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Porphyrins: A solvent free synthesis, combinatorial libraries, a solid phase synthesis, and towards a tetra-Grignard reagent

by

Xianchang Gong

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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Abstract

Porphyrins: A solvent free synthesis, combinatorial libraries, a solid phase synthesis, and towards a tetra-Grignard reagent

By Xianchang Gong

Advisor: Professor Charles M. Drain

Part 1

A new method for the rapid preparation of the commercially important *meso* substituted aryl porphyrins, such as 5,10,15,20-tetraphenylporphyrin (TPP), is presented. This one-step solvent free method involves heating the aromatic aldehyde to ~200 ^{0}C in a vial fitted with a septum cap, followed by addition of the pyrrole and maintaining the temperature for another 20 minutes. The dioxygen in air serves as the oxidant, and the addition of benzoic acid as a catalyst improves the yields of TPP and many other porphyrins to 5-32%. Herein is also presented an examination of the many factors that influence the yield, the ease of purification, and the ability to scale-up the reaction. Since the tarry bi-products from this method are much less soluble than in most other synthetic strategies, much less solvent is required for purification. Thus, this method provides an attractive alternative to others because of its minimal waste generation in terms of both solvent and chromatography support.

Part 2

The synthesis and characterization of combinatorial libraries of *meso*-tetraphenylporphyrin (TPP) derivatives - core structured libraries- is reported. The libraries are readily further derivatized to create libraries of amphipathic porphyrins. These amphipathic porphyrins are then screened for DNA binding, isolated, and

examined for their ability to cleave plasmid DNA. Several novel porphyrins are identified and indicate multifunctional, amphipathic porphyrins bind more strongly than heretofore known homosubsituted porphyrins, thus may be lead compounds for photodynamic therapeutics.

Part 3

A method for the synthesis of porphyrins on a solid support was developed. Using a non-swollen Wang resin, the major product (>90%) is the *trans* substituted ABAB pattern porphyrin. The yield is 4.5% based on the loading of the aldehyde, which is 10mg porphyrin/g resin. When the resin was pre-swollen and a second aldehyde was used, a mixture of porphyrins was resulted. This may arise from the density of active sites on the Wang resin. Thus, at this point in the development process, this is not an attractive route to porphyrin libraries on solid support, but is an unusual alternative to the multi-step procedures to make *trans* substituted porphyrins.

Part 4

A tetra-Grignard reagent zinc-5,10,15,20-Tetrakis-(4-bromomagnesiumphenyl)porphyrin was likely synthesized with a yield of ~33%. This poly-Grignard reagent was used as an attempt to synthesize a porphyrin cube. A interesting porphyrin 5,10,15,20-Tetrakis-(4-diphenylmethanolphenyl)porphyrin, which has 4 triphenyl carbonals was synthesized with a yield of ~37%. This latter compound was made by reaction of excess phenyl Grignard with 5,10,15,20-Tetrakis-(4-methoxycarbonylphenyl)porphyrin.

This work is dedicated to

my parents for their tremendous support and encouragement

Acknowledgments

I wish to express my gratitude to my parents for their love, help and patience without which this work would have never been completed. I also wish to thank all the graduate and undergraduate students who work in my lab. I thank Dr. Clifford E. Soll for the help with Mass spectra and Dr. Michael Blumenstein for the help with NMR spectra. I thank Dr. William F. Berkowitz, Dr. Lynn Francesconi and Dr. Klaus Grohmann for their numerous suggestions, which have improved this work significantly. Finally, I thank my mentor Dr. Charles M. Drain for his teaching, patience and support throughout my research.

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Abbreviations

TFA: Trifluroacetic acid

TPP: 5,10,15,20- Tetraphenylporphyrin

TPC: 5,10,15,20- Tetraphenylchlorin

TTPP: 5,10,15,20- Tetrakis-(4-tolyl)porphyrin

DIC: 1,3-dicyclohexylcarbodiimide

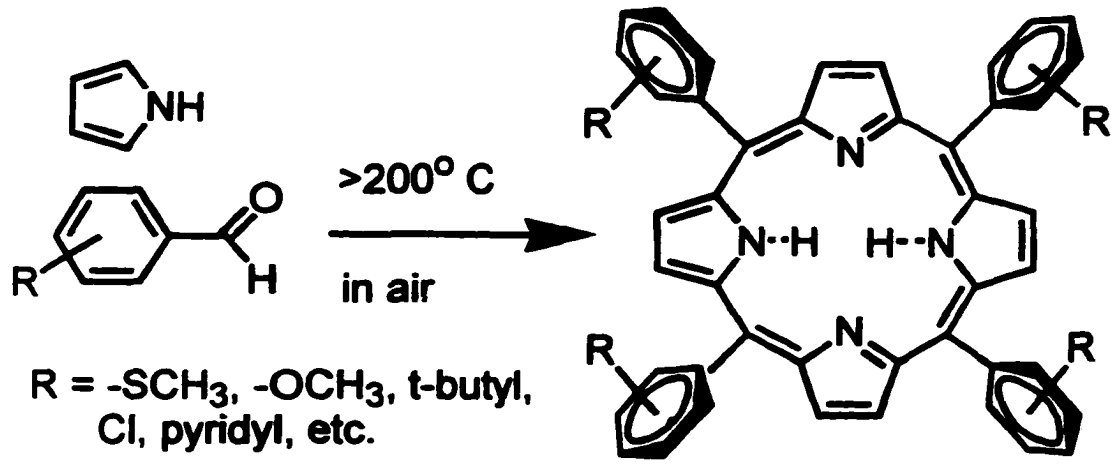
DMAP: 4-(Dimethylamino)-pyridine

Part 1

Solvent free porphyrin synthesis

Introduction

The remarkably diverse photo-, electro-, and bio- chemical properties of the porphyrins continue to attract the attention of researchers even after well over a hundred years. In this century porphyrin research has gone from Hans Fischer's pioneering synthesis of hemin in the 1920s,[1] to their use as redox catalysts,[2] molecular electronic devices,[3] and photodynamic therapy agents. In order to exploit their chemistry and fine tune their properties, a variety of synthetic methods have been developed for non-natural porphyrins, especially for the *meso* tetrasubstituted porphyrins.[4-8] We have developed a simple way to synthesize many *meso* substituted porphyrins without solvents, or man-made oxidants, and the reaction is complete in minutes with yields of 5-32%.[9] This is the solvent free porphyrin synthesis, scheme 1.



Scheme 1.

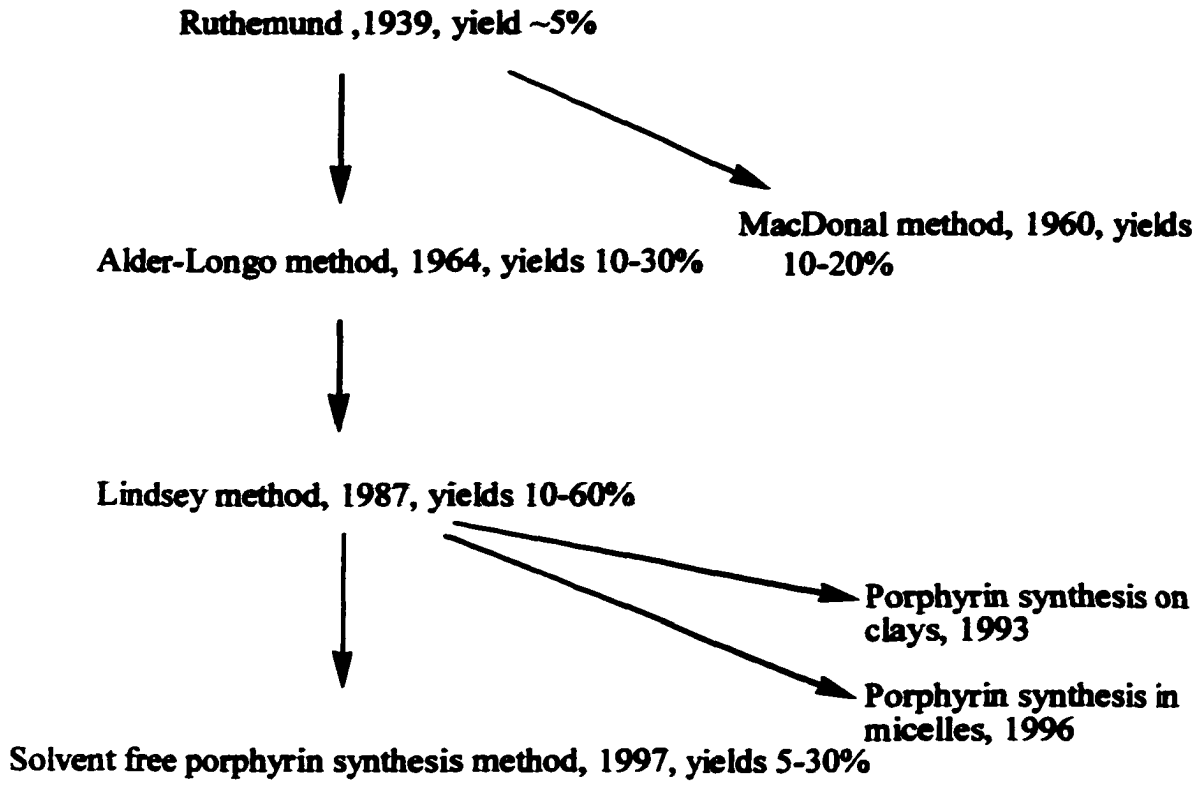
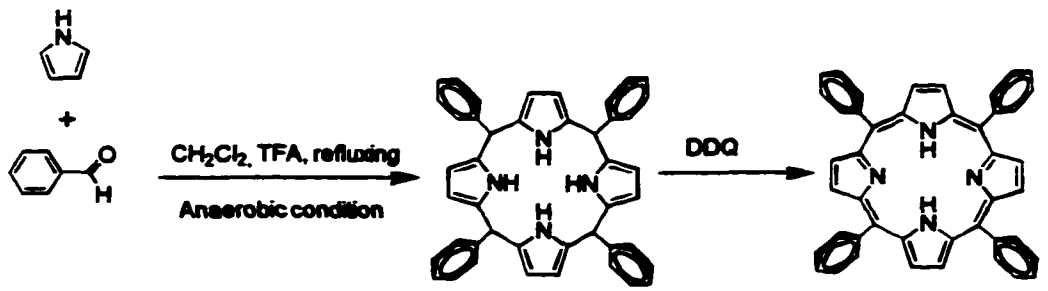


Figure 1. General chronology of porphyrin synthesis

TPP was first synthesized by Ruthemund, figure 1.[10] The reaction was done by mixing of benzaldehyde and pyrrole in a sealed-tube in pyridine heated at 150-200 ^{0}C for two days. The yield was ~5%. Later in 1964, Alder improved the Ruthemund method by refluxing aldehyde and pyrrole together in propionic acid or acetic acid in open air.[4] The optimum concentration of the reactants is 0.1M, and the yields ranged from 10% to 30%. The advantages of the Alder method are that it can synthesize a wide range of porphyrins and can be easily scaled up. The yields of the Adler method can be improved by the template effect through the addition of metal salts to the reaction system.

A major contribution was achieved by Lindsey which has had a broad impact on current porphyrin synthesis.[7] The Lindsey method is a two step reaction sequence. The first step

allows the equilibrium formation of porphyrinogen at room temperature in CH_2Cl_2 by using TFA or BF_3 etherate as a catalyst. The second step is the oxidation of the porphyrinogen, commonly employing DDQ or TCQ as the oxidants. Compared to the Adler method, the Lindsey method has many advantages such as it allows for the synthesis of porphyrins bearing sensitive functional groups. A major draw back of the Lindsey method is that the high dilution condition makes it difficult to prepare porphyrins in large scales. This problem was overcome for several porphyrins by addition of selected salts such as NaCl to the reaction system, and increasing the reaction concentration to 0.1M, such that 50-60% yields are obtained for the synthesis of several porphyrins.[11]



Scheme 2. Lindsey porphyrin synthesis

Other developments that improve the Adler method include:

(1) The removal of water from the reaction system by using a Dean-Stark trap.[12] This method gives an impressive improvement in the synthesis of several tetraalkylporphyrins with yields ~ 20% and an easy work-up process.

(2) By the use of high valent transition metal salts such as $TiCl_4$ or $VOCl_3$ in the Lindsey synthesis, several porphyrins can be synthesized with yields as high as ~60%.[8] The reaction mechanism is believed to be via a free radical reaction in the oxidation of the

porphyrinogen to the porphyrin. Sterically hindered porphyrins such as 5,10,15,25-Tetrakismesitylporphyin can be synthesized by this method with a yield of 50%.

(3) Use of hydrogen peroxide or nitrobenzene as the oxidants besides air in the Alder synthesis.[13] This method has been claimed to improve the isolated yields of several tetrakisarylporphyrins.

(4) Use of solid acids, such as clays, instead of the TFA or BF_3 etherate in Lindsey synthesis conditions. TPP has been synthesized this way with a yield ~30%. The clay was used on a gram scale in the reaction.[14]

(5) In 1996, Bonar-Law reported a porphyrin synthesis using a two step process as the Lindsey synthesis and catalysis by micelles.[15] The report used the same reaction concentrations as the Lindsey synthesis, and had similar kinetics. The advantage of this method is that the polar, functionalized aldehydes can be used directly to synthesize functional porphyrins in high yields typically which are difficult to synthesize by the Lindsey method. The disadvantage of this method is that it uses a large amount of surfactant that might cause both economical and environmental problems, for any large scale procedures.

A solvent free corroles synthesis was reported by Gross in 1999,[16,17] two years after our publication of solvent free porphyrin synthesis. Several corroles were synthesized directly by merely mixing pyrrole and aldehyde on a solid support and heating up in air at 100^0C for several hours followed by oxidation with DDQ. They also mentioned their directed synthesis of TPP with a yield of 5-8% which is much lower than our 1st reported synthesis.

The condensation of pyrrole and aldehyde cannot be used to prepare the *cis-* and *trans-* substituted porphyrins directly. The *trans-* substituted porphyrins were prepared by the multi-step coupling of dipyrroles based on the MacDonald synthesis. Very recently, the Lindsey group reported their synthesis of *trans-* substituted porphyrins based on a one flask dipyrrolemethane synthesis[18,19] and the Dolphin group has reported their synthesis of 5,10- diphenylporphyrin by several different synthetic routes.[20]

Table 1. Comparison of TPP and TTPP yields (%) of different porphyrin synthetic methods

	Ruthemund	Alder	Lindsey	Solvent free	Clay	Highvalent transition metal	Nitrobenzene as oxidant	Micelles catalysis
TPP	5[10]	38[4]	39[7]	32	30[14b]	63[8]	20[13]	18[15]
TTPP	N/A	N/A	35[7]	30	20[14b]	48[8]	N/A	19[15]

Results and discussion

Initial report of the new procedure

The rapid appearance of water and what is now known to be the porphyrinogen[6] in a pseudo zero-order reaction in the Adler-Longo method, the equilibrium conditions of the Lindsey method, and the stability of the porphyrin to high temperatures of the Rothemund method, led us attempt porphyrin synthesis at high temperatures and in the gas phase.

Although solvent free mixtures of the pyrrole and aldehyde rapidly heated to >200° C form the corresponding porphyrins,[21] better yields but similar side product distributions are observed by mixing the two reagents in the gas phase at the same temperature. Whether the porphyrin is formed entirely in the gas phase is unknown at present, but a brown-purple vapor is observed within ~2 seconds of adding the pyrrole to gaseous aldehyde, and a blue-black tar begins to condense on the sides of the reaction vessel soon afterwards. In the rapid heating experiments, porphyrins condense at the top of the reaction vessel, which is below the reaction temperature, with the same purity as that found in the bottom that contained the original solution.

High temperature/gas phase conditions allow for the rapid aldehyde-pyrrole condensation, for the removal of water from the proximity of the intermediate, and for an increase in the rate of oxidation by dioxygen. This latter reaction is proposed to be the rate limiting step in the Adler-Longo method.[4] Examination of yield and products versus time indicates that oxidation of the porphyrinogen, or its intermediates, occurs very rapidly, since we detect no porphyrinogen and no chlorin in the UV-Vis spectra (figure 2) even after reaction times of a few seconds. Anaerobic reaction conditions result in

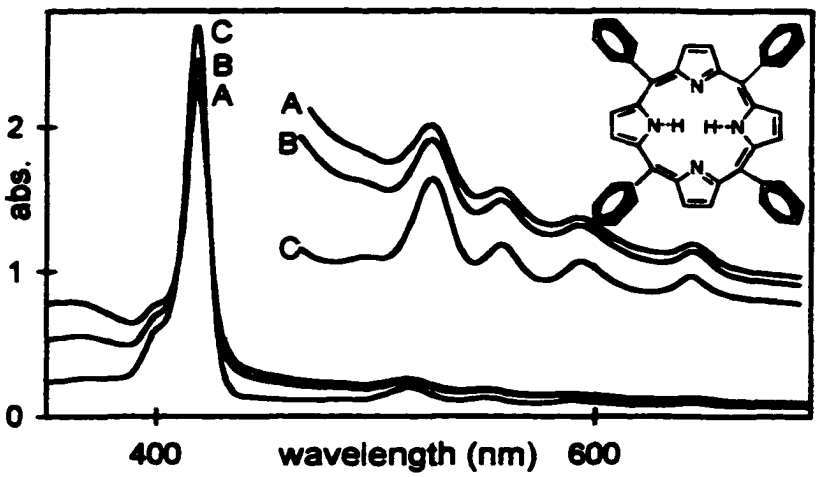
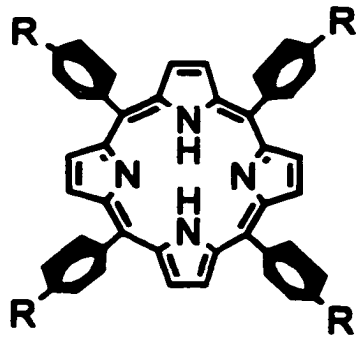


Figure 2. *Unpurified* chloroform rinses from two gas phase reactions (A) a 3:1 mole ratio and (B) a 1:1 mole ratio of benzaldehyde to pyrrole are compared to the UV-Visible absorption spectra of known pure TTP from a large gas phase reaction (C), indicates the purity of the soluble materials and the lack of dipyrromethanes and chlorins in the product mixture. The inset absorbance scale is x10.

very low porphyrin yields and only traces of porphyrinogen, indicating the crucial role of dioxygen in trapping the porphyrin products. Studies show that the reaction temperature must be above the boiling point of benzaldehyde to result in yields of TPP greater than a few percent, but the yield levels off by 210° C. That the rapid removal of water from the condensing porphyrin (intermediates) is one key to these reaction conditions is shown by the decreased yield (~12%) when a 5:1 water:aldehyde mixture is used. Catalytic amounts (<5 mole %) of HCl or NH_3 block porphyrin formation almost entirely, but washing the reaction vessel with 1.0 M HCl or KOH and thorough drying has no effect. Trifluoroacetic (TFA) acid diminishes the formation of TPP, but increases the yield of tetramesitylporphyrin from trace amounts to about 7% for reasons still under investigation.

This method is general enough to make a wide variety of *meso* substituted porphyrins in yields ranging from 5 to 23%, see Table 2. For those reactions using aldehydes that have boiling points greater than ~250° C at 1 atm, a thin film of the melted or liquid aldehyde is coated onto the reaction vessel sides at ~220 ^{0} C before the pyrrole is injected. Thus 15% yields of tetrakis-(4'-*tert*-butylphenyl)porphyrin are obtained.

Table 2. isolated, unoptimized yields of porphyrin derivatives.



R	Yield (%)[a]
phenyl	23
4-tolyl	16
4-isopropylphenyl	12
4-tertbutylphenyl	15
4-chlorophenyl	8
4-methoxyphenyl	12
4-methylthiophenyl	10
Mesityl[b]	7
C_5H_{11}	1-2
4-pyridyl	10[c]

[a] ± 3%. [b] 5 mol% TFA added to reaction. [c] 25 mol % $Zn(Ac)_2$ added to reaction.

We are currently examining the complex balance between temperature, pressure, and concentrations of all three reactants in order to maximize the yield and gain a deeper understanding of the reaction mechanism and thermodynamics. Porphyrin formation is believed to be driven by enthalpic changes[21] and the macrocycle is preferred over the polymer for entropic reasons[6,7,9] (ΔH= -212 kcal/mol, ΔG= -166 kcal/mol, and ΔS= -153 e.u.).[6,7,9] If this data is correct, then the favorable ΔG is dominated by the entropy term, and cooler temperatures should favor porphyrin formation. However, the porphyrin yield increases with temperature up to ~250° C, thus under these conditions the high temperature/gas phase formation of porphyrins represents a kinetically controlled reaction dominated by the reactivity of dioxygen - as the oxidation studies suggest. The glass walls of the vial do not directly participate in the reaction since control experiments, whereby glass wool is inserted into the reaction vial or the vial has been silanated, show similar yields and reactivities. We are further investigating the possibilities of surface catalysis.

Our results show that the commercially important *meso* arylporphyrins may be synthesized without solvent, and using only dioxygen as an oxidant. The polymeric byproducts are insoluble, so washing the reaction vessel with minimal amounts of solvent followed by a short silica gel column results in pure porphyrin. Dioxygen is a key requirement for porphyrin synthesis under these conditions. While the 5-23% isolated yields resulting from our high temperature/gas phase method are typically not as good as the Lindsey method and on a par with the Adler-Longo and MacDonald methods, the great advantages of this method are its simplicity and minimal waste production. Purification is generally easier since there is virtually no chlorin formation and the tarry

byproducts are less soluble than other byproducts from other procedures. We have been able to scale up this method for the synthesis of 0.1 - 0.3 grams of several porphyrins using a glass tube wrapped with heating tape (or in a zone refining furnace) whereby the aldehyde is slowly injected at one end, and the pyrrole is slowly added at the other end, of a 1.5 cm x 60 cm tube closed with glass wool to allow sufficient dioxygen for the reaction. The product is removed with 100 - 200 mL $CHCl_3$ and a very short silica gel column yields pure material. Thus, this method is quite amenable for industrial-type reactors.

Improved yields and mechanism investigation

Herein we describe our exploration of the complex interplay between temperature, pressure, concentration, acid catalysts, and oxidants for this new synthetic strategy for porphyrins, table 3. The present study shows that using benzoic acid as a catalysts substantially improves the yields – from 20% to 32% for 4'-alkylphenyl porphyrins and for many others, table 4. Though the different physical properties of each aldehyde, such as the vapor pressure at 200^0 C, limits our ability to make general rules, there are definite trends and significant considerations that will serve as guides to the utilization of this procedure both in laboratory and industrial scale synthesis of aryl porphyrins. Thus, the relationships between concentration, temperature, pressure and the yield are discussed, as are acid catalysts and other additives.

Temperature, pressure, and aldehyde volatility

Porphyrin formation is believed to be driven by enthalpy changes[21] and the macrocycle is preferred over the polymer for entropic reasons (ΔH= -212 kcal/mol, ΔG= -166 kcal/mol,

Table 3. Salts, templating metals, oxidants, and acid catalysts. Reaction conditions: 3:1 mole ratio of benzaldehyde to pyrrole, 5 minutes preheat, with no additives, the yield is 18%

	Reagent	Mole % relative to pyrrole	Yields TPP (%)
Salts	NaCl	20	14
		100	9
	ZnO	800	11
Template	$Zn(Ac)_2$	25	12 (ZnTPP)
Oxidants	$VOCl_3$	20	0
	$C_6H_5NO_2$	150	5
	$Na_2S_4O_6$	150	<1
	MCPBA	100	6
Acid Catalyst[a]	X-C_6H_5COOH X = H	100	32
	X = 4-Me	100	26
	X = 3-Cl	100	27
	X = 2,6-Me	100	18
	CCl_3COOH	5	15
	CH_3COOH	8.3	14
		16	13
		100	15
		400	15
	CH_3CH_2COOH	100	14
	CF_3COOH	4.5	6
		9.0	3
	C_6H_5OH	100	11
	2-carboxybenzaldehyde	100	6
	BF_3	4	0

[a] 2:1 mole ratio of benzaldehyde to pyrrole

Table 4. Yields of various *meso* aryl porphyrins using new conditions

R (4' unless noted)	Yield with no BA catalyst[1]	Yield with 1:2:1 BA:aldehyde:pyrrole[2]	BP aldehyde °C[3]
H	23	32	178
CH_3	20	30	204
$(CH_3)_2CH$	12	24	235
$(CH_3)_3C$	15	33	~245
Mesityl	7	5[4]	237
CH_3O	20	25	~248
CH_3OOC	--	19	--
CH_3S	10	11	~250
Cl	10	25	--
Br	--	26	--
3,4,5 trimethoxy	--	15	--
3,5 dibenzyloxy	--	15	--
Other porphyrins			
Tetrakis (1'-pyrenyl) porphyrin	--	5	--
Tetrakis (4'-pyridyl) porphyrin	10	7[5]	~200
Tetra n-heptyl porphyrin	--	2[6]	153

[1]From reference 9. [2]Standard reaction conditions. [3]~estimates for 1 atmosphere.

[4] $NH_2OH \bullet HCl$: aldehyde : pyrrole = 1:2:1

[5] propionic acid : aldehyde : pyrrole = 6.8:2:1

[6] 0.35g silica gel added in place of benzoic acid

and ΔS= -153 e.u. for porphine).[6,7,21] If this data is correct, then the favorable ΔG is dominated by the entropy term, and cooler temperatures should favor porphyrin formation. However, under our reaction conditions, the porphyrin yield increases with temperature up to ~250° C (figure 3), thus under these conditions the high temperature/gas phase formation of porphyrins and/or their intermediates appears to represent a kinetically controlled reaction dominated by the reactivity of dioxygen - as the oxidation studies suggest. Coupled with the observation that the polymeric by-

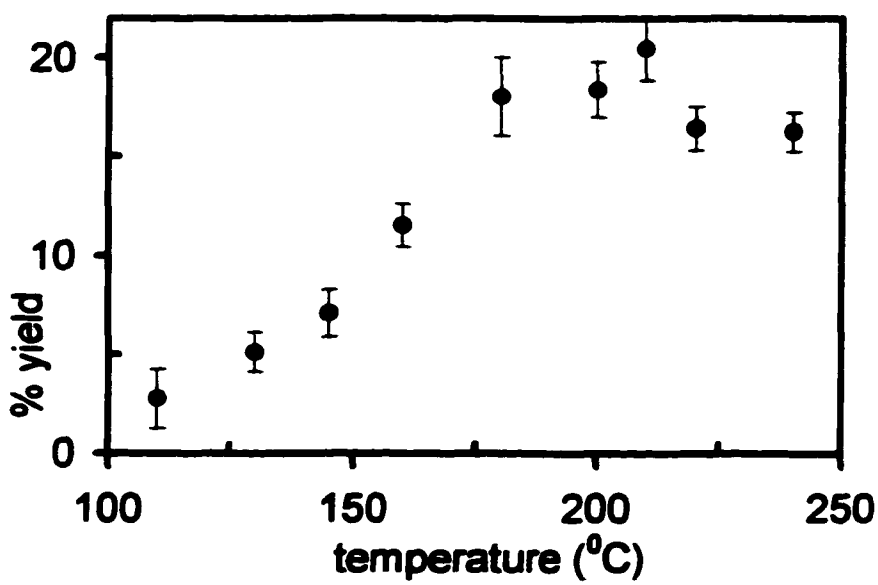


Figure 3. The yield of TPP versus reaction temperature. The reaction utilized a 3:1 mole ratio of benzaldehyde to pyrrole, and 5 minute preheating of the aldehyde

products are much less soluble, indicating the minimal formation of short pyrromethane oligomers, the temperature data may also suggest a change in the complex thermodynamics and equilibria between reactants and the intermediates leading to the products. A series of reactions at different temperatures was done to test the relationship between temperature and yield. As shown for benzaldehyde (figure 3), the yield of TPP[21] increases with temperature, but levels off at ~200^0 C. The pressure was maintained at ~3 atm. Porphyrin formation is very rapid at these temperatures, whereby half of the yield is obtained in the first 1.5 minutes followed by a much slower, ~15 minute, increase such that most of the porphyrin is formed within the first 20 minutes (figure 4). Surprisingly, similar trends for the temperature and the kinetics are observed for the other 4-alkly benzaldehydes, so the reactivity is not determined solely by the volatility of the starting materials, *vide infra.*

Of course, the nature of the aldehyde dictates the amount in the vapor phase; however, in virtually every case, a dark red to brown gas is observed in the first few seconds of the reaction. It is difficult to say how many, if any, succeeding steps after the initial condensation take place before the intermediates liquefy, but it seems likely that condensation to an aerosol dispersion would be virtually instantaneous. This is consistent with the observation of a dark liquid forming on the reaction vessel walls after only ~ two minutes. Possible reactions are occurring in the gas phase are discussed below. Now the role of reaction pressure was examined. In typical conditions, the reaction pressure is ~3 atm. Pressures in the reaction vessel were increased by addition of N_2 or by an inert solvent such as toluene. Using both methods, pressures from ~3 atm to ~10 atm at constant temperature were examined. The concomitant decrease in the partial pressure of

the reactants, as well as the increased amounts of reactants condensed onto the walls of the reaction vessel both correlate with the observed decrease in porphyrin yield with

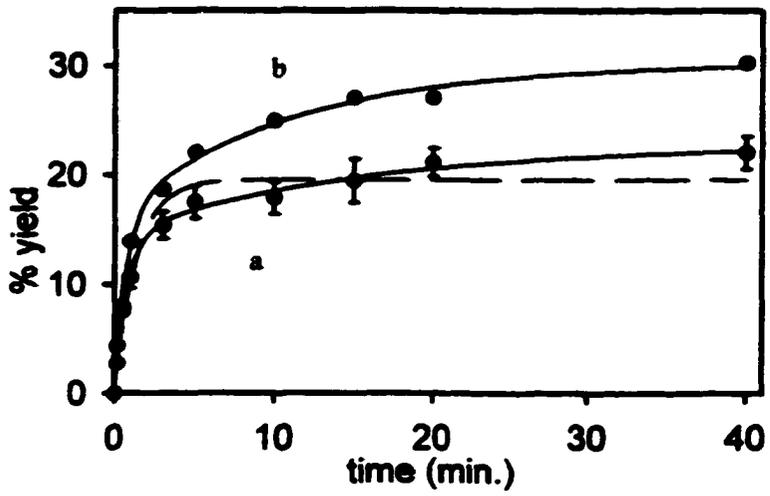


Figure 4. The yield of TPP versus reaction time. a: the conditions used were 2.5:1 benzaldehyde to pyrrole, and 5 minute preheating of the benzaldehyde. The solid line represents a two exponential rise to a constant value (τ_1= 1.3 ± 0.1min., τ_2= 8.3 ± 0.9min.) and the dashed line represents a single exponential rise. b: the conditions used were 1:2.5:1 benzoic acid : benzaldehyde: pyrrole. The fit is for a double exponential rise (τ_1= 1.4 ± 0.2min., τ_2= 13.9 ± 1.5min.). Note that the initial time constants for both reactions are the same, but differ in the second time constant.

increasing pressure. UV-Vis. and NMR results show that the reduced yield of porphyrin was associated with increased yield of polymers – not lower reactivity of the reagents. Under our standard conditions of ~3 atm and 200^{0}C, the concentration of benzaldehyde

and pyrrole in the gas phase is estimated to be ~1.2 mM, remarkably similar to the concentrations used in the Lindsey synthesis.[7a]

It is well recognized that under these conditions, not all the aldehyde is in the gas phase; however, note that the yield is not simply related to the boiling point of the aldehyde at least for the series of 4-alkylphenyl groups (Table 4). The yield is essentially the same when R= H, Me, tBu (boiling point at 1 atm = 178, 204, 245^{0}C, respectively). It is unclear why the yield of the iPr derivative is constantly lower. Other experiments indicate that that reaction is not entirely in the vapor phase. Mixing equimolar amounts of tolyl and tButyl aldehydes results in porphyrins with a statistical distribution of tolyl and tBu groups. If volatility were the primary consideration, the two populations of porphyrins would be observed -- one that contains mostly methyl groups from the gas phase, and one that contains mostly tBu groups from the solution phase. This experiment indicates that the location of the initial condensation step may be irrelevant to the yields observed, and that most of the chemistry take place in the condensed phase. This is also supported by the fact that most of the benzoic acid catalyst remains on the surface of the vial.

Aldehyde and carboxylic acid

There is a complex relationship between the yield and amount of aldehyde used in the reaction. Some of the aldehyde is converted to the corresponding acid under these conditions. As would be expected, the rate of benzaldehyde oxidation is greatly increased when heated in air at 200 ^{0}C for 5 min. such that ~ 1/2 aldehyde is converted to its acid as indicated by ^{1}H-NMR, figure 5. The presence of the benzoic acid does not prevent the

decomposition of the aldehyde, as showed by figure 5. The amount of time of that the aldehyde is preheated prior to pyrrole addition also plays an important role in both

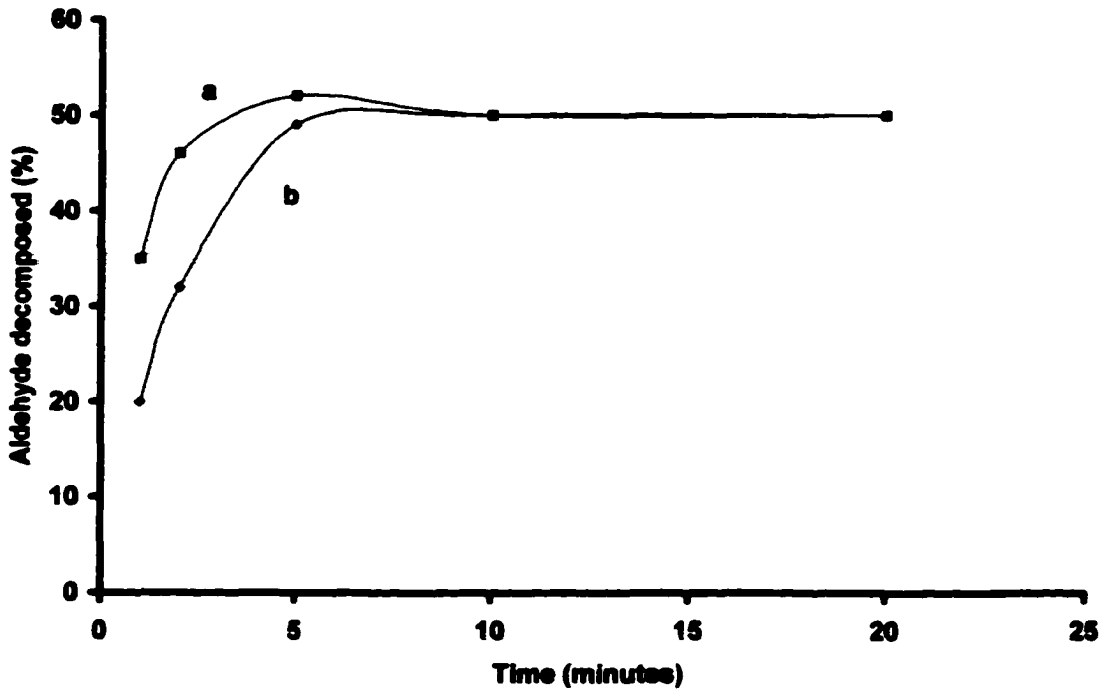
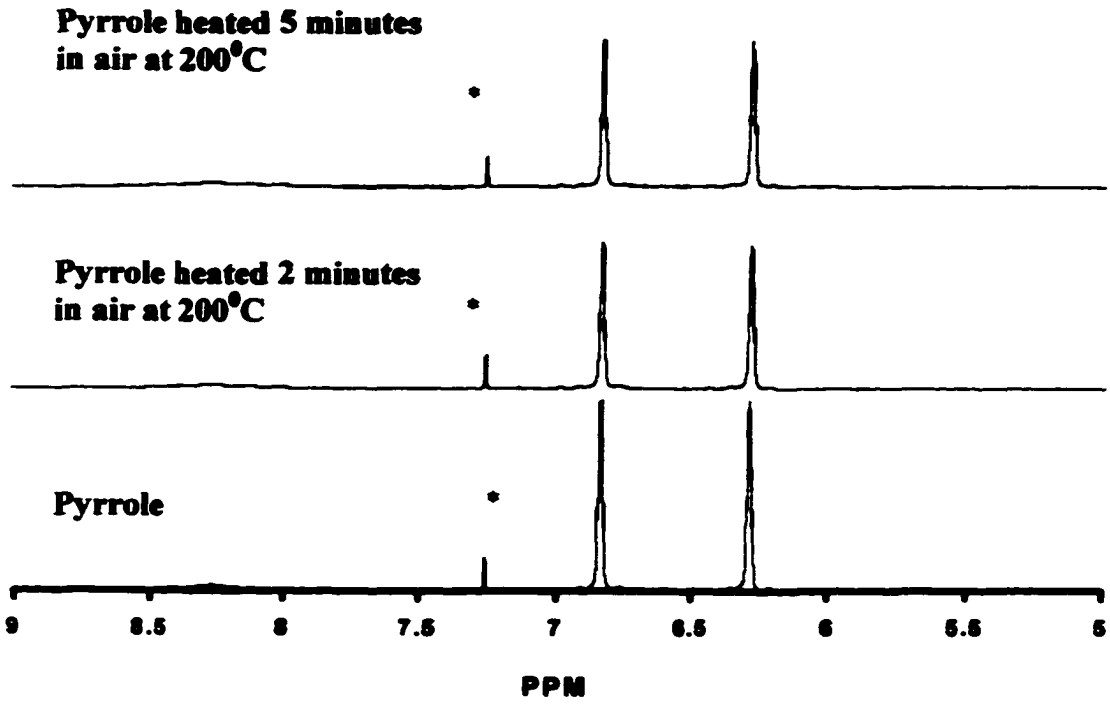


Figure 5. Benzaldehyde decomposition with and with out the present of the benzoicacid. a: With benzoic acid; b: without benzoic acid

porphyrin yields and the ease of purification. Under our standard 3:1 conditions, the yields passes through a maximum where the preheating time is ~ 5 minutes. Increasing the amount of time the aldehyde is preheated increases the amount of benzoic acid formed, thus there is a trade-off between the oxidation of this reactant versus catalysis by the resulting acid. For aldehydes that are difficult to come by, the addition of benzoic acid to the reaction ameliorates this problem somewhat, but the effectiveness of this modification in the procedure depends largely on the relative reactivity, both to oxidation

and porphyrin formation, of the substituted aldehyde. These observations are consistent with the known reactivity of arylaldehydes, many of which oxidize faster than benzaldehyde at these temperatures in air. Thus aldehyde oxidation can be a disadvantage for this synthetic method. Pyrrole is relatively stable when heated in air at these temperatures as demonstrated by the ^{1}H NMR, figure 6. The porphyrin yield vs. the ratio of aldehyde to pyrrole is plotted in figure 7, and shows the optimum to be ~3:1.

Figure 6. Pyrrole heated in air (^{1}H NMR in $CDCl_3$, *: $CHCl_3$).



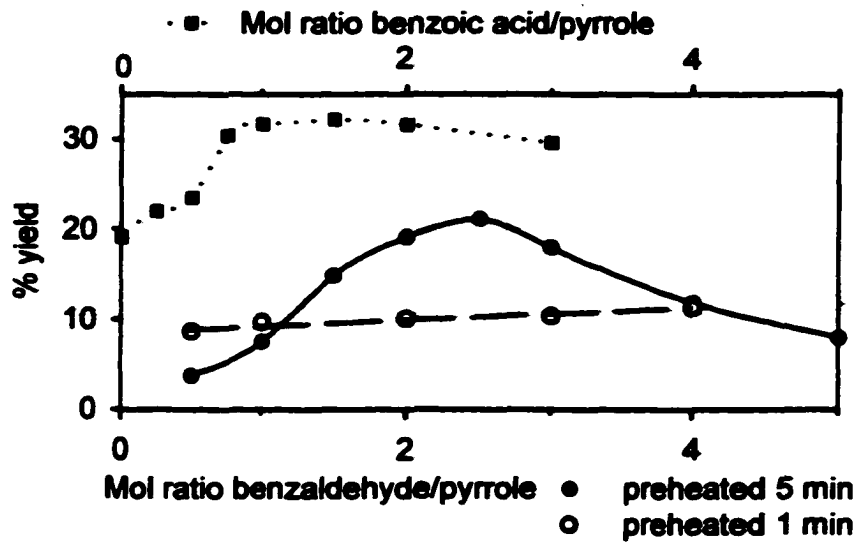


Figure 7. The complex relation between benzaldehyde and benzoic acid. All reactions are at 200 ^{0}C. The lines through the data are for clarity. The ○ shows the yield of H_2TPP versus the mole ratio of benzaldehyde and pyrrole with 1 minute preheating of the aldehyde (minimal benzoic acid formed). The ● show the results when the aldehyde is preheated 5 minutes (~45% conversion to the corrosponding acid). The ■ show the effects of adding benzoic acid to the aldehyde before preheating for 5 minutes, followed by addition of the pyrrole using a constant 2:1 ratio of benzaldehyde to pyrrole.

Interestingly, the longer the aldehyde is incubated before pyrrole addition, the easier it is to purify the porphyrin, because even though the yield of TPP decreases, the formation of less soluble, polymeric by-products increase at the cost of the smaller, soluble materials. Only TPP is observed by ^{1}H-NMR as the product from experiments using ~ 20 mole % 4-methylbenzoic acid with the benzaldehyde, and only tetrakis(4'-methylphenyl)porphyrin (TTP) is observed when ~20 mole % benzoic acid is present with 4-methylbenzaldehyde. Thus, there is no evidence that the benzoic acid is incorporated into any porphyrins, nor

into the side products. The role of benzoic acid in the synthesis of TPP is further discussed in the next section.

Effect of acid catalysis

The role of the excess aldehyde in increasing the yield can arise from: (i) its oxidation to benzoic acid which can act as a catalyst, (ii)from its increased contact with the reaction intermediates. The porphyrin yield increases and then decrease as the amount of benzaldehyde is increased, figure 7. The benzoic acid formed when there is a 3-fold excess of the aldehyde still does not bring the yield up to the level of the reaction with added benzoic acid. The acid might participate in the reaction by activation of the carbonyl in the condensation steps.

Acid catalysis has precedents in other synthetic strategies[4,7] for these compounds. Similar to the observations of Adler and coworkers,[4b] we find that an equal molar quantity of benzoic acid to pyrrole is required to maximize the yield (Table 3, figure 7). Experiments show that the one equivalent of benzoic acid added to two equivalents of benzaldehyde before the preheating increases the yield from ~20% to ~32% (figure 7). Inverting the order, by addition of the aldehyde to the preheated mixture of acid and pyrrole, has only a small effect in increasing the yield from ~18% to ~20%. Again, since most of the benzoic acid remains on the vessel walls even at 200 ^{0}C, it is likely that benzoic acid effects the intermediate steps in porphyrin formation rather than the initial, gas phase reactions.

The presence of the added benzoic acid may inhibit the destruction of porphyrin intermediates by the radicals involved in the benzoic acid oxidation pathway. The small, transient quantities of perbenzoic acid from the decomposition of the aromatic aldehyde

actually inhibits porphyrin formation, table 3. The possibility that some transient intermediate such as a hemiacital is formed between the aldehyde and the acid is supported by the fact that 2,6-dimethylbenzoic acid has virtually no effect on the yield, table 3. As stated above, the benzoic acid is not incorporated into the porphyrin, and there is no NMR evidence at present that it is incorporated into the polymeric by-products. As can be seen in table 3, there is an exquisite balance between pKa, the nature of the acid, and the yield. Aromatic acids are effective reagents, where alkyl acids are not. The reasons for this are unclear, but may have to do with the stability of the intermediate or the pKa of the acids. It is interesting to note that the initial rates for the reaction with and without the benzoic acid catalyst are essentially the same, figure 4.

Other observations that support acid catalysis include the inhibition of the reaction by trace amounts of ammonium hydroxide. Additionally, the pK_a in the gas phase must be considered since catalytic amounts of HCl, trifluoroacetic (TFA), and trichloroacetic acid greatly reduce the yields while propionic, and acetic acids have little effect on the yield, table 3. The Lewis acid, BF_3 (as its etherate) also generally decrease the yields to only trace amounts. In the presence of either TFA or BF_3 there is a substantial increase in the quantity of soluble dipyrromethanes and small polymers in the reaction products as seen from the UV-Vis. spectra.

Oxidation

Figure 4 indicates that porphyrin formation occurs very rapidly. Oxidation of the intermediate polypyrromethanes and/or the porphinogen is the rate limiting step in the propionic acid synthesis. Oxidation must be occurring rapidly and efficiently under these conditions, since we detect no porphyrinogen and no chlorins even after reaction times of

a few seconds. Anaerobic reaction conditions result in trace amounts of porphyrin only, and some dipyrromethane indicating the crucial role of dioxygen in formation of the porphyrin products and that the aldehyde may also serve as an oxidant. Under reaction conditions that use a 3-fold excess of the aldehyde, a small excess of dioxygen exhibits only a weak effect on the porphyrin yield.* However, the use of a dioxygen atmosphere results in the formation of greater quantities of the tarry biproducts and small amounts of (<5%) porphyrin. These byproducts are less-soluble materials. The addition of both volatile and non-volatile oxidants found to be effective in other porphyrin procedures, such as $VOCl_3$,[8] $Na_2S_4O_6$, I_2, H_2O_2 [13]and nitrobenzene,[13] results in little or no detectable porphyrin in the black tarry products, table 3. At these elevated temperatures, these oxidants probably react with the pyrrole, the aldehyde, and even the porphyrin.

Surface

The glass walls of the vial do not directly participate in the reaction since control experiments, whereby glass wool is inserted into the reaction vial or the vial has been silanated, show similar yields and rates.

Unlike the glass surface, a silica gel surface which is weakly acidic catalyzes the formation of meso alkylporphyrins, such as tetraheptaporphyrin.

Water

That the rapid removal of water away from the condensing porphyrin (intermediates) is one key to these reaction conditions is shown by the substantial amount of water needed to reduce porphyrin yields, figure 8. When one equivalent of pyrrole is added to a 15:3 water:benzaldehyde mixture that has been preheated for 5 minutes at 200 ^{0}C, the yield decreases by about a third, from18% to 12%. When a 55-fold excess of water is added

the yield drops to about half that of the anhydrous 1:1 aldehyde:pyrrole reaction (9%) – indicating a relatively weak dependence on water. The presence of 2 equivalents of water essentially quenches porphyrin formation under Lindsey conditions.[7] A stiochiometric amount of water added to the reaction mixture sharply decreases the yields in Alder synthesis too.[4] In our synthesis, some of the reduced yield in the presence of water can be recovered by prolonging the reaction times. Increasing the reaction time from 20 to 40 minutes increases the porphyrin yield from 12% to 16% in the presence of a 15-fold excess of water, using standard reaction conditions. Much longer reaction times, >60 minutes, actually decrease the porphyrin yield. These results support the observation that the dehydration steps are the key part of the mechanism affected by this high-temperature method, shifting the equilibrium towards the products.

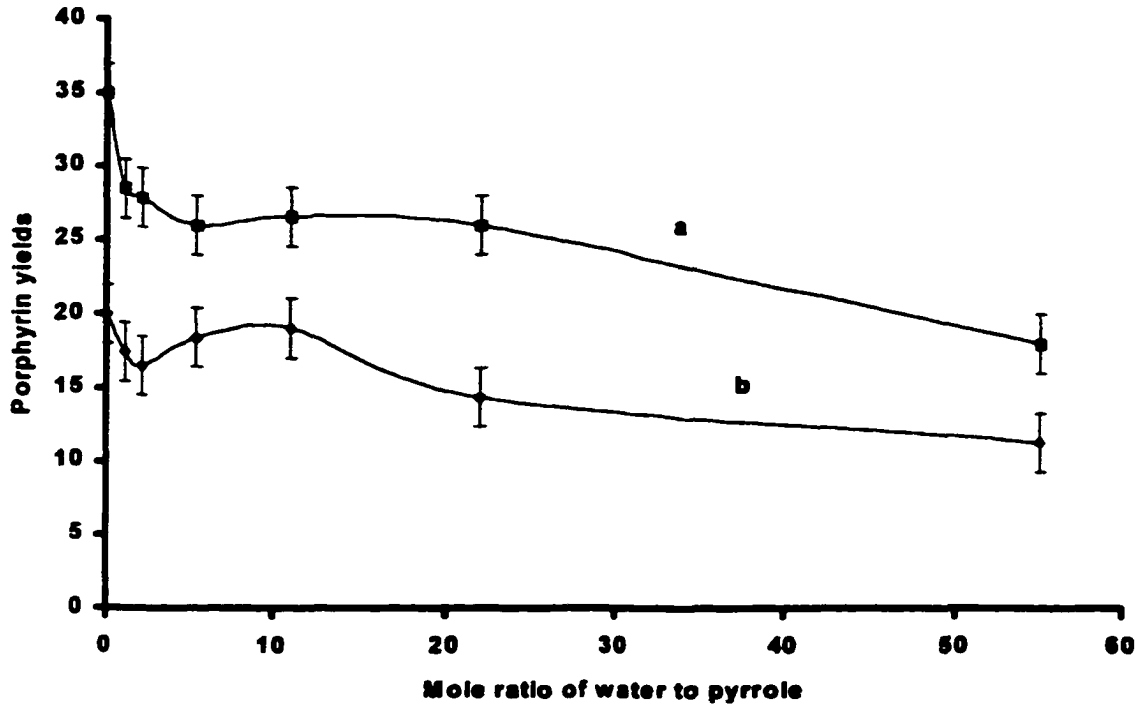


Figure 8. Water effects on porphyrin yields. a: With benzoic acid catalysis, standard conditions; b: without benzoic acid catalysis, mole ratio of benzaldehyde to pyrrole is 3:1, preheat 5 minutes.

Concentration studies

Since there is no solvent, the concentration is defined as the moles of pyrrole or aldehyde inside the vial over the volume of the vial. Standard reaction conditions (a 1:2:1 mole ratio of benzoic acid:benzaldehyde:pyrrole, 5 minutes preheat and 20minutes reaction time at 200^{0}C) were used. The yield of porphyrin vs the reaction concentration is shown in figure 9. From this figure, we can see there is a maxim in porphyrin yield when the concentration of pyrrole is 1.2mM.

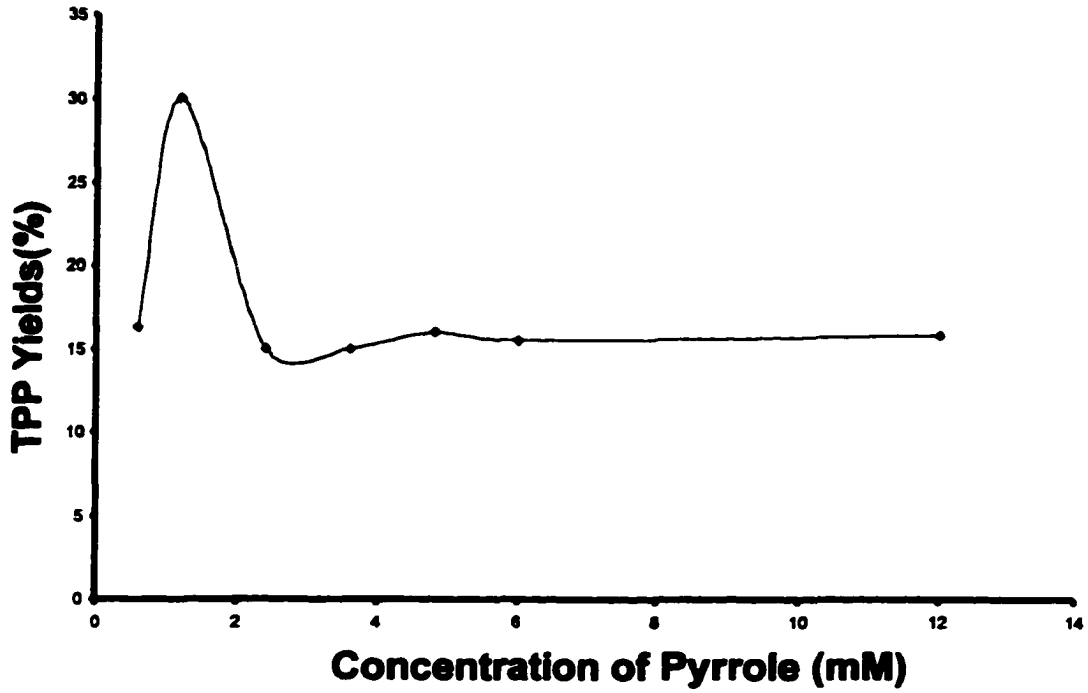


Figure 9. The effects of pyrrole concentration on porphyrin yields. Reaction conditions: mole ratio of benzaldehyde:benzoic acid:pyrrole = 2:1:1, preheat 5 minutes, same vial volume. After ~3 mM pyrrole, O_2 is limiting- assuming all O_2 is available.

Other Reagents

There have been several recent reports that the yield of tetraarylporphyrins can be substantially increased by the addition of various salts or oxidants such as $VOCl_3$,[8] nitrobenzene,[13] and ammonium persulfate.[13] These reagents are ineffective under the present conditions. We find that salts such as NaCl[11] on the bottom or coated as a film on the side of the reaction vial actually decreases the yields of TPP under our standard reaction conditions. These results also indicate that some porphyrin formation, or some step in the process, occurs on the vessel walls. Anhydrous zinc salts or zinc oxide actually decrease the yields of TPP in favor of the soluble bi-products. This observation may be due to adsorption of the zinc by the bi-, tri- or tetra- dentate pyromethane intermediates, which are observed by their broad 500 nm absorption.

