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Approaches to the design of catalytic metallodrugs

Joan J Soldevila-Barreda and Peter J Sadler

Metal ions are known to act as catalytic centres in metallo-enzymes. On the other hand, low-molecular-weight metal complexes are widely used as catalysts in chemical systems. However, small catalysts do not have a large protein ligand to provide substrate selectivity and minimize catalyst poisoning. Despite the challenges that the lack of a protein ligand might pose, some success in the use of metal catalysts for biochemical transformations has been reported. Here, we present a brief overview of such reports, especially involving catalytic reactions in cells. Examples include C–C bond formation, deprotection and functional group modification, degradation of biomolecules, and redox modulation. We discuss four classes of catalytic redox modulators: photosensitizers, superoxide dismutase mimics, thiol oxidants, and transfer hydrogenation catalysts. Catalytic metallodrugs offer the prospect of low-dose therapy and a challenging new design strategy for future exploration.

Address
Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK

Corresponding author: Sadler, Peter J (P.J.Sadler@warwick.ac.uk)

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Introduction

Metal-based catalysts, metallo-enzymes, are well known in natural biological systems. These are often based on transition metal ions surrounded by proteins, with sites carefully designed to allow the selective recognition of substrates, protecting somewhat the metal ion from poisoning. Examples include manganese, iron, copper, zinc and molybdenum in all major classes of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

The potential value of using natural metallo-enzymes or synthetic metal catalysts as drugs has already been recognized. The enzyme SOD for example has been in clinical trials as an oral agent for the treatment of age-related macular degeneration (NCT00800995) [1]. Interestingly the Mn^{II} MRI contrast agent Mangafodipir (MnDPDP) also possesses SOD activity, and Calmangafodipir ($Ca_4Mn(DPDP)_5$) is in Phase II clinical trials (NCT01619423) for treatment of metastatic colorectal cancer [1].

Metallo-drugs with catalytic properties can potentially be administered in smaller doses and with lower toxicity. Furthermore, catalytic drugs are likely to have novel mechanisms of action which might circumvent the development of drug resistance. However, low-molecular-weight catalysts are known to be easily poisoned in the presence of nucleophiles [2]. Retaining the activity of metal-based catalysts in biological media is, therefore, challenging on account of the presence of many biomolecules. Despite the difficulties, four major groups of 'catalytic metallodrugs' have been explored recently with some remarkable successes, those relating to C–C bond formation, deprotection and functional group modification, degradation of biomolecules, and redox modulation. We discuss four classes of catalytic redox modulators: photosensitizers, superoxide dismutase mimics, thiol oxidants and transfer hydrogenation catalysts. The potential of these catalytic systems to progress from model reactions, to cellular and eventually *in vivo* activity and to become approved drugs, is of particular interest (Table 1).

Formation of C–C bonds

Reactions such as azide–alkynyl cycloadditions [3], Suzuki–Miyaura [4–7] or Sonogashira [8] cross-couplings have been explored as synthetic tools for C–C bond formation *in vitro* and *in vivo*. For example, Pd^0 nanoparticles can carry out Suzuki–Miyaura cross-coupling reactions inside living cells. The nanoparticles can be delivered to cells encapsulated in polystyrene microspheres, once inside cells, the nanoparticles can be used for fluorescent labelling [4]. Palladium(II) compounds such as $[Pd(OAc)_2(ADHP)_2]$ (ADHP = 2-amino-4,6-dihydroxypyrimidine), can also catalyse Suzuki–Miyaura cross-coupling and have been used to label modified I-containing proteins on cell surfaces with fluorescent tags bearing boronic acid in *Escherichia coli* [5–7]. Similarly, the complex $[Pd(OAc)_2(N,N\text{-dimethylADHP})_2]$ has been used for fluorescent-labelling of homopropargylglycine-modified ubiquinone and peptides *via* a copper-free Sonogashira reaction [8]. Copper-free Shonogashira in modified *E. coli* and Shigella cells has also been performed using $Pd(NO_3)_2$ to fluorescently label allyl-containing proteins [9]. Copper(I) has been used to perform azyl–alkyne cycloadditions in combination with ligands such as TBTA, THPTA, BTTAA or BTTES (Figure 1). Using such a method, different cell membrane proteins

Table 1

Examples of metal catalysis studied *in cellulo* or *in vivo*

Metal complex	Reaction	Function/use	Cell system
C–C bond formation			
Cu(I)-TBTA (or similar)	Azyl–alkine cycloadditions	Labelling of modified proteins containing alkyne or azyl groups	*E. coli*, HeLa, CHO, Jurkat cells, Zebra fish
$[Pd(OAc)_2(ADHP)_2]$	Suzuki–Miyaura cross coupling	Fluorescence labelling of cell-surface proteins	*E. coli*
$[Pd(OAc)_2(DMDHP)_2]$	Cu-free Sonogashira	Labelling of alkyl-containing proteins	*E. coli*
$Pd(NO_3)_2$	Cu-free Sonogashira	Labelling of alkyl-containing proteins	Shigella cells
Pd^0 nanoparticles	Suzuki–Miyaura cross coupling	Fluorescence labelling	HeLa cells
Deprotection and functional group modifications			
Pd^0 nanoparticles (PET microspheres)	Carbamate cleavage	Activation of pro-fluorophores protected by carbamates	HeLa cells
Pd^0 nanoparticles (PET macrospheres)	Carbamate cleavage Dealkylation of amines	Activation of pro-fluorophores or pro-drugs	Zebra fish
$[Pd(dba)_2]$, $[(Allyl)PdCl]_2$	Carbamate cleavage	Activation of pro-fluorophores protected by carbamates; selective activation of proteins containing lysine protected aminoacids	HeLa, HEK293T, CHO, CaCo-2, A549, NIH3T3 cells
[Fe(TPP)Cl]	Reduction of aromatic azides	Activation of azides/fluorescence imaging	HeLa cells, zebra fish, *Caenorhabditis elegans*
$[Cp^*Ru(\eta^6\text{-pyrene})]PF_6$	Carbamates cleavage	Activation of pro-fluorophores protected by allyl-carbamates	HeLa cells
$[Cp^*Ru(COD)Cl]$	Carbamates cleavage	Activation of pro-fluorophores protected by allyl-carbamates	HeLa cells
$[CpRu(QA)(\eta^3\text{-allyl})]PF_6$	Carbamate cleavage	Activation of pro-fluorophores or pro-drugs protected by allyl-carbamates	HeLa cells
Degradation of biomolecules			
Cu(II)-ATCUN-R	Cleavage of RNA	Hepatitis C and HIV	Huh7 cells (Hepatitis C) Jurkat cells (HIV)
Ni(II)-ATCUN-R	Cleavage of RNA	Hepatitis C and HIV	Huh7 cells (Hepatitis C) Jurkat cells (HIV)

