# Stilbene-cored poly(glutamate) dendrimers

Chikayoshi Mitsuno [a], Atsuya Momotake [a], Yoshihiro Shinohara [b], Kayori Takahashi [c], Ritsuko Nagahata [c], Yoshinobu Nishimura [a], Tatsuo Arai [a,*]

[a] Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8571, Japan
[b] Research Facility Center for Science and Technology, University of Tsukuba, Japan
[c] National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba City, Ibaraki 305-8565, Japan

## ARTICLE INFO

## ABSTRACT

A new class of photoresponsive poly(glutamate) dendrimers based on a stilbene core unit was successfully synthesized. The core stilbene was used to probe the polarity inside the dendrimer based on changes in the absorption and fluorescence spectra under various solvent conditions. Upon acidification of a solution of one of the dendrimers, **wG1**, the absorption band decreased with red-shift and the fluorescence band decreased with blue-shift, indicating that the $-CO_2^-$ groups at dendrimer periphery were protonated, resulting in shrinking of the dendrimer via hydrophobic interactions, and the inside of the dendrimer became more hydrophobic than under neutral pH conditions. In addition, the core stilbene unit underwent photoisomerization to give the cis-isomer with concomitant structural change in the poly(glutamate) dendrimer.

## 1. Introduction

Although the *E–Z* photoisomerization of low-molecular-weight stilbene derivatives is well understood,[1–7] but the photochemistry of molecules with large molecular weights, such as dendrimers,[8–12] still remains a target of intensive study. In this area, the photoisomerization of dendrimers with photoresponsive cores—such as stilbene,[13–21] azobenzene,[22–33] diarylethene,[34–38] intramolecularly hydrogen-bonded chromophores,[39–41] and photolabile chromophores[42–44]—has been extensively investigated. We recently reported that stilbene dendrimers with benzyl ether-type dendrons exhibited a volume-conserving isomerization mechanism and a macromolecular effect (or generation effect) on energy transfer efficiency from the peripheral dendrons to the core stilbene in organic solvents[45–47] and aqueous solution.[48–50] In these cases, the core stilbene unit played an important role as a photo-trigger for large conformational changes (photoisomerization), and as a fluorescent probe for detecting the environment inside the dendrimer. The latter function is based on the high sensitivity to solvent polarity of the 3,3′,5,5′-tetrasubstituted stilbene moiety of which the excited singlet state has a charge transfer (CT) character.[51]

In this Letter, we report the photochemical properties of new stilbene-cored dendrimers with poly(glutamate) dendrons (Scheme 1).[52–55] The purpose of this work was to obtain photoresponsive dendrimers with changeable structures and high biological compatibility, including water solubility, chemical stability in biological buffers, and low toxicity. Strong interactions between the dendrons and the stilbene core—especially in higher generations—and a significant pH effect on the absorption and fluorescence spectra were observed; the dendrimers were shrunk by the interaction among the dendrons triggered by protonation of the peripheral –CO₂ groups. These water-soluble photoresponsive dendrimers may have a role as model compounds for photoreversible biomolecules.

## 2. Results and discussion

### 2.1. Synthesis of stilbene dendrimers with poly(glutamate) dendrons

In order to prepare dendrons based on an L-glutamic acid monomer, a synthesis developed by Mitchell, Twyman et al.[56] was employed. The dendrons were constructed via peptide bond formation using a convergent synthesis; that is, small dendrons were synthesized and then reacted with a core molecule to produce the next-generation dendrimers. Each generation of dendrons was coupled with *trans*-3,3′,5,5′-tetra(4-carboxyphenyl)methoxy-stilbene (core, Scheme 1), benefiting from the use of the same peptide bond formation reaction. Each dendrimer generation terminating with ethyl ester groups (**G1** or **G2**) was purified by flash column chromatography followed by HPLC. Hydrolysis of the ethyl ester groups was carried out using potassium hydroxide in a mixed solvent system, then in water, followed by dialysis to give the potassium salts (**wG1** and **wG2**) of the stilbene dendrimers in quantitative yield.

