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Synthesis and in vitro PDT activity of miscellaneous porphyrins with amino acid and uracil

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Abstract—The synthesis of a series of modularized porphyrins bearing bioactive molecule is described. Starting with *meso*-tetraphenylporphyrin, the compounds with two nitro functional groups were synthesized via regiospecific nitration reaction. After reduction to the amino group and subsequent coupling with L-phenylalanine or 1-carboxylmethyl-5-fluorouracil (5-Fu acid), the functionalized porphyrins were metallized with Co(II) or Mn(II) to form miscellaneous porphyrins in good yields. The spectra of all the porphyrins were furnished. In vitro photodynamic therapy of the porphyrins against Ec9706 cell line was evaluated by standard cytotoxicity assays.

1. Introduction

Photodynamic therapy (PDT), a binary therapy of cancer, is a new modality for treatment of solid tumor based on the selection aggregation of photosensitizers such as porphyrin in tumor tissues.[1–3] Irradiation of the neoplastic region with light leads to activation of photosensitizer. Thereby, the generated singlet oxygen ruins abnormal tissues while sparing healthy adjacent areas. The first generation of pophyrin photosensitizer, Photofrin® is widely used in many countries for treatment of diverse cancers. Further works in developing new photosensitizer with higher photoactivity and higher therapeutic index has resulted in a vast amount of second generation porphyrins.[4–7]

Recently much effort has been aimed at several properties of modified porphyrins. It has been believed that the presence of the carbohydrates moiety,[8–11] amino acidic residues,[12,13] steroid,[14–16] etc., could enhance the membrane interaction to increase tumor selectivity. Porphyrins equipped with chemical therapeutic agents, nitrogen mustard analogues,[17,18] boron neutron capture therapy (BNCT),[19–21]and nitroxyl derivatives[22] were synthesized for better anticancer activity. Texaphytinato-Lu,[23] Chlorin[24] and Bacteriochlorophyll[23] exhibited a strong absorption band in the red wavelength area. Interest has also been focused on porphyrins bearing positively charged groups permitting water solubility.[25,26]

However, a strategy implicated in 'miscellaneous' porphyrins with verified biological potent molecule essence to 'add-on' the sole attributes like intracellular recognition, tissue selectivity, shift chromophores, and aqueous characteristic has accepted little attention.[27] Our report[28] in pophyrins field has suggested that amino acidic porphyrin displayed more PDT efficiency than their precursors, and amino acid has been approved to amplify the affinity of porphyrin with cell membrane.[29] On the other hand, widely used chemical anticancer reagents, such as uracil-containing compounds, suffer from side effects,[30] thus we got better cytotoxicity of 5-fluorouracil derivatives by the high compatibility of the high fatty acid with cell membrane.[31] Therefore it is very promising to synthesize novel compounds that can accumulate higher concentrations in malignant tumors than in normal tissues.

At present, development of new methodologies to functionalize porphyrins at porphyrin periphery, and β of pyrrole and aryl at *meso* sites is still an active and exciting field[32–34] due to providing a variety of new photosensitizers. However, practical and efficient methods for unsymmetrically substituted porphyrins are still

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remarkably absent because of feazing, puzzling heterogeneous condensation of pyrroles and mixed aldehydes. Previously, a vast majority of unsymmentric porphyrins linked with bioactive molecule residues have been synthesized by condensation of several aldehydes with pyrrole[8,12,15,17] followed by tedious chromatographic purification in poor yields. Recently, import of disparate groups to prepared porphyrins[35,36] released the pressing need.

Herein we describe a modified method of nitration of aryl at porphyrin periphery which is convenient for the synthesis of unsymmentric porphyrins. A series of substituted porphyrins with L-phenylalanine or 1-carboxylmethyl-5-fluorouracil (5-Fu acid) as functional substituent were synthesized in good yield, and their metal derivatives were also prepared. The primary in vitro photocytotoxicity of these compounds toward human esophageal cancer cell line was investigated.

2. Results and discussion

2.1. Synthesis of functionalized porphyrins

Porphyrin derivatives **3**–**11** were synthesized via the procedure shown in Figure 1.

So far, functionalization of synthetic porphyrins has been an alternative method for unsymmetrical porphyrins. We chose to improve the nitration reaction for substituting the *para*-H of phenyl at *meso*-tetraphenylporphyrin (TPP), which was readily prepared using Alder–Long method.[37] We have reported regiospecific nitration of *para* position of phenyl at porphyrin.[22] Herein we present a more convenient operation. A_2B_2 porphyrin **2** was obtained in good yield by nitration of TPP with Cl_3CCOOH/ HNO_3 in $CHCl_3$ at room temperature for a few minutes under air. Corresponding amino porphyrin **3** was readily synthesized by the treatment of **2** with $SnCl_2$/HCl at 60 °C over 1 h in yield of 90%.

