Synthesis of cationic porphyrin modified amino acids†

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Received (in Cambridge, MA, USA) 13th June 2005, Accepted 29th July 2005
First published as an Advance Article on the web 23rd August 2005
DOI: 10.1039/b508380j

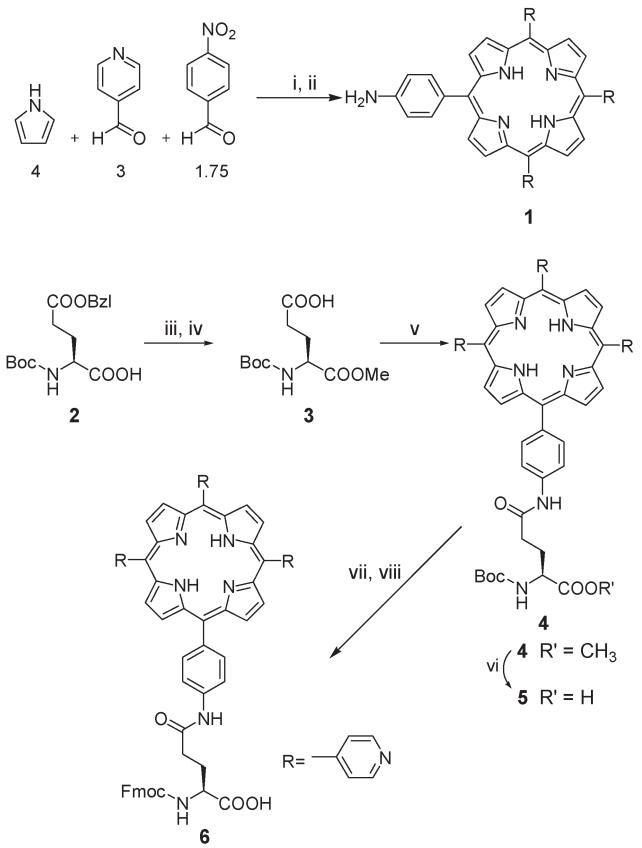
Derivatives of amino acids bearing a porphyrin moiety on a side chain were synthesized by coupling a porphyrin to a glutamic acid side chain; the utility of these compounds was demonstrated by their use in solid-phase synthesis of a peptide bearing a cationic porphyrin and by studying its DNA-binding properties.

In Nature, the task of organizing binding sites and effectors in a defined geometry to achieve exquisite molecular recognition and/or catalysis is performed by polypeptides. Inspired by these biological systems and by important potential applications in many areas, substantial efforts are currently being devoted to the construction of artificial proteins and novel functional supramolecular devices exploiting peptidic architectures.[1–3]

An important prerequisite for such a strategy is the availability of modified amino acids bearing an artificial binding site or an effector in a suitably protected form for direct use in solid-phase peptide synthesis (SPPS). Herein, we describe the synthesis of a cationic porphyrin modified glutamic acid protected either by a *N*-Boc or an *N*-Fmoc group. We also report its compatibility with SPPS in the preparation of a model tripeptide, as well as the efficient solid-phase *N*-methylation of pyridyl groups to yield cationic porphyrins. Such porphyrins are of great interest as they are inhibitors of human telomerases,[4] components of artificial receptors,[5] DNA intercalators,[6] and are commonly used in photodynamic therapy.[7]

The strategy relies on the linkage of the porphyrin to the glutamic acid side chain *via* an amide bond (Scheme 1). First, 5-(4-nitrophenyl)-10,15,20-tris(4-pyridinyl)porphyrin was prepared using the classical Adler–Longo procedure.[8] Reaction of 4-nitrobenzaldehyde (1.75 eq.), 4-pyridinecarboxaldehyde (3 eq.) and pyrrole (4 eq.) in the presence of acetic anhydride in refluxing propionic acid for 1.5 h gave a mixture of porphyrins, which after purification by silica gel chromatography yielded the desired 5-(4-nitrophenyl)-10,15,20-tris(4-pyridinyl)porphyrin in 8% yield. Amino porphyrin **1** was obtained in a 98% yield by reduction of the nitro group by means of stannous chloride in 6 N HCl. On the other hand, *N*-Boc-glutamic acid methyl ester **3** was obtained in high yield by esterification of **2**, followed by a catalytic hydrogenation of the benzyl ester. The coupling reaction of **1** and **3** using dicyclohexylcarbodiimide (DCC) as coupling reagent did not yield the desired compound due to the low nucleophilicity of the aminophenyl group. To overcome this problem, the coupling reaction was achieved by activating the carboxylic function of **3** with ethyl chloroformate in dichloromethane in presence of triethylamine, followed by addition of **1**.[8b] The fully protected modified amino acid **4** was obtained in 80% yield after purification by silica gel chromatography. Methyl ester cleavage was performed with 1 N NaOH in THF to give **5** in 90% yield, ready to use in Boc-SPPS. To obtain the *N*-Fmoc protected analogue, the *N*-Boc protecting group was removed with 4 M HCl in 1,4-dioxane and reprotected using Fmoc-OSu to give **6** in 80% yield after purification by short column silica gel chromatography.