* Corresponding author.
E-mail address: arai@chem.tsukuba.ac.jp (T. Arai).
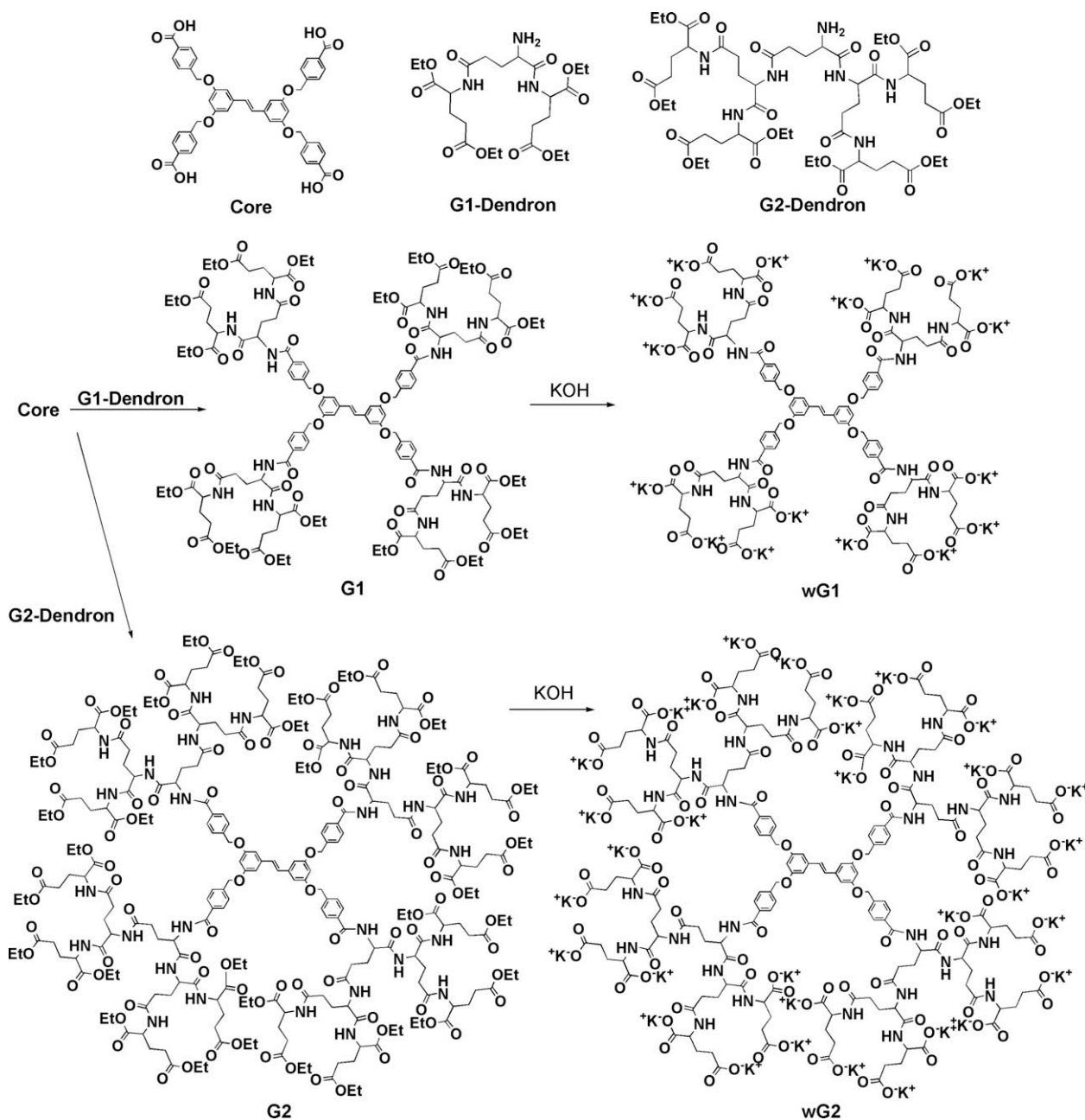
**Scheme 1.** Synthesis of stilbene dendrimers with poly(glutamate) dendrons.

