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Photoactivated Biological Activity of Transition-Metal Complexes

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Dedicated to the memory of Prof. Dr. Hans-Dieter Martin

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Photochemical activation is a very attractive way to achieve precise spatial and temporal control of the biological action of transition-metal complexes that behave as inactive "prodrugs" in the dark. A significant amount of work has been devoted to metal complexes that act on DNA. In this area, focus has been on ruthenium and rhodium polypyridyl compounds, but copper, iron, cobalt, and vanadium complexes also find increasing application as photoactivable DNA cleaving agents, with excitation sometimes even possible in the near IR region. Most often, the activity of these systems is based on the formation of reactive radical species. Another promising approach is the photochemical generation of covalent DNA binders from inactive precursors, as, for example, by some platinum(IV) compounds. The photolytic liberation of biologically active small molecules from inactive metal complex precursors has also become the target of recent research efforts and complements work on purely organic "caged" compounds. The significant progress made on light-induced liberation of neurotransmitters as well as small molecule messengers like nitric oxide (NO) or carbon monoxide (CO) is also summarized here.

1. Introduction

In the manipulation of complex biological systems with externally applied chemical agents, either for fundamental studies or therapeutic purposes, precise spatial and temporal control of their activity is of special importance. Although a significant number of compounds are known to respond to differences in the cellular environment like pH, redox status, and enzyme expression profiles, these are difficult to determine and control and can vary considerably from cell line to cell line. In contrast, the intense focused light sources available today make photoactivation a very attractive way to induce biological activity of otherwise stable precursors in a prodrug approach.

In purely organic systems, the use of photolabile protective groups has found widespread application in "caged" compounds, which are employed to study biological processes down to a very small timescale.[1–6] However, it should be noted that the bioactive compound is very rarely indeed entrapped in the interior of a larger container molecule, but rather, the term "caged" is also applied to compounds inactivated by the covalent attachment of a photolabile protective group. In another approach, the photoinduced generation of reactive singlet oxygen by sensitizers is applied in the clinic in the framework of photodynamic therapy (PDT).[7–10] In this microreview, however, we would like to focus on recent developments in the field of photoac-

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Ulrich Schatzschneider studied chemistry at the Heinrich-Heine-University in Düsseldorf and obtained his PhD at the Max-Planck-Institute for Bioinorganic Chemistry in Mülheim an der Ruhr, Germany, in 2001 under the supervision of Prof. Dr. Karl Wieghardt and Prof. Dr. Eva Rentschler. Supported by a DFG-Forschungsstipendium, he then spent the next two years as a postdoc in the group of Prof. Dr. Jacqueline K. Barton at the California Institute of Technology in Pasadena, USA. In 2004, he returned to Germany to start his independent scientific career, first at the University of Heidelberg and then, since 2006, at the Ruhr-University in Bochum (RUB), where he is currently an independent research group leader within the DFG research group FOR 630 "Biological function of organometallic compounds". His scientific interests are focused on multimodal bioimaging and therapeutic applications of transition-metal complexes and their bioconjugates. Since 2009, he is also a member of the Research Department Interfacial Systems Chemistry (RD IFSC) of the RUB and was selected as one of the 100 members of the Global Young Faculty of the Institute for Advanced Study in Essen. At present, he additionally teaches as a W2-Vertretungsprofessor at the University of Hamburg.

tivable biological activity of transition-metal complexes that go beyond singlet oxygen sensitizers, and thus, we will not cover traditional metal-based PDT agents, although some have advanced to clinical trials like the lanthanide(III) texaphyrins.[11]

With their rich photophysics and photochemistry, which can also easily be tuned by varying the metal center and/or ligands, transition-metal complexes are promising tools for the light-induced liberation of bioactive compounds from the metal coordination sphere as well as more direct action of the metal center on biosystems; these systems also increasingly find interesting applications in both the study of fundamental biological processes as well as novel approaches to photochemotherapy with a precisely controlled spatial and temporal mode of action.[12] Ideally, for such a treatment, one would like to use a prodrug that is stable and nontoxic to cells in the dark but for activation within tumor tissue, for example, shows sensitivity to light of relatively long wavelengths, because for $\lambda_{EX} > 600$ nm, tissue penetration depth is the largest.[13] Also, this minimizes photodamage to healthy cells in the path of the light beam. Finally, if a single active species is to be released from the coordination sphere of a photoactivable metal complex, the byproducts formed should not have any adverse effect on the biosystem under study. In general, two main classes of compounds can be distinguished, those which more or less directly act on DNA,[14] and complexes that liberate small molecule biological effectors inactivated by coordination to a metal center.

2. Photoactivated Modification of DNA by Metal Complexes

2.1 Ruthenium Complexes

Octahedral ruthenium(II) polypyridyl complexes are probably the prototypical transition-metal-based DNA cleaving agents and extensive work on their DNA recognition and photochemical activation has been summarized in a number of reviews.[15–17] In particular, the photoexcited state is a very strong oxidant capable of oxidizing DNA nucleobases, even through extended stretches of interjacent base pairs.[18] A number of organic ligands with an extended aromatic surface area for efficient intercalation into the DNA base stack derived from bpy have been utilized (Figure 1).[19] Especially interesting properties were reported for complexes with two tridentate ligands like pydppz, which can be viewed as a combination of tpy and dppz.[20] These were found to be very efficient singlet oxygen sensitizers with quantum yields for $^1O_2$ generation approaching unity.[21] Recently, the light-induced covalent cross-linking of p53 monomers, a protein very important in maintaining genomic stability, as well as protein–DNA cross-linking by such ruthenium complexes was demonstrated in vitro.[22]

In addition to this oxidative DNA damage, photoactivation of Ru^{II} complexes with highly π-deficient polyazaaromatic ligands like tap or hat leads to covalent adducts with functional groups on the nucleobases, most likely through a radical recombination process (Figure 2a).[23,24] In addition,

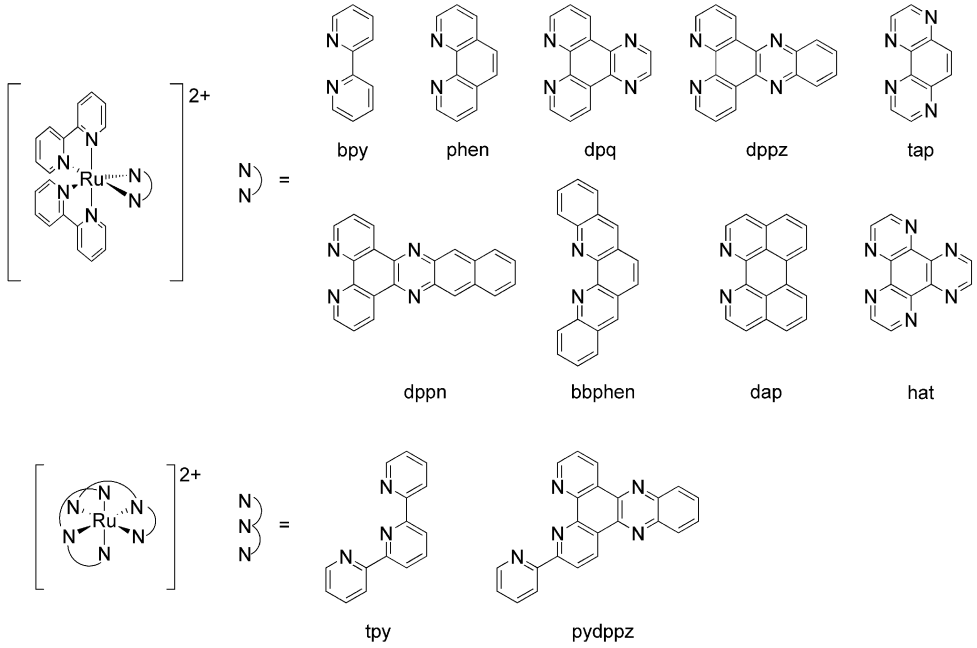


Figure 1. Bis- and trisheteroleptic ruthenium(II) polypyridyl complexes and ligands commonly used as photoactivable DNA cleaving agents. bpy = 2,2′-bipyridine; phen = 1,10-phenanthroline; dpq = dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline; dppz = dipyrido[3,2-*a*;2′,3′-*c*]phenazine; tap = 1,4,5,8-tetraazaphenanthrene; dppn = 4,5,9,16-tetraazadibenzo[*a*,*c*]naphthacene; bbphen = bisbenzo[2,3:9,8]-1,10-phenanthroline; dap = 1,12-diazaperylene; hat = 1,4,5,8,9,12-hexaazatriphenylene; tpy = 2,2′:6′,2′′-terpyridine; pydppz = 2-pyridyldipyrido-[3,2-*a*;2′,3′-*c*]phenazine.

