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Journal of Organometallic Chemistry 692 (2007) 1332–1339
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Evaluation of two chelators for labelling a PNA monomer with the *fac*-[^{99m}Tc(CO)$_3$]$^+$ moiety

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Received 1 September 2006; received in revised form 3 October 2006; accepted 3 October 2006
Available online 12 October 2006

Abstract

A PNA monomer containing thymine as nucleobase (**1**) was synthesized, characterized and coupled to the pyrazolyl containing ligand 3,5-Me$_2$pz(CH$_2$)$_2$N((CH$_2$)$_3$COOH)(CH$_2$)$_2$NHBoc (**2**) and to a modified cysteine *S*-(carboxymethyl-pentafluorphenyl)-*N*-[(trifluor)carbonyl]-L-cysteine methyl ester (**3**) yielding the bifunctional chelators **6** and **7**, respectively. Reactions of **6** and **7** with the Re(I) tricarbonyl starting material [Re(CO)$_3$(H$_2$O)$_3$]Br afforded the complexes *fac*-[Re(CO)$_3$(κ^3-**6**)]$^+$ (**8**) and *fac*-[Re(CO)$_3$(κ^3-**7**)] (**9**), respectively. The identity of **8** and **9** has been established based on IR spectroscopy, elemental analysis, ESI-MS spectrometry and HPLC. The multinuclear NMR spectroscopy (^{1}H, ^{13}C, g-COSY, g-HSQC) has also been very informative in the case of complex **8**, showing the presence of rotamers in solution. For **9** the NMR spectrum was too complex due to the presence of rotamers and diastereoisomers. The radioactive congeners of complexes **8** and **9**, *fac*-[^{99m}Tc(CO)$_3$(κ^3-**6**)]$^+$ (**8a**) and *fac*-[^{99m}Tc(CO)^3(κ^3-**7**)] (**9a**), have been prepared by reacting the precursor *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with the corresponding ligands being their identity established by comparing their HPLC chromatograms with the HPLC of the rhenium surrogates.
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Keywords: PNA; Antisense; Tricarbonyl; Technetium; Rhenium

1. Introduction

Non-invasive molecular imaging of targeted macromolecules and biological processes in living systems is a challenging task and, in this domain, the use of radioactive probes has several advantages when compared with other methodologies [1,2]. According to the state of the art in molecular biology and radiopharmacy, *in vivo* imaging of endogenous gene expression is of major importance as it may provide information on cellular gene expression patterns and detect molecular changes in disease states at relatively early stages [2,3]. The imaging of endogenous gene expression can be directed to transcription of genes into mRNA using a complementary sequence of the target mRNA (antisense approach) [2,3]. Designing a ligand to recognize an overexpressed mRNA is straightforward, in principle, requiring nothing more than a Watson–Crick complementary antisense oligodeoxynucleotide (ODN) [4]. However, naturally occurring ODNs are not very promising for nuclear imaging due to their easy *in vivo* degradation by endo- and exo-nucleases [5]. To increase *in vivo* stability of ODNs many different chemical modifications have been attempted. One of them consisted on the replacement of the sugar-phosphate by a polyamide group leading to peptide nucleic acids (PNAs) (Fig. 1) [6,7].

PNAs are excellent structural mimics of DNA/RNA as they hybridize more strongly and specifically to RNA and DNA than do normal oligonucleotides. Peptide nucleic acids are achiral, neutral, stable over a wide range of pH, resistant to enzymatic degradation and do not activate RNase-H degradation of mRNA [5–11]. The outstanding hybridization properties of PNAs have attracted consider-

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doi:10.1016/j.jorganchem.2006.10.007

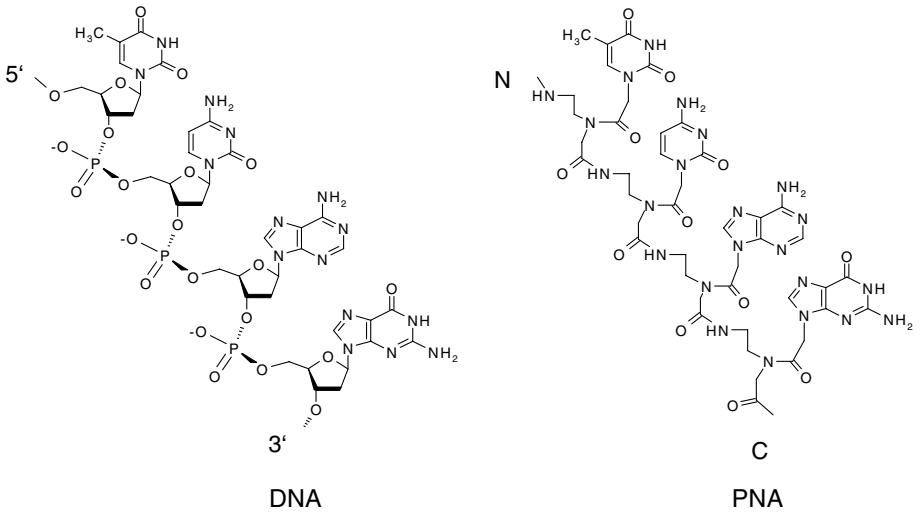


Fig. 1. Structure of DNA and PNA.

able attention for designing radioactive probes using the antisense strategy [5,8,9,12].

Having in mind to contribute for finding novel ^{99m}Tc complexes, potentially useful for antisense imaging, a PNA monomer (**1**) has been synthesized, characterized and coupled to a pyrazolyl containing ligand, 3,5-$Me_2pz(CH_2)_2N((CH_2)_3COOH)(CH_2)_2NHBoc$ (**2**) [13–15], and to a modified cysteine, *S*-(carboxymethyl-pentafluorphenyl)-*N*-[(trifluor)carbonyl]-L-cysteine methyl ester (**3**). The chemistry and radiochemistry of the resulting bifunctional chelators (**6** and **7**) towards the tricarbonyl moiety have also been studied.

