

Facile Solid-Phase Synthesis of Well-Defined Defect Lysine Dendrimers

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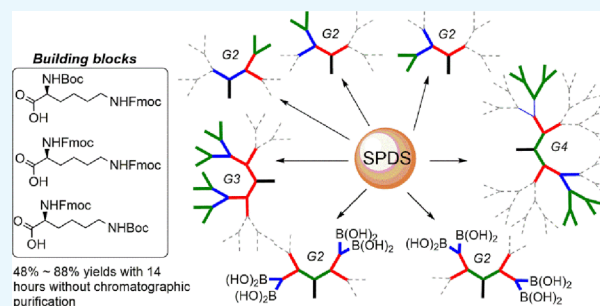
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ABSTRACT: An efficient solid-phase method has been reported to prepare well-defined lysine defect dendrimers. Using orthogonally protected lysine residues, pure G2 to G4 lysine defect dendrimers were prepared with 48–95% yields within 13 h. Remarkably, high-purity products were collected via precipitation without further purification steps. This method was applied to prepare a pair of 4-carboxyphenylboronic acid-decorated defect dendrimers (**16** and **17**), which possessed the same number of boronic acids. The binding affinity of **16**, in which the ϵ -amines of G1 lysine are fractured, for glucose and sorbitol was 4 times that of **17**. This investigation indicated the role of allocation and distribution of peripheries for the dendrimer's properties and activity.



INTRODUCTION

A defect dendrimer is formed by fracturing one or several branches of a perfect dendrimer, resulting in a flexible branched structure, large cavity volume, and different densities of peripheral groups. Defect dendrimers exhibit better efficiency in gene delivery than their perfect analogs.¹ Despite their potential, the lack of structurally well-defined defect dendrimers hampers their further exploration. Although defect dendrimers are inevitably products in the preparation of perfect dendrimers,² the synthesis of well-defined defect dendrimers remains challenging in the conventional dendrimer synthesis.

Heat solvolysis is one traditional method used to prepare defect dendrimers by fracturing the branches of perfect dendrimers. Adding capping reagents or less equivalent substrates in the preparation of dendrimers is an alternative approach to obtain defect dendrimers.³ However, both methods give a mixture of products with randomly distributed fracture sites, and the exact structures of these products remain unclear. In addition, the number of branches and peripheral groups is an important factor in determining the dendrimer properties. Precise analytical methods have been reported to estimate the degree of defects.⁴ In terms of structural determination, nuclear magnetic resonance spectroscopy has been applied; however, its low sensitivity prevents the exact determination of the number of fractured branches⁵ and the structure of the defect dendrimer. Therefore, an approach to prepare defect dendrimers with exact structures should benefit their applications and analysis.

We developed a solid-phase dendrimer synthesis (SPDS) approach in which dendrimers are prepared by iteratively introducing monomers to products anchored on insoluble resins.⁶ Using the designed building blocks, the structures of the dendrimer products can be well regulated.⁷ Meanwhile, monomers with orthogonal functionalities were applied for the accelerated synthesis of perfect dendrimers.⁸ A similar approach has been applied to prepare linear polylysine, either α - or ϵ -amines of the lysine residue to carboxylates. However, this approach has not been applied to the preparation of defect dendrimers.⁹ We envisioned orthogonally protected branched building blocks in SPDS that should provide pure defect dendrimers.

RESULTS AND DISCUSSION

Three defect analogs (**2–4**) of a second-generation (G2) lysine dendrimer (**1**) were designed to demonstrate the feasibility of SPDS for preparing well-defined defect dendrimers (Figure 1). Defect dendrimer **2** lacks one branch at the ϵ -amine of a zero-generation (G0) lysine and one branch at the α -amine of a first-generation (G1) lysine. In **3**, the branches on the α -amine of G0 lysine and G1 lysine are fractured. The branches on the α -amine of G0 lysine and the ϵ -

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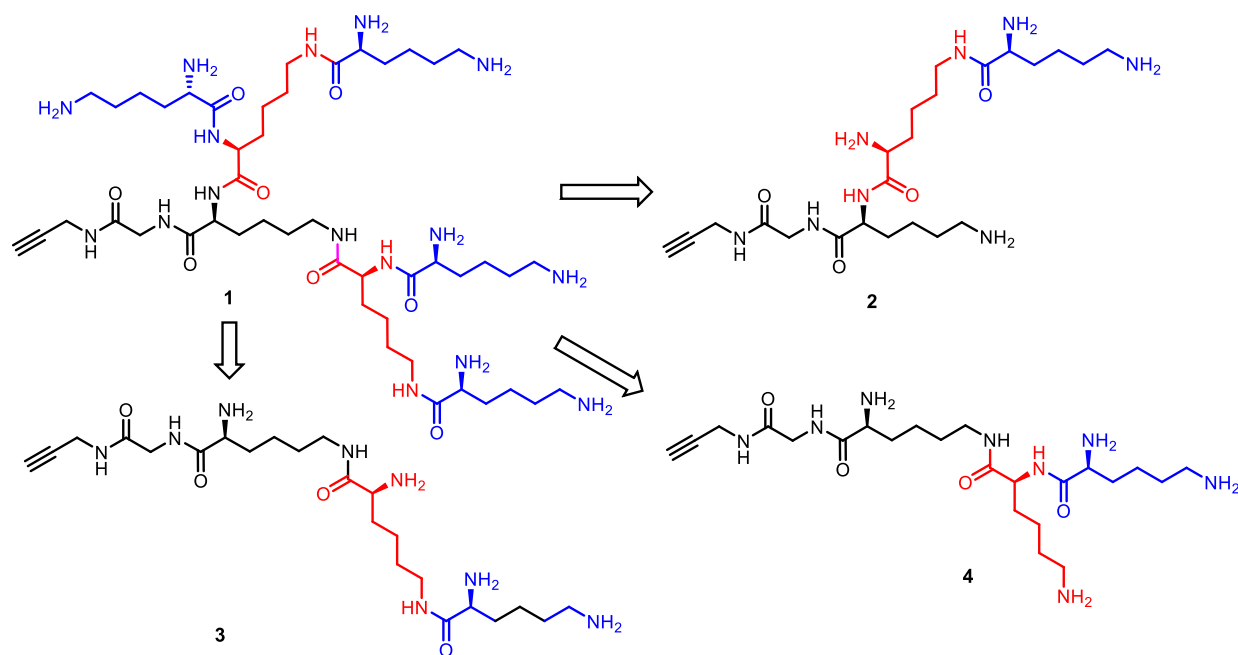
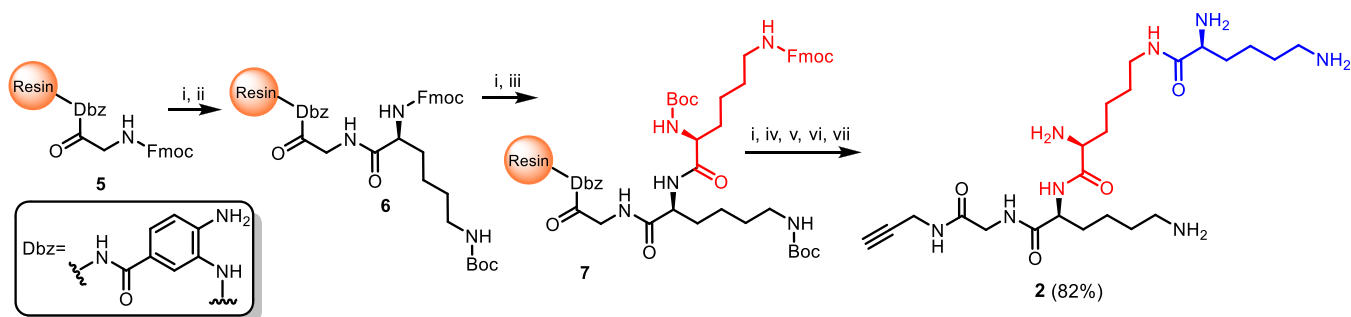


Figure 1. Structures of defect dendrimers 2–4 and full dendrimer 1. Black, red, and blue structures represent the G0, G1, and G2 layers, respectively.

