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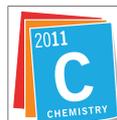
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PAPER

Abiotic formation of uroporphyrinogen and coproporphyrinogen from acyclic reactants†

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Tetrapyrrole macrocycles (*e.g.*, porphyrins) have long been proposed as key ingredients in the emergence of life, yet plausible routes for forming their essential pyrrole precursor have previously not been identified. Here, the anaerobic reaction of δ -aminolevulinic acid (**ALA**, 5–240 mM) with 5-methoxy-3-(methoxyacetyl)levulinic acid (**1-AcOH**, 5–240 mM) in water (pH 5–7) at 25–85 °C for a few hours to a few days affords uroporphyrinogen, which upon chemical oxidation gives uroporphyrin in overall yield of up to 10%. The key intermediate is the α -methoxymethyl-substituted analogue of the pyrrole porphobilinogen (**PBG**). Reaction of **ALA** and the decarboxy analogue of **1-AcOH** (**1-Me**) gave coproporphyrinogen (without its biosynthetic precursor uroporphyrinogen as an intermediate); oxidation gave the corresponding coproporphyrin in yields comparable to those for uroporphyrin. In each case a mixture of porphyrin isomers was obtained, consistent with reversible oligopyrromethane formation. The route investigated here differs from the universal extant biosynthetic pathway to tetrapyrrole macrocycles, where uroporphyrinogen (isomer III) – nature’s last common precursor to corrins, heme, and chlorophylls – is derived from eight molecules of **ALA** (*via* four molecules of **PBG**). The demonstration of the spontaneous self-organization of eight acyclic molecules to form the porphyrinogen under simple conditions may open the door to the development of a chemical model for the prebiogenesis of tetrapyrrole macrocycles.

Introduction

Tetrapyrrole macrocycles play a central role in most present living organisms yet the prebiotic origin of such molecules remains obscure.¹ The dearth of understanding exists despite a recurring view since the 1950s that such molecules were likely to be valuable if not essential for the origin of life.^{2–18} Such views stem from the unique attributes of tetrapyrroles for diverse bioenergetic processes (including photosynthesis) and have been further encouraged by the universality, concision, and molecular logic of the tetrapyrrole biosynthetic pathway. The early steps of the biosynthesis¹⁹ are shown in Fig. 1. Two molecules of **ALA** dimerize to give porphobilinogen (**PBG**), which undergoes tetramerization and macrocyclization (accompanied by inversion of the orientation of one pyrrole) to give uroporphyrinogen III. Uroporphyrinogen III is the last common progenitor of the corrinoid, porphyrin, and (bacterio)chlorin macrocycles known as the pigments of life. Decarboxylation of the four acetic acid side chains of uroporphyrinogen III affords coproporphyrinogen III, which

upon decarboxylation and dehydrogenation of two of the four propionic acid side chains affords protoporphyrinogen IX. Dehydrogenation ($-6e^-$, $-6H^+$) of the protoporphyrinogen IX macrocycle yields protoporphyrin IX, the ligand for heme and a precursor to all chlorophylls and bacteriochlorophylls.

Granick and Mauzerall put forth the view that the biosynthesis provides a “window on evolution” where each molecule served a function in its time and then was replaced with molecules formed later in the biosynthetic pathway as transformations were appended over time.^{3,4,8,18,20–22} The rationale for this view stems from the multifaceted change in features of the molecules in proceeding from the early to later stages of the biosynthesis: (i) from water-soluble compounds to hydrophobic macrocycles, (ii) from saturated to unsaturated molecules, and (iii) from simple to complex molecular architectures. The evolutionary perspective that “biosynthesis recapitulates biogenesis”²⁰ has prompted consideration as to whether the earliest steps might have been operative in the prebiotic era – *via* purely structure-directed processes (*i.e.*, without enzymes or other catalysts) – thereby yielding tetrapyrrole macrocycles that underpinned early catalysis and proto-photosynthesis.

A proposed prebiotic pathway that closely mirrors the extant biosynthesis is outlined in Fig. 2. Examples of transformations and products in each of the steps include the (1)

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† Electronic supplementary information (ESI) available: Mass spectral data for the porphyrinogens, porphyrins, and various pyrroles. See DOI: 10.1039/c0nj00716a

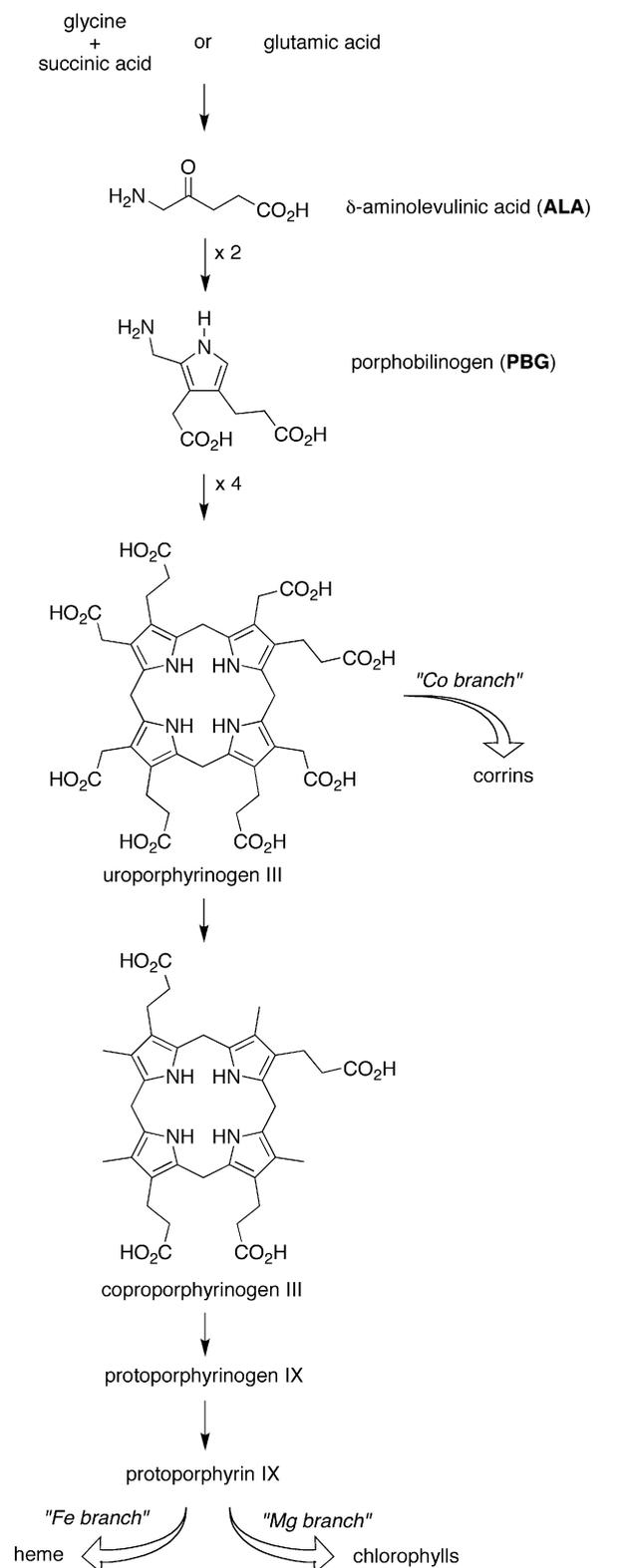


Fig. 1 Contemporary biosynthesis of tetrapyrrole macrocycles.

conversion of early-Earth available substances to **ALA**, (2) dimerization of **ALA** to give **PBG**, (3) tetramerization of **PBG** and macrocyclization to give uroporphyrinogen (four isomers), (4) dehydrogenation to give uroporphyrin (four isomers), and (5) subsequent metalation, decarboxylation, and/or reduction

