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Energy Transfer in β-Turned Peptide-Bridged Porphyrin Dimers

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β-Turned peptide-bridged diporphyrinyl compounds were prepared. Each isomeric monozinc complexes were easily available; metal-free porphyrin–peptide–zinc porphyrin and zinc porphyrin–peptide–metal-free porphyrin. In the isomers, the intramolecular energy transfer efficiencies from zinc porphyrin moiety to metal-free porphyrin moiety were the same from analysis of the steady-state fluorescence spectra, indicating that the efficiencies should be independent upon the linked peptide spacer and dependent upon the distance between the porphyrin moieties in the molecule. Singlet energy might migrate intramolecularly from zinc porphyrin moiety to metal-free one mainly by through-space mechanism.

X-Ray crystallographic analysis of photosynthetic reaction center of several bacteria revealed interaction of photosynthetic dyes (bacteriochlorophylls, bacteriopheophytins, quinones, etc.) with proteins.[1] Especially, the carbonyl groups conjugated with the dyes hydrogen-bonded with OH or NH of amino acid residues in the environmental protein. We have already reported that in synthetic porphyrins with β-turned oligopeptides, intramolecular hydrogen bonding affected their physical properties.[2] The X-ray analysis also clarified the precise distance and orientation among the dyes. It is important to use a rigid peptide spacer in artificial models for photosynthetic reaction center. It is well-known that Pro–Xaa dipeptide sequence favors β-turn conformation[3] and we have already reported the sequence was useful for a rigid spacer.[4] Here, we report preparation of diporphyrinyl compounds derived from β-turned peptides (represented to "dimer" for convenience, hereafter), intramolecular interaction and energy transfer between the porphyrin moieties. Using the β-turned oligopeptides as the spacer linked with porphyrins, we estimate the energy transfer efficiencies on the synthetic dimers with such unsymmetrical peptide spacers and compare the efficiency migrating from the porphyrin of the N-terminal to the porphyrin of the C-terminal with that from C-terminal to N-terminal.

Results and Discussion

Synthesis of β-Turned Peptide-Bridged Porphyrin Dimers. Schemes 1 and 2 show the synthetic route of the β-turn peptide-bridged porphyrin dimers **1**—**3** (see Fig. 1); two *p*-(10,15,20-tri-*p*-tolyl-5-porphyrinyl)phenyl groups were used as the porphyrin part and D-Pro–Gly and D-Pro–L-Asp sequences were used as the β-turned peptide spacer. In these syntheses, conventional methods for peptide synthesis were used for suppression of racemization. For preparation of selectively metallated porphyrin dimers, N-protected prolines with a porphyrin moiety and C-protected glycines or aspartic acids with a porphyrin moiety were coupled in the final step.

Functional groups directly bonded with *meso*-phenyl group of porphyrin moiety were less reactive. The acid **7** was coupled with D-proline in a high yield (>90%) only via acid chloride (see the left line in Scheme 1). The amine **9** was coupled with *t*-butoxycarbonylglycine (Boc–Gly–OH) in a high yield (>95%) by using only dicyclohexylcarbodiimide (DCC) as a coupling reagent (see the middle line in Scheme 1); addition of 1-hydroxybenzotriazole (HOBt) or *N*-hydroxysuccinimide (HONSu) as a coupling promoter suppressed the yield (<10%) and neither $(\text{Boc–Gly})_2O$ (via acid anhydride) nor Boc–Gly–ONSu (via activated ester) were coupled with **9**.

In the last step (see the bottom of left line in Scheme 1), the coupling of acid **8** H_2TTP–CO–D-Pro–OH with amine H–Gly–NH–H_2TTP by DCC–HOBt gave β-turned dipeptide-bridged porphyrin dimer **1HH** H_2TTP–CO–D-Pro–Gly–NH–H_2TTP in a good yield. The dimer **1HH** was easily metallated by excess $Zn(OAc)_2$ to afford dizinc complex **1ZZ**.

For preparation of Boc–L-Asp(NH–H_2TTP)–OH (see the right line in Scheme 1), the DCC-coupling of Boc–L-Asp(OH)–OH with amine **9** was tried and gave only the imide **12** as the isolable product. This result might show that intermolecular dehydration of the β-carboxylic acid of Boc–L-Asp(OH)–OH with **9** should be much slower than undesired successive intramolecular dehydration resulting in the formation of succinimide derivatives **12** (Chart 1). Therefore, the protection of the β-carboxyl group, the successive protection of the α-carboxyl group, following with deprotection of the β-position[5] gave the β-carboxyl-free Boc–L-Asp(OH)–NH–*p*-Tolyl, which was coupled with the amine **9** to afford an aspartic acid derivative with a porphyrinyl-substituted side chain. Similarly to the synthesis of **1**, the coupling of acid **8** with amine **10H** gave **2HH** which

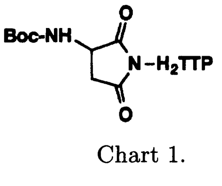

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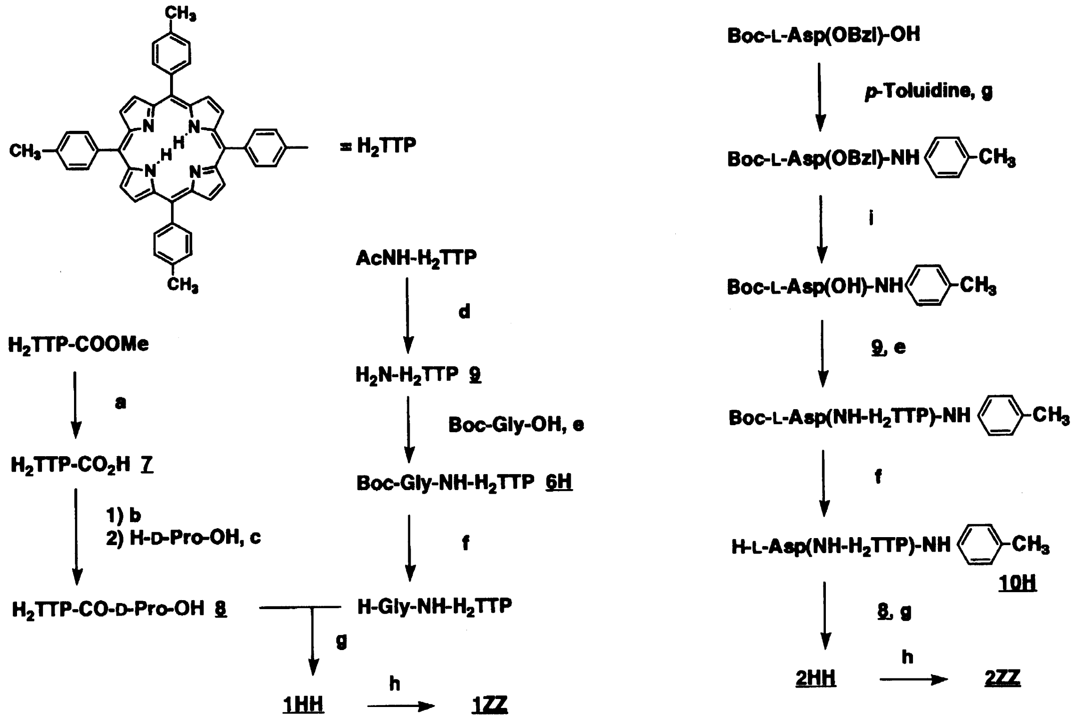
Chart 1.