Herein, we report on the synthesis and characterization of the model compounds **1**, **3**, **6** and **7** as well as on the tricarbonyl complexes *fac*-$[M(CO)_3(\kappa^3$-***6***$)]^+$ (M = Re (**8**), M = ^{99m}Tc (**8a**)) and *fac*-$[M(CO)_3(\kappa^3$-*7*$)]$ (M = Re (**9**), M = ^{99m}Tc (**9a**)) obtained by reacting $[Re(CO)_3(H_2O)_3]Br$ or $[^{99m}Tc(CO)_3(H_2O)_3]^+$ with **6** and **7**, respectively.

2. Experimental part

All chemicals and solvents were of reagent grade and were used without purification unless stated otherwise. The organometallic precursor $[Re(CO)_3(H_2O)_3]Br$ was prepared according to a published method [16]. The radioactive synthon $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared as described elsewhere [17] or using the Mallinckrodt IsoLink kit. $Na^{99m}TcO_4$ in saline solution was eluted from a ^{99}Mo/^{99m}Tc generator Ultra-TechneKow® from Mallinckrodt.

The compounds *N*-*tert*-butoxycarbonyl-1,2-ethanodiamine, methyl *N*-[2-(Boc-amino)ethyl]glycinate, thymin-1-ylacetic acid and 3,5-$Me_2pz(CH_2)_2N((CH_2)_3COOH)(CH_2)_2$-NHBoc (**2**) were synthesized according to procedures previously described in the literature [11,13–15,18,19].

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{13}C chemical shifts were referenced with the residual solvent resonances relative to tetramethylsilane and ^{19}F chemical shifts were referenced relatively to trifluortoluol. In the ^{1}H NMR of the compounds described in this work different rotamers have been observed, appearing as major (ma) or minor (mi) species. IR spectra were recorded as KBr pellets on a Bruker, Tensor 27 spectrometer. C, H and N analyses were performed on an EA110 CE Instruments automatic analyser. Column chromatography was performed using silica gel 60 (Merck). HPLC analyses were performed on a Perkin Elmer LC pump 200 coupled to an LC 290 tunable UV detector and to a Berthold-LB 507A radiometric detector. Separations were achieved on a Macherey-Nagel C18 reversed-phase column (Nucleosil 10 μm, 250 × 4 mm) (analytic) or on a Bondapak C18 reversed-phase column (Waters Associates, 150 × 19 mm) (preparative) using a gradient of MeOH/0.1% CF_3COOH as eluent, and flow rates of 0.5 mL/min (analytic) or 5 mL/min (preparative). *Method 1*: t = 0–3 min: 0% MeOH; 3–3.1 min: 0–25% MeOH; 3.1–9 min: 25% MeOH; 9–9.1 min: 25–34% MeOH; 9.1–18 min: 34–100% MeOH; 18–25 min: 100% MeOH, 25–25.1 min: 100–0% MeOH; 25.1–30 min: 0% MeOH. *Method 2*: t = 0–5 min: 10% MeOH; 5.1–30 min: 10–100% MeOH; 30–34 min: 100% MeOH; 34–35 min: 10% MeOH; 35–40 min: 10% MeOH.

2.1. Synthesis of methyl N-[2-(aminoethyl])-N-(thymin-1-ylacethyl)] glycinate (*1*)

To a solution of methyl *N*-[2-(Boc-amino)ethyl]glycinate (1.7 g, 7.2 mmol) in dry DMF (20 mL) have been added DIPEA (2.5 mL, 14.5 mmol), HBTU (447 mg, 1.2 mmol) and thymin-1-ylacetic acid (1.2 g, 7.2 mmol). The mixture was stirred at room temperature for 19 h and the solvent evaporated under *vacuo*. The residue was dissolved in EtOAc (20 mL) and this solution has been successively washed with $NaHCO_3$, 1 M $NaHSO_4$ and water. The organic phase has been dried over $MgSO_4$, filtered and the solvent evaporated under *vacuo*. A white solid has been

obtained and formulated as methyl *N*-[2-(Boc-amino)ethyl]-*N*-(thymin-1-ylacethyl) glycinate. Yield: 1.8 g, 65%. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.86 (br, N*H*, 1H); 7.01 (mi) and 6.94 (ma) (s, *H*(6)-T, 1H), 5.57 (ma) and 4.96 (mi) (br t, N*H*, 1H); 4.55 (ma) and 4.40 (mi) (s, CH_2, 2H); 4.19 (mi) and 4.03 (ma) (s, CH_2, 2H), 3.79 (mi) and 3.73 (ma) (s, CH_3, 3H); 3.51 (t, CH_2, 2H); 3.31 (q, CH_2, 2H); 1.89 (s, CH_3, 3H); 1.42 (s, 9H, O^{t}Bu). ^{13}C NMR ($CDCl_3$): 170.2 (ma) and 169.9 (mi) (*C*=O); 167.8 (mi) and 167.4 (ma) (*C*=O); 164.4 (*C*=O); 156.1 (*C*=O C(4)T); 151.2 (*C*=O (C(2)T)); 141.1 (*C*(6)T); 110.7 (*C*(5)T); 79.9 (*C*(CH_3)$_3$); 52.2 (mi), 52.9 (mi), 52.5 (ma), 50.2 (mi), 48.9 (ma), 48.6 (ma), 47.9 (ma), 43.2 (mi) (3*C*H$_2$ + *C*H$_3$); 28.3 (C(*C*H$_3$)$_3$); 12.3 (*C*H$_3$-T).

For removing the Boc protecting group, the compound methyl *N*-(2-Boc-aminoethyl)-*N*-(thymin-1-ylacethyl) glycinate (220 mg, 0.5 mmol) has been dissolved in a mixture of CH_2Cl_2/TFA (8:2 mL). The reaction mixture was stirred at room temperature for 4 h and the solvent evaporated under *vacuo*. The obtained crude oil, after being washed with toluene and triturated with diethyl ether, was dried under *vacuo* yielding quantitatively an orange solid which has been formulated as **1**, based on NMR data. ^{1}H NMR (CD_3OD): δ (ppm) 7.36 (mi) and 7.28 (ma) (s, *H*(6)-T, 1H), 4.71 (mi) and 4.58 (ma) (s, CH_2, 2H); 4.38 (ma) and 4.17 (mi) (s, CH_2, 2H), 3.82 (ma) and 3.75 (mi) (s, CH_3, 3H); 3.70 (t, CH_2, 2H); 3.15 (t, CH_2, 2H); 1.87 (s, CH_3, 3H). HPLC (method 1): $R_t = 14.22$ min.

