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Long Alkyl Chain bis-Quaternary Ammonium-based Ionic Liquids as Biologically Active Xanthenes Dyes

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Foram preparados novos exemplos de líquidos iônicos hidrofílicos e hidrofóbicos, estáveis frente ao ar e à umidade, derivados de amônio bi-quaternário. Esses líquidos iônicos foram propostos para atuar como pigmentos biologicamente ativos, com características físico-químicas únicas, como uma alternativa a alguns pigmentos convencionais aniônicos derivados de xanteno, como eosina Y, fluoresceina e eritrosina.

New examples of air- and moisture-stable, hydrophobic and hydrophilic bis-quaternary ammonium derived ionic liquids have been prepared. These ionic liquids have been proposed to act as biological active dyes with characteristic unique physicochemical properties, providing alternatives to some conventional anionic xanthene dyes such as eosine Y, fluorescein and erythrosine.

Keywords: ionic liquids, xanthene dyes

Introduction

Studies of ionic liquids (ILs) are driven by the intrinsic interest as well as by possible applications. The letters give rise to the series of ILs generations. The evolution of ionic liquids proceeds very quickly from the first generation (ILs with unique tunable physical properties), through the second generation (ILs with targeted chemical properties combined with selected physical properties), to the third generation (ILs with targeted biological properties combined with chosen physical and chemical properties).[1] In particular, the air and water stable ILs are considered to be promising solvents for wide variety of applications including organic synthesis,[2-4] catalysis[5-7] (with biocatalysis),[8] biphasic systems,[9] electrochemistry[10-12] and tissues preservation.[13] The vapor pressure of ILs is generally negligible. However, they can be distilled under high vacuum.[14] In addition, ILs have been shown to be non-flammable. The non-flammability of ILs is often highlighted as a safety advantage of these liquids over volatile organic compounds (VOCs). Though many ILs are not flammable themselves, they are not safe to use near fire or heat sources. The decomposition products formed during the thermal decomposition of some IL are sensitive to combustion.[15] The ILs cycle of life has already been established[16] and a brief overview of the ecotoxicological, bioaccumulation, and biological activity of these liquids has appeared in the literature.[17]

ILs are group of quaternary ammonium compounds which represents a cationic surfactant. The nature of interaction of the xanthene dyes with various surfactants is different. They undergo charge transfer interaction with a neutral surfactant, ionic, or electrostatic interaction with a cationic surfactant and no interaction with an anionic surfactant.[18] Hexadecyltrimethylammonium (CTA) bromide provides a good medium for aggregation of xanthene dyes.[19] It has been suggested that both hydrophobic interaction and electrostatic forces favor encounter of anion dye molecules, thus promoting the aggregation. In the literature a sensitive colorimetric method was reported for the analysis of three quaternary ammonium compounds - QAC : 1-hexadecylpyridinium chloride, benzalkonium (BA) chloride and CTA bromide, following ion-pairing with eosin Y in the presence of Triton X-100.[20] QAC–eosin Y equilibrium was examined in aqueous solution in the presence of micelles of Triton X-100.[21] The spectrophotometric methods have been reported for determination of QACs with acid dyes and

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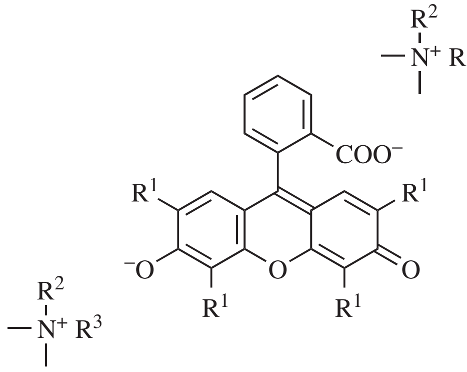
ion pair extraction procedure. Xanthene dyes such as eosin form 1:2 ion associates with quaternary ammonium cations at pH 7-9.[22] Purkait *et al.*[23] separated eosin dye from wastewater with cloud point extraction by using a nonionic surfactant Triton X-100. Recently liquid-liquid extraction with imidazolium IL was proposed for removal of eosin dye from aqueous solutions.[24]

Here, we describe novel bis-quaternary ammonium based ionic liquids as biological active dyes.

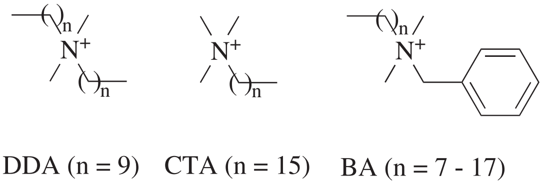
Results and Discussion

Synthesis

Metathesis reactions of long alkyl chain quaternary ammonium chlorides, the commercially inexpensive and broadly used didecyldimethylammonium - DDA, benzalkonium - BA (alkyl group ranging between *n*-C_8H_{17} and *n*-$C_{18}H_{37}$) and hexadecyltrimethylammonium - CTA, were carried out using disodium salts of eosine Y, or fluorescein, or erythrosine. The prepared ion pairs are listed in Scheme 1 and 2 and Table 1.



Scheme 1. Structure of the ion pairs form 1:2 (**1**-**9**).



Scheme 2. Structure of the quaternary ammonium cation.

All isolated bis-salts are nonvolatile, nonflammable, soluble in chloroform, acetone, DMF, DMSO, alcohol (methanol, 1-decanol, cyclohexanol), and insoluble in hexane and CCl_4. These new compounds are insoluble in water, with the exception of the **4**-**6** salts. The hydrophilic and hydrophobic products were dried in vacuum at 80 °C for 24 h and stored over P_4O_{10}. The water contents, determined by Karl-Fischer measurements, were found to be less than 500 ppm. The salts were characterized by ^{1}H and ^{13}C NMR analyses and by elemental analysis.

The thermal properties for the reported ILs were determined using DSC and TG and are presented in Table 2. The salt **4** formed a cooled phase, with a glass transition temperature (T_g) of −41 °C. Melting points (T_m) for **2**, **3**, **5**, **6**, **8** and **9** were observed to be 76, 47, 62, 96, 66, and 76 °C, respectively. Salts **2**, **3**, **8**, **9** were stable up to 210 °C and the onset for thermal decomposition (T_{onset}) amounted to 310 °C. They remained stable in air, in aqueous solution and in common organic solvents and can be assigned to the class of "ionic liquids" (salts that melt at < 100 °C) with very high viscosity.

All prepared ion-pairs were soluble in poly(ethylene glycol), PEG 600. Solutions (1%) proved to be stable, demonstrated a very good lubrication ability and represented colorful materials, being orange (**1**-**3**), yellow (**4**-**6**), or pink (**7**-**9**).

