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Synthesis of mono-, di- and triporphyrin building blocks by click chemistry for photodynamic therapy application

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ARTICLE INFO

In the present work, we describe a successful strategy centered on the use of click chemistry to synthesize mono-, di- and triporphyrin building blocks named [ZnTPP] 1, [ZnTPP] 2, and [ZnTPP] 3. The number of photosensitizers (ZnTPP unit) coupled to the building blocks does not seem to dramatically modify the photophysical properties of the photosensitizer in terms of fluorescence and singlet oxygen formation, quantum yields and lifetime. These building blocks, obtained to high yield, could be combined with a targeting agent (vector) to create new photosensitizer-vector conjugates to investigate the optimum ratio of photosensitizer needed for maximum photodynamic therapy efficiency.

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1. Introduction

Photodynamic therapy (PDT) is a promising treatment involving the concomitant action of: 1) a photoactivatable molecule, the photosensitizer (PS), 2) light of a suitable wavelength, and 3) oxygen present naturally in the biological medium. After photosensitizer excitation by light, energy transfer to the oxygen allows the generation of reactive oxygen species (ROS), and particularly reactive singlet oxygen, which leads to cell death. This technique has proved to be highly effective for the treatment of some types of disease in various therapeutic fields such as oncology, vascular cardiology, dermatology, gynecology or malaria.

Among the various potential photosensitizers that are available, porphyrins have been extensively studied in the field of photodynamic therapy (PDT). Porphyrinic molecules have an aromatic planar architecture capable of developing attractive photophysical properties for PDT applications (Figure 1), and several exist in nature. Photofrin®, a mixture of porphyrin oligomers, is the most popular one used clinically in PDT and corresponds to the first generation photosensitizer. However, first generation photosensitizers present several drawbacks such as poor light absorption in the red, cutaneous photosensitivity and lack of tumor specificity. To overcome these disadvantages, second and third generations of photosensitizer were developed, for example such as in oncology, a new porphyrin (Visudyne®), chlorins (Foscan®, Talaporfin® or Photochlor®) and phthalocyanine (Photosens®).

![Figure 1. Typical structure of a porphyrin that can be functionalized at β- and meso-positions](image)

One of the proposed strategies for increasing the photodynamic efficiency of photosensitizers is to combine them with a vector capable of selectively binding to specific receptors overexpressed on tumor cell membranes. The various vectors which have been used by members of our team are, among others, folic acid (FA). This is specific for the folate receptor alpha (FRα), which is overexpressed in many human cancers (ovary, brain, kidney, breast, myeloid cells, and lung) and peptides (ATWLPPR and DKPPR) for targeting vascular endothelial growth factor (VEGF) receptor or its co-receptor neuropilin-1 (NRP-1), both over-expressed by tumor cells. These new PS-vector conjugates developed by our team are depicted in Figure 2.

![Figure 2. New PS-vector conjugates developed by our team for PDT applications](image)

In the literature, several strategies to synthesize porphyrin conjugates are described but click chemistry appears to be the most effective method and its use in various fields such as biochemistry, and medicinal or surface chemistry, continues to increase year after year.
year. “Click Chemistry” was an idiom introduced in 2001 by Sharpless to cover all organic reactions that could be described as modular, wide in scope, high yielding, easy to perform and stereospecific. The reactions must use harmless or easily disposed solvents and have to generate only by-products that can be removed by simple purification techniques.15

Among the reactions that are considered as “Click Chemistry”, the most commonly used is the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The azide-alkyne cycloaddition was first studied by Huisgen et al. in 1968 and the authors’ findings showed the slow formation of a mixture of both 1,4- and 1,5-disubstituted 1,2,3-triazole regioisomers (Figure 3).16 In 2002, Sharpless and Meldal simultaneously found that the use of the Cu(I) catalyst helps to increase the reaction rate and to obtain only the 1,4-disubstituted regio-isomer even at sub-ambient-temperature (Figure 3).17,18

Figure 3. Huisgen’s reaction

The introduction of the CuAAC reaction has opened up new and diverse opportunities to combine two building blocks that are difficult to connect by conventional coupling techniques. Recently, the use of a microwave was reported, opening wider applications in the field of click chemistry. In 2015 we published a recapitulative review about the synthesis of porphyrin, chlorin or phthalocyanine conjugates by azide-alkyne click chemistry, and their related applications.19

The mild condition of the CuAAC reaction is compatible with the peptidic chemistry and offers the possibility to develop new peptide-based drugs, which could be potentially useful therapeutically.20 Furthermore, the 1,2,3-triazole ring is highly stable against hydrolysis and could ensure delivery of the conjugated drugs to their targeted receptors.21

The main goal of this work is to establish a strategy centered on the use of click chemistry to synthesize mono-, di- and tri-porphyrin building blocks abbreviated to [ZnTPP]1, [ZnTPP]2 and [ZnTPP]3 respectively, for easier reading. These building blocks could be combined with a targeting agent (vector) to create new PS-vector conjugates to investigate the optimum ratio of PS needed for maximum PDT efficiency.

