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Design, Synthesis and Photochemical Properties of Caged Bile Acids

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This paper is dedicated to the memory of the late Professor Yukio Mitsui

Abstract—Photolabile derivatives of bile acids (**8**–**10** and **13**) were synthesized via silver (I) oxide promoted selective etherification of 3α-hydroxyls. Quantitative production of the parent cholic acid was detected from the photolytic mixture of 3-NB-CA (**8**) in Tris buffered solution. Interestingly, the unexpectedly stable nitroso-hemiacetal intermediate (**14**) was detected when the photolysis was conducted in methanol. The enzymatic analysis using 7α-HSDH showed **8** and **9** could serve as caged bile acids that might be able to regulate certain biological processes upon UV irradiation.

Bile acids are the final products of the metabolism of cholesterol, and are essential for the solubilization and transport of dietary lipids. Chenodeoxycholic acid and cholic acid are two major bile acids in humans, and are the primary bile acids formed in the liver.[1] Recently, chenodeoxycholic acids have been found to be ligands for an orphan nuclear receptor (NR), farnesoid X receptor (FXR), which suppresses transcription of the gene encoding cholesterol 7α-hydroxylase when bound to bile acids.[2] The signaling mediated by such NRs are essential for the regulation of endogenous hormones as well as the metabolitic elimination of xenobiotic chemicals.[3] In this report, we sought to synthesize caged bile acids to develop chemical tools which can give us more detailed information about the signaling mediated by steroid hormones.[4] Caged compounds are the artificial molecules in which their biological activities are masked by the covalent attachment of a photoremovable protecting group.[5] Therefore, the use of caged bile acids should enable us to get a rapid concentration jump of the parent bile acids inside live cells or tissues with high spatio-temporal resolution.

To construct caged bile acids, four functional groups can be masked: 3-OH, 7-OH, 12-OH and the side-chain carboxylate. Since chenodeoxycholic acid, which lacks 12-OH, and glyco- and tauro-chenodeoxycholic acids, which have glycine and taurine on the side-chain carboxylate, show substantial activity toward FXR receptors in vitro, 12-OH and the side-chain carboxylate should not be critical to the interaction with FXR receptors.[2] Furthermore, lithocholic acid which lacks both 7- and 12-OH has found to activate other orphan nuclear receptor, such as pregnane X receptor (PXR).[6] Therefore, we targeted 3α-OH moiety to be masked as a 2-nitrobenzyl ether.[7] Among the procedures known to introduce alkyls to a hydroxy functionality, the reaction with 2-nitrobenzyl bromide promoted by silver (I) oxide only gave the desired products in good to modest yields.[8] Thus, we made four derivatives of bile acids, 3-(2-nitrobenzyl)cholic acid (3-NB-CA, **8**), 3-(4,5-dimethoxy-2-nitrobenzyl)cholic acid (3-DMNB-CA, **9**), 3-(2-nitrobenzyl)-chenodeoxycholic acid (3-NB-CDCA, **10**) and 3-(2-nitrobenzyl)-glycochenodeoxycholic acid (3-NB-GCDCA, **13**) as shown in Scheme 1. All of the products were obtained as single regioisomers after chromatographic purification.[9] To confirm which hydroxyls were modified, the ^{1}H NMR spectrum of **5** was compared to that of methyl cholate (**2**). In methyl cholate, three methine protons, C12, C7 and C3, are seen at 3.98, 3.85 and 3.45 ppm, respectively.[10] In compound **5**, an upfield shift of the C3 proton (3.27 ppm)

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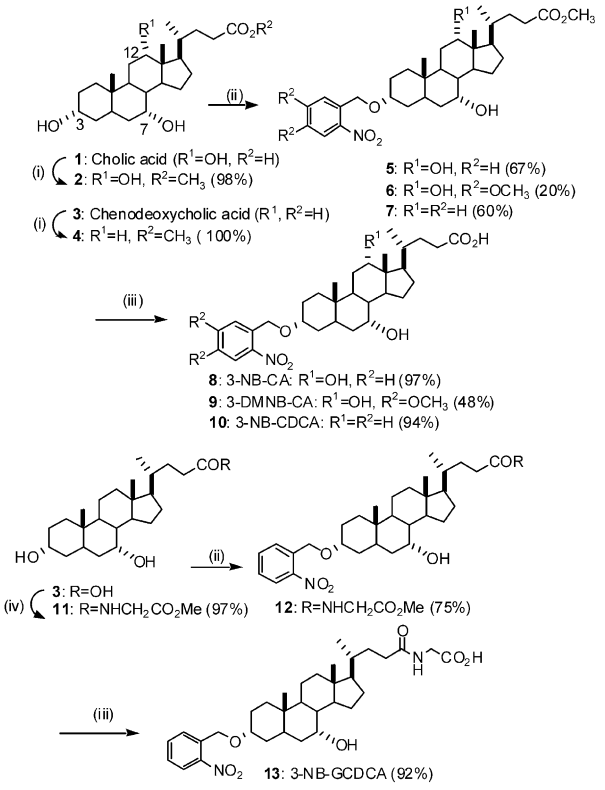


