A Rapid Microwave-Assisted Procedure for Easy Access to N_x Polydentate Ligands for Potential Application in α-RIT

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Received 5 February 2010

Abstract: Heterocycles bearing a hydrazine moiety react with bisaldehydes or bisketones to afford new N_x polydentate ligands suitable for α-radioimmunotherapy. We developed a fast and efficient method using microwave-assisted technology to obtain chelators with variable size and number of coordination centers which were fully characterized. The complexation efficiency with astatine will be assessed.

Key words: astatine, radioimmunotherapy, pyrimidinone, triazinone, chelating agent

Radioimmunotherapy (RIT) is a developing and promising technique for the treatment of small tumors.[1–3] It consists of injecting patients with a radiopharmaceutical able to target and selectively destroy tumor cells. The radionuclides usually used are α or β^- emitters with a short half-life, attached to a vector (antibody or hapten) through a bifunctional chelating agent.[4–6] In order to minimize irradiation of healthy tissue whilst delivering radionuclides to tumors, the metal complex between chelating agent and radionuclide has to be stable in human serum.

Among the potential radionuclides, ^{211}At has attracted interest as a prospective candidate for α-radioimmunotherapy applications due to its adequate physicochemical characteristics: α-emitter with a half-life of 7.2 hours, the emission of high energy α particles (6.8 MeV), and ability to deposit large amounts of energy in a microvolume.[7] Astatine is also an X-ray emitter allowing external imaging of the radiopharmaceutical distribution. Nevertheless, the chemistry of astatine remains in its infancy due to the absence of a stable isotope. The current approach for labeling biomolecules with ^{211}At rests on covalent attachment to a carbon atom.[8] Unfortunately, a significant number of studies has shown that astatinated radiopharmaceuticals can be stable to in vitro conditions, but generally more unstable in vivo.[9–11] Nevertheless, clinical studies are in progress in the US and in Sweden.[12,13] Although it is clear that much of the chemistry ascribed to halogens is applicable, the chemical similarity between astatine and its nearest halogen neighbor, iodine, is not always obvious. The general trend in the periodic system suggests that astatine is more metallic in character than iodine. Indeed, it has been reported that astatine presents a metal-like behavior when existing under the oxidation states +I and +III as At^+ and AtO^+ species.[14,15] Different groups have reported the formation of coordination complexes between AtO^+ and N^- or S^- containing organic chelating agents.[16–18] The stability of these complexes is, however, not sufficient to use this chelation approach in RIT.

These considerations brought us to develop new N_x polydentate ligands with variable size and number of coordination centers. At the same time as a fundamental, theoretical, and pragmatic study around element astatine, we led here a purely empirical approach. This paper reports the synthesis of chelating agents $\mathbf{L_{1–8}}$ containing heterocyclic rings (Figure 1). Our expertise in the synthesis of heterocycles led us to this choice of ligands. They present a semirigid scaffold due to partial conjugation between the linker and heterocycle. This characteristic is essential to preorganization, an important ligand proper-

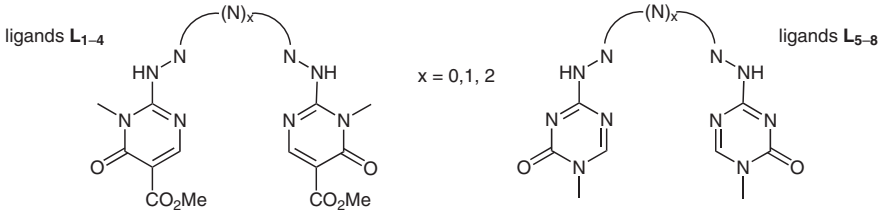
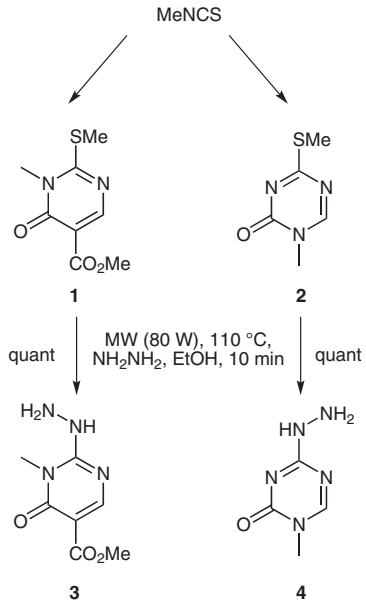