Table 1. Prepared ion pairs

Salts	Anion	Cation		Quaternary ammonium cation	Yield (%)	Color solution	
	R^1	R^2	R^3			Acetone	Chloroform
1[a]	Br	$CH_2C_6H_5$	C_8H_{17}-$C_{18}H_{37}$	BA	99	Bright orange	Red
2[a]	Br	$CH_2(CH_2)_8CH_3$	$CH_2(CH_2)_8CH_3$	DDA	95	Bright orange	Red
3[a]	Br	CH_3	$CH_2(CH_2)_{14}CH_3$	CTA	96	Bright orange	Red
4[b]	H	$CH_2C_6H_5$	C_8H_{17}-$C_{18}H_{37}$	BA	95	Yellow green	Orange
5[b]	H	$CH_2(CH_2)_8CH_3$	$CH_2(CH_2)_8CH_3$	DDA	93	Yellow green	Orange
6[b]	H	CH_3	$CH_2(CH_2)_{14}CH_3$	CTA	85	Yellow green	Orange
7[c]	I	$CH_2C_6H_5$	C_8H_{17}-$C_{18}H_{37}$	BA	91	Bright pink	Pink
8[c]	I	$CH_2(CH_2)_8CH_3$	$CH_2(CH_2)_8CH_3$	DDA	90	Bright pink	Pink
9[c]	I	CH_3	$CH_2(CH_2)_{14}CH_3$	CTA	96	Bright pink	Pink

[a]Red tar with green tinge. [b]Orange wax with green tinge. [c]Pink tar green tinge

Table 2. Thermal properties of the prepared bis-quarternary ammonium ILs

IL	T_g[a]	T_c[b]	T_m[c]	$T_{onset(5\%)}$[d]	T_{onset}[e]
1				184	289
2		18	76	212	309
3			47	220	313
4	−41			120	232
5	−58	−9	62	154	263
6			96	224	265
7				193	310
8			66	217	310
9		58	76	215	313

[a]Glass transition temperature determined by DSC on heating; [b]Crystallization temperature determined by DSC on heating; [c]Melting point determined by DSC on heating; [d]Decomposition temperature determined from onset to 5% mass loss; [e]Decomposition temperature determined from onset to 50% mass loss.

Biological evaluation

The biological activity of the prepared bis-quaternary ammonium ILs was estimated and the minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC) were established by the tube dilution method. MIC and MBC values for 12 strains (rods, cocci, bacilli and fungi) of the prepared ion-pairs and the commercially available BA chloride are compared in Table 3. The results demonstrated that the synthesized ILs were very effective agents against bacteria and fungi. The activity depended upon the kind of cation and anion. The salt **5**, which contains DDA cation, was particularly strongly active against all of the microorganisms and exhibited in this respect a higher efficiency than BA chloride. This salt manifested microstatic activity, *i.e.* MIC values, and microbiocidal activity, measured by MBC values at the same level. Selection of an appropriate ion-pair as an

Table 3. MIC[a] and MBC[a] values of the ILs studied

Strains		Salts									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	BA[f]
M. luteus [b]	MIC	0.2	0.5	1	0.2	< 0.1	< 0.1	0.2	0.5	0.5	0.5
	MBC	0.2	2	8	1	< 0.1	0.5	0.2	0.5	1	4
S. aureus [b]	MIC	2	2	2	1	0.5	1	2	2	1	2
	MBC	31	16	4	4	1	2	8	4	2	8
S. epidermidis [b]	MIC	2	1	0.5	0.2	< 0.1	< 0.1	< 0.1	0.5	0.2	0.5
	MBC	4	2	4	2	0.2	0.5	< 0.1	2	1	2
E. feacium [b]	MIC	2	2	1	2	0.5	0.5	2	4	2	2
	MBC	8	16	4	4	0.5	8	8	4	4	8
M. catarrhalis [b]	MIC	0.5	0.2	1	1	0.2	0.2	1	0.2	0.2	0.2
	MBC	250	4	4	1	1	0.2	4	0.5	2	0.5
E. coli [c]	MIC	0.5	4	2	2	0.2	0.5	0.2	2	4	2
	MBC	1	4	8	2	0.5	2	1	4	4	2
S. marcescens [c]	MIC	250	> 500	> 500	31	16	62	500	> 500	> 500	62
	MBC	250	> 500	> 500	31	31	62	500	> 500	> 500	62
P. vulgaris [c]	MIC	250	31	> 500	31	16	62	500	> 500	> 500	31
	MBC	250	250	> 500	62	16	62	500	> 500	> 500	31
P. aeruginosa [c]	MIC	> 500	> 500	> 500	62	31	> 500	> 500	> 500	> 500	62
	MBC	> 500	> 500	> 500	125	31	> 500	> 500	> 500	> 500	62
B. subtilis [d]	MIC	2	2	2	2	0.2	1	2	2	4	2
	MBC	2	2	2	2	0.2	1	2	2	4	2
C. albicans [e]	MIC	4	16	8	4	2	8	8	62	> 500	4
	MBC	4	31	8	4	4	16	125	125	> 500	31
Rhodothorula Rubra[e]	MIC	4	8	4	4	2	8	16	62	> 500	8
	MBC	8	16	8	8	2	16	62	250	> 500	31

[a]In ppm; [b]Cocci; [c]Rods; [d]Bacillus; [e]Fungi; [f]Benzalkonium chloride.

effective biocidal agent must be preceded by determination of its antimicrobial activity. Results presented in Table 3 showed that despite the generally high activity of **1-9** ILs against microbes, there existed dyes, such as *Serratia marcescens*, *Proteus vulgaris* and *Pseudomonas aeruginosa* in particular, which were insensitive to **1**, **3**, **7**, **8** and **9**.

Spectral, absorption and emission properties

The absorption spectra, fluorescence spectra and fluorescence excitation spectra of **2**, **3**, **5**, and **6** in 1-C_3H_7Cl or DMSO have been measured. For the compounds in concentrations > 10^{-6} mol L^{-1} the spectra revealed considerable changes. With decreasing concentration the maxima of the long-wavelength band in the absorption spectra, fluorescence excitation spectra, and fluorescence spectra were shifted towards longer wavelengths and their shapes changed. In view of the above, the study was performed for IL in low concentrations, ≤ 10^{-6} mol L^{-1} in order to avoid dimer and oligomer formation.[19,22] The exemplary absorption spectra, fluorescence excitation spectra, and fluorescence spectra, recorded for **3** in 1-C_3H_7Cl and for **6** in DMSO are presented in Figures 1 and 2. As the fluorescence excitation spectra are the same as the absorption spectra, we can conclude that the emission comes only and exclusively from the ILs studied and not from the impurities.

Disodium salt of eosin Y or fluorescein can occur in the form of dianion or monoanion. Martin[25] and Petrossyan *et al.*[26,27] proved that these salts in DMF or in DMSO occur in the form of dianion, in whose absorption spectrum the long-wavelength band maximum is at λ^{max}_{AS} = 520 nm. For monoanion in the same solvents λ^{max}_{AS} = 460 nm,[28] so the maximum is strongly hipsochromically shifted relative to its position in the spectrum of dianion. Therefore, measurement of the absorption spectra permits distinction of these two individuals. For **6** in DMSO (Figure 2) λ^{max}_{AS} = 520 nm, which means that it occurs at 1:2 (dianion).

