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Design and photophysical properties of new RGD targeted tetraphenylchlorins and porphyrins

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Abstract

The synthesis, characterization, fluorescence, and singlet oxygen quantum yields of tetraphenylporphyrin and tetraphenylchlorin coupled to RGD type peptide are reported. C-terminus protected RGD derivatives were synthesized in liquid phase at the gram scale via an efficient convergent process. C-terminus unprotected RGD derivatives were synthesized on solid support. The UV–vis, fluorescence, and singlet emission spectra showed that the photophysical properties of the photosensitizers were retained in all compounds and some of them are very promising for potential PDT applications.

Keywords: Porphyrin; Chlorin; Photodynamic therapy; RGD peptide; Cyclic RGD peptide; Fluorescence spectroscopy; Singlet oxygen

1. Introduction

Photodynamic therapy (PDT) is a promising treatment for a variety of diseases mainly in anticancer field.[1] PDT requires a photosensitizer (PS), light, and molecular oxygen, whose combined action results in the formation of reactive oxygen species, singlet oxygen being the main mediator of cellular death.[2] One of the main drawbacks of the PS is their lack of selectivity toward cancer cells. Until now, most of the efforts in the development of tumor targeting photosensitizers have focused on the targeting of markers overexpressed by tumor cells themselves.[3] Nevertheless, the vascular effect is thought to play a major part in the eradication of some vascularized tumors by PDT.[4] Therefore, anti-neovascular PDT could be proposed for a large number of vascularized tumors and is a promising strategy in cancer treatment that has received considerable attention in the recent years.[5]

For this purpose, we have devised a synthetic route to porphyrin derivatives designed for targeting tumors and more specifically neovascularization that feeds cancer cells. We have initiated a program of research aimed at developing photosensitizers targeting endothelial cells. One strategy consists in targeting vascular endothelial growth factor (VEGF) receptor or co-receptor[6] since VEGF is one of the most potent direct-acting angiogenic proteins known,[7] up-regulated in the majority of cancers. Another strategy aims at targeting $\alpha_v\beta_3$ integrin, a heterodimeric transmembrane glycoprotein receptor, which is overexpressed in actively proliferating endothelial cells, in and around tumor tissues.[8]

In the mid 1980s, the H-Arg-Gly-Asp-OH (RGD) tripeptide sequence has been identified as a major cell adhesion recognition motif.[9] This finding, together with the subsequent discovery of the integrin gene family of cell adhesion receptors that recognize the RGD motif has established a highly conserved cell adhesion system regulating cell migration, growth, differentiation, and apoptosis.[10] Not surprisingly, to date a large variety of RGD derivatives[11] and mimetics[12] have been designed

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that are studied as therapeutics for the treatment of thrombosis,[13] tumor metastasis,[14] and infectious diseases,[15] or serve as adhesive devices in tissue engineering, tissue renewal[16] or tissue attachment to prostheses,[17] as well as in tumor diagnosis[18] or photodynamic therapy,[19] or inhibition of secondary cataract.[20] For the latter, we have shown that the protection of terminal NH_2- or/and $-COOH$ groups of RGD by simple derivations such as acetyl, Boc, or benzylamide resulted in interesting evolutions of the inhibitory effect, versus unprotected RGD, and versus the RGDS tetrapeptide, which is commonly used as reference compound in the literature.[9,21]

We have developed rationalized and versatile procedures, to produce these target RGD derivatives. Classical solid-phase synthesis has been used to synthesize the RGD peptide and to couple it to a porphyrin or a chlorin (compounds **5** and **6**, Scheme 1). A possible way of improving the affinity for $\alpha_v\beta_3$ integrin is to protect the C-terminal part of the peptide. In liquid phase, a *C*-benzylamide RGD peptide has been synthesized and coupled to monocarboxylic tetraphenylporphyrin succinimidyl ester (TPP-COOSu **1**) and chlorin (Chl-COOSu **2**) via two different spacers (Ahx **3** and PEG-Su **4**), to evaluate the influence of the type of the linker on the phototoxicity of the new targeted photosensitizers for PDT applications (compounds **19**, **20a**,**b**, **25** and 2**6**, Scheme 3). Another way to improve the affinity of the targeted photosensitizer for $\alpha_v\beta_3$ integrin is to use a cyclic peptide since it is generally accepted that cyclic RGD peptides display a high activity compared to the linear counterparts. Then, a cyclic peptide has been synthesized on solid phase and coupled to a chlorin (compound **33**).[22]

2. Results and discussion

2.1. Syntheses

Aiming at comparing their photophysical properties, we synthesized some RGD targeted photosensitizers for potential PDT applications.

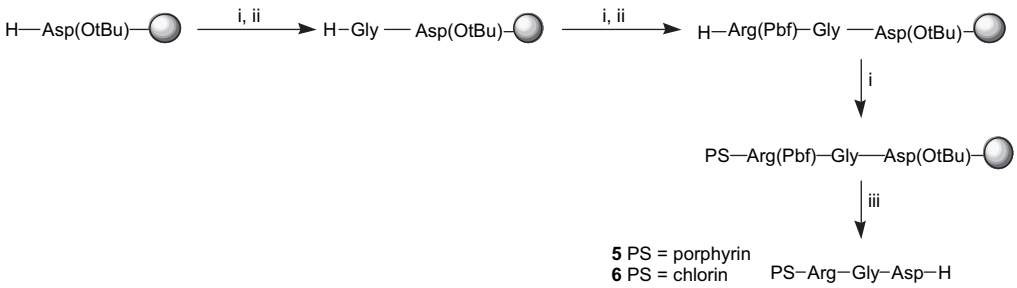
Compounds **5** and **6** were synthesized on a multichannel peptide synthesizer, according to a classical Fmoc/^{t}Bu solid-phase methodology (Scheme 1).

Assembly of the protected peptide chains was carried out using the in situ neutralization protocol described previously.[23] Double coupling was performed using a threefold excess of *N*-Fmoc-amino acid (except during the photosensitizer coupling stage: threefold excess and single coupling) and activation reagents 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium-tetrafluoroborate (TBTU) (3 equiv), 1-hydroxybenzotriazole (HOBt) (3 equiv), and *N*,*N*-diisopropylethylamine (DIEA) (9 equiv) in dimethylformamide (DMF). During the photosensitizer coupling step, light exposure was minimized by sealing the reaction vessel in aluminum foil to limit the occurrence of unwanted side reactions. A standard cleavage with trifluoroacetic acid (TFA) and scavengers afforded the crude peptides or the peptide-conjugated photosensitizer. Both isomers of the chlorin, arising from the asymmetrical character of the molecule, could not be separated due to their similar retention time on reverse phase HPLC (15.1 and 15.5 min).

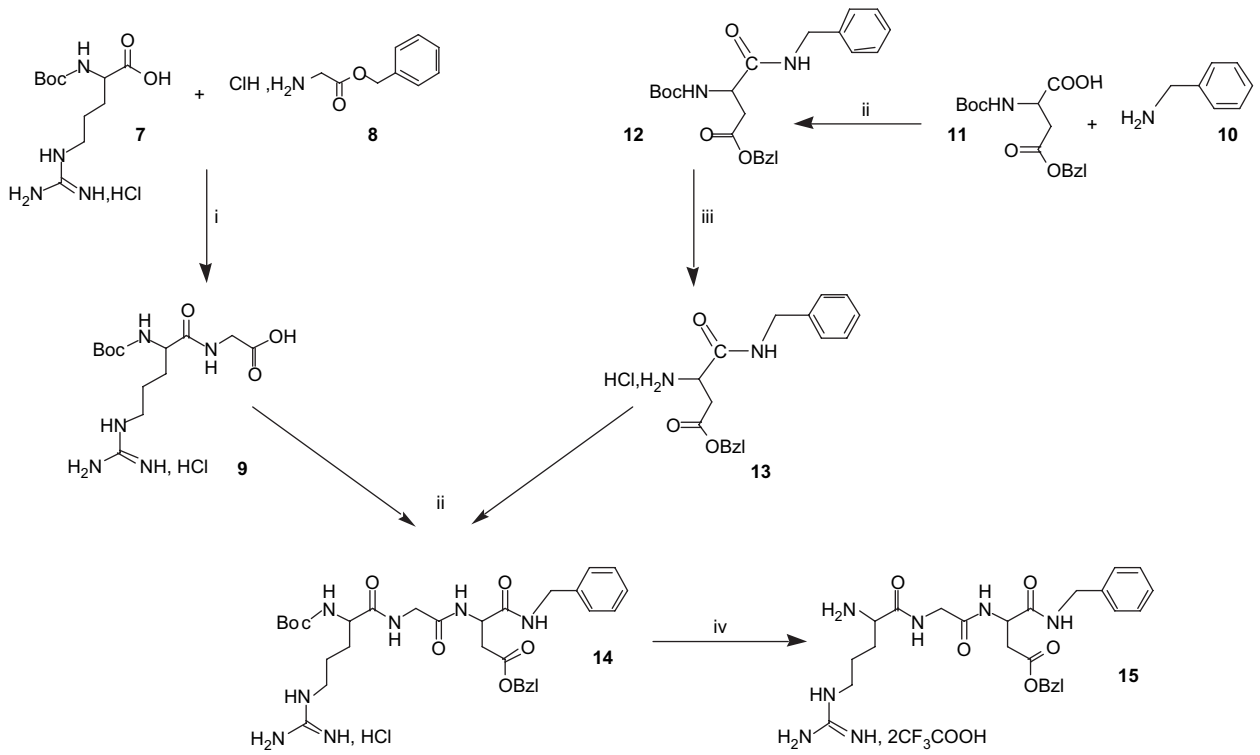
The tripeptide **15** was synthesized in liquid phase at the gram scale using a convergent process resulting in an overall yield of 51% (Scheme 2).