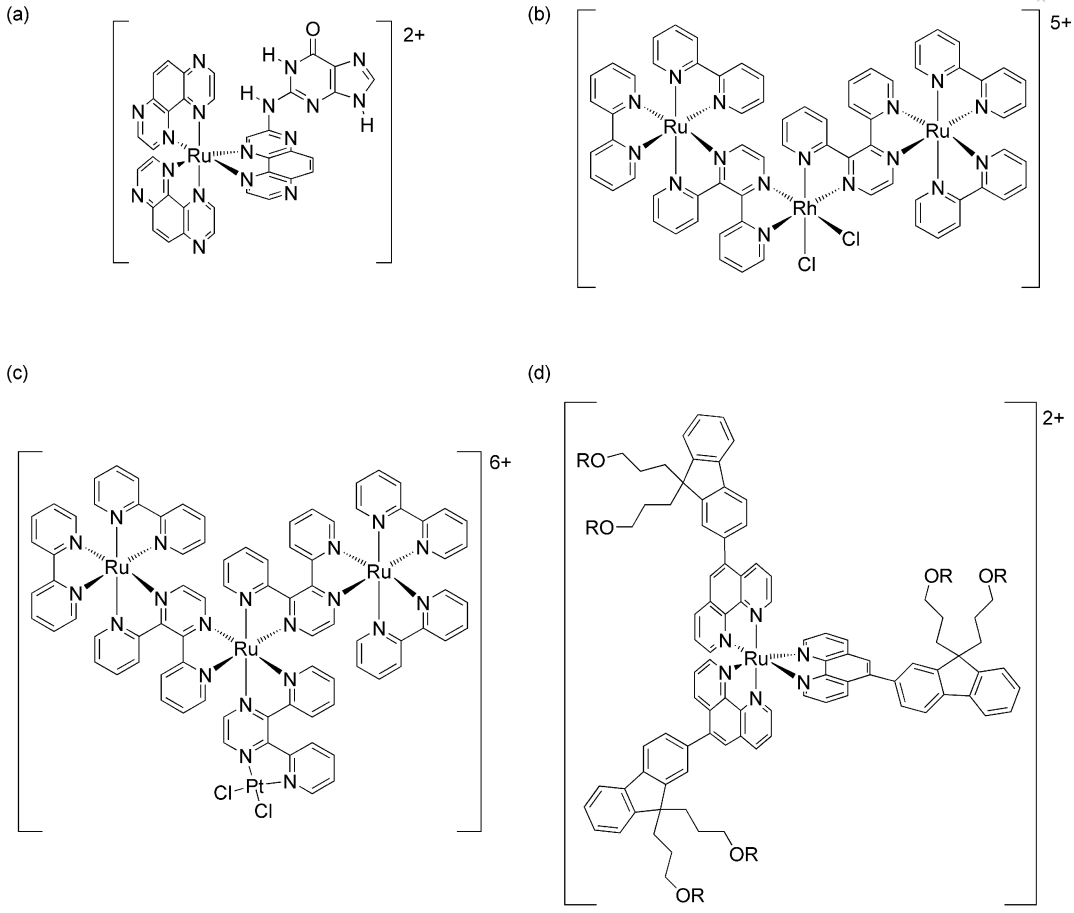


Figure 2. (a) Photoactivated Ru^{II}–tap complexes are able to form covalent adducts with nucleobases; (b) trinuclear Ru_2Rh and (c) tetranuclear Ru_3Pt complexes incorporating both singlet oxygen sensitizing and covalent DNA binding moieties; (d) fluorene-substituted Ru^{II} complex for TPA activation.

interesting polynuclear systems have been developed that are capable of both light-induced DNA cleavage through an oxygen-mediated pathway as well as covalent oligonucleotide binding by a cisplatin-related functional moiety.[25] A related compound with a $RhCl_2$ group complexed by two $Ru(bpy)_2(dpp)$ groups {dpp = 2,3-bis(2-pyridyl)pyrazine} was even tested on Vero cells in vitro and showed evidence for cell death at concentrations above 10 μM after illumination at >460 nm, whereas it was inactive when incubated in the dark (Figure 2b,c).[26,27]

Normally, excitation in metal-to-ligand charge transfer (MLCT) bands below 500 nm is used to initiate photoreactions of Ru^{II} complexes. For therapeutic applications, however, a longer excitation wavelength is highly desirable due to deeper tissue penetration in the near-IR region. Recently, a ruthenium complex with a 5-fluorene-1,10-phenanthroline-derived ligand (Figure 2d) was found to have a large two-photon absorption (TPA) cross-section at 740 nm and could be used in a TPA photodynamic therapy model. F98 glioma cells were irradiated with a pulsed laser source at this wavelength and significant cell damage could be seen in the targeted region.[28]

2.2 Rhodium Complexes

Rhodium(III) complexes with a mixed coordination sphere of polypyridyl and phenanthrene quinone diimine (phi) ligands have also found widespread application in photoinduced DNA cleavage (Figure 3). The cleavage pattern, as determined from autoradiography of ^{32}P-labeled DNA, depends on the ancillary ligands. Due to the site specificity, it is assumed that no diffusible species are involved in the cleavage process and that specific recognition of DNA is due to steric factors and shape complementarity to the local conformation at the DNA binding site.[29] Direct hydrogen abstraction from the ribose sugar after photoexcitation was suggested as the primary step leading to DNA strand cleavage.[30] An intraligand charge transfer (ILCT) state centered on the phi ligands seems to be responsible for the observed photochemistry.[31,32] Rhodium complexes of this type have been extensively used in DNA footprinting experiments. The most interesting applications, however, have resulted from the observation that complexes with the sterically more demanding chrysi ligand site-specifically bind to DNA base mismatches due to a thermo-

dynamic destabilization of the oligonucleotide duplex at these sites.[33] A number of functionalized Rh^{III} metalloinsertors have been prepared, and photolytic DNA cleavage was used to identify the insertion site of the chrysi ligand.[34–36]

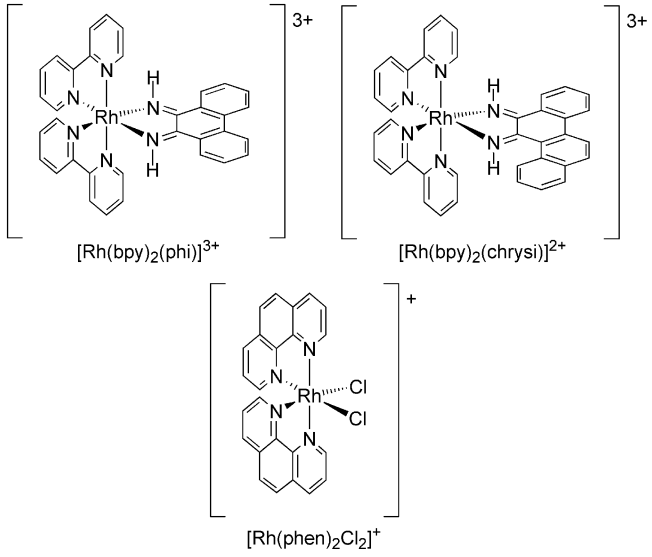


Figure 3. Rhodium(III) complexes for photoactivated DNA cleavage and footprinting. phi = 9,10-phenanthrenequinone diimine; chrysi = 5,6-chrysenequinone diimine.

An alternative mechanism of biological action is operative for the $[Rh(N–N)_2Cl_2]^+$ complexes, which are somewhat misleadingly termed "photocisplatin reagents" due to the light-induced labilization of the chloride ligands. This labilization enables formation of covalent adducts with guanine bases as also observed for cisplatin.[37,38] Dinuclear rhodium complexes with bridging carboxylate ligands also show promising DNA photocleavage and have been employed in live cell cytotoxicity studies (Figure 4). Whereas the dirhodium(II,II) tetraacetates are inactive in the dark, irradiation in the presence of electron acceptors leads to the formation of mixed-valent rhodium(II,III) complexes that efficiently cleave plasmid DNA at excitation wavelengths of up to 610 nm. This reactivity is controlled by the nature of the axial ligand, as it is only observed when it is water, whereas the pyridine- or triphenylphosphane-functionalized compounds are inactive.[39]

The introduction of a dppz ligand to one of the rhodium centers leads to significantly improved properties with a DNA binding constant of 1.8×10^5 M^{-1}.[40] In addition, photocleavage of DNA in the presence or absence of oxygen could now be achieved by using this system with visible light without the need for an additional electron acceptor. In subsequent studies, two extended N–N ligands were introduced into the dinuclear rhodium core, one on each metal center.[41] The mixed ligand complex $[Rh_2(\mu\text{-}OOCCH_3)_2(bpy)(dppz)]^{2+}$ showed an interesting differential cytotoxicity against Hs-27 human skin fibroblast cells. In the dark, it is almost inactive with an IC_{50} values of (208 ± 10) μM, whereas after irradiation, a significant cytotoxicity is observed with an IC_{50} value of (44 ± 2) μM.[42]

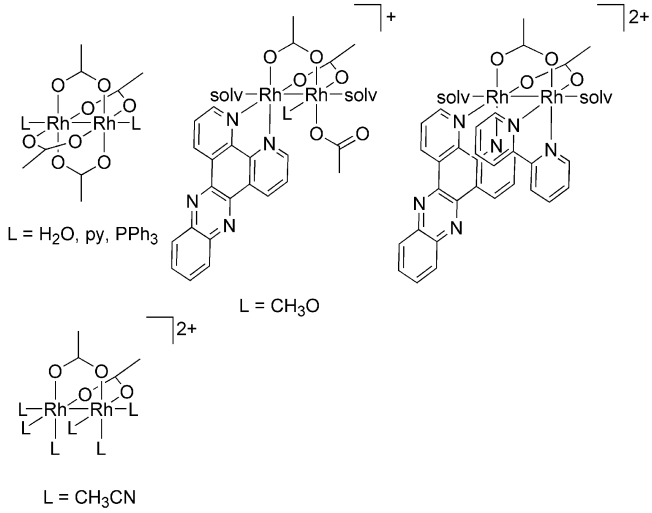


Figure 4. Dinuclear rhodium complexes for photoactivated DNA cleavage.

However, the bidentate ligand is not per se required for high activity. In the bisacetato complex $[Ru_2(\mu\text{-}OOCCH_3)_2(CH_3CN)_6]^{2+}$, for example, only the two axial acetonitrile ligands are readily exchange for water in aqueous solution. Upon photolysis at >455 nm, however, two more CH_3CN molecules are substituted, leading to $[Ru_2(\mu\text{-}OOCCH_3)_2(CH_3CN)_2(H_2O)_4]^{2+}$, which gives rise to three different isomeric species.[43] These cause reduced mobility of linear plasmid DNA in an agarose gel electrophoresis experiment, which is evidence for the formation of covalent adducts. The parent compound is essentially inactive in the dark against Hs-27 cells ($IC_{50} > 400$ μM) but the photoproduct has an IC_{50} value of (12 ± 2) μM, which is an almost 30-fold increase.