Scheme 1. Synthesis of β-turned peptide-bridged porphyrin "homo"-dimers (**HH** and **ZZ**). Reagents; **a**: NaOH/pyridine, Δ, **b**: $(COCl)_2/CH_2Cl_2$, **c**: NEt_3/THF, **d**: HCl/MeOH, Δ, **e**: DCC/$CHCl_3$, **f**: 4 M HCl/AcOEt (1M=1 mol dm^{-3}), **g**: DCC–HOBt/$CHCl_3$, **h**: $Zn(OAc)_2$/MeOH–CH_2Cl_2, **i**: H_2, Pd–C/THF.

was followed with the synthesis of **2ZZ**.

Before the final coupling, a monoporphyrinyl component (represented to "monomer", hereafter) was converted to a monozinc complex. Thus, selectively monometallated dimers **HZ** and **ZH** were synthesized by the coupling of a monozinc monomer and a metal-free monomer, as shown in Scheme 2. In the coupling of zinc porphyrin component with metal-free porphyrin component, neither demetallation nor migration of zinc ion could be observed.

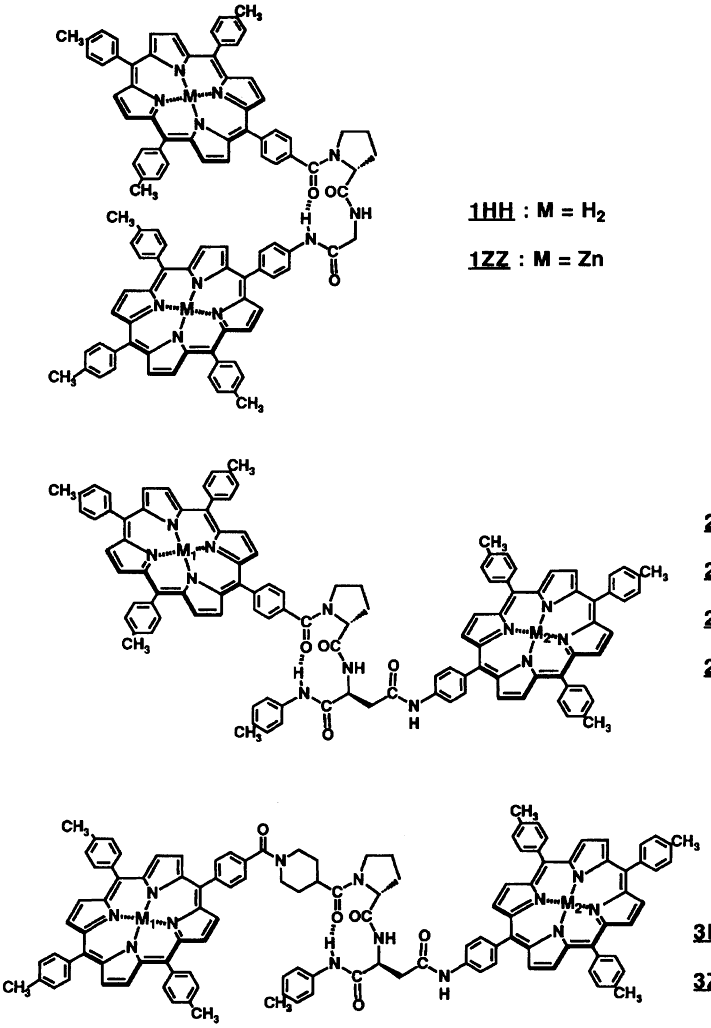
In usual synthetic routes, metallation has been the final step and metal-free, one-side metallated, and both-metallated dimers must be separated. In the systems reported here, the conditions for peptide synthesis in the final step are mild and selectively metallated porphyrin dimers ($M_1 \neq M_2$) are easily prepared without separation.

Intramolecular Interaction and Energy Transfer between Porphyrin Moieties in the Synthetic Dimers. The chemical shifts (δ) characteristic of porphyrin moiety in ^{1}H NMR spectrum of **2HH** (> 7.3 ppm) in $CDCl_3$ were similar to those of equimolar mixture of **4H**+**6H** within 0.04 ppm difference. The δ of **1HH** were broad and complex compared to those of **4H**+**6H**. In zinc complexes, the δ of **2** could be assigned as a summation of the δ of the reference monomers and the δ of **1** were too complex to be assigned clearly. These results indicated that the porphyrin moieties of **1** in $CDCl_3$ interacted in the molecules, the chemical shifts of which were shifted by porphyrin ring current effect[6] and the similar intramolecular interaction in **2** was small.

Table 1 shows the absorption maxima (λ_{max}) of UV-vis spectra and the half-height band-widths (Δ) of the Soret bands in the synthetic dimers and mixtures of reference monomers. The λ_{max} in Q-bands of dimers are identical with those of mixtures of corresponding porphyrin monomers within the errors. Soret bands of the dimers broadened compared to those of the monomer mixtures, which might be due to intramolecular exciton coupling.[6] These results suggest that a porphyrin moiety electronically interacted with the other porphyrin moiety in the molecule of **1** more strongly than in **2** and that both porphyrin moieties of **1** are spatially closer than those of **2**.

^{1}H NMR and UV-vis spectra showed that **1** and **2** should favor the type-II′ β-turn conformation in the solution (see Fig. 1). The porphyrin moieties in **1** spatially interacted each other in the molecule and those in **2** are fairly independent. From their ^{1}H NMR and UV-vis spectra, porphyrin moieties in **3** hardly interacted as well as in **2**.

