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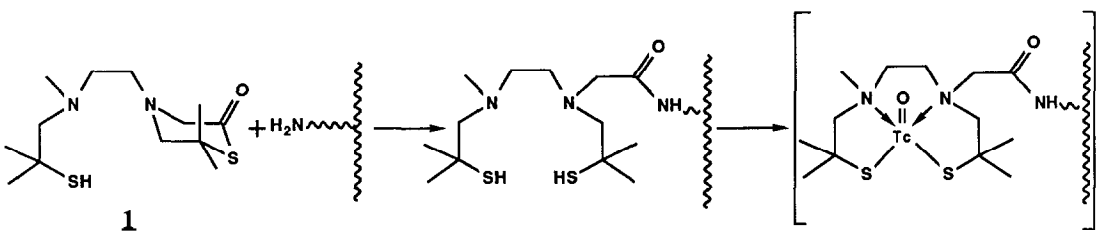
SYNTHESIS OF A NOVEL BIFUNCTIONAL CHELATE DESIGNED FOR LABELING PROTEINS WITH TECHNETIUM-99m.[1]

Susan Z. Lever*, Kwamena E. Baidoo,
Alfred V. Kramer, and H. Donald Burns
Department of Environmental Health Sciences
The Johns Hopkins University, Baltimore, MD 21205, USA

Summary. A novel bifunctional chelate, **1**, was designed and synthesized for the facile and efficient incorporation of technetium-99m into biomolecules under mild conditions. The agent features a thiolactone moiety which upon direct protein coupling *via* amine residues, liberates the diaminedithiol ligand system for chelation with technetium-99m.

The bifunctional chelate approach has proved to be of value for incorporation of metals into proteins.[2-4] When an appropriate ligand is attached *via* a covalent bond to the protein, specific coordination of the metal to the ligand preferentially occurs, yielding a labeled product which generally exhibits enhanced *in vivo* stability.[5-7] The success of this method for a particular purpose relies upon two principal factors. First, the bifunctional chelate must covalently bind to the protein under conditions which do not adversely affect the protein. In addition, it must possess a high affinity for the metal, so that the metal binds specifically to the chelate. In recent years, there has been much interest in the use of the bifunctional chelate approach for the radiolabeling of proteins with technetium-99m in the field of diagnostic Nuclear Medicine.[8] We present the synthesis of a new bifunctional chelate, **1**, designed to couple to free amino groups of proteins and peptides under mild conditions, and permit subsequent specific labeling with technetium-99m (Scheme 1).