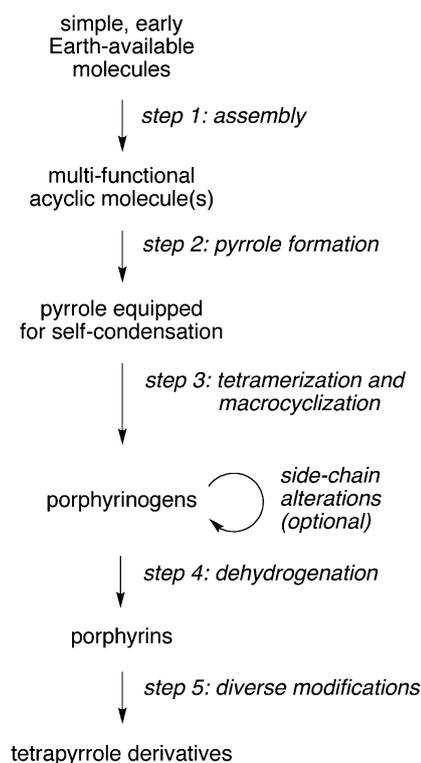


Fig. 2 Conceptual steps in a pre-biosynthetic route to tetrapyrroles.

to form macrocycles such as metalated uroporphyrin, coproporphyrin, and hydrophorphyrin (*e.g.*, chlorin, bacteriochlorin) analogues. Uroporphyrin (and uroporphyrinogen) have been proposed as prebiotic photosensitizers given their water solubility, characteristic reactions, and potential for prebiotic formation.^{9–11,18,22,23} Studies to address the occurrence of the individual steps include the following:

- Diverse modifications (step 5): Uroporphyrin undergoes smooth metalation [*e.g.*, with zinc(II) or copper(II)] in neutral aqueous solution at room temperature;^{24,25} facile photoreduction to give a variety of porphomethenes,^{26,27} and, under exceptionally forcing conditions, decarboxylation of the acetic acid side chains.²⁸

- Dehydrogenation (step 4): The dehydrogenation of a porphyrinogen to give the porphyrin can be achieved with oxidants under a variety of conditions.²⁹ The dehydrogenation of uroporphyrinogen can be achieved in aqueous solution

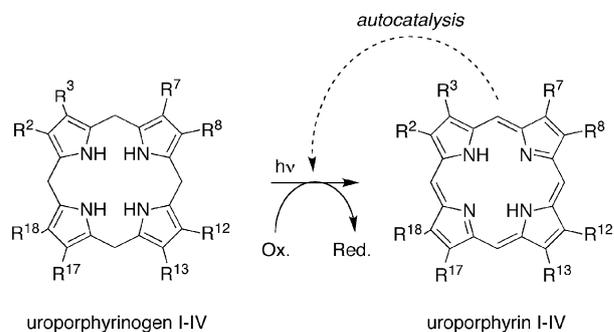


Fig. 3 Step 4: photochemical oxidation of uroporphyrinogen affords uroporphyrin in an autocatalytic manner.

by chemical or photochemical oxidation, and moreover, uroporphyrin can catalyze the dehydrogenation in an autocatalytic manner as shown in Fig. 3.³⁰ The autocatalytic process has been demonstrated with oxygen but is expected to proceed with diverse (prebiotic) oxidants.

- Tetramerization and macrocyclization (step 3): **PBG** undergoes condensation in the absence of enzymes to give the estimated statistical mixture of four uroporphyrinogen isomers as shown in Fig. 4. Examination of the distribution of uroporphyrinogens (inferred upon oxidation to give the corresponding uroporphyrin isomers) indicates that isomer III is the most abundant, as expected on statistical considerations.³¹ Although strong acid was predominantly employed for the condensation, a few reactions have been reported under neutral conditions. At pH 7.6 and 60 °C for 21 h under anaerobic conditions, **PBG** (10 mM) affords the uroporphyrinogen isomers in 70% yield.³¹ Similarly, **PBG** (0.26 mM) at pH 7.4 and 70 °C for 2 h under anaerobic conditions affords the uroporphyrinogen isomers in ~65% yield.³² Treatment of a given uroporphyrinogen isomer to the acidic macrocyclization conditions results in the statistical distribution of isomers, providing evidence for the thermodynamic stability of the macrocycles.³³ These data indicate the robustness of the macrocyclization process under plausible prebiotic conditions.

- Pyrrole formation (step 2): **ALA** in solution affords a dihydropyrazine and pseudo-porphobilinogen rather than **PBG**, the latter outcome owing to the greater reactivity of the δ versus β methylene unit (Fig. 5).³⁴ Despite extensive studies, prebiotically plausible conditions have not been identified for the solution dimerization of **ALA** to give **PBG**.³⁵

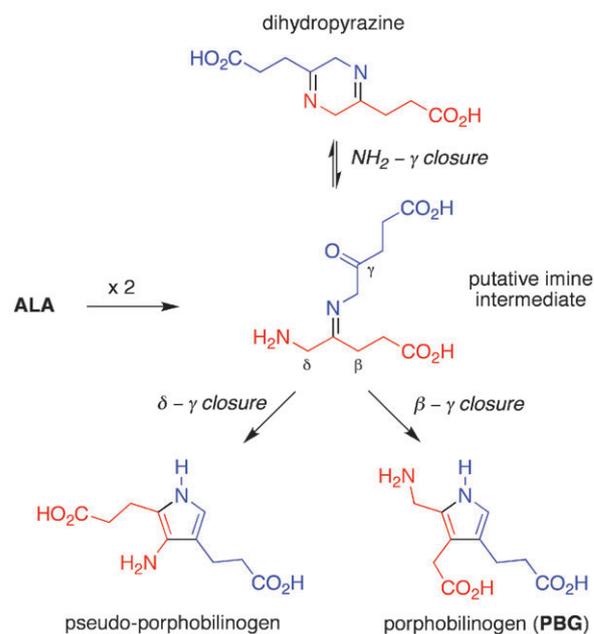


Fig. 5 Attempted step 2: solution self-condensation of **ALA** affords pseudo-porphobilinogen and a dihydropyrazine rather than **PBG**.

The lack of a simple route to **PBG**, while incongruous given the facile occurrence of the seemingly more complex steps 3 and 4 of the pathway, effectively crimps the entire process. The failure to find a viable structure-directed pathway to **PBG** has been referred to as the “Achilles’ heel” of the prebiogenesis of tetrapyrrole macrocycles.^{13,18} The resulting impasse leads to

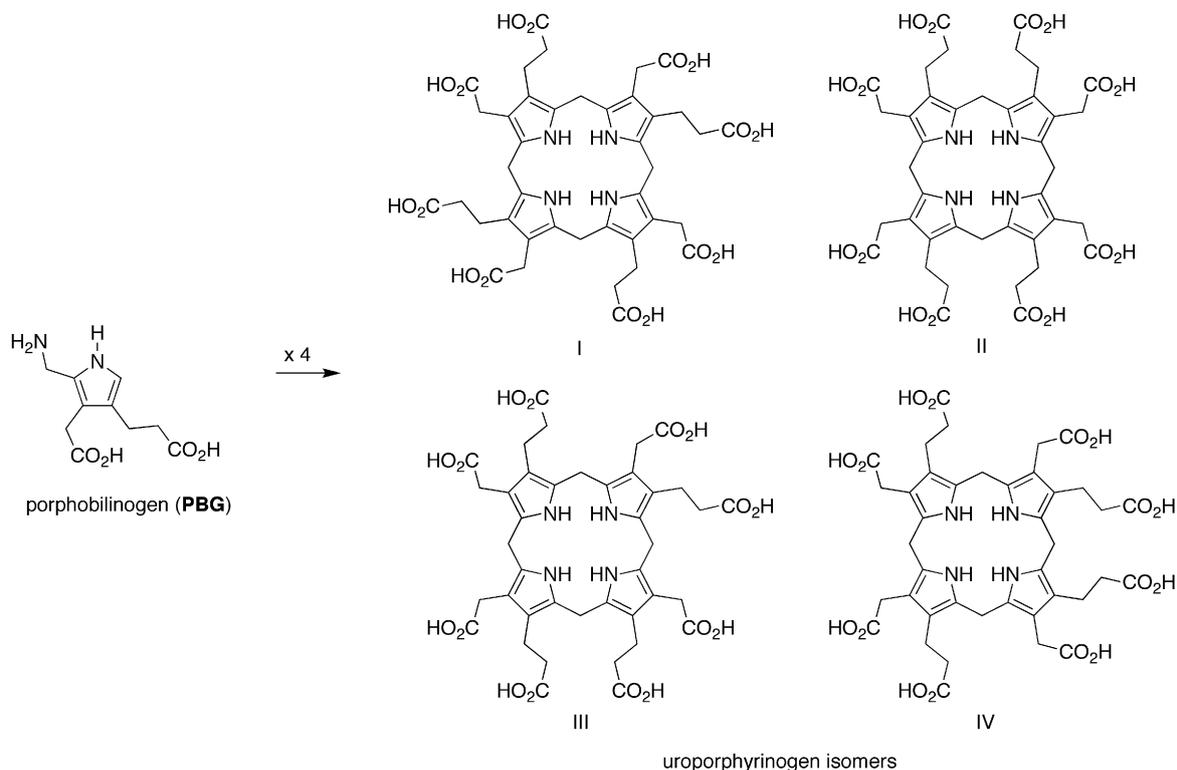


Fig. 4 Step 3: solution condensation of porphobilinogen affords four uroporphyrinogen isomers.

