ORGANIC LETTERS

2005
Vol. 7, No. 4
525−528

Synthesis and Characterization of Polyester Dendrimers from Acetoacetate and Acrylate

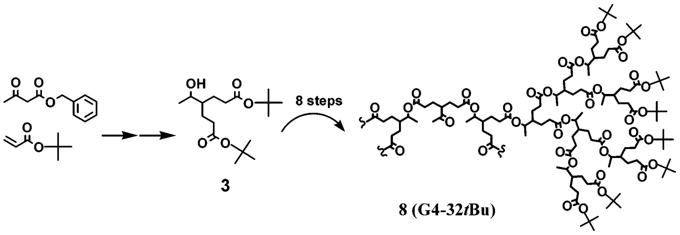
Yuki Hirayama, Taisuke Nakamura, Satoshi Uehara, Yohko Sakamoto,[†]
Kentaro Yamaguchi,[‡] Yoshihisa Sei,[‡] and Michiko Iwamura*

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

michiko@biomol.sci.toho-u.ac.jp

Received September 3, 2004 *(Revised Manuscript Received January 11, 2005)*

ABSTRACT



New aliphatic polyester-type dendrimers were synthesized using a new AB_2-type building block 3, prepared from benzyl acetoacetate and 2 equiv of *tert*-butyl acrylate by acetoacetic acid ester synthesis. The reiterative [deprotection by HCO_2H, then EDCI/DMAP coupling] sequence using divergent growth method gave [G1]-4*t*Bu–[G5]-64*t*Bu dendrimers. ^{13}C NMR relaxation time (T_1) measurements on the carboxy carbons show that the extended chain conformations are predominant in $CDCl_3$.

Dendrimers are globular macromolecules consisting of a core molecule and symmetrically arranged branches with a well-defined number of reactive end groups matching to each generation.[1,2] Among the dendritic branches such as polyamine, polyamide, or polyether, etc., polyester dendrimers attract considerable attention especially for therapeutic applications where biocompatibility and easy hydrolytic nature inside cells are important.[3] In addition, due to high solubility and miscibility in ordinary organic solvents and reactivity of end carboxy or hydroxy groups, they can be used as versatile tools in various fields.[4]

In connection with our continuous effort to develop caged compounds for application to drug delivery systems, we selected dendrimers suitable for a core to attach sugars and caged compounds that could recognize particular cells and release a drug upon irradiation of light.[5] In the course of our study, we were interested to design and synthesize novel polyester-type dendrimers that could be easy to modify the

---

[†] School of Pharmaceutical Sciences.

[‡] Chiba University. Present address: Laboratory of Analytical Chemistry Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Shido, Sanuki-city, Kagawa 769-2193.

(1) (a) Fréchet, J. M. J. *Science* **1994**, *263*, 1710−1715. (b) Tomalia, D. A. *Nature* **1994**, *372*, 617−618.

(2) For recent reviews on dendrimers in general, see: (a) *Dendrimers II*; Vögtle, F., Ed. *Top. Curr. Chem.* **2000**, 210. (b) *Dendrimers and Other Dendritic Polymers*; Fréchet, J. M. J., Tomalia D. A., Eds.; Wiley: New York, 2002. (c) Fréchet, J. M. J. *J. Polym. Sci. Part A: Chem.* **2003**, *41*, 3713−3725. (d) Pyun, J.; Zhou, X.-Z.; Drockenmuller, E.; Hawker, C. J. *J. Mater. Chem.* **2003**, *13*, 2653−2660.

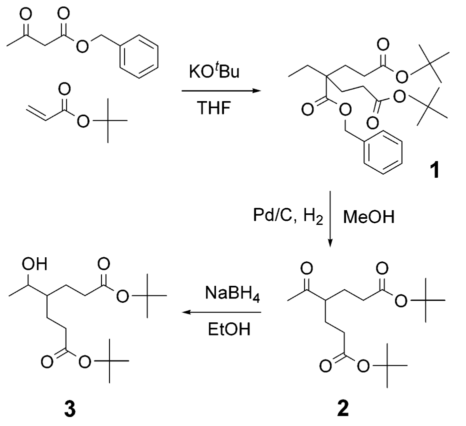
(3) (a) Ihre, H.; Padilla De Jesus, O. L.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2001**, *123*, 5908−5917. (b) Padilla De Jesus, O. L.; Ihre, H.; Gagne, L.; Fréchet, J. M. J.; Szoka, F. C., Jr. *Bioconjugate Chem.* **2002**, *13*, 453−461. (c) Carnahan, M.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2001**, *123*, 2905−2906. (d) Carnahan, M. A.; Middleton, C.; Kim, J.; Kim, T.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2002**, *124*, 5291−5293. (e) Morgen, M. T.; Carnahan, M. A.; Immoos, C. E.; Ribeiro, A. A.; Finkelstein, S.; Lee, S. J.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2003**, *125*, 15485−15489.