The condensation of Boc-Arg-OH **7** with HCl·H-Gly-OBzl **8** using DCC/HOBt afforded Boc-Arg-Gly-OBzl, which was subsequently hydrogenated to give Boc-Arg-Gly-OH **9** with an overall yield of 83% in a one-pot procedure. Coupling Boc-Asp(OBzl)-OH **11** with benzylamine **10** with DCC/HOBt afforded Boc-Asp(OBzl)-NHBzl **12** in 93% yield. Removal of Boc protecting group with HCl gave HCl·H-Asp-(OBzl)-NHBzl **13**[20a] in 93% yield. Coupling of **9** with **13** was achieved by using the same reagents, combined with tributylamine instead of the commonly used triethylamine. By this way the resulting hydrochloride salt, soluble enough in organic solvents, was easily removed by triturating the crude mixture, while the peptide adhered to the flask. The purification process of **14** was achieved by column chromatography. Lyophilization of the aqueous solution of **14** allowed the full removal of residual organic solvents. Thus, **14** was obtained with a yield of 68%. Classical treatment of **14** by trifluoroacetic acid afforded **15** in 91% yield.

Before coupling to the photosensitizers **1** and **2**, **15** had to be linked with apolar (**3**) or polar (**4**) linkers (Fig. 1, Scheme 3). For this purpose, 6-aminohexanoic acid **3** (Ahx) was *N*-protected to give **16**, and then activated to afford *Z*-Ahx-OSu **17**, as previously described.[24] The latter was condensed with **15** and subsequently hydrogenated to give Ahx-RGD-NHBzl **18** in 40% overall yield. Coupling reaction with TPP-COOSu **1** or Chl-COOSu **2** led to the desired products **19**, **20a**, and **20b** in 35, 20, and 20% yield, respectively. It is noteworthy that the two isomers **20a** and **20b** could be separated by HPLC since their retention time on reverse phase HPLC were different enough (15.9 and 16.9 min). In a similar way,

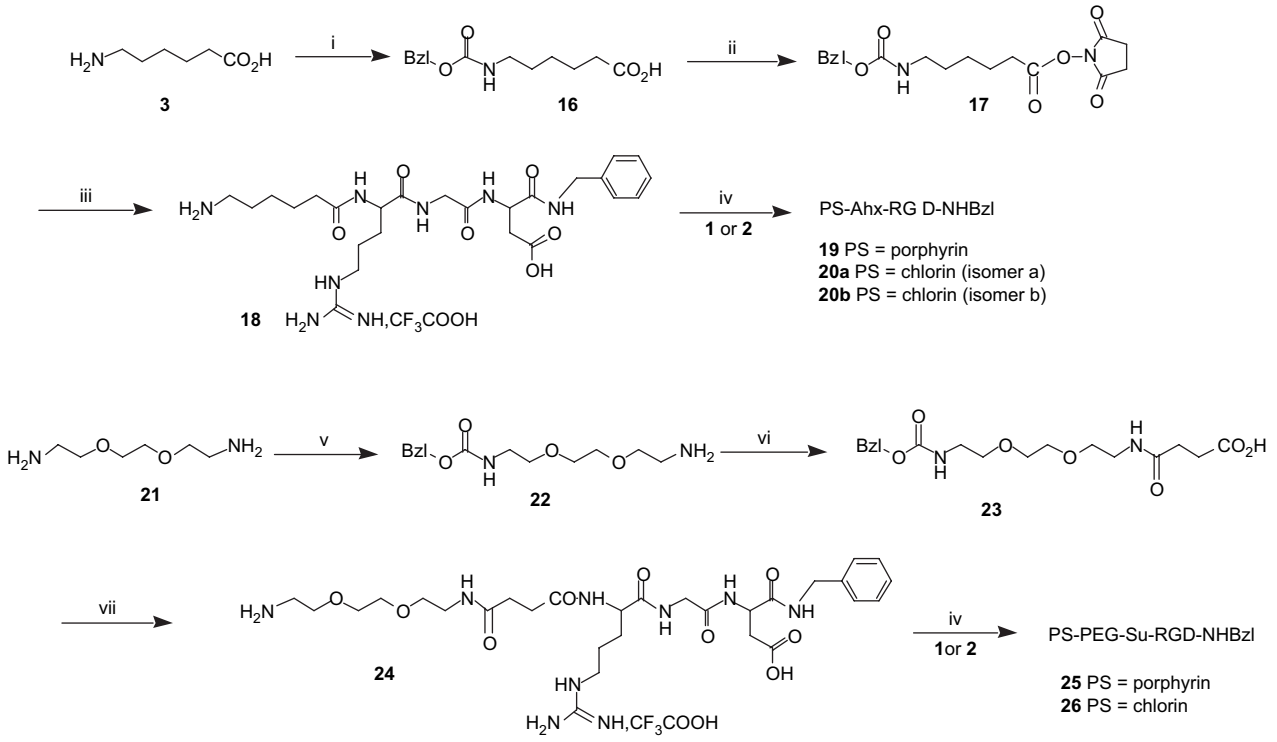


Scheme 1. Reagents and conditions: (i) amino acid or PS, TBTU, HOBt, DIEA (ii) TFA; (iii) TFA, PhOH, EDT, thioanisole, H_2O.



Scheme 2. Reagents and conditions: (i) (1) DCC, HOBt; (2) H_2, Pd/C; (ii) DCC, HOBt; (iii) HCl; (iv) TFA.

2,2′-(ethylenedioxy)bis-ethylamine **21** was mono-*N*-protected to give **22**, which was further reacted with succinic anhydride to give *Z*-PEG-Su **23**, as previously described.[25] The latter was condensed with **15** with DCC/HOBt and tributylamine, and subsequently hydrogenated to give PEG-Su-RGD-NHBzl **24** in 28% overall yield. The latter was too polar to be



Scheme 3. Reagents and conditions: (i) ZCl, NaOH; (ii) DCC, HOSu; (iii) (1) **15**, TEA; (2) H_2, Pd/C; (iv) PS-OSu **1** or **2**, DIEA; (v) ZOSu, NaOH; (vi) succinic anhydride; (vii) (1) **15**, DCC, HOBt, Bu_3N; (2) H_2, Pd/C.

