PERKIN 2

Investigation of porphyrin-forming reactions. Part 1. Pyrrole + aldehyde oligomerization in two-step, one-flask syntheses of *meso*-substituted porphyrins †

G. Richard Geier III and Jonathan S. Lindsey *

Department of Chemistry, North Carolina State University, Raleigh,
North Carolina 27695-8204, USA

Received (in Cambridge, UK) 13th November 2000, Accepted 14th March 2001

A deep understanding of the two-step, one-flask synthesis of *meso*-substituted porphyrins requires characterization of the acyclic oligomers, which typically constitute ≥50% of the products. We have employed laser desorption mass spectrometry (LD-MS) to obtain a qualitative yet high resolution view of the oligomer content of crude oxidized reaction mixtures. This methodology complements other analytical methods which provide information regarding yields of porphyrin, other macrocyclic products, and unreacted aldehyde. Our findings include the following. (1) Crude oxidized porphyrin reaction mixtures provided peaks in the LD-MS spectrum which were readily assigned to oligomers (*m*/*z* 100–2000) derived from pyrrole–aldehyde condensation. The oligomers comprised one of four series depending on the ratio of pyrrole and aldehyde units. (2) Quite disparate reaction conditions that gave good yields of porphyrin also afforded a similar oligomer composition. (3) The change in the nature of the oligomers over time was readily monitored. (4) The maximum yield of porphyrin and the maximum diversity of the oligomer composition were attained at similar times. (5) The decline in yield of porphyrin at long reaction time was accompanied by truncation, not elongation, of oligomers. (6) The onset of the truncation of oligomers and the decline in yield of porphyrin were accompanied by a decrease of the aldehyde to low levels. (7) Pyrrole was incorporated into the growing oligomers more rapidly than aldehyde. Taken together, these studies show how various conditions alter the course of the pyrrole–aldehyde condensation.

Introduction

For some time now we have been working to develop effective methods for the synthesis of porphyrins bearing diverse substituents in desired patterns at the four *meso*-positions. Porphyrins bearing four identical *meso*-substituents can be synthesized by the reaction of an aldehyde and pyrrole in a relatively simple two-step, one-flask procedure.[1] The first step involves acid-catalyzed condensation yielding oligomers and the cyclic porphyrinogen. The second step involves the oxidative conversion of the porphyrinogen to the porphyrin (Scheme 1). The overall process employs mild room-temperature conditions, has broad scope, and is easily implemented. Indeed, the apparent simplicity of this method has perhaps overshadowed the complexity of the underlying reaction processes.

Our previous studies to explore the reaction processes in porphyrin formation have involved measuring the yield of porphyrin as a function of a variety of reaction parameters. With this traditional approach we have made the following findings.

(1) The yield of the porphyrinogen (implied by the formation of the porphyrin upon oxidation) passes through a maximum and declines at long reaction times. In practice this yield trajectory requires that the condensation process be monitored over time so that the oxidation process can be initiated when the yield of porphyrinogen is at a maximum.[2,3]

(2) The yield is sensitive to the concentration of the reactants (aldehyde and pyrrole) and the concentration of acid. The maximum yield occurs with reactant concentrations of ~10 mM.[2,3]

(3) The condensation process is sensitive to the nature of the acid catalyst. Yields of tetraphenylporphyrin (TPP) in the range of 10–58% were observed with 35 of the 45 acids that were examined in a survey.[4] Cocatalytic phenomena leading to enhanced yields have been observed with BF_3–Et_2O and ethanol[5,6] or one of a variety of salts.[7]

(4) The condensation process yields products that undergo reversible interchange to some extent, as determined by double-label crossover (exchange) experiments.[2] In such experiments, the products of two separate condensation reactions (aldehyde A + pyrrole; aldehyde B + pyrrole) were mixed for a period of time prior to oxidation. Examination of the resulting porphyrin products showed the presence of reversible reaction processes. However, the initial condensation of a molecule of aldehyde with pyrrole was found to be irreversible by experiments using an isotopically labeled aldehyde.[7]

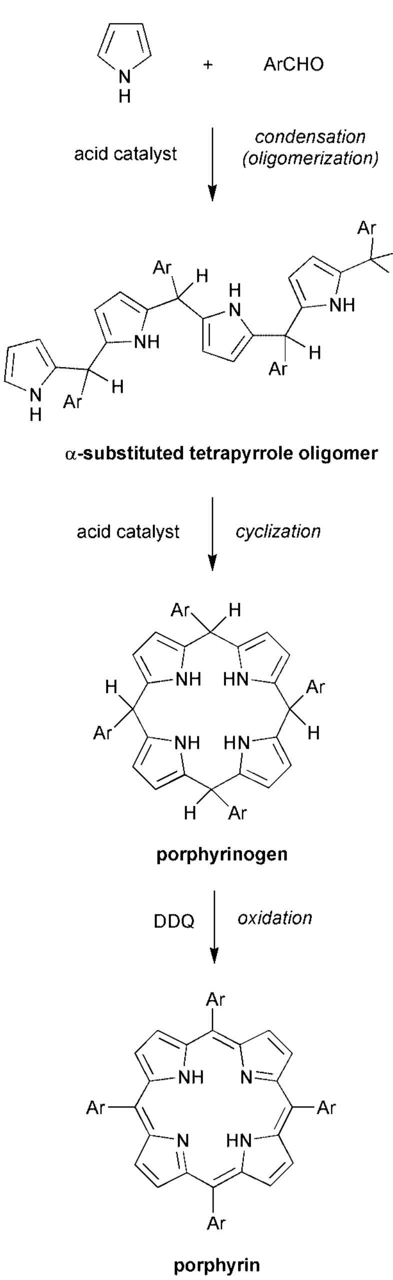
(5) Chromatographic separation of the oxidized reaction products enabled the yields of other macrocycles (N-confused porphyrin,[8,9] sapphyrin[10]) to be characterized.[11] The N-confused porphyrin is a universal byproduct accompanying formation of the porphyrin, while the sapphyrin is at most formed in trace quantities. The existence of these two macrocyclic products indicates (1) the formation of β-linkages and bipyrrolic linkages, respectively, in the condensation process rather than exclusive α-linkages as are present in the porphyrin, and (2) the presence of macrocycles other than the desired porphyrinogen.

While these studies of porphyrin yield under different reaction conditions have been fruitful for optimizing the reaction

---

† Electronic supplementary information (ESI) available: description of experiments probing the robustness and validity of the LD-MS and SEC analyses of the crude oxidized porphyrin reaction mixtures, and illustrative chromatograms from SEC analysis. See http://www.rsc.org/suppdata/p2/b0/b009088n/

DOI: 10.1039/b009088n



Scheme 1 Key stages in the reaction of pyrrole + aldehyde leading to porphyrin.

conditions, much less has been learned about the course of the condensation processes that give rise to these results. Indeed, a tremendous frustration in our studies of these reactions over the years has been the inability to examine the composition of oligomers formed in the condensation process. The oligomers are critical intermediates along the path from starting material to porphyrinogen and also are undesired reaction products. The oligomers are believed to constitute ~50% or more of the material derived from the starting materials. Examples of questions of fundamental importance that remain unanswered are as follows. (1) What is the composition of oligomers (length, end-group, extent of β-linkages and bipyrrolic linkages) and how does the composition change with time? (2) What is the pathway for oligomer growth (*e.g.*, sequential addition of pyrrole and aldehyde subunits or condensation of intermediate oligomers)? (3) Why does the yield pass through a maximum and then decline with time? (4) What is the mechanism by which certain reaction conditions, particularly the inclusion of salts, give rise to increased yields? (5) How does the condensation process change with dipyrromethane and dipyrromethane-carbinol ‡ species under conditions designed to suppress scrambling? (6) What factors affect reaction reversibility and how does reversibility change with different species (*e.g.*, oligomer length, α-linkages and β-linkages)? (7) How does the course of reaction change with different acid catalysts or with different substituents on the aldehyde moiety? (8) In total, what reaction model best describes the aldehyde–pyrrole condensation leading to the porphyrinogen? Clearly, these fundamental questions cannot be addressed by studies of porphyrin yield, but requires a body of data concerning the oligomer composition collected under a wide variety of conditions with various substrates. Our early attempts to examine the oligomers with ^{252}Cf plasma desorption mass spectrometry[12] yielded uninformative spectra,[13] and size exclusion chromatography (SEC) gave broad bands that provided only a very gross view of the general oligomer size.[2]

The relatively new technique of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been used with great efficacy to probe oligomer compositions.[14] Studies have been performed to characterize polymer molecular weight distributions, to analyze the composition of polymer end-groups, and to probe mechanisms of polymerization. We have found that this general mass spectral method can be employed in the absence of a matrix (laser desorption mass spectrometry, LD-MS) for the analysis of *meso*-substituted porphyrins[15] and multiporphyrin arrays of diverse composition.[16] We also have employed LD-MS for the detection of scrambling in syntheses of *trans*-A_2B_2-porphyrins from the condensation of a dipyrromethane + aldehyde.[17] In this series of papers, we have extended the use of LD-MS to the examination of crude, unpurified, oxidized porphyrin reaction mixtures for qualitative assessment of the oligomer composition. It is noteworthy that our use of LD-MS differs in several regards from the use of MALDI-TOF-MS for polymer characterization.[18] Additional analytical techniques previously utilized to quantitate the yields of porphyrin, N-confused porphyrin, sapphyrin (UV–Vis, HPLC)[11] and unreacted aldehyde (TLC)[7] have also been employed.

