ABSTRACT: The development of a simple, low-cost, and robust route to soft nanocapsules is an ever-present hurdle and requirement for their promising use in drug encapsulation and delivery. To date, several elegant strategies for nanocapsule formation have emerged. However, some of them fall short of one or several of the requirements including stability, biocompatibility, antifouling, and low cost which ultimately limit their potential impact. Owing to its inherent biocompatibility, low cost, and high functionalization characteristics, hyperbranched polyglycerol (HPG) derivatives may offer a possible solution. Through esterification and azidation of HPG hydroxyl groups, an intriguing UV-cross-linkable HPG-azidomethylbenzoyl ester (denoted HPG-4-N3-MBE) is yielded. Over short irradiation periods, fair uniform wholly polymeric HPG nanocapsules with diameters less than 100 nm can be formed which exhibit good stability due to abundant cross-linking primarily through nitrene–nitrene coupling to produce azo linkages rather than potentially reactive azidirane ring linkages. This work demonstrates the simple, low-cost, and robust type of chemistry needed for the practical development of polymer nanocapsule-based drug delivery/encapsulation, waste remediation, and surface antifouling strategies.

INTRODUCTION
The development of soft polymeric nanostructures has emerged as a promising approach for drug encapsulation and delivery,1,2 as well as a passivation layer in hard–soft inorganic nanocomposites.3 Owing to the wealth of different polymer types, polymerization techniques, and postpolymerization chemistries, several strategies for translating polymers into nanostructures have been realized. Largely based on the self-assembly of linear block copolymers including poly(lactic acid), poly(e-caprolactone), poly(lactic-co-glycolic acid), poly(N-acryloylactamide), and poly(ethylene glycol), biocompatible liposomes,4 vesicles,5 spherical micelles,6–8 and cylindrical micelles9–11 have been realized. However, the stability of micelles in terms of shelf life and against environmental changes such as temperature, pH, concentration, solution, and ionicity remains a grand challenge.12 One approach to addressing micellar stability involves cross-linking the shape through various means once self-assembled. Specific strategies include photo-cross-linking in nanoscale emulsions,13–15 interfacial silica sol–gel formation,16 and bifunctional covalent cross-linking.17,18 While these strategies do improve the environmental stability, they often suffer from poor size control, large size dispersions, and additional purification steps as precision control of the cross-linking density and emulsion particle sizes is challenging.

Some of these issues noted above have been addressed by using core templating strategies in which copolymers are organized around a surface and cross-linked with the core subsequently removed. The core is most often a rigid inorganic structure, such as Au19,20 and silica,21 that is amenable to chemical ligation and simple etching strategies. The “core” can also be an inner polymeric block of the self-assembled structure that can be removed from the outer cross-linked polymer blocks22 as well as preformed polymer spheres.23,24 In both templating approaches, the size and shape control of the core are more easily controlled through various established wet nanostructure synthesis procedures such as hot injection,25 sol–gel,26 phase transfer/seeded growth,27,28 mesoporous silica,29 and hydrothermal/solvothermal processes.30

A third approach using unimolecular micelles aims to satisfy both the desire for stable, soft nanostructures with more well-defined shapes while also avoiding the use of templating strategies. This approach entails the formation of large globular unimolecular polymers produced by two main routes. The first utilizes multisite initiators including low molecular weight polyols,31,32 polyester polyl dendrimers,33 hyperbranched polyglycerols,34 cyclic sugars (cyclodextrins),35 and inorganic polyhedral oligomeric silsesquioxane (POSS).36 The second utilizes multigeneration dendrimers including poly(ethyleneimine),37 poly(propyleneimine),38 and poly(amicidiamine)39 which can be subsequently modified. Among these effective unimolecular approaches, hyperbranched polyglycerol (HPG) is of particular interest in recent years.
HPG is a hyperbranched polyester polyl that possesses a large number of hydroxyl groups amenable to postpolymerization modification through various means. Only fairly recently has the molecular weight and polydispersity control of HPG been sufficiently improved to allow for systematic investigation into their properties by several approaches including optimized addition reactions \(^{40-44}\) and sequential polymerization \(^{45}\) in the noncatalyzed ring-opening multibranching polymerization (ROMBP) of epoxide-containing glycidol monomers. HPG is also particularly advantageous when applied in bio-related applications. Because of excellent biocompatibility (neutral charge and minimal irritation, cytotoxicity, and genotoxicity)\(^{46}\) and drug uptake potential,\(^{47,48}\) HPG is ideal for applications related to drug delivery. Similarly, it is beneficial as an antifouling coating for use in the body as it does not bind to proteins or biological materials present in the body or within a cell\(^{49}\) which, when coated onto the surface of various inorganic nanostructures, enables the introduction of additional treatment and imaging modes within living systems.\(^{52-55}\)

