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Selective O-Alkylation of 2,2′-Bis(hydroxymethyl)propionic Acid to Synthesize Biodegradable Polymers for Drug Delivery Applications

Santimukul Santra,* Zachary Shaw, Raghunath Narayanam, Vedant Jain, and Tuhina Banerjee

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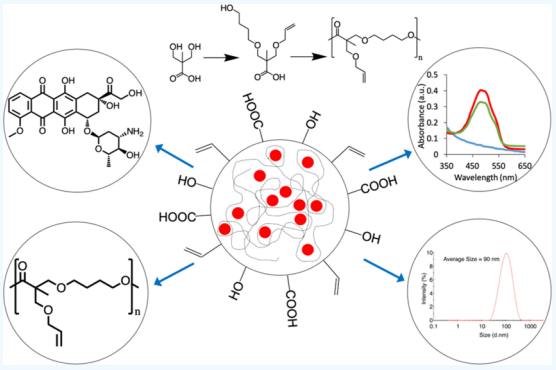
ABSTRACT: A facile synthetic approach to the selective mono- and di-O-alkylation of a β,β-disubstituted hydroxy compound has been demonstrated and applied to the field of polymer chemistry and drug delivery. 2,2′-Bis(hydroxymethyl) propionic acid (bis-MPA) is a β,β-disubstituted hydroxy compound with one carboxylic acid group (AB_2 system); therefore, it is a very important starting material in the synthesis of functional aliphatic macromolecules. The etherification (O-alkylation) of bis-MPA was successful with different alkyl functionalities using this facile approach, where 1,4-diazabicyclo [2.2.2] octane was used as a catalyst. This method successfully prevented the reported internal cyclization of bis-MPA, allowing for selective functionalization. Mono- and di-O-alkylated bis-MPA were synthesized selectively in high yields by controlling the equivalent ratio of the reactants and reaction time. Nonuniform bifunctional O-alkylated bis-MPA with different alkyl halides were prepared using the same reaction conditions and have been used for the synthesis of functional polyester polymers. Postsurface functionalization by the means of bromination was demonstrated on the allyl-functionalized linear polyester. All the compounds were characterized by ^1H and ^{13}C Nuclear Magnetic Resonance, Fourier transform infrared, thermogravimetric analysis, differential scanning calorimetry, size exclusion chromatography, and electrospray and matrix-assisted laser desorption/ionization−time of flight mass spectroscopic techniques. The synthesized functional polyester polymer was able to formulate stable polymeric nanoparticles using the solvent diffusion method and encapsulate therapeutic drug doxorubicin with higher encapsulation efficiency (EE_{Doxo} = 71%). This facile synthetic approach of O-alkylation provides direction for the synthesis of bis-MPA-based functional linear and dendritic macromolecules for drug delivery and other biomedical applications.



KEYWORDS: *bis-MPA, selective O-alkylation, functional polymers, molecular encapsulation, drug delivery*

INTRODUCTION

Aliphatic polymers have a wide variety of applications in the biomedical field, including surgery, pharmacology, and the environment because of their known biodegradability and biocompatibility.[1,2] Both linear and dendritic aliphatic polyester polymers have been prepared toward applications in the biomedical field. Typically, monomers used for synthesizing biodegradable linear polyesters are glycolide (forming polyglycolic acid),[3,4] lactide (forming polylactic acid),[5,6] and caprolactone (forming polycaprolactone).[7,8] Another important polymer derived from glycolide and lactide is poly(lactic-*co*-glycolic acid).[9,10] These monomers are easily polymerized through ring-opening polymerization forming AB intermediates; however, their functionality is limited due to there being only terminal functional groups and have limited solubility in common organic solvents. To overcome these limitations, multifunctional monomers have been used to synthesize functional linear and branched aliphatic polyesters. Typically, these polymers are derived from aliphatic, multifunctional starting materials that upon polymerization provide multifunctional polymers, which include $A_2 + B_3$ systems and A_2B systems, among others. Multifunctional linear polyesters have been synthesized using these systems and generally involve the use of specialized catalysts or the multistep protection and deprotection of functional groups to prevent cross-linking and other unwanted reactions. Typical $A_2 + B_3$ systems include alkyl diacids and triols which have been used to synthesize functional linear polyesters.[11,12] The synthesis of amino acid-based polyesters has also been reported using beta alanine-derived $AB_2 + A_2$ systems.[13,14] These linear and branched architectures obtained were used for effective encapsulation and delivery of theranostic molecules. A_2B and AB_2 monomers are unique in that they are not binary

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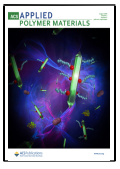

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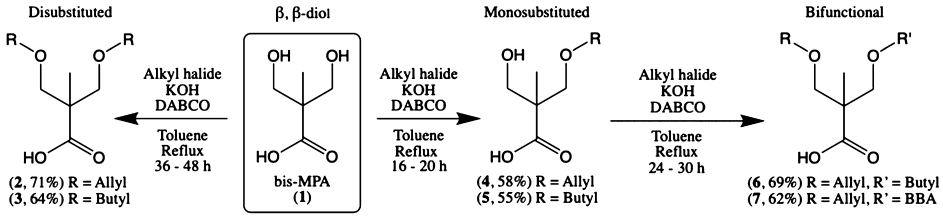
Scheme 1. Simple and Efficient Synthetic Approach for the Selective O-Alkylation of bis-MPA[a]



[a]BBA: 4-bromobutyl acetate.

monomer systems. These systems usually generate hyperbranched polymers forming multifunctional dendritic structures. Functional A_2B compounds such as diethyl malonate-derived monomers form hyperbranched architectures with many cavities and pores, allowing for high encapsulation efficiency, and the polyester backbone provides efficient drug release profiles.[15−17] 2,2′-Bis(hydroxymethyl)propionic acid (bis-MPA) has also been used to synthesize polymers; however, there are some synthetic limitations to overcome in order to synthesize bis-MPA-derived functional polymers.