## 2.2. Absorption spectra

Figure 1a and b show the absorption spectra of ethyl esters **G1** and **G2** in THF and the corresponding potassium salts **wG1** and **wG2** in $2 \times 10^{-3}$ M KOH aqueous solution (pH 10.5), respectively. All dendrimers exhibited an absorption band with $\lambda_{max}$ at 308–315 nm, attributed to the trans-stilbene core. Despite the fact that the structure of the core moiety was the same for all dendrimers, the absorption band appeared in different regions. In THF, the absorption maxima appeared at 308 nm and 313 nm for **G1** and **G2**, respectively. The red-shift of the absorption spectrum of **G2** compared to that of **G1** was quite characteristic, especially in organic solvents. In general, the absorption band of stilbene appears at longer wavelengths when the material is dissolved in polar solvents; for example, the absorption spectrum of 3,3′,5,5′-tetramethoxystilbene appears at different wavelengths with a strong

dependence on solvent polarity. In this experiment, however, the absorption spectra of the glutamate-based stilbene dendrimers appeared at different wavelengths depending on the dendrimer generation, even in the same solvent (in this case THF). This is probably because the interaction between the core and the highly polar poly(glutamate) moiety is stronger in **G2** than in **G1**, leading to the spectral red-shift of **G2** relative to **G1**. In aqueous KOH solution, the absorption bands corresponding to the stilbene moiety appeared at 308 nm and 315 nm, respectively, for **wG1** and for **wG2**, as shown in Figure 1b.

Keeping in view of the tendency of the absorption band to shift depending on solvent polarity, it seemed unusual that the absorption band of **wG1**, whose dendrons were smaller than those of **wG2**, appeared at a shorter wavelength in aqueous solution; that is, the stilbene core of **wG1** was exposed to water molecules because of poor coverage of the core by the dendrons, and therefore
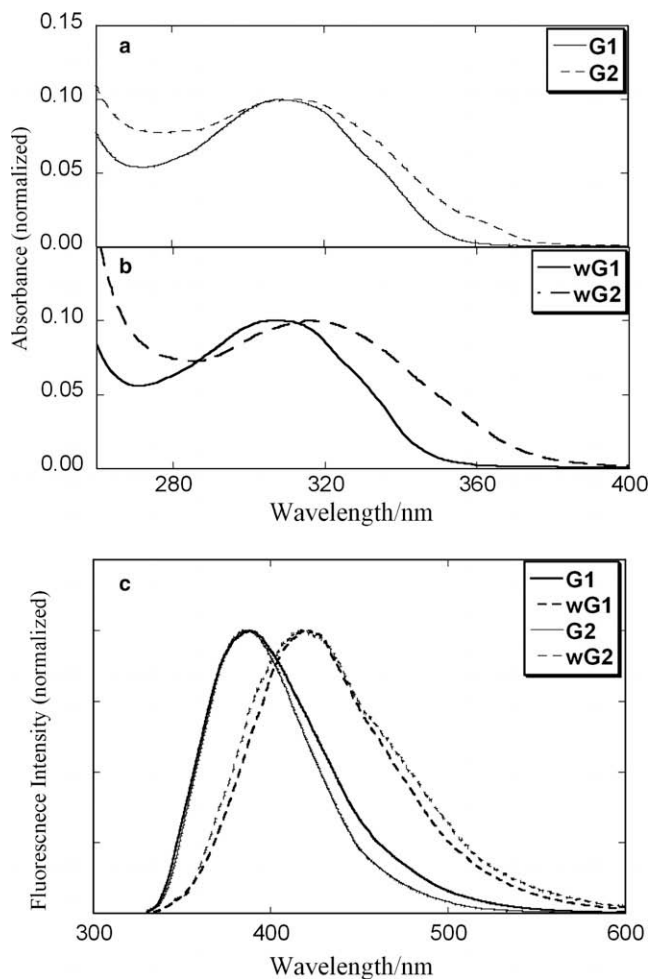
**Figure 1.** Absorption spectra (a and b) and fluorescence spectra (c) of **G1** and **G2** in THF, and **wG1** and **wG2** in $2 \times 10^{-3}$ M KOH aq.

aqueous solution, since their spectra were almost identical. The fluorescence excitation spectra of all dendrimers (data not shown) agreed well with the corresponding absorption spectra, indicating that their emissive electronic states formed similar structures through relaxation from Franck-Condon state, depending not on the dendrimer generation but on the solvent.