have been labelled with fluorescent tags in *E. coli*, HeLa, CHO and Jurkat cells [10,11]. More interestingly, the reaction was also executed successfully in mammalian cells and embryonic zebra fish [12]. This topic has been extensively reviewed recently [13–15].

Deprotection and functional group modification

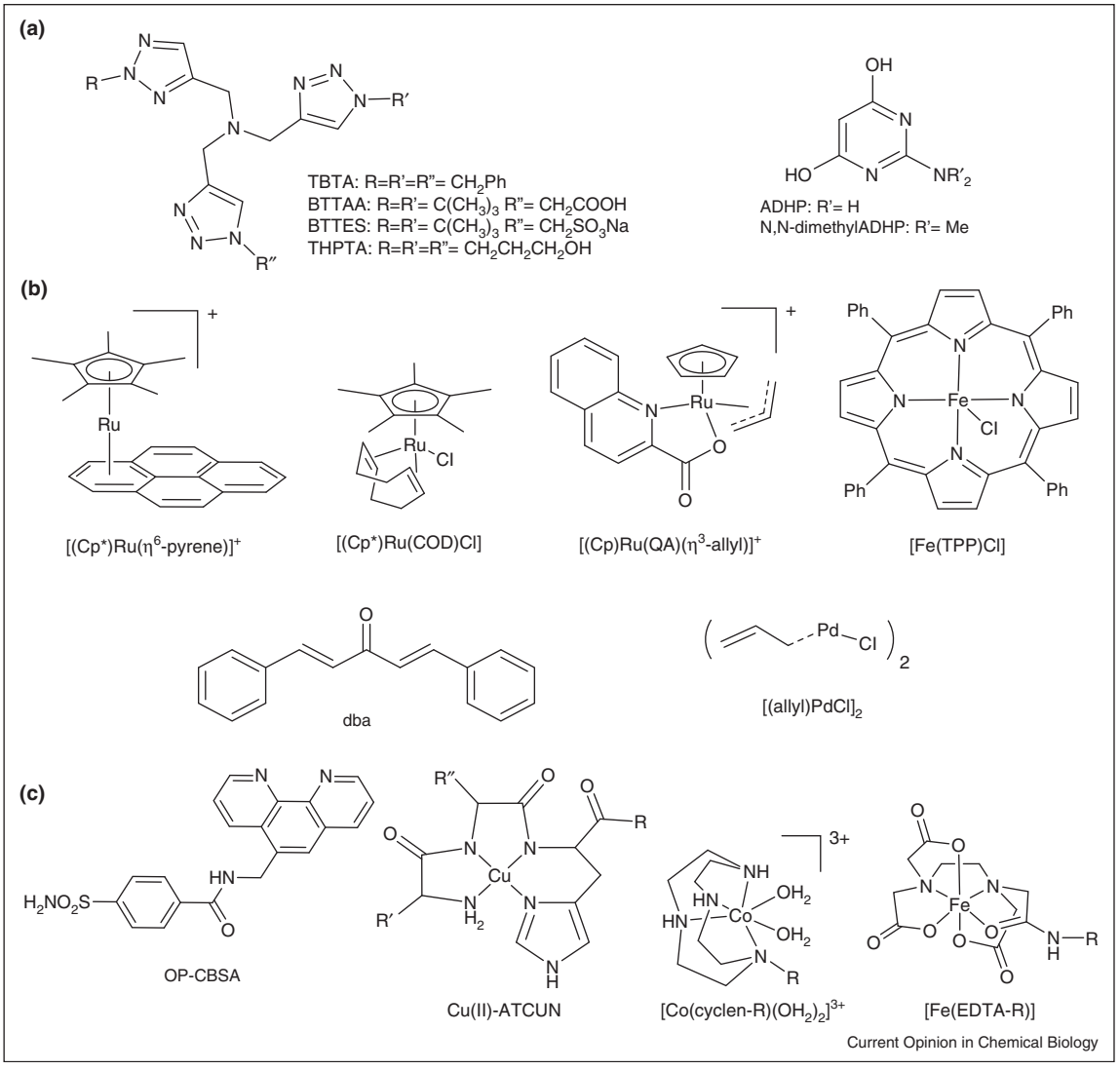
This area of research is remarkably young with only limited examples, but shows much promise. Examples are the Pd^0 nanoparticles mentioned above for the Suzuki–Miyaura reaction [4]. Such particles encapsulated in polystyrene microspheres catalyse the cleavage of allyl-carbamate protected groups [4]. The catalytic reaction is effective in HeLa cells where allyl-carbamate-protected-rhodamine 110 was administered before the administration of the nanoparticles, giving intense fluorescence after the liberation of rhodamine 110 [4].

A similar approach has also been used for the activation of drugs such as modified 5-fluorouracyl, protected at the N1 with allyl, propargyl or benzyl groups. The modified drug was administered and activated in zebra fish by Pd^0 nanoparticles attached to macrospheres of polystyrene (150 μm diameter, larger than human cells). The Pd^0 nanoparticles are capable of catalysing the extracellular dealkylation of the N-alkyl fluoracyl [16••]. The same Pd^0 nanoparticles also activated pro-drugs protected with carbamates such as gemcitabine [17].

