

## Synthesis of Covalently Linked Porphyrin Dimers and Trimers (1)

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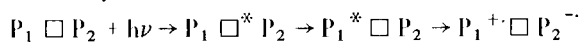
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The synthesis of covalently linked porphyrin dimers and trimers is described. Mono- and dihydroxy porphyrins were synthesized by transesterifying 5,10,15,20-tetra(4-carbomethoxyphenyl)porphyrin with ethylene glycol. The mixture of transesterified porphyrins were separated by preparative thin layer chromatography. Metal derivatives were made of the mono- and dihydroxy porphyrins and these were reacted with the acid chloride of a monocarboxy porphyrin to yield hybrid dimers and trimers containing one metalloporphyrin and either one or two free base porphyrins. The structures and purity of the dimers and trimers were established by measuring the absorbance spectra, nmr spectra, and molecular weight by gel permeation chromatography.

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As progress has been made in identifying the molecules that are responsible for the primary photochemistry in photosynthesis, it has become timely to construct models of the *in vivo* system. Appropriate models are essential to our complete understanding of the mechanism of trapping of the absorbed light energy. When it becomes possible to reproduce in model systems the high efficiency for converting light energy into chemical potential that is exhibited by the *in vivo* system, it may then be possible to construct solar cells of high efficiency and possibly at low cost.

The primary events in photosynthesis may be viewed schematically as:



where  $P_1$  represents the primary electron donor (the first stable species which has lost an electron after the absorption of a quantum of light energy),  $P_2$  is the primary electron acceptor (the first stable species which has gained an electron after the absorption of a quantum of light energy), and the box represents the antenna pigments, protein and whatever other material is necessary for a functioning phototrap. The part of the primary photochemistry which is best understood is the primary electron donor unit. In bacterial photosynthesis, which is the best characterized of all photosynthetic systems,  $P_1$  is a bacteriochlorophyll aggregate which consists of four separate but interacting molecules specifically bound by a protein (3-6). When this aggregate is excited, and an electron is subse-

quently lost, the cation radical thus formed is apparently shared equally over at least two of the bacteriochlorophyll molecules (7-10). This spin delocalization is probably important in both stabilizing the oxidized species and also in providing for the secondary oxidation of cytochrome  $c_2$  at some distance from the location of the reduced primary species, perhaps on the opposite side of the membrane.

Thus,  $P_1$  receives excitation energy from the antenna complex and initiates the photochemistry from its excited state. Progress in constructing appropriate models for this primary electron donor unit has been slow. Aside from an anhydrous chlorophyll *a* dimer (11), it has not been possible to prepare well defined aggregates of chlorophyll in solution.

There are other biological systems that also function through multiple porphyrin centers. For example, it is assumed that electron transport reactions from one heme protein (e.g., cytochrome *c*) to another (e.g., cytochrome oxidase) involve a close approach of iron porphyrin centers either through a shared ligand or of the porphyrin edges. Clearly, model complexes in which two iron porphyrins are covalently linked at selected distances and orientations from each other could be extremely useful in understanding these electron transport reactions.

Schwartz *et al.*, (12) were the first to report synthesis of covalently linked porphyrins and studied excitation energy transfer between the two centers. In unreported work, we synthesized a porphyrin dimer using an amide

