

Tetrapyrroles: Central molecules in life

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Introduction

Life on Earth would be unimaginable without tetrapyrroles. These structurally diverse and functionally rich molecules support numerous physiological processes, forming a crucial bridge between chemistry and biology. Their structural flexibility and functional variety have enabled life to adapt and flourish in diverse environments, ranging from sunlit forests to deep sea vents.

Well-known tetrapyrroles include hemes, which impart the red colour to blood and facilitate oxygen transport; chlorophylls, the central pigments of photosynthesis in plants, algae and cyanobacteria; and cobalamin (vitamin B₁₂), an essential micronutrient in human metabolism. Others, such as siroheme, coenzyme F₄₃₀, heme d1, and bilins, are equally significant across different biological contexts.¹

Beyond their natural biological functions, tetrapyrroles have attracted attention for their promising roles in renewable energy, biotechnology, medicine, and sustainable agriculture.^{2,3}

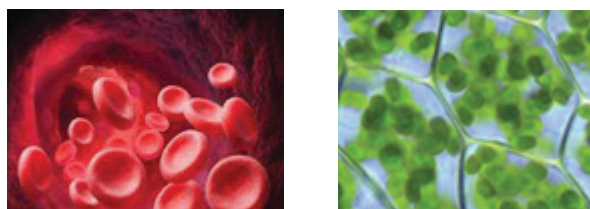


Figure 1: Red blood cells and chloroplasts - two sites of essential tetrapyrroles

Structure and chemistry of tetrapyrroles

As the name implies, tetrapyrroles are built from four pyrrole or pyrrole-like units. These units are interconnected *via* carbon bridges; typically, methine ($-\text{CH}=\text{}$) or methylene ($-\text{CH}_2-$), forming either linear or macrocyclic structures.

The aromaticity of pyrrole arises from six π -electrons delocalized over the ring, complying with Hückel's rule ($4n+2$; $n=1$). The N atom possesses a lone pair of electrons. In pyrrole-based macromolecules, this

lone pair of electrons participates in coordination with metal ions, contributing to the stabilization of metal-ligand complexes.

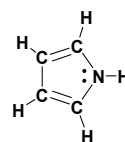
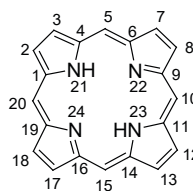


Figure 2: Pyrrole ring - a planar, aromatic heterocycle with six π -electrons

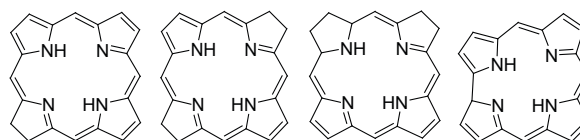
The chemical and structural diversity of tetrapyrroles arises from variations in the ring connectivity, the presence or absence of ring closures, the nature of side-chain substituents, chelated metal ions, and oxidation states of the rings.

Cyclic tetrapyrroles:

Cyclic tetrapyrroles can exist in various forms, like porphyrin, chlorin, bacteriochlorin, isobacteriochlorin and corrin. Substituents at peripheral positions and the coordination of metal ions (e.g., $\text{Fe}^{2+}/\text{Fe}^{3+}$, Mg^{2+} , Co^{3+}) expand the functional diversity of these macromolecules.



Porphin (porphine): The simplest porphyrin



Chlorin Bacteriochlorin Isobacteriochlorin Corrin

Figure 3: Basic skeletons of cyclic tetrapyrroles

a. Porphyrins

In porphyrin, the four pyrrole rings are linked *via* methyne ($-\text{CH}=\text{}$) bridges to form a planar, rigid, highly conjugated 18 π -electron system. Due to the π -electron delocalization, they exhibit strong light-

absorbing properties, particularly in the visible region of the electromagnetic spectrum, making them deeply coloured. The term 'porphyrin' is derived from the Greek word *porphyrá*, meaning purple.⁴

These macrocycles can delocalize π -electrons through six equivalent pathways, contributing to their remarkable aromatic character.

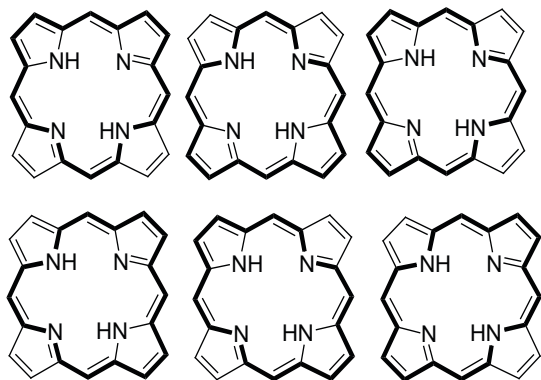


Figure 4: Delocalization pathways in porphyrin

Different substituents can be attached at the eight β -positions of the pyrrole rings or the four meso-positions on the methine bridges.

