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Synthesis and preliminary biological evaluation of a technetium-99m labeled thymidine analog

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Abstract

The synthesis and labeling of ^{99m}Tc-N^3-{N'-[2-sulfanyl-ethylamino)acetyl]-2-aminoethyl-sulfanyl-1-hexanamide}thymidine (^{99m}Tc-NHT) were studied. In the presence of sodium glucoheptonate (GH) and ethylene diamine tetraacetic acid (EDTA), ^{99m}Tc-NHT was obtained by using bisaminoethanethiol (N_2S_2) as a bifunctional coupling agent. The radiochemical purity of the ^{99m}Tc-NHT was over 95%. Biodistribution of ^{99m}Tc-NHT was performed in hepatoma HepA tumor-bearing mice. At 2 h p.i., the ratios of tumor-to-muscle, tumor-to-bone and tumor-to-blood were 4.41 ± 0.32, 2.45 ± 0.24 and 1.51 ± 0.18, respectively.

Keywords: Synthesis; ^{99m}Tc-NHT; Biodistribution; Thymidine analog; Tumor imaging

In clinical oncology, 2′-deoxy-2′-[^{18}F]fluoro-D-glucose (^{18}F-FDG), a glucose derivative, has been widely used for tumor imaging with positron emission tomography (PET) in recent years. However, ^{18}F-FDG is a non-specific tracer for tumor imaging since glucose is highly utilized by many other cells, such as macrophages found in inflammatory lesions [1,2].

To overcome this inconvenience of FDG, many studies have been focus on the development of a variety of DNA precursors [1,3–5]. Specifically, the labeled thymidine analog can target the proliferative activity of malignant lesions [6,7], several useful ligands, such as ^{11}C-labeled nucleoside thymidine [1], 3′-deoxy-3′-[^{18}F]fluoro thymidine ([^{18}F]-FLT) [1,3–5] and its analog ^{18}F-FMAU [8] have been demonstrated their good imaging features. However, these tracers labeled with either ^{11}C or ^{18}F, which were short half-life isotopes produced by a cyclotron, with complicated radiochemical synthesis and the lower radiochemical yield and high cost of PET examination, all these limited their use as tracers in routine clinical studies.

Technetium-99m (^{99m}Tc), the most commonly used radioisotope in SPECT, is continuously available at a reasonable cost in many hospitals and has ideal nuclear properties for imaging ($T_{1/2}$ = 6.02 h, γ = 140 keV). Therefore

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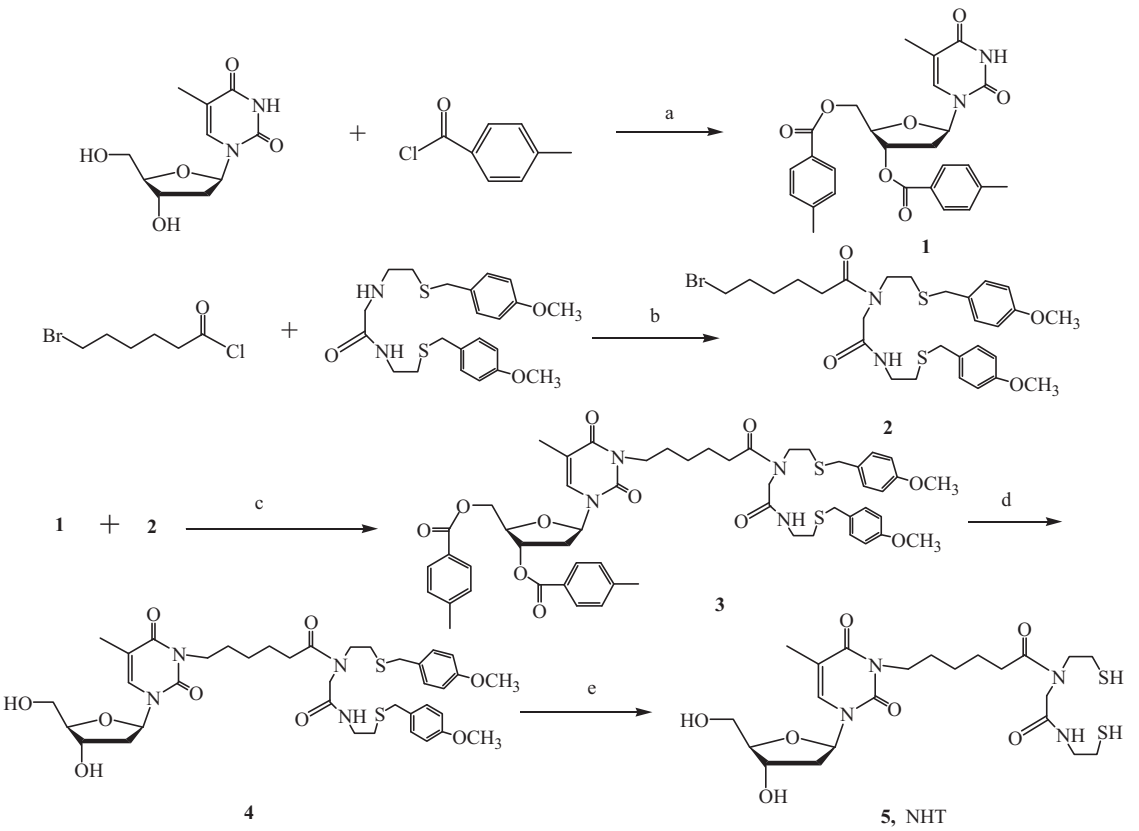
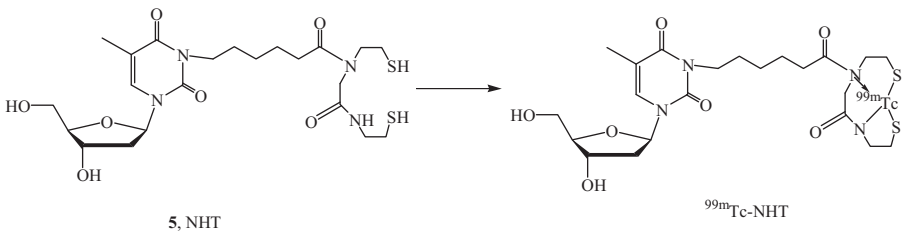


