Efficient synthesis of dendrimers *via* a thiol–yne and esterification process and their potential application in the delivery of platinum anti-cancer drugs†

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A combination of thiol–yne chemistry and esterification reactions were successfully applied for the preparation of dendrimers with an array of terminal functionalities *via* the divergent growth strategy; maximizing the number of reactive chain ends whilst minimizing the number of reaction steps required in the process.

Dendrimers are extremely well-defined, globular, synthetic polymers with a number of characteristics which make them useful in biological systems, especially in the field of drug delivery.[1,2] The nanometric size of dendrimers is beneficial for their entry into hyper-permeable vasculature, while their high molecular weight and lymphatic dysfunction cause their localization, as well as preventing escape *via* the EPR effect. In addition, their surfaces can be elegantly modified so that the drugs can be physically entrapped, encapsulated or conjugated by covalent bonds, hydrogen bonds or ionic interactions.[3] Poly(amidoamine) (PAMAM) dendrimers, the first complete dendrimer family to be synthesized as an example, are widely used in different applications,[4,5] most notably in drug delivery and biomedical applications.[6] The fine structure of dendrimers brings with it excellent properties, but unfortunately synthetic difficulties as well. Dendrimers are synthesized by using repetitive steps of synthetic protocols, either adopting the divergent[7,8] or convergent[9] approach. The synthetic protocols utilized, however, typically require many sequential reaction steps in order to obtain a high number of reactive terminal groups. For example, eight steps are needed to obtain a traditional dendrimer with forty-eight end-group functionalities.[10] To maximize the yield of the desired dendrimers, highly efficient and orthogonal reactions are required. It is also favourable if the number of reaction steps can be reduced, whilst maintaining a high number of functional groups. One good example of this was reported by Majoral and co-workers who employed the Staudinger reaction for the efficient synthesis of dendrimers.[11]

Copper catalyzed azide–alkyne click (CuAAC) chemistry is a robust and efficient reaction, which has been successfully applied to many applications, including dendrimer synthesis.[12–19] Malkoch and co-workers reported accelerated synthesis of dendrimers *via* esterification and CuAAC click reactions.[20] Recently another reaction, emerging as an attractive click process, is the addition of thiols to alkenes, which is called thiol–ene coupling or a thiol–ene click reaction.[21–28] The applicability of this reaction has been highlighted by Hawker and co-workers, whereby they invented a new approach to synthesize dendrimers *via* the thiol–ene chemistry.[25] The process could be carried out in the absence of solvent; it was initiated photochemically and negated the need for a metal catalyst.

Akin to the aforementioned thiol–ene reaction, the thiol addition to alkynes has been exploited as an approach in the field of organic and organometallic chemistry for small molecular synthesis since the reaction was discovered in the 1930s.[29,30] However, this reaction has not been widely used in the field of polymer/material synthesis, with only a few articles published recently,[31–36] none of which cite the synthesis of dendrimers. The process is similar to the thiol–ene click reaction. Like the thiol–ene reaction it is efficient and does not require a metal catalyst but, for the thiol–yne system, the reaction proceeds by reacting two equivalents of thiol with the alkyne, *via* a two-step process.[37] Therefore, after the reaction, each alkyne functional group will be combined with two thiols, which is very attractive and advantageous in the synthesis of dendrimers.

Herein we report a new strategy for synthesizing dendrimers utilizing thiol–yne chemistry. The reactions were very efficient (only ten minutes were required for each thiol–yne reaction) and only a limited number of steps were needed in order to produce macromolecules with a high number of terminal functional groups. For example, a dendrimer with forty-eight end-group functionalities was constructed in three steps from the core (Scheme 1). A benzene-based core was chosen for the dendrimer synthesis with either carboxylic or hydroxyl functional groups on the chain ends. The variety of different terminal groups allows the dendrimers to be complexed or conjugated with a range of different drug molecules. In this communication a preliminary study was undertaken to test if a platinum-based drug could be conjugated with the carboxylate terminated dendrimer.

