In situ fluorescent labeling of highly volatile methylamine with 8-(4,6-dichloro-1,3,5-triazinoxy)quinoline

Huimin Ma,*[a] Ute Jarzak[b] and Wolfram Thiemann*[b]

[a] *Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China. E-mail: mhmliang@public.bta.net.cn*
[b] *Department of Chemistry, University of Bremen, D-28334 Bremen, Germany. E-mail: thiemann@uni-bremen.de*

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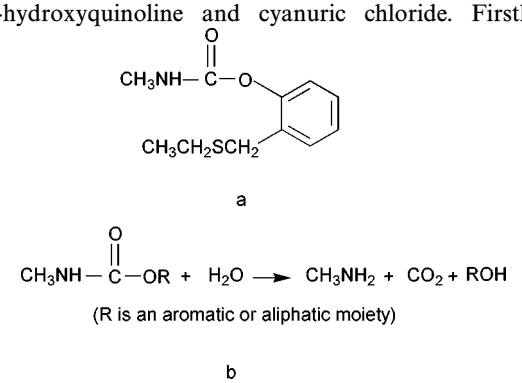
The first *in situ* fluorescent labeling probe for monitoring of highly volatile methylamine, 8-(4,6-dichloro-1,3,5-triazinoxy)quinoline, has been designed, synthesized and evaluated. The probe labels spectroscopically inert methylamine, causing a large change in fluorescence properties through suppression of the internal charge transfer, and thus can serve as an *in situ* labeling probe. This applicability has been demonstrated by measuring methylamine released during hydrolysis of *N*-methylcarbamates such as ethiofencarb.

The residue of *N*-methylcarbamates such as ethiofencarb is of concern for pollution control because of their wide use in agriculture and their more toxic degradation products.[1] This class of pesticides in the environment usually hydrolyzes to generate methylamine, CO_2 and a corresponding alcohol or a phenol (see Scheme 1), hence their residue and degradation process can be determined by monitoring the released methylamine.[2] However, direct detection of the hydrolytic product methylamine is quite difficult, since it escapes readily from the solution during hydrolysis and lacks any easily identifiable property. Hence, it is desirable to develop a method by which *in situ* monitoring of the highly volatile methylamine could be achieved. This has been realized with the aid of electrochemical and optical sensor devices.[3,4] Methylamine contains $-NH_2$ which reacts readily with spectroscopic probes bearing reactive chlorine or formyl groups, another feasible approach to the problem is thus to design appropriate probes for *in situ* labeling of the released methylamine. However, to the best of our knowledge, no fluorescent probes for *in situ* labeling of this volatile compound have been reported, though dansyl chloride, *o*-phthalaldehyde/2-mercaptoethanol and trifluoroacetophenone derivatives have been proposed as derivatization reagents in chromatography.[5–7] In this study, the first *in situ* fluorescent probe for labeling of volatile methylamine, 8-(4,6-dichloro-1,3,5-triazinoxy)quinoline **1**, has been designed and evaluated (Scheme 2).

The design presented here takes advantage of the properties of 8-hydroxyquinoline and cyanuric chloride. Firstly, 8-hydroxyquinoline, as a versatile spectroscopic species, has an electron-rich moiety[8] which could be influenced by distribution of intramolecular electronic charge, causing changes in fluorescence properties. Secondly, cyanuric chloride has three chlorine atoms with different reactivities, which may be replaced sequentially depending upon the temperature and the pH of the medium. It is this property that makes cyanuric chloride so valuable and explains its use for many years as a bridging agent to synthesize reactive dyes[9,10] and labels for corticosteroids,[11] immunoglobulins[12] and amino acids.[13] Since the second chlorine atom of cyanuric chloride is still rather active and often reacts at room temperature after substitution of the first,[14] we chose this moiety to provide active chlorine groups for achieving fast *in situ* labeling of the volatile methylamine released during hydrolysis of *N*-methylcarbamates. Moreover, cyanuric chloride is an electron acceptor, and its electron accepting character can be adjusted by substitution of a nucleophile.[15] This should also lead to changes in the fluorescence properties. We envisioned that 8-hydroxyquinoline could be incorporated into cyanuric chloride so that the resulting compound would alter its fluorescence properties through labeling methylamine which would in turn perturb the photoinduced processes (*e.g.* molecular internal charge transfer), thus allowing the molecule to serve as an *in situ* labeling probe. We now report the results of these studies.

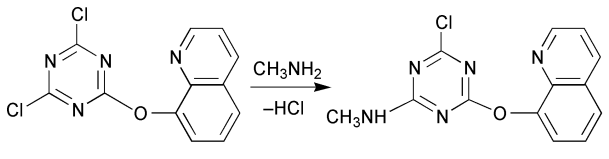


Scheme 1 The structure of ethiofencarb (a) and the hydrolysis of *N*-methylcarbamates (b).

