

Synthesis and degradation of photolabile dendrimers based on *o*-nitrobenzyl ether photolabile cores†

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Two dendrimer cores, **1a** and **2a**, that contain *o*-nitrobenzyl photolabile moieties, lack hydrolytically sensitive ester linkages and possess three and six sites for dendron attachment, respectively, have been alkylated to provide methylated core analogs **1b** and **2b** as well as second-generation benzylaryl ether dendrimer **1c** and third-generation dendrimer **2c**. These dendrimers undergo clean photocleavage as indicated by the evolution of isosbestic points in the UV spectra during photolysis. In addition, the nature of the photodegradation products was confirmed by observing the photolyses by both ¹H NMR and GPC.

Introduction

The ability to degrade dendrimers^{1–5} in a controlled fashion has the potential to impact previously established uses of dendrimers, as well as introduce new paradigms for their roles in chemical systems.^{6–8} Photolabile dendrimers^{9–14} allow the alteration of the nature of a discrete macromolecular system by a non-invasive stimulus, which may have relevance to biological applications.¹⁵ We reported the first examples of photolabile dendrimers that contained specific, photodegradable *o*-nitrobenzyl-based linkages^{16–21} in the interior and demonstrated their fragmentation upon irradiation with ultraviolet light.⁹ However, these dendrimers possessed hydrolytically sensitive ester linkages at the core, and their syntheses, although convergent, involved manipulations on the photolabile moieties prior to the final dendron attachment to the core. We sought to incorporate the photolabile linkage into a core moiety onto which we could attach separately prepared dendrons of varying generation and exterior functionality, thereby minimizing reaction chemistry on the photosensitive units. Accordingly, cores **1a** and **2a** serve this need, as well as meet several design criteria, including containing (a) the well-studied *o*-nitrobenzyl group;¹⁷ (b) several sites (three and six, respectively) for attachment of dendrons; and (c) no hydrolytically sensitive ester linkages.¹⁰ Herein is described the synthesis and degradation of photolabile dendrimers prepared from these cores.

Results and discussion

Synthesis of photolabile dendrimers

Photolabile cores **1a** and **2a** (Scheme 1) were both prepared from readily available piperonal in four and five steps, respectively.¹⁰ Core **1a** exists as a 1 : 3 mixture of homochiral :

heterochiral diastereomers, detectable by NMR but not separable by flash column chromatography, a consequence of the racemic nature of the chiral starting material. Alkylation of core **1a** afforded photolabile dendrimers **1b** and **1c**. Compound **1b** was synthesized by the reaction of photolabile core **1a** with methyl iodide in the presence of K₂CO₃ (Scheme 1). Second-generation dendrimer **1c** was synthesized by treating trifunctional photolabile core **1a** with second-generation benzylaryl ether bromide [G-2]Br²² under similar conditions (Chart 1).

Hexafunctional core **2a** is, in a sense, a design improvement over trifunctional core **1a**. Replacing the methyl group with a second nitrophenyl ring serves three purposes. First, it introduces enhanced photocleavage due to the presence of an additional nitro group.²³ Second, it increases dendritic branching by doubling the number of attachment points. Third, it eliminates the presence of stereoisomers since there are no longer chiral centers in the structure. Two hexafunctional photolabile dendrimers, **2b** and **2c** were synthesized by alkylation of core **2a**. Hexafunctional core **2a** was treated with methyl iodide in the presence of K₂CO₃ and 18-crown-6 to afford first-generation dendrimer **2b** (Scheme 1). Treatment of hexafunctional core **2a** with second-generation benzylaryl ether bromide [G-2]-Br²² under similar conditions provided nominally third-generation dendrimer **2c** (Chart 1).

