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An efficient route to VEGF-like peptide porphyrin conjugates via microwave-assisted 'click-chemistry'

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ABSTRACT

Synthetic cyclopeptides, and particularly those derived from VEGF sequence, present considerable interest for the development of nanodevices devoted to tumour imaging or targeting. In order to provide selective peptide-targeted tetrapyrrolic structures, we designed two *meso*-porphyrin derivatives anchored to a 17-residue-long cyclopeptide, potent antagonist of VEGF receptors, via a flexible tetraethylene glycol chain. Anchoring was achieved by two different strategies: a classical secondary amide bond formation and microwave-assisted Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition ('click-chemistry'). These compounds appear to be promising candidates for applications in PDT.

1. Introduction

Photodynamic therapy (PDT) is an emerging method aimed at destroying diseased tissue or pathogenic organisms.[1] Applied to a variety of human disorders, this curative approach has received considerable attention in recent years for fighting cancer.[2] This technique relies upon accumulation of photosensitizing molecules, such as porphyrins, into tumours followed by exposure of the affected area to visible light. Upon light, the excited state of the photosensitizer generates singlet oxygen that in turn induces cell damage and ultimately leads to cell death.[3] Healthy cells, however, are also able to uptake photosensitizers, leading to systemic and prolonged photosensitization syndromes, and therefore to severe limitations of PDT.[4] As a result, more selective photosensitizers, named third-generation photosensitizers are desired.[5] Until now, most of the efforts in the development of tumour-targeting photosensitizers have focused on the targeting of markers overexpressed by tumour cells themselves.[6] Indeed neoangiogenesis is a key phenomenon in regard to tumour growth. As capillaries supply oxygen and nutrients to cancer cells, destroying neovessels by PDT appears as an attractive goal. To this aim, we have designed synthetic routes to porphyrin derivatives designed for tumour targeting and more specifically neovascularization.

A novel and potentially powerful approach to improving selective delivery of porphyrins consists in conjugating these molecules to oligopeptide vectors,[7] preferably with cyclic structures[8] in order to increase their in vivo half life.[9] Biological functions of vascular endothelial growth factor (VEGF) are mediated through binding to several receptors, among which Type 2 VEGF receptor (VEGF-R2) is the most efficient towards tumoural neoangiogenesis. These interactions are mainly achieved through three basic residues: R82, K84 and H86. We have previously demonstrated that synthetic cyclopeptides, designed from the structural requirements for VEGF activity, present a very high affinity for VEGF-R2 and subsequently a high level of antitumoural in vivo activity.[10] CBO-P11, one of these cyclopeptides, is presently under preclinical development as anticancer agent and studies to use it for anticancer drug delivery or tumour imaging are in progress.

Abbreviations: Abs_{301}, absorbance at 301 nm; AcOH, acetic acid; All, allyl; Boc, *tert*-butoxycarbonyl; Da, dalton; DIEA, *N*,*N*-diisopropylethylamine; DMAc, *N*,*N*-dimethylacetamide; DMF, *N*,*N*-dimethylformamide; EDC, 1-ethyl-3(3′-dimethylaminopropyl)carbodiimide; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MALDI, matrix-assisted laser desorption/ionization; MsCl, mesyl chloride; NHS, *N*-hydroxysuccinimide; NMM, *N*-methylmorpholine; NMR, nuclear magnetic resonance; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PDT, PhotoDynamicTherapy; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; SPPS, solid-phase peptide synthesis; TEG, triethyleneglycol; THF, tetrahydrofuran; TIS, triisopropylsilane; Trt, trityl; VEGF, vascular endothelial growth factor; VEGF-R2, vascular endothelial growth factor receptor 2.

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The main aim of the present work is the design and synthesis of two new bio-conjugate photosensitizers consisting of *meso*-porphyrin derivatives bearing an ethylene glycol spacer arm attached to CBO-P11 (Fig. 1). Two synthetic strategies are depicted in: grafting of the tetraethylene glycol spacer arm by classic peptidic synthetic reaction between carboxyphenylporphyrin **8** and the amino function of spacer (Schemes 1 and 2), and microwave-assisted Cu(I)-catalyzed alkyne dipolar cycloaddition ('*click-chemistry*') (Schemes 1 and 3). These two porphyrins CBO-P11 derivatives were characterized by ^{1}H NMR, MALDI Mass spectrometry, absorption and fluorescence spectroscopies in aqueous solution, and we tested their ability to produce singlet oxygen upon illumination.

2. Results and discussion

2.1. Synthesis of CBO-P11

CBO-P11 was synthesized as previously described, in order to prevent chemical modifications of essential residues occurred in natural VEGF that interacting with VEGF-R2 receptors (Arg82, Lys84 and His86).[11] Accordingly, cyclopeptide **4** (Fig. 1) thus obtained possesses a single deprotected carboxylic group (γ-carboxyl of the glutamate residue) for controlled introduction of molecular decoration. For this purpose, we performed the total synthesis of CBO-P11 on the extremely acid-labile 2-chlorotrityl chloride resin. This resin was loaded by formation of an ester bond

