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Synthesis of the deuterated thymidine-d_9 and deuterated oligonucleotides

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ABSTRACT

The efficient synthesis of the highly-deuterated thymidine-d_9 by the glycosylation reaction between a deuterated nucleobase and deuterated sugar is described. It is also incorporated into the oligonucleotides using a DNA synthesizer. Using this approach, it is possible to make highly-deuterated oligonucleotides for biological studies and structural analyses.

The deuterium-labeled compounds have been used for tracing the pharmacokinetics of drugs, and the deuterated drugs intended for metabolic resistance have recently been designed [1–3]. Furthermore, the deuterated nucleosides are powerful tools for biological studies and structural analyses of high-ordered DNA and RNA structures [4]. However, it is well known that for the partially-deuterated nucleosides, only the nucleobase or sugar part, is used [5,6]. We thought that the fully-deuterated nucleosides have a high utility value. The use of these highly deuterated nucleic acids is particularly useful when local recognition structures such as artificial nucleic acids are clarified by NMR measurement and used for new molecular design. There are several deuteration reaction conditions for aromatic or aliphatic protons by the H-D exchange reaction [7–14]. We also examined the deuteration of the nucleosides, deoxyadenosine and thymidine, by using of Pd/C or Ru/C under H_2 gas in a D_2O solution at 160 °C following the report of Sajiki's group [15]. However, they were decomposed during the reaction or only the nucleobases were deuterated. Therefore, in order to synthesize a highly-deuterated nucleoside derivative, the nucleobase moiety and the sugar moiety were separately deuterated and condensed by the glycosylation reaction. In this study, we have successfully synthesized the deuterated thymidine-d_9 and deuterated oligonucleotides (Figure 1).

The synthesis of thymine-d_4 (**1**) is shown in Scheme 1 [15]. After 24 h, the catalyst was filtered off using a celite pad and washed by hot water to improve the recovery amount of the product. The structure and deuterated percent were determined by ESI-MS and ^{1}H NMR measurements and compared with the spectra of the same thymine concentration (Scheme 1). We also confirmed the deuteration reaction using adenine and cytosine to produce the adenine-d_2 and cytosine-d_2, respectively, under the same conditions. Unfortunately, the guanine did not undergo the deuteration reaction under the same conditions because of its low solubility for D_2O.

We next tried to synthesize the highly-deuterated sugar part from the 1-methoxy-ribose as the starting material (Scheme 2) [16]. The hydrogen adjacent of the hydroxy group of **2** was deuterated using the 10% Ru/C, H_2 D_2O at 80 °C under alkali conditions. All the hydroxyl groups were converted to the acetoxyl group as a coupling intermediate of the deuterated sugar-d_4 (**3**). The glycosylation reaction between the nucleobase part (**1**) and sugar part (**3**) was done under the conventional conditions using a Lewis acid to produce the coupling product **4**. The acetyl groups were removed using NH_3 in methanol to obtain the 2′-hydroxy deuterated thymidine-d_8 (**5**), and the 5′- and 3′-hydroxy groups were protected by the cyclic silyl group. The radical deoxygenation and silyl deprotection reactions were finally accomplished. The protected 2′-hydroxy group of **6** underwent a radical deuteration by means of Bu_3SnD [17] in toluene at 90 °C for 24 h using AIBN as the radical initiator. Although the yield was not sufficient, it successfully synthesized the deuterated thymidine-d_9 (**7**) (Scheme 2). To determine

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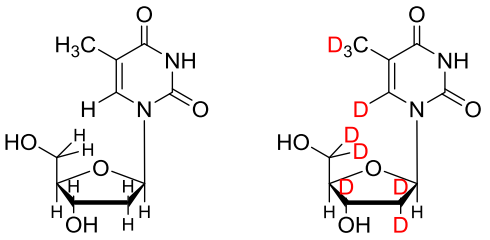
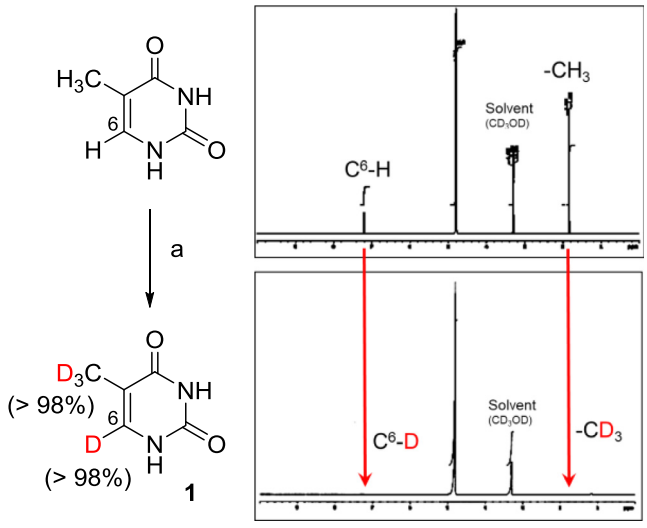