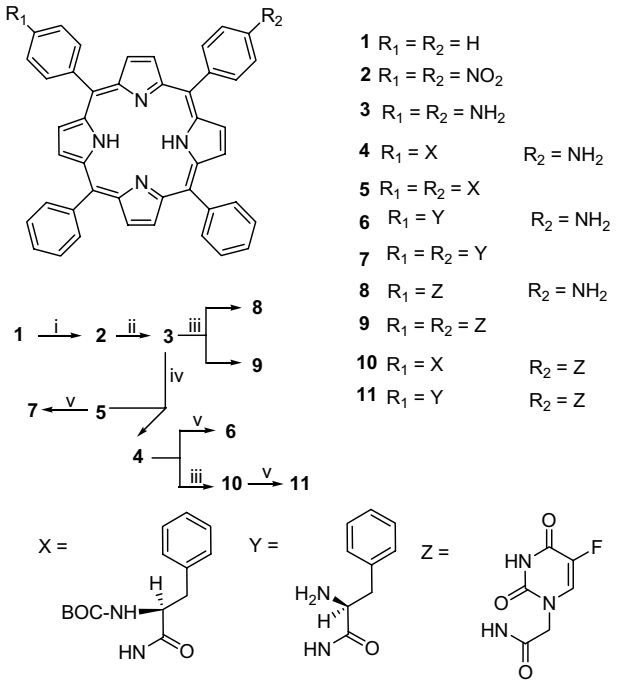

Figure 1. Preparation of porphyrin derivatives. Reaction conditions: (i) CCl_3COOH/HNO_3, $CHCl_3$, 5 min; (ii) $SnCl_2$/HCl, 60 °C, 60 min; (iii) 1-carboxylmethyl-5-fluoro-uracil, DCC, DMAP, DMF, rt, 48 h; (iv) L-phenylalanine, DCC, DMAP, CH_2Cl_2, 48 h; (v) CF_3COOH, CH_2Cl_2, 2 h.

Although the formation of amide bond is very common by condensation of carboxyl acid or carbonyl chloride with amine,[38,39] carboxylations of the amino groups at porphyrin are special due to the step by step procedure. The condensations of the amino porphyrins with carboxyl acid were realized in moderate yield with excess of *N*,*N*′-dicyclohexylcarbodiimide (DCC) as dehydrant, while the attempt was successful to condense monoamino porphyrin with carboxyl of Boc-valine in 90% yield.[28] The exact mechanism of the impediment of the amide bond to subsequent coupling is unknown, but this will benefit the synthesis of 'miscellaneous' porphyrins containing a variety of side chains via a series of functional group conversions step by step.

In this case, we preferred introducing L-phenylalanine moiety first instead of 5-Fu acid moiety, since the poor solubility of the earlier synthesized uracil porphyrin would prevent the subsequent carboxylation. The carboxyl moiety of Boc-L-phenylalanine was activated by DCC, producing an intermediate species which reacted with the amino group of porphyrin **3**. By condensation of Boc-L-phenylalanine with **3**, under the given conditions over a period of 48 h in CH_2Cl_2 at room temperature, 40% of **4** and 46% of **5** were obtained.

In addition, porphyrin **3** was added to the reaction mixture following reaction of Boc-L-phenylalanine with DCC for 30 min at low temperature, and only one Boc-L-phenylalanine was mounted to compound **3**, giving compound **4** in 82% yield.

By application of the similar condensation procedure in DMF, porphyrin bearing one Boc-L-phenylalanine residue was converted into 'miscellaneous' porphyrin **10** with uracil residue on another amino group in 95% yield.

One and double 5-Fu acids were appended on porphyrin to result in **8** and **9** with total yield of 95% by condensation of carboxyl of 5-Fu acid with the amino group of porphyrin **3** in DMF under the given conditions. Separation of the crude porphyrins on silica gel gave **8** in 47% yield, and **9** in 48% yield.

All the porphyrins equipped with Boc-L-phenylalanine were well deprotected by CF_3COOH in DMF for 2 h in good yields.

2.2. Preparation of the metal porphyrins

Metalations were performed in good yield according to the literature method.[38] As shown in Figure 2, mixture

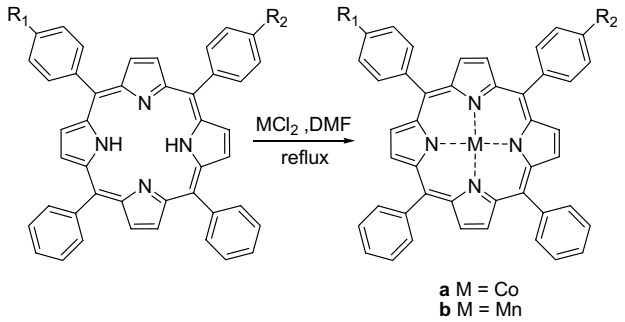

Figure 2. Metallization of porphyrins.

of the relative transition metal chloride and solution of porphyrins in DMF were refluxed for 2 h to give corresponding metallized porphyrins.

2.3. Characterization of the porphyrin derivatives

^{1}HNMR, HRMS, and UV were obtained for all free porphyrins, and the structures of metallized porphyrins were confirmed by these spectra except for ^{1}HNMR.

The synthesized free base porphyrins and their metallized derivatives were characterized with UV–vis spectra, comprising a Soret band and a Q band.

