Synthetic Approaches to Versatile Hemoprotein Model Compounds Built from Porphyrins and Peptides

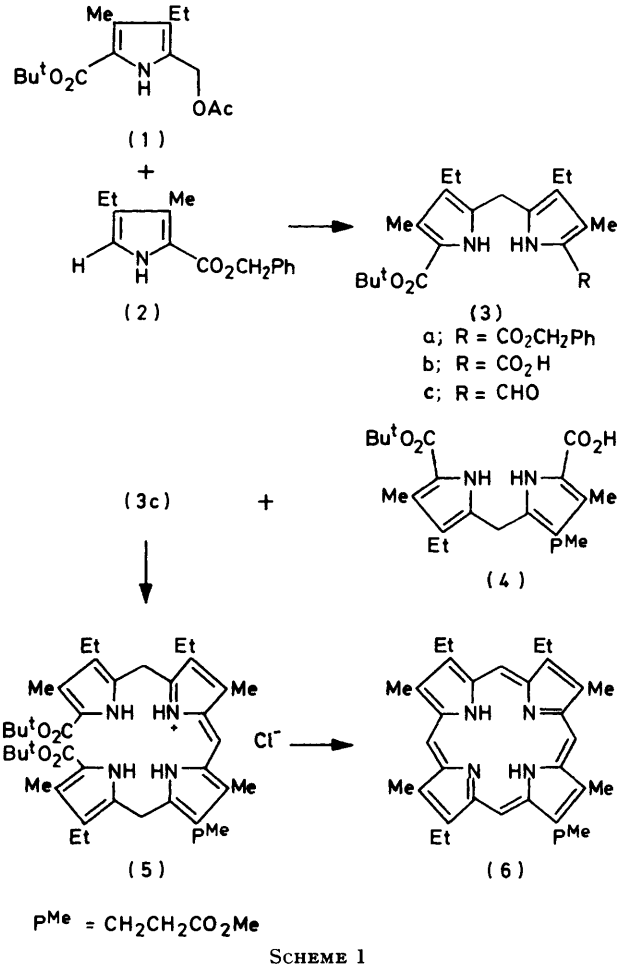
By Anthony H. Jackson,† (the late) George W. Kenner, Kevin M. Smith,‡ and Colin J. Suckling,§ The Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

Several strategies for the preparation of mono- and bi-functional porphyrins (amino and carboxylate) to which oligopeptides can be attached have been investigated. A porphyrinyl amino-acid derivative has been synthesised by coupling a porphyrin monopropionate with phenylalanine methyl ester. Routes to pyrroles bearing butoxycarbonylaminoethyl side-chains *via* Curtius-type degradations, are described, and their potential for elaboration into porphyrins is discussed. The most promising bifunctional porphyrin with differentially protected amino and carboxylate side-chains was found to be the butoxycarbonylhydrazido methyl ester derivative of mesoporphyrin-II.

ONE of the most remarkable features of hemoprotein chemistry is the way in which the electronic structure of the ubiquitous heme prosthetic group is modified by the protein environment around it, such that the various hemoproteins perform widely differing and unique functions. Thus, an iron(II) porphyrin in solution is readily oxidized by oxygen [1] whereas a similar complex embedded in a hydrophobic protein pocket becomes a reversible oxygen-carrier [2] or, if a thiolate is attached as an axial ligand, the complex becomes a vital monooxygenase.[3] Great progress in understanding the versatility of hemes has come from the study of model systems which reproduce, in part, the natural local environment of the protein-bound heme; [4-7] however, in none of these systems is the environment controlled by the natural medium, namely a polypeptide. We wished to synthesize a series of model compounds in which the environment around the prosthetic iron atom could be closely controlled with the wide variation of structures possible using peptides attached as loops to porphyrins. With a series of such compounds to hand, it would be possible to study the fine gradation of properties of the heme as its environment is altered by different peptides. In the present paper we describe routes which are capable of leading to porphyrinyl peptides and of achieving these objectives. However, the versatility of the planned model system imposes a penalty in complexity, and the synthetic programme needed to be approached in stages. Further developments of this work have already led to the stepwise synthesis of dipeptidylporphyrins.[8]

RESULTS AND DISCUSSION

Many of the amino-acids which act as ligands for iron in hemoproteins bear reactive side-chains (histidine, tyrosine, cysteine, and methionine) and, accordingly, mild methods for coupling amino-acids to porphyrins must be established. At the time this work was begun, such reactions were not well known, although some simple peptide derivatives of porphyrins had been reported.[9] We chose to study the coupling reactions of porphyrins and amino-acids initially using monofunctional porphyrins. Accordingly, the synthesis of the monopropionate porphyrin (6) was attempted by the *b*-bilene route (Scheme 1).[10] The 2-acetoxymethylpyrrole



SCHEME 1

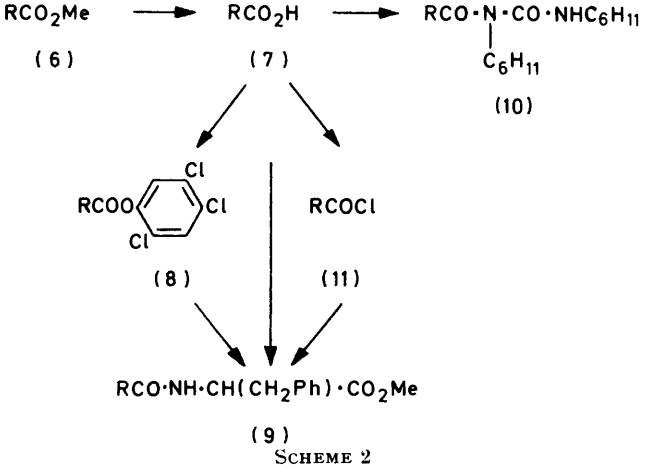
(1) was prepared from the corresponding 2-methylpyrrole [11] by oxidation with lead tetra-acetate in glacial acetic acid and was then coupled with the 2-unsubstituted pyrrole (2) in boiling glacial acetic acid to give the dipyrromethane (3a). Hydrogenolysis of (3a) afforded the

Present addresses: † Department of Chemistry, University College, Cardiff CF1 1XL; ‡ Department of Chemistry, University of California, Davis, CA 95616; § Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1XL.

dipyrromethanecarboxylic acid (3b) which was decarboxylated and formylated (using the Vilsmeier procedure) to give the dipyrromethane (3c). The key tetrapyrrolic intermediate, the *b*-bilene (5), was readily obtained by condensation of dipyrromethanes (3c) and (4) in methylene chloride containing toluene-*p*-sulphonic acid (66% yield). De-blocking of the t-butyl esters was accomplished using cold trifluoroacetic acid, and synthesis of the porphyrin (6) was completed by cyclization with trimethyl orthoformate in the presence of trichloroacetic acid;[10] a 43% yield of porphyrin was obtained from the *b*-bilene (5). Chromatographic purification of this material showed that a mixture of porphyrins had been obtained; 96% of the product was the required monopropionic porphyrin (6), and the by-product (4%) was shown to be mesoporphyrin-IV dimethyl ester by combustion analysis, spectroscopy, and mixed melting point determination. The latter presumably arose from a symmetrical impurity in the bilene derived from the pyrromethane (4).

Coupling of Amino-acids to Monofunctional Porphyrins.—Exploratory coupling reactions were carried out using procedures typical of modern peptide syntheses using the methyl ester of phenylalanine as a representative amino-acid. Because the molecular ion of porphyrins is usually the base peak, and fragmentations are weak,[12] it was convenient to conduct small-scale preliminary reactions using mass spectrometry as the primary analytical tool.

