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Review

Pterin chemistry and its relationship to the molybdenum cofactor

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Contents

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ABSTRACT

The molybdenum cofactor is composed of a molybdenum coordinated by one or two rather complicated ligands known as either molybdopterin or pyranopterin. Pterin is one of a large family of bicyclic N-heterocycles called pteridines. Such molecules are widely found in Nature, having various forms to perform a variety of biological functions. This article describes the basic nomenclature of pterin, their biological roles, structure, chemical synthesis and redox reactivity. In addition, the biosynthesis of pterins and current models of the molybdenum cofactor are discussed.

1. Introduction

1.1. The molybdenum cofactor

The molybdenum cofactor (Moco) from molybdenum enzymes is composed of a molybdenum coordinated by one or two rather complicated ligands known as either *molybdopterin* or *pyranopterin* where both names include a specific reference to *pterin*, a key constituent of the ligand structure (Fig. 1) [1]. The same pyranopterin cofactor is also known to coordinate a tungsten atom in the related tungsten containing enzymes. Three key features of the pyranopterin ligand are the pterin ring system, the pyran ring fused to the pterin structure and the dithiolene moiety that coordinates the metal, Mo or W. A point of variabliity in the ligand is the phosphate terminus, which may be a dinucleotide when the cofactor is from certain bacterial sources. All forms of life possess one or more molybdenum enzymes which participate in a variety of functions, from the global cycling of C, S, N, and As to prodrug metabolism. All such enzymes contain the core Mo cofactor. The molybdenum cofactor is unstable when dissociated from the protein, although several oxidative products have been isolated and characterized [2].

1.2. Pterin nomenclature

Pterin is one of the larger families of bicyclic N-heterocycles named *pteridines*. The pteridine ring system has a pyrimidine joined at the 5,6-position of the pyrimidine ring to a pyrazine (Fig. 2) while *pterin* refers to a pteridine specifically substituted by an amino group at position 2 and a keto group at position 4. The numbering system in a pteridine, as well as the keto-enol tautomerism in pterin, is shown in Fig. 2. The keto-enol equilibrium lies mostly on the keto form, which is designated as 2-amino-4(3*H*)-pteridone. Pterins can exist in several oxidation states indicated by the prefix tetrahydro, trihydro or dihydro-, that specify the number of hydrogen atoms attached to the pyrazine ring and these redox states are discussed in Sections 4.1–4.4. The pterin ring structure is very similar to that of guanine and is closely related to flavin. The four nitrogen atoms within the pteridine bicycle diminish the aromaticity of the system, making it susceptible to nucleophilic reactions [3]. In solution, pteridines are stable to heat, but the addition of acid can lead to ring cleavage forming aminopyrazine carbaldehyde. Unsubstituted pteridine is soluble in water, but common pteridine substituents such as amine and keto groups decrease the solubility through their ability to make intermolecular H-bonds. This characteristic makes pterin manipulation in reactions challenging. Poor solubility can be overcome by substituent modification such as the alkylation of the amine or amide functionality. The field of pterin chemistry is quite mature, and there are several excellent review articles emphasizing different aspects of such chemistry from synthesis, to spectroscopy to biological relevance [3–13].

The role of the pterin component of molybdenum and tungsten enzymes is unknown and remains an active area of research. Potential roles of the pterin portion of the Moco include:

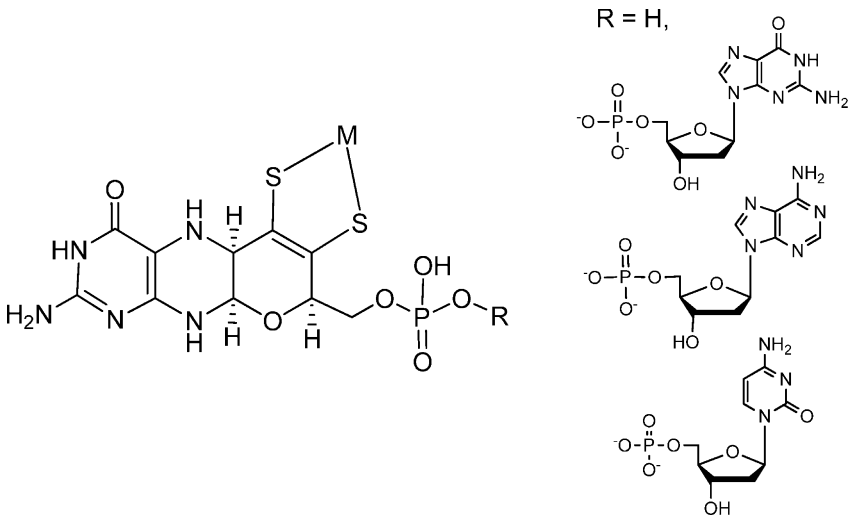


Fig. 1. Structure of the pyranopterin ligand chelating M=Mo in molybdenum enzymes and M=W in tungsten enzymes. Depending on the enzyme source the phosphate terminus may be one of the dinucleotides R, shown to right.

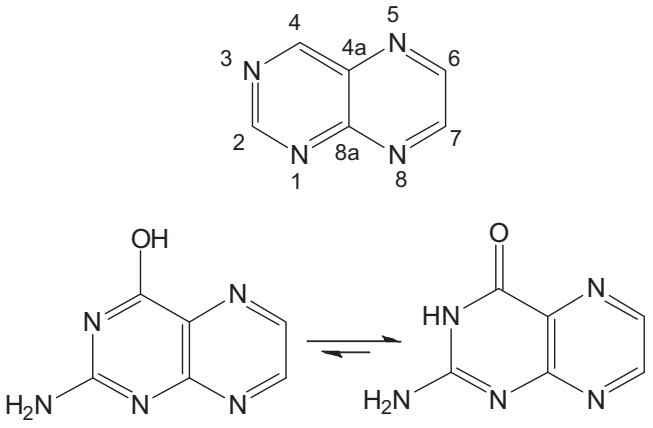


Fig. 2. Numbering pteridine unit and keto-enol equilibrium in pterin.

(a) providing an efficient electron transfer pathway for the regeneration of the catalytically competent metal center; (b) poising the redox potential of the metal center through modulation of the H-bonding, and/or imposing a specific conformation influencing the interaction between the metal and dithiolene sulfur orbitals; (c) providing an anchor for the metal ion. The three-ring pyranopterin structure has been observed in all but three X-ray structures of molybdenum proteins which suggests that it exists preferentially in that tricyclic form. However, the deviating structures exhibit the pyran ring as open, revealing a 6-substituted pterin [14–16]. This has prompted the suggestion that ring opening and closing may be operating during catalysis [17].

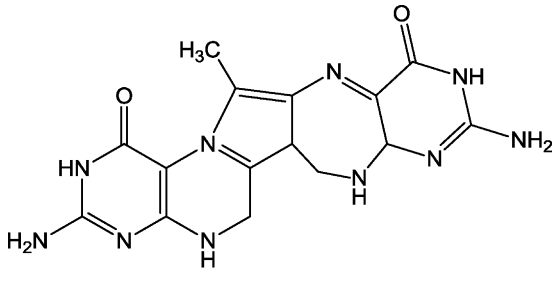
Given the uncertainty concerning the role of pterin, a foray into the larger context of pterin chemistry and biochemistry may prove productive. This is the intent of this chapter which will define the nomenclature of pterin-related chemistry and structures, provide a brief survey of the roles of pterin elsewhere in biology, describe the chief synthetic methods to pterins as well as their complex redox processes, give an overview of the biosynthesis of key pterins, and finish by presenting the state of synthetic models for Moco that include pterin components. The goal of this review article is not to summarize all the historical discoveries but to highlight some of the salient features of pterin chemistry. Special emphasis is given to the pterin in the context of molybdenum cofactors.

2. Biologically derived pterin molecules

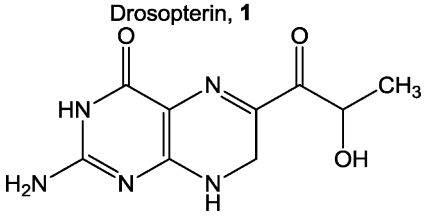
Various substituted pterin derivatives have been isolated from different organisms where these are almost universally substituted at the 6 position. Pterins fulfill a variety of roles in biology including as pigments, one-carbon transfer cofactors and redox cofactors.

2.1. Pterins as pigments

With the exception of tetrapyrroles, pterins are arguably the most important natural monomeric chromophores that are found in many animals and insects. Among the insects perhaps the best documented is *Drosophila melanogaster* (fruit fly) whose eye coloration is due to many pterin molecules. A dimeric pterin found in *D. melanogaster* has been appropriately named as Drosopterin (structure **1**). The drosopterin and its isomeric variations such as isodrosopterin, and neodrosopterin are orange to red in color; the latter two compounds have a similar substitution at position 6 with a three-carbon side chain. There are other pterins present in Drosophila including the yellow sepiapterin (structure **2**), and isosepiapterin. Sepiapterin is also found in vertebrates. Isosepiapterin has the structure of 6-propionyl-7,S-dihydropterin.



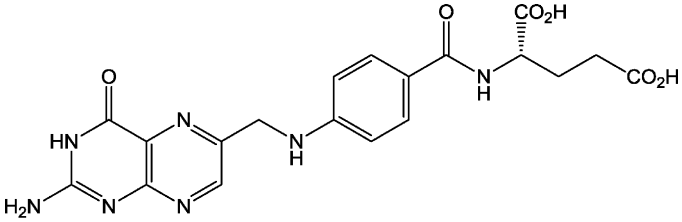
Drosopterin, **1**



Sepiapterin, **2**

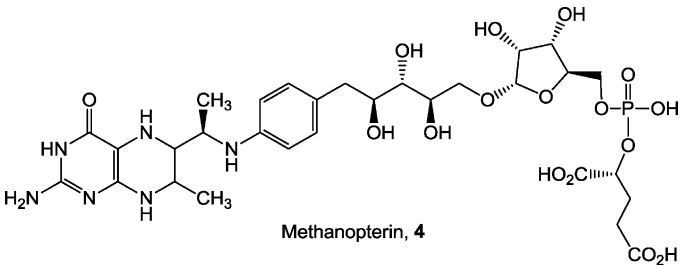
2.2. Pterins in C1 transfer cofactors

Perhaps the most well-known pterin compound is folic acid (structure **3**), commonly known as vitamin B9, also substituted at position 6. The fully reduced form of folic acid, tetrahydrofolate (H_4F), is ubiquitous in living organisms and has three distinct moieties: the pteridine moiety, the *p*-aminobenzoate connected to the pteridine ring at the 6 position, and a glutamate function that is coupled with the carboxylate group of the benzoic acid. While the pterin is biosynthesized by humans, the complete cofactor is not. This molecule plays important roles in the biosynthesis of amino acids and nucleic acids since folate can carry an activated methyl group at N5 that can be transferred to a substrate. The two electron oxidized folate is reduced by dihydrofolate reductase (DHFR) to generate the active state. Because tetrahydrofolate deficiency leads to the retardation of essential biosynthesis in cells, a process that is more important in growing cells, inhibition of DHFR is often used as a strategy for chemotherapy. Folate deficiency leads to many physiological disorders, such as anemia, and it is especially important in pregnant women as neural tube defects have been implicated with folic acid deficiency.



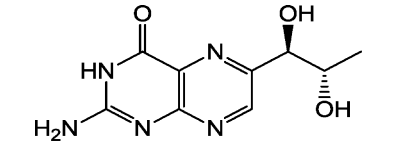
Folic acid, **3**

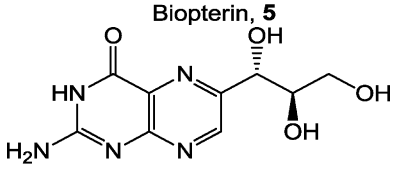
The analog of folate in methanogenic bacteria is methanopterin (structure **4**) which is involved in fixing CO_2 to produce methane. Methanopterin deviates from the typical 6-substitution observed in biological pterins as it is functionalized at both the 6- and 7-positions. In methane biosynthesis, methanopterin is formylated at the N5 position through the reduction of CO_2. The N5-formyl is subsequently converted first to a methyl group then to methane.



2.3. Pterins as redox cofactors

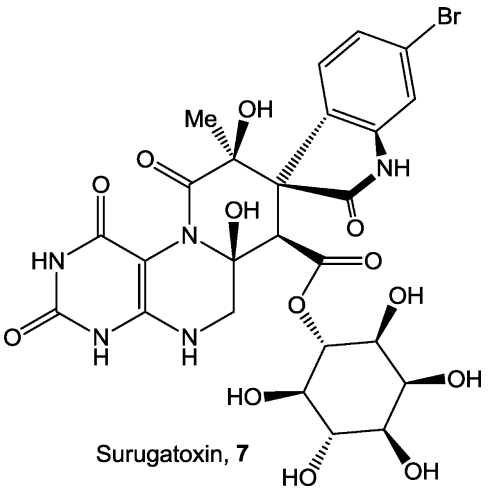
Two important pterins isolated from animals are biopterin (structure **5**) and neopterin (structure **6**). Both pterins are substituted at C6 by a hydroxypropyl moiety. The propyl side group harbors two chiral centers, thus providing possibility of different diastereoisomers. The major isomer in the case of biopterin is an L-isomer while that in neopterin is a D-isomer. In some cases, one of the hydroxy groups is glycosylated to give different derivatives of naturally occurring biopterin and neopterin [18]. The fully reduced form, tetrahydrobiopterin (H4biopterin) was first recognized as the redox cofactor for the aromatic amino acid hydroxylases such as phenylalanine hydroxylase (PAH). More recently H4biopterin has been identified as a required cofactor in nitric oxide synthase (NOS). A biosynthetic defect in H4biopterin has been implicated in phenylketonuria as well as several neurological diseases such as Alzheimer and Parkinson's diseases. Neopterin has been isolated from frog skin and fruits flies, as well as from bacterial sources. In humans neopterin is of interest as it is elevated in cancer patients. Other physiological roles of neopterin include the induction of apoptosis and protection of cellular protein from oxidative damage.

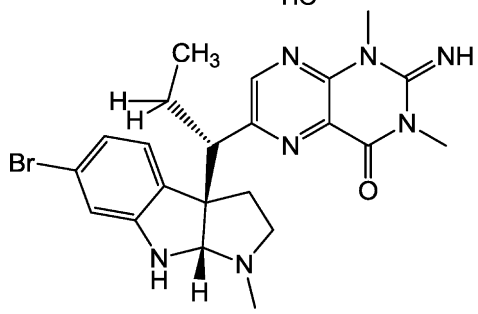


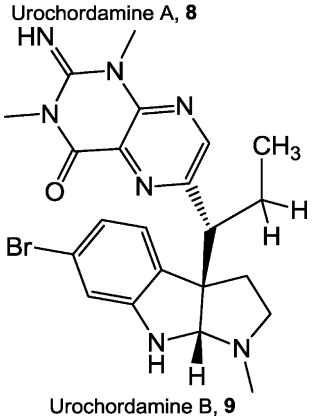


2.4. Pterins as toxins

Not all naturally occurring pterins are physiologically beneficial or aesthetically pleasing from a universal point of view; some of them can cause harmful effects. For example, surugatoxin (structure **7**) is a complicated molecule isolated from the Japanese ivory mollusk (*Babylonia japonica*) found in Suruga Bay [19]. This compound produces a prolonged hypotension in would-be predators of the mollusk, which may be linked to ganglion blocking; i.e., it can function as ganglioplegic. The total synthesis of surugatoxin has been accomplished. Other complex pterin containing natural products include urochordamines, an alkaloid isolated from tunicate, Ciona savignyi [20]. Urochordamine A (structure **8**) has been suggested to promote larvae settlement and metamorphosis of the tunicate at a low concentration, while the stereoisomer urochordamine B (structure **9**) showed no activity at that concentration.



