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ORIGINAL PAPER

Fluorescence Studies of Host–Guest Interaction of a Dansyl Amide Labelled Calix[6]arene

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Abstract The fluorescence behavior of a calix[6]arene with a dansyl group as fluorescence marker (C6-DA) was investigated with respect to the inclusion properties of alkaloids as atropine and cocaine. A strong hypsochromic shift of the fluorescence band and a strong increase in fluorescence intensity is connected with the interaction of atropine to the lower rim of the C6-DA. The fluorescence increase is saturated at an atropine concentration above the 1:1 complex formation. Addition of cocaine to the complexed atropine-C6-DA leads to a decrease of the fluorescence intensity which could be explained by an exchange mechanism of the complexed molecules. The complexation of the atropine (the "belladonna effect") is interpreted by electrostatic interaction (proton transfer from the carboxylic group to the nitrogen) with important contribution from hydrogen bonding by the guest OH-group.

Keywords Belladonna effect · Calix[6]arene · Host–guest complex · Fluorescence marker · Dansyl group · Atropine

Introduction

Fluorescence spectroscopy is a powerful tool to investigate the host–guest molecular systems with both high sensitivity and often high selectivity [1]. Especially in the low concentration range receptor systems with fluorescence markers show remarkable advantages in comparison to other detection methods in general. The sensitivity of the fluorescence detection of host–guest molecular interaction depends upon the electronic or steric changes of the chromophore being responsible for the fluorescence behaviour. On the other hand, the strong environmental dependence the fluorescence behaviour organic or complex inorganic molecule complexes show makes the problem more complicated [2,3]. Only if the complexation are connected with a remarkable change in the fluorescence behaviour, the high sensitivity of fluorescence detection can be used really [4,5]. There already exist lots of systems, especially in the field of ion detection [6–12] and low molecular weight molecules [13–15], where the recognition of the guest molecule of a special kind is neither a general nor a problem of selectivity even at extreme low concentration. In cases when the guest molecules have higher molecular weight and are nonvolatile, electrostatic interactions between guest and host can result in formation of salt bridges in the liquid phase followed by significant fluorescence changes. Remarkable changes of the fluorescence intensity are shown in dansyl-modified cyclodextrin even at high molecular weight molecules like avidin or other steroids [16,17]. In this regard we have investigated a calix[6]arene labelled with dansyl amide with respect to its spectral properties in dependence on the inclusion of alkaloids (atropine, cocaine) to account for the sensory ability.

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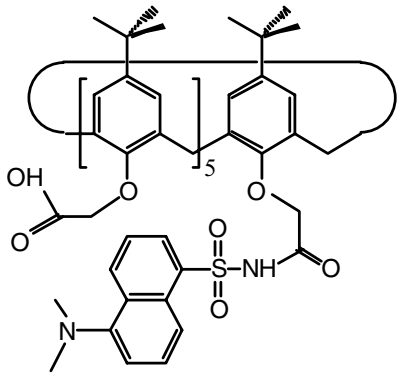
Experimental

Synthesis of the dansyl-linked calix[6]arene

Vacuum-dried 5,11,17,23,29,35-hexa-*tert*-butyl-37,38,39,41,40,42-hexakiscarbomethoxy calix[6]arene [1] (0.4 g,



Scheme 1. Calixarene structure

0.3 mmol) was dissolved in 20 mL dried carbon tetrachloride and treated with oxalyl chloride (1.6 mL, 10 equiv. per −COOH) for 12 hr at reflux temperature. The solvents were then evaporated under N_2-atmosphere. The residue was dissolved in dried tetrahydrofurane (thf). Under cooling with an ice bath, a mixture of triethylamine (0.5 mL) and dansylamide (Acros Organics, 0.5 g) in tetrahydrofuran (20 mL) was added dropwise under stirring. The mixture was stirred for 16 hr at ca. 320 K. After cooling, the precipitate was filtred off and the solvent was evaporated. The residue after evaporation was dissolved in dichloromethane (20 mL) and washed four times with excess amounts of 0.1 M HCl, follwed by repeated washing with H_2O. The organic phase was dried and the residue recrystallized from ethanol to give the mono-substituted product 5,11,17,23,29,35-hexa-*tert*-butyl-37-(5′-dimethyl aminonaphtalene-1′-sulfonylcarbamoylmethoxy)-38,39,41, 40,42-pentakiscarbomethoxycalix[6]arene, Scheme 1 (further called in this paper C6-DA for simplicity) [18a]. The NMR spectroscopy investigations give strong evidence that the synthesis results in the mono-substituted calix[6]arene only [18b].