2. Results and discussion

2.1. Synthesis of clickable porphyrin scaffold bearing a propargyl group

Our approach was first based on the synthesis of a structurally related derivative of zinc tetraphenylporphyrin (ZnTPP) 2 bearing a propargyl group for linkage upon the CuAAC reaction (Scheme 1), using a protocol similar to that which we reported previously with zinc tetraphenylchlorin (ZnTPC).22 The metalization of TPP is essential, to avoid copper metalization of the porphyrin ring during the subsequent CuAAC reaction.

Scheme 1. Synthesis of alkyne-functionalized ZnTPP 2

5-(4-carbonylphenyl)-10,15,20-triphenyl-21H,23H-porphyrin (TPP-COOH) (TPP-COOH) was used as starting material and prepared according to the protocol previously described by our team.23 The TPP-COOH was converted into the propargyl derivative (1) in a one-pot two-step reaction by a classical peptide method (Scheme 1). The first step is the in situ activation of the carboxyl group of TPP-COOH by dicyclohexylcarbodiimide (DCC, 1 equivalent) and N-hydroxysuccinimide (NHS, 2 equivalents) followed by a coupling
reaction with an excess of propargylamine (10 equivalents). Several conditions (temperature and reaction time) were tested and those used are summarized in Table 1. The best yield (96%, trial A6) was obtained after one day at 40°C for both activating and coupling steps. Finally, the porphyrin ring of molecule 1 was metalized with Zn(OAc)_2 to provide the alkyne-functionalized ZnTPP 2 in a quantitative yield.

**Table 1.** Several trials for the one-pot preparation of alkyne-functionalized TPP 1 via Scheme 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Step 1 conditions</th>
<th>Step 2 conditions</th>
<th>Yield (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Reaction time (h)</td>
<td>Temperature °C</td>
</tr>
<tr>
<td>A1</td>
<td>rtb</td>
<td>4</td>
<td>rt</td>
</tr>
<tr>
<td>A2</td>
<td>rt</td>
<td>24</td>
<td>rt</td>
</tr>
<tr>
<td>A3</td>
<td>rt</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>A4</td>
<td>rt</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>A5</td>
<td>40</td>
<td>4</td>
<td>40</td>
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<td>A6</td>
<td>40</td>
<td>24</td>
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</tr>
<tr>
<td>A7</td>
<td>40</td>
<td>0.5</td>
<td>40</td>
</tr>
</tbody>
</table>

a Yields calculated after purification by flash chromatography.
b rt = room temperature

2.2. Synthesis of clickable aromatic scaffolds bearing one, two or three azide groups

To synthesize [ZnTPP]_1, [ZnTPP]_2 and [ZnTPP]_3 building blocks by the CuAAC reaction, we created platforms bearing one, two or three azide groups. To achieve this goal, we chose to use commercially available hydroxy and polyhydroxy derivatives of methyl benzoate (Figure 4).

![Chemical structures of hydroxy and polyhydroxy derivatives of methyl benzoate used in this work](image)

**Figure 4.** Chemical structures of hydroxy and polyhydroxy derivatives of methyl benzoate used in this work

The methyl benzoate derivatives substituted by 1-3 hydroxy groups (Figure 4) were functionalized through their hydroxy groups with an azide functional group 4 derived from diethylene glycol (Scheme 2). We decided to use diethylene glycol as the spacer unit to improve the hydrophilicity of our clickable aromatic scaffolds 6, 8 and 10. 2-(2-azidoethoxy)ethyl methanesulfonate 4 was synthesized in two steps from diethylene glycol with 63% overall yield (Scheme 2). This two-step procedure involved mesylation of both hydroxyl groups using mesyl chloride (4 equivalents) to provide dimesylate intermediate 3, followed by nucleophilic substitution of one mesylate group of 3 with a sodium azide (1 equivalent). The last step has already been described by Sirion et al. under similar conditions but we were able to significantly improve the yield (77% instead of 40%).
Scheme 2. Synthesis of clickable aromatic scaffolds 6, 8 and 10

The resulting azide- and mesylate-functionalized diethylene glycol 4 was used to elaborate clickable aromatic scaffolds 6, 8 and 10 bearing one, two or three azide groups and the same two-step approach was adopted for their synthesis (Scheme 2). To achieve this goal, we decided to use experimental conditions similar to those previously employed by Chen et al.\textsuperscript{25} for the synthesis of products 9 and 10. The first step of this approach is based on the nucleophilic substitution of the mesylate group of product 4 by the hydroxy groups present in the methyl benzoate derivatives represented in Figure 4, to provide the corresponding mono-, di- and triazide derivatives of methyl benzoates 5, 7 and 9 in high yields (Step A in Scheme 2). Finally, the saponification of the ester group of 5, 7 and 9 led to clickable aromatic scaffolds 6, 9 and 10 respectively with yields of up to 90% (Step B in Scheme 2), opening the way for the synthesis of [ZnTPP]\textsubscript{1}, [ZnTPP]\textsubscript{2} and [ZnTPP]\textsubscript{3} building blocks by click chemistry (Figure 5).

Figure 5. Chemical structures of [ZnTPP]\textsubscript{1}, [ZnTPP]\textsubscript{2} and [ZnTPP]\textsubscript{3} building blocks
2.3. Synthesis of [ZnTPP]1, [ZnTPP]2 and [ZnTPP]3 building blocks by click chemistry

The last part of this article is dedicated to the conjugation of the alkyne-functionalized ZnTPP 2 to the three azide-functionalized aromatic scaffolds 6, 8 and 10, through click chemistry. These various conjugations led to the elaboration of the desired [ZnTPP]1, [ZnTPP]2 and [ZnTPP]3 building blocks represented in Figure 5.

