Synthesis and Reactivity of a New Heptacarbon Chain Carboxonium Salt − Access to a New Class of Streptocyanine Dyes

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A new highly reactive heptacarbon chain carboxonium salt **1** was synthesized. It leads, by action of various amines and hydrazone, to a new class of symmetrical heptacarbon chain streptocyanine dyes **2**–**6**. A hemicarboxonium salt **7**, which leads to a dissymmetrical streptocyanine, was also obtained.

Introduction

Polymethinium dyes are cationic conjugated organic compounds containing a chain made up from an odd number of carbon atoms between two nitrogen atoms. They are called cyanine dyes if the nitrogen atoms are part of a conjugated heterocycle, and streptocyanine dyes if not. Among their numerous applications[1] (dyes, photographic sensitizers, recordable laser discs, nonlinear optics, drugs), near-infrared region absorbing compounds are increasingly used as biological fluorescent probes.[2−4] Their peculiar spectral properties in this range (600−1000 nm) allow negligible autofluorescence from biomolecules, which is a great advantage for bioanalytical purposes.[5,6]

Results and Discussion

We have previously developed a synthesis of streptocyanines from the reaction of pentacarbon chain (5-C) carboxonium salts with amines, imines, hydrazines, and hydrazones.[7] 5-C carboxonium salts are obtained by condensation of arylethanones with triethoxymethane in a strongly acidic medium.[8] Unfortunately, the absorption and fluorescence properties of these streptocyanine dyes proved to be still inadequate for near-infrared applications (the maximum absorption wavelength obtained is 600 nm).

It is known that the addition of a vinylene group to the π-electron system of cyanine dyes causes a bathochromic shift of the absorption band by about 100 nm.[9−11] Moreover, pentacarbon and heptacarbon chain cyanines have been synthesized by a condensation reaction between a heterocyclic base (derived from indole or its derivatives) containing an activated methyl group, with triethoxymethane and 1,3,3-trimethoxypropene, respectively, in the presence or absence of a catalyst.[2,12]

Based on these observations, we attempted the synthesis of a new heptacarbon chain (7-C) carboxonium salt **1** (Figure 1) and we report here the development of this original result.

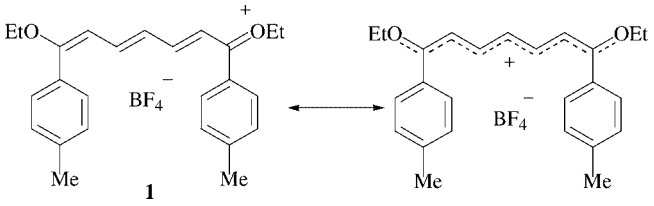

Figure 1. Heptacarbon chain carboxonium salt **1**

The replacement of triethoxymethane, which is used for the synthesis of the 5-C carboxonium salts,[7,8] by its vinylog, 1,3,3-triethoxypropene, does not lead to significant amounts of the expected 7-C carboxonium salt. It appears, surprisingly, that the presence of triethoxymethane is crucial, as we obtained pure 7-C carboxonium salt only with the following stoichiometry: triethoxymethane:4-methylphenylethanone:tetrafluoroboric acid 1:2:1. With less than one equivalent of triethoxymethane, the 7-C carboxonium salt, an unsymmetrical pyrylium salt (Figure 2), and other