Compatibility with solid-phase synthesis conditions was confirmed by incorporating the modified amino acid **6** in a model tripeptide. To do so, we used the Wang resin as solid support



Scheme 1 Reagents, conditions and yields: (i) $CH_3CH_2CO_2H/Ac_2O$, reflux, 1.5 h; 8%; (ii) $SnCl_2$, 6 N HCl; 98%; (iii) a) DCC, HOBt, DCM, 0 °C, 30 min, b) MeOH; 98%; (iv) Pd/C, MeOH; 98%; (v) a) ClCOOEt, TEA, DCM, 0 °C, 30 min, b) **1**, TEA, DCM, 0 °C, 2 h; 80%; (vi) 1 N NaOH, THF, 0 °C, 30 min; 90%; (vii) 4 M HCl/dioxane, 30 min; 99%; (viii) Fmoc-OSu, DIEA, acetonitrile/H_2O (9 : 1), 3 h; 80%.

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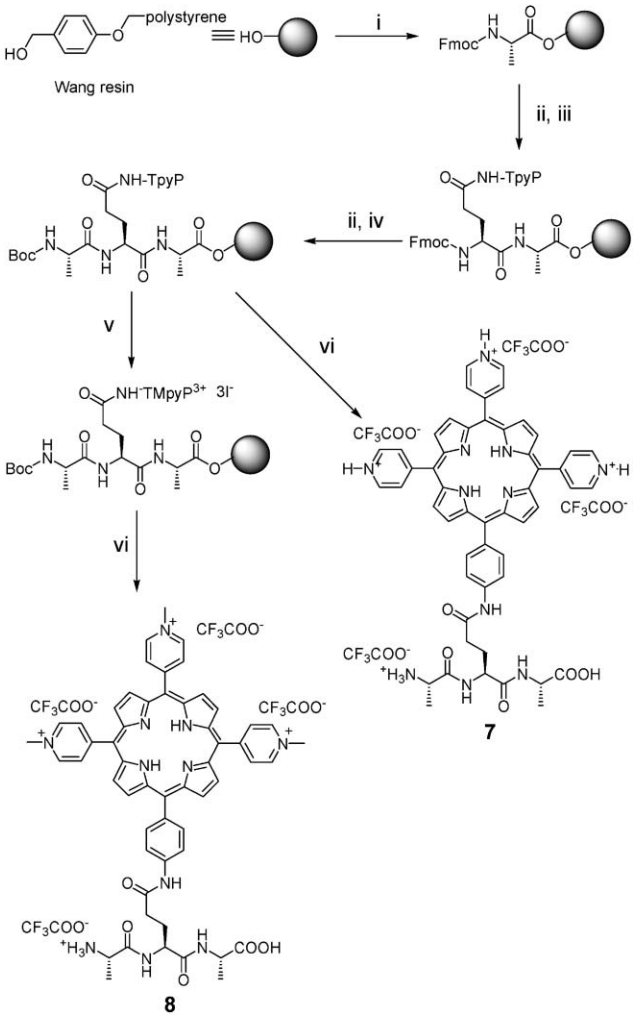
† Electronic supplementary information (ESI) available: Procedures for the synthesis of **1–8**; ^{1}H NMR and MS data for **1–8**; and UV spectra of DNA titration of H_2TMPyP and **8** with poly(dGdC)$_2$ and poly(dAdT)$_2$. See http://dx.doi.org/10.1039/b508380j

(Scheme 2). The first amino acid, *N*-Fmoc-Ala-OH, was attached to the resin *via* activation with diisopropylcarbodiimide (DIC) and hydroxybenzotriazole (HOBt) in DMF. The *N*-Fmoc protecting group was removed with 20% piperidine/DMF. The modified amino acid **6** was coupled to the amino free resin-bound alanine using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) in presence of diisopropylethylamine (DIEA). Finally, the third amino acid, *N*-Boc-Ala-OH, was introduced after removing the *N*-Fmoc protecting group using standard procedure. The final amino acid is *N*-Boc protected to generate free peptides after cleavage under acidic conditions. Most conveniently, *N*-methylation of the pyridyl groups could be carried out directly on solid-phase with an excess of iodomethane in DMF. Finally, methylated and non-methylated peptides were cleaved from the resin using a mixture of trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water (95 : 2.5 : 2.5). Tripeptides **7** and **8** were obtained in good yields (27 and 28% overall yields) and in a highly pure form. Purity was determined by HPLC and peptides were characterized by ^{1}H-NMR and ESI-MS.



