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An efficient route to dimeric porphyrin–RGD peptide conjugates via olefin metathesis

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Abstract

We report the efficient use of cross-metathesis for the solid-phase synthesis of a porphyrin dimer containing as a spacer a pentapeptide moiety with RGD sequence. Such compound appears as a promising candidate for application in PDT.

Keywords: Porphyrin; RGD; Dimers; Metathesis; Solid-phase synthesis

1. Introduction

Metal-catalyzed olefin metathesis has emerged as a powerful tool for carbon–carbon bonding in organic synthesis; more particularly, ring-closing metathesis (RCM) and cross-metathesis (CM) have been thoroughly investigated.[1] Well-defined transition metal catalysts such as the ruthenium carbene complexes (**I**, **II**)[2] developed by the Grubbs' group exhibit good activity; their further commercial availability greatly helped the development of olefin metathesis as an elegant way to achieve molecular complexity.[3] We report in this article the efficient solid-phase synthesis, with the use of cross-metathesis, of a glucosylated porphyrin dimer bearing, between the two macrocycles, a pseudopentapeptide spacer containing an arginyl-glycyl-aspartyl (RGD) sequence. This work has been realized in connection with our research program on photosensitizers and their use in Photodynamic Therapy (PDT).[4] PDT is a local treatment designed to eliminate abnormal tissue, including cancerous tumors and involves the selective accumulation of photosensitizers in tumors and their further irradiation by visible light, leading to the destruction of the treated cells.[5] The present porphyrinic derivative gathers several important features in relation to its potential use in PDT of cancers. First, as a porphyrin dimer, this molecule is prone to an increased light harvesting as compared to porphyrin monomers.[6] Second, the RGD sequence qualifies this photosensitizer to bind $\alpha_v\beta_3$ integrin, an extracellular matrix (ECM) protein highly expressed by many tumor cells such as osteosarcomas, neuroblastomas, and lung carcinomas,[7] and, more importantly, by endothelial cells of tumor neovasculature.[8] Third, glucosylation of the porphyrin macrocycles increases the amphipathic character of the synthesized molecule,[9] and, last, the two allyl glycines that frame the RGD sequence contribute to the conformational flexibility and adaptability of the peptidic moiety of the synthesized photosensitizer.[10] Owing to its design the latter appears as a promising candidate for applications in PDT.

2. Results and discussion

Benzaldehyde **1** was prepared according to a procedure described in our previous papers.[11] Synthesis of the starting porphyrin monomers **2** and **3** was realized according to Lindsey's method.[12] The first step (Scheme 1) consists in the condensation of pyrrole (4 equiv) with 4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)benzaldehyde **1** (3 equiv) and 4-hydroxybenzaldehyde or 4-allyloxybenzaldehyde (1 equiv).

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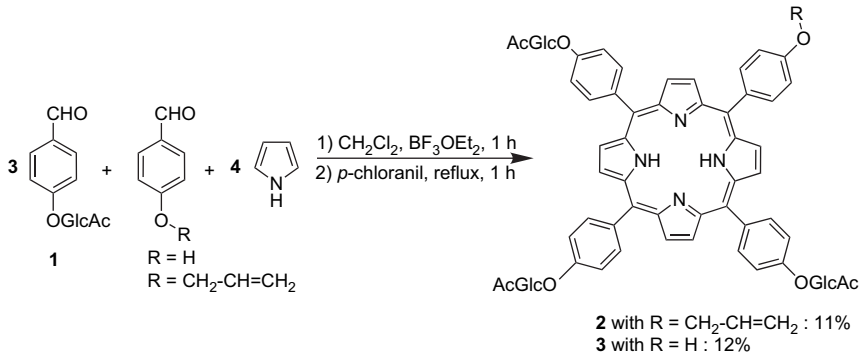
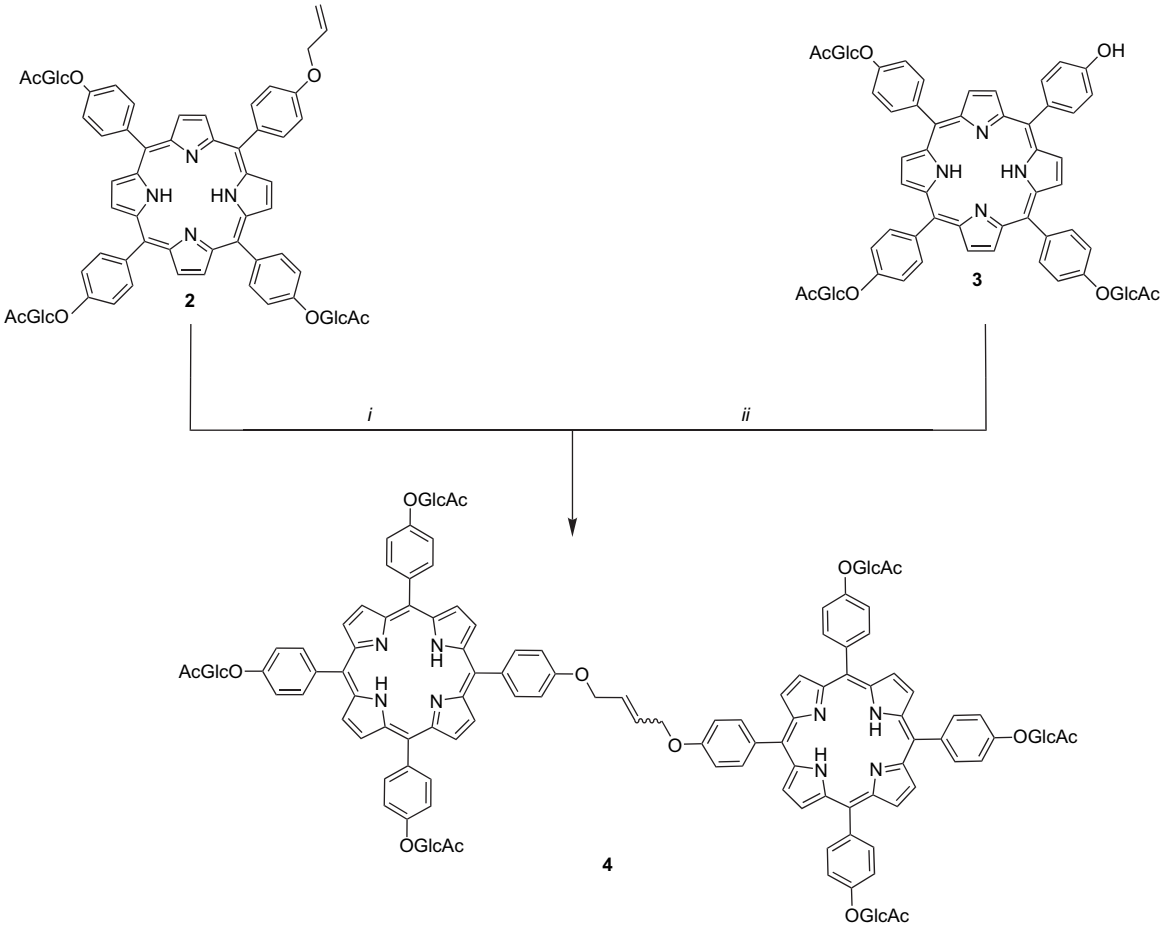


