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Efficient synthesis of new tetradentate ligands with potential applications for ^{64}Cu PET-imaging

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ABSTRACT

We wish to report the synthesis of new tetradentate ligands in less than 3 h in good to excellent yields from a commercially available compound using microwave-assisted technology. First tests of complexation showed a high ability of these ligands to chelate ^{64}Cu(II) in very diluted medium. These new systems have the potential to be used for nuclear medicine and particularly for PET-imaging.

In recent years, advances in imaging technology and more particularly in positron emission tomography (PET) have generated increasing interest.[1–5] PET has numerous advantages over other imagery techniques such as good spatial resolution (better than SPECT—Single Photon Emission Computed Tomography—) and excellent sensitivity (better than all imaging technologies),[2] versatility… Its sensitivity is so high that it allows using only nanomolar to picomolar concentrations of radiopharmaceutical to get an image.[6]

Many positron-emitting radionuclides have been investigated. ^{18}F is by far the most commonly used of the longer-lived PET radionuclides (^{18}F half-life = 110 min).[7] The most famous example is FDG ([^{18}F]-fluorodeoxyglucose) which is widely used for tumor imaging. However, when the pharmacokinetics of the radiopharmaceutical are slow, the short half life of ^{18}F can be inhibitory. In these cases ^{64}Cu (half-life = 761.9 min) seems an excellent alternative.[8–10] Indeed, specific sites imaging can be reached by tethering a copper chelating agent to biologically active molecules (sugar, peptide, antibody…) that selectively bind to certain receptors in vivo.[1,2,11–13] The long half life of copper is particularly important as several hours to several days may be necessary to get a significant accumulation of the injected radionuclide in the tumor.[8,9]

Furthermore, ^{64}Cu displays similarly good spatial resolution, with respect to ^{18}F (around 5.0 mm),[14] some studies have shown that it gives the highest effective dose and above all, that it can be used in preliminary dosimetry studies for radioimmunotherapy (use of radiolabelled antibodies to target and destroy tumor cells).[15–17] In fact, the main problem in radioimmunotherapy is reproducibility in the measurement of be able to measure out the amount of radioactivity bound to the tumor cells (it depends on the kind of cancer, on the vector and even on the patient). One promising solution is to use the dual emission nature of an element such as copper which possess both a beta+ emitter (^{64}Cu) and a beta− emitter (^{67}Cu). With ^{64}Cu, radioactivity can be quantified and as the same vector could be used to transport ^{67}Cu to tumor cells, the pharmacokinetics would be the same and the quantity of ^{67}Cu linked to tumor would be known.[2,18]

These considerations in combination with our groups expertise in chelation chemistry and heterocyclic chemistry led us to explore the design of new copper chelating agents. Copper complexes of bis(thiosemicarbazone) ligands represent an important class of compounds.[19–22] These ligands are particularly interesting due to their rapid complexation kinetics, the ease and the efficiency of their synthesis and their ability to form neutral complexes. Consequently, our attention has been focused on these types of bis(thiosemicarbazone) ligands. Moreover, our strategy allows one to tune the properties of the ligands by varying R^1 and R^2 on the heterocyclic groups (Fig. 1). By this way, we combine both our experience in the synthesis of chelating agents[23–27] and our knowledge in heterocyclic chemistry.[28–30]

Our initial synthetic strategy hinged on of a biscoupling reaction between a dicarbonyl compound, namely isophthalaldehyde, and two thiazolohydrazines. Isophthalaldehyde is commercially available and the thiazolohydrazines were synthesized readily via adaption of our recently reported thiazole synthesis (Scheme 1).[28,29]

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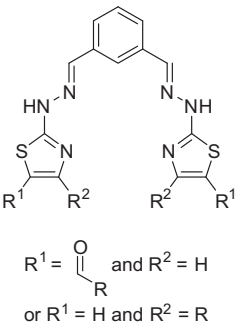


Figure 1. Tetradentate ligands **L1**–**L8**.

