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First organometallic inhibitors for human thymidine kinase: synthesis and in vitro evaluation of rhenium(I)- and technetium(I)-tricarbonyl complexes of thymidine

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Abstract

Six 5′-carboxamide derivatives of 5′-aminothymidine have been synthesized with alkyl chains of various length and a tridentate, imino diacetic acid based chelating system. The thymidine analogues have been reacted with the precursor *fac*-$[M(H_2O)_3(CO)_3]^+$ ($M = {}^{99m}Tc$, Re) in aqueous media to form, water-soluble and stable organometallic complexes in good yields. ^{1}H-NMR and IR spectroscopic analyses confirmed in all cases the tridentate complexation of the metal-tricarbonyl fragment exclusively via the tridentate chelates, and no unspecific interaction with other functional groups of the pharmacophor. The organometallic rhenium–nucleoside complexes have been tested in vitro for competitive inhibition of human cytosolic thymidine kinase (hTK1) and herpes simplex virus thymidine kinase type 1 (HSV1-TK). In case of hTK1 it could be observed, that the inhibition capacity of the complexes improved with increasing spacer length. On the other hand, all six complexes showed no or only slight inhibition of the HSV1-TK. The corresponding radioactive technetium-99m complexes have been prepared and challenged for stability in physiological phosphate buffer and human serum albumin at 37 °C for 24 h. Only minor decomposition of the complexes could be detected under these conditions proving the high kinetic inertness and/or stability of these complexes.

Keywords: Rhenium(I); Technetium(I); Thymidine analogues; Human thymidine kinase; Viral thymidine kinase

1. Introduction

Nucleoside kinases play a pivotal role in the use of nucleosides for cancer and antiviral therapy [1]. In this context the human cytosolic thymidine kinase (hTK1) and the herpes simplex virus type 1 thymidine kinase (HSV1-TK) are important target enzymes. Proliferating cells reveal a dramatically increased hTK1 activity compared to quiescent cells. These characteristics form the base of a selective targeting of growing tumor cells. On the other hand, introduction of reporter genes, such as for the HSV1-TK has emerged as a very powerful tool to monitor the delivery, magnitude, and time variation of therapeutic gene transfer in vivo. These reporter genes can be coupled with a therapeutic gene of interest to indirectly monitor the expression of the therapeutic gene. In case of HSV1-TK a selective targeting is facilitated due to the relaxed substrate specificity of the enzyme as compared with mammalian thymidine kinase. In both respects (tumor proliferation and gene therapy monitoring) radioactive labeled probes allow a non-invasive diagnosis and early assessment of tumor growth and response to therapeutic approaches. Typically, either positron-labeled substrates for intracellular enzymes or positron-labeled ligands for cellular receptors are used as radioactive probes. However, there would be a great interest to develop Tc-99m based diagnostic probes because of the favorable decay properties (γ, 140 keV, $t_{1/2} = 6$ h), low costs and easy availability of this radionuclide. In addition, due to the

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$^{188}W/^{188}Re$-generator technique (Re-188: β^-, 2.1 MeV, $t_{1/2} = 17$ h) a therapeutic pendant of technetium is nowadays also readily available. Particularly organometallic technetium(I) and rhenium(I) modified analogues of thymidine could be attractive candidates for gene therapy monitoring and diagnosis/therapy of proliferating tumor cells. The high kinetic inertness and relatively small size of e.g. the technetium(I)- and rhenium(I)-tricarbonyl core as compared to common Tc(V)/Re(V) complexes of the Werner type is a decisive advantage for bio-medical purposes due to increased in vivo stability and reduced interference with biological activity.

Herein, we report the functionalization, synthesis and structural characterization of a series of novel 5′-aminothymidine analogues and their corresponding technetium-99m(I)/rhenium(I)-tricarbonyl labeled complexes. The biological characterization and evaluation of the organometallic rhenium compounds were performed in vitro against hTK1 respectively HSV1 TK. The Tc-99m analogues have been challenged in vitro in physiological phosphate buffer and human serum albumin.

2. Experimental

Solvents for syntheses were purchased from Aldrich Chemical Co. or Fluka, Buchs, Switzerland and were dried according to standard methods. The organometallic precursor $[NEt_4]_2[ReBr_3(CO)_3]$ and the radioactive precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ were prepared as previously reported [2,3]. 5′-Amino-thymidine [4] and 2-hydroxy-5-amino-pentanoic acid **13** [5] were synthesized according to the literature. The γ-cyano-3-phenylbutanoic acid **10** was synthesized starting from ethyl-α-carbethoxy-γ-cyano-β-phenyl-butyrate [6]. Compounds **2**–**5** have been synthesized by a previously published procedure [7]. $Na[^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$ generator (Mallinckrodt-Tyco, Petten, Netherlands) using 0.9% saline. HPLC analyses of the rhenium and technetium-99m complexes were performed on a Merck-Hitachi L-7000-system equipped with an L-7400 tunable absorption detector and a Berthold LB 506 B radiometric detector using a Macherey-Nagel C-18 reversed phase column (10 μm, 150 × 4.1 mm). HPLC solvents: Aqueous 0.05 M TEAP (triethylammonium phosphate) buffer, pH 2.25 (solvent A), MeOH (solvent B). The HPLC system started with 100% of A from 0 to 3 min. The eluent switched at 3 min to 75% A and 25% B and at 9 min to 66% A and 34% B followed by a linear gradient 66% A/34% B to 100% B from 9 to 20 min. The gradient remained at 100% B for 2 min before switching back to 100% A. The flow rate was 1 ml min^{-1}. Nuclear magnetic resonance spectra were recorded on a 300 MHz Varian Gemini 2000 spectrometer. The ^1H and ^{13}C chemical shifts are reported relative to residual solvent protons as a reference. IR spectra were recorded on a Perkin–Elmer FT-IR 16PC using KBr pellets. Mass and elemental analyses were performed at the Swiss Federal Institute of Technology, ETH, Zurich.

