New Dendritic Caged Compounds: Synthesis, Mass Spectrometric Characterization, and Photochemical Properties of Dendrimers with α-Carboxy-2-nitrobenzyl Caged Compounds at Their Periphery

Soichiro Watanabe, Mika Sato, Shigeru Sakamoto,[†] Kentaro Yamaguchi,[†] and Michiko Iwamura*

Department of Biomolecular Science Faculty of Science, Toho University 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan Chemical Analysis Center, Chiba University 1-33 Yayoicho, Inage-ku, Chiba 263-8522, Japan

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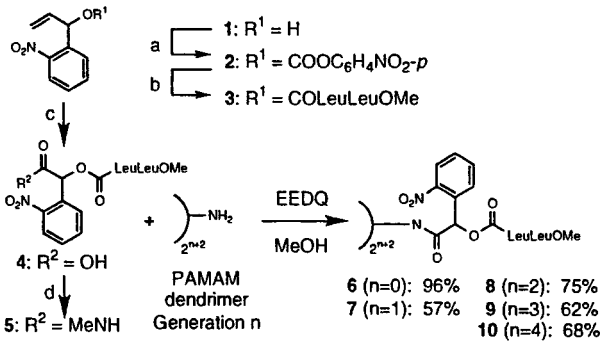
Caged compounds[1] are biologically inert molecules which can release bioactive compounds upon photolysis. In the course of our study on caged compounds for medicinal use, we designed a dendritic caged compound, which may be able to carry a large number of biomolecules without any loss during transport in an inactive form.

Dendrimers, which are hyperbranched artificial polymers with a monodisperse molecular weight and well-controlled shape, have received considerable attention over the past decade.[2] Although dendritic structures are used as the fundamental skeletons of newly developed functional materials, it is often difficult to characterize them by mass spectrometry. Efficient methods for mass spectrometric characterization have to be developed for further progress in dendrimer chemistry.

In this communication, we report the first synthesis, photochemical properties of dendritic caged compounds,[3] and a new efficient method for preparing an α-carboxy-2-nitrobenzyl caged compound[4] of a leucyl leucine methyl ester, which induces apoptosis in immunological cells,[5] as a model substrate. We also describe the mass spectrometric characterization of newly developed dendritic caged compounds with a high molecular weight and a hydrophobic surface.

An α-carboxy-2-nitrobenzyl caged leucyl leucine methyl ester **4** was synthesized by the method shown in Scheme 1.[6] The terminal olefin moiety of **3** was oxidized by reaction with $KMnO_4$ to give **4** as a diastereomeric mixture.[7] Compared with the previously reported method,[8,9] the present method is unique in that α-carboxy-2-nitrobenzyl caged compounds were obtained from a 1-(2-nitrophenyl)allyl alcohol with no chemically sensitive functional groups such as a carboxylic acid or an ester.

Scheme 1. Preparation of the α-Carboxy-2-nitrobenzyl Caged Leucyl Leucine Methyl Ester **4**, Methyl Amide **5**, and Dendritic Caged Compounds **6**−**10**[a]



[a] a: *p*-nitrophenyl chloroformate, DMAP, $CHCl_3$, 1 d, 98%; b: LeuLeuOMe·TFA, DMAP, $CHCl_3$, 2.5 d, 83%; c: $KMnO_4$, $CH_3COOH-H_2O$, 16 h, 98%; d: $MeNH_2$, EEDQ, MeOH, 15 h, 36%.

Methyl amide **5** was prepared as a model compound. Commercially available PAMAM dendrimers were used as a dendritic core. PAMAM dendrimers of generation 0−4, which have 4−64 primary amino groups at their periphery, were treated with **4** in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in methanol (Scheme 1). The yields of the dendritic caged compounds were high enough for use in further chemical and biological applications (57−96%). The structures of dendritic molecules **6**−**10** were confirmed by ^{1}H NMR, ^{13}C NMR, UV/vis, and ESI-MS[10] (vide infra).[11]

These dendritic caged compounds have a hydrophobically functionalized surface and a high molecular weight compared with those in the dendritic core (e.g., PAMAM dendrimer generation 2: MW 3256 vs **8**: MW 10660), which made it difficult to generate ionized species suitable for ESI-MS detection. In the present case, both positive and negative molecular ion peaks of **6**−**8** were observed (Figure 1).[12] As for **9** and **10** (molecular weights of 21716 and 43823, respectively), molecular ion peaks could not be observed.

