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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 $^{1}H/^{13}C$/2D NMR techniques and its photophysical properties evaluated. The ability of **3** to form supramolecular complexes with fullerenes (C_{60} and C_{70}) was evaluated through fluorimetric titration experiments. The value of the binding constants ($K_{3:C_{60}}$ = 1.39x10^5 M^{-1} and $K_{3:C_{70}}$= 6.88x10^4 M^{-1}), and the free energy changes for the inclusion complexation ($\Delta G_{3:C60}$ = −29.33 kJ/mol and $\Delta G_{3:C70}$ = −27.60 kJ/mol), revealed a high sensitivity of the calixarene-carbazole host for both fullerenes. The host molecule shown to be particularly selective towards fullerene C_{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 π–π 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], stands 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 calixarenes 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 have experienced an enhancement in its research particularly in the development of selective methods for their 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 π-electrons, self-assembly through strong π–π interactions may occur between each other [12]. The good compatibility between spherical (C_{60}) or ellipsoid (C_{70}) fullerenes and ball-shaped cavity of calixarenes to form complexes both in solution and in the solid state, have 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 C_{60} and C_{70}.

2. Materials and Methods

2.1. Instruments and Methods

FTIR were measured on a Bruker Vertex 70 as KBr pellets (transmission mode). $^{1}H/^{13}C$ NMR spectra were collected on Brüker AVANCE II+ spectrometers (400 MHz); reported chemical shifts (δ/ppm) are internally referenced to $CDCl_3$ (^{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 in right angle (RA) at 25 °C in air-equilibrated conditions.

Fluorimetric solution experiments were carried out by titration of diluted solutions (6×10^{-7} M) of compound **3** in CH_2Cl_2 with known amounts of the analytes (fullerenes C_{60} and C_{70}) using RA geometry. Fluorescence quantum yields were measured using 9,10-diphenylanthracene as fluorescence reference standard ($\Phi = 0.72$, 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 will be used interchangeable throughout the text) were calculated by solving the following equation, assuming a 1:1 stoichiometry for the complex [21]:

$\Delta F = ½\{\Delta\varepsilon_F([H]_0 + [G]_0 + 1/K_a) - [\Delta\varepsilon_F^2([H]_0 + [G]_0 + 1/K_a)^2 - 4\Delta\varepsilon_F^2[H]_0[G]_0]^{1/2}\}$, where ΔF and $\Delta\varepsilon_F$ 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 $F_{corr} = F_{obs}$ antilog $[(OD_{exc} + OD_{em})/2]$, where OD_{exc} and OD_{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**:C_{60} and **3**: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-tripropoxycalix[4]arene [25] by an adapted synthetic procedure [26] and the details of its synthesis will be presented elsewhere. 3,6-Diethynyl-9-propyl-9*H*-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-C_{60} (Aldrich, 99.5%), Fullerene-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 CaH_2 and distilled under N_2 prior to use. Toluene was previous dried from Na, distilled under N_2 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.

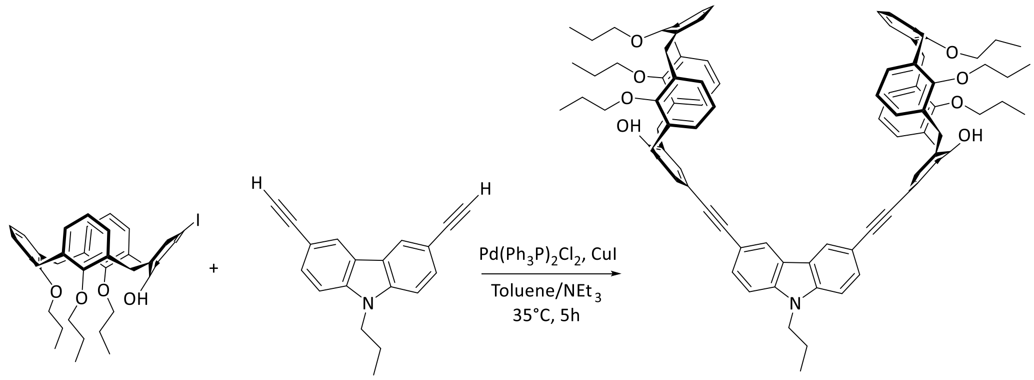
2.3. Synthesis

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, $ArCH_2Ar$, *J* = 13.1 Hz) and 3.34 (d, 4H, $ArCH_2Ar$, *J* = 13.7 Hz)] and axial [4.38 (d, 4H, $ArCH_2Ar$, *J* = 13.9 Hz) and 4.42 (d, 4H, $ArCH_2Ar$, *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-9*H*-carbazole (**2**) [27] in dried toluene and NEt_3, using $PdCl_2(PPh_3)_2$ and CuI as catalytic system under argon (Scheme 1). 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 CH_2Cl_2, $CHCl_3$, THF, cyclohexane and toluene.



