Synthesis and Fluorescence Properties of Novel Transmembrane Probes and Determination of Their Orientation within Vesicles[1])

by **Ernesto Quesada**[a]), **Malin Ardhammar**[b]), **Bengt Nordén**[b]), **Michel Miesch**[c]), **Guy Duportail**[d]), **Yvonne Bonzi-Coulibaly**[e]), **Yoichí Nakatani***[c]), and **Guy Ourisson**[e])

[a]) Instituto de Química Orgánica General C.S.I.C., Juan de la Cierva 3, E-28006 Madrid
[b]) Department of Physical Chemistry, Chalmers University of Technology, SE-41296 Göteborg
[c]) Laboratoire de Chimie Organique Synthétique, associé au CNRS, Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, F-67084 Strasbourg
[d]) Laboratoire de Pharmacologie et Physicochimie, UMR 7034 du CNRS, Faculté de Pharmacie, Université Louis Pasteur, F-67401 Illkirch
[e]) Laboratoire de Chimie Organique des Substances Naturelles, associé au CNRS, Centre de Neurochimie, Université Louis Pasteur, 5 rue Blaise Pascal, F-67084 Strasbourg (tel: (+33) 388456732; fax: (+33) 388607620; e-mail: nakatani@chimie.u-strasbg.fr)

This paper is dedicated to a 45 years' friendship with *Albert Eschenmoser*, not enough made use of. In our next life, I shall better make use of you, *Albert*!

G. Ourisson

Two novel transmembrane fluorescent diester probes **D** and **E** bearing an anthracenediyl moiety in the middle of the molecule have been synthesized. Their absorption and fluorescence spectra in $CHCl_3$ solution as well as their fluorescence characteristics in dimyristoylphosphatidylcholine (DMPC) large unilamellar vesicles were determined. Although their absorption spectra (first transition, $S_0 \rightarrow S_1$) present a good overlap with the fluorescence spectrum of tryptophan, only probe **E** could be a good acceptor for the energy-transfer experiments, since a strong overlap exists between the absorption spectrum of tryptophan and the second transition ($S_0 \rightarrow S_2$) of the absorption spectrum of probe **D**. The *Förster* critical distance R_0 for energy transfer between tryptophan (donor) and probe **E** (acceptor) is found to be 23–24 Å. Finally, linear-dichroism studies on shear-deformed DMPC vesicles show the incorporated probe **E** to lie essentially perpendicular to the bilayer plane. These results establish that probe **E** could be useful in the study of membrane-bound protein topography by the fluorescence-energy-transfer method.

Introduction. – Although there has been considerable progress in crystallizing membrane proteins (for a recent example, see [1]), the determination of protein organization within a membrane is still quite difficult. The *in situ* determination of the topography (localization, orientation, and conformation) of molecules present in biomembranes calls for specific methods involving, *e.g.*, photochemical labelling [2], fluorescence-energy transfer [3], or fluorescence quenching [4]. The fluorescence studies are especially useful to define the position of tryptophan residues of membrane proteins. However, just like for the photolabelling methods, a limitation of the fluorescence methods lies in the difficulty of avoiding extensive disorder of the probe in the liquid-crystalline phase of the phospholipid matrix, leading to a loss of depth selectivity. The concept of transmembrane immobilization of the probe might provide a solution to this problem [5–7].

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[1]) Preliminary results of this work were presented at the XVII International *IUPAC* Meeting on Photochemistry, Sitges, Barcelona, Spain, July, 1998.

We have previously developed the photoactivable membrane probe **A** designed to maintain the photosensitive group near the middle of the membrane [5]. This is a phospholipid characterized by a double-headed system, with the photoactivable group (in our case, a benzophenone) localized in the center of a transmembrane chain. It is easily incorporated into dimyristoylphosphatidylcholine (DMPC) vesicles where, at least in the presence of a high concentration of cholesterol, it spans the membrane. It is also highly regioselective for targets lying near the center of the membrane. We had extended the same principle to fluorescence studies with the novel membrane probes **B** and **C** of similar overall length [8] (*Fig. 1*). It was surprising to find that not only the diphospholipidic fluorene probe **B**, but also the fluorene diester **C**, carrying distal ester groups, are apparently, as judged by their fluorescence properties, well oriented perpendicularly to the bilayer [9].

We have planned to incorporate probes **B** or **C** into membranes so that, if they approached tryptophan residues of a protein, there could be fluorescence-energy transfer between the tryptophan residue and the probe (*Fig. 2*). However, we found that there is an important overlap between the absorption spectra of a tryptophan