2.3 Other Metal Complexes

In addition to the ruthenium and rhodium complexes discussed above, other transition-metal complexes have also been evaluated for their DNA-modifying activity. In particular, the group of Chakravarty has studied numerous copper, iron, cobalt, vanadium, and zinc complexes (Figures 5 and 6). Copper(II) complexes combining both a bidentate phenanthroline ligand and a tridentate functionalized Schiff base were found to bind to DNA through the minor groove.[44–47] In the dark and in the absence of a reducing agent, these complexes show no DNA cleavage. However, when excited into the broad d–d band with a maximum between 640 to 680 nm, the compounds with a thiomethyl or thiophenyl group, in particular, showed efficient photoinduced DNA cleavage. This could be suppressed when the reaction was carried out under an atmosphere of argon or nitrogen protective gas. A rate enhancement resulting from the addition of dimethyl sulfoxide, and inhibition resulting from the addition of azide or histidine, point to the generation of singlet oxygen as the reactive species. In this dual-functional complex, the phenanthroline

ligand is thought to act as the DNA binder, whereas the NSO Schiff base serves as the photosensitizer. The significant cleavage activity at wavelengths near 700 nm makes these compounds interesting candidates for photodynamic therapy (PDT). In the related system $[Cu(dpq)_2(H_2O)]^{2+}$, irradiation at 694 nm from a pulsed ruby laser also led to complete cleavage of double-stranded plasmid DNA in the absence of any external additives. This process is thought to be mediated by metal d–d and dpq quinoxaline triplet states.[48] The corresponding octahedral trisdipyridoquinoxaline complexes $[M(dpq)_3]^{2+}$ with M = Fe^{II}, Co^{II}, Ni^{II}, and Zn^{II} also showed a groove-binding DNA interaction and photoinduced cleavage of double-stranded plasmid DNA, but with distinct reactivity depending on the metal center.[49] The Fe^{II} and Zn^{II} complexes only cleave DNA upon UV light activation, whereas the divalent cobalt compound can also be activated with visible light. In contrast, the Ni^{II} complex is inactive under both conditions. The mechanism of action also shows differences. The cleavage activity of the iron, copper, and zinc compounds involves generation of singlet oxygen, but the cobalt complex generates hydroxyl radicals. The ligand sphere around the copper(II) center allows substantial variation, as long as the *N*,*N*-bidentate DNA-intercalating ligand is retained, as demonstrated also by the L-lysine complexes $[Cu(L\text{-lys})(N–N)(ClO_4)]^+$, which also shows photoinduced DNA cleavage with red light.[50]

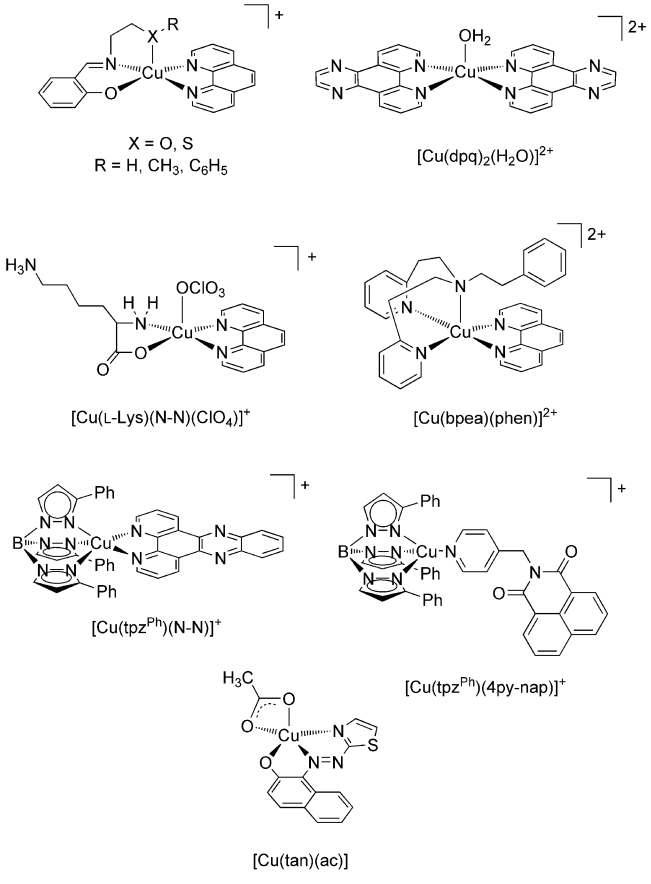


Figure 5. Examples of copper(II) complexes for light-induced oligonucleotide cleavage.

Closely related systems incorporating L-methionine (L-met),[51] L-tryptophan (L-trp), and L-phenylalanine (L-phe) instead of L-lys were also prepared and have a rather similar photonuclease activity.[52] However, with the L-trp ligand, a significant number of double-strand breaks was observed, whereas in the other compounds only single-strand breaks were observed. Other tripodal ligands derived, for example, from bis(2-pyridylethyl)amine (bpea) can also be successfully employed.[53]

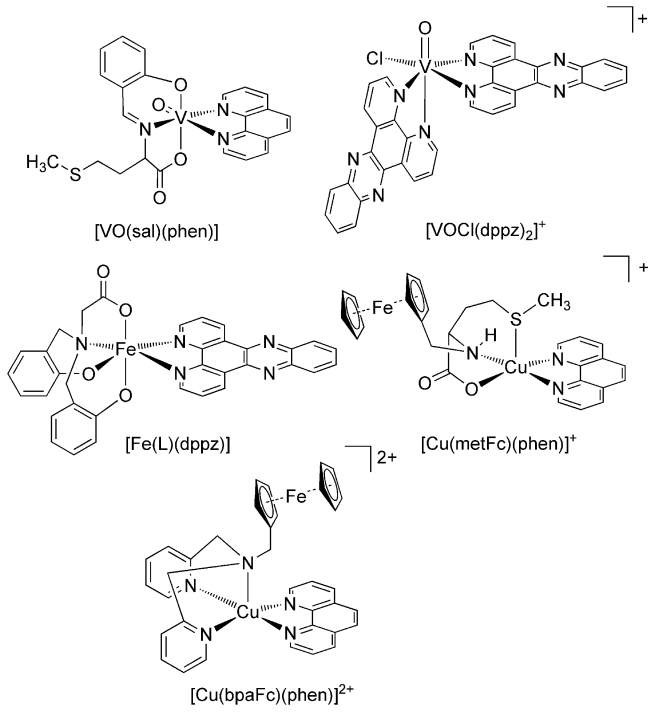


Figure 6. Vanadium, iron, and heterobimetallic iron–copper complexes for photoactivated DNA cleavage.

A significant enhancement in the photonuclease activity could be achieved by protection of the copper(II) center from solvent molecules with a bulky tris(3-phenylpyrazolyl)borate (tpz^{Ph}) ligand as in $[Cu(tpz^{Ph})(N–N)]^+$.[54] Again, singlet oxygen sensitization was shown to be the main pathway for supercoiled DNA cleavage. Work on this ligand system was recently extended to the corresponding cobalt(II), nickel(II), and zinc(II) complexes $[M(tpz^{Ph})(N–N)]^+$.[55] With N–N = dpq, the cobalt compound shows efficient DNA cleavage under visible light activation that is comparable to that of the copper complex, but the zinc compound can only be activated by UV-A light, and the nickel complex turned out to be mostly inactive. In contrast, all phen complexes did not show any DNA cleavage, most likely due to the low affinity for double-helix binding. Replacement of the bidentate N–N ligand by a 4-pyridylmethyl-1,8-naphthalimide (4py-nap) moiety as a photosensitizer led to the four-coordinate copper(II) complex $[Cu(tpz^{Ph})(4py\text{-}nap)]^+$, which was studied together with the corresponding Co^{II} and Zn^{II} compounds.[56] In vitro studies on HeLa cells, however, gave somewhat mixed results, as the copper complex was also active in the dark and the

4py-nap ligand alone also showed light-induced cytotoxic activity. Depending on the choice of ligand system, formation of reactive hydroxyl radical species is also possible, as illustrated by the copper(II) acetato complex of 1-(2-thiazolylazo)-2-naphthol (tan), which cleaves supercoiled plasmid DNA upon activation in the range from 530 to 630 nm.[57] However, the photonuclease activity is not restricted to late-transition-metal complexes. Chakravarty et al. could also show that heteroleptic oxovanadium(IV) compounds with salicylidene and N,N-heterocyclic ligands like phen, dpq, or dppz coordinated to the metal as in [VO(sal)(phen)] also bind to double-stranded DNA with a K_B value in the 10^5 M^{-1} range (Figure 6).[58] Although the chemical nuclease activity in the dark was poor, light-induced double-strand cleavage could be observed upon excitation at both 365 and 750 nm through a singlet oxygen formation pathway, whereas neither the ligands or vanadyl sulfate alone showed any activity.

