

$[\text{M}(\text{CO})_2(\text{NO})]^{2+}$, a new core in bioorganometallic chemistry: model complexes of $[\text{Re}(\text{CO})_2(\text{NO})]^{2+}$ and $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_2(\text{NO})]^{2+}$

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Abstract—In this study selected bidentate (L^2) and tridentate (L^3) ligands were coordinated to the Re(I) or Tc(I) core $[\text{M}(\text{CO})_2(\text{NO})]^{2+}$ resulting in complexes of the general formula *fac*- $[\text{MX}(\text{L}^2)(\text{CO})_2(\text{NO})]$ and *fac*- $[\text{M}(\text{L}^3)(\text{CO})_2(\text{NO})]$ ($\text{M} = \text{Re}$ or Tc ; $\text{X} = \text{Br}$ or Cl). The complexes were obtained directly from the reaction of $[\text{M}(\text{CO})_2(\text{NO})]^{2+}$ with the ligand or indirectly by first reacting the ligand with $[\text{M}(\text{CO})_3]^+$ and subsequent nitrosylation with $[\text{NO}][\text{BF}_4]$ or $[\text{NO}][\text{HSO}_4]$. Most of the reactions were performed with cold rhenium on a macroscopic level before the conditions were adapted to the n.c.a. level with technetium ($^{99\text{m}}\text{Tc}$). Chloride, bromide and nitrate were used as monodentate ligands, picolinic acid (PIC) as a bidentate ligand and histidine (HIS), iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) as tridentate ligands. We synthesised and describe the dinuclear complex $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})_2]$ and the mononuclear complexes $[\text{NEt}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$, $[\text{NEt}_4][\text{ReBr}_3(\text{CO})_2(\text{NO})]$, $[\text{ReBr}(\text{PIC})(\text{CO})_2(\text{NO})]$, $[\text{NMe}_4][\text{Re}(\text{NO}_3)_3(\text{CO})_2(\text{NO})]$, $[\text{Re}(\text{HIS})(\text{CO})_2(\text{NO})][\text{BF}_4]$, $[\text{}^{99\text{m}}\text{Tc}(\text{HIS})(\text{CO})_2(\text{NO})][\text{BF}_4]$, $[\text{}^{99\text{m}}\text{Tc}(\text{IDA})(\text{CO})_2(\text{NO})]$ and $[\text{}^{99\text{m}}\text{Tc}(\text{NTA})(\text{CO})_2(\text{NO})]$. The chemical and physical characteristics of the Re and Tc–dicarbonyl–nitrosyl complexes differ significantly from those of the corresponding tricarbonyl compounds.

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NO is a simple and stable paramagnetic molecule. The unpaired electron in the π^* orbital is easily released, thus creating a nitrosyl cation $[\text{NO}]^+$ with a stronger N–O bond and a higher electron affinity as compared to the original NO molecule. Nitrosylation reactions are well known in organometallic chemistry, but to our knowledge they have not been used for the preparation of $^{99\text{m}}\text{Tc}$ -complexes in the context of potential radiopharmaceutical applications so far.^{1,2}

On the other hand, labelling of various (bio)molecules using *fac*- $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$ has been reported during the last 5 years.^{3,4} One characteristic of the *fac*- $[\text{M}(\text{CO})_3]^+$ moiety is the presence of a ‘soft’ metal centre according to the definitions of Pearson ($\text{M} = \text{Re}$ or Tc).⁵ Replacing one of the three carbonyls in the $[\text{M}(\text{CO})_3]^+$ moiety by an NO^+ group changes the chemical and physical properties of the complex significantly and opens a new field of bioorganometallic chemistry with

low valent Re(I)- and Tc(I)-complexes. The introduced $[\text{NO}]^+$ is isoelectronic with CO, but a stronger π -acceptor and changes the charge of the complex by +1, thus creating a ‘harder’ metal centre as compared to the one in the original *fac*- $[\text{M}(\text{CO})_3]^+$ moiety.