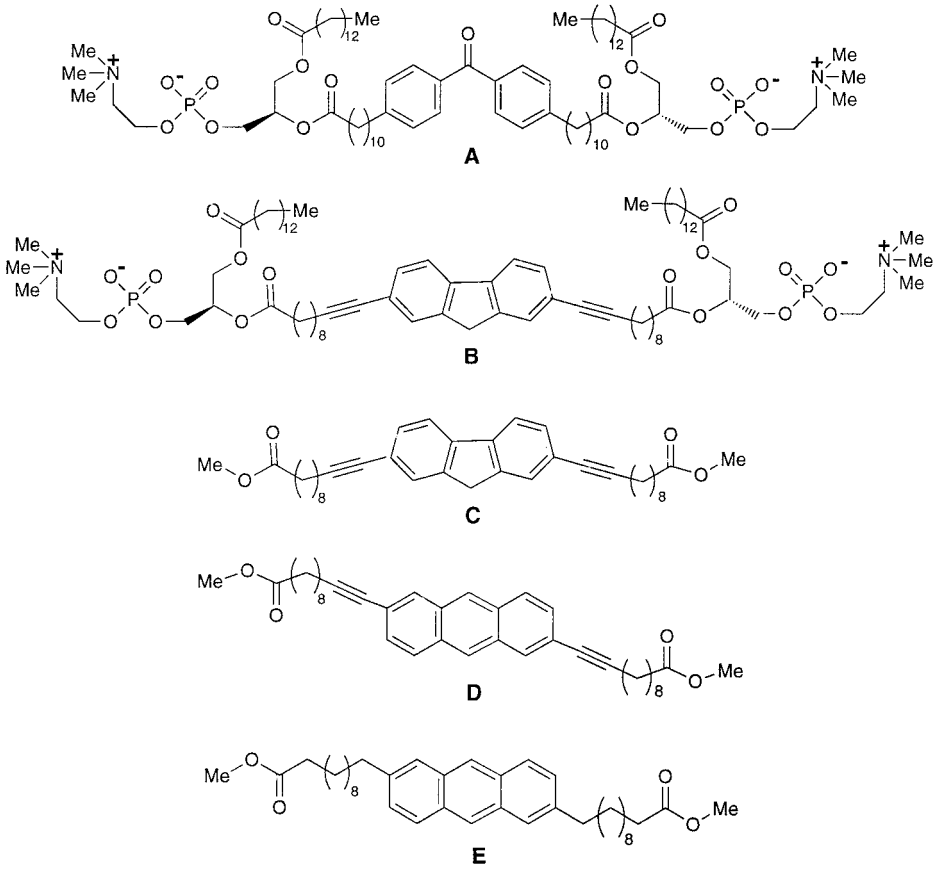


Fig. 1. *Structure of transmembrane photosensitive or fluorescent probes*

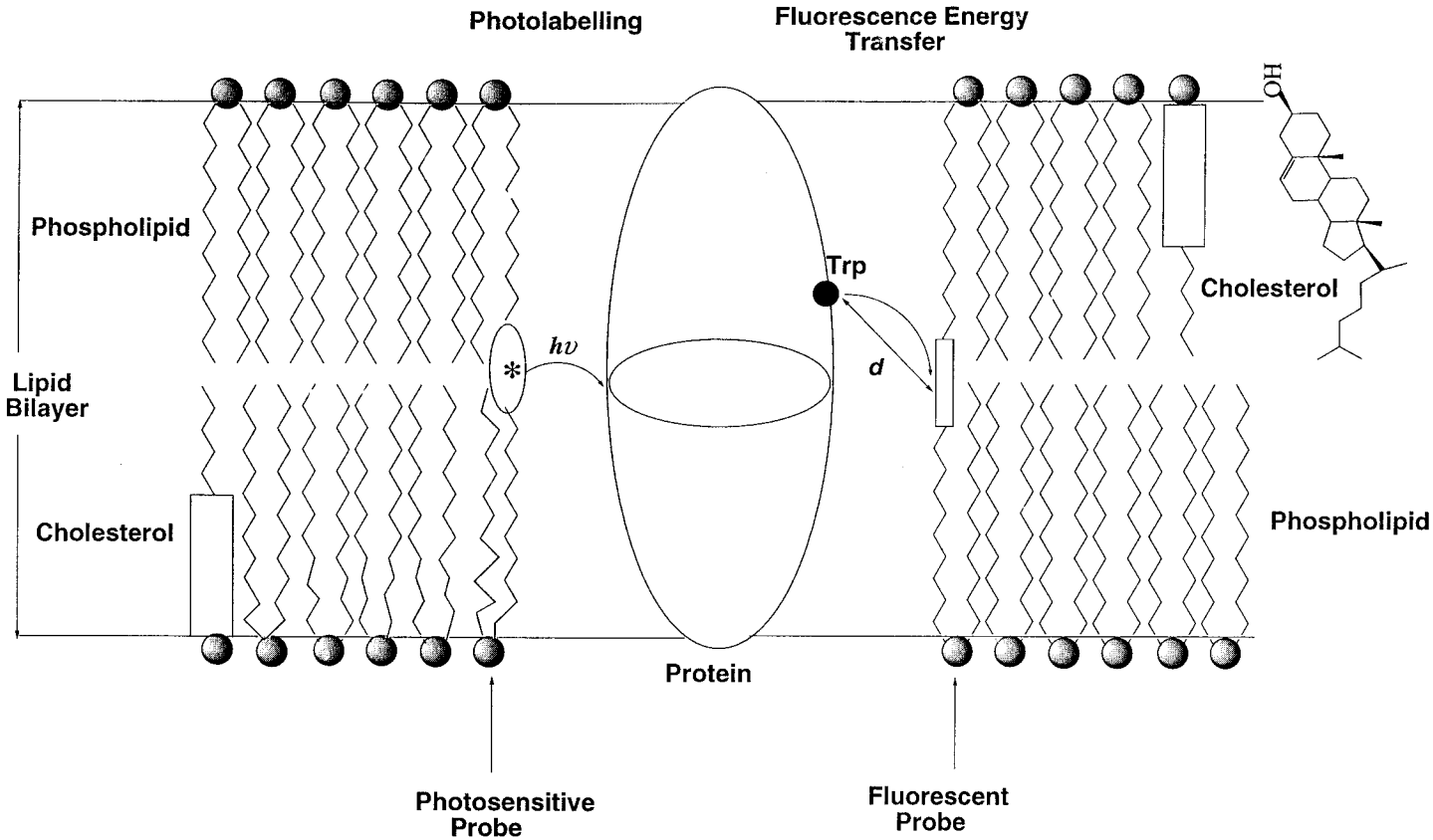
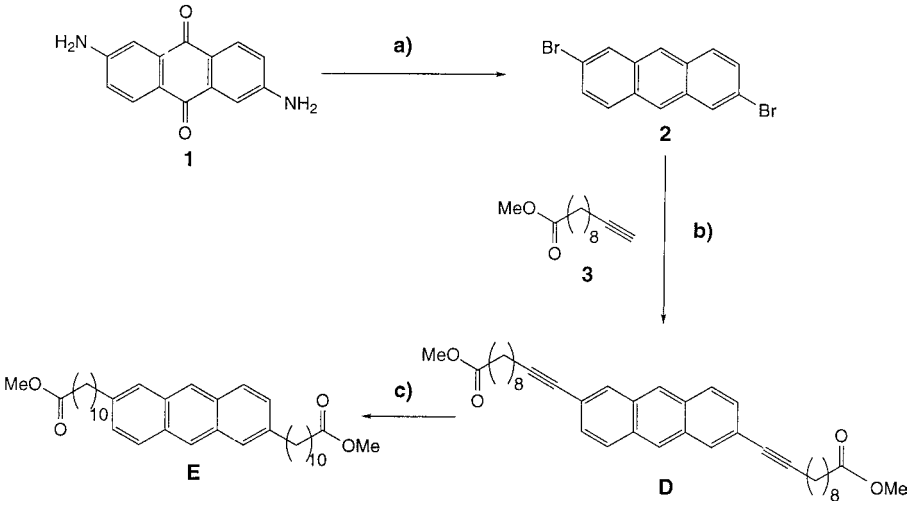


Fig. 2. *Schematic representation of how to determine the topography of proteins in the membrane: photolabelling method* (left) *and fluorescence-energy-transfer method* (right)

(donor) and the diacetylenic fluorene chromophore (acceptor) of both **B** and **C**. This overlap would prevent an accurate evaluation of the distance between donor and acceptor groups. To circumvent this problem, we have now synthesized the anthracene diesters **D** and **E**. We show that probe **E** has favorable properties for fluorescence-energy transfer in membranes. Moreover, linear-dichroism experiments demonstrate that probe **E** is inserted perpendicularly to the bilayer plane. Therefore, this probe might enable us to quantify the donor-acceptor distance and to determine the location of tryptophan residues in membranes. While the corresponding diacids might have been better anchored in the water compartments on both sides of the membranes, it was found unnecessary to study them, as the diesters were apparently already transverse, and furthermore, the diacids were insoluble.

Results and Discussion. – *Synthesis.* We used 2,6-diaminoanthraquinone (**1**) as the starting material to prepare both probes **D** and **E** (*Scheme 1*). Thus, quinone **1** was transformed into 2,6-dibromoanthracene (**2**) [10], and the two unbranched acyl chains were introduced using a *Sonogashira-Hagihara*-type cross-coupling [11] ($[Pd(Ph_3P)_2Cl_2]$/CuI catalysis, Et_2NH) with the acetylenic derivative **3**. Probe **D** was isolated as bright yellow crystals in 70% yield. Reduction of both triple bonds of **D** was best achieved by a slurry of activated *Raney*-Ni, leading to probe **E** in 55% yield.