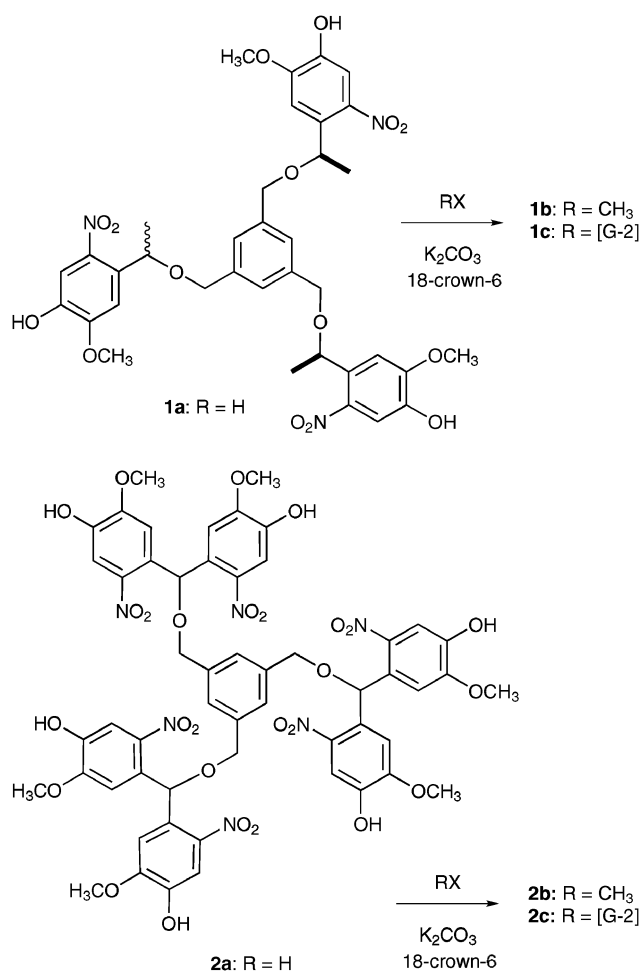
All dendrimers (**1b**, **1c**, **2b** and **2c**) were thermally stable (acetone, 55 °C, 12 h; solid state, rt, three months) and were readily characterized by ¹H and ¹³C NMR, mass spectrometry and elemental analysis.

Degradation of photolabile dendrimers

Photodegradation of dendrimers **1b**, **1c**, **2b** and **2c** occurred readily upon irradiation with UV light. Photolyses were carried out at the λ_{max} of each system, 340–345 nm, with a bathochromic shift in the λ_{max} of the system during photolysis. The degradation of each compound was monitored by UV-Vis spectroscopy and ¹H NMR spectroscopy to assist in elucidation of the photolytic pathway. In the case of the larger compounds **1c** and **2c**, photodegradation was also monitored by gel permeation chromatography (GPC). The photodegradation of all dendrimers was consistent with generation of the

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Scheme 1 Synthesis of photolabile dendrimers from cores **1a** and **1b**.

respective nitroso ketones and the 1,3,5-tris(hydroxymethyl)-benzene core (**3**) (Scheme 2).

UV-Visible spectrophotometry

Compound **1b** was photolyzed at 345 nm in chloroform solution (12 μ M) and monitored by UV-Vis spectroscopy (Fig. 1(a)). Photodegradation was carried out at ambient temperature and there was no noticeable temperature change during the reaction. The evolution of isosbestic points at 280 and 322 nm suggested a clean transformation into photoproduct **1d** according to Scheme 2. The photolysis of **1b** in THF (12 μ M) exhibited a similar spectral evolution (not shown).

Second-generation dendrimer **1c** was photolyzed in chloroform (12 μ M, 343 nm) and monitored by UV-Vis spectroscopy (Fig. 1(b)). The absorbance spectrum of **1c** contained the same bands as that of **1b** with the addition of the benzylaryl ether dendron band at *ca.* 285 nm. The evolution of this band was minor, as would be expected, while the chromophore at 343 nm underwent the bathochromic shift characteristic of *o*-nitrobenzyl degradation. Isosbestic points at 256 and 327 nm was evidence of clean photodegradation into photoproducts **1e** according to Scheme 2. The photolysis of **1c** in THF (12 μ M) exhibited a similar spectral evolution in the UV-Vis (not shown).

