A New Fluorescent Calixarene Dimer: Synthesis, Optical Properties, and Sensory Applications †

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Abstract: A new fluorogenic bis-calix[4]arene-carbazole compound (3) with an enlarged intramolecular cavity able to be involved in host–guest chemistry with large organic guests was designed. Its synthesis was accomplished for the first time using a Sonogashira–Hagihara cross-coupling reaction in the final step. The calixarene receptor was structurally characterized by FTIR and 1H/13C/2D NMR techniques and its photophysical properties evaluated. The ability of 3 to form supramolecular complexes with fullerenes (C\textsubscript{60} and C\textsubscript{70}) was evaluated through fluorometric titration experiments. The value of the binding constants ($K_{3:C60} = 1.39 \times 10^5 \text{ M}^{-1}$ and $K_{3:C70} = 6.88 \times 10^4 \text{ M}^{-1}$), and the free energy changes for the inclusion complexation ($\Delta G_{3:C60} = -29.33 \text{ kJ/mol}$ and $\Delta G_{3:C70} = -27.60 \text{ kJ/mol}$), revealed a high sensitivity of the calixarene-carbazole host for both fullerenes. The host molecule was shown to be particularly selective towards fullerene C\textsubscript{60}.

Keywords: calix[4]arene; carbazole; fluorescent; fullerene; host–guest chemistry

1. Introduction

Supramolecular chemistry has stimulated the attention of the scientific community over the last few decades. Inspired by nature and exploiting the scope of non-covalent interactions such as hydrogen bonding, electrostatics, and $\pi$-$\pi$ stacking, scientists have developed various synthetic systems with potential for application in different fields (e.g., smart materials, catalysts, optical sensors, complexing agents, and nanomedicine) [1,2]. In this context, several calixarene architectures, a benchmark in supramolecular chemistry [3,4], stand out as supramolecular hosts able to act as receptors for a wide range of guests (e.g., neutral and ionic molecular species). Some examples of highly sensitive and selective chemosensors for detection of explosives [5], pollutants [6], and proteins [7] based on fluorescent calixarene structures have been reported by our investigation group.

Stimulated by the increasing use of fullerene molecules in several areas, such as medical and biomedical [8] and advanced materials [9], the supramolecular chemistry of these compounds has experienced an enhancement in its research particularly in the development of selective methods for recognition in solution. Among various hosts for fullerene recognition, calix[n]arenes have played an important role since the pioneering work of Shinkai and Atwood groups [10,11]. Since calixarenes have a cavity composed of aromatic rings and fullerenes are covered by $\pi$-electrons, self-assembly through strong $\pi$-$\pi$ interactions may occur between them [12]. The good compatibility between spherical (C\textsubscript{60}) or ellipsoid (C\textsubscript{70}) fullerenes and ball-shaped cavity of calixarenes to form complexes both in solution and in the solid state has been extensively investigated [13,14].
Calixarene–fullerene complexation studies in solution usually rely on NMR and/or UV–Vis measurements [15–17], although fluorescence spectroscopy also been used [14,18].

In this communication, we report the preliminary results regarding the synthesis, structural characterization, photophysical properties, and fluorescence-based complexation studies of a new fluorescent calixarene-3,6-carbazole dimer (3; Scheme 1) with fullerenes $\text{C}_{60}$ and $\text{C}_{70}$.

2. Materials and Methods

2.1. Instruments and Methods

FTIR were measured on a Bruker Vertex 70 as KBr pellets (transmission mode). The $^1$H/$^{13}$C NMR spectra were collected on Bruker AVANCE II+ spectrometers (400 MHz); reported chemical shifts (δ/ppm) were internally referenced to CDCl₃ ($^1$H NMR, 7.26 ppm; $^{13}$C NMR, 77.16 ppm).

UV-Vis spectra were recorded on a VWR UV 3100PC or on a Jasco J-815 spectrophotometer using 1 cm quartz cells at 25 °C.

Steady-state fluorescence spectra were acquired on a Perkin Elmer LS45 fluorimeter using a 1 cm quartz cuvette at a right angle (RA) at 25 °C in air-equilibrated conditions.

Fluorometric solution experiments were carried out by titration of diluted solutions ($6 \times 10^{-7}$ M) of compound 3 in CH₂Cl₂ with known amounts of the analytes (fullerenes $\text{C}_{60}$ and $\text{C}_{70}$) using RA geometry. Fluorescence quantum yields were measured using 9,10-diphenylanthracene as fluorescence reference standard (Φ = 0.72, EtOH, air equilibrated conditions, RA) [19]. The quantum yields were determined by the slope method [20], keeping the optical densities (ODs) of the sample and reference below 0.05 at the excitation wavelength to prevent inner filter effects (IFEs).

