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Synthesis of chlorophyll–amino acid conjugates as models for modification of proteins with chromo/fluorophores

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ABSTRACT

A chlorophyll-*a* derivative bonded directly with epoxide at the peripheral position of the chlorin π-system was reacted with *N*-urethane and *C*-ester protected amino acids bearing an alcoholic or phenolic hydroxy group as well as a carboxy group at the residue to give chlorophyll–amino acid conjugates. The carboxy residues of *N*,*C*-protected aspartic and glutamic acids were esterified with the epoxide in high yields. The synthetic conjugates in dichloromethane had absorption bands throughout the visible region including intense red-side Qy and blue-side Soret bands. By their excitation at the visible bands, strong and efficient fluorescence emission was observed up to the near-infrared region. The chromo/fluorophores are promising for preparation of functional peptides and modification of proteins.

1. Introduction

Interaction of porphyrinoids (typically, chlorophylls and hemes) with peptides is important for the construction of various functional proteins in photosynthesis, respiration, oxygenative metabolism, and so on. Cyclic tetrapyrroles as their cofactors bind peptides through a covalent bond as well as a non-covalent bond including coordination of any peptidyl residues to the central metals from the axial site. Therefore, a variety of conjugates of porphyrinoids with amino acids, oligopeptides and proteins were prepared and investigated from the viewpoints of functional materials for photoinduced energy/electron transfer and luminescence: artificial photosynthesis, photodynamic therapy, bioimaging, etc.[1] Some of the biocompatible conjugates are utilized for cancer therapy.

Most of the conjugates reported so far are amides of a carboxy group at the peripheral position of porphyrinoids with an *N*-terminal amino group of amino acids and peptides. Some of them are conjugates with the residue of (un)natural amino acids and their peptides.[2] Chlorophylls and their derivatives are useful for photosynthesis and photodynamic therapy,[3] but have been less available for such bioconjugates. Attachment of chlorophyll derivatives to amino acids and peptides at their residues is especially limited.[4] Here, we report preparation of adducts of *N*,*C*-protected amino acids to methyl 3-oxiranyl-pyropheophorbide-*a* (**4**, Fig. 1), one of the chlorophyll-*a* derivatives, through an ether or ester linkage at the residue and their optical properties including visible absorption and fluorescence emission data in a diluted solution.

We recently reported synthesis of epoxide–chlorin **4** and its high reactivity with alcohols and carboxylic acids.[5] No other highly chemically reactive chlorophyll derivatives have been isolated, to our best knowledge, except for activated 17-propionate ester of pyropheophorbide-*a* with *N*-hydroxysuccinimide.[6] In this report, we demonstrate the efficient modification of hydroxy and carboxy groups in the residues of all natural L-α-amino acids by fluorescent chlorophyllous pigment **4**.

2. Results and discussion

2.1. Synthesis of chlorophyll–amino acid conjugates

Commercially available hydroxylated L-amino acids, Fmoc-AA(OH)-OH, protected with a 9-fluorenylmethyloxycarbonyl (Fmoc) group at the *N*-terminal in diethyl ether was treated with ethereal diazomethane to give the corresponding serine (Ser), threonine (Thr) and tyrosine (Tyr) methyl esters, Fmoc-AA(OH)-OMe **5a**–**c**, possessing a hydroxy group at the residue (see the upper drawing of Scheme 1). *tert*-Butoxycarbonylated L-amino acids, Fmoc-AA(O^{t}Bu)-OH, were similarly methylated (see the lower drawing of Scheme 1) and the resulting methyl esters in dichloromethane were subjected to trifluoroacetic acid in the presence of

Abbreviations: APCI, atmospheric pressure chemical ionization; CD, circular dichroism; FCC, flash column chromatography; Fmoc, 9-fluorenylmethyloxycarbonyl; NP, normal phase; RP, reverse phase.

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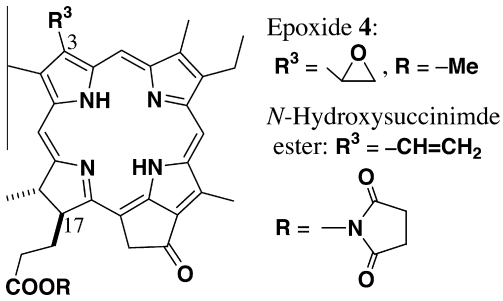


Figure 1. Chemically reactive pyropheophorbides.

triethylsilane to afford *N*,*C*-protected aspartic acid (Asp) and glutamic acid (Glu), Fmoc-AA(OH)-OMe **5d** and **e**, bearing a free carboxy group.

Epoxide **4** in dichloromethane was reacted with 5 equiv of alcohol **5a** in the presence of *p*-toluenesulfonic acid (0.1 equiv) at room temperature in the dark under nitrogen to consume **4** completely after stirring for 2 h and give the corresponding ring-opened adduct (see Scheme 2), confirmed by its mass spectrometry: $m/z = 669.3$ (MH^+). The visible absorption spectra (see Fig. 2A) indicated that the product possessed a 13^1-oxo-chlorin π-system and a 3-CH(OY)CH_2(OZ) moiety:[5,7] $\lambda = 664$ and 410 nm in CH_2Cl_2. The ^{1}H NMR spectra showed that the product was a 1:1 mixture of two stereoisomers. Normal phase flash column chromatography (NP-FCC) on silica gel could partially separate the two isomers and reverse-phase high performance liquid chromatography (RP-HPLC) on octadecylated silica gel achieved their full separation (see the details in Section 4.3). The first-eluting fraction in RP-HPLC (slowly eluting on NP-FCC) gave one set of proton resonance signals and contained a single stereoisomer. From ^{1}H-^{1}H COSY and NOESY, the molecular structure was identified to be 3^1-adduct **1a**. 1D and 2D ^{1}H NMR spectra indicated that the second-eluting fraction in RP-HPLC (fast eluting on NP-FCC) was the other 3^1-adduct **1a**. Hereafter, the former stereoisomer is called **1a1** and the latter 3^1-epimer is referred to as **1a2**.

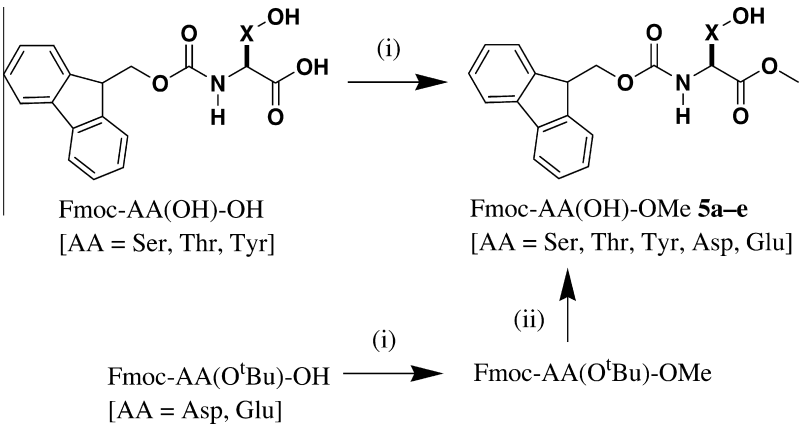
^{1}H NMR spectroscopy and HPLC showed that the above separated samples were diastereomerically pure and thus no racemization occurred at the chiral center in the α-position of L-serine during the acid-catalyzed ring-opening reaction. Circular dichroism (CD) spectra of **1a1** and **1a2** in dichloromethane were slightly but apparently different (see Fig. 2B), confirming them to be 3^1-epimers. The stereoisomers were fully separated by RP-HPLC, but the stereochemistry could not be determined from the present data. Since the product was an equimolar mixture of **1a1** and **1a2**, no remote diastereomeric control at the 3^1-position was observed from the three chiral positions, 17*S* and 18*S* of the chlorin part (situated seven and six covalent-bonds distant from) as well as *S*-stereochemistry at the α-position of the L-serine part (three-bonds apart).