Scheme 1. Synthesis of porphyrins **2** and **3** by Lindsey's method.

In all cases, these reagents were added to dry CH_2Cl_2 under argon at room temperature using BF_3/etherate as a catalyst. Oxidation of the porphyrinogen intermediates with *p*-chloranil, followed by flash chromatography and purification on silica gel TLC gave porphyrins **2** and **3** in 11 and 12% yields, respectively.

Two strategies for the preparation of dimer porphyrin are depicted in Scheme 2. In a first attempt, porphyrin dimer **4** was obtained by cross-metathesis from porphyrin conjugate **2**. In a typical experiment, porphyrin **2** (10^{-2} mol L^{-1}) was dissolved in dry, degassed dichloromethane under argon and then 20 mol % of Grubbs' catalyst **I** or **II** predissolved in CH_2Cl_2 was added in two divided portions with the help of a syringe. Experiments realized under different conditions (nature of catalyst, reaction temperature, see Table 1) show that the best yield of dimer **4** was obtained with Grubbs' catalyst **II** at 40 °C. Under these conditions, compound **4** was isolated, after purification by TLC, in 63% yield (Table 1, entry 4).

The second method involved the synthesis of compound **4** from **3** by ether linkage. The aim of this strategy was to



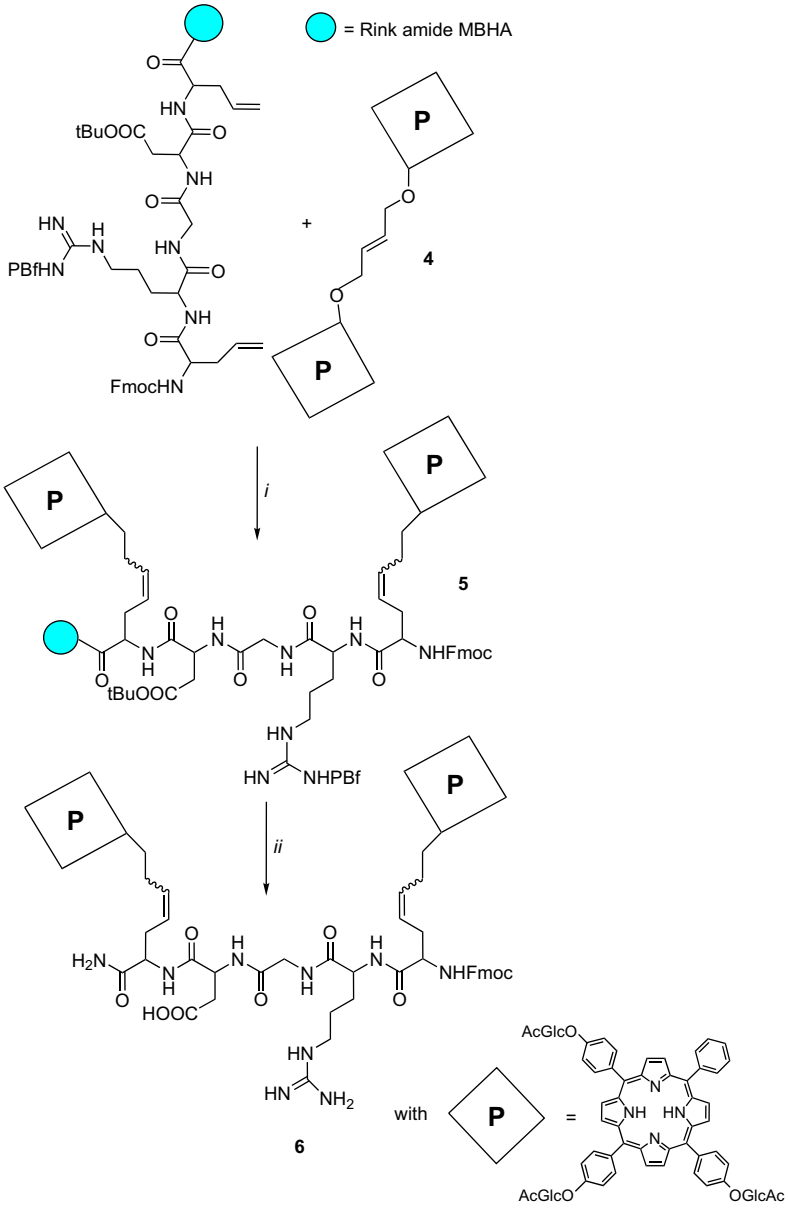
Scheme 2. i) Method 1: Grubbs' catalyst **I** or **II**, CH_2Cl_2; (ii) method 2: $BrCH_2CH{=}CHCH_2Br$ (1 equiv), K_2CO_3 (20 equiv), DMF, rt, 48 h then 30 h.