In this paper, we describe the use of LD-MS to examine the oligomer composition obtained from the reaction of pyrrole and benzaldehyde. The effects of key reaction parameters (reaction time, concentration of reactants, ratio of reactants, and TFA or BF_3–Et_2O concentration) were examined. These experiments provide insight concerning oligomer formation, turnover in porphyrinogen yield, and reactivity of intermediate oligomers. The second paper presents experiments probing reaction reversibility, acid catalyst stability, reaction homogeneity, and the reaction pathway.[19] The third paper investigates the origin of scrambling under different reaction conditions in the reaction of a dipyrromethane and an aldehyde to prepare a *trans*-substituted porphyrin.[20] The fourth paper explores the reactivity of carbinol-derivatized pyrrolic species which are germane to the rational synthesis of a wide variety of porphyrinic compounds,[21] including *trans*-porphyrins,[22] porphyrins bearing up to four different *meso*-substituents,[23] chlorins bearing designated patterns of substituents,[24,25] and heteroatom-substituted porphyrins[26] and corroles.[27] A study of the effects of 45 different acids (including cocatalysis) on the oligomer composition and a comparison of the reaction course with diverse aldehydes will be published elsewhere. Taken together, these studies provide a level of insight into the course of various routes to porphyrins that has not been available by the traditional method involving porphyrin yield determinations or with other analytical methods.

---

‡ The term carbinol has been abandoned by IUPAC; the IUPAC term for carbinol is a substituted methanol.

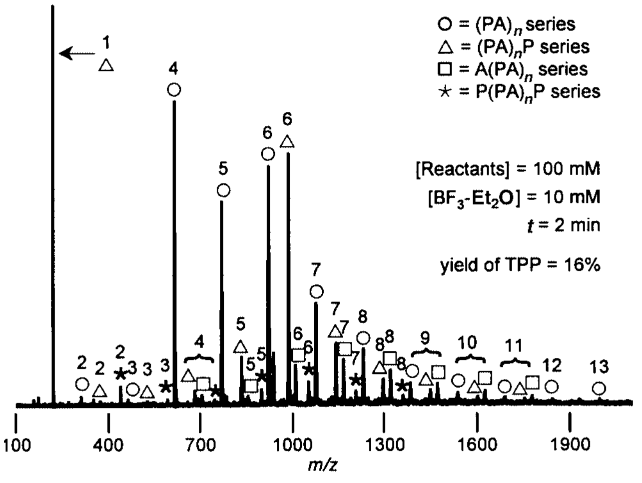


Fig. 1 A representative LD-MS spectrum from a crude oxidized reaction mixture of pyrrole + benzaldehyde (100 mM pyrrole + benzaldehyde, 10 mM BF_3–Et_2O, CH_2Cl_2, room temperature, 2 min). The oligomer length (*n*) is given by the number above the symbol. The yield of TPP (UV–Vis) is noted.

Results and discussion

Examination of oligomers by LD-MS

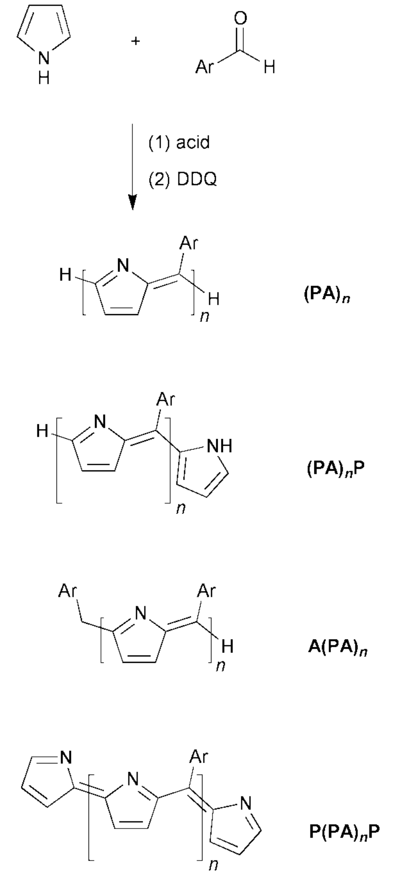
We sought analytical methodology for characterizing the oligomers formed in the condensation of pyrrole + aldehyde that met the following criteria: (1) enable analysis of crude reaction mixtures without work-up so as to not alter the oligomer composition; (2) provide sufficient resolution to distinguish oligomers differing by single pyrrole or aldehyde units; and (3) afford rapid and robust analysis. LD-MS analysis has been found to meet these demands. Sample preparation for LD-MS analysis simply involved spotting an aliquot of the crude, DDQ-oxidized reaction mixture on the LD-MS target.§ The oxidized oligomers are stable while deposited on the target, awaiting LD-MS analysis. The robustness and validity of the LD-MS methodology has been examined thoroughly and found to be excellent (see supplementary information).

A representative LD-MS spectrum of a crude, oxidized sample from a reaction of pyrrole + benzaldehyde ("reactants") is shown in Fig. 1. The reaction was performed at 100 mM reactants in the presence of 10 mM BF_3–Et_2O in CH_2Cl_2 at room temperature for 2 min prior to oxidation with DDQ, affording TPP in 16% yield. Peaks in the range of *m*/*z* 100–2000 can be assigned to oligomers formed from pyrrole–aldehyde condensation (Scheme 2). Three distinct oligomer series are defined based on the groups present at the oligomer chain termini: (1) a pyrrole terminus and an aldehyde terminus, (PA)$_n$ series; (2) pyrrole units at both termini, (PA)$_n$P series; and (3) aldehyde units at both termini, A(PA)$_n$ series; where *n* is the number of repeating pyrrole–aldehyde (PA) units. A handful of unassigned peaks were accounted for by a fourth oligomer series containing alternating pyrrole–aldehyde units, an internal 2,2′-bipyrrole group, and pyrrole groups at both termini, P(PA)$_n$P series. The key distinction between each of the four oligomer series lies in the ratio of pyrrole to aldehyde units. The calculated molecular weights for the first four members of each series are shown in Table 1. The calculated weights are for the fully unsaturated form of each open-chain oligomer. A peak was considered a member of an oligomer series if the *m*/*z* value fell within 0.2% or ±2 of the calculated value.¶

Table 1 Calculated molecular weight of oligomers[a]

	Oligomer series			
n	(PA)$_n$	(PA)$_n$P	A(PA)$_n$	P(PA)$_n$P
1	155	220	245	285
2	308	373	389	438
3	461	526	551	591
4	614	679	704	744

[a] The oligomer masses increase with *n* according to ($MW_{n+1} = MW_n + 153$). The oligomers are fully unsaturated (see Scheme 2).



Scheme 2 Expected oligomers present in crude, oxidized reaction mixtures from the pyrrole + aldehyde reaction.

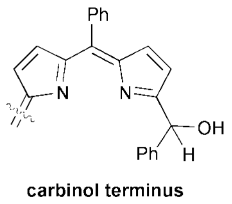
The LD-MS spectra reveal qualitative information concerning the oligomers present in the crude reaction mixture, including (1) the range of oligomer size (peaks up to *m*/*z* 2800 can be reliably detected), and (2) the presence of oligomers from the four series. Nearly all peaks in all LD-MS spectra (>1000 exam-

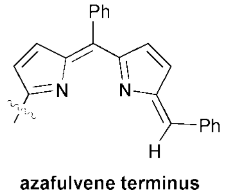
---

§ Analysis of the oligomers without DDQ oxidation was possible. However, the increased conjugation achieved upon DDQ oxidation resulted in increased signal intensity.