The isolated yields of **1a1** and **1a2** were 20% and 21%, respectively, their combined total being 41% (see Table 1). The total yield was comparable to the 43% isolated yield for **3a** obtained from the similar reaction of **4** with one serine analog **6a** lacking a FmocNH group and lower than the 60% yield of **3b** from **4** with the other serine analog **6b** lacking a COOMe group (see Scheme 3). The suppression of the yields in **1a** and **3a** would be ascribable to lower reactivity of **5a** and **6a** through intramolecular six-membered hydrogen bonding between the hydroxy and ester groups, while the intramolecular seven-membered hydrogen bonding between OH and urethane moiety and five-membered H-bond of carbamate NH with serine-residue O might be less effective in **5a** and **6b**. Extensive HPLC gave no 3^2-adducts in any of the three products. The regioselectivity in exclusive formation of such 3^1-adducts **1a**, **3a**, and **3b** is consistent with the previous results in the ring-opening reaction of **4** with various alcohols.[5]

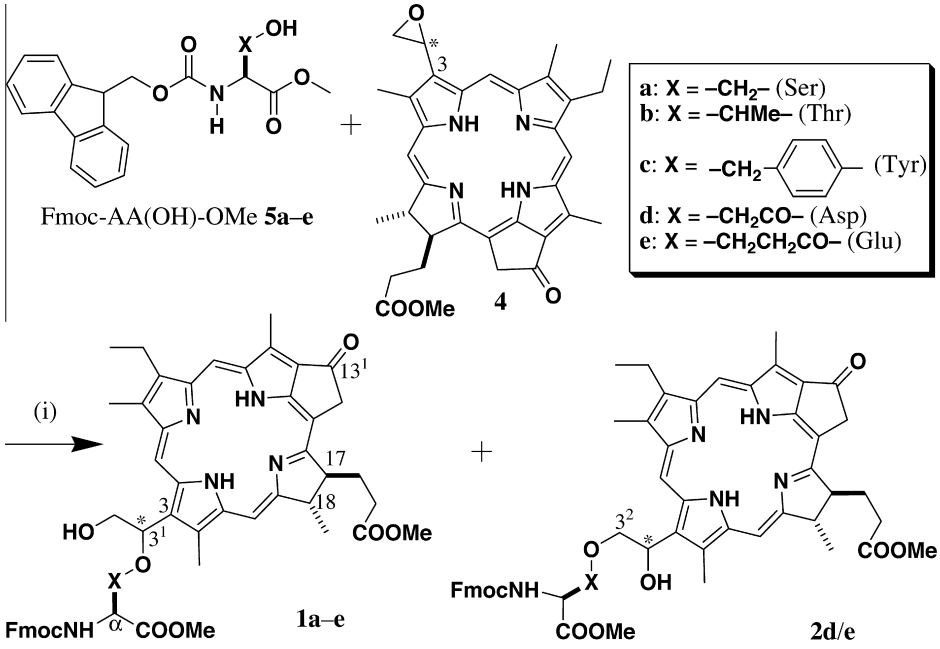
The reaction of **4** with Fmoc-Thr(OH)-OMe **5b** regioselectively afforded 3^1-adducts **1b1** (10% isolated yield) and **1b2** (10%). The total yield (20%) was half of that in **1a** (41%). The reduction was due to the steric hindrance around the secondary alcoholic hydroxy group in Thr residue, compared with the primary OH in Ser. The *R*-configuration at the β-position of Thr was two bonds apart from the 3^1-position in **1b** but did not affect the 3^1-stereoselectivity. It is noteworthy that no racemization of the β-position occurred in the present etheration.

In addition to alcohols, phenols were similarly reacted with **4** to give the corresponding 3^1-adducts. Using Fmoc-Tyr(OH)-OMe **5c** and PhOH **6c** instead of **5a**/**b** and **6a**/**b** led to the sole production of 3^1-equimolar mixtures of **1c** (59% isolated yield) and **3c** (70%), respectively. The comparable yields showed that the reactivity of the phenolic OH in **5c** was less disturbed with FmocNH and COOMe in a molecule. Their 3^1-epimers could not be separated by either HPLC or FCC under the present conditions.

Addition of Fmoc-Asp(OH)-OMe **5d** (5 equiv) to 3-oxiranylchlorin **4** was done without any acid catalyst. After stirring the dichloromethane solution at room temperature for 1 h, epoxide **4** disappeared. The resulting reaction mixture was purified with NP-FCC to give the corresponding adduct as the product. The product was separated by RP-HPLC to give two fractions. Their ^{1}H NMR spectra indicated that the first-eluting fraction was 3^1-adduct **1d** (58% isolated yield) and the second was 3^2-adduct **2d** (35%). Both the isolated products were 3^1-epimeric mixtures (1:1) which could



Scheme 1. Reagents and conditions: Synthesis of *N*,*C*-protected L-amino acids **5**: (i) CH_2N_2/Et_2O; (ii) CF_3COOH, Et_3SiH/CH_2Cl_2.



Scheme 2. Reagents and conditions: Synthesis of *N*,*C*-protected L-amino acids possessing a chlorophyll derivative at the residue **1**/**2**: (i) CH_2Cl_2, cat. *p*-$CH_3C_6H_4SO_3H$ (for **a**–**c**) or no acid catalyst (for **d** and **e**).

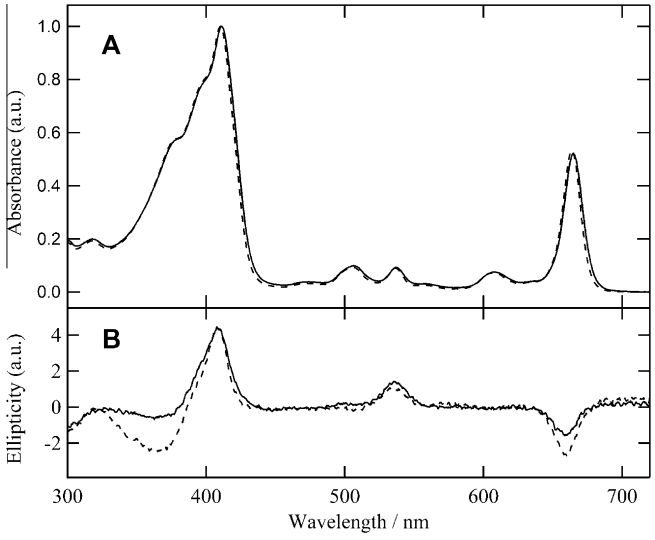
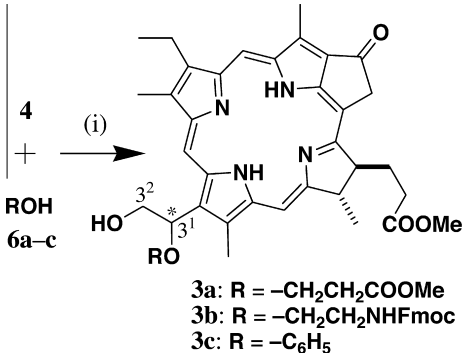


Figure 2. Visible absorption (A) and CD spectra (B) of **1a1** (solid line) and **1a2** (broken line) in dichloromethane. All the spectra were normalized by the intense peaks around 410 nm.