(4) For reviews on polyester dendrimers, see: Nummelin, S.; Skrifvars, M.; Rissanen, K. *Dendrimers II*; Vögtle, F., Ed. *Top. Curr. Chem.* **2000**, *210*, 1−67.

(5) (a) Watanabe, S.; Sato, M.; Sakamoto, S.; Yamaguchi, K.; Iwamura, M. *J. Am. Chem. Soc.* **2000**, *122*, 12588−12589. (b) Watanabe, S.; Iwamura, M. *J. Photochem. Photobiol. A: Chem.* **2003**, *155*, 57−62.

10.1021/ol0482184 CCC: $30.25

Scheme 1. Preparation of Building Block **3**

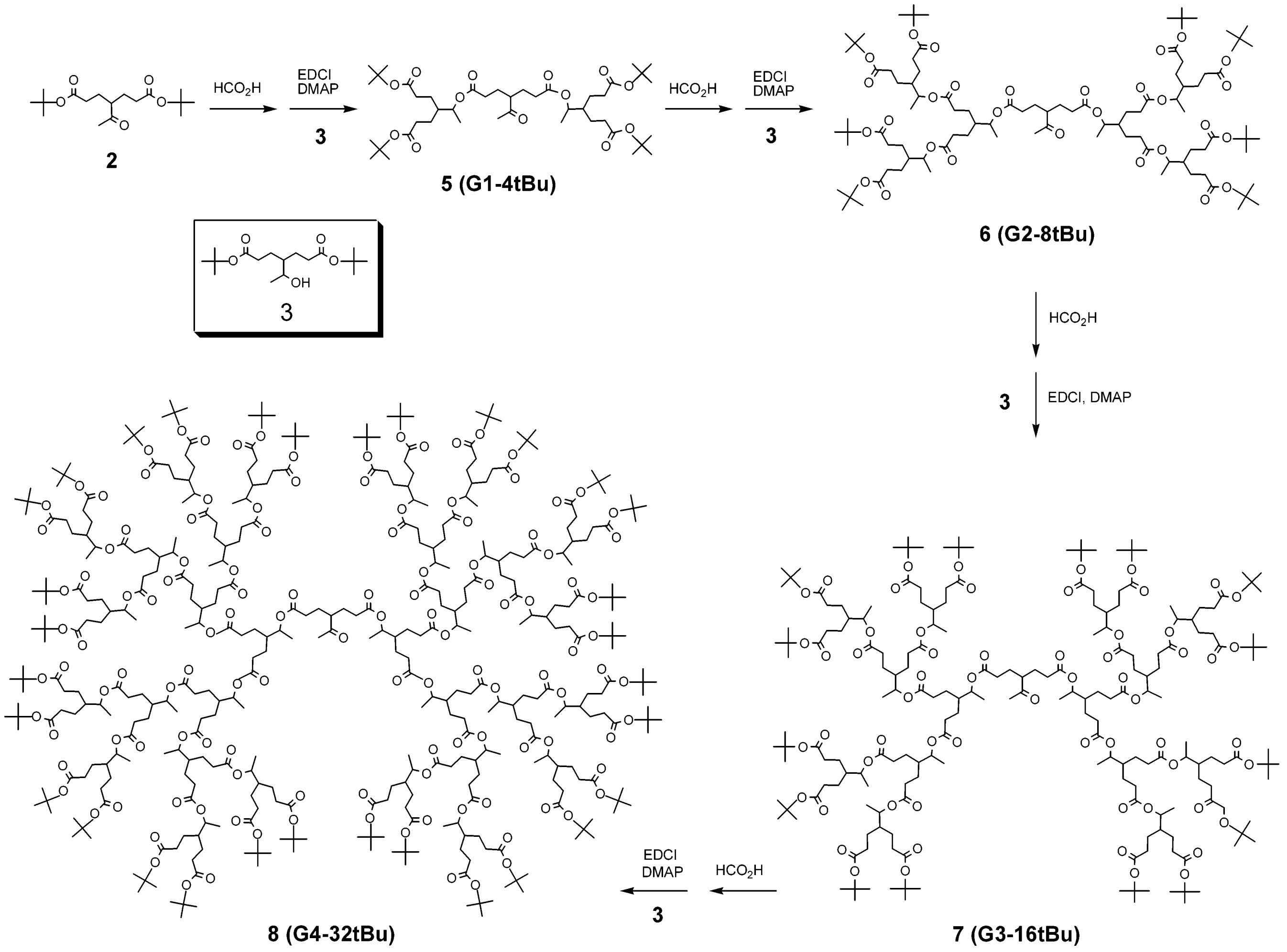


functional groups at the periphery and also to be hydrolyzed in cells by cytosolic esterase. We wish to present here a synthesis of new polyester-type dendrimers using acetoacetic ester synthesis to provide a new AB_2 type building block (Scheme 1).

The reaction of benzyl acetoacetate and 2 equiv of *tert*-butyl acrylate in THF in the presence of catalytic amount of potassium *tert*-butoxide gave the addition product **1** (96%). Hydrogenolysis of **1** in the presence of 5% Pd/C in methanol followed by decarboxylation at 80 °C afforded an almost quantitative amount of **2**. Reduction of **2** by $NaBH_4$ in ethanol gave the building block **3** in 95% yield. Although a combination of *tert*-butyl acetoacetate and benzyl acrylate gave similar yields of both addition and decarboxylation products, reduction by $NaBH_4$ resulted in intramolecular esterification to afford a lactone as a major product. On the other hand, bulky *tert*-butyl groups in **3** prevented cyclization.

