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Journal of Photochemistry and Photobiology A: Chemistry 155 (2003) 57–62

Journal of Photochemistry and Photobiology A:Chemistry

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Dendritic caged compounds

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Received 17 September 2002; accepted 20 September 2002

Abstract

PAMAM and DAB dendrimers with 4–64 terminal caged compounds on their surface were synthesized. They released bioactive compound (LeuLeuOMe) upon irradiation at 350 nm. The amount of LeuLeuOMe released per molecule increased with the increase in the generation of dendrimers. Sugar ball-type caged compounds showed high solubility in PBS and cluster effect of sugars.

Keywords: Caged compounds; Photochemical reactions; Leucyl leucine methyl ester; Dendrimer; Cluster effect; Sugar ball; Cell recognition

1. Introduction

Photochemical reactions are quite unique because they do not require any chemical reagents in the system to obtain the target compounds. In addition, light has other benefits; for example, the wavelength can be easily selected mechanically and light can be focused on an extremely small area by microscopy. Therefore, photochemical reactions are easy to apply in a spatially controlled manner.

This property may make such reactions useful in the study of molecular and cellular biology. To identify the roles of bioactive molecules in biological systems, many chemical reagents are used to elucidate cellular responses. Although these pharmacological methods are widely applied in biology, there are some difficulties in determining the actual target molecule or signal transduction process because many factors may be involved simultaneously. Caged compounds[1] are one of the most promising chemical tools for elucidating the roles of bioactive molecules. They are bioactive molecules that have been modified by attaching a photolabile protecting group that inactivates their function. They can be introduced to the desired system in an inert form, and their activity can then be restored by irradiation. Many bioactive molecules such as hormones, neurotransmitters, peptides, proteins and nucleic acids (DNA and RNA) can be target molecules for caged compounds. Many photolabile protecting groups, such as 2-nitrobenzyl, phenacyl, desyl, coumarinylmethyl and 6-bromo-7-hydroxycoumarinylmethyl groups, have been reported to be useful as caging groups [2]. Moreover, some caged compounds are commercially available [3]. However, the development of methods for their efficient introduction to bioactive molecules as well as more effective caging groups is very important.

We have been studying the synthesis, photochemistry and biological activity of caged compounds of leucyl leucine methyl ester (LeuLeuOMe) [4]. LeuLeuOMe has been reported to induce apoptosis in immunological cells such as NK cells and macrophages [5]. Caged LeuLeuOMes were considered to be useful not only for investigating the signal transduction processes of apoptosis in the cell but also for developing photosensitive medicines for diseases caused by the overexpression of immunological cells such as rheumatoid arthritis. Among the several caged LeuLeuOMes we synthesized, only those modified with 2-nitrobenzyl-type caging groups (Fig. 1) were found to be non-cytotoxic [4]. Although **1** released LeuLeuOMe by irradiation, it was not soluble enough in PBS containing 1% DMSO to satisfy the requirements for an immunological assay (>500 μM). To increase its solubility in water, glucose was used as a hydrophilic substituent on 2-nitrobenzyl caging groups [4c,4f]. The resulting sugar-modified caged LeuLeuOMes **2** (Fig. 1) were highly soluble in PBS, and induced apoptosis in HL60 cells only after irradiation.

Over the past decade, much attention has been paid to dendrimers due to their unique and useful properties.[2] They have a unique molecular weight and a defined chemical structure that depend on the structure of the repeating units and the generation number. One of the most attractive examples of a dendritic compound is a “sugar ball” [7], which has many

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[1] For leading reviews on caged compounds, see [1].

[2] For leading reviews on dendrimers, see [6].

