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Efficient synthesis and evaluation of bimodal ligand NETA

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Abstract—The efficient and short synthetic route to the structurally novel bimodal ligand NETA for antibody-targeted radiation therapy (radioimmunotherapy, RIT) of cancer was developed. The structure of NETA was determined by X-ray crystallography. The arsenazo-based UV spectroscopic complexation kinetics data suggest that NETA is a promising chelator for use in RIT applications of ^{212}Bi, ^{213}Bi, and ^{177}Lu.

Synthetic macrocyclic and acyclic ligands that possess amino and carboxylate groups as metal-binding moieties have been employed for biomedical and radiopharmaceutical applications such as magnetic resonance (MR)[1] and positron emission tomography (PET)[2] imaging and antibody-targeted radiation therapy (radioimmunotherapy, RIT)[3] of cancer. Research efforts have been directed towards development of the adequate metal binding macrocyclic and acyclic ligand, a critical component for enhancing the efficacy of the therapeutic and diagnostic applications.

RIT is a potent cancer therapeutic modality that employs a tumor-targeting mAb for selective delivery of a cytotoxic radioisotope while minimizing exposure of healthy cells.[3] A variety of metallic radionuclides including ^{177}Lu, ^{212}Bi, and ^{213}Bi have been proven effective for RIT.[3] We have recently developed the structurally novel bimodal ligand NETA for use in RIT.[4–7] NETA (2-(4,7-biscarboxymethyl[1,4,7]triazacyclonona-1-yl-ethyl)carbonyl-methylamino]acetic acid) is a hybridized structure of DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid), the most frequently explored polyaminocarboxylates. Macrocyclic DOTA forms a stable complex with various therapeutic metals and is a standard reference ligand for comparison.[8] However, slow formation kinetics of DOTA remains a limitation in RIT applications, particularly involving relatively short-lived radioactive metals such as ^{212}Bi ($t_{1/2}$ = 60.6 m) and ^{213}Bi ($t_{1/2}$ = 45 m).[9] Acyclic DTPA rapidly binds to the metals but produces relatively unstable complexes.[10] NETA containing both macrocyclic and acyclic moieties is proposed to bind the metal ion more rapidly than DOTA[8,11] via dynamic binding while binding the metal stronger than DTPA[10] via three-dimensional binding. NETA complexed with various metals including lanthanides was very stable in human serum or mice.[4–6] NETA was found to bind Y(III) within seconds, while macrocyclic DOTA displays very slow complexation kinetics (>1 h) as determined by a spectroscopic competing reaction with arsenazo III (AAIII).[4] Great potential of NETA for use in RIT prompts us to further investigate the ligand in a unique structural class. Herein, we report the efficient and short synthetic route to NETA and its evaluation as RIT chelators for ^{177}Lu, ^{212}Bi, and ^{213}Bi therapeutic metals. The structure of NETA was determined by X-ray crystallographic analysis.

It was necessary to demonstrate the hypothesized bimodal binding of NETA with various metals by X-ray crystallographic study and measurements of thermodynamic constants that require significant amount of the chelator. The original synthesis of NETA as previously reported involves eight reaction steps including a convenient macrocyclization of the readily available starting materials, ethanol amine and an *N*-tosyl protected ditosylate.[4] We initially attempted preparation of NETA in large quantities by modifying the original