Table 1
Reaction conditions for the synthesis of dimer **4** by cross-metathesis

Entry	Catalyst (20 mol %)	Temperature (°C)	Yield (%)
1	I	20	57
2	I	40	61
3	II	20	59
4	II	40	63

compare its yield with that of method 1. The reaction of an excess of *trans*-1,4-dibromobut-2-ene (added in two separate portions: 1 equiv and then 3 equiv) with monohydroxyphenylglucosylporphyrin **3** in distilled DMF in presence of potassium carbonate under reflux, led to the 2,4-bis-(4(10,15,20-tri[2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)phenyl]-porphyrin-5-yl)phenyloxy)-but-2-ene **4**. After purification by TLC, this compound was obtained in 54% yield.

In the aim to insert the RGD sequence between two units of triglucosylated porphyrin derivatives, cross-metathesis reaction was carried out on a Rink amide MBHA resin bearing the allylGlycyl-RGD-allylGlycine linear peptide (prepared according to conventional solid-phase peptide synthesis using the Fmoc strategy), in which the aspartyl β-carboxyl and the arginyl guanidino functions were, respectively, protected with *tert*-butyl and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) (Scheme 3). In connection with our previous works, we have chosen to use a resin with a substitution of 0.1 mmol g^{-1}.[10] In a typical experiment, the resin beads were allowed to swell in dry, degassed dichloromethane under argon and then Grubbs' catalyst (**I** or **II**) predissolved in dichloromethane was added with the help of a syringe. After completion of the reaction the beads were filtered, rinsed with CH_2Cl_2, DMF, and MeOH and then the product was



Scheme 3. i) Grubbs' catalyst (40 mol %), CH_2Cl_2, 48 h, 40 °C, then rinsing with CH_2Cl_2, DMF, and MeOH; (ii) CH_2Cl_2/TFA (9:1) then TFA/anisole (9:1).

detached and deprotected by treating the reacted resin with TFA/anisole. Different conditions of temperature, reaction time, catalyst or resin substitution were tested. Considering the results previously obtained for the synthesis of porphyrin dimer conjugate **4** by cross-metathesis, we have chosen to use Grubbs' catalyst **II**. Different conditions of temperature and amounts of dimer **4** were tested. Reaction course was monitored by TLC of the reaction mixture and the appearance of final product **6** was checked by TLC after treatment (TFA/anisole) of a small amount of resin (Fig. 1). Under the best conditions, (Table 2, entry 3) we obtained, after deprotection and detachment, the expected compound **6** with 11% yield.

Mass spectrometry of porphyrin derivatives (Figs. 2 and 3) were 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%.

All these products were individually characterized by ^1H and ^{13}C NMR analysis in $CDCl_3$ or $CDCl_3/CD_3OD$ (9:1) (400.13 MHz). The detailed resonance assignments are based on integration and selective homonuclear decoupling, as well as NOE and 2D homonuclear COSY experiments.

The general assignment for starting porphyrin derivative **3** is in agreement with previous works realized in our laboratory. For porphyrin derivative **2** bearing allyl group and in comparison with the ^1H NMR spectrum of **3**, we observed the characteristic signals of protons belonging to β pyrrolic, aromatic, and glucosyl units. Resonance corresponding to CH proton of allyl group was identified at δ 6.17 ppm (double of double of triplet). The two equivalent protons of allylic methylene group were identified as a broad doublet at δ 4.82 ppm. Finally, methylene protons in the terminal position of the vinyl group were not equivalent, with chemical shifts 5.61 (doublet of doublet) and 5.46 ppm (multiplet).

Table 2
Results obtained for synthesis of dimeric porphyrin–RGD peptide conjugate **6**

Entry	Equivalents of compound **4**/peptidic allyl function	Temperature (°C)	Yield after purification
1	3	20	0
2	4	20	Traces
3	4	40	11%
4	6	20	Traces
5	6	40	9%

Comparison of dimer **4** with monomer **2** showed a slipping and/or broadening of signals of glucosyloxyphenyl aromatic protons, H-4 of glucosyl units and of acetyl protons (see Section 4, Fig. 4 in Supplementary data). These phenomena reflect a modification of symmetry of the compound.[13] Protons of the insaturated chain were identified as a triplet at δ 6.53 ppm (2H) and a very broad singlet at 5.00 ppm (4H).

^{13}C NMR spectra recorded at 100 MHz were obtained to confirm the structure of the compounds. Resonance assignments are based on DEPT experiments and $^1H-^{13}C$ shift correlation. For dimer porphyrin derivative **4**, we found the same phenomenon as in ^1H NMR, i.e., splitting or broadening of carbon signals (osidic, acetyl, aromatic groups) that reflects a modified symmetry (see Section 4, Fig. 5 in Supplementary data). In the aim to distinguish between *Z* and *E* isomers of dimer **4** obtained by cross-metathesis (method 1), observation of carbon in α position of insaturation of this later compound is used. However, in our case, inspection of ^{13}C NMR spectra showed only one signal at 128.8 ppm. So, in order to remove all ambiguities, we studied and compared these spectra with those of *E* dimer porphyrin derivative **4** exclusively obtained by the Williamson reaction between the 5-monohydroxyphenyltritolylporphyrine **3** and the *trans*-1,4 dibromobut-2-ene (method 2). The two ^{13}C NMR spectra were similar, displaying the same signal at 128.8 ppm, which confirmed that dimer **4** obtained by cross-metathesis consisted only of a *E* isomer.

