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SYNTHESIS OF A NEW CLASS OF Tc CHELATING AGENTS: N_2S_2 MONOAMINEMONOAMIDE (MAMA) LIGANDS

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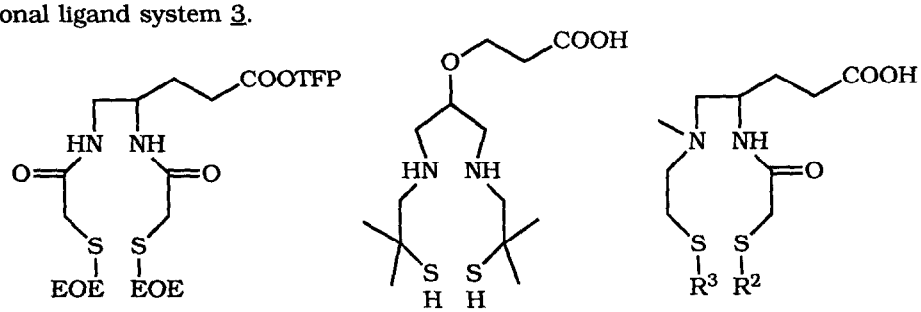
Abstract: A novel N_2S_2-monoamine amide (MAMA) bifunctional chelate was designed and synthesized for labeling of antibodies with Tc-99m. The chelate forms Tc complexes at a range of pH's and shows faster kinetics of complexation than N_2S_2-diamidedithiol (DADS) ligands. The MAMA chelate contains a carboxyl group for covalent attachment to amino groups on proteins.

Monoclonal antibodies are useful for the targeted delivery of radioactivity for diagnostic imaging as well as the delivery of therapeutic radionuclides to tumors.[1] Bifunctional chelates permit the stable functional group directed radiolabeling of proteins.[2,3] In the two step preformed chelate process, the S-protected diamide dithiol (DADS) bifunctional chelating agent 1 forms a highly stable complex with Tc at elevated temperatures (75-100°) and low pH (2-3) and the Tc complex is subsequently conjugated to the antibody.[4,5] This approach provides several advantages: the metal chelate is formed under controlled conditions, is characterizable, and the complexes are highly stable in vitro and in vivo. In the case of antimelanoma Tc-99m dimercapto-acetamidopentanoyl NR-ML-05 Fab, specific localization at sites of melanoma metastases was demonstrated.[6] Since diamine dithiol (DADT) ligand systems such as 2 form Tc complexes at ambient temperature, they show promise for the direct labeling of antibody-ligand conjugates.[7-9] By substituting one of the amide nitrogens of DADS with an amine, we hoped to enhance the kinetics of Tc chelation while maintaining the high stability of Tc-DADS antibody conjugates. Herein we report the synthesis of a new class of chelating agents based on the N_2S_2 monoamine monoamide (MAMA) bifunctional ligand system 3.

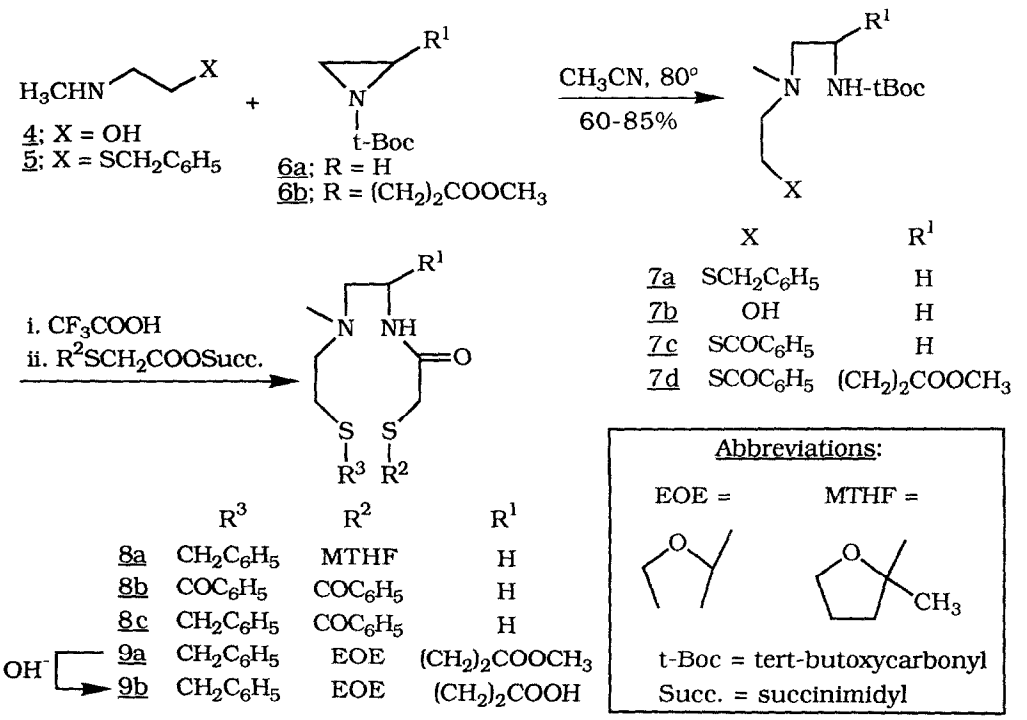


1 [DADS] 2 [DADT] 3 [MAMA]