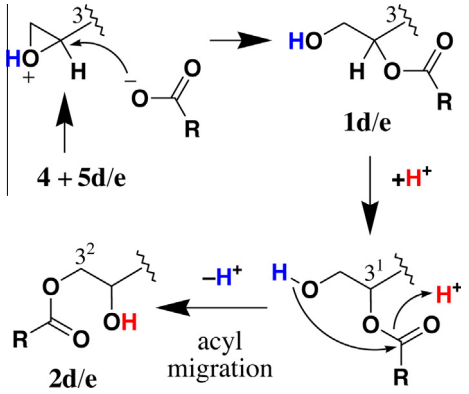
Table 1
Isolated yield of chlorophyll–amino acid conjugates **1**/**2** and their analogs **3** from the reaction of **4** with **5** and **6**, respectively (see Schemes 2 and 3 as well as Section 4.3)

ROH	Yield/% (conjugated product)
5a (Fmoc-Ser-OMe)	41 = 20 (**1a1**) + 21 (**1a2**)
5b (Fmoc-Thr-OMe)	20 = 10 (**1b1**) + 10 (**1b2**)
5c (Fmoc-Tyr-OMe)	59 (**1c**)[a]
5d (Fmoc-Asp-OMe)	93 = 58 (**1d**)[a] + 35 (**2d**)[a]
5e (Fmoc-Glu-OMe)	79 = 33 (**1e1**) + 33 (**1e2**) + 13 (**2e**)[a]
6a ($HOCH_2CH_2COOMe$)	43 (**3a**)[a]
6b ($HOCH_2CH_2NHFmoc$)	60 (**3b**)[a]
6c (HOC_6H_5)	70 (**3c**)[a]

[a] 3^1R:3^1S = 1:1.



Scheme 3. Reagents and conditions: Synthesis of chlorophyll derivatives **3a**/**b** and **3c** as analogs of **2a** and **2c**: (i) CH_2Cl_2, cat. *p*-$CH_3C_6H_4SO_3H$.



Scheme 4. Acid-catalyzed acyl migration of **1d**/**e** to **2d**/**e**.

not be successfully separated under the present HPLC conditions. The carboxylate anion of **5d** first attacked the 3^1-position of protonated **4** at the epoxide oxygen-atom to give 3^1-adduct **1d** (see the upper drawing of Scheme 4), similarly as in the above

reactions of **4** with **5a–c** to **1a–c**. The resulting 3^1-adduct **1d** was transformed through acid-catalyzed acyl migration to regioisomeric 3^2-adduct **2d** (see the lower drawing of Scheme 4). The rearrangement readily occurred at room temperature. Since the reaction completed within 1 h and the total isolated yield was 93%, the addition of **5d** to **4** proceeded smoothly and cleanly.

Similar to the addition of **5d** to **4**, the reaction of Fmoc-Glu(OH)-OMe **5e** with **4** gave **1e1** (33% isolated yield), **1e2** (33%), and **2e** (13%) after purification with FCC followed by HPLC. The total isolated yield was good (79%) and comparable to that in the case of Asp.

2.2. Optical properties of chlorophyll–amino acid conjugates

In a diluted dichloromethane solution (ca. 10 μM), synthetic chlorophyll–amino acid conjugates **1**/**2** gave electronic absorption bands from the visible to ultraviolet regions (see Figs. 2 and 3A and S1–10A). The absorption bands are typical of monomeric species.[7] One of the two intense bands at the red side is called the Qy band, while the other at the blue side is referred to as the Soret band. In the region between these two bands, Qx bands were observed. Both the main Qy and Qx bands had two vibrational bands at their blue side. In **1d**, for example, absorption maxima (λ_{abs}) of Qy(0,0), Qy(0,1), and Qy(0,2) bands were situated at 665, 608, and ≈560 nm, respectively, and Qx(0,0)/(0,1)/(0,2) maxima at 537/506/473 nm (see Fig. 3A and Table 2). The shapes of absorption spectra of all the conjugates are almost the same: typically, $\lambda_{abs}(Qy(0,0)) - \lambda_{abs}(Qy(0,1)) = 57$ nm and relative Qy(0,0) peak absorbances to the Soret maxima (A_{rel}) = 0.525 ± 0.015 (see Table 2). Their peak positions are slightly shifted within 2 nm: $\lambda_{abs}(Qy(0,0)) = 663–665$ nm and $\lambda_{abs}(Soret) = 410–411$ nm. The absorption spectra are little dependent on the substituents Y and Z in 3-CH(OY)CH_2(OZ) of **1**/**2**. It is noted that λ_{abs}(Qy)s of the first fractions of 3^1-adducts **1a1**, **1b1**, and **1e1** are red-shifted slightly but noticeably (1 nm) from those of the second **1a2**, **1b2**, and **1e2**, respectively, and also that those of 3^1-adduct **1d** are larger by 2 nm than those of 3^2-adduct **2d**.

When each highly diluted dichloromethane solution (ca. 1 μM) of **1**/**2** was irradiated with the light at the Soret maxima, strong fluorescence emission was observed at the red side of the Qy region (see Figs. 3B and S1–10B). Fluorescence spectra were nearly mirror images of the Qy bands. The full widths at half maximum of the main emission bands at 668–670 nm were 370–400 cm^{-1} and each value was slightly larger by just 20 cm^{-1} than that of the corresponding Qy(0,0) band (see Table 2). Their Stokes shifts (Δ) were also identical (5 nm) for all the conjugates. Fluorescence emission quantum yields of **1**/**2** were 27 ± 1% and their lifetimes were 6.1 ± 0.3 ns. Although the emission spectra were dependent on the Qy absorption bands, all the synthetic conjugates intensively emitted the light at a visible to near-infrared region to give similar emission spectra.

3. Concluding remarks

Alcoholic and phenolic residues of *N*,*C*-protected amino acids **5a–c** were added to methyl 3-devinyl-3-oxiranyl-pyropheophorbide-*a* (**4**) by the catalytic action of an acid to give epoxide-ring opened 3^1-ethers **1a–c**. Esterification of **4** with carboxy residues of protected amino acids **5d**/**e** without any acid catalyst afforded 3^1-esters **1d**/**e** and their acyl-migrated 3^2-isomers **2d**/**e** in very good yields. The synthetic chlorophyll derivative–amino acid conjugates **1**/**2** absorbed ultraviolet to visible lights and emitted visible to near-infrared lights efficiently. The conjugates could be used for construction of oligopeptides with chemo/fluorophores by conventional peptide synthesis. Epoxide **4** would be useful for modifying and labeling proteins at the residues of aspartic and glutamic acids.

4. Experimental

4.1. General

Visible absorption and CD spectra were measured with a Hitachi U-3500 spectrophotometer and a Jasco J-720 W spectropolarimeter, respectively. Fluorescence emission spectra and quantum yields were obtained by a Hamamatsu Photonics C9920-03G spectrometer. Fluorescence emission lifetimes were determined by a Hamamatsu Photonics C7990S system. ^{1}H NMR spectra in chloroform-*d* were recorded with a JEOL ECA-600 spectrometer (600 MHz); residual non-deuterated chloroform (δ = 7.26 ppm) was used as an internal reference. Time-of-flight mass data were obtained using direct laser desorption/ionization by a Shimadzu AXIMA-CFR plus spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II spectrometer; atmospheric pressure chemical ionization (APCI) and positive mode in an acetonitrile solution. HPLC was performed on a packed octadecylsilylated silica gel column (Cosmosil 5C_{18}-AR-II, Nacalai Tesque), with a Shimadzu LC-10ADvp pump as well as SPD-M10Avp photodiode-array. FCC was carried out on silica gel (Merck, Kieselgel 60, 40–63 μm, 230–400 mesh). Thin layer chromatography (TLC) was performed with silica gel (Merck, Kieselgel 60 F_{254}).

