

A Novel and Efficient Method for the Technetium-99m Labelling of Disulfide Compounds Using a Tetrahydroborate Exchange Resin

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A novel and efficient method for the technetium-99m labelling of disulfide compound **7** was established with high radiochemical purity in which tetrahydroborate exchange resin (BER) simultaneously carries out the reduction of **7**, the reduction of [^{99m}Tc]pertechnetate and chelation of **7** with technetium-99m. The labelling occurs in a one-pot three-step procedure that is amenable to the preparation of ^{99m}Tc-radiopharmaceuticals.

Technetium-99m (^{99m}Tc) is the most widely used radionuclide for diagnostic radiophamarceuticals due to its great advantages in clinical use such as low cost, ready availability, short half-life (6.01 h), and a gamma energy (140 keV) appropriate for obtaining a gamma picture.[1,2] ^{99m}Tc generally forms a complex with compounds having nonbonding electrons such as isocyanates, amines, carboxyls, and thiols, and this complex is then used as a radiopharmaceutical agent for imaging various organs including the lung, liver, and brain, etc. Diamine dithiol has been synthesized and served as a bifunctional chelating agent for the preparation of ^{99m}Tc-radiopharmaceuticals. However, the method for the preparation of the ^{99m}Tc-labelled thiol compound is very limited since the thiol compound is easily oxidized into a disulfide form during the labelling reaction with ^{99m}Tc.[3] To avoid this, the ^{99m}Tc labelling of the thiol compound has been carried out simultaneously by chelating ^{99m}Tc and removing the protecting group of the *S*-protected precursor synthesized in advance. For example, the protection of the 2-aminoethanethiol derivative (**1**) was achieved by reacting it with *p*-methoxybenzyl chloride for 5 h at room temperature in the presence of a basic catalyst, and the deprotection of the *S*-protected precursor (**2**) was achieved by reacting it with mercury(II) acetate at 0 °C for 15 min and H_2S gas for 5 min before labelling ^{99m}Tc in order to form a ^{99m}Tc-labelled thiol compound (**3**) (Scheme 1).[4–6] The previous method has the disadvantage of protection of thiol group and deprotection of the *S*-protected precursor requiring laborious synthetic steps prior to reduction of [^{99m}Tc]pertechnetate. Jeong and coworkers have recently reported the prepartion of ^{99m}Tc-labelled thiol compounds via reduction of disulfide compounds.[7,8] The method also has the disadvantage of reduction of disulfide group and separation of the thiol precursor requiring laborious synthetic steps prior to labelling of ^{99m}Tc. Therefore, there has been a continuous need to develop a one-pot conversion that simultaneously carries out the reduction of disulfide compounds and the reduction of [^{99m}Tc]pertechnetate under mild conditions.

In this paper we report a novel, simple, and efficient method for the ^{99m}Tc labelling of disulfide compounds using tetrahydroborate exchange resin (BER), which can be employed to prepare various types of ^{99m}Tc-radiopharmaceuticals.

Results and Discussion

Preparation and Labelling Efficiency of ^{99m}Tc-Glucoheptonate.[9] ^{99m}Tc-glucoheptonate (^{99m}Tc-GHA, **4**) was prepared by the mixing of lyophilized glucoheptonate and tin(II) chloride with sodium [^{99m}Tc]pertechnetate in saline at room temperature for 20 seconds in order to perform the transchelation (Scheme 2). The assay for formation and structure of $Na^{99m}TcO_4$, $^{99m}TcO_2$, and ^{99m}Tc-GHA, radiolabelled compounds with $[^{99m}Tc^{v}{=}O]^{+3}$ species, was achieved by investigating their position using an instant thin-layer chromatography (ITLC). In this experiment, 98% of labelling efficiency of **4** was determined by performing ITLC. The labelling efficiency (LE) was calculated as follows, LE = 100 − $\%^{99m}TcO_4^-$ − $\%^{99m}TcO_2$. Table 1 shows the results of thin-layer chromatography for **4** by performing ITLC on silica gel impregnated glass fiber sheets using acetone and saline as developing solvents.

