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Novel ^{64}Cu-radiolabeled bile acid conjugates for targeted PET imaging

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ABSTRACT

A promising bifunctional chelate (*N*-NE3TA) was conjugated to bile acids, cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) as tumor targeting vectors. Bile acid conjugates of *N*-NE3TA (CA–*N*-NE3TA, DCA–*N*-NE3TA, and CDCA–*N*-NE3TA) were comparatively evaluated for complexation with ^{64}Cu, an imaging probe for positron emission tomography (PET). *N*-NE3TA–bile acid conjugates were evaluated for radiolabeling kinetics with ^{64}Cu, and the corresponding ^{64}Cu-radiolabeled conjugates were screened for complex stability in human serum and EDTA solution. The NE3TA–bile acid conjugates instantly bound to ^{64}Cu with excellent radiolabeling efficiency at room temperature. All NE3TA–bile acid conjugates radiolabeled with ^{64}Cu remained inert in human serum for 2 days without releasing a considerable amount of the radioactivity. The ^{64}Cu-radiolabeled complexes were further challenged by EDTA in a 100-fold molar excess. Bile acid–*N*-NE3TA conjugates radiolabeled with ^{64}Cu were quite stable with a minimal transfer of ^{64}Cu to EDTA at 4 h time point. The in vitro data indicate that the bile acid–*N*-NE3TA conjugates deserve further biological evaluation for ^{64}Cu-based targeted PET imaging applications.

A sensitive diagnostic modality, positron emission tomography (PET) has been demonstrated to give highly sensitive detection and staging of cancers.[1–3] Metallic radionuclides such as ^{64}Cu, ^{68}Ga, and ^{86}Y have been explored for PET imaging. Among the radionuclides, ^{64}Cu ($t_{1/2}$ = 12.7 h; $E^{\beta+}_{max}$ = 0.655 MeV; $E^{\beta-}_{max}$ = 0.573 MeV; E^{γ}_{max} = 0.511 MeV) possesses half-life and decay property suitable for PET imaging with extended imaging window.[1,4,5] For development of clinically viable ^{64}Cu-based radiopharmaceuticals for targeted PET imaging, it is essential to employ a bifunctional chelate that can rapidly form a stable complex with Cu(II).[5–7] Rapid radiolabeling of ^{64}Cu with a short half-life by a bifunctional chelate attached to a sensitive biomolecule such as antibodies is required for practical preparations of biologically active ^{64}Cu-radiolabeled complexes. ^{64}Cu-radiolabeled complex must be stable in vivo without undergoing transchelation with other metal-binding proteins or biologically important metals. Cu(II) has a relatively small ionic radius (73 ppm) and is known to display a high affinity for nitrogen and oxygen donor atoms. Various acyclic and macrocyclic polyaminocarboxylate-based chelates including DTPA (diethylenetriamine pentaacetic acid), NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), DOTA (1,4,7,10-tetraazacyclododecane tetraacetic acid), and TETA (2-[1,4,8,11-tetraazacyclotetradecane tetraacetic acid) have been explored for PET imaging applications using ^{64}Cu.[1,4,5]

We previously reported a bifunctional chelate *N*-NE3TA (Fig. 1) containing both acyclic and macrocyclic binding moieties as a promising chelate of ^{64}Cu.[8] *N*-NE3TA rapidly bound to ^{64}Cu under mild conditions, and in vitro and in vivo stability of ^{64}Cu-*N*-NE3TA was favorably compared to ^{64}Cu-radiolabeled complex of *C*-DOTA, one of the most frequently used chelate for PET imaging.[8] Encouraged by the complexation kinetics and stability profile of *N*-NE3TA with ^{64}Cu, we were interested in utilizing the bifunctional chelate for targeted PET imaging using a tumor targeting vector. The primary bile acids (cholic acid and chenodeoxycholic acid) and secondary bile acid (deoxycholic acid) are known to target bile acid receptors or carriers overproduced in hepatic and colorectal cancers.[9–12] The amphifacial bile acids were shown to form helical globular aggregates and enter into the cancer cells due to their great cell permeability and have been explored as a delivery shuttle of anti-cancer agents.[10–12]