Meggers *et al.* have reported a series of compounds which are capable of cleaving carbamates in protected amines, using Ru^{II} compounds instead of Pd^0, for example, $[(Cp^*)Ru(COD)Cl]$ (COD = cyclooctadiene) (Figure 1) [18,19] and $[(Cp^*)Ru(\eta^6\text{-pyrene})]PF_6$. The latter is inactive until irradiated with light (λ = 330 nm) and further releases the pyrene moiety. Both complexes activate a derivative of rhodamine 110 protected with allyl-carbamates in HeLa cells (Figure 1). However, the presence of thiols is required [19,20]. More recently, the catalytic cleavage of allyl-carbamates in cells has been greatly improved by the use of the Ru^{IV} complexes such as $[CpRu(QA\text{-}R)(\eta^3\text{-allyl})]PF_6$ (QA = 2-quinolinecarboxylate; R = π-donating groups). These also activate protected fluorophores and protected anticancer drugs such as N-(allyloxycarbonyl) doxorubicin, inside HeLa cells [21].

Chen *et al.* have recently reported the use of four Pd compounds as catalysts for the deprotection of carbamates. These compounds were tested for the deprotection of allyl-carbamate-protected and propargyloxy-carbamate-protected rhodamine 110 and also protected

Figure 1



Chemical structures of metal catalysts. **(a)** Formation of C–C bonds in cells, **(b)** deprotection and functional group modification, and **(c)** degradation of biomolecules.

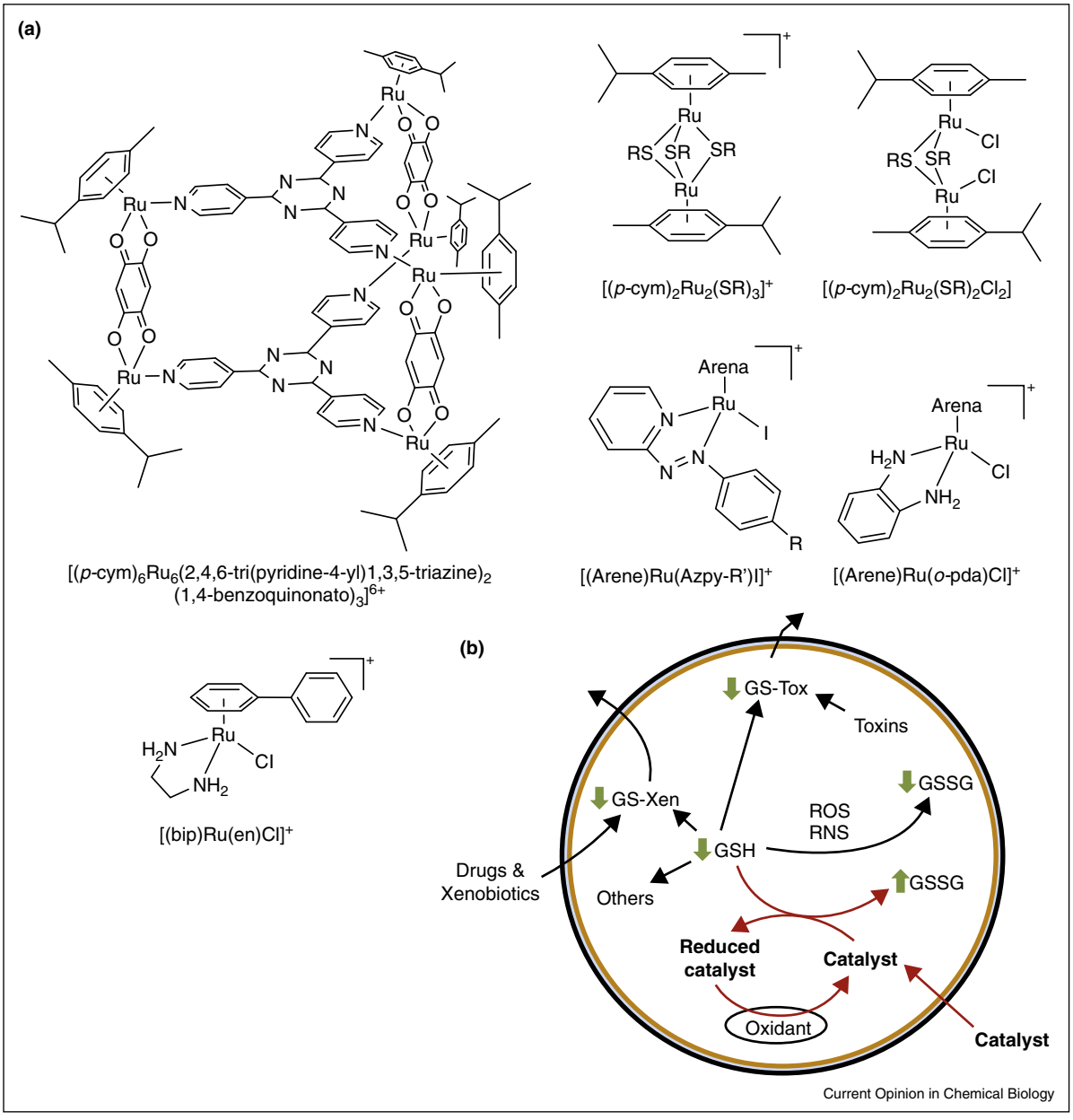
lysine residues. Those experiments were carried out in six different mammalian cell lines. The more effective compounds were $[(allyl)PdCl]_2$ and $[Pd(dba)_2]$ (dba = dibenzylideneacetone) (Figure 1). This method was then readily applied to synthetic proteins containing protected lysine, showing that protein controlled activation can be achieved in cells and *in vivo* [22••].