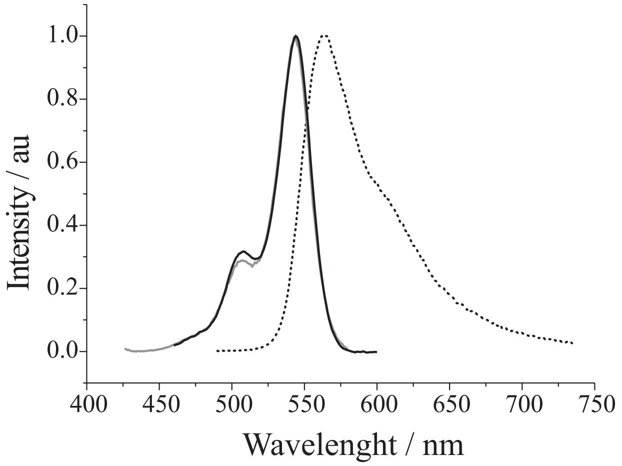


Figure 1. Normalized absorption (solid bold line), fluorescence excitation (λ_{em}= 600 nm) and fluorescence (dotted line) (λ_{exc}= 470 nm) spectra of **3** (≤ 10^{-6} mol L^{-1}) in 1-C_3H_7Cl.

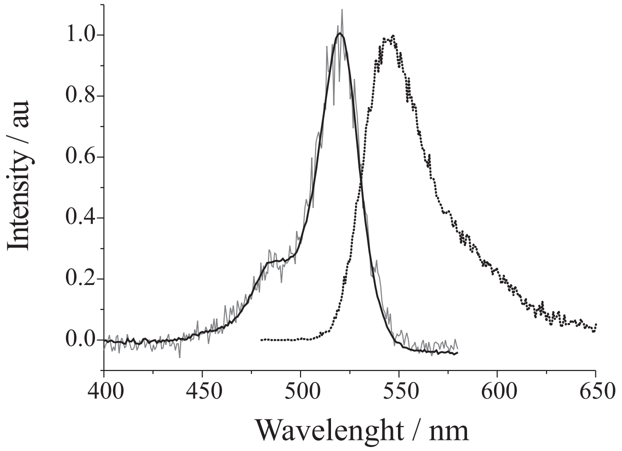


Figure 2. Normalized absorption (solid bold line), fluorescence excitation (λ_{em}= 600 nm) and fluorescence (dotted line), (λ_{exc}= 520 nm) spectra of **6** (*ca.* 10^{-6} mol L^{-1}) in DMSO.

The most important spectral properties of the liquids studied are collected in Table 4. Besides the positions of the maxima in the absorption, $\bar{\nu}^{max}_{AS}$, fluorescence, $\bar{\nu}^{max}_{FS}$, and fluorescence excitation spectra, $\bar{\nu}^{max}_{FES}$, the Table 4

Table 4. Position of the maximum of the long-wavelength band in the absorption spectrum ($\bar{\nu}^{max}_{AS}$) in the fluorescence excitation spectrum ($\bar{\nu}^{max}_{FES}$) and in the fluorescence spectrum ($\bar{\nu}^{max}_{FS}$); the FWHM of the bands ($\Delta\bar{\nu}^{1/2}_{AS}$, $\Delta\bar{\nu}^{1/2}_{FES}$, $\Delta\bar{\nu}^{1/2}_{FS}$) and Stokes shifts ($\Delta\bar{\nu}^{max}$ (AS-FS), $\Delta\bar{\nu}^{max}$ (FES-FS)) for **2**, **3**, **5** and **6** for concentration ≤ 10^{-6} mol L^{-1}

IL	$\bar{\nu}^{max}_{AS}$	$\bar{\nu}^{max}_{FES}$	$\bar{\nu}^{max}_{FS}$	$\Delta\bar{\nu}^{1/2}_{AS}$	$\Delta\bar{\nu}^{1/2}_{FES}$	$\Delta\bar{\nu}^{1/2}_{FS}$	$\Delta\bar{\nu}^{max}$ (AS-FS)	$\Delta\bar{\nu}^{max}$ (FES-FS)
2[a]	18450	18315	17545	1050	975	1690	905	770
3[a]	18380	18380	17760	920	885	1730	620	620
5[a]	19455	19085	18020	2580	1410	2530	1630	1065
6[a]	-	18520	17890	-	960	1515	-	630
6[b]	19230	19230	18480	905	905	1280	750	750

$\bar{\nu}$ and $\Delta\bar{\nu}$ in cm^{-1}; [a]in 1-C_3H_7Cl; [b]in DMSO.

presents full width at half-maximum values, $\Delta\bar{\nu}^{1/2}$ and Stokes shifts, $\Delta\bar{\nu}^{max}$, determined from the absorption and fluorescence spectra as well as for the fluorescence excitation and fluorescence spectra. As follows from the data shown in Table 4, the spectral properties of **6** in DMSO and fluorescein sodium salt in DMSO[25] are very similar, indicating that the absorption and emission properties of these salts are determined by the 1:2 form. An independent confirmation of the lack of the influence of the type of cations on the spectral properties of the IL studied comes from the very similar positions of the maxima $\bar{\nu}_{AS}^{max}$, $\bar{\nu}_{FES}^{max}$ and $\bar{\nu}_{FS}^{max}$ in the spectra of **2** and **3** in 1-C_3H_7Cl.

Experimental

General considerations

^{1}H NMR spectra were recorded on a Mercury Gemini 300 spectrometer operating at 300 MHz, with tetramethylsilane as the internal standard. ^{13}C NMR spectra were obtained with the same instrument at 75 MHz. Elemental analyses were performed at the A. Mickiewicz University, Poznan. The water content was determined by using an Aquastar volumetric Karl Fischer titrator with composite 5 solution as the titrant and anhydrous methanol as the solvent. DSC analyses were measured using a Perkin-Elmer differential scanning calorimeter. Samples were sealed in aluminium pans and scanned at the rate of 10 °C min^{-1} in a nitrogen atmosphere. A Mettler Toledo DA 110 M scale was used for the mass/density measurements. Samples between 2-10 mg were heated under the constant heating at 10 °C min^{-1} in a nitrogen atmosphere. UV-Vis spectra were recorded using Cary 300 Bio (Varian) and V-550 (Jasco) spectrophotometers. The steady-state corrected emission spectra and the corrected excitation spectra were recorded with a modified MPF-3 (Perkin-Elmer) and modified SPF (Aminco-Bowman) spectrofluorimeters with the single-photon counting detection a cooled (T < −20 °C) R-928 (Hamamatsu) PMT. Excitation spectra were corrected using a rhodamine B quantum counter and emission spectra were corrected using set of secondary emission standards.[29]

Synthesis

*General procedure for preparation of hydrophobic ILs (**1**-**3**, **7**-**9**)*

Quaternary ammonium chloride (20 mmol) was dissolved in distilled water (30 mL) and disodium salt of eosin Y or erythrosine (10 mmol) was added. The reaction mixture was stirred at 80 °C for 0.5 h. The product was heavier than water. After cooling to room temperature chloroform (30 mL) was added. The organic phase was washed with distilled water until chloride ions were no longer detected using $AgNO_3$. Chloroform was removed and the obtained product was dried in vacuum.

