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Novel synthetic ligands for targeted PET imaging and radiotherapy of copper

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Abstract—Novel ligands, NBEA, NBPA, NETA, NE3TA, and NE3TA–Bn, were synthesized and evaluated as potential chelators of copper radioisotopes for use in targeted positron emission tomography (PET) imaging or radiation therapy. The new ligands were radiolabeled with ^{64}Cu, and in vitro stability of the radiolabeled complexes was assessed in rat serum. Serum stability results suggest that among the ligands tested, NETA, NE3TA, and NE3TA–Bn form stable complexes with ^{64}Cu.

Among the available copper radioisotpes, ^{64}Cu($t_{1/2} = 12.7$ h, $E^{\beta+}_{\max} = 656$ keV; $E^{\beta-}_{\max} = 573$ keV) is proven to be effective for use in positron emission tomography (PET) imaging and targeted radiation therapy applicable to many types of cancer.[1,2] Bifunctional ligands that possess both binding moieties of Cu(II) and a functional group for conjugation to a targeting moiety are required for the modalities. Research efforts have been directed toward the development of optimal bifunctional ligands that can rapidly form stable complexes with the short-lived ^{64}Cu while being conjugated to a targeting moiety, either peptide or antibody, to provide an efficient way of generating stable and safe copper radioisotope-labeled drugs for cancer therapy and imaging.[3]

Recently, we have developed a structurally novel ligand, NETA, and its analogues.[4] NETA features a parent macrocyclic ligand with a flexible acyclic multidentate pendant arm. DOTA and DTPA are the frequently explored chelators of biologically important metals for biomedical applications. In general, the acyclic ligands such as DTPA possess rapid complex formation kinetics, but weak binding to the metal, whereas the less dynamic macrocyclic ligands such as DOTA display strong binding to the metal, but with relatively slower complex formation kinetics. The idea of designing NETA was to integrate the advantage of both the macrocyclic and acyclic frameworks, that is, both thermodynamic stability and favorable formation kinetics. The bimodal octadentate NETA was found to effectively bind the metallic radionuclides for targeted radiotherapy such as ^{90}Y, ^{177}Lu, and $^{205/6}$Bi.[4,5] Given the capability of octadentate DOTA to tightly bind Cu(II) in vitro and in vivo,[2] we wanted to explore complexation of NETA and NETA analogues, NE3TA and NE3TA–Bn with Cu(II). NE3TA contains four amines and three carboxylates as potential donor groups. NE3TA–Bn is a heptadentate ligand with a benzyl group which can be further modified for conjugation to a targeting moiety. Hexadentate NBEA and NBPA possess three amines, two carboxylates, and a hydroxyl group as the donor groups. Our hypothesis for the design of NBEA and NBPA was that the size-fit between the macrocyclic cavity in NBEA and NBPA and the ionic radius of Cu(II)[6] might provide enhanced radioisotope complex stability while producing a neutral Cu(II) complex that would have an advantage of less protein interaction and a potentially more favorable in vivo tissue distribution. NBPA possesses a longer

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propylene bridge between one of the amino groups and the hydroxyl group compared to the analogous ethylene bridged ligand, NBEA (Fig. 1).

The structures of the novel ligands, NETA, NE3TA, NE3TA–Bn, NBEA, and NBPA, are shown in Scheme 1. These ligands are not functionalized for conjugation to a targeting peptide or antibody. Preparation of the non-functionalized parent ligands is generally relatively straightforward as compared to the bifunctional analogues wherein introduction of an element of asymmetry frequently complicates synthetic efforts. The generally valid assumption is that the bifunctionalization of the ligands usually makes little difference in their metal binding ability. In vitro serum stability is a useful and qualitative screening procedure for identifying unstable metal complexes of non-functionalized chelates by assessment of their potential to maintain stable complexes without dissociation of the metal ion in vitro.[4a] The radiolabeled non-functionalized chelates that demonstrate acceptable in vitro stability qualify for preparation of their respective bifunctional analogues for further evaluation post-conjugation to targeting molecules.

