Substituent-dependent disassembly of self-immolative dendrimers†‡§

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Self-immolative dendrimers are tree-like platforms, which can spontaneously release all their end-group molecules through a single activation event at the dendrimer's focal point. This triggering event induces domino-like fragmentations, which leads to complete disassembly of the dendrimer into its separate building blocks. In this report, we demonstrate a simple approach to control the disassembly-rate of self-immolative dendrimers. Two types of dendrons were synthesized. One with a methyl substituent on the benzene building-block and the other with ethylcarboxy-ester. The dendrons (first- and second-generation) with electron-withdrawing substituents showed approximately 30 times faster disassembly-rate than dendrons with methyl substituents.

Introduction

The structural precision of dendrimers has motivated numerous studies aimed at biological applications, such as, the amplification of molecular effects or the creation of high concentrations of drugs, molecular labels, or probe moieties.[1–3] However, most of the applications of dendrimers rely mainly on the tail-group functionality. An appropriate dendrimer could be structurally designed to conduct a cleavage signal through the molecular dendritic system, similarly to domino bricks falling on each other. Dendritic architectures 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 dendritic architecture with a signal transduction system. Inspired by the dendritic transduction pathways existing in nature, we focused our research on the development of the concept of self-immolative dendrimers.[4] These novel molecular systems were recently introduced as a platform for the amplification of chemical or biological signals. The dendrimers can release all of their tail units through a domino-like chain fragmentation, which is initiated by a single cleavage at the dendrimer's core (Fig. 1).[4–6]

Self-immolative dendrimers have been applied towards the design and evaluation of novel dendritic prodrug systems.[7] We synthesized a single-triggered hetero-dimeric prodrug with the anticancer agents doxorubicin and camptothecin. For the first time it was possible to release two different chemotherapeutic drugs simultaneously at the same location.[8] We have also designed and synthesized fully biodegradable dendrimers that are disassembled through multi-enzymatic triggering followed by self-immolative chain fragmentation.[9] The model of multi-triggered, self-immolative dendron was recently applied to the synthesis of a prodrug activated through a molecular "OR" logic trigger (a dual-trigger activated by either one of two different enzymes).[10]

Like in other drug-delivery systems, we sought to control the disassembly rate of the dendritic platform. Here we present a new study that describes the design and synthesis of two self-immolative dendritic systems with different disassembly rates. The deceleration or acceleration is achieved through a substituent effect on the aromatic building block.

Results and discussion

Design and synthesis of first- and second-generation dendrons with *p*-nitroaniline

In this study, we chose to evaluate dendrons with *p*-nitroaniline (PNA) end-groups that can be released by chemical activation. As a trigger we used the *tert*-butylcarbamate (Boc) group that is removed under acidic conditions. The disassembly mechanism of our self-immolative dendrons is presented in Scheme 1. When dendron **I** is subjected to the acidic environment of TFA (trifluoroacetic acid), amine **II** is formed. The latter is cyclized to generate phenol **III** and a dimethylurea derivative. The phenol undergoes double quinone–methide rearrangement and decarboxylation to release the two molecules of PNA (compound **IV** is formed after the reactive quinone–methide is trapped with methanol).

Earlier studies[11,12] showed that the cyclization is the rate-limiting step of the disassembly process. Therefore, we rationalized that one could gain control of the disassembly rate by changing the substituent R on the aromatic ring. Electron-withdrawing substituents should accelerate the cyclization step since phenol **III** will become a better leaving group in the acyl-substitution of **II** to **III**. Four dendrons were synthesized (Scheme 2).

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‡ Electronic supplementary information (ESI) available: Dendron activation protocol. Scheme S1: Deprotection of dendrons **1–4** with TFA. Fig. S1: Disappearance of dendrons **1** and **2** after chemical activation. Fig. S2: Disappearance of dendrons **3** and **4** after chemical activation. See DOI: 10.1039/b615762a

§ The HTML version of this article has been enhanced with colour images.