*2',4',5',7'-Tetrabromo-3',6'-dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bisbenzalkonium salt (**1**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.6 Hz, 6H); 1.24 (m, 40H); 1.75 (m, 4H); 2.93 (s, 12H); 3.2 (t, *J* 8.3 Hz, 4H); 4.25 (s, 4H); 7.0 (s, 1H); 7.13 (d, *J* 6,9 Hz, 1H); 7.52 (m, 12H); 8.03 (d, *J* 6.9 Hz, 1H); 8.34 (s, 1H); ^{13}C NMR(300 MHz DMSO-d_6) 14.0; 21.8; 25.8; 28.7; 28.8; 28.96; 29.03; 29.07; 29.08; 31.3; 49.0; 63.4; 66.1; 79.2; 99.0; 109.6; 117.8; 128.1; 128.5; 128.7; 128.9; 130.0; 130.6; 132.6; 132.9; 140.8; 152.9; 156.8; 167.0; 168.8.

*2',4',5',7'-Tetrabromo-3',6'-dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(didecyldimethylammonium) salt (**2**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.84 (t, *J* 6.6 Hz, 12H); 1.23 (m, 56H); 1.58 (m, 8H); 2.96 (s, 12H); 3.19 (t, *J* 8.2 Hz, 8H); 7.0 (m, 2H); 7.45 (m, 2H); 8.00 (d, *J* 1.73 Hz, 1H); 8.34 (s, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 13.9; 21.7; 22.1; 25.7; 28.5; 28.7; 28.8; 28.9; 31.1; 31.3; 49.8; 62.7; 79.1; 98.8; 109.4; 117.4; 128.2; 128.4; 129.1; 129.6; 130; 132.5; 142.2; 155.7; 157.4; 167.7. Calcd for $C_{64}H_{102}Br_4N_2O_5$: C, 59.17; H, 7.91; N, 2.16. Found: C, 58.87; H, 7.83; N, 1.81.

*2',4',5',7'-Tetrabromo-3',6'-dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(hexadecyltrimethylammonium) salt (**3**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.89 (t, *J* 6.7 Hz, 6H); 1.29 (m, 52H); 1.69 (m, 4H); 3.1 (s, 18H); 3.28 (t, *J* 8.4 Hz, 4H); 7.08 (s, 1H); 7.16 (d, *J* 6,32 Hz, 1H); 7.55 (m, 2H); 8.09 (d, *J* 6.04 Hz, 1H); 8.42 (s, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 13.9; 22.0; 22.1; 25.8; 28.5; 28.7; 28.8; 28.9; 29.0; 29.06; 31.3; 52.0; 65.2; 98.7; 109.4; 117.5; 127.7; 128.4; 129.9; 132.2; 142.1; 152.7; 157.2; 166.6; 167.8. Calcd for $C_{58}H_{90}Br_4N_2O_5$: C, 57.34; H, 7.47; N, 2.31. Found C, 57.89; H, 7.41; N, 2.03.

*3',6'-Dihydroxy-2',4',5',7'-tetraiodospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bisbenzalkonium salt (**7**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.6 Hz, 6H); 1.23 (m, 40H); 1.75 (m, 4H); 2.92 (s, 12H); 3.39 (t, *J* 5.22 Hz, 4H); 4.53 (s, 4H); 7.03 (d, *J* 8.5 Hz, 1H); 7.21 (s, 1H); 7.46 (m, 12H); 8.0 (d, *J* 9.0 Hz, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 14.0; 21.8; 25.8; 28.5; 28.7; 28.8; 28.9; 29.03; 31.3; 48.9; 63.4; 66.1; 75.0; 95.2; 111.8; 127.7; 128.2;

128.4; 128.6; 128.8; 130.1; 132.9; 137.5; 142.6; 155.8; 157.2; 166.9; 171.1.

*3',6'-Dihydroxy-2',4',5',7'-tetraiodospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(didecyldimethylammonium) salt (**8**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.6 Hz, 12H); 1.24 (m, 56H); 1.6 (m, 8H); 2.96 (s, 12H); 3.16 (t, *J* 8.4 Hz, 8H); 7.01 (d, *J* 8.5 Hz, 1H); 7.25 (s, 2H); 7.44 (m, 2H); 8.00 (d, *J* 9.0 Hz, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 14.0; 21.8; 22.2; 25.8; 28.5; 28.7; 28.8; 29.0; 31.4; 50.0; 62.8; 74.9; 95.0; 111.6; 127.6; 128.2; 128.4; 130.1; 132.5; 137.4; 142.2; 155.6; 157.0; 166.7; 171.0. Calcd for $C_{64}H_{102}I_4N_2O_5$: C, 51.69; H, 6.91; N, 1.88. Found: C, 51.88; H, 6.82; N, 2.01.

*3',6'-Dihydroxy-2',4',5',7'-tetraiodospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(hexadecyltrimethylammonium) salt (**9**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.7 Hz, 6H); 1.23 (m, 52H); 1.60 (m, 4H); 3.02 (s, 18H); 3.21 (t, *J* 8.5 Hz, 4H); 7.02 (d, *J* 8.5 Hz, 1H); 7.24 (s, 2H); 7.32 (m, 2H); 7.97 (d, *J* 8.8 Hz, 1H); ^{13}C NMR (300 MHz DMSO-d_6) δ 14.0; 22.0; 22.1; 25.8; 28.5; 28.7; 28.8; 29.0; 29.03; 29.07; 31.3; 52.0; 65.2; 74.9; 95.1; 111.7; 127.6; 128.3; 128.5; 130.2; 132.5; 137.5; 142.9; 155.9; 157.2; 166.7; 171.1. Calcd for $C_{58}H_{90}I_4N_2O_5$: C, 49.65; H, 6.47; N, 2.00. Found: C, 49.56; H, 6.33; N, 2.19.

*General procedure for preparation of hydrophilic ILs (**4**-**6**)*

Quaternary ammonium chlorides (10 mmol) were dissolved in distilled water (30 mL) and disodium salt of fluorescein (10 mmol) was added. The solution was stirred at 60 ^{o}C for 1 h. Then, water was removed and dry acetone (15 mL) was added. NaCl was filtered and the volatile materials were removed under reduced pressure.

*3',6'-Dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bisbenzalkonium salt (**4**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.5 Hz, 6H); 1.24 (m, 40H); 1.74 (m, 4H); 2.90 (s, 12H); 3.25 (t, *J* 7.9 Hz, 4H); 4.62 (s, 4H); 6.10 (m, 4H); 6.66 (s, 2H); 7.03 (d, *J* 6.9 Hz, 1H); 7.49 (m, 12H); 8.0 (d, *J* 6.8 Hz, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 14.0; 21.9; 22.1; 25.9; 28.6; 28.8; 28.9; 29.03; 29.1; 30.6; 31.3; 48.8; 63.3; 65.9; 102.4; 109.3; 128.1; 121.8; 127.6; 128.2; 128.1; 128.5; 129.2; 129.9; 130.0; 130.6; 132.8; 133.0; 141.6; 157.1; 168.3; 178.7.