2.1. In vitro HPLC assay

Human cytosolic TK was assayed by using a protocol published by Pilger et al. [8] with some modifications. Briefly: hTK1 (2–5 μg) was incubated at 37 °C for 15 min in a mixture (75 μl) containing 50 mM Tris, pH 7.4, 5 mM ATP, 5 mM $MgCl_2$, 1 mM deoxythymidine and different concentrations of the rhenium complexes (0.5–2 mM). The reaction was terminated by adding EDTA (2.5 mM, 675 μl) and analyzed by HPLC. 0.2 M NaH_2PO_4, 25 mM tetrabutylammonium hydrogensulfate and 3% MeOH were used as the mobile phase and the flow rate was 1.1 ml min^{-1}. A Macherey-Nagel C-18 reversed phase column (10 μm, 150 × 4.1 mm) was used. UV-absorption was measured at 254 nm. Samples without substrate (thymidine) or without inhibitor were used as blank reactions. The formation of adenosine diphosphate and thymidine monophosphate was monitored qualitatively. HSV1-TK was assayed under the same reaction conditions.

2.2. Syntheses of compounds 6–9

The syntheses of compounds **6**–**9** was performed according to the following, general procedure: Compounds **2**–**5** (one equivalent) were dissolved in DMF. *N*,*N*′-dicyclohexyl carbodiimide (DCC; 1.1 equivalent) and *N*-hydroxy succinimide (NHS; 1.1 equivalent) were added. The reactions were stirred at 60 °C for 2 h. The formation of the active ester was monitored by TLC. 5′-Aminothymidine (1 equivalent) was added and the reaction stirred over night at 40 °C. When the reaction has finished (monitored by TLC) the solvent was removed under vacuum, and the crude product was purified by chromatography on silicagel (EtOAc–CH_2Cl_2–MeOH: 8/1/1). The saponification of the methyl ester was performed in 1 M NaOH for 3 h at room temperature (r.t.) and monitored by HPLC. Analytical data for **6**: ^1H-NMR (D_2O): δ 1.7 (s, 3H), 1.8 (m, 2H), 2.2 (m, 2H), 2.2 (m, 2H), 3.2 (m, 2H), 3.3 (d, 2H), 3.8 (q, 1H), 4.0 (s, 2H), 4.2 (m, 1H), 6.1 (t, 1H), 7.3 (s, 1H), ^{13}C-NMR (Me_2SO): δ 168.1, 167.2, 160.0, 146.4, 131.9, 105.8, 81.0, 79.7, 67.8, 67.6, 46.5, 36.2, 34.6, 28.5, 21.9, 8.3; HPLC: R_t: 9.3 min.

Analytical data for **7**: ^1H-NMR (D_2O): δ 1.1 (m, 12H), 1.4–1.6 (m, 4H), 1.7 (s, 3H), 2.1–2.2 (m, 4H), 2.9–3.0 (m, 10H), 3.1 (s, 4H), 3.3 (m, 2H), 3.8 (m, 1H), 4.2 (m, 1H), 6.0 (t, 1H), 7.3 (s, 1H); ^{13}C-NMR (CD_3OD): δ 174.3, 169.1, 165.1, 151.1, 137.2, 110.5, 85.9, 85.1, 71.8, 71.5, 57.4, 41.1, 38.6, 34.8, 24.9, 22.3,

11.1; HPLC: $R_t = 10.0$ min. Analytical data for **8**: ^{1}H-NMR (CD_3OD): δ 1.4 (m, 6H), 1.6–1.8 (m, 4H), 2.0 (s, 3H), 2.3 (m, 4H), 2.8 (m, 2H), 3.5 (d, 2H), 3.8 (s, 4H), 4.0 (m, 1H), 4.3 (m, 1H), 6.3 (t, 1H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 175.5, 172.2, 165.1, 151.2, 138.2, 136.1, 110.7, 85.9, 84.5, 72.6, 71.5, 54.6, 54.1, 41.3, 39.0, 36.0, 29.2–25.3, 12.2; HPLC: $R_t = 10.6$ min. Analytical data for **9**: ^{1}H-NMR (D_2O): δ 1.2 (m, 12H), 1.5 (m, 2H), 1.7 (m, 2H), 1.9 (s, 3H), 2.2 (m, 2H), 2.3–2.4 (m, 4H), 3.4 (d, 2H), 3.7 (s, 4H), 4.1 (m, 1H), 4.4 (m, 1H), 6.3 (t, 1H), 7.5 (s, 1H); ^{13}C-NMR (CD_3OD): δ 177.6, 170.7, 166.5, 151.7, 137.5, 111.6, 85.4, 84.4, 71.6, 57.1, 56.3, 40.9, 38.1, 35.9, 32.7, 28.1–29.2, 25.5, 11.7; HPLC: $R_t =$ 16.9 min.

