2 Syntheses of Tetrapyrroles

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1. INTRODUCTION

This chapter addresses basic methodology that can be used to obtain tetrapyrrole macrocycles in the porphyrin and chlorin series from natural materials and some simple methods for the total chemical synthesis of typical pyrroles and porphyrins. The aim is to provide investigators with enough information to decide whether to take on the task of preparing samples of useful porphyrin and chlorophyll derivatives or whether to simply purchase them or collaborate with other individuals more expert in the established synthetic procedures. The procedures reported herein are usually those which are easiest for the nonexpert to perform, while at the same time being sufficient to provide pure samples of the required product.

The porphyrin field has a very rich history; Hans Fischer's books present a laboratory approach to synthesis of porphyrin compounds dating back from the 1930s (20,22,24). In 1975, *Porphyrins and Metalloporphyrins* was published (64); this contained a fairly detailed laboratory methods section, which was useful at that time and is probably still useful to many investigators. An up-to-date and highly detailed description of the synthetic art of porphyrin chemistry can be found in *The Porphyrin Handbook* (39).

At the outset it must be mentioned that a certain degree of expertise in experimental organic chemistry is essential for success in the endeavors described herein; also essential are the appropriate laboratory equipment (fume hoods, rotary evaporators, temperature controlled reaction monitors, chromatographic equipment, etc.) and glassware. Since hazardous waste chemicals and solvents will also need to be disposed of, approved facilities for these responsibilities must also be available.

In terms of chemical technique and procedures, pyrrole and porphyrin derivatives tend to be easy to work with. With the exception of porphyrinogens, they usually do not require stringent exclusion of oxygen and water vapor (as is the case with much of the rest of organometallic chemistry), they are stable at room temperature and higher temperatures, and they can be purified by recrystallization and chromatography in the air at room temperature. As might be expected with any colored compound (which will be absorbing

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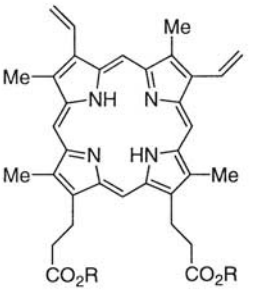
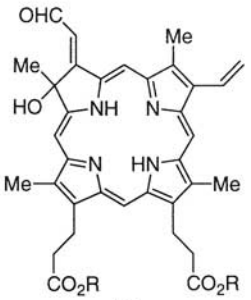
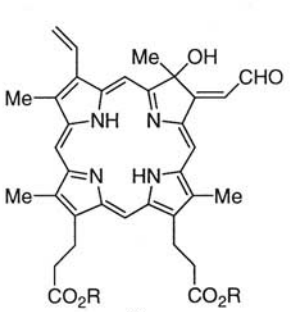
light of various wavelengths and therefore will be accessing excited electronic states—porphyrins fluoresce strongly), attempts should be made routinely to keep porphyrin and chlorin compounds out of the light; this is not difficult, and aluminum foil wrapped around a sample flask or around a chromatography column usually suffices. In the particular case of protoporphyrin IX **[1]** or its dimethyl ester **[2]**, a well-characterized so-called Diels-Alder reaction is known to take place in the presence of oxygen and light to afford a mixture of photoprotoporphyrin and isophotoprotoporphyrin IX dimethyl ester [**3** and **4**, respectively] (7,34); this represents the extreme of normal porphyrin photolability and is caused by the presence of the 3- or 8-vinyl groups. If you can successfully handle protoporphyrin IX without continually



(**1**) R = H (protoporphyrin IX)
(**2**) R = Me (protoporphyrin IX dimethyl ester)



(**3**)



(**4**)

Fischer nomenclature

(**5**)

generating two polar green bands upon chromatography, you should do just fine. Further advice on the specific requirements for handling these molecules can be found in Chapter 3 by Bommer and Hambright.

2. NOMENCLATURE

Over the years, two different schemes for nomenclature of porphyrin systems have been used. The Fischer system for porphyrin nomenclature [structure **5**] provides a link back to the rich history of porphyrin chemistry mentioned above—many trivial names were generated which, particularly in the field of chlorophyll chemistry, are almost impossible to do without. Likewise, in the porphyrin field, there are some names that are indispensable (e.g., protoporphyrin IX, the "first" porphyrin, and deuteroporphyrin IX, the "second" porphyrin); the "IX" given after the porphyrin name refers to the (secondary) type-IX arrangement of the porphyrin substituents. When there are only two types of substituent, for example methyl and ethyl, with one methyl and one ethyl on each pyrrole ring, only four "primary type-isomers" **[6–9]** of the so-called "etio"porphyrins are possible. When there are three kinds of substituent (as with the methyl, vinyl, and propionic substituents in protoporphyrin IX), no less than fifteen "secondary type-isomers" are possible (provided there is one methyl on each pyrrole subunit), and the type-IX isomer is the biologically significant one. In the primary type isomer series, type-III is the biologically significant arrangement. But all that said, and given the near impossibility of naming some porphyrin and chlorophyll derivatives without the use of Fischer's trivial names, the International Union of Pure and Applied Chemistry (IUPAC) system of nomenclature [structure **10**] is the officially adopted nomenclature system, and this will have to be used in this chapter.

3. PREPARATION OF PORPHYRINS AND CHLORINS BY DEGRADATION OF NATURAL PIGMENTS

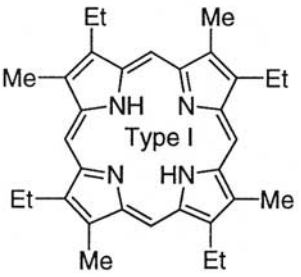
It is truly fortunate that massive amounts of natural products containing both hemin **[11]** and chlorophylls *a* and *b* **[12,13]** can be accessed. Fischer's three volumes (20,22,24), *Die Chemie des Pyrrols*, report an astonishing array of procedures for obtaining tetrapyrrole compounds from natural sources. Thus, large volumes of blood can be processed to provide hemin in kilogram quantities. From hemin, a large number of porphyrins and derivatives can be obtained (see later). Similarly, chlorophyll derivatives in the *a* and *b* series can be obtained by extraction of leaves, usually spinach. But if only chlorophyll *a* derivatives are desired, one can take advantage of the fact that certain algae, such as *Spirulina*, produce only chlorophyll *a*; thus, a laborious separation of the chlorophyll *a* and *b* series can be avoided. If chlorophyll *b* derivatives are required, there used to be no option but to extract plant chlorophylls and perform the chromatographic separation, either by preparative scale high-performance liquid chromatography (HPLC) or by gravity column chromatography on sucrose. Some years ago, *a* chemical derivatization approach was developed to make the chromatographic separations more palatable, and that will be discussed later.

3.1. Porphyrins from Hemoglobin

3.1.1. Hemin [11]

Because of the relative ease with which hemin can be obtained from blood, it can be purchased from a number of chemical companies at costs around a few dollars per gram. The method of choice (19) for preparation of hemin from blood involves addition of heparinized, citrated, or defibrinated blood to hot acetic acid containing sodium

chloride. After cooling and removal of coagulated protein (usually with a wooden stick), the hemin separates and can be collected by filtration. Alternatively, the messy protein can be precipitated by addition of a solution of strontium chloride, followed by concentration to give hemin as above (16,44). Hemes [iron(II) porphyrins] can be obtained from hemins [iron(III) chloride porphyrins] most commonly by reduction with sodium dithionite under nitrogen or argon. Since autoxidation of iron(II) to iron(III) porphyrins is very facile in air, use of nitrogen or (preferably the heavier) argon



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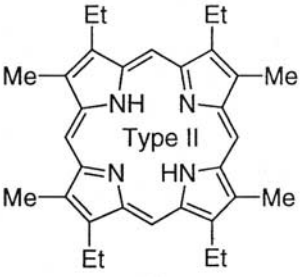
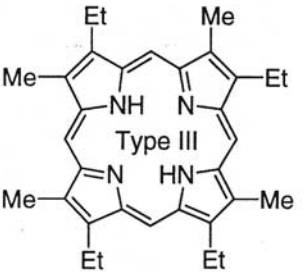


(7)

The four etioporphyrin type-isomers



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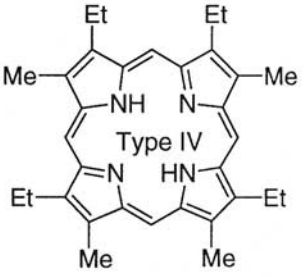

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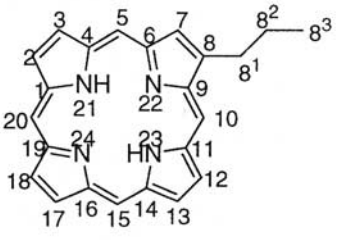


IUPAC nomenclature

(10)

is absolutely essential. Chromatographic purification of hemins is best accomplished on the corresponding (usually methyl) esters; but hemins [e.g., **11**] bearing carboxylic acid groups should not be esterified with diazomethane—a side-reaction takes place with the iron atom. For methyl esters (the simplest and best ester to use under normal circumstances), 5% sulfuric acid in methanol is the best mixture to use (**CAUTION**: take care to gently add the acid to the stirred and cooled alcohol) (66). Hemin esters can be hydrolyzed to the corresponding free carboxylic acids using base (66).