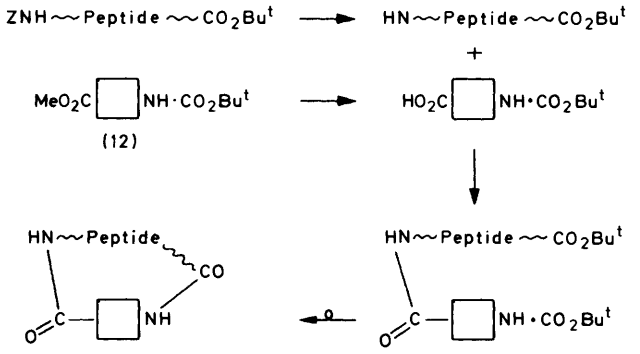
Hydrolysis of the porphyrin ester (6) afforded the corresponding acid (7) (Scheme 2). Although the carboxyl-



SCHEME 2

ate group could be activated *via* the 2,4,5-trichlorophenyl ester (8) or by dicyclohexylcarbodi-imide, very low yields of porphyrinyl amino-acid were obtained, and the acylurea (10) was a major by-product. In contrast to normal peptide synthesis the best results were achieved using the acid chloride (11) derived from the acid (7) with oxalyl chloride, when a 47% yield of the porphyrinyl amide (9) was obtained. Subsequently it was found that porphyrinylacyl imidazolides are superior to acid chlorides for this reaction.[8]

Design of a Versatile Hemoprotein Model System.—Having established in the preliminary studies that porphyrins and amino-acids will couple under mild conditions, it was possible to plan a synthetic route to a hemoprotein model system. Examination of molecular models showed that a pentapeptide would be the shortest polypeptide able to span a porphyrin ring through two ethyl substituents. Such a peptide could be synthesized conveniently with *N*-terminal benzyloxycarbonyl and *C*-terminal t-butyl ester protecting groups so as to be attached to the porphyrin using the data available from our preliminary work. The choice of protecting groups favoured the use of a base-labile blocking group for the *C*-terminus of the porphyrin and an acid-sensitive one (of comparable lability to a t-butyl ester) for the amino terminus (Scheme 3).



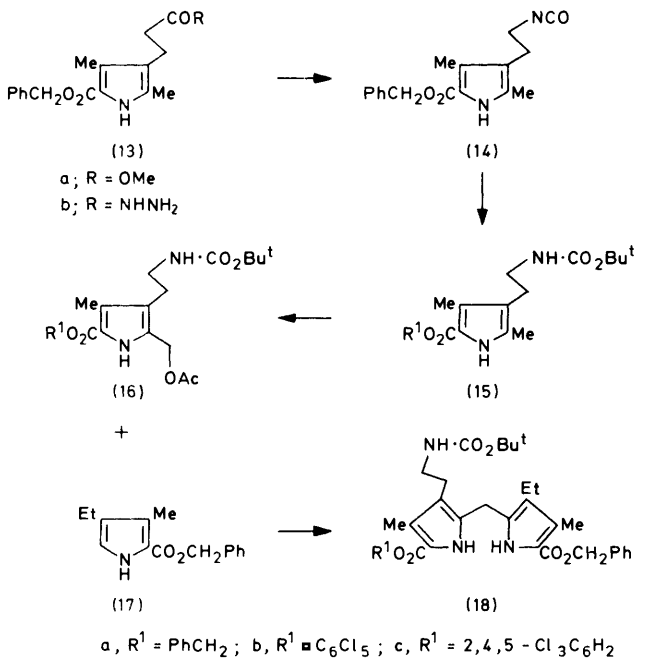
SCHEME 3

Two distinct approaches were available for the synthesis of a bifunctional porphyrin such as that illustrated schematically by (12) in Scheme 3. Firstly, the porphyrin could be constructed from pyrroles which have the required protected side-chains already built in, or, secondly, differential protection of the ' ends ' could be accomplished at a later stage, presumably after formation of the porphyrin macrocycle. Studies of both approaches are discussed below.

Synthesis via *protected pyrroles.* Since a porphyrin methoxycarbonyl-ester substituent is readily available from standard pyrrole syntheses, the key to success in this approach revolved around the preparation of an acid-labile aminoethylpyrrole. Although Fischer[13] had shown that the Curtius degradation applied to 2-methoxycarbonylethylpyrroles readily leads to aminoethylpyrroles and Collier *et al.*[14] had used a more lengthy route *via* 3-formyl- and 3-nitrostyryl-pyrroles, neither approach had led to compounds with the required acid-labile protecting group. We therefore chose to develop a Curtius degradation method to produce t-butoxycarbonylaminoethylpyrroles (Scheme 4). Treatment of the pyrrole diester (13a) with hydrazine hydrate in boiling ethanol afforded the hydrazide (13b) which was converted *via* the azide into the non-crystalline isocyanate (14). The compound was not usually isolated, but was converted directly into the t-butoxycarbonyl-protected aminoethylpyrrole (15a) by acid-catalysed butanolysis.[15]

Dilute benzene solution was used to avoid self-condensation to form a bis-pyrrolylurea.

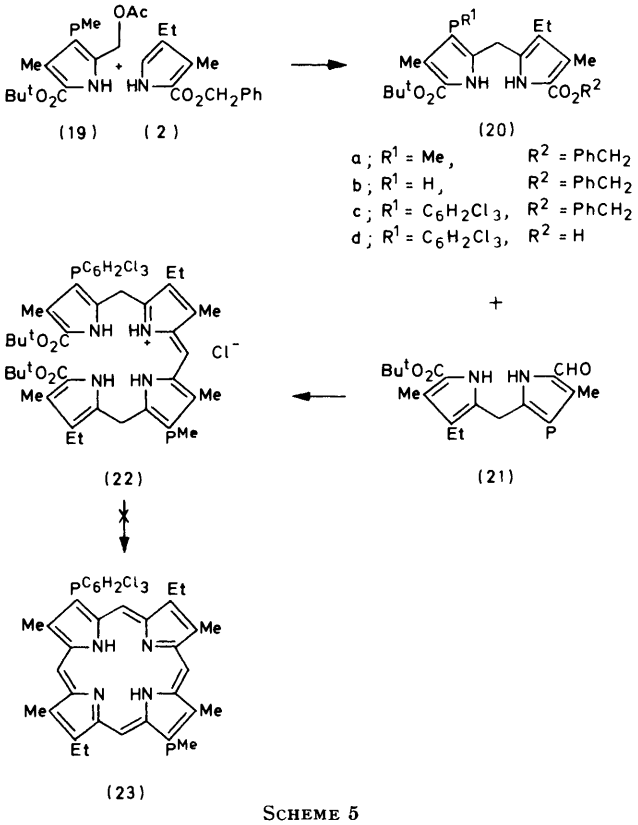
The availability of the pyrrole urethane (15a) prompted an attempt to incorporate it into a stepwise porphyrin synthesis. Routes involving acidic conditions, such as the *b*-bilene [11] or *a,c*-biladiene [16] routes, were obviously inappropriate, but we had previously incorporated a



SCHEME 4

t-butyl ester [17] into a *b*-oxobilane synthesis,[18] so this route was chosen. The remaining problem was the selective protection of the 5,5′-carboxylates in the dipyrromethane intermediate (18) so that a single half-acid (18e) could be prepared. Following previous work,[11] the pentachlorophenyl ester was chosen; the pyrrole urethane (15a) was accordingly hydrogenolysed and the carboxylic acid was converted directly into the pentachlorophenyl ester (15b). This compound was reluctant to undergo activation for dipyrromethane formation, lead tetra-acetate, *N*-bromosuccinimide, and t-butyl hypochlorite all reacting very slowly with the 2-methyl group. However, if the sequence of operations was reversed, namely that activation [(15a) ⟶ (16a)] was carried out prior to transesterification [(16a) ⟶ (16b)], then the required pyrrole (16b) was obtained. Unfortunately, the subsequent coupling of the pyrrole (16b) with the 2-unsubstituted pyrrole (17) to give the pyrromethane (18b) took place in unacceptably low yield (15%). Presumably this was due to the low electrophilicity of the pyrrole (16b) engendered by the strongly electron-withdrawing pentachlorophenyl ester. Unfortunately the related trichlorophenyl ester (16c) fared little better in the coupling reaction, even though it could be prepared directly from the precursor pyrrole (15c). This approach to bifunctional porphyrin synthesis was therefore discontinued.

