Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Organometallic Chemistry 689 (2004) 4712–4721
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Organometallic ^{99m}Tc-technetium(I)- and Re-rhenium(I)-folate derivatives for potential use in nuclear medicine

Cristina Müller [a], Cécile Dumas [a], Ute Hoffmann [a], P. August Schubiger [a,b], Roger Schibli [a,b,*]

[a] Center for Radiopharmaceutical Science ETH-PSI-USZ, Paul Scherrer Institute, OIPA, 5232 Villigen, Switzerland
[b] Department of Chemistry and Applied Biosciences of the ETH, 8056 Zürich, Switzerland

Received 13 August 2004; accepted 27 August 2004
Available online 1 October 2004

Abstract

The folate receptor (FR) is a high affinity membrane protein which is overexpressed on a wide variety of tumor cells, but highly restricted in normal tissues. Therefore folate derivatives labeled with short living isotopes such as ^{99m}Tc (γ, $t_{1/2}$ = 6 h) or ^{188}Re (β^-, $t_{1/2}$ = 17 h) could be used for tumor diagnosis and therapy. In this respect there is a great interest to develop organometallic technetium(I) and rhenium(I) modified folate radiopharmaceuticals. For this purpose folic acid was functionalized with a tridentate picolylamine monoacetic acid chelating system. The chelating system was selectively coupled via an aminohexane spacer to the γ- or α-carboxyl group of the glutamate moiety of folic acid to obtain the corresponding γ- or α-folate derivative or – if directly attached to pteroic acid – the pteroate derivative. The derivatives were reacted with the precursor $[M(OH_2)_3(CO)_3]^+$ (M = ^{99m}Tc, Re) to form uniform organometallic folate complexes under mild reaction conditions. All compounds were chemically characterized by means of NMR, MS, IR and HPLC. The determination of the IC_{50}-values for the PAMA-γ-folate derivative (100 nM) and the corresponding organometallic rhenium complex (110 nM) proved retained receptor binding properties. The radiolabeling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was achieved in excellent yield (>95%) at low ligand concentration (10^{-4} M). The cell binding (>45% of total activity) and internalization (>15% of total activity) of all ^{99m}Tc-complexes was very high and specificity for the FR was proved by their complete displacement with excess folic acid. The ^{99m}Tc-complexes were positively tested for their plasma stability and for the absence of binding to plasma proteins.

Keywords: Rhenium(I); Technetium(I); Folic acid derivatives; Folate radiopharmaceutical; Folate receptor

1. Introduction

The vitamin folic acid is involved in diverse processes during cell proliferation and therefore plays an essential role in the growth of tumors. To cope with this increased requirement for folic acid, tumor cells often overexpress the folate receptor (FR, a high affinity membrane anchored protein, $K_D = 10^{-9}$ M), which mediates the cell uptake of folic acid by endocytosis [1,2]. The reduced folate carrier (RFC) is a low affinity membrane spanning protein that transports preferentially reduced folates directly into the cell cytosol. In normal cells the RFC is ubiquitary existing [3,4] whereas the FR is known to be overexpressed in various neoplastic tissues, including ovarian, breast, cervical, colorectal and nasopharyngeal tumors, but highly restricted in most normal human tissues [5,6]. Folic acid is small in size (441.4 Da), non-immunogenic and has proven its affinity for the FR even after site specific modification. This makes it an ideal tool to be used as a Trojan horse for tumor targeting

* Corresponding author. Tel.: +41 56 310 2837; fax: +41 56 310 2849.
E-mail address: roger.schibli@psi.ch (R. Schibli).

0022-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2004.08.045

[7–9]. Folic acid is structurally composed of pteroic acid and glutamic acid connected via an amide linkage (Fig. 1). Both of the carboxyl groups (α- and γ-carboxyl) of the glutamate moiety are accessible for a potential reaction with an amino-group of any functional ligand. Several studies in recent years describe the derivatization of folic acid at the γ-carboxyl group of the glutamate moiety [10]. To date folate conjugates of chemotherapeutic agents [11,12], antisense oligonucleotides and ribozymes [13,14], proteins and protein toxins [15–17], immunotherapeutic agents [18,19] as well as liposomes with entrapped drugs [20–22], plasmids [23–25] and radiopharmaceutical agents [10,26–28] have been synthesized and successfully tested in cancer cells overexpressing the FR on their surface.

The most thoroughly investigated folate radiopharmaceutical is the ^{111}In-labeled diethylenetriamine pentaacetate-(DTPA)-functionalized folate [29–31]. However ^{99m}Tc-technetium-based radiopharmaceuticals for diagnostic imaging purposes are of great interest because of the more ideal physical decay properties (^{99m}Tc: γ, 140 keV, $t_{1/2}$ = 6 h) than for ^{111}In (^{111}In: γ, 245 keV, $t_{1/2}$ = 2.8 d), lower costs and a generator system which makes it readily available on-site in any hospital. In addition its β^--radiation emitting counterpart ^{188}Re-rhenium with a likewise availability shows the required decay properties for therapy (^{188}Re: β^-, 2.1 MeV, $t_{1/2}$ = 17 h). Previous studies describe folate conjugates for radiolabeling with ^{99m}Tc(V) [10]. Compared to common Tc(V)/Re(V) complexes the use of the high kinetically inert and relatively small Tc(I) or Re(I)-tricarbonyl core is favorable due to an increased in vivo stability and a reduced interference with biological activity [32–35]. Therefore, currently there is a high interest to develop a folate derivative functionalized with the organometallic ^{99m}Tc(I)/Re(I)-tricarbonyl core for potential use in nuclear medicine [36,37].

In the present work we describe the derivatization of folic acid and pteroic acid by a picolylamine monoacetic acid (PAMA) chelating system for tridentate labeling with ^{99m}Tc(I)/Re(I)-tricarbonyl and their in vitro investigation. We established a direct method to synthesize uniform γ- and α-folate derivatives which made the subsequent separating step of the two isoforms – resulting from unspecific coupling procedures – superfluous.

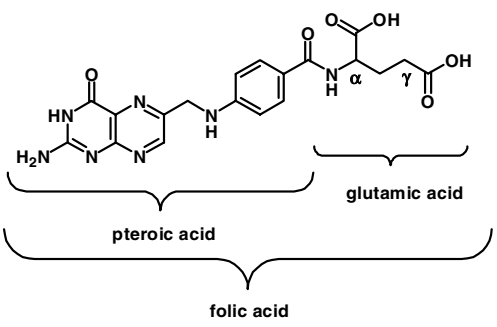


Fig. 1. Structure of folic acid.