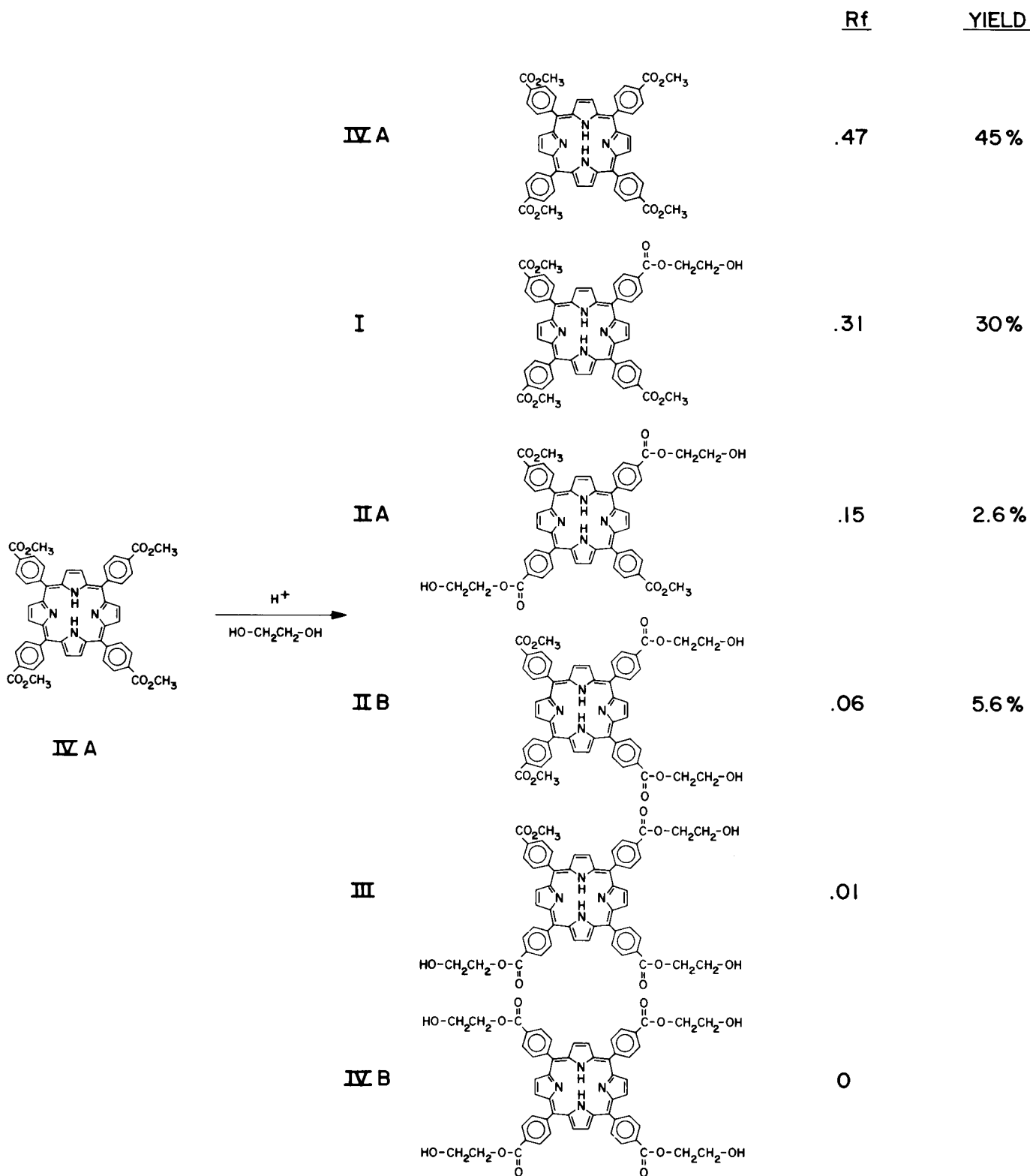


Figure 1. Transesterification of 5,10,15,20-tetra(4-carbomethoxyphenyl)porphyrin (compound IVA) showing Rf's in chloroform and yields. Assignment of Rf's to structures IIA and IIB were arbitrarily made on the basis that we might expect the compound with adjacent diol groups (IIB) to be more polar and therefore migrate more slowly in this system. Also, consistent with this tentative assignment is the yield of IIB which we would expect to be about twice that of IIA on a statistical basis assuming the diol is formed from the mono-substituted analogue.

