Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Organometallic Chemistry 689 (2004) 4757–4763
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

"2 + 1" Dithiocarbamate–isocyanide chelating systems for linking $M(CO)_3^+$ (M = ^{99m}Tc, Re) fragment to biomolecules

N.I. Gorshkov [a], R. Schibli [b], A.P. Schubiger [b], A.A. Lumpov [a,*], A.E. Miroslavov [a], D.N. Suglobov [a]

[a] *Khlopin Radium Institute, St. Petersburg, 194021, 2nd Murinskii Pr., 28, Russia*
[b] *Center of Radiopharmacy, Paul Scherrer Institute, CH-5232 Villigen, Switzerland*

Received 12 August 2004; accepted 10 September 2004

Abstract

"2 + 1" Dithiocarbamate (DTC)–isocyanide (ISO) system was studied to label amino acids and model peptides with $^{99m}Tc(CO)_3^+$ fragment. Two ways were used: (i) conjugation via free carboxy group of bifunctional DTC [$S_2CNMe_2^-$ (L3), $S_2CNHCH_2COO^{2-}$ (L1) and $S_2C(C_4H_7N)COO^{2-}$ (L2)] using pre-labeling procedure and (ii) conjugation via isocyanide ligand [*tert*-BuNC (L4), $EtOC(O)CH_2NHC(O)CH_2NC$ (L5), and gly-gly-gly-$CH_2NHC(O)CH_2NC$ (L6)]. Complexes $M(CO)_3L2L4^-$ (**1a**, **1b**), $M(CO)_3L3L4^-$ (**2a**, **2b**) and bioconjugates $M(CO)_3L1L5$ (**5a**, **5b**), and $M(CO)_3L1L6$ (**6b**) (**a**, M = Re; **b**, ^{99m}Tc) were synthesized. Bioconjugates **4a** and **4b** were prepared by reaction of histidine methyl ester with **2a** and **2b**, respectively. All rhenium complexes were characterized by ^{1}H and ^{13}C NMR, IR, and MS spectroscopy and complexes with ^{99m}Tc, by HPLC using rhenium analogs as references.

Keywords: Technetium; Rhenium; Dithiocarbamates; Isocyanides; 2 + 1 Approach; Biomolecules

1. Introduction

In recent few years researchers working with Tc and Re radiopharmaceuticals have pinned their hopes on the application of organometallic $M(CO)_3(H_2O)_3^+$ (M = Tc, Re) complexes for labeling various biomolecules. As compared to high-valence Tc and Re precursors commonly used in a worldwide practice, this complex shows more promise owing to its relatively compact size, low positive charge (+1), and extreme stability of the $M(CO)_3^+$ core. Moreover, kit for preparing this precursor is commercially available [1]. One of the most important problems in labeling biomolecules with $M(CO)_3^+$ species is to develop chelating cores providing strong complexation of $M(CO)_3^+$ with minimal effect on the biomolecule properties. The chelating units for $M(CO)_3^+$ should meet the following requirements: (1) high affinity to the metal ion; (2) formation of thermodynamically and kinetically stable complexes; and (3) complete binding of ^{99m}Tc at the ligand concentration no higher than 10^{-5}–10^{-4} M. The last requirement is important in labeling of receptor ligands (steroids, small peptides), because at a higher ligand concentration free receptor vacancies can be saturated with unlabeled biomolecules, thus decreasing the selectivity of radionuclide accumulation in the target tissue. Moreover, the resulting complex with $M(CO)_3^+$ core should not contain weak ligands, which can be substituted by stronger electron donors, in the metal coordination environment.

Tridentate chelators often used to incorporate $M(CO)_3^+$ species in biomolecules [2,3] meet all the above requirements, but the synthetic procedure of their

* Corresponding author. Tel./fax: +7 812 2475662.
E-mail addresses: ngorshkov@mail.ru (N.I. Gorshkov), lumpov@atom.nw.ru (A.A. Lumpov).

0022-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2004.09.019

conjugation with biomolecules is rather complex. Bidentate chelators can be more readily derivatized. However, as shown earlier, their complexes with $M(CO)_3^+$ species, except those with diphosphine ligands [4], are unstable in vivo and in vitro [5,6] owing to the presence of coordination site occupied with weak ligands (e.g., solvent molecules).

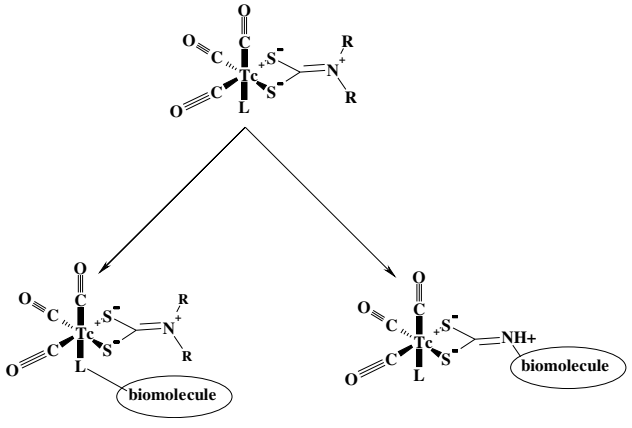
This third coordination vacancy in $M(CO)_3^+$ core can be blocked using "2 + 1" approach (i.e., combination of bidentate and monodentate ligands). Generally, the "n + 1" approach is well developed for high valence Tc and Re [7]. Formation of "2 + 1" complexes of metal tricarbonyls was studied in [8–10] using various heterocyclic bidentate ligands and π-acceptors (isocyanides, cyanide) as monodentate ligands.