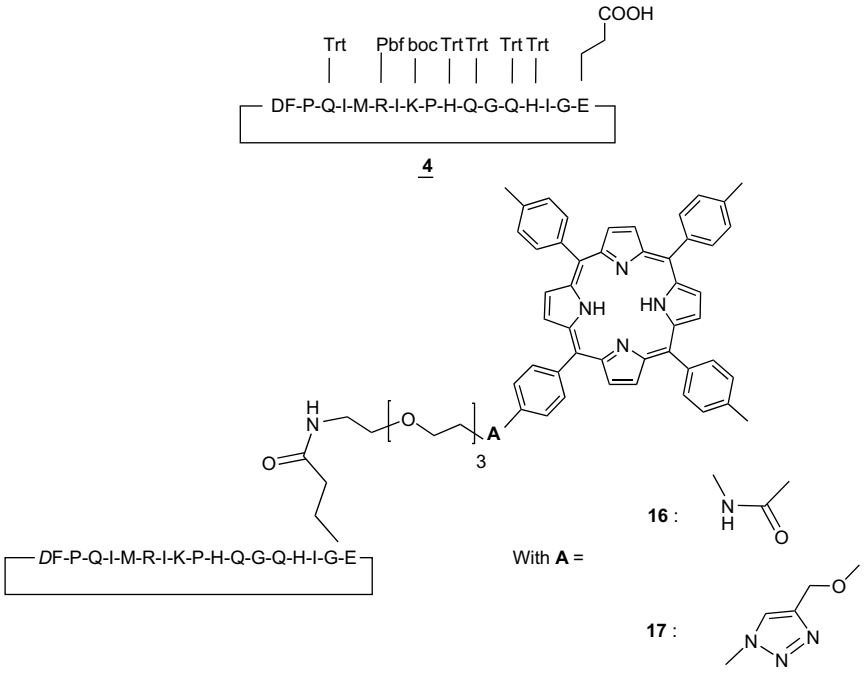
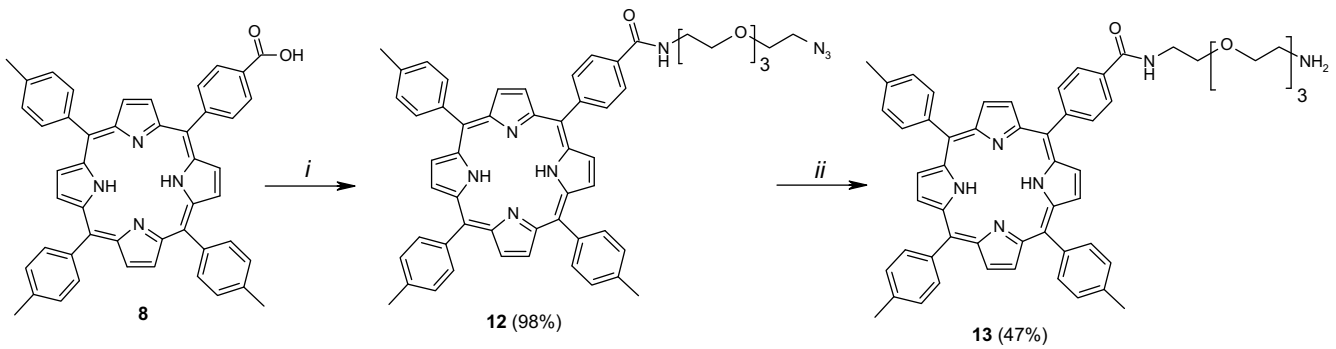
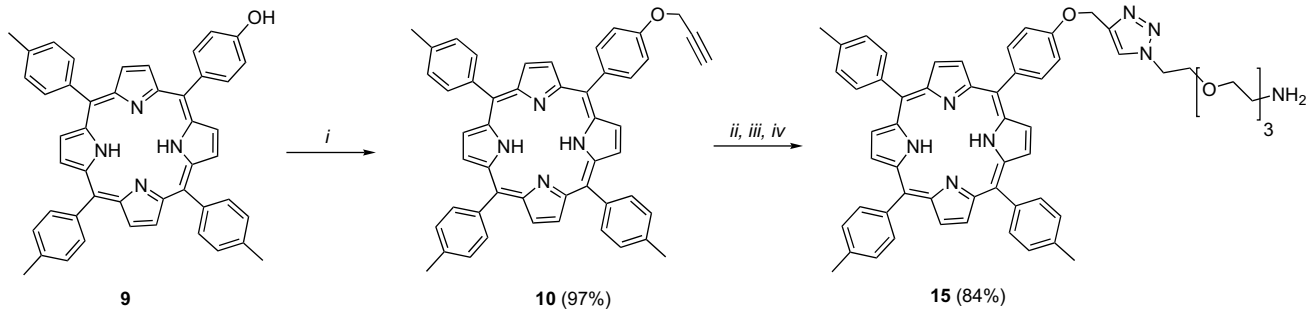


Figure 1. Structures of *cyclo*-VEGI **4** and CBO-11 porphyrins derivatives **16** and **17**.

Scheme 1. Reagents and conditions: (i) MsCl (1.1 equiv), Et_3N in DCM (−5 °C to rt, 90 min); (ii) NaN_3 (4.0 equiv) in EtOH/DMAc (4:1), reflux 6 h; (iii) PPh_3 (1.0 equiv) in Et_2O/HCl (1 M) mixture, rt, 2 h.



Scheme 2. Reagents and conditions: (i) EDC (2.0 equiv), NHS (1.5 equiv) in $CHCl_3$, rt, 45 min, **7** (2.0 equiv), rt, 6 h; (ii) PPh_3 (3.0 equiv) in $CHCl_3$, H_2O (100 μL) in THF (1 mL), rt, 6 h.



Scheme 3. Reagents and conditions: (i) K_2CO_3 (5.0 equiv), propargyl bromide (5.0 equiv) in DMF, rt, 24 h; (ii) AcO_2Zn (3.6 equiv) in THF, reflux 2 h; (iii) **7** (2.3 equiv), $CuSO_4$ (0.5 equiv), sodium L-ascorbate (1.4 equiv) in THF/*t*-BuOH/water (2:1:0.5), MW (60 W, 2 min); (iv) DCM/concd HCl (80:16) mixture, rt, 2 min.

with the γ-COOH group of Fmoc–Glu–OAll, without any coupling reagent in the presence of DIEA. The linear peptide precursor of CBO-P11 was assembled by standard Fmoc chemistry on this loaded resin. Substitution level was monitored by UV quantification and suitable protecting groups were used for every trifunctional amino acid.[12] After SPPS completion and before N-terminal deprotection, removal of allyl (All) protection was carried out according to the method of Kates and co-workers using $Pd(PPh_3)_4$/$CHCl_3$/AcOH/NMM.[13] This reaction was performed under neutral conditions allowing the selective deprotection of the α-COOH group. Treatment of the peptidyl-resin with piperidine removed N-terminal Fmoc protecting group, and allowed on-resin cyclization. The cyclic peptide was then detached from the solid support by repeated incubations in fresh mild-acid medium to prevent any side-chain deprotection stemming from extended exposure of the protected cyclopeptide to residual TFA. In order to establish the yield of peptidic syntheses and to verify its integrity, an aliquot of **4** was deprotected and MALDI analysis was then performed, showing the expected molecular mass (theoretical value: 1998.36 Da, experimental value: 1998.04 Da). Overall yield of the solid-phase synthesis of protected CBO-P11 **4** was established at 65%.

2.2. Synthesis of the linker

The spacer, consisting of hydrophilic oligoethylene glycol chain, was designed in order to minimize steric hindrance between CBO-P11 and porphyrin moieties (**13** and **15**). We first synthesized the hetero-bi-functional linker **7** that possesses a terminal amino group and azido functions (Scheme 1). Azido group allow further coupling options, as the Cu^I-catalyzed Huisgen's cycloaddition with a terminal alkyne, or, after reduction, as amide bond formation with a carboxylic compound. As shown in Scheme 1, linker **7** was prepared in three steps from commercially available tetraethylene glycol (TEG), with an overall yield of 65%. Mesylation of TEG was successfully performed in the presence of triethylamine by using a slight excess of mesyl chloride. Compound **5** was reacted with an excess of sodium azide in a DMAc/ethanol mixture at reflux temperature, providing **6** in quantitative yield. Mono-reduction of the diazido derivative was performed by PPh_3 (1 equiv) in a heterogeneous mixture of Et_2O and acidified water ([HCl]=1 M) providing **7** with good yield (67%).[14]

2.3. Synthesis of *meso*-tritolylporphyrins 8 and 9

Mono-functionalized *meso*-tritolylporphyrins **8** and **9** bearing, respectively, a benzoic acid or phenolic function at one *meso* position of the tetrapyrrolic macrocycle was synthesized by the Little standard method.[15] Condensation of pyrrole (4 equiv) with *para*-tolylaldehyde (3 equiv) and either *p*-carboxybenzaldehyde (1 equiv) or *p*-hydroxybenzaldehyde (1 equiv) in propionic acid; tritolylporphyrins **8** and **9**, which crystallized in the reaction mixture after cooling, were purified and finally obtained with 6% and 4% yield, respectively.