Compound **1** was prepared in 78% yield by a monocondensation of commercially available *N,N*-dimethylformamide dimethyl acetal and thiourea in methanol, and was protected with a *tert*-butyloxycarbonyl group. Protected thiazadiene **2** was treated with an α-bromoketone, to give the corresponding *S*-alkyl salt which underwent a spontaneous cyclization, followed by deamination of the cycloadduct to afford the expected thiazole **3** in good yield. Hydrazine **4** was synthesized by an electrophilic N-amination reaction.[31–33] The presence of the boc group enabled sodium hydride to deprotonate the thiazadiene **2**. The just formed amidure then reacted with *O*-(4-nitrobenzoyl)hydroxylamine to give the hydrazine **4** in 53% yield (the synthesis of *O*-(4-nitrobenzoyl)hydroxylamine was previously reported by Carpino in a two step strategy).[34] The hydrazine was then deprotected in good yield.

Despite a successful test reaction with benzaldehyde, attempts to obtain ligand **L1**, by condensation of hydrazines **4** or **5** with isophthalaldehyde were unsuccessful.

We, thus, decided to change our approach. Instead of coupling the heterocycles at the last step, we chose to start from isophthalaldehyde, and to synthesize in parallel, step by step, the two heterocycles.

The first step was a double condensation reaction of commercially available thiosemicarbazide with isophthalaldehyde. The reaction could be conducted using 'classical heating' to afford **6** in good yield in 10 h. A highly efficient process was essential as purification was challenging due to the poor solubility of the thiosemicarbazide and products. Under microwave irradiation,[35,36] the reaction time was reduced to 15 min and the reaction was cleaner: a simple filtration provided **6** in 98% yield as a pure pale yellow powder. Compound **6** was converted to the bis(thiazadiene) **7** by a double condensation reaction with commercially available *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) in ethanol. Compound **7** could be isolated, but usually engaged directly in the double cyclization with α-bromoketones. This strategy gave the expected ligands in 67–74% overall yields (Scheme 2).

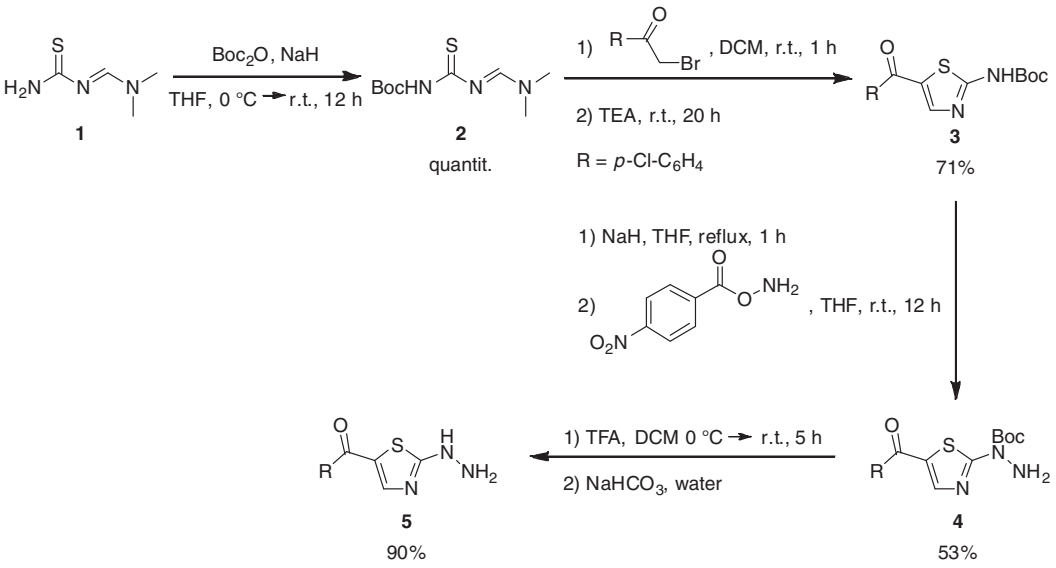
The scope of the reaction and especially the electronic effect of the R group on the α-bromoketones have been studied using various α-bromoketones (electron neutral: *para*-chlorobenzyl, electron donating: *tert*-butyl and electron withdrawing: trifluoromethyl). Ligand **L1–L3** displayed poor water solubility, a very unfortunate property for compounds aimed at biological applications. Consequently the synthesis of the bis(ester) ligand **L4** was targeted in order to open easy access to the corresponding bis(carboxylate). Moreover, modifications of ester functionalities are very versatile; consequently such a ligand would offer a facile route to several other chelating agents.

