# Supplementary materials

(1) X-ray crystallography data

The molecular structures of six isolated porphyrins (including five compounds analyzed isotopically) were determined by X-ray crystallography.   Crystals were prepared from 100–800 µg of each isolated compound.   Dark-red colored crystals of isolated individual vanadyl porphyrins were grown by vapor diffusion (methanol into $CHCl_3$ solution).   Single crystals of various dimensions (as small as $0.1 \times 0.1 \times 0.02$ mm) were subjected to X-ray diffraction analyses. Measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu–Kα radiation.

We obtained conclusive crystallographic information for compounds **1a**, **1b**, **1c**, **1d**, and **1e**, which were determined to be deoxophylloerythroetioporphyrin (DPEP), 3-methyl-DPEP, 3-nor-DPEP, 8-nor-DPEP, and 17-nor-DPEP, respectively.   Given that the structural information for DPEP (**1a**) and 8-nor-DPEP (**1d**) is reported in Kashiyama et al. (in press) and Kashiyama et al. (2007b), respectively, we provide the crystallographic information for the other three compounds below.
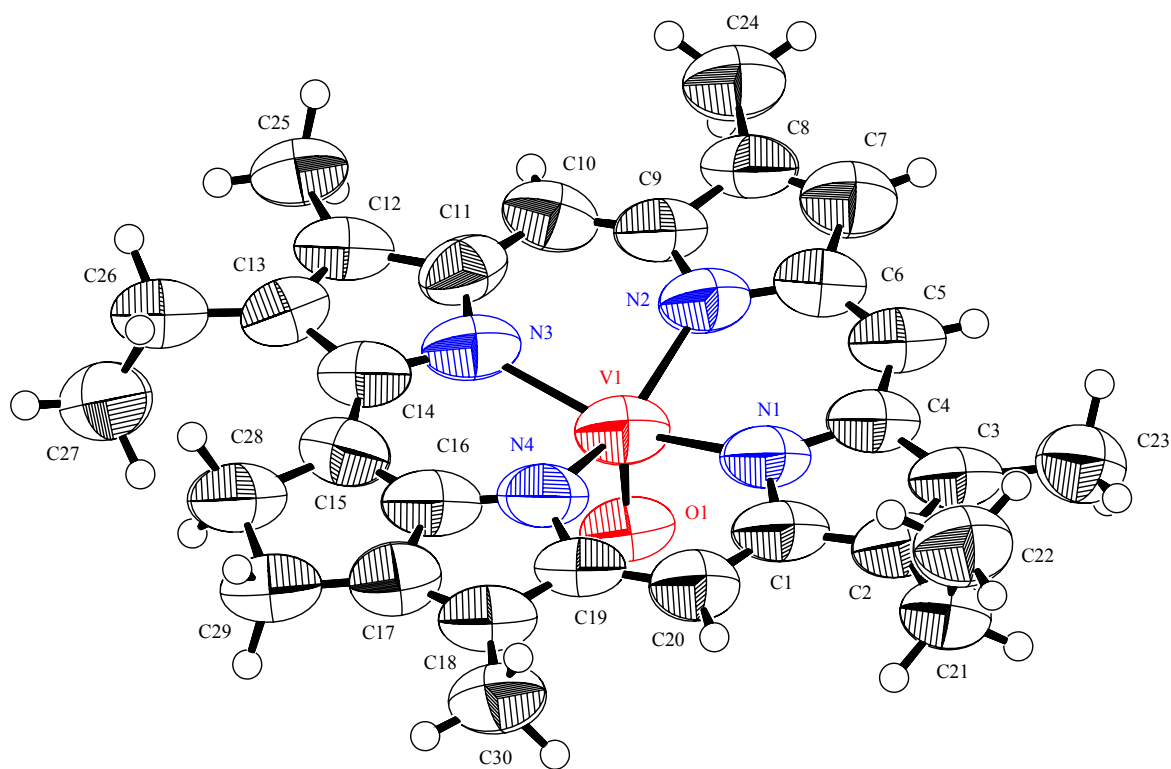
Compound 1b

