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New blue light-emitting isocyanobiphenyl based fluorophores: Their solvatochromic and biolabeling properties

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ABSTRACT

The preparation and optical study of 4-amino-4′-isocyanobiphenyl (ICAB) and its mono-and dialkylated derivatives (monoMICAB and diMICAB) are reported. They were found to be effective blue light emitters with a solvatochromic range of $\Delta\lambda_{max}$ = 104 nm, 92 nm and 90 nm, respectively. All three compounds turned out to be promising candidates in protein-labeling, yielding ~70–190× increase in their fluorescence intensity when mixed with 10× molar excess of bovine serum albumin (BSA). The binding constants for the BSA-fluorophore complexes were found to be $K_{ICAB\text{-}BSA} = 3.8 \times 10^4 \pm 5.7 \times 10^3\ M^{-1}$, $K_{monoMICAB\text{-}BSA} = 6.0 \times 10^4 \pm 3.9 \times 10^3\ M^{-1}$ and $K_{diMICAB\text{-}BSA} = 2.3 \times 10^4 \pm 3.2 \times 10^3\ M^{-1}$. Time resolved fluorescence measurements revealed a nearly uniform ~2 ns lifetime for all three BSA-complexed ICAB derivatives.

1. Introduction

Solvatochromic fluorescent dyes have attracted great attention from both theoretical and practical points of view since the color of their emitted light is sensitive to the polarity of their environment [1–3]. In addition, their optical properties are extremely dependent upon not only the bulk, but even their microenvironment, therefore they are particularly suitable for the use in fluorescent microscopy and other molecular imaging techniques to visually separate the different parts of biomolecules (e.g., cells, proteins, tissues, tumors) [4,5]. As a class of smart materials their field of application also covers optical chemical sensors, molecular electronics, smart display technology, photovoltaics, optical nonlinear systems, etc. Furthermore, fluorescence quenching offers an even more sensitive qualitative and quantitative analysis of appropriate quencher molecules [6] and even structural information can also be obtained [7].

Solvatochromic fluorophores are generally bipolar molecules with charge transfer between a donor (e.g., amino, hydroxyl) and an acceptor (e.g., carbonyl, sulfonyl) group on an aromatic core. Greater difference in the dipole moments of the ground and excited states results in a more pronounced solvatochromic effect owing to the solvent rearrangement around the excited fluorophore. One way to increase this dipole moment difference can be accomplished by elongating the dipole axis by using a larger aromatic core without altering the donor and the acceptor groups as Kucherak et al. demonstrated by exchanging the naphthalene core of PRODAN [8] to fluorene [9]. The Stokes-shift of the resulting dye turned out to be almost double than that of the original.

Solvatochromic dyes are perfect choice for labeling proteins, cells and other biomolecules. They can simply incorporate to a feasible site (binding sites, hydrophobic pockets) of the macromolecule [8,10,11], or can be attached to a specific amino acid unit, such as in the case of acrylodan (the acryl derivative of PRODAN), which forms strong covalent bond with the free thiol group of cysteine [12,13]. The change in the emitted fluorescent light indicates the alteration of the environment around the fluorophore, which is helpful for monitoring conformational changes in labeled biological macromolecules, or to study protein binding sites. Bovine serum albumin (BSA) and human serum albumin (HSA) are widely used model proteins with well-known structures to test the labeling capability of dyes.

Recently we reported the discovery of a new solvatochromic compound family based on 1-amino-5-isocyanonaphthalene (ICAN), where the donor group is amino and the acceptor group is isonitrile [14]. Our aim was to replace the naphthalene ring of ICAN to biphenyl as a cheap and effective alternative to improve the solvatochromic properties. Benzidine (4,4′-diaminobiphenyl)

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was selected as starting material because benzidine and its derivatives were widely used in the industry for the synthesis of dyes and pigments, however their application have been limited due to safety and health regulations since the 2000s. In addition to its easy availability preliminary quantum chemical calculations showed a significantly increased dipole moment in the excited state compared to that of ICAN.

Hereby we report the preparation and optical study of 4-amino-4′-isocyanobiphenyl (ICAB) and its mono-and dialkylated derivatives. In addition the applicability of the ICAB derivatives in the field of biolabeling using BSA as model protein is demonstrated.

2. Experimental

2.1. Materials

Acetone, chloroform, dichloromethane (DCM), ethyl acetate (EtOAc), hexane, toluene, (reagent grade, Molar Chemicals, Hungary) were purified by distillation. Acetonitrile (MeCN), tetrahydrofuran (THF), methanol (MeOH), dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), pyridine (HPLC grade, VWR, Germany), cyclohexane, 1,4-dioxane, 4,4′-diaminobiphenyl (reagent grade, Reanal, Hungary), methyl iodide (Sigma–Aldrich, Germany) were used without further purification. Bovine Serum Albumin (BSA, Cell Culture Grade, pH 7.0, Lyophilized Powder, GE Healthcare Life Sciences, USA) was used as received.

2.2. Synthesis

2.2.1. 4-Amino-4′-isocyanobiphenyl (ICAB)

A 48 ml ACE pressure tube was charged with 4,4′-diaminobiphenyl (2.00 g, 10.9 mmol) dissolved in chloroform (15 ml) and with potassium hydroxide (3.00 g, 53.5 mmol) dissolved in water (10 ml) and vigorously stirred with a magnetic stirrer at 120 °C for 3 days. After cooling down, the organic phase was separated, washed with water 3 times, dried on anhydrous magnesium sulfate and the solvent was removed on a rotary evaporator. The crude product was purified on a column filled with normal-phase silica gel, using dichloromethane as eluent. Yield: 0.53 g, 25% (pale yellow powder).