¶ The appearance of the peak envelope for a given oligomer in a series was consistent with the presence of multiple species. For example, the peak envelope for (PA)$_4$ generally showed peaks of increasing intensity from *m*/*z* 613 to 615, then of decreasing intensity from *m*/*z* 615 to 620. The overall shape of the envelope approximated a Gaussian distribution. This observation contrasts with the peak envelope obtained from pure TPP. In the spectrum of TPP there are no peaks at *m*/*z* < 614, the peak at 614 is the most intense, and peaks at increasing units of *m*/*z* show the characteristic declining intensity of isotope peaks.[15] Although the peak envelopes observed from the crude reaction mixtures suggest the presence of multiple species (attributed to a given oligomer in linear or cyclic form, and the occurrence of such oligomers of the same number of monomer units with different degrees of oxidation), the breadth of the peak envelope does not impede the assignment of the envelope to a particular oligomer. The mass unit difference imparted by slight variability in the number of hydrogens attached to a given oligomer is much smaller than the mass difference that accrues upon incorporation of one monomer unit. Finally, no evidence of multiply charged ions was observed. Peaks of *m*/*z* < 1200 were generally detected with unit resolution and a peak spacing of one mass unit, which is consistent with the predominant presence of singly charged ions.

ined) fit into one of the four series. A small number of minor peaks were observed at 16–18 mass units higher than peaks in the $(PA)_n$ series or 16–18 and 32–36 mass units higher than peaks in the $A(PA)_n$ series. These minor peaks are assigned to carbinol-terminated oligomers, whereas the major peaks bear azafulvene termini. As expected, no similar minor peaks were observed for the $(PA)_nP$ or $P(PA)_nP$ series. The only remaining unassigned peaks appearing with any regularity were found at *m*/*z* 352 and 658.





There are two limitations in interpreting LD-MS spectra. (1) The mass spectral assignments do not imply precise structures. Within each series it is possible to have various orderings of pyrrole and benzaldehyde units, both α- and β-linkages, and cyclic oligomers. For example, the peak from $(PA)_4$ may be due to porphyrin, N-confused porphyrin, and/or other linear products of the same mass. (2) Peak intensities are not necessarily directly proportional to actual oligomer abundance owing to differential ionization efficiencies and detector response.[14] Thus, the interpretation of LD-MS spectra is restricted to qualitative and comparative analyses.

Oligomers in pyrrole + benzaldehyde condensations

The reaction of pyrrole + benzaldehyde providing TPP was performed under conditions previously found to be optimal.[2] The reactions were monitored at time points from 15 s to 24 h. At each time point the reaction was analyzed for the yield of TPP (UV–Vis), amount of unreacted benzaldehyde (TLC), and oligomer content (LD-MS).

Selected time points from a reaction of pyrrole + benzaldehyde (10 mM each) under TFA catalysis are shown in Fig. 2. Initially, the oligomers increased in size as the yield of porphyrin increased and the aldehyde was consumed. The predominant oligomers formed early in the reaction belonged to the $(PA)_n$, $P(PA)_nP$, and $(PA)_nP$ series, indicating the greater reactivity of carbinol end-groups toward pyrrole than of pyrrole end-groups toward the aldehyde. The early appearance of the dipyrromethane, $(PA)_1P$, is consistent with this trend. At 1 h when the yield of porphyrin was near its maximum and ~5% of the benzaldehyde remained, peaks from all four oligomer series were observed. We refer to the presence of the greatest range of different oligomers as the maximum in the oligomer diversity. The increase in oligomer size from 15 s to 1 h occurred without significant peaks from oligomers with $n = 2–3$, raising the question as to whether those oligomers are bypassed (by dipyrromethane + aldehyde condensations) or are so reactive that their concentration remains low. In contrast, oligomer growth from $n = 4$ and above proceeded without skipping any generations. The dominant features at reaction times greater than 1 h are as follows: (1) the aldehyde was below the limit of detection (0.1 mM; *i.e.*, <1%), (2) the yield of TPP declined, (3)

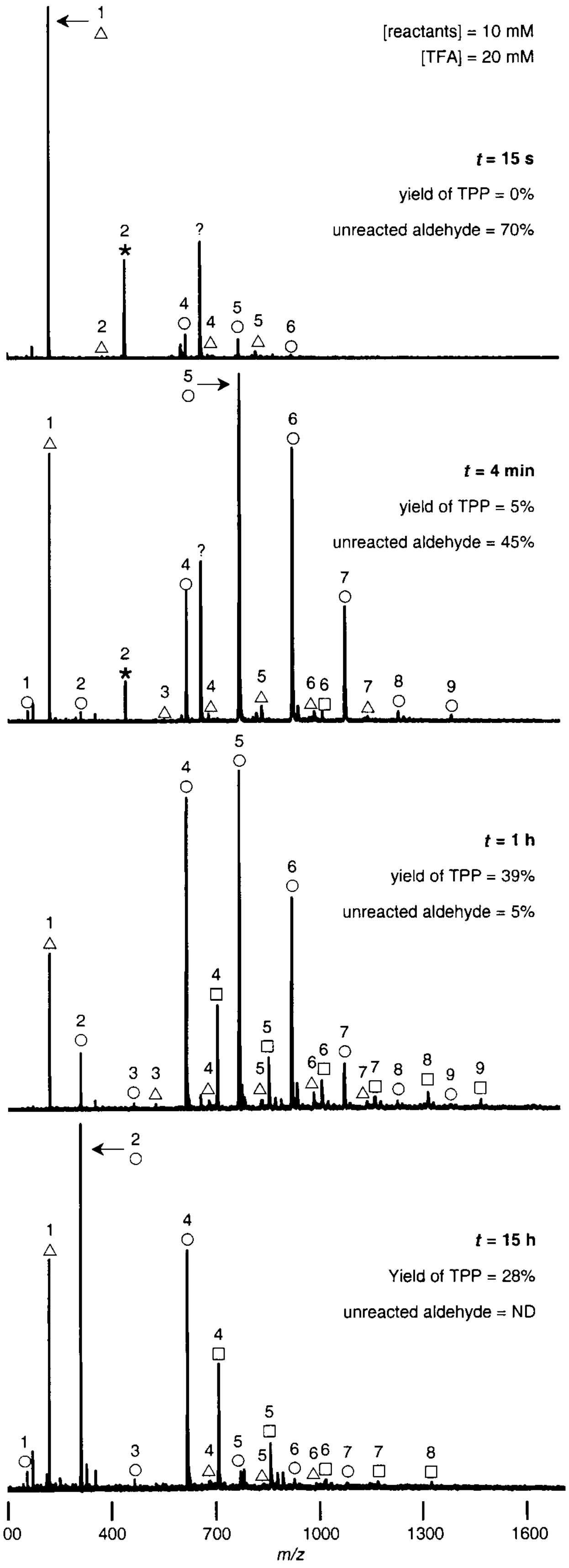


Fig. 2 LD-MS spectra from crude oxidized reaction mixtures obtained over time (15 s, 4 min, 1 h, and 15 h) from the reaction of 10 mM pyrrole + benzaldehyde with TFA catalysis (20 mM TFA, CH_2Cl_2, room temperature). The yield of TPP (UV–Vis) and the level of unreacted benzaldehyde (TLC) are noted for each reaction time. ○ = $(PA)_n$ series, △ = $(PA)_nP$ series, □ = $A(PA)_n$ series, ★ = $P(PA)_nP$ series. Significant peaks that could not be assigned to one of the four oligomer series are denoted with a "?". The oligomer length (*n*) is given by the number above the symbol.

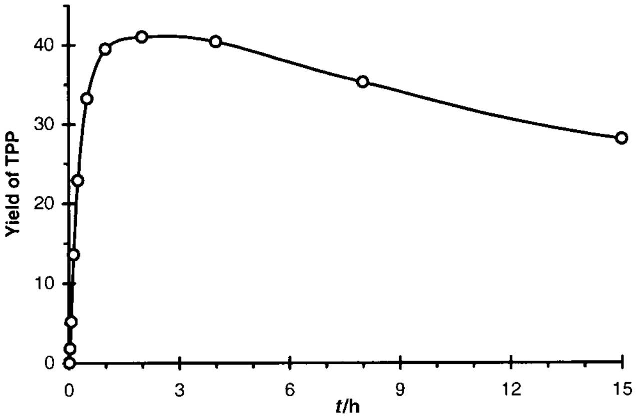


Fig. 3 Yield of TPP as a function of reaction time (10 mM reactants, 20 mM TFA, CH_2Cl_2, room temperature).

the oligomer composition was truncated to shorter species with the major peaks belonging to the $(PA)_n$ and $A(PA)_n$ series, and low *m*/*z* peaks not present earlier in the reaction became dominant. Oligomer growth did not proceed much beyond $n = 9$ in this reaction (control experiments show that oligomers up to $n = 15$ can be readily detected by LD-MS analysis).