Methyl 3-hydroxypropanoate[8] and methyl 3^1,3^2-epoxy-mesopyropheophorbide-*a* (**4**)[5] were prepared according to reported

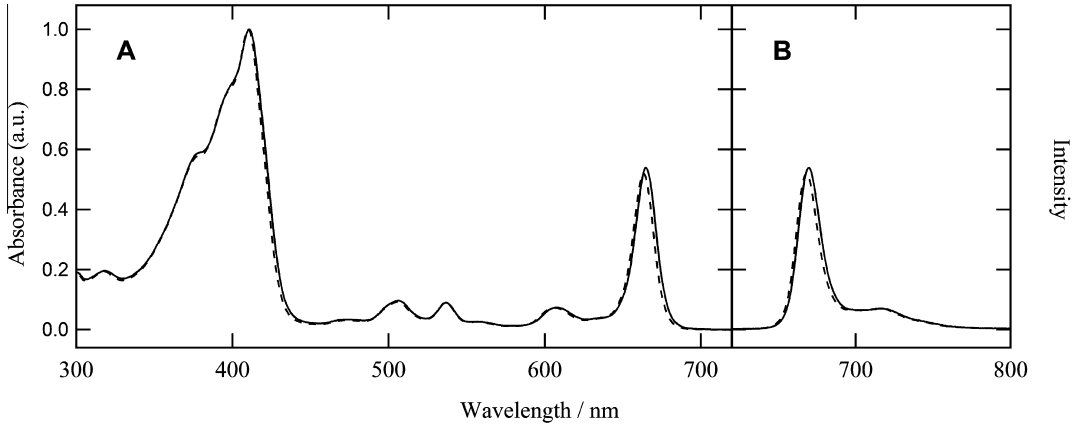


Figure 3. Visible absorption (A) and fluorescence emission spectra (B, excitation at the Soret maxima) of **1d** (solid line) and **2d** (broken line) in dichloromethane. The absorption spectra were normalized by the Soret peaks and each emission maximum around 670 nm was fitted into the corresponding Qy(0,0) peak intensity.

Table 2
Optical properties of **1**/**2** in air-saturated dichloromethane at room temperature[a]

Compound	λ_{abs} (nm)					A_{rel}	λ_{em}	Δ	Φ_{em}	τ_{em}
	Soret	Qx		Qy			(nm)	(nm)	(%)	(ns)
		(0,1)	(0,0)	(0,1)	(0,0)					
1a1 (3^1-Ser*)	411	506	537	608	665 [360]	0.52	670 [380]	5	27	6.4
1a2 (3^1-Ser*)	410	506	537	607	664 [350]	0.53	668 [370]	5	26	6.2
1b1 (3^1-Thr*)	410	505	536	607	664 [350]	0.54	669 [370]	5	27	6.0
1b2 (3^1-Thr*)	410	506	537	606	663 [350]	0.52	668 [370]	5	28	6.3
1c (3^1-Tyr)	410	506	536	607	664 [350]	0.54	669 [370]	5	26	6.0
1d (3^1-Asp)	411	506	537	608	665 [360]	0.54	670 [380]	5	27	6.4
2d (3^2-Asp)	410	506	536	606	663 [360]	0.52	668 [380]	5	27	6.1
1e1 (3^1-Glu*)	410	506	536	607	664 [380]	0.51	669 [400]	5	27	6.0
1e2 (3^1-Glu*)	410	506	537	606	663 [360]	0.51	668 [380]	5	27	5.8
2e (3^2-Glu)	410	506	537	606	663 [360]	0.52	668 [380]	5	27	6.2

[a] λ_{abs}, absorption maxima; A_{rel}, relative Qy(0,0) peak absorbances to the Soret maxima; λ_{em}, emission maxima (excited at Soret maxima); Δ, Stokes shifts = $\lambda_{em} - \lambda_{abs}$(Qy (0,0)); Φ_{em}, emission quantum yields (excited at Soret maxima); τ_{em}, emission lifetimes (excited at 403 nm). The value in a square bracket indicates a full width at half maximum (cm^{-1}).

procedures. *N*-Fmoc-glycinol and *N*-Fmoc-L-amino acids (Fmoc-AA-OH) were purchased from Watanabe Chemical Industries. Commercially available acetonitrile, dichloromethane, diethyl ether, ethyl acetate, hexane, and petroleum ether (Nacalai Tesque) were used without alteration. Other reagents were purchased from commercial suppliers.

Dichloromethane for optical spectroscopy was purchased from Nacalai Tesque as a reagent prepared specially for spectroscopy and used without further purification. Optical properties were measured in air-saturated dichloromethane at room temperature.

4.2. Synthesis of *N*-Fmoc-L-amino acid methyl ester (5, Fmoc-AA(OH)-OMe)

4.2.1. *N*-Fmoc-L-amino acid methyl ester possessing a hydroxy group 5a–c

A diethyl ether solution (100 mL) of Fmoc-AA(OH)-OH (0.8 mmol, AA = Ser, Thr, and Tyr) was treated with ethereal diazomethane (10 mL) at room temperature. After a check of TLC showed that the starting carboxylic acid had disappeared (stirring for 30 min), the reaction was quenched by addition of distilled water. The separated organic phase was washed with water, dried over sodium sulfate, and filtered. All the solvent was evaporated and the residue was purified with FCC (10% Et_2O–CH_2Cl_2) and recrystallization (CH_2Cl_2–hexane) to give **5a**–**c** (ca. 80–90%).

4.2.2. *N*-Fmoc-L-amino acid methyl ester possessing a carboxy group 5d/e

Similar to the synthesis of Fmoc-AA(OH)-OMe, Fmoc-AA(O^{t}Bu)-OH (AA = Asp and Glu) was transformed to Fmoc-AA(O^{t}Bu)-OMe (ca. 90%) after purification of sole recrystallization (AcOEt–petroleum ether).

A dichloromethane solution (10 mL) of Fmoc-AA(O^{t}Bu)-OMe (0.3 mmol) was treated with trifluoroacetic acid (1.5 mL) and triethylsilane (0.25 mL) at room temperature. After a check of TLC showed that the starting *tert*-butyl ether had disappeared (stirring for 2–3 h), the reaction was quenched by addition of distilled water. The separated organic phase was washed with water, dried over sodium sulfate, and filtered. All the solvent was evaporated and the residue was purified with FCC (10% Et_2O–CH_2Cl_2) and recrystallization (CH_2Cl_2–hexane) to give **5d**/**e** (ca. 50–70%).

4.3. Synthesis of conjugates 1–3

A dichloromethane solution (15 mL) of epoxide **4** (28.2 mg, 50 μmol) and Fmoc-AA(OH)-OMe **5** or ROH **6** (250 μmol, 5 equiv) with(out) *p*-toluenesulfonic acid monohydrate (pTSA, 1 mg, 0.1 equiv) was stirred at room temperature in the dark under nitrogen: in the case of carboxylic acids **5d**/**e**, pTSA was not necessary as the catalytic additive. After the disappearance of **4** determined by checking TLC (stirring for 1–3 h), the reaction was quenched by addition of distilled water. The separated organic phase was washed with an aqueous saturated sodium bicarbonate solution and water, dried over sodium sulfate, and filtered. All the solvent was evaporated and the residue was purified with FCC (5–10% Et_2O–CH_2Cl_2 or 50% AcOEt–hexane) and recrystallization (CH_2Cl_2–hexane) to give desired conjugates **1**–**3** as analytically pure black solids. All the samples were purified by HPLC (1% THF-14% H_2O–CH_3CN) just before their optical measurements.

