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Syntheses and biological activities of 5′-*O*-methacryloyl-3′-azido-3′-deoxythymidine and its polymers

Won-Moon Choi [1], Neung-Ju Lee [2], Young-Woo Lee [3], Chang-Sik Ha [1], Won-Jei Cho [1,*]

[1] Department of Polymer Science and Engineering, Pusan National University, Pusan 609-735, Korea
[2] Department of Premedical Sciences, Kosin University, Pusan 602-702, Korea
[3] Department of Neurosurgery, School of Medicine, Pusan National University, Pusan 602-739, Korea

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Summary

The new monomer, 5'-O-methacryloyl-3'-azido-3'-deoxythymidine (MAZT), was synthesized from methacryloyl chloride(MAC) and 3'-azido-3'-deoxythymidine(AZT). The homopolymer of MAZT and copolymers of MAZT with acrylic acid (AA) or exo-3,6-epoxy-1,2,3,6-tetrahydrophthalic glycinylimide (ETGI) were synthesized by radical polymerizations. The structures of MAZT and polymers were confirmed identified by FT-IR and ^{1}H-NMR spectroscopies. The number average molecular weights ($\bar{M}n$) and polydispersity indices of the synthesized polymers were in the range of 4,400~20,400 and 1.2~2.0. The *in vitro* cytotoxicities of polymers against K562 human leukemia and normal cell lines were greater than that of control.

Introduction

It has been known that one of the methods for the reduction of side effects such as the toxicity and delivery problems of low molecular weight drug transforms the drug to polymer containing drug moiety, because polymeric drugs can be expected to have some advantages such as higher specificity of actions, longer duration of actions and lower toxic side effects.[1-3] The polymer drugs containing pyrimidine ring such as 5-fluorouracil have been investigated.[4-9] And also we have been reported on the syntheses and biological activities of polymeric antitumor agents.[10-19]

In this study, we synthesized the new monomer, MAZT, and its homopolymer, and copolymers of MAZT with AA or ETGI by thermal copolymerizations. The synthesized MAZT, poly(MAZT), poly(MAZT-co-AA), and poly(MAZT-co-ETGI) were identified by FT-IR and ^{1}H-NMR spectroscopies. The average molecular weights were determined by GPC. The *in vitro* cytotoxicities were evaluated against K562 human leukemia and normal cell lines.

Experimental

* Corresponding author

Materials

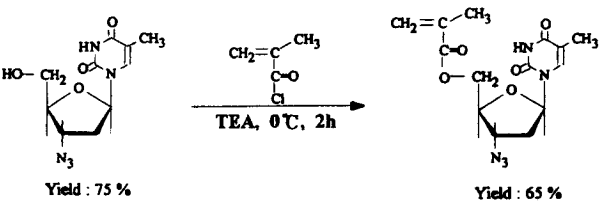
AZT was synthesized by the Horwitz method[20] with slight modification. Methacryloyl chloride (MAC) was used as received from Aldrich Co. without further purification. Triethylamine(TEA; Junsei) was refluxed with acetic anhydride and KOH, and finally distilled. AA (Junsei) was distilled from reduced pressure. Benzoyl peroxide (BPO; Junsei) was purified by conventional recrystallization methods. ETGI was synthesized from the method as described previously.[21] Dimethoxyethane(DME; Fluka), dimethylformamide(DMF; Junsei), dimethylsulfoxide (DMSO; Junsei) and other chemicals were used without further purification.

Instruments

IR spectra were taken on a Jasco FT/IR-5300 spectrophotometer using KBr pellet or liquid cell. ^{1}H-NMR (300 MHz) spectra were recorded on a FT-300 MHz Bruker A-3000 spectrophotometer. Elemental analysis was performed by Carlo Erba Instruments Model EA 1180 elemental analyzer. The average molecular weights were determined by gel permeation chromatography (GPC: Waters 410). DMF was used as an eluent. The morphology was taken on a optical micro-photograph (Carlzeiss) with a magnifying power of 32.