3.1.2. Protoporphyrin IX [1]

Protoporphyrin IX **[1]** is the product obtained by removal of iron from hemin **[11]**, but acid alone does not accomplish this result because iron(III) is very difficult to eject from a porphyrin. Commercial samples of protoporphyrin IX are usually not very pure because of the sensitivity of protoporphyrin to photo-oxygenation at the vinyl groups (see above). The best method for obtaining protoporphyrin IX is to treat hemin **[11]** with ferrous sulfate in hydrochloric acid (46,51,52); the hemin is reduced to heme, and the iron(II), in strict contrast to iron (III), is readily removed by the acid. Commercial hematoporphyrin IX **[14]** is often very pure (unlike protoporphyrin IX), so a method for the preparation of **[1]** by double dehydration of hematoporphyrin IX **[14]** has been reported (40). This involves brief heating of **[14]** with toluene *p*-sulfonic acid in 1,2-dichlorobenzene. The dimethyl ester **[2]** of protoporphyrin IX can be obtained by esterification with either diazomethane (**CAUTION**: diazomethane can be explosive under certain circumstances) or with methanol–sulfuric acid (**CAUTION**) as mentioned above for hemin. The very useful Grinstein method (33) can be used to prepare protoporphyrin IX dimethyl ester **[2]** in one step from hemin **[11]**.

3.1.3. Mesoporphyrin IX [15]

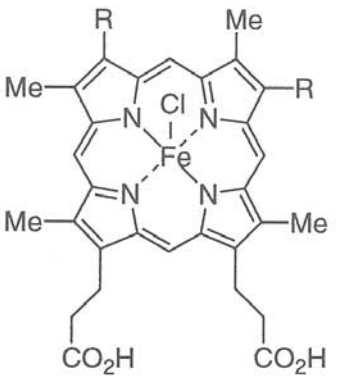
Mesoporphyrin IX **[15]** is related to protoporphyrin IX **[1]** with the important difference that the sensitive 3- and 8-vinyl groups in **[1]** are replaced with durable ethyl groups—hence, mesoporphyrin IX does not undergo the photo-oxygenation reaction mentioned above for protoporphyrin. Early biosynthetic investigations of the metabolism of protoporphyrin IX often used the easy to handle mesoporphyrin IX **[15]**, and so incorporated a hydrogenation step to accomplish reduction of the 3- and 8-vinyl groups in protoporphyrin IX (9); the method of choice (22) is catalytic hydrogenation over palladium in formic acid. Either protoporphyrin IX, its ester, or hemin are used, and the iron in **[11]** is removed concomitantly during the reaction. Mesohemin IX **[16]**, the iron(III) chloride of mesoporphyrin IX, is best obtained by the introduction of iron into **[15]** rather than by reduction of hemin **[11]**. Esterification of mesoporphyrin IX can be carried out using either diazomethane or sulfuric acid acid–alcohol.

3.1.4. Hematoporphyrin IX [14]

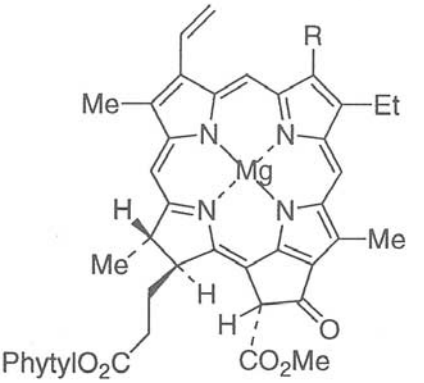
Hematoporphyrin IX [**14**] was the first porphyrin to be isolated (in 1867) (69); it was obtained by treatment of blood with concentrated sulfuric acid. Nominally, hematoporphyrin IX [**14**] is obtained chemically from protoporphyrin by hydration of both of the 3- and 8-vinyl groups. Since the 3^1- and 8^1-carbon atoms are chiral in [**14**], a mixture of four optical isomers (enantiomers and diastereomers) is obtained, and these can be separated by HPLC. Porphyrin [**14**] can also be purchased from commercial sources.

Using protoporphyrin IX [**1**] as the starting material, hematoporphyrin IX is best prepared by treatment with hydrogen bromide in acetic acid, followed by hydrol-

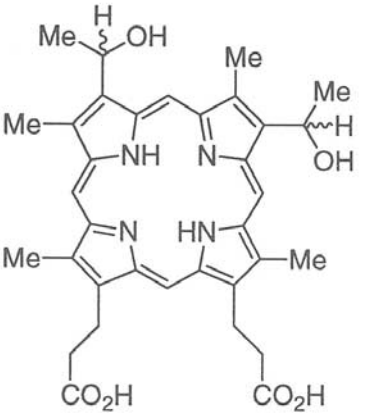
ysis of the resulting 3,8-di(1-bromoethyl)-derivative [**17**] with water (22). If a common alcohol (R^1OH) such as methanol ($R^1 = CH_3$) is used in this last stage, then the 3,8-di(1-alkoxyethyl) analogue [**18**] is produced. Alternatively, reduction of 3,8-diacetyldeuteroporphyrin IX dimethyl ester [**19**] with sodium borohydride affords hematoporphyrin IX dimethyl ester [**20**] [e.g., Reference 66]. 3,8-Diacetyldeuteroporphyrin IX [**21**] can be prepared by oxidation of hematoporphyrin IX (62), or by Friedel-Crafts acetylation of deuterohemin IX [**22**] using acetic anhydride and pyridine, followed by removal of the iron (66). Use of sulfuric acid and methanol to esterify the propionic acids in [**14**] is not advised because acid-catalyzed dehydra-



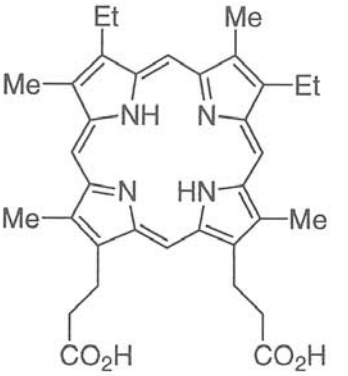
(**11**) R = $CH{=}CH_2$ (hemin)
(**16**) R = Et (mesohemin)



(**12**) R = Me (chlorophyll *a*)
(**13**) R = CHO (chlorophyll *b*)



(**14**) (hematoporphyrin IX)



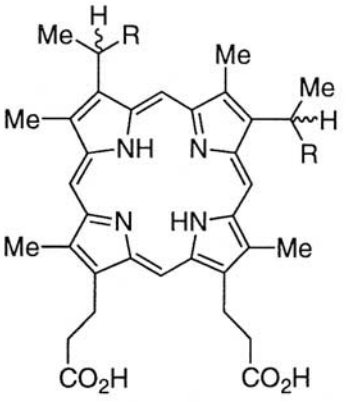
(**15**) (mesoporphyrin IX)

tion, or ether formation, at the 3,8-(1-hydroxyethyl) groups is a problem; it is best to use diazomethane in methanol to obtain the dimethyl ester [**20**] (CAUTION).

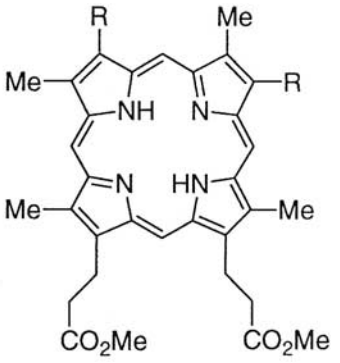
3.1.5. Deuteroporphyrin IX [23]

Deuteroporphyrin IX [**23**] is of significant historical importance because it was the first porphyrin isolated in Fischer's Nobel Prize winning synthesis of hemin [**11**] (29). Deuterohemin [**22**] can be obtained from "proto" hemin by brief heating of [**11**] in a resorcinol melt (60), via the so-called Schumm reaction in which the vinyl groups are replaced by hydrogen atoms (10,12,17,42). Demetalation, as reported above for the preparation of protoporphyrin IX from hemin, then affords deuteroporphyrin IX [**23**].

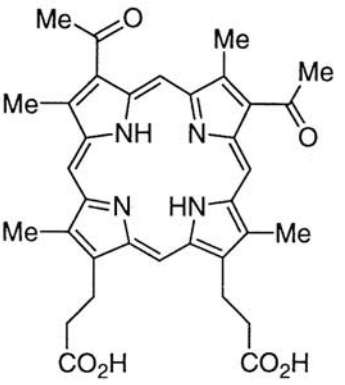
Numerous 3,8-disubstitution products (and 3- or 8-monosubstitution analogues)



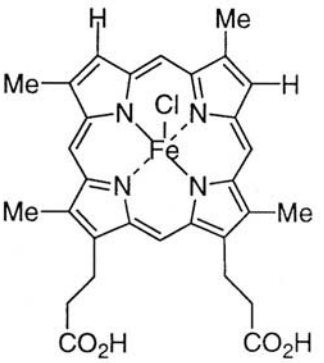
(**17**) R = Br
(**18**) R = OR^1



(**19**) R = COMe
(**20**) R = CH(OH)Me



(**21**) (diacetyldeuteroporphyrin IX)



(**22**) (deuterohemin)

of deuteroporphyrin IX and its esters can be prepared, usually by way of aromatic electrophilic substitution on the hemin or its copper(II) complex. A typical example is 3,8-diacetyldeuteroporphyrin IX [**21**] (see above), which was also an intermediate in the Fischer's hemin total synthesis.