2.4. Coupling *meso*-tritolylporphyrins to the spacer

As shown in Scheme 2, mono-carboxyporphyrin **8** was used to provide the first amino-derivative **13** in two steps by using classical peptidic methodology. Azido derivative **12** was obtained in excellent yield (98%) by coupling **7** to **8** using a slight excess of EDC (2 equiv) and NHS (1.5 equiv). A Staudinger reduction was subsequently performed by treatment of **12** with PPh_3 (3 equiv) providing **13** in 47% yield after flash chromatography.

In order to develop an alternative coupling approach, conjugation of the spacer **7** to the porphyrin macrocycle was performed by '*click-chemistry*' cycloaddition under microwave activation (Scheme 3). Mono-hydroxyphenylporphyrin **9** was *O*-alkylated by Williamson reaction, using propargyl bromide and K_2CO_3 in anhydrous media; by this way, terminal alkyne derivative **10** was obtained in excellent yield (98%). Then **10** was quantitatively converted into **11**, using zinc acetate (3.6 equiv) in THF at reflux in order to avoid copper metallation of porphyrin ring during the subsequent step. Porphyrin derivative **11** was then incubated with azido spacer **7** (Table 1) in presence of $CuSO_4$/sodium L-ascorbate as catalyst.[16] The mixture was activated for 2 min by microwave (60 W) resulting in virtually quantitative formation of 1,2,3-triazole (checked by TLC). Quantitative removal of zinc was achieved by a short acidic treatment of **14**, followed by neutralization provided amino-derivative **15**.

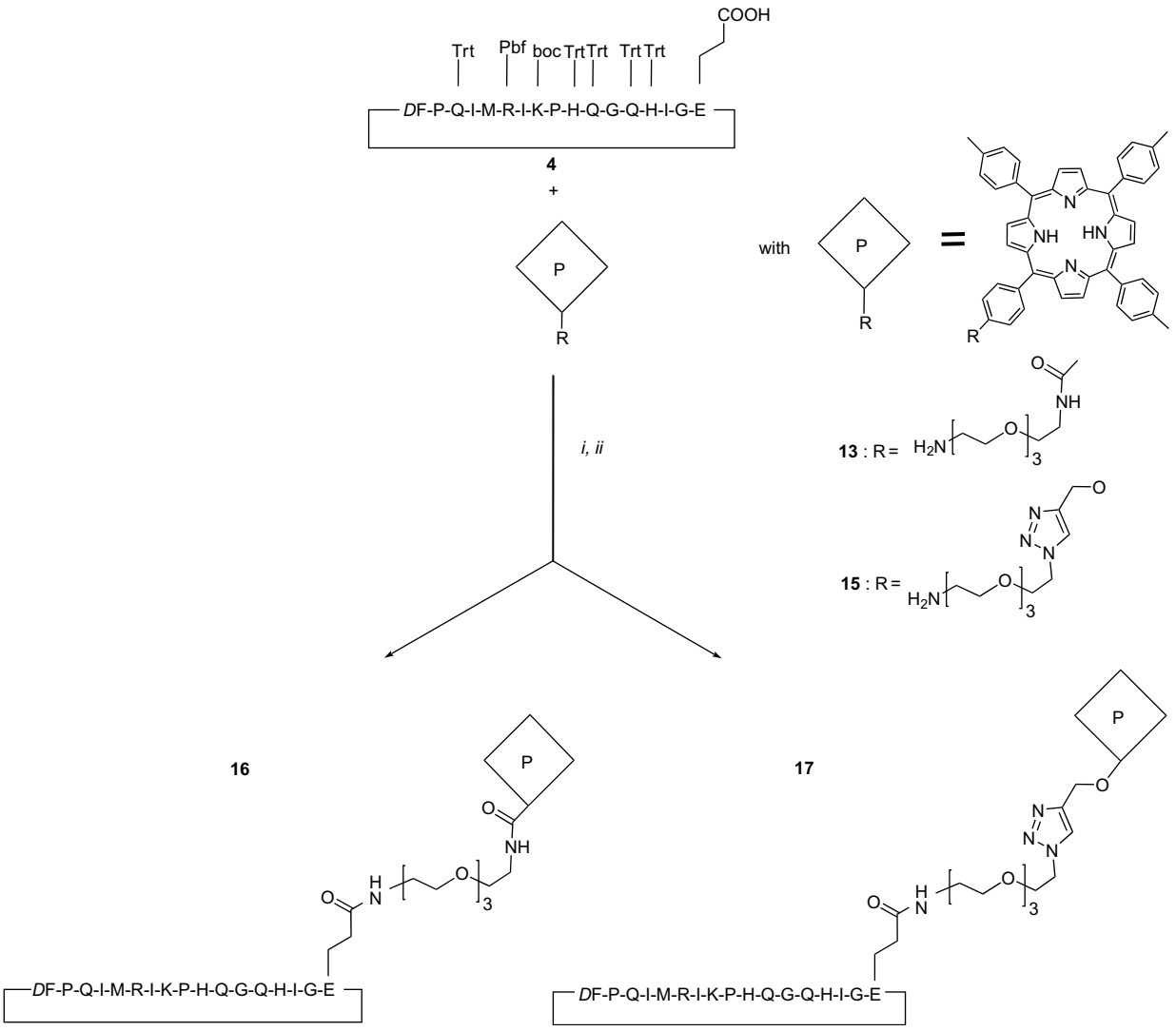
Table 1
Investigation of the Cu(I)-catalyzed 1,3-dipolar cycloaddition of alkyne-functionalized porphyrin **11** (70 μmol) and azide derivative **7** (2.3 equiv) with $CuSO_4$ (0.5 equiv) and sodium L-ascorbate (1.4 equiv) in THF/*t*-BuOH/water (2:1:0.5)

Entry	Temperature	Reaction time	Yield (%)
1	85 °C (60 W)	1 min	52
2	85 °C (60 W)	2 min	84
3[a]	85 °C	24 h	83
4[a]	rt	96 h	77

[a] Without microwave activation.

2.5. Anchoring of cyclopeptide and characterization of the final products

Amino-porphyrin derivatives **13** and **15** were anchored to protected cyclopeptide **4** by amide bond formation, using a fivefold molar excess of EDC and NHS in anhydrous $CHCl_3$. Finally, after deprotection followed by HPLC purification, expected products **16** and **17** were obtained (Scheme 4).



Scheme 4. Reagents and conditions: (i) EDC (5.2 equiv), NHS (5.2 equiv) in $CHCl_3$, rt 30 min., then **13** (or **15**) (1.1 equiv), rt, 40 h.; (ii) phenol (0.75 g) in TFA/thioanisol/water/TIS (10:0.5:0.5:0.25), rt, 3 h, then RP-HPLC.

