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SYNTHESIS OF 2-*p*-AMINOBENZYL-3-METHYL- AND 2-*p*-AMINOBENZYL-3-BENZYL DERIVATIVES OF DIETHYLENETRIAMINEPENTAACETIC ACIDS (DTPA): CARBON BACKBONE MODIFIED BIFUNCTIONAL CHELATING AGENTS.

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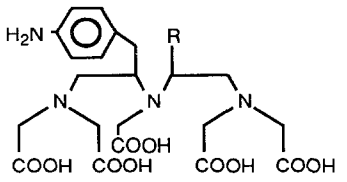
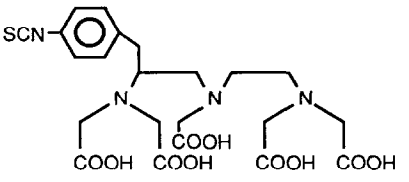
Abstract: A convenient preparation of new bifunctional chelating agents (**1a**) and (**1b**) were achieved based on a practical synthesis of 2-p-nitrobenzyl-3-methyl (**9a**) and 2-p-nitrobenzyl-3-benzyl (**9b**) diethylenetriamines starting from optically pure p-nitro-L-phenylalanine and triflates of 2-hydroxy carboxylic acid ethyl esters. These backbone substituted chelating agents could provide the stable complexation with radiometals.

The attachment of radioactive metal ions to monoclonal antibodies for the diagnosis and treatment of cancer has gained interest in recent years which led to the development of bifunctional chelating agents (BFCA).[1-3] There has been several reports that the carbon backbone of the BFCA can be substituted with various R- groups to create the stability and rigidity in metal-chelate structure once the metal complexation has occurred.[4-6] These derivatives of DTPA have been prepared by the alkylation of substituted diethylenetriamine with halogenated acetic acids.



1a: R=CH_3
1b: R=Bz



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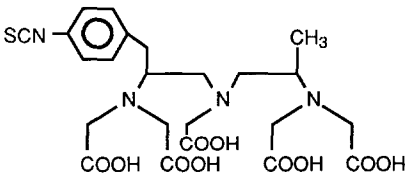

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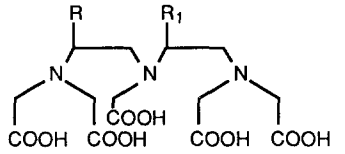
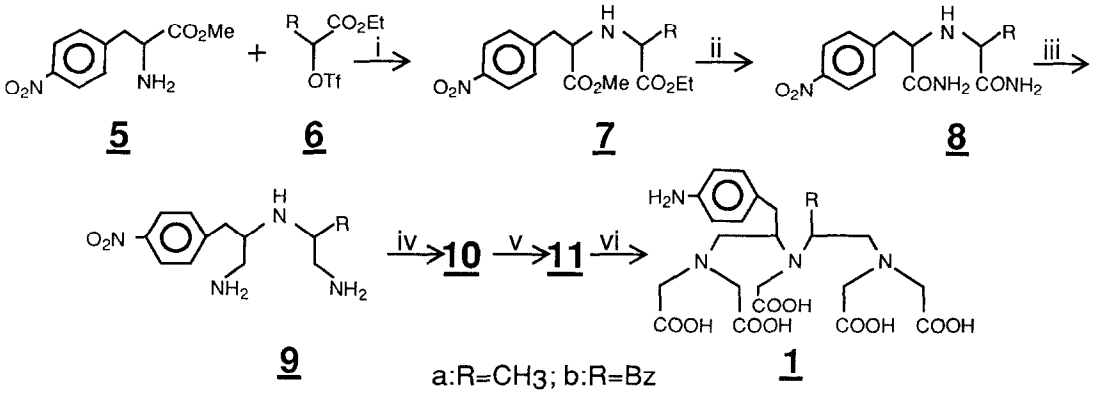


