Synthesis of multidentate ligands with amido or amino donor groups for the preparation of rhenium and technetium radiopharmaceuticals

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Abstract A new method to prepare novel semi-rigid multidentate ligands containing nitrogen atom, to coordinate with rhenium and technetium, was established. The method was based on formylation of substituted anilines, followed by Mannich reaction with glycine and paraformaldehyde. The method was very promising to design ligands of various molecular structures (L1–L5) to coordinate with rhenium metal ions. The complexes were prepared through ligand exchange with the complex ReOCl3(PPh3)2, giving new complex of the structure ReOCl3L (1–5). The prepared ligands and complexes were identified by the use of UV–vis, and infrared absorption spectrometric techniques, elemental analysis, molecular weight determination by depression of freezing point. These ligands were labeled with 99mTc pertechnetate, and the labeling efficiency of the complexes was measured using a well type scintillation gamma counter equipment and obtained a good yield.

Keywords Multidentate ligands · Rhenium and technetium complex ReOCl3(PPh3)2 · ReOCl3L · Labeled with 99mTc pertechnetate · Radioactive purity · Radiopharmaceuticals

Introduction

The coordination chemistry of technetium has rapidly developed, owing to its short half-life, pure photon emission, and suitable energy of 99mTc, make it the best choice for imaging studies [1–3]. The more recent introduction of β-emitting isotopes 188Re and 186Re in diagnostic imaging and radiotherapy boost the chemistry of rhenium as well [4–7]. A great number of chelate ligands for the encapsulation of rhenium and technetium have been prepared in the search of novel, selective, and effective agents for radiodiagnostic imaging and therapy.

Among the first of these ligands is that containing the peptide bonds of glycine and other amino acid derivatives in various molecular design, which were commonly used for imaging of the hepatobiliary system. There are three 99mTc-HIDA (2,6-dimethylphenylcarbamoylmethyliminodiacetic acid) analogues which have been approved for this purpose; 99mTc-Lidofenin, 99mTc-Mebrofenin, 99mTc-Disofenin, and N-(2-pyridylmethyl)iminodiacetic acid. The lipophilic properties of this compound were demonstrated in chloroform extraction studies where more than 80 % of the 99mTc-ligands were extracted into the organic phase from the aqueous phase. The exact nature of the complexes is uncertain but it was proposed to contain two ligands coordinated in an octahedral configuration and bear a single negative charge [8, 9]. Other type of ligands consists of small peptides of glycine and other amino acids, which have proved successful in sequestering these metals.

An example is diethylenetriaminepentaacetic acid (DTPA), mercaptoacetyl triglycerine (known as MAG-3 in the market) etc. The labeling of antibodies with 188Rh using MAG-3 as a bifunctional chelating agent has been optimized and automated [10–16]. Variety of monodentate ligands can be combined with tetradentate Schiff-base ligands to give mixed-ligand rhenium complexes, such as N2O2-calix[4]arene Rhenium Complexes [17].

The present work will focus on the development of a new and simple synthetic procedure of new amino acid
glycine) chelates combined with an aniline substituted moiety through carbamoyl group for labeling with rhenium and technetium metals. Briefly, this study related to their ability to coordinate to rhenium and technetium has shown their potential of using them as new imaging probes.

**Experimental**

**Chemicals and instruments**

Substituted anilines (p-aminobenzoic acid and 2-aminopyrimidine) were purchased from BDH; 4-chloro-2-nitroaniline from Merck) diphenyl amine, and phenylene diamine from Fluka. Formaldehyde and rhenium metal powder purchased from Aldrich, glycine from Riedel de Häen. Melting points were measured with electrothermal melting Point (BUCHI 535). UV–visible spectra were obtained with Shimadzu UV–Visible double beam scanning Spectrophotometer-260. Infrared spectrophotometric spectra were obtained Pye-Unicom-SP3-100 spectrophotometer with KBr disc. Perkin Elmer CHN Elemental Analyzer was used for elemental analyses. Radioactivities were measured by using a well type scintillation gamma counter equipment (berthold MAG 312 West–Germany).

**General formylation procedure**

A mixture of (0.030 mol) of substituted aniline and formic acid (10.0 mL) was refluxed for 8 h. Formic acid was removed by evaporation, and the residue was left over filter paper for 1 h. The residue was transferred a beaker of 100 mL, washed with 10.0 mL distilled water, and then left over watch glass to dry at room temperature.