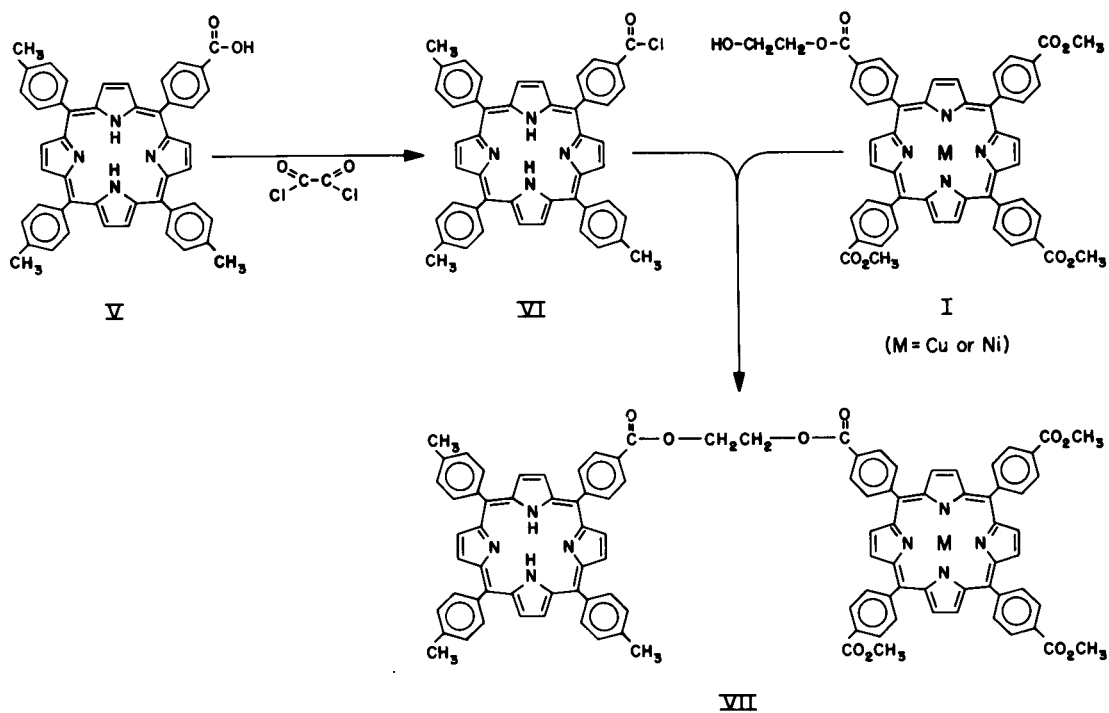


Figure 2. Pathway used for dimer formation.

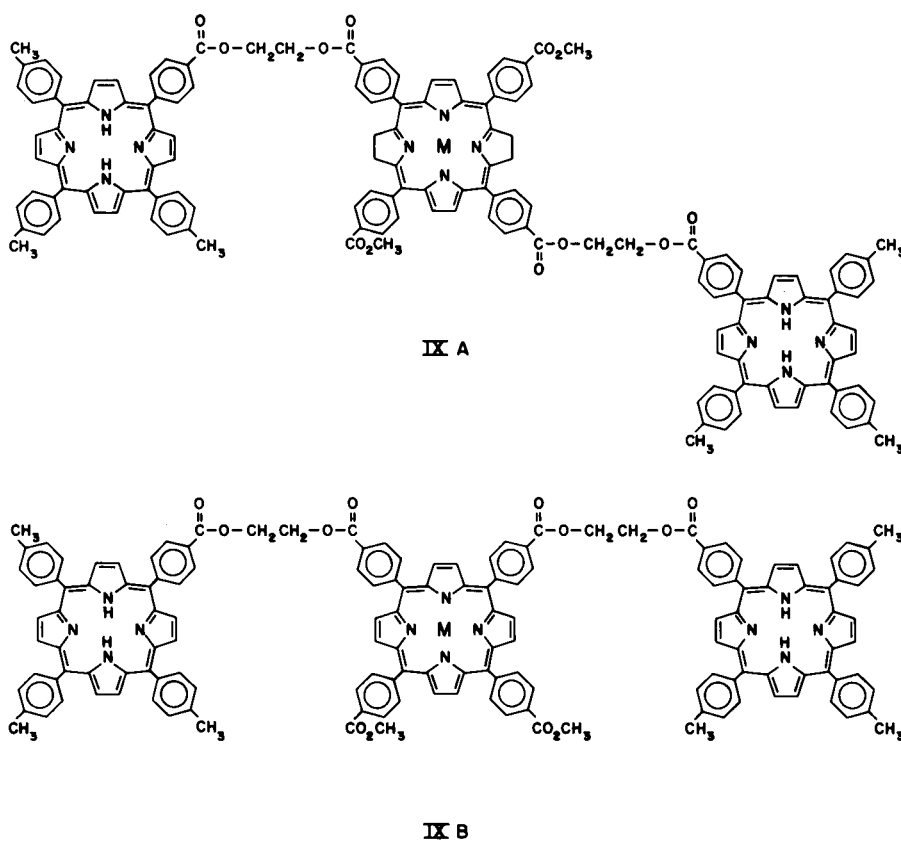


Figure 3. Possible trimer isomers.



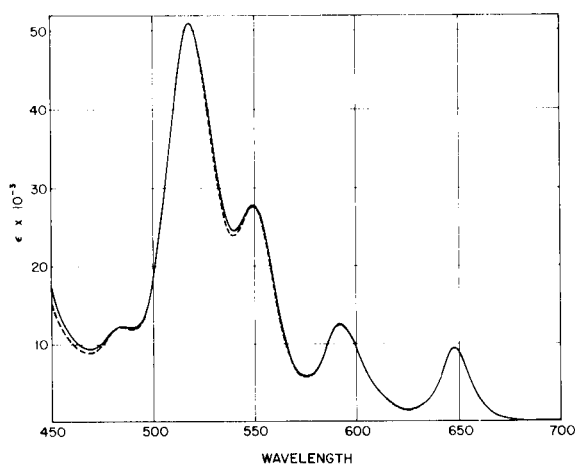


Figure 10. Computer simulation (dashed line) of the trimer absorbance spectrum of compound IX ( $M = Ni$ ) in benzene. Solid line, normalized experimental absorbance spectrum.