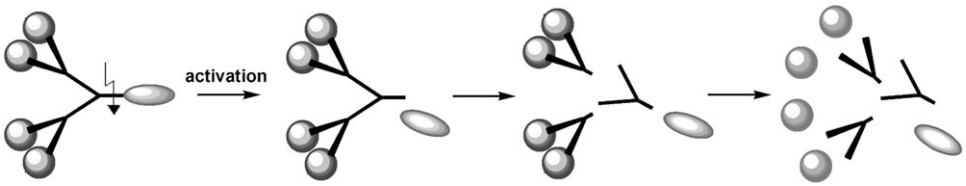


Fig. 1 Self-immolative dendrimers, as shown in the picture, spontaneously release all the end-group molecules following a single activation event. This triggering event induces domino-like fragmentations, which leads to a complete disassembly of the dendrimer into its separate building blocks.

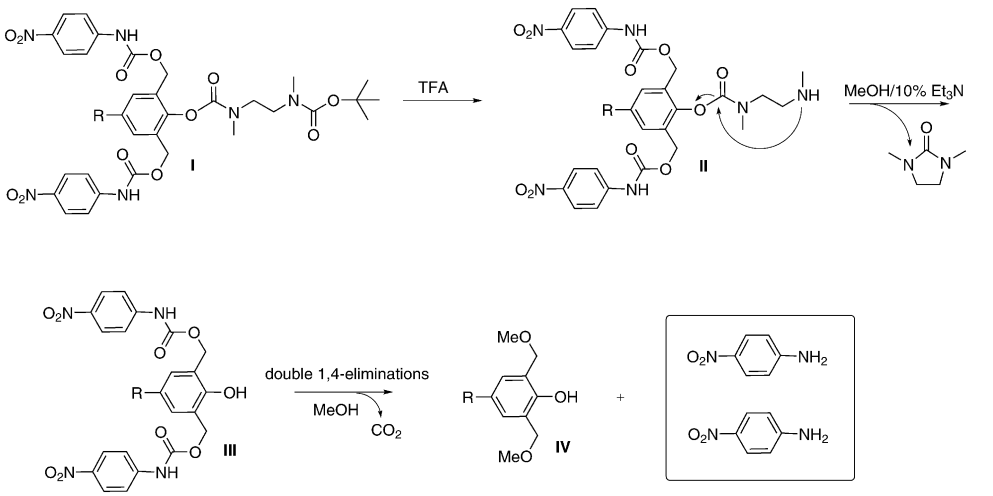
Two dendrons (**1** and **3**) with a methyl substituent and two others with an ethylcarboxy-ester substituent (**2** and **4**) were prepared. All dendrons had the same end-groups (PNA) and activating triggers (Boc).

Synthesis. Dendrons **1** and **3** were synthesized as published before.[4] First-generation dendron **2** was synthesized according to Scheme 3. The core unit **5**[7] was first treated with *tert*-butyldimethylsilyl chloride (TBSCl) to afford phenol **6**,[7] which was acylated with *p*-nitrophenyl chloroformate to give carbonate **7**. Reaction of **7** with mono-Boc-protected *N*,*N*′-dimethylethylenediamine (Boc = *tert*-butyloxycarbonyl) generated compound **8**, which was deprotected in the presence of Amberlyst-15 to give diol **9**. Two equivalents of *p*-nitroaniline were treated with phosgene generating an isocyanate derivative, which conjugated to diol **9**, yielding first-generation dendron **2**.