*3',6'-Dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(didecyldimethylammonium) salt (**5**)*

^{1}H NMR(300 MHz DMSO-d_6) δ 0.83 (t, *J* 6.6 Hz, 12H); 1.24 (m, 56H); 1.59 (m, 8H); 2.96 (s, 12H); 3.2 (t, *J* 8.1 Hz, 8H); 6.03 (m, 4H); 6.61 (s, 2H); 6.96 (d, *J* 5.8 Hz, 1H); 7.37 (m, 2H); 7.81 (d, *J* 8.8 Hz, 1H); ^{13}C NMR(300 MHz DMSO-d_6) δ 14.0; 21.7; 22.2; 25.8; 28.6; 28.8; 28.9; 29.0; 31.4; 49.9; 62.8; 102.5; 109.1; 122.4; 127.2; 128.8; 129.5; 130.3; 133.8; 142.8; 157.7; 168.1; 179.9. Calcd for $C_{64}H_{106}N_2O_5$: C, 78.16; H, 10.86; N, 2.85. Found: C, 77.86; H, 10.91; N, 2.57.

*3',6'-Dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one bis(hexadecyltrimethylammonium) salt (**6**)*

^{1}H NMR(300 MHz DMSO-d$_6$) δ 0.85 (t, J 6.7 Hz, 6H); 1.24 (m, 52H); 1.66 (m, 4H); 3.03 (s, 18H); 3.23 (t, J 8.5 Hz, 4H); 6.0 (m, 2H); 6.05 (m, J 11.5 Hz, 2H); 6.57 (d, J 9.3 Hz, 2H); 7.03 (d, J 8.8 Hz, 1H); 7.42 (m, 2H); 7.92 (d, J 9.0 Hz, 1H); ^{13}C NMR(300 MHz DMSO-d$_6$) δ 14.0; 22.0; 22.1; 25.8; 28.7; 28.9; 29.05; 29.08; 31.3; 52.12; 65.3; 102.5; 109.0; 122.3; 127.8; 128.9; 129.7; 130.2; 134.4; 140.7; 157.5; 158.6; 169.5; 179. Calcd for $C_{58}H_{94}N_2O_5$: C, 77.46; H, 10.53; N, 3.11. Found: C, 77.43; H, 9.87; N, 3.38.

Biological evaluation

The following microorganisms were used: bacteria *Micrococcus luteus* NCTC 7743, *Staphylococcus aureus* NCTC 4163, *Staphylococcus epidermidis* ATCC 49134, *Enterococcus faecium* ATCC 49474, *Moraxella catarrhalis* ATCC 25238, *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 8100, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* NCTC 6749, and fungi *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Rhodothorula rubra* (Demml 1889, Lodder 1934). Standard strains were supplied by the National Collection of Type Cultures (NCTC) and London and American Type Culture Collection (ATCC). *Rhodothorula rubra* was obtained from the Department of Pharmaceutical Bacteriology, University of Medical Sciences, Poznan.

Anti-microbial activity was determined by the tube dilution method. Bacteria strains were cultured in Mueller-Hinton broth for 24 h and fungi were cultured on Sabouraud agar for 48 h. A suspension of the microorganisms, at a concentration of 10^6 cfu mL^{-1} was prepared from each culture. Serial twofold dilutions of a compound (2 mL) were inoculated with a standardized of test microorganism to obtain a final concentration of 1-5×10^5 cfu mL^{-1}. Growth (or lack thereof) of the microorganisms was determined visually after incubation for 24 h at 37 °C (bacteria) or 48 h at 28-30 °C (fungi). The lowest concentration, at which there was no visible growth (turbidity), was taken as the MIC. Then, from each tube, one loopful was cultured on an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80, and 0.1% cysteine L) and incubated for 48 h at 37 °C (bacteria) or for 5 days at 28-30 °C (fungi). The lowest concentration of bis-quaternary ammonium IL supporting no colony formation was defined as the MBC.

Conclusions

We obtained new, more economic, multifunctional bis-quaternary ammonium-based ionic liquids which are easy to prepare. Also, their spectral properties were studied. It was showed that the emission observed comes from their anionic part. Additionally, the potential applications of these ILs are markedly extended because of their very high anti-microbial activities. These ILs are proposed to act as biological active dyes with characteristic unique physicochemical properties, providing alternatives to some conventional anionic xanthene dyes such as eosine Y, fluorescein, and erythrosine.

These materials are expected to open new opportunities and enable advancements in different areas, including biological imaging, fundamental photophysical and photochemical studies, and using them as photosensitizers.

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Supplementary Information

Supplementary information is available free of charge at http://jbcs.org.br, as PDF file.

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Supplementary Information

Long Alkyl Chain bis-Quaternary Ammonium-based Ionic Liquids as Biologically Active Xanthenes Dyes

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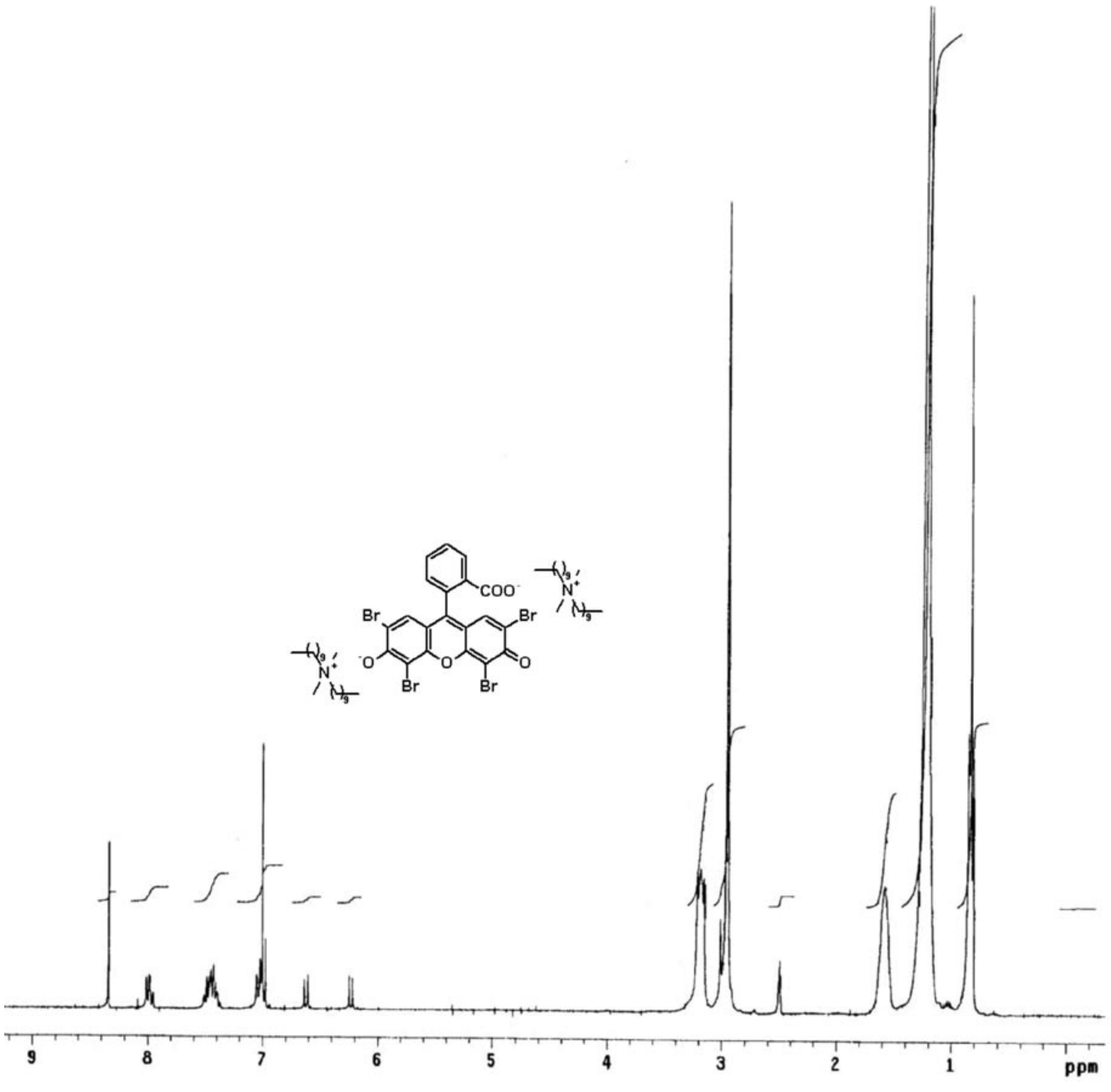

