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A ^{68}Ga complex based on benzofuran scaffold for the detection of β-amyloid plaques

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ABSTRACT

Since the imaging of β-amyloid (Aβ) plaques in the brain is believed to be a useful tool for the early diagnosis of Alzheimer's disease (AD), a number of imaging probes to detect Aβ plaques have been developed. Because the radionuclide ^{68}Ga ($t_{1/2}$ = 68 min) for PET imaging could become an attractive alternative to ^{11}C and ^{18}F, we designed and synthesized a benzofuran derivative conjugated with a ^{68}Ga complex (^{68}Ga-DOTA-C3-BF) as a novel Aβ imaging probe. In an in vitro binding assay, Ga-DOTA-C3-BF showed high affinity for Aβ(1-42) aggregates (K_i = 10.8 nM). The Ga-DOTA-C3-BF clearly stained Aβ plaques in a section of *Tg2576* mouse, reflecting the affinity for Aβ(1-42) aggregates in vitro. In a biodistribution study in normal mice, ^{68}Ga-DOTA-C3-BF displayed low initial uptake (0.45% ID/g) in the brain at 2 min post-injection. While improvement of the brain uptake of ^{68}Ga complexes appears to be essential, these results suggest that novel PET imaging probes that include ^{68}Ga as the radionuclide for PET may be feasible.

The deposition of β-amyloid (Aβ) peptides in the brain is a well-established biomarker of Alzheimer's disease (AD).[1] Since positron emission tomography (PET), which is one of the non-invasive techniques, is superior in terms of quantitative capability and unlimited depth penetration, imaging of Aβ plaques with PET is believed to be an attractive tool for the diagnosis of AD.[2] In the past decade or so, many PET probes, such as [^{11}C]PiB,[3] [^{18}F]florbetaben,[4] [^{18}F]fultemetamol,[5] [^{18}F]florbetapir,[6] and [^{18}F]AZD4694,[7] have been developed and exhibited utility for imaging Aβ plaques in AD brain. In particular, [^{18}F]florbetapir, [^{18}F]flutemetamol, and [^{18}F]florbetaben were approved for clinical use by the U.S. Food and Drug Administration (FDA) in 2012, 2013, and 2014, respectively. However, since ^{11}C ($t_{1/2}$ = 20 min) and ^{18}F ($t_{1/2}$ = 110 min) are produced in a cyclotron, which is an expensive facility, these radionuclides have limited availability for PET. As the world's population ages, the number of patients with AD is expected to increase rapidly. Therefore, attractive Aβ imaging probes for PET, which is valuable in terms of routine use, are strongly needed.

^{68}Ga ($t_{1/2}$ = 68 min) is an attractive alternative to ^{11}C and ^{18}F as a radionuclide for PET because it is obtained from a ^{68}Ge/^{68}Ga generator system, which has the major advantage of clinical use without the need for an on-site cyclotron.[8,9] Recently, many imaging probes conjugated with ^{68}Ga complexes for diagnosing various diseases have been developed.[10] [^{68}Ga]DOTATOC, which targets the somatostatin receptor type 2, has been tested clinically and demonstrated its utility for diagnosing cancer.[11] Recently, some probes conjugated with ^{99m}Tc, ^{64}Cu, and ^{111}In complexes have been developed to detect Aβ plaques.[12–14] However, Aβ imaging probes conjugated with ^{68}Ga complexes have never been reported, neither in clinical trials nor in pre-clinical studies.

In exploratory research for useful imaging probes, we have developed ^{11}C-, ^{18}F-, ^{123}I-, and ^{99m}Tc-labeled benzofuran derivatives that showed high affinity for Aβ aggregates in vitro and in vivo.[15–22] Furthermore, almost all compounds showed desirable pharmacokinetics in the brain to image Aβ plaques. These results strongly indicate that the benzofuran scaffold is useful for the development of Aβ imaging probes. Therefore, we designed and synthesized a novel ^{68}Ga-labeled benzofuran derivative with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), which is one of the most useful chelators to form a complex with Ga.[23] Furthermore, because DOTA is a well-known chelator which can form a complex with various metals, including radionuclides (^{68}Ga, ^{64}Cu, ^{111}In, and ^{90}Y) and non-radionuclides (Gd, Eu, and Ln), a novel benzofuran derivative with DOTA has great potential to develop imaging probes for PET, single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and optical imaging.[14,24,25] Then, we evaluated its binding affinity

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for Aβ aggregates in vitro and pharmacokinetics in normal mice. To our knowledge, this is the first time that an Aβ imaging probe conjugated with a ^{68}Ga complex has been proposed.

A novel benzofuran derivative (Ga-DOTA-C3-BF) was prepared as shown in Scheme 1. The synthesis of 4-(5-(3-bromopropoxy)benzofuran-2-yl)-*N*,*N*-dimethylaniline was achieved by a previously reported method.[20] Then, 4-(5-(3-bromopropoxy)benzofuran-2-yl)-*N*,*N*-dimethylaniline was joined to tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate to generate compound **1**. After deprotection of the *tert*-butyl groups in **1**, **2** (Ga-DOTA-C3-BF) was directly prepared by a reaction with $Ga(NO_3)_3$ at pH 4–5. We synthesized [^{68}Ga]**2** (^{68}Ga-DOTA-C3-BF) according to procedures described previously with some modifications (Scheme 2).[26] After deprotection of the *tert*-butyl groups in **1**, the product in 0.2 M ammonium acetate buffer (pH 5.8) was added to ^{68}Ga solution and heated at 100 °C for 10 min. The radiochemical identity of ^{68}Ga-DOTA-C3-BF was verified by co-injection with non-radioactive compounds from HPLC profiles (Fig. 1). ^{68}Ga-DOTA-C3-BF was obtained in a radiochemical yield of 26% with a radiochemical purity of >99% after purification by HPLC.