As shown in Table 1, side modifications on porphyrin structure have little effect on the photophysical properties of the chromophore, while insertion of a metal into the core of a porphyrin alters the optical spectrum drastically. The result agrees with the literature.[4] Coupling of amino acid or 5-Fu acid with porphyrin makes the change of λ_{max} by a few amounts, for example, a blue shift of 3.0 nm is present between the peak of Soret band of porphyrin **4** and that of the precursor **3**. Metals induce greater shift than the introduction of side chain. The difference between Q band of **3** and its metal derivatives **3a** or **3b** is approximately 30 nm.

Table 1. UV–vis spectra of porphyrin derivatives

Porphyrins	Soret band		Q band
3		422.0	516.0, 557.5, 597.0, 650.0
3a	308.0	430.0	543.0, 581.0
3b	381.5, 399.0	425.5	472.5, 573.5, 612.0
4		419.0	516.0, 554.0, 597.0, 648.0
4a	316.0	430.0	544.0, 582.0
4b	381.0, 401.0	423.0	470.5, 520.0, 564.0, 606.5
5		415.5	513.0, 548.5, 590.0, 645.0
5a		428.5	467.0, 543.5, 582.0
5b	382.5, 400.5	421.5	468.5, 520.0, 565.0, 601.0
6		421.5	516.5, 556.0, 597.0, 648.0
6a	314.0	435.5	549.0, 585.5
6b	381.5, 400.0	422.5	470.5, 522.0, 571.0, 606.0
7		417.5	513.0, 551.5, 593.5, 645.5
7a	323.0	434.0	546.0, 585.0
7b	380.0, 400.5	419.0	468.0, 522.0, 567.5, 601.5
8		416.5	516.0, 553.0, 592.5, 648.0
8a	316.0	433.0	544.0, 585.5
8b	381.0, 401.0	423.0	470.5, 571.0, 607.0
9		416.0	513.0, 553.0, 593.0, 645.0
9a	313.0	428.0	544.0, 585.0
9b	381.5, 401.0	418.5	468.5, 522.0, 565.5, 602.0
10		416.0	513.0, 551.0, 590.0, 645.0
10a	313.5	428.5	543.0, 575.0
10b	381.0, 401.0	421.5	468.5, 518.0, 565.5, 601.5
11		416.5	513.0, 553.0, 594.0, 648.0
11a	315.5	433.5	544.0, 585.0
11b	381.0, 400.5	422.0	468.5, 522.0, 567.0, 601.0

Compared to the metal porphyrins, it was found that substituents took opposite effects on free porphyrins. As can be seen in Table 1, introduction of 5-Fu acid or L-phenylalanine fetches blue shift of Soret band of free porphyrins. In contrast, red shift occurs when side modification on metal porphyrins is carried out.

2.4. In vitro photocytotoxicity of porphyrins

A standard MTT assay was performed at a fixed porphyrin concentration. Inhibition of porphyrin **4**–**12** against Ec9706 (human esophageal cancer cell line) was measured after irradiation for 2.5 h and a further 24 h of incubation in the dark. In vitro Photocytotoxicity data are summarized in Table 2.

As can be seen from Table 2, the photocytotoxicity of the vast majority of free porphyrins was improved by introduction of the bioactive molecules. Unlike Co(II), Mn(II) likely had advantageous effect on the anticancer therapy of porphyrins.

L-phenylalanine took notable effect on photocytotoxicity of porphyrins. As shown in Table 2, inhibition percentage was decreased from 66% to about 40% when single Boc-L-phenylalanine or L-phenylalanine was introduced onto **3**. However, inhibitions were enhanced to over 96% when two Boc-L-phenylalanines or L-phenylalanines were introduced. This result is different from the reported result,[8,12,22] in which inhibitions of porphyrins were enhanced by introduction of Boc-amino acid. Previous reports showed that amino acids, L-valine and L-alanine both evidently enhanced the anticancer activity of porphyrin by membrane recognition.[12,13]

5-Fu acid was confirmed to be profitable to photoactivity of porphyrins. Porphyrins with either one or two 5-Fu acid exhibited higher bioactivity than the precursor, suggesting that 5-Fu acid could increase the bioactivity of porphyrins. Parallel to other chemical

Table 2. Inhibition of porphyrins against Ec9706 (human esophageal cancer cell line)

Porphyrins	Free porphyrins (%)	Co (II) porphyrins (%)	Mn (II) porphyrins (%)
3	66.60	51.98	100.00
4	47.21	55.80	99.81
5	100.00	42.48	42.22
6	37.94	56.82	77.73
7	96.13	74.93	83.31
8	97.09	44.54	57.94
9	100.00	49.32	56.77
10	88.65	20.82	55.29
11	69.45	5	58.00

therapeutic agents,[17–21] 5-fluoro uracil assisted the porphyrin for photocytotoxicity via its strong cytotoxicity. It is possible that the selective congregation of porphyrins in tumor tissue was incorporated with high cytotoxicity of 5-Fu, resulting in magnified inhibitions of porphyrins.