With a similar aim, Meggers *et al.* reported the use of the iron complex [Fe(TPP)Cl] (TPP = 5,10,15,20-tetraphenylporphyrin) as a catalyst for the reduction of azides to imines in cells, in the presence of thiols or other reducing agents (Figure 2) [19,23]. This compound has been studied for the activation of a rhodamine 110 derivative that contains azides instead of amines in HeLa cells, *Caenorhabditis elegans* and zebra fish [23]. The topic has been recently reviewed by Meggers *et al.* [24•].

Degradation of biomolecules

The development of catalytic drugs for the hydrolytic and oxidative cleavage of peptide/proteins has been investigated for several years. A few of these reported metal complexes have shown potential as therapeutic agents. For example Cu^{II}-1,10-phenanthroline-arenesulphonamide (Cu-OP-CBSA) complexes can target carbonic anhydrase and catalyse the hydrolytic cleavage the protein [25]. Similarly, Fe^{III}- or Cu^{II}-containing EDTA–biotin conjugates catalyse the oxidative cleavage

Figure 2



GSH oxidation. **(a)** Chemical structures of the complexes tested for the catalytic oxidation of GSH in cells. **(b)** Effects induced by artificial GSH oxidation in cells. Arene = hmb, *p*-cym or bip. R′ = NMe_2, OH or H; R″ = CH_2Ph, CH_2CH_2Ph or $CH_2C_6H_4$-*p*-tBu.

of streptavidine [26]. The Co^{II} complex, $[Co(cyclen)(OH_2)_2]^{3+}$ (Figure 1) can cleave peptide deformylase under physiologically-relevant conditions, and that could be attractive as an antibiotic agent [27]. Recently, Suh *et al.* have reported catalytic applications of $[Co(cyclen)(H_2O)_2]^{3+}$ for performing hydrolytic cleavage of amyloids at 310 K and pH 7.4 [28–31]. Amyloids are insoluble aggregations of peptides or proteins, and are present in conditions such as Alzheimer's, Parkinson's and Type II diabetes. Ni^{II} and Cu^{II} compounds containing an ATCUN motif (an amino terminal peptide with His in position 3) have also been shown to cleave proteins catalytically. For example, the metal complex of ATCUN-lisinopril catalytically cleaves angiotensin-converting enzyme [32].

Many of the aforementioned compounds can also be conjugated to RNA or DNA targeting vectors, in order to cleave nucleic acid chains specifically. Recent

examples are the Cu^{II} ATCUN compounds of Cowan *et al.* which are designed to target hepatitis C and degrade its RNA [33,34]. Cowan *et al.* have also reported the use of Cu^{II} and Ni^{II} ATCUN compounds to recognize and cleave HIV1 RNA [35,36]. Both ATCUN type compounds were active in cells. Other examples of Ni^{II}, Fe^{II}, Fe^{III}, Co^{III}, Cu^{II} as well as other metals, containing ligands capable of recognizing specific peptides or RNA have been reported and reviewed [37–40].

Modulation of the redox environment

In general, a very tight balance is maintained between the reducing and oxidizing agents present in cells. Oxidative stress caused by the imbalance between reactive oxygen and/or nitrogen species (ROS, RNS) and biological antioxidants can lead to alterations in biomolecules such as DNA, lipids and proteins. The redox status of cells is also critical in the regulation of gene expression and some signalling pathways. Despite the fact that ROS and RNS are necessary for the normal physiological functioning of the cell, over production of redox active species may result in cell dysfunction or death. Alzheimer's, Parkinson's, arteriosclerosis or cancer are some of the diseases that have been linked to oxidative stress [41]. Hence, there is increasing interest in modulating the redox balance. We discuss briefly four different classes of redox modulators.

Photosynthetizers used in photodynamic therapy

PDT relies on the administration of a non-cytotoxic compound (photosensitizer) which can be excited by light irradiation, at a specific wavelength. The excited compound promotes the formation of highly reactive singlet oxygen ($^1O_2$) from ground-state triplet oxygen. Singlet oxygen, like other reactive oxygen species (ROS), causes damage and oxidative stress when in high concentrations [42]. The photosensitizer should ideally be activated at long wavelength in order to achieve deeper penetration in the body. Most photosensitizers are highly conjugated macrocycles such as porphyrins, chlorins or naphthalocyanines. There are a few examples of metal complexes that have been studied for that purpose. Some metallomacrocycles have reached clinical trials, including aluminium sulphonated-phthalocyanine (approved for clinical use in Russia), motexafin lutetium and palladium bacteropheophorbide. This topic has been extensively reviewed [42] and we will not discuss it in the present review.

Superoxide dismutase mimics

Metal-complexes can catalyse the dismutation of superoxide radicals (O_2^{-}), toxic reactive oxygen species generated by different metabolic pathways but mainly in the mitochondrial electron transport chain. Suitable SOD mimics require a redox potential between −180 mV (reduction potential $O_2^{\bullet-}$ referenced to hydrogen electrode) and +890 mV (oxidation potential of $O_2^{\bullet-}$), thus mimicking the thermodynamic and kinetic properties of the original SOD enzymes [43–46]. In mitochondria, SOD is a manganese (Mn^{II}/Mn^{III}) enzyme, and in the cytoplasm a $Cu^{I/II}Zn^{II}$ enzyme. Manganese SOD mimics containing polydentate macrocycles such as porphyrins, corroles, salens, biliverdins or polyamines in particular have shown promise and some have undergone clinical trials (e.g., M40403 for treatment of metastatic melanoma and renal cell carcinoma, currently in clinical trial NCT00033956). There are many reviews related to SOD mimics [43–46] and we will not discuss them further here.