Bis-MPA is a *β,β*-disubstituted hydroxy compound having two primary hydroxyl groups and one carboxylic acid group (AB_2 system); therefore, it has become an important starting material for the synthesis of polyester polymers. Although bis-MPA is an important branching unit in the field of dendrimers and hyperbranched polymers, the O-alkylation (*β,β*-disubstitution) using various alkyl halides is still a major challenge. The inherent difficulties in O-alkylation of bis-MPA are due to (i) lower reactivity of *β,β*-disubstituted primary hydroxyl groups toward the nucleophilic substitution reaction; (ii) intramolecular cyclization[18] of bis-MPA at elevated temperature, and (iii) formation of the cross-linked polymeric material (low-molecular weight).[18]

Hence, it will be highly desirable if one can develop a method for O-alkylation of bis-MPA with various surface functional moieties to generate a library of etherified bis-MPA for the synthesis of a wide range of wholly aliphatic linear and branched polymers. To overcome these drawbacks, scientists have proposed the protection of hydroxyl groups using an acetonide group to synthesize a variety of bis-MPA-based dendritic structures.[19−24] Linear aliphatic polycarbonates have been synthesized from bis-MPA using ethyl chloroformate to protect the hydroxyl groups of bis-MPA, which could be then polymerized to a polycarbonate.[25−28] There are reports available on the etherification of bis-MPA using benzyl chloride by Rehnberg and co-workers,[29] but the principle could not be applied to other alkyl halides because of the inherent cyclization of bis-MPA under the reported reaction conditions. Therefore, there is a need for a facile approach for the selective mono- and di-O-alkylation of bis-MPA to synthesize multifunctional linear and dendritic polymers.

In this direction, we developed a synthetic strategy for the selective mono- and di-O-alkylation of bis-MPA. Our experiments indicated that the use of 1,4-diazabicyclo [2.2.2] octane (DABCO) as a catalyst minimized the intramolecular cyclization of bis-MPA and substantially increases the yield of the reaction. Using this synthetic strategy, we were successful in synthesizing mono-, di-, and bifunctional bis-MPA. These multifunctional bis-MPAs can be used for the synthesis of various linear and branched polymers. Herein, we developed a new linear functional polyester derived from O-alkylated bis-MPA with high-molecular weight. The ease of postfunctionalization was demonstrated using the reactive surface functional groups. In addition, the synthesized new aliphatic polyester polymer was able to encapsulate molecular drugs in the cavity of polymeric nanoparticles (PNPs) for the potential drug delivery applications. Therefore, this new linear polymer opens new routes for the synthesis of a variety of other functional polymers, which can be used as drug delivery systems. Together, our successful O-alkylation of bis-MPA addresses the challenges associated with bis-MPA and consequently opens the field of macromolecular science for developing various linear and dendritic structures for biomedical applications.

RESULTS AND DISCUSSION

This synthetic challenge involves the establishment of an easy and versatile method for preparing etherified bis-MPA with different functional moieties. The reaction conditions were optimized in such a way that led to the formation of desired products while minimizing side reactions such as the formation of cyclic bis-MPA and cross-linked products, as known in the literature.[18] O-alkylated bis-MPA compounds (**2**−**7**) were prepared by reacting several alkyl halides with the free hydroxyl groups of bis-MPA using potassium hydroxide (KOH) as a base and a catalytic amount of DABCO (Scheme 1). DABCO was used as a catalyst because it increases the nucleophilicity of the aliphatic hydroxyl groups and thus facilitates the reaction. In this direction, compounds **2**−**7** were synthesized in high yields by refluxing in toluene in the presence of corresponding halides, and reaction times were adjusted to obtain the highest yields, depending on the reactivity of the various halides used in the reaction.

To this end, di-O-alkylated bis-MPA (**2**−**3**) was prepared by using excess alkyl halides, potassium hydroxide, and a catalytic amount of DABCO in toluene (Scheme 1). The reaction mixture was acidified (pH = 5) with 1 M HCl, and the use of water was avoided during extraction because the products were found to be partially water soluble. The crude mixture obtained after evaporation was directly loaded on a column for purification, and the desired compound was purified using an appropriate mixture of solvents. Both products were characterized by Fourier transform infrared (FT-IR), ^{1}H and ^{13}C NMR spectroscopic techniques, high-resolution mass spectrometry (HRMS), and elemental analysis (Figures 1 and 2; Supporting Information, Figures S1 and S2).

This method was also used for synthesizing mono-O-alkylated products (**4**−**5**; Scheme 1) and was successful by adjusting the molar ratio of the starting materials, which is an important factor in selectivity. The reaction was monitored with respect to time in order to find the duration that provided the highest yield of monoalkylated products and formation of

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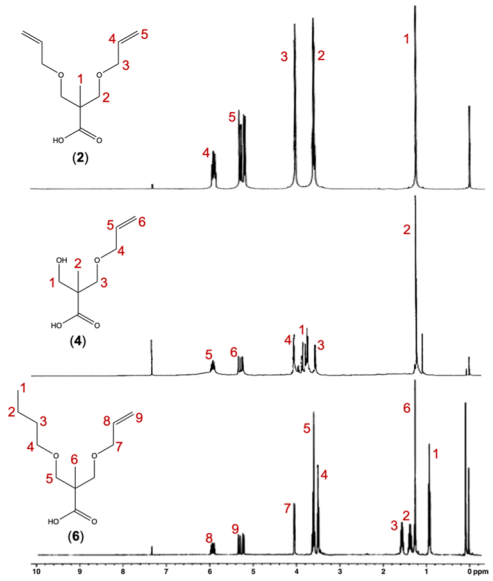

Figure 1. ^{1}H NMR of mono- and dialkylated bis-MPA using allyl chloride and bifunctional butyl-allyl bis-MPA.