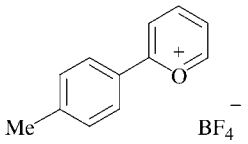

Figure 2. Pyrylium salt

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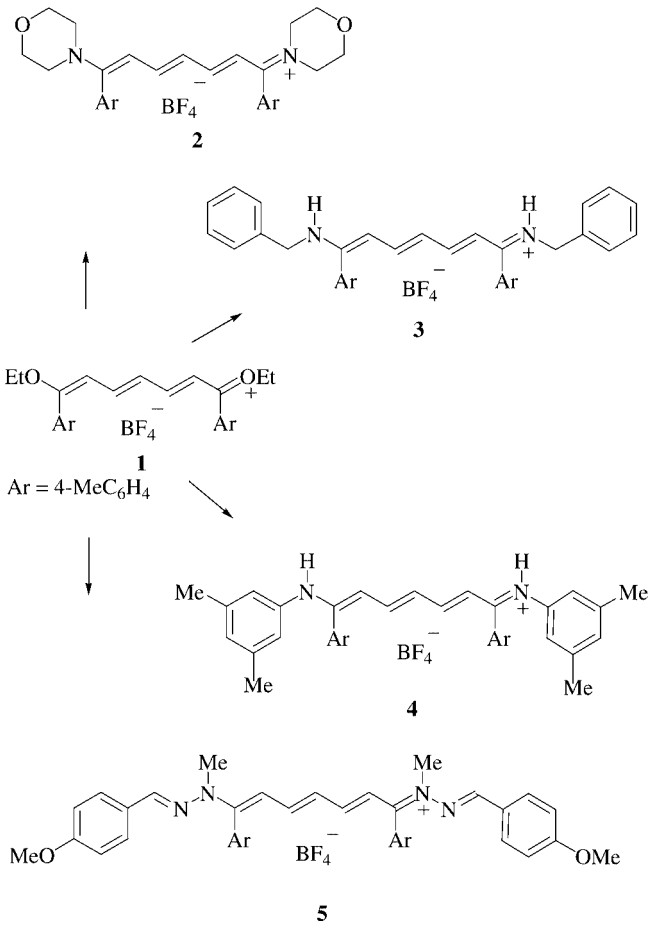
unidentified by-products are obtained, whereas with more than one equivalent, both 5-C and 7-C carboxonium salts are competitively formed.

To explore the ability of the 7-C compound **1** to react, as the 5-C ones do, with various nucleophiles, we used primary and secondary aliphatic amines, a primary aromatic amine, and hydrazone. Likewise, we synthesized the symmetrical streptocyanines **2**, **3**, **4** and **5** from two equivalents of morpholine, benzylamine, 3,5-dimethylaniline, and 4-methoxyphenylmethine-*N*-methylhydrazone, respectively, as representative compounds (Scheme 1).

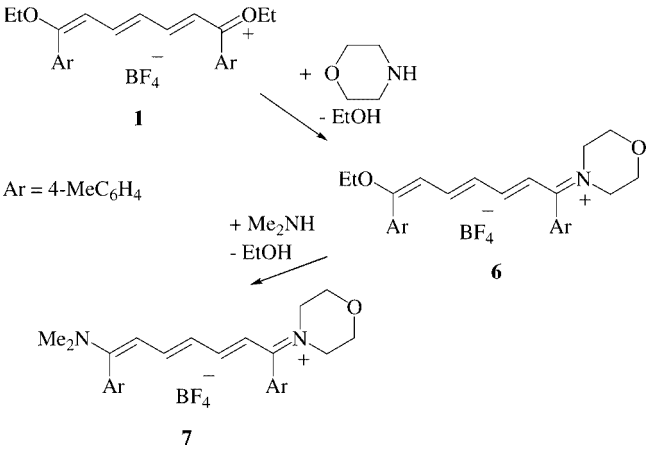


Scheme 1. Syntheses of symmetrical streptocyanine dyes **2**−**5**

The action of only one equivalent of morpholine on **1** leads to the hemicarboxonium salt **6** (Scheme 2). This compound reacts with methylamine to give the dissymmetrical streptocyanine **7**.

The structure of all products was unambiguously established by ^1H and ^{13}C NMR spectroscopy, mass spectrometry (FAB+/mNBA; DCI/NH_3) and for **2** by X-ray diffraction (Figure 3).

The structure of **2** shows an "all *trans*" conjugated carbon chain, which is almost planar, with angles of about 120°. The C−C and C−N bond lengths of the conjugated system have intermediate values between single and double bonds, and the aryl groups are almost perpendicular to the chain plane.



