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Organometallic $[Re(CO)_3]^+$ and $[Re(CO)_2(NO)]^{2+}$ Labeled Substrates for Human Thymidine Kinase 1

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Thymidine was functionalized at position N3 with a tridentate iminodiacetic acid chelating system and a potentially tetradentate mercaptoethyliminodiacetic acid chelating system. Spacers of different lengths (ethyl and butyl) were introduced between the chelators and thymidine. The derivatives were labeled with the $[Re(CO)_2(NO)]^{2+}$ and $[Re(CO)_3]^+$ cores to give isostructural complexes with different overall charges. All complexes were analyzed by NMR, MS, and IR, and in addition, the X-ray structure of a $[Re(CO)_2(NO)]^{2+}$ labeled thymidine derivative functionalized at the N3 position was solved. The ligands incorporating the potentially tetradentate mercaptoethyliminodiacetic acid chelating system coordinated tridentately through iminodiacetic acid to both the $[Re(CO)_2(NO)]^{2+}$ core and the $[Re(CO)_3]^+$ core. This was surprising given that the reaction of $[NEt_4][Re(CO)_2(NO)Br_3]$ with the model ligand ethylmercaptoethyliminodiacetic acid led to dissociation of a carbonyl ligand and formation of a monocarbonyl−mononitrosyl complex, as confirmed by X-ray structure analysis. All of the organometallic thymidine derivatives were substrates for human thymidine kinase 1, a key enzyme in (cancer) cell proliferation. Neutral $[Re(CO)_2(NO)]^{2+}$ labeled thymidine derivatives revealed substrate activity ranging from 24 to 40%, and the structurally analogous anionic $[Re(CO)_3]^+$ labeled thymidine derivatives from 20 to 38% compared with the natural substrate thymidine.

Introduction

Technetium-99m is the mainstay of diagnostic nuclear medicine using single-photon emission computed tomography. The optimal decay properties (γ-emission, 140 keV, $T_{1/2}$ = 6.0 h) and ready availability at low cost are considerable advantages. In addition, there are two medically interesting radionuclides of rhenium, rhenium-186 and rhenium-188, which are suitable for radionuclide therapy (mean β-emission energy of 0.8 and 0.36 MeV, respectively, $T_{1/2}$ = 17 h and 3.7 days, respectively). Over the past decade, our group has put much emphasis on the development and application of the organometallic precursors $[M(CO)_3(H_2O)_3]^+$ (M = ^{99m}Tc, ^{188}Re).[1,2] In fact, two $[^{99m}Tc(CO)_3]^+$ labeled compounds, a tumor affine neurotensin analogue and a vitamin B12 derivative, are currently in clinical phase I trials.[3,4] Buoyed by the success and properties of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, there has been a renewed interest in other low oxidation state organometallic cores of technetium as potential radiolabeling precursors.[5] A prominent example arises from the replacement of a carbonyl ligand by an isolobal nitrosyl ligand to give the $[M(CO)_2(NO)]^{2+}$ core (M = Tc, Re). Not only does the core have an additional positive charge, but the presence of a nitrosyl group is known to considerably effect transition metal centers and their coordination spheres.[6] For potential radiopharmaceutical applications, this may improve the pharmacokinetic profiles of compounds labeled with the $[M(CO)_2(NO)]^{2+}$ core compared to their $[M(CO)_3]^+$ labeled analogues. For example, the Valliant group recently reported a series of metallocarboranes (L), which were evaluated as ligands for the estrogen receptor.[7] Nitrosylation of the negatively charged $[Re(CO)_3L]^-$ compound to give the neutral compound [Re(CO)$_2$(NO)L] resulted in a noticeable increase in receptor affinity.

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The chemistry of mixed carbonyl−nitrosyl complexes of rhenium in organic solvents is well-established,[8−11] and the chemistry in aqueous media has more recently been investigated.[12−15] A comparative study of the ligand exchange reactions of $[NEt_4]_2[Re(CO)_3Br_3]$ and $[NEt_4][Re(CO)_2(NO)Br_3]$ with a series of potential chelating systems revealed that stable complexes are formed with ligands containing carboxylic acids and aromatic and aliphatic amines.[14] A surprising result of this investigation was the dissociation of a carbonyl ligand to give monocarbonyl−mononitrosyl complexes of the form [Re(CO)(NO)L] when the mixed carbonyl−nitrosyl precursor was reacted with ligands with four coordinating groups. Although the survey of ligands was small, the presence of at least two carboxylic acid groups was suggested as a prerequisite for this behavior.

We recently reported the first $[M(CO)_3]^+$ labeled substrates for human thymidine kinase 1 (hTK1).[16,17] Our interest in hTK1 is a result of the potential for selective targeting of proliferating cancer cells due to often dramatically increased hTK1 activity.[18−20] The key to nucleoside metabolism is the rapid enzyme-mediated intracellular phosphorylation of nucleosides to nucleotides, which renders them unable to penetrate biological membranes and thus results in the "trapping" of nucleotides inside cells. Confirming recognition and phosphorylation by hTK1 is therefore the first step in the development of thymidine-based radiopharmaceuticals. In two independent studies with $[M(CO)_3]^+$ labeled thymidine derivatives, we found that, among the compounds tested, neutral and negatively charged thymidine complexes were better substrates than cationic derivatives. However, there were significant differences in the chelating systems of the cationic, neutral, and negatively charged organometallic thymidine derivatives, and variation in their substrate activity as a result of structural differences cannot be excluded. We hypothesized that comparing derivatives labeled with the dicationic *fac*-$[M(CO)_2(NO)]^{2+}$ core with those labeled with the *fac*-$[M(CO)_3]^+$ core, while keeping the chelating systems constant, would provide a more accurate assessment of the influence of the charge of the organometallic thymidine derivative on its phosphorylation rate. For this purpose, thymidine was functionalized with an iminodiacetic acid chelating system and with the mercaptoethyliminodiacetic acid chelating system and reacted with $[NEt_4]_2[Re(CO)_3Br_3]$ and $[NEt_4][Re(CO)_2(NO)Br_3]$. In parallel to these experiments, we investigated the general coordinative behavior of the potentially tetradentate ligand ethylmercaptoethyliminodiacetic acid with the $[Re(CO)_3]^+$ and $[Re(CO)_2(NO)]^{2+}$ cores under aqueous conditions.

Results and Discussion

Functionalization of Thymidine. Iminodiacetic acid (IDA) is a potentially tridentate ligand, which is amenable to incorporation into biomolecules and further functionalization through the secondary amine. Tridentate coordination to the $[M(CO)_3]^+$ and $[M(CO)_2(NO)]^{2+}$ cores gives an anionic complex $[M(CO)_3IDA]^-$, in the case of the tricarbonyl core, and an isostructural but neutral complex $[M(CO)_2(NO)IDA]$ with the dicarbonyl−nitrosyl core. We functionalized thymidine with an iminodiacetic acid chelating system at the N3 position as previously described.[16] Two analogues were prepared with either a two-carbon (L^1) or four-carbon (L^2) spacer between thymidine and the metal chelator, as previous studies by our group and others showed a marked effect of spacer length on the rate of phosphorylation.[16,21]

Ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ were reacted with the precursors $[NEt_4]_2[Re(CO)_3Br_3]$ and $[NEt_4][Re(CO)_2(NO)Br_3]$ in mixtures of methanol and water (Scheme 1). The reactions with $[NEt_4][Re(CO)_2(NO)Br_3]$ proceeded more slowly than those with $[NEt_4]_2[Re(CO)_3Br_3]$, as has been observed with other ligand systems in comparative studies of the reactions of the two precursors.[14] For both $\mathbf{L^1}$ and $\mathbf{L^2}$, formation of the $Re(CO)_2(NO)$ complexes was reliably observed by high-performance liquid chromatography (HPLC) after 6 h of heating at 60 °C. In the case of $[Re(CO)_2(NO)\mathbf{L^1}]$, the pure product precipitated as a pale yellow crystalline solid from the reaction mixture as the volume of the solvent was reduced. The complex $[Re(CO)_2(NO)\mathbf{L^2}]$ was more soluble and could be purified from the reaction mixture by solid-phase extraction. Salts were removed by washing well with water before the product was eluted with a mixture of water and methanol (20%). IR, NMR, mass spectroscopy, and elemental analysis provided clear evidence for the formation of single structural isomers. The IR spectra are typical of dicarbonyl−nitrosyl rhenium complexes, with strong absorption bands at approximately 2100, 2000, and 1800 cm^{-1}, corresponding to the CO ligands and the NO group, respectively. The lack of symmetry in the *fac*-$[Re(CO)_2(NO)]^{2+}$ core caused the NCH_2 protons to appear as four doublets in the ^1H NMR spectrum, two AB spin systems. The complex $[Re(CO)_3\mathbf{L^1}]$ had been prepared and characterized previously.[16] The complex $[Re(CO)_3\mathbf{L^2}]$ was prepared accordingly, and the chemical analyses gave similar results as for compounds $[Re(CO)_3\mathbf{L^1}]$ (see Experimental Details).

