Electronic Supplementary Material (ESI) for Biomaterials Science.

Pseudo-Branched Polyester Copolymer: An Efficient Drug Delivery System to Treat Cancer

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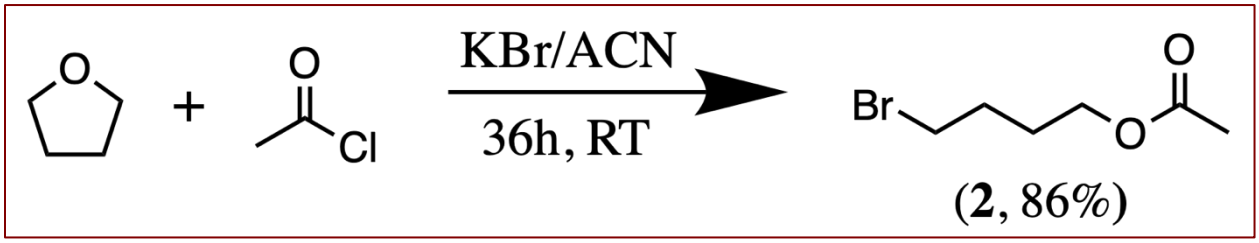
KEYWORDS: pseudo-branched polyester polymer, nanomedicine, theranostics, drug delivery, cancer therapy

Section 1: Synthetic Methods



Scheme S1: Synthesis of 4-bromobutyl acetate from THF and acyl chloride.

*Synthesis of 4-Bromobutyl Acetate (**2**)*. Tetrahydrofuran (12.2 mL, 148.4 mmol) and potassium bromide (21.1 g, 176.5 mmol) were added into a 250 mL round-bottomed flask with 150 mL of acetonitrile. The reaction mixture was cooled to 0 ºC, and by dropwise addition acetyl chloride (11 mL, 155.1 mmol) was added to the RBF. After this addition, the mixture was brought to room temperature and continuously stirred for 36 h. Water was added to the reaction mixture and ethyl acetate was used to extract the product. The organic layer was washed and dried over Na_2SO_4, and concentrated by rotary evaporation to obtain the pure product.

Yield: 24.3 g (85%). BP: >250 ºC. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 1.79 (m, 2H), 1.92 (m, 2H), 2.03 (s, 3H), 3.46 (t, 2H, J = 7.6), 4.08 (t, 2H, J = 6.7). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 20.87, 27.36, 29.36, 33.03, 63.43, 170.95. IR ($CHCl_3$): 3038, 2926, 1352, 1243, 1052 cm^{-1}.

