient binding of molecules with complementary functionalities, cyclodextrins have been modified with specific substituents. In particular, cyclodextrins bearing both positive and negative charges on C_6 carbons of adjacent glucose rings showed enantiomeric recognition toward L- and D-amino acids based on triple recognition (negative, positive, and lipophilic site). [4]

The ability of cyclodextrins to include aromatic fluorophores, inducing an increase of the fluorescence intensity,[5] has been exploited in several analytical applications.[6] The work of Ueno and co-workers has shown how this property could be utilized for achieving shape-selective optical chemosensors based on cyclodextrins[7] modified with several fluorescent groups.[8,9] Among these, the dansyl group has been widely used,[10−13] because of its spectroscopic properties[14] and its affinity for the β-CD cavity. A β-cyclodextrin modified with dansylglycine has been shown to be an optical sensor of remarkable stability once trapped in a sol-gel matrix.[15]

Metallocyclodextrins have also been synthesized by covalently linking metal-binding moieties to the cyclodextrin rim, in order to obtain more specific recognition[16] and to produce catalytic centers for metalloenzyme mimics.[17,18]

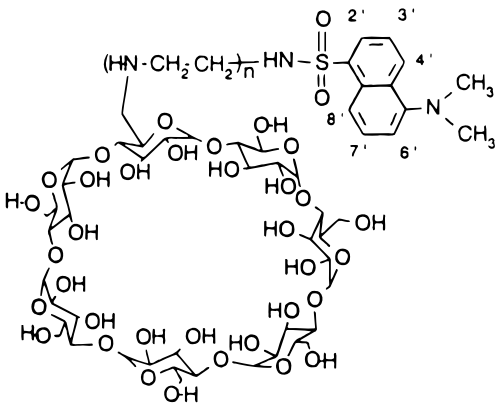
In a general project aimed at studying chiral discrimination by copper(II) complexes, we have recently studied the enantiomeric recognition of aromatic amino acids by the copper(II) complex of a monofunctionalized β-cyclo-

* Corresponding author. Phone: 39-521-905406. Fax: 39-521-905472. E-mail: roberto@vaimo.cce.unipr.it.
† Dipartimento di Chimica Organica e Industriale.
‡ Istituto di Chimica Biologica.
⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1997.

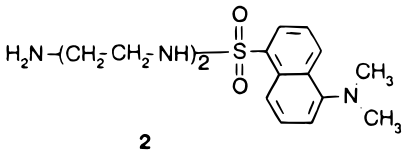
(1) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344−362.
(2) Szejtli, J.; Osa, T. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Elsevier: Oxford, U.K., 1996; Vol. 3.
(3) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803−822.
(4) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514−4518.
(5) Kinoshita, T.; Iinuma, F.; Tsuji, A. *Chem. Pharm. Bull.* **1974**, *22*, 2413−2420.
(6) For a review, see: Li, S.; Pordy, W. C. *Chem. Rev.* **1992**, *92*, 1457−1470.
(7) Ueno, A. *Adv. Mater.* **1993**, *5*, 132−134.
(8) Hamasaki, K.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, F.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1993**, *115*, 5035−5040.
(9) Ueno, A. In *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symposium Series 538; American Chemical Society: Washington, DC, 1992; Chapter 6, pp 74−84.
(10) Ueno, A.; Minato, S.; Suzuki, I.; Fukushima, M.; Ohkubo, M.; Osa, T.; Hamada, F.; Murai, K. *Chem. Lett.* **1990**, 605−608.
(11) Wang, Y.; Ikeda, T.; Ikeda, H.; Ueno, A.; Toda, F. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1598−1607.
(12) Nakamura, M.; Ikeda, T.; Ikeda, A.; Ise, N.; Ikeda, H.; Toda, F.; Ueno, A. *J. Chem. Soc., Chem. Commun.* **1995**, 721−722.
(13) Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno, A. *J. Am. Chem. Soc.* **1996**, *118*, 10980−10988.
(14) Li, Y. H.; Chan, L. M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118−3126.
(15) Narang, U.; Dunbar, R. A.; Bright, F. V.; Prasad, P. N. *Appl. Spectrosc.* **1993**, *47*, 1700−1703.
(16) Impellizzeri, G.; Maccarrone, G.; Rizzarelli, E.; Vecchio, G.; Corradini, R.; Marchelli, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1348−1349.
(17) Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075−1077.
(18) Tabushi, I. *Coord. Chem. Rev.* **1988**, *86*, 1−42 .

S0022-3263(97)00349-6 CCC: $14.00 © 1997 American Chemical Society

Chart 1



1 : n = 1
3 : n = 2



2

dextrin bearing histamine as the binding site. Complexation of the amino acid to the metal ion and inclusion in the cavity were demonstrated by HPLC, circular dichroism, and fluorescence spectra. In particular, with tryptophan, the fluorescence intensity was found to be dependent on the inclusion mechanism, with the isomer (D-Trp) preferentially included in the cavity being more effectively quenched by the copper(II) ion.[19] Thus, in principle, the fluorescence intensity can be regulated both by inclusion and by the presence of suitable binding sites for metal ions such as copper(II).

