Macromolecular Research, Vol. 12, No. 1, pp 63-70 (2004)

Synthesis of Various Polymeric Prodrugs of Ibuprofen with PEG and Its Derivative as Polymeric Carriers

Chan Woo Lee*

Department of Innovative Industrial Technology, Hoseo University, Asan 336-795, Korea

Received Aug. 5, 2003; Revised Dec. 15, 2003

Abstract: We have synthesized various types of poly(ethylene glycol) (PEG)-ibuprofen conjugates by the nucleophilic substitution of bromo-terminated PEG with ibuprofen-Cs salt; PN (Pluronic) was also used in place of PEG. All the bromo-terminated PEGs and PN were obtained in high yield. Conversions of the terminal hydroxyl groups to bromo-termini were quantitative, as were the drug conjugation processes. The I_1/I_3 values obtained from solutions of the ibuprofen-conjugated prodrugs are summarized in relation to those of ibuprofen in water and in aqueous solutions of the original PEG, PN, and several ordinary surfactants. We believe that the fully hydrophilic PEG is completely hydrated and forms no hydrophobic pocket by segment aggregation. These results indicate that the probe environment is significantly hydrophobic, particularly in the solution of prodrug PN, for which the ratio is similar to that obtained from typical micelles of surfactants. The results suggest, therefore, that the present synthetic method is very useful for preparing PEG-based prodrugs from pharmaceuticals having carboxyl functionalities.

Keywords: conjugates, nucleophilic substitution, prodrug, aqueous solution, aggregation, carboxyl functionality.

Introduction

Until now, various polymeric prodrugs have been developed to improve the bioavailability and site specificity of drugs with minimizing the side effects. The ordinary polymeric prodrugs are composed of a polymer carrier to which pharmaceuticals are covalently conjugated. As the polymer carriers, various water-soluble polymers such as poly(vinyl alcohol), dextran, poly(ethylene glycol), and poly(L-lysine) are employed. Among them, poly(ethylene glycol)(PEG) is the most useful polymer in terms of its water solubility, body reservation, and biological safety. It has been reported that the urinary clearance of PEG decreases with increasing molecular weight, while the liver clearance increases with increasing molecular weight.[1] In the chemical conjugations of pharmaceuticals and PEG reported thus far,[2-5] the conjugation yield was not quantitative. Moreover, the separation of the prodrugs from the contaminants was also difficult. Therefore, it has been necessary to develop a facile method for conjugating PEG and drug in which the reaction proceeds at mild conditions in quantitative conversion.

In this study, the author reports on a novel method to directly conjugate unstable pharmaceuticals on PEG terminals that are functionalized with bromide. The conjugation reaction proceeds by the nucleophilic substitution of the bromide. If the drug contains carboxylate groups, the bromide is readily replaced by the drug under mild reaction conditions. Here, 4-isobutylphenyl-2-propanoic acid (Ibuprofen) was employed as a carboxyl-containing drug, which is well known as an anti-inflammatory drug and widely used in therapy. Various PEG-ibuprofen conjugates having different spacer groups were synthesized from PEGs with different molecular weight, and for which the drug-releasing behavior was evaluated.

*e-mail: cwlee@office.hoseo.ac.kr

Experimental

Materials. Poly(ethylene glycol)s having number average molecular weights (M_n) of 7,500 and 20,000 were purchased from Wako Pure Chemical Industry (Osaka, Japan). These PEGs are abbreviated as PEG7.5K and PEG20K, respectively, where the numbers represent their M_n value in kDa. Their molecular weight distribution indexes in weight (M_w)/number (M_n) average molecular weight were 1.07 and 1.06, respectively. Ibuprofen(4-isobutylphenyl-2-propanoic acid) of pharmaceutical grade was purchased from Wako Pure Chemical Industry. Cesium carbonate (Cs_2CO_3), 1,5-dibromopentane, 1,10-dibromodecane, ethanol, sodium hydride (NaH), magnesium chloride hexahydrate, and trifluoroacetic anhydride were of reagent grade and used as received. α,α'-Dibromo-*p*-xylene was purchased from