4.4. Spectral data

4.4.1. Methyl 3^2-hydroxy-3^1-[2-(methoxycarbonyl)ethoxy]-mesopyropheophorbide-*a* (3a)

43% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.51), 606 (0.08), 537 (0.09), 506 (0.09), 474 (0.03), 410 (1.00), 399 (0.80, sh), 379 (0.58, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.67 (1H, s, 5-H), 9.56 (1H, s, 10-H), 8.58 (1H, s, 20-H), 5.984/982 (1H, dd, J = 9, 4 Hz, 3-CH), 5.273/266, 5.127/124 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.60/59 (1H, dt, J = 2, 9 Hz, 3^1-CH), 4.50/49 (1H, dq, J = 2, 7 Hz, 18-H), 4.31 (1H, m, 17-H), 4.15–4.10 (1H, m, 3^1-CH), 4.11–4.06 (1H, m, 3^1-OCH), 4.02/01 (1H, dt, J = 10, 5 Hz, 3^1-OCH), 3.72 (2H, q, J = 8 Hz, 8-CH_2), 3.68 (3H, s, 12-CH_3), 3.624/622 (3H, s, 3^1-$OC_2CO_2CH_3$), 3.604/600 (3H, s, 17^2-CO_2CH_3), 3.430/428 (3H, s, 2-CH_3), 3.27 (3H, s, 7-CH_3), 3.09/07 (1H, br, 3^2-OH), 2.83/81 (1H, dt, J = 8, 5 Hz, 3^1-OCCH), 2.77–2.72 (1H, m, 3^1-OCCH), 2.72–2.65, 2.59–2.52, 2.34–2.21 (1H+1H+2H, m, 17-CH_2CH_2), 1.811/808 (3H, d, J = 7 Hz, 18-CH_3), 1.71 (3H, t, J = 8 Hz, 8^1-CH_3), 0.25, −1.84/85 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 669.3292, calcd for $C_{38}H_{45}N_4O_7$: MH^+, 669.3283.

4.4.2. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-ethoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (3b)

60% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.51), 606 (0.08), 537 (0.09), 506 (0.10), 474 (0.04), 411 (1.00), 399 (0.80, sh), 379 (0.57, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.70 (1H, s, 5-H), 9.48/45 (1H, s, 10-H), 8.58 (1H, s, 20-H), 7.70–7.61, 7.39–7.29, 7.27–7.21, 7.19–7.15, 7.05–7.02, 6.97–6.89 (8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 5.99/97 (1H, dd, J = 6, 4 Hz, 3-CH), 5.25/24, 5.21/07 (each 1H, d, J =19 Hz, 13^1-CH_2), 5.19/17 (1H, t, J = 6 Hz, Fmoc-NH), 4.77–4.71 (1H, m, 3^1-CH), 4.50/48 (1H, dq, J = 2, 7 Hz, 18-H), 4.30 (1H, m, 17-H), 4.21 (1H, m, 3^1-CH), 4.13, 4.03/01 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 3.92–3.85, 3.84–3.79 (2H, m, 3^1-OCH_2), 3.74/73 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.65 (2H, q, J = 7 Hz, 8-CH_2),

3.65–3.58, 3.51–3.46 (2H, m, 3^1-$OCCH_2$), 3.62, 3.60, 3.434/429, 3.284/281 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.88 (1H, br, 3^2-OH), 2.73–2.64, 2.61–2.51, 2.32–2.21 (1H+1H+2H, m, 17-CH_2CH_2), 1.83/79 (3H, d, J = 7 Hz, 18-CH_3), 1.66/64 (3H, t, J = 7 Hz, 8^1-CH_3), 0.17/13, −1.84/86 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 848.4033 calcd for $C_{51}H_{54}N_5O_7$: MH^+, 848.4018.

4.4.3. Methyl 3^2-hydroxy-3^1-phenoxy-mesopyropheophorbide-*a* (3c)

70% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 665 (relative intensity, 0.52), 608 (0.07), 537 (0.09), 506 (0.10), 475 (0.04), 411 (1.00), 399 (0.81, sh), 380 (0.58, sh), 318 nm (0.19); ^{1}H NMR ($CDCl_3$) δ = 9.71 (1H, s, 5-H), 9.554/551 (1H, s, 10-H), 8.56 (1H, s, 20-H), 7.171/169 (2H, d, J = 8 Hz, *o*-H of 3^1-OPh), 7.08/07 (2H, dd, J = 8, 7 Hz, *m*-H of 3^1-OPh), 6.81/80 (1H, t, J = 4 Hz, 3-CH), 6.78/77 (1H, t, J = 7 Hz, *p*-H of 3^1-OPh), 5.254/250, 5.111/108 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.82/80 (1H, ddd, J = 9, 4, 3 Hz, 3^1-CH), 4.47/46 (1H, dq, J = 2, 7 Hz, 18-H), 4.39/37 (1H, dd, J = 9, 4 Hz, 3^1-CH), 4.29 (1H, m, 17-H), 3.72 (2H, q, J = 8 Hz, 8-CH_2), 3.68/67 (3H, s, 12-CH_3), 3.591/590 (3H, s, 17^2-CO_2CH_3), 3.46 (3H, s, 2-CH_3), 3.320/314 (3H, s, 7-CH_3), 2.77/76 (1H, d, J = 3 Hz, 3^2-OH), 2.70–2.63, 2.57–2.49, 2.31–2.19 (1H+1H+2H, m, 17-CH_2CH_2), 1.79/78 (3H, d, J = 7 Hz, 18-CH_3), 1.712/711 (3H, t, J = 8 Hz, 8^1-CH_3), 0.19, −1.90 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 659.3238, calcd for $C_{40}H_{43}N_4O_5$: MH^+, 659.3228.

4.4.4. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)ethoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (Ser-adduct) as the first fraction of HPLC (1a1)

20% isolated yield (3^1-epimerically pure, slow-eluting fraction on FCC with R_f = 0.07); UV–vis (CH_2Cl_2) λ_{max} = 665 (relative intensity, 0.52), 608 (0.08), 537 (0.09), 506 (0.10), 474 (0.04), 411 (1.00), 399 (0.80, sh), 379 (0.58, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.68 (1H, s, 5-H), 9.36 (1H, s, 10-H), 8.59 (1H, s, 20-H), 7.56, 7.47, 7.00, 6.18 (each 1H, d, J = 7 Hz, 1-, 4-, 5-, 8-H of Fmoc), 7.27, 7.10, 7.05, 6.57 (each 1H, t, J = 7 Hz, 2-, 3-, 6-, 7-H of Fmoc), 5.92 (1H, dd, J = 9, 4 Hz, 3-CH), 5.67 (1H, d, J = 8 Hz, Fmoc-NH), 5.23, 5.03 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.78 (1H, dd, J = 13, 9 Hz, 3^1-CH), 4.52 (1H, dt, J = 8, 3 Hz, α-H of Ser), 4.47 (1H, dq, J = 1, 7 Hz, 18-H), 4.29 (1H, m, 17-H), 4.27, 4.04 (each 1H, dd, J = 10, 3 Hz, β-H of Ser), 4.18 (1H, ddd, J = 13, 9, 4 Hz, 3^1-CH), 3.96 (3H, s, Ser-OCH_3), 3.62 (2H, q, J = 8 Hz, 8-CH_2), 3.60 (3H, s, 12-CH_3), 3.58 (3H, s, 17^2-CO_2CH_3), 3.54, 3.24 (each 1H, t, J = 8 Hz, 9-CH_2 of Fmoc), 3.44 (3H, s, 2-CH_3), 3.34 (1H, s, 7-CH_3), 2.75 (1H, d, J = 9 Hz, 3^2-OH), 2.71–2.62 (1H, m, 9-H of Fmoc), 2.71–2.62, 2.57–2.51, 2.29–2.21 (1H+1H+2H, m, 17-CH_2CH_2), 1.86 (3H, d, J = 7 Hz, 18-CH_3), 1.62 (3H, t, J = 8 Hz, 8^1-CH_3), 0.05, −1.87 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 906.4097, calcd for $C_{53}H_{56}N_5O_9$: MH^+, 906.4073.