Copper(II) is known to induce fluorescence quenching of DNS-amino acids and dipeptides;[20] enantioselective fluorescence quenching by a chiral copper(II) complex in aqueous solution has also been reported.[21]

We have recently described the crystal structure of a dansylethylenediamine-modified β-cyclodextrin, CD-en-DNS, **1** (Chart 1), showing that the dansyl group was fully encapsulated in the cyclodextrin cavity and that the high fluorescence observed for this compound was related to its self-included conformation, which was retained in aqueous solutions.[22]

We wish to report here the synthesis and host−guest properties of a β-cyclodextrin covalently linked to *N*-dansyldiethylenetriamine (**2**), CD-dien-DNS (**3**), in which a strong coordination site for the copper(II) ion is present in the proximity of the fluorophore (Chart 1). This molecule was designed to allow self-inclusion of the dansyl moiety and to achieve sensing properties toward several species. Organic lipophilic molecules could affect the fluorescence through competitive inclusion within the cavity. The presence of strong binding sites (amino and sulfonamidate groups) in the cyclodextrin could allow to achieve fluorescence quenching upon addition of metal ions, in particular of copper(II). The quenched copper(II) complex could then interact with bifunctional organic molecules, such as amino acids, through a ligand exchange process, giving rise to changes in the fluorescence response.

In order to evaluate the effect of the length of the spacer on the self-inclusion mode of the DNS group, the preferred conformation of the cyclodextrin **3** in solution has been investigated by circular dichroism, NMR, and fluorescence and compared with the results previously obtained for **1**. Subsequently, the ability of the two CDs, **1** and **3**, to act as host−guest sensory systems has been compared. The coordination ability toward copper(II) has been evaluated, in order to establish if the length and flexibility of the spacer affect the ability of the two cyclodextrins to act as fluorescent sensors for the copper(II) ion. Finally, the effect of metal-coordinating guests on the fluorescence of the cyclodextrin−copper complex has been evaluated.

Results and Discussion

Chemistry. The synthesis of the monodansyl derivative of dien (DNS-dien, **2**) was carried out with dansyl chloride in the presence of excess amine; regioselective sulfonylation at the less-hindered primary amine group was observed, as deduced from the lack of symmetry in the NMR spectrum and from fragments in the mass spectrum.

The synthesis of the monofunctionalized cyclodextrin was carried out by monotosylation in pyridine, substitution of the tosylate by iodide, and finally nucleophilic displacement of iodide with DNS-dien, as previously reported for **1**,[22] with a comparable yield (50%).

Spectroscopic Study of Self-Inclusion of 3 in Aqueous Solution. The conformation of **1** in aqueous solution was fully investigated and compared with the crystal structure.[22] In that study, it was shown that the features of self-inclusion of the dansyl group within the cavity in the solid state were consistent with NMR, circular dichroism, and fluorescence data obtained in solution. Therefore, the same methodologies were utilized for studying the conformation of **3** in aqueous solutions, although for this compound the crystal structure could not be obtained.

The 400 MHz ^{1}H NMR spectrum of **3** in D_2O exhibits severely overlapped peaks in the aliphatic region. However, although the anomeric protons (H_1) of the unmodified β-CD exhibit only one degenerated resonance, those of **3** showed six separated resonances. In the ^{1}H−^{1}H COSY spectra seven cross-peaks, corresponding to the correlation between H_1 and H_2 protons of a monosubstituted cyclodextrin, were observed, allowing the localization of H_2 protons (results not shown).

The combined use of ^{1}H−^{1}H COSY and ROESY allowed to assign all the resonances of the aromatic protons. In particular, two series of signals connected through bonds have been identified by the COSY spectrum, corresponding to the two rings; the connectivity through space with the protons of the dimethylamino group allowed the identification of the protons $H_{4'}$ (8.59 ppm) and $H_{6'}$ (7.33 ppm) of the naphthalene unit and, from these, of the other signals. ROESY spectra were used, instead of NOESY, in order to reveal Overhauser effects arising from through-space interactions among proton nuclei

(19) Corradini, R.; Dossena, A.; Impellizzeri, G.; Maccarrone, G.; Marchelli, R.; Rizzarelli, E.; Sartor, G.; Vecchio, G. *J. Am. Chem. Soc.* **1994**, *116*, 10267−10274.

(20) Lin, W. Y.; Van Wart, H. E. *J. Inorg. Biochem.* **1988**, *32*, 21−38.

(21) Corradini, R.; Sartor, G.; Marchelli, R.; Dossena, A.; Spisni, A. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1979−1983.

(22) Corradini, R.; Dossena, A.; Marchelli, R.; Panagia, A.; Sartor, G.; Saviano, M.; Lombardi, A.; Pavone, V. *Chem. Eur. J.* **1996**, *2*, 373−381.

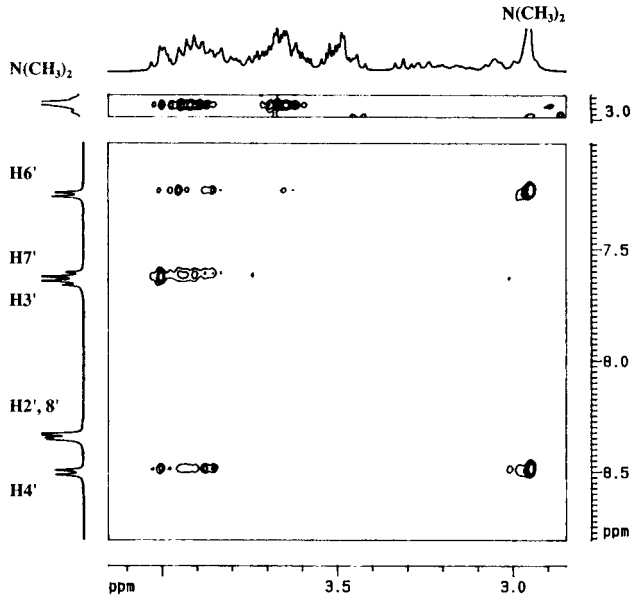


Figure 1. Contour plot of a portion of the ROESY spectrum (400 MHz in D_2O, T = 294 K) of CD-dien-DNS (**3**).