Porphyrins decentralized the cultures with cells at 50 μg/mL. After irradiation for 2 h by red wave, kept at 37 °C in dark for 24 h. Inhibitions were measured by standard MTT methods. **a**: porphyrinate Co(II); **b**: porphyrinate Mn(II).

As shown in Table 2, all of the porphyrin complex with Mn(II) showed higher photocytotoxicity than those with Co (II). Inhibition against Ec9706 cell lines for porphyrins with Co (II) spread from 44% to 74%, while those for porphyrins with Mn(II) from 55% to 100%. It is interesting that insertion of metal ion enhances the activity of mono-substituted porphyrins **4**, **7**, and **9** and in contrast decreases that of di-substituted porphyrins **5**, **6**, **8**.

3. Conclusion

Functionalized porphyrins were synthesized using a modified nitration at *para* position of phenyl at porphyrin, reduction with $SnCl_2$/HCl, and then coupling with some bioactive molecules. A series of porphyrins bearing 5-Fu or L-phenylalanine were synthesized by coupling 5-Fu acid or Boc-L-phenylalanine with amino porphyrins in good yields. The in vitro photocytotoxicities of the functionalized porphyrins against Ec9706 cell lines were tested, and the results suggested that photocytotoxicities of porphyrin were improved remarkably by introduction of 5-fluorouracil and L-phenylalanin, whereas transition metals Co(II) and Mn(II) brought distinct effects on photo activity of porphyrins. Further developments of this strategy are in progress, including other characteristics of photocytotoxicities of porphyrins.

4. Experimental methods

5-Fu acid was synthesized according to the literature method.[40,41] Boc-phenylalanine and pyrrole were purchased from Aldrich, and distilled under reduced pressure before use. All other reagents and solvents were used as received. Solvents were of reagent grade unless otherwise specified and were dried and distilled by the standard method. The UV–vis spectra were obtained on a Perkin-Elmer LS-5B spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AVANCE DPX-400 spectrometer. HRMS (high-resolution mass spectra) were taken with a Q-Tof Micromass spectrometer.

4.1. General metallization of porphyrins

Porphyrins (1 equiv) were dissolved in $CHCl_3$, then MCl_2 (5 equiv) was added. The mixture was stirred, and refluxed for 2 h. Washed with water, the organic layer was dried on magnesium sulfate. Solvent was evaporated in vacuum. The crude product was purified by column chromatography.

4.1.1. 5,10-Di(4-aminophenyl)-15,20-diphenylporphyrin (3). To a solution of porphyrin **1** (1 g, 1.6 mmol) and trichloroacetic acid (50 g) chloroform (100 mL) was dropwise added HNO_3 (7.50 mL, 75%, 15.5 mmol) over 2 min at room temperature under atmosphere. The mixture was stirred for 5 min and then quenched with water, and neutralized with ammonium hydroxide aqueous solution to pH ~7. Chloroform (100 mL) was added, and the organic layer was washed with water (4× 150 mL), and dried over magnesium sulfate. Solvent was removed with evaporation under reduced pressure. The residue was dissolved in concentrated hydrochloric acid (30 mL), to which $SnCl_2 \cdot 2H_2O$ (6 g, 0.028 mol) was added. The mixture was heated to 60 °C and maintained for 1 h. After neutralization with ammonium hydroxide aqueous solution to pH ~7 under cooling, chloroform (200 mL) was added. The organic layer was washed with water (4× 200 mL) and dried over magnesium sulfate. Solvent was evaporated in vacuum. The crude product was purified by column chromatography (silica, eluent/chloroform) to provide **3** (370 mg, 36%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) = 8.92 (s, 4H, β-pyrrole), 8.82 (s, 4H, β-pyrrole), 8.21 (d, 4H, J = 6.2 Hz, 2, 6-H phenyl), 7.98 (d, 4H, J = 8.1 Hz, 2, 6-H aminophenyl), 7.77–7.71 (m, 6H, 3, 5-H phenyl), 7.06 (d, 4H, J = 8.2 Hz, 3, 5-H aminophenyl), 3.67 (s, 4H, NH_2), −2.73 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 422.0, 516.0, 557.5, 597.0, 650.0. HRMS: calcd for $C_{44}H_{32}N_6$: 644.2689; found: 667.2562 $[M+Na]^+$.

4.1.1.1. Compound 3a. Compound **3** (100 mg, 0.16 mmol) and $CoCl_2$ (100 mg, 0.78 mmol) in $CHCl_3$ (10 mL) gave 90 mg of **3a** (83%). UV–vis (λ_{max}, nm) in $CHCl_3$: 308.0, 430.0, 543.0; HRMS: calcd for $C_{44}H_{30}CoN_6$: 701.1864; found: 724.1765 $[M+Na]^+$.

4.1.1.2. Compound 3b. Compound **3** (100 mg, 0.16 mmol) and $MnCl_2$ (97 mg, 0.78 mmol) in $CHCl_3$ (10 mL) gave 99 mg of **3a** (92%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.5, 399.0, 425.5, 472.5, 573.5, 612.0. HRMS: calcd for $C_{44}H_{30}MnN_6$: 697.1912. found: 720.1817 $[M+Na]^+$.