In the fluorescence spectra under the same conditions as measurement of UV-vis spectra, there was no peak shift in dimers **2HZ**, **2ZH**, **3HZ**, and **3ZH** compared to those of mixtures of the corresponding monomer components **5H**+**6Z**, **5Z**+**6H**, **4H**+**6Z**, and **4Z**+**6H** (see Fig. 2) but their intensities of the dimers were differ-



1HH : M = H_2

1ZZ : M = Zn

2HH : M_1 = H_2, M_2 = H_2

2HZ : M_1 = H_2, M_2 = Zn

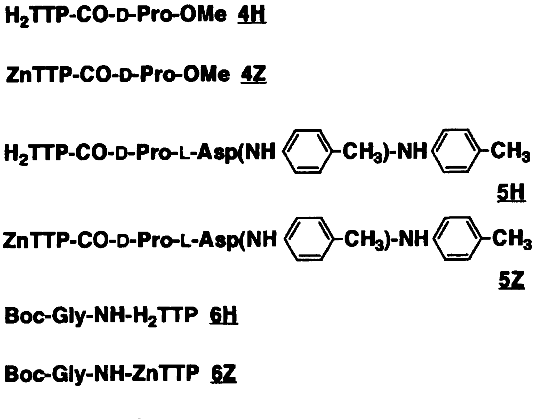
2ZH : M_1 = Zn, M_2 = H_2

2ZZ : M_1 = Zn, M_2 = Zn

3HZ : M_1 = H_2, M_2 = Zn

3ZH : M_1 = Zn, M_2 = H_2

Fig. 1. Synthetic β-turned peptide-bridged porphyrin dimers **1**—**3**.



H_2TTP-CO-D-Pro-OMe **4H**

ZnTTP-CO-D-Pro-OMe **4Z**

H_2TTP-CO-D-Pro-L-Asp(NH-C$_6$H$_4$-CH$_3$)-NH-C$_6$H$_4$-CH$_3$ **5H**

ZnTTP-CO-D-Pro-L-Asp(NH-C$_6$H$_4$-CH$_3$)-NH-C$_6$H$_4$-CH$_3$ **5Z**

Boc-Gly-NH-H_2TTP **6H**

Boc-Gly-NH-ZnTTP **6Z**

Fig. 2. Reference porphyrin monomers **4**—**6**.

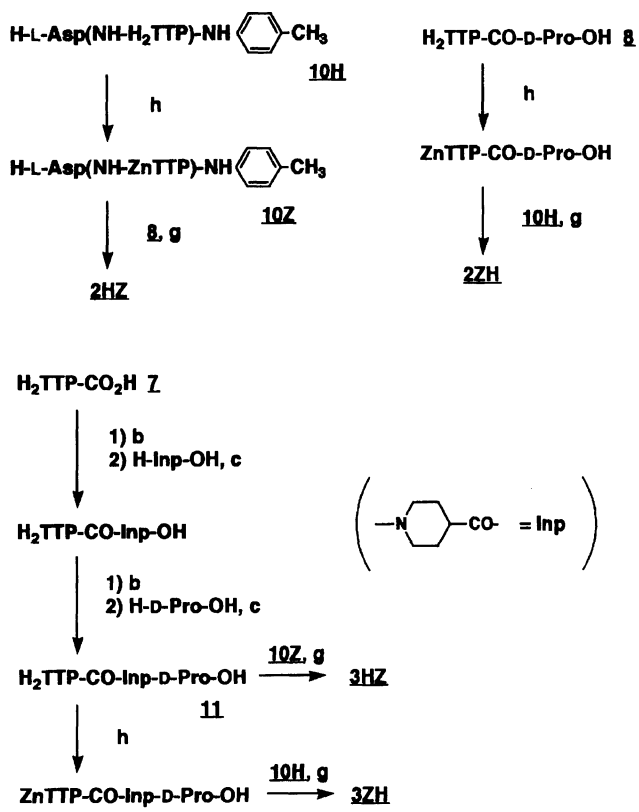
Table 1. UV-vis Absorption Spectral Data of β-Turn Peptide-Bridged Porphyrins in CH_2Cl_2 [a)]

	Soret	Δ[c)]		Q			
1HH (dimer)	418	15.8	(1.26)	517	552	592	647
2HH (dimer)	420	13.1	(1.05)	517	552	592	647
4H+6H[b)]	420	12.5	(1.00)	516	552	592	648
1ZZ (dimer)	419	13.5	(1.26)		550	589	
2ZZ (dimer)	420	12.5	(1.17)		549	588	
4Z+6Z[b)]	420	10.7	(1.00)		549	588	

a) Absorption maxima of Soret and Q-bands and half-height band-width Δ of the Soret band were measured at room temperature, in nm. b) 1 : 1 Mixture of the reference monomers. c) Error was within ±0.1 nm. Parentheses indicated relative values to the reference mixture.

ent from those of the monomer mixtures. It is known that the excited singlet energy state of zinc porphyrin is quenched by the ground state of metal-free porphyrin because of an effective overlap of fluorescence spectra of zinc porphyrins and absorption spectra of metal-free porphyrins.[7)] According to the method reported by Brookfield et al.,[7)] energy-transfer efficiency (*E*) was estimated. The calculated energy-transfer efficiencies are shown in Table 2. There is a trend that energy transfer in **2ZH** and **3ZH** is the same as that in the isomeric **2HZ** and **3HZ**, respectively.

Considering little difference in the spectral overlap



Scheme 2. Synthesis of β-turned peptide-bridged porphyrin "hetero"-dimers (**HZ** and **ZH**). Reagents; **b**: $(COCl)_2/CH_2Cl_2$, **c**: NEt_3/THF, **g**: DCC–HOBt/$CHCl_3$, **h**: $Zn(OAc)_2/MeOH–CH_2Cl_2$.

Table 2. Energy Transfer Efficiencies E in β-Turn Bridged Porphyrin Dimers[a)]

	E/%	Reference mixtures
2HZ	86	**5H**+**6Z**
2ZH	86	**5Z**+**6H**
3HZ	76	**4H**+**6Z**
3ZH	75	**4Z**+**6H**

a) In CH_2Cl_2, at room temperature, ca. 10^{-6} mol dm^{-3}, excitation of 420±5 nm, estimated from fluorescence intensities of zinc porphyrin moiety (600 nm emission peak).

integral[7)] of zinc porphyrin fluorescence with metal-free porphyrin absorption in the isomeric dimers, the agreement of the E-value indicated that intramolecular energy-transfer efficiency in the synthetic unsymmetrical dimers might be dependent upon the distance between the porphyrin moieties in the molecule and independent upon the linked spacer. In the dimers, the singlet-energy should migrate mainly by through-space mechanism.