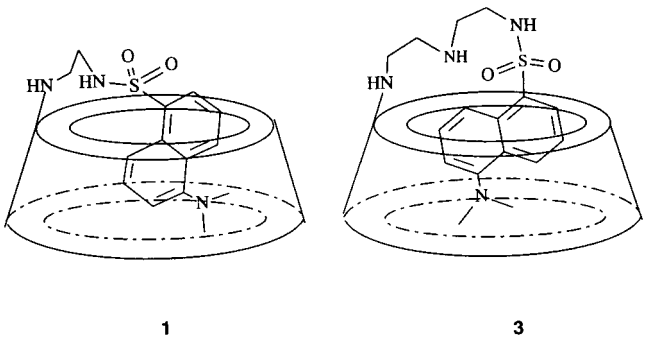


Figure 2. Self-inclusion models for CD-en-DNS (**1**) and CD-dien-DNS (**3**).

within 5 Å, since, for cyclodextrins, the former provides more reliable results than the latter. The contour plots of the portion of the ROESY spectrum of **3** in D_2O showing connectivities of the aromatic and $N(CH_3)_2$ protons with the signals in the aliphatic region are reported in Figure 1.

Connectivities were observed between protons $H_{3'}$, $H_{4'}$, $H_{6'}$, and $H_{7'}$ and the cyclodextrin protons, while no NOE was observed in the same region for protons $H_{2'}$ and $H_{8'}$ of the naphthalene ring. The results are different from those obtained in the ROESY spectrum of **1**, for which an almost axial orientation was observed, and suggest, instead, an equatorial self-inclusion model (Figure 2).

Further insight on the self-inclusion mode was provided by circular dichroism spectra. An achiral guest molecule included in a chiral cyclodextrin cavity may exhibit an induced circular dichroism (ICD) in its absorption regions.

For simple chromophores, such as substituted benzene and naphthalene rings, it has been possible to correlate, on theoretical bases, the sign of the observed ICD with the orientation of the dipole transition moment of a given absorption band relative to the β-cyclodextrin 7-fold axis.[23] For substituted naphthalenes included in cyclodextrins with axial complexation (i.e., with the naphthalene long axis parallel to the CD 7-fold axis), the short wavelength ^1B_b band has a positive sign and the long wavelength ^1L_a has a negative sign; opposite signs are expected for equatorial complexation.[24] This model has been shown to be consistent with the crystal structure of **1**, which, having an orientation with the naphthalene long axis almost parallel to the cyclodextrin axis, showed a negative ^1L_a band and a positive ^1B_b.

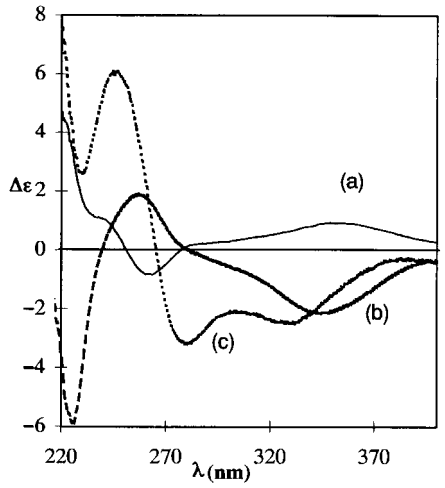


Figure 3. Circular dichroism spectra of (a) CD-dien-DNS (**3**), (b) CD-en-DNS (**1**), (c) CD-dien-DNS; copper(II) = 1:1, pH = 8 in H_2O, T = 294 K.

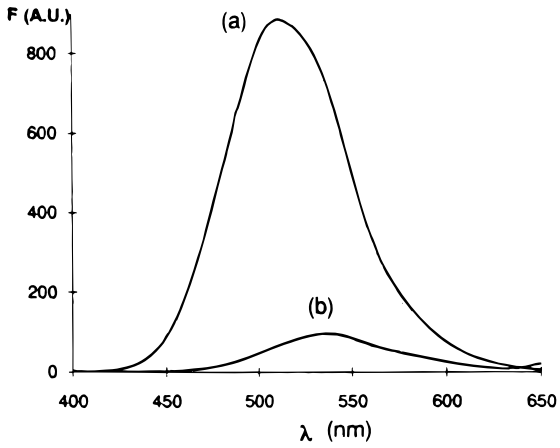


Figure 4. Fluorescence emission spectra of (a) CD-dien-DNS (**3**) (6×10^{-5} M), (b) dien-DNS (**2**) (6×10^{-5} M); in H_2O, pH = 8.0 (λ_{ex} = 345 nm).

For **1** and **3**, CD spectra are reported in Figure 3. Opposite sign of each CD band was observed, suggesting a different type of inclusion for the two derivatives: the results are consistent with an equatorial self-inclusion mode of **3**. Thus, the orientation of the dansyl group inside the cavity seems to be dependent on the length of the spacer: the shorter one induces axial complexation, while the longer one, allowing more rotational freedom, induces equatorial complexation.

Fluorescence measurements were carried out on the dansylated cyclodextrin **3** and on the parent dansylated amine **2** in aqueous solution at pH = 8.0 (Figure 4). The intensity of the cyclodextrin derivative was much higher than that of the reference amine, and the emission maximum of **3** was shifted toward shorter wavelengths,

(23) Kodaka, M. *J. Am. Chem. Soc.* **1993**, *115*, 3702−3705.

(24) Harata, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375−378.