$C_{31}H_{32}N_4OV$, fw 527.56, crystal dimensions 0.20 x 0.10 x 0.03 mm, triclinic, space group P-1 (#2), a = 8.6340(4) Å, b = 12.6127(5) Å, c = 12.9031(5) Å, α = 70.4199(17)°, β = 84.429(2)°, γ = 74.715(2)°, V = 1276.97(9) Å^3, Z = 2, CuKα radiation (λ = 1.54187 Å), D_{calc} = 1.372 g cm^{-3}, μ(CuKα) = 34.974 cm^{-1}, 12807 measured reflections, 4488 unique reflections [R_{int} = 0.072], 4488 reflections included in the refinement, $R1$ = 0.1183 [I > 2.00 σ (I)], $wR2$ = 0.3838 (all reflections). Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 668113).   This information can be obtained free of charge from www.ccdc.cam.ac.uk.
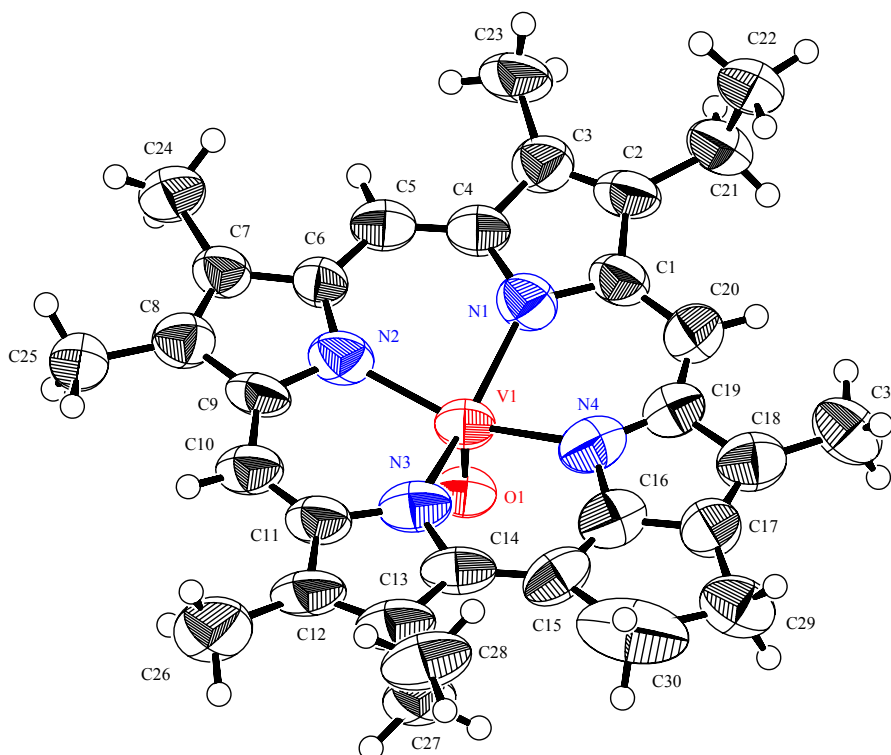
Compound 1c