Prominent examples include heme A, heme B, and siroheme, all of which incorporate iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$) in their core. Their distribution and functions are summarized below in Table 1.¹

b. Chlorins

Chlorins are reduced derivatives of porphyrins (dihydroporphyrin) in which one pyrrole ring is partially saturated. This leads to the loss of aromaticity in that part of the ring but enhances absorption of light

in the red region of the spectrum. Natural examples include chlorophyll a and b, which incorporate a central Mg^{2+} ion and a hydrophobic phytol tail.

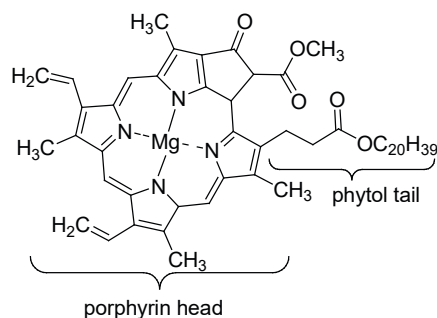


Figure 5: Chlorophyll a with a porphyrin head and phytol tail

c. Bacteriochlorins and isobacteriochlorins

Bacteriochlorins possess two reduced pyrrole rings, further disrupting conjugation. Bacteriochlorophylls, found in photosynthetic bacteria, serve as efficient light-harvesting pigments.

Isobacteriochlorins, such as coenzyme F_{430} (Ni^{2+} center), feature adjacent reduced pyrroles with *cis*-oriented NH groups. Coenzyme F_{430} is essential in methanogenesis.

d. Corrins

Corrins are structurally similar to porphyrins but lack one methine bridge, resulting in a more flexible macrocycle. Cobalamin (vitamin B_{12}), a cobalt-containing corrin, acts as a cofactor in DNA synthesis and methyl group transfers.

Table 1: The structure and occurrence of heme porphyrins

Heme Type	Heme A	Heme B	Siroheme
Occurrence	Cytochrome c oxidase (Complex IV) in mitochondria and bacteria	Hemoglobin, myoglobin, cytochrome b, catalase, peroxidases	Bacteria, fungi and some plants
Structure			

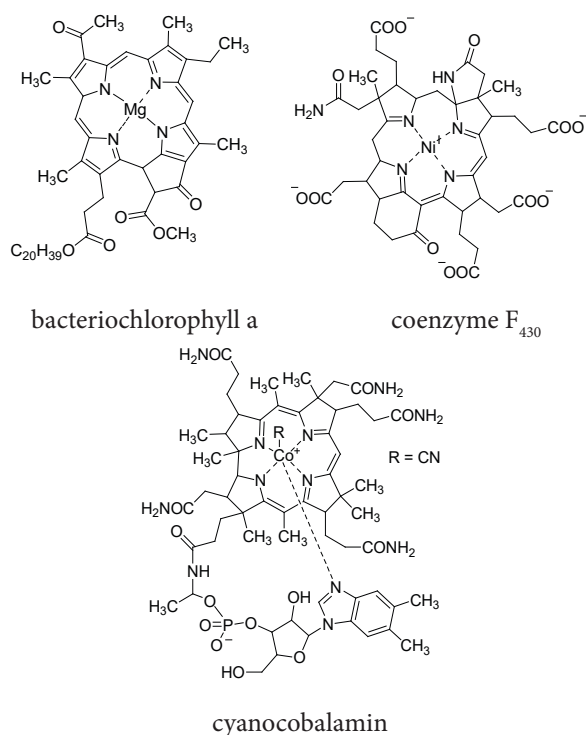


Figure 6: Examples of bacteriochlorin, isobacteriochlorin and corrin

Linear tetrapyrroles:

Linear tetrapyrroles arise from the oxidative cleavage of cyclic forms. They lack extended conjugation over the entire molecule but retain aromaticity within each pyrrole unit. Reduced linear tetrapyrroles are termed bilanes and pyrrole units are interconnected by methylene bridges. Hydroxymethylbilane is a universal biosynthetic intermediate to tetrapyrrole pathways.