linkage as had Schwartz *et al.*, but found the complex was very difficult to solubilize and, therefore, purify or characterize. In our current work involving the construction of model systems we have synthesized some ester linked porphyrin dimers and trimers. The synthetic pathway used was designed to maximize solubility and ease of identification of products. From our previous work (13-14), we knew that the addition of carbomethoxy groups to the tetraarylporphyrin skeleton increased solubility significantly while at the same time decreasing the  $R_f$ 's in the appropriate chromatographic system. Also, the different chemical shifts of the tolyl methyls and carbomethoxy methyls provides a convenient way of determining stoichiometry *via* nmr. Of further usefulness to characterizing the dimer or trimer molecules, the synthesis of hybrid dimers and trimers containing one metalloporphyrin and either one or two free base porphyrins allowed tentative identification *via* visible spectroscopy with a minimal amount of sample and time.

#### EXPERIMENTAL

##### Materials and Methods.

All reactions and chromatography were carried out in the dark. Reagent grade chloroform was used for all chromatography and extractions. The benzene used in the reactions was distilled from phosphorus pentoxide and stored over sodium wire. Pyridine was distilled from *p*-toluenesulfonyl chloride and stored in the dark. The methanol used was reagent grade.

The silica gel used for the dry column chromatography was "Silica Gel Woelm for Dry-Column Chromatography" # 04526 obtained from ICN Pharmaceuticals. The plates used for preparative thin layer chromatography were Quantum 1000  $\mu$  PI, QF silica gel plates.

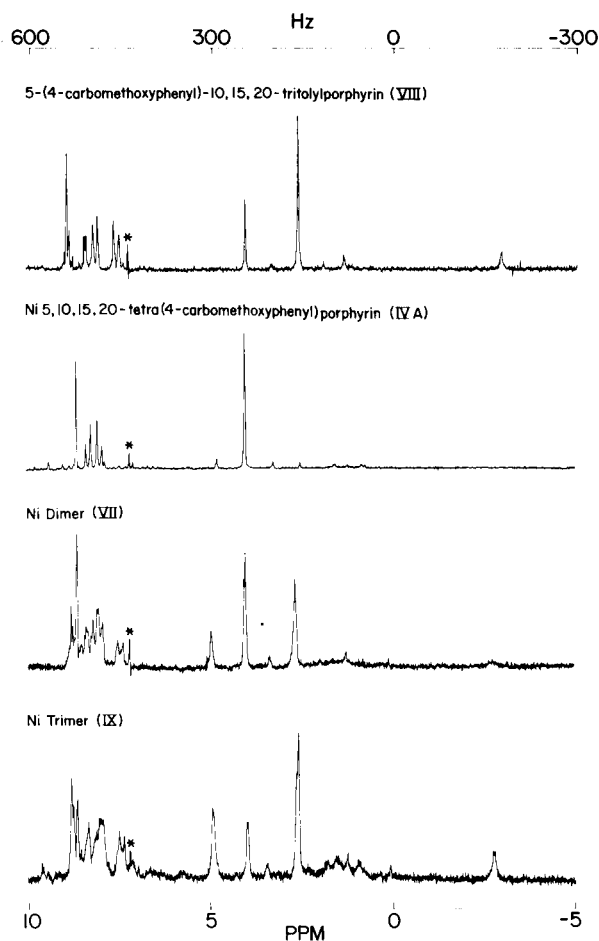


Figure 11. Nmr data for compounds:

VII.  $\Delta = 8.91-8.68$  (m, 8H,  $\beta$ -pyrrole); 8.37 (AB quartet, 4H, carbomethoxyphenyl-2,3,5,6-protons); 8.08 (d, 6H,  $J = 8.0$  Hz, tolyl-2,6-protons); 7.52 (d, 6H,  $J = 8.0$  Hz, tolyl-3,5-protons); 4.05 (s, 3H, methyl ester); 2.63 (s, 9H, methyl); -2.80 (s, 2H, pyrrole N-H).

IV A.  $\Delta = 8.65$  (s, 8H,  $\beta$ -pyrrole); 8.17 (AB quartet, 16H, carbomethoxyphenyl-2,3,5,6-protons); 4.03 (s, 12H, methyl esters).

VII.  $\Delta = 8.88-7.37$  (m, 48H,  $\beta$ -pyrrole and phenyl protons); 4.93 (s, 4H, ethylene); 4.02 (s, 9H, methyl esters); 2.63 (s, 9H, methyls).