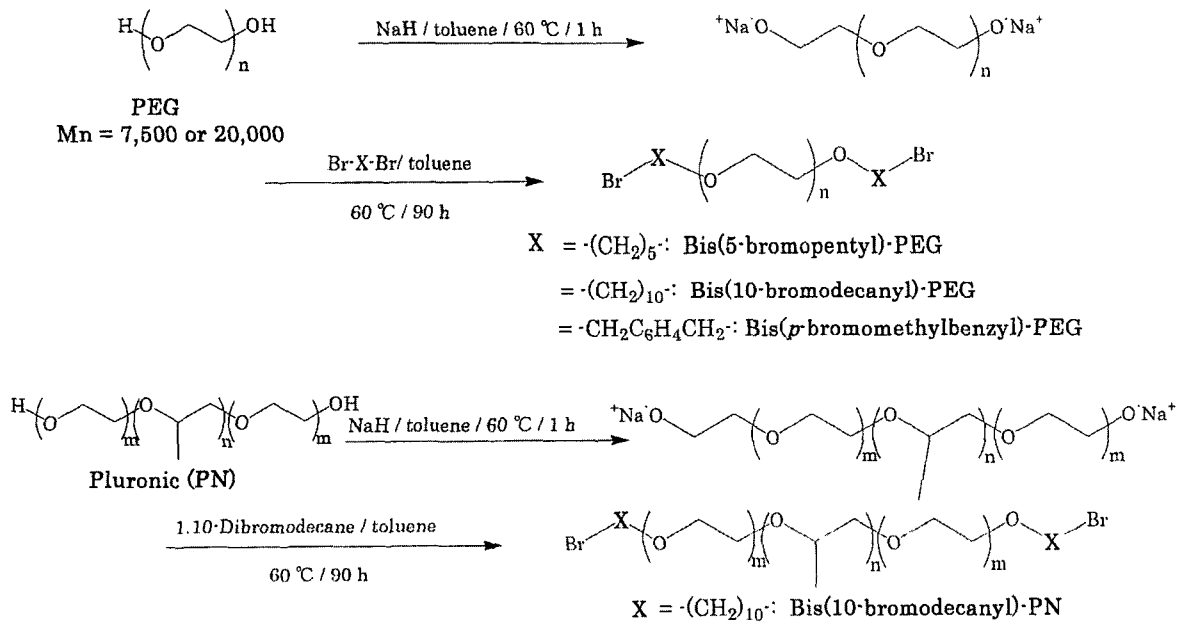
Tokyo Chemical Industry (Tokyo, Japan) and was purified by distillation. Pyrene was purified by recrystallization from ethanol. Distilled water was further purified by filtration using a Milipore Milli Q system. Pluronic F-108™ [PN: a triblock copolymer poly(oxyethylene)-*block*-poly(oxypropylene)-*block*-poly(oxythylene) with an oxyethylene/oxypropylene unit ratio of 20/80)] having a M_n of 15,500 was supplied by Asahi Denka Kogyo (Tokyo, Japan). Its M_w/M_n was 1.06. Rat plasma was supplied by Ono Pharmaceutical Industry (Osaka, Japan).

Measurements. ^{1}H-NMR spectra were recorded in $CDCl_3$ and D_2O with tetramethylsilane (TMS) and 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as the internal standards, respectively, on a Brucker ARX 500 spectrometer operated at 500 MHz. Gel permeation chromatography (GPC) was performed on an analyzer composed of a Shimadzu LC-10AS pump, a Shimadzu RID-10A refractive index detector, a Shimadzu CTO-10A column oven (35 °C), and a Shimadzu C-R4A chromatopac data processor. A Toso TSK gel G4500PW$_{XL}$ column was used together with a guard column, and the eluent was 0.02 M phosphate buffer (pH=7.4) containing 5% acetonitrile. The molecular weight was calibrated with the PEG standards.

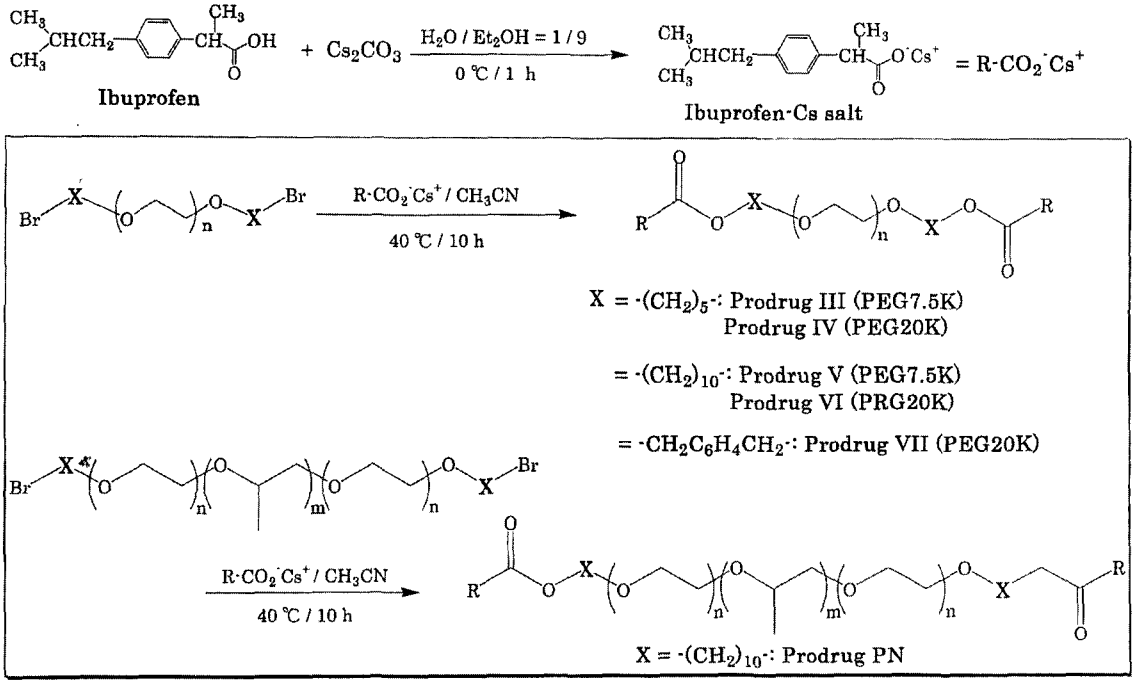
Syntheses of Bromo-Terminated PEG. The reaction procedures are summarized in Scheme I, where all the operations were done in a nitrogen atmosphere unless otherwise noted. A typical example is as follows: a 2.0 g of PEG7.5K (0.34 mmol) was dissolved in a small volume of benzene and subjected to the lyophilization to remove water. The lyophilized PEG was re-dissolved in 20 mL toluene, and a 81 mg of (3.4 mmol) NaH suspended in 10 mL toluene was added to it at 0 °C under nitrogen. Then, the system was stirred at 60 °C for 1 hr to complete the terminal alkoxide formation of PEG and cooled again to 0 °C. 7.7 g of 1,5-dibromopentane was added to this system. After the addition, the mixture was stirred at 60 °C for 90 hrs. The resultant solution was filtered to remove the inorganic by-products, and the filtrate was reprecipitated into a large exess of diethyl ether to isolate the PEG products. The precipitates were filtered and dried under vacuum (**1**, 71%).