Experimental

Instruments and materials

Infrared spectra were taken from KBr disks on a Perkin-Elmer 1000 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-600 spectrometer (^{1}H NMR 600 MHz; ^{13}C NMR 90.5 MHz) in CD_3COCD_3 with tetramethylsilane as internal standard. Mass spectra were measured on a Finnigan



Scheme 2 The labeling of methylamine with the probe **1**.

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MAT 8200 spectrometer (EI, 70 eV). Uncorrected melting points were determined with a Jürgens Electrothermal apparatus. Elemental analysis was carried out by the analytical laboratory of the Chemistry Institute (Beijing). Thin layer chromatographic (TLC) analysis was performed on E. Merck 60 F_{254} silica gel plates (absorbent thickness 0.25 mm), using acetone–chloroform (1 : 1 v/v) as the eluent. Fluorescence spectra were recorded as raw data with an AMINCO SPF-500 spectrofluorimeter in 10 × 10 mm quartz cells. An Ionalyzer 701A pH meter was employed for pH measurements.

Cyanuric chloride, 8-hydroxyquinoline, ethiofencarb and methylamine were obtained from Acros, Merck, Fluka or Riedel-de Haën Chemical Co. and used as received. The solutions of ethiofencarb and the probe **1** were prepared in dry acetone. It should be pointed out that, like many other easy to decompose dichlorotriazine derivatives,[14] solutions of the probe should be kept free from moisture and stored at $\leqslant 25\,^{\circ}C$ to avoid hydrolysis. In this way, no obvious hydrolysis (less than $\pm 5\%$ change in fluorescence intensity for a 3.0×10^{-5} mol dm^{-3} probe solution) was observed within three months at 5–25 °C. All other reagents were of at least analytical grade.

Synthesis of 8-(4,6-dichloro-1,3,5-triazinoxy)quinoline

A solution of 8-hydroxyquinoline (1.45 g, 10 mmol) in acetone (40 mL) and water (10 mL) was mixed with aqueous NaOH (1 mol dm^{-3}, 10 mL) as acid acceptor and cooled to 0–5 °C. Then, the mixture was slowly added to a cold, stirred solution of cyanuric chloride (1.85 g, 10 mmol) in acetone (60 mL) over a period of 5 min. About 8 min later a large quantity of white precipitate was formed. The pH of the reaction mixture was about 8. After stirring at 0–5 °C for an additional 50 min, the reaction mixture was diluted with cold water (100 mL). The precipitated white product was then filtered off, and washed three times with cold water and once with a cold acetone–water mixture (1 : 2 v/v). After drying in a desiccator under reduced pressure at low temperature (*ca.* 5 °C), a light yellow solid (2.54 g, 86% yield) was obtained. The product, which is soluble in acetone but not in water and toluene, is already quite pure at this stage but can be further purified by recrystallization from n-hexane–acetone (1 : 1 v/v). TLC analysis showed a spot with $R_f = 0.92$. Mp 199–200 °C; IR (KBr): 3063m, 1708–1469 (C=C, C=N), 1415s, 1368m, 1305–1087 (C–O, C–Cl), 995s, 852s (C–Cl), 782m cm^{-1}; ^1H NMR (600 MHz, CD_3COCD_3): δ 8.84 (dd, 1H), 8.53 (dd, 1H), 8.05 (dd, 1H), 7.75 (m, 2H), 7.65 (q, 1H); ^{13}C NMR (90.5 MHz, CD_3COCD_3): δ 173.1, 151.2, 137.5, 130.7, 128.0, 127.5, 123.1, 121.6; MS (70 eV, EI): *m/z* (%) 296 (0.9) $[M + 4]^+$, 294 (5.0) $[M + 2]^+$, 292 (8.0) $[M]^+$, 259 (32.0) $[M + 2 - Cl]^+$, 257 (100) $[M - Cl]^+$, 194 (29.1), 128 (8.6), 116 (15.0), 89 (16.0). Elemental analysis calc. for $C_{12}H_6Cl_2N_4O$ (293.1): C 49.17, H 2.06, N 19.12; found C 49.79, H 2.19, N 18.41%.

Fluorescent labeling of methylamine

All labeling reactions were performed in a test tube equipped with a magnetic stirring bar. Unless otherwise noted, the fluorescent labeling of methylamine was carried out by rapidly mixing an acetone solution of the probe **1** with an aqueous solution of methylamine in the presence of sodium carbonate, adjusting the final volume to 10 mL with water and then heating the mixture at 80–95 °C for 40 min in a water bath. After cooling to room temperature, the fluorescence spectra or intensities of the reaction solution were determined in 1 cm quartz cells at $\lambda_{ex} = 340$ nm and/or $\lambda_{em} = 405$ nm. In the meantime, a blank solution containing no methylamine was prepared and measured under the same conditions for comparison purposes.