Synthesis via *differentially protected porphyrins*. The first target in this alternative approach was the differentially protected mesoporphyrin-V di-ester (23) (Scheme 5), which was approached by the *b*-bilene

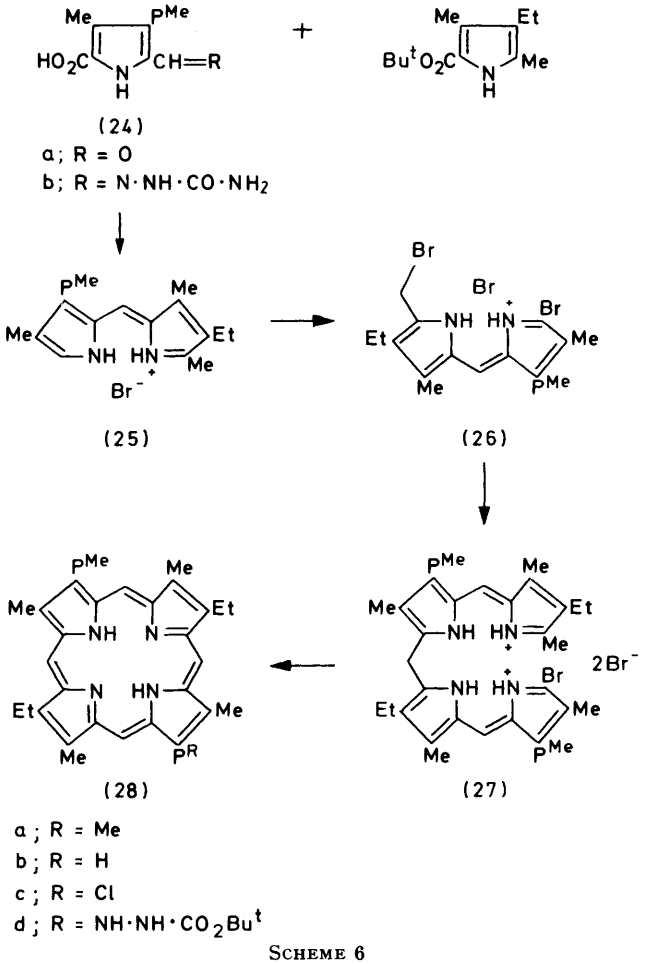


SCHEME 5

route.[10] Since this route to porphyrins utilises t-butyl esters of pyrrole-2-carboxylic acids, selective protection of the propionic side-chain was planned using trichlorophenyl and methyl esters, introduced at the dipyrromethane stage. Dipyrromethane (20a), obtained from the 2-acetoxymethylpyrrole (19) and the 2-unsubstituted pyrrole (2) was hydrolysed specifically at the methyl ester using aqueous dioxan containing sodium hydroxide, and afforded the carboxylic acid (20b) which was re-esterified using dicyclohexylcarbodi-imide, to give the dipyrromethane (20c) required for the *b*-bilene preparation. This was accomplished by condensation of the formyldipyrromethane (21) [10] with the carboxylic acid (20d) (derived by hydrogenolysis of (20c)] to yield the *b*-bilene (22). Disappointingly, cyclization of the *b*-bilene as described previously [10] gave a mixture of porphyrins, most of which did not contain a trichlorophenyl ester; model reactions, reproducing the cyclization conditions, with pyrrole substrates revealed that transesterification of the active ester takes place readily.

At this stage we turned our attention to the *a,c*-biladiene route developed by Johnson and his co-workers.[16] The target was mesoporphyrin-II dimethyl ester, which has since been synthesized more efficiently by Chang [19]

using the dipyrromethane–formic acid procedure.[20] Our synthesis circumvents the problem of the reluctance of pyrroles bearing 2-formyl substituents to decarboxylate and condense to form dipyrromethane salts. Although not the most direct route now available to meso-porphyrin-II, our approach (Scheme 6) possesses



SCHEME 6

generality not otherwise available and is more useful where a less symmetrical porphyrin is required. The pyrrolecarbaldehyde (24a) cannot be decarboxylated directly, but conversion of the aldehyde into its semicarbazone reduces the electron demand sufficiently to allow decarboxylation and condensation to form concomitantly the dipyrromethane in methanolic hydrobromic acid. This dipyrromethene was brominated to give the dibromo-derivative (26) and the two were coupled and cyclised in the usual way [8,16] to give meso-porphyrin-II dimethyl ester (28a) *via* the *a,c*-biladiene dihydrobromide (27).

Conversion of mesoporphyrin-II dimethyl ester into a differentially protected amino-porphyrin-acid required half-hydrolysis. This was easily accomplished using methanolic barium or potassium hydroxide; [8] under these conditions the half-acid (28b) was precipitated. Either the ester or the acid side-chain could be converted into the amino-function, by Curtius degradation, but a shorter route was found. The porphyrin half-acid (28b) was activated as its acid chloride (28c) using oxalyl chloride, and then converted into the substituted hydrazide (28d) in high yield. The butoxycarbonylhydrazide (28d) possesses the required selective protection, a base-labile carboxy-group and an acid-labile amino-group, and is well-suited for elaboration to a versatile porphyrinyl-peptide model system. It remains for the reactions of protected pentapeptides with the porphyrin (28d), after deprotection, to be investigated.

EXPERIMENTAL

M.p.s were measured on a microscopic hot-stage, and are uncorrected. Monitoring of all reactions was performed on glass slides coated with Merck GF 254 silica gel. Column chromatography was carried out using Woelm neutral alumina, Brockman grade III. All reactions were performed under an atmosphere of nitrogen in dry conditions, and usually also in the dark (aluminium foil). Electronic absorption spectra were measured using a Unicam SP-800 spectrophotometer, usually for solutions in methylene dichloride. ^{1}H N.m.r. spectra were measured on a Varian HA-100 or XL-100 instrument, usually with deuteriochloroform as solvent and tetramethylsilane as internal standard. Mass spectra (direct insertion probe, 70 eV, 50 μ A, source temp. *ca.* 200 °C) were measured either on an AEI MS 12 or an AEI MS 902 (high resolution determinations) instrument. Throughout, ether refers to diethyl ether.

t-Butyl 5-Acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (1).—To a solution of t-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (22.3 g) in glacial acetic acid (600 ml) and acetic anhydride (50 ml) was added lead tetra-acetate (48.6 g) in portions with stirring during 4 h. After being allowed to stand at room temperature for 18 h, the solution was added dropwise to stirred water (2 l). The precipitated pyrrole was filtered off and dried *in vacuo*; it recrystallized from hexane as colourless *needles* (25.8 g, 96%), m.p. 115—116 °C (Found: C, 64.2; H, 8.0; N, 5.0. $C_{15}H_{23}NO_4$ requires C, 64.0; H, 8.2; N, 5.0%); τ 0.54 (1 H, br s, NH), 4.95 (2 H, s, CH_2O), 7.56 and 8.90 (2 H, q, and 3 H, t, CH_2CH_3), 7.99 (3 H, s, CH_3CO), and 8.46 (9 H, s, Bu^t).

