Published in final edited form as:

Polym Chem. 2018 May 14; 9(18): 2434–2437. doi:10.1039/c8py00406d.

Cholesterol functionalized aliphatic *N*-substituted 8-membered cyclic carbonate†,‡

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Abstract

Straightforward synthesis of cholesterol functionalized aliphatic *N*-substituted 8-membered cyclic carbonate (Chol-8m) monomer is reported. Well-defined poly(ethylene glycol) (PEG) diblock copolymers were readily accessed via organo catalytic ring opening polymerization. These polymers show promise as building blocks for self-assembled nanostructures and steric stabilizers for liposomes.

Efficient and scalable synthesis of functional monomers is crucial for successful commercialization of degradable polymers for nanobiomedicine.¹ Recently a few aliphatic carbonate platforms have enabled straightforward synthesis of functional monomers.^{2–5} Particularly, amino diol based precursors have emerged as an attractive starting material to access novel functional materials.^{3,4,6} An inherent difference in reactivity of amines and alcohols can be utilized to create functionalized cyclic carbonate monomers, in a chemo-selective and step-efficient manner. Amines in the presence of unprotected alcohols have been shown to react with a wide range of nucleophiles, including alkyl halides, chloroformates and acrylates. This first step results in a functionalized diol that can be further cyclized in an intra-molecular fashion to produce target monomers.^{3,4} With the relative ease in introduction of functionalities and no need for protection and deprotection of alcohols, potential large-scale production of a wide variety of functional monomers, and subsequently polymers, is feasible.

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‡Electronic supplementary information (ESI) available: Details of experimental methods, additional TEM micrograph, NMR spectra and SEC chromatographs. See DOI: [10.1039/c8py00406d](https://doi.org/10.1039/c8py00406d)

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Conflicts of interest

There are no conflicts of interest to declare.

Serinol,^{3,6,7} ammediol,⁸ and diethanolamines^{4,9,10} have all been used to synthesize functionalized carbonate monomers. Depending upon the exact choice of amino diol, 6 or 8-membered cyclic carbonates are produced. Among commercially available amino diols, diethanolamine (DEA) is appealing due its low cost. DEA allows for the synthesis of aliphatic *N*-substituted functional 8-membered cyclic carbonates.^{4,9,11} Moreover, several functionalized diethanolamines are readily available, rendering this an attractive platform. Herein, we report two-step access to Chol-8m from commercially available DEA and cholesteryl chloroformate. Subsequent organo-catalytic ring-opening polymerization of this monomer results in well-defined PEGylated amphiphilic diblock copolymers. The aqueous self-assembly behavior and potential utility of these block copolymers as an inexpensive steric stabilizer for liposomes are also discussed.

Cholesterol, a biologically relevant and hydrophobic moiety has been shown to direct self-assembly and also serve as an excellent handle to encapsulate hydrophobic drugs.^{12,13} Incorporation of cholesterol into biodegradable platforms allows for the development of materials well suited for biomedical applications.^{12,14} In this work, cholesterol-functionalized monomer was synthesized on multigram scale in a two-step process. First, DEA was allowed to react with commercially available cholesterol chloroformate in the presence of Na₂CO₃ in a tetrahydrofuran (THF)–water solvent mixture to result in cholesterol-functionalized diol **1** (Scheme 1). Functional diol was isolated in nearly quantitative yield through solvent–solvent extraction and was then subjected to intramolecular cyclization with ethyl chloroformate as the cyclization reagent to yield target monomer **2** (Scheme 1). The crude product was purified by recrystallization from a dichloromethane (DCM) : diethyl ether mixture (1 : 1 by volume fraction), to result in polymerizable-grade monomer as shiny, flaky crystals and with an overall yield of ≈74%. In contrast to previous reports to synthesize cholesterol-functionalized cyclic carbonate monomers,^{3,5,15,16} the current approach with its straightforward purification (*i.e.*, no need for flash-column chromatography) makes it more commercially practical.