2.2. Synthesis of S-(carboxymethyl-pentafluorphenyl)-N-[(trifluor)carbonyl]-L-cysteine methyl ester (**3**)

2.2.1. S-(tert-Butoxymethyl)-N-(tert-butoxycarbonyl)-L-cysteine methyl ester

To a solution of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (1.2 g, 4.9 mmol) in CH_2Cl_2 (50 mL) has been added NEt_3 (1.0 mL, 7.3 mmol), followed by dropwise addition of *tert*-butylbromoacetate (1.1 mL, 7.3 mmol). The reaction mixture was stirred for 20 h at room temperature. After this time, the mixture was diluted with 170 mL of CH_2Cl_2, washed with H_2O, 1 M HCl and H_2O. The organic phase has been separated and dried over Na_2SO_4, filtered and the solvent evaporated under *vacuo*. The residue was purified by column chromatography, using hexane/EtOAc (7:1) as eluent. Yield: 1.2 g, 70%. ^{1}H NMR ($CDCl_3$): δ (ppm) 5.46 (d br, 1H, N*H*), 4.56 (m, 1H, C*H*), 3.77 (s, 3H, OCH_3), 3.23–3.13 (m, 2H, CH_2CO), 3.11–3.00 (m, 2H, CHCH_2), 1.48 (s, 9H, O^{t}Bu), 1.46 (s, 9H, O^{t}Bu).

2.2.2. S-(Carboxymethyl)-L-cysteine methyl ester

S-(*tert*-Butoxymethyl)-*N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (1.2 g, 3.4 mmol) was dissolved in 7 mL of CH_2Cl_2. After addition of 7 mL of TFA, the resulting mixture was stirred at room temperature. The deprotection was complete after 4 h of reaction, as indicated by HPLC. The solvent was evaporated under *vacuo* and the expected product was recovered almost quantitatively. ^{1}H NMR (D_2O): δ (ppm) 4.30 (m, 1H, C*H*), 3.73 (s, 3H, OCH_3), 3.41–3.30 (m, 2H, CH_2CO), 3.24–2.99 (m, 2H, CHCH_2).

2.2.3. S-(Carboxymethyl-pentafluorphenyl)-N-[(trifluor)carbonyl]-L-cysteine methyl ester

To a solution of *S*-(carboxymethyl)-L-cysteine methyl ester (400 mg, 1.3 mmol) in 2 mL of DMF have been added pyridine (1 mL, 13 mmol) and TFA-PfP (pentafluorphenol trifluoracetic acid) (1.5 mL, 9.1 mmol). The resulting yellow reaction mixture was stirred overnight at room temperature. The solvent was evaporated under high *vacuo* and the residue obtained has been dissolved in 60 mL of EtOAc. After washing the resulting solution with 0.01 N HCl (3 × 10 mL) and 5% $NaHCO_3$ aq. (3 × 10 mL), the organic phase was dried over $MgSO_4$, filtered and the solvent evaporated under *vacuo*. The residue has been washed several times with hexane and dried under *vacuo*, yielding a white powder. Yield: 580 mg, 98%. ^{1}H NMR ($CDCl_3$): δ 7.2 (br, 1H, N*H*), 4.88 (m, 1H, C*H*), 3.82 (s, 3H, OCH_3), 3.64–3.49 (m, 2H, CH_2CO), 3.33–3.16 (m, 2H, CHCH_2). ^{13}C NMR ($CDCl_3$): 169.4 (*C*=O); 166.2 (*C*=O); 157.4 (*C*=O); 142.7, 139.4 and 136.2, (*C*(ar)); 53.4 (*C*H); 51.8 (*C*H$_3$); 33.8 (*C*H$_2$); 32.7 (*C*H$_2$). ^{19}F NMR ($CDCl_3$): −77.5 (s, 3*F*), −154.2 (d, 2*F*), −158.5 (t, 2*F*), −163.3 (t, 1*F*). Anal. Calc. For $C_{14}H_9F_8NO_5S$: C, 36.93; H, 1.99; N, 3.08; S, 7.04. Found: C, 36.99; H, 2.40; N, 3.25; S, 7.40%.

2.3. Synthesis of 2-(N-(2-(4-((2-aminoethyl)(2-(3,5-dimethyl-1H-pyrazol-1- yl)ethyl)amino)butanamido)-ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid (**6**)

To a solution of compound **1** (200 mg, 0.5 mmol) in dry CH_3CN (10 mL) has been added successively NEt_3 (0.2 mL, 1.5 mmol), HBTU (189 mg, 0.5 mmol) and 3,5-$Me_2pz(CH_2)_2N((CH_2)_3COOH)(CH_2)_2NHBoc$ (**2**) (184 mg, 0.5 mmol), and the mixture has been stirred for 19 h. After evaporation of the solvent, the residue obtained has been purified by column chromatography (MeOH (5–13%)/$CHCl_3$). The collected product was washed with water and the Boc protecting group was removed with TFA in CH_2Cl_2. After 1 h at room temperature, the solvent was evaporated, the residue has been dissolved in MeOH and K_2CO_3 added. After stirring overnight, the product has been purified by preparative HPLC (method 2). ***6***: Yield: 174 mg, 65%. ^{1}H NMR (CD_3OD): δ (ppm) 7.30 (ma) and 7.27 (mi) (s, *H*(6)-T, 1H); 5.92 (s, H(4)-pz), 4.69 (ma) and 4.55 (mi) (s, CH_2(k), 2H); 4.38 (t, CH_2(a), 2H); 4.28 (mi) and 4.11 (ma) (s, CH_2(j), 2H); 3.62–3.41 (m, CH_2(b, c, d, h, i), 10H); 3.32 and 3.29 (t, CH_2(g), 1H + 1H); 2.51 and 2.35 (t, CH_2(e), 1H + 1H), 2.27 (s, CH_3-pz, 3H), 2.19 (s, CH_3-pz, 3H); 1.97 (m, CH_2(f), 2H); 1.85 (s, CH_3-T, 3H). ^{13}C NMR (CD_3OD): δ (ppm) 176.1 (*C*OOH); 175.8 (*C*=O); 170.4 (*C*=O); 166.9 (*C*=O (C(4)T)); 153.2 (*C*=O (C(2)T)); 149.8 (*C*(3)pz); 143.7 (*C*(6)T); 142.3 (*C*(5)pz);