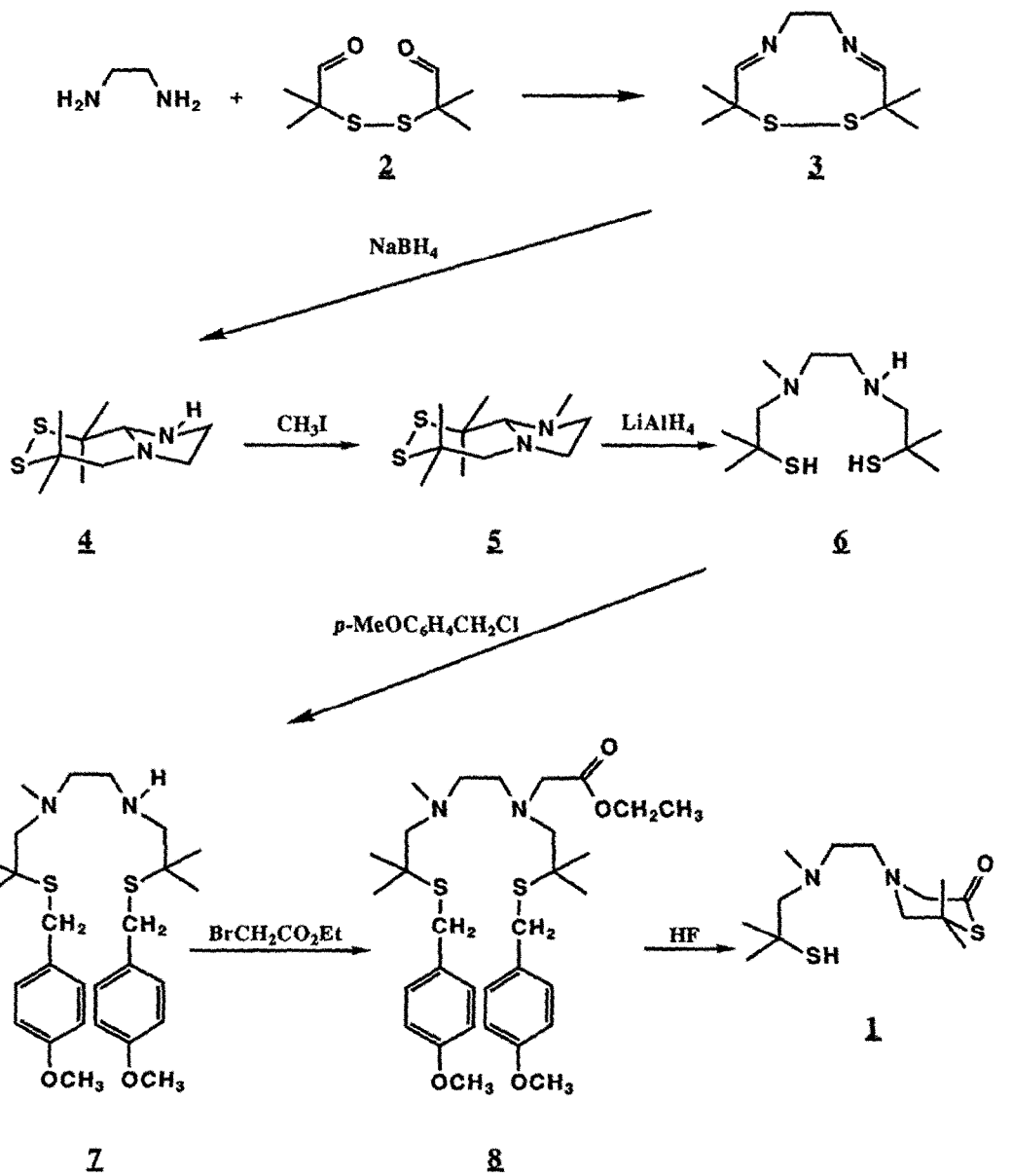
1

Scheme 1.

The six-membered thiolactone portion of the bifunctional chelating agent constitutes a functionality which provides a means for attachment of the agent to proteins *via* amide bond formation without prior activation. There is ample literature precedent for the reactive nature of thioesters and thiolactones with amines,[9-12] and reaction of the thiolactone in this manner releases the previously masked thiol group which completes the tetradentate chelate needed for strong coordination to technetium. The diaminedithiol ligand has been previously shown to form very stable complexes with technetium.[13,14] In order to prevent self-condensation from intra- or intermolecular amide formation, our synthetic strategy included protection of the secondary amine. A simple alkyl substituent was chosen in order to maintain the maximum coordinating ability of the amine.

The synthesis of **1** was accomplished in seven steps in 14% overall yield as shown in Scheme 2.[15] The ligand backbone was constructed as described in the literature through di-imine formation[16] between ethylenediamine and 2,2'-dithiobis(2-methylpropanal) (**2**)[17] to yield **3**. Sodium borohydride reduction, while leaving the disulfide bond intact, provides bicyclic amine **4** in 80% yield *via* intramolecular cyclization of the intermediate mono-imine.[18] This cyclization is advantageous for our synthesis because it permits the differentiation of the two nitrogens for subsequent synthetic elaboration. The bicyclic amine is then alkylated with methyl iodide in the presence of KF/celite[19] which gives 93% conversion to **5**. Reduction of this intermediate with lithium aluminum hydride in refluxing tetrahydrofuran cleaves not only the disulfide bond, but also the transannular C-N bond, affording the open-chained ligand **6** (74% yield, isolated as the di-hydrochloride salt). The thiols are protected as the acid-labile *p*-methoxybenzylthioethers by reaction of dithiol **6** with *p*-methoxybenzylchloride in an aqueous ethanolic solution of sodium hydroxide to give **7** in 76% yield. Incorporation of the two-carbon side-chain which becomes part of the thiolactone is accomplished through the alkylation of the free base with ethyl bromoacetate, to afford **8**, the requisite precursor to the bifunctional chelate, in 55% yield.

The key transformation of precursor **8** to the bifunctional chelating agent is accomplished through hydrolysis with hydrogen fluoride.[20,21] Anhydrous HF is condensed into a Teflon container containing **8** and anisole, present as a free-radical scavenger. After stirring for 1.5 hours in an ice bath, the mixture is allowed to warm to room temperature so that the volatile HF may be flushed carefully into a trap containing excess aqueous potassium hydroxide. Extractive work-up of the residue and short path chromatography on silica provided the thiolactone **1** directly in 70% yield. The isolated material exhibited spectral and analytical data in full agreement with the assigned structure. Infrared spectroscopy showed the carbonyl stretching frequency at 1655 cm^{-1}, which is similar to that of six-membered thiolactones reported in the literature,[22] and did not show evidence of polymerization. Based upon nuclear magnetic resonance (^{1}H, ^{13}C) studies and the precise elemental analysis obtained for **1**, the isolated material is not contaminated with minor products resulting from incomplete hydrolysis of the protecting groups or from hydrolysis of the thioester to the thiol-acid. Although the physical data obtained does not rigorously exclude the presence of nine- or twelve-membered (from



Scheme 2.

dimerization) thiolactones, their formation is highly unlikely based upon literature analogy[23] regarding preparation of medium-sized lactones.

Preliminary evaluation of this bifunctional chelate indicates the thiolactone moiety reacts readily with amine-containing small molecules (*e.g.* benzylamine), proteins (HSA), and antibodies (B72.3). Subsequent incorporation of technetium into the chelate occurs under mild conditions and the resultant labeled products are stable *in vitro* and *in vivo*. The use of this bifunctional chelate as a means for introducing the diaminedithiol ligand system for chelation with other metals such as rhenium is also under investigation.

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