The reaction between **1** and 1-thiolglycerol, in the presence of trace amounts of the photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPA), was carried out at room temperature by irradiation with UV-lamp (λ = 365 nm) for ten minutes. The thiol–yne reaction yielded the first generation of the dendrimer [G1]-OH_{12} (**2**) with twelve hydroxyl functionalities. The subsequent esterification of **2** with acetylene anhydride (**3**)[10] produced the alkyne end-functional dendrimer [G1]-yne_{12} (**4**). The thiol–yne reaction between (**4**) and 1-thiolglycerol

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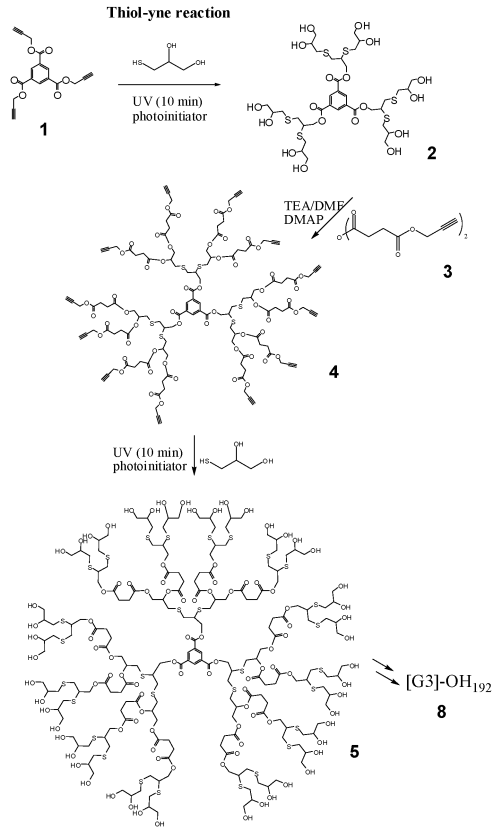
† Electronic supplementary information (ESI) available: Synthetic procedures; GPC, IR, NMR and MALDI-TOF of dendrimers; experimental methods. See DOI: 10.1039/b910340f



Scheme 1 Synthesis of hydroxyl-terminated dendrimer.

afforded the dendrimer (**5**) with forty-eight hydroxyl functionalities. It is worth noting that further purification using chromatography was needed here, after the thiol–yne reaction, due to the impurities in 1-thiolglycerol. Contrary to this, the other reaction using thioglycolic acid afforded the pure product after precipitation. Characterization of the purified products was conducted using NMR spectroscopy, gel-permeation chromatography (GPC) and MALDI-TOF mass spectrometry. A typical NMR spectrum for the dendrimer (**5**) can be seen in Fig. 1. The single peak at 8.43 confirms the symmetrical dendritic framework. The monodisperse nature of each generation of the dendrimer was further demonstrated and complimented by the GPC and MALDI-TOF data. From the MALDI-TOF results, a strong signal for each macromolecule is observed in all cases at the expected molecular weight with only minor contamination from defect structures (Fig. 2). The GPC traces indicated that both the hydroxyl and alkyne-terminal dendrimers are monodisperse, with the expected increase in molecular size as the generation number is increased (Fig. 3). Dendrimer (**5**) was further converted to alkyne functionality and reacted with 1-thiolglycerol to afford a dendrimer (**8**) with 192 hydroxyl functional groups. This demonstrates that the thiol–yne process is a convenient and efficient method to synthesize dendrimers with fewer steps compared with other conventional routes. In addition, dynamic light scattering (DLS) has been used to analyze the size of dendrimers synthesized, which vary between 2 and 4 nm, similar to the traditional PAMAM dendrimer (see ESI†).

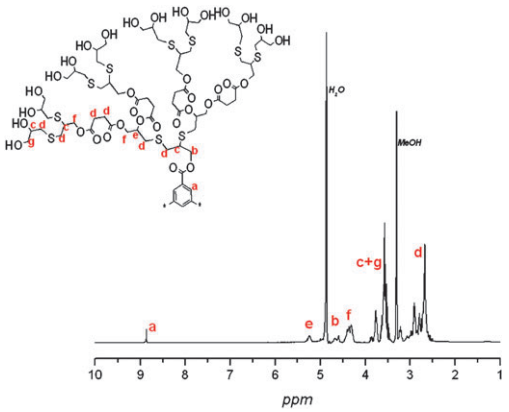


Fig. 1 Representative ^{1}H NMR spectrum of the dendrimer (**5**).