Preparation, Radiochemical Purity, and Labelling Efficiency of ^{99m}Tc-DADS by Transchelation. The strategy followed for the synthesis of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (diamine disulfide, DADS, **7**) is shown in Scheme 3. The IR and ^{1}H NMR spectral data of **7** support the structure of **7** as given in Scheme 3. ^{99m}Tc-GHA (**4**) was reacted with **7** in the presence of BER for transchelation in which the $[^{99m}Tc^{v}{=}O]^{+3}$ species in **4** was transferred to **7** resulting in the formation of ^{99m}Tc-DADS (**3**) (Scheme 4). Radiochemical purity (RCP) and labelling efficiency of **3** were determined by using ITLC and reverse-phase HPLC. ITLC was performed using acetone and a mixture of methanol and HCl (v/v ratio, 99.5:0.5) as developing solvents, and the results are given in Table 2. In addition, reversed-phase HPLC was carried out using a C-18 reverse-phase column as a stationary

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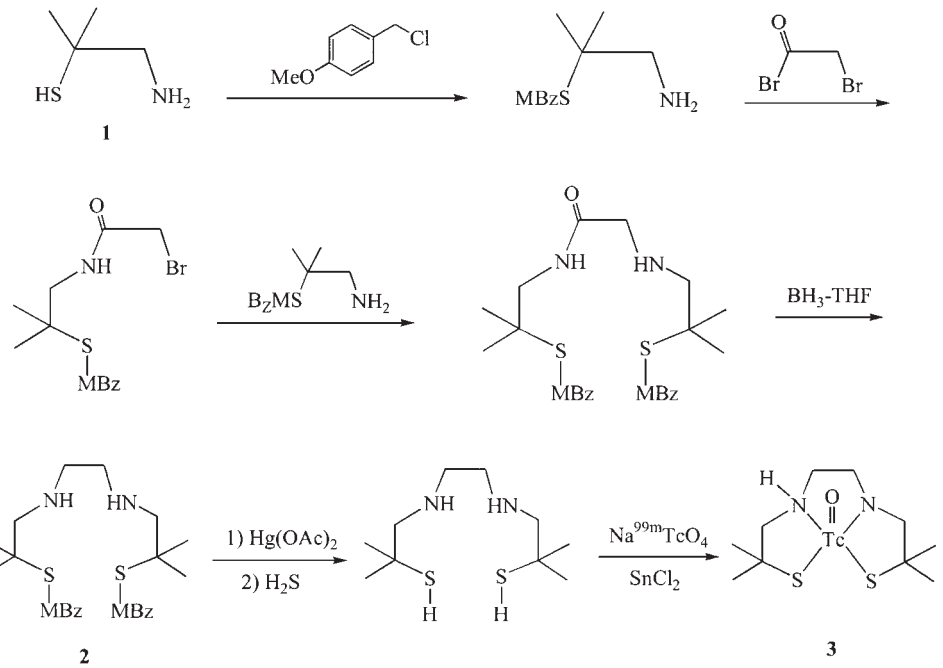
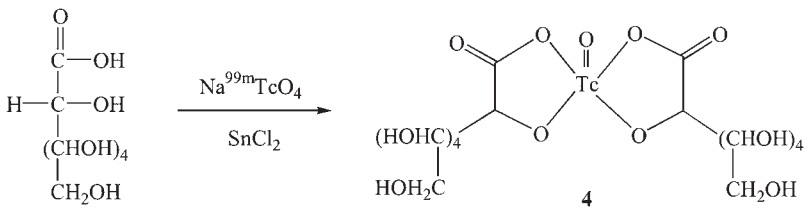


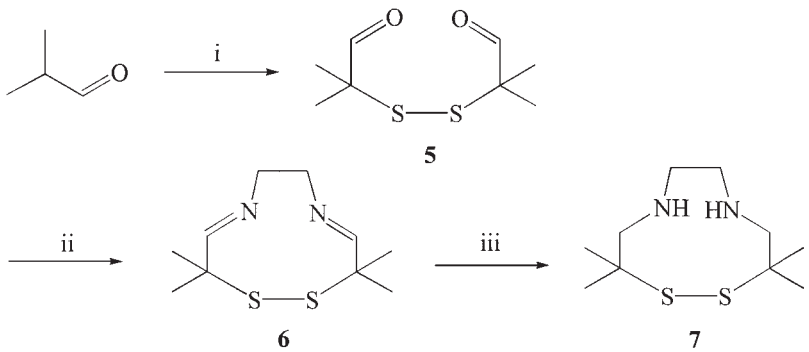
Scheme 1.



Scheme 2.

Table 1. ITLC Analysis of ^{99m}Tc-GHA

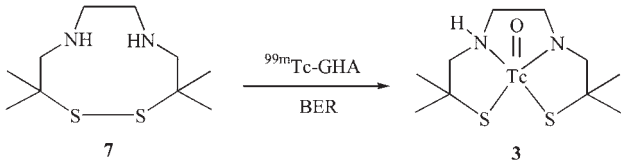
Chromatographic system		^{99m}Tc species at	
Support	Solvent	Orgin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA and $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	2% of $^{99m}TcO_2$	98% of ^{99m}Tc-GHA and $^{99m}TcO_4^-$



Scheme 3. Reagents and conditions: i. S_2Cl_2, CCl_4, 50–55 °C, 3 h; ii. $H_2NCH_2CH_2NH_2$, PTSA, toluene, 2 h, reflux; iii. $Na[BH_3CN]$, CH_3OH, 55–60 °C, 15 min.

