# Biomimetic Chemical Catalysts in the Oxidative Activation of Drugs

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**Abstract:** An overview of the biomimetic catalysts used in the oxidative activation of drugs is given, with an emphasis on the use of synthetic metalloporphyrins as models of cytochrome P450 to mimic the *in vivo* metabolism of pharmaceuticals. In addition, a special focus is directed towards the recent results from the authors' group on the oxidative activation of isoniazid, an antitubercular drug.



**Keywords:** biomimetic catalyst; drug metabolism; isoniazid; metalloporphyrin; oxidation

## 1 Introduction

Drugs are usually highly functionalized compounds and most of them exhibit oxidizable groups. Therefore, they constitute ideal targets for an oxidative metabolism when they are introduced in living organisms. The oxidative metabolism is of major importance since it can significantly affect the drug's safety and efficacy, due to the formation of therapeutically active or toxic metabolites.

Many attempts have been made in the past to develop *biological models* useful to predict the metabolic behavior of drug candidate molecules and, eventually, to prepare drug metabolites which are not easily obtained by conventional routes of organic synthesis. These methods include direct experiments on animals, the use of perfused organs or isolated cells and studies with xenobiotic metabolizing enzymes. Simple heme-enzyme systems, such as peroxidases and cytochrome P450 monooxygenases, have been investigated because of their ability to oxidize a large variety of substrates.[1–5] Cytochrome P450 monooxygenases are multi-enzyme systems which are more sophisticated than simple peroxidases and biotechnological systems based on recombinant human liver cytochromes P450 expressed in a microorganism have been developed.[6] However, several problems are associated with the use of these different systems: (i) animal studies necessitate the sacrifice of animals and are expensive to conduct; (ii) liver preparations (microsomes, cultured hepatocytes) are of variable potency; (iii) the isolation of reactive intermediates which can bind to biological polymers in enzyme-catalyzed oxidations is not so easy and the primary metabolites are often hydrophilic and difficult to isolate, especially for unknown metabolites.

Therefore, it might be convenient to use *biomimetic chemical catalysts* to check the behavior of drugs in oxidative conditions and to prepare some of the different possible metabolites in sufficient amounts for characterization and for pharmacological or toxicological tests.[7–11] In this article, an overview of the biomimetic chemical catalysts used in the oxidative activation of drugs is given, with an emphasis on the large use of synthetic metalloporphyrins as models of cytochromes

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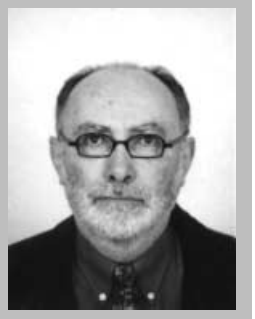

*Jean Bernadou* was born in Villefranche-de-Rouergue (France) in 1947, studied Chemistry, Pharmacy (Doctorate in Pharmaceutical Sciences in 1975) and Medicine (Doctorate in 1985) at the University Paul Sabatier of Toulouse, where he was appointed Professor of Medicinal Chemistry in 1987. His collaboration with the group of Bernard Meunier started in 1980 with his research interests in the mechanism of action of antitumor drugs. His current scientific interests focus on biological applications of metalloporphyrins, in particular oxidative DNA cleavage, biomimetic oxidation of drugs and characterization of reactive metal-oxo intermediates. His most recent research concerns the design of new analogues of the antitubercular drug isoniazid.

*Bernard Meunier* was born in 1947 and educated at the Universities of Poitiers (B.Sc.), Montpellier (with R. J. P. Corriu) and Orsay (with H. Felkin, ICSN-CNRS). After a post-doc at the University of Oxford, he joined the "Laboratoire de Chimie de Coordination du CNRS" in Toulouse in 1979. His current research interests include catalytic oxidations, oxidative DNA cleavage, oxidation of pollutants and the mechanism of action of antimalarial drugs. He is the author of 305 publications and 25 patents. He was elected as a Member of the French Academy of Sciences in 1999.

P450 to mimic the *in vivo* metabolism of pharmaceuticals. As we will see from the reported examples in the present review, one of the main drawbacks of these biomimetic systems is the absence of regioselective oxidation, unlike what is observed with cytochrome P450 enzymes and unlike what is expected to be obtained in the future with catalytic antibodies. So, the main scope of the first part of this article is to provide a survey on the different structures or functional groups present in a large range of pharmaceuticals that are oxidized by biomimetic metalloporphyrin systems. In addition, in a second part, a special focus is directed towards the recent results from the authors' group on the oxidative activation of isoniazid, an antitubercular drug. The knowledge of the essential metabolic oxidative pathway of isoniazid allowed us to elect manganese(III) pyrophosphate as an appropriate non-heme biomimetic system. The use of this chemical model allowed us to gain a better knowledge of the reactive species involved in the oxidative activation of isoniazid and a to make a significant contribution to the elucidation of its mechanism of action.

## 2 Synthetic Metalloporphyrins as Models of P450 and Peroxidases to Mimic the *in vivo* Metabolism of Pharmaceuticals and some other Xenobiotics

The oxidative metabolism of drugs, either exogenous or endogenous, by cytochrome P450[12,13] and peroxidases[14,15] has been extensively studied. A better understanding of biosynthetic pathways (e.g., side-chain degradation of cholesterol, aromatization of the A-ring in estrogen biosynthesis) and of the production of (i) mutagenic intermediates in the biotransformation of polyaromatic molecules and (ii) toxic or active drug metabolites by heme enzymes is now accessible. In this context, synthetic metalloporphyrins have been intensively developed as model systems for studies of the oxidative metabolism and for the synthesis of potential metabolites of drugs and some other xenobiotics (mutagens, agrochemicals).[4] The first metalloporphyrins used, such as iron and manganese tetraphenylporphyrins, were structurally simple (Figure 1A) but they were found to be fragile: the rapid destruction of the porphyrin macrocycle allowed a only limited number of catalytic cycles. Considerable advances have been made with the development of robust metalloporphyrins (sterically hindered or fluorinated derivatives) and electronically activated metalloporphyrins (introduction of halogens, for example, on the aryl groups of *meso*-tetraarylporphyrins and on the β-pyrrolic positions of the porphyrin ring, Figure 1B) with the following advantages: reduction of the porphyrin destruction allowed us to increase the catalytic efficiency and electronegativity of the substituents enhanced the reactivity of the high-valent oxo species involved in these oxidation reactions. Furthermore, sophisticated model systems have been developed combining cavities based on cyclodextrins and utilizing the interactions between host and guest to generate substrate selectivity and stereoselectivity in product formation.[5] At the same time, since most of the drugs are water-soluble chemicals, water-soluble metalloporphyrins were also developed (Figure 1C).

The structures of some of the porphyrin ligands used as biomimetic models in drug metabolism studies are shown in Figure 1 with their chemical denominations and usual abbreviations listed in Table 1. The iron

  