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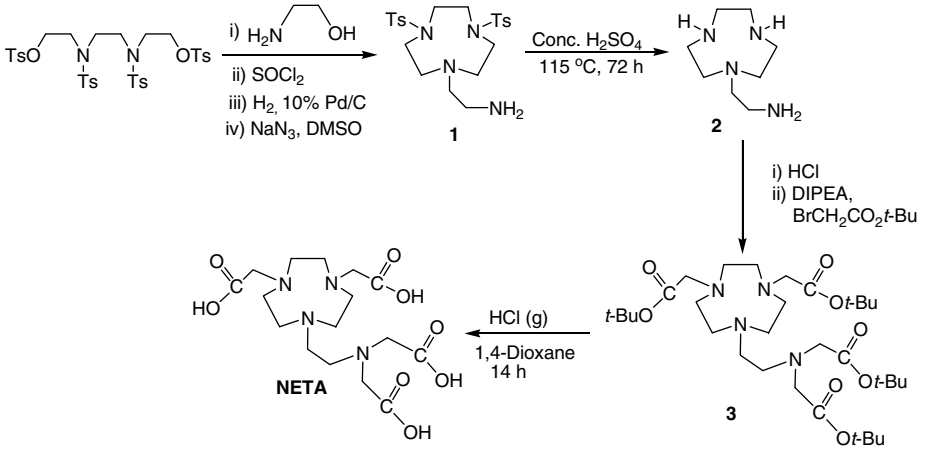
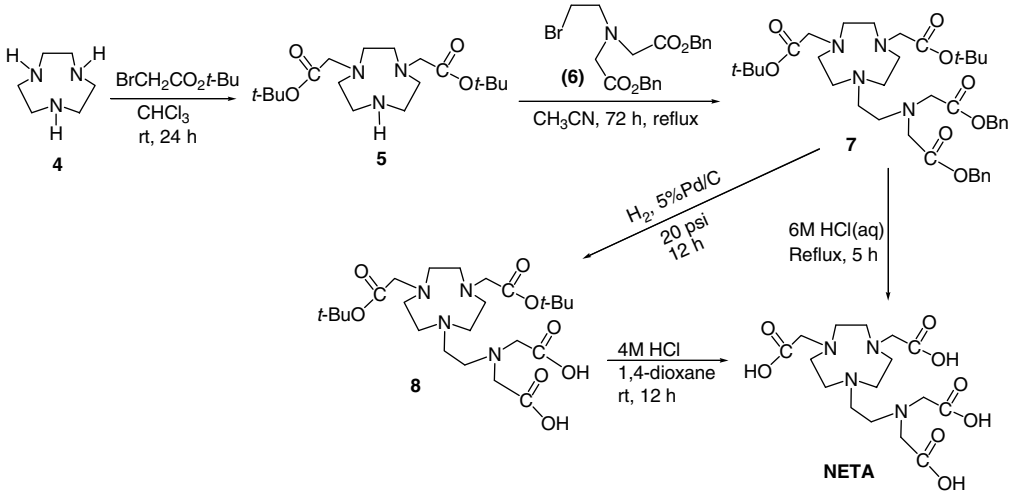


Scheme 1. Modification to original synthesis[4] of NETA.

synthetic route (Scheme 1). We found that the original synthetic route is quite lengthy and not efficient for large-scale preparation of NETA. The detosylation and alkylation reaction steps to produce **2** and **3**, respectively, significantly lower an overall yield (<20%) of the synthetic route (Scheme 1). We observed that preparation of precursor molecule **1** in large quantities (>30 g) was practically and efficiently carried out in excellent yield (>86%). All of the compounds except the first macrocyclization step were isolated after simple work-up or recrystallization and did not require column chromatographic purification. However, scale-up deprotection of compound **1** was problematic providing **2** in a low yield, despite working very well in small scale (<1 g) and nearly 90% yield. Furthermore, detosylation of **1** in large quantities required tedious work-up involving stepwise neutralization and extraction generating significant amount of toxic waste. We found that free amine **2** oxidizes in less than 12 h at both room temperature and −20 °C as monitored by NMR, while the oxidation can be prevented by immediately treating **2** with HCl to afford an acidic salt (Scheme 1). The alkylation reaction of **2** as an acidic salt in large quantity produced large amount of polyalkylated byproducts along with the desired compound **3** in a very poor yield (11%). Optimization of the alkylation reaction to exclusively produce **3**, which does not contain a UV chromophore, was quite challenging. The polar-tailing compound **3** and byproducts possess concentration-dependent R_f values. Therefore, TLC analysis of fractions eluted from flash chromatography on normal phase silica gel is not very informative in distinguishing the targeted product from byproducts that possess very similar R_f value(s). This also makes the desired **3** using flash silica gel (or alumina) column chromatography extremely complicated. This difficulty in producing NETA analogue **3** in large quantities prompted us to develop more practical and short synthetic route.

Since Prep-HPLC or other purification methods are not helpful for purification of NETA without a strong UV chromophore, we have attempted different reaction routes in efforts to develop a synthetic route to NETA avoiding harsh detosylation and complicated alkylation reaction steps. An efficient and short method to prepare NETA is shown in Scheme 2. The key step is the coupling reaction of bisubstituted macrocyclic TACN (1,4,7-Triazacyclononane) **5** with acyclic *N*-dialkylated bromide **6** in an equimolar ratio. The starting material, bisubstituted TACN (**5**),[12] was efficiently prepared by



Scheme 2. Short and efficient synthesis of NETA.