Table 2. ITLC Analysis of ^{99m}Tc-DADS by Transchelation

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Orgin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA and $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Methanol and HCl (99.5:0.5, v/v)	4% of $^{99m}TcO_2$	96% of ^{99m}Tc-GHA and $^{99m}TcO_4^-$



Scheme 4.

phase and a mixture of tetraethylammonium phosphate buffer solution and methanol as a mobile phase, while maintaining a flow rate of 1 mL/min. The results are given in Fig. 1. As shown in Fig. 1, only one peak was observed with a retention time of 19.4 min, which is the retention time of the compound of interest, demonstrating the formation of **3** having over 99% of RCP. The RCP was determined from relative peak areas of HPLC chromatograms with monitoring at 254 nm.

Preparation, Radiochemical Purity, and Labelling Efficiency of ^{99m}Tc-DADS by Tetrahydroborate Exchange Resin. Tetrahydroborate exchange resin (BER) is comprised of a tetrahydroborate ion (BH_4^-) bound to a quaternary ammonium cation supported on a polymer. BER played an essential role in reducing the disulfide compound to a thiol compound, as well as the [$^{99m}Tc^{\text{Ⅶ}}$]pertechnetate to the [$^{99m}Tc^{\text{Ⅴ}}$=O]$^{+3}$ species with low oxidation state simultaneously affording a ^{99m}Tc-labelled compound directly from the disulfide compound. The tetrahydroborate exchange resin was used enough to reduce both the disulfide compound and pertechnetate.

DADS (**7**) was reacted with $Na^{99m}TcO_4$ in the presence of BER resulting in the formation of ^{99m}Tc-DADS (**3**) (Scheme 5). 99% of RCP and 97% of labelling efficiency of **3** were also determined by using ITLC and reverse-phase HPLC. ITLC was performed using acetone and a mixture of methanol and HCl (99.5:0.5, v/v) as developing solvents, with the results given in Table 3. In addition, reversed-phase HPLC was carried out using a C-18 reverse-phase column as the stationary phase, and a mixture of tetraethylammonium phosphate buffer solution and methanol as the mobile phase, while maintaining a flow rate of 1 mL/min. The results are as same as those of Fig. 1. Only one peak was observed with a retention time of 19.4 min, which is the retention time of the compound of interest, demonstrating the formation of **3** with a labelling efficiency of over 99%. In the case of the conventional method using tin(Ⅱ) chloride as a reducing agent, the formation of **3** was not observed (Scheme 5).

The key step in this new procedure involves the introduction of BER followed by the simultaneous reduction of the disulfide bond and pertechnetate. In this procedure, BER provides not only the reduction of ^{99m}Tc, but also the cleavage of the disulfide bond, providing mercapto groups for chelating with ^{99m}Tc resulting in high labelling efficiency and radiochemical purity. Generally, the reaction was completed within 30 min depending on the ligand and conditions used. Based on the preferred embodiment of the present study, we have recognized that the ^{99m}Tc-DADS (**3**) has more than 99% of RCP and 98% of labelling efficiency.

Conclusions

We have established a novel and efficient method in which BER simultaneously carries out the reduction of disulfide compounds and the reduction of pertechnetate under mild conditions, so that the labelling of technetium with sulfide com-