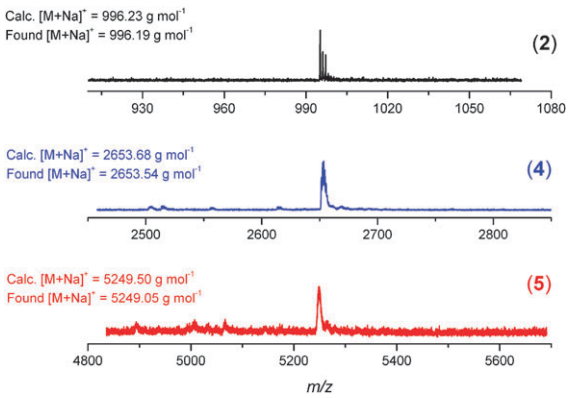


Fig. 2 MALDI-TOF spectra of the dendrimers (**2**), (**4**) and (**5**).

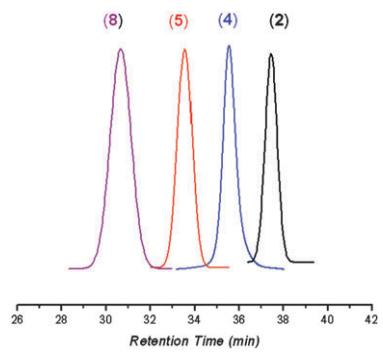
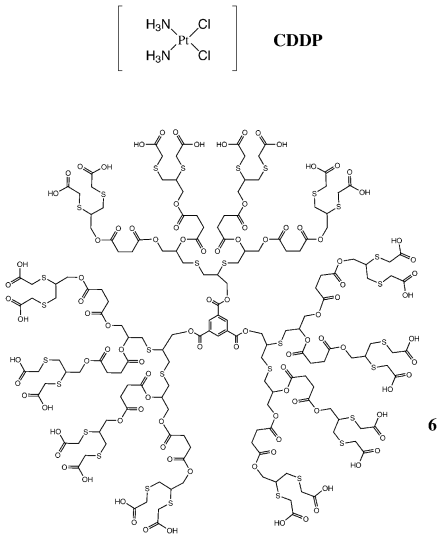


Fig. 3 GPC traces of the dendrimers (**2**), (**4**), (**5**) and (**8**).

To further demonstrate the efficiency of the thiol–yne reaction in the construction of the dendritic backbone with other chain end functionalities, a second thiol–yne reaction to introduce carboxyl functionalities onto the chain ends was carried out. Previous research has shown that anionic carboxyl functionalized dendrimers have a significantly lower cytotoxicity compared to the cationic amino-terminated dendrimers[1] and that carboxylated terminal half-generation PAMAM can be conjugated to platinum-based drugs.[38] In this communication, [G1]-yne$_{12}$ (**4**) was reacted with thioglycolic acid to afford a dendrimer with twenty-four carboxylic acid terminal functionalities (**6**). *cis*-Dichlorodiammineplatinum(II) (**CDDP**) was then used to test the viability of the new carboxylic terminal dendrimer (**6**) as a carrier (Scheme 2). The amount of **CDDP**



Scheme 2 The chemical structures of the cisplatin (**CDDP**) and the carboxylic terminal dendrimer (**6**).

in the conjugate was determined by a method described in a previous study,[39] and a loading of 1.37 mmol g^{-1} of platinum was found in reference to standard solutions of free **CDDP**, which corresponds to a loading of approximately 86%, based on the theoretic value of 1.59 mmol g^{-1} whereby all the platinum species present have been conjugated to the dendrimer.

In summary, the thiol–yne reaction was successfully applied to the synthesis of dendritic macromolecules *via* a divergent approach. Dendrimers with one hundred and ninety-four hydroxyl functionalities and twenty-four carboxyl groups were synthesized, maintaining a high number of reactive chain ends with the bare minimum of reactions. The latter system was further tested as a possible drug delivery vehicle with the successful conjugation of the dendrimer with cisplatin. The metal-free and benign reaction conditions allow for an environmentally friendly process to be developed which is more attractive for drug delivery related applications. The platinum conjugation experiment, although only a preliminary study, has shown a possible application for these macromolecules hence, further investigations in regard to the dendrimer acting as a drug carrier are currently being carried out. Most importantly, we believe this approach featuring the robust and efficient nature of thiol–yne reaction will not only benefit dendrimer-related synthesis but shows great potential as a versatile synthetic tool for the fabrication of different well-defined functional macromolecules.

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