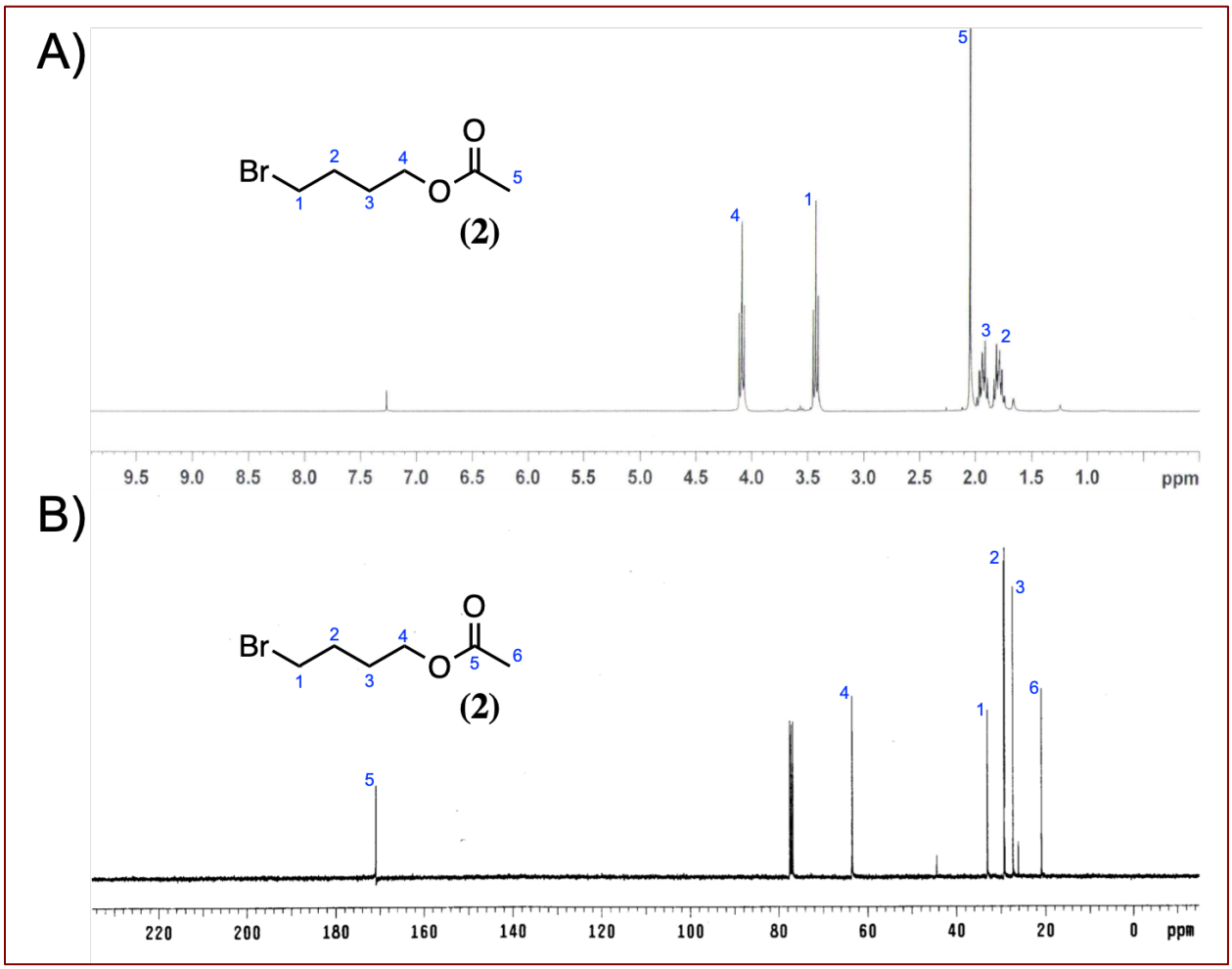


Figure S1: A) ^{1}H NMR and B) ^{13}C NMR of 4-bromobutyl acetate (**2**).

*Synthesis of 2-(4-Acetoxybutyl)malonic Acid Diethyl Ester (**3**)*. Diethyl malonate (**1**) (10 g, 62.5 mmol) and 4-bromobutyl acetate (**2**) (15.84 g, 81.3 mmol) were combined in a round-bottomed flask with acetonitrile (120 mL) and stirred for 2 min at room temperature.{Santra:2010ik} Potassium carbonate (34.5 g, 250.1 mmol) was added and the mixture was refluxed for 36 h. The resulting mixture was filtered and concentrated via rotary evaporation to obtain a yellow liquid, extracted with ethyl acetate, and washed with water. The organic layers were combined and dried over Na_2SO_4, and purified by column chromatography using 4% ethyl acetate in petroleum ether as the eluent.

Yield: 13.02 g (76%). BP: 250 ºC. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 1.28 (t, 6H, J = 7.6), 1.38 (m, 2H), 1.62 (q, 2H, J = 7.2), 1.98 (q, 2H, J = 7.7), 2.05 (s, 3H), 3.34 (t, 1H, J = 7.7), 4.09 (t, 2H, J = 6.6), 4.22 (q, 4H, J = 7.2). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 14.06, 20.79, 23.74, 28.25,

28.25, 51.84, 61.27, 63.89, 169.31, 171.11. IR ($CHCl_3$): 2982, 1728, 1463, 1367, 1233, 1151, 1029, 860 cm^{-1}.