Figure 1. Structures of thymidine and deuterated thymidine-d_9.

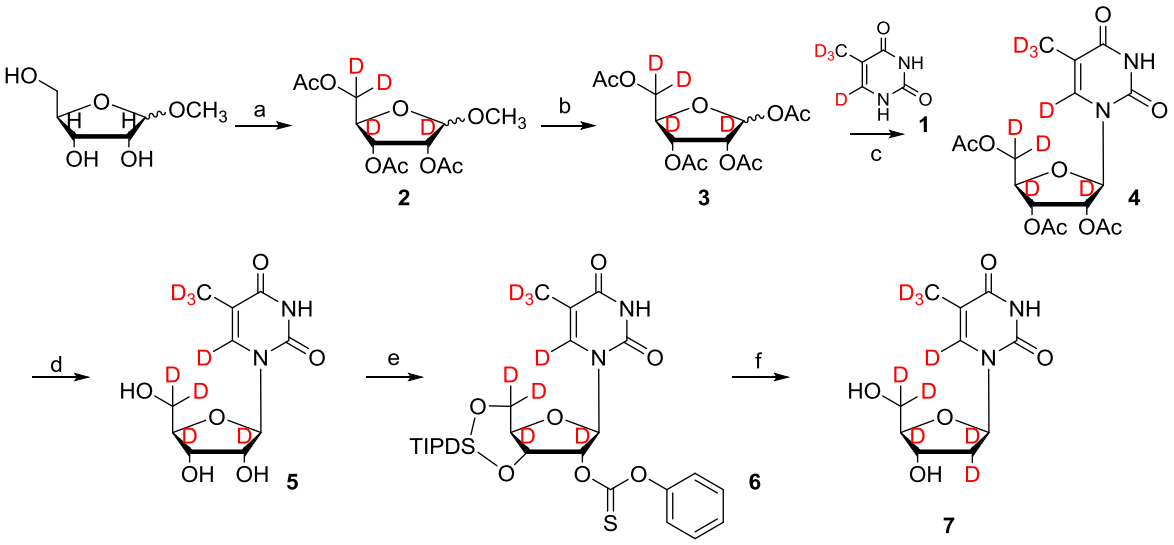


Scheme 1. Synthesis of the thymine-d_4. Reagents and Conditions: (a) 10% Pd/C, H_2, D_2O, 110 °C, 24 h, 92%

the structure and deuterated percent of compound **7**, we measured the high-resolution ESI-MS (HR-ESI-MS) and ^{1}H NMR (Figure 2) [18]. Based on the results of the NMR spectrum, the deuterium efficiencies of **7** were very good (5-CD_3 was over 98%, 6-D was over 98%, 2′-Ds were about 80% (average), 3′-D was 80% and 5′-Ds were about 96% (average)) except for the 1′ and 4′-protons of the sugar part.

In order to incorporate into the oligonucleotides (ODNs), the deuterated thymidine-d_9 (**7**) was converted to the corresponding phosphoramidite compound (**8**). This monomer unit of **8** was applied using an automated DNA synthesizer (Scheme 3). First, we synthesized the trimer oligonucleotides containing one thymidine-d_9 in the middle. After cleavage from CPG, it was treated with a 28% ammonia solution and concentrated under reduced pressure. The ^{1}H NMR spectrum of **ODN1** (T^dTT-trimer) in D_2O was measured without further purification and its downfield region is shown in Figure 3. As compared to the spectrum of **ODN**-T (TTT-trimer), three anomeric protons appeared around 6.2 ppm. Furthermore, two protons in the 6-position of thymine were observed around 7.6 ppm, because one was converted to deuterium. These results indicated that the deuterated thymidine-d_9 was successfully incorporated into the oligonucleotides. We next tried the synthesis of the 8-mer (**ODN2**) and 11-mer (**ODN3**) oligo ^dT sequences including one 2′-deoxyadenosine (dA) in each sequence. After cleavage from CPG treated with the 28% ammonia solution, the synthesized ODNs were purified by HPLC. The structures were confirmed by the MALDI-TOF mass measurements before and after removing the DMTr group at the 5′ end of the synthesized **ODN2**, **ODN3** and **ODN4** as the control sequence (Table 1). We measured the ^{1}H NMR spectrum of **ODN3** and **ODN4** in D_2O [19]. Apparently, the spectrum of **ODN3** was more clear than that of the non-deuterated sequence, but further studies, such as 2D NMR measurements, are needed to show the advantage of the deuterated conversion.