These findings provide insight into the origin of the decline in yield of TPP at long reaction times in the TFA-catalyzed reaction. As shown in Fig. 3, the yield of TPP (and hence the porphyrinogen precursor) increases, reaches a plateau, then declines. The origin of the decline in yield of the porphyrinogen has been the subject of speculation. We proposed that the porphyrinogen is a thermodynamically stable product formed under equilibrium conditions, coupled to which are irreversible side reactions.[28] An alternative proposal is that the porphyrinogen is a kinetically trapped intermediate that is eventually converted to long, linear oligomers.[29] LD-MS analysis indicates that under these reaction conditions, oligomers undergo truncation at long reaction times rather than extended polymerization. Furthermore, the truncated oligomers are new species not present to a large extent prior to the turnover in the yield of TPP (*e.g.*, $(PA)_2$). Analysis by low resolution SEC supports these findings (see supplementary information). These data are consistent with our hypothesis that irreversible side reactions are the source of turnover in yield of porphyrin. The identity of the side reactions is not known but they likely involve damage to the ends of the oligomers, thereby preventing condensation and/or cyclization.

The BF_3–Et_2O catalyzed reaction of pyrrole + benzaldehyde (10 mM each) was monitored over time (Fig. 4). In accord with the TFA reaction, benzaldehyde appeared to react more slowly than pyrrole with the growing oligomers, the dipyrromethane species $(PA)_1P$ was a key peak, oligomer generations $n = 2$–3 were not observed, and the maximum yield of TPP and the maximum diversity of oligomer content were obtained at the same time. In contrast with the TFA reaction, benzaldehyde reacted more slowly (~30% remained at 1 h), the decline in yield of porphyrin was only ~2%, and the oligomer content changed little from 1 to 15 h.

Though the data in Figs. 2 and 4 are drawn from only two experiments, the results show the outline of two correlations that emerge from the more extensive studies reported in the subsequent sections. (1) The maximum yield of TPP and the maximum diversity of oligomer content (particularly regarding the relative intensities of the $A(PA)_n$ and $(PA)_nP$ series peaks) are attained at similar times. (2) The onset of the truncation of oligomers and the decline in yield of TPP are accompanied by the decrease of the aldehyde to low levels.

Oligomer formation as a function of reaction parameters

Understanding the effects of key reaction parameters on the product distribution is essential for refining synthetic methodology in a rational manner. We previously have made several observations concerning the effects of reaction parameters on

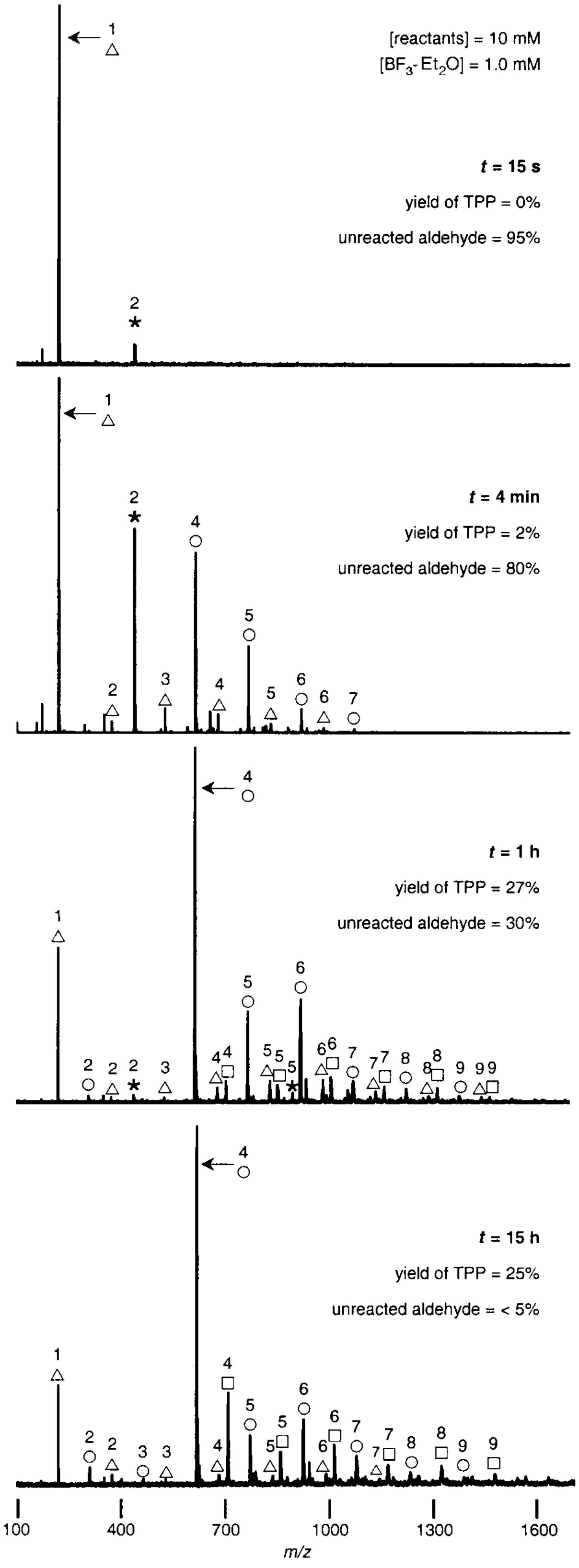


Fig. 4 LD-MS spectra from crude oxidized reaction mixtures obtained over time (15 s, 4 min, 1 h, and 15 h) from the reaction of 10 mM pyrrole + benzaldehyde with BF_3–Et_2O catalysis (1.0 mM BF_3–Et_2O, CH_2Cl_2, room temperature). The yield of TPP (UV–Vis) and the level of unreacted benzaldehyde (TLC) are noted for each reaction time. ○ = $(PA)_n$ series, △ = $(PA)_nP$ series, □ = $A(PA)_n$ series, ★ = $P(PA)_nP$ series. The oligomer length (*n*) is given by the number above the symbol.

the rate and yield of the porphyrin-forming reaction. (1) The yield of porphyrin is quite sensitive to the concentration of reactants, with the highest yield at intermediate concentration (10 mM) and low yields at 1 mM or 100 mM.[2] (2) The falloff in yield at high concentration can be largely ameliorated with a higher concentration of acid.[3] (3) The rate of reaction increases with concentration and also is dependent on acid concentration.[3] (4) The yield of porphyrin passes through a maximum and declines, though the time at which the optimal yield is obtained varies with the concentration of the reactants and the acid concentration.[2,3] While our prior studies examined primarily the yield of porphyrin, experiments performed herein examined the effects on the oligomer composition of the concentration of reactants, the ratio of reactants, and the acid concentration for both TFA catalysis and BF_3–Et_2O catalysis.

(i) Concentration of reactants. Data from reactions performed for 1.0, 10, 32, and 100 mM reactants (equimolar pyrrole and benzaldehyde) with 1.0 mM BF_3–Et_2O in CH_2Cl_2 at 1 h are shown in Fig. 5. In comparing the four reactions, the oligomer content measured at 1 h was shifted to longer species with increasing concentration of reactants. An intermediate concentration (32 mM) gave oligomers of intermediate size as well as the highest yield of TPP. The increase in oligomer size as a function of the concentration of reactants occurred despite the observation of progressively more unreacted benzaldehyde at the higher reactant concentrations (none detected with 1.0 mM and 40% detected with 100 mM reactants; limits of detection = 0.1 mM).

The reactions at the four different concentrations were monitored over time. The reaction at 1.0 mM did not provide any detectable quantity of TPP over the course of 8 h. Although oligomers with up to $m/z = 1000$ did eventually form, lower molecular weight material appeared to dominate. The reaction at 10 mM yielded little change in oligomer content and a slight decrease in the yield of TPP, even though the quantity of unreacted benzaldehyde declined from ~30% to <5% from 1 to 8 h. The reaction at 32 mM behaved similarly with the exception of a slight increase in the yield of TPP (to 39%) at 8 h accompanied by a decrease in unreacted benzaldehyde to ~5%. In none of the previous three reactions did the oligomer content ever resemble that of the 100 mM reaction, even at long reaction times. The reaction at 100 mM provided a yield of TPP of 24% with ~30% unreacted benzaldehyde, both of which remained unchanged from 2 to 8 h. These results support the conclusion that the optimal yield of porphyrin is obtained under reaction conditions which (1) establish an oligomer composition of appropriate length and diversity, and (2) enable the efficient utilization of aldehyde. Furthermore, the oligomer content depends on the initial concentration of reactants in a predictable way, despite the changing ratio of reactants to acid and the differences in the level of unreacted aldehyde.