1 **2** **3**

Scheme 1. Cross-coupling of calix[4]arene-tripropyl-mono-iodo derivative (**1**) and 3,6-diethynyl-9-propyl-9*H*-carbazole (**2**).

The structural characterization by FTIR and $^1H/^{13}C$ and 2D NMR analysis fully corroborated the proposed structure for compound **3**. From FTIR analysis the absence of terminal ethynylic 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 showed 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 (Φ_F) of **3** is strongly dependent on the solvent nature. The lowest Φ_F was retrieved for $CHCl_3$ (Φ_F = 0.028), followed by CH_2Cl_2 (Φ_F = 0.13), THF (Φ_F = 0.18) and cyclohexane (Φ_F = 0.20). A great stability toward photobleaching was witnessed for compound **3** in CH_2Cl_2, the solvent used in the titration experiments with fullerenes.

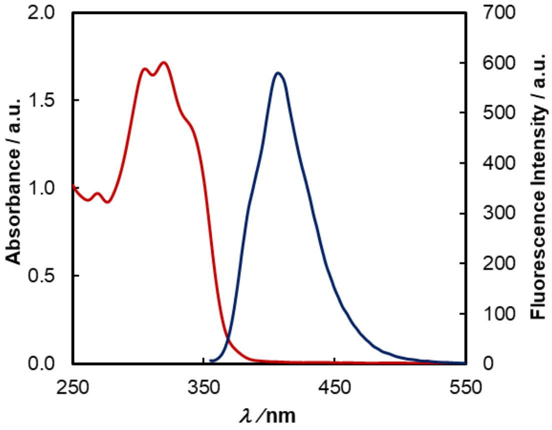
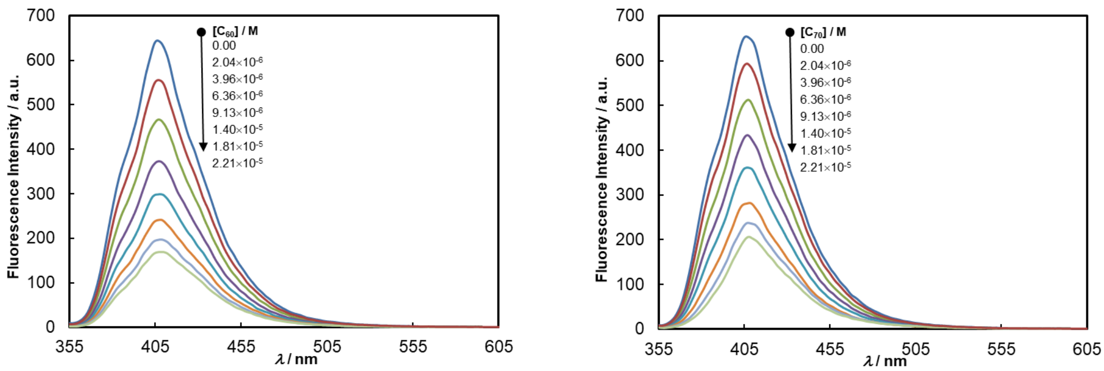


Figure 1. Absorption (2.5×10^{-5}M) and fluorescence (6.0×10^{-7} M, λ_{exc} = 340 nm) spectra of dimer **3** in CH_2Cl_2.

