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Synthesis and evaluation of a bifunctional chelate for development of Bi(III)-labeled radioimmunoconjugates

Mamta Dadwal [a], Chi Soo Kang [a], Hyun A. Song [a], Xiang Sun [a], Anzhi Dai [a], Kwamena E. Baidoo [b], Martin W. Brechbiel [b], Hyun-Soon Chong [a,*]

[a] Chemistry Division, Biological, Chemical, and Physical Sciences Department, Illinois Institute of Technology, 3101 S. Dearborn St., LS 182, Chicago, IL 60616, USA
[b] Radioimmune and Inorganic Chemistry Section, Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA

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ABSTRACT

A new bifunctional ligand *C*-DEPA was designed and synthesized as a component for antibody-targeted radiation therapy (radioimmunotherapy, RIT) of cancer. *C*-DEPA was conjugated to a tumor targeting antibody, trastuzumab, and the corresponding *C*-DEPA-trastuzumab conjugate was evaluated for radiolabeling kinetics with $^{205/6}$Bi. *C*-DEPA-trastuzumab conjugate rapidly bound $^{205/6}$Bi, and $^{205/6}$Bi-*C*-DEPA-trastuzumab conjugate was stable in human serum for 72 h. The in vitro radiolabeling kinetics and serum stability data suggest that *C*-DEPA is a potential chelate for preclinical RIT applications using ^{212}Bi and ^{213}Bi.

Radioimmunotherapy (RIT) using α-particle emitting radionuclides has been proven to be effective in treatment of leukemia, lymphoma, and micrometastatic cancers.[1–3] Among those radionuclides for α-RIT, ^{212}Bi ($t_{1/2}$ = 60.6 m) and ^{213}Bi ($t_{1/2}$ = 45 m) with high α energy (5–8 MeV) and a short emission range (50–80 μm) were proposed to selectively and effectively kill tumor cells with a minimum damage to normal cells.[3] Preclinical and clinical studies have demonstrated therapeutic efficacy of α-particle emitting radionuclides in treatment of the blood-born cancers.[4–7] In particular, RIT using monoclonal antibody (mAb) labeled with ^{213}Bi was found to be effective in the treatment of chemo- and external radiation-resistant leukemia cells.[8] An effective bifunctional chelator of ^{212}Bi or ^{213}Bi possessing a functional group for conjugation to a tumor targeting antibody is an essential component for α-RIT.[1] Several less optimal bifunctional ligands including *C*-DOTA(2-(4-nitrobenzyl)-1,4,7,10-tetraazacyclotetradecane-1,4,7,10-tetra-acetic acid) and *C*-DTPA ((2-(4-nitrobenzyl)-diethylenetriamine pentaacetic acid) have been explored for RIT of ^{213}Bi and ^{212}Bi.[1,9–11] *C*-DTPA displays rapid and high yield radiolabeling with Bi(III), while the chelator produces an unstable complex both in vitro and in vivo.[1] The excellent in vitro or in vivo stability of Bi(III) complex of DOTA is reported.[1] However, slow kinetics of DOTA in binding Bi(III) under mild conditions limits RIT application of the ligand.[1] Development of a superior bifunctional ligand that can tightly and rapidly bind the short-lived radioisotopes is required for safe and potent α-RIT application.

We previously reported that decadentate DEPA (7-[2-(bis-carboxymethyl-amino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraazacyclododec-1-yl-acetic acid) is a promising chelator for the radioisotopes of Bi(III).[12] DEPA was designed to possess a donor system integrating both macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid) and acyclic DTPA (diethylenetriamine pentaacetic acid) and expected to rapidly form a stable complex with a metal having relatively large ionic radii such as Bi(III), and Ac(III) based on cooperative binding of both the macrocyclic and acyclic moieties. $^{205/6}$Bi-DEPA was stable in human serum without any loss of the radioactivity for two weeks and displayed excellent in vivo stability in mice.[12] We herein report synthesis and evaluation of the bifunctional analog, *C*-DEPA, as a potential chelator of the Bi(III) radionuclides.