4.1.2. 5-{4-[*N*-(*t*-Butyloxycarbonyl)-3-phenyl-L-alanylamido]phenyl}-10-(4-aminophenyl)-15,20-diphenylporphyrin (4). To a solution of Boc-L-phenylalanine (41 mg, 0.155 mmol) in dried chloroform (30 mL) were added DCC (17 mg, 0.35 mmol) and DMAP (20 mg, 0.35 mmol). The mixture was stirred at 0–5 °C for 60 min, and **3** (100 mg, 0.155 mmol) was subsequently added. The mixture was maintained at room temperature for 24 h, and then in ice bath overnight. Most of DCU was removed by filtration, oxalic acid was added to consume the remaining DCC. Solvent was evaporated in vacuum. The crude product was purified by column chromatography (silica, eluent: chloroform/methanol = 10:1) to give **4** in 82% yield. ^{1}H NMR ($CDCl_3$) δ 8.83–8.80 (m, 8H, β-pyrrole), 8.22–8.14 (m, 6H, 3,5-H amidophenyl and 2,6-H phenyl), 7.88 (d, 2H, J = 8.12 Hz, 3, 5-H amidophenyl), 7.80 (d, 2H, J = 8.04 Hz, 2, 6-H aminophenyl), 7.75–7.71 (m, 6H, 3, 4, 5-H phenyl), 7.40 (d, 4H, J = 8.25 Hz, 2, 3, 5, 6-H alkylphenyl), 7.24 (s, 1H, 4-H alkylphenyl), 6.98 (s, 2H, 3, 5-H aminophenyl), 5.32 (s, 1H, N-CH), 3.31 (m,

2H, Ph-CH_2), 1.51(s, 9H, C-CH_3), −2.73 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 419.0, 516.0, 554.0, 597.0, 648.0. HRMS: calcd for $C_{58}H_{49}N_7O_3$: 891.3897. found: 914.3791 $[M+Na]^+$.

4.1.2.1. Compound 4a. Compound **4** (100 mg, 0.11 mmol) and $CoCl_2$ (72 mg, 0.56 mmol) in $CHCl_3$ (10 mL) gave 90 mg of **4a** (85%). UV–vis (λ_{max}, nm) in $CHCl_3$: 316.0, 430.0, 544.0, 582.0; HRMS: calcd for $C_{58}H_{47}CoN_7O_3$: 948.3072. found: 971.2972 $[M + Na^+]$.

4.1.2.2. Compound 4b. Compound **4** (100 mg, 0.11 mmol) and $MnCl_2$ (70 mg, 0.56 mmol) in $CHCl_3$ (10 mL) gave 94 mg of **4b** (92%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.0, 401.0, 423.0, 470.5, 520.0, 564.5, 606.5. HRMS: calcd for $C_{58}H_{47}CoN_7O_3$: 944.3121. found: 967.3025 $[M+Na^+]$.

4.2. General procedure for synthesis of porphyrins (5), or (8),(9), (10) containing with amino acid and/or uracil

Porphyrin **3** was dissolved in CH_2Cl_2, and DCC (1–2.3 equiv), DMAP (1–2.3 equiv), Boc-L-phenylalanine (2.5 equiv) or 5-Fu acid (1–2 equiv) were added. The mixture was stirred at room temperature for 48 h, then DCU was removed by filtration followed by addition of oxalic acid. Solvent was evaporated in vacuum. The product was obtained by purification on silica gel.

4.2.1. 5,10-Di{4-[*N*-(*t*-butyloxycarbonyl)-3-phenyl-L-alanylamido]phenyl}-15,20-di-phenylporphyrin (5). Compound **3** (100 mg, 0.155 mmol), Boc-L-phenylalanine (100 mg, 0.377 mmol), DCC (43 mg, 0.35 mmol), DMAP (68 mg, 0.35 mmol) in CH_2Cl_2 (3 mL) gave 157 mg of **5** (89%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 6:1). ^1H NMR ($CDCl_3$) δ 8.83–8.80 (m, 8H, β-pyrrole), 8.19–8.11 (m, 6H, 3, 5-H amidophenyl and 2, 6-H phenyl), 7.79–7.68 (m,10H, 2, 5-H amidophenyl, 3, 4, 5-H phenyl), 7.41 (d, 8H, J = 5.6 Hz, 2, 3, 5, 6-H alkylphenyl), 7.25 (d, 2H, J = 5.6 Hz, alkylphenyl), 5.33 (s, 1H, N-CH), 3.30 (m, 2H, Ph-CH_2), 1.51 (s, 18H, CCH_3), −2.73 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 415.5, 513.0, 548.5, 590.0, 645.0. HRMS: calcd for $C_{72}H_{66}N_8O_6$: 1138.5105. found: 1161.5006 $[M+Na]^+$.

4.2.1.1. Compound 5a. Compound **5** (100 mg, 0.088 mmol) and $CoCl_2$ (57 mg, 0.44 mmol) in $CHCl_3$ (5 mL) gave 86 mg of **5a** (82%). UV–vis (λ_{max}, nm) in $CHCl_3$: 428.5, 467.0, 543.5, 582.0; HRMS: calcd for $C_{72}H_{64}CoN_8O_6$: 1195.4281, found: 1218.4171 $[M+Na]^+$.

