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Receiver–Amplifier, Self-Immolative Dendritic Device

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Abstract: Self-immolative dendrimers disassemble through a domino-like chain fragmentation initiated by a single cleavage at the dendrimer core. We have designed and synthesized dendritic molecules that resemble dendritic architectures present in nature. The unique design allows a cleavage signal received by any one of the multiple triggers on one side of the dendrimer to be transferred convergently to a focal point. The signal is divergently amplified through to the other side of the dendrimer, reporter units are released, and fluorescence is emitted. During signal propagation, the dendritic molecule disassembles in a self-immolative manner into small fragments. These compounds are the longest dendritic system ever reported to disassemble through sequential self-immolative reactions. The synthesized dendritic molecules have an architecture and signal-conducting activity related to that of neurons.

Keywords: dendrimers · enzymes · fluorescence · prodrugs · self-immolative

Introduction

Dendritic architectures[1] are often used in nature to achieve divergent or convergent conducting effects. For example, the structural properties of a tree allow it to transfer water and nutrients from the trunk toward the branches and the leaves. The structural design of nerve cells is another striking example of a dendritic architecture that acts as a signal transduction system. Neurons are known to send out a series of long specialized processes that will either receive electrical signals (dendrites) or transmit these electrical signals (axons) to their target cells (Figure 1).

The dendritic architecture of neurons inspired us to design dendrimers with a signal transduction pathway similar to that of a nerve cell. Here we report the design and synthesis of a self-immolative dendritic molecule that is capable of transferring a cleavage signal in a convergent manner to the core and then amplifying it divergently to the periphery. This synthetic system is analogous to the signal transduction pathway of a neuron.[2]

Self-immolative dendrimers have recently been introduced as a potential platform for single-triggered, multiple-release prodrugs.[3–5] These unique dendrimers are designed to release all of their tail units through a domino-like chain-fragmentation process that is initiated by a single cleavage at the dendrimer core.[6–9] Incorporation of drug molecules as the tail units and use of an enzyme substrate as the trigger[10] generates a multiple-release prodrug unit that is initiated by a single enzymatic cleavage. Dendritic prodrugs have a significant advantage in tumor-cell-growth inhibition compared with classic monomeric prodrugs.[11,12] We have also designed and synthesized fully biodegradable dendrimers that are disassembled through multienzymatic triggering followed by self-immolative chain fragmentation.[13] The concept of a multitriggered, self-immolative dendron was recently applied to the synthesis of a prodrug activated

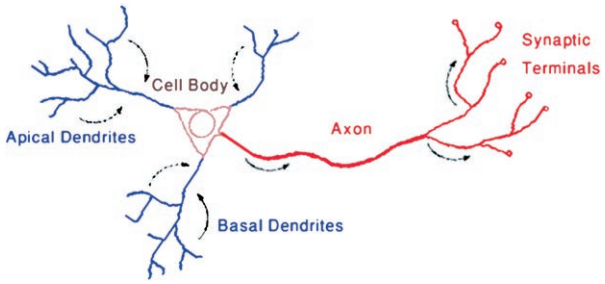

Figure 1. Schematic representation of the dendritic architecture of a neuron. The electrical signal is transferred in a convergent manner from the dendrites towards the axon where it diverges to the synaptic terminals.

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through a molecular "OR" logic trigger (a dual-input trigger activated by either one of two different enzymes).[14]

Results and Discussion

Molecular design of the dendritic system: To construct a dendritic architecture with signal-conducting activity similar to that of a neuron, we used a multitriggered self-immolative dendron[13] as a receiver and linked it through a short spacer to a single-triggered self-immolative dendron[3] that acts as an amplifier. In this design (shown schematically in Figure 2), a signal is received through activation of either of the triggers. The signal is transferred to the focal point, where it is divergently amplified through one of the dendrons, and both reporter units are released. During signal propagation, the dendritic molecule is disassembled into small fragments.

Based on the design illustrated in Figure 2, we synthesized two dendritic molecules: compound **1** (first generation) and compound **2** (second generation) shown in Figure 3. In each molecule, the signal transduction is programmed to initiate through enzymatic cleavage of the phenylacetamide trigger by using penicillin G amidase (PGA). 6-Aminoquinoline was used on one end as a reporter unit because its release can be monitored by employing fluorescence spectroscopy. Upon release of 6-aminoquinoline from the dendrimer, the conjugation of its amine functional group with the quinoline π system is increased and a new band at $\lambda = 460$ nm appears in the fluorescence emission spectrum.[15] Polyethylene glycol 400 (PEG-400) oligomers were attached to the other end (right-hand side in Figure 2) of the dendritic molecules to make them sufficiently soluble in aqueous solution to allow enzymatic activation.

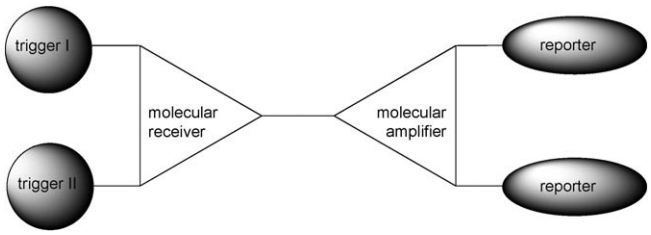


Figure 2. Graphical illustration of a receiver–amplifier dendritic molecule.

The signal-transfer mechanism of the first-generation dendritic molecule (**1**) is illustrated in Scheme 1. Enzymatic cleavage of either one of the phenylacetamide groups by PGA gives intermediate **3**, which contains an exposed amine group. The amine group is then cyclized to initiate a series of self-immolative fragmentations that releases phenol **4** along with several other short fragments. Phenol **4** is disassembled through a double quinone methide type rearrangement to release carbon dioxide, compound **5**, and most importantly free the two fluorescent molecules of 6-aminoquinoline. The second-generation dendritic molecule **2** disassembles by employing a similar mechanism (Scheme 2). Enzymatic cleavage of one of the four phenylacetamide groups by PGA releases amine intermediate **6**, which initiates the signal transfer through self-immolative fragmentations. The output is expressed in the form of a fluorescence signal as a result of the release of four 6-aminoquinoline molecules.

Synthesis of the dendritic molecules 1 and 2: First-generation dendritic molecule **1** was synthesized according to