111.1 (*C*(4)pz); 107.0 (*C*(5)T); 50 (CH_2), 49.7 (CH_2); 48.0 (CH_2); 44.0 (CH_2); 38.2 (CH_2); 36.1 (CH_2); 33.8 (CH_2); 21.0 (CH_2); 13.2 (pz-CH_3); 12.2 (CH_3-T); 10.7 (pz-CH_3). HPLC (method 1): $R_t = 19$ min. Anal. Calc. for $C_{24}H_{38}N_8O_6 \cdot 3CF_3COOH$: C, 41.08; H, 4.67; N, 12.78. Found: C, 42.14; H, 3.53; N, 12.94%.

2.4. Synthesis of 2-amino-3-(2-(2-(N-(carboxymethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)ethylamino)-2-oxoethylthio)propanoic acid (7)

NEt_3 (0.2 mL, 1.5 mmol) has been added to a solution of **1** (78 mg, 0.3 mmol) in dry CH_3CN (10 mL). After stirring for 2 h at room temperature, the solution was cooled to 0 °C and **3** (148 mg, 0.26 mmol) has been added. The mixture has been stirred for 12 h at room temperature, the solvent removed under *vacuo* and the resulting residue purified by column chromatography (MeOH (3–7%)/$CHCl_3$). Yield: 37 mg (0.07 mmol, 25%). HPLC (method 1): $R_t = 21.23$ min. The product was dissolved in MeOH and 10 equiv of K_2CO_3 were added. After stirring overnight the mixture was purified by preparative HPLC (method 1). Yield: 20 mg (0.05 mmol, 19%). ^{1}H NMR (D_2O): δ (ppm) 7.20 (ma) and 7.18 (mi) (s, *H*(6)-T, 1H); 4.61 (ma) and 4.47 (mi) (s, CH_2(g), 2H); 4.18 (mi) and 4.01 (ma) (s, CH_2 (f), 2H); 4.03–3.99 (m, C*H* (a), 1*H*); 3.45 (q, CH_2 (d), 2H); 3.39 (t, CH_2 (e), 2H); 3.29 (m, CH_2 (c), 2H), 3.07 (m, C*H* (b), 1H); 2.95 (m, C*H* (b), 1H); 1.71 (s, CH_3-T, 3H). ^{13}C NMR (D_2O): δ (ppm) 181.0 (C=O); 178.1 (C=O); 174.9 (C=O); 172.3 (C=O); 165.7 (C=O); 160.6 (C=O); 144.6 (*C*(6)T); 112.9 (*C*(5)T); 58.4 (CH_2); 53.6 (CH_2); 52.5 (CH_2); 51.7 (CH_2); 49.1 (CH_2); 39.5 (CH_2); 37.7 (CH_2); 14.6 (CH_3-T). HPLC (method 1): $R_t = 13.82$ min.

2.5. Synthesis of [Re(CO)$_3$(κ^3-6)]$^+$ (8) and [Re(CO)$_3$(κ^3-7)] (9)

Reaction of $[Re(CO)_3(H_2O)_3]Br$ with equimolar amounts of **6** or **7** in methanol/reflux overnight (**8**) or in water/75 °C/5 h (**9**) yielded, respectively, complexes **8** and **9**, after removing the solvent and purification by preparative HPLC: **8**, method 2; **9**, method 1. Yields prior to purification were higher than 70%, according to analytical HPLC.

Characterization of **8**. Yield: 11 mg, 20%. ^{1}H NMR (CD_3OD): (four CH_2 are under the solvent peak) δ (ppm) 7.31 (ma) and 7.30 (mi) (s, *H*(6)-T, 1H); 6.2 (s, *H*(4)pz, 1H); 5.63 (br, NH_2, 1H); 4.73 (ma) and 4.57 (mi) (s, CH_2, 2H); 4.47 (m, CH_2, 1H); 4.21 (m, CH_2, 1H); 4.30 (mi) and 4.13 (ma) (s, CH_2, 2H); 4.05 (br, NH_2, 1H); 3.65 (m, CH_2, 1H); 3.60–3.40 (m, CH_2, 5H); 3.15 (m, CH_2, 1H); 2.85 (m, CH_2, 2H); 2.65 (m, CH_2, 1H); 2.51 (m, CH_2, 1H); 2.43 (s + m, $CH_3 + CH_2$, 3H + 1H); 2.36 (s, CH_3, 3H); 2.26 (m, CH_2, 1H); 2.17 (m, CH_2, 1H); 2.05 (m, CH_2, 1H); 1.86 (s, CH_3, 3H). ^{13}C NMR (CD_3OD): δ (ppm) 195.2 (Re*C*O); 194.9 (Re*C*O); 193.8 (Re*C*O); 175.2 (*C*OOH); 172.5 (*C*=O); 169.8 (*C*=O); 167.0 (*C*=O (C(4)T)); 155.1 (*C*(3)pz); 153.0 (*C*=O (C(2)T)); 145.4 (*C*(5)pz); 143.8 (*C*(6)T); 111.0 (*C*(5)T); 109.2 (*C*(4)pz); 67.3 (CH_2), 62.5 (CH_2); 53.8 (CH_2); 43.7 (CH_2); 37.9 (CH_2); 33.2 (CH_2); 21.0 (CH_2); 16.1 (pz-CH_3); 12.2 (pz-CH_3); 11.6 (CH_3-T). IR (KBr) (cm^{-1}): ν(C≡O) 2029, 1912; ν(C=O) 1678. ESI-MS (referenced to the species with ^{187}Re) *m*/*z*: 805.5 $[M]^+$, 827.5 $[(M-H)+Na]^+$ and 828.5 $[M+Na]^+$, $[C_{27}H_{38}N_8O_9Re]^+$, Calc. 805.23. Anal. Calc. for $[C_{27}H_{38}N_8O_9Re]^+(CF_3COO)^- \cdot 2CF_3COOH$: C, 34.59; H, 3.52; N, 9.78. Found C, 35.42; H, 3.95; N, 9.16%. HPLC: $R_t = 27.13$ min (method 2); 22.35 min (method 1).