Dendrimer synthesis was carried out by a divergent method using **2** as a core molecule. Synthesis of [G3]-16*t*Bu, **6**, is shown in Scheme 2. Since the use of trifluoroacetic acid sometimes cleaved the ester bond, formic acid was used for the deprotection of *tert*-butyl group. Compound **2** was deprotected by HCO_2H at room temperature followed by complete evaporation of the acid by azeotropic distillation with 1,2-dichloroethane under reduced pressure. Deprotected **2** was reacted with 2.05 equiv of **3** in the presence of EDCI (2.1 equiv) and DMAP (1.0 equiv) in anhydrous dichloromethane to give **4** ([G1]-4*t*Bu) in 90% yield after purification by silica gel chromatography. Compound **4** was subsequently deprotected by HCO_2H and then reacted with 4.1

Scheme 2. Divergent Synthesis of G1, G2, G3, and G4 Dendrimers



equiv of **3** in the presence of EDCI (5.2 equiv) and DMAP (1.8 equiv) to produce **5** ([G2]-8*t*Bu) in 82% yield. Compounds **6** ([G3]-16*t*Bu), **7** ([G4]-32*t*Bu), and **8** ([G5]-64*t*Bu) were synthesized by repetition of the [deprotection by HCO_2H then EDCI/DMAP coupling] sequence. **8** was obtained in 49% yield after a nine-step synthesis starting from **2** as shown in Table 1.

Table 1. Total Yields, ESI-MS, and Elemental Analysis Data for the Dendrimers

	yield (%) from **2**	MW calcd	MS from ESI-MS	elemental analysis[e]
4	88	799.2	798[a]	C, 64.49 (64.64); H, 9.37 (9.33)
5	81	1767.1	1768.6[a]	C, 64.38 (64.53); H, 9.30 (9.23)
6	69	3704.3	3704.9[b]	C, 64.38 (64.48); H, 9.06 (9.19)
7	60	7578.7	7571.1[c]	C, 64.00 (64.46); H, 9.10 (9.17)
8	49	15327.6	15342[d]	C, 64.43 (64.45); H, 9.19 (9.16)

[a] Observed as $[M + Na]^+$. [b] Observed as $[M + 2Na]^{2+}$. [c] Observed as $[M + 3Na]^{3+}$. [d] A weak ion-peak corresponding to $[M + 3Na]^{3+}$ was observed. [e] Calculated values in parentheses.