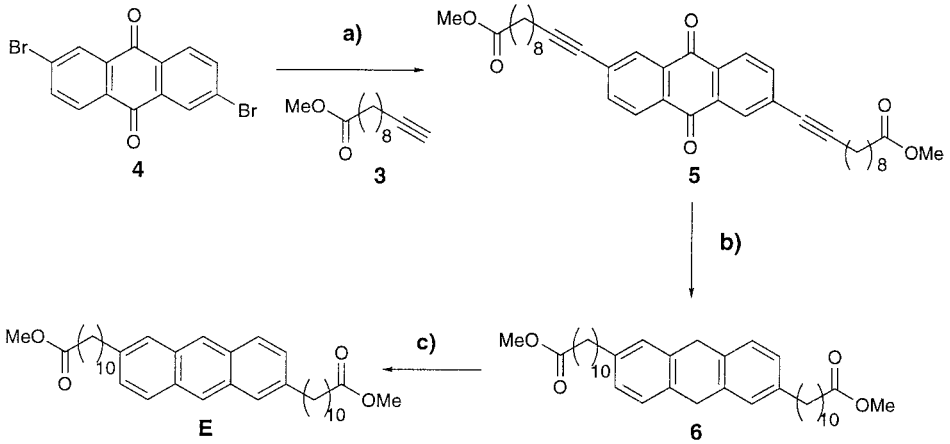
Scheme 1. *Synthesis of the Fluorescent Probe* **E**



a) 1. $CuBr_2$, MeCN, 80°; 2. $NaBH_4$, MeOH; 3. 5M HCl, 90°, 6 h; 4. $NaBH_4$, ^{i}PrOH. *b*) $[Pd(Ph_3P)_2Cl_2]$, CuI, Et_2NH, reflux, 4 h. c) *Raney*-Ni, THF, r.t.

Alternatively, we ran first a *Sonogashira-Hagihara*-type cross-coupling between 2,6-dibromoanthraquinone (**4**) and the acetylenic derivative **3** (*Scheme 2*). Compound **5** was obtained in 73% yield. The reduction was carried out in AcOH/$CHCl_3$ 1:1 in the presence of PtO_2 as catalyst under H_2 at 10 bar; this gave the dihydro derivative **6** in 31% yield. Finally, a DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone = 4,5-dichloro-3,6-dioxocyclohexadiene-1,2-dicarbonitrile) oxidation of compound **6** led to probe **E** in 50% yield.

Scheme 2. *Alternative Synthesis of the Fluorescent Probe* **E**



a) [Pd(Ph$_3$P)$_2$Cl$_2$], CuI, Et$_2$NH, reflux, 8 h. *b*) H$_2$, PtO$_2$, AcOH/CHCl$_3$, 10 bar, r.t. *c*) DDQ, dioxane, reflux, 6 h.

Absorption and Fluorescence Characterization. Spectra were first measured in $CHCl_3$ solution, and comparison was systematically made with anthracene, considered as a reference compound for this kind of derivatives. Absorption spectra are shown in *Fig. 3* for the first absorption band ($S_0 \rightarrow S_1$) and in *Fig. 4* for the second absorption band ($S_0 \rightarrow S_2$). Concerning the first absorption band, both probes show properties

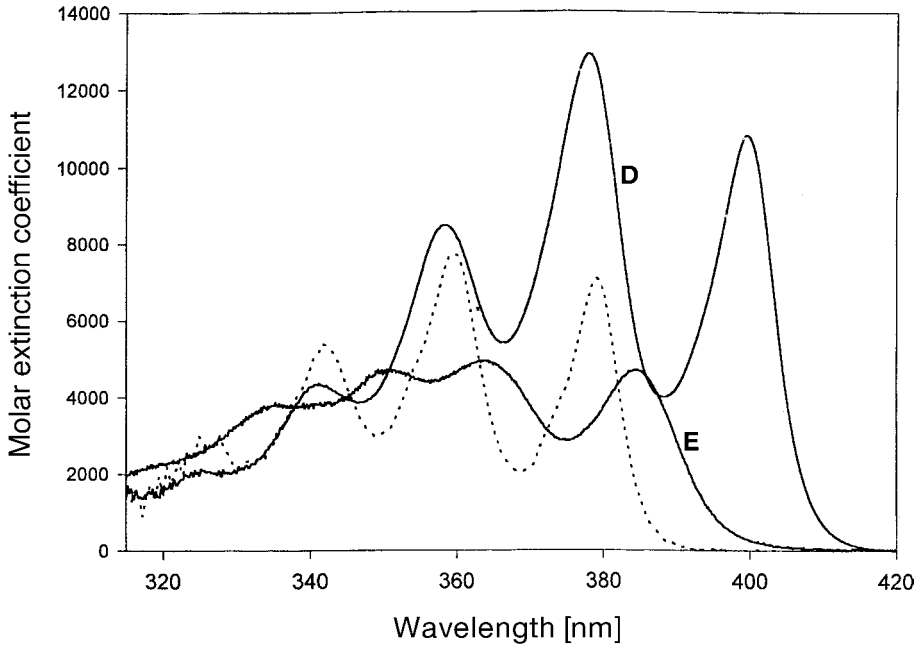


Fig. 3. *Absorption spectra* ($S_0 \rightarrow S_1$ electronic transition) *of anthracene* (·····) *and probes* **D** *and* **E** *in* $CHCl_3$

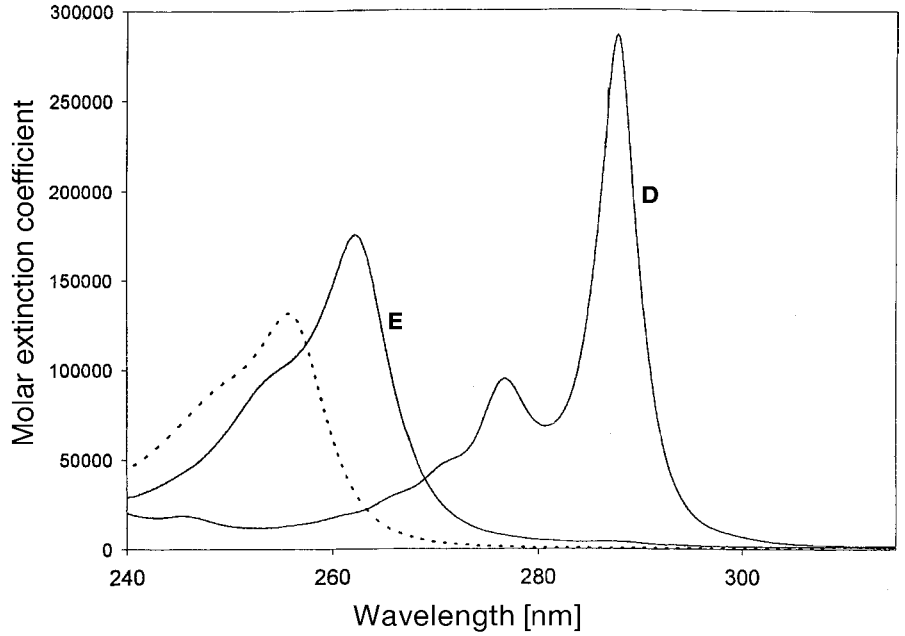


Fig. 4. *Absorption spectra* ($S_0 \rightarrow S_2$ electronic transition) *of anthracene* (·····) *and probes* **D** *and* **E** *in* $CHCl_3$