Benzyl 4-(2-Hydrazidoethyl)-3,5-dimethylpyrrole-2-carboxylate (13b).—Benzyl 4-(2-methoxyoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (4.7 g) was dissolved in absolute ethanol (3 ml) containing hydrazine hydrate (1.2 g; 95%) and the solution was heated under reflux for 3 h. The solution was cooled and ether added to produce a white crystalline precipitate; this was filtered off, washed successively with ethanol and ether, and then dried *in vacuo*. The required hydrazide was obtained as colourless *needles* (4.7 g, 100%), m.p. 154—155 °C, after recrystallization from aqueous ethanol (Found: C, 65.0; H, 6.9; N, 13.2. $C_{17}H_{21}N_3O_3$ requires C, 64.7; H, 6.7; N, 13.3%); τ (CF_3CO_2H) 1.01 (1 H, br s, NH); 2.62 and 4.65 (5 H, s and 2 H, s, $PhCH_2$), 6.9 and 7.5 (4 H, m, CH_2CH_2), and 7.70 and 7.76 (each 3 H, s, CH_3).

Benzyl 4(2-t-Butoxycarbonylaminoethyl)-3,5-dimethylpyrrole-2-carboxylate (15a).—Benzyl 4-(2-hydrazidoethyl)-3,5-dimethylpyrrole-2-carboxylate (13b) (6.26 g) was suspended in dilute hydrochloric acid (0.5M; 50 ml) and the mixture was cooled to 0 °C whilst being stirred. A cold

solution of sodium nitrite (1.16 g) in water (20 ml) was added dropwise to the suspension during 30 min, and the precipitated azide was extracted into methylene chloride (50 ml); this was then washed with water (100 ml), dried ($MgSO_4$), and evaporated to dryness. The pale brown syrupy residue was redissolved in dry benzene (400 ml), and the solution was heated under reflux for 2 h. t-Butyl alcohol (3 g) and trifluoroacetic acid (230 mg) were added and the solution was heated under reflux until the i.r. spectrum showed complete decomposition of the isocyanate (2—4 h). The brown solution was evaporated to dryness to give a solid which recrystallized from methylene dichloride–hexane as fluffy white *needles* (4.66 g, 63%), m.p. 124—125 °C (Found: C, 67.6; H, 7.5; N, 7.9. $C_{21}H_{28}N_2O_4$ requires C, 67.7; H, 7.6; N, 7.5%); τ 0.79 (1 H, br s, NH), 2.71 and 4.74 (5 H, s and 2 H, s, $PhCH_2$), 5.40 (2 H, br s, CH_2NH), 6.82 and 7.43 (2 H, q and 2 H, t, CH_2CH_2), 7.69 and 7.80 (each 3 H, s, CH_3), and 8.58 (9 H, s, Bu^t).

Pentachlorophenyl 3,5-Dimethyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (15b).—Benzyl 3,5-dimethyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (15a) (4.7 g) was dissolved in tetrahydrofuran (250 ml) and hydrogenated over 10% palladised charcoal (1 g) in the presence of triethylamine (0.5 ml) for 2 h. The catalyst was then filtered off onto Celite and the residue was evaporated to dryness under reduced pressure. The residual colourless solid foam was dissolved in dry methylene chloride (150 ml) and the solution was cooled to 0 °C with stirring. Pentachlorophenol (3.0 g) and dicyclohexylcarbodi-imide (2.6 g) were added and the solution was stirred at 0 °C for a further 40 h. The precipitated dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. Trituration with hexane crystallized a small quantity of pyrrolyl-acylurea by-product, and the remaining oil was filtered through alumina (100 g), the required pentachlorophenyl ester being eluted with benzene. Recrystallization from methylene dichloride–hexane gave colourless *plates* (4.91 g, 73%) (Found: C, 44.8; H, 4.0; N, 5.1. $C_{20}H_{23}Cl_5N_2O_4$ requires C, 45.1; H, 4.3; N, 5.3%); τ 0.48 (1 H, br s, NH), 5.30 (1 H, br s, NHCO), 6.78 and 7.37 (2 H, q and 2 H, t, CH_2CH_2), 7.64 and 7.79 (each 3 H, s, CH_3), and 8.57 (9 H, s, Bu^t).

2,4,5-Trichlorophenyl 3,5-Dimethyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (15c).—This compound was prepared in the same manner as the one above, but using 2,4,5-trichlorophenol. The yield was 76% and reaction for only 20 h at 0 °C was required. The *trichlorophenyl ester* was recrystallized from methanol, m.p. 152—153 °C (Found: C, 52.0; H, 5.3; N, 5.8. $C_{20}H_{25}Cl_3N_2O_4$ requires C, 51.8; H, 5.4; N, 6.0%); τ 2.45 and 2.60 (each 1 H, s, phenyl-H), 6.83 and 7.37 (2 H, q and 2 H, t, CH_2CH_2), 7.62 and 7.74 (each 3 H, s, CH_3), and 8.57 (9 H, s, Bu^t).

Pentachlorophenyl 5-Acetoxymethyl-3-methyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (16b).—This compound was prepared in an analogous manner to the above pyrroles from benzyl 5-acetoxymethyl-3-methyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (16a) in 70% yield; 48 h were required for the esterification and the product recrystallized from methanol as colourless *prisms*, m.p. 180—181 °C (Found: C, 45.1; H, 4.3; N, 4.6. $C_{22}H_{23}Cl_5N_2O_6$ requires C, 44.9; H, 3.9; N, 4.8%); τ 0.50 (1 H, br s, NH), 4.89 (2 H, s, CH_2O), 5.30 (1 H, br s, NHCO), 6.72 and 7.28 (2 H, q and 2 H, t, CH_2CH_2), 7.61 (3 H, s, CH_3), 7.89 (3 H, s, CH_3CO), and 8.57 (9 H, s, Bu^t).

Benzyl 5-Acetoxymethyl-3-methyl-4-(2-t-butoxycarbonylaminoethyl)pyrrole-2-carboxylate (16a).—To a solution of benzyl-3,5-dimethyl-4-(2-t-butoxycarbonylaminoethyl)-pyrrole-2-carboxylate (13b) (4.8 g) in glacial acetic acid (75 ml) containing acetic anhydride (2 ml) was added lead tetra-acetate (6.3 g) in small portions with stirring during 2 h. The solution was set aside overnight and was then poured into water (500 ml) and chloroform (500 ml). The mixture was shaken and separated and the organic phase was then washed with saturated aqueous sodium carbonate (400 ml) and water (500 ml) and finally dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was crystallized by trituration with hexane, and then recrystallized from methylene dichloride–hexane to give the *acetoxymethylpyrrole* (4.8 g, 87%) as colourless needles, m.p. 115—116 °C (Found: C, 64.0; H, 7.2; N, 6.6. $C_{23}H_{30}N_2O_6$ requires C, 64.2; H, 7.0; N, 6.5%); τ 2.71 and 4.69 (5 H, s and 2 H, s, $PhCH_2$), 4.97 (2 H, s, CH_2O), 6.79 and 7.38 (2 H, q and 2 H, t, CH_2CH_2), 7.71 (3 H, s, CH_3), 7.96 (3 H, s, CH_3CH_3CO), and 8.58 (9 H, s, Bu^t).

