Bioorganic & Medicinal Chemistry Letters 21 (2011) 4724–4727

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Improved performance by replacing iminodiacetic residues with glyceryl residues in symmetrically branched oligoglycerols

Hisao Nemoto [a,*], Atsushi Ishihara [b], Takaaki Araki [a], Ayato Katagiri [a], Masaki Kamiya [a], Tsuyoshi Matsushita [a], Hatsuhiko Hattori [a], Yukiteru Mimura [b], Yutaka Tomoda [b], Motoo Yamasaki [c]

[a] Department of Pharmaceutical Chemistry, Institute of Health Biosciences, Graduate School of The University of Tokushima, 1-78-1 Sho-machi, Tokushima 770-8505, Japan
[b] Drug Formulation Research and Development Laboratories, Production Division, Kyowa Hakko Kirin Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan
[c] Innovative Drug Research Laboratories, Research Division, Kyowa Hakko Kirin Co., Ltd, 3-6-6 Asahi-machi, Machida, Tokyo 194-8533, Japan

ARTICLE INFO

Article history:
Received 15 April 2011
Revised 9 June 2011
Accepted 16 June 2011
Available online 25 June 2011

Keywords:
Symmetrically branched oligoglycerols
Dendron
Water-solubility
Liposome
Duration period

ABSTRACT

Synthesis of a symmetrically branched diglycerol (BGL002, involving one iminodiacetic residue) as a G2 dendron, and the tetradecaglycerol (BGL014, involving one iminodiacetic residue) as a G4 dendron, is described. Several members of the BGL family of G2–G4 dendrons were assembled, with G2 bearing four hydroxyl groups at the terminus region, G3 bearing eight, and G4 bearing sixteen. It is noteworthy that triglycerol (BGL003, including no iminodiacetic residue), has a water-solubility ten times higher than BGL002, and the liposome surrounded by BGL014 has a duration period in blood vessel roughly two times longer than the liposome surrounded by dodecaglycerol (BGL012, including three iminodiacetic residues).

Derivates of medicinal compounds with polyethylene glycol (PEG) group attached via covalent bonding (PEGylation) have been reported in the recent decades.[1] Such PEGylation often provides the derivatives with greater in vivo biologically or thermally stability than the original molecules and/or a greater duration period in blood vessels.

To enhance such biologically favorable properties, glyceryl oligomers or polymers, mainly involving dendric skeletons, have also been studied as rival candidates to PEG.[2] As shown in Figure 1, three basic types of oligomers **1**, **2**, and **3**, can be listed. Although a vague mixture probably containing **1** and **2** was reported in an earlier papers,[3] practical methods to produce oligomers with known chemical structure came later with the synthesis of **1** by Koma in 1986,[4] and of **2** by Vanlerberghe in 1969,[5] and also by Yoshii et al.[6] and Yasukochi et al.[7] in 1997. In contrast, no reports were made on symmetrically branched oligoglycerols **3** (BGL) before our publication in 1992.[8]

The synthesis we developed for **3** yielded a single product (no statistical mixture) with a peculiar symmetrical cascade-shape, and *no asymmetric center*. Therefore **3** may be more potentially applicable for medicinal use than **1** and **2**. We have previously reported the preparation of a trimer (BGL003) as a second generation (G2) of dendron,[8] a hexamer (BGL006)[9,10] and a heptamer (BGL007)[11] as third generation (G3) of dendrons, and dodecamer (BGL012)[12] as a fourth generation (G4) of dendron,

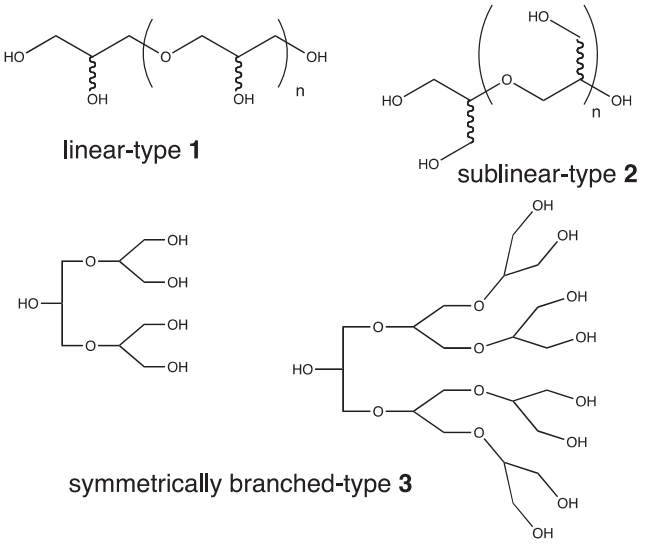


Figure 1. Various oligoglycerols including our developed symmetrical ones.