Other Observations

The porphyrin and other products are found to be distributed throughout the reaction vessel. When the products at various places in the vial are carefully removed and analyzed, it is found that the porphyrin is found both at the top and the bottom of the vial. In fact, very thin 1-4 mm branched leaflets of nearly pure porphyrin are found on the sides of the glass reaction vessel. Small purple islands of porphyrin in the midst of dark tar are also observed. Merely heating benzoic acid and pyrrole results in neither porphyrin nor pyrromethanes. Alternative methods to heat the reaction vial by a standard microwave oven, or sonnication, yields only trace amounts of porphyrin.

Under the standard condition, no traces of corrole[16,17] was found by the ESI-MS of the crude reaction mixture when reacting pentalfluroaldehyde with pyrrole, only porphyrin and some polypyrroles were found. The structure of the polypyrroles was not further studied.

Porphyrin formation

The mechanism of porphyrin formation has been well reviewed,[1,6,7] and we have no evidence that the basic condensation reactions or oxidation steps are different under the conditions of this synthesis. In this procedure, there are several pathways to porphyrin: (a) the porphyrin forms in the gas-phase then condenses on the reaction vessel sides; (b) the porphyrin forms in a film coated onto the reaction vial surface; (c) the initial condensation step(s) occur in the gas-phase and the final steps on the surface, the latter may be both condensation and oxidation.

Several observations imply that at least the initial reactions take place in the gas phase. The atmosphere in the vial containing only benzaldehyde remains clear after it is heated for 5 minutes at 210^0 C, but a brown-purple gas forms immediately upon addition of the pyrrole. There are minimal effects of surface area and surface modification on reaction yields. Experiments where the reactants are mixed as gasses at 210^0 C by diffusion from opposited side of a reaction tube show similar results as when the liquid pyrrole is injected to a preheated vapor of the aldehyde. Quenching the reaction just after addition of the pyrrole indicates that the dark vapor consists of both reactants, dipyrromethanes, TPP, and trace amounts of the chlorin indicated by UV-Vis. and ^{1}H NMR spectra.

The second, liquid phase only, pathway can be eliminated because of the above observations and the volatility of the reactants and temperature dependence of porphyrin formation. Assuming ideal solutions, the concentration of the reactants approaches 3 M in this condensed film. The great increase in insoluble polymer formation at the expense of TPP when the reactants are mixed at room temperature and flash heated to 210^0 C,[21] coupled with the observation of porphyrin, dipyrromethanes, trace amounts of chlorin,

and virtually no insoluble polymer just after the reaction is initiated, suggests that the porphyrin can indeed be formed in the gas phase. Increasing the pressure of the reaction by increasing the amount of air in the system, results in the formation of an equilibrium between the liquid and vapor state for both reactants. The relative product distribution at elevated pressure is quite similar to the flash heating experiments.

The third pathway, where the initial reactions take place in the gas phase and the intermediates condense on the walls of the reaction vessel where the porphyrin is formed, is most consistent with our experiments. A picture of the mechanism for this porphyrin synthesis emerges from the above data. Most of the initial condensation reactions between pyrrole and aldehyde occurs in the vapor phase. The pyrro- and dipyrro-methanes immediately begin to aerosolize into minute droplets while the water remains gaseous. The small amounts of benzoic acid that is in the gas phase catalyzes the condensation reactions either as transient complexes with the reactants or in the droplets. While oxidation probably occurs concomitantly to the condensation, the components of these droplets further oxidize to the methylenes and react with each other to form larger species, including the porphyrin, which then condense on the surface of the reaction vessel. This idea has precedence in the MacDonald "2+2" and "3+1" coupling reactions to form porphyrins. The high temperatures drive the condensation reactions by removal of water from the droplets and liquids on the surface. This accounts for the 1.5 minute time constant seen in reactions with and without the benzoic acid. The second time constant represents further reactions of non-porphyrinic products condensed on the vessel walls that can form polymers or porphyrin, and the benzoic acid catalyzes the formation of the later by a process yet to be determined. Again, the porphyrinogen instability

towards oxidation, argues against large amounts of this species being formed in any phase of the reaction. Note that the time constants for the bi-phasic increases are essentially the same for both the benzaldehyde/pyrrole reactions (τ_1= 1.3 ± 0.1min., τ_2= 8.3 ± 0.9min.), as well as the benzoic acid catalyzed reaction (τ_1= 1.4 ± 0.2min., τ_2= 13.9 ± 1.5min.). The observation that the yields continue to increase with increasing temperature up to ~250^0 C is consistent with keeping more of the small pyrrolic multimers (the initial intermediates) in the gas phase. The small islands of pure TPP in black tar indicate that the porphyrin can crystalize out of the hot reaction solution. The branched leaflets of pure porphyrin on the vial wall with no indication that they came from solution, may arise from sublimation processes.

*: In an 8.3 mL vial, there is ~6.8 x 10^{-5} mol of O_2. A typical experiment uses 10^{-4} mol of pyrrole and 2 x 10^{-4} mol of aldehyde for a theoretical yield of 2.5 x 10^{-5} mol of porphyrin. Since 2 mol of O_2 are consumed in the oxidation steps, and 4 mol of H_2O produced, the theoretical requirement for O_2 is 5 x 10^{-5} mol. Therefore the amount of O_2 is sufficient for the reaction. However, O_2 reacts with the aldehyde, this reduces the amount available to form porphyrin, unless a transient intermediate in this process serves as an oxidant, such as the peracid.

Conclusion

A new synthetic method has been developed to synthesize commercially important porphyrins. The method tolerates a variety of functional groups, but the specific reaction conditions vary depending on the nature of the aldehyde. Mixed, or combinatorial libraries of porphyrins can be made using this strategy; however as in the traditional Adler synthesis of these libraries, the product distribution is influenced by the relative reactivity of the aldehydes. The optimum reaction conditions for TPP are a 1:2:1 mole ratio of benzoic acid:benzaldehyde:pyrrole, preheating the benzoic acid/aldehyde mixture for 5 minutes before pyrrole addition, and a reaction time of 20 minutes at 200° C. This results in an average spectroscopic yields of 30 ± 1.5 and isolated yields of 32 ± 2% of TPP. While the mechanism of the benzoic acid catalyzed reaction remains unclear, it greatly enhances the yield up to 150% of many of the *meso* tetraaryl porphyrins studied where R= CH_3, OCH_3, $COOCH_3$, Cl, and SCH_3. Since the reaction utilizes no solvent, and the purification is substantially simplified due to the insolubility of the tarry by-products, there is minimum waste generated – both in terms of solvent and in silica-gel for purification. The utilization of air in the oxidative steps also substantially reduces the cost of the procedure. This synthesis utilizes low pressure, aerobic conditions, whereas the Rothemund synthesis[10] is done in pyridine, at high pressure and anaerobic conditions and the yield is <5%.

Experiment section

Aldehydes were run over a short pipet columns of basic alumina before used. Pyrrole wsa purified by a short pipet silica gel column. All other reagents were used as received from Aldrich. ^{1}H- NMR were obtained on a 300MHz GE or 400 MHz Varian spectrometer. UV-Vis absorption spectra were obtained on a Carey 1 spectrophotometer and taken in $CHCl_3$ if not specified.

General method:

5,10,15,20-Tetraphenylporphyin was used to demonstrate the general procedure of the solvent free porphyrin synthesis and the spectroscopic yields determination:

The reactions were performed in a 8.3mL vial closed by a cap fitted with a gas tight rubber septum. Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and benzaldehyde (20 μL, 0.2 mmol, 2eq) were placed in a septum capped vial, The vial is placed in a temperature controlled sand bath which had a temperature of 200 (± 5) ^{0}C. After 5 minutes, pyrrole (7μL, 0.1 mmol, 1eq) was injected and the vial was kept at 200°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. Once at room temperature, a minimum amount of chloroform (~ 1-2 mL) was used to wash the vial – sonication facilitates the extraction of all the porphyrin. The rincate was loaded directly onto a pipet column loaded with ~ 1 g flash silica, and eluted with another 5 mL chloroform. Yields were determined spectroscopically[7a] and compared to the isolated yields - typically the latter were greater. ^{1}H-NMR in $CDCl_3$ (ppm, mult, int): 8.85, s, 8H pyrrole βH; 7.77, m, 12H 2,4,6-phenyl; 8.24, m, 8H 3,5-phenyl; -2.75, s, 2H pyrrole NH. UV-visible in CH_2Cl_2 [λ max (nm), log ε ($M^{-1}cm^{-1}$)]: 418(5.66), 514(4.20), 549(3.81), 595(3.60), 645(3.49). ESI-MS of the H_2TPP complex in

acetonitrile (m/e, rel. % abundance.): 615, 100%; 616, 45%; 617, 15%. (Figure A1, A2, A3 and A5)

Spectroscopic yield determination[7a]

The spectroscopic yield was calculated by subtracting the baseline absorbence at 437 nm (typically <0.1 O.D.) from that at λ_{max} at 418 nm (typically ~ 1.7 O.D.), Δabs. Since there are no added oxidants such as DDQ, chlorin, or much else that absorbs in this region, these calculations are reasonably accurate and are consistently within a few percent of the isolated yields. For analytical purposes, the vial from the above described reaction (1:2:1 benzoic acid:benzaldehyde:pyrrole) was rinsed thoroughly with several 1-2 mL aliquots of chloroform, transferd to a small test-tube, and diluted to 10 mL. If any solid was visible, sonication was used to fully solublize the reaction mixture. 25 μL of this solution was then dilluted to 5 mL and the spectra taken in a 1 cm cuvette. For a typical reaction Δabs = 1.64 O.D. and by using $\varepsilon = 4.27 \times 10^5\ M^{-1}\ cm^{-1}$ for the Soret band in Beer's law, the concentration of the cuvette solution was 3.8 μM. Factoring in the dilutions, the 25 μ mol theoretical yield, and multiplying by 100 gives a 30.9 % yield.

General procedure for scale up

Method 1:

Benzoic acid (24.4 mg, 0.2 mmol, 1eq) and benzaldehyde (40mL, 0.4 mmol, 2eq) were placed in a septum capped 1cm x 12 cm test tube which have a volume of 17mL. The test tube was put into a sand bath which had a temperature of 200°C. After 5 minutes, pyrrole (14μL, 0.2 mmol, 1eq) was injected and the test tube kept at 200°C for another 20 minutes. Afterwards, the test tube was removed from the sand bath and cooled down in

ambient air. 10mL $CHCl_3$ was used to wash the test tube and the yield was determined spectroscopically (yields: try 1: 37%, try 2: 32%).

Method 2:

Benzoic acid (0.66 g, 5.35 mmol, 1eq) and benzaldehyde (1.142g, 10.7 mmol, 2eq) were placed in a 1.1 x 7.5 test tube. The test tube was placed inside a 6.2cm x 14.8cm reaction jar that has a volume of 447mL. pyrrole (0.361g, 5.35 mmol, 1eq) was placed inside the jar but outside the test tube. The jar was capped and carefully placed vertically in a big sand bath which had a temperature of 200^{0}C. The jar was keep inside the sand bath for 30 minutes. Afterwards, the jar was removed from the sand bath and cooled down in ambient air. 30mL $CHCl_3$ was used to wash the jar and the yield was determined spectroscopically (yields: try 1: 14.8%, try 2: 14.3%).

Preparation of 5,10,15,20-Tetrakis(4-tolyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-tolualdehyde 23μL (0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath that had a temperature of 210^{0}C. After 5 minutes, 7μL (0.1 mmol, 1 eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yields were determined spectroscopically (30%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.77(s,2H), 2.71(s, 12H), 7.55(d, 8H, J=8.1Hz), 8.09(d, 8H, J=8.1Hz), 8.85(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 418.6, 515, 550, 590, 648; ESI-MS [(m+H)/z$^+$, % relative intensity] 671, 100%. (Figure A4, A6, A7 and A8)

Preparation of 5,10,15,20-Tetrakis(4-methoxyphenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-anisaldehyde 26μL (0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial is removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (25%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.75(s,2H), 4.1(s, 12H), 7.28(d, 8H, J=8.4Hz), 8.12(d, 8H, J=8.4Hz), 8.86(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 421.8, 518, 556, 592.5, 650; ESI-MS [$(m+H)/z^+$, % relative intensity] 735, 100%

Preparation of 5,10,15,20-Tetrakis(4-t-butylphenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-t-butylbenzaldehyde (32μL, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 220°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 220°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (32.5%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.74(s,2H), 1.61(s, 36H), 7.75(d, 8H, J=8.1Hz), 8.15(d, 8H, J=8.1Hz), 8.87(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 421, 517, 554, 592, 648; ESI-MS [$[m+Co(III)]/z^+$, % relative intensity] 895, 100%. (Figure A9, A10 and A11)

Preparation of 5,10,15,20-Tetrakis(3,4,5-trimethoxyphenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 3,4,5-trimethoxybenaldehyde (39.2mg, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 220^{0}C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 220°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (15%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.75(s,2H), 3.97(s, 24H), 4.18(s, 12H), 7.47(s, 8H), 8.96(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 423, 517,554,591, 647; ESI-MS [(m+H)/z$^+$, % relative intensity] 975, 100%. (Figure A12, 13 and A14)

Preparation of 5,10,15,20-Tetrakis(4-chlorophenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-chlorobenzaldehyde 28mg (0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210^{0}C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (25%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.85(s,2H), 7.75(d, 8H, J=8.1Hz), 8.13(d, 8H, J=8.1Hz), 8.84(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 419, 515, 550, 590, 647; ESI-MS [(m+H)/z$^+$, % relative intensity] 753, 100%

Preparation of 5,10,15,20-Tetrakis(4-bromophenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-bromobenzaldehyde 0.037g (0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (25%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.89(s,2H), 7.90(d, 8H, J=8.4Hz), 8.07(d, 8H, J=8.4Hz), 8.84(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 419, 514, 549, 590, 648; ESI-MS [$(m+H)/z^+$, % relative intensity] 931, 100%

Preparation of 5,10,15,20-Tetrakis(4-isopropiphenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-isopropibenzaldehyde 30μL (0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (25%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.75(s,2H), 1.55(d,24H, J=6.9Hz), 3.26(q, 4H, J=6.9Hz), 7.60(d, 8H, J=8.1Hz), 8.13(d, 8H, J=8.1Hz), 8.86(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 420, 517, 553, 591, 647; ESI-MS [$(m+H)/z^+$, % relative intensity] 783, 100%

Preparation of 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-methylxycarbonylbenzaldehyde (0.033g, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (19%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.81(s,2H), 4.12(s, 12H), 8.29(d, 8H, J=8.1Hz), 8.45(d, 8H, J=8.1Hz), 8.82(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 420, 516, 549, 592, 652; ESI-MS [$(m+H)/z^+$, % relative intensity] 847, 100%

Preparation of 5,10,15,20-Tetrakis(4-methylthiophenyl)porphyin

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 4-methylthiobenzaldehyde (30μL, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (11%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.78(s,2H), 2.76(s, 12H), 7.63(d, 8H, J=8.4Hz), 8.12(d, 8H, J=8.4Hz), 8.87(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 424, 518, 556, 592, 649; ESI-MS [$(m+H)/z^+$, % relative intensity] 799, 100%

Preparation of 5,10,15,20-Tetramesitylporphyin

NH_2OH HCl (10 mg, 0.14 mmol, 1eq) and mesitylaldehyde (30μL, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (5%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.5(s,2H), 1.85(s, 24H), 2.62(s, 12H), 7.27(s, 8H), 8.6(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 418.6, 514, 548, 590, 645; ESI-MS [$(m+H)/z^+$, % relative intensity] 783, 100%

Preparation of 5,10,15,20-Tetrakis(4-pyridyl)porphyin

Propionic acid (50μL, 0.68mmol, 6.8eq) and 4-pyridinecarboxaldehyde (20μL, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 200°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 200°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (7%). After removing the solvent, the crude was purified by flash chromatography (methanol) to give the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.92(s, 2H), 8.19(d, J=5.7Hz, 8H), 8.87(s, 8H), 9.09(d, J=5. 7Hz, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 417, 512, 547, 587, 644; ESI-MS [$(m+H)/z^+$, % relative intensity] 619, 100%

Preparation of 5,10,15,20-Tetraheptaporphyin

4-hyptaldehyde 20μL (0.2 mmol, 2eq), pyrrole 7μL (0.1 mmol, 1eq) and 0.35g silica gel were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 160°C. The vial kept at 160°C for another 5 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (2.7%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -2.6(s, 2H), 0.94(t, 12H, J=6.9Hz), 1.5(m, 8H), 1.55(m, 8H), 1.82(m,8H), 2.50(m, 8H), 4.94(t, 8H, J=7.5Hz), 9.47(s, 8H); UV-Vis. in $CHCl_3$ [λ max (nm)] 418.5, 520, 556, 601, 659; ESI-MS [(m+H)/z^+, % relative intensity] 703, 100%. (Figure A15, A16 and A17)

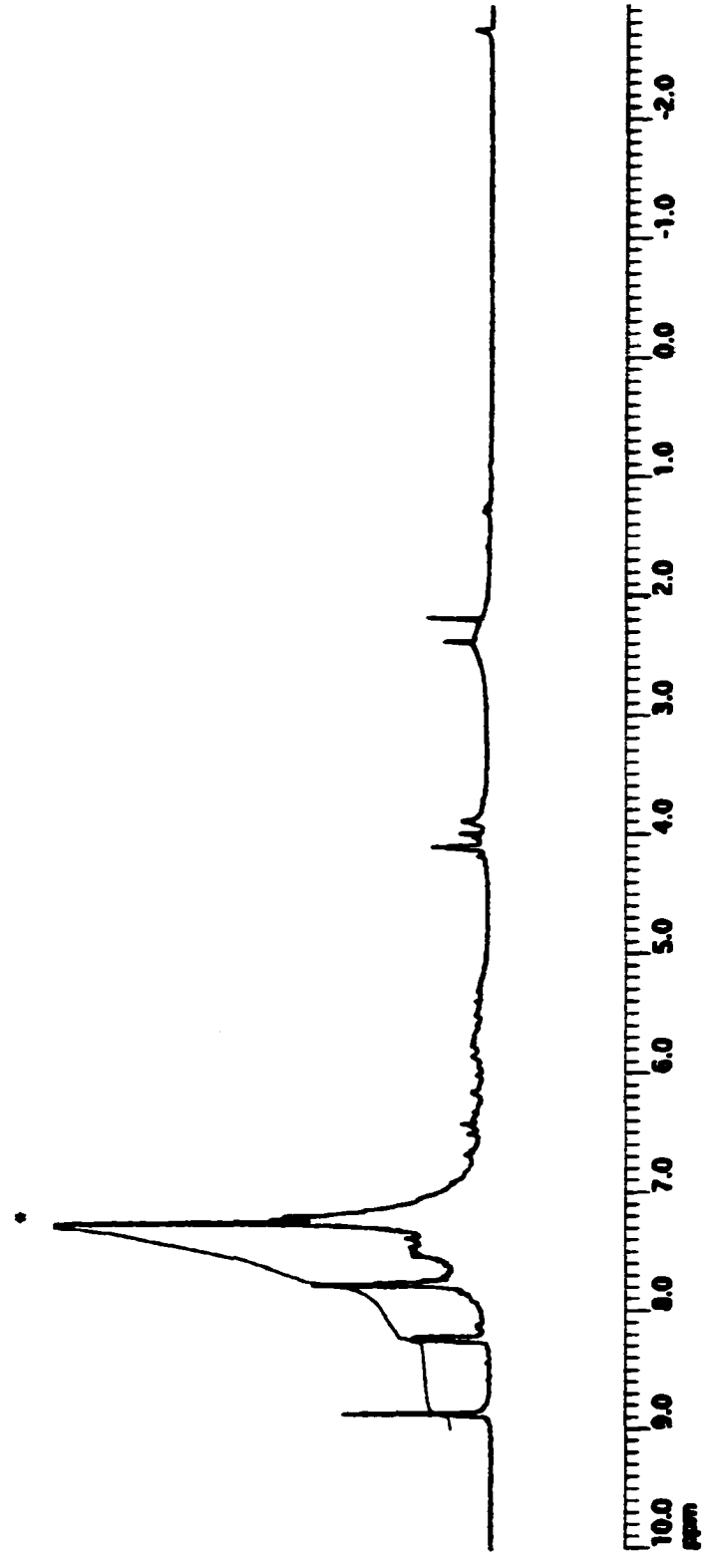
Preparation of 5,10,15,20-Tetrakis(1-pyrenyl)porphyin (mixture of rotamers)

Benzoic acid (12.2 mg, 0.1 mmol, 1eq) and 1-pyrenealdehyde (64mg, 0.2 mmol, 2eq) were placed in a septum capped vial. The vial was put into a sand bath which had a temperature of 210°C. After 5 minutes, 7μL (0.1 mmol, 1eq) of pyrrole was injected and the vial kept at 210°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled down in ambient air. 10mL $CHCl_3$ was used to wash the vial and the yield was determined spectroscopically (5%). After removing the solvent, the crude was purified by flash chromatography ($CHCl_3$) to give the pure product.

^1H NMR(300 MHz, $CDCl_3$) δ -1.9(s, 2H), 7.55(m, 4H), 7.72(m, 4H), 8.05(m, 8H), 8.3(m, 12H), 8.4(m, 12H), 8.8(m, 4H); UV-Vis. in $CHCl_3$ [λ max (nm)] 433.6, 520, 556, 594, 653; ESI-MS [[m+Co(III)]/z^+, % relative intensity] 1167, 100%. (Figure A18-20)

Appendix

Figure A 1



^{1}H NMR of crude TPP in a standard reaction after removing benzoic acid in $CDCl_3$. *: $CHCl_3$

Figure A 2

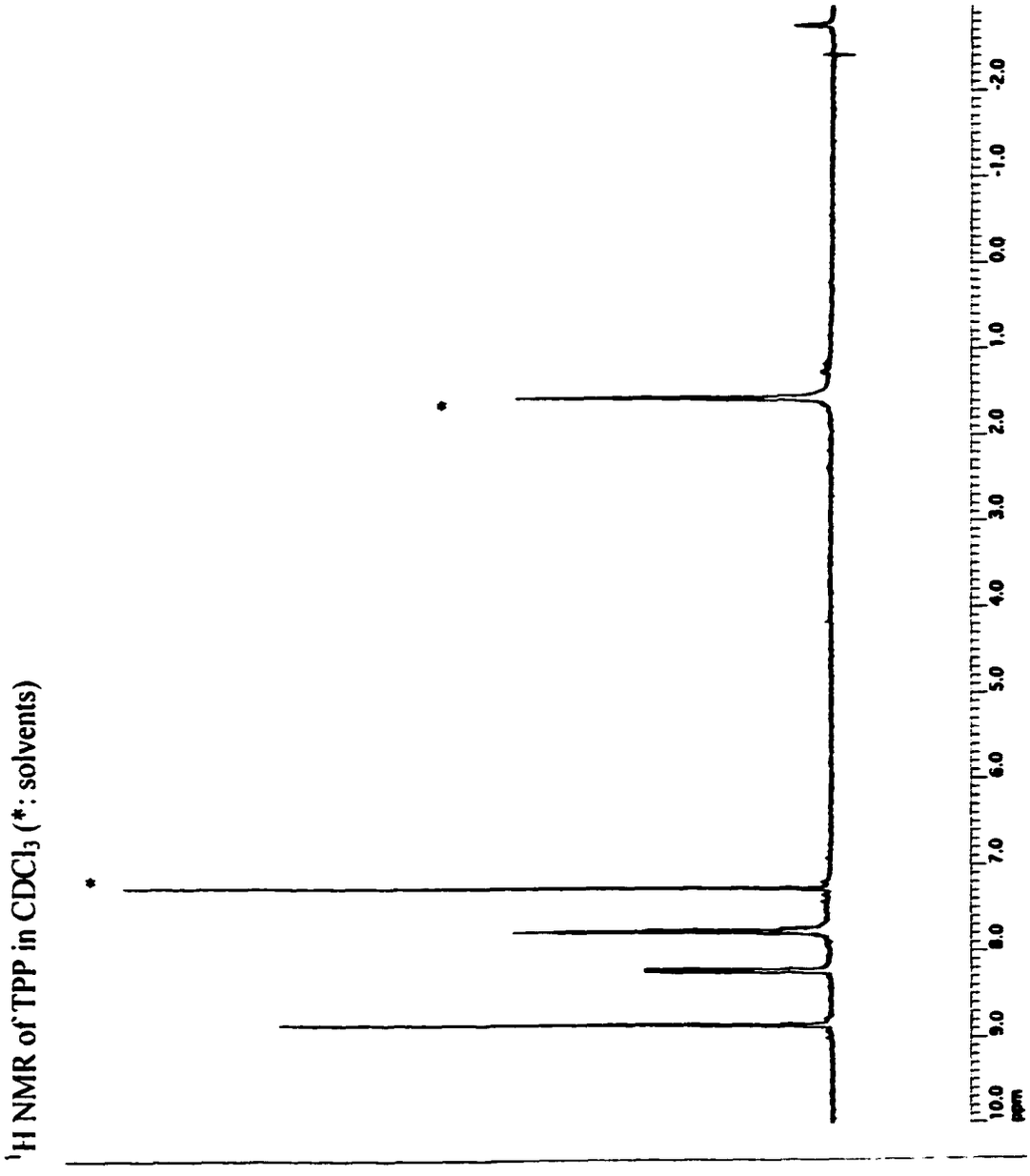


Figure A 3

a: Pure TPP in $CHCl_3$
b: Crude reaction mixture of a standard solvent free TPP synthesis in $CHCl_3$

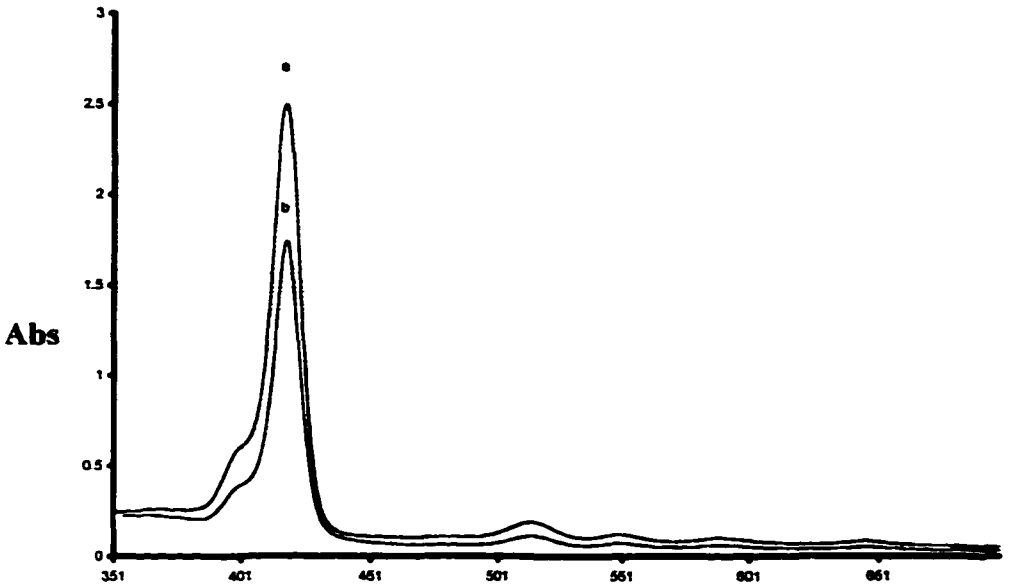


Figure A4. a: Pure TTPP in $CHCl_3$
b: Crude reaction mixture of a standard solvent free TTPP synthesis in $CHCl_3$

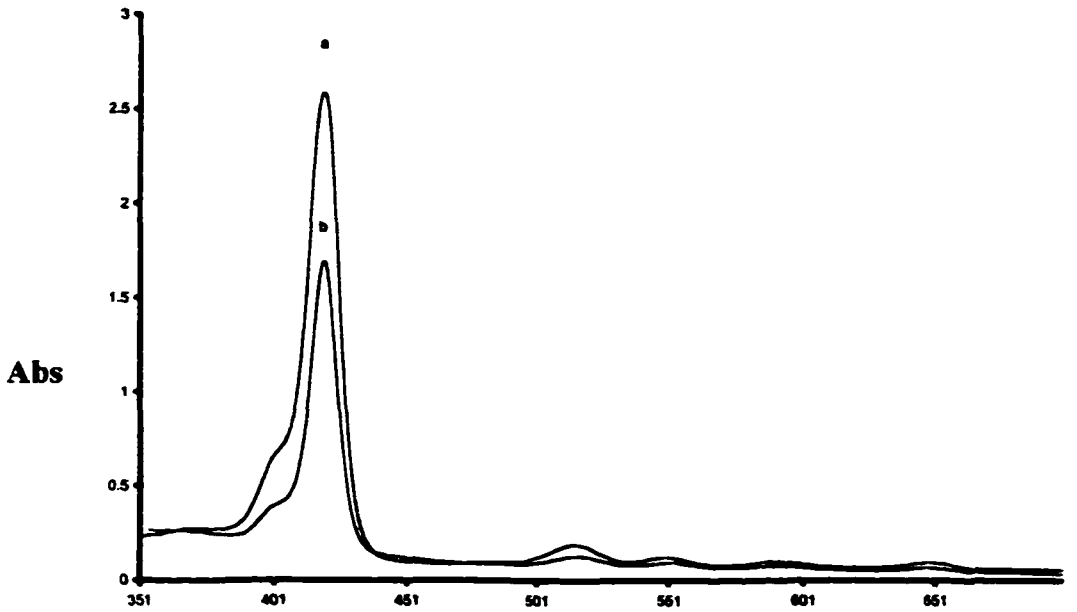


Figure A5 ESI-MS of TPP

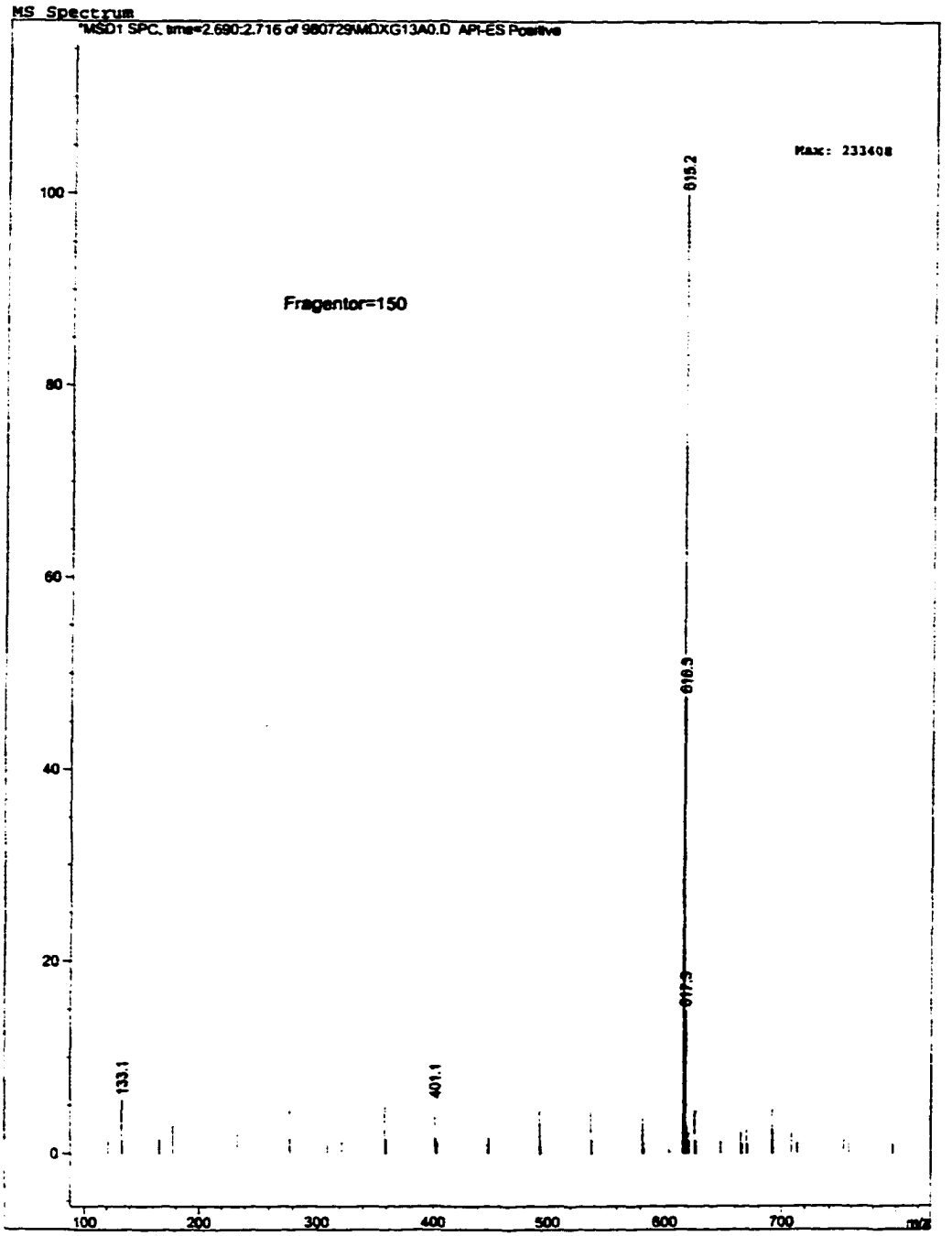


Figure A 6

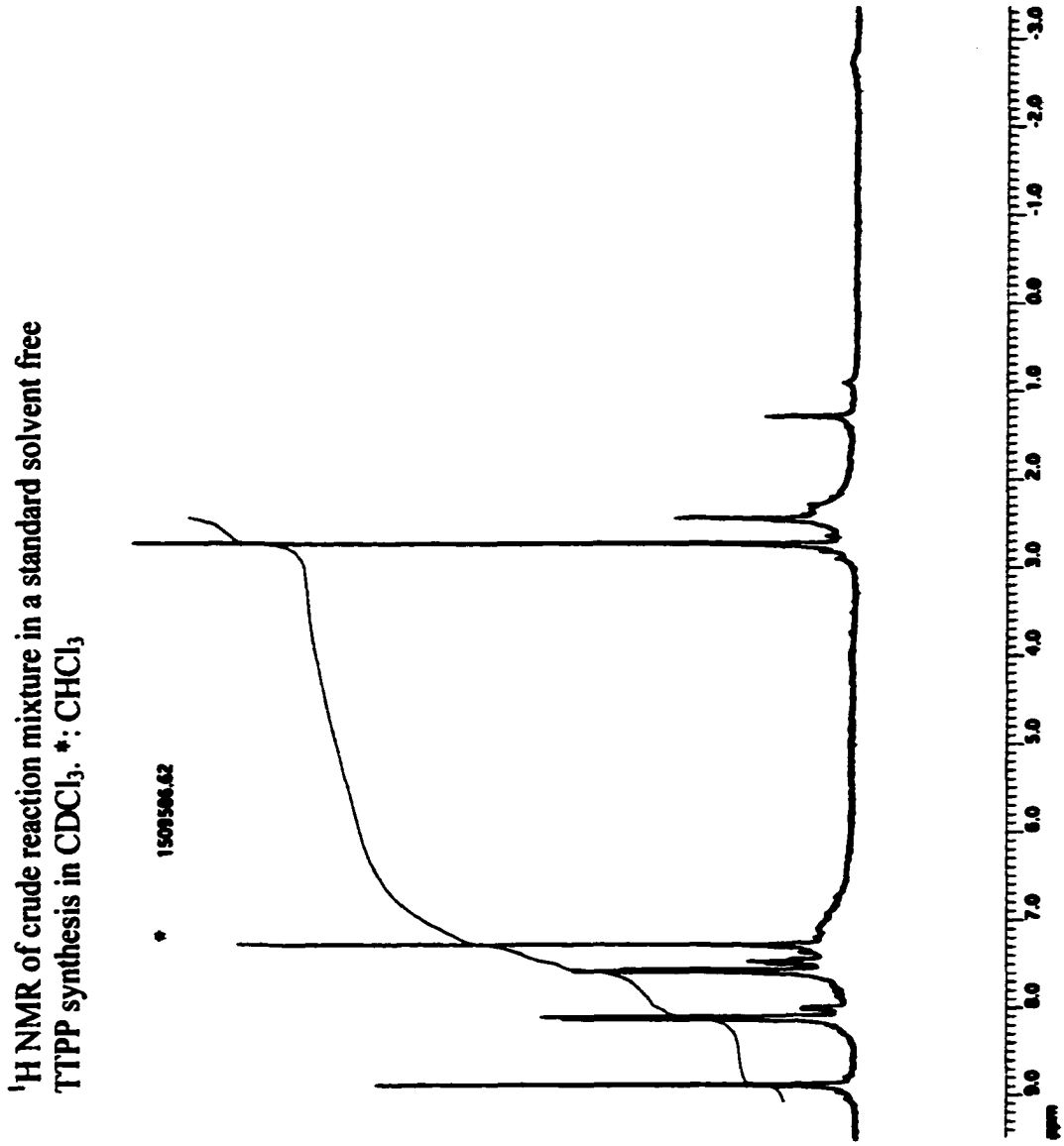


Figure A 7

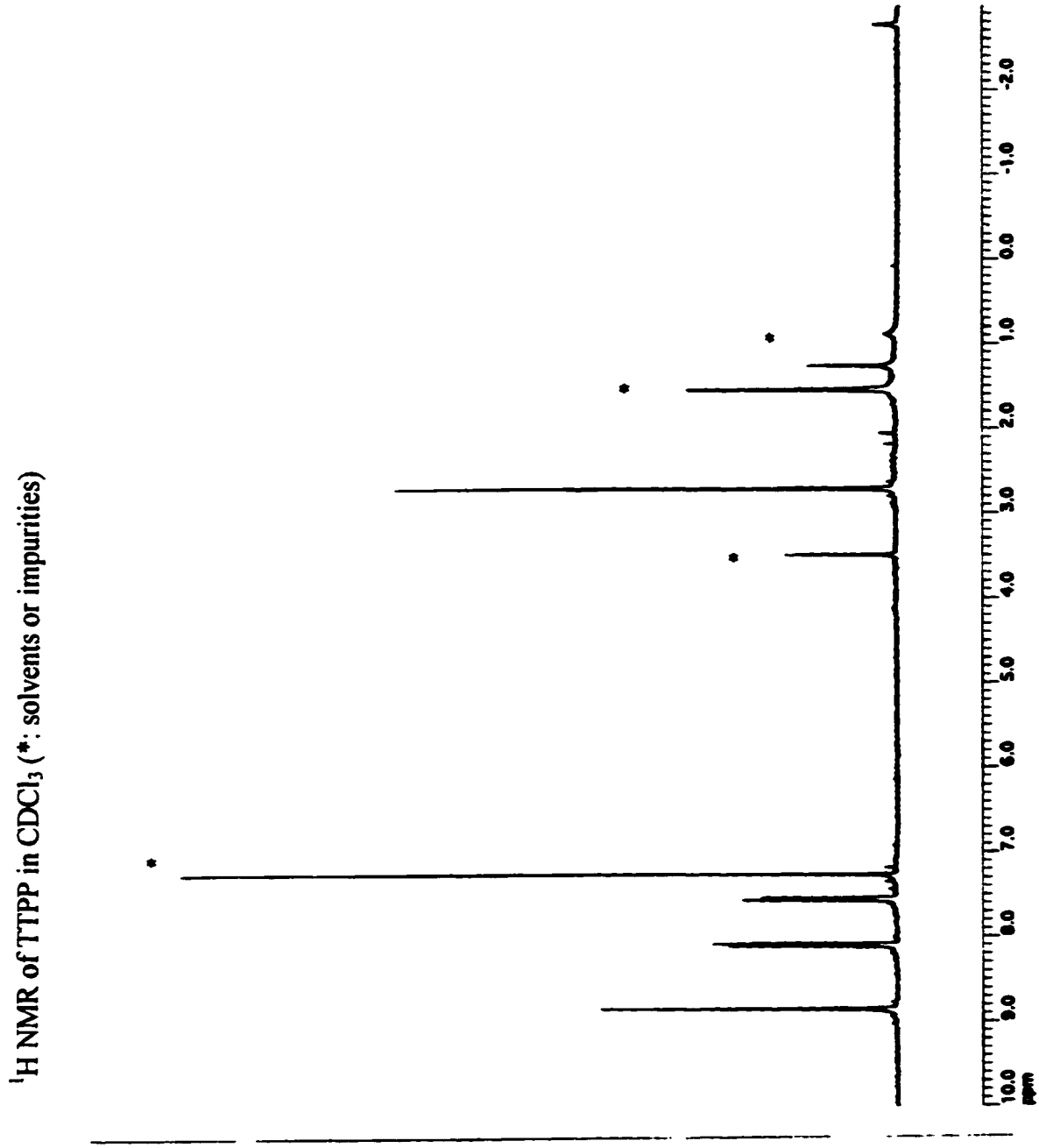


Figure A 8

ESI-MS of TTPP

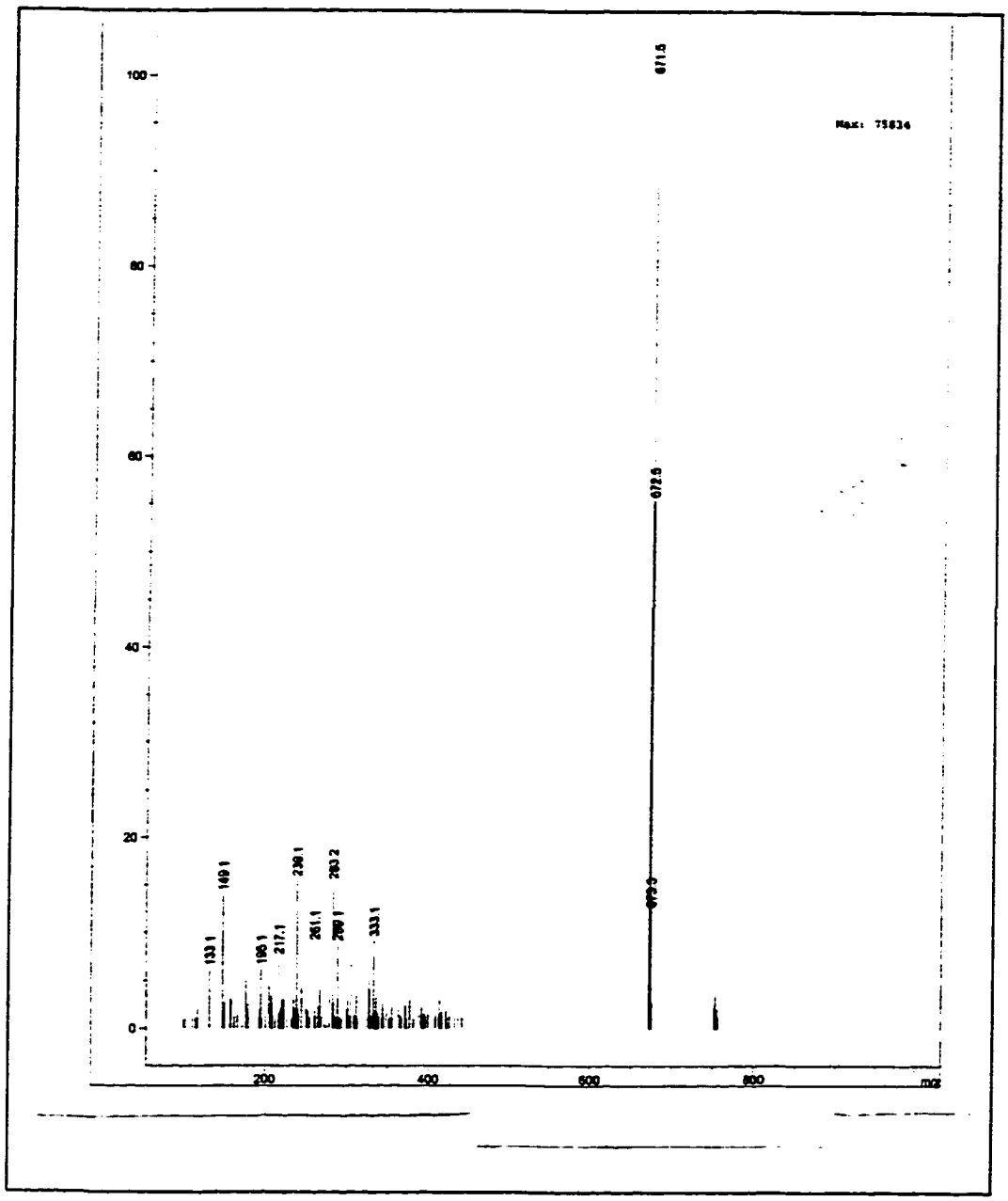


Figure A 9

^1H NMR of 5,10,15,20-Tetrakis(4-t-butylphenyl)porphyin in $CDCl_3$ (*: solvents)