Scheme 1. Synthetic Route for 2^a



^aReaction conditions: (i) 20% piperidine in DMF, 2 × 10 min; (ii) Fmoc-Lysine(Boc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 1 h, rt; (iii) Boc-Lysine(Fmoc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 1 h, rt; (iv) Boc-Lysine(Boc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 2 h, rt; (v) isoamyl nitrite (125 mM, 10 equiv), DMF, 1.5 h, rt; (vi) propargylamine (53 mM, 4 equiv), DIPEA in DMF (8 equiv), 6 h, rt; (vii) TFA:H₂O (95:5).

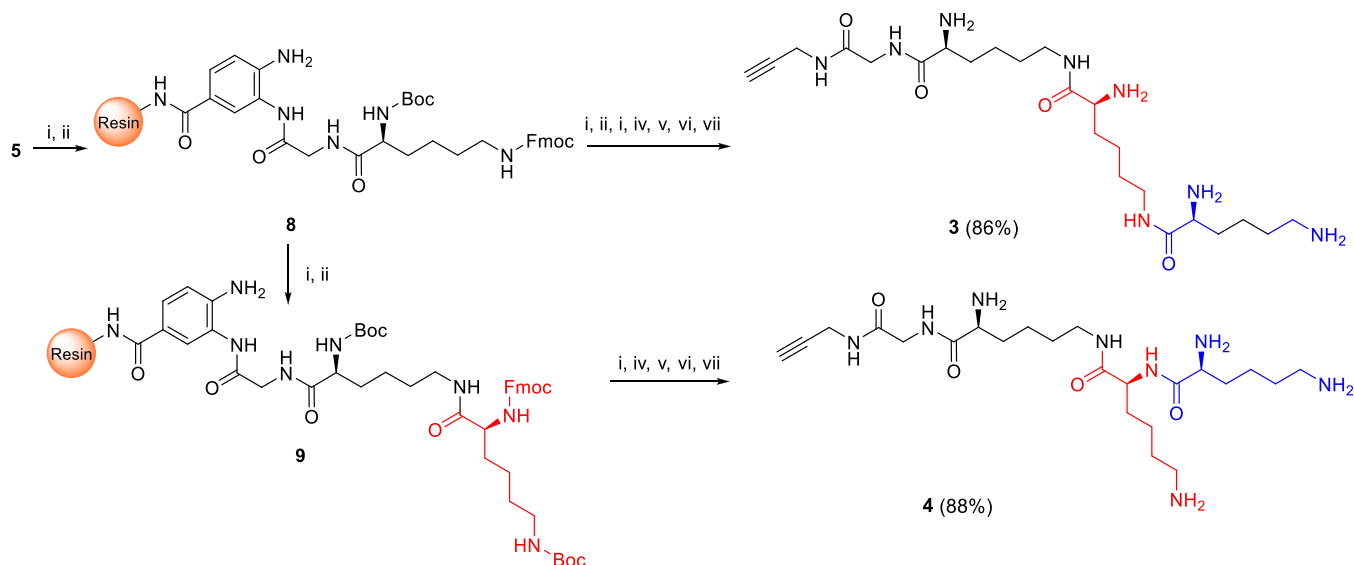
amine of G1 lysine are missing in 4. Each compound demonstrates one type of branch fracturing pattern.

The Fmoc solid-phase peptide synthesis removed the Fmoc groups under basic conditions after each coupling step. In contrast, the Boc groups remained intact until the cleavage step. Therefore, Fmoc and Boc groups were selected as two orthogonal protective groups in each lysine residue. Herein, diamino benzoic acid (Dbz)-loaded resin, a safety-catch resin, was selected to further functionalize at the C-terminal.¹⁰ The preparation of dendrimer 2 involved the introduction of Fmoc-Lys(Boc)-OH to the Dbz-loaded resin (Scheme 1). For fracturing the ϵ -position at the first lysine, Fmoc-Lys(Boc)-OH was needed to synthesize 2. The removal of Fmoc at the α -amine allowed the introduction of Boc-Lys(Fmoc)-OH to give 7. The final lysine residue was introduced to the ϵ -amine of G1 lysine using Boc-Lys(Boc)-OH through a similar reaction. Finally, consecutive treatment with isoamyl nitrite and propargyl amine gave the final product 2 in 82% yield within

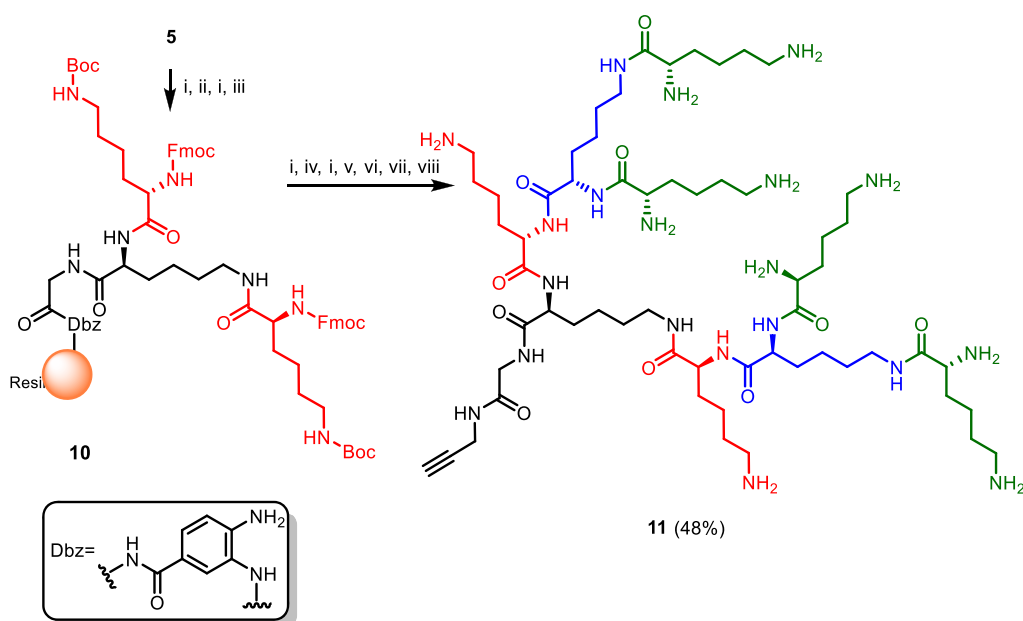
13 h. Propargyl amine could be replaced with other nucleophiles for diverse functionalities.

A similar approach was used to prepare 3 and 4 (Scheme 2). The sequential introduction of Boc-Lys(Fmoc)-OH, Fmoc-Lys(Boc)-OH, and Boc-Lys(Boc)-OH afforded dendrimer 3 in 86% yield. Starting from 5, the sequential incorporation of Boc-Lys(Fmoc)-OH and Boc-Lys(Boc)-OH gave dendrimer 4 in 88% yield.