* Corresponding author.
E-mail address: nem@ph.tokushima-u.ac.jp (H. Nemoto).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.06.064

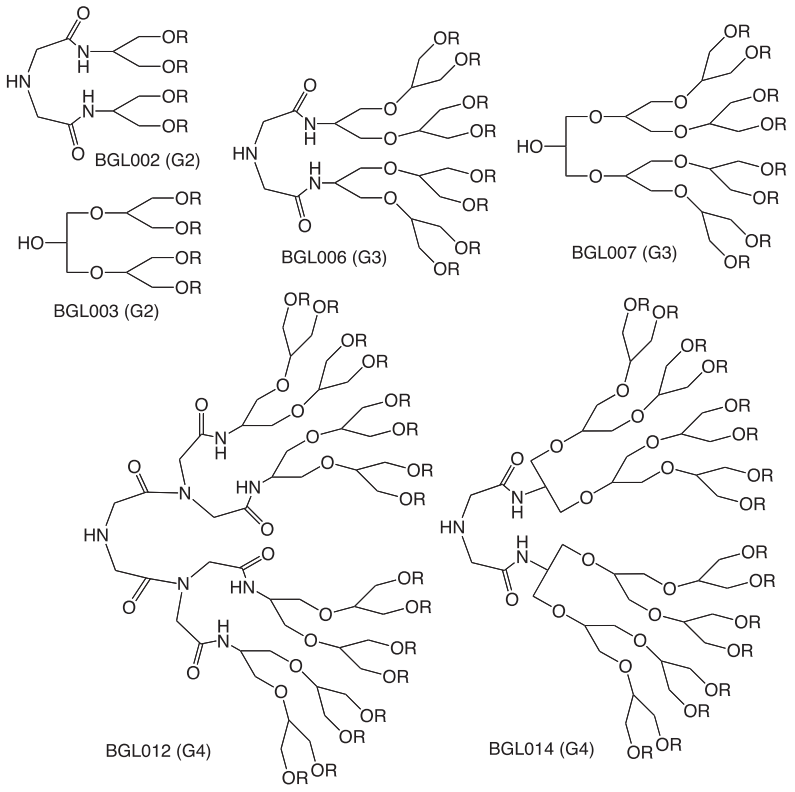
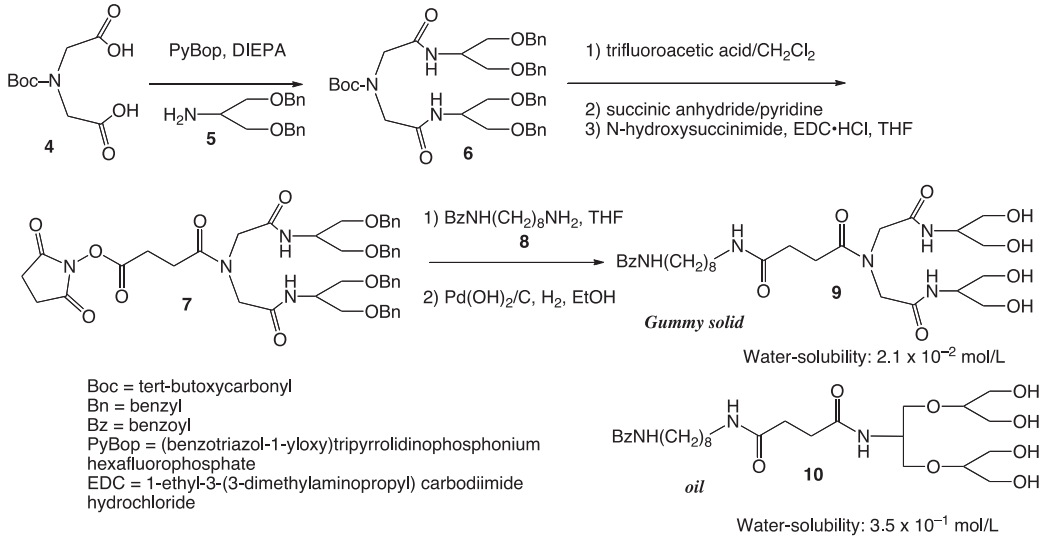


Figure 2. Previously prepared BGL family and newly disclosed two BGL (BGL002 and BGL014).

