Synthesis and Photophysical Properties of Fluorescent Derivatives of Methylmercury

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Summary: New fluorescent methylmercury acetylides carrying the anthracene, acridinone, and dansyl frameworks have been prepared and photophysically characterized. These compounds, which partially retain the fluorescence properties of the parent alkynes, suggest a promising use as luminescent labels for the analysis of methylmercury.

Within the family of species which take part in the biogeochemical cycle of mercury,[1] methylmercury (CH_3-Hg^+) is the most toxic species which can be detected in the environment and in biological tissues.[2] Major efforts are presently devoted to the search for new rapid, sensitive, and reliable separation and detection procedures for methylmercury.[3]

We have recently reported that the reaction of CH_3-HgCl (**1**) with phenylacetylene in alkaline aqueous solution affords $CH_3HgC{\equiv}CPh$. The reaction is fast, and a new method of analysis of **1** was proposed, based on extraction of $CH_3HgC{\equiv}CPh$ followed by HPLC separation and UV detection.[4] Here we wish to broaden the scope of the reaction, showing that the reaction of **1** with 1-alkynes (i) is general, since other aromatic and heteroaromatic 1-alkynes can be used,[5] and (ii) represents a very simple way to link a fluorescent label to **1** by exploiting properly tailored alkynes.

A luminescent label is defined as a signal-generating molecule (fluorophore) which can be effectively and selectively coupled to the analyte without changing significantly its photophysical properties. The properly derivatized molecule is then detected in very low concentration, thanks to the extraordinary improvement of present photon measuring techniques, by exploiting the fluorophore properties.[6]

We report the preparation and photophysical properties of some fluorescent methylmercury acetylides which could be useful in the development of new analytical methodologies for methylmercury in environmental and biological samples. To this purpose, we envisioned alkynes **2a**−**c** as attractive candidates, since they carry auxiliary groups which are well-known to possess high luminescence quantum yields[7] and, as a further advantage, synthesis of **2a**−**c** was straightforward and required simple routine chemistry (see Experimental Section). Moreover, **2a**−**c** smoothly reacted with **1** in THF/H_2O or CH_3OH/H_2O solution to give **3a**−**c** on a preparative scale (Scheme 1).

The photophysical properties of chromophores **2** and complexes **3** have been studied in CH_2Cl_2 and CH_3CN/H_2O (8/2)[8] solvents. In Table 1 we collect the most relevant data on the absorption and emission spectra of **2** and **3** together with the values of emission lifetimes and quantum yields. The spectra of **3c** are shown, as an example, in Figure 1. From the experimental data no significant differences in the absorption and emission spectra between uncomplexed and complexed chromophore can be noted in all the cases studied. This suggests that the metal does not have a strong electronic interaction with the various chromophores.

On the other hand, significant but not dramatic quenching effects are noted in going from **2** to **3** species when emission quantum yields and lifetimes are considered. Due to the lack of available low-lying metal-localized excited states and to the values of redox potentials of both metal and chromophores, we can exclude energy and electron transfer quenching mechanisms.[9] We think that the quenching can be reasonably attributed to the heavy-atom effect which catalyses the nonradiative deactivation of the excited states of the fluorophore. Only for compounds **2b** and **3b** does the emission quantum yield strongly depend on the polarity of the solvent. This behavior, which was already observed in the parent compound 10-methylacridinone,[7] limits the use of this compound in nonpolar solvents.

By exploiting the differences in terms of photophysical properties between **3** and **2**, it is possible to gain a deeper insight into the mechanism of the acetylide formation reaction. In fact, carrying out a derivatization reaction of **1** into **3c** under high-dilution conditions ([**1**] $= 10^{-4}$ M; [**2c**] $= 10^{-6}$ M) in aqueous 1 M NaOH,[10] it is

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(5) Aliphatic alkynes exhibit the same reactivity; in particular, 1-pentyne quantitatively reacts with **1** in water under heterogeneous conditions within 1 h (Trombini, C. Unpublished results).