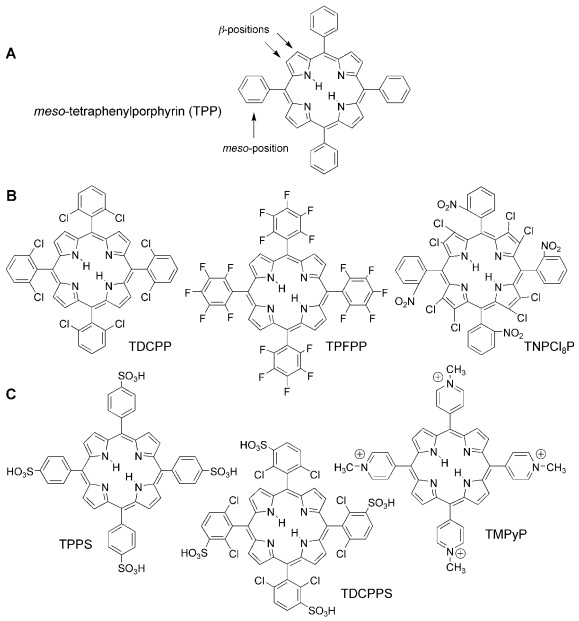


**Figure 1.** Structures of some porphyrin ligands.

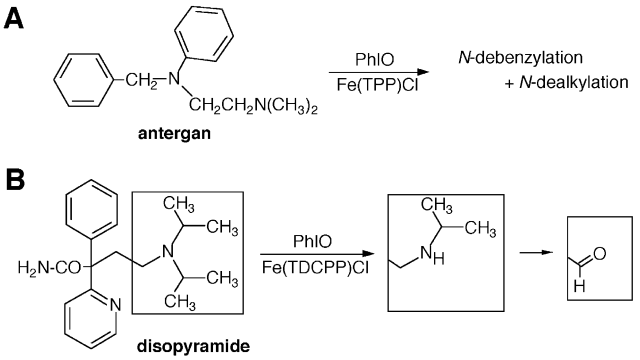


**Figure 2.** *N*-Dealkylation and *N*-debenzylation reactions.[16,29]

complexes are very usual but manganese porphyrins, although not directly biologically relevant as models of heme oxygenases, behave also as efficient catalysts. The catalytic reactions have been mainly performed in dichloromethane, benzene, toluene, methanol (pure solvent or mixtures) and also in aqueous solutions. Unlike cytochromes P450 that use molecular oxygen as a donor of oxygen atom, the synthetic metalloporphyrins require single oxygen atom donors (also named oxygen surrogate). The most common ones are sodium hypochlorite (NaOCl), iodosylbenzene (PhIO), potassium monoperoxysulfate ($KHSO_5$), magnesium monoperoxyphthalate (MMPP), hydrogen peroxide ($H_2O_2$), *tert*-butyl hydroperoxide (*t*-BuOOH), cumene hydroperoxide (CumOOH). Molecular oxygen associated to a reductant-like borohydride derivative could also be used. Lastly, a nitrogen base is sometimes used as proximal ligand to increase the reactivity or to modify the selectivity of the reaction.

### 2.1 Iron or Manganese Complexes of Simple Non-Water Soluble Porphyrins: Fe(TPP)Cl and Mn(TPP)Cl

The Fe(TPP)Cl/PhIO system oxidizes antergan (an antihistaminic drug) to *N*-dealkylated and *N*-debenzylated products (Figure 2A),[16] sulfides to *S*-oxidized and *S*-dealkylated compounds (Figure 3A, B),[17] the anesthetic agent phencyclidine to a piperidine-3-oxo compound *via* an iminium intermediate (Figure 4A),[18,19] and effects the aromatization of tetralone derivatives.[20] Activated with $H_2O_2$, Fe(TPP)Cl reacts on all-(*E*)-retinol to give hydroxylated, oxo-, epoxy- and dehydro-compounds (Figure 4B).[21]

The complex Mn(TPP)Cl catalyzes the PhIO oxidation of nicotine to cotinine and 3-hydroxycotinine, two

**Table 1.** Abbreviations and chemical denominations of different porphyrin ligands.

| Abbreviation | Chemical denomination |
|---|---|
| $Cl_8$TDCPP | *meso*-tetrakis(2,6-dichlorophenyl)-β-octachloroporphyrin dianion |
| $Cl_8$TDCPPS | *meso*-tetrakis(2,6-dichloro-3-sulfonatophenyl)-β-octachloroporphyrin ligand |
| $Cl_8$TPP | *meso*-tetraphenyl-β-octachloroporphyrin dianion |
| TDCPP | *meso*-tetrakis(2,6-dichlorophenyl)porphyrin dianion |
| TDCPPS | *meso*-tetrakis(2,6-dichloro-3-sulfonatophenyl)porphyrin ligand |
| TDFPP | *meso*-tetrakis(2,6-difluorophenyl)porphyrin dianion |
| TMP | *meso*-tetramesitylporphyrin dianion |
| TMPyP | *meso*-tetrakis(4-*N*-methylpyridinumyl)porphyrin tetracation |
| $TNPCl_8P$ | *meso*-tetrakis(2-nitrophenyl)-β-octachloroporphyrin dianion |
| TPFPP | *meso*-tetrakis(pentafluorophenyl)porphyrin dianion |
| $TPFPS_4P$ | *meso*-tetrakis(pentafluorophenyl)-β-tetrasulfonatoporphyrin dianion |
| TPP | *meso*-tetraphenylporphyrin dianion |
| TPPS | *meso*-tetrakis(4-sulfonatophenyl)porphyrin ligand |
| TTMPP | *meso*-tetrakis(2,4,6-trimethoxyphenyl)porphyrin dianion |
| TTPPP | *meso*-tetrakis(2,4,6-triphenylphenyl)porphyrin dianion |

  

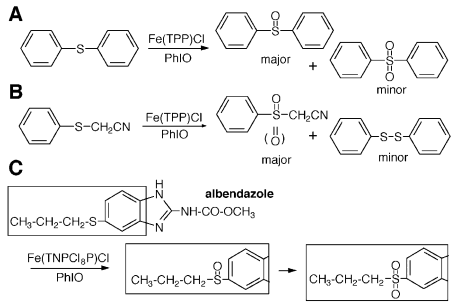


**Figure 3.** Biomimetic *S*-oxidation (to give sulfoxide and sulfone products) and *S*-dealkylation reactions.[17,29]

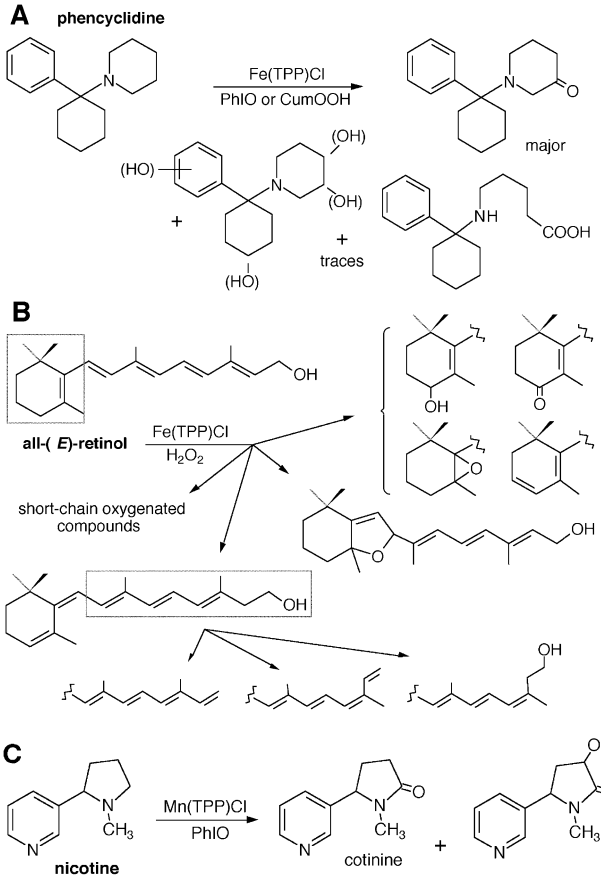


**Figure 4.** Examples of *C*-oxidation reactions.[18,19,21,22]

products identical to those obtained from *in vivo* metabolism (Figure 4C).[22]

This Mn(TPP)Cl/PhIO system also oxidizes KRP-197, a dual antagonist of the muscarinic acetylcholine receptors $M_1$ and $M_3$, to give a mono-oxidized product at position 5 of the imidazole ring (which is the putative precursor of the main metabolites), giving an example of application of these biomimetic systems to the *in vitro* metabolic studies of heterocyclic drugs (Figure 5A).[23] The Mn(TPP)Cl/NaOCl system was used to couple phenolic benzylisoquinoline alkaloids.[24] For biomimetic activation of mutagens catalyzed by TPP complexes, see Section 2.2.

