Synthesis of a Novel γ-Folic Acid–N^{τ}-Histidine Conjugate Suitable for Labeling with ^{99m}Tc and ^{188}Re

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Abstract: Radiolabeled folate derivatives have the potential to target folate receptor-positive tumor cells for noninvasive diagnosis and therapy. We report the synthesis of a novel γ-folic acid–N^{τ}-histidine conjugate **1** wherein the N^{τ}-histidine is suitable for radiolabeling with isotopes ^{99m}Tc (diagnosis) and ^{188}Re (therapy). A modular synthetic strategy was applied: N^{α}-Boc-α-carboxy-protected glutamic acid was amidically linked to N^{τ}-(functionalized aminoalkyl)histidine via the γ-carboxy group to form building block **8**. Intermediate **8** was coupled to protected pteroic acid to give **1** in two steps in 47% yield. N^{τ}-(Functionalized aminoalkyl)histidine was synthesized by two different routes. The preferred route starting from Boc-His-OMe led in two steps to the N^{τ}-(functionalized aminoalkyl)histidine in 36% yield.

Key words: alkylations, amino acids, regioselectivity, organometallic, receptor

The folate receptor (FR) is a promising tumor target for folate conjugates since it is overexpressed on a wide variety of cancer cells.[1] A number of folate conjugates have been synthesized for application in tumor therapy and diagnosis (e.g., of chemotherapeutic agents,[2] antisense oligonucleotides,[3] antibodies,[4] protein toxins,[5] liposomes[6]). For the diagnostic application some folate-based $^{66/67/68}$Ga, ^{99m}Tc, and ^{111}In radiopharmaceuticals have been developed.[7] Folate derivatives suitable for labeling with the radionuclides ^{99m}Tc (^{99m}Tc: 6 h half-life, 140 keV γ-radiation) and ^{188}Re (^{188}Re: 17 h half-life, 2.12 MeV β^-_{max}-radiation) are especially attractive since these isotopes are readily available and exhibit excellent decay properties for radiodiagnosis and radiotherapy.[8] Recently, a series of novel, organometallic Tc/Re-folate derivatives comprising different chelating systems have been reported.[9c] 2-Picolylamine-*N*-acetic acid (PAMA) for example has been conjugated to the γ-carboxy group of folic acid, successfully labeled with ^{99m}Tc and ^{188}Re and tested in vivo.[9] Van Staveren et al. have shown that N^{τ}-functionalized histidine derivatives are superior with respect to stability and/or chelating capacity for the tridentate $M(CO)_3$ core (M = Tc, Re) compared to PAMA.[10] We wanted to explore the possibility of coupling an N^{τ}-functionalized histidine derivative to folic acid for subsequent radiolabeling with ^{99m}Tc. One focus of this work was the development of the regioselective synthesis of an N^{τ}-(4-aminoalkyl)histidine synthon for further coupling to the γ-carboxy group of folic acid or glutamic acid, respectively (Scheme 1). Two synthetic strategies were assessed and reported in this work. The final γ-folic acid–histidine conjugate **1** was reacted with the organometallic $[M(CO)_3]$ synthons (M = ^{99m}Tc, Re) and spectroscopically and radiochemically analyzed.

In the present work, two approaches (route A and route B, Scheme 2) were investigated for the preparation of the N^{τ}-functionalized histidine building block. It is known that alkylation of the imidazole group of histidine derivatives gives in general a mixture of τ- and π-regioisomers (route A).[11] Usually the τ-isomer is predominant and is formed exclusively in cases of very bulky alkylating reagents like trityl halides.[12] For route A, the alkylation of Boc-His-OMe (**2**) with 1-azido-4-chlorobutane[13] in refluxing acetone and sodium iodide/potassium carbonate as catalyst/base gave the N^{τ}-(4-azidobutyl)histidine **3** in 36% yield after purification by flash chromatography.

One way to prevent π-alkylation is protection via the cyclic urea **5** (route B). The cyclic urea **5** was prepared according to the literature.[14] Reaction with 1-azido-4-chlorobutane was performed under similar conditions as for **2**. The crude product **6** was directly treated with *tert*-butyl alcohol/*N*,*N*-diisopropylethylamine to give the N^{τ}-(4-azidobutyl)histidine **3**. Reduced nucleophilicity of the τ-imidazole nitrogen in **5** and the sluggish ring-opening reaction of the alkylated urea **6** with *tert*-butyl alcohol resulted in an overall yield of only 10% for compound **3** over two steps starting from urea **5**.

The NMR data of the N^{τ}-(4-azidobutyl)histidine **3**, prepared by the two independent routes A and B, were identical and confirmed the correct assignment of the structure of **3**. In addition, by comprehensive NOE measurements on **3**, a clear and distinct NOE signal was observed for the $C(1)H_2$ group of the butyl side chain and the C(5)H of the histidine. Reduction of the azido group was achieved by catalytic hydrogenation with platinum(IV) oxide as catalyst in methanol to give the N^{τ}-(4-aminobutyl)histidine building block **4** in quantitative yield.