None of the dendritic caged compounds obtained here gave significant fluorescent adducts after reaction with fluorescamine, suggesting the absence of primary amino groups which were present on the surface of PAMAM dendrimers. The caged compounds obtained above were used for the following experiments as mixtures of diastereomers, since we did not expect the diastereomers to have significantly different photochemical properties.

The molar extinction coefficients of dendrimers **6**−**10** as well as those of monomers **4** and **5** at both λ_{max} and 350 nm are almost

* To whom correspondence should be addressed. Telephone/Fax: +81-47-472-7562. E-mail: michiko@biomol.sci.toho-u.ac.jp.

[†] Chiba University.

(1) For leading reviews on caged compounds, see: (a) Kaplan, J. H. *Annu. Rev. Physiol.* **1990**, *52*, 897. (b) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755. (c) *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley & Sons: 1993; Vol. 2.

(2) For leading reviews on dendrimers, see: (a) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Molecules: Concepts, Syntheses, Perspectives*; VCH: Weinheim, 1996. (b) Fischer, M.; Vögtle, F. *Angew. Chem., Int., Ed.* **1999**, *38*, 884−905. (c) Bosman, A, M.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665−1688.

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(5) (a) Sakauchi, H.; Furuta, T.; Kobayashi, Y.; Iwamura, M. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 211−215. (b) Watanabe, S.; Iwamura, M. *J. Org. Chem.* **1997**, *62*, 8616−8617.

(6) The allyl alcohol **1** was prepared as reported, see: Marriot, G.; Ottl, J. *Methods Enzymol.* **1998**, *291*, 155−175.

(7) α-Carboxy-2-nitrobenzyl caged leucyl leucine methyl ester **4**: pale yellow crystals; mp: 82.0−84.0 °C; ^{1}H NMR (270 MHz, CD_3OD): δ 0.60−1.05 (m, 12H), 1.34−1.87 (m, 6H), 3.67 and 3.69 (s, 3H), 4.00−4.34 (m, 1H), 4.34−4.55 (m, 1H), 6.71 and 6.74 (s, 1H), 7.36−7.63 (m, 1H), 7.63−7.80 (m, 2H), 7.90−8.10 (m, 1H), 8.30 (br s, 1H); UV/vis (MeOH): 257 nm (ϵ 4040). Anal. Calcd for $C_{22}H_{31}N_3O_9$: C, 54.88; H, 6.49; N, 8.73. Found: C, 55.17; H, 6.73; N, 8.33%.

(8) Caged compounds derived from α-carboxy-2-nitrobenzylamine, see: (a) Cohen, B. E.; Stoddard, B. L.; Koshland, Jr., D. E. *Biochemistry* **1997**, *36*, 9035−9044. (b) England, P. M.; Lester, H. A.; Davidson, N.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11025−11030 and references therein.

(9) Caged compounds derived from α-carboxy-2-nitrobenzylbromide, see: (a) Curley, K.; Lawrence, D. S. *J. Am. Chem. Soc.* **1998**, *120*, 8573−8574. (b) Niu, L.; Gee, K. R.; Schaper, K.; Hess, G. P. *Biochemistry* **1996**, *35*, 2030−2036 and references therein.

(10) Although molecular ion peaks of dendritic caged compounds **6** and **7** could be observed by MALDI-TOF MS, those of **8**−**10** were not observed in this method.

(11) See Supporting Information.

(12) Detection of negative molecular ion peaks of dendritic caged compounds were carried out as a method described in the previous report, see: Cheng, X.; Gao, Q.; Smith, R. D. *J. Org. Chem.* **1996**, *61*, 2204−2206.