IX.  $\Delta = 8.88-7.37$  (m, 72H,  $\beta$ -pyrrole and phenyl protons); 4.93 (s, 8H, ethylenes); 3.98 (s, 6H, methyl esters); 2.63 (s, 18H, methyls).

Absorbance spectra were taken using a Cary 14 R Recording Spectrophotometer. All spectra taken utilized reagent grade benzene as solvent. Nmr data were obtained using a Varian T-60 Spectrometer and chloroform-D 99.8% as solvent. The chloroform-H peak was used as an internal standard, assigned a  $\Delta$  value of 7.2, and is marked with an asterisk in Figure 11.

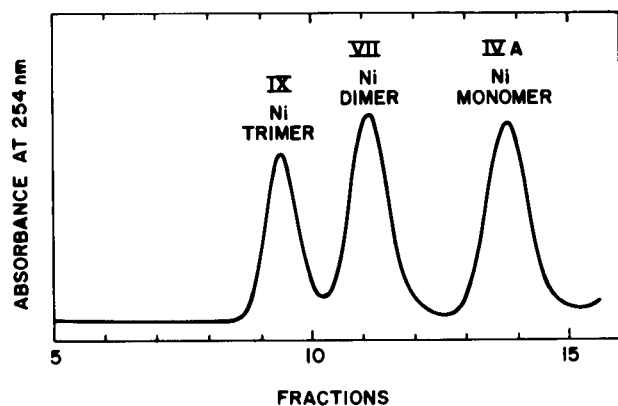


Figure 12. Chromatographic elution pattern for the Ni trimer, Ni dimer and Ni monomer.

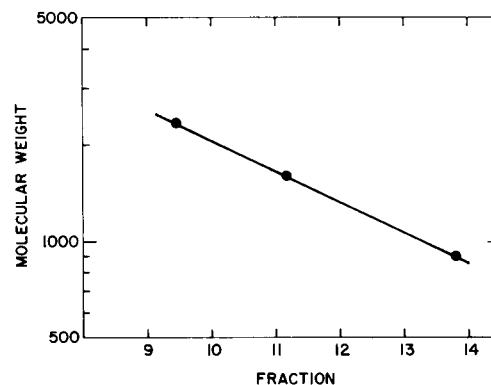


Figure 13. Least squares plot of log molecular weight vs. elution volume for the Ni trimer, Ni dimer and Ni monomer for the gel permeation separation shown in Figure 12.

Table 1

Extinction Coefficients and Relative Intensities of Absorbance Bands

Compound								
IVA (M = Ni)	$\lambda$		528					417
(Ni Monomer)	$\epsilon$		19.9					268
IVA (M = Cu)	$\lambda$		541					419
(Cu Monomer)	$\epsilon$		23.0					472
VIII	$\lambda$	648	592	551	515	483		421
(Free base Monomer)	$\epsilon$	4.66	5.78	10.3	19.4	4.02		481
Relative Intensity	R.I.	1	1.24	2.21	4.16	0.86		103
VII (M = Ni)	$\lambda$	649	592	522				421
(Ni Dimer)	$\epsilon$	4.75	6.30	32.2				635
Computer Simulation	$\lambda$	648	592	520				420
	$\epsilon$	4.82	6.54	32.7				729
VII (M = Cu)	$\lambda$	650	590	543	518	483		420
(Cu Dimer)	$\epsilon$	5.39	6.73	28.8	23.3	7.66		855
Computer Simulation	$\lambda$	648	591	543	517	482		420
	$\epsilon$	4.73	6.82	29.3	23.7	7.64		935
IX (M = Ni)	$\lambda$	648	592	549	518	485		
(Ni Trimer)	R.I.	1	1.34	2.98	5.50	1.31		
Computer Simulation	$\lambda$	648	592	550	518	484		421
	$\epsilon$	9.48	12.4	27.5	51.1	12.1		1208
	R.I.	1	1.31	2.90	5.39	1.28		127.4
IX (M = Cu)	$\lambda$	649	590	546	516	485		
(Cu Trimer)	R.I.	1	1.30	3.58	4.31	1.19		
Computer Simulation	$\lambda$	648	591	547	517	483		420
	$\epsilon$	9.39	12.6	37.4	43.0	11.6		1412
	R.I.	1	1.34	3.98	4.58	1.24		150.4

Gel permeation chromatography was carried out in THF solutions using a 0.8 x 110 cm column of Bio Beads SX-1. Bands were detected by continuously monitoring the ultraviolet absorption at 254 nm.