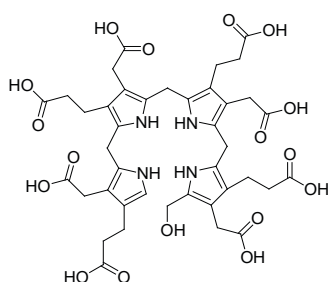


Figure 7: Hydroxymethylbilane

Stabilized linear tetrapyrroles with extended conjugation are known as bilins. Examples include:

- Bilirubin: yellow bile pigment
- Phycobilin: accessory pigments in cyanobacteria and red algae
 - o Phycocyanobilin (blue)

- o Phycoerythrobilin (red)
- o Phycourobilin (orange)

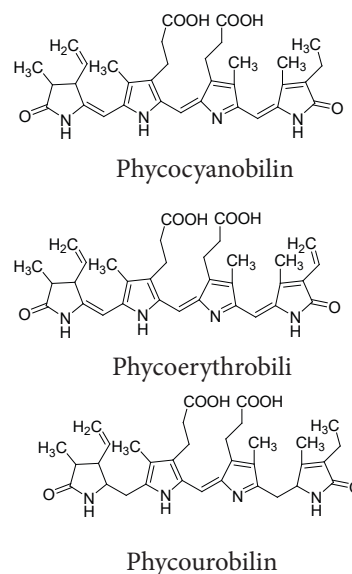


Figure 8: Structures of some major bilins

Biological roles of tetrapyrroles^{1,5,6}

Tetrapyrroles are a biologically indispensable group of organic compounds. Their structural versatility underlies a wide range of biological functions such as,

- **Oxygen Transport and Storage** – Heme: Central to hemoglobin and myoglobin, binds oxygen reversibly, enabling aerobic respiration, fueling cellular metabolism.
- **Photosynthesis** – Chlorophyll: Converts solar energy into chemical energy via light absorption and electron transfer.
- **Electron Transfer and Enzymatic Catalysis** – Cytochromes and catalases: Facilitate electron transfer and protect cells from oxidative stress.
- **Vitamin B₁₂ Function** – Cobalamin: Acts in DNA synthesis, methylation, and fatty acid metabolism.
- **Nitrogen and Sulfur Metabolism** – Siroheme: Involved in nitrite and sulfite reduction in bacteria and plants.

Biosynthesis of tetrapyrroles

Tetrapyrroles are ancient molecules considered to have emerged 3 billion years ago. The biosynthetic pathways of tetrapyrroles share a common evolutionary

origin. All major branches -heme, chlorophyll, and vitamin B₁₂ - begin with 5-aminolevulinic acid (5-ALA), which condenses to form porphobilinogen (PBG). Four PBG units polymerize into hydroxymethylbilane, which cyclizes to form uroporphyrinogen III, a universal precursor.¹

The major pathways, along with their key intermediates, can be summarized as follows. Each of these pathways involves multiple enzymatic steps and is highly regulated.

- **Heme pathway:**

- o Uroporphyrinogen III → decarboxylated to coproporphyrinogen III → oxidized to protoporphyrin IX → insertion of Fe²⁺ to yield heme

- **Chlorophyll pathway:**

- o Protoporphyrin IX → Mg insertion to give Mg-protoporphyrin IX → cyclization to give chlorin rings to yield chlorophyll a/b

- **Vitamin B₁₂ pathway:**

- o Uroporphyrinogen III → methylation to give precorrin-2 → oxidation and isomerization to Factor II → cobalt insertion to give cobalt-Factor II → multiple methylation and ring contraction → adenosylation to generate vitamin B₁₂

Tetrapyrroles and disease

Defects in tetrapyrrole metabolism can lead to serious disorders. Porphyria, which is a group of rare metabolic diseases, is caused by deficiencies or dysfunction in heme synthesis. Accumulation of porphyrin intermediates in various places in the body causes neurological (abdominal pain, seizures, mental changes), dermatological (blistering, photosensitivity) or urinary (red or brown urine) symptoms.⁷



Figure 9: Symptoms of dermatological porphyria

Vitamin B₁₂ Deficiency affects DNA synthesis, red

blood cell formation, and neurological function, causing symptoms such as anemia, numbness (paresthesia), visual disturbances, depression, memory loss and confusion.⁸

Tetrapyrroles for the future

Research into tetrapyrroles has revealed their importance in various fields.

