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The Foundations of the Development of Technologies of the Synthesis of Radiopharmaceuticals

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Abstract. The selection of precursors (for example chelating agents) and development of a technique of chemical modification of the target molecules retaining its ability to bind to specific receptors are very important in the synthesis of radiopharmaceuticals. As some important precursors for target radiopharmaceuticals omega-iodo-aliphatic carboxylic acids and their esters can be used. We have developed an environmentally safe process for producing omega-iodo-aliphatic carboxylic acids and their esters of the available, inexpensive and low toxic aliphatic cyclic ketones. We proposed a new method for the synthesis of the chelating agents omega-thia- or (bis(2-hydroxyethyl)amino)- aliphatic carboxylic acids (chelate 1 and chelate 2), which was caused by the existing disadvantages in the existing methods. Thus, based on our method the precursors (chelates) with yield of over 70–90% on the final stage were synthesized, and then the high effectiveness in producing target radiopharmaceuticals using different biomolecules was showed. ^{99m}Tc-chelates complexes were prepared with radiochemical purity >91% and found to be stable at room temperature for six hours.

INTRODUCTION

Radiopharmaceuticals are drugs containing a radionuclide, and they are used routinely in nuclear medicine for diagnosis or therapy of diseases [1]. Diagnostic radiopharmaceuticals are predominantly metal complexes with an organic chelator for metal-essential agents or a chelator-biomolecule conjugate for target-specific radiopharmaceuticals. Radiopharmaceuticals can be macromolecules such as monoclonal antibodies, antibody fragments, small peptides and hormones. This class is often named target-specific radiopharmaceuticals. Generally, a target-specific radiopharmaceutical is based on the receptor binding a radiolabeled receptor ligand in the diseased tissue [2–6]. In general, a target-specific radiopharmaceutical can be divided into four parts: targeting biomolecule (BM), pharmacokinetic modifying linker, bifunctional coupling or chelating agent (BFC), and radionuclide [7].

BFC is needed for radiolabeling biomolecules with a metallic radionuclide (for example, ^{99m}Tc and $^{188/186}$Re) [8]. BFC is covalently attached to the targeting molecule either directly or through a linker, and strongly coordinates to the radiometal. The choice of BFC is largely determined by the nature and oxidation state of the radiometal. Different radiometals require BFCs with different donor atoms and chelator frameworks. Therefore, it is important to understand the coordination chemistry of BFCs with any given radiometal to be labeled [9–13].

Nearly 80% of radiopharmaceuticals currently available in clinical nuclear medicine are ^{99m}Tc compounds due to ideal nuclear properties of ^{99m}Tc. The 6 h half-life is long enough to allow a radiopharmacist to carry out radiosynthesis and prepare the dose, and for nuclear medicine practitioners to collect clinically useful images. At the same time, it is short enough to permit administration of millicurie amounts of ^{99m}Tc radiopharmaceutical without causing a significant radiation dose to the patient. The monochromatic 140 KeV photons are readily collimated to

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give images of high spatial resolution. Furthermore, ^{99m}Tc is readily available from the ^{99}Mo-^{99m}Tc generators at low cost [14].

An ideal BFC is that which is able to form a stable ^{99m}Tc complex in high yield at very low concentration of the BFC-BM conjugate. To achieve this goal, the BFC must selectively stabilize an intermediate or lower oxidation state of Tc so that the ^{99m}Tc complex is not subject to redox reactions. Oxidation state changes are often accompanied by transchelation of ^{99m}Tc from a ^{99m}Tc-BFC-BM complex to the native chelating ligands in biological systems. The BFC should form a ^{99m}Tc complex which has thermodynamic stability and kinetic inertness with respect to dissociation or release of ^{99m}Tc. The BFC should form the ^{99m}Tc complex with a minimum number of isomers since different isomeric forms of the ^{99m}Tc-chelate may result in significantly different biological and pharmacokinetic characteristics of the ^{99m}Tc-BFC-BM complex. Finally, the conjugation group should be easily attached to the targeting biomolecule [7].

The particularly important tasks in radiochemical synthesis are the selection of a chelating agent and the development of a technique for chemical modification of the peptide retaining its ability to bind to specific receptors.

We proposed a new method for the synthesis of the chelating agents, which was caused by the existing disadvantages in the existing synthetic methods. We have developed an environmentally safe process to produce ω-iodohexanoic acid and its esters of the available, inexpensive and low toxic aliphatic cyclic ketones [13]. Methyl 6-iodohexanoate was an important precursor for the synthesis of the chelating agents.