3.1.6. Coproporphyrin III [24]

Coproporphyrin III [**24**] is a biologically significant porphyrin because its hexahydroderivative, coproporphyrinogen III [**25**], is a colorless intermediate on the pathway between uroporphyrinogen III [**26**], protoporphyrinogen IX [**27**], and protoporphyrin IX [**1**] in normal porphyrin metabolism. Under normal circumstances, the amount of **[25]** present at steady state is small. However, biological oxidation of coproporphyrinogen III yields the colored coproporphyrin III, which takes it out of the normal metabolic sequence. Hence, certain diseases of porphyrin metabolism can result in a buildup of excess photochemically active porphyrins in tissues; such diseases are known collectively as porphyrias. Chemically, porphyrinogens can be oxidized very efficiently to porphyrins by use of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). If biosynthetic work using porphyrinogens is to be carried out, the corresponding porphyrin can usually be reduced to porphyrinogen using sodium amalgam or catalytic hydrogenation (15). When vinyl groups are present on the porphyrin macrocycle, of course, only the sodium amalgam route is recommended—catalytic hydrogenation will probably reduce the vinyls to ethyls. It must be kept in mind when handling porphyrinogens, that oxygen and light can efficiently oxidize the hexahydro material to the porphyrin level, which will make it inactive in biosynthetic investigations—the first true porphyrin in porphyrin biosynthesis is protoporphyrin IX itself.

3.2 Porphyrins and Chlorins from Plants and Algae

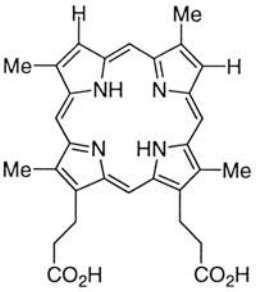
In this section, some simple degradation reactions, which furnish porphyrins and chlorins in useful quantities from plants and algae, will be described.

The traditional source for chlorophylls *a* [**12**] and *b* [**13**], usually present in a ratio of about 3:1, was leaf tissue, usually spinach (25,68). A very useful chemical adjunct for simplification of the mandatory chromatographic separation of the chlorophyll *a* and *b* pigments has been reported (41); it employs the Girard reagent T as a means of dramatically increasing the polarity of the series *b* component in the mixture. For example, reaction of methyl pheophorbide *a* [**28**] and *b* [**29**] mixture (see above) with Girard's reagent T gives a mixture consisting of unreacted *a* series compound, i.e., methyl pheophorbide *a* [**28**], and the salt [**30**] from the *b* series. Column chromatography then achieves a very simple separation in which [**30**] remains adsorbed to the top of the column, whereas the relatively nonpolar *a* series compound [**28**] is eluted quickly. Use of a polar solvent then elutes the *b* series salt, which can be hydrolyzed to give pure methyl pheophorbide *b* [**29**].

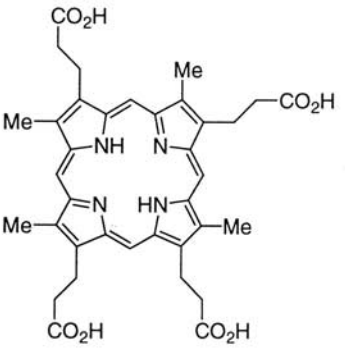
Investigators wishing only to deal with chlorophyll derivatives in the *a* series were advantaged when it was shown that *Spirulina maxima* (from Mexico) or *S. pacifica* (from Hawaii) contain only the chlorophyll *a* series of pigments. On account of the fairly drastic extraction conditions, chlorophyll *a* itself is usually not obtained directly from the alga, but large quantities of pheophytin *a* [**31**] and methyl pheophorbide *a* [**28**] (up to 0.4% measured by dry weight) can be obtained (67).

Treatment of the plant chlorophylls (either separately or as a mixture) with mild acid gives the metal-free pheophytins *a* [**31**] and *b* [**32**]; this, as a dried paste, is

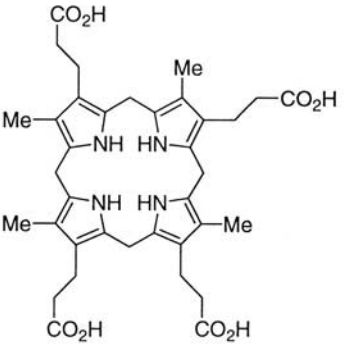
usually the form in which the pigments are stored prior to further degradation to useful materials. Hydrolysis of the pheophytins gives the corresponding pheophorbides *a* [**33**] and *b* [**34**]; (note that the pheophorbides still contain one ester, and that hydrolysis of this ester will cause concomitant decarboxylation on ring E). Alternatively, and preferably (for ease of handling), methanolysis of pheophytin *a*



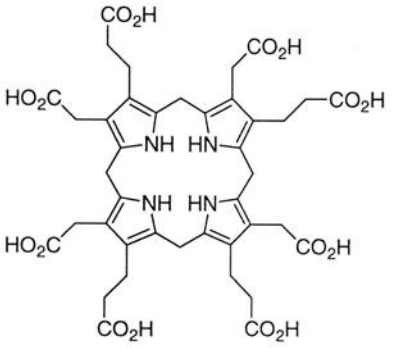
(**23**) (deuteroporphyrin IX)



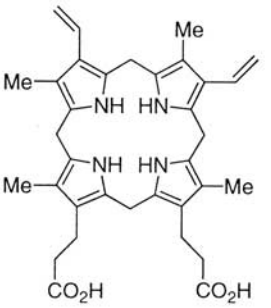
(**24**) (coproporphyrin III)



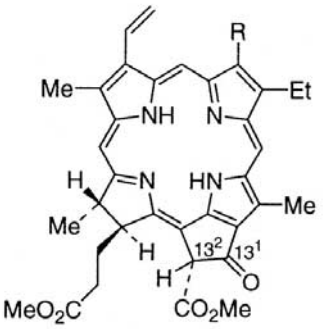
(**25**) (coproporphyrinogen III)



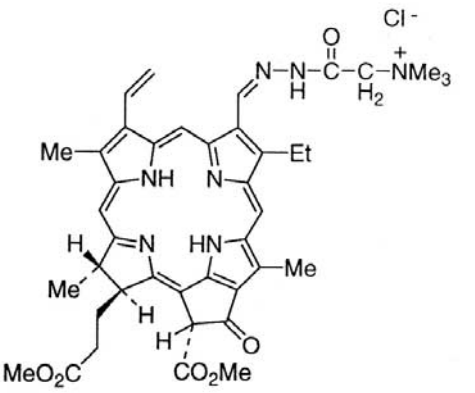
(**26**) (uroporphyrinogen III)



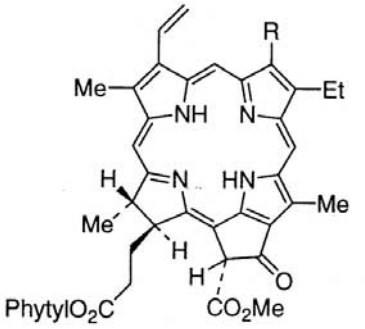
(**27**) (protoporphyrinogen IX)



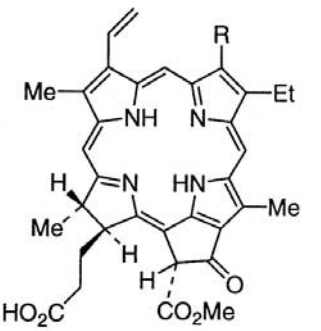
(**28**) R = Me (methyl pheophorbide *a*)
(**29**) R = CHO (methyl pheophorbide *b*)



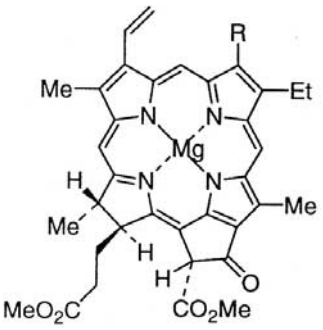
(**30**)



(**31**) R = Me (pheophytin *a*)
(**32**) R = CHO (pheophytin *b*)



(**33**) R = Me (pheophorbide *a*)
(**34**) R = CHO (pheophorbide *b*)

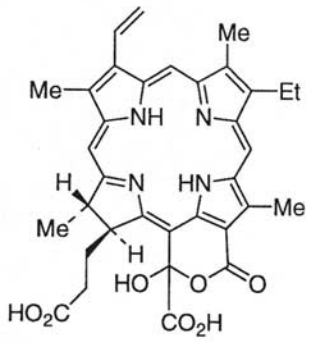


(**35**) R = Me
(**36**) R = CHO

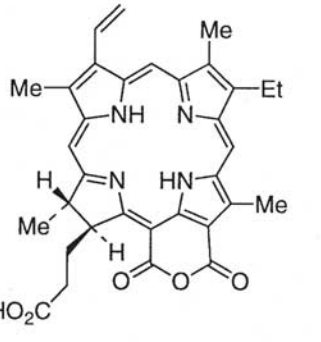
or *b* provides the corresponding methyl pheophorbides *a* or *b* [**28** or **29**, respectively]—these contain two methyl esters. Transesterification of the phytyl ester for methyl, without removal of the magnesium atom, can be accomplished to afford the methyl chlorophyllides [**35**] and [**36**] (26).

A number of simple to perform but mechanistically complex reactions can be carried out on chlorophyll derivatives. For example, oxidation of pheophytin *a* [**31**] under highly alkaline conditions accomplishes cleavage the 13^1-13^2 bond in the β-ketoester ring E, with hydrolysis of the of phytyl ester, to give Fischer's "unstable chlorin" [**37**] (28). Simple evaporation of the solution affords the so-called purpurin 18 [**38**], which bears a very useful anhydride ring [**45**]. On the other hand, diazomethane esterification (**CAUTION**) yields purpurin 7 trimethyl ester [**39**] (26–28,45). Heating of [**39**] in collidine gives a diversely substituted porphyrin, 3-vinylrhodoporphyrin XV dimethyl ester [**40**] (28). If the so-called "meso" (i.e., 3-ethyl instead of 3-vinyl) series of pigments is used, another porphyrin, rhodoporphyrin XV dimethyl ester [**41**], is obtained.