similar to those of anthracene, with four well-defined vibronic peaks (although somewhat smoothed for probe **E**). Compared to anthracene, an overall red-shift is observed, more pronounced for **D** (*ca.* 15 nm) than for **E** (*ca.* 6 nm), and accompanied, in the case of probe **D**, by a strong hyperchromism. Both absorption spectra present a good overlap with the fluorescence-emission spectrum of tryptophan.

Concerning the second absorption band, it is important to note the strong absorbance of probe **D** in the range 275–295 nm, with a huge maximum of the molar extinction coefficient ($2.86 \cdot 10^5$ $mol^{-1}\,cm^{-1}$ at 288 nm). This wavelength region, unfortunately, corresponds to the absorption spectrum of tryptophan, which would preclude any use of this probe as an acceptor in fluorescence-energy-transfer experiments with tryptophan-containing peptides or proteins, since donor (tryptophan) and acceptor would be excited by the same wavelengths. Contrary to probe **D**, probe **E** presents only a weak absorbance in this wavelength range, and consequently could be a good acceptor for tryptophan fluorescence.

Fluorescence-emission spectra (*Fig. 5*) were obtained by exciting solutions of identical optical densities ($OD = 0.1$) at the excitation wavelength (360 nm), so that these spectra show the relative fluorescence quantum yields of both probes and anthracene in $CHCl_3$. By considering the areas under these emission spectra (with the scale expressed in wavenumbers rather than in wavelengths), the fluorescence quantum yield of probe **D** is 2.1 times higher than the anthracene one, while that of probe **E** is only 1.4 times higher. The absolute quantum yields, determined with quinine hydrogen sulfate as a reference (in 0.05M H_2SO_4, quantum yield = 0.51) [12] are found

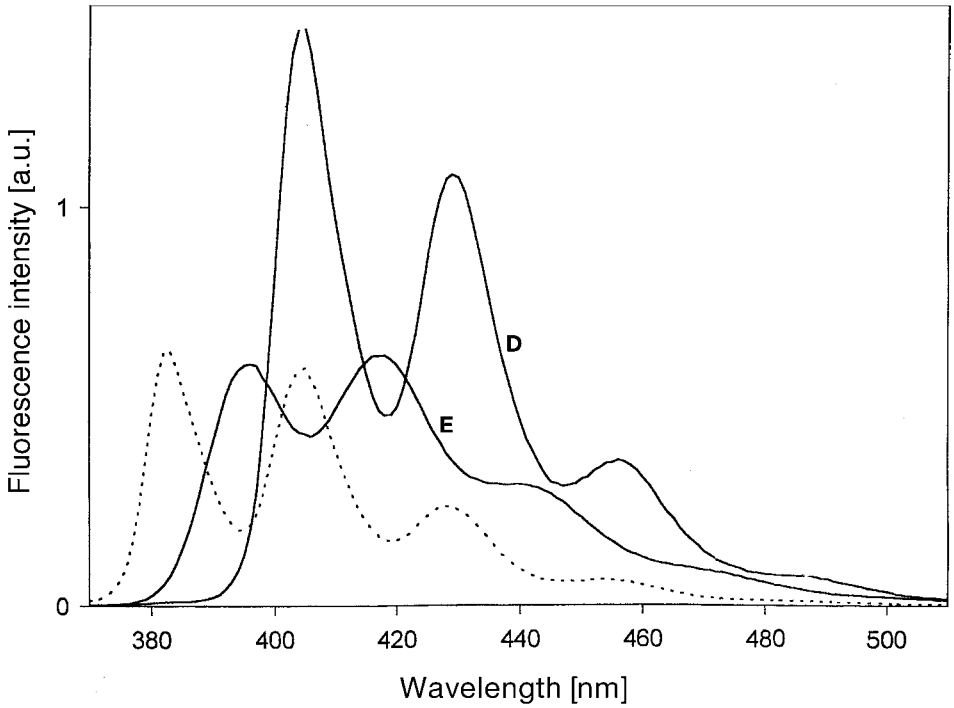


Fig. 5. *Fluorescence-emission spectra of anthracene* (·····) *and probes* **D** *and* **E** *in* $CHCl_3$ (excitation wavelength 360 nm; optical density 0.1)

to be 0.28 for **D** and 0.19 for **E**. Like for the first-band absorption spectra, the emission spectra show four well-defined vibronic peaks, with an overall red-shift of *ca.* 24 nm for probe **D** but only 13 nm for probe **E**. The higher quantum yield and red-shift observed for probe **D** are probably due to the presence of the triple bonds, leading to a stronger conjugation of the molecular orbitals of the excited molecules. Only a single fluorescence lifetime was measured for both probes in $CHCl_3$, namely 3.1 ns for probe **D** and 3.9 ns for probe **E**.

To appraise the suitability of probe **E** for its use as an energy-transfer acceptor and to investigate spatial relationships with tryptophan residues of membrane proteins, we evaluated the critical distance R_0 between a tryptophan fluorophore and probe **E**, according to *Förster*'s equation [13] (see *Eqn. 1*), where R_0 is the distance for which 50% of the energy transfer is efficient, κ^2 the orientation factor between donor and acceptor (averaged as 2 : 3), Φ_D the absolute quantum yield of the donor in the absence of acceptor, and n the refraction index of the medium. J is the overlap integral between donor and acceptor, according to *Eqn. 2*, where F_D is the peak-normalized emission spectrum of the donor and $\varepsilon_A(\lambda)$ the molar extinction coefficient *vs.* wavelength of the acceptor.

$$R_0^6 = 8.785 \cdot 10^{-5} \kappa^2 \Phi_D J / n^4 \tag{1}$$

$$J = \int F_D(\lambda)\ \varepsilon_A(\lambda)\ \lambda^4\ d\lambda \tag{2}$$

R_0 was determined for two limiting cases, namely a buried (in our case inside the membrane) or an exposed tryptophan (at the lipid-water interface). These limiting cases may be mimicked by *N*-acetyl-L-tryptophanamide either dissolved in dioxane (buried; blue-shifted emission spectrum, $\Phi_D = 0.30$) or in H_2O (exposed; red-shifted emission spectrum, $\Phi_D = 0.14$). Very close values of R_0 were found: 24 Å for a buried tryptophan and 23 Å for an exposed one. Such values indicate that probe **E** should be able to localize a tryptophan moiety whatever its position within the membrane bilayer.