two opposing views concerning the origin of uroporphyrinogen in the prebiotic era: (i) uroporphyrinogen formed *via* structure-directed pathways through use of precursors other than **PBG**,^{13,18} or (ii) uroporphyrinogen did not form until a later era when appropriate catalysts (*e.g.*, enzymes) were present to direct conversion of **ALA** to **PBG**. A parallel possibility is that diverse abiotic porphyrinogens – distinct from (and prebiotic predecessors to) uroporphyrinogen – formed in a structure-directed manner prior to the emergence of catalysts.

An investigation of the chemical foundation for the latter possibility revealed a new process that encompasses two of the distinct reaction steps (2 and 3) shown in Fig. 2: **ALA** and a 4-methoxy-substituted β -ketoester (**2**) react to yield a porphobilinogen analogue (**3**) that self-condenses to give the corresponding porphyrinogen (**4**, Fig. 6). The reaction proceeds under plausible prebiotic conditions [anaerobic reaction of **ALA** (1–5 mM) with **2** (2–40 mM) in water (pH 5–7) at 70–100 °C for >6 h]; upon oxidation the porphyrin is obtained in up to 10% yield.³⁶ The 1,3-dicarbonyl motif directs enamine formation to the *meso*-carbon (C2), whereas the 4-methoxymethyl unit is untouched and becomes the α -methoxymethyl unit that equips the pyrrole for self-condensation. The resulting porphyrinogen and porphyrin

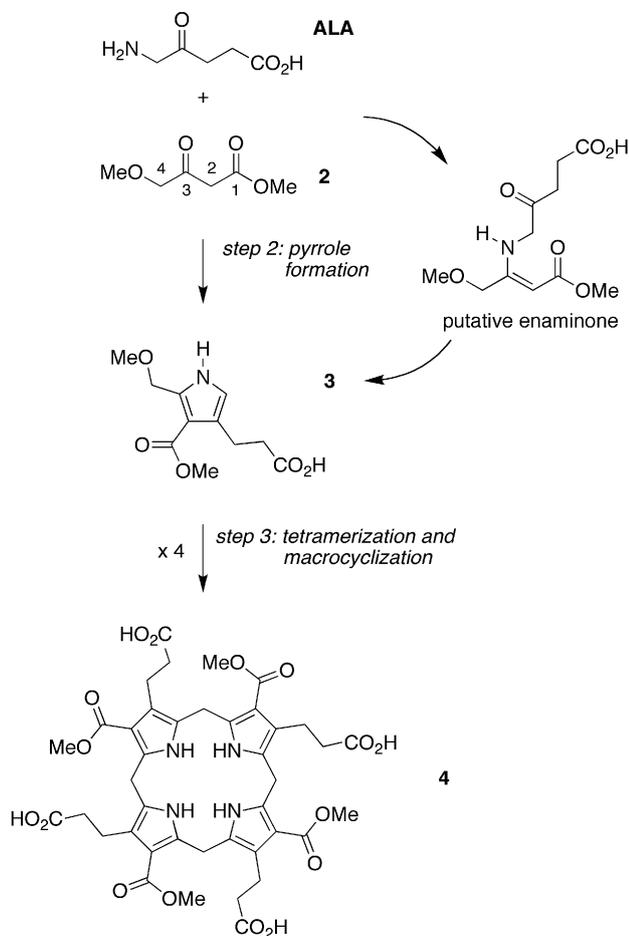


Fig. 6 Integration of steps 2 and 3: hybrid condensation of **ALA** and a 4-methoxy- β -ketoester (**2**) affords pyrrole **3** equipped for self-condensation to give the non-biological porphyrinogen **4**.

are not found in contemporary living organisms; however, the success of this route provided a conceptual foundation for addressing the uroporphyrinogen problem. Here we describe a structure-directed route to uroporphyrinogen that entails reaction of acyclic species in anaerobic aqueous solution under mild conditions of temperature and pH. The same route with a different precursor affords coproporphyrinogen without the intermediacy of uroporphyrinogen, its contemporary biosynthetic precursor.

Results and discussion

Uroporphyrinogen

The conceptual approach we investigated to form a **PBG** analogue from acyclic materials is shown in Fig. 7. A 2,4-diketone (**1-AcOH**) was chosen for reaction with **ALA**. Compound **1-AcOH** is a hybrid of 5-methoxylevulinic acid and the simple 2,4-diketone acetylacetonone. As a 5-methoxylevulinic acid species, **1-AcOH** bears a methoxyacetyl unit at

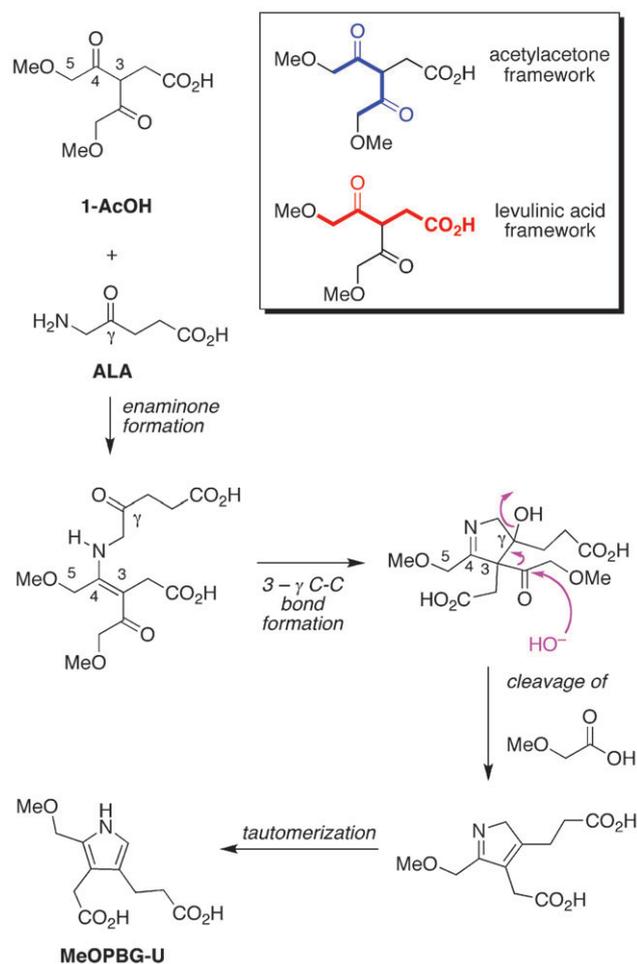


Fig. 7 Compound **1-AcOH** is a hybrid of the 2,4-diketone acetylacetonone and the monoketone levulinic acid. Reaction with **ALA** is expected to preferentially yield the enaminone whereupon 3- γ bond formation closes the ring. Loss of the sacrificial methoxyacetyl unit (upon attack of HO^- , H_2O , or other nucleophile) followed by tautomerization affords the porphobilinogen analogue **MeOPBG-U**. Other pathways and products are possible (*vide infra*).

the 3-position. As an acetylacetonate species, **1-AcOH** bears methoxy groups at the two terminal methyl groups and an acetic acid substituent at the *meso*-carbon (C3). Thus, **1-AcOH** is an **ALA** analogue yet differs in that **1-AcOH** (i) does not self-condense; (ii) is expected to form the enamine wherein the carbon-carbon double bond encompasses the *meso* carbon (3-position), and thereby direct carbon-carbon bond formation at the 3- γ positions; (iii) contains an α -methoxyacetyl unit that is lost upon pyrrole formation; and (iv) contains a methoxy substituent destined to be the leaving group at the α -methylpyrrole site upon reaction with **ALA**. The resulting pyrrole (**MeOPBG-U**) is an analogue of **PBG** that is equipped for self-condensation, bears the same β -substituents as **PBG**, and differs only in the presence of an α -methoxymethyl group rather than an α -aminomethyl unit.

