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Synthesis and biological evaluation of a novel decadentate ligand DEPA

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ABSTRACT

An efficient and short synthetic route to a novel decadentate ligand 7-[2-(bis-carboxymethyl-amino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetic acid (DEPA) with both macrocyclic and acyclic binding moieties is reported. A reproducible and scalable synthetic method to a precursor molecule of DEPA, 1,4,7-tris(*tert*-butoxycarbonylmethyl)tetraazacyclododecane was developed. DEPA was evaluated as a chelator of ^{177}Lu, ^{212}Bi, and ^{213}Bi for potential use in an antibody-targeted cancer therapy, radioimmunotherapy (RIT) using Arsenazo III based spectroscopic complexation kinetics, in vitro serum stability, and in vivo biodistribution studies.

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, iron depletion therapy (IDT),[3] and radioimmunotherapy (RIT).[4] Recently, we have developed novel bimodal polyaminocarboxylate ligands in the NETA ({4-[2-(bis-carboxymethyl-amino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid) and NE3TA ({4-carboxymethyl-7-[2-(carboxymethyl-amino)-ethyl]-[1,4,7]triazonan-1-yl}-acetic acid) series (Fig. 1) possessing both macrocyclic and acyclic moieties.[5–8] The bimodal ligands NETA and NE3TA were evaluated as chelators of various metals such as ^{90}Y, $^{205/6}$Bi, ^{203}Pb, ^{177}Lu, and ^{64}Cu and showed their potential for use in cancer therapeutic and diagnostic applications.[5–7] In particular, NETA was proven to be an effective chelator for RIT, an antibody-targeted radiation therapy[4] that employs a tumor-targeting mAb for selective delivery of a cytotoxic radioisotope.[5] Previous studies have suggested that ^{177}Lu ($t_{1/2}$ = 6.7 d), ^{212}Bi ($t_{1/2}$ = 60.6 m), and ^{213}Bi ($t_{1/2}$ = 45.7 m) are promising radioisotopes for RIT.[9,10] For a safe and potent RIT, a ligand that can form a stable complex with the radioisotope with clinically acceptable complexation kinetics is required.

As an ongoing effort to develop an effective chelator for use in RIT, we designed decadentate DEPA (Fig. 1) having a larger macrocyclic cavity (12-membered ring) than NETA. The most frequently explored polyaminocarboxylates in RIT are 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid (DOTA) and diethylene triamine pentaacetic acid (DTPA) derivatives. The novel bimodal ligand DEPA is proposed to rapidly form a stable complex with a metal having relatively large ionic radii such as Lu(III), Bi(III), and Ac(III) using the donor system integrating both macrocyclic DOTA and acyclic DTPA.

Herein, we report an efficient and short synthetic route to the novel bimodal ligand DEPA ({7-[2-(bis-carboxymethyl-amino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl}-acetic acid, Fig. 1). The precursor molecule to DEPA, 1,4,7-tris(*tert*-butoxycarbonylmethyl)tetraazacyclododecane was prepared from cyclen using a convenient synthetic method involving a simple pH-controlled work-up. The structurally new ligand DEPA was evaluated as a potential chelator of ^{177}Lu, ^{212}Bi, and ^{213}Bi for RIT applications.

Synthesis and isolation of polar macrocyclic polyaminocarboxylates remain challenging. Chromatographic purification of the polar macrocycles is often complicated due to the formation of polar polyalkylated by-products which are quite indistinguishable from the desired product by TLC. An efficient and short method to prepare DEPA (Scheme 1) is based on a coupling reaction of prealkylated precursor molecules **2** and **3**. Reaction of trisubstituted cyclen derivative **2**[13] and *N*,*N*-dialkylated bromide **3**[14] was expected to provide the desired macrocycle **4** while minimizing the formation of polyalkylated by-products. A short and reproducible