$C_{30}H_{30}N_4OV$, fw 513.53, crystal dimensions 0.10 x 0.10 x 0.02 mm, triclinic, space group P-1 (#2), a = 9.4833(6) Å, b = 11.1878(6) Å, c = 12.2682(7) Å, α = 82.029(3)°, β = 71.105(3)°, γ = 82.285(3)°, V = 1214.03(11) Å^3, Z = 2, CuKα radiation (λ = 1.54187 Å), D_{calc} = 1.405 g cm^{-3}, μ(CuKα) = 36.638 cm^{-1}, 10950 measured reflections, 4238 unique reflections [R_{int} = 0.116], 4238 reflections included in the refinement, $R1$ = 0.1030 [I > 2.00 σ (I)], $wR2$ = 0.3441 (all reflections). Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 668112).   This information can be obtained free of charge from www.ccdc.cam.ac.uk.
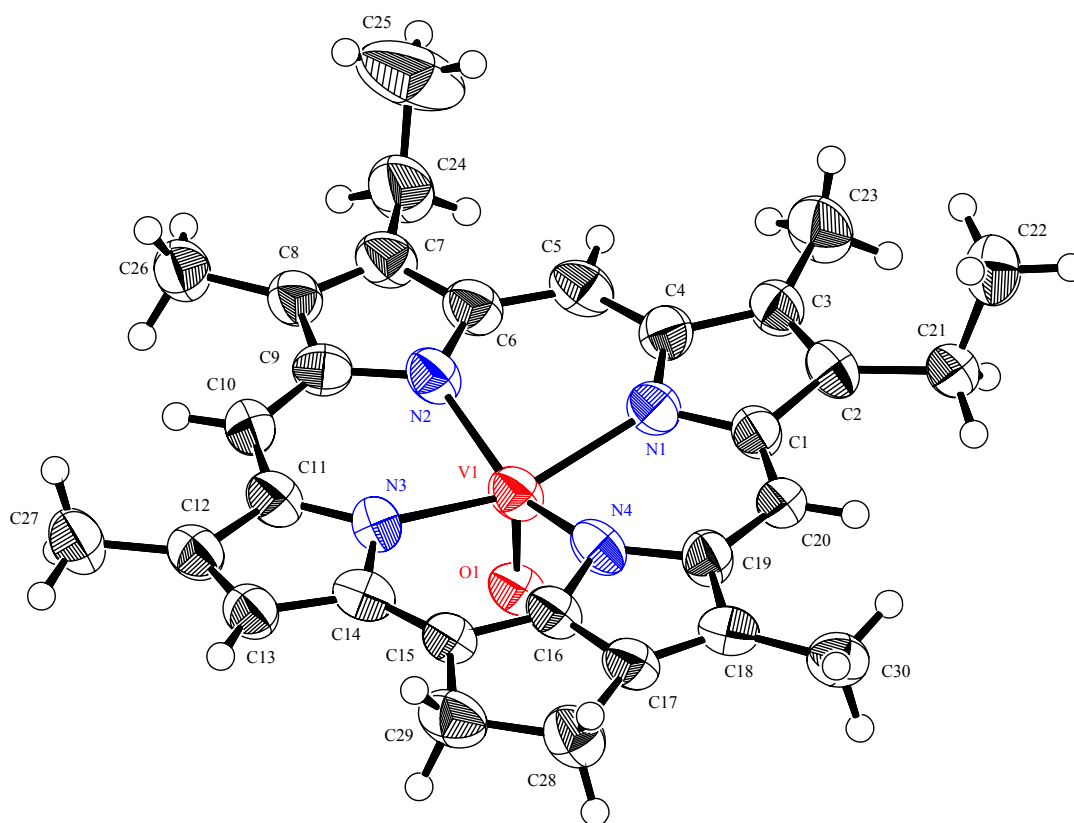
Compound 1e

$C_{30}H_{30}N_4OV$, fw 513.53, crystal dimensions 0.10 x 0.10 x 0.04 mm, triclinic, space group P-1 (#2), $a = 9.6551(3)$ Å, $b = 10.9130(3)$ Å, $c = 12.2787(3)$ Å, $\alpha = 81.7071(17)°$, $\beta = 74.0418(15)°$, $\gamma = 82.7403(18)°$, $V = 1225.77(6)$ Å^3, $Z = 2$, CuKα radiation ($\lambda = 1.54187$ Å), $D_{calc} = 1.391$ g cm^{-3}, $\mu(CuK\alpha) = 36.287$ cm^{-1}, 13897 measured reflections, 4387 unique reflections [$R_{int} = 0.062$], 4387 reflections included in the refinement, $R1 = 0.0664$ [$I > 2.00\ \sigma\ (I)$], $wR2 = 0.1934$ (all reflections). Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 668111).   This information can be obtained free of charge from www.ccdc.cam.ac.uk.