4a: R=p-SCNBz; R_1=CH_3
4b: R=CH_3; R_1=p-SCNBz

Brechbiel et al[7] have reported the synthesis of ligand **2**, a DTPA based BFCA, from the reaction of p-nitrophenylalanine methyl ester with ethylenediamine. Cummins et al[5] have reported a preparation of ligands **2** and **3** by a modification of the previously reported procedure[7]. Later, Brechbiel and Gansow reported the synthesis of methyl substituted ligands **4** by utilizing a peptide approach.[4] Ligands **3** and **4** conjugated with monoclonal antibodies and labeled with radiometals after methyl substitution on their backbone have shown to be superior to ligand **2** in tumor uptake and biodistribution studies.[8-10] We, therefore, have attempted the synthesis of modified ligands **1a** and **1b** having methyl or benzyl groups on C-3 and p-aminobenzyl substituent on C-2 of DTPA backbone, not reported previously, in order to investigate the in vivo stability of their monoclonal antibodies conjugates labeled with radiometals (Yttrium or Indium).

The basic strategy we choose here for the construction of backbone modification of DTPA is based on the stereospecific displacement of triflate group of a 2-hydroxycarboxylic acid ethyl ester with amino group of p-nitro-L-phenylalanine methyl ester under complete inversion of configuration[11] to give iminodiacetic acid diesters **7** which can be utilized for the synthesis of **1a** and **1b** as depicted in Scheme-1.

Scheme 1



i) Et_3N(1.1 equiv), CH_2Cl_2, r.t., 48-72 hr (a 85%, b 77%); ii) NH_3, CH_3OH,-15°C, one week (a 99%, b 98%); iii) THF,0°C,BH_3. THF(1M in THF, 10 equiv), 2hr then reflux 6-8 hr, EtOH-HCl, reflux 4-6 hr, pH 14(50% NaOH), workup($CHCl_3$)(a 70%, b 67%); iv) $BrCH_2CO_2t$-Bu (5.5 equiv), KI (5.5 equiv), THF,r.t., 1hr, then K_2CO_3 (10 equiv), triamine **9** (1 equiv) in THF,r.t., 24-72 hr (a 52%, b 62%); v) H_2 10%Pd/C, EtOAC, r.t., 4-6hr (a 95%, b 96%); vi) Conc. HCl,2hr (a 94%, b 95%)

Preparation of requisite diethylenetriamine derivatives **9** was achieved from p-nitro-L-phenylalanine methyl ester hydrochloride **5**[12] and alkyl triflate ethyl esters **6**.[13] S_N2 displacement of triflate group of **6** with an amino group of **5** gave diastereospecifically pure **7** [14] with inversion of configuration in 85-91% yields. Amidation of **7** with saturated ammonical solution of MeOH at -15°C for one week produced diamide **8**[14] (98.0-99.0%). Reduction of diamide **8** with BH_3.THF produced desired backbone of C-2 and C-3 disubstituted diethylenetriamine **9**[14](67-70%). Conversion of triamines **9** into their corresponding DTPA analogues was accomplished by alkylation with t-butylbromoacetate to give t-butylpentaesters **10**[14] (52-62 %). Catalytic hydrogenation of p-nitro group of **10** into amino group to afford t-butylpentaesters **11**[14] (95-96%) which after hydrolysis with conc. HCl gave DTPA analogues **1**[14] in 94-95% yields. These ligands can be easily converted to their corresponding isothiocyanato compounds suitable for the conjugation with proteins.

The inclusion of the iminodiacetic acid ester synthesis allows different substitution at C-3 on the backbone by selecting the appropriate 2-hydroxy acid ethyl esters. The loss of enantiomeric purity is unlikely under the reaction conditions utilized.[11,15] The analysis of compound **7** showed consistent results (90% inversion) with previously reported data[11]. The synthesis of ligands **1a** and **1b** will allow investigation of the structural and conformational influences toward the stability of metal complex containing methyl (**1a**) or benzyl (**1b**) substituents at C-3 and p-aminobenzyl substituent at C-2 of DTPA backbone, and to compare the ligand stability of **1a** and **1b** with ligand **2** that has a substituent at carbon no. 1. The added substituents in these BFCA will probably restrict the release of radiometal from the metal-ligand complex and could provide the optimal results in vivo. Conversion of amino group of **1a** and **1b** into their corresponding isothiocyanato group, conjugation with antibody, and coordination with ^{90}Y and ^{111}In is in progress and will be published in due course elsewhere.

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