Synthesis of MAZT

The MAZT was prepared by the reaction of AZT and MAC in the presence of TEA as shown in Scheme 1. A mixture of 1.5 g (5.6 mmole) of AZT synthesized from thymidine, 0.9 mL (8.6 mmole) of TEA, and 80 mL of DME were allowed to reflux for 3 h with precautions being taken to exclude moisture from the system. The resultant clear solution was cooled to -5℃ in an ice-salt bath. And the MAC solution (0.9 mL, 8.6 mmole) with a trace amount of sulfur as an inhibitor in 10 mL dry DME was then slowly added with vigorous stirring over a period of 2 h, and then allowed to stand overnight at 0℃. The triethylamine hydrochloride salt was filtered and the filtrate was concentrated on a rotary evaporator. The concentrated solution was slowly poured into 1000 mL of diethyl ether. The diethyl ether solution was filtered and the filtrate was absolutely concentrated under reduced pressure condition. The remaining viscous liquid was washed three times with n-hexane and the residue was dried under vacuum to obtain pure viscous product of 1.22 g (65%).



Scheme 1

Homopolymerization of MAZT

A solution of 1.0 g (3.0 mmole) of MAZT and 0.0073 g (3.0×10^{-2} mmole) of BPO in 10 mL of dry 2-butanone was introduced into a dry polymerization tube. The solution was degassed twice by purging with purified N_2 gas. The tube was sealed and placed in a regulated thermostatic bath at 70±0.05℃ for 68 h under dark surroundings. The obtained polymer solution was precipitated in 100 mL n-hexane. The precipitate was collected by filtration and then dried at room temperature under vacuum. The solid product was washed several times with diethyl ether and the residue was dried under reduced pressure to a constant weight. The conversion of homopolymer was 51%.

Copolymerization of MAZT and AA

A solution of 1.0 g (3.0 mmole) of MAZT, 0.2 g (3.0 mmole) of AA and 0.0073 g (3.0×10^{-2} mmole) of BPO in 10 mL of dry 2-butanone was introduced into a dry polymerization tube. The synthetic procedure of the poly(MAZT-co-AA) was the same method as that of homopolymer except monomer pairs. The conversion of obtained copolymer was 81%.

Copolymerization of MAZT and ETGI

The procedure of thermal copolymerization of MAZT and ETGI was the same method as that of poly(MAZT-co-AA) except monomer pairs. The conversion of obtained poly(MAZT-co-ETGI) was 73%.

In vitro *cytotoxicity test*

K562 human leukemia cell line (K562-HL) as a cancer cell line and fibroblast cells obtained from 14-day chick embryos as a normal cell line (NCL) were used for cytotoxicity test. The cell lines were continuously cultured in a 50 μL RPMI 1640 (Gibco) medium supplemented with 10% fetal calf serum (Santa Ana.), 2 mM glutamine, 100 units penicillin, 100 μg streptomycin, and 0.25 μg amphotericin. Stock solutions of the compounds were prepared in DMSO, and then diluted in the medium to give the desired concentration. The maximal concentration of DMSO in the solutions was less than 0.1%, and then placed in 96-well flat bottomed plates and cultured for 48 h at 37℃ in a 5% CO_2 incubator. After adding trypan blue (0.4%, 50 μL), the number of cells were counted by using a hemocytometer. The percentage of cytotoxicity was calculated by the following equation :

$$\text{Cytotoxicity (\%)}=\frac{\text{cell number of control-cell number of treated}}{\text{cell number of control}}\times 100$$

Results and discussion

Identifications of MAZT and poly(MAZT)