In conclusion, we successfully synthesized the deuterated thymidine-d_9 compound and identified its properties by ^{1}H NMR measurements. Its phosphoramidite unit was also possible to be incorporated into the oligonucleotides. Further studies, the synthesis of other deuterated nucleosides and measurement of their 2D NMR spectra, are currently underway.



Scheme 2. Synthesis of the thymidine-d_9. Reagents and Conditions: (a) 1) 10%Ru/C, H_2, NaOH, D_2O, 80 °C, 2) Ac_2O, dry pyridine; (b) Ac_2O, HOAc, H_2SO_4, r.t.,3.5 h, 69%; (c) BSA, TMSOTf, dry CH_3CN, 60 °C, 15 h, 94%; (d) NH_3 in CH_3OH, r.t., 64 h; (e) 1) $[(i\text{-}Pr)_2SiCl]_2O$, dry pyridine, r.t., 13 h, 51% for 2 steps, 2) PhOC(S)Cl, DMAP, dry CH_3CN, r.t., 11 h, 59%; (f) 1) n-Bu_3SnD, AIBN, dry toluene, 90 °C, 23 h, 50%, 2) TBAF in THF, r.t., 2 h, 90%.

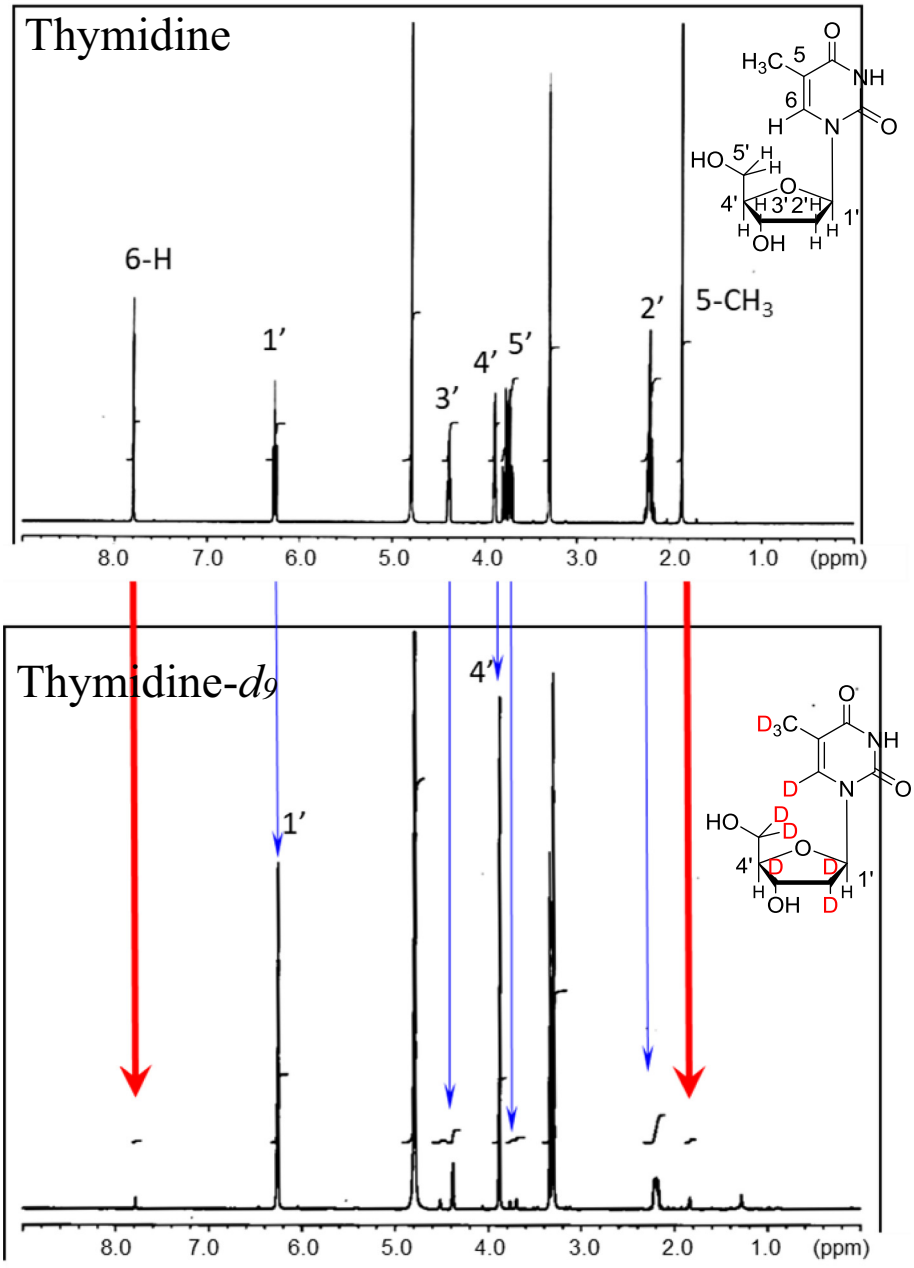
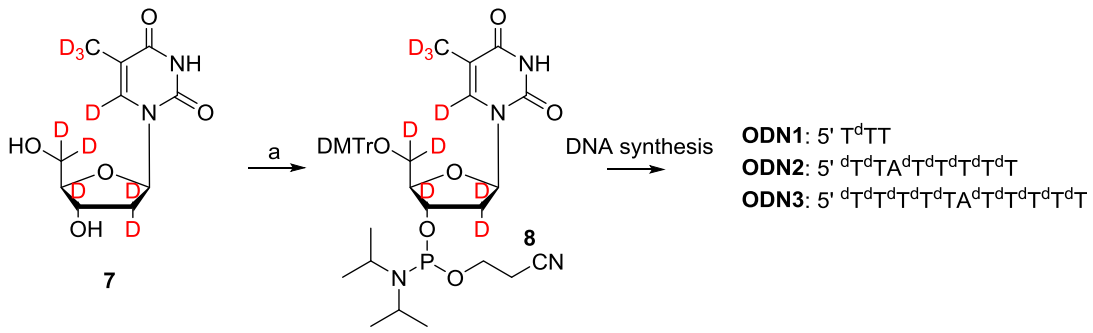


Figure 2. The ^{1}H NMR spectra of thymidine and deuterated thymidine-d_9 in CD_3OD.



Scheme 3. Synthesis of the thymidine-d_9 phosphoramidite and oligonucleotides. Reagents and Conditions: (a) 1) DMTrCl, dry pyridine, r.t., 3.5 h, 89%, 2) 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DIPEA, dry CH_2Cl_2, 0 °C, 2 h, 88%. DNA synthesis was done using standard protocol.