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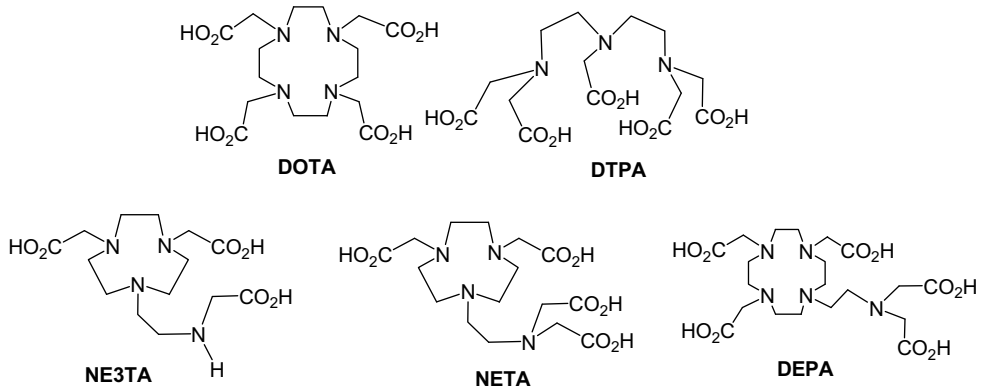
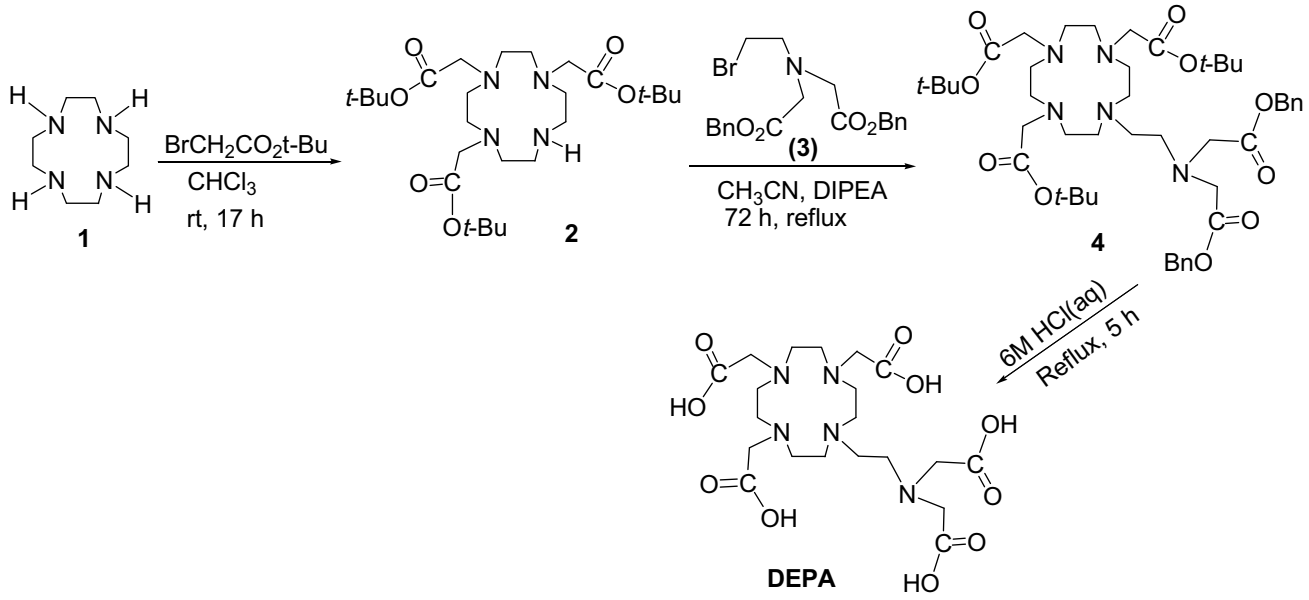


Figure 1. Ligands in preclinical evaluation for RIT.