Scheme 2. Syntheses of the hemicarboxonium **6** and the dissymmetrical heptacarbon chain streptocyanine dyes **7**

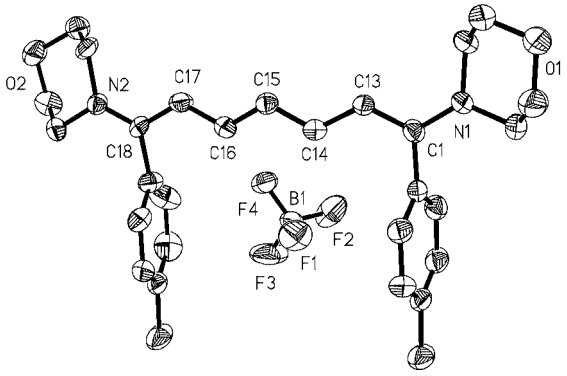


Figure 3. X-ray crystal structure of **2**; selected bond lengths [Å] and angles [°]: N1−C1 1.360(3), C1−C13 1.386(3), C13−C14 1.401(3), C14−C15 1.378(3), C15−C16 1.393(4), C16−C17 1.377(3), C17−C18 1.405(15), C18−N2 1.337(9); N1−C1−C13 122.2(3), C1−C13−C14 123.7(3), C13−C14−C15 124.9(3), C14−C15−C16 121.9(3), C15−C16−C17 125.5(3), C16−C17−C18 125.6(5), C17−C18−N2 126.5(11); deviation of the chain carbons from the mean plane: 0.127 Å

As expected, an average bathochromic effect of more than 100 nm is observed for the new heptacarbon chain streptocyanines relative to the pentacarbon ones; large molar extinction coefficients are also observed (Table 1).

Table 1. Absorption and fluorescence data of the 7-C streptocyanines (CH_2Cl_2, 23 °C) (in brackets, values for the corresponding 5-C compound)

	$\lambda_{max.abs}$ [nm]	ε_{max} [$M^{-1}\cdot cm^{-1}$]	$\lambda_{max.em}$ [nm]
2	566 (453)	183600	606 (518)
3	552	118000	592
4	599	40000	687
5	679 (558)	137000	720 (608)
6	501	55000	[a]
7	559	158000	599

[a] No fluorescence emission.

The absorption and emission spectra of **2** in CH_2Cl_2 are shown in Figure 4. The Stokes shift is 40 nm.

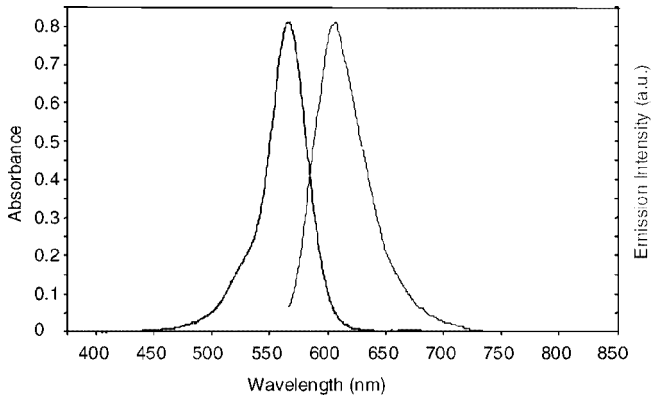


Figure 4. Absorption and emission spectra of **2** in CH_2Cl_2; the absorption spectrum was obtained with a 1 cm path length quartz cell and a concentration of **2** of 4.41×10^{-6} M.