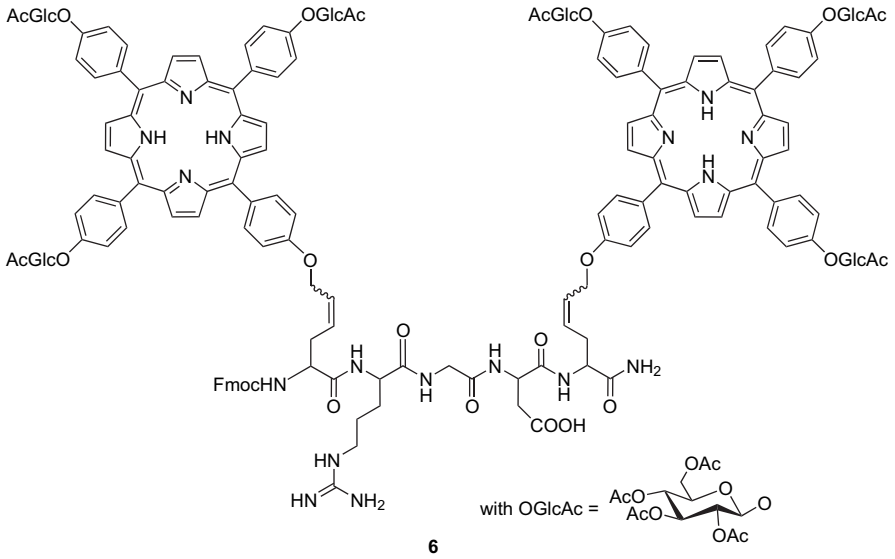


Figure 1. Schematic representation of the molecular structure of dimeric porphyrin–RGD peptide conjugate **6**.

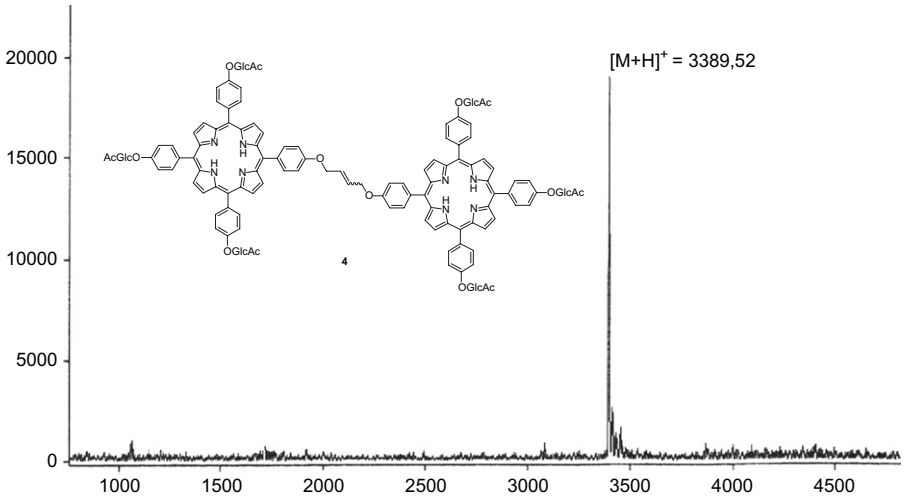


Figure 2. MALDI spectrum of dimer **4**.

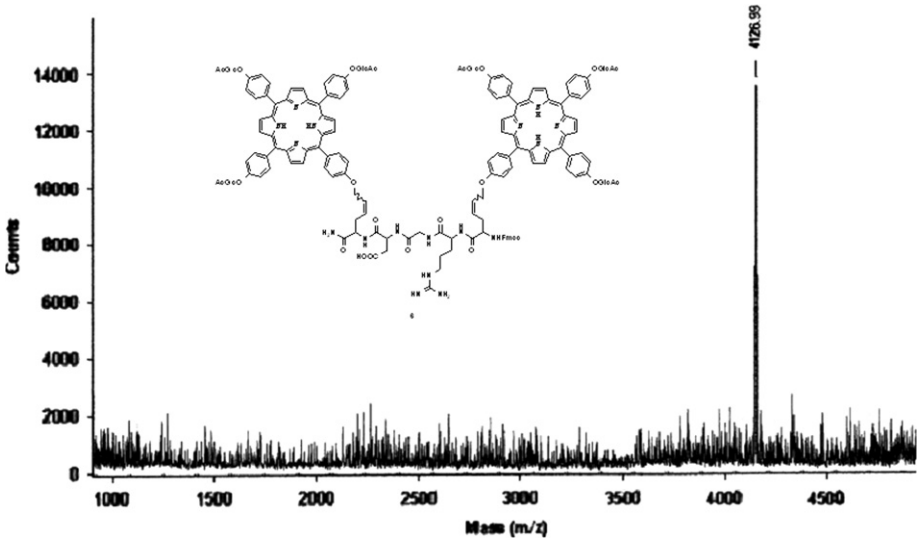


Figure 3. MALDI spectrum of dimeric porphyrin–RGD peptide conjugate **6**.