Analyses were performed at Micro-Tech Laboratories, Inc.,

Skokie, Illinois, Trace Elements, Inc., Park Ridge, Illinois, Chemalytics, Inc., Tempe, Arizona, and Schwarzkopf Microanalytical Laboratory, Woodside, New York.

Transesterified Porphyrins - Compounds I, IIA, IIB, III and IVB.

A solution of 268 mg. of 5,10,15,20-tetra(4-carbomethoxyphenyl)porphyrin in a mixture of 75 ml. of chloroform, 85 ml. of ethylene glycol, 15 ml. of methanol, and 2 ml. of concentrated sulfuric acid was refluxed overnight. The following morning it was poured into 500 ml. of water, neutralized with sodium bicarbonate, and extracted with chloroform. The chloroform layer containing the porphyrins was washed well with water and then the chloroform was removed under reduced pressure. The mixture of transesterified porphyrins was separated using preparative thin layer chromatography and recrystallized from chloroform-methanol. The yields, structures, and Rf values are given in Figure 1. The Roman numerals were assigned on the basis of the compounds containing one ethylene glycol linkage (compound I), two ethylene glycol linkages (compound II), etc. Roman numeral IV is used for compounds with four identical para substituents.

Analytical data were obtained for compound I only.

*Anal.* Calcd. for  $C_{53}H_{40}N_4O_9$ : C, 72.59; H, 4.60; N, 6.39. Found: C, 71.77; H, 4.72; N, 6.46.

#### Compound I (M = Cu).

A solution of 156 mg. of compound I and 139 mg. of cupric acetate in 50 ml. of pyridine was refluxed for 20 minutes. Conversion to the copper complex was followed by visible spectroscopy. The solvent was removed under reduced pressure and the Cu porphyrin was then dissolved in chloroform. The chloroform solution was washed with water, 2% sulfuric acid, water, 8% sodium bicarbonate, and water. The chloroform was then removed under reduced pressure and the Cu porphyrin was purified further by preparatory thin layer chromatography. It was removed from the silica gel with chloroform containing 5% methanol. The material was recrystallized from chloroform-methanol giving 129 mg. for a yield of 77%.

*Anal.* Calcd. for  $CuC_{53}H_{38}N_4O_9$ : C, 67.83; H, 4.08; N, 5.97. Found: C, 67.65; H, 4.26; N, 5.81.

#### Compound V.

*p*-Carboxybenzaldehyde (15.0 g., 0.1 mole) and *p*-tolualdehyde (60.0 g., 0.5 mole) were heated to reflux in 2.5 liters of propionic acid. Pyrrole (40.3 g., 0.6 mole) was then added and reflux was continued for ½ hour. The reaction mixture was allowed to cool and kept at 4° overnight. The following morning it was filtered and the purple crystals obtained were washed well with methanol and finally hot water. The yield was 15.7 g. of mixed porphyrins.

The mixed porphyrins were separated by dry column chromatography using 1.5 kg. of "Woelm Silica Gel for Dry Column Chromatography" and chloroform as eluent. The first band to be eluted moved with the solvent front and contained tetratolylporphyrin. This was followed by a very slow moving thin dark band of impurity. Directly behind the dark band came compound V (see Figure 2 for structure). It took approximately 14 hours to run the column. The product was then recrystallized from chloroform-methanol to yield 3.60 g. (5.1%). An analytical sample was recrystallized from chloroform-tetrahydrofuran.

*Anal.* Calcd. for  $C_{48}H_{36}N_4O_2$ : C, 82.26; H, 5.18; N, 7.99. Found: C, 81.89; H, 5.40; N, 8.01.

#### Compound VI.

The acid chloride was prepared by dissolving 569 mg. of compound V in a mixture of 125 ml. of benzene and 10 ml. of oxalyl chloride. It was stirred at room temperature for three hours and then the solvent was removed under reduced pressure. The acid chloride was redissolved in about 50 ml. of benzene and once again taken to dryness under reduced pressure to remove traces of oxalyl chloride. The acid chloride was then used immediately without

further purification.

#### Compound VII (M = Cu).

A solution of 123 mg. of compound I (M = Cu) in 2 ml. of pyridine and 125 ml. of benzene was added to compound VI prepared from 569 mg. of compound V (see Figure 2 for reaction). The solution was refluxed overnight and the following morning 2 ml. of ethylenediamine were added and reflux was continued for ½ hour to use up the excess acid chloride. The solution was cooled, and the solvent removed under reduced pressure. The residue was dissolved in chloroform and washed with water, 2% sulfuric acid, water, 8% sodium bicarbonate, and water. The chloroform was removed under reduced pressure and the sample applied to a 100 g. silica gel dry column with chloroform as eluent. The Cu dimer porphyrin moved quickly down the column followed by several bands of impurities, and the very slowly moving porphyrinamide. Further purification was achieved by preparatory thin layer chromatography and recrystallization from chloroform-methanol giving 109 mg. of product with a yield of 51%.

