Construction of well-defined multifunctional dendrimers using a trifunctional core†

Cátia Ornelas and Marcus Weck*

Received (in Austin, TX, USA) 2nd July 2009, Accepted 28th July 2009
First published as an Advance Article on the web 18th August 2009
DOI: 10.1039/b913139f

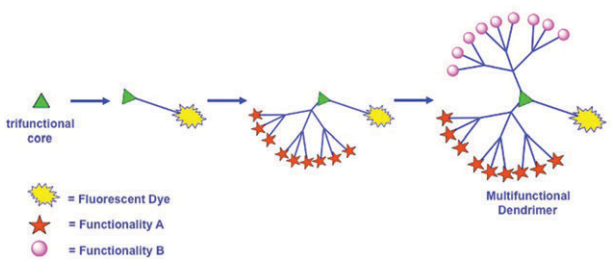
A simple synthetic strategy was developed for the synthesis of well-defined multifunctional poly(amide)-based dendrimers using a trifunctional core.

Dendrimers are perfectly branched macromolecules possessing a high number of active termini that are partially responsible for their properties and functions.[1–3] Synthetic methodologies for the construction of dendrimers are categorized into either divergent[4] or convergent[5] strategies, both consisting of stepwise, iterative reaction processes based on branched monomers. Therefore, appropriate selection of monomers, cores, and mode of assembly ultimately defines the physical properties of dendrimers, such as size, shape, molecular weights, chemical properties, solubility, viscosity, polydispersities, thermal behaviors, and internal and surface functionalization.[6]

Traditionally, dendrimers are synthesized from AB_x monomers, resulting in symmetrical structures with B terminal groups.[4] As dendritic materials migrate into new research areas, the demand on their structural complexity is increasing. For example, the use of dendrimers in theranostics requires multiple functionalities for drug delivery, imaging and targeting. This has led to the partial elucidation of general design principles for the relationship between dendrimer architecture, biocompatibility, retention and drug release.[7] The "ideal" dendritic drug carrier for cancer therapy should contain several functionalities including: (i) hydrophilic groups such as PEG chains in order to increase water solubility and biocompatibility; (ii) imaging agents such as fluorescent dyes or gadolinium complexes in order to monitor the trajectory of the dendrimer *in vitro* or/and *in vivo*; (iii) targeting groups, such as folic acid, biotin or antibodies, to increase binding specificity to cancer cells; and finally (iv) the drug, either encapsulated in the dendritic structure or covalently attached by hydrolysable bonds.[7–11] Pertinent to the realization of such a complex dendrimer-based drug carrier is the multifunctionalization of a dendrimer.

Strategies to multifunctionalize dendrimers are limited. Multifunctionalization of PAMAM dendrimers, the leading dendrimer scaffold used for biological applications, is mainly achieved using a random statistical approach, *i.e.* by successive partial functionalization of the total amine termini[9] with a lack of control over the final structures. There are a few reports on the synthesis of well-defined bifunctional dendrimers either by combination of two different functionalized dendrons[12,13] or by using AB_xC_y-type dendrons,[4,14–16] where $x \geq 1$ and $y = 1$, and A is the functional group at the focal point. These strategies, however, are limited to two functionalities and do not allow for the controlled incorporation of multiple functionalities. Introduction of more than two functionalities was only achieved for benzyl ether-based[17,18] and triazine-based[19] dendrimers. We have reported the synthesis of trifunctional poly(amide)-based dendrimers containing 16 protected acid groups, one azide and one aldehyde groups and have demonstrated their orthogonal functionalization by using the copper-catalyzed 1,3 dipolar cycloaddition (CuAAC) and Schiff base coupling.[20,21] While highly successful, our initial strategy requires extensive synthesis potentially limiting the general use of this strategy. Here we introduce a new concept towards multifunctionalized dendrimers starting with a trifunctional core which can be monofunctionalized selectively. Dendrons bearing different functionalities can be added to the trifunctional core, affording a well-defined multifunctional dendrimer. To demonstrate our new strategy, we describe the synthesis of a dendrimer containing one fluorescent dye, nine azide groups and nine acid groups that are available for further functionalization (Scheme 1).