In the first phase, we investigated the synthesis of the [ZnTPP]1 building block by appending the alkyne-clickable porphyrin 2 (1 equivalent) onto the monoazide-functionalized aromatic scaffold 6 (1 equivalent) through the CuAAC reaction. The active Cu(I) catalyst, needed to drive the reaction, is generated from CuSO4·5H2O (0.1 equivalent) and sodium ascorbate (0.5 equivalent). With the aim of obtaining the best conditions for this reaction, several trials were conducted by changing 5 parameters i.e. solvent (DMF or THF/H2O), method (magnetic stirring or microwave), temperature (rt, 50°C or 80°C), time of reaction and under nitrogen or not. The results of these trials are summarized in Table 2.

An examination of the results shown in Table 2 demonstrated that the reaction yield is better with the microwave technique than with magnetic stirring, giving higher yields in a shorter time (compare B3/B7 or B4/B8 trials), in THF/H2O than in DMF (compare B4/B12 or B6/B14 trials), at 80°C than 50°C (compare B10/B11 and B5/B6 trials) and under nitrogen (compare B5/B7 or B6/B8 trials). Moreover, the addition of a second dose of CuSO4·5H2O and sodium ascorbate contributes positively to increase the reaction yield (compare B13/B14 or B15/B16 trials). In summary, the [ZnTPP]1 building block is provided at a very high yield (97%, trials B14 and B16).

Table 2. Several trials for the synthesis of [ZnTPP]1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Solvent Method</th>
<th>Temperature °C</th>
<th>Reaction time</th>
<th>Nitrogen</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>DMF Magnetic stirring</td>
<td>Rt</td>
<td>Overnight</td>
<td>No</td>
<td>7</td>
</tr>
<tr>
<td>B2</td>
<td>DMF Magnetic stirring</td>
<td>Rt</td>
<td>Overnight</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>B3</td>
<td>DMF Magnetic stirring</td>
<td>50</td>
<td>Overnight</td>
<td>Yes</td>
<td>75</td>
</tr>
<tr>
<td>B4</td>
<td>DMF Magnetic stirring</td>
<td>80</td>
<td>Overnight</td>
<td>Yes</td>
<td>75</td>
</tr>
<tr>
<td>B5</td>
<td>DMF Microwave</td>
<td>50</td>
<td>2 rounds of 45 minutes</td>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td>B6</td>
<td>DMF Microwave</td>
<td>80</td>
<td>2 rounds of 45 minutes</td>
<td>No</td>
<td>60</td>
</tr>
<tr>
<td>B7</td>
<td>DMF Microwave</td>
<td>50</td>
<td>2 rounds of 45 minutes</td>
<td>Yes</td>
<td>86</td>
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<tr>
<td>B8</td>
<td>DMF Microwave</td>
<td>80</td>
<td>2 rounds of 45 minutes</td>
<td>Yes</td>
<td>89</td>
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<tr>
<td>B9</td>
<td>THF/H2O (4:1) Magnetic stirring</td>
<td>Rt</td>
<td>Overnight</td>
<td>No</td>
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<tr>
<td>B10</td>
<td>THF/H2O (4:1) Magnetic stirring</td>
<td>Rt</td>
<td>Overnight</td>
<td>Yes</td>
<td>20</td>
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<tr>
<td>B11</td>
<td>THF/H2O (4:1) Magnetic stirring</td>
<td>80</td>
<td>3 hours</td>
<td>Yes</td>
<td>55</td>
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</table>
Table 3. Several trials for the synthesis of [ZnTPP]$_3$

| Trial | Solvent Method | Temperature °C | Reaction time | Nitrogen | Yield (%) $^a$
|-------|----------------|----------------|---------------|----------|------------------------|
| B4    | DMF Magnetic stirring | 80 | Overnight   | Yes | nd $^c$
| B8    | DMF Microwave | 80 | 2 rounds$^b$ of 45 minutes | Yes | nd $^c$
| B12   | THF/H$_2$O (4:1) Magnetic stirring | 80 | Overnight | Yes | nd $^c$
| B14   | THF/H$_2$O (4:1) Microwave | 80 | 2 rounds$^b$ of 45 minutes | No | nd $^c$
| B17   | B14 followed by magnetic stirring at rt for 5 days under nitrogen atmosphere | rt | 5 days | Yes | 18

$^a$Yields calculated after purification by flash chromatography.

$^b$CuSO$_4$.5H$_2$O (0.1 equiv.) and Na ascorbate (0.5 equiv.) were added again at the end of the first round microwave.

$^c$nd = not determined.

Table 4. Several trials for the synthesis of [ZnTPP]$_2$

| Trial | Solvent Method | Temperature °C | Reaction time | Nitrogen | Yield (%) $^a$
|-------|----------------|----------------|---------------|----------|------------------------|
| B4    | DMF Magnetic stirring | 80 | Overnight | Yes | 59
| B8    | DMF Microwave | 80 | 2 rounds$^b$ of 45 minutes | Yes | 38
| B12   | THF/H$_2$O (4:1) Magnetic stirring | 80 | Overnight | Yes | nd $^c$
| B14   | THF/H$_2$O (4:1) Microwave | 80 | 2 rounds$^b$ of 45 minutes | No | nd $^c$
| B19   | B4 followed by magnetic stirring at rt for 3 days under nitrogen atmosphere | 93
| B20   | B8 followed by magnetic stirring at rt for 3 days under nitrogen atmosphere | 81

$^a$Yields calculated after purification by flash chromatography.