Additionally, we investigated other protected or masked aminobutyl synthons in the alkylation of Boc-His-OMe

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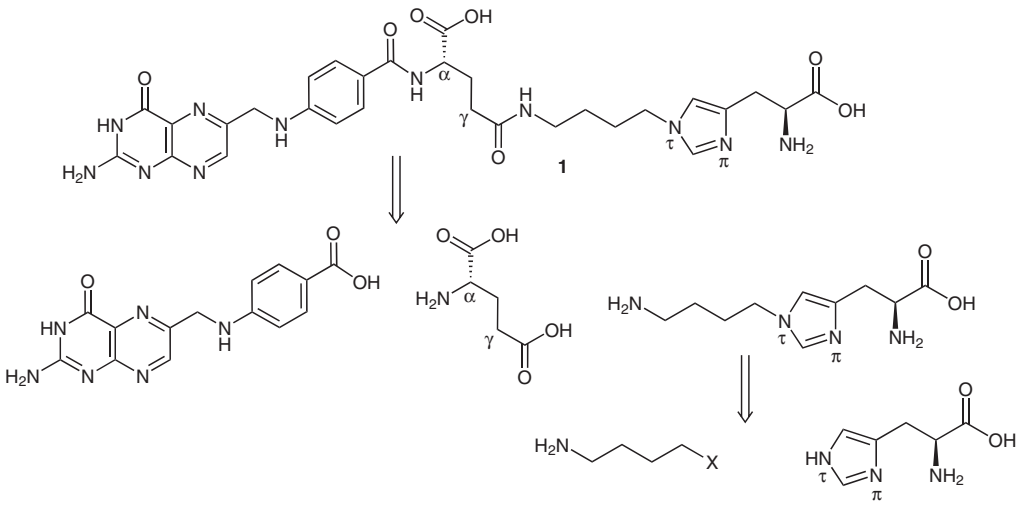


Scheme 1 Retrosynthesis for the γ-folic acid–histidine conjugate **1**

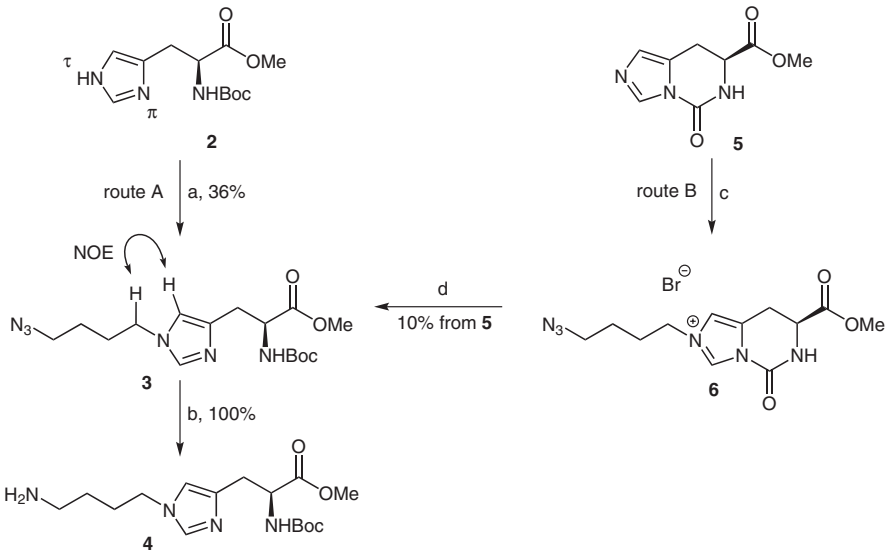
(**2**) or cyclic urea **5**. The use of Fmoc- and Cbz-protected halobutylamine derivatives on either route A or B resulted in the decomposition of the alkylating reagent by intramolecular 1,5-cyclization leading to the formation of the corresponding pyrrolidine derivatives. On the other hand, the alkylation of cyclic urea **5** with 3-bromopropanenitrile yielded the desired τ-isomer, but all attempts to reduce the nitrile group to the desired amine **4** were unsuccessful.

The final steps towards the target molecule **1** are shown in Scheme 3. The N^{τ}-(4-aminobutyl)histidine derivative **4** was reacted with Fmoc-Glu(OSu)-O*t*-Bu to give the protected γ-glutamate histidine building block **7** in 29% yield. Fmoc deprotection was achieved by standard procedures[15] to give **8** in 50% yield. The relatively low yield in the preparation of **7** and **8** is attributed to losses during chromatographic purification. The coupling of the protected pteroic acid **10** with **8** was achieved best with 4-(4,5-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)[16] as coupling reagent. Selective amide bond formation and workup by simple precipitation from water provided fully protected γ-folic acid–histidine conjugate **9** in 62% yield.