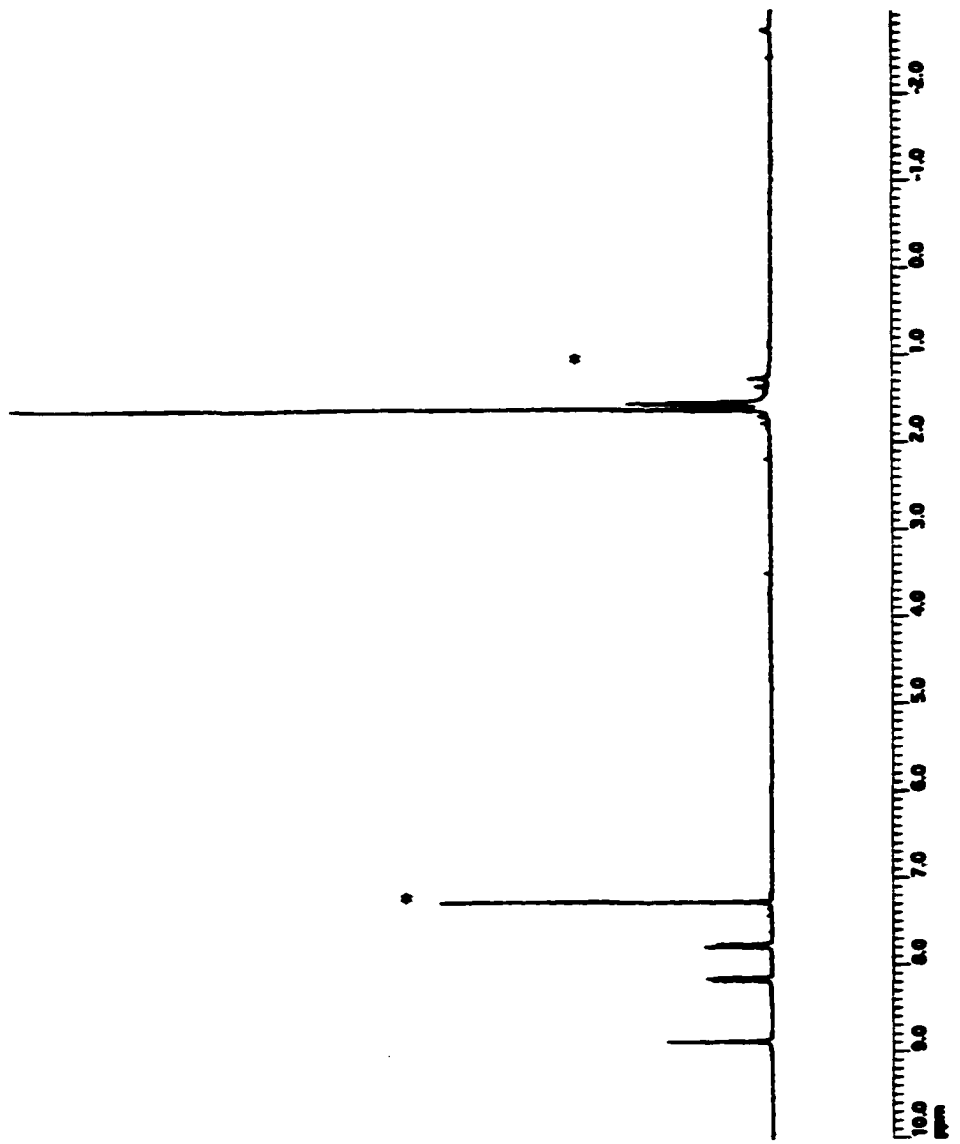


Figure A 10

ESI-MS of Co(III)-Tetrakis-(4-t-butylphenyl)porphyrin

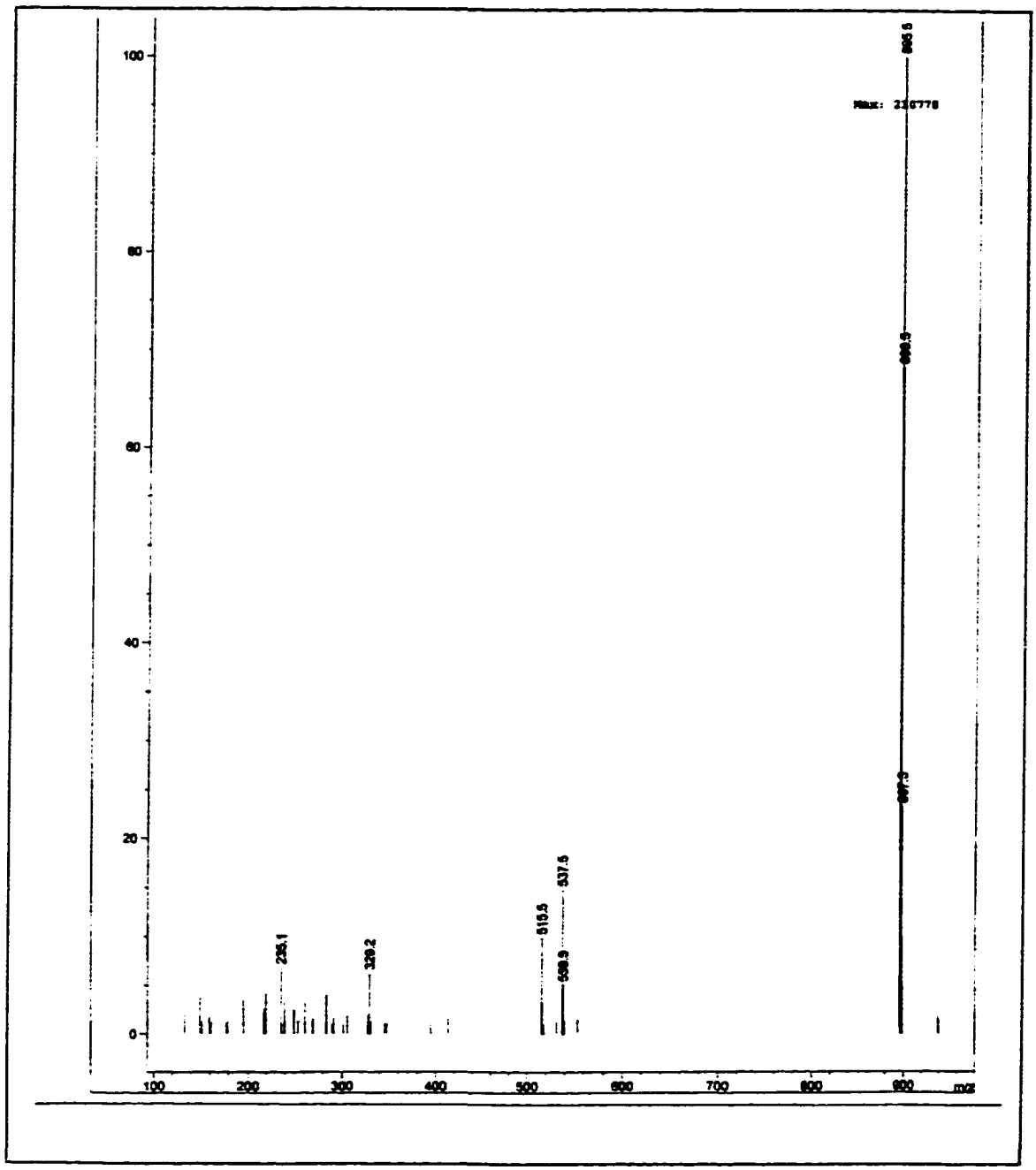


Figure A11. 5,10,15,20-Tetrakis(4-t-butylphenyl)porphyin in $CHCl_3$

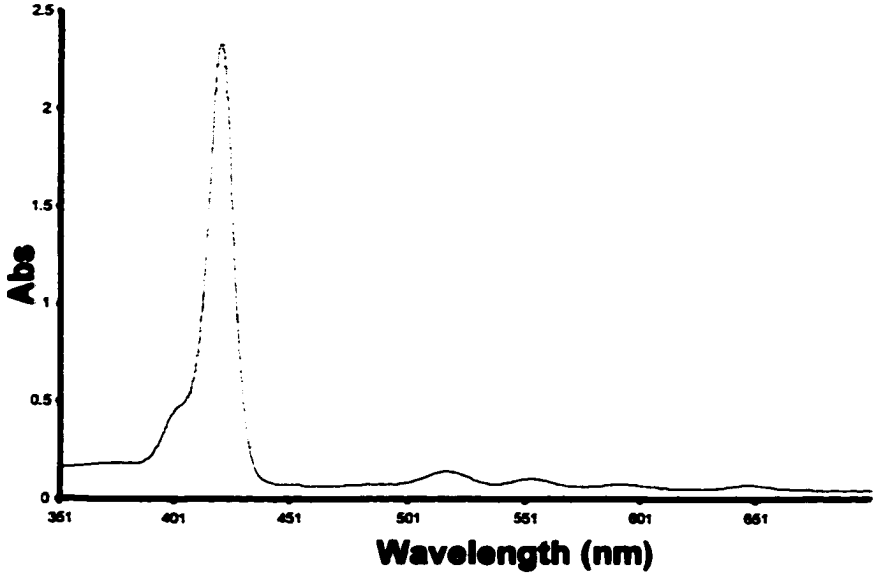


Figure A 12

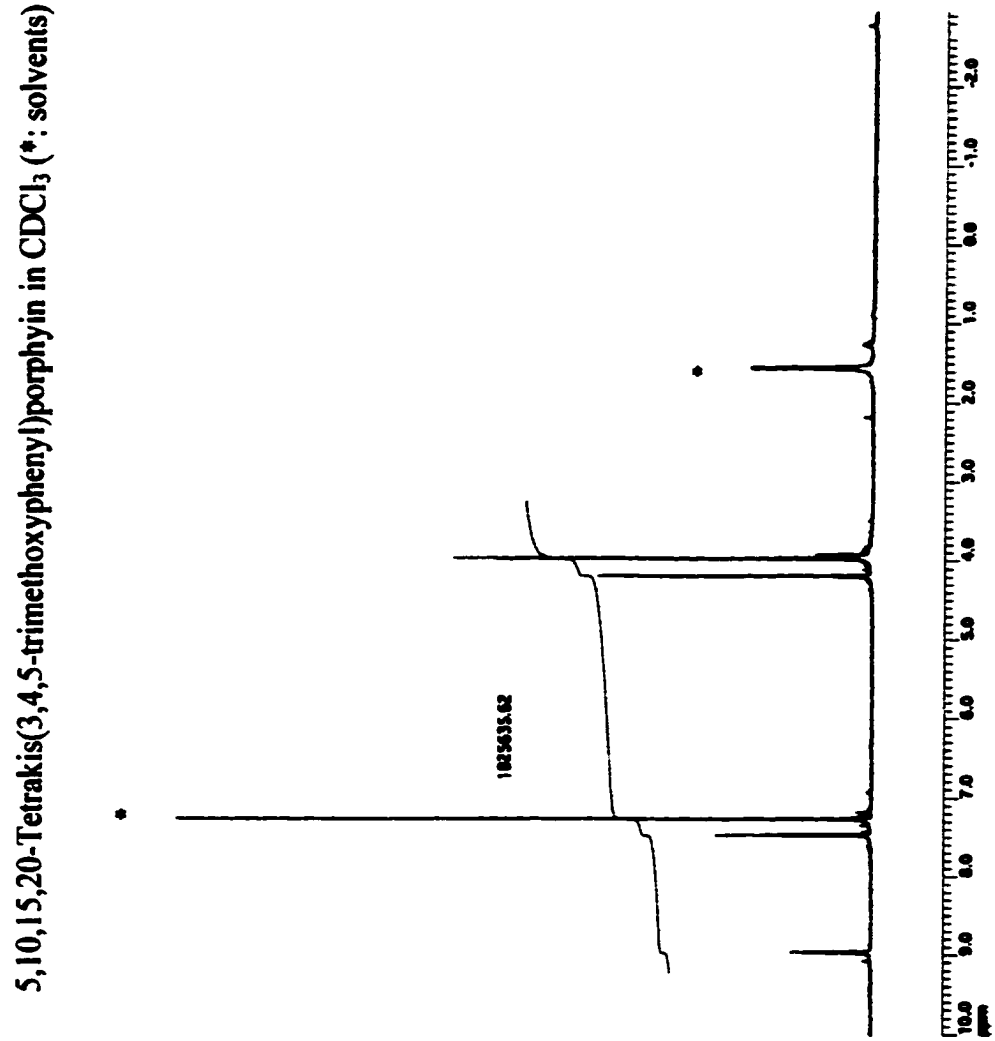


Figure A13. 5,10,15,20-Tetrakis(3,4,5-trimethoxyphenyl)porphyin in $CHCl_3$

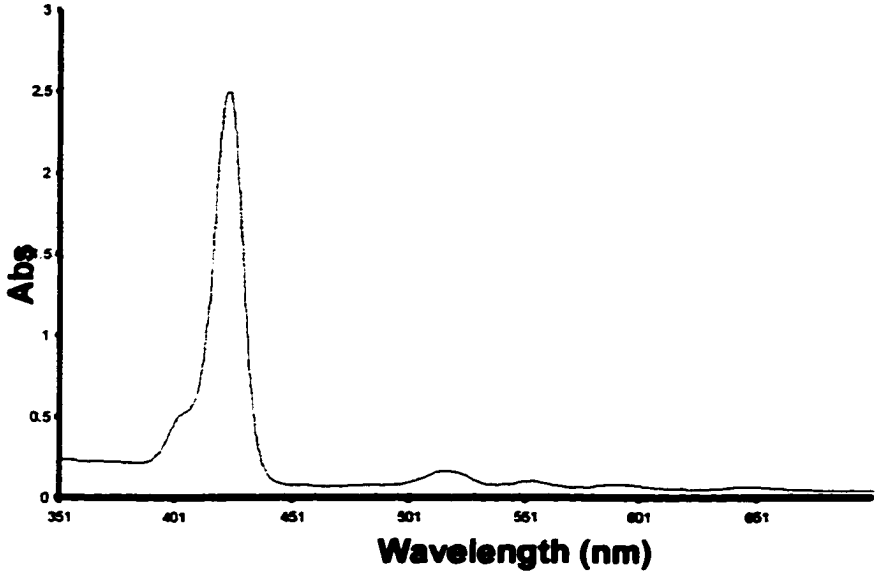


Figure A14. ESI-MS of 5,10,15,20-Tetrakis(3,4,5-trimethoxyphenyl) porphyin

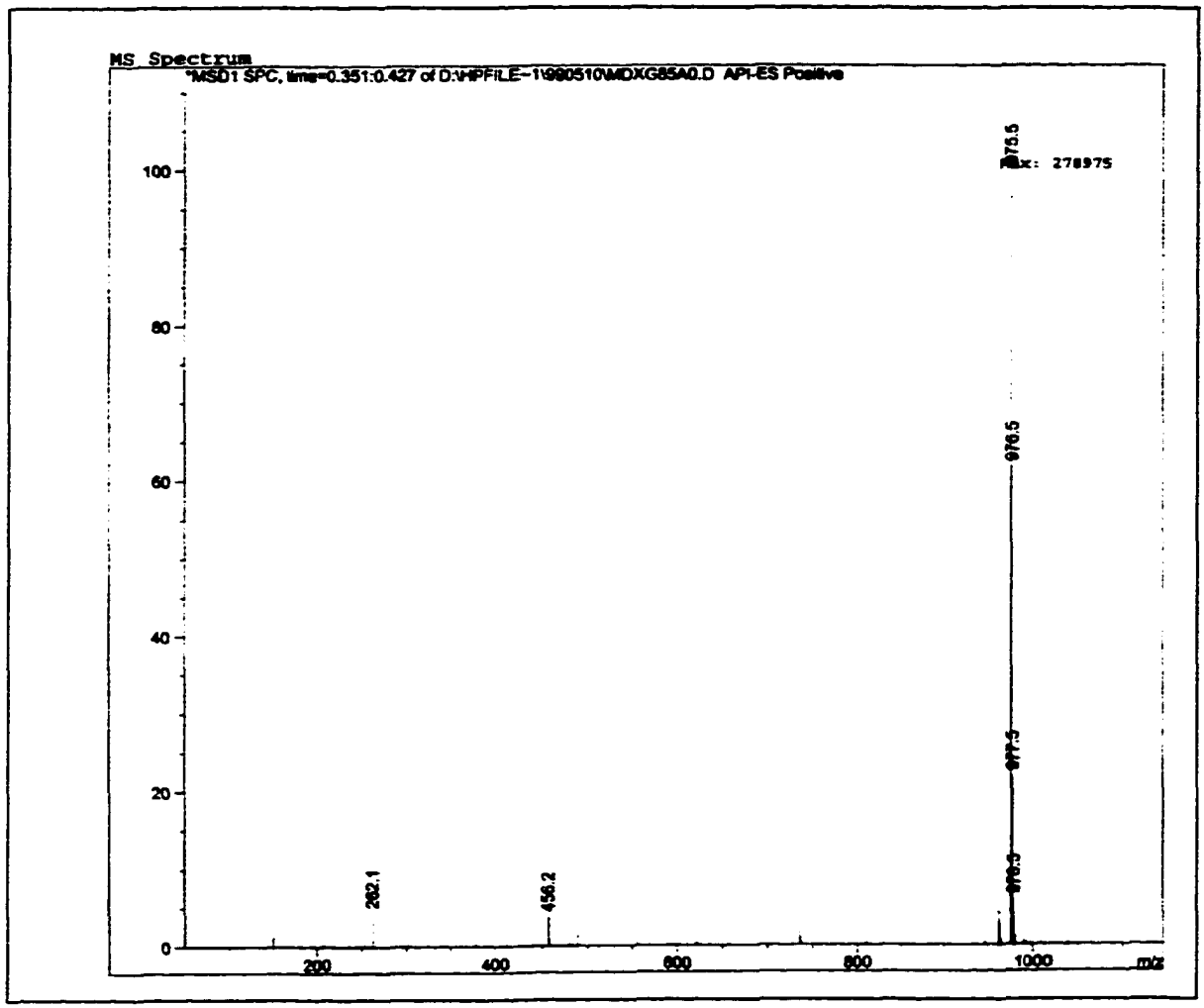


Figure A 15.

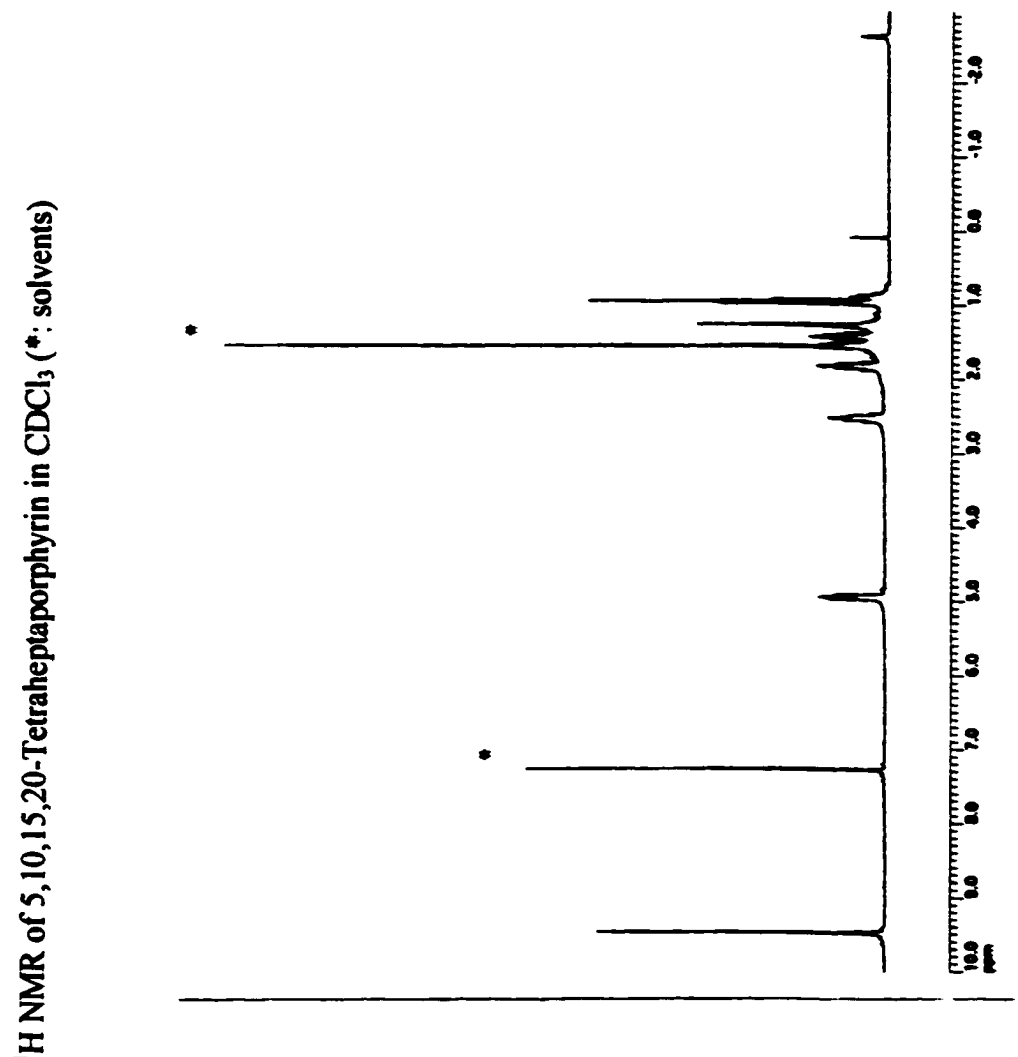


Figure A16. 5, 10, 15, 20-Tetraheptaporphyrin in $CHCl_3$

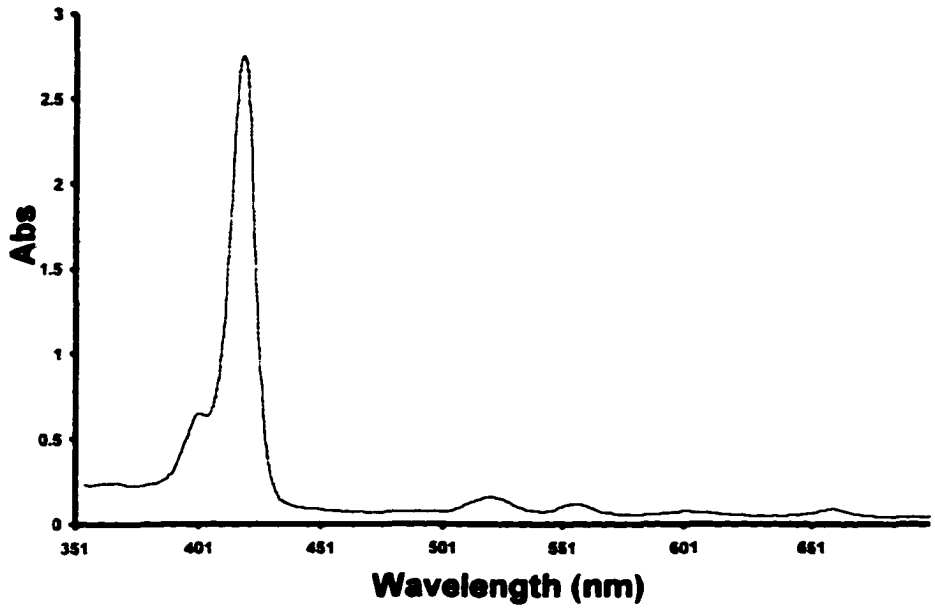


Figure A17. ESI-MS of 5, 10, 15, 20-Tetraheptaporphyrin

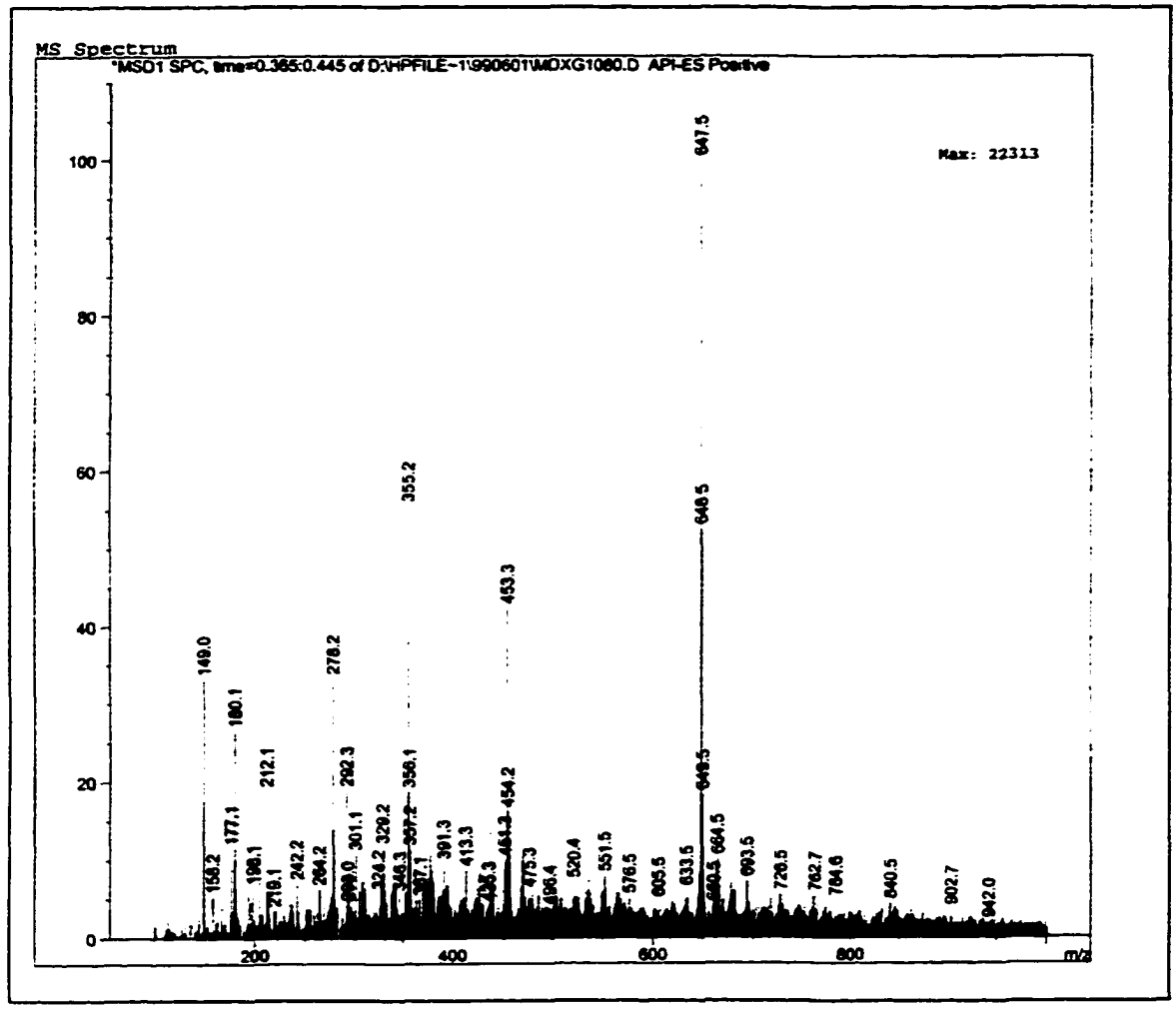


Figure A 18.

^{1}H NMR of 5,10,15,20-Tetrakis(1-pyrenyl)porphyin (mixture of rotamers) in $CDCl_3$ (*: $CHCl_3$)

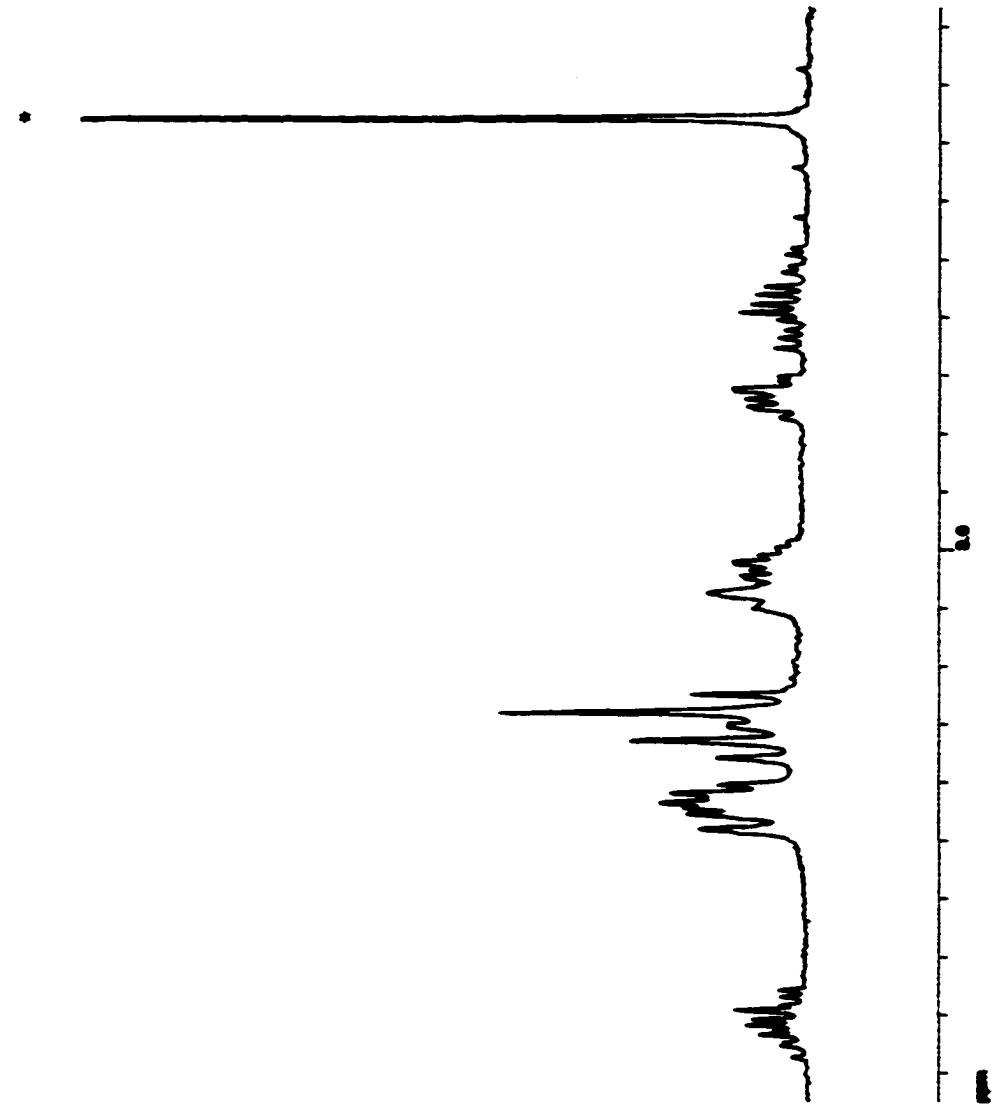


Figure A19. ESI-MS of Co(III)-5,10,15,20-Tetrakis(1-pyrenyl)porphyrin

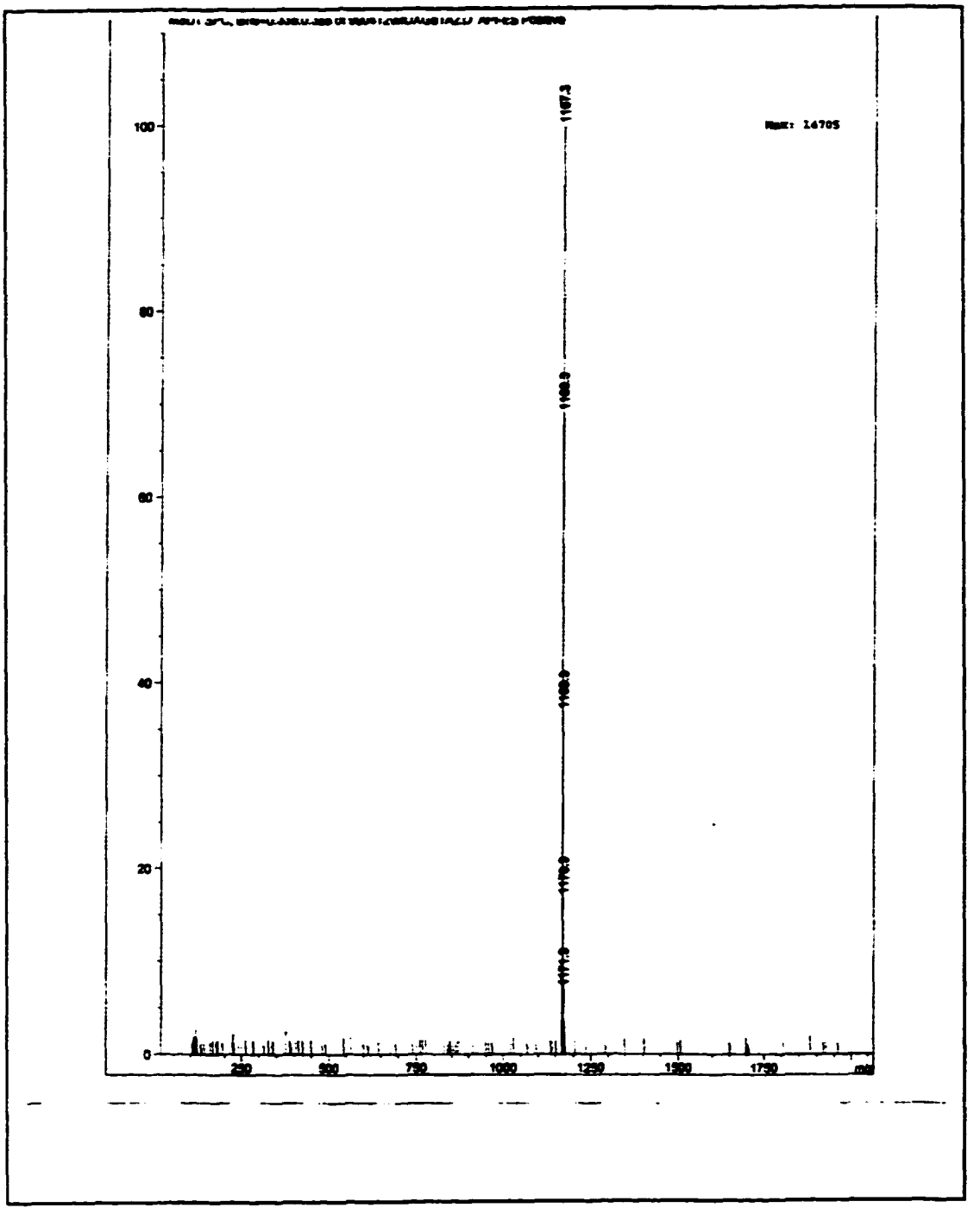
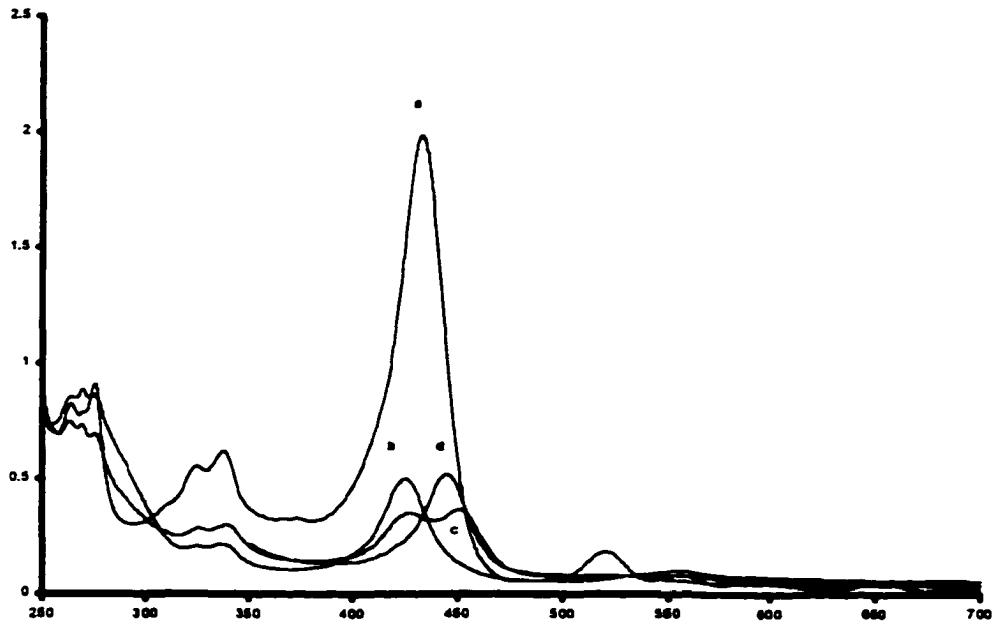


Figure A 20.

a: 5,10,15,20-Tetrakis(1-pyrenyl)porphyrin in $CHCl_3$
b: Co(II)- 5,10,15,20-Tetrakis(1-pyrenyl)porphyrin in $CHCl_3$
c: A mixture of Co(II)- 5,10,15,20-Tetrakis(1-pyrenyl)porphyrin and Co(III)- 5,10,15,20-Tetrakis(1-pyrenyl)porphyrin in $CHCl_3$
d: Co(III)- 5,10,15,20-Tetrakis(1-pyrenyl)porphyrin in $CHCl_3$



An undergraduate lab design

Compared to other porphyrin synthetic methods, our synthetic method is more suitable for an undergraduate lab. First, it is a solvent free process, so it minimizes the waste of the solvent; second, it is a very inexpensive experiment and requires only a small vial for glassware. The work up process is very easy and does not require a chromatography process for several porphyrins. The experiment's time is about 45 minutes so students should have enough time to run this reaction twice in a three hour lab class, or to do other experiments on the compound.

Experimental Procedure

Preparation of 5,10,15,20- Tetraphenylporphyrin

A 8.3mL vial contained benzaldehyde(20μL, 0.2mmol, 2eq) and benzoic acid(0.0122g, 0.1mmol, 1eq) was closed by a cap fitted with a rubber septum (there was a small hole in the cap in order to inject sample) and placed in a sand bath. The temperature of the sand bath was adjusted to ~200^{0}C. After heating the vial for 5 minutes at 200^{0}C, a stirring rod was used to lift the vial a little bit above the sand surface. Pyrrole(7μL, 0.1mmol, 1eq) was injected to the vial by a microsyringe and the vial was pushed back to the sand bath. The vial was kept in the sand bath for another 20 minutes. The vial was then removed and left in air to cool. Three clean test tubes were marked 1, 2 and 3. 10 mL $CHCl_3$ was placed in test tube 1 and 5 mL $CHCl_3$ was placed in test tube 3. The 10mL $CHCl_3$ in test tube 1 was used to wash the reaction vial. The wash-out was placed in test tube 2. A microsyringe was used to transfer 25μL solution from test tube 2 to test tube 3. A pipette was used to transfer the solution from test tube 3 to a 1x1cm cuvette to measure the

absorbence of the Soret band at 419nm. The yield can be calculated this way: Yield (%) = Abs (at 419 nm) X 19 (%). After the yield was determined, a 5mL 0.1 M KOH solution was added to the 10mL crude reaction mixture to extract the benzoic acid. The organic layer was separated and dried by anhydrous Na_2SO_4 and the salt was removed by vacuum filtration. 0.5g silica gel was added to the filtrate and the solvent was removed under vacuum. 5mL methanol was used to wash the porphyrin from the silica gel and the methanol was removed under vacuum. Thus, pure tetraphenyl porphyrin was obtained. Yield: 30%, ^{1}H NMR ($CDCl_3$, 300MHz):δ -2.75(s, 2H, pyrrole NH), 7.77(m, 12H, 2,4,6-phenyl), 8.24(m, 8H, 3,5-phenyl), 8.85(s, 8H, pyrrole βH)

Preparation of 5,10,15,20-tetrakis(4'-methyl)phenylporphyrin

Same procedure as above except using 23μL 4-methylbenzaldehyde instead of the 20μL benzaldehyde. Yields: 28%, ^{1}H NMR ($CDCl_3$, 300MHz): δ -2.75(s, 2H, pyrrole NH), 2.72 (s, 12H, $-CH_3$), 7.58(d, J=8.1 Hz, 8H, 2,4-phenyl), 8.08(d, J=8.1 Hz, 8H, 3,5-phenyl), 8.85(s, 8H, pyrrole βH)

Notes:

1: The rapid formation of the gases and the flash points of the aldehydes and pyrrole should be considered.

2: High temperature is the key for a higher yield, so make sure the vial is deeply buried inside the sand bath.

3: Ethyl acetate can be used instead of $CHCl_3$ to wash the vial, but purification is more tedious.

4: Yield = moles of porphyrin formed/ (moles of pyrrole/4)

$$= \left[\frac{\text{Abs(at 419nm)}}{(4.27 \times 10^5\ M^{-1}cm^{-1}) \times 1cm} \times \frac{5 \times 10^3 mL}{25mL} \times 0.01L \right] \Big/ 2.5 \times 10^{-5} mol$$

$$= \text{Abs(at 419nm)} \times 19\%$$

Part 2

Solution phase combinatorial synthesis and modification of porphyrin libraries

Introduction

In the last few years there has been an explosive growth in the development of chemically diverse libraries of molecules. Combinatorial libraries have proven to be a powerful method to examine structure-function relationships, and the synthesis and screening of small molecule libraries is emerging as an important strategy for drug discovery.[22-24] Several strategies have been employed to create and characterize these linear- and core- structured combinatorial libraries including: (i)solid phase and solution phase synthesis and, (ii)grid, deconvolution, and tagging methods to identify compounds.[25]

The history of combinatorial chemistry can be traced back to 1963, when R.B. Merrifield at Rockfeller University developed the first solid phase peptides synthesis.[26] The construction of the first combinatorial library was done by H. Mario Geysen in 1984 using "pins" technology.[27] In 1985, R.A. Houghten developed the "tea bags" technology.[28] The developing of these two techniques was aimed at increasing the diversity of the molecules. The " tea bags" technique allows the synthesis of multiple peptide in preparative amount. For the coupling reaction, each "tea bag" is separated according to the amino acids and reacted in parallel with the amino acids in separate bottles. After the coupling reactions, the tea begs are collected and washed together. The

great advantage of the "tea bags" technique is that it is cost effective and easy to use. In 1991, Furka developed the "combine and split" method that is currently adopted by most researchers using a solid phase synthetic method to construct libraries.[29] This method allows us to synthesize libraries on a one bead/one compound basis. Recent advances in combinatorial chemistry include encoding and tag technologies to identify the compounds.[25,30-32]

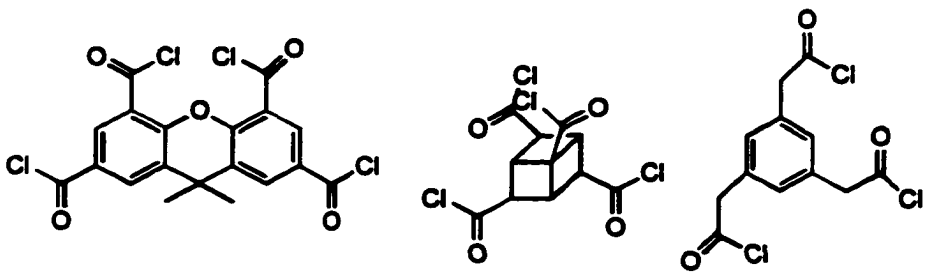
Solid phase synthesis allows for the rapid separation of the product and the generation of large diversity libraries. The reaction is driven to completion by using a large excess of the reactants. The excess reactants can be washed away after the reaction is finished so there is no tedious separation process required. In theory, if we use 10 building blocks for each reaction, a four step sequence of solid phase reactions can generate a library containing 10,000 compounds while carrying out only 40 reactions. The advantage of a "combine and split" solid phase synthesis strategy is that it is about 250 times more efficient compared to a parallel solution phase synthesis in terms of library size.

The kinetics of the reaction on a solid surface is similar to the kinetics of the same reaction taking place in the solution phase.[33-35] The reaction rate can be either somewhat faster or slower compared to the reaction in the solution phase depending on the choice of the solid support and the type of reaction. The environment of the polymer surface acts like a solvent. Researchers can mimic the solid phase reaction through the study of solution phase synthesis and can accelerate the reaction through the selection of the solid support.

Solution phase library synthesis is another important method for combinatorial chemistry. Initially, solution phase synthesis was done by reaction of two kinds of building blocks in

a one, two or three step sequence of reactions.[36,37] A library of 1600 compounds has been made this way.[37] Three building blocks reacted in one sequence with linear addition of one to the other have been developed by Ugi.[38] Most of the liner structured solution phase libraries can be modified to parallel synthesis and "combine and split" solid phase synthetic methods.

Compared to the linear structured libraries, core structured library are not as thoroughly studied. There are only few reports of core structured combinatorial libraries. Rebek, Jr. and coworkers reported the first core structured library.[39] A library of 65,341 members has been prepared this way. Reports of core structured porphyrin libraries comes from Berlin's group.[40,41] Libraries of 6, 21, 55, 120 and 666 alkly substituted porphyrins have been made. The libraries were characterized by MS, NMR and UV-Vis. spectra. Only a single step modification which was the cleavage of the acetoxy groups was done to their library after the purification.



Scheme 1. Building blocks used by Rebek, Jr and coworkers for the core structed libraries.

There are both advantages and disadvantages to solid phase synthesis and solution phase synthesis. Compared to solid phase synthesis, solution phase chemistry has been well studied and can generate large member libraries as a mixture, but the screening process

including isolation and purification is very difficult. Solid phase synthesis requires a linker and an extra cleavage step. Solid phase synthesis can use the "combine and split" method to easily generate very large member libraries on a one compound per bead basis. The characterization and monitoring methods for solid phase synthesis are not well developed compared to solution phase synthesis.

We describe herein the synthesis and characterization of directed combinatorial libraries of *meso*-tetraphenylporphyrin (H_2TPP) derivatives[42] - core structured libraries- where the largest contains 1540 compounds (including isomers) figure 1. The derivitization of entire libraries to make them amphipathic (figure 1), and the iterative selection of winning compounds by DNA binding is described. As a demonstration of the methodology, we find that the di-positively charged porphyrins bearing at least one hydroxyl group to be the most efficacious in the photo initiated cleavage of plasmid DNA.

Both naturally occurring and synthetic porphyrins have long been known to exist in a large variety of isomers. For example, there are 60 possible isomers for protoporphyrin due to the four methyl, two vinyl, and two propionate groups on the eight pyrrole positions, and there are 420 possible isomers of the heme *a* prosthetic group in cytochrome *a*.[43] Similarly, we and others have exploited the six possible compounds and isomers when two different arylaldehydes are employed in the synthesis of *meso*-substituted porphyrins,[3, 44] and libraries of chemically inert, lipophilic, alkly substituted H_2TPPs have been made.[40,41] The Photo Dynamic Therapeutic (PDT) Photofrin® is a mixture of monomers and multimers of protoporphyrin IX linked by ester and ether bonds. The advantages and drawbacks of PDT has been extensively reviewed.[45-48]

Although the binding mode is still controversial,[49-54] 5,10,15,20-tetrakis(4-methypyridinium)porphyrin has been known to strongly bind DNA for over 20 years, but negatively charged tetrabenzoate porphyrins do not bind strongly. Similarly, the mechanism of action of these compounds is also under debate, but probably arises from the formation of singlet oxygen and subsequently a combination of damage to lipids, proteins, and nucleic acids, as well as hypoxia.[45-48, 55-58] The purpose of this study is to identify new combinations of functional groups that enhance porphyrin binding to biomolecules relative to the homosubstituted parent molecules, *not* to get embroiled into mechanistic debates. Since porphyrins have been shown to congregate in a variety of cell structures,[55,59,60] it is likely that different substitution patterns will target different tissues or cellular components. *In vivo* experiments show that the greater the amphipathicicity the greater the selectivity towards tumor cells.[55-62] The assays chosen herein (DNA binding and cleavage, and water-octanol partition coefficient) are currently standards used to identify potential new PDT agents.[45-62] Therefore a directed porphyrin library is made in order to bind or cross the cell membrane (which generally contain negatively charged lipids), be reasonably soluble under physiological conditions, and to bind nucleic acids. Thus, the members of the libraries should be amphipathic with some positive charge. [63]

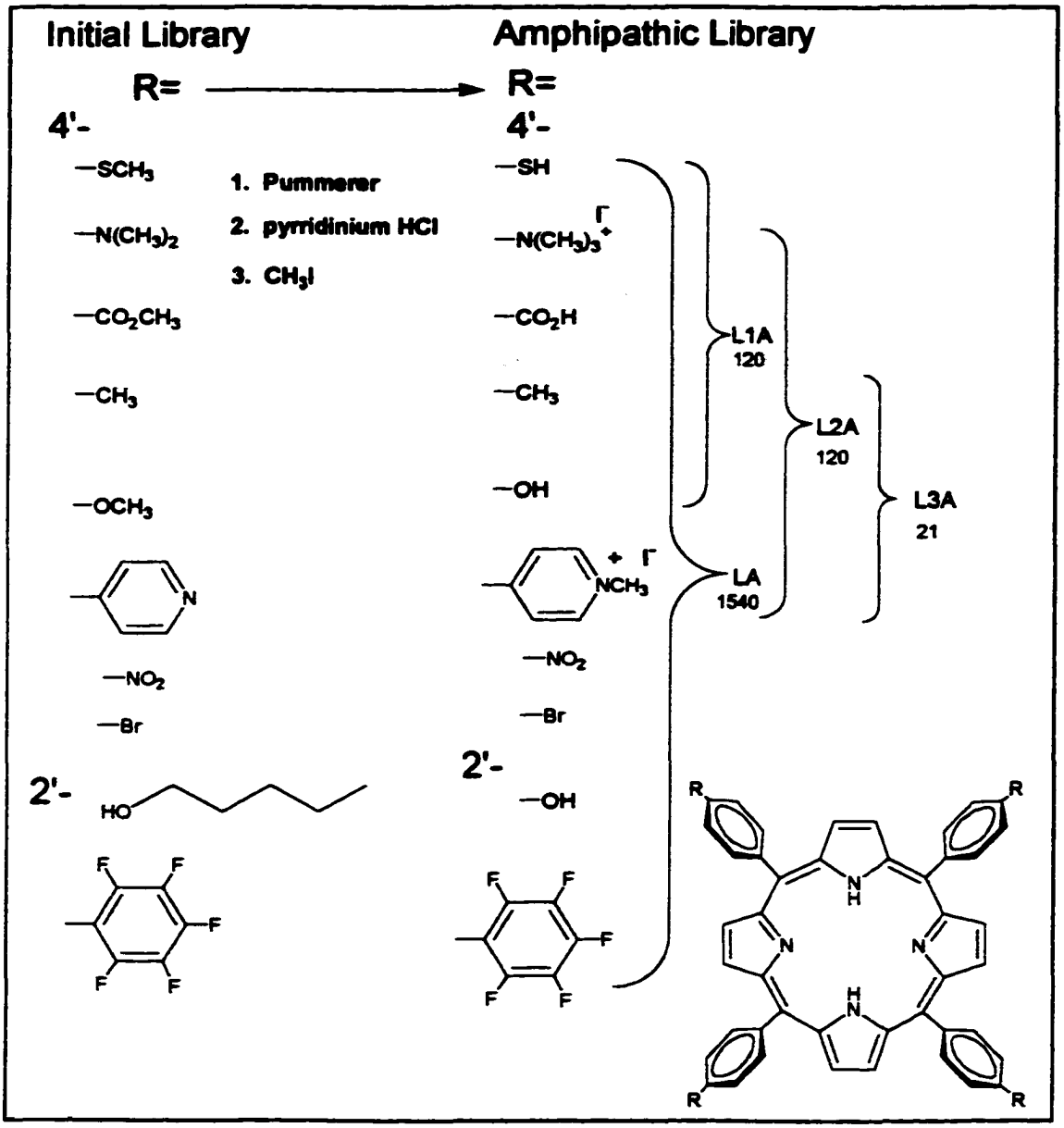
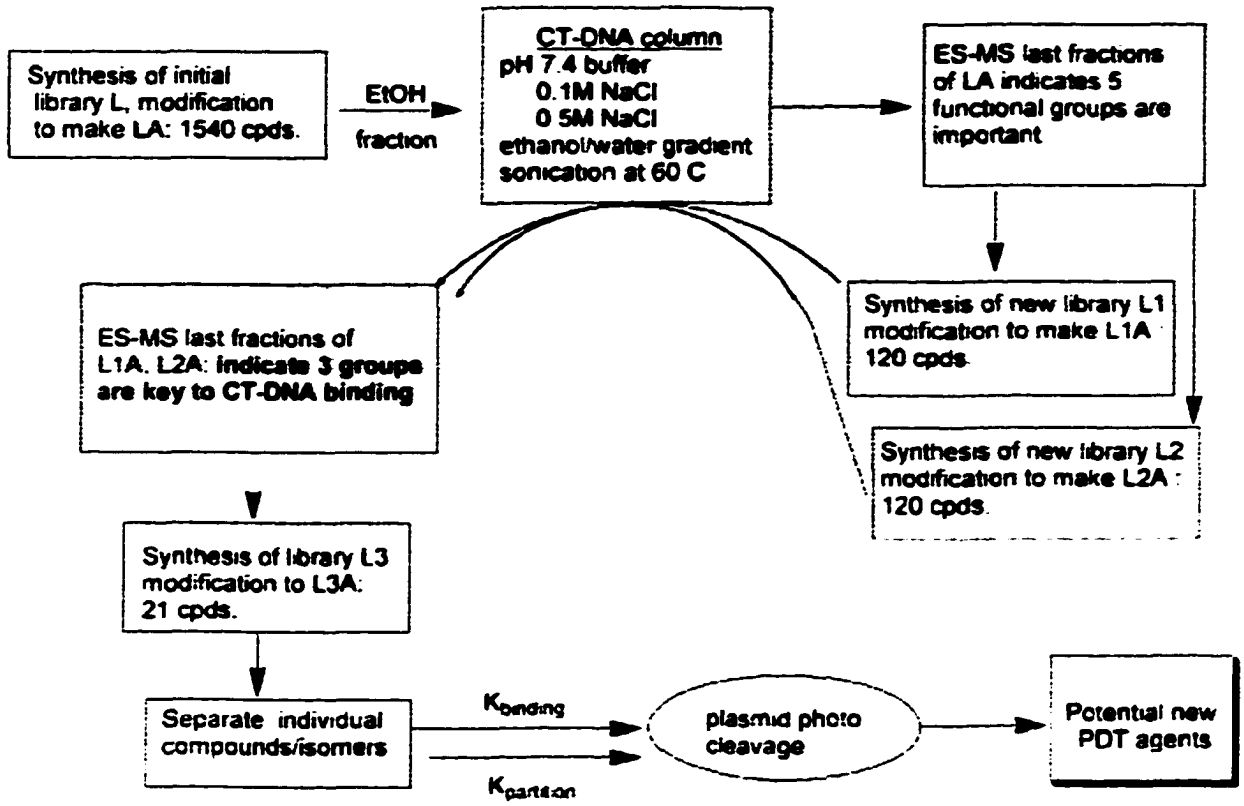


Figure 1. Porphyrin libraries bearing small functional groups, with the number of compounds in the library indicated under the library name. Polar functional groups are protected as the methyl derivative for efficient combinatorial synthesis and characterization of the initial hydrophobic libraries (L). These are then deprotected and the amines quaternized to make the amphiphilic libraries (LA). All libraries, L, LA, L1, L1A, L2, L2A, L3, L3A are fully characterized by ESI-MS, ^{1}H-NMR, and UV-Vis.

Results and discussion

The standard Adler,[4] Lindsey,[7] and solvent free[9] methods of porphyrin synthesis result in low or erratic yields when the aldehyde bears most highly polar or charged groups such as alcohols, thiols, carboxylic acids, pyridinium, and quaternary ammonium moieties. Therefore the starting aryl aldehydes bearing the methyl protected precursors of the above groups were used in the Adler synthesis of combinatorial libraries L, L1, L2, L3 – libraries of 1540, 120, 120, and 21 porphyrins, respectively. Subsequent removal of the methyl groups from the ether, thioether, and ester, followed by careful methylation of the amine groups resulted in the desired amphipathic porphyrin libraries, LA, L1A, L2A, and L3A, figure 1 and scheme 2. This is a directed library since the rationale for choosing the aldehydes for this study includes: (i)the substituted aldehyde must yield the tetra derivative in normal yields, (ii)cleavage of the protecting group must go near quantitatively, (iii) the resulting functional group should impart some amphipathic character to the macrocycle,[46,50,51] (iv)positively charged moieties are preferable to negatively charged ones for membrane, polysaccharide, and nucleic acid binding, (v)maximize the number and kinds of derivatives. Functional groups were chosen for their hydrophobic, electrostatic, and hydrogen-bonding potentials. The resulting libraries contain a variety of amphipathic, hydrophobic, and hydrophilic porphyrins. After partitioning the 1540 member LA into water, ethanol, and ethyl acetate fractions, the water and ethanol fractions are screened for DNA binding by elution through a calf-thymus (CT)-DNA column. Electrospray mass spectra (ESI-MS), ^{1}H-NMR, and UV-Vis then characterize the last fractions off the column. The results from this first screening



Scheme 2.

show a paucity of 2'-hydroxy, 4-nitro, 4-bromo, perfluoro, and 4-thio groups. To further identify those porphyrins that bind strongest to DNA, it was decided to make smaller libraries, L1 and L2, scheme 2.

Characterization of the libraries and their diversity is accomplished by a variety of spectroscopic techniques. Characterization of the initial library, L, and the modified, amphipathic library, LA, follow the same course. A clean UV-Visible spectra indicates the lack of chlorins, polypyromethanes, and other side products of porphyrin synthesis. ^{1}H-NMR spectra also demonstrates the lack of side products and starting materials. The methyl resonances can be used as a handle to characterize the library since they should represent a statistical distribution of compounds, and therefore a broader peak width. Most importantly, the ESI-MS can be used to identify the number of isobarric species for small libraries of <60 components, and as a highly sensitive fingerprint in the characterization of all libraries.[40, 41]

The characterization of the largest libraries, L and LA, is described, and is generally applicable to the smaller libraries as well. A statistical mixture of 1540 porphyrins in L is clearly demonstrated by the UV-Vis (figure A1), ESI-MS (figure 2) (L has 715 isobaric species, L1 & L2 have 70, and L3 has 15 isobaric species), and ^{1}H-NMR spectra (figure A2). The absorption spectra shows a substantially broadened Soret peak and that there are no porphyrin side products such as chlorins, dipyromethanes. The ^{1}H-NMR spectra of L shows no starting aldehydes or pyrrole, all five of the different methyl group resonances between 2 and 5 ppm, (there are 5 for L1, and 4 for L2), a highly complex aromatic region, and the characteristic absorption of the pyrrole NH at about -2.75 ppm. The half-widths of the methyl resonances of L are about 2.5 times as broad as those of the

individual tetra substituted porphyrins, and have a much broader base. There is a substantial increase in the ^{1}H- NMR methyl peak half-widths upon going from 1, 6, 21, 55, 120, 1540 member libraries, figure 3, table 1. Metallating the libraries with high spin cobalt (II) expands the methyl resonances in the ^{1}H-NMR such that they are ~3 times broader than those for the individual Co(II)porphyrins. NMR spectra of the free-base and Co(II) L1 and L2 are also consistent with a fully diverse libraries. The ESI-MS of L (figure 2) is remarkably similar to the calculated spectra. The calculated and observed ESI-MS spectra of the Co(III) metallated library L-Co [Co(III) imparts a + charge on all porphyrins] ensures the MS results of the free-base L. Since these are core-structured libraries, the range of molecular weights is limited. Thus, L was chromatographed on a silica gel column, and the ESI-MS and ^{1}H-NMR of each fraction taken. In this way, all 715 of the isobaric compounds are identified, and >93% of the compounds/isomers found based on relative intensities compared to known unique m/z peaks, table A1. There is a random distribution of functional groups on the porphyrins not identified by these procedures, table A2 – assuring the reactivity of the aldehydes are similar under the synthetic conditions. The 70 isobaric compounds/isomers in L1 and L2 are readily observed by ESI-MS (figure 4) using the same variety of instrumental methods, including the Co(III) metallated libraries (figure A6, A9). Libraries of L1 and L2 were characterized by the ^{1}H NMR, figure A4. ^{1}H NMR experiment at 500MHz in C_6D_6 reveals no further differentiation of the methyl resonance, but does show a dramatic increase in the area of pyrrole NH resonance, figure A5. The ESI-MS of the Co(III) L1(figure A6) shows similar results as the standard ESI-MS (figure 4) validating the method using the free base libraries. The mass spectra of the amphipathic libraries are

much more complex due to the presence of multiple charged groups on many of the porphyrins (LA, figure A3; L1A, figure A7). The purification of the libraries can remove a few molecules as shown in table A3 for L1. In an attempt to further differentiate the methyl groups, L2 was metallated with high spin Co(II). As seen in figure A8, the resonance are much broader, but no increased resolution is observed. The ESI-MS of Co(III) L2 (figure A9) also is shown. The UV-Vis. of L1, L2, L1A and L2A show that they are the free bases, and no chlorin is present, figure A10. Taken together, these results indicate that the expected diversity of the libraries is maintained throughout the synthesis and purification.

Upon completion of the quaternization and cleavage reactions, the ^{1}H-NMR exhibits the ammonium and tolyl methyl groups, the thiol, alcohol, and acid protons, in addition to the resonances due to the macrocycle. Integration of these resonances yield the expected ratios and indicate that each modification reaction proceeds with > 90% efficiency. Unreacted groups may be considered to further diversify the library. ESI-MS of amphipathic libraries LA, L1A, and L2A are essentially similar to their calculated spectra. Thus 6, 21, 120, and 1540 member libraries have been constructed and characterized by ^{1}H- ^{13}C- NMR, UV-Vis, and ESI-MS, and indicate that the library's diversity is maintained upon modification.