Bis(5-bromopentyl)-PEG20K (**2**), bis(10-bromodecanyl)-PEG7.5K (**3**), PEG20K (**4**), bis(10-bromodecanyl)-PN (**5**), and bis(*p*-bromomethyl benzyl)-PEG20K (**6**) were also prepared likewise (2: 87%, 3: 82%, 4: 83%, 5: 80%, 6: 81%).

Syntheses of PEG-Ibuprofen Conjugates. Conjugation of ibuprofen with bromo-terminated PEG was performed as shown in Scheme II. Ibuprofen (8.0×10^{-2} mmol) and cesium carbonate (0.24 mmol) were dissolved in 1 mL of a water/ethanol (1/9 in vol.) mixture and stirred at 0 °C for 1 hr. The solution was then evaporated under vacuum to obtain the ibuprofen-Cs salt as the residue. It was mixed with a bromo-terminated PEG (2.0×10^{-2} mmol) in a small volume of benzene and lyophilized. The mixture was subsequently dissolved in 1 mL acetonitrile and stirred at 40 °C for 10 hrs. After the reaction being over, the reaction system was filtered to remove the abundant ibuprofen-Cs salt, and the filtrate was poured into 50 mL *t*-butanol containing 5 g magnesium chloride hexahydrate. The resultant suspension was stirred at 30 °C for 3 hrs. This procedure was effective for removing the residual ibuprofen -Cs salt by adsorption. The suspension was finally evaporated under vacuum, and the residue was subjected to extraction with a dichloromethane/water system



Scheme I. Syntheses of bromo-terminated PEG and PN.



Scheme II. Syntheses of various types of ibuprofen-conjugated prodrugs.

to recover the product in dichloromethane. The dichloromethane extract was dried over sodium sulfate, filtered, and evaporated under vacuum. The residue was purified by reprecipitation using a benzene/diethyl ether (solvent/precipitant) system. The finally obtained precipitates were filtered and dried under vacuum. The ibuprofen-PEG conjugates derived from 1, 2, 3, 4, and 6 by this method are named as prodrugs III (7, 0.085 g, 53%), IV (8, 0.23 g, 56%), V (9, 0.073 g, 45%), VI (10, 0.20 g, 58%), and VII (11, 0.20 g 49%), respectively. The ibuprofen-PN conjugate derived from 5 is named as prodrug PN (12, 0.15 g, 45%).

Fluorescence Measurements. A stock solution of pyrene was prepared by dissolving a freshly recrystallized pyrene in ethanol in a concentration of 1.0×10^{-4} M. Each prodrug was dissolved in a distilled water to have a concentration of 0.1 or 0.6 wt%. 10, 40 and 100 μL portions or the stock solution of pyrene were charged into three separate reaction vials and dried up under a reduced pressure. Then, a 1 mL volume of the prodrug solution was charged into each of the vials and incubated at 37 °C for 24 hrs. The concentrations of pyrene in the prodrug solutions in the vials were 1.0, 4.0 and 10 μM, respectively. Each solution in the vial was then subjected to the fluorescence measurement using a Simadzu RF-5300PC spectrofluorophotometer. The fluorescence emission spectra of pyrene were recoded in a wavelength range of 350-600 nm at an excitation wavelength of 338 nm. The intensities of the first emission band I_1 (at 374 nm) and the third band I_3 (at 385 nm) were determined. Their ratio (I_1/I_3) was used as a measure of the polarity of the microenvironment where the hydrophobic pyrene molecule was located.[6]

Results and Discussion

Preparation of Bromo-Terminated PEG. The bromo-terminated PEGs (1, 2, 3, 4 and 6) and PN (5) were synthesized according to Scheme I. These reactions relied on the nucleophilic substitution between the dibromides and the alkoxides formed on the PEG terminals. All the bromo-terminated PEGs and PN were obtained in high yield. Figures 1 (A) and (B) show the ^{1}H-NMR spectra of bis(5-bromopentyl)-PEG7.5K (1) and PEG20K (2), respectively. The enlarged spectra (A′) and (B′) around δ4.5 ppm are for their reaction products with trifluoroacetic anhydride (TFAn). The original spectra (A) and (B) involve multiplet signals due to the terminal bromomethylene groups on the foot of the large signal due to the oxyethylene groups of PEG. Addition of TFAn to the PEG made a signal (g) appear at δ4.5 ppm as shown in the enlarged spectrum (A″). This signal (g) can be assigned to the trifluoroacetylated oxymethylene groups that were derived from the terminal hydroxyl groups of PEG. The addition of TFAn to the bromoterminated PEG gave no signal due to the trifluoroacetylated oxymethylene groups as shown in the enlarged spectra (A′) and (B′), suggesting that the terminal conversion in both 1 and 2 was quantitative.