Fluorescence characteristics of probes **D** and **E** embedded in large unilamellar vesicles of DMPC (dimyristoylphosphatidylcholine) were also determined. Excitation and emission fluorescence spectra obtained in the L_α liquid-crystalline phase are reported in *Fig. 6*, and are similar to those obtained in $CHCl_3$; quite identical spectra are obtained in the L_β gel phase. Absolute quantum yields and fluorescence-decay parameters are reported in *Table 1*. Both quantum yields and average decay times are found to be higher than in $CHCl_3$ solution; these fluorescence characteristics do not appear to be very sensitive to the phase transition of DMPC vesicles, which is consistent with the hypothesis of a transmembrane topography of such diesters, as was previously shown with the fluorene probe **C** [9].

Linear-Dichroism Measurements on Shear-Deformed Liposomes. To obtain clear indications concerning the transmembrane localization of probes **D** and **E**, we performed linear-dichroism (*LD*) experiments on shear-deformed DMPC unilamellar vesicles. We chose to examine the linear dichroism of the long axis of the compounds, the short-axis transition generally being too weak to be observable at the concentrations used here (see *Exper. Part*). All compounds show negative values of the linear dichroism (*LD*) and reduced linear dichroism (LD^r) (*Table 2*, *Fig. 7*). The diacetylenic diesters **C** and **D** have the lowest values of the reduced linear dichroism, followed by the free anthracene and fluorene molecules, whereas the saturated diester **E** has a reduced linear dichroism about five times as large as the LD^r of the other compounds. In contrast, the diesters all show high orientation parameters in stretched polyethylene film ($S_{zz} \approx 0.7$), whereas the free anthracene and fluorene have poorer orientation.

The results in stretched polyethylene films showed that all three diesters behave as rod-shaped molecules with high orientation parameters [14]. In bilayers, all compounds exhibit negative linear dichroism in the region of the long-axis transition, and are thus oriented with their long axes perpendicular to the bilayer plane [15]. The saturated diester **E** has a much better orientation than the diacetylenic diesters **C** and **D**, which are only oriented to the same degree as the free fluorene and anthracene molecules. There are several possible explanations for the behavior of probe **D** in the bilayer. The anthracene is substituted in 2 and 6 positions, imposing a certain tilt on the long axis of the anthracene, especially in the case of the rigid diacetylenic diester **D**, and thus lowering its LD^r. However, such a tilt should lower the LD^r only by 25–30%. Rather, the answer could be that the diester **D** with triple bonds is slightly too short to span the DMPC membrane adequately. In this case, the moderately hydrophilic ester groups, which are intended to have an anchoring effect, could not anchor the probe with its two heads at the interface. Hence, one end of the diester may be drifting inside the bilayer, making it possible for the molecule not to remain perpendicularly oriented and thereby lowering the *LD*.

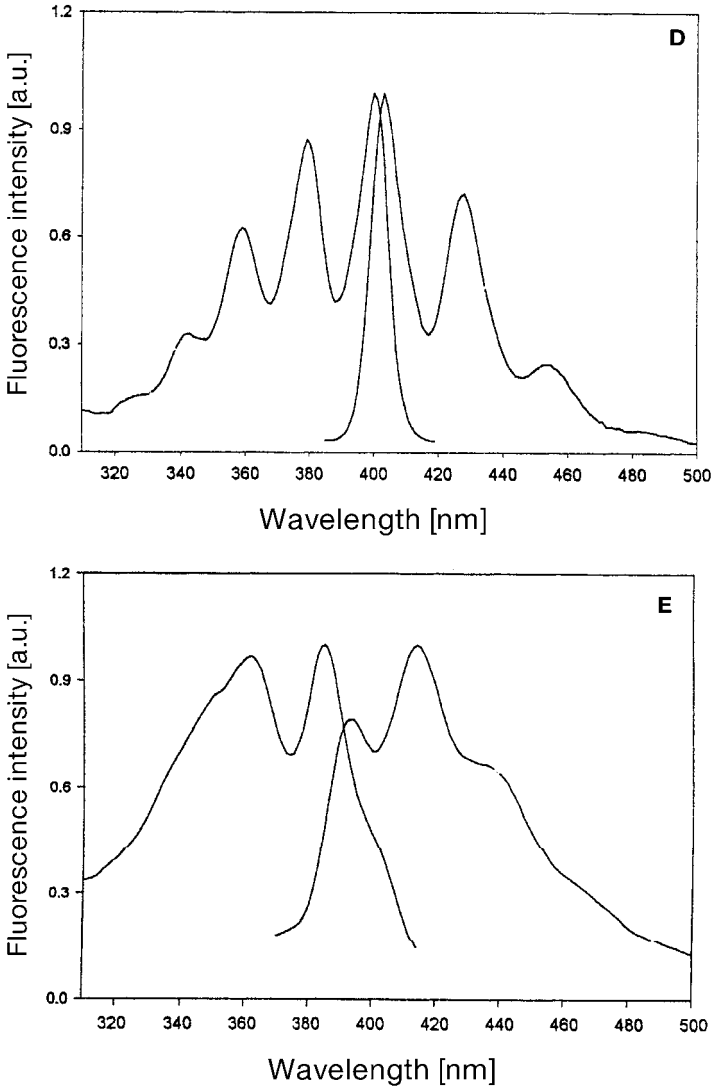


Fig. 6. *Excitation and emission fluorescence spectra of probes* **D** *and* **E** *in DMPC large unilamellar vesicles, with a lipid-to-probe ratio of 100 : 1*

These results lead to the conclusion that the saturated diester **E**, possessing appropriate fluorescence properties to be an acceptor in fluorescence-energy-transfer experiments with tryptophan as a donor, presents a far better transmembrane orientation within the membrane than the unsaturated diesters **C** and **D**. To the best of our knowledge, this is the first time that a simple long-chain molecule like **E**, terminated by ester groups, is shown to be oriented perpendicularly to the bilayer plane with its fluorophore positioned near the center of the bilayer. Therefore, this probe

Table 1. *Absolute Quantum Yields* (Φ; ±0.005) *and Fluorescence Lifetimes* (τ) *for Probes* **D** *and* **E** *Embedded in DMPC Large Unilamellar Vesicles in* L_α (30°) *and* L_β (15°) *Phases, at a Lipid-to-Probe Ratio of 100 : 1*

	T [°C]	Φ	τ_1[a]) [ns]	α_1[b])	τ_2[a]) [ns]	α_2[b])	$\langle\tau\rangle$[c]) [ns]
D	15	0.36	6.60	0.92	1.85	0.08	6.20
D	30	0.35	6.50	0.93	1.60	0.07	6.15
E	15	0.35	7.95	0.53	1.45	0.47	4.90
E	30	0.34	7.45	0.60	1.45	0.40	5.05

[a]) Fluorescence lifetimes τ_i (± 0.05) were obtained by the phase method, with frequencies of the exciting light scanned from 3 to 90 MHz. Diphenylhexatriene in hexane was chosen as fluorescence-lifetime reference (τ 6.75 ns) [17]. [b]) α_i (± 0.001): Relative contribution of each lifetime component. [c]) $\langle\tau\rangle$: Average fluorescence lifetime.

