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COMMUNICATION

Amphiphilic poly(aminoester) dendrimers: Click reaction-enabled synthesis, structure-governed self-assembling and degradation

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Poly(aminoester) (PAE) dendrimers are attractive materials for biomedical applications owing to their biodegradability and precise structure, provided effective synthesis and favourable safety profile. We report here a click chemistry–enabled divergent–convergent strategy for the efficient synthesis of amphiphilic PAE dendrimers. These dendrimers exhibit negligible cytotoxicity, generation-dependent degradation behaviour, and tunable self-assembly controlled by hydrophobic chain length. This study offers accessible synthesis of PAE dendrimers for advancing them in future biomedical applications.

Biodegradable biomaterials, particularly poly(aminoesters) (PAEs), have gained considerable attention for biomedical applications owing to their intrinsic biodegradability.[1] PAEs combine hydrolysable ester bonds with ionizable amine functionalities, facilitating efficient drug and nucleic acid delivery.[2,3] However, their inherent polydispersity poses a significant challenge to their clinical translation.[2] Dendrimers with well-defined structures and highly branched architectures,[4] are unique precision materials for biomedical advantages.[5] Yet, synthesizing high-generation dendrimers, in particular PAE dendrimers,[6] is challenging due to structural defects and purification difficulties, hindering their clinical progress.[7]

To address these challenges, we have pioneered a novel synthetic approach for constructing supramolecular dendrimers via the self-assembling of small amphiphilic dendrimers.[8] Expanding on this concept, a series of amphiphilic dendrimers with varied hydrophilic-hydrophobic structures have been designed for diverse biomedical applications.[9-12] These amphiphilic dendrimers were synthesized by coupling hydrophilic dendrons to hydrophobic entities via click chemistry—a method known for efficiency, simplicity, and mild reaction conditions.[13] Leveraging this approach, we have synthesized a small amphiphilic PAE dendrimer **I** via a click reaction between an azido-functionalized hydrophobic C_{18} alkyl chain and an alkyne-bearing hydrophilic PAE dendron with four terminal groups (Figure 1A).[14] This dendrimer seamlessly integrates the structural features of lipid and dendrimer vectors. It incorporates hydrolysable ester bonds and ionizable amine functionalities (e.g., tertiary and primary amines). The presence of tertiary amines in the dendrimer interior imparts pH-responsive behavior, enabling endosomal escape via the "proton sponge effect" and facilitating drug release under acidic conditions. Moreover, the ester bonds confer greater hydrolytic lability than the amide linkages in polyamidoamine (PAMAM) dendrimers, enhancing degradability and promoting carrier clearance and cargo release under physiological conditions. All these features enable this amphiphilic PAE dendrimer to achieve effective delivery of small interfering RNA (siRNA) alongside with excellent biodegradability.

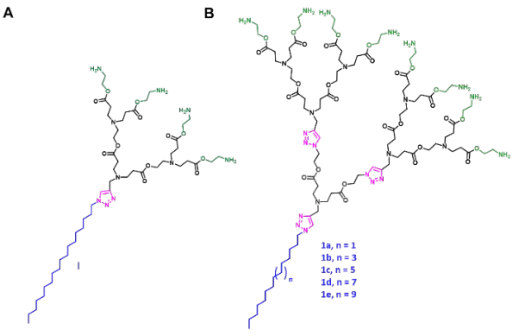

Fig. 1 The molecular structure of amphiphilic PAE dendrimers: A) lower-generation amphiphilic PAE dendrimer bearing four amine terminals (**I**); B) higher-generation amphiphilic PAE dendrimer featuring hydrophobic alky chains of varying lengths (C_{14} to C_{22}) and hydrophilic PAE dendrons with eight amine terminals (**1a-1e**).