2.3. Synthesis of compound *11*

The γ-cyano-3-phenylbutanoic acid **10** (1.7 mmol) was solved in DMF (10 ml) and DCC (1.8 mmol) and NHS (1.8 mmol) were added. The solution was stirred for 90 min at 50 °C. 5′-Aminothymidine (1.6 mmol) was added in portions to the reaction solution. The DMF was removed in vacuum and the product was purified by chromatography on silica gel (EtOAc–MeOH: 9/1) and checked via ^{1}H-NMR spectroscopy. Yield: 284 mg (52%). ^{1}H-NMR (CD_3OD): δ 2.0 (d, 3H), 2.3 (m, 2H), 2.8 (m, 4H), 3.5 (m, 3H), 3.9 (m, 1H), 4.3 (m, 1H), 6.2 (m, 1H), 7.4–7.5 (m, 5H), 7.6 (s, 1H). The cyano group was reduced in dry MeOH with HCl conc. (100 μl) and Pd/C 10%. H_2 was bubbled through the solution over night. The solution was filtered over celite and the product (301 mg, 98%) was used without further purification. ^{1}H-NMR (CD_3OD): δ 2.0 (d, 3H), 2.1 (m, 2H), 2.3 (m, 2H), 2.7 (m, 4H), 3.5 (m, 3H), 3.9 (m, 1H), 4.3 (m, 1H), 6.2 (m, 1H), 7.4–7.5 (m, 5H), 7.6 (s, 1H).

2.4. Syntheses of compounds *12* and *15*

To a solution of **11** or **14** (0.6 mmol) in MeOH, NEt_3 (1.9 mmol) and methylbromoacetate (1.3 mmol) was added drop wise under an atmosphere of nitrogen. The reaction mixture was refluxed over night, the solvent removed and the product was purified by chromatography on silica gel (EtOAc–MeOH: 9/1). The saponification of the ester groups was performed in 1 N NaOH for 3 h at r.t. (Scheme 2). Analytical data for **12**: ^{1}H-NMR (CD_3OD): δ 2.0 (s, 3H), 2.2 (m, 2H), 2.6 (m, 2H), 2.6 (m, 4H), 3.2 (m, 1H), 3.4 (d, 2H), 3.5 (s, 4H), 3.9 (m, 1H), 4.2 (m, 1H), 6.2 (t, 1H), 7.2–7.4 (m, 5H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 172.8, 169.8, 165.3, 151.2, 142.2, 137.1, 128.8–127.1, 110.3, 85.7, 84.9, 71.7, 71.6, 57.5, 41.0, 40.6, 38.6, 30.2, 10.9; HPLC: $R_t = 15.4$ min. Analytical data for **15**: ^{1}H-NMR (CD_3OD): δ 1.8–1.9 (m, 4H), 1.9 (s, 3H), 2.4 (m, 2H), 3.4 (m, 2H), 3.6 (d, 2H), 4.1 (m, 1H), 4.1 (s, 4H), 4.2 (m, 1H), 4.4 (m, 1H), 6.2 (t, 1H), 7.5 (s, 1H); ^{13}C-NMR (D_2O): δ 176.6, 169.3, 166.9, 152.1, 139.1, 136.9, 112.0, 85.8, 85.0, 71.7, 70.9, 57.7, 55.7, 38.2, 31.8, 20.1, 12.9; HPLC: $R_t = 9.9$ min.

2.5. Syntheses of the complexes *16a–21a*

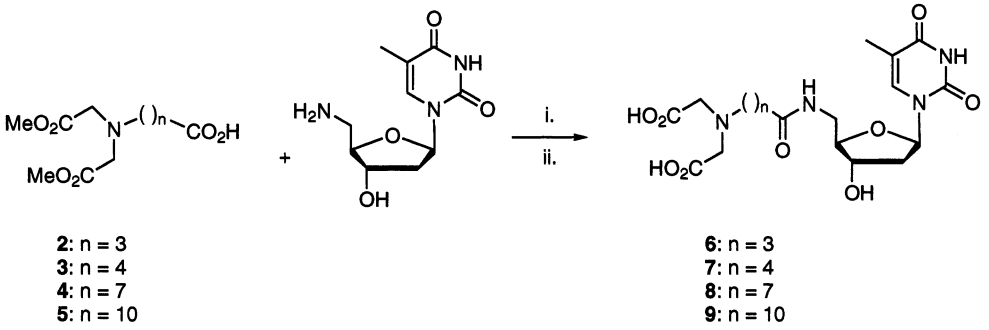
The complexes **16a–21a** were prepared according to the following general procedure: 900 μl of a solution of *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ (1.8–8 GBq) and 100 μl of a 10^{-3} M solution of the corresponding ligand in PBS buffer (0.1 M NaCl/0.05 M sodium phosphate buffered, pH 7.4) were placed in a 10-ml glass vial under nitrogen. The vial was sealed and the reaction heated to 75 °C for 60 min and cooled on an ice bath. The complex formation was checked by HPLC.

2.6. Syntheses of complexes *16b–21b*

The Re(CO)$_3$–thymidine-complexes were synthesized according to the following general procedure: (NEt$_4$)$_2$[ReBr$_3$(CO)$_3$] and the ligands **6–9**, **12** and **15** in equimolar amounts were solved in a H_2O–MeOH mixture (1/1) (5 ml) and stirred at 60 °C till the disappearance of the starting material (monitored by means of HPLC). The solution was evaporated to dryness and resolved in a small amount of water. The resulting complexes were purified by chromatography on SepPak®-cartridge (Waters, C18-cartridges) using a water–MeOH gradient. Analytical data for complex **16b**: Yield: 64 mg (76%); ^{1}H-NMR (D_2O): δ 1.12 (t, 4H), 1.78 (s, 3H), 1.8 (m, 2H), 2.2 (m, 2H), 2.2 (m, 2H), 3.0 (q, 3H), 3.2 (m, 2H), 3.4 (d, 2H), 3.4–3.6 (AB, 4H), 3.8 (q, 1H), 4.2 (m, 1H), 6.1 (t, 1H), 7.4 (s, 1H), ^{13}C-NMR (D_2O): δ 192.4 (CO), 191.8 (CO), 178.4, 170.7, 162.1, 146.9, 133.1, 106.8, 81.0, 79.7, 66.8, 63.6, 57.5, 47.2, 36.6, 33.5, 27.9, 16.1, 6.3, 1.9; Anal. Calc. for $C_{21}H_{24}N_4O_{12}Re(C_8H_{20}N)_{0.3}Na_{3.2}Br_{2.5}\cdot 0.5H_2O$: C, 27.23; H, 3.03; N, 5.83. Found: C, 27.51; H, 3.98; N, 5.72%; IR (KBr): 3750 br, 2014 s, 1890 vs, 1684 s, cm^{-1}; HPLC: R_t: 18.5 min; MS (ESI): *m*/*z* (%) 709 (60), 711 (100) [M$^-$].