Renewable energy: One of the most promising future applications of tetrapyrroles lies in their use in renewable energy production. Porphyrin and chlorin derivatives play a key role in converting solar energy into chemical fuel in artificial photosynthetic systems. Porphyrin-sensitized solar cells have achieved over 15% efficiency by optimizing light-harvesting and charge transfer processes. Additionally, researchers have developed microbial electrosynthesis systems in which bacteria, engineered to enhance tetrapyrrole biosynthesis, convert CO₂ into biofuels using electrical currents.⁹

Medicine: Heme-based compounds are used as photosensitizers in photodynamic therapy (PDT). It is a treatment that uses light to activate a drug, selectively targeting and destroying cancer cells. Additionally, bilin-based neuroprotectants from cyanobacteria that reduce oxidative stress in neuronal cells have shown promise for Alzheimer's treatment.¹⁰

Food and nutraceuticals: Cyanobacterial tetrapyrroles are gaining popularity as nutraceuticals. Phycocyanin has shown higher antioxidant capacity than vitamin E. Iron supplements made from microbial heme are better absorbed by the body than plant-based iron. This helps treat anemia without side effects. Some supplements containing biliverdin activate genes related to longer life and improved health, as shown in lab studies.¹¹ Chlorophyll and its derivatives are used as natural colorants and antioxidants in food and nutraceutical industries.²

Biotechnology: Using synthetic biology, scientists have increased tetrapyrrole production in microbes. By using modified *Corynebacterium glutamicum* strains, 5-aminolevulinic acid production was increased 50 times more than in the normal strains.¹² CRISPR techniques (genetic engineering tools) have enabled *Propionibacterium freudenreichii* to produce vitamin

B₁₂ at an industrial scale, making vitamin supplements cheaper.¹³

The ability of metalated tetrapyrroles to undergo redox transformations and bind specific substrates makes them valuable as biosensors for detecting environmental pollutants and biological analytes with high specificity and sensitivity.¹⁴

Agriculture: Utilizing tetrapyrroles in enhancing crop productivity and stress resistance in sustainable agricultural practices is also being researched.¹⁵

Conclusion

Tetrapyrroles are fundamental to the chemistry of life, serving as indispensable cofactors in a vast array of biological processes, such as oxygen transport, photosynthesis, electron transfer, and nitrogen metabolism. Their unique structures enable remarkable chemical versatility, facilitating critical functions across diverse organisms and environments. Beyond their natural roles, tetrapyrroles have become valuable tools in renewable energy, medicine, biotechnology, and agriculture. Further studies on tetrapyrrole analogues and biomimetic complexes promise exciting breakthroughs in both fundamental science and applied innovation.

References

- Bryant, D. A., Hunter, C. N., & Warren, M. J. (2020). Biosynthesis of the modified tetrapyrroles—the pigments of life. *Journal of Biological Chemistry*, 295(20), 6888–6925. <https://doi.org/10.1074/jbc.REV120.006194>
- Mansur, M., Colleagues. (2022). Therapeutic and nutraceutical roles of cyanobacterial tetrapyrrole pigments. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2022.932459>
- Pierri, A. E., Cacavelli, L. M., & San Román, M. (2020). Peptide–Tetrapyrrole Supramolecular Self Assemblies: State of the Art. *Molecules*, 26(3), 693. <https://doi.org/10.3390/molecules26030693>
- Wikipedia, <https://en.wikipedia.org/wiki/Porphyrin>
- Larkin, R. M. (2016). Tetrapyrrole signaling in plants. *Frontiers in Plant Science*, 7, 1586. <https://doi.org/10.3389/fpls.2016.01586>
- Banerjee, R., & Ragsdale, S. W. (2003). The many faces of vitamin B12: Catalysis by cobalamin-dependent enzymes. *Annual Review of Biochemistry*, 72, 209–247. <https://doi.org/10.1146/annurev.biochem.72.121801.161828>
- Puy, H., Gouya, L., & Deybach, J.-C. (2010). Porphyrins. *The Lancet*, 375(9718), 924–937. [https://doi.org/10.1016/S0140-6736\(09\)61925-5](https://doi.org/10.1016/S0140-6736(09)61925-5)
- O'Leary, F., & Samman, S. (2010). Vitamin B12 in health and disease. *Nutrients*, 2(3), 299–316. <https://doi.org/10.3390/nu2030299>
- Zhang, W., Lai, W., & Cao, R. (2017). Energy-related small molecule activation reactions: Oxygen reduction and hydrogen and oxygen evolution reactions catalyzed by porphyrin- and corrole-based systems. *Molecules*, 22(7), 1168. <https://pubmed.ncbi.nlm.nih.gov/28222601/#:~:text=Abstract,catalyzed%20by%20porphyrins%20and%20corroles>
- Agostinis, P., Berg, K., Cengel, K. A., Foster, T. H., Girotti, A. W., Gollnick, S. O., ... & Krammer, B. (2011). Photodynamic therapy of cancer: An update. *CA: A Cancer Journal for Clinicians*, 61(4), 250–281. <https://doi.org/10.3322/caac.20114>
- Grover, P., Bhatnagar, A., Kumari, N., Bhatt, A. N., Nishad, D. K., & Purkayastha, J. (2021). C-Phycocyanin—a novel protein from *Spirulina platensis*: In vivo toxicity, antioxidant and immunomodulatory studies. *Saudi Journal of Biological Sciences*, 28(3), 1853–1859. <https://doi.org/10.1016/j.sjbs.2020.12.037>
- Chen, J., Wang, Y., Guo, X., Rao, D., Zhou, W., Zheng, P., Sun, J., & Ma, Y. (2020). Efficient bioproduction of 5-aminolevulinic acid, a promising biostimulant and nutrient, from renewable bioresources by engineered *Corynebacterium glutamicum*. *Biotechnology for Biofuels*, 13, Article 41. <https://doi.org/10.1186/s13068-020-01680-3>
- Tripathi, A., Pandey, V. K., Panesar, P. S., Taufeeq, A., Mishra, H., Rustagi, S., Malik, S., Kovács, B., Suthar, T., & Shaikh, A. M. (2024). Fermentative production of vitamin B₁₂ by *Propionibacterium shermanii* and *Pseudomonas denitrificans* and its promising health benefits: A review. *Food Science & Nutrition*, 12(11), 8675–8691.