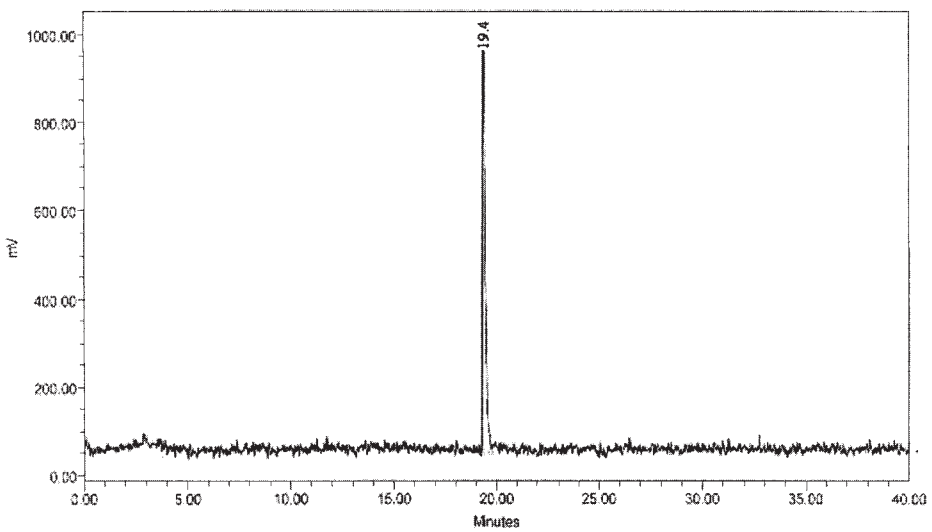
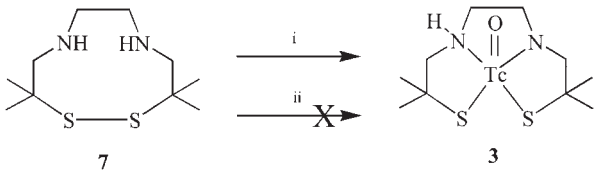


Fig. 1. Elution profile of ^{99m}Tc-DADS (**3**) by transchelation at 30 min post labelling from a C_{18} RP-HPLC column.

Table 3. ITLC Analysis of ^{99m}Tc-DADS by BER

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Orgin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA and $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Methanol and HCl (99.5:0.5, v/v)	3% of $^{99m}TcO_2$	97% of ^{99m}Tc-GHA and $^{99m}TcO_4^-$



7 3

Scheme 5. Reagents and conditions: i. $Na^{99m}TcO_4$, BER, r.t., 30 min; ii. $Na^{99m}TcO_4$, $SnCl_2$, r.t., 30 min.

pounds with high radiochemical purity and high yield can be carried out. This novel labelling method could be applicable not only to the ^{99m}Tc labelling, but also to the $^{186/188}$Re labelling. In this study, we first introduced BER as a new reducing agent and established a new labelling method for the facile preparation of ^{99m}Tc-complexes. This new labelling method, employing simple and mild labelling conditions, is useful for the preparation of ^{99m}Tc-radiopharmaceuticals by ^{99m}Tc labelling of biologically active molecules containing disulfide bonds. Therefore, we have developed a method in which the reduction of a disulfide compound, the reduction of $^{99m}TcO_4^-$, and labelling with ^{99m}Tc occur in a one-pot three-step procedure that is amenable to 'kit' formation. Currently, we are in the process of using this method for the preparation of ^{99m}Tc labelling of a disulfide compound (N_2S_2),[10] a potential probe for the β-amyloid protein of Alzheimer's disease, the results of which will be reported in due course.

Experimental

Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. $Na^{99m}TcO_4$ was obtained by the solvent extraction method from ^{99}Mo produced by a 30-MW multi-purpose research nuclear reactor (HANARO) in the Korea Atomic Energy Research Institute, Daejeon, Korea. Lyophilized glucoheptonate was obtained from the Korea Atomic Energy Research Institute, Daejeon, Korea. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck) and all chromatographic separations were monitored by TLC analyses, performed using glass plates precoated with 0.25-mm, 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm). IR spectra were recorded on a Bomen MB154 FTIR (KBr pellets). ^{1}H NMR was recorded on a Bruker 500-MHz FTNMR spectrometer in $CDCl_3$, and chemical shifts were recorded in ppm units using $SiMe_4$ as an internal standard. Labelling efficiency and radiochemical purity were determined by an instant thin-layer chromatography (ITLC) and reversed phase high performance liquid chromatography (RP-HPLC). The ITLC system consists of a ITLC scanner and 1-dimentional analysis of Berthold chroma program. The RP-HPLC system was equipped with a μBondapak C-18 column (3.9 × 300 mm, 10 μm, Waters, USA), ultraviolet detector and gamma-ray detector.

Preparation of Tetrahydroborate Exchange Resin (BER). The tetrahydroborate Exchange Resin (BER) was prepared by the reported method with a minor modification.[11] 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, aqueous sodium tetrahydroborate solution (200 mL, 0.25 M) was slowly passed through the resin over a period of 30 minutes. The resulting resins were washed thoroughly with distilled water until free of excess, and finally with ethanol (10 mL × 3). The tetrahydroborate form anion exchange resin (BER) was then partially air-dried by removing the ethanol on the surface of BER. This resin was analyzed for tetrahydroborate content by hydrogen evolution upon acidification with 0.08 M HCl, and the average capacity of BER was found to be 2.5 meq of tetrahydroborate ion per gram. BER was stored under nitrogen at 4 °C. The hydride content was constant over 5 weeks.