Crystals of $[Re(CO)_2(NO)\mathbf{L^1}]$ suitable for X-ray analysis were obtained by dissolving the complex in hot water and allowing the solution to cool slowly to room temperature. The structure is shown in Figure 1

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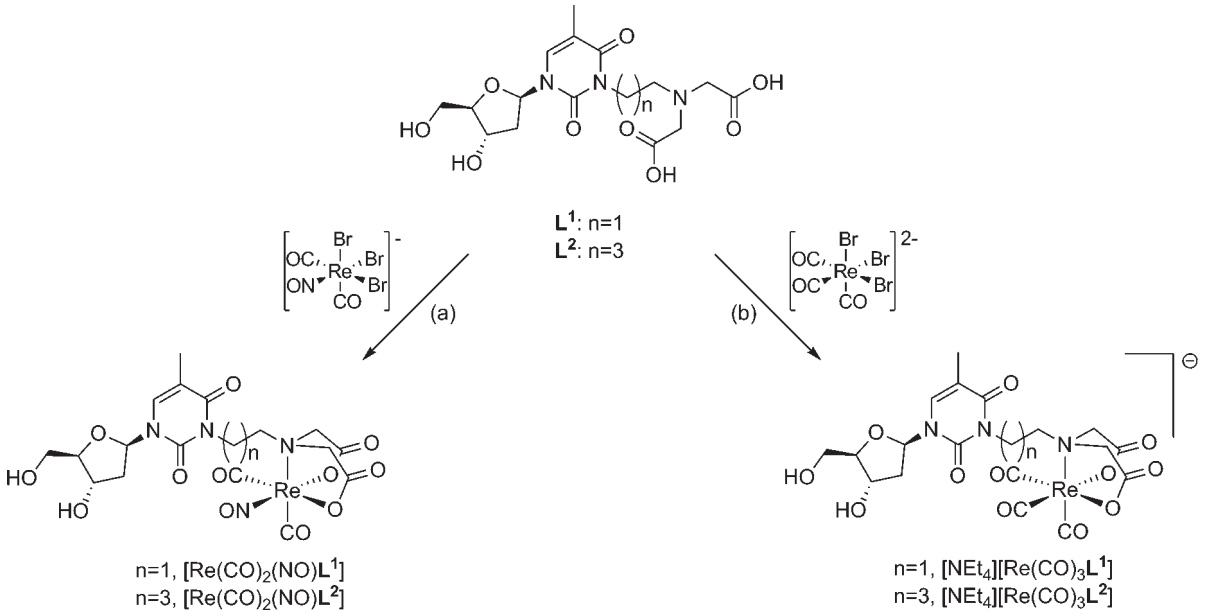
Scheme 1. Reactions of the Thymidine Derivatives $\mathbf{L^1}$ and $\mathbf{L^2}$ with the Precursors $[NEt_4]_2[Re(CO)_3Br_3]$ and $[NEt_4][Re(CO)_2(NO)Br_3]$[a]



[a] (a) MeOH/H_2O, 60 °C, 2 h; (b) MeOH/H_2O, 60 °C, 12 h.

(crystallographic data are reported in Table 1). The complex crystallized in the $P2_12_12_1$ space group, with four molecules in the unit cell. The distorted octahedral environment of Re(I) is defined by the facial coordination of the CO and NO groups of the $[Re(CO)_2(NO)]^{2+}$ core, and the amine and two carboxylic acids of the iminodiacetic acid chelating system. The coordination of the ligand to the metal core is in agreement with the published structure of $[Re(CO)_2(NO)IDA]$.[14] The NO ligand is linearly coordinated to the rhenium center (O(10)−N(10)−Re angle: 177.1°). Coordination of one of the CO ligands trans to the tertiary amine is assumed from the noticeably longer Re−C(30) bond (2.00 Å) compared with the shorter Re−N(10) and Re−C(20) bonds. The positions of the NO and second CO ligand (both coordinated trans to π-donating carboxylic acids) cannot be unambiguously assigned since the differences in length are not significant (1.855 Å and 1.866 Å, respectively). The assignment of the positions of the NO and CO ligands represents the best R value, but the relatively large thermal ellipsoids along the Re−N(10) and Re−C(20) bonds indicate a disorder in the structure. While the *fac*-$Re(CO)_2(NO)$ core has bond angles close to 90° (90.6−93.1°), the steric strain of the tridentate chelate ligand reduces the octahedral bond angles within the five-membered chelate rings. This results in N(11)−Re−O(14) and N(11)−Re−O(14) angles of 79.6° and 79.8°, respectively. The thymidine group is directed away from the metal center, and therefore no direct or indirect interaction with the rhenium chelate was observed.

Synthesis of Ligand $\mathbf{L^3}$ and Thymidine Derivatives $\mathbf{L^4}$ and $\mathbf{L^5}$. Ethylmercaptoethyliminodiacetic acid $\mathbf{L^3}$ (Scheme 2) is reported in the literature as a ligand for technetium(V),[24] and also as a bifunctional chelator for the $M(CO)_3$ core,[25] where it adopts $\kappa S,\kappa N,\kappa O$-coordination when it is amidically coupled through one of the carboxylic acids to a biomolecule. Until now, however, the coordinative behavior of $\mathbf{L^3}$ has not been investigated with either the $[M(CO)_3]^+$ core or the $[M(CO)_2(NO)]^{2+}$ core to model the situation where the chelator is coupled to a biomolecule through the thioether rather than through a carboxylic acid group. Functionalization of thymidine with the mercaptoethyliminodiacetic acid chelating system and either a two-carbon ($\mathbf{L^4}$) or four-carbon ($\mathbf{L^5}$) spacer was achieved using a procedure similar to the synthesis of $\mathbf{L^1}$ and $\mathbf{L^2}$ (Scheme 3).[16] Thymidine was protected with the TBDMS protecting group to allow selective alkylation with a dibromoalkane at the N3 position and more ready purification of intermediates. Nucleophilic substitution with *N*-Boc-aminoethanethiol installed the thioether and amine components of the chelating system. The intermediates were then fully deprotected in a mixture of methanol and concentrated HCl. Selective alkylation of the primary amines was achieved with methyl bromoacetate in a mixture of acetonitrile and methanol. Ligands $\mathbf{L^4}$ and $\mathbf{L^5}$ were obtained by saponification with aqueous NaOH in methanol. The products were purified by solid-phase extraction. Full details of the synthesis of all thymidine-containing ligands can be found in the Supporting Information.

Reaction of the $[Re(CO)_3]^+$ and $[Re(CO)_2(NO)]^{2+}$ Cores with $\mathbf{L^3}$ and Thymidine Derivatives $\mathbf{L^4}$ and $\mathbf{L^5}$. First, we synthesized and characterized the complex $[Re(CO)_3\mathbf{L^3}]^-$. Data from mass spectroscopy were in agreement with the data already published.[25] However, from NMR spectroscopic analyses, we concluded that the ligand coordinates to the metal preferentially through iminodiacetic acid (adopting $\kappa O,\kappa N,\kappa O'$-coordination) and not through the thioether, amine, and one of the

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carboxylic acids as previously suggested. Assignment of the nature of the coordination of the ligand is based on a comparison of the ^{1}H NMR spectra of the uncoordinated ligand and the complex. The ^{1}H NMR spectrum of the uncoordinated ligand $\mathbf{L^3}$ has a singlet corresponding to the four equivalent CH_2 protons of the acetic acid groups, which is replaced by two doublets in the spectrum of the complex, as the CH_2 protons are no longer equivalent (see also Figure 3 and the Supporting Information).

When ligand $\mathbf{L^3}$ was reacted with $[NEt_4][Re(CO)_2(NO)Br_3]$ in a mixture of methanol and water at 60 °C for 12 h, the dissociation of one of the carbonyl ligands and formation of the neutral complex $[Re(CO)(NO)\mathbf{L^3}]$ was observed (Scheme 2). The formation of a monocarbonyl−mononitrosyl complex was immediately apparent from the IR spectrum of the product, which shows only one strong CO stretching band at 1971 cm^{-1} and a NO stretching band at 1709 cm^{-1}. As for the tricarbonyl complex, the ^{1}H NMR spectrum is consistent with coordination of both carboxylic acids since the four equivalent NCH_2 protons of the iminodiacetic part of the ligand all become distinguishable in the spectrum of the complex, which in this case lacks symmetry. Similarly, coordination of the sulfur is assumed by a more complicated coupling pattern for the protons in the two CH_2 groups of the ethyl spacer between the amine and the thioether. The composition of the product was confirmed by mass spectroscopy and X-ray crystallography (Figure 2).