We explored organo-catalytic ring opening polymerization (ROP) of monomer **2**, by using commercially available macroinitiator, polyethylene glycol monomethyl ether (mPEG-OH, 2.4 and 5.0 kDa). The ROP reaction was carried out at room temperature for about 2 h using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5% by mol with respect to monomer) as the catalyst and DCM as the solvent. By varying the mPEG-OH and by changing the feed ratio of monomer *versus* initiator, a series of diblock copolymers, **3a–k** (mP(EG)_{*m*}-*b*-P(**2**)_{*n*}), was readily synthesized (Table 1). In general the observed degree of polymerization (DP_{*n*}) as determined by ¹H nuclear magnetic resonance (NMR) spectroscopy (by comparing resonances due to signals from cholesteryl protons to that of signals from PEG protons), demonstrated very good agreement to the initial feed ratio (mPEG-OH : monomer **2**). The series of synthesized block copolymers has amphiphilic balance tuned over a broad range (PEG mass fractions *f* ranging from 0.87 to 0.18). All polymers have a relatively low polydispersity ($M_w/M_n < 1.2$), suggesting that the polymerization reaction proceeded with excellent control.

Nanostructures of various shape, obtained from block copolymers, find multitude of applications in medicine and consumer-care domains.¹⁷ Engineered block copolymer

compositions, covering broad range of amphiphilic balance, allows for modulation of interfacial curvature and thereby accessing nanostructures of different shapes.¹⁸ Selected representative examples of block copolymers were investigated for their aqueous self-assembly *via* solvent exchange by dialysis (from THF to water) at room temperature (Fig. 1). As observed by conventional transmission electron micrographs (negatively stained with phosphotungstic acid), polymer **3a** was found to form discrete disk-like micelles, whereas polymer **3e**, was found to form a mixture of disk-like micelles and tape-like nanostructures. Depending upon orientation of nanostructures on the TEM grid, disks are observed in edge-on, face-on and intermediate orientations. Similar disk-like micelles and the transformation of disks-to-tapes have been observed for cholesterol-containing PEGylated systems.^{15,19} It is important to note that disk-like micelles are not commonly encountered and in most instances, they require sophisticated polymer design or processing conditions.²⁰ It is remarkable that disk-like micelles can be accessed with these simple diblock copolymers. Two-dimensional sheet or membrane-like structures were formed by polymer **3k** and these membranes were found to have internal striped patterns, presumably due to the ordering of cholesteryl side-chains as noted earlier for other cholesterol functionalized block copolymers (Fig. S1†).¹⁶ Apart from exploring the self-assembly of these block copolymers, we are also interested in using them as an additive to impart stability to liposomes by the formation of *hybrid stealth liposomes*.

Stealth liposomes are polymer coated lipid vesicles, wherein the water-soluble polymers anchor into vesicle bilayers through a covalently attached lipid (*e.g.*, dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[mPEG-5000] (DPPE-PEG)).^{21–23} The polymer coating prevents liposome bilayer contact and consequently fusion, therefore sterically stabilizing liposomes in solution. Through their polymer coating, stealth liposomes dramatically outperform their unfunctionalized analogues in numerous attributes, including *in vitro* shelf-life and *in vivo* circulation times.²² Currently, these liposomes form a significant fraction of nanoformulations for a variety of therapeutics.^{23,24} However, the expensive nature of lipid-anchored polymers may restrict their wide-spread application.²⁵ To address this limitation, we explored the use of block copolymer **3** as a liposome additive.