Scheme 1. Synthesis of DEPA.

synthetic route to trisubstituted cyclen **2** which appears as a non-UV responsive tailing spot on TLC was developed since the known syntheses required either a lengthy procedure or complicated column chromatographic purification of the polar macrocycle.[12,13] The efficient synthetic procedure reported herein provides for isolation of **2** by a simple pH-controlled work-up without complicated column chromatography in highly reproducible isolated yield. When no base and an equimolar molar quantity of *tert*-butyl bromoacetate (3.0 equiv) were employed, **2** was actually produced in a higher yield (47%). The base-promoted coupling reaction of **2** and *N*,*N*-dialkylated bromide **3** in CH_3CN successfully provided **4** in moderate yield (56%). Isolation of **4** having benzyl groups which could be monitored by HPLC and TLC analysis was achieved by flash column chromatography. Both benzyl and *tert*-butyl groups in **4** were removed by treatment with 6 M HCl(aq) to afford the desired chelator DEPA.

The complexation kinetics of the ligand DEPA with Bi(III) and Lu(III) was determined using a well-known spectroscopic competing reaction with Arsenazo III (AAIII) according to a modification of a previously reported procedure.[5] AAIII is known to form a weak complex with many different metals producing a UV–vis absorbance maximum at ~652 nm,[15] while uncomplexed AAIII absorbs little at this wavelength. The absorbance (A_{652}) for the AAIII–Lu(III) or AAIII–Bi(III) complex was measured in the absence and in the presence of the ligands over 1 h at room temperature. The complexation kinetics of Lu(III) and Bi(III) with DEPA was determined

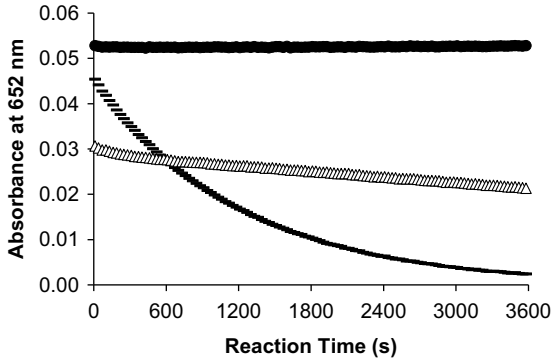


Figure 2. Plot of absorbance (652 nm) versus time of Lu(III)–AAIII (●), DOTA (-), and DEPA (△) at pH 4.5 (0.15 M NH_4OAc) and 25 °C.

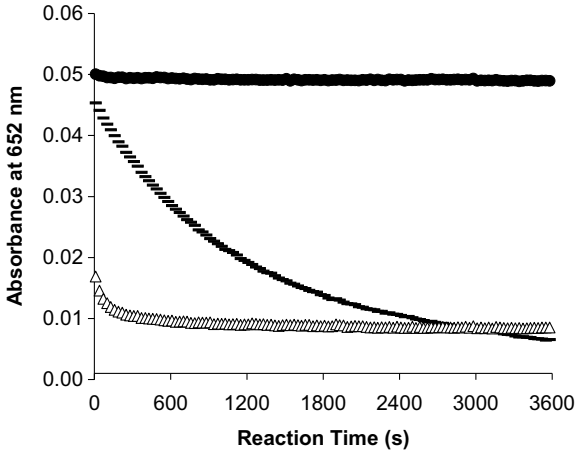


Figure 3. Plot of absorbance (652 nm) versus time of Bi(III)–AAIII (●), DOTA (-), and DEPA (△) at pH 4.0 (0.15 M NH_4OAc) and 25 °C.

