Pergamon

Tetrahedron Letters 43 (2002) 9295–9297

TETRAHEDRON LETTERS

A simplified route to the synthesis of new ^{99m}Tc-specific tetradentate ligands

Julien Le Gal,[a] Eric Benoist,[a,*] Marie Gressier,[a] Yvon Coulais[b] and Michèle Dartiguenave[a]

[a]*Laboratoire de Chimie Inorganique, Université Paul Sabatier, Bat. IIR1, 118, route de Narbonne, 31062 Toulouse, France*
[b]*Service de Médecine Nucléaire, Hôpital Purpan, Place du docteur Baylac, 31059 Toulouse, France*

Received 24 September 2002; accepted 23 October 2002

Abstract—The synthesis of two new N_2S_2 or N_2SO tetradentate ligands and the preparation of their technetium-99m complexes are reported. Each ligand leads to a unique ^{99m}Tc-complex with an excellent radiochemical yield. The in vivo stability of these complexes was determined by serum stability and cysteine challenge studies.

The appropriate physical properties ($T_{1/2}=6$ h, 140 KeV gamma emitter) and widespread availability of ^{99m}Tc continue to make it the most attractive candidate for use in formulating diagnostic radiopharmaceuticals for SPECT imaging studies in patients.[1] Efforts to design new chelate systems for this nuclide and for rhenium for subsequent uses, respectively, in diagnostic and therapy have led us[2] and others[3] to the development of new N_2S_2 tetradentate ligands. These ligand systems are one of the most commonly used ligands which form stable technetium(V) or rhenium(V) complexes.

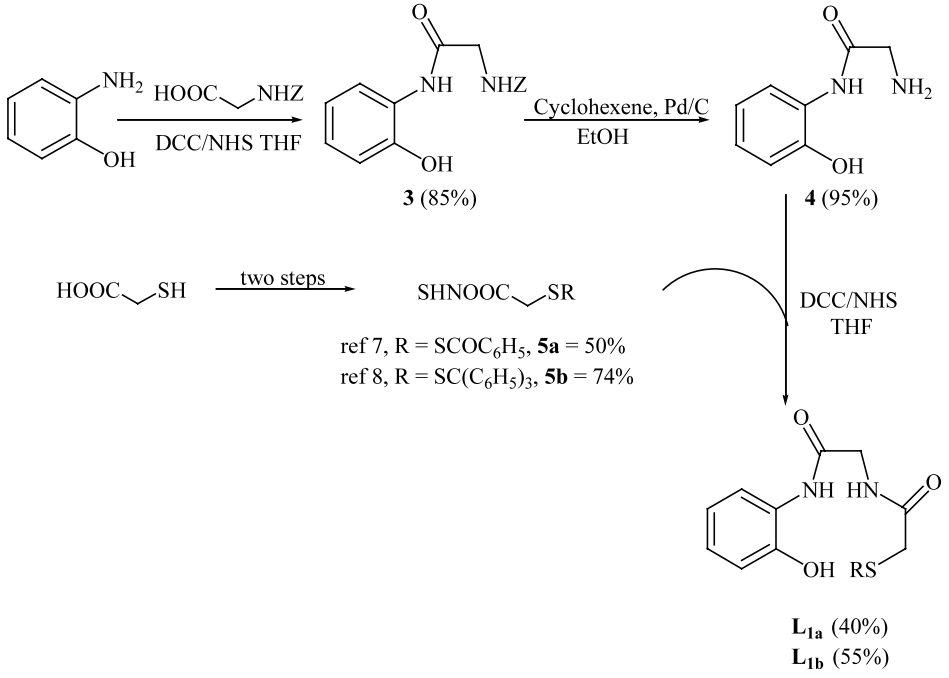
One of the most important criteria in radiopharmaceutical design is the in vivo stability of radiopharmaceuticals. In order to limit metal dissociation, we initially reported the synthesis of a new class of tetradentate ligands incorporating an aromatic ring to induce a rigidity to the cavity and facilitate complexation notably by reducing the reaction entropy.[4] The rigidity of the ligands was introduced to improve the stability of the corresponding complexes.[5] But, our first stability investigations showed us a fairly stability in human serum after 2 h but a significative dissociated ^{99m}Tc ratio after 6 h ($\approx$50%). The objective of the present study was to develop and to test two new aromatic bridged diamide–dithiol (DADT) or diamide–thiol–alcohol (DATA) chelators which, while still maintaining a simplicity of synthesis, will be able to increase the in vivo stability of ^{99m}Tc-complexes.

The ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ were synthesised with a benzoyl or a triphenylmethyl (trityl) protected mercapto group, in order to inhibit the oxidation of the thiol functionality upon a long storage. They can be easily removed during the ^{99m}Tc radiolabelling reaction. Ligands were prepared by a multi-step synthesis, starting from *o*-substituted anilines as outlined in Schemes 1 and 2. Ligand $\mathbf{L_1}$ was synthesised according to a previously reported procedure[3] but with major modifications. The first step involves a conventional carbodiimide amide coupling of *N*-carbobenzyloxyglycine and 2-aminophenol followed by the removal of the carbobenzyloxy protecting group by catalytic hydrogenation using cyclohexene as a source of hydrogen and palladium on charcoal (80% for the two steps).[6] Acylation with the appropriate synthesised activated ester **5a**[7] or **5b**[8] afforded the desired ligands $\mathbf{L_{1a}}$ and $\mathbf{L_{1b}}$, respectively, in 40 and 55% yield.

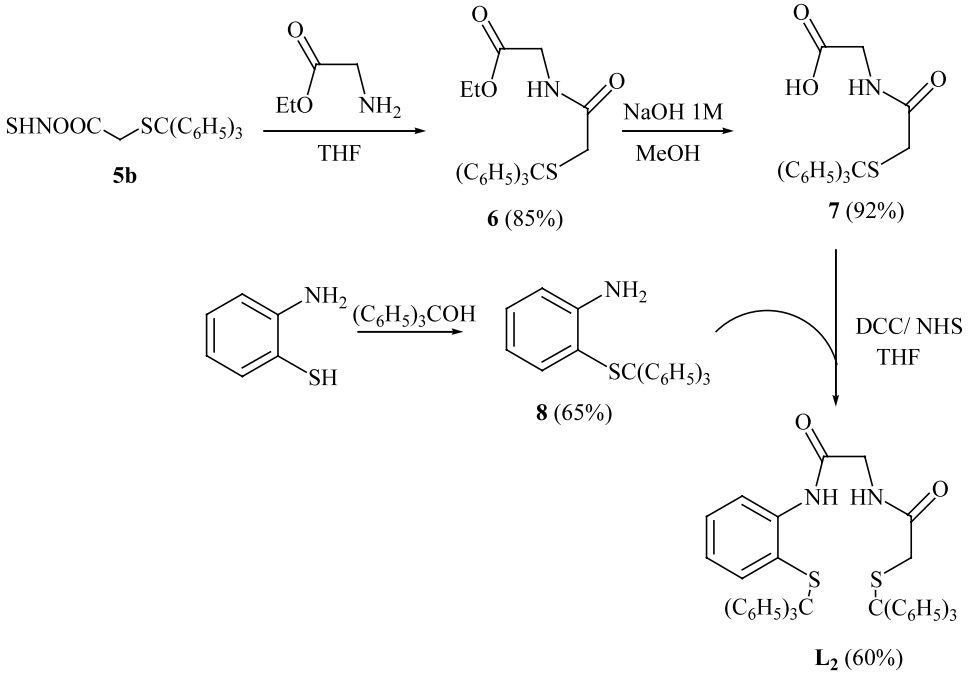
Heterogeneous catalysts like palladium on charcoal are usually poisoned by small amounts of sulfur such as thiols or sulfides. The obtention of the sulfur analog of compound **3** is impossible using a catalytic hydrogenation step. To circumvent this problem, we adopted a different synthetic way to obtain $\mathbf{L_2}$ (Scheme 2). This route needs to use a base-stable sulfur protecting group. Thus, we firstly synthesised the succinimidyl-*S*-triphenylmethylthioglycolate **5b**.[7] Reaction of ethyl glycinate with **5b** in THF yielded to the compound **6** (85%). Hydrolysis of the ester function of **6** in basic media (NaOH in methanol) afforded the corresponding acid **7** in excellent yield (92%). Then, $\mathbf{L_2}$ was obtained by a classical peptidic coupling of the acid **7** with the 2-(*S*-trityl)aniline **8**[9] in the presence of dicyclohexylcarbodiimide. The same reaction attempted with 2-

* Corresponding author. Fax: 33 561 55 61 18; e-mail: benoist@chimie.ups-tlse.fr

0040-4039/02/$ - see front matter
PII: S0040-4039(02)02385-7



Scheme 1. Synthetic pathway for $\mathbf{L_1}$.



