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Divergent synthesis of novel unsymmetrical dendrons containing photosensitizing units

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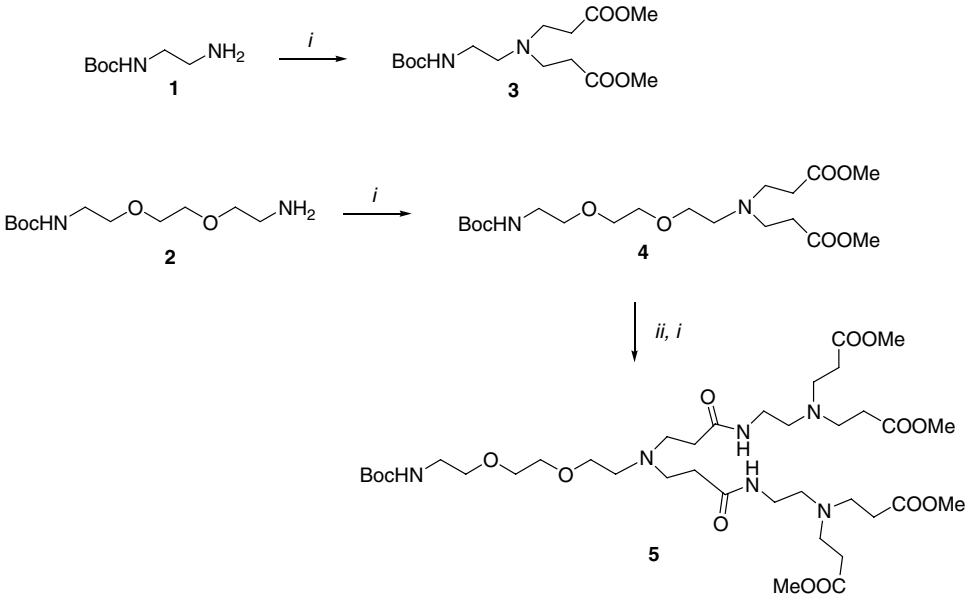
Abstract—The synthesis, characterization and singlet oxygen quantum yield of novel unsymmetrical dendrons containing photosensitizing units at their periphery is reported. Boc-protected 2,2′-(ethylenedioxy)-bis-ethylamine reacted with methyl acrylate and ethylene diamine to give half-dendrimers **4** and **5** with 2 and 4 end groups, respectively. Amine-tethered porphyrins and compounds **4** and **5** were efficiently coupled in DMF at 100 °C to give the functionalized dendrons. UV–visible and fluorescence emission spectra showed that the photophysical properties of the porphyrins were retained in the dendrimers.

Many types of dendrimers have been designed and utilized in many applications, including chemistry and pharmaceutics.[1] Among them, poly(amidoamine) (PAMAM) dendrimers have demonstrated great promise for a variety of biomedical applications. This class of polymers has a great number of favourable properties



Scheme 1. Synthesis of the ester-terminated half dendrimers. Reagents and conditions: (i) methyl acrylate (3.0 equiv/NH_2), MeOH, 24 h, rt and (ii) ethylene diamine (5.0 equiv/COOMe), MeOH, 5 days, rt.