2. Experimental

Solvents for syntheses were purchased from Aldrich Chemical Co. or Fluka, Buchs, Switzerland and were dried according to standard methods. The chemical substances were purchased from Aldrich Chemical Co. or Fluka, Buchs, Switzerland. The Boc-protected α- and γ-glutamate methyl esters were purchased from Bachem AG, Bubendorf, Switzerland. The N2,N10-protected pteroic acid precursor was generously provided by Merck Eprova AG, Schaffhausen, Switzerland and [3′,5′,7,9-^{3}H]Folic acid potassium salt (1 mCi/ml, 24 Ci/mmol) was purchased from Amersham Biosciences, Buckimghamshire, UK. The scintillation solution Ultima Gold™ high flash-point LSC-cocktail was purchased from Packard Company, Groningen, Netherlands. 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 [32,38]. [Na][^{99m}TcO$_4$] was eluted from a ^{99}Mo/^{99m}Tc generator (Mallinckrodt-Tyco, Petten, Netherlands) using a 0.9% saline solution. KB cells (CCL-17) were purchased from ATCC (American Type Culture Collection, Manassas, USA). Special RPMI cell culture medium (without folic acid, vitamin B_{12}, phenol red) was purchased from Cell Culture Technologies GmbH, Gravesano. HPLC analyses were performed on a Merck-Hitachi L-6200A-system equipped with an L-3000 tunable absorption detector and a Berthold LB 508 radiometric detector using an uptisphere C-18 reversed phase column (5 μm, 250 × 4.0 mm). HPLC solvents: Aqueous 0.05 M TEAP (triethylammonium phosphate) buffer, pH 7.0 (solvent A), MeOH (solvent B). The HPLC system started with 100% A with a linear gradient to 20% A and 80% B over 30 min followed by 10 min of 100% A. (If the HPLC analyses were performed with the Xterra™ MS C-18 reversed phase column (5 μm, 150 × 4.6 mm) the program was shortened to 20 min (Fig. 2)). The flow rate was 1 ml/min for both programs. Plasma protein binding studies were performed using a Superdex 200 size-exclusion column (13–15 μm, 300 × 10 mm) with PBS pH 7.4 containing 0.05% Tween20 as the mobile phase and a flow rate of 0.9 ml/min. If not else defined TLC analyses of organic synthesis were performed with silica gel 60 aluminium sheets (5 × 7.5 cm), F254, Merck, using CH_2Cl_2/MeOH = 9/1 as an eluent. TLC analyses for plasma stability of the ^{99m}Tc-complexes were performed with silica gel plates (5 cm × 20 cm), F254, Merck, using MeOH/conc. HCl = 99/1 as an eluent and the activity was measured by a RAYTEST RITA-3200 radioanalyzer. Nuclear magnetic resonance spectra were recorded on a 300 MHz Varian Gemini 2000 spectrometer. The ^{1}H and ^{13}C chemical shifts were reported

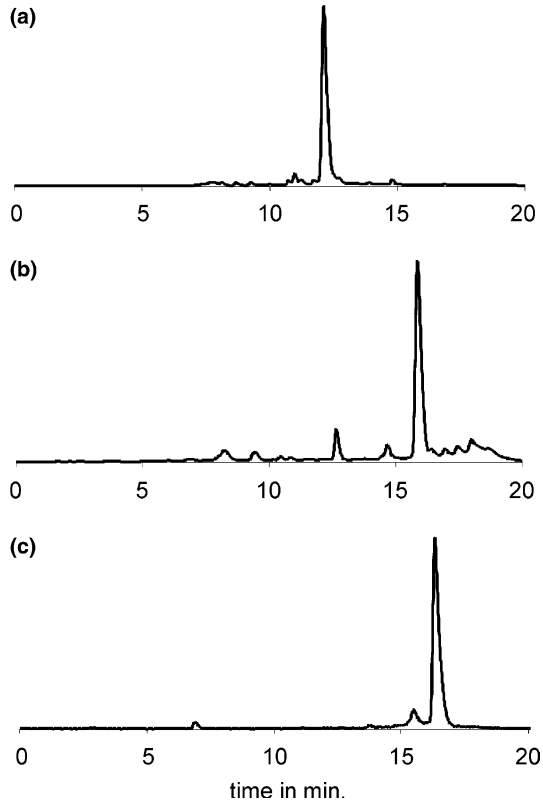


Fig. 2. HPLC traces (Xterra™ MS C-18 column): (a) UV-trace (254 nm) of compound **8**; (b) UV-trace (254 nm) of the rhenium complex **9a**; (c) γ-trace of the radioactive ^{99m}Tc-complex **9b**.

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. Radioactivity (^{99m}Tc, ^{3}H) was measured with a γ-counter (Cobra™ II, Model B 5003, Packard) or a β-counter (TRI-CARB, 1900 TR, Liquid Scintillation Analyzer, Packard). Protein concentrations for the in vitro experiments were measured with a microplate reader (Bio-Rad, Model 550), using a Micro BCA™ Protein Assay Kit (Prod # 23235), Socochim.

2.1. Synthesis of compound *1*

Compound **1** has been synthesized in a 80% yield by a previously published procedure and the analyses were in agreement with the literature [39]. Analytical data for **1**: ^{1}H NMR ($CDCl_3$): δ 1.38–1.50 (m, 6H), 1.50–1.66 (m, 2H), 1.55 (s, 9H), 2.79 (t, J = 6.9 Hz, 2H), 3.16–3.29 (m, 2H).

2.2. Synthesis of compound *2*

The following procedure is based on literature procedures with a slight modification [40]. Molecular sieve and pyridine-2-carboxaldehyde (198 mg; 1.85 mmol; 1 eq.) were added to a solution of compound **1** (400 mg; 1.85 mmol; 1 eq.) in dry toluol (15 ml) and stirred for 30 min at rt. After 4 h reflux the solution was filtered, evaporated and dried in vacuum. The yellow oil of the imine was dissolved in methanol (15 ml) and the reduction was performed by $NaBH_4$ (70 mg; 1.85 mmol; 1 eq.), stirred at rt over night. After addition of water to destroy residual $NaBH_4$, the organic solvent was removed in vacuum. The aqueous solution was extracted three times with EtAc, the organic layer dried over Na_2SO_4, evaporated and dried in vacuum to obtain a 68% yield of a yellow oil **2**. Analytical data for **2**: ^{1}H NMR ($CDCl_3$): δ 1.38–1.66 (m, 8H), 1.55 (s, 9H), 2.79 (t, J = 7.1 Hz, 2H), 3.16–3.29 (m, 2H), 4.01 (s, 2H), 4.78 (s, 1H), 7.23–7.30 (m, 1H), 7.39–7.42 (m, 1H), 7.75 (ddd, 1H), 8.64–8.69 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 26.8, 27.1, 28.6 (3C), 30.1, 30.2, 40.6, 49.7, 55.5, 79.1, 122.0, 122.4, 136.5, 149.4, 156.1, 160, 0, 171.3; IR (KBr): 1697 cm^{-1}; MS (ESI): *m/z* (%) 308 [M^+ + H] (100).

2.3. Synthesis of compound *3*

The following procedure is based on literature procedures with a slight modification [40]. Compound **2** (386 mg; 1.26 mmol; 1 eq.) was dissolved in 20 ml dry THF. Methyl bromoacetate (350 μl; 3.78 mmol; 3 eq.) and NEt_3 (530 μl; 3.78 mmol; 3 eq.) were added and refluxed for 4 h. After cooling to rt the reaction solution was filtered and evaporated. The crude product was purified by chromatography on silica gel using CH_2Cl_2/MeOH (9/1) as an eluent. After drying in high vacuum the product **3** was obtained in a 70% yield. Analytical data for **3**: ^{1}H NMR ($CDCl_3$): δ 1.34–1.44 (m, 4H), 1.50–1.66 (m, 4H), 1.53 (s, 9H), 2.75 (t, J = 7.2 Hz, 2H), 3.12–3.22 (m, 2H), 3.51 (s, 2H), 3.79 (s, 3H), 4.03 (s, 2H), 7.26 (ddd, 1H), 7.58–7.64 (m, 1H), 7.77 (ddd, 1H), 8.60–8.64 (m, 1H); IR (KBr): 1742, 1708 cm^{-1}; MS (ESI): *m/z* (%) 402 [M^+ + Na] (100).

2.4. Synthesis of compound *4*

The Boc-deprotection of compound **3** (334 mg; 0.88 mmol; 1 eq.) was perfomed quantitatively in 6 N HCl (10 ml) at rt, monitored by TLC/ninhydrine. The solvent was evaporated and the compound **4** · HCl was dried in vacuum. Analytical data for **4**: ^{1}H NMR (CD_3OD): δ 1.52–1.66 (m, 4H), 1.79–1.92 (m, 2H), 1.92–2.09 (m, 2H), 3.09 (t, J = 7.2 Hz, 2H), 3.42–3.48 (m, 2H), 3.97 (s, 3H), 4.53 (s, 2H), 5.06 (s, 2H), 8.11–8.21 (m, 1H), 8.31–8.41 (m, 1H), 8.61–8.70 (m, 1H), 9.03–9.10 (m, 1H); ^{13}C NMR (CD_3OD): δ 23.7, 25.5, 26.8, 39.3, 52.8, 53.0, 53.4, 54.6, 56.4, 128.3, 130.2, 143.9, 144.4, 146.7, 166.5; IR (KBr): 1747 cm^{-1}; MS (ESI): *m/z* (%) 280 [M^+ + H] (100).