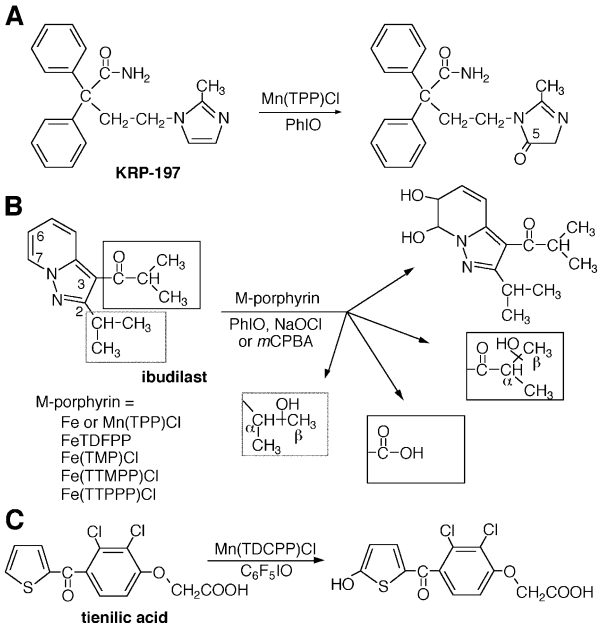


**Figure 5.** Biomimetic oxidation of heterocyclic drugs.[23,30,32,42,43]

## 2.2 Sterically Protected and Electronically Activated Metalloporphyrins

### *2.2.1 Iron Complexes*

Robust iron porphyrins associated with *m*-CPBA have been used to oxidize polycyclic aromatic hydrocarbons yielding mixtures of phenols and quinones,[25,26] *p*-substituted phenols to give *p*-benzoquinone[27] or steroid aldehydes to afford the corresponding acid derivatives.[28]

Associated to PhIO, Fe(TDCPP)X (i) oxidizes the antiarrythmic drug disopyramide[29] to mono-*N*-deisopropyldisopyramide and to the aldehyde resulting of its oxidative deamination (Figure 2B) and (ii) cyclizes $\gamma,\delta$-unsaturated carboxylic acids to give $\delta$-hydroxylated $\gamma$-butyrolactone derivatives (Figure 6A); indomethacin, a $\beta,\gamma$-unsaturated carboxylic acid, lactonized in the same manner to afford $\beta$-hydroxy-$\gamma$-lactone (Figure 6B) competitively with the formation of decarboxylated products depending on the nature of the porphyrin derivatives (Figure 6).[30,31]

$Fe(TNPCl_8P)Cl$ associated to PhIO oxidizes the anthelmintic drug albendazole to form the same sulfoxide and sulfone metabolites as those obtained *in vivo* (Figure 3C).[29]

A range of selected metalloporphyrins catalyzes the oxidation by PhIO, NaOCl or *m*-CPBA of the anti-asthma agent and cerebral vasodilatator ibudilast

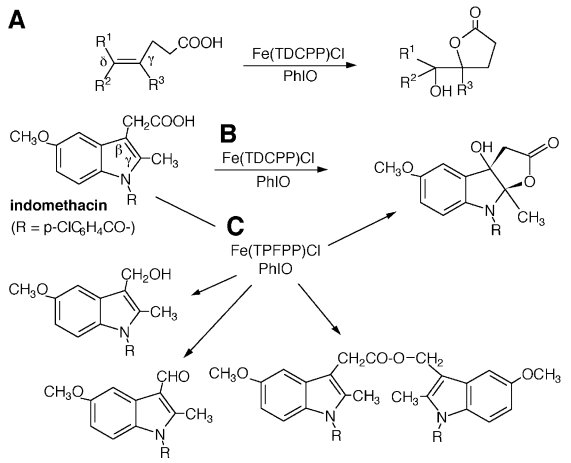


**Figure 6.** Hydroxylactonization of γ,δ- and β,γ-unsaturated carboxylic acids.[30,31,33]

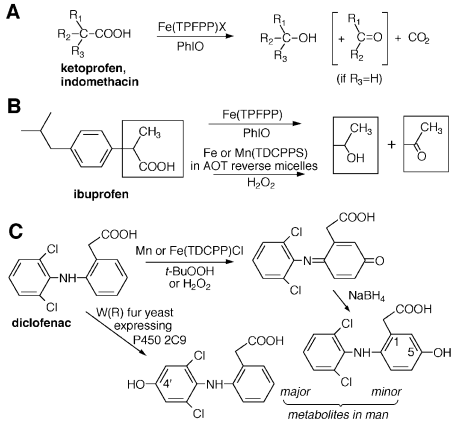


**Figure 7.** Biomimetic oxidation of arylacetic and arylpropionic drugs.[33,34,50]

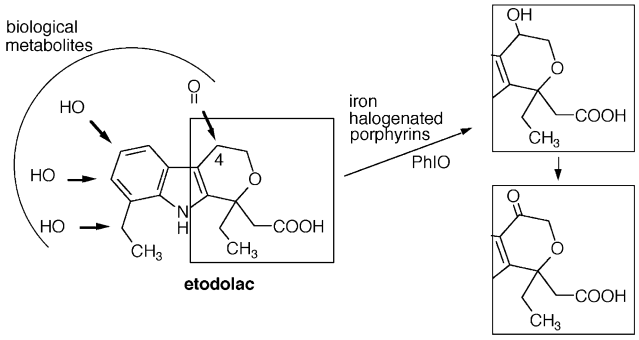


**Figure 8.** Biomimetic oxidation of etodolac in comparison with its *in vivo* metabolism[36] [porphyrins = Fe(TPP)Cl, $Fe(Cl_8TPP)Cl$, Fe(TPFPP)Cl, $Fe(Cl_8TDCPP)Cl$].

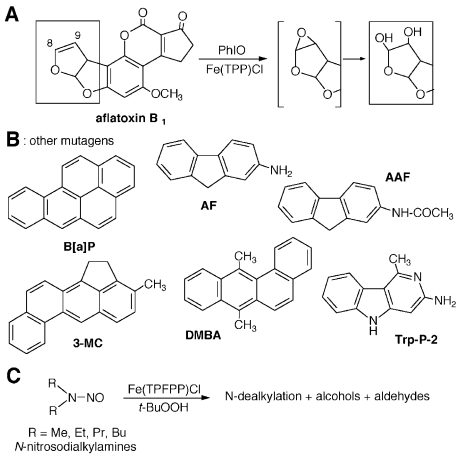


**Figure 9.** Biomimetic metabolic activation systems in mutation assays.[37–41]

(IBPP) to afford side-chain- and heterocycle-hydroxylated products (Figure 5B).[30,32]

The Fe(TPFPP)X/PhIO system can decarboxylate non-steroidal and anti-inflammatory drugs belonging to the family of arylacetic and arylpropionic acids to give the corresponding alcohol and carbonyl derivatives (Figures 6C and 7A,B).[33] Similarly the oxidation of ibuprofen with $H_2O_2$ catalyzed by either Fe- or Mn(TDCPPS) in AOT reverse micelles gives 2-(4′-isobutylphenyl)ethanol and 4-isobutylacetophenone (Figure 7B),[34] and ketoprofen was directly oxidized to 3-benzoylacetophenone by Mn-TMPyP/$KHSO_5$.[35]

A range of halogenated and perhalogenated iron(III) porphyrins associated to PhIO has been used as efficient catalysts in the regioselective synthesis of two metabolites, 4-hydroxyetodolac and 4-oxoetodolac, by oxidation of the analgesic/anti-inflammatory agent etodolac (Figure 8).[36]

