JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 319–331.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.673

Research Article

Synthesis and characterization of new ^{99m}Tc-radiopharmaceuticals with dithiobenzoate derivatives for the study of septic inflammatory processes

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Summary

Improved methods for the preparation of ^{99m}Tc-radiopharmaceuticals containing dithiobenzoate ligands, in sterile and pyrogen free conditions, are described. These procedures are based on the reaction of these ligands either with [^{99m}Tc] pertechnetate in the presence of a strong reducing agent (HCl/tertiary phosphine, $SnCl_2 \cdot 2H_2O$), or with pre-reduced complexes obtained from different kit formulations. All the preparations led to the high-yield formation of the neutral and lipophilic ^{99m}Tc-complex [^{99m}Tc][Tc(S_3CPh)$_2$ (S_2CPh)], which is analogous to the corresponding compounds obtained with rhenium and the long-lived β-emitting isotope technetium-99 g recently described. HPLC analysis and thin layer chromatography were used to confirm the characterisation of the resulting ^{99m}Tc-radiopharmaceutical which was found to be potentially suitable for blood-cell labelling as applied to the

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Received 13 April 2002
Revised 13 September 2002
Accepted 3 October 2002

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Key Words: Technetium-99m; radiopharmaceuticals; dithiobenzoate ligands

Introduction

Nuclear medicine is an area of research having a specific interest in the design, synthesis and clinical application of new radiopharmaceuticals for diagnosis and therapy.[1] In particular, there is a growing interest for the development of new tracers for blood-cell labelling which may be useful in the study of the mechanism of immune response or to visualize inflammatory lesions.[2] This approach has been successfully applied during the last 20 years using ^{111}In-oxine and ^{99m}Tc-HMPAO (HMPAO = hexamethylpropyleneamine oximate).[3] However, these well-known blood-cell labelling agents suffer from various disadvantages, the most significant being their lack of selectivity, which necessarily requires a first separation step of the target blood cells before labelling. Thus, current efforts are focused on the design and the preparation of more specific blood-cell labelling agents. In our laboratory, we found that the class of nitrido technetium-99m complexes with dithiocarbamate ligands showed promising results for this purpose. Specifically, the complex bis-(*N*-ethoxy, *N*-ethyldithiocarbamate)nitridotechnetium(V) [^{99m}TcN(NOET)$_2$] allows *in vitro* specific polynuclear cells labelling in the whole blood.[4] whereas similar disubstituted complexes with dithiocarboxylates having aliphatic linear side-chains induce a high lymphocyte selectivity in the same conditions.[5]

[^{99m}TcN(NOET)$_2$][6] is an example of a large class of Tc-99m complexes, including also xanthates[7] and dithiocarboxylates,[8] characterised by the presence of a terminal Tc≡N multiple bond. It was found that [^{99m}TcN(NOET)$_2$] exhibits high myocardial localization both in animals and in humans, and it is currently under phase III clinical trials for receiving final approval as heart imaging agent.[9] The efficient preparation of the Tc≡N group, at no *carrier-added* level and under sterile and pyrogenic free conditions, has been recently made possible after the introduction by Pasqualini *et al.*[10] of a suitable kit formulation based on the initial reaction of pertechnetate ([^{99m}Tc]TcO_4^-) with *N*-methyl-*S*-methyl dithiocarbazate [MDTCZ = H_2N–N(Me)C(=S)SMe] or, alternatively, with succinic dihydrazide [SDH = H_2N–NH–C(=O)–$(CH_2)_2$–(O=)C–NH–NH_2] as sources of nitride nitrogen atoms, in the

presence of a reducing agent such as $SnCl_2{\cdot}2H_2O$ or HCl and tertiary phosphines. The initial step is then followed by the addition of the appropriate ligand to afford the final radiopharmaceutical having the nitrido core.

In this paper, we report the synthesis of the complex $[^{99m}Tc(S_3CPh)_2(S_2CPh)]$ using different kit methods and by varying the nature of the counter ion in the starting ligand $PhCS_2^-X^+$ **L1**–**L4** [X = MgBr, Na, $NH_2(CH_2)_5$, NMe_4]. The preparation and characterization of the complexes $[M(S_3CPh)_2(S_2CPh)]$ (M = ^{99g}Tc, Re) in macroscopic amounts, using the long-lived isotope Tc-99 g and the stable rhenium congener, has been recently described by our group.[11] These latter species have been utilized here as reference compounds to obtain the full elucidation of the chemical nature of the new ^{99m}Tc-radiopharmaceutical by HPLC analysis and thin layer chromatography.

