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# Preparation and biological evaluation of $^{111}$In-, $^{177}$Lu- and $^{90}$Y-labeled DOTA analogues conjugated to B72.3

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**Abstract**

Three 1,4,7,10-tetraazacyclododecane-*N,N′ ,N″,N‴* -tetraacetic acid (DOTA) analogues were evaluated for relative in vivo stability when radiolabeled with $^{111}$In, $^{90}$Y and $^{177}$Lu and conjugated to the monoclonal antibody B72.3. The DOTA analogues evaluated were "NHS-DOTA" [*N*-hydroxysuccinimdyl (NHS) group activating one carboxylate], "Arm-DOTA" (also known as MeO-DOTA; with a *p*-NCS, *o*-MeO-benzyl moiety on the methylene group of one acetic acid arm) and "Back-DOTA" (with a *p*-NCS-benzyl moiety on a backbone methylene group of the macrocycle). The B72.3 was conjugated to the DOTA analogues to increase the retention time of the radioloabeled conjugates in vivo in mice. The serum stability of the various radiometalated DOTA conjugates showed them to have good stability out to 168 h (all >95% except $^{111}$In-NHS-DOTA-B72.3, which was 91% stable). Hydroxyapatite stability for the $^{111}$In and $^{177}$Lu DOTA-conjugates was >95% at 168 h, while the $^{90}$Y DOTA-conjugates were somewhat less stable (between 90% and 95% at 168 h). The biodistribution studies of the radiometalated DOTA-conjugates showed that no significant differences were observed for the $^{111}$In and $^{177}$Lu analogues; however, the $^{90}$Y analogues showed lower stabilities, as evidenced by their increased bone uptake relative to the other two [2–20% injected dose per gram (% ID/g) for $^{90}$Y and 2–8% ID/g for $^{111}$In and $^{177}$Lu]. The lower stability of the $^{90}$Y analogues could be due to the higher beta energy of $^{90}$Y and/or to the larger ionic radius of $Y^{3+}$. Based on the bone uptake observed, the $^{177}$Lu-NHS-DOTA-B72.3 had slightly lower stability than the $^{177}$Lu-Arm-DOTA-B72.3 and $^{177}$Lu-Back-DOTA-B72.3, but not significantly at all time points. For $^{90}$Y, the analogue showing the lowest stability based on bone uptake was $^{90}$Y-Arm-DOTA-B72.3, perhaps because of the metal's larger ionic radius and potential steric interactions minimizing effective complexation. The $^{111}$In analogues all showed similar biological distributions at the various time points. This study suggests that care must be taken when evaluating $^{90}$Y-labeled antibodies and in using NHS-DOTA-antibody conjugates with $^{177}$Lu. All evaluations should be extended to time points relevant to the half-life of the radiometal and the therapy applications.


## 1. Introduction

Radiolabeled antibodies have shown promise for cancer diagnosis and therapy. Several $^{111}$In-labeled antibodies (OncoScint, MyoScint and ProstaScint) are approved by the United States Food and Drug Administration (FDA) for imaging colorectal and ovarian cancer, necrotic myocardial tissue and prostate-specific membrane antigen-positive prostate cancer [1–3]. All three of these approved agents use a diethylenetriamine-*N,N,N′ ,N″,N‴*-pentaacetic acid (DTPA) chelator to complex the $^{111}$In. Two $^{99m}$Tc based antibody agents, Tc-99m-LeuTech and Tc-99m Arcitumomab (CEA-Scan), were FDA-approved for infection imaging and colorectal imaging, respectively [4,5]. Recently, two radiotherapeutic agents (Zevalin and

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Bexxar) were approved for the treatment of refractory non-Hodgkin's lymphoma [6,7]. Zevalin uses the MX-DTPA chelate to complex $^{90}Y$, while Bexxar contains the nonradiometal $^{131}I$ for therapy [6,7].

Targeted radiopharmaceuticals, whether designed for diagnostics or therapy, often involve the use of a radiometal [8–11]. This requires that the radiometal be stably attached to the targeting moiety, often a peptide or antibody, and this is generally accomplished using a bifunctional chelate. The choice of the appropriate bifunctional chelate is radiometal-dependent, as the coordination requirements of the particular metal must be considered (i.e., denticity, donor atoms, etc.) to achieve a kinetically inert and thermodynamically stable radiopharmaceutical complex [12,13]. The amine carboxylate ligands such as DTPA and 1,4,7,10-tetraazacyclododecane-*N,N′ ,N″,N‴* -tetraacetic acid (DOTA) and their analogues have been the bifunctional chelates of choice for the +3 radiometal ions such as $^{111}In$, $^{90}Y$ and the radiolanthanides [13]. Conjugation of these chelates to the biomolecule can be accomplished through activation of one of the carboxylic acid groups or through a functional group attached to one of the methylene carbons in the molecules. The DOTA analogues have generally resulted in more stable radiometal bioconjugates, probably through the macrocyclic effect coupled with the hepta- or octadenticity of these chelates [14].