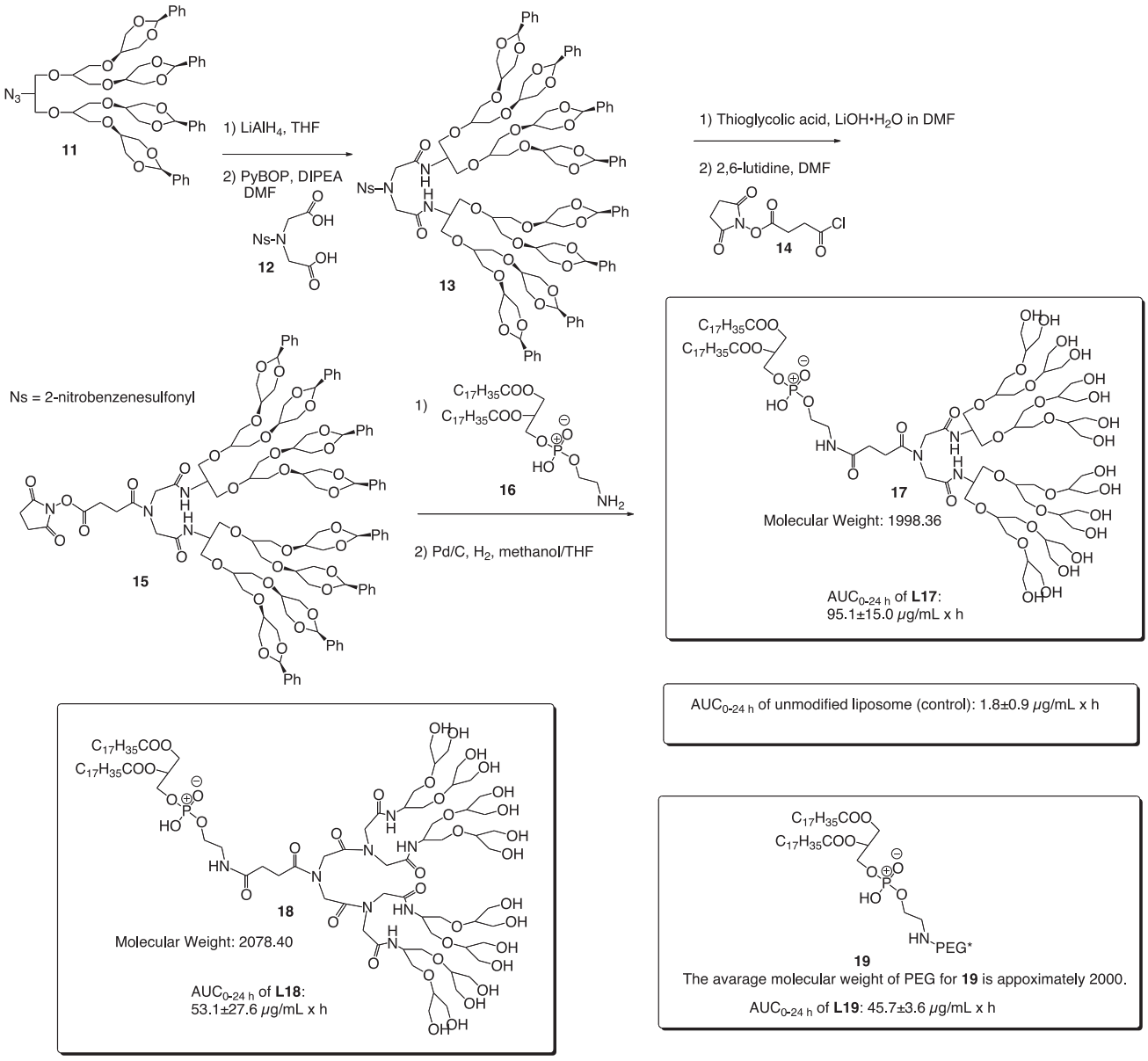


Scheme 1.

including the molecules with protecting groups (R) at the terminus region and those with free hydrogens (R = H) (Fig. 2).

