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Fluorescence and UV/Vis spectroscopic behaviour of novel biindolizines

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Abstract

Indolizines, known as a useful class of fluorophores, were bridged yielding biindolizines. We intended to obtain long wavelength absorbing and emitting fluorescent systems suitable for fluorescence labeling of biomolecules. The influence of the different kinds of coupling on the absorption and fluorescence behaviour of the resulting biidolizines was studied. The new fluorophore systems were characterized spectroscopically by their absorption and emission maxima and their quantum yields.

Keywords: Dimerisation; Bridged indolizines; Extended π-systems; Fluorophores; UV/Vis; Fluorescence spectroscopy

1. Introduction

During the last few years, considerable attention has been devoted to molecular systems containing switchable functionalities [1]. These functionalities enable, for example, supramolecules to react reversibly on a photochemical or electrochemical input by changing characteristics such as size, shape, electronic structure and complexation behaviour [2,3].

Recently, we reported on biindolizines as stable, two-step redox systems [4] with well defined electrochemical behaviour as part of such macrocyclic systems [5,6]. Considering the well known fluorescence properties of indolizines [7] and the increasing importance of fluorescence spectroscopy in both biological and environmental analysis, we were interested in the synthesis of biindolizine systems suitable for use in fluorometric analysis. In particular, interest focused on derivatives bearing reactive substituents as binding sites for further functionalisation that might be useful as building blocks. This paper concerns the synthesis of bis (hydroxyethyl)biindolizines and their derivatives (Fig. 1) to obtain long wavelength absorbing and emitting systems with high quantum efficiencies.

2. Experimental

2.1. General

NMR data were recorded using a Unity Plus-500 spectrometer ($SIMe_4$ as internal standard, coupling constants were taken directly from the

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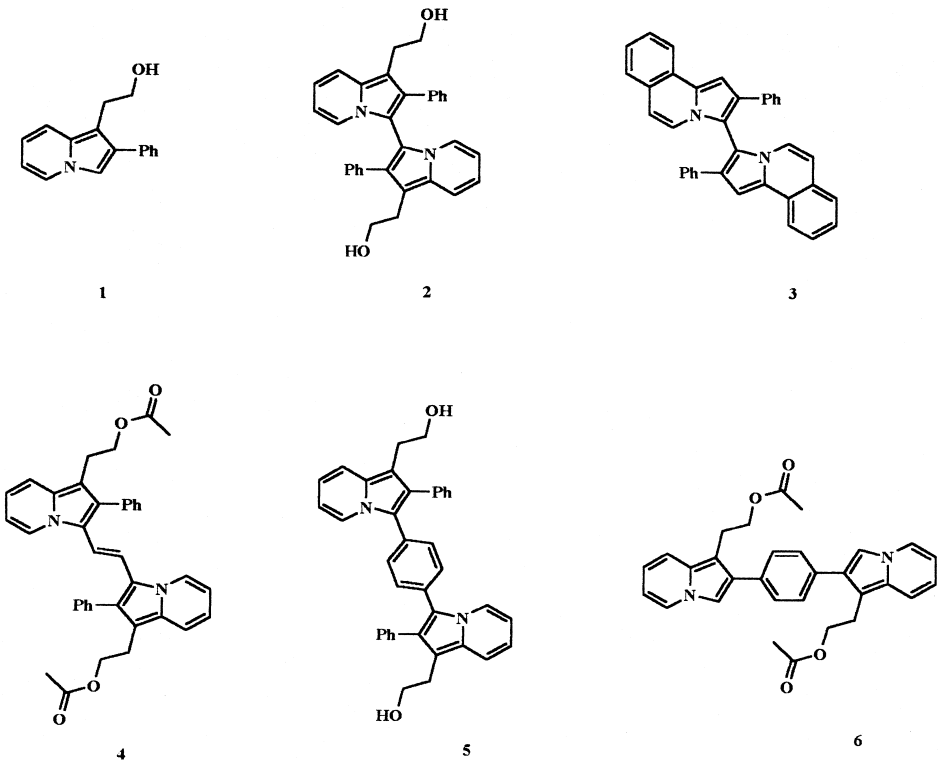