2.5. Synthesis of compound **5**

HOBt · H_2O (142 mg; 1.93 mmol; 1 eq.) was added to a solution of Boc–Glu–OMe (243 mg; 0.93 mmol; 1 eq.) in dry CH_2Cl_2 to form a suspension and after addition of EDC · HCl (194 mg; 1.01 mmol; 1.1 eq.) a transparent solution. After 4 h stirring at rt, the UV-active product was detected by TLC analysis ($R_f = 0.9$). The reaction solution was washed three times with water to remove impurities, the organic layer was dried over Na_2SO_4, evaporated and dried in vacuum to obtain a white solid in a 95% yield, directly processed in the further coupling reaction. Compound **4** · HCl (278 mg; 0.88 mmol; 1 eq.) and N-ethyldiisopropylamine (300 μl; 1.76 mmol; 2 eq.) dissolved in DMF were added in two portions to a solution of the activated Boc–Glu–OMe (333 mg; 0.88 mmol; 1 eq.) in dry DMF. After a night at 50 °C, the solution was distilled to remove DMF and the crude product was purified by chromatography on silica gel using CH_2Cl_2/MeOH (9/1) to obtain the product **5** in a 75% yield. Analytical data for **5**: ^{1}H NMR ($CDCl_3$): δ 1.26–1.43 (m, 4H), 1.48–1.61 (m, 2H), 1.51 (s, 9H), 1.96–2.12 (m, 1H), 2.16–2.30 (m, 1H), 2.35 (t, 2H), 2.46–2.56 (m, 2H), 2.66–2.76 (m, 2H), 3.27 (q, 2H), 3.48 (s, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 4.00 (s, 2H), 4.25–4.50 (m, 1H), 7.44–7.62 (m, 1H), 7.59–7.65 (m, 1H), 7.73–7.85 (m, 1H), 8.59–8.65 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5 (3C), 26.5 (2C), 27.0, 28.0, 30.2, 52.2, 52.3, 53.0, 54.1, 54.8, 59.8, 61.0, 122.0, 123.1, 136.6, 148.9, 171.0, 171.8, 172.0; IR (KBr): 1711, 1737 cm^{-1}; MS (ESI): *m*/*z* (%) 545 [M^+ + Na] (100).

2.6. Synthesis of compound **6**

Compound **5** (345 mg; 0.66 mmol; 1 eq.) was quantitatively deprotected in CH_2Cl_2/TFA (9/1) (10 ml) at rt over night, monitored by TLC/ninhydrine. The reaction solution was evaporated and the crude product **6** dried in vacuum. Analytical data for **6**: ^{1}H NMR (CD_3OD): δ 1.44–1.70 (m, 6H), 1.78–1.94 (m, 2H), 2.18–2.48 (m, 3H), 2.50–2.78 (m, 2H), 3.26–3.42 (m, 3H), 3.95 (s, 3H), 3.98 (s, 3H), 4.18–4.30 (m, 1H), 4.32 (s, 2H), 4.74 (s, 2H), 7.68–7.83 (m, 2H), 8.11–8.25 (m, 1H), 8.80–8.88 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 26.5 (2C), 27.0 (2C), 27.4, 29.1, 51.4, 51.6, 53.0, 54.3, 54.8, 59.8, 60.1, 122.0, 123.1, 136.6, 148.9, 171.0, 171.8, 171.0; IR (KBr): 1741, 1678, 1653 cm^{-1}; MS (ESI): *m*/*z* (%) 423 [M^+ + H] (100).

2.7. Synthesis of compound **8**

N2-*N,N*-dimethylaminomethylene-10-formylpteroic acid **7** (261 mg; 0.66 mmol; 1 eq.) was suspended in dry CH_2Cl_2. CDI (428 mg; 2.64 mmol; 4 eq.) was added and stirred under N_2 for 2 h to obtain a yellow solution. The reaction solution was washed four times with water, the organic layer was dried over Na_2SO_4 and filtered to obtain a transparent slightly yellow colored solution. The formation of the activated protected pteroic acid was monitored by HPLC ($R_t = 25$ min). Compound **6** · TFA (354 mg; 0.66 mmol; 1 eq.) was dissolved in dry CH_2Cl_2, neutralized by N-ethyldiisopropylamine (115 μl; 0.66 mmol; 1 eq.) (HPLC: $R_t = 28$ min) and added in two portions. The coupling reaction, monitored by HPLC ($R_t = 29.9$ min), was stirred at rt over night. The reaction solution was washed once with water, the organic layer dried over Na_2SO_4, filtered and evaporated for direct use of the following deprotection reaction. The fully protected PAMA-aminohexane-γ-folate was dissolved in 1 N NaOH (10 eq.) and stirred for 5 h, monitored by HPLC ($R_t = 18.9$ min). After neutralization with 1 N HCl, the pH was decreased to 3 by adding 0.1 N HCl. The yellow product precipitated by adding EtOH. After centrifugation the solid was washed three times with EtOH and dried in vacuum to obtain an orange powder of the product **8** in a 10% yield. Analytical data for **8**: ^{1}H NMR (DMSO): δ 1.10–1.62 (m, 8H), 1.92–2.35 (m, 4H), 2.68–2.81 (m, 2H), 3.01–3.19 (m, 2H), 3.43 (s, 2H), 4.04 (s, 2H), 4.30–4.45 (m, 1H), 4.61 (s, 2H), 6.65–6.82 (m, 2H), 7.24–7.46 (m, 2H), 7.70–7.82 (m, 2H), 7.84–8.00 (m, 1H), 8.54–8.66 (m, 1H), 8.77 (s, 1H); IR (KBr): 1687, 1651, 1632, 1608 cm^{-1}; MS (ESI): *m*/*z* (%) 687 [$M^+ - H$] (100); 688 [M^+] (40).

2.8. Synthesis of compound **10**

Boc–Glu(OMe)–OH · DCHA (530 mg; 1.20 mmol) was dissolved in CH_2Cl_2 (40 ml) and washed twice with $KHSO_4$ 10% (15 ml). The organic layer was dried over Na_2SO_4, filtered and dried in vacuum to obtain a 80% yield of the free Boc–Glu(OMe)–OH. The activation of Boc–Glu(OMe)–OH (243 mg; 0.93 mmol; 1 eq.) with HOBt · H_2O (142 mg; 0.93 mmol; 1 eq.) and EDC · HCl (194 mg; 1.01 mmol; 1.1 eq.) was performed as described for the Boc–Glu–OMe in a 95% yield. Compound **10** has been synthesized according to the above described procedure for compound **5** using the activated Boc–Glu(OMe)–OH (333 mg; 0.88 mmol; 1 eq.) to obtain the purified product **10** in a 75% yield. Analytical data for **10**: ^{1}H NMR ($CDCl_3$): δ 1.28–1.60 (m, 8H), 1.51 (s, 9H), 1.92–2.10 (m, 1H), 2.10–2.28 (m, 1H), 2.40–2.62 (m, 2H), 2.78 (t, 2H), 3.24–3.34 (m, 2H), 3.57 (s, 2H), 3.76 (s, 3H), 3.79 (s, 3H), 4.12 (s, 2H), 4.18–4.35 (m, 1H), 7.32–7.40 (m, 1H), 7.67–7.73 (m, 1H), 7.76–7.82 (m, 1H), 8.58–8.64 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 26.7, 26.8, 27.3, 28.2, 28.4 (3C), 29.4, 30.4, 39.5, 51.6, 51.8, 53.7, 54.2, 54.7, 60.0, 68.2, 122.3, 123.3, 136.8, 148.9, 155.8, 159.1, 171.5, 171.7, 173.8; IR (KBr): 1737, 1715, 1667, cm^{-1}; MS (ESI): *m*/*z* (%) 545 [M^+ + Na] (100), 523 [M^+ + H] (20).