The anaerobic reaction of **ALA** and **1-AcOH** (120 mM each) at 60 °C and pH 5 for 24 h yielded the uroporphyrinogen [ESI-MS analysis gave $m/z = 837.2873$, $(M + H)^+$, Fig. S1†] as shown in Fig. 8. The uroporphyrinogen was accompanied by a small amount of uroporphyrin (owing to background oxidation). Also present upon ESI-MS examination was a peak attributed to the formation of a pyrrole *via* the Fischer-Fink pathway, termed **MeO-FF-U** [$m/z = 314.1234$, $(M + H)^+$; Table S1†]; *vide infra*. Treatment of an aliquot of the reaction mixture with iodine in aqueous acid, a standard technique developed for conversion of uroporphyrinogen to uroporphyrin,^{31,33} enabled spectrophotometric quantitation on the basis of the characteristic Soret absorption band (Fig. 9A). Extraction of the acidic oxidized mixture with cyclohexanone gave uroporphyrin in crude form, which upon dissolution in neutral aqueous solution gave Soret and visible

absorption bands (Fig. 9B) as well as fluorescence emission and excitation spectra (Fig. 9C), all of which were almost identical with that of an authentic sample of uroporphyrin (isomer III). ESI-MS analysis gave the uroporphyrin molecular ion [$m/z = 831.2350$, $(M + H)^+$, Fig. S1†]. HPLC analysis with mass or absorption detection of the crude uroporphyrin gave three peaks, two of which exhibited retention times identical with those of commercially available samples of two uroporphyrin isomers (I and III) (Fig. 9D). Note that chromatographic conditions have only been developed for separation of three of the four uroporphyrin isomers.³⁷ The ratio of uroporphyrin isomers observed from reaction of **ALA** and **1-AcOH** resembled the equilibrium distribution obtained upon non-enzymic condensation of **PBG** (Fig. 9D).³¹

The reaction of **ALA** and **1-AcOH** was examined under a range of conditions spanning concentration (5–240 mM), time (6–96 h), temperature (25–85 °C), and pH (5–7). Illustrative examples are as follows.

The reaction at 60 °C for 24 h afforded yields that increased from <0.5% to 7.9% (pH 5) or 10.7% (pH 7) upon going from dilute (5 mM) to concentrated (240 mM) reactions, with yields peaked in the 120–170 mM region (Fig. 10).

The reaction for 72 h at 60 °C gave uroporphyrin in 10.6% (120 mM, pH 7) or 4.3% yield (120 mM, pH 5).

The reaction at 25 °C (120 mM, pH 7) afforded uroporphyrin in 0.9% and 4.1% yield after 1 and 4 days, respectively, whereas at 85 °C for 6 h the yield was 2.2%.

In summary, while the interplay of concentration, pH, temperature and time are not yet fully delineated, a broad range of plausible early-Earth conditions³⁸ was found to give successful condensation with yields of up to 10% of uroporphyrin. These

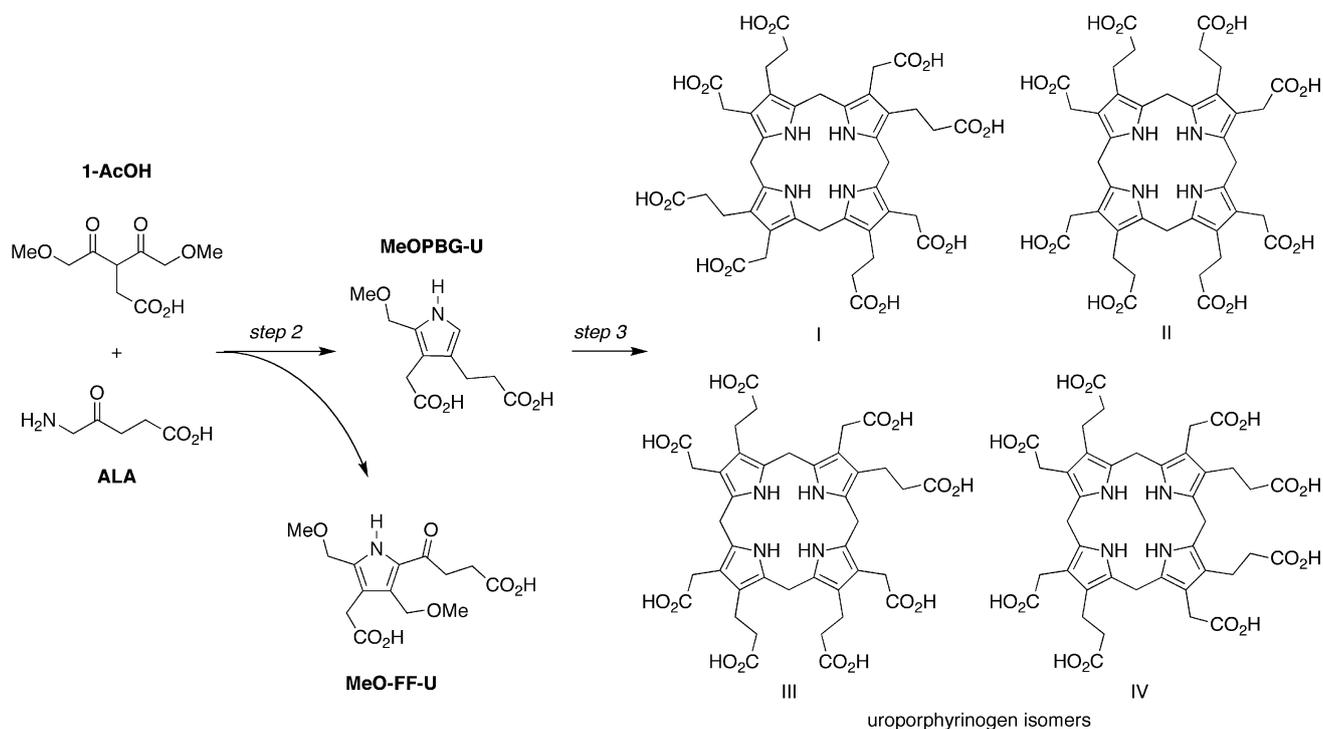


Fig. 8 Condensation of **ALA** and **1-AcOH** affords a porphobilinogen analogue (**MeOPBG-U**) that self-condenses to give uroporphyrinogen isomers (all four are shown). The competing Fischer-Fink pathway affords a fully substituted pyrrole (**MeO-FF-U**).

results constitute the first laboratory demonstration of the structure-directed formation of uroporphyrinogen from acyclic reactants.

Coproporphyrinogen, a biosynthetically advanced tetrapyrrole macrocycle

The scope was examined by reaction of a substrate other than **1-AcOH** under conditions identical to those to form uroporphyrinogen (Fig. 11). Thus, the reaction of the decarboxy analogue of **1-AcOH** (**1-Me**) gave a molecular ion [$m/z = 661.3236, (M + H)^+$] consistent with coproporphyrinogen (Fig. S2†). Use of LC-ESI-MS revealed the presence of four peaks as expected for the four possible coproporphyrinogen isomers (Fig. 12). Examination of the crude reaction mixture also revealed the presence of the Fischer-Fink pyrrole **MeO-FF-C** [$m/z = 292.1161, (M + Na)^+$; Table S1†]; *vide infra*.