Table 2 also shows that the efficiencies in **3HZ** and **3ZH** were smaller than those in **2HZ** and **2ZH**. This suppression indicated the center-to-center distance (d) of porphyrin moieties in **3** was longer than that in **2**. Therefore, the d in the dimers decreased in the order, **3**>**2**>**1** and a β-turn conformation fixed intramolecular distance of chromophores, to be available for a rigid spacer as reported before.[4)]

Experimental

Apparatus. All melting points were measured with a Yanagimoto micro melting point apparatus and were uncorrected. Ultraviolet and visible spectra were measured with a Shimadzu UV-3000 spectrometer. Fluorescence spectra were measured with a Shimadzu RF-502A spectrometer. ^{1}H NMR spectra were recorded on a JEOL JMN-FX 400 instrument; chemical shift (δ) are expressed in parts per million relative to Me_4Si (0.00 ppm) or $CHCl_3$ (7.26 ppm) as an internal reference. FAB mass spectra were measured with a JEOL JMS-DX-300 spectrometer; samples were dissolved in $CHCl_3$ and m-nitrobenzyl alcohol was used as the matrix.

Materials. CH_2Cl_2 and DMF were distilled from CaH_2, stored over molecular sieves and used. THF and Et_2O were dried over $CaCl_2$, distilled from benzophenone ketyl, stored over Na wire and used. 5-(4-Methoxycarbonylphenyl)-10,15,20-tri-p-tolylporphyrin,[6,8)] 5-(4-acetamidophenyl)-10,15,20-tri-p-tolylporphyrin,[9)] Boc–Gly–OH,[10)] Boc–D-Pro–OH,[10)] and Boc–L-Asp(OBzl)–OH[10)] were synthesized according to the procedure in the literature. Other reagents and solvents were commercially available and used without purification.

General Remark. All reactions were done under N_2 in the dark. All porphyrins were purified by flash column chromatography on silica gel (Merck Kieselgel 60H 7736 or Wakogel FC-40) with CH_2Cl_2–MeOH as eluants and recrystallization from CH_2Cl_2–MeOH and given as purple solid (mp>300°C) except specially mentioned.

5-(4-Carboxylphenyl)-10,15,20-tri-*p*-tolylporphyrin: $H_2TTP–COOH$ (7). 5-(4-Methoxycarbonylphenyl)-10,15,20-tri-p-tolylporphyrin (168 mg, 0.234 mmol) was added in pyridine and aq 4 mol dm^{-3} NaOH (8 : 1, 90 ml). After refluxed overnight, the reaction mixture was neutralized with $KHSO_4$ and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue gave **7**[2)] (99%); ^{1}H NMR ($CDCl_3$) $\delta=-2.77$ (2H, s), 2.71 (9H, s), 7.56 (6H, d, J=8 Hz), 8.09 (6H, d, J=8 Hz), 8.34 (2H, d, J=8 Hz), 8.48 (2H, d, J=8 Hz), 8.77 (2H, d, J=4 Hz), 8.86 (4H, s), and 8.88 (2H, d, J=4 Hz); MS m/z 701 (MH$^+$).

$H_2TTP–CO–D-Pro–OH$ (8). A dry CH_2Cl_2 (50 ml) solution of **7** (502 mg, 0.717 mmol) and oxalyl dichloride (1 ml) was stirred at room temperture for 6 h and concentrated in vacuo. The residue was dissolved in THF (100 ml) and added dropwise to the ice-cooled DMF (8 ml) solution of D-proline (47 mg, 0.409 mmol) and NEt_3 (0.200 ml). After sitrred overnight, the solution was washed with aq 5% $KHSO_4$ and brine, dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue gave **8**[2)] (91%); ^{1}H NMR ($CDCl_3$) $\delta=-2.78$ (2H, s) 2.11 (1H, m), 2.22 (1H, m), 2.32 (1H, m), 2.68 (1H, m), 2.70 (9H, s), 3.94 (1H, m), 4.01 (1H, m), 5.02 (1H, dd, J=5 and 9 Hz), 7.55 (6H, d, J=8 Hz), 7.99 (2H, d, J=8 Hz), 8.09 (6H, d, J=8 Hz), 8.30 (2H, d, J=8 Hz), 8.78 (2H, br-s), and 8.86 (6H, br-s); MS m/z 798 (MH$^+$).

5-(4-Aminophenyl)-10,15,20-tri-*p*-tolylporphyrin:

H_2N–H_2TTP (9). 5-(4-Acetamidophenyl)-10,15,20-tri-*p*-tolylporphyrin (1.07 g, 1.50 mmol) was added in concd HCl and MeOH (8 : 1, 90 ml). After refluxed overnight, the reaction mixture was neutralized with Na_2CO_3 and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Recrystallization from CH_2Cl_2–MeOH gave **9** (70%); ^{1}H NMR ($CDCl_3$) $\delta=-2.77$ (2H, s), 2.70 (9H, s), 4.03 (2H, s), 7.06 (2H, d, $J=8$ Hz), 7.53 (6H, d, $J=8$ Hz), 7.94 (2H, d, $J=8$ Hz), 8.08 (6H, d, $J=8$ Hz), 8.82 (2H, d, $J=4$ Hz), 8.84 (4H, s), and 8.90 (2H, d, $J=4$ Hz); MS *m*/*z* 672 (MH^+).

Boc–Gly–NH–H_2TTP (6H). Boc–Gly–OH (26.1 mg, 0.149 mmol), **9** (100.0 mg, 0.149 mmol) and DCC (33.6 mg) were dissolved in ice-cooled CH_2Cl_2 (30 ml). After stirred overnight, the solution was concentrated in vacuo and EtOAc was added to the residue. After removal of insoluble dicyclohexylurea by filtration, the solution was washed with aq 5% $KHSO_4$, aq 4% $NaHCO_3$, and brine, dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue gave **6H** (98%); UV (CH_2Cl_2) 420, 516, 552, 592, and 649 nm; ^{1}H NMR ($CDCl_3$) $\delta=-2.78$ (2H, s), 1.57 (9H, s), 2.71 (9H, s), 4.10 (2H, d, $J=6$ Hz), 5.30 (1H, br-s), 7.55 (6H, d, $J=8$ Hz), 7.91 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.18 (2H, d, $J=8$ Hz), 8.46 (1H, br-s), 8.80 (2H, d, $J=5$ Hz), 8.83 (2H, d, $J=5$ Hz), and 8.85 (4H, s); MS *m*/*z* 829 (MH^+). Found: *m*/*z* 829.3897. Calcd for $C_{54}H_{48}N_6O_3\cdot H^+$: MH^+, 829.3866.

H_2TTP–CO–D-Pro–Gly–NH–H_2TTP (1HH). An ice-cooled 4 mol dm^{-3} HCl–AcOEt (20 ml) of **6H** (107 mg, 0.129 mmol) was stirred for 1 h and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with aq 4% $NaHCO_3$, brine and dried over Na_2SO_4 and concentrated in vacuo. Recrystallization from CH_2Cl_2–MeOH gave H–Gly–NH–H_2TTP (99%); ^{1}H NMR ($CDCl_3$) $\delta=-2.77$ (2H, s), 2.70 (9H, s), 3.66 (2H, s), 7.55 (6H, d, $J=8$ Hz), 8.01 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.18 (2H, d, $J=8$ Hz), 8.85 (8H, s), and 9.78 (1H, br-s); MS *m*/*z* 729 (MH^+).