The synthetic route to the new bifunctional ligand *C*-DEPA is outlined in Schemes 1 and 2. We wanted to prepare the macrocyclic backbone compound **5** via nucleophilic ring opening reaction of *N*-BOC protected aziridine **2** by cyclen (1,4,7,10-tetraazacyclododecane) **1** followed by removal of the BOC group in **4** (Scheme 1). Alkylation of **5** was expected to provide the key precursor molecule **10** for the synthesis of *C*-DEPA (Scheme 2). As expected, selective mono-substitution of **1** with aziridine derivative **2** produced **4** by the opening of the aziridine ring in **2** at the less hindered methylene carbon. However, the isolated yield of **4** was low as the

* Corresponding author. Tel.: +1 312 567 3235; fax: +1 312 567 3494.
E-mail address: Chong@iit.edu (H.-S. Chong).

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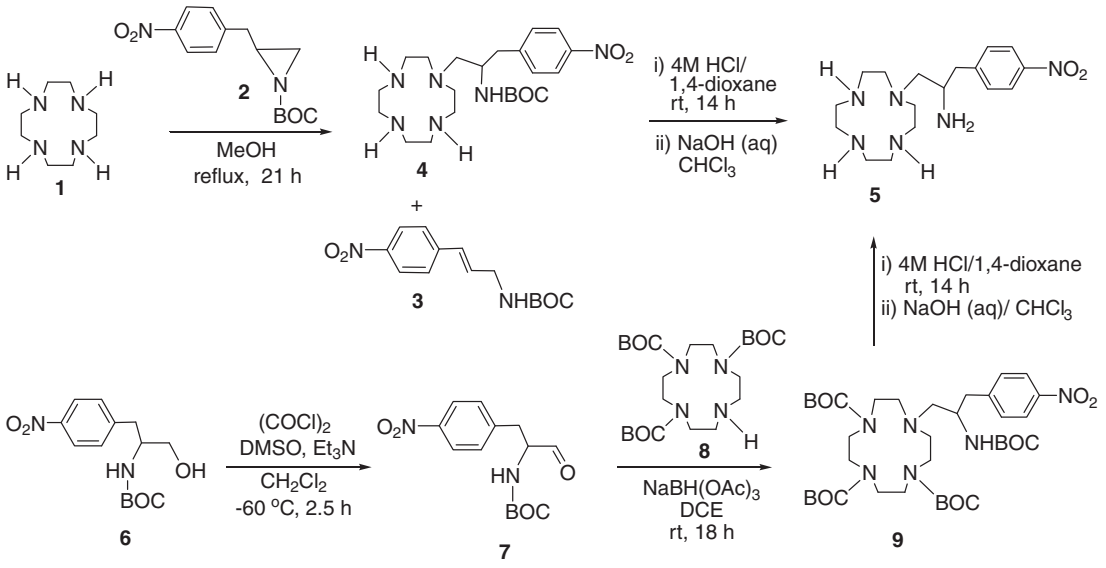
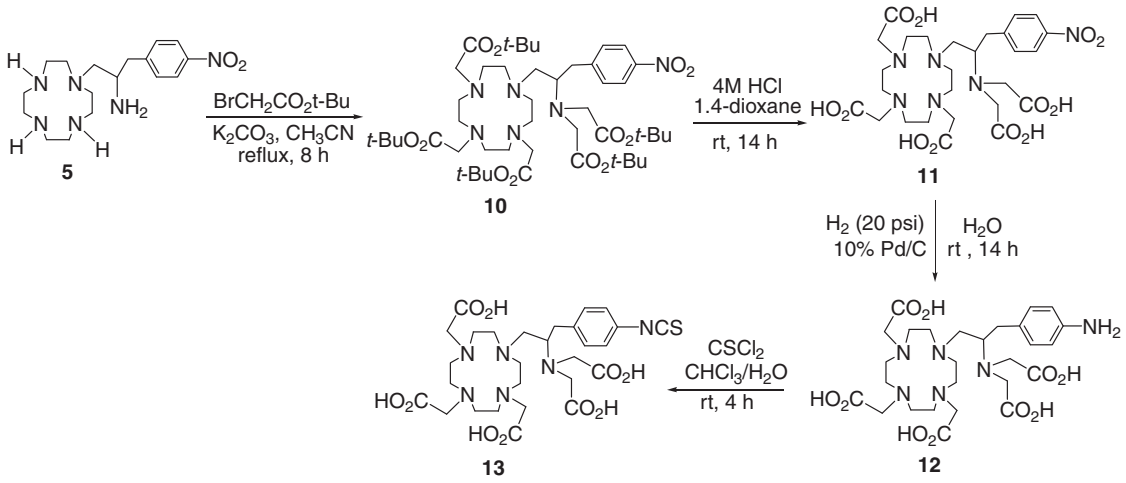


Scheme 1. Synthesis of key intermediate compound **5**.