We herein report synthesis of bile acid conjugates of *N*-NE3TA and evaluation of the corresponding bile acid–NE3TA conjugates for complexation with ^{64}Cu for targeted PET imaging. The bifunctional chelate *N*-NE3TA was conjugated to tumor-targeting bile

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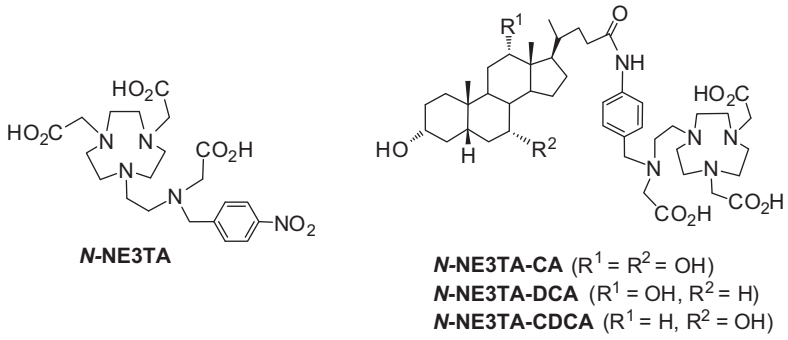
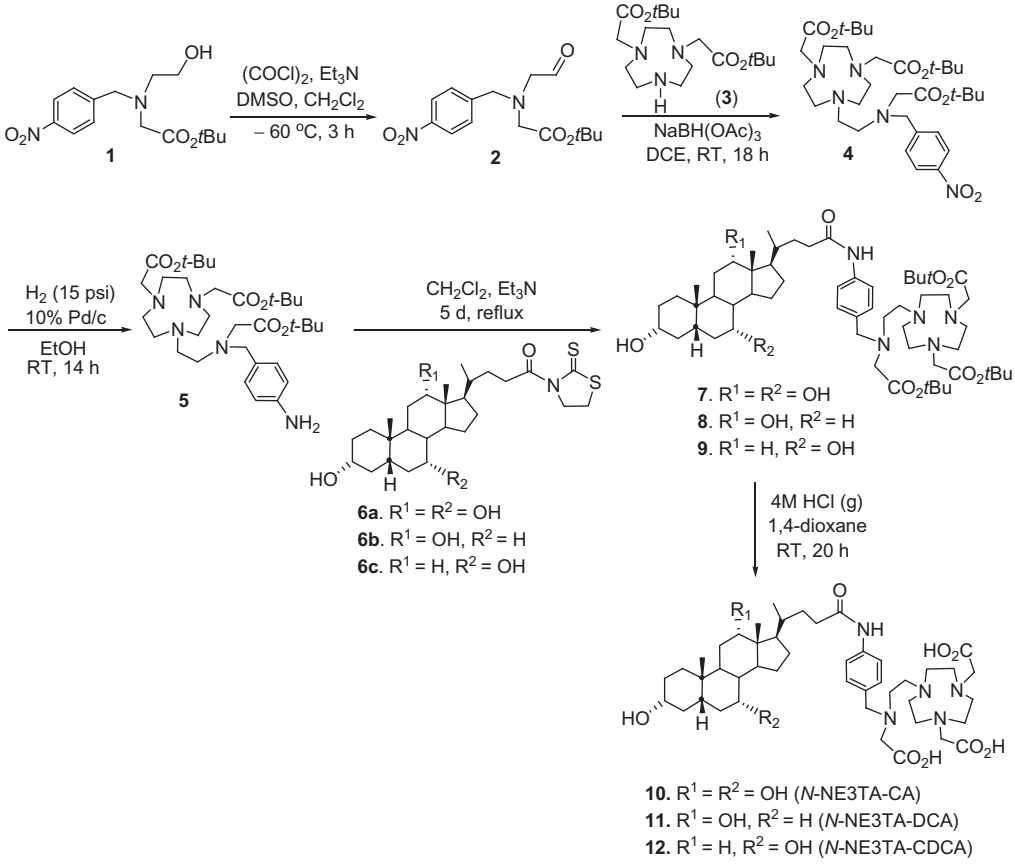


Figure 1. Structure of *N*-NE3TA and *N*-NE3TA–bile acid conjugates.