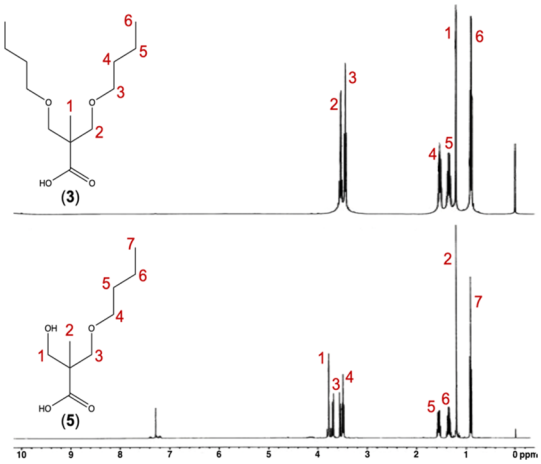

Figure 2. ^{1}H NMR of mono- and dialkylated bis-MPA using butyl bromide.

no to minimal disubstituted product. In the case of monoalkylation, only 1.2 equiv of alkyl halides were used with 1.0 equiv of bis-MPA to minimize the probability of forming disubstituted products during the reaction. The advantage of mono-alkylation is that it can be further used for synthesizing heterogeneously bifunctional bis-MPA, which can be used to synthesize functional polymers. These compounds were found to be soluble in water because of the presence of one free aliphatic hydroxyl group, making the mono O-alkylated compounds more hydrophilic. Therefore, purification has been carried out directly by using column chromatography (after acidification), without adopting the work-up process to avoid water. In ^{1}H NMR at δ = 3.78 ppm (in the case of compound **5**), a distinct doublet peak indicated the presence of a methylene group attached to the hydroxyl group in the structure, confirming the successful synthesis of the monoalkylated product. Both monoalkylated products (**4**–**5**) were confirmed by FT-IR, ^{1}H and ^{13}C NMR, and mass spectroscopy, as shown in Figures 1, 2 and Supporting Information, Figures S3 and S4.

Nonuniformly di-O-alkylated bis-MPA is very important in the field of functional linear and dendritic polymer chemistry and in the field of catalysis.[30−33] Etherification has been carried out one by one with two different alkyl groups to get bifunctional bis-MPA (Scheme 1). Mono-O-allylated bis-MPA (**4**) reacted with butyl bromide to form butyl-allyl bifunctional bis-MPA compound **6**, and a similar procedure using bromobutyl acetate (BBA) was followed for the synthesis of BBA-allyl bifunctional bis-MPA compound **7** (Scheme 1). Product (**6**) obtained was purified by applying high vacuum to remove excess of *n*-butyl bromide, while compound **7** was purified by column chromatography with 25% ethyl acetate in petroleum ether. Both compounds were characterized by FT-IR, ^{1}H and ^{13}C NMR, and mass spectroscopy (Figures 1 and 3; Supporting Information, Figures S5−S6). Compound **7** can be used as an important synthon for making functional linear polyesters.

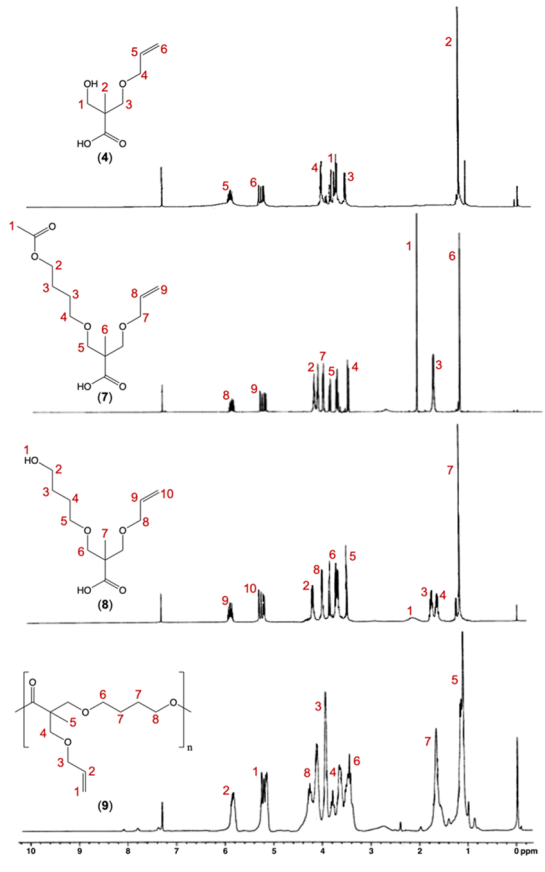

Figure 3. ^{1}H NMR of the synthetic route to linear polyester (**9**).

To make an allyl functional AB-type monomer (Scheme 2), compound **7** was hydrolyzed in the presence of a base to remove the acetyl group, resulting in monomer **8**. This compound was subsequently characterized by ^{1}H and ^{13}C NMR, FT-IR, and mass spectroscopy (Figure 3; Supporting Information, Figure S7). To obtain a polyester polymer, compound **8** was polymerized in the presence of a catalytic amount of *p*-tolulenesulfonic acid (*p*TSA) at 150 °C under reduced vacuum [size exclusion chromatography (SEC): M_w = 24,800, PD = 1.5 and thermogravimetric analysis (TGA) 10% weight loss at 243 °C] to obtain polymer **9** and was characterized by spectroscopic analyses (Figures 3 and 5). This polymer can be used to synthesize a library of functional