1010-6030/02/$ – see front matter
PII: S1010-6030(02)00369-6

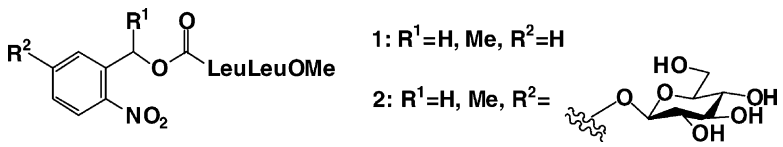

Fig. 1. Prototypes of caged LeuLeuOMes **1** and sugar-modified compound **2**.

sugar units in the peripheral region of the dendrimer. Since sugars play an important role in biological processes such as signal transduction and cell recognition, sugar balls may be useful in biological science. The ability of sugars to recognize cells increases dramatically upon clustering [8] and the density and intersugar distance in a sugar ball can be controlled by selecting the generation of dendrimers. This unique property can be effectively applied in a drug delivery system because they are expected to carry an appropriate drug to target region.

This concept led us to use dendrimers as an attractive scaffold [4e]. If sugar ball-type caged compounds can be obtained, they should offer several benefits: (1) they can transport many bioactive compounds at a time in an inactive form; (2) they may be able to recognize cells due to the cluster effect of sugars attached to the dendrimer; (3) another bioactive compound or fluorophore can be accommodated by host–guest interaction between such molecules and the interior of the dendrimer. This concept is schematically depicted in Fig. 2.

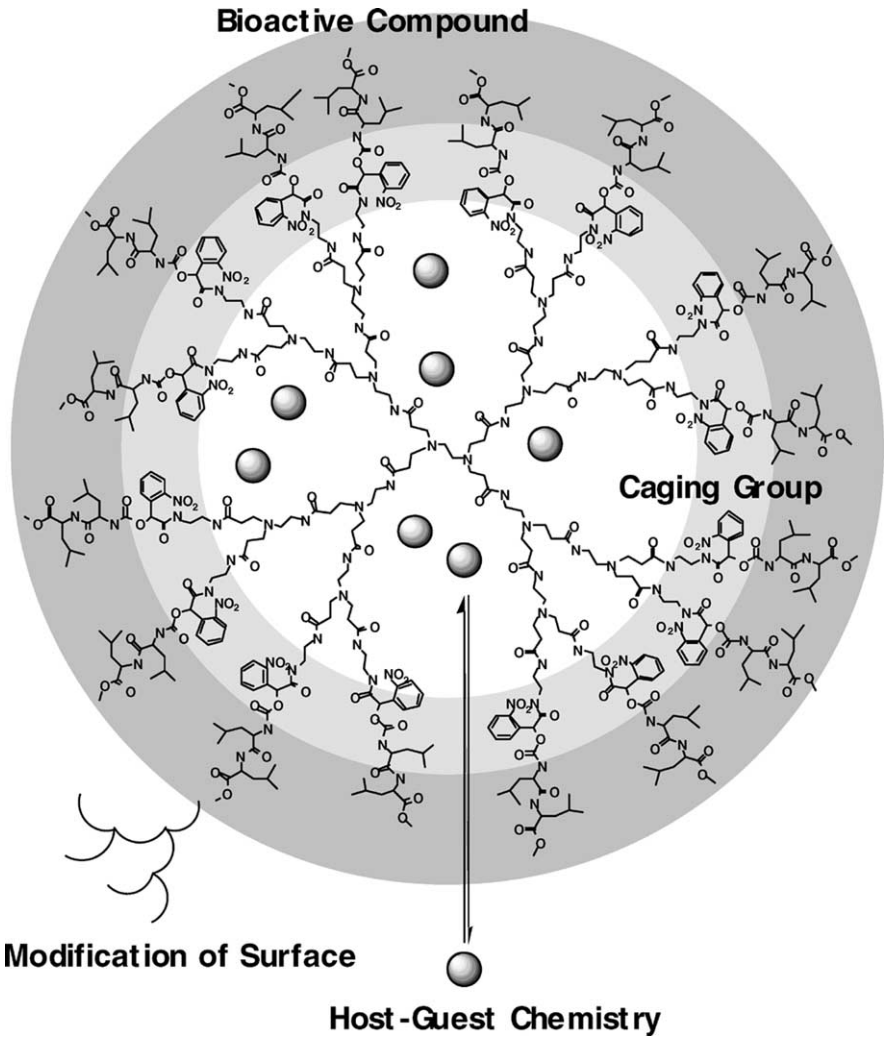

Fig. 2. Schematic representation of dendritic caged compounds and their expected functions.