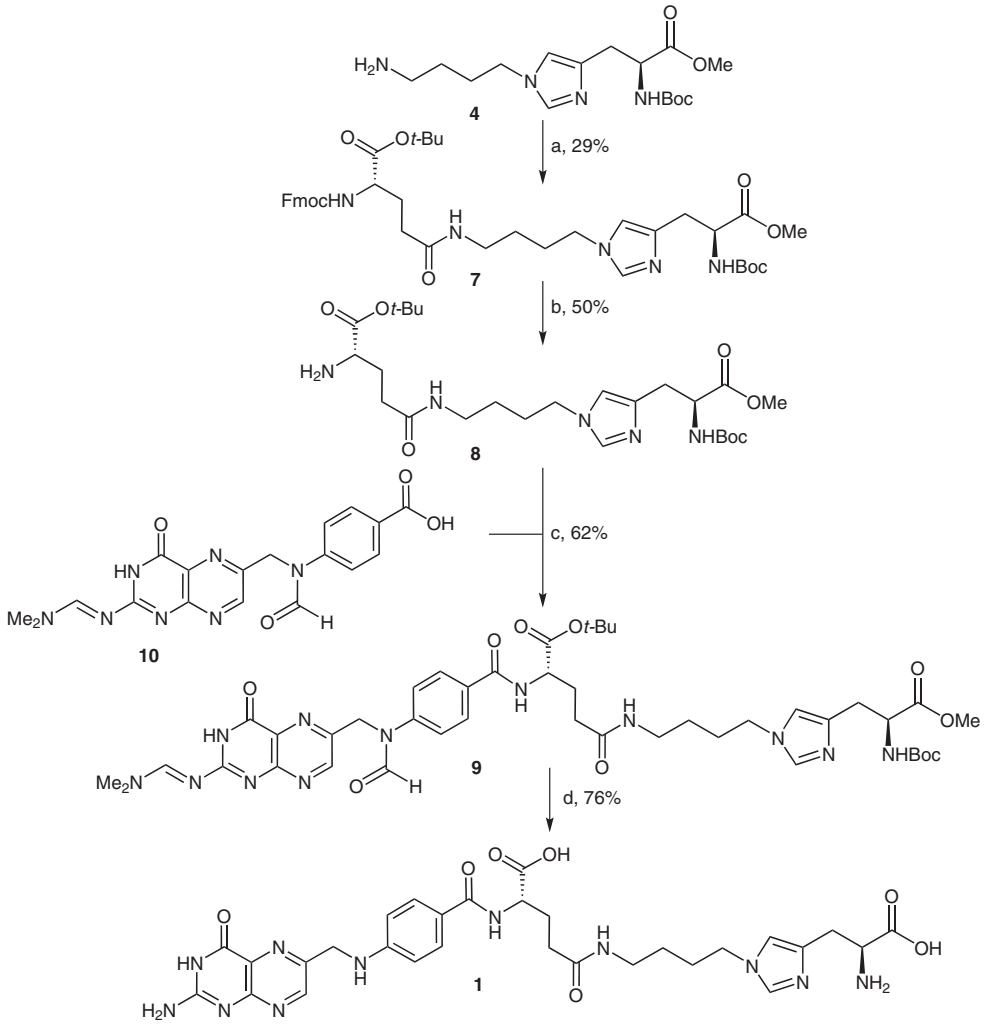
Finally, global deprotection was performed in two steps: After acidic hydrolysis of the *tert*-butyl ester, Boc, and (dimethylamino)methylene protecting groups, the methyl ester and the formyl group were cleaved under basic conditions.[17] Precipitation of the crude product at pH 2.5 and further purification by preparative reversed phase chromatography gave **1** in 76% yield.

The metal labeling of the folic acid conjugate **1** was achieved with $[Re(Br)_3(CO)_3][Et_4N]_2$ or $[^{99m}Tc(CO)_3(OH_2)_3]^+$ according to the procedure described by Müller et al[9b,c] (Scheme 4).

The rhenium complex **11** was purified by HPLC (Figure 1) and characterized by HRMS and NMR spectroscopy. Analysis of the ^{1}H NMR spectra proved triden-

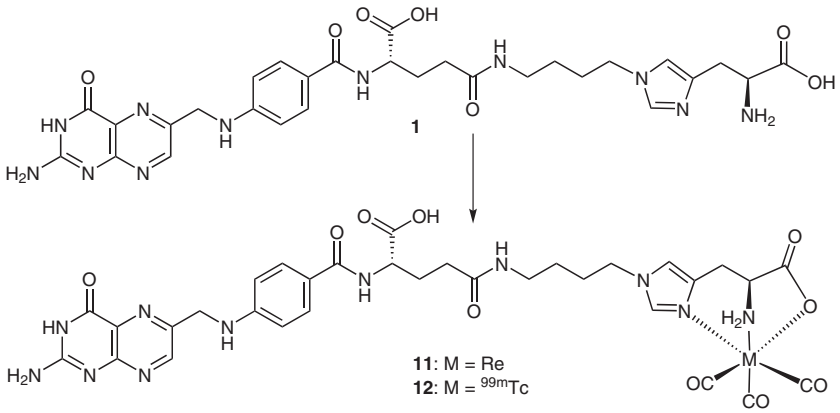


Scheme 2 Synthesis of the N^{τ}-(4-aminobutyl)histidine building block **4**. *Reagents and conditions*: (a) $Cl(CH_2)_4N_3$,[13] NaI, K_2CO_3, acetone, 65 °C, 48 h; (b) H_2, PtO_2, MeOH, r.t., 10 bar, 24 h; (c) $Cl(CH_2)_4N_3$, NaI, acetone, 65 °C, 16 h; (d) *t*-BuOH, *i*-Pr_2NEt, 85 °C, 20 h.