Table 2. *Linear Dichroism* (*LD*) *of Compounds Dissolved in the Bilayer of Shear-Deformed Vesicles at 35° and 3100* s^{-1} *and Orientation Parameters* S_{zz} *in Stretched Polyethylene Film*

	Long-axis transition [nm]	Linear dichroism $LD \cdot 10^3$	Absorption A_{iso}	Reduced linear dichroism[a]) $LD^r \cdot 10^3$	S_{zz} in stretched polyethylene film
Anthracene	252	– 4.8	0.68	– 7.1	0.3
Diacetylenic anthracene diester **D**	285	– 2.1	0.37	– 5.7	0.7
Sat. anthracene diester **E**	260	– 2.1	0.048	– 44	0.6
Fluorene	266	– 2.9	0.35	– 8.3	0.22
Diacetylenic fluorene diester **C**	330	– 2.0	0.74	– 2.7	0.7

[a]) Reduced linear dichroism $LD^r = LD/A_{iso}$.

might enable us to quantify the donor-acceptor distance and to determine the location of tryptophan residues in membranes.

Professor *P. Hodges* is gratefully thanked for providing us the experimental procedure leading to the synthesis of 2,6-dibromoanthracene. *E. Quesada* is grateful for grants from the *Ministerio de Educación y Cultura* of Spain and the *Société des Amis des Sciences* of France. *Y. Bonzi-Coulibaly* is indebted to the *Société des Amis des Sciences* of France for a grant. We also wish to thank Prof. *H. Bouas-Laurent*, Université de Bordeaux 1, and Dr. *L. Désaubry*, Université Louis Pasteur, for stimulating discussions. This work was supported in part by the *Dirección General de Investigación Científica y Técnica* of Spain (projects PB93-0126 and PB96-0852) and by the *European Union* (DG XII, contract PL950990).

Experimental Part

General. All solvents were freshly distilled prior to use. THF and Et_2O were distilled from sodium metal-benzophenone ketyl, and MeOH, EtOH, ^{i}PrOH, MeCN and 1,4-dioxane from CaH_2. Air- or moisture-sensitive reactions were conducted in dried glassware and under a positive pressure of dry Ar. All reagents were commercial products purchased from *Aldrich*, *Fluka*, *Lancaster*, *Acros*, or other suppliers. 2,6-Dibromoanthracene (**2**), methyl undec-10-ynoate (**3**), and 2,6-dibromoanthraquinone (**4**) were prepared according to recently published procedures [8][10]. All org. extracts were dried ($MgSO_4$) and concentrated in a rotary evaporator. TLC: Precoated aluminium silica-gel plates *60 F254* (*Merck*, 0.25 mm); detection under UV lamps at 254 or 360 nm, by I_2 vapors, or by dipping into a soln. of vanillin (1 g) in EtOH/H_2SO_4 95 : 5 (1 l) or phosphomolybdic acid (5 g) in EtOH (250 ml), followed by heating on a hot plate. Prep. medium-pressure column chromatography (CC): pressure 0.5 – 1.1 bar, silica-gel columns (40 – 63 mm, *Merck*); according to the standard procedure [16]. M.p.: *Reichert-Kofler* hot-stage microscope; uncorrected. IR Spectra: KBr pellets; *Perkin-Elmer-881* or *-681* IR spectrophotometers; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-SY-200*

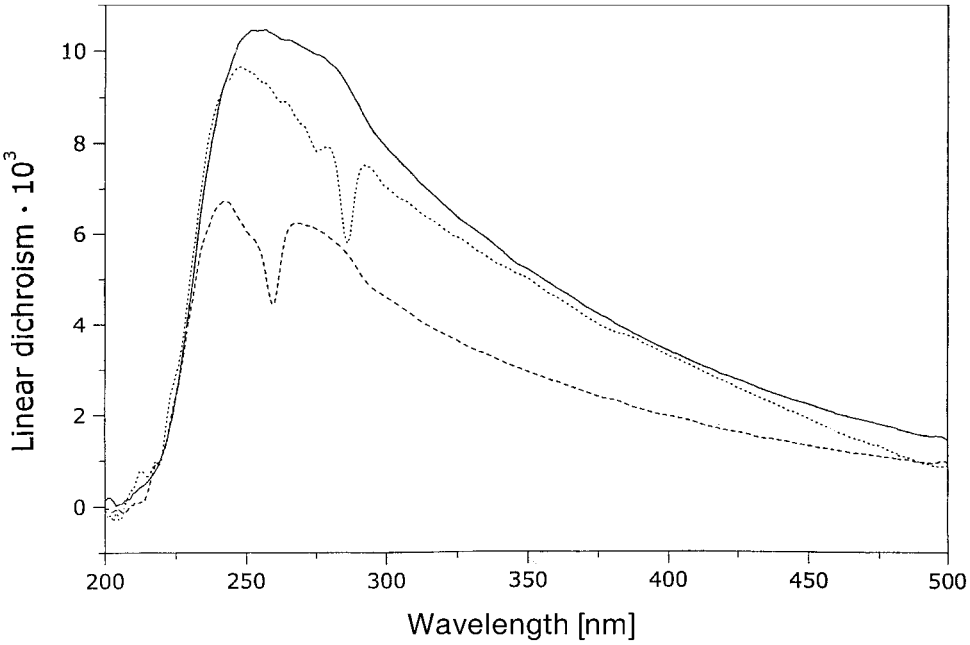


Fig. 7. *Linear dichroism* (*LD*) *of shear-deformed DMPC vesicles at 35° and 3100 s^{-1}: DMPC vesicles* (—), *DMPC vesicles with probe* **E** (⋯) *and DMPC vesicles with probe* **D** (---). Probe/lipid ratio 1:100. The blank DMPC vesicles exhibit strong scattering with increasing positive *LD* when passing from 500 to 250 nm; the negative *LD* at shorter wavelength is due to lipid absorption. The two peaks at 285 and 260 nm show that their anthracenediyl long axes are preferentially oriented along the lipid chains (for theory of interpretation of shear *LD* of liposomes, see [14]).

(200 MHz), *Bruker-AM-200* (200 MHz), *Bruker-AM-400* (400 MHz), *Varian-Gemini-200* (200 MHz), *Varian-INOVA* (300 MHz), or *Varian-INOVA-400* (400 MHz) spectrometers; signal of the residual non-deuterated solvent as internal reference: δ(H) 7.26 or 2.49 ppm and δ(C) 77.0 or 39.7 ppm for spectra in $CDCl_3$ or (D_6)DMSO, resp., *J* values in Hz; ^{13}C-assignments by ^{13}C,^{1}H polarization transfer (DEPT) and ^{13}C,^{1}H heteronuclear single quantum correlation (HSQC) experiments. MS: Direct injection on a *Trio-Fisons* or a *Hewlett-Packard-5973* spectrometer, electron impact (EI) technique; in *m*/*z* (% of basis peak). Elemental analyses were carried out on a *Carlo Erba 1108* elemental analyzer.