MAZT was identified from its FT-IR spectrum at 2080 cm^{-1} (N_3), 1650 cm^{-1} (vinyl stretching) and 900 cm^{-1}(vinyl out of plane), with disappearance of hydroxy

absorption at 3420 cm^{-1} of AZT. In ^{1}H-NMR spectrum [Fig. 1(A)] of MAZT, several characteristic peaks were appeared at δ 1.75 (3H, s, CH_3), 1.87 (3H, s, =C-CH_3), 3.2 (2H, s, H2'), 3.64 (2H, d, H5'), 4.0 (1H, m, H4'), 4.42 (1H, m, H3'), 6.05 (1H, m, H1'), 6.0~6.21 (2H, d, CH_2=), 7.48 (1H, s, H6) and 11.3 ppm (1H, br, NH). In identification of poly(MAZT), several IR characteristic peaks were appeared at 2110 cm^{-1} (N_3) and 1710 cm^{-1} (C=O) with disappearance of vinyl absorption at 1650 cm^{-1}. In ^{1}H-NMR spectrum [Fig. 1(B)], several peaks were observed at δ 1.6~2.0 (-CH_3, MAZT), 3.0~3.6 (-CH_2-), 3.7~4.6 (AZT moiety), 6.05 (1H, m, H4') and 7.48 (1H, br, H6), with disappearance of vinyl moiety at δ 6.0~6.21 ppm.

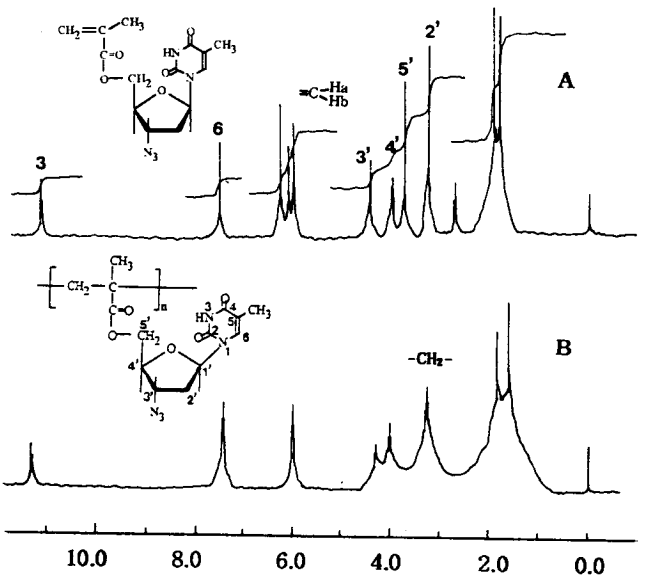


Figure 1. ^{1}H-NMR spectra of MAZT [A] and poly(MAZT) [B].

Identifications of the synthesized copolymers

The FT-IR spectrum of poly(MAZT-co-AA) indicated several absorption peaks at 3400~2400 cm^{-1} (COOH, AA), 2110 cm^{-1} (N_3, MAZT), and 1520 cm^{-1}(-CH_2-, AA). In ^{1}H-NMR spectrum[Fig. 2(A)] of poly(MAZT-co-AA), several characteristic peaks were observed at δ 0.8~1.4 (-CH-, AA), 1.6~2.0 (-CH_3, MAZT), 3.0~3.6 (-CH_2-, MAZT, AA), 3.7~4.6 (AZT moiety), 6.05 (1H, m, H4'), 7.48 (1H, br, H6), 10.6 (br, COOH) and 11.3 ppm (1H, br, NH). In the Identification of poly(MAZT-co-ETGI), the FT-IR spectrum of poly(MAZT-co-ETGI) indicated absorption peaks at 2700~3600 cm^{-1} (-COOH, ETGI), 2110 cm^{-1} (N_3, MAZT), and 1180~1200 cm^{-1} (C-N, ETGI). In ^{1}H-NMR spectrum [Fig. 2(B)], several characteristic peaks were appeared at δ 1.4~2.2 (-CH-, ETGI), 3.2~3.8 (-CH_2-, MAZT), 4.7 (N-CH_2-, ETGI), 6.2 (1H, m, H4', MAZT) and 10.7 ppm (-COOH, ETGI).