2.9. Synthesis of compound **11**

The deprotection reaction of compound **10** (345 mg; 0.66 mmol; 1 eq.) to obtain compound **11** has been performed according to the above described deprotection reaction of compound **5**. Analytical data for **11**: ^{1}H NMR (CD_3OD): δ 1.44–1.59 (m, 4H), 1.59–1.75 (m, 2H), 1.75–1.93 (m, 2H), 2.18–2.45 (m, 2H), 2.50–2.78 (m, 2H), 3.25–3.45 (m, 4H), 3.81 (s, 3H), 3.93 (s, 3H), 4.02–4.11 (m, 1H), 4.29 (s, 2H), 4.74 (s, 2H), 7.68–7.89 (m, 2H), 8.16–8.28 (m, 1H), 8.80–8.90 (m, 1H); MS (ESI): *m/z* (%) 423 [M^+ + H] (100), 445 [M^+ + Na] (95).

2.10. Synthesis of compound **12**

Compound **12** has been synthesized and purified according to the procedure described for compound **8**, but using compound **11** · TFA (354 mg; 0.66 mmol; 1 eq.) as a ligand for coupling with the N2-*N,N*-dimethylaminomethylene-10-formylpteroic acid **7** (261 mg; 0.66 mmol; 1 eq.), which was previously activated with CDI. The coupling reaction and the in situ deprotection to obtain the product **12** in a 7% yield were monitored by HPLC (R_t = 29.9 min, R_t = 18.9 min (**12**), respectively). Analytical data for **12**: ^{1}H NMR (DMSO): δ 1.10–1.70 (m, 8H), 1.79–2.15 (m, 4H), 2.25–2.50 (m, 2H), 2.86–3.25 (m, 4H), 4.33 (s, 2H), 4.40–4.51 (m, 1H), 4.62 (s, 2H), 6.65–6.85 (m, 2H), 7.02–7.40 (m, 2H), 7.72–7.89 (m, 2H), 7.93–8.05 (m, 1H), 8.65–8.75 (m, 1H), 8.78 (s, 1H); MS (ESI): *m/z* (%) 689 [M^+ + H] (40); 710 [M^+ − H + Na] (50).

2.11. Synthesis of compound **14**

Compound **14** has been synthesized and purified by the above described procedure for compound **8** using directly compound **4** · HCl (208 mg; 0.66 mmol; 1 eq.) as a ligand for coupling with the N2-*N,N*-dimethylaminomethylene-10-formylpteroic acid **7** (261 mg; 0.66 mmol; 1 eq.), which was previously activated. The coupling reaction and the in situ deprotection to obtain the product **14** in a 15% yield were monitored by HPLC (R_t = 32.8 min, R_t = 20.0 min (**14**), respectively). Analytical data for **14**: ^{1}H NMR (DMSO): δ 1.10–1.80 (m, 8H), 3.08–18 (m, 2H), 3.18–3.32 (m, 2H), 4.06 (s, 2H), 4.50 (s, 2H), 4.59 (s, 2H), 6.68–6.80 (m, 2H), 6.88–7.20 (m, 1H), 7.26–7.36 (m, 1H), 7.72–7.78 (m, 2H), 7.96–8.06 (m, 1H), 8.68–8.80 (m, 2H); IR (KBr): 1703, 1688, 1605 cm^{-1}; MS (ESI): *m/z* (%) 560 [M^+ + H] (100), 582 [M^+ − H + Na] (30).

2.12. Synthesis of the complexes **9a, 13a, 15a**

The complexes **9a**, **13a** and **15a** were prepared according to the following general procedure: A slight excess of the precursor $(NEt_4)_2[ReBr_3(CO)_3]$ (1.1–1.5 eq.) and the ligands **8**, **12** or **14** (1 eq.) were dissolved in a H_2O/MeOH (2/1) and stirred at rt for 2 h. An aqueous solution of 1 M $NaHCO_3$ was dropwise added increasing the pH value to 8–9. The formation of the rhenium-complex was monitored by HPLC (**9a**: R_t = 26.3 min; **15a**: R_t = 27.2 min). The reaction solution was acidified with 1 N HCl to a pH of 3 to precipitate an orange solid which was centrifuged, washed several times with H_2O and dried in vacuum. Analytical data for **9a**: ^{1}H NMR (DMSO): δ 1.25–1.95 (m, 8H), 1.95–2.13 (m, 1H), 2.13–2.26 (m, 1H), 2.26–2.44 (m, 2H), 3.00–3.6 (m, 5H), 3.94 (d, J = 16.8 Hz, 1H), 4.33–4.55 (m, 1H), 4.61 (s, 2H), 4.65 (d, J = 16.2 Hz, 1H), 4.88 (d, J = 15.9 Hz, 1H), 6.68–6.88 (m, 2H), 7.65–7.90 (m, 3H), 7.90–8.08 (m, 1H), 8.22–8.40 (m, 1H), 8.78 (s, 1H), 8.88 (d, J = 5.4 Hz, 1H); IR (KBr): 2024, 1899, 1638 cm^{-1}; MS (ESI): *m/z* (%) 957 [M^+ − H], 958 [M^+] (40). Analytical data for **13a**: ^{1}H NMR (DMSO): δ 1.17–1.85 (m, 8H), 3.15–3.65 (m, 5H), 3.82 (d, J = 16.2 Hz, 1H), 4.52 (d, J = 16.0 Hz, 1H), 4.08–4.16 (m, 2H), 4.76 (d, J = 16.5 Hz, 1H), 6.50–6.95 (m, 2H), 7.50–7.68 (m, 3H), 7.68–7.80 (m, 1H), 7.96–8.20 (m, 1H), 8.69 (s, 1H), 8.72–8.80 (m, 1H); IR (KBr): 2025, 1898, 1610 cm^{-1}; MS (ESI): *m/z* (%) 852 [M^+ + Na] (100), 874 [M^+ + 2 Na] (50). Analytical data for **15a**: ^{1}H NMR (DMSO): δ 1.15–1.85 (m, 8H), 3.10–3.60 (m, 5H), 3.80 (d, J = 16.5 Hz, 1H), 4.50 (d, J = 15.9 Hz, 1H), 4.06–4.16 (m, 2H), 4.75 (d, J = 16.5 Hz, 1H), 6.48–6.90 (m, 2H), 7.50–7.66 (m, 3H), 7.66–7.75 (m, 1H), 7.94–8.20 (m, 1H), 8.68 (s, 1H), 8.70–8.78 (m, 1H); IR (KBr): 2025, 1897, 1608 cm^{-1}; MS (ESI): *m/z* (%) 852 [M^+ + Na] (100), 874 [M^+ + 2 Na] (50).

2.13. Synthesis of the complexes **9b, 13b, 15b**

The complexes **9b**, **13b**, **15b** were prepared according to the following general procedure: *fac*-[^{99m}Tc$(OH_2)_3$-$(CO)_3]^+$ (100 μl; ~1 GBq/ml), PBS pH 7.4 (350 μl) and the stock solution (10^{-3} M) of the compound (**8**, **12**, **14**) in PBS buffer (50 μl) were placed in a sealed glass vial. After 30 min reaction time at 75 °C the vial was cooled on ice. The formation of the complexes were monitored by HPLC (**9b**: R_t = 26.3 min, **13b**: R_t = 26.3 min, **15b**: R_t = 27.2 min).