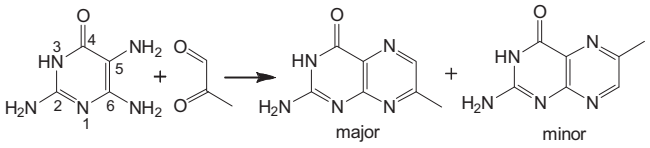




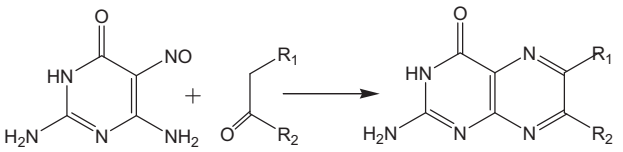
The two isomers differ in the stereochemistry of the carbon that links to the pyrazine ring.

3. Chemical synthesis of pterins

Chemical synthesis of the two heterocycles in pteridine, i.e., pyrimidine and pyrazine, can be accomplished in any order, i.e., starting with a pyrazine and building on the pyrimidine ring or from a pyrimidine and constructing the pyrazine ring. The pteridine substituents characteristic of the pterin structure can be introduced through derivatizing a pteridine or by carrying the functionality through the formation of the pterin system. Chemical synthesis of the pyranopterin cofactor poses an additional challenge since a pyran ring must be included in the ligand framework. In this section we discuss some of the strategies of pterin synthesis as well as several synthetic approaches to the pyranopterin unit of the molybdenum cofactor.



Scheme 1. Gabriel–Isay condensation.



Scheme 3. Stereoselective synthesis of pterin by Timmis reaction.

3.1. Synthesis via the formation of a pyrazine ring starting with a pyrimidine ring

3.1.1. Gabriel–Isay condensation

A popular methodology for synthesizing pterin involves the Gabriel–Isay condensation reaction. This method involves condensation of 5,6-pyrimidinediamines with compounds containing a 1,2-dicarbonyl functionality. Various types of 1,2-dicarbonyl compounds such as dialdehye, ketoaldehyde, aldehydoester, diketone and diesters have been successfully used in synthesizing pterin using Gabriel–Isay condensation. Because the pyrimidine ring is unsymmetrical, reactions with unsymmetrical diketones lead to the formation of regioisomers. In the traditional Gabriel–Isay condensation, the major product is the 7-substituted isomer (Scheme 1). Mechanistically, this reaction thought to involve an initial nucleophilic attack of the more reactive amino group at the more reactive carbonyl functionality followed by a ring closure via a similar condensation reaction. Thus there are two dehydration steps in the reaction sequence and the isomeric product distribution is dependent on relative reactivity of the amine and carbonyl functionalities. Various modifications of this basic reaction have modulated the regioselectivity by taking advantage of the mechanistic description. For example, the nucleophilic character of the amine groups in positions 5 and 6 are different because the amine on C5 is more nucleophilic, and reacts preferentially with the carbonyl group. The aldehyde carbonyl is in turn more reactive than the keto, and reacts preferentially with the amine at C5 position yielding the 7-substituted compound as the preferred regioisomer. Under strongly acidic conditions the C5 amine nitrogen is protonated and therefore nucleophilic advantage is lost, leading to the 6-substituted isomer [21–23]. However, when one of the carbonyl functionalities is from an aldehyde the reaction is less predictable, and is dependent on exact conditions [24–26].

The influence of reaction conditions has also been investigated, particularly with respect to the regiospecific/regioselective formation of a particular isomer. Thus, under neutral conditions reaction of methylglyoxal with 2-phenylpyrimidine-4,5,6-triamine yields 7-methyl derivative, however, in the presence of hydrazine the 6-methyl derivative is isolated as the only product indicating the regiospecificity of the reaction (Scheme 2) [27]. Condensations of methylglyoxal with a diamino pyrimidine in the presence of sodium hydrogen sulfite at pH ∼ 4 allowed the ready separation of the two isomers formed.

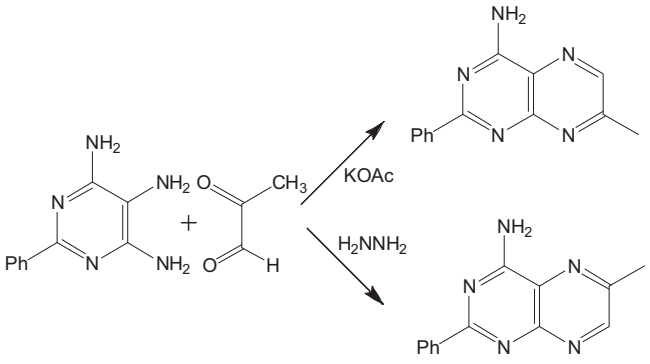
Unlike in the previous case, where reactivity of the amine was modulated to obtain a specific regioisomer, both regioisomers are formed under these conditions. However, $NaHSO_3$ forms adducts in different ways with the 6- and 7-isomers such that the 6-substituted pterin precipitates from the solution as pure 6-substituted isomer. The 7-substituted isomer can be isolated from the filtrate of the reaction mixture. In this case, the 6-substituted isomer was isolated in a higher yield, almost in 2:1 ratio [28]. Other attempts to improve the synthesis of 6-methyl pterin in a one pot synthesis involved condensing 2,5,6-triaminopyrimidin-4(3H)-one with methylglyoxal pretreated with $NaHSO_3$, at a lower temperature 0–5 °C [29].

A microwave assisted Gabriel–Isay condensation reaction has been reported to yield 6-substituted pterin regiospecifically in good yield, and with significantly reduced reaction time [30]. Pterins have also been synthesized through solid state support very effectively. The basic strategy involves the functionalization of a pyrimidine moiety onto a polystyrene resin, cyclization of the pyrazine ring followed by oxidative cleavage of the target compound from the resin through nucleophilic substitution [31,32].

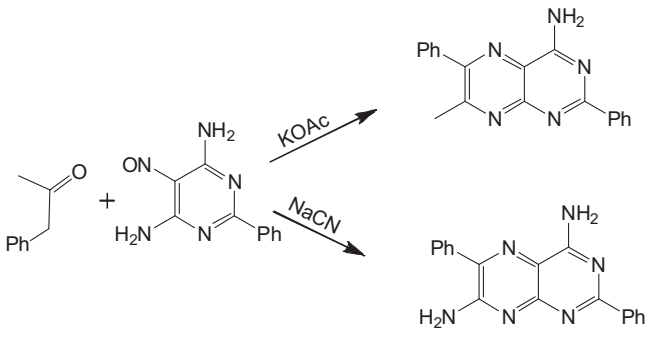
3.1.2. Timmis reaction

The stereoselective synthesis of pteridine achieved by condensing 5-nitroso-6-aminopyrimidine with an active methylene-containing compound is called the Timmis reaction (Scheme 3) [33]. Here the nitroso group serves as a precursor to the amine functionality, and an active methylene group containing compound is coupled with the pyrimidine. The active methylene containing compound may be keto, aldehyde, ester or nitrile, and the condensation reaction is usually carried out in the presence of a base. The first step of this condensation process is the reaction between the keto group and the amine group connected to C6. The second step involves the condensation of the active methylene group with the nitroso group, thus yielding a regiospecific product. The regioselective nature of this reaction makes it a very versatile method of synthesizing pterins [34].

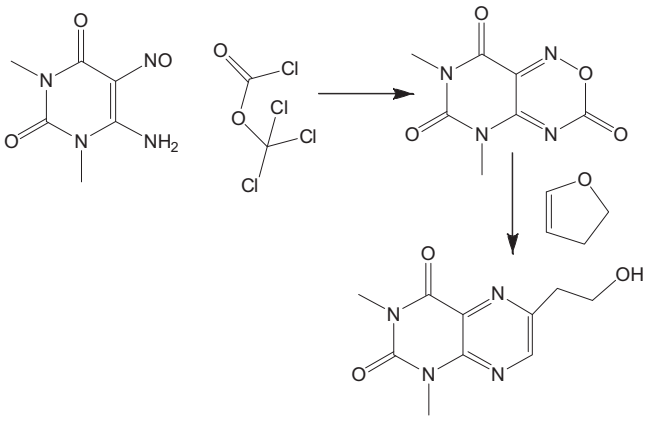
The generic Timmis reaction has been modified in a variety of ways by Pachter et al. to obtain different products; Scheme 4 shows two examples [35–38]. The reactants in the presence of the mild base KOAc yield the methyl-substituted product.



Scheme 2. Condition dependent Gabriel–Isay condensation.



Scheme 4. Condition dependent stereoselectivity of Timmis reaction.



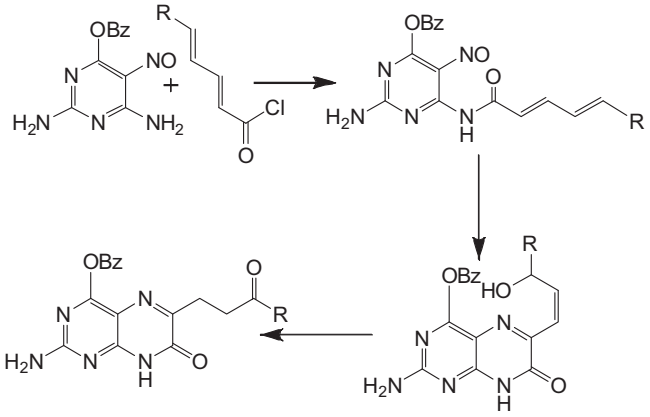
Scheme 5. Modified Timmis reaction with Diels-Alder type condensation.

Under both conditions i.e., either in the presence of a base or cyanide, the reaction presumably proceeds through a hydroxylamine intermediate which under basic conditions can be dehydrated yielding the expected phenyl substitution at C8. Instead of dehydration the hydroxylamine intermediate can undergo oxidation and the oxidized product can react with the cyanide producing a nitrone which subsequently cyclize to the amine derivative.

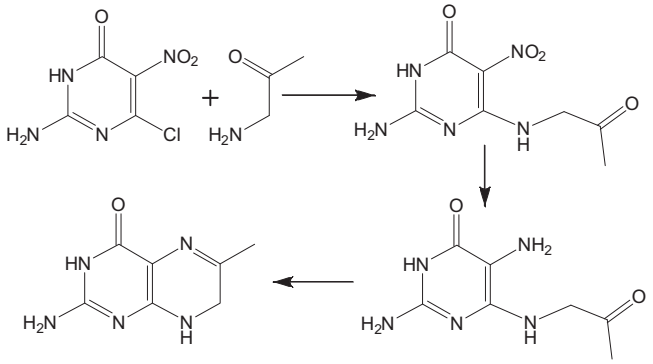
Intramolecular cyclization of oxadiazinone with dienophiles serves as another approach for constructing the pyrazine ring [39,40]. The oxadiazinone can be synthesized from the nitroso amine derivative as in the Timmis reaction. Because one can manipulate the dienophile easily, a wide variety of substitutions can be made on the pyrazine ring (Scheme 5) [41]. An interesting modification of this reaction was reported by Xu et al. where intramolecular Diels-Alder reaction leads to the formation of a pterin derivative (Scheme 6) [42].

3.1.3. Polonovski–Boon reaction

Because nitro groups are readily reduced to amines, the pyrazine ring of the pterin has also been formed by condensing 6-chloro-5-nitro-pyrimidine with α-amino carbonyl compounds, in a reaction, known as the Polonovski–Boon cyclization (Scheme 7) [43,44]. An advantage of this reaction is an entry to the reduced dihydro form of pterin, which can also be readily converted to the fully oxidized pterin. The strongly electron withdrawing nitro group makes the carbon attached to the chloride susceptible to nucleophilic attack by the nitrogen lone pair of the amino carbonyl compound leading to a carbon–nitrogen bond formation with the loss of chloride.



Scheme 6. Intramolecular Diels-Alder reaction incorporating the Timmis reaction.



Scheme 7. Polonovski–Boon reaction.

Reduction of the nitro group with subsequent formation of a second carbon–nitrogen bond leads to a dihydropterin which can be oxidized by an oxidizing agent to afford fully oxidized pterin. This regiospecific reaction has been used in synthesizing functionalized tetrahydrobiopterin [45,46].

3.1.4. Viscontini reaction

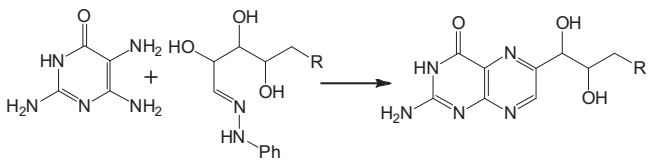
The Gabriel–Isay condensation reaction used with α-oxo oximes affords regioselective products. A similar methodology with sugar derivatives is known as the Viscontini reaction (Scheme 8; additional details are provided in Section 3.4.1). Condensation of 2,5,6-triaminopyrimidine-4(3H)-one with the phenylhydrazone derivative of a sugar in mildly acidic solution leads to the 6-substituted 2-aminopteridin-4(3H)-one. This is a regioselective reaction where, depending on the reactants, an Amadori rearrangement may take place. This methodology was applied in a large scale synthesis of biopterin and neopterin using phenylhydrazone derivatives of the corresponding pentose [9,47–49]. The same methodology was applied by Pfleiderer to obtain a pyranopterin compound that has been crystallographically characterized [50].

3.2. Synthesis via the formation of a pyrimidine ring starting with a pyrazine ring

3.2.1. Taylor method

Pterin synthesis starting from a pyrazine ring onto which is constructed a pyrimidine ring is an alternative approach when the construction of a pyrazine ring onto an existing pyrimidine is not straightforward. This method can be limited by the availability of the desired substitution on the pyrazine reagent. The basic premise of this approach is the formation of a pyrimidine ring where the nitrogen is incorporated from an amine such as guanidine, urea or thiourea [51,52]. In these reactions at least one nitrogen atom of the pyrimidine comes from the guanidine or its analogs forming a carbon–nitrogen bond. The insertion reaction to form C2–N3 fragment into a pyrazine ring is known as the Taylor synthesis. This method has been used in preparing different pterin derivatives as schematically shown in Fig. 3.

The two nitrogen atoms of the pyrimidine ring can originate as substituents on the pyrazine ring. There are a variety of ways to incorporate the additional carbon atom into the pyrimidine ring.



Scheme 8. The Viscontini reaction..