Dendrimers **2b** and **2c** were similarly irradiated separately in chloroform and the degradations were followed by UV-Vis spectroscopy (Fig. 1(c) and (d)). Isosbestic points were again evident for both **2b** (257, 268 and 354 nm) and **2c** (258, 269 and 352 nm). The photodegradation of dendrimers **2b** and **2c** should result in release of nitronitroso benzophenones **2d** and **2e**, respectively, and the corresponding core **3** (Scheme 2, bottom). The photolysis of both compounds in THF (12 μ M) again exhibited similar spectral evolution with isosbestic points in the UV-Vis (not shown).

The logical existence of two intermediates in the stepwise degradation of **1b**, **1c**, **2b** and **2c** (*i.e.*, after one and two equivalents of their respective photoproducts **1d**, **1e**, **2d**, or **2e** have been cleaved from the core) and the appearance of isosbestic points, implies that these systems may be considered “pseudo-two-component” or a depolymerization process (*i.e.*, $P_n \rightarrow nM$) where polymer subunits and monomer units are distinct chromophores.^{9,10,24}

¹H NMR

Supporting evidence for the identity of the photocleavage products was obtained by following the photolyses by ¹H NMR in CDCl₃ (Fig. 2). Photolysis of **1b** was accompanied by the decrease in intensity of the methoxy singlets (δ 3.92–3.96) corresponding to the six diastereomerically related methoxy groups of **1b**⁹ and the evolution of two new methoxy peaks (δ 4.08 and 3.88) corresponding to **1d**.[‡] The correlation between disappearance of **1b** and appearance of **1d** can be seen readily in terms of percentage conversion as measured by NMR integration (Fig. 3).

In comparing the ¹H NMR of the photolysis of **1b** with that of second-generation **1c** (Fig. 2(b)), the replacement of the methyl *meta* to the nitro group in **1b** with a benzylaryl ether dendron in **1c** is readily apparent. The three (rather than six) diastereomerically related methoxy groups of **1c** appear as three singlets in a 1 : 2 : 1 ratio, consistent with a statistical mixture of 1 : 3 homochiral/heterochiral diastereomers, at 3.87–3.89 ppm. Photodegradation was accompanied by the decrease in intensity of these peaks and the evolution of the methoxy peak corresponding to photoproduct **1e** at 4.03 ppm, with a good correlation according to integration data (Fig. 3). The apparent doublet at 3.90 ppm and singlet at 3.93 ppm are attributed to partially degraded dendrimer with one and two equivalents of **1e** removed from the core, respectively.⁹ These peaks were not present after complete photodegradation.

The photocleavage of **2b** was followed by ¹H NMR (Fig. 2(c)) in CDCl₃ and indicated the disappearance of both methoxy peaks (δ 3.96 and 3.76) of **2b** and the appearance of four methoxy peaks (δ 4.16, 3.95, 3.94 and 3.85) consistent with the structure of the unsymmetrical benzophenone **2d**. The appearance of several other peaks throughout the ¹H NMR during the photolysis is attributed to photolytic intermediates during the reaction, as these peaks disappear upon complete photolysis.

[‡] The identity of **1d** was verified by comparison to the ¹H NMR spectrum, provided by Dr C. P. Holmes of Affymax Research Institute, Palo Alto, CA, of an authentic sample.