Analytical data for complex **17b**: Yield: 57.8 mg (61%); ^{1}H-NMR (CD_3OD): δ 1.3–1.4 (m, 12H), 1.75–1.85 (m, 4H), 2.0 (s, 3H), 2.3–2.4 (m, 4H), 3.3–3.4 (m, 10H), 3.5 (d, 2H), 3.5–3.8 (AB, 4H), 4.0 (q, 1H), 4.4 (m, 1H), 6.3 (t, 1H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 197.8 (CO), 196.9 (CO), 181.8, 174.7, 165.3, 151.2, 137.0, 110.6, 86.0, 85.4, 71.7, 63.0, 52.0, 52.5, 50.8, 41.4, 38.0, 35.3, 24.4, 22.9, 11.7; Anal. Calc. for $C_{22}H_{26}N_4O_{12}Re(C_8H_{20}N)_{1.5}Na_{1.5}Br_{1.3}Cl_{0.7}\cdot H_2O$: C, 37.08; H, 5.31; N, 6.99. Found: C, 37.37; H, 5.38; N, 6.16%; IR (KBr): 3418 br, 2014 s, 1998 vs, 1874 w, 1684 s, 1654 m cm^{-1}; HPLC: $R_t = 16.1$ min; MS (ESI): *m*/*z* (%) 725.2 (100), 723.2 (60) [M$^-$].

Analytical data for complex **18b**: Yield: 76 mg (86%); ^{1}H-NMR (CD_3OD): δ 1.3–1.4 (m, 18H) 1.7–1.8 (m,



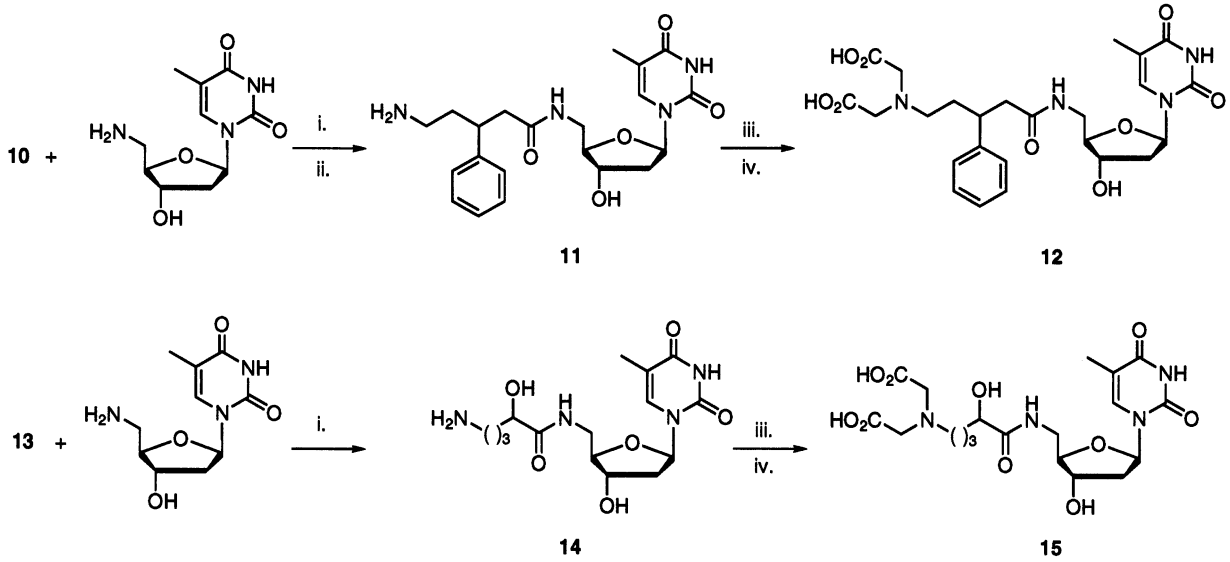
Scheme 1. (i) DCC, NHS in DMF, 60 °C, 2 h; (ii) 1 N NaOH, r.t., 3 h.

4H), 2.0 (s, 3H), 2.3 (m, 4H), 3.3–3.4 (m, 10H), 3.5 (d, 2H), 3.6–3.8 (AB, 4H), 4.0 (m, 1H), 4.3 (m, 1H), 6.3 (t, 1H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 199.0 (CO), 198.0 (CO), 183.0, 176.8, 166.0, 151.2, 139.6, 137.4, 112.0, 88.0, 85.5, 74.0, 71.0, 64.4, 52.0, 42.7, 40.2, 37.3, 30.0–26.0, 13.4; Anal. Calc. for $C_{25}H_{32}N_4O_{12}Re(C_8H_{20}N)_{0.6}Na_{0.4}\cdot 1.5H_2O$: C, 40.62; H, 5.38; N, 7.31. Found: C, 40.19; H, 5.45; N, 6.88%; IR (KBr): 3384 br, 2020 s, 1880 vs, 1700 w, 1684 s, 1652 s, 1646 m, cm^{-1}; HPLC: $R_t = 20.9$ min; MS (ESI): *m/z* (%) 766.8 (100), 764.8 (60) [M^-].