This method was applied to the preparation of a third-generation (G3) lysine defect dendrimer 11, in which the ϵ -amines of G1 lysine are fractured, and the outer layer of lysine is implanted on the α -amines (Scheme 3). This defect dendrimer 9 is difficult to prepare because of the peripheral residues located at hindered positions. Fmoc-Lys(Boc)-OH was used as the G1 residue. Fmoc was removed to allow for the incorporation of Fmoc-Lys(Fmoc)-OH and the construction of the G2 layer. The same procedure was repeated to construct the G3 layer. The final nucleophilic cleavage to introduce the propargyl group and the acidic removal of Boc gave the final

Scheme 2. Synthetic Routes for **3** and **4**^a

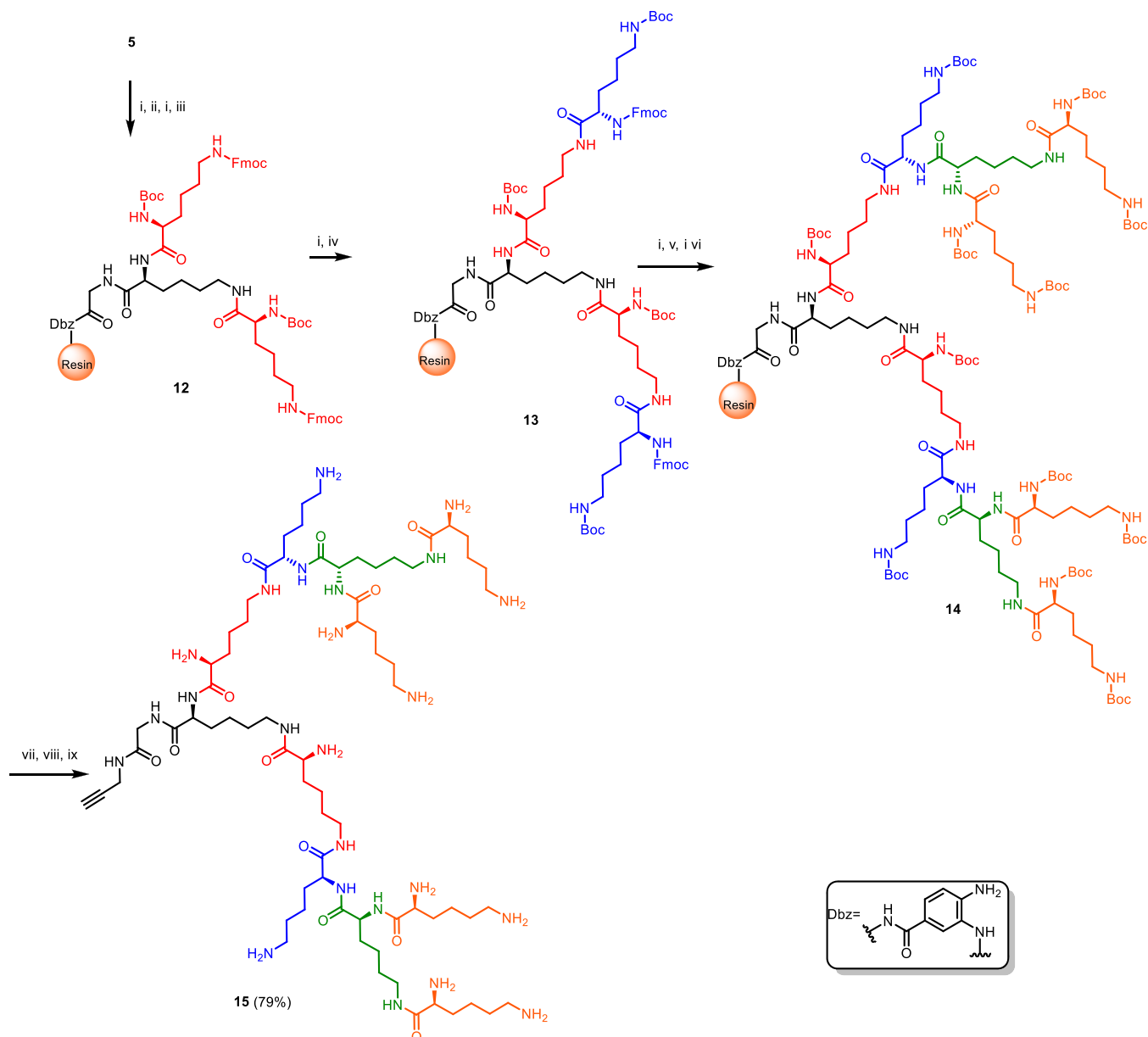
^aReaction conditions: (i) 20% piperidine in DMF, 2 × 10 min; (ii) Boc-Lys(Fmoc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 1 h, rt; (iii) Fmoc-Lys(Boc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 1 h, rt; (iv) Boc-Lys(Boc)-OH (29 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 2 h, rt; (v) isoamylnitrite (125 mM, 10 equiv), DMF, 1.5 h, rt; (vi) propargylamine (53 mM, 4 equiv), DIPEA in DMF (8 equiv), 6 h, rt; (vii) TFA:H₂O (95:5).

Scheme 3. Synthesis of **11**^a

^aReaction conditions: (i) 20% piperidine in DMF, 2 × 10 min; (ii) Fmoc-Lys(Fmoc)-OH (116 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 15 min, rt; (iii) Fmoc-Lys(Boc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (iv) Fmoc-Lys(Fmoc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (v) Boc-Lys(Boc)-OH (233 mM, 14 equiv), HBTU (14 equiv), 10% NMM in DMF, 45 min, rt; (vi) isoamylnitrite (333 mM, 10 equiv) in DMF, 1.5 h, rt; (vii) propargylamine (133 mM, 4 equiv), DIPEA in DMF (8 equiv), 6 h, rt; (viii) TFA:H₂O (95:5).

product in a 48% yield. Remarkably, higher reagent and substrate concentrations (0.1–0.2 M) in this synthesis allowed each reaction to be completed in 15–45 min, and **11** was prepared within 12 h. Compared to the previous conditions (Schemes 1 and 2), the reaction time for each coupling took 60–120 min, while the concentration of reagents was around 29–125 mM.

In addition, the yield of **11** was relatively lower than in other examples. This low yield might be blamed on the steric hindrance from the branch structure. Compared to other compounds reported here, **11** possesses a branch at the α -position of the residue at the G1 layer (red), and the residue at the G2 layer (blue) was fully functionalized. This fact hampered the efficiency of the nucleophilic addition at the cleave step and led to the low yield. Furthermore, in an earlier investigation,¹¹

Scheme 4. Synthesis of **15**^a

^aReaction conditions: (i) 20% piperidine in DMF, 2 × 10 min; (ii) Fmoc-Lys(Fmoc)-OH (117 mM, 3.5 equiv), HBTU (3.5 equiv), 5% NMM in DMF, 15 min, rt; (iii) Boc-Lys(Fmoc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (iv) Fmoc-Lys(Boc)-OH, (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (v) Fmoc-Lys(Fmoc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (vi) Boc-Lys(Boc)-OH (233 mM, 14 equiv), HBTU (14 equiv), 10% NMM in DMF, 45 min, rt; (vii) isoamyl nitrite (333 mM, 10 equiv), DMF, 1.5 h, rt; (viii) propargylamine (133 mM, 4 equiv), DIPEA in DMF (8 equiv), 6 h, rt; (ix) TFA:H₂O (95:5).

longer reaction time leads to hydrolytic product accumulation, which increases the purification effort. Therefore, no further optimization proceeded.