The bis-dppz complex $[VOCl(dppz)_2]^+$ could even be activated with near-IR light at >750 nm. Both singlet oxygen and hydroxyl radical formation was identified in mechanistic studies with various quenchers and radical scavengers.[59] In vitro studies on human cervical cancer HeLa cells showed that the complex was inactive in the dark but after irradiation in the presence of increasing concentrations of compound, a dose-dependent decrease in cell viability with an IC_{50} value in the low micromolar range was observed. This is probably due to photoinduced DNA and protein damage by the generated reactive oxygen species (ROS). Also, high-spin iron(III) complexes incorporating both a tetradentate NO_3 ligand derived from bis(2-hydroxyphenyl)aminoacetic acid and a planar heteroaromatic N,N-ligand like dppz cleave supercoiled DNA through a photoredox process involving the formation of hydroxyl radicals, whereas the constituents alone are inactive as is the complex in the absence of light (Figure 6).[60] It is proposed that LMCT excitation leads to a charge-separated Fe^{II}-phenoxyl radical state, which first produces superoxide followed by generation of hydroxyl radicals by the reaction $3\ O_2^{\cdot-} + 2\ H^+ \rightarrow OH^{\cdot} + OH^- + 2\ O_2$. Cytotoxicity studies on HeLa and HaCaT cell lines demonstrated that the dppz complex was inactive in the dark in both cases up to 100 μM. In contrast, visible light irradiation in the 400 to 700 nm range led to a concentration-dependent decrease in cell viability with IC_{50} values in the low micromolar range. Further experiments revealed the onset of apoptotic processes with caspase 3/7 activation after irradiation in the presence of the metal complex. This is most likely due to extensive generation of ROS leading to direct DNA damage.[61] Very recently, an interesting bimetallic ferrocene–copper(II) compound with a metFc ligand has been reported, which shows two quasireversible redox waves due to the $Cu^{II/I}$ and Fc^+/Fc couples. Over 90% cleavage efficiency for double-stranded plasmid DNA could be achieved upon irradiation at 633 nm for the dppz complex.[62] A related series of Cu^{II} compounds in which the ferrocene (Fc) moiety was attached to a bis(2-pyridylmethyl)amine (bpa) ligand through a methylene group was also investigated and led to photoinduced generation of hydroxyl radicals, which gave rise to both DNA cleavage as well as protein degradation, as demonstrated in irradiation experiments in the presence of bovine serum albumin (BSA).[63]

2.4 Organometallic Complexes

In contrast to the coordination compounds described in the previous section, only a very limited number of purely organometallic photonucleases have been reported so far. Work in the group of Mohler has focused on alkyl–metal complexes like the half-sandwich compounds shown in Figure 7. Upon irradiation, these air- and water-stable compounds produce alkyl radicals, which induce the formation of single-strand breaks in plasmid DNA. The involvement of alkyl- instead of oxygen-centered radicals, as often encountered in DNA cleavage by coordination compounds, was demonstrated by inhibition of the process by addition of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), which only captures carbon- but not oxygen-centered radicals.[64] More detailed studies of the reaction mechanism showed that strand scission was initiated by hydrogen atom abstraction from the 4′- and 5′-positions of the deoxyribose moiety of the backbone of DNA. In addition, methylation of all four nucleobases was found to occur.[65] To increase the DNA affinity and induce formation of more lethal double-strand breaks, the $CpW(CO)_3CH_3$ building block was attached to polyamine dendrimers related to spermine (Figure 7a). Although the di- and tetrametallic systems led to photoinduced double-strand scission as expected, the use of more complex systems was hampered by competing DNA aggregation and precipitation.[66,67] To achieve sequence-selective recognition and cleavage of DNA, the polyamine derivatives were replaced by analogues of the minor groove binders netropsin and distamycin (Figure 7b). In a first series of compounds, these were attached to the Cp ring through an amide bond. Because these systems generate diffusible methyl radicals upon irradiation, only nonspecific DNA strand scission was observed. Thus, in an alternative approach, a phenyltungsten complex was prepared to which the minor groove binder was attached through a carboxylate in the *para* position to the carbon–tungsten bond. In this case, the phenyl radical remains connected to the DNA recognition element after photolysis, and therefore, specific cleavage at T residues within the binding site was achieved.[68] A comparison of the group VI compounds $CpM(CO)_3R$ (R = Cr, Mo, W) with $CpFe(CO)_2R$ (R = methyl or phenyl) showed that only the tungsten and iron complexes gave DNA cleavage upon irradiation (Figure 7c,d). The Fe complexes were more active than the W compounds and also gave double-strand cleavage. In addition, an effect of the R group was noticed: the phenyl compounds produced more damage than the methyl systems.[69] Subsequent work thus focused on the phenyliron complexes and a number of homodinuclear systems related to enediyne anticancer antibiotics was prepared and studied.[70] Photoactivation of $CpFe(CO)_2C_6H_5$ could even

be achieved by ambient light in the presence of hydrogen peroxide but also led to the formation of reactive oxygen species in addition to carbon-centered radicals.[71,72] In addition to the cyclopentadienyl half-sandwich complexes described above, dual photoinduced backbone cleavage and a base modification effect on DNA by the molybdenum allyl complex [Mo(η^3-allyl)(phen)(CO)$_2$CH$_3$] was described (Figure 7e). Whereas the first process leads to nonspecific cleavage through abstraction of H1′ and/or H5′ from the deoxyribose moiety, the second results in G-specific cleavage through the formation of the base-labile residues methylguanine, methoxyguanine, and 8-oxoguanine.[73] Methylcobalt(III) complexes with a cyclam ligand in the equatorial plane have also been evaluated for light-activated liberation of methyl radicals with concurrent reduction of the metal to cobalt(II).[74]

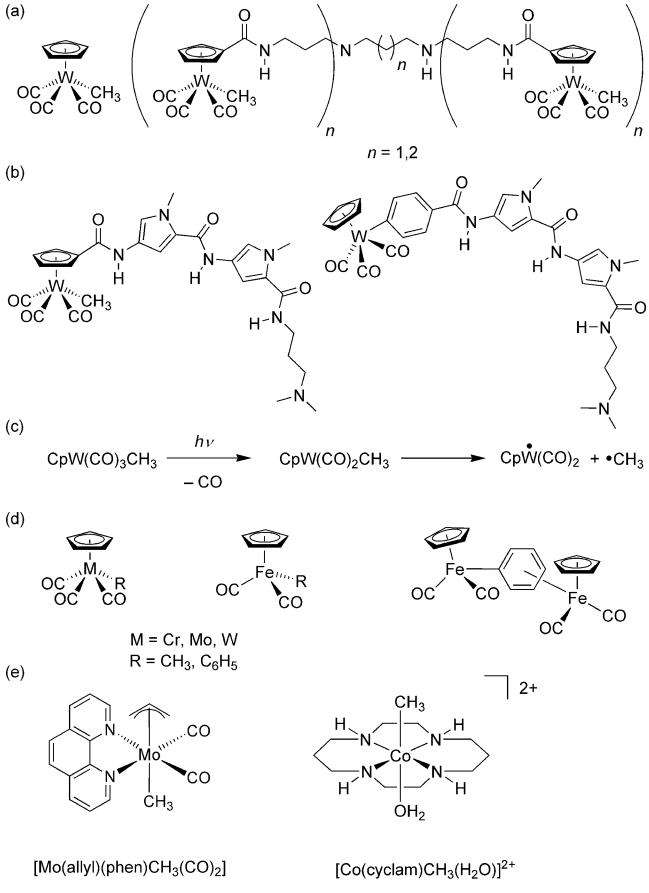


Figure 7. Photoactivated homolytic cleavage of the alkyl–metal bond in the organometallic compounds leads to generation of alkyl radicals that induce single-strand cleavage in plasmid DNA. (a) CpW(CO)$_3$CH$_3$ parent compound and polyamine conjugates; (b) CpW(CO)$_3$ fragment functionalized with DNA minor groove binder netropsin; (c) mechanism of photoactivation; (d) comparison of Cr, W, W, and Fe organometallic photonucleases; and (e) Mo^{II} and Co^{III} methyl complexes for light-induced DNA modification.

2.5 Photoactivable Platinum Prodrugs

In contrast to the complexes described in the previous section, which usually generate diffusible reactive species able to modify DNA, photoactivation of transition-metal compounds for covalent binding to bio(macro)molecules requires a different strategy. An interesting approach is to use inert metal complexes that, after photoexcitation, become susceptible to ligand exchange and redox state changes. This concept has been applied by the groups of Sadler and Bednarksi for the generation of square-planar platinum(II) complexes related to the well-established clinical anticancer agent cisplatin {*cis*-[PtCl$_2$(NH$_3$)$_2$]}[75–78] from inert octahedral platinum(IV) compounds bearing photolabile ligands.[79,80] It was found that *trans*,*cis*-[Pt(OAc)$_2$I$_2$(en)] (Figure 8) shows no covalent binding to nucleotides when incubated in the dark, whereas irradiation at >375 nm resulted in the covalent attachment of the platinum(II) species to GMP (guanosine monophosphate).[81] Cytotoxicity studies on TCCSUP human bladder cancer cells showed a small but statistically significant reduction in the IC$_{50}$ values and thus establish this complex as a light-sensitive prodrug.[82] The axial and equatorial anionic ligands allow some modulation of the general lead structure, and for *trans*,*cis*-[Pt(N$_3$)$_2$(OH)$_2$(en)], it was found that their rate of photolysis closely parallels that of DNA platination, indicating that the photolysis products interact directly and rapidly with DNA. The response of the cell to photoactivation, however, was different to that induced by cisplatin, and although the formation of platinum(II) species is thought to follow photoactivation, this indicates a mechanism of action different from that of cisplatin.[83] Unlike other agents for photodynamic therapy, these platinum diazide complexes do not rely on the presence of oxygen, which is a potential advantage due to the anoxic environment that is often found in tumor tissue.

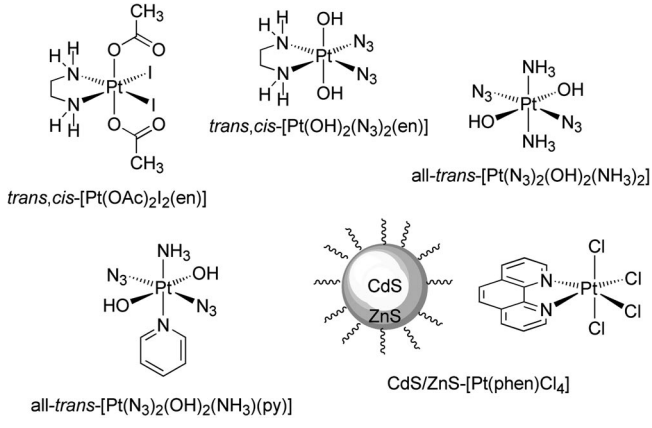


Figure 8. Photoactivable cytotoxic platinum(IV) prodrugs.