Analytical data for complex **19b**: Yield: 74.2 mg (79%); ^{1}H-NMR (CD_3OD): δ 1.4 (m, 18H), 1.7 (m, 2H) 1.8 (m, 2H), 2.0 (s, 3H), 2.3–2.4 (m, 4H), 3.4 (m, 4H), 3.5 (d, 2H), 3.6–3.8 (AB, 4H), 4.0 (m, 1H), 4.3 (m, 1H), 6.3 (t, 1H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 197.6 (CO), 197.1 (CO), 181.7, 175.3, 165.0, 151.2, 136.9, 110.5, 85.6, 85.2, 71.8, 69.5, 62.8, 51.9, 46.8, 46.9, 41.1, 38.8, 35.8, 29.2–28.8, 26.6–24.9, 11.1; Anal. Calc. for $C_{28}H_{38}N_4O_{12}Re(C_8H_{20}N)_{0.3}Na_{0.7}\cdot 2H_2O$: C, 40.57; H, 5.38; N, 6.69. Found: C, 40.00; H, 5.41; N, 6.63%. IR (KBr): 3550 br, 2015 s, 1888 vs, 1672 s, cm^{-1}; HPLC: $R_t = 20.5$ min; MS (ESI): *m/z* (%) 809.0 (100), 807.0 (60) [M^-].

Analytical data for complex **20b**: Yield: 87.8 mg (75%); ^{1}H-NMR (CD_3OD): δ 1.3–1.4 (m, 5H), 2.0 (s, 3H), 2.15–2.3 (m, 4H), 2.6 (m, 2H), 3.0–3.25 (m, 3H), 3.3–3.5 (m, 3H), 3.5 (d, 2H), 3.6–3.8 (AB, 4H), 3.84 (m, 1H), 4.23 (m, 1H), 6.2 (t, 1H), 7.3–7.4 (m, 5H), 7.5 (s, 1H); ^{13}C-NMR (CD_3OD): δ 197.3 (CO), 196.9 (CO), 181.3, 173.0, 165.3, 151.2, 142.5, 137.1, 128.6–126.8, 110.4, 85.9, 85.0, 71.7, 67.6, 62.9, 51.9, 43.0, 40.9, 38.8, 30.9, 11.1, 6.3; Anal. Calc. for $(C_{28}H_{30}N_4O_{12})_{1.17}Re(C_8H_{20}N)_{0.4}Na_{0.6}$: C, 44.47; H, 4.47; N, 7.32. Found: C, 44.51; H, 5.17; N, 7.15%; IR (KBr): 3739 br, 2014 s, 1892 vs, 1703 s, 1671 s, cm^{-1}; HPLC: $R_t = 20.2$ min; MS (ESI): *m/z* (%) 801.2 (100), 799.2 (60) [M^-].

Analytical data for complex **21b**: Yield: 45 mg (92%); ^{1}H-NMR (CD_3OD): δ 1.3–1.4 (m, 6H), 1.78–1.9 (m, 4H), 2.0 (s, 3H), 2.3 (m, 2H), 3.3–3.4 (m, 6H), 3.6 (d, 2H), 3.6–3.8 (AB, 4H), 4.0 (m, 1H), 4.2 (m, 1H), 4.39 (m, 1H), 6.28 (t, 1H), 7.6 (s, 1H); ^{13}C-NMR (CD_3OD): δ 197.8 (CO), 196.9 (CO), 181.8, 176.0, 165.3, 151.2, 138.5, 136.2, 110.6, 86.0, 85.4, 72.3, 71.5, 63.0, 52.0,



Scheme 2. (i) DCC, NHS in DMF, 60 °C, 2 h; (ii) methanol, conc. HCl, 10% Pd/C, H_2, r.t., 12 h; (iii) $BrCH_2CO_2CH_3$, methanol, NEt_3, reflux, 12 h; (iv) 1 N NaOH, r.t., 3 h.

41.4, 38.6, 31.8, 20.1, 12.0; Anal. Calc. for $C_{22}H_{26}N_4O_{13}Re(C_8H_{20}N)_{0.7}Na_{0.3}$: C, 39.52; H, 4.81; N, 7.85. Found: C, 38.71; H, 5.29; N, 8.07%; IR (KBr): 3408 br, 2020 s, 1878 vs, 1646 s, cm^{-1}; HPLC: R_t: 14.9 min; MS (ESI): *m/z* (%) 741.1 (100), 739.1 (60) [M^-].

3. Results and discussion

3.1. Syntheses

Martin et al. recently presented a series of carboxamide derivatives of 5′-amino-2′,5′-dideoxy-5-ethyluridine and could show, that they are highly potent inhibitors of HSV1-TK and HSV2-TK [9]. The IC_{50} values for these enzymes were found to range from 1 μM to 1 nM which is orders of magnitude more potent than e.g. the antiviral drug Acyclovir ($IC_{50} = 28$ μM). The design of these HSV TK inhibitors was based on isosteric and isoelectronic analogues of thymidine monophosphate (Fig. 1). Based on the X-ray structure of HSV-1 TK and molecular modeling calculation, there is evidence that the aromatic residues of these compounds are located outside the binding site of the enzyme [10,11]. Other X-ray structure analyses of the viral enzyme predicted, that functionalization of thymidine/uridine at positions other than C-5′ causes serious reduction of binding to the enzyme [12]. Thus, it would be interesting and reasonable to develop synthetic strategies for high affinity, C5′-functionalized and ^{99m}Tc-labeled thymidine/uridine analogues for potential use in radiopharmacy.