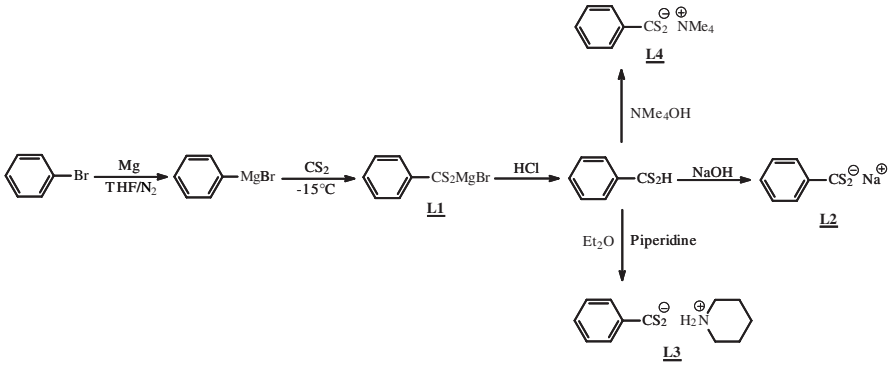
Experimental

Materials

Tetrahydrofuran (THF) was distilled twice under nitrogen from sodium/benzophenone before use, while diethyl ether, dichloromethane and petroleum ether were distilled using the same procedure from calcium chloride. Bromobenzene, magnesium turnings, carbon disulfide, tetramethylammonium hydroxyde, piperidine, triphenylphosphine and stannous chloride dihydrate were commercially available (Aldrich, St Quentin Fallavier, France) and used as purchased. Sodium tris (*m*-sulfonatophenyl)phosphine $[P(m\text{-}C_6H_4SO_3)_3]Na_3$ (TPPTS) was synthesized as reported previously.[12] Sodium [^{99m}Tc]pertechnetate, in physiological solution, was obtained from a ^{99}Mo/^{99m}Tc generator ELU III (CIS bio international/Schering SA, Gif sur Yvette, France). All other chemicals were of laboratory grade and used without further purification. 1,2-diaminopropane-*N*-*N*-*N*′-*N*′-tetraacetic acid (PDTA) and kits for the preparation of the Tc≡N group (^{99m}Tc-MDTCZ and ^{99m}Tc-SDH) and of ^{99m}Tc-Gluconate were generously offered by CIS bio international/Schering SA (Gif-sur-Yvette, France).

Ligands

The magnesium bromide salt, $PhCS_2^-MgBr^+$ **L1**, was prepared in anhydrous THF as reported previously,[13] and isolated by filtration at



Scheme 1. Synthesis of dithiobenzoic ligands

Table 1. Ligand synthesis

Ligands	Conter ion	Rf[a]	M.p. (°C)	yield(%)
L1	MgBr	0.85	Oxidation in air	82
L2	Na	0.81	92-94	94
L3	$H_2N(CH_2)_5$	0.83	96-98	76
L4	NMe_4	0.74	98-100	52

[a] eluent : acetone

–15°C, washed with cold diethyl ether and dried under reduced pressure. The tetramethylammonium salt **L4**, $PhCS_2^-NMe_4^+$, was precipitated by addition of an equivalent amount of tetramethylammonium hydroxide to a pink solution of dithiobenzoic acid, under vigorous stirring, and then isolated by filtration, washed with diethyl ether and dried under reduced pressure. $PhCS_2H$ was previously obtained by addition of 20 ml of HCl ($1\,mol^{-1}$) to the reaction solution containing the magnesium bromide salt at 0°C and then isolated after extraction with diethyl ether. The sodium salt **L2**, $PhCS_2^-Na^+$, was prepared using a similar procedure by addition of an equivalent amount of aqueous sodium hydroxide ($1.25\,mol\,dm^{-3}$), and precipitated after concentration of the resulting solution. Similarly, the piperidinium salt **L3**, $PhCS_2^-(C_5H_{10})NH_2^+$, was precipitated from $PhCS_2H$, by addition of an equivalent amount of piperidine, and then isolated by filtration, washed with diethyl ether and dried under vacuum. The preparations of the various salts of the ligand $PhCS_2^-$ are summarized in Scheme 1. Analytical data are reported in Tables 1–3. All the products were stored at –12°C under a nitrogen atmosphere.