Although a combination of DCC and DPTS can be used instead of EDCI, the yield of the dendrimer was lower than that by using EDCI due to the difficulty in separating the byproduct DCU from the product.

Convergent synthesis or a combination of divergent and convergent synthesis were also attempted. For example, **7** was synthesized from [G2]-8COOH and OH-[G1] in 52%. However, because the use of $NaBH_4$ for reduction of a focal carbonyl group also reduced the ester bonds even under careful and mild conditions, divergent method was preferred.

All the *t*-Bu-capped dendrimers are colorless viscous liquids which are soluble in halogenated solvents, methanol, or ethyl acetate and insoluble in water. They were mostly purified by silica gel chromatography using a mixture of hexane and ethyl acetate (1:1 v/v) as an eluent. The elemental analysis showed good agreement with the calculated values, however, ESI-MS offered more reliable molecular weight data as shown in Table 1.[6] Whereas the molecular ion peaks of **4**−**7** were clearly observed, only a very weak molecular ion peak assigned to $[M + 3Na]^{3+}$ was found among a number of weak ion peaks for **8**. The ESI-MS spectra of **6** and **7** are shown in Figure 1. GPC measurements confirmed the low polydispersities of 1.08, 1.07, and 1.09 for **6**, **7**, and **8**, respectively.

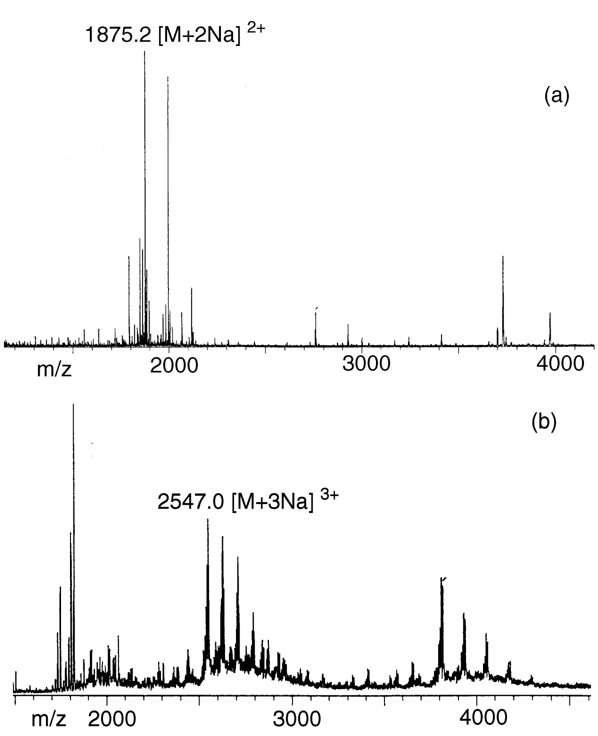


Figure 1. ESI-MS spectra of (a) **6** and (b) **7**.

^1H and ^{13}C NMR spectra are almost the same regardless of the generations only differing in the integration ratios of the signals. For example, while the integration ratio of a multiplet centered at 4.8 ppm (CH_3CH-), a singlet at1.36 ppm ($(CH_3)_3C-$) and a doublet at 1.11 ppm (CH_3CH-) is 1:12:3 for **5**, and the ratio for **8** is 1:9.27:3. Complete assignments of all the ^1H and ^{13}C signals of **6** and **7** were carried out using versatile NMR techniques such as TOCSY, HOHAHA, HMQC, etc.[7] In particular, for the assignment of the ^{13}C signals of the carboxys, "^{13}C gated decoupling without NOE technique" was very useful (Figure 2). From the intensity ratios, the three closely adjacent signals, 172.68, 172.77, and 172.83 ppm, were assigned for the outermost, the second from the outermost, and the rest of the carboxyl carbons, respectively.