Discovered for radiopharmaceutical application, the water-soluble, organometallic complex $[M(H_2O)_3(CO)_3]^+$ (M = Tc, Re), **1**, has gained considerable attention in the past years. The high kinetic inertness, its small size and the versatility of appropriate chelating systems make this organometallic core particularly suitable for in vivo stable radiolabeling of small biomolecules. Mundwiler et al. have recently presented C-5 functionalized organo-metallic thymidine complexes of Tc-99m and Re [13]. However, to our knowledge, there exists no other systematic in vitro investigation of

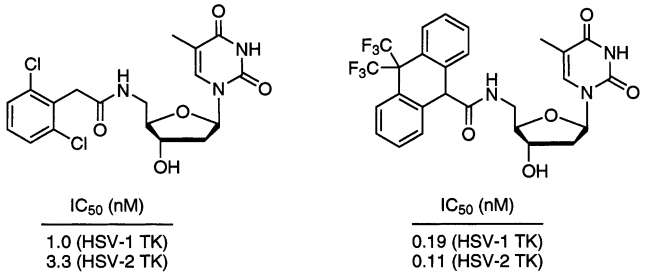
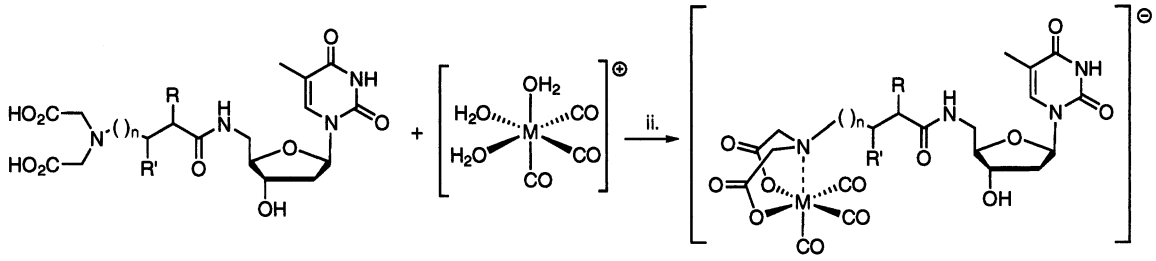


Fig. 1. Examples of a new generation of potent inhibitors of herpes simplex virus thymidine kinase [9].

biologically active technetium and rhenium based thymidine analogues in the literature. In order to elucidate the potential of organometallic technetium and rhenium-tricarbonyl complexes of thymidine (or uridine) for radiopharmaceutical application, we are currently focusing on the functionalization and labeling of thymidine at the C-5′-position. From our previous studies we have recognized, that poly amino poly carboxylic acid based ligand systems react readily with the precursor **1** to form stable and hydrophilic complexes [14–16]. Thus, we synthesized a series of 5′-carboxamide derivatives of 5′ amino thymidine with an imino diacetic acid chelating moiety and alkyl spacers of variable chain lengths and with various side chains. The thymidine derivatives **6**–**9**, **12** and **15** were obtained through coupling with the corresponding *N*,*N*-dicarboxy methyl amino carboxylic acids **2**–**5** in the presence of 1,3-dicyclohexyl carbodiimide (DDC) and *N*-hydroxysuccinimid (NHS) in DMF as shown in Scheme 1. The compounds were purified over silica gel (ethylacetate–methanol: 8/1). Yields varied between 64 and 79% for the coupling reaction. For the derivatives **12** and **15** with additional side chains the chelating moiety was build up after coupling of the spacers to 5′-amino-thymidine. *N*-Boc-5-amino-2(*S*)-hydroxy-pentanoic acid [17] or 4-cyano-3-phenyl butanoic acid **10** were amidically linked to 5′-amino-thymidine (Scheme 2). After reduction of the cyano group or cleavage of the Boc group, the pendent primary amino function of compounds **11** and **14** were double N-alkylated with methyl bromo acetate in presence of NEt_3 to form the nucleoside derivatives **12** and **15** in yields of 38–59% after chromatographic purification on silica gel (ethylacetate–methanol: 8/2, ethylacetate–methanol: 9/1, respectively). All nucleoside derivatives have been unambiguously characterized by means of ^{1}H-, ^{13}C-NMR and mass spectroscopy.

The reactions with the organometallic precursor **1b** have been performed in a water–methanol mixture (1/1) at 60 °C (Scheme 3). The crude products were purified over SepPak® reversed phase columns using a water–methanol gradient. The complexes **16b**–**21b** were obtained in good yields (61–84%) as a mixture of the corresponding Na^+ and NEt_4^+ salts as evident from the elemental analyses. The IR spectra of all complexes revealed the typical *fac*-$M(CO)_3$ pattern of a symmetrically coordinated complex with significantly blue-shifted CO stretch frequencies compared to the starting material $[NEt_4]_2[ReBr_3(CO)_3]$ (2000 and 1868 cm^{-1}). In the ^{1}H-NMR spectra of complexes **16b**–**21b**, the four protons of the coordinated IDA moiety showed the typical pattern of an AB spin system (Fig. 2B). The singlet of the previously identical NCH_2CO_2 protons in **6**–**9**, **12** and **15** (Fig. 2A) became non-equivalent upon rigid coordination to the metal center by virtue of their axial and equatorial orientation. The same spectroscopic features have been previously reported for rhenium-



6: n = 1; R = H; R' = H
7: n = 2; R = H; R' = H
8: n = 5; R = H; R' = H
9: n = 8; R = H; R' = H
12: n = 2; R = H; R' = C_6H_5
15: n = 2; R = OH; R' = H

16: n = 1; R = H; R' = H
17: n = 2; R = H; R' = H
18: n = 5; R = H; R' = H
19: n = 8; R = H; R' = H
20: n = 2; R = H; R' = C_6H_5
21: n = 2; R = OH; R' = H

Scheme 3. (i) M = ^{99m}Tc: PBS buffer, pH 7.4, 75 °C, 1 h; M = Re: methanol–H_2O, 60 °C, 5 h.