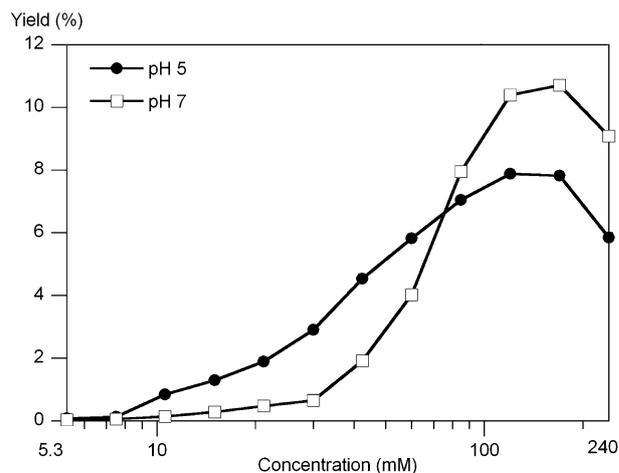
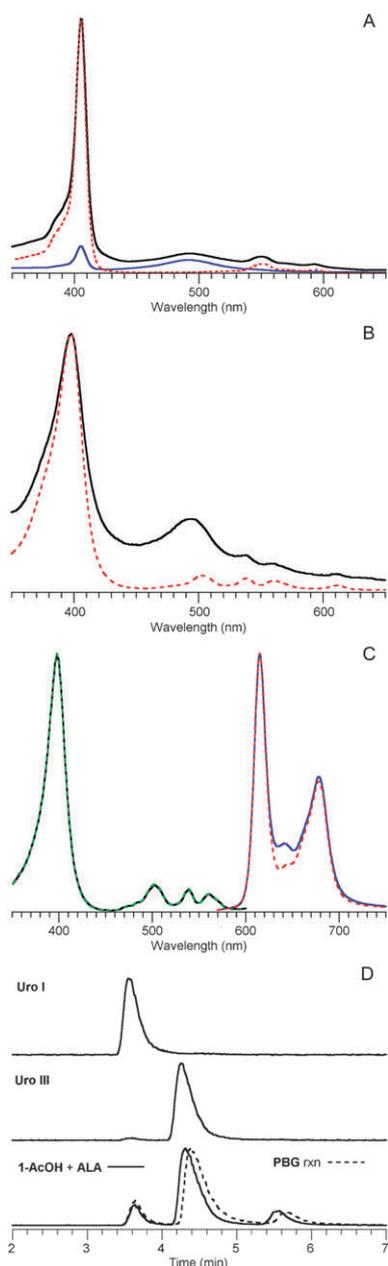


Fig. 10 The yield of uroporphyrin as a function of the concentration of **1-AcOH** and **ALA** (equimolar) upon reaction for 24 h at 60 °C at pH 5 or pH 7 determined upon oxidation of reaction aliquots. The data points are positioned at factors of $2^{-1/2}$ from the highest concentration (240 mM) examined.

Oxidation of the reaction mixture obtained from **1-Me** and **ALA** gave coproporphyrin in 10.6% overall yield (upon reaction at 120 mM, pH 7, 60 °C, 24 h). The absorption spectrum, fluorescence excitation spectrum, and fluorescence emission spectrum were nearly identical with those from an authentic sample of coproporphyrin III (Fig. 13A,B). Characterization of the coproporphyrin sample by mass spectrometry gave the expected molecular ion, and LC-ESI-MS showed three coproporphyrin isomers (Fig. S3†). HPLC with absorption detection also showed three coproporphyrin isomers (Fig. 13C). The same reaction at 25 °C for 4 days afforded coproporphyrin in 3.1% yield, whereas at 85 °C for 6 h the yield was 6.4%.

Fig. 9 Characterization data for the uroporphyrin product obtained from reaction of **1-AcOH** and **ALA** (120 mM each, pH 5, 60 °C, 24 h). (A) Absorption spectra in 0.1 M HCl. An aliquot from the reaction mixture diluted 850-fold into a 2.6 mL cuvette (solid blue line) shows a trace of porphyrin (Soret band at 405 nm) and dipyrin species (490 nm) owing to background oxidation. Upon addition of excess I_2 to the cuvette (followed by discharging with $Na_2S_2O_3$) the colorless porphyrinogen³⁰ is converted to the porphyrin (solid black line). The spectrum of an authentic sample of uroporphyrin III is included for comparison (dotted red line, normalized at the Soret band of the crude sample). (B) Absorption spectra in 0.1 M sodium phosphate (pH 7). The crude uroporphyrin extracted from acidic brine (solid black line) is compared with an authentic sample of uroporphyrin III (dotted red line). (C) Fluorescence excitation (λ_{em} 678 nm) and emission spectra (λ_{exc} 398 nm) in 0.1 M sodium phosphate (pH 7) of the crude uroporphyrin (solid lines) are compared with those of an authentic sample of uroporphyrin III (dashed lines, normalized at the peak band of the crude sample). (D) HPLC with mass spectral detection [$m/z = 831 \pm 2, (M + H)^+$] of the crude uroporphyrin (showing three putative isomers) is compared with data from authentic samples of uroporphyrin I and uroporphyrin III. The chromatogram from the acidic condensation (90 °C, 1 h, air oxidation) of **PBG** is shown in overlay. Slight variation in retention times (*e.g.*, 6 s at 4.4 min) occurred for a given sample on multiple runs. See Materials and methods for HPLC conditions.

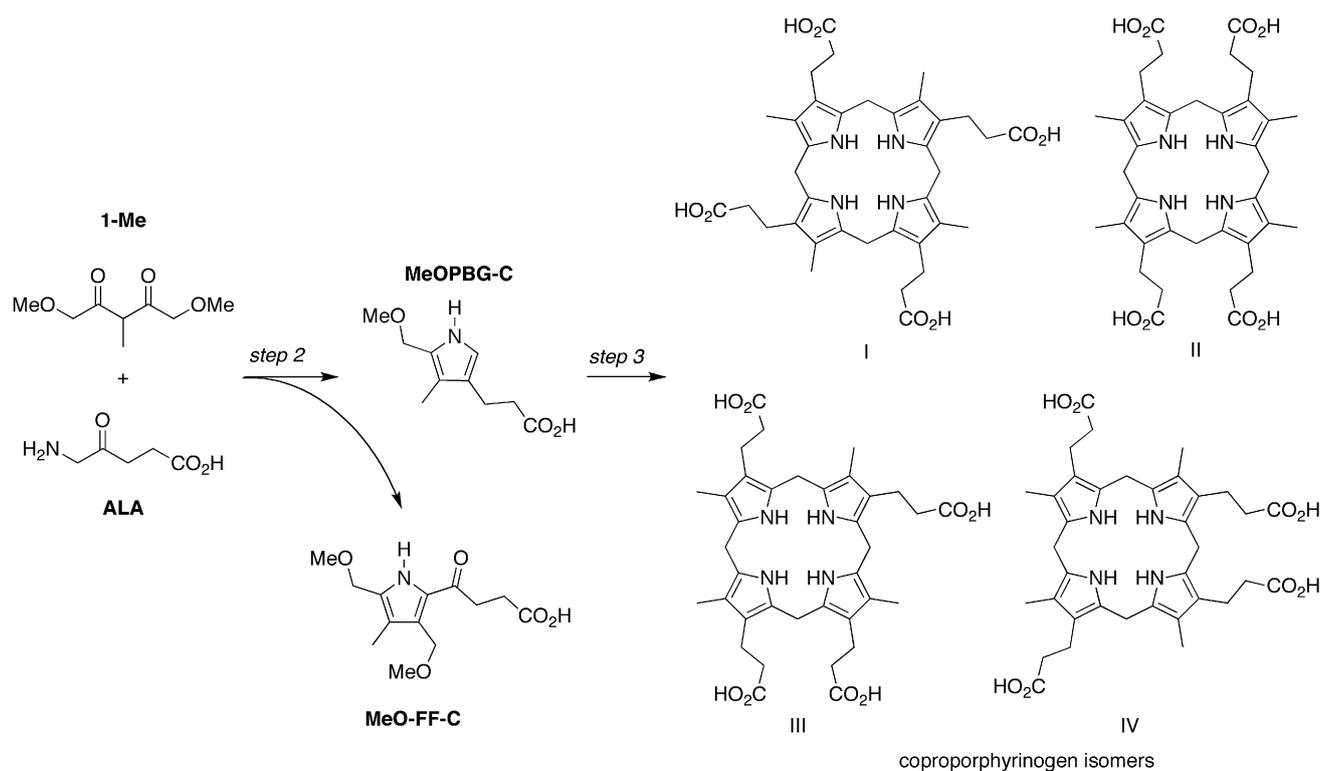


Fig. 11 Condensation of **1-Me** and **ALA** provides direct access to coproporphyrinogen isomers. The competing Fischer-Fink pathway affords a fully substituted pyrrole (**MeO-FF-C**).