Fig. 1. Fluorophore systems **1**–**6**.

obtained spectra). Mass spectra were run on a MAT-95 with a ESI II/APCI (Finnegan MAT, Bremen) or on a Hewlett-Packard 5985 B (electron impact, 70 eV, FAB–MS). Melting points are uncorrected (Boëtius apparatus). TLC was carried out on plastic plates coated with silica gel 60 F_{254} (5735) (Merck) and flash chromatography was undertaken using Merck silica gel 60 (7731).

2.2. Spectroscopic studies

Fluorescence spectra in acetonitrile were measured using a Perkin-Elmer LS5O spectrometer (slit width 5 nm for excitation and emission), utilising a four-sided 1 cm quartz cell at room temperature using right angle geometry, and are corrected for spectral response of the detection system. Absorption spectra of solutions in acetonitrile were recorded on a Carl Zeiss Specord M400. For the determination of quantum yields, quinine bisulfate in 0.1 M H_2SO_4 was used as reference. The absorbances at the excitation wavelengths, 350 nm for quinine bisulfate and the respective biindolizine, were in the range of 0.01–0.02 for both the reference and the respective biindolizine.

2.3. Materials

Compounds **1**, **4**, **5** and **6** were prepared using a Chichibabin-type reaction [8], starting from the commercially available 2-pyridinepropanol and the corresponding bromo ketones. Dimerisation of **1** by the use of $K_3Fe(CN)_6$ yielded compound **2** in reported manner [9].

2.3.1. 3,3′-Bis-(2-phenyl-benzo[g]indolizine) (3)

Compound **3** was prepared according to the described procedure by dimerisation of 2-phenyl-pyrrolo [2,1-*a*] isochinolin [10] on platinum [11]. mp 140–142°C. MS *m*/*z* (ESI-HRMS):

Found 485.2007, calcd. for $C_{36}H_{24}N_2$: 485.2017. ^{1}H NMR ($CDC1_3$) δ (ppm): 6.73 (d, J = 7.4 Hz, 2 H); 7.1–7.76 (m, 20 H); 8.06 (dd, J = 7.9 Hz,

$J = 0.7$ Hz, 2H). ^{13}C NMR ($CDCI_3$) δ (ppm): 99.4; 111.2; 112.8; 122.1; 122.2; 125.9; 126.1; 126.5; 126.6; 126.9; 127.5; 128.7; 128.8; 129.2; 131.3; 135.1. X-ray analysis is shown in Fig. 2.

2.3.2. 1,2-Bis-[1-(2-acetoxyethyl)-2-phenyl-indolizin-3-yl]-ethylen (**4**)

Compound **4** was prepared according to the procedure described by Fraser and Reid [12] starting from l-hydroxyethyl-2-phenyl-indolizine perchlorate [13] and glyoxal in acetic acid. mp 188–191°C. Found: C, 78.58; H, 5.85; N, 4.57, calcd. for $C_{38}H_{34}N_2O_4$: C, 78.33; H, 5.88; N, 4.81. MS *m/z* (FAB): 582 (M^+). ^{13}C NMR (DMSO-d_6, 100°C) δ (ppm): 20.2; 22.9; 63.3; 108.6; 111.1; 113.3; 116.7; 117.0; 119.2; 122.0; 126.6; 128.1; 128.5; 129.7; 130.6; 137.2. X-ray analysis is shown in Fig. 3.

2.3.3. 1,4-Bis[1-(2-hydroxyethyl)-2-phenylindolizin-3-yl]- benzene (**5**).

