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A Direct Synthesis of a Bifunctional Chelating Agent for Radiolabeling Proteins

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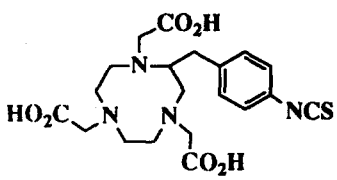
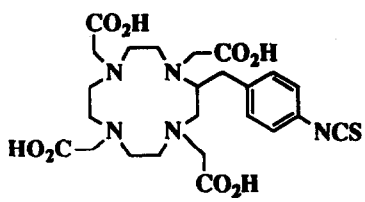
Abstract: A convenient synthesis of a bifunctional derivative of the ligand NOTA is described. The synthesis offers advantages over current literature methods for making similar molecules by starting with the easily prepared ortho-amide of 1,4,7-triazacyclononane which can be sequentially alkylated to provide a bifunctional NOTA derivative in 9% yield overall. The replacement of preparative HPLC with simple ion-exchange chromatography provides analytically pure ligand suitable for clinical applications.

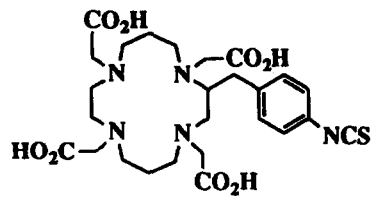
The potential usefulness of chelating agents for the modification of proteins for radioimmmunodiagnosis and therapeutic applications has been well discussed.[1] Successful clinical utility demands that the bifunctional chelating reagent be both capable of maintaining a stable complex *in vivo* while possessing a functional group which can be used for protein modification without deleterious effects.[2] We have attempted to fulfill these requirements by synthesizing polyaminocarboxylate ligands suitable for complexing radionuclides of interest (^{111}In, ^{90}Y, ^{212}Bi, etc).[3] While the backbone-substituted derivatives of DTPA have been shown useful in the clinic,[4] demonstrable advantages of the bifunctional NOTA, DOTA, and TETA ligands indicate their potential superiority *in vivo* [5].



p-NCS-Bz-NOTA



p-NCS-Bz-DOTA

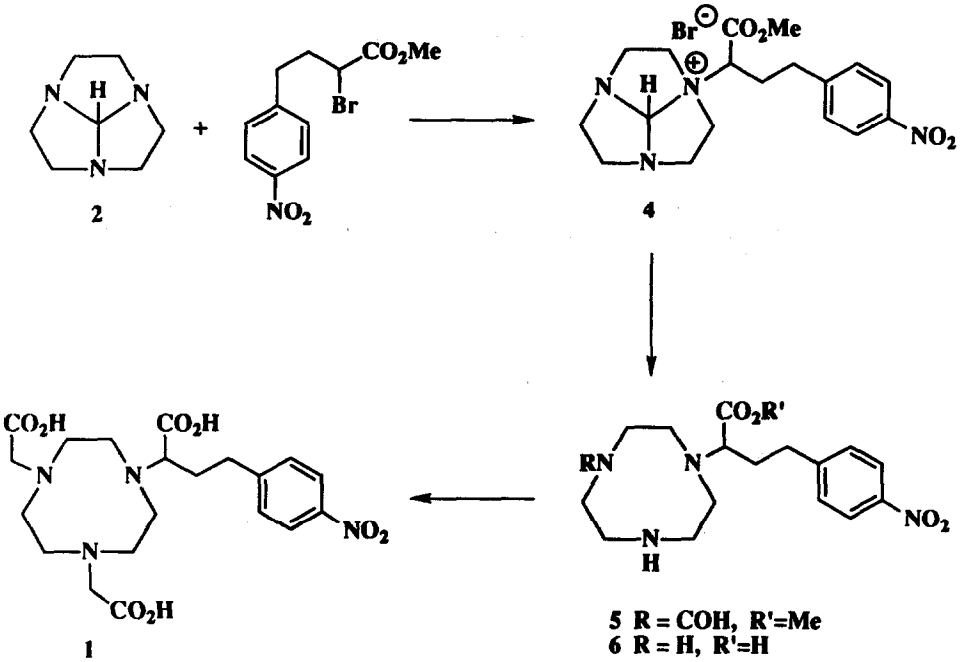


p-NCS-Bz-TETA

Three syntheses of substituted NOTA ligands have been reported. Parker and co-workers[6] have prepared a benzamide protected C-substituted NOTA based on (S)-lysine forming the 9-membered ring by Richman-Atkins conditions in 7 steps and 7.7% yield. In a second synthesis by this same group,[6] triazacyclononane was first directly mono-alkylated with a benzamide protected bromo aminoester followed by alkylation with ethyl bromoacetate to generate an N-substituted triester benzamide NOTA in 12% yield. Recently, Studer and Meares[7] reported a unimolecular cyclization route which derived the requisite framework from a tripeptide to generate a *para*-nitrobenzyl C-substituted NOTA in 7 steps at less than 2% yield from *tert*-butoxycarbonyl-*para*-nitrophenylalanine.

