HETERICYCLES, Vol.55, No.3, pp.526 - 533, Received, 29th November, 2000

SYNTHESIS AND SOLUTION BEHAVIOR OF WATER-SOLUBLE BIS-PORPHYRINS

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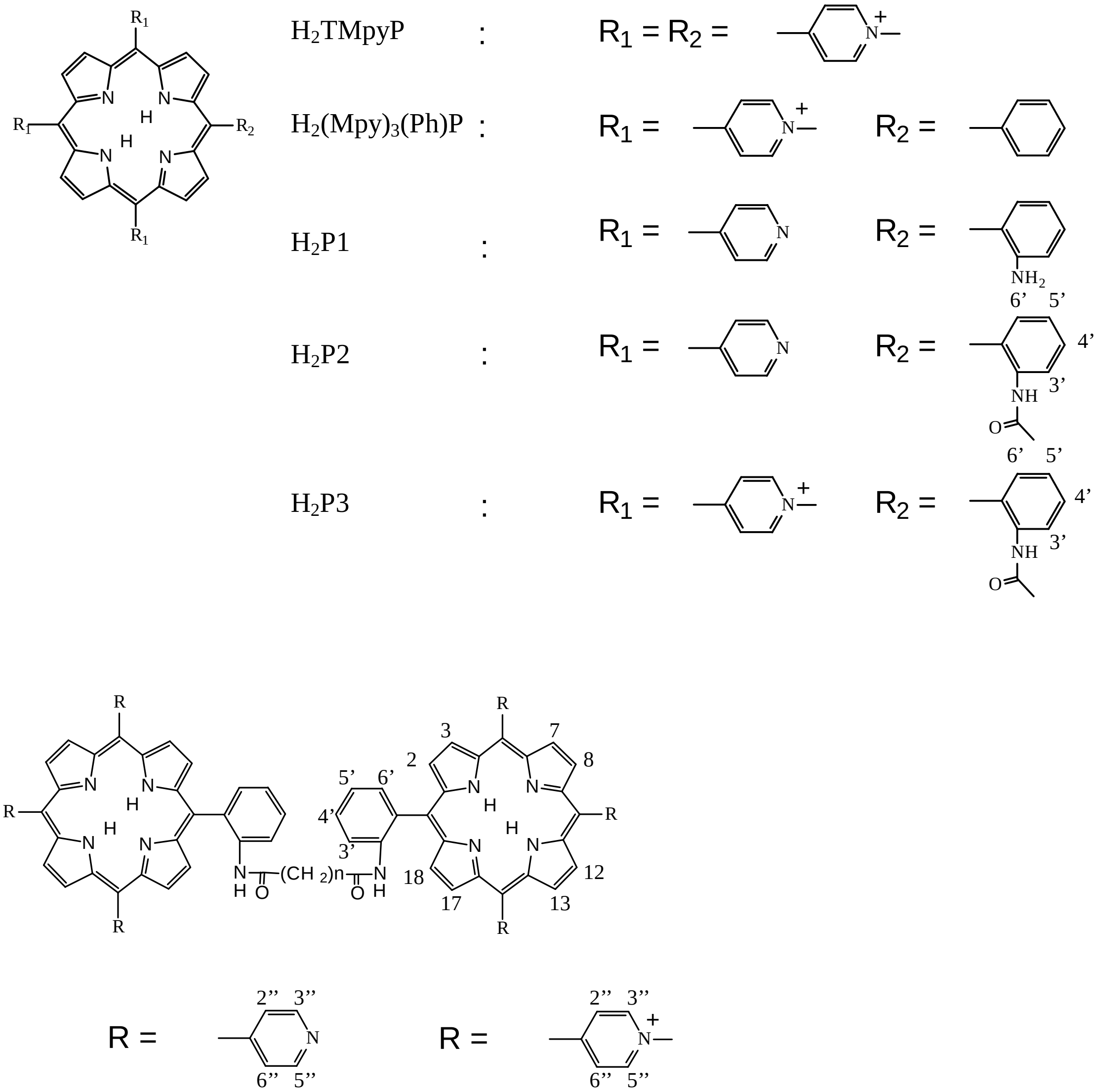
Abstract - A series of water-soluble bis-porphyrins in which two porphyrin (5-(2-amidophenyl)-10,15,20-tris(*N*-methyl-4-pyridiniumly)porphyrin) units were linked by a methylene chain with various lengths (-NHCO-$(CH_2)_n$-CONH-, n = 1, 3, 5, 7 and 11) were synthesized and characterized by UV-visible and ^{1}H-NMR spectroscopy. The conformations of the bis-porphyrins in solution were estimated from calculation on the basis of their ring-current shifts. The self-association behavior of the bis-porphyrins in aqueous solution (at 25℃, pH 7.0, μ = 0.045) was also reported.

INTRODUCTION

Since Fiel *et al.*[1] have reported that a typical cationic porphyrin *meso*-tetrakis(4-*N*-methylpyridyl)-porphyrin (H_2TMpyP, Scheme 1) can intercalate into calf thymus DNA, interactions of cationic porphyrins with DNA or nucleic acids have been studied by several workers.[2-16] In addition, cationic porphyrins have been reported to show anti-tumor activity by their accumulation in tumor cells and their subsequent photosensitization effects.[17,18] The free base porphyrin, H_2TMpyP and its four-coordinate metal complexes have been found to show intercalative binding to GC-rich regions and groove binding to AT-rich regions in DNA.[19-21] Meunier *et al*. have reported that, hybrid molecules “metalloporphyrin-ellipticine” show intercalative binding at the elliptieine site and groove binding at the metalloporphyrin site with DNA.[22] Thus, it may be expected that dimerized cationic porphyrins (bis-porphyrins) exhibit a new type of binding mode as a result of a collaboration effect of the two binding sites with DNA. Further, the new binding mode may reveal a specific recognition property for a certain array of nucleic acids. On the basis of this idea, we designed and synthesized of a series of water-soluble bis-porphyrins by tethering two porphyrins *via* various lengths of linker (Scheme 1) that are capable of showing a collaboration effect. The present paper describes the syntheses and self-aggregation behavior of the bis-porphyrins.