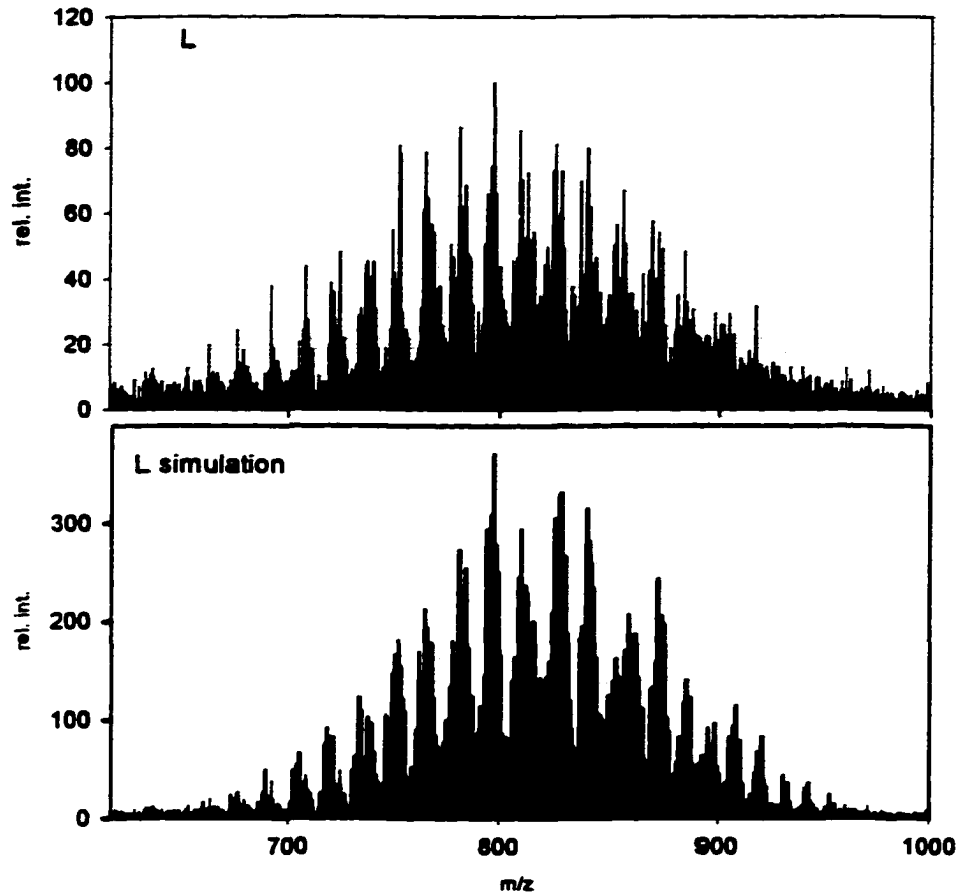


Figure 2. ESI-MS of 1540 member library L and its simulation. The sample (~10^{-6}M) was dissolved acetonitrile/water (75/25) containing 1% trifluoroacetic acid. The spectrum was taken in positive ion mode and the fragmentor voltage ramped from 25 to 150 V.

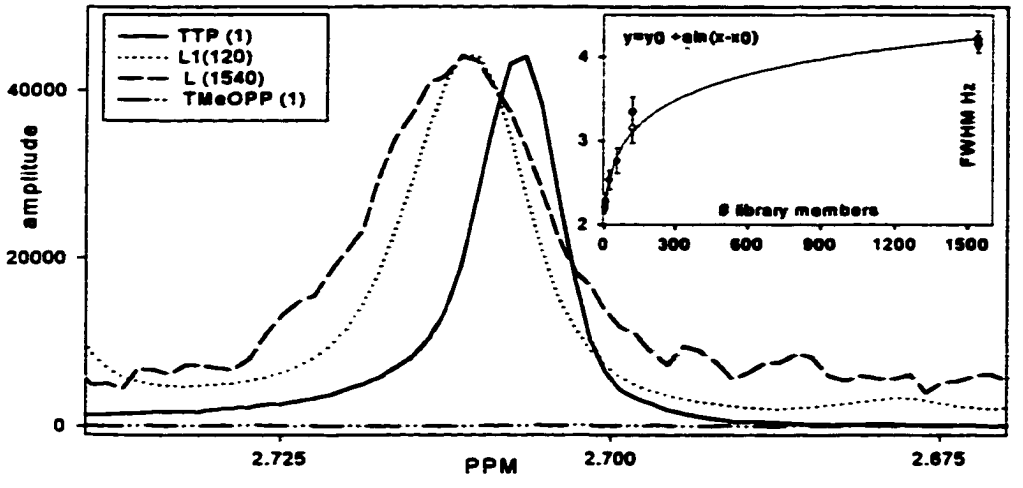


Figure 3. tolyl methyl region of the ^{1}H NMR spectra showing the peak shape differences between an individual porphyrin and that of library L and L1. The small peaks disposed about the main methyl peak are presumed to be due to small sets of isomers or compounds. Note the relationship between the numbers of the library and the full width at half maximum (FWHM) in the inset; the line has no physical meaning.

FWHM methyl peak widths (Hz)

Library/porphyrin	Me	COOMe	SMe	NMe_2	4'-OMe	2'-OPe
L	4.15	3.35	2.53	4.17	3.40	3.47 (center res.)
L1	3.19	2.34	2.23	2.74	2.25	
L2	3.12	2.35		2.64	2.24	
L3	2.33				1.83	
$TTolPH_2$	2.21					
$TMeOPPH_2$					1.42	
$TMeBzPH_2$		1.86				

Table 1. The FWHM peak widths (Hz) of the methyl resonances of the three parent libraries normalized to the ¼ width of residual $CHCl_3$ to account for differences between experiments (shimming, concentration, etc.). Though the instrument was carefully shimmed, as judged by the $CHCl_3$ peak (<5% differences), there is a broad base for each of these peaks. $TTolPH_2$ = 5,10,15,20-tetrakis(4'-methylphenyl)porphyrin; $TMeOPPH_2$ = 5,10,15,20-tetrakis(4'-methoxyphenyl)porphyrin; $TMeBzPH_2$ = 5,10,15,20-tetrakis(4'-methylbenzoate)porphyrin. Chemical shifts of the tetra-substituted compounds are consistent with literature values.

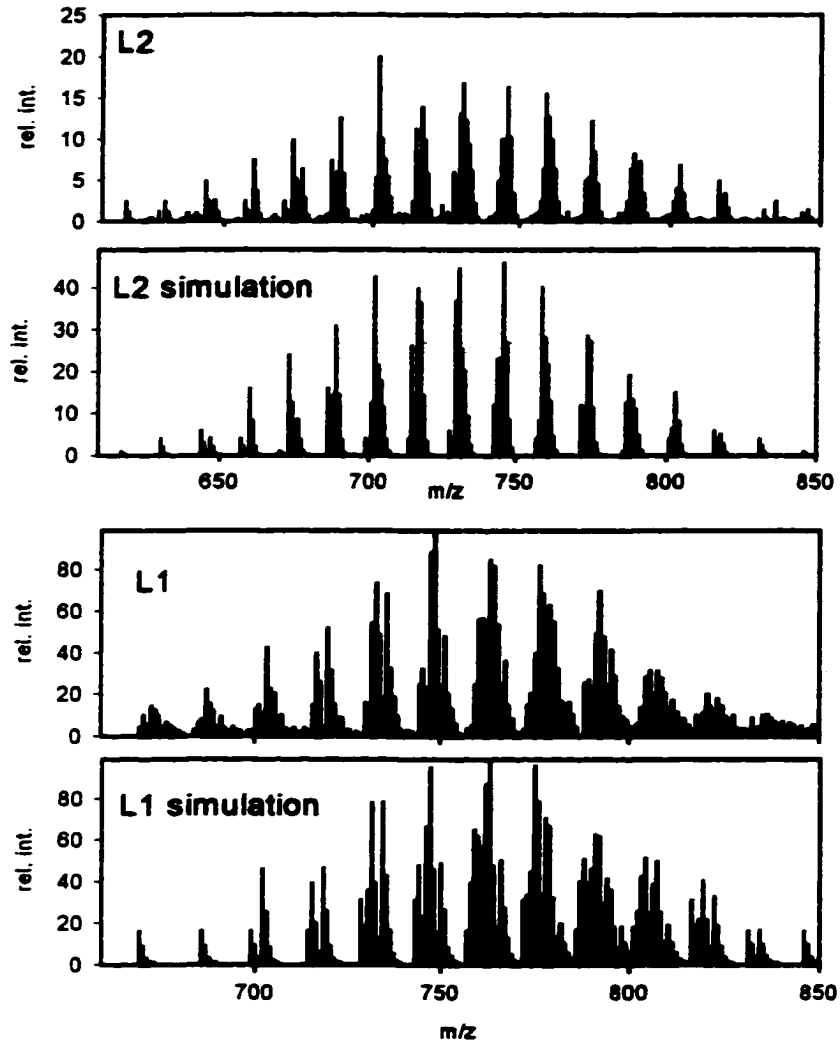


Figure 4. ESI-MS of libraries L1, and L2 with a simulated spectra. The simulated spectra were calculated using a program written by us using quick basic, and confirmed by using the MASP program.[40]

We initially differentiate the resultant amphipathic porphyrin libraries by solubility: by exhaustively extracting solid LA, or L1A, or L2A with water, the remaining solid with ethanol, and finally with ethyl acetate. This yields three "fractions" (hydrophilic, amphipathic, hydrophobic) of which the ethanol fraction is by far the largest of all three. ESI-MS of all three fractions of the three libraries using methods described above, further assist in characterization of the libraries, with the water and ethyl acetate fractions yielding members that would be expected or are known to be hydrophilic and hydrophobic, respectively.

Elution of the ethanol and water soluble fractions, from LA, over a column of calf-thymus DNA absorbed onto glass wool further differentiates the porphyrins and selects those that tightly bind DNA. The eluent is a salt gradient followed by a water-to-ethanol gradient. In control experiments, we find that the tetracationic species are the predominant ones that bind to the glass wool, and since these are known compounds they are not used in further analysis. The last two salt and last two ethanol fractions from L showed very little or no thio-, nitro-, bromo-, or perfluoro- moieties by ESI-MS. Therefore L1 and L2 were constructed. ESI-MS analysis finds about 8 porphyrins from L2A constitute the major part of the fractions that binds most strongly to the DNA (see table 2 and scheme 2), although we can detect small amounts of ~8 others. While the pyridinium groups were expected there are a surprising number of methyl- and hydroxy-groups present.

These results led to the construction of L3 and L3A (bearing only hydroxy-, methyl- and methylpyridinium- groups) to further delineate the members of the library with the desired properties. Chromatographic separation of all 21 members of this library is

accomplished by several MPLC columns. Each compound is then fully characterized by ESI-MS, ^{1}H-NMR, and UV-Vis. The DNA binding constants, and the octanol-water partition coefficient are then measured for each, table 2. The best of these are used in DNA photo cleavage experiments.

Table 2. Characterization of selected porphyrin compounds (from L3A)

Porphyrin	R	R'	R''	R'''	K_{B1} x10^{-6} M	K_{B2}	$K_{Oct/Water}$	ϕX174 photo cleavage
1	OH	Me	Me	PyMe+	0.3	179	>2500	Poor
2	OH	Me	PyMe+	PyMe+	1.5	46	7.23	Excellent
3	OH	PyMe+	Me	PyMe+	2.3	55	16.5	Good
4	OH	OH	PyMe+	PyMe+	0.8	86	4.02	Excellent
5	OH	PyMe+	OH	PyMe+	0.8	75	6.25	Poor
6	OH	OH	OH	PyMe+	1.7	245	>2500	Good
7	OH	PyMe+	PyMe+	PyMe+				Good
8	OH	OH	Me	$N(Me)_3$+	--	--	---	
9	PyMe+	PyMe+	PyMe+	PyMe+	12	>1000	<0.0004	Fair
10	Me	Me	PyMe+	PyMe+	0.8	237	>100	Fair
11	Me	PyMe+	Me	PyMe+	1.8	453	38.5	Poor

Assays are all compared to the extensively studied, tetrapyridiniumporphyrin #9. Though the mode of binding and mechanism of action for even extremely well studied porphyrins, #9 and Photofrin®, are still controversial, a well accepted indicator of the potential efficacy is the rough binding constants measured by changes in the visible spectra[58] of the porphyrin upon DNA addition. The octanol-water partition coefficient is an indicator of the amphiphilicity of the compound and of lipid bilayer binding. The cleavage reactions on ϕx174 or PUC-19 supercoiled plasmids follows standard literature procedures.[55-60] Changes in the appearance of the plasmid on an agarose gel after incubation, irradiation, and treatment with S1 nuclease (degrades single strand DNA). Poor=only small changes, fair=noticable changes only after S1 amplification, Good=can be visualized without S1 nuclease treatment. Excellent=complete degradation without S1 nuclease treatment, see figure 5.

As an indicator of bioactivity,[45-51, 54-62] the eight novel porphyrins thus found were incubated with a supercoiled plasmid DNA (ϕ-X174) for 2-12 hours in the dark at room temperature, and then irradiated with continuous 50 foot-candles white light with a 450 nm cut-off filter that eliminates blue light for up to 90 minutes at 35 °C. To amplify the signal, single strand nicks, some samples were treated with S1 nuclease. Gel electrophoresis shows that the plasmid DNA treated with the selected porphyrins derivatives has been degraded, figure 5. Further studies are under way to examine the mode of binding of these selected porphyrins to small DNA fragments.

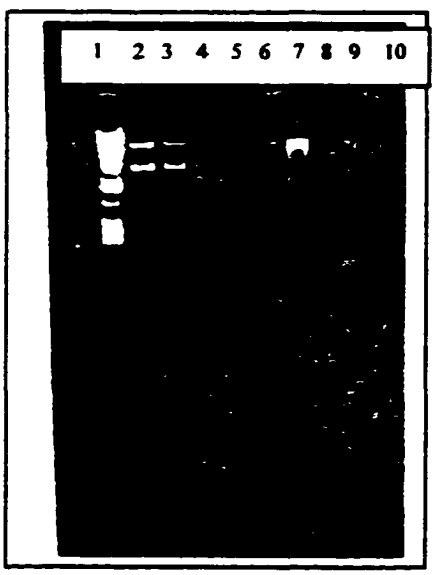


Figure 5. Lane 1: α1Kb molecular marker; lane 2: ϕ–x174 plasmid DNA; lane 3: plasmid with ~ 4 μM porphyrin 3; lanes 4, 5, 6: irradiation with 50 Lux light (450-800 nm) for 30, 60, 90 minutes, respectively. Lane 7: same plasmid treated with S1 nuclease; lane 8: with ~ 0.8 μM of porphyrin 4 irradiated for 15 minutes; irradiation for 30 and 60 minutes of the plasmid porphyrin 4 complex, lanes: 9, 10 respectively.

Conclusion

This report demonstrates that (i)a large porphyrin libraries, containing 1540 different members, may be formed and subsequently modified to yield libraries with a wide range of solubilities and functionalities; (ii)the libraries may be differentiated into hydrophilic, amphipathic, and hydrophobic fractions; and (iii)the resultant fractions screened for DNA binding. Since the mode of DNA binding (electrostatic, hydrogen bond, intercalation, etc.) of even the simplest porphyrins is controversial at best, and these multifunctional porphyrins can potentially bind in a variety of ways, we cannot as yet speculate on how the tight-binding porphyrins bind to DNA. Since the mode of action and the localization of PDT agents is also under intense investigation,[45-62] these libraries may serve as a means to probe these questions. However, the optical spectra of the selected porphyrin(s) exhibit a 3-10 nm red shift,[58, 64] and a substantial broadening with a concomitant decrease in ε of the porphyrin Soret bands upon addition of CT-DNA. The fluorescence emission and excitation spectra are dramatically altered, and there is a substantial fluorescence anisotropy observed upon DNA addition (all at ~1μM). Upon excitation in the nucleotide region at 270nm where the absorbance of DNA is at least 10x that of the porphyrin, the porphyrin fluoresces. These observed spectral changes are consistent with those observed for most other porphyrin-DNA complexes.[45-62] Together with the photo cleavage experiments, the evidence that this small cadre of porphyrins (~0.5% of the parent library) bind to DNA is unequivocal. Since the mechanism, the mode of binding, and the localization of PDT agents is under intense investigation, libraries such as these may aid in the determination of what factors direct porphyrins to certain cellular structures and tissues. Once compounds have been identified or selected, they maybe readily reduced to

the corresponding chlorin to enhance their absorption in the >700 nm region where biofluids are more transparent.[62] We find that a specific set of motifs or substituents bind quite strongly to CT-DNA and cause strand scission upon illumination with white light. Notably, these substituents are a combination of polar (alcohol), nonpolar (methyl), and cationic (pyridininium) moieties indicating that a combination of hydrophillic, hydrophobic, and electrostatic effects are key considerations in the binding of porphyrins to biomolecules such as lipids[3d, 3e] and nucleic acids. The powerful analytical techniques based on mass spectrometry allow for the characterization of exquisitely small amounts of material from these selection methods.

Lastly, we and others[40,41] have developed a simple computer program that makes a table of all the possible derivatives and their associated molecular weights that results in a simulation of the mass spectra. This invaluable aid compliments the deconvolution in identifying which compounds (from libraries with 7260 members for a core with 4 positions and 15 functionalities) in a library are selected by an assay. This program will be placed on our home page for public use.[63]

Experimental

General Experimental: Pyrrole was purified by a short pipet silica gel column and the aldehydes were run over a short pipet columns of basic alumina before used. All other reagents were used as received from Aldrich ^{1}H- NMR were obtained on a 300MHz GE, 400 MHz Varian or a 500MHz Varian Unity Plus spectrometer. UV-Vis absorption spectra were obtained on a Carey 1 spectrophotometer and taken in $CHCl_3$ if not specified (at μM concentrations, even LA, L1A and L2A are soluble in $CHCl_3$). ESI-MS were obtained on a Hewlett-Packard HP 1100 LC/MSD spectrophotometer.

General ESI-MS method: ~10^{-6} M solutions in acetonitrile:water (75:25) containing 1% trifluoroacetic acid, positive ion mode, and the fragmentor voltage ramped from 25-150 V. The positively charged Co(III) L, assists in the characterization since all porphyrins have a +1 charge.

Synthesis of Library L and modification of L

The synthesis of L was accomplished by adding equimolar (0.001mol each) amounts of the ten aldehydes (shown in figure 1) to 200mL propionic acid (0.005M, each), bring the solution to 80-90 ^{0}C, and adding pyrrole (0.01mol, 0.05M) while vigorously stirring the solution. The mixture is then refluxed for 90 minutes. These lower concentrations (usual Alder conditions are 0.1-0.2M) assure the statistical distribution of functional groups and the maximum number of members of the library. The reaction mixture is cooled and the solvent removed. The resultant crude material was dissolved in a minimal amount of chloroform, and 15g Flurosil added and the solvent removed. A slurry of 2g of the Florosil in toluene was loaded onto a flash silica gel column (35g), and eluted using a

solvent gradient from toluene to chlorofrom to ethyl acetate to methanol. The porphyrin yield was 23% based on standard UV-Visible analysis.

Modification of Library L to LA (to remove Me groups from $-SCH_3$, $-OCH_3$, $-CO_2CH_3$ and –OPe): 3g Florosil that absorbed the crude product was added to 75mL chloroform. From the UV-Visible spectrum, there was about 1.065 x 10^{-4}mols of porphyrin in the mixture. To deprotect the thio group, a Pummer reaction was used. MCPBA (0.0424g, 0.246mmol) was added to the reaction mixture. The reaction mixture was stirred at 0^0C for 3 hours. The Florosil was filtered from the solution and 10mL ~ 0.6N KOH water solution was added to the filtrate. The mixture was then stirred at room temperature for 20 minutes. The two phases were separated and 52g Na_2SO_4 was added to the organic layer to absorb water. Then the solution was decanted and the solvent was removed under vacuum. 10mL chloroform was added to dissolve the porphyrin followed by 10mL TFAA. The mixture was refluxed for 30 minutes. The solvent was removed under vacuum. 20mL chloroform was added to the flask to dissolve the residue then 2.3g K_2CO_3 was added to the solution. The mixture was stirred at room temperature for 1 hour. K_2CO_3 was filtered and the solvent was removed under vacuum. In order to deprotect the $-OCH_3$, $-CO_2CH_3$ and –OPe groups, 5g pyrridium HCl was added to the residue and the mixture was refluxed for 40 minutes. When the reaction mixture cooled, 200mL water was added then 200mL ethyl acetate was used to extract the porphyrin by adding K_2CO_3 to the water layer. We found only porphyrins were extracted by ethyl acetate, all other by products from the synthesis remain in the water layer. After the porphyrin was recovered, the water layer should be light yellow and with a pH value between 5-6. The ethyl acetate layer was separated and the solvent was removed under

vacuum. 1mL methanol was added to the residue followed by adding 2ml ethyl acetate. 2mL CH_3I was added to the solution and the solution was stirred at 0^0C for two days. After removing the solvent, LA was obtained.

Synthesis of library L1, L2 and L1A, L2A

L1 synthesis: In a 1000mL flask, a mixture was made of 500mL acetic acid with 4-dimeylamino-benzaldehyde (1.49 g, 10 mmol, 1eq), 4-methylthio-benzaldehyde (1.52 g, 10mmol, 1eq), 4-tolualdehyde (1.2g, 10mmol, 1eq), 4-anisaldehyde (1.36g, 10mmol, 1eq) and 4-formalbenzoate (1.64g, 10mmol, 1eq). The mixture was refluxed at 160 ^0C for 5 minutes then pyrrole (3.35g, 50mmol, 5eq) was added to the reaction mixture. This reaction mixture was refluxed at 160 ^0C for 2 hours under air. The porphyrin yield was ~ 7.5% based on standard UV-Visible analysis. The crude product was stored at 0^oC for two days to see if the porphyrin would precipitate, but this failed. So the solvent was removed under vacuum and the crude product was loaded onto a flash silica-gel column. The column was developed first by chloroform and then by 50% ethyl acetate and 50% chloroform, following by a mixture of 5% methanol in 95% chloroform.

Library L1: ^1H NMR (300MHz, $CDCl_3$), figure A3: δ=-2.75 (br, m, pyrrole N-H), 2.71 (s, $-CH_3$), 2.75 (s, $-SCH_3$), 3.23 (s, $-N(CH_3)_2$), 4.09 (s, $-OCH_3$), 4.12 (s, $-COOCH_3$), 7.1 (d, J = 8.4 Hz), 7.3 (d, J = 7.5 Hz), 7.57 (d, J = 7.5 Hz), 7.63 (d, J = 8.1 Hz), 8.11 (m), 8.32 (d, J = 7.8 Hz), 8.46 (d, J = 7.8 Hz), 8.90 (m). ^{13}C NMR (75MHz, $CDCl_3$): δ= 16.6 ($-SCH_3$), 22.3 ($-CH_3$), 41.5 ($-N(CH_3)_2$), 53.1 ($-COOCH_3$), 56.3 (-OCH3), 111.4, 112.9, 120 (m, br, meso-C), 125.2, 128.1, 128.5, 132 (m, br, pyrrole-C), 135.1, 135.3, 136.2, 136.4, 160.1 (m), 167.8 (m). UV-Visible ($CHCl_3$, nm), figure A10: 420.8, 518.1, 554.7, 592.8, 651.2.

L2 synthesis: In a 100mL flask, a mixture was made of 30ml acetic acid with 4-dimeylamino-benzaldehyde (0.18 g, 1mmol, 1eq), 4-tolualdehyde (144μL, 1mmol, 1eq), 4-anisaldehyde (165μL, 1mmol, 1eq), 4-pyridinecarboxaldehyde (115μL, 1mmol, 1eq) and 4-formalbenzoate (0.195g, 1mmol, 1eq). The mixture was refluxed for 5 minutes then pyrrole (420μL, 5mmol, 5eq) was added. This mixture was refluxed for 2 hours under air. The porphyrin yield was ~ 7.5% based on standard UV-Visible analysis. The solvent was removed under vacuum and the crude product was loaded onto a flash silica gel column. The column was developed first by $CHCl_3$ and then by 50% ethyl acetate and 50% chloroform, following by a mixture of 5% methanol in 95% chloroform.

Library L2: ^1H NMR (300MHz, $CDCl_3$), figure A4: δ=-2.57 (br, m, pyrrole N-H), 2.73 (s, $-CH_3$), 3.19 (s, $-N(CH_3)_2$), 4.03 (s, $-OCH_3$), 4.16 (s, $-COOCH_3$), 7.1 (d, J = 8.4 Hz), 7.3 (d, J = 7.5 Hz), 7.56 (d, J = 6.6 Hz), 8.11 (m), 8.31 (d, J = 8.1 Hz), 8.45 (d, J = 8.1 Hz), 8.90 (m). ^{13}C NMR (75MHz, $CDCl_3$): δ= 22.3 ($-CH_3$), 41.4 ($-N(CH_3)_2$), 53.2 ($-COOCH_3$), 56.3 (-OCH3), 111.4, 112.9, 120 (m, br, meso-C), 128.1, 128.5, 132 (m, br, pyrrole-C), 135.2, 136.2, 136.4, 147.5 (m), 148.8, 150.6 (m), 160.1 (m), 167.9 (m). UV-Visible ($CHCl_3$, nm), figure A10: 420.8, 518.0, 555.9, 592.4, 651.3.

Derivation of L1: To deprotected the thio group, a Pummer reaction was used. To a solution of 0.0605g L1 dissolved in 10mL $CHCl_3$, MCPBA (0.0243g, 0.14mmol) was added and stirred at 0^0C for 2hours. KOH (0.01176g, 0.21mmol, 1.5eq) was added to the reaction mixture and the mixture was stirred for 15 minutes at room temperature. The solution was filtered and the filtrate was evaporated under vacuum. The residue was dissolved in 1-2 mL $CHCl_3$ and 10mL TFAA was added. This solution was refluxed for 2 hours and then dried under vacuum. A saturated K_2CO_3 water solution was used to wash

the residue. The residue was dissolved in 1-2 mL $CHCl_3$, and a mixture of 5 mL CH_3OH and 5mL $N(C_2H_5)_3$ was added. The mixture was then dried under vacuum. In order to deprotect the $-OCH_3$, $-COOCH_3$ groups, 16g pyridine hydrochloride was added to the mixture. The mixture was refluxed for 30 minutes. The mixture was diluted with 100mL water and then extracted with 160mL ethyl acetate. K_2CO_3 powder was added to the aqueous phase during the extraction. The solution was extracted until there was no purple color left in the aqueous phase. The ethyl acetate phase was washed with a 0.1M HCl water solution to remove the pyridine. The residue was dried under vacuum and then redissolved in 1-2 mL acetone. 1 mL CH_3I was added to the solution and the mixture was stirred at 0^0C for 2 days. The product was dried under vacuum at room temperature.

L1A: ^1H NMR (300MHz, d-Acetone): δ=-2.75 (br, m, pyrrole N-H), 2.68 (s, $-CH_3$), 4.26 (s, $-N^+(CH_3)_3$), 7.32 (d, J = 8.4 Hz), 7.63 (d, J = 8.1 Hz), 7.69 (d, J = 7.5 Hz), 8.11 (m), 8.37 (d, J = 8.1 Hz), 8.48 (d, J = 8.1 Hz), 8.90 (m). UV-Visible ($CHCl_3$, nm), figure A10: 421.7, 517.6, 554.2, 592.2, 648.5.

Derivation of L2: A mixture of 0.02g L2 and 16g pyridine hydrochloride was made and the mixture was refluxed for 30 minutes to deprotect the $-OCH_3$, $-COOCH_3$ groups. The mixture was diluted with 100mL water and extracted with 160mL ethyl acetate. K_2CO_3 powder was added to the aqueous phase during the extraction. The extraction was conducted until the aqueous phase was no longer purple. The ethyl acetate phase was washed with 0.1M HCl water solution to remove the pyridine. The organic phase was dried under vacuum and the residue was dissolved in 1-2 mL acetone. 1 mL CH_3I was added to the solution and the mixture was stirred at 0^0C for 2 days. The solvent was removed under vacuum at room temperature.

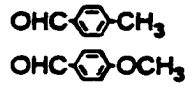
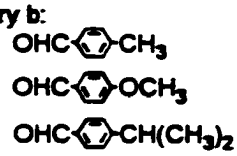
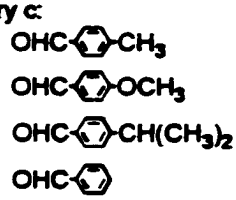
L2A: ^{1}H NMR (300MHz, d-Acetone): δ=-2.75 (br, m, pyrrole N-H), 2.68 (s, -CH_3), 4.26 (s, -$N^+(CH_3)_3$), 4.98 (s, Me of pyrridinium), 7.32 (d, J = 8.4 Hz), 7.63 (d, J = 8.1 Hz), 8.11 (m), 8.37 (d, J = 8.1 Hz), 8.48 (d, J = 8.1 Hz), 8.90 (m). UV-Visible ($CHCl_3$, nm), figure A10: 421.7, 517.7, 554.9, 591.7, 648.5.

Libraries synthesized for ^{1}H NMR line width studies.

Libraries of 6, 21 or 55 (libraries a, b, c) members porphyrin were made to examine the relationship of ^{1}H NMR peak shape with the # of porphyrins inside the library (Figure 3, table 3).

General procedure for preparing library a to library c (Table 3): The reaction was performed in a 8.3mL vial closed by a cap fitted with a gas tight rubber septum. The vial was placed in a temperature controlled sand bath. After heating the vial to 200 (± 5) ^{0}C, 0.2mmole each aldehyde was injected into the system. After 5 minutes, pyrrole (one third of the total moles of the aldehydes) was injected and the vial kept at 200°C for another 20 minutes. Afterwards, the vial was removed from the sand bath and cooled. Once at room temperature, $CDCl_3$ was added directly to the vial and the solution was used as the sample of ^{1}H- NMR spectrum. For this line width study, where we were only interested the tolyl methyl group, the by products do not interfere. The yields were not measured.

Table 3. Library designed for $W_{1/2}$ study

Aldehyed employed in the library	The number of porphyrin inside the library
Library a: 	6
Library b: 	21
Library c: 	55

Synthesis Co (II), Co (III) libraries and single porphyrins

Preparation of Co(II)-L2

A solution of 0.0179 g $CoCl_2$ dissolved in 2 mL acetone was added to a mixture of 0.0552g L2 and 50mL chloroform. The solution was refluxed two hours in open air to form Co (II)-L2. Excess $CoCl_2$ was removed by water extraction. The final product was obtained by removed the solvent under vacuum. UV-Visible ($CHCl_3$, nm), figure A11: 413, 531, 610.

Preparation Co (III)-L1

0.0129g $Co(Ac)_2$ was added to a mixture of 0.0268 g L1 and 50 mL toluene. The solution was refluxed for 3 hours. After refluxing, the solution was left in air overnight for the

slow oxidation of Co (II) to Co (III). Water was used to extract the excess $Co(Ac)_2$. The organic phase was evaporated to dryness to obtain the final product. UV- Visible ($CHCl_3$, nm): 438, 554, 598.

Preparation Co (III)-L2

0.0129g $Co(Ac)_2$ was added to a mixture of 0.0268 g L2 and 50 mL toluene. The solution was refluxed for 3 hours. After refluxing, the solution was left in air overnight for the slow oxidation of Co (II) to Co (III). Water was used to extract the excess $Co(Ac)_2$. The organic phase was evaporated to dryness to obtain the final product. UV- Visible ($CHCl_3$, nm): 436(11.5), 531(1), 594(0.48)

Preparation of Co (II)-TPP

4.4mg Co $(Ac)_2$ was added to a mixture of 3.8 mg H_2TPP and 20mL toluene. The solution was refluxed for 2 hours under N_2. Water was used to extract the excess $Co(Ac)_2$. The organic phase was evaporated to dryness to obtain the final product. UV – Visible ($CHCl_3$, nm): 410, 528, 610.

Library L3 synthesis and separation

Synthetic procedure: A mixture of 1.2g 4-tolualdehyde, 1.36g 4-anisaldehyde and 1.06g 4-pyridinecarboxaldehyde was refluxed in 300mL propionic acid for 5 minutes, 2.1g pyrrole was added to the mixture. After refluxing two hours, the reaction was stopped and the mixture was cooled to room temperature. Silica gel was added to the mixture and the solvent was removed by vacuum.

Separation (Table 4):

Library L3 was loaded onto a flash silica gel column. The column was first developed by $CHCl_3$ to elute the six porphyrins (Comp# 1-6) that do not contain a pyridyl group (see

table 4). By increasing the polarity of the elution solvent to 50% ethyl acetate and 50% $CHCl_3$, the 6 porphyrins which contain only one pyridyl group (Comp# 7-12) were eluted out first and followed by the *trans* compounds which have two pyridyl groups (Comp# 14, 16, 18). The *cis* isomers (Comp # 13, 15, 17) were obtained by eluting with a mixture of 5% methanol and 95% $CHCl_3$. The tetra substituted pyridyl porphyrin (Comp# 21) and the tri-sustitute pyridyl porphyrin (Comp# 19, 20) were eluted out with pure methanol.

The fraction containing comp# 7, 8, 9, 10, 11 was first cleaved by pyrridium HCl and the residue (comp# 7, 8', 9', 10', 11') was loaded onto a silica gel column. By eluting with a mixture of 5% ethyl acetate and 95% chloroform, comp. # 7 was isolated first. A mixture of 10% ethyl acetate and 90% chloroform, eluted comp. # 8', 9', 10'. (R_f: in 5% ethyl acetate and 95% chloroform, Comp# 7: 0.5, Comp# 9': 0.33; in 10% ethyl acetate and 90% chloroform, Comp# 9': 0.44, Comp# 8': 0.13, Comp# 10': 0)

The fraction containing two pyridyl groups in the *trans* position (comp# 14, 16, 18) was first cleaved by pyrridium HCl and the product (comp# 14, 16', 18') was again loaded on to silica gel column. All three isomers were isolated by developing the column with a solvent containing 5% methanol and 95% chloroform (R_f: Comp# 14: 0.54, Comp# 18': 0.47, Comp# 16': 0.37).

The fraction containing two pyridyl groups in the *cis* position (comp# 13, 15, 17) was first cleaved by pyrridium HCl and the product (Comp# 13, 15', 17') was loaded on to silica gel column. All three isomers were isolated by developing the column with a solvent of 5% methanol and 95% chloroform (R_f: Comp# 13: 0.55, Comp# 17': 0.42, Comp# 15': 0.3).

After separation of all the target compounds, each compound was treated with CH_3I individually to make the final compounds. The selected compound were then characterized by UV-Vis., figure A12; ESI-MS, figure A13; and ^1H NMR, figure A14.

Table 4. Separation of L3

Comp#	R1	R2	R3	R4	Comp#	R1	R2	R3	R4
1	Me	Me	Me	Me					
2	Me	Me	Me	OMe					
3	Me	Me	OMe	OMe					
4	Me	OMe	Me	OMe					
5	Me	OMe	OMe	OMe					
6	OMe	OMe	OMe	OMe					
7	Me	Me	Me	Py					
8	Me	Me	OMe	Py	8'	Me	Me	OH	Py
9	Me	OMe	Me	Py	9'	Me	OH	Me	Py
10	Me	OMe	OMe	Py	10'	Me	OH	OH	Py
11	OMe	Me	OMe	Py	11'	OH	Me	OH	Py
12	OMe	OMe	OMe	Py	12'	OH	OH	OH	Py
13	Me	Me	Py	Py					
14	Me	Py	Me	Py					
15	OMe	OMe	Py	Py	15'	OH	OH	Py	Py
16	OMe	Py	OMe	Py	16'	OH	Py	OH	Py
17	Me	OMe	Py	Py	17'	Me	OH	Py	Py
18	Me	Py	OMe	Py	18'	Me	Py	OH	Py
19	Me	Py	Py	Py					
20	OMe	Py	Py	Py	20'	OH	Py	Py	Py
21	Py	Py	Py	Py					

5,10-bis(4-tolyl)-15,20-bis(4-pyridyl)porphyrin ^{1}H NMR (300MHz, $CDCl_3$): δ=-2.84 (br, pyrrole N-H, 2H), 2.72 (s, $-CH_3$, 6H), 7.58 (d, J = 8.1 Hz, 4H, *o*-Ph), 8.08 (d, J = 8.1 Hz, 4H, *m*-Ph), 8.17 (d, J = 6.0 Hz, 4H, pyridyl), 8.79 (d, J = 5.1 Hz, 2H, β-pyrrole), 8.83 (s, 2H, β-pyrrole), 8.89 (s, 2H, β-pyrrole), 8.93 (d, J = 5.1 Hz, 2H, β-pyrrole), 9.04 (d, J = 6.0 Hz, 4H, pyridyl), ESI-MS[(m+H)/z$^+$, % relative intensity]: 645, 100%.

5,15-bis(4-tolyl)-10,20-bis(4-pyridyl)porphyrin ^{1}H NMR (300MHz, $CDCl_3$): δ=-2.84 (br, pyrrole N-H, 2H), 2.72 (s, $-CH_3$, 6H), 7.56 (d, J = 8.1 Hz, 4H, *o*-Ph), 8.08 (d, J = 8.1 Hz, 4H, *m*-Ph), 8.17 (d, J = 6.0 Hz, 4H, pyridyl), 8.78 (d, J = 4.8 Hz, 4H, β-pyrrole), 8.92 (d, J = 4.8 Hz, 4H, β-pyrrole), 9.04 (d, J = 6.0 Hz, 4H, pyridyl). ESI-MS [(m+H)/z$^+$, % relative intensity]: 645, 100%.

5-(4-tolyl)-10-(4-hydroxylphenyl)-15,20-bis(4-pyridyl)porphyrin ^{1}H NMR (300MHz, $CDCl_3$): δ=-2.84 (br, pyrrole N-H, 2H), 2.72 (s, $-CH_3$, 3H), 7.27 (d, J = 8.4 Hz, 2H, *o*-Ph, phenol), 7.56 (d, J = 7.8 Hz, 2H, *o*-Ph, tolyl), 8.06 (d, J = 7.8 Hz, 2H, *m*-Ph, tolyl), 8.10 (d, J = 8.4 Hz, 2H, *m*-Ph, phenol), 8.15 (d, J = 5.4 Hz, 4H, pyridyl), 8.78 (d, J = 5.1 Hz, 2H, β-pyrrole), 8.81 (s, 2H, β-pyrrole), 8.90 (s, 2H, β-pyrrole), 8.95 (d, J = 5.1 Hz, 2H, β-pyrrole), 8.97 (d, J = 5.4 Hz, 4H, pyridyl), ESI-MS [(m+H)/z$^+$, % relative intensity]: 646, 100%.

5-(4-tolyl)-15-(4-hydroxylphenyl)-10,20-bis(4-pyridyl)porphyrin ^{1}H NMR (300MHz, $CDCl_3$): δ=-2.84 (br, pyrrole N-H, 2H), 2.72 (s, $-CH_3$, 3H), 7.34 (d, J = 8.1 Hz, 2H, *o*-Ph, phenol), 7.56 (d, J = 7.8 Hz, 2H, *o*-Ph, tolyl), 8.10 (m, 4H), 8.17 (d, J = 6.0 Hz, 4H, pyridyl), 8.78 (d, J = 4.8 Hz, 4H, β-pyrrole), 8.93 (d, J = 4.8 Hz, 4H, β-pyrrole), 9.04 (d, J = 6.0 Hz, 4H, pyridyl), ESI-MS, [(m+H)/z$^+$, % relative intensity]: 646, 100%.

5,10-bis(4-tolyl)-15-(4-hydroxylphenyl)-20-(4-methylpyrridinium)porphyrin ^1H NMR (300MHz, Acetone-d_6) δ –2.75 (br, pyrrole N-H, 2H) 2.70 (s, 6H, -CH_3, tolyl), 5.0 (s, 3H, -CH_3, pyrridinium), 7.3 (d, J = 8.4 Hz, 2H, *o*-Ph, phenol), 7.66 (d, J = 7.8 Hz, 4H, *o*-Ph, tolyl), 8.05 (d, J = 8.4 Hz, 2H, *m*-Ph, phenol), 8.2 (m, 4H, *m*-Ph, tolyl), 9.1-8.8 (m, br, 8H, β-pyrrole), 9.12 (d, J = 6.6 Hz, 2H, pyrridinium), 9.62 (d, J = 6.6 Hz, 2H, pyrridinium). UV-Vis. in CH_3OH [λ max (nm), relative intensity]: 419(15.28), 517.4(1), 557(0.73), 591(0.42), 649(0.31). ESI-MS [$(m+H)/z^+$, % relative intensity]: 674, 100%.

5-(4-tolyl)-10,15-bis(4-hydroxylphenyl)-20-(4-methylpyrridinium)porphyrin ^1H NMR (300MHz, Acetone-d_6) δ –2.75 (br, pyrrole N-H, 2H) 2.70 (s, 3H, -CH_3, tolyl), 5.0 (s, 3H, -CH_3, pyrridinium), 7.3 (d, J = 8.4 Hz, 4H, *o*-Ph, phenol), 7.66 (d, J = 7.5 Hz, 2H, *o*-Ph, tolyl), 8.05 (d, J = 8.4, 4H, *m*-Ph, phenol), 8.2 (d, J = 7.5 Hz, 2H, *m*-Ph, tolyl), 9.1-8.8 (m, br, 8H, β-pyrrole), 9.09 (d, J = 6.3 Hz, 2H, pyrridinium), 9.63 (d, J = 6.3 Hz, 2H, pyrridinium). UV-Vis. in CH_3OH [λ max (nm), relative intensity]: 421(14.12), 519(1), 559(0.74), 591(0.42), 651(0.35). ESI-MS [$(m+H)/z^+$, % relative intensity]: 676, 100%.

5,10-bis(4-tolyl)-15,20-bis(4-methylpyrridinium)porphyrin ^1H NMR (300MHz, Acetone-d_6) δ -2.75 (s, br, pyrrole N-H, 2H), 2.70 (s, 6H, -CH_3, tolyl), 5.0 (s, 6H, -CH_3, pyrridinium), 7.67 (d, J = 7.8 Hz, 4H, *o*-Ph, tolyl), 8.14 (d, J = 7.8 Hz, 4H, *m*-Ph, tolyl), 9.1-8.8 (m, br, 8H, β-pyrrole), 8.97 (d, J = 6.6 Hz, 4H, pyrridinium), 9.39 (d, J = 6.6 Hz, 4H, pyrridinium). UV-Vis. in CH_3OH [λ max (nm), relative intensity]: 420(11), 516(1), 555(0.69), 590(0.44), 647(0.19) ESI-MS [(m- Me)/z^+, % relative intensity]: 659, 30%; [m/z^{2+}, % relative intensity]: 337, 100%.

5,15-bis(4-tolyl)-10,20-bis(4-methylpyrridinium)porphyrin ^1H NMR (300MHz, DMSO-d_6) δ -2.75 (s, br, pyrrole N-H, 2H), 2.60 (s, 6H, -CH_3, tolyl), 5.0 (s, 6H, -CH_3,

pyrridinium), 7.65 (d, J = 8.1 Hz, 4H, *o*-Ph, tolyl), 8.07 (d, J = 8.1 Hz, 4H, *m*-Ph, tolyl), 9.0-8.8 (m, br, 8H, β-pyrrole), 8.94 (d, J = 6.9 Hz, 4H, pyrridinium), 9.39 (d, J = 6.9 Hz, 4H, pyrridinium), ESI-MS [m/z^{2+}, % relative intensity]: 337, 100%.

5-(4-tolyl)-10-(4-hydroxylphenyl)-15,20-bis(4-methylpyrridinium)porphyrin ^{1}H NMR (300MHz, Methanol-d_4) δ -2.75 (s, br, pyrrole N-H, 2H), 2.75 (s, 3H, -CH_3, tolyl), 5.0 (s, 6H, -CH_3, pyrridinium), 7.29 (d, J = 8.4 Hz, 2H, *o*-Ph, phenol), 7.70 (d, J = 8.1 Hz, 2H, *o*-Ph, tolyl), 8.07 (d, J = 8.4 Hz, 2H, *m*-Ph, phenol), 8.2 (d, J = 8.1 Hz, 2H, *m*-Ph, tolyl), 9.2-8.9 (m, br, 8H, β-pyrrole), 8.97 (d, J = 5.7 Hz, 4H, pyrridinium), 9.39 (d, J = 5.7 Hz, 4H, pyrridinium). UV-Vis. in CH_3OH [λ max (nm), relative intensity]: 423(10.8), 520(1), 555(0.6), 591(0.4), 648(0.2) ESI-MS [(m- Me)/z^+, % relative intensity]: 661, 15%; [m/z^{2+}, % relative intensity]: 338, 100%.

5,10-bis(4-hydroxylphenyl)-15,20-bis(4-methylpyrridinium)porphyrin ^{1}H NMR (300MHz, Methanol-d_4) δ -2.75 (s, br, pyrrole N-H, 2H), 5.0 (s, 6H, -CH_3, pyrridinium), 7.70 (d, J = 8.1 Hz, 4H, *o*-Ph, phenol), 8.14 (d, J = 8.1 Hz, 4H, *m*-Ph, phenol), 9.1-8.8 (m, br, 8H, β-pyrrole), 8.98 (d, J = 5.7 Hz, 4H, pyrridinium), 9.4 (d, J = 5.7 Hz, 4H, pyrridinium). UV-Vis. in CH_3OH [λ max (nm), relative intensity]: 423(10.4), 518(1), 554(0.79), 590(0.46), 650(0.2). ESI-MS [(m- Me)/z^+, % relative intensity]: 663, 100%; [m/z^{2+}, % relative intensity]: 339, 70%.

DNA binding and octanol/water partition coefficient study:

Porphyrin was titrated with CT-DNA in distilled water at 50 ^{0}C with stirring. The concentration of DNA base pairs was up to 2.0mM and the concentration of porphyrins were between 2 - 6μM. The volume of the porphyrin solution was 3ml. A small stir bar was placed inside the cuvette. Data was collected 10 minutes after adding DNA. Figure

A16 and A17 show an example of this data, and analysis. Possibly due to the porphyrin aggregation, the absorption data of the Soret peak droped very fast first and then slowly increased. The binding constants were thus obtained by plotting ΔA vs. DNA concentration[52,54,60] using the equation as below:

$$f = -\frac{ax}{K1+x} + \frac{bx}{K2+x}$$

Octanol/water partition coefficient measurements were done as follows. A small amount porphyrin was dissolved in 3mL octanol in a 8.3mL vial, 3mL water was added to the octanol solution. The mixture was shaken very hard and then centrifuged for 10 minutes to accelerate the separation. The Soret absorption in those two layers was measured, and the ratio of the absorption in octanol over the absorption in water is the partition coefficient.