Figure 1 Polydentate chelators prepared in the study

SYNLETT 2010, No. 8, pp 1215–1218
Advanced online publication: 07.04.2010
DOI: 10.1055/s-0029-1219813; Art ID: G04610ST

ty.[19,20] The degree of freedom is minimized, so that the structure of the chelate before complexation is similar to that in the complex. Preorganization provides high thermodynamic stability as well as increased kinetic inertness of the metal complex.

Commercially available methylisothiocyanate was used as the starting reagent for the synthesis of chelating agents $\mathbf{L_{1-8}}$. The key step in this synthetic process was the preparation of the two corresponding hydrazino heterocyclic rings **3** and **4** (Scheme 1). The intermediary pyrimidinone **1** and triazinone **2** were obtained in high yields according to our previously described methodology.[21,22] Nucleophilic displacement of the methylsulfanyl group of **1** and **2** with hydrazine was carried out using ethanol as solvent to afford 2-hydrazonopyrimidine **3** and 2-hydrazonotriazine **4**.[23] The reaction was performed under microwave irradiation in order to minimize reaction time and secondary reactions. Indeed, with pyrimidinone **1**, at room temperature and after 20 hours, a byproduct (20%) resulting from double attack of hydrazine onto two heterocycles, was observed.



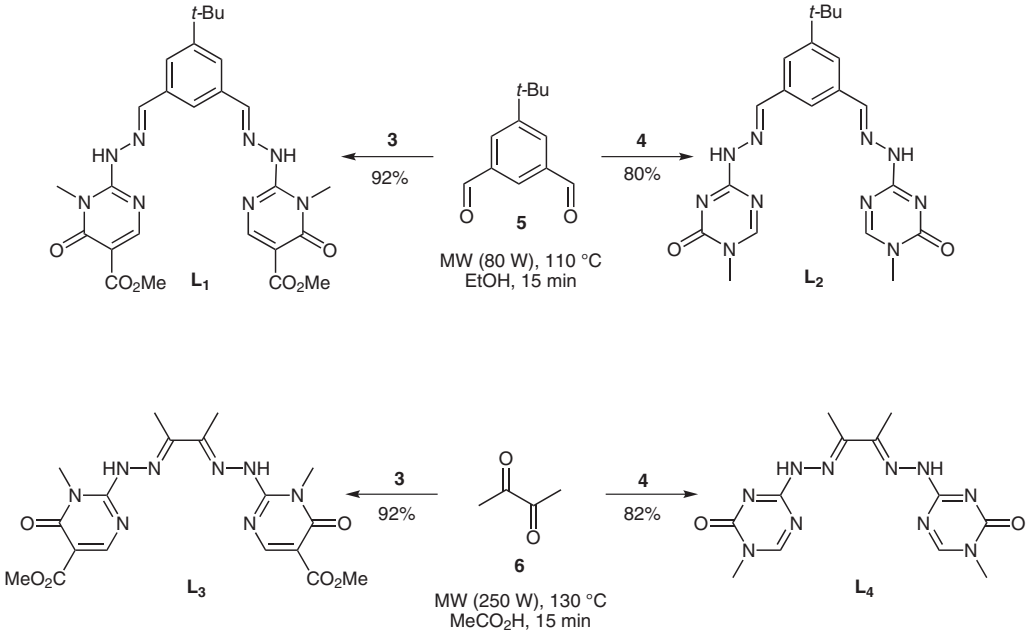
Scheme 1 Synthetic route to heterocyles **3** and **4**

Finally, the heterocyclic rings **3** and **4** were converted into the new polydentate ligands $\mathbf{L_{1-8}}$ with 4–6 coordination centers (N_4, N_5, and N_6) by a double imination reaction with dialdehydes **5**, **8** or diketones **6**, **7**.[24] These carbonyl compounds were commercially available except **5**. This latter compound was prepared by initial reduction (using BH_3·THF) of 5-*tert*-butylisophtalic acid to give the corresponding diol, which was subsequently oxidized (PCC/CH_2Cl_2) into the dialdehyde.[25]