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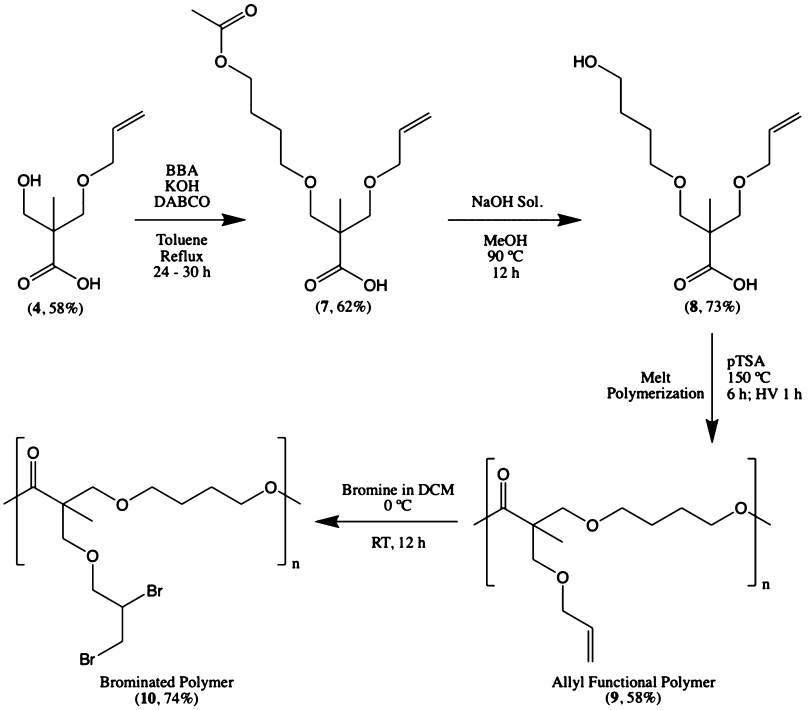
Scheme 2. Synthesis of Bifunctional O-Alkylated Bis-MPA and Their Functional Linear Polyesters



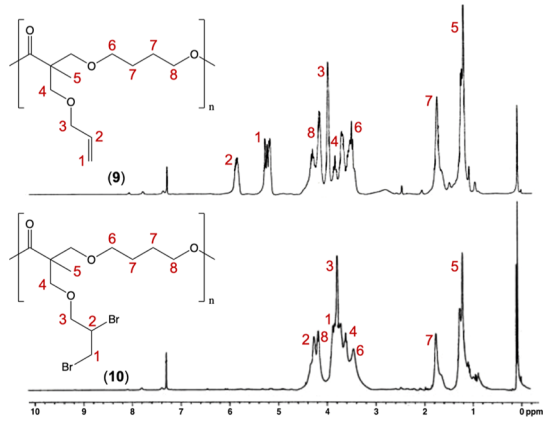


Figure 4. ^{1}H NMR of linear polyester **9** and brominated linear polyester **10**.

linear polymers using common organic synthesis. For instance, a linear polyester with pendant alkyl bromide groups (**10**) (SEC: M_w = 29,300, PD = 1.4; TGA: 10% weight loss at 241 °C) has been synthesized by bromination of the allyl functionalized polymer (**9**) and fully characterized by spectroscopic analysis (Figures 4 and 5). The successful bromination was confirmed by NMR spectroscopy (Figure 4) and FT-IR spectroscopy (Figure 5A). Matrix-assisted laser desorption/ionization−time of flight (MALDI-TOF) confirmed the formation of high-molecular-weight polymers with results showing 22,026 Da for polymer **9** and 25,593 Da for polymer **10** (Figure 6). Low-molecular-weight fragments are expected in MALDI-TOF because of the dissociation of alkoxy chains in the polymer backbone. These results are also supported by performing SEC, as shown in Figure 5B. TGA indicated moderate thermal stability for these two aliphatic polyester polymers (Figure 5C). Low-glass-transition temperatures (−40 °C) were observed for these two polymers, as expected (Figure 5D). This type of functional polyester polymer has potential applications in the biomedical field, where receptor-targeting compounds can be attached covalently to the functional groups to be used in the field of targeted drug delivery. In addition, the amphiphilic nature of the polymeric backbone due to the presence of aliphatic butyl chain and polar ester functional groups allows for the encapsulation of therapeutic compounds.[34,35]

The unique structure of aliphatic polyester polymer **9** gives a suitable platform for the delivery of therapeutic compounds for biomedical applications. The synthesized polymer being amphiphilic in nature was found to be soluble in polar solvents, including chloroform and dimethyl sulfoxide (DMSO), and suitable for the delivery of hydrophobic and amphiphilic therapeutic drugs. For effective encapsulation, the solvent diffusion method was used, and doxorubicin (Doxo) was selected as a model therapeutic drug for one-pot encapsulation. To this end, a solution of polymer **9** in DMSO (25 mg/mL, Figure 7A) was briefly mixed with Doxo solutions of various concentrations ($1-9 \times 10^{-6}$ M in DMSO) and drop-wise added to deionized (DI) water (4 mL) with continuous mixing. In this process, Doxo is forced to encapsulate within hydrophobic cavities of polymer **9** in order to avoid an outer aqueous environment, while hydrophilic functional groups of the polymer exposed to the water environment, forming stable PNPs (**11**, PNP-Doxo) via hydrogen bonding. The resulting PNPs were purified from free Doxo and organic solvents using dynamic dialysis technique against DI water. We observed an absorbance maximum at 480 nm in the aqueous PNP solution, confirming the presence of Doxo inside the nanoparticles (Figure 7B). As the concentration of Doxo loading increased during this encapsulation process, there was a linear increase in the absorbance intensity of the Doxo-encapsulated PNP solution up to the saturation point (Figure 7C). The encapsulation efficiency was calculated using the equation EE % = [(Doxo

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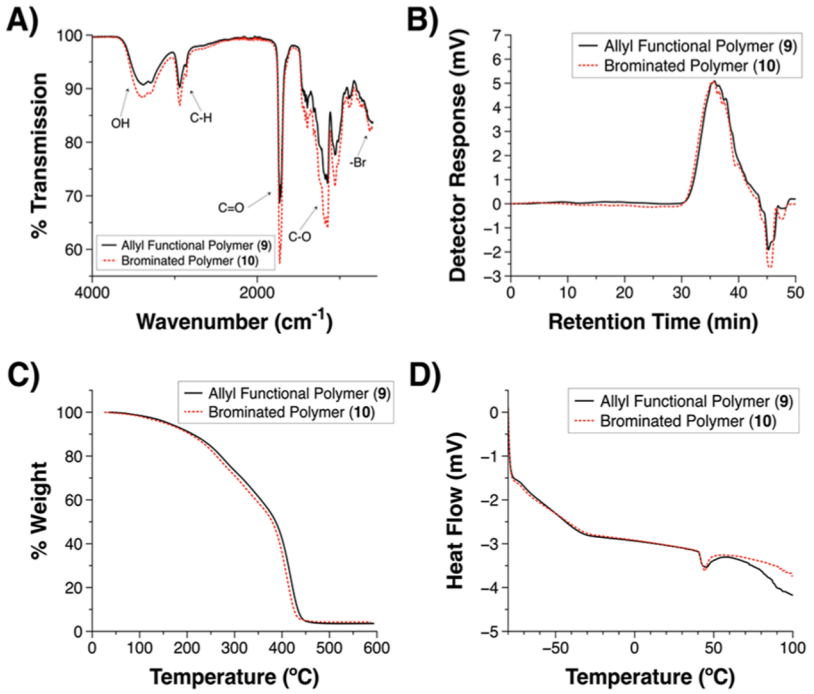