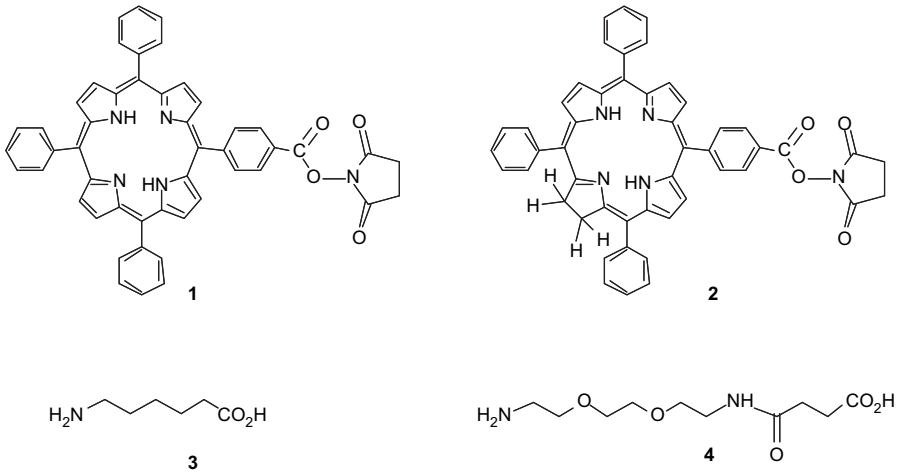
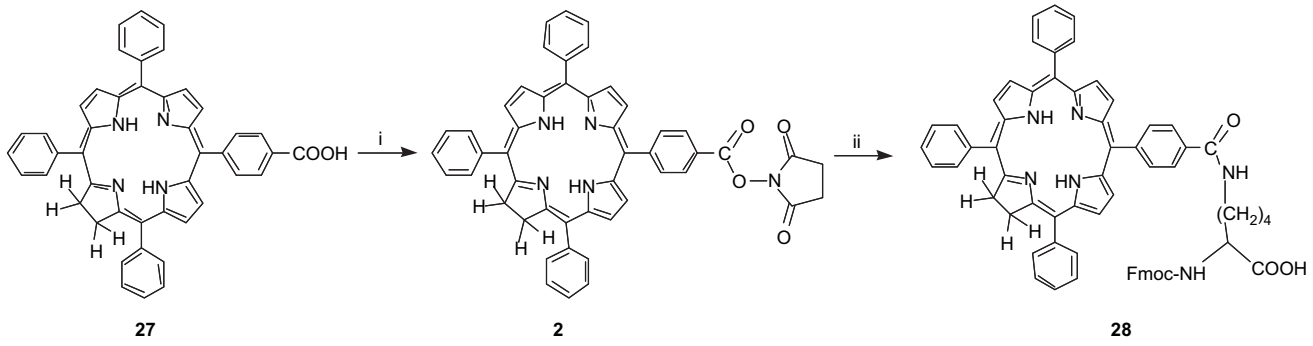


Figure 1.

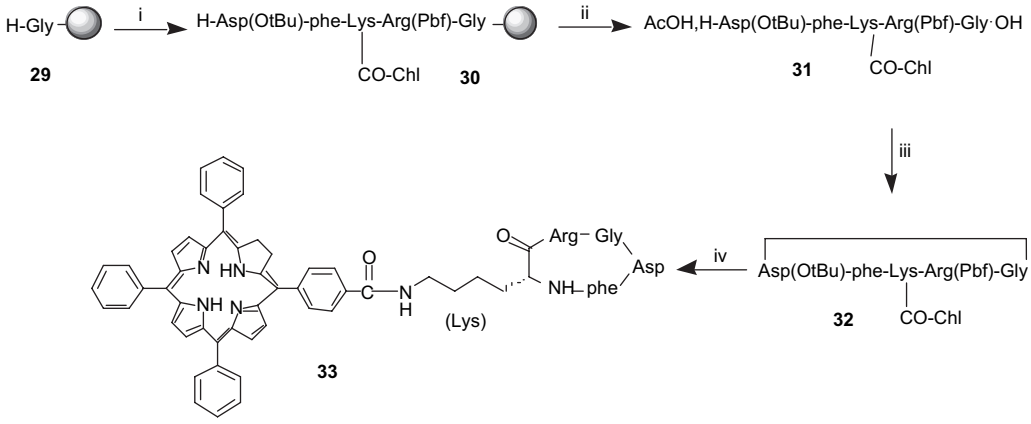
analytically pure after normal phase column chromatography. Crude **24** was then directly coupled with TPP-COOSu **1** or Chl-COOSu **2** to afford the corresponding PS-PEG-Su-RGD-NHBzl **25** and **26** in 58 and 60% yield, respectively.

For the synthesis of cyclo[RGDfK(CO-Chl)] **33**, we followed and adapted the improved synthesis based on Kessler's procedure developed by Dai et al.[26] We chose to couple first in liquid phase the monocarboxylic tetraphenylchlorin succinimidyl ester (Chl-COOSu) **2** to the amine function of the lysine lateral side chain (Scheme 4) as it has been already described.[19c]

This site of attachment on the peptide was chosen because the lateral side chain of the lysine is not essential for activity and thus, the coupling preserves the specificity of the cyclopeptide for $\alpha_v\beta_3$ integrin and can also act as a spacer. Coupling of Fmoc-Lys-OH with *N*-hydroxysuccinimide activated Chl-COOSu **2** afforded Fmoc-Lys(CO-Chl)-OH **28**, which can be coupled on solid-phase support to the resin-Gly-Arg(Pbf)-NH_2 (Scheme 5).



Scheme 4. Reagents and conditions: (i) HOSu; (ii) Fmoc-Lys-OH·HCl, TEA.



Scheme 5. Reagents and conditions: (i) amino acid, TBTU, HOBt, DIEA; (ii) CH_3COOH, TFE, CH_2Cl_2; (iii) T3P, AcOEt; (iv) TFA, EDT, phenol, thioanisole, H_2O.

The linear RGDfK **31** peptide was cleaved from the resin without affecting other protecting groups with a mixture of acetic acid, 2,2,2 trifluoroethane (TFE), and DCM (1:1:3). The head-to-tail cyclization was performed by treatment with 1-propane-phosphonic acid cyclic anhydride (T3P) to afford the chlorin coupled to the protected cyclic peptide **32**. The other protecting groups of the above cyclic peptide were removed with trifluoroacetic acid (TFA) leading to the desired product **33** with an overall yield of 5%.

Compound **33** was obtained with a final purity greater than 95%, as assessed by analytical RP-HPLC. Two isomers, corresponding to the reduction of a double bond on either opposing side of the tetrapyrrolic macrocycle could be observed by RP-HPLC but not separated (17.7 and 18.2 min). Identities of the compounds were confirmed by MALDI-TOF mass spectrometry and NMR experiments.

2.2. Photophysical properties

Table 1 shows the compared photophysical properties of the RGD targeted photosensitizers synthesized by our group.

Room temperature absorption and fluorescence emission spectra of targeted porphyrins **19** and **25** and chlorins containing **20a**,**b** and **26** were recorded in ethanol. All the compounds show the Soret band ($414<\lambda_{max}<420$ nm) and the four Q bands around 515, 545, 596, and 650 nm common for porphyrins and chlorins in the UV–vis region. To have a better penetration of the light in the tissues, the QI band (around 650 nm) is used for PDT applications. It is interesting to notice that, at this specific wavelength, the porphyrinated compounds **5**, **19**, and **25** present the same low absorption whatever the spacer is. In contrast, we can notice a high influence of the type of the arm on the extinction coefficient of the chlorin containing ones. Compound **26** presents the best absorption, which is around four times better than the chlorin with the Ahx spacer **20** and almost five times better than the chlorin without any spacer **6**. This may be explained by the increased solubility of the compound due to the PEG spacer and this is in good agreement with previous study. 4-Carboxyphenylporphyrin was coupled to folic acid via 1,6-diaminohexane or 2,2′-(ethylenedioxy)-bis(ethylamine) spacer. The structure of the linker influenced the absorption properties, and the compound with the PEG arm displayed an higher extinction coefficient value at 650 nm (2373 M^{-1} cm^{-1} vs 1266 M^{-1} cm^{-1}).[3c] To our knowledge, no systematic study has been done concerning the influence of the spacer on the photochemical and biological properties of the photosensitizers. In a recent publication, Maillard et al.[27] reported the synthesis and in vitro photoactivity of a series of glycol conjugated porphyrins and chlorins on the 179 retinoblastoma cell line. The sugar component was directly attached to the *p*-hydroxyphenyl moiety or through a diethylene glycol linker for one porphyrin TPP(*p*-*O*-Deg-*O*-β-D-GluOH)$_3$. No difference in absorption was observed. Nevertheless, the porphyrin monosaccharide conjugates, in which the sugar and porphyrin moieties were separated by diethylene glycol spacer, were essentially non cytotoxic and displayed good to excellent phototoxicity.

Table 1
Photophysical properties of photosensitizers-RGD compounds

Compound	ε_{Soret}[a]	λ_{Soret}[b]	ε_{QI}[c]	λ_{QI}[d]	Φ_f[e]	Φ^1O_2[f]
5	136,000	415	1520	649	0.05	0.60
6	19,600	415	1800	649	0.18	0.50
19	54,352	414	1668	650	0.09	0.77
20a	39,220	418	7809	650	0.25	0.72
20b	32,817	420	5938	651	0.23	0.60
25	85,876	414	1673	647	0.09	0.58
26	52,232	415	8323	651	0.29	0.57
33	53,300	415	3400	649	0.18	0.82

[a] Molar extinction coefficient of Soret band (10^3 cm^3 mol^{-1}).
[b] Soret band wavelength.
[c] Molar extinction coefficient of QI band.
[d] QI wavelength.
[e] Fluorescence quantum yield.
[f] Singlet oxygen formation quantum yield.