2.6. Product characterization

All synthesized products (except) were individually characterized by ^{1}H NMR analysis in $CDCl_3$ or DMSO-d_6 (400.13 MHz). The detailed resonance assignments are based on integration and 2D homonuclear COSY experiments. The general assignment for starting porphyrins **8** and **9** are in agreement with data previously described, as well as for compound **7**.[17] For porphyrin derivative **10** bearing a propargyl group and in comparison with the ^{1}H NMR spectrum of **9**, we observed the characteristic signals of protons belonging to β pyrrolic, aryl and tolyl units. Resonance corresponding to CH proton in the terminal position of the propargyl group was identified as a triplet at δ 2.68 ppm. The 2 equiv protons of propargylic methylene group were identified as a broad doublet at δ 4.98 ppm. For compound **15**, the most characteristic signal was the broad singlet of the proton on the triazole ring. ^{1}H NMR spectra of CBO-P11 porphyrin derivatives **16** and **17** have shown the characteristic signals of protons belonging to β pyrrolic, aryl and tolyl units, between 7.20 and 8.85 ppm. We also observed signals attributable to methyl (tolyl units) and NH pyrrole protons. Signals from protons belonging to spacer or peptidic units showed up between 1.2 and 8.4 ppm.

Mass spectrometry of all porphyrin derivatives was performed using the MALDI–TOF (matrix-assisted laser desorption ionization–time-of-flight) technique. Positive ion mass spectra exhibited a base peak corresponding to the intact porphyrin and no fragment ion was detected. Analysis of the isotopic components indicated the presence of a protonated species $[M+H]^+$ with a minor contribution of the radical cation $M^{+\bullet}$, allowing the determination of the molecular mass with an accuracy generally around 0.001%.

UV–vis spectra of all *meso* substituted porphyrin derivatives in their free base form (Table 2) display a Soret band near 420 nm and four less intense visible Q bands with an etio outline. Comparison of

Table 2
UV–visible spectra [λ_{max} ($\epsilon\times10^{-3}$ L mol^{-1} cm^{-1})] of porphyrin derivatives

Compounds	Soret	Q IV	Q III	Q II	Q I
8[a]	420 (689.3)	517 (23.2)	553 (10.6)	592 (5.1)	648 (4.7)
9[a]	418 (363.0)	516 (13.5)	552 (7.4)	592 (4.0)	648 (4.3)
10[a]	427 (380.2)	526 (14.7)	562 (8.2)	603 (4.8)	659 (4.8)
11[a]	425 (460.0)	554 (15.6)		596 (6.2)	
12[a]	420 (189.4)	517 (7.6)	552 (4.1)	591 (2.4)	647 (2.3)
13[a]	420 (185.8)	517 (7.8)	552 (3.8)	591 (2.1)	647 (1.9)
14[a]	430 (336.1)	562 (27.3)		605 (23.0)	
15[a]	420 (572.4)	517 (25.2)	553 (15.0)	592 (8.0)	648 (7.5)
16[b]	438 (181.1)	519 (6.7)	556 (3.0)	589 (1.9)	646 (1.4)
17[b]	431 (334.3)	517 (19.8)	554 (12.4)	592 (6.7)	655 (6.0)

[a] Solvent: $CHCl_3$.
[b] Solvent: H_2O.

UV–vis spectra of porphyrin derivatives **8**–**17** shows that absorption coefficients can be affected by peripheral substituents, which influence the macrocycle (chromophore) delocalized system by the means of various inductive polarization effects, leading to valence tautomerisms.[18] Accordingly, addition of linkers (PEG or triazole-PEG) to initial photosensitizers (**8** and **9**) brought changes in ϵ values of resulting porphyrin-linker derivatives (**13** and **15**). We also observed that these changes depended on the nature of linkers: porphyrin derivatives with the triazole-PEG linker (**15** and **17**) displayed extinction coefficient values larger than those of compounds bearing PEG spacer only (**13** and **16**). Nevertheless, compounds **12** and **13**, bearing the same spacer (PEG) with N_3 (**12**) or NH_2 (**13**) as terminal function, presented similar ϵ values. These behaviours are in agreement with results from Schneider et al., who reported that different spacers (1,6-diaminohexane or 2,2′-(ethylenedioxy)-bis(ethylamine)) grafted on macrocycles induced different extinction coefficient values.[19] Final compounds **16** and **17** displayed a decrease in absorptivity accompanied by a broadening and a red shift of the Soret band. According to the classical excitonic interaction model,[20] the red shift could be attributed to edge-to-edge interaction of the chromophores. These observations imply that aggregation of compounds **16** and **17** occurs in aqueous solutions even at low concentrations, a behaviour shared by numerous porphyrin derivatives.[21] Molar extinction coefficients of these compounds were deduced from slopes of the linear regions of Beer–Lambert plots.

2.7. Singlet oxygen production

Determination of singlet oxygen quantum yield ($\Phi(^1O_2)$) of **16** and **17** was estimated from $^1O_2$ luminescence at 1272 nm in EtOH. Rose Bengal was used as the standard reference.[22] The quantum yield of $^1O_2$ production was calculated to be 0.85 (**16**) and 0.80 (**17**). Production of $^1O_2$ is compatible with stacking effect between macrocycle units as we have showed in previous papers. [7,21] The two compounds present an excellent singlet oxygen quantum yields and appearing as promising candidates for application in PDT. Biological evaluations of these products, such as VEGF-R2 binding tests, intracellular internalization and subcutaneous implanted tumour targeting studies, are in progress.

3. Conclusion

We have presented for the first time a novel procedure to synthesize original photosensitizing bio-conjugates aimed at VEGF-R2 and tumour neovasculature targeting. To this goal, 'Click-Chemistry' cycloaddition is an attractive approach; in addition, we have shown that microwave activation significantly shortens the reaction time compared to classical heating conditions. This methodology provides a versatile approach for the efficient synthesis of various porphyrinic hybrid systems. These compounds can therefore be considered as the first members of a new class of potential antivascular derivatives.