Conclusion

In conclusion, the synthesis of a heptacarbon chain carboxonium salt was achieved. Its reactivity towards amines and hydrazones is identical to that of the pentacarbon one. This allows us to obtain a new class of symmetrical heptacarbon chain streptocyanines, with absorption and emission wavelengths shifted toward the near-IR region. The ethoxy group of the hemicarboxonium salt is always reactive and can give, by action of a different nucleophile, dissymmetrical streptocyanines, or could be grafted onto the amino moieties of biomolecules.[13]

Experimental Section

General Remarks: All experiments were performed under dry conditions (argon atmosphere, anhydrous solvents) to avoid degradation of the carboxonium salt.

Melting points were determined with Büchi capillary apparatus. ^{1}H NMR and ^{13}C NMR (63 MHz) spectra were recorded on Bruker AC 250. Chemical shifts are expressed in ppm relative to TMS. Mass spectra were obtained on Nermag R10−10H apparatus for FAB positive mode (mNBA) and DCI/NH_3. UV/Vis spectra were obtained on a Hewlett−Packard 8453 UV/Vis spectrometer. Excitation and fluorescence emission spectra were obtained with a Perkin−Elmer LS 50B spectrofluorimeter fitted with a Xenon lamp (20 kW, flash-time 8 μs) and a Hamamatsu R 928 photomultiplicator. X-ray data were collected on a Bruker-AXS CCD 1000 diffractometer. Elemental analyses were realised at the Microanalysis Interuniversity Service of the Chemistry School of Toulouse (ENSIACET).

1,7-Diethoxy-1,7-bis(4-methylphenyl)hepta-1,3,5-trienylium Tetrafluoroborate (1): A mixture of 4-methylphenylethanone (2.81 mL, 21.08 mmol) and 54% tetrafluoroboric acid in diethyl ether (1.45 mL, 10.54 mmol) was added dropwise to a solution of 1,3,3-triethoxypropene (1.837 g, 10.54 mmol; synthesized according to Lounasmaa[14]), and triethoxymethane (1.75 mL, 10.54 mmol). The solution was stirred one hour, turning progressively more purple. Diethyl ether (250 mL) was then added and the solution was stirred for one hour more. The product was filtered off and dried under reduced pressure to give **1** as a violet powder (2.31 g, 49%). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.52 (t, ^3J = 7.0 Hz, 6 H, CH_3CH_2O), 2.43 (s, 6 H, CH_3-Ar), 4.51 (q, ^3J = 7.0 Hz, 4 H, CH_3CH_2O), 6.72 (d, ^3J = 12.8 Hz, 2 H, $H_{2,6}$), 7.31 (part A of AB syst., $^3J_{AB}$ = 8.1 Hz, 4 H, H_{arom}), 7.37 (t, ^3J = 12.8 Hz, 1 H, H_4), 7.45 (part B of AB syst., $^3J_{AB}$ = 8.1 Hz, 4 H, H_{arom}), 7.67 (t, ^3J = 12.8 Hz, 2 H, $H_{3,5}$) ppm. ^{13}C NMR Jmod (63 MHz, $CDCl_3$, 25 °C): = 14.3 (CH_3CH_2O), 21.7 (CH_3-Ar), 69.5 (CH_3CH_2O), 111.1 ($C_{2,6}$), 129.8 (C_{arom}), 129.9 (C_{arom}-$C_{1,7}$), 130.5 (C_{arom}), 131.0 (C_4), 144.8 (C_{arom}-CH_3), 168.4 ($C_{3,5}$), 184.5 ($C_{1,7}$) ppm.

Preparation of 2−4: The amine (morpholine, benzylamine, or 3,5-dimethylphenylamine; 2.08 mmol) was added dropwise to a solution of **1** (0.447 g, 0.99 mmol) in 60 mL of acetonitrile. After stirring for 24 h, the solvent was removed under reduced pressure and the precipitate was washed with pentane, and dried. The crude streptocyanine was recrystallized from ethanol.