Sample preparation and experimental details of the fluorescence investigations

The free dye, dansyl amide for reference measurements was purchased from ACROS Organics. Host–guest interaction was examined with atropine, an alkaloid which was purchased from Sigma Aldrich. Based on stock solutions of 0.1 mM calix[6]arene and atropine as well as cocaine hydrochloride of 20 mM prepared in acetonitrile the following investigations were accomplished.

First, a comparison of fluoresence intensity of bounded fluorophor on calix[6]arene with free dansyl amide was carried out to characterize the fluorescence behaviour of these. Absorption and emission were measured with same concentrations of 1.5×10^{-5} M in acetonitrile and are depicted in Fig. 1.

To examine alkaloid interactions with C6-DA a solution of 3 mL acetonitrile and 0.3 mL C6-DA of 0.1 mM were mixed and used as emission basic signal. Investigations with alkaloids were carried out with these solutions by adding 10–200 μL of 20 mM atropine or cocaine stock solution in acetonitrile. The fluorescence behaviour was measured with respect to the fluorescence intensity of the C6-DA basic signal. Furthermore, it was necessary to investigate the saturation effect of atropine on C6-DA. Therefore, atropine was added to the C6-DA basic signal up to a concentration where no changes in the fluorescence characteristics were measured.

To determine the equilibrium constant of atropine or cocaine complexation with C6-DA as well as the mechanism of the release of atropine from C6-DA by adding cocaine concentration, a series of measurements were made leaving the concentration of one analyte (atropine or cocaine) constant and changing the concentration of the other partner. The changed concentrations were chosen around the concentration of the other analyte.

Absorption spectra were taken on a Perkin–Elmer Lambda 2 UV-Vis spectrometer using quartz cuvettes with an optical pathway of 10 mm. Fluorescence measurements were carried out with a Perkin–Elmer LS-50B fluorescence spectrometer at room temperature using an excitation wavelength of 350 nm.

Results and discussion

The fluorescence behaviour of dansyl amide

Dansyl amide in solution shows an absorption maximum at 335 nm and an unstructured fluorescence band with a maximum at 505 nm. Binding of the dansyl amide to the calix[6]arene via a carbonyl group results in a bathochromic shift of the absorption by ca. 10 nm and a bathochromic shift of the fluorescence of about 20 nm leading to a fluorescence maximum of 525 nm as depicted in Fig. 1. The fluorescence intensity of C6-DA is decreasing to around 50% compared to the fluorescence intensity of the free dansyl amide taken at equal concentration. Adding atropine to a dansyl amide solution at equal and higher concentrations leads to no change in the fluorescence behaviour of the dansyl amide at all.

Calixarene–alkaloid interaction

The inclusion of atropine at the C6-DA-complex was measured in acetonitrile solution. Whereas the fluorescence of the dansyl marker at C6-DA concentrations around 10^{-5} M is at 525 nm (see Fig. 1), complexation with atropine leads to a strong hypsochromic shift with a fluorescence maximum at 470 nm connected with a further strong enhancement of

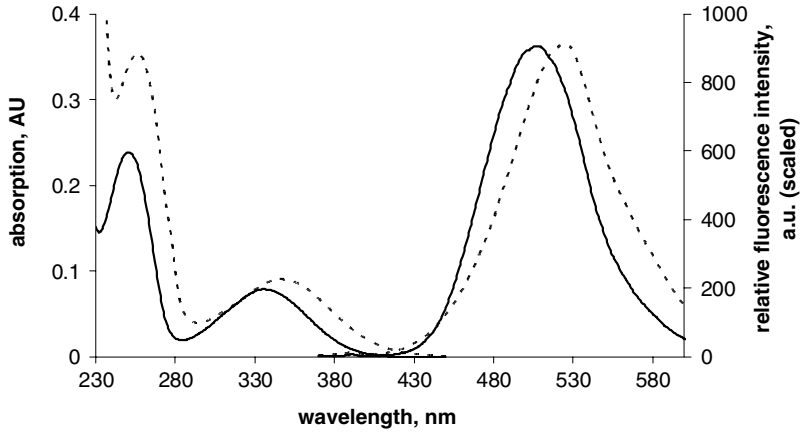


Fig. 1 Absorption and fluorescence spectra of the used calix[6]arene – dansyl complex (C6-DA) (fluorescence excitation wavelength – 350 nm)