Finally ^{1}H NMR spectrum of porphyrin **6**, gave the expected signals, although somewhat broadened (see Section 4, Fig. 6 in Supplementary data). The comparison between the number of protons for Fmoc signals and the number of protons of aromatic groups confirmed the presence of the two macrocycles. Interestingly, these spectra displayed split signals for the aromatic protons of glucosyloxyphenyl substituents along with complex signal pattern of β-pyrrolic protons; these features reflect a reduced symmetry of dimer **6**. We also noted a downfield chemical shift of Fmoc protons resulting from the proximity of a porphyrin ring.

The electronic spectra of all protected *meso* substituted porphyrin derivatives **2**–**6** (Table 2) are very similar to those of various glycosylated porphyrins.[11,13] They display a Soret band near 420 nm and four less intense visible Q bands with an *etio* outline. We also observed an increased ε for porphyrin dimer conjugates **4** and **6**.

In order to determine the photosensitizing properties of dimeric porphyrin–RGD peptide conjugate **6**, the trapping reactions of $^{1}O_2$ with ergosterol acetate were carried out. Reference experiments with eosin, rose bengal or haematoporphyrin (HP), known as singlet oxygen producers, gave ergosterol acetate epidioxide with nearly quantitative yields.[14] In the same experimental conditions, porphyrin **6** showed the same efficiency for $^{1}O_2$ production than HP. So dimer **6** appears to be a promising candidate for application in PDT. Biological evaluation of compounds **6** and the corresponding deprotected product for their anticancer activity by PDT is currently in progress in our laboratory against the promyelocyte K562 cell line. Preliminary results obtained shows that the photocytotoxicity of these synthetic dimers against K562 cells indicated that the dead cell counts were lower than those observed with Photofrin® in the following conditions: porphyrins dimers (final concentration 1.25 μg mL^{-1}) are added to the wells and the plates are incubated for 18 h in the dark before illumination, then cells are irradiated during 30, 60, 90, and 120 min (fluence rate=10 mW cm^{-2}) and cell survival is measured immediately after irradiation or after a further 24 h

incubation in the dark using MTT assay.[13] These, preliminary, in vitro biological data suggest that amphiphilic characters are essential factors for an efficient photodynamic activity. In addition, the peptidic moieties could play a significant role in the photosensitizing properties and so a particular interest will be devoted to the role of the RGD groups on cellular uptake and on binding to specific cellular targets.[7b] Current studies are underway to elucidate these points (others cells lines) and to determine possible interactions with $\alpha_5\beta_3$ integrins.

3. Conclusion

We have presented for the first time a novel procedure to synthesize a new glycosyl bis-porphyrin derivative with the RGD peptidic sequence between two units of porphyrin through cross-metathesis reactions using Grubbs' catalyst, which appears as a new candidate for application in PDT. This methodology provides a versatile approach for the efficient synthesis of various porphyrinic hybrid systems. Further studies will be focused on the synthesis of more photosensitizer derivatives (as dissymmetric dimer, chlorins–porphrin dimers, bis-chlorins dimers, etc.) and examine their physicochemical (log *P*, singlet oxygen production) and biological properties (binding affinities, PDT activities).

4. Experimental

4.1. General experimental information

All solvents and reagents were purchased from Aldrich, Acros, or VWR. The grafted allylGlycyl-RGD-allylGlycine peptide was purchased from NEOSYSTEM. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Dimethylformamide was distilled over CaH_2 under reduced pressure and stored under argon. Methylene chloride was distilled over P_2O_5 and then over CaH_2. Analytical thin-layer chromatography (TLC) was performed on silica gel Merck 60 F_{254} or RP-18 F_{254S}. Column chromatography was carried out with silica gel (60 ACC; 15–40 μm, Merck) or LiChroprep® RP-18 (5–20 μm, Merck). Solid support functionalization was carried out in a small glass reactor (20 mL) equipped with a sintered glass filter, a stopcock, and a cap. ^1H and ^{13}C NMR spectroscopies were performed with a Brücker DPX-400 spectrometer. Chemical shifts are reported as δ in parts per million, downfield from internal TMS and are listed according to the standard numbering of *meso*-arylporphyrins and glucopyranose. UV–visible spectra were recorded on a Perkin–Elmer Lambda 25 double-beam spectrophotometer using 10 or 50 mm quartz cells. Infra-Red spectra were recorded on a Perkin–Elmer spectrum 1000 with KBr pellets.

4.2. Synthesis

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide and glucosylaldehyde **1** were synthesized according to the literature as described in a previous paper.[11b]

Table 3
UV–visible spectra [λ_{nm} ($\varepsilon\times10^{-3}$, $cm^{-1}\,mol^{-1}\,L$)] of porphyrin conjugates in organic solvents

Derivatives	Soret	Q IV	Q III	Q II	Q I
2[a]	421 (341.5)	517 (10.5)	553 (8.6)	592 (4.4)	648 (4.2)
3[a]	420 (422.0)	516 (14.2)	552 (8.2)	592 (4.0)	650 (4.4)
4[a]	421 (718.2)	517 (28.1)	553 (17.4)	592 (9.3)	649 (9.2)
6[b]	420 (686.2)	516 (27.2)	553 (15.1)	592 (10.4)	649 (8.7)

[a] Solvent: $CHCl_3$.
[b] Solvent: $CHCl_3$/MeOH (90:10).