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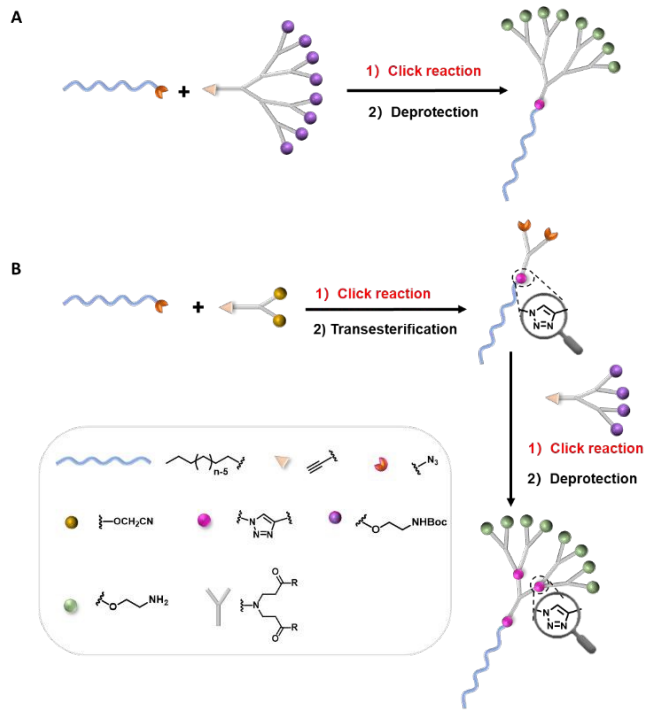
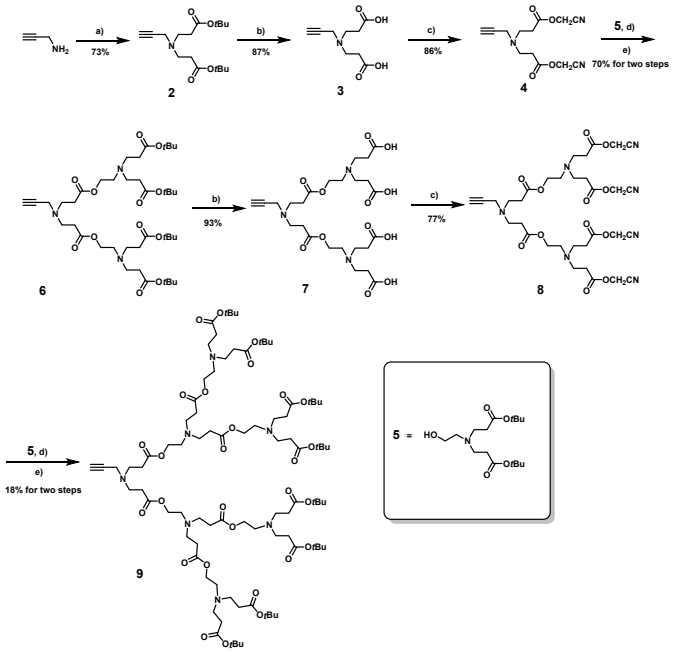


Fig. 2 Modular strategies for efficient synthesis of amphiphilic PAE dendrimers: (A) previously reported approach; (B) approach developed in this study.