Scheme 3 Synthesis of the γ-folic acid–histidine conjugate **1**. *Reagents and conditions*: (a) Fmoc-Glu(OSu)-O*t*-Bu, DME, r.t., 2 h; (b) piperidine, CH_2Cl_2, r.t., 3,h. (c) **10**, DMTMM, THF, r.t., 3 h. (d) 1. 1 M HCl, 50 °C, 2 h; 2. NaOH, r.t., 1 h.

date coordination (π-N, α-NH_2, COOH) of the metal center with histidine as chelator since the signals of the histidine protons were typically broader and shifted to low field compared to the unlabeled compound **1**. Due to the low concentration of the radioactive ^{99m}Tc complex (~10^{-9} M) NMR measurement was not possible and therefore the (structural) identity of the corresponding ^{99m}Tc complex **12** was confirmed by comparison of the retention time (radioactive HPLC trace) with that of the corresponding rhenium complex (Figure 1).



Scheme 4 Synthesis of the $M(CO)_3$ complexes (M = Re, ^{99m}Tc) of the γ-folic acid–histidine conjugate **1**. *Reagents and conditions*: **11**: $[ReBr_3(CO)_3][Et_4N]_2$ (1.1 equiv) MeOH–H_2O (1:1), $NaHCO_3$, pH = 8, 50 °C, 1.5 h, **12**: *fac*-$[^{99m}Tc(CO)_3(OH_2)_3]^+$ (100 μL; ~1 GBq/mL), PBS buffer pH 7.4 (350 μL), 0.001 M **1** in PBS buffer (50 μL), 75 °C, 30 min.

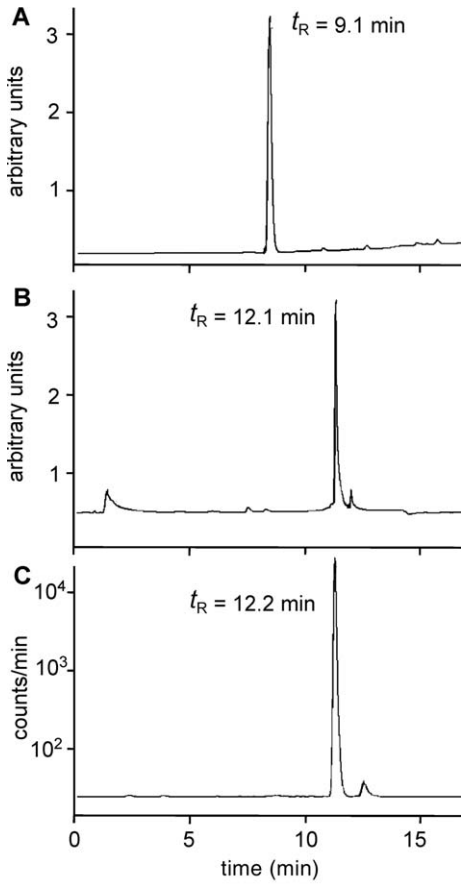


Figure 1 HPLC traces (Xterra™ MS C-18-column): (a) UV trace (254 nm) of **1**; (b) UV trace (254 nm) of the $Re(CO)_3$ complex **11**; (c) γ-trace of the radioactive ^{99m}Tc complex **12**. Differences between the t_R of the ^{99m}Tc and Re complex are due to the sequential setup of the UV/Vis and radioactive detector.

The complex **12** proved to be stable in vitro and in vivo. Detailed in vitro and in vivo results are described by Müller et al.[18]

In conclusion, we have identified the most suitable synthetic pathway for the preparation of a novel γ-folic acid–N^{τ}-histidine conjugate as potential precursor for (radio)labeling with the (radio)metal precursors of technetium and rhenium. Preliminary in vitro and in vivo experiments performed in folate receptor positive cell lines have demonstrated the superior characteristics of the novel folate derivatives compared to previously published organometallic folate derivatives.[18] Further experiments will be needed to assess the potential of this radiofolate for use in diagnosis (and therapy) of folate receptor positive tumor cells.

All experiments were carried out under an argon atmosphere. All commercially available reagents were used as received. The N^2-[(dimethylamino)methylene]-10-formylpteroic acid (**10**) was provided by Merck Eprova AG, Schaffhausen, Switzerland. TLC was performed on Merck silica gel 60 F254 glass-plates, flash-column chromatography on Fluka silica gel 60. IR spectra were recorded on a Perkin Elmer Spectrum 100. NMR spectra were recorded on a Bruker Avance 300; TMS was used as internal reference unless otherwise mentioned, in D_2O–NaOD the internal reference was $\delta(H_2O) = 4.79$ unless otherwise stated. HRMS (ESI) were recorded with a Bruker FTMS 4.7 T BioAPEXII (ESI) spectrometer.