the fluorescence intensity. The increase of the fluorescence intensity at an evaluation wavelength of 450 nm is appr. 30-fold (see Fig. 2). Such effects have never been described before in the literature on fluorescent calix[6]arenes. The saturation effect of the atropine inclusion into C6-DA is shown in Fig. 3. At increasing atropine concentration (at a C6-DA concentration of 10^{-5} M), a continuous shift and increase of the fluorescence yield is seen. In the insert the fluorescence intensity at 450 nm is depicted in dependence on the atropine concentration. The saturation is reached at the highest concentration. This is at much higher concentrations (200-fold excess) of atropine compared to the C6-DA concentration. At an 1:1 relation between the C6-DA molecule and atropine saturation is not yet seen. This result is in agreement with the NMR investigations of the complexation, where even at the highest atropine concentrations no unbounded atropine was found in the solution [15]. It seems that more than one atropine molecule could be attached to the rim of the C6-DA complex. Possible reasons for this behaviour are (i) the number of five carboxylate groups, each interacting with the *N*-atom of atropine, and (ii) the built-up of self-assembled, supramolecular structures to which the conformational flexibility of the host contribute. NMR investigations [18] show that C6-DA in the free and complexed state exists in alternate conformation.

Competition effect in calixarene–alkaloid interaction

Despite the structural similarity of atropine and cocaine only atropine can be recognized by fluorescence changes of the calixarene complex. Cocaine as guest molecule shows no changes in the emission spectrum (for comparison see Fig. 4 dashed line).

However, dissolving cocaine in an atropine-calixarene solution a bathochromic shift towards the basic signal of C6-DA is shown (see Fig. 5). It is assumed that cocaine also interacts by means of electrostatic and hydrophobic interactions with the host molecule. It can displace the COO^- NH^+ binding of atropine. However, cocaine does not cause a change in the fluorescence signal. We conclude, that one important binding site, that for signalling via H–bonding, is not available. Probably the lack of –OH group combined

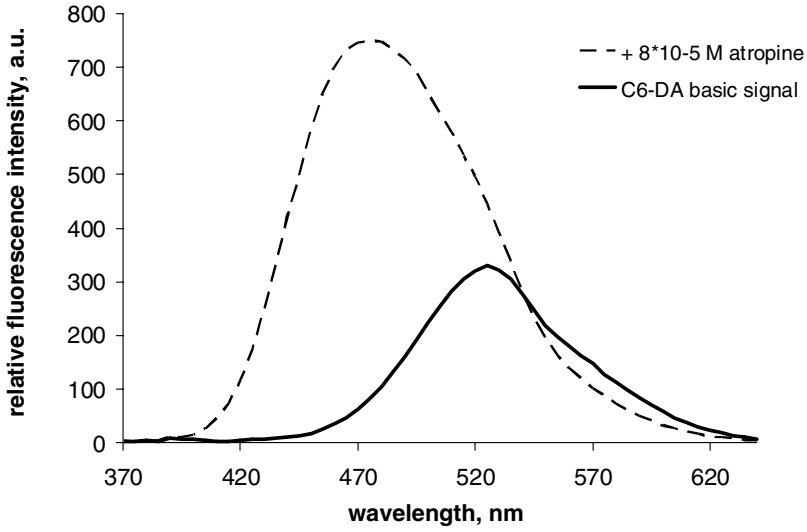


Fig. 2 Comparison of C6-DA spectrum (excitation wavelength 350 nm) (a) C6-DA basic signal (*full line*, fluorescence maximum at 525 nm) and (b) with 8 $\times$ 10^{-5} M atropine (*broken line*, fluorescence maximum at 470 nm)