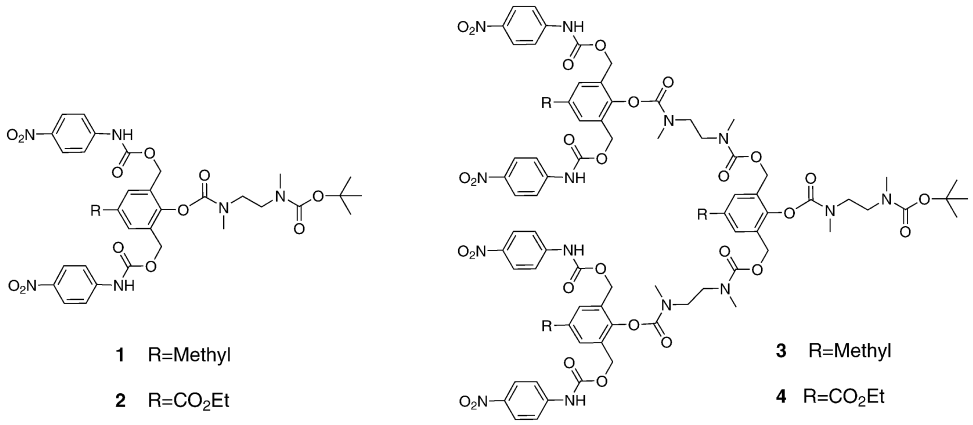
The synthesis of second-generation dendron **4** is shown in Scheme 4. Acylation of diol **9** with two equivalents of *p*-nitrophenyl chloroformate afforded dicarbonate **10**. Two equivalents of dendron **2** were deprotected with TFA to afford the salt of amine **11**, which treated *in situ* with compound **10** to give dendron **4** (the intramolecular cyclization of amine **11** to give a five-membered cyclic urea derivative is slower than cross-linked reaction with dicarbonate **11**).

Chemical activation of dendritic molecules 1–4. The dendrons were initially incubated with TFA to afford their amine salts, which dissolved in dimethyl sulfoxide (DMSO). Then, the solutions were diluted into methanol with 10% triethylamine. The sequential fragmentation of compounds **1–4** was monitored by reverse-phase analytical HPLC, using a C-18 analytical column, at a wavelength of 348 nm. The release of PNA from dendrons **1** and **2** is presented in Fig. 2. The disassembly of dendron **2** occurred within a little more than 1 h, while 25 h were needed to complete the disassembly of dendron **1**. Similarly, Fig. 3 shows that second-generation dendron **4** disassembled much faster than dendron **3**; 50 h for dendron **3** *cf*. 3 h for dendron **4** (the disassembly rate was quantified based on the peak area of the HPLC chromotograms). The data were normalized to percentage units where the sum of all peaks is equal to 100%. We observed 100% conversion of the starting material to PNA).

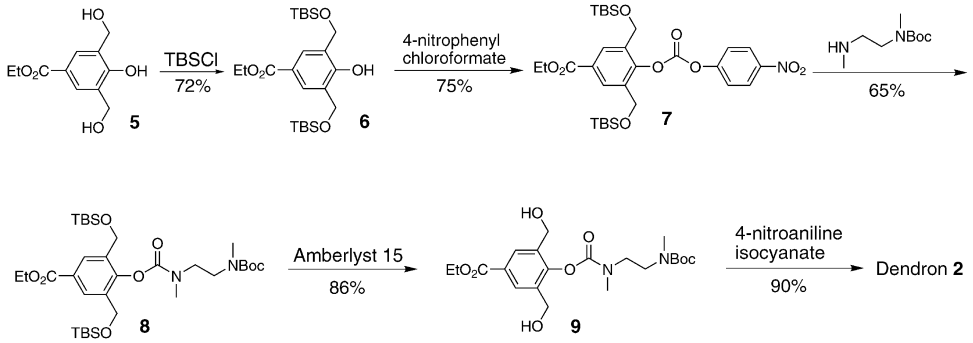
Kinetic studies. The dendritic platform is designed to disassemble upon triggering, through a process of self-immolative chain fragmentation, based on cyclization and elimination reactions. We have previously reported a kinetic model that explains the release mechanism from dendrons with a methyl substituent.[13] The model showed that the amine cyclization is the rate-limiting step in the overall release pathway.[4] In order to provide a quantitative evidence for the increase of disassembly rate of dendrons with a carboxy-ester substituent, we calculated the rate constants of the first cyclization step (**II** → **III**, Scheme 1) for both types of dendrons. Since the disassembly of amine intermediate **II**



Scheme 1 Disassembly mechanism of a first-generation self-immolative dendron.



Scheme 2 Chemical structures of first- and second-generation self-immolative dendrons.