N^{τ}-(4-Azidobutyl)-N^{α}-(*tert*-butoxycarbonyl)-L-histidine Methyl Ester (3)

Boc-His-OMe (**2**, 24.24 g, 90 mmol, 1.0 equiv) was stirred in acetone (50 mL). After the addition of K_2CO_3 (13.68 g, 99 mmol, 1.1 equiv), 1-azido-4-chlorobutane[13] (13.22 g, 99 mmol, 1.1 equiv), and NaI (3.75 g, 25 mmol, 0.28 equiv), the mixture was heated to reflux. After 2 d, TLC indicated a conversion of ~85% [R_f = 0.58 (**3**), 0.37 (**2**) (CH_2Cl_2–MeOH, 9:1)]. The product was isolated by chromatography (EtOAc–*n*-hexane, 4:1) to give **3** (11.74 g, 36%) as a yellow-brownish viscous oil; purity: ~95% (NMR).

IR (Golden Gate/ATR): 3345, 2930, 2095, 1745, 1705, 1495, 1365, 1160 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9 H, *t*-Bu), 1.5–1.6 [m, 2 H, C(3)H_2, Bu], 1.8–1.9 [m, 2 H, C(2)H_2, Bu], 3.00 [dd, ^2J = 14.4 Hz, ^3J = 4.7 Hz, 1 H, C(β)H_A, His], 3.08 [dd, ^2J = 14.8 Hz, ^3J = 5.5 Hz, 1 H, C(β)H_B, His], 3.31 [t, ^3J = 6.6 Hz, 2 H, C(4)H_2, Bu], 3.70 (s, 3 H, OCH_3, His), 3.90 [t, ^3J = 7.0 Hz, 2 H, C(1)H_2, Bu], 4.5–4.6 [m, 1 H, C(α)H, His], 5.9 (d, ^3J = 8.2 Hz, 1 H, NH, His), 6.68 [s, 1 H, C(5)H, His], 7.37 [s, 1 H, C(2)H, His].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.9, 28.2, 28.3, 30.3, 46.4, 50.8, 52.0, 53.6, 79.5, 116.2, 136.8, 137.9, 155.4, 172.5.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{27}N_6O_4$: 367.2094; found: 367.2087.

N^{τ}-(4-Aminobutyl)-N^{α}-(*tert*-butoxycarbonyl)-L-histidine Methyl Ester (4)

To a soln of **3** (7.0 g, 19.1 mmol, 1.0 equiv) in MeOH (140 mL) PtO_2·x H_2O (0.14 g, 81% Pt, 0.58 mmol) was added. The mixture was hydrogenated at r.t. using H_2 (10 bar) for 24 hours. During hydrogenation the H_2 atmosphere was replaced three times to avoid an accumulation of N_2 from the azide. After addition of cellulose (0.7 g) and charcoal (0.7 g) the mixture was stirred at r.t. for 5 min, the solids were filtered off and washed with MeOH (20 mL). The filtrate was concentrated under vacuum to give **4** (6.5 g, 100%), which was used directly in the next step.

IR (Golden Gate/ATR): 3305, 2935, 1745, 1705, 1495, 1365, 1160 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, *t*-Bu), 1.5–1.6 [m, 2 H, C(3)H_2, Bu], 1.7–1.8 [m, 2 H, C(2)H_2, Bu], 2.1–2.2 (br s, 2 H, NH_2, Bu), 2.71 [t, ^3J = 6.6 Hz, 2 H, C(4)H_2, Bu], 3.00 [dd, ^2J = 14.4 Hz, ^3J = 4.8 Hz, 1 H, C(β)H_A, His], 3.08 [dd, ^2J = 14.6 Hz, ^3J = 6.6 Hz, 1 H, C(β)H_B, His], 3.70 (s, 3 H, OCH_3, His), 3.90 [t, ^3J = 7.0 Hz, 2 H, C(1)H_2, Bu], 4.5–4.6 [m, 1 H, C(α)H, His], 5.95 (d, ^3J = 7.9 Hz, 1 H, NH, His), 6.68 [s, 1 H, C(5)H, His], 7.35 [s, 1 H, C(2)H, His].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.3, 28.5, 30.3, 30.4, 40.4, 46.9, 52.1, 53.6, 79.6, 116.4, 136.8, 137.7, 155.6, 172.6.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{29}N_4O_4$: 341.2189; found: 341.2183.

N^{α}-(*tert*-Butoxycarbonyl)-N^{τ}-(4-{[O^{α}-*tert*-butyl-N^{α}-(9*H*-fluoren-9-ylmethoxycarbonyl)-γ-glutamyl]amino}butyl)-L-histidine Methyl Ester (7)

To the soln of Fmoc-Glu(OSu)-O*t*-Bu (5.23 g, 10 mmol, 1.0 equiv) in DME (40 mL), **4** (6.88 g, 10 mmol, purity ~50% w/w, 1.0 equiv) was added. The mixture was stirred at r.t. for 2 h and then it was concentrated to ~15 g and the product was purified by flash chromatography (CH_2Cl_2–MeOH, 25:1) to give **7** (2.29 g, 29%) as a yellowish foam; purity: 95% (NMR); R_f = 0.46 (CH_2Cl_2–MeOH, 9:1)