2.14. Plasma stability and protein binding of the ^{99m}Tc-complexes

The ^{99m}Tc-radiolabeled complexes **9b**, **13b** and **15b** were purified by HPLC to separate them from the unlabeled compounds **8**, **12**, **14**. Each of the collected solutions was diluted in PBS pH 7.4 to a concentration of ≈65 MBq/ml and mixed with 100 μl human plasma (1:1). The samples were incubated at 37 °C and analyzed after 1, 4 and 24 h either by TLC to determine decomposition rate or by HPLC on a Superdex size-exclusion

column to determine the fraction of the complexes bound to plasma proteins. For the TLC analyses it is known that pertechnetate migrates with an $R_f \approx 0.7$, whereas the pure ^{99m}Tc-complexes (**9b**, **13b**, **15b**) were detected with an $R_f \approx 0.2$. No decomposition could be observed up to 4 h incubation time and only <10% of the ^{99m}Tc-complexes were decomposed to pertechnetate after 24 h. For the HPLC analyses it is known that the plasma proteins are detected after 12 and 18 min in the UV trace. The ^{99m}Tc-complexes **9b** and **13b** were detected after a retention time of 25 min, whereas the complex **15b** was detected after 55 min. HPLC analysis for **9b**: after 1 h, $R_t = 25$ min (100%); after 4 h, $R_t = 25$ min (100%); after 24 h, $R_t = 15$ min (1%) and $R_t = 25$ min (99%); HPLC analysis for **13b**: after 1 h, $R_t = 25$ min (100%); after 4 h, $R_t = 25$ min (100%); after 24 h, $R_t = 15$ min (1%) and $R_t = 25$ min (99%); HPLC analysis for **15b**: after 1 h, $R_t = 55$ min (100%); after 4 h, $R_t = 55$ min (100%); after 24 h, $R_t = 15$ min (10%) and $R_t = 55$ min (90%).

2.15. Cell culture

KB cells, a human nasopharyngeal epidermal carcinoma cell line, overexpressing the folate receptor (FR), were cultured continuously as a monolayer at 37 °C in a humidified atmosphere containing 5% CO_2 in a special RPMI 1640 medium without folic acid, vitamin B_{12} and phenol red, supplemented with 10% fetal calf serum (FCS), L-glutamine and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml, fungizone 0.25 μg/ml). Eighteen to twenty hours prior to each experiment, the cells were seeded in 12-well plates (8×10^5 cells per well) to form confluent monolayers over night.

2.16. Determination of IC_{50}-values

Cell binding experiments to calculate IC_{50} values were performed according to the following general procedure: The monolayers were rinsed with ice-cold PBS pH 7.4. Eight concentrations (0–10 μM) of cold folic acid, the PAMA-γ-folate derivative **8** and the corresponding rhenium complex **9a** were prepared in PBS pH 7.4. Pure special RPMI medium (without FCS/L-glutamine/antibiotics) (475 μl) and the corresponding solutions of the compounds (500 μl) were added and the well plates were pre-incubated at 37 °C for 40 min. The ^{3}H-folic acid solution (25 μl, 0.8 μM; ~0.02 μCi/μl) was added to each well and the well plates incubated again at 37 °C for 1 h and then rinsed three times with ice-cold PBS pH 7.4. The monolayers were dissolved in 1 N NaOH (1000 μl) and after transfer in 4 ml-tubes homogenized by vortex. The concentration of the proteins for each sample was determined. Scintillation solution (4 ml) was added to the cell suspensions, homogenized and transferred in a scintillation flacons to be counted for radioactivity using a β-counter.

2.17. Cell binding experiment

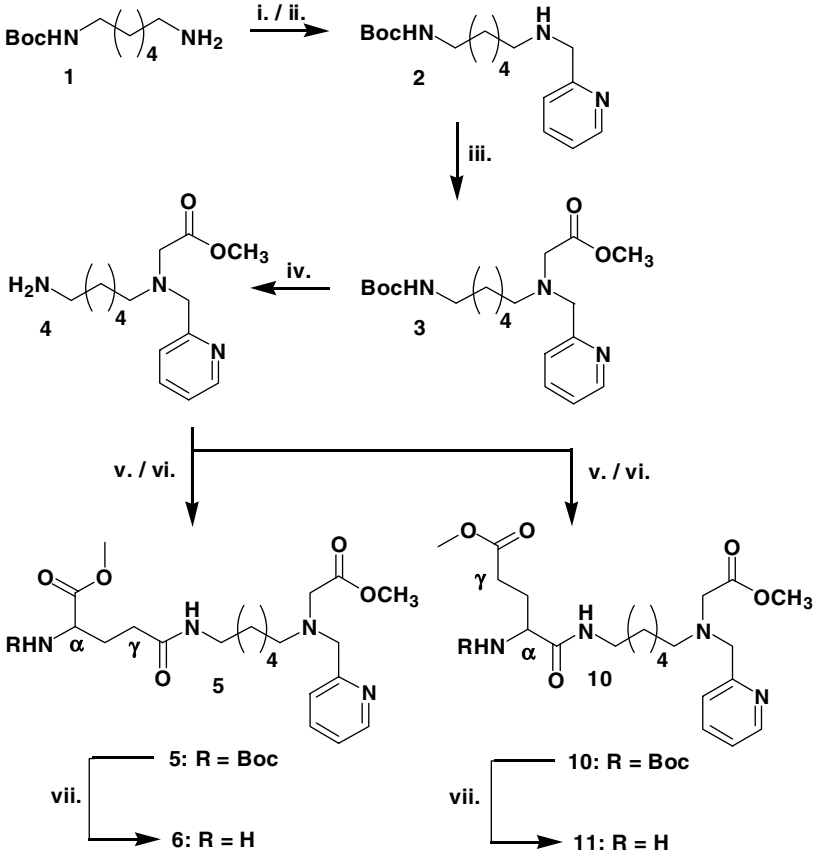
Cell binding experiments were performed according to the following general procedure: The monolayers were rinsed with ice-cold PBS pH 7.4. Pure special RPMI medium (without FCS/L-glutamine/antibiotics) only (975 μl), or medium (475 μl) and a folic acid solution (200 μM, 500 μl) were added into the corresponding wells. The well plates were pre-incubated at 37 °C for 40 min. The solutions of the complexes **9b**, **13b**, **15b** (25 μl, 1 MBq/ml), purified by HPLC, were added and the well plates incubated again at 37 °C for 1 h and then rinsed three times with ice-cold PBS pH 7.4 or twice with PBS pH 7.4 and once with a stripping buffer (aqueous solution of 0.1 M acetic acid and 0.15 M NaCl), respectively, to remove bound complex from the FR on the cell surface [11]. The monolayers were dissolved in 1 N NaOH (1000 μl) and after transfer in 4 ml-tubes homogenized by vortex. They were counted for radioactivity using a Cobra^{TM} II γ-counter. The concentration of proteins for each sample was determined.

3. Results and discussion

3.1. Syntheses

Both of the carboxyl groups (α- and γ-carboxyl) of folic acid are accessible for a potential modification and thus reaction with an amino-group of any functional ligand (Fig. 1). The necessity of a free α-carboxyl group for retained binding to the FR is controversially discussed in the literature [10,41,42]. We therefore reasoned the synthesis of clearly defined γ- or α-folate derivatives. Since both carboxyl groups of the glutamate moiety have similar reactivities unspecific coupling reactions result in a mixture of γ- and α-derivatives and require a subsequent cumbersome separation step [10,43]. To avoid this disadvantage the synthesis of folate derivatives via solid phase chemistry has been described [28,41]. We developed and applied a straight forward method for synthesizing uniform γ- and α-folate derivatives via a converging synthetic strategy.