EXPERIMENTAL

Materials and Methods



compound	n
bisC1	1
bisC3	3
bisC5	5
bisC7	7
bisC11	11

compound	n
bisMC1	1
bisMC3	3
bisMC5	5
bisMC7	7
bisMC11	11

Scheme 1

All chemicals were purchased commercially and were used as received without further purification unless otherwise noted. Malonyl dichloride, glutaryl dichloride, pimeloyl dichloride and azelaoyl dichloride were used after distillation. Tridecanyl dichloride,[23] $H_2(Mpy)_3(Ph)P$[24] and H_2P1[25,26] were prepared according to the publish methods (Scheme 1). H_2TMpyP was purchased from Aldrich, and was used without further purification.

Measurements

The UV-visible spectra were recorded on a Hitachi U3000 spectrophotometer. The ^{1}H NMR spectra were recorded on a JEOL JNM-GSX-400 spectrometer. The ring-current shifts were calculated using the double-loop model of Abraham *et al.* [27,28] The detailed procedures for the calculation of ring-current shifts and the determination of the conformation for the porphyrins have been described earlier.[29]

Synthesis of bisC1

A CH_2Cl_2 solution (15 mL) containing malonyl dichloride (7.5 μL, 77 μmol) was added slowly to a refluxed 1 : 1 CH_2Cl_2 / benzene solution (100 mL) containing H_2P1 (103 mg, 0.163 mmol) under N_2 atmosphere, then the solution was refluxed for 5 h. After cooled to rt, a 10% NaOH solution (100 mL) was added to the reactant solution. The organic layer was separated and the aqueous layer was extracted twice with $CHCl_3$ (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and was evaporated to dryness. The resultant solid was dissolved in $CHCl_3$ and chromatographed on a silica gel column (2 × 15 cm). The column was eluted with $CHCl_3$ / EtOH (100 : 5). The eluate was evaporated to dryness and was crystallized from CH_2Cl_2 / ether, yielding 18 mg (17%). $C_{85}H_{56}N_{16}O_2$ FAB MS: m/z (relative intensity, %) 1332 (57), 1333 (95), 1334 (100 M + $H^+)^+$, 1335 (72), 1336 (40). ^{1}H NMR (DMSO-d_6, TMS): δ = -3.01 (s, 4H, pyrrole-NH), 1.90 (s, 2H, -CH_2-), 6.72 {t, 2H, *J* = 7.3 Hz, phenyl H (4')}, 6.82 {d, 2H, *J* = 8.8 Hz, phenyl H (3')}, 7.29 {t, 2H, *J* = 7.3 Hz, phenyl H (5')}, 7.85 {d, 2H, *J* = 7.3 Hz, phenyl H (3')}, 7.91 {s, 4H, α-pyridine H (3'', 5'')}, 8.20 {s, 4H, α pyridine H (3'', 5'')}, 8.29 {d, 4H, *J* = 5.9 Hz, β-pyridine H (3'', 5'')}, 8.35 {d, 4H, *J* = 4.4 Hz, pyrrole H (2, 18)}, 8.62 {s, 4H, pyrrole H (3, 17)}, 8.72 {s, 4H, α-pyridine H (2'', 6'')}, 8.76 (s, 2H, -NHCO-), 8.67 {d, 4H, *J* = 4.9Hz, pyrrole H (7, 13)}, 8.91 {d, 4H, *J* = 4.9Hz, pyrrole H (8, 12)}, 8.99{s, 4H, α-pyridine H (2'', 6'')}, 9.08 {s, 4H, β-pyridine H (2'', 6'')}.

BisC3, bisC5, bisC7 and bisC11 were similarly prepared as the case of bisC1 by use of glutaryl dichloride, pimeloyl dichloride, azelaoyl dichloride and tridecanyl dichloride, respectively, instead of malonyl dichloride.

BisC3: yield was 39%. $C_{87}H_{60}N_{16}O_2$ FAB MS: m/z (relative intensity, %) 1361 (77), 1362 (100 M + $H^+)^+$, 1363 (88), 1364 (61). ^{1}H NMR (DMSO-d_6, TMS): δ = -3.03 (s, 4H, pyrrole-NH), 0.14 (m, 2H, -CH_2-), 0.71 (m, 4H, -CH_2-), 7.45 {t, 2H, *J* = 9.3 Hz, phenyl H (4')}, 7.51 {m, 4H, phenyl H (3', 5')}, .92 {d, 2H, *J* = 5.9 Hz, phenyl H (6')}, 8.17 {s, 4H, α-pyridine H (3'', 5'')}, 8.21 {s, 4H, α-pyridine H (3'', 5'')}, 8.32 {d, 4H, *J* = 5.9 Hz, β-pyridine H (3'', 5'')}, 8.50 (s, 2H, -NHCO-), 8.58 {d, 4H, *J* = 4.4 Hz, pyrrole H (2, 18)}, 8.71 {d, 4H, *J* = 3.9 Hz, pyrrole H (3, 17)} 8.83 {s, 4H, α-pyridine H (2'', 6'')}, 8.86 {d, 4H, *J* = 4.9Hz, pyrrole H (7, 13)}, 8.89 {d, 4H, *J* = 4.9Hz, pyrrole H (8, 12)}, 9.00 {s,4H, α-pyridine H (2'', 6'')}, 9.07 {d, 4H, *J* = 4.4Hz, β-pyridine H (2'', 6'')}.