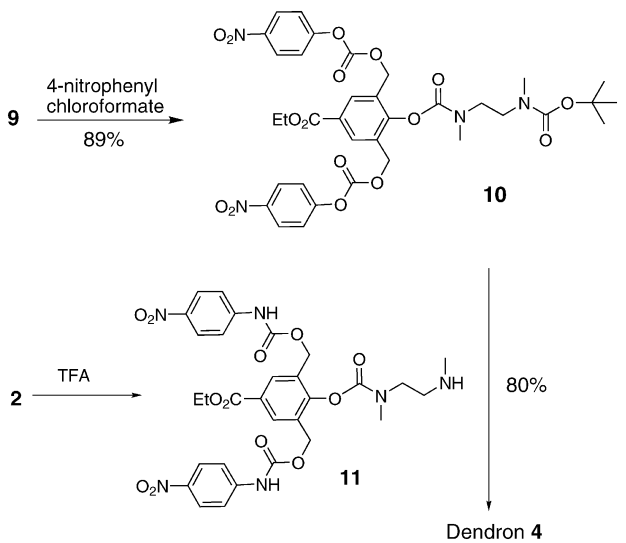
Scheme 3 Synthesis of first-generation self-immolative dendron **2**.

(Scheme 1) to give a phenol and a urea derivative is a first-order reaction, the equation for the disassembly is described as

$$\partial/\partial t\,[\text{Dendron}] = -k_n[\text{Dendron}]_{(t)} \qquad (1)$$

where k_n (n is the generation number) is the cyclization rate constant. The solution of eqn (1) is given by

$$[\text{Dendron}]_{(t)} = [\text{Dendron}]_{(t=0)}\exp(-k_n t) \qquad (2)$$



Scheme 4 Synthesis of second-generation self-immolative dendron **4**.

Therefore, a plot of the natural logarithm of the dendron concentration ($[\text{Dendron}]_{(t)}$), as a function of time (t) should present a good correlation with a straight line ($y = ax + b$), as derived from eqn (3):

$$\ln[\text{Dendron}]_{(t)} = k_n t + \ln[\text{Dendron}]_{(t=0)} \qquad (3)$$

The results for the first- and second-generation dendrons are presented in Fig. 4 and 5, respectively. Excellent correlations were found for all four structures. The cyclization rate constants for the first- and second-generation dendrons **1** and **3**

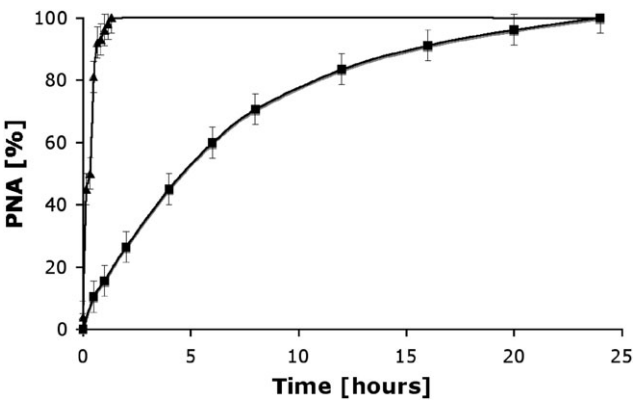


Fig. 2 Release of PNA from dendrons **1** (▲) and **2** (■) after chemical activation. Starting concentration: 500 μM; λ = 348 nm; 90% MeOH–10% Et_3N; room temperature.

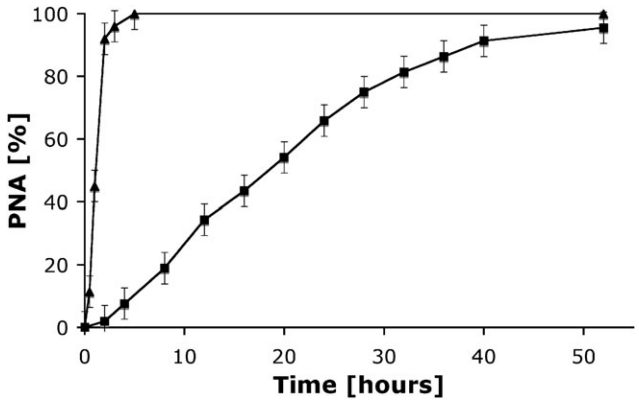


Fig. 3 Release of PNA from dendrons **3** (▲) and **4** (■) after chemical activation. Starting concentration: 500 μM; λ = 348 nm; 90% MeOH–10% Et_3N; room temperature.

