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## Highly branched poly( $\beta$ -amino ester)s with thiolated branching units for gene delivery

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Highly branched poly( $\beta$ -amino ester)s (HPAEs) are among the most promising candidates for gene delivery. However, so far, all HPAEs have been synthesized primarily via Michael addition with triacrylates, tetraacrylates or diamines as branching monomers. Here we report the development of HPAEs with thiolated branching units. By using pentaerythritol tetrakis(3-mercaptopropionate) (PTMP) as the branching monomer, two HPAEs with similar molecular weights but different branching degrees were synthesized. The results show that both HPAEs effectively condense DNA into nanosized polyplexes with positive zeta potentials. HPAE-1/DNA polyplexes were capable of transfecting both the human cervical cancer cell line (HeLa) and the difficult-to-transfect human bladder transitional cell carcinoma (UM-UC-3). The maximum transfection efficiency of HPAE-1 was lower than that of linear poly( $\beta$ -amino ester) (L-C32) and HPAE containing tetraacrylate branching units (ER-HPAE-1), but comparable to that of star-shaped PAE with a diamine core (SPAE-1-5 h) and branched PEI 25k, with high cell viability. This study develops a new class of HPAEs with thiolated branching units, expanding the chemical diversity of the HPAE family.

## Introduction

Many diseases are associated with disease-causing genes. By delivering functional genetic material to replace faulty genes and regulate the expression of specific proteins, these diseases can potentially be treated at the genetic level.<sup>1</sup> However, the

translation of gene therapy is currently hindered by the lack of efficient and safe gene delivery vectors.<sup>2</sup> Ideal gene vectors are expected to achieve high levels of gene transfection efficiency while minimizing cytotoxicity and immune responses.<sup>3</sup> Over the past three decades, significant efforts have been devoted to developing gene delivery vectors. While nonviral gene vectors (e.g., cationic lipids, polymers) generally exhibit lower transfection efficiency, they are safer, structurally flexible, and have a higher gene packaging capacity, which has led to growing interest in the development of clinically reliable nonviral gene vectors.<sup>4–6</sup>

Among the various nonviral gene vectors, HPAEs have shown considerable promise compared to other cationic polymers including PEI, poly(dimethylaminoethyl methacrylate) (PDMAEMA), and polyamidoamine (PAMAM) due to multiple advantages such as broad monomer availability, ease of synthesis, structural diversity, and hydrolytic degradability.<sup>7–11</sup> Moreover, the three-dimensional (3D) structure and multivalency of the terminal groups facilitate multiple steps in the gene delivery process, such as DNA binding and polyplex cellular uptake.<sup>11–14</sup> In addition, the terminal groups offer multiple sites for further functionalization.<sup>15</sup> In 2015, Zhou *et al.* developed an “A2 + B3 + C2” type Michael addition strategy for synthesizing HPAEs by introducing B3-type triacrylate trimethylolpropane triacrylate (TMPTA) into the A2/C2 monomer combinations for linear poly( $\beta$ -amino ester) (LPAE) synthesis.<sup>9,16</sup> Gene transfection studies revealed that the HPAEs mediated up to an 8521-fold higher transfection efficiency compared to corresponding LPAEs and commercial gene transfection reagents.<sup>10</sup> Later, B4-type tetraacrylate pentaerythritol tetraacrylate (PET4A), diamines 1,3-diaminopropane (DAP), ethylenediamine (EDA) and hexamethylene diamine (HDA) were further employed to synthesize HPAEs.<sup>17–21</sup> Despite the great progress, however, the branching monomers B3 and B4 have been predominantly limited to triacrylates, tetraacrylates and diamines. Moreover, the conjugation of amines to acrylates results in the formation of tertiary amines, which become protonated under physiological conditions. This increases the positive

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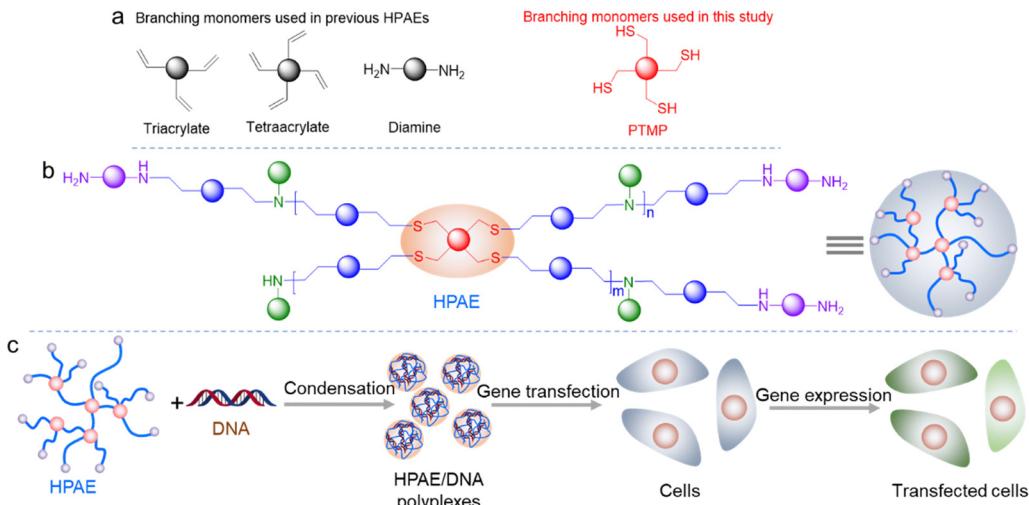
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**Scheme 1** Synthesis of HPAEs with thiolated branching units for DNA transfection. (a) Chemical structures of various branching monomers used for the synthesis of HPAEs; (b) a depiction of the chemical structure of HPAE featuring thiolated branching units; (c) HPAEs with thiolated branching units effectively condense DNA to form polyplexes, achieving high transfection efficiency.

charge density and enhances adsorption to negatively charged biomacromolecules. Therefore, the synthesis of HPAEs using new branching monomers could further expand the chemical diversity of this family.