Figure 2 (A) and (B) show the ^{1}H-NMR spectra of bis(5-bromo decanyl)-PEG7.5K (3) and PEG20K (4), respectively. The signals due to the decamethylene moiety introduced were

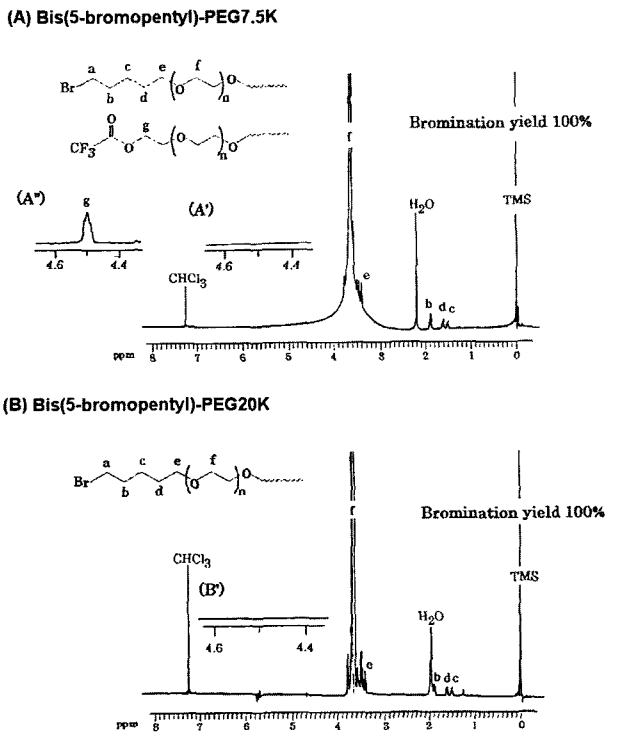


Figure 1. 500 MHz ^{1}H-NMR spectra of bis(5-bromopentyl)-PEG7.5K (A) and PEG20K (B). The partial spectra of (A′) and (B′) are for the corresponding samples trested with TFAn.

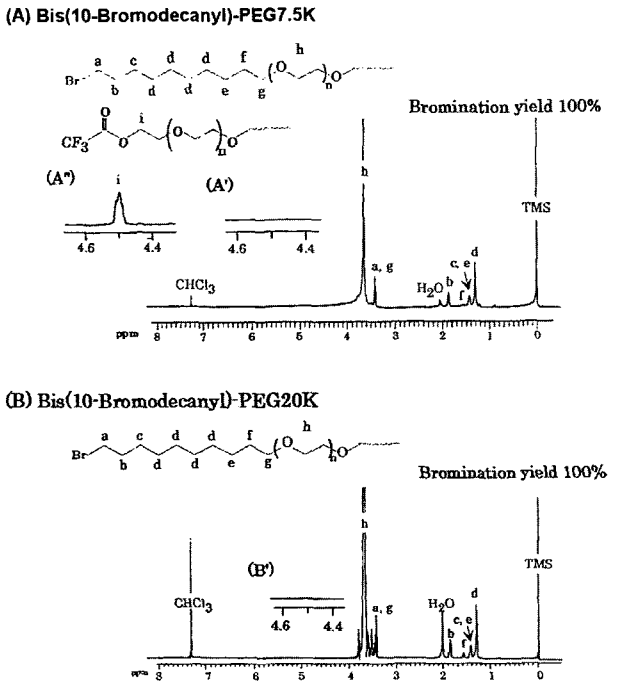


Figure 2. 500 MHz ^{1}H-NMR spectra of bis(10-bromodecanyl)-PEG7.5K (A) and PEG20K (B). The partial spectra of (A′) and (B′) are for the corresponding samples trested with TFAn. The partial spectrum (A″) is for the original PEG treated with TFAn.

detected with a reasonable integral ratio. Absence of the trifluoroacetylated oxymethylene signal around δ4.5 ppm in the expanded spcetrum (A′) or (B′) supported the quantitative conversion of the terminal groups. Figure 3 (A) and (B) show the ^{1}H-NMR spectra of bis(5-bromodecanyl)-PN (5) and bis(*p*-bromomethylbenzyl)-PEG20K (6), respectively. In the spectrum (A) the signals due to the decamethylene moiety were detected, while in the spectrum (B) the signals of *p*-bromomethylbenzyl groups were detected around δ4.5, δ4.6 and δ7.4 ppm. Trifluoroacetylation of these products gave no signal at δ4.5 ppm, supporting the perfect terminal introduction of the bromo groups. In the case of 6, the signal due to the trifluoroacetylated oxymethylene signal overlapped with the signals a and c, the quantitative conversion was confirmed for prodrug III (Figure 6(B)) shown in the later section. Consequently, it was shown that the terminal conversion was quantitative and that all the bromo-terminated PEG were free from residual hydroxyl groups.