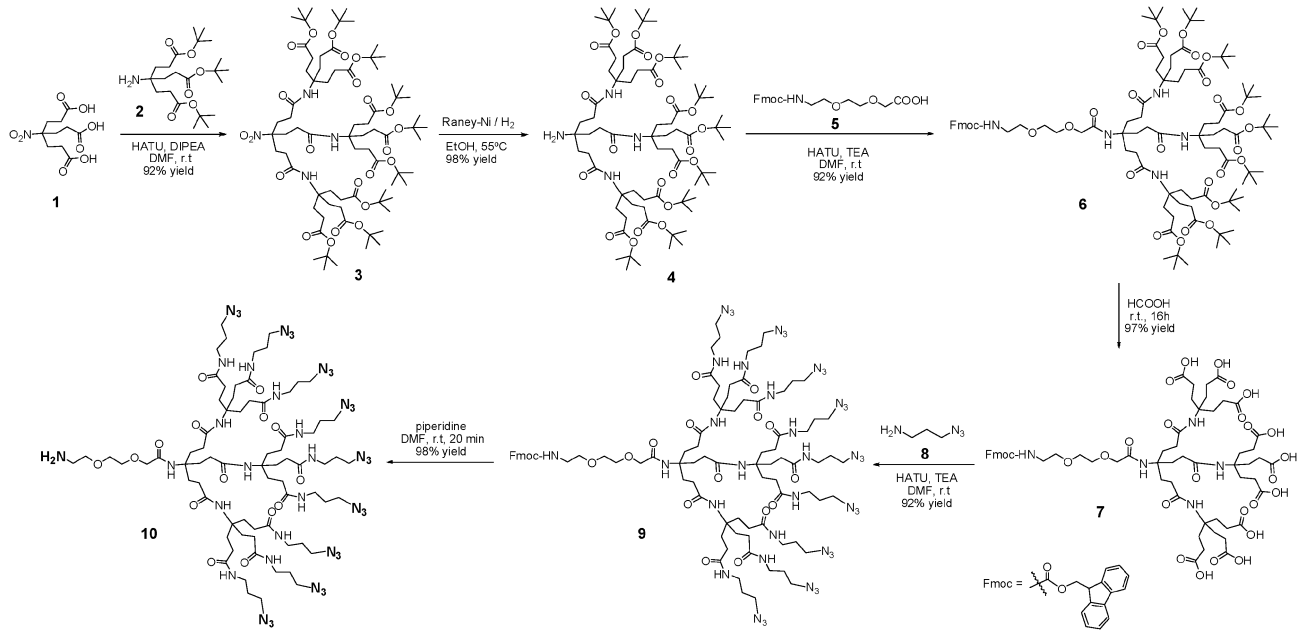
The dendrons used in our strategy are poly(amide)-based to assure biocompatibility and follow the $1 \rightarrow 3$ connectivity pioneered by Newkome *et al.*[22] The synthesis of the dendrons was carried out using peptide coupling steps between commercially available monomers **1** and **2** to yield dendron **3** (Scheme 2).[21,22] After reduction of the nitro group in **3** affording the dendron amino-nona-ester **4**, a PEG spacer (**5**) containing a fmoc protected amine was introduced at the focal point to enhance chemical accessibility of the amine group and to increase water solubility. This coupling reaction



Scheme 1 Schematic representation of our strategy towards well-defined multifunctional dendrimers using dendrons bearing different functionalities that are selectively attached to a trifunctional core.

Molecular Design Institute, Department of Chemistry, New York University, New York, NY 10003-6688, USA. E-mail: Marcus.weck@nyu.edu; Tel: +1 212-992-7968

† Electronic supplementary information (ESI) available: Detailed experimental procedures, characterization data, and ^{1}H NMR and mass spectra. See DOI: 10.1039/b913139f