A more complicated fourth-generation (G4) lysine defect dendrimer **15** was designed and prepared based on this encouraging result (Scheme 4). Lysine residues with various patterns of protecting groups were used to demonstrate the diversity of this synthetic approach. A similar approach was applied for **11**, with Fmoc-Lys(Boc)-OH used as the G1 residue. Removal of the Fmoc group to incorporate a Fmoc-Lys(Boc)-OH on the side chain of each lysine residue afford **13**. The following removal of Fmoc allowed the incorporation of Fmoc-Lys(Fmoc)-OH on the α -amine of lysine. Removal of

the Fmoc group allowed the subsequent incorporation of two Fmoc-Lys(Fmoc)-OH groups to give **14**. The final cleavage procedure gave the designed product **15** (79%) in 14 h. This synthesis demonstrates the flexibility of this method, which allows substitution at any given position. Two significant advantages of this method are its efficiency and ability to give pure products. The products were collected by simple filtration; chromatographic purification was not necessary.

The number of peripheral groups is a vital feature for dendrimers' activities. One representative example is the boronic acid-modified defect dendrimers reported as carbohydrate sensors and for biomedical applications, including bacterial detection.¹² Currently, results indicated that the size

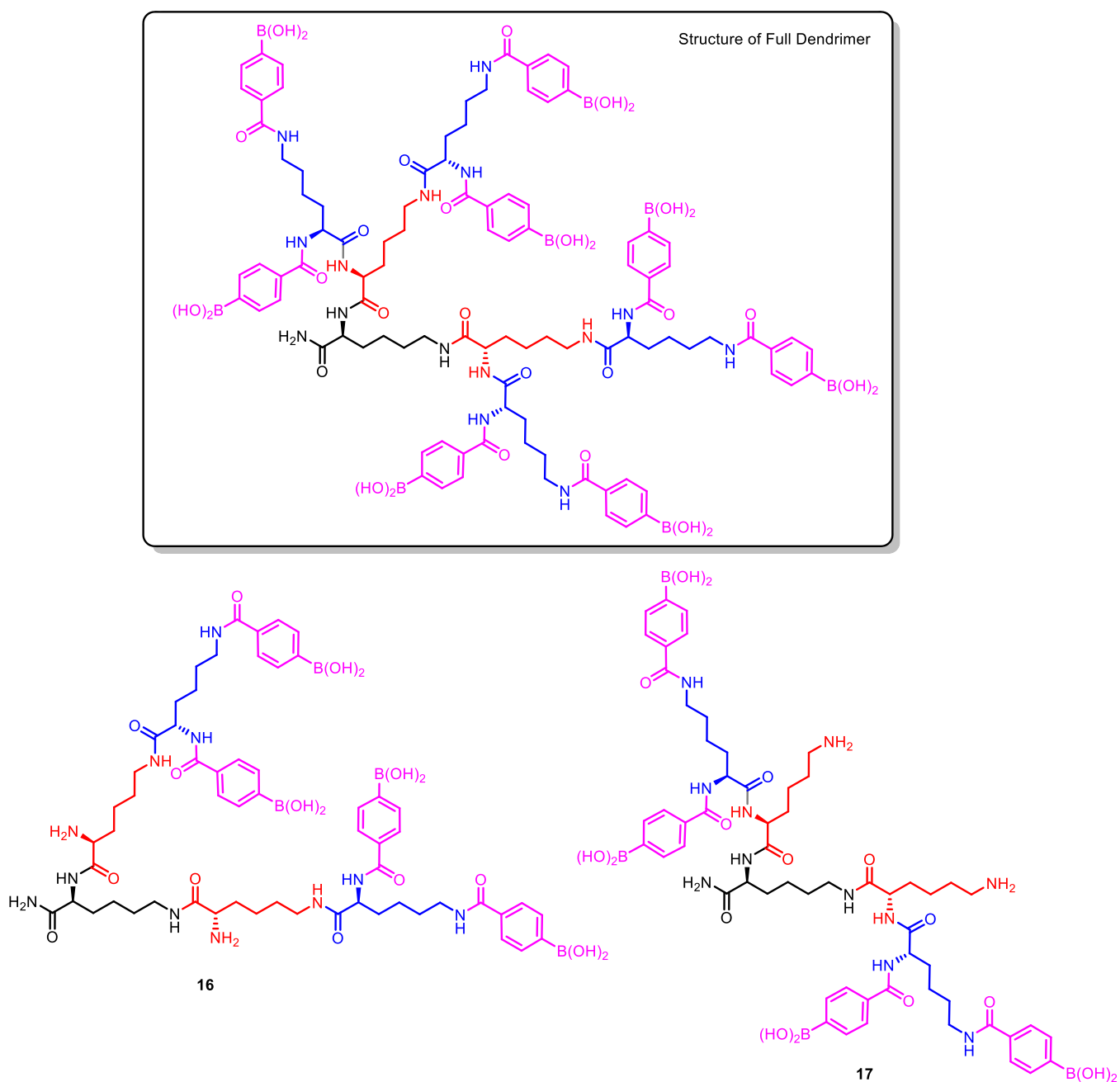


Figure 2. Structure of defect dendrimers **16** and **17**. Black, red, and blue structures represent G0, G1, and G2 layers, respectively.

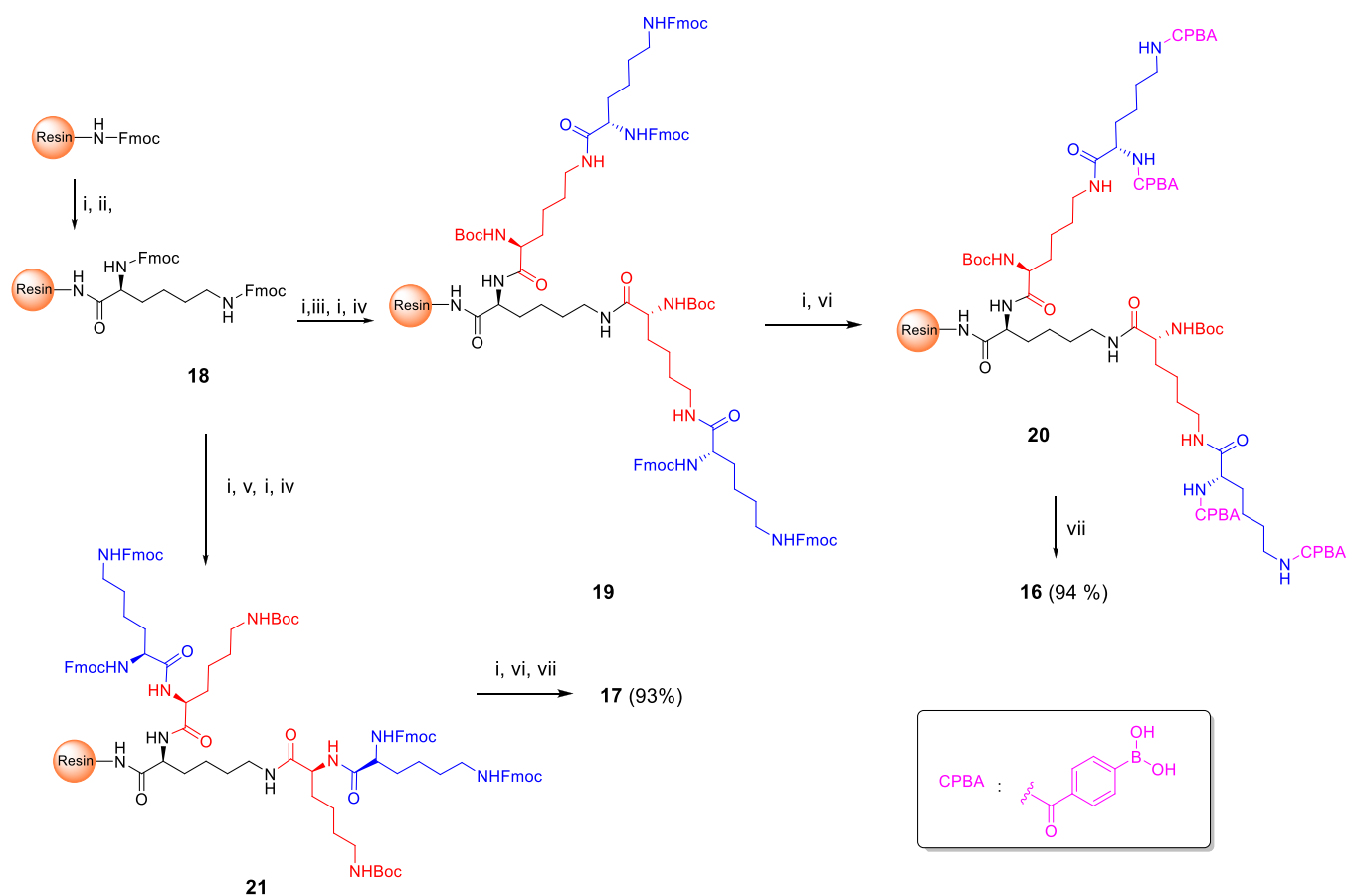
of the dendrimer is a decisive factor in their selective binding to glucose.¹³ However, the binding mechanism for selective affinity to a given carbohydrate remains unclear.¹⁴ The requirement of boronic acids' allocation was not studied well due to the difficulty of synthesizing the designed products. Herein, a pair of defect dendrimers with the same number and different distribution of phenylboronic acid was designed to reveal the potential mechanism. We expected that the flexibility of the dendrimers' structure caused by the fracture branch contributed to the carbohydrates binding.