Most carcinogens are promutagens and require oxidation by cytochrome P450 to be converted to their active form(s).[37] Therefore, microsomes (rat liver homogenate) have been incorporated into *in vitro* mutation assays. However, promutagens can alternatively be activated with a combination of iron porphyrin and an oxygen surrogate as a biomimetic model of cytochrome P450. Aflatoxin $B_1$ ($AFB_1$) has been shown to be converted by Fe(TPP)Cl/PhIO to $AFB_1$-8,9-diol, probably *via* an 8,9-epoxide, a possible carcinogenic and mutagenic product of $AFB_1$ (Figure 9A).[38] Fe(TPP)Cl/PhIO system also activates benzo[*a*]pyrene (B[a]P) and 2-aminofluorene (AF) (Figure 9B) to mutagens in the Ames assay.[39,40] Later, the same metalloporphyrin has been used for the activation of other promutagens (3-methylcholanthrene [3-MC]; 7,11-dimethylbenz[*a*]anthracene [DMBA]; 2-acetylaminofluorene [2-AAF]; 2-amino-3-methylimidazo[4,5-*f*]quinoline [IQ]) (Figure 9B) in the presence of various oxygen donors (PhIO, CumOOH, *t*-BuOOH, $H_2O_2$). Fe(TPFPP)Cl associated to *t*-BuOOH also mimics the metabolic activation of several *N*-nitrosodialkylamines, in a reaction that produced the same alcohols and aldehydes as the microsomal reaction (Figure 9C);[41] Fe(TPP)Cl catalyzes more efficiently than Mn(TPP)Cl the oxidative dealkylation of the carcinogenic *N*-nitrosodialkyl-

  

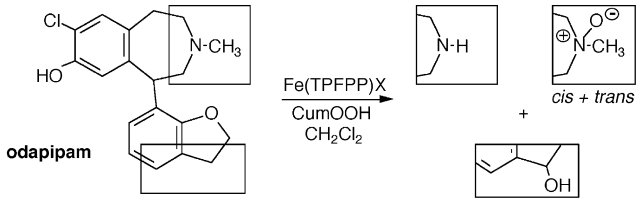


**Figure 10.** Biomimetic oxidation of odapipam.[9]

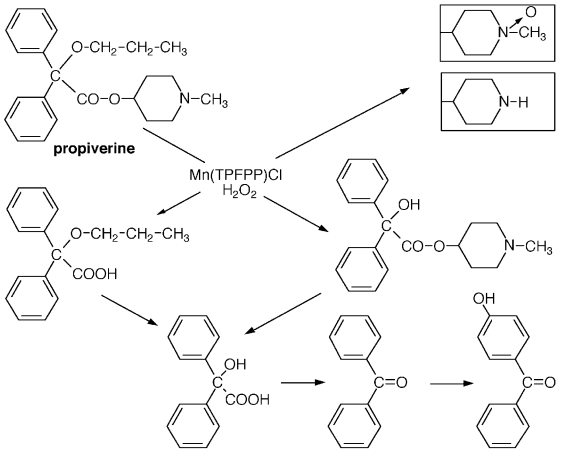


**Figure 11.** Biomimetic oxidation of propiverin.[46]

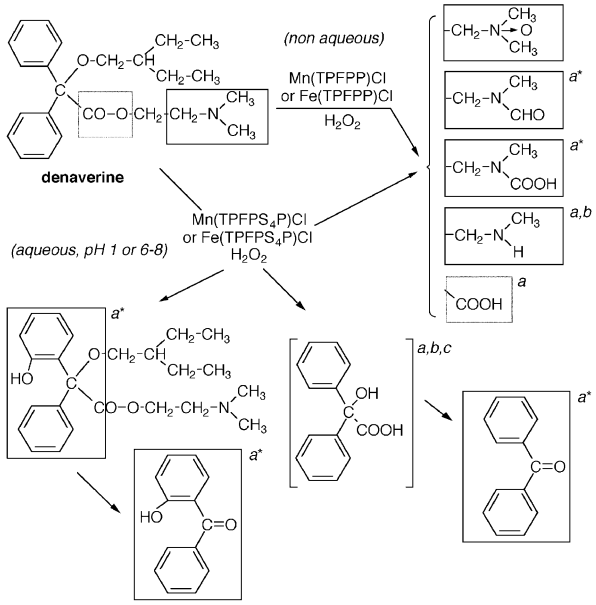


**Figure 12.** Biomimetic oxidation of denaverin in comparison with its *in vivo* metabolism.[49]
[a] Metabolism in rat.
[b] Metabolism in human.
[c] Detected as methyl ester due to the use of methanol during the work-up.
* Detected, but not identified.

amines by PhIO, *t*-BuOOH or CumOOH.[41] In an extended study of the use of biomimetic models in the Ames test, a series of promutagens (AF, B[a]P, the tryptophan pyrolysate 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole [Trp-P-2], and 2-acetylaminofluorene [AAF]) have been shown to become mutagenic in the presence of Fe(TPFPP)Cl plus an oxidant (*t*-BuOOH, *m*-CPBA, or MMPP); the order of effectiveness of three solvents (acetonitrile, 1,4-dioxane, and *N*,*N*-dimethylformamide) has been determined in the AF activation (acetonitrile > dioxane > DMF).[37]

The perfluorinated iron porphyrin Fe(TPFPP)X activated with CumOOH has been used in $CH_2Cl_2$ to study *in vitro* the metabolism of odapipam, an anti-psychosis drug (Figure 10).[9]

### 2.2.2 Manganese Complexes

Manganese porphyrin complexes have also been used in metabolic studies. The Mn(TDCPP)Cl/PhIO system is able to mimic the physiological hydroxylation of the thiophene ring of tienilic acid in a highly regioselective manner (Figure 5C).[42,43] With the aim to predict the oxidative metabolism of drugs having the diphenylmethane structural backbone, the system Mn-polyhalogenated porphyrin/$H_2O_2$/pyridine was found to give the best yields for aromatic ring hydroxylation, an important cytochrome P450-dependent metabolic pathway.[44]

Mn(TDCPP)Cl/CumOOH oxidized 1,3-dimethyluracil to 5-hydroxy-1,3-dimethyluracil.[45] The reaction of propiverin and 1-methyl-4-piperidyl benzilate with Mn(TPFPP)X/$H_2O_2$/pyridine affords about fifteen metabolites found to be in nearly conformity with *in vivo* rat metabolism data (Figure 11);[46] when performed in an aqueous phase and with the corresponding sulfonated fluorinated metalloporphyrin, the *in vitro* metabolic profile correlated well with the studies of metabolism in man.[47] The biomimetic oxidation of the antihistaminic drug clemastine in a non-aqueous system generates *O*-dealkylation, *N*-demethylation and *N*-oxidation products as well as aromatic hydroxylation metabolites.[48]

### 2.2.3 Iron and Manganese Complexes

The behavior of denaverine, a spasmolytic drug, has been studied in non-aqueous medium with the iron or the manganese derivatives of the robust porphyrins TPFPP or in aqueous medium (pH 1 or 6–8) with TPFPS$_4$P. The catalyzed oxidation was performed using either imidazole or pyridine as co-catalyst and either $H_2O_2$ or PhIO as oxygen donor (see Figure 12 and Section 2.3 for more detailed description).[49]

The oxidation profile of lidocaine using a large range of iron- or manganese-metallated porphyrins activated with $H_2O_2$, PhIO, *t*-BuOOH, CumOOH or MMPP was

  