4. Experimental

4.1. General information

Mass spectra were run using a matrix-assisted laser desorption ionization–time-of-flight (MALDI–TOF) Reflex III Brüker apparatus for MALDI spectra and a Micromas VGAutospec-Q for high-resolution mass spectra (HRMS). For the nuclear magnetic resonance (NMR) spectra a Brüker Avance 300 was used. Chemicals shifts are reported in parts per million relative to tetramethylsilane as an internal standard (in NMR description, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad peak). A Genesys 5 Spectronic and a Perkin–Elmer LS-5B spectrophotometer were used for UV measurements. 63–200 mesh silica gel 60 (VWR International) was used as the stationary phase for column chromatography. Infrared (IR) spectra were recorded on a Brüker Tensor 27 (Germany, Ettlingen) spectrometer equipped with an HTS-XT autosampler, a Globar (MIR) source (7 V), a KBr beam splitter, and a DTGS/B detector (18-36-C). The beam diameter at the sample location was 6 mm. In all experiments, a 2.0 cm^{-1} resolution was used and acquisitions were performed using 32 scans in transmittance. The *N*-9-fluorenylmethoxy-carbonyl (Fmoc)-protected amino acids and 2-chlorotrityl chloride resin were purchased from Advanced Chemtech. All trifunctional amino acids were modified by suitable protecting groups: α-carboxyl of glutamic acid by allyl (*All*), ϵ-amino of lysine by *tert*-butyloxycarbonyl (*Boc*), histidine imidazole and glutamine amide group by triphenylmethyl (trityl, *Trt*)[23] and the guanidine function of arginine by 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (*Pbf*).[24] A 0.45 M solution of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in 1-hydroxybenzotriazole (HOBt) was purchased from Applied Biosystems. *N*,*N*-Diisopropylethylamine (DIEA), magnesium sulfate, ninhydrine, phosphorus pentoxide, potassium carbonate, potassium hydroxide, sodium azide, sodium hydrogenocarbonate, thioanisole, trifluoroacetic acid (TFA), triisopropylsilane (TIS) and triphenyl-phosphine were purchased from Aldrich. Diethyl ether, *N*-methylmorpholine (NMM), piperidine, sodium hydroxide, sodium diethyldithiocarbamate and triethylamine were purchased from Avocado. *N*,*N*-Dimethylacetamide (DMAc), *N*-methylpyrrolidone (NMP) and N,*N*-dimethylformamide (DMF) were purchased from Acros. Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), hydrochloric acid, tetrakis-(triphenyl-phosphine)palladium ($Pd(PPh_3)_4$), mesyl chloride (MsCl) and phenol were purchased from Lancaster. Acetonitrile, dichloromethane, absolute ethanol, methanol and petroleum ether [Bp 40–65 °C] were purchased from VWR. During porphyrin synthesis and all photosensitizer coupling steps, light exposure was minimized by wrapping reaction vessels in aluminium foils to limit the occurrence of unwanted side reactions.

4.2. Syntheses

*4.2.1. N-(9-Fluorenylmethoxycarbonyl)-glutamic acid allyl ester anchored to the 2-chlorotrityl chloride resin (**1**)*

This was accomplished by a general procedure recommended by Novabiochem. Fmoc–Glu–OAll (638.2 mg, 1.56 mmol) was dissolved in 10 mL of CH_2Cl_2 with DIEA (1.08 mL, 6.29 mmol). The solution was added to the 2-chlorotrityl chloride resin at room temperature. After 2 h, the resin was filtered off and washed with 3×10 mL of CH_2Cl_2–MeOH–DIEA (17:2:1), followed by CH_2Cl_2 (3×10 mL), DMF (2×10 mL) and then CH_2Cl_2 (2×10 mL). Resin was dried over KOH under vacuum. Then substitution level was determined spectrophotometrically by Fmoc cleavage. An aliquot (5.1 mg) of Fmoc–Glu(resin)–OAll was introduced into a test tube, followed by a solution of 20% piperidine in DMF (500 μL). A same volume of this solution was also added to an empty test tube to serve as a blank. Over the next 15 min, the test tube with the resin was swirled several times to insure a total deprotection. Then DMF was added to both tubes to reach a 50 mL volume. The blank was used as zero at 301 nm with the UV spectrophotometer. Then absorbance of the solution was measured (Abs found=0.501) in order to calculate the substitution level from the formula: $Abs_{301}\times Vol_{(mL)}/[7800\times m_{(g)}]$. Several assays have given consistently the value of 0.63 mmol/g.

4.2.2. Solid-phase peptide synthesis

The linear sequence D-FPQIMRIKPHQGQHIGE was synthesized by Fmoc/*t*-Bu batch solid-phase synthesis on an automated peptide synthesizer (*Applied Biosystems 433A*) starting from the preloaded

Fmoc–Glu(2-ClTrt resin)–OAll (**1**) (397 mg, 0.25 mmol). Subsequent Fmoc amino acids were coupled using a fourfold excess of amino acids activated as HOBt ester by means of a 0.45 M HBTU–HOBt solution.

4.2.3. 'Head to tail' cyclization

Removal of the allyl protecting group was performed before N-terminal Fmoc deprotection. The peptidyl-resin **2** (1.59 g, 0.25 mmol) was dried at 40 °C under vacuum. The reaction vessel was flushed with a stream of argon. $Pd(PPh_3)_4$ (965.83 mg, 0.83 mmol) was dissolved in a solution of $CHCl_3$–AcOH–NMM (37:2:1) (15 mL/g of resin) by bubbling a stream of argon through the mixture. Thereafter this solution was added to the peptidyl-resin under argon and was manually swirled every 15 min for 3 h at room temperature. Then peptidyl-resin was filtered and washed consecutively with 50 mL of 0.5% DIEA–NMP (v:v), 50 mL of 0.5% sodium diethyldithiocarbamate–NMP (w:w), 50 mL of CH_2Cl_2, 50 mL of 1 M HOBt in NMP, 50 mL of NMP, and 50 mL of CH_2Cl_2. The resin was dried overnight under vacuum (over KOH). Fmoc removal was achieved with a 20% piperidine solution in NMP. The peptidyl resin was newly washed with 40 mL of 1 M HOBt in NMP, 40 mL of NMP, and 40 mL of CH_2Cl_2, and was dried overnight under vacuum. Peptidyl resin was weighed (1.11 g, 0.20 mmol) and mixed using a solution of PyBOP (312.18 mg, 0.60 mmol), HOBt (81.07 mg, 0.60 mmol), and DIEA (207.63 mL, 1.2 mmol) in 20 mL of NMP. The mixture was allowed to proceed at room temperature until Kaiser's test was negative. The peptidyl resin (**3**) was washed with 50 mL portions of NMP, CH_2Cl_2, MeOH and was dried under vacuum.

*4.2.4. Synthesis of the protected CBO-P11 free from resin (**4**)*

Final detachment of CBO-P11 from the resin without loss of any side-chain protecting group was performed with a solution of 1% TFA in CH_2Cl_2 (10 mL/g of resin). Cyclopeptidyl resin (1.02 g) was mixed with this solution, and shaken for 2 min. Then the solution was filtered and the filtrate was collected in a flask containing a solution of 10% pyridine in MeOH (20 mL). This was repeated 10 times and cyclopeptidyl resin was successively washed with CH_2Cl_2 (3×30 mL), MeOH (3×30 mL), CH_2Cl_2 (2×30 mL) and MeOH (3×30 mL). Then filtrate was evaporated under reduced pressure to 5% of the volume. Thereafter, 40 mL of cold water were added to the residue to aid precipitation of the product, which was subsequently isolated by filtration through a sintered glass funnel. Product was washed three times with fresh water and dried in a dessiccator under vacuum over KOH, and later over P_2O_5. Then 580 mg (65%) of protected CBO-P11 (**4**) were obtained as a white solid without further purification. A portion of the initial product was treated with 0.75 g of phenol in a TIS–thioanisole–H_2O–TFA solution (0.25:0.50:0.50:10.0) for 3 h at room temperature. The product was precipitated from cold diethyl ether and filtered. MALDI mass spectrometry analysis gave the expected molecular mass for deprotected CBO-P11 (theoretical value: 1998.36 Da; experimental value: 1998.04 Da).