Scheme 2. Synthesis of *C*-DEPA (**11**) and *C*-DEPA-NCS (**13**).

reaction also formed polysubstitution byproducts and elimination product **3**, and purification of the polar intermediate compound **4** was challenging. Different reaction conditions at varying temperature, time, or solvent were examined to optimize the isolated yield of **4**. The reaction of **1** (1 equiv), **2** (1 equiv), and DIPEA (3 equiv) in DMF (45 °C, 25 h) provided elimination product **3** as the exclusive product. The reaction of **1** with **2** in CH_3CN for 10–24 h provided **4** in poor yield (12–21%). When the reaction was refluxed in CH_3OH for 48 h, a mixture of **3** and **4** in a 1:1 mole ratio was obtained as evidenced by analytical HPLC. The best isolated yield of **4** (56%) was achieved from the reaction of **1** (1 equiv) and **2** (2 equiv) in MeOH under reflux for 21 h. The BOC group in **4** was removed by the treatment of **4** with 4 M HCl in 1,4-dioxane to provide **5**. Regiochemistry in the ring opening of aziridine analog **2** was confirmed by independently preparing compound **5** starting from **6** (Scheme 1). Swern oxidation of **6** provided **7** in good isolated yield (78%), and BOC-protected macrocyclic compound **9** was obtained from reductive amination of **7** with **8**[13] using sodium triacetoxyborohydride provided in excellent isolated yield (85%). Removal of the BOC groups in **9** using 4 M HCl in 1,4-dioxane provided **5**. Preparation of **5** via this alternative route was found to be more reproducible and practical as compared to the original synthetic route via selective substitution of **1** which we initially accomplished. The ^{1}H and ^{13}C NMR spectral data of **5** obtained from two different synthetic routes were essentially identical and confirms the regiochemistry observed in the ring opening of the aziridine compound **2**. Synthesis of the desired bifunctional ligands *C*-DEPA and *C*-DEPA-NCS is shown in Scheme 2. Reaction of **5** with *tert*-butyl bromoacetate in acetonitrile produced compound **10**. This substitution reaction turned out to be very challenging, and **10** was isolated in poor yield (10%) using semi-prep HPLC purification, and the isolated yield of the reaction is yet to be improved. Subsequent removal of the *tert*-butyl groups in **10** using HCl(g) in 1,4-dioxane provided *C*-DEPA (**11**). The nitro group in **11** was transformed into the amino group to provide **12**. Reaction of **12** with thiophosgene provided the bifunctional ligand *C*-DEPA-NCS (**13**) containing the isothiocyanate group for conjugation to an antibody.

C-DEPA-NCS was conjugated to a tumor targeting antibody, trastuzumab which was approved by the FDA for the treatment

Table 1
Radiolabeling efficiency (%) of *C*-DEPA-trastuzumab, *C*-DTPA-trastuzumab, and *C*-DOTA-trastuzumab conjugates with $^{205/6}$Bi (pH 5.5, RT)[a]

Time (min)	*C*-DEPA-trastuzumab	*C*-DTPA-trastuzumab	*C*-DOTA-trastuzumab[b]
1	88.7 ± 1.4	93.6 ± 0.5	8.4 ± 1.9
5	90.4 ± 1.0	95.1 ± 0.3	17.2 ± 4.3
10	91.2 ± 1.2	95.3 ± 0.6	28.7 ± 4.5
20	91.1 ± 1.4	96.0 ± 0.3	38.0 ± 4.9
30	92.6 ± 0.8	95.9 ± 0.3	49.7 ± 9.0
60	92.7 ± 0.8	95.8 ± 0.1	60.2 ± 8.0

[a] SE-HPLC (mobile phase: PBS, pH 7.4). Radiolabeling efficiency (mean ± standard deviation %) was measured in triplicate.
[b] The data was cited for comparison (Ref. 16)