*Anal.* Calcd. for  $Cu_2C_{101}H_{72}N_8O_{10}$ : C, 74.82; H, 4.48; N, 6.91; Cu, 3.92. Found: C, 74.31; H, 4.54; N, 6.79; Cu, 4.13.

#### Compound VII (M = Ni).

The same general procedure was used as for the formation of the Cu hybrid dimer. The only difference was that Ni was inserted using nickel acetate and DMF as the solvent. The yield for the Ni insertion was somewhat lower than that for the Cu insertion presumably due to acetylation and to the higher temperature required.

*Anal.* Calcd. for  $Ni_2C_{101}H_{72}N_8O_{10}$ : C, 75.05; H, 4.49; N, 6.93; Ni, 3.63. Found: C, 74.21; H, 4.45; N, 6.36; Ni, 3.6.

#### Results and Discussion.

The starting point for the synthesis of the dimers and trimers was 5,10,15,20-tetra(4-carbomethoxyphenyl)porphyrin (15) which was obtained by treating 5,10,15,20-tetra(4-carboxyphenyl)porphyrin (16) with diazomethane. It was first purified by dry column silica gel chromatography followed by preparative thin layer chromatography and recrystallization from chloroform-methanol.

Compounds I, IIA, IIB and III were obtained by transesterifying the starting material with ethylene glycol. The transesterified porphyrins were separated using preparative thin layer chromatography. Yield and Rf's are shown in Figure 1. Attempts to increase the yields by increasing the ethylene glycol concentration or reflux time were unsuccessful.

Metal derivatives were made of the mono- and dihydroxyporphyrins and their metal derivatives were reacted with the acid chloride of compound V to form the hybrid porphyrin dimers and trimers.

Compound V was obtained by a mixed aldehyde approach (13,14). The reaction path for formation of the dimer is shown in Figure 2. The formation of the trimers shown in Figure 3 were carried out by a procedure analogous to that for the dimers except that the porphyrin derivatives with two primary alcohol groups (IIA and IIB) were used instead of compound I. The yields were somewhat lower than for the dimer preparation. The assign-

ment of the trimer isomer is uncertain because of the uncertainty in assigning structures IIA and IIB. It, of course, must be either IXA or IXB.

In the experimental section, the synthesis of a Cu hybrid dimer having an ethylene linkage was described. The size of the linkage in the dimers has also been varied by using 1,5-pentanediol in place of ethylene glycol. Somewhat lower yields than those obtained for the ethylene analogue were obtained for all reaction steps. We have also prepared free base dimers and hybrid dimers with an ethylene linkage containing one Ni or one Zn. The yield of the coupling reactions were approximately the same in all cases. Spectral data for characterization of the Cu hybrid dimer and the Ni hybrid dimer are presented in Table I and Figures 5 and 6. Extinction coefficients were determined for compounds IVA, VII and VIII and are also shown in Table I and Figures 5 and 6. The observed extinction coefficients for compounds IVA and VIII were not significantly different from the extinction coefficients previously determined for TPP and its Ni and Cu derivatives (17). The absorbance spectra obtained for the porphyrin dimers appear to be simply the sum of the free base porphyrin spectra and the metallo porphyrin spectra. A computer addition of the spectra of compounds IVA and VII is shown in Figures 7 and 8 along with the experimentally obtained spectra for the Cu hybrid dimer and the Ni hybrid dimer. There appears to be little electronic interaction between the covalently linked porphyrin dimers and trimers. Extinction coefficients were not obtained for the porphyrin trimers but rather the spectra were normalized at the peak in the visible region which had maximum intensity. A qualitative examination of the visible spectra showed close correlation of the relative peak intensities of the computer simulations. Figures 9 and 10 show the computer simulation of the Cu hybrid and Ni hybrid porphyrin trimers and the experimentally measured spectra of the porphyrin trimers. A close spectral match is found at most wavelengths. These spectral data are also summarized in Table I.