$^b$CuSO$_4$.5H$_2$O (0.2 equiv.) and Na ascorbate (1 equiv.) were added again at the end of the first round microwave.

The successful synthesis of [ZnTPP]$_1$, [ZnTPP]$_2$, and [ZnTPP]$_3$ building blocks through the CuAAC reaction paves the way for the development of new PS-vector conjugates. These new conjugates may be synthesized via a peptide coupling reaction between the carboxylic acid function of [ZnTPP]$_1$, [ZnTPP]$_2$, and [ZnTPP]$_3$ building blocks and a targeting agent (vector) such as folic acid or ATLWLPR and DKPPR peptides (Figure 2) to investigate the optimum ratio of PS needed for maximum PDT efficiency. However, to take the best advantage of the click chemistry, it would be interesting to develop new PS-vector conjugates through the CuAAC reaction. To reach that goal, we have shown that it is possible to functionalize the carboxylic acid moiety of our building blocks (only [ZnTPP]$_1$ is used as an example) with commercially available 3-azido-1-propanamine. The azide-functionalization of [ZnTPP]$_1$ provides the corresponding [ZnTPP]$_1$-N$_3$ in a 72% yield (Scheme 3).
2.4. Photophysical properties of [ZnTPP], [ZnTPP]: and [ZnTPP]: building blocks

Absorption presents a typical porphyrin absorption spectrum with a Soret band at 414 nm and four Q-bands. The fluorescence spectrum is typical of a porphyrin with two maxima at around 650 nm and 720 nm. As we already showed in a previous study, the addition of Zn induced a shift of 1) the Soret band from 414 nm to 421 nm and 2) the maximum of fluorescence from 650 nm to 610 nm for the first and 720 nm to 660 nm for the second band. It also induced a decrease of the fluorescence lifetime from 10.0 ns to 2.1 ns. Surprisingly, the fluorescence quantum yield did not decrease as has often been described in the literature. DeRosa et al. reported that the introduction of Zn into TPP increased the singlet oxygen quantum yield in benzene from 0.63 to 0.83. In our case, the introduction of Zn into TPP-propargyl increases the singlet oxygen quantum yield in ethanol from 0.49 to 0.72.

The addition of a Zn-porphyrin to the structure does not seem to modify drastically the photophysical properties of the photosensitizer in terms of fluorescence and singlet oxygen formation quantum yields or lifetime. However, [ZnTPP]: presents two different fluorescence lifetimes. This might be due to a dimer formation.

Scheme 3. Azide-functionalization of [ZnTPP]$_1$
Figure 6. Photophysical properties of TPP-COOH, [ZnTPP]_1, [ZnTPP]_2, [ZnTPP]_3 and [ZnTPP]_1-N_3. (a) Absorption spectra at a single concentration of DMSO (1 mg in 1 mL of DMSO) and (b) fluorescence emission spectra at a single concentration of EtOH (equal Soret bands at 0.2 ± 0.05 by dilution). (c) Fluorescence decay, (d) singlet oxygen emission spectra and (e) singlet oxygen decay after excitation (λ_{exc.} = 414 nm and 421 nm) at a constant EtOH concentration (equal Soret bands at 0.2 ± 0.05 by dilution).
3. Conclusion

Our goal was to synthesize three building blocks named [ZnTPP]1, [ZnTPP]2, and [ZnTPP]3 having one, two, or three PS units in their structures, respectively. Starting from the use of click chemistry (and more specifically the CuAAC reaction), we described the synthesis of these three building blocks with high yields. The presence of one or more PS units did not seem to result in profound changes in the photophysical properties of the PS (i.e. fluorescence and singlet oxygen formation, quantum yields and lifetime). These photophysical results pave the way for the potential conjugation of [ZnTPP]1, [ZnTPP]2, and [ZnTPP]3 building blocks with a targeting agent (vector) for the elaboration of new PS-vector conjugates to investigate the optimum ratio of PS needed for maximum PDT efficiency.

4. Experimental

4.1. General

Unless otherwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purifications. All reactions involving porphyrin compounds were performed in the dark. All microwave reactions were conducted in oven-dried glass tube using CEM Discover microwave synthesizer (North Carolina, USA). Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed silica gel plates (Macherey-Nagel ALUGRAM® SIL >G/UV254). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with a staining solution of phosphomolybdic acid. Product purifications were performed using Silica Gel 60 (230-400 mesh) for column chromatography and Geduran 60 H Silica Gel (63-200 mesh) for flash column chromatography. 1H NMR spectra were recorded on a Bruker Advance spectrophotometer operating at 300 MHz. The spectra were recorded in CDCl3 and DMSO-d6 solvents at room temperature (T = 298 K) using TMS (δ = 0 ppm) or DMSO residual peak (δ ≈ 2.5 ppm) as internal references respectively. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). Multiplicities are reported as follow: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad and Ar. = aromatic. Mass spectra were recorded on a Shimadzu LCMS 2010 EV apparatus (Champs-sur-Marne, France). Absorption spectra were recorded on a Perkin-Elmer Lambda EZ210 (Courtaboeuf, France) double beam UV-visible spectrophotometer. 