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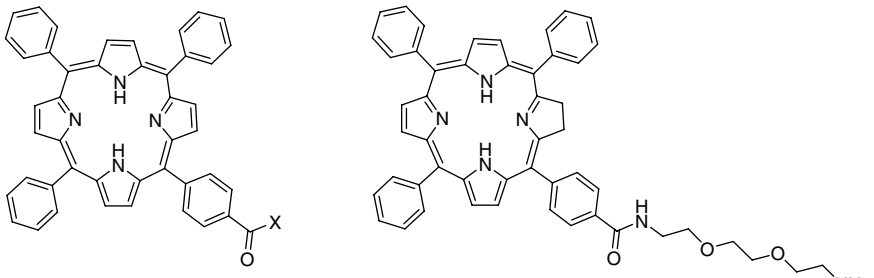
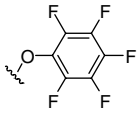
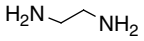
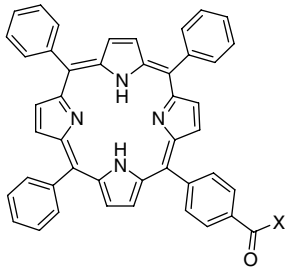
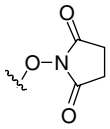
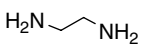
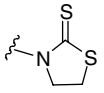
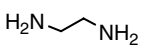
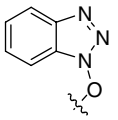
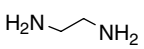
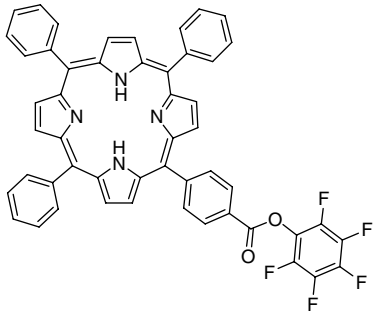
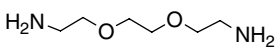
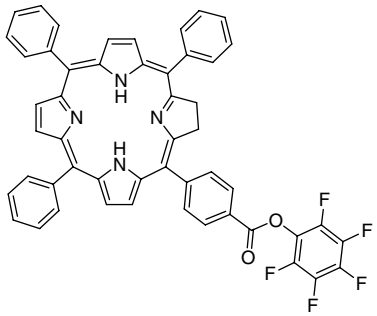
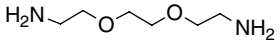


Figure 1.

Table 1. One-pot preparation of amine-functionalized porphyrins and chlorins[a]

Starting material	X	Amine[b]	Product	Yield[c] (%)
			**6**	93
			**6**	82
			**6**	29
			**6**	74
			**7**	95
			**8**	89

[a] Reactions were conducted in CH_2Cl_2 between 0 and 25 °C for 1 h.
[b] 10 equiv of diamine were used.
[c] Isolated yields after column chromatography.

including well-defined chemical structure, globular shape, low polydispersity index (close to 1.0), biocompatibility, and controlled terminal functional groups. Modification of the PAMAM dendrimer surface functional groups with targeting compounds, fluorescent dyes, and drugs is an interesting method to produce new imaging and therapeutic agents.[1c,2] However, for some applications, it would be advantageous to use an unsymmetrical dendrimer containing more than one terminal functional group.

Photodynamic therapy (PDT) is a relatively new cytotoxic treatment used mainly in anticancer approaches.[3] PDT requires a photosensitizer (PS), light of an appropriate wavelength and molecular oxygen. Upon photoactivation, the generation of cytotoxic species, such as singlet oxygen ($^1O_2$), induces the destruction of cancer cells. Nevertheless, one of the main drawbacks of the PS tested is their lack of selectivity with regard to uptake and retention by tumour cells versus normal ones, which leads to a prolonged skin photosensitization.[4]

We have initiated a programme of research aimed at developing dendrimers that contain PS's like porphyrins and chlorins on one surface, and cell recognition groups such as folic acid or peptides to generate delivery systems able to target specific cells.[3b,c] The produced macromolecules will be used for targeted PDT with the goal to selectively accumulate the PS within the diseased tissue with little or non-uptake by non-cancerous cells. Towards this end, a challenging target is the construction of PAMAM dendrimers possessing a well-defined number of photosensitizing units at their periphery.

We report herein, for the first time, the successful preparation of PAMAM dendrimers functionalized with PS using a divergent/divergent approach.[5] The ability of these dendrimers to produce $^1O_2$ was investigated by monitoring $^1O_2$ emission at 1272 nm after 418 nm excitation in toluene.

Boc-protected ethylene diamine **1** and 2,2′-(ethylenedioxy)-bis-ethylamine **2** used as cores for the divergent/divergent PAMAM synthesis were prepared according to published procedure.[3b] 2,2′-(ethylenedioxy)-bis-ethylamine was chosen as the core since block copolymers containing poly(ethylene glycol) (PEG) show high solubility in water, non-immunogenicity and improved biocompatibility.[6] Compounds **1** and **2** were subjected to a standard PAMAM synthesis[7] using ethylene diamine and methyl acrylate to afford the ester-terminated half dendrimers **3**, **4** and **5**, in overall yields of 62, 63 and 57%, respectively (Scheme 1).

Dendrimers **3**, **4** and **5** were purified by column chromatography on silica gel using MeOH/CH_2Cl_2 (1/5) as the eluant. Their structures were confirmed by ^1H and ^{13}C NMR spectroscopy, IR spectroscopy and HR-MS.