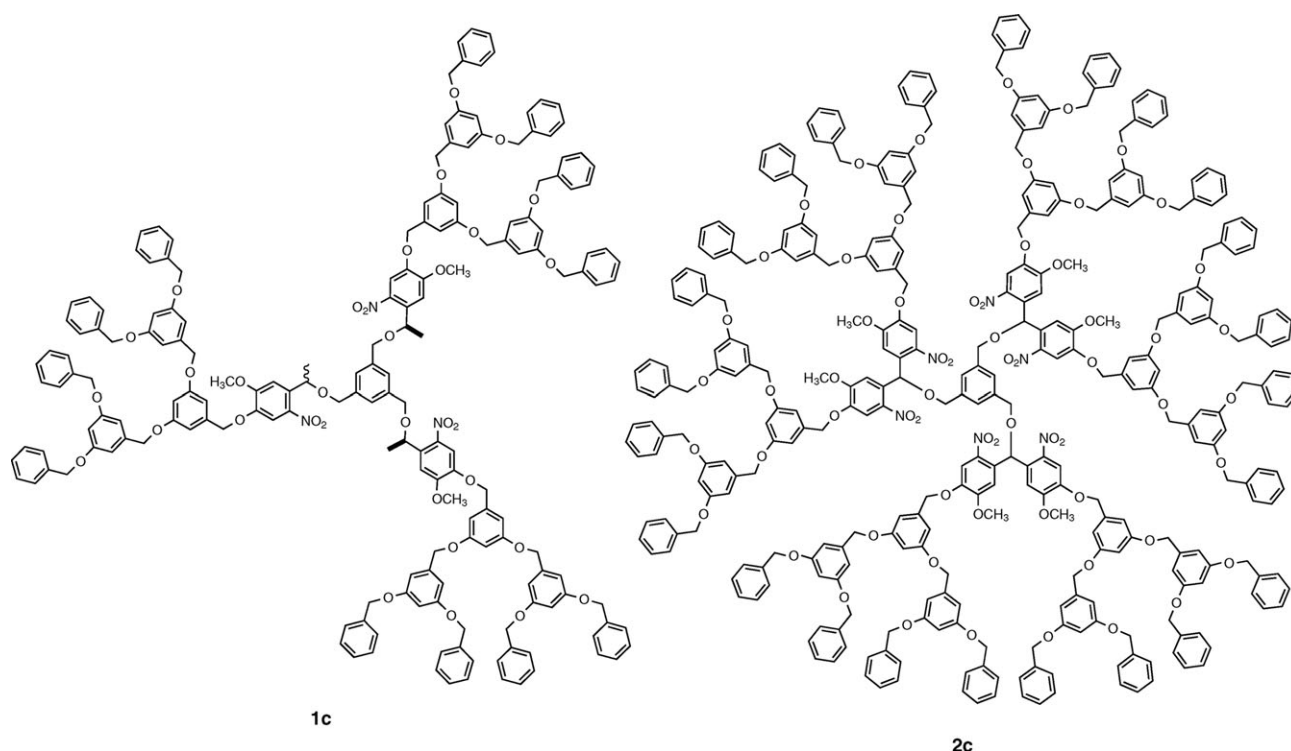
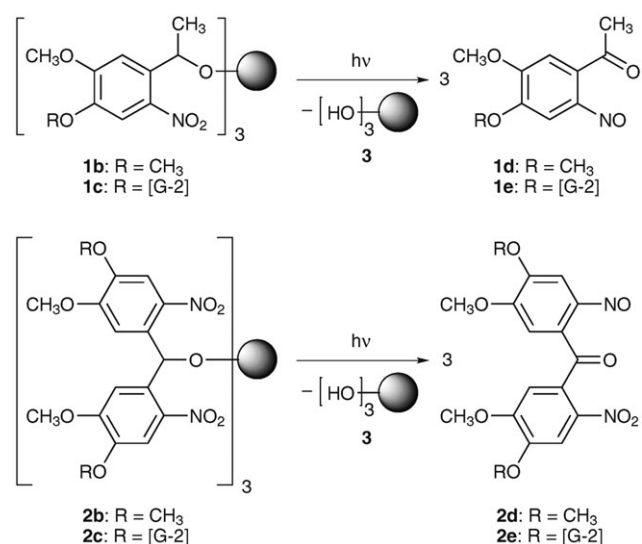


Chart 1 Structural formulas of second-generation dendrimer **1c** and third-generation dendrimer **2c**.