For $^{99\text{m}}\text{Tc}$ compounds, $[\text{}^{99\text{m}}\text{Tc}(\text{OH}_2)_3(\text{CO})_3]^+$, generated directly from pertechnetate,⁶ serves as starting material to form the new *fac*- $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_2(\text{NO})]^{2+}$ moiety.⁷ Here we present complex formations of selected bidentate (L^2) and tridentate (L^3) ligands with Re and Tc via this ‘nitrosyl-approach’, which extends the number of potentially useful Tc-complexes for biological applications. The resulting complexes with the general formula *fac*- $[\text{MX}(\text{L}^2)(\text{CO})_2(\text{NO})]$ or *fac*- $[\text{M}(\text{L}^3)(\text{CO})_2(\text{NO})]$ ($\text{X} = \text{Br}$ or Cl) are described in detail.

For analytical reasons, radioprotection concerns and to further compare rhenium and technetium complexes, most of the reactions were performed with Re on a macroscopic level before the conditions were adapted to the n.c.a. level with $^{99\text{m}}\text{Tc}$. The starting material for this kind of rhenium chemistry was the complex $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})_2]$, which was described by Uguagliati and co-workers already in the seventies.⁸ However, the conversion of $[\text{ReCl}(\text{CO})_4]_2$ with NO-gas and HCl in

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boiling benzene to the desired NO-complex was performed in poor yields only. Berke and co-workers optimised the synthesis: a solution of $[\text{Re}_2(\text{CO})_{10}]$ in CCl_4 was flushed with Cl_2 -gas affording $[\text{ReCl}(\text{CO})_5]$, which was converted with $[\text{NEt}_4]\text{Cl}$ to $[\text{NEt}_4]_2[\text{ReCl}(\mu\text{-Cl})(\text{CO})_3]_2$. Subsequent nitrosylation with $[\text{NO}][\text{BF}_4]$ gave $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})]_2$. The nitrosylation reaction takes place selectively *trans* to the chlorides not involved in bridge formation, thus creating a single product with one NO group bound to each Re centre.⁹

In the present study, we cleaved the dinuclear compound $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})]_2$ with an excess of $[\text{NEt}_4]\text{Cl}$, resulting in the mononuclear complex $[\text{NEt}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$. In a typical procedure $[\text{NEt}_4]_2[\text{ReCl}(\mu\text{-Cl})(\text{CO})_3]_2$ (6.5 g, 6.9 mmol) was dissolved in 150 mL CH_2Cl_2 and $[\text{NO}][\text{BF}_4]$ (1.62 g, 13.84 mmol) was added. The inhomogeneous mixture became orange and clear within 1 h. After 48 h at room temperature, the solvent was removed, the residue washed with water and again dried, yielding 4.17 g (6.07 mmol, 88%) of the yellow $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})]_2$ (a dimer containing only one NO-group was not detected). 200 mg (0.29 mmol) of this dinuclear complex in 50 mL CH_2Cl_2 was stirred with $[\text{NEt}_4]\text{Cl}$ (96 mg, 0.058 mmol) overnight at room temperature to form the mononuclear complex. Crystallisation from CH_2Cl_2 /hexane (1:1) yielded 255 mg (0.501 mmol, 86%) of $[\text{NEt}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$, which was characterised by IR-spectroscopy, mass spectroscopy (MS-FAB⁺=379) and X-ray structure analysis. IR-spectroscopy is a fast and convenient method to follow the nitrosylation process, as for example, the broad bands of $[\text{ReCl}_3(\text{CO})_3]$ (2004 cm^{-1} , 1884 cm^{-1}) are replaced by three sharper bands of $[\text{ReCl}_3(\text{CO})_2(\text{NO})]$ at 2093, 2018 and 1770 cm^{-1} . The NO-band in the region of 1770 cm^{-1} is very characteristic for all complexes with the $[\text{M}(\text{CO})_2(\text{NO})]^{2+}$ moiety and well separated from the CO-bands. Complexes of the type *fac*- $[\text{Re-X}_3(\text{CO})_2(\text{NO})]^-$ (X = Br or Cl) served as precursors for all experiments with rhenium.