'Surface' functionalization of ester-terminated half dendrimers **3**, **4** and **5** with photoactive units was evaluated using ethylene diamine and 2,2′-(ethylenedioxy)-bis-ethylamine tethered 5,10,15-(triphenyl)-20-(4-carboxyphenyl)porphyrin (compounds **6** and **7**) and 5,10,15-(triphenyl)-20-(4-carboxyphenyl)chlorin (compound **8**) (Fig. 1).

Compound **6** was previously prepared using Boc-protected ethylene diamine[8] but, in our hands, degradation made the final purification procedure extremely difficult, resulting in a low yield (31%). A direct synthesis using an active ester of the 5,10,15-(triphenyl)-20-(4-carboxyphenyl)porphyrin or chlorin and an excess diamine was therefore developed since ethylene diamine and 2,2′-(ethylenedioxy)-bis-ethylamine are water-soluble and very easy to remove. Results obtained with the pentafluorophenol active ester[9] were found superior to *N*-hydroxysuccimide,[10] 1,3-thiazoline-2-thione[11] or by activation of the carboxyl function using DCC and HOBt.[12] The results are summarized in Table 1.[13]

Amines **6** and **7** were subjected to amide bond formation with ester-terminated half dendrimers **3**, **4** and **5**. An examination of the literature shows that such functionalizations are very limited and required a high excess of amine (generally more than 12 equiv per ester function) and long reaction times (20 days at room temperature) to convert all the ester functions at the periphery.[5] The use of a large excess of amine tethered porphyrins for the amidation of dendrons **3**, **4** or **5** is not feasible. After some experimentation, we found that good results for the amidation step could be obtained using only a slight excess of **6** or **7** (1.3 equiv per ester function) in DMF at 100 °C.

Although several conditions were attempted (use of higher temperatures and/or extended reaction times), the coupling of **3** with aminoporphyrins **6** or **7** was unsatisfactory and yielded only monoamidation products **9** and **10** in 54% and 84% yields, respectively (Fig. 2).

Much better results were obtained using half dendrimers **4** and **5** and amine tethered porphyrin **7**. Complete func-

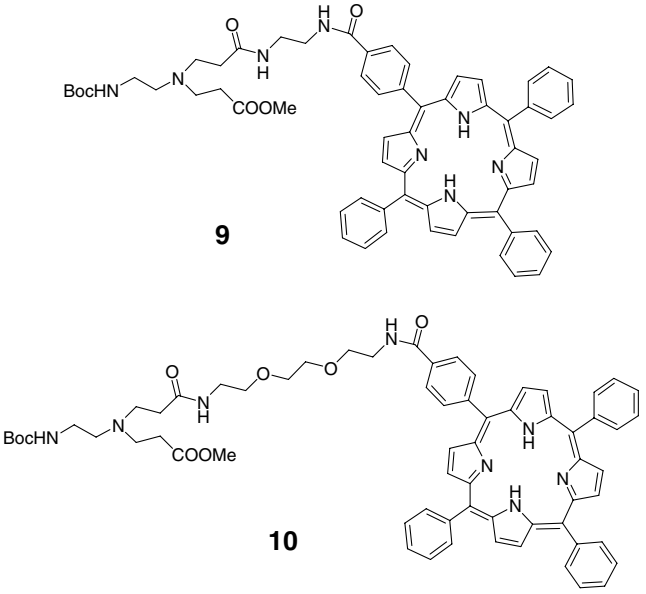


Figure 2.

tionalization of ester functions could be achieved and compounds **11** and **12** containing 2 and 4 porphyrin end groups were obtained in 42% and 37% yields, respectively (Fig. 3).[14] Monoamidation compound was obtained as the by-product in a 28% yield starting from **4**. FT-IR, ^{1}H NMR, UV–visible spectroscopy studies and HR-MS analysis confirmed the chemical structure of final products for both generations.