Table 1
In vitro serum stability of ^{177}Lu–DOTA, ^{177}Lu–DEPA, and $^{205/6}$Bi–DEPA at pH 7 and 37 °C

Time (h)	Radiolabeled complex		
	^{177}Lu–DOTA	^{177}Lu–DEPA	$^{205/6}$Bi–DEPA
0	100	100	100
0.25	100.5	100	100
0.5	99.0	100	100
1	100.1	100	100
2	99.3	100	100
4	97.0	100	100
6	97.5	100	100
24	98.2	100	100
48	91.2	100	100
96	89.6	100	100
120	—	—	100
192	—	—	100
288	—	—	100
336	—	—	100

at pH 4.5 or 4.0, respectively, as hydrolysis occurs at a higher pH and radiolabeling reaction are routinely performed at this pH.[7,16] The complexation of the new ligands studied herein was compared to that of DOTA, which is known to form an inert complex with metallic radionuclides, but with extremely slow kinetics.[5] A plot of absorbance at 652 nm versus time is shown in Figures 2 and 3. The data in Figure 2 indicate that decadentate DEPA is quite slow and inefficient in binding Lu(III) resulting in slight decrease in the absorbance (~0.02) for AAIII–Lu(III) (Fig. 2). At this point, it is not clear why the more flexible DEPA with 10 donor groups displayed slow complexation kinetics with Lu(III) versus the octadentate DOTA ligand. The spectroscopic kinetics data indicate that DOTA displayed sluggish complexation with both Lu(III) and Bi(III), while DEPA showed enhanced complexation kinetics with Bi(III) as compared to DOTA. The complexation of DEPA with Bi(III) was almost complete at the starting point (To), although the ligand produces a reaction equilibrium curve at the absorbance (A_{652} = ~0.01).

The new ligand DEPA was radiolabeled with ^{177}Lu and $^{205/6}$Bi (a surrogate of ^{212}Bi and ^{213}Bi) and the corresponding radiolabeled complexes were evaluated for in vitro serum stability as described previously.[6,17,18] For comparison, DOTA was also radiolabeled with ^{177}Lu. DEPA and DOTA (0.25 M NH_4OAc buffer, pH 4.0) was radiolabeled with ^{177}Lu at 45 °C for 0.5 h to afford ^{177}Lu–DEPA (R_f = 0.6) and ^{177}Lu–DOTA (R_f = 0.4) in respective radiochemical yields of 90% and 95% as determined by radio-TLC. DEPA (0.25 M NH_4OAc buffer, pH 5.0) was successfully radiolabeled with $^{205/6}$Bi at room temperature for 1 h to afford $^{205/6}$Bi–DEPA in 96% yield (radio-TLC).[6,18] ^{177}Lu–DOTA, ^{177}Lu–DEPA, and $^{205/6}$Bi–DEPA were purified from unbound ^{177}Lu or $^{205/6}$Bi by ion-exchange chromatography using a Chelex-100 column (1 mL volume bed, 100–200 mesh, Na^+ form, Bio-Rad, Richmond, CA) eluted with PBS (pH 7.4). In vitro serum stability of the purified radiolabeled complexes was performed to determine if DEPA or DOTA radiolabeled with ^{177}Lu or $^{205/6}$Bi remained stable without loss of the radionuclide in human serum. This was assessed by measuring the transfer of radionuclide from the complex to serum proteins. The data in Table 1 indicate that $^{205/6}$Bi–DEPA was extremely stable in serum, and no radioactivity was released over 14 days (Table 1). ^{177}Lu–DEPA remained intact without being dissociated in serum. However, 10% of the radioactivity was released from ^{177}Lu–DOTA in 4 days.