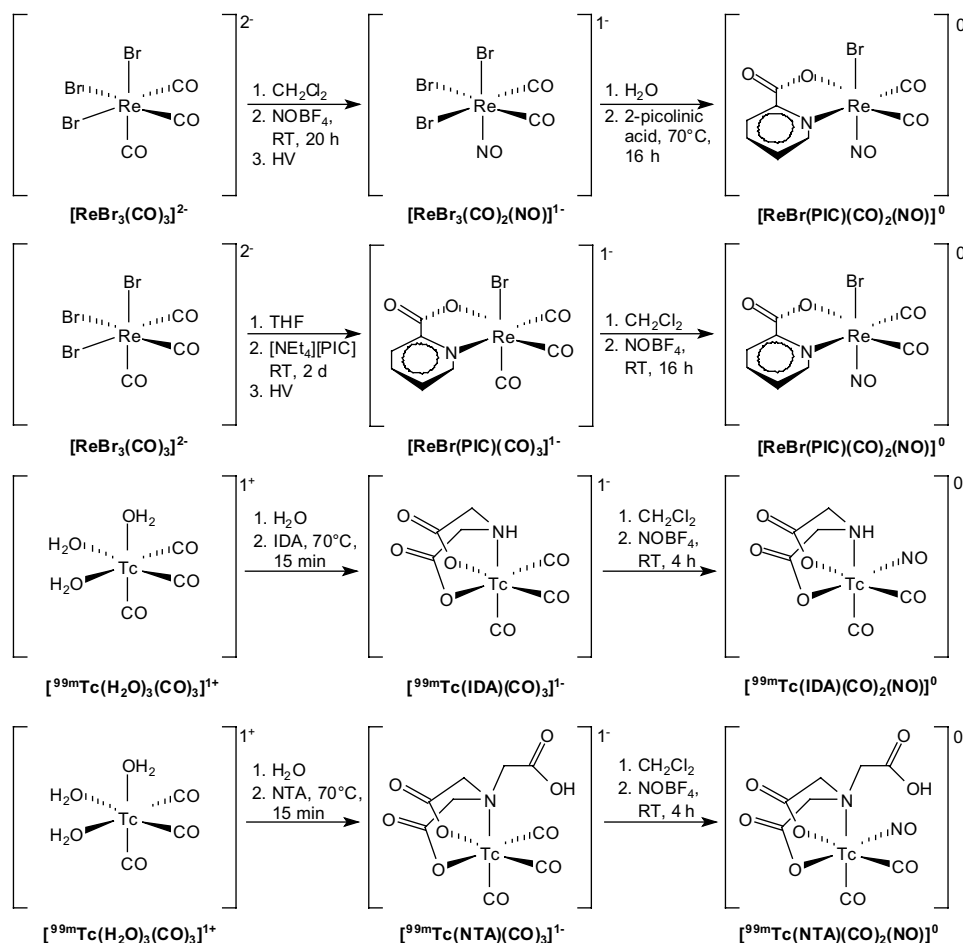
For the conversion of $[\text{M}(\text{CO})_3]^+$ to $[\text{M}(\text{CO})_2(\text{NO})]^{2+}$ (M = Re, Tc) we explored two different sources of $[\text{NO}]^+$. $[\text{NO}][\text{BF}_4]$ was used in dichloromethane or acetonitrile at room temperature, $[\text{NO}][\text{HSO}_4]$ in water at 70 °C. The yields were higher with $[\text{NO}][\text{BF}_4]$, but the change to an organic solvent in the synthesis is a drawback for potential future radiopharmaceutical applications.