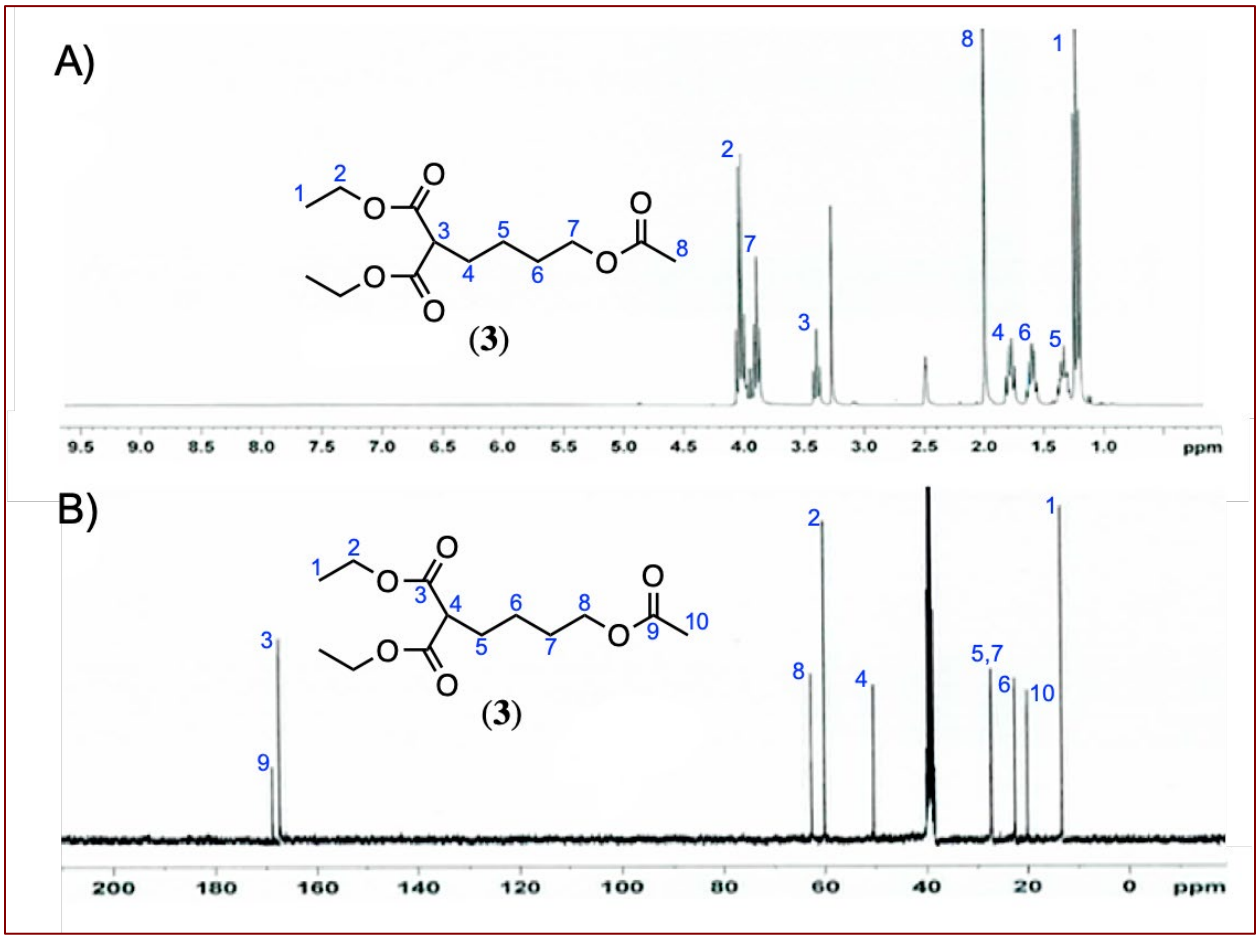


Figure S2: A) ^{1}H NMR and B) ^{13}C NMR of 2-(4-acetoxybutyl)malonic acid diethyl ester (**3**).

*Synthesis of 2-(4-Hydroxybutyl)malonic Acid (**4**)*. 2-(4-Acetoxybutyl)malonic acid diethyl ester (**3**) (5.0 g, 18.25 mmol) was added to a 100 mL round-bottomed flask with 50 mL of methanol, and stirred at room temperature for 2 min. To this, NaOH (2.1 g, 54.74 mmol) in water (7 mL) was added and the reaction was stirred at 90 ºC for 8 h. The reaction mixture was then brought down to room temperature and acidified (pH 2-3) with dilute hydrochloric acid while stirring at room temperature. The resulting mixture was concentrated using a rotary evaporator. The mixture was diluted with chloroform (50 mL) and nitrogen gas was bubbled through the mixture at 60 ºC to remove HCl, filtered, and then concentrated. The concentrated solution was purified by column chromatography using 35% ethyl acetate in petroleum ether as eluent.