4.2.1. 5-(4-Allyloxyphenyl)-10,15,20-tri[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]porphyrin (2)

Pyrrole (0.618 mL, 8,94 mmol, 4 equiv), per-*O*-acetyl glucosylated benzaldehyde **1** (3 g, 6.7 mmol, 3 equiv), and 4-allyloxybenzaldehyde (362 mg, 2.23 mmol, 1 equiv) were added to methylene chloride (500 mL) purged with argon for 30 min. The mixture was stirred and purged with argon for a further 10 min after which a $BF_3\cdot$etherate solution (0.5 mL, 0.2 M) in methylene chloride was added. This reaction mixture was stirred overnight at room temperature. *p*-Chloranil (0.75 equiv/pyrrole) was then added and stirred under reflux for 1 h. The solvent was evaporated to dryness and the porphyrin mixture was purified by column chromatography ($CHCl_3$/EtOH, 100:0–98:2) and TLC ($CHCl_3$/EtOH 98:2). Compound **2** of 190 mg was obtained (yield 11%). R_f 0.52 (chloroform/ethanol 98:2). UV–visible (see Table 3). ^1H NMR ($CDCl_3$, 400.13 MHz): δ (ppm)=8.88 (d, 2H, J=4.5 Hz, H-2,8 β-pyrrole), 8.86 (s, 4H, H-12,13,17,18 β-pyrrole), 8.83 (d, 2H, J=4.5 Hz, H-3,7 β-pyrrole), 8.14 (d, 6H, J=8.4 Hz, H-2,6 aryl), 8.10 (d, 2H, J=8.6 Hz, H-2,6 phenyl), 7.38 (d, 6H, J=8.4 Hz, H-3,5 aryl), 7.29 (d, 2H, J=8.5 Hz, H-3,5 phenyl), 6.27 (ddd, 1H, J=17.2–10.2–6.8 Hz, O–CH_2–CH=CH_2), 5.61 (dd,1H, J=17.2–1.3 Hz, O–CH_2–CH=HCH), 5.46 (m, 10H, H-1′,2′,3′ ose and O–CH_2–CH=HCH), 5.31 (t, 3H, J=9.4 Hz, H-4′ ose), 4.82 (br d, 2H, O–CH_2–CH=CH_2), 4.42 (dd, 3H, 12.0–5.1 Hz, H-6′a ose), 4.30 (br d, 3H, J=12.0 Hz, H-6′b ose), 4.06 (ddd, 3H, J=9.6–5.1–2.3 Hz, H-5′ ose), 2.22 (s, 9H, CH_3CO), 2.12 (s, 9H, CH_3CO), 2.11 (s, 9H, CH_3CO), 2.10 (s, 9H, CH_3CO), −2.78 (br s, 2H, NH–pyrrole). ^{13}C NMR ($CDCl_3$, 100.13 MHz): 170.6 (CH_3CO), 170.3 (CH_3CO), 169.5 (CH_3CO), 158.5 (1C, C-4 phenyl), 156.6 (3C, C-4 aryl ose), 146.0 (8C, C_α pyrrole), 137.2 (3C, C-1 aryl ose), 135.5 (8C, C-2,6 aryl ose and C-2,6 phenyl), 134.5 (1C, C-1 phenyl), 133.3 (1C, −O–CH_2–CH=CH_2), 131.1 (8C, C_β pyrrole), 120.2 (1C, C *meso* porphyrin), 119.2 (3C, C *meso* porphyrin), 115.1 (6C, C-3,5 aryl ose), 115.0 (1C, −O–CH_2–CH=CH_2), 99.1 (3C, C-1′ ose), 72.9 (3C, C-3′ ose), 72.3 (3C, C-5′ ose), 71.4 (3C, C-2′ ose), 69.1 (1C, −O–CH_2–CH=CH_2), 68.4 (3C, C-4′ ose), 62.1 (3C, C-6′ ose), 20.8 (CH_3CO), 20.7 (CH_3CO). MS (MALDI) m/z=1701.61 ($[M+H]^+$ monoisotopic).

4.2.2. 5-(4-Hydroxyphenyl)-10,15,20-tri[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]porphyrin (3)

Pyrrole (0.454 mL, 6.56 mmol, 4 equiv), per-*O*-acetyl glucosylated benzaldehyde **1** (2.2 g, 4.92 mmol, 3 equiv), and

4-allyloxybenzaldehyde (200 mg, 1.64 mmol, 1 equiv) were added to methylene chloride purged with argon for 30 min. The mixture was stirred and purged with argon for a further 10 min after which a BF_3·etherate solution (0.01 equiv) in methylene chloride was added. This reaction mixture was stirred overnight at room temperature. *p*-Chloranil (0.75 equiv/pyrrole) was then added and stirred under reflux for 1 h. The solvent was evaporated to dryness and the porphyrin mixture was purified by column chromatography ($CHCl_3$/EtOH 100:0–98:2) and thin-layer chromatography ($CHCl_3$/EtOH 95:5). Compound **3** (325 mg) was obtained (yield 12%). R_f 0.43 (chloroform/ethanol 95:5). UV–visible (see Table 3). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm)=8.89 (d, 2H, J=4.5 Hz, H-2,8 β-pyrrole), 8.86 (s, 4H, H-12,13,17,18 β-pyrrole), 8.83 (d, 2H, J=4.5 Hz, H-3,7 β-pyrrole), 8.15 (d, 6H, J=8.4 Hz, H-2,6 aryl), 8.15 (d, 2H, J=8.6 Hz, H-2,6 phenyl), 7.40 (d, 6H, J=8.4 Hz, H-3,5 aryl), 7.40 (d, 2H, J=8.5 Hz, H-3,5 phenyl), 5.48 (m, 9H, H-1′,2′,3′ ose), 5.35 (m, 3H, H-4′ ose), 4.44 (dd, 3H, 12.0–5.1 Hz, H-6′a ose), 4.31 (br d, 3H, J=12.0 Hz, H-6′b ose), 4.06 (ddd, 3H, J=9.6–5.1–2.3 Hz, H-5′ ose), 2.23 (s, 9H, CH_3CO), 2.13 (s, 9H, CH_3CO), 2.12 (s, 9H, CH_3CO), 2.10 (s, 9H, CH_3CO), −2.78 (br s, 2H, NH–pyrrole). ^{13}C NMR ($CDCl_3$, 100.13 MHz): 170.6 (CH_3CO), 170.3 (CH_3CO), 169.5 (CH_3CO), 156.6 (3C, C-4 aryl ose), 156.0 (1C, C-4 phenyl), 146.5 (8C, C_α pyrrole), 137.2 (3C, C-1 aryl ose), 135.7 (2C, C-2,6 phenyl), 135.5 (6C, C-2,6 aryl ose), 134.4 (1C, C-1 phenyl), 130.9 (8C, C_β pyrrole), 120.2 (1C, C *meso* porphyrin), 119.2 (2C, C *meso* porphyrin), 119.1 (1C, C *meso* porphyrin), 115.1 (6C, C-3,5 aryl ose), 113.7 (2C, C-3,5 phenyl), 99.2 (3C, C-1′ ose), 72.9 (3C, C-3′ ose), 72.3 (3C, C-5′ ose), 71.4 (3C, C-2′ ose), 68.1 (3C, C-4′ ose), 62.3 (3C, C-6′ ose), 20.8–20-7 (12C, CH_3CO). MS (MALDI) m/z=1671.4 ($[M+H]^+$ monoisotopic). Anal. Calcd (found) for $C_{86}H_{84}N_4O_{31}$: C, 61.87 (61.85); H, 5.07 (5.13); N 3.36 (3.31).