BisC5: yield was 37%. $C_{89}H_{64}N_{16}O_2$ FAB MS: m/z (relative intensity, %) 1389 (84), 1390 (100 M +

$H^+)^+$, 1391 (94), 1392 (69). ^{1}H NMR (DMSO-d_6, TMS): δ = -3.07 (s, 4H, pyrrole-NH), -0.35 (m, 2H, -CH_2-), 0.08 (m, 4H, -CH_2-), 0.77 (m, 4H, -CH_2-), 7.54 {t, 2H, *J* = 7.3 Hz, phenyl H (5')}, 7.73 {t, 2H, *J* = 7.8 Hz, phenyl H (4')}, 7.85 {d, 2H, *J* = 8.3 Hz, phenyl H (3')}, 7.95 {d, 2H, *J* = 7.8 Hz, phenyl H (6')}, 8.08 {s, 4H, pyridine H (3'', 5'')}, 8.19 {s, 4H, pyridine H (3'', 5'')}, 8.23 {d, 4H, *J* = 5.9 Hz, pyridine H (3'', 5'')}, 8.53 (s, 2H, -NHCO-), 8.66 {d, 4H, *J* = 4.9 Hz, pyrrole H (2, 18)}, 8.70 {d, 4H, *J* = 4.9 Hz, pyrrole H (3, 17)}, 8.80 {d, 4H, *J* = 4.4Hz, pyrrole H (7, 13)}, 8.84 {d, 4H, *J* = 4.4Hz, pyrrole H (8, 12)}, 8.90 {s, 4H, α-pyridine H (2'', 6'')}, 8.99 {s, 4H, α-pyridine H (2'', 6'')}, 9.03 {s, 4H, β-pyridine H (2'', 6'')}.

BisC7: yield was 36%. $C_{91}H_{68}N_{16}O_2$ FAB MS: m/z (relative intensity, %) 1417 (52), 1418 (100 M + $H^+)^+$, 1419 (73), 1420 (46). ^{1}H NMR (DMSO-d_6, TMS): δ = -3.09 (s, 4H, pyrrole-NH), -0.79 (m, 6H, -CH_2-), -0.29 (m, 4H, -CH_2-), 0.75 (m, 4H, -CH_2-), 7.57 {t, 2H, *J* = 7.8 Hz, phenyl H (5')}, 7.79 {t, 2H, *J* = 7.8 Hz, phenyl H (4')}, 7.93 {d, 2H, *J* = 7.8 Hz, phenyl H (3')}, 7.98 {d, 6H, *J* = 7.3 Hz, phenyl H (6'), α-pyridine H (3'' or 5'') }, 8.05 {s, 2H, β-pyridine H (3'', 5'')}, 8.12 {s, 4H, α-pyridine H (3'', 5'')}, 8.20 {s, 2H, β-pyridine H (3'', 5'')}, 8.69-8.71 {m, 12H, pyrrole H (2, 3, 7, 13, 17, 18)}, 8.76 {d, 4H, pyrrole H (8, 12)}, 8.83 {s, 6H, α-pyridine H (2'', 6''), β-pyridine H (2'', 6'')}, 8.93 {s, 4H, α-pyridine H (2'', 6'')}, 9.01 {s, 2H, β-pyridine H (2'', 6'')}.

BisC11: yield was 34%. $C_{95}H_{76}N_{16}O_2$ FAB MS: m/z (relative intensity, %) 1472 (43), 1473 (86), 1474 (100 M + $H^+)^+$, 1475 (75), 1476 (52). ^{1}H NMR (DMSO-d_6, TMS): δ = -3.01 (s, 4H, pyrrole-NH), -0.79 (m, 4H, -CH_2-), -0.69 (m, 2H, -CH_2-), -0.45 (m, 4H, -CH_2-), -0.20 (m, 4H, -CH_2-), 0.23 (m, 4H, -CH_2-), 1.26 (m, 4H, -CH_2-), 7.50 {t, 2H, *J* = 7.8 Hz, phenyl H (5')}, 7.81 {t, 2H, *J* = 7.8 Hz, phenyl H (4')}, 7.90 {s, 4H, pyridine H (3'', 5'')}, 8.01 {d, 6H, *J* = 9.3 Hz, phenyl H (5'), pyridine H (3'', 5'')}, 8.15 {s, 6H, pyridine H (3'', 5'')}, 8.50 {s, 2H, pyridine H (2'', 6'')}, 8.63 {s, 4H, pyridine H (2'', 6'')}, 8.75 (s, 16H, pyrrole H), 8.86 (s, 2H, -NHCO-), 8.93 {s, 4H, pyridine H (2'', 6'')}, 8.97 {s, 2H, pyridine H (2'', 6'')}.