The complex crystallized in the $P2_1/c$ space group with four molecules in the unit cell. The structure shows that the nitrosyl group is linearly coordinated with a Re−N(10)−O(10) bond angle of 179.2° and is distin-

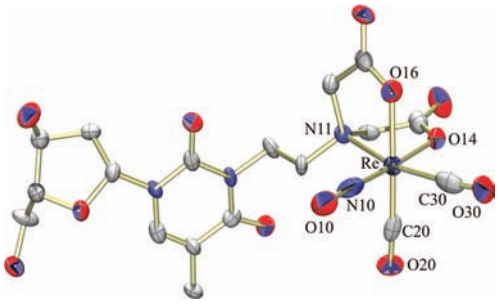
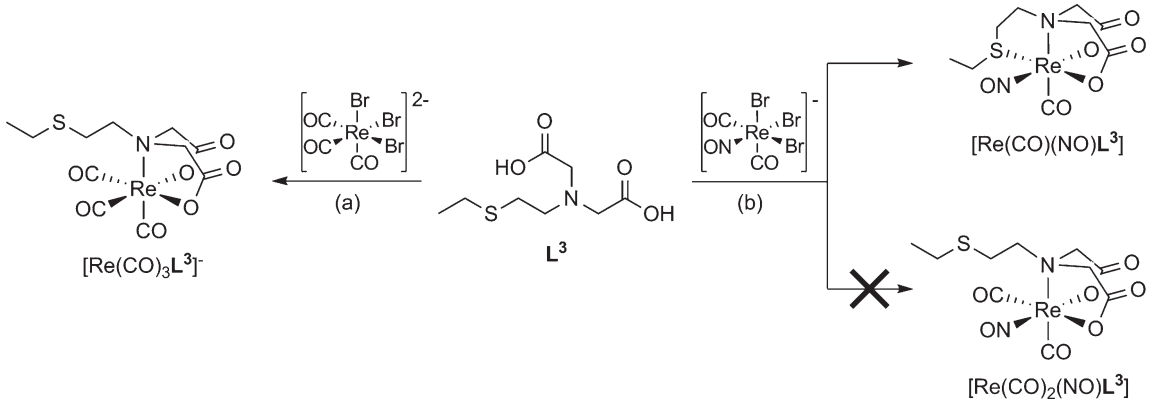


Figure 1. ORTEP-3[23] representation of the neutral complex $[Re(CO)_2(NO)\mathbf{L^1}]$ with thermal ellipsoids shown at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Re−N(10) 1.86(1), Re−C(20) 1.87(2), Re−C(30) 2.00(2), Re−O(14) 2.049(9), Re−O(16) 2.063(9), Re−N(11) 2.210(8), N(10)−Re−C(20) 91.1(5), N(10)−Re−C(30) 90.6(5), C(20)−Re−C(30) 93.1(5), N(10)−Re−O(14) 176.8(4), C(20)−Re−O(14) 92.1(4), C(30)−Re−O(14) 89.7(4), N(10)−Re−O(16) 93.5(4), C(20)−Re−O(16) 173.2(4), C(30)−Re−O(16) 91.9(5), O(14)−Re−O(16) 83.3(3), N(10)−Re−N(11) 99.8(5), C(20)−Re−N(11) 94.4(4), C(30)−Re−N(11) 167.1(5), O(14)−Re−N(11) 79.5(4), O(16)−Re−N(11) 79.9(3).

Table 1. Crystal Data and Structure Refinement for $[Re(CO)_2(NO)\mathbf{L^1}]$ and $[Re(CO)(NO)\mathbf{L^3}]$

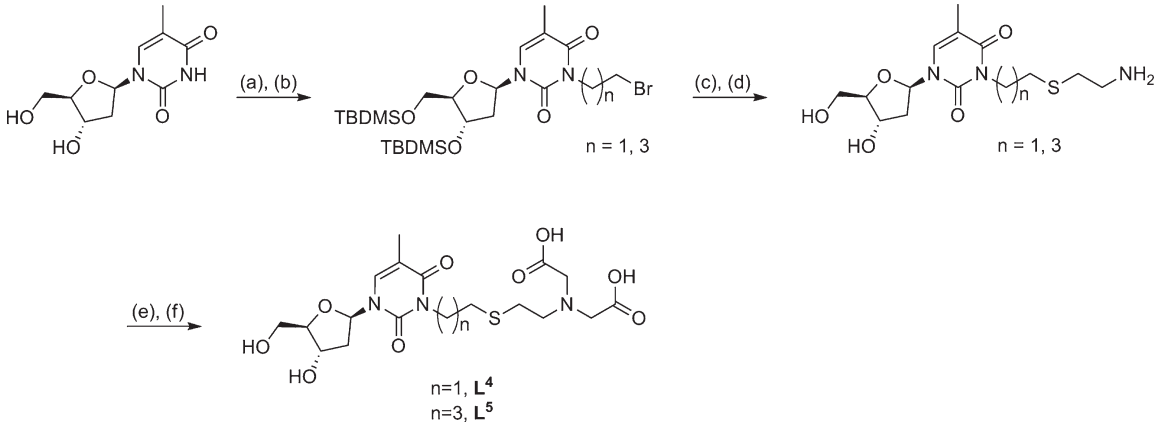
	$[Re(CO)_2(NO)\mathbf{L^1}]$	$[Re(CO)(NO)\mathbf{L^3}]$
formula	$C_{18}H_{21}N_4O_{12}Re$	$C_9H_{13}N_2O_6ReS$
Mr	671.59	463.47
temp [K]	173(2)	200(2)
wavelength [Å]	0.71069	0.71073
cryst size [mm^3]	0.18 × 0.077 × 0.020	0.4 × 0.05 × 0.05
cryst syst	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1/c$
a [Å]	6.991(5)	13.419(1)
b [Å]	9.738(5)	6.827(1)
c [Å]	32.511(5)	14.785(1)
α [deg]	90	90
β [deg]	90	106.83(1)
γ [deg]	90	90
volume [$Å^3$]	2213(2)	1296.5(2)
Z	4	4
D_{calcd} [Mg m^{-3}]	2.015	2.375
abs coeff [mm^{-1}]	5.564	9.558
F(000)	1312	880
θ range for data collection [deg]	2.44 − 26.77	2.88 − 29.48
reflns collected	16321	9112
independent reflns	4687 [R(int) = 0.0827]	3462 [R(int) = 0.0747]
completeness to θ [%]	99.3 (θ = 26.77°)	95.8 (θ = 29.48°)
absorption correction	integration	integration
max. and min. transmission	0.8874 and 0.6506	0.847 and 0.328
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/params	4687/0/317	3462/0/172
goodness-of-fit on F^2	0.998	0.858
final R indices [$I > 2\sigma(I)$]]	R1 = 0.0538, wR2 = 0.1214	R1 = 0.0377, wR2 = 0.0918
R indices (all data)	R1 = 0.0804, wR2 = 0.1330	R1 = 0.0686, wR2 = 0.1054
absolute structure param	0.00(2)	
largest diff. peak and hole [e $Å^{-3}$]	1.154 and −1.358	1.444 and −3.167

Scheme 2. Reaction of $\mathbf{L^3}$ with the Precursors $[NEt_4]_2[Re(CO)_3Br_3]$ and $[NEt_4][Re(CO)_2(NO)Br_3]$[a]



[a] (a) MeOH/H_2O, 60 °C, 2 h; (b) MeOH/H_2O, 60 °C, 12 h.

Scheme 3. Synthesis of Thymidine-Containing Ligands $\mathbf{L^4}$ and $\mathbf{L^5}$[a]



[a] (a) TBDMSCl, imidazole, DMF; (b) $Br(CH_2)_nBr$, Cs_2CO_3, DMF; (c) $HSCH_2CH_2NHBoc$, Cs_2CO_3, DMF; (d) HCl, MeOH; (e) $BrCH_2CO_2Me$, TEA, MeCN; (f) NaOH, H_2O.

guishable from the carbonyl group by the bond length of the Re−N(10) bond (1.773 Å), which is significantly shorter than the Re−C(20) bond (1.955 Å). As expected from comparison with published structures of other Re(CO)(NO) and $Re(CO)_2(NO)$ complexes, both the Re−N(10) and Re−C(20) bond lengths are shorter in $[Re(CO)(NO)\mathbf{L^3}]$ than in $[Re(CO)_2(NO)\mathbf{L^2}]$.[14] The iminodiacetic part of the ligand has facial coordination, as is the case in $Re(CO)_3$ and $Re(CO)_2(NO)$ complexes with this chelating system.[14,22] The thioether is coordinated trans to a carboxyl oxygen atom. The Re−S bond length is shorter (2.39 Å) than in $Re(CO)_3$ complexes with tridentate thioether containing ligands (2.47−2.48 Å), but in those cases the sulfur atom is invariably coordinated trans to a carbonyl ligand.[26−28] As expected, coordination of a tetradentate ligand results in a greater distortion from octahedral geometry than in $Re(CO)_3$ and $Re(CO)_2(NO)$ complexes with tridentate chelating systems. Whereas the N(10)−Re−C(20) bond angle is 91.6°, within the three five-membered chelate rings, the N−Re−O(1), N−Re−O(2), and N−Re−S have angles of 79.7°, 76.7°, and 84.6°, respectively. Crystallographic data are reported in Table 1.