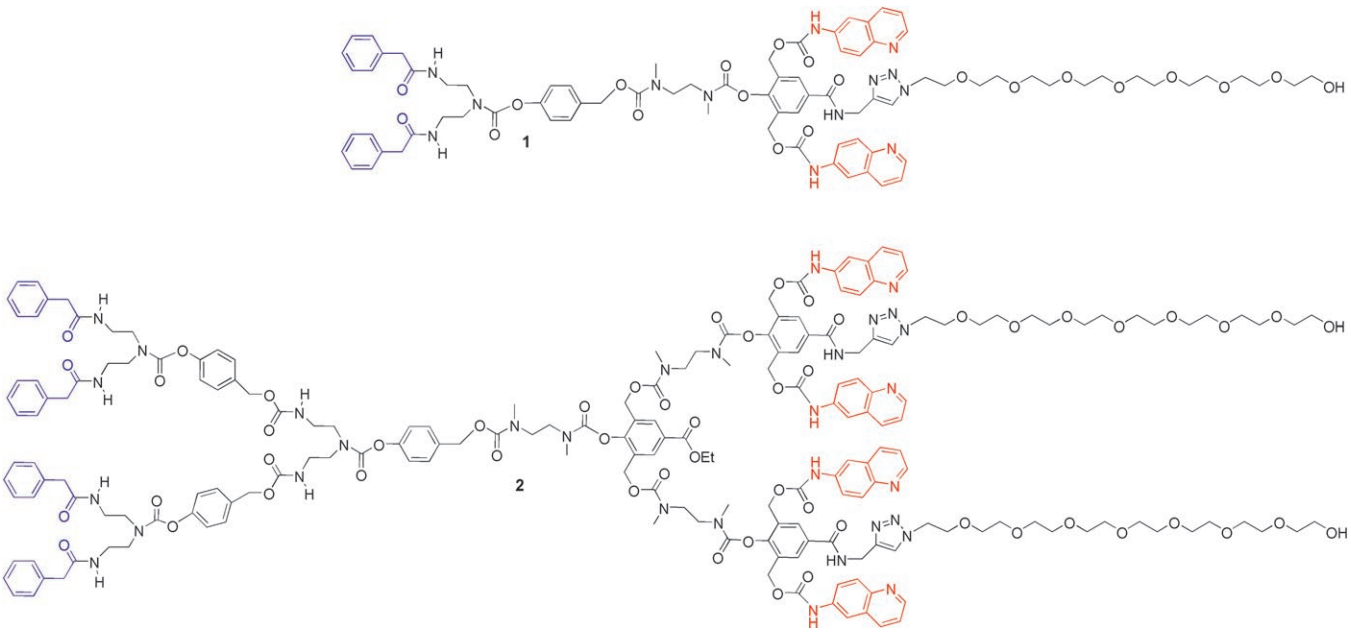
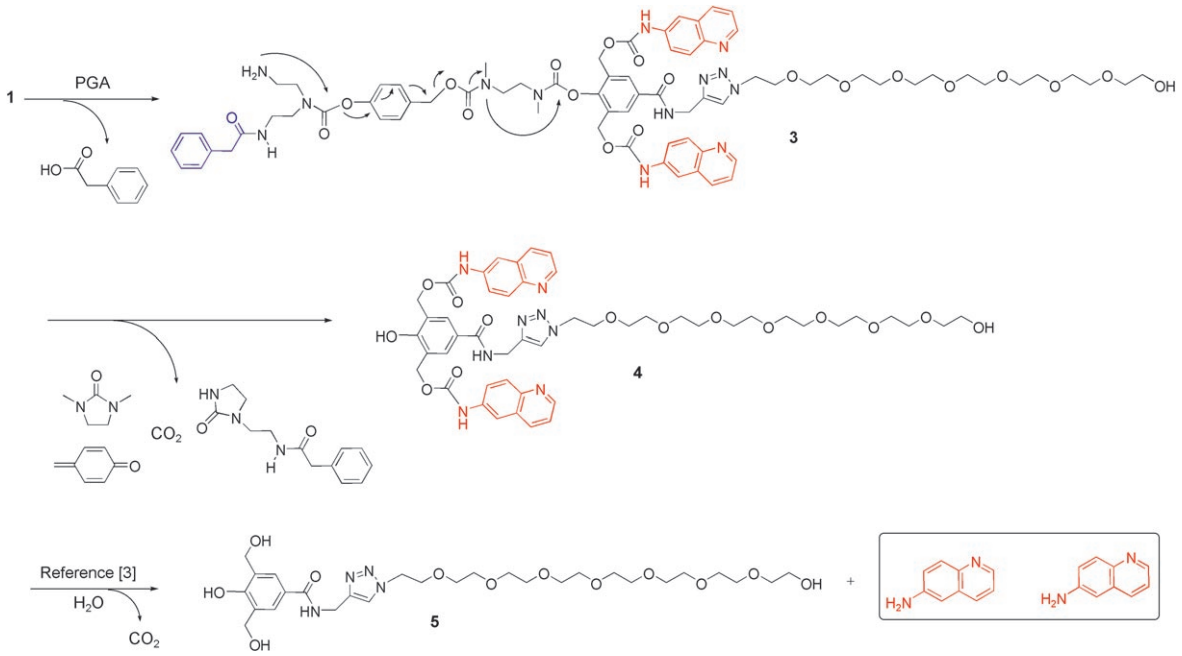
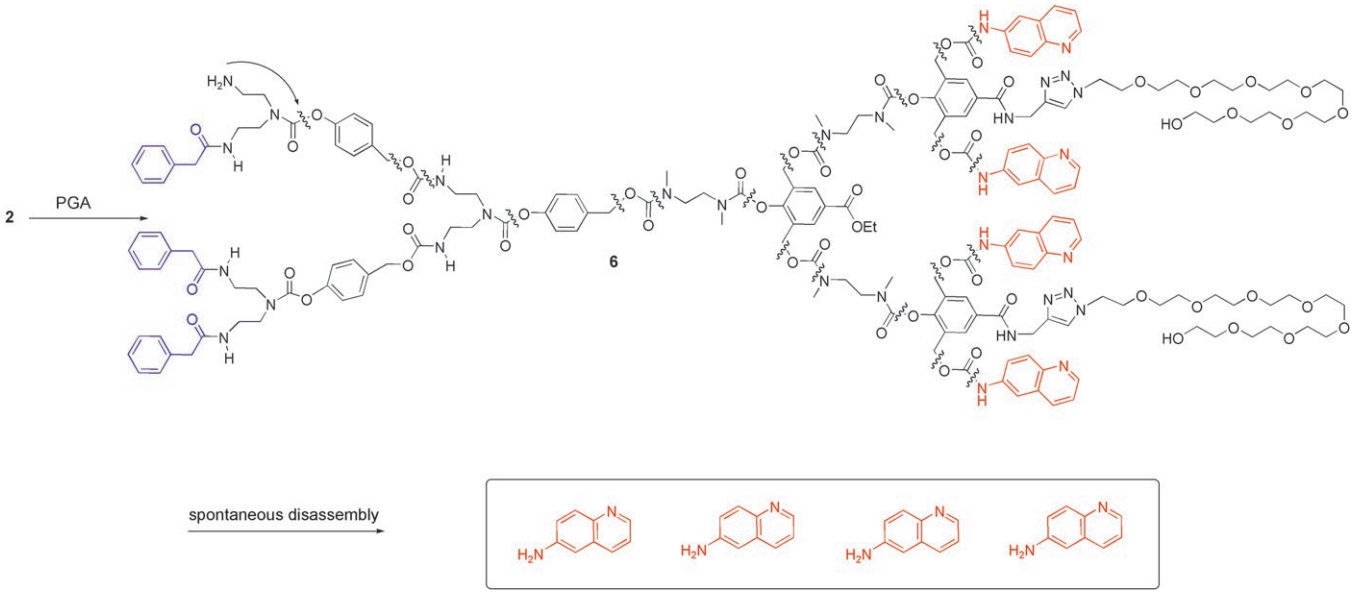


Figure 3. Chemical structures of first-generation (**1**) and second-generation (**2**) self-immolative, receiver–amplifier dendritic molecules with an enzymatic trigger (blue), cleaved by PGA and 6-aminoquinoline (red) reporter groups.

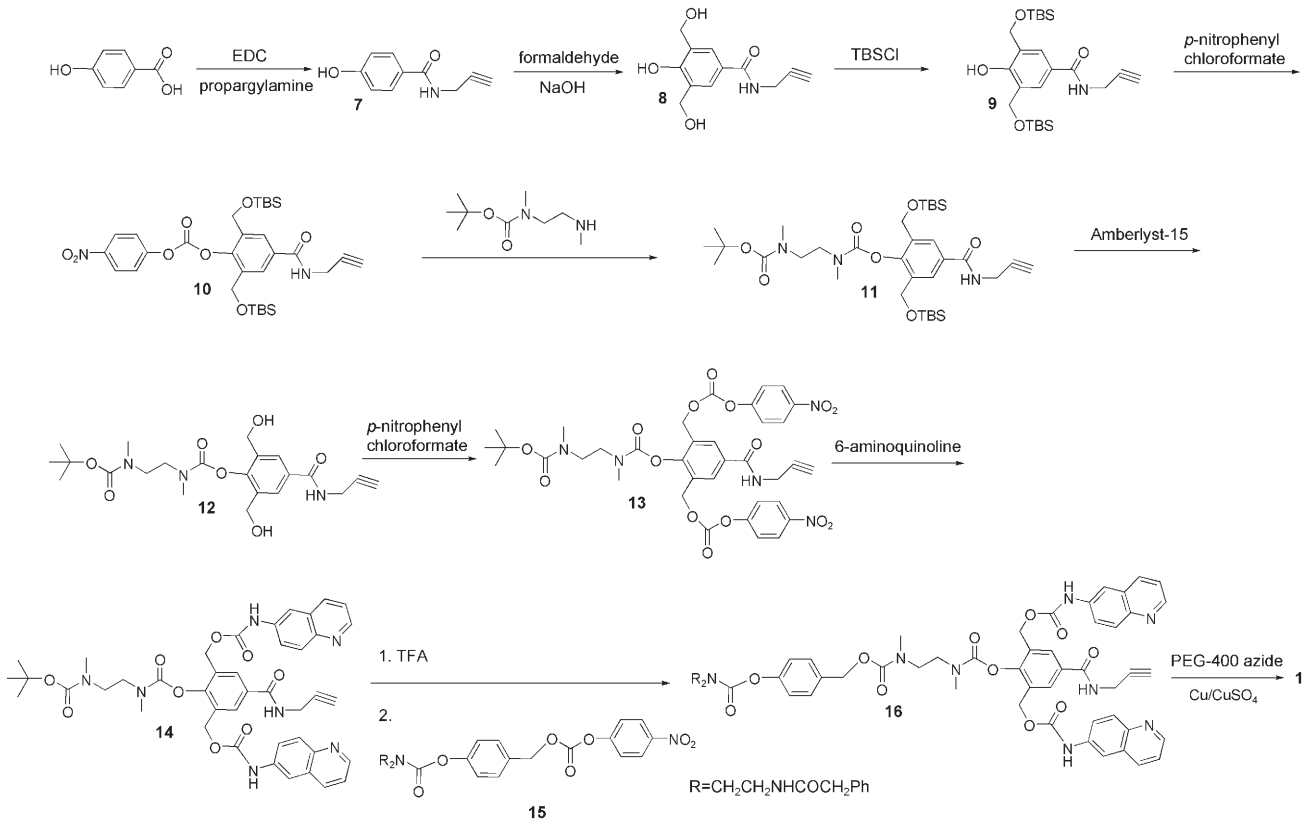


Scheme 1. Signal transduction mechanism for dendritic molecule **1** by using a self-immolative reaction sequence.