the reaction of TACN **4** with *tert*-butylbromoacetate. It was necessary to develop a short and reproducible synthetic route to **5** since the known procedures either involve lengthy and poor total synthetic yield or require column chromatographic purification of the polar tailing macrocycle.[12,13] In the present study, **5** was isolated from a reaction mixture by pH controlled stepwise neutralization without column chromatography. The best yield of **5** was obtained from the reaction of TACN (1 equiv) and *tert*-butylbromoacetate (2.2 equiv) without the addition of base. In the presence of base (triethylamine or diisopropylethylamine), the mixture of the desired bisubstituted TACN and trisubstituted and/or monosubstituted TACN was obtained (supplementary data, Table 1). The coupling reaction of **5** with **6**[16] provided the desired product **7** (Scheme 2). Since each of the precursor molecules was alkylated prior to the coupling reaction, the formation of polyalkylation byproducts was minimized. Isolation of tailing polar macrocycle **7**, which can be monitored by HPLC and TLC analysis due to the presence of the UV chromophore, was achieved by flash column chromatography eluting with 5–6% CH_3OH/CH_2Cl_2. Most importantly, with the development of TLC and analytical and preparative HPLC methods based on benzyl UV chromophore, the reaction condition could be readily modified if an improvement of the reaction yield is required. Compound **7** was efficiently prepared in a good and highly reproducible yield (>60%). Both benzyl and *tert* -butyl protection groups in **7** were removed by refluxing **7** in 6 M HCl(aq). The benzyl groups in **7** were also selectively removed by hydrogenation and provided *tert* -butyl group containing chelate **8**. The partially deprotected compound **8** may be a useful backbone molecule which can be selectively reacted with a variety of amino-containing peptides and antibodies to generate a viable chelator for biomedical applications. NETA was efficiently prepared in two steps and large quantities and good overall yield (61%).

The structure of NETA as an acidic salt (HCl) was successfully determined via X-ray crystallography (Fig. 1). Crystals of NETA for X-ray analysis were obtained by slow evaporation of $H_2O/EtOH/Et_2O$ in a 1:1:2 ratio. Two of the carboxylate groups on the macrocyclic backbone are protonated (CO_2H), while the other two carboxylate groups on the acyclic moiety exist as anion (CO_2^-). The C–O bond distances of the protonated and anionic carboxylates indicate that O_5/O_6 and O_7/O_8 are in a resonance form (CO_2^-), while O_1/O_2 and O_3/O_4 have one bond longer than the other (CO_2H). It is interesting to note that hydrogen bonding exists between the protonated nitrogen atoms N_1, N_2, and N_4 in both macrocyclic and acyclic moiety and the chlorine atom Cl_1; this may be a good entry point to demonstrate the proposed bimodal binding hypothesis. Cl_1 is bound to the protonated nitrogens N_1, N_2, and N_4 with respective bond distances of 3.22, 3.14, and 3.04 Å.

We previously reported that NETA labeled with the cancer therapeutic metal, ^{177}Lu or $^{205/6}$Bi, was extremely stable in human serum without leaking the radionuclide.[5] With the promising data, we wanted to further

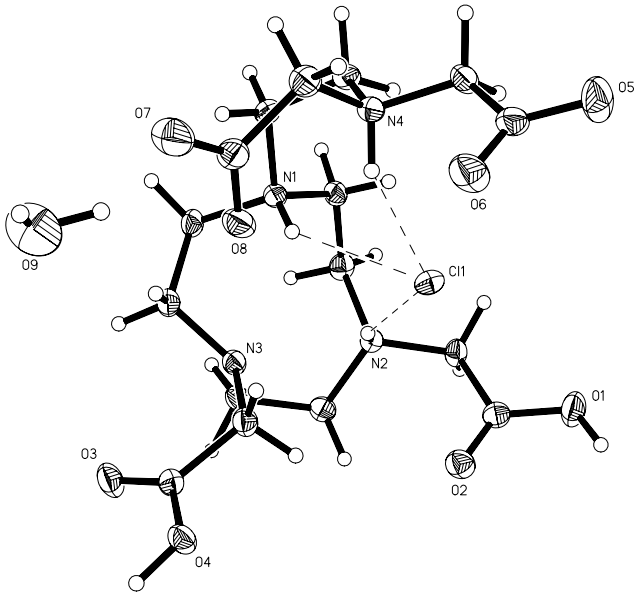


Figure 1. XRD structure of NETA.