Oxidation of thiols

Thiol groups play critical roles in the folding and stability of proteins and enzymes. Reduced cysteines and oxidized cystine disulphide cross-links are redox pairs, although there are several other important oxidation states of sulphur (including sulfenate, sulfinate, and sulfonate). Thus, thiols play an important role in controlling and maintaining the redox homeostasis of cells, and can provide protection against elevated levels of reactive oxygen species. The redox state of sulphur is involved in cellular signalling.

Thiol groups are therefore targets in the development of catalytic drugs. For example, anti-cancer half-sandwich Ru^{II} complexes containing azopyridine (Azpy) chelating ligands (Figure 2), can oxidize glutathione (GSH) to GSSG catalytically [47••]. Glutathione is a cysteine-containing tripeptide, γ-L-Glu-L-Cys-Gly, which is of critical importance for the maintenance of the redox homeostasis of the cell. GSH can act as an antioxidant and prevent damage by reactive oxygen and nitrogen species (ROS, RNS) [48,49]. As a consequence of the treatment of cancer cells with azopyridine-Ru^{II} half sandwich compounds, the levels of GSH in cells are lowered and the redox balance of the cell disrupted. A549 cancer cells show an increase of ROS levels upon 24-hour exposure to the complex. Interestingly, the Os^{II} analogues show very different reactivity and do not oxidize GSH [50].

In addition to the effects on the redox balance, the depletion of GSH may also interfere with other cellular processes since GSH is not only an antioxidant but is also involved in detoxification. Many internal and external toxins react with GSH to form GS-X adducts, that are excreted from the cell or the body [48,49]. GSH is also involved in some metabolic processes, including storage and transport of nitric oxide, metabolism of estrogens, leukotrienes, and prostaglandins, reduction of ribonucleotides; maturation of iron–sulphur clusters of diverse proteins, and operation of some transcription factors [49].

To explore this approach further, a series of Ru^{II} complexes containing redox-active *o*-phenylenediamine (*o*-pda) chelating ligands have been designed (Figure 2). These complexes undergo ligand oxidation in the presence of oxygen, generating [(η^6-arene)Ru(o-benzoquinonediimine)Cl]$^+$

(arene = *p*-cym, hmb or bip) which is readily reduced by GSH to regenerate the complex. These complexes can also catalytically oxidize up to 15 mol equivalents of GSH in 72 hours, under biologically relevant conditions [51]. The compound showed no antiproliferative activity in cells, perhaps because the catalysis is not efficient enough [51].

Surprisingly, the ruthenium complex $[(bip)Ru(en)Cl]^+$ (en = ethylenediamine) also reacts with GSH at pH 7 in the presence of oxygen to give the sulphenato-GS adduct (Figure 2) [52,53]. Bound sulfonate appears to be more readily displaced (e.g., by G on DNA) than the thiolate, but this reaction has yet to be studied as a catalytic process.

Thiolate-bridged ruthenium(II) arene dimers of the type $[(\eta^6\text{-arene})_2Ru_2(SR)_3]^+$ and $[(\eta^6\text{-arene})_2Ru_2(SR)_2Cl_2]$ are known to oxidize thiols (Figure 4). Recently, Therrien *et al.* have shown that catalysis can occur under biological conditions. The Ru^{II} dimers exhibit high antiproliferative activity against a range of cancer cell lines, including cisplatin-resistant cells, however the anticancer activity does not correlate with the catalytic activity of the dimers [54–56]. The catalytic activity may not be the main anticancer mechanism but could contribute. The Rh^{III} and Ir^{III} analogues of the Ru^{II} dimer show similar antiproliferative activity [54], although the catalytic activity towards oxidation of GSH is markably lower than with Ru^{II} compounds.

The Ru^{II} cages $[(p\text{-cym})_6Ru_6(2,4,6\text{-tri(pyridine-4-yl)}1,3,5\text{-triazine})_2(1,4\text{-benzoquinonato})_3]^{6+}$ and $[(p\text{-cym})_6Ru_6(2,4,6\text{-tri(pyridine-4-yl)}1,3,5\text{-triazine})_2(5,8\text{-dihydroxy-}1,4\text{-naphthoquinonato})_3]^{6+}$ synthesized as carriers for transport of cytotoxic molecules into cancer cells, also oxidize GSH. However, the active species is the product of the reaction between the $\{(p\text{-cym})Ru\}^{2+}$ and GSH or some amino acids. The metallo-cages undergo ligand substitution and form Ru^{II} species capable of oxidizing GSH under biological conditions. Interestingly, in competition reactions involving various amino acids and cysteine or GSH (Figure 2), the catalytic properties are retained [57,58].

Despite the success of some complexes in oxidizing thiols, they are non-specific and many other sulphur-containing molecules and reducing species often react with the catalysts. However, some compounds appear to show a preference for oxidation of thiols in cells which is encouraging for future tuning and improvement of their properties.