Synthesis of bisMC1

Iodomethane (100 μL, 1.61 mmol) was added to a DMF solution (20 mL) containing bisC1 (17 mg , 13 μmol) at 50 ℃ and the solution was stirred for 3 h at that temperature. After cooled to rt, ether was added to the solution to precipitate a solid. After filtration, the resultant solid was dissolved in MeOH and chromatographed on a cation-exchange resin (Amberlist A-21, Cl^- form) column (2 × 20 cm) to give the porphyrin as chloride. The column was eluted with MeOH, then the eluate was evaporated to dryness. The solid was recrystallized from MeOH / acetone, yielding 15 mg (72%). $C_{91}H_{74}N_{16}O_2Cl_6$ FAB MS: m/z (relative intensity, %) 1416 (50), 1417 (82), 1418 (91, M -6Cl^- -5$H^+)^+$, 1419 (100), 1420 (84), 1421 (84), 1422 (64). Anal. Calcd for $C_{91}H_{74}N_{16}O_2Cl_6 \cdot 18H_2O$: C, 55.75; H, 5.65; N, 11.43. Found: C, 55.52; H, 5.15; N, 11.33. ^{1}H NMR (DMSO-d_6, TMS): broad.

BisMC3, bisMC5, bisMC7, bisMC11 and H_2P3 were similarly synthesized from the corresponding bis-porphyrins or porphyrin.

BisMC3: yield was 61%. $C_{93}H_{78}N_{16}O_2Cl_6$ FAB MS: m/z (relative intensity, %) 1446 (56, M $-6Cl^- -5H^+)^+$, 1447 (77), 1448 (100), 1449 (91), 1450 (72), 1451 (53), 1452 (38). Anal. Calcd for $C_{93}H_{78}N_{16}O_2Cl_6 \cdot 17H_2O$: C, 56.68; H, 5.73; N, 11.37. Found: C, 56.36; H, 5.73; N, 11.35. ^{1}H NMR ($DMSO-d_6$, TMS): δ = -3.08 (s, 4H, pyrrole-NH), 0.21 (m, 2H, $-CH_2-$), 0.71 (m, 4H, $-CH_2-$), 4.72 (s, 12H, α-CH_3), 4.74 (s, 6H, β-CH_3), 7.22 {d, 2H, *J* = 9.1 Hz, phenyl H (3')}, 7.40 {t, 2H, *J* = 8.6 Hz, phenyl H (4')}, 7.47 {t, 2H, *J* = 8.6 Hz, phenyl H (5')}, 7.83 {d, 2H, *J* = 9.1 Hz, phenyl H (6')}, 8.66{d, 4H, *J* = 5.7 Hz, pyrrole H (2, 18)}, 8.72 (s, 2H, -NHCO-), 8.91 (m, 12H, pyrrole H (3, 17), α-pyridine H (3'', 5'')}, 8.95 {d, 4H, *J* = 5.7 Hz, β-pyridine H (3'', 5'')}, 9.13 {s, 8H, pyrrole H (7, 8, 12, 13)}, 9.46 {d, 8H, *J* = 5.7 Hz, α-pyridine H (2'', 6'')}, 9.51 {s, 4H, β-pyridine H (2'', 6'')}.

BisMC5: yield was 75%. $C_{95}H_{82}N_{16}O_2Cl_6$ FAB MS: m/z (relative intensity, %) 1474 (45, M $-6Cl^- -5H^+)^+$, 1475 (78), 1476 (91), 1477 (100), 1478 (87), 1479 (65), 1480 (48). Anal. Calcd for $C_{95}H_{82}N_{16}O_2Cl_6 \cdot 16H_2O$: C, 57.61; H, 5.80; N, 11.31. Found: C, 57.40; H, 5.59; N, 11.26. ^{1}H NMR ($DMSO-d_6$, TMS): δ = -3.07 (s, 4H, pyrrole-NH), -0.05 (m, 2H, $-CH_2-$), 0.22 (m, 4H, $-CH_2-$), 0.91 (m, 4H, $-CH_2-$), 4.71 (s, 12H, α-CH_3), 4.74 (s, 6H, β-CH_3), 7.58 {t, 2H, *J* = 8.0 Hz, phenyl H (5')}, 7.76 {t, 2H, *J* = 8.6 Hz, phenyl H (4')}, 7.84 {d, 2H, *J* = 8.6 Hz, phenyl H (3')}, 7.90 {d, 2H, *J* = 6.3 Hz, phenyl H (6')}, 8.79 {d, 8H, *J* = 4.6 Hz, pyrrole H (2, 3, 17, 18)}, 8.87 (s, 2H, -NHCO-), 8.93 (m, 12H, pyridine H (3'', 5'')}, 9.11 {s, 8H, pyrrole H (7, 8, 12, 13)}, 9.48 {d, 8H, *J* = 6.3 Hz, α-pyridine H (2'', 6'')}, 9.51 {d, 4H, *J* = 4.0 Hz, β-pyridine H (2'', 6'')}.