In this Letter, we describe the preparation of an alternative G2 dendron, BGL002 derivative **9** (Scheme 1) and a G4 dendron, BGL014 derivative **17** (Scheme 2), to complement the existing G2–G4 series of BGL family. Moreover, we report on the improved performance obtained by replacing iminodiacetic residues with glyceryl residues in the dendric BGL.

Because we have previously measured the water-solubility of BGL003 derivative **10**,[9] an equivalent BGL002 derivative **9** was prepared for comparison. Condensation of **4**[13] and **5**[14] using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) in DMF in the presence of diisopropylethylamine (DIPEA) at room temperature for 9.5 h afforded **6** in 80% yield. The Boc group of **6** was removed by trifluoroacetic acid (TFA)/dichloromethane at room temperature for 30 h, then treatment of succinic anhydride in pyridine at 100 °C for 15 h, and condensation of the resulting acid and *N*-hydroxysuccinimide (HOSu) with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) in the presence of triethylamine (TEA) in THF at reflux for 18 h gave **7** in 68% yield from **6**. Finally, condensation between **7** and **8**[15] in THF at room temperature for 40 h, followed



Scheme 2.

by deprotection of the four benzyl groups in ethanol in the presence of palladium hydroxide on charcoal under a hydrogen atmosphere for 7 h afforded **9** as a gummy solid in 71% from **7**.

It is noteworthy that the water-solubility[9] of **10** was significantly higher than that of **9** (more than ten times), despite the fact that both **9** and **10** have four hydroxyl groups. Consequently, the number of primary hydroxyl groups at the terminus site was not an exclusive factor in determining the water-solubility, which was also strongly dependent on the apex region of the molecular block comprising the dendron.

Based on the results described above, the water-solubility of BGL014 is probably much higher than that of BGL012. However, we did not obtain values for the water-solubility of the BGL012-**8** and BGL014-**8** conjugates because their water-solubility was too great to measure. In fact, even the water-solubility of the BGL006-**8** conjugate was too high to be measured.[9]

Next, we prepared a liposome including the 1,2-distearoylphosphatidylethanolamine (DSPE)–BGL014 conjugate **17** because we have previously studied the properties of an equivalent liposome including the DSPE–BGL012 conjugate **18**[12] (Scheme 2). The conjugate **17** was synthesized from a BGL007 derivative **11**,[11] which has an azido group at the apex region and bears four benzylidene protecting groups at the terminus region. To orthogonally deprotect the benzylidene groups of **11**, an iminodiacetic acid bearing the 2-nitrobenzenesulfonyl (Ns) group[16] **12** was prepared. After the azido group of **11** was reduced with lithium aluminum hydride in THF, condensation of the resulting amine with **12** afforded **13** in 86% yield by using PyBOP and DIPEA in DMF at 50 °C for 18 h. After the deprotection of the Ns group[16] of **13** by thioglycolic acid and lithium hydroxide monohydrate in DMF at room temperature for 2 h, condensation between the resulting amine and 2,5-dioxopyrrolidin-1-yl 4-chloro-4-oxobutanoate (**14**)[17] afforded **15** in 56% yield using 2,6-lutidine[18] in DMF at 0 °C for 2 h. Finally, condensation of **15** and DSPE (**16**) in chloroform/methanol in the presence of TEA at room temperature for 7 days, followed by deprotection of the eight benzylidene groups by palladium charcoal in methanol/THF under hydrogen atmosphere at 50C for 6.5 h afforded **17** in 64% yield.

We disclose herein preliminary data on the duration in blood vessels for the liposomes containing **17**, **18**[12] and **19**[12] (**L17**, **L18**,

and **L19**, respectively) although a detailed comparison of various properties for **L17–L19** is currently underway.[19] These three liposomes were prepared according to our previous paper.[12] The values as determined by analytical ultracentrifugation over 24 h (AUC_{0-24}) obtained for the control (neither PEG nor BGL), **L17**, **L18**, and **L19** liposomes were 1.8 ± 0.9, 95.1 ± 15.0, 53.1 ± 27.6, and 45.7 ± 3.6 μg mL^{-1} h^{-1}, respectively. It is noteworthy that the AUC_{0-24} value of **L17** was larger than that of **L18** (BGL014 versus BGL012), and was also larger than that of **L19** (BGL versus PEG).