Three DOTA analogues that have been developed as bifunctional chelates include the *N*-hydroxysuccinimdyl (NHS) ester of DOTA ("NHS-DOTA"), the *p*-NCS-benzyl-DOTA ("Back-DOTA") and the *p*-NCS, *o*-methoxy-benzyl-DOTA ("Arm-DOTA"), and the structures are shown in Fig. 1 [15]. The three DOTA analogues differ in their coordination denticity to the metal and their site of attachment to the biomolecule. NHS-DOTA may act as a heptadentate or octadentate chelate, coordinating to metals via four amines, three carboxylate groups and, potentially, an amide. The fourth carboxylate group of DOTA has been derivatized with an active ester to yield an amide group on

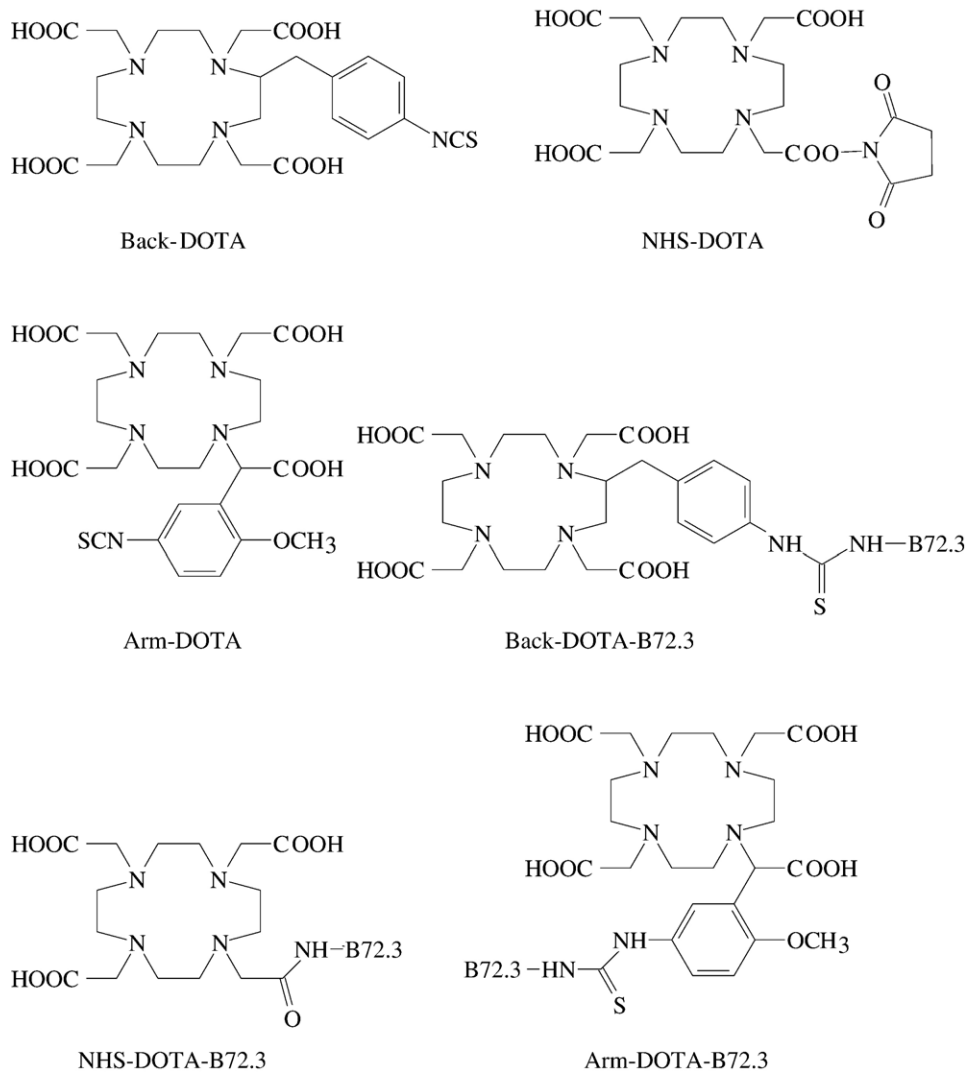


Fig. 1. Structures of chelators and their B72.3 antibody conjugates.