To an ice-cooled $CHCl_3$ (20 ml) solution of **8** (20 mg; 25 μmol), the above amine (18 mg; 25 μmol) and HOBt (3 mg; 25 μmol) was added DCC (10 mg; 33 μmol). After stirred for 5 h, similar work-up as in **6H** gave **1HH** (76%); UV (CH_2Cl_2) 418, 517, 552, 592, and 647 nm; ^{1}H NMR ($CDCl_3$) $\delta=-2.83$ (2H, s), −2.81 (2H, s), 1.96—2.08 (1H, m), 2.17—2.30 (1H, m), 2.25—2.38 (2H, m), 2.47 (6H, s), 2.49 (6H, s), 2.69 (6H, s), 3.93—4.07 (3H, m), 4.45 (1H, m), 4.57 (1H, dd, $J=8$ and 17 Hz), 6.78 (1H, br-s), 7.0—8.3 (32H, m), 8.6—8.9 (16H, m), and 9.28 (1H, s); MS *m*/*z* 1508 (MH^+). Found: *m*/*z* 1508.6584. Calcd for $C_{102}H_{81}N_{11}O_3\cdot H^+$: MH^+, 1508.6601.

ZnTTP–CO–D-Pro–Gly–NH–ZnTTP (1ZZ). To the solution of **1HH** (11.8 mg, 10.3 μmol) in CH_2Cl_2 (10 ml), a MeOH solution (2 ml) saturated by $Zn(OAc)_2\cdot 2H_2O$ was added. The solution was stirred at room temperature for 1 h and then washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue gave **1ZZ** (91%); UV (CH_2Cl_2) 419, 550, and 589 nm; ^{1}H NMR ($CDCl_3$) $\delta=2.0$—2.3 (1H, m), 2.3—2.6 (3H, m), 2.6 (18H, br-s), 2.6—3.0 (2H, m), 3.8—4.0 (1H, m), 3.4—3.5 (1H, m), 3.9—4.0 (1H, m), 6.3 (1H, br-s), 7.51—7.57 (12H, m), 7.71 (2H, br-d), 7.78 (2H, br-d), 7.99 (2H, br-s), 8.10 (4H, d, $J=8$ Hz), 8.13 (4H, d, $J=8$ Hz), 8.36 (2H, br-d), 8.56 (2H, br-d), 8.64 (2H, br-d), 8.77 (2H, br-d), 8.84 (2H, br-d), 8.93 (6H, br-d), 8.98 (6H, br-d), and 9.5 (1H, br-s); MS *m*/*z* 1632 (MH^+, for ^{64}Zn). Found: *m*/*z* 1632.4888. Calcd for $C_{102}H_{77}N_{11}O_3{}^{64}Zn_2\cdot H^+$: MH^+, 1632.4872.

H–L-Asp(NH–H_2TTP)–NH–*p*-Tolyl (10H). Similarly to the synthesis of **1HH**, DCC–HOBt coupling of Boc–L-Asp(OBzl)–OH and *p*-toluidine (H_2N–*p*-Tolyl) and recrystallization from AcOEt–hexane gave the Boc–L-Asp(OBzl)–NH–*p*-Tolyl (98%) as white solid, mp 115—116 °C, ^{1}H NMR ($CDCl_3$) $\delta=1.48$ (9H, s), 2.32 (3H, s), 2.78 (1H, dd, $J=4$ and 17 Hz), 3.08 (1H, dd, $J=4$ and 17 Hz), 4.06 (1H, br-s), 5.13 (1H, d, $J=10$ Hz), 5.15 (1H, d, $J=10$ Hz), 5.80 (1H, br), 7.10 (2H, d, $J=8$ Hz), 7.34 (5H, s), 7.36 (2H, d, $J=8$ Hz), and 7.37 (1H, br-s); MS *m*/*z* 413 (MH^+).

A THF suspension (30 ml) of the above benzyl ester (221 mg, 0.524 mmol) and 10% Pd–C (20 mg) was stirred under H_2. After disappearance of the starting material, the catalyst was removed by filtration and the solvent was removed in vacuo. Recrystallization from AcOEt–hexane gave Boc–L-Asp(OH)–NH–*p*-Tolyl (86%) as white solid, mp 128—133 °C, ^{1}H NMR ($CDCl_3$) $\delta=1.47$ (9H, s), 2.30 (3H, s), 2.79 (1H, dd, $J=4$ and 17Hz), 3.02 (1H, dd, $J=4$ and 17 Hz), 4.64 (1H, br-s), 5.84 (1H, br), 7.09 (2H, d, $J=8$ Hz), 7.36 (2H, d, $J=8$ Hz), and 8.64 (1H, br-s); MS *m*/*z* 323 (MH^+).

Similarly to the synthesis of **6H**, DCC-coupling of the above acid and **9** gave Boc–L-Asp(NH–H_2TTP)–NH–*p*-Tolyl (69%) as purple solid, mp 232—236 °C, ^{1}H NMR ($CDCl_3$) $\delta=-2.79$ (2H, s), 1.54 (9H, s), 2.32 (3H, s), 2.69 (6H, s), 2.70 (3H, s), 2.97 (1H, dd, $J=7$ and 15 Hz), 3.30 (1H, dd, $J=3$ and 15 Hz), 4.80 (1H, br-s), 6.38 (1H, br), 7.21 (2H, d, $J=8$ Hz), 7.49 (2H, d, $J=8$ Hz), 7.53 (4H, d, $J=8$ Hz), 7.55 (2H, d, $J=8$ Hz), 7.89 (2H, d, $J=8$ Hz), 8.07 (4H, d, $J=8$ Hz), 8.09 (2H, d, $J=8$ Hz), 8.16 (2H, d, $J=8$ Hz), 8.36 (1H, br-s), 8.82 (2H, d, $J=4$ Hz), 8.84 (2H, d, $J=4$ Hz), 8.85 (4H, s), and 9.09 (1H, br-s); MS *m*/*z* 976 (MH^+).

Similarly to the synthesis of H–Gly–NH–H_2TTP, deprotection and neutralization of the above amide gave **10H** (90%); ^{1}H NMR ($CDCl_3$) $\delta=-2.78$ (2H, s), 2.33 (3H, s), 2.70 (9H, s), 3.02—3.15 (2H, m), 4.07 (1H, br-s), 7.18 (2H, d, $J=8$ Hz), 7.54 (8H, m), 7.89 (2H, d, $J=8$ Hz), 8.08 (6H, d, $J=8$ Hz), 8.15 (2H, d, $J=8$ Hz), 8.58 (1H, br-s), 8.85 (8H, s), and 9.64 (1H, br-s); MS *m*/*z* 876 (MH^+).