In a typical labeling experiment, the reaction solution (10 mL) comprised 0.2 mL of 0.2 mol dm^{-3} Na_2CO_3, 0.1 mL of 3.4×10^{-3} mol dm^{-3} probe (in acetone) and no more than 3.4×10^{-4} mmol of methylamine. For the hydrolytic analysis of ethiofencarb with the probe **1**, the same procedure given above was employed except that methylamine solution was replaced by 0.05 mL of 4.4×10^{-3} mol dm^{-3} ethiofencarb (in acetone).

Results and discussion

As discussed previously, *in situ* monitoring of highly volatile methylamine is a challenging subject due to its spectroscopic inertness and the absence of any easily identifiable property. Fortunately, methylamine contains an amino group that can react with an active chlorine group, therefore an effective method would be to develop *in situ* spectroscopic probes bearing active chlorine atoms and then to label it with the probes. In an ideal scenario the probe itself would have no spectroscopic response but would fluoresce after reacting with the target. However, in many cases the probe has its own spectroscopic response, but its spectroscopic properties are often changed through disturbance of related photophysical processes after the labeling reaction.[16,17] This change can take several forms: (1) an increase or decrease in the spectroscopic response intensity of the probe with little change in either the absorption or fluorescence spectra; (2) a shift of the absorption, and therefore the excitation, spectrum to shorter or longer wavelengths with little shift in the emission maximum; or (3) a shift in both the absorption (excitation) and emission maximum.

In the case of wavelength change, the use of strongly fluorescent moieties such as quinoline would be beneficial to sensitive labeling. On the other hand, there are a lot of compounds which can provide an active chlorine group for labeling methylamine, but cyanuric chloride is our choice because its three chlorine atoms are easily controlled for stepwise substitutions. Thus, the probe **1** was readily prepared in high yield (86%) by linking the spectroscopic moiety 8-hydroxyquinoline to cyanuric chloride in the presence of NaOH. It is important that the synthetic operation and, especially, the drying of the product should be conducted at low temperature (*ca.* 5 °C) to avoid hydrolysis. Ordinarily, the as-obtained product is essentially pure, and can give satisfactory spectral (IR, NMR, MS) data. For instance, the EI mass spectrum of probe **1** shows the molecular ion peak at *m/z* 292 $[M]^+$, and two distinct isotope peaks at *m/z* 294 $[M + 2]^+$ and 296 $[M + 4]^+$, with intensities in a ratio of 9 : 6 : 1 ($[M]^+ : [M + 2]^+ : [M + 4]^+$), according very well with that of a typical compound containing two chlorine atoms. The base peak occurred at *m/z* 257, resulting from the loss of one chlorine atom, and thus meaning that one chlorine atom in the probe should be active.

Several possible factors affecting the labeling of methylamine were examined, including the pH of the reaction media, temperature, time, *etc.* The reaction of methylamine with active chlorine groups releases hydrogen chloride, so the base Na_2CO_3 was used as acid acceptor; the strongest fluorescence intensities were observed within the pH range 9–11. In a medium of less than pH 6, no obvious labeling reaction occurred and a large decrease in the fluorescence intensity of the probe itself took place, presumably resulting from protonation of the N atoms. In order to capture the volatile methylamine released during hydrolysis and to achieve the *in situ* labeling, it is necessary to simultaneously conduct the hydrolytic release and fluorescent labeling of methylamine. The hydrolysis of *N*-methylcarbamates is often carried out at 80–95 °C,[18,19] this temperature was therefore used in our labeling reactions. The reaction time for labeling at 80–95 °C can be set to 40 min, since at this time the changes in fluorescence properties reached a maximum. Fig. 1 shows such changes after labeling methylamine with the probe. The fluorescence

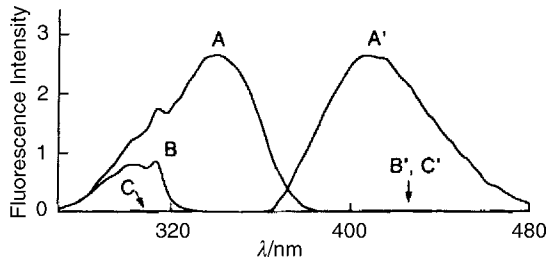

Fig. 1 Fluorescence spectra of the probe **1** and methylamine solutions (pH 11). Excitation spectra of (A) the reaction solution of **1** (3.4×10^{-4} mmol) with methylamine (3.4×10^{-4} mmol), (B) the solution of **1** alone (3.4×10^{-4} mmol) and (C) the solution of methylamine alone (3.4×10^{-4} mmol). Emission spectra (A′, B′, C′) corresponding to the above solutions are also shown. Bandpass of 2 nm at the entrance slit and 4 nm at the exit slit.