Previously [11], we reported that dithiocarbamates form strong complexes with $M(CO)_3^+$ fragment, which are quite stable in a wide pH range. Dithiocarbamates, being "soft" Lewis bases, have high affinity for "soft" acids [like Tc(I) and Re(I)] and are promising as bidentate ligands for "2 + 1" approach. It should be noted that they can be easily modified to form dithiocarbamates with free carboxy group readily coupling with amino acid residue. Though bifunctional dithiocarbamates are well known [13], no published data on their use as linkers for biomolecules are known. As for conjugation of 2 + 1 complexes with biomolecules, two ways are possible: via monodentate and via bidentate ligands (Scheme 1).

In the present work, we studied dithiocarbamate–isocyanide system for labeling model amino acids and peptides with Re and ^{99m}Tc and proposed synthetic strategy for coupling reactions.

2. Experimental

All the reagent grade chemicals were purchased from Fluka and used without further purification. Complexes



Scheme 1.

$(NEt_4)[ReBr_3(CO)_3]$ and $[Re,Tc(CO)_3(H_2O)_3]Cl$ were synthesized according to known procedures [12,13]. $Na^{99m}TcO_4$ was eluted from a $^{99}Mo/^{99m}Tc$ generator (Mallinckrodt, Petten) with 0.9% saline. $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared using the boranocarbonate kit [1]. HPLC analysis was performed on a Merck–Hitachi L-7000-system equipped with an L-7400 tunable absorption detector, a Berthold LB 506 B radiometric detector, and a Macherey-Nagel C-18 reverse-phase (10 pm, 150 × 44 mm) column. Aqueous 0.05 M TEAP (triethylammonium phosphate) (solvent A, pH 2.25) and methanol (solvent B) were used as HPLC solvents. The HPLC system started with 100% A from 0 to 3 min. The eluent switched on at 3 min to 75% A and 25% B and at 9 min to 66% A and 34% B followed by a linear gradient 66% A and 34% B to 100% B from 9 to 20 min. The gradient remained at 100% B for 2 min before switching back to 100% A. The flow rate was 1 ml/min. The NMR spectra were recorded on a 300 MHz Varian Gemini 2000 spectrometer. The IR spectra were registered on a Perkin–Elmer FT-IR 16PC using KBr pellets.

2.1. Synthesis of bifunctional dithiocarbamates

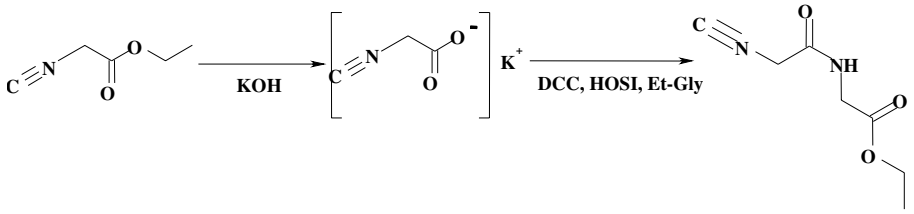
The dithiocarbamate ligands were prepared by reaction of corresponding amines with CS_2 in the alkaline medium.

Ligand (L1) [14]. ^1H (D_2O, ppm), 4.06 (s) (CH_2); IR (KBr, cm^{-1}), 1650 (COO), 1475 (CN), 1058, 1035 (CSS). *Ligand (L2)* [16]. IR (KBr, cm^{-1}) 1475 (CN); 980 (CSS); ^1H NMR (D_2O, ppm) 5.52 (t) CH(COO), 4.18 (t) CH_2 (NCSS), 1.93(m) $2CH_2$; MS (FAB) *m/z* 189 $[C_6H_7NO_2S_2]$. *Ligand (L3)* [15]. ^1H (D_2O, ppm), 2.64 s (CH_3); IR (KBr, cm^{-1}), 1480 (CN), 1035, 995 (CSS).