Scheme 1. Synthesis of *N*-NE3TA analogues and *N*-NE3TA–bile acid conjugates.

acids, cholic acid (CA), deoxycholic acid (DCA), or chenodeoxycholic acid (CDCA). The bile acid conjugates were evaluated for radiolabeling kinetics with ^{64}Cu for PET imaging application. ^{64}Cu-radiolabeled bile acid conjugates were evaluated for complex stability in human serum and a solution of EDTA.

Synthesis of bifunctional *N*-NE3TA analogue **5** and *N*-NE3TA–bile acid conjugates **10**–**12** is shown in Scheme 1. Compound **2** was readily prepared from Swern oxidation of **1**,[13] and reductive amination of **2** with **3**[14] provided the key precursor macrocyclic compound **4**.[13] The nitro group in **4** was converted to the amino group in **5** which further was reacted with an activated bile acid analogue **6a**, **6b**, or **6c** which were prepared from reaction of bile acid with 2-mercaptothiazoline as reported previously.[15] *tert*-Butyl *N*-NE3TA-NH$_2$ (**5**) was reacted with the preactivated cholic acid analogue (CA, **6a**) in the presence of triethylamine under reflux to provide *N*-NE3TA–CA conjugate **7**. Similarly, *N*-NE3TA-DCA and *N*-NE3TA-CDCA analogues **8** and **9** were prepared from reaction of **5** with **6b** and **6c**, respectively. The removal of *tert*-butyl protecting groups in **7**–**9** using 4 M HCl (g) in 1,4 dioxane provided *N*-NE3TA–CA (**10**), *N*-NE3TA–DCA (**11**) and *N*-NE3TA–CDCA (**12**), respectively.

The new *N*-NE3TA–bile acid conjugates were evaluated for radiolabeling reaction kinetics with ^{64}Cu at room temperature (Table 1, Fig. 2, and Supporting information). Each conjugate (20 μg) in 0.25 M NH$_4$OAc (pH 5.5) was radiolabeled with ^{64}Cu (60 μCi) at room temperature. During the reaction time (30 min), the components were withdrawn at the designated time points (1 min, 10 min, and 30 min), and the radiolabeling efficiency (%) was determined using ITLC (20 mM EDTA in 0.15 M NH$_4$OAc). ^{64}Cu-EDTA migrated with the solvent front on TLC (R_f = 0.88), while

Table 1
Evaluation of bile acid conjugates for radiolabeling efficiency (%) with ^{64}Cu (RT, 0.25 M NH_4OAC, pH 5.5) using ITLC*

Time (min)	*N*-NE3TA–CA	*N*-NE3TA–DCA	*N*-NE3TA–CDCA
1	99.7 ± 0.3	89.6 ± 3.8	97.6 ± 0.3
10	99.9 ± 0.1	97.0 ± 0.4	98.2 ± 0.4
30	99.9 ± 0.1	98.9 ± 0.5	99.2 ± 0.1

* Radiolabeling efficiency (mean ± standard deviation %) was measured in triplicate using ITLC (eluent: 20 mM EDTA in 0.15 M NH_4OAc).

Table 2
Evaluation of ^{64}Cu-radiolabeled bile acid conjugates for in vitro stability in serum (37 °C, pH 7) using radio-HPLC*

Day	*N*-NE3TA–CA	*N*-NE3TA–DCA	*N*-NE3TA–CDCA
0	98.0 ± 0.3	97.3 ± 0.8	97.7 ± 0.2
1	97.7 ± 0.3	95.5 ± 1.3	98.7 ± 0.1
2	99.0 ± 0.4	97.0 ± 1.0	99.7 ± 0.3

* Bound complex (mean ± standard deviation %) was measured in duplicate using radio-HPLC (solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN, 0–100% B/15 min, flow rate: 1 mL/min).