This approach allowed the three step synthesis of ligands **L1**–**L4** in good yield.[37,38] Furthermore, under microwave irradiation, the overall synthesis was achieved in less than 3 h including all work-ups and purifications (Table 1).

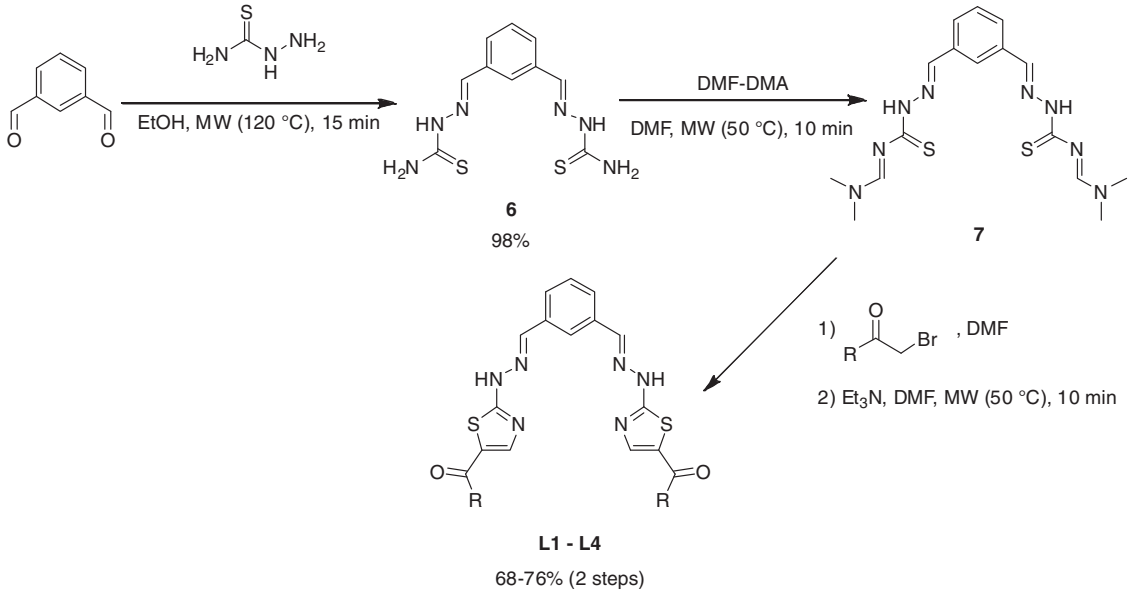
To study the influence of the position of the R group on the heterocycle, ligands with a substituent on position 5 of the thiazole rings were synthesized. These chelating agents **L5**–**L8** were efficiently prepared from bis(thiosemicarbazone) **6** and the different previously used α-bromoketones (Scheme 3). *S*-Alkyl intermediate **8**, underwent in situ cyclization via condensation of the generated imines with the ketones. The ligands **L5**–**L8** were obtained in good to excellent overall yields (70–96% from isophthalaldehyde) (Table 2), the difference of yield being due to a more or less poor solubility in organic solvent.

For these kinds of chelating agents the ability to use various α-bromoketones with electronically different R was important. As the R groups are directly bound to the heterocyclic rings of the ligand, they should influence the electron density located on chelating atoms. Thus, the chelation properties of the ligand may be tuned by changing of the α-bromoketone.

Preliminary complexation tests were performed with **L1** using $^{64}CuCl_2$. To form the ^{64}Cu-chelated complexes, 1 μL of an aqueous ammonium acetate solution (2.5 M) was added to a $^{64}CuCl_2$ solution (10^{-7} M) in aqueous hydrochloric acid (0.1 M) providing a $^{64}Cu(OAc)_2$ solution. To this medium were added 3 μL of an



Scheme 1. The synthesis of thiaziolohydrazines **5**.