In this work, we describe the processes for the synthesis of the chelating agents 6-thiahexanoic acid (Chelate 1) and methyl 6-(bis(2-hydroxyethyl)amino)hexanoate (Chelate 2) and demonstrate their high efficiency in the production of a conjugate with ^{99m}Tc.

EXPERIMENT

Reagents and Instrumentation

All commercial reagents were ACS reagent grade and were used without further purification. All other reagents and solvents were of commercial quality from freshly opened containers. NMR spectra were recorded with a Bruker AM400 and a Varian Unity Inova 300 MHz NMR spectrometer at 300 MHz (^{1}H NMR); chemical shifts are reported in parts per million (ppm).

Technetium eluate for research was obtained from technetium generators "^{99m}Tc-GT-TOM".

Radiochemical purity (RCP) and radiochemical yield (RCY) of the produced pharmaceuticals were determined by the method of thin layer chromatography (TLC), using plates PTLC silicagel-AF-A-UV (Sorbfil), the mm size 20×100. The analyzed sample with the volume of 5 μL was applied to the plate with a thin silica layer of PTLC silicagel-AF-A-UV (Sorbfil), size 20×100 mm within the start line. The dried plate was placed in two prepared chromatographic chambers with acetone (1) and mix $C_2H_5OH : 25\%NH_4OH : H_2O \div 2 : 5 : 5$ (2) for 10–40 min, depending on the used mix. With acetone, the free pertechnetate migrates with the solvent front, whereas colloidal ^{99m}Tc and ^{99m}Tc–Chelate both remain at the application point. With mix (2) as the mobile phase, colloidal ^{99m}Tc remains at the application point, whereas free pertechnetate and ^{99m}Tc–Chelate move with the solvent front.

To the mixture a chelates 100 μl of 1 mg/ml of solution (in water), 120 μl sodium citrate solution at a concentration of 100 mg/ml, 100 μl of a solution of $SnCl_2 \cdot 2H_2O$ at a concentration of 7 mg/ml and 1 ml of a ^{99m}Tc eluate with activity of 0.5–3.7 GBq were added in a 10 mL bottle without inserting an air needle. The solution is ready for use after mixing and incubation for 40 minutes at room temperature.

Synthesis of Methyl 6-Iodohexanoate

To a solution of cyclohexanone (0.294 g, 3 mmol) and iodine (0.381 g, 1.5 mmol) in MeOH (10 mL), copper(I) chloride (0.03 g, 0.3 mmol) was added, and then hydrogen peroxide solution (6 mmol, 0.638 g, 32% H_2O_2, d 1.125g/mL) in methanol (4 mL) was added dropwise during 2 h at stirring. After that the solution was stirred at room temperature for 10 h, and then hydrogen peroxide (3 mmol, 0.319 g) was added again dropwise during 6 h. An aqueous solution of Na2SO3 (1 mL) was added to the reaction mixture and the resulting solution was filtered. Dichloromethane (15 mL) was added to the filtrate and the organic layer was washed with saturated aqueous $NaHCO_3$ (15 mL), water, brine and dried (Na_2SO_4). The solvents were removed in vacuum to give methyl 6-iodohexanoate as a colourless oil (yield 0.599 g, 78%); Rf 0.82 (1:5 ethylacetate:hexane). ^{1}H NMR (300 MHz,

CDCl3): δ 3.67 (s, 3H, OCH3), 3.18 (t, 2H, 6-H), 2.32 (t, 2H, 2-H), 1.84 (m, 2H, 4-H), 1.62 (m, 2H, 3-H), 1.43 (m, 2H, 5-H) ppm.

Synthesis of Methyl 6-Thiahexanoate

To a solution of methyl 6-iodohexanoate (0.180 g, 0.7 mmol) in MeOH (1 mL) sodium hydrosulfide ($NaSH \cdot nH_2O$) (0.100 g) was added and the reaction mixture was stirred at room temperature for 30 min. Then 1 mL of dichloromethane was added, precipitate was removed by filtration. The solvent was evaporated under reduced pressure and the residue dried in vacuum to give methyl 6-thiahexanoate as an yellow solid: yield 0.111 g (98%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 3H, OCH_3), 2.67 (t, 2H, 6-H), 2.32 (t, 2H, 2-H), 1.58-1.74 (m, 4H, 3-H, 5-H), 1.39-1.46 (m, 2H, 4-H) ppm.