Appendix

Figure A1. a: L in $CHCl_3$
b: LA in $CHCl_3$

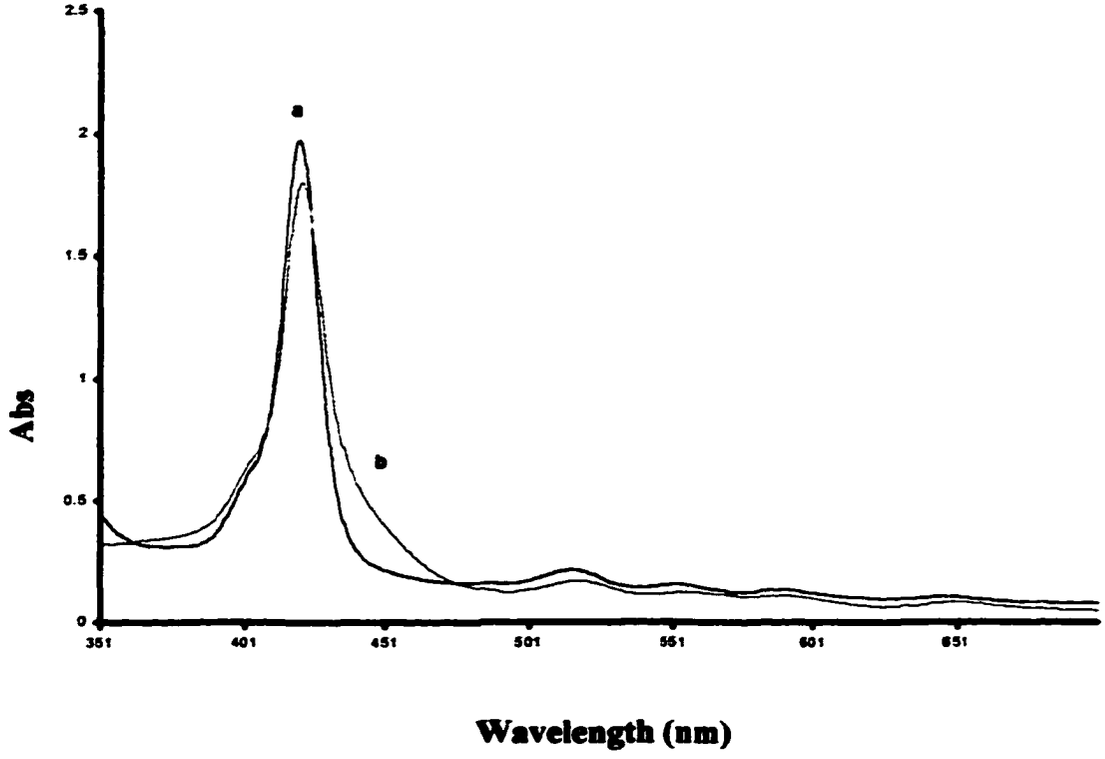


Figure A2

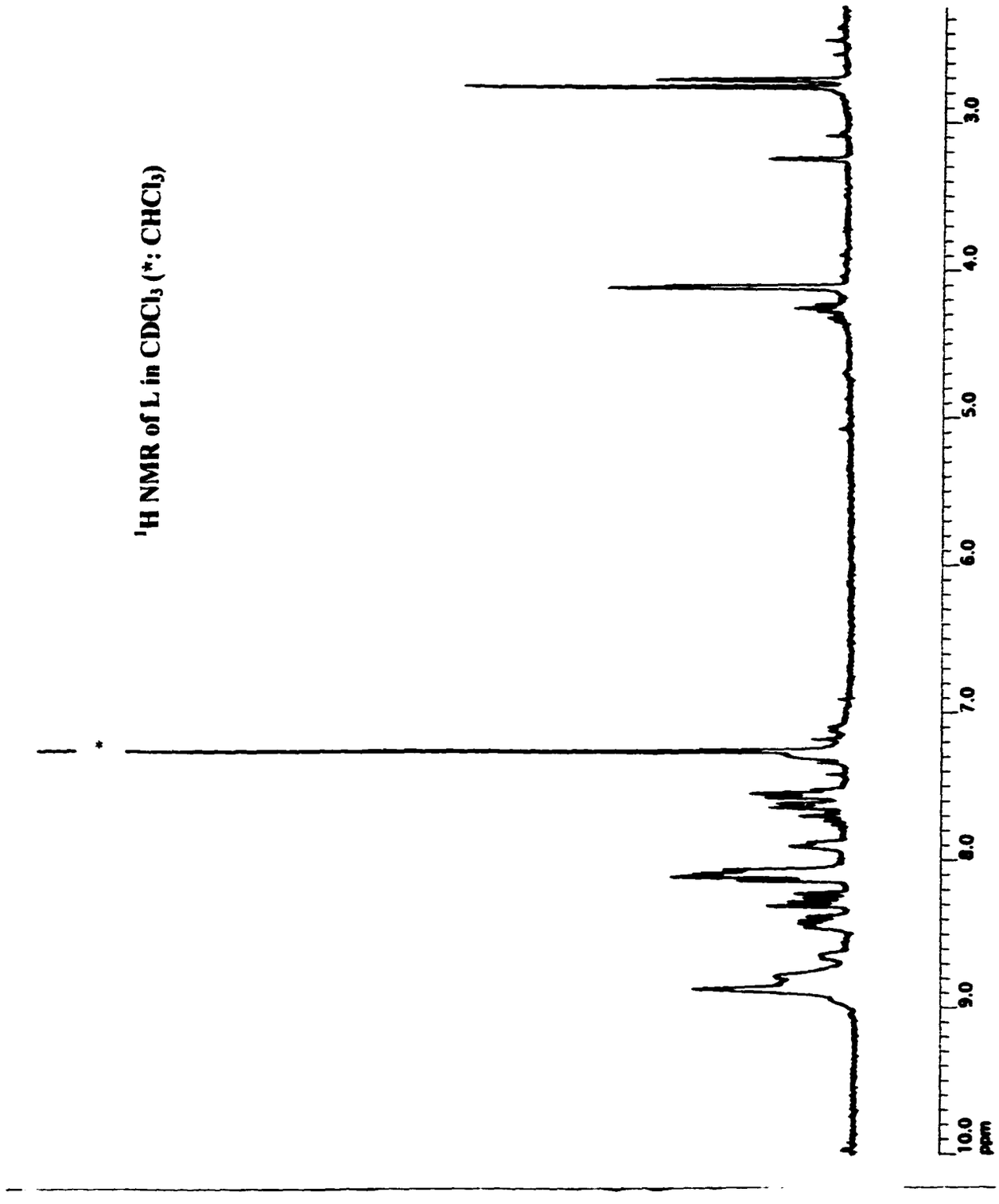


Table A1. Porphyrin identified in different fractions of library L from ESI-MS:

$CHCl_3$ fraction #	Compounds
1	Me/Me/F_5/F_5, OMe/F_5/OMe/CO_2CH_3, OMe/OPe/Br/Br, Me/OPe/OPe/OPe, OMe/OMe/F_5/SCH_3, Me/Me/Me/F_5, Me/Me/F_5/Br, NO_2/Py/Py/Py, Me/F_5/F_5/F_5, OMe/Br/CO_2CH_3/Br, OMe/CO_2CH_3/F_5/OPe, , Me/OPe/CO_2CH_3/OPe, Me/F_5/F_5/OPe, OMe/OPe/CO_2CH_3/OPe, F_5/F_5/Br/Br
2	Me/Me/F_5/F_5, OMe/OMe/F_5/CO_2CH_3, Me/F_5/Br/Br, OMe/Br/CO_2CH_3/Br, Me/OPe/CO_2CH_3/OPe, Me/OPe/OPe/OPe, OMe/OPe/Br/Br, Me/F_5/F_5/OPe, OMe/CO_2CH_3/F_5/OPe, Me/F_5/F_5/F_5, OMe/F_5/Br/SCH_3, F_5/OPe/Br/Br, OMe/OMe/F_5/SCH_3, OMe/OMe/OMe/F_5, Me/Me/Me/F_5, Me/OMe/OPe/Br, Me/Me/F_5/Br
5	OMe/OMe/F_5/CO_2CH_3, OMe/OMe/F_5/F_5, Me/OPe/OPe/OPe, OMe/OPe/Br/Br, Me/F_5/Br/Br, Me/F_5/F_5/F_5, Me/OPe/CO_2CH_3/OPe, OMe/CO_2CH_3/F_5/OPe, F_5/F_5/Br/Br, Me/F_5/OPe/OPe, OMe/OMe/F_5/SCH_3, Me/OMe/OPe/Br, Me/Me/Me/F_5, Me/Me/F_5/Br, OMe/OMe/OMe/F_5
7	Me/F_5/OMe/F_5, OMe/SCH_3/F_5/CO_2CH_3, F_5/SCH_3/SCH_3/SCH_3, SCH_3/OPe/Br/Br, CO_2CH_3/OPe/CO_2CH_3/OPe, OMe/OPe/OPe/OPe, OMe/OMe/F_5/F_5, Me/Br/SCH_3/Br, OMe/OMe/Br/Br, Me/F_5/Br/Br, Me/OPe/OPe/OPe, OMe/OMe SCH_3/CO_2CH_3, Me/OMe/OMe/F_5, Me/F_5/Me/SCH_3, Me/Me/F_5/OPe, OMe/OMe/OMe/F_5, OMe/OMe/F_5/SCH_3, OMe/OMe/OMe/OMe, Me/Me/F_5/Br
9	OMe/F_5/SCH_3/SCH_3, Me/OPe/OPe/OPe, Me/F_5/F_5/F_5, OMe/OMe/F_5/SCH_3, OMe/Me/F_5/Br, OMe/OMe/OMe/F_5, Me/F_5/OMe/SCH_3, Me/OMe/CO_2CH_3/OPe, Me/Me/Me/F_5
11	Me/OMe/Me/F_5, OMe/OMe/OMe/F_5, Me/F_5/OMe/SCH_3, Me/OMe/Br/CO_2CH_3, OMe/OPe/CO_2CH_3/OPe, Me/F_5/F_5/F_5, Me/OPe/OPe/OPe, OMe/OMe/F_5/SCH_3, Me/Me/F_5/Br
13	Me/F_5/OMeSCH_3, OMe/OMe/F_5/SCH_3, Me/Me/F_5/Br, Me/OMe/OPe/F_5, OMe/OPe/CO_2CH_3/OPe, Me/F_5/F_5/F_5, Me/OPe/OPe/OPe
15	Me/F_5/OMe/SCH_3, Me/OMe/Br/OMe, OMe/OMe/F_5/SCH_3, Me/Me/Me/Br, Me/F_5/CO_2CH_3/F_5, OMe/F_5/SCH_3/OPe, OMe/OMe/F_5/F_5, Me/CO_2CH_3/OPe/Br, OMe/OPe/CO_2CH_3/OPe, Me/F_5/F_5/F_5
17	Me/F_5/OMe/SCH_3, OMe/OMe/F_5/SCH_3, Me/Me/F_5/Br, Me/Me/SCH_3/OPe, Me/OMe/OMe/OPe, Me/F_5/F_5/F_5, F_5/SCH_3/F_5/Co_2CH_3, Me/CO_2CH_3/CO_2CH_3/Br, CO_2CH_3/CO_2CH_3/Br/Br, Me/OPe/OPe/OPe, F_5/F_5/SCH_3/SCH_3, OMe/OPe/CO_2CH_3/OPe

19	Me/CO_2CH_3/CO_2CH_3/Br, Me/OPe/OPe/OPe, F_5/F_5/SCH_3/SCH_3, CO_2CH_3/CO_2CH_3/Br/Br, F_5/SCH_3/F_5/CO_2CH_3
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23	Me/F_5/SCH_3/F_5, OMe/OMe/CO_2CH_3/Br, Me/CO_2CH_3/CO_2CH_3/Br, F_5/SCH_3/OPe/CO_2CH_3, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, CO_2CH_3/CO_2CH_3/Br/Br, F_5/SCH_3/F_5/CO_2CH_3, Me/OPe/CO_2CH_3/OPe
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29	Me/OMe/CO_2CH_3/OMe, Omd/OMe/CO_2CH_3/Br, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, SCH_3/SCH_3/OPe/OPe, Me/CO_2CH_3/CO_2CH_3/Br, F_5/SCH_3/OPe/CO_2CH_3, F_5/SCH_3/F_5/SCH_3, CO_2CH_3/CO_2CH_3/Br/Br
31	Me/OMe/CO_2CH_3/OMe, OMe/OMe/CO_2CH_3/Br, OMe/OMe/OMe/CO_2CH_3, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, SCH_3/OPe/SCH_3/OPe, Me/CO_2CH_3/CO_2CH_3/Br, F_5/SCH_3/OPe/CO_2CH_3, F_5/F_5/SCH_3/SCH_3, F_5/SCH_3/F_5/CO_2CH_3
33	Me/OMe/CO_2CH_3/OMe, Me/Me/SCH_3/CO_2CH_3, OMe/OMe/CO_2CH_3/Br, OMe/SCH_3/SCH_3/CO_2CH_3, SCH_3/OPe/SCH_3/OPe, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, Me/CO_2CH_3/CO_2CH_3/Br, F_5/F_5/SCH_3/SCH_3, Me/OPe/CO_2CH_3/OPe
35	OMe/SCH_3/SCH_3/CO_2CH_3, OMe/OMe/CO_2CH_3/Br, Me/SCH_3/CO_2CH_3/Br, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, SCH_3/OPe/SCH_3/OPe, Me/CO_2CH_3/CO_2CH_3/Br, F_5/SCH_3/OPe/CO_2CH_3, F_5/F_5/SCH_3/SCH_3
37	Me/OMe/CO_2CH_3/OMe, Me/Me/SCH_3/CO_2CH_3, OMe/OMe/CO_2CH_3/Br, Me/SCH_3/CO_2CH_3/Br, OMe/SCH_3/SCH_3/CO_2CH_3, Me/CO_2CH_3/CO_2CH_3/Br, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, SCH_3/OPe/SCH_3/OPe, F_5/SCH_3/F_5/CO_2CH_3, F_5/F_5/SCH_3/SCH_3, Me/OPe/CO_2CH_3/OPe
39	Me/OMe/CO_2CH_3/OMe, Me/Me/SCH_3/CO_2CH_3, OMe/OMe/CO_2CH_3/Br, Me/SCH_3/CO_2CH_3/Br, Me/Me/CO_2CH_3/CO_2CH_3, Me/Me/OMe/OPe, OMe/SCH_3/SCH_3/CO_2CH_3, Me/CO_2CH_3/CO_2CH_3/Br, Me/OMe/Br/CO_2CH_3, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, Me/F_5/SCH_3/F_5, F_5/SCH_3/F_5/CO_2CH_3, SCH_3/OPe/Sche/OPe, Me/OPe/CO_2CH_3/OPe
41	OMe/SCH_3/SCH_3/CO_2CH_3, Me/OMe/CO_2CH_3/OMe, Me/Me/SCH_3/CO_2CH_3, OMe/OMe/CO_2CH_3/Br,

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43	OMe/SCH_3/SCH_3/CO_2CH_3, Me/OMe/CO_2CH_3/OMe, Me/Me/SCH_3/CO_2CH_3, OMe/OMe/F_5/SCH_3, Me/F_5/SCH_3/SCH_3, OMe/OMe/CO_2CH_3/Br, Me/SCH_3/CO_2CH_3/Br, OMe/OMe/CO_2Ch_3/OPe, NO_2/OMe/CO_2CH_3/Br, Me/CO_2CH_3/CO_2CH_3/Br, F_5/SCH_3/F_5/CO_2Ch_3, F_5/F_5/SCH_3/SCH_3, Me/OPe/CO_2CH_3/OPe
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51	Me/F_5/SCH_3/SCH_3, OMe/SCH_3/SCH_3/CO_2CH_3, OMe/CO_2CH_3/CO_2CH_3/CO_2CH_3, Me/SCH_3/CO_2CH_3/OPe, Me/CO_2CH_3/CO_2CH_3/Br, Me/OPe/CO_2CH_3/OPe, NO_2/OMe/F_5/Br
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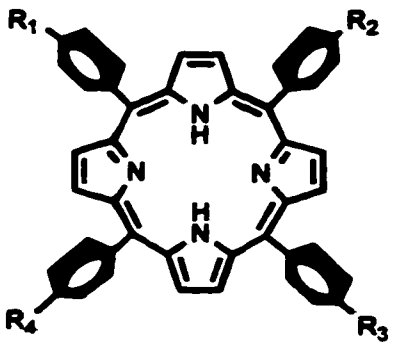
57	OMe/SCH_3/SCH_3/CO_2CH_3, Me/CO_2CH_3/OMe/CO_2CH_3, NO_2/OMe/OMe/F_5, Me/NO_2/No_2/CO_2CH_3, NO_2/NO_2/NO_2/CO_2CH_3, Me/SCH_3/CO_2CH_3/Br, F_5/SCH_3/CO_2CH_3/CO_2CH_3, Me/CO_2CH_3/CO_2CH_3/CO_2CH_3, Me/F_5/SCH_3/SCH_3, SCH_3/SCH_3/SCH_3/CO_2CH_3, OMe/Py/Br/Br, CO_2CH_3/CO_2CH_3/CO_2CH_3/Br, Me/OMe/Br/CO_2CH_3, NO_2/NO_2/OMe/F_5, NO_2/NO_2/NO_2/OPe, NO_2/F_5/NO_2/CO_2CH_3, Py/Br/Br/Br, F_5/CO_2CH_3/CO_2CH_3/OPe, F_5/SCH_3/SCH_3/CO_2CH_3

EtOAc fraction	compounds
	NMe_2/Py/Py/Py, NMe_2/Me/Py/Py, NMe_2/Me/Py/Me, NMe_2/OMe/Py/Py, NMe_2/Py/NMe_2/Py, NMe_2/Me/Py/OMe, NMe_2/Py/Py/SMe, NMe_2/Me/Me/OMe, NMe_2/Me/NMe_2/Py, NMe_2/Py/Py/CO_2Me, NMe_2/OMe/OMe/Py, NMe_2/Py/Me/SMe, NMe_2/Me/CO_2Me/Py, NMe_2/OMe/Me/OMe, NMe_2/Me/SMe/Me, NMe_2/NMe_2/OMe/Py, NMe_2/OMe/Py/SMe, NMe_2/Me/Me/CO_2OMe, NMe_2/NMe_2/NMe_2/Py, NMe_2/NMe_2/Me/OMe, NMe_2/Py/Py/OPe, NMe_2/OMe/Py/CO_2Me, NMe_2/OMe/OMe/OMe, NMe_2/NMe_2/Py/SMe, NMe_2/Me/OMe/SMe, NMe_2/Py/Py/F_5, NMe_2/Py/SMe/SMe, NMe_2/Me/Py/Br, NMe_2/Py/NMe_2/CO_2Me, NMe_2/Me/OMe/CO_2Me, NMe_2/Me/NMe_2/SMe, NMe_2/NMe_2/OMe/OMe, NMe_2/Py/Me/F_5, NMe_2/Py/SMe/CO_2Me, NMe_2/Py/SMe/CO_2Me, NMe_2/Me/SMe/SMe, NMe_2/OMe/OMe/SMe, NMe_2/Me/Me/Br, NMe_2/Py/OMe/Br, NMe_2/Me/OPe/Me, NMe_2/NMe_2/NMe_2/OMe, NMe_2/OMe/OPe/Py, NMe_2/Py/CO_2Me/CO_2Me, NMe_2/OMe/CO_2Me/OMe, NMe_2/Me/F_5/Me, NMe_2/SMe/Me/CO_2Me, NMe_2/OMe/NMe_2/SMe, NMe_2/Py/OMe/F_5, NMe_2/SMe/OMe/SMe, NMe_2/Me/Br/OMe, NMe_2/NMe_2/Py/Br, NMe_2/SMe/Py/Br, NMe_2/NMe_2/NMe_2/NMe_2, NMe_2/OMe/Me/OPe, NMe_2/Me/CO_2Me/CO_2Me, NMe_2/NMe_2/NMe_2/SMe, NMe_2/Py/OPe/SMe, NMe_2/OMe/SMe/CO_2Me, NMe_2/Py/NMe_2/F_5, NMe_2/OMe/Me/F_5, NMe_2/SMe/NMe_2/SMe, NMe_2/Me/NMe_2/Br, NMe_2/Py/F_5/SMe, NMe_2/Py/CO_2Me/Br, NMe_2/SMe/SMe/SMe, NMe_2/Me/SMe/Br, NMe_2/OMe/OMe/Br, NMe_2/Me/NMe_2/OPe, NMe_2/NMe_2/NMe_2/CO_2Me, NMe_2/Py/CO_2Me/OPe, NMe_2/Me/SMe/OPe, NMe_2/OMe/OMe/OPe, NMe_2/CO_2Me/OMe/CO_2Me, NMe_2/NMe_2/Me/F_5, NMe_2/NMe_2/SMe/CO_2Me, NMe_2/Py/F_5/CO_2Me, NMe_2/Me/F_5/SMe, NMe_2/SMe/SMe/CO_2Me, NMe_2/OMe/F_5/OMe, NMe_2/NMe_2/OMe/Br, NMe_2/SMe/OMe/Br, NMe_2/CO_2Me/Me/OPe, NMe_2/NMe_2/CO_2Me/CO_2Me, NMe_2/NMe_2/OMe/OPe, NMe_2/OMe/OPe/SMe, NMe_2/SMe/CO_2Me/CO_2Me, NMe_2/Me/F_5/CO_2Me, NMe_2/OMe/NMe_2/F_5, NMe_2/NMe_2/NMe_2/Br, NMe_2/OPe/Py/Br, NMe_2/F_5/OMe/SMe, NMe_2/OMe/CO_2Me/Br, NMe_2/SMe/NMe_2/Br, NMe_2/Py/Br/F_5, NMe_2/SMe/SMe/Br, NMe_2/Br/Me/Br, NMe_2/NMe_2/NMe_2/OPe, NMe_2/OPe/Py/OPe, NMe_2/OMe/CO_2Me/OPe, NMe_2/CO_2Me/CO_2Me/CO_2Me, NMe_2/NMe_2/SMe/OPe, NMe_2/NMe_2/NMe_2/F_5, NMe_2/Py/F_5/OPe, NMe_2/OMe/F_5/CO_2Me, NMe_2/SMe/OPe/SMe, NMe_2/Me/OPe/Br, NMe_2/NMe_2/F_5/SMe, NMe_2/NMe_2/CO_2Me/Br, NMe_2/F_5/Py/F_5, NMe_2/Me/F_5/Br, NMe_2/SMe/Br/CO_2Me, NMe_2/OPe/Me/OPe, NMe_2/NMe_2/CO_2Me/OPe, NMe_2/Me/F_5/OPe, NMe_2/SMe/OPe/CO_2Me, NMe_2/F_5/NMe_2/CO_2Me, NMe_2/Me/F_5/F_5, NMe_2/SMe/F_5/CO_2Me, NMe_2/OPe/OMe/Br, NMe_2/CO_2Me/CO_2Me/Br, NMe_2/F_5/OMe/Br, NMe_2/Br/NMe_2/Br, NMe_2/Br/SMe/Br, NMe_2/OMe/F_5/OPe, NMe_2/OPe/NMe_2/Br, NMe_2/OMe/F_5/F_5, NMe_2/NMe_2/F_5/Br, NMe_2/NMe_2/OPe/OPe, NMe_2/F_5/SMe/Br, NMe_2/Br/CO_2Me/Br, NMe_2/SMe/OPe/OPe, NMe_2/NMe_2/F_5/OPe, NMe_2/F_5/OPe/SMe, NMe_2/CO_2Me/OPe/Br, NMe_2/F_5/NMe_2/F_5, NMe_2/Br/OPe/Br, NMe_2/OPe/F_5/Br, NMe_2/F_5/Br/F_5, NMe_2/F_5/OPe/OPe, NMe_2/F_5/F_5/OPe, NMe_2/F_5/F_5/F_5
CH_3OH fraction	compounds
	Py/Py/Py/Py, NMe_2/OMe/Py/CO_2Me, Me/Py/Py/SMe, OMe/OMe/Py/Py

Table A2. Porphyrins not observed in L:



R_1 to R_4(including all the isomers):

MePyPyPy, MePyBrPy, MePyPyBr, NO_2PyBrPy, NO_2PyPyBr, MeNO_2SMeBr, PySMeBrCO_2Me, NO_2OMeBrOMe, MePyBrF_5, NO_2OMeBrSMe, OMePyBrF_5, $NO_2NO_2NO_2F_5$, PyOPeF_5Br, NO_2SMeOPeBr, NO_2SMeF_5Br, PyF_5BrF_5, $NO_2NO_2F_5F_5$, $NO_2F_5CO_2$MeBr, NO_2F_5OPeCO_2Me, $NO_2F_5CO_2$MeF_5, NO_2BrF_5Br, NMe_2MeMeMe, NMe_2MeNMe_2Me, NMe_2PyBrPy, $NMe_2NMe_2NMe_2$Me, NMe_2SMeF_5SMe, NMe_2BrOMeBr, NMe_2CO_2MeOPeCO_2Me, NMe_2OPeOMeOPe, NMe_2SMeOPeBr, $NMe_2F_5F_5$SMe, NMe_2CO_2MeF_5Br, NMe_2OPeCO_2MeOPe, $NMe_2F_5CO_2$MeOPe

Distribution of not observed groups (%):

NO_2: 12, Py: 9, NMe_2: 11, Br: 18, F_5: 15, Me: 7, OMe: 5, OPe: 8, SMe: 7, CO_2Me: 8

Figure A3. ESI-MS of LA. Note: some of the porphyrins have 2, 3 or 4 positive charges, so the peaks observed should be $m/2^+$, $m/3^+$ or $m/4^+$

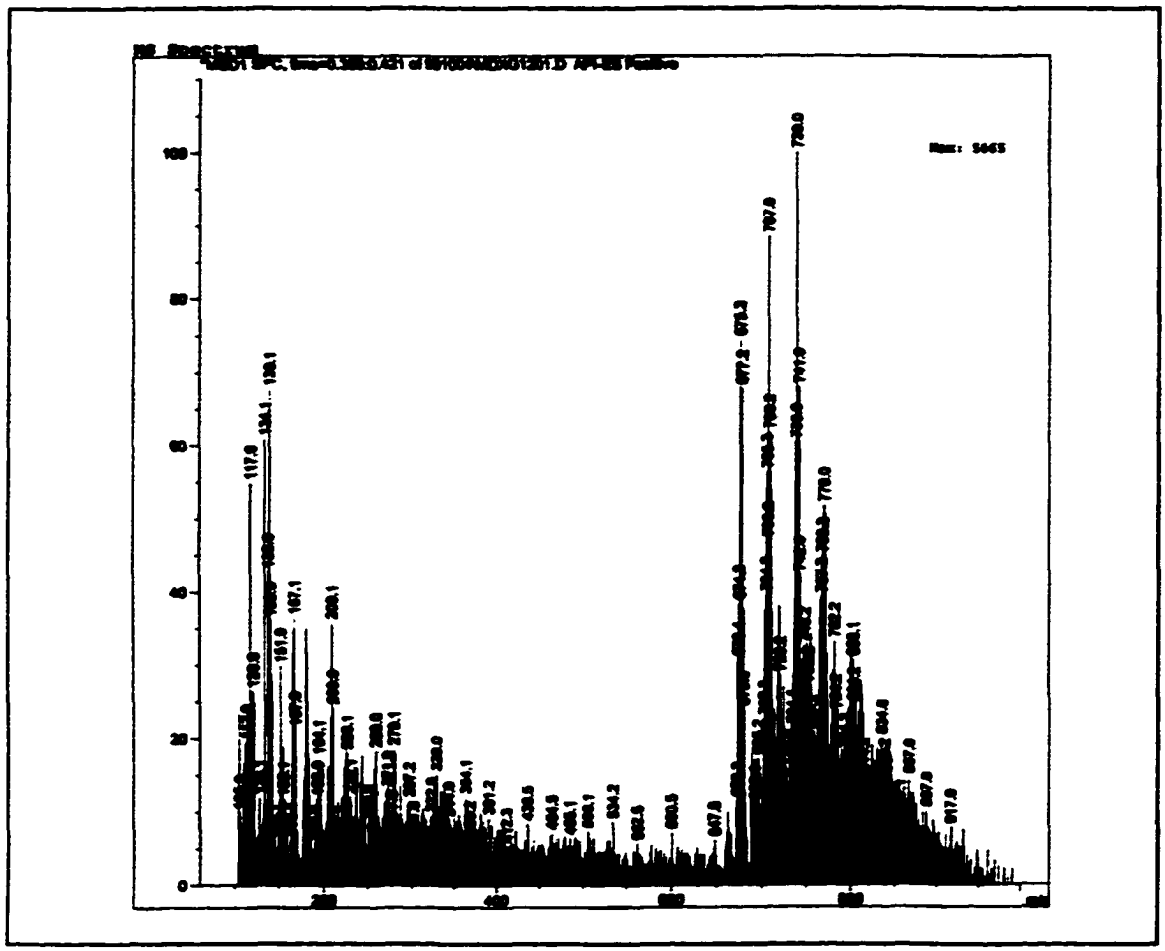
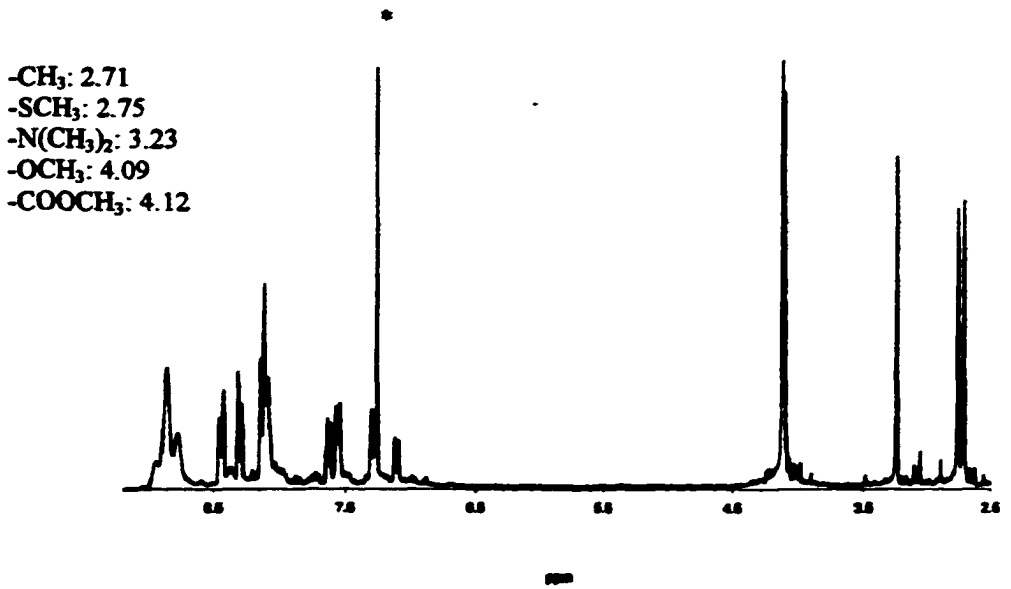


Figure A4.

^{1}H NMR of L1 in $CDCl_3$. (*: $CHCl_3$)



^{1}H NMR of L2 in $CDCl_3$. (*: $CHCl_3$)

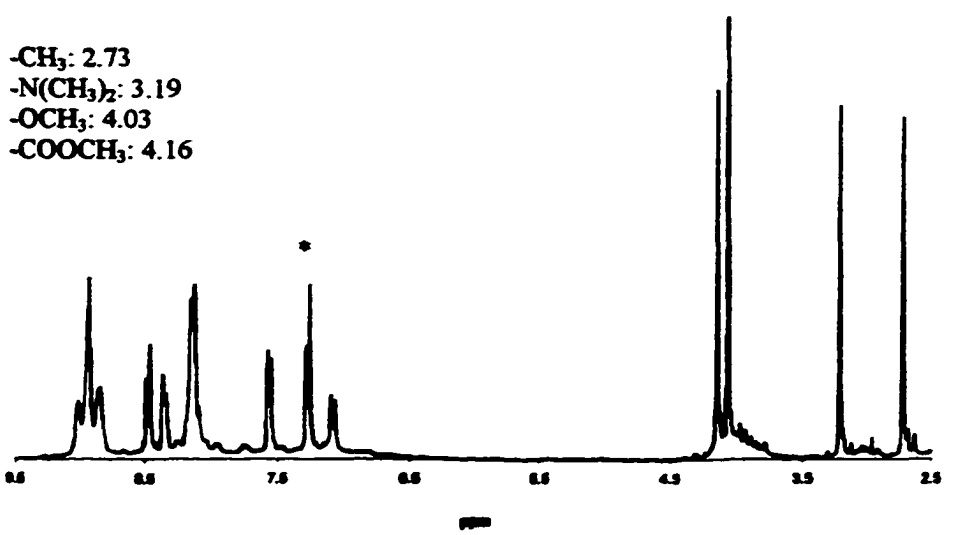


Figure A5.

^{1}H NMR of L1 (500MHz, Benzene-d_6, *: solvents). In order to investigate the distribution of the methyl groups in the NMR, the spectra was taken in C_6D_6 – conditions known to maximizing the chemical shift differences. Note that we observe different line shapes only, but the pyrrole NH are further differentiated.

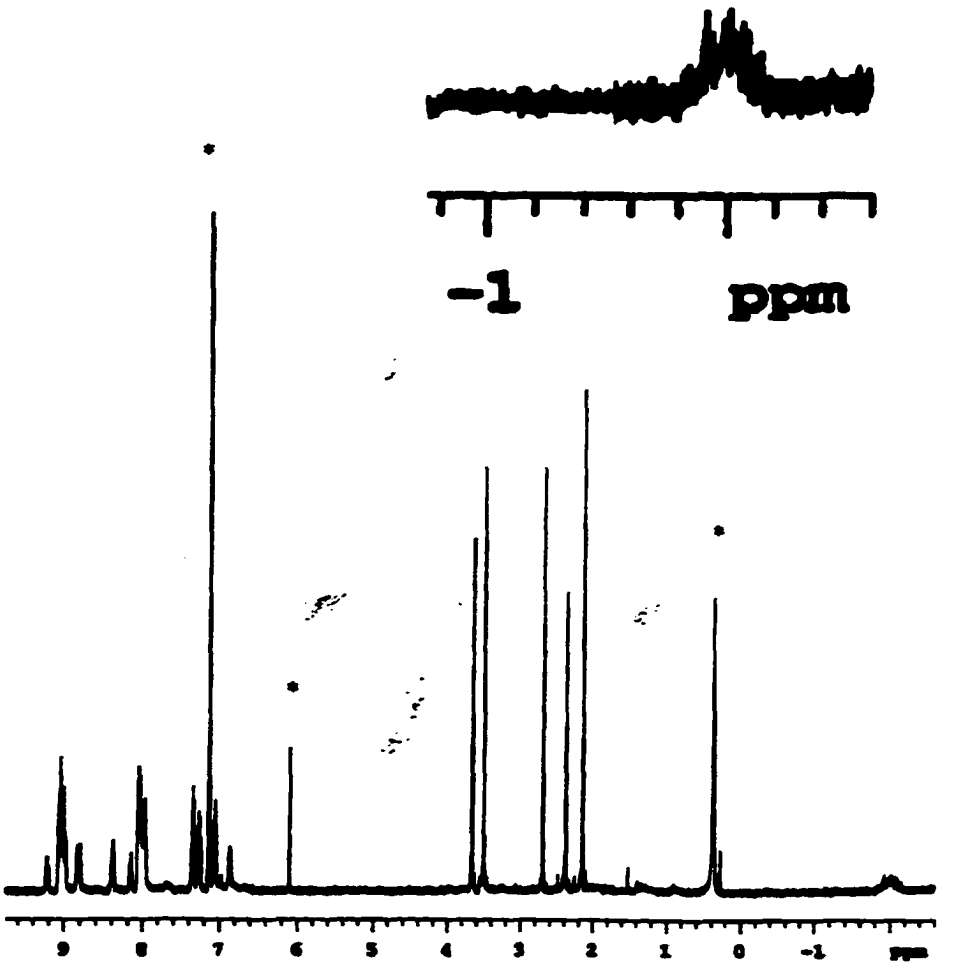
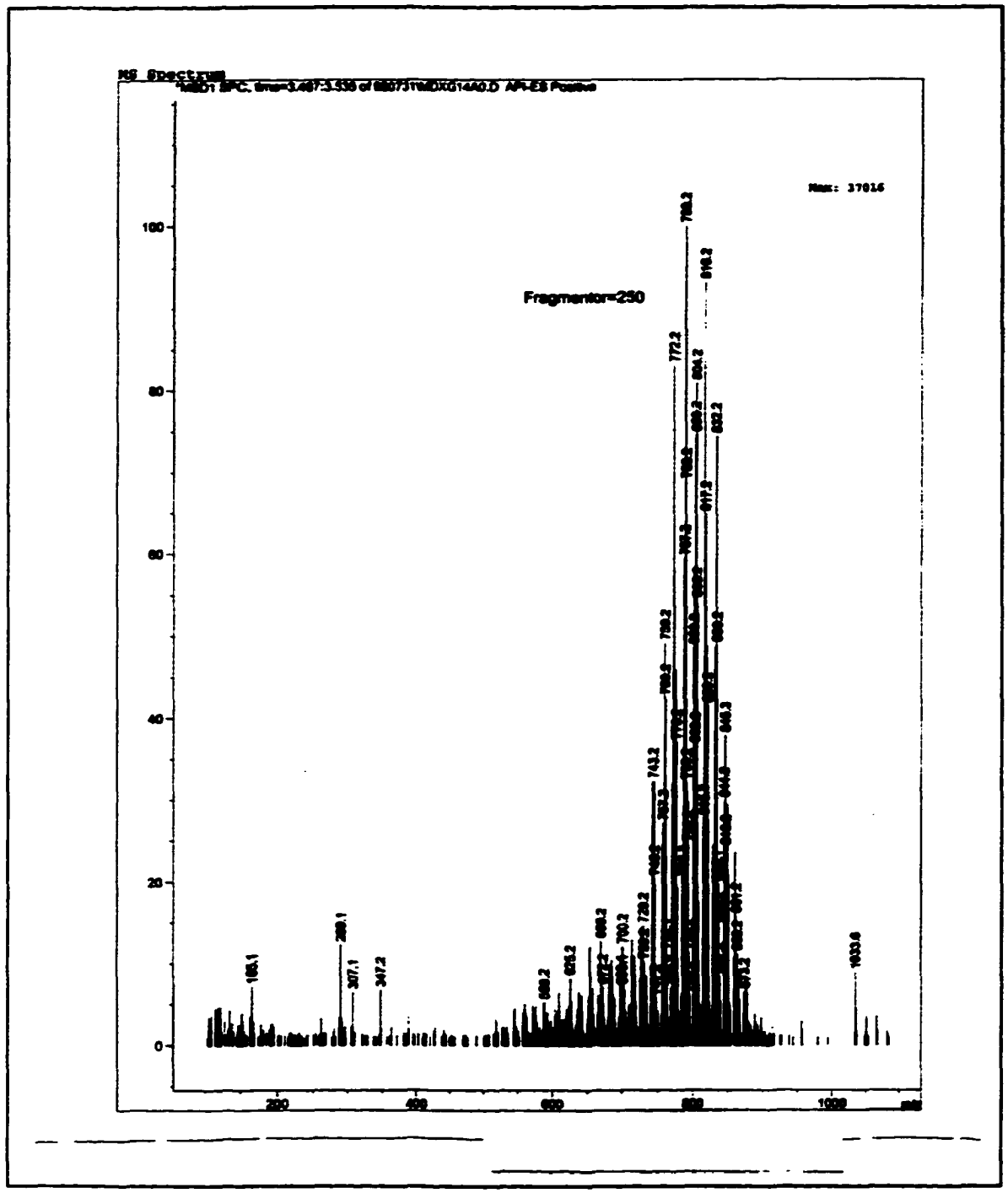


Figure A6. ESI-MS of Co(III)-L1



Figuire A7.

ESI-MS of L1A. Note: some of the porphyrins have 2, 3 or 4 positive charges, so the peaks observed should be $m/2^+$, $m/3^+$ or $m/4^+$

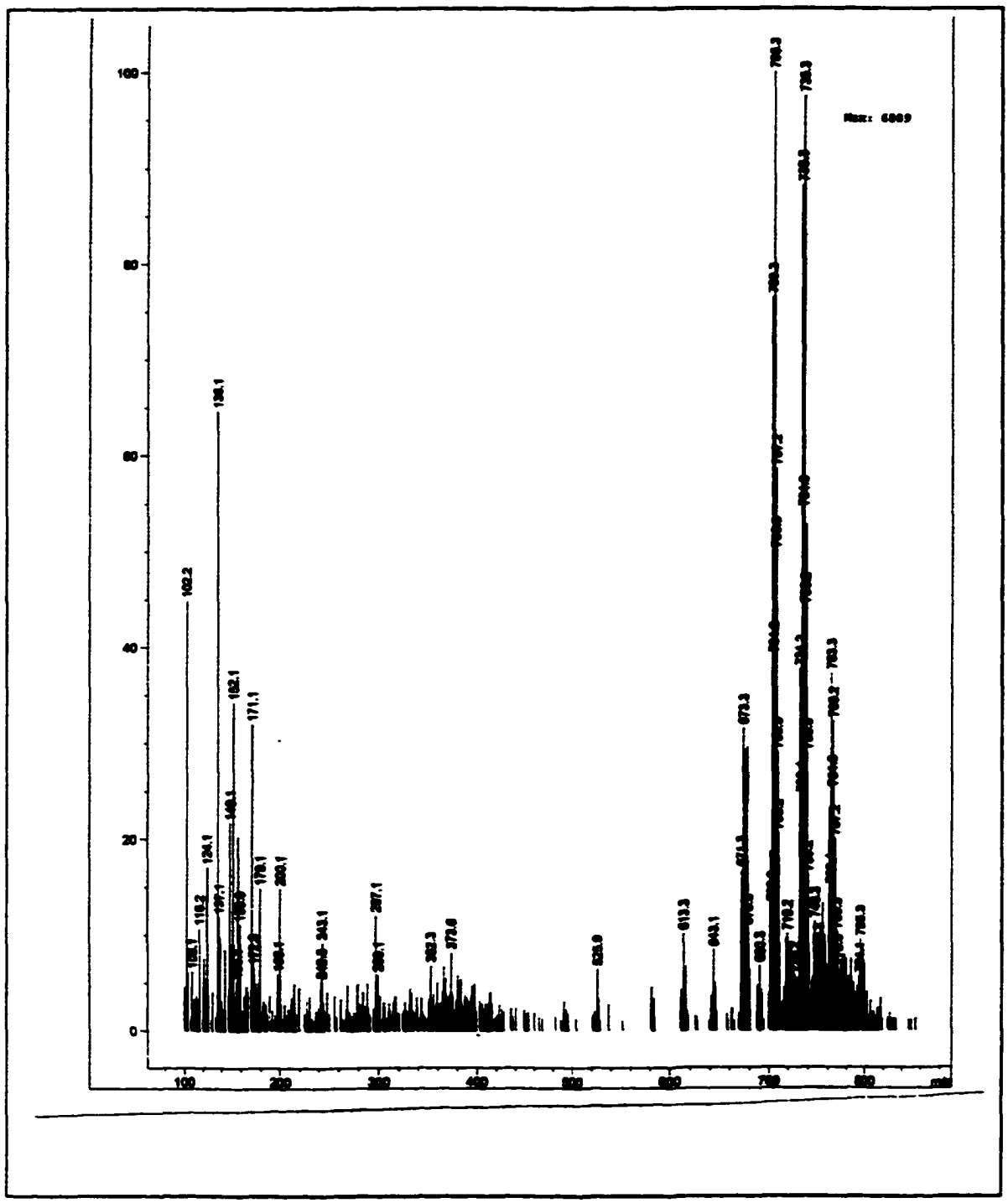
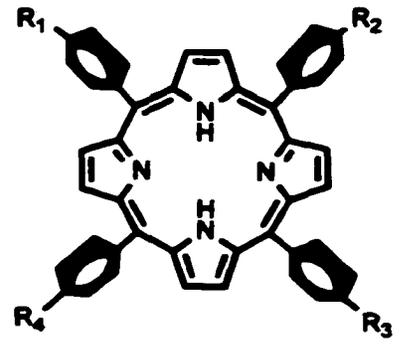


Table A3.



Porphyrins not observed in crude L1:

R_1 to R_4 (including all the isomers):

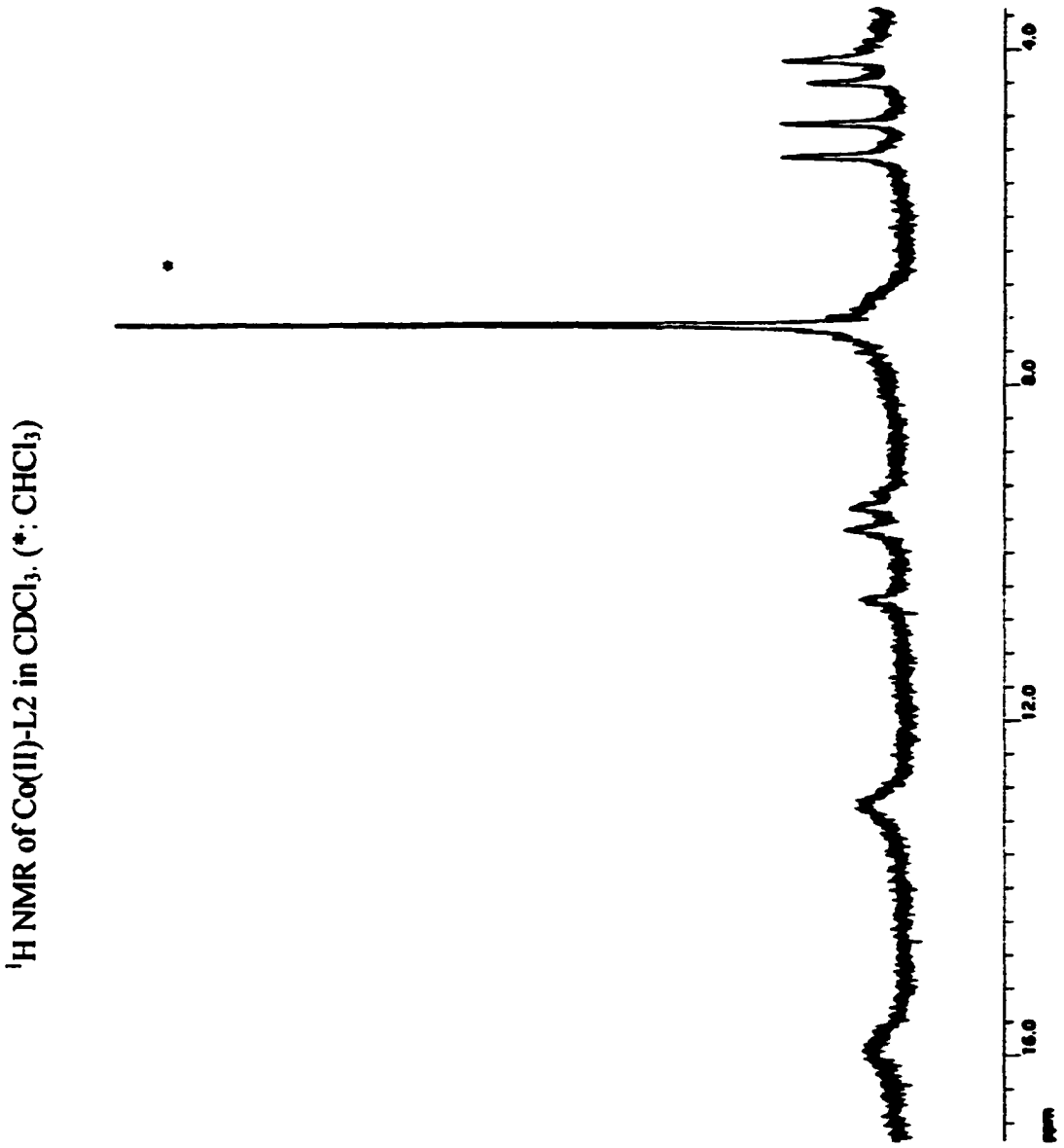
$NMe_2NMe_2NMe_2NMe_2$, $NMe_2NMe_2NMe_2Me$, $CO_2MeCO_2MeCO_2MeCO_2Me$

Porphyrins not observed in pure L1 compared to crude L1:

R_1 to R_4 (including all the isomers):

$CO_2MeCO_2MeCO_2MeOMe$, $CO_2MeCO_2MeCO_2MeSMe$, $NMe_2NMe_2NMe_2CO_2Me$, $CO_2MeCO_2MeCO_2Me\ NMe_2$, $CO_2MeCO_2MeCO_2MeMe$

Figure A8.



^{1}H NMR of Co(II)-L2 in $CDCl_3$. (*: $CHCl_3$)

Figure A9.

ESI-MS of Co(III)-L2

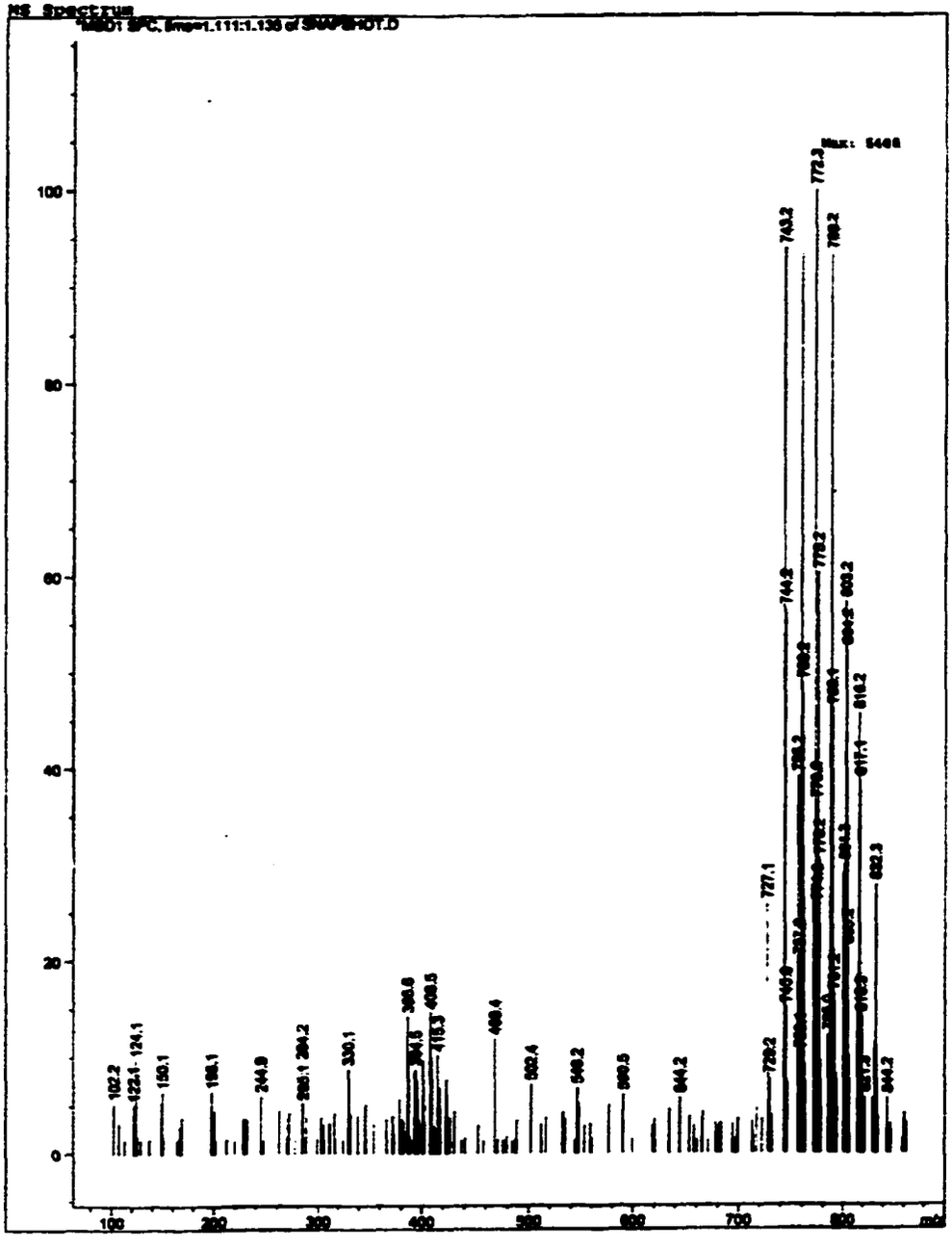
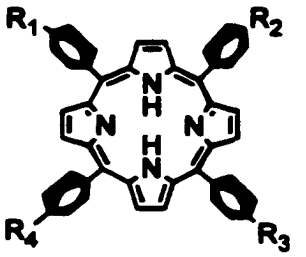


Table A4.

Porphyrins not observed in pure L2

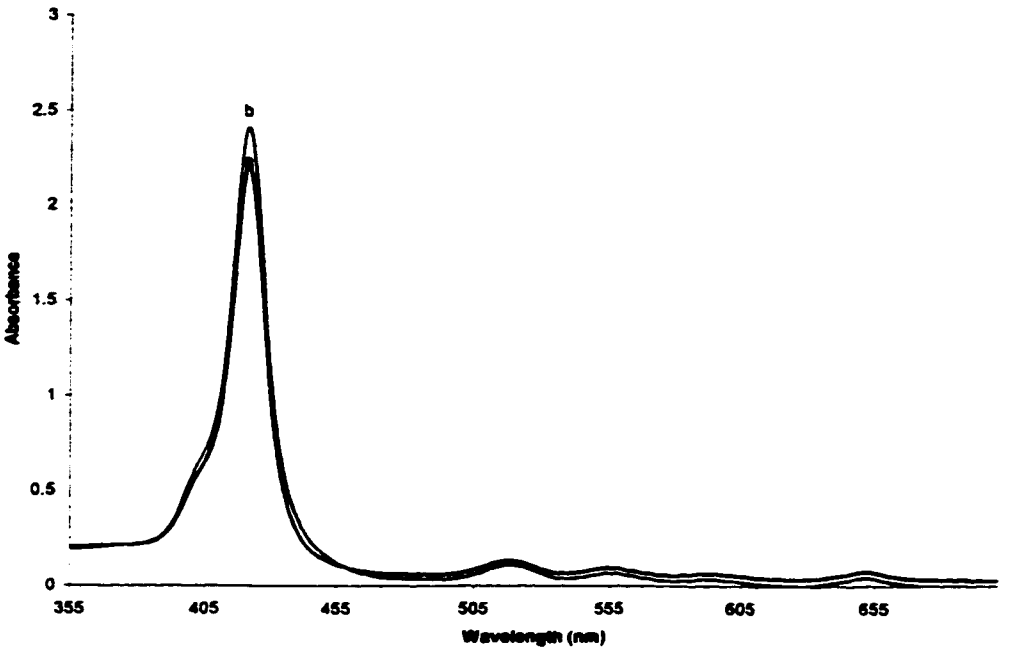


R_1 to R_4:

Py/Py/Py/Py
CH_3/Py/Py/Py
OCH_3/Py/Py/Py
CH_3/$N(CH_3)_2$/$N(CH_3)_2$/$N(CH_3)_2$
OCH_3/$N(CH_3)_2$/$N(CH_3)_2$/$N(CH_3)_2$
$N(CH_3)_2$/$N(CH_3)_2$/$N(CH_3)_2$/$N(CH_3)_2$
CH_3/$COOCH_3$/$COOCH_3$/$COOCH_3$
OCH_3/$COOCH_3$/$COOCH_3$/$COOCH_3$
$N(CH_3)_2$/$COOCH_3$/$COOCH_3$/$COOCH_3$
$COOCH_3$/$COOCH_3$/$COOCH_3$/$COOCH_3$
OCH_3/$COOCH_3$/$COOCH_3$/$N(CH_3)_2$
$COOCH_3$/$COOCH_3$/$N(CH_3)_2$/$N(CH_3)_2$

Figure A10.

UV-Vis spectra of L1:a and L2:b in $CHCl_3$



UV-Vis spectra of L1A:a and L2A:b in $CHCl_3$

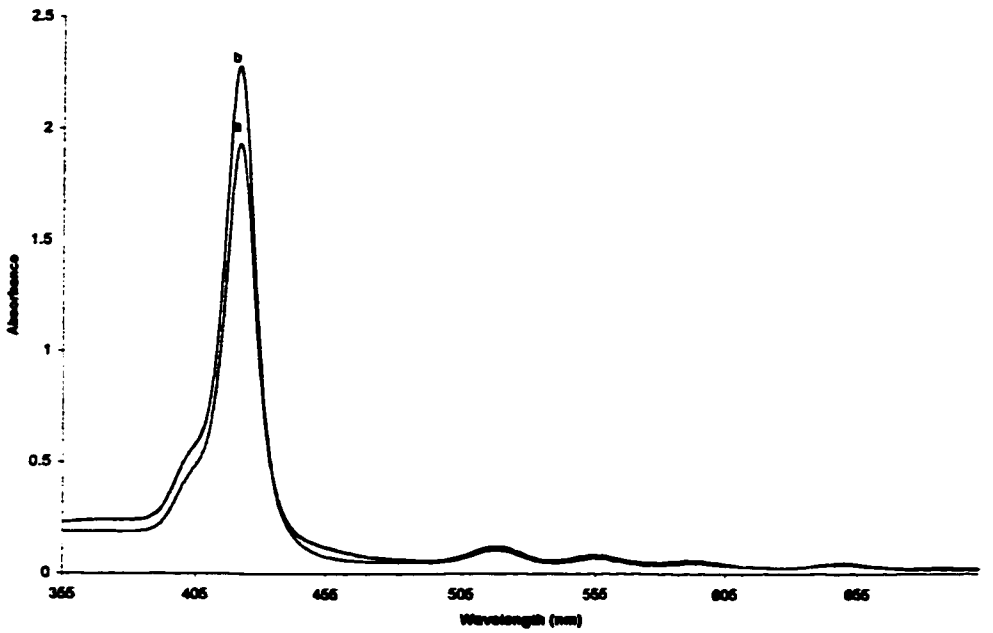
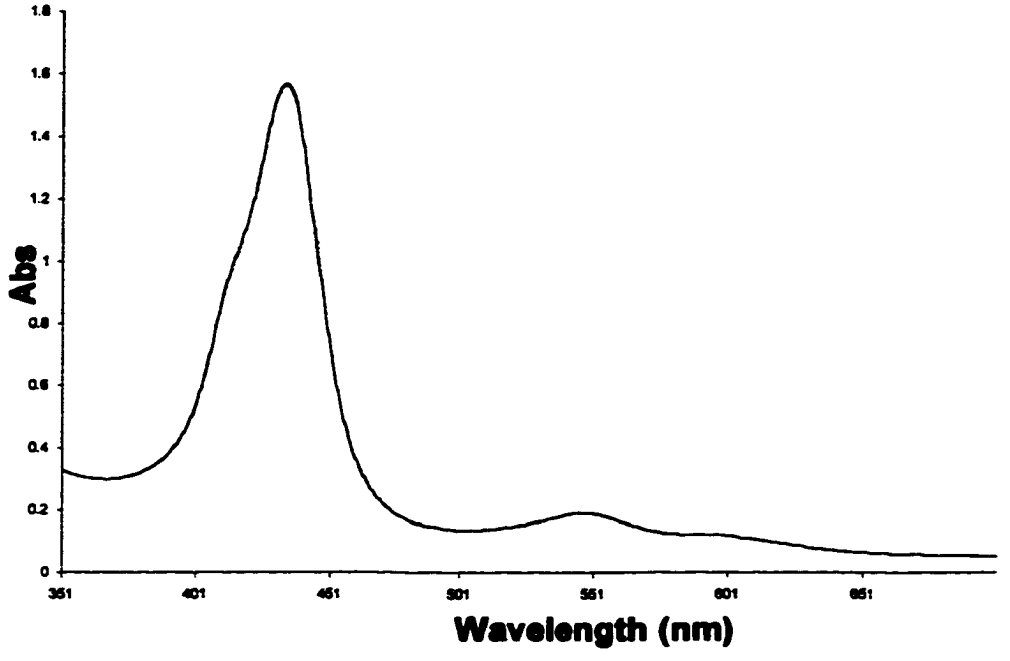


Figure A11.