3,3′-Diethyl-4,4′-dimethyl-5′-t-butoxycarbonyldipyrromethane-5-carboxylic Acid (3b).—Benzyl 3-ethyl-2-iodo-4-methylpyrrole-5-carboxylate (16.3 g) was hydrogenated in methanol (250 ml) containing anhydrous sodium acetate (15 g) and Adams platinum oxide catalyst (200 mg) at room temperature for 3 h. The methanol was evaporated and the residue was extracted with methylene dichloride (400 ml) after addition of water (400 ml). The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness to give benzyl 3-ethyl-4-methylpyrrole-5-carboxylate as an oil. This was heated with t-butyl 2-acetoxymethyl-3-ethyl-4-methylpyrrole-5-carboxylate (9 g) and anhydrous sodium acetate (15 g) in glacial acetic acid (200 ml) at 120—130 °C for 45 min. The mixture was then cooled, poured into water (500 ml), and extracted with methylene dichloride (500 ml); this was then washed with water (3 × 200 ml), dried ($MgSO_4$), and evaporated to dryness to give *benzyl 3,3′-diethyl-4,4′-dimethyl-5′-t-butoxycarbonyldipyrromethane-5-carboxylate* (3a) (9.8 g, 71%) as an oil which could not be induced to crystallize [τ 0.63 and 1.01 (each 1 H, br s, NH), 2.71 and 4.77 (5 H, s and 2 H, s, Ph-CH_2), 6.17 (2 H, s, methane-CH_2), 7.54 and 7.76 (each 3 H, s, CH_3), 7.60 and 9.10 (4 H, q and 6 H, t, CH_2–CH_3), and 8.51 (9 H, s, Bu^t)].

A solution of the dipyrromethane (3a) (8.9 g) in tetrahydrofuran (1 250 ml) and triethylamine (0.8 ml) was hydrogenated at room temperature for 3 d over 10% palladised charcoal (1.8 g). The catalyst was filtered off onto Celite and the filtrate was evaporated to dryness to give an oily residue which crystallized upon trituration with ether. The *dipyrromethane* recrystallized from methylene dichloride–ether–benzene as colourless needles (7.2 g, 98%), m.p. 179—180 °C (dec.) (Found: C, 67.3; H, 7.9; N, 7.6. $C_{21}H_{30}N_2O_4$ requires C, 67.4; H, 8.1; N, 7.5%); τ −0.84 and −1.38 (each 1 H, br s, NH), 6.25 (2 H, s, methane-CH_2), 7.51 and 8.93 (4 H, m and 6 H, m, CH_2CH_3), 7.60 and 7.78 (each 3 H, s, CH_3), and 8.45 (9 H, s, Bu^t).

t-Butyl 3,3′-Diethyl-5-formyl-4,4′-dimethyldipyrromethane-5′-carboxylate (3c).—The dipyrromethanecarboxylic acid (3b) (7.0 g) was dissolved in methylene dichloride (400 ml) and a solution of toluene-*p*-sulphonic acid hydrate (7.0 g) in methanol (100 ml) was added. After being stirred for 1 h at room temperature, the solution was washed with aqueous sodium carbonate (2%; 400 ml), dried ($MgSO_4$), and then evaporated to dryness. The red residue was dissolved in dry methylene dichloride (400 ml) and added to a stirred

suspension of calcium carbonate (AnalaR; 1 g) and dimethylformamide (DMF)–phosphoryl chloride complex (1.25 g, DMF and 2.6 g $POCl_3$), and then stirred for a further 20 min at room temperature. A solution of sodium acetate in water (15%; 400 ml) was slowly added to the mixture which was stirred vigorously for 40 min. It was then carefully neutralized with solid sodium carbonate and the organic layer was separated and washed with water (3 × 500 ml), dried ($MgSO_4$), and evaporated to dryness. The residual oil was chromatographed on alumina (300 g, elution with benzene) and the appropriate eluates were evaporated to dryness. The residue recrystallized from hexane as colourless *needles* (3.6 g, 50%), m.p. 168—170 °C (Found: C, 70.4; H, 8.4; N, 7.8. $C_{21}H_{30}N_2O_3$ requires C, 70.4; H, 8.4; N, 7.8%), τ −0.41 and 0.53 (each 1 H, br s, NH), 0.53 (1 H, s, CHO), 6.08 (2 H, s, methane-CH_2), 7.46 and 8.95 (4 H, q and 6 H, t, CH_2CH_3), and 7.72 and 7.77 (each 3 H, s, CH_3), and 8.50 (9 H, s, Bu^t).

Benzyl 3-Ethyl-4,4'-dimethyl-3'-(2-t-butoxycarbonylaminoethyl) 5'-(2,4,5-tricholorophenoxycarbonyl)dipyrromethane-5-carboxylate (18b).—2,4,5-Trichlorophenyl 2-acetoxymethyl-4-methyl-3-(2-t-butoxycarbonylaminomethyl)pyrrole-5-carboxylate (16c) was prepared in 95% yield by treatment of the corresponding 2-methylpyrrole (15c) with lead tetraacetate in acetic acid and acetic anhydride, as described above for compound (16a). This material was not fully characterized, but its n.m.r. spectrum was compatible with the proposed structure [τ 0.46 (1 H, br s, NH), 2.49 and 2.63 (each 1 H, s, phenyl-H), 4.98 (2 H, s, CH_2O), 5.20 (1 H, t, NHCO), 6.79 and 7.34 (2 H, q and 2 H, t, CH_2CH_2), 7.67 (3 H, s, CH_3), 7.94 (3 H, s, $COCH_3$), and 8.59 (9 H, s, Bu^t)]. Compound (16c) (10.4 g), benzyl 2-ethyl-3-methylpyrrole-5-carboxylate (4.84 g), and sodium acetate (12 g) were dissolved in glacial acetic acid (200 ml) and the solution was heated under reflux for 40 min. It was cooled, poured into water (1 l), and then extracted with methylene dichloride (500 ml). The organic phase was washed with water (1 l), saturated aqueous sodium carbonate (1 l), with water again (1 l), and dried ($MgSO_4$). Evaporation gave a brown oily residue which was chromatographed on alumina (elution with 10% ethyl acetate in benzene) to give the dipyrromethane as a light brown solid foam (5.7 g, 40%), which could not be induced to crystallize; τ 0.05 and 0.22 (each 1 H, br s, NH), 2.69 (2 H), 2.72 (5 H), and 4.80 (2 H), (each s, phenyl-H, Ph, and $PhMCH_2$), 5.12 (1 H, s, NHCO), 6.11 (2 H, s, methane-CH_2), 6.6—7.8 (6 H, m, CH_2CH_2 and CH_2CH_3), 7.73 and 7.75 (each 3 H, s, CH_3), 8.61 (9 H, s, Bu^t), and 9.00 (3 H, t, CH_2CH_3).

Benzyl 3-Ethyl-3'-(2-methoxycarbonylethyl)-4,4'-dimethyl-5'-t-butoxycarbonyldipyrromethane-5-carboxylate (20a).—This compound was prepared in an analogous manner to that described above from benzyl 3-ethyl-4-methylpyrrole-5-carboxylate (6.0 g) and t-butyl 2-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylate (8.6 g) in 71% yield. It was recrystallized from methylene dichloride–methanol and had m.p. 104—105 °C (Found: C, 68.4; H, 7.1; N, 5.5. $C_{30}H_{38}N_2O_6$ requires C, 68.9; H, 7.3; N, 5.4%); τ 0.43 and 1.04 (each 1 H, br s, NH), 2.70 and 4.78 (5 H, s and 2 H, s, $PhCH_2$), 6.11 (2 H, s, methane-CH_2), 6.42 (3 H, s, OCH_3), 7.2—7.8 (6 H, m, CH_2CH_2 and CH_2CH_3), 7.71 and 7.79 (each 3 H, s, CH_3), 8.53 (9 H, s, Bu^t), and 8.90 (3 H, t, CH_2CH_3).