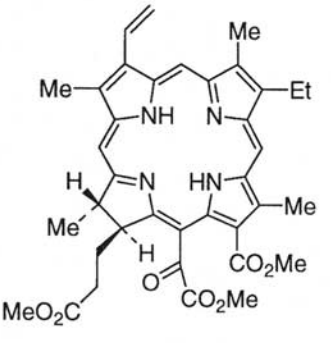
The isocyclic ring (E) in chlorophylls and their derivatives contains a β-ketoester function which imparts a high degree of chemical reactivity upon the compounds containing it. Such lability is often a disadvantage in the use of chlorophyll derivatives



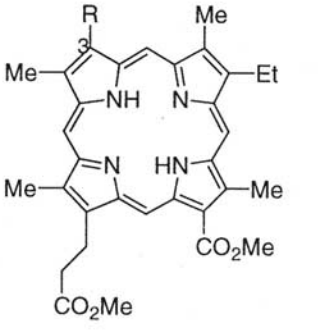
(**37**) ("unstable chlorin")



(**38**) (purpurin 18)

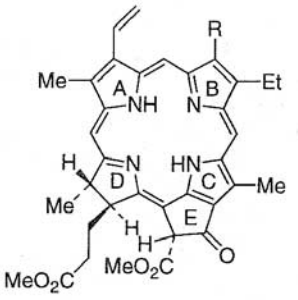


(**39**) (purpurin 7 trimethyl ester)

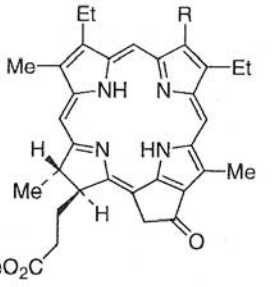


(**40**) R = $CH{=}CH_2$ (3-vinylrhodoporphyrin XV dimethyl ester)
(**41**) R = Et (rhodoporphyrin XV dimethyl ester)

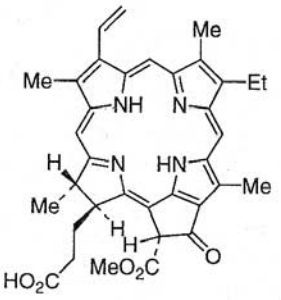
for specific purposes; the spectrum of chemical reactivity in the ring E portion of the pigments can be dramatically decreased by removal of the 13^2-CO_2Me group. When the 13^2-CO_2Me group is removed, the so-called "pyro" series of chlorophyll derivatives are obtained. Basically, ketones are much less reactive than are conjugated ketoesters. Thus, heating of methyl pheophorbide *a* [**28**] (or *b* [**29**]) in collidine (30) gives methyl pyropheophorbide *a* [**42**] (or *b* [**43**]) in virtually quantitative yield; use of collidine is a yield-enhancing improvement upon the classical method (28) which uti-



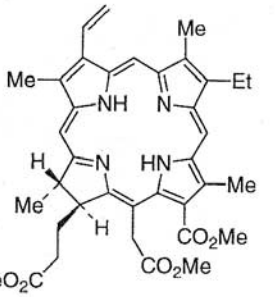
(**28**) R = Me
(**29**) R = CHO



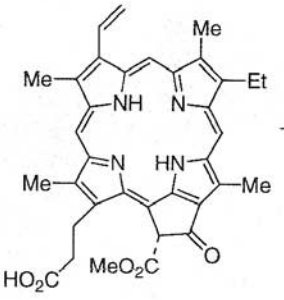
(**42**) R = Me
(**43**) R = CHO



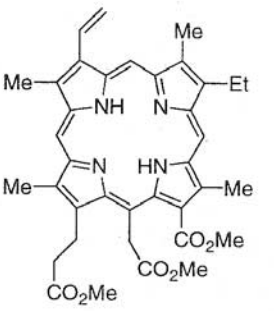
(**33**)



(**45**) (chlorin e_6 trimethyl ester)



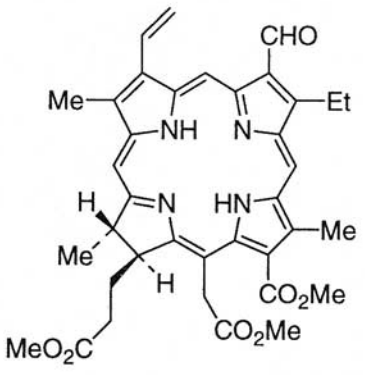
(**44**)



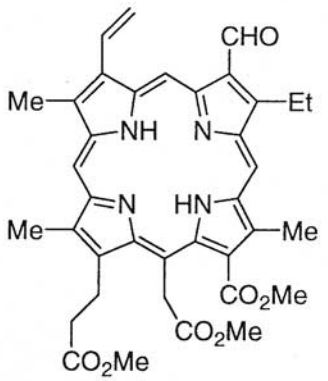
(**46**) (chloroporphyrin e_6 trimethyl ester)

lized pyridine. Identical demethoxycarbonylation reactions take place with the so-called meso- (3-ethyl) series of compounds.

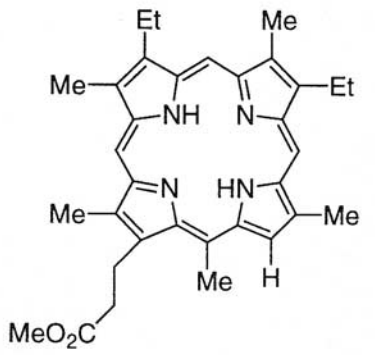
The 5-membered isocyclic ring in the pyro-series of chlorophylls cannot be cleaved, but the ring E in its β-ketoester form can be readily opened since the highly reactive conjugated functionality provides a handle for chemical elaboration of ring E. For example, pheophorbide a [**33**] and 3-vinylpheoporphyrin a_5 [**44**, vide infra] can be treated with alkali to give, after esterification with diazomethane, chlorin e_6 trimethyl ester [**45**] and chloroporphyrin-e_6 trimethyl ester [**46**], respectively (24). Methanolysis of pheophorbide *a* also affords [**45**]. This reaction can be reversed, and ring E is reformed either by treatment with methoxide (24), with *tert*-butoxide (65), or best of all using triphenylphosphine and bis(trimethylsilyl) amide (31).



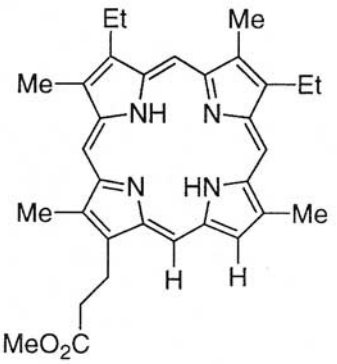
(47) (rhodin g_7 trimethyl ester)



(48) (rhodinporphyrin g_7 trimethyl ester)



(49) (phylloporphyrin XV methyl ester)



(50) (pyrroporphyrin XV methyl ester)

Although the chlorophyll *b* series of pigments is less accessible than those from chlorophyll *a* (and indeed, as mentioned above, *Spirulina* algae contains no chlorophyll *b*) a series of reactions parallel to those described above also occurs in the *b* series; the analogue of chlorin e_6 trimethyl ester in the *b* series is called rhodin g_7 trimethyl ester [**47**] and of chloroporphyrin e_6 trimethyl ester is rhodinporphyrin g_7 trimethyl ester [**48**].

Chlorins can be converted into porphyrins by using DDQ as a dehydrogenation agent. The β-ketoester functionality does not take kindly to oxidative stress, so methyl pheophorbide *a* [**28**] gives only a low yield of 3-vinylpheoporphyrin a_5 dimethyl ester [**44**]. Using a "sledgehammer" approach to preparation of porphyrins from chlorophyll derivatives, chlorophyll *a* under very vigorous basic conditions followed by esterification (methanolysis), affords phylloporphyrin XV methyl ester [**49**] and pyrroporphyrin XV methyl ester [**50**] (23).

❖ Procedure 1. Isolation of Methyl Pheophorbide *a* [28] from *S. maxima* (67)

1. About 500 g of dried *S. maxima* alga is slurried in 2 L of acetone, and then liquid nitrogen is added to form a frozen slush.
2. After transferring to a 5-L 3-necked round-bottom flask, the mixture is heated at reflux with mechanical stirring for 2 hours. The supernatant is filtered through a Whatman filter paper (Whatman, Clifton, NJ, USA) using a Buchner funnel, and more acetone is added to the solid debris.
3. The extraction process, with refluxing, is repeated twice more—note that the debris retains its deep green color, but amounts of additional chlorophyll obtained are marginal.
4. The green filtrate is evaporated and then purified by flash chromatography on Grade V neutral alumina, eluting first with n-hexane to remove a fast running yellow band, with dichlormethane to remove the major blue-gray pheophytin *a* band, and finally with 97:3 dichloromethane:tetrahydrofuran to remove some bright green magnesium-containing pigments.
5. The evaporated pheophytin *a* fraction is treated with 500 mL of 5% sulfuric acid in methanol (degassed by bubbling with nitrogen gas) for 12.5 hours at room temperature in the dark (aluminum foil) under nitrogen, followed by dilution with dichloromethane, and rinsing with water.
6. The mixture is rinsed with 10% saturated aqueous sodium bicarbonate, the organic layer is dried over anhydrous sodium sulfate, followed by evaporation and crystallization of the residue from dichloromethane:methanol. This gives methyl pheophorbide *a* [**28**] (average yield 1.8 g).