The fluorescence lifetime of **G1** in THF under an argon atmosphere was determined to be 14.2 ns with single exponential decay. The fluorescence decay curves did not fit a single exponential function, but rather a bi-exponential function, giving 11.6 and 2.4 ns for **G2**, 14.1 and 3.4 ns for **wG1**, and 10.5 and 1.0 ns for **wG2**, respectively. These results indicate that the structure and environment were not uniform in **G2**, **wG1**, and **wG2**. The fluorescence quantum yields for **G1** (0.44) and **wG1** (0.34) were higher than for **G2** (0.17) and **wG2** (0.07), suggesting that the peripheral dendrons in higher generations may give more specialized environment due to the surrounding poly(glutamate) dendrons. In other words, poly(glutamate) dendrons can change the excited state of stilbene core, which is different from the experimental results that the generation scarcely affected the excited state properties such as fluorescence lifetime and fluorescence quantum yield in stilbene dendrimers with benzyl ether-type dendrons.[45,49]

### 2.4. pH effect

The absorption and fluorescence spectra of **wG1** and **wG2** varied under different pH conditions. Figure 2 shows changes in the absorption (**2a**) and fluorescence spectra (**2b**) of **wG1** in buffer at different pH values. Upon acidification, the absorption band decreased significantly and was slightly red-shifted, particularly when the pH changed from 6 to 2. The fluorescence spectra of **wG1** also changed upon acidification, but in a slightly different manner from the absorption spectra. The fluorescence intensity decreased with bathochromic shift under acidic conditions. These spectral changes depending on pH were expected to correspond
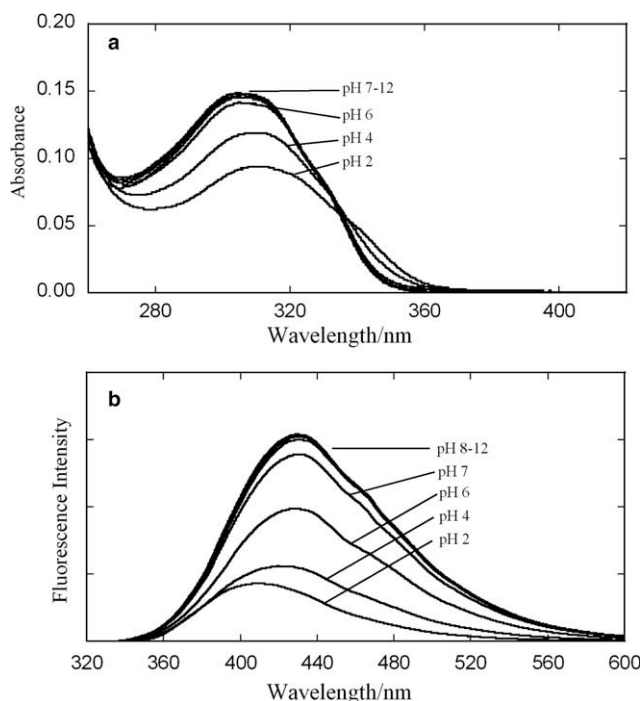
the absorption band of **wG1** should appear at longer wavelength than that of **wG2**. This result was not thought to be due to the hydrophobic environment around the core in **wG1**. One possible reason for the blue-shift observed for **wG1** relative to **wG2** is that the core may have been slightly distorted and unable to adopt a planar conformation due to intramolecular hydrophobic interactions. Such a phenomenon has been observed in water-soluble azobenzene dendrimers.[30]