4.2. Synthesis of clickable porphyrin scaffold bearing a propargyl group (2)

4.2.1. 5-(4-propargylamidocarboxyphenyl)-10,15,20-triphenyl-21H,23H-porphyrin (I). In the absence of light and under N2, dicyclohexyl carbodiimide (DCC, 31 mg, 0.15 mmol, 1.0 equiv) and N-hydroxysuccinimide (NHS, 35 mg, 0.30 mmol, 2.0 equiv) were added successively to a solution of TPP-COOH (100 mg, 0.15 mmol, 1.0 equiv) in CH2Cl2 (20 mL). The mixture was stirred at room temperature during a specified period of time (see Table 1) to afford the succinimide intermediate. An excess of propargylamine (100 μL, 1.5 mmol, 10 equiv) was then added and the reaction was allowed to continue for several hours (see Table 1). The solvent was evaporated in vacuo and the resulting crude material was purified by flash chromatography (CH2Cl2/1-propanol, 97/3) to afford I (101 mg, 0.145 mmol) as purple solid in 96% of maximum yield (Trial A6). TLC (CH2Cl2/1-propanol, 97/3): Rf = 0.72. 1H NMR (300 MHz, CDCl3) δ = 2.89 (s, 2H, 2 NH2), 2.38 (t, J = 2.7 Hz, 1H, CH alkylene), 4.39 (dd, J = 5.1, 2.7 Hz, 2H, CH2), 6.55 (t, J = 5.4 Hz, 1H, NH), 7.70-7.79 (m, 9H, 9 CH), 8.14-8.22 (m, 8H, 8 CH), 8.33 (d, J = 8.1 Hz, 2H, 2 CH), 8.77 (d, J = 4.8 Hz, 2H, 2 CH), 8.83-8.90 (m, 6H, 6 CH); MS calculated for C80H52N12O6 [M+H]+ m/z 696.27, found 696.22.

Table 5. Photophysical data for the porphyrin derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption λmax (nm) (ε) - Soret band</th>
<th>ϕf</th>
<th>εf</th>
<th>τf</th>
<th>τe</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP-COOH</td>
<td>414 (166993)</td>
<td>0.08</td>
<td>0.60</td>
<td>10.1</td>
<td>16</td>
</tr>
<tr>
<td>TPP-propargyl 1</td>
<td>414 (126289)</td>
<td>0.09</td>
<td>0.49</td>
<td>10.0</td>
<td>16</td>
</tr>
<tr>
<td>ZnTPP-propargyl 2</td>
<td>421 (290200)</td>
<td>0.07</td>
<td>0.72</td>
<td>2.1</td>
<td>15</td>
</tr>
<tr>
<td>[ZnTPP]1</td>
<td>421 (211587)</td>
<td>0.08</td>
<td>0.54</td>
<td>1.9</td>
<td>18</td>
</tr>
<tr>
<td>[ZnTPP]2</td>
<td>421 (226551)</td>
<td>0.07</td>
<td>0.43</td>
<td>1.9</td>
<td>16</td>
</tr>
<tr>
<td>[ZnTPP]3</td>
<td>421 (329375)</td>
<td>0.06</td>
<td>0.46</td>
<td>2.2-10.6</td>
<td>16</td>
</tr>
</tbody>
</table>

a) Maximum absorption or fluorescence in EtOH.
b) ε in L/mol cm.

4.3. In vivo photodynamic therapy (PDT)

The photophysical results pave the way for the potential conjugation of [ZnTPP]1, [ZnTPP]2, and [ZnTPP]3 building blocks with a targeting agent (vector) for the elaboration of new PS-vector conjugates to investigate the optimum ratio of PS needed for maximum PDT efficiency.
4.2.2. Zinc 5-(4-propargylaminocarboxyphenyl)-10,15,20-triphenyl porphyrin (2). In the absence of light and under N2, zinc acetate dehydrate (114 mg, 0.51 mmol, 3.6 equiv) was added to a solution of 1 (100 mg, 0.14 mmol, 1 equiv) in THF (15 mL) and refluxed for 2 hours. The reaction was monitored by UV-visible spectrometry (350 to 800 nm) and TLC (CH2Cl2/EtOH, 97/3). After cooling to room temperature, the THF was removed in vacuo and the crude material was dissolved in CH2Cl2 (50 mL), washed with water (5 x 20 mL) and dried over MgSO4. After evaporation of CH2Cl2, compound 2 was obtained as purple microcrystalline solid in quantitative yield (106 mg, 0.14 mmol). TLC (CH2Cl2/EtOH, 97/3): Rf = 0.81; 1H NMR (300 MHz, CDCl3) δ 2.39 (t, J = 4.7 Hz, 1H, CH3), 3.77 (t, 2H, J = 4.8 Hz, CH2-O), 4.23 (t, 2H, J = 4.5 Hz, 2CH2-OMe); MS calculated for C33H23N2O12 [M+H]+ m/z 524.22, found 524.18.