UV-Vis. of Co(III)-L2 in $CHCl_3$



UV-Vis. of Co(II)-TPP in $CHCl_3$

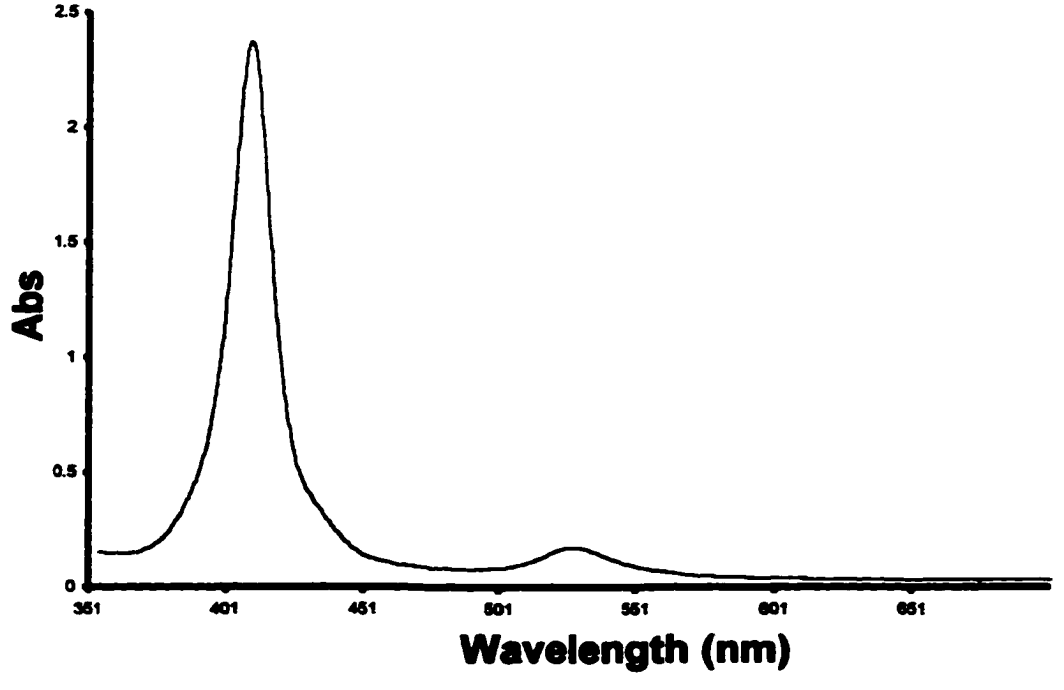
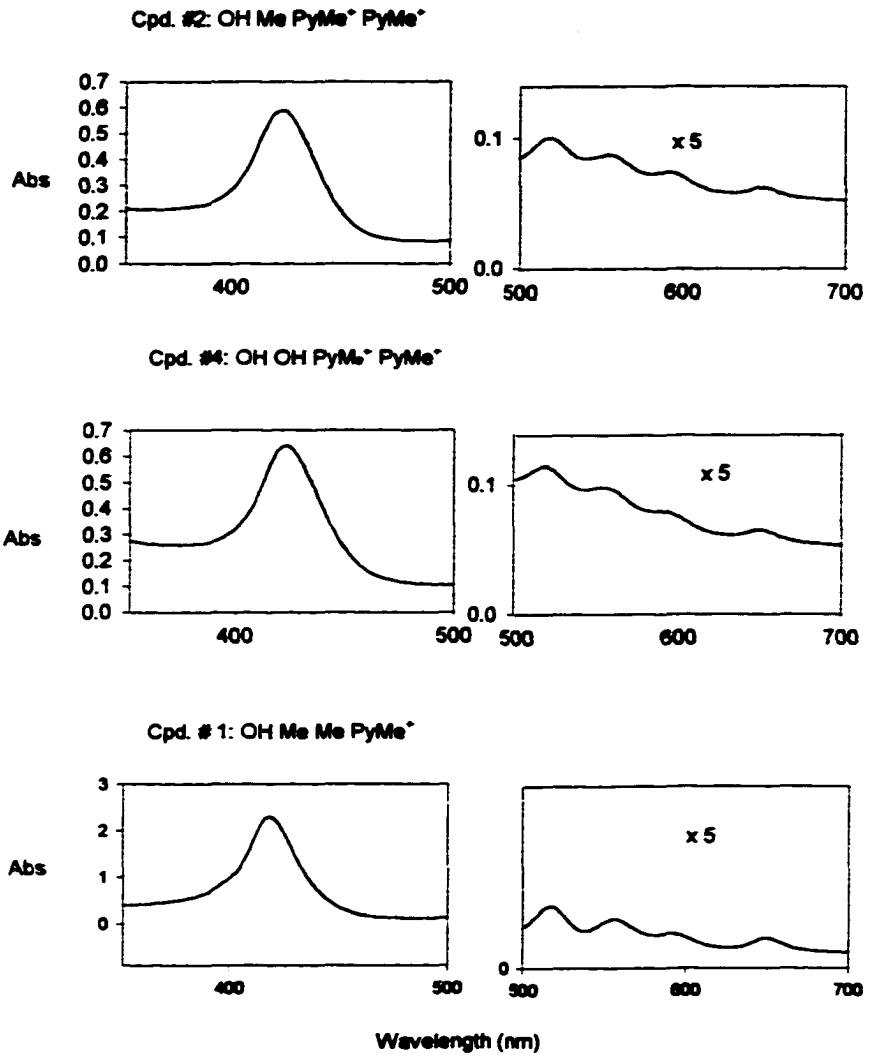


Figure A12.



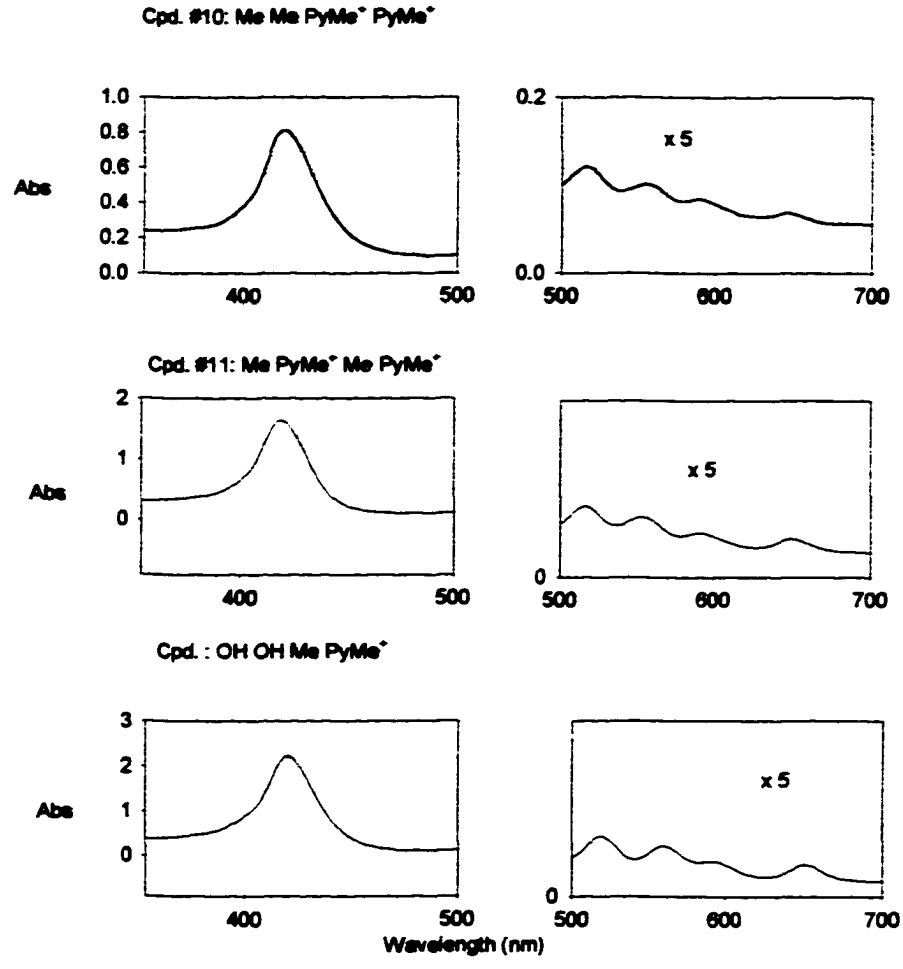


Figure A13.

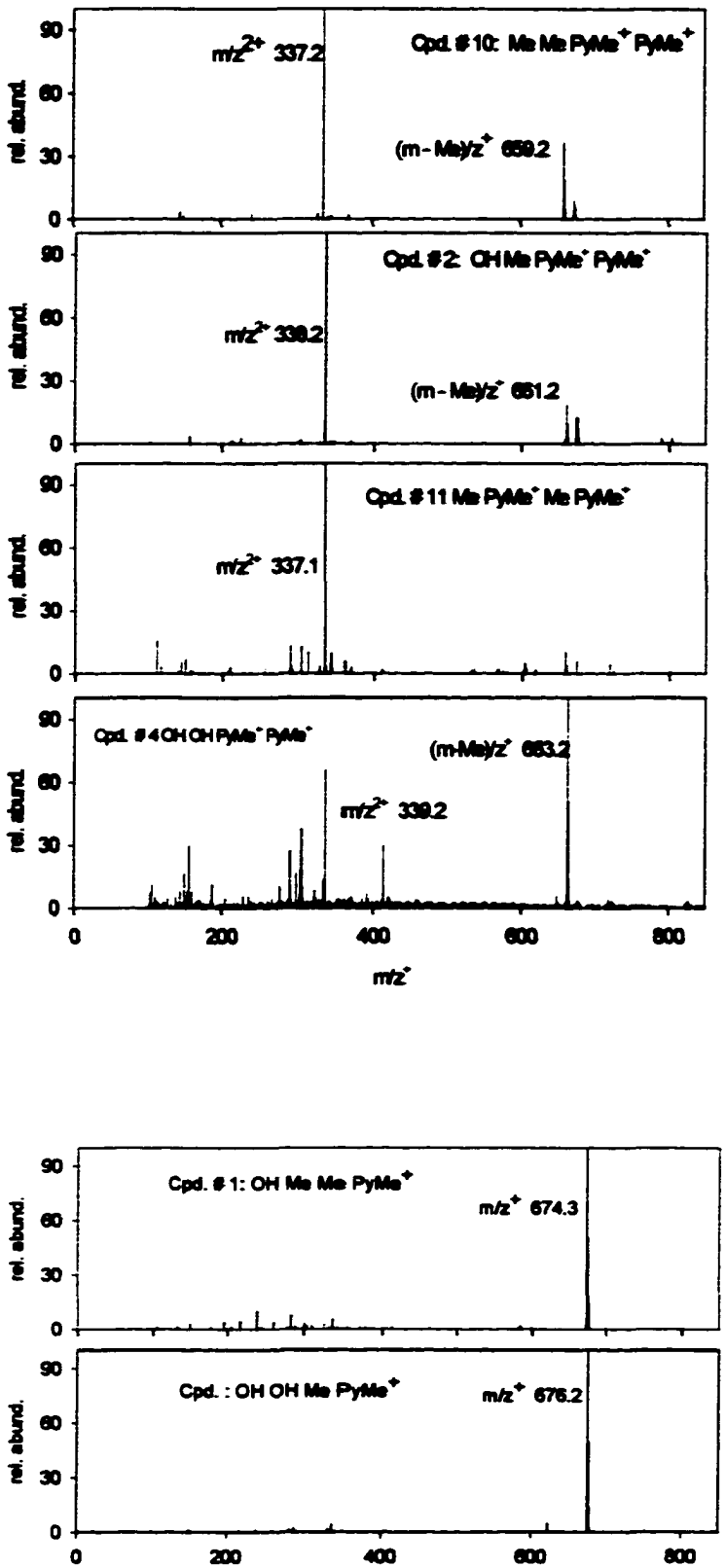
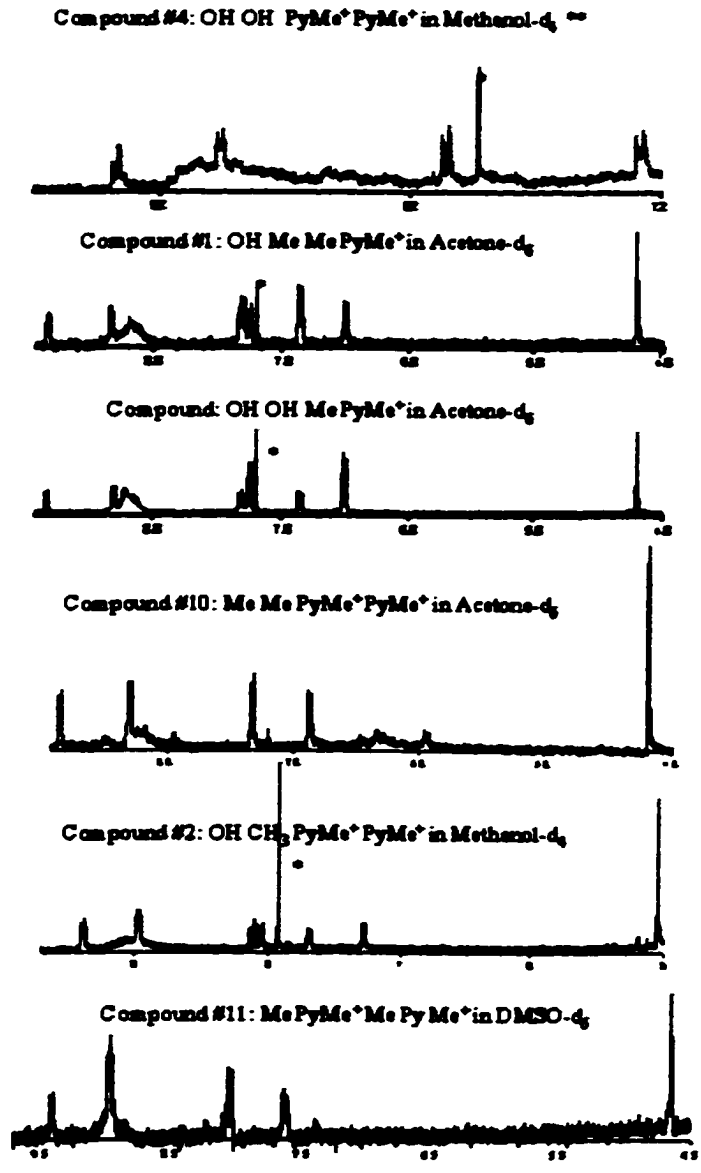


Figure A14.



*: Solvent Peak
**: the methyl peak buried in solvent

Figure A15.

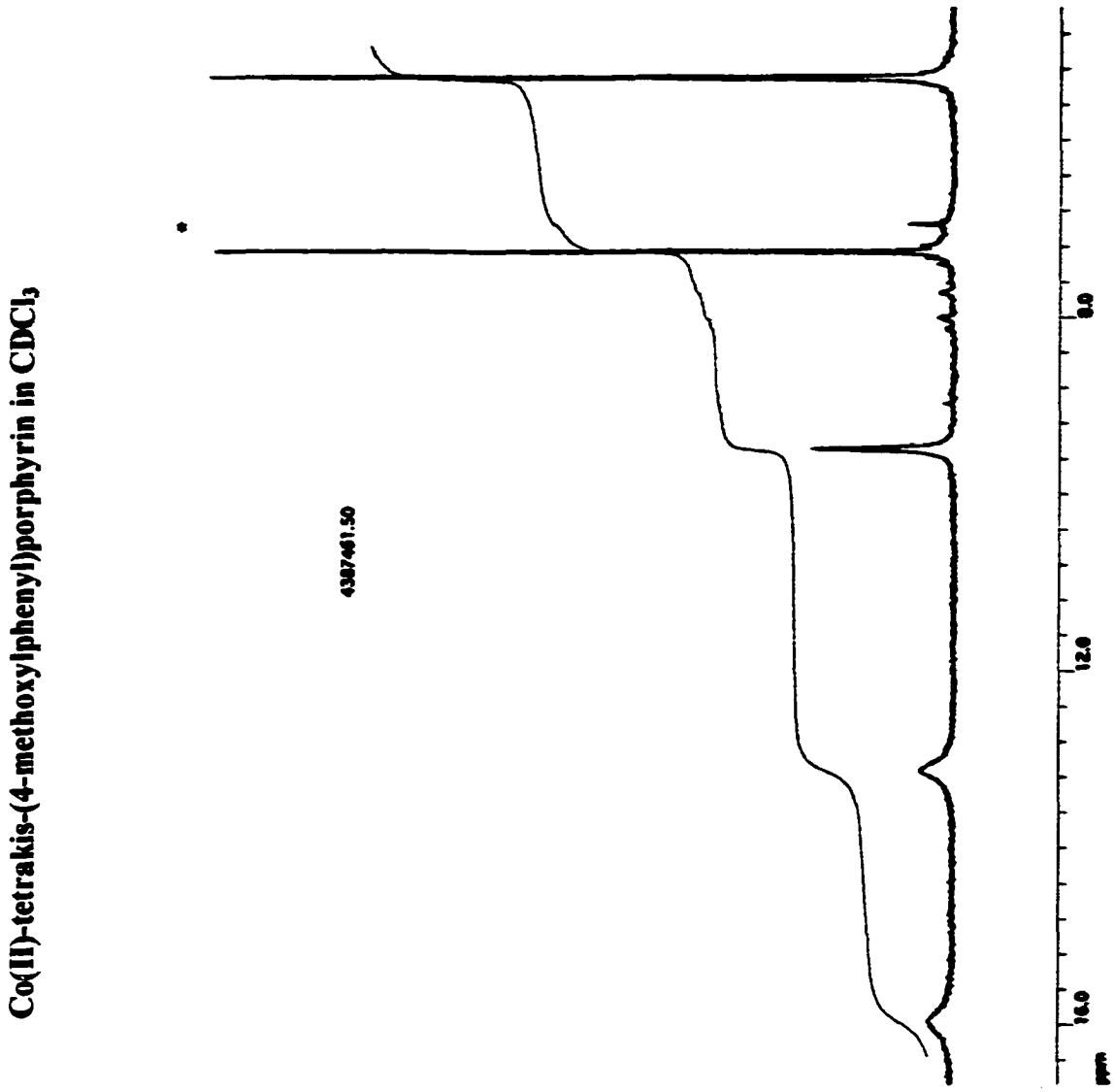
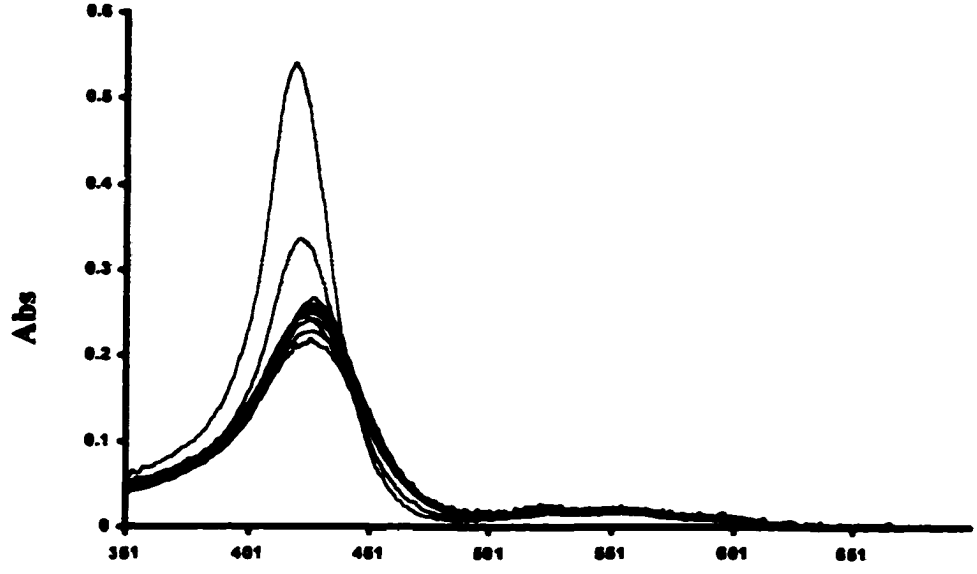


Figure A16.

Cpd. #2: OH Me PyMe$^+$ PyMe$^+$ titrated by CT-DNA



Wavelength (nm)

[DNA base pairs] (μM)

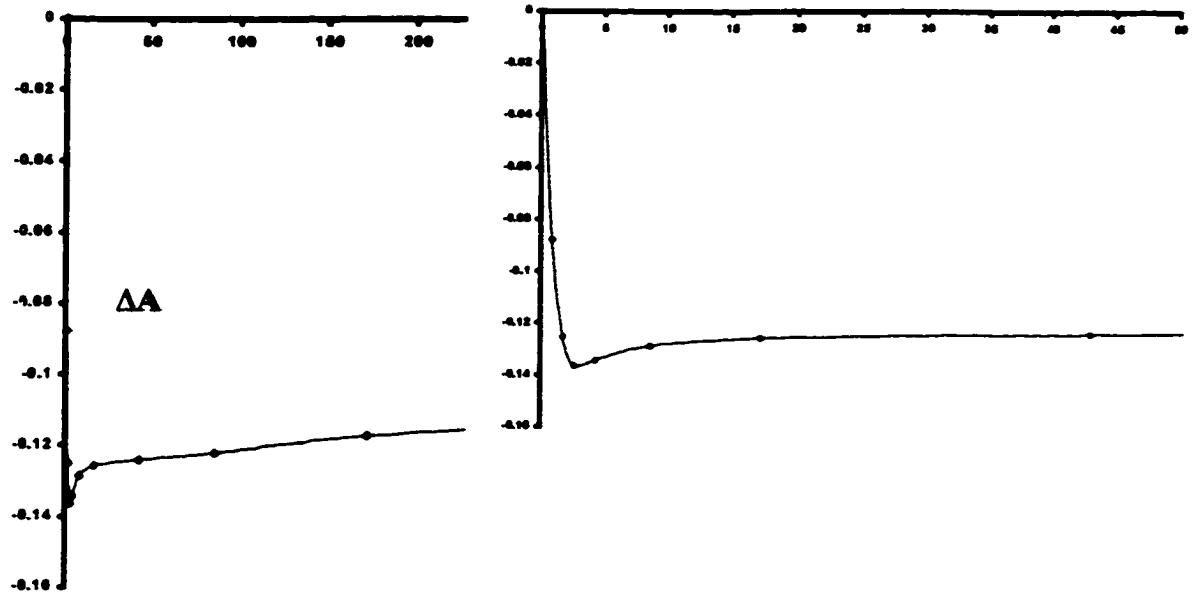
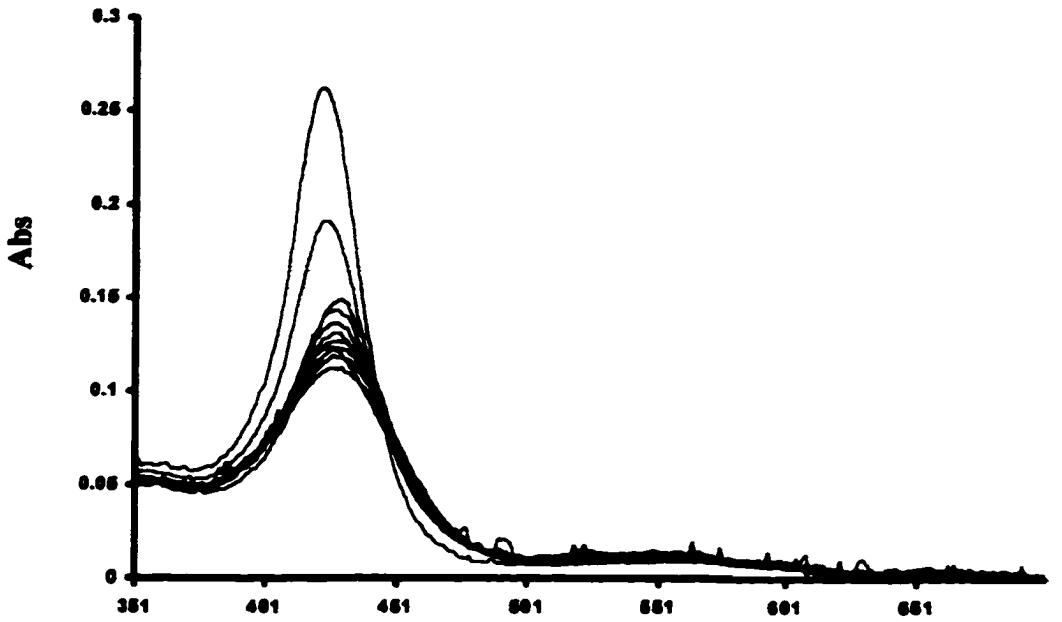


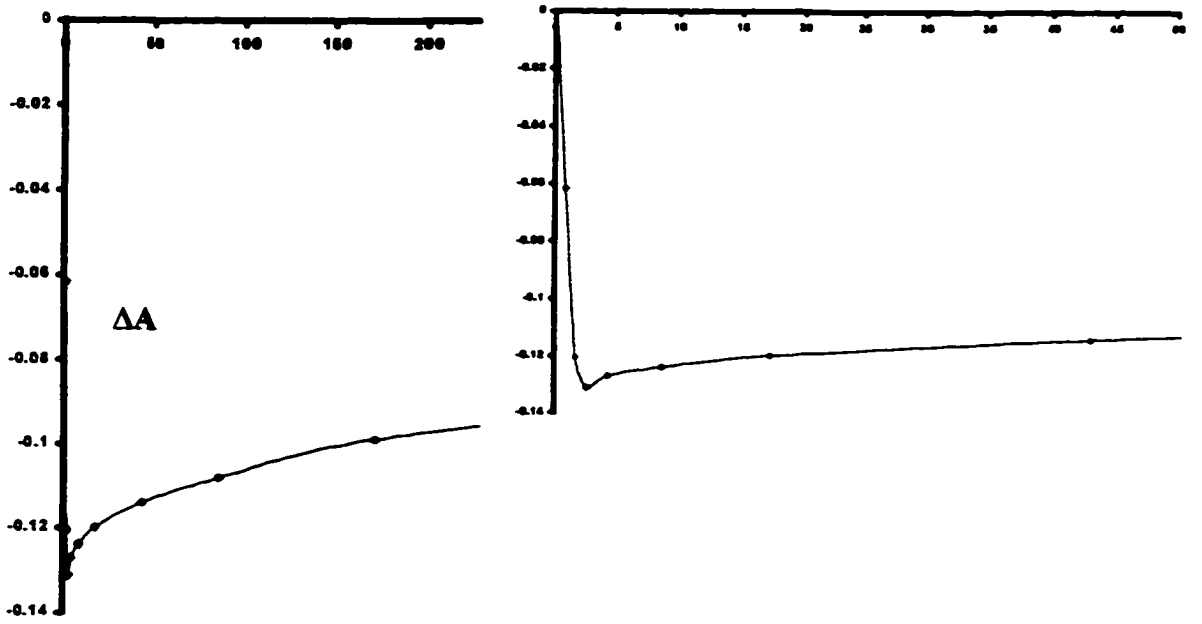
Figure A17.

Cpd. #4: OH OH $PyMe^+$ $PyMe^+$ titrated by CT-DNA



Wavenumbers (nm)

[DNA base pairs] (μM)



Part 3

Solid Phase Porphyrin Synthesis

Introduction

Although a solution phase synthesis of porphyrin libraries can afford large diverse libraries, there are several problems with this approach. These include the fact that the library is formed as a mixture and characterization of the library relies on statistical means that compare spectral fingerprints to a calculated or model spectra. Selection of new compounds from the library can present technical problems. Purification of the library is difficult: even if there are no side products, it still requires substantial time. Synthesis of libraries on a solid support addresses many of these problems.[65] To our best knowledge there is no report on the synthesis of porphyrins on solid supports which is suitable for preparing combinatorial libraries.[66] We propose the first route for solid phase porphyrin synthesis aimed in combinatorial synthesis, scheme 1.

The advantages of porphyrins synthesis on the solid supports are:

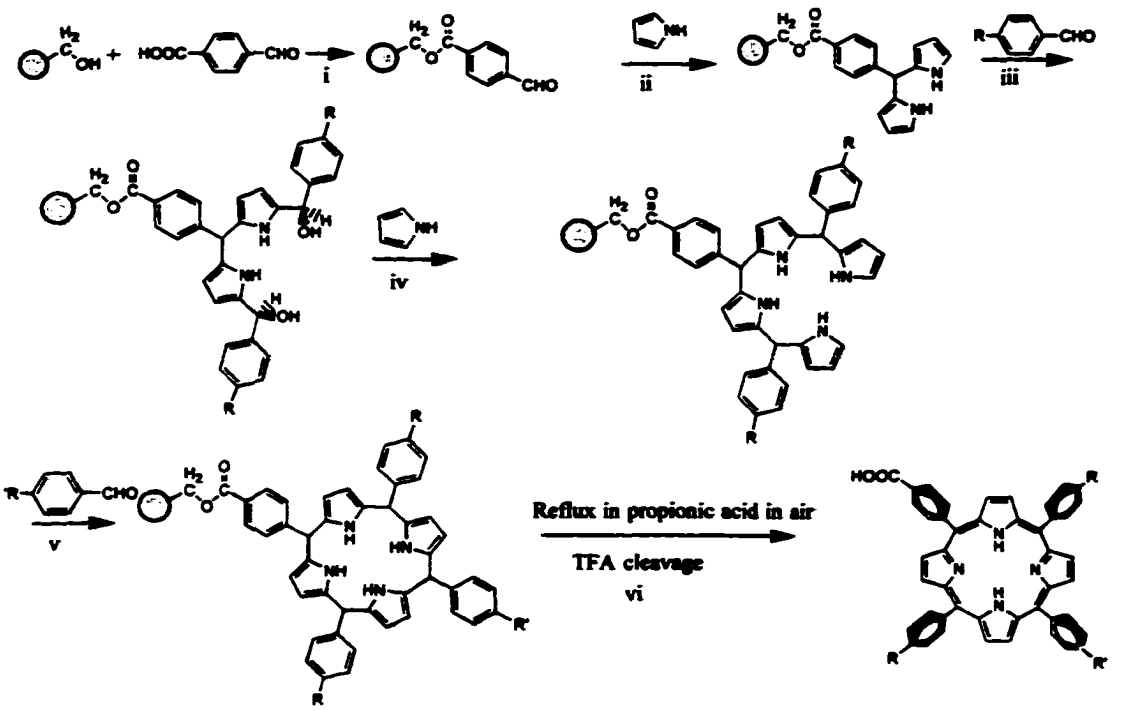
1: Building porphyrin libraries.

2: One bead one compound.

3: Easy work up process.

4: Direct synthesis of the ABCB pattern porphyrins.

5: An unexpected result is that this method can be used to form the *trans*- substituted ABAB pattern porphyrin in a reasonable yield and purity if non-swollen resin was used in the synthesis.

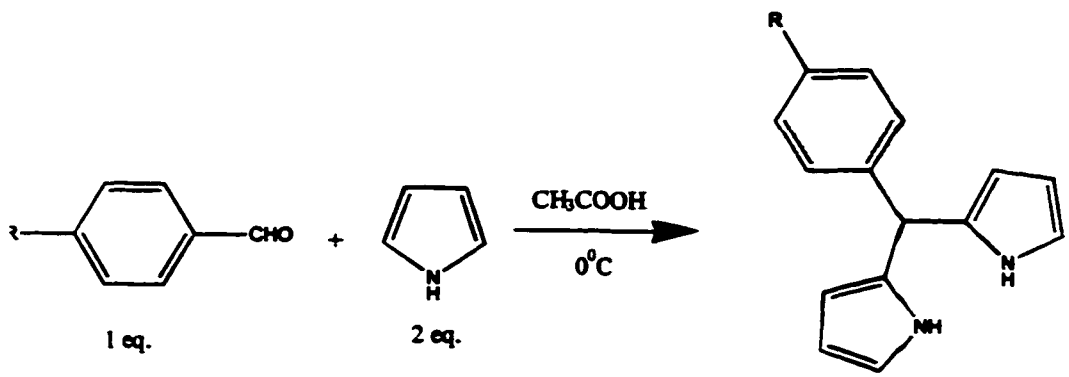


Scheme 1.

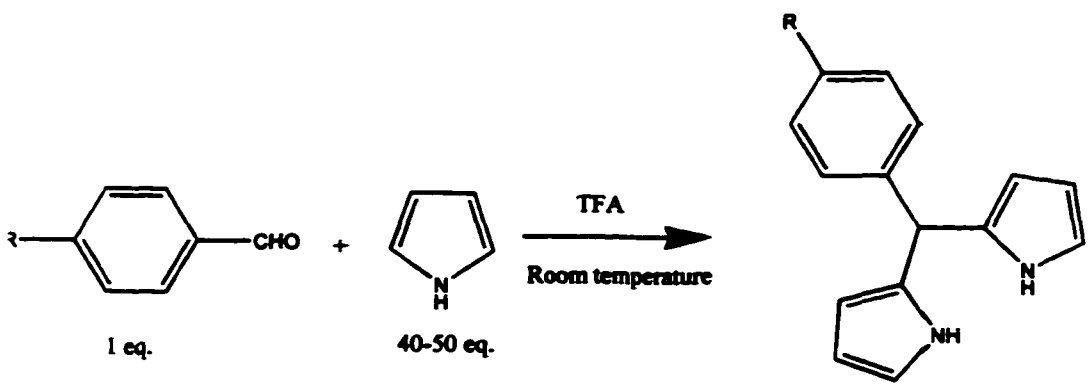
Most of current solid phase synthesis focuses on the liner addition of the fragments step by step. Porphyrins are a closed-ring system and are large molecules compared to the non-polypeptide target molecules in most solid phase synthesis, reported to date.[65] According to previous solution phase studies, the porphyrin precursor is a porphyrinogen, which is fromed in one reaction step. However porphyrin synthesis on a solid support may be different from the solution phase, scheme 1.

This solid phase synthesis is partly based on previous solution phase work on dipyrrolemethane synthesis.[67,68] In one reported method for the synthesis of dipyrrolemethane by S.J. Vigmond et al[67], scheme 2, the reaction was done in acetic acid.

Another report by C.H. Lee and J.S. Lindsey[68] used a large excess pyrrole as the solvent and TFA was used to catalyze the reaction, scheme 3. To our knowledge there are no reports on the formation and reaction of dipyrrolemethane on solid supports.



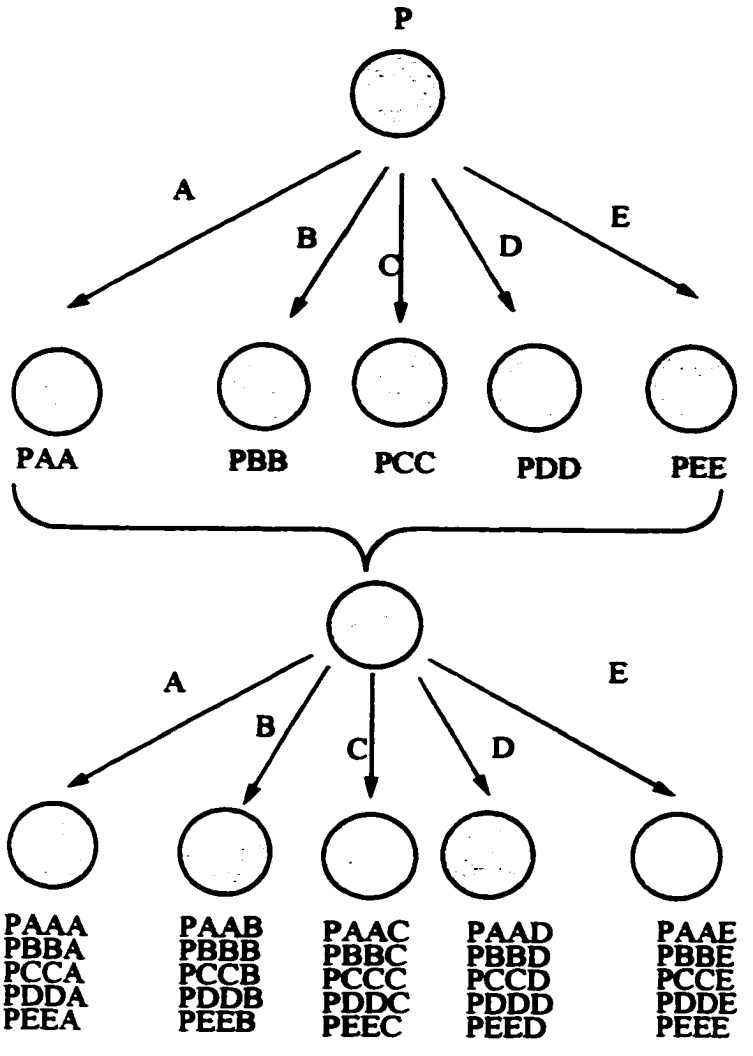
Scheme 2.



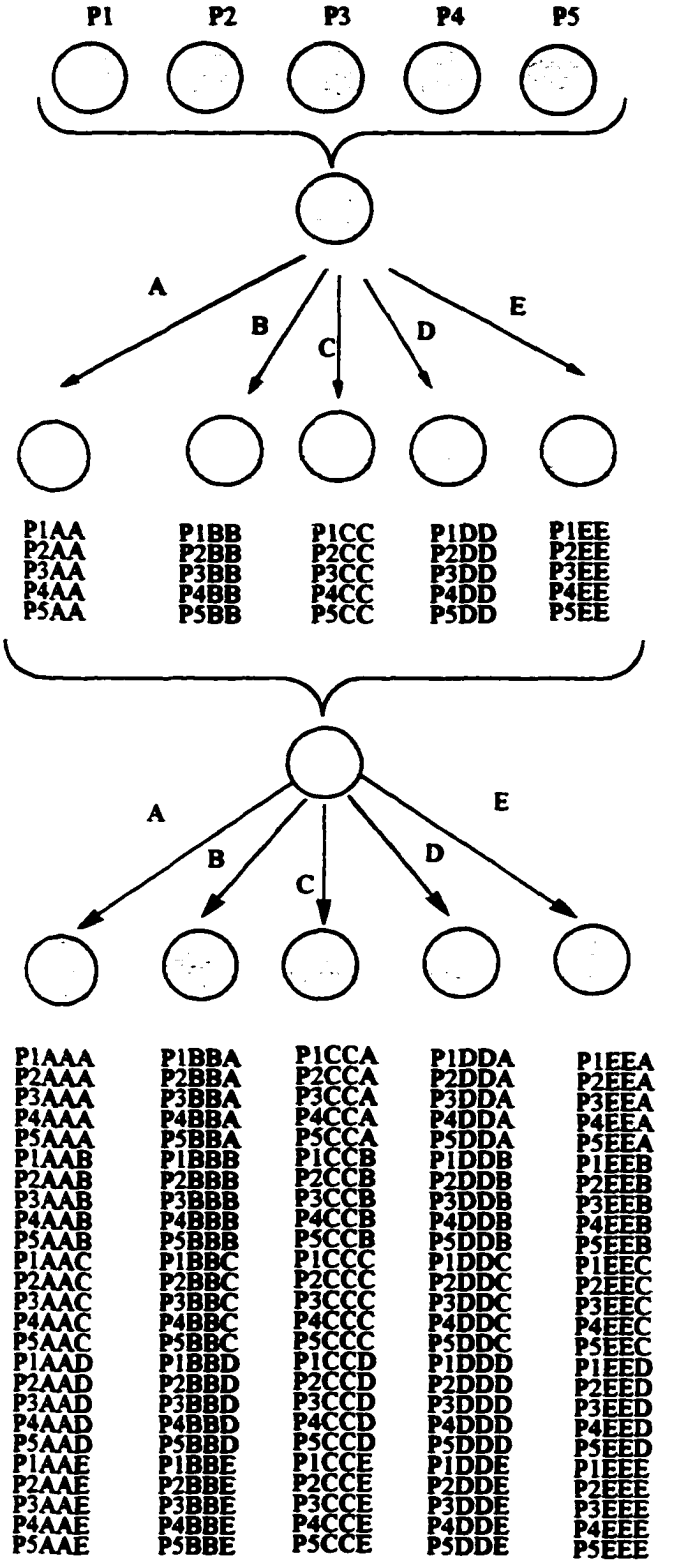
Scheme 3.

The potential development of this solid phase strategy into a solid phase porphyrin library is shown in scheme 4. The library diversity based on scheme 4 is very limited compared to solution phase synthesis. For example, including the pyrrole addition and the linkage steps, only 25 porphyrins are formed in 13 reactions using 5 additional aldehydes, and 16 porphyrins are formed in 11 reactions using 4 additional aldehydes. In the solution phase porphyrin library synthesis, 5 aldehydes generate a library of 120 porphyrins and 4 aldehydes generate a library of 55 porphyrins. In principle, If m additional aldehydes are used in this synthesis, m^2 compounds can be generated through a 2m+3 reactions, including the two pyrrole additions and the aldehyde linkage steps. In solution phase porphyrin library synthesis, the number of compounds can be generated is $C^1_m + 4C^2_m + 6C^3_m + 3C^4_m$. This limited diversity problem can be overcome by linking different functional groups to the resin beads, scheme 5. The library diversity based on scheme 5 is much larger than the library diversity based on scheme 4. If there are n aldehydes used to link to the polymer and m different aldehydes(m≠n) were used in the subsequent reactions, n x m^2 porphyrins can be obtained through 2m+n+2 reactions. For example, when n=5, m=5, 125 porphyrins can be obtained through 17 steps.

At present the goal is to develop the solid phase chemistry of porphyrin synthesis, not to generate porphyrin libraries.



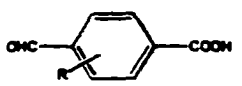
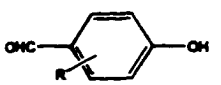
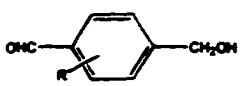
Scheme 4. Solid phase library diversity where P is the aldehyde linked to the polymer and A, B, C, D, E are the aldehydes employed in the combinatorial synthesis.



Scheme 5. Solid phase library diversity where P1, P2, P3, P4, P5 are aldehydes linked to the polymer and A, B, C, D, E are aldehydes employed in the combinatorial synthesis.

Besides the carboxylic acids, phenols and alcohols can be linked to the Wang resin with a high yield and are easy to cleave, table 1. There are lots of commercially available carboxylic acids, phenols and alcohols bearing different substituent groups that can be used to linked to the Wang resin.

Table 1. Wang resin coupling

R	Reference	Comments
	69	Condensation is easy and cleavage is easy
	70	Condensation uses Mitsunobu reaction condition and cleavage is easy
	71	Condensation is easy and cleavage is easy

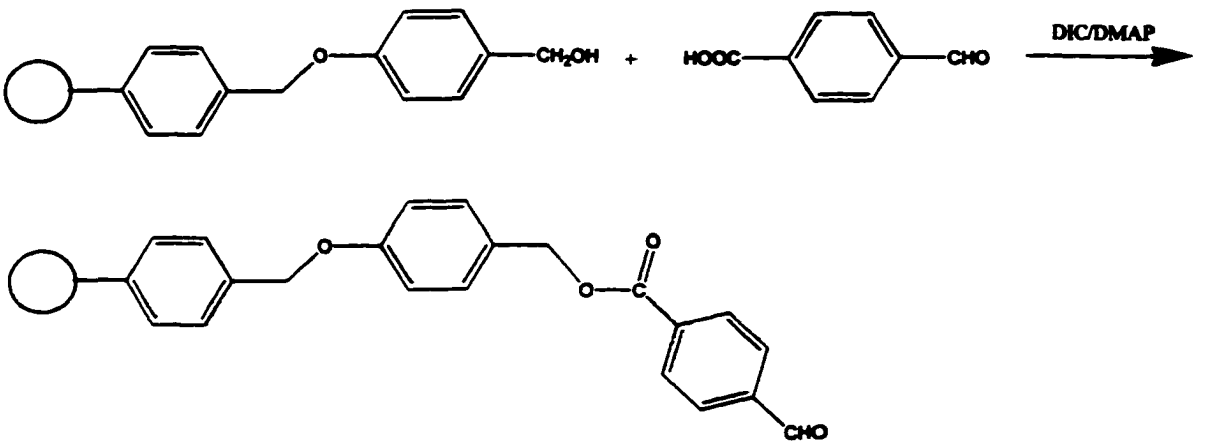
Both solution phase porphyrin library synthesis and solid phase porphyrin library synthesis have advantages and disadvantages, table 2. The combination of these two methods may be the best choice. Herein we report our recent progress on solid phase porphyrin synthesis as a complementary strategy to solution phase porphyrin synthesis.

Table 2. Comparison of the solid phase porphyrin library synthesis and the solution phase library synthesis

	Solid Phase	Solution Phase
Yield	Poor	Fair
Diversity	Fair	Good
Purification	Easy	Difficult
Resolution of 1 compound	Easy	Difficult
Reaction steps	Depend on the diversity	One pot synthesis

Results and discussion

Link the aldehyde to the resin



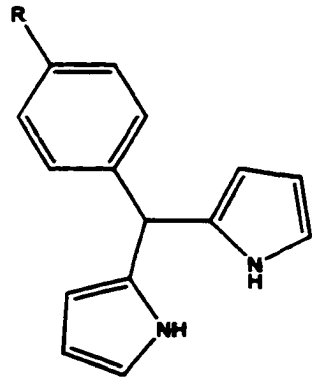
Scheme 5.

4-carboxybenzaldehyde was linked to the Wang resin by an acetate linkage using DIC and a catalytic amount DMAP as the activating agents, scheme 5. The resin was first swollen in CH_2Cl_2, then a mixture of 4-carboxybenzaldehyde, DIC and DMAP in CH_2Cl_2 was added to the slurry. The slurry was stirred overnight for a complete loading. The loading of the resin was estimated by cleavage of aldehyde from a certain amount of polymer then weighing the cleaved aldehyde. A loading of ~0.33mmol/g was obtained for this procedure.

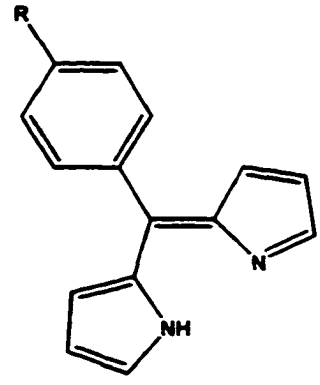
Synthesis 5, 15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin without swelling the resin

The Lindsey conditions in scheme 3 were adapted for all subsequent reactions (addition of pyrrole and aldehydes). The second reaction was to form the dipyrrolemathane on the polymer. By using a large excess pyrrole and TFA as a catalyst, the reaction was

complete in 30 minutes. The polymer surface remained colorless. After the polymer was washed, benzaldehyde was added to react with the dippyrolemathane. Once the polymer contacted the aldehyde, even without adding of TFA, the polymer surface began to change to purple. Since there are no reports on the reaction of dipyrrolemathane with a large excess aldehyde in the solution phase, we cannot draw analogies to solution phase chemistry. After adding TFA, the polymer surface changed to a deeper purple. This color may be due to the formation of the dipyrrolemethenes, or the porphyrin, see below.



Dipyrolemethane



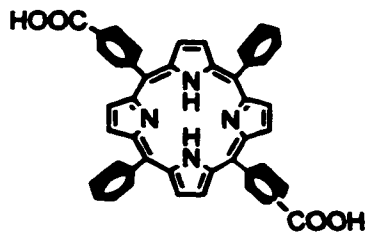
Dipyrrolemethene

In step iv and step v there are no further visual changes. The polymer surface remained a dark purple. As in scheme 1, after the ring closing reaction was finished, the polymer was added to propoinic acid and refluxed for an hour, to assure oxidation of the porphyrinogen or other intermediates to the porphyrin. Then the porphyrin and other products were cleaved from the polymer by using a solution of 50% TFA and 50% $CHCl_3$ (figure A1).

From the ^1H NMR (figure 1) and the ESI-Mass (figure 2) spectrum, we found that the main product is 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin, table 3. The yield based on the aldehyde loading was very low, ~ 4.5%, which is ~ 10mg porphyrin/g resin.

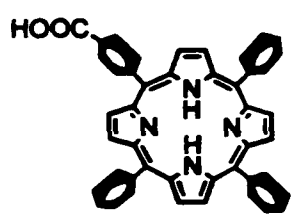
The purity of porphyrin in the crude mixture was ~37% based on the ^{1}H NMR of the crude cleavage product. The yields of the by-products, including our target product 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin are extremely low. The relative abundance in the ESI-MS are all lower than 5% compared to the abundance of 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin of 100%. This is confirmed by TLC, ^{1}H NMR. Considering the route we designed, this result is very surprising.

Major product:



100%

Other products:



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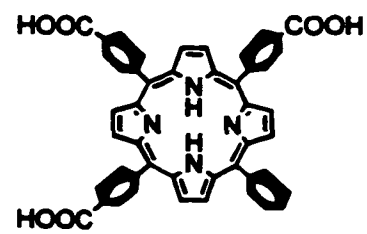

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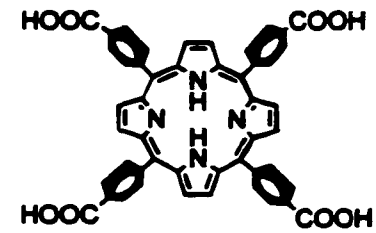


<5%

Table 3. Products and relative abundance from the ESI-MS of the crude reaction mixture, a non-swollen resin was used to form the porphyrin.

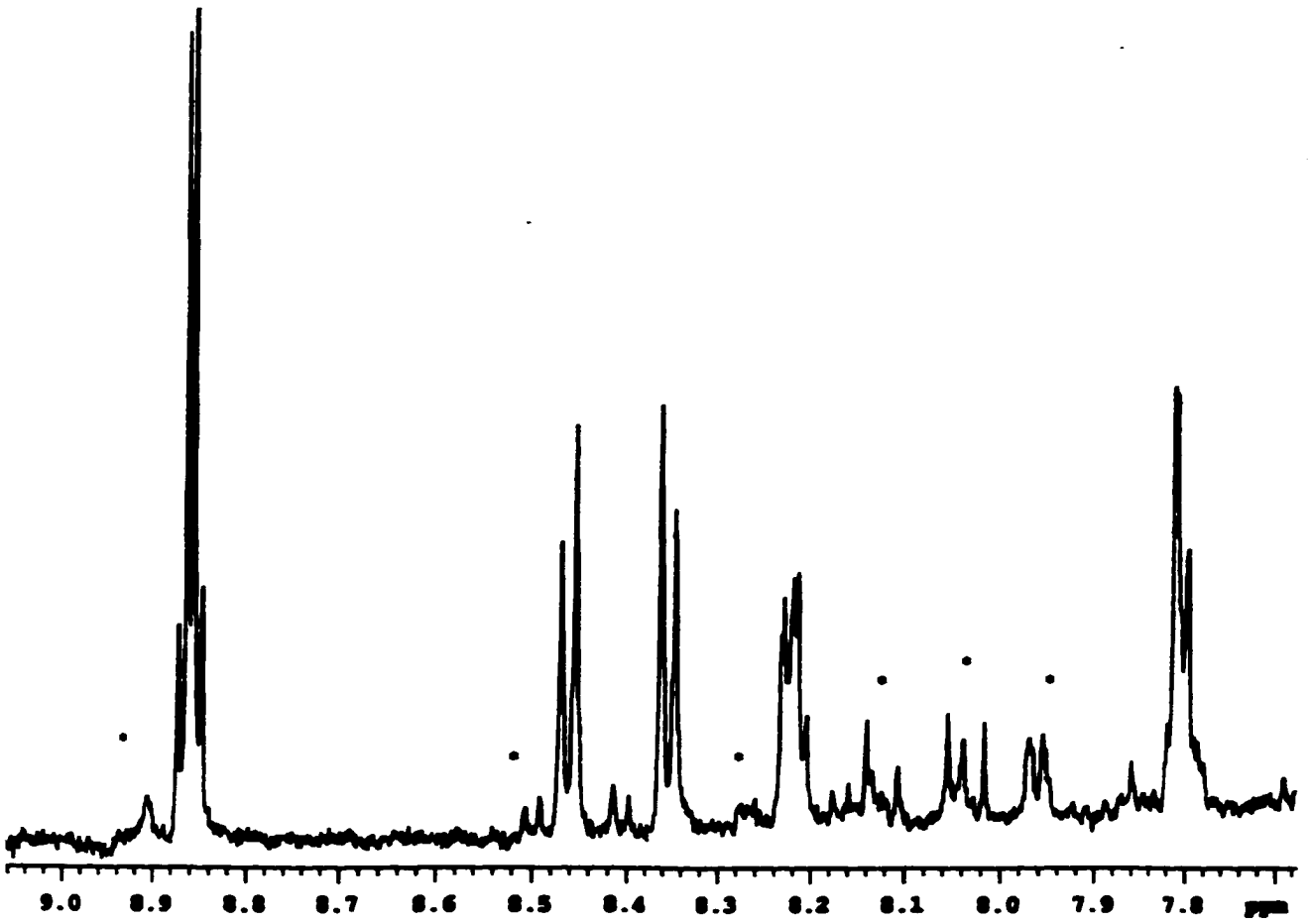


Figure 1. ^{1}H NMR (500MHz, Acetone-d_6) of 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin (*: the traces of other porphyrins). The pyrrole resonance at ~8.86ppm are diagnostic for the 5,15- substituted porphyrin, where a doublet of doublets is expected and observed. The 5,10- substituted porphyrin results in two singlets and a doublet of doublets for the pyrrole resonance.