4.2.1.2. Compound 5b. Compound **5** (100 mg, 0.088 mmol) and $MnCl_2$ (55 mg, 0.44 mmol) in $CHCl_3$ (5 mL) gave 99 mg of **5b** (95%). UV–vis (λ_{max}, nm) in $CHCl_3$: 382.5, 400.5, 421.5, 468.5, 520.0, 565.0, 601.0. HRMS: calcd for $C_{72}H_{64}MnN_8O_6$: 1191.4329. found: 1214.4231 $[M+Na]^+$.

4.2.2. 5-{4-[2-(5-Fluoro-uracil)-acetylamido]phenyl}-10-(4-aminophenyl)-15,20-diphenylporphyrin (8) 5,10-di{4-[2-(5-fluoro-uracil)-acetylamido]phenyl}-15,20-diphenylporphyrin (9). Compound **3** (200 mg, 0.31 mmol), 5-Fu acid (113 mg, 0.6 mmol) and DCC (85 mg, 0.7 mmol), and DMAP (135 mg, 0.7 mmol) in CH_2Cl_2 (6 mL) gave 100 mg of **8** (40%) and 140 mg of **9** (46%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 9:4). **8** ^1H NMR ($CDCl_3$) δ 10.87 (s, 1H, NH-uracil), 8.97 (s, 4H,β-pyrrole), 8.80 (d, 4H, J = 4.9 Hz, β-pyrrole), (m, 4H,β-pyrrole), 8.23–8.17 (m, 6H, 3, 5-H amidophenyl and 2, 6-H phenyl), 8.06 (d, 2H, J = 9.6 Hz, 2, 6-H amidophenyl), 7.88–7.82 (m, 10H, 2, 3, 5, 6-H aminophenyl and 3, 4, 5-H phenyl), 4.71 (s, 2H, N-CH_2), −2.84 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$:416.5, 516.0, 553.0, 592.5, 648.0. HRMS: calcd for $C_{50}H_{35}FN_8O_3$: 814.2816. found: 837.2718 $[M+Na]^+$.

4.2.2.1. Compound 8a. Compound **8** (80 mg, 0.098 mmol) and $CoCl_2$ (63 mg, 0.49 mmol) in $CHCl_3$ (5 mL) gave 68 mg of **8a** (80%). UV–vis (λ_{max}, nm) in $CHCl_3$: 316.0, 433.0, 544.0, 585.5; HRMS: calcd for $C_{50}H_{33}CoFN_8O_3$: 871.1992, found: 894.1896 $[M+Na]^+$.

4.2.2.2. Compound 8b. Compound **8** (80 mg, 0.098 mmol) and $MnCl_2$ (61 mg, 0.49 mmol) in $CHCl_3$ (5 mL) gave 78 mg of **8b** (91%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.5, 401.0, 423.0, 470.5, 571.0, 607.0; HRMS: calcd for $C_{50}H_{33}MnFN_8O_3$: 867.2040. found: 890.1945 $[M+Na]^+$.

4.2.2.3. Compound 9. Compound **9** ^1H NMR ($CDCl_3$) δ 10.86 (2H, s, NH-uracil), 10.86 (s, 2H, NH-uracil), 8.88–8.82 (m, 8H,β-pyrrole), 8.25–8.18 (m, 10H, 3, 5-H amidophenyl-H and 2, 6-H phenyl), 8.06 (d, 4H, J = 8.44 Hz, 2, 6-H amidophenyl), 7.87–7.80 (m, 6H, 3, 4, 5-H phenyl), 4.71 (s, 4H, N-CH_2), −2.90 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 416.0, 513.0, 553.0, 593.5, 645.0. HRMS: calcd for $C_{56}H_{38}F_2N_{10}O_6$: 984.2944. found: 1007.2835 $[M+Na]^+$.

4.2.2.4. Compound 9a. Compound **9** (80 mg, 0.081 mmol) and $CoCl_2$ (52 mg, 0.41 mmol) in $CHCl_3$ (5 mL) gave 71 mg of **9a** (84%). UV–vis (λ_{max}, nm) in $CHCl_3$: 313.0, 428.0, 544.0, 585.0; HRMS: calcd for $C_{56}H_{36}CoF_2N_{10}O_6$: 1041.2119. found: 1064.2025 $[M+Na]^+$.

4.2.2.5. Compound 9b. Compound **9** (80 mg, 0.081 mmol) and $MnCl_2$ (51 mg, 0.41 mmol) in $CHCl_3$ (5 mL) gave 76 mg of **9b** (90%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.5, 401.0, 418.5, 468.5, 522.0, 565.5, 602.0; HRMS: calcd for $C_{56}H_{36}MnF_2N_{10}O_6$: 1037.2168. found: 1060.2068 $[M+Na]^+$.