The equilibrium constants for the supramolecular complexation (association or binding constants are referred to interchangeably throughout the text) were calculated by solving the following equation, assuming a 1:1 stoichiometry for the complex [21]:

$$
\Delta F = \frac{1}{2}\Delta \epsilon_F \left[\left[H\right]_0 + [G]_0 + 1/K_a\right] - \left[\Delta \epsilon_F^2 \left[\left[H\right]_0 + [G]_0 + 1/K_a\right]^2 - 4 \Delta \epsilon_F \left[\left[H\right]_0 [G]_0\right]\right]^{1/2},
$$

where $\Delta F$ and $\Delta \epsilon_F$ are the changes in fluorescence intensity and molar fluorescence intensity of the host upon complexation with fullerenes, $K_a$ is the association constant, and $[H]_0$ and $[G]_0$ denote the initial concentrations of the host and the guest, respectively. All input data from fluorescence titration measurements were previously corrected at the excitation and emission wavelengths for the hetero-inner-filter effects (h-IFEs) resulting from the analytes using the expression $F_{\text{corr}} = F_{\text{obs antilog}} \left[\frac{\text{OD}_{\text{exc}} + \text{OD}_{\text{em}}}{2}\right]$, where $\text{OD}_{\text{exc}}$ and $\text{OD}_{\text{em}}$ are the optical densities of the solutions at the excitation and emission wavelengths [22]. Calculations were performed by a non-linear regression analysis using the Solver function in Microsoft Excel [23], with a non-linear generalized reduced gradient (GRG) algorithm and a convergence criterion for $R^2 < 10^{-9}$.

The lowest-energy conformers for 3:$\text{C}_{60}$ and 3:$\text{C}_{70}$ complexes were obtained from a molecular mechanics method (Monte Carlo method, MMFF94 force field) as implemented in Spartan’18 computational software [24].

2.2. Materials

Calix[4]arene-tripropyl-mono-iodo derivative 1 was obtained by selective mono-iodination of the corresponding 25-hydroxy-26,27,28-tripropoxy-calix[4]arene [25] by an adapted synthetic procedure [26], and the details of its synthesis will be presented elsewhere. The 3,6-Diethynyl-9-propyl-9H-carbazole (2) [27] was synthesized according to our reported method. Both compounds were fully characterized by FTIR, UV-Vis, and NMR spectroscopies.

Dichlorobis(triphenylphosphine)palladium (II) (98%, Aldrich), copper(I) iodide (98%, Aldrich), Fullerene-$\text{C}_{60}$ (Aldrich, 99.5%), Fullerene-$\text{C}_{70}$ (Aldrich, 98%), and 9,10-diphenylanthracene (scintillation grade, Nuclear Enterprises Ltd.) were used as received.
Triethylamine (99%, Riedel-de-Haën) was previously dried from CaH₂ and distilled under N₂ prior to use. Toluene was previously dried from Na, distilled under N₂, and stored over Na. All other reagents and solvents were reagent grade and were purified and dried by standard methods. Organic extracts were dried over anhydrous magnesium sulphate.

2.3. Synthesis

The synthesis of 3 was accomplished by a Sonogashira–Hagihara cross-coupling methodology. Full experimental details will be presented elsewhere. After purification by flash chromatography, compound 3 was isolated as a light-yellow solid. The isolated fraction exhibits a cone conformation as shown by the NMR duplets of the equatorial [3.22 (d, 4H, ArCH₂Ar, J = 13.1 Hz) and 3.34 (d, 4H, ArCH₂Ar, J = 13.7 Hz)] and axial [4.38 (d, 4H, ArCH₂Ar, J = 13.9 Hz) and 4.42 (d, 4H, ArCH₂Ar, J = 13.2 Hz)] protons. The NMR spectrum of the crude reaction mixture also revealed the presence of other conformers, namely the partial cone conformer, which are currently being investigated.

3. Results and Discussion

3.1. Synthesis and Structural Characterization

The new fluorogenic calix[4]arene-carbazole dimer 3 was synthesized from calix[4]arene-tripropyl-mono-iodo derivative 1 [25,26], by a Sonogashira–Hagihara cross-coupling reaction with 3,6-diethynyl-9-propyl-9H-carbazole (2) [27] in dried toluene and NEt₃, using PdCl₂(PPh₃)₂ and CuI as catalytic system under argon (Scheme 1).

![Scheme 1](image)


To minimize the self-condensation of carbazole units, the compound was slowly added to 1 from the onset of the reaction. By this procedure, cleaner reaction mixtures and higher isolated yields were obtained. The resultant solid is freely soluble in CH₂Cl₂, CHCl₃, THF, cyclohexane, and toluene.