*4.2.5. 1,11-Bis[(methanesulfonyl)oxy]-3,6,9-trioxaundecane (**5**)*

Tetraethyleneglycol (19.5 g; 100 mmol) was dissolved in CH_2Cl_2 (100 mL) with triethylamine (90 mL, 646 mmol). The mixture was cooled to −5 °C and mesyl chloride (17.0 mL; 220 mmol) in CH_2Cl_2 (10 mL) was added drop wise until the temperature of the reaction did not exceed 10 °C. Reaction mixture was then heated at room temperature for 90 min, and poured into water (1000 mL). The organic layer was washed with aqueous hydrochloric acid (1 M, 400 mL), then with saturated aqueous $NaHCO_3$ (3×400 mL), dried over $MgSO_4$ and filtrated. The solvent was evaporated under reduced pressure to give a dark oily residue that was purified over silica gel by flash chromatography (CH_2Cl_2/methanol, 95:5). Compound **5** (34.3 g, 98%) was thus obtained as a brownish oil.

^{1}H NMR ($CDCl_3$, 400.13 MHz, 25 °C) δ: 3.08 (s, 6H, C*H*$_3$), 3.62–3.68 (m, 8H, O–C*H*$_2$–C*H*$_2$–O), 3.76 (t, 4H, J_{H-H}=4.6 Hz, O–C*H*$_2$–CH$_2$–OMs), 4.37 (t, 4H, J_{H-H}=4.4 Hz, C*H*$_2$–OMs).

^{13}C NMR ($CDCl_3$, 100.62 MHz, 25 °C) δ: 37.59 (*C*H$_3$), 68.94 (*C*H$_2$–OMs), 69.47 (O–*C*H$_2$–CH$_2$OMs), 70.42 and 70.55 (O–*C*H$_2$–*C*H$_2$–O).

IR (cm^{-1}): 2912, 2873, 1678, 1134.

*4.2.6. 1,10-Diazido-3,6,9-trioxaundecane (**6**)*

To a solution of compound **5** (34.3 g, 98 mmol) in absolute ethanol (200 mL) and DMAc (50 mL) sodium azide (26.0 g, 400 mmol) was added. The mixture was refluxed for 6 h, and then poured onto water (200 mL) and CH_2Cl_2 (200 mL) with good stirring. The decanted organic layer was washed with water (3×200 mL), brine (200 mL), dried over $MgSO_4$ and filtered. Solvent was evaporated under reduced pressure to give 23.9 g (quantitative yield) of compound **6** as a brownish oil.

^{1}H NMR ($CDCl_3$, 400.13 MHz, 25 °C) δ: 3.39 (t, 4H, J_{H-H}=5.1 Hz, –C*H*$_2$N$_3$), 3.65–3.69 (m, 12H, C*H*$_2$–CH$_2$N$_3$ and –OC*H*$_2$C*H*$_2$–).

^{13}C NMR ($CDCl_3$, 100.62 MHz, 25 °C) δ: 50.30 (–*C*H$_2$N$_3$), 69.95 (–CH$_2$*C*H$_2$N$_3$), 70.49 (OCH$_2$*C*H$_2$–).

IR (cm^{-1}): 2912, 2873, 2106, 1678, 1134.

*4.2.7. 1-Amino-11-azido-3,6,9-trioxaundecane (**7**)*

To a solution of **6** (10.98 g, 45 mmol) in Et_2O (150 mL) and THF (20 mL) was added aqueous HCl (1 M, 200 mL). With good stirring, a solution of PPh_3 (11.84 g, 45 mmol, 1.0 equiv) in Et_2O (100 mL) was added drop wise upon 60 min. After 2 h, the organic layer was decanted, and extracted with aqueous HCl (1 M, 2×50 mL). Aqueous layers were saturated with NaCl, and then made basic (to pH=14) by addition of NaOH pellets and extracted with toluene (3×70 mL). Pooled organic layers were washed with brine and dried over KOH pellets. After evaporation under reduced pressure, the oily residue was purified by flash chromatography over silica gel (CH_2Cl_2–MeOH, 100:0 to 90:10), giving 6.57 g (67%) of **7** as a yellowish oil.

^{1}H NMR ($CDCl_3$, 400.13 MHz, 25 °C) δ: 2.71 (t, 2H, J_{H-H}=5.2 Hz, C*H*$_2$NH$_2$), 3.25 (t, 2H, J_{H-H}=5.1 Hz, C*H*$_2$N$_3$), 3.37 (t, 2H, J_{H-H}=5.0 Hz, C*H*$_2$CH$_2$NH$_2$), 3.48–3.72 (m, 10H, C*H*$_2$C*H*$_2$O, C*H*$_2$CH$_2$N$_3$).

^{13}C NMR ($CDCl_3$, 100.62 MHz, 25 °C) δ: 41.2 (*C*H$_2$NH$_2$), 50.3 (*C*H$_2$N$_3$), 69.9–70.3 (CH$_2$–*C*H$_2$–O and *C*H$_2$CH$_2$N$_3$), 72.8 (*C*H$_2$CH$_2$NH$_2$).

IR (cm^{-1}): 3360, 2909, 2870, 1774, 2110, 1124.

HRMS (FAB$^+$): calculated for $C_8H_{19}N_4O_3$ (MH)$^+$ 219.1457, found 219.1462.

*4.2.8. 5-(4-Carboxyphenyl)-10,15,20-tris(4-methylphenyl)-porphyrin (**8**)*

This compound was obtained by the Little's method.[15] 4-Carboxybenzaldehyde (4.06 g, 27 mmol, 1 equiv) and 4-tolylaldehyde (9.6 mL, 81 mmol, 3 equiv) were dissolved in propionic acid (300 mL) by stirring for 30 min at reflux. To this solution, pyrrole (7.5 mL, 108 mmol, 4 equiv) was added drop wise over 15 min and the mixture was refluxed for an additional 90 min. After cooling, the crude that crystallized out was filtered off, washed with ethanol (3×20 mL), and then purified by flash chromatography on silica gel ($CHCl_3$/abs EtOH, 100–0 to 90–10) to afford 800 mg (4.2%) of product **8** as a purple solid.

UV/visible ($CHCl_3$): λ ($\epsilon\times10^{-3}$) 420 (689.3), 517 (23.2), 553 (10.6), 592 (5.1), 648 (4.7).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ: −2.77 (br s, 2H, N*H*), 2.71 (s, 9H, C*H*$_3$), 7.55 (d, 2H, J_{H-H}=7.8 Hz, H$_{3,5}$ Ar), 7.53 (d, 6H, J_{H-H}=7.4 Hz, H$_{3,5}$ tolyl), 8.33 (d, 2H, J_{H-H}=7.8 Hz, H$_{2,6}$ Ar), 8.48 (d, 6H, J_{H-H}=7.4 Hz, H$_{2,6}$ tolyl), 8.78 (d, 2H, J_{H-H}=4.5 Hz, H$_\beta$ pyr), 8.89 (br s, 6H, H$_\beta$ pyr).

SM (MALDI): m/z=701.6 [M+H]$^+$.