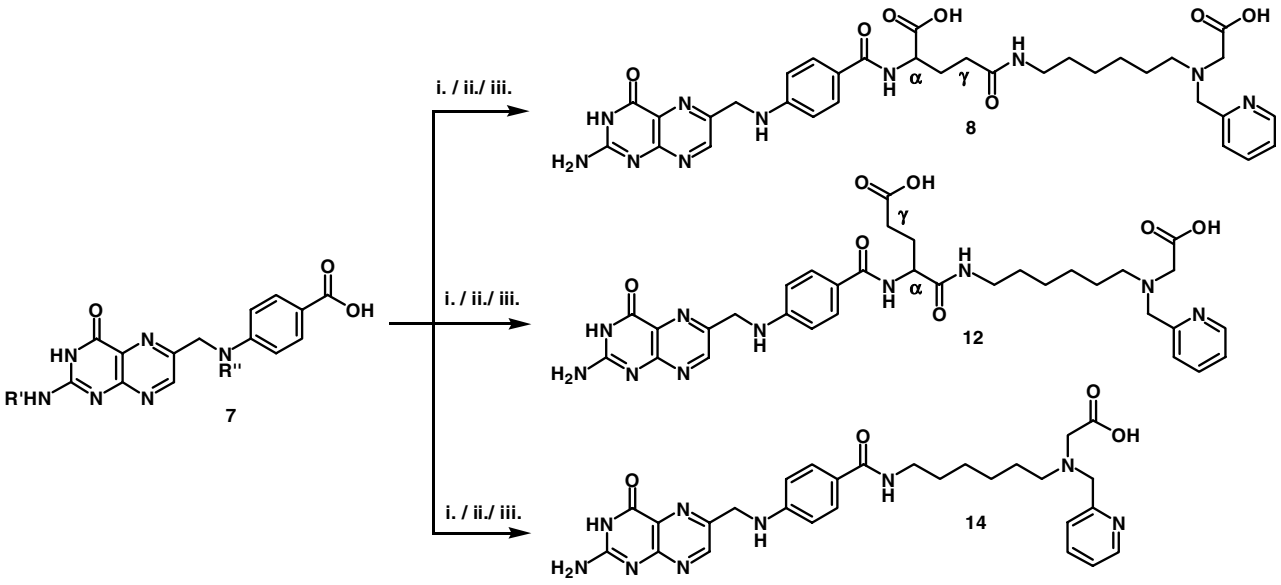
The bifunctional, tridentate metal chelating system was synthesized according to a previously described procedure, starting from a mono-Boc protected diaminohexane **1** which was reacted with pyridine-2-carboxaldehyde to form a Schiff base, followed by an in situ reduction [40]. The alkylation of the secondary amine by bromo methylacetate and the following Boc-deprotection resulted in the PAMA-ligand **4** with a primary amino group for coupling with any carboxyl group of a biomolecule. Compound **4** was attached either to the



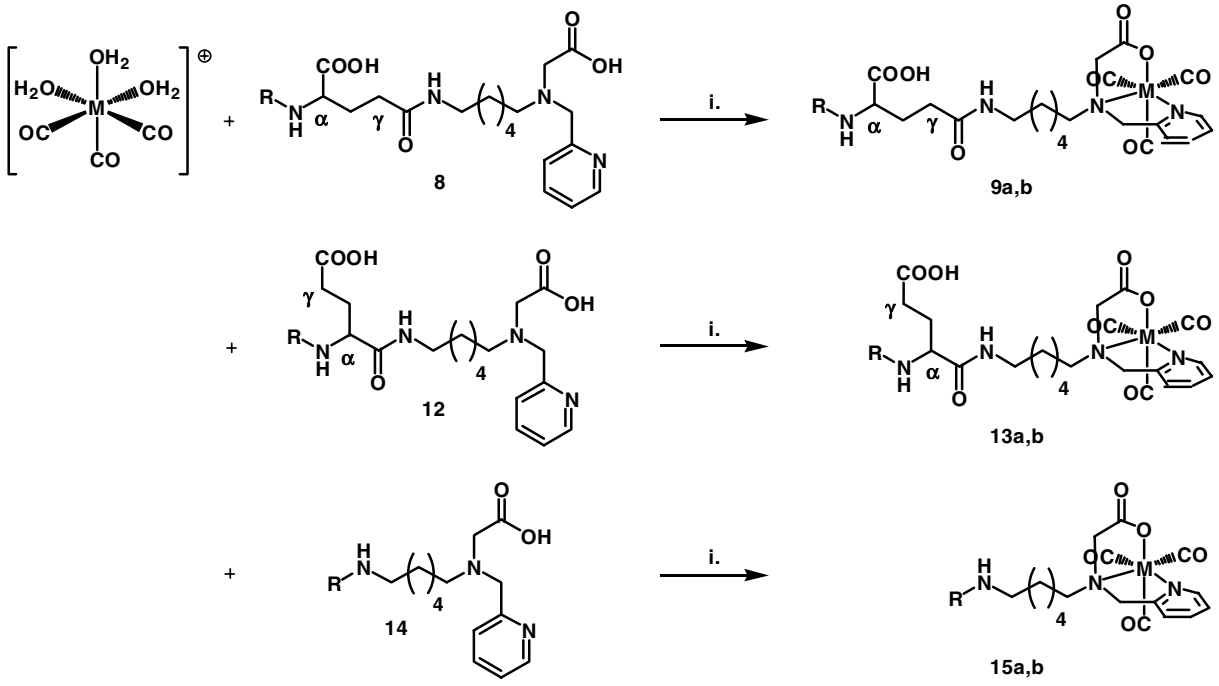
Scheme 1. (i) Pyridine-2-carboxaldehyde, toluol, reflux 3 h; (ii) $NaBH_4$, MeOH, rt, over night; (iii) methyl bromoacetate, Et_3N, THF, reflux, 5 h; (iv) HCl 6 N, rt, 3 h; (v) Boc–Glu–OMe or Boc–Glu(OMe)–OH, HOBt, EDC, CH_2Cl_2, rt, 4 h; (vi) N-ethyldiisopropylamine, DMF, 50 °C, over night; (vii) TFA/CH_2Cl_2 (9:1), rt, over night.

activated γ-carboxyl group of the Boc-protected glutamate α-methylester or the α-carboxyl group of the Boc-protected glutamate γ-methylester (Scheme 1). This coupling reactions monitored by TLC resulted in the formation of the PAMA-γ-glutamate **5** or a PAMA-α-glutamate derivative **10**, respectively. The reactions were relatively fast and the products **5** and **10** received in good yields of 75% after chromatographic purification. The following deprotection reaction performed under acidic conditions ensured intact ester protecting groups. The NMR spectra proved selectivity of the Boc-deprotection at the α-amino-group of these compounds (**5**, **10**) by disappearance of the singlet at 1.5 ppm representing nine protons of the *tert*-butyl group. Compounds **6** and **11** where then coupled via the α-amino group to N2,N10-protected pteroic acid, activated by carbonyldiimidazole (CDI) as previously described (Scheme 2) [44]. These reactions were followed by HPLC analyses whereby after 12 h the HPLC trace showed a peak with a retention time of ≈30 min, clearly indicating the formation of a novel product (starting material: R_t = 25 min and 28 min, respectively). The deprotection reactions were performed under alkaline conditions. The final product showed a significantly shifted peak detected after ≈18 min. The PAMA-γ- (**8**) or PAMA-α-folate (**12**) derivatives, respectively, were unambiguously identified by means of NMR- and mass spectroscopy. The coupling of the ligand **4** directly to the activated N2,N10-protected pteroic acid as well as the deprotection (yielding in compound **14**) were performed in the same way as described for the folate derivatives. Based on the significantly longer retention time of compound **14** (≈33 min before and 20 min after deprotection) it can be concluded that the pteroate derivative was more lipophilic than the folate derivatives **8** and **12**. The yields of the final coupling reaction to obtain **8**, **12** and **14** were generally low (10% (**8**), 7% (**12**) and 15% (**14**), respectively). Incomplete coupling as well as the purification and isolation of the products by extraction and precipitation are reasons for these low yields. However our method exhibits the important advantage of well defined derivatives accessible without the necessity of arduous separating steps.