Photocleavage of **2c** was similarly monitored by following the photolysis by ^1H NMR in CDCl_3 (Fig. 2(d)) and a comparison with the data from the photocleavage of **2b** reflects the replacement of two methoxy groups with a benzyl aryl ether dendron. The single methoxy peak corresponding to dendrimer **2c** at 3.64 ppm decreased in intensity with concomitant evolution of two chemical shift nonequivalent methoxy peaks corresponding to photoproduct **2e** at 4.09 and 3.87 ppm. Photolytic intermediates are observed in this case as well, largely between 3.8–3.9 ppm, that subside upon complete photodegradation.



Scheme 2 Proposed degradation products from the photolysis of dendrimers **1b**, **1c**, **2b** and **2c**.

Gel permeation chromatography

Evidence for the existence of the photolytic intermediates observed in the NMR experiments was obtained by monitoring the photolyses by GPC. A solution of dendrimer **1c** in CH_2Cl_2 was photolyzed in intervals and run through a GPC column (Fig. 4). Initially, the only species present is dendrimer **1c**. Upon photolysis, it was evident that the high molecular

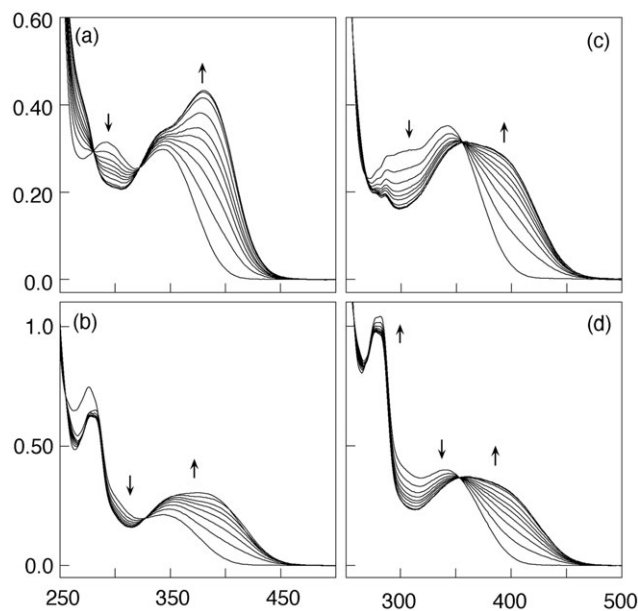


Fig. 1 UV-Vis spectra of the photolysis of 12 μM solutions (CHCl_3) of (a) **1b**, (b) **1c**, (c) **2b** and (d) **2c** at $t = 0, 2, 4, 6, 8, 10, 15, 25, 35, 45$ min.

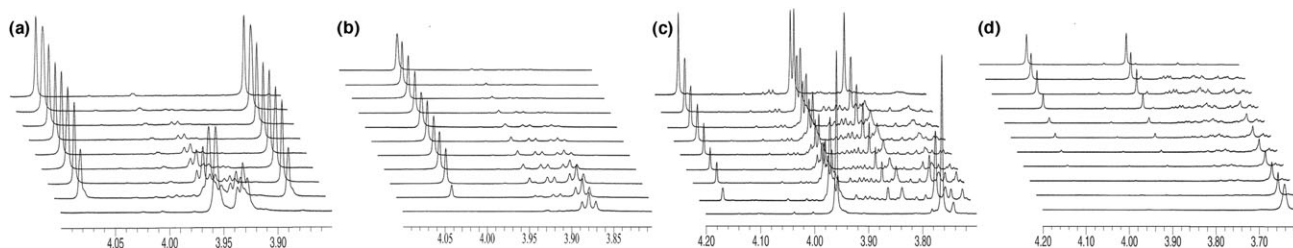


Fig. 2 ^1H NMR spectra of the photolysis of 5 mM solutions (CDCl_3) of (a) **1b**, (b) **1c**, (c) **2b** and (d) **2c** at $t = 0, 10, 20, 30, 40, 50, 60, 90, 120$ (**1c** and **2c** only), 150 (**1c** and **2c** only), 180 min (proceeding upwards).