H_2TTP–CO–D-Pro–L-Asp(NH–H_2TTP)–NH–*p*-Tolyl (2HH). Similarly to the synthesis of **1HH**, DCC–HOBt coupling of **8** and **10H** gave **2HH** (66%); UV (CH_2Cl_2) 420, 517, 552, 592, and 647 nm; ^{1}H NMR ($CDCl_3$) $\delta=-2.78$ (4H, s), 1.96—2.08 (1H, m), 2.25—2.38 (3H, m), 2.27 (3H, s), 2.68 (6H, s), 2.69 (6H, s), 2.70 (6H, s), 3.01 (1H, d, $J=16$ Hz), 3.66 (1H, d, $J=16$ Hz), 4.03—4.09 (1H, m), 4.11—4.17 (1H, m), 4.76 (1H, m), 5.30 (1H, br), 7.17 (2H, d, $J=8$ Hz), 7.50—7.56 (12H, m), 7.83 (2H, d, $J=8$ Hz), 7.93 (2H, d, $J=8$ Hz), 8.03 (2H, d, $J=8$ Hz), 8.07 (8H, d, $J=8$ Hz), 8.09 (4H, d, $J=8$ Hz), 8.18 (2H, d, $J=8$ Hz), 8.19 (1H, br), 8.29 (1H, br), 8.30 (2H, d, $J=8$ Hz), 8.84 (16H, m), and 9.74 (1H, s); MS *m*/*z* 1655 (MH^+). Found: *m*/*z* 1655.7272. Calcd for $C_{111}H_{90}N_{12}O_4\cdot H^+$: MH^+, 1655.7286.

ZnTTP–CO–D-Pro–L-Asp(NH–ZnTTP)–NH–*p*-Tolyl (2ZZ). Similarly to the synthesis of **1ZZ**, metallation of **2HH** gave **2ZZ** (58%); UV (CH_2Cl_2) 420, 549, and 588 nm; ^{1}H NMR ($CDCl_3$) $\delta=2.08$—2.17 (1H, m), 2.24—2.48 (3H, m), 2.17 (3H, s), 2.68 (6H, s), 2.70 (6H, s), 2.71 (6H, s), 2.94 (1H, d, $J=16$ Hz), 3.46 (1H, d, $J=16$ Hz), 4.02—4.15 (2H, m), 4.61—4.68 (1H, m), 5.13 (1H, br), 7.15

(2H, d, $J=8$ Hz), 7.50 (13H, m), 7.78 (2H, d, $J=8$ Hz), 7.90 (2H, d, $J=8$ Hz), 8.01 (2H, d, $J=8$ Hz), 8.08 (12H, m), 8.17 (2H, d, $J=8$ Hz), 8.22 (1H, br-s), 8.29 (2H, d, $J=8$ Hz), 8.90 (2H, d, $J=4$ Hz), 8.93 (4H, s), 8.94 (4H, s), 8.95 (4H, s), 8.97 (2H, d, $J=4$ Hz), and 9.53 (1H, br-s); MS m/z 1779 (MH$^+$, for ^{64}Zn). Found: m/z 1779.5601. Calcd for $C_{111}H_{86}N_{12}O_4{}^{64}Zn_2{\cdot}H^+$: MH$^+$, 1779.5556.

H–L-Asp(NH–ZnTTP)–NH–*p*-Tolyl (10Z). Similarly to the synthesis of **1ZZ**, metallation of **10H** gave **10Z** (100%); ^{1}H NMR (CDCl$_3$) $\delta=2.31$ (3H, s), 2.70 (9H, s), 3.05—3.20 (2H, m), 4.05 (1H, br-s), 6.42 (2H, br-s), 7.19 (2H, d, $J=8$ Hz), 7.57 (8H, d, $J=8$ Hz), 7.90 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.52 (1H, br-s), 8.95 (8H, s), and 9.12 (1H, br-s); MS m/z 938 (MH$^+$, for ^{64}Zn).

H_2TTP–CO–D-Pro–L-Asp(NH–ZnTTP)–NH–*p*-Tolyl (2HZ). Similarly to the synthesis of **1HH**, DCC–HOBt coupling of **8** and **10Z** gave **2HZ** (66%); UV (CH$_2$Cl$_2$) 421, 516, 550, 590, and 647 nm; ^{1}H NMR (CDCl$_3$) $\delta=-2.79$ (2H, s), 2.10—2.20 (1H, m), 2.28 (3H, s), 2.30—2.55 (3H, m), 2.68 (6H, s), 2.69 (6H, s), 2.71 (6H, s), 3.00 (1H, d, $J=16$ Hz), 3.60 (1H, d, $J=16$ Hz), 4.15 (1H, m), 4.25 (1H, m), 4.67 (1H, m), 5.28 (1H, br), 7.15 (2H, d, $J=8$ Hz), 7.53 (12H, m), 7.82 (2H, d, $J=8$ Hz), 7.92 (2H, d, $J=8$ Hz), 8.08 (15H, m), 8.18 (2H, d, $J=8$ Hz), 8.26 (1H, br-s), 8.29 (2H, d, $J=8$ Hz), 8.80 (2H, d, $J=4$ Hz), 8.85 (4H, s), 8.87 (2H, d, $J=4$ Hz), 8.94 (2H, d, $J=4$ Hz), 8.95 (4H, s), 8.95 (2H, d, $J=4$ Hz), and 9.63 (1H, br-s); MS m/z 1717 (MH$^+$, for ^{64}Zn). Found: m/z 1717.6380. Calcd for $C_{111}H_{88}N_{12}O_4{}^{64}Zn{\cdot}H^+$: MH$^+$, 1717.6421.

ZnTTP–CO–D-Pro–L-Asp(NH–H_2TTP)–NH–*p*-Tolyl (2ZH). Similarly to the synthesis of **1ZZ**, metallation of **8** gave ZnTTP–CO–D-Pro–OH (88%); ^{1}H NMR (CDCl$_3$) $\delta=2.10$ (1H, m), 2.22 (1H, m), 2.33 (1H, m), 2.66 (1H, m), 2.69 (9H, s), 3.93 (1H, m), 4.02 (1H, m), 4.94 (1H, br-s), 7.55 (6H, d, $J=8$ Hz), 7.97 (2H, d, $J=7$ Hz), 8.09 (6H, d, $J=7$ Hz), 8.30 (2H, d, $J=7$ Hz), 8.87 (2H, d, $J=4$ Hz), and 8.96 (6H, br-s); MS m/z 860 (MH$^+$, for ^{64}Zn).