Preparation of PEG-Ibuprofen Conjugates. Ibuprofen was readily conjugated by the nucleophilic substitution of the bromo-terminated PEG and PN with ibuprofen-Cs salt. The reaction proceeded under mild conditions in all runs. Figure 4 (A) and (B) show the ^{1}H-NMR spectra of the resultant prodrugs III and IV having pentamethylene spacers, respectively. A small signal (h) at δ4.1 ppm is assigned to

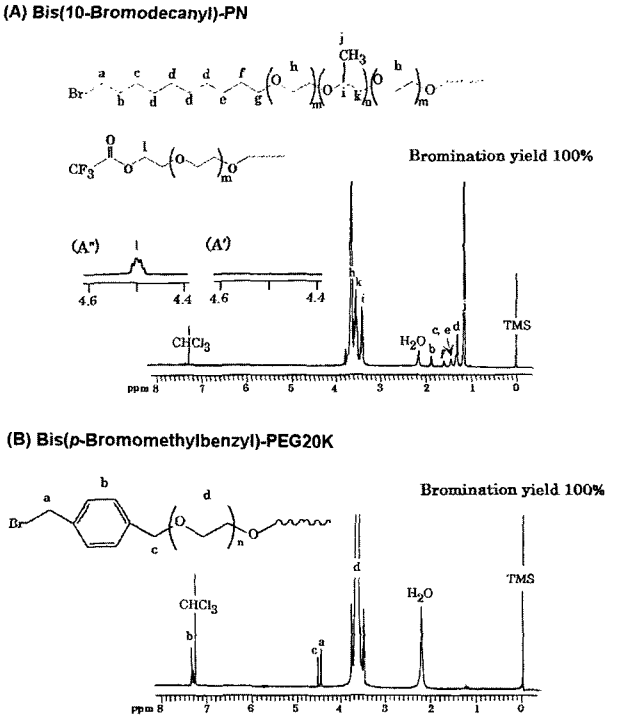


Figure 3. 500 MHz ^{1}H-NMR spectra of bis(10-bromodecanyl)-PN (A′) and bis(*p*-bromomethylbenzyl)-PEG20K (B). The partial spectra of (A′) and (A″) are for the bromo-functionalized PN and the original PN treated with TFAn, respectively.

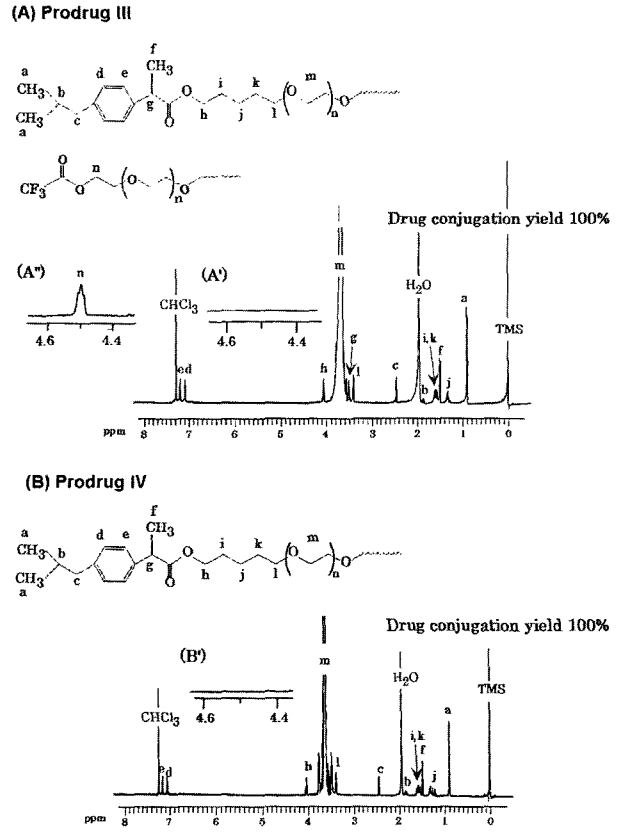


Figure 4. 500 MHz ^{1}H-NMR spectra of prodrug III (A) and prodrug IV (B). The partial spectra of (A') and (B') are for the corresponding samples treated with TFAn, The partial spectrum (A") is for the original PEG treated with TFAn.

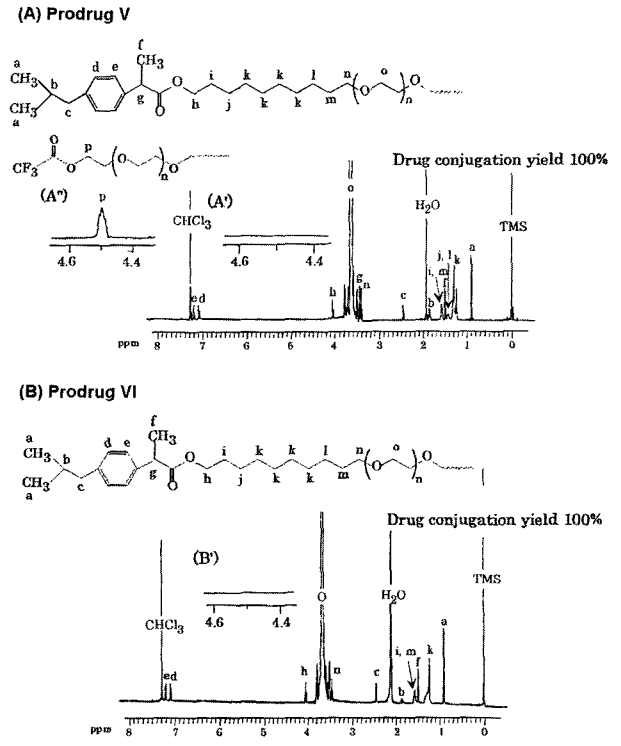


Figure 5. 500 MHz ^{1}H-NMR spectra of prodrug V (A) and prodrug VI (B). The partial spectra of (A') and (B') are for the corresponding samples treated with TFAn, The partial spectrum (A") is for the original PEG treated with TFAn.

the oxymethylene groups that were linked with ibuprofen. It is known that the β-methylene signal (b) shown at δ1.85 ppm in Figure 1 shifted to δ1.6 ppm in Figure 4. This fact supports the quantitative conjugation of ibuprofen at the terminals of the bromo-terminated PEG. The integral ratio of the all signals are also compatible with the structure of prodrug III. Trifluoroacetylation made no signal to appear around δ4.5 ppm as shown in the expanded spectra (A') and (B') to suggest that the conjugation reaction proceeded without hydrolysis of the ester groups. Figures 5 and 6 show the ^{1}H-NMR spectra of the conjugated prodrugs V, VI, VII and PN. The signal assignment confirmed the quantitative conjugation of ibuprofen.