In this work, we report the use of **PTMP** as the branching monomer for synthesizing HPAEs *via* an “A2 + B4 + C2” Michael addition strategy (Scheme 1). By adjusting the feed ratio of **PTMP**, we synthesized HPAE-1 and HPAE-2 with different branching degrees but similar molecular weights. Both HPAEs exhibit high DNA binding affinity (>75%) and are capable of condensing DNA into polyplexes with a diameter of less than 300 nm and a zeta potential of over +12 mV. In HeLa cells, HPAE-2 achieved comparable or even up to 2.04-fold higher DNA transfection efficiency compared to the corresponding LPAE (L-C32), HPAE with tetraacrylate branching units (ER-HPAE-1), and star-shaped HPAE with a diamine core (SPAE-1-5 h). In the difficult-to-transfet UM-UC-3 cells, HPAE-2 demonstrated up to a 9.1-fold higher gene transfection efficiency than the commercial reagent PEI 25k, while maintaining high cell viability. This study demonstrates the feasibility of using thiolated branching monomers in HPAE synthesis, greatly expanding the chemical diversity and family of HPAEs.

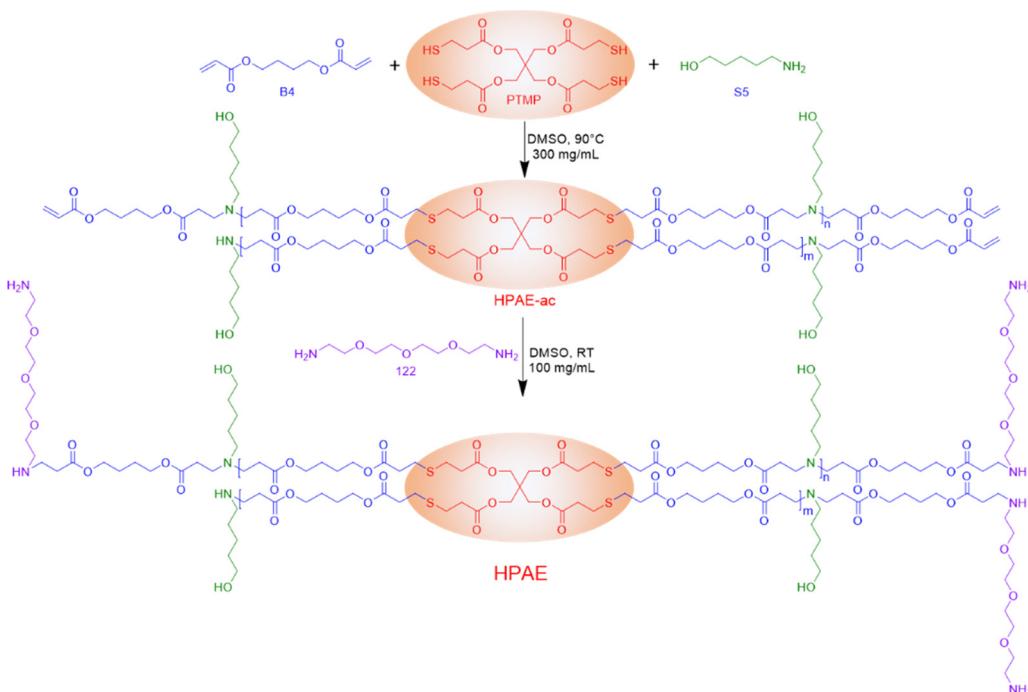
## Results and discussion

### Synthesis of HPAEs using PTMP as the branching monomer

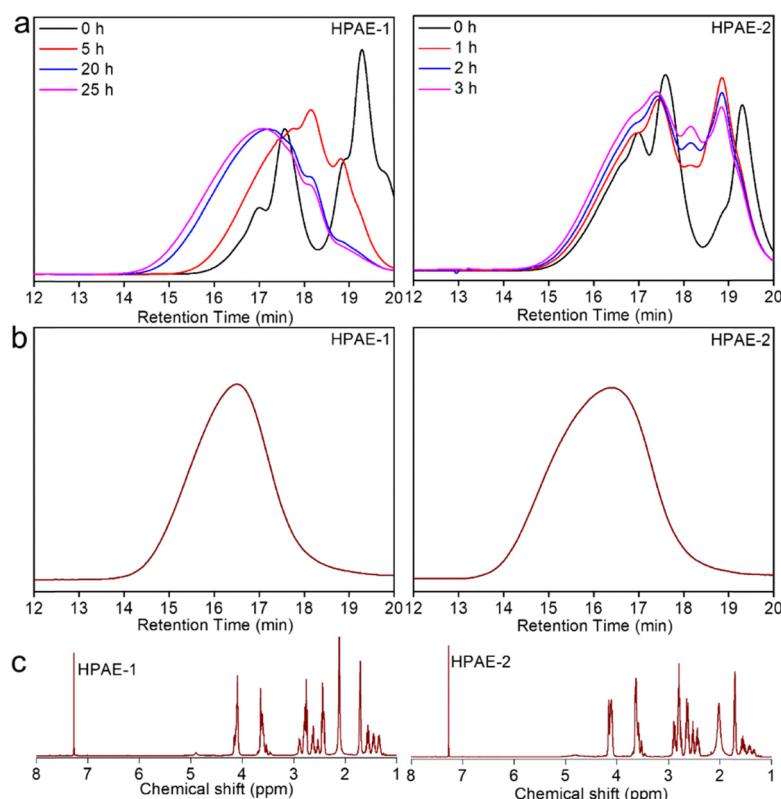
Previously, HPAEs were primarily synthesized through conjugation addition of amines to diacrylates, triacrylates, or tetraacrylates.<sup>13,18,20-30</sup> These branching monomers, such as triacrylates, tetraacrylates, or diamines, share similar reactivity with functional diacrylates or amines. They branch out the linear segments, resulting in the formation of a branched topological structure for HPAEs. The polymerization process was typically carried out at 90 °C. However, excess endcapping