Complexes for transfer hydrogenation reactions

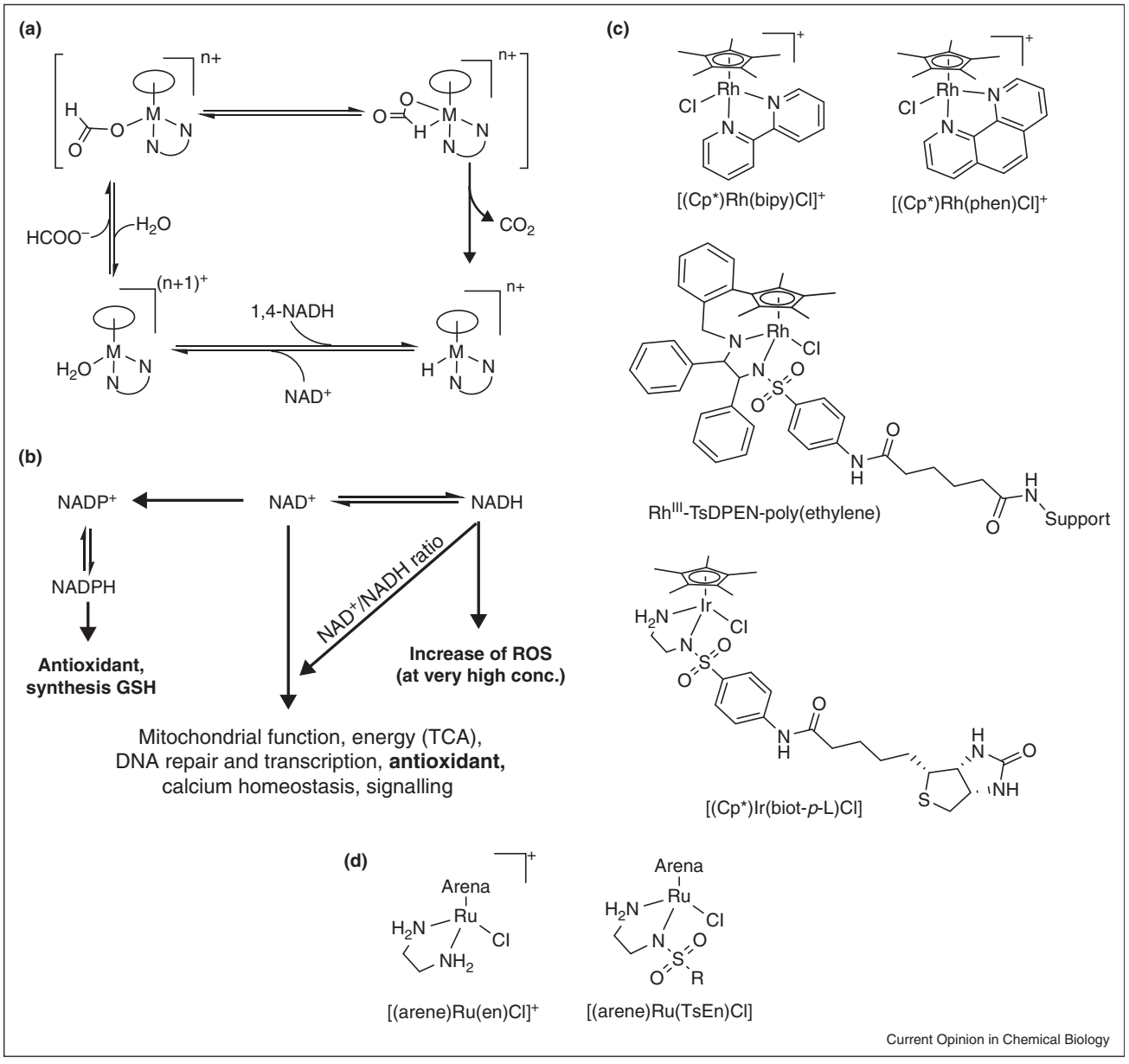
Transfer hydrogenation reactions are usually defined as the reduction process by which a catalyst can promote the transfer of a hydride ion from a donor to a molecule containing a multiple bond. Traditionally, this reaction has been used for the reduction of ketones, imines and C=C double bonds in non-aqueous (usually alcoholic) media, using 2-propanol/KOH or formic acid/triethylamine as hydride donors[59]. However, the reductions can be carried out in aqueous media using sodium formate as a hydride source [60]. Such mild conditions allow transfer hydrogenation reactions to be carried out with biomolecules, such as nicotinamide adenine dinucleotide or pyruvate.

In the field of hydrogenation and transfer hydrogenation reactions, regeneration of NADH and NAD^+ has been intensively studied, mainly for enzymatic reduction in organic synthesis [61]. The first series of complexes reported which were capable of reducing NAD^+ to NADH via transfer hydrogenation was that of Steckhan *et al.* Organometallic Rh^{III} bipyridine complexes were shown to reduce NAD^+ efficiently with high turnover frequencies. The mechanism and applications for the reduction of ketones (complex-NAD-enzymatic system) using $[(Cp^*)Rh(bipy)Cl]^+$ (Figure 3) have been well studied by Fish *et al.* [62–67]. Süss-Fink *et al.* reported the catalytic reduction of NAD^+ by a series of phenanthroline-containing Ru^{II}, Rh^{III} and Ir^{III} catalysts [68]. The Ru^{II} half sandwich compounds were shown to be much less active than their Rh^{III} and Ir^{III} analogues. The complex $[(Cp^*)Rh(phen)Cl]^+$ (Figure 3) achieved conversions and turnover frequencies of up to twice those obtained previously with $[(Cp^*)Rh(bipy)Cl]^+$ in aqueous media, at 333 K and pH 7 [68]. Subsequently the catalytic regeneration of 1,4-NADH by some Ru^{II} complexes containing ethylenediamine or acetylacetonate ligands was studied (Figure 3), but the turnover frequencies were low [69]. Salmain *et al.* prepared a series of Rh^{III} and Ru^{II} complexes using dipyridyl amine ligands functionalized by maleimide. The complexes were not as active as the bipyridine-containing complex $[(Cp^*)Rh(bipy)Cl]^+$, but comparable turnover frequencies and conversions were achieved with a Rh^{III} complex containing a 2,2-dipyridylamine-maleimide ligand [70].

Poisoning of the metal catalysts when the system is coupled to alcohol dehydrogenase or other enzymes, mainly by reaction with thiol groups, has been reported and is often a feature of such systems [71]. A common approach to avoid catalyst inactivation has been to attach the catalyst to a solid support. For example, Hollmann *et al.* used a tethered Rh^{III}-Noyori type complex immobilized on a poly(ethylene) polymer (Figure 3c) [72]. The catalytic activity of the heterogeneous catalyst was lower than that of other soluble Rh^{III} complexes. This area has been reviewed [71].