4.4.5. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)ethoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (Ser-adduct) as the second fraction of HPLC (1a2)

21% isolated yield (3^1-epimerically pure, fast-eluting fraction on FCC with R_f = 0.12); UV–vis (CH_2Cl_2) λ_{max} = 664 (relative intensity, 0.53), 607 (0.07), 537 (0.09), 506 (0.09), 473 (0.03), 410 (1.00), 399 (0.81, sh), 379 (0.58, sh), 318 nm (0.19); ^{1}H NMR ($CDCl_3$) δ = 9.54 (2H, s, 5-, 10-H), 8.56 (1H, s, 20-H), 7.78 (2H, d, J = 7 Hz, 4-, 5-H of Fmoc), 7.76, 7.61 (each 1H, d, J = 7 Hz, 1-, 8-H of Fmoc), 7.42, 7.41 (each 1H, t, J = 7 Hz, 3-, 6-H of Fmoc), 7.34, 7.30 (each 1H, t, J = 7 Hz, 2-, 7-H of Fmoc), 6.11 (1H, d, J = 8 Hz, Fmoc-NH), 5.97 (1H, dd, J = 9, 4 Hz, 3-CH), 5.28, 5.13 (each 1H, d, J =19 Hz, 13^1-CH_2), 4.62 (1H, dt, J = 8, 4 Hz, 3^1-CH), 4.62 (1H, dt, J = 8, 4 Hz, α-H of Ser), 4.51 (1H, dq, J = 2, 7 Hz, 18-H), 4.48, 4.44 (each 1H, dd, J = 10, 7 Hz, 9-CH_2 of Fmoc), 4.38 (1H, dt, J = 9, 2 Hz, 17-H), 4.25 (1H, t, J = 7 Hz, 9-H of Fmoc), 4.18 (1H, dd, J = 9, 8 Hz, 3^1-CH), 4.10, 4.06 (each 1H, dd, J = 4, 10 Hz, β-H of Ser), 3.70 (2H, q, J = 7 Hz, 8-CH_2), 3.68, 3.62, 3.40, 3.27, 3.17 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Ser-OCH_3), 3.15 (1H, br, 3^2-OH), 2.74–2.67, 2.61–2.55, 2.35–2.24 (1H+1H+2H, m, 17-CH_2CH_2), 1.81 (3H, d, J = 7 Hz, 18-CH_3), 1.68 (3H, t, J = 7 Hz, 8^1-CH_3), 0.20, −1.86 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 906.4063, calcd for $C_{53}H_{56}N_5O_9$: MH^+, 906.4073.

4.4.6. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)-1-methylethoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (Thr-adduct) as the first fraction of HPLC (1b1)

10% isolated yield (3^1-epimerically pure, slow-eluting fraction on FCC with R_f = 0.14); UV–vis (CH_2Cl_2) λ_{max} = 664 (relative intensity, 0.54), 607 (0.07), 536 (0.09), 505 (0.10), 475 (0.04), 410 (1.00), 399 (0.80, sh), 379 (0.58, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.55 (1H, s, 10-H), 9.48 (1H, s, 5-H), 8.59 (1H, s, 20-H), 7.79, 7.77 (each 1H, d, J = 7 Hz, 4-, 5-H of Fmoc), 7.62, 7.50 (each 1H, d, J = 7 Hz, 1-, 8-H of Fmoc), 7.42, 7.38 (each 1H, t, J = 7 Hz, 3-, 6-H of Fmoc), 7.32, 7.20 (each 1H, t, J = 7 Hz, 2-, 7-H of Fmoc), 6.00 (1H, m, 3-CH), 5.80 (1H, d, J = 9 Hz, Fmoc-NH), 5.28, 5.14 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.82 (1H, m, 3^1-CH), 4.52 (1H, dq, J = 1, 7 Hz, 18-H), 4.42, 4.40 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 4.35–4.30 (1H, m, 17-H), 4.32 (1H, dq, J = 2, 6 Hz, β-H of Thr), 4.23 (1H, dd, J = 9, 2 Hz, α-H of Thr), 4.18 (1H, t, J = 7 Hz, 9-H of Fmoc), 4.17 (1H, m, 3^1-CH), 3.72 (2H, q, J = 8 Hz, 8-CH_2), 3.69, 3.62, 3.49, 3.42, 3.30 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Thr-OCH_3), 2.75–2.69 (1H, br, 3^2-OH), 2.75–2.69, 2.63–2.56, 2.35–2.28 (1H+1H+2H, m, 17-CH_2CH_2), 1.80 (3H, d, J = 7 Hz, 18-CH_3), 1.69 (3H, t, J = 8 Hz, 8^1-CH_3), 1.59 (3H, d, J = 6 Hz, β-CH_3 of Thr), 0.20, −1.85 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 920.4261, calcd for $C_{54}H_{58}N_5O_9$: MH^+, 920.4229.

4.4.7. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)-1-methylethoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (Thr-adduct) as the second fraction of HPLC (1b2)

10% isolated yield (3^1-epimerically pure, fast-eluting fraction on FCC with R_f = 0.16); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.52), 606 (0.07), 537 (0.09), 506 (0.09), 475 (0.04), 410 (1.00), 399 (0.81, sh), 379 (0.58, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.68 (1H, s, 5-H), 9.58 (1H, s, 10-H), 8.60 (1H, s, 20-H), 7.71 (2H, d, J = 7 Hz, 4-, 5-H of Fmoc), 7.56, 7.54 (each 1H, d, J = 7 Hz, 1-, 8-H of Fmoc), 7.30 (2H, t, J = 7 Hz, 3-, 6-H of Fmoc), 7.18, 7.15 (each 1H, t, J = 7 Hz, 2-, 7-H of Fmoc), 6.02 (1H, dd, J = 10, 4 Hz, 3-CH), 5.88 (1H, d, J = 9 Hz, Fmoc-NH), 5.28, 5.14 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.72 (1H, dd, J = 9, 2 Hz, α-H of Thr), 4.63 (1H, dd, J = 13, 10 Hz, 3^1-CH), 4.51 (1H, dq, J = 2, 7 Hz, 18-H), 4.49 (1H, dq, J = 2, 6 Hz, β-H of Thr), 4.45, 4.39 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 4.32 (1H, ddd, J = 9, 3, 2 Hz, 17-H), 4.22 (1H, t, J = 7 Hz, 9-H of Fmoc), 4.05 (1H, m, 3^1-CH), 4.02, 3.70, 3.60, 3.45, 3.31 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Thr-OCH_3), 3.71 (2H, q, J = 8 Hz, 8-CH_2), 3.13 (1H, br, 3^2-OH), 2.73–2.67, 2.58–2.53, 2.34–2.22 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, J = 7 Hz, 18-CH_3), 1.69 (3H, t, J = 8 Hz, 8^1-CH_3), 1.06 (3H, d, J = 6 Hz, β-CH_3 of Thr), 0.21, −1.87 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 920.4262, calcd for $C_{54}H_{58}N_5O_9$: MH^+, 920.4229.

4.4.8. Methyl 3^1-[4-{2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)ethyl}phenoxy]-3^2-hydroxy-mesopyropheophorbide-*a* (Tyr-adduct, 1c)

59% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 664 (relative intensity, 0.54), 607 (0.07), 536 (0.09), 506 (0.09), 474 (0.04), 410 (1.00), 399 (0.81, sh), 380 (0.59, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.69 (1H, s, 5-H), 9.561/555 (1H, s, 10-H), 8.57/56 (1H, s, 20-H), 7.57–7.48, 7.41, 7.36–7.32, 7.28–7.10, 7.01–6.97

(8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 7.074/071 (2H, d, J = 9 Hz, 3-H of 3^1-OPh), 6.77/75 (2H, d, J = 9 Hz, 2-H of 3^1-OPh), 6.76 (1H, m, 3-CH), 5.26, 5.12/11 (each 1H, d, J = 19 Hz, 13^1-CH_2), 5.06/4.99 (1H, d, J = 8 Hz, Fmoc-NH), 4.82–4.76 (1H, m, 3^1-CH), 4.51 (1H, dq, J = 2, 8 Hz, 18-H), 4.45 (1H, m, α-H of Tyr), 4.38/36 (1H, dt, J = 10, 3 Hz, 3^1-CH), 4.30 (1H, m, 17-H), 4.27, 4.18/13 (each 1H, dd, J = 10, 7 Hz, 9-CH_2 of Fmoc), 3.99/89 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.72 (2H, q, J = 8 Hz, 8-CH_2), 3.69/68, 3.590/586, 3.51/49, 3.45/44, 3.31/30 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Tyr-OCH_3), 2.83 (2H, m, β-H of Tyr), 2.71/69 (1H, d, J = 3 Hz, 3^2-OH), 2.69–2.64, 2.56–2.50, 2.31–2.21 (1H+1H+2H, m, 17-CH_2CH_2), 1.79/77 (3H, d, J = 8 Hz, 18-CH_3), 1.71 (3H, t, J = 8 Hz, 8^1-CH_3), 0.17, −1.90 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 982.4400, calcd for $C_{59}H_{60}N_5O_9$: MH^+, 982.4386.