Scheme 1. Reagents and conditions: (a) DMAP, CH_2Cl_2, ref; (b) DMAP, CH_2Cl_2, 0 °C; (c) K_2CO_3, DMF/acetone (1/1, v/v), 50 °C; (d) CH_3ONa, methanol, rt; (e) CF_3COOH, $Hg(CH_3COO)_2$, H_2S, 0 °C.

it is important to develop a ^{99m}Tc labeled thymidine analog so as to provide the ideal characteristics needed for routine clinical studies [9–12].

In this communication, we report the synthesis of a thymidine analog, N^3-{N'- [2-sulfanylethylamino)acetyl]-2-amino-ethylsulfanyl-1-hexanamide}thymidine (NHT), which could be labeled easily by technetium-99m and explored the primary labeling conditions. Biodistribution of ^{99m}Tc-NHT was performed in hepatoma HepA tumor-bearing mice. The purpose of this study is to conjugate thymidine analog with chelating agent and evaluate the feasibility of technetium-99m-labeled thymidine analog as candidate for tumor imaging agent.

The labeled precursor NHT was synthesized through a multiple-step reaction using thymidine as a starting material and the total yield was 34.68%. The synthesis procedure is outlined in Scheme 1. Thymidine was protected at the 3′,5′-*O*-position with *p*-toluoyl chloride in CH_2Cl_2 to give compound **1**, and 6-bromohexanoyl chloride was coupled with *N*-[2-((2-*S*-(4-methoxybenzyl)sulfanyl)ethyl)amino]acetyl-*S*-(4-methoxybenzyl)-2-aminoethanethiol (N_2S_2) in CH_2Cl_2 to give compound **2**. The subsequent synthesis of compound **3** was through substitution reaction using compound **1**



Scheme 2. Reagents and conditions: $SnCl_2$, GH, EDTA, $Na^{99m}TcO_4$, 100 °C, 30 min.

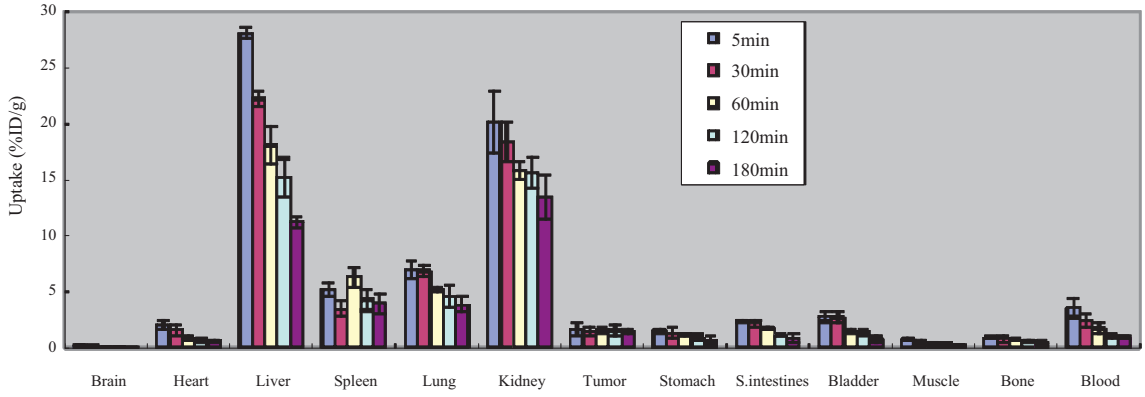


Fig. 1. Biodistribution of ^{99m}Tc-NHT in tumor-bearing mice ($x \pm \sigma$, $n = 5$, %ID/g).