evaluate NETA for the complexation kinetics with Lu(III) and Bi(III). The complexation kinetics of NETA with Lu(III) and Bi(III) was determined using a well-known spectroscopic competing reaction with AAIII according to a modification of a previously reported procedure.[7] AAIII is known to form a weak complex with many different metals, which produce a UV–Vis absorbance maximum at ~652 nm.[14] However, uncomplexed AAIII absorbs weakly at this wavelength. When introduced to a solution containing the AAIII–metal complex, a chelate can compete with AAIII for the metal. The idea is that if the chelate is more capable of binding the metal than AAIII, the metal will dissociate from the AAIII complex and form a complex with the chelate leading to the decrease in the absorbance at the wavelength. The absorbance (A_{652}) for the Bi(III)–AAIII or Lu(III)–AAIII complex was measured in the absence and in the presence of the ligands over 1 h at rt. The complexation kinetics of NETA with Lu(III) and Bi(III) was determined at pH 4.5 or 4.0, respectively, as hydrolysis occurs at a higher pH.[7,15] The complexation result of the new ligands studied herein was compared to that of DOTA and DTPA, which are known to form a complex with metallic radionuclides with extremely slow and fast kinetics, respectively.[7] A plot of absorbance at 652 nm versus time is shown in Figures 2 and 3. The data in Figure 2 indicate that NETA displayed fast complexation kinetics with Lu(III), while DOTA is sluggish in binding Lu(III). As expected, DTPA was very fast in binding to Lu(III). NETA was also shown to instantly bind to Bi(III), and its complexation with Bi(III) was essentially complete very shortly after the starting point of the measurement (Fig. 3). DOTA consistently exhibited slow complexation kinetics with Bi(III), and DTPA displayed very fast complexation kinetics with Bi(III). The kinetics data indicate that DOTA displayed sluggish complexation with Lu(III) and Bi(III), and the new ligand NETA formed a complex with the metals at a much greater rate than DOTA and a rate comparable to DTPA.

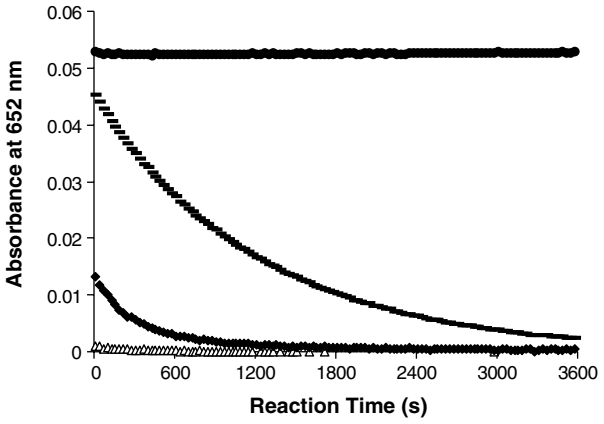


Figure 2. Plot of absorbance (652 nm) versus time of Lu(III)–AAIII (●), NETA (⧫), DOTA (–), and DTPA (Δ) at pH 4.5 (0.15 M NH_4OAc) and 25 °C.

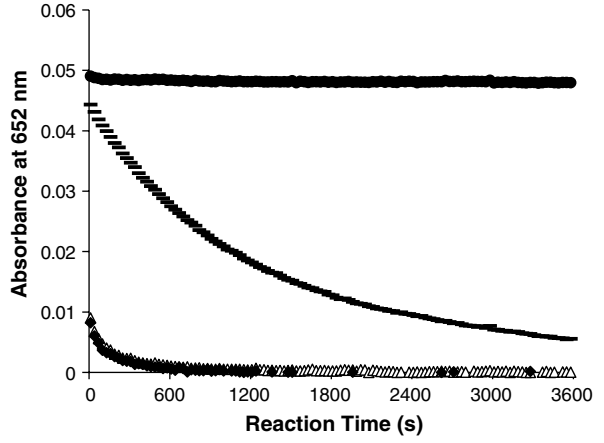


Figure 3. Plot of absorbance (652 nm) versus time of Bi(III)–AAIII (●), NETA (⧫), DOTA (–), and DTPA (Δ) at pH 4.0 (0.15 M NH_4OAc) and 25 °C.

In summary, the efficient and short synthetic route to the bimodal ligand NETA and partially protected NETA analogue **8**, which allows for their preparation in large quantities, is described. The structure of NETA was determined via X-ray crystallographic analysis. The efficient synthetic route to NETA can be applied to preparation of various polyazamacrocyclic ligands for biomedical and radiopharmaceutical applications. The AA(III)-based spectroscopic complexation kinetics data suggest that NETA is a viable chelator for use in RIT applications of ^{177}Lu, ^{212}Bi, and ^{213}Bi.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.084.

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