EOE = 1-ethoxyethyl; TFP = 2,3,5,6-tetrafluorophenyl

Protection of the sulfur atoms of the MAMA bifunctional chelate prior to antibody conjugation minimizes the potential for aggregation of the protein by free thiols. However the sulfur protecting group must cleave and the sulfur simultaneously complex Tc under conditions compatible with preservation of antibody reactivity. To determine which sulfur protecting groups are cleaved under mild Tc labeling conditions, methods were developed for the synthesis of model MAMA ligands with sulfur protecting groups belonging to three categories: acid labile hemithioacetals, base labile S-acyl groups and metal labile benzyl thioethers. This method was extended to the preparation of a novel MAMA bifunctional chelate from a protected glutamic acid derivative.

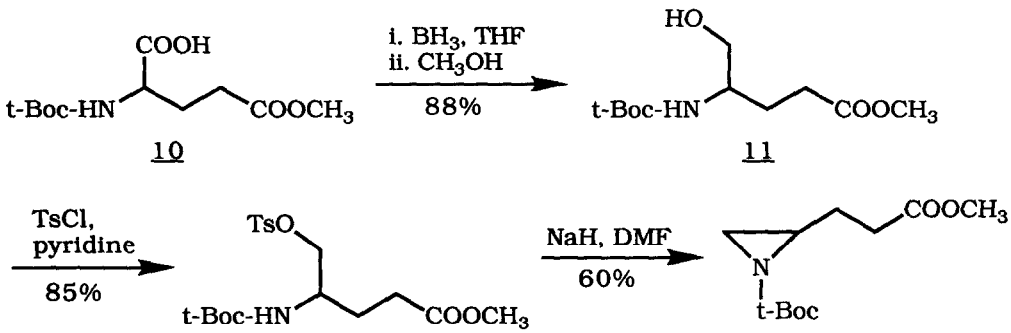
A general route to the model MAMA ligands 8a-c and the MAMA ligand functionalized with a carboxylate side chain 9b is shown below.[10] Differentiation of the two nitrogens of the protected diamine 7 was accomplished by opening of the activated aziridine 6 with a β-hydroxy- or S-protected-β-mercaptoamine.[11] Acid cleavage of the t-butylcarbamate 7 followed by acylation provided the MAMA ligands 8a-9b.[12] The thiobenzoate 7c was prepared indirectly by tosylation of the alcohol 7b and subsequent in situ displacement with potassium thiobenzoate in pyridine. Direct opening of an aziridine with 2-S-benzoylmethylaminoethane to prepare 7c was not investigated because of the likelihood of S- to -N acyl transfer.[13]



	X	R^1
7a	$SCH_2C_6H_5$	H
7b	OH	H
7c	$SCOC_6H_5$	H
7d	$SCOC_6H_5$	$(CH_2)_2COOCH_3$

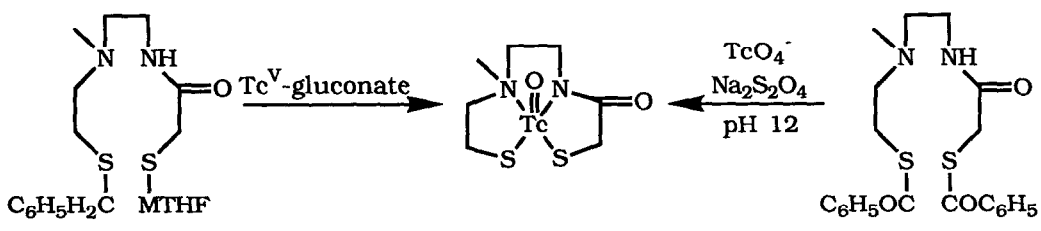
	R^3	R^2	R^1
8a	$CH_2C_6H_5$	MTHF	H
8b	COC_6H_5	COC_6H_5	H
8c	$CH_2C_6H_5$	COC_6H_5	H
9a	$CH_2C_6H_5$	EOE	$(CH_2)_2COOCH_3$
9b	$CH_2C_6H_5$	EOE	$(CH_2)_2COOH$

A procedure for the conversion of a functionalized α-aminoacid to the aziridine 6b was developed. Borane reduction of N-t-Boc glutamic acid-δ-methyl ester with 1.0 M borane in THF provided alcohol 11 which was tosylated in pyridine to give 12. Ring closure of the tosylate with sodium hydride in DMF provided aziridine 6b. Further conversions to the MAMA ligand 9b were the same as for the synthesis without a side chain.



10 11 12 6b

Since the N_2S_2 amine-amide ligands are a new class of chelating agents, the identity of the Tc complex was confirmed by two independent syntheses. Thus complexation of the acid labile S-hemithioacetal ligand 8a by exchange with Tc(V) gluconate gave the same product as obtained by reduction of pertechnetate by sodium dithionite in the presence of the base labile thiobenzoate ligand 8b.