Owing to its unimolecular shape, biocompatibility, and high degree of functionality, HPG is an ideal candidate for developing soft cross-linked nanostructures. Previous work in this area has largely relied on cross-linking through diacrylate-functionalized HPG\(^{56}\) with only a few reports describing enzymatic cross-linking.\(^{57}\) Such chemical and biochemical approaches are fairly complex and require precise control of the chemical cross-linker or enzyme chemistry and often lead to large multimolecular aggregates of poorly controlled shape. Herein, we report on the synthesis of novel cross-linked azido-hyperbranched polycrylcerol nanocapsules through a combination of ROMBP, esterification, and UV-induced azide–azide homocoupling. Notably, the advantage of this strategy is that azidation and cross-linking require no additional chemical cross-linker; nanocapsule formation occurs quickly under mild conditions driven only by UV light with only \(\text{N}_2\) gas as a byproduct.\(^{58-60}\) The abundance of surface azide groups present on HPG enables the predominant coupling between the nitrene intermediates to create intramolecular azo linkages, which is in sharp contrast to nitrene double bond coupling to form aziridines.\(^{61}\) The synthesis of the azido-hyperbranched polycrylcerol and their subsequent nanocapsule formation (15–25 nm diameters) are systematically monitored over the course of UV irradiation. This simple, three-step strategy for nanocapsule formation may provide a platform for many promising functionalized derivatives for applications in tailored drug encapsulation and delivery.

### Experimental Section

**Materials.** 4-Bromomethylbenzoyl chloride (4BMBC, 98%), 1-methyl-2-pyrrolidone (NMP, 98%), and glycidol (96%) were obtained from Sigma-Aldrich and used as received unless otherwise specified. NMP was stirred overnight with \(\text{CaH}_2\) and distilled prior to use. Glycidol was vacuum distilled immediately before use. Sodium methoxide (\(\text{NaOMe}\), 98%), 1,1,1-tris(hydroxymethyl)propane (TMP, 98%), sodium bicarbonate (\(\text{NaHCO}_3\), 98%), magnesium sulfate (\(\text{MgSO}_4\) 98%), sodium azide (\(\text{NaN}_3\) 99%) and phosphotungstic acid (97%) were obtained from Alfa Aesar and used as received. Diethyl ether (solvent grade), dichloromethane (DCM, solvent grade), dimethylformamide (DMF, solvent grade), chloroform (CHCl\(_3\) solvent grade), tetrahydrofuran (THF, solvent grade), and toluene (solvent grade) were purchased from BDH and used as received unless otherwise specified. THF was dried by stirring with sodium metal and naphthalene and distilled under vacuum prior to use. Bis(2-methoxyethyl) ether (diglyme, 98%) was purchased from Acrros Organics.