Characterization of **9**. Yield: 15 mg, 69%. ESI-MS (referenced to the species with ^{187}Re) *m*/*z*: 716.2 $[M+H]^+$, $[C_{19}H_{23}N_5O_{11}SRe]^+$, calc. 716.07. IR (KBr) (cm^{-1}): ν(C≡O), 2039, 1913; ν(C=O) 1673. Anal. Calc. for $C_{19}H_{22}N_5O_{11}ReS \cdot 2CF_3COOH$: C, 29.30; H, 2.57; N, 7.43; S, 3.40. Found: C, 29.88; H, 3.50; N, 7.62; S, 3.33%. HPLC (method 1): $R_t = 19.90$, 19.70 min.

2.6. Synthesis of the ^{99m}Tc(I) complexes (8a and 9a)

General method. In a glass vial under nitrogen, 100 μL of a 10^{-3} M aqueous solution of **6** or **7** were added to 900 μl of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in NaCl. The reaction was incubated at 100°C for 45 min in the case of **8a**, and at 75 °C for 45 min in the case of **9a**. The resulting complexes were analyzed by HPLC. HPLC (method 1): R_t for **8a** = 23 min; R_t for **9a** = 19.88, 20.14 min.

3. Results and discussion

Taking into account the interest on designing ^{99m}Tc radioactive probes for antisense imaging and also the advantages of using PNAs relatively to normal oligonucleotides, we have decided to prepare two bifunctional chelators bearing a PNA monomer and to explore their suitability to the *fac*-$[M(CO)_3]^+$ moiety (M = Re, ^{99m}Tc). The evaluation of these model compounds would be helpful for choosing the most suitable chelator to label clinical relevant PNAs sequences with *fac*-$[^{99m}Tc(CO)_3]^+$. Based on previous results, pyrazolyl- and cysteine-containing ligands, namely 3,5-Me_2pz$(CH_2)_2$N$((CH_2)_3$COOH$(CH_2)_2)$-NH_2 and $RSCH_2CHNH_2COOH$, have been selected [13–15,20]. By choosing these potentially tridentate chelators, which have different donor atom sets, size and physicochemical properties, we could explore the effect of such differences on the labeling of the same biomolecule.

3.1. Synthesis of bifunctional chelators bearing a PNA monomer

The PNA monomer (**1**) was synthesized by reacting the previously described compounds *N*-[2-(Boc-amino)ethyl]-glycinate and thymin-1-ylacetic acid [18,19]. This reaction was performed in DMF, in the presence of NEt_3 and HBTU, yielding compound methyl *N*-[(2-aminoethyl)]-*N*-

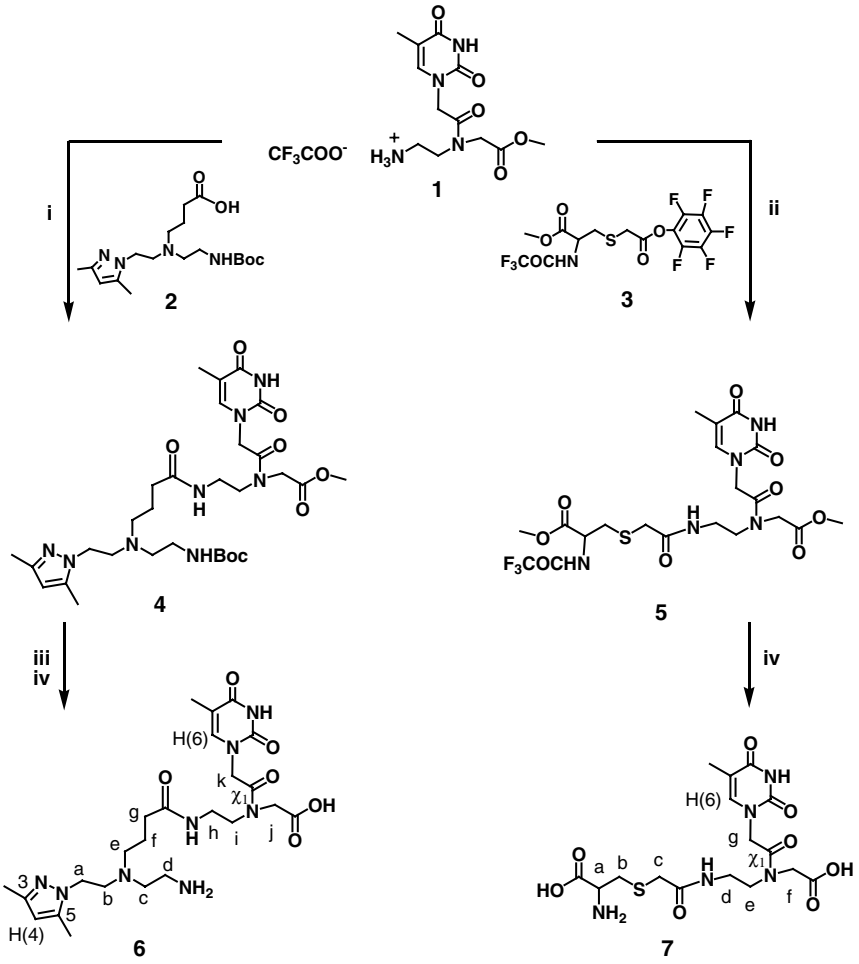
(thymin-1-ylacethyl) glycinate (**1**) in 65% yield, after removing the Boc protecting group with TFA.