Benzyl 3-Ethyl-4,4'-dimethyl-5'-t-butoxycarbonyl-3'-[2-(2,4,5-trichlorophenoxycarbonylethyl)]dipyrromethane-5-carboxylate (20c).—Benzyl 3-ethyl-3'-(2-methoxycarbonylethyl)-4,4'-dimethyl-5'-t-butoxycarbonyldipyrromethane-5-carboxylate (20a) (5 g) was dissolved in dioxan (150 ml) and a solution of sodium hydroxide (1.2 g) in water (150 ml) was added. The solution was stirred at room temperature for 3 h and then neutralized with dilute hydrochloric acid. The pyrromethane was extracted into methylene dichloride (100 ml) and the extract was washed with water, dried ($MgSO_4$), and then evaporated to dryness. The residue was then treated in either of two ways:

(a) It was dissolved in ethyl acetate (200 ml) and 2,4,5-trichlorophenol (1.9 g) was added. After being cooled to 0 °C with stirring, dicyclohexylcarbodi-imide (2 g) was added and the solution was stirred at 0 °C for a further 30 min. The solution was allowed to warm to room temperature and was then further stirred for 3 h, after which time analytical t.l.c. showed the reaction to be complete. Glacial acetic acid (1.0 ml) was added and, after being stirred for 10 min, the precipitate of dicyclohexylurea was filtered off. The filtrate was evaporated to dryness and the residual oil was chromatographed on alumina (150 g, elution with benzene). Evaporation of the appropriate eluates gave the dipyrromethane which recrystallized from methylene dichloride–hexane as colourless needles (1.6 g, 20%), m.p. 70—71 °C; τ 0.42 and 0.62 (each 1 H, br s, NH), 2.72 and 4.75 (5 H, s and 2 H, s, $PhCH_2$), 2.53 and 2.90 (each 1 H, d, phenyl-H), 6.12 (2 H, s, methane-CH_2), 7.07—7.85 (6 H, m, CN_2CH_2 and CH_2CH_3), 7.64 (6 H, s, CH_3), 8.51 (9 H, s, Bu^t), and 9.01 (3 H, t, CH_2CH_3).

(b) The residue was dissolved in dry benzene (50 ml) and oxalyl chloride (5 ml) was added. After 1 h the mixture was evaporated to dryness and then further portions of benzene (3 × 20 ml) were added and evaporated successively. The residue was dissolved in carbon tetrachloride (AnalaR; 200 ml) and 2,4,5-trichlorophenol (1.69 g) and *NN*-dimethylaniline (1.22 g, redistilled) were added. The solution was heated under reflux for 4 h, cooled, washed with dilute sulphuric acid (2 × 100 ml; 6N) and water (3 × 1 l), and then dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on alumina (150 g, elution with benzene) and evaporation of the appropriate eluates gave the dipyrromethane (2.4 g, 25%) after recrystallization as described above. The material from methods (a) and (b) was identical by m.p., n.m.r., and t.l.c. comparison.

4-Ethyl-3'-(2-methoxycarbonylethyl)-3,4',5-trimethyldipyrromethene Hydrobromide (25).—4-(2-Methoxycarbonylethyl)-3-methyl-5-semicarbazidomethylpyrrole (24b) (12 g) was suspended in methanol (250 ml) and t-butyl 3-ethyl-2,4-dimethylpyrrole-5-carboxylate (9.2 g) and aqueous hydrobromic acid (48%; 12 ml) were added. The suspension was heated under reflux for 5 h before being concentrated and chilled. The dipyrromethene salt slowly crystallized in many crops and was filtered off and dried. An alternative, more rapid work-up was developed whereby the reaction mixture was evaporated to dryness and then extracted with ethyl acetate (100 ml). The undissolved semicarbazide salts were filtered off and the solution was concentrated until crystallization began. The crystals were filtered off and were dried under vacuum. This material was usually pure enough to use directly in subsequent reactions and the yield (17 g) was virtually quantitative. A small sample recrystallized from ethyl acetate–hexane as black *needles*, m.p. 118—119 °C (Found: C, 56.8; H, 6.4; N, 7.3. $C_{18}H_{25}BrN_2O_2$ requires C, 56.7; H, 6.6; N, 7.4%); τ (CF_3CO_2H), 2.44 and 2.49 (each 1 H, s, 5'-H and methene-H), 6.18 (3 H, s, OCH_3), 6.58—7.60 (6 H, m, CH_2CH_2 and CH_2CH_3), 7.34

(3 H, s, 5-CH_3), 7.57 and 7.75 (each 3 H, s, 3,4'-CH_3), and 8.80 (3 H, t, CH_2CH_3).

5'-Bromo-5-bromomethyl-3-(2-methoxycarbonylethyl)-4'-ethyl-3',4-dimethyldipyrromethene Hydrobromide (26).—4-Ethyl-3'-2(2-methoxycarbonylethyl)-3,4',5-trimethyldipyrromethene hydrobromide (25) (5 g) was suspended in formic acid (AnalaR; 7 ml) and bromine (1.5 ml) was added. The suspension was allowed to stand at room temperature for 5 min and was then heated on a water-bath at 80—100 °C for 10 min. On cooling a red solid separated and, after addition of ether (10 ml) the solid was filtered off, washed with ether and then dried *in vacuo*. It was recrystallized from anhydrous formic acid as rust brown *rhombs* (4.5 g, 66%), m.p. 156—158 °C (Found: C, 38.9; H, 4.4; N, 4.9. $C_{17}H_{21}Br_3N_2O_2$ requires C, 38.8; H, 4.0; N, 5.3%); τ (CF_3CO_2H) 2.31 (1 H, s, methane-H), 5.29 (2 H, s, CH_2Br), 6.55—7.60 (6 H, m, CH_2CH_2, CH_2CH_3), 7.59 and 7.81 (each 3 H, s, CH_3), and 8.72 (3 H, t, CH_2CH_3).

Di-t-butyl 2,3,7-Triethyl-6-(2-methoxycarbonylethyl)-1,4,5,8-tetramethyl-b-bilene-1',8'-dicarboxylate Hydrochloride (5).—t-Butyl 3,3'-diethyl-5-formyl-4,4'-dimethyldipyrromethane-5-carboxylate (3c) (2.9 g) and 3-ethyl-3'-(2-methoxycarbonylethyl)-4,4'-dimethyl-5'-t-butoxycarbonyldipyrromethane-5-carboxylic acid (3b) (2.98 g) were dissolved in methylene dichloride (700 ml) and a solution of toluene-*p*-sulphonic acid monohydrate (5.2 g) in methanol (180 ml) was added. The mixture was stirred at 20 °C for 30 min, then washed with aqueous sodium carbonate (2%; 1 l) and water (1 l), dried ($MgSO_4$), and evaporated to dryness. Dry benzene (2 × 300 ml) was added and then evaporated. The residual brown oil was dissolved in methylene dichloride (50 ml) and hydrogen chloride gas was blown through the solution until it was deep red (5 s). Benzene (300 ml) was again added and the solution was evaporated to dryness. The red oil crystallized on trituration with ether to give orange-red needles (3.4 g, 66%). Recrystallization from methylene dichloride–hexane gave the b-*bilene salt*, m.p. 147—148 °C (decomp.) (Found: C, 67.7; H, 8.0; N, 7.1. $C_{43}H_{60}ClN_4O_6$ requires C, 67.6; H, 7.9; N, 7.3%); τ −0.82 and 0.27 (each 2 H, br s, NH), 2.52 (1 H, s, methene-H), 5.66 (2 H, s, methane-CH_2), 6.06 (3 H, s, OCH_3), 7.36—7.71 (10 H, m, CH_2CH_2, CH_2CH_3), 7.70 and 7.75 (each 6 H, s, CH_3), 8.60 (18 H, s, Bu^t), and 8.94 (9 H, t, CH_2CH_3); $\lambda_{max.}$ 516 nm (ε 44 000).