4.4.9. Methyl 3^1-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)ethyl]carbonyloxy-3^2-hydroxy-mesopyropheophorbide-*a* (Asp-adduct, 1d)

58% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 665 (relative intensity, 0.54), 608 (0.07), 537 (0.09), 506 (0.10), 473 (0.04), 411 (1.00), 399 (0.81, sh), 380 (0.59, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.60/57 (1H, s, 5-H), 9.50/37 (1H, s, 10-H), 8.60/59 (1H, s, 20-H), 7.65, 7.59, 7.42, 7.35–7.17, 7.11–7.07, 7.03, 6.97, 6.72, 6.55, 6.41 (8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 7.31 (1H, m, 3-CH), 5.78/66 (1H, d, J = 8 Hz, Fmoc-NH), 5.28/27, 5.12/11 (each 1H, d, J = 18 Hz, 13^1-CH_2), 4.94/73 (1H, dt, J = 8, 4 Hz, α-H of Asp), 4.85/78, 4.33–4.27 (each 1H, m, 3^1-CH_2), 4.50/49 (1H, dq, J = 2, 7 Hz, 18-H), 4.33–4.27 (1H, m, 17-H), 4.26/15, 3.94/70 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 3.82, 3.67/65, 3.59, 3.49/48, 3.31 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Asp-OCH_3), 3.80/18 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.70/48 (2H, q, J = 8 Hz, 8-CH_2), 3.42–38/25–21, 3.27–24/13–08 (each 1H, m, β-H of Asp), 2.95/53 (1H, br, 3^2-OH), 2.79–2.54, 2.42–2.22 (each 2H, m, 17-CH_2-CH_2), 1.82/78 (3H, d, J = 7 Hz, 18-CH_3), 1.70/67 (3H, t, J = 8 Hz, 8^1-CH_3), 0.07/−0.13, −1.96/−2.08 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 934.4002, calcd for $C_{54}H_{56}N_5O_{10}$: MH^+, 934.4022.

4.4.10. Methyl 3^2-[2-(9-fluorenylmethoxycarbonyl)amino-2-(methoxycarbonyl)ethyl]carbonyloxy-3^1-hydroxy-mesopyropheophorbide-*a* (Asp-adduct, 2d)

35% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.52), 606 (0.07), 536 (0.09), 506 (0.09), 473 (0.03), 410 (1.00), 398 (0.80, sh), 379 (0.58, sh), 318 nm (0.19); ^{1}H NMR ($CDCl_3$) δ = 9.69/65 (1H, s, 5-H), 9.48 (1H, s, 10-H), 8.56/55 (1H, s, 20-H), 7.64, 7.53, 7.32–7.22 (8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 6.50/45 (1H, m, 3-CH), 5.94/93 (1H, d, J = 8 Hz, Fmoc-NH), 5.23/21, 5.08/07 (each 1H, d, J = 19 Hz, 13^1-CH_2), 5.02/4.96 (1H, dd, J = 12, 9 Hz, 3^1-CH), 4.84 (1H, ddd, J = 8, 5, 4 Hz, α-H of Asp), 4.82/80 (1H, m, 3^1-CH), 4.48/47 (1H, dq, J = 3, 7 Hz, 18-H), 4.44–4.32 (2H, m, 9-CH_2 of Fmoc), 4.26 (1H, m, 17-H), 4.13/09 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.81/80, 3.65, 3.62, 3.45/44, 3.25 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Asp-OCH_3), 3.69 (2H, q, J = 8 Hz, 8-CH_2), 3.65/44 (1H, br, 3^1-OH), 3.14/11 (1H, dd, J = 16, 5 Hz, β-H of Asp), 3.08/06 (1H, dd, J = 16, 4 Hz, β-H of Asp), 2.71–2.63, 2.59–2.53, 2.32–2.22 (1H+1H+2H, m, 17-CH_2CH_2), 1.80/79 (3H, d, J = 7 Hz, 18-CH_3), 1.693/691 (3H, t, J = 8 Hz, 8^1-CH_3), 0.17, −1.89 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 934.4030, calcd for $C_{54}H_{56}N_5O_{10}$: MH^+, 934.4022.

4.4.11. Methyl 3^1-[3-(9-fluorenylmethoxycarbonyl)amino-3-(methoxycarbonyl)propyl]carbonyloxy-3^2-hydroxy-mesopyropheophorbide-*a* (Glu-adduct) as the first fraction of HPLC (1e1)

33% isolated yield (3^1-epimerically pure, slow-eluting fraction on FCC with R_f = 0.23); UV–vis (CH_2Cl_2) λ_{max} = 664 (relative intensity, 0.51), 607 (0.07), 536 (0.09), 506 (0.10), 473 (0.03), 410 (1.00), 399 (0.82, sh), 379 (0.59, sh), 319 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.60 (1H, s, 5-H), 9.53 (1H, s, 10-H), 8.60 (1H, s, 20-H), 7.65, 7.39–7.28, 7.22–7.16 (8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 7.34 (1H, m, 3-CH), 5.41 (1H, d, J = 8 Hz, Fmoc-NH), 5.25, 5.10 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.88–4.82 (1H, m, 3^1-CH), 4.59 (1H, m, α-H of Glu), 4.47 (1H, dq, J = 2, 7 Hz, 18-H), 4.33 (1H, ddd, J = 13, 8, 4 Hz, 3^1-CH), 4.28 (1H, m, 17-H), 4.23, 4.20 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 3.86 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.70 (2H, q, J = 8 Hz, 8-CH_2), 3.67, 3.66, 3.60, 3.49, 3.27 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Glu-OCH_3), 2.80 (1H, br, 3^2-OH), 2.76–2.69 (2H, m, γ-H of Glu), 2.71–2.64, 2.57–2.51, 2.28–2.21 (1H+1H+2H, m, 17-CH_2CH_2), 2.35–2.29, 2.11–2.05 (each 1H, m, β-H of Glu), 1.78 (3H, d, J = 7 Hz, 18-CH_3), 1.70 (3H, t, J = 8 Hz, 8^1-CH_3), 0.11, −1.94 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 948.4185, calcd for $C_{55}H_{58}N_5O_{10}$: MH^+, 948.4178.