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(7) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*; Dekker: New York, 1993.

(8) Acetonitrile−water mixtures were used as eluents in HPLC analysis.

(9) For example, cyclic voltammetry of **3c** in acetonitrile solution shows in the range −2.0 to +2.0 V only one peak at 0.9 V *vs* SCE, due to the irreversible oxidation of the dansyl chromophore. This locates an hypothetical charge-transfer state at an energy above 2.9 eV, much higher than the energy (2.4 eV) of the excited state responsible for the emission.

(10) The choice of this reaction medium was dictated by the solubility of **2c** and **3c** in the form of deprotonated sulfonamides.

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Scheme 1

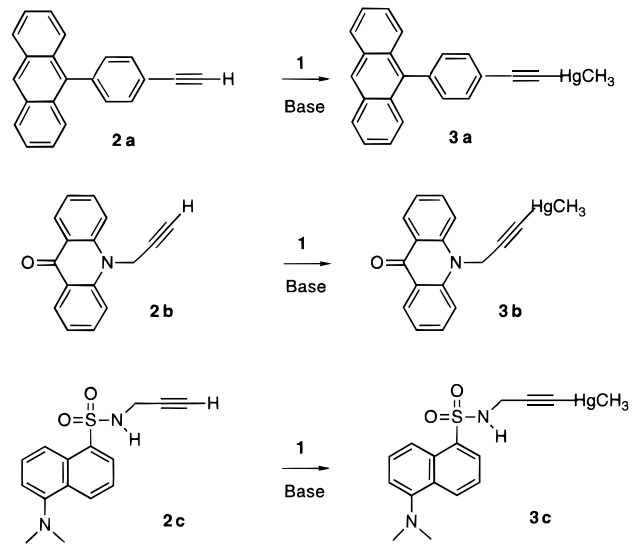

Table 1. Photophysical Properties of Methylmercury Products and Their Parent Alkynes[a]

		absorption		luminescence		
	solvent	λ_{max} (nm)[b]	ϵ (M^{-1} cm^{-1})	λ_{max} (nm)[b]	ϕ_{em}	τ (ns)
2a	CH_2Cl_2	258	122 000	405	0.52	4.6
	CH_3CN/H_2O (8/2)	255	105 000	406	0.45	4.0
3a	CH_2Cl_2	258	135 000	410	0.29	2.4
	CH_3CN/H_2O (8/2)	255	132 000	407	0.29	2.0
2b	CH_2Cl_2	254	36 500	407	0.008	1.2
	CH_3CN/H_2O (8/2)	251	45 200	416	0.58	13.0
3b	CH_2Cl_2	254	30 100	407	0.007	2.0
	CH_3CN/H_2O (8/2)	251	36 200	418	0.50	7.7
2c	CH_2Cl_2	256	12 400	501	0.45	18.4
	CH_3CN/H_2O (8/2)	251	11 000	534	0.25	11.4
3c	CH_2Cl_2	256	12 000	497	0.30	11.9
	CH_3CN/H_2O (8/2)	252	11 500	528	0.12	5.4

[a] At room temperature. [b] Data referring to the highest peak in the spectrum.

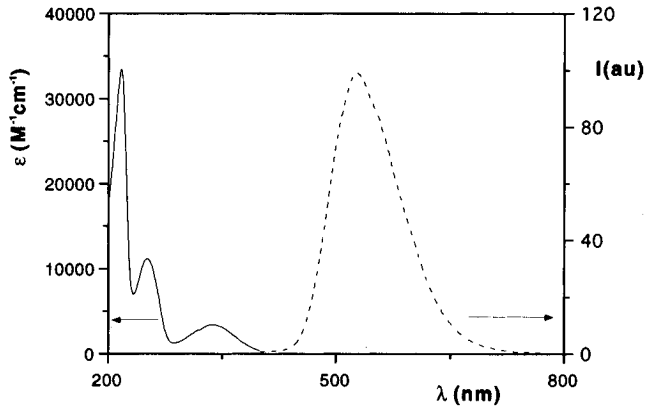

Figure 1. Absorption (—) and emission (- - -) spectra of **3c** in CH_3CN/H_2O (8/2) at room temperature.