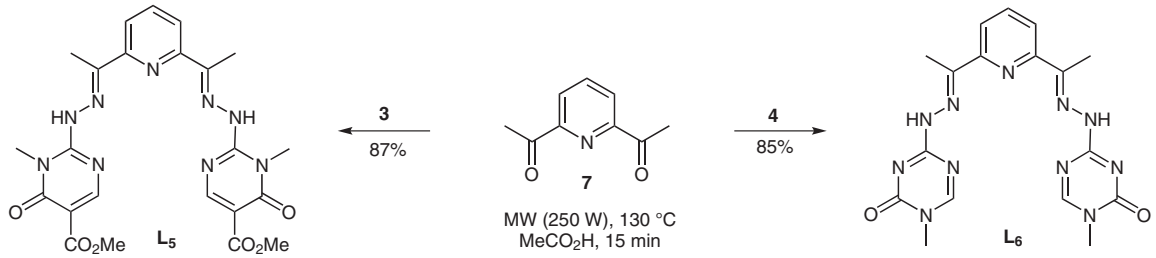
To prepare N_4-tetradentate ligands $\mathbf{L_{1-4}}$, two equivalents of heterocyclic ring **3** or **4** were reacted with 1,3-bis(formyl)-5-*tert*-butylbenzene (**5**) or butane-2,3-dione (**6**, Scheme 2). The syntheses were performed under microwave irradiation at 110 °C in ethanol for dialdehyde **5** and at 130 °C in acetic acid for diketone **6** and afforded the N_4 ligands in high yields.[24] Microwave irradiation significantly improved yields and condensation reaction kinetics compared to conventional thermal protocol.

The N_5-pentadentate ligands were prepared in a similar way. Reaction of heterocyclic ring **3** or **4** with diacetylpyrimidine **7** led to the formation of ligands $\mathbf{L_5}$ and $\mathbf{L_6}$ (Scheme 3). The reaction proceeded at 130 °C in acetic acid under microwave irradiation affording the ligands in high yields.

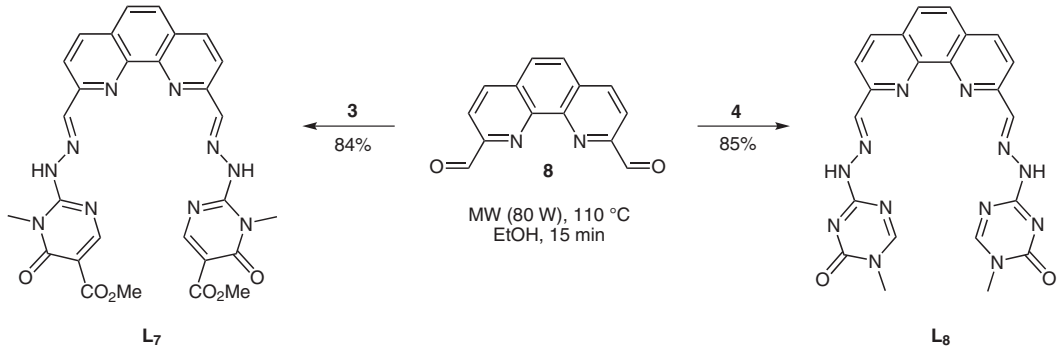
Finally, N_6-hexadentate ligands $\mathbf{L_7}$ and $\mathbf{L_8}$ were analogously prepared by condensation of 2,9-diformylphenanthroline (**8**) and pyrimidine **3** or triazine **4** (Scheme 4). These N_6 ligands posses the rigidity imposed by the cen-



Scheme 2 Tetradentate ligands $\mathbf{L_{1-4}}$ with pyrimidinone and triazinone scaffolds



Scheme 3 Pentadentate ligands $\mathbf{L_5}$ and $\mathbf{L_6}$ with pyrimidinone and triazinone scaffolds



Scheme 4 Hexadentate ligands $\mathbf{L_7}$ and $\mathbf{L_8}$ with pyrimidinone and triazinone scaffolds

tral phenantroline ring and the flexibility of the heterocyclic arms.