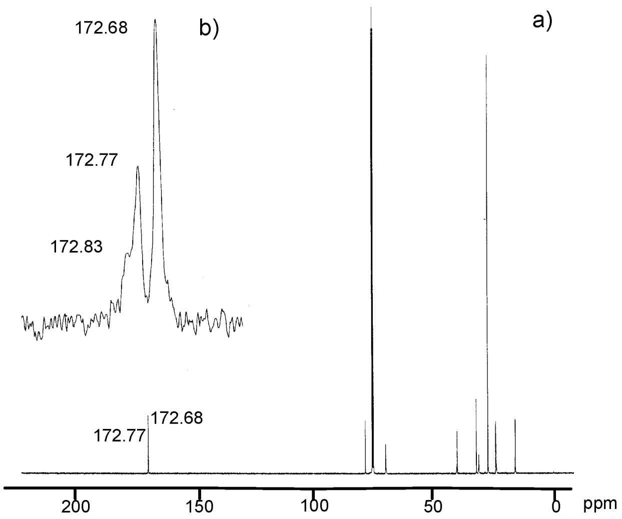
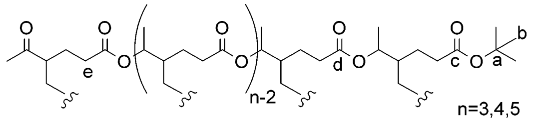


Figure 2. (a)^{13}C NMR of **8** ([G5]-64tBu) in $CDCl_3$; (b) spectra of carboxy carbons using ^{13}C gated decoupling without NOE technique.

---

(6) Due to our instrumental convenience, ESI and CSI (cold spray ionization) MS were used instead of most commonly used MALDI-MS.

Table 2. ^{13}C NMR Chemical Shifts (ppm) and T_1 Relaxation Time Constants (s) for the Dendrimers [G*n*] in $CDCl_3$ at 300 K



carbons	δ_{TMS}	T_1([G3]-16*t*Bu)	T_1([G4]-32*t*Bu)	T_1([G5]-64*t*Bu)
a	80.27	10.97	9.83	9.67
b	28.20	1.34	1.31	1.23
c	172.80	6.61	5.64	5.31
d	172.89	1.57	1.62	1.62
e	23.90	0.37	0.37	0.33

^{13}C NMR relaxation time (T_1) measurements were performed in $CDCl_3$ to give the results shown in Table 2. As the dendrimer generation increases, a slight decrease in T_1 is observed for the outermost carboxyl carbons **c**, methyl carbons **b** and quaternary carbons **a** of *tert*-butyls, suggesting the decrease in the mobility at the periphery of the dendrimer molecules due to the increasing congestion of the *tert*-butyl groups. The second from the outermost carboxy carbons **d** always show smaller T_1 than those of **c**. These results are consistent with those previously reported.[8,9]

Interestingly, T_1 values of the interior carboxy carbons **d** are almost constant in **6**, **7**, and **8**, which indicates that the bulky *tert*-butyls provide mobility around the carbons **d** almost to the same extent in regardless of generation from [G3] to [G5]. These observations suggest that the ester skeleton of these dendrimers takes predominantly extended chain conformation at least in $CDCl_3$.

In summary, an efficient divergent procedure for synthesizing new aliphatic polyester-type dendrimers from acetoacetic acid and acrylic acidhas been established. The reiterative [deprotection by HCO_2H then EDCI/DMAP coupling] sequence gave dendrimers ([G1]−[G5]) in fairly good yield. The *tert*-butyl groups at the periphery can be deprotected easily by formic acid to afford the dendrimers with free COOH, which can be easily modified with various kinds of functional groups. Studies on biocompatibility of these new dendrimers for application as a novel drug delivery system with sugars and caged compounds, or modification of the periphery with various functionalities are now in progress.

Acknowledgment. We thank Drs. Haruhisa Akiyama and Nobuyuki Tamaoki of Molecular Smart System Group, Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), and Dr. Ken-ichi Aoki of Faculty of Science, Toho University, for the GPC measurements. This work was partly supported by a Grant-in-Aid for Scientific Research (No. 13680677) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: Experimental procedures for the synthesis of **3**−**8** and ^{1}H and ^{13}C NMR of **6**−**8** and deprotected **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0482184

---

(7) The detailed analysis of these NMR spectra will be reported elsewhere in the near future.

(8) (a) Jiang, D.-L.; Aida, T. *J. Am. Chem. Soc.* **1998**, *120*, 10895−10901. (b) Hecht, S.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1999**, *121*, 4084−4085. (c) Meier, H.; Lehmann, M.; Kolb, U. *Chem. Eur. J.* **2000**, *6*, 2401−2406.

(9) Chai, M.; Niu, Y.; Youngs, W. J.; Rinaldi, P. L. *J. Am. Chem. Soc.* **2001**, *123*, 4670−4678.