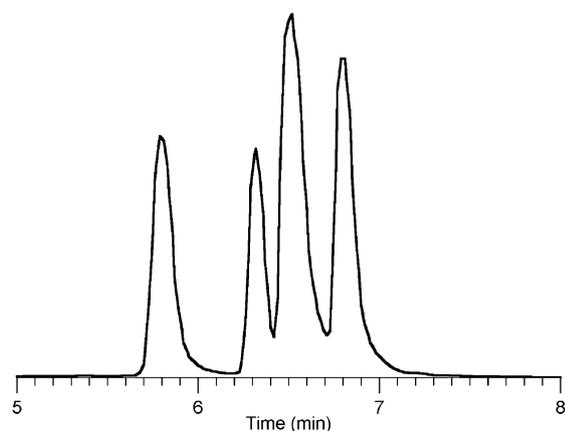


Fig. 12 LC-ESI-MS analysis of a sample from the reaction of **1-Me** and **ALA** with detection at the mass channel m/z 659.4–663.1 shows four putative coproporphyrinogen isomers $[M + H]^+$. See Materials and methods for HPLC conditions.

It is noteworthy that the pyrrole (**MeOPBG-C**) obtained in the reaction of **1-Me** and **ALA** is not equipped to form a lactone with the β -alkylcarboxylic acid, as can occur with the pyrrole (**MeOPBG-U**) derived from **1-AcOH** and **ALA**; with **PBG**,³¹ and perhaps with an α -(methoxymethyl)pyrrole used in a chemical synthesis of coproporphyrin³⁹ (Fig. 14). Thus, the formation of such a lactone intermediate and/or intramolecular catalysis by the β -acetic acid unit is not essential to promote the condensation in step 3 (tetramerization and macrocyclization). Whether there are reaction conditions that

proceed beneficially *via* a lactone intermediate remains to be determined.

Competing pathways to pyrroles

The reaction of an α -aminoketone and a β -diketone can proceed *via* multiple pathways, including here the desired Knorr and undesired Fischer-Fink routes (Fig. 15). Indeed, examination of a variety of reactions of **ALA** and **1-AcOH** (or **1-Me**) by ESI-MS revealed the presence of a peak due to the porphyrinogen (derived from the porphobilinogen-like Knorr pyrrole) as well as a peak due to the Fischer-Fink pyrrole (Table S1[†]). The Knorr and Fischer-Fink routes have their branch point at the step where the initial enaminone undergoes cyclization.

The Fischer-Fink route stems from condensation at the δ -methylene of **ALA** with the 2-carbonyl of **1-AcOH** (or **1-Me**) followed by dehydration. The Fischer-Fink pyrrole (**MeO-FF-U** or **MeO-FF-C**) is expected to be relatively unreactive owing to the deactivating effect of the α -acyl substituent. The Knorr pathway entails condensation of the enaminone C3-carbon with the **ALA** γ -ketone to yield the pyrrole intermediate (see Fig. 7). Because the enaminone nucleophile is a tertiary carbon, cleavage of the methoxyacetyl unit at the resulting quaternary carbon is required (followed by loss of hydroxide and tautomerization) to give the pyrrole equipped for self-condensation (**MeOPBG-U** or **MeOPBG-C**). Thus, one methoxyacetyl unit becomes an integral part of the structure (of **MeOPBG-U** or **MeOPBG-C**) while the second serves only as a temporary aide to direct enamine formation at the C3 site

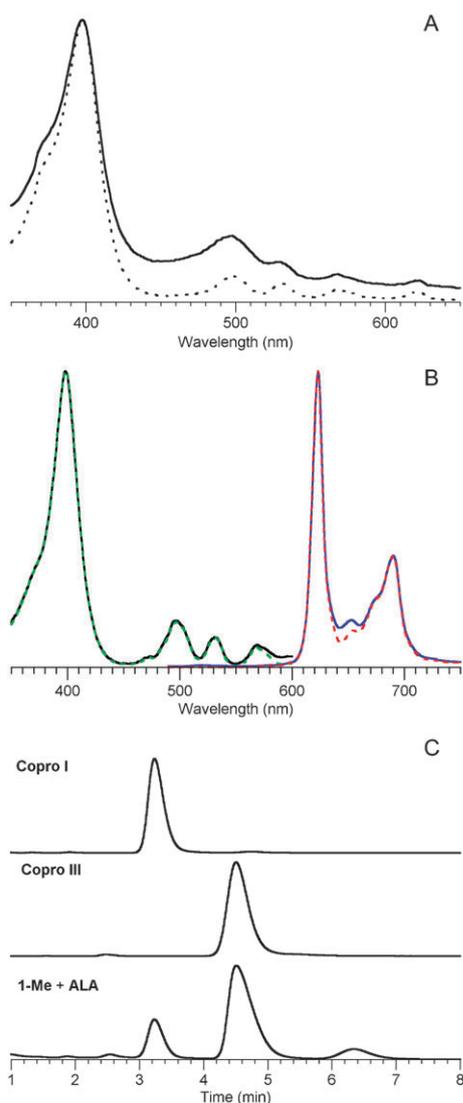


Fig. 13 Data for coproporphyrin obtained upon oxidation of a sample from the reaction of **1-Me** and **ALA** (pH 7, 60 mM **ALA** and 120 mM **1-Me**, 24 h, 60 °C). (A) Absorption spectra in *n*-propanol of the crude porphyrin extract (solid line) and comparison with the spectrum of an authentic sample of coproporphyrin III (dashed line); the spectra are normalized at the Soret band. (B) Fluorescence excitation (λ_{em} 678 nm) and emission spectra (λ_{exc} 398 nm) in *n*-propanol of the crude coproporphyrin (solid lines) obtained upon oxidation and extraction are compared with an authentic sample of coproporphyrin III (dashed lines, normalized at the peak bands). (C) HPLC with absorption spectral detection of the crude porphyrin extract compared with authentic samples of coproporphyrin I and coproporphyrin III. Three putative coproporphyrin isomers are observed in the crude sample. See Materials and methods for HPLC conditions.

(Fig. 7). Other pyrroles are possible (*e.g.*, by cleavage of the succinyl moiety along the Fischer-Fink route) but were not observed upon ESI-MS analysis of the reaction mixtures. Isomers of the Fischer-Fink pyrroles formed by δ -2 closure (rather than δ -2 closure as shown in Fig. 15) cannot be discriminated by nominal mass analysis, and while considered to be less likely, cannot at present be ruled out.