^{64}Cu-radiolabeled chelator complexes travel slower on the TLC (R_f = 0.54). The ^{64}Cu-radiolabeled complexes of the conjugates and ^{64}Cu-EDTA were well separated on the ITLC. All *N*-NE3TA bile acid conjugates instantly bound to ^{64}Cu with excellent radiolabeling efficiency (>90%, 1 min time point, Table 1, Fig. 2a) at room temperature. *N*-NE3TA–DCA was slightly slower in binding ^{64}Cu as compared to *N*-NE3TA–CA and *N*-NE3TA–CDCA, although radiolabeling of the conjugate with ^{64}Cu was nearly complete at 30 min time point. All ^{64}Cu-radiolabeled complexes were shown to be stable against EDTA present in the eluent of TLC (Supporting information).

In vitro serum stability of the radiolabeled *N*-NE3TA–bile acid conjugates was performed to determine if the ^{64}Cu-radiolabeled conjugates remained stable without loss of the radioactivity in human serum. This was assessed by measuring the transfer of ^{64}Cu from the complex to human serum proteins using radio-HPLC (Table 2, Fig. 2d, and Supporting information). A fresh solution of ^{64}Cu-radiolabeled conjugates were readily prepared from the reactions of *N*-NE3TA–bile acid conjugates with ^{64}Cu at room temperature and directly used for serum stability studies (pH 7, 37 °C) using radio-HPLC by radio-HPLC analysis (solvent A: 0.1% TFA in H_2O, solvent B: 0.1% TFA in CH_3CN, 0–100% B/15 min, flow rate: 1 mL/min). The trace related to ^{64}Cu bound to serum (t_R = 2.5 min) was clearly distinguished from the peaks of the ^{64}Cu-*N*-NE3TA–bile acid conjugate (t_R = 10–11 min for *N*-NE3TA–CA and t_R = 11–12 min for *N*-NE3TA–DCA and *N*-NE3TA–CDCA). All ^{64}Cu-radiolabeled conjugates remained quite stable in human serum for 2 days as evidenced by radio-HPLC. While a tiny amount of ^{64}Cu (<1.0%) was detected from ^{64}Cu-*N*-NE3TA–CA and ^{64}Cu-*N*-NE3TA–CDCA over 2 days, ^{64}Cu-*N*-NE3TA–DCA released ~3% of the radioactivity at 48 h time points (Fig. 2d) and appears to be least stable in serum among the ^{64}Cu-radiolabeled conjugates tested.

^{64}Cu-radiolabeled *N*-NE3TA–bile acid conjugates were further challenged for complex stability in an excess amount of EDTA solution. ^{64}Cu-radiolabeled complexes were treated with a solution of EDTA at a 100-fold molar excess, and the resulting solution (pH 5.5) was incubated at 37 °C for 24 h. A sample was withdrawn at