For the synthesis of complexes with the general formula *fac*- $[\text{M}(\text{L}^3)(\text{CO})_2(\text{NO})]$ or *fac*- $[\text{MX}(\text{L}^2)(\text{CO})_2(\text{NO})]$, two different procedures have been reported: nitrosylation of the metal-tricarbonyl precursor followed by reaction with the ligand or formation of the metal-tricarbonyl-ligand complex and subsequent nitrosylation.¹⁰ We found that the latter approach works best if the $[\text{M}(\text{L}^3)(\text{CO})_3]$ or *fac*- $[\text{MX}(\text{L}^2)(\text{CO})_2(\text{NO})]$ complex is negatively charged, as demonstrated for iminodiacetic acid (IDA), nitrilotriacetic acid (NTA) and 2-picolinic acid (PIC) (Scheme 1).

The ^{99m}Tc-complexes were analysed by reversed phase HPLC (X-Terra RP-18 column, 250 mm × 4.6 mm/Waters; gradient elution from 0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in acetonitrile in 20 min; flow rate 1 mL/min) and LC-MS (Waters separation module, XTerra MS C18 column 50 mm × 2.1 mm, 3-inch NaI(Tl) radiation detector, Micromass LCT mass spectrometer and MassLynx software/Waters-Micromass). The Re-complexes were characterised by IR (Bio-Rad FTS-45 or Perkin-Elmer FT-IR-16PC spectrometer), mass spectroscopy (Finnigan MAT-8320) and for some of the compounds also by X-ray structure analysis (Siemens Nicolet R3m/V2000 diffractometer).

The ^{99m}Tc-tricarbonyl complexes $[\text{sup}99\text{mTc}(\text{IDA})(\text{CO})_3]^-$ (HPLC retention time = 7.2 min) and $[\text{sup}99\text{mTc}(\text{NTA})(\text{CO})_3]^-$ (t_R = 12.1 min) were formed with yields >90% at pH 8–9. The conversion to the corresponding ^{99m}Tc-dicarbonyl-nitrosyl complexes was carried out using $[\text{NO}][\text{BF}_4]$ in dichloromethane. Results of the analyses by LC-MS support the supposed structures of both Tc-dicarbonyl-nitrosyl complexes shown above (Scheme 1). Generator eluate with a higher amount of ^{99m}Tc (0.1–0.3 ng/MBq) was used for the synthesis of the complexes to enhance the signal in mass spectroscopy. We isolated the compounds by HPLC and found a mass of 315.5 Da (theoretical: 315.9) for the IDA-complex and 375.2 Da (theoretical: 374.0) for the NTA-complex. Both $[\text{sup}99\text{mTc}(\text{IDA})(\text{CO})_2(\text{NO})]^\circ$ and $[\text{sup}99\text{mTc}(\text{NTA})(\text{CO})_2(\text{NO})]^\circ$ showed a clearly different behaviour in HPLC (t_R = 8.2 and 7.4 min, respectively) as compared to the corresponding Tc-tricarbonyl complexes. The influence of the different lipophilicity on the biological properties will be studied by biodistribution experiments in animals.

In aqueous solution, the reactive metal-tricarbonyl species is the tris-aquo complex $[\text{M}(\text{OH}_2)_3(\text{CO})_3]^+$. In the metal-dicarbonyl-nitrosyl precursor, however, one halide remains bound to the metal centre, generating a $[\text{MX}(\text{OH}_2)_2(\text{CO})_2(\text{NO})]$ -complex. The halide is located *trans* to the NO-group of the molecule due to stronger π -backbonds as compared to the CO-groups.⁷ As a consequence, the $[\text{MX}(\text{CO})_2(\text{NO})]$ -moiety has only two easily accessible coordination sites as compared to three in tricarbonyl compounds. Tridentate ligands have to replace the remaining halide in order to form a $[\text{Re}(\text{L}^3)(\text{CO})_2(\text{NO})]$ -complex. To enhance the rate of complex formation, the halides were removed by precipitation with AgNO_3 before the corresponding ligand was added. Although the formation of stereoisomers with the tridentate ligands presented in this study is theoretically possible, IR-analysis gave no evidence for such formation. Typically, $[\text{NMe}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$ (50 mg, 0.11 mmol) was dissolved in 10 mL ethanol and AgNO_3 (56 mg, 0.33 mmol) was added. The filtered solution was evaporated to dryness and the residue crystallised from CH_2Cl_2 /hexane (1:1) to yield 47 mg (0.088 mmol, 80%) of the complex $[\text{NMe}_4][\text{Re}(\text{NO}_3)_3(\text{CO})_2(\text{NO})]$ as yellow crystals (Fig. 1).¹¹ In the IR-spectrum (KBr) of this novel complex the Re-NO and Re-CO bands showed a significant shift to higher wave numbers as compared to the complex with