The removal of the Boc-protecting group of compounds **11** and **12** was finally achieved using CF_3CO_2H/CH_2Cl_2 (1/1) to give the free amine dendrons **13** and **14** in 64%

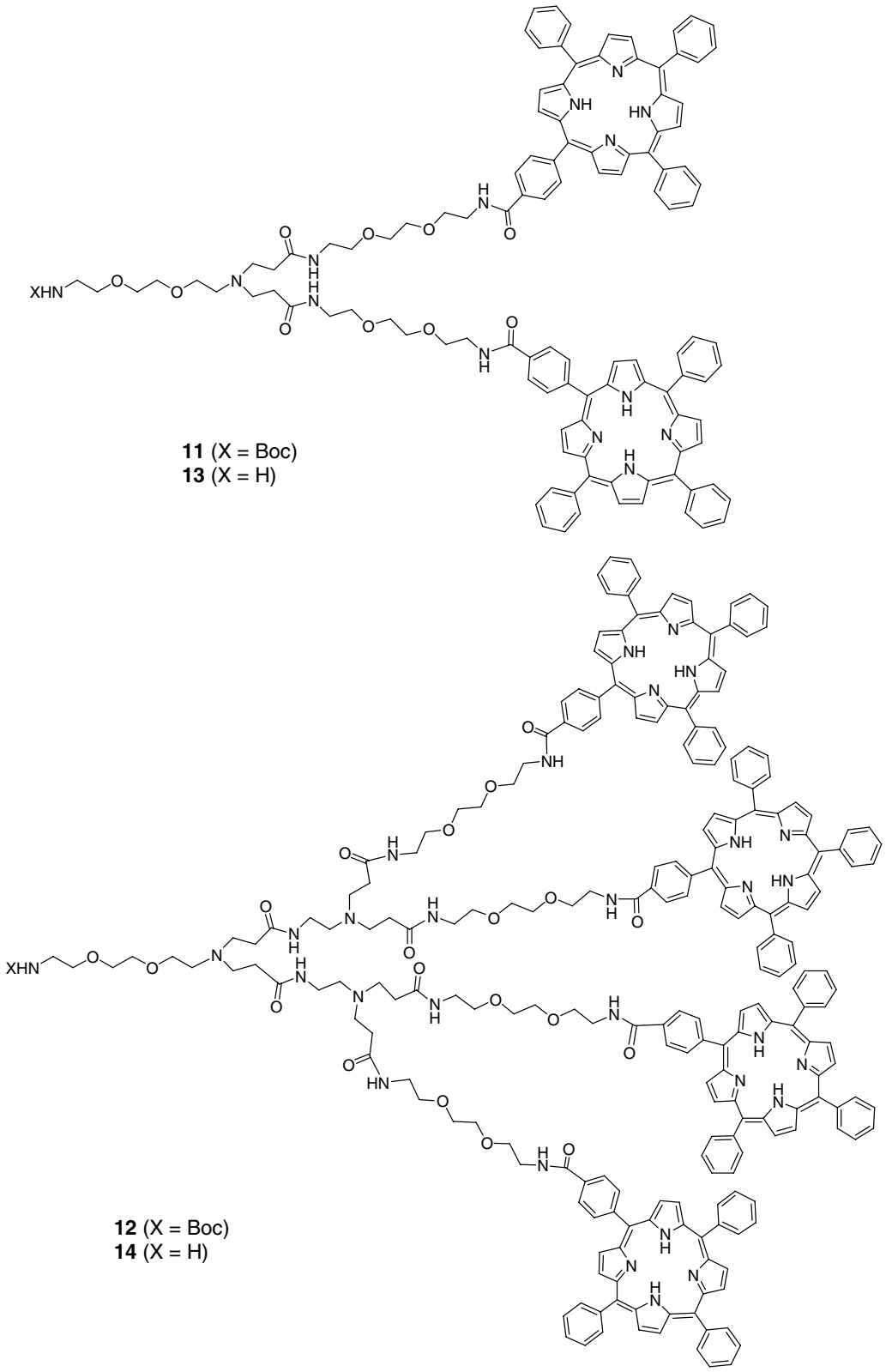


Figure 3.

and 58% yields, respectively. The use of HCl in Et_2O did not lead to an improvement of the deprotection and larger amounts of by-products with concomitant lower isolated yield (31%) were observed.