Ligands $\mathbf{L^4}$ and $\mathbf{L^5}$ were also reacted with the precursor $[NEt_4][Re(CO)_2(NO)Br_3]$ in a mixture of water and methanol (Scheme 4). The reaction mixtures were heated at 60 °C for 6 h and followed by HPLC. In the reaction with $\mathbf{L^4}$, a single product formed in quantitative yield, which could be isolated by solid-phase extraction of the reaction mixture. In light of the model complex formed with $\mathbf{L^2}$, we had expected the product of the reaction with $\mathbf{L^4}$ to be a monocarbonyl−mononitrosyl complex. However, IR and mass spectroscopic analyses revealed the product complex to have retained the $Re(CO)_2(NO)$ core. Elemental analysis confirmed the composition of a neutral complex, suggesting the ligand was coordinated through the iminodiacetic acid part of the chelator ($\kappa N,\kappa O,\kappa O'$). This was supported by NMR analysis, which showed the four CH_2 protons of the acetic acid groups had all become distinguishable as a result of coordination to the metal center. Furthermore, IR analysis of the product showed no evidence of an uncoordinated carboxylic acid. We postulated that a ligand with a longer spacer between thymidine and the metal chelating system might be more amenable to tetradentate coordination on steric grounds. However, in the reaction with $\mathbf{L^5}$, which has a butyl rather than an ethyl spacer between thymidine and the potentially tetradentate chelating system, the tridentate $\kappa N,\kappa O,\kappa O'$ coordinated complex, $[Re(CO)_2(NO)\mathbf{L^5}]$, was again the major product of the reaction. Characterization and analysis of the complex was consistent with a structure analogous to that of $[Re(CO)_2(NO)\mathbf{L^4}]$.

We investigated whether the dissociation of a carbonyl ligand was pH-dependent in the cases of $\mathbf{L^4}$ and $\mathbf{L^5}$. Initially, the reactions were carried out at pH 5, which led to the products described above. Both reactions were also performed at pH 3 and pH 8; however, in neither case

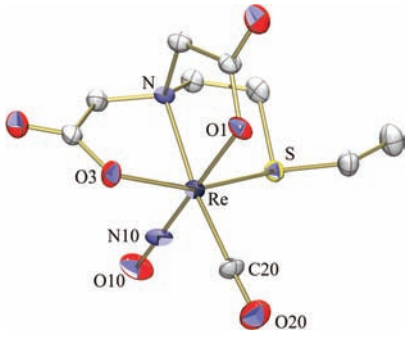


Figure 2. ORTEP-3[23] representation of the neutral complex [Re(CO)(NO)$\mathbf{L^3}$] with thermal ellipsoids shown at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Re−N(10) 1.773(6), Re−C(20) 1.955(7), Re−O(1) 2.059(5), Re−O(3) 2.099(5), Re−N 2.161(6), Re−S 2.39(2), N(10)−Re−C(20) 91.6(3), N(10)−Re−O(1) 179.0(2), C(20)−Re−O(1) 88.6(3), N(10)−Re−O(3) 93.9(3), (20)−Re−O(3) 102.3(3), O(1)−Re−O(3) 87.0(2), N(10)−Re−N 100.1(3), C(20)−Re−N 168.3(3), O(1)−Re−N 79.7(2), O(3)−Re−N 76.7(2), N(10)−Re−S 90.3(2), C(20)−Re−S 95.8(2), O(1)−Re−S 88.75(16), O(3)−Re−S 161.29(15), N−Re−S 84.58(16).

(26) Karagiorgou, O.; Patsis, G.; Pelecanou, M.; Raptopoulou, P.; Terzis, A.; Siatra-Papastaikoudi, T.; Alberto, R.; Pirmettis, I.; Papadopoulos, M. *Inorg. Chem.* **2005**, *44*, 4118–4120.
(27) Lazarova, N.; Babich, J.; Valliant, J.; Schaffer, P.; James, S.; Zubieta, J. *Inorg. Chem.* **2005**, *44*, 6763–6770.
(28) van Staveren, D. R.; Benny, P. D.; Waibel, R.; Kurz, P.; Pak, J. K.; Alberto, R. *Helv. Chim. Acta* **2005**, *88*, 447–460.

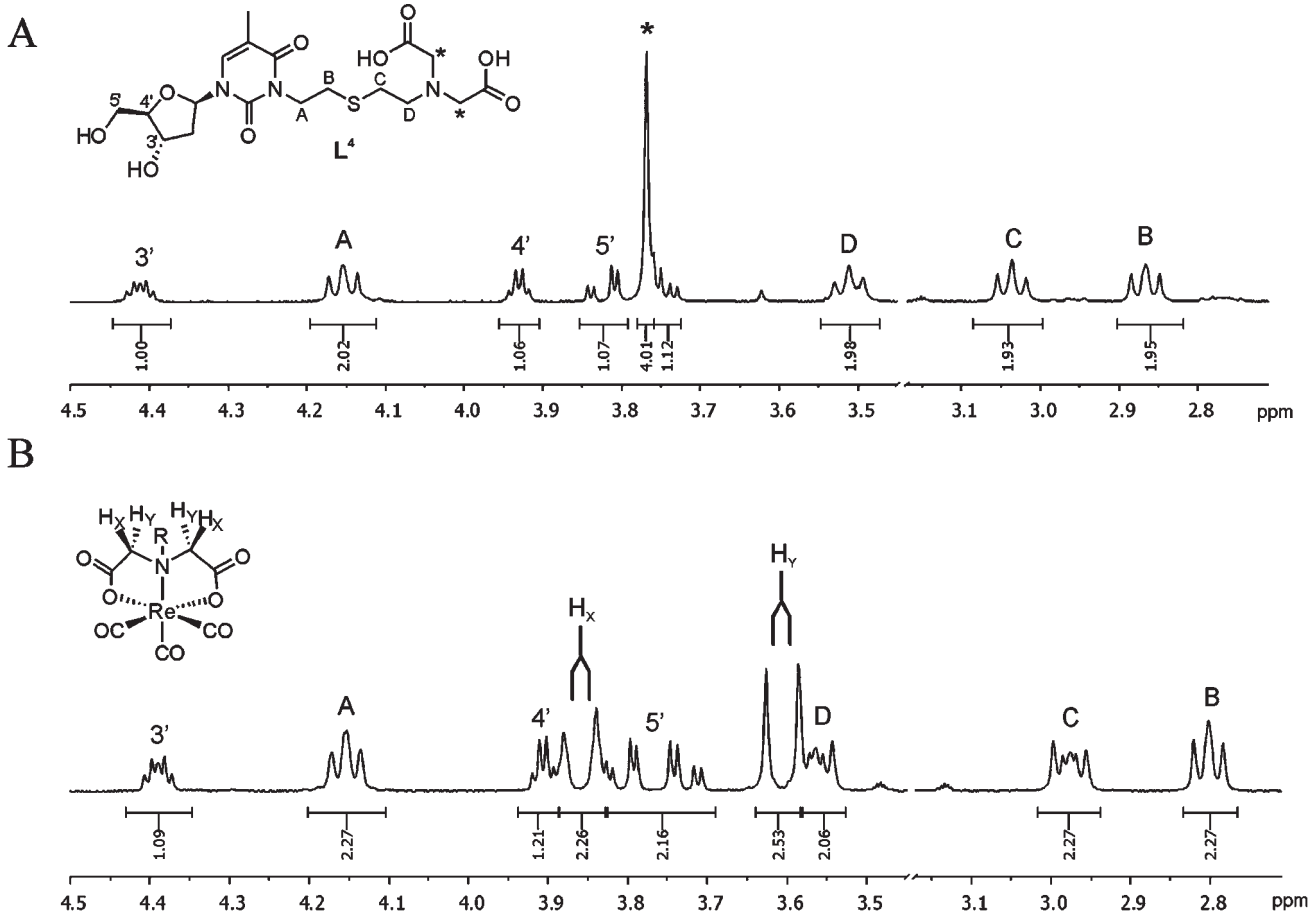


Figure 3. (A) Aliphatic region of the ^{1}H NMR spectrum of $\mathbf{L^4}$ with numbering scheme and (B) aliphatic region of the ^{1}H NMR spectrum of $[Re(CO)_3\mathbf{L^4}]^-$ and scheme for coordination of the ligand. The solvent signals ([D$_4$]MeOH) have been omitted for clarity.

did this have any impact on the HPLC trace of the reaction. Increasing the pH from 5 to 8 led only to a decrease in the yield of the reaction, as a result of the instability of the dicarbonyl−nitrosyl precursor in solution at higher pH, which is apparent from the darkening of the initially bright yellow reaction solution.

