A Liposome-Forming Caged Compound: Synthesis and Photochemical Properties of a Caged L-Leucyl-L-leucine Methyl Ester with a Steroid Skeleton

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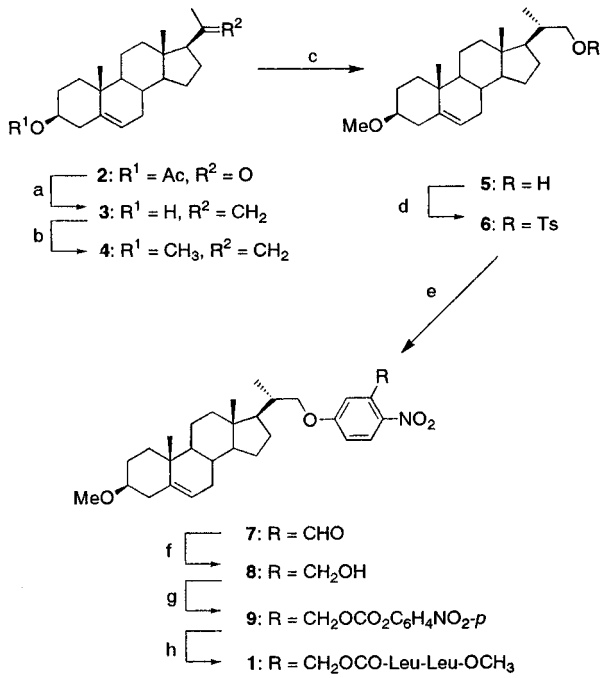
Caged compounds[1] are biologically inert molecules that can release bioactive compounds upon photolysis. They are useful for investigating the mechanisms of very fast biological reactions. Such compounds can also serve as key substrates that control the function of biological systems. Several kinds of caged compounds of biologically important molecules such as ATP, cAMP, peptides, nucleosides, and neurotransmitters have been synthesized and applied to biological systems. We have been studying the caged compounds of L-leucyl-L-leucine methyl ester,[2] which has been reported to induce apoptosis in NK cells and macrophages.[3]

One of the most important factors in the application of a caged compound to a biological system is the facility of its incorporation into a target cell. The introduction of a water-soluble substituent into the molecule is a promising design strategy for improving the absorption of caged compounds, and we recently reported the chemical and biological properties of a glucose-containing caged compound using this concept.[2c] Although this highly water-soluble molecule showed excellent properties as a caged compound, other strategies need to be developed to broaden the scope of biological application. In this paper, we report the synthesis and photochemical properties of a novel lipophilic caged L-leucyl-L-leucine methyl ester.

Macrophages are known to have the special function of phagocytosis, and some kinds of liposomes have been reported to be incorporated efficiently via endocytosis.[4] By using this property, we intended to develop useful caged compounds that could be expected to be easily incorporated by forming liposomes with phospholipids. From a clinical point of view, liposomes have been widely used as drug delivery systems, which suggests the possibility that a liposome-forming caged compound may be used as a photochemically controlled drug delivery system. Since phospholipids form stable liposomes with cholesterol, we designed a caged L-leucyl-L-leucine methyl ester with a steroid skeleton **1**. The caging group, a 2-nitrobenzyl group, is widely used as a photoremovable protecting group.[1] Our system reduces the biological effects of photobyproducts by allowing the released L-leucyl-L-leucine methyl ester to immediately diffuse into the aqueous phase to function as a bioactive compound, while 2-nitrosobenzaldehyde, the byproduct of photolysis, would remain in the liposomes and have little chemical or biological reactivity.

Scheme 1[a]



[a] Reagents and conditions: (a) Ph_3PCH_3Br, *t*-BuOK, THF, reflux, 12 h, and then K_2CO_3, MeOH, rt, 15 h, 95%; (b) MeI, KOH, DMSO, rt, 10 h, 88%; (c) 9-BBN, THF, reflux, 13 h, and then H_2O_2, NaOH (aq), rt, 16 h, 90%; (d) TsCl, pyridine, rt, 12 h, 96%; (e) 5-hydroxy-2-nitrobenzaldehyde, K_2CO_3, DMF, 110 °C, 15 h, 77%; (f) $NaBH_4$, EtOH, rt, 20 min, 95%; (g) 4-nitrophenyl chloroformate, DMAP, $CHCl_3$, rt, 30 h, 61%; (h) Leu-Leu-OMe·TFA, DMAP, $CHCl_3$, rt, 4 d, 58%.