All new ligands $\mathbf{L_{1–8}}$ were isolated with a high degree of purity by simple filtration after microwave heating in glass vial. Their structure was determined unequivocally by complementary $^1H/^{13}C$-2D NMR techniques (COSY, HMQC, and HMBC).[26]

In conclusion, the synthesis of N_x-polydentate ligands (N_4, N_5, and N_6) was efficiently and simply performed by use of microwave-assisted technology. These chelators were prepared in 50–80% overall yield from methylisothiocyanate. The described way of synthesis will be easily adaptable to various starting heterocyclic structures. Study of the complexing ability of these new chelating agents toward astatine, suitable for α-radioimmunotherapy, is in progress. According to the results of this study we envision the synthesis of various analogues of ligands $\mathbf{L_{1–8}}$ in order to adjust their structure (size, nature, number of coordination centers, and heterocyclic ring) to finally find out the ligand which would be best suited for astatine.

Acknowledgment

The authors thank the French National Research Agency (ANR) (JCJC06_137852), the French Ministry of Education and CNRS for financial support.

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(24) **General Procedure for Ligands $L_{1–8}$**
To a 10 mL microwave reaction vessel was placed heterocyclic ring **3** or **4** (0.5 mmol) and dialdehyde **5** or **8** or diketone **6** or **7** (0.25 mmol) in MeOH for $L_{1,2,7,8}$ (4 mL) or in AcOH for $L_{3–6}$ (4 mL). The vial was heated (internal temperature measured by fibre optic) in a microwave synthesizer (MultiSYNTH®, Milestone S.r.l.) to 110 °C [2 min (110 W) ramp + 15 min irradiation (80 W)] for $L_{1,2,7,8}$ or to 130 °C [2 min (300 W) ramp + 15 min irradiation (250 W)] for $L_{3–6}$. The solvent was removed under vacuum, and the solid residue was washed with Et_2O. The product was filtered and dried under vacuum to afford ligands $L_{1–8}$.
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(26) Spectroscopic Data of Ligands $L_{1–8}$
L_1: mp 243–244 °C. IR (KBr): 3245, 1733, 1628, 1480, 1193, 1147 cm^{-1}. ^1H NMR (TFA): δ = 1.23 [s, 9 H, $C(CH_3)_3$], 3.61 (s, 6 H, NCH_3), 3.87 (s, 6 H, OCH_3), 7.78 (s, 2 H, H_{ar}), 8.02 (s, 1 H, H_{ar}), 8.52 (s, 2 H, CH), 8.59 (s, 2 H, CH_{pyr}). ^{13}C NMR (TFA): δ = 27.3, 28.4, 33.4, 52.1, 106.5, 122.4, 130.3, 145.6, 148.9, 153.8, 155.7, 156.4, 162.7. MS (MALDI): *m/z* = 551.42 $[M + H]^+$, 573.31 $[M + Na]^+$.