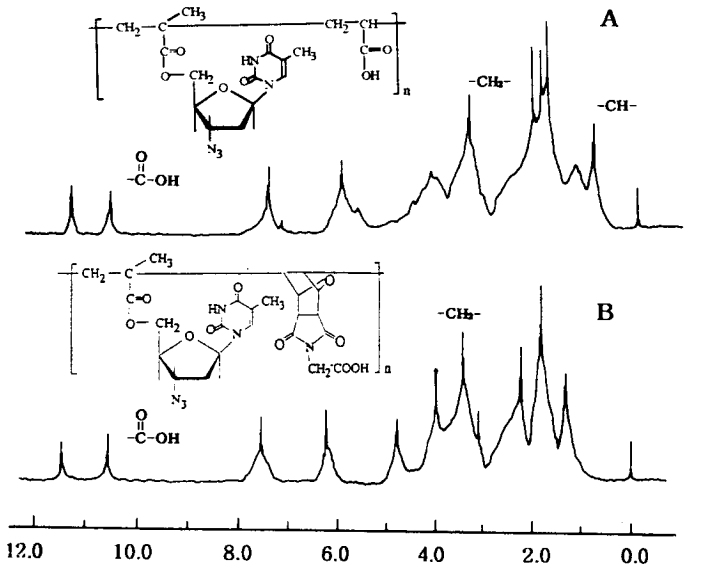


Figure 2. ^{1}H-NMR spectra of poly(MAZT-co-AA) [A] and poly(MAZT-co-ETGI) [B].

Solubility of monomer and polymers

The solubilities of synthesized MAZT and polymers were listed in Table 1. MAZT was good soluble in organic solvents but insoluble in water. MAZT and polymers were good soluble in DMF and DMSO, but polymers were insoluble or poor soluble in water, 2-butanone and diethyl ether.

Table 1. Solubility of MAZT and Polymers

Sample	Solvent						
	Water	Acetone	2-Butanone	Ethanol	Diethyl ether	DMF	DMSO
MAZT	I.S[a]	S[b]	S	P.S[c]	S	S	S
poly(MAZT)	I.S	P.S	I.S	I.S	I.S	S	S
poly(MAZT-co-AA)	I.S	P.S	I.S	P.S	I.S	S	S
poly(MAZT-co-ETGI)	I.S	S	P.S	P.S	I.S	S	S

[a]I.S, Insoluble; [b]S, soluble; [c]P.S, poorly soluble.

Contents and average molecular weights

The contents and average molecular weights of the synthesized polymers were shown in Table 2. The contents of MAZT unit in poly(MAZT-co-AA) and poly(MAZT-co-ETGI) determined by elemental analysis and integration of characteristic peaks on the ^{1}H-NMR spectrum were 20.3 and 28.9 mole%,

respectively. The average molecular weights and polydispersity indices were as follows: $\bar{M}n$=4,400, $\bar{M}w$=8,800, $\bar{M}w/\bar{M}n$=2.0 for poly(MAZT); $\bar{M}n$=19,500, $\bar{M}w$= 23,400, $\bar{M}w/\bar{M}n$=1.2 for poly(MAZT-co-AA); $\bar{M}n$=20.400, $\bar{M}w$=32,600, $\bar{M}w/\bar{M}n$= 1.6 for poly(MAZT-co-ETGI). Ottenbrite et al[22] has been reported that the optimum antitumor activity can be obtained in the range of the average molecular weights from 10,000 to 30,000 depending on polymers. Therefore, the average molecular weights of the synthesized polymers were in the reasonable ranges to exhibit the antitumor activity.