The synthesis of the target molecule **1** is shown in Scheme 1.[5] The structure of the target molecule **1** was confirmed by its ^1H and ^{13}C NMR spectra, and a solution of **1** in methanol exhibited an absorption maximum at 309 nm (ϵ 12 400) in the UV−vis spectrum.[6]

Photolysis of the caged L-leucyl-L-leucine methyl ester **1** in methanol was carried out with a Rayonet photochemical reactor (RPR 3500 Å × 4). The time-dependent change in the UV−vis absorption spectrum of the caged L-leucyl-L-leucine methyl ester **1** during 40 min of irradiation is depicted in Figure 1. The intensity of absorption around 310 nm due to **1** decreased with a concomitant increase in absorption around 350 nm, probably caused by a 2-nitrosobenzaldehyde derivative, the photolytic side product.[1] The UV−vis spectrum shown in Figure 1 has an isosbestic point, indicating that only a desirable photocleavage occurred under 40 min of irradiation.

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(5) For the details, see the Supporting Information.

(6) **1**: white crystals; mp 79.0−81.0 °C; ^1H NMR (270 MHz, CD_3OD) δ 0.78 (s, 3H), 1.02 (s, 3H), 1.16 (d, J = 6.4 Hz, 3H), 0.85−2.16 (m, 38H), 2.36 (m, 1H), 3.06 (m, 1H), 3.34 (s, 3H), 3.69 (s, 3H), 3.91 (m, 1H), 4.01 (m, 1H), 4.22 (t, J = 7.4 Hz, 1H), 4.46 (dd, J = 5.4, 9.4 Hz, 1H), 5.36 (d, J = 4.0 Hz, 1H), 5.49 (s, 2H), 6.99 (d, J = 9.2 Hz, 1H), 7.14 (s, 1H), 8.18 (d, J = 9.2 Hz, 1H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 12.4 (q), 17.9 (q), 19.9 (q), 21.8 (q), 22.1 (q), 22.2 (t), 23.4 (q), 23.5 (q), 25.4 (t), 25.9 (d × 2), 28.8 (t), 29.0 (t), 33.0 (t), 33.3 (d), 37.7 (d), 38.0 (s), 38.4 (t), 39.7 (t), 41.0 (t), 41.3 (t), 42.0 (t), 43.6 (s), 51.7 (d), 52.1 (d), 52.6 (q), 53. 8 (d), 54.7 (d), 55.9 (q), 57.9 (d), 64. 6 (t), 75.1 (t), 81.9 (d), 114.3 (d × 2), 122.7 (d), 128.9 (d), 138.2 (s), 140.9 (s), 141.8 (s), 157.8 (s), 165.5 (s), 174.5 (s), 175.3 (s); UV λ_{max} (methanol) 309 nm (ϵ 12 400).

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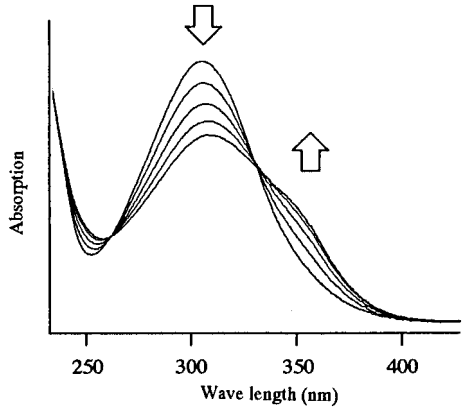

Figure 1. Time-dependent change in the UV−vis absorption spectra of a solution of the caged L-leucyl-L-leucine methyl ester **1** in methanol at 0, 10, 20, 30, and 40 min irradiation.