Compound 2

We obtained preliminary crystallographic information for Compounds **2**, which suggests that the crystal consists of a one-to-one pair of diastereomers of C_{33} bicycloalkanoporphyrin (biCAP). Namely, the two structures illustrated below are suggested to be present in the analyzed crystal with equal probability.   The analysis also indicates that carbon atoms at C-13 and C-13A, as well as those at C-15 or C-15A, are present with equal probability, and that each diastereomer has only one of each pair (C-13 or C-13A, and C-15 or C-15A) in its structure.   Considering the subcultures of naturally occurring tetrapyrroles, we concluded the chemical structure of Compounds **2** to be that shown in the Appendix figures.
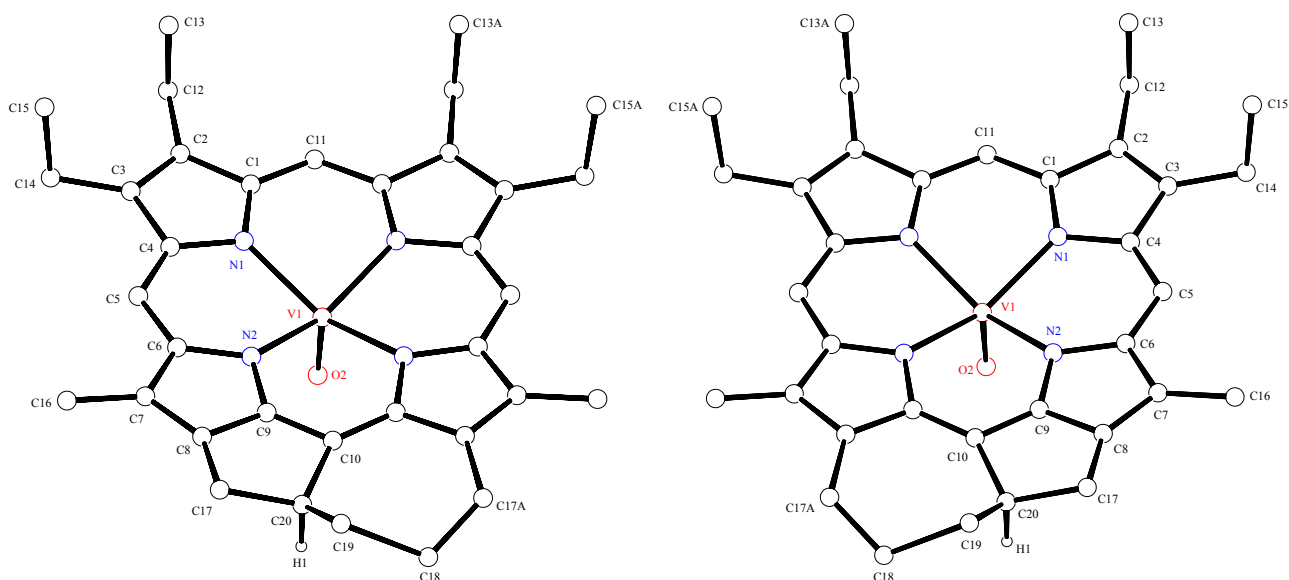
(2)   Student's *t* test

Table S1. Student's *t* test examining if $\delta^{15}N$ and $\delta^{13}C$ values of porphyrins (**1c-e** and **2**; numbers referring to structures in Appendix A) are different from those of DPEP (**1a**) at a significance level of 0.05. The test indicates that: (1) Compound **1c** are not statistically distinguished from DPEP in both $\delta^{15}N$ and $\delta^{13}C$; (2) Compound **1d** and **1e** are statistically distinguished from DPEP in $\delta^{13}C$; and (3) Compound **2** are statistically distinguished from DPEP in both $\delta^{15}N$ and $\delta^{13}C$.