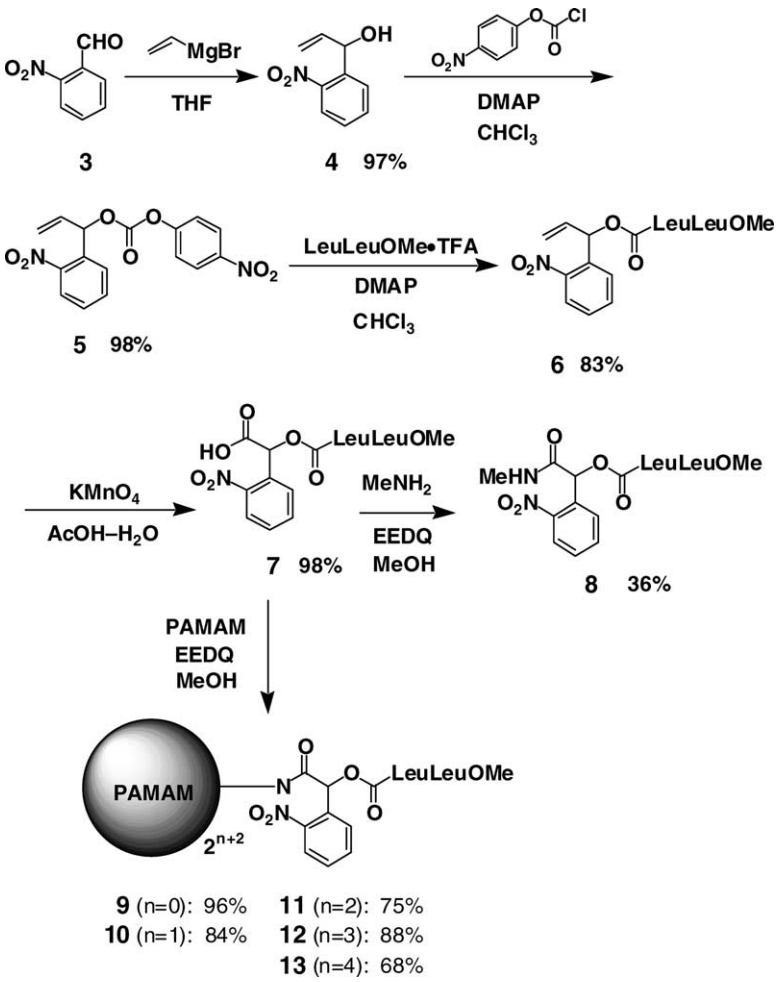

Fig. 3. Synthesis of dendritic caged compounds with a PAMAM dendrimer core unit.

2. Dendritic caged compounds

As a first step, we attempted to synthesize dendrimers with caged units but without sugar units at peripheral positions to establish a synthetic route for dendritic caged compounds. We used 2-nitrobenzaldehyde **3** as a starting material. The reaction of **3** with vinyl magnesium bromide gave benzylalcohol derivative **4**, which was converted to activated carbonate **5** by the reaction with 4-nitrophenyl chloroformate. A LeuLeuOMe was introduced to give the carbamate derivative **6**. The olefin moiety of **6** was converted to carboxylic acid as a linking part with a dendrimer. Commercially available PAMAM (G0–4) and DAB (G1–5) dendrimers with 4–64 terminal amino groups were used as core units. The condensation reactions were carried out in methanol with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as a condensation reagent (Fig. 3).