Table 1. Fluorescence Lifetimes (τ) and Normalized Molar Fractions (A) for DNS-dien (2) and CD-dien-DNS (3) in Aqueous Solution (pH = 8.0, 0.1 M tetraborate buffer)

compd	guest	equiv	τ_1	A_1	τ_2	A_2	τ_3	A_3	χ^2
2			3.3	0.40	2.2	0.40	1	0.20	1.25
3			19.1	0.65	5.4	0.28	0.9	0.14	1.10
3	ACA	100	15.1	0.15	6.7	0.61	1.72	0.24	1.14
3	Cu(II)	0.5	19.5	0.71	6.6	0.23	1.4	0.06	1.10

Table 2. Fluorescence Intensity of 1 and 3 (normalized) in the Absence and Presence of Excess (100:1) of Various Guests in Aqueous Solution at pH = 8.0 (0.1 M tetraborate buffer)

guest	**1**[a]	**3**
none	1.000	1.000
ACA[b]	0.311	0.303
(*R*)-camphor	0.514	0.561
(*S*)-camphor	0.518	0.561
L-fenchone	0.673	0.734
borneol	0.370	0.364
L-menthol	0.658	0.634
L-menthyl acetate	0.698	0.806
cholesterol	0.872[c]	0.904[c]

[a] From ref 22. [b] ACA = adamantanecarboxylic acid. [c] Cyclodextrin:cholesterol = 1:3. Standard deviations are in the range 0.001−0.005.

suggesting that the fluorophore experienced a less polar environment.

Correspondingly, time-resolved fluorescence experiments showed for CD-dien-DNS (**3**) the presence of a component having a longer (19.1 ns) lifetime, which was not observed in the case of DNS-dien (**2**) (Table 1). Both observations are in agreement with the previously reported studies on the complexation of fluorescent guests by β-cyclodextrin,[25] with the results obtained on *N*-dansylglycine-[26] or *N*-dansylleucine-modified β-cyclodextrins,[13] and with our previous results from CD-en-DNS (**1**).[22]

The higher fluorescence intensity is correlated with a longer fluorescence lifetime, since the steady state fluorescence of the *i*th component can be expressed as $F_i = \alpha_i\tau_i$, where α_i is the fraction of fluorophore with lifetime τ_i. Thus, it seems reasonable to propose that also in the case of **3**, as for **1**, the fluorophore is included in the cavity and protected from the quenching of solvent molecules, giving rise to a component with a longer lifetime. Therefore, all the above spectroscopic evidences strongly suggest self-inclusion of the dansyl moiety in the cyclodextrin cavity and a different orientation for **1** and **3**.

Sensing Properties of 3 for Organic Guests. In order to establish if the different orientation inside the cavity affects the host−guest sensory properties, we measured the fluorescence intensity of **3** in the presence of an excess (1:100) of several guests at pH = 8. The results, reported in Table 2, are comparable to those obtained with the analogous cyclodextrin **1**.

For both cyclodextrins, the most effective guest was adamantanecarboxylic acid (ACA), which is known to form stable inclusion complexes with β-CD, due to the size and tricyclic rigid structure well fitting within the cavity. Furthermore, at the pH utilized both **1** and **3** were presumably in their protonated form (and hence cationic), while ACA was under the anionic form, giving rise to additional electrostatic interactions which stabilized the complex. The fluorescence decrease for camphor (which is bicyclic) and menthol (monocyclic) was less pronounced. Correspondingly, the fluorescence lifetime distribution changed upon addition of ACA: the component τ_1 with a longer lifetime (15.1 ns) showed a fraction α_1 smaller than in free **3** (Table 1). This means that the fraction of molecules having the dansyl group inside the cavity had decreased. Accordingly, the circular dichroism bands of **3** progressively decreased in intensity upon addition of ACA, as previously observed for **1**.[22] All the above spectroscopic evidences are consistent with a model of "in−out" movement of the dansyl moiety induced by the guest, as previously proposed for analogous fluorescent cyclodextrins.

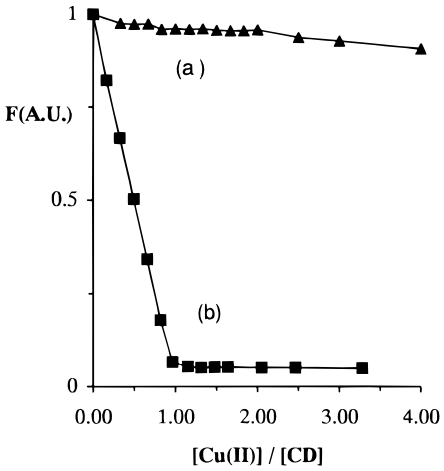


Figure 5. Variation of the fluorescence intensity of (a) CD-en-DNS (**1**) (6×10^{-5} M) and (b) CD-dien-DNS (**3**) (6×10^{-5} M) upon addition of copper(II) ion; T = 294 K.

The length of the spacer which connects the cyclodextrin to the dansyl group has little effect on the ability of the molecule to act as a fluorescent sensor for organic guests. Probably the spacers of both **1** and **3** are long enough to allow inclusion and flexible enough to allow displacement of the dansyl group by a guest molecule.

Copper(II)-Sensing Properties of 1 and 3. A completely different behavior for **1** and **3** was observed in the presence of copper(II) ion (Figure 5) at pH = 8: CD-en-DNS underwent only negligible changes; the lack of quenching was attributed to the constrained conformation of the ethylenediamine linker, which prevented the formation of a chelate ring, since instead the parent dansylethylenediamine was quenched by copper(II) ion under the same conditions. On the contrary, the fluorescence of CD-dien-DNS (**3**) was almost completely quenched, the titration curve showing the formation of a complex of 1:1 stoichiometry. On these bases, the occurrence of a copper(II)/cyclodextrin 1:2 complex could be ruled out. Dilution experiments indicate that also a 2:2 complex is unlikely to be formed.