Scheme 2. Signal transduction pathway for dendritic molecule **2** by employing a self-immolative reaction sequence.

Scheme 3. 4-Hydroxybenzoic acid was coupled with propargylamine to form amide **7**, which was treated with paraformaldehyde to generate dibenzyl alcohol **8**. The latter was subsequently treated with two equivalents of *tert*-butyldimethylsilyl chloride (TBSCl) to afford phenol **9**, which was acylated with *p*-nitrophenyl chloroformate to give carbonate **10**. Reaction of **10** with mono-Boc-protected *N,N′*-dimethylethylenediamine (Boc = *t*-butoxycarbonyl) generated compound **11**, which was deprotected in the presence of Amberlyst-15 to give diol **12**. Acylation of diol **12** with two equivalents of *p*-nitrophenyl chloroformate afforded dicarbonate **13**, which was then treated with two equivalents of 6-aminoquinoline to give compound **14**. Deprotection with trifluoroacetic acid (TFA) afforded an amine salt, which was treated in situ with compound **15**[13] to yield dendritic molecule **16**. Compound **16** was treated with commercially available PEG-400 azide to perform a copper(I)-catalyzed Huisgen cycloaddition[16] reaction to generate first-generation dendritic molecule **1**.

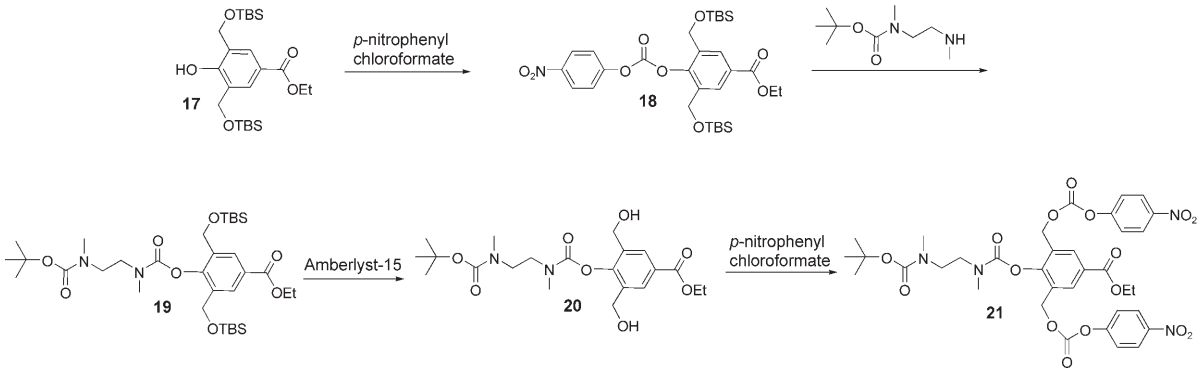


Scheme 3. Synthesis of first-generation dendritic molecule **1**.

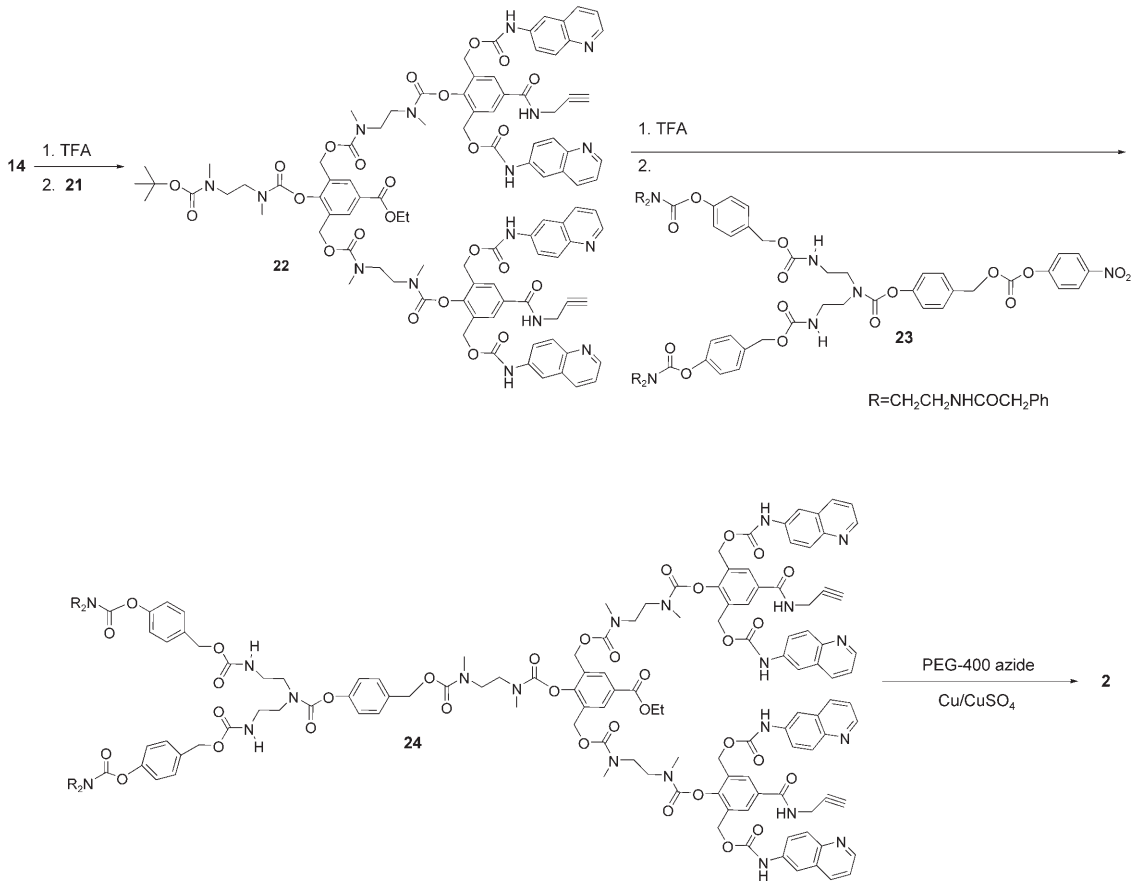
The synthesis of second-generation dendritic molecule **2** is shown in Schemes 4 and 5. Acylation of phenol **17**[12] with *p*-nitrophenyl chloroformate afforded carbonate **18**. Reaction of **18** with mono-Boc-protected *N,N'*-dimethylethylenediamine generated compound **19**, which was deprotected in the presence of Amberlyst-15 to give diol **20**. Acylation of diol **20** with two equivalents of *p*-nitrophenyl chloroformate afforded dicarbonate **21**.

Two equivalents of dendron **14** were deprotected with TFA to afford an amine salt, which was treated in situ with compound **21** to yield dendritic molecule **22**. The latter was treated with TFA to afford an amine-salt that was treated in situ with compound **23** (see Experimental Section) to yield dendritic molecule **24**. Compound **24** was treated with commercially available PEG-400 azide to perform a copper(I)-catalyzed Huisgen cycloaddition reaction to generate second-generation dendritic molecule **2**.

Enzymatic activation of dendritic molecules 1 and 2: To prepare aqueous solutions of **1** and **2**, the compounds were initially dissolved in dimethylsulfoxide (DMSO)/Cremophor EL (4:1) and then added to water. The final composition of the solution was 10% organic and 90% aqueous. Dendritic molecules **1** and **2** were then incubated with PGA in phos-



Scheme 4. Synthesis of intermediate molecule **21**.



