Cholesterol Analogs Labeled with Novel Silylated Fluorescent Compounds

Tomohisa Moriguchi,[1] Masahiro Hosaka,[2] Akihito Yoshinari,[1] Akiko Ozaki,[1]
Toshiyuki Takeuchi,[2] and Kazuo Shinozuka*[1]
[1]*Department of Chemistry and Chemical Biology, Graduate School of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu 376-8515*
[2]*Department of Molecular Medicine Institute for Molecular and Cellular Regulation, 3-39-15 Showa-machi, Maebashi 371-8512*

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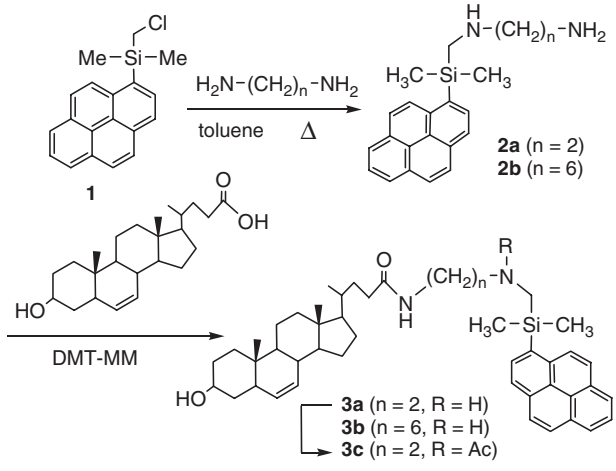
Novel fluorescent cholesterol derivatives bearing dimethylsilylated fluorescent molecules were prepared. The derivatives exhibit markedly enhanced fluorescence compared to that of an analogous derivative bearing unmodified pyrene in living cells.

Visualizing biologically important molecules such as proteins, lipids, and nucleic acids, via fluorescent labeling is a convenient and widely used technique in biological study.[1] Pyrene and its derivatives have been used as versatile fluorescent labeling agents for such study because of their chemical stability, low toxicity, and long fluorescent lifetimes.[2] At the same time, pyrene and its derivatives have some unfavorable photochemical characteristics, such as low fluorescent quantum yield and relatively short absorption wavelength. Meanwhile, the introduction of trimethylsilyl groups to polycyclic aromatic compounds seems to be an effective technique to enhance the fluorescent quantum yield of the compounds, as it was reported by Kyushin et al.[3] Indeed, a series of studies carried by Mizuno et al.[4] showed that the introduction of a trimethylsilyl functionality to pyrene brings about enhancement of fluorescent quantum yield. At the same time, both absorption maxima and emission maxima of the silylated pyrene derivatives shift to longer wavelength compare to those of unmodified pyrene. These are assumed to be a result of Si-associated σ–π interaction.[3] Although the trimethylsilylation improves photochemical properties of pyrene as described above, the silyl group itself does not have the ability to be connected to other functional groups, such as –OH, $-NH_2$, and –COOH, commonly found in biologically important molecules. Recently we have reported the preparation of novel chloromethyldimethylsilylated pyrene **1** through the lithiation of bromopyrene followed by the silylation with chloromethyldimethylchlorosilane.[5] After several steps, the chlorine of compound **1** was converted to a phosphoramidite group, and the resulting reactive trivalent phosphorous derivative was utilized to functionalize oligoDNA through the formation of a phosphodiester linkage via the 5′-terminal hydroxy group.

To seek out further utility of the silylated pyrene molecule, we converted chloromethyldimethylsilylated pyrene to the corresponding primary amine-bearing derivative **2a** and **2b** and incorporated it into a biologically important molecule, cholesterol through the formation of an amide linkage. Although several cholesterol analogs bearing a fluorescent group, such as NBD,[6] BODIPY,[7] fluorescein-PEG[8] were reported previously, the biological behaviors of these analogs were somewhat different from natural cholesterol in living cells. On the other hand, dehydroergosterol,[9] a fluorescent analog of cholesterol reportedly behaves as natural cholesterol, exhibits only a faint fluorescent signal. Therefore, a novel fluorescent analog of cholesterol possessing both the nature of natural cholesterol and strong fluorescence would be highly desirable and could be used as a probe to trace the behavior of cholesterol in living cells. The novel cholesterol analog bearing a silylated pyrene moiety described here (**3a** and **3b**) exhibits more distinctive fluorescent signal compared to the corresponding derivative with an unmodified pyrene, as well as dehydroergosterol in living cells. Here, we would like to report the synthesis and properties of these fluorescently labeled cholesterol analogs.

Synthesis of the fluorescent cholesterol derivative bearing silylated pyrene **3** is summarized in Scheme 1. In brief, a mixture of chloromethyldimethylsilylpyrene, prepared by the reaction of bromopyrene and chloromethyldimethylchlorosilane, and an appropriate diaminoalkane in toluene was refluxed for 12 h. After washing the reaction mixture with water, the separated organic layer was evaporated, and the residue was purified by silica gel column chromatography to give compounds **2**. The primary amine-bearing silylated pyrene derivatives **2** were further reacted with β-hydroxy-Δ^5-cholenic acid in the presence of condensing agent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM),[10] in methanol at room temperature for 12 h. After the evaporation of the volatile material, the residue was purified by column chromatography to give **3a** and **3b** with 18 and 35%, yields respectively.[13]

UV–vis absorption spectra of compounds **3** were recorded in degassed methanol and those are depicted in Figure 1, along with unmodified pyrene. As it is shown in Figure 1, compounds **3** give



Scheme 1.

