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Technetium-99m-labeling and synthesis of thymidine analogs: Potential candidates for tumor imaging

Bao Teng, Yunpeng Bai, Yu Chang, Shizhen Chen and Zhaolong Li*

Department of Chemistry, Tsinghua University, Beijing 100084, PR China

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Abstract—The technetium-99m-labeling and synthesis of a series of thymidine analogs were studied. The target molecules were obtained by using 6-hydrazinopyridine-3-carboxylic acid (HYNIC) as a bifunctional coupling agent and using *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine (tricine) and ethylenediamine-*N*,*N*′-diacetic acid (EDDA) as coligands. The effects of different spacers between thymidine analog with HYNIC on radiochemical yield were also studied.

Nuclear medicine imaging of tumor proliferation has gained broad interest. Proliferation is one of the greatest targets to differentiate between carcinoid and malignant tumor. Proliferation is one of the tumor characteristics and thymidine is utilized by proliferating cells for DNA replication, the determination of cell proliferation with radiolabeled thymidine.[1,2] has been a well-established method in life sciences. Positron isotope labeled thymidine analog is an important branch of radiolabeled thymidine analogs.[3,4] 3-Deoxy-3-[^{18}F]fluorothymidine ([^{18}F]FLT) is one of the most promising PET tracers.[5,6] The labeled thymidine analog targets specifically the proliferative activity of malignant lesions.[7,8] However, the short half-life, complicated radiochemical synthesis, and the lower radiochemical yield have become main obstacles for its production. In addition, the high cost of PET examination has also become a large difficulty for its clinical application, especially in developing country. Technetium-99m-labeled thymidine analogs would settle the concerns because of its convenience of production and optimal physical characteristics[9] ($T_{1/2}$ = 6 h, 140 keV).

Toward this goal, it would be desirable to explore some technetium-labeled thymidine analogs for tumor imaging. We have designed four precursors which are alleged to permit label with isotopes, such as technetium and rhenium. We synthesized these thymidine analogs which could be labeled easily by technetium-99m and explored the primary labeling conditions. Up to now, these compounds have not been reported in previous references. The labeling of these innovative compounds is the first time to be reported. The purpose of this study is to conjugate thymidine analogs with chelating agent and evaluate the feasibility of technetium-99m-labeled thymidine analogs as candidates for tumor imaging agents.

The 3′-amino-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-thymidine (compound **5**) is a significant intermediate which was synthesized through a multiple-step reaction using thymidine as a starting material. The synthesis procedure is outlined in Scheme 1. First, thymidine was protected at the 5′-*O*-position with dimethoxytrityl chloride in dry pyridine to give compound **2**. The subsequent synthesis of compound **3** was an inversion of the 3′-carbon from S-configuration (ribose like) to R-configuration (lyxose like);[10] following that compound **4** was obtained through Nucleophilic Substitution Reaction using sodium azide as nucleophile.[11] Finally, thymidine analog 4 was reduced to obtain 3′-amino-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-thymidine using triphenyl phosphine as a reducing agent.[12]

The metal chelating moiety was 6-hydrazinopyridine-3-carboxylic acid (HYNIC). Succinimidyl 6-Boc-hydrazinopyridine-3-carboxylic acid (Boc-HYNIC-NHS) was an active ester which was prepared according to the method previously described.[13] HYNIC-thymidine was synthesized according to procedure outlined in Scheme 2. The intermediate compound **5** was reacted with this

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* Corresponding author. Tel.: +86 10 62796753; fax: +86 10 62771149; e-mail: zhlli@mail.tsinghua.edu.cn