**Synthesis of Hyperbranched Polyglycerol (HPG).** HPG is polymerized through a ring-opening multibranching polymerization (ROMBP) of an epoxide-containing monomer (i.e., glycidol) slowly added to a reactor to minimize the cyclization and coupling of neighboring polymers. As the reaction proceeded, the viscosity of the polymer-containing solution increases rapidly. The ROMBP reactor setup is designed to minimize the impact of viscosity on the molecular weight and PDI control. In a general setup, an external power source was attached to a Teflon stirring rod and placed in a three-port flask in a thermostated oil bath. A syringe pump was attached to the second port charged with a 50/50 (v/v) of dried NMP and glycidol monomer and the third port for argon atmosphere supply. The main reactor was charged with TMP and KOMe basic initiator. The reactor was heated to 80 °C for 1 h to drive off methanol formed during initiator formation. Benzene was then added and evaporated to remove any residual water. The initiator was then dissolved with NMP and diglyme and stirred at a set speed. Monomer was injected at a specific rate and left to react for a period of time. The crude product was then purified by precipitation dissolution three times in acetone and ethanol, respectively (HPG yield: 30%). IG \(^{13}\)C NMR (DMSO-d\(_6\) \(\delta\) (ppm): 79.5–80.4 (L\(_{13}\)); 78–79 (D); 72–73 (2L\(_{14}\)); 70.4–71.4 (2D, 2T); 68–70 (L\(_{15}\); L\(_{14}\)); 62–63.5 (T); 61–62.5 (L\(_{16}\)). HI NMR (DMSO-d\(_6\) \(\delta\) (ppm): 0.91 (s, 3H, TMP methyl group); 1.45 (s, 2H, –CH\(_2\)– group in TMP); 3.4–4.0 (m, 5H, backbone of pure HPG). Details of HPG synthesis can be found in Table S1 of the Supporting Information.Synthesis details and sample calculations for HPG characterization can be found in our previous work.\(^{34}\)

**Synthesis and Purification of Hyperbranched Polyglycerol-4-bromomethyl Benzyl Ester (HPG-4BMBE).** The brominated HPG nanocapsule precursor is formed by esterification of the hydroxyl groups of HPG with the acid chloride unit of 4BMBC. In a typical reaction, 0.1 g of HPG10 (1.35 mmol of –OH) was azetropically dried with toluene (20 mL) to remove all water. HPG10 was dissolved in dry NMP (10 mL) and cooled in an ice bath (0 °C). Then 0.94 g of 4BMBC (3.38 mmol) dissolved in 6 mL of dry NMP was added dropwise to the stirred HPG10 solution over 30–60 min in ice and allowed to react 24 h at room temperature. The crude product was precipitated in diethyl ether and dissolved in DCM three times. Product was washed with \(\text{NaHCO}_3\) (5 wt % in DI water) three times and then pure DI water three times and dried over MgSO\(_4\) for 12 h. Product was filtered and dried overnight (60 °C) to produce a viscous brown liquid (yield: 95% for HPG-4BMBC). We note that 4BMBC was added in a 1:2.5 molar ratio of –OH:4BMBC. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) (ppm): 3.2–4 (4H, HPG backbone); 4.3–4.7 (4H, –CH\(_3\)Br); 5.2–5.4, 5.45–5.6 (m, 6H, –COO–CH\(_2\)– and –CH\(_2\)–CH\(_2\)–(–COO–)– and –COO–CH\(_2\)–CH\(_2\)–(–COO–)–CH\(_2\)– protons in HPG backbone next to esterification sites).

**Synthesis of Hyperbranched Polyglycerol-4-azidomethylbenzyl Ester (HPG-4-N\(_3\)-MBE).** In a small vial, 0.2 g of HPG-4BMBC (0.93 mmol) was dissolved in 6 mL of dry DMF. To this, 0.12 g of \(\text{NaH}(1.86\text{ mmol})\) dissolved in 7 mL of dry DMF was added. The mixture was allowed to react under vigorous stirring under ambient conditions for 72 h. It was then precipitated in water, centrifuged and dissolved in DCM, washed with water in a separatory funnel, filtered, and dried overnight (60 °C). FT-IR (cm\(^{-1}\)) 1050 (C–O–C–); 1720 (–COO–), 2100 (–N=), which is the indirect evidence of azidation in \(^1\)H NMR where the peak shift of the methylene protons adjacent to the bromine in HPG-4BMBC was seen. This peak shift is consistent with that observed for conversion of benzoyl bromide to benzyl azide.