Scheme 2 Synthesis of peptides bearing a porphyrin side chain: Reagents and conditions: i) Fmoc-Ala-OH, DIC, HOBt, DIEA, DMF; ii) 20% piperidine/DMF; iii) **6**, HATU, DIEA, DMF; iv) Boc-Ala-OH, DIC, HOBt, DIEA, DMF; v) CH_3I, DMF, 5 h; vi) TFA/TIS/H_2O (95 : 2.5 : 2.5).

DNA binding studies were performed to demonstrate that the attached cationic porphyrin maintains its DNA binding properties. The modes of interaction of porphyrins with DNA have been studied by several groups and their results established three types of binding modes: intercalative binding, groove binding, and outside binding.[9] Intercalative binding has been found to occur predominantly at GC-rich regions, groove binding at AT-rich regions, and outside binding at both GC-rich and AT-rich regions.[6d,10] During spectrophotometric titration with DNA, the intercalated porphyrin species has the following characteristics: (i) a large red shift of the Soret band ($\geqslant$ 15 nm),[11] (ii) substantial hypochromicity ($\geqslant$ 35%),[11] and (iii) an induced negative CD band in the Soret region.[6d,12] In contrast, the groove binding porphyrin species has the following characteristics: (i) a small red shift in the Soret band ($\leqslant$ 8 nm),[11] (ii) little hypochromicity or hyperchromicity of the Soret maximum,[11] and (iii) an induced positive CD band in the Soret region.[6d,12] On the other hand, the outside binding porphyrin species is characterized by an induced conserved Soret region.[6d,12] Association of peptide **8** with various types of DNA (calf thymus DNA (CT-DNA), poly(dAdT)$_2$ and poly(dGdC)$_2$) was examined by spectrophotometric titration in the Soret region (UV and CD) and compared with the *meso*-tetrakis(*N*-methyl-4-pyridyl)porphyrin (H_2TMPyP). The high extinction coefficient of the Soret band for the cationic porphyrins allowed spectrophotometric detection of porphyrin–DNA interaction at very low concentration (5 μM). Visible spectra of H_2TMPyP and compound **8** were recorded in the presence of an increasing amount of DNA in a buffer (TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0) at 1/*R* values up to 50, where *R* denotes input ratio of ([porphyrin]/[base pairs]).[9d,13]

During titration with CT-DNA, which contains 42% GC base pairs, the intensity of the Soret band of H_2TMPyP decreased with one set of isosbestic points as shown in Fig. 1A. Furthermore, the λ_{max} shifted to longer wavelength (bathochromic shift: $\Delta\lambda$ = 16 nm) and showed large hypochromicity (H = 44%). The hypochromicity was determined by the equation $H = (A_f - A_b)/(A_f) \times 100$, where A_f and A_b represent the Soret absorbances of the free and bound porphyrins, respectively. Compound **8** showed a similar behavior during titration with CT-DNA with a bathochromic shift ($\Delta\lambda$) of 20 nm, an important hypochromicity (H) of 49% and an isosbestic point (Fig. 1B). Results for both compounds correspond to an intercalative binding and because of the presence of an isosbestic point throughout the titration, the optical contribution certainly came from two distinct species, free and bound porphyrin chromophore.[11]

In contrast, when poly(dAdT)$_2$ was used the intensity of the Soret band decreased much less for both studied compounds. For H_2TMPyP a bathochromic shift ($\Delta\lambda$) of 8 nm and a hypochromicity (H) of 26% were observed. In the same way a bathochromic shift ($\Delta\lambda$) of 6 nm and a hypochromicity (H) of 4% were observed for compound **7**. On the other hand, during titration with poly(dGdC)$_2$, the intensity of the Soret band of H_2TMPyP and compound **8** decreased with one set of isosbestic points. For H_2TMPyP a bathochromic shift ($\Delta\lambda$) of 22 nm and a substantial hypochromicity (H) of 52% were observed. Similarly a bathochromic shift ($\Delta\lambda$) of 20 nm and an hypochromicity (H) of 54% were observed for compound **8**. These results clearly demonstrate that the attached porphyrin maintains its DNA-binding properties.