Figure 5. Characterization data for allyl functional polymer (**9**) and brominated polymer (**10**) displaying (A) FT-IR, (B) SEC, (C) TGA, and (D) differential scanning calorimetry (DSC).

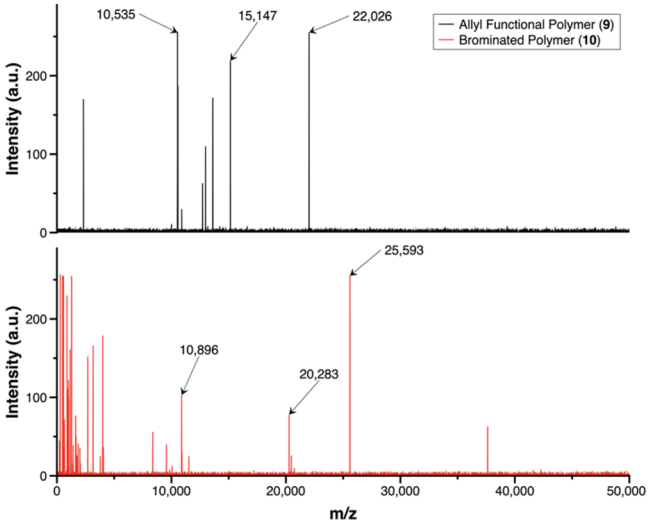

Figure 6. MALDI-TOF spectra of allyl functional polymer (**9**) and brominated polymer (**10**).

added − free Doxo)/Doxo added] × 100 and found to be EE % = 71, with an optimum loading of 5 μM. The size and surface charge of the PNPs were found to be D = 90 nm with zeta potential ζ = −21 mV (Figures 7D,E), and this result is supported by the scanning transmission electron microscopy (STEM) data. These data show that therapeutic drugs can successfully be encapsulated within the aliphatic polyester polymer **9** matrix with the potential for drug delivery applications.

To summarize, we were successful in avoiding intermolecular cyclization of bis-MPA by using a catalytic amount of DABCO in the O-alkylation of bis-MPA. A facile approach for the selective mono-, di-, and bifunctional O-alkylation of bis-MPA has been demonstrated successfully, confirmed by spectroscopic analysis. This synthetic method was used to generate a variety of functional bis-MPA compounds, applicable to other areas in the chemical sciences. Bifunctional bis-MPA has been synthesized which was applied to the field of polymer chemistry through the development of functional linear polyesters, which were then used to successfully encapsulate the therapeutic drug Doxo, giving rise to potential applications in the field of biomedical sciences as a drug delivery system. The polyesters formed were of high-molecular weight and were confirmed by spectroscopic methods. Furthermore, this synthetic strategy can be adapted for bis-MPA-based branched polymers, including hyperbranched and dendritic structures with potentials in the fields of catalysis, drug delivery, and other biomedical applications.

EXPERIMENTAL SECTION

Materials. Bis(hydroxymethyl)propionic acid (bis-MPA), 1,4-diazabicyclo[2.2.2]octane (DABCO), *p*TSA, allyl chloride, butyl bromide, potassium hydroxide, toluene, trifluoroacetic acid, and sodium hydroxide were purchased from Aldrich Chemicals Co. and used as received. Normal solvents such as chloroform, hexane, tetrahydrofuran (THF), and ethyl acetate were bought from Fisher Scientific. Deuterated chloroform ($CDCl_3$) used in ^{1}H NMR and ^{13}C NMR spectroscopies were bought from Cambridge Isotope Laboratories. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix for MALDI-TOF mass spectroscopy and purchased from Bruker. Toluene was dried by refluxing over sodium wire and distilled under nitrogen prior to use.

Instrumentations. FT-IR spectra of monomers and polymers were recorded on a PerkinElmer's Spectrum TWO infra-red spectrometer. ^{1}H NMR spectra were recorded on either VXR 300 NMR and 400 NMR spectrometers using the tetramethylsilane/solvent signal as an internal reference. Chemical shift is reported in δ scale. The abbreviations s, d, t, and m stand for singlet, doublet, triplet, and multiplet, respectively. Q-tof micro mass experiments were carried out using Waters Q-Tofmicro-YA-105. MALDI-TOF was executed using the Bruker microflex LRF MALDI-TOF. A matrix solution was prepared for the samples being analyzed using Bruker's protocol, as described in the user manual. To begin with, acetonitrile