Scheme 5. Synthesis of second-generation dendritic molecule **2**.

phate-buffered saline (PBS, pH 7.4) solution at 37 °C. Control solutions were incubated in buffer without the enzyme. The sequential fragmentation illustrated in Schemes 1 and 2 was monitored through the release of 6-aminoquinoline. Free 6-aminoquinoline is generated upon addition of PGA to a solution of **1** or **2**, as shown in Figure 4. The fluorescence spectra of **1** and **2** exhibit one emission band at $\lambda = 390$ nm that disappeared during dendrimer fragmentation. The generation of a new band at $\lambda = 460$ nm indicates the formation of free 6-aminoquinoline. To evaluate the kinetic behavior of the sequential fragmentation, the intensities of the bands at 390 and 460 nm were plotted as a function of time (Figure 4b and d). The release of 6-aminoquin-

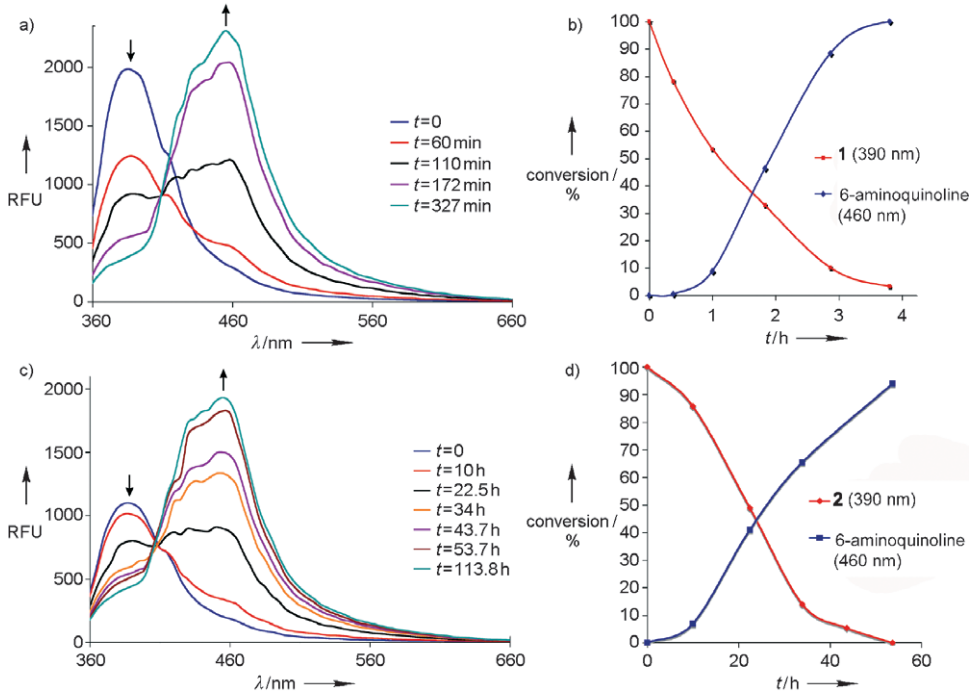


Figure 4. Emission fluorescence spectra ($\lambda_{ex} = 250$ nm) of **1** (a, b) and **2** (c, d) upon addition of PGA (0.1 mg mL^{-1}). The concentrations of dendritic molecules **1** and **2** were 25 and 12 μM, respectively.

oline from first-generation dendritic molecule **1** was complete in approximately 4 h, whereas the fragmentation of second-generation dendritic molecule **2** required over 50 h. No release of 6-aminoquinoline was observed when compounds **1** and **2** were incubated in the buffer without PGA (data not shown). The fragmentation mechanism was previously described for both the receiver[13] and the amplifier dendrons.[3]

PGA is a specific proteolytic enzyme that selectively cleaves phenylacetamide groups.[17] Dendritic molecule **25** with *t*-butylcarbamate as a trigger instead of the phenylacetamide group was evaluated for activation with PGA. Compound **25** was found to be stable when incubated with PGA and no degradation was observed. However, when **25** was first deprotected by treatment with TFA and then incubated in PBS at pH 7.4 the release of 6-aminoquinoline was observed (Scheme 6). This result clearly proves that activation of dendrimers **1** and **2** by PGA is specific and that the release process is indeed trigger-dependent.

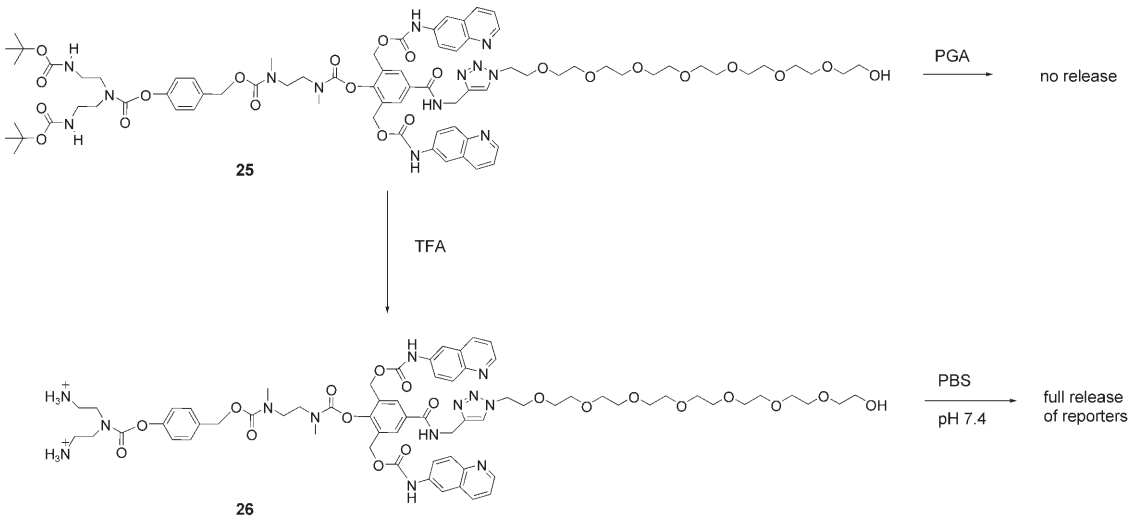
Dendrimer fragmentation occurs through enzymatic cleavage, followed by cyclization, quinone methide type rearrangement, and decarboxylation. Previous studies have shown that the slow step in self-immolative reactions is the cyclization.[3] The signal transfer is significantly slower in **2** than in **1**. This observation is not unexpected because four cyclization steps are needed to complete the disassembly of the second-generation molecule, whereas only two are needed in the first-generation dendrimer. In addition, the trigger groups in **2** are less accessible to the activating enzyme owing to increased steric hindrance in comparison with **1**. This phenomenon was observed in dendrimer-bound peptides that had increased stability towards enzymatic degradation when compared with monomeric peptides.[18] We are currently attempting to synthesize dendritic molecules that will disassemble without a cyclization step. The signal transfer is expected to be significantly faster in the absence of this slow step.

Conclusion

In conclusion, we designed and synthesized new dendritic molecules that act as receiver–amplifier devices. A cleavage signal received by one side of the dendritic molecule is transferred in a convergent manner to the core and then amplified divergently toward the other side. The signal is propagated through self-immolative sequential fragmentations to release reporter molecules that are observed by means of fluorescence spectroscopy. There are similarities between this system and the dendritic architecture and function of neurons and other dendritic transduction pathways in nature. Dendritic molecule **2** is the longest dendritic system ever reported to date to disassemble through sequential self-immolative reactions. Learning to control and optimize the kinetics of self-immolative reactions is currently a major focus of our laboratory.

Experimental Section

General: All reactions requiring anhydrous conditions were performed under an argon or nitrogen atmosphere. Chemicals and solvents were either A.R. grade or purified by employing standard techniques. Thin-layer chromatography (TLC) was performed on silica gel plates Merck 60 F_{254}; compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid (25 g), $Ce(SO_4)_2{\cdot}H_2O$ (10 g), concd H_2SO_4 (60 mL), and H_2O (940 mL), and developed by heating. Flash chromatography was performed by using silica gel Merck 60 (particle size 0.040–0.063 mm) and the eluent given in parentheses. ^{1}H NMR spectroscopy was performed by using a Bruker AMX 200 or 400 instrument. The chemical shifts are expressed in δ relative to tetramethylsilane (TMS) ($\delta = 0$ ppm) and the coupling constants J in Hz. The spectra were recorded by using $CDCl_3$ or CD_3OD as a solvent at RT. All reagents, including salts and solvents, were purchased from Sigma-Aldrich. PEG-400 azide was purchased from Polypure (Norway). tris(1-Benzyl-1*H*-[1,2,3]triazol-4-ylmethyl)amine (TBTA) was kindly received from the Sharpless laboratory (The Scripps Research Institute, La Jolla, USA).