Although transplatin {*trans*-[PtCl$_2$(NH$_3$)$_2$]} shows a much lower biological activity than cisplatin in the dark (although it can be photoactivated, too),[84] cytotoxicity studies on *trans*,*trans*,*trans*-[Pt(N$_3$)$_2$(OH)$_2$(NH$_3$)$_2$] revealed that although the compound is nontoxic to keratinocytes in the dark, it becomes as active as cisplatin after irradiation. Thus, unlike the Pt^{II} complexes where only the *cis* compounds show high activity, both *trans*- and *cis*-diamine

platinum(IV) diazide complexes have potential as photochemotherapeutic agents.[85] The exchange of one amine ligand to pyridine improved the biological properties even further. The complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(NH_3)(py)]$ was up to 80 times more cytotoxic than cisplatin and also showed significant activity against a cisplatin-resistant human ovarian cancer cell line. Instead of the intrastrand 1,2-GG cross-links usually found for Pt^{II} complexes, the photolysis products of this compound led to 1,3-GXG cross-links on the same oligonucleotide strand. Also, the activation profile of a number of cell death-signaling pathways was different from that induced by cisplatin.[86] Further variation of the ligand framework in these Pt^{IV} complexes and detailed studies of their photophysics and photochemistry will likely bring up more interesting octahedral photoactivable platinum(IV) prodrugs.[87] A very interesting supramolecular system for the release of bioactive platinum(II) complexes from Pt^{IV} precursors with visible light was recently reported by Marque-Rivas et al.[88] They combined hydrophobic colloidal core-shell CdSe-ZnS quantum dots (QDs) with $[PtCl_4(bpy)]$ and showed that the system is stable in the dark. However, upon irradiation at 390 nm, formation of $[PtCl_2(bpy)]$ could by demonstrated by ^1H NMR spectroscopy. About 2000 to 2200 molecules of the Pt^{II} complex could be generated per QD.

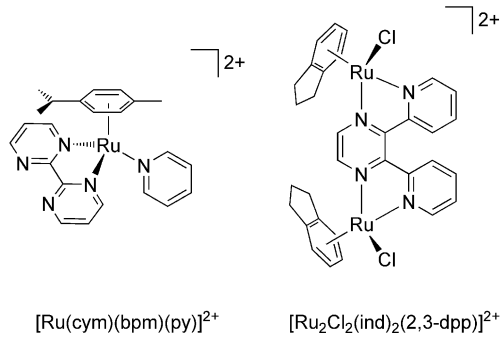


Figure 9. Organometallic Ru^{II} arene complexes with light-induced activity against cancer cells.

2.6 Photoactivation of Ruthenium Anticancer Agents

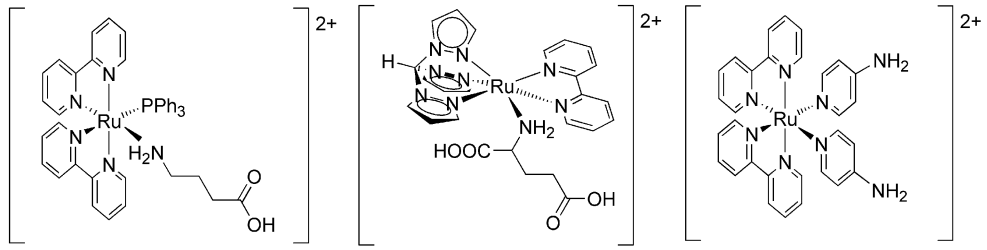
Established arene–ruthenium anticancer agents have also served as starting structures for the development of interesting photoactivable cytotoxic metal complexes.[89–92] In complexes of the general formula $[Ru(\eta^6\text{-arene})(L–L)(X)]^{n+}$, the central activation step is the aquation of the Ru–X bond to form more reactive species. Careful tuning of the hydrolysis rate was found to be crucial to achieve high in vitro cytotoxicity. An attractive approach would be the preparation of complexes with a Ru–X bond stable in solution in the dark but with a photolabile ligand X, giving rise to the active aquo species upon irradiation. Using 2,2′-bipyrimidine (bpm) as L–L and X = pyridine, Sadler et al. obtained a complex stable in the dark in aqueous solution over a wide pH range for up to two months (Figure 9). Upon photolysis at 400 to 600 nm, however, characteristic changes in the UV/Vis and NMR spectra could be observed. The photoproduct was found to bind to 9-ethylguanine (9-EtG) and will possibly open the way to compounds with phototriggered direct covalent binding to DNA.[93]

For a detailed understanding of the mode of biological action of anticancer agents, the study of cellular uptake and intracellular distribution is of pivotal importance. An interesting compound that combines photoinducible cytotoxic activity with a "turn-on" fluorescence marker is the binuclear ruthenium(II) indane complex with a bridging 2,3-bis(2-pyridyl)pyrazine (2,3-dpp) ligand shown in Figure 9.[94] Initial hydrolysis gives rise to substitution of the two chloride ligands by water, but otherwise the compound is stable in the dark in aqueous solution. Irradiation at 360 nm, however, leads to pronounced changes in the absorption spectra, and interestingly, an increasing fluorescence signal from the compound was observed, whereas it is nonemissive in the dark. Because the fluorescence spectrum of the irradiated product is similar to that of indan, photolytic liberation of the arene ligand occurs, as also demonstrated by NMR spectroscopy. The photoproduct efficiently formed DNA interstrand cross-links, whereas the nonirradiated complex was less active.

3. Photochemical Release of Bioactive Small Molecules from Metal Complexes

3.1 "Caging" of Neuroactive Amines in the Coordination Sphere of Metal Complexes

In addition to the photoreactive ruthenium complexes described in the previous section, in which the metal center is directly involved in the biological mechanism of action, coordination compounds have also been used to inactivate organic bioactive molecules through binding in the coordination sphere of the metal. In analogy to the purely organic "caged" compounds mentioned in the introduction, in this approach, the metal complex fragment acts as a photolabile protective group. An important field of research in which such "caged" metal compounds are applied is neuroscience. The rapid and localized release of neuroactive molecules from inactive precursors is used to study receptor distribution, ion channel kinetics, and other processes. Because many organic "caging" groups can only be photolyzed off at wavelengths below 400 nm, it appears quite promising to evaluate metal complexes in such a context. The $[Ru(bpy)_2(PPh_3)(GABA)]^{2+}$ complex shown in Figure 10 was found to release the neurotransmitter γ-aminobutyric acid (GABA) with a quantum yield of 0.2 upon excitation at 450 nm. This complex was able to induce membrane ionic current changes in frog oocytes presenting the $GABA_c$ receptor after photostimulation in much the same way as the addition of free GABA.[95] This concept could also be extended to additional bioactive amines like tryptamine, serotonin, tyramine, and butylamine.[96]



$[Ru(bpy)_2(PPh_3)(GABA)]^{2+}$ $[Ru(tpm)(bpy)(GlutH_2)]^{2+}$ $[Ru(bpy)_2(4\text{-}AP)_2]^{2+}$

Figure 10. Ruthenium polypyridyl "cage" compounds for photorelease of neuroactive amines. GABA = γ-aminobutyric acid; $GlutH_2$ = glutamate; 4-AP = 4-aminopyridine.

Other bioactive amino acids require a different metal complex design, as α-amino acids are able to strongly bind to a metal center in a bidentate way. Thus, to prepare a "caged" complex of glutamate ($GlutH_2$), which is the major excitatory neurotransmitter in the central nervous system of mammals and one of the most important biomolecules to be caged, a [3+2] approach was used in which five coordination sites on a ruthenium(II) center were occupied by a tridentate tris(pyrazolyl)methane (tpm) and a bidentate 2,2′-bipyridine ligand, thus leaving only one binding site for coordination of $GlutH_2$ through the amino group. From $[Ru(tpm)(bpy)(GlutH_2)]^{2+}$, photochemical release of glutamate was possible upon excitation at 450 nm.[97] A very important factor in such systems is the speed of release of the bioactive compound from the metal coordination sphere, as it sets the timeframe for the processes to be studied or controlled. Very recently, Etchenique et al. made significant progress when they were able to report a ruthenium–polypyridyl complex that releases glutamate in less than 50 ns after excitation at 532 nm. In addition, this system could also be activated by two-photon excitation at 800 nm.[98] Another interesting organic neurochemical inactivated by coordination to a ruthenium(II) center is 4-aminopyridine (4-AP), which acts as a potassium channel blocker. When the dark-stable complex $[Ru(bpy)_2(4\text{-}AP)_2]^{2+}$ was applied to a preparation of a central ganglion of the medicinal leech *Hirudo medicinalis*, the single-cell transmembrane potential showed characteristic action potentials (peaks) in response to light flashes from a Xe flashlamp. This stimulation of neuronal response only occurred with the 4-AP complex, whereas the related $[Ru(bpy)_3]^{2+}$ complex showed no activity.[99] Still, the 473 nm excitation wavelength used is not optimal for many biological studies. However, it was found that the use of two-photon excitation from a high-power laser source at 730 nm allows the uncaging of 4-AP from this ruthenium polypyridyl complex under physiological conditions.[100]