**General Mannich reaction**

A mixture of a formyl derivative of substituted aniline (0.006 mol), paraformaldehyde (0.18 g, 0.006 mol), glycine (0.46 g, 0.006 mol), distilled water (10.0 mL) and 95 % ethanol (25.0 mL) in 100 ml r.b.f, was refluxed for 10 h. The mixture was left to cool, filtered, and then dried at room temperature overnight.

**Preparation of the complexes ReOCl₃L**

An amount of the complex ReOCl₃(PPh₃)₂ (0.20 g, 0.04 mmol) was placed in 100.0 mL r.b.f, and treated with a mixture of the ligand (0.40 mmol) and 95 % ethanol (2.0 mL). The mixture was refluxed for 90 min and color change was observed. The flask was cooled and the precipitate was filtered with filter paper and then, dried at room temperature overnight.

**Radiochemical purity**

For labeling, ligand solution (0.20 mg in 0.4 mL of saline solution) was mixed with freshly prepared solution of hydrated stannous chloride (containing 0.30 mg SnCl₂·2H₂O in 0.20 mL of 0.2 N HCl). The resulting mixture was labeled by adding a suitable volume 2.0–5.0 mL of ⁹⁹ᵐ⁹⁹ᵐTc-pertechnetate (0.5–10 mCi) eluate from ⁹⁹ᵐ⁹⁹ᵐMo to ⁹⁹ᵐ⁹⁹ᵐTc generator (CIS–biointernational, France). Radiochemical labeling analysis was performed by adding a suitable volume (0.10–0.30 mL) of the above labeled preparation on the top of a column (1 × 20 cm) packed with Sephadex-25-fine (Pharmacia, Sweden). The column was eluted with normal saline solution, and (3.0 mL) fractions were collected and the radioactivity of each fraction was counted with a well type scintillation counter to obtain the labeling efficiency of each ligand.

**Results and discussion**

The formyl derivatives and Mannich reaction substituted anilines were prepared following the general procedures mentioned in the experimental part. They were obtained in good purity and radiolabeling yields (~70 % in general). Their physical, UV–visible, and IR absorption spectroscopic properties of the formyl derivatives (I–V) and their Mannich reaction products with glycine (L1–L5) as well as the 1:1 coordination products ReOCl₃L (C1–C5), were presented in Tables 1, 2, and 3. The proposed chemical structure of the Mannich reaction products were presented in Fig. 1. These results were in good agreement with the proposed chemical structure of the products. All formyl derivatives showed two new absorption bands at 1,668–1,735 and at 2850–2750 cm⁻¹ in the FT-IR spectra corresponding the
attachment of formyl group on anilines. The first one was due to the C=O stretching, while the second one was due to the C–H aliphatic stretching. The second absorption band disappeared upon Mannich reaction substitution. $\lambda_{\text{max}}$ of the UV–visible absorption spectra of the substituted anilines used as starting materials showed clear shift to higher wave

| Chemical formula | M. wt (theor.) | m. p. (°C) | UV–visible | Color | Yield % |
|------------------|---------------|------------|------------|-------|---------|
|                  | (meas.)       |            | $\lambda_{\text{max}}$ (nm) | $\varepsilon$ (l mol$^{-1}$ cm$^{-1}$) |         |
| I C$_4$H$_7$NO$_3$ | 165           | 166.5      | 225–227 | 296   | 4,256   | Purple | 70 |
| II C$_7$H$_7$NO  | 197           | 196        | 70–73   | 246   | 9,700   | Gray   | 69 |
| III C$_7$H$_7$N$_2$O$_3$Cl | 200 | 198 | 142–145 | 247 | 12,750 | Yellow | 73 |
| IV C$_3$H$_5$N$_2$O$_3$ | 123 | 122 | 166–169 | 232 | 16,000 | Brown | 71 |
| V C$_8$H$_9$N$_2$O$_2$ | 164 | 163 | 170–174 | 208 | 11,400 | Purple | 76 |
| L1 C$_{11}$H$_{12}$N$_2$O$_5$ | 252.22 | 250.2 | 171–175 | 296 | 4,256 | Brown | 70 |
| L2 C$_{16}$H$_{16}$N$_2$O$_3$ | 284 | 287.5 | 67–70 | 246 | 9,700 | Brown | 69 |
| L3 C$_{10}$H$_{10}$ClN$_3$O$_5$ | 287.66 | 289 | 136–139 | 266 | 9,200 | Orange | 73 |
| L4 C$_8$H$_{10}$N$_2$O$_3$ | 210.19 | 212 | 151–153 | 297 | 2,950 | Brown | 71 |
| L5 C$_{14}$H$_{18}$N$_4$O$_6$ | 338.32 | 342 | 162–165 | 264 | 5,657 | Purple | 76 |