For these diblock copolymers to serve as a steric-stabilizing additive, their tendency to self-assemble into liposome bilayers (rather than reside in preformed micelles) is crucial.²⁶ At lower DP_n^{Chol} , copolymer **3** will have a higher propensity to integrate into lipid membranes through insertion of the cholesteryl moieties. As a proof-of-concept study, liposomes were prepared from dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) by thinfilm rehydration and extrusion. Then copolymer **3a** or DPPE-PEG was introduced at 5% by mol relative to the lipid. Hydrodynamic diameter of resultant stealth and hybrid stealth liposomes were monitored as a function of time by dynamic light scattering (DLS). From DLS data it is obvious that steric stabilizers are crucial for extended stability of liposomes (Fig. 2). Large aggregates that appear in a few days in the absence of steric stabilizers are not observed even up to 30 days with addition of 5% by mol of copolymer **3a** or DPPE-PEG (Fig. 2D–F).

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These findings highlight the capability of the current block copolymers as a shelf-life extending additive for liposomes.²⁷

In summary, Chol-8m (**2**), was synthesized through a commercially relevant synthetic approach. By using polyethylene glycol monomethyl ether as a macroinitiator, organo-catalytic ROP of monomer **2** was conducted to result in a series of PEGylated diblock copolymers, **3** (mPEG-*b*-P(**2**)) with tunable amphiphilic balance. These block copolymers were shown to undergo aqueous self-assembly to result in unique nano-structures, including disk-like micelles. It has also been demonstrated that synthesized diblock copolymers can serve as inexpensive steric stabilizers for liposomes. Owing to their biocompatible constituents and biodegradable hydrophobic block, these functional polymers are amenable for biomedical and consumer-care applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore). K. P. M. acknowledges postdoctoral research support from the National Research Council Research Associateship Program.

Notes and references

1. Tian H, Tang Z, Zhuang X, Chen X and Jing X, Prog. Polym. Sci, 2012, 37, 237–280; Feng J, Zhuo R-X and Zhang X-Z, Prog. Polym. Sci, 2012, 37, 211–236; Tempelaar S, Mespouille L, Coulembier O, Dubois P and Dove AP, Chem. Soc. Rev, 2013, 42, 1312–1336. [PubMed: 23151841]
2. Pratt R, Nederberg F, Waymouth RM and Hedrick JL, Chem. Commun, 2008, 114–116; Sanders DP, Fukushima K, Coady DJ, Nelson A, Fujiwara M, Yasumoto M and Hedrick JL, J. Am. Chem. Soc, 2010, 132, 14724–14726; [PubMed: 20883030] Mindemark J and Bowden T, Polym. Chem, 2012, 3, 1399–1401.
3. Venkataraman S, Veronica N, Voo ZX, Hedrick JL and Yang YY, Polym. Chem, 2013, 4, 2945–2948.
4. Venkataraman S, Ng VW, Coady DJ, Horn HW, Jones GO, Fung TS, Sardon H, Waymouth RM, Hedrick JL and Yang YY, J. Am. Chem. Soc, 2015, 137, 13851–13860. [PubMed: 26456146]
5. Olsson JV, Hult D, Cai Y, Garcia-Gallego S and Malkoch M, Polym. Chem, 2014, 5, 6651–6655.
6. Sanda F, Kamatani J and Endo T, Macromolecules, 2001, 34, 1564–1569.
7. Qiu F-Y, Song C-C, Zhang M, Du F-S and Li Z-C, ACS Macro Lett, 2015, 4, 1220–1224; Qiu F-Y, Zhang M, Du F-S and Li Z-C, Macromolecules, 2017, 50, 23–34; Hu X, Chen X, Xie Z, Cheng H and Jing X, J. Polym. Sci., Part A: Polym. Chem, 2008, 46, 7022–7032.
8. Dong P, Sun H and Quan D, Polymer, 2016, 97, 614–622.
9. Tan JPK, Coady DJ, Sardon H, Yuen A, Gao S, Lim SW, Liang ZC, Tan EW, Venkataraman S, Engler AC, Fevre M, Ono R, Yang YY and Hedrick JL, Adv. Healthcare Mater, 2017, 6, 1601420; Venkataraman S, Tan JPK, Ng VWL, Tan EWP, Hedrick JL and Yang YY, Biomacromolecules, 2017, 18, 178–188; [PubMed: 28064501] Qiu F-Y, Yu L, Du F-S and Li Z-C, Macromol. Rapid Commun, 2017, 38, 1700400.
10. Chang YA, Rudenko AE and Waymouth RM, ACS Macro Lett, 2016, 5, 1162–1166.
11. Pascual A, Tan JPK, Yuen A, Chan JMW, Coady DJ, Mecerreyes D, Hedrick JL, Yang YY and Sardon H, Biomacromolecules, 2015, 16, 1169–1178. [PubMed: 25764341]
12. Zhou Y, Briand VA, Sharma N, Ahn S. k. and Kasi RM, Materials, 2009, 2, 636–660.