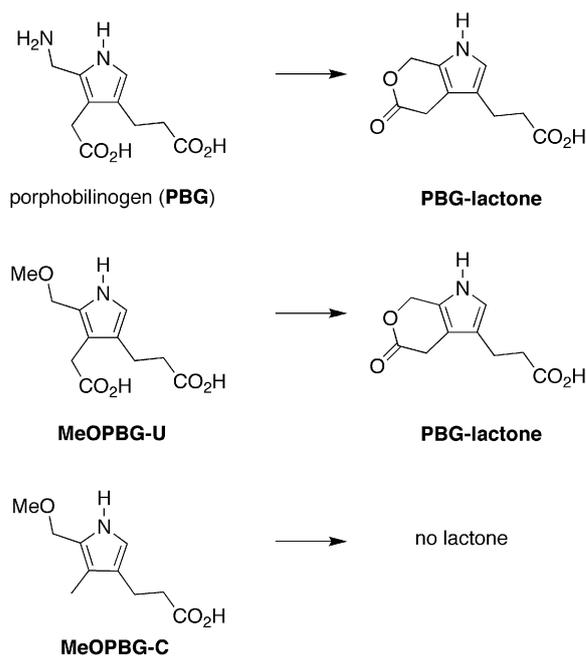


Fig. 14 Possible lactone formation in step 3 (tetramerization and macrocyclization).

Butler and George carried out the reaction of **ALA** and 3-methylpentan-2,4-dione (**5-Me**), a substrate closely resembling the core structure of **1-AcOH** or **1-Me**.⁴⁰ The Fischer-Fink pyrrole (**6-Me**) was the only solid product isolated, albeit in 16.7% yield.⁴⁰ Were the Fischer-Fink pathway the only pathway available, of course, no porphyrinogen would result upon reaction of **ALA** and **1-AcOH** or **1-Me**. Because the result of Butler and George taken on face value indicated the reaction of **ALA** and **1-AcOH** or **1-Me** would not lead to porphyrinogen, we repeated the reaction of **ALA** and 3-substituted pentan-2,4-diones to assess the products under the exact conditions employed here that led to porphyrinogens. The 3-substituted pentan-2,4-diones lack the 1,5-dimethoxy substituents of **1-AcOH** or **1-Me** and hence are arrested with regards to subsequent condensations (Fig. 16). To assess the products unbiased by chemical separation methods, we employed ESI-MS analysis of the crude reaction mixtures (a measurement method not commonly available at the time when Butler and George performed their study).

The reaction of **ALA** and 3-methylpentan-2,4-dione (**5-Me**, 60 mM) at 60 °C and pH 5 for 24 h upon ESI-MS analysis showed peaks for both the Fischer-Fink pyrrole **6-Me** and the Knorr pyrrole **7-Me** (Table S2†). Identical reaction of **ALA** and 3-acetyllevulinic acid (**5-AcOH**) showed peaks for both the Fischer-Fink pyrrole **6-AcOH** and the Knorr pyrrole **7-AcOH**. Whether the isolation of pyrrole **6-Me** and not also **7-Me** by Butler and George reflects relative yield or relative stability of the two pyrroles remains to be determined. In summary, the competing formation of the Fischer-Fink and Knorr pyrroles appears to be intrinsic to the reaction of a 3-substituted 2,4-diketone with **ALA**. Quantitation of the two pathways and examination of simple catalysts or conditions that might suppress the Fischer-Fink shunt require further study.

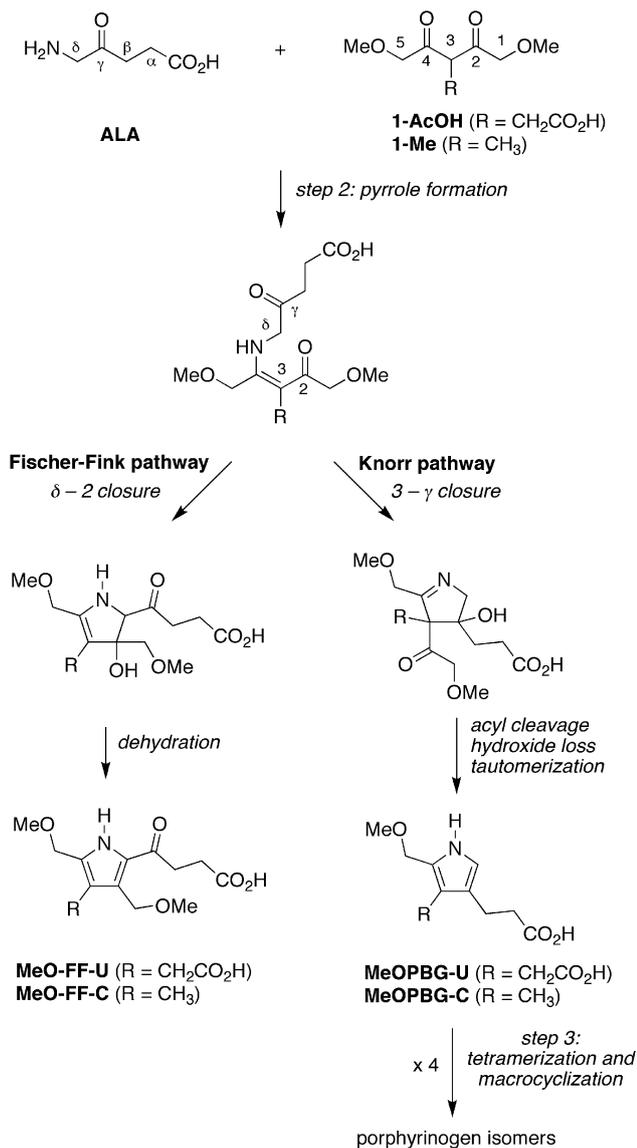


Fig. 15 Competing Fischer-Fink and Knorr routes on the pathway to porphyrinogens.

Outlook

The prior impasse concerning plausible prebiotic routes to tetrapyrrole macrocycles stemmed from inability to form the pyrrole **PBG** or equivalent thereof. Numerous routes are known for the chemical synthesis of pyrroles,⁴¹ including **PBG**;^{42–45} however, none employs relatively simple substances that spontaneously give rise to a pyrrole that contains all of the essential features of **PBG**. **PBG** (i) is water-soluble owing to the two carboxylic acid side chains and the aminomethyl unit, (ii) reacts as a 1° electrophile (or azafulvene equivalent) at the α-methyl site, and (iii) undergoes electrophilic substitution at the open α-site, a process potentiated by the two activating β-alkyl groups. Together, such features enable self-condensation of **PBG** in aqueous solution under mild conditions.

The reaction pathway identified herein to form **PBG** analogues is fundamentally different from both the (efficient) enzymatic transformation and the (failed) solution dimerization of two

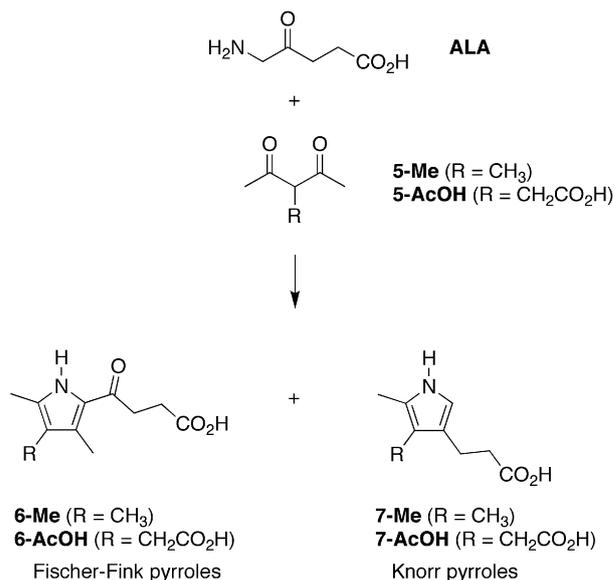


Fig. 16 Competing Fischer-Fink and Knorr routes with 3-substituted pentan-2,4-diones.

molecules of **ALA**. The enzyme porphobilinogen synthase is believed to direct an aldol condensation (to set the critical β-γ bond, Fig. 5) between two molecules of **ALA** followed by imine formation to give the 5-membered ring.⁴⁶ The dimerization of **ALA** in solution, which presumably entails initial formation of the imine, is thwarted by the greater reactivity of the δ *versus* β site for aldol condensation.³⁴ The **ALA**-dimerization problem has been sidestepped here through a hybrid reaction of **ALA** with a 2,4-diketone bearing a 3-substituent and 1,5-dimethoxy units; one keto moiety functions sacrificially to direct reaction at the 3-position (corresponding to the β-site in **ALA**, which is comparatively unreactive in solution) and then is lost upon pyrrole formation. Other sacrificial directing groups (*e.g.*, cyano) at the 3-position might serve equally well. The simplicity, structure-directed transformations, mild conditions, and non-negligible yields for the multistep process identified herein would appear to meet many criteria for a plausible prebiotic process.