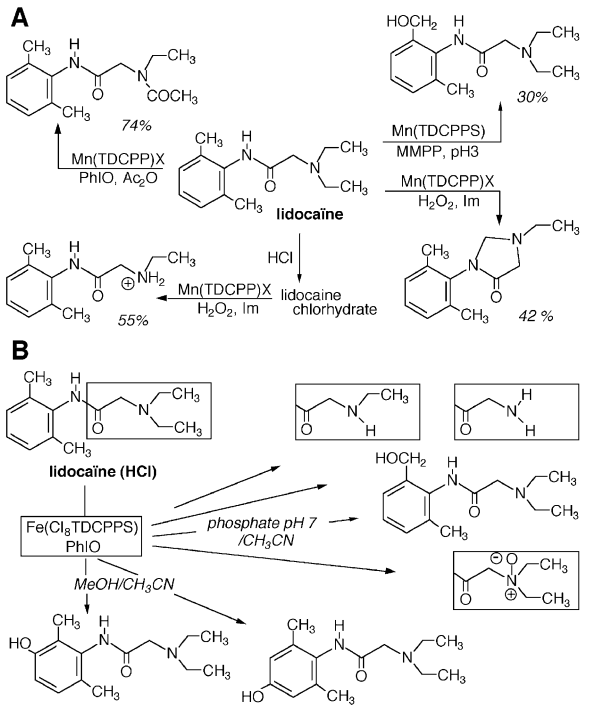


**Figure 13.** Selective biomimetic oxidation of lidocaine.[9,10]

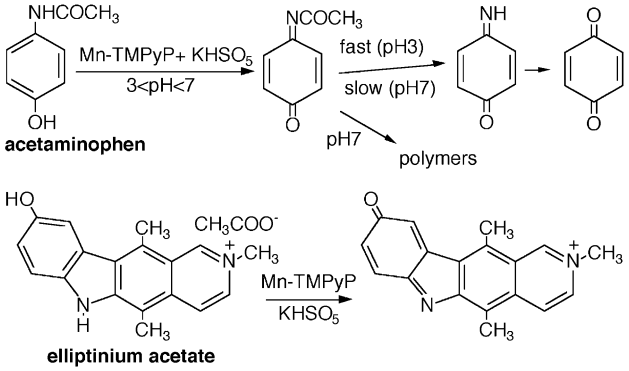


**Figure 14.** Biomimetic oxidation of acetaminophen and elliptinium acetate.[7,8]

studied by two groups.[9,10] A number of reaction products has been isolated and identified, showing that a proper choice of the model system and adjusted reaction conditions allow the synthesis of selected metabolites (Figure 13).

Metabolism of diclofenac in humans mainly leads to the two 5-hydroxylated (minor metabolites) and 4′-hydroxylated (major metabolite) products (Figure 7C). The complementary roles of chemical and biotechnological model systems has been illustrated with this case since the oxidation with Mn or Fe complexes of TDCPP in the presence of $H_2O_2$ or *t*-BuOOH gives mainly the quinone-imine precursor of the 5-hydroxylated metabolite whereas the bioconversion with yeast expressing

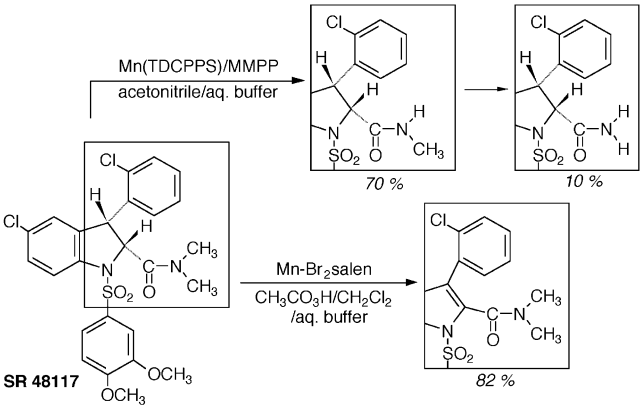


**Figure 15.** Modulation of the oxidation of SR 48117 on different sites depending on the experimental conditions.[51]

the human cytochrome P450 2C9 allows specific hydroxylation of diclofenac at position 4′.[50]

## 2.3 Water-Soluble Iron and Manganese Porphyrins

More recently, we have shown that water-soluble iron and manganese porphyrins, M-TPPS, M-TMPyP and some robust derivatives, associated with potassium monopersulfate, are able to mimic efficiently the peroxidase oxidation of acetaminophen, a well-known analgesic, as well as those of antitumoral ellipticine derivatives (Figure 14).[7,8] In both cases, the expected quinone-imines are formed in high yields (40 – 90%) with initial turnover rates similar to those observed with horseradish peroxidase/$H_2O_2$. Therefore, these electrophilic drug metabolites are easily prepared by using biomimetic catalysts, since they are not quenched by nucleophilic sites of the protein outside, a result usually observed in enzyme-catalyzed oxidations. The oxidation of SR 48117, an antagonist of vasopressin V1a receptors, by biomimetic catalysts based on metalloporphyrin [such as Mn(TDCPPS)] or Schiff-base complexes (Mn-$Br_2$salen) indicates that modulation of the reaction conditions (catalyst, oxygen donor, nature of the reaction medium,...) leads to different reaction compounds in various yields and thus allows access to selected metabolites (Figure 15).[51]

Water-soluble, sterically protected and electronically activated metalloporphyrins have been used in the biomimetic oxidation of aminopyrine giving a series of potential metabolites (Figure 16) with variation of the reaction conditions allowing here also the specific synthesis of selected metabolites.[10] Hydroxylation of adenosine 5′-monophosphate has been observed with $Mn^{III}$TMPyP/$KHSO_5$ giving 8-hydroxyadenosine 5′-monophosphate.[52] This reaction illustrates the hydroxylase activity of this system that has been extensively developed as artificial nuclease for DNA.[53] Epoxidation of drugs can also be performed as shown with the

  

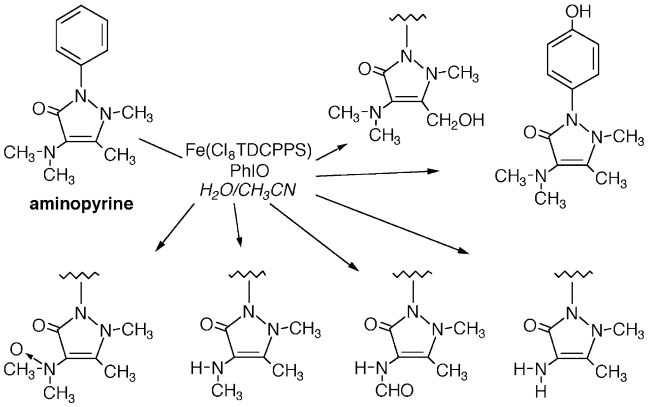


**Figure 16.** Biomimetic oxidation of aminopyrine.[10]

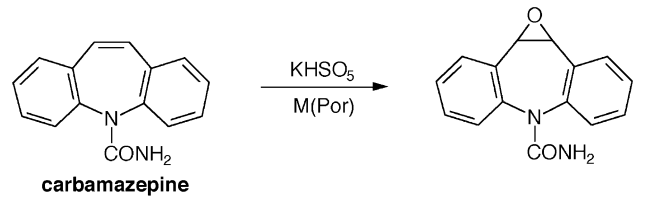


**Figure 17.** Biomimetic oxidation of carbamazepine.[54a]

antiepileptic drug carbamazepine which oxidation by $KHSO_5$, $H_2O_2$ or *t*-BuOOH and different metalloporphyrin catalysts can afford up to 90% of the 10,11-epoxide, the main metabolite observed *in vivo* (Figure 17).[54]

The behavior of the spasmolytic drug denaverine in aqueous and non-aqueous biomimetic systems based on manganese and iron porphyrins/$H_2O_2$/imidazole or pyridine has been investigated and the resulting oxidation profile compared to the *in vivo* metabolism in rat and human.[49] Besides oxidation at the tertiary amine giving *N*-demethylation or *N*-oxidation and some extent of *C*-oxidation at the *N*-methyl group, cleavage of the ester and ether bonds was observed as well as a very low amount of hydroxylation on one of the phenyl groups (Figure 12).