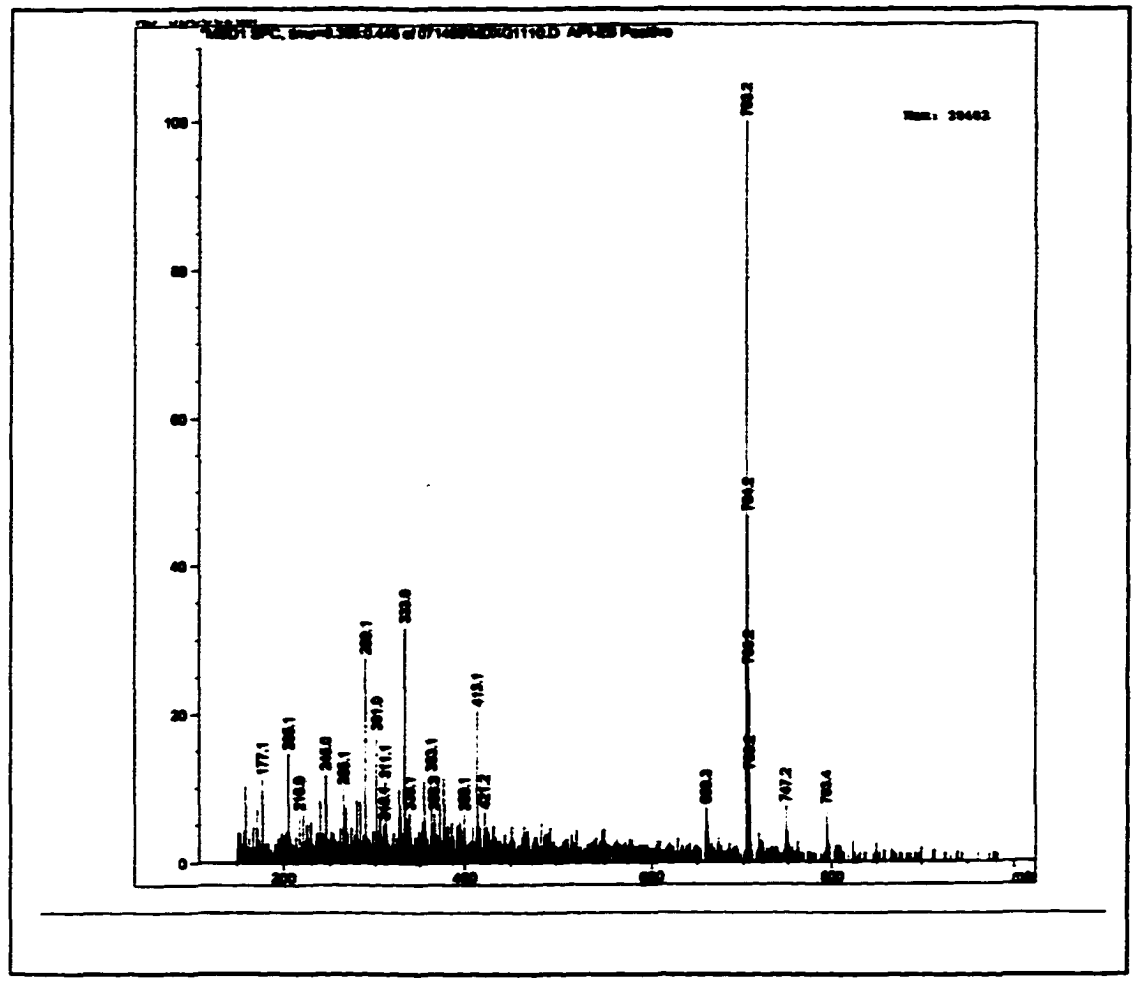
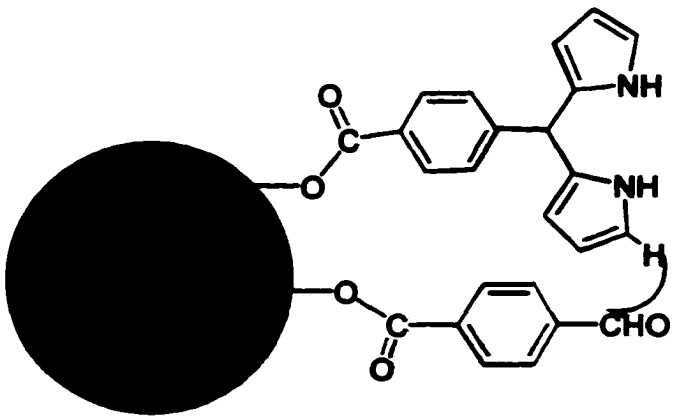


Figure 2. ESI-MS of the crude reaction mixture of a porphyrin synthesis on non-swollen Wang resin shown that the 5,15-bis(4- carboxyphenyl)-10,20-diphenylporphyrin is the major product.



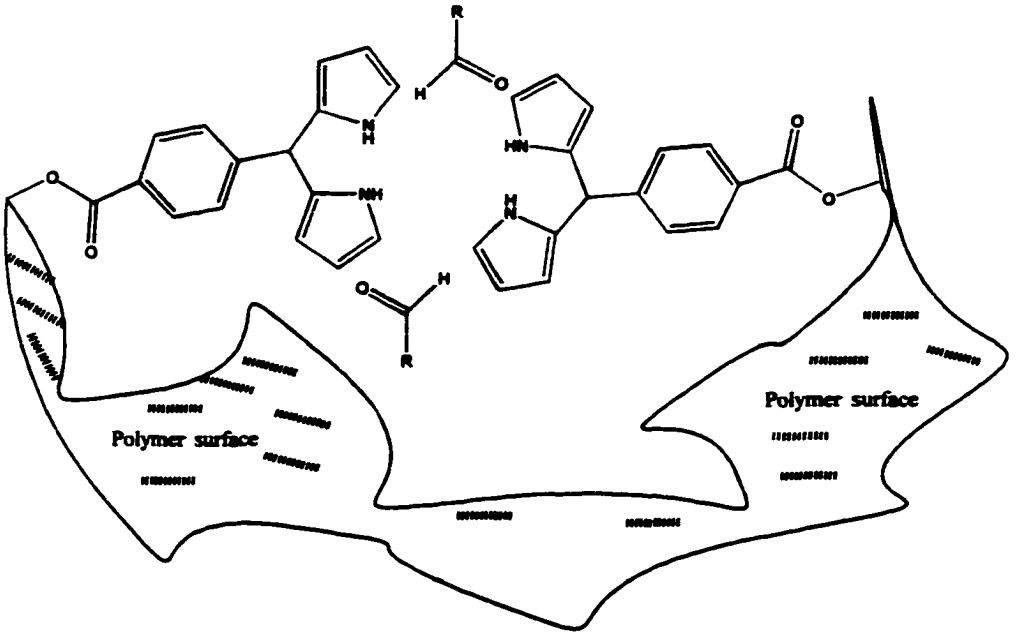
Scheme 6.

Due to the very high density of reactive sites on the Wang resin, neighboring aldehydes can react with dipyrrolemethanes formed in step ii of scheme 1. If this were the case, a 5,10- substituted porphyrin would result (scheme 6) and no porphyrin would be formed after the step iii. Since we observed none of the "*cis*" product, another mechanism must be considered. The only way the 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin can formed is that two intermediate dipyrrolemethanes react as shown in scheme 7. In this latter mechanism, the formation of porphyrinegon and porphyrin would then be observed upon the first addition of the benzaldehyde- step iii in scheme 1. This is exactly what we observed in that the color began to change to the characteristic porphyrin purple upon the first benzaldehyde addition.

We confirmed the above analysis by doing only reactions i, ii and iii in scheme 1 on non-swollen resin, followed by the high temperature aerobic oxidation. A mixture of porphyrins is formed with a total yield of 16% by the standard UV-Visible analysis,

which is 41mg porphyrin/g resin, table 4. The reason for the formation of the mixture is not clear so far, but the presence of the 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin indicate scheme 7 is correct. The reason for the domination of the 5,15-bis(4-carboxyphenyl)-10,20-diphenylporphyrin in the previous synthesis is not clear too, but may be due to the thermodynamic stability of the *trans* (2+2) porphyrinegons compared to the 1+3, and the *cis* (2+2) porphyrinegons. The lower symmetry "*cis*" species is usually formed in solution phase synthesis for entropic reasons.[‡] So this must result from forces exerted by the resin. After porphyrinegon formation in step iii, further pyrrole and aldehyde addition may open the ring of the porphyrinegons to form polymers. This is supported by the decreased yield of the previous synthesis compared to this.

Several other aldehydes were used in the synthesis by doing only reactions i, ii and iii in scheme 1 on non-swollen resin, followed by the high temperature aerobic oxidation. A porphyrin mixture was obtained in each case. The yields of the porphyrin mixtures are shown in table 5.



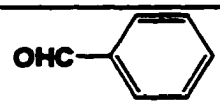
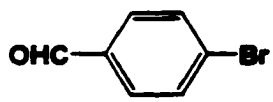
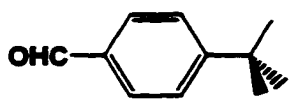
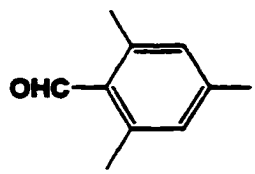
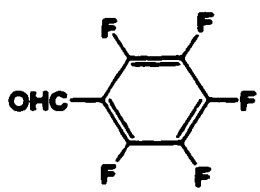
Scheme 7

Control experiments showed that 5μL TFA in these 1mL reactions did not result in the cleavage of the aldehydes from the resin, under similar conditions (15 minutes at room temperature).

Table 4. Summary of the solid phase porphyrin synthesis.

Synthetic procedure in scheme 1	State of resin	Results (R_1 to R_4, A= 4- carboxyphenyl B= phenyl C= 4-tolyl)	Characterization
Step i to vi	Non swollen	ABAB: >90%	TLC, ^{1}H NMR and ESI-MS
Step i, ii , iii followed by oxidation and cleavage	Non swollen	ABBB: 12% ABAB: 36% AABB: 36% AAAB: 16%	TLC, ^{1}H NMR and ESI-MS
Step i to vi	Swollen	AABB + ABAB: 43% AABC + ABAC: 24% AAAB: 11% AACC + ACAC: 22%	TLC and ESI-MS
Step i, ii , iii followed by oxidation and cleavage	Swollen	--- ---	--- ---

Table 5 Yield as a porphyrin mixture when using various aldehydes

Aldehyde	Yields(%)
	16
	9
	15
	10
	12
	6
	10
	0.5

Synthesis with swelling the resin and using two different aldehydes

In order to further examine the reaction mechanism and increase the yield, the resin was swollen with CH_2Cl_2 before each reaction step and 4-tolualdehyde was used instead of the benzaldehyde in step v according to scheme 1.

The purity of the crude cleaved product was relatively low compared to the previous synthesis. The formation of the porphyrins was about 10mg/g determined by a standard UV-Vis. analysis. The ESI-MS showed that there were at least four porphyrins in the crude reaction mixture, table 6:

a: 5,10-bis(4-carboxylphenyl)-15,20-diphenylporphyrin (or its isomer)

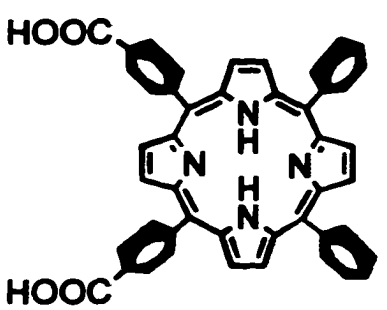
b: 5,10-bis(4-carboxylphenyl)-15-(4-tolyl)-20-phenyl-porphyrin(or its isomer)

c: 5,10,15-tris(4-carboxylphenyl)-20-phenyl-porphyrin

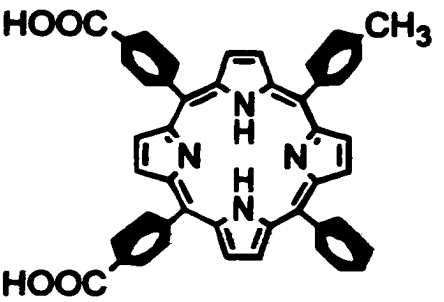
d: 5,10-bis(4-carboxylphenyl)-15,20-(4-tolyl)-porphyrin (or its isomer).

The separation of the various products was not finished and no further ^1H NMR experiment has been done to assign the porphyrin isomers. Compared to the non-swollen resin synthesis, this result indicates the amount of swelling of the resin during the reactions plays a crucial role in the product distribution. In order to make the porphyrin on the resin, a much lower loading for the resin is probably necessary.

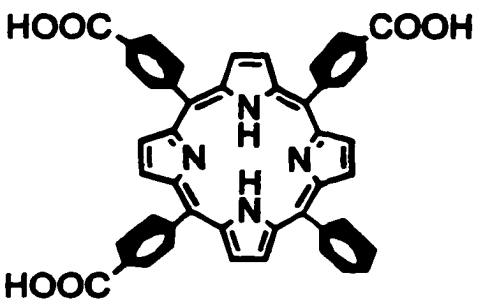
Due to the low yields and the product scrambling of this solid phase porphyrin synthesis, a better way to built a solid phase porphyrin library may be to link a tetra-substituted porphyrins, such as the tetrakis-(4-carboxylphenyl)porphyrin to a low loading Wang resin first, followed by the modification of the substituted groups using known solid phase chemistry. In this way, a higher yield and larger diversity porphyrin library can be made on a solid support.



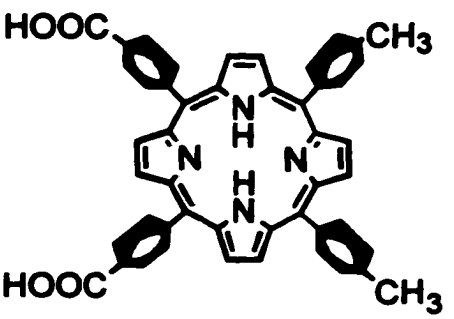
(or its isomer)
27%



(or its isomer)
15%



7%



(or its isomer)
14%

Table 6. Products and relative abundance from the ESI-MS of the crude reaction mixture, when a swollen resin was used to form the porphyrin.

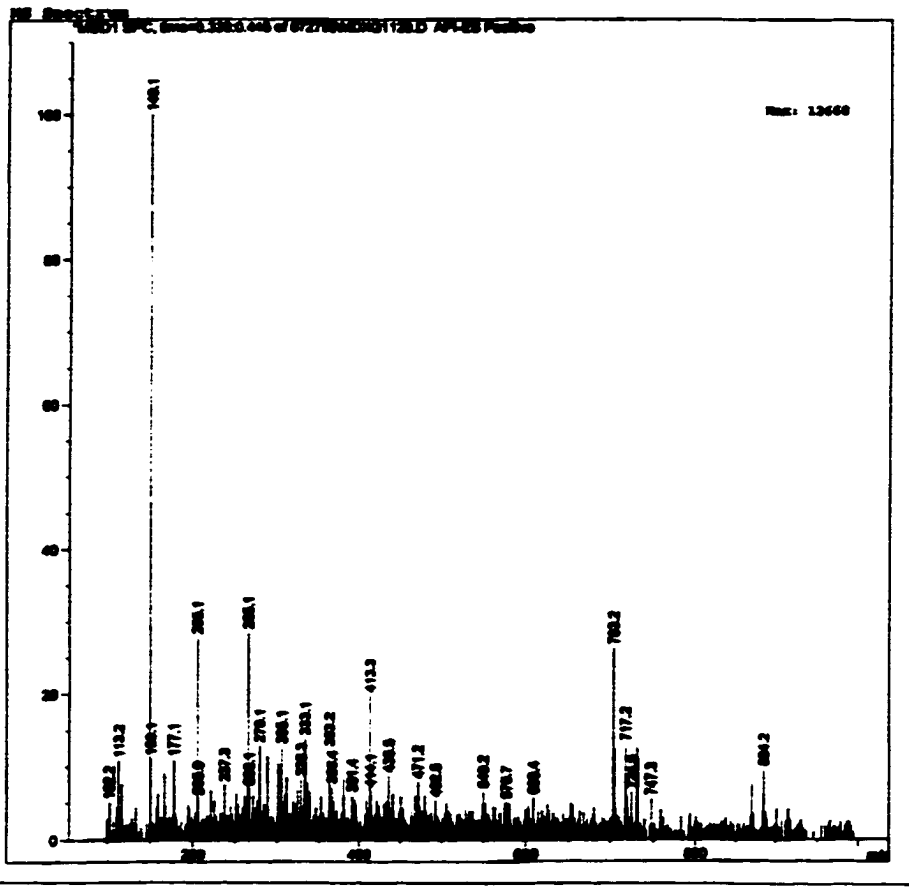


Figure 3. ESI-MS of the crude reaction mixture of a synthesis using swollen Wang resin and mixed aldehydes. (R_1 to R_4, A= 4- carboxyphenyl, B= phenyl, C= 4-tolyl)
AABB+ABAB: 703, AABC+ACAB: 717, AACC+ACAC: 731, AAAB: 747.

‡: The product ratio of a 1:1 mixture of 2 aldehydes with equal reactivity has the following composition: A_4(1); A_3B(4); AABB(4); ABAB(2); AB_3(4); B_4(1). Note that the "*cis*" AABB is twice as abundant as the other "*trans*" ABAB isomer.

Conclusion:

1: 5,15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin has been successful synthesized by solid phase with reasonable selectivity, but with a low yield of ~ 4.5%, which is 10mg porphyrin/g resin. A non-swollen Wang resin was employed as the solid support.

2: This solid phase synthesis opens a new way for the direct synthesis of *trans*-substituted ABAB patterned porphyrins in reasonable purity in 4 steps. The previous methods for synthesizing *trans*-substituted ABAB patterned porphyrins always involved muti-column separation of a porphyrin mixture or muti-step reactions which costs much more labor and time.[18-20]

3: Further development of this synthesis method may lead to the direct synthesis of the *trans*- substituted ABAC patterned porphyrins.

4: The amount of resin swelling plays a crucial role in the product distribution and the yield. A low loading and higher surface area Wang resin is required for the synthesis of the target porphyrins in scheme 1.

Experimental

General experimental: Aldehydes were run over a short pipet columns of basic alumina before used. Pyrrole was purified by a short pipet silica gel column. Wang resin was bought from Advanced Chemtech (loading 1.1mmol/g, 100-200 mesh). All other reagents were used as received from Aldrich. ^{1}H- NMR were obtained on a 300MHz GE, 400 MHz Varian or a 500MHz Varian Unity Plus spectrometer. UV-Vis absorption spectra were obtained on a Carey 1 spectrophotometer and taken in $CHCl_3$ if not specified. ESI-MS were obtained on a Hewlett-Packard HP 1100 LC/MSD spectrophotometer.

General apparatus for all solid phase reactions: The reactions were carried out in a 35mL round bottom flask fitted with a silicon rubber septum. The nitrogen purging was accomplished by a needle inserted into the solution through the septum and a venting needle. The flask contained a stir bar.

Linkage 4- carboxybenzaldehyde to Wang resin:

10 g Wang resin (0.011 eq) was swollen in 80mL CH_2Cl_2 for 10 minutes and a mixture of 4-carboxybenzaldehyde (10 g, 0.066mol), DIC (1,3-dicyclohexylcarbodiimide) (6.8 g, 0.033mol), catalytic amount of DMAP and 20mL CH_2Cl_2 was added. This mixture was stirred for 24 hours and then washed with THF (2 x 100mL), methanol (2 x 100mL), chloroform (2 x 100mL), hexane (2 x 100mL). The resin was dried under vacuum.

Loading determination:

0.5g loaded Wang resin was added to a mixture of 5mL TFA and 5mL $CHCl_3$. The mixture was refluxed for 30minutes. The resin was filtered and the filtrate was transferred to a 3mL round bottom flask. The solvent was removed under vacuum. The residue was

dried in a oven and had a weight of 20mg. Thus the loading of 4-carboxybenzaldehyde on the Wang resin was calculated to be ~ 1.1mmol/g.

Synthesis 5,15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin without swelling the resin

0.1g loaded Wang resin was suspended in 1 mL pyrrole, the suspension was bubbled with N_2 for 10 minutes and then 5μL TFA was injected into the system. The system was stirred at room temperature for 15 minutes. Then the polymer was filtered and washed by a standard wash procedure (75mL acetone, 50mL methanol, 50mL ethyl acetate). After this reaction, the polymer still looks white. The polymer was re-suspended in 1mL benzaldehyde and N_2 bubbled for 10 minutes. Once the polymer contacted the aldehyde, its surface started to change to purple and this continued for the rest of the reaction. 5μL TFA was injected to the system after N_2 purging was stopped and stirred at room temperature for 15 minutes. After this step, the surface of the polymer turned its color to a deeper purple. Then the standard filter and wash procedure was applied. The polymer was re-suspended in 1mL pyrrole and N_2 bubbled through for another 10 minutes. Another 5μL TFA was added into the system followed by 15 minutes stirring at room temperature. No further visual changes of the color of the polymer surface were observed. The polymer was filtered and washed again and then re-suspended in 1mL benzaldehyde. After 10 minutes bubbling with N_2, 5μL TFA was injected to the reaction system and then followed by 15 minutes stirring at room temperature. The polymer was filtered and washed, then refluxed in 5mL propioinc acid for one hour. The polymer was once again filtered, washed and cleaved by a mixture of 2mL TFA and 2mL chloroform. The color of the solution changed to green very quickly, indicating the cleavage of the

free base porphyrin from the resin and the formation of the porphyrin dication in the solution. The yield (4.5%, based on the initial loading) was decided by the absorption at 438nm of the porphyrin dication solution in $CHCl_3$ (about ~80% of the Soret absorption of the free base), assume $\varepsilon = 3.42 \times 10^5\ M^{-1}cm^{-1}$. The resin was filtered and the filtrate was transferred to a flask. Solvent was removed. The crude product was purified by a flash silica gel column (CH_3OH: EtOAc: $CHCl_3$, 1:2:2), 5,15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin was obtained.

^1H NMR (500MHz, Acetone-d_6), figure 1: δ -2.75(b, 2H, pyrrole N-H), 7.8(m, 6H, *m*, *p*-phenyl), 8.21(m, 4H, *o*-phenyl), 8.34(d, 4H, J=8.0Hz, *m*-carboxyphenyl), 8.45(d, 4H, 8.0Hz, *o*-carboxyphenyl), 8.85(d, 4H, J=5.0Hz, β-pyrrole), 8.87(d, 4H, J=5.0Hz, β-pyrrole). ESI-MS [(m+H)/z^+, % relative intensity], figure 2: 703, 100%

Synthesis with swelling the resin and using two different aldehydes

0.1g loaded Wang resin was swollen with 1mL CH_2Cl_2 and then suspended in 1mL pyrrole, the suspension was bubbled with N_2 for 10 minutes and then 5μL TFA was injected to the system. The system was stirred at room temperature for 30 minutes. Then the polymer was filtered and washed by a standard wash procedure (75mL acetone, 50mL methanol, 50mL ethyl acetate). After this reaction, the polymer still looked white. The polymer was swollen by 1mL CH_2Cl_2 again and re-suspended in 1mL benzaldehyde. The mixture was bubbled with N_2 for 10 minutes. Once the polymer contacted the aldehyde, the surface of the polymer started to change to purple. After stopping the N_2 purge, the mixture was stirred at room temperature for 2 hours without adding TFA. After this step, the surface of the polymer turned to a deeper purple. Then the standard filter and wash procedure was applied. The polymer was re-swollen, re-suspended in 1mL pyrrole, and

N_2 bubbled through for another 10 minutes. Another 5μL TFA was added into the system followed by overnight stirring at room temperature. No further visual changes of the color of the polymer surface were observed. The polymer was filtered, washed, re-swollen and re-suspended in 1mL tolualdehyde. After 10 minutes purging with N_2, 5μL TFA was injected to the reaction system and then followed by 15 minutes stirring at room temperature. The polymer was filtered and washed, then refluxed in 5mL propioinc acid for one hour. The polymer was filtered and washed and then cleaved by a mixture of 2mL TFA and 2mL chloroform. The color of the solution changed to green very quickly, indicating the cleavage of the free base porphyrin from the resin and the formation of the porphyrin dication in the solution. The resin was filtered, and the filtrate was transferred to a flask, and the solvent was removed. The resulting residue was not purified. The crude product was examined by TLC, ^{1}H NMR and ESI-MS.

ESI-MS [$(m+H)/z^+$, % relative intensity] 703, 27%; 717, 15%; 731, 14%; 747, 5%. (figure 3).

Appendix

Figure A1: Crude reaction mixture of a synthesis of 5,15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin using non-swollen Wang resin directly after the cleavage step in $CHCl_3$. The TFA in the solution protonates the porphyrin, causing a red-shift of the Soret band to 438nm and the increase in the Q band intensity at 650nm.

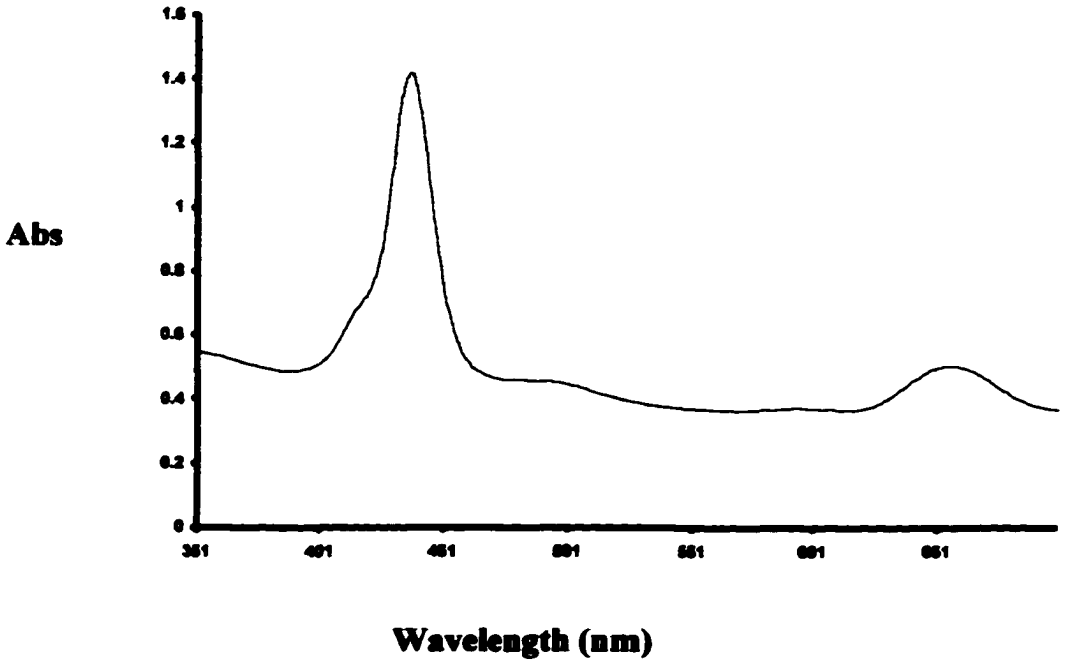
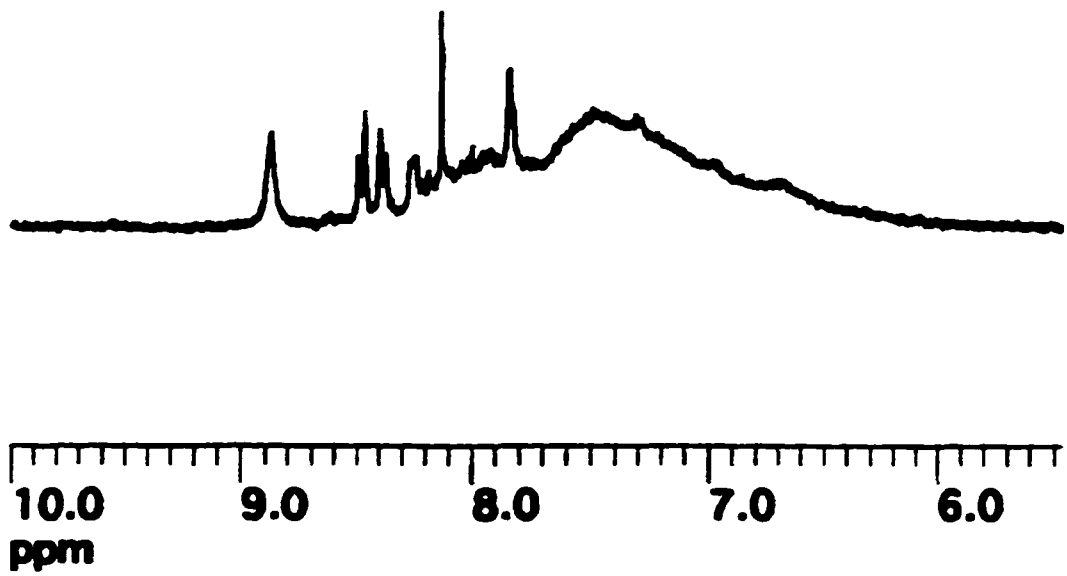


Figure A2: ^{1}H NMR (300MHz, $CDCl_3$) of the crude reaction mixture of 5,15-bis(4-carboxyphenyl)-10,20-diphenyl-porphyrin using non-swollen Wang resin.

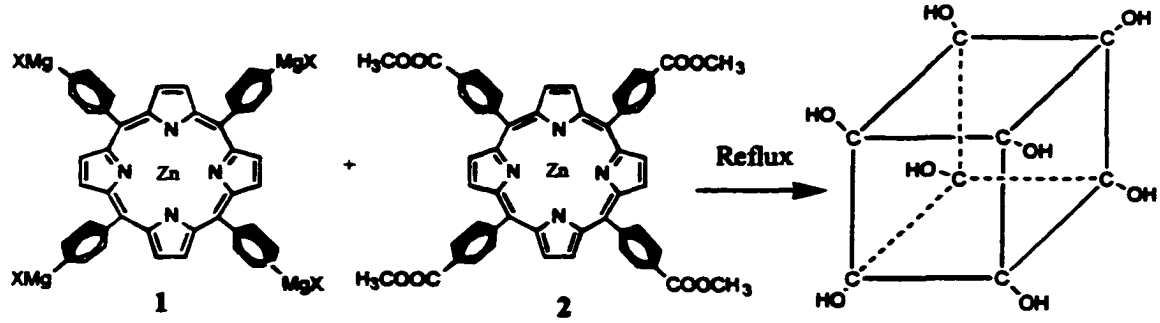


Part 4

Synthesis of poly-Grignard of porphyrins: as a strategy towards synthesis of a porphyrin cube?

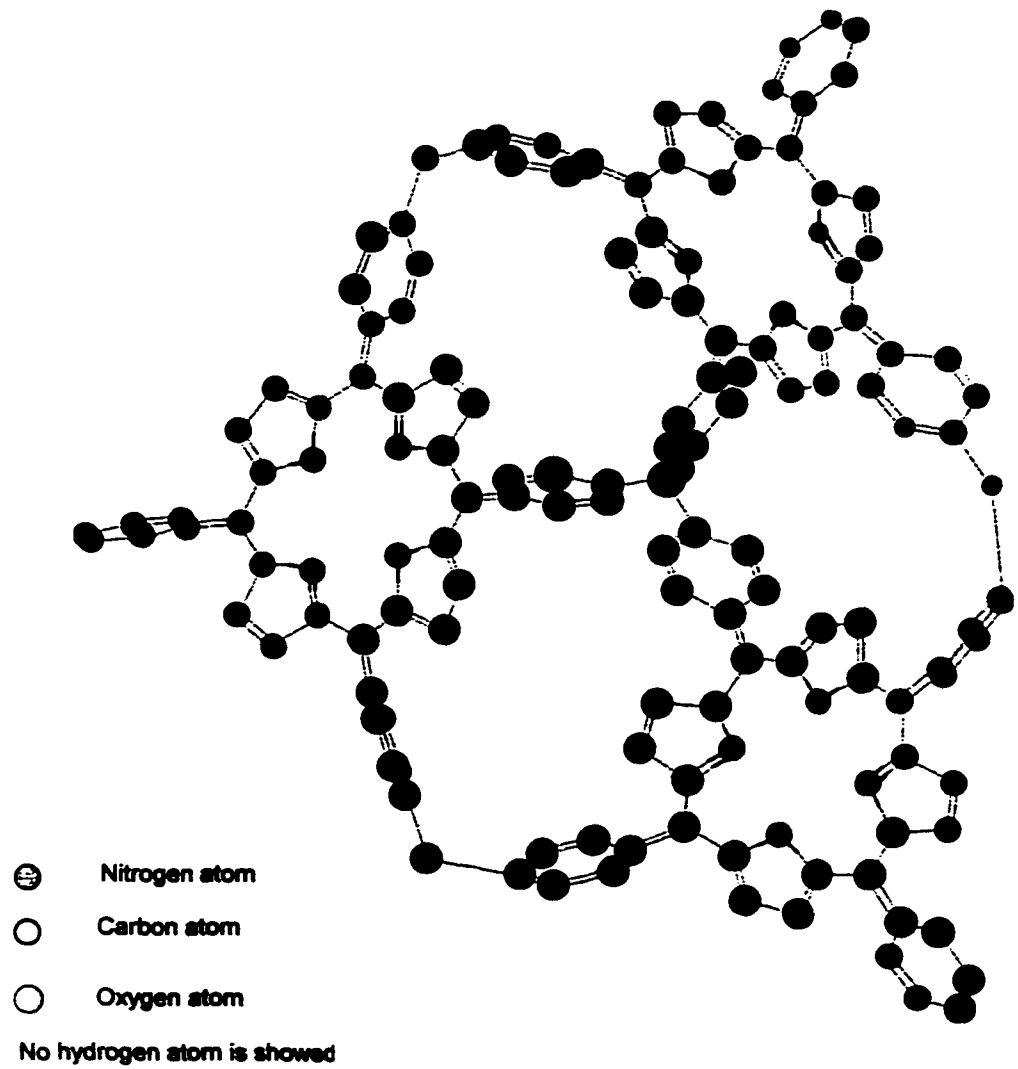
Introduction

Electronic energy transfer in and between molecules is a very interesting phenomenon in today's chemistry. Linked porphyrin systems have been widely used as a model system for the study of this important phenomenon. These model systems ranged from covalently linked porphyrin arrays to hydrogen bonds linked porphyrins.[3,72-80] The Grignard reaction is an important method for the formation of C-C bonds. The high versatility of the Grignard reactions makes it an attractive method for the formation of linked porphyrin system. Grignard reagents have been used to react with porphyrins in privious studies.[81-83] To our knowledge there is no report on the direct formation of a



Scheme 1.

Grignard reagents on the para- position of porphyrin's 5,10,15,20- substitutents. Herein we describe our synthetic efforts toward the formation of a porphyrin tetra-Grignard reagent and its use toward the synthesis of our target molecule – a porphyrin cube that is like a huge cubane, scheme 1. A corner view of the porphyrin cube is showed in scheme 2.

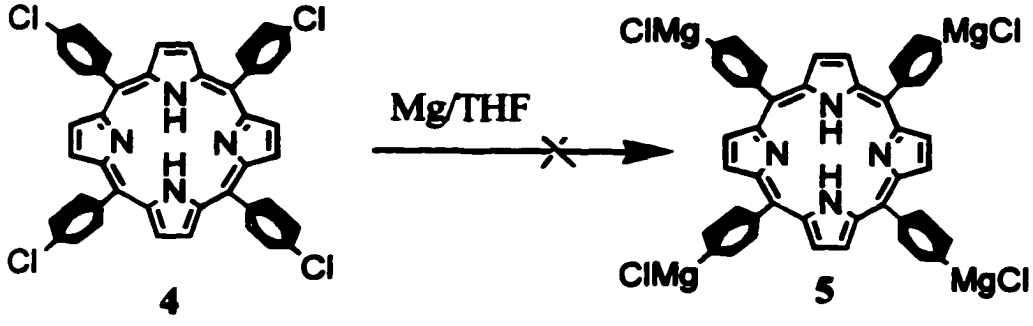


Scheme 2.

Results and discussion

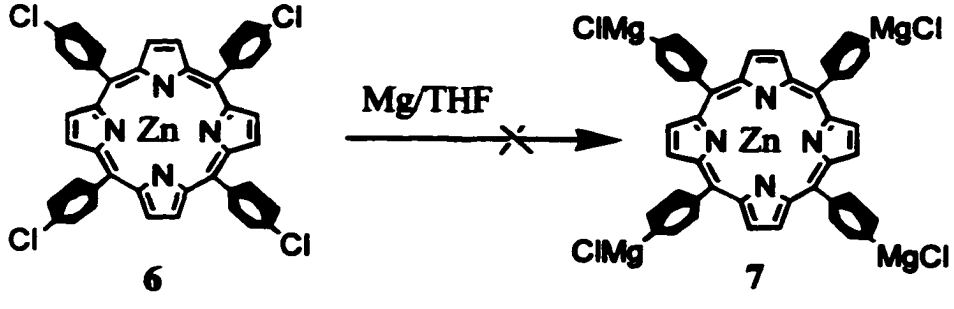
Preparation of the tetra-Grignard reagents

Compound 4 was first used to try to synthesize the tetra-Grignard compound 5 in THF, scheme 3. The reaction was initiated by adding a few drops of $BrCH_2CH_2Br$. Compound 5 was not formed in detectable amount either by reacting at room temperature or reflux at 160^0C. Notice that compound 4 is a free base porphyrin and the inside pyrrole N-H protons are reasonably acetic, which could interfere with the formation of the Grignard. Thus the zinc derivatives compound 6 was made by standard procedures to avoid this complication. However, compound 7 could not be made in detectable amounts, and only the starting material was recovered, scheme 4.



4 5

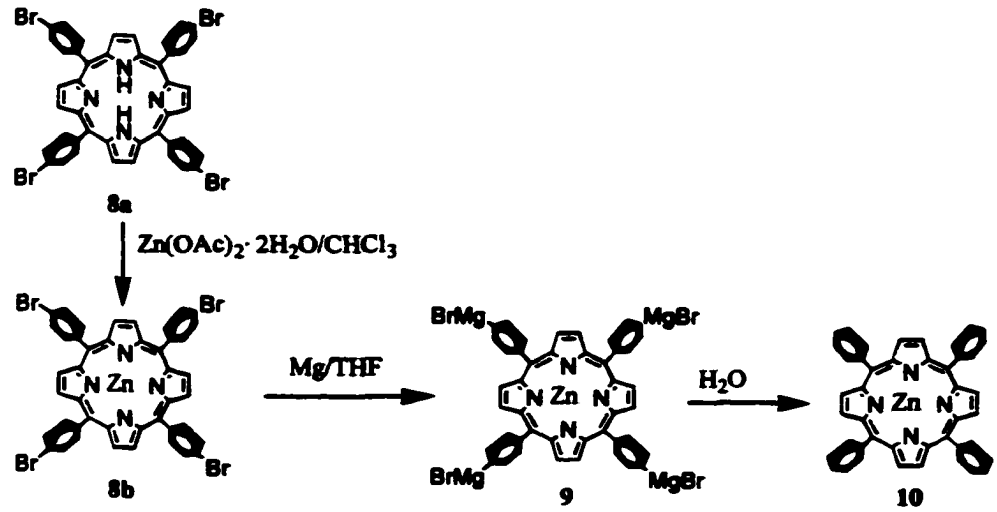
Scheme 3.



6 7

Scheme 4.

When using the bromonated derivatives compound 8b in the preparation of the Grignard reagent, satisfactory results were obtained, scheme 5. After initiating the Grignard reaction, glass wool was wrapped around the reaction vessel to maintain the temperature of the reaction, and no heat was applied. The reaction was stopped 10 minutes after it was started. The yield of 9 was determined by adding water to an aliquot of the reaction mixture and by the integration of the phenyl doublets at 8.2ppm and 8.1ppm for 10 vs. 8b in the crude ^{1}H NMR spectrum, figure 1. The crude reaction mixture indicated ~ 33% of the tetra-Grignard, a small amount (<2%, according to the ^{1} H NMR) of the reduced product— zinc tetraphenylchlorin and the lack of mono, di and tri derivatives. The crude product was demetalized by diluted HCl and then sampled for the ESI-MS study where the detection limit for free base porphyrin is 5μM. Only TPP, tetraphenylchlorin and compound 8a were identified from the ESI-MS spectrum.



Scheme 5.

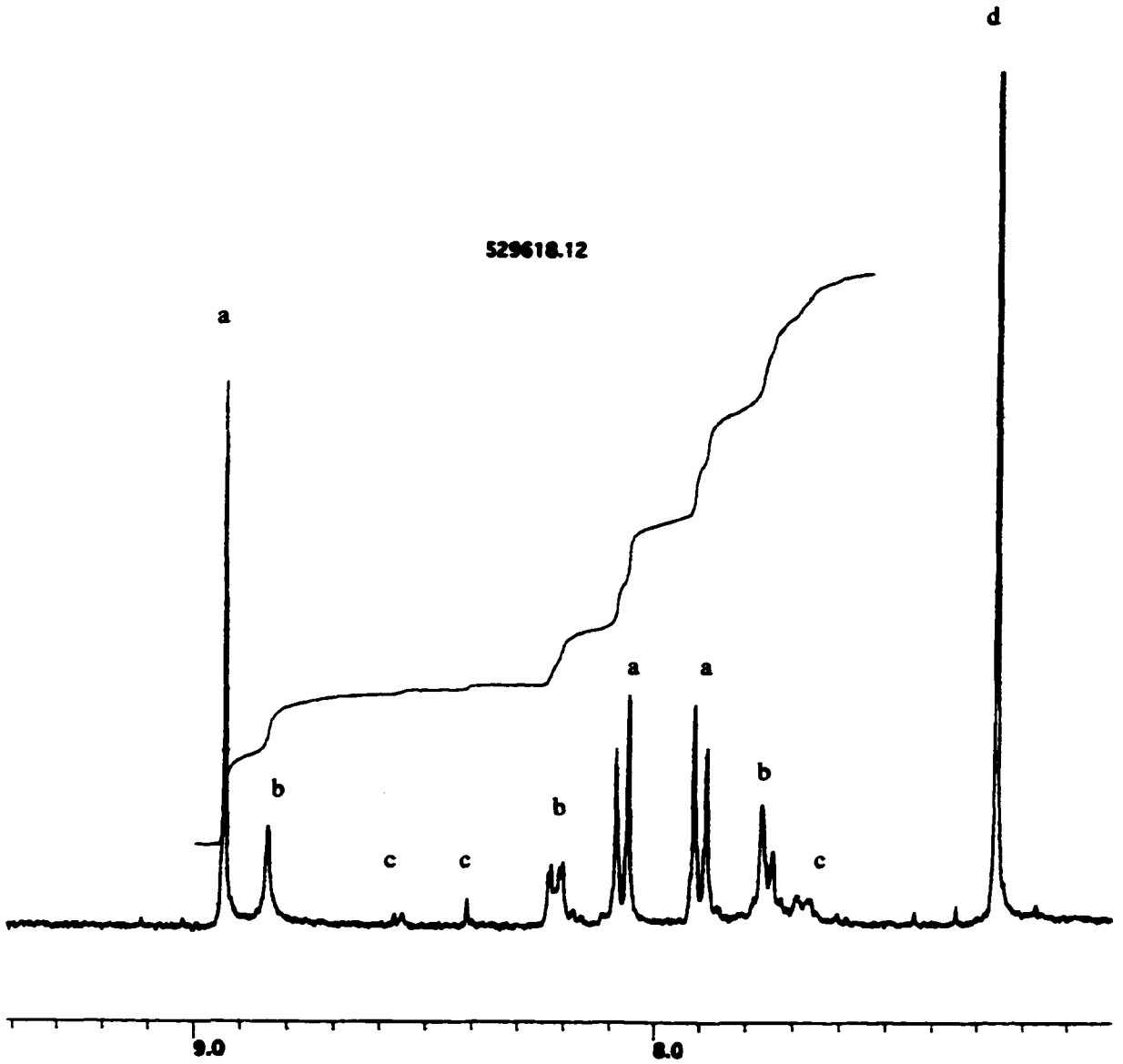
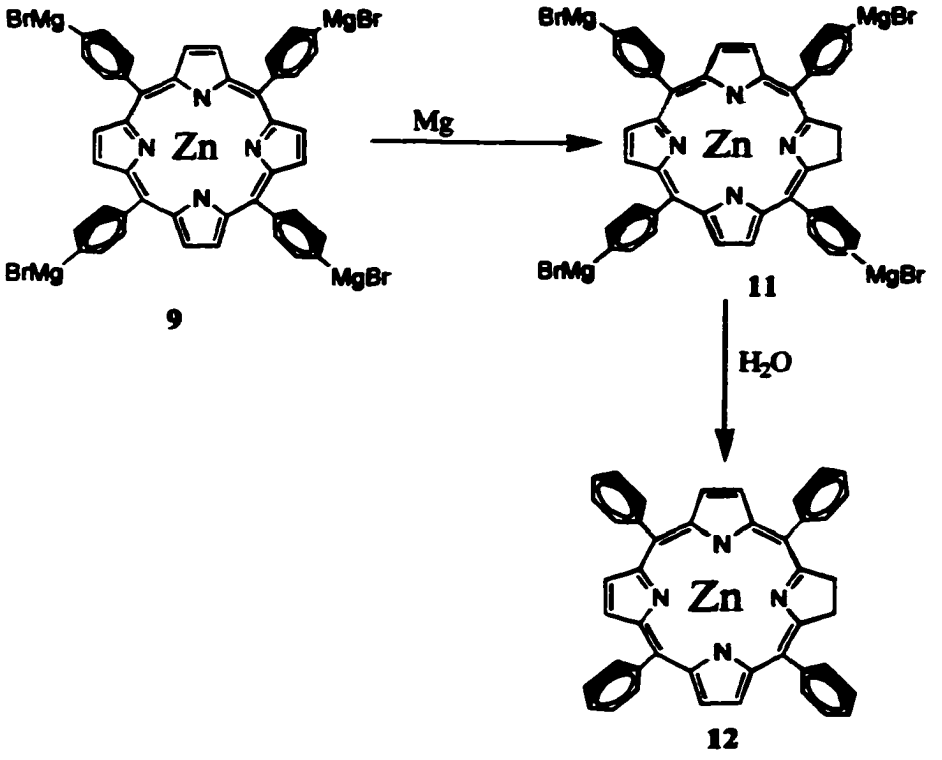


Figure 1. ^{1}H NMR of the crude reaction mixture of a tetra-Grignard reaction after quenched with water. a: 8a, b: ZnTPP, c: ZnTPC, d: $CHCl_3$.

The lack of mono, di and tri derivatives may suggest the reaction is a self-catalyzed process or more likely, each subsequent step is thermodynamically more favorable until the tetra-Grignard is formed. Once the mono Grignard formed, the di, tri and tetra-Grignard are formed in a much faster rate one after the other.

Since the reaction mixture was turning greenish upon extend reaction times, the chlorin derivatives must be formed after the Grignard and before the hydrolysis step, scheme 6. The mechanism for this reaction is unknown, neither is the source of the protons. The protons may come from minute traces of water or be extracted from the solvent.



Scheme 6.

When we extended the reaction time to 30 minutes or to 1 hour, the yield of 9 was not improved as indicated by the ^{1}H NMR. The UV-Visible spectrum of the crude reaction mixture in dry THF showed a 5nm red shift in the Soret and a 16nm red shift in the Q band absorptions compared to the starting porphyrin, figure 2. This observation may

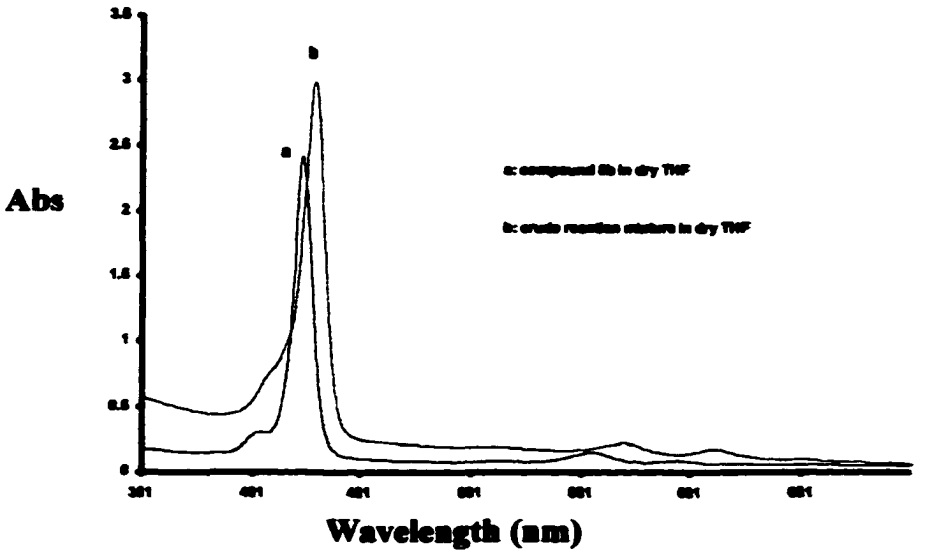
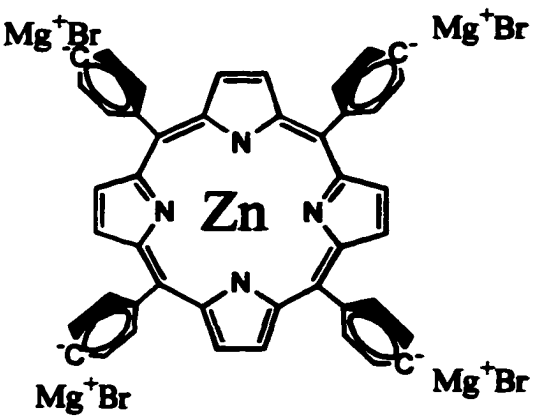


Figure 2. UV-Vis. of 8b and the crude reaction mixture in THF before hydrolysis.

indicate the electronic communication between the carbon atoms connected to the magnesium which builds up some negative charge on the porphyrin ring system, scheme 7.



Scheme 7.

D_2O was used to quench the reaction directly after the tetra-Grignard formation. Zinc-5,10,15,20-Tetrakis(4-deuteriumphenyl)porphyrin was detected from the ^1H NMR of the

crude product, figure 3. The multiple peaks at 7.7ppm and 8.2ppm indicate the formation of the Zinc-5,10,15,20-Tetrakis(4-deuteriumphenyl)porphyrin. The crude product was demetalized by dilute HCl and then sampled for the ESI-MS analysis. 5,10,15,20-Tetrakis(4-deuteriumphenyl)porphyrin was identified from the ESI-MS spectrum, figure 4.

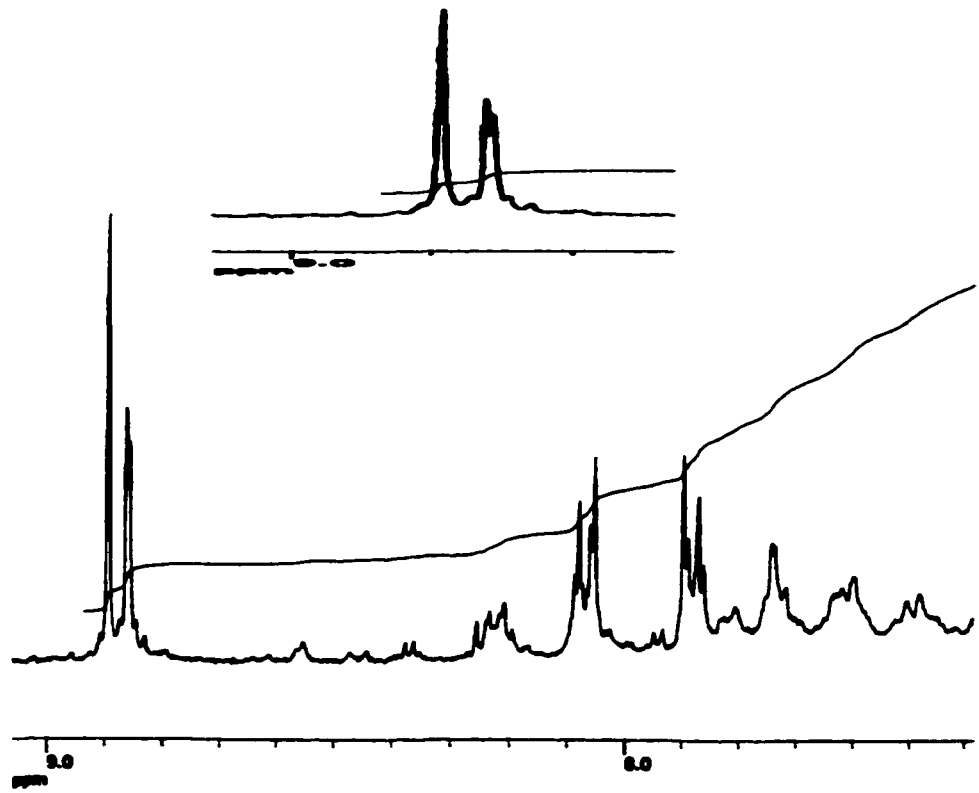


Figure 3. ^{1}H NMR of the crude reaction mixture of a tetra-Grignard reaction after quenched with D_2O.