4.3. Synthesis of azide-terminated diethylene glycol linker (4)

4.3.1. 2-(2-methylsulfonyloxyethoxy)ethyl methanesulfonate (3). To a solution of diethylene glycol (10.6 g, 100 mmol, 1.0 equiv) in CH2Cl2 (200 mL) at -10°C was added Et3N (22.3 g, 220 mmol, 2.2 equiv) and the mixture was stirred during 1 hour. Methanesulfonyl chloride (34.3 g, 340 mmol, 3.0 equiv) was then added dropwise over a period of 0.5 hour and the resulting mixture was stirred for 24 hours at room temperature under nitrogen atmosphere. The reaction progress was monitored by TLC and the crude material was concentrated under reduced pressure and compound 3 was recrystallized in hot MeOH and white crystals were obtained with a 82% yield (21.5 g, 82 mmol). TLC (CH2Cl2/MeOH, 95/5): Rf = 0.67; 1H NMR (300 MHz, CDCl3) δ 3.06 (s, 6H, 2 CH3), 3.79 (t, 4H, J = 4.5 Hz, 2 CH2-O), 4.37 (t, 4H, J = 4.5 Hz, 2 CH2-OMe); MS calculated for C19H23N2O8 [M+H]+ m/z 265.04, found 264.98.

4.3.2. 2-(Azidoethoxy)ethyl methanesulfonate (4). To a solution of compound 3 (5.24 g, 20 mmol, 1.0 equiv) in CH2Cl2 (50 mL) was added NaN3 (1.30 mg, 20 mmol, 1.0 equiv), and the mixture was stirred at 70°C for 40 h under nitrogen atmosphere. The precipitate was cooled at room temperature, filtered off, and the filtrate was evaporated and purified by flash column chromatography (EtOAc/petroleum ether, 40/60) to give 4a in 77% yield (3.22 g, 15.4 mmol) as a colorless oil. TLC (CH2Cl2/MeOH, 95/5): Rf = 0.69; 1H NMR (300 MHz, CDCl3) δ 3.04 (s, 6H, 2 CH3), 3.79 (t, 4H, J = 4.5 Hz, 2 CH2-O), 3.89 (t, 2H, J = 4.8 Hz, CH2-OMe); MS calculated for C19H27N9O8 [M+H]+ m/z 381.15, found 381.09.

4.4. Synthesis of clickable aromatic scaffolds bearing one, two or three azide groups (6, 8 and 10)

4.4.1. General method for nucleophilic substitution reaction (Step A).

4.4.1.1. Methyldi-4,5,6,7-tetrahydro[2-(Azidoethoxy)-ethoxy]-benzoate (5). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.71; 1H NMR (300 MHz, CDCl3) δ 3.20 (t, 2H, J = 4.8 Hz, CH2-N), 3.78 (t, 2H, J = 4.8 Hz, CH2-O), 3.87 (t, 2H, J = 4.8 Hz, CH2-OMe), 3.88 (t, 2H, J = 4.8 Hz, CH2-OMe), 4.20 (t, 2H, J = 4.8 Hz, CH2-O), 4.21 (t, 2H, J = 4.5 Hz, CH2-OMe), 4.27 (t, 2H, J = 4.5 Hz, CH2-0), 4.35 (t, 2H, J = 4.5 Hz, CH2-OMe); MS calculated for C21H21N2O8 [M+H]+ m/z 395.16, found 395.12.

4.4.1.2. Methyl 3,5-Bis-[2-(Azidoethoxy)-ethoxy]-benzoate (7). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.60; 1H NMR (300 MHz, CDCl3) δ 3.23 (t, 2H, J = 4.8 Hz, CH2-N), 3.37 (t, 2H, J = 4.8 Hz, CH2-O), 3.36 (t, 2H, J = 4.8 Hz, CH2-OMe), 3.88 (t, 2H, J = 4.8 Hz, CH2-OMe), 3.88 (t, 2H, J = 4.8 Hz, CH2-OMe), 4.14 (t, 2H, J = 4.8 Hz, CH2-O), 6.71 (t, 1H, J = 2.1 Hz, CHortho Ar), 7.20 (3H, J = 2.1 Hz, CHortho Ar); MS calculated for C19H21N2O8 [M+H]+ m/z 395.16, found 395.12.

4.4.1.3. Methyl 3,4,5-Tris-[2-(Azidoethoxy)-ethoxy]-benzoate (8). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.58; 1H NMR (300 MHz, CDCl3) δ 3.37-3.42 (m, 6H, 3 CH2-N), 3.89-3.91 (m, 6H, 3 CH2-O, OCH3), 4.18-4.26 (m, 6H, 3 CH2-O), 7.30 (2H, 2 CHortho Ar); MS calculated for C21H21N2O8 [M+H]+ m/z 524.22, found 524.18.

4.4.2. General method for saponification reaction (Step B). To a solution of methyl ester derivative 5, 7 or 9 (3 mmol, 1.0 equiv) in MeOH (30 mL) was added KOH (6 mmol, 2.0 equiv) and the mixture was refluxed for 24 hours. The crude material was cooled at room temperature, neutralized with Amberlite IR 120 ion exchange resin. The reaction was filtered and the resin was washed with MeOH (3 x 10 mL). The filtrate was concentrated under reduced pressure to give the desired clickable aromatic scaffold (6, 8 or 10) as light-yellowish oil in yield up to 93%. Compounds 5, 7 and 9 were characterized as reported below.

