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Fluorescent Properties of 9-Anthracenecarboxamides

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Abstract—A number of new 9-anthracenecarboxamides are synthesized in order to create new fluorescent probes for studying biological systems. The parameters of their fluorescence in organic solvents of various polarities are investigated, and possible mechanisms of internal quenching of fluorescence of these compounds are discussed. One of the compounds, 4-ethoxycarbonylphenylamide of 9-anthracenecarboxylic acid, is shown to be a promising basis for the development of a new fluorescent probe.

Key words: 9-anthracenecarboxamides, fluorescence

INTRODUCTION

9-Anthracenecarboxylic acid and its esters (mainly 9-anthroyloxyfatty acids) have received wide acceptance as fluorescent probes for studying biological and model membranes.[2] Up to now, properties of these compounds have been much studied [1, 2]. Aliphatic amides of AC received no application as fluorescent probes probably due to their low quantum yields in most cases. For example, we found [3] that the quantum yield of 11-(9-anthroylamino)undecanoic acid is 1–3% in various media. Both aliphatic and aromatic amides of AC have scarcely been studied. Their photophysical properties are interesting, because the parameters of their fluorescence are in a complex dependence on the medium polarity and the fluorophore conformation [4, 5]. The mechanisms of relaxation of the excited state of AC aromatic amides attract attention [5] (see below). In addition, these substances and their analogues may be applied in fluorescent sensors for the determination of a number of ions [5, 6]. Our preliminary results indicate that anthracene-9-carboxamides may be prospective probes for studying biopolymers and membrane systems, since the emission parameters of some of them (position of maximum, quantum yield, etc.) largely depend on the polarity of medium and pH value. The properties of fluorescent probes available to researchers are far from optimal; new claims are being laid to them; and, therefore, the need in new probes of various types does not decrease [7–9].

This study is undertaken in order to comparatively study some fluorescent properties of an AC aliphatic amide and a number of AC aromatic amides.

RESULTS AND DISCUSSION

We describe here the fluorescence parameters (positions of emission maxima and quantum yields in a number of organic solvents) for methyl (11-(9-anthroylamino)undecanoate (**VII**) [3] and five aromatic amides (**VIII**)–(**XII**) (see scheme). The derivatives (**VIII**)–(**XII**) were obtained by one method, the reaction of 9-anthroyl chloride with the corresponding amine (**I**)–(**VI**) in the presence of an HCl acceptor. The following AC derivatives with an electron donor group in arylamide substituent were synthesized by this way: 4-methylphenylamide (**VIII**), 4-methoxyphenylamide (**IX**), 2-dodecanoylaminophenylamide (**X**), and isomeric 4-dodecanoylaminophenylamide (**XI**); 4-ethoxycarbonylphenylamide (**XII**) with an electron acceptor group in arylamide residue was also obtained. The 2- and 4-dodecanoylaminophenylamines (**IV**) and (**V**), synthons necessary for the synthesis of diamides (**X**) and (**XI**), were obtained by monoacylation of 1,2- and 1,4-phenylenediamines, respectively.

Note that 9-anthroyl chloride has rather low acylating ability toward low basic aromatic amines. Long reaction times (or heating) and the use of *N*-ethyldiisopropylamine for binding HCl were necessary to achieve satisfactory yields in this case. Triethylamine and pyridine were unsatisfactory as the HCl-binding agents: their use resulted in low yields of target products and polar side products probably due to the amine acylation with 9-anthroyl chloride to form quaternary ammonium salts and products of their further degradation.

Structures of the synthesized 9-anthroylamide derivatives were confirmed by UV, mass, and ^{1}H NMR spectra. The signal of anthracene H10 (singlet at δ 8.4–8.7 ppm) is characteristic of 9-anthroyl residue [5]. UV spectra of all the AC amides display an intensive short-wave peak at 250–270 nm (ε 6–7 $\times$ 10^4 M^{-1} cm^{-1}) and a triplet at 340–350, 360–370, and 380–390

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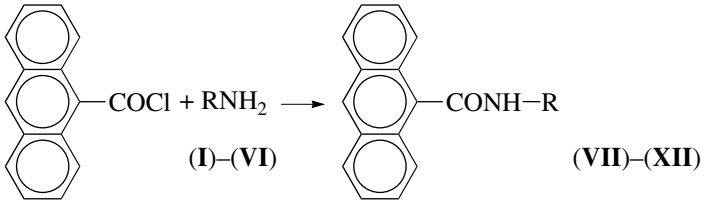
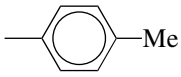
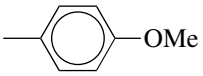
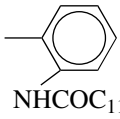
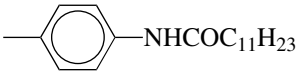
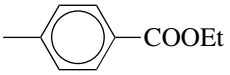
[2] Abbreviations: AC, 9-anthracenecarboxylic acid; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; PET, photoinduced electron transfer; and TICT, twisted internal charge transfer.