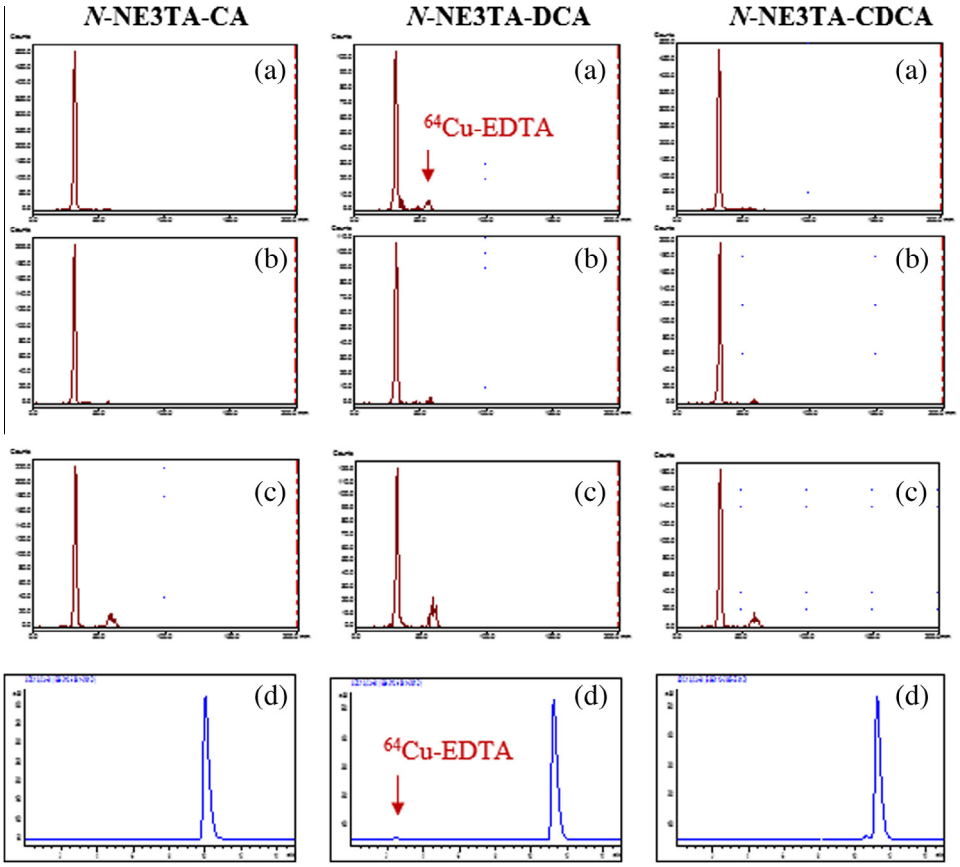


Figure 2. (a) TLC chromatogram of ^{64}Cu-radiolabeled complexes at 1 min time point of radiolabeling; (b) TLC chromatogram of ^{64}Cu-radiolabeled complexes in a solution of EDTA at 100-fold molar excess (1 h time point); (c) TLC chromatogram of ^{64}Cu-radiolabeled complexes in a solution of EDTA at 100-fold molar excess at 24 h; (d) HPLC chromatogram of ^{64}Cu-radiolabeled complexes in human serum at 48 h.

Table 3
Stability of ^{64}Cu-radiolabeled complexes against EDTA at a 100-fold molar excess (37 °C)*

Time (h)	*N*-NE3TA–CA	*N*-NE3TA–DCA	*N*-NE3TA–CDCA
0	100.0 ± 0.1	99.8 ± 0.1	99.5 ± 0.6
1	99.4 ± 0.0	97.5 ± 0.1	97.9 ± 0.3
4	98.5 ± 0.4	95.2 ± 0.9	97.1 ± 0.2
24	88.4 ± 0.4	80.4 ± 1.3	88.9 ± 0.4

* Bound complex (mean ± standard deviation %) was measured in duplicate using ITLC (eluent: 20 mM EDTA in 0.15 M NH_4OAc).

different time points (0 h, 1 h, 4 h, and 24 h) and analyzed using ITLC (Table 3, Fig. 2b and c, and Supporting information). All ^{64}Cu-radiolabeled conjugates remained bound against EDTA challenge up to 4 h time point (Supporting information), although slow release of the radioactivity was observed over 24 h. A small portion of the activity (<5%) was transferred from the complexes to EDTA (99.4% for *N*-NE3TA–CA, 97.9% for *N*-NE3TA–CDCA, 97.5% for *N*-NE3TA–DCA) at 1 h time point (Fig. 2b). ^{64}Cu-*N*-NE3TA–DCA was shown to be less tolerant of EDTA treated. ~20% of ^{64}Cu was dissociated from the complex at 24 h time point (Fig. 2c). Approximately 10% of the radioactivity was leaked from ^{64}Cu-*N*-NE3TA–CA and ^{64}Cu-*N*-NE3TA–CDCA at 24 h time point (Fig. 2c).

In summary, *N*-NE3TA–bile acid conjugates were evaluated for complexation kinetics and stability with ^{64}Cu for potential use in targeted PET imaging. All *N*-NE3TA–bile acid conjugates rapidly and almost completely bound to ^{64}Cu. The corresponding ^{64}Cu-radiolabeled complexes remained quite stable in human serum, and no considerable release of the radioactivity was observed with the complexes. When rigorously challenged by excess amount of EDTA at 37 °C for 24 h, a small amount of the radioactivity (>10%) was dissociated from ^{64}Cu-radiolabeled *N*-NE3TA–bile acid conjugates. The in vitro complexation kinetics and stability data suggest that the *N*-NE3TA–bile acid conjugates can be further evaluated for targeted PET imaging using animals.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.008.

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