4. CHEMICAL SYNTHESES OF PORPHYRINS

Porphyrin chemical synthesis will be discussed here in connection with two series of compounds: *(i)* those porphyrins which have been most often used in connection with model studies, e.g., 5,10,15,20-tetraphenylporphyrin (TPP) [**51**] and 2,3,7,8,12,13,17,18-octaethylporphyrin (OEP) [**52**]; and *(ii)* those related to protoporphyrin IX [1]. Simply based on the symmetry in the substituent arrays of [**51**] and [**52**] and the lack of symmetry in [**1**], it is obvious that it would be a waste of time to approach the synthesis of both series of compounds using the same strategy. To attempt the synthesis of OEP [**52**] by labo-

rious multistep construction of an open-chain tetrapyrrolic intermediate would be plainly unwise—such symmetrically substituted compounds are most efficiently obtained by tetramerization of a suitable monopyrrole (see below). On the other hand, there is no way (in the absence of enzymes) that protoporphyrin IX [**1**] can be synthesized chemically by monopyrrole chemical self-condensation, so a more sophisticated chemical approach is essential. As it happens, porphyrins [**51**] and [**52**] can be synthesized by self-condensation of monopyrroles, while protoporphyrin IX [**1**] can be accessed by a number of routes, the most simple (and the one to be used as an example in this chapter) being from dipyrroles.

4.1. Syntheses of Pyrroles

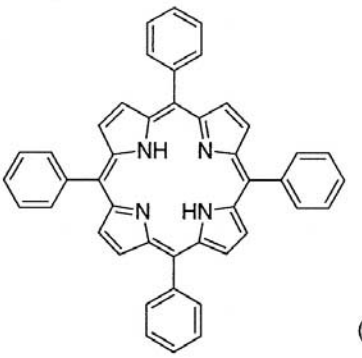
For both series of compounds mentioned above, it is first essential to synthesize monopyrroles. Pyrrole itself [**53**] is commercially available. Syntheses of two common examples of useful pyrroles (from the many dozens in the literature) (5,20,32,37,38) will be illustrated here.

Pyrroles bearing peripheral substituents are those which are most useful for application to dipyrrole and porphyrin synthesis. The Johnson–Kleinspehn synthesis (11,43) is perhaps the most useful for tetra-substituted pyrroles. For example, pyrrole [**54**], bearing very useful methyl and propionate groups, is prepared by the reaction of dione [**55**] with benzyl oximinoacetoacetate [**56**]—compound [**56**] is in turn obtained by the reaction of benzyl acetoacetate [**57**] with sodium nitrite in the presence of acetic acid. Slow admixture of equimolar amounts of [**55**] and [**56**] and excess zinc powder and sodium acetate in hot acetic acid results in reduction of the oximinoderivative [**56**] to the amine, followed by in situ condensation with [**55**] to give pyrrole [**54**]. Simply pouring the cooled reaction mixture into iced water causes precipitation of the product pyrrole. The reaction works with a variety of substituents on the central (i.e., 2-) carbon of the 1,3-dione and with a variety of esters on the acetoacetate. The reaction described above (using acetoacetates) is the Johnson version, while the Kleinspehn modification employs oximinomalonic esters in place of the acetoacetates.

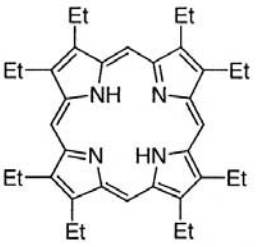
Compared with the above synthesis of pyrroles, methodology for preparing pyrroles such as [**58**] is relatively new. A major advance in the field was made when the Barton–Zard pyrrole synthesis was reported (8); the importance of this route was related to the substituent patterns which could be accessed using it. Thus, treatment of a nitroalkene [**59a**] or its synthetic precursor, an acetoxynitroalkane [e.g., **59b**], with an isocyanoacetate [e.g., **60**] affords excellent yields of pyrroles such as [**58**].

❖ Procedure 2. Synthesis of Ethyl 3,4-Diethylpyrrole-2-Carboxylate [58] (55)

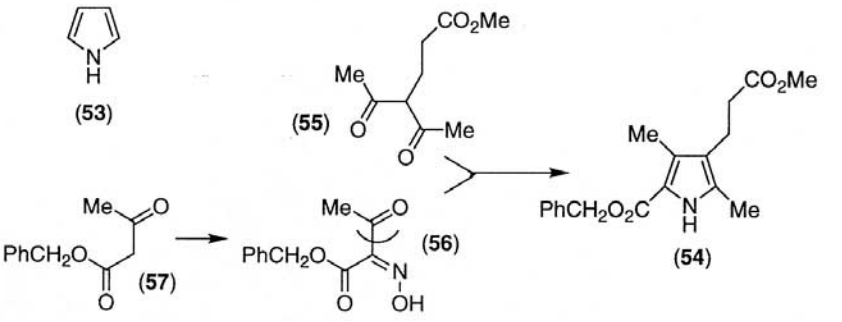
1. A mixture of 3-acetoxy-4-nitrohexane [**59b**] (8)(16.3 g), ethyl isocyanoacetate [**60**] (9.8 g; Sigma, St. Louis, MO, USA), and 1,8-diazabicyclo[5.4.0] undec-7-ene (26.4 g; Sigma) in tetrahydrofuran (100 mL) is stirred at 20°C for 12 hours.
2. The mixture is poured into water containing 1 M HCl and then extracted with ethyl acetate.
3. The extracts are washed with water and dried over anhydrous magnesium sulfate.
4. After evaporation of the solvents, the residue is chromatographed on a column of silica gel eluted with hexane: dichloromethane mixtures.

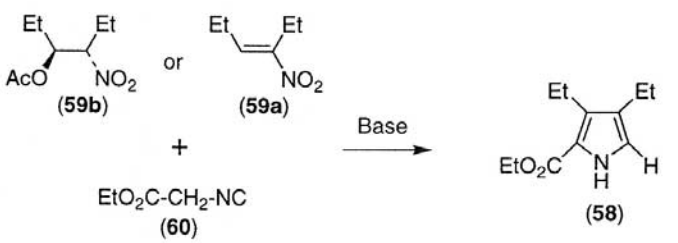


(**51**) (5,10,15,20-tetraphenylporphyrin, "TPP")



(**52**) (2,3,7,8,12,13,17,18-octaethylporphyrin "OEP")

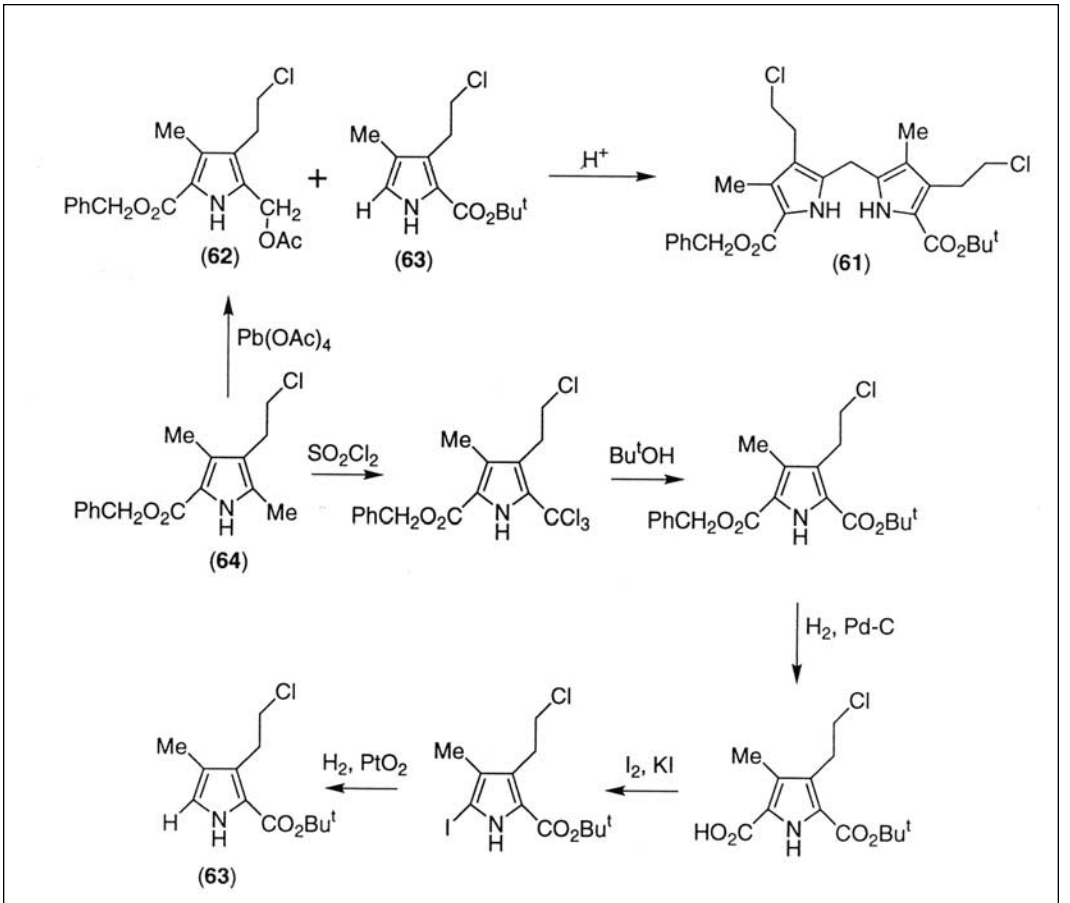




5. Evaporation of the eluates containing the red band will give the required pyrrole [**58**], with an average yield of 14.2 g.