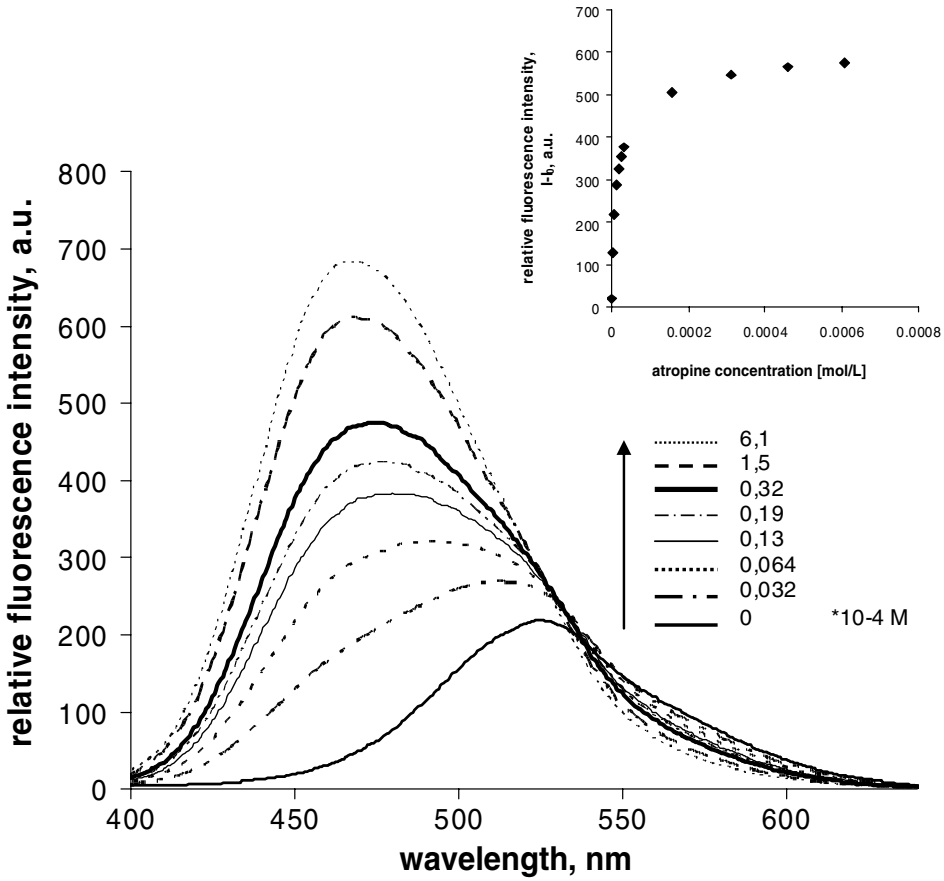


Fig. 3 Saturation effect of atropine inclusion: (a) C6-DA without atropine (*full line* below); (b) C6-DA with 0.32×10^{-5} M atropine (*dashed-dotted line*, below); (c) C6-DA with 0.64×10^{-5} M atropine (*dotted line*, below; (d) C6-DA with 1.3×10^{-5} M atropine (*continuous line* weak); (e) C6-DA with 1.9×10^{-5} M atropine (*dashed-dotted line* weak); (f) C6-DA with 3.2×10^{-5} M atropine (*continuous line* strong); (g) C6-DA with 1.58×10^{-4} M atropine (*dashed line*); and (h) C6-DA with 6.1×10^{-4} M atropine (*dotted line* weak) In the insert the fluorescence intensities at 450 nm is depicted showing the saturation effect more clearly

with the steric requirements of the ester group is responsible for the absence of fluorescence changes with cocaine as analyte. This effect is even more pronounced when tuning the cocaine concentration at fixed atropine concentration. At very high concentrations of cocaine ($>5 \times 10^{-4}$ M) the fluorescence spectrum is comparable to the uncomplexed C6-DA spectrum. On the other hand, changing the atropine concentration at fixed cocaine concentration leads to the usual saturation effect with reduced fluorescence intensity (see Fig. 4). Plotting the concentration dependence for both inclusion partners at fixed concentration of the other one gives two saturation curves with intercept point at an 1:1 complex (see Fig. 6). Work is under progress to estimate the complex formation constants of the