* By depression of freezing point

| Chemical formula | M. wt | Carbon | Hydrogen | Nitrogen |
|------------------|-------|--------|----------|----------|
|                  |       | Theo.  | Det.     | Theo.    | Det.    |
| I C$_4$H$_7$NO$_3$ | 165.15 | 58.18 | 58.00 | 5.62 | 4.10 | 8.48 | 8.50 |
| II C$_{11}$H$_7$NO | 197.23 | 79.16 | 57.20 | 5.62 | 5.50 | 7.10 | 7.20 |
| III C$_8$H$_7$ClN$_2$O$_3$ | 206.58 | 41.92 | 42.0 | 2.51 | 2.40 | 13.97 | 14.00 |
| IV C$_8$H$_7$N$_2$O$_3$ | 167.12 | 43.12 | 42.95 | 3.02 | 2.95 | 25.14 | 26.20 |
| V C$_8$H$_7$N$_2$O$_2$ | 164.16 | 58.53 | 58.40 | 4.91 | 4.90 | 17.06 | 17.10 |
| L1 C$_{11}$H$_{12}$N$_2$O$_5$ | 252.22 | 52.38 | 52.35 | 4.80 | 4.65 | 11.11 | 11.30 |
| L2 C$_{16}$H$_{16}$N$_2$O$_3$ | 284.31 | 67.59 | 67.62 | 5.67 | 5.50 | 9.85 | 10.20 |
| L3 C$_{10}$H$_{10}$ClN$_3$O$_5$ | 287.66 | 47.15 | 41.55 | 3.50 | 3.40 | 14.61 | 14.90 |
| L4 C$_8$H$_{10}$N$_2$O$_3$ | 210.19 | 45.71 | 45.55 | 4.80 | 4.75 | 26.66 | 26.60 |
| L5 C$_{14}$H$_{18}$N$_4$O$_6$ | 338.32 | 18.52 | 18.40 | 1.94 | 1.85 | 10.80 | 10.50 |
| C1 C$_{11}$H$_{12}$N$_2$O$_5$Cl$_3$Re | 592.87 | 25.62 | 25.60 | 2.90 | 2.85 | 8.66 | 8.70 |
| C2 C$_{16}$H$_{16}$N$_2$O$_3$Cl$_3$Re | 592.3 | 32.41 | 31.95 | 2.72 | 2.60 | 4.73 | 4.60 |
| C3 C$_{10}$H$_{10}$ClN$_3$O$_5$Cl$_3$Re | 594.9 | 20.14 | 31.88 | 1.69 | 1.65 | 7.05 | 6.98 |
| C4 C$_{14}$H$_{18}$N$_4$O$_6$Cl$_3$Re | 518.8 | 18.52 | 18.40 | 1.94 | 1.85 | 10.80 | 10.50 |
| C5 C$_{14}$H$_{18}$N$_4$O$_7$Cl$_3$Re | 646.9 | 25.99 | 25.85 | 2.80 | 2.90 | 8.66 | 8.70 |
Table 3  The IR absorption band of the formyl derivatives, Mannich reaction products with glycine, and the 1:1 coordination products ReOCl₃L