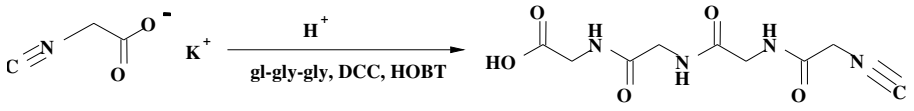
2.2. Synthesis of isocyanide ligands

Ligand (L5) [16] (Scheme 2). IR (KBr, cm^{-1}) 2928 (CN), 1626, 1576 (COOEt); ^1H NMR ($CDCl_3$, ppm) 4.54 (s) CH_2 (CN), 4.35 (q) CH_2 (Et), 4.13 (s) CH_2 (NH), 1.41 (t) CH_3 (Et); MS (FAB) *m/z* 170 $[C_7H_{10}N_2O_3]$.

Ligand (L6) (Scheme 3). Potassium isocyanoacetate (20 mg, 0.1 mmol) was dissolved in water (0.5 ml), and acidified with an equivalent amount (0.0083 ml) of concentrated hydrochloric acid. Then the solution of HOBT (27 mg), DCC (40 mg), and triglycine in a mixture of acetonitrile and phosphate buffer (1:1 v/v) was added. The heterogeneous mixture was stirred overnight. The reaction mixture was analyzed by HPLC. The water–acetonitrile phase contained L6 (90%), and unchanged HOBT (10%). This solution was evaporated to dryness. The residue was dissolved in acetonitrile and L6 was precipitated with water. Yield: 9 mg. IR (KBr, cm^{-1}) 2928 (C≡N), 1643, 1572 (NHCO); ^1H NMR (D_2O)



Scheme 2.



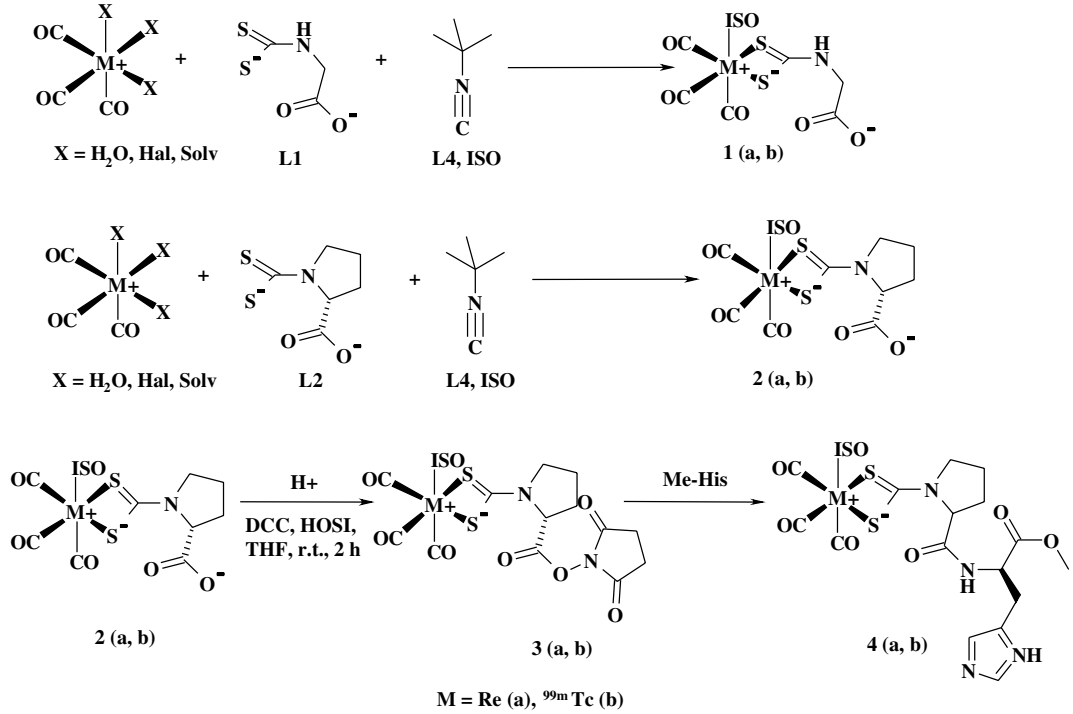
Scheme 3.

ppm, 5.64 (br. s) (NH), 3.91 (dq), 3.86 (dq) CH_2; MS (FAB) *m/z* 256 [$C_9H_{12}N_4O_5$].

2.3. Synthesis of rhenium complexes

*Complex (**1a**)* (Scheme 4). [$Re(CO)_3Br_3$][NEt_4]$_2$ (240 mg, 0.3 mmol) was dissolved in methanol (5 ml), L5 (0.34 ml, 0.3 mmol) and L1 (60 mg, 0.3 mmol) were added dropwise. The reaction mixture was stirred at 50 °C for 3 h. The solvent was removed in vacuum. The oily residue was extracted with THF to remove [NEt_4]Br. After solvent removal the reaction mixture was purified with Sep-Pak C18 cartridge using an aqueous methanol eluent. The fractions 70:30 and 80:20 (MeOH:H_2O) were collected and analyzed by HPLC. A single peak with RT 22 min was observed. Yield: 160 mg. IR (KBr, cm^{-1}) 2185 (C≡N), 2017, 1972, 1907 (CO), 1650, 1564 (COO), 1506 (C=N), 1002 (CSS); ^{1}H NMR ($CDCl_3$, ppm), 7.01 (br. s) (NH), 4.54 (s) (CH_2),1.65 (s) (CH_3)$_3$MS (FAB) *m/z* 476 [$C_{11}H_{11}NO_5ReS_2$].