Similarly to the synthesis of **1HH**, DCC–HOBt coupling of the above acid and **10H** gave **2ZH** (55%); UV (CH$_2$Cl$_2$) 420, 517, 550, 590, and 649 nm; ^{1}H NMR (CDCl$_3$) $\delta=-2.80$ (2H, s), 2.09—2.18 (1H, m), 2.28 (3H, s), 2.37—2.52 (3H, m), 2.67 (3H, s), 2.69 (6H, s), 2.70 (6H, s), 2.71 (3H, s), 2.92 (1H, d, $J=16$ Hz), 3.51 (1H, d, $J=16$ Hz), 4.10 (2H, m), 4.60 (1H, m), 5.18 (1H, br), 7.15 (2H, d, $J=8$ Hz), 7.49 (12H, m), 7.82 (2H, d, $J=8$ Hz), 7.92 (2H, d, $J=8$ Hz), 8.07 (15H, m), 8.17 (2H, d, $J=8$ Hz), 8.23 (1H, br-s), 8.29 (2H, d, $J=8$ Hz), 8.82 (4H, br-s), 8.83 (4H, br-s), 8.90 (2H, d, $J=5$ Hz), 8.96 (4H, s), 8.97 (2H, d, $J=5$ Hz), and 9.65 (1H, br-s); MS m/z 1717 (MH$^+$, for ^{64}Zn). Found: m/z 1717.6423. Calcd for $C_{111}H_{88}N_{12}O_4{}^{64}Zn{\cdot}H^+$: MH$^+$, 1717.6421.

H_2TTP–CO–Inp–D-Pro–OH (11). Similarly to the synthesis of **8**, coupling of **7** and 4-piperidinecarboxylic acid (isonipecotic acid; H–Inp–OH) gave H_2TTP–CO–Inp–OH (65%); ^{1}H NMR (CDCl$_3$) $\delta=-2.78$ (2H, s), 1.93—2.07 (2H, m), 2.12—2.28 (2H, m), 2.70 (9H, s), 2.72—2.86 (2H, m), 3.22—3.34 (2H, m), 4.64—4.78 (1H, m), 7.55 (6H, d, $J=8$ Hz), 7.80 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.27 (2H, d, $J=8$ Hz), 8.81 (2H, d, $J=5$ Hz), 8.86 (4H, s), and 8.88 (2H, d, $J=5$ Hz).

Similarly to the synthesis of **8**, coupling of the above acid and D-proline gave **11** (65%); ^{1}H NMR (CDCl$_3$) $\delta=-2.78$ (2H, s), 1.93—2.20 (5H, m), 2.30—2.56 (3H, m), 2.71 (9H, s), 2.80—2.90 (2H, m), 3.16—3.24 (2H, m), 3.62—3.78 (2H, m), 4.16—4.42 (1H, m), 4.94—5.00 (1H, m), 7.56 (6H, d, $J=8$ Hz), 7.81 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.27 (2H, d, $J=8$ Hz), 8.71 (2H, d, $J=5$ Hz), 8.86 (4H, s), and 8.88 (2H, d, $J=5$ Hz).

H_2TTP–CO–Inp–D-Pro–L-Asp(NH–ZnTTP)–NH–*p*-Tolyl (3HZ). Similarly to the synthesis of **1HH**, DCC–HOBt coupling of **11** and **10Z** gave **3HZ** (57%); UV (CH$_2$Cl$_2$) 420, 516, 549, 590, and 649 nm; ^{1}H NMR (CDCl$_3$) $\delta=-2.78$ (2H, s), 1.88—2.38 (8H, m), 2.17 (3H, s), 2.57 (1H, dd, $J=6$ and 14 Hz), 2.65 (3H, s), 2.69 (3H, s), 2.70 (6H, s), 2.71 (6H, s), 2.80—2.88 (2H, m), 3.05—3.38 (1H, m), 3.48 (1H, dd, $J=4$ and 14 Hz), 3.72—3.87 (2H, m), 4.08—4.33 (1H, m), 4.34 (1H, t, $J=7$ Hz), 4.28—4.43 (1H, m), 5.08 (1H, m), 7.14 (2H, d, $J=8$ Hz), 7.46 (2H, d, $J=8$ Hz), 7.54 (6H, d, $J=8$ Hz), 7.56 (4H, d, $J=8$ Hz), 7.73 (2H, m), 7.76 (2H, d, $J=8$ Hz), 7.83 (2H, d, $J=8$ Hz), 7.91 (1H, d, $J=9$ Hz), 8.03 (4H, d, $J=8$ Hz), 8.08 (6H, d, $J=8$ Hz), 8.09 (2H, d, $J=8$ Hz), 8.10 (1H, s), 8.13 (2H, d, $J=8$ Hz), 8.22 (2H, d, $J=8$ Hz), 8.79—8.93 (16H, m), and 9.47 (1H, br-s); MS m/z 1827 (M$^+$, for ^{64}Zn).

ZnTTP–CO–Inp–D-Pro–L-Asp(NH–H_2TTP)–NH–*p*-Tolyl (3ZH). Similarly to the synthesis of **1ZZ**, metallation of **11** gave ZnTTP–Inp–D-Pro–OH (94%).

Similarly to the synthesis of **1HH**, DCC–HOBt coupling of the above acid and **10H** gave **3ZH** (50%); UV (CH$_2$Cl$_2$) 420, 516, 550, 590, and 648 nm; ^{1}H NMR (CDCl$_3$) $\delta=-2.81$ (2H, s), 1.92—2.38 (8H, m), 2.17 (3H, s), 2.62 (1H, m), 2.65 (3H, s), 2.69 (9H, s), 2.71 (3H, s), 2.86 (2H, m), 3.1—3.3 (1H, m), 3.47 (1H, m), 3.77 (2H, m), 3.82 (1H, m), 4.37 (1H, t, $J=7$ Hz), 5.07 (1H, m), 5.12 (1H, m), 7.15 (2H, d, $J=8$ Hz), 7.49 (4H, d, $J=8$ Hz), 7.52—7.57 (10H, m), 7.77 (2H, d, $J=8$ Hz), 7.83 (2H, d, $J=8$ Hz), 7.90 (1H, d, $J=9$ Hz), 8.04 (4H, d, $J=8$ Hz), 8.10 (9H, m), 8.13 (2H, d, $J=8$ Hz), 8.24 (2H, d, $J=8$ Hz), 8.78—8.82 (8H, m), 8.90 (2H, d, $J=4$ Hz), 8.97 (4H, s), 8.98 (2H, d, $J=4$ Hz), and 9.47 (1H, s); MS m/z 1828 (MH$^+$, for ^{64}Zn).