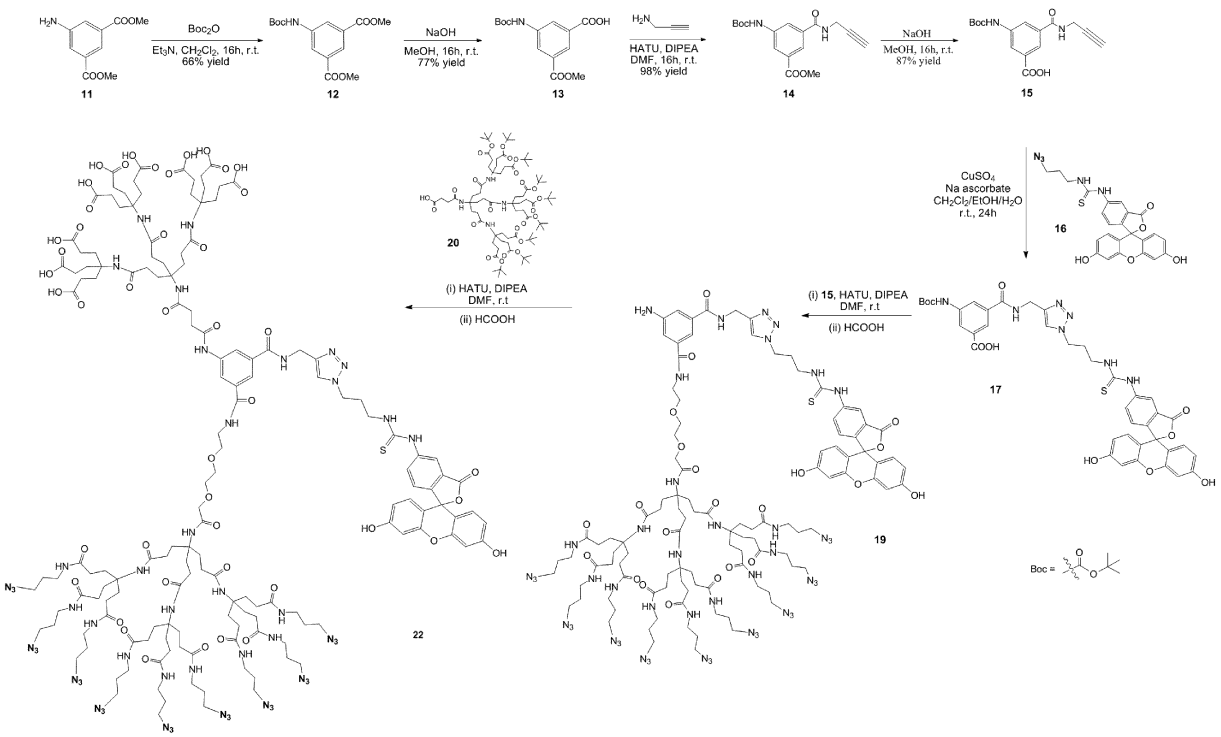


Scheme 2 Synthesis of the poly(amide)-based dendrons.

was performed using HATU as the coupling agent. While most peptide couplings involving HATU use diisopropylethylamine (DIPEA) as the base, it resulted in fmoc deprotection during the coupling reaction. Therefore, triethylamine was successfully used as base for the coupling reactions that involve molecules containing fmoc groups. Dendron **6** was purified by column chromatography using silica gel and ethyl acetate as the eluent. The acid groups of **6** were selectively deprotected in the presence of the fmoc-amine group on the focal point, using formic acid, affording dendron **7**. The azide functionality was introduced into the dendron by coupling **7** with 3-aminopropyl azide (**8**) to obtain dendron **9**. Deprotection of the fmoc-amine group at the focal point was performed in 20 min, using piperidine in DMF at room temperature to yield the final target structure **10**. Dendron **10** was purified by dialysis against water using a Spectra-Por® MWCO (molecular weight cut-off) 500–1000 dialysis membrane and was obtained as a colorless waxy product (Scheme 2). The mass spectrum of dendron **10** shows its molecular peak $(M+H)^+$ at 1819.4 m/z (calcd for $C_{73}H_{128}N_{41}O_{15}$: 1819.1), confirming the expected structure. Every step of the synthesis was carried out in above 90% yield making the synthetic strategy highly efficient and usable on the larger scale.

Our trifunctional core **15** was synthesized from dimethyl 5-aminoisophthalate **11** in four steps in 44% overall yield (Scheme 3) and contains (i) an alkyne group suitable for 1,3 dipolar cycloaddition using copper catalysts (CuAAC), (ii) a carboxylic acid available for amide or ester formation and (iii) a Boc-protected amine. The structure of core **15** was confirmed by ^{1}H NMR spectroscopy that shows the aromatic C*H* at 8.07 and 8.02 ppm, the $CH_2C{\equiv}CH$ at 4.15 ppm, $CH_2C{\equiv}CH$ at 2.62 ppm and the *t*Bu protons of the Boc group at 1.52 ppm, and by mass spectroscopy showing the molecular ion peak $(M-H)^-$ at 317.4 m/z (calcd for $C_{15}H_{17}N_2O_5$: 317.1).

The attachment of the functionalities to the trifunctional core was carried in a stepwise fashion by first introducing a fluorescent dye (fluorescein) *via* CuAAC, an efficient click reaction.[23] The reaction occurred in an orthogonal fashion between the alkyne of **15** and the azide derivative of fluorescein **16** affording compound **17** that was purified by reverse-phase column chromatography and was obtained as a yellow solid in 62% yield. Dendron **10** was then attached to the acid group of **17** using peptide coupling affording, after purification by dialysis against methanol using a Spectra-Por® MWCO 2000 dialysis membrane, compound **18** in 72% yield. The ^{1}H NMR spectrum of **18** shows the signals corresponding to the fluorescein and the trifunctional core in the aromatic region, the Boc group at 1.52 ppm, the PEG linker between 3.5 and 4.0 ppm, and the peaks corresponding to the dendron, including the CH_2N_3 at 3.36 ppm. After deprotection of the amine group using formic acid (the disappearance of the Boc protons was followed by ^{1}H NMR spectroscopy) dendron **20** containing one acid group at the focal point and nine protected acid groups at the periphery was attached to the amino group of the trifunctional core. Dendrimer **21** was obtained in 81% yield after purification by dialysis against methanol using a Spectra-Por® MWCO 2000 dialysis membrane. Due to the addition of dendron **20** that contains *t*Bu termini, we observed a significant increase in the hydrophobicity of dendrimer **21** compared to **19** resulting in good solubility in methylene chloride. This fact, together with NMR spectroscopy and mass spectrometry, confirmed the successful attachment of the third functionality to the trifunctional core. The final step of the synthesis was the deprotection of the acid groups of **21** to afford the multifunctional target dendrimer **22** containing one fluorescent dye, nine azide groups and nine acid groups. The mass spectrum of **22** shows its molecular ion peak $(M+H)^+$ at 3524.2 (calcd for $C_{152}H_{219}N_{52}O_{45}S$: 3524.6) confirming the structure of the final well-defined multifunctional dendrimer (see ESI† for experimental details, characterization data and ^{1}H NMR and mass spectra of all compounds).