Additional experiments performed with reactant concentrations ranging from 0.32 to 320 mM in the presence of BF_3–Et_2O (10 mM) or TFA (20 mM, 64 mM) supported these conclusions. A key difference between TFA and BF_3–Et_2O was that with TFA catalysis, the level of unreacted benzaldehyde was <10% by 1 h under any reactant concentrations examined. Thus, with TFA the correlation of the oligomer content and the yield of TPP was not complicated by the presence of variable quantities of unreacted aldehyde as occurred with BF_3–Et_2O. With BF_3–Et_2O catalysis, the lower yield of porphyrin could stem from insufficient extent of reaction (measured by the utilization of the aldehyde) or an inappropriate oligomer composition.

(ii) Acid concentration. The concentration of the acid catalyst is a key parameter in the optimization of porphyrin syntheses. Knowing the oligomer content at different acid concentrations would help understanding of how the acid concentration affects

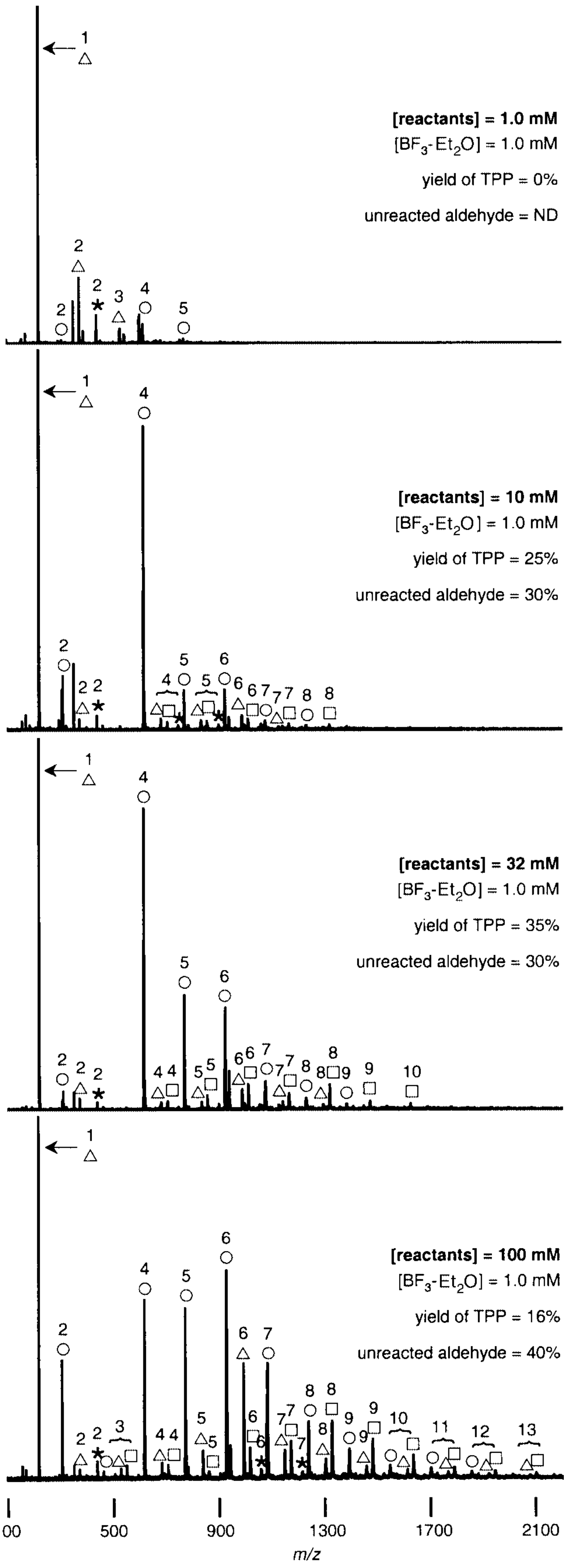


Fig. 5 LD-MS spectra of crude oxidized reaction mixtures showing the effect of concentration of reactants (pyrrole + benzaldehyde) on the oligomer composition for condensations catalyzed by 1.0 mM BF_3–Et_2O. Reactions were performed in CH_2Cl_2 at room temperature for 1 h. The yield of TPP (UV–Vis) and the level of unreacted benzaldehyde (TLC) are noted for each concentration of reactants. ○ = $(PA)_n$ series, △ = $(PA)_nP$ series, □ = $A(PA)_n$ series, ★ = $P(PA)_nP$ series. The oligomer length (n) is given by the number above the symbol.

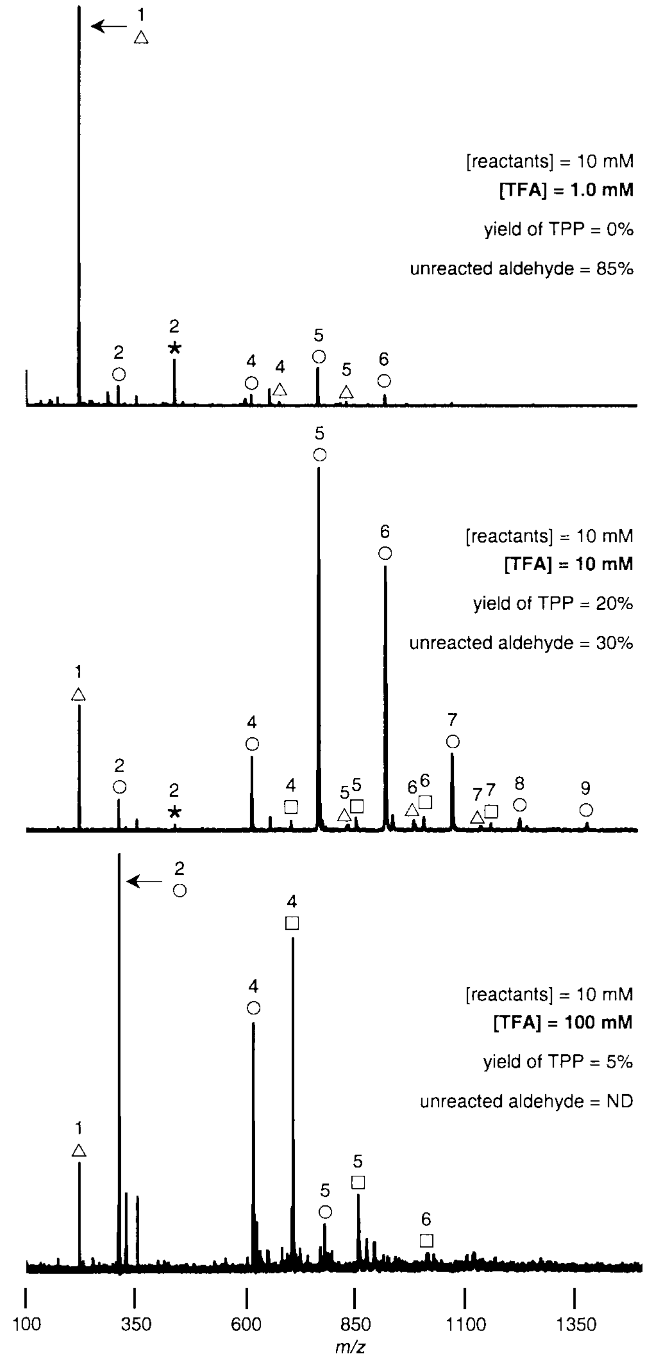


Fig. 6 LD-MS spectra of crude oxidized reaction mixtures showing the effect of TFA concentration on the oligomer composition obtained from the condensation of 10 mM pyrrole + benzaldehyde. Reactions were performed in CH_2Cl_2 at room temperature for 1 h. The yield of TPP (UV–Vis) and the level of unreacted benzaldehyde (TLC) are noted for each TFA concentration. ○ = $(PA)_n$ series, △ = $(PA)_nP$ series, □ = $A(PA)_n$ series, ★ = $P(PA)_nP$ series. The oligomer length (*n*) is given by the number above the symbol.

the course of reaction. The oligomer content from reactions of 10 mM reactants with 1.0, 10, and 100 mM TFA at 1 h is shown in Fig. 6. At low acid concentration (1.0 mM), the oligomers were generally of low molecular weight, most of the aldehyde was unreacted, and no TPP was formed. At 10 mM TFA, longer oligomers were present, ~30% unreacted aldehyde remained, and a good yield of TPP was obtained (~20%). At 100 mM TFA, truncated oligomers were present (predominately $(PA)_n$ and $A(PA)_n$ series peaks), no unreacted aldehyde was detected, and a low yield of TPP was obtained (~5%). The three LD-MS spectra mirror the time-evolution data from experiments at fixed acid concentration (Figs. 2 and 4).