Scheme 2. The synthesis of ligands **L1**–**L4**.

Table 1
Yields of ligands **L1**–**L4**

Entry	R	Overall yield (%)
L1	*p*-Cl-C_6H_4-	69
L2	*t*Bu-	67
L3	F_3C-	74
L4	EtO_2C-	71

Table 2
Yields of ligands **L5**–**L8**

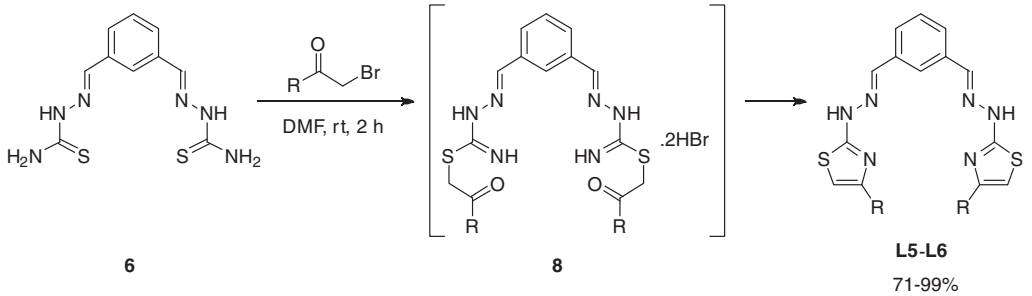
Entry	R	Overall yield (%)
L5	*p*-Cl-C_6H_4-	96
L6	*t*Bu-	97
L7	F_3C-	70
L8	EtO_2C-	93

aqueous sodium acetate buffer and 4 μL of dimethylformamide. 1 μL of a solution of **L1** in DMF (1 or 10 equiv) was finally added. The solution was incubated at room temperature for 30 min. The chelation yield was measured on a phosphorimager apparatus after TLC on silica gel plates.

The experimental results revealed a high chelation rate of 70% when one equivalent of **L1** was used, and more than 80% when 10 equiv were used. These results compare in comparison with currently used chelation agents.[2] These results are very exciting as they suggest that the complexation kinetics are very fast even in hostile conditions: room temperature, acid buffer and in very diluted medium (10^{-9} M).

The composition of the synthesized complex **CuL1** was established by IR, UV, mass spectrometry (MALDI) and elemental analysis with stable isotope of copper.[39] The NMR spectrum was presented broad peaks due to this paramagnetic configuration. Energy-dispersive X-ray spectroscopy (EDXS) indicated a copper to ligand **L1** ratio of 1:1. The different results show that is not a binuclear complex like is classically described in the literature.[40–44] We obtained a single copper centre with four donor atoms from the chelator.

In conclusion, we have designed new tetradentate ligands and more particularly, we have developed two fast and efficient original methods for their microwaved assisted synthesis. In two or three steps, these methods furnished quite sophisticated ligands in less than 3 h and in good to excellent yields (from 67% to 97%). Furthermore, the versatility of our two synthetic methods should allow us to study the influence that the nature and position of substituents position on the heterocyclic rings has on the chelation properties. A preliminary complexation test using radioactive copper demonstrated the ability of **L1** to chelate this metal very efficiently both kinetically and thermodynamically. These results encourage us to envision the synthesis of functionalized analogues of ligands **L1–L8** giving chelates that could be grafted to chemical or biological vectors.



Scheme 3. The synthesis of ligands **L5**–**L8**.