4.2.3. 5-{4-[2-(5-Fluoro-uracil)-acetylamido]phenyl}-10-{4-[N-(*t*-butyloxycarbonyl)-3-phenyl-L-alanylamido]phenyl}-15,20-diphenylporphyrin (10). Compound **4** (100 mg, 0.11 mmol), 2-(5-Fu acid)-acetic acid (13 mg, 0.11 mmol) and DCC (23 mg, 0.12 mmol), and DMAP (15 mg, 0.12 mmol) in CH_2Cl_2 (3 mL) gave 110 mg of **10** (95%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 5:1). ^1H NMR ($CDCl_3$) δ 10.80 (s, 1H, NH-uracil), 8.90 (d, 4H, J = 11.7 Hz, β-pyrrole), 8.83 (s, 4H,β-pyrrole), 8.24–8.18 (m, 8H, 3, 5-H amidophenyl and 2, 6-H phenyl), 8.10–8.05 (m, 4H, amidophenyl), 7.84 (d, 8H, J = 6.04 Hz, 3,

4, 5-H phenyl), 7.37–7.47 (m, 4H, 2, 3, 5, 6-H alkylphenyl), 7.27 (s, 1H, 4-H alkylphenyl), 4.71 (s, 2H, N-CH_2), 4.53 (1H, s, N-CH), 3.21–3.17 (m, 2H, Ph-CH_2), 1.40 (s, 9H, C-CH_3), −2.90 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 416.0, 513.0, 551.0, 590.0, 645.0. HRMS: calcd for $C_{64}H_{52}FN_9O_6$: 1061.4025. found: 1084.3926 $[M+Na]^+$.

4.2.3.1. Compound 10a. Compound **9** (80 mg, 0.075 mmol) and $CoCl_2$ (48 mg, 0.38 mmol) in $CHCl_3$ (5 mL) gave 71 mg of **9a** (84%). UV–vis (λ_{max}, nm) in $CHCl_3$: 313.5, 428.5, 543.0, 575.0; HRMS: calcd for $C_{64}H_{50}CoFN_9O_6$: 1118.3200. found: 1141.3093 $[M+Na]^+$.

4.2.3.2. Compound 10b. Compound **9** (80 mg, 0.075 mmol) and $MnCl_2$ (47 mg, 0.38 mmol) in $CHCl_3$ (5 mL) gave 74 mg of **9a** (88%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.0, 401.0, 421.5, 468.5, 518.0, 565.5, 601.5. HRMS: calcd for $C_{64}H_{50}MnFN_9O_6$: 1114.3249. found: 1137.3142 $[M+Na]^+$.

4.3. General deprotection of Boc

To a solution of Porphyrins (1 equiv) with Boc group in dried DMF was added trifluoro-acetic acid (TFA, 20–63 equiv) at 0–5 °C. The mixture was kept at room temperature for 2 h. After being neutralized with ammonium hydroxide aqueous solution, the mixture was extracted by CH_2Cl_2. Combined organic layer was washed with saturated brine, and then dried over magnesium sulfate. Solvent was removed by evaporation under reduced pressure. The product was obtained by purification on silica gel.

4.3.1. 5-[4-(-3-Phenyl-L-alanylamido)phenyl]-10-(4-aminophenyl)-15,20-diphenyl-porphyrin (6). Compound **4** (50 mg, 0.056 mmol) with TFA (0.4 mL, 4.2 mmol) gave 40 mg of **6** (91%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 7:1). ^1H NMR ($CDCl_3$) δ 8.92 (s, 2H, β-pyrrole), 8.87 (s, 2H, β-pyrrole), 8.80 (d, 4H, J = 5.3 Hz, β-pyrrole), 8.33–8.20 (m, 4H, 3, 5-H phenyl), 8.16 (2H, d, J = 8.64 Hz, 3, 5-H amidophenyl), 8.10 (d, 2H, J = 8.48 Hz, 2, 6-H amidophenyl), 7.88–7.83 (m, 6H, 2, 6-H aminophenyl and 3, 4, 5-H phenyl), 7.37 (d, 4H, J = 6.64 Hz, 2, 3, 5, 6-H alkylphenyl), 7.27 (s, 1H, 4-H phenyl), 7.01 (d, 2H, J = 8.24 Hz, 3, 5-H aminophenyl), 3.80–3.77 (m, 1H, N-CH), 3.20–3.16 (m, 2H, Ph-CH_2), −2.90 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 421.5, 516.5, 556.0, 597.0, 648.0. HRMS: calcd for $C_{53}H_{41}N_7O$: 791.3373. found: 814.3274 $[M+Na]^+$.

4.3.1.1. Compound 6a. Compound **6** (80 mg, 0.1 mmol) and $CoCl_2$ (65 mg, 0.5 mmol) in $CHCl_3$ (5 mL) gave 69 mg of **6a** (81%). UV–vis (λ_{max}, nm) in $CHCl_3$: 314.0, 435.5, 549.0, 585.5; HRMS: calcd for $C_{53}H_{39}CoN_7O$: 848.2548. found: 871.2453 $[M+Na]^+$.