Scheme 1. Reagents and conditions: (i) $SOCl_2$, CH_3OH; (ii) 2-nitrobenzyl bromide (for **5**, **7** and **12**) or 4,5-dimethoxy-2-nitrobenzyl bromide (for **6**)/Ag_2O/CH_2Cl_2; (iii) NaOH/CH_3OH-THF; (iv) Methyl glycinate hydrochloride/EDCI/HOBt/DMF.

was observed, while the signals of the other protons were unchanged, suggesting the introduction of a 2-nitrobenzyl group to the C3 hydroxyl. This is the case for DMNB-CA (**9**). The upfield shift of the C3 proton (3.30 ppm) was observed in its methyl ester **6**. Since 3-OH is known to be the most reactive among the three hydroxyls in cholic acids,[11] selective derivatization of this group is possible without the need to protect the others. However, it was reported that the direct alkylation of cholic acid or methyl cholate could not be realized in reasonable yield,[12] the present method would offer a short and direct synthetic method for 3-OH modified cholic acid.

Next, the photolysis of **8** was examined in Tris-buffered solution (pH 8.5). Figure 1 showed the time course for the consumption of the starting material determined by HPLC. The quantitative production of cholic acid was detected concomitant with the consumption of **8** upon irradiation (350 nm). Photolysis of **8** was also performed in two different types of solvents, an aprotic organic solvent (chloroform) and a protic organic solvent (methanol). Almost the same efficiency of photolysis was observed for the three solvents tested, suggesting the photolytic consumption of the starting **8** might not be affected by the nature of the solvents. This should especially be useful for biological applications, because the nature of the microenvironment in which the caged compounds would be used should not necessarily affect the photochemical reactivity of **8**.

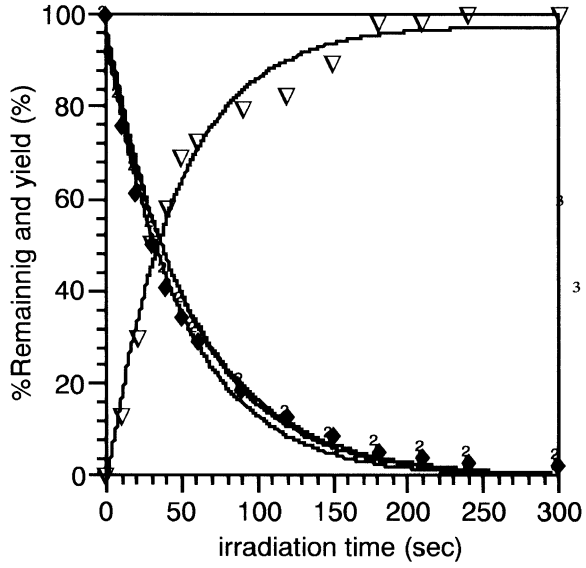
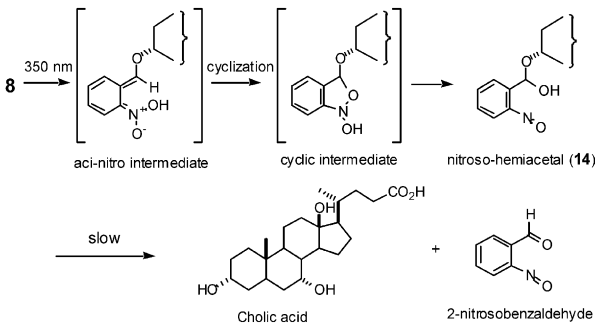


Figure 1. Time course for the photolytic consumption of 3-NB-CA (**8**) and production of cholic acid. Samples (100 μM) were irradiated with RPR 350 nm×16 lamps. Consumption of 8 in Tris buffer (pH 8.5, -△-), methanol (-◆-) and chloroform (-◇-) and production of cholic acid in tris buffer (-▽-) were determined from HPLC traces.