The system Fe(TPPS)/$H_2O_2$ has been shown to be a good model for enzymatic activation and DNA cleavage of the genotoxic ochratoxin A, a mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi.[55]

## 2.4 More Sophisticated Porphyrin Catalysts

A polypeptide-bound iron porphyrin activated with $NaBH_4$/$O_2$/tetramethylammonium hydroxide or CumOOH and an iron porphyrin bearing an imidazole or an alkyl thiolate as axial ligand activated with *m*-CPBA have been used as biomimetic models to study the oxidative metabolism of drugs such as phencyclidine

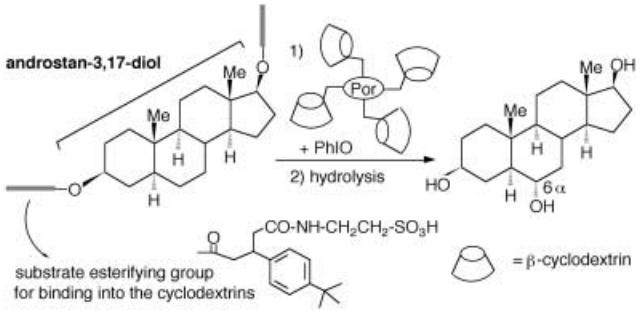


**Figure 18.** Conversion of a doubly bound steroid substrate to the 6α-hydroxy derivative by an artificial cytochrome P450.[58]

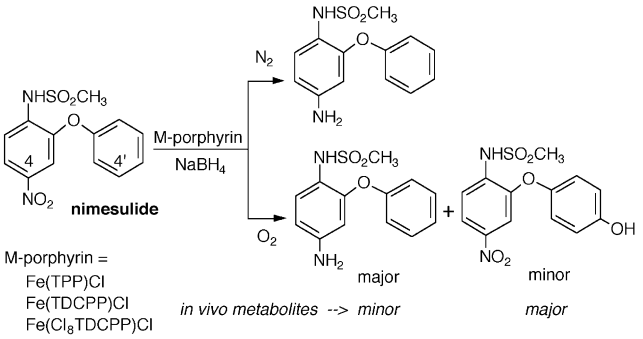


**Figure 19.** Treatment of nimesulide with sodium borohydride and metalloporphyrins under anaerobic and aerobic conditions as a mimic of the *in vivo* cytochrome P450/NAD(P)H transformation.[59]

(formula depicted in Figure 4A) or ibudilast (formula in Figure 5B).[30,56]

An iron membrane-spanning porphyrin introduced in bilayer vesicles has been shown to catalyze the regioselective epoxidation of steroids: for example, the $\Delta^{24}$ double bond of desmosterol, intrinsically less reactive than the $\Delta^5$ double bond, is selectively epoxidized because this $\Delta^5$ double bond is not accessible to the high-valent metal-oxo species.[57] As another example in this field, a manganese fluorinated tetraphenylporphyrin bearing four attached cyclodextrins can regio- and stereoselectively hydroxylate at position 6α a steroid substrate which has been previously esterified for an adequate binding into the cyclodextrins (Figure 18).[58]

The combination of sodium borohydride and metalloporphyrins used in the absence or presence of molecular oxygen have been shown to mimic the various redox transformations of organic substrates by cytochrome P450 and NAD(P)H. As an example, the non-steroidal anti-inflammatory, analgesic and antipyretic agent nimesulide (a selective inhibitor of cyclooxygenase-2) could give under aerobic conditions 4-amino-nimesulide and 4′-hydroxynimesulide, which are the two reduced and oxidized metabolites found in the *in vivo* metabolic profile of this drug (Figure 19).[59]

  

### 2.5 Miscellaneous

Ruthenium porphyrin serves as example of the use of other metallated porphyrins in biomimetic oxidations which can catalyze the oxidation of aromatic compounds by heteroaromatic *N*-oxides leading to selective quinone formation.[60]

The use of the poly(pyrrole-manganese porphyrin) electrode represents an alternative modality to homogeneous electrocatalytic systems or chemical catalysts. It gives good results for the oxidation of antisecretory and antiulcer thioacetamide derivatives, and so appears to be a convenient tool in the preparation of oxidized metabolites.[61]

The biomimetic oxidation approach used for drugs or carcinogens could obviously be used for other xenobiotics like agrochemicals (e.g., insecticides) for the prediction of metabolic profiles in relation to some toxicity aspects or to mechanisms of action in a host (humans or animals).[62] Suitable chemical models of the cytochrome P450-catalyzed oxidative metabolism of carbamate insecticides have been developed and their effectiveness demonstrated in the synthesis of potential metabolites. Depending on the appropriate choice of metalloporphyrin and oxygen surrogate, they allow one to reproduce their *in vivo* metabolic profiles (see Figure 20A,B for two examples of biomimetic oxidation of carbamate insecticides).[62a] The organochlorinated insecticide dieldrin is quite recalcitrant towards oxidation and the common porphyrin catalysts are usually destroyed before sufficient substrate oxidation can occur. However, highly halogenated metalloporphyrins, either in aqueous medium [e.g., the sulfonated porphyrins M($Cl_8$TDCPPS)] or in organic medium [e.g., M($Cl_8$TDCPP)], are effectively able to catalyze the oxidation of dieldrin to give the same products as those previously identified from mammalian metabolism studies (Figure 20C).[62b] Depending on a careful choice of catalyst, oxidant and reaction conditions, the production of natural metabolites can be mimicked and reasonable amounts of such metabolites can be produced at the bench.

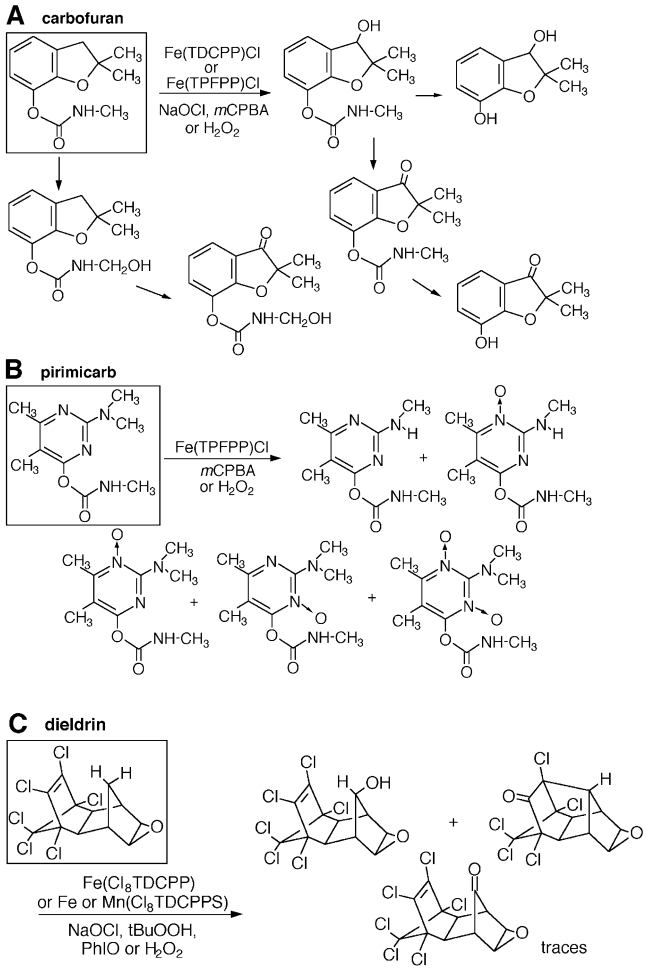


**Figure 20.** Biomimetic oxidation of insecticides.[62]

## 3 Other Chemical Models: the Case of Oxidative Activation of Isoniazid by Mn(III) Pyrophosphate

Although cytochrome P450 monooxygenases and, at a lesser extent, peroxidases and catalases are the main enzymes involved in the oxidative metabolism of xenobiotics *in vivo*, their chemical models based on metalloporphyrin associated to an oxygen atom donor can behave sometimes as too strong oxidant systems and then recourse to more mild oxidants becomes necessary. To illustrate this point, we now report some recent results developed in our laboratory on the oxidative activation of the antituberculosis agent isoniazid (INH).