(**I**)–(**VI**) (**VII**)–(**XII**)

Compound	R
(**I**), (**VII**)	$C_{11}H_{23}COOMe$
(**II**), (**VIII**)	
(**III**), (**IX**)	
(**IV**), (**X**)	
(**V**), (**XI**)	
(**VI**), (**XII**)	

Scheme.

(ε 5–9 $\times$ 10^3 M^{-1} cm^{-1}) characteristic of 9-anthroyl derivatives [5, 10]; the corresponding maxima are clearly visible in the excitation spectra of (**VII**), (**XI**), and (**XII**) (see Fig. 1).

The fluorescence spectra of (**VII**)–(**XII**) were recorded for their solutions in media of various polarities: apolar (hexane, chloroform), polar aproton (acetonitrile), and polar proton (methanol). The excitation and emission spectra of the most interesting three amides (**VII**), (**XI**), and (**XII**) are shown in the figure. The most important parameters of the emission spectra of all the studied compounds are summarized in the table.

A close similarity of the absorbance and excitation spectra of all the studied substances is readily explained by the fact that, in the main state, the carbonyl group of anthroyl residue is oriented perpendicularly to the anthracene ring and its electrons are not conjugated with the aromatic system (see [5] and references therein).

Unlike 9-anthroyl esters [2], the emission spectra of AC amides (table, Fig. 1) do not show the same unequivocal dependence of the positions of their fluorescence maxima and quantum yields (Φ) on the polarity of medium. Obviously, all the amides have their quantum yields lower in polar solvents than in apolar ones; this effect is more pronounced for aromatic amides (**VIII**)–(**XII**) than for aliphatic (**VII**). In this case, aromatic substituents at the amide nitrogen atom very actively participate in the processes of fluorophore excitation and relaxation of the excited state, which is evident from the table. The aromatic amides with the electron donor substituents (**VIII**)–(**XI**) display substantially lower Φ values than the aliphatic amide (**VII**) or the aromatic (**XII**) with an electron donor substituent. The AC esters exhibit a clear dependence of the position of emission maximum on the polarity of medium (the more the polarity the more the shift of the maximum to the long-wave area and the less the quantum yield [2]), whereas the AC amides do not demonstrate such a direct dependence. Undoubtedly, this is a consequence of a complex character of the interaction