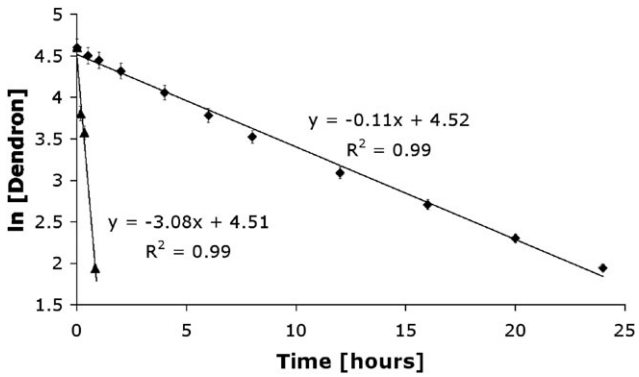


Fig. 4 First-order kinetic plots for the disassembly of first-generation self-immolative dendrons **1** (◆) and **2** (▲).

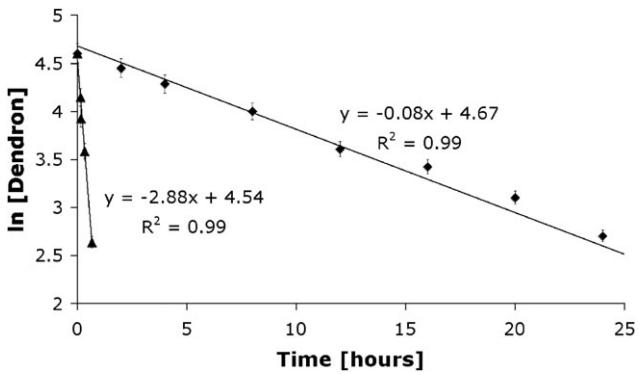


Fig. 5 First-order kinetic plots for the disassembly of second-generation self-immolative dendrons **3** (◆) and **4** (▲).

were similar (0.110 ± 0.002 and 0.089 ± 0.002 h^{-1}, respectively) and in good agreement with our previously reported results. The same phenomenon was observed for dendrons **2** and **4** (k_n = 3.08 ± 0.06 and 2.88 ± 0.06 h^{-1}, respectively).

The acceleration of disassembly of dendrons **2** and **4** can be explained by the stabilization of the phenolate species, which is released upon the intra-cyclization of amine intermediate **II** (Fig. 2). While the ester-substituent (an electron-withdrawing group) oriented *para* to the phenol, stabilizes the negative charge on the phenolic-oxygen, the methyl substituent destabilizes it through an electron-donating inductive effect. The kinetic measurements indicate that dendrons **2** and **4** disassembled approximately 30 times faster than dendrons **1** and **3**. This result is further supported by the pK_a values difference of cresol and 4-hydroxybenzoic acid, ethyl ester (10.26 and 8.34, respectively).[14] The increase of the disassembly rate by one and a half orders of magnitude is proportional to difference in the pK_a values.

Conclusions

In summary, we have demonstrated a simple approach to control the disassembly-rate of self-immolative dendrimers. Two types of dendrons were synthesized; one with a methyl substituent on the benzene building-block and the other with ethylcarboxy-ester. The dendrons (first- and second-generation) with electron-withdrawing substituents showed faster disassembly rate than dendrons with methyl substituents. Gaining control of self-immolative dendrimer disassembly could be especially applicable in drug-delivery systems, when a specific release-rate is needed to control the dose of an active drug.