BisMC7: yield was 66%. $C_{97}H_{86}N_{16}O_2Cl_6$ FAB MS: m/z (relative intensity, %) 1501 (42), 1502 (40, M $-6Cl^- -5H^+)^+$, 1503 (96), 1504 (100), 1505 (97), 1506 (80), 1507 (58), 1508 (38). Anal. Calcd for $C_{97}H_{86}N_{16}O_2Cl_6 \cdot 16H_2O$: C, 58.00; H, 5.92; N, 11.16. Found C, 57.77; H, 5.71; N, 11.25. ^{1}H NMR ($DMSO-d_6$, TMS): δ = -3.06 (s, 4H, pyrrole-NH), 0.03 (m, 4H, $-CH_2-$), 0.39 (m, 4H, $-CH_2-$), 1.05 (m, 4H, $-CH_2-$), 1.23 (m, 2H, $-CH_2-$), 4.71 (s, 12H, β-CH_3), 4.73 (s, 6H, β-CH_3), 7.60 {t, 2H, *J* = 7.4 Hz, phenyl H (5')}, 7.82 {t, 2H, *J* = 7.4 Hz, phenyl H (4')}, 7.94 {t, 4H, *J* = 6.7 Hz, phenyl H (3', 6')}, 8.83 {d, 4H, *J* = 4.6 Hz, pyrrole H (2, 18)}, 8.85-8.89 {m, 6H, -NHCO-, pyrrole H (3, 17)}, 8.94 {d, 12H, *J* = 5.1 Hz, pyridine H (3'', 5'')}, 9.10 {s, 8H, pyrrole H (7, 8, 12, 13)}, 9.47 {d, 8H, *J* = 6.9 Hz, α-pyridine H (2'', 6'')}, 9.50 {d, 4H, *J* = 6.3 Hz, β-pyridine H (2'', 6'')}.

BisMC11: yield was 64%. $C_{101}H_{94}N_{16}O_2Cl_6$ FAB MS: m/z (relative intensity, %) 1557 (38), 1558 (56, M $-6Cl^- -5H^+)^+$, 1559 (88), 1560 (94), 1561 (100), 1562 (88), 1563 (63), 1564(44). Anal. Calcd for $C_{101}H_{94}N_{16}O_2Cl_6 \cdot 17H_2O$: C, 58.24; H, 6.19; N, 10.76. Found: C, 57.93; H, 5.77; N, 10.83. ^{1}H NMR ($DMSO-d_6$, TMS): δ = -3.02 (s, 4H, pyrrole-NH), 0.29 (m, 6H, $-CH_2-$), 0.35 (m, 8H, $-CH_2-$), 0.68 (m, 4H, $-CH_2-$), 1.29 (m, 4H, $-CH_2-$), 4.72 (s, 12H, α-CH_3), 4.73 (s, 6H, β-CH_3), 7.62 {t, 2H, *J* = 7.2 Hz, phenyl H (5')}, 7.85 {t, 2H, *J* = 7.8 Hz, phenyl H (4')}, 7.96 {d, 2H, *J* = 8.3 Hz, phenyl H (6')}, 8.00{d, 2H, *J* = 8.3 Hz, phenyl H (3')}, 8.85-9.00 {m, 20H, pyrrole H (2, 3, 17, 18), pyridine H (3'', 5'')}, 9.05

(s, 2H, -NHCO-), 9.11 {s, 8H, pyrrole H (7, 8, 12, 13)}, 9.49 {d, 8H, J = 6.5 Hz, α-pyridine H (2'', 6'')}, 9.51 {d, 4H, J = 4.3 Hz, β-pyridine H (2'', 6'')}.

Synthesis of H_2P2

An acetyl chloride was added to a ice cooled CH_2Cl_2 solution (10 mL) containing H_2P1 (20 mg, 32 μmol) and triethylamine (a few drop) under N_2 atmosphere, then the solution was stirred for 20 min. A 10% NaOH solution (20 mL) was added to the reactant solution. The organic layer was separated and the aqueous layer was extracted twice with $CHCl_3$ (20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and was evaporated to dryness. The resultant solid was dissolved in $CHCl_3$ and chromatographed on a silica gel column (1.5 × 10 cm). The column was eluted with $CHCl_3$. The eluate was evaporated to dryness and was crystallized from CH_2Cl_2 / hexane, yielding 15 mg (69%). ^{1}H NMR (DMSO-d_6, TMS) : δ = -2.86 (s, 2H, pyrrole-NH), 1.21 (s, 3H, -CH_3), 7.58 {t, 1H, J = 7.8 Hz, phenyl H (5')}, 7.84 {t, 1H, J = 7.8 Hz, phenyl H (4')}, 7.98 {d, 1H, J = 7.3 Hz, phenyl H (6')}, 8.13 {d, 1H, J = 8.3 Hz, phenyl H (3')}, 8.25 {q, 6H, J = 2.0, 4.2 Hz, pyridine H (3'', 5'')}, 8.79 {d, 2H, J = 4.9 Hz, pyrrole H (2, 18)}, 8.85 {d, 2H, J = 4.9 Hz, pyrrole H (3, 17)}, 8.89 (s, 4H, pyrrole), 8.92 (s, 1H, -NHCO-), 9.06 {d, 6H, J = 5.4 Hz, pyridine H (2'', 6'')}.

H_2P3: ^{1}H NMR (DMSO-d_6, TMS): δ = -2.98 (s, 2H, pyrrole-NH), 1.20 (s, 3H, -CH_3), 4.73 (s, 9H, N-CH_3), 7.63 {t, 1H, J = 8.2 Hz, phenyl H (5')}, 7.88 {t, 1H, J = 8.2 Hz, phenyl H (4')}, 7.98 {d, 1H, J = 7.7 Hz, phenyl H (6')}, 8.09 {d, 2H, J = 7.7 Hz, phenyl H (3')}, 8.91 {d, 2H, J = 6.4 Hz, pyrrole H (2, 18)}, 8.97 {d, 6H, J = 7.3 Hz, pyridine H (3'', 5'')}, 8.99 {d, 2H, J = 4.3 Hz, pyrrole H (3, 17)}, 9.08 (s, 1H, -NHCO-), 9.13 {s, 4H, pyrrole H (7, 8, 12, 13)}, 9.49 {d, 4H, J = 6.4 Hz, α-pyridine H (2'', 6'')}, 9.50 {d, 2H, J = 6.4 Hz, β-pyridine H (2'', 6'')}.