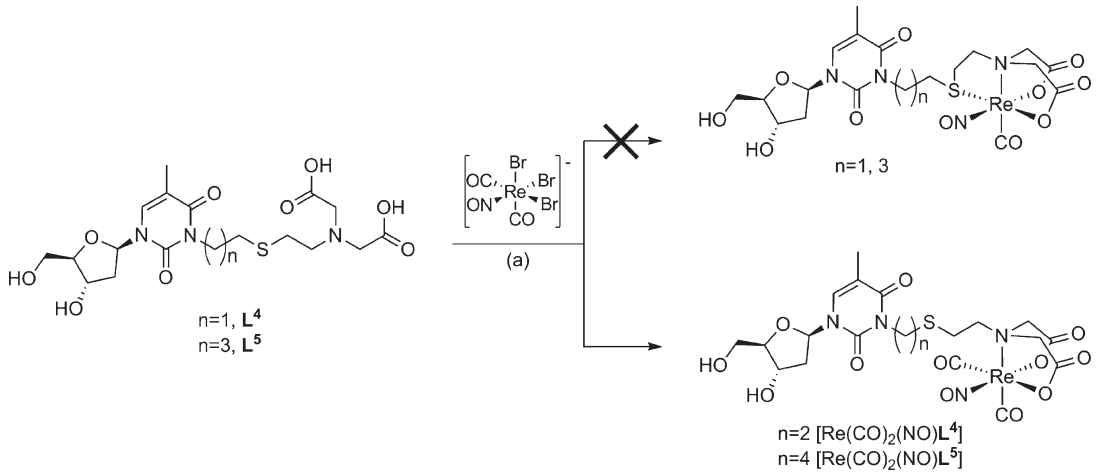
The $Re(CO)_3$ complexes of $\mathbf{L^4}$ and $\mathbf{L^5}$ were also synthesized, and as expected from the complex formed with the model ligand $\mathbf{L^3}$, the ligands were coordinated in both cases through iminodiacetic acid to give anionic species. As for the model compound $[Re(CO)_3\mathbf{L^3}]^-$, the most convincing evidence for this mode of coordination came from the ^{1}H NMR spectra of the complexes. As shown in Figure 3, the ^{1}H NMR spectrum of the uncoordinated ligand has a singlet at 3.77 ppm, with a relative intensity of 4, corresponding to the four equivalent CH_2 protons of the acetic acid groups. On the other hand, in the complex, the two protons of each CH_2 group are distinguishable. The protons appear as two doublets, each with an intensity of 2 as a result of the C_s symmetry of the complex, and have a ^2J coupling constant of 16.4 Hz. Identical coupling patterns were observed in the ^{1}H NMR spectra of complexes $[NEt_4][Re(CO)_3\mathbf{L^1}-\mathbf{L^3}]$.

In Vitro Evaluation of the Organometallic Complexes of the Thymidine Derivatives $\mathbf{L^1}$, $\mathbf{L^2}$, $\mathbf{L^4}$, and $\mathbf{L^5}$. A fundamental aspect of this study was the assessment of the influence of the overall charge of isostructural thymidine complexes on their hTK1 substrate activity. Thus, the substrate activities of anionic $[Re(CO)_3]^+$ labeled complexes were compared with the substrate activities of neutral $[Re(NO)(CO)_2]^{2+}$ labeled complexes. In addition, the influence of the spacer entity between thymidine and the metal chelate was investigated. The hTK1 substrate activities of the organometallic thymidine derivatives $[NEt_4][Re(CO)_3\mathbf{L^1}/\mathbf{L^2}]$, $[Re(CO)_2(NO)\mathbf{L^1}/\mathbf{L^2}]$, $[NEt_4][Re(CO)_3\mathbf{L^4}/\mathbf{L^5}]$, and $[Re(CO)_2(NO)\mathbf{L^4}/\mathbf{L^5}]$ were assessed using a coupled thymidine kinase−pyruvate kinase−lactate dehydrogenase UV assay (λ = 340 nm), as previously described (Scheme 5; see the Supporting Information for more details).[29,30] The results are presented in Table 2. The phosphorylation of thymidine (dT) was arbitrarily set to 100%.

The organometallic complex of $\mathbf{L^1}$, $[Re(CO)_3\mathbf{L^1}]^-$, showed a relative phosphorylation of 29.9 ± 2.0% compared to the natural substrate thymidine, which was slightly lower (33.8 ± 2.4%) than had been measured in our previous studies.[16] The isostructral neutral complex $[Re(CO)_2(NO)\mathbf{L^1}]$ revealed a very similar phosphorylation rate of 28.9 ± 2.4%. A more significant difference in substrate activity was observed between the anionic $[Re(CO)_3]^+$ labeled derivative $[Re(CO)_3\mathbf{L^2}]^-$ (27.6 ± 2.7%) and the neutral $[Re(CO)_2(NO)]^{2+}$ labeled

(29) Byun, Y.; Thirumamagal, B. T. S.; Yang, W.; Eriksson, S.; Barth, R. F.; Tjarks, W. *J. Med. Chem.* **2006**, *49*, 5513–5523.
(30) Kornberg, A.; Pricer, W. E. *J. Biol. Chem.* **1951**, *193*, 481–495.

Scheme 4. Reactions of $\mathbf{L^4}$/$\mathbf{L^5}$ with $[NEt_4][Re(CO)_2(NO)Br_3]$[a]



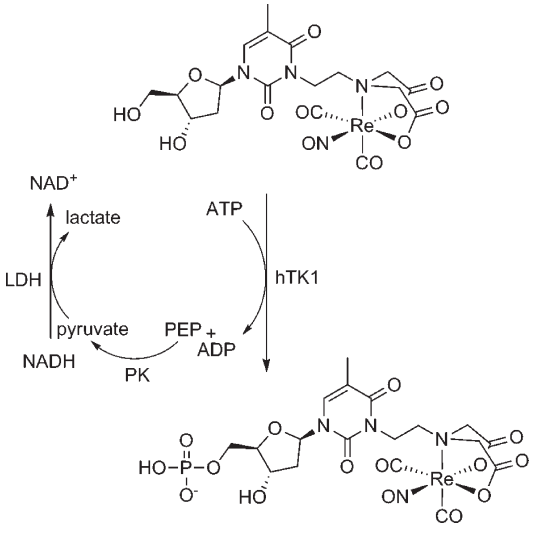
[a] (a) $MeOH/H_2O$, 60 °C, 12 h.

Table 2. Phosphorylation of Organometallic Thymidine Derivatives Relative to dT [%][a]

compound	$[NEt_4][Re(CO)_3\mathbf{L}]$	$[Re(CO)_2(NO)\mathbf{L}]$
$\mathbf{L^1}$	29.9 ± 2.0	28.9 ± 2.4
$\mathbf{L^2}$	27.6 ± 2.7	40.2 ± 1.6
$\mathbf{L^4}$	19.9 ± 2.4	24.4 ± 0.6
$\mathbf{L^5}$	38.3 ± 1.3	27.8 ± 2.1

[a] Mean ± SD values are based on three experiments per compound. The phosphorylation of dT was arbitrarily set to 100%.

Scheme 5. Phosphorylation of $[Re(CO)_2(NO)\mathbf{L^1}]$ and Coupled hTK1, PK, and LDH Assay



derivative $[Re(CO)_2(NO)\mathbf{L^2}]$ (40.2 ± 1.6%), which have a butyl spacer separating the metal complex and thymidine. This is presumably because the length of the spacer determines with which amino acid residues of the enzyme the metal complex interacts. On the other hand, the neutral derivatives $[Re(CO)_2(NO)\mathbf{L^4}/\mathbf{L^5}]$ (phosphorylation 24.4 ± 0.6% and 27.8 ± 2.1%, respectively) with the mercaptoethyliminodiacetic acid chelating system showed comparatively low relative phosphorylation compared to $[Re(CO)_2(NO)\mathbf{L^2}]$. This is contrary to the results obtained for the series of anionic $Re(CO)_3$−IDA−thymidine complexes $[Re(CO)_3\mathbf{L^1}/\mathbf{L^2}/\mathbf{L^5}]^-$ and a series of neutral $Re(CO)_3$−Cys−thymidine complexes, where a continuous increase in substrate activity was observed as the spacer was elongated.[16] Whether the differences in the chelating system, spacer, or the metal core are responsible for these controversial results needs to be investigated further. For reasons which are not yet known, the phosphorylation of complex $[Re(CO)_3\mathbf{L^4}]^-$ is not consistent with this trend.

Conclusions

The use of isostructural but differently charged organometallic precursors and complexes is a valuable tool for the evaluation of the influence of charge on substrate affinity, since structural differences arising from the use of different ligand systems can be avoided. Isostructural neutral and anionic organometallic $[Re(CO)_2(NO)]^{2+}$ and $[Re(CO)_3]^+$ labeled complexes of N3-functionalized thymidine derivatives were synthesized and characterized. In the case of the tridentate IDA-functionalized derivative $\mathbf{L^1}$, both the neutral and anionic thymidine derivatives were equally good substrates for hTK1, with relative phosphorylation rates of 28.9% and 29.9% compared with the value for the natural substrate. The neutral derivatives $[Re(CO)_2(NO)\mathbf{L^4}/\mathbf{L^5}]$, which are structurally similar to $[Re(CO)_2(NO)\mathbf{L^1}/\mathbf{L^2}]$, but have a longer, sulfur-containing spacer, were also substrates for hTK1. However, increasing the spacer length did not lead to higher substrate activity. Thus, the substrate activity of organometallic rhenium thymidine derivatives appears to be influenced by a combination of multiple parameters including charge, chelating system, spacer length, and spacer composition, as well as other factors such as subtle changes of the metal core. Derivatives $\mathbf{L^4}$ and $\mathbf{L^5}$, functionalized with a potentially tetradentate mercaptoethyliminodiacetic acid chelator, revealed exclusively tridentate coordination through the iminodiacetic acid part of the chelating system with both the tricarbonyl and the dicarbonyl−mononitrosyl precursor. This is surprising in the case of the $[Re(CO)_2(NO)]^{2+}$ core, since the reaction of the model ligand ethylmercaptoethyliminodiacetic acid, $\mathbf{L^3}$, with $[NEt_4][Re(CO)_2(NO)Br_3]$ induced dissociation of one of

the carbonyl ligands and led exclusively to the formation of the complex [Re(CO)(NO)$\mathbf{L^3}$].