Compound **5** was obtained by reaction of 2-bromo-2-[4-(l-bromo-2-oxo-2-phenyl-ethyl)-phenyl]-1-phenyl-ethanone with 3-(2-pyridyl)-1-propanol. mp 240–245°C. MS *m/z*: found (CI-HRMS): 549.25357 ($M + H^+$), Calc, for $C_{38}H_{32}N_2O_2 + H^+$: 549.2542). ^1H NMR: (DMSO-d_6) δ (ppm) 2.87 (t, $J = 7.4$ Hz, 4H); 3.4–3.5 (m, 4H); 4.62 (OH, t, $J = 5.3$ Hz, 2H); 6.56 (t, $J = 6.7$ Hz, 2H); 6.74 (t, $J = 7.5$ Hz, 2H); 7.15–7.35 (m, 14 H); 7.53 (d, $J = 8.7$ Hz, 2H); 8.10 (d, $J = 7.3$ Hz, 2H). ^{13}C NMR: (DMSO-d_6) δ (ppm) = 27.6; 61.7; 108.6; 110.9; 116.6; 117.8; 120.5; 121.8; 126.2; 127.8; 127.9; 129.4; 130.2; 134.9.

2.3.4. 1,4-Bis[1-(2-acetyoxyethyl)-indolizin-2-yl]-benzene (**6**).

Compound **6** was prepared by the reaction of 1, 4-bis-bromoacetyl-benzene with 1-acetoxy-3-pyridin-2-yl-propane. mp 178–180°C. Found: C, 74.70; H, 5.80; N, 5.47, calcd. for $C_{30}H_{28}N_2O_4$: C, 74.98; H, 5.87; N, 5.83. MS: *m/z* (FAB): 480 (M^+) ^1H NMR (DMSO-d_6) δ (ppm): 1.91 (s, 6H); 3.19 (t, $J = 7.0$ Hz, 4H); 4.14 (t, $J = 7.2$ Hz, 4H); 6.55 (dt, $J = 7.1$, 1.2 Hz, 2H); 6.71 (dt, $J = 6.5$, 1.2 Hz, 2H); 7.49 (d, $J = 8.9$ Hz, 2H); 759 (s, 4H); 7.72 (s, 2H); 8.21 (d, $J = 6.8$, 2H). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.6; 23.4; 64.2; 104.9; 110.3; 110.9; 116.6; 117.0; 125.5; 128.0; 128.5; 130.9; 133.5; 170.2.

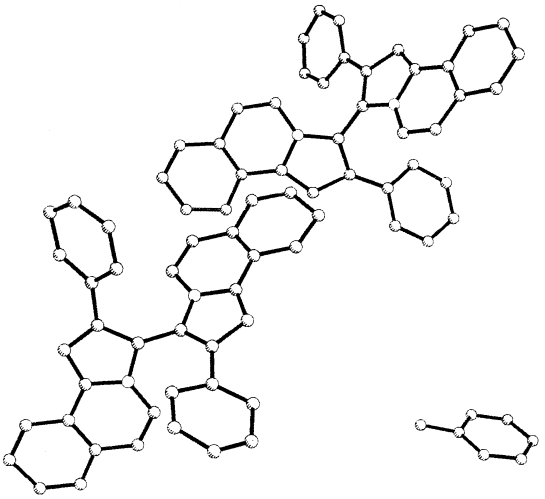

Fig. 2. Crystal structure of **3**. The plot shows two molecules of **3** and one solvent molecule (toluene).