1,7-Bis(4-methylphenyl)-1,7-bis(di-*N*-morpholino)hepta-1,3,5-trienylium Tetrafluoroborate (2): Violet spangles with blue metallic glints (74%). m.p.: 204 °C (dec.). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 2.39 (s, 6 H, CH_3Ar), 3.53 (m, 8 H, CH_2N), 3.76 (m, 8 H, CH_2O); 6.29 (m, 2 H, $H_{2,6}$), 6.55 (m, 3 H, $H_{3,4,5}$), 7.09 (part A of AB syst., 4 H), 7.27 (part B of AB syst., $^3J_{AB}$ = 7.8 Hz, 4 H, H_{arom}) ppm. ^{13}C NMR Jmod (63 MHz, $CDCl_3$, 25 °C): δ = 21.6 (CH_3Ar), 50.3 (CH_2N), 66.7 (CH_2O), 109.5 ($C_{2,6}$), 124.5 (C_4), 129.1 (C_{arom}), 129.6 (C_{arom}-$C_{1,7}$), 129.9 (C_{arom}), 141.1 (C_{arom}-CH_3), 158.6 ($C_{3,5}$), 167.9 ($C_{1,7}$) ppm. MS (DCI/NH_3): *m*/*z* (%) = 443 (10.2) $[M]^+$, 204 (100). $C_{29}H_{35}BF_4N_2O_2$ (530.27) calcd. C 65.67, H 6.65, N 5.28; found C 65.63, H 5.93, N 5.10. UV/Vis (23 °C, CH_2Cl_2): λ_{max} (ε_{max}) = shoulder at 528 (nm), 566 (183600 $M^{-1}\cdot cm^{-1}$). Fluorescence spectrometry (23 °C, CH_2Cl_2): $\lambda_{ex}/\lambda_{em}$ = 569/604 nm.

1,7-Bis(benzylamino)-1,7-bis(4-methylphenyl)hepta-1,3,5-trienylium Tetrafluoroborate (3): blue-violet crystals (67%). m.p.: 187−189 °C (dec.). ^{1}H NMR (250 MHz, CD_3CN, 25 °C): δ = 2.30 (s, 6 H, CH_3Ar), 4.63 (s, 4 H, CH_2NH), 5.98 (d, ^3J = 13.0 Hz, 2 H, $H_{2,6}$), 6.22 (t, ^3J = 12.7 Hz, 1 H, H_4), 6.75 (t, ^3J = 12.9 Hz, 2 H, $H_{3,5}$), 7.21 (s, 8 H, H_{arom}), 7.36 (m, 10 H, H_{arom}), 7.67 (s, 2 H, NH) ppm. ^{13}C NMR Jmod (63 MHz, CD_3CN, 25 °C): δ = 21.6 (CH_3Ar), 48.9 (CH_2NH), 105.7 ($H_{2,6}$), 122.1 (C_4), 128.6, 129.0, 130.0, 130.1, 130.5 (C_{arom}), 132.0 (C_{arom}-CH_2), 137.6 (C_{arom}-$C_{1,7}$), 142.9 (C_{arom}-CH_3), 160.5 ($C_{3,5}$), 169.0 ($C_{1,7}$) ppm. MS (FAB+, MNBA): *m*/*z* (%) = 483 (100) $[M]^+$. $C_{35}H_{35}BF_4N_2$ (570.28): calcd. C 73.69, H 6.18, N 4.91; found C 73.65, H 6.14, N 4.83. UV/Vis (23 °C, CH_2Cl_2), λ_{max} (ε_{max}) = 318 nm (9000 $M^{-1}\cdot cm^{-1}$), 392 (7000), 552 (118000). Fluorescence spectrometry (23 °C, CH_2Cl_2): $\lambda_{ex}/\lambda_{em}$ = 552/592 nm.