4.2.9. 5-(4-Hydroxyphenyl)-10,15,20-tris(4-methylphenyl)-porphyrin (**9**)

This compounds was synthesized according to the literature as described in previous paper.[25]

4.2.10. 5-[4-(3-Propynyloxy)phenyl]-10,15,20-tris(4-methylphenyl)porphyrin (**10**)

Under argon, compound **9** (130 mg, 193 μmol, 1 equiv) was dissolved in anhydrous DMF (8 mL), then anhydrous K_2CO_3 (140 mg, 970 μmol, 5 equiv) and propargyl bromide (110 μL; 970 μmol, 5 equiv) were added. After 24 h at rt, 50 mL of $CHCl_3$ were added and organic layer was washed with water (3×75 mL), brine (75 mL) and dried over $MgSO_4$. Solvent was evaporated under reduced pressure, giving a solid residue that was purified by flash chromatography over silica gel ($CHCl_3$/EP, 80:20) to afford 133 mg (97%) of **10** as a purple solid.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 427 (380.2), 526 (14.7), 562 (8.2), 603 (4.8), 659 (4.8).

Microanalysis calcd for $C_{50}H_{38}N_4O\cdot 2H_2O$: C, 80.40; H, 5.67; N, 7.50. Found C, 80.32; H, 5.63; N, 7.56.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ: −2.77 (br s, 2H, N*H*), 2.68 (s, 9H, C*H*$_3$), 2.86 (t, 1H, J_{H-H}=2.5 Hz, H propargyl), 4.98 (d, 2H, J_{H-H}=2.2 Hz, OC*H*$_2$), 7.15 (d, 2H, J_{H-H}=8.2 Hz, $H_{3,5}$ Ar), 7.53 (d, 6H, J_{H-H}=7.7 Hz, $H_{3,5}$ tolyl), 8.03 (d, 2H, J_{H-H}=8.2 Hz, $H_{2,6}$ Ar), 8.08 (d, 6H, J_{H-H}=7.7 Hz, $H_{2,6}$ tolyl), 8.84 (br s, 6H, H_β pyr), 8.93 (d, 2H, J_{H-H}=4.4 Hz, H_β pyr).

4.2.11. 5-[4-(3-Propynyloxy)phenyl]-10,15,20-tris(4-methylphenyl)porphyrinato zinc(II) (**11**)

To a well stirred solution of **10** (50 mg, 70 μmol) in THF (25 mL) was added zinc diacetate (56 mg, 255 μmol) and the mixture was refluxed for 2 h. Solvent was evaporated under reduced pressure, then $CHCl_3$ (40 mL) was added to the crude. Organic layer thus obtained was washed with water (3×30 mL) dried over $MgSO_4$ and evaporated to dryness under reduced pressure, affording 54 mg (quantitative yield) of **11** as a purple solid.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 425 (460.0), 554 (15.6), 596 (6.2).

4.2.12. 5-[N-(11-Azido-3,6,9-trioxaundecyl)-4-carboxamidophenyl]-10,15,20-tris(4-methylphenyl)-porphyrin (**12**)

Compound **8** (155 mg, 221 μmol) was dissolved in dry $CHCl_3$ (5 mL), then EDC (90 mg, 442 μmol, 2.0 equiv) and NHS (38 mg, 331 μmol, 1.5 equiv) were added. After 45 min under argon, compound **7** (100 mg, 459 μmol, 2.0 equiv) was introduced and the mixture was stirred for an additional 6 h. Then, 15 mL of $CHCl_3$ were added and organic layer was washed with water (3×20 mL), brine (20 mL) and dried over $MgSO_4$. After evaporation under reduced pressure, the solid residue was purified by flash chromatography on silica gel (CH_2Cl_2 to CH_2Cl_2–MeOH 90:10), affording 195 mg (98%) of compound **12** as a purple solid.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 420 (189.4), 517 (7.6), 552 (4.1), 591 (2.4), 647 (2.3).

Microanalysis calcd for $C_{56}H_{52}N_8O_4\cdot 2H_2O$: C, 71.77; H, 6.02; N, 11.95. Found C, 71.73; H, 5.98; N, 12.01.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ: −2.77 (br s, 2H, N*H*), 2.70 (s, 9H, C*H*$_3$), 3.39 (t, 2H, J_{H-H}=5.2 Hz), 3.48 (t, 2H, J_{H-H}=5.2 Hz), 3.62–3.65 (m, 2H), 3.68–3.70 (m, 2H), 3.72–3.76 (m, 4H), 3.79–3.85 (m, 4H), 7.16 (d, 2H, J_{H-H}=8.3 Hz, $H_{3,5}$ Ar), 7.55 (d, 6H, J_{H-H}=7.8 Hz, $H_{3,5}$ tolyl), 8.08 (d, 6H, J_{H-H}=7.8 Hz, $H_{2,6}$ tolyl), 8.25 (d, 2H, J_{H-H}=8.3 Hz, $H_{2,6}$ Ar), 8.78 (d, 2H, J_{H-H}=4.7 Hz, H_β pyr), 8.86 (br s, 6H, H_β pyr).

SM (MALDI): *m*/*z*=902.10 $[M+H]^+$.

4.2.13. 5-[N-(11-Amino-3,6,9-trioxaundecyl)-4-carboxamidophenyl]-10,15,20-tris(4-methylphenyl)-porphyrin (**13**)

To a solution of **12** (195 mg, 216 μmol) in $CHCl_3$ (5 mL), PPh_3 (170 mg, 3 equiv) was added, followed by water (100 μL, 5.5 mmol) in THF (1 mL). After 6 h at rt, the mixture was evaporated to dryness under reduced pressure. Purification was performed by flash chromatography over silica gel (CH_2Cl_2 to CH_2Cl_2–MeOH 90:10) affording 89 mg (47%) of compound **13** as a purple solid.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 420 (185.8), 517 (7.8), 552 (3.8), 591 (2.1), 647 (1.9).

Microanalysis calcd for $C_{56}H_{54}N_{20}O_4\cdot H_2O$: C, 75.31; H, 6.32; N, 9.41. Found C, 75.28; H, 6.29; N, 9.48.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ: −2.77 (br s, 2H, N*H*), 2.70 (s, 9H, C*H*$_3$), 2.81 (br s, 2H), 3.48 (t, 2H, J_{H-H}=5.2 Hz), 3.62–3.65 (m, 2H), 3.68–3.70 (m, 2H), 3.72–3.76 (m, 4H), 3.79–3.85 (m, 4H), 4.86 (br s, 2H), 7.55 (d, 6H, J_{H-H}=7.8 Hz, $H_{3,5}$ tolyl), 8.08 (d, 6H, J_{H-H}=7.8 Hz, $H_{2,6}$ tolyl), 8.25 (d, 2H, J_{H-H}=8.3 Hz, $H_{2,6}$ Ar), 8.78 (d, 2H, J_{H-H}=4.7 Hz, H_β pyr), 8.86 (br s, 6H, H_β pyr).

SM (MALDI): *m*/*z*=876.16 $[M+H]^+$.

