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Clickable polyglycerol hyperbranched polymers and their application to gold nanoparticles and acid-labile nanocarriers†‡

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A one-step, large-scale preparation of alkyne-containing hyperbranched polyglycerols (HPG) is reported. The HPGs undergo click reactions to organic azides allowing a range of applications.

Polyglycerol dendrimers and hyperbranched polyglycerols (HPGs) have a number of attractive properties that have led to their use in a broad range of applications from drug delivery devices to catalysis to diversity platforms for synthesis.[1] Arguably, the most important property is the biocompatibility afforded by the water-soluble, highly branched polyether–polyol structure. Indeed, HPGs resist protein adsorption and show toxicity profiles in cell studies that are at least as good as polyethylene glycol (PEG). Further, no toxicity in mice was observed even at high doses for a prolonged period.[2] Beyond their biocompatibility, HPGs can be prepared on kilogram scale[3] in one step from commercial reagents.

Because of these favourable features, much effort has focused on specific functionalization of HPGs.[4] For example, 8 step routes to azide and alkyne-cored polyglycerol dendrons and their subsequent click reaction were recently reported.[5] Herein we report the straight forward and scalable preparation of alkyne-cored HPG. Both high ($>50\,000$ M_n) and low ($<10\,000$ M_n) molecular weight polymers could be accessed with higher M_n material synthesized using an emulsion polymerization method.[6] Details on the preparation, characterization, and chemistry of the high M_n HPG can be found in the ESI.‡ The utility of the low M_n HPGs was shown by clicking them to fluorescein and biotin, as well as their use in stabilizing gold nanoparticles (NP) and in a pH-sensitive nanocarrier to catch and release small molecules.

Two routes to alkyne-functionalized HPG are outlined in Fig. 1. The first uses triol initiator **1**, prepared in three steps from pentaerythritol. Thus, a diglyme solution of **1** was treated with 10 mol% sodium hydride and glycidol was slowly added to produce HPG **2a**. Typical M_n and PDI values (M_w/M_n) for purified polymers are shown in Table 1 with representative SEC traces presented in Fig. 2a. An alternative synthesis uses propargyl alcohol as an alkyne source allowing the HPG to be prepared in a single step. The precipitation process used to purify the polymers reduced the PDI and increased molecular weights by removing lower molecular weight polymer. This process was further refined and used to fractionate polymer **2b** giving material with a lower PDI and 77% mass recovery overall (Table 2 and Fig. 2b). The presence of the propargyl group was confirmed using MALDI (inset to Fig. 2b) and NMR, (see ESI‡).

Inverse gated ^{13}C NMR confirmed the branched structure of HPG **2b** and indicated a degree of branching of 58%.[3a] As has been observed previously for HPGs,[3a] M_n values determined by SEC are overestimated under these conditions.

By virtue of the single alkyne group at the focal point, these HPGs can be covalently linked to a broad range of compounds and materials using standard click chemistry.[7] To illustrate this potential in a chemical biology context, **2b** was reacted with biotin azide **3** to give **4** and also clicked to fluorescein azide **5** giving fluorescent PG **6** (Scheme 1).[8] SEC traces of **5** and **6** showed no sign of cross-linking as a result of the orthogonal nature of the click reaction.

Because of their biocompatibility and resistance to protein adsorption,[9] it is expected that HPG **2b** will also be useful as a NP based surface coating. This is shown in Scheme 2 for the synthesis of HPG-capped gold NP **2b**. This scheme also shows the utility of the click reaction to introduce other functional groups at the focal point of the HPG polymer. Thus, rather than linking **2b** to the NP by a click reaction, amine functionalized HPG **8** was synthesized using azide **9**. Thiols and amines are well-known to undergo the exchange reaction shown in Scheme 2.[10] Thus **8** was used to coat 13.5 ± 1.1 nm diameter, citrate-capped gold NPs **10**, prepared using the Frens method.[11]

Addition of HPG **8** to an aqueous suspension of NP **10** led to aggregation, which was confirmed by dynamic light scattering (DLS). However, when **10** was suspended in DMF and treated

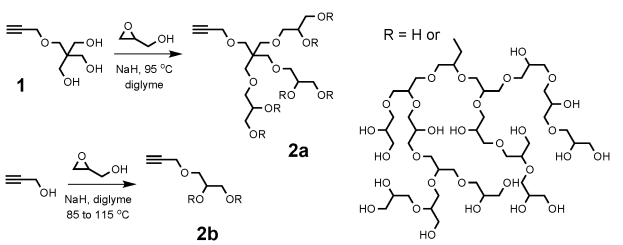

Fig. 1 Hyperbranched polymerization to form PGs **2a** and **2b**. Structure of PG is representative.