The fluorescence emission spectra of all the compounds display two bands at around 650 and 717 nm. As previously observed,[3b] fluorescence quantum yields are higher for chlorins than for porphyrins, 20–29% against 5–9%, respectively. This is not in favor of good photosensitizers since we expect the compounds to produce singlet oxygen.

Singlet oxygen quantum yields $\Phi(O_2)$ were determined using tetraphenylporphyrin (TPP) as reference solution ($\Phi(^1O_2)$ [TPP]=0.68, in toluene) and were estimated from $^1O_2$ luminescence at 1272 nm. All the compounds present good to excellent singlet oxygen quantum yields compatible for a use in PDT. If compound **26** is the best candidate for PDT application in terms of absorption at 650 nm, it is not the best for singlet oxygen production. Porphyrins reveal better singlet oxygen production than their chlorin homologues as previously observed.[3b]

Surprisingly, both isomers **20a** and **20b** exhibit different photophysical properties that is to say a difference of 10% for singlet oxygen quantum yield and 20% in absorption. To the best of our knowledge, it is the first time that both isomers have been separated by HPLC and it has been shown that the photophysical properties are slightly different.

3. Conclusion

New targeted photosensitizers for anti-vascular photodynamic therapy have been synthesized with success in good yields. The photophysical properties of the photo-activable moiety are conserved after the coupling of the vector. In conclusion, if chlorins present the best absorption at 650 nm, porphyrins have very good singlet oxygen quantum yields. A PEG spacer increases the hydrophily of the targeted photosensitizers. This preliminary in vitro results concerning chlorin or porphyrin coupled to RGD are very promising.[19c] The in vitro study of the new compounds is under progress and will be described elsewhere.

4. Experimental

4.1. General

Unless otherwise stated, reagents were purchased from chemical companies and used without prior purification.

Reagent grade solvents were used as received. 5-(4-Carboxyphenyl)-10,15,20-triphenylchlorin (TPC) was purchased from Frontier Scientific (Logan, Utah). 5-(4-Carboxyphenyl)-10,15,20-triphenylporphyrin was synthesized as previously described.[3b] The Fmoc-Asp(O^{t}Bu)-Wang and H-Gly-2-chlorotrityl resins, and 9-fluorenyl-methoxy-carbonyl (Fmoc)-aminoacids were from Senn Chemicals International (Dielsdorf, Switzerland). Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates (Merck Chimie S.A.S., Fontenay Sous Bois, France) and developed with the appropriate solvents. The TLC spots were visualized either by UV light or by heating plates sprayed with a solution of phosphomolybdic acid (5% ethanolic solution). Chromatography column was carried out on Merck silica gel (40–63 μm; 230–400 mesh ASTM). Assembly of compounds **5**, **6**, and **33** was carried out on a multichannel peptide synthesizer PSP 4000, according to a classical Fmoc/^{t}Bu solid-phase methodology using the in situ neutralization protocol.[23] For the attachment of each amino acid, double couplings (20 and 40 min, respectively) were performed using a threefold excess of *N*-Fmoc-amino acid and activation reagents 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumtetrafluoroborate (TBTU) (3 equiv), 1-hydroxybenzotriazole (HOBt) (3 equiv), and *N*,*N*-diisopropylethylamine (DIEA) (9 equiv) in dimethylformamide (DMF). Monitoring of the reaction was performed by the 2,4,6-trinitrobenzenesulfonic acid test. For the attachment of the photosensitizer, only one coupling step using twofold excess was applied. During the photosensitizer coupling stage and all the next steps, light exposure was minimized by sealing the reaction vessel in aluminum foil to limit the occurrence of unwanted side reactions. Before cleavage, the peptide-resin was washed extensively with dichloromethane and dried in vacuo. A semi-preparative Alltech Apollo C18, 5 μm column (250×10 mm ID, Alltech, Lokeren, Belgique) was used for purification. Fluorescence detection was optimized with excitation and emission wavelengths of 415 and 650 nm, respectively, and coupled with UV absorption (350 nm). All the targeted photosensitizers were eluted with a gradient of methanol–H_2O (75:25, v/v) for 15 min, followed by 100% methanol for the last 15 min. Room temperature and a flow-rate of 4.0 mL min^{-1} were maintained throughout the purification. A volume of 1 mL of the samples in methanol was injected into the column. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advancer 300 (300.130 and 75.475 MHz, respectively), or on a Bruker DRX 400 (400.130 and 100.612 MHz, respectively). Multiplicities are reported as follow: s=singlet, t=triplet, q=quadruplet, and m=multiplet. Mass spectra analyses (MALDI-TOF) were carried out on a Bruker Reflex IV time-of-flight mass spectrometer (Bruker-Daltonic, Bremen, Germany) equipped with the SCOUT 384 probe ion source, and electospray (ESI-MS) on a Platform Micromass apparatus. Melting points (°C, uncorrected) were determined on a Electrothermal 9100 Capillary apparatus. Infrared spectra were recorded on a Bruker Vector 22 apparatus (KBr, ν in cm^{-1}), and elemental analyses were performed on a Thermofinnigan FlashEA 1112 apparatus, at the Service Commun de Microanalyse, Nancy.

4.2. Syntheses

4.2.1. 5-(4-Carboxy-RGD-phenyl)-10,15,20-triphenylporphyrin **5** and 5-(4-carboxy-RGD-phenyl)-10,15,20-triphenylchlorin **6**

The synthesis was performed using the preloaded Fmoc-Asp(O^{t}Bu)-Wang (capacity, 0.79 mmol/g) on a 0.15 g scale. The side chains of arginine and aspartic acid were, respectively, protected by Pbf (2,2,5,7,8-pentamethylchroma-6-sulfonyl) group and ^{t}Bu (*tert*-butyl). The successive coupling of Fmoc-Gly-OH (106 mg) and Fmoc-Arg(Pbf)-OH (230 mg) in the presence of TBTU (114 mg), HOBt (54 mg), and 0.18 mL of DIEA in 5 mL of DMF was achieved. After the removal of the Fmoc group from arginine, the photosensitizer was coupled by using the same protocol. The photosensitizer–peptide–resin was washed with CH_2Cl_2 (6×5 mL) and then dried in vacuo overnight. A standard cleavage with a mixture of 0.75 g of crystalline phenol, 0.25 mL of 1,2-ethanedithiol, 0.5 mL of thioanisole, 0.5 mL of deionized H_2O, and 10 mL of trifluoroacetic acid (TFA) for 1.5 h afforded the targeted photosensitizer, which was lyophilized. The compounds were purified by RP-HPLC. After removal of the solvents, the purified compounds were lyophilized and analyzed by ^{1}H NMR and mass spectroscopy.

4.2.2. 5-(4-Carboxy-RGD-phenyl)-10,15,20-triphenylporphyrin **5**

Yield 55%. ^{1}H NMR (300 MHz, DMSO-d_6): δ −2.91 (s, 2H, NH-pyrrole), 1.58 (m, 3H, 2γ,1βArg), 1.70 (m, 1H, βArg), 2.54, 2.74 (ABX, J_{AB}=16.2 Hz, J_{Ax}=5.7 Hz, J_{Bx}=7.9 Hz, 2H, βAsp), 3.12 (m, 2H, δArg), 3.59, 3.98 (ABX, J_{AB}=16.9 Hz, J_{Ax}=5.8 Hz, J_{Bx}=5.3 Hz, 2H, αGly), 4.28 (br t, 1H, αArg), 7.22 (m, 1H, εN*H*Arg), 8.05 (br d, 1H, N*H*Arg), 8.25 (br t, 1H, N*H*Gly), 7.23–8.83 (m, 27H, Ar*H*, pyrrole). MS (MALDI-TOFMS): *m*/*z* found: 987.78 [M+H]$^+$.