IR (Golden Gate/ATR): 3300, 2935, 1710, 1650, 1500, 1440, 1365, 1155, 1050 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, *t*-Bu), 1.47 (s, 9 H, *t*-Bu), 1.3–1.4 [m, 2 H, C(2)H_2, Bu], 1.63–1.75 [m, 2 H, C(3)H_2, Bu], 1.85–1.95 [m, 1 H, C(β)H_A, Glu], 2.1–2.3 [m, 3 H, C(β)H_B, Glu, C(γ)H_2, Glu], 2.95–3.05 [m, 2 H, C(β)H_2, His], 3.15–3.25 [m, 2 H, C(1)H_2, Bu], 3.68 (s, 3 H, OCH_3, His), 3.86 [t, ^3J = 6.9 Hz, 2 H, C(4)H_2, Bu], 4.15–4.25 [m, 2 H, C(9)H, Fmoc and C(α)H, Glu], 4.39 [t, ^3J = 5.0 Hz, 2 H, C(9)CH_2, Fmoc], 4.45–4.55 [m, 1 H, C(α)H, His], 5.82 [d, ^3J = 7.6 Hz, 1 H, C(α)NH, Glu], 5.95 [d, ^3J = 8.1 Hz, 1 H, C(α)NH, His], 6.4 [s, 1 H, C(4)NH, Bu], 6.66 [s, 1 H, C(5)H, His], 7.32 [s, 1 H, C(2)H, His], 7.3–7.75 [m, 8 H, Fmoc].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6, 28.0, 28.1, 28.3, 30.2, 32.5, 38.6, 46.5, 47.1, 52.1, 53.7, 54.0, 67.0, 79.6, 82.5, 116.4, 120.0, 125.2, 127.1, 127.8, 136.8, 137.5, 141.3, 143.6, 143.9, 155.6, 156.5, 171.1, 172.2, 172.6.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{40}H_{54}N_5O_9$: 748.3922; found: 748.3921.

N^{α}-(*tert*-Butoxycarbonyl)-N^{τ}-{4-[(O^{α}-*tert*-butyl-γ-glutamyl)amino]butyl}-L-histidine Methyl Ester (8)

To a mixture of **7** (2.29 g, 3.06 mmol, 1.0 equiv) and CH_2Cl_2 (50 mL), piperidine (3.0 mL, 30.6 mmol, 10.0 equiv) was added. The mixture was stirred at r.t. for 3 h and then the product was isolated directly by flash chromatography (CH_2Cl_2–MeOH, 4:1) to give **8** (892 mg, 50%) as a pink foam; purity: 90% (NMR); R_f = 0.20 (CH_2Cl_2–MeOH, 9:1).

IR (Golden Gate/ATR): 3300, 2935, 1710, 1650, 1500, 1435, 1365, 1155, 1050 cm^{-1}.

^{1}H NMR (300 MHz, DMSO): δ = 1.43 (s, 9 H, *t*-Bu), 1.46 (s, 9 H, *t*-Bu), 1.35–1.55 [m, 2 H, C(2)H_2, Bu], 1.75–1.9 [m, 3 H, C(3)H_2, Bu and C(β)H_A, Glu], 2.05–2.2 [m, 1 H, C(β)H_B, Glu], 2.35 [t, ^3J = 7.2 Hz, 2 H, C(γ)H_2, Glu], 2.95–3.1 [2 dd, 2 H, C(β)H_2, His], 3.22 [q, ^3J = 6.4 Hz, 2 H, C(1)H_2, Bu], 3.41 [dd, ^3J = 8.4 Hz, ^3J = 4.3 Hz, 1 H, C(α)H, Glu], 3.67 (s, 3 H, OCH_3, His), 3.90 [t, ^3J = 6.9 Hz, 2 H, C(4)H_2, Bu], 4.45–4.55 [m, 1 H, C(α)H, His], 5.94 [d, ^3J = 8.4 Hz, 1 H, C(α)NH, His], 6.65–6.7 [2 s, 2 H, C(γ)CONH, Glu and C(5)H, His], 7.42 [s, 1 H, C(2)H, His].

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6, 28.0, 28.1, 28.2, 28.3, 30.3, 32.9, 38.5, 44.5, 46.5, 52.1, 53.7, 54.4, 59.0, 79.6, 81.4, 116.4, 136.9, 137.6, 155.6, 172.2, 172.8, 174.8.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{25}H_{44}N_5O_7$: 526.3241; found: 526.3239.

N^{α}-(*tert*-Butoxycarbonyl)-N^{τ}-[4-({O^{α}-*tert*-butyl-N^2-[(dimethylamino)methylene]-10-formylfolyl}amino)butyl]-L-histidine Methyl Ester (9)

To a soln of **10** (391 mg, 0.90 mmol, 1.0 equiv) in THF (3 mL) was added DMTMM (274 mg, 0.99 mmol, 1.1 equiv) and **8** (473 mg, 0.99 mmol, 1.1 equiv]. The suspension was stirred at r.t. for 3 h and then concentrated under vacuum to ~1.5 g. After addition of H_2O (2 mL) a precipitate was formed which was separated from the soln by centrifugation. The precipitate was washed with H_2O (0.5 mL), separated by centrifugation, and dried under vacuum to give **9** (526 mg, 62%) as a yellow foam; purity: 95% (NMR).