Molecular Aggregation of the Prodrugs. Figures 7 and 8 show the ^{1}H-NMR spectra of the ibuprofen-conjugated prodrugs in D_2O. In these spectra, the signals are broader in comparison with those observed in $CDCl_3$ (Figures 4, 5, and 6). Prodrugs V-VII and PN show two separate signals for the ibuprofen-derived phenyl protons, while prodrugs III and IV show one multiplet peak. This fact suggests that in the former cases the hydrophobic ibuprofen moieties are placed in a local pocket that may be formed by aggregation of the hydrophobic polymer tails and backbone. For analyzing such a microenvironment formation, the pyrene fluorescence spectra were measured in the aqueous solution of the prodrugs.

Pyrene is a strongly hydrophobic molecule whose solubility in water is very low (2-3 μM). It has been known that when it is dispersed in micelles, pyrene is preferentially captured in the interior hydrophobic region of the micelles to change its fluorescence nature. Pyrene has, therefore, been utilized as a probe (chromophore) for studying the micro-heterogeneity of the solution systems, such as micellar[6] and polyamphiphile solutions.[7] The measurable indicator is the probe polarity that is evaluated by the intensity ratio (I_1/I_3) or the first (λ_{max} = 374 nm, intensity: I_1) to the third peaks (λ_{max} = 385 nm, intensity: I_3) in the fluorescence emission spectrum of pyrene (see Figure 9(A)). Figure 9 shows the fluorescence emission spectra of pyrene in the aqueous solutions of the respective prodrug as compared with that in water. At higher concentrations of pyrene more than one chromophoric probe molecule can occupy the same micelle particle to likely form an excimer. In fact, the excimer peak was observed at 480 nm in the spectra taken in the prodrug solutions, but not observed in water. When a 0.2 wt% sodium dodecyl sulfate (SDS) solution was added to the prodrug solutions, the excimer peak disappeared, and the absorption peak at 350-430 nm

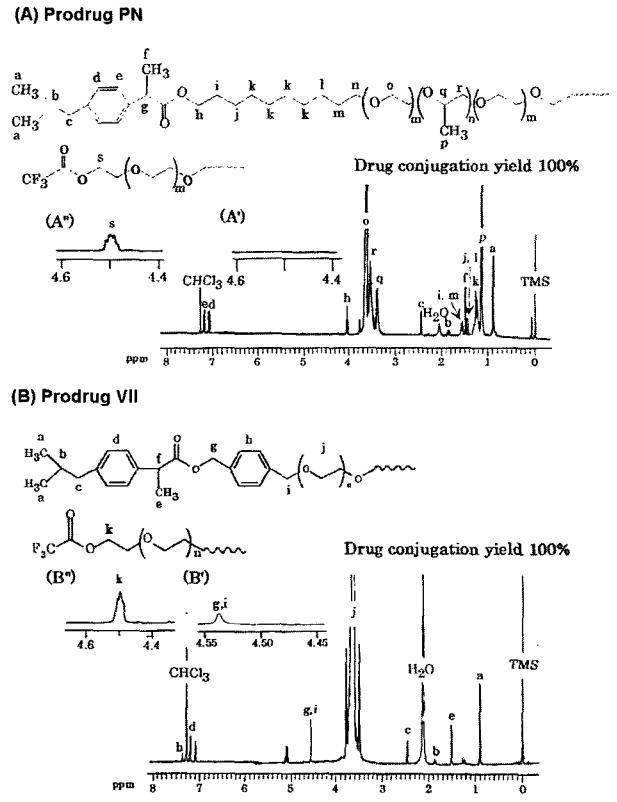


Figure 6. 500 MHz ^{1}H-NMR spectra of prodrug PN (A) and prodrug VII (B). The partial spectra of (A′) and (B′) are for the corresponding samples treated with TFAn, The partial spectra (A″) (B″) are for the original PN and PEG treated with TFAn, respectively.

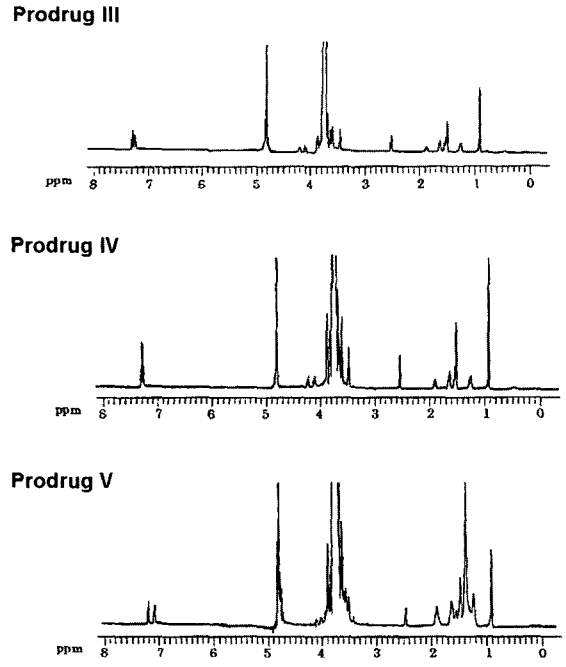


Figure 7. 500 MHz ^{1}H-NMR spectra of prodrugs III, IV, and V in D_2O.