The structures of these compounds were determined by ^{1}H and ^{13}C NMR spectra. The molecular ion peak of PAMAM G0–2 caged LeuLeuOMe was observed by ESI mass spectrometric analysis. The observed molecular ion peaks of **9**, **10** and **11** were 2405.9 (calcd. for $[\mathbf{9} + Cl]^{-}$: 2405.1),

Table 1
UV-Vis spectral data for caged compounds, monomer and PAMAM dendrimers

	Compounds						
	7	**8**	**9**	**10**	**11**	**12**	**13**
Molecular weight	481	494	2,368	5,132	10,660	21,716	43,828
Number of nitrobenzyl groups	1	1	4	8	16	32	64
λ_{max} (nm)	257	256	257	258	259	258	259
$\varepsilon_{\lambda_{max}}$ ($M^{-1}\,cm^{-1}$)	4040	4500	17,900	33,600	53,400	129,000	249,000
ε_{350} ($M^{-1}\,cm^{-1}$)	279	279	1,320	2,330	3,780	9,570	18,000

2604.5 (calcd. for $[\mathbf{10} + 2\mathrm{Cl}]^{2-}$: 2604.3) and 3592.8 (calcd. for $[\mathbf{11} + \mathrm{Cl}]^{3-}$: 3592.8), respectively. Although we did not observe the molecular ion peak of PAMAM G3–4, a molecular ion peak of more than 10,000 (G2) could be successfully observed by the method we developed [4e]. In the UV-Vis spectra, the molar absorption coefficients of a series of dendritic caged compounds were proportional to the number of aromatic rings in the molecule (Table 1). This result supports the notion that all of the terminal amino groups are modified by the caged compounds. The absorption maxima of these compounds are almost identical in the same series. This indicates that the electronic environment of the caging groups is not influenced by the dendrimer structure, whereas they are in close proximity to the aromatic rings in larger dendrimers. Dendritic caged compounds with the DAB dendrimer were also synthesized in the same way.

The photochemical reactivity of these caged compounds in methanol was investigated with a Rayonet Photochemical Reactor using four RPR3500 Å lamps. The wavelength of 350 nm was selected by considering both the efficiency of the photoreaction and the lack of effect on cells. (Previously, we examined the viability of HL60 cells versus the duration of irradiation under the same conditions and found that more than 90% of the cells remained intact within 10 min of irradiation.) First, we used UV-Vis spectroscopy to observe the time-dependent change in monomer **7** upon irradiation. The absorption maxima of the starting material at ca. 260 nm decreased with a concomitant increase in the maxima at ca. 300 nm, probably due to a nitrosoacetophenone-type byproduct. During 50 min of irradiation, an isosbestic point was observed, indicating that the photoreaction proceeded cleanly during the first 50 min. This result should satisfy the requirement for the photoreaction to be clean, since the duration of irradiation in cell experiments is expected to be about 5 min.

A decrease in the starting material was estimated by ^{1}H NMR spectra. It is well-known that the 2-nitrobenzyl protecting group decomposes to afford a 2-nitrosobenzoyl derivative (see footnote 1). A decrease in the integration

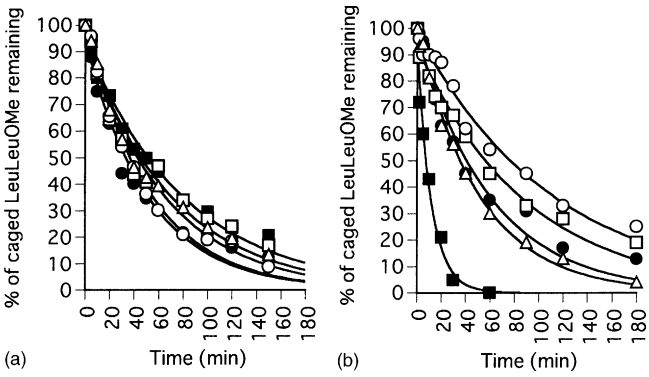

Fig. 4. Time-dependent decrease in caged compounds. (a) Dendritic caged compounds with a PAMAM-core: (■) G0, (●) G1, (□) G2, (○) G3, (△) G4. (b) Dendritic caged compounds with a DAB-core: (■) G1, (●) G2, (□) G3, (○) G4, (△) G5.

values of the benzyl proton in caged compounds was considered to correspond to the amount of starting material, and their time-dependent decrease is shown in Fig. 4. In the case of PAMAM dendrimer G0–4, all of the compounds decomposed in the same manner (Fig. 4a). Interestingly, although DAB dendrimer G2–5 decreased in the same manner, the DAB G1 dendrimer had the fastest reaction rate (Fig. 4b). The relationship between the reaction rate and the generation or structure of dendrimers should be clarified by a detailed investigation.