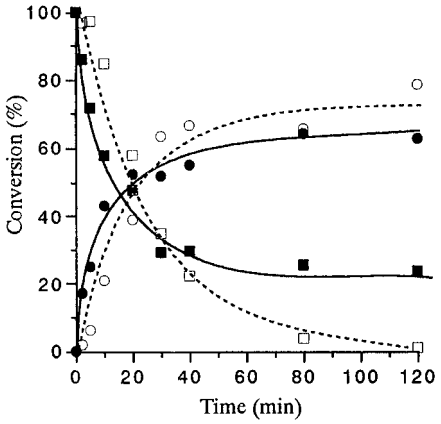

Figure 2. Decrease of the caged L-leucyl-L-leucine methyl ester **1** and the increase of a L-leucyl-L-leucine methyl ester by photolysis confirmed by HPLC analysis. □: Caged Leu-Leu-OMe in MeOH. ■: Caged Leu-Leu-OMe in liposomes. ○: released Leu-Leu-OMe in MeOH. ●: released Leu-Leu-OMe in liposomes.

To investigate the details of this photoinduced removal of the protecting group, the time-dependent change in the amount of the caged L-leucyl-L-leucine methyl ester **1** and the L-leucyl-L-leucine methyl ester released during photolysis was estimated by HPLC analysis, and the latter was monitored by the intensity of fluorescence derived from the adduct of fluorescamine[7] with a released L-leucyl-L-leucine methyl ester. Although the photochemical reaction became ineffective after prolonged irradiation, probably because of a filter effect of the byproducts, a nearly quantitative release of a L-leucyl-L-leucine methyl ester with a decrease in the caged compound was observed during the initial 40 min (Figure 2), which is consistent with the results obtained in the time-dependent UV−vis absorption spectrum during photolysis. The reaction rate for the photolysis of **1** is almost the same as that for a caged L-leucyl-L-leucine methyl ester with a glucose unit which we previously reported.[2c]

Liposomes containing the caged L-leucyl-L-leucine methyl ester, phosphatidyl choline, and dicetyl phosphate in phosphate-buffered saline (PBS) were prepared by the usual method.[5] This combination of phospholipids was selected for liposome formation because liposomes consisting of these phospholipids have been reported to be effectively incorporated into macrophage via phagocytosis.[4]

The caged compound **1** embedded in liposomes is fairly stable. According to an HPLC analysis of suspension-containing liposomes, more than 95% of the caged L-leucyl-L-leucine methyl ester molecules remained intact even after standing for 10 days in the dark. To estimate the amount of caged compound incorporated into liposomes, the suspension of liposomes in PBS was centrifuged and its supernatant was analyzed by HPLC. This analysis showed that 20% or less of the caged compound was not included in liposomes. These results indicate that the caged compound forms stable liposomes efficiently with phospholipids, as expected.

The decomposition of the caged L-leucyl-L-leucine methyl ester in liposomes as well as the release of L-leucyl-L-leucine methyl ester was estimated by a procedure similar to those in methanol, and the results are shown in Figure 2. Interestingly, the rate of its photocleavage was faster in liposomes than in methanol during the initial 30 min, although the intensity of UV light is considered to be reduced in the former case because of the scattering of light by phospholipids in liposomes. On the basis of HPLC analysis, the photoreaction proceeded cleanly in liposome as well as in methanol, indicating that caged compound **1** works well as an effective precursor of the bioactive molecule even in the presence of phospholipids. Application of the caged L-leucyl-L-leucine methyl ester to biological systems is currently under investigation.

In summary, we have synthesized a novel caged L-leucyl-L-leucine methyl ester with a steroid skeleton. This compound was shown to release a L-leucyl-L-leucine methyl ester by irradiation at 350 nm, and this was confirmed by time-dependent UV−vis spectroscopy and HPLC analysis. Clean generation of a L-leucyl-L-leucine methyl ester by photolysis of the caged compound **1** in liposomes indicates that the present molecule should be a useful probe for investigating the mechanism of the induction of apoptosis in NK cells and macrophages by a L-leucyl-L-leucine methyl ester. Furthermore, it is possible that this caged L-leucyl-L-leucine methyl ester will be useful for controlling the function of immunocytes and may also have clinical applications.

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Supporting Information Available: Synthetic procedures, physical and spectral data for **1** and **3**−**9**, preparation of liposomes, and procedure for the photolysis of **1** (6 pages).

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