amines, high temperatures, and prolonged endcapping duration would lead to aminolysis of the HPAE base polymers (HPAE-ac). In contrast, thia-Michael addition has been widely used in organic synthesis due to its mild reaction conditions and high reaction rates, which can even be performed in physiological environments. To test our hypothesis, we employed **PTMP** with four thiol groups as the branching monomer, while 1,4-butanediol diacrylate (**B4**) and 5-amino-1-pentanol (**S5**) which have demonstrated to be effective for gene delivery as the functional monomers (Fig. 1)<sup>23</sup>. Dimethyl sulfoxide (DMSO) was used as the solvent. To synthesize HPAE-1, which has a relatively low degree of branching, the molar feed ratio of **[B4]:[S5]:[PTMP]** was set at 5.28:4.0:0.24 (Table S1), corresponding to a stoichiometric ratio of **[B4]/[S5 + PTMP]** close to 1.2:1, to favor the formation of acrylate-terminated HPAE-ac. The total monomer concentration was 300 mg mL<sup>-1</sup>, and the polymerization was carried out in a one-pot manner at 90 °C. Under these conditions, **B4** reacts concurrently with both **PTMP** and **S5**. Because **PTMP** exhibits higher reactivity toward **B4** than **S5** does, the reaction results in the formation of a branched polymer containing multiple **PTMP**-derived branching units, **B4-S5** linear segments, and terminal acrylate groups. Gel permeation chromatography (GPC) equipped with a refractive index (RI) detector was used to monitor the evolution of molecular weight during the polymerization process, with molecular weight determined relative to poly(methyl methacrylate) (PMMA) standards. After 5 hours of polymerization, the weight average molecular weight ( $M_w$ ) of the reaction mixture increased to 4020 g mol<sup>-1</sup>, with a polydispersity index ( $D$ ) of 1.32 (Fig. 2a and Table S2). When the reaction time was extended to 20 hours, the  $M_w$  steadily increased to 6280 g mol<sup>-1</sup>, with a  $D$  of 1.67. Further extending the reaction to 25 hours led to a slight increase in  $M_w$ , reaching 6560 g mol<sup>-1</sup>, and the  $D$  remained below 2.0. Previous studies have demonstrated that HPAEs with a  $M_w$  around 10 000 g mol<sup>-1</sup>





**Fig. 1** Synthesis of HPAEs with thiolated branching units via an “A2 + B4 + C2” type Michael addition. **B4**, **PTMP**, and **S5** were first copolymerized to produce HPAE-ac with acrylate terminal groups, which were then endcapped with **122** to generate the final HPAEs containing thiolated branching units.



**Fig. 2** Characterization of HPAEs. (a) Evolution of the molecular weight of HPAE-1 and HPAE-2 during the polymerization process; (b) GPC traces of HPAE-1 and HPAE-2 after endcapping and purification; (c) <sup>1</sup>H NMR spectra of HPAE-1 and HPAE-2 following endcapping and purification.

exhibit high DNA transfection efficiency without significant cytotoxicity.<sup>10,11,13</sup> Therefore, the reaction was terminated by diluting with DMSO and cooling to room temperature. Excess endcapping amine, 3,6,9-trioxaundecamethylenediamine (122), was then added to endcap the HPAE-1 base polymer (HPAE-1-ac) for 48 hours. After precipitation with diethyl ether three times, the purified HPAE-1 has a  $M_w$  of 9460 g mol<sup>-1</sup> and a  $D$  of 1.74 (Fig. 2b and Table S3). By weighing the final products and comparing them to the starting materials, we determined that the yields for HPAE-1 and HPAE-2 were approximately 37% and 48%, respectively.

To investigate the effect of branching degree on gene transfection performance, we synthesized HPAE-2 with a higher degree of branching by increasing the molar feed ratio of [B4]:[S5]:[PTMP] to 6.24:4.0:0.72, corresponding to a stoichiometric ratio of [B4]/[S5 + PTMP] of 1:1.15, while keeping the other polymerization parameters constant (Table S4). GPC results showed that with the increased PTMP content in the feed ratio, the  $M_w$  growth of reaction mixture was faster than that in the synthesis of HPAE-1 (Fig. 2a and Table S5). After 1 hour of polymerization, the  $M_w$  reached 5066 g mol<sup>-1</sup> with a  $D$  of 1.60, as more PTMP branching monomers rapidly combined with the linear segments to generate a branched structure. After 2 hours, the  $M_w$  increased to 5260 g mol<sup>-1</sup> with a  $D$  of 1.59. Extending the reaction to 3 hours led to a slight increase in  $M_w$  to 5500 g mol<sup>-1</sup> with a  $D$  of 1.64. After endcapping with 122 and purification by precipitation with diethyl ether, HPAE-2 with a  $M_w$  of 10 880 g mol<sup>-1</sup> and a  $D$  of 3.51 was synthesized (Fig. 2b and Table S6). Proton nuclear magnetic resonance (<sup>1</sup>H NMR) characterization confirmed the purity and chemical composition of the HPAEs (Fig. 2c and Fig. S1, S2). These results demonstrate that HPAEs with thiolated branching units can be effectively synthesized in a controlled manner with respect

to molecular weight, chemical composition, and branching degree, without the occurrence of gelation. It should be noted that, due to the step-growth polymerization mechanism and the broad  $D$ , it is difficult to exclude the presence of LPAE in the final products. For comparison, a LPAE, L-C32, was synthesized according to previous studies with slight modifications (Fig. S3–S5).<sup>31–33</sup> L-C32 has a similar composition to HPAE-1 and HPAE-2 but lacks the PTMP branching units. L-C32 has a  $M_w$  of 9460 g mol<sup>-1</sup> and a  $D$  of 1.74 (Tables S7 and S8).