Table 2. ^{1}H NMR and ^{13}C NMR of ligands

Ligands (solvent)	^{1}H NMR [ppm, peak multiplicity, (number of hydrogens)][a]
L1 : dmso-d^6	Aromatic hydrogens: 7.19, t, J = 7.6 Hz(2 H) 7.29, t, J = 7.6 Hz(1 H) 8.15, d, J = 7.6 Hz(2 H)
L2 : D_2O	Aromatic hydrogens: 7.28, t, J = 7.1 Hz (2 H) 7.39, t, J = 7.1 Hz (1 H) 7.89, d, J = 7.1 Hz (2 H)
L3 : $CDCl_3$	CH_2: p, J = 5.0 Hz(2 H) CH_2: p, J = 6.0 Hz(4 H) NCH_2: 3.22, s (12 H) Aromatic hydrogens: 7.35, t, J = 7.5 Hz(2 H) 7.46, t, J = 7.0 Hz(1 H) 8.46, d, J = 7.5 Hz(2 H) NH: 8.12,s(1 H)
L4 : dmso-d^6	NCH_3: 3.10, s, 12 H Aromatic hydrogens: 7.14, t, J = 7.1 Hz(2 H) 7.29, t, J = 7.1 Hz(1 H) 8.15, d, J = 7.1 Hz(2 H)
	^{13}C NMR (ppm)
L1 : dmso-d^6	Aromatic carbons : 125.8, 125.9, 128.5, 152.7 CS_2: 231.0
L2 : D_2O	Aromatic carbons : 126.3, 127.9, 130.6, 153.9 CS_2: 228.4
L3: $CDCl_3$	CH_2 : 22.2 ; 22.8 NCH_2 : 45.4 Aromatic carbons: 126.9, 127.3, 130.8, 150.8 CS_2: 254.8
L4 : dmso-d^6	NCH_3 : 54.3 Aromatic carbons: 126.2, 126.3, 128.0, 152.9 CS_2: 250.2

[a] m = multiplet, t = triplet, d = doublet and p = pentuplet

Methods

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, D_2O or dmso-d^6 solutions on a Bruker ARX 400 instrument. Values for the chemical shift were determined using $CHCl_3$ as a reference. Elemental analysis of C, H and S were performed by I.C.S.N. (Gif-sur-Yvette, France) on a Carlo Erba 1106 analyser. FT IR spectra were recorded on a Nicolet 205 instrument in KBr pellets within the range 4000–500 cm^{-1}.

Radiochemical purity (RCP) of radioactive compounds was measured by thin-layer chromatography (TLC) on aluminium-backed silica-gel plates (F_{254}, Merck) using a mixture of petroleum ether and dichloromethane (60:40) as mobile phase. Quantitative evaluation of radioactivity profiles was obtained by applying the following method. After development of the chromatogram, the plates were dried under an air stream and protected with an adhesive tape to avoid contamination. The chromatograms were then placed in close contact with a Fuji imaging plate (BAS-IIIS), in a dark box, for 5–10 min depending on the initial activity. Location of the radioactivity as a dark spot was accomplished with a Fujix Bas 1000 bio-imaging analyser (Fujix Bas 1000). The original chromatographic strip was then cut in correspondence of each spot and radioactivity was measured with a Kontron GAMMAMATIC γ-counter. RCP values were calculated as the ratio of migrated radioactivity to the total radioactivity on the strip.