Di-t-butyl 3,7-Diethyl-6-(2-methoxycarbonylethyl)-1,4,5,8-tetramethyl 2-[2-(2,4,5-trichlorophenoxycarbonyl)ethyl]-b-bilene-1',8'-dicarboxylate Hydrochloride (22).—This compound was prepared in an analogous manner to the above *b*-bilene (5) in 73% yield from 3'-ethyl-4,4'-dimethyl-5-t-butoxycarbonyl-3-[2-(2,4,5-trichlorophenoxycarbonyl)-ethyl)dipyrromethane-5'-carboxylic acid (20d) (720 mg) and t-butyl 3-ethyl-5'-formyl-3'-(2-methoxycarbonylethyl)-4,4'-dimethyldipyrromethane-5-carboxylate (21) (480 mg). Recrystallization from ether gave orange-red needles of the b-*bilene salt*, m.p. 101 °C (Found: C, 60.7; H, 6.4; N, 5.6. $C_{50}H_{62}Cl_4N_4O_8$ requires C, 60.7; H, 6.3; N, 5.7%); τ −0.50 and 0.89 (each 2 H, br s, NH), 2.48 (2 H, s, phenyl-H), 2.54 (1 H, s, methene-H), 6.08 (2 H, s, methane-H), 6.33 (3 H, s, OCH_3), 7.08—7.89 (12 H, m, CH_2CH_2, CH_2CH_3), 7.70 (12 H, m, CH_3), 8.51 (18 H, s, Bu^t), and 8.92 (6 H, t, (6 H, t, CH_2CH_3); $\lambda_{max.}$ 508 nm (ε 54 000).

2,3,7-Triethyl-6-(2-methoxycarbonylethyl)-1,4,5,8-tetramethylporphyrin (6).—Di-t-butyl 2,3,7-triethyl-6-(2-methoxycarbonylethyl)-1,4,5,8-tetramethyl-*b*-bilene-1',8'-dicarboxylate hydrochloride (5) (2.1 g) was dissolved in trifluoroacetic acid (30 ml) and nitrogen gas was bubbled through the solution for 20 min. The solvent was then evaporated under reduced pressure and traces of it were removed by evaporation of benzene (50 ml). The residual red oil was dissolved in methylene dichloride (1 020 ml) and triethylamine (4.2 ml) was added. After a few minutes, trimethyl orthoformate (6.3 ml) was added and the solution was treated with a solution of trichloroacetic acid in dry methylene chloride (1N; 180 ml). After being stirred overnight at room temperature, the solution was washed with aqueous sodium carbonate (2%; 400 ml), dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on alumina (100 g, elution with 1 : 1 benzene–methylene chloride). The first band to be eluted yielded the *porphyrin monopropionate* (667 mg, 43%), m.p. 263 °C, after recrystallization from methylene dichloride–methanol (Found: C, 76.1; H, 7.5; N, 10.3. $C_{34}H_{40}N_4O_2$ requires C, 76.1; H, 7.5; N, 10.4%); τ (0.1M), 0.00 (4 H, s, *meso*-H), 5.66 (2 H, m, Ar-CH_2CH_2), 6.34 (3 H, s, OCH_3), 6.73 (2 H, m, CH_2CH_2CO), 6.40 (12 H, s, CH_3), and 5.91 and 8.15 (6 H, q and 9 H, t, 3 × CH_2CH_3); $\lambda_{max.}$ 400 (ε 123 000), 500 (10 300), 530 (6 700), 568 (3 900), and 621 nm (1 000); $\lambda_{max.}$ (CH_2CCl_2–5% CF_3CO_2H), 416 (ε 268 000), 554 (11 800), and 597 nm (5 400); *m/z* 536 (100%), 521, 504, 478, 463, 268, 239, and 231.5.

The second band to be eluted from the column was shown to contain mesoporphyrin-IV dimethyl ester (34 mg, 2%), and was crystallized from methylene dichloride–methanol, m.p. 241—242 °C (lit.,[21] 238 °C; mixed m.p. with an authentic sample 242—244 °C, corrected) (Found: C, 72.5; H, 7.2; N, 9.0. Calc. for $C_{36}H_{42}N_4O_4$: C, 72.7; H, 7.1; N, 9.4%), τ (0.1M) 0.05 (4 H, s, *meso*-H), 5.57—6.20 (8 H, m, Ar-CH_2CH_2, CH_2CH_3), 6.37 (3 H, s, OCH_3), 6.45 and 6.49 (each 6 H, s, CH_3), 6.80 (4 H, t, CH_2CH_2CO) and 8.16 (6 H, t, CH_2CH_3); $\lambda_{max.}$ 399 (ε 133 000), 500 (11 200), 532 (7 600), 567 (5 000), and 620 nm (3 100); $\lambda_{max.}$ (CH_2Cl_2–5% CF_3CO_2-H) 417 (ε 279 000), 554 (12 800), and 597 nm (5 900); *m/z* 594 (100%), 577, 561, 534, 520, 478, 463, 297, and 296.

2,6-Diethyl-4,8-di-(2-methoxycarbonylethyl)-1,3,5,7-tetramethylporphyrin, Mesoporphyrin-II Dimethyl Ester (28a).—1'-Bromo-6-(2-carboxyethyl)-4,8-diethyl-2-(2-methoxycarbonylethyl)-1,3,5,7,8'-pentamethyl-*a,c*-biladiene (27) (3.0 g) was finely powdered and suspended in dry *o*-dichlorobenzene (300 ml) before being heated under reflux for 15 min. After cooling the solvent was evaporated to dryness and the residue was treated with methanol containing sulphuric acid (5% v/v; 50 ml) for 16 h. Water (200 ml) and ether (200 ml) were added and the mixture was shaken vigorously to remove traces of *o*-dichlorobenzene. The layers were separated and the aqueous phase was poured into chloroform (200 ml) and the mixture was carefully neutralized with saturated aqueous sodium carbonate (200 ml). The organic layer was separated, washed with water (200 ml), dried ($MgSO_4$), and then evaporated to dryness. The residue was chromatographed on alumina (200 g, elution with methylene dichloride) and evaporation of the appropriate eluates gave a residue which crystallized from methylene dichloride–methanol as purple needles, m.p. 223—234 °C (lit.,[22] 233 °C) Found: C, 72.8; H, 6.9; N, 9.1. Calc. for $C_{36}H_{42}N_4O_4$: C, 72.7; H, 7.1; N, 9.4%), τ (0.1M), 0.07 (4 H, s, *meso*-H), 5.56—6.17 (8 H, m, CH_2CH_2CO, CH_2CH_3), 6.34 (6 H, s, OCH_3), 6.45 and 6.49 (each 6 H, s, CH_3), 6.80 (4 H, t, CH_2CO), and 8.19 (6 H, t, CH_2CH_3); *m/z* 594 (100%), 577, 561, 534, 520, 478, 297, and 286; $\lambda_{max.}$ 400 (ε 133 000), 500 (10 500), 532 (7 600), 568 (5 000), and 622 nm (2 500); $\lambda_{max.}$ ($CHCl_3$–5%

CF_3CO_2H), 416 (ε 282 000), 555 (12 400), and 598 nm (5 400).