The time course of the reaction at each acid concentration was monitored from 1 min to 8 h. The reaction with 1 mM TFA eventually produced an oligomer content at 4 h similar to that with 10 mM TFA at 1 h. Nevertheless, ~80% of the aldehyde was still unreacted and the yield of TPP was only ~2%. This result showed that long oligomers can form even when most of the aldehyde has not reacted, indicating that the intermediate oligomers are more reactive than the starting material.|| The yield of TPP obtained in the reaction with 10 mM TFA increased to 35% by 8 h while the unreacted aldehyde decreased to <5% over the same period. At the longer reaction times the oligomer content showed truncation to shorter oligomers and a shift from $(PA)_nP$ to $A(PA)_n$ peaks similar to that shown in Fig. 2. The reaction with 100 mM TFA also provided a high yield of TPP (38%), but at a much earlier stage in the reaction (4 min). At that time the oligomer content was similar to that at the 1 h time point in Fig. 4, and ~5% unreacted aldehyde was present. At longer reaction times, the yield of TPP declined to 0% (at 2 h), the aldehyde level was below the limit of detection (at 15 min), and the oligomer content showed dramatic truncation until the $(PA)_2$ and $A(PA)_4$ peaks were essentially the only peaks in the mass spectrum (8 h). Thus, under these conditions, the yield of TPP appeared to reach its maximum at the point when the level of unreacted aldehyde declined to <5–10%. As the aldehyde level decreased further, the $A(PA)_n$ series peaks began to increase in intensity and the yield of TPP declined. Additional experiments at 10 and 100 mM reactants with variable concentrations of BF_3–Et_2O and TFA were consistent with these results.

These observations indicate that a range of acid concentrations can provide good yields of porphyrin at a given concentration of reactants provided the reaction time is adjusted, with low acid concentration requiring longer times than higher acid concentration. But at very low acid concentration, little reaction occurs and no porphyrin is produced, and at very high acid concentration, the yield of porphyrin spikes quickly then falls quickly to 0%. The acid concentration affects the kinetics of the pyrrole–aldehyde reaction and the formation of truncated oligomers. Nevertheless, whether the reaction is fast or slow, the maximum yield of porphyrin, concentration of unreacted aldehyde, and oligomer content obtained tend to exhibit the same respective features at the time of maximal yield of porphyrin.

(iii) Ratio of reactants. Aldehyde and pyrrole are generally reacted in a 1 : 1 ratio in porphyrin syntheses. However, the use of an excess of pyrrole to achieve the synthesis of N-confused porphyrin[8] and sapphyrin[10] prompted examination of the effect of altered ratios on the reaction. The oligomer composition at 1 h for reactions with 1.0 mM BF_3–Et_2O, 10 mM pyrrole, and 5.0, 10, or 20 mM benzaldehyde is shown in Fig. 7. All conditions produced yields of porphyrin ranging from ~20% to ~40% (based on the limiting reactant). The oligomer content shifted from $(PA)_n$ and $(PA)_nP$ peaks when pyrrole was in excess to $(PA)_n$ and $A(PA)_n$ peaks when benzaldehyde was in excess. At an 8-fold excess of benzaldehyde (not shown), significant $(PA)_n$ peaks and oligomers up to *m*/*z* 1500 were observed, though the yield of TPP was decreased to 25%. Reactions performed at 10 mM benzaldehyde and 5.0, 10, and 20 mM pyrrole (Fig. 8) showed a shift in the oligomer content toward $(PA)_1P$ as the ratio of pyrrole to aldehyde increased. The yield of TPP was initially insensitive to the change, but at 4-fold and 8-fold excesses of pyrrole a sharp decline was observed (15% and 4%, respectively). Experiments performed with the fixed reactant held at 100 mM were also consistent with these

---

|| This finding illustrates that LD-MS only assesses the oligomer content, not the extent of reaction. Other analytical methods are needed to determine whether the oligomer content represents 10% or 100% of the starting materials.

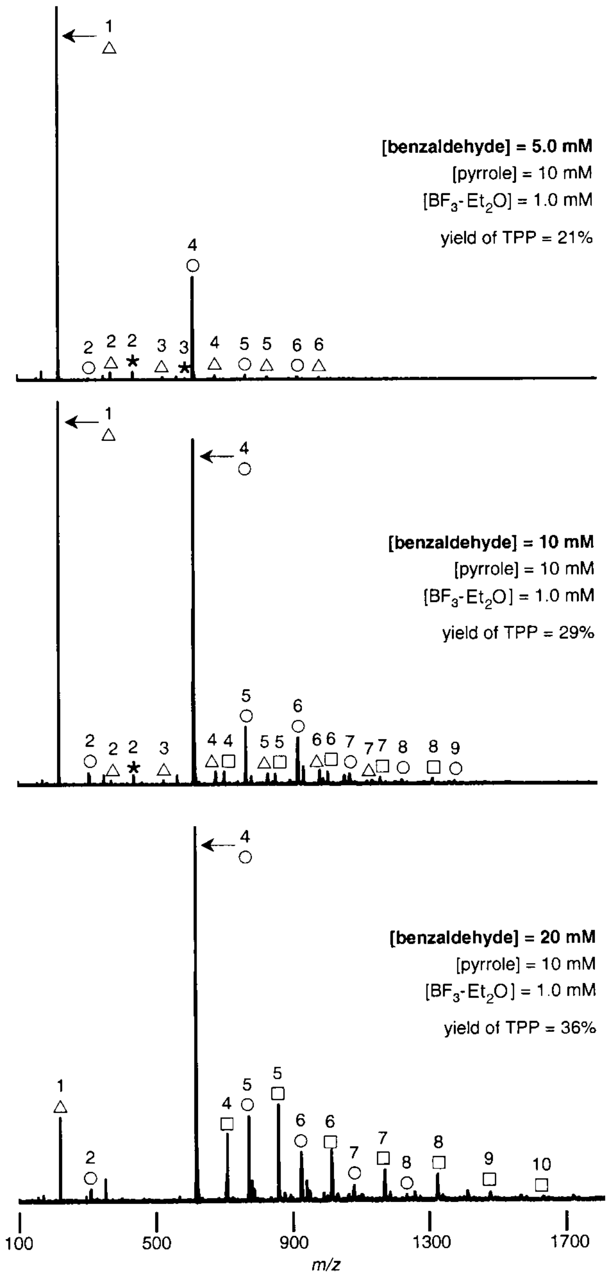


Fig. 7 LD-MS spectra of crude oxidized reaction mixtures showing the effect of the pyrrole : benzaldehyde ratio (fixed pyrrole) on the oligomer composition. Reactions were performed with 1.0 mM BF$_3$–Et$_2$O in CH$_2$Cl$_2$ at room temperature for 1 h. The yield of TPP (UV–Vis) is noted for each benzaldehyde concentration. ○ = (PA)$_n$ series, △ = (PA)$_n$P series, □ = A(PA)$_n$ series, ★ = P(PA)$_n$P series. The oligomer length (n) is given by the number above the symbol.

results, though generally the maximum yield of TPP was obtained at equimolar pyrrole and benzaldehyde.