4.2.3. 5-(4-Carboxy-RGD-phenyl)-10,15,20-triphenylchlorin **6**

Yield 58%. ^{1}H NMR (300 MHz, DMSO-d_6): δ −1.52, −1.58 (s, 2H, NH-pyrrole), 1.50 (m, 3H, 2γ,1βArg), 1.72 (m, 1H, βArg), 2.58, 2.82 (ABX, J_{AB}=16.1 Hz, J_{Ax}=5.6 Hz, J_{Bx}=8.0 Hz, 2H, βAsp), 3.11 (m, 2H, δArg), 3.62, 4.02 (ABX, J_{AB}=16.9 Hz, J_{Ax}=5.7 Hz, J_{Bx}=5.4 Hz, 2H, αGly), 4.10 (br t, 1H, αAsp), 4.14 (m, 4H, CH_2-chlorin), 4.32 (br t, 1H, αArg), 7.23 (br t, 1H, εN*H*Arg), 7.98 (br d, 1H, N*H*Arg), 8.20 (br t, 1H, N*H*Gly), 7.23–8.57 (m, 23H, ArH, pyrrole). MS (MALDI-TOFMS): *m*/*z* found: 989.34 [M+H]$^+$.

4.2.4. tert-Butyloxycarbonylarginylglycine hydrochloride **9**

A solution of *tert*-butyloxycarbonylarginine **7** (1.163 g, 4.24 mmol), dicyclohexylcarbodiimide (874 mg, 4.24 mmol), hydroxybenzotriazole hydrate (649 mg, 4.24 mmol), and glycine benzylester hydrochloride **8** (854 mg, 4.24 mmol) in DMF (30.00 mL) was stirred at room temperature under argon for 12 h. The precipitate was filtered and washed with EtOAc (3×5 mL). The solution was concentrated under reduced

pressure to give a yellow gum. Deprotection of the benzylester: the residue was dissolved in MeOH (50 mL) and to the resulting solution was added 10% Pd/C (250 mg). The mixture was stirred under H_2 at atmospheric pressure at room temperature for 12 h. The suspension was filtered through Celite® and the solvent was evaporated. The resulting oil was dissolved in 20 mL of MeOH and the title compound was precipitated as a gum by the addition of Et_2O (300 mL). The suspension was let for half an hour, then the mixture of solvents was removed, while the compound sticked to the flask. The dissolution/precipitation process was renewed three more times, using 25 mL of MeOH for the last dissolution. The final residue was carefully dried under reduced pressure to give 1.332 g (3.54 mmol, 83.5% yield) of white material. Mp 108–110 °C. IR (KBr): ν=3343, 2979, 1165, 1525, 1368, 1251, 1165. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H, ^{t}Bu), 1.50 (m, 3H, 2γ,1βArg), 1.67 (m, 1H, βArg), 3.08 (m, 2H, δArg), 3.70, 3.80 (ABX, J_{AB}=17.6 Hz, J_{Ax}=5.7 Hz, J_{Bx}=5.7 Hz, 2H, αGly), 3.96 (m, 1H, αArg), 6.93 (d, J=8.2 Hz, 1H, N*H*Boc), 7.00–7.75 (br s, 4H, guanidinium), 7.78 (m, 1H, N*H*Arg), 8.16 (t, J=7.5 Hz, 1H, N*H*Gly), 12.60 (COOH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.3 (γArg), 28.4 (*C*H$_3$ Boc), 29.3 (βArg), 40.5, 40.9 (δArg, αGly), 53.8 (αArg), 78.3 (*C*$_{quat}$ Boc), 155.5 (*C*O Boc), 157.2 (guanidinium), 171.4, 172.4 (*C*O Arg, *C*OOH Gly). ESI-MS (pos. mode): *m/z* 354.2 $[M+Na]^+$, 332.1 $[M+H]^+$. Anal. Calcd for $C_{13.25}H_{27}N_{5.25}O_5Cl$ (Boc-RG-OH·HCl, 0.25MeOH: 375.85): C, 42.34; H, 7.24; N, 18.64. Found: C, 42.22; H, 7.31; N, 18.73.

4.2.5. *tert*-Butyloxycarbonylbenzylaspartamide-β-benzylester **12**

A solution of Boc-Asp(OBzl)-OH (4.000 g, 12.37 mmol), DCC (2.552 g, 12.37 mmol), and HOBt hydrate (1.894 g, 12.37 mmol) in THF (100 mL) was stirred at room temperature for 15 min. Benzylamine (1.49 mL, 13.60 mmol) was then added to the resulting suspension and stirring was maintained for 5 h. The mixture was filtered and washed with EtOAc (2×10 mL). The solution was concentrated under reduced pressure and to the residue was added CH_2Cl_2 (50 mL). The insoluble material was filtered off and washed with CH_2Cl_2 (2×5 mL). The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (eluent: CH_2Cl_2, then CH_2Cl_2/MeOH, 99:1) to give 4.739 g of white solid (93% yield). Mp 91 °C. IR (KBr): ν=1687, 1667. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, ^{t}Bu), 2.78 (dd, J_1=17.4 Hz, J_2=5.7 Hz, 1H, βAsp), 3.13 (dd, J_1=17.2 Hz, J_2=4.4 Hz, 1H, βAsp), 4.38–4.55 (quartet d+m, 3H, αAsp+benzylamide C*H*$_2$), 5.12 (s, 2H, benzylester C*H*$_2$), 5.69 (br d, 1H, N*H*Boc), 6.81 (br d, 1H, benzylamide N*H*), 7.26–7.40 (m, 10H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.7 (^{t}Bu CH$_3$), 36.5 (CH$_2$ of Asp), 43.9 (benzylamide CH$_2$), 51.2 (CH of Asp), 67.3 (benzylester CH$_2$), 81.0 (^{t}Bu C$_{quat}$), 127.9 (Ph CH), 127.9 (Ph CH), 128.7 (Ph CH), 128.8 (Ph CH), 129.0 (Ph CH), 129.1 (Ph CH), 135.8 (Ph C$_{quat}$), 138.3 (Ph C$_{quat}$), 156.0 (carbamate CO), 171.0 (ester CO), 172.2 (amide CO). ESI-MS (pos. mode): *m/z* 413.24 $[M+H]^+$, 435.24 $[M+Na]^+$, 451.21 $[M+K]^+$. Anal. Calcd for $C_{23}H_{28}N_2O_5$ (Boc-Asp(OBzl)-NHBzl: 412.48): C, 66.97; H, 6.84; N, 6.82. Found: C, 67.13; H, 7.06; N, 6.93.

4.2.6. Benzylaspartamide-β-benzylester hydrochloride **13**

A stirred solution of **12** (4.739 g, 11.49 mmol) in EtOAc (150 mL) was cooled in an ice bath. Hydrogen chloride gas was let bubble in the solution for 2 h, then stirring was maintained for 5 h at the same temperature. The resulting suspension was filtered and washed with Et_2O (2×15 mL) to give 3.383 g of white powder. Filtration and washing of the filtrate with Et_2O (2×5 mL) gave another amount (0.329 g) of the same material. Total yield of **13**: 3.712 g (93% yield). Mp 157 °C. IR (KBr): ν=1730, 1666. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.01 (m, 2H, βAsp), 4.18 (t, J=6.9 Hz, 1H, αAsp), 4.33 (d, J=5.9 Hz, 2H, benzylamide C*H*$_2$), 5.15 (s, 2H, benzylester C*H*$_2$), 7.24–7.40 (m, 10H, Ar*H*), 8.43 (br s, 3H, N*H*$_3$), 9.06 (t, J=5.8 Hz, 1H, benzylamide N*H*). ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.0 (CH$_2$ of Asp), 43.3 (benzylamide CH$_2$), 49.8 (CH of Asp), 67.1 (benzylester CH$_2$), 127.8 (Ph CH), 128.2 (Ph CH), 128.9 (Ph CH), 129.0 (Ph CH), 129.1 (Ph CH), 129.3 (Ph CH), 136.5 (Ph C$_{quat}$), 139.3 (Ph C$_{quat}$), 168.0 (ester CO), 169.9 (amide CO). ESI-MS (pos. mode): *m/z* 313.23 $[M+H]^+$. Anal. Calcd for $C_{18}H_{21}N_2O_3Cl$ (H-Asp(OBzl)-NHBzl·HCl: 348.82): C, 61.98; H, 6.07; N, 8.03. Found: C, 61.76; H, 6.17; N, 8.00.[20b]

4.2.7. *tert*-Butyloxycarbonylarginylglycylbenzylaspartamide-β-benzylester hydrochloride **14**

