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SYNTHESIS OF A NOVEL DIAMINODITHIOL LIGAND FOR LABELING PROTEINS AND SMALL MOLECULES WITH TECHNETIUM-99M

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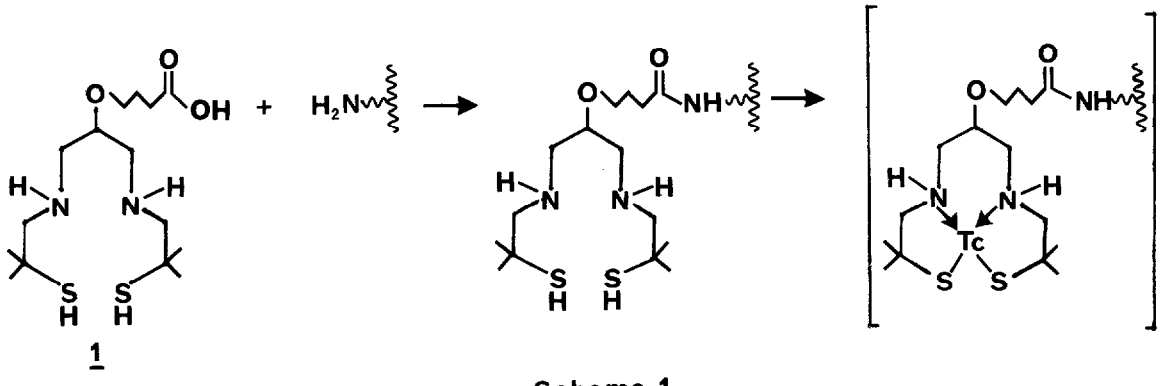
Summary: A novel bifunctional chelate, 1, was designed and synthesized for labeling of proteins and small molecules with technetium-99m. The new chelate contains a free carboxyl group which is available for covalent attachment to free amino groups on biologically-important molecules. The resulting chelator may bind reduced technetium-99m such that the label is stable in vitro and in vivo.

The labeling of biologically important molecules by means of bifunctional chelating agents has become routine since its introduction by Sundberg et al.[1] The success of this technique relies upon a bifunctional chelating agent which possess both a powerful metal chelating group and a second functional group that covalently binds to protein or other molecules of interest without adversely altering their properties. Radioactive metal ions attached by chelation to proteins such as monoclonal antibodies have been used clinically for diagnosis of cancer.[2,3] Recent applications of bifunctional chelating agents in the medical sciences have been described.[4,5] As is described therein several diaminodithiol ligands have been used as chelators to carry technetium-99m across the normal blood-brain barrier to image normal brain.[6-8] In the present report we describe the synthesis of a new diaminodithiol ligand, 1, design to couple with free amino groups of proteins and small molecules and to permit subsequent specific binding with technetium-99m as shown in Scheme 1.



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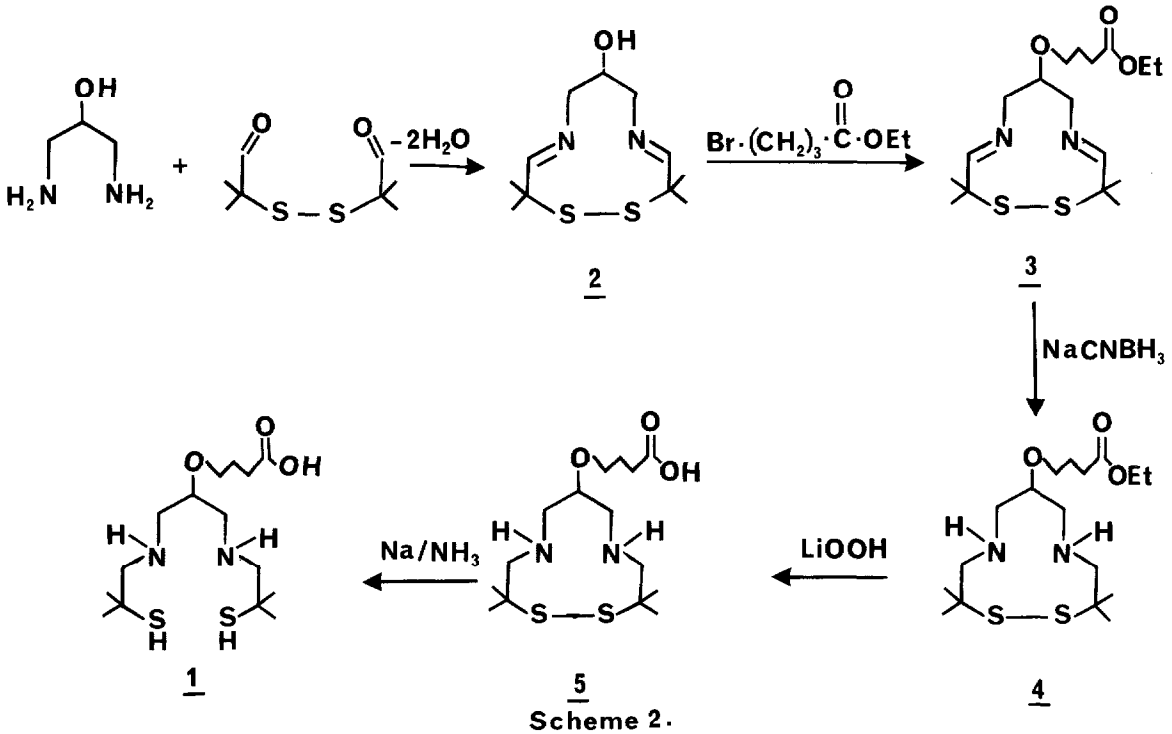
Scheme 1.