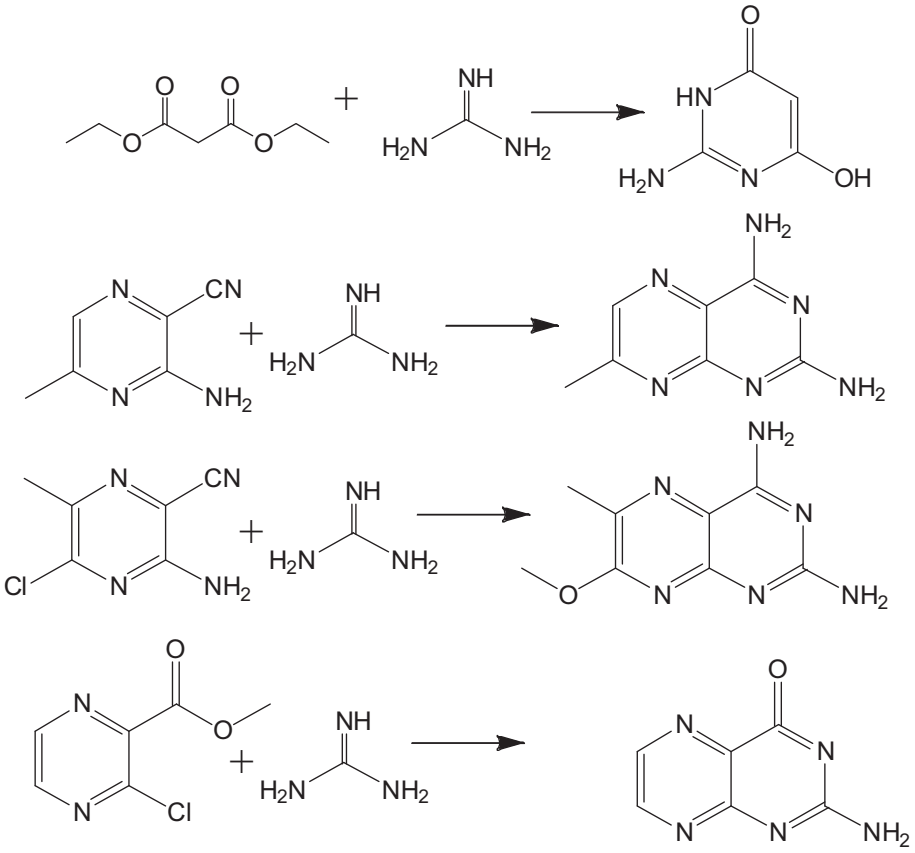


Fig. 3. Schematic representations of different approaches to construct the pyrimidine ring of the pterin developed by Taylor.

Use of orthoformate as a source of one carbon yields a reduced pteridine, which can be oxidized by MnO_2 to the corresponding aromatic compound [53]. An intramolecular aza-Wittig reaction has been used to cyclize a substituted pyrazine to yield a pterin [54]. Ammonia can also be used as a source of the nitrogen atom of the pyrimidine ring of the pterin. These reactions are summarized in Fig. 4 [55]. Asymmetric pterins are produced from asymmetrically substituted pyrazine precursors, a method used to synthesize the biomolecules urothione (Scheme 9) [56] and neopterin (Scheme 10) [49].

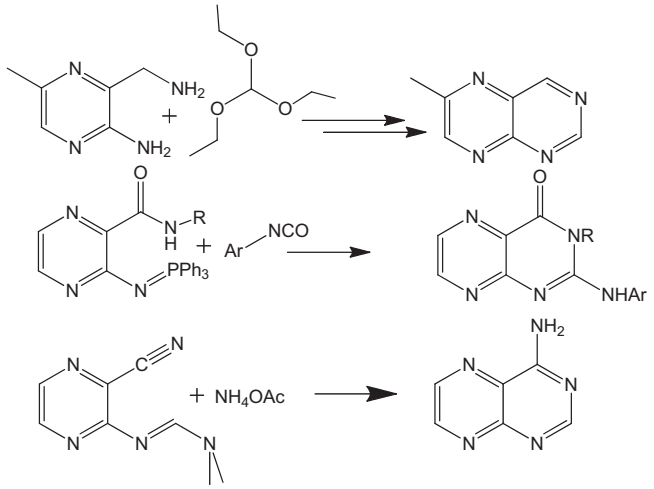
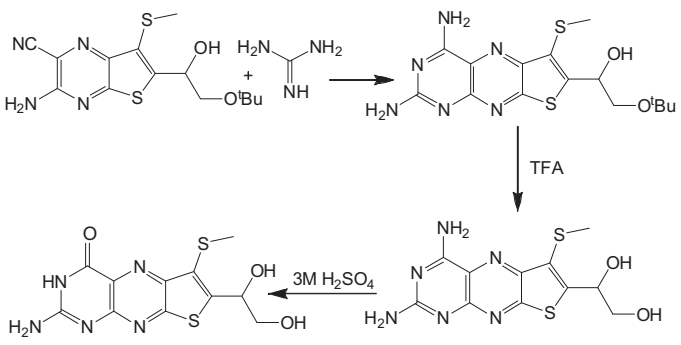


Fig. 4. Formation of the pyrimidine ring from pyrazine derivatives.

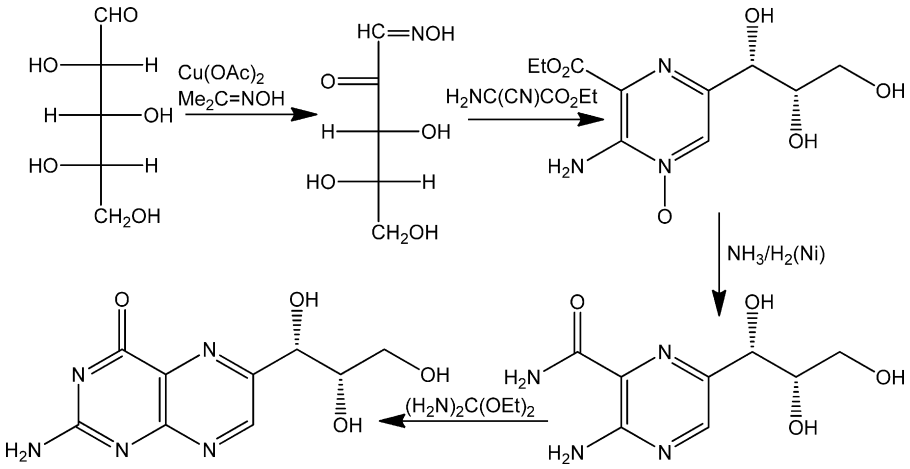
3.3. Pterin side chain modification

Side chain modification on a pterin can be accomplished either after the construction of the pterin ring system or by carrying a specifically substituted pyrazine on to a pterin through the Taylor reaction. For example, a halogenated (chloro or bromo) pyrazine and pterin ring can undergo Pd catalyzed C–C bond formation (Heck coupling) with alkynes (Scheme 11).

In the case of pyrazine the pyrimidine ring can be formed subsequently by reaction with guanidine (Taylor reaction). This methodology is particularly useful in the functionalization of the alkyne during the preparation of model molybdenum cofactors. The chloropteridines have also been used in preparing thione derivative by reacting with sodium hydrogen sulfide. Use of alkyl sulfide affords alkyl sulfinyl pteridines [3].



Scheme 9. Scheme for total synthesis of urothione.



Scheme 10. Synthetic route to neopterin.

3.4. Synthesis of pyranopterin

The pterin cofactor of the molybdenum and tungsten enzymes is distinctive from all other pterins used in biology because there is a pyran ring fused at the C6 and C7 positions of the pterin system. This structure, referred to as a "pyranopterin", changes not only the numbering system of the three ring structure (Fig. 1) but more importantly, it will impact the relevant redox chemistry discussed for pterins in Section 4. There are only a handful of reports on pyranopterin synthesis. These can be separated into three categories as discussed below.

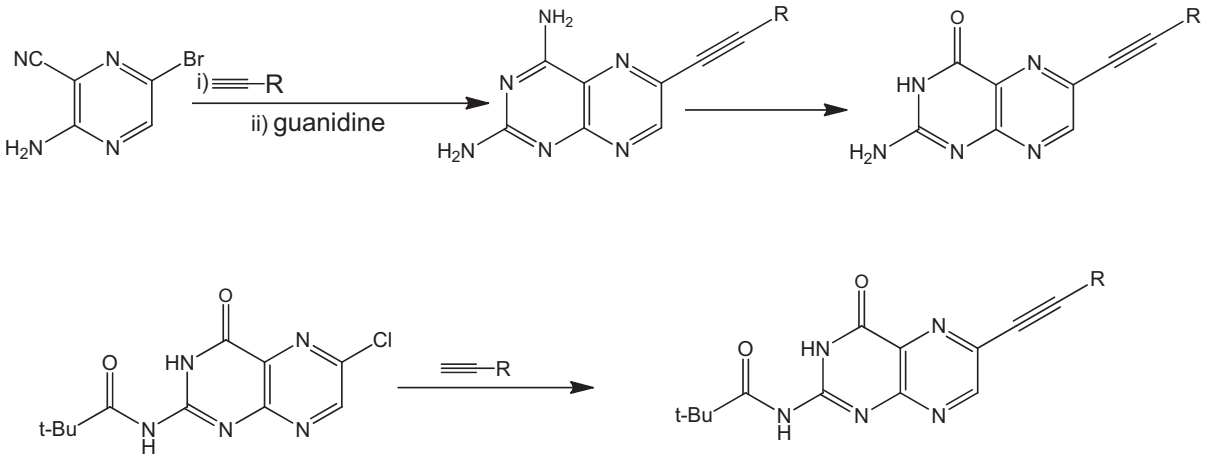
3.4.1. Pyranopterins via 5,6-dihydropterins

The first report of a synthesis leading to a pyranopterin appeared in 1985 as part of a series of studies directed towards regiospecific formation of 6-substituted pterins. Viscontini had reported the synthesis of L-biopterin starting from a condensation of the phenylhydrazone of 5-deoxy-L-arabinose with a 4-hydroxy-2,5,6-triaminopyrimidine [57]. The possibility of an unusual 5,6-dihydrobiopterin intermediate in this reaction sequence was later pursued by Soyka and Pfleiderer through an investigation of the reaction of the phenylhydrazone of D- or L-arabinose and 4-hydroxy-2,5,6-triaminopyrimidine (Scheme 12) [50]. The formation of 5,6-dihydro-L-neopterin **1** after the elimination of phenyl hydrazine was presumed to precede the subsequent oxidation (with O_2, MnO_4^-) that yielded L-neopterin **2**.

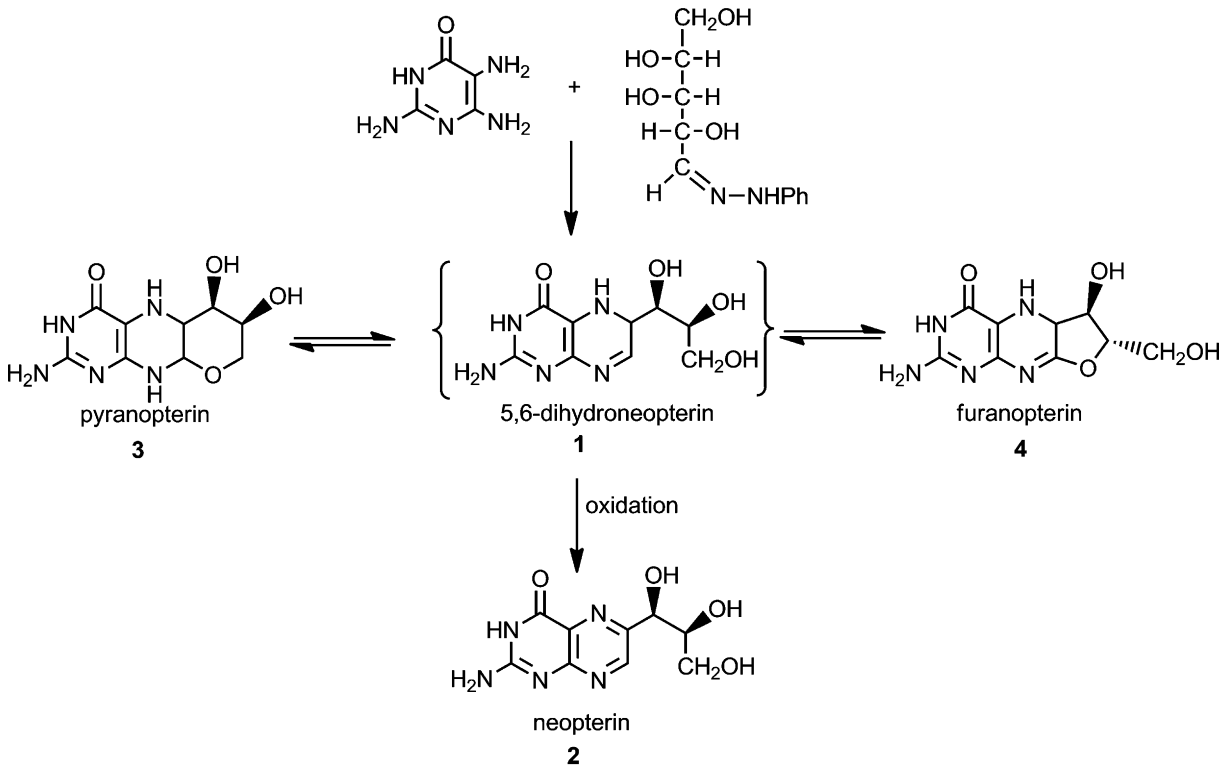
It was suggested that the hydroxyl group in the C3′ position is involved in the regiospecific cyclization process leading to one diastereoisomer. However these researchers discovered that the isolated dihydropterin intermediate existed not as **1** but in two cyclized forms, the pyranopterin **3** and the furanopterin **4**. The pyrano- and furano-rings in **3** and **4** result from nucleophilic attack of the side chain hydroxyls at C3′ and C2′, respectively, and these structural isomers exist in solution as an equilibrium mixture of ratio 3:1 where the pyrano-form predominates over the furanopterin form. The pyranopteridine structure was later confirmed by X-ray crystallography on a 2-thiomethyl, 4-amino-derivative related to **3** [58].

3.4.2. Pyranopterins from pyrazine ring alkylation

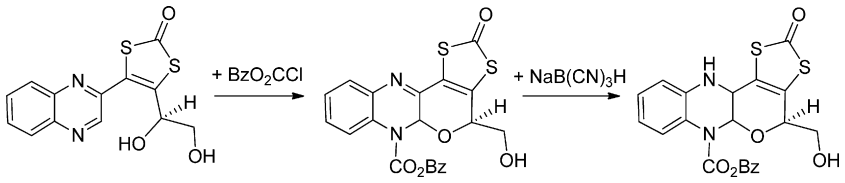
The efforts of several groups notably Garner, Joule, and Burgmayer to develop synthetic analogs of Moco have produced other examples of pyran ring formation fused to pterins and quinoxaline. Joule and co-workers first demonstrated that alkylation at a quinoxaline N4 site reduction promotes pyran ring cyclization, and that subsequent pyrazine ring reduction by cyanoborohydride creates the fused pyranopyrazine structure observed in the molybdopterin ligand of Moco [59,60]. The reaction scheme in Scheme 13 illustrates this strategy operating on a quinoxaline bearing a protected form of dithiolene at position 2. Choice of alkylating agent is critical; use of chloroformate leads to the alkylation of the side chain hydroxyl, but benzylchloroformate



Scheme 11. Pterin synthesis via ring functionalization.