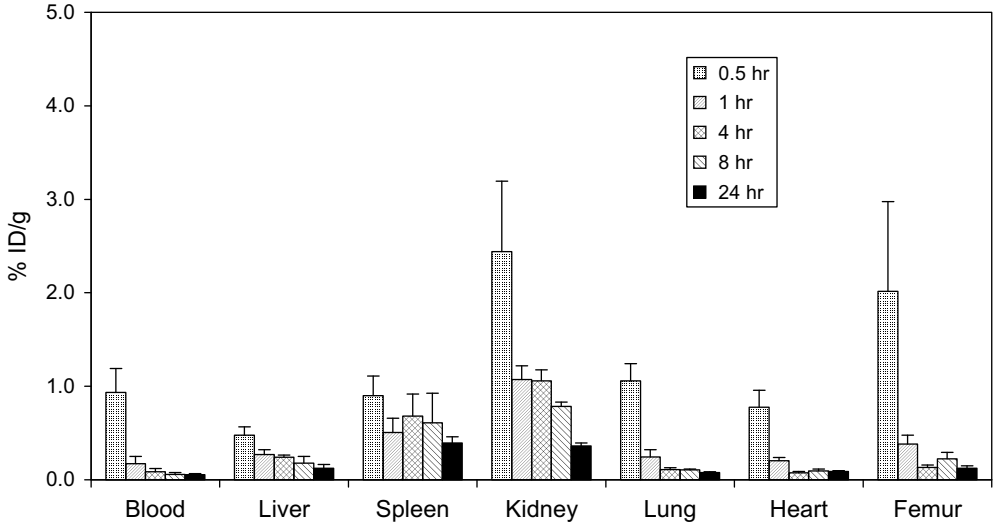


Figure 4. Biodistribution of 205/6Bi–DEPA (iv injection) in non-tumor bearing athymic mice.

Based on the promising data obtained from the AAIII based spectroscopic complexation kinetics and serum stability experiments, the stability of $^{205/6}$Bi–DEPA was further evaluated by performing a biodistribution study in normal athymic mice as described previously.[8] Blood levels and organ uptake of the radiolabeled complexes in mice were measured at five time points, 0.5, 1, 4, 8, and 24 h post-injection of $^{205/6}$Bi–DEPA. The data in Figure 4 illustrate that DEPA radiolabeled with $^{205/6}$Bi was essentially inert in vivo and rapidly cleared from the body. Radioactivity that was detected in the blood and the organs was less than 2.44 %ID/g at all points. At 24 h post-injection, the %ID/g in the kidneys and spleen was 0.36 ± 0.03% and 0.39 ± 0.07%, respectively, which was slightly higher than that observed in other organs. The bone accumulation of the radioactivity was 2.02 ± 0.96 %ID/g at 0.5 h which rapidly decreased to 0.38 ± 0.09 %ID/g at 1 h. Previously, we reported that ^{177}Lu–NETA displayed very low organ uptake and rapid blood clearance, while $^{205/6}$Bi–NETA exhibited very high retention in liver at the longer time intervals (5.93 ± 0.78 %ID/g at 0.5 h and 7.31 ± 1.521 %ID/g at 24 h) due to possible dissociation of the complex in vivo. Although NETA formed a stable complex with Lu(III) (89 pm), the ligand seems to be inadequate for larger metal Bi(III) (117 pm) due to its smaller cavity size compared to the macrocyclic ring in DEPA. The in vivo biodistribution result suggest that the enhanced in vivo stability of $^{205/6}$Bi–DEPA compared to $^{205/6}$Bi–NETA may result from size-match between Bi(III) and macrocyclic DOTA backbone.[19]

In summary, the novel decadentate ligand DEPA having both macrocyclic and acyclic metal binding moieties was efficiently prepared. The complexation stability and kinetics data suggest that DEPA displayed more rapid and substantial complexation with Bi(III) as compared to DOTA, but appears to be slow in binding Lu(III). ^{177}Lu–DEPA was found to be stable in serum, while considerable amount of ^{177}Lu (10%) was released from ^{177}Lu–DOTA over 4 days. DEPA radiolabeled with $^{205/6}$Bi was very stable in human serum for 2 weeks and displayed excellent in vivo stability. The complexation kinetics, serum stability, and in vivo biodistribution data confirm the potential of DEPA as a viable chelator of ^{212}Bi and ^{213}Bi and validate the synthesis of a bifunctional derivative for RIT.

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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.2008.09.063.

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