**UV-Induced Azide Homocoupling of HPG-4-N\(_3\)-MBE.** In a small vial, 2 mg of HPG-4-N\(_3\)-MBE was dissolved in 3 mL of \(\text{CHCl}_3\) and passed through a 0.45 μm syringe filter. Samples were then vigorously stirred at room temperature, and subjected to UV light irradiation (\(\lambda = 254 \text{ nm}\)) in an enclosed dark container for various times (i.e., no ambient visible light exposure) to generate reactive nitrene intermediates that subsequently couple together to form azo cross-linkers. Samples were then dried and dissolved in dry THF and
cast on TEM grids to dry overnight. Some samples were also stained with phosphotungstic acid (0.2 wt % aqueous solution).

**Characterizations.** The absolute molecular weight of HPG was evaluated by $^1$H NMR using a Varian VXR-400 Unity Inova spectrometer (400 MHz) with a quad probe from Nalorac. Time inversion recovery and inverse-gated carbon NMR (IG $^{13}$C NMR) analyses were performed on the same instrument to determine the necessary scan times, degree of branching (DB), and structural breakdown of HPG. All sample concentrations were 10 mg/mL.

The gel permeation chromatography (GPC) analysis was performed on a Shimadzu system (CTO-20A column oven, LC-20A pump, and RID-10A refractive index detector). The mobile phase was DMF stabilized with LiCl and calibrated against linear PEO standards. A Phenomenex linear column with mixed pore sizes (range 20–2 × 10$^6$ g mol$^{-1}$) was used.

FT-IR spectroscopy measurement was performed on a Shimadzu IRT-Tracer-100. Samples were dispersed in KBr discs.

Transmission electron microscopy (TEM) imaging was performed on a Jeol 100 CX-II (acceleration voltage 100 keV). Samples were drop cast on square mesh copper grids (CF-400) from Electron Microscopy Sciences and stained with phosphotungstic acid (0.2 wt % aqueous solution).

**RESULTS AND DISCUSSION**

Scheme 1 depicts the synthesis route to cross-linked HPG-4-N3-MBE nanocapsules in three consecutive steps. The mild and simple reaction steps required are ideal from a safety and energy standpoint, making this route fairly practical and green.

**HPG Synthesis, Bromination, and Azidation.** Among several batches of HPG synthesized, HPG10 was chosen due to its fairly low PDI (1.38), intermediate molecular weight ($M_n = 9.3$ kg mol$^{-1}$), and sufficiently large number of hydroxyl groups ($n_{OH} = 126$ on average). This high degree of $−$OH and thus azide ($N_3$) functional groups is ideal for creating intramolecular cross-links within individual molecules via azide–azide (i.e., nitrene–nitrene) homocoupling. Details of HPG synthesis, including molecular weight, degree of functionalization, and structural breakdown, are summarized in Table 1. Synthesis details and reaction conditions are summarized in Table S1 (see Supporting Information). A representative IG $^{13}$C NMR plot is shown in Figure S1 (see Supporting Information).

The degree of polymerization (DP) and degree of branching (DB) were determined using eqs 1 and 2 with the HPG molecular characterization summarized in Table 1:

$$\text{DP} = \frac{A_4}{A_{\text{methyl}}} - 2A_{\text{methyl}}$$

$$\text{DB} = \frac{2D}{2D + L_{13} + L_{14}}$$

where $A_4$ is the area of the HPG backbone signal, $A_{\text{methyl}}$ is the area of the methyl group from the TMP initiator, and $D$, $L_{13}$, and $L_{14}$ are the areas of the peaks in the IG $^{13}$C NMR HPG spectrum. Maximizing the dendritic (D) and terminal (T) units at the expense of linear units ($L_{13}$, $L_{14}$) is ideal as it raised the degree of branching, enabling a larger number of hydroxyl groups for the same molecular weight as well as a more spherical shape.