RESULTS AND DISCUSSION

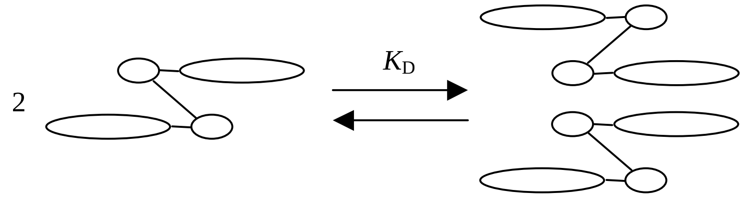
Synthesis

To synthesize bis-porphyrins, one of the four pyridinium groups of H_2TMpyP was replaced with a phenyl group bearing an amino group for constructing an amide bond bridge between the two porphyrin units. Based on a model building for these bis-porphyrins, we predicted that if the amide bond position on the phenyl group was *para*-, the two porphyrin units would be placed as a flat side-to-side structure, but that if it was *ortho*-, the two units would be a parallel side-to-side or face-to-face structure. We chose the *ortho*- linking form for the preference of intercalative binding in a face-to-face structure into DNA, because the face-to-face structure has been expected to have a stronger collaboration effect than the side-to-side structure in interacting with DNA. Further, intercalative binding is known to provide high array specificity compared to other binding modes.[14,20] The subsequent amide bridging of the two porphyrins by several diacid chlorides gave the desired bis-porphyrins (Scheme 1).

In the synthesis of bisC1, the coupling of H_2P1 with malonic acid chloride was not successful under refluxing in CH_2Cl_2, probably due to the low boiling point. Our attempts using benzene, THF, DMF, DMSO, chlorobenzene, or CCl_4 as the solvent were also unsuccessful because undesired precipitates appeared as the reaction proceeded. Consequently, we used a mixed solvent of CH_2Cl_2 : benzene = 1 : 1 that allowed a higher reaction temperature than that of CH_2Cl_2 and gave no precipitate.

Visible spectroscopic study

Some of cationic porphyrins have a tendency to dimerize in aqueous solution depending on the porphyrin concentration.[30,31] The association behavior of two porphyrin units is known to be reflected to their visible spectral properties.[32,33] Hence, we examined the solution properties of the bis-porphyrins by visible spectroscopy. Beer's law experiments for the bis-porphyrins were carried out at 520 nm, 25 ℃, pH 7.0 buffered by tris-HCl (15 mM tris and 15 mM HCl) containing NaCl (ionic strength μ = 0.045). The bis-porphyrins except bisMC1 obeyed Beer's law in the concentration range 10^{-6} < [bis-porphyrin as porphyrin unit] < 10^{-4} M, suggesting that these bis-porphyrins did not show intermolecular self-association in this concentration range. Contrary to this, bisMC1 obeyed Beer's law (R = 0.999) in the concentration range between 10^{-6} and 10^{-5} M but deviated from Beer's law above 10^{-5} due to intermolecular association. From the spectral results at low porphyrin concentration, ε_M for non self-associated species of bisMC1 at 520 nm was estimated to be 3.34×10^4 $M^{-1}cm^{-1}$. The intermolecular self-association constant (K_D, see Scheme 2) of bisMC1 was calculated by using the method by Pasternack *et al.*,[30,31] giving the K_D and the ε_D (520 nm) value of 3.9×10^2 M^{-1} and 2.62×10^4 $M^{-1}cm^{-1}$, respectively (Figure 1). The intermolecular self-association form of bisMC1 was estimated to be less than 1% in the concentration range for the spectroscopic measurements in the Soret region. However, the molar absorption coefficient for the Soret band of bisMC1 is about half of those of porphyrin monomers, H_2TMpyP and $H_2(Mpy)_3(Ph)P$ (Table 1, Figure 2), suggesting that the two porphyrin units in bisMC1 interact intramolecularly with each other in a partially stacked manner (Scheme 2 left). Indeed, the bridged chain length between, the two porphyrin units in bisMC1 was estimated to be 8.8 Å by CPK models and this value is short enough to allow the stacked conformation. Furthermore the Soret band is red-shifted compared with that of the corresponding monomer, $H_2(Mpy)_3(Ph)P$. This result suggests that the two porphyrin units of bisMC1 exist in a J-type (side-to-side, Scheme 3) conformation because the Soret band of porphyrins was reported to show a red shift with J-type self-association and a blue shift with H-type (face-to-face) self-association.[32,33] On the other hand, the Soret band of the other bis-porphyrins exhibits slight blue shifts compared to that of $H_2(Mpy)_3(Ph)P$, but the differences in molar absorption coefficient of the Soret band are small. The lengths of the bridged chains for bisMC3, MC5, MC7 and MC11 were estimated to be 11.2, 13.6, 16.0 and 20.8 Å, respectively, by CPK models and these values are too long to associate the two porphyrin units in a molecule. Consequently, the two porphyrin units in these bis-porphyrins are not stacked with each other.



Scheme 2.