1,7-Bis(3,5-dimethylphenylamino)-1,7-bis(4-methylphenyl)hepta-1,3,5-trienylium Tetrafluoroborate (4): Green crystals with gold glints (13%). m.p.: 184−187 °C (dec.). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C), δ = 2.25 (s, 12 H, CH_3Ar), 2.40 (s, 6 H, CH_3Ar), 6.29 (d, ^3J = 13.0 Hz, 2 H, $H_{2,6}$), 6.34 (t, ^3J = 13.0 Hz, 1 H, H_4), 6.78 (s, 4 H, H_{arom}), 6.84 (s, 2 H, H_{arom}), 7.05 (t, ^3J = 13.0 Hz, 2 H, $H_{3,5}$), 7.27 (part A of AB syst., 4 H), 7.33 (part B of AB syst., $^3J_{AB}$ = 8.1 Hz, 4 H, H_{arom}), 8.59 (s, 2 H, NH) ppm. ^{13}C NMR Jmod (63 MHz, $CDCl_3$, 25 °C): δ = 21.3 [(CH_3)$_2$ArNH], 21.6 (CH_3Ar), 110.1 ($C_{2,6}$), 121.5 (C_{arom}), 124.1 (C_4), 128.7 (C_{arom}), 129.7 (C_{arom}), 129.8 (C_{arom}), 130.5 (C_{arom}-$C_{1,7}$), 137.5 (C_{arom}-NH), 139.3 [C_{arom}-CH_3, of (CH_3)$_2$ArNH]; 142.4 (C_{arom}-CH_3), 157.3 (C_{3-5}), 164.9 ($C_{1,7}$) ppm. MS (DCI/NH_3): *m*/*z* (%) = 511 (1.4) $[M^+]$, 238 (100). $C_{37}H_{39}BF_4N_2$ (598.31): calcd. C 74.25, H 6.57, N 4.68; found C 73.88, H 6.41, N 4.56. UV/Vis (23 °C, CH_2Cl_2): λ_{max} (ε_{max}) =

423 nm (24000 $M^{-1}\cdot cm^{-1}$), 599 (40000). Fluorescence spectrometry (23 °C, CH_2Cl_2): $\lambda_{ex}/\lambda_{em}$ = 594/687 nm.

1,7-Bis(4-methoxyphenylmethine-*N*-methylhydrazono)-1,7-bis(4-methylphenyl)hepta-1,3,5-trienylium Tetrafluoroborate (5): The 4-methoxyphenylmethine-*N*-hydrazone (1.01 g, 6.13 mmol) was added to a solution of **1** (1.310 g, 2.92 mmol) in acetonitrile (150 mL) and triethylamine (0.42 mL, 2.92 mmol). After 48 h of stirring, the solvent was removed and the crude reaction mixture was dissolved in dichloromethane and then precipitated with petroleum ether before being recrystallized from acetonitrile. Green crystals with gold glints were obtained (24%). m.p.: 212 °C (dec.). ^{1}H NMR (250 MHz, [D$_6$]DMSO, 25 °C): δ = 2.30 (s, 6 H, CH_3Ar), 3.37 (s, 6 H, N-CH_3), 3.85 (s, 6 H, O-CH_3), 6.64 (m, 2 H), 6.88 (m, 1 H), 7.13 (m, 6 H), 7.32 (m, 8 H), 7.81 (m, 21 H, H_{arom} and H_{2-6}), 8.39 (s, 2 H, CH=N) ppm. ^{13}C NMR Jmod, (63 MHz, [D$_6$]DMSO, 25 °C): δ = 21.0 (CH_3Ar), 37.1 (N-CH_3), 55.4 (O-CH_3), 112.0 ($CH_{2,6}$), 114.5 (C_{arom}), 126.1 (C_4), 126.3 (C_{arom}-$C_{1,7}$), 128.1 (C_{arom}), 129.8 (C_{arom}), 140.2 (C_{arom}-CH_3), 148.1 (CH=N), 156.3 ($C_{3,5}$), 161.7 (C_{arom}-OCH_3), 164.2 ($C_{1,7}$) ppm. MS (FAB>0, MNBA): *m*/*z* (%) = 597 (85.5) [M]$^+$, 327 (100). UV/Vis (23 °C, CH_2Cl_2): λ_{max} (ε_{max}) = 272 nm (25000 $M^{-1}\cdot cm^{-1}$), 380 (33000), shoulders at 581 and 630, 679 (137000). Fluorescence spectrometry 23 °C, CH_2Cl_2), $\lambda_{ex}/\lambda_{em}$ = 666/720 nm.

