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Double exponential growth of aliphatic polyamide dendrimers *via* AB_2 hypermonomer strategy†

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Received (in Cambridge, UK) 25th February 2009, Accepted 1st June 2009
First published as an Advance Article on the web 17th June 2009
DOI: 10.1039/b903948a

Published on 17 June 2009. Downloaded by McMaster University on 29/10/2014 17:43:50.

We report for the first time a novel strategy for the synthesis of aliphatic polyamide dendrimers based on a hypermonomer (1) starting from allylamine.

Interest in dendrimers,[1,2] since their introduction, has grown considerably owing to their uniqueness in synthesis and applications. It is attractive for researchers to exploit the distinctive and invaluable properties of dendrimers, as many of these compounds find use in medicine,[3] semiconductor,[4] and other fields.[5] Whether it is molecular architecture[6] or complex supramolecular assembly,[7] multi-fold strategies for their synthesis have been reported. Two main synthetic strategies remained unchallenged, namely convergent synthesis developed by Frechet and Hawker[8] and Moore and Xu[9] and divergent synthesis developed by Tomalia *et al.*,[10] Newkome and Shreiner[1]. The main drawbacks associated with both methods are iterative steps, lengthy syntheses requiring substantial amounts of time and their regular purifications of most of the intermediates, thus leading to typically poor overall yields.

Polyamido amine (PAMAM) dendrimers[10] are one of the important classes of dendrimers. The presence of multiple arms in PAMAM dendrimers enhances the availability and use of terminal functionalities.[11] Owing to multiple synthetic steps, it is often difficult to synthesize monodisperse higher generation dendrimers without truncated arms. In order to improve the efficiency of dendrimer synthesis and reduce the time taken to prepare higher generations, several methods to shorten these syntheses have been reported, including double stage, double exponential growth, hypermonomer, and orthogonal coupling strategies. We are particularly interested in the double exponential growth strategy. In this method hyperbranched monomers[12,13] (AB_2) can be utilized to make higher generation products in a fewer number of steps. Use of orthogonal functionalities for AB_2 type monomers can improve many of the shortcomings imposed by conventional dendrimer synthesis. However, an easy and convenient synthetic route to aliphatic polyamide dendrimers using AB_2 hypermonomer has not yet been reported.

A variety of biological functions depend on host–guest interactions.[14] Applications of various synthetic molecules to probe or detect the actions of enzymes, proteins, and other biomolecules are gaining attention. The avidin/streptavidin–biotin system has become a universal tool in many biotechnological applications[13] owing to the stability of these complexes over a wide range of temperature and pH conditions. Unlike avidin, streptavidin can bind four biotin units for enhanced signal intensity; however, the signal intensity obtained with many systems leaves much to be improved. In order to enhance signal-to-noise ratios for diverse and advanced applications, we envisioned building a new biotin–dendrimer complex for general use in biochemical detection. The use of multiple end groups per biotin molecule has the potential to increase the level of sensitivity for various biosensing applications. Since biotin can be efficiently coupled with various amino acids using standard solid-phase peptide chemistry, we chose polyamide units as building blocks. Thus the target molecule will have biotin at the core and extend its dendritic arms that terminate with markers or labels. Since biotin is sensitive to various conditions required for exponential polyamide dendrimer synthesis, we choose to introduce the biotin in the last step of our approach. Thus our strategy is broken into a three-part synthesis consisting of building the dendrimer scaffold, attaching the biotin unit and adding the end groups. The last two components can be interchanged.

A survey of the literature provided very little information on compounds similar to our target polyamide dendrimers. Initially one of the related methods[15,16] was followed, which used 6-aminohexanoic acid as the starting point for the polyamide compound. However, this method was found to be not only lengthy but also associated with poor yields and long reaction times. Moreover, the alkylation step proved very difficult to achieve. It required 3 days of reflux and resulted in a very poor yield. Hence it was decided to abandon this method.

At this juncture a totally different approach to this problem was intriguing. A simple retrosynthetic analysis yielded an AB_2 type monomer precursor. It could be envisioned that, if both A and B are orthogonal functionalities, then selective manipulation of either side will lead to the desired product in fewer steps. In theory any amine can be sourced back to ammonia. Thus, an ammonia equivalent to make an AB_2 monomer became the strategic focus. Various ammonia equivalents are reported in the literature.[17] Most of them,[18] including electrophilic amination reagents,[19] employ strong bases that are incompatible with esters. Some of them require very expensive catalysts.[20] In searching for an easy alternative for the ammonia source, allylamine appeared promising. The allyl group can be orthogonally employed with various acid sensitive and/or base sensitive groups. Esters were chosen as