The fluorescence quenching induced by copper(II) on DNS-amino acids is most likely promoted by the complexation of the copper(II) ion by a donor group adjacent to the dansyl group, with subsequent abstraction of the sulfonamide hydrogen; coordination of the deprotonated sulfonamidate group to copper(II) has been observed both in polarimetric studies[27] and in crystal structures.[28]

(25) Bright, F. V.; Catena, G. C.; Huang, J. *J. Am. Chem. Soc.* **1990**, *112*, 1343−1346.
(26) Dunbar, R. A.; Bright, F. V. *Supramol. Chem.* **1994**, *3*, 93−99.
(27) Battistuzzi Gavioli, G.; Grandi, G.; Menabue, L.; Pellacani, G. C.; Sola, M. *J. Chem. Soc., Dalton Trans.* **1985**, 2363−2368.
(28) Antolini, L.; Menabue, L.; Sola, M.; Battaglia, L. P.; Bonamartini Corradi, A. *J. Chem. Soc., Dalton Trans.* **1986**, 1367−1373.

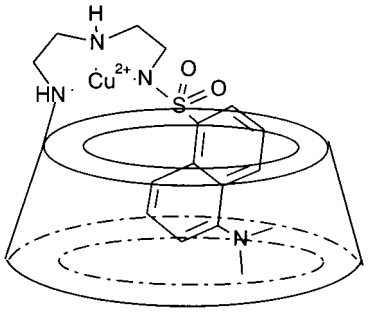


Figure 6. Copper(II) complexation model for CD-dien-DNS (**3**).

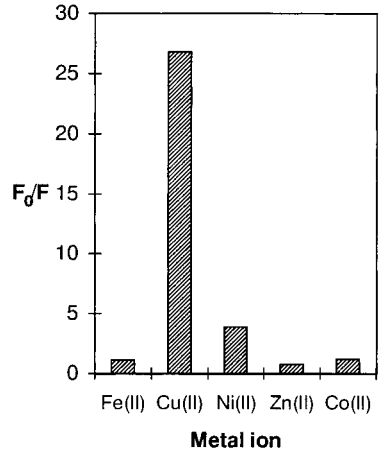


Figure 7. Fluorescence quenching (F_0/F) for CD-dien-DNS (**3**) (6×10^{-5} M) in the presence of divalent transition metal ions in a 1:1 ratio in H_2O (0.1 M borate buffer, pH = 8.0) at 294 K.

Time-resolved fluorescence measurements reported in Table 1 also indicate that quenching is due to complex formation: in fact, in the presence of 0.5 equiv of Cu(II), the fluorescence was quenched to one-half of that observed in free **3**, while lifetimes were unchanged, in agreement with the static quenching model.

The flexibility of the diethylenetriamino spacer in **3** allows the donor atoms to coordinate to copper(II) in a multidentate fashion, through the amine nitrogen atoms and the deprotonated sulfonamide, giving rise to fluorescence quenching (Figure 6). The titration experiment reported in Figure 5 shows that **3** can be utilized as a fluorescent chemosensor for the copper(II) ion, since the fluorescence intensity decreased linearly up to a 1:1 ratio. A similar effect has been observed for dien-DNS (**2**), but since the initial fluorescence intensity was quite low, the absolute decrease of the fluorescence intensity was much lower, leading to a less efficient chemosensor.

CD-dien-DNS (**3**) was also tested against other divalent transition metal ions, in order to study if selectivity could be obtained. Figure 7 reports the quenching ratio F_0/F, where F_0 is the fluorescence of free **3** and F is the corresponding fluorescence in the presence of the metal ion in a 1:1 molar ratio; it is evident that fluorescence quenching was much higher for Cu(II) ion, while it was weaker in the case of Ni(II) and very low for Co(II) and Fe(II). Zn(II) also gave a very low reponse, with slight increase of fluorescence. It is beyond the scope of the present work to discuss in detail the mechanism of interaction of **3** with these and other metal ions.

Upon addition of copper(II), the CD spectrum of **3** drastically changed, giving rise to a negative band with a minimum at 335 nm, similar to that observed for **1** (Figure 3, curve c). Thus, probably the copper(II) complexation induced an axial inclusion of the dansyl group, similar to that observed for **1**, due to the more rigid conformation of the linking moiety on the rim.

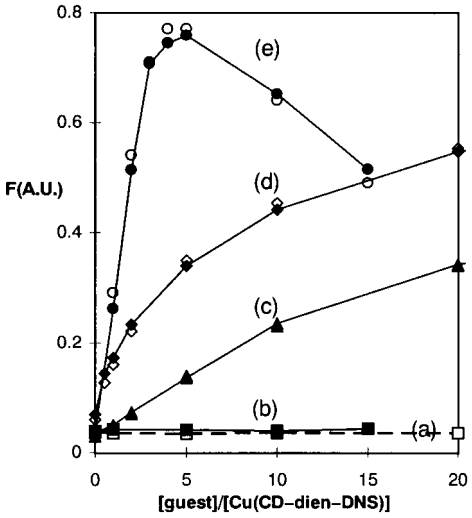


Figure 8. Effect of several potentially coordinating molecules on the fluorescence of [Cu(CD-dien-DNS)] complex (6×10^{-5} M): (a) L-mandelic acid (□), (b) (*S*)-naphthylethylamine (■), (c) D-alanine (▲) and L-alanine (△), (d) D-tryptophan (◆) and L-tryptophan (◇), (e) D-thyroxine (●) and L-thyroxine (○) in 0.1 M tetraborate buffer, pH = 8; T = 294 K.