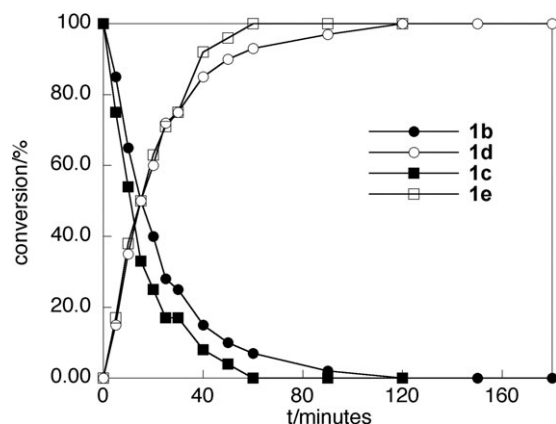


Fig. 3 Conversion of **1b** to **1d** and **1c** to **1e** as monitored by integration of the appropriate peaks in the ^1H NMR (CDCl_3) spectra (**1b**: 3.97–3.91 ppm; **1d**: sum of 4.09–4.06 and 3.90–3.87 ppm; **1c**: 3.90–3.86 ppm; **1e**: 4.05–4.02 ppm; TMS peak used for integration reference).

weight dendrimer degrades into smaller molecular weight components (Scheme 3). Photodegradable dendrimer **1c** (29.18 min) degraded by loss of one dendron to give a two-arm dendrimer corresponding to **B** in Scheme 3 (30.32 min) as well as dendritic fragment **1e** (32.72 min). Subsequently, **B** degraded by further loss of dendron to give a one-arm dendrimer corresponding to **C** (33.70 min) and release of another equivalent of fragment **1e**. Finally, photolysis was complete when dendrimer **C** released the final equivalent of fragment **1e** as well as core **3**. Core **3** was not seen in the GPC trace.

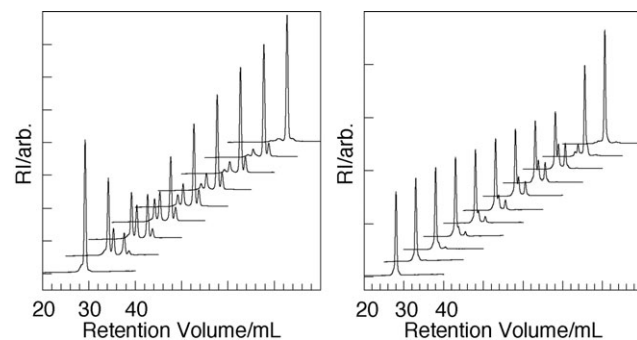
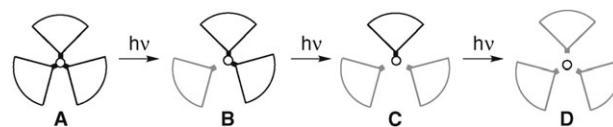


Fig. 4 GPC in CH_2Cl_2 of conversion of (left) **1c** to **1e** ($t = 0, 5, 10, 15, 20, 25, 30, 35, 170$ min) and (right) **2c** to **2e** ($t = 0, 5, 10, 15, 20, 25, 30, 35, 40, 90, 140$ min).



Scheme 3 Schematic representation of the sequential photodegradation of a three-arm dendrimer (**A**) into a two-arm dendrimer (**B**), a one-arm dendrimer (**C**) and full degradation (**D**).