IR (Golden Gate/ATR): 3280, 2935, 1630, 1605, 1540, 1500, 1455, 1420, 1330, 1155, 1115 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 9 H, *t*-Bu), 1.3–1.45 [m, 2 H, C(3)H_2, Bu], 1.46 (s, 9 H, *t*-Bu), 1.7–1.8 [m, 2 H, C(2)H_2, Bu], 1.8–1.9 [m, 1 H, C(β)H_A, Glu], 2.1–2.4 [m, 3 H, C(β)H_B and C(γ)H_2, Glu], 2.95–3.05 [2 dd, 2 H, C(β)H_2, His], 3.14 (s, 3 H, CH_3, DMAM), 3.22 (s, 3 H, CH_3, DMAM), 3.2–3.3 [m, 2 H, C(4)H_2, Bu], 3.69 (s, 3 H, CH_3), 3.8–3.9 [m, 2 H, C(1)H_2, Bu], 4.4–4.6 [m, 2 H, C(α)H, His and Glu], 5.29 [s, 2 H, C(9)H_2, Pte], 5.60 (d, 1 H, NH), 5.94 (d, 1 H, NH), 6.70 [s, 1 H, C(5)H, His], 6.95 (s, 1 H, NH), 7.40 [d, ^3J = 8.6, 2 H, C(3′,5′)H, Pte], 7.48 [s, 1 H, C(2)H, His], 7.87 [d, ^3J = 8.6, 2 H, C(2′,6′)H, Pte], 8.70 (s, 1 H, CH, DMAM), 8.80 [s, 1 H, N(10)CHO, Pte], 8.93 [s, 1 H, C(7)H, Pte].

^{1}H–^{1}H COSY-90 correlations (300 MHz, MeOD): δ (F2/F1) = 1.41/1.72 [C(2)H_2/C(3)H_2, Bu], 1.41/3.20 [C(2)H_2/C(1)H_2, Bu], 1.72/3.95 [C(3)H_2/C(4)H_2, Bu], 2.02/2.22 [C(β)H_A/C(β)H_B, Glu], 2.02/2.35 [C(β)H_A/C(γ)H_2, Glu], 2.22/2.35 [C(β)H_B/C(γ)H_2, Glu], 2.02/4.42 [C(β)H_A/C(α)H, Glu], 2.22/4.42 [C(β)H_B/C(α)H, Glu], 2.92/4.35 [C(β)H_2/C(α)H, His)], 6.9/7.58 [C(5)H/C(2)H, His], 7.54/7.9 [C(3′,5′)H/C(2′,6′)H, Pte).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.5, 28.1, 28.2, 28.5, 30.4, 32.8, 35.6, 38.8, 41.9, 46.8, 48.5, 52.3, 53.6, 54.1, 56.0, 79.8, 82.4, 116.8, 122.6, 123.2, 129.1, 130.2, 132.1, 137.2, 143.6, 147.8, 149.9, 155.8, 158.3, 159.3, 154.0, 162.5, 162.6, 166.5, 168.6, 171.3, 172.9, 173.1.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{43}H_{59}N_{12}O_{10}$: 903.4477; found: 903.4489.

N^{τ}-[4-(Folylamino)butyl]-L-histidine (1)

To **9** (200 mg, 0.22 mmol, 1 equiv) aq 1 M HCl (22 mL) was added and the mixture was stirred at 50 °C for 2 h. After cooling to ~15 °C, solid NaOH (1.76 g) was added, the soln was stirred at r.t. for 1 h. The pH was adjusted to 2.5 by addition of HCO_2H and the precipitate was isolated by filtration. The product was purified by RP-MPLC (solid phase: Europrep 60-60 C-18, 60 μ, 35–70 μm, 140 g; 36 cm × 26 mm, liquid phase: 0–10 min. 99.9% H_2O–0.1% HCO_2H, 10–40 min. 34.9% MeOH–65% H_2O–0.1% HCO_2H) to give **1** (120 mg, 76%) as a yellowish solid; purity: 91% (HPLC).

^{1}H NMR (300 MHz, D_2O–10% D_2SO_4): δ = 0.4–0.6 [m, 2 H, C(2)H_2, Bu], 0.75–0.9 [m, 2 H, C(3)H_2, Bu], 1.1–1.25 [m, 1 H, C(β)H_A, Glu], 1.25–1.4 [m, 1 H, C(β)H_B, Glu], 1.50 [t, ^3J = 7.1 Hz, 2 H, C(γ)H_2, Glu], 2.1–2.3 [m, 2 H, C(1)H_2, Bu], 2.4–2.5 [2 dd, 2 H, C(β)H_2, His], 3.1–3.3 [m, 2 H, C(4)H_2, Bu], 3.42 [t, ^3J = 6.7 Hz, 1 H, C(α)H, His], 3.6 [dd, ^3J = 4.6 Hz, ^3J = 9.44 Hz, 1 H, C(α)H, Glu], 4.08 [s, 2 H, C(9)H_2, Pte], 6.5 [s, 1 H, C(5)H, His], 6.65 [d, ^3J = 6.7 Hz, 2 H, C(3′,5′)H, Pte], 6.95 [d, ^3J = 6.1 Hz, 2 H, C(2′,6′)H, Pte], 7.68 [s, 1 H, C(2)H, His], 7.83 [s, 1 H, C(7)H, Pte).