The BOC protected 3,5-$Me_2pz(CH_2)_2N((CH_2)_3COOH)$-$(CH_2)_2NHBoc$ (**2**) has been synthesized, as previously described [13–15] and coupled to compound **1**. This coupling was performed in CH_3CN in the presence of NEt_3 and HBTU and the bifunctional chelator **6** was obtained in 65% yield, after removing the BOC and the ester protecting groups with TFA and K_2CO_3, respectively (Scheme 1).

The cysteine-containing ligand (**7**) has been prepared by a multistep synthetic procedure, which involved the preparation of *S*-(carboxymethyl-pentafluorphenyl)-*N*-[(trifluor)carbonyl]-L-cysteine methyl ester (**3**), followed by its coupling to **1.** The coupling was performed in CH_3CN and in the presence of NEt_3. The cysteine chelator **7** was obtained in a relatively low yield (≈20%), after removing the protecting groups with potassium carbonate (Scheme 1).

Compounds **6** and **7** are air and water stable and are soluble in water and in most common polar organic solvents. These compounds have been characterized by multinuclear NMR spectroscopy, by HPLC and by elemental analysis in the case of **6**.

The ^1H and ^{13}C NMR spectra obtained for **6** in CD_3OD and for **7** in D_2O presented the expected resonances, which have been easily assigned. The main feature of these spectra is the presence of two resonances with different intensities for some of the protons of the PNA monomer, indicating the existence of rotamers in solution. These rotamers are formed due to the high barrier of rotation and low rate of exchange around the tertiary amide bond (χ_1) of the PNA monomer (Scheme 1) [21]. In both cases, the intensity ratio of the two resonances assigned to the H(6) and to the methylenic protons of the PNA monomer indicated the presence of a major (ma) species, which may be the *cis*- or *trans*-isomer [21]. As an example, in Fig. 2 (top) is shown the ^1H NMR spectrum obtained for **6**, where two resonances are clearly assigned to the H(6) proton of the thymine (T) nucleobase and to the methylenic protons k and j of the PNA monomer.



Scheme 1. Synthesis of the bifunctional chelators **6** and **7**. (i) CH_3CN, NEt_3, HBTU; (ii) CH_3CN, NEt_3; (iii) CH_2Cl_2, TFA; (iv) MeOH, K_2CO_3.

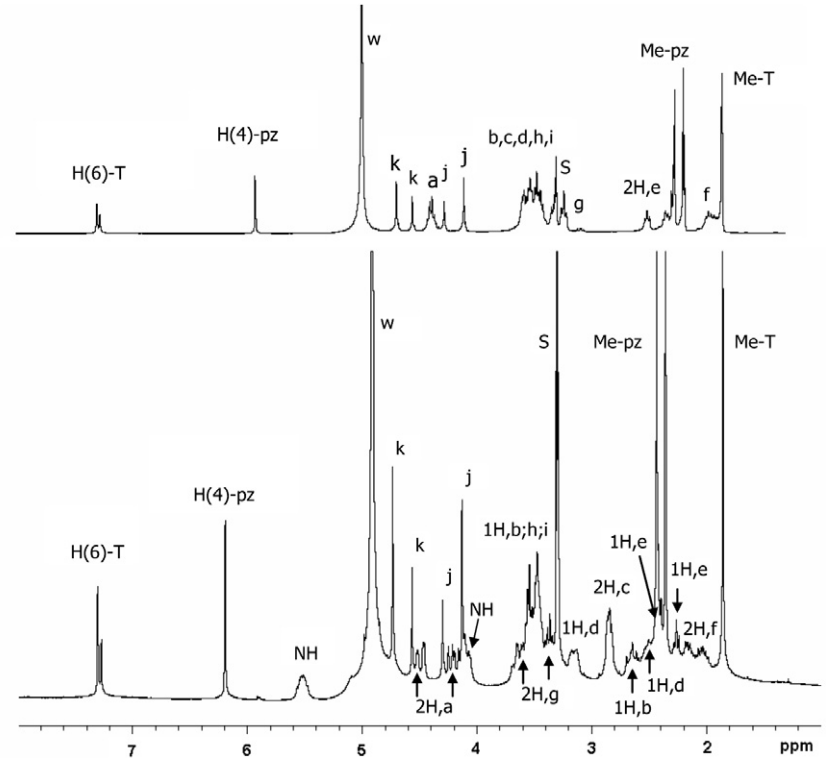


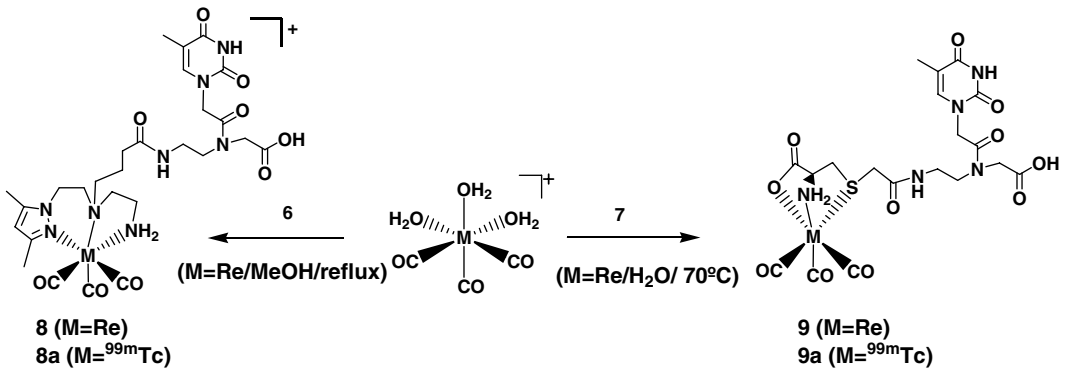
Fig. 2. ^{1}H NMR spectra of **6** (top) and **8** (bottom) in CD_3OD. S denotes the solvent signal and w denotes the water signal.