The nmr data shown in Figure 11 is consistent with what would be expected for the porphyrin dimers and trimers. The Ni hybrids were chosen for the nmr measurements because this metal derivative did not degrade readily in light and nmr measurements could be made without interference from a paramagnetic metal. For the dimer, the integrated areas for the ethylene, tolyl methyl, and carbomethoxy protons were in the expected ratio of 4:9:9, while in the trimer they were found to be in the expected ratio of 4:9:3. The chemical shifts for the tolyl methyls and the carbomethoxy protons were essentially the same for the monomers, dimers and trimers. The ethylene protons also had essentially the same shifts when incorporated into the dimers and trimers. Thus, as would be

expected by considering the steric restraints for the dimer and trimer molecules which were constructed with a short (2-carbon) linkage, there is no evidence from the nmr for porphyrin-porphyrin interaction due to the molecule folding back on itself.

Attempts to determine molecular weights by mass spectrometry were unsuccessful, presumably because the high temperature required (above 200°) led to degradation of the sample.

To obtain confirmatory information on the size of the prepared porphyrins, a solution containing the Ni monomer, dimer and trimer (compounds IVA, VII and IX) in THF was applied to a gel permeation column equipped with an ultraviolet detector. As shown in Figure 12, the compounds eluted in the expected order (their absorbance spectra were measured after they came off the column) and with good separation. A plot of log molecular weight vs. elution volume is shown in Figure 13. A least squares analysis gave a straight line with a correlation coefficient of -0.9995.

The availability of the molecules whose syntheses are described in this paper clearly indicates that the way is open for an extensive systematic study of porphyrin-porphyrin interaction at the level of excited states, energy transfer, and trapping, as well as at the level of ground state redox chemistry. We plan to continue our studies of these structures by physical characterization of any photochemistry exhibited as well as oxidation-reduction reactions between the porphyrin centers.

#### Acknowledgments.

Spectra were recorded on a Cary 14R Recording Spectrophotometer at Argonne National Laboratory where the instrument was on line with a computer. We are grateful to Dr. J. R. Norris and Therese Cotton for their help in taking these spectra. We also wish to thank Dr. Chris Bull for his help in the computer simulations and Mr. Steve Jacobs for assistance in the gel permeation work.

#### REFERENCES

- (1) This investigation was supported by research grants from the National Science Foundation (GB-42860) and the National Institutes of Health (GM 11741).
- (2) Recipient of a Research Career Development Award from the U. S. Public Health Service (5 K04GM-70133).
- (3) P. A. Loach, "Chemical Properties of the Phototrap in Bacterial Photosynthesis" in *Progress in Bioorganic Chemistry*, E. T. Kaiser and F. J. Kezdy, Eds., Wiley Interscience, N. Y., Vol. 4, Chapter 2, 1976, p. 91.
- (4) W. W. Parson and R. J. Cogdell, *Biochim. Biophys. Acta*, **416**, 105 (1975).
- (5) P. A. Loach and B. J. Hales, "Free Radicals in Photosynthesis," in *Free Radicals in Biology*, W. A. Pryor, Ed., Academic Press, N. Y., 1976, Chapter 5, p. 199.
- (6) R. K. Clayton, *Ann. Rev. Biophys. Bioeng.*, **2**, 131 (1973).
- (7) J. R. Norris, R. A. Uphaus, H. L. Crespi, and J. J. Katz, *Proc. Natl. Acad. Sci. U. S.*, **68**, 625 (1971).



- (8) J. J. Katz, in *Inorganic Biochemistry*, Vol. II, G. I. Eichhorn, Ed., Elsevier, N. Y., 1973, p. 1022.
- (9) G. Feher, A. J. Hoff, R. A. Isaacson, and L. C. Ackerson, *Ann. N. Y. Acad. Sci.*, **244**, 239 (1975).
- (10) P. A. Loach, M. Chu Kung, and B. J. Hales, *ibid.*, **244**, 297 (1975).
- (11) K. Ballschmitter, K. Truesdell, and J. J. Katz, *Biochim. Biophys. Acta*, **184**, 604 (1969).
- (12) F. P. Schwarz, M. Goutterman, Z. Muljiani, and D. H. Dolphin, *Bioorg. Chem.*, **2**, 1 (1972).
- (13) R. G. Little, J. A. Anton, P. A. Loach, and J. A. Ibers, *J. Heterocyclic Chem.*, **12**, 343 (1975).
- (14) J. A. Anton and P. A. Loach, *ibid.*, **12**, 573 (1975).
- (15) N. Datta-Gupta and T. Bardos, *ibid.*, **3**, 495 (1966).
- (16) F. R. Longo, M. G. Finarelli, and J. B. Kim, *ibid.*, **6**, 927 (1969).
- (17) G. D. Dorough, J. R. Miller, and F. M. Huennekens, *J. Am. Chem. Soc.*, **73**, 4315 (1951).