Scheme 1. Synthesis of the complexes $[\text{ReBr(PIC)(CO)}_2(\text{NO})]^0$, $[\text{}^{99\text{m}}\text{Tc(IDA)(CO)}_2(\text{NO})]^0$ and $[\text{}^{99\text{m}}\text{Tc(NTA)(CO)}_2(\text{NO})]^0$ starting from Re and Tc-tricarbonyl precursors (HV = high vacuum; RT = room temperature).

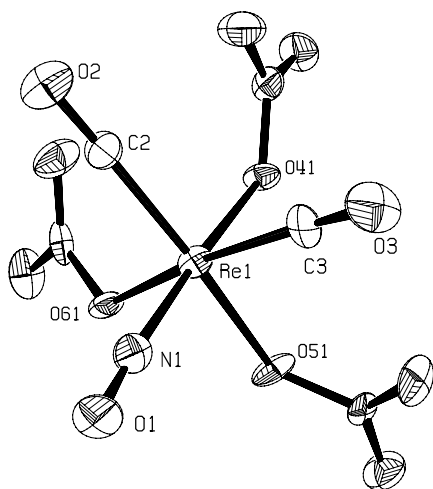


Figure 1. ORTEP drawing of $[\text{Re}(\text{NO}_3)_3(\text{CO})_2(\text{NO})]^-$.

three halides: $2114\text{ cm}^{-1}(\text{CO})$, $2047\text{ cm}^{-1}(\text{CO})$ and $1794\text{ cm}^{-1}(\text{NO})$. Also the bond lengths varied from the trichloro to the trinitrato complex: the Re–NO-bond with a $[\text{Cl}]^-$ in *trans* position to it was with 1.715 \AA significantly shorter than the 1.81 \AA for the Re–NO bond in *trans* position to a $[\text{NO}_3]^-$, while the Re–CO-

bond remained almost unchanged in both complexes (1.94 \AA in average). In the trinitrato complex, the Re–ONO₂ bond in *trans* position to the nitrosyl group was shorter (2.08 \AA) compared to both Re–ONO₂ bonds in *trans* position to the carbonyl groups (2.11 and 2.10 \AA , respectively). Again, the crystal structure demonstrated the changes in the complex caused by the replacement of a carbonyl by a nitrosyl group. The three weakly bound monodentate NO₃-groups of the complex could easily be replaced by chelating ligands, making it a convenient precursor for the reaction with various target molecules.

Histidine is known as an excellent ligand for the $[\text{M}(\text{CO})_3]$ -system, so we selected it as the model of a tridentate ligand for the reaction with the $[\text{M}(\text{CO})_2(\text{NO})]^{2+}$ moiety. In a typical procedure to obtain $[\text{Re}(\text{HIS})(\text{CO})_2(\text{NO})][\text{BF}_4]$, $[\text{NEt}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$ (50 mg , 0.078 mmol) was dissolved in 3 mL ethanol and the halides were precipitated with AgBF_4 (45 mg , 0.231 mmol) and filtered off (filtrate = solution A). Separately, a suspension of L-histidine (12.1 mg , 0.078 mmol) in $52\text{ }\mu\text{L}$ of a 1.5 M solution of $[\text{NEt}_4]\text{OH}$ in methanol (0.078 mmol) was stirred until complete dissolution and then evaporated to dryness. The solution of this residue in 3 mL ethanol was added to solution A. After 5 days at room temperature the reaction mixture