Room temperature absorption and fluorescence emission spectra of half-dendrimers **11** and **12** were recorded in toluene. Compounds **11** and **12** show the Soret band ($\lambda_{max} = 419$ nm) and the four Q bands at 515, 550, 590 and 650 nm common for porphyrins in the UV–visible region. The fluorescence emission spectra ($\lambda_{exc} = 419$ nm) of **11** and **12** display two bands at 650 and 717 nm. The similarities of the absorption and the fluorescence spectra of 5,10,15-(triphenyl)-20-(4-carboxyphenyl)porphyrin and compounds **11** and **12** demonstrate that the photophysical properties of the porphyrin were preserved in the dendrimer. Singlet oxygen quantum yields ϕ_Δ for **11** and **12** were determined using tetraphenylporphyrin (TPP) as the reference solution (ϕ_Δ [TPP] = 0.70, in toluene) and were estimated from $^1O_2$ luminescence at 1272 nm. For **11** and **12**, ϕ_Δ values are, respectively, equal to 0.21 and 0.23, pointing out aggregates the formation in toluene solution. Their dissociations could be achieved by the addition of cetyltrimethylammonium bromide in concentration as low as 0.2% (the concentrations of **11** and **12** were 10^{-6} mol L^{-1}) and a value of 0.68 for ϕ_Δ was then reached for both.

In conclusion, a novel series of unsymmetrical dendrons containing photosensitizing units at their periphery has been prepared in good yields using a divergent/divergent approach. The synthesis strategy was based on the polyamidation of ester-terminated half dendrimers **4** and **5** with amine-tethered porphyrins **6** or **7**. Subjecting the primary amine group at the focus of compounds **13** and **14** to a second PAMAM synthesis for constructing unsymmetrical dendrimers with terminal groups suitable for targeted PDT will be reported in due course.

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13. *Typical experimental procedure for the synthesis of* **7**. Under a nitrogen atmosphere and in the darkness, the pentafluorophenol ester of 5,10,15-(triphenyl)-20-(4-carboxyphenyl)porphyrin (82 mg, 0.1 mmol) in 10 mL anhydrous CH_2Cl_2 was added dropwise over a period of 30 min to a solution of 2,2′-(ethylenedioxy)-bis-ethylamine (148 mg, 1 mmol) in 10 mL CH_2Cl_2 cooled to 0 °C. The reaction mixture was stirred at room temperature for 1 h. The organic layer was then washed with water (2×10 mL), dried (Na_2SO_4) and concentrated in vacuo. Column chromatography (Acetone/MeOH gradient, 1:0–7:3) afforded **7** as a purple film in a 95% yield. $R_f = 0.58$ (Acetone/MeOH, 4:1); IR (NaCl, cm^{-1}): 3237, 3124 and 1691; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.89$–8.77 (m, 8H), 8.27 (d, $J = 7.6$ Hz, 2H), 8.24–8.17 (m, 8 H), 7.79–7.70 (m, 9H), 7.47 (br t, NH), 3.88–3.53 (m, 10H), 2.92 (br td, 2H), 2.34 (br s, NH_2), −2.78 (s, 2H, NH); UV/vis(CH_2Cl_2): $\lambda_{max} = 415$, 514, 552, 590 and 649 nm.
14. *Typical experimental procedure for the synthesis of* **11**. Under a nitrogen atmosphere and in the darkness, to a solution of ester terminated half dendrimer 4 (10 mg, 0.024 mmol) in 1.5 mL DMF was added aminoporphyrin 7 (50 mg, 0.063 mmol) in 1.5 mL DMF and the reaction mixture was heated to 100 °C. After stirring for 15 h, the reaction mixture was concentrated to dryness. Column chromatography (Acetone/hexanes gradient: 1:10–7:3) afforded **11** as a purple film in 42% yield. $R_f = 0.68$ (Acetone/hexanes, 7:3); IR (NaCl, cm^{-1}): 3117 and 1682; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.89$–8.82 (m, 12H), 8.79 (d, $J = 4.8$ Hz, 4H), 8.29 (d, $J = 8.0$ Hz, 4H), 8.24–8.20 (m, 12H), 8.17 (d, $J = 8.0$ Hz, 4H), 7.81–7.69 (m, 18H), 7.52 (br t, 1H, NH), 6.95 (br t, 1H, NH), 3.91–3.25 (m, 30H), 2.83 (t, $J = 6.4$ Hz, 4H), 2.67 (t, $J = 6.4$ Hz, 2H), 2.46 (t, $J = 6.8$ Hz, 4H), 2.29 (t, $J = 6.4$ Hz, 2H), 2.16 (t, $J = 6.8$ Hz, 4H), 1.44 (s, 9H), −2.78 (s, 4H, NH); UV/vis(CH_2Cl_2): $\lambda_{max} = 417$, 513, 549, 588 and 649 nm. MALDI-TOF: found, $m/z = 1134.491$.