The release of LeuLeuOMe by irradiation was estimated using HPLC by the fluorescamine method, in which primary amines are converted to fluorescent derivatives. Fluorescent derivatives were not observed without irradiation, indicating that dendritic terminal amino units were completely modified by caged units. In these experiments, the concentration of caged units was identical for all samples. Fig. 5a shows the amount of LeuLeuOMe released in the molecule. Dendrimers with a lower generation release LeuLeuOMe more efficiently. Fig. 5b shows the amount of LeuLeuOMe released per starting molecule. In this respect, dendritic caged compounds with a higher generation can transport

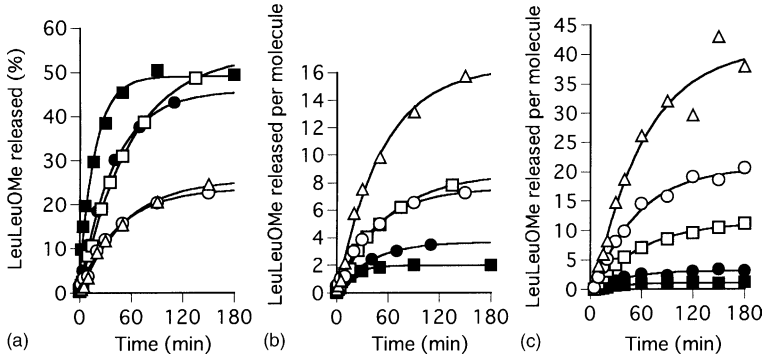

Fig. 5. Time-dependent release of LeuLeuOMe from dendritic caged compounds upon irradiation. (a) The amount of LeuLeuOMe released per terminal unit from dendritic caged compounds with a PAMAM dendrimer, core. (b) The amount of LeuLeuOMe released per starting molecule from dendritic caged compounds with a PAMAM dendrimer core: (■) G0, (●) G1, (□) G2, (○) G3, (△) G4. (c) Amount of LeuLeuOMe released per starting molecule from dendritic caged compounds with a DAB dendrimer core: (■) G1, (●) G2, (□) G3, (○) G4, (△) G5.

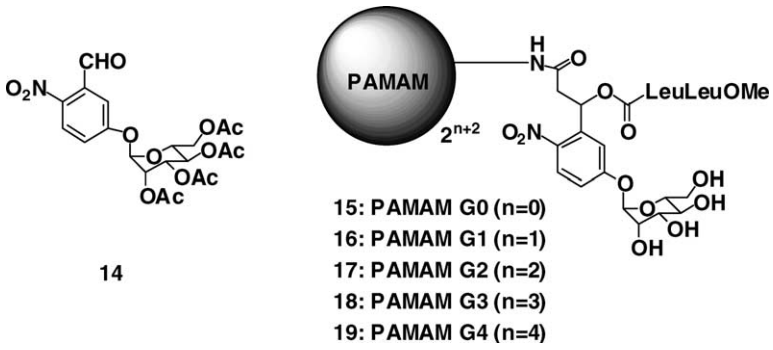

Fig. 6. Schematic representation of sugar ball-type caged compounds.

Table 2
UV-Vis spectral data for sugar ball-type caged compounds

	Compounds				
	15	**16**	**17**	**18**	**19**
Number of nitrobenzyl groups	4	8	16	32	64
λ_{max} (nm)	299	301	300	299	299
$\varepsilon_{\lambda_{max}}$ (M^{-1} cm^{-1})	23,700	41,200	82,500	214,000	370,000
Saturated concentration (mM)	382	33.2	18.0	2.22	0.806

more bioactive compounds at a time. As for dendritic caged compounds with a DAB-core unit, LeuLeuOMe is released far more efficiently than with PAMAM-core dendrimers (Fig. 5c). Since there was little difference in the rate of the decrease in the starting materials between PAMAM- and DAB-core dendritic caged compounds, this result seems to mean that the present detection method may be influenced by the structure of the dendritic scaffold. Host–guest complexation between the dendrimer and released LeuLeuOMe may be influenced by the structure of the dendrimer core because a PAMAM dendrimer with a larger inner space may incorporate more LeuLeuOMes than a DAB dendrimer.