### Physiological properties of HPAEs and HPAE/DNA polyplexes

Effective condensation of DNA into nanoscale polyplexes is a key prerequisite for high-performance gene transfection.<sup>2</sup> To evaluate this, we first assessed the DNA binding affinity of HPAE-1 and HPAE-2. Commercial gene transfection reagent branched polyethyleneimine (PEI 25k,  $M_w$  = 25 000 g mol<sup>-1</sup>) was used as the control.<sup>28,34</sup> As shown in Fig. 3a and Table S9, at a PEI/DNA weight/weight (w/w) ratio of 5:1, PEI 25k demonstrated a relative DNA binding affinity of 95%. This high DNA binding capability is attributed to high nitrogen content of PEI 25k, which facilitates strong electrostatic interactions with the negatively charged DNA. The DNA binding capability of HPAE-1 and HPAE-2 was measured across w/w ratios ranging from 10:1 to 60:1. At the lowest w/w ratio of 10:1, both HPAE-1 and HPAE-2 displayed high DNA binding affinity of >76%, with HPAE-2 showing a slightly higher DNA binding affinity than HPAE-1. As the w/w ratio increased, both HPAE-1 and HPAE-2 exhibited a modest increase in DNA binding affinity, and consistently, HPAE-2 showed a slightly stronger binding affinity with DNA than HPAE-1. This can be attributed to the higher branching degree of HPAE-2, which provides more terminal groups, thus enhancing DNA binding. Consequently, at the w/w ratio of 10:1, HPAE-1/DNA polyplexes have an average diameter

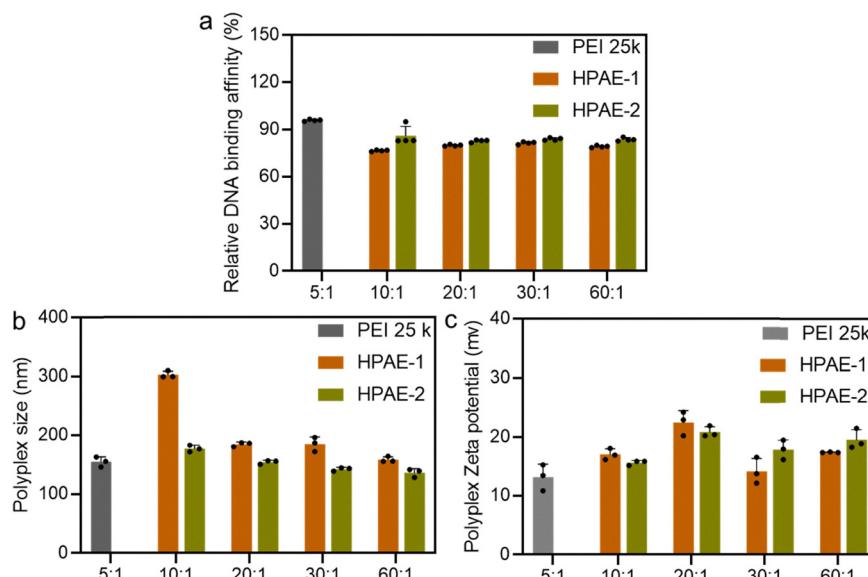


Fig. 3 Physiological properties of HPAEs and HPAE/DNA polyplexes. (a) Relative DNA binding affinity of HPAE-1 and HPAE-2 at w/w ratios ranging from 10:1 to 60:1; (b) the corresponding sizes of HPAE-1/DNA and HPAE-2/DNA polyplexes; (c) the corresponding zeta potentials of HPAE-1/DNA and HPAE-2/DNA polyplexes.



of approximately 299 nm (Fig. 3b, Fig. S6 and Table S10). In contrast, HPAE-2/DNA polyplexes are much smaller, with a size of around 183 nm, indicating that HPAE-2 condenses DNA into more compact polyplexes. As the w/w ratio increased, both HPAE-1 and HPAE-2 formed more compact polyplexes, with sizes decreasing to below 180 nm. Again, across all w/w ratios, HPAE-2/DNA polyplexes are consistently smaller than the HPAE-1/DNA counterparts. Moreover, all the polyplexes exhibit a low polydispersity index (Fig. S7 and Table S11), indicating a high degree of uniformity. Zeta potential measurements further reveal that all HPAE/DNA polyplexes exhibited high zeta potentials of  $>+12$  mV, which were even higher than those of the PEI/DNA polyplexes, especially at the w/w ratio of 20:1 (Fig. 3c and Table S12). The small sizes and high positive zeta potentials of the polyplexes are expected to be favorable for efficient DNA transfection.