*Complex (**2a**)* (Scheme 4). Solid L3 (70 mg, 0.215 mmol) and solution of L5 (0.215 mmol, 0.0024 ml) in methanol (4 ml) were added to [$Re(CO)_3(H_2O)_3$]Cl (1 ml of 0.215 M solution). The reaction mixture was stirred for 2 h at 70 °C and the solvent was removed on a



Scheme 4.

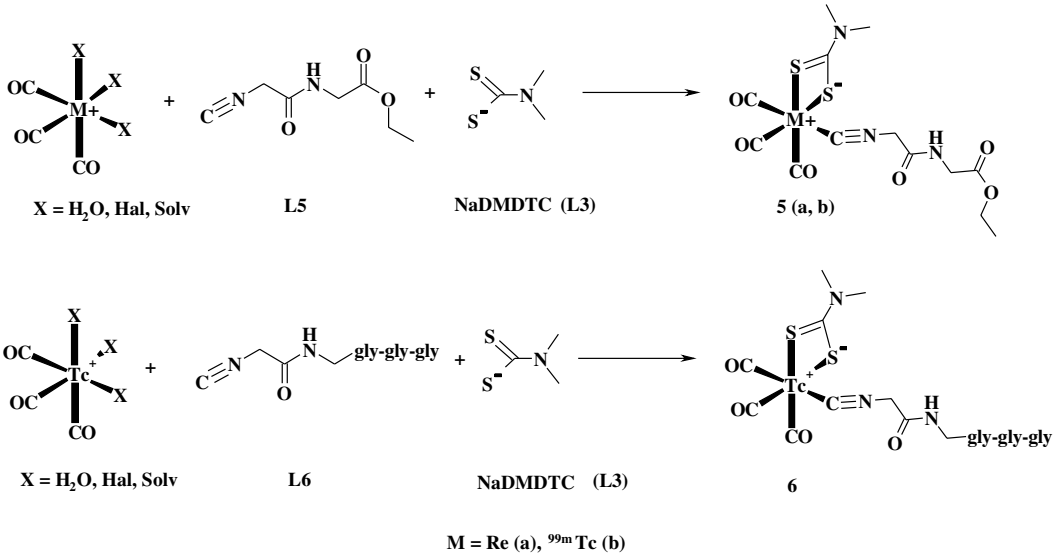
rotary evaporator. The white powder was recrystallized from chloroform/hexane mixture. Yield: 80 mg IR (KBr, cm^{-1}) 2185 (C≡N), 2017, 1929 (CO), 1650, 1564 (COO), 1512 (C=N), 1002 (CSS). ^1H NMR ($CDCl_3$, ppm) 5.52 (m), 4.18 (m), 1.92 (m) CH_2, 1.53 (s) CH_3. MS (FAB) M/Z 516 [$C_{13}H_{16}NO_5ReS_2$].

*Complex (**4a**)* (Scheme 4). Complex (**2a**) (113 mg, 0.21 mmol) was dissolved under argon in dry THF; the equivalent amounts of the concentrated acetic acid, HOSI (29 mg, 0.25 mmol), and DCC (54 mg, 0.26 mmol) were added. The reaction mixture was stirred for 2 h at 50 °C. The urea precipitate was filtered off and the solution was evaporated to dryness to give intermediate complex (**3a**). The reaction was monitored by TLC (CH_2Cl_2). The intermediate complex (**3a**) was dissolved in dry THF; methyl histidine hydrochloride (51 mg) and triethylamine (0.029 ml) were added. The reaction mixture was stirred for 3 h at 50 °C. The solvent was evaporated in a vacuum, and the residue was extracted with methylene dichloride. The extract was filtered and evaporated. The oily residue was dissolved in THF, and the resulting solution was poured in water. The aqueous layer was treated with ethyl acetate. The extract was washed with brine and water, dried over anhydrous calcium chloride, and evaporated to dryness under a reduced pressure. The residue was recrystallized from a chloroform/hexane mixture. Yield: 55 mg. IR (KBr, cm^{-1}) 2187 (C≡N), 2017, 1929 (CO), 1745, 1693 (COO), 1520 (C=N), 1024 (CSS); ^1H NMR ($CDCl_3$, ppm), 9.10 (br. s) (NH, im), 7.74, 7.60 (d) (CH, im), 5.50 (br. s) NH, 4. 93 (m) CH(NH) + CH (CO), 3.87 (t) CH_2 (NCSS), 3. 55 (s) OCH_3, 2.21, 2.08 (m) CH_2 (proline residue);MS (FAB) *m/z* 679 [$C_{20}H_{25}N_5O_6ReS_2$].