4.2. Syntheses of Dipyrromethanes

Unsymmetrically substituted dipyrromethanes, [e.g., **61**], can be prepared by condensation of 2-acetoxymethylpyrroles [**62**] with 2-unsubstituted pyrroles [**63**] in acetic acid containing a catalytic amount (<0.1 equiv.) of toluene *p*-sulfonic acid (13). Montorillonite K-10 clay has also been shown to be a very useful acid catalyst in dipyrromethane syntheses (30,36); the advantage of using the clay is that it can be removed simply by filtration after the reaction is complete. Compound [**62**] is obtained from the corresponding methylpyrrole [**64**] simply by treatment with lead tetra-acetate. Note that pyrrole [**64**] possesses the same "symmetry pattern" as does pyrrole [**54**]; its synthesis is relatively straightforward (21). Pyrrole [**63**] can be obtained from [**64**] by following a sequence of reactions as shown in Scheme 1. The dipyrromethane [**61**] will form rings A and B of protoporphyrin IX dimethyl ester [**2**] in the synthesis, which will be described later. The 1- and 9-car-



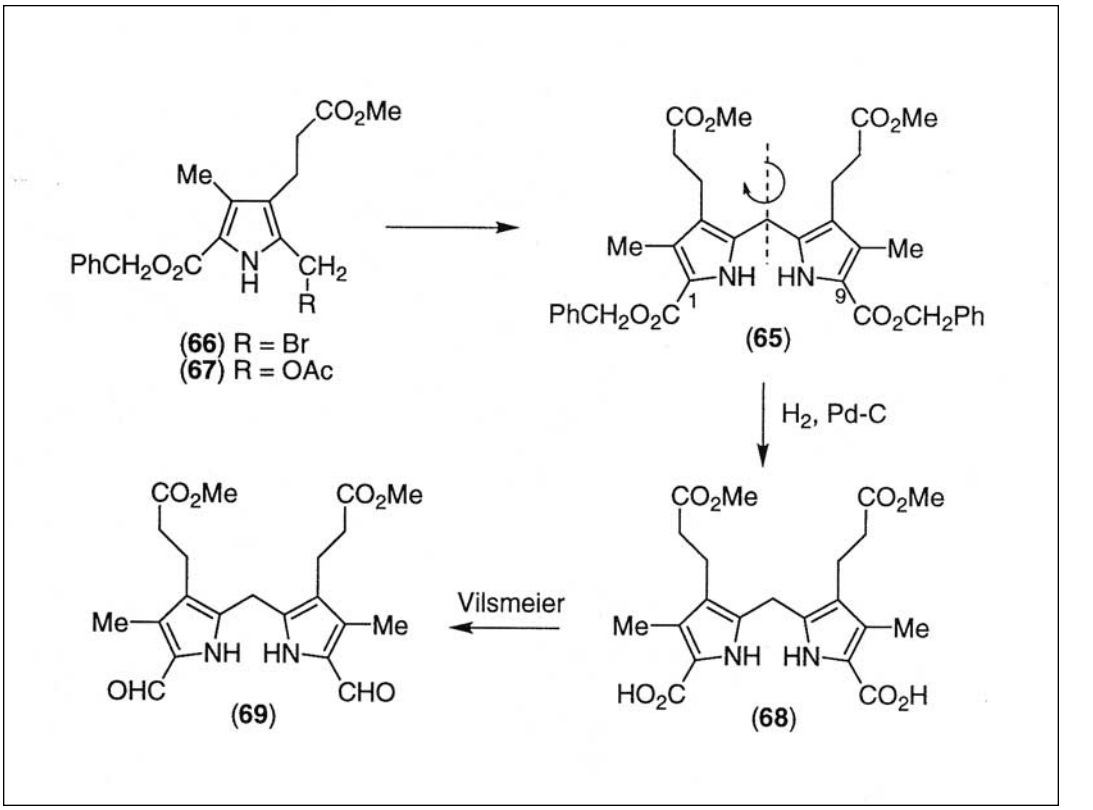
Scheme 1

boxylate substituents are differentially protected, and the future vinyl groups are protected as 2-chloroethyls.

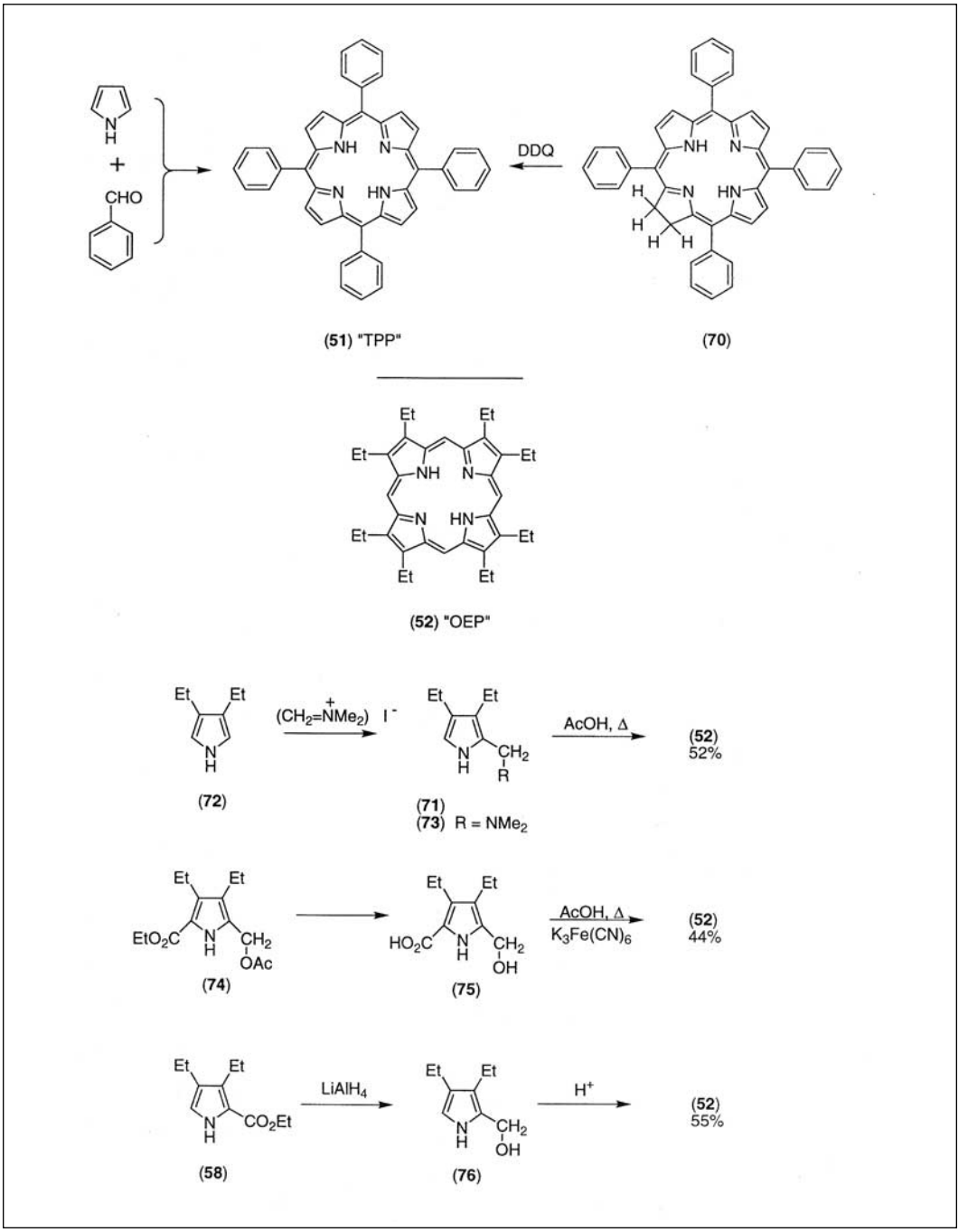
Symmetrically substituted dipyrromethanes [e.g., **65**] are best prepared in one step by self-condensation of bromomethylpyrroles [e.g., **66**] in hot methanol (21), or by heating 2-acetoxymethylpyrroles [e.g., **67**] in methanol-hydrochloric acid (50). The 1- and 9-benzyl esters can be cleaved using catalytic hydrogenation with hydrogen gas and 5% (or 10%) palladium–carbon as catalyst. The resulting 1,9-dicarboxylic acid [**68**] can then be formylated using the Vilsmeier reagent (phosphoryl chloride or benzoyl chloride mixed with equimolar amounts of dimethylformamide) to give [**69**]. The 1- and 9-formyl groups serve as the bridging carbons in the MacDonald porphyrin macrocyclization, which will be described later.