Isoniazid (INH), an antibiotic used in the treatment of tuberculosis,[63] is considered as a prodrug requiring activation by the *Mycobacterium tuberculosis* KatG, a catalase-peroxidase enzyme.[64–66] However, none of the stable derivatives observed in KatG-dependent INH conversion, i.e., isonicotinic acid, isonicotinamide and isonicotinaldehyde (Figure 21) have demonstrated any bactericidal effect.[67] Recent studies[68–70] have suggested that the active form of INH responsible for the lethal effects on bacterial cells, probably an isonicotinoyl radical, is capable of reacting with the β-nicotinamide adenine dinucleotide ($NAD^+$/NADH), which is the cofactor of the long-chain 2-*trans*-enoyl-acyl carrier protein reductase InhA, a key enzyme involved in the biosynthesis of long-chain fatty acids and of mycolic acids, specific components of the mycobacterial cell wall.[71–74] The formation of covalent adduct(s) INH-NAD as competitive inhibitors might explain the loss of InhA activity.[68–75] More recently, another potential INH target, the NADPH-dependent β-ketoacyl reductase MabA, was identified in the fatty acid elongation

  

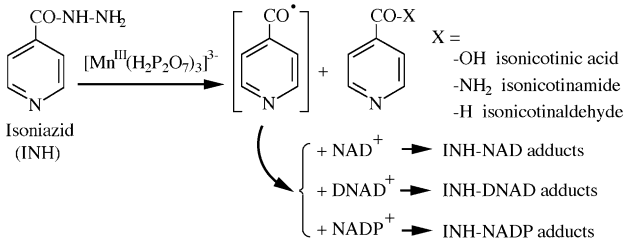


**Figure 21.** Isoniazid oxidation products and adducts formed in the presence of NAD(H), DNAD(H) or NADP(H) and Mn(III) pyrophosphate.[70,76]

system FAS-II (MabA catalyzes the second step of FAS-II elongation cycle and InhA the fourth). The enzyme MabA is efficiently inhibited by isoniazid *in vitro* by a mechanism similar to that of InhA, involving the formation of adducts INH-NADP (Figure 21).[76]

In spite of recent efforts, the activation mechanism of INH, the nature of the active form of this drug and the exact nature of the INH-NAD or NADP adducts are still matter of debate. Several studies have focused on the role of the catalase-peroxidase KatG,[65,66,77–79] a hemoprotein with manganese-dependent peroxidase activity.[80,81] KatG catalyzes the conversion of an Mn(II) chelate to an Mn(III) chelate (for this reason it is called manganese peroxidase, MnP), the Mn(III) chelate being able to oxidize INH. This mode of activation is reminiscent of the mechanism of manganese-peroxidase MnP of the white rot fungus *Phanerochaete chrysosporium* (see Figure 22 for a simplified scheme of the mechanism of MnP).[82] The role of chelating agents in *Phanerochaete chrysosporium* (α-hydroxy acids like lactate, succinate, malonate) is to stimulate the MnP catalytic activity by facilitating the dissociation of the enzyme-manganese complex by chelating Mn(III) and to stabilize manganese at the oxidation state III.

### 3.1 Chemical Systems as Models to Mimic Manganese Peroxidase Activity of the Catalase-Peroxidase KatG

To correctly model the manganese peroxidase activity of KatG, a chemical model must be able to selectively oxidize Mn(II) salts in the presence of chelating agents and of easily oxidized substrates. The catalytic oxidation of Mn(II) to Mn(III) chelates, i.e., the key step performed by MnP, has been mimicked using sulfonated iron porphyrin complexes (FeTMPS, FeTDCPPS, $FeBr_8$TMPS, $FeCl_{12}$TMPS) as catalyst and $KHSO_5$ as primary oxidant[83] or various water-soluble metallophthalocyanine/*t*-BuOOH systems,[84,85] but with catalytic activities far below the activity of MnP itself.

Our aim was to mimic the INH activation by KatG with chemical models in the presence of the two cofactors (NADH or NADPH) of the enzymes InhA

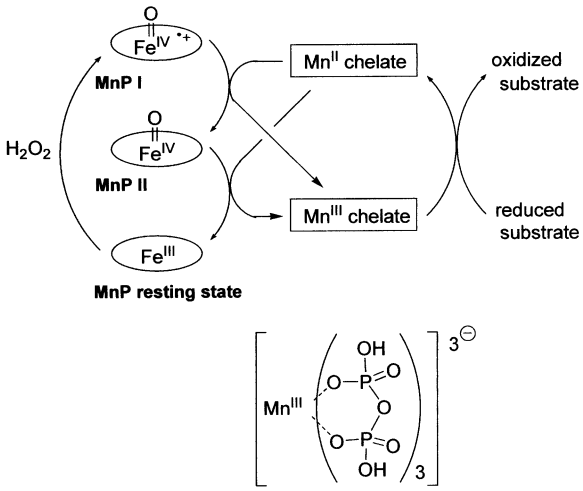


**Figure 22.** Catalytic oxidation of a substrate by manganese peroxidase (MnP) coupled to the oxidation of Mn(II) chelates, and structure of Mn(III) pyrophosphate as a stable complex for mimicking MnP.

or MabA in order to form, isolate and characterize the covalent INH-cofactor adducts as potential inhibitors of these two enzymes. Drug-activating systems based on metalloporphyrins (e.g., Mn-TMPyP/$KHSO_5$) have been shown to be too strong oxidants for the substrate INH, affording mainly the usual and non-active INH oxidation products (isonicotinic acid, isonicotinamide and isonicotinaldehyde) and only a very low amount of INH-cofactor adducts, even when using the mild, biologically relevant oxidant system $Na_2SO_3/O_2$.[70,86] Since Mn(II) is only slowly converted into Mn(III) in aerobic conditions, the alternative Mn(II)/$O_2$ system behave as a poor activating system.[80,81] Direct INH activation by Mn(III) salts appears to be more attractive since Mn(III) malonate or Mn(III) pyrophosphate has been shown to be capable of activating this drug to form isonicotinic acid and isonicotinamide[87] or to induce DNA breaks.[81] The retained system consisted of Mn(III) pyrophosphate (Figure 22),[70,88,89] a stable form of Mn(III) ions in aqueous solution which has been previously used in our model studies on manganese peroxidase.[53,83]

Pyrophosphate is an oxidant-resistant ligand able to stabilize Mn(III) in the pH range of 4 – 6. Stoichiometric oxidation of INH by Mn(III) pyrophosphate gave high conversions of INH within short reaction times, afforded the same stable oxidation products as in the KatG protein assay and, finally, was able to produce short-lived reactive intermediates that can react on the $NAD^+$/NADH or $NADP^+$/NADPH cofactors of InhA or MabA, respectively, to generate inhibitors of these enzymes. The yield of adducts (35 – 45% depending on the reaction conditions) was sufficient for further structural studies and some biochemical evaluation as inhibitors.

  

### 3.2 The Nature of the Reactive Species Involved in the Isoniazid-NAD Adducts Formation

In previous reports, the mechanism for the oxidation of isoniazid[66] and for the formation of adduct(s) between an activated form(s) of INH and $NAD^+$ or NADH[68–70,90] has been discussed. It has been proposed that the adducts can be formed either by attachment of an isonicotinoyl anion to $NAD^+$[68] or more likely by direct addition of an isonicotinoyl radical to an $NAD^{\bullet}$ radical[68] or to $NAD^+$ and subsequent reduction of the radical-cation.[69] Our proposed mechanism for the oxidation of INH by Mn(III) pyrophosphate is shown in Figure 23.[70,90] The formation of the isonicotinoyl radical is a key intermediate in the formation of isonicotinic acid, isonicotinaldehyde and, in the presence of $NAD^+$, in the formation of INH-NAD adducts. Di-4-pyridylglyoxal, formed in some amount by recombination of two isonicotinoyl radicals, plays the role of a radical marker and was effectively detected during LC-MS analyses. The transient existence of the isonicotinoyl radical was also suggested by INH oxidation experiments performed in $H_2{}^{18}O$ leading to about 20% of the formed isonicotinic acid without [$^{18}$O] label, which was indicative of the reaction of the isonicotinoyl radical with dioxygen; in similar experiments performed with the KatG enzyme, this percentage was 33%.[66] In the presence of $NAD^+$,[69,70,90] addition of the isonicotinoyl radical to $NAD^+$ should gives a pyridinium radical (Figure 23) with creation of a new chiral carbon in position 4 of the nicotinamide ring. Its fast one-electron reduction, possibly by INH itself, gives adducts with an open structure which are in slow equilibrium with cyclized adducts. Slow oxidation of these different adducts can give a common oxidized compound.