Experimental Details

General Methods. All chemicals were purchased from Sigma-Aldrich or Fluka, Buchs, Switzerland. All chemicals and solvents were of reagent grade and were used without further purification unless otherwise stated. The precursors $[NEt_4][Re(CO)_2(NO)Br_3]$[8−11] and $[NEt_4]_2[Re(CO)_3Br_3]$[31] were prepared according to published procedures. Reactions were monitored by HPLC or by thin layer chromatography using precoated silica gel 60 F_{254} aluminum sheets (Merck) and visualized by UV absorption or stained with a solution of ninhydrin in EtOH. Column chromatography was performed using silica gel 60 (Fluka; particle size 0.040−0.063 mm). Analytical and semipreparative HPLC were performed using a Merck-Hitachi L-7000 system equipped with an L-7400 tunable absorption detector and either an XBridge C-18 reverse phase column (5 μM, 4.6 × 150 mm, Waters) or an XBridge Prep C-18 reverse phase column (5 μM, 10 × 150 mm, Waters). HPLC solvents were water with 0.1% TFA (solvent A) and MeCN (solvent B) with a flow rate of 1 mL/min for analytical HPLC and 3 mL/min for semipreparative HPLC. The analytical system was as follows: 0−15 min, gradient from 95% A to 20% A; 15−20 min, gradient from 20% A to 95% A; 20−25 min, 95% A. The semipreparative system was as follows: 0−14 min, gradient from 85% A to 40% A; 14−16 min, gradient from 40% A to 85% A; 16−17 min, 85% A. Sep-Pak columns (Waters) were washed with methanol and water prior to use. Nuclear magnetic resonance spectra were recorded on a 400 MHz Bruker spectrometer. ^{1}H and ^{13}C chemical shifts are reported relative to residual solvent peaks or water as a reference. The chemical shifts of complex multiplets are given as the range of their occurrence. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR, with a universal ATR sampling accessory. Low-resolution mass spectra were recorded with a Micromass Quattro micro API LC-ESI using either the negative or positive ionization mode. High-resolution mass spectra were recorded with a Bruker FTMS 4.7T BioAPEXII.

Ligand Synthesis. The syntheses of $\mathbf{L^1}$, $\mathbf{L^2}$, $\mathbf{L^4}$, and $\mathbf{L^5}$ are outlined in Scheme S1 of the Supporting Information. Ethylmercaptoethyliminodiacetic acid $\mathbf{L^3}$ was prepared according to a literature procedure.[25]

3-[2-[Bis(carboxymethyl)amino]ethyl]thymidine ($\mathbf{L^1}$). The synthesis of the iminodiacetic acid functionalized thymidine derivative $\mathbf{L^1}$ was recently reported.[16] ^{1}H NMR (D_2O): δ 7.73 (s, 1H), 6.33 (t, J = 6.6 Hz, 1H), 4.51 (m, J = 10.1, 5.2 Hz, 1H), 4.40 (m, J = 5.7, 2.7 Hz, 2H), 4.07 (m, J = 8.4, 4.4 Hz, 1H), 3.90 (s, 3H), 3.88 (dd, J = 12.6, 3.5 Hz, 1H), 3.81 (dd, J = 12.6, 5.2 Hz, 1H), 3.54 (t, J = 5.6 Hz, 2H), 2.46 (t, J = 5.6 Hz, 2H), 1.98 (s, 3H) ppm. ^{13}C NMR (D_2O): δ 170.1, 165.7, 152.0, 136.2, 110.6, 86.6, 86.2, 70.2, 61.1, 57.0, 54.6, 46.7, 38.7, 12.2 ppm. HRMS: m/z 400.1362 $[C_{16}H_{22}N_3O_9]^-$ (calcd 400.1360).

3-[4-[Bis(carboxymethyl)amino]butyl]thymidine ($\mathbf{L^2}$). Ligand $\mathbf{L^2}$ was prepared using the same procedure as for $\mathbf{L^1}$, but alkylating with *N*-Boc-amino-4-bromobutane rather than *N*-Boc-amino-2-bromoethane. ^{1}H NMR (D_2O): δ 7.69 (d, J = 1.0, 1H), 6.36 (t, J = 6.6, 1H), 4.54−4.48 (m, 1H), 4.08 (dd, J = 8.9, 3.9, 1H), 4.01 (t, J = 6.7, 2H), 3.90 (dd, J = 12.4, 3.6, 1H), 3.86−3.78 (m, 5H), 3.37−3.30 (m, 2H), 2.50−2.37 (m, 2H), 1.97 (d, J = 1.0, 3H), 1.86−1.69 (m, 4H) ppm. ^{13}C NMR (D_2O): δ 170.5, 165.7, 151.7, 135.6, 110.6, 86.5, 85.9, 70.4, 61.1, 57.1, 55.6, 40.6, 38.7, 23.8, 21.2, 12.3 ppm. HRMS: m/z 428.1659 $[C_{18}H_{26}N_3O_9]^-$ (calcd 428.1675).

3-[2-[S-(Bis(carboxymethyl)aminoethylthio)]ethyl]thymidine ($\mathbf{L^4}$). Thymidine derivatives $\mathbf{L^4}$ and $\mathbf{L^5}$ incorporating a mercaptoethyliminodiacetic acid chelating system were prepared by a procedure analogous to that reported for $\mathbf{L^1}$. Full details of the synthetic procedure can be found in the Supporting Information. ^{1}H NMR (CD_3OD): δ 7.87 (d, J = 1.0, 1H), 6.32 (t, J = 6.7, 1H), 4.43 (m, 1H), 4.15 (m, 2H), 3.93 (q, J = 3.5, 1H), 3.82 (dd, J = 12.0, 3.2, 1H), 3.77 (s, 4H), 3.74 (dd, J = 12.0, 3.7, 1H), 3.51 (t, 2H), 3.04 (t, J = 7.3, 2H), 2.85 (t, 2H), 2.31 (m, 1H), 2.23 (m, 1H), 1.93 (d, J = 1.0, 3H) ppm. ^{13}C NMR (CD_3OD): δ 170.1, 165.6, 152.5, 136.8, 111.2, 88.6, 87.3, 71.9, 62.6, 56.9, 55.7, 41.2, 41.0, 29.3, 26.8, 13.3 ppm. HRMS: m/z 460.1398 $[C_{18}H_{26}N_3O_9]^-$ (calcd 460.1395).

3-[4-[S-(Bis(carboxymethyl)aminoethylthio)]butyl]thymidine ($\mathbf{L^5}$). Full details of the synthesis of $\mathbf{L^5}$ can be found in the Supporting Information. ^{1}H NMR (CD_3OD): δ 7.71 (s, 1H), 6.38 (t, J = 6.5, 1H), 4.53 (m, 1H), 4.10 (m, 1H), 4.00 (t, J = 6.6, 2H), 3.88 (s, 3H), 3.94−3.81 (m, 2H), 3.54 (t, J = 6.6, 2H), 3.00 (t, J = 6.8, 2H), 2.73 (t, J = 6.7, 2H), 2.52−2.35 (m, 2H), 1.98 (s, 3H), 1.77 (m, 2H), 1.70 (m, 2H) ppm. ^{13}C NMR (CD_3OD): δ 171.0, 165.7, 152.5, 136.6, 111.1, 88.6, 87.2, 72.0, 62.7, 58.4, 55.4, 41.9, 41.1, 31.9, 27.7, 27.6, 27.2, 13.4 ppm. MS: m/z 487.98 $[C_{20}H_{30}N_3O_9]^-$.

Complex Formation. The synthesis and characterization of $[NEt_4][Re(CO)_3\mathbf{L^3}]$ have been reported previously.[16] Mixed carbonyl−nitrosyl complexes were prepared using the following general procedure unless stated otherwise. One equivalent of the ligand $\mathbf{L^1}$−$\mathbf{L^5}$ was dissolved in a 2:1 mixture of MeOH and water to form a 0.1 M solution. One equivalent of $[NEt_4][Re(CO)_2(NO)Br_3]$ was added, and the reaction mixtures were stirred at 65 °C. The reactions with thymidine-containing ligands $\mathbf{L^1}$, $\mathbf{L^2}$, $\mathbf{L^4}$, and $\mathbf{L^5}$ were followed by HPLC. After 6 h, the solvents were removed under vacuum conditions, and the residue was purified by solid-phase extraction using a Sep-Pak column with a water−MeOH gradient. The fractions containing the product were evaporated under reduced pressure. Tricarbonyl complexes of ligands $\mathbf{L^1}$−$\mathbf{L^5}$ were prepared according to the following general procedure unless stated otherwise. One equivalent of the ligand $\mathbf{L^1}$−$\mathbf{L^5}$ was dissolved in a 1:1 mixture of MeOH and water to form a 0.1 M solution. One equivalent of $[NEt_4]_2[ReBr_3(CO)_3]$ was added, and the reaction mixtures were stirred at 65 °C. All complexation reactions with thymidine-containing ligands $\mathbf{L^1}$, $\mathbf{L^2}$, $\mathbf{L^4}$, and $\mathbf{L^5}$ were followed by HPLC. After 2 h, the solvents were removed under vacuum conditions, and the residue was purified by solid-phase extraction using a Sep-Pak column with a water−MeOH gradient. The fractions containing the product were evaporated under reduced pressure.