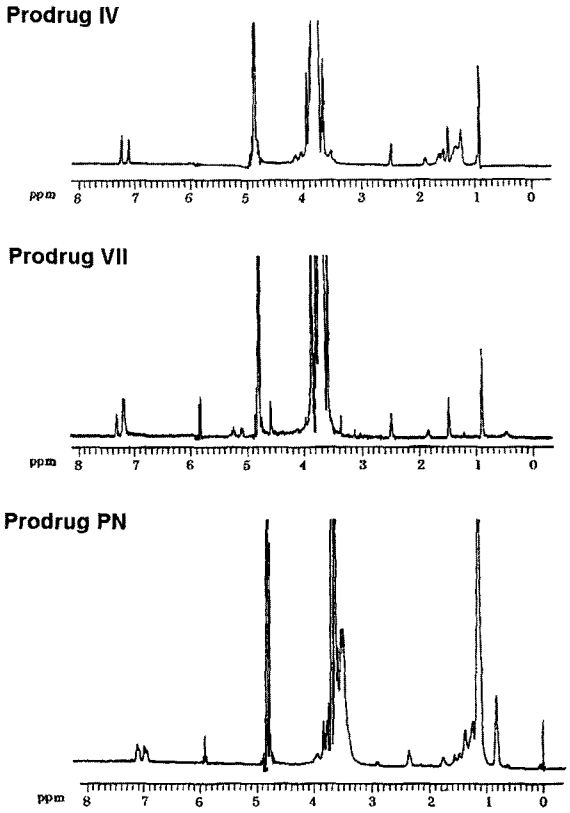


Figure 8. 500 MHz ^{1}H-NMR spectra of prodrugs VI, VII, and PN in D_2O.

was enhanced. This may be because the probe molecules were completely distributed in the newly formed micelles with SDS.

Table I summarizes the I_1/I_3 values in the solutions of the ibuprofen-conjugated prodrugs as compared with those in water and aqueous solutions of the original PEG, PN, and several ordinary surfactants.[8-13] In water, the I_1/I_3 ratio was 1.83, whereas in nonpolar solvents it was well-below 1 (data are not shown, but $I_1/I_3 = 0.61$ in *n*-hexane[6]). In the aqeous solutions of PEG7.5K and 20K, the ratios were 1.81 and 1.82, and no excimer formation was observed. In the aqueous solution of PN, on the other hand, the ratio was 1.56, and the excimer was formed. Therefore, the fully hydrophilic PEG is completely hydrated to form no hydrophobic pocket by the segment aggregation. Since the oxypropylene segments contained in PN is rather hydrophobic, they aggregate by hydrophobic interaction in aqueous environment to form a hydrophobic microenvironment in which pyrene is accommodated. In the aqueous solutions of prodrugs III, IV, and VII, I_1/I_3 were 1.62, 1.64 and 1.59, respectively, being between the values in the PEG and PN were ever lower than that observed in the PN solutions. These results indicate that the probe environment is significantly hydrophobic, particularly in the solution of prodrug PN, for which the ratio was

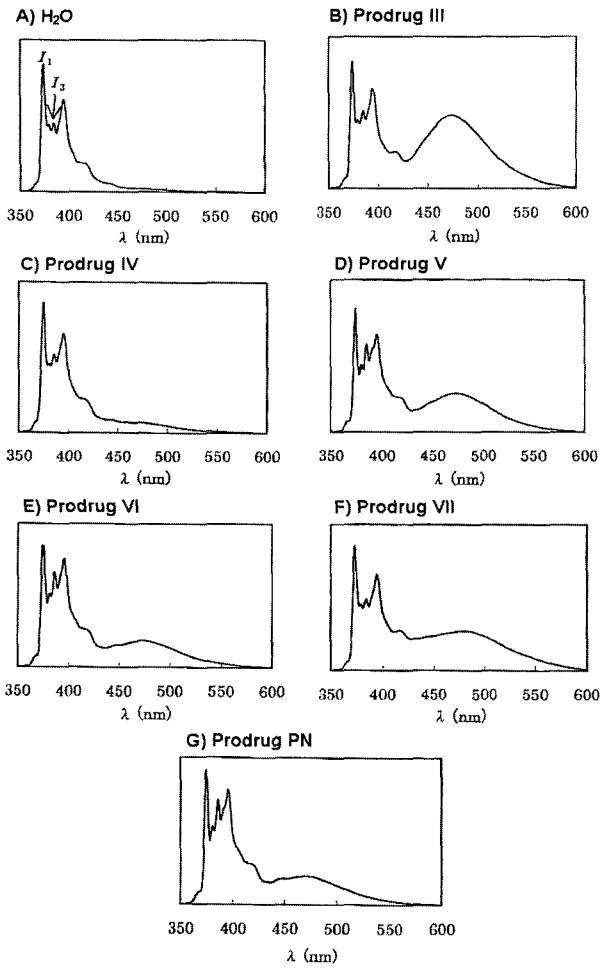


Figure 9. Fluorescence emission spectra of pyrene solubilized in aqueous solutions of 0.6 wt% prodrugs; pyrene = 10 μM.