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Table 1 Molecular weights of HPGs **2a** prepared from **1** and various equivalents of glycidol followed by acetone precipitation. SEC determined in DMF with polystyrene calibration

Equivalents	M_n (theor)	M_n (SEC)	M_n/M_w
25	2027	7100	1.36
50	3878	11 600	1.39
75	5729	13 100	1.49
100	7580	14 500	1.54

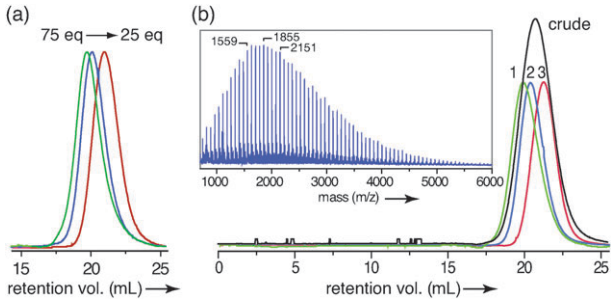
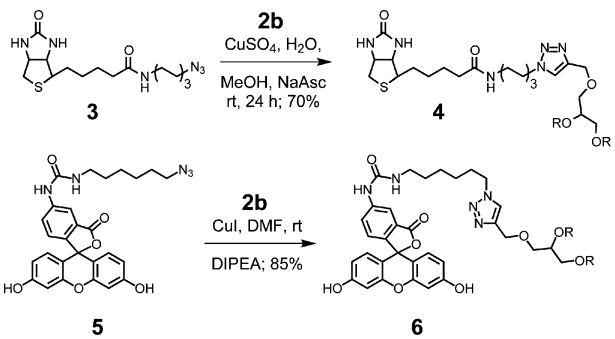


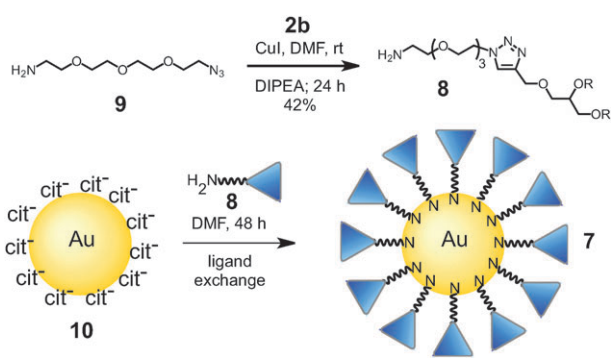
Fig. 2 (a) SEC traces of HPG **2a** prepared using 25, 50, and 75 equivalents of glycidol. (b) SEC traces of **2b**: crude and fractionated (fractions 1–3). Inset shows MALDI of **2b**. Adjacent peaks separated by m/z 74.03, the MW of glycidol. Within 0.1%, (b) indicated peaks correspond to initiator + 20, 24, and 28 glycidol units + Na.

Table 2 Molecular weights of HPG **2b** prepared from propargyl alcohol. SEC determined in DMF with polystyrene calibration

Polymer	M_n (theor)	M_n (SEC)	M_n (NMR)	M_n/M_w	Mass (g)
Crude	2054	8200		1.5	90
1st fraction		14 000	7000	1.35	20.1
2nd fraction		11 600	6000	1.26	29
3rd fraction		7500	3000	1.25	34.5



Scheme 1 Click chemistry of HPGs.



Scheme 2 Synthesis of HPG coated gold nanoparticles.

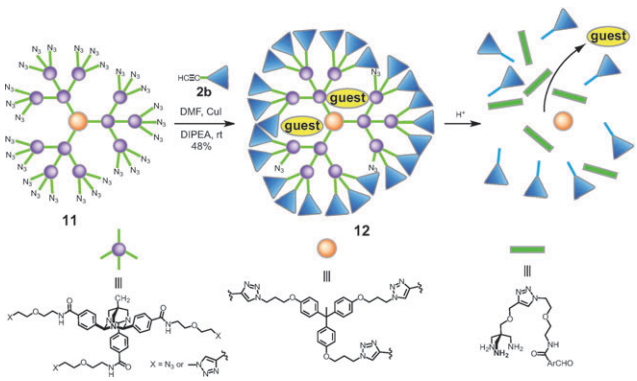
with **8**, a stable solution formed. This allowed the ligand exchange to take place and stable HPG-capped gold NPs were isolated without aggregation and resuspended in DI water.

Functionalization of the NPs was confirmed by an increase of hydrodynamic diameter of 2.7 nm and a change in surface effective charge (zeta potential) from -30.2 ± 0.6 mV to -0.01 ± 4 mV for HPG-capped NPs. Previous reports of HPG-capped gold NPs used random and highly amine functionalized HPG which produced positively charged nanoparticles.[12] HPG-capped NPs exhibited enhanced stability to salt-induced aggregation compared to citrate-capped NPs as judged by DLS and UV-Vis spectroscopy (see ESI). The enhanced stability of **7** likely involves a steric protection of the NP surface that is independent of solution ionic strength, thus mimicking the classic PEG NP coating.[13]

As with NPs, drug delivery agents such as unimolecular micelles rely on a biocompatible surface coating for extended circulation in the body and for minimizing toxicity.[14] Recently we reported a method for the synthesis of degradable dendrimers and hydrogels utilizing 1,3,5-triazaadamantane (TAA) monomers.[15] These hydrophobic dendrimers are acid-labile but lack water solubility. Because they are built through a series of click reactions, they present an excellent synthetic scaffold for the synthesis of acid-sensitive unimolecular micelles using alkyne-functionalized HPGs.