Based on the observation that a BGL002 derivative **9** was more easily solidified than a BGL003 derivative **10**, the conformational rigidity of the iminodiacetic residue was thought to be higher than that of the glyceryl residue. It is considered that BGL003 can flexibly interact with more water molecules via hydrogen bond interactions than BGL002. Similarly, BGL014 can also interact with more water molecules than BGL012.

In conclusion, we have successfully obtained various members of the BGL family (G2–G4), and observed significant differences resulting from the dendritic block unit between tetrahydroxyl derivatives (BGL002 versus BGL003) and between hexadecahydroxyl derivatives (BGL012 versus BGL014). Various studies utilizing covalent bond formation with the BGL family (BGLation) including liposome chemistry are in progress.

Acknowledgments

This work was partly supported by the Japan Science and Technology Agency (JST), Adaptable and Seamless Technology Transfer Program through Target-driven R&D in 2009-2012.

Supplementary data

Supplementary data (procedures for synthesis of **9** and **17** are available including ^{1}H NMR, ^{13}C NMR, IR, and high resolution mass spectra data for **6**, **7**, **9**, **13**, and **15**, and 1H NMR and low mass spectrum date for **17**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.064.

References and notes

1. (a) Allen, T. M.; Hansen, C. *Biochim. Biophys. Acta* **1991**, *1068*, 133; (b) Woodle, M. C.; Lasic, D. D. *Biochim. Biophys. Acta* **1992**, *1113*, 171; (c) Gabizon, A.; Isacson, R.; Libson, E.; Kaufman, B.; Uziely, B.; Catane, R.; Ben-Dor, C. G.; Rabello, E.; Cass, Y.; Peretz, T.; Sulkes, A.; Chisin, R.; Barenholz, Y. *Acta Oncol.* **1994**, *33*, 779; (d) Abuchowski, A.; Davis, F. F. *Biochim. Biophys. Acta* **1979**, *578*, 41; (e) Monkarsh, S. P.; Ma, Y.; Aglione, A.; Bailon, P.; Ciolek, D.; DeBarbieri, B.; Graves, M. C.; Hollfelder, K.; Michel, H.; Palleroni, A.; Porter, J. E.; Ussoman, R. E.; Roy, S.; Pan, Y. E. *Anal. Biochem.* **1997**, *247*, 434; (f) Esposito, P.; Barbero, L.; Caccia, P.; Caliceti, P.; D'Antonio, M.; Piquet, G.; Veronese, F. M. *Adv. Drug Deliv. Rev.* **2003**, *55*, 1279; (g) Suzawa, T.; Nagamura, S.; Saito, H.; Ohta, S.; Hanai, N.; Kanazawa, J.; Okabe, M.; Yamasaki, M. *J. Control Release* **2002**, *79*, 229; (h) Suzawa, T.; Nagamura, S.; Saito, H.; Ohta, S.; Hanai, N.; Yamasaki, M. *J. Control Release* **2000**, *69*, 27; (i) Suzuki, T.; Kanbara, N.; Tomono, T.; Hayashi, N.; Shinohara, I. *Biochim. Biophys. Acta* **1984**, *788*, 248; (j) Chapman, A. P.; Antoniw, P.; Spitali, M.; West, S.; Stephenes, S.; King, D. *J. Nat. Biotechnol.* **1999**, *17*, 780; (k) Zalipsky, S. *Bioconjugate Chem.* **1995**, *6*, 150; (l) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Adv. Drug Deliv. Rev.