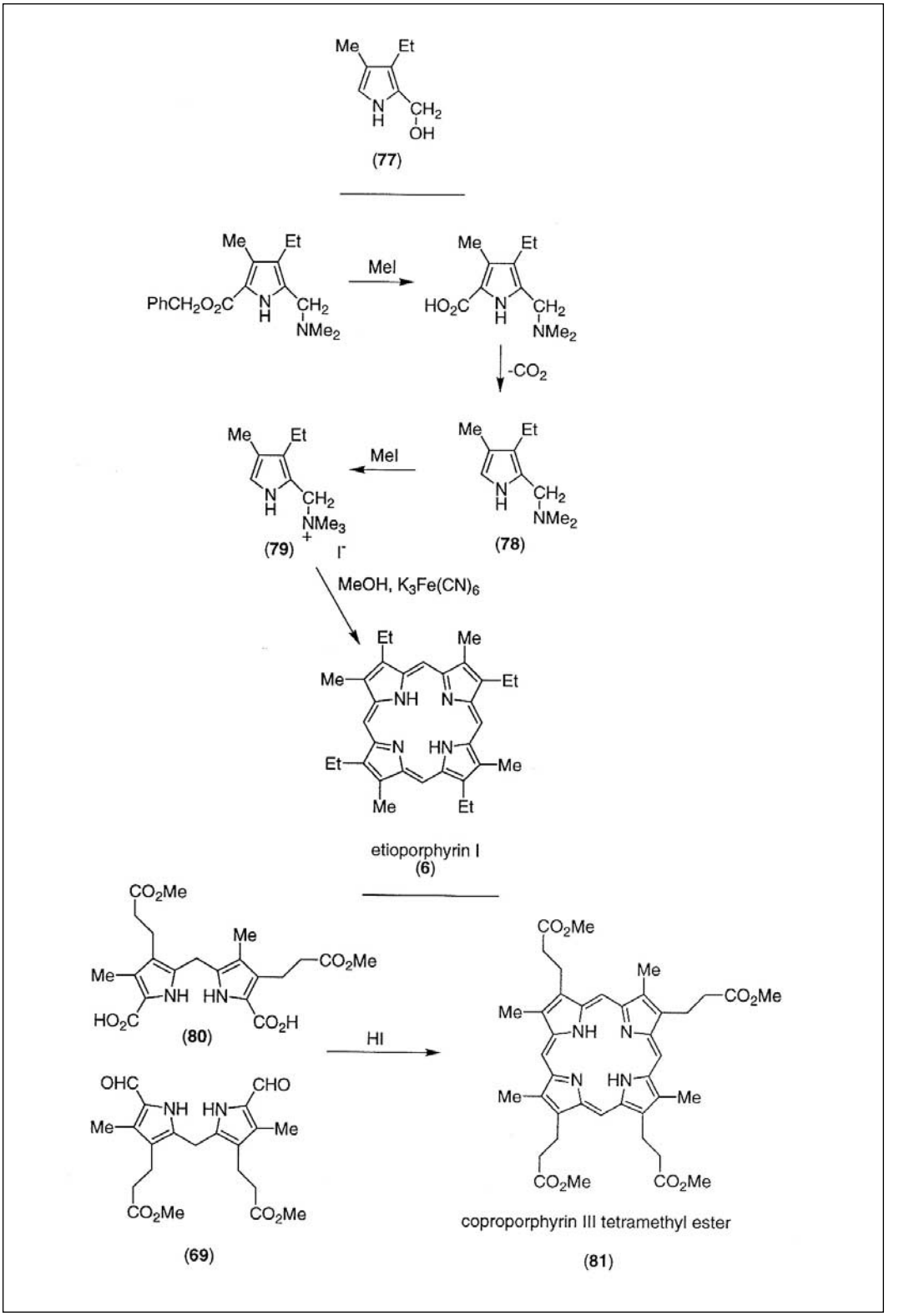
4.3. Porphyrins via Monopyrrole Tetramerization

By definition, tetramerization of monopyrroles must result either in a single symmetrically substituted porphyrin (if the 3- and 4-substituents are identical) or in a mixture of porphyrins (if the 3- and 4-substituents are different—see later). By far, the easiest way to prepare a porphyrin involves the reaction of pyrrole [**53**] with benzaldehyde. The product is the almost legendary TPP [**51**]. This simple route was first reported by Rothemund (57,58) and, after modification by Adler, Longo and colleagues (involving use of refluxing propionic acid instead of sealed tube chemistry) (1), was finally optimized as a two-step procedure by Lindsey's group (48). The nonexpert procedure that is easiest to follow for the synthesis of [**51**] involves addition of equimolar amounts of crude (undis-



tilled) pyrrole [**53**] and benzaldehyde to refluxing propionic acid. After heating for about 30 minutes, the mixture is allowed to cool, and the TPP is filtered off, usually in 20% to 22% yield. The propionic acid can be recovered by distillation and then reused. Higher yields of TPP can be obtained by use of more elaborate and expensive chemistry, but, for TPP, quick and dirty seems to work well. The product




Me
Et
N
H
CH2
OH
(77)
Me
Et
PhCH2O2C
N
H
CH2
NMe2
MeI
Me
Et
HO2C
N
H
CH2
NMe2
-CO2
Me
Et
N
H
CH2
NMe3
+
I-
(79)
MeI
Me
Et
N
H
CH2
NMe2
(78)
MeOH, K3Fe(CN)6
Et
Me
Me
NH
N
Et
N
HN
Et
Me
Me
Et
etioporphyrin I
(6)
CO2Me
Me
Me
NH
HN
CO2Me
HO2C
CO2H
(80)
OHC
CHO
NH
HN
Me
Me
CO2Me
CO2Me
(69)
HI
CO2Me
Me
Me
NH
N
CO2Me
N
HN
Me
Me
CO2Me
CO2Me
coproporphyrin III tetramethyl ester
(81)

from the Rothemund and Adler–Longo (propionic acid) approaches is somewhat impure (though highly crystalline) and contains (4) about 5% or less of meso-tetraphenylchlorin [**70**]. Brief treatment (6) of the crude product with DDQ accomplishes transformation of [**70**] into [**51**]; earlier methodology involved the separation of these two components on a chromatography column, but transformation of [**70**] into [**51**] instead of separation of [**51**] from [**70**] is much more sensible. Using this kind of methodology, literally kilograms of TPP can be prepared. TPP can, in any case, be purchased either "chlorin-free" or crude. Additionally, with only relatively few exceptions, the reaction tolerates substitution of other arylaldehydes for benzaldehyde, and good yields of a variety of tetra-arylporphyrins can be obtained (47).

❖ Procedure 3. Synthesis of Chlorin-Free TPP [51] (6)

1. Benzaldehyde (66.5 mL) and pyrrole (46.5 mL) are simultaneously added to refluxing propionic acid (2.5 L), and the mixture is refluxed for a further 30 minutes before being allowed to cool overnight to room temperature.
2. The crude TPP is filtered off, washed with hot water, and then washed with methanol until the filtrate is colorless, to give 20.4 g (20% yield) of purple glistening crystals.
3. Concentration of the propionic acid filtrate affords a second crop of crystals.
4. The crude TPP (20 g) is dissolved in refluxing ethanol-free chloroform (2.5 L) before addition of DDQ (5 g) in dry toluene (150 mL).
5. The mixture is refluxed for 3 hours before filtration of the yellowish solution, under suction, through a sintered glass funnel containing Grade I alumina (300 g).
6. The alumina is washed with dichloromethane (200 mL), and the combined filtrates are concentrated to approximately 200 mL before addition of 200 mL of methanol.
7. Filtration results in the chlorin-free product as glistening purple crystals, with an average yield of 19.2 g.

Approaches to so-called octaalkylporphyrins (such as OEP [**52**]) are a little more complicated, but only with regard to the difficulty of preparing the pyrrole starting materials. In this case, the future meso- (i.e., interpyrrolic) carbons can either be present already on the pyrrole, or as in the case with TPP (wherein the meso-carbons were provided by the formyl carbon in benzaldehyde), the meso-carbons can be added separately from the pyrrole. A primary stricture, as mentioned above, is that a monopyrrole tetramerization approach can only be used to give structurally unique product if the substituents at positions 3- and 4- in the monopyrrole are identical.

Thus, fully symmetrical porphyrins such as octaethylporphyrin [**52**] can be prepared easily using two major routes. The first approach, which chronologically was developed first, involves the tetramerization of pyrroles [**71**] bearing 2-CH_2R substituents; the "R" group must be a good leaving group, and the methylene carbon of the 2-substituent will eventually be the source of the 5,10,15, and 20-carbons of the product porphyrin. After the condensation reaction, an oxidation step is necessary to afford good yields of symmetrical porphyrin. Useful examples for attachment of CH_2R groups to pyrroles are *(i)* the Mannich reaction of pyrrole [**72**] with formaldehyde and dimethylamine [or better, with commercially available (N,N-dimethylmethylene)ammonium iodide, Eschenmoser's reagent (56,59)] to give the 2-(N,N-dimethylaminomethyl)pyrrole [**73**]; heating of this in acetic acid gives a 52% yield of [**52**]

(18,70); and *(ii)* hydrolysis of the pyrrole [**74**] to give pyrrole [**75**] which is tetramerized to give [**52**] in 44% yield by heating in acetic acid containing potassium ferricyanide (35,63). Most recently, the Barton–Zard pyrrole synthesis (see above) (8) has greatly simplified preparative approaches to monopyrroles of the type [**58**]; lithium aluminum hydride (**CAUTION**: reacts violently with moisture) reduction, followed by tetramerization of the resulting pyrrole-2-carbinol [**76**] under acidic conditions, gives [**52**] in 55% yield (2,55).

❖ Procedure 4. Synthesis of OEP [52] from Pyrrole [58] (54)

1. Ethyl 3,4-diethylpyrrole-2-carboxylate [**58**] (see above, 657 mg) is added dropwise at 0°–5°C to a stirred solution of lithium aluminum hydride (320 mg; Sigma) in dry tetrahydrofuran (15 mL). The mixture is stirred for 2 hours at 0°–5°C before destroying the excess lithium aluminum hydride by addition of ethyl acetate.
2. It is then poured into saturated aqueous ammonium chloride, extracted with ethyl acetate (3 times 10 mL), washed with aqueous sodium chloride, and dried over anhydrous magnesium sulfate.
3. The solution is evaporated to dryness under vacuum before addition of dichloromethane (15 mL).
4. To this solution is added dimethoxymethane (0.7 mL; Sigma) and toluene *p*-sulfonic acid (110 mg), and the mixture is stirred for 12 hours at room temperature. Aerial oxidation occurs under these conditions, but chloranil (Sigma) can also be used, although without any improvement in yield.
5. The mixture is washed with aqueous sodium bicarbonate, and the organic layer is dried over anhydrous magnesium sulfate.
6. Evaporation gives a residue which is chromatographed on silica gel, eluted with dichloromethane to give OEP [**52**], with an average 55% yield (240 mg).