The cyclization procedure developed in our laboratory for C-substituted DOTA and TETA ligands provided the 12- and 14-membered ring systems in good yields, however, attempts to prepare the 9-membered ring were plagued by a transannular condensation which produced a mixture of fused ring 5,6-acylamidines.[8] While pursuing the analagous C-substituted NOTA derivative via an alternate route,[9] we have developed a synthesis of an N-substituted NOTA derivative which exploits ortho-amide chemistry in the sense of the recent report that the ortho-amide of 1,4,7-triazanonane can be selectively converted into a mono-alkylated derivative.[10]

Scheme 1



The synthesis of ligand **1** was achieved as outlined in Scheme 1. The readily available orthoamide **2** was prepared as described by Atkins.[11] Alkylation of 1.00 g of **2** with methyl 2-bromo-3-(p-nitrophenyl)propionate in refluxing dry toluene for 18 hr resulted in formation of a red-brown semi-solid **4** which was recovered by decanting the solution, washing the residue with diethyl ether, and vacuum drying. The literature reports this reaction employing benzyl bromides proceeded at *room temperature* to give precipitated product after 2 hr.[10] Presumably, steric hindrance of the less reactive secondary bromide necessitated the forcing conditions required to yield the bromide salt **4**. The crude salt was hydrolysed according to the literature by addition of water followed by refluxing the suspension for 8 hr to yield formamide **5**.[10b] The pH was adjusted to ca. 12 and the solution of amide **5** was refluxed for 12 hr which in our hands yielded a mixture of varying proportions of **5** and **6**. Refluxing this mixture in 6M $HCl_{(aq)}$ for 8 hr completed the hydrolysis to the desired mono-alkylated triamine **6**. Progress of the reaction was conveniently monitored by M.S. and/or by HPLC. Note that compounds **5** and **6** were not isolated except to provide a M.S. sample thus eliminating tedious and time-consuming purification procedures.[12]

The crude triamine was alkylated with excess bromoacetic acid at 45°C maintaining the pH at 8.5 using 5M NaOH via a pHstat and autoburette for 12 hr after which the solution pH was adjusted to ca. 2.0. The product was purified by first de-salting with an AG50 H^+ cation exchange column and eluting the crude tri-acid from the resin with 2M NH_4OH. After taking the solution to dryness, the material was applied to an AG1 acetate anion exchange column from which the product was eluted with a 0.0-2.0M acetic acid gradient. The solution containing the product was first concentrated, then lyophilized to an off-white solid which was dried at 70°C, 0.05 mm to yield 280 mg of analytically pure NOTA 1 in 9% overall yield. The ligand was characterized by routine spectroscopic methods (^1H- and ^{13}C-NMR, FAB-MS, and elemental analysis) and found to be totally in agreement with the proposed structure.[13] Preliminary studies in our laboratories have demonstrated that ligand 1 forms a complex with ^{111}In.

In conclusion, the methodology presented herein provides a simple direct route to an N-substituted NOTA ligand reducing tedious multistep synthesis and replacing often required preparative HPLC with convenient ion-exchange chromatography. Routine hydrogenation of the aryl nitro group to an aniline provides entry into a variety of reactive groups for protein conjugation,[3,8,14] and will allow complete evaluation through both *in vitro* and *in vivo* stability and tumor targeting studies with ^{111}In, $^{66,67}Ga$, and ^{67}Cu for both radioimmunoimaging and therapy.

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12. Intermediates **5** and **6** were minimally characterized by positive FAB-MS *m/e* 365 and 337, respectively, in a glycercol matrix to determine completion of reaction. Additionally, analytical HPLC for crude **5** and **6** were recorded using the system and method described in reference 3c to give retention times of **16.5 and 15.7** min., respectively.

13. ^{1}H NMR (300 MHz, D_2O, pH 1.0) δ 8.185 (d,2H,J=8.7), 7.511 (d,2H,J=8.7), 4.103 (s,4H), 3.872 (dd,1H,J=9.0,4.5), 3.65-3.28 (m,12H), 3.12-2.87 (m,2H), 2.46-2.29 (m,1H), 2.29-2.13 (m,1H); ^{13}C NMR (300 MHz, D_2O, pH 1.0) δ 177.71, 174.02, 152.42, 149.43, 132.69, 127.00, 67.74, 59.40, 54.69, 53.74, 50.48, 35.28, 32.25; ^{1}H NMR (300 MHz, D_2O, pH 12.0) δ 8.185 (d,2H,J=8.4), 7.501 (d,2H,J=8.4), 3.242 (dd,4H,J=20.1,16.2), 3.049 (br.t,1H,J=8.1), 2.89-2.45 (m,14H), 2.07-1.96 (m,2H); ^{13}C NMR (300 MHz, D_2O, 12.0) δ 183.99, 182.28, 153.28, 148.21, 131.80, 125.99, 73.40, 64.00, 54.63, 53.59, 51.17, 34.91, 33.77; FAB-MS (glycerol) *m/e* 453 (M+1). Chemical Analysis; Calc. for $C_{20}H_{28}N_4O_8$; C, 54.78; H, 6.45; N, 9.59: Found; C, 54.53; H, 6.47; N, 9.62 (Atlantic Microlabs). The reference for the NMR spectra was TSP.

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