^{1}H NMR (360 MHz, $CDCl_3$) δ = 7.52 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 4H), 6.75 (d, J = 8.4 Hz, 2H), 3.81 (s, 2H) ppm.

^{13}C NMR (95 MHz, $CDCl_3$) δ = 160.1 (C_{NC}), 143.6 ($C_{4,1'}$), 139.4 (C_1), 127.0 ($C_{4'}$), 125.8 ($C_{3',5'}$), 124.7 ($C_{2',6'}$), 124.5 ($C_{3,5}$), 113.6 ($C_{2,6}$) ppm.

ESI-TOF MS (m/z): calculated for $C_{13}H_{10}N_2$: 195.092; found: 195.092 ($[M+H]^+$).

2.2.2. 4-Isocyano-4′-methylaminobiphenyl (monoMICAB) and 4-dimethylamino-4′-isocyanobiphenyl (diMICAB)

A 50 ml round-bottom flask was charged with 4-amino-4′-isocyanobiphenyl (0.200 g, 1.03 mmol), methyl iodide (192 μl, 3.09 mmol), potassium hydroxide (0.28 g, 5.00 mmol) dissolved in dry dimethyl formamide (20 ml), and the reaction mixture was stirred with a magnetic stirrer for 4 days at room temperature. The solution was diluted with dichloromethane, and washed with brine 3 times. The organic phase was dried on anhydrous magnesium sulfate and the solvent was removed on a rotary evaporator. The crude product was purified on a column filled with normal-phase silica gel, using dichloromethane:hexane 1:1 (V/V) as eluent. Yield: 60 mg monoMICAB (27%) and 62 mg diMICAB (28%). Both compounds were obtained as white powder.

monoMICAB: ^{1}H NMR (360 MHz, $CDCl_3$) δ = 7.53 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 3.89 (s, 1H), 2.88 (s, 3H).

^{13}C NMR (91 MHz, $CDCl_3$) δ = 163.8 (C_{NC}), 149.5 ($C_{4,1'}$), 142.5 (C_1), 128.8 ($C_{4'}$), 128.1 ($C_{3',5'}$), 127.8 ($C_{2'}$), 126.9($C_{6'}$), 126.6 ($C_{3,5}$), 112.7 ($C_{2,6}$), 30.6 (C_{Me}).

ESI-TOF MS (m/z): calculated for $C_{14}H_{12}N_2$: 209.107; found: 209.107 ($[M+H]^+$).

diMICAB: ^{1}H NMR (360 MHz, $CDCl_3$) δ = 7.57 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 6.82 (d,J = 8.6 Hz, 2H), 3.03 (s, 6H).

^{13}C NMR (91 MHz, $CDCl_3$) δ = 163.8 (C_{NC}), 150.5 ($C_{4,1'}$), 142.4 (C_1), 127.85 ($C_{3',5'}$), 127.60 ($C_{2'}$), 126.86 ($C_{6'}$), 126.59 ($C_{3,5}$), 125.83 ($C_{4'}$), 112.61 ($C_{2,6}$), 40.37 (C_{Me}).

ESI-TOF MS (m/z): calculated for $C_{15}H_{14}N_2$: 223.123; found: 223.123 ($[M+H]^+$).

3. Methods

3.1. NMR

^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 25 °C on a Bruker AM 360 spectrometer at 360 MHz (^{1}H) or 90 MHz (^{13}C) with tetramethylsilane as the internal standard.

3.2. ESI-TOF MS

ESI-TOF MS measurements were performed with a BioTOF II instrument (Bruker Daltonics, Billerica, MA). The concentration of the samples were 0.02 mg/ml. The solutions were introduced directly into the ESI source with a syringe pump (Cole-Parmer Ins. Comp.) at a flow rate of 2 μl/min. The temperature of the drying gas (N_2) was maintained at 100 °C. The voltages applied on the capillary entrance, and the second skimmer were −4500 V and 30 V, respectively.

3.3. UV–vis

The UV–vis spectra were recorded on a HP 8453 diode array spectrophotometer in a quartz cuvette of 1 cm optical length. For UV–vis measurements the investigated compounds were dissolved in the solvents at a concentration of 0.20 mg/ml and was diluted to 0.004 mg/ml and 0.0008 mg/ml. 3.00 ml solution was prepared from the sample.

3.4. Fluorescence measurements

Steady-state fluorescence measurements were carried out using a Jasco FP-8200 fluorescence spectrophotometer equipped with a Xe lamp light source. The excitation and emission spectra were recorded at room temperature, using 2.5 nm excitation, 5.0 nm emission bandwith, and 200 nm/min scanning speed. 3.00 ml solution was prepared from the sample with a concentration of 0.0008 mg/ml. Fluorescence quantum yields were calculated by using quinine sulfate in 0.1 mol/l sulfuric acid as the reference absolute quantum efficiency (Φ_n = 55%).

Laser flash photolysis experiments have been carried out in an Applied Photophysics LKS.60 nanosecond transient absorption spectrometer, equipped with a Quantel Brilliant Nd:YAG laser along with its second and third harmonic generator. Third harmonic was used, which emits at 355 nm.

3.5. Determinations of the binding constants

1.373×10^{-4} M BSA stock solution was prepared in distilled water at 25 °C. Proper amounts of the BSA stock solution were diluted to a final volume of 3 ml. The concentration range of BSA was set between (3.43×10^{-6} and 3.43×10^{-5} M) to be in ∼1–10 molar equivalent range relative to the dyes. Next 10 μl acetonitrile stock solution (0.2 mg/ml) of the dyes were added, were equilibrated for 15 min and their fluorescence spectra were recorded. The excitation wavelengths were 314 nm, 335 nm and