The predominance of (PA)$_1$P in excess pyrrole is reminiscent of the one-flask synthesis of dipyrromethanes.[30] This observation indicates that the pyrromethanecarbinol (formed upon pyrrole–aldehyde reaction) undergoes rapid reaction with pyrrole, while the pyrrole + aldehyde addition is slower. In the case of excess benzaldehyde, significant growth of oligomers was still observed even with a large excess of benzaldehyde (4- to 8-fold).** This observation also reflects the faster reaction of the growing oligomers bearing pyrrole-carbinol end-groups or equivalent with pyrrole than of oligomers bearing pyrrole end-groups with free benzaldehyde. The greater facility of the pyrrole-carbinol + pyrrole condensation (compared with the aldehyde + pyrrole addition) has been exploited in porphyrin chemistry under mild reaction conditions: pyrrole-carbinol species provide the foundation for the rational synthesis of porphyrins bearing up to four different *meso*-substituents,[23] the self-condensation of dipyrromethane-monocarbinols yielding *trans*-substituted porphyrins,[22] and the synthesis of chlorin building blocks.[24,25]

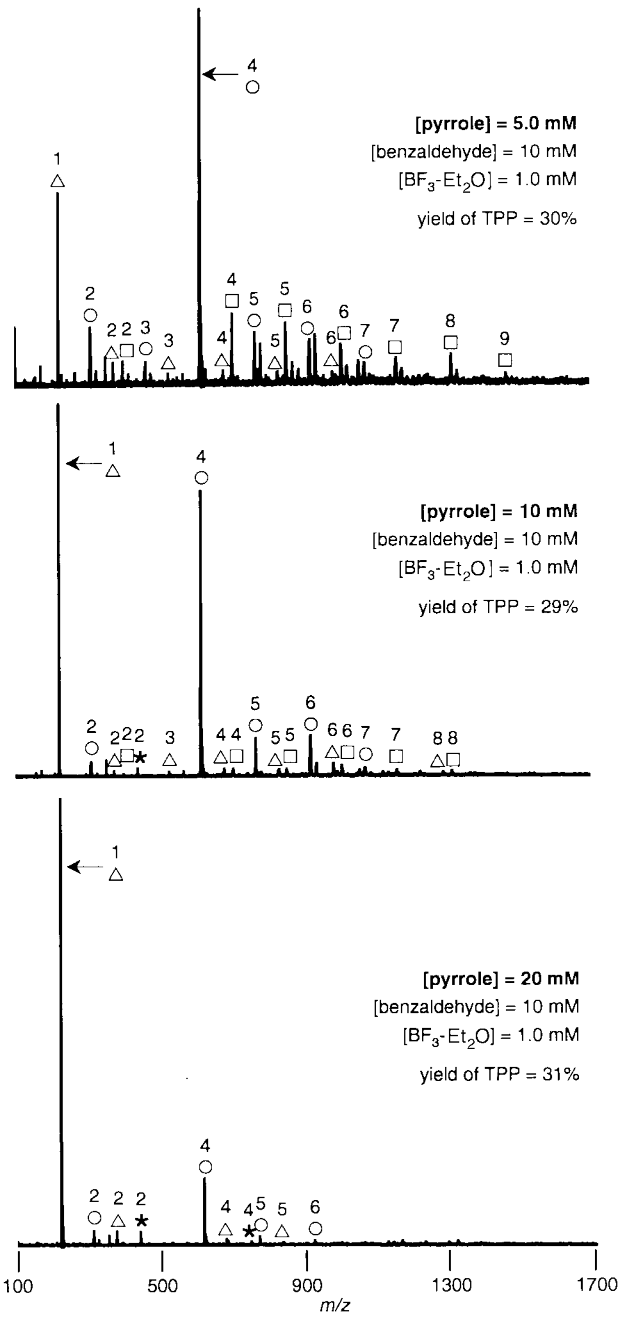


Fig. 8 LD-MS spectra of crude oxidized reaction mixtures showing the effect of the pyrrole : benzaldehyde ratio (fixed benzaldehyde) on the oligomer composition. Reactions were performed with 1.0 mM BF$_3$–Et$_2$O in CH$_2$Cl$_2$ at room temperature for 1 h. The yield of TPP (UV–Vis) is noted for each pyrrole concentration. ○ = (PA)$_n$ series, △ = (PA)$_n$P series, □ = A(PA)$_n$ series, ★ = P(PA)$_n$P series. The oligomer length (n) is given by the number above the symbol.

Conclusions

Examination of the oligomers formed upon pyrrole–aldehyde

** Peaks due to oligomers (PA)$_n$ (n = 4–8) were observed in reactions even with an 8-fold excess of aldehyde, indicating the inability of an excess of aldehyde to cap the pyrrolic termini of such oligomers. One interpretation is that those oligomers exhibited conformations (*e.g.*, cyclic or coiled) that precluded reaction of the terminus with excess aldehyde.

condensation has revealed a number of facets of the porphyrin reaction not available from studies where the yield of porphyrin was the only quantifiable parameter. Evidence has been provided that the turnover in porphyrin yield at long reaction times stems from truncation of oligomers rather than from formation of longer oligomers. Experiments examining key reaction parameters for a given concentration of reactants revealed that most conditions affording good yields of porphyrin gave similar oligomer compositions. Thus, there appears to be a number of reaction conditions involving the interplay of acid concentration, acid identity, concentration of reactants, and time that afford similar, suitable yields of porphyrin, rather than a single optimal set. Two striking correlations noted throughout this work are as follows. (1) The maximum yield of TPP and the maximum diversity of oligomer content (particularly in regard to the relative intensities of $A(PA)_n$ and $(PA)_nP$ series peaks) were attained at similar times. (2) The onset of the truncation of oligomers and the decline in yield of TPP were accompanied by the decrease of the aldehyde to low levels. The origin of these correlations is not clear. Perhaps the truncation of oligomers is due to acid-mediated processes that become competitive only when the free aldehyde has fallen to a low concentration. These insights have come from use of LD-MS to examine the oligomer content of crude oxidized mixtures from two-step, one-flask syntheses of *meso*-substituted porphyrins. The following paper[19] extends this approach to probe features of the reaction course more complicated than the general analysis of oligomers described herein.

Experimental

Materials

Pyrrole (Acros) was distilled from calcium hydride and stored at −15 °C until use. The distilled pyrrole was used prior to the appearance of any discoloration. CH_2Cl_2 (Fisher, ACS grade) was distilled from potassium carbonate and stored over 4 Å Linde molecular sieves. Benzaldehyde (Aldrich, 99.5%), TFA (Aldrich), BF_3–Et_2O (Aldrich, redistilled grade), DDQ (Aldrich), and α-cyano-4-hydroxycinnamic acid (Aldrich, 97%) were used as received. All other materials and solvents were used as received.

General reaction conditions

CH_2Cl_2, pyrrole, and benzaldehyde were dispensed into 20 mL vials containing a micro stir bar. The volume of CH_2Cl_2 was selected so that the final volume of the reaction mixture would be 10 mL, assuming additivity of volumes. Benzaldehyde and pyrrole were dispensed as neat reagent, or as 1 M or 0.1 M stock solutions in CH_2Cl_2 depending on the desired final concentration. The reactions were initiated by addition of TFA or BF_3–Et_2O (neat acid, 1 M, or 0.1 M stock solutions in CH_2Cl_2). The reaction mixtures were stirred in tightly capped vials shielded from light for the desired length of time. The reactions were terminated by addition of DDQ. For 10 mM reactions, a 1.5 molar excess of DDQ (35 mg, 0.15 mmol) was used. For reactions at higher concentration, 0.85 equiv. of DDQ (based on limiting pyrrole or benzaldehyde; 200 mg for 100 mM reactions) was used. An LD-MS target was spotted with 1 µL of the crude reaction mixture (no matrix) 1–5 min after DDQ addition. After acid neutralization by 8 equiv. of triethylamine, the yield of TPP was determined spectroscopically by UV–Vis analysis as described previously.[2] The level of unreacted benzaldehyde was estimated by TLC as described previously [silica, CH_2Cl_2–hexanes, (1 : 1)].[7] GC Analysis of the crude, filtered reaction mixtures was used to confirm selected TLC values [35 °C (5 min) to 310 °C (2 min) at 40 °C min^{-1}, HP-5, 30 m × 0.32 mm × 0.25 µm]. Extensive GC analysis was not done due to inlet contamination by non-volatile components.

LD-MS Analysis

LD-MS Spectra were recorded using a linear TOF instrument (Bruker Proflex II) equipped with a nitrogen laser (337 nm, 3 ns pulse width), 1.2 m flight tube, dual multichannel plate detector, and a 1 GHz digitizer. Mass accuracy is 0.1% with external standards. Instrument calibration was performed each day immediately after data acquisition using α-cyano-4-hydroxycinnamic acid (*m*/*z* 379.09, $[2M + H]^+$), angiotensin II or angiotensin III (respectively *m*/*z* 1046.54 and 931.51, $[M + H]^+$), and bombesin (*m*/*z* 1619.82, $[M + H]^+$). Crude reaction samples were analyzed in the absence of added matrix. Spectra were averaged over 150 to 200 shots from at least two locations on the target. Samples were analyzed after all reactions for one day were completed. Samples spent between 3 and 10 h on the target prior to LD-MS analysis. A description of control and validation studies is provided in the supplementary information.

Kinetics experiments

Reactions were performed on a 45 mL scale as described (see General reaction conditions above) with the exception that a 100 mL round-bottomed flask under argon was used rather than a capped vial. At each time point, 1 mL of the crude reaction mixture was removed (by a 5 mL adjustable pipetter equipped with a Pasteur pipette) and transferred to a 1 dram vial containing DDQ (4 mg for 10 mM reactions and 20 mg for 100 mM reactions). The LD-MS target was spotted with 1 µL of the crude reaction mixture; UV–Vis and TLC analyses were done as described above.