enhancement and the shift to a longer wavelength may result from the introduction of the methylamino substituent, which behaves as a strong electron donor.[20] As is well known, when a fluorescent molecule contains an electron-donating group conjugated to an electron-withdrawing group, it undergoes internal charge transfer from the donor to the acceptor upon excitation by light. The consequent change in dipole moment results in a Stokes shift that is sensitive to environmental stimuli.[17] Also, it is generally accepted that when the internal charge transfer is perturbed by some factor (*e.g.* by chemical reactions), the photophysical properties of the fluorescent molecule are often altered. The probe **1** is a donor–acceptor molecule, and the internal charge transfer occurs from the donating oxyquinoline[8] to the electron accepting 1,3,5-triazinyl group. Upon replacement of the second chlorine atom of the probe by the strong electron-donating group $-NHCH_3$,[20] which reduces the electron acceptor character of the triazinyl ring system,[15] the internal charge transfer is suppressed, thus resulting in photophysical changes. As expected, the probe **1** labels methylamine resulting in significant changes to its fluorescence properties, and the labeled methylamine, representing a new compound, exhibits an excitation peak at 340 nm and emission peak at 405 nm (Fig. 1), respectively. This new compound can be obtained nearly quantitatively by reacting equimolar portions of the probe **1** and methylamine at 30–50 °C in the presence of NaOH and was identified as 8-(4-chloro-6-methylamino-1,3,5-triazinoxy)quinoline **2** by MS analysis {300 (18.5) $[M + H + 2]^+$, 288 (52.6) $[M + H]^+$, 252 (12.6) $[M - Cl]^+$}. Furthermore, the fluorescence spectrum of **2** is consistent with that of the *in situ* labeling solution of methylamine in Fig. 1.

For a sensitive assay of methylamine with low background fluorescence, 340 and 405 nm were employed as excitation and emission wavelengths in this system, respectively. With the present labeling procedure, the linear correlation between the fluorescence intensity (F) and the methylamine concentration (C) in the range 3.2–34 μM was found to be $F = 0.067C + 0.169$ ($n = 5$, $r = 0.9804$), and a detectable signal change ($S/N = 3$) was produced down to 0.064 μM of methylamine. The rather poor correlation coefficient ($r = 0.9804$) may be attributed to the fact that, besides the major reaction product **2**, minor trisubstituted and hydrolytic products of the probe can also be formed at a high labeling temperature of 80–95 °C. Obviously, the probe cannot distinguish methylamine from a mixture of amines owing to the nonspecific labeling reaction of its active chlorine atoms, and the reactivity of other low molecular weight amines with the probe might follow their basicity [*e.g.* $(CH_3)_2NH > CH_3NH_2 > NH_3$]. Nevertheless, this property would enable the probe to find a use in pre- or post-column derivatization of chromatographically separable compounds such as mixed amines, and perhaps also phenols.

In order to test the applicability of the probe to the *in situ* fluorescent labeling of volatile methylamine, the hydrolytic yield of an important insecticide, ethiofencarb, was determined by measuring the released methylamine. The results showed that 59 ± 6% ($n = 3$) of the ethiofencarb can be hydrolyzed under the given conditions (pH 11, temperature 80 °C, hydrolysis time 40 min), which was obtained by comparing the fluorescence signal of the labeled released methylamine with that of a standard labeled methylamine solution. This finding was also confirmed by analyzing the ethiofencarb remaining in the hydrolytic solution by HPLC (Kromasil KR100-5 C18, 250 × 4.6 mm), which showed 64% hydrolysis of ethiofencarb. These results demonstrate that the present method can be applied to the *in situ* monitoring of highly volatile methylamine.

In conclusion, although a variety of fluorescent *ion* sensing molecules have been designed based on the perturbation of photophysical processes, less attention has been paid to *in situ* fluorescent labeling probes for *organic* molecules. In this study we have demonstrated the first *in situ* fluorescent labeling of highly volatile methylamine with the donor–acceptor molecule **1**. The probe labels spectroscopically inert methylamine causing a significant change in the fluorescence properties through suppression of the internal charge transfer, and can thereby serve as an *in situ* labeling probe, overcoming the problem of the ready escape of methylamine from solution during hydrolysis of *N*-methylcarbamates such as ethiofencarb. Further work in this area may be beneficial to the development of a new generation of different *in situ* labeling probes for other organic targets.

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