Scheme 6. Activation studies for dendritic molecules **25** and **26**.

Synthesis of first-generation self-immolative dendritic molecule 1

Compound ***7***: Commercially available 4-hydroxybenzoic acid (2.0 g, 14.5 mmol) was dissolved in DMF (10 mL) before *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) (3.3 g, 17.4 mmol), 1-hydroxybenzotriazole (HOBT) (1.0 g, 7.3 mmol), and propargylamine (1.0 mL, 14.5 mmol) were added. The mixture was stirred overnight and monitored by TLC (EtOAc/Hex 2:3). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 2:3) to give **7** as a yellowish oil (1.8 g, 70%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.70 (d, J = 6.8 Hz, 2H), 6.81 (d, J = 6.8 Hz, 2H), 4.11 (d, J = 2.5 Hz, 2H), 2.71 ppm (t, J = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.9, 160.6, 128.8, 124.4, 114.5, 79.5, 70.3, 28.3 ppm; MS (FAB): m/z: 176.0 $[M+H]^+$.

Compound ***8***: Compound **7** (1.8 g, 10.2 mmol) was added to a cold (0 °C) 12% aqueous NaOH (12 mL) solution. The mixture was kept at 0 °C and formaldehyde (37% in water; 10 mL) was added. The reaction was stirred at 55 °C for 3 d and monitored by TLC (EtOAc/MeOH 95:5). Upon completion of the reaction, the solution was diluted with EtOAc and washed with saturated ammonium chloride solution. The aqueous layer was washed twice with EtOAc. The combined organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/MeOH 19:1) to give **8** as a white solid (1.9 g, 80%). ^{1}H NMR (200 MHz, CD_3OD): δ = 7.80 (s, 2H), 4.91 (s, 4H), 4.26 (d, J = 2.5 Hz, 2H), 2.70 ppm (t, J = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 168.1, 156.7, 126.8, 126.0, 124.4, 79.4, 70.2, 60.3, 28.3 ppm; MS (FAB): m/z: 236.0 $[M+H]^+$.

Compound ***9***: Compound **8** (713 mg, 3.0 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C before imidazole (408 mg, 6.0 mmol) and TBS-Cl (910 mg, 6.0 mmol) were added. The reaction was stirred at RT for 2 h and monitored by TLC (EtOAc/Hex 2:8). Upon completion of the reaction, the solution was diluted with diethyl ether and washed with saturated ammonium chloride solution. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 15:85) to give **9** as a colorless oil (1.12 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 (s, 2H), 4.87 (s, 4H), 4.23 (dd, J = 5.0, 2.5 Hz, 2H), 2.17 (t, J = 2.5 Hz, 1H), 0.95 (s, 18H), 0.13 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.7, 156.4, 126.1, 124.5, 79.6, 71.7, 62.7, 29.6, 25.8, 25.6, 18.2, −5.5 ppm; MS (FAB): m/z: 464.2 $[M+H]^+$.

Compound ***10***: Compound **9** (1.12 g, 2.4 mmol) was dissolved in dry THF (20 mL) before NEt_3 (1.0 mL, 7.2 mmol) was added and the mixture was cooled to 0 °C. *p*-Nitrophenyl chloroformate (581 mg, 2.9 mmol) dissolved in dry THF (10 mL) was then added dropwise before the reaction was stirred for 1 h at RT and was monitored by TLC (EtOAc/Hex 2:8). Upon completion of the reaction, the solution was filtered, the solvent was evaporated, and the crude product was purified by using column chromatography on silica gel (EtOAc/Hex 15:85) to give **10** as a colorless oil (1.35 g, 90%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.43 (d, J = 8.1 Hz, 2H), 8.02 (s, 2H), 7.63 (d, J = 8.1 Hz, 2H), 7.01 (m, 1H), 4.91 (s, 4H), 4.38 (dd, J = 2.5, 2.6 Hz, 2H), 2.41 (t, J = 2.5 Hz, 1H), 1.08 (s, 18H), 0.29 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.4, 155.2, 149.4, 147.7, 145.5, 133.9, 132.2, 126.3, 125.3, 121.5, 79.2, 71.8, 60.3, 31.5, 25.8, 18.2, −5.5 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{31}H_{44}N_2O_8Si_2$: 651.2528; found: 651.2562 $[M+Na]^+$.

Compound ***11***: Compound **10** (1.5 g, 2.3 mmol) was dissolved in DMF (7 mL). Previously described mono-Boc-protected *N,N'*-dimethylethylenediamine[3] (541 mg, 2.9 mmol) was added. The reaction was stirred at RT for 1 h and monitored by TLC (EtOAc/Hex 1:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc/Hex 2:8) to give **11** as a colorless oil (1.45 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (s, 2H), 6.32 (m, 1H), 4.68–4.67 (m, 4H), 4.27–4.25 (m, 2H), 3.61–3.43 (m, 4H), 3.24 (s, 2H), 3.12 (s, 1H), 2.96 (s, 3H), 2.32 (brs, 1H), 1.51–1.46 (m, 9H), 0.92 (s, 18H), 0.08 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.2, 153.1, 153.0, 134.6, 130.8, 125.1, 80.2, 78.8, 72.0, 59.9, 46.4, 46.1, 36.4, 35.9, 35.1, 29.8, 28.3, 25.7, 18.2, −5.5 ppm; MS (FAB): m/z: 700.4 $[M+Na]^+$.

Compound ***12***: Compound **11** (1.5 g, 2.2 mmol) was dissolved in MeOH (10 mL) and Amberlyst-15 was added. The reaction was stirred at RT for 2 h and monitored by TLC (EtOAc). Upon completion of the reaction, the solution was filtered to remove Amberlyst-15 and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/MeOH 19:1) to give **12** as a white solid (500 mg, 56%). ^{1}H NMR (200 MHz, CD_3OD): δ = 7.78 (s, 2H), 4.57 (brs, 4H), 4.25–4.23 (m, 2H), 3.60–3.40 (m, 4H), 3.20 (s, 2H), 3.10 (s, 1H), 2.96 (s, 3H), 2.40 (brs, 1H), 1.59–1.54 ppm (m, 9H); ^{13}C NMR (100 MHz, CD_3OD): δ = 169.8, 158.9, 157.5, 152.1, 134.1, 130.3, 130.0, 83.2, 83.0, 74.4, 62.5, 50.3, 49.0, 38.7, 37.9, 37.6, 31.3 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{22}H_{31}N_3O_7$: 472.2015; found: 472.2059 $[M+Na]^+$.

Compound ***13***: Compound **12** (300 mg, 0.67 mmol) was dissolved in dry THF (6 mL) and was cooled to 0 °C before diisopropyl ethyleneamine (DIPEA) (945 μL, 5.4 mmol) followed by *p*-nitrophenyl chloroformate (800 mg, 4.0 mmol) and pyridine (27 μL, 0.33 mmol) were added. The reaction was allowed to warm to RT and was monitored by TLC (EtOAc/Hex 3:1). Upon completion of the reaction, the solution was diluted with EtOAc and washed with saturated aqueous NH_4Cl and $NaHCO_3$ solutions. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 7:3) to give **13** as a white solid (430 mg, 82%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.23 (d, J = 9.0 Hz, 4H), 7.94 (s, 2H), 7.34 (d, J = 9.0 Hz, 4H), 5.28 (s, 4H), 4.23 (m, 2H), 3.62–3.43 (m, 4H), 3.18–3.00 (m, 3H), 2.92–2.83 (m, 3H), 2.27 (brs, 1H), 1.45–1.42 ppm (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.2, 156.6, 154.1, 153.1, 146.3, 132.9, 130.4, 130.0, 126.1, 122.6, 122.5, 80.8, 78.9, 73.0, 66.2, 48.5, 47.8, 46.8, 36.1, 35.6, 30.7, 29.2 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{36}H_{37}N_5O_{15}$: 802.2178; found: 802.2112 $[M+Na]^+$.