was evaporated to dryness, the residue washed twice with dichloromethane and again evaporated to dryness. $[\text{Re}(\text{HIS})(\text{CO})_2(\text{NO})][\text{BF}_4]$ was isolated as an orange solid with a yield of 30.8 mg (0.060 mmol, 77%) and characterised. Besides the high bands of CO (2112, 2040 cm^{-1} , very strong) and NO (1790 cm^{-1} , very strong), additional bands for the CO-group of the amino acid (1666 cm^{-1} , strong) and for the counter ion BF_4^- (1068 cm^{-1} , very strong, broad) characterised the complex in the IR-spectrum. Mass analysis (FAB⁺, DMSO) showed the calculated peak at 427 (M⁺). ¹H NMR spectroscopy (*d*₆-DMSO) also supported the suggested structure: δ (ppm) = 8.87 (s, 1H, imidazole-HIS-C5), 7.34 (s, 1H, imidazole-HIS-C6), 4.26 (m, 1H, HIS-C2), 3.38–3.26 (m, 2 H, HIS-C3).

The corresponding Tc-complex $^{99}\text{Tc}(\text{HIS})(\text{CO})_2(\text{NO})[\text{BF}_4]$ was obtained the same way in 56% yield, starting from $[\text{NEt}_4][^{99}\text{TcCl}_3(\text{CO})_2(\text{NO})]$.

In conclusion, the syntheses of the complexes $[\text{ReCl}(\mu\text{-Cl})(\text{CO})_2(\text{NO})]_2$, $[\text{NEt}_4][\text{ReCl}_3(\text{CO})_2(\text{NO})]$, $[\text{NEt}_4][\text{ReBr}_3(\text{CO})_2(\text{NO})]$, $[\text{NMe}_4][\text{Re}(\text{NO}_3)_3(\text{CO})_2(\text{NO})]$ and $[\text{NEt}_4][^{99}\text{TcCl}_3(\text{CO})_2(\text{NO})]$ confirm that Re(I) and Tc(I)-dicarbonyl-nitrosyl complexes can conveniently be obtained. All tested metal-dicarbonyl-nitrosyl complexes showed a high stability, comparable to the corresponding tricarbonyl compounds. Depending on the ligands, the conversion to the nitrosyl complex can be done using either nitrosylation of the $[\text{MX}_3(\text{CO})_3]$ -complex followed by reaction with a ligand or formation of the $[\text{MX}(\text{L}^2)(\text{CO})_3]$ or $[\text{M}(\text{L}^3)(\text{CO})_3]$ complex followed by nitrosylation with $[\text{NO}][\text{BF}_4]$. The latter method offers the possibility to introduce the NO-group even in a later stage of a labelling procedure.

Based on these results the NO-chemistry was also adapted to the n.c.a. level with ^{99m}Tc. First experimental data show formation of the intended $^{99m}\text{Tc}(\text{L}^3)(\text{CO})_2(\text{NO})$ complexes and biodistribution

studies are in progress. The investigated bidentate and tridentate ligands are suitable models for (larger) biomolecules or can serve as bifunctional chelating ligands. In this way the 'nitrosyl-approach' offers a new synthetic tool for the labelling of biomolecules and extends the possibilities of the already established metal tricarbonyl chemistry.

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11. Experimental data for $[\text{NMe}_4][\text{Re}(\text{NO}_3)_3(\text{CO})_2(\text{NO})]$: MS (FAB⁻, acetone): 459 (M⁺), 394, 332, 241; ¹³C NMR (D₂O): δ (ppm) = 187.2; Crystal data: MW = 532.38, orthorhombic, *Pbca*, *a* = 10.558(1) Å, *b* = 10.7853(9) Å, *c* = 26.742(3) Å, *V* = 3045.2(6) Å³, *Z* = 8, *D*_{calc} = 2.32 g/cm³, μ = 81.6 mm⁻¹, Mo-K_α radiation (λ = 0.71073 Å), 3077 reflections, 2347 with *F* > 2σ(*F*) used for refinement, *R* = 0.068, w*R* = 0.048.