*Complex (**5a**)* (Scheme 5). [$Re(CO)_3Br_3$] [NEt_4]$_2$ (123 mg, 0.16 mmol) was mixed with ligand L5 (25 mg, 0.16 mmol) in methanol (5 ml). The reaction mixture was stirred for 1 h under a nitrogen flow at 50 °C. The reaction was monitored by IR spectroscopy (N and CO absorption). After reaction completion, NaDDTC (L3) (23 mg, 0.16 mmol) was added and the mixture was additionally stirred at 50 °C for 2 h. The solvent was evaporated. The dark green oil was extracted with THF to remove NEt_4Br. TLC analysis (hexane/methylene dichloride/acetone 1:1:0.5) showed the presence of unchanged compound and the target product with $R_f = 0.5$. The target product was isolated on a 20×150 mm silica gel chromatographic column using eluent mentioned above. The middle band was collected. The solvent was evaporated to dryness in vacuum. The residue was recrystallized from a diethyl ether–hexane mixture. Yield: 60 mg. IR (KBr, cm^{-1}) 2185 (C=N), 2017, 1929 (*fac*-CO), 1650, 1564 (COO), 1512 (C=N), 1002 (CSS); ^1H NMR ($CDCl_3$, ppm), 5.01 (br. s) (NH), 4.54 CH_2(CN), 4.35 (q) (CH_2 (Et)), 4.13 (s) CH_2(NH), 3.16 (d) N(CH3)2, 1.41 (t) CH_3 (Et); MS (FAB) *m/z* 560 [$C_{13}H_{16}N_3O_6ReS_2$].

2.4. Labeling of model amino acids (Me-His) and peptides (triglycine) with $^{99m}Tc(CO)_3$

Model amino acid (Me-His) and peptide (triglycine) were labeled with $^{99m}Tc(CO)_3$ fragment (complexes **5b**, **6**) by the following procedure. $^{99m}Tc(CO)_3$ solution (100 μl, 1.2–1.5 GBq/ml) were neutralized with 1 M HCl to pH 6, and the phosphate buffer (350 μl, pH 5.5) and 25–50 μl of dithiocarbamate (L1, L2) and isocyanide ligands (L4, L5, L6) were added. The ligand



Scheme 5.

concentration was 10^{-4}–10^{-2} M. The reaction mixture was incubated at 70 °C for 25 min. The reaction was monitored by HPLC.

Prelabeling (complexes **2b**, **3b**, **4b**) was performed as follows. First, complex **2b** was prepared according to the above procedure. A 0.001 M DCC solution in THF (30 μl) and an equimolar amount of HOSI was added, and the reaction mixture was incubated for 30 min at 50 °C to give complex **3b**. Then, a 10^{-4} M solution (30 μl) of amino acid (Me-His) in the phosphate buffer was added and the mixture was incubated for 30 min at 50 °C to give complex **4b**. The reaction was monitored by HPLC.

3. Results and discussion

3.1. Reaction of $M(CO)_3(L1)(H_2O)$ with monodentate ligands and histidine challenge tests

Previously [11,18] we studied a series of monodentate ligands (L) to block the third coordination site in the $M(CO)_3(L1)(H_2O)$ complex. The behavior of the resulting complexes in histidine challenge reaction was taken as a main criterion of their stability. The study was carried out by ^{99}Tc NMR spectroscopy in 10^{-3}M $^{99}Tc(CO)_3^+$ solutions at the Tc:L1:L molar ratio = 1:1:1. Thiols, thioethers, primary and secondary amines, phosphines, isocyanides and imidazole were studied, and it was found that only mixed-ligand complexes $M(CO)_3(L1)L$ (L = imidazole, phosphine and isocyanide) appeared to be stable to histidine challenge.

As for technetium-99m, complexation of $^{99m}Tc(CO)_{3aq}^+$ with L1–L system yields three complexes, namely: $Tc(CO)_3(L1)L$, $Tc(CO)_3(L1)(H_2O)$, and $Tc(CO)_3(L)_3$; and only in the case of ISO ligand, pure $^{99m}Tc(CO)_3(L1)ISO$ was formed. The minimal concentration of L1 and ISO providing quantitative binding of $Tc(CO)_3(H_2O)_3^+$ was 10^{-4} M. It should be noted that the sequence of addition of these ligands to $Tc(CO)_3(H_2O)_3^+$ does not affect the complexation. The HPLC patterns are shown in Fig. 1. Corresponding rhenium analogs were used as references. It should be noted, that the resulting complex is stable in mice serum at 37 °C at least for 24 h.

Thus, the DTC–ISO systems show promise for labeling biomolecules with $^{99m}Tc(CO)_3^+$.