1-Ethoxy-1,7-bis(4-methylphenyl)-7-morpholinohepta-1,3,5-trienylium Tetrafluoroborate (6): At −15 °C, morpholine (0.094 mL, 1.08 mmol) was added dropwise to a solution of **1** (0.487 g, 1.08 mmol) in acetonitrile (40 mL). After 1 h of stirring, the solvent was removed. After crystallization from toluene/dichloromethane, red-orange crystals were obtained (31%). m.p.: 70−75 °C. The ^{1}H and ^{13}C NMR spectra are complex, therefore only the ^{1}H NMR spectrum is described. ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 1.41 (m, 3 H, C$H_3$$CH_2$O), 2.36, 2.41 (2 s, 6 H, CH_3Ar), 3.34 to 4.26 (m, 8 H, CH_2), 5.86 to 7.84 (m, 13 H, H_{arom} and H_{2-6}) ppm. MS (DCI/NH_3): *m*/*z* (%) = 402 (100) [M]$^+$.UV/Vis (23 °C, CH_2Cl_2): λ_{max} (ε_{max}): 309 nm (8000 $M^{-1}\cdot cm^{-1}$), 501 (55000). No fluorescence emission.

7-Dimethylamino-1,7-bis(4-methylphenyl)-1-morpholinohepta-2,4,6-trienylium Tetrafluoroborate (7): Dimethylamine (0.150 g, 0.641 mmol) was added to a solution of **6** (0.261 g, 0.534 mmol) in 20 mL of acetonitrile. After 24 h of stirring, the solvent was evaporated, the precipitate washed with diethyl ether, then dried under reduced pressure. The crude product was crystallized from ethanol to give silver-blue crystals (0.189 g, 73%). m.p.: 217 °C (dec.). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): δ = 2.36 (s, 6 H, CH_3Ar), 3.10−3.37 (m, 10 H, CH_3N and CH_2N), 3.71 (m, 4 H, CH_2O), 5.99 (m, 1 H, $H_{2\ or\ 6}$), 6.20 (m, 1 H, $H_{2\ or\ 6}$), 6.42 (m, 3 H, $H_{3,4,5}$), 7.05 (m, 4 H, H_{arom}), 7.23 (m, 4 H, H_{arom}) ppm. ^{13}C NMR Jmod (63 MHz, $CDCl_3$, 25 °C): δ = 21.5 (CH_3Ar), 49.6 (CH_2N), 66.6 (CH_2O), 106.9 ($C_{2\ or\ 6}$), 110.9 ($C_{2\ or\ 6}$), 123.1 (C_4), 128.6 (C_{arom}), 129.2 (C_{arom}), 129.2 (C_{arom}-$C_{1,7}$), 129.8 (C_{arom}), 130.1 (C_{arom}-$C_{1,7}$), 140.7 (C_{arom}-CH_3), 141.0 (C_{arom}-CH_3), 156.4 ($C_{3\ or\ 5}$), 159.0 ($C_{3\ or\ 5}$), 165.5 ($C_{1\ or\ 7}$), 171.1 ($C_{1\ or\ 7}$) ppm. MS (DCI/NH_3): *m*/*z* (%) = 401 (17) [M]$^+$, 162 (100). $C_{27}H_{33}BF_4N_2O$ (488.26): calcd. C 66.40, H 6.81, N 5.74; found C 66.15, H 6.68, N 5.61. UV/Vis (23 °C, CH_2Cl_2): λ_{max} (ε_{max}): shoulders at 522 nm, 559 (158000 $M^{-1}\cdot cm^{-1}$). Fluorescence spectrometry (23 °C, CH_2Cl_2): $\lambda_{ex}/\lambda_{em}$: 554/599 nm.