Scheme 12. Formation of a pyranopterin from cyclization of 5,6-dihydroneopterin and its equilibrium with a furanopterin.



Scheme 13. Pyran ring cyclization on quinoxaline.

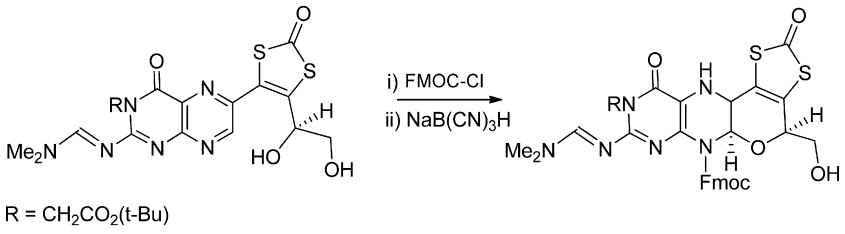
was successful in pyrano-cyclization on quinoxaline. This approach extrapolated to the pterin system (Scheme 14) was also successful if Fmoc-Cl (Fmoc = fluoren-9-ylmethyl chloroformate) is used as the alkylating agent to initiate pyrano ring formation. The deprotection of the 2-amino group under basic condition can provide the complete cofactor [61].

3.4.3. *Pyranoquinoxalines via the Gabriel–Isay condensation*

Synthetic efforts directed at the pyranopterin ligand of Moco often use a quinoxaline as a simpler N-heterocycle to test the method. Here such an approach was used in combination with the Gabriel–Isay condensation of a protected pyranodithiolene diketone to produce a pyranoquinoxaline system. The condensation with the amine and the ketone functionalities leads primarily to an open ring compound as the major product. The ring closure forming a pyran quinoxaline was achieved by subsequently reacting with benzyl chloroformate (Scheme 15). The dithiolene moiety can be deprotected under basic conditions. The compound itself is slightly fluorescent, but when it binds to Pb^{2+} it exhibits enhanced fluorescence thus functioning as a 'turn on' sensor. The sensor is exquisitely sensitive and can detect ppb levels of Pb^{2+} even in the presence of other metal ions. This compound was appropriately named as 'leadglow' [62].

4. Pterin redox chemistry

Participation in redox reactions of organic molecules is one of the fundamental roles of pterins in biology. The N-heterocyclic structure of the bicyclic pterin system exhibits a wealth of redox reactions in ways similar to the related



Scheme 14. Pyran ring cyclization on pterin.

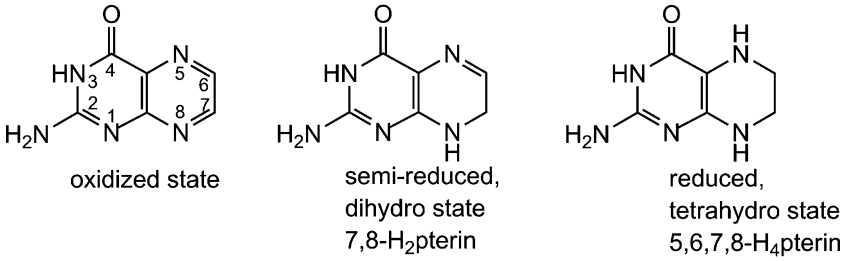
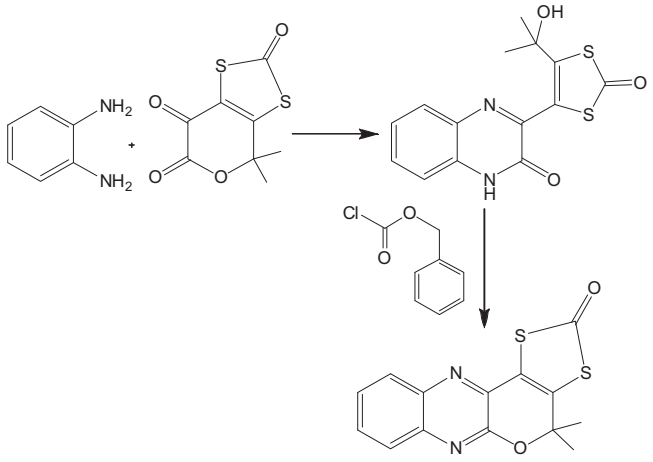


Fig. 5. The three oxidation states of pterin.



Scheme 15. Synthesis of fluorogenic pyranopterin using Gabriel–Isay condensation reaction.

N-heterocycle isoalloxazine in FAD. Unlike FAD where redox reactions are limited to $2e^-$, $2H^+$ processes, pterins are able to transfer up to $4e^-$, $4H^+$ units in sequential reactions. This section describes the different redox states of pterins, how they are produced chemically and detected electrochemically, how they are known to function in biological redox processes and how metals influence the redox chemistry of pterins.

4.1. Pterin redox states

There are three main redox states of pterins: the fully oxidized state, the semi-reduced or dihydro state and the fully reduced or tetrahydro state (Fig. 5). These are interconverted by $2e^-$, $2H^+$ reactions as described in more detail below. The greater complexity of pterin redox chemistry (as compared to flavin redox chemistry) becomes apparent when the tautomers of the semi-reduced state are considered. Choice of reduction method determines which semi-reduced pterin is initially produced (Fig. 6). Regardless, all dihydropterin tautomers, unless highly substituted, will eventually rearrange to the most thermodynamically stable form, the 7,8-dihydropterin.

4.2. Redox chemistry

4.2.1. Synthetic methods for reduced pterins

Synthetic procedures for preparing pterins by the condensation of diaminopyrimidines and α,β-dicarbonyl reagents (Gabriel–Isay synthesis) will yield pterin in the fully oxidized state. During pterin synthesis by other methods (e.g., Polonovski–Boon synthesis), the semi-reduced 7,8-dihydro-form is the immediate product of ring closure but if the reaction is performed in air, oxidation by O_2 may produce the fully oxidized pterin. Otherwise oxidants such as hydrogen peroxide, permanganate or iodine are used to produce the oxidized pterin. Under the tightly controlled environment of biological pterin synthesis, biosynthetic processes produce pterin in one of the tautomeric forms of the semi-reduced state. This dihydro state of the pterin ring system persists through subsequent side chain manipulations to give the final species. Synthetic preparation of reduced 5,6,7,8-tetrahydropterins from oxidized pterins most often uses catalytic hydrogenation or borohydride reagents. Many different pterins have been reduced from the oxidized state to the tetrahydro state by reaction with H_2 gas in acidic solution (pH < 1), either aqueous or methanolic, in the presence of palladium metal on carbon (Scheme 16) [63]. Tetrahydropterins are generally unstable in air towards oxidation to a dihydro state followed by rapid

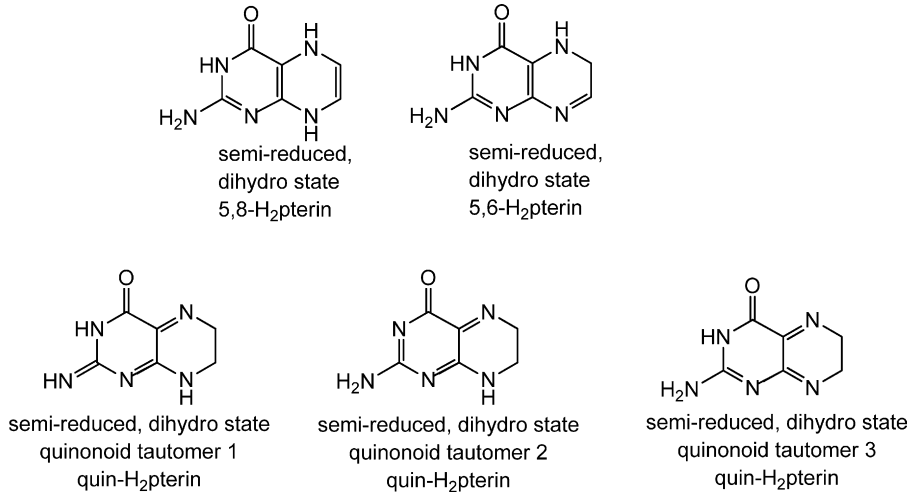
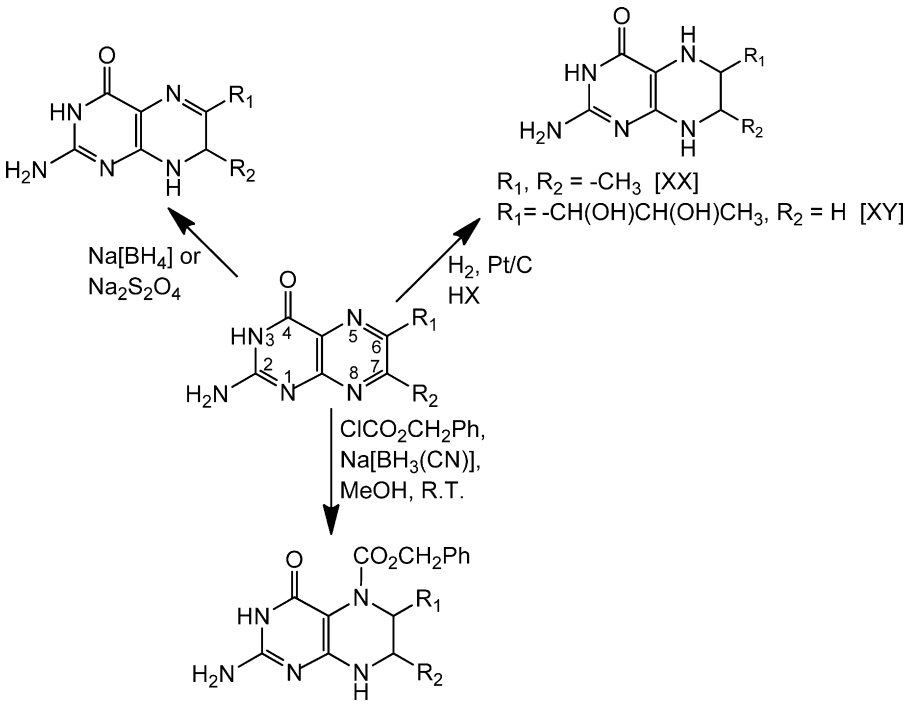


Fig. 6. Less thermodynamically stable dihydropterin tautomers.



Scheme 16. Different synthetic approaches to reduce the pterin ring from the fully oxidized pterin.

rearrangement to the stable 7,8-dihydro tautomer. It has been observed that the rearrangement is blocked by disubstitution at the 6 position permitting isolation of a quinonoid tautomer of dihydropterin [64,65].

Sodium dithionite will partially reduce oxidized pterin to 7,8-H_2pterins [66]. Borohydride reductants of the general type $M[BHR_3]$ (M = Li, Na, K; R = H, alkyl, CN) can be selected to partially reduce oxidized pterins to the semi-reduced state, usually obtained as the stable 7,8-dihydropterin tautomer (Scheme 16). Combining a borohydride reductant with an alkylating reagent (benzylchloroformate or FMOC) accomplishes reduction of pterins to the 5,6,7,8-tetrahydro reduced state with mono-alkylation (Scheme 16) while di-alkyl protection at N5 and N8 is required for quinoxaline reduction [67]. In simple pterins the reduction proceeds by first alkylation at N5 followed by hydride addition from the borohydride reagent. It should be noted that N-alkylation generally has a stabilizing effect. Alkylation at N3 has been observed to stabilize quinonoid tautomers against rearrangement to 7,8-dihydropterins [68].

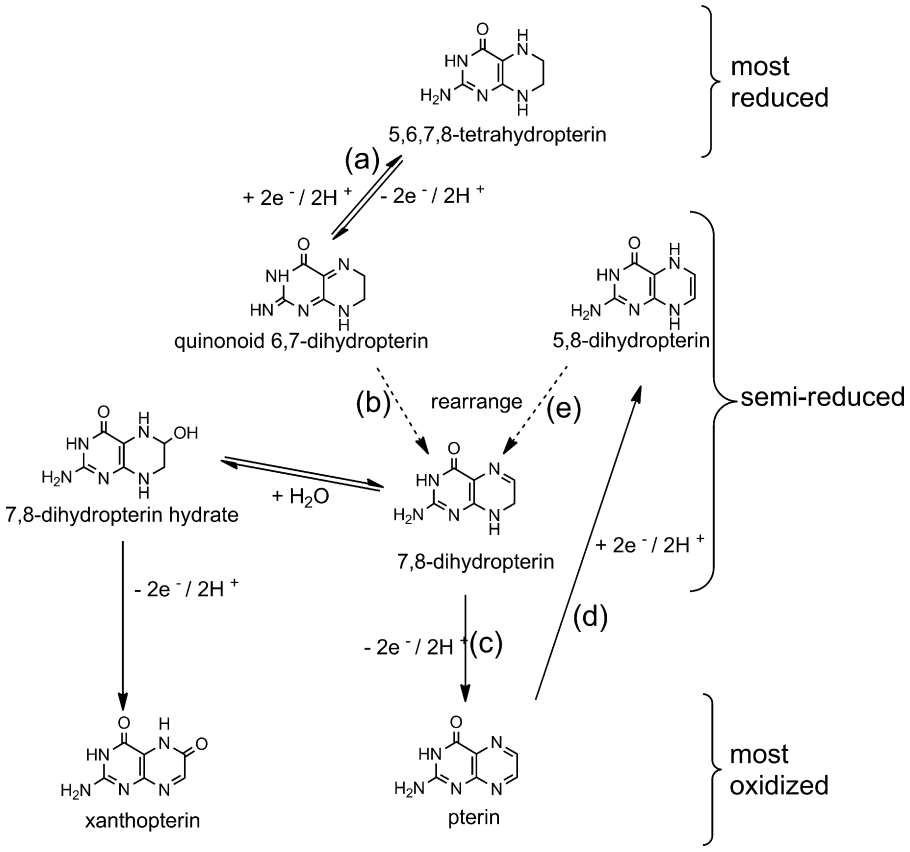
4.2.2. Electrochemical methods

The most thorough investigation of the redox processes of pterins has been accomplished in a series of studies by Dryhurst using cyclic and linear sweep voltammetry combined with thin-layer spectroelectrochemistry [69–74]. The processes determined for unsubstituted pterin illustrated in Scheme 17 reveal the key features of pterin redox reactions, which were generally applicable to 6- and 6,7,-methylated pterins. The scheme is arranged with the most reduced species at the top, the most oxidized species at the bottom and semi-reduced species in the middle of the diagram. Tetrahydropterins undergo reversible oxidation/reduction to an unstable quinonoid tautomer of dihydropterin in step (a) which can be observed under rapid scanning of potential. If not rapidly re-reduced, the quinonoid dihydropterin rearranges to the 7,8-dihydro tautomer in step (b). The 7,8-dihydropterin is oxidizable to pterin in step (c) but at potentials ~500 mV more positive than that for the reversible tetrahydro/quinonoid oxidation. Likewise 7,8-dihydropterin is reducible to tetrahydropterin but at potentials over 1 V more negative than reversible quinonoid/tetrahydro reduction. Fully oxidized pterin may be reduced by $2e^-$, $2H^+$ in step (d) to generate another unstable dihydropterin, the 5,8-dihydro tautomer. This is unstable and rearranges to 7,8-dihydropterin in step (e) before reduction to tetrahydropterin can occur. Using methylated tetrahydropterins which "locked" the initially formed quinonoid tautomers allowed determination of quinonoid tautomer 1 (f) in Fig. 6 as the most likely product formed upon tetrahydropterin oxidation.