possible to determine the rate constant of **3c** by following the decrease in emission intensity of the reaction mixture due to the transformation of **2c** ($\Phi_{em} = 0.25$) into **3c** ($\Phi_{em} = 0.12$). The kinetic equation obtained was

$$d[\mathbf{3c}]/dt = k[\mathbf{2c}][\mathbf{1}]$$

and the value of k was 15 M^{-1} s^{-1}. This value indicates that more than 95% conversion is achieved after 20 min using a 10^{-6} M solution of **1** and 100 equiv of the derivatizing alkyne **2c**.

The high emission quantum yields of the mercuric complexes **3** appear to be appropriate for fluorometric analytical detection. This was clearly confirmed by preliminary HPLC measurements. Dose−response curves for methylmercury complexes **3a**−**c** were measured, and a good proportionality exists between the amount of analyte injected (0.1−100 ng as Hg) and peak areas. The correlation coefficients (r) of these graphs lie between 0.9998 and 1; excellent fit and linearity indicate that this method is suitable for quantitative analysis.

We believe that the high value of the kinetic rate constant measured for the formation of **3c** and the investigated photophysical properties of **3a**−**c** represent a promising starting point for the development of a novel, rapid, and sensitive method for the analysis of methylmercury, the most toxic form of mercury detectable in the environment. A more detailed study on analytical applications of **2a**−**c** as fluorescent labels for methylmercury will be reported in due course.

Experimental Section

Equipment. Absorption spectra were recorded with a Perkin-Elmer Lambda 16 spectrophotometer. Uncorrected emission and corrected excitation spectra were obtained with a Perkin-Elmer LS50 spectrofluorometer.

The fluorescence lifetimes (uncertainty ±5%) were obtained with an Edinburgh single-photon counting apparatus, in which the flash lamp was filled with N_2. Each sample gave rise to single-exponential decays. Luminescence quantum yields (uncertainty ±15%) were determined using quinine sulfate in 1 N H_2SO_4 aqueous solution ($\Phi = 0.546$) as a reference.[11]

Analytical HPLC was performed on a Hewlett-Packard Series 1050 instrument connected to an HP Model 386s/20 integrator and an HP Model 1046A fluorescence detector using a reversed-phase column (Hypersil ODS, 150 × 0.46 i.d., 5 mm diameter particles).

9-(4-Ethynylphenyl)anthracene (2a). To a THF (20 mL) solution of the Grignard reagent obtained from 2-(4-bromophenyl)-1,3-dioxane (1 g, 0.4 mmol) after sonication at 70 °C was added 9(10*H*)-anthraquinone (4.79 g, 24.7 mmol) in THF (30 mL) dropwise; the solution rapidly turned from colorless to fluorescent green and finally to orange. Hydrolysis of the acetal group was performed by stirring the crude reaction mixture with 6 M HCl (20 mL); after standard workup and flash chromatography on silica gel (cyclohexane/ether 95:5) 4-(9-anthracenyl)benzaldehyde was isolated (5.5 g, 79%) as a yellow solid; mp 126−130 °C; IR (Nujol) 1690 cm^{-1}; MS (70 eV) *m/z* (relative intensity) 282 (M, 100), 253 (55), 252 (68), 250 (30), 126 (13); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 10.21 (s, 1H, CHO), 8.55 (s, 1H, H-10), 8.11 (t, 4H, $J = 8.5$ Hz), 7.62 (t, 4H, $J = 7.5$ Hz), 7.54−7.36 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 192.0 (CO), 145.7, 135.6, 135.2, 132.1, 131.2, 129.8, 128.5, 127.3, 126.1, 125.8, 125.2.