A solution of *tert*-butyloxycarbonylarginylglycine hydrochloride (1.385 g, 3.68 mmol), dicyclohexylcarbodiimide (760 mg, 3.68 mmol), hydroxybenzotriazole hydrate (564 mg, 3.68 mmol), and benzylaspartamide-β-benzylester hydrochloride **13**[20b] (1.285 g, 3.68 mmol) in DMF (50.00 mL) was stirred at room temperature under argon for 0.5 h. To the resulting suspension was added tributylamine (877 µL, 3.68 mmol) and the stirring was maintained for 12 h. The precipitate was filtered and washed with EtOAc (3×5 mL). The solution was concentrated under reduced pressure to give a yellow gum, which was dissolved in CH_2Cl_2 (70 mL). The desired compound was precipitated as a gum by addition of Et_2O (200 mL). The suspension was let for half an hour, then the mixture of solvents was removed, while the compound sticked to the flask. The dissolution/precipitation process was renewed once and column chromatography (eluent: CH_2Cl_2/MeOH, 19:1 then 18:2, then 17:3) afforded a white solid material, which was dissolved in CH_3CN (30 mL) and H_2O (30 mL), and then lyophilized to give 1.672 g (68% yield) of white powder. Mp 100–110 °C. IR (KBr): ν=3324, 2978, 1735, 1662, 1527, 1366, 1251, 1168, 738, 699. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H, ^{t}Bu), 1.50 (m, 3H, 2γ,1βArg), 1.67 (m, 1H, 1βArg), 2.68, 2.88 (ABX, J_{AB}=16.2 Hz, J_{Ax}=5.8 Hz, J_{Bx}=7.8 Hz, 2H, βAsp), 3.08 (m, 2H, δArg), 3.72, 3.79 (ABX, J_{AB}=16.7 Hz, J_{Ax}=5.2 Hz, J_{Bx}=5.4 Hz, 2H, αGly), 3.95 (m, 1H, αArg), 4.28 (d, J=5.9 Hz, 2H, NHC*H*$_2$Ph), 4.72 (m, 1H, αAsp), 5.09 (s, 2H, OC*H*$_2$Ph), 7.01 (d, J=7.4 Hz, 1H, N*H*Arg), 7.02–7.65 (m, 14H, H$_{arom}$+guanidinium), 8.19 (br t, 1H, N*H*Gly), 8.33

(d, J=8.2 Hz, 1H, NH-Asp), 8.49 (br t, 1H, N*H*CH_2Ph). ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.2 (γArg), 28.4 (*C*H_3 Boc), 29.0 (βArg), 36.5 (βAsp), 40.5 (δArg), 42.3 (NH*C*H_2Ph+αGly), 49.7 (αAsp), 54.0 (αArg), 65.8 (O*C*H_2Ph), 78.4 (C_{quat} Boc), 126.8, 127.2, 128.0, 128.1, 128.3, 128.5 (*C*H Ph), 136.2, 139.3 (C_{quat} Ph), 155.6 (*C*O Boc), 157.1 (guanidinium), 169.0, 170.2 (2 pics), 172.5 (*C*O Gly, β*C*O Asp, *C*O Arg, *C*O Asp). ESI-MS (pos. mode): *m/z* 626.31 $[M+H]^+$. Anal. Calcd for $C_{31}H_{44}N_7O_7Cl$ (662.18): C, 56.22; H, 6.70; N, 14.81. Found: C, 55.95; H, 6.77; N, 14.63.

4.2.8. Arginylglycylbenzylaspartamide-β-benzylester bis-trifluoroacetate monohydrate **15**

To a solution of **14** (1.622 g, 2.45 mmol) in CH_2Cl_2 (160 mL) was added trifluoroacetic acid (53 mL). The solution was stirred 0.75 h at room temperature, then concentrated in vacuo. To the residue was added Et_2O (210 mL). The resulting white precipitate was filtered, washed with Et_2O (3×30 mL), and dried to give a white powder, which was dissolved in H_2O (45 mL). The solution was lyophilized to give 1.714 g (91% yield) of white powder. Mp 65–70 °C. IR (KBr): ν=3423, 3068, 2929, 1735, 1672, 1204, 1135, 723. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.54 (m, 2H, 2γArg), 1.71 (m, 2H, 2βArg), 2.68, 2.86 (ABX, J_{AB}=16.1 Hz, J_{Ax}=5.7 Hz, J_{Bx}=8.2 Hz, 2H, βAsp), 3.11 (m, 2H, δArg), 3.70–3.92 (m, 3H, αArg, αGly), 4.28 (d, J=5.7 Hz, 2H, NHC*H*$_2$Ph), 4.73 (m, 1H, αAsp), 5.1 (s, 2H, OC*H*$_2$Ph), 6.80–7.60 (m, 14H, H_{arom}+guanidinium), 7.70 (br t, 1H, εNHArg), 8.15 (br s, 3H, NH_3 Arg), 8.47 (d, J=8.0 Hz, 1H, N*H*-Asp), 8.54 (t, J=5.8 Hz, 1H, N*H*CH_2Ph), 8.71 (br t, 1H, N*H*Gly). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.9 (γArg), 29.2 (βArg), 37.3 (βAsp), 41.0 (δArg), 42.7 (αGly), 43.1 (NH*C*H_2Ph), 50.4 (αAsp), 52.6 (αArg), 66.6 (O*C*H_2Ph), 118.0 (q, J=297 Hz, CF_3), 127.6, 127.9, 128.7, 128.9, 129.1, 129.3 (*C*H Ph), 136.9, 140.0 (C_{quat} Ph), 157.8 (guanidinium C), 159.6 (q, J=31 Hz, CF_3*C*OOH), 169.1, 169.6, 170.8, 170.9 (*C*O Gly, β*C*O Asp, *C*O Arg, *C*O Asp). ESI-MS (pos. mode): *m/z* 526.3 $[M+H]^+$, 436.3 $[M-Bn+H]^+$. Anal. Calcd for $C_{30}H_{39}N_7O_{10}F_6$ (HRGD(OBn)NHBn, 2CF_3COOH, H_2O: 771.68): C, 46.69; H, 5.09; N, 12.71. Found: C, 46.80; H, 5.20; N, 12.60.

4.2.9. 6-Aminohexanoylarginylglycylbenzylaspartamide trifluoroacetate dihydrate **18**

To a solution of **15** (426 mg, 0.55 mmol) in DMF (20 mL) was added a solution of 6-(benzyloxycarbonylamino)hexanoic acid succinimidyl ester (200 mg, 0.552 mmol) in CH_2Cl_2 (20 mL) and triethylamine (0.077 mL, 0.552 mmol). The mixture was stirred at room temperature for 12 h and concentrated under reduced pressure. To the residue was added H_2O (10 mL). The resulting gel was sonicated, filtered, and washed with water (3×10 mL). After drying (Na_2SO_4), it was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 95:5 then 90:10, then 85:15) to give 222 mg of white solid material. Deprotection: to a solution of the latter compound in MeOH (20 mL) was added 10% Pd/C (55 mg). The mixture was stirred under H_2 at atmospheric pressure at room temperature for 12 h. The suspension was filtered through Celite®, and the solvent was evaporated to give a white solid, which was dissolved in H_2O (10 mL) and lyophilized to afford 154 mg (40% yield over the two steps) of white powder. Mp 222 °C. IR (KBr): ν=3378, 3070, 2941, 1662 (br peak), 1538, 1393, 1200, 1130, 722. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (m, 2H, CH_2 Ahx), 1.47 (m, 7H, 2CH_2 Ahx+2γ,1βArg), 1.99 (m, 1H, 1βArg), 2.12 (t, J=7.2 Hz, 2H, CH_2CO Ahx), 2.23, 2.64 (ABX, J_{AB}=16.1 Hz, J_{Ax}=3.4 Hz, J_{Bx}=5.1 Hz, 2H, βAsp), 2.72 (m, 2H, CH_2NH Ahx), 2.94 (m, 1H, δArg), 3.11 (m, 1H, δArg), 3.49, 3.83 (ABX, J_{AB}=16.9 Hz, J_{Ax}=5.8 Hz, J_{Bx}=5.3 Hz, 2H, αGly), 4.13, 4.39 (ABX, J_{AB}=15.6 Hz, J_{Ax}=6.8 Hz, J_{Bx}=5.5 Hz, 2H, CH_2Ph), 4.28 (m, 2H, αAsp+αArg), 7.08 (br s, 2H, guanidinium), 7.25 (s, 5H, Ph), 7.57 (br t, 1H, N*H*CH_2Ph), 7.77 (br s, 3H, guanidinium), 7.94 (d, J=7.6 Hz, 1H, N*H*Arg), 8.86 (br t, 1H, N*H*Gly), 8.98 (d, J=8.0 Hz, 1H, N*H*-Asp), 10.28 (br s, 1H, COO*H*). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.8, 25.0, 25.8 (*C*H_2 Ahx, γArg), 27.1 (βArg), 29.8 (*C*H_2 Ahx), 35.0 (*C*H_2CO Ahx), 37.8 (βAsp), 39.1 (*C*H_2NH_2), 40.6 (δ Arg), 42.3 (αGly), 43.4 (NH*C*H_2Ph), 50.2 (αAsp), 51.9 (αArg), 117.6 (q, J=297 Hz, CF_3), 126.8, 126.9, 128.3 (*C*H Ph), 139.6 (C_{quat} Ph), 157.8 (guanidinium C), 158.5 (q, J=31 Hz, CF_3*C*OOH), 168.7, 171.6, 172.5, 174.1, 176.0 (*C*O Gly, β*C*O Asp, *C*O Arg, *C*O Ahx, *C*O Asp). ESI-MS (pos. mode): *m/z* 571.1 $[M+Na]^+$, 549.1 $[M+H]^+$, 275.3 $[M+H]^{2+}$. Anal. Calcd for $C_{27}H_{45}N_8O_{10}F_3$ (H-Ahx-RGD-NHBzl, CF_3COOH, 2H_2O): C, 46.41; H, 6.49; N, 16.04. Found: C, 46.15; H, 6.25; N, 15.95.