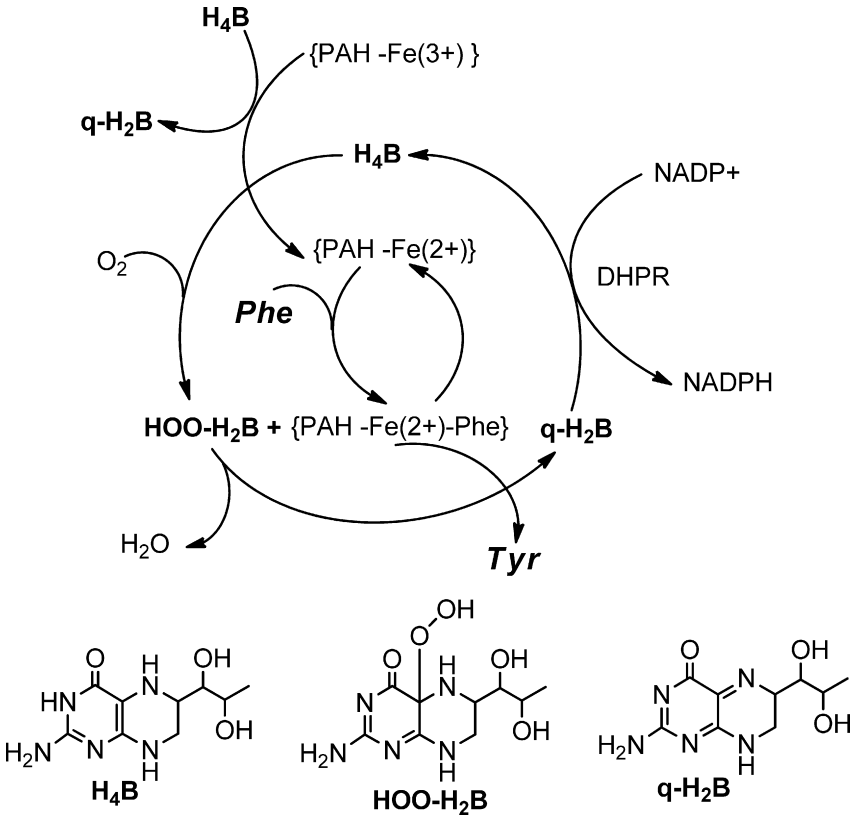
Detection of pterin cofactors in proteins by electrochemical techniques is limited. Protein film voltammetry studies on *E. coli* respiratory nitrate reductase showed catalytic processes occurring at two different potentials with unique reactivity profiles [75]. One possible explanation proposed for multiple catalytically active species was that the pterin component of the cofactor might have undergone a redox or rearrangement process such as a pyran ring scission during the voltammetric experiment, creating a state of Moco active at a different potential.

4.3. Pterin redox chemistry in enzyme catalysis and pterin radicals

Tetrahydrobiopterin (H_4biopterin) is involved in redox reactions in both enzymes where it functions as a cofactor. In the aromatic amino acid hydroxylases H_4biopterin has two jobs: first, the reduction of ferric to ferrous ion in the resting state of the enzyme and second, as the initial reductant for dioxygen. The sequences of the early mechanistic steps of phenylalanine hydroxylase (PAH) have recently been elucidated (Scheme 18). Following reduction to active {PAH-Fe(II)} enzyme, a quaternary complex must be formed where all the constituents, {PAH-Fe(II)-Phe-H_4biopterin-O_2} (Phe = phenylalanine) come together before O_2 attacks the C4a of H_4biopterin to form a 4a-peroxydihydropterin. This triggers a dissociation of water from Fe(II) opening a coordination site and allowing formation of a peroxo-bridged Fe–OOH–H_2biopterin that then hydrolytically cleaves to yield the active hydroxylating species Fe(IV)-O and a 4a-hydroxypterin. The 4aOH-H_2biopterin dehydrates yielding a quinonoid-dihydropterin



Scheme 17. Reaction scheme relating the various redox species generated from unsubstituted pterin.



Scheme 18. Proposed catalytic mechanism of PAH involving pterin-metal redox chemistry (B refers to biopterin).

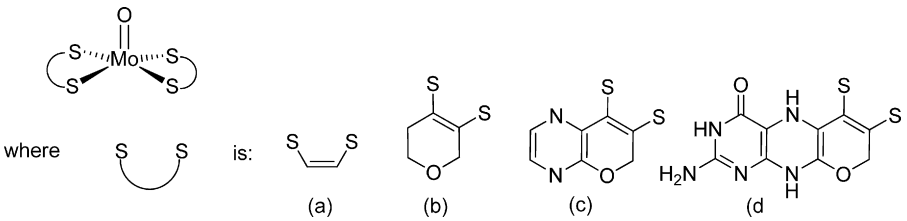


Fig. 7. The series of dithiolene ligands used in DFT calculations of [MoO(dithiolene)$_2$] complexes to investigate the importance of fusing pyran, pyranopyrazine and pyranopterin systems to the dithiolene chelate.

(q-H_2biopterin), the pterin product of the catalytic cycle. This unstable semi-reduced pterin is reduced by dihydropteridine reductase (DHPR) to regenerate H_4biopterin [10,76]. Tetrahydrobiopterin is also the redox cofactor for the nitric oxide synthase (NOS) family of enzymes that catalyze the formation of NO from L-arginine, NADPH, and O_2 [77–79]. Here H_4biopterin participates in an unprecedented role for a pterin where it is involved in one-electron redox cycles during both steps of the catalytic reaction sequence. In the first step, H_4biopterin bound near one of the heme propionate groups donates an electron to a P450-type heme in the active site, yielding a one-electron trihydropterin radical that is subsequently re-reduced. In the second step the BH_3 radical removes an electron from the active site to generate NO rather than NO^-. In the aromatic amino acid hydroxylases H_4biopterin undergoes two-electron oxidation, so it is curious how NOS adjusts the pterin environment to stabilize a bound trihydropterin radical. Recent EPR, ENDOR and DFT calculations led to the conclusion that the pterin radical is maintained in a protonated form, as cationic H_4biopterin$^{\bullet+}$ [76,77]. In NOS, the H_4biopterin+ radical is anchored by hydrogen bonding to several amino acids through protons at N2 and N8 H-bonded to backbone carbonyl oxygen atoms of Trp457 and Ile456. The H_4biopterin$^{\bullet+}$ is further stabilized by pi-stacking between the indole ring of Trp457 that sits over the H_4biopterin pyrimidine ring, and by Phe470 that sits over the pyrazine ring. Nature's use of pterin protonation as a ploy to control the radical behavior is consistent with previous observations of pterin radicals generated and observed transiently only under strongly acidic conditions [77,79,80]. A radical role is not out of the question for molybdopterin in Moco in molybdenum enzymes, since a trihydropterin radical was detected by EPR in aldehyde dehydrogenases [81].

4.4. Computational studies on pterins

Computational tools have been brought to bear on the problem of determining what pterin chemistry could be occurring in enzyme catalytic sites. Early *ab initio* studies focused on the quality of structural modeling dependent on the basis set [82], protonation energies and calculated pK_a's [83] and relative energies of dihydropterin tautomers [84] relevant for the enzyme mechanism of dihydrofolate reduction to tetrahydrofolate. The plethora of tautomeric structures possible for the semi-reduced state of pterin was more recently explored through *ab initio* calculations to address the possible structures of molybdopterin in the molybdenum cofactors, and to determine which are most energetically feasible [85,86]. A polarizable continuum model (PCM) was employed in an attempt to obtain comparative energies in a polar environment more similar to that in an enzyme, though this still fails to model the contributions of specific H-bonding interactions which are certainly critical in pterin cofactors with multiple H-bonds anchoring them into the protein. Forty dihydropterin structures were evaluated at various calculation levels from which the main conclusions were that the 2-amino, 4(3-H)-one is the most stable tautomer of the pyrimidine ring and that the 7,8-, the 5,6-, the 6,7- and the para-quinonoid forms of dihydropterin (Figs. 5 and 6) are nearly isoenergetic, implying that interconversions between these are energetically reasonable to consider. Therefore pyran ring scission exposing a double bond in the pyrazine ring could subsequently be redistributed in the pterin system to access conjugated structures with the dithiolene chelate. The critical consequence of this would be electronic communication distributed from the Mo atom, through the dithiolene into the pterin system.

Density functional theory was used to evaluate the importance of including the pterin in computational studies of the molybdenum cofactor [86]. Comparing structural details (bond lengths, angles, dihedral fold angles), charge distribution and redox potentials for increasingly complex MoO(dithiolene)$_2$ structures (Fig. 7), the researchers concluded that the simplest enedithiolate (a) was insufficient to accurately describe the molecular properties while including the full pterin-dithiolene structure (d) of molybdopterin made a significant difference. However, including only the pyrazine ring (c) of the pterin (not the pyrimidine) did reproduce rather closely the values obtained using the full molybdopterin ligand.

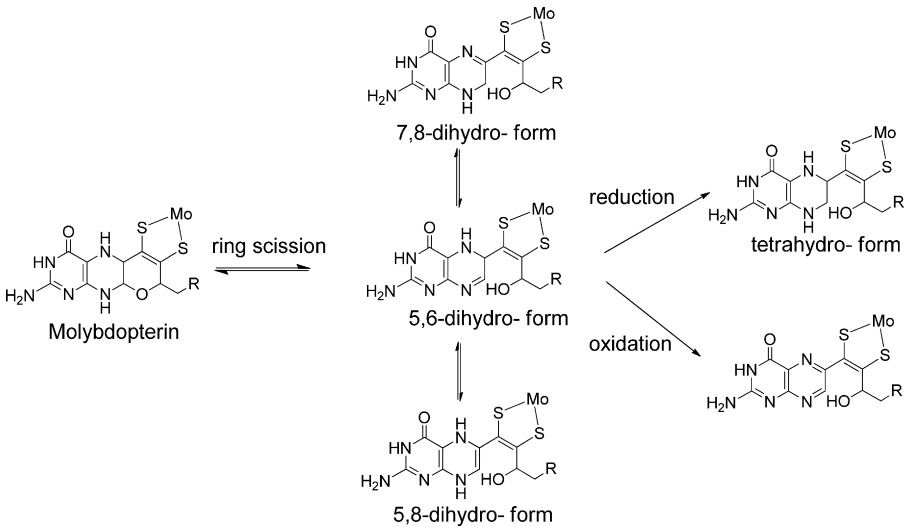
4.5. Redox state of the pyranopterin system

A close reading of the literature concerning the pyranopterin ligand on Moco reveals some confusion about how to consider its redox state. The saturated, quaternary carbons of the middle pyrazine ring in the pyranopterin seem similar to the structure of a tetrahydropterin but reversing the pyran ring cyclization reaction reveals a 5,6-dihydropterin (Scheme 19). The ring-opened, 5,6-dihyropterin form of molybopterin might then be anticipated to tautomerize to other dihydro-structures, and further reduced to a true tetrahydropterin or oxidized to an oxidized pterin.

Burgmayer investigated the redox behavior of pyranopterin system using Pfleiderer's polyhydroxylated pyranopterin as a model for molybdopterin [87]. This study confirmed that pyranopterin behaves as a dihydropterin under oxidative conditions, undergoing a $2e^-$, $2H^+$ oxidation to yield the fully oxidized neopterin (Fig. 8). Kinetic analysis showed this pyranopterin oxidation to neopterin was slower than tetrahydropterin oxidation to 7,8-dihydropterin, thereby confirming that the fused pyran ring contributes a stabilizing effect. The oxidation reaction of pyranopterin exhibits a minimal pH and solvent dependence, in marked contrast to tetrahydropterin oxidation that is strongly pH and solvent dependent. The pyranopterin in this study resisted further reduction to a tetrahydropterin using a variety of reducing agents known to reduce oxidized and 7,8-dihydropterins to tetrahydropterins. This experimental work proves that pyranopterin possesses distinctive redox chemistry unlike that of the simple pterin system, a result that may be critical to its use as part of the molybdopterin ligand for molybdenum and tungsten in enzymes.

4.6. Pterin redox with transition metal complexes

The rich redox activity of pterins suggests a natural match for transition metals. The metal–pteridine pair exhibits a variety



Scheme 19. A few of many possible outcomes of redox reactions at molybdopterin after pyran ring scission.

of chemical behaviors and has been previously reviewed [88]. Metal–pterin redox reactions span the gamut of complete transfer of one or more electrons to a partial transfer of electron density producing intermediate redox states for both the metal and pterin. The partial redox has been observed in both directions: metal oxidized with pterin reduction and metal reduction with pterin oxidation. This behavior places metal–pteridine complexes among the growing numbers of metal compounds having non-innocent ligands.

4.6.1. Metal–pterin reactions resulting in complete electron transfer

Complete electron transfer between pterin and metal is exhibited by the reaction between tetrahydropterins and ferricyanide producing 7,8-dihydropterin and ferrocyanide. The H_4pterin is oxidized by two electrons requiring two ferricyanide molecules be reduced for each pterin. This reaction was used to quantify the reduction state of the molybdenum cofactor. The redox titration of Moco to yield oxidized pterin required two equivalents of ferricyanide and led to the proposal that molybdopterin was at a dihydropterin reduction level [89–91].