Table 3. Elemental analysis and Infrared of dithiobenzoate ligands

Ligands	Assayed values (%)			Calculated values (%)		
	C	H	S	C	H	S
L1 : $C_7H_5BrMgS_2$	32.54	2.12	24.81	32.70	2.00	24.90
L2 : $C_7H_5NaS_2$	47.73	2.92	36.38	47.70	2.90	36.40
L3 : $C_{12}H_{15}NS_2$	60.17	7.16	26.71	60.20	7.16	26.70
L4 : $C_{11}H_7NS_2$	58.34	7.54	27.13	58.10	7.50	28.20
	Infrared (KBr pellets) : cm^{-1} (intensity)					
L1	1609(m), 1444(m), 1304(w), 1216(s), 1173(m), 1077(m), 1008(s, ν_{C-S}), 980(s), 967(m), 904(s), 757(s), 683(s), 653(m).					
L2	1629(s), 1438(m), 1316(w), 1223(s), 1168(m), 1077(m), 1014(s, ν_{C-S}), 910(m), 906(m), 765(s), 692(m), 655(m)					
L3	2955(w), 1584(f), 1443(m), 1384(w), 1305(w), 1263(w), 1208(m), 1177(w), 1082(m), 1004(s, ν_{C-S}), 988(s), 944(w), 926(w), 906(w), 767(m), 694(m), 655(m)					
L4	1487(s), 1437(m), 1403(w), 1216(w), 1168(m), 1077(m), 1014(s, ν_{C-S}), 956(s), 948(s), 906(m), 759(s), 694(m)					

The complexes $[M(S_3CPh)_2(S_2CPh)]$ ($M = {}^{99m}Tc$, ${}^{99g}Tc$ and Re) chromatograms were performed by HPLC (System Gold, Beckman), equiped with a Spectra-Physics SP 8800 ternary pump. Injections were performed automatically with a Spectra-Physics autosampler with 20-µl Rheodine injector. Elutions were run in the isocratic mode on a ODS Beckman Column (4.6×250 mm, d.p. 5 µm) equiped with a ODS pre-column (4.6×45 mm, dp 5 µm) using He-degassed tetrahydrofuran (THF)/H_2O gradient (0–3 min 70% H_2O; 3.1–17 min 100% THF ; 17.1–30 min 70% H_2O). The flow was 1 ml/min and the injection volume varied between 10 and 100 µL depending on the activity containing in the vial. The detector consist of a ABI Kratos UV-Vis detector (Spectroflow 783) coupled to a 10-µl loop flow-through gamma detector (LB2040 Berthold Spectrometer). Output signals were analysed by a Spectra-Physics SP4290 dual-channel integrator. The software program controlling the apparatus is from Beckman (System Gold Package). Retention time differences (16.1 and 15.6 min) between gamma emitting compounds and UV-Vis complexes is due to the answer delay resulting from the coupling through a loop-flow of two different detectors.

Elution yields of the different radioactive entities were satisfactory ($> 95\%$), thus indicating that these compounds were not retained in the column or the HPLC system (Table 4).

Table 4. HPLC and TLC analysis of $[M(S_3CPh)_2(S_2CPh)]$ (M = ^{99m}Tc, ^{99g}Tc, $^{185/187}$ Re)

	$[^{99m}TcO_4]$	$[^{99m}Tc(S_3CPh)_2(S_2CPh)]$	$[^{99g}Tc(S_3CPh)_2(S_2CPh)]$	$[Re(S_3CPh)_2(S_2CPh)]$
RCP (%)[a]	> 95	> 95	> 90	> 90
R_t (min)[b]	3.44	16.1	15.6	15.6
R_f[c]	0	0.62	0.62	0.62

[a] Chromatographic purity determined by HPLC analysis.
[b] retention time.
[c] TLC analysis on SiO_2 normal plates eluated with CH_2Cl_2/petroleum ether 30/70.

Preparation of $[^{99m}Tc(S_2CPh)(S_3CPh)_2]$

The title compound was prepared by reacting the dithiobenzoate ligand DTCX [$PhCS_2X$ **L1**–**L4**, X = MgBr, NMe_4, Na $(C_5H_{10})NH_2$] with two different kit formulations for producing the Tc≡N triple bond at carrier-free level which have been described previously.[6, 9, 10, 14] The two formulations, ^{99m}Tc-MDTCZ and ^{99m}Tc-SDH, differ in the nature of the donor of N^{3-} groups. In ^{99m}Tc-MDTCZ this reagent is *N*-methyl *S*-methyl dithiocarbazate [MDTCZ = H_2N–N(Me)C(=S)SMe] while in ^{99m}Tc-SDH it is succinic dihydrazide [SDH = $H_2N.NH$–C(=O)–$(CH_2)_2$–(O=)C–NH–NH_2].