Scheme 3 Synthesis of the multifunctional dendrimer **22**.

In conclusion, in this contribution, we introduce a new multifunctionalization strategy in dendrimer chemistry. All steps have been carried out in high yields and allow for the introduction of a wide variety of functional groups into dendrimers. We suggest that this strategy permits for synthesis of highly functionalized dendrimer-based materials for a number of applications including theranostics. For example, the dendrimer reported here might potentially be used in anti-cancer technology by attaching (i) targeting moieties using CuAAC reaction, (ii) drugs *via* the unprotected acid groups on the surface of the dendrimer as well as (iii) different imaging agents. Therefore, this strategy can be viewed as a ''toolbox'' of drug delivery carriers by varying the dendron functionalities and size. Further development of this ''toolbox'' as well as its application to anti-cancer studies are currently being carried out in our group.

Notes and references

1 G. R. Newkome, C. N. Moorefield and F. Vögtle, *Dendrimers and Dendrons: Concepts, Synthesis, Applications*, Wiley-VCH, Weinheim, 2001.
2 D. A. Tomalia, A. M. Naylor and W. A. Goddard, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 138–175.
3 Dendrimers and Nanosciences, (ed. D. Astruc), *C. R. Chim.*, 2003, **6**, issues 8–10.
4 G. R. Newkome and C. D. Shreiner, *Polymer*, 2008, **49**, 1–173.
5 C. Hawker and J. M. J. Fréchet, *J. Chem. Soc., Chem. Commun.*, 1990, 1010–1013.
6 A. W. Bosman, H. M. Janssen and E. W. Meijer, *Chem. Rev.*, 1999, **99**, 1665–1688.
7 J. B. Wolinsky and M. W. Grinstaff, *Adv. Drug Delivery Rev.*, 2008, **60**, 1037–1055.
8 E. R. Gillies and J. M. J. Fréchet, *Drug Discovery Today*, 2005, **10**, 35–43.
9 R. K. Tekade, P. V. Kumar and N. K. Jain, *Chem. Rev.*, 2009, **109**, 49–87.
10 I. J. Majoros, C. R. Williams and J. R. Baker, *Curr. Top. Med. Chem.*, 2008, **8**, 1165–1179.
11 E. Hill, R. Shukla, S. S. Park and J. R. Baker, *Bioconjugate Chem.*, 2007, **18**, 1756–1762.
12 J. W. Lee, B. K. Kim, J. H. Kim, W. S. Shin and S. H. Jin, *J. Org. Chem.*, 2006, **71**, 4988–4991.
13 G. Franc and A. Kakkar, *Chem. Commun.*, 2008, 5267–5276.
14 G. R. Newkome, H. J. Kim, C. N. Moorefield, H. Maddi and K. S. Yoo, *Macromolecules*, 2003, **36**, 4345–4354.
15 A. P. Goodwin, S. S. Lam and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2007, **129**, 6994–6995.
16 P. Antoni, Y. Hed, A. Nordberg, D. Nystrom, H. von Holst, A. Hult and M. Malkoch, *Angew. Chem., Int. Ed.*, 2009, **48**, 2126–2130.
17 K. Sivanandan, D. Vutukuri and S. Thayumanavan, *Org. Lett.*, 2002, **4**, 3751–3753.
18 K. Sivanandan, B. S. Sandanaraj and S. Thayumanavan, *J. Org. Chem.*, 2004, **69**, 2937–2944.
19 W. Zhang, D. T. Nowlan, L. M. Thomson, W. M. Lackowski and E. E. Simanek, *J. Am. Chem. Soc.*, 2001, **123**, 8914–8922.
20 K. Yoon, P. Goyal and M. Weck, *Org. Lett.*, 2007, **9**, 2051–2054.
21 P. Goyal, K. Yoon and M. Weck, *Chem.–Eur. J.*, 2007, **13**, 8801–8810.
22 G. R. Newkome, K. K. Kotta and C. N. Moorefield, *J. Org. Chem.*, 2005, **70**, 4893–4896.
23 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.