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† Electronic supplementary information (ESI) available: Experimental details, NMR and mass data of compounds **1–10**. See DOI: 10.1039/b903948a

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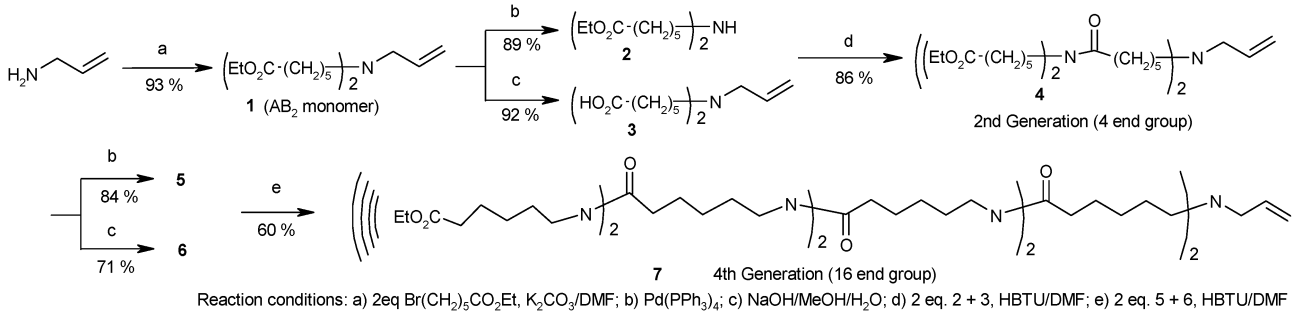

Scheme 1 Reaction conditions: (a) 2 eq. $Br(CH_2)_5CO_2Et$, K_2CO_3–DMF; (b) $Pd(PPh_3)_4$; (c) NaOH–MeOH–H_2O; (d) 2 eq. (**2**) + (**3**), HBTU–DMF; (e) 2 eq. (**5**) + (**6**), HBTU–DMF.

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counterparts to the allyl group, owing to their ease of removal. The first alkylation of allylamine was attempted using ethyl bromohexanoate under various conditions. It was found that K_2CO_3–DMF gave the highest yield and the most pure product. Pure product was obtained either by column chromatography (35–40% EtOAc in hexane) or by vacuum distillation. This AB_2 monomer was obtained in a single step in 93% yield (Scheme 1).

The next task was to remove selectively either of the protecting groups. For removal of the ester group, NaOH-based hydrolysis gave the best results (yield 92%).‡ For the allyl group removal, Pd-catalyzed deallylation was chosen.[21] However, purification was problematic after the deallylation reaction. Neither the reported purification method nor column chromatography was successful. However, the issue was circumvented by selectively choosing the proper purification method[22] (which relies on subtle changes in basicity). This finding not only gave very pure material (yield 89%) but also significantly reduced the purification time. The purification was achieved in less than 30 min. The newly developed method for the AB_2 monomer synthesis has various advantages over the existing methods: (a) it involves only one step to make the monomer, (b) manipulation of orthogonal functionalities are straightforward and simple, (c) the yields are higher, and (d) purifications can be achieved in a relatively short period of time.

After successful preparation of these hypermonomers, steps to make the dendrimer could be pursued. Solid-phase methods were attempted first. Various attempts to couple the deallylamino ester (**2**) to 2-chlorotrityl chloride resin were not successful. Even though the resin-based amino ester could be made by changing the starting material to 6-bromohexanoic ester on Rink amide, the resin-based hydrolysis was not successful. In order to perform hydrolysis under mild conditions, synthesis of TBDMS ester or trichloroacetyl ester, instead of ethyl ester for the AB_2 monomer, was attempted. However, neither change afforded the desired AB_2 monomer.

Next solution-based methods for building the dendrimer were investigated. The deallylamino ester (**2**) and allylic diacid (**3**) were subjected to amide coupling conditions[23] and the tetrameric dendrimer (**4**) was obtained in high yield (86%). The product was split into two fractions. Deallylation was carried out on one to obtain the deallyl tetra ester (**5**, yield 84%), and the other was hydrolyzed to obtain the allyl tetra acid (**6**, yield 71%). Repetition of the amide coupling of these two products, **5** and **6**, led to the hexadecamer dendrimer (**7**, yield 60%). Thus, by using simple reactions, the hexadecamer was obtained in five steps. Extension of this exponential synthesis to larger dendrimers, such as a 256-mer, might be hindered by steric effects, observed in dendrimers,[24] and may require additional modifications of the AB_2 hypermonomer strategy, in order to achieve optimal synthesis.