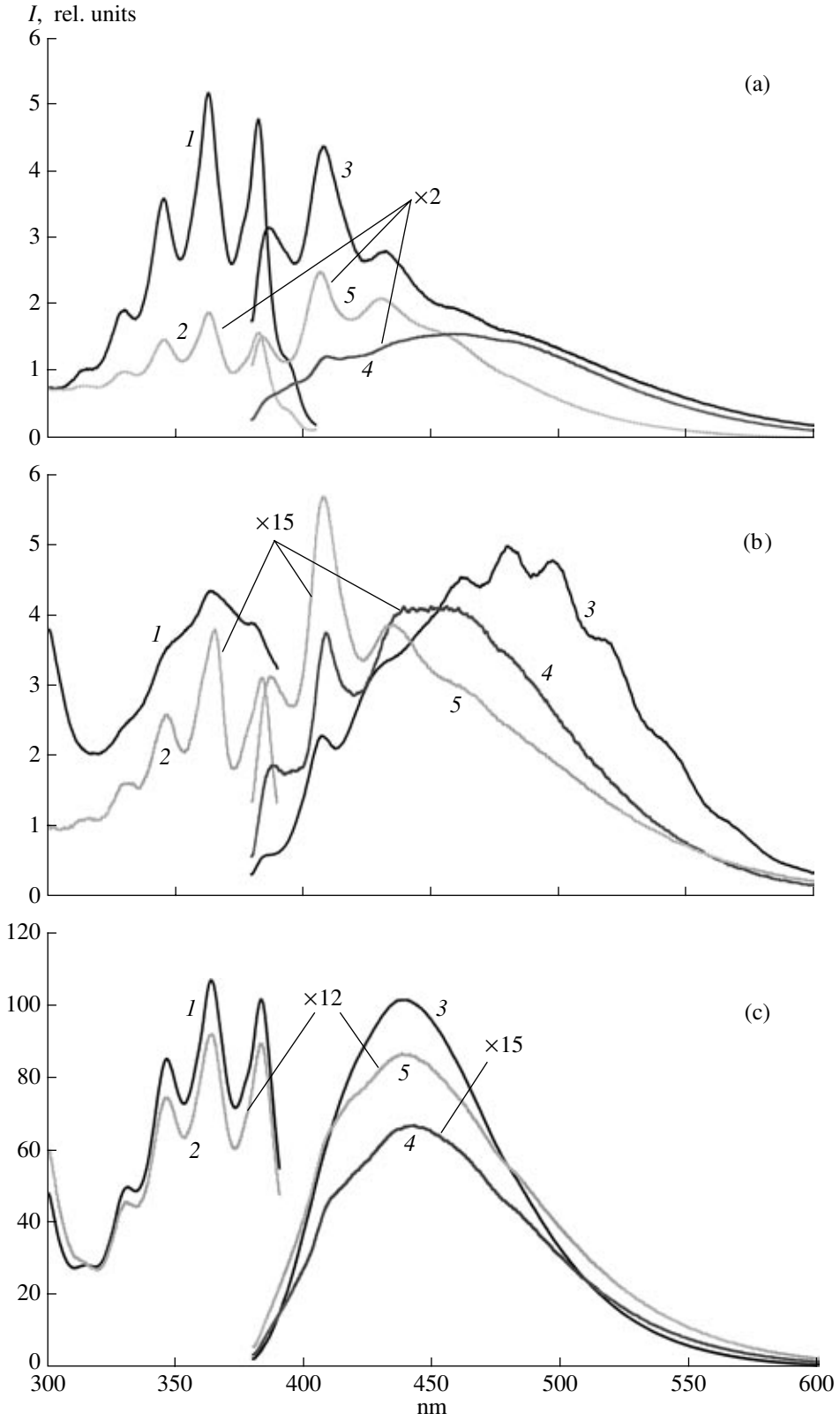


Fig. 1. The (*1*, *2*) excitation and (*3*, *4*, *5*) emission spectra of AC amides (a) (**VII**), (b) (**XI**), and (c) (**XII**). The concentration of substances is approximately 20 μM and temperature 20°C. The spectra are measured in (*1*, *3*) hexane, (*4*) acetonitrile, and (*2*, *5*) methanol. The emission spectra are recorded at λ_{em} (a, b) 410 and (c) 440 nm; all the excitation spectra, at λ_{ex} 365 nm.

Maxima of fluorescence and quantum yields of *N*-anthroyl derivatives in media of various polarities

Substance	Solvent			
	hexane	chloroform	acetonitrile	methanol
(**VII**)	384.5, **407**, 431 9	391, 413.5, **454.5** 15	409.5, **458** 5	386.5, **407.5**, 432.5 5
(**VIII**)	408, **435** 0.2	416.5, **436.5** 0.1	409, **433** 0.07	**408**, 432 0.07
(**IX**)	409, **444** 0.02	414.5, **437** 0.01	**410**, 436 0.005	**408**, 431 0.005
(**X**)	**408**, 430 0.5	416.5, **439** 0.4	**409.5**, 434 0.3	**451** <0.001
(**XI**)	408, 463, **480**, 497.5 5	392, 413, **461.5** 0.4	388, **409**, 455 0.3	387.5, 408, **434** 0.3
(**XII**)	**438.5** 18	**456** 9	**442.5** 1	**438** 1

Note: The wavelengths of emission maxima (the most intensive is given in boldface) are listed in numerator; the quantum yields (%), in denominator. The excitation wavelength is 365 nm.

of amide grouping and the substituent at it with the basic (anthroyl) fluorophore.