Photolytic degradation **2c** in CH_2Cl_2 was also monitored by GPC. Conversion into smaller molecular weight components is also evident (Fig. 4). Dendrimer **2c** (28.03 min) degraded by loss of one dendron to give two-arm dendrimer **B** (28.03 min) as well as dendritic fragment **2e** (30.57 min) (Scheme 2). Dendrimer **2c** and partially intact dendrimer **B** appear to coelute in the GPC because complete photolysis results in the disappearance of the peak at 28.03 min and no other peaks in the GPC can be attributed to **B**. Subsequently, **B** degraded by further loss of dendron to give one-arm dendrimer **C** (28.84 min) and release of another equivalent of fragment **2e**. Finally, photolysis was complete when dendrimer **C** underwent further photolysis to release the final equivalent of fragment **2e** as well as core **3**. Core **3** was not seen in the GPC.

Conclusions

In conclusion, we have presented the synthesis and demonstrated the photodegradation of photolabile dendrimers based on cores that contain *o*-nitrobenzyl photolabile moieties, lack hydrolytically sensitive ester linkages and possess three and six sites for dendron attachment, respectively. A combination of UV-Vis, NMR and GPC studies established the clean character of the photocleavage, the identity of the photolysis products and the sequential nature of the photodegradation.

Experimental

General

NMR and mass spectra (MS) were obtained using commercially available instrumentation. Tetrahydrofuran (THF) was distilled under N_2 from potassium-benzophenone ketyl. Acetone, DMF, DMSO and methanol were dried over crushed 3 Å molecular sieves. Potassium carbonate (granular, J. T. Baker) was dried at 100°C at reduced pressure and stored in a vacuum oven. Compounds **1a**, **1b**, **2a** and **2b** were synthesized as previously.¹⁰ Compound [G-2]Br was prepared according to

the literature.²⁵ All other needed reagents were purchased from commercial suppliers and used as received. Flash chromatography was performed by the method of Still *et al.*²⁶ using silica gel (32–63 μm , Scientific Adsorbants, Inc., Atlanta GA). Thin-layer chromatography (TLC) was performed on precoated plates (Silica Gel HLO, F-254, Scientific Adsorbants, Inc.).

Photolysis procedure

UV spectra were recorded in a quartz cuvette in distilled chloroform or spectral grade THF at concentrations that afforded absorbances of less than 5. Irradiation for UV-Vis experiments was carried out in a quartz cuvette using a Photon Technology International Xenon arc lamp at the appropriate λ_{max} (~340–345 nm) and an irradiation (inlet and outlet) slit width of 10 nm. Photolyses monitored by ^1H NMR were irradiated in a Kontes Glass Company 5 mm \times 8 NMR tube in a Rayonet with mercury lamps (350 nm irradiation). Photolyses monitored by GPC were carried out in glass scintillation vials with a mercury pen lamp (365 nm irradiation). Throughout the course of photolyses no noticeable temperature change was observed.