However, unlike the photolysis in aqueous solution, the production of cholic acid was not seen with the photolytic consumption of **8** in methanol. In this case, photochemical transformation of **8** was completed within 5 min and gave a single product, which is considered to be a nitroso-hemiacetal (**14**) (Scheme 2). Several hours after photolysis was complete, the slow production of cholic acid and 2-nitrosobenzaldehyde was observed by both HPLC and ^1H NMR analysis. Typical HPLC traces are shown in Figure 2.[13] Two peaks with retention times around 4.1 and 4.3 min in panel (b) are assigned as the both diastereomers of nitroso-hemiacetal **14**, which are completely disappeared 20 h after the photolysis of **8** was completed (panel d). Isolation and characterization of a *o*-nitrosobenzyl alcohol intermediate from 2-nitrobenzylamide has been reported in which they reported to measure the ^1H NMR spectrum of *N*-(α-hydroxy-2-nitrosobenzyl)-1-naphthamide at low temperature and assigned a doublet at δ 6.21 (1H, $J=8$ Hz) as an aromatic proton *ortho* to a nitroso substituent and a double doublet at δ 8.52 (1H, $J=8.3$, 4.8 Hz) as the benzylic proton.[14] In compound **14**, the similar absorptions are seen at δ 6.13 (1H, d, $J=8$ Hz) and 7.83 (1H, s) which should correspond to an aro-



Scheme 2. Proposed mechanism for the photolysis of **8** in methanol.

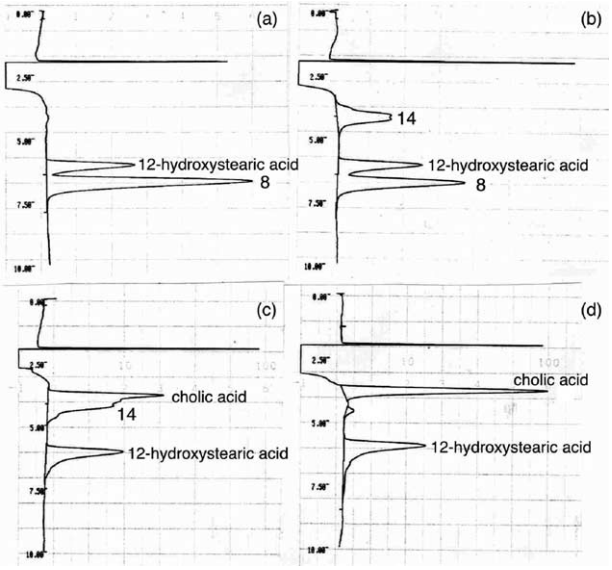


Figure 2. HPLC traces for the photolysis of **8** in methanol taken after 0 min (a), 1 min (b), and 5 min (c) irradiation. The trace (d) was taken from the sample after 20 h from the sample (c). 12-Hydroxystearic acid was used as the internal standard.

matic H3′ *ortho* to a nitroso and the benzylic proton, respectively, when the photolysis was performed in CD_3OD.

Interestingly, photolysis of other caged bile acids (**9**, **10** and **13**) produced the parent bile acids concomitant with photolysis even in methanol. Although the reason for this unusual phenomenon is unclear, the nitroso-hemiacetal **14** is stable enough to exist over several hours in methanol. One possible explanation is that the intermediate hemiacetal functionality might be protected from the nucleophilic attack of a solvent molecule. Molecular model analysis suggests that the 3′-hemiacetal moiety must be located inside the concave face of the cholic acid skeleton. It might be supported by the fact that the cholic acid derivatives are known to act as good host molecules that can trap small molecules inside their concave face. As far as I know, very few examples have been reported for the measurement of NMR spectrum of nitroso-alcohol intermediate in 2-nitrobenzyl-type photochemistry, even though the mechanism for the photolysis of compounds of this type has been extensively studied by absorption spectroscopy,[15] ESR spectroscopy[16] and time-resolved FT-IR spectroscopy.[17] The compound **8** would offer a good model for a detailed mechanistic study of this type photochemistry.

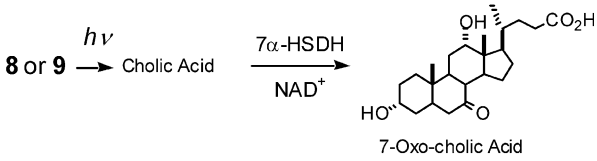
Selected physical and chemical data for the caged bile acids **8**–**10** and **13** are shown in Table 1. Although there are large difference in the photolytic quantum yield (Φ) and molar absorptivity (ε) between NB and DMNB-cholic acid (**8** vs **9**), the overall photosensitivity, expressed as the product of Φ and ε, turned out to be almost the same. All of the compounds showed satisfactory photosensitivity and appropriate solubility in aqueous solution for use in caging chemistry.