Tetrahydropterin reactions with cupric and ferric ions was first reported by Scrimgeour and Vonderschmitt and these reactions have been later reinvestigated by several groups to gain insight into aspects of the PAH mechanism [92]. The outcome of H_4pterin and Cu(II) reactions is highly dependent on the ancillary ligands. Simple cupric salts (e.g., cupric nitrate) undergo rapid one-electron redox with H_4pterins yielding initially a trihydropterin radical that quickly disproportionates to 5,6,7,8-tetrahydro- and

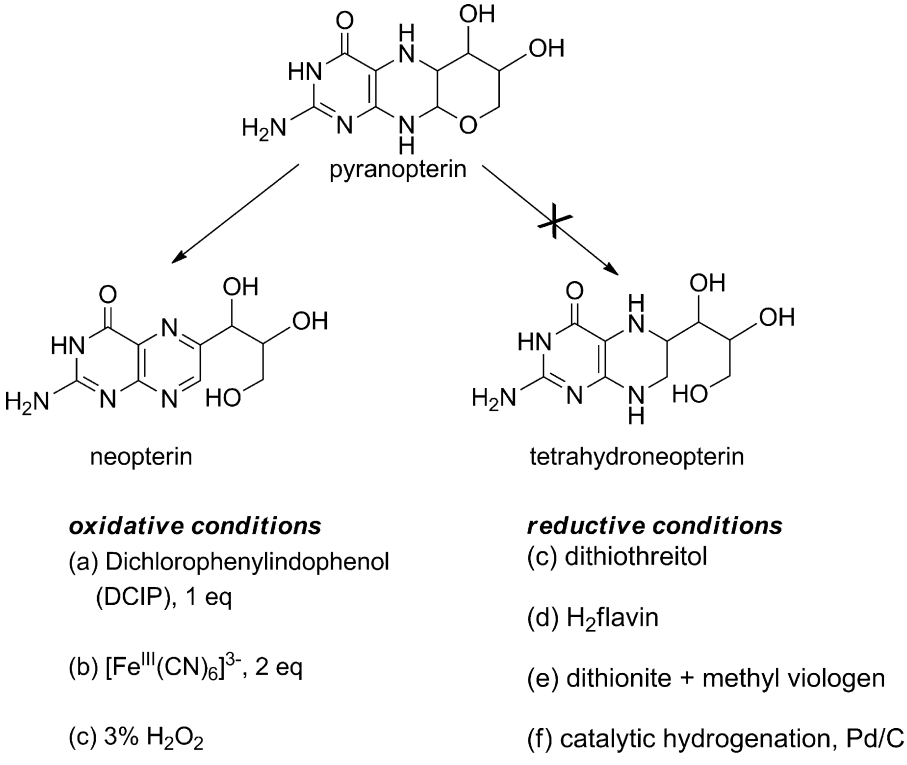
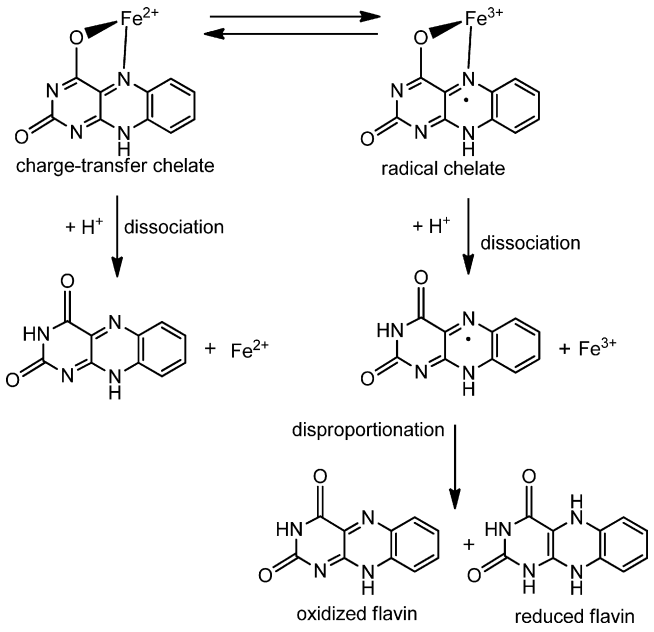


Fig. 8. The redox reactivity of a model pyranopterin.



Scheme 20. Hemmerich's depiction of the delocalized, i.e., non-innocent, behavior of a flavin chelate.

7,8-dihydro-pterin [93]. Copper(II)-bipyridine complexes react with H_4pterins to generate hydroxyl radical capable of aromatic hydroxylation [94]. Copper(II) complexes of tridentate hydro-*tris*-pyrazolylborate ligands stabilize a short lived H_4pterin chelate that then undergoes one-electron redox yielding trihydropterin radical that disproportionates to H_4- and H_2pterin [88,95]. Copper(II) complexes of dipeptides also chelate H_4pterin weakly without radical formation [93]. Reactions of ferric ions with tetrahydropterins were also interpreted as yielding trihydropterin-Fe(II) complexes antiferromagnetically coupled making them EPR silent, analogous to Mo(6+) complexes (see below) albeit much less stable and unisolable [96,97]. Fischer et al. reported that pyranopteridine is oxidized by two electrons when reacted with Mo(VI) and Fe(III) complexes with opening of the pyrano ring. This reactivity parallels that of a fully reduced pterin [98].

4.6.2. Pterins as non-innocent ligands for metals

The majority of metal–pterin studies have demonstrated the non-innocent nature of pterin ligands. The term "non-innocent" refers to a highly covalent interaction between a ligand and a metal such that the redox state of metal and ligand are often ambiguous. A non-innocent ligand is often described as the one participating in partial electron transfer between metal and ligand. Hemmerich was perhaps the first to recognize this characteristic of pteridine chelates (specifically flavins and isoalloxazines) bound to biologically relevant redox-active metals when he attempted to depict a non-innocent ferrous complex in the manner shown in Scheme 20 [99].

4.6.2.1. Pterin complexes of Mo(+4,+5,+6). Prior to the X-ray structure that gave a definitive confirmation of the dithiolene chelate of molybdopterin in Moco [100], there were speculations whether a reduced tetrahydropterin might react with an oxidized Mo(6+) center. Studies showed that a variety of Mo(+6) complexes reacted with tetrahydropterins to produce intensely colored mono-oxo Mo complexes (Fig. 9) [101–103]. These Mo-pterin products revealed considerable ambiguity in the oxidation state assignments of the metal and pterin ligand as illustrated by the three resonance structure options in Fig. 10 consistent with the non-innocent nature of pterin ligands. This ambiguity was finally resolved through a combination of X-ray photoelectron spectroscopy (XPS) and the Bond Valance Sum (BVS) method which determined the preferred electronic structure description of an antiferromagnetically coupled Mo(5+) coordinated by a Mo=N bond to a trihydropterin radical [104]. One significant consequence of reduced pterin coordination to Mo is it changes pterin redox reactivity. So for example, when H_4pterin chelates to Mo to electronically resemble H_3pterin, it is no longer reactive to the oxidant DCIP.

4.6.2.2. Pterin and flavin complexes of Ru(+2). Clarke's work on Ru(+2)-flavin reactions first suggested radical character on flavin arising from intramolecular, electron transfer. The evidence was structural distortion of flavin observed crystallographically, consistent with partial Ru(+3)-flavinsemiquinone character [105]. Clarke's group also determined that Ru-pterin complexation shifted subsequent protonation from N1 to N8, due to electronic delocalization from Ru(+2) to the pterin anti-bonding pi system (i.e., back bonding), making the pyrazine more electron rich and basic [106]. A similar intramolecular electron transfer interpretation was made for Mo(4+) complexes of oxidized pteridine ligands including alloxazine, flavin and pterin [101]. Here it was argued that the appropriate electronic description should include resonance contribution from a Mo(5+)-(Hpteridine$^{\bullet-}$) structure. The anionic pteridine radical, Hpteridine$^{\bullet-}$, results from electron transfer from Mo(4+) to partially reduce the pteridine (as alloxazine, flavin or pterin). Like the Ru-flavin complex reported by Clarke, the Mo complexes display short M=N5 bonds and bent flavin or pterin planes, consistent with a similar delocalized electronic structure.

More recent work on a Ru(L-N_4)(pterin) shows that methylation at pterin N2 can shift the relative contribution of the quinonoid resonance by changing participation of N2 amino in the para-quinonoid form. Ru(+2) coordination makes the pterin ligand more basic so that it can be protonated twice [107,108]. The doubly protonated species can be 1e$^-$ reduced to a H_2pterin radical, observable by EPR. This Ru(pterin) system was studied within the thermochemical cycle for Proton Coupled Electron Transfer (PCET), and demonstrated to do H-atom abstraction from phenolic substrates. A kinetic analysis shows that the PCET H-abstraction follows an associative mechanism via a pre-equilibrium step where the substrate H-bonds to the 2-amino end of the pterin ligand on Ru(+3) [107].

4.7. Biosynthesis of pterins

4.7.1. Biosynthesis of H_4biopterin and neopterin

The biosynthesis of pterin has been investigated for many years, particularly in the context of tetrahydrobiopterin and folate biosynthesis. The biosynthetic pathway for biopterin is schematically shown in Fig. 11. The pterin moiety is formed from the guanosine triphosphate (GTP). Three different enzymes are needed to convert the GTP into 6*R*-l-erythro-5,6,7,8-tetrahydrobiopterin. The conversion proceeds through the intermediate formation of 7,8-dihydroneopterin triphosphate and 6-pyruvoyl-5,6,7,8-tetrahydropterin, both which have been isolated.

The first step of the process is hydrolytic release of formate from imidazole ring of the GTP, which is catalyzed by a Zn-containing enzyme called GTP cyclohydrolase I (GTPCH) [109,110]. The GTPCH enzymes isolated from phylogenetically different sources share a high degree of sequence similarity, indicating a conserved role of this enzyme [111]. The hydrolysis reaction is followed by the pyrazine ring formation, which completes formation of the pterin system in dihydroneopterin triphosphate. All but two carbon atoms in the pterin come from the purine, whereas the two additional carbon atoms are supplied by the ribose moiety.

The GTPCH catalyzed reaction is complex. The formation of the formamide intermediate has been confirmed by spectroscopy

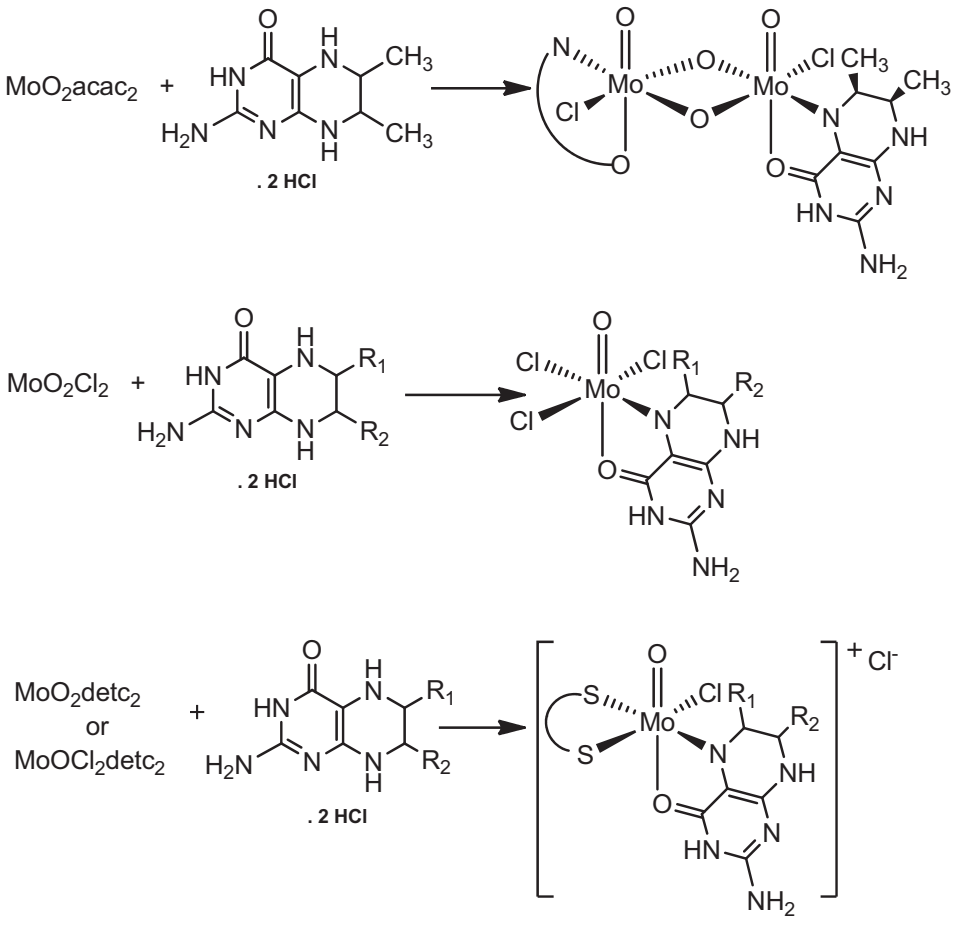


Fig. 9. Examples of Mo(6+) reactions with tetrahydropterins.

[112]. The carbohydrate is rearranged for the introduction of the two carbon atoms to the pyrazine ring. The GTPCH active site has a Zn-center coordinated by a histidine and two cysteine residues [113]. A water molecule is activated through Zn(II) coordination to hydrate the imidazole ring. This initial step is followed by the opening of the imidazole and ribose rings. Subsequent release of formate is also assisted through coordination to Zn. Amadori rearrangement and subsequent intramolecular ring closure leads to the formation of the 7,8-dihydroneopterin triphosphate. This hydrolysis reaction is faster than the carbohydrate rearrangement reaction. The active site topology is independent of Zn coordination, and the presence of the Zn presumably influences the orientation of the GTP through deformation of the ring which may influence the rate of the hydrolysis reaction [110].

The regulatory aspect tetrahydrobiopterin biosynthesis is complex, and a universal regulatory scheme for all pterins in all tissues does not exist. Expression of GTPCH is inducible. The rate limiting step for the synthesis of tetrahydrobiopterin is the hydrolysis by GTPCH [114]. The cellular concentration of tetrahydrobiopterin is regulated by GTPCH-I. The activity of GTPCH can be regulated at the transcriptional and post translation levels. Various cytokines, phytohaemagglutinin, endotoxin and hormones induce the GTPCH gene resulting in a higher level of tetrahydrobiopterin. At the post transcriptional level GTPCH is also regulated through the cleavage of N-terminal amino acid and phosphorylation. The higher the phosphorylation level, the higher the enzymatic activity, which results in an increased amount of cellular tetrahydrobiopterin. It appears that the one of the serine residues in the GTPCH is modified by the casein kinase II and/or protein kinase C [5].

GTPCH is also regulated by its end product, tetrahydrobiopterin, and by the substrate GTP. Cellular GTP cooperatively binds the enzyme and influences the kinetics. Tetrahydrobiopterin and

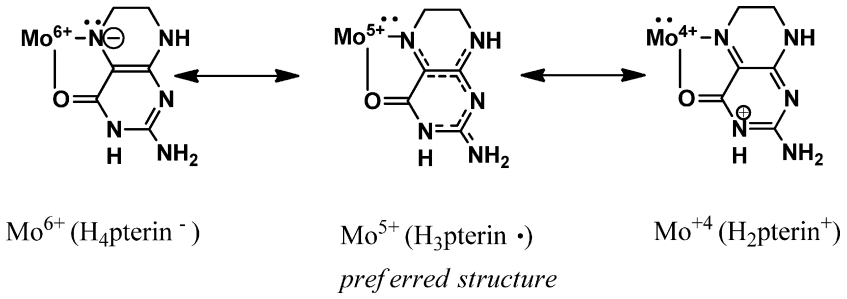


Fig. 10. Three possible resonance structures of Mo-pterin complex.