After successful synthesis of the dendrimer core, attention shifted to preparation and attachment of a biotin unit as the basis for a chemical probe or sensor.[25] Even though biotin can be added in a single step to the dendrimer unit, doing so will not make the target compound stable under *in vivo* or *ex vivo* conditions.[15] Biotinidase selectively cleaves the secondary amide bond connecting biotin to other molecules. This enzymatic degradation will lead to the loss of biotin from the dendritic unit. In order to avoid biotinidase-mediated decomposition, the secondary amide nitrogen has to be protected. A potentially simple step to protect the amide is to use alkyl-substituted amino acids. Owing to very poor solubility in many organic solvents, a solution-based amide coupling using unmodified biotin never proceeded to completion. Moreover, removing excess biotin from the desired product was not practical. Next, solid-phase techniques for making the biotin moiety were explored in order to circumvent the removal of excess of biotin. A C_5 or C_6 linker would position the biotin further from the dendrimer unit, thereby alleviating steric hindrance and making biotin available for complexation with avidin or streptavidin. However, a suitable C_5 or C_6 *N*-alkyl amino acid could not be obtained easily. In order to overcome this problem the simplest *N*-methyl amino acid, sarcosine, was incorporated between the linker and biotin. The present solid-phase synthesis began by linking *N*-fmoc 6-aminohexanoic acid to 2-chlorotrityl chloride resin, using standard techniques and standard, sequential coupling of sarcosine and biotin (Scheme 2). Final cleavage and purification gave the required product in high yield (77%). Using this solid-phase technique we have successfully attached commercially available under-ivatized biotin.

After synthesizing the dendrimer unit and biotin moiety, methods to join them were investigated next. The tetramer unit was chosen for proof-of-principle. Owing to poor solubility of biotin in DMF the amide coupling was carried out in NMP. After the reaction was over (TLC analysis) NMP was removed using high vacuum, and the residue was purified using RP-HPLC. The resulting biotin tetra ester (**9**) was hydrolyzed using a

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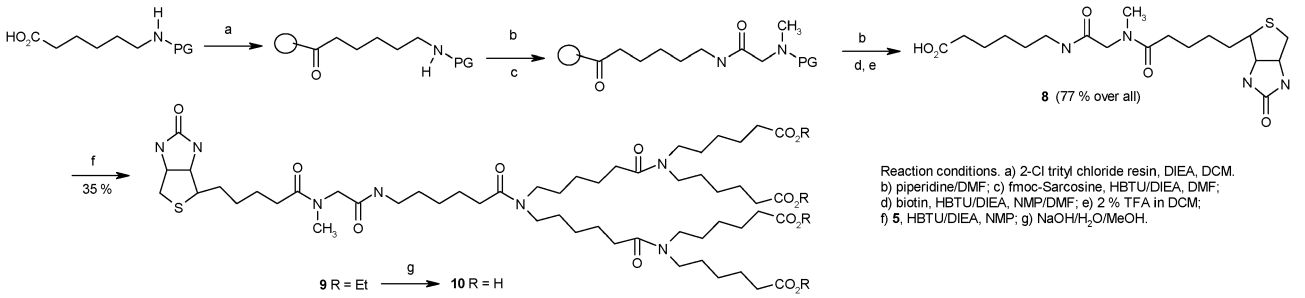

Scheme 2 Reaction conditions: (a) 2-Cl trityl chloride resin, DIEA, DCM; (b) piperidine–DMF; (c) fmoc-sarcosine, HBTU–DIEA, DMF; (d) biotin, HBTU–DIEA, NMP–DMF; (e) 2% TFA in DCM; (f) **5**, HBTU–DIEA, NMP; (g) NaOH–H_2O–MeOH.

NaOH–MeOH–H_2O mixture. Following the work-up method mentioned above the biotin tetra acid was obtained as a white solid (**10**). Work is currently under way to attach various markers and radiolabels to this product, with the future goal of comparing various signal-to-noise enhancements, *e.g.* streptavidin immunohistochemistry, with respect to singly modified biotin derivatives.

In conclusion, for the first time successful application of double exponential growth for the synthesis of aliphatic polyamide dendrimers has been achieved. Judicious choices of purification methods not only afforded pure products but also reduced purification times, thereby enabling faster assembly of these dendrimer molecules. It was also shown that unactivated biotin can be efficiently coupled to amino acids in high yields, using solid-phase techniques. Further work is under way to couple various markers and labels to the biotin–dendrimer units, so that their use in various biotechnological applications can be studied.

Notes and references

‡ Owing to trace amount of solubility of NaCl in methanol[26] an analytically pure sample could not be obtained. However, H, C and mass analysis confirmed the product identity.

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