Preparation of 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodecane (7, diamine disulfide; DADS). 3,3,10,10-Tetramethyl-1,2-dithia-5,8-diazacyclodecane (**7**) was prepared by the reported method with minor modifications.[12]

Synthesis of 2,2′-dimethyl-2,2′-dithiadipropanal (5): A solution of isobutyraldehyde (353 g, 4.89 mol) in carbon tetrachloride (350 mL) was heated to 50 °C and disulfur dichloride was added dropwise under nitrogen atmosphere in order to keep the evolution of hydrogen chloride under control. Upon completing the addition, the solvent was removed by distillation under suction at 60–80 °C. Vacuum distillation gave a 56% yield of the title compound. ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 12H, 4 CH_3), 9.1 (s, 2H, 2 CHO).

Synthesis of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-4,8-diene (6): Ethylenediamine (3.6 g, 60 mmol) was added to a solution of **5** (10.3 g, 50 mmol) in ethanol (70 mL), and the reaction mixture was heated to reflux for 1 h. The precipitate formed at room temperature and was allowed to stand at 5 °C overnight and was then filtered and recrystallized from ethyl acetate in order to give 90% yield of the title compound: mp 163–164 °C; IR (cm^{-1}, KBr pellet) 3420, 2949, 2842, 1672, 1448, 1373; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H, 2 CH_3), 1.46 (s, 6H, 2 CH_3), 3.26 (d, J = 6.39 Hz, 2H, 2 NCH), 4.16 (d, J = 6.3 Hz, 2H, 2 NCH), 6.88 (s, 2H, 2 N=CH).

Synthesis of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (7): To a solution of **6** in ethanol (120 mL) was added sodium tetrahydroborate (6.1 g, 160 mmol). The reaction mixture was heated to reflux for 1 h and allowed to stand at room temperature for 18 h. The solution was evaporated to dryness and the residue was extracted with dichloromethane. After washing with water (50 mL) twice, the organic layer was dried over anhydrous magnesium sulfate and evaporated. Recrystallization from ethanol gave 82% of the title compound (7.7 g): mp 57–59 °C; IR (cm^{-1}, KBr pellet) 3450, 2964, 2964, 2735, 2734, 2456, 1560, 1468, 1388; ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 6H, 2 CH_3), 1.29 (s, 6H, 2 CH_3), 1.75 (bs, 2H, 2 NH), 2.4–3.0 (m, 8H, 4 NCH_2).

Preparation of ^{99m}Tc-Glucoheptonate (4). $Na^{99m}TcO_4$ in saline (0.5 mL, 25 mCi) was added to a vial containing lyophilized glucoheptonate and tin(II) chloride. The reaction mixture was stirred for about 20 s at room temperature under nitrogen atmosphere, and the reaction was carried out until the glucoheptonate powder was completely dissolved. It was filtered through a membrane filter having a pore size of 0.22 μm and gave a 98% labelling efficiency of the title compound.

Preparation of ^{99m}Tc-DADS (3) by Transchelation. To a vacuum vial containing 5.0 mg of BER were simultaneously added prepared ^{99m}Tc-glucoheptonate and a solution of **7** (1 mg, diamine disulfide; DADS) in 0.1 mL of distilled water. The reaction mixture was stirred for about 20 min at room temperature under nitrogen atmosphere. It was filtered through a membrane filter having a pore size of 0.22 μm and gave a 96% labelling efficiency of the title compound as a technetium-labelled compound of interest.

Preparation of ^{99m}Tc-DADS (3) by Using BER. To a vial containing tetrahydroborate exchange resin (5 mg) were injected simultaneously a solution of **7** (0.1 mg, diamine disulfide; DADS) in distilled water (0.9 mL) and an aqueous solution of sodium pertechnetate, $[Na^+][^{99m}TcO_4^-]$ (0.1 mL, 5 mCi). After stirring for 30 min, 97% of labelling efficiency of the title compound was obtained by filtering through a membrane filter having a pore size of 0.22 μm.

Attempted Preparation of ^{99m}Tc-DADS (3) by the Conventional Method. An aqueous solution of sodium pertechnetate, $[Na^+][^{99m}TcO_4^-]$ (0.1 mL, 5 mCi) was injected in to a vial containing a solution of **7** (1 mg) in distilled water (0.8 mL) and tin(II) chloride dihydrate (0.5 mg) in 0.005 M HCl (0.1 mL). After stirring for 30 min, no technetium labeled complex, [^{99m}Tc-S], was obtained. The mixture was then heated in boiling water for 15 min and cooled to room temperature. After cooling to room temperature, no technetium labeled complex, [^{99m}Tc-S], was obtained.

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