To investigate the allocation of peripheral boronic acids, we synthesized a pair of G2 lysine defect dendrimers (**16** and **17**) that possess four peripheral 4-carboxyphenylboronic acids (CPBA) but with different allocations based on the skill developed in this investigation (Figure 2). The following

binding experiments with various carbohydrates reveal the importance of the allocation of peripheral groups.

The same procedure used to prepare **11** was applied to prepare **16**, except that a rink amide resin was used. After the basic removal of ϵ -Fmoc groups of **18** (Scheme 5), the introduction of Fmoc-Lys(Fmoc)-OH gave **19**, which was subjected to basic conditions to remove Fmoc groups and incorporate CPBA. The final cleavage and deprotection of Boc gave the desired product **16** in 94% yield. A similar approach with Fmoc-Lys(Boc)-OH as the G1 residue gave **17** in 84% yield (Scheme 5).

Compounds **16** and **17** were subjected to binding experiments with six carbohydrates. Dendrimers were mixed with each carbohydrate for 5 min, and the solution was ultrafiltered to remove dendrimers and binding carbohydrates. The remaining carbohydrate concentration was determined by

Scheme 5. Synthesis of **16** and **17**^a

^aReaction conditions: (i) 20% piperidine in DMF, 2 × 10 min; (ii) Fmoc-Lys(Fmoc)-OH (117 mM, 3.5 equiv), HBTU (3.5 eq), 5% NMM in DMF, 15 min, rt; (iii) Boc-Lys(Fmoc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (iv) Fmoc-Lys(Fmoc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (v) moc-Lys(Boc)-OH (233 mM, 7 equiv), HBTU (7 equiv), 5% NMM in DMF, 30 min, rt; (vi) CPBA (466 mM, 14 equiv), PyBOP (14 equiv), 5% NMM in DMF, 2 h, rt; (vii) TFA:H₂O (95:5).

high-performance liquid chromatography (HPLC) equipped with evaporative light scattering detectors (ELSD) and evaluated the abilities of **16** and **17** to bind to the carbohydrates (Figure 3). Compounds **16** and **17** showed

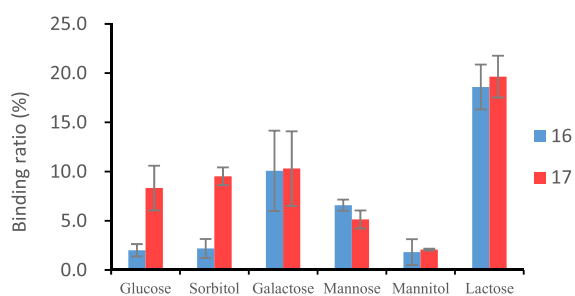


Figure 3. Binding affinities of **16** and **17** to carbohydrates ($n = 3$).

similar binding affinities for the four tested carbohydrates except for glucose and sorbitol; the binding affinity of **16** for glucose and sorbitol was 4 times that of **17**. Although the underlying mechanism remains under investigation, this experiment demonstrates the proximity of boronic acid to selective carbohydrate sensing. Meanwhile, this observation also suggested the contribution of defect dendrimers in the investigation of dendrimers' applications.

CONCLUSIONS

Using orthogonally protected lysine residues, G2 lysine defect dendrimers (**2–4**) with various configurations were prepared in 82–88% yield within 13 h. The same method with higher reagent concentrations was applied to prepare G3 (**11**) and G4 (**15**) analogs in 48 and 79% yields, respectively. Remarkably, higher reactant concentrations effectively shortened the preparation time. Moreover, this method gave high-purity products that could be collected via precipitation without further purification steps. Two types of resins were used to prepare defect dendrimers with various arrangements of branches. When using the Dbz-resin, a propargyl group could be introduced in the core structure, further increasing the diversity of this approach. This approach was also applied to prepare the CPBA-decorated defect dendrimers **16** and **17**. Although **16** and **17** have the same number of boronic acids in one molecule, they exhibited different binding affinities for glucose and sorbitol. This observation indicates the importance of the dendrimer structure in carbohydrate recognition. For the first time, this study indicated that the positions of peripheral groups are one critical factor for the selective binding of guest molecules by dendrimers. We are currently pursuing this research direction by studying a family of CPBA-decorated defect dendrimers.

The precise distribution of peripheral groups is crucial for the dendrimer's properties and activity. The ability to prepare well-defined defect dendrimers allows intensive investigation of the relationship between the branch and periphery and the biomedical activity of dendrimers and graft polymers. Moreover, the precise control of functional groups at a given position is necessary for the multivalent effect of polymers.¹⁵ The method reported herein should benefit the preparation of dendrimers with various allocations and distributions of branches and peripheral groups. In addition, the findings shed light on the investigation of dendritic molecules from tailor-made well-defined defect products.

EXPERIMENTAL SECTION

General Information. All commercial materials were used without further purification. Peptides were synthesized on a Rink amide resin (100–200 mesh, 1% DVB cross-linking, 0.3 mmol/g) from Advanced ChemTech Inc. NMR spectra were obtained on a Joel 400 MHz spectrometer. Peptides were characterized using liquid chromatography-electrospray ionization-mass spectrometry (LC/ESI MS) (Agilent Technologies 1100 Series equipped with SHIMADZU LCMS-2020). Molecular weight was calculated from the experimental mass to charge (m/z) ratios.

(NH₂)₂Lys-(^εNH₂)Lys-(^εNH₂)Lys-Gly-Propargylamine (2). A rink amide resin (167 mg, 0.3 mmol/g loadings, 0.05 mmol) was swollen in DMF (3.0 mL) for 1 h. After removing DMF, the resulting resin was shaken in 20% piperidine/DMF (3.0 mL) for 10 min twice. After filtration, the residue was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times for the following coupling of residues. To the resin was added the solution of Fmoc-Gly-DBz-OH (86 mg, 0.2 mmol, 4 equiv, 0.033 M) and HBTU (76 mg, 0.2 mmol, 4 equiv, 0.033 M) in 5% NMM/DMF (6.0 mL) and shaken for 2 h for the coupling of the first residue. After removing the solvent, the resin was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times. To the resulting resin was added 20% piperidine/DMF (3.0 mL) and shaken for 10 min twice to remove the Fmoc group. The resin was washed with DMF (3.0 mL) and DCM (3.0 mL) separately three times for the following synthesis.