Sensing Properties of the [Cu(CD-dien-DNS)] Complex. The metal complexes of ligand-appended cyclodextrins can be used as luminescent chemosensors for organic molecules, provided that the addition of the analytes induces a large modification of the photophysical properties of either the ligand or the metal ion, as recently reported in several examples.[29,30]

In the titration of CD-dien-DNS with copper(II), the quenching process is consistent with the coordination of the deprotonated sulfonamidate nitrogen atom, which is assisted by the anchoring effect of the two amino groups, leading to a terdentate complex (Figure 6). Since copper(II) shows preferential tetracoordination with longer axial contacts, the addition of a bidentate ligand could displace one of the three donor groups in the [Cu(CD-dien-DNS)] complex, giving rise to a change in the fluorescence intensity.

In Figure 8 the effect on fluorescence of the addition of different molecules containing donor groups (mono- or bidentate) is reported.

As reported above, the 1:1 mixture of copper(II) and **3** showed very little fluorescence. The addition of a monodentate molecule, such as (*R*)- or (*S*)-naphthylethylamine, which contained an aromatic group potentially fitting the cavity, did not affect the fluorescence intensity. The same was observed for bidentate molecules with poorly coordinating groups, such as D- or L-mandelic acid and D- or L-lactic acid, whereas a sharp increase in fluorescence was observed upon addition of the amino acids D- or L-alanine and D- or L-tryptophan, and it was especially strong for D- or L-thyroxine (the human thyroid hormone T4). The increase in fluorescence can be attributed to the displacement of the dansyl group from the copper ion,

(29) Nakamura, A.; Okutsu, S.; Oda, Y.; Ueno, A.; Toda, F. *Tetrahedron Lett.* **1994**, *39*, 7241−7244.
(30) Mortellaro, M. A.; Nocera, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 7414−7415.

while the decrease observed in the presence of excess thyroxine is most likely due to the quenching effect of its iodine substituents ("heavy atom effect"). The fact that tryptophan and alanine gave different fluorescence responses seems to indicate that the cavity could be involved in the complexation process. However, no enantioselectivity was observed for these amino acids. The fluorescence intensity was "switched on" also in the case of the copper(II) complex of the parent amine **2**, although the total intensity was much lower.

The copper(II) complex acts therefore as a "back-titration" chemosensor able to discriminate between molecules of different affinity for the copper(II) ion, being particularly sensitive, among the analytes tested in the present work, for thyroxine. Further work is in progress to understand the actual nature of the amino acid-sensing process, decomplexation, or ternary complex formation: in the former case the sensing process should require reloading of copper(II) ion, while in the latter, the copper(II) complex could be used without loss of the metal ion.

Conclusions

Detailed spectroscopic studies allowed to produce direct evidence of the inclusion of the dansyl group of CD-dien-DNS within the cyclodextrin cavity in aqueous solution and suggest that the self-inclusion mode of the dansyl group is dependent on the spacer length, being almost axial in the case of **1** and equatorial in the case of **3**. However, the different conformations of **1** and **3** did not affect their ability to act as fluorescence chemosensors for many organic guests but produced different binding ability toward the copper(II) ion. The fluorescence of **1** was not quenched by the metal ion, on account of the unfavorable conformation of the linker that places the donor groups apart from each other. The length of the linker is therefore critical, since with the longer flexible spacer of **3** inclusion of the naphthyl group can be achieved without losing much of the conformational freedom of the spacer itself, allowing coordination of the copper(II) ion and fluorescence quenching. This quenching process was found to be selective, when compared with the effect produced by other transition metal ions.

Thus the behavior of compound **3** allows to envisage a strategy aimed at obtaining receptors with a fluorophore and a metal ion-binding site mantained in a fixed position with respect to other functionalities. Any event occurring on the metal ion could then, in principle, have a consequence on the photophysical properties of the fluorophore, allowing to study interactions with other molecules, as shown in the present studies for the amino acids.

Experimental Section

General. The following materials were used: β-cyclodextin hydrate (Janssen Chemical Co.), dansyl chloride (Sigma Chemical Co.), diethylenetriamine (Merck). The latter reagent was distilled prior to use. Anhydrous copper(II) sulfate, (*R*)- and (*S*)-naphthylethylamine, adamantanecarboxylic acid, (*R*)- and (*S*)-camphor, and L-menthol were from Aldrich. Nickel(II), iron(II), and cobalt(II) sulfates heptahydrates were from Carlo Erba (Italy); zinc sulfate heptahydrate was from Merck. D- and L-mandelic acid, L-fenchone, borneol, cholesterol, L-menthyl acetate, and L-menthol were from Fluka. D- and L-lactic acid, D- and L-alanine, D- and L-tryptophan, and D- and L-thyroxine were from Sigma.

Thin layer chromatographic analyses were conducted using precoated TLC silica gel plates (60 F-254, Merck). The detection of cyclodextrin derivatives on SiO_2 thin layer chromatography plates was achieved by UV irradiation ($\lambda = 254$ nm) or by using the anisaldehyde test.

All IR spectra were recorded at room temperature from 4000 to 600 cm^{-1}. Melting points are uncorrected. ^1H NMR spectra were obtained at 20 °C on either a 400 or a 100 MHz spectrometer. Tetramethylsilane was used as reference in $CDCl_3$. The NMR spectra of **3** were recorded in D_2O (external reference: 3-(trimethylsilyl)propionic acid, sodium salt). Optical rotations were recorded with a sodium D-line source (589 nm), in a 10 cm cell.

