# Microwave Assisted Facile One-Pot Synthesis of $^{188}$Re-Complex Using a Tetrahydroborate Exchange Resin. A Bifunctional Chelating Agent for Radiopharmaceuticals

Sang Hyun Park,* Hui Jeong Gwon, and Kyung Bae Park
*Division of Radioisotope Production and Application, HANARO Application Research, Korea Atomic Energy Research Institute, 150 Deokjin-dong, Yuseong-gu, Daejeon 305-353, Korea*

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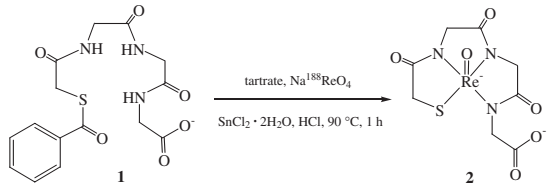
A facile one-pot synthesis of $^{188}$Re-complex as a bifunctional chelating agent for the preparation of therapeutic radiopharmaceuticals was accomplished with good labeling yields and radiochemical purity by using a tetrahydroborate exchange resin as a reducing agent for a disulfide ligand as well as the [$^{188}$Re]perrhenate ion under microwave irradiation.

Rhenium-188 ($^{188}$Re) is currently obtained from the parent nuclide tungsten-188 ($^{188}$W) through a generator system.[1] Due to its easy availability and suitable nuclear properties ($E_{\beta max} = 2.1$ MeV, $t_{1/2} = 16.9$ h), $^{188}$Re is one of the widely used radionuclides for therapeutic radiopharmaceuticals.[2–8] Furthermore, the associated $\gamma$-emission ($E_\gamma = 155$ keV) could be conveniently utilized for imaging and dosimetric purposes. The $^{188}$W/$^{188}$Re generator provides the Re-188 radionuclide in the form of tetraoxo anion [$^{188}ReO_4^-$]. The rhenium complex between the reduced $^{188}$Re, prepared by a stannous reduction of the perrhenate ion, and a ligand is a bifunctional chelating agent which can be conjugated to biologically active molecules.[9]

Guhlke and co-workers[10] reported that $^{188}$Re-MAG$_3$ (**2**) was prepared by adding sodium [$^{188}$Re]perrhenate into a vial containing Bz-MAG$_3$ (**1**), potassium sodium tartrate, $SnCl_2 \cdot 2H_2O$, and HCl (pH = about 3.8), and then by heating the reaction mixture at 90 °C for 1 h (Scheme 1).

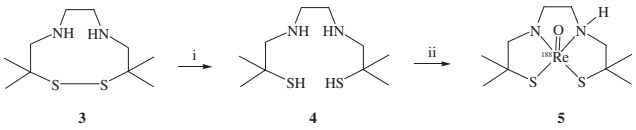



1 2

**Scheme 1.** Synthesis of $^{188}$Re-MAG$_3$ (**2**).

The method has the disadvantage of the protection of the thiol group and the deprotection of the S-protected precursor **1** prior to the reduction of [$^{188}$Re]perrhenate.

Recently, the preparation of a $^{188}$Re-labeled thiol compound has been developed via the reduction of a disulfide compound. Jeong and co-workers[11] reported that $^{188}$Re-complex **5** was prepared by adding [$^{188}$Re]perrhenate into a vial containing 2,9-dimethyl-4,7-diazadecane-2,9-dithiol (TDD, **4**), $SnCl_2 \cdot 2H_2O$ and tartaric acid, and then by heating the reaction mixture at 90 °C for 30 min (Scheme 2). However, the method involves the reduction of **3** and the separation of **4** prior to the $^{188}$Re labeling, resulting in laborious synthetic steps. Furthermore, it is difficult to isolate and store the deprotected ligand **4**. The time between deprotection and labeling with $^{188}$Re should be kept as short as possible to avoid a disulfide formation.




3 4 5

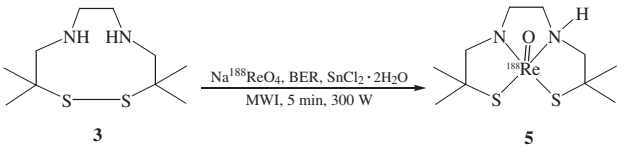
**Scheme 2.** Synthesis of $^{188}$Re-TDD (**5**). Reaction conditions: i) LAH/THF, ii) Na$^{188}ReO_4$, $SnCl_2 \cdot 2H_2O$, potassium sodium tartrate, 90 °C, 30 min.

In the previous preparations, heat treatment should be performed to reduce the perrhenate ion or to react the reduced rhenium with the ligand. Such a high temperature reaction may cause a deterioration of the antibodies and proteins bonded to the ligand and may cleave chemical bonds. Consequently, antibodies and proteins weak against heat cannot be used. In addition, difficulties are involved in controlling the conditions of a refluxing process for use in the treatment of radioactive materials at high temperatures. Therefore, there is a continuous need to develop a one-pot conversion that simultaneously carries out the reduction of both the disulfide compounds and sodium [$^{188}$Re]perrhenate under mild conditions.

In this paper, we report a facile synthesis of the $^{188}$Re-complex in which the deprotection of the ligand and labeling with $^{188}$Re occur in a one-pot one-step procedure under microwave irradiation (MWI).