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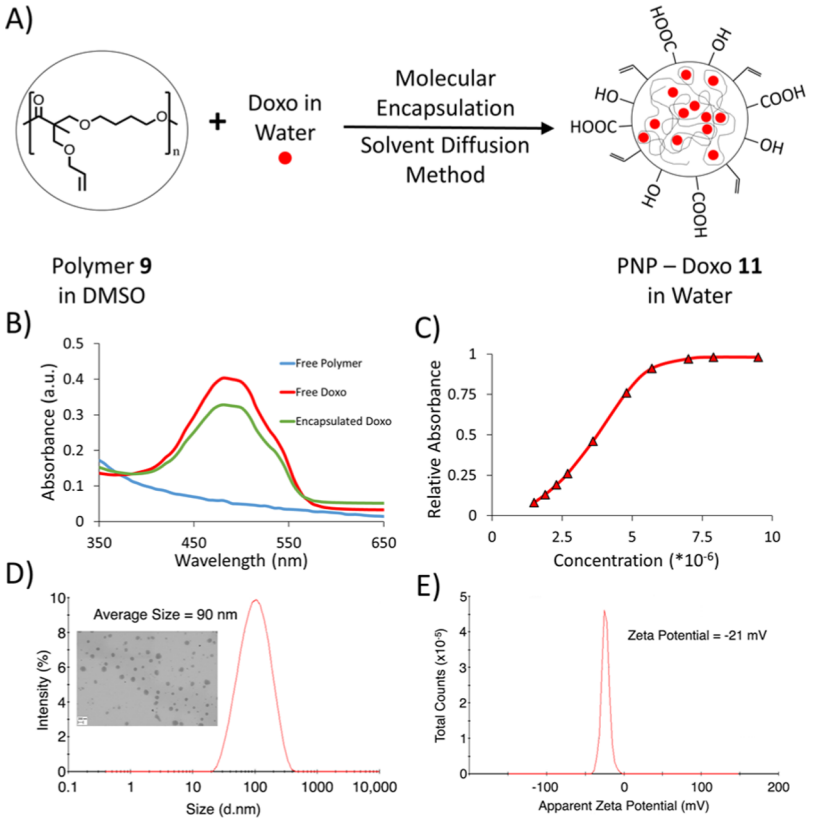

Figure 7. (A) Polymer **9** used to encapsulate Doxo with (B) UV−vis data confirming its successful encapsulation and (C) absorbance at various stock solutions of Doxo in the polymer. (D) Polymer nanoparticle's size distribution revealed by dynamic light scattering and STEM studies (inset, scale bar 200 nm), and (E) zeta potential of the PNP surface.

and DI water were mixed in a 30:70 volume ratio with 0.1% trifluoroacetic acid so that the total volume became 100 μL. Then, DHB (2 mg) was added to the TA30 to finish the matrix solution. Next, the polymer sample being analyzed (2 mg) was dried under vacuum and dissolved in 100 μL of THF. The prepared solutions (the polymer solution and the TA30 matrix solution) were added to a 1 mL Eppendorf tube and mixed (1000 rpm) for 2 min. The resulting solution was then plated (1 μL drop) to a ground steel MALDI target plate. The spots were dried completely (approximately 6 h) and added to the mass spectrometer for analysis. For gel permeation chromatography (GPC) experiments, a Waters 2410 DRI gel permeation chromatograph, consisting of four phenogel 5 μL columns filled with cross-linked polystyrene-divinylbenzene beads was used. The polymer samples (20 mg) were vacuum-dried and dissolved in stabilized THF (1 mL), filtered (0.2 μm pores), and moved to a GPC vial. The flow rate of THF was dialed to 1 mL/min at 30 °C for 50 min and calibrated against a polystyrene standard. The thermal stability of the synthesized polymers was analyzed on a TA Instruments Q50 thermogravimetric analyzer. Polymer samples (between 5 and 10 mg) were heated under a nitrogen atmosphere at a rate of 10 °C/min for 60 min from room temperature (25 °C) to 600 °C. For calorimetry measurements, polymer samples (10 mg) were analyzed on a TA Instruments Q100 DSC. The samples underwent one thermo-cycle from −80 to 100 °C at a rate of 10 °C/min. A Malvern ZS90 zetasizer was used for measuring the overall size of the PNPs, whereas Zeiss's scanning transmission electron microscope was used for determining the shape and size of the nanoparticles. UV−vis experiments were carried out using TECAN's infinite M200 Pro microplate reader.

3-Allyloxy-2-allyloxymethyl-2-methylpropionic Acid (2). Bis-MPA **1** (1 equiv), allyl chloride (5 equiv), potassium hydroxide (8 equiv), and a catalytic amount of DABCO (0.01 mole %) were taken in a round-bottom flask containing toluene, and the mixture was then refluxed for 36 h. The reaction was brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered. The filtrate was then concentrated and purified by column chromatography using 5% ethyl acetate in petroleum ether as an eluent.

Yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.22 (s, 3H), 3.58 (dd, 4H, J_1 = 3.6, J_2 = 9.7), 4.01 (d, 4H, J = 3.6), 5.22 (q, 4H, J_1 = 11.0, J_2 = 24.4), 5.88 (m, 2H), 10.5 (br s, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.98, 48.22, 71.89, 72.45, 116.94, 134.64, 180.54. IR ($CHCl_3$): 2920 (broad), 1709, 1458, 1350, 1250, 1097 cm^{-1}. Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.80; H, 8.65. Q-tof HRMS: mass determined for $C_{11}H_{18}O_4Na$ is 237.1103; found, 237.1102.

3-Butoxy-2-butoxymethyl-2-methyl Propionic Acid (3). Bis-MPA **1** (1 equiv), butyl bromide (5 equiv), potassium hydroxide (8 equiv), and a catalytic amount of DABCO (0.01 mole %) were taken in a round-bottom flask containing toluene, and the mixture was then refluxed for 48 h. The reaction was brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered. The filtrate was then concentrated and purified by column chromatography using 3% ethyl acetate in petroleum ether as an eluent.

Yield: 64%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 0.90 (t, 6H, J = 4.1), 1.20 (s, 3H), 1.38 (m, 4H), 1.54 (m, 4H), 3.38 (t, 4H, J = 6.4), 3.46 (dd, 4H, J_1 = 3.9, J_2 = 8.8), 10.5 (br s, 1H). ^{13}C NMR (75.43 MHz, $CDCl_3$, δ ppm): 13.79, 17.68, 19.26, 31.43, 50.31, 71.17, 72.35, 180.51. IR ($CHCl_3$): 2920, 1716, 1453, 1185, 1036 cm^{-1}. Anal. Calcd for $C_{13}H_{26}O_4$: C, 63.30; H, 10.64. Found: C, 63.63; H, 10.26. Q-tof HRMS: mass determined for $C_{13}H_{26}O_4Na$ is 269.1729; found, 269.1719.