Alkyne **2a** was prepared by the following modification of the Corey procedure:[12] to a solution of CBr_4 (11.1 g, 3.35 mmol) in anhydrous CH_2Cl_2 (5 mL) cooled to 0 °C was slowly added triphenylphosphine (1.76 g, 6.71 mmol) with efficient stirring. After 10 min 4-(9-anthracenyl)benzaldehyde (0.80 g, 2.83 mmol) was added slowly and the reaction mixture was stirred for 20 min at 0 °C. Cyclohexane was added (20 mL), and the solid residue was filtered over Celite. The organic phase was evaporated to dryness, affording pure 9-[4-(2,2-dibromoethenyl)phenyl]anthracene (1.12 g, 90%): mp 171−173 °C; ^{1}H

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NMR (300 MHz, $CDCl_3$) δ (ppm) 8.52 (s, 1H, H-10), 8.06 (d, 2H, J = 8.5 Hz), 7.79 (d, 2H, J = 8.0 Hz), 7.68−7.64 (m, 3H), 7.50−7.44 (m, 4H), 7.40−7.30 (m, 2H). To a solution of 9-[4-(2,2-dibromoethenyl)phenyl]anthracene (0.876 g, 2 mmol) in anhydrous THF (6 mL) cooled to −78 °C was slowly added *t*-BuOK (0.246 g, 2.2 mmol), and the reaction mixture was stirred at −78 °C for 1 h. To the same solution was added BuLi (2.5 M in hexane, 0.8 mL, 2 mmol), and the reaction mixture was stirred 1 h more at −78 °C and then quenched with phosphate buffer (pH 7, 2 mL). After extraction with ether **2a** (0.50 g, 90%) was obtained as a yellow solid: mp 168−170 °C (cyclohexane); HPLC (CH_3CN/H_2O, 80/20) t_R = 3.8 min; IR (KBr) 3310, 2100, 1250, 630 cm^{-1}; MS (70 eV) *m*/*z* (relative intensity) 278 (M, 100), 277 (35), 276 (52), 274 (18), 138 (19), 124 (7); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.52 (s, 1H, H-10), 8.07 (d, 2H, J = 8.5 Hz), 7.75 (d, 2H, J = 8.2 Hz), 7.67 (d, 2H, J = 8.9 Hz), 7.51−7.36 (m, 6H), 3.21 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 139.7, 136.0, 132.2, 132.1, 131.4, 130.1, 128.4, 126.9, 126.5, 125.6, 125.1, 123.2, 121.5, 83.7 (*C*CH), 77.6 (C*C*H).

9-(4-Ethynylphenyl)anthracene, Methylmercury Complex (3a). To a solution of **1** (Alfa, 95%, 0.90 g, 0.36 mmol) in 3 mL of basic MeOH (prepared by dissolving 0.1 g of NaOH in 30 mL of MeOH) was added a solution of **2a** (0.10 g, 0.36 mmol) in THF (3 mL). After a few minutes a light yellow solid precipitated from the homogeneous solution and the reaction mixture became colorless. The solid was separated by filtration and washed with pentane (20 mL) to afford 0.11 g (62%) of **3a** as a white solid that was purified by recrystallization from CH_2Cl_2. Anal. Calcd for $C_{23}H_{16}Hg$: C, 56.04; H, 3.27. Found: C, 55.9; H, 3.32. Mp: decomposition at 270−275 °C. HPLC: (CH_3CN/H_2O 80/20) t_R = 4.9 min. MS (70 eV): *m*/*z* (relative intensity) 494 (M relative to ^{202}Hg, 8), 358 (12), 279 (25), 278 (100), 277 (34), 274 (16), 266 (53), 217 (CH_3Hg, 5), 202 (11), 138 (21), 42 (10). ^1H NMR (300 MHz, $CDCl_3$, T = 50 °C): δ (ppm) 8.52 (s, 1H, H-10), 8.05 (d, 2H, J = 8.5 Hz), 7.75−7.65 (m, 4H), 7.49−7.28 (m, 6H), 068 (s, 3H, CH_3), 0.68 (d, 3H, J(relative to ^{199}Hg) = 148 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, T = 50 °C): δ (ppm) 139.6, 136.1, 132.5, 132.2, 131.5, 130.2, 128.4, 127.0, 126.7, 125.6, 125.2, 122.7, 121.4, 106.0 (C−Hg), 77.2 (*C*C−Hg), 7.0 (Hg−CH_3).