Compound ***14***: Compound **13** (430 mg, 0.55 mmol) was dissolved in DMF (2 mL) before 6-aminoquinoline (320 mg, 2.2 mmol), a catalytic amount of HOBT (5 mg), and DIPEA (240 μL, 1.4 mmol) were added. The reaction was heated to 50 °C, stirred overnight, and monitored by TLC (MeOH/EtOAc 1:9). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (MeOH/EtOAc 2:8) to give **14** as a white solid (270 mg, 62%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.69–8.67 (m, 2H), 7.98–7.88 (m, 8H), 7.54–750 (m, 2H), 7.25–7.22 (m, 2H), 5.08 (brs, 4H), 4.13 (s, 2H), 3.52–3.36 (m, 4H), 3.05–2.76 (m, 6H), 2.17 (brs, 1H), 1.38–1.30 ppm (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8, 154.4, 154.1, 149.8, 149.7, 145.9, 137.0, 136.3, 132.3, 131.1, 130.9, 129.9, 129.6, 123.4, 122.3, 114.8, 81.2, 80.1, 72.7, 63.3, 47.6, 46.4, 36.6, 35.2, 30.6, 29.2 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{42}H_{43}N_7O_9$: 812.3061; found: 812.3014 $[M+Na]^+$.

Compound ***16***: Compound **14** (64 mg, 0.08 mmol) was dissolved in TFA (1 mL) and stirred for few minutes before the excess acid was removed under reduced pressure and the crude amine salt was dissolved in DMF (0.5 mL). Compound **15**[13] (53 mg, 0.08 mmol) and NEt_3 (0.1 mL) were then added, and the reaction was monitored by TLC ($MeOH/CH_2Cl_2$ 1:9). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (MeOH/EtOAc 1:9) to give **16** as a white solid (45 mg, 46%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.85–8.55 (m, 2H), 8.10–7.80 (m, 10H), 7.67–7.45 (m, 2H), 7.29–6.71 (m, 14H), 5.09–5.01 (m, 6H), 4.13–4.05 (m, 2H), 3.67–3.30 (m, 16H), 3.04–2.90 (m, 6H), 2.23 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1, 166.5, 155.3, 153.7, 153.5, 150.9, 148.9, 145.0, 136.6, 135.8, 135.0, 133.6, 103.7, 130.5, 130.2, 129.9, 129.4, 129.3, 129.0, 128.9, 128.8, 128.5, 127.3, 122.9, 121.8, 121.7, 80.0, 71.9, 71.8, 62.2, 53.6, 48.5, 43.6, 38.8, 32.1, 31.7, 30.0, 29.8 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{66}H_{64}N_{10}O_{13}$: 1227.4547; found: 1227.4656 $[M+Na]^+$.

Compound ***1***: Compound **16** (16 mg, 0.013 mmol) was dissolved in DMF (0.5 mL) before PEG-400 azide (6.3 mg, 0.016 mmol), copper sulfate (2 mg, 0.013 mmol), and TBTA (7.5 mg, 0.0133 mmol) were added. Final-

ly, a few copper turnings were added before the reaction was stirred overnight at RT. The reaction was monitored by HPLC, and upon completion of the reaction the solution was filtered and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel ($MeOH/CH_2Cl_2$ 1:9) to give **1** as a white solid (17.7 mg, 83%). HPLC: C18 reverse-phase column; $\lambda = 250$ nm; flow: 1 mL min^{-1}; gradient program: $t = 0$ (30% MeCN/70% H_2O), $t =$ 20–25 min (100% MeCN); $t_R = 8.26$ min (**16**); $t_R = 7.38$ min (**1**). HRMS (MALDI-TOF): m/z calcd for $C_{82}H_{97}N_{13}O_{21}$: 1622.6814; found: 1622.6797 $[M+Na]^+$.

Synthesis of second-generation self-immolative dendritic molecule 2

*Compound **18***: Compound **17**[12] (780 mg, 1.7 mmol) was dissolved in CH_2Cl_2 (20 mL) before NEt_3 (870 μL, 6.0 mmol) and a catalytic amount of dimethylaminopyridine (DMAP; 5 mg) were added. The reaction was cooled to 0°C and *p*-nitrophenyl chloroformate (520 mg, 2.6 mmol) was added. The reaction was stirred at RT for 1 h and monitored by TLC (EtOAc/Hex 1:9). Upon completion of the reaction, the solution was diluted with CH_2Cl_2, and washed with saturated aqueous NH_4Cl and with brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 5:95) to give compound **18** as a colorless oil (790 mg, 75%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.29$ (d, $J = 9.0$ Hz, 2H), 8.11 (s, 2H), 7.45 (d, $J = 9.0$ Hz, 2H), 4.75 (s, 4H), 4.35 (q, $J = 7.0$ Hz, 2H), 1.36 (t, $J = 7.0$ Hz, 3H), 0.90 (s, 18H), 0.07 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.5$, 156.1, 150.16, 149.26, 146.4, 134.5, 129.9, 129.6, 126.2, 122.1, 61.9, 61.2, 26.7, 19.1, 15.1, −4.5 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{30}H_{45}NO_9Si_2$: 642.2525; found: 642.2482 $[M+Na]^+$.

*Compound **19***: Compound **18** (750 mg, 1.2 mmol) was dissolved in DMF (5 mL) and mono-Boc-protected *N,N′*-dimethylethylenediamine[3] (280 mg, 1.45 mmol) was added. The reaction was stirred at RT and monitored by TLC (EtOAc/Hex 1:3). Upon completion of the reaction, the solution was diluted with EtOAc, and washed with saturated aqueous NH_4Cl solution and with brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 1:4) to give **19** as a viscous oil (630 mg, 78%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.10$ (s, 2H), 4.64 (s, 4H), 4.32 (q, $J = 7.0$ Hz, 2H), 3.55–3.42 (m, 4H), 3.12–3.00 (m, 3H), 2.92–2.89 (m, 3H), 1.53–1.45 (m, 9H), 1.38 (t, $J = 7.0$ Hz, 3H), 0.9 (s, 18H), 0.07 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.0$, 153.7, 149.4, 135.1, 128.8, 126.8, 116.3, 80.6, 61.6, 60.2, 48.1, 47.1, 36.2, 35.9, 29.2, 26.6, 19.1, 14.9, −4.5 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{33}H_{60}N_2O_8Si_2$: 691.3780; found: 691.3748 $[M+Na]^+$.

*Compound **20***: Compound **19** (570 mg, 0.85 mmol) was dissolved in methanol (15 mL) and Amberlyst-15 was added. The reaction was stirred at RT for 5 h and monitored by TLC (EtOAc). Upon completion of the reaction, the solution was filtered to remove Amberlyst-15 and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc) to give **20** as a white solid (270 mg, 71%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.03$ (s, 2H), 4.55 (s, 4H), 4.35 (q, $J = 7.0$ Hz, 2H), 3.59–3.44 (m, 4H), 3.13–3.00 (m, 3H), 2.90–2.85 (m, 3H), 1.44–1.39 (m, 9H), 1.34 ppm (t, $J = 7.0$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.6$, 156.8, 155.6, 155.4, 135.1, 131.5, 129.2, 81.2, 61.9, 61.0, 47.5, 47.1, 36.9, 35.8, 29.1, 15.1 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{21}H_{32}N_2O_8$: 463.2051; found: 463.2087 $[M+Na]^+$.

*Compound **21***: Compound **20** (75 mg, 0.17 mmol) was dissolved in dry THF (2 mL) and was cooled to 0°C before DIPEA (270 μL, 1.44 mmol), *p*-nitrophenyl chloroformate (220 mg, 1.1 mmol), and pyridine (7 μL, 0.09 mmol) were added. The reaction was allowed to warm up to RT and was monitored by TLC (EtOAc/Hex 1:1). Upon completion of the reaction, the solution was diluted with EtOAc and washed with saturated aqueous NH_4Cl and $NaHCO_3$ solutions. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 2:3) to give **21** as a white solid (100 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.24$–8.20 (m, 6H), 7.36 (d, $J = 7.0$ Hz,4H), 5.31 (s, 4H), 4.38 (q, $J = 7.0$ Hz, 2H), 3.60–3.45 (m, 4H), 3.20–3.02 (m, 3H), 2.94–2.85 (m, 3H), 1.43–1.41 (m, 9H), 1.38 ppm (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.8$, 156.2, 154.1, 153.0, 152.5, 152.4, 146.3, 132.0, 129.6, 126.1, 122.8, 122.6, 80.7, 66.4, 62.3, 48.3, 46.9, 35.6, 32.3, 29.1, 14.9 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{35}H_{38}N_4O_{16}$: 793.2175; found: 793.2148 $[M+Na]^+$.