Yield: 2.31 g (72%). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 1.41 (m, 2H), 1.59 (m, 2H), 1.91 (q, 2H, J_1 = 7.3, J_2 = 7.8), 3.37 (t, 1H, J = 7.4), 3.64 (t, 2H, J = 6.5), 5.54 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 23.53, 28.52, 31.75, 52.64, 62.11, 170.55. IR ($CHCl_3$): 3507, 2941, 1710, 1626, 1459, 1438, 1391, 1198, 1157, 1050, 947, 772, 741, 664 cm^{-1}.

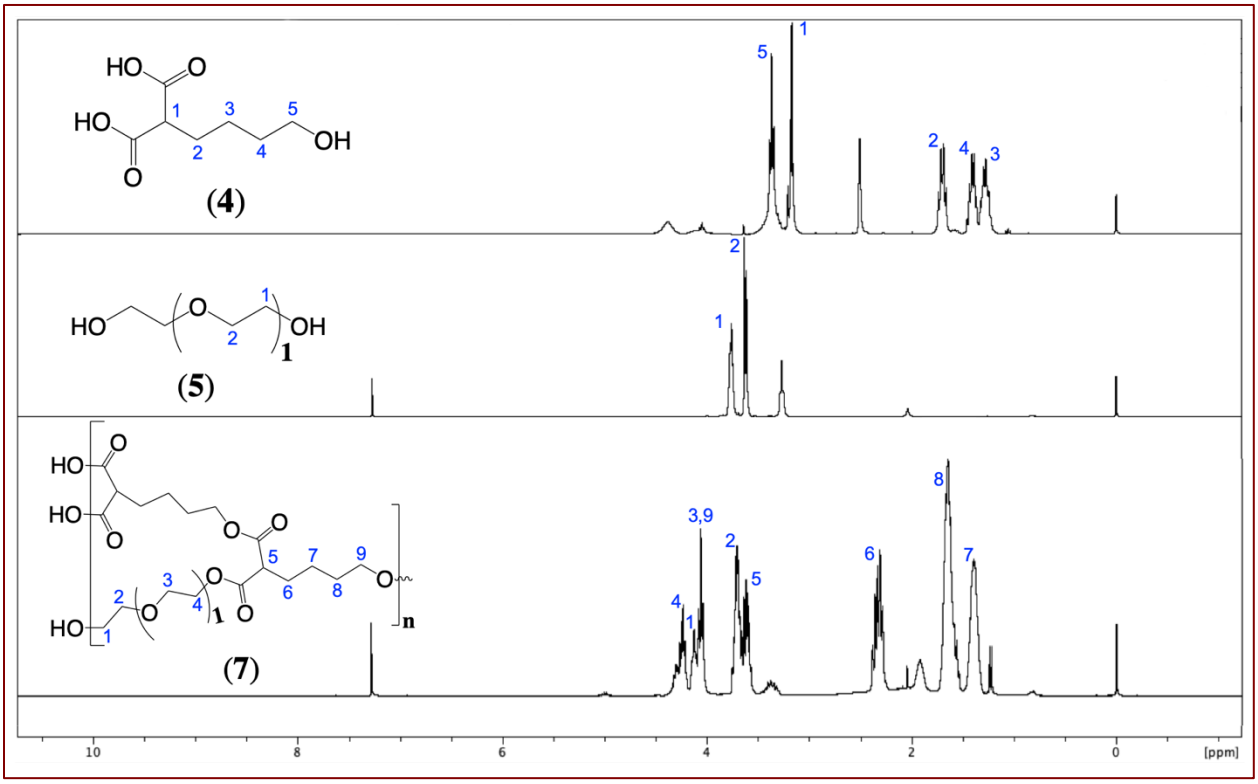


Figure S3: ^{1}H NMR spectra of the monomers: A_2B monomer (**4**), triethylene glycol (**5**), and subsequent PBPE co-polymer (**7**).