*4.2.3. 2,4-Bis-(4(10,15,20-tri[2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)phenyl]porphyrin-5-yl)phenyloxy)-but-2-ene (**4**)*

4.2.3.1. Method 1. Porphyrin **2** (100 mg, 0.06 mmol, 1 equiv) was dissolved under argon in freshly distilled CH_2Cl_2 (5 mL). Grubbs' catalyst **I** (bis-tricyclohexylphosphinedichlororuthenium benzylidene) or **II** ((4,5-dihydro-1,3-dimesityl-imidazolin-2-ylidene)tricyclohexylphosphine)-dichlororuthenium(IV) benzylidene) (9.6 mg, 0.0116 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was added to the solution. The reaction mixture was stirred under reflux for 18 h. Then, the solvent was evaporated in vacuo and the residue was purified by TLC ($CHCl_3$/EtOH 98:2). Dimer **4** was obtained with 63% yield (125 mg).

4.2.3.2. Method 2. Porphyrin **3** (50 mg, 0.03 mmol, 1 equiv) was dissolved in freshly distilled DMF (5 mL). Anhydrous K_2CO_3 (80 mg, 0.60 mmol, 20 equiv) was added and the mixture was heated to 60 °C for 30 min. *trans*-1,4-Dibromobut-2-ene (7 mg, 0.03 mmol, 1 equiv) was added and the reaction was stirred under room temperature for 48 h then supplementary 150 mg of *trans*-1,4-dibromobut-2-ene (0.09 mmol, 3 equiv) was added. The reaction was monitored by TLC. After an additional 30 h, the solvent was evaporated in vacuo and the reaction mixture was dissolved in 50 mL of $CHCl_3$ and washed several times with water (3×75 mL). The organic extract was then dried on $MgSO_4$, filtered, and concentrated under reduced pressure. The pure product **4** (55 mg) was obtained after purification on thin-layer chromatography ($CHCl_3$/EtOH 98:2) (yield: 54%).

R_f 0.47 (chloroform/ethanol 98:2). UV–visible (see Table 3). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm)=8.91 (d, 4H, J=4.7 Hz, H-2,8 β-pyrrole), 8.85 (d, 4H, J=4.7 Hz, H-3,7 β-pyrrole), 8.84 (s, 8H, H-12,13,17,18 β-pyrrole), 8.16 (d, 4H, J=8.5 Hz, H-2,6 phenyl), 8.14 (d, 6H, J=8.4 Hz, H-2,6 aryl), 8.12 (d, 6H, J=8.4 Hz, H-2,6 aryl), 7.38 (d, 6H, J=8.4 Hz, H-3,5 aryl), 7.37 (br d, 10H, J=8.5 Hz, H-3,5 phenyl and H-3,5 aryl), 6.53 (t, 2H, J=3.8 Hz, O–CH_2–C*H*=C*H*–CH_2–O), 5.47 (m, 18H, H-1′,2′,3′ ose), 5.32 (t, 3H, J=9.4 Hz, H-4′ ose), 5.30 (t, 3H, J=9.3 Hz, H-4′ ose), 5.00 (br s, 4H, O–CH_2–CH=CH–CH_2–O), 4.42 (m, 6H, H-6′a ose), 4.30 (dd, 6H, J=12.0–2.2 Hz, H-6′b ose), 4.05 (ddd, 6H, J=9.9–5.4–2.3 Hz, H-5′ ose), 2.22 (s, 9H, CH_3CO), 2.21 (s, 9H, CH_3CO), 2.12 (s, 9H, CH_3CO), 2.11 (s, 9H, CH_3CO), 2.10 (s, 9H, CH_3CO), 2.09 (s, 9H, CH_3CO), −2.78 (br s, 2H, NH–pyrrole). ^{13}C NMR ($CDCl_3$, 100.13 MHz): 170.6 (CH_3CO), 170.3 (CH_3CO), 169.5 (CH_3CO), 158.6 (2C, C-4 phenyl), 156.6 (6C, C-4 aryl ose), 146.5 (16C, C_α pyrrole), 137.2 (6C, C-1 aryl ose), 135.5 (16C, C-2,6 aryl ose and C-2,6 phenyl), 134.7 (2C, C-1 phenyl), 131.0 (16C, C_β pyrrole), 120.1–119.3 (8C, C *meso* porphyrin), 128.8 (2C, –O–CH_2–C*H*=C*H*–CH_2–O–), 115.1 (12C, C-3,5 aryl ose), 99.2 (6C, C-1′ ose), 72.8 (6C, C-3′ ose), 72.3 (6C, C-5′ ose), 71.3 (6C, C-2′ ose), 68.4 (6C, C-4′ ose), 68.2 (2C, –O–CH_2–CH=CH–CH_2–), 62.1 (6C, C-6′ ose), 20.8–20.6 (12C, CH_3CO). MS (MALDI) m/z=1701.61 ($[M+H]^+$ monoisotopic).