[$Re(CO)_2(NO)\mathbf{L^1}$]. This complex was synthesized as per general procedure, except that the pure product is poorly soluble in both water and methanol and was isolated by precipitation from the reaction solution. Yield: 46%. Anal. calcd for $C_{18}H_{21}N_4O_{12}Re$: C, 32.19; H, 3.15; N, 8.34. Found: C, 31.78; H, 3.34; N, 8.21. ^{1}H NMR (CD_3OD): δ 7.84 (d, J = 1.1, 1H), 6.21 (t, J = 6.7, 1H), 4.31 (d, J = 16.9, 1H), 4.25 (m, 1H), 4.22−4.14 (m, 3H), 4.07 (dd, J = 16.0, 2.0, 1H), 4.01 (dd, J = 16.0, 4.4, 1H), 3.79 (q, J = 3.7, 1H), 3.71 (m, 1H), 3.66−3.52 (m, 4H), 2.13 (m, 2H), 1.86 (d, J = 1.1, 3H) ppm. ^{13}C NMR (CD_3OD): δ 188.9, 187.9, 178.2, 177.1, 162.63, 150.3, 135.2, 108.5, 87.4, 84.9, 70.1, 65.7, 62.3, 61.9, 61.1, 36.9, 12.9 ppm. IR: ν 3309, 2939, 2112, 2029, 1782, 1712, 1687, 1655, 1627, 1462, 1434, 1357, 1335, 1321, 1236, 1177, 1109, 1059, 1031, 999, 981, 943, 927, 911, 850, 788, 769, 649 cm^{-1}. MS: m/z 672.94 $[C_{18}H_{21}N_4O_{12}Re]H^+$.

[NEt_4][$Re(CO)_3\mathbf{L^1}$]. This complex was synthesized as per general procedure. Yield: 63%. ^{1}H NMR (CD_3OD): δ 7.88 (s, 1H), 6.33 (t, J = 6.6 Hz, 1H), 4.41 (m, 1H), 4.29 (m, J = 6.4 Hz, 2H), 3.93 (m, J = 3.4 Hz, 1H), 3.86 (t, J = 7.3 Hz, 2H), 3.76 (m, 4H), 3.55 (t, J = 7.3 Hz, 2H), 3.31 (m, 8H, NEt_4^+), 2.32 (m, 1H), 2.24 (m, 1H), 1.94 (s, 3H), 1.31 (m, 12H, NEt_4^+) ppm.

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^{13}C NMR (CD_3OD): δ 198.7, 197.9, 182.6, 165.2, 152.2, 137.0, 110.8, 89.0, 87.3, 72.1, 66.9, 63.8, 62.8, 53.3 (NEt_4), 41.4, 38.6, 13.2, 7.7 (NEt_4) ppm. IR: ν 3385, 2932, 2017, 1863, 1697, 1624, 1466, 1375, 1320, 1272, 1185, 1173, 1092, 1057, 999.6, 946.3, 908, 770, 752, 660, 643, 608 cm^{-1}. HRMS: m/z 670.0675 $[C_{19}H_{21}N_3O_{12}Re]^-$ (calcd 670.0689).

[Re(CO)$_2$(NO)L^2]. This complex was synthesized as per general procedure. Yield: 77%. Anal. calcd for $C_{20}H_{25}N_4O_{12}Re$: C, 34.33; H, 3.60; N, 8.01. Found: C, 34.10; H, 3.86; N, 7.85. ^{1}H NMR (CD_3OD): δ 7.87 (d, J = 0.8, 1H), 6.32 (t, J = 6.7, 1H), 4.40 (dt, J = 6.4, 3.4, 1H), 4.26 (d, J = 16.7, 1H), 4.16 (d, J = 16.7, 1H), 4.02 (t, J = 6.6, 3H), 3.98 (m, 1H), 3.92 (m, 1H), 3.81 (dd, J = 12.1, 3.2, 1H), 3.73 (dd, J = 12.1, 3.7, 1H), 3.68 (m, 1H), 3.64−3.53 (m, 1H), 2.33−2.24 (m, 1H), 2.24−2.17 (m, 1H), 1.92 (d, J = 0.8, 3H), 1.86−1.76 (m, 2H), 1.77−1.67 (m, 2H) ppm. ^{13}C NMR (CD_3OD): δ 182.1, 180.6, 165.7, 152.6, 136.8, 110.9, 102.4, 89.1, 87.3, 72.3, 71.0, 63.9, 63.6, 62.9, 41.4, 41.2, 25.7, 23.0, 13.3 ppm. IR: ν 3409, 2941, 2107, 2030 1779, 1684, 1660, 1626, 1469, 1357, 1311, 1276, 1194, 1090, cm^{-1}. MS: m/z 700.87 $[C_{20}H_{25}N_4O_{12}Re]H^+$.

[NEt$_4$][Re(CO)$_3$L^2]. This complex was synthesized as per general procedure. Yield: 55%. ^{1}H NMR (CD_3OD): δ 7.84 (d, J = 0.9, 1H), 6.30 (t, J = 6.6, 1H), 4.43−4.35 (m, 1H), 4.00 (t, J = 6.5, 2H), 3.92 (q, J = 3.4, 1H), 3.80 (dd, J = 12.0, 3.2, 1H), 3.74 (d, J = 16.2, 2H), 3.73 (dd, J = 12.0, 4.3, 2H), 3.51 (d, J = 16.2, 2H), 3.40−3.36 (m, 2H), 3.30 (m, 8H, NEt_4^+), 2.30 (m, 1H), 2.21 (m, 1H), 1.91 (d, J = 0.9, 3H), 1.72 (m, 4H), 1.36−1.23 (m, 12H, NEt_4^+) ppm. ^{13}C NMR (CD_3OD): δ 199.0, 183.0, 165.7, 152.6, 136.7, 110.8, 89.0, 87.3, 72.2, 70.3, 64.2, 62.9, 53.4 (NEt_4), 41.5, 26.0, 23.3, 13.3, 7.7 (NEt_4) ppm. IR: ν 3363, 2952, 2019, 1865, 1693, 1624, 1575, 1467, 1393, 1366, 1279, 1185, 1173, 1092, 1055, 1000, 912, 771, 660, 643 cm^{-1}. HRMS: m/z 698.0987 $[C_{21}H_{25}N_3O_{12}Re]^-$ (calcd 698.0991).

[Re(CO)(NO)L^3]. This complex was synthesized as per general procedure, except that the pH of the reaction solution was increased to 7 with 1 M NaOH after dissolution of the ligand. Yield: 40%. ^{1}H NMR ($CDCl_3$): δ 3.63 (d, J = 15.8, 1H), 3.61 (d, J = 5.2, 1H), 3.56 (d, J = 5.2, 1H), 3.53 (d, J = 15.8, 1H), 3.45−3.29 (m, 2H), 2.84−2.66 (m, 2H), 2.54 (q, J = 7.4, 2H), 1.23 (t, J = 7.4, 3H) ppm. ^{13}C NMR ($CDCl_3$): δ 179.8, 176.9, 67.7, 64.4, 62.9, 27.1, 26.5, 14.7 ppm. IR: ν 2981, 1971, 1709, 1655, 1593, 1486, 1457, 1394, 1362, 1327, 1173, 1137, 1083, 1002, 925, 908, 786, 740 cm^{-1}. MS: m/z 464.69 $[C_9H_{13}N_2O_6ReS]H^+$.

[NEt$_4$][Re(CO)$_3$L^3]. This complex was synthesized as per general procedure, except that the pH of the reaction solution was increased to 7 with 1 M NaOH after dissolution of the ligand, and the product was isolated by precipitation as the reaction solution was concentrated. Yield: 65%. ^{1}H NMR (CD_3OD): δ 3.76 (d, J = 16.5, 2H), 3.56 (d, J = 16.5, 2H), 3.52−3.45 (m, 2H), 3.29 (m, 9H), 2.95−2.82 (m, 2H), 2.63 (q, J = 6.9, 2H), 1.31−1.28 (m, 15H) ppm. ^{13}C NMR (CD_3OD): δ 182.6, 70.1, 64.0, 53.3 (NEt_4), 27.6, 27.0, 15.4, 7.6 (NEt_4) ppm. IR: ν 2944, 2707, 2507, 2025, 1941, 1917, 1890, 1738, 1667, 1616, 1590, 1457, 1433, 1423, 1382, 1346, 1264, 1230, 1205, 1191, 1102, 1058, 990, 959, 919, 883, 794, 764, 702, 649, 630, 610 cm^{-1}. MS: m/z 489.67 $[C_{11}H_{13}NO_7ReS]^-$.