4.2.10. N-Succinimidylarginylglycylbenzylaspartamide-2,2′-(ethylenedioxy)bis(ethylamine) trifluoroacetate **24**

Coupling: to a solution of **23** (148 mg, 0.39 mmol) in DMF (10 mL) were added **15** (299 mg, 0.39 mmol), DCC (80 mg, 0.39 mmol), and HOBt hydrate (59 mg, 0.39 mmol). The solution was stirred for 0.3 h at room temperature, then tributylamine (0.092 mL, 0.39 mmol) was added, and stirring was maintained for 12 h. The resulting suspension was filtered and the precipitate was washed with CH_2Cl_2 (2×4 mL). The mixture of solvent was evaporated and the residue was dissolved in CH_2Cl_2 (20 mL). The desired compound was precipitated as a gum by addition of Et_2O (20 mL). The suspension was let for 1 h, then the mixture of solvents was removed, while the compound sticked to the flask. The dissolution/precipitation process was renewed once and column chromatography (eluent: CH_2Cl_2/MeOH, 19:1 then 18:2, then 17:3) afforded 110 mg of white solid material. Deprotection: to a solution of the latter compound in MeOH (12 mL) was added 10% Pd/C (40 mg). The mixture was stirred under H_2 at atmospheric pressure at room temperature for 12 h. The suspension was filtered through Celite®, and the solvent was evaporated to give a white solid, which was dissolved in H_2O (10 mL) and lyophilized to afford 83 mg (28% yield over the two steps) of white powder. This crude compound was used without further purification. Mp 98 °C. ESI-MS (pos. mode): *m/z* 705.0 $[M+K]^+$, 688.0 $[M+Na]^+$, 666.30 $[M+H]^+$, 352.7 $[M+K]^{2+}$, 333.8 $[M+H]^{2+}$.

*4.2.11. 5-(4-Carboxyphenyl succinimidyl ester)-10,15,20-triphenylporphyrin **1** and 5-(4-carboxyphenyl succinimidyl ester)-10,15,20-triphenylchlorin **2***

In the dark under a nitrogen atmosphere, *N*-hydroxysuccinimide (0.23 mmol) and dicyclohexylcarbodiimide (DCC) (0.23 mmol) were added to a solution of 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin or 5-(4-carboxyphenyl)-10,15,20-triphenylchlorin (0.23 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred 4 h at room temperature. The solvent was evaporated and the crude material was purified by column chromatography using EtOH/CH_2Cl_2 4:96 (v/v) as the eluent. The fractions were tested by TLC and the pure compound was isolated as a purple solid (112 mg, 65% for **1**, 127 mg, 73% for **2**).

Compound **1**: ^{1}H NMR (300 MHz, DMSO-d_6): δ −2.75 (s, 2H, NH-pyrrole), 2.97 (s, 4H, CH_2 Su), 7.27−8.91 (m, 27H, ArH, pyrrole).

Compound **2**: ^{1}H NMR (300 MHz, DMSO): δ −1.50, −1.65 (s, 2H, NH-pyrrole), 2.99 (s, 4H, CH_2 Su), 4.17 (s, 4H, CH_2-chlorin), 7.26−8.85 (m, 27H, ArH, pyrrole).

*4.2.12. 5-(4-Carboxy-Ahx-RGD-NHBzl-phenyl)-10,15,20-triphenylporphyrin **19***

General procedure for the coupling of the photosensitizers: compound **18** (51 mg, 0.093 mmol) was added to a solution of 5,10,15,tri(*p*-tolyl)-20-(*p*-carboxyphenyl succinimidyl ester)-porphyrin **1** (70 mg, 0. 0.093 mmol) in DMF/CH_2Cl_2 (v/v 10 mL) and DIEA (16 μL, 0.093 mmol). The mixture was stirred at room temperature for five days and concentrated under reduced pressure. The crude product was lyophilized and purified by RP-HPLC to give 38.70 mg (35% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ −2.90 (s, 2H, N*H*-pyrrole), 1.09 (m, 2H, CH_2 Ahx), 1.38 (m, 7H, 2CH_2 Ahx+2γ,1βArg), 1.79 (m, 1H, 1βArg), 2.28 (br s, 2H, CH_2CO Ahx), 2.36, 2.68 (ABX, J_{AB}=16.1 Hz, J_{Ax}=3.4 Hz, J_{Bx}=5.1 Hz, 2H, βAsp), 3.12 (m, 2H, δArg), 3.41, 3.79 (ABX, J_{AB}=16.3 Hz, J_{Ax}=7.8 Hz, J_{Bx}=5.3 Hz, 2H, αGly), 3.55 (m, 2H, CH_2NH Ahx), 4.19 (m, 1H, αArg), 4.23 (d, J=5.6 Hz, 2H, CH_2Ph), 4.29 (m, 1H, αAsp), 7.13 (br s, 1H, εN*H*Arg), 7.24−8.84 (m, 27H, Ar*H*, pyrrole), 7.57 (br t, 1H, N*H*CH_2Ph), 7.77 (br, 3H, guanidinium), 7.89 (d, J=7.5 Hz, 1H, N*H*Arg), 8.79 (br t, 1H, N*H*Gly), 8.83 (br, 1H, N*H*-Asp). MS (MALDI-TOFMS): *m/z* 1190.421 $[M+H]^+$.

*4.2.13. 5-(4-Carboxy-Ahx-RGD-NHBzl-phenyl)-10,15,20-triphenylchlorin **20a** and **20b***

The coupling was performed as describe for **19** (vide supra) on a 0.1 mmol scale with an overall yield of 40% for the mixture of **20a** and **20b**.

Compound **20a**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ −1.52 (s, 2H, N*H*-pyrrole), 1.26 (m, 2H, CH_2 Ahx), 1.45 (m, 7H, 2CH_2 Ahx, 2γ,1βArg), 1.98 (m, 1H, 1βArg), 2.13 (br s, 2H, CH_2CO Ahx), 2.39, 2.70 (ABX, J_{AB}=16.1 Hz, J_{Ax}=3.4 Hz, J_{Bx}=5.1 Hz, 2H, βAsp), 3.09 (m, 2H, δArg), 3.43, 3.73 (ABX, J_{AB}=16.2 Hz, J_{Ax}=7.7 Hz, J_{Bx}=5.4 Hz, 2H, αGly), 3.57 (m, 2H, CH_2NH Ahx), 4.25 (m, 4H, αArg, αAsp, CH_2Ph), 7.13 (br s, 1H, εN*H*Arg), 7.24−8.84 (m, 27H, Ar*H*, pyrrole), 7.59 (br t, 1H, N*H*CH_2Ph), 7.80 (br s, 3H, guanidinium), 7.86 (br d 1H, N*H*Arg), 8.83 (br t, 1H, N*H*Gly), 8.98 (br d, 1H, N*H*-Asp). MS (MALDI-TOFMS): *m/z* found: 1191.196 $[M+H]^+$.

Compound **20b**: ^{1}H NMR (DMSO-d_6, 300 MHz): except the N*H*-pyrrole (δ −1.54), the spectrum is superimposable to that of **20a**. MS (MALDI-TOFMS): *m/z* 1191.325 $[M+H]^+$.

*4.2.14. 5-(4-Carboxy-PEG-Su-RGD-NHBzl-phenyl)-10,15,20-triphenylporphyrin **25***

The coupling was performed as describe for **19** (vide supra) on a 0.1 mmol scale with an overall yield of 58%.