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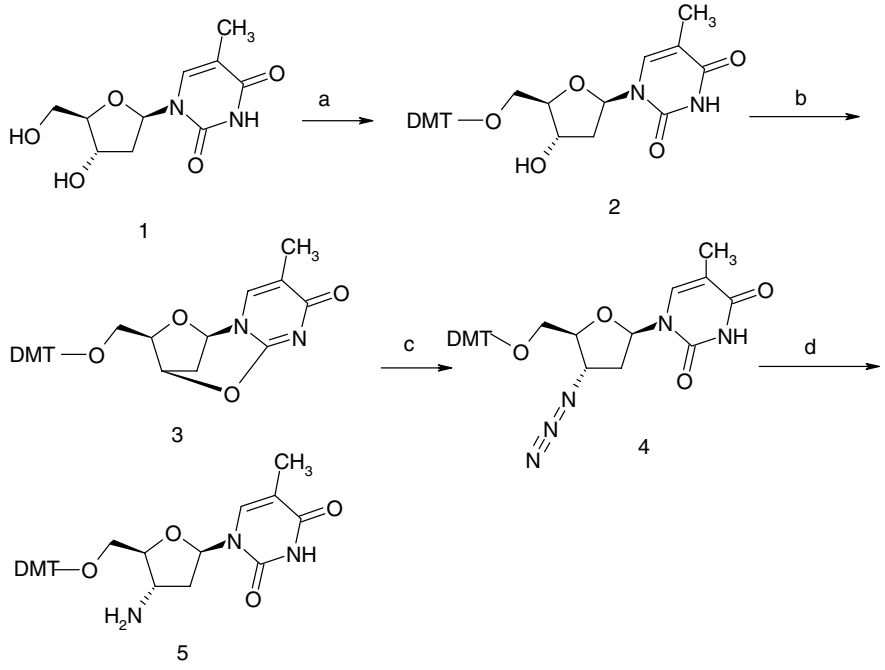