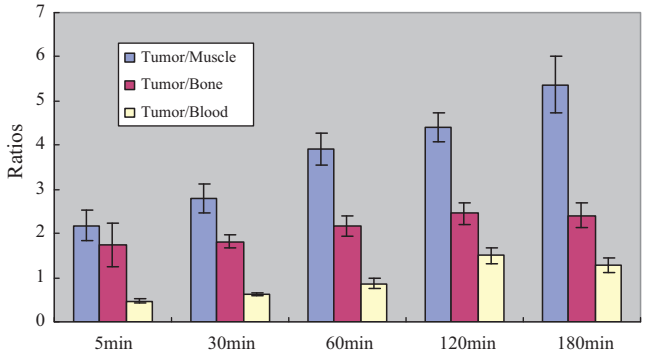


Fig. 2. Ratios of tumor-to-muscle, tumor-to-bone and tumor-to-blood.

and compound **2** in an acetone/DMF mixed solvent. The compound **4** was obtained by removing the toluoyl protecting groups of compound **3** with sodium methoxide in methanol. The thiol protecting groups, 4-methoxybenzyl, of compound **4** were removed with $Hg(OAc)_2$ in trifluoroacetic acid to give compound **5** (NHT) [13].

Using $SnCl_2$ as reducing agent, and in the presence of sodium glucoheptonate (GH) and ethylene diamine tetraacetic acid (EDTA), a series of studies were performed to optimize labeling efficiency of ^{99m}Tc-NHT, as show in Scheme 2. When the reaction temperature was set at 100 °C and kept for 30 min, the labeled yield and radiochemical purity (RCP) were over 95%, which determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) [14].

RCP of freshly prepared ^{99m}Tc-NHT was evaluated every hour at room temperature to determine whether it was stable within 6 h. The results showed that the ^{99m}Tc-NHT had good stability in vitro.

Biodistribution of ^{99m}Tc-NHT was performed in hepatoma HepA tumor-bearing mice showed that the high uptake of ^{99m}Tc-NHT in liver and kidney, which means that the clearance of ^{99m}Tc-NHT was mainly through the hepatobiliary pathway and the renal pathway, as show in Fig. 1. At 2 h post injection, the ratios of tumor-to-muscle, tumor-to-bone and tumor-to-blood were 4.41 ± 0.32, 2.45 ± 0.24 and 1.51 ± 0.18, respectively, as show in Fig. 2.

In summary, we have synthesized a novel thymidine analog NHT, which can be easily labeled with ^{99m}Tc. Biodistribution of ^{99m}Tc-NHT in tumor-bearing mice showed that the high uptake of ^{99m}Tc-NHT in liver and kidney. At 2 h p.i., the ratios of tumor-to-muscle, tumor-to-bone and tumor-to-blood were 4.41 ± 0.32, 2.45 ± 0.24 and 1.51 ± 0.18, respectively. This indicated that ^{99m}Tc-NHT might be a potential SPECT imaging agent for tumor study.

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[13] Analytical data for NHT. IR (film, cm^{-1}): (3360, 2926, 2835, 1680, 1663, 1629, 1556, 1470, 1363, 1271; ^{1}H NMR (400 MHz, CD_3OD): δ 8.25 (s, 1H), 7.82 (m, 1H), 6.31 (t, 1H, J = 6.6 Hz), 4.37–4.42 (m, 1H), 4.16 (s, 1H), 3.97 (m, 2H), 3.92 (m, 2H), 3.73–3.80 (m, 2H), 3.61 (m, 2H), 3.50 (m, 1H), 3.33–3.43 (m, 4H), 3.22 (m, 1H), 2.70–2.74 (t, 1H, J = 7.2 Hz), 2.59–2.69 (m, 3H), 2.51–2.56 (t, 1H, J = 7.6 Hz), 2.41–2.44 (d, 1H, J = 6 Hz), 2.17–2.33 (m, 2H), 1.91 (s, 3H), 1.58–1.74 (m, 4H), 1.35–1.42 (m, 2H); ^{13}C NMR (400 MHz, CD_3OD): δ 175.1, 170.0, 164.5, 151.5, 135.4, 110.2, 87.8, 86.5, 78.0, 71.2, 61.8, 49.0, 41.5, 41.0, 33.1, 31.2, 29.5, 26.8, 26.5, 26.0, 25.0, 13.2; ESI–MS (m/z): 533.1.($[M+H]^+$).
[14] HPLC of ^{99m}Tc-NHT: Lichrospher C18 column (150 mm × 4.6 mm, 5 μm), eluted with water/methanol (85/15, v/v); flow rate: 1.0 mL/min, retention time: 6.70 min.