For the incorporation of the second residue, Fmoc-Lys(Boc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M) and HBTU (66 mg, 0.175 mmol, 3.5 equiv, 0.029 M) were subjected to the same procedure of the coupling of the first residue. For the incorporation of the third residue, Boc-Lys(Fmoc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M) and HBTU (66 mg, 0.175 mmol, 3.5 equiv, 0.029 M) were subjected to the same procedure of the coupling of the first residue. For the incorporation of the fourth residue, Boc-Lys(Boc)-OH (61 mg, 0.175 mmol, 3.5 equiv, 0.029 M) and HBTU (66 mg, 0.175 mmol, 3.5 equiv, 0.029 M) were subjected to the same procedure of the coupling of the first residue with 2 h of shaking.

To the resulting resin in DMF (4.0 mL) was added isoamyl nitrite (68 μL, 0.5 mmol, 10 equiv, 0.125 M) and shaken at rt for 1.5 h. After filtration, the filtrate was consecutively washed with DMF (5.0 mL) and DCM (5.0 mL) separately three times. The resulting mixture was dissolved in DMF (3.0 mL) and bubbled with N₂ for 20 min. To the mixture was added DIPEA (70 μL, 0.4 mmol, 8 equiv) and propargylamine (13 μL, 0.2 mmol, 4 equiv, 0.053 M) and shaken at rt for additional 6 h. The resulting mixture

was filtrated, and the residue was washed with DCM (5.0 mL) and DMF (2.0 mL) twice. The combined filtrate was concentrated *in vacuo* to give a crude product. To the resulting mixture was added the mixture of TFA:H₂O (95:5, 2.0 mL) and stirred at rt for 30 min. After removal of the solvent *in vacuo*, to the resulting mixture was added to cold ether and stood for 30 min. After centrifugation (5500 rpm, 5 min), the supernatant was decanted to collect the product. This procedure was repeated to collect the final product (20 mg, 82%). ¹H NMR (400 MHz, D₂O): δ 4.39 (t, $J = 7.2$ Hz, 1H; CH), 4.06 (t, $J = 6.8$ Hz, 1H; CH), 4.02 (d, $J = 2.4$ Hz, 2H; CH₂), 3.99–3.95 (m, 3H; CH, CH₂), 3.33–3.20 (m, 2H), 3.03 (t, $J = 7.6$ Hz, 4H; CH₂), 2.67 (t, $J = 2.4$ Hz, 1H; CH), 1.98–1.89 (m, 4H), 1.87–1.80 (m, 2H), 1.77–1.69 (m, 4H), 1.63–1.55 (m, 2H), 1.53–1.38 (m, 6H); ¹³C NMR (101 MHz, D₂O, as a TFA salt, the signal of TFA was not included): δ 174.0, 171.0, 170.0, 169.4, 79.6, 71.9, 54.1, 53.2, 52.9, 42.5, 39.2, 39.0, 30.6, 30.4, 30.3, 28.8, 28.0, 26.4, 26.4, 22.0, 21.4, 21.4; **MS (ESI⁺)** calcd for [C₂₃H₄₆N₈O₄]²⁺, (M + 2H)²⁺: 249. Found: 249 (100%); calcd for [C₂₃H₄₅N₈O₄]⁺, (M + H)⁺: 497. Found: 497 (30.22%); **HRMS (ESI⁺)** calcd for [C₂₃H₄₅N₈O₄]⁺, (M + H)⁺: 497.3558. Found: 497.3559.

(NH₂)₂Lys-(^εNH₂)Lys-(^εNH₂)Lys-Gly-Propargylamine (3). The same procedure to produce **2** was applied with Boc-Lys(Fmoc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M), Boc-Lys(Fmoc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M), and Boc-Lys(Boc)-OH (61 mg, 0.175 mmol, 3.5 equiv, 0.029 M) consecutively to give **3** (21 mg, 86%). ¹H NMR (400 MHz, D₂O): δ 4.08 (t, $J = 6.8$ Hz, 1H; CH), 4.01 (d, $J = 2.8$ Hz, 2H; CH₂), 4.00 (s, 2H; CH₂), 3.96 (t, $J = 6.8$ Hz, 1H; CH), 3.30–3.24 (m, 4H), 3.03 (t, $J = 8.0$ Hz, 2H; CH₂), 2.65 (t, $J = 2.4$ Hz, 1H; CH), 1.98–1.87 (m, 6H), 1.77–1.69 (m, 2H), 1.64–1.55 (m, 4H), 1.50–1.36 (m, 6H); ¹³C NMR (101 MHz, D₂O, as a TFA salt, the signal of TFA was not included): δ 170.6, 170.4, 169.6, 169.4, 79.4, 72.0, 53.3, 53.2, 42.3, 39.2, 39.1, 39.0, 30.5, 30.4, 30.4, 28.8, 28.0, 27.9, 26.4, 21.7, 21.5, 21.4; **MS (ESI⁺)** calcd for [C₂₃H₄₆N₈O₄]²⁺, (M + 2H)²⁺: 249. Found: 249 (100%); calcd for [C₂₃H₄₅N₈O₄]⁺, (M + H)⁺: 497. Found: 497 (31.23%); **HRMS (ESI⁺)** calcd for [C₂₃H₄₅N₈O₄]⁺, (M + H)⁺: 497.3558. Found: 497.3558.

(NH₂)₂Lys-(^εNH₂)Lys-(^εNH₂)Lys-Gly-Propargylamine (4). The same procedure to produce **2** was applied with Boc-Lys(Fmoc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M), Fmoc-Lys(Boc)-OH (82 mg, 0.175 mmol, 3.5 equiv, 0.029 M), and Boc-Lys(Boc)-OH (61 mg, 0.175 mmol, 3.5 equiv, 0.029 M) consecutively to afford **4** (22 mg, 88%). ¹H NMR (400 MHz, D₂O): δ 4.14 (t, $J = 7.2$ Hz, 1H; CH), 3.95 (t, $J = 6.4$ Hz, 1H; CH), 3.92 (t, $J = 6.4$ Hz, 1H; CH), 3.88 (d, $J = 2.4$ Hz, 2H; CH₂), 3.87 (s, 2H; CH₂), 3.17–3.03 (m, 2H), 2.88 (q, $J = 7.6$ Hz, 4H; CH₂), 2.51 (t, $J = 2.8$ Hz, 4H; CH₂), 1.84–1.77 (m, 4H), 1.69–1.54 (m, 6H), 1.49–1.42 (m, 2H), 1.39–1.23 (m, 6H); ¹³C NMR (101 MHz, D₂O, as a TFA salt, the signal of TFA was not included): δ 173.2, 170.6, 170.5, 169.6, 79.5, 72.0, 54.2, 53.2, 52.8, 42.4, 39.2, 39.0, 39.0, 30.6, 30.5, 30.4, 28.8, 27.9, 26.4, 26.4, 22.2, 21.4, 21.2; **MS (ESI⁺)** calcd for [C₂₃H₄₆N₈O₄]²⁺, (M + 2H)²⁺: 249. Found: 249 (100%); calcd for [C₂₃H₄₅N₈O₄]⁺, (M + H)⁺: 497. Found: 497 (23.56%); **HRMS (ESI⁺)** calcd for [C₂₃H₄₅N₈O₄]⁺, 571.4654. Found: 571.4656.