4.4.12. Methyl 3^1-[3-(9-fluorenylmethoxycarbonyl)amino-3-(methoxycarbonyl)propyl]carbonyloxy-3^2-hydroxy-mesopyropheophorbide-*a* (Glu-adduct) as the second fraction of HPLC (1e2)

33% isolated yield (3^1-epimerically pure, fast-eluting fraction on FCC with R_f = 0.30); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.51), 606 (0.07), 537 (0.09), 506 (0.10), 474 (0.03), 410 (1.00), 398 (0.80, sh), 379 (0.58, sh), 318 nm (0.19); ^{1}H NMR ($CDCl_3$) δ = 9.59 (1H, s, 5-H), 9.54 (1H, s, 10-H), 8.57 (1H, s, 20-H), 7.74 (2H, d, J = 8 Hz, 4-, 5-H of Fmoc), 7.54 (2H, d, J = 7 Hz, 1-, 8-H of Fmoc), 7.38 (2H, t, J = 7 Hz, 2-, 7-H of Fmoc), 7.29 (2H, t, J = 7 Hz, 3-, 6-H of Fmoc), 7.43 (1H, dd, J = 9, 3 Hz, 3-CH), 5.67 (1H, d, J = 8 Hz, Fmoc-NH), 5.26, 5.12 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.84 (1H, ddd, J = 13, 9, 4 Hz, 31-CH), 4.62 (1H, m, α-H of Glu), 4.48 (1H, dq, J = 2, 8 Hz, 18-H), 4.41, 4.32 (each 1H, dd, J = 11, 7 Hz, 9-CH_2 of Fmoc), 4.29 (1H, dt, J = 8, 2 Hz, 17-H), 4.24 (1H, ddd, J = 13, 9, 3 Hz, 3^1-CH), 4.11 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.77, 3.69, 3.60, 3.46, 3.19 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Glu-OCH_3), 3.76 (1H, dd, J = 9, 4 Hz, 3^2-OH), 3.68 (2H, q, J = 8 Hz, 8-CH_2), 2.82–2.76, 2.59–2.52 (each 1H, m, γ-H of Glu), 2.72–2.66, 2.57–2.52, 2.31–2.23 (1H+1H+2H, m, 17-CH_2CH_2), 2.43–2.37 (2H, m, β-H of Glu), 1.79 (3H, d, J = 8 Hz, 18-CH_3), 1.69 (3H, t, J = 8 Hz, 8^1-CH_3), 0.16, −1.93 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 948.4187, calcd for $C_{55}H_{58}N_5O_{10}$: MH^+, 948.4178.

4.4.13. Methyl 3^2-[3-(9-fluorenylmethoxycarbonyl)amino-3-(methoxycarbonyl)propyl]carbonyloxy-3^1-hydroxy-mesopyropheophorbide-*a* (Glu-adduct, 2e)

13% isolated yield ($3^1R/S$ = 1/1); UV–vis (CH_2Cl_2) λ_{max} = 663 (relative intensity, 0.52), 606 (0.07), 537 (0.09), 506 (0.10), 475 (0.04), 410 (1.00), 398 (0.80, sh), 379 (0.58, sh), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.73/67 (1H, s, 5-H), 9.50/49 (1H, s, 10-H), 8.549/546 (1H, s, 20-H), 7.66–7.54, 7.34–7.21, 7.03 (8H, m, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-H of Fmoc), 6.58/50 (1H, dd, J = 9, 3 Hz, 3-CH), 5.64/60 (1H, d, J = 8 Hz, Fmoc-NH), 5.25/24, 5.10/09 (each 1H, d, J = 19 Hz, 13^1-CH_2), 5.16/4.74 (1H, dd, J = 12, 9 Hz, 3^1-CH), 4.89/62 (1H, dd, J = 12, 3 Hz, 3^1-CH), 4.68–4.62 (1H, m, α-H of Glu), 4.54–4.44 (2H, m, 9-CH_2 of Fmoc), 4.48 (1H, m, 18-H), 4.28 (1H, m, 17-H), 4.25/14 (1H, br, 3^1-OH), 4.18/17 (1H, t, J = 7 Hz, 9-H of Fmoc), 3.83/81, 3.653/651, 3.613/610, 3.45/44, 3.25/23 (each 3H, s, 2-, 7-, 12-CH_3, 17^2-CO_2CH_3, Glu-OCH_3), 3.69/68 (2H, q, J = 8 Hz, 8-CH_2), 2.72–2.52, 2.35–2.24 (each 2H, m, 17-CH_2CH_2), 2.49–2.34 (2H, m, γ-H of Glu), 2.07–1.93 (2H, m, β-H of Glu), 1.80/79 (3H, d, J = 7 Hz, 18-CH_3), 1.694/687 (3H, t, J = 8 Hz, 8^1-CH_3), 0.30/24, −1.82/85 (each 1H, s, NH × 2); HRMS (APCI) found: m/z = 948.4187, calcd for $C_{55}H_{58}N_5O_{10}$: MH^+, 948.4178.

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Supplementary data

Supplementary data (optical spectra of **1** and **2**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.059.

References and notes

1. (a) Tamiaki, H.; Suzuki, S.; Maruyama, K. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2633; (b) Tamiaki, H.; Nomura, K.; Maruyama, K. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 3062; (c) Tamiaki, H.; Nomura, K.; Maruyama, K. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1863; (d) Alonso, C.; Boyle, R. W. In *Handbook of Porphyrin Science*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Singapore, 2010; p 121. Vol. 4, Chapter 17; (e) Reddy, K. R.; Jiang, J.; Krayer, M.; Harris, M. A.; Springer, J. W.; Yang, E.; Jiao, J.; Niedzwiedzki, D. M.; Pandithavidana, D.; Parkes-Loach, P. S.; Kirmaier, C.; Loach, P. A.; Bocian, D. F.; Holten, D.; Lindsey, J. S. *Chem. Sci.* **2013**, *4*, 2036; (f) Sakai, S.; Noji, T.; Kondo, M.; Mizuno, T.; Dewa, T.; Ochiai, T.; Yamakawa, H.; Itoh, S.; Hashimoto, H.; Nango, M. *Langmuir* **2013**, *29*, 5104; (g) Giuntini, F.; Boyle, R.; Sibrian-Vazquez, M.; Vicente, M. G. H. In *Handbook of Porphyrin Science*; Ferreira, G. C., Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Singapore, 2014; p 303. Vol. 27, Chapter 135.
2. Recent reports: (a) Tamiaki, H.; Kozawa, T.; Kitamoto, R.; Kunieda, M. *J. Porphyrins Phthalocyanines* **2010**, *14*, 375; (b) Chaleix, V.; Sol, V.; Krausz, P. *Tetrahedron Lett.* **2011**, *52*, 2977; (c) Melomedov, J.; von Leupoldt, A. W.; Meister, M.; Laquai, F.; Heinze, K. *Dalton Trans.* **2013**, *42*, 9727.
3. Kashiyama, Y.; Tamiaki, H. *Chem. Lett.* **2014**, *43*, in press.
4. (a) DelGovernatore, M.; Hamblin, M. R.; Piccinini, E. E.; Ugolini, G.; Hasan, T. *Br. J. Cancer* **2000**, *82*, 56; (b) Rau, H. K.; Snigula, H.; Struck, A.; Robert, B.; Scheer, H.; Haehnel, W. *Eur. J. Biochem.* **2001**, *268*, 3284; (c) Anatelli, F.; Mroz, P.; Liu, Q.; Yang, C.; Castano, A. P.; Swietlik, E.; Hamblin, M. R. *Mol. Pharm.* **2006**, *3*, 654; (d) Oba, T. *Curr. Bioact. Compd.* **2007**, *3*, 239; (e) Bhatti, M.; Yahioglu, G.; Milgrom, L. R.; Garcia-Maya, M.; Chester, K. A.; Deonarain, M. P. *Int. J. Cancer* **2008**, *122*, 1155; (f) Srivatsan, A.; Ethirajan, M.; Pandey, S. K.; Dubey, S.; Zheng, X.; Liu, T.-H.; Shibata, M.; Missert, J.; Morgan, J.; Pandey, R. K. *Mol. Pharm.* **2011**, *8*, 1186.
5. Machida, S.; Isoda, Y.; Kunieda, M.; Tamiaki, H. *Tetrahedron Lett.* **2012**, *53*, 6277.
6. Helmreich, M.; Ermilov, E. A.; Meyer, M.; Jux, N.; Hirsch, A.; Röder, B. *J. Am. Chem. Soc.* **2005**, *127*, 8376.
7. Tamiaki, H.; Kunieda, M. In *Handbook of Porphyrin Science*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Singapore, 2011; p 223. Vol. 11, Chapter 51.
8. Hollowood, C. J.; Yamanoi, S.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 1664.