10-(2-Propynyl)-9(10*H*)-acridinone (2b). The following modified literature procedure[13] was adopted: propargyl bromide (0.385 mL, 80% solution in toluene, 2.58 mmol) was directly added to a solid potassium salt prepared from 9(10*H*)-acridinone (0.50 g, 2.58 mmol) and *t*-BuOK (0.29 g, 2.58 mmol). After vigorous stirring for 12 h GC−MS analysis revealed the formation of two products, namely **2b** and 10-(2-propadienyl)-9(10*H*)-acridinone[14] in a 10:1 ratio. The mixture was quenched with phosphate buffer (pH 7, 2) and extracted with CH_2Cl_2 (3 × 5 mL). Recrystallization from toluene gave **2b** as a yellow solid (0.360 g, 63%): mp 212−215 °C; HPLC (CH_3CN/H_2O 50/50) t_R = 2.3 min; MS (70 eV) *m*/*z* (relative intensity) 233 (M, 100), 232 (43), 204 (65), 194 (82), 166 (81), 140 (58), 102 (14), 75 (16), 69 (13); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.55 (dd, 2H, J = 8.0, 1.7 Hz), 7.77 (dt, 2H, J = 8.0, 1.7 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.33 (t, 2H, J = 7.9 Hz), 5.22 (d, 2H, J = 2.5 Hz, N−CH_2), 2.43 (t, 1H, J = 2.5 Hz, CCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 178.1 (CO), 141.7, 134.1, 127.9, 122.7, 121.8, 114.5, 77.2 (*C*CH), 73.9 (C*C*H), 36.8 (N−CH_2).

10-(2-Propynyl)-9(10*H*)-acridinone, Methylmercury Complex (3b). According to the same procedure described for the synthesis of **3a**, we obtained **3b** in 65% yield after recrystallization from toluene. Anal. Calcd for $C_{17}H_{13}NOHg$: C, 45.59; H, 2.93; N, 3.13. Found: C, 45.9; H, 2.8; N, 3.0. Mp: decomposition at 155−160 °C. HPLC (CH_3CN/H_2O 50/50): t_R = 3.8 min. MS (70 eV): *m*/*z* (relative intensity) 449 (M relative to ^{202}Hg, 23), 255 (5), 232 (100), 217 (CH_3Hg, 6), 204 (50), 194 (60), 166 (52), 140 (26). ^1H NMR (300 MHz, $CDCl_3$, T = 50 °C): δ (ppm) 8.57 (dd, 2H, J = 8.0, 1.6 Hz), 7.83−7.64 (m, 4H), 7.38−7.27 (m, 2H), 5.0 (s, 2H, N−CH_2), 0.63 (s, 3H, CH_3), 0.63 (d, 3H, J(relative to ^{199}Hg) = 147.7 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, T = 50 °C): δ(ppm) 178.2 (CO), 141.9, 133.9, 127.9, 122.9, 121.6, 114.8, 98.8 (C−Hg), 77.2 (*C*C−Hg), 37.6 (N−CH_2), 6.7 (CH_3−Hg).