(((NH₂)₂Lys)₂Lys)-(^εNH₂)Lys)₂Lys-Gly-Propargylamine (11). **5** was shaken in the solution of 20% piperidine in DMF (1.5 mL) for 10 min twice for deprotection. After removing the solvent, the resulting resin was consecutively washed with

DMF (3.0 mL) and DCM (3.0 mL) three times. To the resulting resin was added the solution of Fmoc-Lys(Fmoc)-OH (108 mg, 0.175 mmol, 3.5 equiv, 0.116 M) and HBTU (68 mg, 0.175 mmol, 3.5 equiv, 0.116 M) in 5% NMM/DMF (1.5 mL) and shaken for 15 min for coupling of the first residue.

For the second residue, the same procedure, including deprotecting, washing, and coupling steps, was repeated with Fmoc-Lys(Boc)-OH (166 mg, 0.35 mmol, 7 equiv, 0.233 M), HBTU (132 mg, 0.35 mmol, 7 equiv, 0.233 M), and 30 min of coupling time. For the third residue, the same procedure for the coupling of the first residue was repeated with Fmoc-Lys(Fmoc)-OH (208 mg, 0.35 mmol, 7 equiv, 0.233 M), HBTU (133 mg, 0.35 mmol, 7 equiv, 0.233 M), and 30 min. The same procedure for coupling the first residue was repeated with 10% NMM in DMF as the solvent and 45 min of coupling time for the fifth residue. The reagents are Boc-Lys(Boc)-OH (249 mg, 0.7 mmol, 14 equiv, 0.233 M) and HBTU (266 mg, 0.7 mmol, 14 equiv, 0.233 M).

To the resulting resin was added DMF (1.5 mL) and isoamyl nitrite (70 μ L, 0.5 mmol, 10 equiv, 0.333 M). After shaken for 1.5 h, the resulting resin was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times. To the resulting resin was added DMF (1.5 mL) and bubbled with N₂ for 20 min. To the mixture was added DIPEA (70 μ L, 0.4 mmol, 8 equiv, 0.166 M) and propargylamine (13 μ L, 0.2 mmol, 4 equiv, 0.133 M). After shaken for 4 h, the solvent was removed *in vacuo* and added the mixture of TFA and H₂O (95:5, 2.0 mL). After being stirred for 30 min, the resulting solution was concentrated *in vacuo*. The resulting mixture was added to cold ether and stood at 0 °C for 30 min. After centrifugation (0 °C, 6000 rpm, 15 min), the supernatant was removed by decantation. The residue was subjected to the precipitation again, and the collected precipitate was lyophilized to give the product (30 mg, 48%). ¹H NMR (400 MHz, D₂O): δ 4.35 (t, *J* = 5.6 Hz, 1H; CH), 4.33 (t, *J* = 5.6 Hz, 2H; CH), 4.24 (t, *J* = 7.4 Hz, H; CH), 4.22 (t, *J* = 7.2 Hz, H; CH), 4.05 (t, *J* = 6.8 Hz, 2H; CH), 4.00 (d, *J* = 2.4 Hz, 2H; CH₂), 3.95 (t, *J* = 6.8 Hz, 2H; CH), 3.93 (d, *J* = 8.8 Hz, 2H; CH₂), 3.30–3.10 (m, 6H), 3.04–2.95 (m, 12H), 2.66 (t, *J* = 2.6 Hz, H; CH), 2.00–1.85 (m, 8H), 1.85–1.67 (m, 22H), 1.60–1.33 (m, 24H); ¹³C NMR (101 MHz, D₂O, as a TFA salt, the signal of TFA was not included): δ 174.4, 173.6, 173.5, 173.5, 173.3, 171.0, 169.7, 169.4, 79.5, 72.0, 54.0, 53.9, 53.5, 53.2, 52.8, 42.5, 39.4, 39.2, 39.1, 39.0, 30.7, 30.5, 30.4, 28.8, 28.1, 27.9, 26.4, 26.4, 22.5, 22.4, 22.2, 22.1, 21.4, 21.3; MS (ESI⁺) calcd for [C₅₉H₁₁₉N₂₀O₁₀]³⁺, (M + 3H)³⁺: 423. Found: 423; calcd for [C₅₉H₁₂₀N₂₀O₁₀]⁴⁺, (M + 4H)⁴⁺: 317. Found: 317.

(((NH₂)₂-Lys)₂-Lys-(^εNH₂)Lys-(^αNH₂)Lys)₂-Lys-Gly-Propargylamine (15). The same procedure for synthesizing **13** was used with the Fmoc-Lys(Fmoc)-OH (105 mg, 0.175 mmol, 3.5 equiv, 0.117 M) and HBTU (66 mg, 0.175 mmol, 3.5 equiv, 0.117 M) and shaken for 15 min for coupling of the first residue; Boc-Lys(Fmoc)-OH (165 mg, 0.35 mmol, 7 equiv, 0.233 M) and HBTU (135 mg, 0.35 mmol, 7 equiv, 0.233 M) and shaken for 30 min for the second residues; Fmoc-Lys(Boc)-OH (166 mg, 0.35 mmol, 7 equiv, 0.233 M), HBTU (132 mg, 0.35 mmol, 7 equiv, 0.233 M), and 30 min of coupling time for the third residue; Fmoc-Lys(Fmoc)-OH (208 mg, 0.35 mmol, 7 equiv, 0.233 M), HBTU (133 mg, 0.35 mmol, 7 equiv, 0.233 M), and 30 min of coupling time for the fourth residue; and Boc-Lys(Boc)-OH (248 mg, 0.7 mmol, 14

equiv, 0.233 M), HBTU (266 mg, 0.7 mmol, 14 equiv, 0.233 M), and 45 min of coupling time for the fifth residue. The coupling reaction proceeded in 10 NMM in DMF. To the resulting resin was added DMF (1.5 mL) and isoamyl nitrite (70 μ L, 0.5 mmol, 10 equiv, 0.333 M). After shaking for 1.5 h, the resulting resin was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times. To the resulting resin was added DMF (1.5 mL) and bubbled with N₂ for 20 min. To the mixture was added DIPEA (70 μ L, 0.4 mmol, 8 equiv, 0.166 M) and propargylamine (13 μ L, 0.2 mmol, 4 equiv, 0.133 M). After being shaken for 4 h, the solvent was removed *in vacuo* and added to the mixture of TFA and H₂O (95:5, 2.0 mL). After being stirred for 30 min, the resulting solution was concentrated *in vacuo*. The resulting mixture was added to cold ether and stood at 0 °C for 30 min. After centrifugation (0 °C, 6000 rpm, 15 min), the supernatant was removed by decantation. The residue was subjected to precipitation again, and the collected precipitate was lyophilized to give the product (60 mg, 79%). ¹H NMR (400 MHz, D₂O): δ 4.35 (t, *J* = 8.0 Hz, 1H; CH), 4.33 (t, *J* = 7.6 Hz, 2H; CH), 4.21 (t, *J* = 7.0 Hz, 2H; CH), 4.05 (t, *J* = 6.8 Hz, 2H; CH), 4.00 (d, *J* = 2.4 Hz, 2H; CH₂), 3.95 (t, *J* = 6.8 Hz, 3H; CH), 3.93 (d, *J* = 6.4 Hz, 2H; CH₂), 3.32–3.12 (m, 10H), 3.05–2.95 (m, 12H), 2.64 (t, *J* = 2.4 Hz, H; CH), 2.00–1.85 (m, 12H), 1.86–1.67 (m, 22H), 1.62–1.33 (m, 34H); MS (ESI⁺) calcd for [C₇₁H₁₄₃N₂₄O₁₂]³⁺, (M + 3H)³⁺: 508. Found: 508; calcd for [C₇₁H₁₄₄N₂₄O₁₂]⁴⁺, (M + 4H)⁴⁺: 381. Found: 381; calcd for [C₇₁H₁₄₅N₂₄O₁₂]⁵⁺, (M + 5H)⁵⁺: 305. Found: 305.