Compounds		BA138b	BA140a III	BA140a IV	BA140a V	GJ01-7	GJ01-3	GJ01-G	$\bar{x}$	s^2	s'^2	t_{calc}	n-1	t (P=0.05)	Choice of hypotheses*
1c	$\delta^{15}N$	0.0	-0.9	0.4	0.6	(n/a)	(n/a)	-0.4	-0.1	0.39	0.30	-0.28	4	2.78	H_0
	$\delta^{13}C$	0.1	0.8	-0.1	0.5	(n/a)	(n/a)	0.0	0.3	0.15	0.06	2.42	4	2.78	H_0
1d	$\delta^{15}N$	2.3	4.0	-1.0	-0.3	(n/a)	(n/a)	3.1	1.6	4.73	4.64	1.68	4	2.78	H_0
	$\delta^{13}C$	-4.1	-1.9	-4.2	-2.5	(n/a)	(n/a)	-1.6	-2.9	1.54	1.45	-5.30	4	2.78	H_1
1e	$\delta^{15}N$	-0.1	-0.5	1.4	-0.5	2.8	-0.1	-0.7	0.3	1.65	1.56	0.73	6	2.45	H_0
	$\delta^{13}C$	0.4	0.6	0.9	1.3	0.5	0.2	0.5	0.6	0.13	0.04	8.49	6	2.45	H_1
2	$\delta^{15}N$	-1.5	-1.7	-1.3	-0.7	-0.7	-0.9	0.2	-0.9	0.41	0.32	-4.43	6	2.45	H_1
	$\delta^{13}C$	-2.5	-2.1	-1.9	-1.6	-3.0	-2.7	-2.5	-2.3	0.23	0.14	-16.19	6	2.45	H_1

*Hypotheses tested herein are as follows:

$H_0: \mu = \mu_0 = 0$ (null hypothesis)

$H_1: \mu \neq \mu_0 = 0$ (alternative hypothesis)

where μ denotes the population mean (the "true" mean) of the *difference* in isotopic values between each compound and DPEP. Therefore, H_0, indicating that null hypothesis was not rejected, suggests that the isotopic value is statistically indistinguishable from that of DPEP; H_1, indicating that null hypothesis was rejected, suggests that the isotopic value is statistically distinguished from that of DPEP.

**Definition of symbols:

$\bar{x}$: sample mean.

s^2 : sample variance including the variance of analytical error.

s'^2 : estimated sample variance that is attributed to the population variance (the "true" variance) after excluding the variance of analytical error ($s^2_{error} = 0.3^2$) because $s^2 = s'^2 + s^2_{error}$.

t_{calc} : Student's *t* variable calculated from $\bar{x}$ and s'^2: $t_{calc} = (\bar{x} - \mu_0)/(s'^2/n)^{0.5}$, where $\mu_0 = 0$.

n -1: degree of freedom.

t (P=0.05): the critical value of *t* corresponding to given v at a significance level of 0.05. H_0 would be rejected if $|t_{calc}| > t$ (P=0.05).

Table S2. Student's t test examining if $\delta^{15}N$ and $\delta^{13}C$ values of porphyrins (**1a**, **c**, **d** and **2**; numbers referring to structures in Appendix A) are different from those of 17-nor-DPEP (**1e**) at a significance level of 0.05. The test indicates that: (1) Compound **1a**, **c**, **d** are statistically distinguished from 17-nor-DPEP in $\delta^{13}C$; and (3) Compound **2** are statistically distinguished from 17-nor-DPEP in both $\delta^{15}N$ and $\delta^{13}C$.