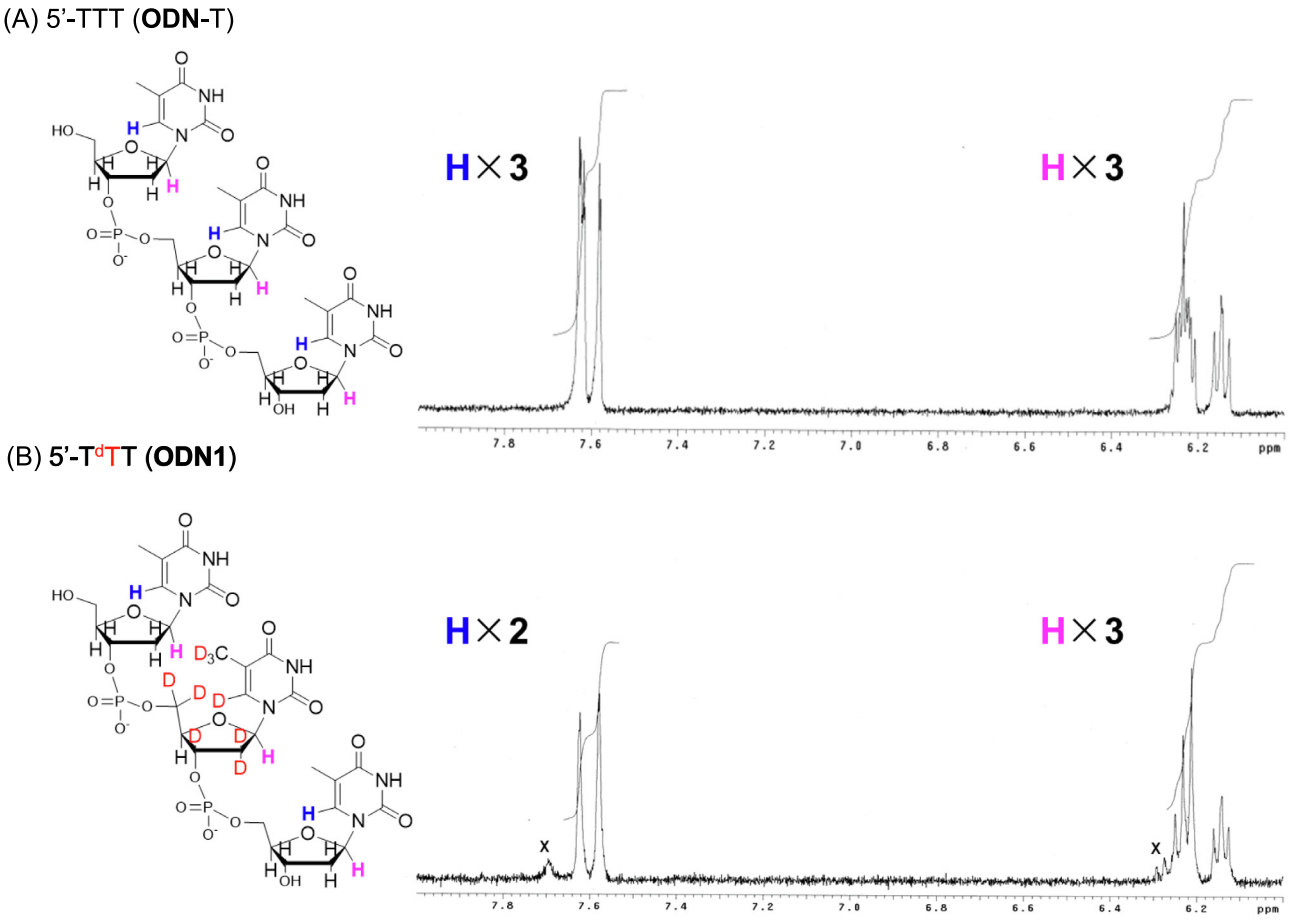


Figure 3. The downfield region of ^{1}H NMR spectra of trimer of (A) TTT and (B) T^{d}TT in D_2O, ^{d}T is a thymidine-d_9. X is an impurity.

Table 1
MALDI-TOF Mass results (*m*/*z*, measured in negative mode).

	calcd. for	found
ODN2-DMTr	2737.91	2736.47
ODN2	2435.78	2434.15
5′ (DMTr)-^{d}T^{d}T^{d}TA^{d}T^{d}T^{d}T^{d}T^{d}T		
ODN3-DMTr	3683.26	3682.35
ODN3	3382.14	3378.15
5′ (DMTr)-^{d}T^{d}T^{d}T^{d}T^{d}T^{d}TA^{d}T^{d}T^{d}T^{d}T^{d}T		
ODN4-DMTr	3593.30	3593.31
ODN4	3291.67	3289.30
5′ (DMTr)-TTTTTATTTTT		

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151037.

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[18] Compound data of **7** (white powder). $C_{10}H_5D_9N_2O_5$ (MW: 251.28); HR-ESI-MS (m/z) calcd. for: 274.1360 $[M+Na]^+$, found: 274.1360. IR (cm^{-1}): 3400, 1676, 1466, 1316, 1054; ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 7.79 (s, 0.02 H), 6.26 (d, 1H, *J* = 4.9 Hz), 4.38 (d, *J* = 3.0 Hz), 3.88 (d, , 1H, *J* = 12.0 2.8 Hz), 3.76 (d, 0.04H, *J* = 2.8 Hz), 3.70 (d, 0.05H, *J* = 3.9 Hz), 2.26-2.14 (m, 0.4H), 2.09 (s, 0.06H); ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 166.5, 152.4, 138.0, 111.2, 88.7, 86.2, 72.1, 62.1, 40.8, 11.6.
[19] See a supporting information.