3.2. Conjugation of $M(CO)_3^+$ to amino acid residue through bifunctional bidentate dithiocarbamates

As mentioned above, two possible ways for attaching "2 + 1" complexes to biomolecules are possible (Scheme 1). In this work we studied both approaches using DTC–ISO ligand system. For this purpose, we derivatized these ligands with free carboxy group,

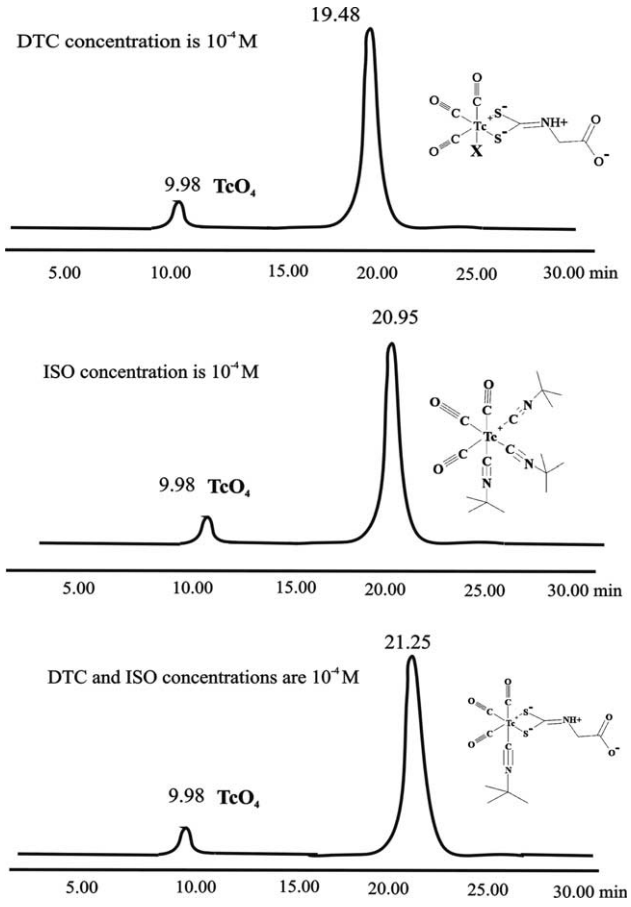


Fig. 1. HPLC γ-ray patterns of ^{99m}Tc complexes: [$^{99m}Tc(CO)_3(L1)(L)$], [$^{99m}Tc(CO)_3(ISO)_3]^+$, [$^{99m}Tc(CO)_3(L1)(ISO)$].

which can be attached to amino group of biomolecule using standard methods of peptide synthesis. Both dithiocarbamates and isocyanides containing free carboxy group were prepared.

First, we performed coupling reaction of L1 with model amino acid (glycine ethyl ester) using the activated ester method. However, dithiocarbamate ligand was completely decomposed under these conditions. This is probably due to two reasons: (1) decomposition of dithiocarbamates in the acidic media [13], which is required to protonate the carboxy group in the coupling reaction, and (2) activation of the CSS with DCC with precipitation of corresponding thiourea.

Thus, it is necessary to protect the CSS group from the carbodiimide attack. For this purpose we used the prelabeling procedure involving preparation of the metal complex with bifunctional ligand containing free carboxy group and then its coupling via the amino group to a biomolecule (Scheme 4). To study this approach we prepared and characterized complex $Re(CO)_3(L1)(ISO)$ (**1a**). Previously we found [17] that dithiocarbamate complexes with $M(CO)_3^+$ fragment are stable in a wide pH range including slightly acidic

solutions. The coupling reaction was carried out in THF at room temperature. The TLC data showed fast formation of several products. These products were separated using a Sep-Pak cartridge (see Section 2). The IR spectra of these products exhibited characteristic absorption bands of tricarbonyl fragment and coordinated C=N group. This confirms, that $M(CO)_3(ISO)$ core is stable under the experimental conditions. However, no signals of the desired conjugate were observed in the ^1H NMR spectra. All isolated complexes can be probably assigned to the mixed thiourea–isocyanide compounds. Thus, this procedure also causes decomposition of the dithiocarbamate moiety due to attack of DCC on both the CSS and carboxy groups.

It is known that dithiocarbamate complexes of the secondary amines are more thermodynamically stable then that of primary amines. To increase the stability of dithiocarbamate complex, we prepared dithiocarbamate derivative of proline simulating natural peptide fragment. Barium salt of L2 was prepared in methanol. Its complex $Re(CO)_3(L2)(ISO)$ (complex **2a**) was prepared in aqueous solution and purified by recrystallization. Then, we obtained the activated ester of complex **2a** (complex **3a**) with HOSI (Scheme 4). The solid complex was isolated from the reaction mixture and coupled with histidine methyl ester (a representative amino acid) at room temperature to form the desired conjugate **4a**.

Thus, in contrast to primary amine derivatives, dithiocarbamates of secondary amines can be used to conjugate $M(CO)_3(DTC)ISO$ complexes with biomolecules containing free amino group by conventional coupling procedure.