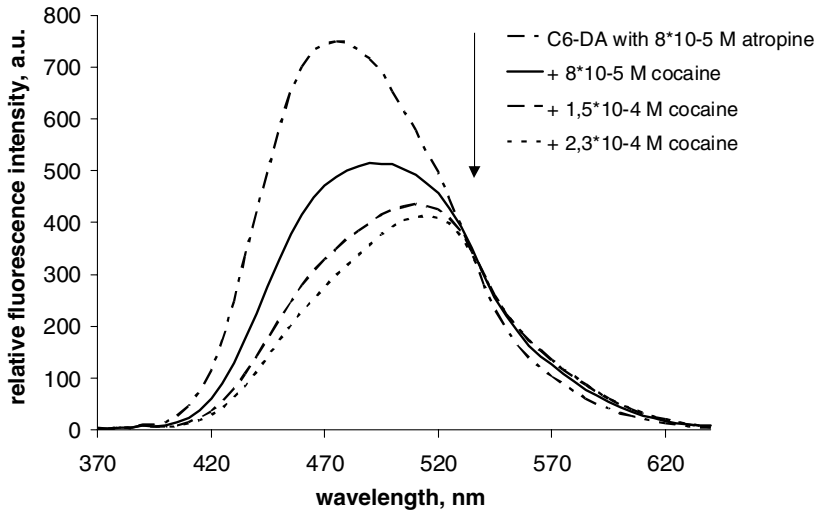


Fig. 4 Saturation effect of cocaine addition to the C6-DA complex: (a) C6-DA with 8×10^{-5} M atropine; (b) complex under (a) with 8×10^{-5} M cocaine; (c) complex under (a) with 1.5×10^{-4} M cocaine; (d) complex under (a) with 2.3×10^{-4} M cocaine

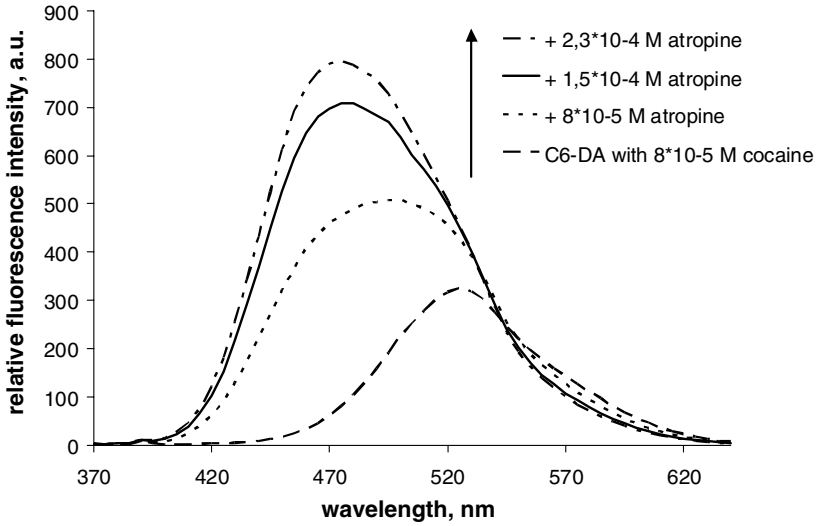


Fig. 5 C6-DA complex with cocaine and addition of various atropine concentrations: (a) C6-DA with 8×10^{-5} M cocaine; (b) complex under (a) with 8×10^{-5} M atropine; (c) complex under (a) with 1.5×10^{-4} M atropine; (d) complex under (a) with 2.3×10^{-4} M atropine

C6-DA–atropine complex as well as the respective C6-DA–cocaine complex [19].

Structural characteristics in alkaloid–calixarene interaction

In Fig. 7 the assumed structure of the complex between C6-DA and atropine is shown schematically, taking into consideration the COO^- NH^+ salt bridge formation between carboxylate and atropine, hydrogen bonding to the atropine –OH and C=O group, hydrophobic interactions and sterical conditions. Due to the lack of –OH group, the presence of an ester group and the different polarity (for general polarity effects upon the host–guest interaction see [20]) of the cocaine with respect to the atropine the displacement reaction produces no fluorescence change. The results can be understood in terms of an interaction between alkaloid NH^+ interaction with $-COO^-$, accompanied by hydrogen bonding to dansyl in case of atropine. In case of cocaine, hydrogen bonds are not formed and signalling of the complex formation does not occur. Under such a condition the partial release of atropine in presence of cocaine can be explained. Whereas the inclusion of atropine at the calixarene exceeds a 1:1-complex as concluded from the effect that even at the highest atropine concentrations no free atropine could be detected in solution, the release curves of atropine by competition of cocaine and vice-versa have a crossing at equal concentrations.

These results of atropine and cocaine inclusion at the calixarene host molecule give a hint of what kind the inclusion site and the complexation structure could be built of. While host–guest salt bridge formation is discussed to be mainly responsible for binding, double hydrogen bonding between guest and dansyl group is considered to be responsible for