On the other hand, one criterion that has not yet been met is a prebiotic route to **1-AcOH** (or **1-Me**), or for that matter to **ALA**. Ultimately, a claim to prebiotic relevance for the proposed route is contingent on substantiation of routes to such precursors from early Earth-available substances. With regards to the β-diketones (**1-AcOH**, **1-Me**), β-dicarbonyl compounds are central to fatty acid biosynthesis/degradation and microbial polyketide synthesis.⁴⁷ In addition, the α-methoxyacetyl unit is employed in polyketide synthesis.⁴⁸ While it may never be possible to prove the occurrence of a prebiotic process, at a minimum, the study herein may reveal “indispensable chemical requirements that restrict the range of boundary conditions compatible with a chemistry of biogenesis.”⁴⁹ In this context, the process highlights a number of issues central to studies of the origin of life.

A key issue concerns proto-metabolism *versus* extant metabolism, which de Duve has emphasized may be broadly parallel yet have distinct features.¹⁴ The following points deserve comment in this regard.

• The macrocycles uroporphyrinogen and coproporphyrinogen are 'generationally simple yet architecturally complex'⁴⁹ and form spontaneously from acyclic reactants; however, structure-directed reactions in general are far slower than enzymatically catalyzed processes.⁵⁰ Here, the obvious limitations of the structure-directed processes include the competing formation of the undesired Fischer-Fink pyrrole, the formation of four porphyrinogen isomers, the requirement for somewhat high concentrations, and the comparatively low yields. It remains unknown how/when processes dictated solely by the intrinsic molecular structure of reactants gave way to more efficient and selective catalyzed reaction processes.

• Uroporphyrin, derived oxidatively from uroporphyrinogen, has been proposed as an ideal prebiotic photosensitizer,^{9–11,18,22,23} as has coproporphyrin.¹⁰ Both can now be readily formed from acyclic reactants yet neither is a constituent of extant biochemistry.^{4,19}

• Coproporphyrinogen is a more advanced intermediate than uroporphyrinogen along the extant biosynthetic path to hemes and chlorophylls. The facile formation of coproporphyrinogen without uroporphyrinogen as a precursor raises questions concerning how/why the extant biosynthesis originated with reliance on **ALA** as the sole precursor followed by decarboxylation of the acetic acid side chains of uroporphyrinogen, *versus* employing two distinct substrates to directly form coproporphyrinogen.

• The yields to date are not high, but tetrapyrrole macrocycles persist for hundreds of millions of years in anaerobic environments,⁵¹ and hence would be expected to accumulate under prebiotic conditions.

The demonstration of a route from acyclic materials to porphyrinogens opens the door to the development of a chemical model for the prebiogenesis of tetrapyrrole macrocycles. Ultimately, such a model must begin with simple, early Earth-available precursors to **ALA** and **1-AcOH** (or analogues) and integrate multiple successive transformations (steps 1–5, Fig. 2). Concerning step 1, we note that both **ALA** and **1-AcOH** are of structural complexity intermediate between that of accepted prebiotic species such as amino acids and nucleosides. Steps 2 and 3 have been demonstrated herein, and precedent exists for step 4 (albeit as a stand-alone process).³⁰ Toward greater molecular complexity, the reaction of diverse analogues of **ALA** and of **1-AcOH** alone or in combinatorial processes, as well as *in situ* modification processes (step 5: side-chain alteration, reduction, metalation), are expected to yield diverse biotic and abiotic macrocycles encompassing a range of polarity and functionality.

The availability of tetrapyrrole macrocycles *via* model prebiotic processes enables examination of a variety of catalytic and energy-utilization processes central to origin-of-life problems. For example, the question of whether the earliest cells⁵² were chemotrophic or phototrophic has animated the life sciences since the time of Oparin and Haldane. The facile formation of diverse tetrapyrrole macrocycles from simple precursors may provide an opportunity to explore systematically the *de novo* emergence of proto-photosynthetic processes.

Materials and methods

Reactants

ALA is commercially available. The syntheses of compounds **1-AcOH** and **1-Me** (by alkylation of the known 1,5-dimethoxy-pentan-2,4-dione⁵³) and **5-AcOH** will be reported elsewhere.

Reactions

All experiments were carried out at 0.5 mL scale under anaerobic conditions following techniques that have been described in detail for the reaction of **ALA** and **2**.³⁶ Reactions were performed in 0.5 M sodium acetate (pH 5) or 0.5 M 3-(*N*-morpholinyl)propylsulfonic acid (pH 7), respectively, with pH values determined at 25 °C.

Porphyrin yields

The yield of porphyrin was determined by addition of a reaction aliquot (50 µL of a 30 mM reaction) to 0.1 M HCl (2.6 mL) in a cuvette under aerobic conditions, to which was then added 56 µL of I₂ solution (50 mM in ethanol); subsequent treatment with 112 µL of Na₂S₂O₃ (100 mM in water) discharged excess iodine. The absorption spectrum was quantitated using $\epsilon_{\text{Soret}} = 505\,000\text{ M}^{-1}\text{ cm}^{-1}$.³³ The oxidation process was scaled linearly for reactions at other concentrations, and for oxidations at larger scale. Treatment of the oxidized reaction mixture with acidic brine (3.6 M NaOAc, pH 3.2), extraction into cyclohexanone,⁵⁴ and concentration of the organic extract afforded the crude porphyrin mixture.

HPLC conditions

HPLC of the uroporphyrin sample with mass spectral detection was carried out at 50 °C on a C18 column (50 × 2.1 mm, 2.7 µm particles) with the following solvents [A = aqueous NH₄OAc (1.0 M, pH 5.15); B = 98% CH₃CN, 2% water, and 0.3% formic acid; the CH₃CN itself contained 2% water] and flow rate 0.35 mL min⁻¹. The elution regimen consisted of 7% B for 4 min followed by transition to 10% B over 6 min. See Fig. 9D.

HPLC of the coproporphyrinogen sample with mass spectral detection was carried out at 50 °C on a C18 column (50 × 2.1 mm, 2.7 µm particles) with the following solvents [A = 98% water and 2% CH₃CN; B = 98% CH₃CN, 2% water, and 0.3% formic acid; the CH₃CN itself contained 2% water] and flow rate 0.4 mL min⁻¹. The elution regimen consisted of 25% B for 2 min, transition to 75% B over 8 min, then transition to 25% B over 3 min. See Fig. 12.

HPLC of the coproporphyrin sample with absorption spectral detection ($\lambda_{\text{det}} = 405\text{ nm}$) was carried out at 25 °C on a C18 column (100 × 2.1 mm, 5 µm particles) in isocratic mode (flow rate 0.8 mL min⁻¹) using 26% of CH₃CN and 74% of aqueous NH₄OAc (1.0 M, pH 5.15). See Fig. 13C.

Fluorescence spectroscopy

The fluorescence spectra were obtained at room temperature with excitation and emission slit widths of 1.5 nm, and a sweep rate of 1 nm/2 s. The spectra were corrected for excitation intensity and instrument response on emission. The fluorescence emission spectra were obtained upon excitation at the

peak of the Soret band with samples having absorbance values <0.06 in 1 cm pathlength cuvettes. Fluorescence excitation spectra were obtained upon emission detection at the Q(0,1) peak of the same samples.

Mass spectrometry

High resolution exact mass measurements of the porphyrins and porphyrinogens were carried out using electrospray ionization (ESI) time-of-flight on an Agilent Technologies 6224 LC-TOF mass spectrometer operated in positive-ion mode (Duke University, with assistance of Dr George Dubay). High resolution exact mass measurements of the pyrroles were carried out using electrospray ionization (ESI) time-of-flight on an Agilent Technologies 6210 LC-TOF mass spectrometer operated in positive-ion mode (NC State University, with assistance of Ms. Danielle Lehman).

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