2-Allyloxymethyl-3-hydroxy-2-methyl Propionic Acid (4). Bis-MPA **1** (1 equiv), allyl chloride (1.2 equiv), potassium hydroxide (4 equiv), and a catalytic amount of DABCO (0.01 mole %) were taken in a round-bottom flask containing toluene, and the mixture was then refluxed for 16 h. The reaction was brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered.

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The filtrate was then concentrated and purified by column chromatography using 20% ethyl acetate in petroleum ether as an eluent.

Yield: 58%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.21 (s, 3H), 3.53 (dd, 2H, J_1 = 3.4, J_2 = 8.2), 3.72 (m, 2H), 4.02 (d, 2H, J = 3.8), 5.21 (q, 2H, J_1 = 9.2, J_2 = 14.1), 5.86 (m, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.81, 48.67, 66.34, 72.67, 73.37, 117.47, 134.31, 179.68. IR ($CHCl_3$): 3468 (broad), 3018, 2945, 1719, 1459, 1418, 1366, 1217, 1091, 1043, 930, 869, 759 cm^{-1}. Q-tof HRMS: mass determined for $C_8H_{14}O_4Na$ is 197.0790; found, 197.0798.

2-Butoxymethyl-3-hydroxy-2-methyl Propionic Acid (5). Bis-MPA **1** (1 equiv), butyl bromide (1.2 equiv), potassium hydroxide (4 equiv), and a catalytic amount of DABCO (0.01 mole %) were taken in a round-bottom flask containing toluene, and the mixture was then refluxed for 20 h. The reaction was brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered. The filtrate was then concentrated and purified by column chromatography using 15% ethyl acetate in petroleum ether as an eluent.

Yield: 55%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 0.91 (t, 3H, J = 7.3), 1.20 (s, 3H), 1.34 (m, 2H), 1.56 (m, 2H), 3.48 (t, 2H, J = 6.7), 3.53 (dd, 2H, J_1 = 3.1, J_2 = 9.2), 3.76 (d, 2H, J = 2.5). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 13.85, 17.76, 19.28, 31.51, 48.59, 66.43, 71.77, 74.12, 179.72. IR ($CHCl_3$): 3483 (broad), 3016, 2935, 2868, 1708, 1465, 1415, 1377, 1303, 1216, 1099, 1043, 760 cm^{-1}. Q-tof HRMS: mass determined for $C_9H_{18}O_4Na$ is 213.1103; found, 213.1100.

2-Allyloxymethyl-3-butoxy-2-methyl Propionic Acid (6). 2-Allyloxymethyl-3-hydroxy-2-methyl propionic acid **4** (0.5 g, 2.87 mmol), butyl bromide (1.18 g, 8.62 mmol), potassium hydroxide (0.65 g, 11.49 mmol), and a catalytic amount of DABCO (0.01 mole %) were added to a round-bottom flask with toluene (25 mL), and the mixture was then refluxed for 30 h. The reaction was brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered. The filtrate was then concentrated and purified via column chromatography with 16% ethyl acetate in petroleum ether.

Yield: 0.45 g (69%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 0.91 (t, 3H, J = 7.4), 1.23 (s, 3H), 1.36 (m, 2H), 1.54 (m, 2H), 3.46 (t, 2H, J = 6.4), 3.55 (q, 4H, J_1 = 8.2, J_2 = 8.6), 4.01 (d, 2H, J = 5.5), 5.18 (q, 2H, J_1 = 9.2, J_2 = 24.5), 5.89 (m, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 13.95, 18.11, 19.38, 31.63, 48.23, 71.63, 72.19, 72.57, 72.61, 117.03, 134.67, 179.83. IR ($CHCl_3$): 2969, 2868, 1709, 1459, 1261, 1215, 1099, 1017, 930, 792, 759 cm^{-1}. Q-tof HRMS: mass determined for $C_{12}H_{22}O_4Na$ is 253.1416; found, 253.1415.

3-(4-Acetoxy-butoxy)-2-allyloxymethyl-2-methyl Propionic Acid (7). 2-Allyloxymethyl-3-hydroxy-2-methyl propionic acid **4** (0.25 g, 1.43 mmol), BBA (0.85 g, 4.31 mmol), potassium hydroxide (0.32 g, 5.74 mmol), and a catalytic amount of DABCO (0.01 mole %) were added to a round-bottom flask with toluene (20 mL), and the mixture was then refluxed for 24 h. The reaction was then brought to room temperature (25 °C), acidified (pH = 5) drop-wise with diluted HCl, and filtered. The filtrate was then concentrated and purified via column chromatography with 22% ethyl acetate in petroleum ether.

Yield: 0.26 g (62%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.18 (s, 3H), 1.72 (m, 4H), 2.05 (s, 3H), 3.45 (d, 2H, J = 9.2), 3.69 (t, 2H, J = 8.3), 3.83 (d, 2H, J = 11.3), 3.97 (d, 2H, J = 5.5), 4.09 (t, 2H, J = 5.8), 5.19 (q, 2H, J_1 = 9.6, J_2 = 23.9), 5.88 (m, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.61, 20.82, 25.15, 25.23, 48.89, 63.86, 66.22, 72.19, 72.33, 73.37, 116.80, 134.44, 171.01, 175.10. IR ($CHCl_3$): 2960, 1732, 1646, 1471, 1368, 1248, 1145, 1091, 1045, 925, 756 cm^{-1}. Q-tof HRMS: mass determined for $C_{14}H_{24}O_6Na$ is 311.1471; found, 311.1469.