Acid halides (typically bromine and chlorine varieties) are advantageous for functionalizing polymers containing hydroxyl groups as they react irreversibly through the formation of HBr or HCl, respectively. In this work, bifunctional 4-bromomethylbenzoyl chloride (4BMBC) contains an acid chloride end for attachment to HPG and a primary bromine in the para position amenable to displacement by azide groups. Functionalization of HPG with 4-bromomethylbenzoyl chloride (4BMBC) to yield HPG-4BMBC proceeded with high conversion to produce a highly esterified (i.e., brominated) product. HPG-4BMBC is the precursor polymer amenable to intramolecular azide homocoupling possessing a relatively hydrophilic HPG core and relatively hydrophilic cross-linked shell. This esterification is analogous to traditional ATRP functionalization with α-bromoalkoxy bromide (BIBB) with the major difference being a tertiary bromine amenable to ATRP initiation rather than simple displacement. The high degree of esterification (98%) is attributed to the NMP solvent which served as both a solvent and a scavenger for HCl formed during reaction. The successful esterification was verified by $^1$H NMR (Figure 1) and clearly shows attachment of the aromatic unit (7–8 ppm) as well as the characteristic methylene peak adjacent to the primary bromine (4.2–4.7 ppm).

The emergence of new peaks around 5.2–5.7 ppm can be attributed to the shift of HPG protons adjacent to the 4BMBC

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_{n,\text{GPC}}$ a (g mol$^{-1}$)</th>
<th>$M_{w,\text{GPC}}$ b (g mol$^{-1}$)</th>
<th>$M_{w,\text{GPC}}$ b (g mol$^{-1}$)</th>
<th>$M_{n,\text{GPC}}$ b (g mol$^{-1}$)</th>
<th>$D_{\text{GPC}}$ a (%)</th>
<th>$L_{13}$ a (%)</th>
<th>$L_{14}$ a (%)</th>
<th>$L_{c}$ a ($)</th>
<th>$\text{DB} c$ (%)</th>
<th>$\text{%E} d$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPG10</td>
<td>9300</td>
<td>7200</td>
<td>10000</td>
<td>138</td>
<td>126</td>
<td>23</td>
<td>34</td>
<td>18</td>
<td>25</td>
<td>52</td>
</tr>
</tbody>
</table>

Determined by integrating the HPG backbone proton peak and dividing by integrated initiator peak area using eq 1. aMobile phase is DMF with 0.1 M LiCl stabilizer and linear PEG standards. bHPG dendritic (D), terminal (T), linear type 1 ($L_{13}$), and linear type 2 ($L_{14}$) determined by integrating peaks in IG $^{13}$C NMR (Figure S1 is a representative HPG spectrum). cDegree of branching (DB) is calculated from eq 2 introduced by Frey. dDegree of esterification (i.e., bromination) via 4-bromomethylbenzoyl chloride (4BMBC) is calculated by eq 3. More details regarding HPG synthesis and characterization can be found in our previous work. 34
formation sites. A similar peak appearance is observed when HPG is functionalized with 4BIBB to act as an ATRP macroinitiator. The primary bromine is amenable to displacement by various species, including azide.

The degree of esterification (i.e., bromination) of HPG10 was calculated using eq 3 based on the $^1$H NMR peaks of HPG-4BMBE.

$$%E = \frac{A_c}{A_d} \times 5$$

where $A_c$ is the peak area of the methylene group in HPG-4BMBE and $A_d$ is the peak area of the HPG10 backbone.

It was found that the degree of esterification was nearly quantitative (98%). This is a necessary condition for having a large number of azide groups for intramolecular cross-linking. Azidation was also quantitative as evidenced by the complete peak shift of nearby proton signals in the formed HPG-4-N$_3$-MBE (Figure 2) in conjunction with the excess NaN$_3$ employed and long reaction time. Having a large number of azide groups is essential for the intramolecular cross-linking to proceed for two reasons: (1) a large number of cross-link sites gives the formed nanocapsules a more rigid shape that can survive through purification and imaging, and (2) a large number of azide groups promotes homocoupling of the azide intermediates (nitrenes) into stable azo cross-linkers rather than other coupling reactions including aziridine formation (nitrene addition to alkenes) which can further react through other pathways.61

**UV-Induced Nanocapsule Formation.** The primary reaction pathway for the formation of wholly polymeric HPG-based nanocapsules is through nitrene homocoupling under UV irradiation. The reaction can be followed directly by FT-IR and TEM and indirectly by $^1$H NMR studies. Since azide has a very distinctive FT-IR absorbance at around 2100 cm$^{-1}$, it was possible to track the disappearance of azide groups as they react to form azo cross-links (Figure 3). Prior to azidation, there is no azide peak detected (Figure 3a). Nanocapsule formation occurs over 70 min of UV irradiation, and the intensity of the azide peak gradually reduces relative to the other unaffected peaks (Figures 3c–e).