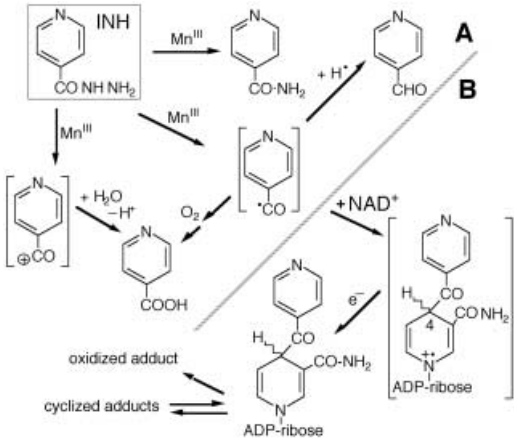


**Figure 23.** Proposed mechanism for the Mn(III)-mediated oxidation of INH alone (**A**) and formation of INH-NAD adducts in the presence of $NAD^+$ (**B**).[70,90]

### 3.3 The Precise Structure of the Isoniazid-NAD Adducts

A single INH-NAD adduct has been characterized within the active site of InhA by X-ray crystallography,[68] and this enzyme-bound adduct has been interpreted as the open epimer 4*S* (Figure 24). In contrast, on the basis of mass analyses and by using Mn(III) pyrophosphate as INH activation system in the presence of $NAD^+$, the chemical oxidation generated a family of INH-NAD adducts interpreted as a mixture of six open or cyclized adducts with a dihydropyridine structure concomitantly formed in solution, and one oxidized pyridinium adduct (Figure 24). The alkylation of $NAD^+$ at C4 by the isonicotinoyl radical in a non-chiral environment explains the formation of two 4*R*/4*S* epimeric open structures; a further cyclization process, giving hemiamidal structures, creates a second new chiral center at C7 and explains why these cyclized dihydropyridine derivatives are obtained as four diastereoisomers. Most likely, the enantiomeric ratio *R*/*S* at carbon 4 of the isonicotinamide is close to 1/1 (the isonicotinoyl radical can attack equally the two faces of the nicotinamide ring to give the two epimeric open structures) but the cyclization giving rise to the hemiamidal structures is selective enough to produce preferentially the two diastereoisomers with opposite configurations at C4 and the two bulky substituents at C4 and C7 (dihydropyridine and pyridine nuclei, respectively) being in opposite sides of the pyrrolidinone ring (the two other cyclized diastereoisomers are only observed in much lower amounts). When nicotinic adenine diclucleotide (deamido-$NAD^+$ or $DNAD^+$) instead of $NAD^+$ is used as starting material, a cyclization process cannot be observed and only two open reduced forms and one open oxidized form are obtained (Figure 24). All these adducts have been fully characterized by MS and isotopic experiments,[70] and by $^1$H and $^{13}$C NMR spectroscopy.[91] On the basis of partial structural characterization, the adducts INH-NADP have similar behavior and similar structures.[76]

### 3.4 Some Biochemical Results Obtained with Isoniazid-NAD and -NADP Adducts

An isolated 100 nM pool of INH-NAD adducts has been shown to give a 78% inhibition of the InhA activity (enzyme concentration was 77 nM).[75] This efficient competitive inhibition can probably be related, on the basis of crystallographic results,[68] to the structure of these adducts which are able to bind both to the NADH binding site by the NAD part and to the substrate binding site by the isonicotinoyl moiety. In addition, preliminary results indicate a different level of activity for each of the two main cyclic adducts, consistent with somewhat different binding constants of these diaster-

  

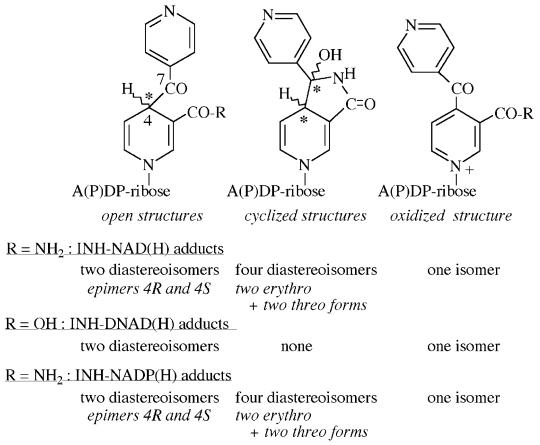


**Figure 24.** Structures of the INH-NAD, INH-DNAD and INH-NADP adducts.[70,91]

eoisomers, depending on the stereochemistry of the chiral centers at C4 and C7.[75] With INH-NADP adducts that can adopt multiple chemical forms in a dynamic equilibrium, kinetic experiments with isolated forms and purification of the enzyme-ligand complex suggested that the molecules active against MabA activity are the oxidized derivative and a major cyclic form.[76] Concerning the case of InhA and although the structure of the bound inhibitor interpreted on the basis of a X-ray crystallography study was an open structure,[68] it is now legitimate to raise the question about the effective active form(s) of INH-NAD adduct(s): could cyclic hemiamidal diastereoisomers, which are the main products present in aqueous solution, behave as the real InhA inhibitors? These studies will also be useful to help further design of specific inhibitors for the development of new antituberculosis agents efficient against drug resistant tubercle bacilli.

## 4 Conclusion

In addition to biological systems involving animal or human samples and cytochrome P450 human monooxygenases produced in microorganisms, the use of biomimetic chemical catalysts based on metalloporphyrins is a complementary promising approach to study the oxidative metabolism of drugs or drug candidates. The lack of selectivity of these chemical models is a drawback of these systems but it must be relativized since human liver contains many cytochrome P450 isozymes and the hepatic oxidative metabolism of a drug generally leads to a series of metabolites derived from the oxidation of different regions of the molecule. Metalloporphyrin systems do not *a priori* involve specific sites for selective binding of the drug and so they should oxidize it at all positions that are chemically reactive and not too hindered. Moreover, by varying the structure of the metalloporphyrin, the nature of its environment (organic solvent, water, homogeneous catalyst or catalyst inserted into organic or inorganic polymers) or of the oxidant, it is possible to modulate the regioselectivity of the oxidation and generate systems adapted for the regioselective oxidation of the drug. When the drug is too easily oxidizable, then the metalloporphyrin systems may behave as too strong oxidants giving rise directly to stable overoxidized products and possible reactive intermediates can be by-passed. Then, a reasonable knowledge of the *in vivo* metabolic pathways of the drug may help in the choice of a convenient mild oxidant. That approach has been developed in our studies on the biomimetic oxidation of INH where the modeling of the catalase peroxidase KatG with Mn(III) pyrophosphate allowed us to contribute to an improved knowledge of the activation pathways of INH and to characterize the different reactive species and key products involved in the mechanism of action of isoniazid in relation with its antituberculous properties.

Finally, using biomimetic chemical models in the oxidative activation of xenobiotics represents today a useful tool in early research and development of biologically active molecules that could become drugs or agrochemicals for (i) identification of metabolic sensitive functional groups, (ii) modeling *in vivo* metabolic profiles, (iii) prediction of oxidative metabolites, (iv) production of natural metabolites at a scale of some milligrams and even larger if necessary for pharmacological and toxicological tests, (v) identification and sometimes isolation of transient reactive species and instable metabolites under selected and controlled reaction conditions, (vi) contribution to the understanding of the mechanism of action of drugs, (vii) detecting unstable activated mutagens, and finally (vii) reducing the number of animal experiments.

## Acknowledgements

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