L_2: mp 245–247 °C. IR (KBr): 3302, 1674, 1493, 1429, 1303, 1193 cm^{-1}. ^1H NMR (TFA): δ = 1.37 [s, 9 H, $C(CH_3)_3$], 3.68 (s, 6 H, NCH_3), 8.12 (s, 2 H, H_{ar}), 8.30 (s, 1 H, H_{ar}), 8.50 (s, 2 H, CH), 8.52 (s, 2 H, CH_{tri}). ^{13}C NMR (TFA): δ = 28.5, 33.6, 33.7, 125.7, 130.1, 130.9, 146.3, 153.9, 154.4, 157.0, 161.3. MS ($CI^–$): *m/z* = 436.2 $[M]^+$.
L_3: mp 197–199 °C. IR (KBr): 3304, 1723, 1652, 1480, 1189, 1149 cm^{-1}. ^1H NMR (TFA): δ = 2.40 (s, 6 H, CH_3), 3.66 (s, 6 H, NCH_3), 3.87 (s, 6 H, OCH_3), 8.57 (s, 2 H, CH_{pyr}). ^{13}C NMR (TFA): δ = 10.7, 27.5, 52.3, 108.4, 145.0, 149.8, 155.1, 160.1, 162.3. MS (CI^+): *m/z* = 447.3 $[M + H]^+$.
L_4: mp >250 °C. IR (KBr): 3434, 3071, 1691, 1622, 1494, 1291 cm^{-1}. ^1H NMR (TFA): δ = 2.52 (s, 6 H, CH_3), 3.61 (s, 6 H, NCH_3), 8.53 (s, 2 H, CH_{tri}). ^{13}C NMR (TFA): δ = 10.7, 33.9, 146.4, 155.8, 161.0, 162.2; MS (CI^+): *m/z* = 333.0 $[M + H]^+$.
L_5: mp 247–249 °C. IR (KBr): 3216, 1734, 1627, 1481, 1189, 1148 cm^{-1}. ^1H NMR (TFA): δ = 2.73 (s, 6 H, CH_3), 3.75 (s, 6 H, NCH_3), 3.95 (s, 6 H, OCH_3), 8.45 (d, 2 H, *J* = 8.0 Hz, H_{ar}), 8.64 (s, 1 H, H_{pyr}), 8.81 (t, 1 H, *J* = 8.0 Hz, H_{ar}). ^{13}C NMR (TFA): δ = 12.8, 27.9, 52.2, 104.9, 126.4, 146.3, 146.5, 148.1, 150.1, 154.1, 157.7, 163.4. MS (MALDI): *m/z* = 524.52 $[M + H]^+$, 546.33 $[M + Na]^+$.
L_6: mp 211–213 °C. IR (KBr): 3220, 1634, 1489, 1303, 1187 cm^{-1}. ^1H NMR (TFA): δ = 2.74 (s, 6 H, CH_3), 3.66 (s, 6 H, NCH_3), 8.55 (d, 2 H, *J* = 8.0 Hz, H_{ar}), 8.65 (s, 1 H, H_{tri}), 8.85 (t, 1 H, *J* = 8.0 Hz, H_{ar}). ^{13}C NMR (TFA): δ = 12.4, 34.0, 126.9, 145.0, 146.1, 148.6, 150.9, 157.5, 162.9. MS ($CI^–$): *m/z* = 409.2 $[M]^+$.
L_7: mp >250 °C. IR (KBr): 1733, 1627, 1495, 1191, 1146 cm^{-1}. ^1H NMR (TFA): δ = 3.66 (s, 6 H, NCH_3), 3.86 (s, 6 H, OCH_3), 8.18 (s, 2 H, H_{ar}), 8.69 (s, 2 H, H_{pyr}), 8.72 (d, 2 H, *J* = 8.5 Hz, H_{ar}), 8.84 (d, 2 H, *J* = 8.5 Hz, H_{ar}), 9.15 (s, 2H, CH). ^{13}C NMR (TFA): δ = 28.7, 53.2, 107.8, 123.7, 129.0, 131.9, 137.5, 143.2, 146.4, 147.7, 150.0, 150.9, 151.0, 163.4. MS (MALDI): *m/z* = 597.41 $[M + H]^+$, 619.22 $[M + Na]^+$.
L_8: mp >250 °C. IR (KBr): 3212, 1623, 14932, 1306, 1186 cm^{-1}. ^1H NMR (TFA): δ = 3.69 (s, 6 H, NCH_3), 8.27 (s, 2 H, H_{ar}), 8.69 (s, 2 H, H_{ar}), 8.61 (s, 2 H, H_{tri}), 8.89 (d, 2 H, *J* = 8.6 Hz, H_{ar}), 9.06 (s, 2 H, CH), 9.29 (d, 2 H, *J* = 8.6 Hz, H_{ar}). ^{13}C NMR (TFA): δ = 33.9, 123.2, 128.0, 130.9, 136.3, 141.8, 147.1, 147.3, 149.7, 156.1, 162.7. MS ($CI^–$): *m/z* = 482.2 $[M]^+$.