4.3.1.2. Compound 6b. Compound **6** (80 mg, 0.1 mmol) and $MnCl_2$ (63 mg, 0.5 mmol) in $CHCl_3$ (5 mL) gave 79 mg of **6a** (93%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.5, 400.0, 422.5, 470.5, 522.0, 571.0, 606.0; HRMS: calcd for $C_{53}H_{39}MnN_7O$: 844.2597. found: 867.2498 $[M+Na]^+$.

4.3.2. 5,10-Di[4-(3-phenyl-L-alanylamido)phenyl]-15,20,-diphenyl-porphyrin (7). Compound **4** (50 mg, 0.056 mmol) with TFA (0.8 mL, 8.5 mmol) gave 48 mg of **7** (91%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 4:1). ^1H NMR ($CDCl_3$) δ 8.89(s, 4H, β-pyrrole), 8.83 (s, 4H, β-pyrrole), 8.24–8.22 (4H, m, 2, 6-H phenyl), 8.16 (d, 4H, J = 8.52 Hz, 3, 5-H amidophenyl), 8.11(d, 4H, J = 8.52 Hz, 2, 6-H amidophenyl-H at meso), 7.85–7.83 (m, 6H, 3, 4, 5-H phenyl), 7.40–7.35 (m, 8H, 2, 3, 5, 6-H alkylphenyl), 7.27 (s, 2H, 4-H alkylphenyl), 3.77–3.81 (m, 1H, N-CH), 3.24–3.16 (m, 4H, Ph-CH_2), −2.90 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 417.5, 513.0, 551.5, 593.5, 645.5. HRMS: calcd for $C_{62}H_{50}N_8O_2$: 938.4057. found: 961.3958 $[M+Na]^+$.

4.3.2.1. Compound 7a. Compound **7** (80 mg, 0.085 mmol) and $CoCl_2$ (55 mg, 0.43 mmol) in $CHCl_3$ (5 mL) gave 68 mg of **7a** (80%). UV–vis (λ_{max}, nm) in $CHCl_3$: 323.0, 434.0, 546.0, 585.0; HRMS: calcd for $C_{62}H_{48}CoN_8O_2$: 995.3232, found: 1018.3134 $[M+Na]^+$.

4.3.2.2. Compound 7b. Compound **7** (80 mg, 0.085 mmol) and $MnCl_2$ (65 mg, 0.43 mmol) in $CHCl_3$ (5 mL) gave 74 mg of **7 b** (88%). UV–vis (λ_{max}, nm) in $CHCl_3$: 380.5, 400.5, 419.0, 468.0, 522.0, 567.5, 601.5. HRMS: calcd for $C_{62}H_{48}MnN_8O_2$: 991.3281. found: 1014.3186 $[M+Na]^+$.

4.3.3. 5-{4-[2-(5-Fluoro-uracil)-acetylamido]phenyl}-10-[4-(3-phenyl-L-alanylamido)phenyl]-15,20-diphenylporphyrin (11). Compound **4** (90 mg, 0.085 mmol) with TFA (0.4 mL, 8.5 mmol) gave 78 mg of **11** (96%). The product was obtained by column chromatography (silica, eluent: chloroform/methanol = 3:1). ^1H NMR ($CDCl_3$) δ 10.94 (s, 1H, NH-uracil), 8.89(s, 4H, β-pyrrole), 8.83 (s, 4H, β-pyrrole), 8.24–8.06 (m, 12H, amidophenyl and 2, 6-H phenyl), 7.84–7.80 (m, 6H, 3, 4, 5-H phenyl), 7.40–7.34 (m, 4H, 2, 3, 5, 6-H alkylphenyl), 7.27 (s, 1H, 4-H alkylphenyl), 4.69 (s, 2H, C-CH_3), 3.80–3.77 (m, 1H, N-CH), 3.23–3.16 (m, 2H, Ph-CH_2), −2.90 (s, 2H, NH-pyrrole). UV–vis (λ_{max}, nm) in $CHCl_3$: 416.5, 513.0, 553.5, 594.0, 648.0. HRMS: calcd for $C_{59}H_{44}FN_9O_4$: 961.3500. found: 984.3404 $[M+Na]^+$.

4.3.3.1. Compound 11a. Compound **11** (80 mg, 0.083 mmol) and $CoCl_2$ (53 mg, 0.42 mmol) in $CHCl_3$ (5 mL) gave 72 mg of **11a** (85%). UV–vis (λ_{max}, nm) in $CHCl_3$: 315.5, 433.5, 544.0, 585.0; HRMS: calcd for $C_{59}H_{42}CoFN_9O_4$: 1018.2676. found: 1041.2576 $[M+Na]^+$.

4.3.3.2. Compound 11b. Compound **11** (80 mg, 0.083 mmol) and $MnCl_2$ (52 mg, 0.42 mmol) in $CHCl_3$ (5 mL) gave 74 mg of 11b (88%). UV–vis (λ_{max}, nm) in $CHCl_3$: 381.0, 400.5, 422.0, 468.5, 522.0, 567.0, 601.0; HRMS: calcd for $C_{59}H_{42}MnFN_9O_4$: 1014.2724. found: 1037.2628 $[M+Na]^+$.

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