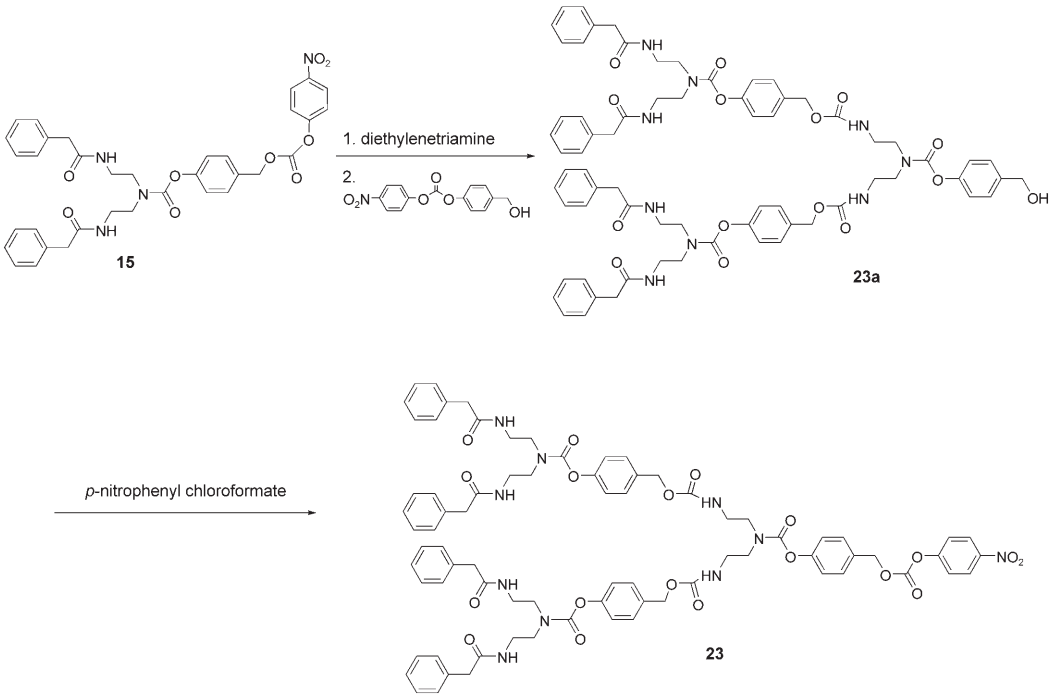
*Compound **22***: The Boc group of compound **14** (200 mg, 0.25 mmol) was removed with TFA (1 mL). The excess TFA was removed under reduced pressure and the residue was dissolved in DMF (1 mL). Compound **21** (90 mg, 0.12 mmol) and NEt_3 (1 mL) were then added and the solution was stirred for 3 h. Upon completion of the reaction, DMF was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to give **22** as a white powder (130 mg, 58%). ^{1}H NMR (200 MHz, CD_3OD): $\delta = 8.59$ (brs, 4H), 8.02–7.34 (m, 22H), 7.34–7.28 (m, 4H), 5.11–5.00 (m, 16H), 4.09 (brs, 4H), 3.57–3.41 (m, 12H), 3.10–2.57 (m, 18H), 1.82 (brs, 1H), 1.24–1.14 ppm (m, 9H); HRMS (MALDI-TOF): m/z calcd for $C_{97}H_{98}N_{16}O_{24}$: 1893.6832; found: 1893.6937 $[M+Na]^+$.

*Compound **23a** (Scheme 7)*: Compound **15**[13] (587 mg, 1 mmol) was dissolved in DMF (3 mL) before diethylenetriamine (51.6 mg, 0.5 mmol) was added and the reaction was stirred at RT for several hours. Upon completion of the reaction, *p*-hydroxybenzyl *p*-nitrophenyl carbonate (150 mg, 0.52 mmol) and NEt_3 (65 μL, 0.5 mmol) were added. The reaction was monitored by TLC (EtOAc). Once the reaction was complete, the solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc) to give **23a** as a white powder (332 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.32$–7.06 (m, 26H), 6.94 (d, $J = 8.2$ Hz, 4H), 6.86 (d, $J = 8.3$ Hz, 2H), 6.58 (brs, 2H), 5.9–5.5 (m, 2H), 5.01 (s, 2H), 4.98 (s, 2H), 4.49 (s, 2H), 3.45–3.24 ppm (m, 32H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.8$, 172.6, 157.4, 156.0, 151.7, 151.0, 139.5, 135.8, 135.7, 130.7, 130.1, 129.5, 128.5, 127.9, 122.5, 122.3, 66.8, 65.0, 49.1, 49.0, 44.21, 40.6, 39.4 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{70}H_{77}N_9O_{15}$: 1306.5431; found: 1306.5529 $[M+Na]^+$.

*Compound **23***: Compound **23a** (125 mg, 0.098 mmol) and DIPEA (25 mg, 0.195 mmol) were dissolved in CH_2Cl_2 (3 mL). *p*-Nitrophenyl chloroformate (39 mg, 0.195 mmol) and DMAP (3 mg) were then added before the reaction was monitored by TLC (EtOAc). Upon completion of the reaction, the solution was diluted with EtOAc and washed with saturated aqueous NH_4Cl solution and brine. The organic layer was dried over $MgSO_4$, the solvent removed under reduced pressure, and the crude product was purified by using column chromatography on silica gel (EtOAc) to give **23** as a yellowish powder (92 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.24$ (d, $J = 8.2$ Hz, 2H), 7.41–7.18 (m, 28H), 7.10 (d, $J = 8.4$ Hz, 2H), 6.99 (d, $J = 7.5$ Hz, 4H), 6.60 (brs, 2H), 6.31 (brs, 2H), 5.23 (s, 2H), 5.04 (s, 2H), 5.02 (s, 2H), 3.49–3.29 ppm (m, 32H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.7$, 172.4, 157.3, 156.2, 156.0, 153.2, 152.4, 151.8, 151.7, 146.2, 135.7, 135.6, 130.8, 130.7, 130.3, 130.1, 129.6, 128.0, 126.0, 122.8, 122.6, 71.1, 66.9, 49.4, 49.2, 44.3, 40.6, 39.5 ppm; HRMS (MALDI-TOF): m/z calcd for $C_{77}H_{80}N_{10}O_{19}$: 1471.5493; found: 1471.5544 $[M+Na]^+$.

*Compound **24***: Compound **22** (100 mg, 0.053 mmol) was dissolved in TFA (1.5 mL) and stirred for a few minutes before excess TFA was removed under reduced pressure and then the crude amine salt was redissolved in DMF (0.5 mL). Compound **23** (77.5 mg, 0.053 mmol) and NEt_3 (0.1 mL) were added and the reaction was monitored by TLC ($MeOH/CH_2Cl_2$ 1:9). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel ($MeOH/CH_2Cl_2$ 1:9) to give **24** as a white powder (65 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.71$ (s, 4H), 8.04–7.76 (m, 16H), 7.70–7.28 (m, 4H), 7.27–7.15 (m, 26H), 7.14–6.70 (m, 12H), 5.15–4.90 (m, 18H), 4.30–4.02 (m, 6H), 3.63–3.19 (m, 44H), 3.07–2.70 (m, 18H), 2.25 (brs, 2H), 0.89–0.85 ppm (m, 3H); HRMS (MALDI-TOF): m/z calcd for $C_{163}H_{165}N_{25}O_{38}$: 3103.1640; found: 3103.1723 $[M+Na]^+$.

*Compound **2***: Compound **24** (15 mg, 4.9 μmol) was dissolved in DMF (0.5 mL), and PEG-400 azide (4.6 mg, 11.7 μmol), copper sulfate (1.6 mg, 9.7 μmol), and TBTA (5.5 mg, 9.7 μmol) were added. Subsequently a few copper turnings were added and the reaction was stirred overnight at RT.



Scheme 7. Synthesis of intermediate molecule **23**.

The reaction was monitored by HPLC and upon its completion, the mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel ($MeOH/CH_2Cl_2$ 2:9) to give **2** as a white solid (16 mg, 85%). HPLC: C18 reverse-phase column; $\lambda = 250$ nm; flow: 1 mL min^{-1}; gradient program: $t = 0$ (10% MeCN/90% H_2O), $t = 23$–27 min (100% MeCN); $t_R = 15.66$ min (**24**); $t_R = 15.01$ min (**2**); HRMS (MALDI-TOF): m/z calcd for $C_{195}H_{231}N_{31}O_{54}$: 3893.6175; found: 3893.6046 $[M+Na]^+$.