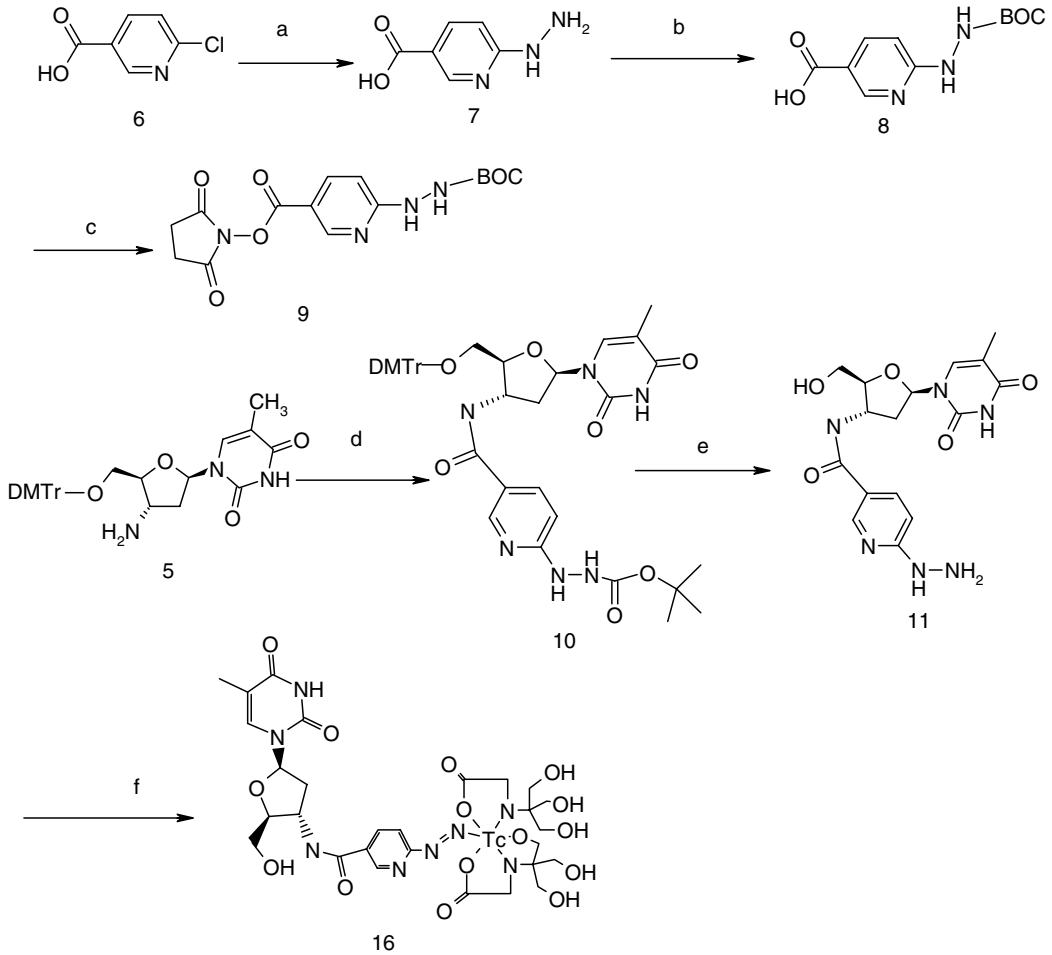
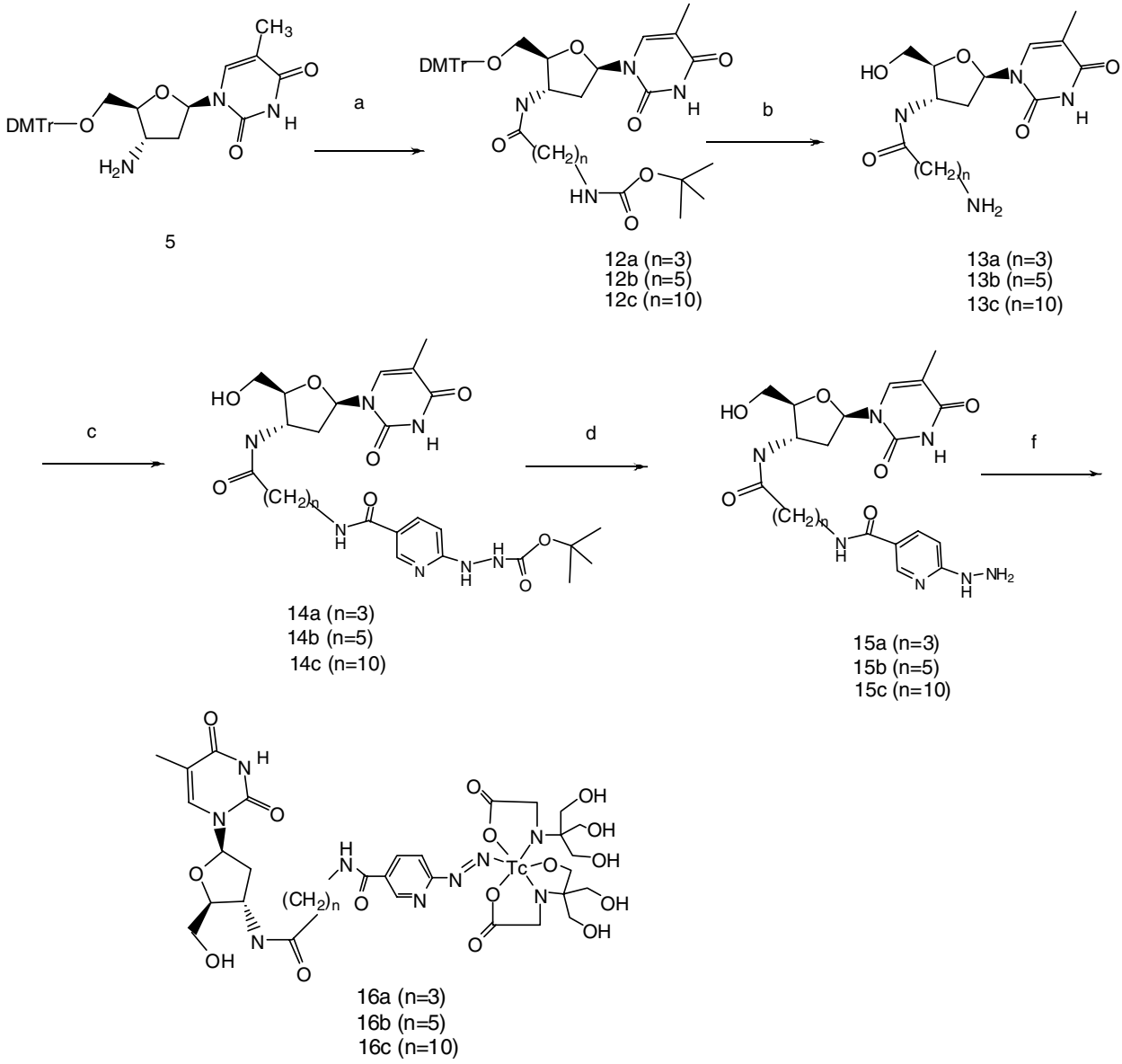