Acknowledgments

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37. *General procedure for ligands* **L1**–**L4**: Thiosemicarbazide (2.19 mmol, 2 equiv) and isophtalaldehyde (1.10 mmol, 1 equiv) were suspended in 4 mL of ethanol in a 10 mL-microwave reactor. The reactor was placed in the microwave oven. The reactor was heated from room temperature to 120 °C (OF) on 2 min by monomode microwaves irradiation (power: 150 W, stirring: 50%, ventilation: 1/3), maintained at 120 °C for 15 min (power: 50 W, stirring: 50%, ventilation: 1/3) and let cooled down to room temperature (power: 0 W, stirring: 50%, ventilation: 3/3). The mixture was filtered and washed with ethanol to give **6**. Compound **6** (0.71 mmol, 1 equiv) and DMF-DMA (1.78 mmol, 2.5 equiv) were dissolved in 4 mL of DMF in a 10 mL-microwave reactor. After total solubilisation, the reactor was placed in the microwave oven. The reactor was heated from room temperature to 50 °C (OF) over 1 min by monomode microwaves irradiation (power: 80 W, stirring: 30%, ventilation: 1/3), maintained at 50 °C for 8 min (power: 50 W, stirring: 30%, ventilation: 1/3) and let cooled down to room temperature (power: 0 W, stirring: 30%, ventilation: 3/3). The appropriate α-bromoketone (1.42 mmol, 2 equiv) was added in the previous reactor. The solution turned to orange. After 2 min of stirring, distilled triethylamine (2.13 mmol, 3 equiv) was added. The solution turned to dark red. The reactor was heated from room temperature to 50 °C (OF) over 1 min by monomode microwaves irradiation (power: 70 W, stirring: 30%, ventilation: 1/3), maintained at 50 °C for 8 min (power: 30 W, stirring: 50%, ventilation: 1/3) and let cooled down to room temperature (power: 0 W, stirring: 50%, ventilation: 3/3). The solution was concentrated under reduced pressure. The residue was suspended in 20 mL of dichloromethane, washed five times with 30 mL of water. The organic layer was evaporated under reduced pressure. The residue was suspended in dichloromethane, filtered and washed with dichloromethane.
38. *Spectroscopic data of ligand* **L1**: pale yellow powder; yield: 70%; mp: 204–206 °C; ^{1}H NMR (300 MHz, DMSO-d_6): 7.56 (t, 1H, *H*ar, J_3 = 7.7 Hz), 7.60 (d, 4H, *H*ar, J_3 = 8.5 Hz), 7.81 (m, 2H, *H*ar), 7.83 (d, 4H, *H*ar, J_3 = 8.5 Hz), 7.94 (s, 2H, *H*thiaz), 7.97 (s, 1H, *H*ar), 8.24 (s, 2H, *CH*=N), 12.90 (br s, 2H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): 125.6, 127.2, 127.6, 127.9, 128.6, 130.3, 134.4, 136.4, 136.8, 145.2, 150.1, 173.2, 184.4; MS (EI) *m*/*z* (%): 604 (14, M^+), 367 (10), 238 (86), 139 (100), 111 (73), 75 (55); IR (KBr): 3451 (w), 3187 (w), 3059 (w), 2761 (w), 1614 (s), 1588 (s), 1568 (s), 1507 (s), 1485 (m), 1438 (m), 1397 (m), 1323 (s), 1278 (m), 1257 (m), 1200 (m), 1174 (m), 1100 (s), 1090 (s), 1013 (m), 933 (w), 906 (w), 879 (m), 842 (w), 792 (w), 750 (m), 687 (m), 628 (w), 596 (w). UV–vis (DMF): λ_{max} (10^{-3} ε) 395 nm (46 L mol^{-1} cm^{-1}), 505 (15).
39. *Spectroscopic data of complex* **CuL1**: Brown powder; yield: 95%; MS (MALDI) *m*/*z* (%): 666 (M+H$^+$); IR (KBr): 3446 (w), 3062 (w), 1588 (m), 1526 (s), 1496 (s), 1477 (s), 1398 (m), 1348 (m), 1288 (m), 1255 (s), 1199 (m), 1174 (m), 1088 (m), 1013 (m), 958 (w), 918 (w), 888 (m), 840 (w), 748 (m), 686 (m), 628 (w), 600 (w); UV–vis (DMF): λ_{max} (10^{-3} ε) 440 nm (33 L mol^{-1} cm^{-1}); EDXS: Cu:Cl and Cu:S ratios were 1:1.
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