^{99m}Tc-MDTCZ. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial containing 1.0 mg MDTCZ, 0.1 mg of $SnCl_2 \cdot 2H_2O$ and 10.0 mg of PDTA in a freeze-dried form. The mixture was heated at 100°C for 30 min and then cooled to room temperature. 20.0 mg of the appropriate dithiobenzoate ligand DTCX, dissolved in 1.0 mL of physiological serum, were finally added and the solution was heated at 100°C for 45 min.

^{99m}Tc – SDH. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial containing 5.0 mg of SDH, 0.1 mg of $SnCl_2 \cdot 2H_2O$ and 5.0 mg of PDTA in a freeze-dried form. The mixture was stirred at room temperature for 30 min. 20.0 mg of the appropriate DTCX ligand, dissolved in 1.0 mL of physiological serum were finally added, and the solution was heated at 100°C for 45 min.

The following procedures have been also used to prepare the same complex $[^{99m}Tc(S_2CPh)(S_3CPh)_2]$ using different reducing agents and from the commercial kit ^{99m}Tc-Gluconate.[15]

PPh_3/HCl. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial containing 0.2 ml of an ethanolic solution of triphenylphosphine (2×10^{-2} mol dm^{-3}), 0.2 mL of aqueous HCl (0.1 mol dm^{-3}) and 0.6 ml of physiological serum. The mixture was vortexed and then heated at 100°C for 15 min. 10.0 mg of the appropriate ligand DTCX, dissolved in 1.0 ml of physiological serum, were finally added to the hot solution and the heating was continued for 30 min.

TPPTS/HCl. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial containing 0.2 mL of an aqueous solution of TPPTS (2×10^{-2} mol dm^{-3}), 0.2 mL of aqueous HCl (0.1 mol dm^{-3}) and 0.6 mL of physiological serum. The mixture was vortexed and then heated at 100°C for 15 min. 10.0 mg of the appropriate ligand DTCX, dissolved in 1.0 ml of physiological serum, were finally added to the hot solution and the heating was further continued for 30 min.

$SnCl_2$. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial 0.1 mg of $SnCl_2{\cdot}2H_2O$ and 10 mg of 1,2-diaminopropane-*N*-*N*-*N*′-*N*′-tetraacetic acid (PDTA). The mixture was vortexed and then heated at 100°C for 15 min. 20.0 mg of the appropriate DTCX ligand, dissolved in 1.0 mL of physiological serum, were finally added to the hot solution and the heating was further continued for 45 min.

^{99m}Tc-Gluconate. [^{99m}Tc]Pertechnetate (0.4–0.8 GBq) was added to a vial containing 75.0 mg of calcium gluconate dissolved in 0.1 ml of water, 0.75 mg of $SnCl_22H_2O$ and 25.0 mg of sodium chloride. The mixture was stirred for 10 min at room temperature. 20.0 mg of the appropriate DTCX ligand, dissolved in 1.0 mL of physiological serum, were finally added and the solution was heated at 100°C for 15 min.

Results and discussion

Ligands

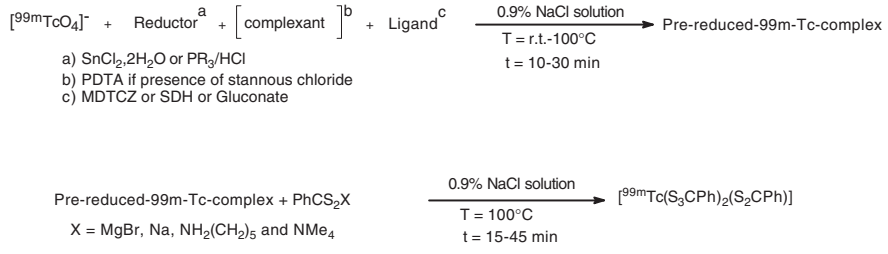
Various salts of dithiobenzoic acid $PhCS_2X$ **L1**–**L4** (DTCX, X = MgBr, Na, $NH_2(C_5H_{10})$, NMe_4) were synthesized using well-established procedures. Analytical, spectral and chromatographic data for these compounds are given in Tables 1–3.