3.2 Photoactivated NO Release from Metal Complexes

Probably the smallest bioactive molecules to form photoactivable prodrugs by incorporation in the coordination sphere of a metal are the diatomic gases carbon monoxide and nitric oxide. Both are also endogenously produced in organisms including humans and have important functions in cytoprotection and as small-molecule messengers. Since the identification of NO as the endothelium-derived relaxing factor (EDRF),[101] a lot of work has focused on its intracellular production and biological mode of action.[102–104] The generation of nitric oxide in higher organisms is a two-step process starting from the amino acid L-arginine. Both steps are catalyzed by the heme enzyme NO synthase (NOS), which leads to the formation of L-citrulline in addition to NO. Three different isoforms of NO synthase are expressed depending on the tissue. Neuronal NOS (nNOS) is found in neuronal cells and skeletal muscles, whereas the endothelial NOS (eNOS) is mostly localized in endothelial and epithelilal cells, but also some neurons. Finally, the inducible NOS (iNOS) is active in many cells like macrophages, hepatocytes, astrocytes, and smooth muscle cells. To investigate the physiological role of NO and for therapeutic applications, several classes of NO-releasing drugs have been introduced over the years. Classical nitrovasodilators are based on organic nitrate and nitrite esters and require enzymatic metabolization to generate bioactive NO.[105] However, nitrosyl-metal complexes have also been employed as a source of NO for a long time.[106] For example, sodium nitroprusside (SNP, Figure 11) is in clinical use for over 70 years now to reduce blood pressure in hypertensive emergencies.[107,108] The NO release from SNP can either be induced photochemically or by one-electron reduction. Because this simple compound lacks options for further functionalization and tuning of the NO release profile, more elaborate compounds have been developed in recent years. The group of Mascharak, for example, has pioneered the use of ruthenium nitrosyl complexes with functionalized pyridine carboxamide ligands.[109,110] It was found that upon exposure to low-intensity UV light, $[Ru(PaPy_3)(NO)]^{2+}$ rapidly looses NO on a second-timescale, which can be detected through binding to heme proteins like myoglobin (Mb) and cytochrome *c* oxidase (cyt *c*).[111–113]

A related Ru–bpb complex with an axial 4-vinylpyridine (4-vpy) coligand could be covalently attached to a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel during radical polymerization with ethylene glycol dimethacrylate (EGDMA). Because only NO can diffuse out of the macroporous hydrogel, it is very reasonable to expect that such

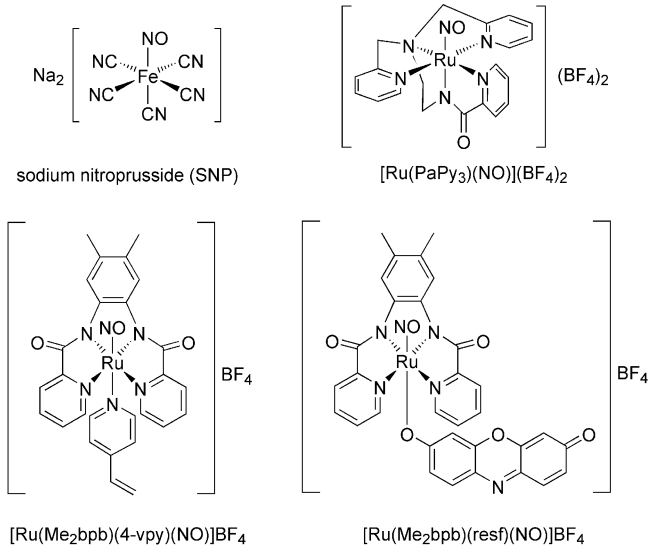


Figure 11. Photoactivable metal–nitrosyl complexes as a source for nitric oxide in biological systems.

material could be useful for the photodelivery of NO to biological targets.[114] Other polymeric hybrid materials incorporating metal-based NO donors were also investigated.[115] A common problem with light-activable metal complexes is the need to use low-wavelength UV radiation to initiate a physiological response. This reduces the tissue penetration depth and might cause photodamage of integral cell constituents. It is therefore highly desirable to prepare nitrosyl complexes from which NO can be released with visible-light irradiation. Thus, Ru–bpb complexes tethered to organic dye sensitizers like resorufin were investigated for their ability to promote Ru–NO bond cleavage upon energy transfer from the visible-light-absorbing pendant chromophore. Some of the compounds were found to rapidly release nitric oxide upon exposure to visible light ($\lambda_{EX} > 455$ nm), and because they are fluorescent, they could be tracked inside MDA-MB-231 human mammary cancer cells. NO release from these compounds then triggered apoptosis in tumor cells.[116,117] The manganese analogue of the Ru–$PaPy_3$ complex was even found to release NO upon near-IR excitation when one of the pyridyl groups was replaced by a 2-quinoline moiety (λ_{EX} = 810 nm).[118] Alternatively, "heavy-atom" substitution of the central oxygen atom in resorufin to sulfur (thionol) or selenium (selenophore) was employed to obtain Ru–bpb complexes that show a systematic redshift in the absorption spectrum from λ_{max} = 500 nm for O to λ_{max} = 535 nm for Se.[119] Recently, this work was extended to modulate enzyme activity with a photolabile {Mn-NO}^{6} complex. Nitric oxide photochemically released from $[Mn(PaPy_3)(NO)]^+$ was found to inhibit the cysteine protease papain by *S*-nitrosylation at Cys25 in the active site. In addition, the enzyme–NO adduct generated could be identified by ESI mass spectrometry.[120] Also, activation of soluble guanylate cyclase (sGC), the major cellular target of nitric oxide, was demonstrated for NO released photochemically from Ru and Mn $PaPy_3$ complexes, and its vasorelaxant action on cultured smooth muscle cells was studied in tissue bath experiments.[121,122] Replacing the carboxamide group of $PaPy_3$ by an imino moiety, {Mn-NO}^{6} complexes could be prepared that even release nitric oxide exposure to near-IR light at up to 950 nm.[123]

In addition to the ruthenium and manganese nitrosyl complexes with functionalized pyridine carboxamide ligands described above, other preorganized ligand systems with a mixed N,O or N,S donor set have also been employed as photolabile NO donors (Figure 12). For example, irradiation of $[Ru(py^{Si}S_4)(NO)]Br$ at $\lambda_{EX} > 455$ nm leads to liberation of nitric oxide, while the bromide counterion binds to the vacant coordination site generated on the metal

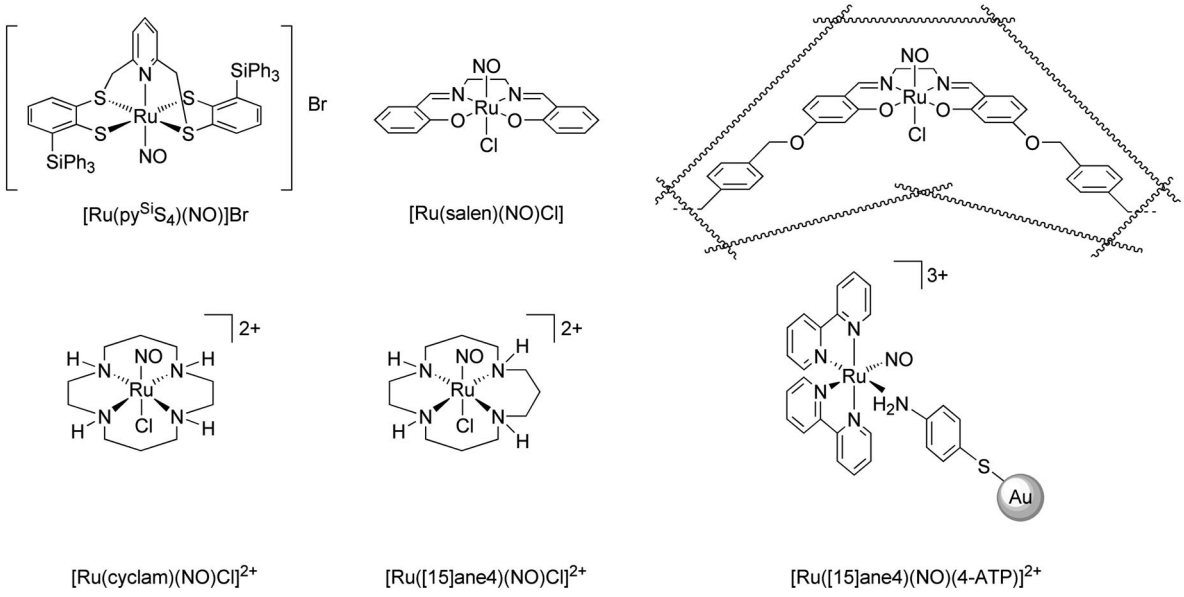


Figure 12. Ruthenium–NO complexes and supramolecular NO releasing systems.

center.[124] Also, ruthenium–salen–NO complexes were studied as analogues to ruthenium–nitrosyl porphyrins.[125] An interesting extension of this work is the copolymerization of styryloxy-functionalized Ru(salen)(NO) complexes with ethylene glycol dimethacrylate (EGDMA) to form a porous material that retains its photoactivated NO delivery properties.[126] Instead of incorporation in a polymer matrix, ruthenium nitrosyl complexes have also been attached to the surface of gold nanoparticles. NO could be liberated from these nanometer-sized structures upon irradiation with visible light at 430 nm.[127] Ruthenium nitrosyl complexes with macrocyclic ligands like cyclam ([14]ane4) were also studied for their photoinduced NO release. It was found that the efficiency of nitrosyl liberation depended on the size of the macrocyclic ring, as the complex $[RuCl(cyclam)(NO)]^{2+}$ showed a much lower quantum yield than that of the corresponding [15]ane4 compound, which could also induce relaxation of aortic rings, whereas the former was inactive.[128] Another class of metal complexes with photolabile NO groups but different general structure are the iron–sulfur nitrosyl clusters of the Roussin's salt type (Figure 13). A particularly attractive feature of these compounds is their strong absorption in the visible region, which, for the Roussin's black salt (RBS), appears at up to 580 nm.[129] The related "red salt esters" (RSE) are obtained by alkylation of the μ-sulfido groups in Roussin's red salts (RRS), which allows tuning of properties like solubility, reactivity, photochemical behavior, and biological specificity by variation of the substituents.[130–133]

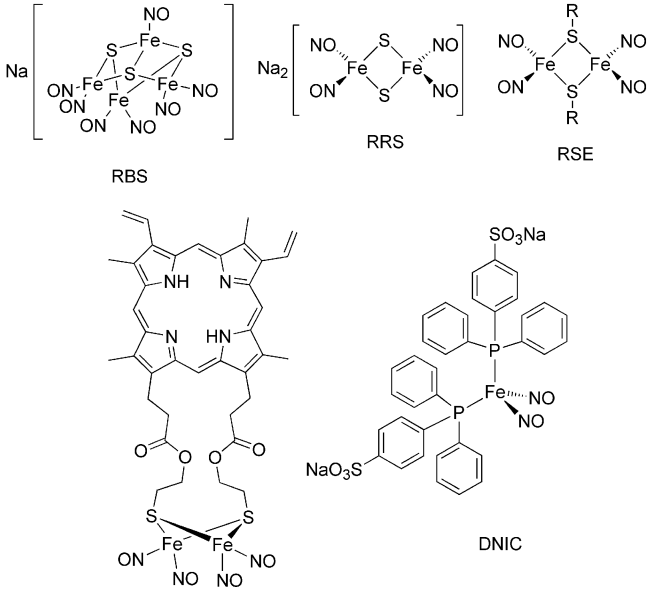


Figure 13. Iron–sulfur nitrosyl clusters and dinitrosyl iron complexes (DNIC) used as photoactivable NO sources. RBS = Roussin's black salt; RRS = Roussin's red salt; RSE = Roussin's salt ester.