It is accepted [5] that carbonyl remains perpendicular to fluorophore in the photoexcited state I of AC amide. The emission from this state is short-wave and resembles that from anthracene (see spectrum *3* in Fig. 1a or spectrum *5* in Fig. 1c). The subsequent relaxation into the excited state II leads to the conjugation of the carbonyl with the anthroyl fluorophore; this state has a longer-wave light emission (like that of spectrum *4* in Fig. 1a), if an aromatic substituent is present at the NH group. Its conjugation through the amide bond additionally shifts the emission maximum to the red side of spectrum (see spectrum *3* in Fig. 1c). The conjugation degree of the amide grouping bearing an aromatic substituent with the anthroyl fluorophore may be different, and therefore, the emission spectrum of the AC amides may be structured to a lesser degree than that of anthracene or even be completely unstructured. In the case of aromatic amides, one more excited state with the separation of charges of fluorophore and aromatic substituent is formed due to the rotation of C(O)–NH bond by the TICT mechanism. The recovery from this state into the main state is not emissive; i.e., a quenching of fluorescence occurs and the TICT structures are stabilized by polar solvents with a low viscosity [5, 11]. It is highly probable that the fluorescence quenching by the TICT mechanism is the cause of low quantum yields of (**VIII**)–(**XI**), particularly, in acetonitrile and methanol (see the table).

The observed dependences of the positions of λ_{em} and Φ values on the AC amide structures are probably determined (at least, partially) by another mechanism, PET. According to PET, the quenching of the excited fluorophore state occurs due to the photoexcited unshared electron pair at the nitrogen atom in the side chain at fluorophore. However, until now, the PET mechanism is only ascribed to the fluorescence quenching of aromatic fluorophores with an amine group in side chain [12]. We can presume that the same mechanism is also inherent in the photophysical processes in the case of AC amides under consideration. The following observation could confirm this presumption. An addition of DBU, a strong base, up to the concentration of 1 mM to a 20 μM solution of (**VII**) in acetonitrile (spectrum *4*, Fig. 1a) results in significant changes in the fluorescence spectrum of the solution: it becomes structured, its maximum is shifted to short-wave area, and it becomes practically identical to the spectrum in methanol (*5*). A similar, but a weaker, effect of DBU was also observed in the case of other AC amides (data not shown). This effect can be attributed to the change in the charge on the amide nitrogen atom under the action of DBU; amide group is known to have the properties of both a weak acid and a weak base [13]. However, this effect may also be explained within the frames of TICT mechanism; the choice could obviously be made after the further thorough studies.

Among the AC amides described here, (**XII**) attracts a particular interest. It displays the highest quantum yield in comparison with the other AC amides, and its fluorescence maximum and the form of spectrum little depend on the polarity of medium, whereas its Φ value largely depends on this parameter (see the table and Fig. 1c). Obviously, this effect is due to the electron-acceptor action of ethoxycarbonyl group, which reduces the participation of the electrons of phenylene ring in the quenching of anthroyl fluorescence. On the

other hand, the interaction of solvent dipoles with ester group leads to a decrease in the electron-acceptor effect and an increase in the quenching presumably by the TICT mechanism (see the table). Amide (**XII**) has satisfactory chemical and photostabilities: its alcoholic solution does not undergo a marked degradation in the dark at room temperature for more than 15 days (TLC) and irradiation of its hexane solution with the excitation light in a fluorimeter (see the Experimental section) for 1 h does not change its emission spectrum.

We believe that the parameters of (**XII**) (fluorescence parameters, the dependence of Φ value on the polarity of medium, stability, and a convenient method of synthesis) allow its use for the design of fluorescent probes suitable for studying biological systems.

EXPERIMENTAL

We used dodecanoyl chloride, DBU, *N*-ethyldiisopropylamine, and ethyl *p*-aminobenzoate from Fluka (Switzerland), 9-anthracenecarboxylic acid and 1,4-phenylenediamine dihydrochloride from Merck (Germany), and the other reagents and solvents from Reakhim (Russia). Dry chloroform was obtained by distillation over phosphorus pentoxide, other solvents were used after the purification by usual procedures. Kieselgel 60 (Merck, Germany) was used for column chromatography. Precoated plates with fluorescent indicator (Kieselgel 60 F_{254}) and without the indicator (Kieselgel 60) were from (Merck, Germany). Substance spots were detected by phosphomolybdic acid (A), ninhydrin (B), and by visualization under UV light (C). Anthracene-9-carboxylic acid chloride was obtained by boiling of the acid with thionyl chloride excess in chloroform containing 0.05% DMF; mp 95–98°C (decomp., from chloroform–toluene). Methyl 11-(9-anthroylamino)undecanoic acid (**VII**) was synthesized as described previously [3].

Mass spectra were measured under the electron impact ionization (70 eV) on a SSQ-710 instrument (Finnigan MAT, United States); UV spectra, on a Ultraspec II spectrophotometer (LKB, Sweden); fluorescence spectra, on a Hitachi F-4000 spectrofluorimeter (Japan); and ^{1}H NMR spectra (δ, ppm, relative Me_4Si, spin coupling constants, *J*, Hz), on a Bruker WM 500 spectrometer (Germany).