The disappearance of the azide peak is direct evidence of the formation of azo cross-links through a nitrene–nitrene coupling intermediate. The likely reason for a small remnant azide peak is that not all of the azides were able to couple in the fairly short irradiation time, thus leaving dangling groups.

Further indirect evidence of nanocapsule formation can be found in $^1$H NMR spectra measured over the same series of irradiation times (Figure 2). Upon azidation, there is a small shift in the peak positions of the nearest protons (i.e., the methylene protons and aromatic protons of the 4-azidomethylbenzoyl unit). This peak shift is analogous to that observed in
the azidation of benzyl bromide and is consistent with what is expected of the slightly nucleophilic terminal nitrogen of the azide group. Longer irradiation times were not performed as local heating may destroy the formed nanocapsules and possibly lead to some intermolecular coupling. It was also clear that the nitrene–nitrene intermediate coupling was the dominant pathway for cross-linking as there are no additional peaks forming in the aromatic region. If aziridine cross-linking was present, there would be reaction between the phenyl groups of HPG-4-N$_3$-MBE and nitrene intermediates as well as the potential for additional side reactions through aziridine ring-opening. However, this was not seen.

Direct evidence of nanocapsule formation was also visualized by TEM. Over the course of UV irradiation, nanocapsule formation was clearly present and dominant (Figure 4). The diameter of the formed nanocapsules was approximately 20 nm with a fairly uniform spherical shape and with no large scale aggregation. There is no apparent change in size or overall shape of the formed nanocapsules between 20 and 40 min UV irradiation. This further supports that intramolecular cross-linking is primarily occurring during this stage of the reaction (2 mg/mL). With longer irradiation times, larger multimolecular nanocapsules formed as a result of intermolecular cross-linking reactions (Figure 4C). For comparison, a TEM control was performed in which HPG-4-N$_3$-MBE was drop-cast in the dark. As expected, no nanocapsule formation was observed for the control as there was no nitrene intermediate formation. This led to the formation of essentially large film-like regions of un-cross-linked HPG-4-N$_3$-MBE. The nanocapsules formed rapidly and remained stable in solution for several weeks.

## CONCLUSIONS

A simple, clean, and green approach for crafting nanocapsules based on biocompatible hyperbranched polyglycerol (HPG) is described. This three-step route to nanocapsules involves the polymerization of well-defined HPG first, followed by its esterification with a bromine-containing acid halide (HPG-4BMBE) and subsequent azidation via bromine displacement to produce a new nanocapsule-forming material HPG-4-N$_3$-MBE. Unlike previous methods to wholly polymeric nanocapsule synthesis, our approach offers a fast, mild, UV-cross-linkable strategy for forming inexpensive, biocompatible nanocapsules with diameters below 100 nm. When reacted in a dilute regime with brief irradiation times, intermolecular cross-linking can be suppressed. Our strategy requires minimal purification as it avoids the use of chemical cross-linkers and metallic catalysts with only gaseous nitrogen as the byproduct of nanocapsule formation. Taking full advantage of the abundance of azide groups present, the stability of nanocapsules is preserved via preferential intramolecular cross-linking through intermediate nitrene–nitrene coupling to produce azo linkages rather than aziridine linkages which may participate in additional ring-opening reactions. It is interesting to note that HPG-4BMBE may serve as an attractive starting material for creating other UV-cross-linkable nanocapsules for use in drug encapsulation/delivery, waste remediation, and...
surface antifouling coating. This will be the focus of future investigations.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.7b01074.

Reaction details for ROMBP synthesis of HPG including reaction conditions, reactants and reactor setup, sample IG $^{13}$C NMR plot of HPG (PDF)

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REFERENCES