Experimental

General

All reactions requiring anhydrous conditions were performed under an Ar or N_2 atmosphere. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): 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 (20% wt. in ethanol), followed by heating. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040–0.063 mm), eluent given in parentheses. ^{1}H NMR: Bruker AMX 200 or 400 instrument. The chemical shifts are expressed in δ relative to TMS (δ = 0 ppm) and the coupling constants J in Hz. The spectra were recorded in $CDCl_3$ or CD_3OD as a solvent at room temperature. All reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Syntheses

Compound 7. Compound **6**[7] (1.62 g, 3.56 mmol) was dissolved in 50 ml of THF, Et_3N (1.7 ml, 12.4 mmol) and a catalytic amount of DMAP (5 mg) were added. The reaction was cooled to 0 °C, and a solution of 4-nitrophenyl chloroformate (1.07 g, 5.34 mmol) in 20 ml THF was added dropwise. The reaction was stirred in room temperature and monitored by TLC (EtOAc–hexane 10 : 90). After 3 h the reaction was complete and diluted with EtOAc and washed with saturated NH_4Cl solution. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc–hexane = 1 : 19) to give compound **7** (1.65 g, 75%) as a colorless oil.

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.32 (2H, d, J = 9.2); 8.14 (2H, s); 7.48 (2H, d, J = 9.2); 4.78 (4H, s); 4.38 (2H, q,

J = 7.1); 1.37 (3H, t, J = 7.1); 0.94 (18H, s); 0.09 (12H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 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. HRMS (MALDI-TOF): calc. for $C_{30}H_{45}NO_9Si_2$ 642.2525 [M + Na^+], found 642.2482.

Compound 8. Compound **7** (1.17 g, 1.92 mmol) was dissolved in 30 ml DMF and mono-Boc-protected *N*,*N*′-dimethylethylenediamine (543 mg, 2.88 mmol) was added. The reaction was stirred in room temperature and was monitored by TLC (EtOAc–hexane = 1 : 3). After 1 h 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–hexane = 15 : 85) to give compound **8** (835 mg, 65%) as a viscous oil.[1]

^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10 (2H, s); 4.64 (4H, s); 4.32 (2H, q, J = 7.0); 3.55–3.42 (4H, m); 3.12–3.00 (3H, m); 2.92–2.89 (3H, m); 1.53–1.45 (9H, m); 1.38 (3H, t, J = 7.0); 0.9 (18H, s); 0.07 (12H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ 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. HRMS (MALDI-TOF): calc. for $C_{33}H_{60}N_2O_8Si_2$ 691.3780 [M + Na^+], found 691.374.

Compound 9. Compound **8** (800 mg, 1.19 mmol) was dissolved in 10 ml MeOH and Amberlyst-15 was added. The reaction was stirred in room temperature for 2 h and was monitored by TLC (EtOAc–hexane = 3 : 1). After completion, the Amberlyst-15 was filtered out and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc–hexane = 9 : 1) to give compound **9** (455 mg, 86%) as a white powder.

Mp 100 °C (decomp.). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08 (2H, s); 4.61 (4H, s); 4.36 (2H, q, J = 7.1); 3.7–3.4 (4H, m); 3.17 (3H, s); 3.04 (3H, s); 2.94 (3H, s); 1.48–1.42 (9H, m); 1.35 (3H, t, J = 7.1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 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. HRMS (MALDI-TOF): calc. for $C_{21}H_{32}N_2O_8$ 463.2051 [M + Na^+], found 463.2087.

Dendron 2. *p*-Nitroaniline (342 mg, 2.48 mmol) was dissolved in 20 ml of dry THF and a solution of 20% phosgene in toluene (2.6 ml, 4.956 mmol) was added. The reaction was refluxed for 2 h and monitored by ^{1}H NMR (200 MHz, DMSO). After the isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **9** (364 mg, 0.826 mmol) in 10 ml THF followed by Et_3N (0.42 ml, 3 mmol) was added to the residue. The reaction was stirred in room temperature and was monitored by TLC (EtOAc–hexane = 1 : 1). After 1 h the reaction was completed and the solvent was removed under reduced pressure. The crude product was diluted with DCM, salts were filtered out and the crude was washed again with DCM. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc–hexane = 2 : 3) to give compound **2** (573 mg, 90%) as a yellow powder.