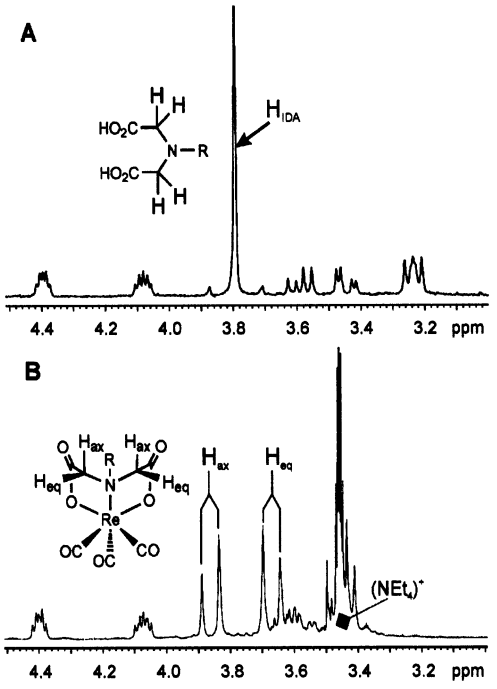


Fig. 2. Section of the ^{1}H-NMR spectra between 3.0 and 4.5 ppm of the thymidine analogue **9** (A) and of the corresponding complex [NEt$_4$][Re(**9**)(CO)$_3$], **19b** (B) (R = 5′-amidodecyl thymidine). Only NCH_2 protons of the IDA-moiety and of the counter ion NEt_4^+ (in case of complex **19b**) are labeled.

tricarbonyl complexes of IDA-functionalized desoxyglucose and glucose derivatives [16]. This proved the desired symmetric, tridentate coordination of the metal-tricarbonyl core via the IDA chelate. There was no significant chemical shift observed in the ^{1}H-NMR spectra of complexes **16b**–**21b** for protons, which are not in close vicinity of the chelate. Therefore, unspecific coordination or interaction of the metal center via other functional groups of thymidine can largely be excluded. All complexes revealed a good solubility in water and polar organic solvents, which was a prerequisite for enzymatic studies in vitro.

The corresponding radioactive technetium-99m complexes **16a**–**21a** have been almost quantitatively prepared in physiological phosphate buffer (PBS; pH 7.4) at concentration of 10^{-4} M of compounds **6**–**9**, **12** and **15** after 60 min at 75 °C (Scheme 3). Specific activities between 18 and 180 MBq μmol^{-1} could be achieved under these labeling conditions. Single species have been produced with all nucleoside derivatives. The characterization of complexes **16a**–**21a** was accomplished by comparison of the retention times of the γ-traces with the UV-traces (254 nm) of the corresponding rhenium complexes (Fig. 3). The organometallic ^{99m}Tc-complexes were challenged in vitro in PBS buffer and human serum albumin for 24 h at 37 °C. Decomposition or dissociation of the complexes to either TcO_4^- or **1a** was less than 8% of the initial activity. This stabilities and specific activities would be sufficient for a potential radiopharmaceutical application of such compounds.

3.2. In vitro evaluation

For an initial evaluation of the biological activity, the rhenium complexes **16b**–**21b** have been screened and tested in vitro for inhibition of HSV-1 TK as well as human TK 1 according to the procedure described by Pilger et al. [8]. The inhibition of the enzymatic reaction

$$\mathrm{ATP} + \mathrm{dT} \xrightarrow{\mathrm{TK}} \mathrm{ADP} + \mathrm{dTMP}$$

was monitored. The results of these in vitro experiments are depicted in Fig. 4. The formation of ADP or dTMP in presence of hTK 1 at various concentrations of complexes **16b**–**21b** was measured. The organometallic rhenium–thymidine complexes revealed competitive inhibition of hTK 1. One could observe a correlation between length of the spacer and the inhibitorial effect of the rhenium complexes (Fig. 4A). Inhibition was found to be increased for complexes with longer alkyl chains. On the other hand, the introduction of an additional hydrophobic (complex **20b**) or hydrophilic

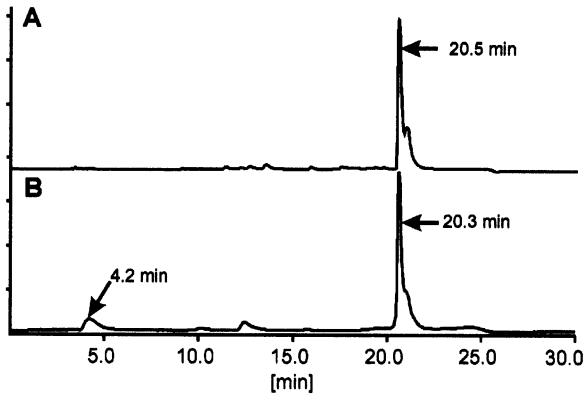


Fig. 3. (A) HPLC trace of complex **19b** (254 nm); (B) γ-trace of the radioactive complex **19a**. Peak at 4.2 min represent unreacted $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (< 5% of total activity).