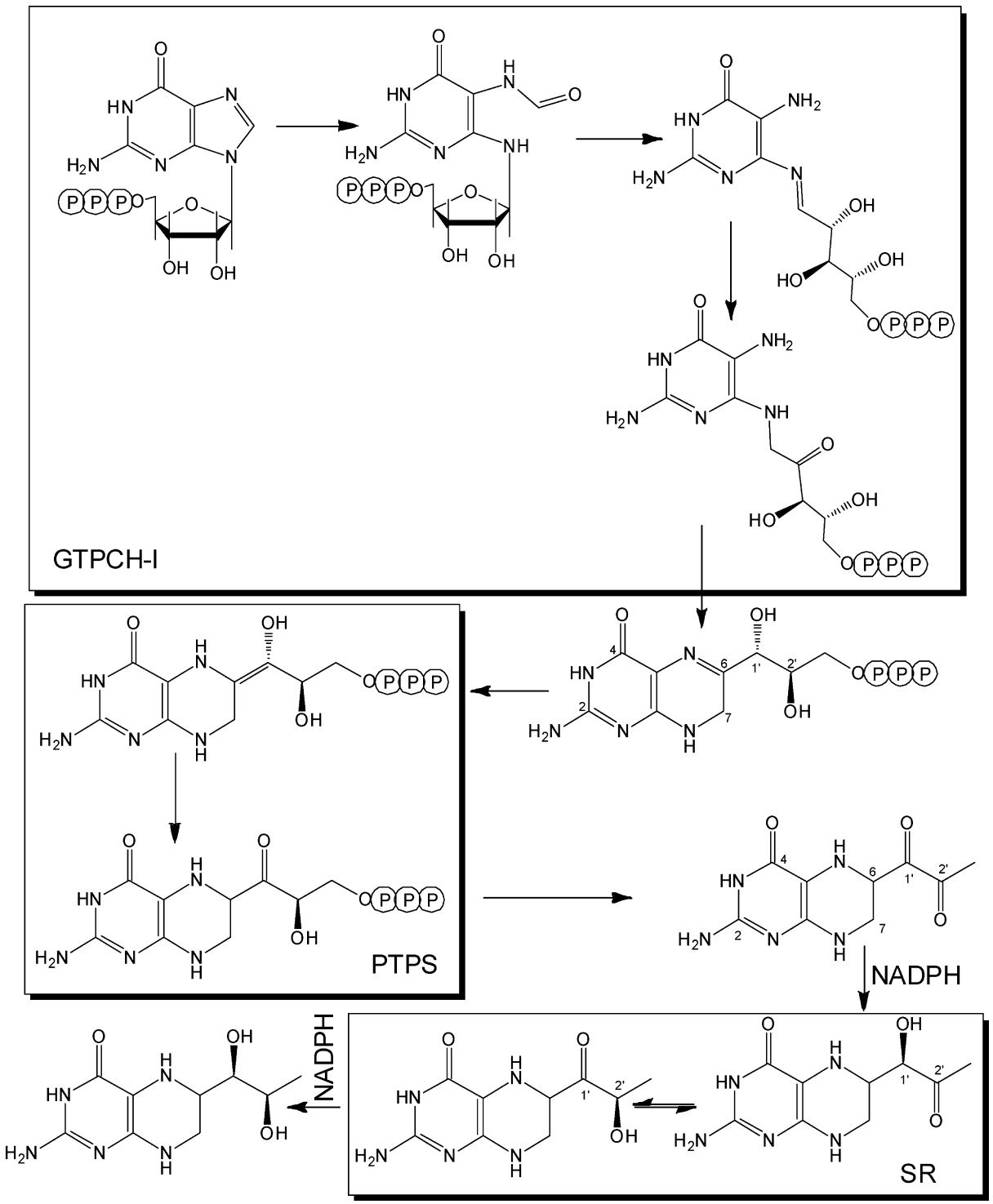


Fig. 11. Biosynthetic scheme for biopterin.

phenylalanine affect the activity of this enzyme through GTPCH-I feedback regulatory protein (GFRP). The tetrahydrobiopterin inhibits GTPCH-I activity, presumably through GFRP binding to GTPCH-I that induces conformational changes. The change can be reversed by phenylalanine and the activity can be restored.

The triphosphate group from 7,8-dihydroneopterin triphosphate is removed enzymatically by 6-pyruvoyl-tetrahydropterin synthase (PTPS), which also reduces the pterin to 6-pyruvoyl-tetrahydropterin that is subsequently converted to tetrahydrobiopterin by sepiapterin reductase (SR). PTPS is found in humans and is generally expressed at a very low level, making it the rate limiting step in the biopterin biosynthesis in humans. PTPS is constitutively expressed and the level of expression, compared to GTPCH-I, is unaffected or marginally increased by external stimulant. While the regulation of PTPS is not completely clear, PTPS deficiency is the most frequent of the pterin biosynthetic disorders [115].

Functionally, the PTPS is essentially a carbon-oxygen lyase that acts on the phosphate group of the 7,8-dihydroneopterin triphosphate, releasing the phosphate and 6-pyruvoyl

tetrahydropterin. The overall reaction involves a rearrangement of the substrate, dihydroneopterin triphosphate, internal redox chemistry and finally the elimination of the phosphate to 6-pyruvoyl tetrahydropterin [116]. PTPS is a Zn and Mg dependent enzyme that accomplishes a stereospecific reduction. The Zn binding site consists of three histidine residues and the hydroxyl groups from the substrate, neopterin [117]. When the substrate is not bound the coordinated hydroxy groups are replaced with a water molecule. In addition there exist an intersubunit catalytic site formed cysteine, an aspartate and a histidine residue. Coordination to the Zn-center stabilizes the conformation, suitably activating the proton of the neopterin side chain via a cysteine residue. The proposed reaction mechanism involves protonation of the pyrazine nitrogen, abstraction of hydrogen from the neopterin side chain by the cysteine thiolate, formation of the carbonyl group at the C1 of the side chain, and release of the phosphate. The Zn atom remains coordinated by the neopterin side chain either through the hydroxyl or keto functionality [118]. It is also interesting to note that the triphosphate functionality is required for enzymatic activity. Its presence in an optimal concentration is also required for proper functioning of the enzyme.

In eukaryotic systems, dihydroneopterin is converted to the dihydrobiopterin which is further reduced to tetrahydrobiopterin by sepiapterin reductase (SR) [115]. SR plays an important role in the biosynthesis of biopterin by catalyzing the NADPH dependent reduction of carbonyl groups of the pteridine side chains. There is no indication that SR controls the rate of biopterin biosynthesis, and a defect in SR synthesis is not associated with an increase of the phenylalanine level. This enzyme catalyses a two-step reaction and, in physiological conditions, is the only enzyme required to complete the synthesis of biopterin. Other reductases such the aldose reductase can catalyze the first step of the reaction in the event of SR deficiency; the product of this reaction can be reduced by dihydrofolate reductase in an alternate pathway of biopterin biosynthesis called a 'salvage pathway'. This alternate pathway can operate in the liver, kidney, but not in the brain as dihydrofolate reductase activity is low there. This leads to the accumulation of sepiapterin there, which serves as a marker for sepiapterin reductase deficiency disease [119,120]. There are other diseases such as Parkinson's disease has been linked with the differential expression of sepiapterin [121,122].

The SR is capable of stereospecific reduction of both keto groups of 6-pyruvoyl tetrahydropterin in an NADPH dependent pathway to tetrahydrobiopterin. The stereospecific reduction is accomplished by proper orientation of the substrates through the interaction of amino acid residues appropriately placed in the catalytic pocket [123]. Of the two keto functionalities the position 1′ is reduced first, with the second reduction proposed to take place again at the same carbon but after an isomerization step. This isomerization occurs via an ene-diol intermediate that shifts the carbonyl group from the second carbon to the first carbon [112]. Nearly, all SR activity in the brain or erythrocyte is compromised by N-acetyl serotonin or melatonin. Both of these compounds are synthesized by tryptophan hydroxylase that has tetrahydrobiopterin as a required cofactor. Because the overall expression of pterin in brain is low, it may provide a controlling avenue for developing therapeutics.

4.8. Biosynthesis of Moco

Although widespread in all kingdoms, Moco is synthesized by a conserved biosynthetic pathway divided into four steps according to the products [124–127]. They are cyclic pyranopterin monophosphate (cPMP) also known as precursor Z, molybopterin (MPT), adenylated MPT and finally Moco. There are six gene products that have been identified in the biosynthesis of Moco. In humans four genes, *mocs1*, *mocs2*, *mocs3* and *geph,* are required to generate the six gene products while six genes, *cnx1–cnx6*, are needed to carry out the same function in plants, *mocs1* and *mocs2* encode for two gene products each.

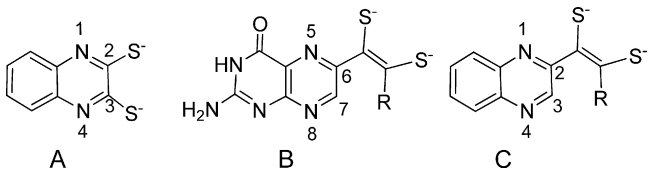


Fig. 12. A: quinoxaline-2,3-dithiolate, B: pterin-6-ene-dithiolate, C: quinoxaline-2-enedithiolate.

The first step of the biosynthesis involves the conversion of GTP into cPMP (also known as precursor Z) catalyzed by a 4Fe4S containing protein involved in radical SAM generation, and a protein involved in pyrophosphate release. These two proteins are encoded by the *mocs1* gene. Each carbon of the ribose and purine is incorporated into the cPMP [128]. Structurally, the cPMP is a reduced pyranopterin with a terminal cyclic phosphate and geminal diol, which is different from the precursor Z that has a keto functionality instead of a geminal diol [129]. The sulfur atoms are inserted into the cPMP by heterotetrameric MPT synthase (encoded by *mocs2*, and *mocs3*), and coordinate to a Cu ion. In the last step of Moco biosynthesis, a Mo atom is transferred to MPT enedithiolates, resulting in the formation of Moco. This process is catalyzed by gephyrin (encoded by *geph*) which also adenylates MPT and releases AMP and Cu [130]. Structurally, there are three stereocenters in pyranopterin cofactor, and in all cases, the hydrogen atoms are on the same side of the ring, suggesting a regiospecific synthesis. Much of the biosynthetic pathway of this cofactor is now known, and the final product indicates that stereochemistry in the biosynthetic pathway is tightly controlled. It is however, not known whether other diastereoisomers have any activity, as these compounds have not yet been chemically synthesized.

5. Cofactor models including pterins or pterin analogs

Many models of the molybdenum cofactor have been synthesized and studied over several decades [131,132]. Early examples among these models resulted from efforts to reproduce the inner coordination sphere of Mo, the spectroscopic features of Moco or to provide examples of substrate reactivity and mechanism. Following the identification of the dithiolene tether of molybdopterin for Mo, synthetic analog work was directed at dithiolene complexes, but these typically employed dithiolenes substituted by simple groups such as methyl [133,134], cyanide [135–137] or as part of a ring system (e.g., 1,2-benzenedithiolate, bdt) [138–140]. Only a few groups have tackled the problem of placing a pterin on the dithiolene chelate to access both essential components of molybdopterin. This section is devoted to a survey of model complexes of Moco which contain the dithiolene chelate substituted by pterin or other closely related N-heterocycles, such as quinoxaline.

Most of the research directed at the synthesis of pterin-substituted dithiolenes begins with exploratory reactions using quinoxaline derivatives due to their considerably easier preparation. A quinoxaline attached via C2 to a dithiolene resembles molybdopterin in positioning a pyrazine ring in conjugation with the dithiolene (Fig. 12C). This approach has been valuable not only as a synthetic stepping stone to pterin dithiolenes but also in providing useful information about how the heterocycle can modulate the electronic structure of the metal–dithiolene unit and alter its chemistry.

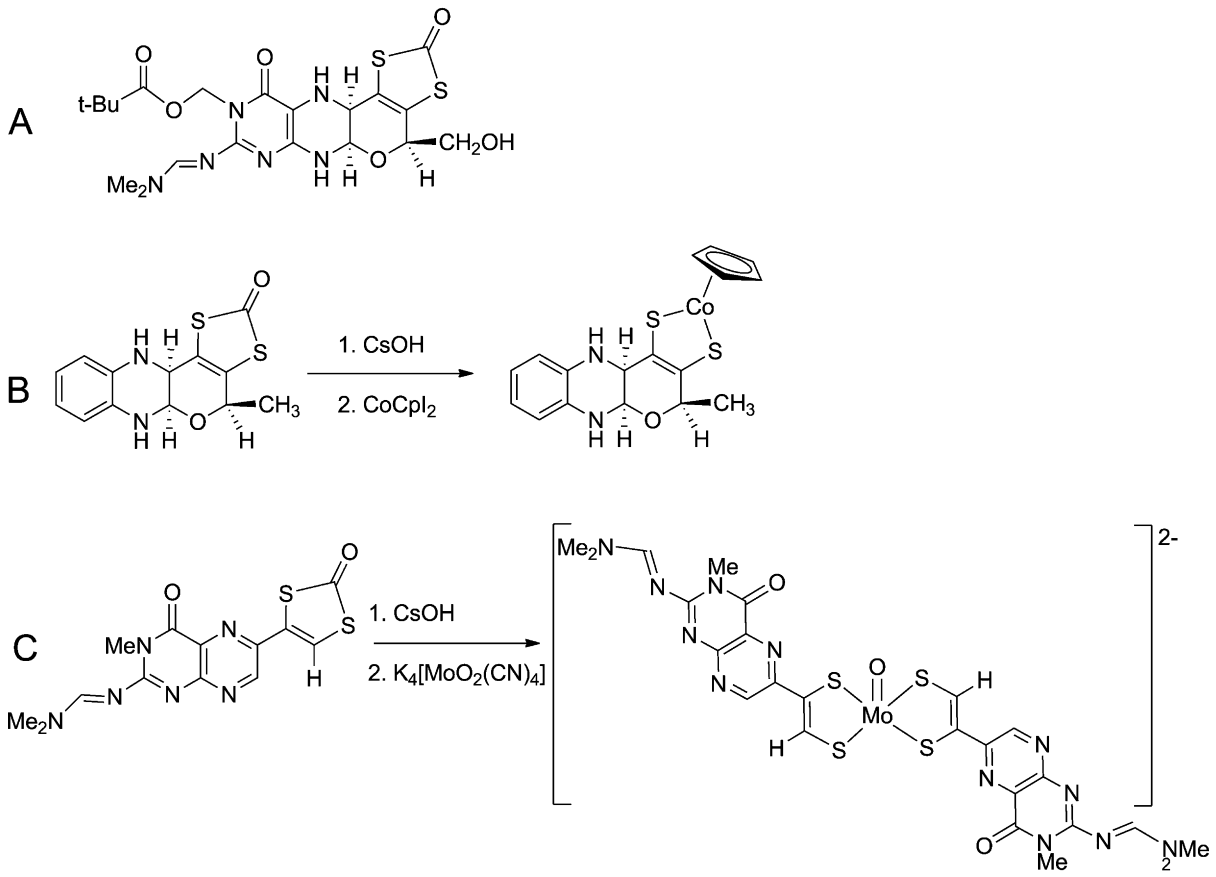


Fig. 13. A: target model complex for Moco; B: synthetic method demonstrated for a quinoxalyl dithiolene installed on Cp_2Co; C: synthetic method applied to formation of a *bis*-pterinyldithiolene model complex.