Recently, Ward *et al.* incorporated biotin–streptavidin into Noyori-type Ir^{III} complexes, thus creating artificial metalloenzymes with higher activities than the well-established [Cp*Rh(bipy)Cl]. The conjugate was

Figure 3



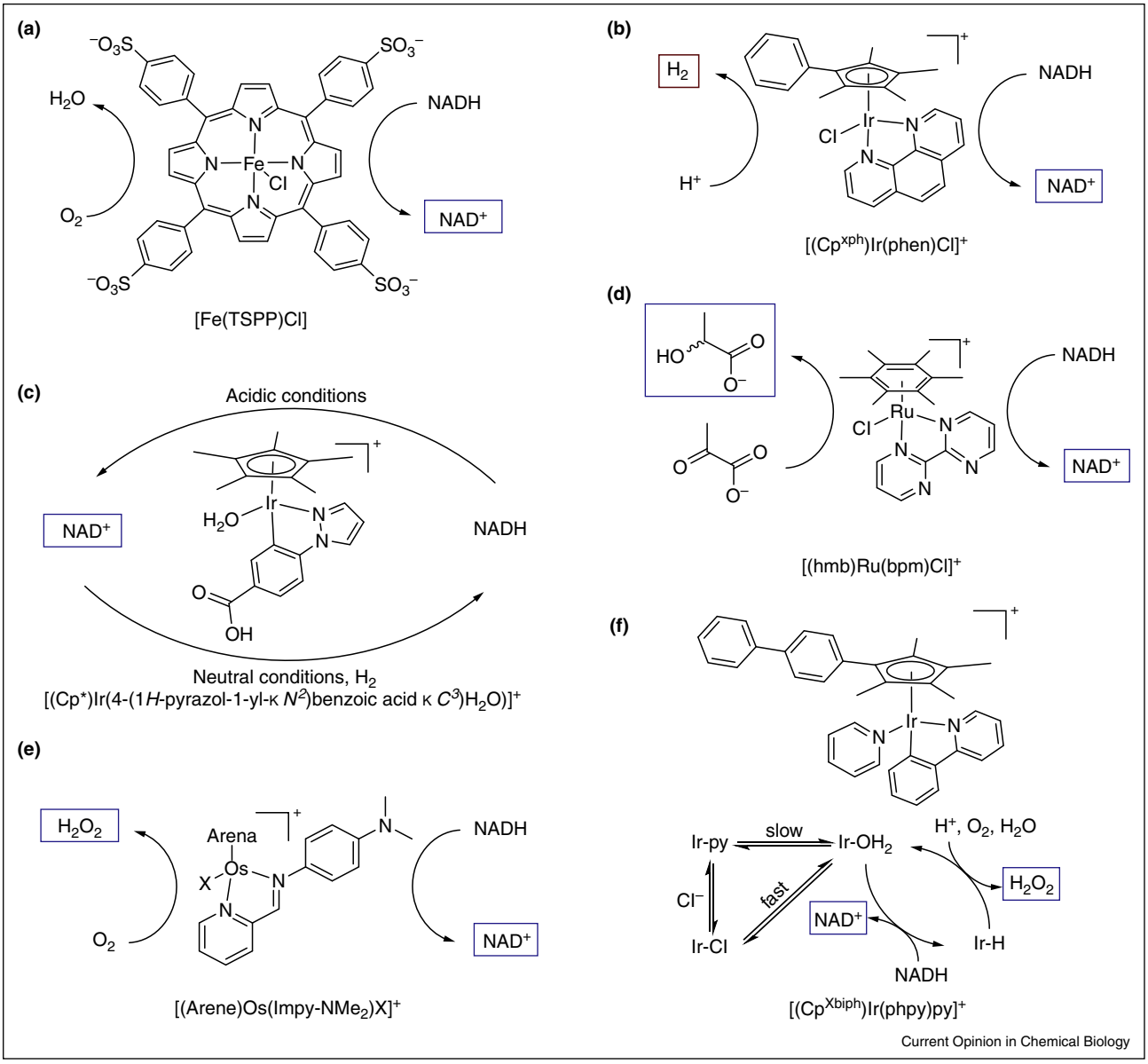
Regeneration of NADH. **(a)** Scheme for the catalytic process inside cells. **(b)** Summary of some of the relevant functions of NAD^+ in cells. **(c)** Chemical structures of some of the complexes studied for regeneration of NADH. **(d)** Chemical structures of the ruthenium catalysts that have been studied in cells. Arene = bn, hmb, p-cym or bip. R = *p*-tosyl, methyl or *p*-trifluoromethylbenzene.

designed to circumvent catalyst poisoning. Although the Ir^{III} was still poisoned by the addition of nucleophiles such as glutathione, co-administration of the compound with oxidizing agents, capable of oxidizing GSH, resulted in a significant increase in the catalytic activity of the complex [73]. They also demonstrated that [Cp*Ir(biot-*p*-L)Cl] anchored within streptavidin can be used as an asymmetric transfer hydrogenase with a variety of redox enzymes relying on NADH, $FADH_2$ and haem cofactors [74].

In our more recent work, we have focussed on the modulation of the NAD^+/NADH ratio in cells using organometallic complexes such as $[(hmb)Ru(en)Cl]^+$ or [(*p*-cym)Ru(TsEn)Cl] (TsEn = N-(2-aminoethyl)-4-toluenesulphonamide) [69]. The concentration of NAD^+ as well as the NAD^+/NADH ratio play crucial roles in many cellular metabolic processes such as regulation of energy metabolism, DNA repair and transcription, immunological functions and cell death [61,75,76]. Furthermore, changes in metabolism result in fluctuations

in the NAD^+/NADH ratio, and, conversely, changes in the ratio can produce metabolic changes [61,75,76]. In some cases alterations in the cellular redox status play an important role in cell death, making the coenzymes potential drug targets for chronic or auto-immune diseases such as Parkinson's, hepatitis C, diabetic vascular dysfunction, hyperglycaemia and cancer [61,75,76]. Cancer cells, due to their increased rate of metabolism, generate high levels of oxidizing species and, therefore, are under constant oxidative stress [77]. This makes cancer cells more dependent on redox regulatory systems, and more sensitive to variations in the NAD^+/NADH ratio. In addition, NAD^+ is also required as a substrate for the synthesis of ADP-ribose, ADP-ribose polymers and cyclic ADP-ribose which are crucial for genome stability, DNA repair and maintenance of calcium homeostasis [76].