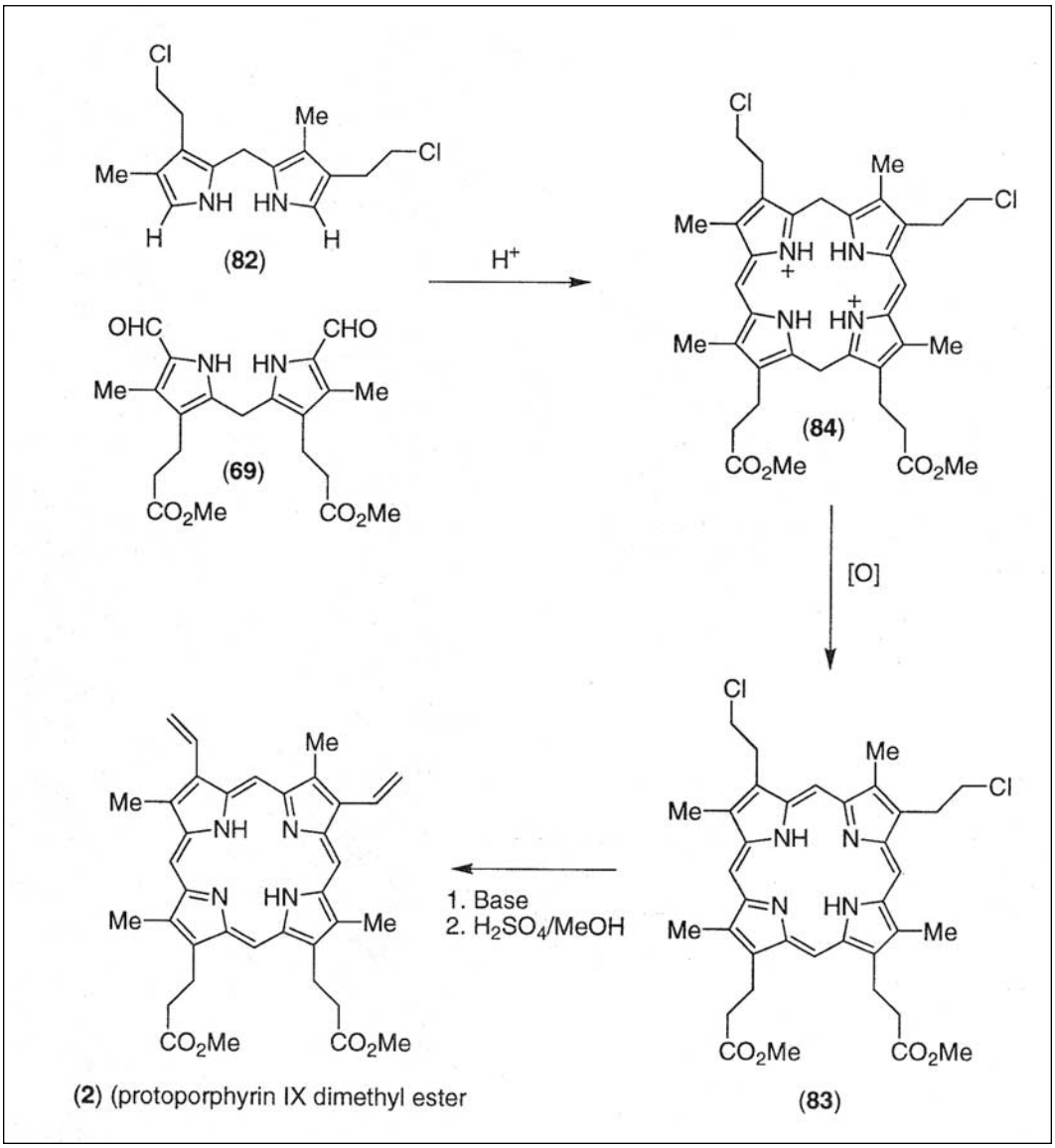
Alternatively, tetramerization of 2,5-diunsubstituted pyrroles [e.g., **72**] in the presence of reagents that can provide the four meso-methine carbons of the product can be used. Cyclization of 3,4-diethylpyrrole [**72**] with formaldehyde affords 55%–75% yields of OEP [**52**] (61).

If the 3- and 4-substituents on the monopyrrole component are not identical, mixtures will usually result due to acid catalyzed pyrrole ring scrambling reactions (49). Thus, acid catalyzed tetramerization of pyrrole [**77**] will result in production of a mixture of the four etioporphyrin type isomers [**6–9**]. However, a method has been devised which does produce only etioporphyrin I [**6**] from tetramerization of a pyrrole related to [77]; treatment of 2-(N,N-dimethylaminomethyl)pyrroles [e.g., **78**] with methyl iodide gives [**79**], which has a leaving group that is labile even under neutral conditions (i.e., no acid), which would cause pyrrole ring scrambling, is present (53,54). This, quaternized in methanol containing potassium ferricyanide (to accomplish rapid in situ oxidation of the labile porphyrinogen intermediate), gives a good yield of pure etioporphyrin I [**6**].

❖ Procedure 5. Synthesis of Etioporphyrin I [6] from Pyrrole [78]

1. Benzyl 4-ethyl-5-(N,N-dimethylaminomethyl)-3-methylpyrrole-2-carboxylate (53,54) (1.88 g) is dissolved in tetrahydrofuran (600 mL), and 10% palladium on carbon (500 mg; Sigma) is added.
2. The resulting mixture is stirred under hydrogen at room temperature for 12 hours before the catalyst is removed, and the solvent is evaporated to dryness.

3. Recrystallization from dichloromethane/hexane affords 4-ethyl-5-(N,N-dialkyl-aminomethyl)-3-methylpyrrole-2-carboxylic acid as an off-white powder in quantitative yield. Note that because of spontaneous decarboxylation at room temperature to give [**78**], this must be used immediately.
4. The pyrrole carboxylic acid (1.29 g) is dissolved in a solution of methanol (200 mL) and triethylamine (2 mL) and heated under reflux for 15 minutes.
5. Potassium ferricyanide (3.8 g) is added, and the reaction is continued at reflux for another 10 hours.
6. After removal of the solvent, the residue is redissolved in chloroform, the insoluble material filtered off, and the red solution is passed through a short column of silica gel (eluted with chloroform).
7. The solvent is evaporated, and the residual porphyrin is recrystallized from



dichloromethane/methanol to afford etioporphyrin-I in 36% yield (284 mg).

4.4. Porphyrins via Dipyrromethane Intermediates

If two dipyrromethane units with appropriate bridging carbons are condensed together, there are three possible products because the dipyrromethanes can either react with themselves or with each other. If the dipyrromethanes individually possess an unsymmetrical array of substituents, even greater mixtures can occur because there is no control over which end of one dipyrromethane reacts with the end of another. These symmetry limitations are common with all so-called "2 + 2" syntheses; in a porphyrin synthesis involving an A-B and a C-D dipyrromethane is to be condensed, the symmetry problems can be avoided if the A-B or C-D dipyrromethane unit is symmetrical about the interpyrrolic (5-) carbon atom. Arsenault, MacDonald, and coworkers showed (3) that a 1,9-diformyldipyrromethane, [e.g., **69**], can be condensed with a 1,9-di-unsubstituted dipyrromethane or its 1,9-dicarboxylic acid [**80**] in the presence of an acid catalyst to give pure porphyrin [e.g., **81**], often in high yields. MacDonald used hydriodic acid, but since that time, toluene *p*-sulfonic acid has been shown (14,15) to be a much better choice and more convenient too.

This 2 + 2 route using dipyrromethanes is probably the most widely used pathway to synthetic porphyrins. Thus, for example, treatment of dipyrromethane [**82**] (obtained from [**61**] by catalytic debenzylation followed by treatment with trifluoroacetic acid) with 1,9-diformyldipyrromethane [**69**] gives a good yield of porphyrin [**83**] after oxidation of the intermediate porphodimethene [**84**]; no mixtures are produced because both of the future linking meso-carbons are sited on the same dipyrromethane (preventing either of the two individual dipyrromethanes from reacting with themselves) and dipyrromethane [**69**] is symmetrical about its 5-carbon. Conversion of the 3,8-bis(2-chloroethyl)porphyrin [**83**] into protoporphyrin IX dimethyl ester [**2**] is accomplished simply by treatment with base (40)—just in case this base treatment also accomplished hydrolysis of the methyl esters, the product is then set aside in methanol containing 5% sulfuric acid (**CAUTION**: add the acid to the methanol, cooled and slowly). Workup and chromatography [**NOTE**: protoporphyrin is photolabile (see above), so the column should be run in the dark or with aluminum foil wrapped around it] then produces the product, [**2**].

It has been my intention to provide a summary of fairly simple procedures that can be used, given a certain competence in synthetic organic chemistry, to obtain by extraction or by total synthesis some useful porphyrins with defined symmetry and structural characteristics. But competence in organic chemistry is not easily earned. If the procedures still look too complex, or (more likely) if you do not have the basic laboratory equipment with which to carry out the procedures described, then the best bet is to collaborate. Just remember, most organic chemists would not know where to start if they needed to run a gel or if they needed to do a northern blot. They will want to collaborate also if they need these things.

ABBREVIATIONS

DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; OEP, 2,3,7,8,12,13,17,18-octaethylporphyrin; TPP, 5,10,15,20-tetraphenylporphyrin.

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