Scheme 1. Reagents and conditions: (a) DMTrCl, Pyridine, rt, 2 h; (b) Mesyl chloride, THF, −8–0 °C; Ethyl alcohol, 1 M NaOH, 80 °C, reflux, 16 h; (c) DMF/HMPA = 1:1, sodium azide, 100 °C; (d) PPh3, Pyridine, NH_4OH.



Scheme 2. Reagents and conditions: (a) 85% NH_2NH_2; (b) $(t\text{-BuOCO})_2O$; (c) NHS, DCC; (d) Compound **9**, DMF, rt; (e) 100% TFA, rt; (f) EDDA/Tricine.



Scheme 3. Reagents and conditions: (a) Boc-4-aminobutanoic acid, Boc-6-aminocaproic acid or Boc-11-amnioundecanoic acid, DCC, CH_2Cl_2, 0 °C; (b) 100% TFA, rt; (c) Boc-HYNIC-NHS, DMF, rt; (d) 100% TFA, rt; (f) EDDA/Tricine.

active ester to give compound **10**. Following that, DMTr and Boc protecting groups were removed in one pot by addition of trifluoroacetic acid (TFA) for 30 min to give 3′-(hydrazinopyridine-3-carbonyl-amino) thymidine (compound **11**).

Between the metal chelating moiety and 3′-amino-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-thymidine, we designed some flexible spacers with different alkyl chain lengths. The thymidine analogs with different alkyl chain lengths were prepared by reaction with Boc-protected

Table 1. R_f values of ^{99m}Tc-species on TLC

^{99m}Tc-species	System 1[a]	System 2[b]	System 3[c]
^{99m}Tc-compound **11**	0.9–1.0	0.0–0.2	0.0–0.2
^{99m}Tc-compound **15a**	0.9–1.0	0.0–0.2	0.0–0.2
^{99m}Tc-compound **15b**	0.9–1.0	0.0–0.2	0.0–0.2
^{99m}Tc-compound **15c**	0.9–1.0	0.0–0.2	0.0–0.2
$^{99m}TcO_4^-$	0.9–1.0	0.0–0.1	0.9–1.0
$^{99m}TcO_2{\cdot}H_2O$	0.0	0.0	0.0

[a] Xinhua No. 1 paper developed by 0.1 N citrate buffer (pH 5).
[b] Xinhua No. 1 paper developed by acetone/water (9:1, v/v).
[c] Merck silica gel 60 on aluminum sheets developed by dichloromethane/methanol (1:1, v/v).

Table 2. The radiochemical yields of Tc-99m-labeled thymidine analogs obtained by using tricine as coligand

Compound	Radiochemical yield (%)/temperature (°C)								
	25 °C/time (min)			50 °C/time (min)			100 °C/time (min)		
	5	15	30	5	15	30	5	15	30
11	31	34	43	42	50	51	43	57	62
15a	32	37	45	32	45	52	31	46	67
15b	23	31	35	26	37	59	28	50	71
15c	19	24	29	15	27	31	19	25	35

Table 3. The radiochemical yields of Tc-99m-labeled thymidine analogs obtained by EDDA/tricine exchange labeling