5.1. Syntheses of pterin-substituted dithiolenes

There are several synthetic routes to pterin-dithiolenes illustrated in Fig. 13 which were in every case first demonstrated using their quinoxaline analogs. One approach is to build the entire pterin-dithiolene ligand as a protected trithiocarbonate (Fig. 13A) [141]. The protecting moiety can be removed under basic conditions in the presence of appropriate metal reagents to trap the revealed dithiolene chelate (Fig. 13B and C) [142,143]. The other main approach has been to prepare pterin molecules having an alkyne functionality that reacts with Mo tetrasulfide reagents to generate the pterin-dithiolene within the coordination sphere of the Mo (Scheme 21) [144]. Both sulfido and oxo forms of Tp*MoO (pterin-dithiolene) complexes were obtained. The Mo(5+) complexes of both systems in Scheme 21, Tp*MoO (pterin-dithiolene) and Cp_2Mo (pterin-dithiolene) were produced by oxidation with ferrocenium or iodine. Other methods for producing quinoxaline and pyridine-dithiolenes through reactions of *a*-bromo or tosyl N-heterocycles with Mo-(SH_2) have not yet been applied to the formation of pterin dithiolenes [145,146].

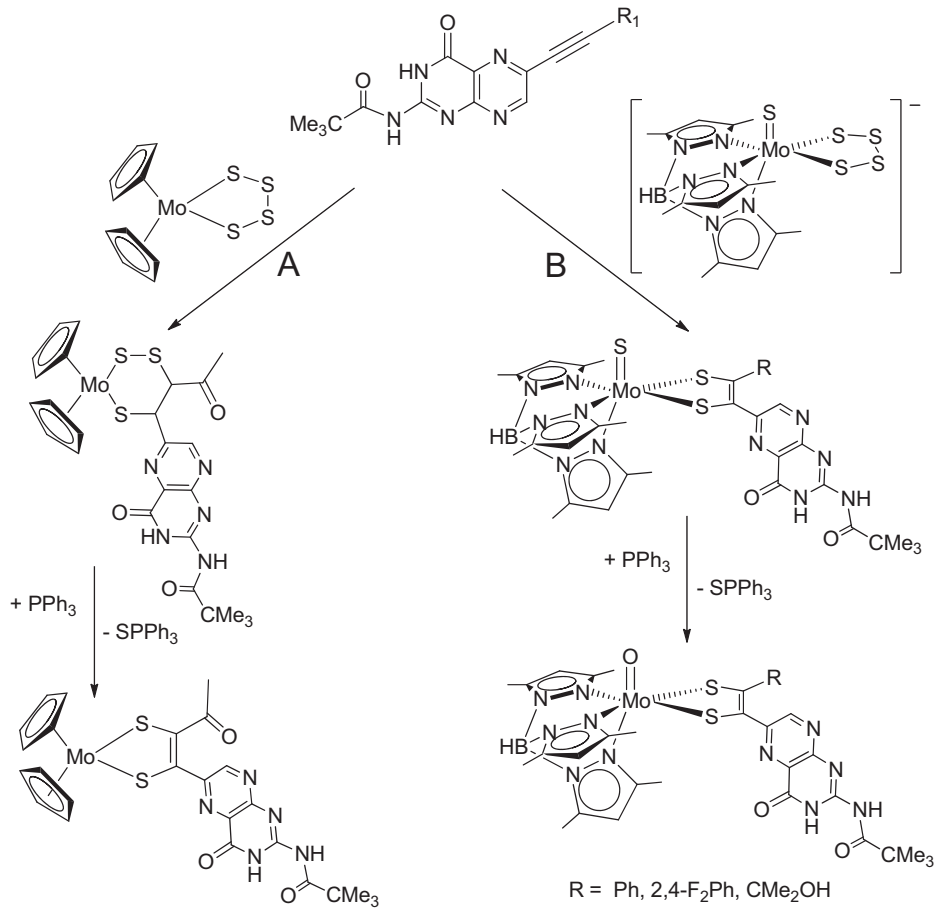
5.2. Consequences of N-heterocycle substitution on dithiolene

One of the earliest studies to explore the effects of N-heterocyclic substitution on a dithiolene at molybdenum was reported from Garner's lab. The tris-dithiolene Mo(5+) complex of 2,3-quinoxalyldithiolate (qdt) (Scheme 22A) was structurally characterized by X-ray crystallography [147,148] for both Mo(4+) and Mo(5+) then the effect of N-protonation on redox processes was monitored using cyclic voltammetry [149].

The key findings were that protonation at quinoxaline made reduction more favorable and that the reduced species should be described as containing quinoxalyl dithiolene radicals represent an early awareness of the non-innocent chemistry of a dithiolene on an N-heterocycle.

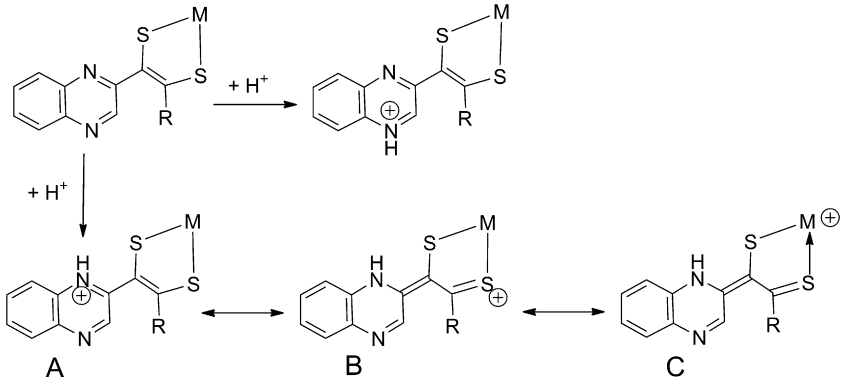
The effect of N-heterocycle protonation on the electronic structure of the dithiolene was revisited in a number of studies. Protonation of 2-substituted quinoxaline, pyrazine and pyridines attached to a dithiolene chelate on a metal can induce a reorganization of electron density throughout the heterocycle in conjugation with the dithiolene as shown in Scheme 22. The distinction between tautomers B and C is a subtle shift of electron density such that the S atom in tautomer B is electron deficient and oxidized compared to tautomer A whereas in tautomer C the Mo atom is oxidized relative to A. Evidence for multiple tautomers of protonated quinoxaline N atoms was observed through the electrochemical behavior of $Cp_2Co(S_2C_2H(quin))$ monitored by cyclic voltammetry [149] Other investigations of $Cp_2Mo(S_2C_2H(N\text{-cycle}))$ [145] have shown that the pK_a values for protonated N-cycles (N-cycle is either quinoxaline or pyridine) on dithiolene chelated to Mo and Pt increase by 1–3 units as compared to the free heterocycles due to the resonance stabilization by the metallo-1,2-enedithiolate.

N-heterocycle protonation also has a strong effect on electronic transitions in these complexes where the relative energies of d-based and ligand-based unoccupied orbitals can be switching, accessing luminescent emissive Inter Ligand Charge Transfer (ILCT) excited states [150]. Similar effects can be expected for complexes of pterin-dithiolenes, though parallel studies have not yet been accomplished. Data available for the few molybdenum complexes of pterin-dithiolenes includes redox potentials, electronic, Magnetic Circular Dichroism (MCD) and Electron

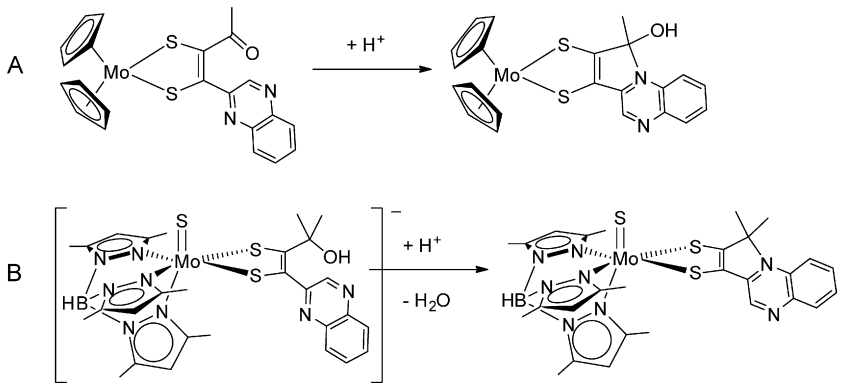


Scheme 21. Pterin dithiolene complexes of molybdenum formed by coupling reactions of pterinyl alkynes and molybdenum tetrasulfide reagents [152,144].

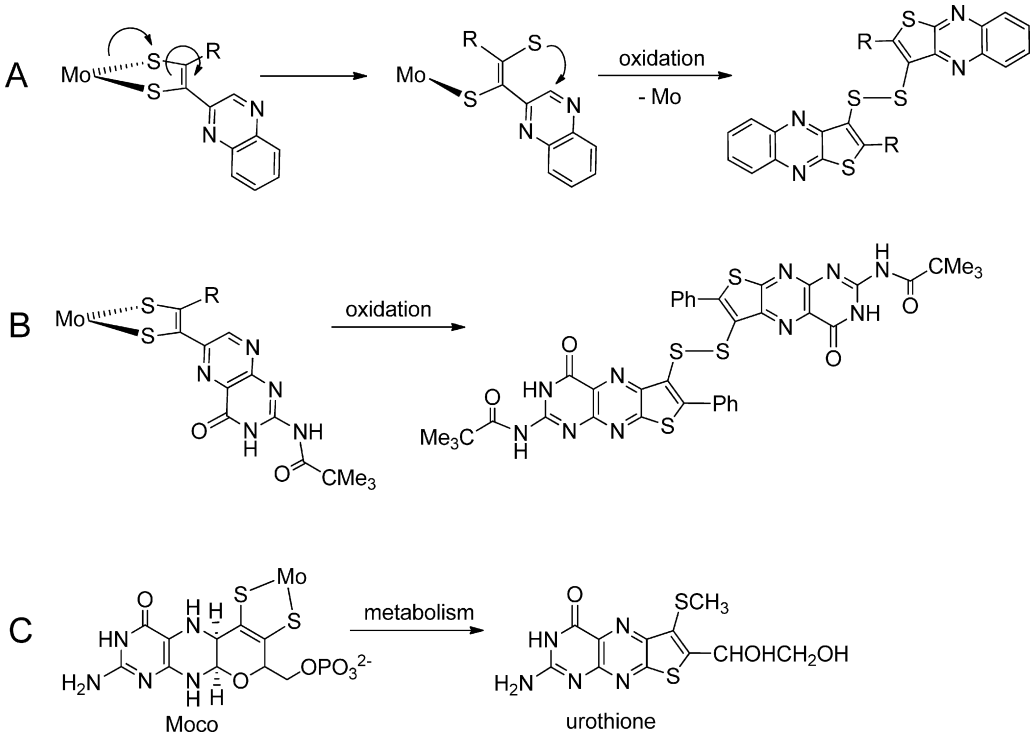
Paramagnetic Resonance (EPR) spectra. In the case of the Tp*MoX (pterin-dithiolenes) (X = S,O), it was concluded that the most significant effect of pterin (or quinoxaline) substitution was to shift the Mo(5+/4+) redox potential considerably in the positive direction compared to simpler model complexes bearing bdt or edt [144]. Surprisingly, the EPR and MCD parameters exhibited by Tp*MoO(bdt) and Tp*MoO(pterin-dithiolene) complexes are nearly identical, but have ^{95,97}Mo hyperfine values considerably larger (A_{ave} = 37G) than observed for Cp_2Mo(pterin-dithiolene) (A_{ave} = 11G). This smaller ^{95,97}Mo *A* value in Cp_2Mo(pterin-dithiolene) has been interpreted as reflecting the composition of the HOMO being based on the C and S atoms of the dithiolene [144,147]. None of the reported pterin-dithiolene complexes are fluorescent. The introduction of pterin- and quinoxaline-substituents on dithiolenes induces reactivity at the pyrazine N atoms. In both Cp_2Mo(quinoxaline-dithiolene) and Tp*MoO(N-cycle-dithiolene) (N-cycle is pterin or quinoxaline) complexes, electrophilic attack at atom N1 of the pyrazine ring results in an intramolecular cyclization producing a pyrrole-like ring (Scheme 23) [144,147]. While this cyclization does not, on the surface, appear to have any relevance to the possible roles of pterin on the dithiolene in Moco, the X-ray structures and an analysis



Scheme 22. Quinoxaline protonation can access other resonance forms including thiolate, thione chelates on Mo.



Scheme 23. Electrophilic reactions of quinoxalyldithiolenes cause cyclization.



Scheme 24. Oxidative products of quinoxaline and pterin dithiolene originating from dechelation of the metal and the metabolic product of the cofactor degradation.

of the electronic structure of this type of ligand provide evidence of tautomers such as in Scheme 22. In both cyclized products in Scheme 23, the dithiolene chelate is asymmetrically bound, exhibiting asymmetric Mo–S and S–C bond lengths. Resonance Raman, EPR and Density Functional Theory (DFT) calculations on the product in Scheme 23B are consistent with an admixture of a thione–thiolate structure which can be considered to result from electron density distributed into the quinoxaline structure from partial thiolate to thione oxidation.

This electronic delocalization into the quinoxaline system makes the structure in Scheme 23B on the right significantly electron-withdrawing as compared to the structure on the left and this is manifested as a 300 mV positive shift in the Mo(5+/4+) potential where the Mo(4+) state is more stabilized. Both of these cyclized structures are characterized by intense absorption in the visible electronic spectrum that have been assigned to intramolecular ligand charge transfer transitions. Dechelation of one S atom of a dithiolene chelate can lead to S attack on a pyrazine carbon and cyclization to form thiophenes fused to quinoxaline and pterin (Scheme 24A and B) [151]. This observation was an early confirmation of the correctness of the proposed dithiolene in Moco before X-ray protein structure was available since urothione, a natural metabolite of Moco, had previously been identified as a pterinylthiophene (Scheme 24C).

6. Summary

We have presented here the breadth of pterin chemistry, illustrating through selected examples that pterins do a variety of things. From their roles in biology to their chemical behavior in organic and inorganic systems, it should be clear that a pterin is generally not a spectator group like a methyl, but that it contributes to the chemistry of the system. Pterins are most often involved in redox processes so it is likely the same will be true of the pterin part of the molybdenum cofactor. It has already been noted that Moco stands as the most redox rich biological cofactor since it has three redox active units: the Mo center, the dithiolene and the pterin [148]. The pterin is likely to work in concert with the dithiolene and the molybdenum center to create the unique reactivity of Moco. Speculations have been made here and elsewhere regarding the

possible ways that these three units could play roles in the catalytic reaction of Moco.

Acknowledgments

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