Figure S1. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **2**.

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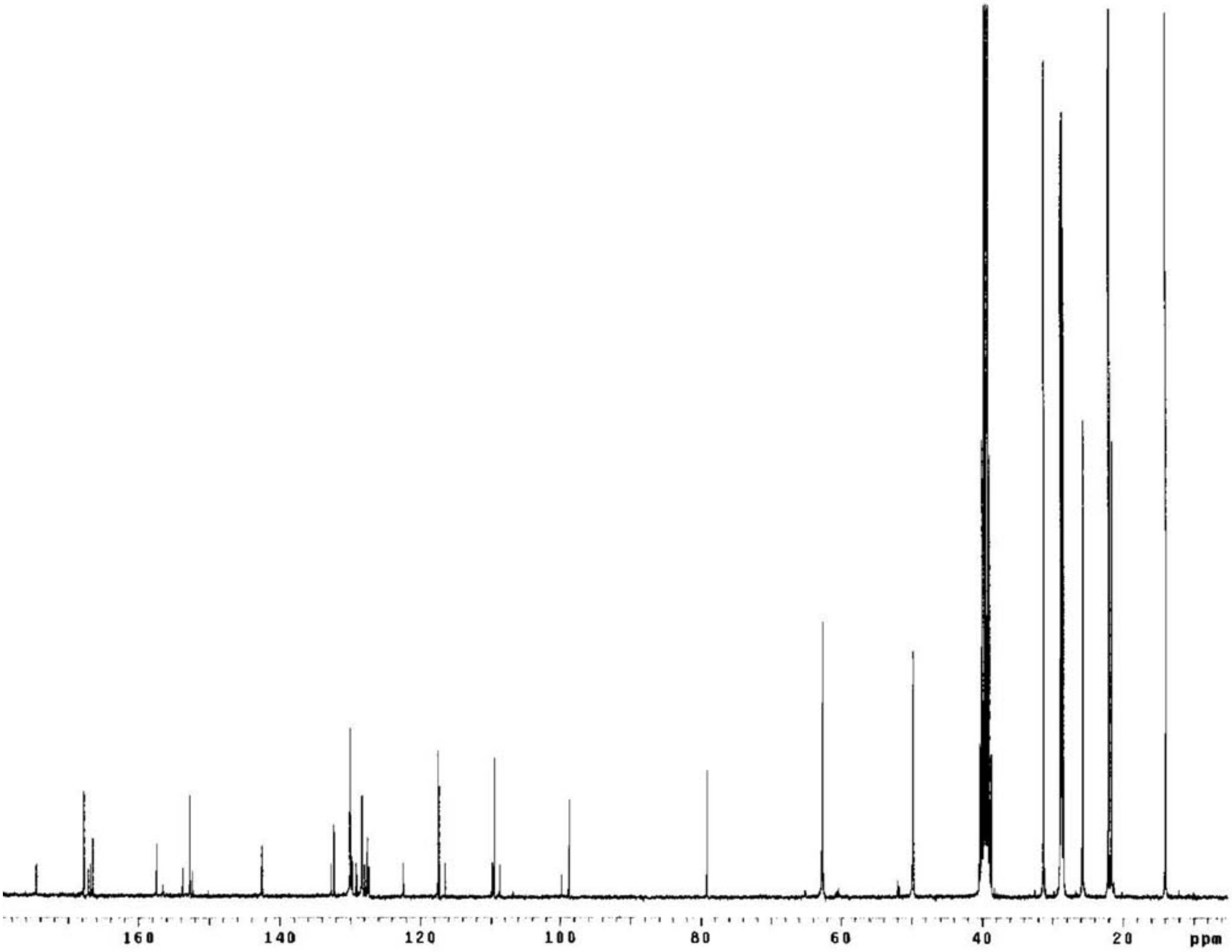

Figure S2. ^{13}C NMR spectrum (300 MHz, DMSO-d_6) of compound **2**.