H_2TTP–CO–D-Pro–OMe (4H). To the THF (30 ml) solution of **8** (29.5 mg, 36.9 μmol), Et$_2$O solution of CH$_2$N$_2$ (excess) was added. The solution was stirred at room temperature for 6 h and then concentrated in vacuo. Purification of the residue gave **4H** (49%); UV (CH$_2$Cl$_2$) 419, 516, 551, 591, and 647 nm; ^{1}H NMR (CDCl$_3$) $\delta=-2.78$ (2H, s), 2.09—2.25 (3H, m), 2.48 (1H, m), 2.71 (9H, s), 3.77 (3H, s), 3.93 (1H, m), 4.04 (1H, m), 4.87 (1H, dd, $J=6$ and 8 Hz), 7.55 (6H, d, $J=8$ Hz), 7.99 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.27 (2H, d, $J=8$ Hz), 8.80 (2H, d, $J=5$ Hz), 8.86 (4H, s), and 8.88 (2H, d, $J=4$ Hz); MS m/z 812 (MH$^+$).

ZnTTP–CO–D-Pro–OMe (4Z). Similarly to the synthesis of **1ZZ**, metallation of **4H** gave **4Z** (59%); UV (CH$_2$Cl$_2$) 420, 549, and 587 nm; ^{1}H NMR (CDCl$_3$) $\delta=$ 2.09—2.55 (3H, m), 2.48 (1H, m), 2.71 (9H, s), 3.77 (3H, s), 3.95 (1H, m), 4.05 (1H, m), 4.86 (1H, dd, $J=6$ and 8 Hz), 7.56 (6H, d, $J=8$ Hz), 8.00 (2H, d, $J=8$ Hz), 8.09 (6H, d, $J=8$ Hz), 8.27 (2H, d, $J=8$ Hz), 8.83 (2H, d, $J=4$ Hz), 8.88 (4H, s), and 8.91 (2H, d, $J=4$ Hz); MS m/z 874 (MH$^+$, for ^{64}Zn). Found: m/z 874.2743. Calcd for $C_{54}H_{43}N_5O_3{}^{64}Zn{\cdot}H^+$: MH$^+$, 874.2736.

H_2TTP–CO–D-Pro–L-Asp(NH–*p*-Tolyl)–NH–*p*-Tolyl (5H). Similarly to the synthesis of **6H**, DCC-coupling of Boc–L-Asp–OH and *p*-toluidine gave Boc–

L-Asp(NH-*p*-Tolyl)-NH-*p*-Tolyl (67%), mp 185—190 °C, ^{1}H NMR (CDCl$_3$) δ=1.47 (9H, s), 2.30 (6H, s), 2.78 (1H, br-d, J=13 Hz), 3.19 (1H, br-d, J=13 Hz), 4.65 (1H, br-s), 6.27 (1H, br-s), 7.10 (4H, d, J=8 Hz), 7.34 (2H, d, J=8 Hz), 7.38 (2H, d, J=8 Hz), 7.89 (1H, br-s), and 9.01 (1H, br-s).

Similarly to the synthesis of H-Gly-NH-H$_2$TTP, deprotection and neutralization of the above amide gave H-L-Asp(NH-*p*-Tolyl)-NH-*p*-Tolyl (63%); mp 227—237 °C.

Similarly to the synthesis of **1HH**, DCC-HOBt coupling of **8** and the above amine gave **5H** (56%); UV (CH$_2$Cl$_2$) 419, 516, 552, 590, and 646 nm; ^{1}H NMR (CDCl$_3$) δ=−2.78 (2H, s), 2.00—2.12 (1H, m), 2.25 (3H, s), 2.27 (3H, s), 2.21—2.45 (3H, m), 2.71 (9H, s), 2.83 (1H, dd, J=6 and 15 Hz), 3.38 (1H, dd, J=4 and 15 Hz), 4.06 (1H, m), 4.09 (1H, m), 4.69 (1H, t, J=8 Hz), 5.17 (1H, m), 7.10 (4H, d, J=8 Hz), 7.37 (2H, d, J=8 Hz), 7.58 (6H, d, J=8 Hz), 7.73 (2H, d, J=8 Hz), 7.83 (1H, s), 8.03 (2H, d, J=8 Hz), 8.10 (6H, d, J=8 Hz), 8.17 (1H, d, J=8 Hz), 8.27 (2H, d, J=8 Hz), 8.79 (2H, d, J=4 Hz), 8.87 (4H, s), 8.88 (2H, d, J=4 Hz), and 9.55 (1H, s); MS m/z 1091 (MH$^+$).

ZnTTP-CO-D-Pro-L-Asp(NH-*p*-Tolyl)-NH-*p*-Tolyl (5Z). Similarly to the synthesis of **1ZZ**, metallation of **5H** gave **5Z** (79%); UV (CH$_2$Cl$_2$) 422, 550, and 590 nm; ^{1}H NMR (CDCl$_3$) δ=2.07 (1H, m), 2.25 (3H, s), 2.26 (1H, m), 2.28 (3H, s), 2.37 (2H, m), 2.71 (9H, s), 2.72 (1H, dd, J=5 and 14 Hz), 3.31 (1H, dd, J=4 and 14 Hz), 4.04 (2H, m), 4.62 (1H, m), 5.03 (1H, br), 7.09 (2H, d, J=8 Hz), 7.10 (2H, d, J=8 Hz), 7.36 (2H, d, J=8 Hz), 7.50 (6H, d, J=8 Hz), 7.62 (1H, s), 7.69 (2H, d, J=8 Hz), 7.99 (2H, d, J=8 Hz), 8.05 (1H, d, J=9 Hz), 8.09 (6H, d, J=8 Hz), 8.22 (2H, d, J=8 Hz), 8.89 (2H, d, J=4 Hz), 8.97 (4H, s), 8.98 (2H, d, J=4 Hz), and 9.47 (1H, s); MS m/z 1152 (MH$^+$, for ^{64}Zn).

Boc-Gly-NH-ZnTTP (6Z). Similarly to the synthesis of **1ZZ**, metallation of **6H** gave **6Z** (100%); UV (CH$_2$Cl$_2$) 421, 549, and 588 nm; ^{1}H NMR (CDCl$_3$) δ=1.56 (9H, s), 2.70 (9H, s), 3.55 (2H, s), 4.28 (1H, br-s), 7.58 (6H, d, J=8 Hz), 7.93 (2H, d, J=8 Hz), 8.10 (6H, d, J=8 Hz), 8.20 (2H, d, J=8 Hz), 8.95 (8H, s), and 9.88 (1H, br-s); MS m/z 891 (MH$^+$, for ^{64}Zn). Found: m/z 891.3004. Calcd for $C_{54}H_{47}N_6O_3{}^{64}Zn{\cdot}H^+$: MH$^+$, 891.3001.

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