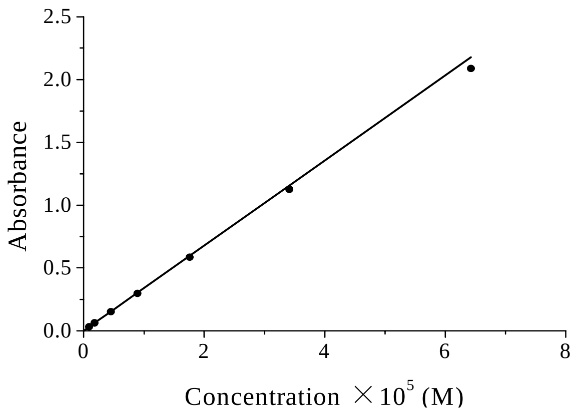


Figure 1. Beer's law experiment for bis MC1 in 15 mM tris-HCl buffer. At 520 nm, 25.0 ℃, pH 7.0, μ = 0.045. The line marked ε 3.34×10^4 $M^{-1}cm^{-1}$ defined Beer's law behavior for bisMC1. The filled circles represent the experimental data.

Table 1. Visible Absorption Spectral Data of Porphyrins in Tris-HCl Buffer[a].

		λ_{max}, ($\varepsilon \times 10^{-4}$ $M^{-1}cm^{-1}$)			
porphyrin	Soret band	Q bands			
H_2TMpyP	422 (22.4)	518 (1.53)	554 (0.555)	585 (0.645)	642 (0.196)
$H_2(Mpy)_3(Ph)P$	422 (26.1)	519 (1.58)	558 (0.709)	584 (0.741)	639 (0.258)[c]
$H_2(Mpy)_3(Ph)P$	ND[b]	520 (1.52)	558 (0.689)	583 (0.718)	640 (0.240)[d]
bisMC1	425 (10.7)	521 (1.70)	557sh (1.05)	585 (0.945)	656 (0.648)[e]
bisMC1	ND[b]	520 (1.62)	557sh (1.00)	586 (0.893)	655 (0.619)[f]
bisMC3	421 (20.7)	520 (1.45)	557 (5.86)	587 (0.629)	646 (0.214)
bisMC5	419 (19.1)	518 (1.41)	555 (5.81)	586 (0.572)	655 (0.407)
bisMC7	419 (17.4)	520 (1.18)	558 (5.05)	587 (0.520)	643 (0.160)
bisMC11	420 (20.2)	520 (1.38)	557 (5.88)	588 (0.591)	648 (0.225)

[a] At 25℃, pH 7.0, μ = 0.045. [b] Could not be determined. [c] Concentration of $H_2(Mpy)_3(Ph)P$ = 1.90 μM. [d] Concentration of $H_2(Mpy)_3(Ph)P$ = 134 μM. [e] Concentration of bisMC1 = 1.82 μM as porphyrin unit. [f] Concentration of bisMC1 = 129 μM as porphyrin unit.

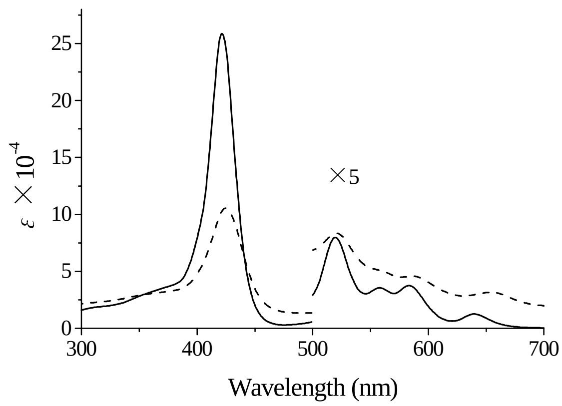
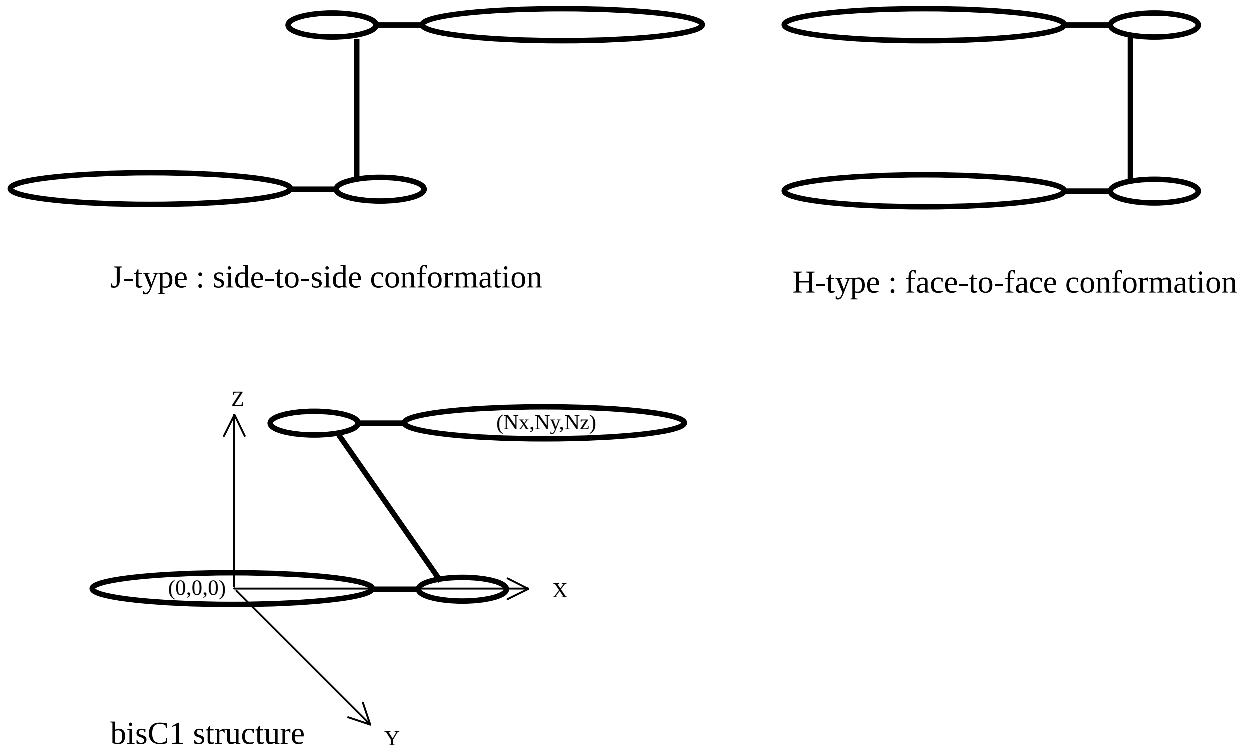