similar to that in the typical micelles consisting of surfactants. These data do support the fact that the prodrugs readily aggregate in water to form the hydrophobic pockets, in particular, prodrugs V, VI, and PN aggregate strongly. Figure 10 shows the variation of I_1/I_3 as a function of the concentration of prodrugs VI and PN (log scale). At a prodrug concentration of 0.1 wt%, the I_1/I_3 ratios of both prodrugs were similar to those at 0.6 wt%. It was previously reported[6] that pyrene is captured in the hydrophobic pocket above the critical micelle concentration (cmc) of a surfactant to result in the decrease in I_1/I_3. Judging from the curves in Figure 10, it was known that the cmc value of prodrugs VI and PN are between 10^{-3} and 10^{-4} M.

Conclusions

Various types of PEG-ibuprofen conjugates were synthesized by the nucleophilic substitution of the bromo-terminated PEG with ibuprofen-Cs salt. PN was also used instead of PEG. The terminal conversion from the hydroxyl to the bromo-terminal was quantitative, and the drug conjugation proceeded in quantitative yield as well. It was therefore suggested that the present synthetic method is very useful for preparing the PEG-based prodrugs with pharmaceuticals having carboxyl functionality. If the drug contains carboxylate groups, the bromide is readily replaced by the drug under mild reaction conditions.

Table I. I_1/I_3 Ratios of Various Aqueous Solutions[a]

Solute	I_1/I_3
None	1.83
PEG20K	1.82
PEG7.5K	1.81
Pluronic (PN)	1.56
Prodrug III	1.62
Prodrug IV	1.64
Prodrug V	1.36
Prodrug VI	1.40
Prodrug VII	1.59
Prodrug PN	1.27
NaLS [b]	1.14
TritonX-100 [c]	1.32
SDS [d]	1.14

[a] Containing 0.6 wt% solute and 10 μM of pyrene. [b] Sodium lauryl sulfate. [c] Polyoxyethylene(10) octylphenyl ehter. [d] Sodium dodecyl sulfate.

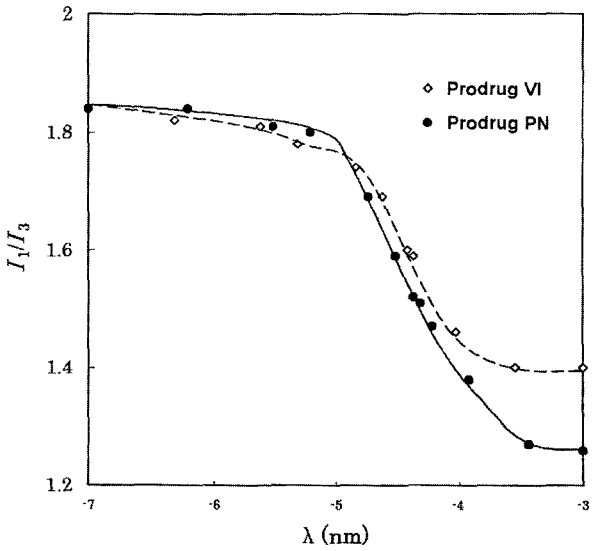


Figure 10. I_1/I_3 vs. the prodrug concentration (C) of prodrug IV and PN.

Acknowledgements. This work was supported by Korea

Research Foundation Grant (KRF-2002-D00148).

References

(1) T. Yamaoka, Y. Tabata, and Y. Ikada, *J. Pharm. Sci.*, **83**, 601 (1994).
(2) L. Sartore, I. Peroni, P, Ferruti, R. Latini, and R. Bernasconi, *J. Biomater. Sci., Polym. Edn.*, **8**, 741 (1997).
(3) M. Nichifor, E. H. Schacht, and L. W. Seymour, *J. Control. Release*, **48**, 165 (1997).
(4) R. B. Greenwald, C. W. Gilbert, A. Pendri, C. D. Conover, J. Xis, and A. Martinez, *J. Med. Chem.*, **39**, 424 (1996).
(5) R. B. Greenwald, C. W. Gilbert, A. Pendri, C. D. Conover, C. Lee, Y. H. Choe, C. Gilbert, A. Martinez, J. Xis, D. Wu, and M. Hsue, *Bioorganic & Medical Chem.*, **6**, 551 (1998).
(6) K. kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1997).
(7) C. Maltesh, Q. Xu, P. Somasundaran, W. J. Benton, and H. Nguyen, *Langmuir*, **8**, 1511 (1992).
(8) O. Vorobyova, A. Yekta, and M. A. Winnik, *Macromolecules*, **31**, 8998 (1998).
(9) C. W. Lee, Y. G. Kang, and K. Jun, *Macromol. Res.*, **9**, 84 (2001).
(10) T. Ouchi, *Koubunshi Kakou*, **34**, 18 (1985).
(11) T. Ouchi, A. Fujino, K. Tanaka, and T. Banba, *J. Control. Release*, **12**, 143 (1990).
(12) C. W. Lee, *Macromol. Res.*, **9**, 259 (2001).
(13) C. W. Lee, *Macromol. Res.*, **11**, 42 (2003).