Syntheses

1,3,5-Tris((1-(4-(benzyloxy)-5-methoxy-2-nitrophenyl)ethoxy)methyl)benzene (1c). To a solution of **1a** (259 mg, 0.34 mmol) in dry acetone (15 mL) was added K_2CO_3 (436 mg, 3.16 mmol), 18-crown-6 (55 mg, 0.21 mmol) and $[\text{G}-2]\text{Br}$ (830 mg, 1.03 mmol). The mixture was allowed to reflux in the absence of light for 6 days. The mixture was removed from heat and concentrated. The dark red solid was dissolved in CH_2Cl_2 (15 mL) and washed with water (3×15 mL). The organic layer was dried over MgSO_4 , filtered, concentrated and purified by column chromatography (mobile phase 4 : 5 : 1 CH_2Cl_2 –hexanes– Et_2O) to yield **1c** (0.624 g, 62%) as a bright yellow foam: UV-Vis: λ_{max} 344 nm, ϵ $1.58 \times 10^4 \text{ L cm}^{-1} \text{ mol}^{-1}$; ^1H NMR (CDCl_3): δ 7.62 (s, 3 H), 7.32 (m, 20 H), 7.13 (s, 3 H), 6.66 (m, 6 H), 6.55 (m, 3 H), 5.28 (d, $J = 6.1$ Hz, 3 H), 5.22 (s, 2 H), 5.00 (s, 8 H), 4.94 (s, 4 H), 4.29 (dd, $J = 11.8$ Hz, 6 H), 3.88 (t, $J = 3.9$ Hz, 9 H), 1.53 (d, $J = 6.1$ Hz, 9 H); ^{13}C NMR (CDCl_3): δ 160.1, 154.4, 146.7, 140.3, 139.1, 138.1, 136.7, 135.6, 128.5, 128.0, 127.5, 109.7, 108.9, 106.5, 106.3, 101.7, 101.5, 73.3, 71.1, 70.9, 70.0, 56.4, 23.5; MS (MALDI in dithranol) m/z 2956 ($\text{M} + \text{Na}$, $\text{C}_{183}\text{H}_{165}\text{N}_3\text{O}_{33}\text{Na}$ requires 2955.12). Anal. Calc. for $\text{C}_{183}\text{H}_{165}\text{N}_3\text{O}_{33}$: C, 74.41; H, 6.67; N, 1.43; Found: C, 74.08; H, 6.49; N, 1.68%.

1,3,5-Tris((bis(4-(3,5-bis(3,5-bis(benzyloxy)benzyloxy)benzyloxy)-5-methoxy-2-nitrophenyl)methoxy)methyl)benzene (2c). To a solution of **2a** (49 mg, 0.04 mmol) in dry acetone (5 mL) was added K_2CO_3 (0.120 g, 0.87 mmol), 18-crown-6 (33 mg, 0.12 mmol) and $[\text{G}-2]\text{Br}$ (204 mg, 0.25 mmol). The mixture was allowed to reflux in the absence of light for 3 days. The mixture was removed from heat and concentrated.

The resulting orange oil was poured over water (30 mL) and extracted with CH_2Cl_2 (3×10 mL). The organic layers were combined, dried (MgSO_4), filtered, concentrated and the residue was purified by column chromatography (SiO_2 3 : 6 : 1 gradient to 5 : 4 : 1 CH_2Cl_2 –hexanes– Et_2O) to yield **2c** (178 mg, 79%) as a bright yellow solid: UV-Vis: λ_{max} 339 nm, ϵ $3.28 \times 10^4 \text{ L cm}^{-1} \text{ mol}^{-1}$; ^1H NMR (CDCl_3) δ 7.65 (s, 6 H); 7.37–7.21 (m, 123 H); 7.00 (s, 3 H); 6.86 (s, 6 H); 6.64–6.63 (d, $J = 2.0$ Hz, 12 H); 6.62–6.61 (d, $J = 2.0$ Hz, 24 H); 6.52–6.51 (t, $J = 2.0$ Hz, 12 H); 6.50–6.49 (t, $J = 2.0$ Hz, 6 H); 4.98 (s, 12 H); 4.94 (s, 48 H); 4.87 (s, 24 H); 4.52 (s, 6 H); 3.64 (s, 18 H); ^{13}C NMR (CDCl_3) δ 160.4, 153.9, 147.6, 141.4, 139.3, 138.1, 137.0, 130.5, 128.8, 128.2, 127.8, 110.9, 110.5, 106.9, 106.6, 102.1, 101.8, 71.4, 70.4, 70.3, 70.2, 56.6; MS (MALDI in dithranol) m/z 5536 ($\text{M} - \text{CH}_3\text{OH}$, $\text{C}_{347}\text{H}_{296}\text{N}_6\text{O}_{62}$ requires 5538.019). Anal. Calc. for $\text{C}_{348}\text{H}_{300}\text{N}_6\text{O}_{63}$: C, 74.98; H, 5.42; N, 1.51; Found: C, 74.75; H, 5.69; N, 1.88%.

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