An in vitro binding experiment to quantify the affinity of Ga-DOTA-C3-BF (**2**) for Aβ(1-42) aggregates was carried out with [^{125}I]6-iodo-2-(4′-dimethylamino)phenylimidazo[1,2]pyridine (IMPY) as the competitive ligand (Fig. 2). IMPY is the only

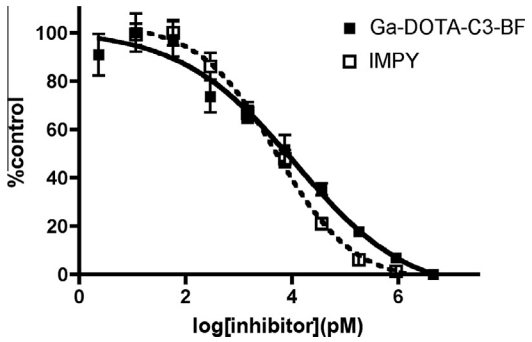
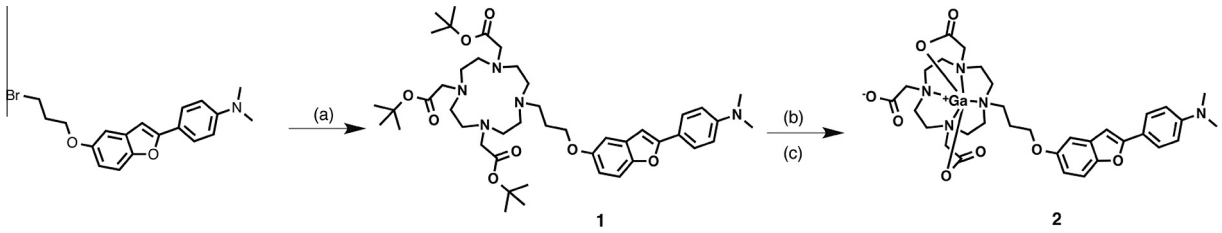


Figure 2. Inhibition curves of Ga-DOTA-C3-BF and IMPY for the binding of [^{125}I]IMPY to Aβ(1-42) aggregates.

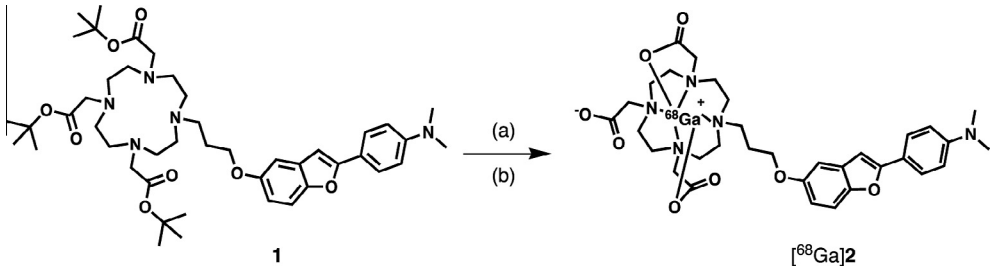
Table 1
Inhibition constants (K_i) for binding of Ga-DOTA-C3-BF determined using [^{125}I]IMPY as the ligand in Aβ(1-42) aggregates

Compound	K_i[a] (nM)
Ga-DOTA-C3-BF (**2**)	10.8 ± 0.57
IMPY	4.47 ± 1.80

[a] Values are the mean ± standard error of the mean for 3 independent experiments.



Scheme 1. Reagents: (a) tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate, K_2CO_3, CH_3CN. (b) TFA. (c) $Ga(NO_3)_3{\cdot}10H_2O$, H_2O.



Scheme 2. Reagents: (a) TFA. (b) ^{68}Ga solution, 0.2 M NH_4OAc.

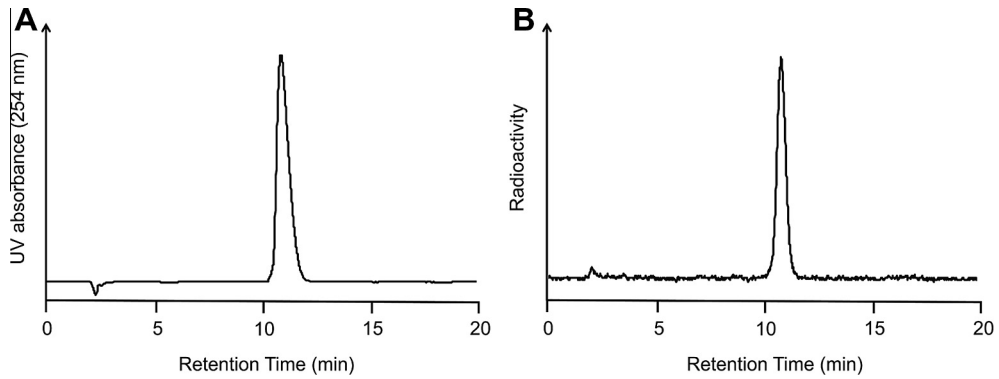


Figure 1. Typical HPLC profiles of Ga–DOTA-C3-BF (A) and ^{68}Ga-DOTA-C3-BF (B).