3. Sugar ball-type caged compounds

Next, sugar units were added to our caged dendrimers to make sugar ball-type caged compounds. Since one of the target cells of LeuLeuOMe is a macrophage that has a mannose receptor on the cell surface [9], we designed mannose-modified dendritic caged LeuLeuOMes. The target materials were synthesized by the modified method shown in Fig. 3. In this case, a mannosylated compound **14** was used instead of **3** and the target materials **15**–**19** are depicted schematically (Fig. 6).

UV-Vis spectral data are shown in Table 2 together with solubility in PBS. These compounds were far more soluble in PBS than dendritic caged compounds without a sugar moiety. Sugar ball-type caged compounds with a higher generation were less soluble in PBS. This is probably due to the combined influence of the hydrophilic nature of sugar and the hydrophobic character of the dendrimer skeleton, the latter of which is dominant for the whole molecule. Fortunately, even the dendritic caged compound with the lowest solubility in PBS (G4) soluble enough for a bioassay.

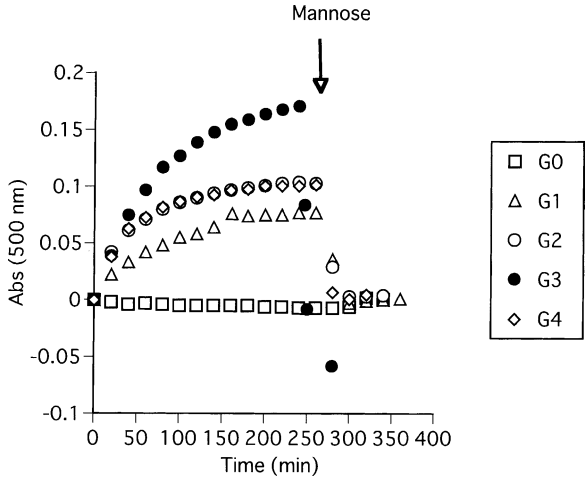

Fig. 7. UV-Vis monitoring of the cross-linking reaction between dendritic caged compounds and Con A.

The photoreaction of sugar ball-type caged compounds proceeded in almost the same way as described above for dendritic caged compounds to give LeuLeuOMe. Since the cluster effect is one of the most attractive properties of sugar balls, we performed a lectin binding experiment with Concanavalin A (ConA), which is a well-known mannose-binding protein with high specificity. The time-dependent change in turbidity due to cross-linking

between ConA and sugar ball-type caged compounds was monitored by absorbance at 500 nm (Fig. 7). We kept the concentration of the sugar moiety constant throughout the experiment. Sugar ball-type caged compounds with a higher generation form aggregates more efficiently than those with a lower generation except for G4, implying the existence of a cluster effect in these compounds. For G4, either a negative cluster effect is present or a low molar concentration of sugar ball molecule may lower the cross-linking ability in this case. The solution became clear when a solution of mannose in PBS was added, indicating that this cross-linking reaction was specific to mannose.

4. Conclusion

In conclusion, we designed new dendritic caged compounds with PAMAM and DAB dendrimer core units and successfully synthesized these target materials. These compounds decompose to afford bioactive compounds upon irradiation at 350 nm. Dendrimers with a higher generation gave more biomolecules, indicating the possibility of mass transport. We also synthesized sugar ball-type caged compounds with mannose terminal units. These sugar-modified compounds were sufficiently soluble in PBS to use for cell experiments. A lectin binding experiment clearly showed a cluster effect of mannose, which may offer a new strategy for drug delivery. These results are attractive for application to a biological study. We are now trying to assay the efficiency of these compounds in living cells to investigate their abilities to induce apoptosis as caged compounds.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research (No 13740364 and 13680677) from the Ministry of Education, Science, and Culture, Japan. We thank all of the graduate and undergraduate students who contributed to this study for their hard work.

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