Figure 2. Visible absorption spectra of $H_2(Mpy)_3(Ph)P$ (——) and bisMC1 (-----) in 15 mM tris-HCl buffer. At 25.0 ℃, pH 7.0, μ = 0.045.



Scheme 3.

^{1}H NMR study

Proton NMR spectra of the bis-porphyrins were measured in DMSO-d_6, from which their conformations were presumed with the ring-current calculation described in the EXPERIMENTAL. For the bis-porphyrins, two extreme conformations are possible as shown in Scheme 3 (upper). Based on the ring-current calculation for the face-to-face conformation, the ring-current shift for H4’ of the phenyl group (numbering is shown in Scheme 1) should be smaller than those for H (2, 18) and H (3, 17) of the pyrrole groups, while for the side-to-side conformation, that for H4’ of the phenyl group should be greater than those for H (2, 18) and H (3, 17) of the pyrrole groups. The observed signal for H4’ of the phenyl group in bisC1 shifts to a higher field by 1.12 ppm compared to that of H_2P2. Similarly, the signals for H (2, 18) and H (3, 17) of the pyrrole groups in bisC1 shift to a higher field by 0.44 and 0.23 ppm, respectively. These results indicate that the conformation of bisC1 should be side-to-side. Furthermore only one signal was observed for H4' of the phenyl groups and each signal for H2 and H18, H3 and H17 of the pyrrole group also appeared as one signal. Thus bisC1 is suggested to have C_{2h} symmetry (two porphyrin planes are parallel and Ny = 0 in Scheme 3). The coordinate of the porphyrin (Nx, Ny = 0, Nz) was evaluated to give a best fit for the observed ring current shifts ($\Delta\delta$) with the calculated values by using a least-squares method and a solver program in Microsoft® Excel. The coordinates of the two porphyrin centers were evaluated to be (0, 0, 0 Å) and (8.6, 0, 7.3 Å, Scheme 3 low) (Table 2). In bisC3, the signals of the phenyl H4’ and the pyrrolic H (2, 18) and H (3, 17) shift to higher fields by 0.39, 0.21 and 0.14 ppm, respectively. Similarly the conformation of bisC3 was assigned to be side-to-side where the coordinates of the two porphyrin centers were (0, 0, 0 Å) and

(9.4, 0, 10.7 Å), respectively. Since the ring-current shifts for bisC5, bisC7, and bisC11 were small, we could not determine the conformations of these porphyrins.

The conformations of the water-soluble bis-porphyrins in aqueous solution could not be estimated because of broadening of the pyrrolic protons. Hence, we tried to determine the conformations of the bis-porphyrins in DMSO-d_6. Unfortunately, we could not determine the conformations of bisMC5, bisMC7 and bisMC11, because of the small ring-current shifts. In addition, the spectrum for bisMC1 showed broad unresolved signals then we could not determine the conformation. As a result, only the conformation of bisMC3 could be estimated. The signals of the phenyl H4' and the pyrrolic H (2, 18) and H (3, 17) in bisMC3 shift to higher fields by 0.49, 0.44 and 0.19 ppm, respectively. The conformation of bisMC3 was estimated to be side-to-side where the coordinates of the two porphyrin centers are (0, 0, 0 Å) and (9.7, 0, 9.9 Å), respectively. The coordination difference between bisMC3 and bisC3 was (0.4, 0, -0.8 Å), suggesting that the conformation of bisC3 was retained after methylation of the pyridine groups. In addition, the conformation of bisMC3 in DMSO may be similar to that observed in aqueous solution with visible spectroscopy where the two porphyrin units are enough separated and, hence, do not interact with each other.

Table 2. Experimental and Calculated $\Delta\delta$ Values of Bis-Porphyrins in DMSO-d_6

porphyrin	phenyl H4'[a]		pyrrole H (2, 18)		pyrrole H (3, 17)	
	$\Delta\delta$ exptl[b]	$\Delta\delta$ calcd[c]	$\Delta\delta$ exptl	$\Delta\delta$ calcd	$\Delta\delta$ exptl	$\Delta\delta$ calcd
bisC1	-1.12	-1.07	-0.44	-0.48	-0.23	-0.15
bisC3	-0.39	-0.40	-0.21	-0.22	-0.14	-0.11
bisMC3	-0.48	-0.49	-0.25	-0.24	-0.09	-0.11

[a] For numbering system, see Scheme 1. [b] $\Delta\delta$ exptl = δ (proton in bisC1) - δ (proton in H_2P2), δ (proton in bisC3) - δ (proton in H_2P2) or δ (proton in bisMC3) - δ (proton in H_2P3). [c] Side-to-side conformation.

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