2-Allyloxymethyl-3-(4-hydroxy-butoxy)-2-methyl Propionic Acid (8). 3-(4-Acetoxy-butoxy)-2-allyloxymethyl-2-methyl propionic acid **7** (1.0 g, 3.47 mmol) was added to a round-bottom flask with methanol (20 mL) and was stirred at room temperature (25 °C) for 2 min. Then, NaOH (0.21 g, 5.21 mmol) in water (4 mL) was added and stirred for 12 h at 90 °C. The reaction temperature was adjusted to room temperature (25 °C) and acidified (pH 2−3) drop-wise with dilute hydrochloric acid at room temperature (25 °C) with constant stirring. The resulting mixture was concentrated, reconstituted in chloroform (50 mL), and sparged with Argon gas at 60 °C to remove HCl. The resulting mixture was filtered, concentrated, and purified via column chromatography with 30% ethyl acetate in petroleum ether.

Yield: 0.62 g (73%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.17 (s, 3H), 1.64 (m, 2H), 1.73 (m, 2H), 2.18 (br s, 1H), 3.46 (d, 2H, J = 9.1), 3.69 (m, 2H), 3.82 (d, 2H, J = 11.0), 3.97 (d, 2H, J = 5.1), 4.18 (t, 2H, J = 6.1), 5.16 (m, 2H), 5.87 (m, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.83, 25.14, 29.09, 48.96, 62.11, 64.73, 66.58, 72.52, 73.63, 117.17, 134.47, 175.37. IR ($CHCl_3$): 3429, 2933, 2863, 2243, 1721, 1463, 1317, 1231, 1137, 1092, 1046, 991, 909, 732 cm^{-1}. Q-tof HRMS: mass determined for $C_{12}H_{22}O_5Na$ is 269.1365; found, 269.1373.

Polymer of 2-Allyloxymethyl-3-(4-hydroxy-butoxy)-2-methyl Propionic Acid (9). 2-Allyloxymethyl-3-(4-hydroxy-butoxy)-2-methyl propionic acid (**8**) and the catalyst *p*TSA (100:1 molar ratio) were taken in a polymerization tube and were dried under high vacuum followed by the release of vacuum using dry argon gas. Then, the tube was slowly heated to 150 °C under an argon atmosphere using an oil bath, and it was maintained at this temperature for 6 h. The melt was evacuated at 0.2 mm/Hg for 1 h while maintaining the same polymerization temperature. The polymer was purified by reprecipitating into methanol from chloroform solution of the polymer. This was then centrifuged, washed with solvent, and dried in a vacuum oven, resulting in a pure polymer.

Yield: 58%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.11 (s, 3H), 1.14 (s, 2H), 1.65 (s, 2H), 3.41 (m, 4H), 3.61 (m, 2H), 3.89 (br s, 2H), 4.07 (br s, 2H), 5.15 (m, 2H), 5.79 (m, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.86, 25.25, 29.14, 47.65, 48.93, 49.24, 62.25, 64.25, 66.21, 66.86, 72.53, 73.69, 117.16, 134.87, 175.33. IR ($CHCl_3$): 3498, 2956, 2857, 2248, 1721, 1462, 1377, 1256, 1124, 1093, 1041, 990, 908, 732, 646 cm^{-1}.

Bromination of the Allyl Functionalized Linear Polyester (10). Allyl functionalized linear polyester **9** (0.08 g) was added to a round-bottom flask with dichloromethane (DCM) (10 mL) at 0 °C. A solution of bromine in DCM (1:1 ratio) was then added drop by drop till the color of bromine just remained in the solution while maintaining the same reaction temperature with continuous stirring. The reaction mixture was then brought to room temperature and stirred for 12 h. The DCM layer was concentrated under reduced pressure to get the brominated polymer as a highly viscous liquid. The polymer was then purified by reprecipitating in methanol from its chloroform solution.

Yield: 74%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, J Hz): 1.20 (s, 3H), 1.25 (s, 2H), 1.74 (br s, 2H), 3.44 (s, 2H), 3.60 (m, 4H), 3.77 (m, 4H), 4.16 (s, 2H), 4.25 (s, 1H). ^{13}C NMR (100.56 MHz, $CDCl_3$, δ ppm): 17.79, 25.30, 29.73, 3296, 47.59, 48.86, 49.02, 64.48, 64.77, 66.18, 72.30, 73.15, 74.35, 175.06. IR ($CHCl_3$): 3441, 2923, 2863, 1728, 1633, 1460, 1361, 1297, 1258, 1235, 1123, 1039, 796, 656, 605 cm^{-1}.

Synthesis of Doxo-Encapsulating PNP Nanoparticles (11): Solvent Diffusion Method. The polyester polymer (**9**, 300 μL, 25 mg/mL of DMSO) solution was transferred to an Eppendorf tube, and various concentrations of Doxo (1−9 × 10^{-6} M in DMSO) were added and vortexed. The polymer−drug mixture was then added drop-wise to 4 mL DI water taken in a 15 mL Eppendorf tube with continuous mixing. The resulting mixture was pipetted off into a dialysis bag (MWCO = 6−8 K) for purification using DI water for 24 h. The fluorescence spectroscopy method was used for the calculation of encapsulation efficiency of the synthesized polymer using the equation EE % = [(cargo added − free cargo)/cargo added] × 100. The synthesized PNPs (**11**, PNP-Doxo, Figure 7) appeared as a pink colored milky solution, and the PNPs were found to be stable in aqueous solution and stored at 4 °C for further characterizations.

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■ ASSOCIATED CONTENT

Supporting Information

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Characterization data: ^{13}C NMR of functional bis-MPA compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Santimukul Santra − *Department of Chemistry, Pittsburg State University, Pittsburg, Kansas 66762, United States;* orcid.org/0000-0002-5047-5245; Email: ssantra@pittstate.edu

Authors

Zachary Shaw − *Department of Chemistry, Pittsburg State University, Pittsburg, Kansas 66762, United States*

Raghunath Narayanam − *Department of Chemistry, Pittsburg State University, Pittsburg, Kansas 66762, United States*

Vedant Jain − *Department of Chemistry, Pittsburg State University, Pittsburg, Kansas 66762, United States*

Tuhina Banerjee − *Department of Chemistry, Pittsburg State University, Pittsburg, Kansas 66762, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsapm.0c00509

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