4.2.14. 5-[1-(11-Amino-3,6,9-trioxaundecyl)-4-(4-methyleneoxytriazolyl)phenyl]-10,15,20-tris(4-methylphenyl)porphyrinato zinc(II) (**14**)

In the cylindrical reactor of a *Synthewave 402* apparatus were introduced solutions of **11** (70 μmol) in THF (2 mL), **7** (35 mg, 161 μmol, 2.3 equiv) in *t*-BuOH (1 mL), sodium L-ascorbate (20 mg, 98 μmol, 1.4 equiv) in water (0.2 mL) and copper sulfate (10 mg, 40 μmol, 0.57 equiv) in water (0.3 mL). The mixture was heated by microwave irradiation (60 W, 2 min) then allowed to cool to rt. Addition of water (50 mL) precipitated out a purple solid. It was collected by filtration over a sintered funnel, washed with water (3×20 mL), MeOH (3×20 mL) then Et_2O (3×20 mL). After drying overnight under high vacuum, 59 mg (84%) of pure compound **14** were obtained as a purple solid without further purification.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 430 (336.1), 562 (27.3), 605 (23.0).

4.2.15. 5-[1-(11-Amino-3,6,9-trioxaundecyl)-4-(4-methyleneoxytriazolyl)phenyl]-10,15,20-tris-(4-methyl-phenyl)porphyrin (**15**)

To the solution of **14** (45 mg, 43 μmol) in CH_2Cl_2 (80 mL), concentrated HCl (12 M, 16 mL) was added with good stirring. After 2 min at rt, the mixture was decanted and the aqueous acid layer was discarded. The organic layer was washed with water (2×80 mL) then with saturated aqueous $NaHCO_3$ (50 mL) and dried over Na_2SO_4. After evaporation to dryness under high vacuum, 42 mg (quantitative yield) of **15** were obtained as a purple solid without further purification.

UV/visible ($CHCl_3$): λ (ϵ×10^{-3}) 420 (572.4), 517 (25.2), 553 (15.0), 592 (8.0), 648 (7.5).

Microanalysis calcd for $C_{58}H_{56}N_8O_4\cdot 2H_2O$: C, 72.17; H, 6.26; N, 11.61. Found C, 72.12; H, 6.19; N, 11.69.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ: −2.77 (br s, 2H, N*H*), 2.70 (s, 9H, C*H*$_3$), 2.81 (br s, 2H), 3.48 (t, 2H, J_{H-H}=5.0 Hz), 3.62–3.65 (m, 2H), 3.68–3.70 (m, 2H), 3.72–3.76 (m, 4H), 3.79–3.85 (m, 4H), 4.36 (s, 2H), 4.88 (br s, 2H, OC*H*$_2$), 7.19 (s, 1H triazole), 7.38 (d, J_{H-H}=8.5 Hz, 2H, $H_{3,5}$ Ar), 7.55 (d, 6H, J_{H-H}=7.8 Hz, $H_{3,5}$ tolyl), 8.09 (d, 2H, J_{H-H}=8.5 Hz, $H_{2,6}$ Ar), 8.13 (d, 6H, J_{H-H}=7.8 Hz, $H_{2,6}$ tolyl), 8.84 (br s, 6H, H_β pyr), 8.92 (d, 2H, J_{H-H}=4.4 Hz, H_β pyr).

SM (MALDI): *m*/*z*=930.20 $[M+H]^+$.

4.2.16. General procedure followed for syntheses of **16** and **17**

Under argon, EDC (20 mg, 110 μmol, 5 equiv) and NHS (12 mg, 110 μmol, 5 equiv) were added with good stirring to a solution of **4** (77 mg, 21 μmol) in $CHCl_3$ (6 mL). After 30 min at rt, a solution of porphyrin **13** or **15** (1.1 equiv) in $CHCl_3$ (3 mL) was added to the mixture and completion of the reaction was checked by TLC. The mixture was then diluted with $CHCl_3$ (30 mL), washed with water (3×30 mL), brine (2×30 mL) and dried over anhydrous K_2CO_3. Evaporation under vacuum afforded the crude, which was treated with phenol (0.75 g) in a TIS–thioanisole–H_2O–TFA (1:2:2:40)

mixture for 3 h at rt. After concentration under reduced pressure the residue was precipitated from cold diethyl ether and filtered. The product was loaded onto a preparative Hibar Purosphere C18 column. The elution was achieved under the following conditions: eluent A, 0.05% TFA in water; eluent B, 0.05% TFA in CH_3CN/H_2O (70:30); A/B gradient 60/40 to 0/100 over 40 min; flow rate, 4 mL/min; detection was performed at 214 nm and 438 nm for **16** or 431 nm for **17**.

4.2.16.1. Compound ***16***. Reaction with **13** (20 mg, 24 μmol, 1.1 equiv) has afforded the crude (96 mg) as a purple solid. After deprotection, RP-HPLC (detections at 214 and 438 nm, retention time 34 min) and lyophilization, 14 mg (23%) of the expected compound **16** were obtained as a purple solid.

UV/visible (H_2O): λ ($\epsilon\times10^{-3}$) 438 (181.1), 519 (6.7), 556 (3.0), 589 (1.9), 646 (1.4).

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ: −2.90 (br s, 2H, N*H*), 2.59 (s, 9H, CH_3 tolyl), 1.20–8.50 (m, H spacer and H amino acids), 7.20–8.85 (m, 24H, H tolyl, H Ar, H_β pyr).

SM (MALDI): mass spectrometry analysis shows the expected molecular mass (theoretical value: 2854.28 Da; experimental value: 2855.67 Da).

4.2.16.2. Compound ***17***. Reaction with **15** (25 mg, 24 μmol, 1.1 equiv) has afforded the crude (110 mg) as a purple solid. After deprotection, RP-HPLC (detections at 214 and 431 nm, retention time 37 min) and lyophilization, 19 mg (26%) of the expected compound **17** were obtained as a purple solid.

UV/visible (H_2O): λ ($\epsilon\times10^{-3}$) 431 (334.3), 517 (19.8), 554 (12.4), 592 (6.7), 655 (6.0).

^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ: −2.96 (br s, 2H, N*H*), 2.59 (s, 9H, CH_3 tolyl), 1.20–8.40 (m, H spacer and H amino acids), 7.20–8.85 (m, 24H, H tolyl, H Ar, H_β pyr).

SM (MALDI): mass spectrometry analysis gave the expected molecular mass (theoretical value: 2908.14 Da; experimental value: 2909.54 Da).

4.3. Determination of singlet oxygen quantum yield $\Phi(^1O_2)$

Quantum yield of $^1O_2$ production was determined by direct analysis of the $^1O_2$ near-infrared luminescence at 1270 nm. Excitation occurred with a Xe-arc, the light was separated in a SPEX Fluorolog-3 F222, 0.22 μm double monochromator. A high pass filter (1060 nm) was placed between the sample and the detector. The detection at 1270 nm was done through a PTI S/N 1565 monochromator, and the emission was monitored by a liquid nitrogen-cooled Ge-detector model (EO-817L, North Coast Scientific Co). The absorbance of the reference solution (Bengal pink in EtOH Φ_F ($^1O_2$)=0.68)[26] and the sample solution (at 415 nm) were set equal (between 0.2 and 0.5) by dilution.

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