Dimethyl 11,11′-(Anthracene-2,6-diyl)bis[undec-10-ynoate] (**D**). To freshly crystallized 2,6-dibromoanthracene (**2**; 100 mg, 0.298 mmol, 1 equiv.), methyl undec-10-ynoate (**3**; 175.2 mg, 0.893 mmol, 3 equiv.), [Pd(Ph_3P)$_2Cl_2$] (41.8 mg, 59.6 mmol, 0.20 equiv.), and CuI (5.7 g, 29.8 mmol, 0.10 equiv.) in a *Schlenk*-type flask under continuous Ar stream, freshly distilled deoxygenated Et_2NH (15 ml) was added, and the mixture was stirred and heated under reflux for 4 h. The brown crude soln. was cooled and filtered with CH_2Cl_2 through a short *Celite* pad, and the soln. was evaporated. The residual solid was washed with pentane (5 × 25 ml portions) until disappearance of non-fluorescent by-products (TLC), and further purified by CC (hexane/AcOEt 9:1, then hexane/Et_2O 3:2). The crude product was recrystallized from acetone: 118 mg (70%) of **D**. Bright yellow crystals. M.p. 101–103°. R_f 0.25 (hexane/AcOEt 9:1), 0.5 (hexane/Et_2O 3:2). IR (KBr): 2890, 2810, 2182, 1712, 1605, 1448, 1395, 1358, 1300, 1262, 1227, 1187, 1150, 879, 779. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.35 (br. *s*, 12 H, CH_2); 1.54 (*m*, 4 H, CH_2); 1.63 (*m*, 4 H, CH_2); 1.65 (*m*, 4 H, CH_2); 2.31 (*t*, ^3J = 7.32, 4 H, CH_2); 2.47 (*t*, ^3J = 7.17, 4 H, CH_2); 3.66 (*s*, 2 Me); 7.38 (*dd*, ^4J = 1.31, ^3J = 8.94, H–C(3), H–C(7)); 7.86 (*d*, ^3J = 8.9, H–C(4), H–C(8)); 8.03 (*s*, H–C(1), H–C(5)); 8.27 (*s*, H–C(9), H–C(10)). ^{13}C-NMR (50 MHz, $CDCl_3$): 19.57; 24.91; 28.75; 28.88; 28.94; 29.11 (2×); 34.09; 51.40; 81.19; 91.65; 121.06; 125.81; 128.04; 128.37; 130.82; 131.19; 131.41; 174.26.

EI-MS (70 eV): 566 (100, M^+), 409 (12), 295 (6), 279 (15), 265 (17), 252 (36), 239 (16), 229 (20), 215 (11), 74 (24). Anal. calc. for $C_{38}H_{46}O_4$: C 80.53, H 8.18; found: C 80.64, H 8.21.

Dimethyl 11,11′-(Anthracene-2,6-diyl)bis[undecanoate] (**E**). *Procedure A: Triple-Bond Reduction of* **D** *by* Raney-*Ni Catalysis. Caution:* The catalyst must never be dried and must be handled carefully. A slurry of activated ('ready-to use') *Raney*-Ni in H_2O was successively washed and decanted with *Milli-Q* water, EtOH, and ^{i}PrOH (4 ×) in an ultrasound bath. The *Raney*-Ni slurry was then added to **D** (51 mg, 90 mmol) in THF (15 ml) under Ar at r.t. under efficient stirring (TLC (hexane/Et_2O 3:2; det. 360 nm) monitoring and color change from yellow (**D**) to colorless (**E**); the catalyst surface taking a characteristic blue color). After *ca.* 1 h, the stirring was stopped and the soln. decanted. The remaining solid catalyst was washed with CH_2Cl_2 (3 × 20 ml) and the combined decanted phase filtered through a *Celite* pad, dried ($MgSO_4$), and evaporated to afford a white-yellowish solid (50 mg) which was purified by recrystallization from acetone (20°): 28 mg (55%) of **E**.

Procedure B: DDQ Oxidation of 9,10-Dihydro Derivative **6**. A soln. of dimethyl 11,11′-(9,10-dihydroanthracene-2,6-diyl)bis[undecanoate] (**6**, 8 mg, 14 mmol) in dry 1,4-dioxane (3 ml) was added through a canula into a flask containing DDQ (freshly recrystallized from $CHCl_3$; 4.5 mg, 20 mmol) under Ar, with protection from light, and heating under reflux for 6 h. The solid $DDQH_2$ formed was separated by filtration through a *Celite* pad (CH_2Cl_2 as eluent), the filtrate evaporated, and the crude solid purified by CC (hexane/AcOEt 3:2) yielding **E** (7.6 mg, 94%) as a yellowish solid. Further recrystallization from acetone afforded anal. pure **E** (4.2 mg, 50%). M.p. 108–110°. R_f 0.55 (hexane/Et_2O 3:2). IR (KBr): 3400, 2887, 2818, 1721, 1638, 1502, 1438, 1394, 1212, 1181, 1146, 887, 865. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.28 (br. *s*, 20 H, CH_2); 1.35 (*m*, 4 H, CH_2); 1.61 (*quint.* ^3J = 7.6, 4 H, CH_2); 1.73 (*quint.*, ^3J = 7.6, 4 H, CH_2); 2.29 (*t*, ^3J = 7.47, 4 H, CH_2); 2.78 (*t*, ^3J = 7.47, 4 H, CH_2); 3.66 (*s*, 2 Me); 7.30 (*dd*, ^4J = 1.47, ^3J = 8.64, H–C(3), H–C(7)); 7.72 (*s*, H–C(1), H–C(5)); 7.90 (*d*, ^3J = 8.64, H–C(4), H–C(8)); 8.29 (*s*, H–C(9), H–C(10)). ^{13}C-NMR (50 MHz, $CDCl_3$): 24.93; 29.12; 29.23; 29.33; 29.41; 29.49 (2 ×); 30.98; 34.09; 36.24; 51.41; 125.00; 125.75; 127.37; 127.85; 130.65; 131.50; 139.29; 174.33. EI-MS (70 eV): 574 (100; M^+); 389 (13), 231 (6), 217 (18), 204 (33), 191 (17), 74 (16), 69 (6). Anal. calc. for $C_{38}H_{54}O_4$: C 79.45, H 9.47; found: C 79.28, H 9.28.