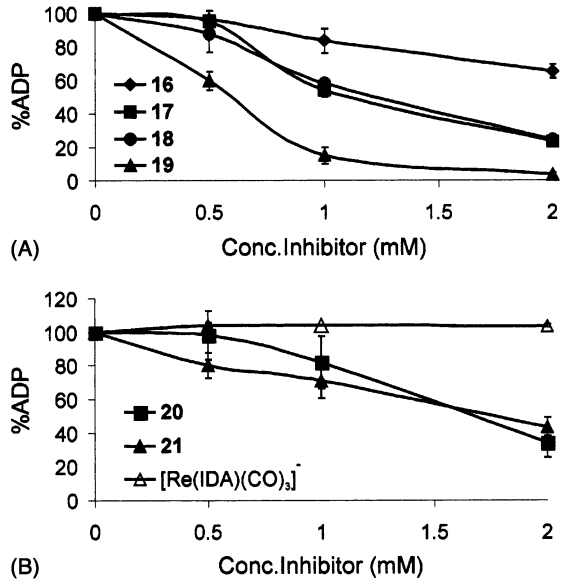


Fig. 4. Enzymatic formation of ADP at various concentrations of complexes **16b**–**19b** (A) and of complexes **20b** and **21b** (B) in presence of hTK 1. Complex $[Re(IDA)(CO)_3]^-$ was used as a negative control.

(complex **21b**) group does not significantly change the inhibitory activity (Fig. 4B). The complex $[NEt_4]$-$[Re(IDA)(CO)_3]$ without the pharmacophoric group was used as a negative control (Fig. 4B). It is reasonable to assume, that a better spacial separation of the pharmacophor (thymidine) and the complex moiety reduce potential steric and/or electrostatic interference with the protein. Using the viral enzyme, only insignificant inhibition of dTMP/ADP formation could be detected in presence of the complexes. These findings are surprising since the thymidine moiety should be recognized by both human and viral TK. In case of the inhibitors shown in Fig. 1 the selectivity for HSV1-TK, respectively, is not related to the organic residues introduced at position C-5′ but by substitution of the methyl group at the C-5 position by an ethyl functionality [1]. In case of the organometallic complexes **16**–**21** the selectivity for hTK 1 is presumably related to the introduction of the organometallic complex moiety at the C-5′ position of 5′-amino-thymidine. To the best of our knowledge, such an effect has never been observed and there are no other compounds reported in the literature, which reveal a comparable TK selectivity. Till now, we do not have a concluding explanation for these results. It can be speculated that steric interference of the rhenium-tricarbonyl complex moiety with the viral enzyme prohibits a binding of the complexes to the active site. Computer-aided docking studies based on the X-ray structure of HSV1-TK are in progress and might help to understand the absence of inhibition of the viral enzyme.

4. Conclusion

In conclusion, the first series of organometallic technetium and rhenium–thymidine complexes have been synthesized and fully characterized. The biological affinity of the rhenium-tricarbonyl compounds was tested in vitro with hTK 1 and HSV1-TK. The selective and exclusive inhibition of hTK 1 prohibits the use of the corresponding radioactive technetium-tricarbonyl complexes for gene therapy monitoring. On the other hand, with the current functionalization and labeling strategy at position C-5′ thymidine derivatives could be produced, which might have the potential to serve as proliferation markers for tumor diagnosis.

Acknowledgements

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References

[1] D. Baker, C. Chu, Nucleosides and Nucleotides as Antitumor and Antiviral Agents, Kluwer Academic/Plenum, New York, 1993.
[2] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A.P. Schubiger, J. Am. Chem. Soc. 123 (2001) 3135.
[3] R. Alberto, R. Schibli, A. Egli, P.A. Schubiger, W.A. Herrmann, G. Artus, U. Abram, T.A. Kaden, J. Organomet. Chem. 493 (1995) 119.
[4] W. Bannwarth, Helv. Chim. Acta 71 (1988) 1517.
[5] D.S. Karanewsky, M.C. Badia, D.W. Cushman, J.M. Deforrest, T. Dejneka, V.G. Lee, M.J. Loots, E.W. Petrillo, J. Med. Chem. 33 (1990) 1459.
[6] A.P. Krapcho, Synthesis (1982) 805.
[7] R. Schibli, R. Schwarzbach, R. Alberto, K. Ortner, H. Schmalle, C. Dumas, A. Egli, A.P. Schubiger, Bioconjugate Chem. (2002) in press.

[8] B.D. Pilger, R. Perozzo, F. Alber, C. Wurth, G. Folkers, L. Scapozza, J. Biol. Chem. 274 (1999) 31967.
[9] J.A. Martin, R.W. Lambert, J.H. Merrett, K.E.B. Parkes, G.J. Thomas, S.J. Baker, D.J. Bushnell, J.E. Cansfield, S.J. Dunsdon, A.C. Freeman, R.A. Hopkins, I.R. Johns, E. Keech, H. Simmonite, A. Walmsley, P.W. Kai-In, M. Holland, Bioorg. Med. Chem. Lett. 11 (2001) 1655.
[10] L. Scapozza, personal communication.
[11] J. Vogt, R. Perozzo, A. Pautsch, A. Prota, P. Schelling, P. Pilger, G. Folkers, L. Scapozza, G.E. Schulz, Proteins 41 (2000) 545.
[12] K. Wild, T. Bohner, G. Folkers, G.E. Schulz, Protein Sci. 6 (1997) 2097.
[13] S. Mundwiler, R. Alberto, J. Labelled Compd. Radiopharm. 44 (2001) S494.
[14] R. Schibli, R. La Bella, R. Alberto, E. Garcia-Garayoa, K. Ortner, U. Abram, P.A. Schubiger, Bioconjugate Chem. 11 (2000) 345–351.
[15] R. Waibel, I. Novak-Hofer, R. Schibli, P. Blauenstein, E. Garcia-Garayoa, R. Schwarzbach, K. Zimmermann, R. Pellikka, O. Gasser, A. Blanc, M. Bruhlmeier, P.A. Schubiger, Chimia 54 (2000) 683.
[16] J. Petrig, R. Schibli, C. Dumas, R. Alberto, P.A. Schubiger, Chem.-Eur. J. 7 (2001) 1868.
[17] I. Shin, M.R. Lee, J. Lee, M. Jung, W. Lee, J. Yoon, J. Org. Chem. 65 (2000) 7667.