SEC Analysis

Analytical SEC was performed using a Hewlett-Packard 1100 series HPLC equipped with a quaternary pump, autosampler, column compartment heater, and UV–Vis diode array detector as described previously.[2] A 50 Å organic SEC column was used (PLgel, 5 µm, 7.5 × 300 mm, Hewlett-Packard) with THF at 1 mL min^{-1} at 35 °C. The detection wavelengths were 275, 325, 375, 425, and 475 nm; each with a bandwidth of 50 nm. Each sample was analyzed within 5 min of the completion of the reaction. A description of control and validation studies is provided in the supplementary information.

Acknowledgements

This work was funded by the NIH (GM36238). Mass spectra were obtained at the Mass Spectrometry Laboratory for Biotechnology. Partial funding for the Facility was obtained from the North Carolina Biotechnology Center and the National Science Foundation.

References

1 J. S. Lindsey, in *The Porphyrin Handbook*, eds. K. M. Kadish, K. M. Smith and R. Guilard, Academic Press, San Diego, CA, 2000, vol. 1, p. 45.

2 J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *J. Org. Chem.*, 1987, **52**, 827.

3 J. S. Lindsey, K. A. MacCrum, J. S. Tyhonas and Y.-Y. Chuang, *J. Org. Chem.*, 1994, **59**, 579.

4 G. R. Geier III, Y. Ciringh, F. Li, D. M. Haynes and J. S. Lindsey, *Org. Lett.*, 2000, **2**, 1745.

5 J. S. Lindsey and R. W. Wagner, *J. Org. Chem.*, 1989, **54**, 828.

6 R. W. Wagner, F. Li, H. Du and J. S. Lindsey, *Org. Process Res. Dev.*, 1999, **3**, 28.

7 F. Li, K. Yang, J. S. Tyhonas, K. A. MacCrum and J. S. Lindsey, *Tetrahedron*, 1997, **53**, 12339.

8 P. J. Chmielewski, L. Latos-Grazynski, K. Rachlewicz and T. Glowiak, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 779.

9 H. Furuta, T. Asano and T. Ogawa, *J. Am. Chem. Soc.*, 1994, **116**, 767.

10 P. J. Chmielewski, L. Latos-Grazynski and K. Rachlewicz, *Chem. Eur. J.*, 1995, **1**, 68.

11 G. R. Geier III and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 1596.

12 (*a*) R. D. Macfarlane and D. F. Torgerson, *Science*, 1976, **191**, 920; (*b*) B. T. Chait, W. C. Agosta and F. H. Field, *Int. J. Mass Spectrom. Ion Phys.*, 1981, **39**, 339; (*c*) J. S. Lindsey, T. Chaudhary and B. T. Chait, *Anal. Chem.*, 1992, **64**, 2804.

13 J. S. Lindsey and B. T. Chait, unpublished data, 1984.

14 (*a*) H. J. Räder and W. Schrepp, *Acta Polym.*, 1998, **49**, 272; (*b*) M. W. F. Nielen, *Mass Spectrom. Rev.*, 1999, **18**, 309; (*c*) H. Pasch and R. Ghahary, *Macromol. Symp.*, 2000, **152**, 267.

15 N. Srinivasan, C. A. Haney, J. S. Lindsey, W. Zhang and B. T. Chait, *J. Porphyrins Phthalocyanines*, 1999, **3**, 283.

16 D. Fenyo, B. T. Chait, T. E. Johnson and J. S. Lindsey, *J. Porphyrins Phthalocyanines*, 1997, **1**, 93.

17 B. J. Littler, Y. Ciringh and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 2864.

18 Our use of LD-MS in studies of the porphyrin-forming reaction and the use of MALDI-TOF-MS in studies of polymer distributions differ in the following ways. (1) Polymer studies typically (but not always)[18a] have employed a cationization reagent (*e.g.*, a silver salt) in addition to a matrix.[18b] In our studies, neither a cationization reagent nor a matrix is employed. (2) Polymer studies typically have examined a large distribution of molecular weights, ranging up to 10^6 Da or more.[18c] Our studies focus on oligomers with $m/z \leq 2000$. (3) The objectives of polymer studies often are constrained by instrumental limitations.[18d] For example, mass-dependent detection efficiency, stemming from detector saturation in the low mass regime (typically due to matrix) and decreased analyte velocity in the high mass regime, can lead to inaccurate molecular weight distributions.[18d,e] These instrumental limitations occur in addition to the unavoidable mass-dependent differences in efficiency of desorption and ionization.[18f] In addition, the formation of aggregates of polymers as well as multiply charged polymers, which complicate interpretation of mass spectral data, generally occurs only in the higher mass regime.[18f] In contrast, in our studies of porphyrin-forming reactions, the absence of a matrix and the relatively narrow mass distribution ($m/z \leq 2000$) examined are expected to minimize the complications that are typically encountered in many studies of high molecular weight polymers; (*a*) J. Liu, R. S. Loewe and R. D. McCullough, *Macromolecules*, 1999, **32**, 5777; (*b*) R. Knochenmuss, E. Lehmann and R. Zenobi, *Eur. Mass Spectrom.*, 1998, **4**, 421; (*c*) D. C. Schreimer and L. Li, *Anal. Chem.*, 1996, **68**, 2721; (*d*) D. C. Schreimer and L. Li, *Anal. Chem.*, 1997, **69**, 4176; (*e*) H. Rashidzadeh and B. Guo, *Anal. Chem.*, 1998, **70**, 131; (*f*) D. C. Schreimer and L. Li, *Anal. Chem.*, 1997, **69**, 4169.

19 Part 2. G. R. Geier III and J. S. Lindsey, *J. Chem. Soc.*, *Perkin Trans. 2* (DOI: 10.1039/b009092l).

20 Part 3. G. R. Geier III, B. J. Littler and J. S. Lindsey, *J. Chem. Soc. Perkin Trans. 2* (DOI: 10.1039/b009098k).

21 Part 4. G. R. Geier III, B. J. Littler and J. S. Lindsey, *J. Chem. Soc.*, *Perkin Trans. 2* (DOI: 10.1039/b009101o).

22 P. D. Rao, B. J. Littler, G. R. Geier III and J. S. Lindsey, *J. Org. Chem.*, 2000, **65**, 1084.

23 P. D. Rao, S. Dhanalekshmi, B. J. Littler and J. S. Lindsey, *J. Org. Chem.*, 2000, **65**, 7323.

24 J. P. Strachan, D. F. O'Shea, T. Balasubramanian and J. S. Lindsey, *J. Org. Chem.*, 2000, **65**, 3160.

25 T. Balasubramanian, J. P. Strachan, P. D. Boyle and J. S. Lindsey, *J. Org. Chem.*, 2000, **65**, 7919.

26 W.-S. Cho, H.-J. Kim, B. J. Littler, M. A. Miller, C.-H. Lee and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 7890.

27 C.-H. Lee, W.-S. Cho, J.-W. Ka, H.-J. Kim and P. H. Lee, *Bull. Korean Chem. Soc.*, 2000, **21**, 429.

28 One readily observed side reaction is the formation of dipyrrin chromophores, which can occur *via* oxidation or tautomerization.[5] The yield of dipyrrins is known to increase over time, reaching 0.25% (BF_3–Et_2O) or 3.9% (TFA) after 1 h for a condensation of 10 mM benzaldehyde and pyrrole.[2] Dipyrrins are intensely colored and are responsible for the observation of a colored reaction mixture during the condensation (pyrrole, aldehyde, pyrromethane oligomers and the porphyrinogen are colorless). Dipyrrins are unreactive toward electrophilic aromatic substitution[28a,b] and the presence of a dipyrrin at the end of an oligomer effectively would cap chain growth. (*a*) A. Gossauer and J. Engel, in *The Porphyrins*, ed. D. Dolphin, Academic Press, New York, 1978, vol. II, p. 197; (*b*) see supporting information of ref. 24.

29 S. G. DiMagno, R. A. Williams and M. J. Therien, *J. Org. Chem.*, 1994, **59**, 6943.

30 (*a*) B. J. Littler, M. A. Miller, C.-H. Hung, R. W. Wagner, D. F. O'Shea, P. D. Boyle and J. S. Lindsey, *J. Org. Chem.*, 1999, **64**, 1391; (*b*) C.-H. Lee and J. S. Lindsey, *Tetrahedron*, 1994, **50**, 11427.