Use of the tetrahydroborate exchange resin (BER) as a reducing agent results in a direct formation of the rhenium complex from a disulfide without the synthesis of a thiol-protected precursor. BER was prepared by the reported method.[12,13] Chloride-form resin (Amberlite® ion exchange resin, 12.5 g) was slurry-packed with water into a 30-mL fritted glass funnel mounted on a filter flask. Then, an aqueous sodium tetrahydroborate solution (200 mL, 0.25 M) was slowly passed through the resin over a period of 30 min. The resulting resins were washed thoroughly with distilled water until free of excess, and finally with ethanol. The tetrahydroborate-form anion exchange resin was then partially air-dried by removing ethanol on the surface of the BER. This resin was analyzed for its tetrahydroborate content by hydrogen evolution upon an acidification with 0.08 M HCl, and the average capacity of BER was found to be 2.5 m equiv of tetrahydroborate ion per gram.

Radiolabeling of TDD (**3**) with reduced $^{188}$Re, the formation of $^{188}$Re-TDD (**5**), was achieved by the new method as follows. To a vial containing 5 mg of BER, 0.1 mL of Na$^{188}ReO_4$ (185 MBq) and a solution of **3** (1 mg) and $SnCl_2 \cdot 2H_2O$ (0.1 mg) in 0.9 mL of physiological saline were added at a time. After irradiation of the mixture to 90 °C for 5 min at the power of 300 W in a microwave oven with temperature and pressure control (Milestones, Italy), it was cooled to room temperature. And it was then filtrated by a membrane filter (0.22 μm) (Scheme 3).




**Scheme 3.** Microwave assisted synthesis of $^{188}$Re-TDD (**5**).

Unreacted tetrahydroborate ion was removed by a simple membrane filtration, regardless of its used amount.

The key step in this new procedure involves the introduction of MWI and BER followed by the simultaneous reduction of the disulfide bond of **3** and [$^{188}$Re]perrhenate. In this procedure, use of microwave led to a huge reduction of the reaction time (5 min). The very rapid and direct energy absorption of reactants due to the characteristic heating properties of microwave favored reaction pathway over others and thus led to a high labeling efficiency and radiochemical purity.[14,15]

The assay for the formation and structure of the $^{188}$Re-complex, reduced hydrolyzed $^{188}$Re, and [$^{188}$Re]perhenate ion can be achieved by investigating their positions using an instant thin-layer chromatography (ITLC) with a gamma-ray scanner (RITA TLC Analysis, Raytest, Germany) and HPLC equipped with a gamma-ray detector (IsoScan LC gamma, Biostep, Germany). Table 1 shows the results of the thin-layer chromatography for **5** by performing ITLC on silica gel impregnated glass fiber sheets using distilled water as a developing solvent.

**Table 1.** ITLC Analysis of $^{99m}$Re-TDD (**5**)

| Chromatographic System | | $^{188}$Re Species at | |
|---|---|---|---|
| Support | Solvent | Origin | Solvent Front |
| ITLC-SG | Water | 100% of $^{188}$Re-TDD | 2% of $^{188}ReO_4^-$ |

As apparent in Table 1, showing the result of the ITLC-SG of **5** using distilled water as a development solvent, there was no observation of a peak of [$^{188}$Re]perhenate ion at the solvent front, which is expected to migrate with the solvent front. Radiochemical purity of **5** was determined using HPLC equipped with Jupiter 5$\mu$ C-18 300A column (250 × 4.6 mm, 5 micron, Phenomenex, USA), applying a gradient system with 0.05 *M* tetraethylammoniumphosphate (TEAP) buffer and 100% methanol at a flow rate of 1 mL/min. As shown in Figure 1, only one peak was seen at a retention time of 15.36 min due to the compound of interest, indicating a formation of **5** with more than a 98% radiochemical purity. These results indicate that **5** having more than a 98% labeling efficiency was formed. In order to estimate the stability of the radiolabeled compound **5**, it was stored in closed vials at room temperature and the labeling efficiency was determined at 1, 2, 4, 8, 12, 24 h, respectively. It was found to be over 96% till 24 h.

In conclusion, attempts to improve the overall efficiency of this process have focused on use of MWI and BER as a solid phase reducing agent. Contrary to the conventional usage of a long reflux under an acidic condition for the deprotection of the thiol groups of **1** and the reduction of [$^{188}$Re]perrhenate (Scheme 1) {or a laborious reduction of **3**, separation of **4** and

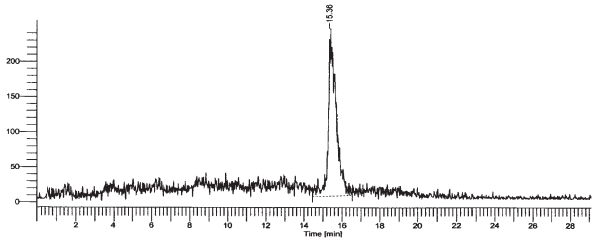



**Figure 1.** HPLC chromatogram of $^{188}$Re-complex **5**.

reflux for the reduction of [$^{188}$Re]perrhenate (Scheme 2)}, $^{188}$Re-complex **5** was prepared directly from a disulfide ligand **3** by employing a simple and convenient labeling condition (BER, 5 min-MWI, and a membrane filtration) (Scheme 3). This paper describes the first use of MWI and BER as a new reducing agent for the facile preparation of $^{188}$Re-complexes that are expected to have a therapeutic potential in nuclear medicine.

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