8a 8b

Investigation of the radiolabeling with ^{99m}Tc at 37° for 30 minutes revealed that the substitution of the amide nitrogen in the DADS ligand 1 with an amine nitrogen in 3 resulted in a threefold increase in radiochemical yield of Tc complex.[14] The S-benzyl-S-methylfuranyl ligand 8a formed Tc complexes at pH 3.0-7.0 while the S-benzyl-S-acyl ligand 8c was complexed with Tc at pH 7.0-10.0.

In conclusion, we have developed methods for the synthesis of MAMA N_2S_2 ligands differentially protected with S-acyl, hemithioacetal, and arylthioether protecting groups as well as a MAMA bifunctional chelate containing a carboxylic acid. These ligands form Tc-complexes at a range of pH's in moderate yield at 37°.

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10. A typical procedure for the preparation of MAMA ligands is as follows (ligand 9a): A solution of 2-S-benzylmethylaminoethane (360 mg, 2.00 mmol) and 6b (160 mg, 0.70 mmol) in acetonitrile (2.0 mL) was heated under reflux for 24 hours. Evaporation and chromatography on silica gel gave 7d as an oil (240 mg, 84%). A solution of 7d (240 mg, 0.58 mmol) in CH_2Cl_2 (2.76 mL) and trifluoroacetic (0.47 mL, 3.47 mmol) was stirred for 40 minutes at 21°. The solution was evaporated. The residue was dissolved in DMF (5.5 mL). To this solution at 0° was added succinimidyl 2-S-ethoxyethylmercaptosuccinate (179 mg, 0.69 mmol) followed by triethylamine (0.47 mL, 3.47 mmol). The solution was stirred for 24 hours at 21°and then extracted with CH_2Cl_2. Chromatography gave 9a as a yellow oil (193 mg, 80%).
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12. Purity of the MAMA ligands as assessed by HPLC analysis revealed each of the ligands 8a-9b to be a single peak. Spectral data for the new compounds including M.S. and ^1H NMR are in accordance with sturctures assigned, and only selected data are cited below.
    8a: ^1H NMR δ 1.55 (s, 3 H, CH_2), 1.70-2.00 (m, 4 H, CH_2CH_2C), 2.10 (s, 3 H, NCH_3) 2.20-2.60 (m, 6 H, CH_2NCH_2, CH_2NCO), 3.15-3.35 (m, 4 H, $COCH_2S$, SCH_2), 3.70 (s, 2 H, $SCH_2C_6H_5$), 3.70-4.05 (m, 2 H, CH_2O), 7.25 (br s, 5 H, C_6H_5). LRCIMS: 383 (M + 1), 299 (M - MTHF). 8b: ^1H NMR δ 2.30 (s, 3 H, NCH_3), 2.60 (t, J = 6 Hz, 2 H, NCH_2), 2.65 (t, J = 7 Hz, 2 H, NCH_2), 3.18 (t, J = 7 Hz, 2 H, $COSCH_2$), 3.38 (q, J = 6 Hz, NCH_2), 3.78 (s, 2 H, $COSCH_2CO$) 7.42-7.60 (m, 4 H, Ar), 7.58-7.62 (m, 2 H, Ar), 7.8-8.2 (m, 4 H, Ar). LRCIMS:417 (M + 1). 8c: ^1H NMR δ 2.15 (s, 3 H, NCH_3), 2.35-2.55 (m, CH_2NCH_2, 4 H), 2.90 (t, J = 7, 2 H), 3.27 (q, J = 7, SCH_2, 2 H), 3.63 (s, 2 H, SCH_2CO), 3.75 (s, 2 H, $SCH_2C_6H_5$), 6.90 (broad, s, 1 H, NH), 7.20-8.20 (m, 10 H, 2 Ar). Anal. Calcd. for $C_{21}H_{26}N_2O_2S_2$: C, 62.65; H, 6.51; N, 6.96. Found: C, 62.93; H, 6.66; N, 7.06. 9a: ^1H NMR δ 1.20 (t, J = 7 Hz, 3 H, CH_3), 1.50 (d, J = 7 Hz, 3 H, CH_3). 1.62-1.80 (m, 2 H, CH_2), 1.90-2.05 (m, 2 H, CH_2), 2.20 (s, 3 H, NCH_3), 2.25-2.45 (m, 4 H, CH_2NCH_2), 2.47-2.60 (m, 4 H, CH_2S, CH_2CO_2), 3.25 (s, 2 H, $COCH_2S$), 3.40-3.52 (m, 1 H, one of CH_2O), 3.65 (s, 3 H, OCH_3), 3.60-3.70 (m, 1 H, one of CH_2O), 3.72 (s, 2 H, $SCH_2C_6H_5$), 3.95 (broad s, 1 H, CHN), 4.70 (q, J = 7 Hz, 1 H, CHO), 6.80 (broad s, 1 H, NH), 7.20-7.70 (m, 5 H, Ar). HRMS (CI): Calcd for $C_{22}H_{37}N_2O_4S_2$: 457.2194 ; Obsd: 457.2209.
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