Synthesis of 6-Thiahexanoic Acid

The acid was prepared according to Minisci (1959) from 0.0656 g (0.27 mmol) of 6-iodohexanoic acid, 0.042 g (0.27 mmol) of sodium thiosulfate, 1.5 mL of water, and 1 M NaOH (sufficient to attain pH 7) under reflux for 1 h at 50°C. The mixture was then acidified to pH 1 with concentrated HCl and boiled for 2 h. After cooling, the reaction mixture was washed with dichloromethane (3×5 mL). The aqueous layer was evaporated under reduced pressure and the residue dried in vacuum, which on removal provided the acid as the white crystals: 0.036 g (0.24 mmol, 90%); TLC: R_f 0.48 (ethyl acetate-hexane, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69 (t, 2H, 6-H), 2.38 (t, 2H, 2-H), 1.63-1.81 (m, 4H, 3-H, 5-H), 1.43-1.51 (m, 2H, 4-H) ppm.

Synthesis of Methyl 6-(Bis(2-Hydroxyethyl)Amino)Hexanoate

To a solution of methyl 6-iodohexanoate (0.2136 g, 0.83 mmol) in isopropanol (2 mL), diethanolamine (0.149 g, 1 mmol) and triethylamine (0.14 mL, 1 mmol) were added. After stirring at 50°C for 48 h, isopropanol was evaporated and 3 mL of water was added. Then the reaction mixture was extracted with portions of dichloromethane (3×5 mL), organic extracts were combined and washed with water (5 mL), brine (5 mL), and dried with Na_2SO_4. The solvents were removed in vacuum and the residue was purified by column chromatography (hexane-EtOAc = 1:1 v/v). Methyl 6-(bis(2-hydroxyethyl)amino)hexanoate was isolated as an yellow oil (69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ3.72 (s, OH, CH_3), 3.63 (t, CH_2), 2.64 (t, CH_2), 2.53 (t, CH_2), 2.32 (т, CH_2), 1.48 (m, CH_2), 1.39 (m, CH_2), 1.26 (m, CH_2) ppm.

In Vitro Stability of ^{99m}Tc–Chelates

The stability of the freshly prepared ^{99m}Tc–Chelates was assessed by measuring the RCP through TCL at 1, 2, 3, 4, 5 and 6 h at room temperature (25°C) after preparation.

RESULTS AND DISCUSSION

We have developed a new method for the synthesis of effective chelating agents 6-thiahexanoic acid (Chelate 1) and methyl 6-(bis(2-hydroxyethyl)amino)hexanoate (Chelate 2). At present, various methods are known to be used for synthesis of the chelating agent which is used to obtain radioconjugates. However, the existing methods for producing such agents have drawbacks.

We have developed an optimized general procedure for the preparation of Chelate 1 and Chelate 2. Previously, we have reported an environmentally safe way to obtain ω-iodoalconoic acids and their esters starting from readily available, low-cost and low-toxic aliphatic cyclic ketones. This method is based on the Bayer-Villiger reaction (*Baeyer-Villiger oxidation*) and the methods proposed by Nikishin et al. [15].

Starting from cyclohexanone, we have developed a simple scheme for the synthesis of the chelating agents. The scheme of synthesis includes two steps (Fig. 1). In the first step, the intermediate substrates methyl 6-iodohexanoate and 6-iodohexanoic acid are prepared by oxidative cleavage of cyclohexanone.

In the second step, methyl 6-(bis(2-hydroxyethyl)amino)hexanoate is prepared from methyl 6-iodohexanoate via diethanolamine. It was found experimentally that in order to increase the yield of the desired product, synthesis must be carried out in the presence of triethylamine (for coupling with HI) by heating to 50°C for 24 hours in isopropyl alcohol.