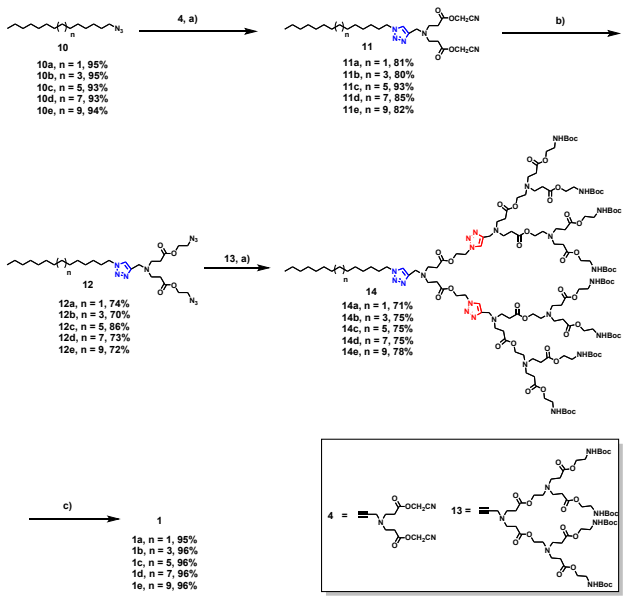
Inspired by the promising feature of this PAE dendrimer, we aimed to construct higher-generation amphiphilic PAE dendrimers **1a-1e** with varied hydrophobic chain lengths (Figure 1B and Figure S1) for potential application in drug delivery. To this end, we sought to extend our previous strategy[14] by coupling a higher-generation hydrophilic PAE dendron bearing eight terminal groups to hydrophobic alkyl chains of varying lengths via click chemistry (Figure 2A). Although the previously reported method employing reactive cyanomethyl ester intermediates successfully constructed four-terminal PAE-based hydrophilic dendron, extending this approach to eight-terminal analogs was not satisfactory, giving higher-generation dendrimers in very low yields. Specifically, a transesterification reaction between the four terminal cyanomethyl ester intermediate **8** and the branched alcohol **5** containing two tert-butyl ester termini afforded the target dendron **9** with eight tert-butyl ester termini in an extremely low yield of only 18% (Scheme 1). This can be ascribed to the steric hindrance and incomplete coupling efficiency during the assembly of higher generation dendron. Also the increased number of ester bonds made higher generation PAE dendrimers more susceptible to nucleophilic attack or degradation during prolonged synthesis conditions and purification steps, leading to further structural defects, hence low yields.

To overcome these limitations, we were committed to developing novel approaches for robust and efficient construction of amphiphilic PAE dendrimers, particularly those of higher generation. We envisioned a divergent-convergent strategy combined with click chemistry for synthesizing the PAE dendrimers (**1a-1e**) (Figure 2B). Specifically, an azide-functionalized hydrophobic alkyl chain was first conjugated to a smaller alkynyl-bearing PAE dendron with two terminal groups via a Cu(I)-catalyzed Huisgen cycloaddition. The resulting intermediate was then subjected to transesterification to yield an amphiphilic dendrimer bearing two terminal azide groups. This intermediate was subsequently coupled with, via a second click reaction, an alkynyl-terminated PAE dendron containing four terminal groups, followed by deprotection to afford the amphiphilic PAE dendrimer with eight terminal groups. Using this approach, the amphiphilic PAE dendrimers (**1a–1e**) with various chain length were successfully synthesized. We present below their synthesis and evaluation on their degradation and self-assembly behaviour.



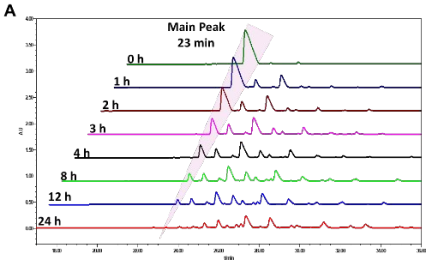
Scheme. 1 Synthesis of PAE dendron **9** with with eight tert-butyl ester termini. a) tert-Butyl Acrylate, MeOH, 30°C, 72 h, b) TFA, DCM, 30°C, 48 h, c) Chloroacetonitrile, NEt_3, DMF, 30°C, 24 h, d) DBU, MeCN, 30°C, 48 h, e) Bz_2O, DMAP, DCM, 30°C, 2 h.