6-Deoxy-6-*N*-(*N*′-((5-(dimethylamino)-1-naphthyl)sulfonyl)diaminoethyl)β-cyclodextrin (1): synthesized as reported previously.[15]

***N*^{1}-Dansyldiethylenetriamine (2).** Diethylenetriamine (11.7 mL, 109 mmol) was cooled to 0 °C; dansyl chloride (2.95 g, 10.9 mmol) in 290 mL of CH_3CN was then added under stirring. The mixture was allowed to stir at room temperature for 3 h. Acidification (pH = 4) and extraction with ethyl ether allowed to eliminate dansyl chloride unreacted. The aqueous layer was basified with aqueous NaOH, extracted with $CHCl_3$, and dried over Na_2SO_4, and the solvent was removed by rotary evaporation to provide 2.46 g of a yellow solid (yield, 67%).

2: mp 117−119 °C; IR (KBr) 3340, 3200, 2840, 1570, 1320, 1150, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (t, 2H, J = 6.0 Hz), 2.57 (t, 2H, J = 5.9 Hz), 2.58 (t, 2H, J = 5.5 Hz), 2.89 (s, 6H, NH(CH_3)$_2$), 2.93 (t, 2H, J = 5.5 Hz), 7.18 (d, 1H, J = 7.6 Hz), 7.52 (dd, 1H, J = 7.2, 8.4 Hz), 7.57 (dd, 1H, J = 8.8, 7.6 Hz), 8.26 (d, 1H, J = 7.2 Hz), 8.31 (d, 1H, J = 8.8 Hz), 8.53 (d, 1H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.47, 42.60, 47.63, 51.32, 115.15, 118.81, 123.20, 129.27, 129.65, 129.85, 130.29, 134.70, 151.99; MS (CI, CH_4) *m*/*e*(relative intensity) 337 (M^+, 100), 306 (9), 250 (6), 234 (4), 170 (3), 73 (4). Anal. Calcd for $C_{16}H_{24}N_4O_2S\cdot{}^1/_2H_2O$: C, 55.63; H, 7.29; N, 16.22. Found: C, 55.38; H, 6.94; N, 16.13.

6-*O*-(*p*-Tosyl)-β-cyclodextrin (CDOTs) and 6-Deoxy-6-iodo-β-cyclodextrin (CDI): synthesized according to a literature procedure.[31]

6-Deoxy-6-*N*-(*N*′′-dansyldiethylenetriamino)-β-cyclodextrin (CD-dien-DNS, 3). Dried CDI (0.150 g, 0.12 mmol) was dissolved in anhydrous DMF (14 mL), and dien-DNS (**2**) (0.811 g, 2.41 mmol) was added. The reaction was carried out at 50 °C under nitrogen. After 3 days the DMF was evaporated under vacuum at 40 °C. The yellow syrup obtained was washed with acetone. The solid obtained was dissolved in the minimum amount of water and precipitated again with acetone. The precipitate collected by suction was dissolved in water, and the solution was applied to a column of CM-Sephadex C-25 resin (in NH_4^+ form). The column was eluted initially with water (120 mL) and then with a gradient from 0 to 0.2 M aqueous ammonium hydrogen carbonate (800 mL total volume). The collected fractions were assayed by TLC. Fractions that gave only one spot with R_f = 0.7 (eluent 5:3:1 propanol−water−ammonia) were combined and evaporated to dryness at 40 °C under vacuum to decompose ammonium hydrogen carbonate. A yellow solid was obtained (yield, 50% based on CDI).

3: $[\alpha]_D^{21}$ = 141 (c = 0.21, D_2O); MS (FAB) *m*/*e* 1454 (M + 1)$^+$; IR (KBr) 3600−2800, 3060, 2920, 1580−1630, 1400, 1320, 1150, 1020 cm^{-1}; ^1H NMR (400 MHz, D_2O, assignments made on the basis of COSY and ROESY experiments) δ 1.87 (m, 2H, N-CH_2), 2.01 (m, 1H, N-CH), 2.20 (m, 1H, N-CH), 2.58 (m, 3H, H_6(A) + N-CH_2), 2.75 (d, 1H, ^2J = 13 Hz, H_6(A)), 3.05 (s, 6H, N(CH_3)$_2$), 2.95−4.01 (m, 42 H, H_{2-6} glucose + S0_2N-CH_2), 4.96 (d, 1H, ^3J = 3.0 Hz, H_1), 5.00 (d, 1H, ^3J = 2.9 Hz, H_1), 5.06 (d, 1H, ^3J = 2.8 Hz, H_1), 5.14 (d, 1H, ^3J = 3.0 Hz, H_1), 5.18 (m, 2H, H_1), 5.22 (d, 1H, ^3J = 2.9 Hz, H_1), 7.33 (d, 1H, ^3J = 7.7 Hz, $H_{6'}$ arom), 7.70 (dd, 1H, ^3J = 8.5, 7.7 Hz, $H_{7'}$ arom), 7.74 (dd, 1H, ^3J = 8.4, 7.1 Hz, $H_{3'}$ arom), 8.45 (d, 1H, ^3J = 8.5 Hz, $H_{8'}$ arom), 8.46 (d, 1H, ^3J = 7.1 Hz, $H_{2'}$ arom), 8.59 (d, 1H, ^3J = 8.4 Hz, $H_{4'}$ arom); ^{13}C NMR (100 MHz, D_2O) δ 47.71, 49.76, 62.16, 74.44, 75.48, 82.44, 83.54, 86.64, 103.77, 104.48, 116.70,

(31) Bonomo, R. P.; Cucinotta, V.; D'Alessandro, F.; Impellizzeri, G.; Maccarrone, G.; Vecchio, G.; Rizzarelli, E. *Inorg. Chem.* **1991**, *30*, 2708−2713.

121.10, 126.18, 130.80, 131.52, 131.93, 132.14, 132.55, 136.86, 154.66. Anal. Calcd for $C_{58}H_{92}N_4O_{37}S\cdot 14H_2O$: C, 40.46; H, 7.03; N, 3.25. Found: C, 40.39; H, 6.80; N 3.31.