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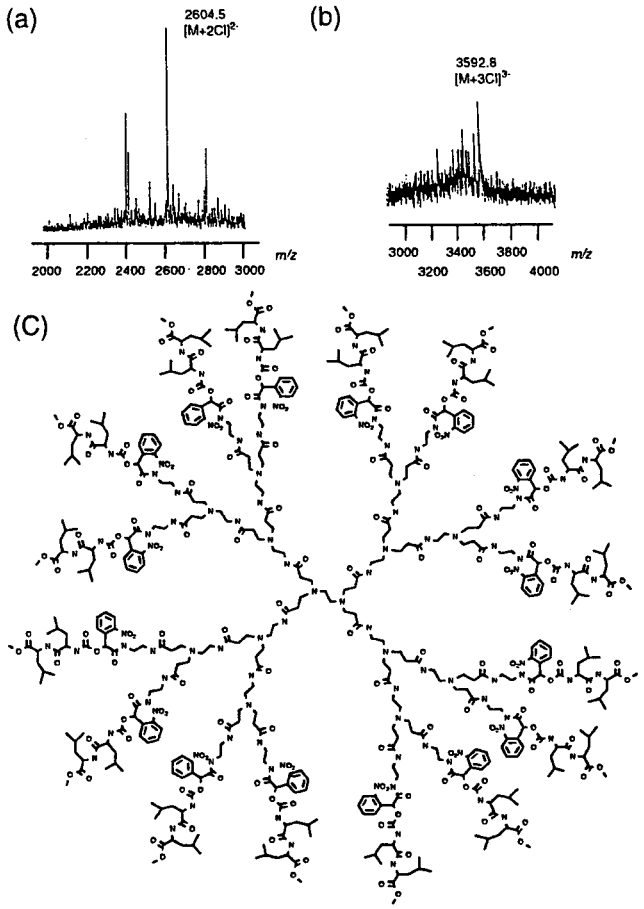

Figure 1. (a) ESI-MS spectra of **7**, (b) ESI-MS spectra of **8**, (c) chemical structure of **8**.

Table 1. Absorption Maxima and Molar Extinction Coefficients of **4**−**10** in Methanol

	λ_{max} (nm)	$\epsilon_{\lambda max}$ (M^{-1} cm^{-1})	ϵ_{350} (M^{-1} cm^{-1})
4	257	4040	279
5	256	4650	246
6	257	17900	1320
7	258	33600	2330
8	259	53400	3780
9	258	127000	8480
10	259	262000	16900

proportional to the number of aromatic rings per molecule (Table 1), indicating complete modification of all of the amino groups. The absorption maxima show only a very slight red-shift with increasing generation, implying that the electronic environment of the nitrobenzene moiety in these compounds is scarcely influenced by the dendritic structure.

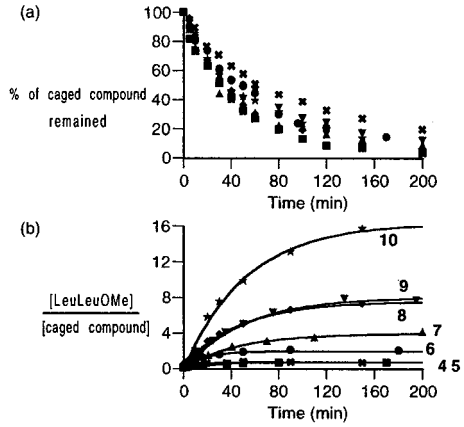

Figure 2. Time-dependent decrease in caged compounds (a) and release of LeuLeuOMe (b). (■) **4**; (×) **5**; (●) **6**; (▲) **7**; (▼) **8**; (◆) **9**; (★)**10**.

The progress of the photolytic reactions of caged compounds **4**−**10** was estimated by irradiation of the compounds in methanol at 350 nm using a Rayonet Photochemical Reactor (RPR 3500 Å ×4).[11] The reaction profiles of caged compounds are shown in Figure 2. The following trends are noted: (1) the photoreaction proceeded smoothly, and about 50% of the caged compounds decomposed after 40 min irradiation in almost all cases, and (2) the molar ratio of LeuLeuOMe released to caged compound used increased with an increase in the generation of the dendritic core. The latter result indicates that dendritic caged compounds with higher generation have desirable properties for the mass transportation of drugs. The details of these photoreactions are now under investigation.

In this communication, we have reported a new synthetic strategy for preparing an α-carboxy-2-nitrobenzyl caged compound. We have also presented the efficient synthesis of a new type of polyfunctional molecule with a dendritic core and peripheral caged moieties. This dendritic caged compound with a molecular weight of more than 10 000 was successfully characterized by ESI-MS as both positive and negative molecular ion peaks. The molar ratio of LeuLeuOMe released after irradiation to caged compound used increased with an increase in the generation of the dendritic core, suggesting that these new compounds are promising candidates for new caged drugs.

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Supporting Information Available: Experimental procedures for the synthesis of dendritic caged compounds, ^{1}H and ^{13}C NMR spectra of **6**−**10**, ESI-MS spectra of **6**−**8**, time-dependent change of ^{1}H NMR spectra of **4**−**10** during photolysis, photoreaction profiles of caged compounds **4**−**10** (PDF). This material is available free of charge via Internet at http://www.pubs.acs.org.

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