The new ligands NBEA and NBPA are efficiently synthesized starting from **1** and **2**, respectively. We previously reported the efficient and convenient synthetic route to the starting materials **1** and **2** from the readily available ditosylate[4b] using a convenient cyclization and detosylation process.[7] The base-promoted reaction of **1** or **2** with *tert*-butylbromoacetate afforded alkylated ligand **3** or **4** in good yield (~50%).[8] Removal of *tert*-butyl groups in **3** and **4** was efficient with TFA (room temperature, 4 h) to provide NBEA and NBPA in high yields (>90%) without any further purification.[9] Synthesis of NE3TA and NETA, and NE3TA–Bn involves a coupling reaction between two precursor molecules, a pre-alkylated amino ethyl bromide and a bisubstituted 1,4,7-triazatricyclononane (tacn) derivative. The key coupling step was very efficiently achieved while minimizing formation of polyalkylated byproducts.[5]

The radiolabeling reactions of the new ligands with ^{64}Cu were performed at elevated temperatures to ensure complete complex formation.[10] At the no-carrier-added (NCA) level, all ligands were successfully labeled with ^{64}Cu in quantitative radiochemical yields (100%) as determined by radio-TLC. The ligands displayed rapid

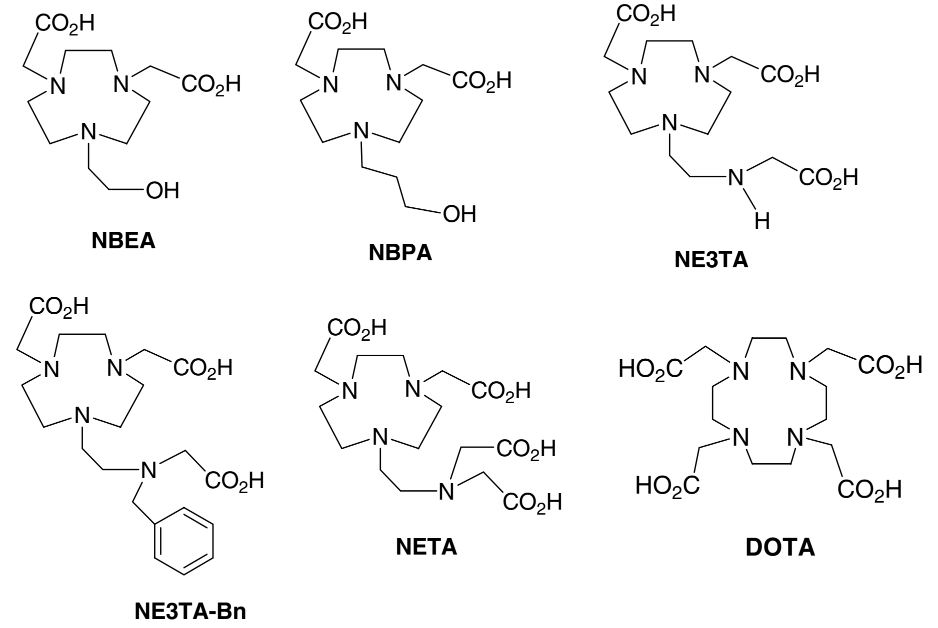
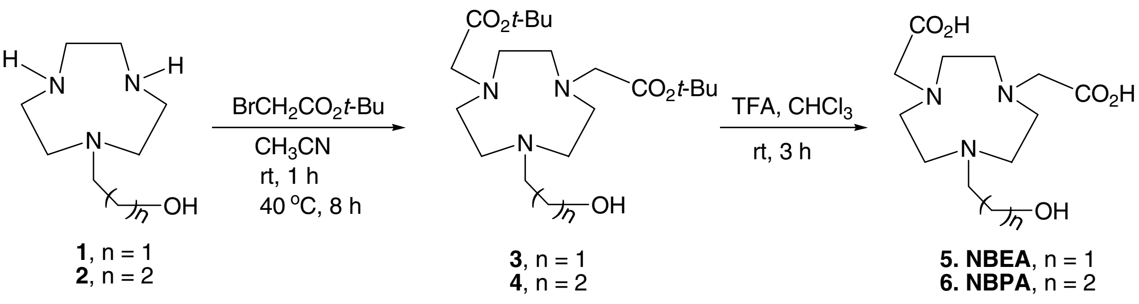


Figure 1. Potential ligands for use in PET imaging and radiotherapy of copper.



Scheme 1. Synthesis of new hexadentate ligands, NBEA and NBPA.