Figure 4



Regeneration of NAD^+. **(a)** Oxidation of NADH by [Fe(TSPP)Cl], **(b)** formation of H_2 via transfer hydrogenation using NADH as a hydride source, **(c)** NADH oxidation by [(Cp*)Ir(4-(1H-pyrazol-1-yl-κN^2)benzoic acid κC^3)H_2O)]$_2SO_4$, **(d)** reduction of pyruvate by [(hmb)Ru(bpm)]$^+$ using NADH as a hydride source; this reaction also occurs with [(Cp^{xph})Ir(phen)Cl]$^+$. **(e)** Formation of H_2O_2 and oxidation of NADH by [(arene)Os(Impy-NMe_2)X]PF_6 (X = Cl^-, I^-; arene = *p*-cym, bip). **(f)** Mechanism of action of [(Cp^{Xbiph})Ir(phpy)py]$^+$. Compounds in (b), (e) and (f) have been studied in cells.

Human lung A549 cancer cells for example, can tolerate millimolar levels of formate, but in the presence of $[(hmb)Ru(en)Cl]^+$ no significant effect on cell growth was observed [69]. We hypothesized that, in order to catalyse the reduction of NAD^+ in cells, more active catalysts are required. Recently, water-soluble Ru^{II} Noyori-type complexes such as [(*p*-cym)Ru(TsEn)Cl] (Figure 3) were shown to reduce NAD^+ to NADH, with turnover frequencies of up to 10 times higher than their ethylenediamine analogues [78]. These Noyori-type complexes are capable of reducing the levels of NAD^+ in A2780 ovarian cancer cells when co-administered with sodium formate, potentiating the antiproliferative activity of the complexes, such that their activity is comparable with the clinical drug cisplatin [79••].

Regeneration of NAD^+ by transfer hydrogenation and oxidation of NADH has also been studied. The water-soluble iron(III) porphyrin [Fe(TSPP)Cl] (TSPP = meso-tetrakis(4-sulphonatophenyl)porphyrin) catalyses the oxidation of NADH using O_2 as an electron acceptor, at pH 7 and ambient temperature (Figure 4) [80]. Fukuzumi *et al.* reported the regeneration of NADH using $[(Cp^*)Ir(4\text{-}(1H\text{-}pyrazol\text{-}1\text{-}yl\text{-}\kappa N^2)benzoic\ acid\ \kappa C^3)H_2O)]_2SO_4$ and H_2 in neutral or slightly basic media. Interestingly, the Ir^{III} compound regenerated NAD^+ under acidic conditions (pH ≈ 3; Figure 4) [81]. The complexes $[(\eta^6\text{-arene})Ru(bpm)Cl]^+$ and $[Cp^x)Ir(phen)H_2O]^{2+}$ (bpm = bipyrimidine) can utilize NADH as a hydride source for transfer hydrogenation reactions involving important biomolecules such as quinones and pyruvate [82•].

Incubation of A2780 ovarian cancer cells with $[Cp^x)Ir(phen)H_2O]^{2+}$ increases the NAD^+/NADH ratio after 24 hours exposure [82•]. Similarly, the Ir^{III} complex $[(Cp^{Xbiph})Ir(phpy)py]^+$ (phpy = phenylpyridine), can utilize NADH as a biological hydride donor, to generate an iridium-hydride complex capable of increasing the levels of reactive oxygen species in cancer cells [83••]. Thus, offering an alternative mode of action compared to cisplatin [83••].

We have also shown that Os^{II} compounds such as $[(arene)Os(Impy\text{-}NMe_2)X]PF_6$ (X = Cl^-, I^-; arene = *p*-cym, bip; Impy = iminopyridine) can oxidize NADH via transfer hydrogenation reactions, possibly generating hydrogen peroxide (Figure 4) [84]. The NAD^+/NADH ratio in A2780 cancer cells increases upon treatment with the osmium compound as do the ROS levels. Oxidation of NADH may be involved in the mechanism of action of these complexes [84].

Currently, transfer hydrogenation reactions in cells appear to be promising as part of the mechanism of action of metal anticancer agents. The next challenge will be to introduce more selectivity into substrate recognition.

Concluding remarks

Catalysis by metal complexes is well known in chemical systems and widely used. Whether the chemical catalysis is homogeneous or heterogeneous, it is usually carried out in well-defined media so as to avoid catalyst poisoning. Control over the type of transformation and, in particular the stereochemical course of the reaction, is often achieved by the choice of metal, types and number of ligands and coordination geometry of the complex.

Our brief review illustrates that catalysis with low-molecular-weight metal complexes can successfully be achieved in biological systems. Some remarkable recent examples of metal-based catalytic drugs include the use of Cu^I/TBTA (or similar) compounds to label proteins *in vivo*, Pd^0 nanoparticles that activate anti-cancer drugs, the controlled activation of proteins by using Pd^0 complexes, Cu^{II}-ATCUN compounds which target and degrade hepatitis C and HIV RNA, and the achievement of transfer hydrogenation reactions by Ir^{III} and Ru^{II} half sandwich complexes. Altogether, this suggests that further progress can be achieved in this field, with the prospect of leading to novel drugs for clinical trials.

Of special interest to our research is the development of redox-modulating drugs. Such approach has already been shown to be successful in areas such as the clinical use of aluminium sulphonated phthalocyanine for PDT, and Mangafodipir or Calmangafodipir as SOD mimics. It is apparent that other approaches such as thiol oxidation and transfer hydrogenation reactions are also promising. However, these approaches are still all in their infancy, and will be aided by future advances in design which increase substrate selectivity and efficiency.

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