Starting from the compounds **8**, **12** and **14** the Re-complexes **9a**, **13a**, **15a** have been synthesized on the macroscopic level using the organometallic precursor $(NEt_4)_2[ReBr_3(CO)_3]$ (Scheme 3). Due to the fact that the folate (**8**, **12**) and especially the pteroate (**14**) deriva-



Scheme 2. R′ = N2-*N,N*-dimethylamino-group, R″ = formyl-group: (i) CDI, rt, 3h; (ii) **6**, **11** or **4** in CH_2Cl_2, rt, over night; (iii) NaOH, rt, 4–5 h.



Scheme 3. R = pteroic acid; (i) **9a**, **13a**, **15a**, M = Re: MeOH/H_2O (2/1), rt, 3 h; **9b**, **13b**, **15b**: M = ^{99m}Tc: PBS pH 7.4, 75 °C, 30 min.

tives were poorly soluble in neutral or acidic aqueous solutions, but reasonably soluble under alkaline conditions, the formation of the desired rhenium complexes competed with the formation of the cluster $[Re(OH)(CO)_3]_4$ formed under likewise conditions [45]. Therefore a slight excess of $(NEt_4)_2[ReBr_3(CO)_3]$ (1.1 or 1.5 eq., respectively) was necessary in particular for the formation of the pteroate complex **15a**. After completion of the reaction the complexes could be isolated from the acidified reaction solutions. The precipitates were centrifugated and washed several times with water. An additional washing step with ether was necessary in order to eliminate the rhenium clusters. The IR spectra of the rhenium complexes (**9a**, **13a**, **15a**) revealed the characteristic *fac*-$[M(CO)_3]$ pattern (2024–2025 cm^{-1} and 1897–1899 cm^{-1}, respectively) with significantly blueshifted CO stretch frequencies compared to the starting material $(NEt_4)_2[ReBr_3(CO)_3]$ (2000 and 1868

cm^{-1}). In the ^1H NMR spectra the typical patterns of AB spin systems (J = 15–17 Hz) of the methylene groups of the PAMA chelator were observed. This proved the desired tridentate coordination of the metal-tricarbonyl core via the PAMA chelator as previously described by our group [40]. Compared to the unlabeled derivatives (**8**, **12**: R_t = 18.9 min; **14**: R_t = 20.0 min) the UV-HPLC-traces of the rhenium-complexes **9a**, **13a** and **15a** showed a significant shift to a longer retention time (**9a**, **13a**: R_t = 27.2 min; **15a**: R_t = 26.3 min). Similar retention times were observed for the corresponding ^{99m}Tc-radiolabeled complexes (**9b**, **13b**, **15b**) (Fig. 2).

The radiolabeling with ^{99m}Tc-tricarbonyl under mild and aqueous conditions was achieved (>95%) for all of the derivatives **8**, **12** and **14** in a concentration of 10^{-4} M and a reaction time of 30 min at 75 °C to obtain the ^{99m}Tc-complexes **9b**, **13b**, **15b**. No "unspecific" radiolabeling via functional groups of the pterin-moiety could be observed, indicating the proposed characteristics of the PAMA-system as an effective chelator.

3.2. In vitro evaluation

To investigate specific FR-binding of the derivatives we performed in vitro experiments with KB cells, a human nasopharyngeal carcinoma cell line overexpressing the FR. The cells were cultured in folate deficient medium with a concentration similar to that of human plasma to provoke up-regulation of the FR expression.

The ^{99m}Tc-radiolabeled folate (**9b**, **13b**) and pteroate (**15b**) derivatives, separated from unlabeled substance (**8**, **12**, **14**) by HPLC, evidenced an unexpected high cell binding of 48 ± 7% (**9b**), 57 ± 1% (**13b**), 60 ± 2% (**15b**) of total activity (Fig. 3). The internalized fractions were approximately one third of total binding (15 ± 2% (**9b**), 16 ± 1% (**13b**), 21 ± 2% (**15b**) of total activity). If the cells were pre-incubated with excess folic acid almost complete displacement of the activity could be observed (0.05 ± 0.01% (**9b**), 0.02 ± 0.01% (**13b**), 0.27 ± 0.01% (**15b**)), indicating that the binding of all of the tested derivatives was highly specific. The amount of cell binding and internalization were in the same range for the γ-, α-folate and pteroate derivatives which clearly disproved the necessity of a free carboxyl group or the glutamate moiety for FR binding. This could be important considering the future development of potential folate or pteroate (radio)pharmaceuticals.

Inhibition experiments with KB cells (Fig. 4), performed with ^3H-folic acid in order to determine the IC_{50}-values of the unlabeled PAMA-γ-folate derivative **8** and the rhenium–tricarbonyl complex **9a** resulted in 100 nM (**8**) and 110 nM (**9a**), respectively, and were in the same range as found for other folate derivatives published in the literature [28]. These experiments demonstrated full retention of receptor binding of the γ-folate derivative and the corresponding rhenium–tricarbonyl complex. Due to equal cell binding properties of the γ- and α-folate derivative as well as of the pteroate derivative as earlier described it can be assumed that the IC_{50}-values of the α-folate and the pteroate derivative are in the same range as for the γ-folate derivative.

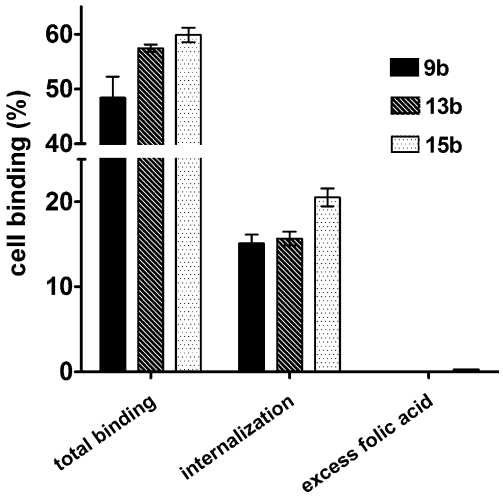


Fig. 3. In vitro experiment of the ^{99m}Tc-complexes **9b**, **13b**, **15b** with KB cells (human nasopharyngeal carcinoma cell line, overexpressing the FR); cell binding, cell internalization and displacement by excess folic acid (<1% bound of total activity).

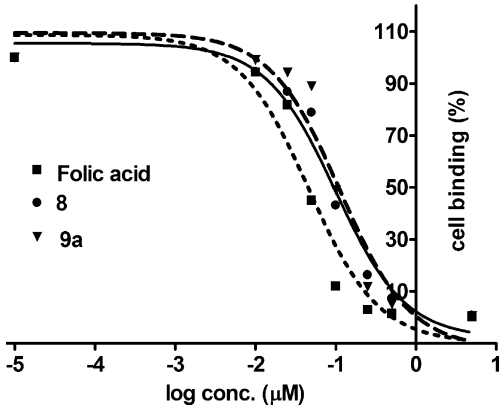


Fig. 4. Determination of the IC_{50}-values, using KB cells (human nasopharyngeal carcinoma cell line overexpressing the FR): Inhibition of ^3H-folic acid with cold folic acid (IC_{50} = 50 nM), compound **8** (IC_{50} = 100 nM) and the corresponding rhenium complex **9a** (IC_{50} = 110 nM).

^{99m}Tc-radiolabeld complexes (**9b**, **13b**, **15b**) were tested for plasma stability by means of TLC analyses. Up to 4 h incubation in plasma at 37 °C no decomposition of the complexes could be observed and after 24 h <10% complexes were decomposed to pertechnetate. The derivatives (**9b**, **13b**, **15b**) were tested for their unspecific binding to plasma proteins (R_t = 12–18 min) over 24 at 37 °C. After 24 h a small amount (≈1%) of the activity of both folate derivatives (**9b**, **13b**) and 10% for the pteroate derivative **15b** were bound to plasma proteins (R_t = 15.5 min). Slightly higher and

therefore unfavorable plasma protein binding for the pteroate derivative is presumably due to its higher lipophilicity which generally favors unspecific binding.