2-Dodecanoylaminophenylamine (IV). A solution of dodecanoyl chloride (0.22 g, 1 mmol) in chloroform (1 ml) was added in five portions at 5-min intervals to a solution of 1,2-phenylenediamine (0.52 g, 4.2 mmol) and *N*-ethyldiisopropylamine (0.2 ml) in dry chloroform (10 ml). The solution was kept for 12 h, diluted with ether (100 ml), washed with water (3 × 15 ml), dried with Na_2SO_4, and evaporated. Chromatography on a silica gel column eluted with 5 ⟶ 10% ethyl acetate in chloroform led to pure (**IV**) as a white powder homogeneous according to TLC; yield 152 mg (52%); R_f 0.7 (80 : 17 : 1 chloroform–ethyl acetate–methanol; A, B); mp 93–94°C (from chloroform–hexane); MS, *m*/*z*: 290 $[M]^+$; ^{1}H NMR ($CDCl_3$): 0.89 (3 H, t, *J* 7.1, CH_3), 1.28 [16 H, broad m, $(C\underline{H}_2)_8CH_3$], 1.76 (2 H, m, $COCH_2C\underline{H}_2$), 2.41 (2 H, t, *J* 7.6, $COCH_2$), 3.86 [bs, (NH_2)], 6.81 (2 H, d, *J* 7.1, arom. H), 7.07 (1 H, t, *J* 7.8, arom. H), 7.10 (1 H, bs, NH), and 7.19 (1 H, d, *J* 8.3, arom. H).

4-Dodecanoylaminophenylamine (V) was obtained as described for (**IV**) by a reaction of a solution of 1,4-phenylenediamine dihydrochloride (0.7 g, 3.9 mmol) and *N*-ethyldiisopropylamine (1 ml) in chloroform (25 ml) with dodecanoyl chloride (0.22 g); chromatographically homogeneous cream-white powder; yield 20%; R_f 0.5 (80 : 17 : 1 chloroform–ethyl acetate–methanol; A, B); mp 150–153°C (sintered at about 140°C, from chloroform–hexane); MS, *m*/*z*: 290 $[M]^+$.

Anthroylamides (general procedure). 9-Anthracenecarboxylic acid chloride (1.5 equiv) was added to a stirred solution of an aromatic amine or a derivative (**IV**) or (**V**) (0.2–1 μmol) and *N*-ethyldiisopropylamine (3–5 equiv) in dry chloroform (5–15 ml). The mixture was stirred until the complete dissolution, allowed to stand for 2 days, diluted with ethyl acetate, treated with water, and intensively stirred for 6 h. Then it was twice washed with water and a saturated NaCl solution (10 ml each), and dried with Na_2SO_4. The target substance was isolated from the extract by column chromatography on silica gel in a step gradient of ethyl acetate in benzene or chloroform (99 : 1 to 9 : 1) monitoring the separation by TLC (detection A–C). In this manner, there were obtained:

9-Anthracenecarboxylic acid 4-methylphenylamide (VIII); yield 40%; R_f 0.5 (19 : 1 benzene–ethyl acetate, A, C); mp 202–205°C (with sublimation, from chloroform–methanol); MS, *m*/*z*: 311 $[M]^+$, 205 $[C_{14}H_9CO]^+$; ^{1}H NMR ($CDCl_3$): 2.40 (3 H, s, CH_3), 7.26 (2 H, d, *J* 8.6, arom. H), 7.53 (4 H, m, arom. H), 7.64 (1 H, bs, NH), 7.67 (2 H, d, *J* 8.6, arom. H), 8.05 (2 H, d, *J* 8.1, arom. H), 8.19 (2 H, d, *J* 8.1, arom. H), and 8.54 (1 H, s, arom. H).

9-Anthracenecarboxylic acid 4-methoxyphenylamide (IX); yield 35%; R_f 0.4 (19 : 1 benzene–ethyl acetate, A, C); mp 219–222°C (from chloroform–methanol); MS, *m*/*z*: 327 $[M]^+$, 205 $[C_{14}H_9CO]^+$; ^{1}H NMR ($CDCl_3$): 3.77 (3 H, s, CH_3), 6.89 (2 H, d, *J* 10.9, arom. H), 7.43 (4 H, m, arom. H), 7.51 (1 H, bs, NH), 7.60 (2 H, d, *J* 10.9, arom. H), 7.95 (2 H, d, *J* 10.6, arom. H), 8.09 (2 H, d, *J* 10.6, arom. H), and 8.44 (1 H, n, arom. H).