A second generation TAA dendrimer **11** (MW = 11 402; 27 azide groups) was used as the core and was reacted with one equivalent of HPG **2b** (fraction 3, M_n = 3000, PDI = 1.25) in DMF with CuI and DIPEA (Scheme 3). The reaction was monitored by SEC and was stopped after 24 h. The polymer obtained, **12**, was fully water soluble despite the IR spectrum indicating the presence of unreacted azide groups. DLS measurements of concentrated solutions of **12** (2.5 mg/mL) indicated that the polymer is monomeric in phosphate buffer with an average hydrodynamic diameter of 8.4 nm. In addition, a cell viability assay showed that the polymer is well-tolerated by cells at concentrations up to 0.75 mg/mL with a toxicity profile similar to HPG alone.[2]

Titration of 1-anilino-8-sulfonic acid (1,8-ANS) was used to verify that **12** could encapsulate non-polar guest molecules. The 1,8-ANS probe is well-established to show enhanced fluorescence in non-polar environments. A plot of fluorescence against [**12**] exhibits a 1 : 1 binding isotherm with an apparent



Scheme 3 Surface functionalization of a TAA dendrimer using **2b** to produce water-soluble, acid-labile unimolecular micelle.

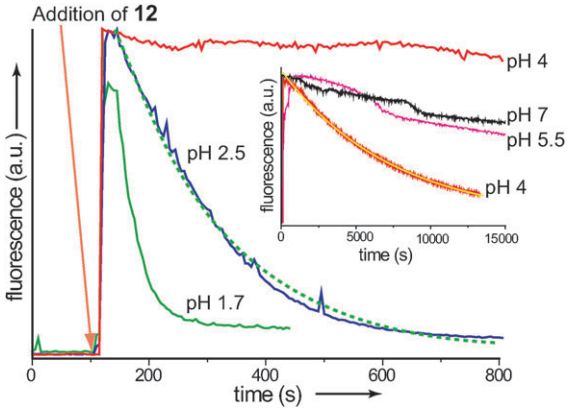


Fig. 3 Fluorescence degradation of ANS in the presence of **12**. Inset, fluorescence response at higher pH values. Green dashed and yellow solid lines represent a fit to a first order decay curve. Degradation at pH 4 shown in both plots.

$K_a = 2.0 \times 10^4\ M^{-1}$. To further verify the guest is encapsulated in a single polymer and not in polymer aggregates, the fluorescence enhancement at lower concentrations was measured. Under these conditions, a linear dependence of fluorescence was observed down to 20 nM with no critical micelle concentration, indicating that **12** does not aggregate under these conditions.

The enhanced fluorescence observed for encapsulated 1,8-ANS provides a convenient method of monitoring dendrimer degradation (Fig. 3). Thus, the fluorescence of 1,8-ANS at various pH values was monitored during the addition of 2.4 equivalents of **12**. Time-dependent fluorescence plots show a spike with addition of **12** as a result of the uptake of the dye into the hydrophobic interior. This is followed by a first order decay of the fluorescence as the dendrimer degrades and the dye is released back into solution. The decay is pH dependent with steady state fluorescence reached in 5 min at pH 1.7 and over 4 h at pH 4. When the same experiment was conducted in the absence of 1,8-ANS a marginal increase in fluorescence was observed which quickly reached a steady state indicating that the fluorescence is due to dye encapsulation and not the fluorescence of the polymer itself. The degradation of the TAA core was also monitored by ^{1}H NMR through the appearance of the aldehyde peak at 10.5 ppm and an overall sharpening of the peaks in the aromatic region. A comparison of the dendrimer degradation and the fluorescence decay of ANS showed similar rates (see ESI‡). The results indicate that the two processes are occurring on a similar time scale and that the fluorescence decay is due to dendrimer degradation and not fluorophore degradation.

Herein we have outlined a simple procedure for the synthesis of alkyne functionalized HPG. These polymers facilitated the preparation of potential biological imaging agents such as monofunctionalized fluorescein and biotin HPG, the stabilization of gold nanoparticles, and the synthesis of unimolecular acid-labile micelles. Because of the diverse applications involving HPG, there is a need for robust chemistry for their synthesis. With this in mind, the preparation of alkyne-cored HPG directly from commercial starting materials is particularly appealing. We are continuing to investigate the use of these polymers as a method for the functionalization and stabilization of nanoparticles, as well as stabilizing agents for water-soluble fluorescent dyes.

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