4. Conclusion

In conclusion, a novel method for synthesizing uniform PAMA-γ- and α-folate and PAMA-pteroate derivatives has been established. The folate and pteroate derivatives have been successfully labeled with $[M(OH_2)_3(CO)_3]^+$ (M = ^{99m}Tc, Re). IC_{50}-values of the PAMA-γ-folate derivative and the corresponding rhenium complex were similar to folic acid which proves their retained receptor binding properties. The cell binding and internalization studies showed a high and specific binding to the FR for all three ^{99m}Tc-complexes. Therefore the glutamate moiety of folic acid seems not to be essential for FR binding in vitro. However in vivo experiments are ongoing and will provide evidence of potentially varying pharmacokinetics of the γ- and α-folate or pteroate derivatives, respectively.

Acknowledgements

We thank Merck Eprova AG, for providing us with the pteroic acid precursor and Judith Stahel and Christine De Pasquale for assisting the in vitro experiments. This work was financially supported by Mallinckrodt-Tyco Inc.

References

[1] C.P. Leamon, P.S. Low, Proc. Natl. Acad. Sci. USA 88 (1991) 5572.
[2] C.P. Leamon, P.S. Low, Biochem. J. 291 (1993) 855.
[3] A.C. Antony, Blood 79 (1992) 2807.
[4] A.C. Antony, Annu. Rev. Nutr. 16 (1996) 501.
[5] S.D. Weitman, R.H. Lark, L.R. Coney, D.W. Fort, V. Frasca, V.R. Zurawski, B.A. Kamen, Cancer Res. 52 (1992) 3396.
[6] G. Toffoli, C. Cernigoi, A. Russo, A. Gallo, M. Bagnoli, M. Boiocchi, Int. J. Cancer 74 (1997) 193.
[7] J. Sudimack, R.J. Lee, Adv. Drug Deliv. Rev. 41 (2000) 147.
[8] C.P. Leamon, P.S. Low, Drug Discov. Today 6 (2001) 44.
[9] C.Y. Ke, C.J. Mathias, M.A. Green, Nucl. Med. Biol. 30 (2003) 811.
[10] W.J. Guo, G.H. Hinkle, R.J. Lee, J. Nucl. Med. 40 (1999) 1563.
[11] C.A. Ladino, R.V.J. Chari, L.A. Bourret, N.L. Kedersha, V.S. Goldmacher, Int. J. Cancer 73 (1997) 859.
[12] J.W. Lee, J.Y. Lu, P.S. Low, P.L. Fuchs, Bioorg. Med. Chem. 10 (2002) 2397.
[13] J. Matulic-Adamic, M. Sanseverino, L. Beigelman, Tetrahedron Lett. 43 (2002) 4439.
[14] C.P. Leamon, S.R. Cooper, G.E. Hardee, Bioconjugate Chem. 14 (2003) 738.
[15] C.P. Leamon, I. Pastan, P.S. Low, J. Biol. Chem. 268 (1993) 24847.
[16] J.Y. Lu, D.A. Lowe, M.D. Kennedy, P.S. Low, J. Drug Target. 7 (1999) 43.
[17] C.M. Ward, N. Acheson, L.W. Seymour, J. Drug Target. 8 (2000) 119.
[18] D.M. Kranz, T.A. Patrick, K.E. Brigle, M.J. Spinella, E.J. Roy, Proc. Natl. Acad. Sci. USA 92 (1995) 9057.
[19] B.K. Cho, E.J. Roy, T.A. Patrick, D.M. Kranz, Bioconjugate Chem. 8 (1997) 338.
[20] A. Gabizon, A.T. Horowitz, D. Goren, D. Tzemach, F. Mandelbaum-Shavit, M.M. Qazen, S. Zalipsky, Bioconjugate Chem. 10 (1999) 289.
[21] W.J. Guo, T. Lee, J. Sudimack, R.J. Lee, J. Liposome Res. 10 (2000) 179.
[22] G.F. Shi, W.J. Guo, S.M. Stephenson, R.J. Lee, J. Control. Release 80 (2002) 309.
[23] C.P. Leamon, D. Weigl, R.W. Hendren, Bioconjugate Chem. 10 (1999) 947.
[24] J.A. Reddy, P.S. Low, J. Control. Release 64 (2000) 27.
[25] H.E.J. Hofland, C. Masson, S. Iginla, I. Osetinsky, J.A. Reddy, C.P. Leamon, D. Scherman, M. Bessodes, P. Wils, Mol. Ther. 5 (2002) 739.
[26] S. Ilgan, D.J. Yang, T. Higuchi, F. Zareneyrizi, H. Bayhan, D.F. Yu, E.E. Kim, D.A. Podoloff, Cancer Biother. Radiopharm. 13 (1998) 427.
[27] C.J. Mathias, D. Hubers, P.S. Low, M.A. Green, Bioconjugate Chem. 11 (2000) 253.
[28] C.P. Leamon, M.A. Parker, I.R. Vlahov, L.C. Xu, J.A. Reddy, M. Vetzel, N. Douglas, Bioconjugate Chem. 13 (2002) 1200.
[29] C.J. Mathias, S. Wang, D.J. Waters, J.J. Turek, P.S. Low, M.A. Green, J. Nucl. Med. 39 (1998) 1579.
[30] C.J. Mathias, M.A. Green, Nucl. Med. Biol. 25 (1998) 585.
[31] B.A. Siegel, F. Dehdashti, D.G. Mutch, D.A. Podoloff, R. Wendt, G.P. Sutton, R.W. Burt, P.R. Ellis, C.J. Mathias, M.A. Green, D.M. Gershenson, J. Nucl. Med. 44 (2003) 700.
[32] R. Alberto, R. Schibli, A. Egli, P.A. Schubiger, W.A. Herrmann, G. Artus, U. Abram, T.A. Kaden, J. Organomet. Chem. 492 (1995) 217.
[33] R. Alberto, R. Schibli, A. Egli, A.P. Schubiger, U. Abram, T.A. Kaden, J. Am. Chem. Soc. 120 (1998) 7987.
[34] R. Alberto, R. Schibli, R. Waibel, U. Abram, A.P. Schubiger, Coord. Chem. Rev. 192 (1999) 901.
[35] R. Schibli, P.A. Schubiger, Eur. J. Nucl. Med. Mol. Imaging 29 (2002) 1529.
[36] D.P. Trump, C.J. Mathias, Z.F. Yang, P.S. Low, M.E. Marmion, M.A. Green, J. Nucl. Med. 41 (2000) 1014.
[37] D.P. Trump, C.J. Mathias, Z.F. Yang, P.S.W. Low, M. Marmion, M.A. Green, Nucl. Med. Biol. 29 (2002) 569.
[38] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A.P. Schubiger, J. Am. Chem. Soc. 123 (2001) 3135.
[39] J.F. Pons, J.L. Fauchere, F. Lamaty, A. Molla, R. Lazaro, Eur. J. Org. Chem. (1998) 853.
[40] A. Stichelberger, R. Waibel, C. Dumas, P.A. Schubiger, R. Schibli, Nucl. Med. Biol. 30 (2003) 465.
[41] C.P. Leamon, R.B. DePrince, R.W. Hendren, J. Drug Target. 7 (1999) 157.
[42] C.Y. Ke, C.J. Mathias, M.A. Green, J. Nucl. Med. 42 (2001) 427.
[43] S. Wang, J. Luo, D.A. Lantrip, D.J. Waters, C.J. Mathias, M.A. Green, P.L. Fuchs, P.S. Low, Bioconjugate Chem. 8 (1997) 673.
[44] M. Nomura, S. Shuto, A. Matsuda, J. Org. Chem. 65 (2000) 5016.
[45] A. Egli, K. Hegetschweiler, R. Alberto, U. Abram, R. Schibli, R. Hedinger, V. Gramlich, R. Kissner, P.A. Schubiger, Organometallics 16 (1997) 1833.