Compounds		BA138b	BA140a			GJ01-7	GJ01-3	GJ01-G	$\bar{x}$	s^2	s'^2	t_{calc}	n-1	t (P=0.05)	Choice of hypotheses*
			III	IV	V										
1a	$\delta^{15}N$	0.1	0.5	-1.4	0.5	-2.8	0.1	0.7	-0.3	1.65	1.56	-0.73	6	2.45	H_0
	$\delta^{13}C$	-0.4	-0.6	-0.9	-1.3	-0.5	-0.2	-0.5	-0.6	0.13	0.04	-8.49	6	2.45	H_1
1c	$\delta^{15}N$	0.1	-0.4	-1.0	1.1	(n/a)	(n/a)	0.3	0.0	0.61	0.52	0.00	4	2.78	H_0
	$\delta^{13}C$	-0.3	0.2	-1.0	-0.8	(n/a)	(n/a)	-0.6	-0.5	0.20	0.11	-3.24	4	2.78	H_1
1d	$\delta^{15}N$	2.3	4.5	-2.4	0.2	(n/a)	(n/a)	3.8	1.7	7.87	7.78	1.35	4	2.78	H_0
	$\delta^{13}C$	-4.5	-2.5	-5.1	-3.8	(n/a)	(n/a)	-2.1	-3.6	1.65	1.56	-6.43	4	2.78	H_1
2	$\delta^{15}N$	-1.4	-1.2	-2.7	-0.2	-3.5	-0.8	0.9	-1.3	2.17	2.08	-2.36	6	2.45	H_1
	$\delta^{13}C$	-2.9	-2.7	-2.7	-2.9	-3.5	-2.9	-3.0	-3.0	0.06	0***	∞	6	2.45	H_1

*Hypotheses tested herein are as follows:

$H_0: \mu = \mu_0 = 0$ (null hypothesis)

$H_1: \mu \neq \mu_0 = 0$ (alternative hypothesis)

where μ denotes the population mean (the "true" mean) of the *difference* in isotopic values between each compound and 17-nor-DPEP. Therefore, H_0, indicating that null hypothesis was not rejected, suggests that the isotopic value is statistically indistinguishable from that of 17-nor-DPEP; H_1, indicating that null hypothesis was rejected, suggests that the isotopic value is statistically distinguished from that of 17-nor-DPEP.

**Definition of symbols refer to those in Table S1.

***Because s^2 is smaller than estimated variance of the analytical error, the true variance is estimated to be null.

(3) Estimation of the degree of overall isotopic fractionation during photosynthesis (ε_p)

In the present work, ε_p, the overall isotopic fractionation, is defined as:

$$\varepsilon_p = 10^3[(\delta^{13}C_{cell} + 1000)/(\delta^{13}C_{CO2aq} + 1000) - 1] \approx \delta_{cell} - \delta_d \ (‰)$$

where δ_{cell} and δ_d denote $\delta^{13}C$ values of the whole cell of photoautotrophs and CO_2aq, respectively. δ_d can be calculated as follows:

$$\delta_d = \delta_c + (\varepsilon_{b/c} + \varepsilon_{d/b}) \ (‰)$$

where δ_c, $\varepsilon_{b/c}$, and $\varepsilon_{d/b}$ denote $\delta^{13}C$ values of carbonate (CO_3^{2-}), equilibrium isotopic discrimination between CO_3^{2-} and HCO_3^{-}, and equilibrium isotopic discrimination between HCO_3^{-} and CO_2aq, respectively. $\varepsilon_{b/c}$ and $\varepsilon_{d/b}$ are functions of temperature (T), and the following relationships are known (Thode et al., 1965; Mook et al., 1965):

$$\varepsilon_{b/c} = [653.627/(T - 233.45)^2] + 0.22 \ (‰)$$
$$\varepsilon_{d/b} = 24.12 - 9866/T \ (‰)$$

Therefore, δ_d can be estimated from δ_c and T as follows:

$$\delta_d = \delta_c + ([653.627/(T - 233.45)^2] + 0.22) + 24.12 - 9866/T \ (‰)$$

δ_c for surface water in the Onnagawa basin is difficult to explicitly evaluate because primary carbonate is not preserved. The δ_c of shallow-dwelling planktonic foraminifera of the middle Miocene age (9-13Ma) indicates that the δ_c was approximately 1‰ higher than that from the modern surface ocean (e.g., Kennett, 1986; Woodruff and Savin, 1989, 1991; Hodell and Vayavananda, 1994; Pagani et al., 1999a, 1999b). T in the Onnagawa basin (i.e., sea surface temperature; SST) was assumed to be in the range of 10-20°C. The results of calculation are shown in the Table 4 of the main text.