Scheme 2. $\mathbf{L_2}$ synthesis.

aminothiophenol instead of 2-(*S*-trityl)aniline failed, giving a mixture of unseparable products.

The new ligands $\mathbf{L_{1a}}$, $\mathbf{L_{1b}}$ and $\mathbf{L_2}$, obtained with a good overall yield, have been satisfactorily characterised by usual spectroscopic methods.[10] Thiol deprotection and ^{99m}Tc-labelling of the ligands were performed in situ in a mixture methanol/buffer solution pH 8.6 (1/4) by direct reduction of sodium pertechnetate in the presence of tin chloride at 80°C during 30 min with a radiochemical yield of 90%. Purification was accomplished by C-18 reverse phase HPLC and resulted in only a component for each ligand. The large difference obtained in the retention time of the two complexes could result in the formation of a $\mathbf{L_2M}$ species for ^{99m}Tc-$\mathbf{L_2}$.[11] Rhenium chemistry is under investigation to confirm this hypothesis.

The radiolabelled chelators were subject to metathesis in the presence of an excess of cysteine as an assay of

label stability.[12] After 12 h (two ^{99m}Tc periods), about 45% of ^{99m}Tc dissociated were observed for ^{99m}Tc-**L_2** while ^{99m}Tc-**L_1** exhibited a great stability (less than 3% of ^{99m}Tc dissociated). The excellent behaviour for the latter compound was confirmed by a serum stability study. After 12 h of incubation in fresh human serum, no significative technetium dissociation of the complex was observed.

In conclusion, a simplified route has been developed for the synthesis of two new tetradentate ligands. Their corresponding ^{99m}Tc-complexes were achieved with a good radiochemical yield. Serum stability and cysteine challenge assay showed a great stability for one of the two ^{99m}Tc-complexes, which makes it a promising chelator of technetium. Biodistribution studies are currently under progress.

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10. Only selected data are cited below for **L_{1a}** and **L_2** characterisation: **L_{1a}**: $^{13}C\{^1H\}$ NMR (50.32 MHz, $CDCl_3$ *JMOD*) δ_C (ppm): 37.7 (SCH_2), 48.6 (NCH_2), 120.7 (CH_{Ar}), 124.3 (CH_{Bz}), 127.1, 129.9 ($2CH_{Ar}$), 131.2 (C_{Ar}), 132.3, 134.5 ($4CH_{Bz}$), 139.5 (CH_{Ar}), 141.3 (C_{Bz}), 152.9 (C_{Ar}), 172.8, 172.9 (2CO), 195.6 (COS). IR (KBr): $\nu_{NHC=O}=1674$ cm^{-1}. *m/z* (DCI NH_3): 345 ($M+H^+$); **L_2**: $^{13}C\{^1H\}$ NMR (50.32 MHz, $CDCl_3$ *JMOD*) δ_C (ppm): 35.5 (SCH_2), 43.7 (NCH_2), 60.5, 67.9 (2C-S), 119.4 (CH_{Ar}), 120.4 (C_{Ar}), 123.6 (CH_{Ar}), 127.1, 127.2, 127.9, 128.3, 129.5, 129.6 ($30CH_{Tr}$), 131.2, 137.4 ($2CH_{Ar}$), 141.5 (C_{Ar}), 143.6, 143.9 ($6C_{Tr}$), 165.5, 168.4 (2CO). IR (KBr): $\nu_{NHC=O}=1677$ cm^{-1}. *m/z* (DCI NH_3): 741 ($M+H^+$).
11. **HPLC conditions**: Satisfaction RP18AB column, eluent: MeOH/H_2O/TFA: 45/55/0.1; $\lambda=270$ nm; Tr (^{99m}Tc-**L_1**) = 9.40 min; Tr (^{99m}Tc-**L_2**) = 17.00 min. Electrophoresis of each purified complex showed that both are negatively charged complexes.
12. **Cysteine challenge assay**: In each case, an aliquot of a fresh prepared solution of L-cysteine at 1 mg/mL was added to each purified complex, in a 500:1 cysteine/complex molar ratio. Each solution was incubated at 20°C and samples were removed for analysis. Metal dissociation was measured at various times from 2 to 12 h on a LB 2832 linear analyser (Berthold) after thin layer chromatography on nano-sil C18 plates (Macherey-Nagel) by elution with MeOH/CH_3CN/H_2O/TFA 20/15/65/0.1. R_f (free technetium) = 1; R_f (^{99m}Tc-**L_1**) = 0.5; R_f (^{99m}Tc-**L_2**) = 0.25.