Synthesis of first-generation dendritic molecule 25 (Scheme 8)

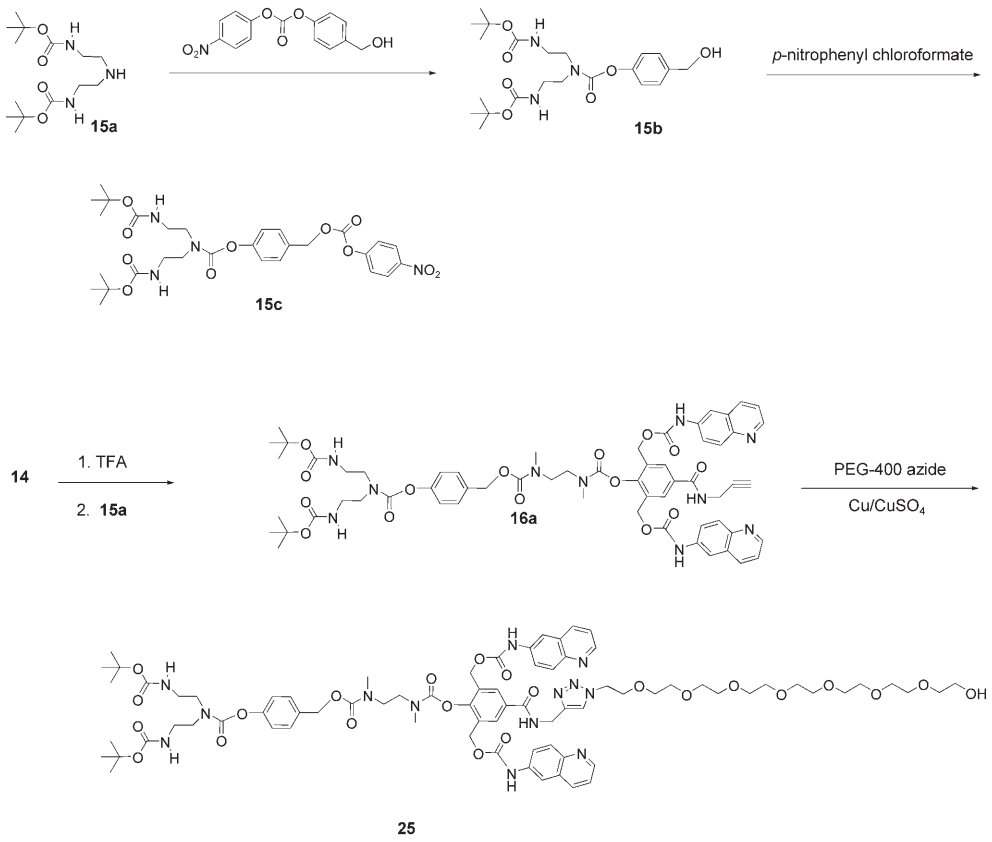
*Compound **15b***: Compound **15a**[19] (1.14 g, 3.75 mmol) was dissolved in DMF (10 mL) before the *p*-nitrophenyl carbonate of 4-hydroxybenzyl alcohol (1.64 g, 5.62 mmol) and NEt_3 (780 μL, 5.62 mmol) were added. The reaction was stirred at RT for 2 h and monitored by TLC (EtOAc/Hex 3:1). Upon completion of the reaction, the solution was diluted with EtOAc and washed with water and with brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 3:1) to give compound **15b** as a white solid (1.20 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.27$ (d, $J = 8.4$ Hz, 2H), 7.03 (d, $J = 8.4$ Hz, 2H), 5.24 (br s, 2H), 4.57 (s, 2H), 3.48–3.25 (m, 8H), 1.39 (s, 9H), 1.37 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 157.2$, 156.9, 156.3, 151.1, 139.3, 128.5, 122.4, 80.2, 80.1, 65.1, 48.8, 40.0, 29.2, 29.1 ppm.

*Compound **15a***: Compound **15b** (122 mg, 0.27 mmol) was dissolved in THF (5 mL) before DIPEA (188 μL, 1.1 mmol), 4-nitrophenyl chloroformate (163 mg, 0.81 mmol), and a catalytic amount of pyridine (5.4 μL, 0.07 mmol) were added. The reaction was stirred at RT for 1 h and monitored by TLC (EtOAc/Hex 1:1). Upon completion of the reaction, the solution was diluted with EtOAc and washed with 1 M aqueous HCl solution and with brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/Hex 1:1) to give **15c** as a white powder (97 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.26$ (d, $J = 9.1$ Hz, 2H), 7.44 (d, $J = 8.4$ Hz, 2H), 7.37 (d, $J = 9.1$ Hz, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 5.28 (s, 2H), 5.09 (br s, 2H), 3.57–3.37 (m, 8H), 1.44 (s, 9H), 1.41 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 157.1, 156.8, 156.3, 156.0, 153.2, 152.5, 146.2, 132.1, 130.7, 126.1, 122.9, 122.6, 80.2, 71.1, 49.0, 40.1, 29.2, 29.1 ppm.

*Compound **16a***: Compound **14** (100 mg, 0.13 mmol) was dissolved in TFA (1.5 mL) and stirred for few minutes. The excess acid was then removed under reduced pressure and the crude amine salt was dissolved in DMF (0.5 mL). Subsequently, compound **15c** (78 mg, 0.13 mmol) and NEt_3 (0.1 mL) were added and the reaction was monitored by TLC (EtOAc). Upon completion of the reaction, the solution was diluted with EtOAc and washed with saturated NH_4Cl solution and with brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc) to give **16a** as a white solid (131 mg, 88%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.78$–8.77 (m, 2H), 8.10–7.96 (m, 8H), 7.63–7.60 (m, 2H), 7.34–7.32 (m, 2H), 7.05–6.95 (m, 4H), 5.12–4.97 (m, 6H), 4.28–4.23 (m, 2H), 3.55–3.38 (m, 12H), 3.17–2.97 (m, 6H), 2.31 ppm (br s, 1H); HRMS (MALDI-TOF): m/z calcd for $C_{60}H_{68}N_{10}O_{15}$: 1191.4758; found: 1191.4767 $[M+Na]^+$.

*Compound **25***: Compound **16a** (28 mg, 0.024 mmol) was dissolved in DMF before PEG-400 azide (11.4 mg, 0.029 mmol), copper sulfate (4 mg, 0.024 mmol), and TBTA (13 mg, 0.024 mmol) were added. Subsequently, a few copper turnings were added and the reaction was stirred overnight at RT. The reaction was monitored by HPLC and upon its completion, the mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel ($MeOH/CH_2Cl_2$ 1:9) to give **25** as a white solid (16.7 mg, 44%). HPLC: C18 reverse-phase column; $\lambda = 250$ nm; flow: 1 mL min^{-1}; gradient program: $t = 0$ (30% MeCN/70% H_2O), $t = 20$–25 min (MeCN); $t_R = 16.70$ min (**16a**); $t_R = 15.60$ min (**25**); HRMS (MALDI-TOF): m/z calcd for $C_{76}H_{101}N_{13}O_{23}$: 1586.7026; found: 1586.7300 $[M+Na]^+$.

Dendrimer activation protocol and fluorescence measurements for 6-aminoquinoline: PGA (56 mg mL^{-1}) was purchased from Sigma. Stock solutions of dendrimers **1**, **1a**, and **2** were prepared in DMSO with 20% Cremophor EL to yield 250 mM stock solutions of **1** and **1a**, and a 100 mM stock solution of **2**. The stock solutions (100 mL) were diluted with either 900 mL PBS at pH 7.4 (control) or with 882 mL PBS at pH 7.4 and 18 mL PGA (56 mg mL^{-1}) to give final concentrations of 25 mM of dendrimers **1** and **1a** and 10.0 mM of dendrimer **2**. The final concentration of PGA was 1.0 mg mL^{-1} (14 mM). All solutions were kept at 37 °C and their fluorescence spectra were measured by using a SpectraMax M2 spectrophotometer (Molecular Devices). Standard Costar 96-well plates were



Scheme 8. Synthesis of first-generation dendritic molecule **25**.

used with sample volumes of 150 mL. The spectra were measured by excitation at 250 nm and the emitting fluorescence between 360–660 nm was recorded. The relative fluorescence units (RFU) values at 390 and 460 nm were used for the kinetic analysis of the release of 6-aminoquinoline from the dendrimers.

Acknowledgements

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