- <https://doi.org/10.1002/fsn3.4428>
14. Rao, Y., Sun, Y., Wang, D., & Zhang, Q. (2020). Metalloporphyrin-based electrochemical sensors for analytical applications. *TrAC Trends in Analytical Chemistry*, 124, 115814. <https://doi.org/10.1016/j.trac.2019.115814>
 15. Prasad, R., Bhattacharyya, A., & Nguyen, Q. D. (2017). Nanotechnology in sustainable agriculture: Recent developments, challenges, and perspectives. *Frontiers in Microbiology*, 8, 1014. <https://doi.org/10.3389/fmicb.2017.01014>



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Guest Articles

Biosynthetic Potential and metabolites of the Bioherbicide *Colletotrichum spinosum*

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Introduction

C. spinosum was first found and reported in Queensland, Australia, as *Colletotrichum* sp.¹ and later discovered in New South Wales.² In the late 1940s, this fungus caused severe damage (50–80% mortality) in the *Xanthium spinosum* population.^{3,4} *C. spinosum* was recently identified as a species within the *C. orbiculare* complex,⁴ and has previously been investigated as a targeted bioherbicide for *X. spinosum* based on its ability to kill 6-week-old plants in 14 days using spore concentrations of 107 mL⁻¹.⁵ Since there have been no reports on secondary metabolites (natural products) isolated from *C. spinosum*, the aim of this study was to discover the chemical potential of *C. spinosum* CBS 515.97. In this study, we performed genome mining on the publicly available genome 6 while also screening chemical extracts obtained from different cultivation conditions. The detailed isolation and structural elucidation of 3"-demethylthielavin M (**1**), a polyketide of the tridepside family, and seven other known compounds (**2-8**) that were unknown to be produced by *C. spinosum* was recently published. In addition, detailed bioinformatics analysis enabled us to identify a putative biosynthetic gene cluster (BGC) encoding the biosynthesis of **1**.⁷

Genome Mining

Using fungiSMASH version 7.1.0,⁸ on the publicly available genome (NCBI accession number QAPG000000000.1), we determined that *C. spinosum* CBS 515.97 possesses 74 BGCs likely to encode secondary metabolites. Of the 74 BGCs identified, 15 encode Type I polyketide synthases (PKSs), one encodes a Type III PKS, 17 encode non-ribosomal peptide synthetases (NRPS) or NRPS-like enzymes, 16 are identified as potentially encoding ribosomally synthesized and post-translationally modified peptides (fungal RiPPs) or fungal RiPP-like enzymes, and the remainder are comprised of hybrid combinations, as well as seven miscellaneous BGC types. Most of the BGCs detected by fungiSMASH show little similarity to characterized BGCs from other fungi, with only four of these BGCs sharing significant homology to known BGCs (Table 1).