Scheme 2 illustrates the synthesis of amphiphilic PAE dendrimers (**1a–1e**) step-by-step in details. Azide-functionalized hydrophobic alkyl chains (**10a-10e**) (Scheme S1), and the alkynyl-containing hydrophilic PAE dendrons **4** (Scheme 1) and **13** (Scheme S2) were prepared according to previously reported procedures.[14] Then (**10a-10e**) were respectively coupled with **4** via a $CuSO_4 \cdot 5H_2O$/sodium ascorbate-mediated copper-catalyzed azide–alkyne cycloaddition (CuAAC), affording the corresponding (**11a-11e**). Subsequent transesterification of (**11a-11e**) with 2-azidoethanol under DBU-promoted alkaline conditions yielded the azide-functionalized intermediates (**12a-12e**). A second click reaction between (**12a-12e**) and dendron **13** under identical CuAAC conditions produced dendrimers (**14a-14e**). Further deprotection of Boc groups with trifluoroacetic acid (TFA) furnished the target PAE dendrimers (**1a-1e**) in yields exceeding 95%. The structures and purities of the final dendrimers (**1a-1e**) were confirmed using ^{1}H NMR and ^{13}C NMR spectroscopy, high-resolution mass spectrometry (HRMS), and high-performance liquid chromatography (HPLC) (Fig. S2-S6, ESI†).



Scheme. 2 Synthesis of amphiphilic PAE dendrimers (**1a-1e**) with different hydrophobic chain lengths. a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, 50°C, 3 h, b) 2-Azidoethanol, DBU, MeCN, 30°C, 48 h, c) TFA/DCM, 30°C, 6 h.

The PAE dendrimers (**1a-1e**), featuring ester functionalities within their backbone, are expected to be susceptible to hydrolytic degradation. We therefore assessed their degradation behaviour in aqueous solution at 25°C using HPLC. Taking **1c** as a representative example, the peak with retention time of 23 min in HPLC featuring the intact dendrimer **1c** steadily decreased and vanished completely after 24 h, whereas



B

1c

t (h)	Main Peak Area	Degradation Percentage
0 h	15754656	0
1 h	12558939	20.3
2 h	8196468	48.0
3 h	4112689	73.9
4 h	2737096	82.6
8 h	1256892	92.0
12 h	661958	95.8
24 h	0	100

Fig. 3 Degradation of amphiphilic PAE dendrimer **1c** at room temperature: A) Temporal changes in the main HPLC peak corresponding to the intact dendrimer; B) Quantification of the main peak area over time, monitoring the degradation of PAE dendrimer **1c**.

numerous peaks with longer retention times appeared corresponding to the degradation products (Fig.3A**,** Fig. S7, ESI†). Integration of the peak at 23 min revealed a sharp decline in area within 12 h, with full disappearance by 24 h (Fig. 3B), highlighting rapid degradation.

Similarly, the other dendrimers exhibited comparable degradation profiles to **1c**, with complete degradation observed within 24 h at room temperature (Fig. S8–S11, ESI†). Further quantitative analysis confirmed that all dendrimers share a similar degradation pattern (Fig. 4A), despite variations in hydrophobic alkyl chain length ranging from C14 to C22. This consistent degradation behaviour can be attributed to their identical hydrophilic dendrons, indicating that hydrolytic degradation is governed primarily by dendritic architecture rather than alkyl chain length. These results highlight the pivotal role of the dendron structure in dictating the stability and degradation of amphiphilic PAE dendrimers.

Dendrimers often exhibit a "dendritic effect," wherein their properties change with generation and size.[15,16] This effect encompasses multivalency—absent in monomeric analogues but present across all generations—and generation-dependent property evolution, both of which modulate dendrimer characteristics. To probe the influence of this dendritic effect on degradation, we compared **1c** with a previously reported lower-generation amphiphilic PAE dendrimer with four terminal groups (**I**) and two terminal groups ($\mathbf{I_0}$), which shares the same hydrophobic alkyl chain length but has a lower ester bond density (Fig. 4B, Fig. S7, S12 and S13, ESI†). Notably, **1c** degraded faster than its lower generation analogues **I** and $\mathbf{I_0}$, consistent with generation-dependent degradation. This may be attributed to the increasing dendrimer generation, which creates steric hindrance, making the ester linkage unstable hence ready for hydrolysis. This "dendritic effect" thus accelerates degradation in the higher-generation PAE dendrimers, which can be explored for generation-dependent drug release.