X-ray Crystallographic Study of 2: $C_{29}H_{35}BF_4N_2O_2$, M = 530.40, monoclinic, $P2_1/n$, a = 10.098(1), b = 27.502(3), c = 10.427(1) Å, β = 108.949(3)°, V = 2738.6(3) Å^3, Z = 4, ρ_c = 1.286 Mg·m^{-3}, $F(000)$ = 1120, λ = 0.71073 Å, T = 173(2) K, μ(Mo-K_α) = 0.098 mm^{-1}, crystal size 0.05 × 0.1 × 0.4 mm, 1.48° ≤ Θ ≤ 23.25°, 12328 reflections (3942 independent, R_{int} = 0.0682) were collected at low temperature using an oil-coated, shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer. The structure was solved by direct methods (SHELXS-97)[15] and 428 parameters were refined using the least-squares method on F^2.[16] Largest electron density residue: 0.180 e·Å^{-3}, R_1 [for $I > 2\sigma(I)$] = 0.0506 and wR_2 = 0.1022 (all data) with $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $wR_2 = [\Sigma w (F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{0.5}$.

CCDC-193199 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

[1] A. Mishra, R. K. Behera, P. K. Behera, B. K. Mishra, G. B. Behera, *Chem. Rev.* **2000**, *100*, 1973−2011 and references therein.
[2] S. R. Mujumdar, R. B. Mujumdar, C. M. Grant, A. S. Waggoner, *Bioconjugate Chem.* **1996**, *7*, 356−362.
[3] A. L. Ellis, J. C. Mason, H. W. Lee, L. Strekowski, G. Patonay, H. Choi, J. J. Yang, *Talanta* **2002**, *56*, 1099−1107.
[4] K. Licha, C. Hessenius, A. Becker, P. Henklein, M. Bauer, S. Wisniewski, B. Wiedenmann, W. Semmler, *Bioconjugate Chem.* **2001**, *12*, 44−50.
[5] G. Patonay, M. D. Antoine, *Anal. Chem.* **1991**, *63*, 321−327A.
[6] N. Narayanan, G. Little, R. Raghavachary, G. Patonay, *Proc. SPIE -Int. Soc., Opt. Eng.* **1995**, *2388*, 6−15.
[7] N. Obaya, C. Payrastre, Y. Madaule, *Tetrahedron* **2001**, *57*, 9137−9147, and references therein.
[8] A. L. Pikus, V. M. Feigel′man, V. V. Mezheritskii, *Zh. Org. Khim.* **1989**, *25*, 2333−2337.
[9] W. Z. König, *Z. Angew. Chem.* **1925**, *38*, 743−748.
[10] S. Dähne, F. Moldenhauer, in *Progress in Physical Organic Chemistry* (Ed.: R. W. Taft), John Wiley & Sons: New York, 1985; Vol.15, pp1−130.
[11] J. Fabian, H. Hartmann, in *Reactivity and Structure* (*Light Absorption of Organic Colorants)*; Springer-Verlag, Berlin, 1980; Vol. 12, chapter 12, pp162−197.
[12] R. B. Mujumdar, L. A. Ernst, S. R. Mujumdar, A. S. Waggoner, *Cytometry* **1989**, *10*, 11−19.
[13] Y. Madaule, C. Payrastre, A. Izquierdo, WO 02/046139, **2002**, (*Chem. Abstr.* **2002**, *137*, 17444).
[14] M. Lounasmaa, P. Hanhinen, R. Jokela, *Tetrahedron* **1995**, *51*, 8623−8648.
[15] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **1990**, *46*, 467−473.
[16] G. M. Sheldrick, *SHELXL-97, Program for Crystal Structure Refinement*, Göttingen **1997**.

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