4-(2-*Carboxyethyl*-2,6-*diethyl*-8-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethylporphyrin* (28b).—Mesoporphyrin-II dimethyl ester (28a) (110 mg) was dissolved in tetrahydrofuran (11 ml) and ether (110 ml). A solution of barium hydroxide in dry methanol (saturated; 5.5 ml) and barium acetate in dry methanol (saturated; 11 ml) was added and the solution was heated under reflux until all of the porphyrin had precipitated from solution (4—6 h). Hydrochloric acid (2N; 120 ml) was added and the aqueous porphyrin-containing layer was separated. Methylene dichloride (200 ml) was added and the solution was neutralized with solid sodium carbonate. The organic layer was separated, washed with water (200 ml), dried ($MgSO_4$), and the solvent was evaporated. The residue was crystallized from tetrahydrofuran–benzene (or hexane) to give the *half-acid* (28b) as its trihydrate (101 mg, 96%), m.p. >300 °C (Found: C, 66.6; H, 7.3; N, 8.8. $C_{35}H_{40}N_4O_4 \cdot 3H_2O$ requires C, 66.2; H, 7.3; N, 8.6%; τ (CF_3CO_2H), −0.96 and −0.81 (each 2 H, s, *meso*-H), 5.26—6.02 (8 H, m, CH_2CH_2CO, CH_2CH_3), 6.22 (15 H, s, CH_3 and OCH_3), 6.67 (4 H, t, CH_2CO), and 8.15 (6 H, t, CH_2CH_3); $\lambda_{max.}$ 400 (ε 133 000), 501 (10 000), 571 (4 500), and 622 nm (2 000).

2,6-*Diethyl*-8-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethyl*-4-(2-t-*butoxycarbonylhydrazidoethyl*)*porphyrin* (28d).—The porphyrin half-acid (28b) (137 mg) was suspended in dry methylene chloride (30 ml) and oxalyl chloride (5 ml) was added. The solution was set aside at room temperature for 1 h and then evaporated to dryness under reduced pressure. Residual traces of oxalyl chloride were removed by evaporation with dry ethanol-free chloroform. The residue was redissolved in dry methylene dichloride (100 ml) and a solution of di-isopropylethylamine (66 mg) in methylene dichloride (10 ml) was added. A solution of t-butoxycarbonylhydrazine (35 mg) in methylene chloride (10 ml) was finally added and the solution was stirred at room temperature for 3 h. The solution was heated under reflux for 10 min and then evaporated to dryness under reduced pressure. The residue crystallized from tetrahydrofuran–cyclohexane to give the *porphyrin* (28d) as flocculent needles (152 mg, 92%), m.p. >300 °C (Found: C, 68.2; H, 7.5; N, 12.1. $C_{39}H_{50}N_6O_5$ requires C, 68.6; H, 7.4; N, 12.3%); τ ([$^2H_5$]-pyridine) 0.65 (4 H, s, *meso*-H), 5.74—6.17 (8 H, m, CH_2CH_2CO, CH_2CH_3), 6.39 and 6.46 (9 H and 6 H, each s, CH_3,OCH_3), 7.02 (4 H, t, CH_2CO), 8.14 (6 H, t, CH_2CH_3), and 8.54 (9 H, s, Bu^t); *m/z* 594 (100%; M^+ − BOC), 579, 521, 297, 276, and 260; $\lambda_{max.}$ 400 (ε 156 000), 501 (13 000), 568 (6 000), and 621 nm (3 500).

2,3,7-*Triethyl*-1,4,5,8-*tetramethyl*-6-(2-L-*phenylalanylcarbamoyl Methyl Ester* (9).—6-Carboxyethyl-2,3,7-triethyl-1,4,5,8-tetramethylporphyrin (10 mg) was dissolved in oxalyl chloride (1 ml) and the solution set aside at room temperature for 1 h. The excess of oxalyl chloride was evaporated off under reduced pressure and the last traces were removed by evaporation of benzene (20 ml). The porphyrin was redissolved in methylene dichloride and a solution of L-phenylalanine methyl ester hydrochloride (18 mg) in chloroform (5 ml) through which gaseous ammonia had been bubbled, was added. After 30 min the solution was evaporated to dryness and the residue was chromatographed on alumina (50 g, elution with methylene dichloride). Evaporation of the appropriate eluates gave a residue which was crystallized from methylene dichloride methanol to give the *amide* (6.4 mg, 47%) as purple needles, m.p. 258—259 °C (Found: M^+, 683.385. Calc. for $C_{43}H_{49}N_5O_3$: *M*, 683.384); *m/z* 683 (100%), 549, 478, 463, and 341.5; $\lambda_{max.}$ 399 (ε 165 000), 500 (15 700), 532 (11 300), 568 (7 100), and 630 nm (4 400); $\lambda_{max.}$ ($CHCl_3$–5% CF_3CO_2H) 417 (ε 318 000), 555 (16 000), and 597 nm (8 000).

[1/1618 *Received*, 19*th October*, 1981]

REFERENCES

1 J. M. Pratt in ' Techniques and Topics in Bioinorganic Chemistry,' ed. C. A. McAuliffe, Macmillan, London, 1975, p. 146.

2 R. E. White and M. J. Coon, *Ann. Rev. Biochem.*, 1980, **49**, 315; C. K. Chang and D. Dolphin, *J. Am. Chem. Soc.*, 1976, **98**, 1607; J. P. Collman, T. N. Sorrell, and B. M. Hoffman, *J. Am. Chem. Soc.*, 1975, **97**, 913; D. Mansuy, J.-P. Battioni, J.-C. Chottard, and V. Ullrich, *J. Am. Chem. Soc.*, 1979, **101**, 3971.

3 J. P. Collman, R. R. Gagne, C. A. Reed, J. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, 1975, **97**, 1427.

4 A. R. Battersby, D. G. Buckley, S. G. Hartley, and M. D. Turnbull, *J. Chem. Soc., Chem. Commun.*, 1976, 879; A. R. Battersby and A. D. Hamilton, *ibid.*, 1980, 117.

5 H. Ogoshi, H. Sugimoto, and Z-i Yoshida, *Tetrahedron Lett.*, 1976, 4477 and 4481.

6 J. E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, 1973, **95**, 5757; J. Almog, J. E. Baldwin, R. L. Dyer, and M. Peters, *ibid.*, 1975, **97**, 226.

7 T. Mashiko, J.-C. Marchon, D. T. Musser, and C. A. Reed, *J. Am. Chem. Soc.*, 1979, **101**, 3653.

8 K. M. Smith, L. R. Milgrom, and G. W. Kenner, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2065.

9 P. K. Warme and L. P. Hager, *Biochemistry*, 1970, **9**, 1599.

10 A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc. C*, 1971, 502.

11 P. J. Crook, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc. C*, 1971, 474.

12 A. H. Jackson, G. W. Kenner, K. M. Smith, R. T. Aplin, H. Budzikiewicz, and C. Djerassi, *Tetrahedron*, 1965, **21**, 2913.

13 H. Fischer, O. Süs, and F. G. Weilguny, *Annalen*, 1930, **481**, 159.

14 G. L. Collier, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc. C*, 1967, 66.

15 B. Loer and M. F. Kormendy, *J. Org. Chem.*, 1963, **28**, 3421.

16 A. W. Johnson, R. L. N. Harris, and I. T. Kay, *J. Chem. Soc. C*, 1966, 22.

17 T. Lewis, Research report, University of Liverpool, 1970.

18 A. H. Jackson, G. W. Kenner, G. McGillivray, and K. M. Smith, *J. Chem. Soc. C*, 1968, 294.

19 C. K. Chang, *J. Am. Chem. Soc.*, 1977, **99**, 2819.

20 K. M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1471.

21 H. Fischer and H. Orth, ' Die Chemie des Pyrrols,' vol. II, part 1, Akademische Verlag, Leipzig, 1937, p. 438.

22 H. Fischer and H. Orth, ' Die Chemie des Pyrrols,' vol. II, part 1, Akademische Verlag, Leipzig, 1937, p. 436.