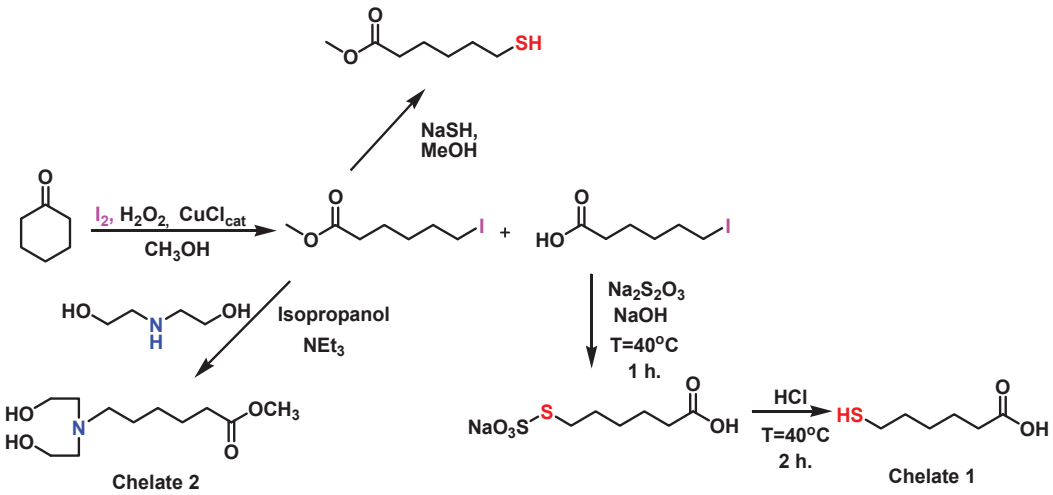

FIGURE 1. The scheme for synthesis of methyl 6-thiahexanoate, 6-thiahexanoic acid and methyl 6-(bis(2-hydroxyethyl)amino)hexanoate

In the second step, 6-thiahexanoic acid is prepared from 6-iodohexanoic acid via sodium thiosulfate.

Also, methyl 6-thiahexanoate is prepared from methyl 6-iodohexanoate via sodium hydrosulfide. However, the use of sodium hydrosulfide is accompanied by the preparation of a by-product—thioether. To suppress this process, the reaction was carried out in methyl alcohol for 30 min without heating. The selected conditions allowed us to significantly reduce the by-product content (up to 5–7%). The resulting ester looks like a light yellow crystal with a characteristic smell of mercaptans. The next aim was to prepare 6-thiahexanoic acid. For this purpose, we used methyl 6-thiahexanoate. Hydrolysis was carried out in a mixture of acetonitrile–water under the action of hydrochloric acid at room temperature. In this case, the oxidation of the ether was observed with the formation of a by-product (disulphide, up to 20%). If you use 6-iodohexanoic acid to produce 6-thiahexanoic acid via sodium hydrosulfide, it increases the formation of a by-product—thioether (up to 40–50%).

Because of this, we proposed to use 6-iodohexanoic acid and sodium thiosulfate to prepare 6-thiahexanoic acid, during the interaction of which S-alkylthiosulphates (Bunt salts) are formed. Then S-alkylthiosulphates can be hydrolyzed to 6-thiahexanoic acid. In this synthesis we used different reaction conditions (solvents, time, temperature and excesses of reagents) and developed the effective procedure. The resulting acid looks like a white crystal with a weak odor, turning yellow in the light.

The results of binding chelates with technetium-99m depending on the amount of relucting agent are showed in Table 1. As can be seen from the presented data, the largest radiochemical yield with a low impurity content of unreduced and hydrolyzed technetium-99m was observed in case of adding 100 µl solution of stannum chloride to 100 µl solution of chelates and 120 µl sodium citrate solution, it was more than 91.0%. Radiochemical yield was dropping as a large amount of stannum chloride was added due to the increase in formed hydrolyzed technetium-99m colloid. Thus the optimal conditions were proposed: 100 µl solution of chelates in concentration of 1.0 mg/ml (in water), 120 µl solution of sodium citrate in concentration of 100 mg/ml, 100 µl solution of $SnCl_2$ $2H_2O$ with concentration of 7 mg/ml and ^{99m}Tc with activity of 0.5–3.7 GBq.

The complexes ^{99m}Tc–Chelates prepared under optimal radiolabeling conditions were found to be stable at room temperature during 6 h after preparation. At 1 h, the RCP reached a maximum of 96.1%. Then, the RCP was found to be 91.1%, 91.3%, 91.4%, 91.0% and 90.7% at 2, 3, 4, 5 and 6 h post-labeling, respectively.

TABLE 1. Radiochemical yield of ^{99m}Tc–Chelate 1 and ^{99m}Tc–Chelat 2 complexes

Labeled compound	Radiochemical yield, %	Percentage of ^{99m}Tc–Sn colloid, %
^{99m}Tc–Chelate 1	94.0	2.0–3.0
^{99m}Tc–Chelate 2	92.0	1.0–3.0

The labeling efficiency of ^{99m}Tc–Chelates were high and its stability duration was long enough to allow further biodistribution and imaging studies.

Thus, based on our method we have developed the synthesis of the chelating agents with yield over 70% in the final stage and then have demonstrated high effectiveness in producing a conjugate chelating agents with ^{99m}Tc.

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