[Re(CO)$_2$(NO)L^4]. This complex was synthesized as per general procedure. Yield: 39%. Anal. calcd for $C_{20}H_{25}N_4O_{12}ReS$: C, 32.83; H, 3.44; N 7.66. Found: C, 32.31; H, 3.74; N 7.42. ^{1}H NMR (CD_3OD): δ 7.88 (s, 1H), 6.33 (t, J = 6.7, 1H), 4.46−4.36 (m, 2H), 4.31 (d, J = 16.6, 1H), 4.17 (t, J = 6.9, 2H), 4.09 (m, J = 16.6, 2H), 3.91 (m, 2H), 3.85−3.71 (m, 3H), 3.09 (t, 2H), 2.85 (m, 2H), 2.37−2.17 (m, 2H), 1.93 (s, 3H) ppm. ^{13}C NMR (CD_3OD): δ 190.0, 187.0, 181.9, 180.3, 165.3, 152.4, 136.8, 110.9, 89.0, 87.2, 72.2, 70.4, 63.9, 63.5, 62.8, 41.5, 41.5, 29.5, 26.7, 13.2 ppm. IR ν 3283, 2940, 2108, 2027, 1781, 1711, 1687, 1651, 1625, 1461, 1434, 1357, 1334, 1320, 1260, 1236, 1176, 1162, 1108, 1096, 1058, 1031, 998, 981, 943, 927, 911, 881, 850, 788, 768, 753, 649, 631 cm^{-1}. HRMS: m/z 733.0824 $[C_{20}H_{25}N_4O_{12}ReS]H^+$ (calcd 733.0819).

[Na][Re(CO)$_3$L^4]. This complex was synthesized as per general procedure. Yield: 85%. ^{1}H NMR (CD_3OD): δ 7.84 (d, J = 1.2, 1H), 6.30 (t, J = 6.6, 1H), 4.43−4.35 (m, 1H), 4.15 (t, 2H), 3.91 (q, J = 3.5, 1H), 3.86 (d, J = 16.4, 2H), 3.81 (dd, J = 12.1, 3.2, 1H), 3.73 (dd, J = 12.1, 3.7, 1H), 3.61 (d, J = 16.4, 2H), 3.56 (m, 2H), 3.02−2.94 (m, 2H), 2.80 (t, 2H), 2.30 (m, 1H), 2.22 (m, 1H), 1.92 (d, J = 1.2, 3H) ppm. ^{13}C NMR (CD_3OD): δ 182.7, 165.1, 136.6, 110.7, 88.8, 87.2, 71.9, 63.8, 62.7, 53.2, 41.4, 41.2, 29.4, 26.7, 13.1 ppm. IR: ν 3387, 2938, 2027, 1877, 1731, 1698, 1624, 1467, 1361, 1280, 1229, 1187, 1092, 1056, 995, 919, 784, 767, 648 cm^{-1}. HRMS: m/z 777.0581 $[C_{21}H_{26}N_3O_{12}ReSNa]Na^+$ (calcd 777.0584).

[Re(CO)$_2$(NO)L^5]. This complex was synthesized as per general procedure, except that the product was purified by semipreparative HPLC. Yield: 47%. ^{1}H NMR (CD_3OD): δ 7.80 (d, J = 0.9, 1H), 6.28 (t, J = 6.7, 1H), 4.36 (dt, J = 6.7, 3.5, 1H), 4.31 (d, J = 17.2, 1H), 4.22 (d, J = 16.3, 1H), 4.00 (m, 3H), 3.92 (t, J = 7.2, 2H), 3.88 (m, 1H), 3.77 (m, 2H), 3.72−3.61 (m, 2H), 2.91 (t, 3H), 2.66 (t, J = 7.2, 2H), 2.29−2.19 (m, 1H), 2.19−2.10 (m, 1H), 1.87 (d, J = 0.9, 3H), 1.71 (m, 2H), 1.61 (m, 2H) ppm. ^{13}C NMR (CD_3OD): δ 171.1, 170.2, 165.6, 152.5, 136.6, 110.9, 89.0, 87.2, 72.3, 62.9, 41.8, 41.4, 32.7, 28.3, 27.7, 27.6, 13.4 ppm. IR: ν 3397, 2936, 2109, 2034, 1784, 1743, 1688, 1662, 1627, 1469, 1358, 1313, 1268, 1192, 1177, 1131, 1268, 1192, 1177, 1131, 1093, 1053, 984, 915, 834, 798, 770, 752, 720, 636 cm^{-1}. MS: m/z.760.95 $[C_{22}H_{29}N_4O_{12}ReS]H^+$.

[NEt$_4$][Re(CO)$_3$L^5]. This complex was synthesized as per general procedure. Yield: 40%. ^{1}H NMR (CD_3OD): δ 7.74 (d, J = 1.1, 1H), 6.22 (t, J = 6.6, 1H), 4.31 (dt, J = 6.7, 3.5, 1H), 3.88 (t, J = 6.9, 2H), 3.83 (m, 1H), 3.75−3.60 (m, 5H), 3.47 (d, J = 16.0, 2H), 3.42−3.36 (m, 2H), 2.84−2.75 (m, 2H), 2.56 (t, J = 7.1, 2H), 2.25−2.06 (m, 2H), 1.82 (d, J = 1.1, 3H), 1.69−1.62 (m, 3H), 1.61−1.49 (m, 1H) ppm. ^{13}C NMR (CD_3OD): δ 182.8, 163.4, 163.1, 119.8, 116.9, 89.0, 87.3, 72.3, 70.2, 64.1, 62.9, 41.8, 41.4, 32.8, 28.5, 28.1, 27.0, 13.4 ppm. IR: ν 3374, 2932, 2286, 2026, 1881, 1692, 1623, 1469, 1365, 1266, 1189, 1092, 1053, 991, 918, 769, 649, 624, 608 cm^{-1}. MS: m/z 757.96 $[C_{23}H_{29}N_3O_{12}ReS]^-$.

X-Ray Crystallography. The intensities for the X-ray determinations were collected on a STOE IPDS 2T instrument with Mo Kα radiation. Standard procedures were applied for data reduction and absorption correction. Structure solution and refinement were performed with SHELXS97 and SHELXL97.[32] Hydrogen atom positions were calculated for idealized positions and treated with the "riding model" option of SHELXL.

Phosphorylation Transfer Assays. Thymidine and the organometallic thymidine derivatives [NEt$_4$][Re(CO)$_3$**L^1/L^2**], [Re(CO)$_2$(NO)**L^1/L^2**], [NEt$_4$][Re(CO)$_3$**L^4/L^5**], and [Re(CO)$_2$(NO)**L^4/L^5**] were assayed at 25 °C for 15 min in 200 μL of a mixture containing 155.38 μL of water; 10 μL of 1 M HEPES buffer (pH 7.5); 0.2 μL of 1 M DTT; 0.42 μL of 100 mM PEP; 0.5 μL of 1 M $MgCl_2$; 7.2 μL of 5 mM NADH; 2 μL of 100 mM ATP; 0.6 μL of pyruvate kinase (1350 U/mL); 0.7 μL of lactate dehydrogenase (1420 U/mL); 5 μL of hTK1 (~0.5 mg/mL); and 20 μL of a 10 mM 1:1 DMSO/water solution of thymidine, [NEt$_4$][Re(CO)$_3$**L^1/L^2**], [Re(CO)$_2$(NO)**L^1/L^2**], [NEt$_4$][Re(CO)$_3$**L^4/L^5**], or [Re(CO)$_2$(NO)**L^4/L^5**]. For each substrate, the linear decrease in UV absorption at 340 nm was measured from 0 to 15 min. The gradients of the regression lines between 6 and 12 min were compared to thymidine, the phosphorylation of which was assumed to be 100%.

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Supporting Information Available: Experimental procedures and analytical data for target compounds $\mathbf{L^1}-\mathbf{L^5}$, $[Re(CO)_2(NO)\mathbf{L^1}/\mathbf{L^2}/\mathbf{L^4}/\mathbf{L^5}]$, $[Re(CO)(NO)\mathbf{L^3}]$, $[Re(CO)_3\mathbf{L^1}-\mathbf{L^5}]^-$, and all intermediates. Table of bond lengths and bond angles for complexes $[Re(CO)_2(NO)\mathbf{L^1}]$ and $[Re(CO)(NO)\mathbf{L^3}]$. CIF files of the complexes $[Re(CO)_2(NO)\mathbf{L^1}]$ and $[Re(CO)(NO)\mathbf{L^3}]$. Protocols and analysis of the phosphorylation experiments. This material is available free of charge via the Internet at http://pubs.acs.org. Supplementary crystallographic data for this paper has been further deposited under 713659 ($[Re(CO)_2(NO)\mathbf{L^1}]$) and 713658 ($[Re(CO)(NO)\mathbf{L^3}]$) at the Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk/data_request/cif).