In order to allow for precise spatial control of the cellular activity of NO, interesting RSE derivatives in which chromophores with high one- and two-photon absorption cross-sections are attached to the bridging sulfido groups have been prepared. Two-photon excitation (TPE) at near-IR wavelengths is particularly appealing, because tissue penetration is high at such low energies and absorption by biomolecules is negligible.[134–137] Recently, some RSEs and related dinitrosyl–iron complexes (DNICs) have also been evaluated in the context of NO-induced protein regulation and cellular uptake properties on immortalized vascular endothelial cells. These compounds had low cytotoxicity at submillimolar concentrations and were able to induce protein *S*-nitrosylation in vitro.[138]

3.3 Light-Inducible CO Releasing Molecules (CORMs)

In contrast to the large amount of information on the biological activity of nitric oxide, the physiological role of carbon monoxide is somewhat less understood at the molecular level. About 80% of the CO endogenously produced in higher organisms results from the enzymatic degradation of heme by the isoenzymes of the heme oxygenase (HO) family.[139] In this interesting reaction, the heme group acts as the cofactor in its own regioselective conversion to α-*meso*-hydroxyheme by an iron–hydroperoxido species. In two subsequent steps, the tetrapyrrole ring system is cleaved to biliverdin with concurrent liberation of CO and iron(II) under further consumption of dioxygen and NADPH reducing equivalents.[140–143] The carbon monoxide released induces a number of physiological responses including vasodilatory effects and shows beneficial cytoprotective activity.[144–148] Because carbon monoxide is a colorless and odorless gas that is highly toxic when overdosed, it has found only limited application in both basic research and clinical studies. Therefore, there is a steadily increasing interest in metal–carbonyl complexes as solid storage forms for carbon monoxide.[149–153] Most of these CO releasing molecules (CORMs), however, only liberate carbon monoxide upon hydrolysis in aqueous buffer on a rather short timescale (Figure 14).[154–160] The half-life of these compounds sets the limit for their in vivo application, as target structures in the body have to be reached before a significant amount of CORM has decayed.

Photoinducible CO releasing molecules, on the other hand, will allow selective enrichment of a dark-stable CORM prodrug at the biological target site from which carbon monoxide is only released upon irradiation. This will allow precise spatial and temporal control of the biological action of CO. Initially, commercially available metal–carbonyl complexes like iron pentacarbonyl or dimanganese decacarbonyl (CORM-1) were studied that require activation by light.[161] Because the bioavailability of these nonpolar organometallic compounds is very poor and they lack options for tuning of physical and chemical properties, interest has mostly shifted to ruthenium-, iron-, and molybdenum-based CORMs with hydrolytic activation. On the basis of our interest in the evaluation of novel photoactivable CORMs with potential chemotherapeutic applications against cancer, we recently started to evaluate a number of metal–carbonyl complexes that will allow easy tuning of

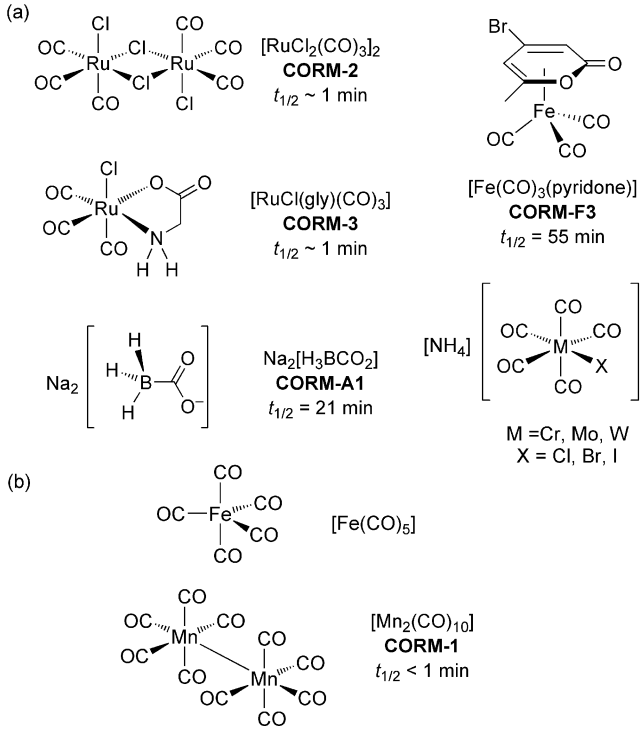


Figure 14. CO releasing molecules activated (a) by hydrolysis in aqueous buffer and (b) by irradiation.

photophysical and photochemical properties as well as functionalization with bio(macro)molecules for targeted delivery to cancer cells.

Initial work in our group focused on functionalized cymantrenes {Cym, $CpMn(CO)_3$} and their bioconjugates with cell-penetrating peptides (CPPs), as the Cym moiety is well known to photolytically liberate at least one,[162–168] and under certain conditions all three, carbonyl ligands.[169,170] Functionalized cymantrenes are easily accessible by Friedel–Crafts acylation, and the $CpMn(CO)_3$ group was found to be fully compatible with microwave-assisted solid-phase peptide synthesis (SPPS), even withstanding the final cleavage step of the modified peptide from the resin with concentrated trifluoroacetic acid (TFA).[171,172] Although such bioconjugates (Figure 15) showed promising cytotoxic activity against HT-29 and MCF-7 human cancer cells when conjugated to complex carrier peptides in the dark,[173,174] photolytic CO release from the cymantrene moiety was very slow under irradiation conditions compatible with biosystems, with a half-life of several hours in our setup. Because significant general photodamage to cells is to be expected under such conditions, these compounds do not offer much promise in this context. Thus, our interest shifted to the closely related $[Mn(tpm)(CO)_3]^+$ complexes in which the manganese(I) tricarbonyl unit is coordinated by a tridentate facial tpm {tris(pyrazolylmethane)} coligand (Figure 16). This class of compounds is an attractive target, as the other group VII metals should show similar chemical behavior, opening the way to radioactive technetium(I)[175] and possibly fluorescent rhenium(I) complexes.[176,177] In addition, the tpm ligand is easily accessible and offers significant flexibility for modification at both the pyrazole rings and the central methine carbon atom.[178–180] In a myoglobin(Mb)-based assay, which spectrophotometrically detects binding of CO to reduced myoglobin on the basis of changes in the Q-band region upon formation of $MbFe^{II}CO$ from $MbFe^{II}$, it was found that the parent compound $[Mn(tpm)(CO)_3]^+$ releases two equivalents of carbon monoxide upon irradiation at 365 nm and the reaction is complete after about 20 min under our conditions. TDDFT calculations showed that the primary photophysical process is a MLCT transition from manganese t_{2g}-type orbitals to unoccupied orbitals with mixed metal–CO character. Due to promising CO release properties, the bioavailability of $[Mn(tpm)(CO)_3]^+$ was studied with an AAS-based cell uptake assay. Incubation of HT-29 human colon cancer cells with the manganese tricarbonyl complex showed significant intracellular accumulation as manifested in a concentration-dependent increase in the intracellular Mn content.[181] As no saturation was observed, a passive diffusion process is assumed to take place. Apparently, the monocationic complex offers a good balance between lipophilicity and hydrophilicity to cross the cell membrane. Very recently, we were able to use confocal Raman microscopy at submicrometer resolution to study the intracellular distribution of $[Mn(tpm)(CO)_3]^+$ in HT-29 cells. Using the intrinsic vibrational signature of the $Mn(CO)_3$ moiety, we could show that the compound specifically accumulates in the nuclear membrane and the nucleolus.[182] Therefore, the effect of this complex on the viability of HT-29 human colon cancer cells with and without irradiation was studied with the crystal violet assay. $[Mn(tpm)(CO)_3]^+$ had no adverse effect on the cells at 100 μM after incubation for up to 48 h in the dark. In contrast, when the cell culture was irradiated in the middle of the 48 h incubation period for 10 min after washing away excess noninternalized metal complex, a significant reduction in the cell biomass to about 25% of the negative control was observed. This significant light-induced cytotoxic effect is comparable to that observed for 5-fluorouracil (5-FU) employed as a positive control and establishes

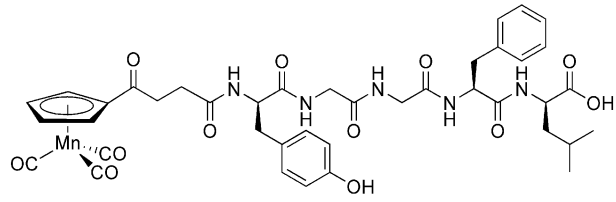


Figure 15. Cymantrene-[Leu^5]enkephalin peptide bioconjugate.