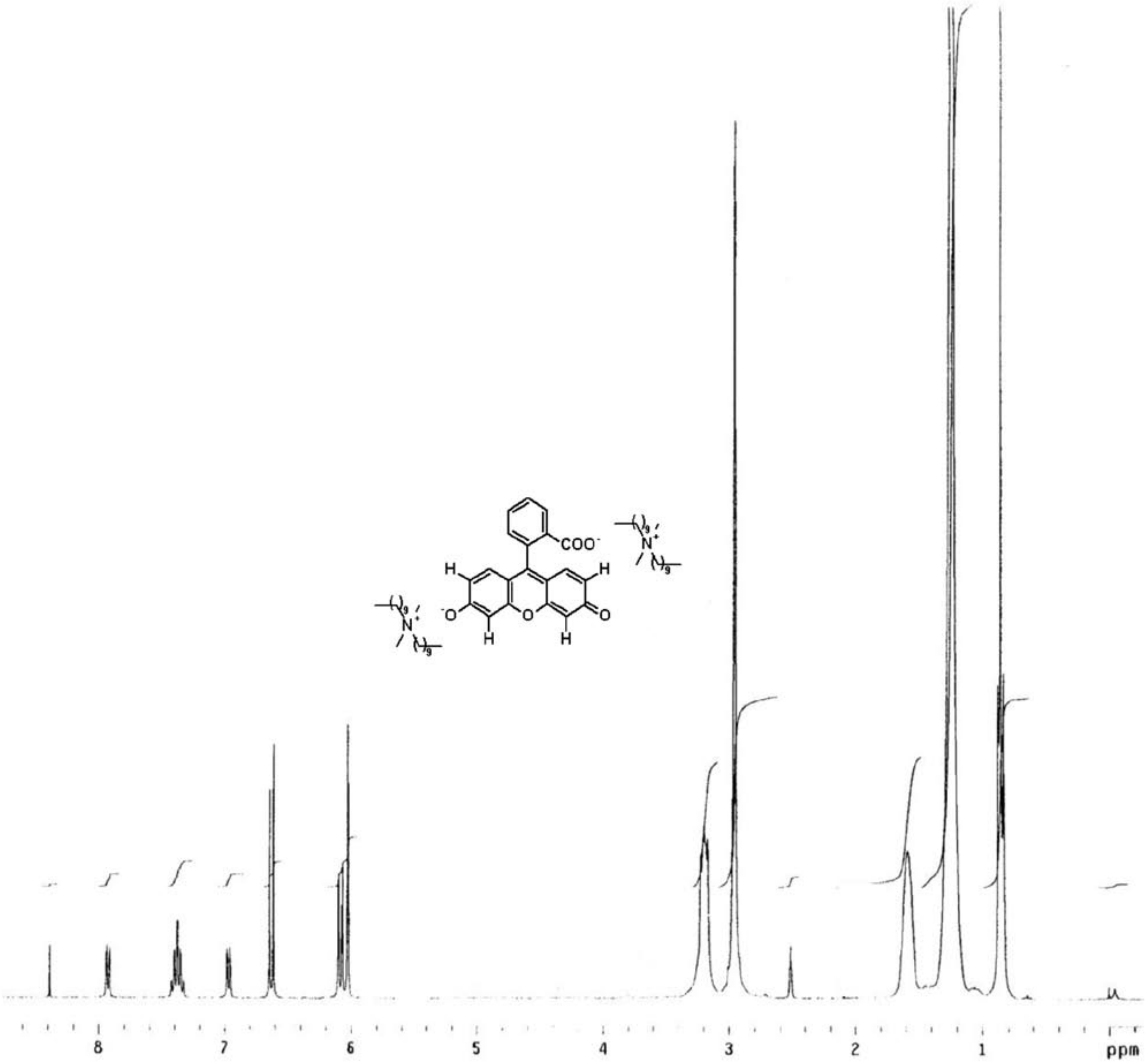

Figure S3. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **5**.

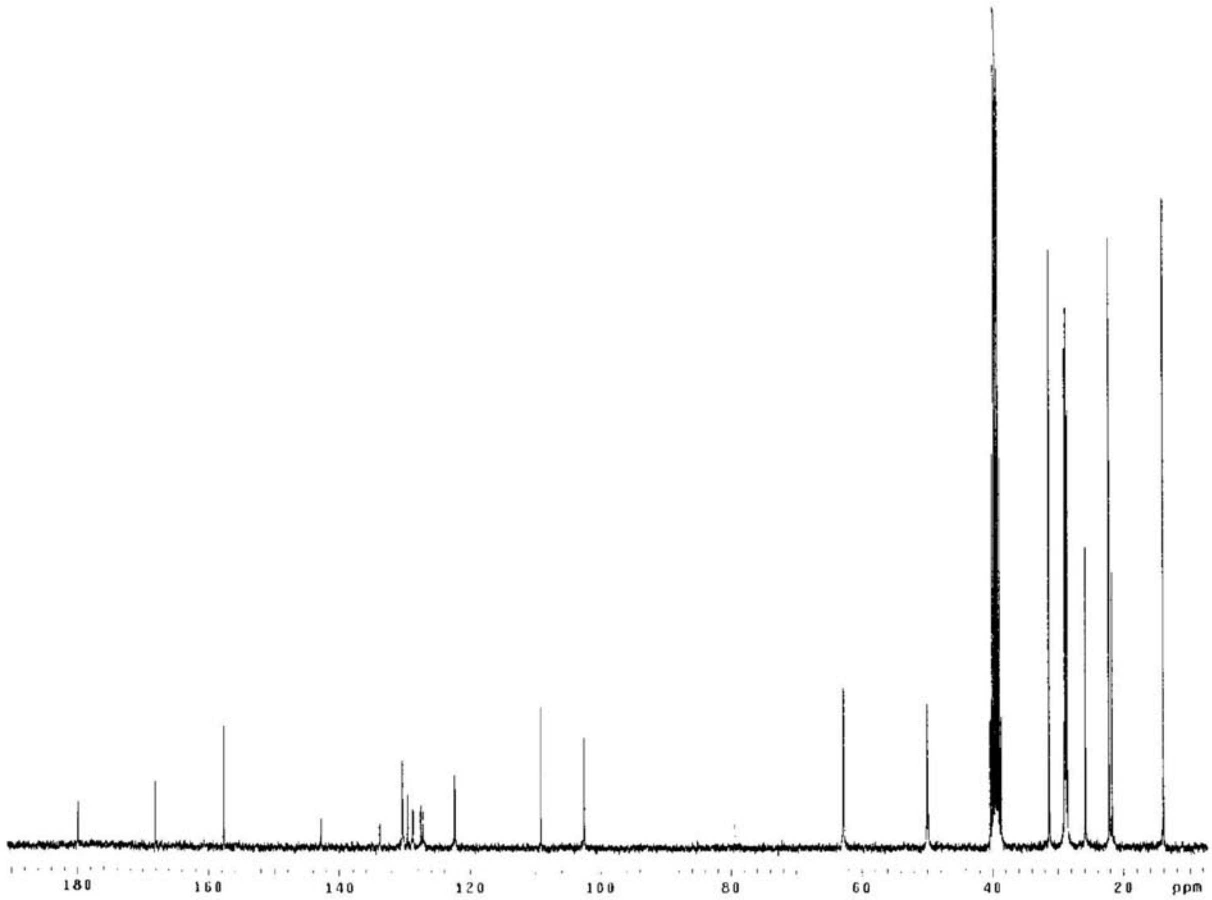

Figure S4. ^{13}C NMR spectrum (300 MHz, DMSO-d_6) of compound **5**.