3.2. Synthesis and characterization of *fac*-[M(CO)$_3$(κ^3-**6**)]$^+$ (M = Re (**8**), ^{99m}Tc (**8a**)) and *fac*-[M(CO)$_3$(κ^3-**7**)] (M = Re (**9**), ^{99m}Tc (**9a**)) complexes

The identity of the ^{99m}Tc complexes is established by comparing the HPLC profile of these complexes with the HPLC chromatogram of the corresponding Re surrogates, which can be fully characterized at the macroscopic level. To achieve this goal we have studied the chemistry of the pyrazolyl- and cysteine-containing ligands, **6** and **7**, with the rhenium starting material $[Re(CO)_3(H_2O)_3]Br$. These reactions were studied using equimolar amounts of the reagents and using methanol (**6**) or water (**7**) as solvents. After purification by preparative HPLC, complexes *fac*-[Re(CO)$_3$(κ^3-**6**)]$^+$ (**8**) and *fac*-[Re(CO)$_3$(κ^3-**7**)] (**9**) were obtained as white microcrystalline solids (Scheme 2).

Compounds **8** and **9** are soluble in water and in the most common polar organic solvents. Compound **8** is stable towards air oxidation but compound **9** is more sensitive, most probably due to the oxidation of the sulfur atom. This conclusion has been based on ESI-MS analyses of samples which have been left under air for some time. For **9**, besides the molecular ion, it was found a second peak which has been assigned to $[M+H+O]^+$.

In the IR spectra of **8** and **9** two strong ν(CO) bands in the 1912–2039 cm^{-1} range could be observed, a typical pattern for complexes having the "*fac*-Re(CO)$_3$" moiety [13–15,21]. These bands in complex **9** are shifted to higher frequencies than the corresponding bands in **8**.



Scheme 2. Synthesis of rhenium and technetium complexes.

This is probably because the cysteine-containing ligand is a poor σ-donor and a better acceptor than the pyrazolyl chelator. The IR spectra of these compounds also present medium to strong bands in the 1678–1673 cm^{-1} range, assigned to the ν(CO) stretching mode associated to the carboxylic functions. The proposed formulation of **8** and **9** was also based on the ESI-positive mass spectra. In the spectrum of **8** three main peaks were found at $m/z = 805.5$, 827.5 and 828.5 corresponding to $[M]^+$, $[(M-H)+Na]^+$ and $[M+Na]^+$, respectively. For **9** only one peak was found at $m/z = 716.2$ ($[M+H]^+$), with the expected isotopic pattern. So far, no solid state structure has been obtained for **8** but the NMR studies are consistent with a tridentate coordination mode for the pyrazole-diamine ligand (**6**). As shown in Fig. 2 (bottom), in complex **8** the NH_2 and the methylenic protons of **6** became diastereotopic and the H(4) and Me protons of the pyrazolyl ring are significantly downfield shifted relatively to the same resonances in the corresponding free ligand (**6**). As previously described, this pattern is consistent with a tridentate coordination mode of the pyrazolyl containing ligand, through the nitrogen atom of the azole ring and through the two nitrogen atoms of the tertiary and primary amines [13–15]. For **8** the pattern obtained for the H(6) proton of the thymine (T) nucleobase and for the methylenic protons k and j of the PNA monomer compares well with the pattern found for **6** (Fig. 2, top), being consistent with the presence of rotamers. The assignment of the resonances in the NMR spectra of **6** and **8** (Fig. 2) was based in g-COSY and g-HSQC NMR experiences (results not shown).

Relatively to complex **9** its formulation has been mainly based on ESI-MS and on elemental analyses of the HPLC purified product. In fact, as just referred, the ^1H and ^{13}C NMR spectra of **9** were very complex and no clear assignment has been possible, mainly in the region where the protons of the cysteine fragment may appear. This complexity was certainly due to the presence of different species in solution, as indicated by the number of signals due to the H(6) proton of the thymine base (three resonances with different intensities at 7.31, 7.26 and 7.22 ppm). It may be anticipated that existing in the bifunctional chelator **7** other potential coordination sites than the cysteine fragment, the structure proposed for complex **9** (Scheme 2) is not the only one possible. However, it is well documented and recognized the affinity of the *fac*-$[M(CO)_3]^+$ moiety for cysteine and cysteine-containing ligands [20]. Any other possibility would require coordination of the amide groups to the metal fragment and this would lead certainly to a less stable complex. Due to the splitting observed for the H(6) proton of the thymine base and also due to the existence of a prochiral center in the molecule (coordinated thioether S-atom), the complexity of the NMR spectra of **9** may be due to the presence of isomers and/or rotamers in solution. The presence of such isomers, which do not interconvert at room temperature, may also justify the two peaks found in the HPLC chromatogram of **9/9a** (**9**: $R_t = 19.90$ and 19.70 min; **9a**: $R_t = 20.14$ and 19.88 min) (Fig. 3(e) and (f)). The ^{99m}Tc complexes (**8a** and **9a**) were prepared by reacting the *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ with a solution of **6** or **7** ($[L] = 10^{-4}$ M) in water. Complexes **8a** and **9a** were obtained in quantitative yield, with high radiochemical purity and were characterized by comparing their HPLC profiles with that of the corresponding Re(I) tricarbonyl complexes (**8**and **9**) (Fig. 3).