2D NMR Spectra. COSY and ROESY spectra (2K, 512 experiments) were obtained with the pulse programs COSYTP and ROESYTP, supplied by Bruker, with TPPI phase cycling. ROESY spectra were obtained using a 250 ms spin−lock time (20 dB).

Fluorescence Measurements. Fluorescence spectra were measured using a JASCO FP 770 instrument in a 0.2 × 1 cm quartz cell. Concentrated stock solutions of **1** and **3** (6×10^{-3} M) and various guests (60×10^{-3} M) were prepared at pH = 8 in a 0.1 M aqueous tetraborate buffer. Aqueous solutions of $CuCl_2$, $CuSO_4$ (anhydrous), $FeSO_4\cdot 7H_2O$, $CoSO_4\cdot 7H_2O$, $NiSO_4\cdot 7H_2O$, and $ZnSO_4\cdot 7H_2O$ were prepared at a 6×10^{-3} M concentration and used immediately after preparation. Fe(II) solution was kept under nitrogen in order to avoid oxidation to Fe(III). Solutions for fluorescence measurements were prepared by diluting standard solutions of **2** and **3** to a final concentration of 6×10^{-5} M; 0.5 mL of these solutions was put in the cell and measured, and then small amounts of concentrated solution of guest or metal ion were added with a Koehln 10 or 100 μL syringe.

In titration experiments, fluorescence intensity corresponding to maximum emission intensity was used instead of the area, since all samples showed the same peak shape and maxima. Alternate measurements of samples and a reference solution of **3** (6×10^{-5} M) were done, in order to compensate for lamp fluctuations. Three measurements of each sample and the reference were made for each point.

In the selectivity study three independent samples were prepared. Correction of the fluorescence intensity of all the samples (F_i) was made according to the expression: $(F_i)_{corr} = (F_i)/F_{ref}$, where F_i is the observed fluorescence intensity and F_{ref} is the intensity of the reference solution of **3**, both measured at the same excitation and emission wavelengths (corresponding to maximum fluorescence). In each titration, the corrected fluorescence intensity was normalized for F_0, the fluorescence of the fluorophore in the absence of any guest or metal ion.

In dilution experiments the fluorescence of 6×10^{-5}, 6×10^{-6}, 6×10^{-7} M solutions was measured. Then 1 equiv of copper(II) was added to each solution, and the corresponding quenching (F_0/F ratio) was measured.

Oxygen was not removed, since the quantum yield of the dansyl moiety has been reported to be unaffected by it; moreover, only relative fluorescence and lifetimes were compared. Other experimental details were as previously described.[22]

Titrations of [Cu(CD-dien-DNS)] and [Cu(dien-DNS)] were carried out with 6×10^{-5} M solutions of the complex in 0.1 M tetraborate buffer. To 0.5 mL of this solution were added aliquots of D- or L-tryptophan (6×10^{-2} M in the same buffer), D- and L-alanine (6×10^{-2} M in the same buffer), D- or L-mandelic acid (6×10^{-2} M in the same buffer), D- or L-thyroxine (1×10^{-2} M in DMSO), and L-naphthylethylamine (6×10^{-2} M in ethanol). The fluorescence was measured and corrected as reported above. For strong absorbimg amino acids (tryptophan and thyroxine) fluorescence was then corrected for absorbance by means of the expression: $F_{corr} = F10^{(A_{ex}+A_{em})/2}$, where A_{em} and A_{ex} are the absorbances of the solutions at excitation and emission wavelengths, respectively. The effect of DMSO or ethanol was checked by titration with the same amounts of pure solvent and was found to be negligible.

Time-Resolved Experiments. Lifetime measurements were carried out on a single-proton-counting instrument equipped with a nanosecond pulse flash lamp (Edimburg Instrument, model F199), modified in order to allow N_2 flux at a rate of 1 L/min; Jasco and Farrand monochromators and Philips XP20202Q photomultiplier were used for fast detection. Fast NIM electronics were from EG&G, Tennelec, and Silena. Decays were recorded on a Silena BS27n multichannel analyzer (512 channels). Alternate measurements of the sample and the scattering solution (glycogen) were made in order to compensate for lamp fluctuations and drift. The excitation wavelength was set at 337 nm, and measurements for each sample were made at different wavelengths, from 450 to 580 nm every 10 nm, in order to cover the emission spectrum. Deconvolution of the decay profile from the lamp profile was carried out as reported in the literature.[32] Fluorescence lifetimes were calculated from decay data, utilizing a global approach[33] by means of a three-lifetime model.

UV and CD Measurements. UV spectra are obtained with a Kontron Uvicon 860 spectrophotometer, and CD spectra were recorded with a Jasco 500A spectropolarimeter. Solutions of **3** (1×10^{-4} M) were obtained by dilution of freshly prepared concentrated solutions (1×10^{-3} M). In the displacement experiments, to 2.5 mL of these solutions were added aliquots of a concentrated solution of adamantanecarboxylic acid (1×10^{-2} solution in ethanol) up to a final 10:1 ACA/cyclodextrin ratio (solution in 10% ethanol). In the complexation experiment to 2.5 mL of the same solution was added 0.25 mL of $CuCl_2$ (1×10^{-2} M in water).

Acknowledgment. We thank the Centro Interfacoltà di Misure of the University of Parma for allowing us to use the CD and NMR facilities. This work was partially supported by MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica).

JO970349D

(32) O'Connor, D. V.; Phillips, D. *Time Correlated Single Photon Counting*; Associated Press: London, U.K., 1984.

(33) Knutson, J. R.; Beechem, J. M.; Brand, L. *Chem. Phys. Lett.* **1983**, *102*, 501−507.