^{1}H NMR (300 MHz, DMSO-d_6): δ −2.91 (s, 2H, NH-pyrrole), 1.45 (m, 3H, 2γ,1βArg), 1.97 (m, 1H, 1βArg), 2.18−2.64 (m, 6H, Su, βAsp), 2.88 (br t, 2H PEG), 3.12 (m, 2H, δArg), 3.31 (td, J=5.0 Hz, 2H PEG), 3.43, 3.78 (m, 8H, PEG, 2H αGly), 4.12, 4.43 (ABX, J_{AB}=15.7 Hz, J_{Ax}=6.8 Hz, J_{Bx}=5.4 Hz, 2H, CH_2Ph), 4.28 (m, 2H, αAsp+αArg), 7.13 (s, 1H, εNHArg), 7.19 (s, 5H, Ph), 7.23−8.83 (m, 27H, ArH, pyrrole), 7.12 (br t, 1H, N*H*CH_2Ph), 7.77 (br s, 3H, guanidinium), 7.28 (d, J=7.6 Hz, 1H, NHArg), 8.75 (br t, 1H, NHGly), 8.95 (d, J=8.0 Hz, 1H, NH-Asp). MS (MALDI-TOFMS): *m/z* 1307.274 $[M+H]^+$.

*4.2.15. 5-(4-Carboxy-PEG-Su-RGD-NHBzl-phenyl)-10,15,20-triphenylchlorin **26***

The coupling was performed as describe for **19** (vide supra) on a 0.1 mmol scale with an overall yield of 60%.

^{1}H NMR (300 MHz, DMSO-d_6): δ −1.58. −1.53 (s, 2H, NH-pyrrole), 1.33 (m, 3H, 2γ,1βArg), 1.87 (m, 1H, 1βArg), 2.25−2.69 (m, 6H, Su, βAsp), 3.36 (m, 2H, δArg), 3.62, 3.89 (ABX, J_{AB}=16.7 Hz, J_{Ax}=5.8 Hz, J_{Bx}=5.3 Hz, 2H, αGly), 4.13 (s, 4H, CH_2-chlorin), 4.15, 4.48 (ABX, J_{AB}=15.6 Hz, J_{Ax}=6.9 Hz, J_{Bx}=5.5 Hz, 2H, CH_2Ph), 4.41 (m, 2H, αAsp+αArg), 7.18 (br s, 7H, N*H*CH_2Ph, εNHArg, Ph), 7.20−8.84 (m, 23H, ArH, pyrrole), 7.28 (d, J=7.6 Hz, 1H, NHArg), 8.75 (br t, 1H, NHGly), 7.77 (br s, 3H, guanidinium), 8.98 (d, J=8.0 Hz, 1H, NH-Asp). MS (MALDI-TOFMS): *m/z* calculated 1307.48; found: 1308.221 $[M+H]^+$. MS (MALDI-TOFMS) *m/z* 1308.221 $[M+H]^+$.

*4.2.16. 2-(9H-Fluoren-9-ylmethoxy-carbonylamino)-6-[5,10,15,tri(p-tolyl)-20-(p-carboxyamino)-chlorin]-hexanoic acid (Fmoc-Lys(CO-Chl)-OH) **28***

In the dark under a nitrogen atmosphere, to a solution of 81 mg of Fmoc-Lys-OH·HCl (0.20 mmol) in a minimum of DMF were added *N*-hydroxysuccinimide activated chlorin **2** (152 mg, 0.20 mmol) and triethylamine (0.20 mmol, 28.3 mL) in 10 mL CH_2Cl_2. After been stirred at ambient temperature for 24 h, the solvent was evaporated and the crude material was purified by column chromatography using EtOH/CH_2Cl_2 5:95 (v/v) as the eluent. The fractions were tested by TLC and the pure compound was isolated as a purple solid by column chromatography (152 mg, 75%).

^{1}H NMR (300 MHz, DMSO-d_6): δ −1.52, −1.58 (s, 2H, NH-pyrrole), 1.57 (m, 2H, γLys), 1.75 (m, 2H, δLys), 1.85, 2.00 (m, 2H, βLys), 3.57 (m, 1H, εLys), 4.11 (s, 4H, CH_2-chlorin), 4.14 (m, 1H, CH-Fmoc), 4.40 (m, 1H, αLys),

4.36 (s, 2H, CH_2-Fmoc), 5.76 (m, 1H, NH-Lys), 6.70 (m, 1H, ζNH-Lys), 7.18–8.82 (m, 31H, ArH, pyrrole, Fmoc).

4.2.17. Cyclo[RGDfK(CO-Chl)] 33

The synthesis was performed using the preloaded H-Gly-2-chlorotrityl PS resin (capacity: 0.85 mmol/g) on a 0.115 g scale. The side chains of arginine and aspartic acid were, respectively, protected by Pbf and ^{t}Bu groups. The successive coupling of Fmoc-Arg(Pbf)-OH (190 mg), Fmoc-Lys-(CO-Chl)-OH **28** (152 mg), Fmoc-(D)Phe-OH (114 mg), and Fmoc-Asp(O^{t}Bu)-OH (121 mg) in the presence of TBTU (94 mg), HOBt (45 mg), and 0.15 mL of DIEA were achieved. After the final removal of the Fmoc group, the peptide-resin was washed with CH_2Cl_2 (6.5 mL) and then dried in vacuo overnight. The linear H-RGDfK(CO-Chl)-OH peptide **31** was cleaved from the resin without affecting other protecting groups with 10 mL of a mixture of acetic acid, 2,2,2 trifluoroethane (TFE) and CH_2Cl_2 (1:1:3) for 1 h at room temperature. The resin was washed three times with acetic acid and then lyophilized. The head-to-tail cyclization was performed by slowly adding a solution of the linear peptide acetate salt **31** in 20 mL of CH_2Cl_2 to a solution of 50% 1-propane-phosphonic acid cyclic anhydride (T3P) in EtOAc (3 mL), triethylamine (3 mL), and DMAP (10 mg) in 400 mL of CH_2Cl_2. After stirring overnight, the reaction mixture was concentrated and purified by chromatography (MeOH/AcOEt, 10:90) to afford 115 mg of the chlorin coupled to the protected cyclic peptide **32**. A standard cleavage with a mixture of 0.75 g of crystalline phenol, 0.25 mL of 1,2-ethanedithiol, 0.5 mL of thioanisole, 0.5 mL of deionized H_2O, and 10 mL of trifluoroacetic acid (TFA) for 1.5 h afforded the crude peptide **33**, which was lyophilized and was purified by RP-HPLC to give 56 mg (45% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ −1.53, −1.58 (s, 2H, N*H*-pyrrole), 1.20 (m, 2H, δLys), 1.42 (m, 2H, γArg), 1.53, 1.72 (m, 2H, βArg), 1.55 (m, 2H, δLys), 1.80 (m, 2H, βLys), 2.40, 2.81 (m, 2H, β(D)Phe), 2.84, 2.94 (m, 2H, βAsp), 3.12 (m, 2H, δArg), 4.01–4.15 (m, 4H, αLys, αGly, αArg), 4.52 (br d, 1H, αAsp), 4.70 (br d, 1H, α(D)Phe), 4.13 (m, 4H, C*H*$_2$-chlorin), 6.77 (m, 1H, ζN*H*-Lys), 7.48 (br t, 1H, εN*H*Arg), 7.65 (br d, 1H, N*H*Arg), 8.05 (br d, 1H, N*H*-Asp), 8.08 (br d, 1H, (D)Phe-N*H*), 8.12 (br d, 1H, N*H*-Lys), 8.43 (br t, 1H, NHGly), 7.19–8.84 (m, 30H, ArH, pyrrole). MS (MALDI-TOFMS): *m/z* 1246.33 $[M+H]^+$. (MALDI-TOFMS): *m/z* 1246.33 $[M+H]^+$.

4.3. Absorption and fluorescence

Absorption spectra were recorded on a Perkin–Elmer (Lambda 2, Courtaboeuf, France) UV–vis spectrophotometer. Fluorescence spectra were recorded on a SPEX Fluorolog-3 (Jobin Yvon, Longjumeau, France) equipped with a thermostated cell compartment (25 °C), using a 450 W Xenon lamp. Fluorescence quantum yields (Φ_f) were determined using a TPP solution as a fluorescence standard (Φ_f=0.11,[28] in toluene, taking into account solvent refractive index and absorption efficiencies).

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

Excitation occurred with a Xe-arc, the light was separated in a SPEX 1680, 0.22 μm double monochromator. 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) $\Phi_f(^1O_2)$=0.68[29] and the sample solution (at 515 nm) was set equal (between 0.2 and 0.5) by dilution.

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