#### HPAEs mediate effective gene transfection in the difficult-to-transfect UM-UC-3

The gene transfection efficiency of HPAEs with thiolated branching units was first evaluated in UM-UC-3 cells. PEI 25k was used as a positive control at a w/w ratio of 5:1, in accordance with previous studies.<sup>28,34</sup> In general, HPAEs mediate effective gene transfection at w/w ratios ranging from 10:1 to 60:1; therefore, this range was employed in our experiments.<sup>9-13</sup> At a w/w ratio of 5:1, after 48 hours of transfection in the presence of serum, almost no UM-UC-3 cells were transfected by PEI 25k, as evidenced by the absence of green fluorescence protein (GFP)-positive cells (Fig. 4a). Flow cytometry confirmed that the transfection efficiency was only around 1.5% (Fig. 4b and Table S13). This low efficiency is likely due to the inherent difficulty of transfecting UM-UC-3 cells.<sup>34</sup> At w/w ratios ranging from 10:1 to 50:1, HPAE-1 successfully mediated GFP expression, with visible

GFP-positive cells detected. However, increasing the w/w ratio did not result in a significant improvement in transfection efficiency (Fig. S8). Flow cytometry measurements showed that the overall transfection efficiency remained around 3%. Similarly, HPAE-2 also exhibited low gene transfection efficiency within the same w/w ratio range. Correspondingly, alamarBlue assays indicated that cell viability remained above 100% throughout the experiments, which may be attributed to the high degradability of poly( $\beta$ -amino ester)s under physiological conditions.<sup>15,20,23</sup> However, when the w/w ratio was increased to 60:1, a substantial improvement in transfection efficiency was observed with HPAE-1. Flow cytometry revealed that nearly 7% of the cells were GFP-positive, which was significantly higher than the transfection efficiency of the positive control (PEI 25k). Notably, 100% cell viability was maintained as measured by alamarBlue assay (Fig. 4c and Table S14). In contrast, at the same w/w ratio of 60:1, HPAE-2 achieved 14% transfection efficiency, as indicated by the significantly greater number of GFP-positive cells. However, as determined by alamarBlue assays, only around 60% of the cells remained viable, therefore the high transfection efficiency is possibly due to cell apoptosis. These results indicate that while HPAEs with a higher branching degree exhibit enhanced gene transfection capability, they also cause more severe cytotoxicity. The branching degree significantly affects the overall gene transfection performance of HPAEs with thiolated branching units.

#### Comparison of the gene transfection efficiency of HPAEs with L-C32, ER-HPAE-1, and SPAE-1-5 h

To further validate the transfection capability of HPAE-1 and HPAE-2, we synthesized L-C32, a LPAE widely used for DNA transfection due to its excellent efficiency.<sup>31-33</sup> Notably, L-C32 has a similar composition to HPAE-1 and HPAE-2 but lacks PTMP branching units. Additionally, we used ER-HPAE-1 (with

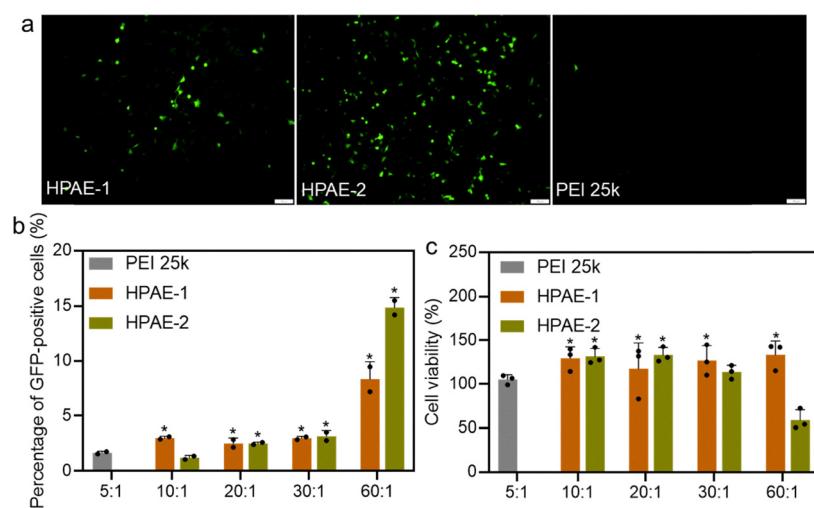
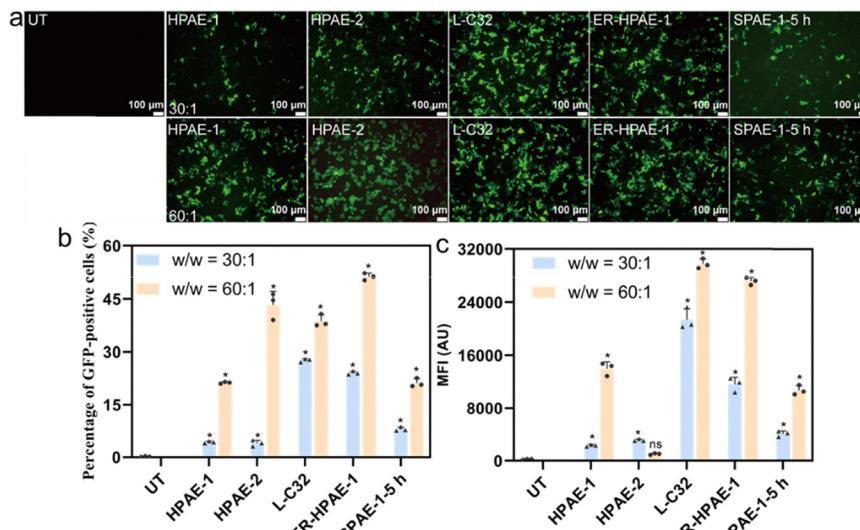


Fig. 4 Gene transfection efficiency in UM-UC-3 mediated by HPAEs and PEI 25k. (a) GFP images of UM-UC-3 cells 48 hours after transfection with HPAE-1 and HPAE-2 at a w/w ratio of 60:1, and PEI 25k at a w/w ratio of 5:1. The scale bars present 100  $\mu$ m; (b) percentage of GFP-positive UM-UC-3 cells measured by flow cytometry 48 hours post-transfection. \* $p < 0.05$  indicates superior transfection efficiency compared to the PEI 25k group,  $n = 2$ ; (c) viability of UM-UC-3 cells measured by alamarBlue assay 48 hours after transfection. \* $p < 0.05$  indicates superior cell viability compared to the PEI 25k group,  $n = 3$ .