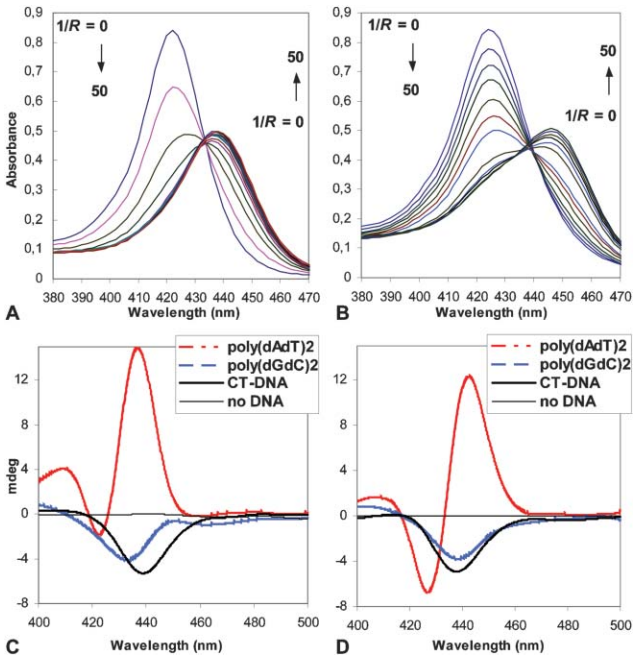


Fig. 1 Spectrophotometric titrations of H_2MTPyP and **8** with various DNAs. A) UV titration of H_2TMPyP with CT-DNA; B) UV titration of **8** with CT-DNA; C) Induced CD of H_2TMPyP with CT-DNA, poly(dAdT)$_2$ and poly(dGdC)$_2$ at $1/R = 6$; D) Induced CD of **8** with CT-DNA, poly(dAdT)$_2$ and poly(dGdC)$_2$ at $1/R = 6$.

Induced circular dichroism (ICD) in the Soret region is very helpful for analysis of the binding mode of an achiral porphyrin to chiral DNA.[6d,14] Cationic porphyrins, H_2TMPyP and compound **8**, did not show any ICD in the absence of duplex DNA, but characteristic spectra in the Soret region were induced with addition of DNA in buffer (TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0). Fig. 1C shows the ICD spectra of H_2TMPyP bound to CT-DNA, poly(dAdT)$_2$ and poly(dGdC)$_2$ at $1/R = 6$. In the presence of poly(dAdT)$_2$ the ICD spectrum comprised a small negative peak at 427 nm and a large positive peak at 437 nm corresponding to groove binding. In the presence of poly(dGdC)$_2$ and CT-DNA a negative peak was induced at 439 and 433 nm, respectively, corresponding to intercalative binding. ICD spectra of compound **8** bound to various DNAs showed a similar profile (Fig. 1D) with a positive peak at 442 nm in presence of poly(dAdT)$_2$ and a negative peak at 438 nm in presence of poly(dGdC)$_2$ and CT-DNA. The obtained results confirmed that the modified porphyrin maintains its DNA-binding properties when introduced into a peptide structure and clearly show that intercalative binding occurs at a GC-rich region and groove binding at an AT-rich region.[6d,12–14]

In conclusion, cationic porphyrin modified amino acids were rapidly and efficiently prepared in both *N*-Fmoc and *N*-Boc protected forms. The *N*-Fmoc protected modified amino acid was successfully used in solid-phase peptide synthesis, demonstrating the compatibility of porphyrin residues with Fmoc-SPPS conditions. We also showed that DNA-binding properties of the cationic modified porphyrin are maintained when it is introduced into a peptidic sequence compared to the cationic porphyrin analogue H_2TMPyP. With the possibility of solid-phase quaternization, modified amino acids **5** and **6** are useful building blocks for the development of novel peptidic nanostructures with interesting biological and functional properties, including electron/energy transfer systems, artificial host compounds, DNA polyintercalating molecules and telomerase inhibitors. Efforts along these lines are currently being pursued in our laboratory.

This research was supported by the NSERC of Canada and the FQRNT of Quebec. E.B. thanks the NSERC of Canada and the FQRNT of Quebec for postgraduate scholarships.

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