Dimethyl 11,11′-(9,10-Dioxoanthracene-2,6-diyl)bis[undec-10-ynoate] (**5**). A mixture of 2,6-dibromoanthraquinone (**4**) (500 mg, 1.37 mmol), **3** (805 mg, 4.1 mmol, 3 equiv.), $[Pd(Ph_3P)_2Cl_2]$ (96 mg, 0.137 mmol, 0.1 equiv.), CuI (26.1 mg, 0.137 mmol, 0.1 equiv.), and degassed Et_2NH (50 ml) in a *Schlenk*-type flask was heated under reflux and under Ar for 8 h. The crude mixture was then cooled to r.t. and filtered with CH_2Cl_2 through a short *Celite* pad, and the soln. was evaporated. The crude solid was washed with pentane (5 × 25 ml) and the remaining solid purified by CC (hexane/Et_2O/CH_2Cl_2 3:2:0.2 → 1:4:0.5). The obtained yellow solid was washed with pentane and dried: **5** (598 mg, 73%). Light yellow crystals. M.p. 111–113°. R_f 0.45 (hexane/Et_2O/CH_2Cl_2 3:2:0.2). IR (KBr): 3420, 2890, 2820, 2190, 1715, 1658, 1578, 1452, 1409, 1289, 1245, 1210, 1150, 959, 903, 897, 831, 719. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.34 (br. *s*, 12 H, CH_2); 1.46 (*m*, 4 H, CH_2); 1.63 (*quint.*, ^3J = 6.9, 8 H, CH_2); 2.31 (*t*, ^3J = 7.5, 4 H, CH_2); 2.46 (*t*, ^3J = 6.9, 4 H, CH_2); 3.66 (*s*, 2 Me); 7.75 (*dd*, ^4J = 1.8, ^3J = 8.1, H–C(3), H–C(7)); 8.22 (*d*, ^3J = 8.1, H–C(4), H–C(8)); 8.27 (*d*, ^3J = 1.5, H–C(1), H–C(5)). ^{13}C-NMR (50 MHz, $CDCl_3$): 19.57; 24.91; 28.42; 28.85; 28.91; 29.08; 34.06; 51.40; 79.75; 96.37; 127.25; 130.17; 130.70; 131.81; 133.41; 136.59; 174.22; 182.16. EI-MS (70 eV): 596 (53, M^+), 564 (34), 532 (12), 504 (8), 476 (7), 311 (11), 297 (11), 259 (8), 226 (16), 123 (10), 95 (29), 55 (100). Anal. calc. for $C_{38}H_{44}O_6$: C 76.48, H 7.43; found: C 76.70, H 7.24.

Dimethyl 11,11′-(9,10-Dihydroanthracene-2,6-diyl)bis[undecanoate] (**6**). Through a soln. of **5** (108 mg, 0.19 mmol) AcOH/$CHCl_3$ 1:1 (10 ml) in a high-pressure hydrogenation flask, Ar was bubbled for 15 min. Then, PtO_2 (49 mg, 0.21 mmol) was added under Ar, and the system was closed. After 3 vacuum/H_2 cycles, the mixture was stirred under H_2 (10 bar) at r.t. for 16 h. The resulting org. layer was washed with H_2O, dried ($MgSO_4$), and evaporated. The white solid (90 mg) was further purified by CC (hexane/Et_2O 3:2): pure **6** (34.4 mg, 31%). Colorless crystals. M.p. 65–67°. R_f 0.59 (hexane/Et_2O 3:2). IR (KBr): 3400, 2885, 2818, 1730, 1452, 1400, 1247, 1217, 1179, 1151, 812, 781. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.27 (br. *s*, 24 H, CH_2); 1.61 (*quint.*, ^3J = 7.2, 4 H, CH_2); 2.30 (*t*, ^3J = 7.5, 4 H, CH_2); 2.57 (*t*, ^3J = 7.8, 4 H, CH_2); 3.66 (*s*, 2 Me); 3.88 (*s*, 4 H, CH_2); 6.99 (*dd*, ^4J = 1.8, ^3J = 7.5, H–C(3), H–C(7)); 7.10 (*d*, ^4J = 1.8, H–C(1), H–C(5)); 7.19 (*d*, ^3J = 7.5, H–C(4), H–C(8)). ^{13}C-NMR (50 MHz, $CDCl_3$): 24.96; 26.98; 29.15; 29.22; 29.33; 29.47; 31.69; 34.12; 35.63; 35.8; 36.61; 51.39; 126.04; 127.21; 127.42; 133.94; 136.72; 140.67; 174.30. EI-MS (70 eV): 576 (50, M^+), 544 (68), 516 (87), 345 (36), 205 (56), 191 (100), 179 (33), 69 (16).

Vesicle Preparations for Fluorescence and Linear-Dichroism Experiments. Large unilamellar vesicles (LUV) were prepared by the extrusion method: DMPC and the various probes (diesters, anthracene, or fluorene) were dissolved in $CHCl_3$ and mixed in appropriate proportions (50:1 to 100:1). The $CHCl_3$ was then evaporated. The liquid film thus obtained was hydrated with an aqueous buffer (2 mM, TES (=2-

{[tris(hydroxymethyl)methyl]amino}ethane-1-sulfonic acid), 2 mM L-histidine, 0.1M NaCl, pH 7.4 for fluorescence experiments; 0.15M PBS for linear-dichroism experiments) and vortexed vigorously for 5 min to give a soln. of multilamellar vesicles (MLV). LUV were obtained by extrusion of the previous MLV soln. through polycarbonate filters (*Nucleopore*) at 35° by means of a thermostated extruder (*Lipex Biomembranes*). For fluorescence experiments (concentration of lipids *ca.* 1 mM), a first extrusion was carried out on 200 nm filters (7 passages) and a second one on 100-nm filters (11 passages); for linear-dichroism experiments (concentration *ca.* 10 mM), only the extrusion step on 100-nm filters (11 passages) was carried out, but preceded by freeze-thawing in liq. N_2 (5 cycles) to homogenize the MLV soln. The method provided homogeneous vesicles (110–120 nm diameter), as checked by light scattering with a *N4SD Coultronics Nanosizer*.

Absorption and Fluorescence Spectra. UV Spectra: *Kontron-Uvikon-810* or *Cary-4* UV/VIS spectrophotometer. Fluorescence-emission spectra: *SLM-48000* spectrofluorimeter in the steady-state mode; fluorescence lifetimes by the phase and modulation method in the time-resolved mode.

Linear-Dichroism Measurements. Linear-dichroism (*LD*) measurements were performed at 35° in a *Couette* cell with a *Jasco-500* instrument. A baseline was recorded at low shear gradient, typically 100 s^{-1}, while the measurement was performed with a shear gradient of 3100 s^{-1}. The isotropic absorption (A_{iso}) of the soln. was measured, and the reduced linear dichroism (LD^r) could be calculated as LD/A_{iso}. Stretched polyethylene films were prepared following the standard procedure: after stretching the film to five times its initial length, it was allowed to swell with $CHCl_3$, and baselines were collected for the absorption parallel and perpendicular to the stretching direction. Anthracene or fluorene diesters in $CHCl_3$ were then applied onto the film, the $CHCl_3$ was evaporated, and excess sample was washed off the film with EtOH. Polarized absorption spectra – parallel and perpendicular to the stretching direction – were collected. The reduced linear dichroism could be calculated as $LD^r = 3 \cdot (A_{par} - A_{perp})/(A_{par} + 2 \cdot A_{perp})$. For a pure long-axis-polarized transition, $LD^r = 3 \cdot S_{zz}$, where S_{zz} is the orientation parameter [15].

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