### 2.3. Fluorescence properties

Unlike the absorption spectra, the fluorescence spectra of dendrimers showed no dependence on the dendrimer generation. However, they were affected by the solvent used, as shown in Figure 1c. Fluorescence maxima appeared at 389 nm and 386 nm, respectively, for **G1** and **G2** in THF and at 421 nm and 419 nm, respectively, for **wG1** and **wG2**. In previous work it was found that for a stilbene dendrimer with benzyl ether dendrons, the fluorescence maximum from the stilbene core was blue-shifted for increasing generations ($\lambda_{max}$: 424 nm, 411 nm and 389 nm for G1, G2 and G3, respectively).[48] In that case, greater generations of water-soluble dendrimers resulted in a decrease in interaction between the stilbene core and water due to their extended hydrophobic dendron groups, which diminished the stabilization of the excited state of the stilbene core by water. However, it seems the surrounding solvent molecules affected the singlet excited state of the stilbene core in **wG2** to the same extent as **wG1** in KOH



**Figure 2.** pH dependence of absorption (a) and fluorescence (b) spectra of **wG1** in buffered solution.

to the p$K_a$ value of the carboxylate moieties on the periphery of the dendrimer. Since the two –CO$_2$H groups of parent glutamic acid have p$K_a$ values of 2.19 and 4.25,[55] all the –CO$_2$H groups at the periphery of the dendrimer were expected to be in the form of –CO$_2^-$ anions at neutral pH, but mostly protonated at pH 2.0, with the whole dendrimer shrinking as a result of hydrophobic interactions. From pH 3 to pH 4, the –CO$_2$H groups were expected to be partially protonated, and probably moved to the hydrophobic interior of the dendrimer. The hypsochromic and the bathochromic shift of the absorbance and the fluorescence band, respectively, upon the acidification of **wG1** can be interpreted by the strength of the hydrophobic or hydrophilic environment depending on pH of the aqueous solution. In acidic solution, the dendrimer tends to shrink to exclude the water from the interior to make the environment around stilbene core hydrophobic. In this case, the molecular shape does not change much upon the excitation. On the other hands, in the high-pH environment, water may penetrate into the core to make highly polar environment and the molecular shape in the excited state changes more compared to that at low pH. Thus, the pH induces the change of dendrimer terminal to include or exclude water molecules to affect the core stilbene environment, which leads to the hypsochromic and the bathochromic shift of the absorbance and the fluorescence band. In addition, one can explain the decrease of the absorption intensity of **wG1** upon the
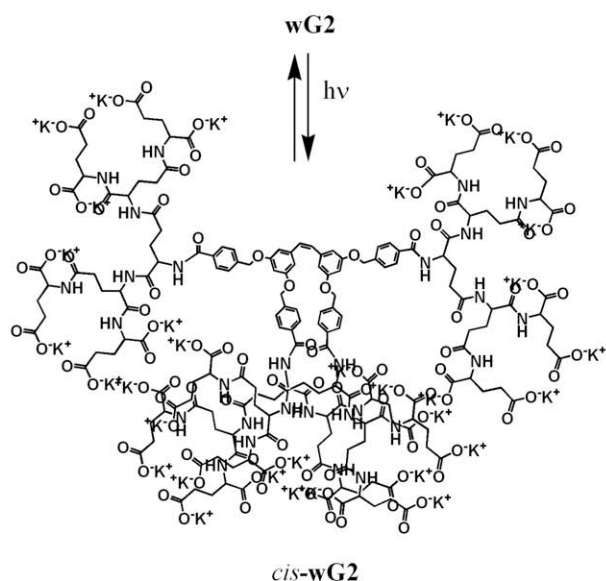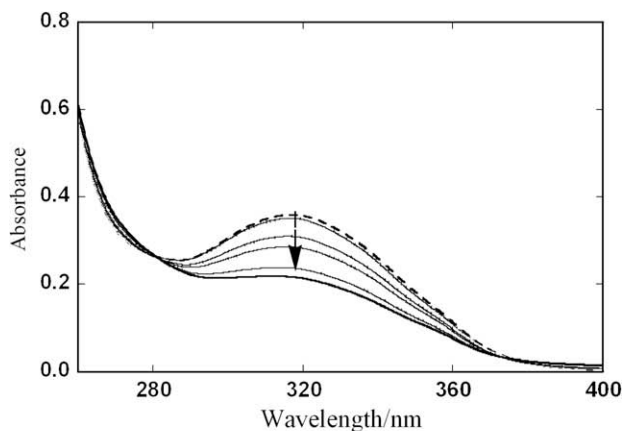
**Table 1**
Absorption maxima, fluorescence maxima, Stokes shifts, fluorescence quantum yields, fluorescence lifetimes, and quantum yields for photoisomerization of stilbene-cored poly(glutamate) dendrimers **G1** and **G2** in THF, and **wG1** and **wG2** in $2 \times 10^{-3}$ M KOH aq