3.3. Conjugation of $M(CO)_3^+$ to amino acid residue through bifunctional monodentate isocyanide ligands

Attaching via the monodentate ligand is the other way to conjugate "2 + 1" complexes with biomolecules. In this case, dithiocarbamate ligand blocks two coordination sites in the $M(CO)_3^+$ core. The third coordination site can be occupied by isocyanide ligands containing free carboxy group, which can be easily attached to the amino group of biomolecule by the one-step standard coupling procedure. In contrast to multi-step attaching of complex tridentate chelation systems, this is more convenient and simpler way to modify biomolecules.

We studied this approach by an example of the L5–L3 system. It is known [16] that commercially available ethyl ester of isocyanoacetic acid can be readily conjugated with various amino acids. Glycine ethyl ester was chosen as a simplest example of biomolecule containing free amino group and L5 was prepared according to Scheme 2.

However, simultaneous addition of NaDMDTC (L3) and isocyanide derivative (L5) to rhenium tricarbonyl precursor (Scheme 5) under aerobic conditions results in formation of a mixture of nonseparable products, probably due to the ligand oxidation. To prevent oxidation, we performed this reaction under anaerobic conditions. The target product (the main fraction) was isolated by column chromatography and characterized by spectral methods.

Thus, we succeeded in attaching "2 + 1" $Re(CO)_3^+$ species via monodentate bifunctional isocyanide ligand to model amino acid (glycine ethyl ester), and this approach can be probably used in the case of real peptides.

3.4. Labeling of model amino acids and peptides (triglycine) with $^{99m}Tc(CO)_3$ species

"2 + 1" Dithiocarbamato–isocyanido ligand system was used to label model amino acids and peptides with $^{99m}Tc(CO)_3^+$. The reactions were followed by HPLC with γ-ray detection using rhenium analogs as references. To simulate functionalized real peptide, we prepared isocyanide derivative of triglycine (L6) using coupling reaction with glycine ethyl ester (Scheme 3).

Though L1–ISO system gave one complex **1b** (single peak in the HPLC)f γ-ray pattern with RT 21.25 min (Fig. 1), $^{99m}Tc(CO)_3^+$ reacted with isocyanide ligands L5 and L6, and L3 (dithiocarbamate blocking ligand) to form complexes **5b** and **6** (Scheme 5) contaminated with $^{99m}Tc(CO)_3(L3)(X)$ (X = H_2O, halide ion) in ratio 3:1, respectively [e.g., complex **6** (Fig. 2)]. Complexes **5b** and **6** had nearly similar RT values.

Conjugation of $^{99m}Tc(CO)_3^+$ with histidine methyl ester was performed by prelabeling procedure described previously for rhenium analog. Complexation of L2–ISO system with $^{99m}Tc(CO)_3$ (Scheme 4) yielded two products with RT 20.85 and 21.25, respectively. One of these peaks can be assigned to complex **2b**, and the other, to $[^{99m}Tc(CO)_3(ISO)_3]^+$. The ratio of these species is 1:3. The concentration of both ligands and the order of their addition had virtually no effect on this ratio. Assuming that $Tc(CO)_3(ISO)_3$ is not involved into the

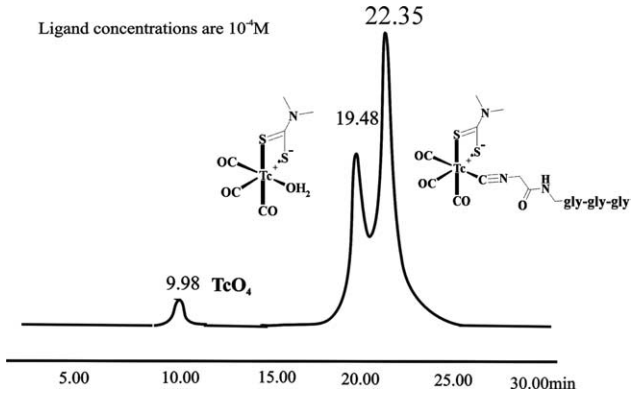


Fig. 2. HPLC γ-ray pattern of the reaction mixture for preparing bioconjugate **6**.

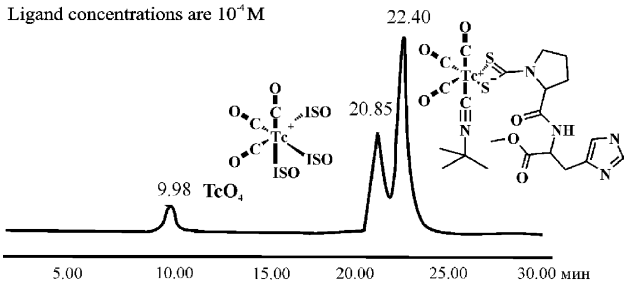


Fig. 3. HPLC γ-ray pattern of the reaction mixture for preparing bioconjugate **4b**.

coupling reaction, we used this mixture without further purification. Activation of **2b** with HOSI (complex **3b**) and than coupling with histidine methyl ester (Scheme 4) resulted in quantitative conversion of **2b** into the desired bioconjugate **4b** (Fig. 3). In both cases purification of the target bioconjugate is required.