13. Ercole F, Whittaker MR, Quinn JF and Davis TP, *Biomacromolecules*, 2015, 16, 1886–1914. [PubMed: 26098044]
14. Lee AL, Venkataraman S, Sirat SB, Gao S, Hedrick JL and Yang YY, *Biomaterials*, 2012, 33, 1921–1928; [PubMed: 22137125] Coady DJ, Ong ZY, Lee PS, Venkataraman S, Chin W, Engler AC, Yang YY and Hedrick JL, *Adv. Healthcare Mater*, 2014, 3, 882–889; Lee ALZ, Venkataraman S, Fox CH, Coady DJ, Frank CW, Hedrick JL and Yang YY, *J. Mater. Chem. B*, 2015, 3, 6953–6963; [PubMed: 32262545] Yang C, Liu SQ, Venkataraman S, Gao SJ, Ke X, Chia XT, Hedrick JL and Yang YY, *J. Controlled Release*, 2015, 208, 93–105.
15. Venkataraman S, Lee AL, Maune HT, Hedrick JL, Prabhu VM and Yang YY, *Macromolecules*, 2013, 46, 4839–4846.
16. Zhou L, Zhang D, Hocine S, Pilone A, Trepout S, Marco S, Thomas C, Guo J and Li M-H, *Polym. Chem*, 2017, 8, 4776–4780.
17. Venkataraman S, Hedrick JL, Ong ZY, Yang C, Ee PLR, Hammond PT and Yang YY, *Adv. Drug Delivery Rev*, 2011, 63, 1228–1246.
18. Mai Y and Eisenberg A, *Chem. Soc. Rev*, 2012, 41, 5969–5985; [PubMed: 22776960] Epps TH III and O'Reilly RK, *Chem. Sci*, 2016, 7, 1674–1689. [PubMed: 30155013]
19. Danino D, Abezgauz L, Portnaya I and Dan N, *J. Phys. Chem. Lett*, 2016, 7, 1434–1439. [PubMed: 27031669]
20. Holder SJ and Sommerdijk NAJM, *Polym. Chem*, 2011, 2, 1018–1028; Li Z, Chen Z, Cui H, Hales K, Qi K, Wooley KL and Pochan DJ, *Langmuir*, 2005, 21, 7533–7539; [PubMed: 16042491] Li Z, Chen Z, Cui H, Hales K, Wooley KL and Pochan DJ, *Langmuir*, 2007, 23, 4689–4694; [PubMed: 17397196] Lin Z, Sun J, Zhou Y, Wang Y, Xu H, Yang X, Su H, Cui H, Aida T, Zhang W and Cheng SZD, *J. Am. Chem. Soc*, 2017, 139, 5883–5889; [PubMed: 28388040] Lodge TP, Hillmyer MA, Zhou Z and Talmon Y, *Macromolecules*, 2004, 37, 6680–6682; Edmonds WF, Li Z, Hillmyer MA and Lodge TP, *Macromolecules*, 2006, 39, 4526–4530; Zhu J, Zhang S, Zhang K, Wang X, Mays JW, Wooley KL and Pochan DJ, *Nat. Commun*, 2013, 4, 2297; [PubMed: 23921650] Venkataraman S, Chowdhury ZA, Lee AL, Tong YW, Akiba I and Yang YY, *Macromol. Rapid Commun*, 2013, 34, 652–658. [PubMed: 23483622]
21. Lasic DD and Needham D, *Chem. Rev*, 1995, 95, 2601–2628.
22. Immordino ML, Dosio F and Cattel L, *Int. J. Nanomed*, 2006, 1, 297–315.
23. Barenholz Y, *J. Controlled Release*, 2012, 160, 117–134.
24. Allen TM and Cullis PR, *Adv. Drug Delivery Rev*, 2013, 65, 36–48.
25. For example: 1,2-dipalmitoyl-sn-glycero-3-phosphoethano-lamine-N-[methoxy(polyethylene glycol)-5000] (ammonium salt), 880200P-1 g, \$ 980 <https://avantilipids.com/product/880200/>, (accessed Jan 11, 2018).
26. Edwards K, Johnsson M, Karlsson G and Silvander M, *Biophys. J*, 1997, 73, 258–266; [PubMed: 9199790] Rovira-Bru M, Thompson DH and Szleifer I, *Biophys. J*, 2002, 83, 2419–2439. [PubMed: 12414678]
27. Mineart KP, Venkataraman S, Yang YY, Hedrick JL and Prabhu VM, *Macromolecules*, 2018, DOI: 10.1021/acs.macromol.8b000361.