**Fig. 5** HPAEs mediate high gene transfection efficiency in HeLa cells. (a) GFP images of HeLa cells after 48 hours of transfection by HPAE-1, HPAE-2, L-C32, ER-HPAE-1 and SPAE-1-5 h at the w/w ratios of 30 : 1 and 60 : 1. The scale bars present 100  $\mu$ m; (b) percentage of GFP-positive cells quantified by flow cytometry. \* $p$  < 0.05 indicates superior transfection efficiency compared to the UT group,  $n$  = 3; (c) MFI of cells after 48 hours of transfection measured by flow cytometry. \* $p$  < 0.05 indicates superior transfection efficiency compared to the UT group,  $n$  = 3.

tetraacrylate branching units) and SPAE-1-5 h (with a diamine core), both previously synthesized by our group for high-performance DNA transfection, as benchmarks.<sup>20,35</sup> As shown in Fig. 5a, in robust HeLa cells, untreated cells (UT) showed no GFP expression after 48 hours of transfection. At a w/w ratio of 30 : 1, L-C32 and ER-HPAE-1 mediated intense GFP expression, with flow cytometry showing 27.6% and 24.0% transfection efficiency (Fig. 5b, Fig. S9 and Table S15). The corresponding mean fluorescence intensity (MFI) values were 21 369 AU and 11 601 AU (Fig. 5c and Table S16). In contrast, HPAE-1, HPAE-2, and SPAE-1-5 h showed similar, but lower, transfection efficiency at this w/w ratio. As the w/w ratio was increased to 60 : 1, the percentage of GFP-positive cells for L-C32, ER-HPAE-1, and SPAE-1-5 h increased to 38.7%, 51.2%, and 21.2%, respectively. Importantly, the transfection efficiency of HPAE-1 and HPAE-2 increased substantially to 21.3% and 43.3%. Specifically, the percentage of GFP-positive cells after transfection by HPAE-2 is 1.12- and 2.04-fold higher than that achieved with L-C32 and SPAE-1-5 h, respectively. However, the MFI of the cells transfected by HPAE-2 is substantially lower than that mediated by L-C32 and SPAE-1-5 h. Collectively, these results demonstrate that, at the optimal w/w ratio, HPAE-1 exhibits gene transfection comparable to or even greater than that of the widely used L-C32. It is worth noting that Santangelo *et al.* previously synthesized and screened a series of HPAEs, finding that thiol-containing HPAEs exhibited superior mRNA transfection efficiency compared to formulations lacking thiol component.<sup>36</sup> These HPAEs were successfully used to deliver an mRNA-expressed Cas13a-mediated treatment in a SARS-CoV-2 challenge model, achieving similar efficacy to a 20-fold higher dose of a neutralizing antibody. Based on these findings, we speculate that HPAEs with thiolated branching units may also offer similar advantages for mRNA delivery; however, this is beyond the scope of the current study. Given the wide availability of

diverse thiolated branching monomers, their incorporation into HPAEs could significantly expand the HPAE family and facilitate the development of new, high-performance HPAEs for both DNA and mRNA transfection.

## Conclusions

In this study, for the first time, **PTMP**, instead of triacrylates, tetraacrylates and diamines, was used as the branching monomer in the synthesis of HPAEs for gene delivery. With the increase of **PTMP** in monomer feed ratio, the polymerization was significantly accelerated, two HPAEs with well-controlled molecular weight, chemical composition, and branching degrees were synthesized successfully in a one-pot reaction without gelation. HPAEs effectively condensed DNA into polyplexes with diameters of less than 300 nm and zeta potentials greater than +12 mV. In the challenging UM-UC-3 cell line, HPAE-1 exhibited significantly higher gene transfection efficiency at a high w/w ratio of 60 : 1 compared to PEI 25k at its optimal ratio of 5 : 1, while maintaining high cell viability. Given the mild reaction conditions and the commercial availability of thiolated branching monomers, this study presents a new strategy for the synthesis of HPAEs, significantly expanding the potential applications of this polymer family.

## Conflicts of interest

The authors declare no conflicts of interest.

## Data availability

All data relevant to this study are available from the corresponding author upon reasonable request. will be made available on request.



The supplementary information includes sections on materials, polymer synthesis and characterization, polyplex formulation and characterization, and evaluation of transfection efficiency and cytotoxicity. See DOI: <https://doi.org/10.1039/d5ma00056d>.