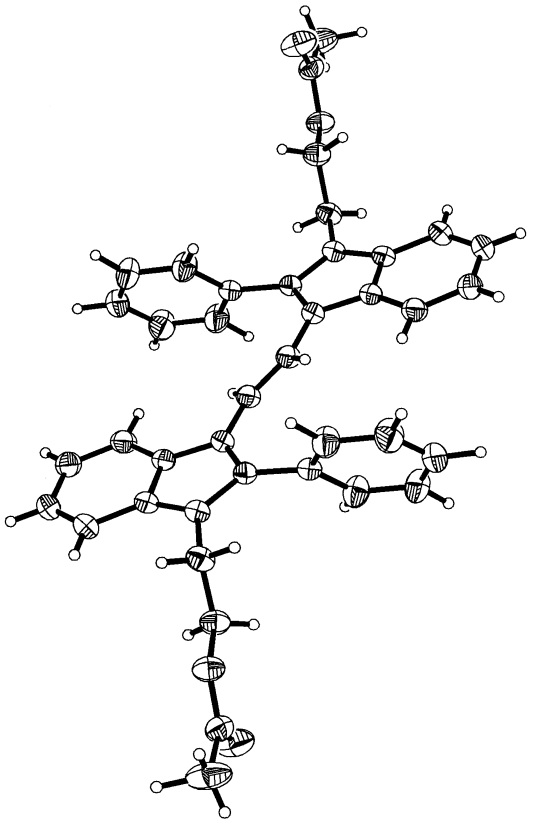

Fig. 3. Crystal structure of **4**.

2.4. X-ray structure analysis

The X-ray data of **3** and **4** were collected on a Siemens Smart diffractometer at 293 K using MoK_α radiation ($\lambda = 0.71073$ Å) monochromatized by a graphite crystal. The structures were solved by direct methods and refined by full-matrix least squares calculations (SHELXTL). The relative high *R*-value of **3** is caused by the poor quality of the available crystals. Nevertheless, the electron density distribution allowed an unequivocal assignment of the structure reported here.

2.4.1. Crystal data of **3**

A colorless, prismatic crystal of dimensions $0.6\times0.3\times0.15$ mm^3 was used for data collection. Crystal system: triclinic, space group P1-(No2), $a = 12.85(3)$ Å, $b = 15.90(5)$ Å, $c = 16.3\ 1(2)$ Å, $\alpha = 71.5(2)^\circ$, $\beta = 71.2(1)^\circ$, $\gamma = 89.7(2)^\circ$, $V = 2977(12)$ Å^3, $Z = 4$, d (cal) $= 1.184$ Mg m^{-3}, linear absorption coefficient $\mu = 0.069$ mm^{-1} and $Fw = 530.64$ for $C_{36}H_{24}N_2x(C_7H_8)_{0.5}$, $F(000) = 1116$. The total number of reflections was 3349, 2553 independent, 1266 observed [$I > 2\sigma(I)$]. The number of refined parameters was 334; the final R (on F) $= 0.177$.

2.4.2. Crystal data of **4**

A light yellow, prismatic crystal with the dimensions $0.85\times0.42\times0.28$ mm^3 was used for data collection. Crystal system: monoclinic, space group $P2_1/n$ (No14), $a = 15.2722(3)$ Å, $b = 5.74700$ (10) Å, $c = 18.5486(4)$ Å, $\beta = 103.318(1)^\circ$, $V = 1584.22$ (5) Å^3, $Z = 2$, d (cal) $= 1.221$ Mg m^{-3}, $\mu = 0.079$ mm^{-1} and $Fw = 582.67$ for $C_{38}H_{34}N_2O_4$, $F(000) =$ 616. The total number of reflections was 6741, 2276 independent, 2147 observed [$I > 2\sigma(I)$]. The number of refined parameters was 268; final R (on F) = 0.0422.

3. Results and discussion

By comparison of the readily available 3,3′-bis[1(2-hydroxyethyl)-2-phenylindolizine] [13] **2** with the corresponding monomer **1**, it was evident that the directly coupled 3,3′ dimer had a significant lower quantum yield ($\phi = 0.09$) than the starting material ($\phi = 0.26$). Obviously, the hindered rotation around the bond connecting both indolizines [9] prevented an efficient conjugation of the heteroaromatic systems. Additionally, a new channel for non radiative deactivation of the excited state was created by direct 3,3′ coupling of two indolizines. Nevertheless, a remarkable bathochromic shift of the emission maximum of **2** to 480 nm was observed, compared to the monoindolizine **1** (445 nm).