Scheme 1.

Synthesis of PEGylated amphiphilic diblock copolymers (**3**) from Chol-8m (**2**). A photograph of monomer **2** crystals is included.

Fig. 1.

Aqueous self-assembly of amphiphilic diblock copolymers **3**. (A) **3a**, discrete disk-like micelles; (B) **3e**, mixture of disk-like micelles and elongates tape-like structures and (C) **3k**, membrane like structures.

Fig. 2.

Application of amphiphilic block copolymer **3a** in the preparation of hybrid stealth liposomes. Schematic representations of a (A) standard liposome and (B) sterically-stabilized stealth liposomes; (C) chemical structure of liposome components, their corresponding schematic representations and bilayer configurations; and size distribution evolutions as a function of time for each type of liposome: (D) standard, (E) stealth, and (F) hybrid stealth. Formulations containing block copolymer **3a** demonstrate temporal stability comparable to that of pre-existing stealth liposome technology.

Table 1

Characterization of amphiphilic diblock copolymers (**3**)

S. no.	Code	mPEG-OH M_n^a (kDa)	DP _n ^{Chol} Target ^b	DP _n ^{Chol} Observed ^c	M_n^c (kDa)	$\frac{d}{M}$	f^e
1	3a	5.0	2.1	1.4	5.7	1.11	0.87
2	3b	5.0	2.5	2.2	6.2	1.11	0.81
3	3c	5.0	3.0	2.8	6.5	1.12	0.77
4	3d	5.0	3.5	3.3	6.8	1.13	0.74
5	3e	5.0	4.0	3.7	7.0	1.12	0.71
6	3f	5.0	5.0	4.8	7.6	1.13	0.66
7	3g	2.4	5.1	3.9	4.5	1.12	0.53
8	3h	5.0	10.0	9.8	10.3	1.15	0.49
9	3i	5.0	15.0	14.1	12.7	1.16	0.40
10	3j	5.0	19.6	18.6	15.1	1.18	0.33
11	3k	2.4	20.4	19.8	13.1	1.17	0.18

^aRelative number-average molecular mass as provided by the manufacturer.^bBased on the initial molar feed ratio with respect to the mPEG-OH.^cBased on ¹H-NMR spectroscopy.^dDispersity index (M_w/M_n) as determined by size-exclusion chromatography (SEC).^ePEG mass fraction based upon: $f = M_n(\text{PEG})/[M_n(\text{PEG}) + M_n^{(\text{NMR})}\text{P}(2)]$.