Mp 100 °C (decomp.). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.16 (4H, d, J = 8.2); 7.66 (2H, s); 7.56 (4H, d, J = 8.2); 5.16 (4H, s); 4.40 (2H, q, J = 6.8); 3.7–3.3 (4H, m); 3.2 (3H, s); 2.94 (3H, s); 1.41 (9H, s); 1.27 (3H, t, J = 6.8). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 158.2, 154.8, 146.5, 144.6, 135.1, 133.2, 131.6, 126.9, 119.6, 82.7, 63.7, 63.4, 49.1, 48.5, 38.2, 37.5, 30.4, 16.247. HRMS (MALDI-TOF): calc. for $C_{35}H_{40}N_6O_{14}$ 791.2494 [M + Na]$^+$, found 791.2470.

Compound 10. Compound **9** (103 mg, 0.234 mmol) was dissolved in 10 ml dry THF and the solution was cooled to 0 °C. Then DIPEA (0.33 ml, 1.872 mmol), was added followed by PNP-chloroformate (283 mg, 1.4 mmol) and a catalytic amount of pyridine. The reaction was stirred in room temperature and monitored by TLC (EtOAc–hexane = 1 : 1). After 15 min the reaction was completed and the solvent was removed under reduced pressure. The crude product was diluted with EtOAc and washed with saturated NH_4Cl and with saturated $NaHCO_3$ solutions. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc–hexane = 1 : 1) to give compound **10** (160 mg, 89%) as a white solid.

Mp 100 °C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24–8.20 (6H, m); 7.36 (4H, d, J = 7.0); 5.31 (4H, s); 4.38 (2H, q, J = 7.0); 3.60–3.45 (4H, m); 3.20–3.02 (3H, m); 2.94–2.85 (3H, m); 1.43–1.41 (9H, m); 1.38 (3H, t, J = 7.0). ^{13}C NMR (100 MHz, $CDCl_3$): δ 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. HRMS (MALDI-TOF): calc. for $C_{35}H_{38}N_4O_{16}$ 793.2175 [M + Na^+], found 793.2148.

Dendron 4. Compound **2** (250 mg, 0.32 mmol) was dissolved in 1 ml TFA and stirred for a few minutes, the excess of acid was removed under reduced pressure and the crude amine salt was dissolved in 2 ml DMF. Compound **10** (100 mg, 0.13 mmol) was added followed by the addition of 1 ml Et_3N. The reaction mixture was stirred in room temperature and monitored by TLC (EtOAc–hexane = 75 : 25). After 2 h the reaction was completed and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc–hexane = 70 : 30) to give compound **4** (189 mg, 80%) as a yellowish powder.

Mp 100 °C (decomp.). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.20–8.06 (14H, m); 7.48 (6H, m); 5.13 (12H, m); 4.38 (6H, m); 3.61–2.61 (30H, m); 1.40 (9H, m); 1.31–1.23 (9H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.13, 154.75, 146.68, 144.49, 131.9, 131.69, 130.39, 126.84, 119.59, 64.15, 63.43, 48.67, 46.94, 36.75, 33.35, 30.19, 22.94. HRMS (MALDI-TOF): calc. for $C_{83}H_{92}N_{14}O_{34}$ 1851.5792 [M + Na]$^+$, found 1851.5638.

Dendron activation protocol

Dendrons **1–4** were deprotected with trifluoroacetic acid (TFA) and the corresponding salts were used for the preparation of 10.0 mM stock solutions in DMSO. The stock solutions were diluted with MeOH and 10% triethylamine to give final concentrations of 500 μM of dendrons **1–4**. The release of *p*-nitroaniline from the dendron stock solutions was monitored by an HPLC assay using C-18 reverse-phase analytical

column; λ = 348 nm; flow: 1 mL min^{-1}; eluent: MeCN–H_2O; gradient program: t = 0 (30% MeCN–70% H_2O), t = 20 (100% MeCN); t_R = 12.22 min (TFA salt of **1**); t_R = 12.16 min (TFA salt of **2**); t_R = 18.34 min (TFA salt of **3**); t_R = 18.42 min (TFA salt of **4**); t_R = 8.67 min (*p*-nitroaniline). The relative peak areas (in %) were used for the kinetic analysis.

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