4.4.2.1. 4-[2-(Azidoethoxy)-ethoxy]-benzoic acid (6). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.46; 1H NMR (300 MHz, CDCl3) δ 3.43 (t, 2H, J = 4.8 Hz, CH2-N), 3.77 (t, 2H, J = 4.8 Hz, CH2-O), 3.91 (t, 2H, J = 4.8 Hz, CH2-OMe), 2.39 (t, 2H, J = 4.8 Hz, CH2-OMe), 6.98 (d, 2H, J = 9.0 Hz, 2 CHmeta Ar), 8.07 (d, 2H, J = 9.0 Hz, 2 CHortho Ar); MS calculated for C13H11N2O5 [M+H]+ m/z 252.09, found 252.04.

4.4.2.2. 3,5-Bis-[2-(Azidoethoxy)-ethoxy]-benzoic acid (8). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.38; 1H NMR (300 MHz, CDCl3) δ 3.43 (t, 4H, J = 4.8 Hz, 2 CH2-N), 3.76 (t, 4H, J = 4.8 Hz, 2 CH2-O), 3.88 (t, 4H, J = 4.5 Hz, 2 CH2-OMe), 4.17 (t, 4H, J = 4.5 Hz, 2 CH2-OMe), 6.77 (3H, 1H, CHpara Ar), 7.27 (2H, 2 CHortho Ar); MS calculated for C15H13N2O7 [M+H]+ m/z 381.15, found 381.09.

4.4.2.3. 3,4,5-Tris-[2-(Azidoethoxy)-ethoxy]-benzoic acid (10). TLC (EtOAc/petroleum ether, 40/60): Rf = 0.32; 1H NMR (300 MHz, CDCl3) δ 3.37-3.43 (m, 6H, 3 CH2-N), 3.73-3.77 (m, 6H, 3 CH2-O), 3.83-3.91 (m, 6H, 3 CH2-O), 4.22-4.30 (m, 6H, 3 CH2-O), 7.38 (s, 2H, 2 CHortho Ar); MS calculated for C19H19N2O8 [M+H]+ m/z 510.20, found 510.16.
4.5.1. Magnetic stirring method (Tables 2-4). To a solution of clickable aromatic scaffold 6, 8 or 10 (0.1 mmol, 1.0 equiv.) and ZnTPP 2 (1.0 equiv per azide function) in appropriate solvent (5 mL) was added CuOxSO₅·H₂O (0.1 equiv per azide function) and Na ascorbate (0.5 equiv per azide function). The mixture was stirred at appropriate temperature for some time under a nitrogen atmosphere or not (see Tables 2-4). At the end of the reaction and after cooling to room temperature, the mixture was washed with water (3 x 20 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography using a stepwise gradient of MeOH in CH₂Cl₂ (2-10%, v/v) to provide the corresponding building block ([ZnTPP], [ZnTPP]; or [ZnTPP]) as dark purplish powder.

4.5.2. Microwave method (Tables 2-4). Clickable aromatic scaffold 6, 8 or 10 (0.05 mmol, 1.0 equiv.), ZnTPP 2 (1.0 equiv per azide function), CuOxSO₅·H₂O (0.1 equiv per azide function) and Na ascorbate (0.5 equiv per azide function) and appropriate solvent (2.5 mL) were mixed in a microwave tube with a magnetic stir bar. The reaction tube was then fitted with its screw cap, and stirred under a nitrogen atmosphere or not (see Tables 2-4). The reaction was carried out under microwave irradiation at 300 W and appropriate temperature for 45 or 90 minutes per round (see Tables 2-4). After cooling to room temperature, the crude reaction mixture was treated and purified in the same way as magnetic stirring method.

4.5.3. [ZnTPP], [ZnTPP] and [ZnTPP] building blocks were characterized as reported below.

4.5.3.1. [ZnTPP] building block. TLC (CH₃Cl/MeOH, 95/5): Rf = 0.38; ¹H NMR (300 MHz, DMSO-d₆): δ 3.83 (t, 2H, J = 5.1 Hz, CH₂-nitro), 3.94 (t, 2H, J = 5.1 Hz, CH₂-O), 4.18 (t, 2H, J = 5.1 Hz, CH₂-O-Ar), 4.66 (t, 2H, J = 5.1 Hz, O-CH₂-CH₂-triazole), 4.68 (d, 2H, J = 5.7 Hz, triazole-CH₂-NH), 7.04 (d, 2H, J = 8.7 Hz, 2 CHortho of the benzoic acid unit), 7.78-7.81 (m, 9H, 9 CH), 7.89 (d, 2H, J = 8.7 Hz, 2 CHortho of the benzoic acid unit), 8.16-8.33 (m, 11H, 10 CH, CH triazole ring), 8.74-8.79 (m, 8H, 8 CH), 9.38 (t, 1H, J = 5.7 Hz, NH-CH₂-triazole), 12.4 (br s, 1H, CO₂O), MS calculated for C₆₂H₅₀N₁₂O₄Zn [M+H]+, m/z 948.26, 632.51 and 474.43.