Table 2. Content of MAZT unit in Copolymers and Average Molecular Weights

Polymer	Content of MAZT unit in copolymer (mole%)	Molecular weight		$\bar{M}w/\bar{M}n$
		$\bar{M}n$	$\bar{M}w$	
poly(MAZT)	--	4,400	8,800	2.0
poly(MAZT-co-AA)	20.3	19,500	23,400	1.2
poly(MAZT-co-ETGI)	28.9	20,400	32,600	1.6

Cytotoxicity against K-562 human leukemia and normal cell lines

In order to determine the cytotoxicity on the concentration of samples, K562 human leukemia cell line (K562-HL) and normal cell line (NCL) were incubated with varying sample concentrations (1～100 μg/mL) for 48 h. As shown in Table 3, the *in vitro* cytotoxicities at 100 μg/mL were increased in the following order: for K562-HL, poly(MAZT-co-AA) > poly(MAZT-co-ETGI) > poly(MAZT) > MAZT > control > AZT; for NCL, AZT > poly(MAZT-co-ETGI) > poly(MAZT) > control > MAZT > poly(MAZT-co-AA). The cytotoxicities of polymers against K562-HL increased with increasing concentration. Among synthesized polymers, the cytotoxicity of poly(MAZT-co-AA) showed the highest value against K562-HL and the lowest value against NCL. This means that the synthesized poly(MAZT-

Table 3. Effect of Sample Concentration on Cytotoxicity against K562-Human Leukemia and Normal Cell Lines

Cell line / Sample	Cytotoxicity(%)					
	1 (μg/mL)		10 (μg/mL)		100 (μg/mL)	
	K562-HL[a]	NCL[b]	K562-HL	NCL	K562-HL	NCL
control	0.0	0.0	0.0	0.0	0.0	0.0
AZT	27.3	74.4	-70.0	84.9	-87.3	89.5
MAZT	-24.5	95.3	-14.5	30.2	36.4	-40.0
poly(MAZT)	-6.4	84.8	9.1	58.1	49.1	17.4
poly(MAZT-co-AA)	76.4	35.1	78.0	32.8	90.9	-46.5
poly(MAZT-co-ETGI)	45.5	82.9	72.7	67.4	76.4	39.5

[a]K562-HL was cultured in a 50 μL RPMI-1640 medium. [b]NCL was examined by use of fibroblast cells obtained from 14-day chick embryos.

co-AA) has a strong destruct power against K562-HL and extremely low toxicity against NCL. Figure 3 showed the optical microphotographs of K562-HL treated [Fig. 3 (B)] with poly(MAZT-co-AA) at 100 μg/mL and that of control cell line [Fig. 3 (A)]. In Figure 3, the small ring vesicles denote the leukemia cells. The number of the small ring vesicle was drastically decreased in Figure 3(B) compared with Figure 3(A). This implies that leukemia cells are effectively destructed by the synthesized poly(MAZT-co-AA).

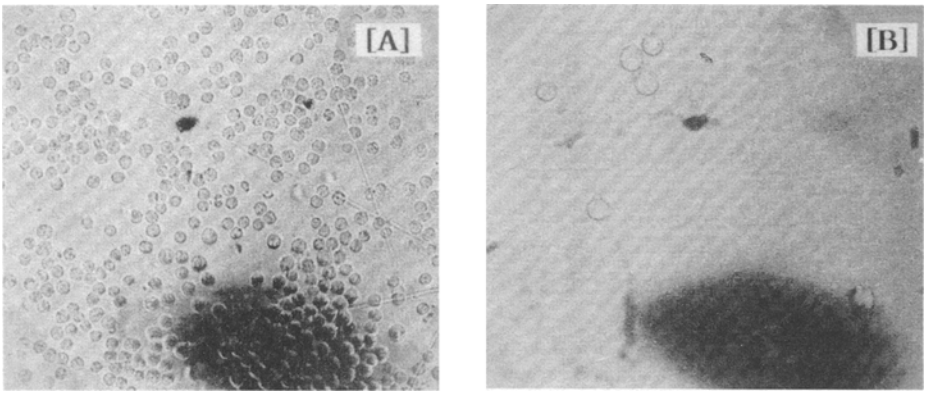


Figure 3. Optical microphotographs of K562-HL treated (B) with poly(MAZT-co-AA) at 100 μg/mL and control cell line (A) [original magnification×32].

Acknowledgement

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