Thus, "2 + 1" dithiocarbamate–isocyanide system can be used for labeling biomolecules with $^{99m}Tc(CO)_3^+$ fragment. Attaching via bifunctional dithiocarbamate ligand is possible using prelabeling procedure, in which the $M(CO)_3^+$ fragment acts as the protecting group. This procedure can be used for labeling heavy biomolecules containing free amino groups (e.g., large peptides, antibodies, [19,20]) with subsequent purification of the resulting material by preparative HPLC.

Attaching via bifunctional monodentate isocyanide ligands is also possible; it is preferable for conjugation with various small biomolecules containing free amino groups (e.g., steroids, small peptides, brain receptor ligands, etc.). This single-step procedure is very simple and convenient. Labeling of real small biomolecules using of this approach is in progress.

4. Conclusions

1. Dithiocarbamato–isocyanide chelation systems were used to conjugate $M(CO)_3^+$ species with the amino acid residue.
2. Model amino acids were attached to the "2 + 1" chelation system in both monodentate and bidentate fashion using post- and pre-labeling procedures. Corresponding rhenium complexes were isolated and characterized.
3. These procedures can be used for labeling biomolecules with $^{99m}Tc(CO)_3^+$ fragment. Purification of the final complex by preparative HPLC is required.

Acknowledgement

This work is financially supported by ISTC Project No. 1723.

References

[1] R. Alberto, K. Ortner, N. Wheatley, R. Shibli, A.P. Schubiger, J. Am. Chem. Soc. 123 (2001) 3135.
[2] R. Alberto, R. Schibli, R. Waibel, U. Abram, A.P. Schubiger, Coord. Chem. Rev. 190–192 (1999) 901.
[3] H.-J. Pietzch, A. Gupta, M. Reisgys, A. Drews, S. Seifert, R. Syhre, H. Spies, R. Alberto, U. Abram, A.P. Schubiger, B. Johannsen, Bioconjugate Chem. 11 (2000) 414.
[4] R. Schibli, K.V. Katti, C. Higginbotham, W.A. Volkert, R. Alberto, Nucl. Med. Biol. 25 (1999) 711.
[5] R. Schibli, R. La Bella, R. Alberto, E. Garcia-Garayoa, K. Ortner, Bioconjugate Chem. 11 (2000) 345.
[6] H.-J. Pietzch, A. Gupta, M. Reisgys, A. Drews, S. Seifert, R. Syhre, H. Spies, R. Alberto, U. Abram, A.P. Schubiger, B. Johannsen, Bioconjugate Chem. 11 (2000) 414.
[7] S.S. Jurisson, J.D. Lydon, Chem. Rev. 99 (1999) 2205.
[8] P. Kurz, B. Spingler, R. Alberto, in: Technetium Rhenium and Other metals in chemistry and nuclear medicine, SGEditorial, Padova, 2002, pp. 115–117.
[9] K. Ortner, M. Kunding, S. Mundwiler, R. Alberto, J. Labelled Compd. Rad. 44 (suppl. 1) (2001) S504.
[10] S. Mundwiler, M. Kundig, K. Ortner, R. Alberto, Dalton Trans. 7 (2004) 1320.
[11] N.I Gorshkov, J.A. Katzenellenbogen, L.G. Luyt, A.A. Lumpov, A.E. Miroslavov, D.N. Suglobov, in: Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, SGEditorial, Padova, 2002, pp. 127–130.
[12] A.E. Miroslavov, N.I. Gorshkov, A.A. Lumpov, D.N. Suglobov, Radiokhimiya 42 (2000) 213.
[13] R. Alberto, R. Schibli, A.P. Schubiger, U. Abram, T.A. Kaden, Polyhedron 15 (1996) 1079.
[14] V.M. Byr'ko, Ditiocarbamaty (Dithiocarbamates), M. Nauka, 1984.
[15] B. Macias, M.V. Villa, E. Chiote, S. Martin-Velasco, A. Castinerais, J. Borras, Polyhedron 21 (2002) 1899.
[16] K. Nunami, M. Suzuki, K. Matsumoyo, N. Yoneda, K. Takiguchi, Agric. Biol. Chem. 48 (1984) 1073.
[17] N.I. Gorshkov, J.A. Katzenellenbogen, L.G. Luyt, A.A. Lumpov, A.E. Miroslavov, D.N. Suglobov, J. Labelled Compd. Rad. 44 (suppl. 1) (2001) S486.
[18] N.I. Gorshkov, A.A. Lumpov, A.E. Miroslavov, D.N. Suglobov, Radiokhimiya 45, 2005, in press.
[19] D. Parker, Chem. Soc. Rev. 19 (1990) 271.
[20] M.R. Zalutsky, J.S. Lewis, in: Handbook of Radiopharmaceuticals. Radiochemistry and Application, Wiley, New York, 2003, pp. 685–715.