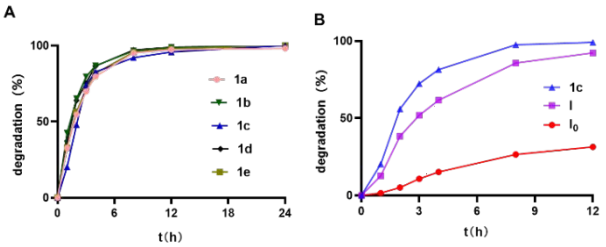


Fig. 4 Quantitative analysis of the degradation behaviour of amphiphilic PAE dendrimers at room temperature: A) dendrimers (**1a-1e**); B) 1c and amphiphilic PAE dendrimer bearing four terminal groups (**I**) and two terminal groups ($\mathbf{I_0}$).

Owing to their amphiphilic nature, (**1a-1e**) are expected to self-assemble in aqueous environment. We therefore evaluated their critical aggregation concentration (CAC) utilizing a fluorescence assay with Nile red as a probe at room temperature. As summarized in Table 1 and shown in Fig. S14, all dendrimers exhibited pronounced self-assembly. Notably, the CAC decreased from 53.7 µM for **1a** to 5.01 µM for **1e** with increasing alkyl chain length, revealing a clear chain length-dependent aggregation behaviour. This trend can be attributed to strengthened hydrophobic interactions within increasing alkyl chain length among (**1a-1e**). These results align with our previous observation[17] and demonstrate that tuning the balance between hydrophilic and hydrophobic segments modulates self-assembly, providing a foundation for the

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rational design of modular PAE dendrimers for the desired application.

Table. 1 Critical aggregation concentration (CAC) of PAE dendrimers (**1a-1e**) at room temperature.

Dendrimer	CAC (μM)
1a	53.7
1b	20.3
1c	11.5
1d	6.16
1e	5.01

We further assessed the cytotoxicity of dendrimers (**1a-1e**) across multiple cell lines, including mouse fibroblast L929, human liver cell L02, and Madin-Darby canine kidney cell MDCK. All dendrimers exhibited half maximal inhibitory concentration (IC_{50}) exceeding 100 μM, indicating negligible cytotoxicity (Table 2 and Fig S15). Given that the dendrimers underwent completely degradation within 24 h at room temperature (25°C) (Fig. 3 and S7, ESI†) and within 8 h at 37 °C (Fig. S16, ESI†), these finding demonstrate that both the intact dendrimers and their degradation products showed no cytotoxic effects. This favourable safety profile supports the potential of these dendrimers for further biomedical applications.

Table. 2 IC_{50} of PAE dendrimers in normal cells (L929 cells, L02 cells, MDCK cells) determined by MTT assay after 48 h incubation at 37°C.

Dendrimer	IC_{50} (μM)		
	L929	L02	MDCK
1a	> 100	> 100	> 100
1b	> 100	> 100	> 100
1c	> 100	> 100	> 100
1d	> 100	> 100	> 100
1e	> 100	> 100	> 100

In summary, we have developed a divergent-convergent strategy combined with click chemistry for the rapid and efficient synthesis of higher-generation amphiphilic PAE dendrimers. This method streamlines the synthesis of higher-generation PAE dendrimers by reducing synthetic steps and reaction times, and improving overall yields. They exhibit excellent and fast degradability alongside safety profiles, with degradation performance governed by a generation-dependent "dendritic effect". Additionally, their self-assembly behavior is tunable through variations in hydrophobic chain length highlighting their potential as modular platform for future biomedical applications. Nevertheless, the rapid degradation of PEA dendrimers must be carefully assessed to ensure their effective use in biomedical applications without compromising their advantageous properties. We are actively working in this direction.

L. P., X. L.: project conceptualization and supervision; Y. M., J. Z., D. Z., A. M., D. Y., C. M., X. L.: methodology, investigation, data analysis; H. D.: data analysis; Y. M., D. Z., L. P., X. L.: wrote the paper, all approved the manuscript

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI).

Acknowledgements

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Data Availability Statement

The data supporting this article have been included as part of the supplementary information (SI).