[^{99m}Tc(S_2CPh)(S_3CPh)$_2$]

The preparation of the complex bis(trithioperoxybenzoate)(dithiobenzoate)technetium-99m, [^{99m}Tc(S_2CPh)(S_3CPh)$_2$], was accomplished using various procedures and different salts of the ligand $PhCS_2^-$ **L**. This study was undertaken with the aim to find the more suitable procedure for obtaining this radiopharmaceutical in high yield and in conditions as close as possible to physiological conditions. The main interest in the compound [^{99m}Tc(S_2CPh)(S_3CPh)$_2$] comes from the fact that in vitro experiments showed that it is very efficiently and selectively retained into leukocytes when incubated with whole blood. Further, it was found that the activity was stably incorporated thus suggesting that this tracer has a peculiar affinity for these blood constituents. The solid structure of the analogous complexes, [M(S_2CPh)(S_3CPh)$_2$], prepared in macroscopic amounts respectively, with the long-lived Tc-99 isotope and cold rhenium, has been determined by X-ray diffraction methods.[11] The central metal lies in a distorted trigonal prism environment made up by the six terminal sulfur atoms. These complexes were utilized to establish the chemical identity of the corresponding Tc-99m compound through the comparison of their chromatographic properties. HPLC and TLC chromatographic data are illustrated in Table 4.

The need to investigate different reaction procedures for obtaining an efficient method for the preparation of the complex [^{99m}Tc(S_2CPh)(S_3CPh)$_2$], at no *carried-added* level, was dictated by the very complicated reactivity of dithiocarboxylate ligands which may give rise to a wide variety of final products.[12] A first attempt was undertaken by reacting the ligands **L1**–**L4** with the $[^{99m}Tc{\equiv}N]^{2+}$ core prepared through a well-established procedure described a few years ago.[10] Such an attempt was justified by the fact that it was previously observed that thiol and dithiol ligands were able to remove easily the Tc≡N multiple bond to afford the corresponding S-substituted complexes.[16] Thus, the reaction was carried out by first producing the Tc≡N group through the reaction of [^{99m}Tc]pertechnetate with *S*-methyl-*N*-methyldithiocarbazate (MDTCZ), or alternatively succinic dihydrazide (SDH), as source of nitride nitrogen atoms (N^{3-}), in the presence of a reducing agent such as $SnCl_2.2H_2O$ or a combination of HCl and a tertiary phosphine.

Another approach to the synthesis of the complex [^{99m}Tc(S_2CPh)(S_3CPh)$_2$] was undertaken when ^{99m}Tc-gluconate was used as pre-reduced substrate in the reaction with DTCX. In this preparation, a commercial kit formulation for ^{99m}Tc-gluconate[15] was utilized, and successively mixed with the ligands DTCX. Finally,



Scheme 2. Synthesis of [^{99m}Tc(S$_3$CPh)$_2$(S$_2$CPh)]

Table 5. Influence of the chemical nature of the ligand on RCP using the MDTCZ/PDTA Kit

Ligand	NOET[a]	$C_{10}MgBr$[a]	**L1**	**L2**	**L3**	**L4**
R_f	0.62[b]	0.75[b]	0.62[c]	0.62[c]	0.62[c]	0.62[c]
RCP[d] (%)	>90	70	70	90	70	40

[a] Ligands used as reference (NOET: Et(OEt)NCS$_2$Na and $C_{10}MgBr$: $CH_3(CH_2)_8CS_2MgBr$).
[b] TLC (SiO_2), eluent CH_2Cl_2, R_f ($\pm$ 0.12).
[c] TLC (SiO_2), eluent petroleum ether/CH_2Cl_2 : 70/30, R_f ($\pm$ 0.12).
[d] Radiochemichal purity estimated.

[^{99m}Tc(S$_2$CPh)(S$_3$CPh)$_2$] was prepared through a simpler route involving the reaction of DTCX with [^{99m}Tc]pertechnetate in the presence of $SnCl_2$ or HCl/tertiary phosphine as reductants. All the procedures are summarised in Scheme 2. It should be noted that, in all preparations using stannous chloride, 1,2-diaminopropane-*N*-*N*-*N*′-*N*′-tetraacetic acid (PDTA) was always added as complexing agent to avoid precipitation of Sn^{2+} ions co-ordinated to the ligand DTCX.