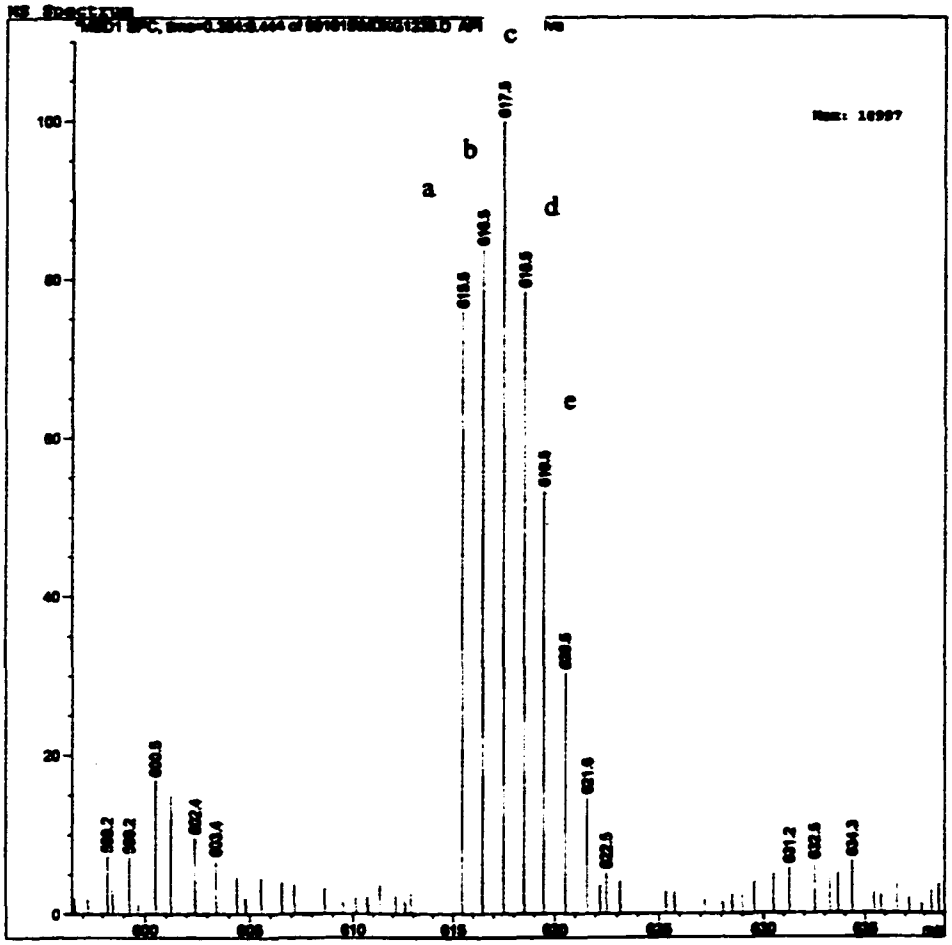


Figure 4. ESI-MS of the crude reaction mixture of a tetra porphyrin Grignard synthesis after quenching with D_2O. a: TPP, b: TPP-d_1, c: TPP-d_2, d: TPP-d_3, e: TPP-d_4,

Since 99.96% D_2O was used in the reaction, we would expect >99% of the reaction product to be TPP-d_4, but this is not what was observed. The product distribution may arise from several facts: (1) The tetra-Grignard in fact is not formed, but a distribution of mono, di and tri Grignard are formed, with the remaining Br groups undergoing a simple reductive reaction. (2) The tetra-Grignard is formed but reacted with trace amounts of water in the system. The second process is likely due to the small scale of the reaction. The reactivity of the supposed zinc-5,10,15,20-Tetrakis(4-bromomagnesium phenyl)porphyrin molecule (compound 9) may not be as high as other known Grignard

reagents. The precursor, 8b is only slightly soluble (<10mM) in THF, so the tetra-Grignard 9 can not be formed in high concentration. The electron withdrawing effects of the porphyrin may make the Grignard less nucleophilic. Further examination of this supposed Grignard product show that it is quite unreactive or has already reacted with trace water. We tried several standard reactions, table 1.

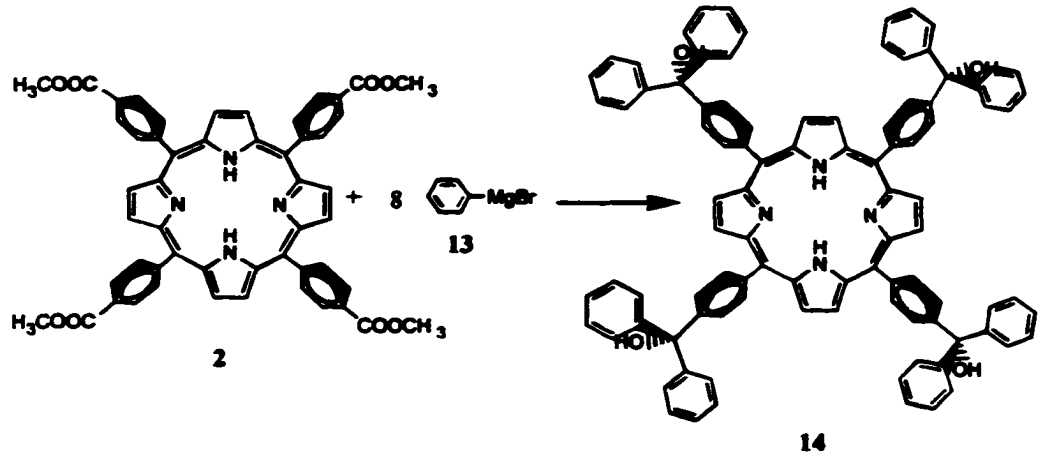
Table 1. Summary of the porphyrin Grignard reaction

Porphyrin Grignard	Reagent	Result
9	Benzaldehyde	No reaction
9	Heptaldehyde	No reaction
Mono Grignard	Diphenylketone	No reaction
Mono Grignard	5,10,15,20-Tetrakis(4-methoxycarbonylphenyl) porphyrin	No reaction

A mono porphyrin Grignard zinc-5,10,15-triphenyl-20-(4-bromomagnesiumphenyl) porphyrin was prepared by reacting zinc-5,10,15-triphenyl-20-(4-bromophenyl)porphyrin with magnesium in dry THF. Like the tetra porphyrin Grignard, no interesting reactivity of this mono porphyrin Grignard has been found so far, table 1.

Synthesis of 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin

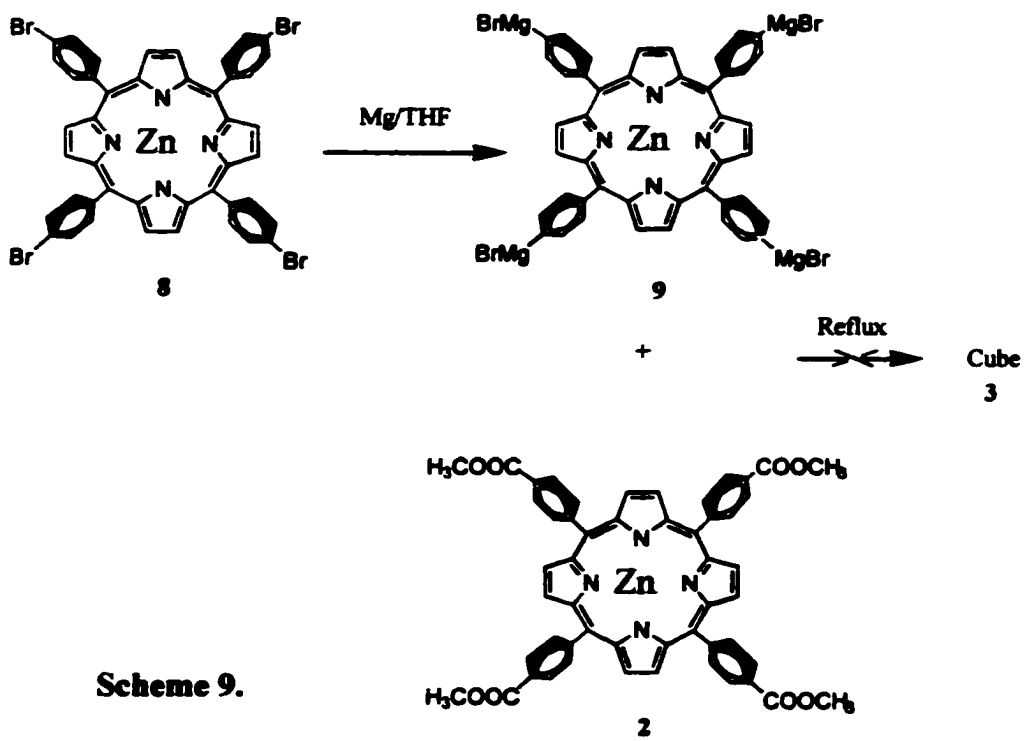
In order to have a better understanding of the cube corner formation process, compound 2 was reacted with a large excess 13 to form compound 14 with a yield of 30%, scheme 8. The formation of the triphenylcarbonyl group connected to the porphyrin ring indicates the cube corners may be formed by Grignard reactions, through this is not a porphyrin Grignard.



Scheme 8.

Attempt to synthesize of the porphyrin cube

Compound 9 and 2 was refluxed overnight in dry THF in an attempt to form the porphyrin cube, scheme 9. By analyzing the ESI-MS (figure 5) of the demetalized crude reaction mixture, we found TPP (615), free base 5,10,15,20-Tetrakis(4-bromophenyl)porphyrin (930.8) and free base 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin (847). There was no indication of the formation of the cube, or any polymeric products.



Scheme 9.

By chromatographic separation of the crude reaction mixture we isolated the ZnTPP and recovered both the starting materials, and no other interesting products. The reason for the failure of the cube synthesis may be due to:

(i) The concentration of the zinc-5,10,15,20-Tetrakis(4-bromomagnesiumphenyl) porphyrin is very low, requiring much longer reaction time;

(ii) The possible steric problems. It may be too crowed for three porphyrins join together in one corner with this fixed geometry.

(iii) The Grignard reactivity is very low.

(iv) The Grignard reacts rapidly with traces of water much faster than react with the zinc 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin

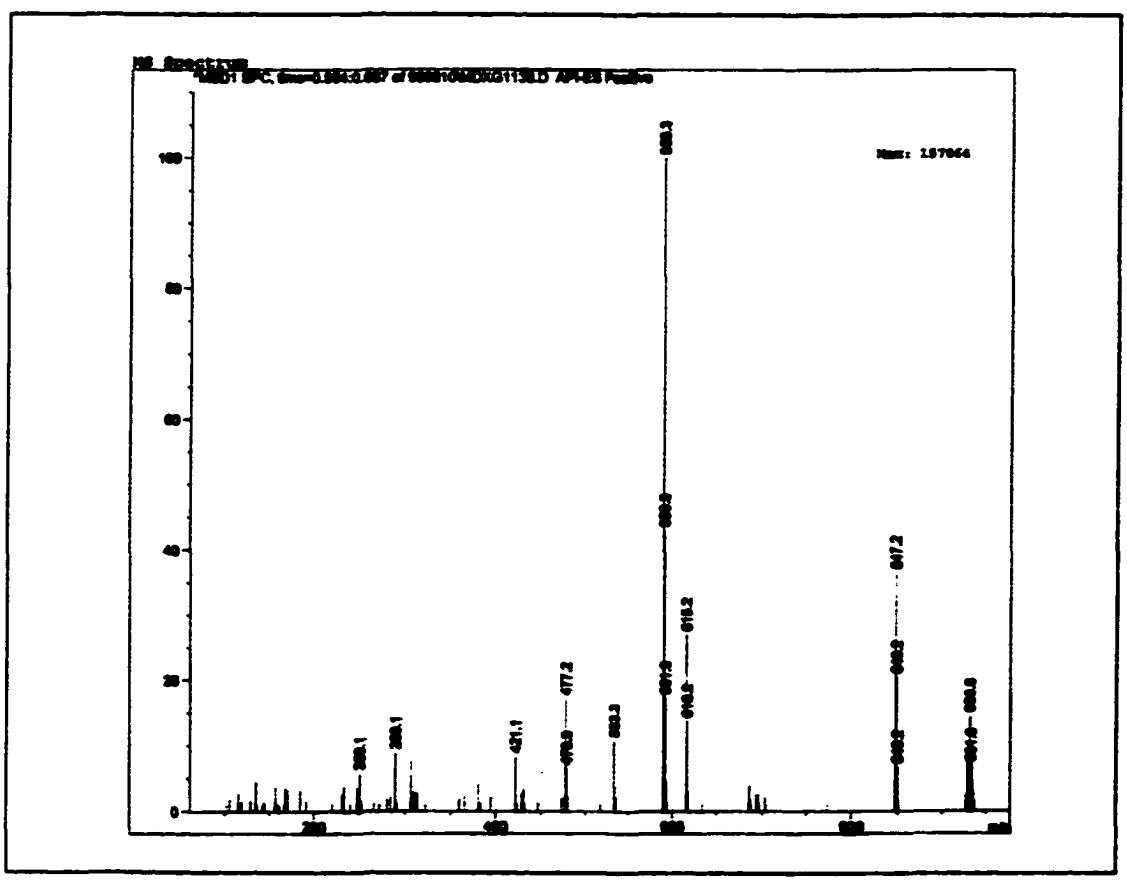


Figure 5. ESI-MS of the crude reaction mixture of an attempt cube synthesis

Conclusion

1: Zinc-5,10,15,20-Tetrakis(4-bromomagnesiumphenyl)porphyrin is likely made with a yield ~33%. The probable formation of the tetra-Grignard is shown by the formation of the TPP after quenched the reaction with water. But on the small scales used, the Grignard reacted with residual water thus preventing full characterization of the tetra-Grignard or explanation of its reactivity. Larger scales or more carefully procedures to exclude water will be necessary to investigate the reactivity of what we assume to be the tetra-Grignard.

2: Using phenyl Grignard and the 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl) porphyrin, 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin has been made with a yield ~30%.

3: The target molecule- the porphyrin cube has not been made using this method, at present.

Experimental

General: $BrCH_2CH_2Br$ and aldehydes were run over a short pipet column of basic alumina before used. Pyrrole was purified by a short pipet silica gel column. All other reagents were used as received from Aldrich. Dry THF was obtained by refluxing over sodium metal with benzenophenone as indicator. ^1H NMR were obtained on a 300MHz GE or 400 MHz Varian spectrometer. UV-Vis absorption spectra were obtained on a Carey 1 spectrophotometer and taken in $CHCl_3$ if not specified. ESI-MS were obtained on a Hewlett-Packard HP 1100 LC/MSD spectrophotometer. The zinc porphyrin derivatives were changed to the free base using diluted HCl before examined by the ESI-MS spectrophotometer.

General method for metalization porphyrins.

Free base porphyrin was dissolved in $CHCl_3$, 2-3 eq. of $Zn(Ac)_2 \bullet 2H_2O$ (premixed with 1mL CH_3OH) was added. The mixture was reflux for 30minutes. Water was used to extract the excess salt and the organic phase was dried over $MgSO_4$ followed by evaporating to obtain the pure product.

General method for the Grignard reaction.

The reaction flask with the added magnesium and porphyrin were flame dried first. After the flask cooled, minimal amount of dry THF was added to dissolve the porphyrin. 0.1-0.3 mL $BrCH_2CH_2Br$ was used to initiated the reaction. The reaction was started by carefully heating with a heat gun. Then glass wool was used to maintain the temperature of the reaction.

Preparation of the 5,10,15,20-Tetrakis(4-bromophenyl)porphyrin (8a)

A mixture of 50mL propionic acid and 4-bromobenaldehyde (1.85g, 0.01 mol, 1eq) was refluxed at 141 ^{0}C for 5 minutes, pyrrole (0.7mL, 0.01mol, 1eq) was added. The mixture was refluxed at 141 ^{0}C for 1 hour. The yield (29.4%) was determined spectropictly[7a] before the solvent was removed. The solvent was removed under vacuum and the crude was purified through flash chromatography using silica gel ($CHCl_3$) to give pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ -2.89(s, b, pyrrole N-H, 2H), 7.90(d, 8H, J=8.4Hz, *o*-phenyl), 8.07(d, 8H, J=8.4Hz, *m*-phenyl), 8.84(s, 8H, β-pyrrole); UV-Vis. in $CHCl_3$ [λ max (nm)] 419, 514, 549, 590, 648

Preparation of the zinc-5,10,15,20-Tetrakis(4-bromophenyl)porphyrin (8b)

To a mixture of 5,10,15,20-Tetrakis(4-bromophenyl)porphyrin (0.35g, 4.25mmol, 1eq) and 50mL $CHCl_3$, $Zn(OAc)_2$• $2H_2O$ (0.33g, 0.0015mol, 4eq, premixed with 1mL methanol) was added. The mixture was refluxed at 60^{0}C for 30 minutes. Water was used to extract the excess salt and the organic phase was dried over $MgSO_4$ followed by evaporating to obtain the pure product.

^{1}H NMR(300 MHz, $CDCl_3$) δ 7.88(d, 8H, J=8.1 Hz, *o*-phenyl), 8.05(d, 8H, J=8.1 Hz, *m*-phenyl), 8.92(s, 8H, β-pyrrole); UV-Vis. in THF [λ max (nm)] 423.4, 556, 595.

Preparation of the zinc-5,10,15,20-Tetrakis(4-bromomagnesiumphenyl)porphyrin (9)

To a mixture of zinc-5,10,15,20-Tetrakis(4-bromophenyl)porphyrin(0.015g), 7mL dry THF and 0.5 g magnesium, 0.1 mL $BrCH_2CH_2Br$ was added. The reaction was started by carefully heat the mixture with a heat gun. The reaction was stopped 10 minutes after it was started.

Yield determination: Water was added to an aliquots of the reaction mixture. Ethyl acetate was used to extract the porphyrin. The organic layer was separated and the solvent was removed. $CDCl_3$ was added to the resulting residue. The yield (33%) was determined by the integration of the ZincTPP vs. zinc-5,10,15,20-Tetrakis(4-bromophenyl)porphyrin in the ^1H NMR spectrum of the crude product.

Preparation of 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin (14)

To a mixture of 0.3629g Mg and 10 mL dry THF, C_6H_5Br (0.6mL, 3.9mmol, 10eq) was added and the reaction was initiated by gently heating. After the reaction is finished, the warm Grignard solution was added to a mixture of 10mL dry THF and 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin (0.33g, 0.39mmol, 1eq). The resulting mixture was refluxed at 66^0C for 3 hours. The reaction mixture was quenched by water and ethyl acetate was added to extract the product. The organic phase was separated and the solvent was removed. The crude was purified by flash chromatography using silica gel ($CHCl_3$) to give pure product (0.17g, 30%).

^1H NMR(300 MHz, Acetone-d_6) δ -2.75(b, pyrrole N-H, 2H), 5.8(s, 4H, -OH), 7.42(m, 8H, *p*-phenyl), 7.52(m, 16H, *o*-phenyl), 7.60(m, 16H, *m*-phenyl), 7.70(d, 8H, J= 8.1Hz, C_6H_4), 8.17(d, 8H, J= 8.1Hz, C_6H_4), 8.90(s, 8H, β-pyrrole); ^{13}C NMR(75 MHz, $CDCl_3$) δ 100.3, 119.7, 126.2, 127.5, 128.0, 128.1, 128.6, 134.0, 141.0; UV-Vis. in $CHCl_3$ [λ max (nm)] 422, 517.5, 552.8, 592.4, 647.8. ESI-MS [Positive APCI, $(m+H)/z^+$, % relative intensity] 1343, 93.7%

Attempted preparation of a porphyrin cube (3)

A three neck 200mL flask was connected to a condenser with one neck, while another neck connected to a 100mL separatory funnel. Some glass wool was added to the neck of

the funnel to prevent tiny pieces of magnesium from falling into the flask in the later reactions. 0.5 g Mg and 5,10,15,20-Tetrakis(4-bromophenyl)porphyrin (0.0611g, 0.0615mmol, 4eq) were added to the separation funnel. 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin (0.0134g, 0.0149mmol, 1eq) was added to the three-neck flask. The glassware was flame dried first. The other neck of the flask was sealed with a rubber septum. Two drying test tubes were connected separately to the tops of the condenser and the separation funnel with rubber septum. 10mL anhydrous THF was injected to the separation funnel and 5mL anhydrous THF was injected to the flask. Solutions in the separation funnel and in the flask were heated very carefully with a heating gun to accelerate the dissolving of the porphyrins. After the porphyrins were dissolved, 0.3mL $BrCH_2CH_2Br$ was injected to the hot THF solution in the separation funnel to start the reaction. Aluminum foil was placed around the separatory funnel to maintain the temperature of the exothermic reaction. After 10 minutes reaction, the contents of the separatory funnel was added to the 3-neck flask. By applying pressure through the end of the drying tube, the THF solution in the separatory funnel was pushed to the flask in seconds while the solution in the flask was stirring. The separatory funnel was closed and the reaction mixture was stirred in room temperature for 2 hours then refluxed overnight. The reaction was stopped and 100mL water was added to hydrolyze the crude mixture. 100mL ethyl acetate was used to extract the product. After extraction, the solvent was removed under vacuum. The crude reaction mixture was purified through flash chromatography using silica gel ($CHCl_3$) and no compound 3 was isolated.

Appendix

Figure A1: Tetraphenylchlorin in $CHCl_3$

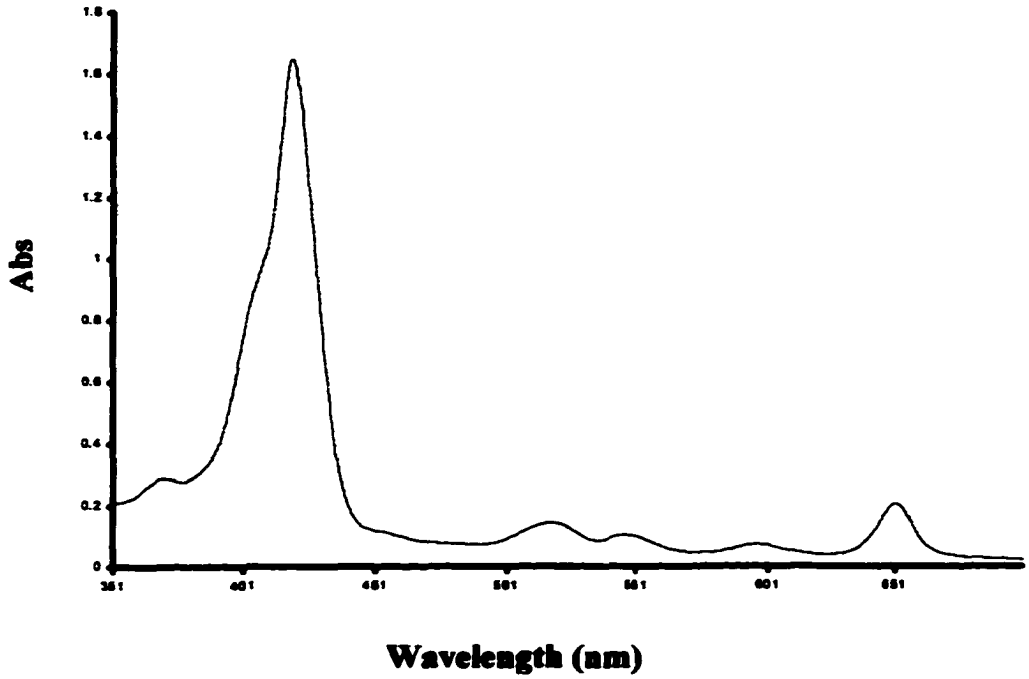


Figure A2. a: UV-Vis. spectrum of zinc-5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin(2) in dry THF
b: UV-Vis. spectrum of crude reaction mixture of zinc-5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin (2) reacted with phenyl Grignard in dry THF

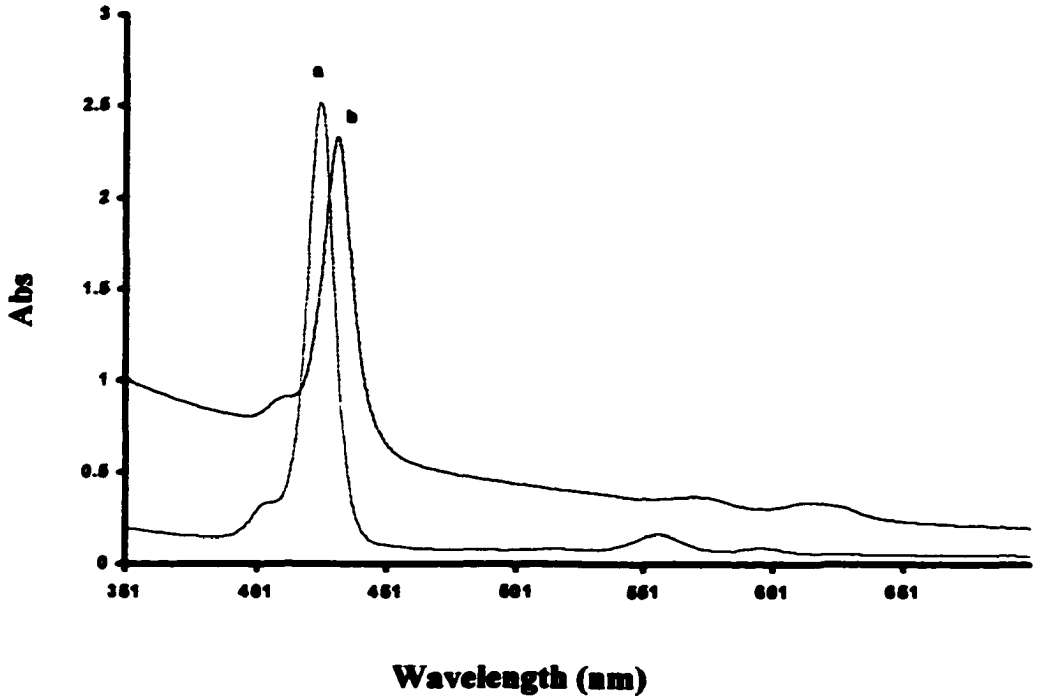


Figure A3. UV-Visible spectrum of 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin (14) in $CHCl_3$.

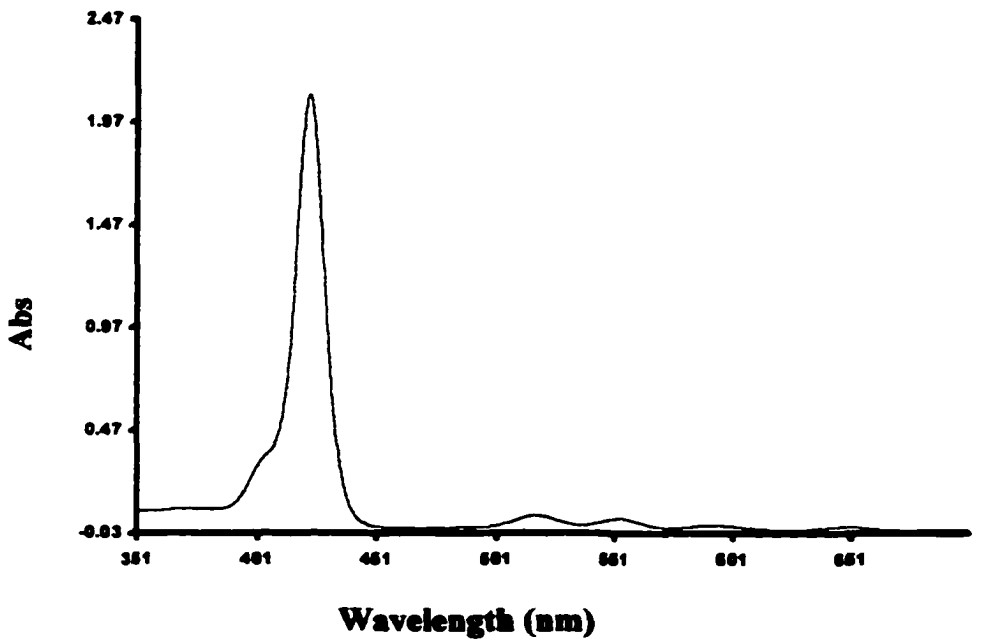


Figure A4. ^{1}H NMR(300 MHz, Acetone-d_6) of 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin (14) (*: $CHCl_3$)

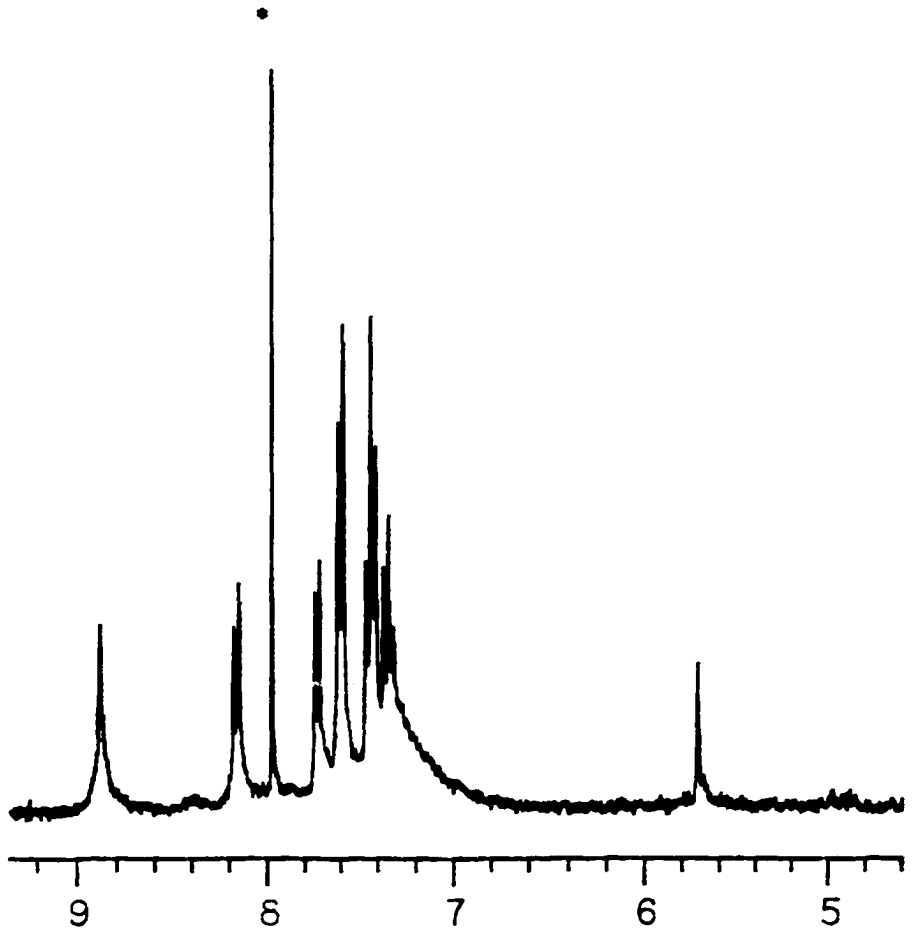
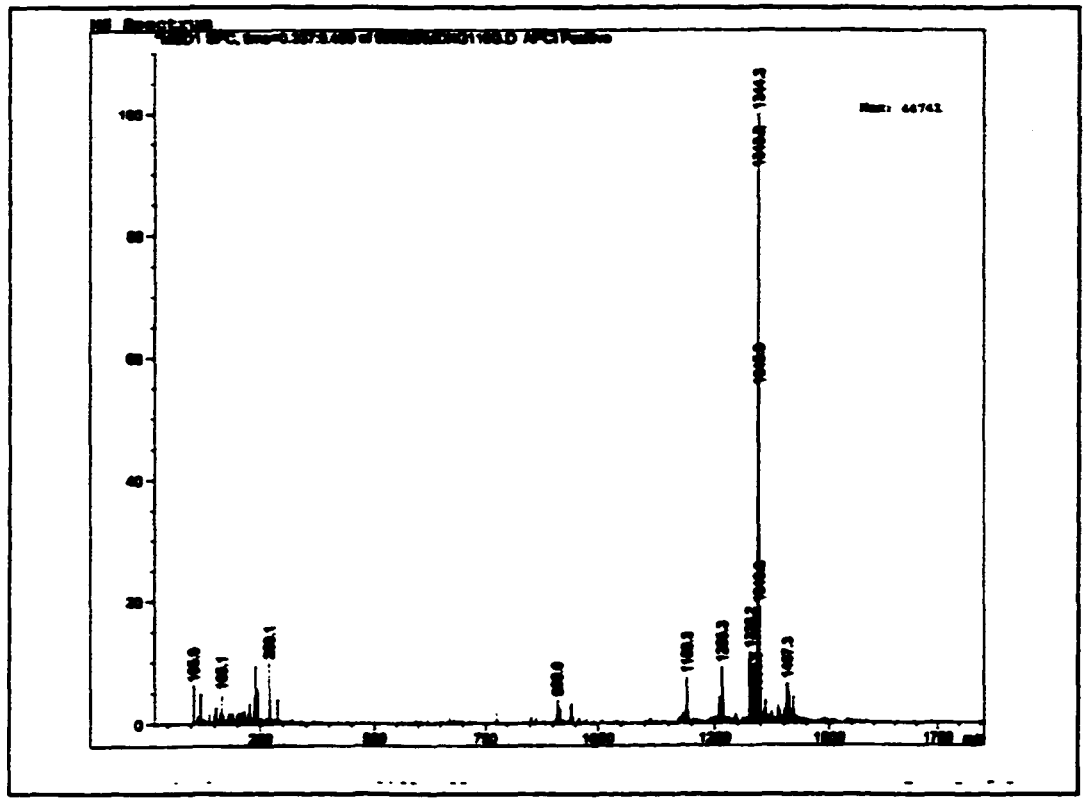


Figure A5. ESI-MS of 5,10,15,20-Tetrakis(4-diphenylmethanolphenyl)porphyrin (14)



Bibliography

1. Adler, A.D. Ed, *The Chemical & Physical Behavior of Porphyrin Compounds & Related Structures; Annal. N.Y. Acad. Sci.*: **1973**, Vol. 206.

2. For a review: **Mlodnicka**, "Metalloporphyrins as catalyst in autoxidation processes: A review", T. *J. Mol. Catal.* **1986**, *36*, 205-242.

3. (a) Drain, C.M.; Nifiatis, F.; Vasenko, A.; Batteas, J.D., "Porphyrin tessellation by design: Metal mediated self-assembly of large arrays and tapes", *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2344-2347. (b) Drain, C.M.; Lehn, J.-M. "Self-assembly of square multiporphyrin arrays by metal ion coordination", *J. Chem. Soc., Chem. Commun.*, **1994**, 2313-2315. (c) Drain, C.M.; Russell, K.C.; Lehn, J.-M. "Self-assembly of a multi-porphyrin supramolecular macrocycle by hydrogen bond molecular recognition", *J. Chem. Soc., Chem. Commun.* **1996**, 337-338. (d) Drain, C.M.; Mauzerall; D. "Photogating of ionic currents across lipid bilayers", *Biophys. J.* **1992**, *63*, 1556-1555. (e) Drain, C.M.; Mauzerall, D. "An example of a working molecular charge sensitive ion conductor", *Bioelectrochem. Bioenerg.* **1990**, *24*, 263-268.

4. (a) Adler, A.D.; Longo, F.R.; Shergalis, W. "Mechanistic investigation of porphyrin synthesis. I. Preliminary studies on *meso*-Tetraphenylporphyrin", *J. Am. Chem. Soc.* **1964**, *86*, 3145-3149. (b)Adler, A.D., "A simplified synthesis for *meso*-Tetraphenyl porphyrin", *J. Org. Chem.* **1967**, *32*, 476.

5. (a)Arsenault, G.P.; Bullock, E.; MacDonald; S.F., "Pyrromethanes and porphyrins there from", *J. Am. Chem. Soc.* **1960**, *82*, 4384-4389. (b)Nguyen, L.T.; Senge, M.O.; Smith, K.M. "Simple methodology for syntheses of porphyrins possessing multiple peripheral substituents with an element of symmetry", *J. Org. Chem.* **1996**, *61*, 998-1003.

6. Mauzerall, D. in *The Porphyrins;* Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 2.

7. (a) Lindsey, J.S.; Schreiman, I.C.; Hsu, H.C.; Kearney, P.C.; Marguerettaz, A.M. "Rothemund and Adler-Longo reactions revisited: Synthesis of tetraphenylporphyrins under equilibrium conditons", *J. Org. Chem.* **1987**, *52*, 827-836. (b) Lindsey, J.S.; MacCrum, K.A.; Tyhonas, J.S.; Chuang, Y.-Y. "Investigation of a synthesis of *meso*-porphyrins employing high concentration conditions and an electron transport chain for aerobic oxidation", *J. Org. Chem.* **1994**, *59*, 579-587. (c) Ravikanth M.; Achim, C.; Tyhonas, J.S.; Munck, E.; Lindsey, J.S. "Investigation of phthalocyanine catalysts for the aerobic synthesis of *meso*-substituted porphyrins", *J. Porphyrins and Phthalocyanines* **1997**, *1*, 1385-1394 and references therein.

8. Gradillas, A.; Campo, C.; Sinisterra, J.V.; Llama, E.F. "Novel synthesis of 5,10,15,20-tetraarylporphyrins using high-valent transition metal salts", *J. Chem. Soc. Perkin Trans. 1* **1995**, 2611-2613.

9. Drain, C.M.; Gong, X. "Synthesis of meso substituted porphyrins in air without solvents or catalysts", *J. Chem. Soc., Chem. Commun.* **1997**, 2117-2118.

10. Rothemund, P.; Menotti, A.R., "Porphyrin studies: IV. the synthesis of α, β, γ, δ-tetraphenylporphyrin", *J. Am. Chem. Soc.* **1941**, *63*, 267-270.

11. Li, F.; Yang, K.; Tyhonas, J.S.; MacCrum, K.A.; Lindsey, J.S. "Beneficial effects of salts on an acid-catalyze condensation leading to porphyrin formation", *Tetrahedron* **1997**, *53*, 12339-12360.

12. Crossley, M.J.; Thordarson, P; Bannerman, J.P.; Marnard, P.J. "A convenient procedure for moderate-scale Rothemund synthesis of lipophilic porphyrins: an alternative to the Adler-Longo and Lindsey methodologies", *J. Porphyrins and Phthalocyanines* **1998**, *2*, 511-516.

13. Johnstone, R.W.; Nunes, M.L.G.; Pereira, M.; Gonsalves, A.R.; Serra, A. "Improved synthesis of 5,10,15,20-tetrakisaryl- and tetrakisalkylporphyrins", *Hererocycles* **1996**, *43*, 1423-1437.

14. (a) Shinoda, T.; Izumi, Y.; Onaka, M. "FSM-16: A recyclable mesoporous acid promoter for mesotetraaryporphyrin synthesis", *J. Chem. Soc., Chem. Commun.* **1995**, 1801-1802. (b) Onaka, M.; Shinoda, T.; Izumi, Y.; Nolen, E. "Clay –mediated meso-tetraarylporphyrin synthesis", *Tetra. lett.* **1993**, *34*, 2625-2628. (c) Laszlo, P.; Luchetti, J. " Porphyrin synthesis using clays. Taking advantage of statistical product distributions", *Chem. Lett.* **1993**, 449-452.

15. Bonar-Law, R. P. "Porphyrin synthesis in surfactant solution: multicomponent assembly in micelles", *J. Org. Chem.* **1996**, *61*, 3623-3634.

16. Gross, Z.; Galili, N.; Simkhovich, L.; Saltsman, I.; Botoshansky, M.; Blaser, D.; Boese, R..; Goldberg, I. "Solvent-free condensation of pyrrole and pentafluorobenzaldehyde: A novel synthetic pathway to corrole and oligopyrromethenes", *Org. Lett*, **1999**, *1*, 599-602.

17. Gross, Z.; Galili, N.; Saltsman, I. "The first direct synthesis of corroles from pyrrole", *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 1427-1429.

18. Littler, B. J.; Ciringh, Y.; Lindsey, J. S. "Investigation of conditions giving minimal scrambling in the synthesis of *trans*-porphyrins from dipyrromethanes and aldehydes", *J. Org. Chem.* **1999**, *64*, 2864-2872.

19. Littler, B. J.; Miller, M. A.; Hung, C.-H.; Wagner, R. W.; O'Shea, D. F.; Boyle, P. D.; Lindsey, J. S. "Refined synthesis of 5-substituted dipyrromethanes", *J. Org. Chem.* **1999**, *64*, 1391-1396.

20. Bruckner, C.; Posaknoy, J.J.; Johnson, C.K.; Boyle, R.W.; James, B.R.; Dolphin, D. " Novel and improved syntheses of 5, 15-diphenylporphyrin and its dipyrrolic precursors", *J. Porphyrins and Phthalocyanines*, **1998**, *2*, 455-465.

21. (a) George, P. page 84 in ref. 1. (b)Trace amounts of porphyrin are formed at room temperature and pressure. Hodgson, G.W. *Annal. N.Y. Acad. Sci.* **1972**, *194*, 87-97.

22. Terrett, N.K. *Combinatorial Chemistry* Oxford University Press: New York, 1998

23. Borman, S., "Combinatorial chemistry", *C&EN*, **1998**, *April 6*, 47-67.

24. Borman, S., "Reducing time to drug discovery", *C&EN*. **1999**, *March 8*, 33-48.

25. Shipps, G. W.; Pryor, K. E.; Xian, J.; Skyler, D. A.; Davidson, E. H.; Rebek Jr., J.. "Synthesis and screening of small molecule libraries active in binding to DNA", *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11833-11838.

26. Merrifield, R. B., "Solid phase peptide synthesis. I. The synthesis of a tetrapeptide", *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

27. Geysen, H. M.; Meloen, R. H.; Barteling, S. J. "Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid", *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3998-4002.

28. Houghten, R. A. "General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen-antibody interaction at the level of individual amino acids", *Proc. Natl. Acad. Sci. USA*, **1985**, *82*, 5131-5135.

29. Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. "General method for rapid synthesis of multicomponent peptide mixtures", *Int. J. Pept. Protein Res.* **1991**, *37*, 487-493.

30. Xiao, X.-Y.; Parandoosh, Z.; Nova, M. P., "Design and synthesis of a taxoid library using radiofrequency encoded combinatorial chemistry", *J. Org. Chem.*, **1997**, *62*, 6029-6033.

31. Brenner, S.; Lerner, R.A.; "Encoded combinatorial chemistry", *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5181-5183.

32. Needeles, M. N.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersperger, L. M.; Dower, W. J.; Barrett, R. W.; Gallop, M. A. "Generation and screening of an oligonucleotide-encoded synthetic peptide library", *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10700-10704.

33. Czarnik, A. W. "Solid-phase synthesis supports are like solvents", *Biotech. Bioeng. (combinatorial chemistry)*, **1998**, *61*, 77-79.

34. Li, W.; Yan, B., "Effects of polymer supports on the kinetics of solid-phase organic reactions: A comparison of polystyrene- and TentaGel-based Resins", *J. Org. Chem.*, **1998**, *63*, 4092-4097.

35. Li, W.; Xiao, X.; Czarnik, A. W. "Kinetic comparison of amide formation on various cross-linked polystyrene resins", *J. Combin. Chem.*, **1999**, *1*, 127-129.

36. Pirrung, M. C.; Chau, J. H-L.; Chen, J. "Discovery of a novel tetrahydroacridine acetylcholinesterase inhibitor through an indexed combinatorial library", *Chem. Biol.* **1995**, *2*, 621-626.

37. Balkenhohl, F.; Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. Combinatorial synthesis of small organic molecules", *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288-2337.

38. Goebel, M.; Ugi, I. "Beyond peptide and nucleic acid combinatorial libraries: Applying unions of multicomponent reactions towars the generation of carbohydrate combinatorial libraries", *Tetra. Lett.* **1995**, *36*, 6043-6046.

39. Carell, T.; Wintner, E. A.; Bashir-Hashemi, A; Rebek, J. "A novel procedure for the synthesis of libraries containing small organic molecules", *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2159-2161.

40. Berlin, K.; Jain, R. K.; Tetziaff, C.; Steinbeck, C.; Richert, C. "Spectrometrically monitored selection experiments: Quantitative laser desorption mass spectrometry of small chemical libraries", *Chem. & Biol.* **1997**, *4*, 63-67.

41. Berlin, K.; Jani, R. K.; Richert, C.. "Are porphyrin mixtures favorable photodynamic anticancer drugs: A model study with combinatorial libraries of tetraphenylporphyrins", *Biotech. Bioeng.* **1998**, *61*, 107-118.

42. A simple algorithm to calculate the molar masses and the number of isobaric species will be made available on the internet. The number of compounds for *meso* substituted porphyrins (not including possible rotameric forms) is found by the following formula: $N = C^1_m + 4C^2_m + 6C^3_m + 3C^4_m$ where $C^n_m = m!/(n!(m-n)!)$, n = positions on the *meso*-porphyrin (n=1 when all the substituents are the same to n=4 when all are different), and m = the number of starting aldehydes. For 10 starting aldehydes there are 1540 compounds of which 715 are isobaric.

43. Tapscott, R. E.; Marcovich, D. "Enumeration of permutational isomers: the porphyrins", *J. Chem. Educ.* **1978**, *55*, 446-447.

44. Fleischer, E.B.; Shachter, A.M. "Coordination oligomers and a coordination polymer of zinc tetraarylporphyrins", *Inorg. Chem.* **1991**, *30*, 3763-3769.

45. Sternberg, E. D.; Dolphin, D.; Brücker, C. "Porphyrin-based photosensitizers for use in photodynamic therapy", *Tetrahedron* **1998**, *54*, 4151-4202.

46. Armitage, B. "Photocleavage of nucleic acids", *Chem. Rev.* **1998**, *98*, 1171-1200.

47. Bonnet, R. "Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy", *Chem. Soc. Rev.* **1995**, 19-32.

48. Rouhi, A.M. "Let there be light and let it heal", *C&E News*, **1998**, *Nov. 2*, 22-27.

49. Sari, M. A.; Battioni, J. P.; Dupre, D.; Mansuy, D.; Pecq, J. B. L. "Interaction of cationic porphyrins with DNA: Importance of the number and position of the charges and minimum structural requirements for intercalations", *Biochem.*, **1990**, *29*, 4205-4215.

50. Meng, G.G.; James, B. R.; Skov, K. A.; Korbelik, M. "Porphyrin chemistry pertaining to the design of anti- cancer drugs; part 1, the synthesis of porphyrins containing meso--pyridyl and meso--substituted phenyl functional groups", *Can. J. Chem.* **1994**, *72*, 1894-1909.

51. Meng, G.G.; James, B. R.; Skov, K. A.; Korbelik, M. "Porphyrin chemistry pertaining to the design of anti-cancer drugs: part 2, the synthesis and in vitro testes of water-soluble porphyrins containing, in the meso positions, the functional groups: 4-methylpyridinium, or 4-sulfantophenyl, in combination with phenyl, 4-pyridyl, 4-nitrophenyl, or 4-aminophenyl". *Can. J. Chem.* **1994**, *72*, 2447-2457.

52. Uno, T.; Hamasaki, K.; Tanigawa, M.; Shimabayashi, S. "Binding of meso-tetrakis(N-methylpyridinium-4-yl)porphyrin to double helical RNA and DNA-RNA hybrids", *Inorg. Chem.* **1997**, *36*, 1676-1683.

53. Lipscomb, L. A.; Zhou, F. X.; Presnell, S. R.; Woo, R. J.; Peek, M. E.; Plaskon, R. R.; Williams, L. D. "Structure of a DNA-porphyrin complex", *Biochem.* **1996**, *35*, 2818-2823.

54. Mettath, S.; Munsun, B.R.; Pandey, R.K. "DNA interaction and photocleavage properties of porphyrins containing cationic substituents at the peripheral position", *Bioconj. Chem.*, **1999**, *10*, 94-102.

55. Takemura, T.; Ohta, N.; Nakajima, S.; Sakata, I. "The mechanism of photosensitization in photodynamic therapy: chemiluminescence caused by photosensitization of porphyrins in saline containing human serum albumin", *Photochem. Photobiol.* **1992**, *55*, 137-140.

56. Oulmi, D.; Maillard, P.; Vever-Bizet, C.; Momenteau, M.; Brault, D. "Glycosylated porphyrins: characterization of association in aqueous solutions by absorption and fluorescence spectroscopies and determination of singlet oxygen yield in organic media", *Photochem. Photobiol.* **1998**, *67*, 511-518.

57. Valenxeno, D.P "Photomodification of biological membranes with emphasis on singlet oxygen mechanisms", *Photochem. Photobiol.* **1987**, *46*, 147-160.

58. Pasternack, R.F.; Garrity, P.; Ehrlich, B.; Davis, C.B.; Gibbs, E.J.; Orloff, G.; Giartosio, A.; Turano, C. "The influence of ionic strength on the binding of a water soluble porphyrin to nucleic acids", *Nucl. Acids Res.* **1986**, *14*, 5919-5931.

59. Giulio, J. "Far-red absorbing photosensitizers: their use in photodynamic therapy", *Photochem. Photobiol., A.* **1992**, *62*, 371-378.

60. Woodburn, K.W.; Vardaxis, N.J.; Hill, J.S.; Kaye, A.H.; Reiss, J.A. Phillips, D.R. "Evaluation of porphyrin characteristics required for photodynamic therapy", *Photochem. Photobiol.* **1992**, *55*, 697-704.

61. Wainwright, M. "Non-porphyrin photosensitizers in biomedicine", *Chem. Soc. Rev.* **1996**, 351-359.

62. Adams, K.R.; Berenbaum, M.C.; Bonnett, R.; Nizhnik, A.N.; Salgado, A.; Valles, M.A. "Second generation tumor photo sensitizers: the synthetic and biological activity of octaalkyl chlorins and bacteriochlorins with graded amphiphilic character", *J. Chem. Soc., Perkin Trans. 1* **1992**, 1465-1470.

63. Drain, C.M.; Gong, X.; Ruta, V.; Soll, C. and Chicoineau, P. "Combinatorial synthesis of functional porphyrin libraries: Identification of New, Amphipathic Motifs for Biomolecule Binding", *J. Combin. Chem.*, **1999**, *1*, 286-290.

64. Pasternack, R.F.; Gibbs, E.J.; Collings, P.J.; dePaula, J.C.; Turzo, C.L.; Terracina, A. "A nonconventional approach to supramolecular formation dynamics. The kinetics of assembly of DNA-bound porphyrins", *J. Am. Chem. Soc.* **1998**, *120*, 5873-5878.

65. Fruchtel, J.S.; Jung, G. "Organic chemistry on solid supports", *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 17-42.

66. For a direct synthesis of porphyrins on a solid support: Leznoff, C.C.; Svirskaya, P.I. "The synthesis of unsymmetrical tetraarylporphyrins on solid phase", *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 947.

67. Vigmond, S.J.; Chang, M.C.; Kallury, K.M.; Thompson, M. "Direct synthesis of aryldipyrromethanes", *Tet. lett.* **1994**, 2455-2458.

68. Lee, C.H.; Lindsey, J.S. "One-flask synthesis of meso-substituted dipyrromethanes and their application in the synthesis of trans-substituted porphyrin building blocks", *Tetrahedron*, **1994**, *50*, 11427-11440.

69. Hermkens, P. H.; Ottenheijim, H. C.; Rees, D., "Solid-Phase organic reactions: a review of the recent literature", *Tetrahedron*, **1996**, *52*, 4527-4554.

70. Richter, L.S.; Gadek, T.R. "A surprising observation about Mitsunobu reactions in solid phase synthesis", *Tet. Lett.*, **1994**, *35*, 4705-4706.

71. Hanessian, S.; Xie, F. "Exploring functional and molecular diversity with polymer-bound p-alkoxybenzyl ethers: Scope and applications of preparatively useful organic reactions", *Tet. Lett.*, **1998**, *39*, 733-736.

72. Anderson, H. L. "Conjugated porphyrin ladders", *Inorg. Chem.* **1994**, *33*, 972-981.

73. Wagner, R. W.; Johnson, T. E.; Lindsey, J. S., "Soluble synthetic multiporphyrin arrays. 1. Modular design and synthesis", *J. Am. Chem. Soc.*, **1996**, *118*, 11166-11180.

74. Hsiao, J.-S.; Krueger, B. P.; Wagner, R. W.; Johnson, T. E.; Delaney, J. K.; Mauzerall, D. C.; Fleming, G. R.; Lindsey, J. S.; Bocian, D. F.; Donohoe, R. J., "Soluble synthetic multiporphyrin arrays. 2. Photodynamics of energy-transfer processes", *J. Am. Chem. Soc.*, **1996**, *118*, 11181-11193.

75. Seth, J.; Palaniappan, V.; Wagner, R. W.; Johnson, T. E.; Lindsey, J. S.; Bocian, D. F., "Soluble synthetic multiporphyrin arrays. 3. Static spectroscopic and electrochemical probes of electronic communication", *J. Am. Chem. Soc.*, **1996**, *118*, 11194-11207.

76. Osuka, A.; Liu, B.; Maruyama, K. "Synthesis of a 1,3,5-triporphyrinylbenzene", *J. Org. Chem.*, **1993**, *58*, 3582-3585.

77. Osuka, A.; Shimidzu, H. "*meso*, *meso*-Linked porphyrin arrays", *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 135-137.

78. Ogawa, T.; Nishimoto, Y.; Yoshida, N.; Ono, N.; Osuka, A. "One port electrochemical formation of *meso*, *meso*-Linked porphyrin arrays", *J. Chem. Soc., Chem. Commun.* **1998**, 337.

79. Jiang, B.; Yang, S.; Barbini, D. C.; Jones Jr, W. E. "Synthesis of soluble conjugated metalloporphyrin polymers with tunable electronic properties", *J. Chem. Soc., Chem. Commun.* **1998**, 213-214.

80. Crossley, M. J.; Prashar, J. K. "Thiophene-appended porphyrin systems", *Tet. lett.* **1997**, *38*, 6751-6754.

81. Chmielewski, P.J.; Latos-Grazynski, L.; Rachlewicz, K., " ^{1}H nuclear magnetic resonances studies of the reaction of iron (III) porphyrin cation radical with aryl grignard reagents", *Magn. Reson. Chem.* **1993**, *31*, S47-S52.

82. Crossley, M.J.; Harding, M.; Tansey, C.W., " A convenient synthesis of 2-alkyl-5,10,15,20-tetraphenylporphyrins: reaction of metallo-2-nitro-5,10,15,20-tetraphenylporphyrins with Grignard and organolithium reagents", *J. Org. Chem.*, **1994**, *59*, 4433-4437.

83. Jiang, X.; Nurco, D.; Smith, K.M., "Direct meso-alkylation of meso-formylporphyrins using Grignard reagents", *J. Chem. Soc., Chem. Commun.*, **1996**, *15*, 1759-1760.