Table 2
In vitro serum stability (SE-HPLC) of $^{205/6}$Bi-radiolabeled trastuzumab-ligand conjugates (pH 7 and 37 °C)

Radioimmunoconjugate	Time (h)	$^{205/6}$Bi-bound conjugate complex (%)
$^{205/6}$Bi-*C*-DEPA-trastuzumab	0	100.0
	24	100.0
	48	100.0
	72	100.0
$^{205/6}$Bi-*C*-DTPA-trastuzumab	0	100.0
	24	73.4
	48	76.7
	72	77.0

of metastatic breast cancer and is known to selectively target the HER2 protein over-expressed in various tumors including colon and breast cancers.[14,15] Protein concentration in the *C*-DEPA-trastuzumab conjugate was quantified using the UV spectroscopic method as described in the experimental.[16] The Pb(II)-AAIII based UV–Vis spectrophotometric assay as previously reported[16,17] was used for the determination of the number of *C*-DEPA ligand linked to trastuzumab (L/P ratio). The spectroscopic assay data indicate that *C*-DEPA was conjugated to trastuzumab with a L/P ratio of 0.84:1 (Supplementary data).

The purified *C*-DEPA-trastuzumab conjugate was further evaluated for radiolabeling reaction kinetics with $^{205/6}$Bi ($t_{1/2}$ = 15.3 d for ^{205}Bi; $t_{1/2}$ = 6.24 d for ^{206}Bi), a surrogate of ^{212}Bi and ^{213}Bi at room temperature for 1 h. For comparison, *C*-DTPA-trastuzumab conjugate was prepared and radiolabeled with $^{205/6}$Bi. During the incubation time (1 h), the radiolabeling reaction kinetics was determined by taking aliquots of the reaction solutions at six different time points (1, 5, 10, 20, 30, and 60 min). The components were analyzed using SE-HPLC after challenging the reaction mixture with 10 mM DTPA, and the radiolabeling efficiency (%) at each time point was determined (Table 1 and Supplementary data). The data in Table 1 indicate that *C*-DEPA-trastuzumab conjugate was rapid in binding $^{205/6}$Bi (1 min, 89%; 60 min, 93%) at room temperature which was comparable to labeling of *C*-DTPA-trastuzumab with $^{205/6}$Bi (1 min, 94%; 60 min, 96%). However, *C*-DOTA-trastuzumab conjugate displayed slow complex formation kinetics with $^{205/6}$Bi (60 min, 60%) as previously reported.[16] The result confirms that *C*-DOTA is not qualified for use in RIT applications using the short-lived radioisotopes ^{212}Bi and ^{213}Bi.

In vitro serum stability of the radiolabeled complexes was performed to determine if $^{205/6}$Bi-*C*-DEPA-trastuzumab conjugate remained stable without loss of $^{205/6}$Bi in human serum (Table 2 and Supplementary data). $^{205/6}$Bi-*C*-DTPA-trastuzumab conjugate was evaluated for comparison. The $^{205/6}$Bi-*C*-DEPA-trastuzumab conjugate was prepared at 37 °C and pH 5.5, purified on a PD-10 column, and incubated in human serum (37 °C). At each time point (0, 24, 48, and 72 h), aliquots of the reaction mixture were analyzed using SE-HPLC (PBS, pH 7.4). $^{205/6}$Bi-*C*-DEPA-trastuzumab was stable without release of the radioactivity into serum, and no transchelation of $^{205/6}$Bi from the complex to the serum protein was observed over the course of 3 days. However, ~23% of the radioactivity was released from $^{205/6}$Bi-*C*-DTPA-trastuzumab conjugate in 72 h.

In summary, the new bifunctional ligand *C*-DEPA was prepared, characterized, and conjugated to a tumor-targeting antibody, trastuzumab. The corresponding *C*-DEPA-trastuzumab conjugate was evaluated for complexation kinetics with $^{205/6}$Bi. *C*-DEPA-trastuzumab conjugate rapidly formed a complex with $^{205/6}$Bi with an excellent radiolabeling efficiency, and $^{205/6}$Bi-*C*-DEPA-trastuzumab conjugate was stable in human serum for 3 days. Based on the promising result of the in vitro evaluations, *C*-DEPA will be further evaluated for RIT with ^{212}Bi and ^{213}Bi using tumor bearing mice.

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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.2011.06.107.

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