4.5.3.2. [ZnTPP] building block. TLC (CH₃Cl/MeOH, 95/5): Rf = 0.26; ¹H NMR (300 MHz, DMSO-d₆): δ 3.72 (t, 4H, J = 5.1 Hz, 2 CH₂-O), 3.87 (t, 4H, J = 5.1 Hz, 2 CH₂-O), 4.09 (t, 4H, J = 5.1 Hz, 2 CH₂-O-Ar), 4.53 (t, 4H, J = 5.1 Hz, 2 O-CH₂-CH₂-triazole), 4.64 (d, 4H, J = 5.7 Hz, 2 triazole-CH₂-NH), 6.72 (br s, 1H, CHortho of the benzoic acid unit), 7.08 (br s, 2H, 2 CHortho of the benzoic acid unit), 7.76-7.80 (m, 18H, 18 CH), 7.95-8.31 (m, 22H, 20 CH, 2 CH triazole ring), 8.72-8.77 (m, 16H, 16 CH), 9.41 (t, 2H, J = 5.7 Hz, 2 NH-CH₂-triazole); MS calculated for C₁₁₁H₆₅N₁₀O₇Zn [M+2H]⁺, [M+3H]³⁺ and [M+4H]⁴⁺, m/z 948.26, 632.51 and 474.43.

4.5.3.3. [ZnTPP] building block. TLC (CH₃Cl/MeOH, 95/5): Rf = 0.21; ¹H NMR (300 MHz, DMSO-d₆): δ 3.73-3.84 (m, 6H, 3 CH₂-O), 3.89-3.98 (m, 6H, 3 CH₂-O), 4.08-4.19 (m, 6H, 3 CH₂-O-Ar), 4.53-4.60 (m, 6H, 3 O-CH₂-CH₂-triazole), 4.62-4.69 (m, 6H, 3 triazole-CH₂-NH), 7.25 (s, 2H, 2 CHortho of the benzoic acid unit), 7.72-7.79 (m, 27H, 27 CH), 8.09-8.30 (m, 33H, 30 CH, 3 CH triazole ring), 8.74-8.77 (m, 24H, 24 CH), 9.36 (t, 3H, J = 5.7 Hz, 3 NH-CH₂-triazole); MS calculated for C₁₆₈H₁₀₄N₁₂O₇Zn [M+2H]²⁺, [M+3H]³⁺ and [M+4H]⁴⁺, m/z 1391.38 and 927.92 found 1391.40 and 927.90.

4.6. Azide-functionalization of [ZnTPP] ([(ZnTPP)-N3]. [ZnTPP] (101 mg, 1.0 equiv, 0.1 mmol), EDC.HCl (38 mg, 2.0 equiv, 0.2 mmol) and NHS (46 mg, 4.0 equiv, 0.4 mmol) were dissolved in 5 mL DCM/methanol (4:1) and microwaved at 300 W, 40°C during 30 minutes. The coupling agents, EDC.HCl (19 mg, 1.0 equiv, 0.1 mmol) and NHS (23 mg, 2.0 equiv, 0.2 mmol), were added in extra and the microwave reaction was continued for 60 minutes. The reaction was monitored by TLC [Rf: 0.65 in DCM/methanol (93:7) for P1-clicked-NHS] and subsequently 3-azido-1-propanamine (50 mg, 0.5 equiv, 0.5 mmol) was added into the reaction vessel and the coupling was also conducted in microwave (300 W, 60°C, 120 minutes). The crude product was washed with 1M HCl (5 mL), saturated NaHCO₃ (5 mL) and water (3 x 5 mL). The crude mixture was dried over MgSO₄ and purified by flash column chromatography (CH₂Cl₂/MeOH, 95/5) to give [ZnTPP]-N₃ in 72% yield (79 mg, 0.072 mmol) as a purple powder. TLC (CH₂Cl₂/MeOH, 95/5): Rf = 0.56; ¹H NMR (300 MHz, DMSO-d₆): δ 1.49-1.56 (m, 2H, 2 CH₂-CH₂-CH₂), 3.28-3.40 (m, 4H, 4 CH₂-CH₂-CH₂-N₃), 3.80 (t, 2H, J = 5.1 Hz, CH₂-O), 3.94 (t, 2H, J = 5.1 Hz, CH₂-O), 4.16 (t, 2H, J = 5.1 Hz, CH₂-O-Ar), 4.60 (t, 2H, J = 5.1 Hz, O-CH₂-CH₂-triazole), 4.68 (d, 2H, J = 5.7 Hz, triazole-CH₂-NH), 7.02 (d, 2H, J = 8.7 Hz, 2 CHortho of the p-hydroxybenzamide unit), 7.77-7.84 (m, 11H, 9 CH, 2 CHortho of the p-hydroxybenzamide unit), 8.12-8.35 (m, 12H, 10 CH, CH triazole ring, NH-CH₂-CH₂), 8.75-8.78 (m, 8H, 8 CH), 9.41 (t, 1H, J = 5.7 Hz, NH-CH₂-triazole); MS calculated for C₁₁₁H₆₅N₁₀O₇Zn [M+H]+, m/z 1091.34, found 1091.38.

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6. Supplementary data

Supplementary data related to this article can be found in the online version, at doi:10.1016/j.tet.2016.xxx.xxx.

7. References and notes