|        | O–H Stretch | N–H Stretch | N–H Stretch | C=O Stretch | C–N Aliphatic Stretch | C–H Aliphatic Stretch | C–H Aromatic O.O.P | N–H Bend | N–H O.O.P | Others N=O |
|--------|-------------|-------------|-------------|-------------|------------------------|-----------------------|-------------------|-----------|-----------|-----------|
| I      | 2,507       | 3,311       | 1,668       | 1,556       | 1,170                  | 2798                  | 665               | 1,490     | 806       | –         |
|        | 3,400       |             |             |             |                        |                       |                   |           |           |           |
| II     | –           | –           | 1,672       | 1,589       | 1,180                  | 2800                  | 692               | 1,490     | 842       | –         |
| III    | –           | 3,282       | 1,679       | 1,571       | 1,149                  | 2922                  | 646               | 1,502     | 788       | 1,342     |
| IV     | –           | 3,344       | 1,710       | 1,523       | 1,112                  | 2790                  | 640               | 1,454     | 804       | 1,502     |
| V      | –           | 3,398       | 1,735       | 1,569       | 1,132                  | 2781                  | 710               | 1,569     | 777       | –         |
| L1     | 2,500       | 3,309       | 1,697       | 1,562       | 1,172                  | –                     | 607               | 1,498     | 812       | –         |
|        | 3,400       |             |             |             |                        |                       |                   |           |           |           |
| L2     | 2,341       | 3,108       | 1,672       | 1,593       | 1,182                  | –                     | 611               | 1,492     | 844       | –         |
|        | 3,350       |             |             |             |                        |                       |                   |           |           |           |
| L3     | 2,611       | 3,182       | 1,679       | 1,573       | 1,151                  | –                     | 648               | 1,500     | 796       | 1,340     |
|        | 3,367       |             |             |             |                        |                       |                   |           |           |           |
| L4     | 2,374       | 3,560       | 1,695       | 1,525       | 1,118                  | –                     | 640               | 1,454     | 804       | –         |
|        | 3,442       |             |             |             |                        |                       |                   |           |           |           |
| L5     | 2,542       | 3,210       | 1,740       | 1,506       | 1,130                  | –                     | 702               | 1,506     | 746       | –         |
|        | 3,450       |             |             |             |                        |                       |                   |           |           |           |

Fig. 1  Chemical structure of the new ligands: (L1) N-Glycylacetyl p-aminobenzoic acid, (L2) N-Glycylacetyl diphenylamine, (L3) N-Glycylacetyl 4-chloro-2-nitroaniline, (L4) N-Glycylacetyl 2-pyrimidine, and (L5) Bis(N-Glycylacetyl) phenylene diamine

Fig. 2  The UV–visible spectrum of p-aminobenzoic acid (dashed line), N-formyl p-aminobenzoic acid (dot-dashed line), and N-Glycylacetyl p-aminobenzoic acid (continuous line)
Fig. 3 The UV–visible spectrum of diphenyl amine (dashed line), N-formyl diphenyl amine (dot-dashed line), and N-Glycylacetyl diphenylamine (continuous line).

Fig. 4 The UV–visible spectrum of phenylene diamine (dashed line), N,N’-diformyl phenylene diamine (dot-dashed line), and Bis-N,N’(Glycylacetyl) phenylene diamine (continuous line).

Fig. 5 Chromatography separation profile of the labeled ligands on a Sephadex G-25 column of $^{99m}$Tc pertechnetate labeling with (L1) N-Glycylacetyl p-aminobenzoic acid, (L2) with N-Glycylacetyl diphenylamine, (L3) N-Glycylacetyl 4-chloro-2-nitroaniline, (L4) with N-Glycylacetyl 2-pyrimidine, and (L5) with Bis(N-Glycylacetyl) phenylene diamine.
length upon substitution with the formyl group, and with methyl glycine after Mannich reaction. Generally, this shift is accompanied with increase in the value of $k_{\text{max}}$ of the products due to the hyperconjugation of the amine proton with benzene ring (Figs. 2, 3, 4). This new method will offer reliable procedure to design ligands of the following general structure.

\[
\begin{align*}
\text{HN} & \quad \text{C} & \quad \text{H}_2 & \quad \text{COOH} \\
\text{HN} & \quad \text{C} & \quad \text{NH} & \quad \text{CH} & \quad \text{R} \\
\text{O} & \quad \text{HN} & \quad \text{C} & \quad \text{H}_2 & \quad \text{COOH} \\
\end{align*}
\]

This structure will contain a lipophilic part of aromatic nucleus, and the hydrophilic part which can be any other amino acids. Rhenium complexes of these complexes were prepared by ligand substitution with the rhenium complex, oxotrichloro(triphenyl phosphine)rhenium(V) \([\text{ReOCl}_3\text{-(PPh}_3)_2]\) with 1:1 mol ratio of the metal:ligand. Chromatography profile of the labeled ligands on a Sephadex G-25 column shows that high percentage of the radioactivity was recovered in the void volume associated with the ligand fraction (Fig. 5). It gives good indication about the efficiency of labeling these ligands with Na$^{99m}$TcO$_4$. Future work will be directed towards the direct application of these ligands in radiopharmaceutical imaging.

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