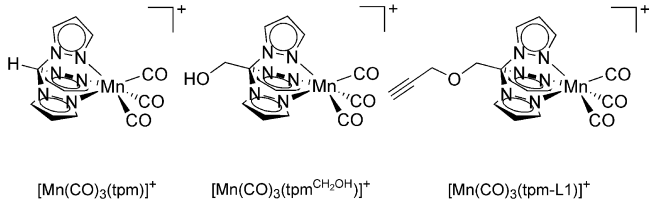


Figure 16. Photoactivable CORMs derived from $[Mn(tpm)(CO)_3]^+$. tpm = tris(pyrazolyl)methane.

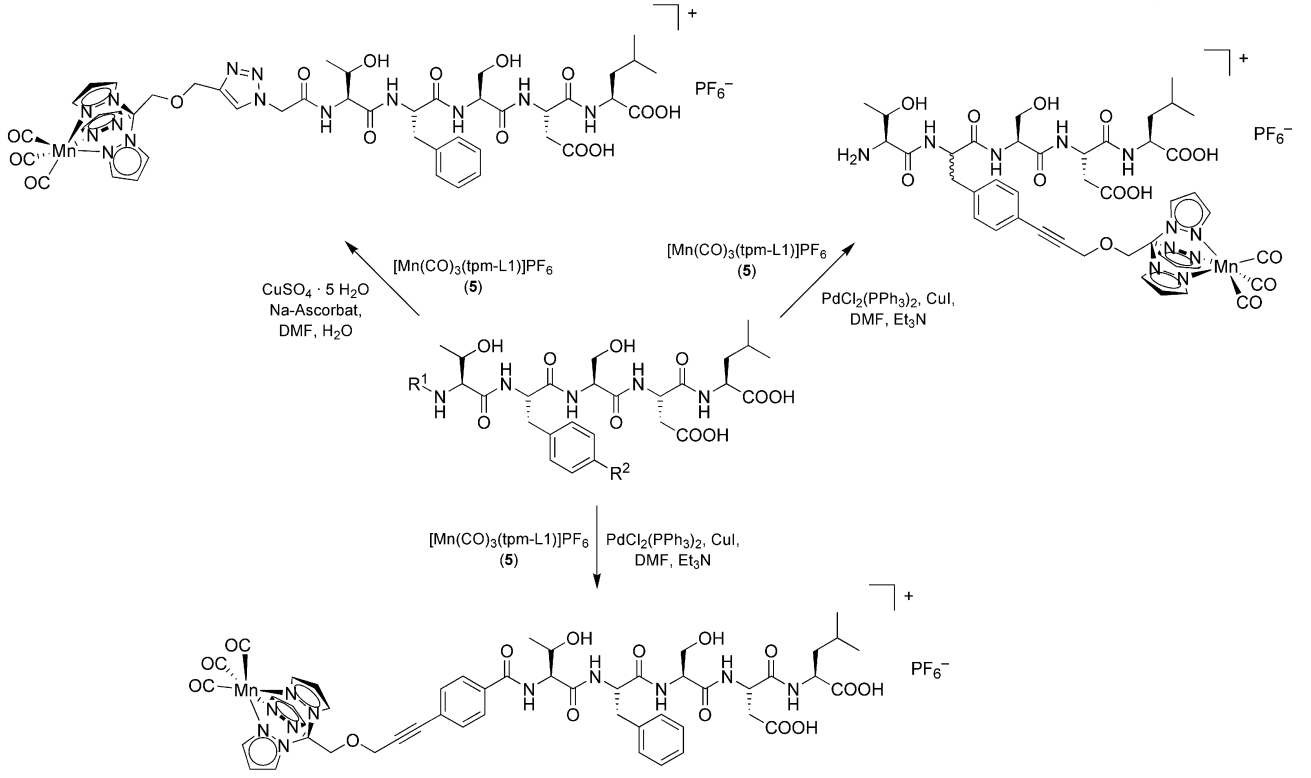


Figure 17. Different synthetic post-labeling strategies to prepare *N*-terminally or side-chain functionalized peptide bioconjugates of a photoactivable CORM derived from $[Mn(tpm)(CO)_3]^+$.

$[Mn(tpm)(CO)_3]^+$ as a new light-activable prodrug with activity against cancer cells comparable to that of an established clinical agent.[181]

The most common intermediate on the way to functionalized tpm ligands is 1,1,1-tris(pyrazoyl)ethanol $tpmCH_2OH$.[178] The esterification of the hydroxy group, however, turned out to be difficult, and in the sole case where the benzoic acid ester of the $[Mn(tpm^{CH_2OH})(CO)_3]^+$ complex could be isolated, it quickly hydrolyzed in methanol to give back the starting material. Thus, the tpm–alcohol was converted into the propargyl ether (Figure 16), which proved to be a versatile starting material in the preparation of peptide bioconjugates by bioorthogonal coupling methods.[179] Both Sonogashira cross-coupling with a 4-iodophenylalanine residue in the middle of the amino acid sequence or an *N*-terminal iodobenzoic acid as well as copper-catalyzed 1,3-dipolar cycloaddition "click" reaction (CuAAC) with *N*-terminal azido acetic acid gave, for the first time, peptide bioconjugates of a CO releasing molecule (Figure 17). The "post-labeling" strategy with the metal complex fragment was necessary, as the tpm ligand itself is not stable under SPPS conditions, in particular towards the strongly acidic final cleavage step of the peptide from the resin. Also, the catalytic copper(I) species formed during both the Sonogashira and 1,3-dipolar azide–alkyne cycloaddition reaction bind to the tris(pyrazolyl)methane, preventing introduction of the $Mn(CO)_3$ group in the final step.[183]

To further explore the utility of manganese(I) tricarbonyl complexes with tripodal ligands as CORMs and elucidate some of the structure–activity relationships that govern CO release properties in these compounds, we very recently also started to study the tris(2- and 4-imidazolyl)phosphane (tip) analogues of the tpm complexes with various substituents on both the imidazole rings as well as the bridging phosphorus atom (Figure 18).[184] All compounds are stable in the dark for an extended period of time and, thus, do not release CO spontaneously in aqueous solution in the absence of light, as also observed for the related $[Mn(tpm)(CO)_3]^+$ systems. Upon photoactivation at 365 nm, all five complexes studied showed CO release in

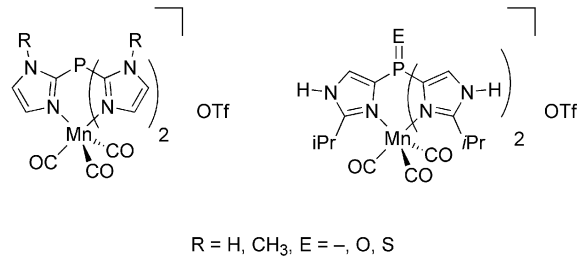


Figure 18. Mn^ICO_3 complexes with 2-tip and 4-tip ligands show light-induced CO release with the number of carbon monoxide equivalents released determined by the substituent R on the nitrogen atom, whereas P-substitution does not have any effect. 2-tip = tris(2-imidazolyl)phosphane; 4-tip = tris(4-imidazolyl)phosphane.

the myoglobin assay and thus represent an additional class of light-inducible CORMs. Interestingly, however, there were pronounced difference with regard to both the number of CO equivalents released per mol of metal complex as well as the half-life of the compounds under irradiation. In the case of the 2-imidazolyl ligands, two mol of CO were released per mol of metal complex, regardless of the substituent on the noncoordinated nitrogen atom, whereas in the 4-imidazolyl complexes, only one of the three carbon monoxide ligands was liberated even after extended irradiation, whereas substitution at the phosphorus atom had no noticeable effect. Apparently, the intermediate formed after liberation of the first carbon monoxide has a different reactivity towards further photolytic release of the remaining CO ligands depending on the tip ligand. Also, the half-lives of the two classes of compounds were different.

Thus, manganese(I) tricarbonyl complexes with tripodal ligands represent a new class of photoactivable CO releasing molecules (CORMs) with potential in the chemotherapy of cancer. In particular, tris(pyrazolyl)methane compounds offer interesting opportunities to prepare functionalized bioconjugates. At present, the major drawback of these systems is the rather low excitation wavelength of 365 nm. Thus, in the future, the potential of a redshifted excitation in CORM-photosensitizer conjugates needs to be explored. Also, although some evidence indeed points to carbon monoxide as the cytotoxic agent, potentially by disruption of the mitochondrial electron-transfer chain due to CO binding to heme proteins,[185] at present an important role of the coligand–manganese fragment cannot be totally ruled out. Thus, attachment of the CORM to a nonmembrane permeable support or nanomaterial might give some useful insight, as it would retain the metal–ligand fragment after photoactivation while CO is generated as the only diffusible species able to cross the cell membrane and reach its intracellular target structures. Work is under way in this direction.

4. Conclusions and Outlook

Photoactivation of transition-metal complexes otherwise stable under physiological conditions in the absence of light allows very precise spatial and temporal control of their biological activity. This can be utilized in fundamental studies on the behavior of biological systems but might also find application in novel photochemotherapeutic approaches. Here, a dark-stable prodrug that can accumulate in the target tissue to only exert its cytotoxic activity upon irradiation of a well-defined area is particularly appealing. The mechanism of action of such systems on bio(macro)molecules, cells, and tissues ranges from DNA cleavage by generation of reactive radical species to the release of bioactive molecules from the coordination sphere of the metal. The latter includes neurotransmitters as well as small-molecule messengers like the diatomic gases nitric oxide and carbon monoxide. It is this area that will likely be the most promising, as these bioactive molecules have well-defined cellular target structures, whereas DNA photonuclease activity is usually rather unspecific. The field of research spans the whole range from synthesis of metal complexes and bioconjugates to detailed studies of the photophysical properties of the compounds and the evaluation of their biological activity on in vitro systems. Certainly, further research in this area has a bright future.

Acknowledgments

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