^{1}H–^{1}H COSY-90 correlations (300 MHz, D_2O–D_2SO_4): δ (F2/F1) = 0.50/0.84 [C(2)H_2/C(3)H_2, Bu], 0.50/2.19 [C(2)H_2/C(1)H_2, Bu], 0.84/3.18 [C(3)H_2/C(4)H_2, Bu], 1.18/1.34 [C(β)H_A/C(β)H_B, Glu], 1.18/1.5 [C(β)H_A/C(γ)H_2, Glu], 1.18/3.57 [C(β)H_A/C(α)H, Glu], 1.34/3.57 [C(β)H_B/C(α)H, Glu], 2.4/2.5 [C(β)H_A/C(β)H_B, His], 2.4/3.45 [C(β)H_A/C(α)H, His], 2.5/3.45 [C(β)H_B/C(α)H, His], 6.5/7.7 [C(5)H/C(2)H, His], 6.65/6.95 [C(3′,5′)H/C(2′,6′)H, Pte).

^{13}C NMR (75 MHz, D_2O–10% D_2SO_4, TSP-d_4): δ = 28.1, 28.2, 29.3, 29.6, 35.2, 37.4, 41.6, 52.1, 52.6, 54.8, 55.8, 121.7, 124.0, 130.0, 130.2, 132.4, 138.0, 149.8, 151.8, 152.7, 154.5, 162.8, 168.7, 172.5, 173.1, 178.0.

HRMS (ESI): *m*/*z* [M – H]$^-$ calcd for $C_{29}H_{34}N_{11}O_7$: 648.2643; found: 648.2630.

Rhenium Complex 11

The synthesis of **11** was achieved in analogy to the literature procedure.[9b] Briefly: His-folate **1** (15.0 mg, 23 μmol) and $[ReBr_3(CO)_3][Et_4N]_2$ (20.0 mg, 26 μmol) were suspended in H_2O–MeOH (1:1, 4 mL) and the pH was adjusted to pH 8 with dil $NaHCO_3$. The resulting yellow soln was stirred at 50 °C for 1.5 h after which HPLC indicated complete conversion of His-folate **1**. The mixture was cooled to r.t. and the pH adjusted to pH 2–3 by addition of 0.1 M HCl. The precipitate was isolated by centrifugation (10 min, 3500 rpm) and dried under reduced pressure to provide Re complex **4** as a brown solid (HPLC purity: ~70%). The crude product was purified via HPLC (XTerra® column, MSC18, 5μm, 4.6 × 150 mm, Waters; liquid phase: solvent A = 0.1% aq TFA, solvent B = MeCN; 0–15 min 5%→80% B; 15–20 min 95% B): t_R = 12.1 min.

^{1}H NMR (300 MHz, D_2O–NaOD): δ = 0.4–0.6 [m, 2 H, C(2)H_2, Bu], 0.85–1.00 [m, 2 H, C(3)H_2, Bu], 1.28–1.32 [m, 1 H, C(β)H_A, Glu], 1.25–1.4 (m, 1 H,), 1.50 (t, ^3J = 7.1 Hz, 2 H), 2.1–2.3 [m, 5 H, C(γ)H_2, C(β)H_B, Glu; C(1)H_2, Bu], 2.58 [br s, 2 H, C(β)H_2, His], 3.1–3.3 [m, 2 H, C(4)H_2, Bu], 3.54 [t, ^3J = 6.7 Hz, 1 H, C(α)H, His], 4.00–4.09 [m, 1 H, C(α)H, Glu], 4.25 [s, 2 H, C(9)H_2, Pte], 6.30 [s, 1 H, C(5)H, His], 6.48 [d, ^3J = 7.5 Hz, 2 H, C(2′,6′)H, Pte], 7.33 [d, ^3J = 7.5 Hz, 2 H, C(3′,5′)H, Pte], 7.40 [s, 1 H, C(7)H, Pte], 8.26 [s, 1 H, C(2)H, His).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{32}H_{35}N_{11}O_{10}Re$: 920.2126; found: 920.2131.

^{99m}Tc(CO)$_3$ Complex 12

The radiotracer was prepared according to the literature procedure.[9b] Briefly: $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (100 μL), phosphate buffered saline (PBS pH 7.4, 350 μL) and 0.001 M **1** in PBS buffer (50 μL) were placed in a sealed glass vial. After 30 min at 75 °C, the vial was cooled on ice; yield: 92% (based on HPLC; t_R = 12.2 min). The radioactive precursor $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was synthesized according to the literature procedure.[19]

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