The structural characterization by FTIR and ¹H/¹³C and 2D NMR analysis fully corroborated the proposed structure for compound 3. From FTIR analysis the absence of terminal ethynyllic C≡C-H stretching vibrations characteristic of 3,6-CBZ carbazole unit [27] and the simultaneous presence of internal alkyne frequencies at 2203 cm⁻¹ was discernible. The cone conformation of the calixarene units in dimer 3 was ascertained by the presence of a set of characteristic resonances for the protons of bridged methylene groups in the calixarene skeleton in the ¹H NMR spectrum [3.22 ppm (4H, d, J = 13.1 Hz), 3.34 ppm (4H, d, J = 13.7 Hz) for equatorial protons and 4.38 ppm (4H, d, J = 13.9 Hz), 4.42 ppm (4H, d, J = 13.2 Hz) for axial protons].
3.2. Photophysical Properties

The photophysical properties of dimer 3 were studied by UV-Vis and fluorescence spectroscopies, and its ground-state absorption and steady-state luminescence spectra are shown in Figure 1. The absorption profile exhibits a peak around 320 nm at its absorption maxima, with a shoulder near 344 nm. The emission spectra revealed the most prominent band peak at 407 nm. The quantum yield ($\Phi_F$) of 3 is strongly dependent on the solvent nature. The lowest $\Phi_F$ was retrieved for CHCl₃ ($\Phi_F = 0.028$), followed by CH₂Cl₂ ($\Phi_F = 0.13$), THF ($\Phi_F = 0.18$), and cyclohexane ($\Phi_F = 0.20$). A great stability toward photobleaching was witnessed for compound 3 in CH₂Cl₂, the solvent used in the titration experiments with fullerenes.

Figure 1. Absorption ($2.5 \times 10^{-5}$ M) and fluorescence ($6.0 \times 10^{-7}$ M, $\lambda_{ex} = 340$ nm) spectra of dimer 3 in CH₂Cl₂.

3.3. Complexation Studies with Fullerenes

The ability of host 3 to form inclusion complexes with two fullerenes (C₆₀ and C₇₀) was assessed through fluorometric titrations in CH₂Cl₂ (a solvent choice trade-off between securing a reasonable quantum yield for 3 and solubilizing the fullerenes). The experiments were conducted with the guests in a concentration range of $2.04 \times 10^{-6}$–$2.21 \times 10^{-5}$ M, while keeping that of the fluorophore constant ($6.0 \times 10^{-7}$ M) (Figure 2). Since both guests display absorption of radiation at the excitation (340 nm) and emission wavelengths (407 nm), correction for h-IFEs was applied (cf. Experimental section for details). Raw titration data for 3 with C₆₀ and C₇₀, and the corresponding curve-fitting plots, are shown in Figure 2.

Considering a neglectable dynamic quenching component for the system, and a 1:1 host-to-guest equilibrium, the association constants of the complexes were retrieved. In either case, the goodness of the fits ($R^2 = 0.9806$ for C₆₀ and 0.9852 for C₇₀) indicates that a 1:1 stoichiometry was attained in the supramolecular complexes. Several remarks are in order. First of all, the binding affinities of calixarene 3 to both fullerenes are remarkable and likely the highest ever reported for calix[4]arene-based hosts. Secondly, the binding of C₆₀ to 3 is considerably larger ($K_a = 1.39 \times 10^5$ M⁻¹) than that of C₇₀ ($K_a = 6.88 \times 10^4$ M⁻¹), making 3 a selective host for C₆₀ by a factor of around two. The favorable interaction of 3 with C₆₀ may be also evaluated by the free energy change ($\Delta G$) associated with the complex formation ($\Delta G = -29.33$ kJ/mol for 3:C₆₀ complex and $\Delta G = -27.60$ kJ/mol for 3:C₇₀ complex).
Figure 2. Emission spectra of 3 (6.0 × 10^{-7} M in CH2Cl2) after successive additions (2.04 × 10^{-6} - 2.21 × 10^{-5} M) of fullerene C60 (a) and C70 (b); curve-fitting plots for C60 (c) and C70 (d) derived from a non-linear regression analysis of the fluorescence data (λ_{exc} = 340 nm).

The putative structures of the complexes of 3 with the fullerene guests, obtained from conformational searches (Monte Carlo method, MMFF94 force field), are depicted in Figure 3.

Figure 3. Best conformers of C60 (a) and C70 (b) complexes with calixarene 3, after Monte Carlo/MMFF94 molecular mechanics calculations [24].

4. Conclusions

A new molecular receptor-based fluorescent bis-calix[4]arene-carbazole compound was synthesized, and its ability to selectively binding C60 and C70 fullerenes was evaluated. A remarkable affinity for both fullerenes was found, as determined by fluorescence assays. It was also unveiled that the host 3 is quite sensitive to the molecular properties
(molecular volume and electronic environment) of the two fullerenes. As a result, 3 can discriminate between the two fullerenes (selectivity ratio $C_{60}/C_{70} = 2$).

No rationale for the higher binding affinity of compound 3 to $C_{60}$ over $C_{70}$ is presented here. Density functional theory (DFT) calculations at a significant level of theory (e.g., B3LYP-D3, wB97X-V, and M06-2X functionals with a large basis set (6-311+G(2df, 2p)) are currently under investigation to enlighten such observed selectivity. Moreover, the current study has already been extended to other bis-calix[4]arene-carbazole conjugates, namely those synthesized from 2,7-diethynyl-9-propyl-9H-carbazole units, which will certainly lead to different calixarene architectures (larger available space between the expected binding sites of calixarene moieties), with new foreseen supramolecular inclusion properties towards a variety of large organic and organometallic guests. All the above results will be published elsewhere.

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