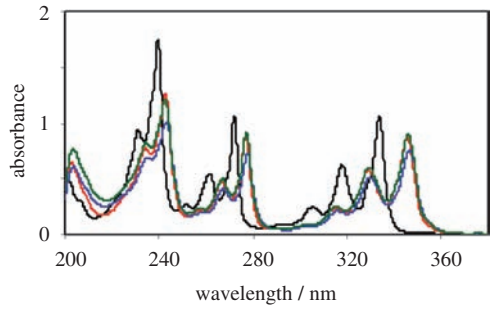


Figure 1. UV–vis absorption spectra of silylated pyrene conjugated cholesterol analogs **3**. (—) Pyrene, (—) **3a**, (—) **3b**, and (—) **3c** in MeOH (30 μM) at room temperature.

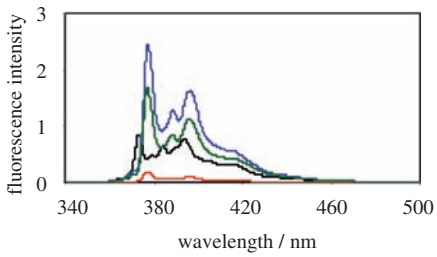


Figure 2. Fluorescent spectra of silylated pyrene conjugated cholesterol analogs **3**. (—) Pyrene, (—) **3a**, (—) **3b**, and (—) **3c** in MeOH (0.1 OD_{332}/mL at room temperature). Excitation wavelength is 332 nm.

almost identical spectra as unmodified pyrene; however, the absorption maxima of compounds **3** exhibited marked bathochromic shift (ca. 10 nm), particularly in the wavelength of 250–360 nm. Also, absorption coefficients were slightly increased. These could be due to the σ–π interaction between the silyl and pyrene moieties. Similar observations have been reported in the case of trimethylsilylated pyrene by Mizuno et al.[4]

Fluorescent spectra of the compounds **3** were also measured in the same conditions and those are shown in Figure 2. As it is shown in Figure 2, compound **3b** bearing a long alkyl linker (C6) between the silylated pyrenyl moiety and the cholenic acid moiety gives an enhanced fluorescent signal compared to the pyrene. The estimated fluorescence quantum yield of **3b** was 0.65.[13] Meanwhile, the fluorescent signal of **3a** bearing a short alkyl linker (C2) was dramatically decreased, and the fluorescent quantum yield of **3a** was only 0.03. Actually, it is even smaller than that of the unmodified plain pyrene ($\phi = 0.32$).[11] Since compound **3a** was prepared from strongly basic ethylenediamine, we speculated that the basicity of the amino function in **3a** is considerably higher than that of **3b** and, therefore, that in **3a** the photo-induced electron transfer (PET)[12] of the lone pair in the NH-function to the pyrene moiety in relatively polar solvent (methanol) might take place. Based on this assumption, we carried out an acetylation reaction of **3a** to decrease the basicity of the NH-group and obtained **3c** with 30% yield.[13] As expected, the fluorescent signal of **3c** was dramatically recovered by the acylation, and the estimated quantum yield of **3c** was 0.61.[13] Thus, the observed low fluorescence of **3a** in methanol could be due to the PET-effect mentioned above.

To estimate the utility of **3a** as a feasible fluorescent cholesterol probe, we monitored uptake and localization of **3a** in cultured insulin-secreting pancreatic cells (MIN-6 cells) using a fluorescent microscope. The monitoring was carried out along with dehydroergosterol (DHE), a frequently used standard fluorescent cholesterol analog, and the results are depicted in Figure 3. Similarly to DHE, the uptake of compound **3a** commenced within 1 to 2 h. The detection of compound **3a** is, however, much easier compared to DHE as it is clear from Figure 3. Meanwhile, the localization of **3** in cell compartments (organelles) was almost the same as that of DHE. It should be noted that cholesterol seems to be localized in hydrophobic lipid membranes in which the possible PET on **3a** would be effectively prohibited. Thus, the new fluorescent cholesterol analog behaves almost the same as that of standard fluorescent cholesterol analogs, yet it is much easier to be detected in fluorescent microscopic observation.

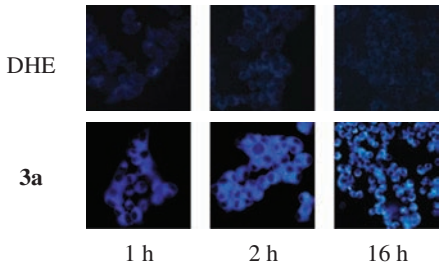


Figure 3. Fluorescent images of MIN-6 cells after incubation with compound **3a** and dihydroergosterol (DHE).

In conclusion, we have prepared novel fluorescent analogs of cholesterol bearing a silylated pyrene moiety. The basic behavior of the analogs seems to mimic natural cholesterol in living cells, yet the detectability of the analog is much higher than that of DHE, the standard fluorescent cholesterol analog, under observation using fluorescent microscopy. This would make the novel pyrene-bearing cholesterol as a feasible bioprobe for cholesterol study.

References and Notes

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13 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem.-lett/index.html.

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