The results are reported in Tables 5 and 6. The complex [^{99m}Tc(S$_2$CPh)(S$_3$CPh)$_2$] was obtained as the main product in all preparations, with a radiochemical purity (RCP) depending on both the chemical nature of the ligand DTCX and the reaction procedure performed. Starting with a precursor containing the Tc≡N multiple bond, the highest yield (approximately 90%) was obtained with MDTCZ as donor of N^{3-} groups and using the sodium salt **L2** of the ligand DTCX (X = Na). Others salts gave lower RCP values. Similarly, the preparation containing SDH as N^{3-} donor produced unsatisfactory results with a final yield lower than 60%.

The complex [^{99m}Tc(S$_2$CPh)(S$_3$CPh)$_2$] could not be easily obtained starting from [^{99m}Tc]pertechnetate and $SnCl_2$ (RCP < 20%). Nevertheless, the use of a tertiary phosphine and aqueous hydrochloric acid

Table 6. Different kit formulations for the synthesis of [^{99m}Tc(S_3CPh)$_2$(S_2CPh)] with $PhCS_2Na$ L2

KIT	medium / pH	T (°C)	t (min)	R_f[g]	PRC (%)
MDTCZ/PDTA[a]	pH4	100	45	0.62	85
HCl/PPh_3[b]	Serum φ	100	30	0.62	71
HCl/TPPTS[c]	Serum φ	100	30	0.62	94
$SnCl_2$/Gluconate[d]	Serum φ	100	15	0.62	> 95
SDH[e]	Serum φ	100	45	0.62	55
$SnCl_2$/ PDTA[f]	Serum φ	100	45	0.62	< 20

[a] MDTCZ/PDTA kit (CIS bio international) and $PhCS_2NH_2(CH_2)_5$ L3.
[b] HCl 0.1 N (0.2 ml), PPh_3 2.10^{-2} M / EtOH (0.2 ml).
[c] HCl 0.1 N (0.2 ml), TPPTS 2.10^{-2} M / H_2O (0.2 ml).
[d] $SnCl_2$/gluconate kit (CIS bio international) diluted 1/10 (1.0 ml).
[e] SDH kit (CIS bio international).
[f] $SnCl_2$/ PDTA kit.
[g] TLC (SiO_2), eluent petroleum ether/CH_2Cl_2 : 70/30, $R_f \pm 0.12$.

gave the ^{99m}Tc-radiopharmaceutical in higher yield (71% for PPh_3 and 94% for TPPTS). The most quantitative reaction was obtained using the ^{99m}Tc-gluconate kit. [^{99m}Tc(S_3CPh)$_2$(S_2CPh)] was prepared with the highest radiochemical yield (> 95%) through the reaction of ^{99m}Tc-gluconate with $PhCS_2Na$ at 100°C for 15 min. In this reaction, the use of the other salts of the same ligand produced lower yields.

Conclusion

In this work, the ^{99m}Tc-labelled compound [^{99m}Tc(S_3CPh)$_2$(S_2CPh)] analogous to the compounds [M(S_3CPh)$_2$(S_2CPh)] (M = ^{99}Tc, Re), previously isolated and characterized in macroscopic amounts, was prepared using several procedures. The most efficient method was based on the reaction of a lyophilized formulation of ^{99m}Tc-gluconate with the sodium salt **L2** of phenyldithiocarboxylic acid. The neutral and lipophilic [^{99m}Tc(S_3CPh)$_2$(S_2CPh)] was obtained in very high yield (> 95%) and characterized by TLC and HPLC chromatography. The similarity between its chromatographic behaviour and that of the analogous ^{99g}Tc and Re complexes clearly established the chemical nature of this new radiopharmaceutical. The aim of these experiments will be in carefully evaluating the possibility to introduce the new ^{99m}Tc-labelled compound into the clinical practice as an attractive alternative to the current employed leukocyte labelling agents.

Acknowledgements

We thank Mrs A. Trichet and Mrs S. Abgrall for technical assistance in the radiopharmaceuticals preparation. CIS bio international/Schering SA and Region Bretagne for financial support for this work. We are also grateful to Dr. A. Boschi for HPLC analysis and the International Society of Radiolabelled Blood Elements (ISORBE).

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