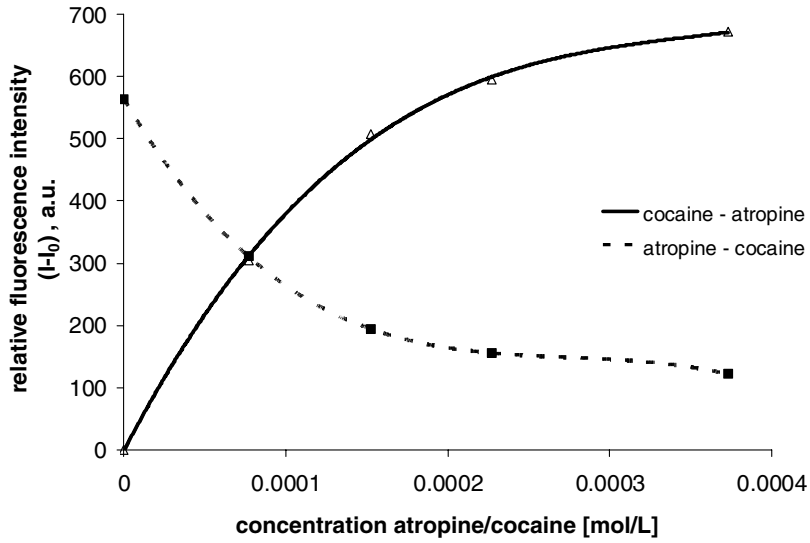


Fig. 6 Fluorescence intensity of the C6-DA complex with regard to the competition effect: (a) C6-DA complex with 8×10^{-5} M cocaine and various atropine concentrations (*full line*) and (b) C6-DA complex with 8×10^{-5} M atropine and various cocaine concentrations (*dashed line*)

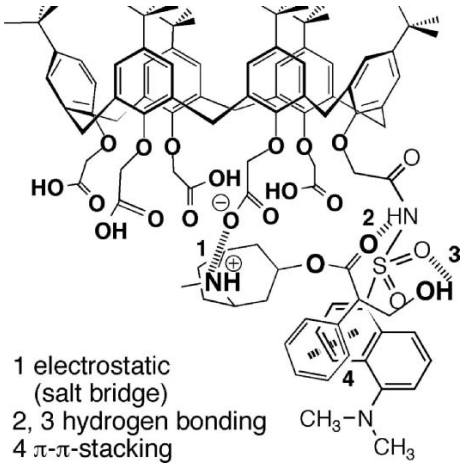


Fig. 7 Scheme of the C6-DA complex indicating the supposed sterical configuration of the C6-DA–atropine complex

the increasing fluorescence signal with increasing atropine concentration. In case of cocaine, the ester group may interfere with the hydrogen-bonding to the dansyl group, thus no change in fluorescence takes place. Or more general, among alkaloids, the host turned out to selectively respond to atropine, which seems to be due to the combination of multiple interactions at different binding sites. The proton transfer (between organic bases like atropine and calixarenes), followed by strong intermolecular electrostatic attraction seems to be a new possible kind of complex formation strategies in sensing of organic higher molecular compounds. This behaviour is even more pronounced when the calixarene is immobilized in polymer matrices to build-up a sensor chip [21]. Under such conditions the conformational behaviour of the complex is dependent on the free volume and the space for inclusion of guest molecules the host molecule has.

Summary

The investigation of the host–guest behaviour of C6-DA-alkaloid complexes shows quite different inclusion properties with respect to alkaloid molecules (atropine, cocaine). Among alkaloids, the C6-DA complex turned out to selectively respond to atropine, which seems to be due to the binding in terms of electrostatic interaction with a remarkable contribution from hydrogen-bonding by the guest OH-group. In our case, the electrostatic interaction seems to be connected with a proton transfer from the carboxylic group of the linking group to the nitrogen of the atropine. The multiple interaction with binding and signalling sites is a new kind of selective recognition system with high adaptability to the sensing of organic molecules.

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18a. Yield 93%; m.p. 170 - 173 °C; t.l.c. (SiO_2 , $CHCl_3$ /EtOH 9/1) R_f 0.4; NMR (ppm): σ_H 0.9 and 1.3 (br m, 54H, ^{t}Bu), 2.9 (br s, 6H, N-CH_3), 3.4 (br s) and 4.24 (br sh) CH_2-Ar (12H), 4.5 (br sh, 12H, O-CH_2), 6.75 to 7.6 (br) and 8.6 (br) H_{Ar} (18H); selected σ_C 44.9 (N-CH_3), 71.0 (O-CH_2-amide); MS (MALDI-TOF,) 1554 (LH^+); $C_{90}H_{108}N_2O_{19}$ S calc. C 69.6, H 7.0, N 1.8, S 2.06%, found C 69.3, H 6.96, N 2.0, S 2.1%.
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