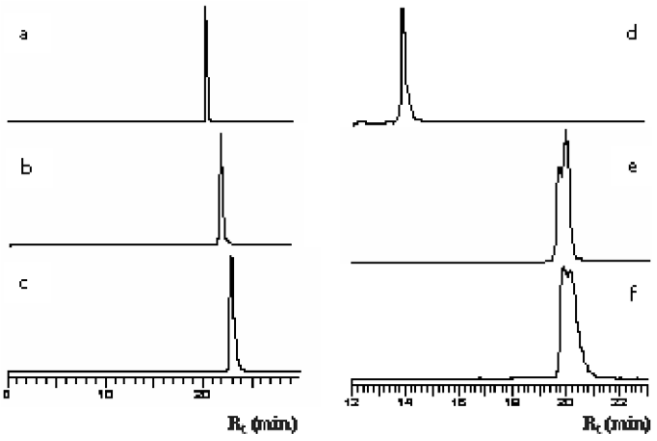


Fig. 3. HPLC chromatograms of chelators **6** (a) and **7** (d) (254 nm), Re complexes **8** (b) and **9** (e) (254 nm), and corresponding ^{99m}Tc complexes **8a** (c) and **9a** (f) (γ trace).

4. Concluding remarks

To evaluate the possibility of labeling with *fac*-$[^{99m}Tc(CO)_3]^+$ clinically interesting PNAs, two potentially tridentate chelators bearing a PNA monomer containing thymine as nucleobase (**6**, **7**) have been synthesized and characterized. The characterization of these two bifunctional chelators by multinuclear NMR spectroscopy has shown the presence of rotamers, due to the low rate of exchange around the tertiary amide bond of the PNA monomer. Compounds **6** and **7** react with *fac*-$[M(CO)_3(H_2O)_3]^+$ yielding the complexes *fac*-$[M(CO)_3(\kappa^3$-**6**$)]^+$ (M = Re (**8**) and ^{99m}Tc (**8a**)) and *fac*-$[M(CO)_3(\kappa^3$-**7**$)]$ (M = Re (**9**) and ^{99m}Tc (**9a**)), respectively. For complex **8** NMR studies have been very informative and a complete assignment of the resonances was possible using different techniques. It was clear the tridentate coordination mode of the pyrazolyl-containing ligand and also the presence of rotamers in solution. For **9** the NMR data was too complex and not so informative, possibly due to the presence in solution of isomers and/or rotamers, which do not interconvert at room temperature. The presence of a prochiral center in the molecule (thioether S-atom) may be responsible for the formation of diastereoisomers, which also appear in the HPLC chromatogram of **9a**. The formation of the stable model complexes **8a** and **9a**, in high yield, confirmed the possibility of attaching **6** or **7** to PNA sequences complementary of a target mRNA, aiming their evaluation as antisense nuclear imaging probe.

Acknowledgements

Catarina Xavier thanks Fundação para a Ciência e Tecnologia (FCT) for a PhD grant (SFRH/BD/16680/2004). Mallinckrodt Tyco Healthcare is also acknowledged by providing IsoLink kits.

References

[1] H.R. Herschman, Science 302 (2003) 605.
[2] C.Y. Shiue, S. Eck, in: M.J. Welch, C.S. Redvanly (Eds.), Handbook of Radiopharmaceuticals, John Wiley & Sons Ltd, 2003, pp. 467–479.
[3] M.R. Lewis, F. Jia, J. Cell. Biochem. 90 (2003) 464.
[4] H. Fang, X. Yue, X. Li, J.-S. Taylor, Nucleic Acids Res. 33 (2005) 6700.
[5] X. Sun, H. Fang, X. Li, R. Rossin, M.J. Welch, J.-S. Taylor, Bioconjugate Chem. 16 (2005) 294.
[6] A. Kumar, Eur. J. Org. Chem. (2002) 2021.
[7] S. Basu, E. Wickstrom, Bioconjugate Chem. 8 (1997) 481.
[8] V. Menchise, G. De Simone, C. Pedone, P. Natl. Acad. Sci. USA 100 (2003) 12021.
[9] H. Rasmussen, J. Sandholm, Nat. Struct. Biol. 4 (1997) 98.
[10] A. Ray, B. Nordén, FASEB J. 14 (2000) 1041.
[11] P. Clivio, D. Guillaume, M.T. Adeline, J. Hamon, C. Riche, J.L. Fourrey, J. Am. Chem. Soc. 120 (1998) 1157.
[12] D.J. Hnatowich, K. Nakamura, Ann. Nucl. Med. 18 (2004) 363.
[13] S. Alves, A. Paulo, J.D.G. Correia, A. Domingos, I. Santos, J. Chem. Soc. Dalton (2002) 4714.
[14] S. Alves, A. Paulo, J.D.G. Correia, L. Gano, C.J. Smith, T.J. Hoffman, I. Santos, Bioconjugate Chem. 16 (2005) 438.
[15] S. Alves, J.D.G. Correia, I. Santos, B. Veerendra, G.L. Sieckman, T.J. Hoffman, T.L. Rold, S.D. Figueroa, L. Retzloff, J. McCrate, A. Prasanphanich, C.J. Smith, Nucl. Med. Biol. 33 (2006) 625.
[16] N. Lazarova, S. James, J. Babich, J. Zubieta, Inorg. Chem. Commun. 7 (2004) 1023.
[17] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, P.A. Schubiger, J. Am. Chem. Soc. 123 (2001) 3135.
[18] A.P. Krapcho, C.S. Kuell, Synth. Commun. 20 (1990) 2559.
[19] K. Dueholm, M. Egholm, O. Buchardt, J. Org. Chem. 59 (1994) 5767.
[20] (a) D. Staveren, P.D. Benny, R. Waibel, P. Kurz, J-K Pak, R. Alberto, Helv. Chim. Acta 88 (2005) 447;
(b) O. Karagiorgou, G. Patsis, M. Pelecanou, C.P. Raptopoulou, A. Terzis, T. Siatra-Papastaikoudi, R. Alberto, I. Pirmettis, M. Papadopoulos, Inorg. Chem. 44 (2005) 4118;
(c) H. He, M. Lipowska, X. Xu, A.T. Taylor, M. Carlone, L.G. Marzilli, Inorg. Chem. 44 (2005) 5437.
[21] G.J. Sanjayan, V.R. Pedireddi, K.N. Ganesh, Org. Lett. 2 (2000) 2825.