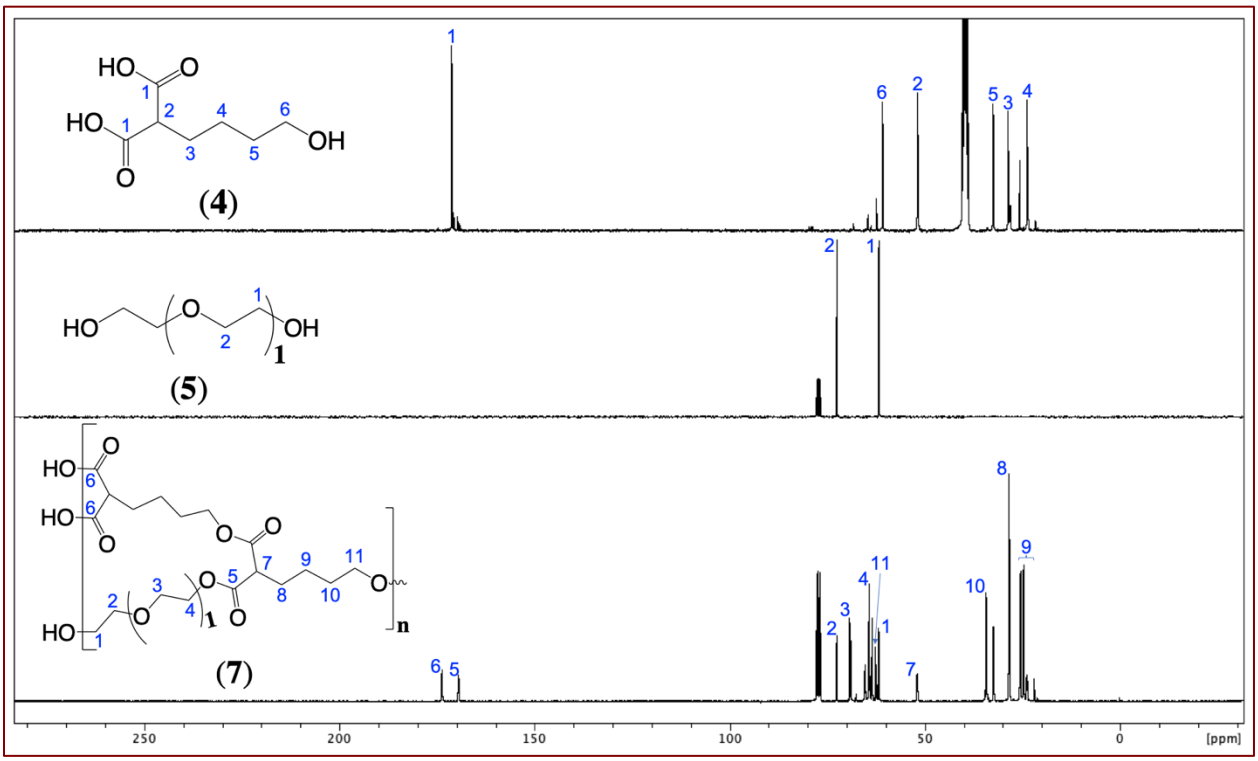
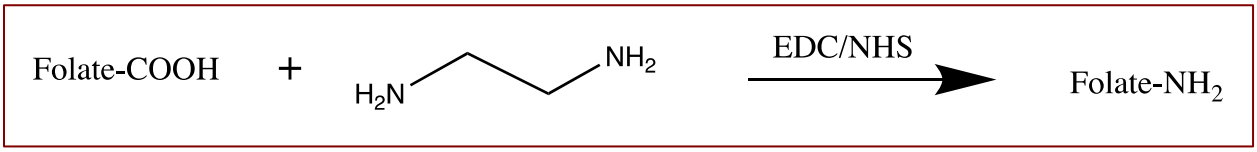


Figure S4: ^{13}C NMR spectra of the monomers: A_2B monomer (**4**), triethylene glycol (**5**), and subsequent PBPE co-polymer (**7**).



Scheme S2: Synthesis of folate-amine from ethylenediamine using EDC/NHS chemistries.

Synthesis of folate-amine. Our PBPE nanoparticles use folate as a targeting ligand for LNCaP cells. To prepare folic acid for conjugation, we prepared four solutions: (1) EDC (0.005 g) in 100 µL of MES buffer, (2) NHS (0.004 g) in 100 µL of MES buffer, (3) EDA (0.001 g) in 100 µL of DMF, and (4) folic acid (0.050 g) in 100 µL of PBS buffer. Solution (1) was added to solution (4) and allowed to mix for 10 seconds, followed by the addition of solution (2) with 3 minutes of mixing, and finally the dropwise addition of solution (3) followed by mixing on a table mixer at room temperature overnight, forming a solution of aminated folic acid.