## Acknowledgements

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## References

- 1 J. J. Green, R. Langer and D. G. Anderson, A Combinatorial Polymer Library Approach Yields Insight into Nonviral Gene Delivery, *Acc. Chem. Res.*, 2008, **41**(6), 749–759.
- 2 D. W. Pack, A. S. Hoffman, S. Pun and P. S. Stayton, Design and Development of Polymers for Gene Delivery, *Nat. Rev. Drug Discovery*, 2005, **4**(7), 581–593.
- 3 M. A. Mintzer and E. E. Simanek, Nonviral Vectors for Gene Delivery, *Chem. Rev.*, 2009, **109**(2), 259–302.
- 4 D. Zhou, C. Li, Y. Hu, H. Zhou, J. Chen, Z. Zhang and T. Guo, PLL/pDNA/P(His-*co*-DMAEL) Ternary Complexes: Assembly, Stability and Gene Delivery, *J. Mater. Chem.*, 2012, **22**(8), 10743–10751.
- 5 B. Newland, Y. Zheng, Y. Jin, M. Abu-Rub, H. Cao, W. Wang and A. Pandit, Single Cyclized Molecule Versus Single Branched Molecule: A Simple and Efficient 3D “knot” Polymer Structure for Nonviral Gene Delivery, *J. Am. Chem. Soc.*, 2012, **134**(10), 4782–4789.
- 6 D. Zhou, C. Li, Y. Hu, H. Zhou, J. Chen, Z. Zhang and T. Guo, The Effects of A Multifunctional Oligomer and Its Incorporation Strategies on The Gene Delivery Efficiency of Poly(L-lysine), *Chem. Commun.*, 2012, **48**(38), 4594–4596.
- 7 T. Zhao, H. Zhang, B. Newland, A. Aied, D. Zhou and X. Wang, Significance of Branching for Transfection: Synthesis of Highly Branched Degradable Functional Poly(dimethylaminoethyl methacrylate) by Vinyl Oligomer Combination, *Angew. Chem., Int. Ed.*, 2014, **53**(24), 6095–6100.
- 8 M. Cortez, W. Godbey, L. Fang, M. Payne, B. Cafferty, K. Kosakowska and S. Grayson, The Synthesis of Cyclic Poly(ethylene imine) and Exact Linear Analogues: An Evaluation of Gene Delivery Comparing Polymer Architectures, *J. Am. Chem. Soc.*, 2015, **137**(20), 6541–6549.
- 9 L. Cutlar, D. Zhou, Y. Gao, T. Zhao, U. Greiser, W. Wang and W. Wang, Highly Branched Poly(β-amino esters): Synthesis and Application in Gene Delivery, *Biomacromolecules*, 2015, **16**(9), 2609–2617.
- 10 D. Zhou, L. Cutlar, Y. Gao, W. Wang, J. O’Keeffe-Ahern, S. McMahon, B. Duarte, F. Larcher, B. Rodriguez, U. Greiser and W. Wang, The Transition From Linear to Highly Branched Poly(β-amino ester)s: Branching Matters for Gene Delivery, *Sci. Adv.*, 2016, **2**(6), e1600102.
- 11 S. Liu, Y. Gao, D. Zhou, M. Zeng, F. Alshehri, B. Newland, J. Lyu, J. O’Keeffe-Ahern, U. Greiser, T. Guo, F. Zhang and W. Wang, Highly Branched Poly(β-amino ester) Delivery of Minicircle DNA for Transfection of Neurodegenerative Disease Related Cells, *Nat. Commun.*, 2019, **10**, 3307.
- 12 A. Patel, J. Kaczmarek, S. Bose, K. Kauffman, F. Mir, M. Heartlein, F. DeRosa, R. Langer and D. Anderson, Inhaled Nanoformulated mRNA Polyplexes for Protein Production in Lung Epithelium, *Adv. Mater.*, 2019, **31**(8), 1805116.
- 13 M. Zeng, D. Zhou, F. Alshehri, I. Lara-Sáez, Y. Lyu, J. Creagh-Flynn, Q. Xu, A. Sigen, J. Zhang and W. Wang, Manipulation of Transgene Expression in Fibroblast Cells by a Multi-functional Linear-Branched Hybrid Poly(β-amino ester) Synthesized through an Oligomer Combination Approach, *Nano Lett.*, 2019, **19**(1), 381–391.
- 14 S. Liu, Z. Sun, D. Zhou and T. Guo, Alkylated Branched Poly(β-amino ester)s Demonstrate Strong DNA Encapsulation, High Nanoparticle Stability and Robust Gene Transfection Efficacy, *J. Mater. Chem. B*, 2017, **5**(27), 5307–5310.
- 15 S. Liu, Y. Gao, S. A, D. Zhou, U. Greiser, T. Guo, R. Guo and W. Wang, Biodegradable Highly Branched Poly(β-amino ester)s for Targeted Cancer Cell Gene Transfection, *ACS Biomater. Sci. Eng.*, 2017, **3**(7), 1283–1286.
- 16 J. Huang, Y. Gao, L. Cutlar, J. O’Keeffe-Ahern, T. Zhao, F. Lin, D. Zhou, S. McMahon, U. Greiser, W. Wang and W. Wang, Tailoring Highly Branched Poly(β-amino ester)s: A Synthetic Platform for Epidermal Gene Therapy, *Chem. Commun.*, 2015, **51**(40), 8473–8476.
- 17 Q. Xu, L. Guo, S. A, Y. Gao, D. Zhou, U. Greiser, J. Creagh-Flynn, H. Zhang, Y. Dong, L. Cutlar, F. Wang, W. Liu, W. Wang and W. Wang, Injectable Hyperbranched Poly(β-amino ester) Hydrogels with On-demand Degradation Profiles to Match Wound Healing Processes, *Chem. Sci.*, 2018, **9**(8), 2179–2187.
- 18 Q. Xu, M. Venet, W. Wang, J. Creagh-Flynn, X. Wang, X. Li, Y. Gao, D. Zhou, M. Zeng, I. Lara-Sáez, S. A, H. Tai and W. Wang, Versatile Hyperbranched Poly(β-hydrazide ester) Macromers as Injectable Antioxidative Hydrogels, *ACS Appl. Mater. Interfaces*, 2018, **10**(46), 39494–39504.
- 19 Y. Wang, C. Wang, M. Lie, D. Zhou, W. Huang and W. Wang, Effects of Branching Strategy on the Gene Transfection of Highly Branched Poly(β-amino ester)s, *Chin. J. Polym. Sci.*, 2020, **38**(8), 830–839.
- 20 C. Wang, W. He, R. Guo, C. Pan, H. Yong, T. Bo, Y. Zhao, Z. Li, F. Wang, W. Xu, D. Yao, S. Zhang, M. Li and D. Zhou, Endoplasmic Reticulum-Targeting Highly Branched Poly(β-amino ester)s for Skin Gene Delivery, *ACS Mater. Lett.*, 2024, **6**(11), 5048–5057.
- 21 Y. Zhou, H. Yong, R. Guo, K. Wang, Z. Li, W. Hua and D. Zhou, Self-reporting and Biodegradable Thermosetting Solid Polymer Electrolyte, *Angew. Chem., Int. Ed.*, 2024, **63**(7), e202319003.
- 22 D. Zhou, Y. Gao, A. Aied, L. Cutlar, O. Igoucheva, B. Newland, V. Alexeeve, U. Greiser, J. Uitto and W. Wang, Highly Branched Poly(β-amino ester)s for Skin Gene Therapy, *J. Controlled Release*, 2016, **244**, 336–346.