|  | Abs $\lambda_{max}$/ nm | FL $\lambda_{max}$/ nm | $\Delta E_{ss}$/ cm$^{-1}$ | $\Phi_f$ | $\tau_s$/ns (%) | $\Phi_{tc}$ |
|---|---|---|---|---|---|---|
| **G1** | 308 | 389 | 6768 | 0.44 | 14.2(1.00) | 0.18 |
| **G2** | 313 | 386 | 6042 | 0.17 | 11.6(0.27), 2.4(0.73) | 0.16 |
| **wG1** | 306 | 421 | 8949 | 0.34 | 14.1(0.39), 3.4(0.61) | 0.25 |
| **wG2** | 315 | 417 | 7765 | 0.07 | 10.5(0.14), 1.0(0.86) | 0.06 |

acidification (Fig. 2a) by considering aggregation of dendrimers. In acidic pH, peripheral carboxyl groups take –CO$_2$H form which reduces the charge repulsion of dendrimers. Another reason may be distortion of stilbene moiety originated by shrinkage of dendrimer due to hydrophobic interaction at low pH.

It is noteworthy that **wG1** and **wG2** remained soluble throughout the entire pH range.

### 2.5. Photoisomerization

On irradiation with light at 330 nm under argon, the trans-isomer of **wG2** underwent isomerization around the double bond to give the corresponding cis-isomer. The absorption changes upon irradiation of **wG2** are shown in Figure 3. The isomer ratios at the photostationary state ([t]/[c])$_{pss}$ were determined to be 20:80, 26:74, 16:84, and 15:85 for **G1**, **G2**, **wG1**, and **wG2**, respectively. Thus, the cis-isomer composition in the photostationary state slightly increased in both **wG1** and **wG2** in aqueous solution. The quantum yield of the trans-to-cis isomerization was almost constant for **G1** and **G2** in THF—0.18 for **G1** and 0.16 for **G2**—while those for the water-soluble dendrimers depended on the generation and were determined as 0.25 and 0.06 for **wG1** and **wG2**, respectively. On direct irradiation, cis-isomer produced by photoisomerization generally undergoes either isomerization to the trans-isomer or cyclization reaction to give dihydrophenanthrene (DHP)-type compound in the excited singlet state with nearly equal probability. Because the photocyclization of 3,3′,5,5′-tetramethoxystilbene is quite efficient,[57] cyclization was expected to take place especially in the **G1** dendrimers. In the case of the higher generation dendrimers **G2** and **wG2**, however, photocyclization did not take place during irradiation because of the bulky poly(glutamate) dendrons. These results are consistent with the photochemical cyclization of other series of stilbene-cored dendrimers,[45,49] where the stilbene core in higher generation did not undergo photocyclization. It should be important for photochemical cyclization to take the planar conformation in the excited singlet state, and the bulky dendrons in higher generation probably disturb it (Table 1).

### 3. Conclusion

A new series of photoresponsive stilbene-cored dendrimers with poly(glutamate)dendrons was successfully synthesized up to the second generation. A generation effect was observed in the absorption spectra, probably due to the stronger interaction of the dendrons with the stilbene core in higher generations. Fluorescence spectra revealed that solvent effect was exhibited more strongly in the excited state dendrimers. Upon acidification of **wG1** solution, the absorption and fluorescence spectra changed significantly, probably due to protonation of the –CO$_2^-$ groups and shrinking of the dendrimer due to hydrophobic interactions.



**Figure 3.** Change in the absorption spectra of **wG2** upon irradiation with light at 320 nm, and the structure of the product, cis-**wG2**.

## Acknowledgment

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.004.

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