The annelated biindolizine **3** was preparated by dimerisation of the known 2-phenyl-benzo[g]indolizine [10] in the presence of platinum on carbon as a model substance with a more extended π-systems. Indeed, compound **3** showed an extraordinary high quantum yield of 0.87. However, this effect can be attributed to the existence of the condensed heteroaromatic systems, rather than to the dimerisation of the two monomers: X-ray analysis confirmed that, in **3**, the two heterocycles were twisted towards each other (at an angle of 60° as depicted in Fig. 2). Additionally, in solution, steric hindrance is to be expected, preventing an efficient conjugation of the indolizines across the 3,3′ bond axis in the same way as observed for compound **2** [13]. Therefore the coupling of the two heterocycles by the use of a spacer was regarded as a means of obtaining biindolizines that can adopt an overall planar conformation in order to obtain higher quantum yields.

In **4** and **5**, the two 3 positions of indolizines were bridged via a vinylene or a phenylene moiety, respectively. The incorporation of the vinylene bridge in **4** resulted in relatively long absorption and emission wavelengths [λ_{max}(abs) = 419 nm, λ_{max}(em) = 509 nm], indicating conjugation of the π-electrons over the connecting double bond. As in the case of polymethine dyes [14], the lack of rigidity of the bridged molecule lowered the quantum yield ($\phi = 0.11$). The X-ray analysis of **4** (see Fig. 3) shows two parallel indolizines twisted out of the π-plane of the ethylene bridge and the phenyl substituents not located in the planes of the heterocyclic systems.

However, in solution, the lack of steric hindrance should prevent planarisation of the whole biindolizine system in the cases of **4** and **5**. The phenylene-bridged biindolizine **5** ($\phi = 0.15$) had a higher quantum yield compared to **2** ($\phi = 0.09$)

Table 1
Absorption and fluorescence spectral data of the compounds **1**–**6**[a]

	Absorption		Fluorescence		
	λ_{ex} (nm)	($\log_{10}\epsilon$)	λ_{ex} (nm)	λ_{em} (nm)	ϕ
1	302 (3.45),	362 (3.36)	362	445	0.26
2	253 (4.73),	348 (3.32)	342	480	0.09
3	281 (4.67),	332 (3.80)	334	448	0.87
4	284 (3.80),	419 (3.82)	437	509	0.11
5	249 (4.54),	282 (4.04), 362 (3.84)	364	450	0.15
6	268 (4.76),	356 (3.85)	356	441	0.13

[a] Biindolizines in acetonitrile solutions. Quantum yields were determined using methods described before in accordance to the literature [15].

and **4** (ϕ= 0.11) which was still much lower here than the ϕ-value of the monomer **1** (ϕ = 0.26). The absorption and emission wavelengths were very similar to those of the monomer.

A different approach to obtain an extended, conjugated π-system was realised by synthesis of compound **6**. In this compound, a phenylene-bridge connects the 2 positions of the two 1-acetyloxyethylindolizines. In contrast to the biindolizines described above, here the 3,3′ positions are unsubstituted. The 1,4.-bis-[1-(2-acetyloxyethyl)-indolizin-2-yl]-benzene (**6**) showed a quantum yield of ϕ = 0.13. The results of spectroscopic examination of the compounds **1** to **6** are summarised in Table 1.

4. Conclusions

In the case of the influence of dimerisation of indolizines, in a series of differently coupled biindolizines, on their fluorescence properties, it is evident that the direct, covalent linking of two 1-(2-hydroxyethyl)-2-indolizines **1** at the 3 position (compound **2**) lead to a bathocromic shift of the emission maximum and a drastic decrease of the quantum yield in comparison to the monomer. The coupling of two monomers by spacer groups (compounds **4**–**6**) resulted in a moderate enhancement of the emission intensity, but none of the biindolizines achieved the quantum yields of compound **1**. Only in the case of the annelated compound **3**, was a biindolinzine with a remarkable high quantum yield obtained. In the attempt to obtain long wavelength, absorbing and emitting fluorophores it has to be noted especially that biindolizine **4** showed a significant bathocromic shift in both absorption [λ_{max}(abs) = 337 nm] and emission [λ_{max}(em) = 509] compared to the monomer compound **1**.

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