This laboratory earlier designed a novel tetradentate (2-hydroxy-N,N'-bis(2-methyl-2-mercaptopropyl)propylenediamine (DADT-3C) in which the usual ethylene bridge of diaminodithiol ligand was replaced with a propylene backbone between the two amino groups.[9] Radiolabeling studies of the free chelator demonstrated its favourable properties in forming stable chelates with technetium-99m which avoid the nonspecific binding of the label to other chelators in solution.[9] As such, DADT-3C has been considered as an attractive alternative chelator for small molecules but especially for proteins such as anti-tumor monoclonal antibodies to be used in the detection of cancer. With a view to coupling the DADT-3C chelator to proteins, a novel chelator [DADT-3C-$(CH_2)_3$-COOH] has been synthesized in which a carboxyl group is attached to carbon-2 via a spacer (to reduce steric hindrance) such that the free carboxyl group will be used to couple the chelator to biological molecules of interest.

The synthesis of ligand 1 was achieved in five steps as shown in Scheme 2. The ligand backbone was constructed as described in the literature,[7,8] through diimine formation by condensation of 1,3-diamino-2-propanol and 2,2'-dithio-bis(2-methylpropanal)[10] in anhydrous benzene. The cyclized product 2 was isolated as a colorless crystalline product in 87% yield, m.p. (C_2H_5OH) 86-87°C, ^{1}H-NMR (300 MHz; $CDCl_3$) δ:7.26 (s, N=CH, 1H), 4.19 (m, 2H), 3.87 (d, 1H), 3.55 (dd, 1H), 3.30 (dd, 1H), 3.17 (dd, 1H), 2.5 (br, OH, exchangeable with D_2O), 1.5 (s, 6H, $2CH_3$), 1.33 (s, 6H, $2CH_3$); ^{13}C-NMR (75.5 MHz; $CDCl_3$) δ:170.66 (C=NH), 75.01 (CHOH), 63.02 and 50.45 (2 CH_2), 28.78, 24.93, 23.90, and 22.48 (4 CH_3); MS (EI) m/e 260.10 (1.5%), 186.15 (9%), 171.15, (5.2%), 154.2, (13.7); Anal. Calc. for $C_{11}H_{20}N_2OS_2$: C (50.62), H (7.64), N (10.55), found: C (50.76), H (7.74), N (10.76). Alkylation of the hydroxyl group of compound 2 was carried out by the procedure of Jones et al.[11] by reacting equimolar amount of 2 and ethyl 4-bromobutyrate in anhydrous DMF under N_2 in presence of K_2CO_3 at 65°C for 60 hrs. The ester 3 was isolated in 82% yield (m.p. 52-53°C) after purification by silica gel open column chromatography. ^{1}H-NMR (300 MHz; $CDCl_3$) δ: 7.28 (s, N=CH, 1H), 5.16 (s, 1H), 4.04 (m, 8H, $4CH_2$), 3.50 (m, 1H, CHOH), 2.39 (t, 2H, CH_2), 1.98 (m, 2H, CH_2), 1.41 (s, 6H, CH_3), 1.30 (m, 9H, $3CH_3$); MS (EI) m/e 374.10 (0.2% M^+), 375.2 (0.1% M^++1), 344.15 (23.6%), 312.20 (22.9%), 287.05 (13.2%), 141.10 (49.0%), 115.15 (100%). Compound 3 was selectively reduced with sodium cyanoborohydride in acetic acid at 15°C leaving the disulfide bond intact. After column chromatography over a silica gel column compound 4 was isolated in 89% yield and was characterized by ^{1}H-NMR (200 MHz; $CDCl_3$). A characteristic

singlet due to -NH was observed at 2.05 ppm (exchangeable with D_2O) and a loss of the imine (-C=N) peak strongly suggested that the reduction was complete without loss of the ester group, since the presence of ester group was observed by ^1H-NMR and IR. Further the structure of compound 4 was confirmed by exact mass determined by high resolution mass spectroscopic data. The hydrolysis of the compound 4 was achieved by using lithium hydroperoxide as described by Evans,[12] since this reagent found to be highly regio-selective for the hydrolysis of a range of carboximides,[12] which generated in situ by reacting lithium hydroxide with hydrogen peroxide at 5°C. Reaction completed within an hour and compound 5 was obtained in 88% yield after column chromatography over a silica gel column. The structure of 5 was characterized by spectroscopic (NMR, IR and MS) data.



Scheme 2.

The reduction of the disulfide bond of compound 5 was achieved in a solution of NH_3 in the presence of sodium metal (freshly cut) under a N_2 stream at -70°C. Extractive work-up of the residue and short column chromatography on a silica gel column provide the bifunctional chelating agent 1 in 62% yield. Compound 1 was subsequently converted into the

hydrochloride salt by addition of HCl(g). The salt was filtered and washed with dry ether at 25°C and dried overnight (25°C 0.4 mmHg). The crystallizing ability of the salt was poor due to its strong hygroscopic nature. The salt is very pungent in odor, soluble in water, heat sensitive, and migrates on TLC (Whatman, silica gel in 12:2:1; chloroform: methanol: acetic acid, Rf 0.12, positive with Ellman's Reagent). IR (free base, film) 3450 cm^{-1} (NH), 2550 cm^{-1} (SH), ^{1}H-NMR (D_2O) δ:5.5(s, H), 4.0 (m, 6H), 3.05 (m, 4H, $2CH_2$), 2.3 (m, 4H), 1.8 (2H, SH), 1.5 (s, 6H, $2CH_3$), 1.35 (s, 6H, $2CH_3$).

Preliminary studies showed that labeling of chelate with technetium-99m occurs under mild conditions and the resultant labeled product is stable in vitro in 37°C human serum. The use of this bifunctional chelate as a means of introducing technetium-99m into biologically important molecules is under investigation.

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