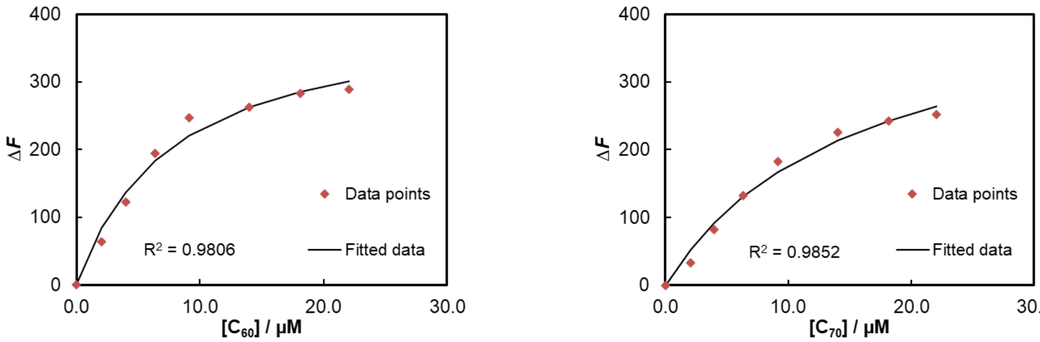
3.3. Complexation Studies with Fullerenes

The ability of host **3** to form inclusion complexes with two fullerenes (C_{60} and C_{70}) was assessed through fluorimetric titrations in CH_2Cl_2 (a solvent choice trade-off between securing a reasonable quantum yield for **3** and get the fullerenes solubilized). The experiments were conducted with the guests in a concentration range of 2.04×10^{-6}–2.21×10^{-5} M, while keeping that of the fluorophore constant (6.0×10^{-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 C_{60} and C_{70}, 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 C_{60} and 0.9852 for C_{70}) 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 higher ever reported for calix[4]arene-based hosts. Secondly, the binding of C_{60} to **3** is considerable larger ($K_a = 1.39 \times 10^5$ M^{-1}) than that of C_{70} ($K_a = 6.88 \times 10^4$ M^{-1}), making **3** a selective host for C_{60} by a factor around two. The favourable interaction of **3** with C_{60} may be also evaluated by the free energy change (ΔG) associated with the complexes' formation ($\Delta G = -29.33$ kJ/mol for **3**:C_{60} complex and $\Delta G = -27.60$ kJ/mol for **3**:C_{70} complex).



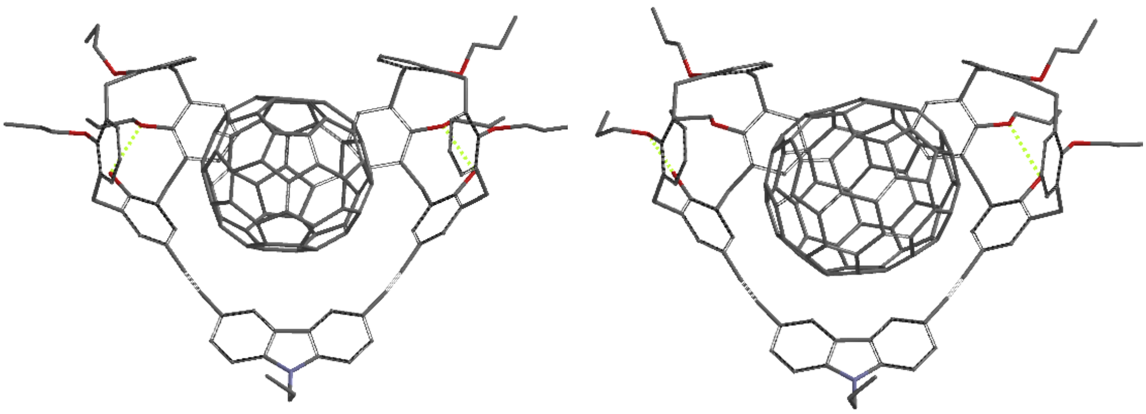
(**a**) (**b**)



(**c**) (**d**)

Figure 2. Emission spectra of **3** (6.0×10^{-7} M in CH_2Cl_2) after successive additions (2.04×10^{-6}–2.21×10^{-5} M) of fullerene C60 (**a**) and C70 (**b**); Curve-fitting plots for C_{60} (**c**) and C_{70} (**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.



(**a**) (**b**)

Figure 3. Best conformers of C_{60} (**a**) and C_{70} (**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 C_{60} and C_{70} 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} was 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-9*H*-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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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text. For example, explanations of experimental details that would disrupt the flow of the main text, but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Appendix B

All appendix sections must be cited in the main text. In the appendixes, Figures, Tables, etc. should be labeled starting with 'A', e.g., Figure A1, Figure A2, etc.

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