*4.2.4. Resin (**5**)*

Resin of 80 mg (Rink amide MBHA) bearing allylGlycyl-RGD-allylGlycine peptide (1 equiv) and 216 mg of **4** (0.0064 mmol, 8 equiv) were added to methylene chloride (5 mL) purged with argon for 30 min. Then Grubbs' catalyst (I or II) predissolved in dichloromethane with the help of a syringe. After 24 h, 1 mL of this solution was added again. After reaction (48 h), the beads were filtered, rinsed with CH_2Cl_2 (1×10 mL), MeOH (2×20 mL) then CH_2Cl_2 (2×10 mL). The incorporation yield was estimated, after drying under reduced pressure, by measurement of mass increase of the treated resin and was found around 85.1 mg (yield: 19%).

*4.2.5. N-[N-[N-[N-[N-(Fmoc)-5-(10,15,20-Tri[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy]phenyl)porphyrin-5-ylphenyloxyethyl)-4,5-didehydro-L-norvalyl]-L-arginyl]-glycyl]-L-aspartyl]-5-(10,15,20-tri[4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy]phenyl)porphyrin-5-ylphenyloxyethyl)-4,5-didehydro-L-norvalinamide (**6**)*

Resin **5** (amount containing 81.5 mg (0.00152 mmol) of protected dimeric porphyrin–RGD conjugate) was placed in

reactor system 45®, and 10 mL of 10% TFA in CH_2Cl_2 (v/v) were added, followed by 60 mL of the same mixture and the resin was allowed to react for 30 min. After solvent evaporation, reaction products were mixed with 5 mL of CH_2Cl_2 and treated with TFA (4.5 mL) and anisole (0.5 mL) After reaction completion (1 h), solvents were evaporated under reduced pressure. The mixture was suspended in $CHCl_3$ (20 mL) and washed with saturated $NaHCO_3$ (15 mL) and water (3×40 mL) until pH=7. After purification on silica gel plates ($CHCl_3$/EtOH 80:20) and filtration on a Sephadex LH20 column, 4 mg of compound **6** was obtained (yield 11%).

R_f: 0.41 ($CHCl_3$/EtOH 80:20). UV–visible (see Table 3). ^{1}H NMR ($CDCl_3/CD_3OD$ 9:1, 400.13 MHz): δ (ppm)= 8.96–8.84 (m, 16H, H β-pyrrole), 8.06 (br d, 4H, J=8.0 Hz, H-2,6 phenyl), 8.12 (br d, 12H, J=8.0 Hz, H-2,6 aryl ose), 7.71 (d, 2H, J=7.6 Hz, Fmoc), 7.55 (d, J=7.2 Hz, 2H, Fmoc), 7.39 (d, 6H, J=8.4 Hz, H-3,5 aryl ose), 7.38 (d, 6H, J=8.4 Hz, H-3,5 aryl ose), 7.24 (br d, 4H, J=8.0 Hz, H-3,5 phenyl), 7.05 (m, 4H, Fmoc), 6.09 (m, 2H, CH allylglycine), 5.71 (m, 2H, CH allylglycine), 5.48 (t, 12H, J=7.0 Hz, H-2′,3′ ose), 5.47 (d, 6H, J=7.0 Hz, H-1′ ose), 5.30 (m, 3H, H-4′ ose), 5.30 (t, 3H, J=9.3 Hz, H-4′ ose), 4.73 (m, 4H, phenyl–O–CH_2), 4.45 (m, 1H, CH aspartic acid), 4.41 (m, 1H, CH allylglycine), 4.40 (dd, 6H, J=12.1–5.6 Hz, H-6′a ose), 4.30 (m, 3H, CH_2 Fmoc and CH allylglycine), 4.29 (dd, 6H, J=12.1–2.0 Hz, H-6′b ose), 4.22 (br d, 2H, J=5.0 Hz, CH_2 glycine), 4.21 (br t, J=5.8 Hz, 1H, CH Fmoc), 4.15 (m, 1H, CH arginine), 4.02 (m, 6H, H-5′ ose), 3.55 (m, 2H, CH_2 arginine), 2.57 (m, CH_2 aspartic acid), 2.48 (m, 4H, CH_2 allyl glycine), 2.22–2.01 (m, 72H, CH_3CO), 1.80 (m, 2H, CH_2 arginine), 1.55 (m, 2H, CH_2 arginine). MS (MALDI) m/z=4126.99 ($[M+H]^+$ monoisotopic).

4.3. Singlet oxygen production

Photosensitizers (10^{-5} M) and ergosterol acetate were dissolved in DMF. The mixture was illuminated during 30 min with two white bulbs (30 W each, output 400–800 nm) giving a light fluence of 10 mW cm^{-2}, under oxygen atmosphere and room temperature. Then, the appearance of ergosterol acetate epidioxide (EEP) was monitored by TLC (R_f=0.3 eluent $CHCl_3$).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.10.092.

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