Table 1. Selected physical and chemical properties

Compd	λ_{max}[a] (ε[b])	ε_{350}	Φ_{350}[c]	$\Phi\varepsilon_{350}$	Solubility[d]
8	259 (4,500)	240	0.13	30	2×10^{-3}
9	342 (5,400)	5,240	0.006	30	1×10^{-3}
10	263 (6,300)	680	0.06	40	5×10^{-5}
13	258 (4,300)	530	0.12	64	4×10^{-3}

[a]Absorption maximum (nm) measured in methanol.
[b]Molar absorptivity (M^{-1} cm^{-1}).
[c]Quantum yields for the disappearance of the starting materials upon irradiation (350 nm). Samples (100 μM) in Tris buffer (pH 8.5) were photolysed with RPR 350 nm×4 lamps.
[d]The molar concentration (mol dm^{-3}) of the saturated solution in 100 mM Tris (pH 8.5)/30% PEG 6000/200 mM NaOAc.

Next, enzymatic tests were done to see whether the synthetic 3-OH modified bile acids can serve as caged bile acids. Thus, enzymatic activities of **8** and **9** using 7α-HSDH, which oxidizes cholic acid to 7-oxo-cholic acid,[18] were examined before and after 350-nm irradiation (Scheme 3). Figure 3 represents the recovery of the enzymatic activity of **8** and **9** as a function of irradiation time. Neither compounds showed residual activity against the enzyme before photolysis. On the other hand, almost quantitative recovery of the activities was observed with the progress of photolysis until about 40% of the starting material was converted into the product. During the initial 40-s irradiation periods, the relative enzymatic activities were reached to around 40% for both **8** and **9** (Fig. 3), indicating good agreement with the amount of the released cholic acid determined by the HPLC analysis shown in Figure 1. The fact that the maximum enzymatic activities could not be



Scheme 3. Enzymatic transformations of caged bile acids.

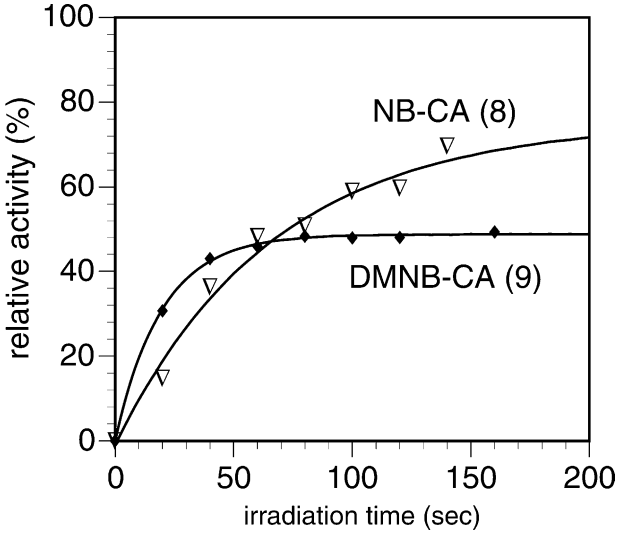


Figure 3. Recovery of the enzymatic activity of caged bile acids upon irradiation (350 nm).

obtained (70% recovery for **8** and 50% for **9**) after the photolysis was complete wouldn't cause any severe drawbacks when the compound is subjected to live cell applications, because a photolysis is typically performed in a small volume and the complete conversion into the product is not necessary. The present results demonstrate that the 3-OH modified bile acids act as caged bile acids even for the reaction in which the enzymatic transformation takes place at the functional group other than 3-OH.

In conclusion, four photolabile derivatives of bile acids were synthesized through the silver oxide-mediated selective introduction of a 2-nitrobenzyl group to 3α-OH. All of the derivatives showed satisfactory photoreactivity upon irradiation (350 nm) in aqueous solution. Photolysis of 3-NB-CA (**8**) in methanol produced an unprecedentedly stable nitroso-hemiacetal intermediate (**14**) whose NMR spectrum could be measured at room temperature. Enzymatic assays of **8** and **9** revealed that these compounds would be useful as chemical tools to investigate biological processes in which bile acids play key roles.

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