Compound	Radiochemical yield (%)/temperature (°C)								
	25 °C/time (min)			50 °C/time (min)			100 °C/time (min)		
	5	15	30	5	15	30	5	15	30
11	45	48	52	86	75	79	88	95	95
15a	30	45	54	60	65	74	79	89	92
15b	36	45	61	72	74	81	84	94	90
15c	24	25	31	39	43	42	59	63	62

ω-amino acids (Scheme 3). First, 3′-amino-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-thymidine reacted with Boc-protected ω-amino acids to give compound **12a**–**c**, following that DMTr and Boc protecting groups were removed in one pot by addition of TFA at 0 °C for 30 min to give compound **13a**–**c**. And then, thymidine analogs with different chain lengths were conjugated with Boc-HYNIC-NHS to give compound **14a**–**c**. Finally, the deprotection of compound **14a**–**c** was performed with the same procedure of HYNIC-thymidine to give 3′-{[(hydrazinopyridine-3-carbonyl-amino)-1-oxobutyl]-amino} thymidine (compound **15a**), 3′-{[(hydrazinopyridine-3-carbonyl-amino)-1-oxohexyl]-amino}thymidine (compound **15b**), and 3′-{[(hydrazinopyridine-3-carbonyl-amino)-1-oxoundecanyl]-amino} thymidine (compound **15c**).

These HYNIC-thymidine analogs were conjugated with technetium-99m to give compounds **16**, **16a**–**c**. We explored two different labeling methods including direct labeling with *N*-(2-hydroxy-1,1-bis(hydroxymethyl) ethyl)glycine (tricine) and Ethylenediamine-*N*,*N*′-diacetic acid (EDDA)/tricine exchange labeling.

The reaction time (5–30 min) and temperature (25–100 °C) were varied to optimize the labeling reaction conditions. The amount of thymidine analogs (compounds **11**, **15a**–**c**) (10 μg), tricine (40 mg) and EDDA (1.5 mg), $Na^{99m}TcO_4$ (150 MBq) were kept constant. The labeled mixture was passed through a Sep-Pak Plus C_{18} cartridge and then the final labeling product was eluted by alcohol. The radiochemical yields and radiochemical purity of technetium-99m-labeled thymidine analogs were determined by TLC on three systems and the R_f values of technetium-99m-species are listed in Table 1.

The radiochemical purity of all labeled thymidine analogs was over 97% through purification using Sep-Pak Plus C_{18} cartridge. The radiochemical yields obtained by different labeling methods are, respectively, shown in Tables 2 and 3.

It has been shown that the choice of chelating system has profound influence upon the radiochemistry and labeling conditions. EDDA/tricine exchange labeling could produce better radiochemical yield than the other labeling methods. With increase in reaction temperature, the radiochemical yield obviously increased in the radiolabeling experiments. The best radiochemical yields (95%, 89%, 94% and 63%) were obtained by using compounds **11**, **15a**–**c**, respectively, as precursors when the reaction temperature was set at 100 °C. The optimized labeling time was 15 min when the reaction temperature was 100 °C; the prolonged reaction time could not increase obviously the radiochemical yield. On the other hand, the spacer is an important factor which influences the radiochemical yield. Overlong spacer decreased obviously the radiochemical yield in labeling experiment.

In summary, we have synthesized several novel thymidine analogs which could be labeled easily with technetium-99m. We suggested that it is advisable when the length of alkyl spacers was set as $n = 3$, 5 or none spacer. Additionally, we have explored the technetium-99m-labeled conditions of these thymidine analogs and found that the optimized labeling conditions were the reaction temperature 100 °C and reaction time 15 min. The biodistribution and imaging of these labeled thymidine analogs in tumor-bearing mice are underway in our laboratory. The effect of different spacers between thymidine analogs with HYNIC on biological function of these labeled thymidine analogs will also be published elsewhere.

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Supplementary data

Experimental details associated with the synthesis and labeling of thymidine analogs can be found in the online version via internet.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.03.086.

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