23 D. Zhou, Y. Gao, J. O'Keeffe Ahern, S. A, Q. Xu, X. Huang, U. Greiser and W. Wang, Development of Branched Poly(5-amino-1-pentanol-*co*-1,4-butanediol diacrylate) with High Gene Transfection Potency Across Diverse Cell Types, *ACS Appl. Mater. Interfaces*, 2016, **8**(50), 34218–34226.

24 M. Zeng, F. Alshehri, D. Zhou, I. Lara-Sáez, X. Wang, X. Li, S. A, Q. Xu, J. Zhang and W. Wang, Efficient and Robust Highly Branched Poly(β-amino ester)/Minicircle *COL7A1* Polymeric Nanoparticles for Gene Delivery to Recessive Dystrophic Epidermolysis Bullosa Keratinocytes, *ACS Appl. Mater. Interfaces*, 2019, **11**(34), 30661–30672.

25 Q. Li, L. Sun, X. Huang, S. Liu, H. Yong, C. Wang, J. Li and D. Zhou, Genetic Engineering of Adipose-Derived Stem Cells Using Biodegradable and Lipid-Like Highly Branched Poly(β-amino ester)s, *ACS Macro Lett.*, 2022, **11**(5), 636–642.

26 D. Che, C. Wang, Z. Li, K. Wang, S. Sun, X. Zhang, Y. Li, Z. Chen, L. Guo, Y. Hou, D. Zhou and S. Geng, Efficient Gene Transfection of Suspension Cells by Highly Branched Poly(β-amino ester), *Chin. Chem. Lett.*, 2023, **34**(7), 108066.

27 Y. Zhang, J. Shi, B. Ma, H. Yong, Z. Li, Y. N. Zhou, J. Li, L. Liang and D. Zhou, Phosphocholine-Functionalized Zwitterionic Highly Branched Poly(β-amino ester)s for Cytosolic Protein Delivery, *ACS Macro Lett.*, 2023, **12**(5), 626–631.

28 Z. Li, Q. Wo, D. Huang, D. Zhou, L. Guo and Y. Mao, Improving Gene Transfection Efficiency of Highly Branched Poly(β-amino ester)s Through The In-situ Conversion of Inactive Terminal Groups, *Chin. Chem. Lett.*, 2024, **35**(8), 109737.

29 T. Bo, C. Wang, D. Yao, Q. Jiang, Y. Zhao, F. Wang, W. He, X. Wang, H. Zhou, M. Li, S. Zhang and R. Xue, Efficient Gene Delivery by Multifunctional Star Poly (β-amino ester)s Into Difficult-to-Transfect Macrophages for M1 Polarization, *J. Controlled Release*, 2024, **368**(13), 157–169.

30 H. Yong, L. Lin, Z. Li, R. Guo, C. Wang, S. Liu and D. Zhou, Tailoring Highly Branched Poly(β-amino ester)s for Efficient and Organ-Selective mRNA Delivery, *Nano Lett.*, 2024, **24**(30), 9368–9376.

31 A. Eltoukhy, D. Siegwart, C. Alabi, J. Rajan, R. Langer and D. Anderson, Effect of Molecular Weight of Amine End-modified Poly(β-amino ester)s on Gene Delivery Efficiency and Toxicity, *Biomaterials*, 2012, **33**(13), 3594–3603.

32 J. Green, G. Zugates, N. Tedford, Y. Huang, L. Griffith, D. Lauffenburger, J. Sawicki, R. Langer and D. Anderson, Combinatorial Modification of Degradable Polymers Enables Transfection of Human Cells Comparable to Adenovirus, *Adv. Mater.*, 2007, **19**(19), 2836–2842.

33 D. Zhou, Y. Gao, J. O'Keeffe-Ahern, S. A, Q. Xu, X. Huang, U. Greiser and W. Wang, Development of Branched Poly(5-amino-1-pentanol-*co*-1,4-butanediol diacrylate) with High Gene Transfection Potency Across Diverse Cell Types, *ACS Appl. Mater. Interfaces*, 2016, **8**(50), 34218–34226.

34 Z. Li, R. Guo, Z. Zhang, H. Yong, L. Guo, Z. Chen, D. Huang and D. Zhou, Enhancing Gene Transfection of Poly(β-amino ester)s Through Modulation of Amphiphilicity and Chain Sequence, *J Controlled Release*, 2024, **368**, 131–139.

35 C. Wang, F. Wang, Y. Zheng, T. Bo, Y. Zhao, H. Yong, Z. Li, W. Xu, Q. Zhao and D. Zhou, Enhancing Gene Transfection Efficiency of Star-shaped Poly(β-amino ester)s via “Top-down” Hydrolysis, *ACS Macro Lett.*, 2024, **13**(9), 1218–1225.

36 L. Rotolo, D. Vanover, N. Bruno, H. Peck, C. Zurla, J. Murray, R. Noel, L. O'Farrell, M. Arainga, N. Orr-Burks, J. Joo, L. Chaves, Y. Jung, J. Beyersdorf, S. Gumber, R. Cuerrero-Ferreira, S. Cornejo, M. Thoresen, A. Oliver, K. Kuo, J. Cumbart, A. Wolums, F. Villinger, E. Lafontaine, R. Hogan, M. Finn and P. Santangelo, Species-agnostic Polymeric Formulations for Inhalable Messenger RNA Delivery to the Lungs, *Nat. Mater.*, 2023, **22**(3), 369–379.