* **2003**, *55*, 217; (m) Kochendoerfer, G. G.; Chen, S.; Mao, F.; Sressman, S.; Traviglia, S.; Shao, H.; Hunter, C. L.; Low, D. W.; Cagle, E. N.; Carnevali, M.; Gueriguian, V.; Keogh, P. J.; Porter, H.; Stratton, S. M.; Wiedeke, M. C.; Wilken, J.; Tang, J.; Levy, J. J.; Miranda, L. P.; Crnogorac, M. M.; Kalbag, S.; Bottei, P.; Schindler-Horvat, J.; Savatski, L.; Adamson, J. W.; Kung, A.; Kent, S. B. H.; Bradburne, J. A. *Science* **2003**, *299*, 884; (n) Tsunoda, S.; Ishikawa, T.; Yamamoto, Y.; Kamada, H.; Koizumi, K.; Matsui, J.; Tsutsumi, Y.; Hirano, T.; Mayumi, T. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 368–372.
2. Recent examples (a) Weinhart, M.; Grunwald, I.; Wyszogrodzka, M.; Gaetjen, L.; Hartwig, A.; Haag, R. *Chem. Asian J.* **2010**, *5*, 1992; (b) Calderón, M.; Quadir, M. A.; Sharma, S. K.; Haag, R. *Adv. Mater.* **2010**, *22*, 190; (c) Ooya, T.; Lee, J.; Park, K. *Bioconjugate Chem.* **2004**, *15*, 1221.
3. The oldest publication obtained in SciFinder™ with 'polyglycerol' as the keyword: Zajic, J. *Sb. Vysoke Skoly Chem.-Technol. Praze, Potravinarske Technol.* **1963**, *7*, 170.
4. Koma, T. Patent JP61238749, 1986.
5. Vanlerberghe, G. Patent DE1813226, 1969.
6. Yoshii, T.; Suzuki K.; Ueda, S. Patent JP09235247, 1997.
7. Yasukochi, T; Maruyama, T.; Enomoto, H. Patent JP09188754, 1997.
8. Nemoto, H.; Wilson, J. G.; Nakamura, H.; Yamamoto, Y. *J. Org. Chem.* **1992**, *57*, 435.
9. Nemoto, H.; Araki, T.; Kamiya, M.; Kawamura, T.; Hino, T. *Eur. J. Org. Chem.* **2007**, 3003.
10. Yamaguchi, H.; Suzawa, T.; Araki, T.; Kamiya, M.; Nemoto, H.; Yamasaki, M. *Biochim. Biophys. Acta* **2008**, *1780*, 680.
11. Nemoto, H.; Kamiya, M.; Minami, Y.; Araki, T.; Kawamura, T. *Synlett* **2007**, 2091.
12. Ishihara, A.; Yamauchi, M.; Kusano, H.; Mimura, M.; Nakakura, M.; Kamiya, M.; Katagiri, A.; Kawano, M.; Nemoto, H.; Suzawa, T.; Yamasaki, M. *Int. J. Pharm.* **2010**, *391*, 237.
13. Wang, Y. A.; Li, J. J.; Chen, H.; Peng, X. *J. Am. Chem. Soc.* **2002**, *124*, 2293.
14. Nemoto, H.; Cai, J.; Yamamoto, Y. *J. Med. Chem.* **1995**, *38*, 1673.
15. Zaragoza-Dörwald, F.; Von Kiedrowski, G. *Synthesis* **1988**, 917.
16. Fukuyama, T.; Cheung, M.; Kan, T. *Synlett* **1999**, 1301.
17. Charbonnel-Jobic, G.; Guémas, J.-P.; Adelaere, B.; Parrain, J.-L.; Quintard, J.-P. *Bull. Soc. Chim. Fr.* **1995**, *132*, 624.
18. Our attempts for the preparation of **13** using various tertiary amines were unsuccessful with the exception of 2,6-lutidine because the *N*-hydroxysuccinimide ester moiety was decomposed by relatively unhindered amines such as TEA, DIPEA, 4-(*N*,*N*-dimethylamino)pyridine, and 1,8-diazabicyclo[5.4.0]undec-7-ene. In contrast, condensation reaction of the resulting amine and acid chloride **14** was too slow with weak base such as pyridine or *N*-methylmorpholine. The success with 2,6-lutidine was probably due to its appropriate basicity and very hindered circumstance around the amino group.
19. Ishihara, A.; Yamauchi, M.; Tsuchiya, T.; Mimura, M.; Tomoda, Y.; Katagiri, A.; Kamiya, M.; Nemoto, H.; Suzawa, T.; Yamasaki, M. in preparation.