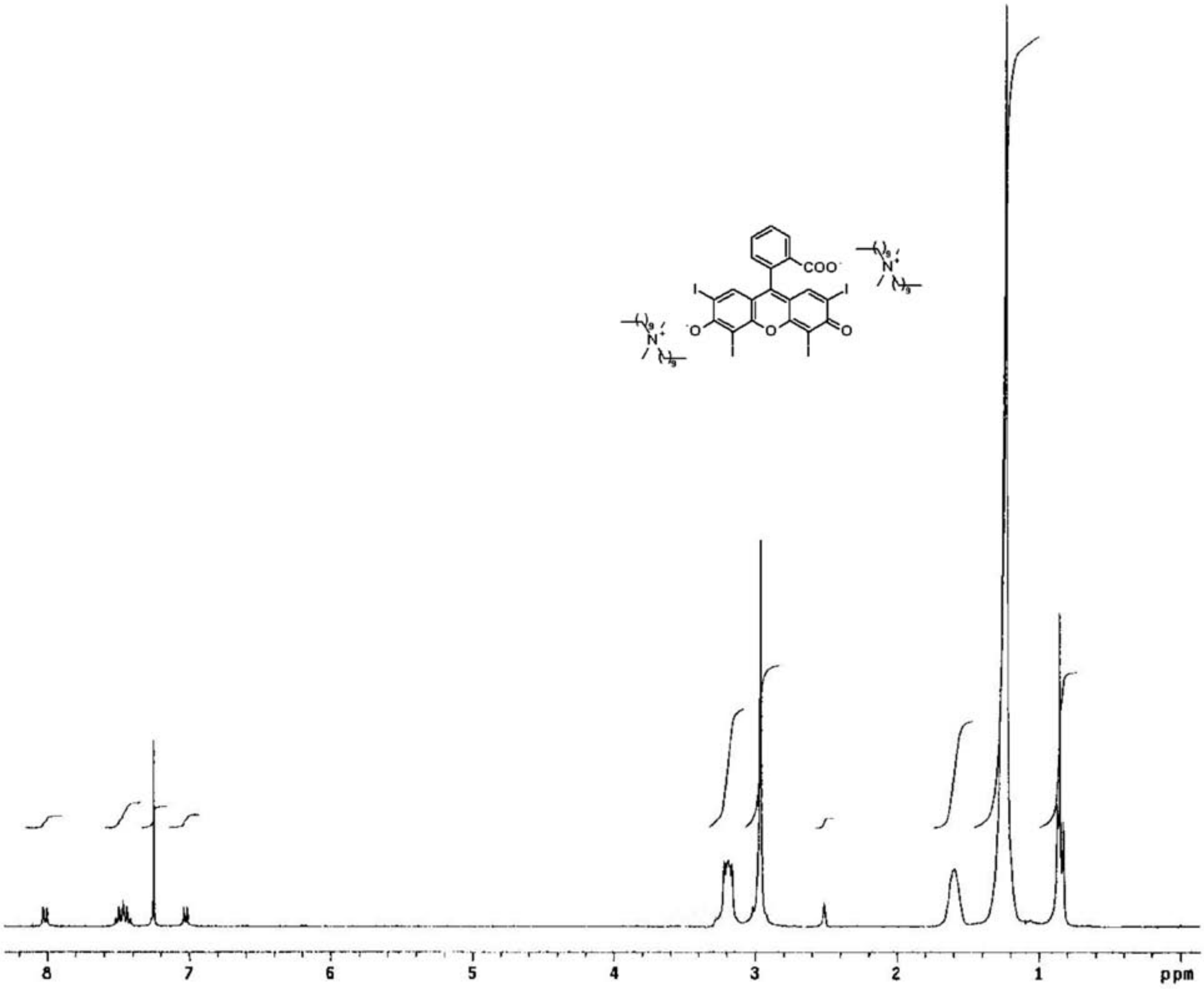

Figure S5. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of compound **8**.

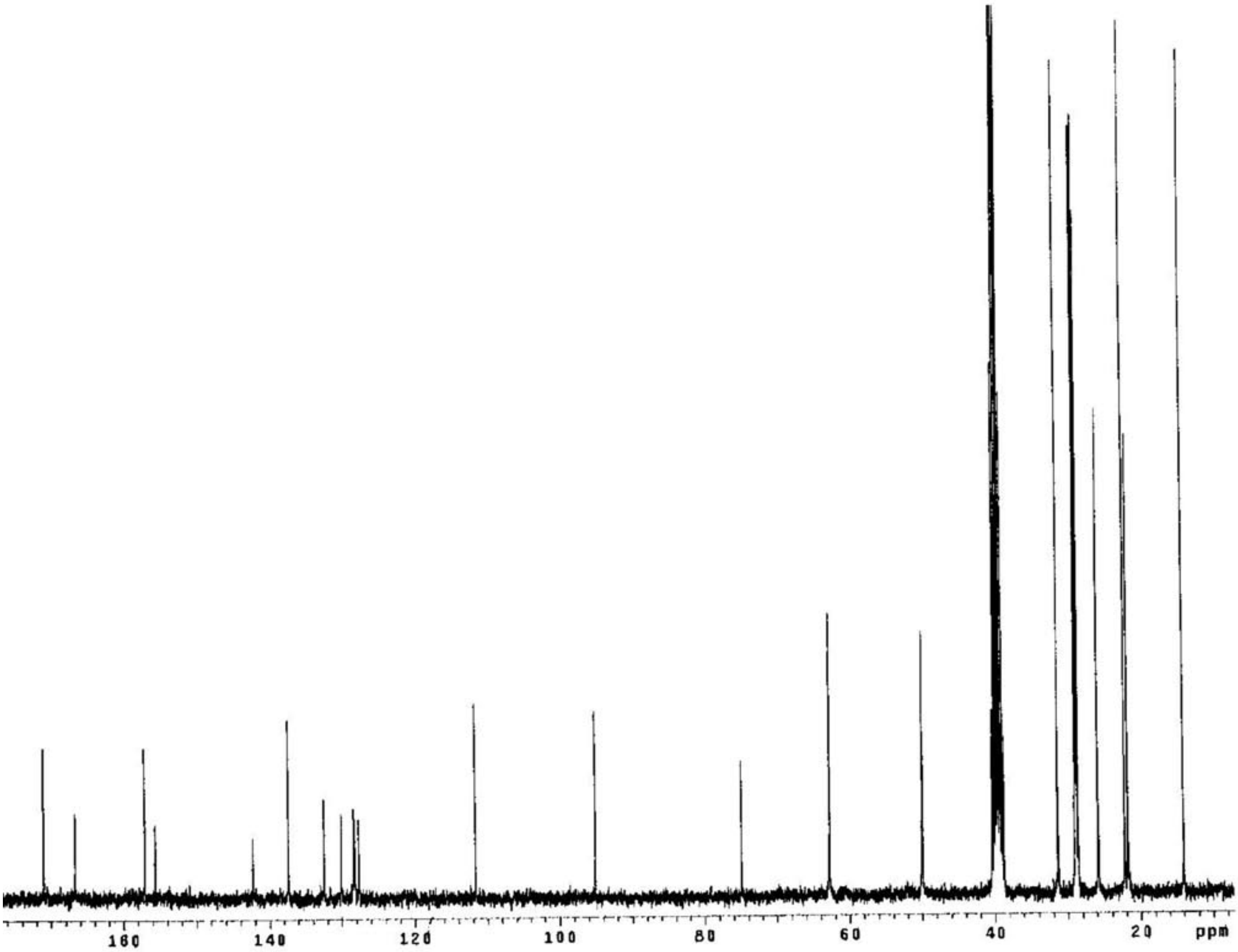

Figure S6. ^{13}C NMR spectrum (300 MHz, DMSO-*d*$_6$) of compound **8**.

Figure S7. 1% solution of compound **1** in chloroform.

Figure S10. 1% solution of compound **1** in acetone.

Figure S8. 1% solution of compound **4** in chloroform.

Figure S11. 1% solution of compound **4** in acetone.

Figure S9. 1% solution of compound **7** in chloroform.

Figure S12. 1% solution of compound **7** in acetone.