((CPBA)₂-Lys-(^εNH₂)Lys)₂-Lys-CONH₂ (16). A rink amide resin (169 mg, 0.3 mmol/g loadings, 0.05 mmol) was swollen in DMF (1.5 mL) for 1 h. After removing DMF, the resulting resin was shaken in 20% piperidine/DMF (1.5 mL) for 10 min twice. After filtration, the residue was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times for the following coupling of residues. To the resin was added the solution of Fmoc-Lys(Fmoc)-OH (105 mg, 0.175 mmol, 3.5 equiv, 0.113 M) and HBTU (68 mg, 0.175 mmol, 3.5 equiv, 0.117 M) in 5% NMM/DMF (1.5 mL) and shaken for 15 min for coupling of the first residue. After removing the solvent, the resin was consecutively washed with DMF (3.0 mL) and DCM (3.0 mL) three times. To the resulting resin was added 20% piperidine/DMF (1.5 mL) and shaken for 10 min twice to remove the Fmoc group. The resin was washed with DMF (3.0 mL) and DCM (3.0 mL) separately three times for the following synthesis.

For the incorporation of the second residue, the solution of Boc-Lys(Fmoc)-OH (165 mg, 0.35 mmol, 7 equiv, 0.233 M) and HBTU (135 mg, 0.35 mmol, 7 equiv, 0.233 M) in 5% NMM in DMF (1.5 mL) was subjected to the same procedure of the coupling of the first residue with 30 min of coupling time. For the incorporation of the third residue, Fmoc-Lys(Fmoc)-OH (208 mg, 0.35 mmol, 7 equiv, 0.233 M) and HBTU (132 mg, 0.35 mmol, 7 equiv, 0.233 M) in 5% NMM in DMF (1.5 mL) were subjected to the same procedure of the coupling of the first residue with 30 min of coupling time. To the resulting resin was added the solution of 4-carboxyphenylboronic acid (117 mg, 0.7 mmol, 14 equiv, 0.466 M) and PyBOP (365 mg, 0.7 mmol, 14 equiv, 0.46 M) in 5% NMM/DMF (1.5 mL), and the resulting mixture was shaken for additional 2 h. After removing the solution by filtration, the resulting mixture was washed with DMF (3.0 mL \times 3) and DCM (3.0 mL \times 3). To the resulting mixture was added the mixture of TFA and H₂O (95:5, 1.0 mL) and shaken for 2 h.

After removing the solution by filtration, the resulting filtrate was mixed with cold ether and stood at 0 °C for 30 min. After centrifugation (6000 rpm, 15 min), the supernatant was decanted to collect the product. This procedure was repeated to collect the final product (59 mg, 94%). ¹H NMR (400 MHz, D₂O): δ 7.68 (d, *J* = 8.0 Hz, 8H, CH), 7.55 (d, *J* = 8.0 Hz, 4H, CH), 7.49 (d, *J* = 8.0 Hz, 4H, CH), 4.38 (t, *J* = 8.0 Hz, 2H, CH), 4.20 (t, *J* = 8.0 Hz, 1H, CH), 3.96 (t, *J* = 8.0 Hz, 1H, CH), 3.83 (t, *J* = 8.0 Hz, 1H, CH), 3.37–3.29 (m, 4H), 3.20–3.03 (m, 6H), 1.88–1.77 (m, 8H), 1.68–1.58 (m, 6H), 1.47–1.43 (m, 10H), 1.35–1.27 (m, 6H). MS (ESI⁺) calcd for [C₅₈H₈₄B₄N₁₁O₁₇]⁺, (*M* + 1H)⁺: 1251, found: 1251. calcd for [C₅₈H₈₅B₄N₁₁O₁₇]²⁺, (*M* + 2H)²⁺: 626, Found: 626.

((CPBA)₂-Lys-(^εNH₂)Lys)₂-Lys-CONH₂ (17). The same procedure for 16 was used to prepare 17, but Fmoc-Lys(Boc)-OH (163 mg, 0.35 mmol, 7 equiv, 0.233 M) and HBTU (135 mg, 0.35 mmol, 7 equiv, 0.233 M) were used as the second residue instead of Boc-Lys(Fmoc)-OH to give the desired product (58 mg, 93%). ¹H NMR (400 MHz, D₂O): δ 7.64 (d, *J* = 8.0 Hz, 8H, CH), 7.59 (d, *J* = 8.0 Hz, 4H, CH), 7.53 (d, *J* = 8.0 Hz, 4H, CH), 4.48 (t, *J* = 8.0 Hz, 2H, CH), 4.36 (t, *J* = 8.0 Hz, 1H, CH), 4.24 (t, *J* = 8.0 Hz, 1H, CH), 4.19 (t, *J* = 8.0 Hz, 1H, CH), 3.38–3.30 (m, 4H), 3.14–3.06 (m, 2H), 2.95–2.90 (m, 4H), 1.89–1.62 (m, 18H), 1.45–1.26 (m, 12H), MS (ESI⁺) calcd for [C₅₈H₈₅B₄N₁₁O₁₇]²⁺, (*M* + 2H)²⁺: 626, Found: 626.

Binding Experiments of Compounds 16 and 17 to Carbohydrates. To the stock solution of carbohydrate in 50% aqueous MeOH (1 mL, 100 mM) was added the solution of 16 or 17 in MeOH (1 mL, 0.1 mM). After being shaken for 5 min, the resulting solution was subjected to ultrafiltration (cutoff: 1 K Dalton, 5500 rpm, 30 min). Next, the filtration was subjected to the HPLC analysis. The intensity of the signal represented the remaining amount of carbohydrate after binding.

Meanwhile, the stock solution in 50% aqueous MeOH (1 mL, 100 mM) was added MeOH (1 mL). The resulting solution was subjected to the HPLC analysis. The intensity of the signal represented the original amount of carbohydrates.

HPLC (Agilent Technologies 1100 Series) was equipped with a light scattering detector (SofTA Corporation, Model 400 ELSD) with the following parameters: Temp_{column}: 60 °C; Temp_{drift tube}: 70 °C; Temp_{spray chamber}: 50 °C; Temp_{exhaust tube}: 70 °C; Temp_{Optical Cell}: 70 °C; gas pressure: 48 psi. The eluent conditions are 88% of acetonitrile in water to 78% of acetonitrile in 17 min, rate = 0.7 mL/min. Each sample was diluted 20 times with water, and 10 μL of the resulting sample was injected for analysis. The area of each peak was subjected to eq 1 to give the binding ratio, and the result is shown in Table S1.

$$\text{binding ratio (\%)} = \frac{A_{\text{saccharide}} - A_{\text{saccharide-CPBA}}}{A_{\text{saccharide}}} \times 100\% \quad (1)$$

where *A*_{saccharide} is the peak area of each carbohydrate solution before mixing with 16 or 17 and *A*_{saccharide-CPBA} is the peak area of each carbohydrate solution after mixing with 16 or 17.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c02708>.

¹H and ¹³C NMR spectra for all the compounds (PDF)
MS data for all the compounds (ZIP)

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Notes

The authors declare no competing financial interest. FAIR Data is available as Supporting Information for Publication and includes the primary NMR FID files.

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