5-(Dimethylamino)-*N*-(2-propynyl)-1-naphthalenesulfonamide (2c). To a solution of 5-(dimethylamino)naphthalene-1-sulfonyl chloride (1 g, 3.7 mmol) in anhydrous CH_2Cl_2 (10 mL) were added triethylamine (0.55 mL, 4 mmol) and propargylamine (0.275 mL, 4 mmol); the solution immediately turned from dark yellow to fluorescent. After quenching (phosphate buffer, pH 7) and extraction (CH_2Cl_2) **2c** was obtained as a yellow solid (0.95 g, 89%): mp 93−95 °C; HPLC (CH_3CN/H_2O 50/50) t_R = 3.3 min; MS (70 eV) *m*/*z* (relative intensity) 288 (M, 21), 171 (100), 154 (22), 127 (16); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.55 (d, 1H, J = 7.1 Hz), 8.26 (t, 2H, J = 7.1 Hz), 7.54 (quintet, 2H, J = 7.1 Hz), 7.19 (d, 1H, J = 7.1 Hz), 4.85 (t, 1H, J = 6.0 Hz, NH), 3.77 (dd, 2H, J = 6, 2.6 Hz, N−CH_2), 2.90 (s, 6H, N−CH_3), 1.92 (t, 1H, J = 2.6 Hz, CCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 152.0, 134.1, 130.8, 129.8, 128.5, 123.1, 118.5, 115.2, 77.6 (*C*CH), 72.6 (C*C*H), 45.4 (N−CH_3), 32.9 (N−CH_2).

5-(Dimethylamino)-*N*-(2-propynyl)-1-naphthalenesulfonamide, Methylmercury Complex (3c). To a solution of **1** (0.252 g, 1 mmol) in water (10 mL, NaOH 1 M, NaCl 0.35 M) was added **2c** (0.288 g, 1 mmol); within a few minutes a yellow solid precipitated out. After adjustment to pH 6 with dilute HCl, extraction (CH_2Cl_2), and recrystallization (CH_2Cl_2/*n*-hexane), **3c** (0.42 g, 84%) was obtained as a light yellow solid. Anal. Calcd for $C_{16}H_{18}N_2SO_2Hg$: C< 38.21; H, 3.61; N, 5.57. Found: C, 38.0; H, 3.5; N, 5.6. Mp: decomposition at 140−145 °C. HPLC (CH_3CN/H_2O 50/50): t_R = 4.8 min. MS (70 eV): *m*/*z* (relative intensity) 504 (M relative to ^{202}Hg, 5.2), 387 (5.5), 288 (34.4), 217 (CH_3Hg, 4), 171 (100), 170 (49.3), 168 (28), 154 (16.8), 127 (21), 42 (15.3). ^1H NMR (300 MHz, $CDCl_3$, T = 50 °C): δ (ppm) 8.55 (d, 2H, J = 8.6 Hz), 8.33−8.23 (m, 2H), 7.63−7.51 (m, 2H), 7.19 (d, 1H, J = 7.6 Hz), 4.82 (t, 1H, J = 6.0 Hz, NH), 3.75 (d, 2H, J = 6.0 Hz, N−CH_2), 2.9 (s, 6H, N−CH_3), 0.55 (s, 3H, CH_3), 0.55 (d, 3H, J(relative to ^{199}Hg) = 147.0 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, T = 50 °C): δ (ppm) 152.0, 134.8, 130.5, 129.7, 128.5, 123.3, 118.7, 115.1, 99.2 (C−Hg), 77.3 (*C*C−Hg), 45.4 (N−CH_3), 33.8 (N−CH_2), 6.7 (CH_3−Hg).

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(14) R_f = 0.38 (cyclohexane/ethyl acetate 8/2). GC: t_R = 22.78 min. MS: *m*/*z* (relative intensity) 233 (M, 33), 232 (100), 204 (87), 140 (14), 102 (12). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.55 (d, 2H, J = 8.6 Hz), 7.72−7.70 (m, 4H), 7.35−7.30 (m, 2H), 6.62 (t, 1H, J = 6.2 Hz), 5.42 (d, 2H, J = 6.2 Hz).