9-Anthracenecarboxylic acid 2-dodecanoylaminophenylamide (X); yield 70%; R_f 0.55 (83 : 15 : 2 benzene–ethyl acetate–acetic acid, A, C); mp 151−153°C (from chloroform–methanol); MS, *m*/*z*: 494 $[M]^+$, 476 $[M - H_2O]^+$, 311 $[M - C_{11}H_{23}CO]^+$, 293 $[M - C_{11}H_{23}CO - H_2O]^+$, 205 $[C_{14}H_9CO]^+$; ^{1}H NMR ($CDCl_3$): 0.89 (3 H, t, *J* 6.8, CH_3), 1.22 [16 H, bm,

$(CH_2)_8CH_3$], 1.55 (2 H, m, $COCH_2C\underline{H}_2$), 2.32 (2 H, t, *J* 7.5, $COCH_2$), 7.31 (2 H, m, arom. H), 7.51 (4 H, i, arom. H), 7.56 (1 H, m, NH), 7.63 (1 H, m, NH), 8.03 (2 H, d, *J* 7.8, arom. H), 8.13 (2 H, d, *J* 7.8, arom. H), 8.34 (1 H, s, arom. H), 8.45 (1 H, s, arom. H), and 8.52 (1 H, s, arom. H).

9-Anthracenecarboxylic acid 4-dodecanoylaminophenylamide (XI); yield 45%; R_f 0.5 (83 : 15 : 2 benzene–ethyl acetate–acetic acid, A, C); mp 195–200°C (sintered at ~160°C, from chloroform–methanol); MS, *m*/*z*: 494 $[M]^+$, 205 $[C_{14}H_9CO]^+$; ^{1}H NMR ($CDCl_3/CD_3OD$): 1.06 (3 H, t, *J* 6.4, CH_3), 1.45 (16 H, bm, $(C\underline{H}_2)_8CH_3$)), 1.90 (2 H, m, OCH_2CH_2), 2.55 (2 H, t, *J* 7.5, OCH_2), 7.66–7.72 (5 H, bm, arom. H + NH), 7.78 (2 H, d, *J* 8.1, arom. H), 7.94 (2 H, d, *J* 8.1, arom. H), 8.22 (2 H, d, *J* 8.2, arom. H), 8.29 (2 H, d, *J* 8.2, arom. H), and 8.71 (1 H, s, arom. H).

9-Anthracenecarboxylic acid 4-ethoxycarbonylphenylamide (XII). Due to the slow reaction, the reaction mixture was kept for 2 days at 50°C; yield of (**XII**) 46%; R_f 0.6 (19 : 1 chloroform–ethyl acetate, A, C); mp 218–220°C (from chloroform–methanol); MS, *m*/*z*: 369 $[M]^+$, 324 $[M - OC_2H_5]^+$, 296 $[M - COOC_2H_5]^+$, 205 $[C_{14}H_9CO]^+$; ^{1}H NMR ($CDCl_3$–CD_3OD): 1.51 (3 H, t, *J* 14.7, CH_3), 4.48 (2 H, q, CH_2), 7.61 (5 H, m, NH, arom. H), 8.00 (2 H, d, *J* 8.2, arom. H), 8.14 (2 H, d, *J* 8.2, arom. H), 8.17 (2 H, d, *J* 9.3, arom. H), 8.19 (2 H, d, *J* 9.3, arom. H), and 8.63 (1 H, s, arom. H).

Fluorescent measurements were carried out on a Hitachi F-4000 fluorimeter in quartz cuvettes 10 × 10 mm. The corrected spectra of the fluorescence emission were registered at the excitation wavelength of 365 nm; the excitation spectra, at the wavelength of the main emission maximum. The slit width was 3 nm for the excitation and 5 nm for the emission; temperature was 20 ± 1°C. The optical absorption of sample did not exceed 0.1.

The quantum yields were determined by the known formula using 1,8-anilinonaphthalenesulfonate as a standard:

$$\Phi = \Phi_{st}\frac{IA_{st}}{I_{st}A},$$

where A and A_{st} are the optical absorbances, and I and I_{st} are the integral intensities of fluorescence of the sample and the standard, respectively; the quantum yield of standard $\Phi_{st} = 0.2$ in methanol [14].

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