

Supplementary Information

Facile synthesis of hydroxymethylcytosine-containing oligonucleotides and their reactivity upon osmium oxidation

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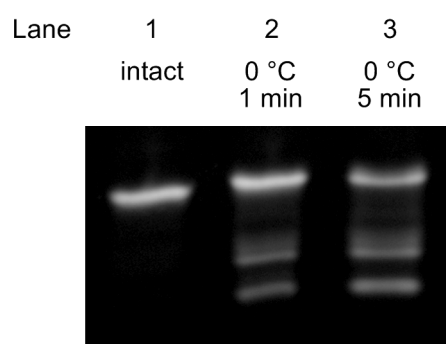


Fig. S1 Osmium oxidation of **ODN3^{hmC}**, 5'-Fluo-AAA^{hm}CGAG^{hm}CGAAAAA-3'. The fluorescein-labeled **ODN3^{hmC}** (5 μ M) to be examined was incubated in a solution of 5 mM potassium osmate, 100 mM potassium hexacyanoferrate(III), 100 mM bipyridine, 0.5 mM EDTA, and 1 M sodium chloride in 50 mM Tris-HCl buffer (pH 7.7) and 10% acetonitrile at 0 °C for 1 min (Lane 2) or 5 min (Lane 3). The reaction sample was heated in 50 μ L of 10% piperidine at 90 °C for 20 min to cleave the strand at oxidation site. Lane 1, intact **ODN3^{hmC}**.

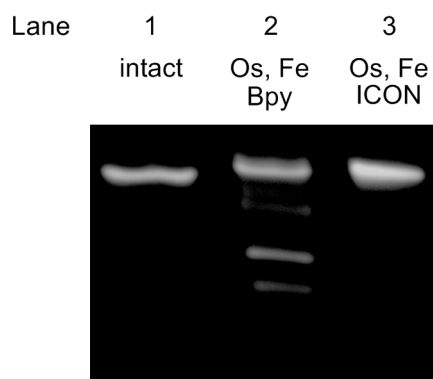


Fig. S2 Effect of hybridization with the ICON probe on osmium oxidation. The fluorescein-labeled **ODN4**^(^hmC) 5'-Fluo-AGCAA^hmCGAAGCAAAA-3' (5 μ M) to be examined was incubated in a solution of 5 mM potassium osmate, 100 mM potassium hexacyanoferrate(III), 100 mM bipyridine, 0.5 mM EDTA, and 1 M sodium chloride in 50 mM Tris-HCl buffer (pH 7.7) and 10% acetonitrile at 0 °C for 5 min (Lane 2) or in a solution of 5 μ M **ODN2'**(**ICON**), 5 mM potassium osmate, 100 mM potassium hexacyanoferrate(III), 0.5 mM EDTA, and 1 M sodium chloride in 50 mM Tris-HCl buffer (pH 7.7) at 25 °C for 10 min (Lane 3). The reaction sample was heated in 50 μ L of 10% piperidine at 90 °C for 20 min to cleave the strand at oxidation site. Lane 1, intact **ODN3**^(^hmC).

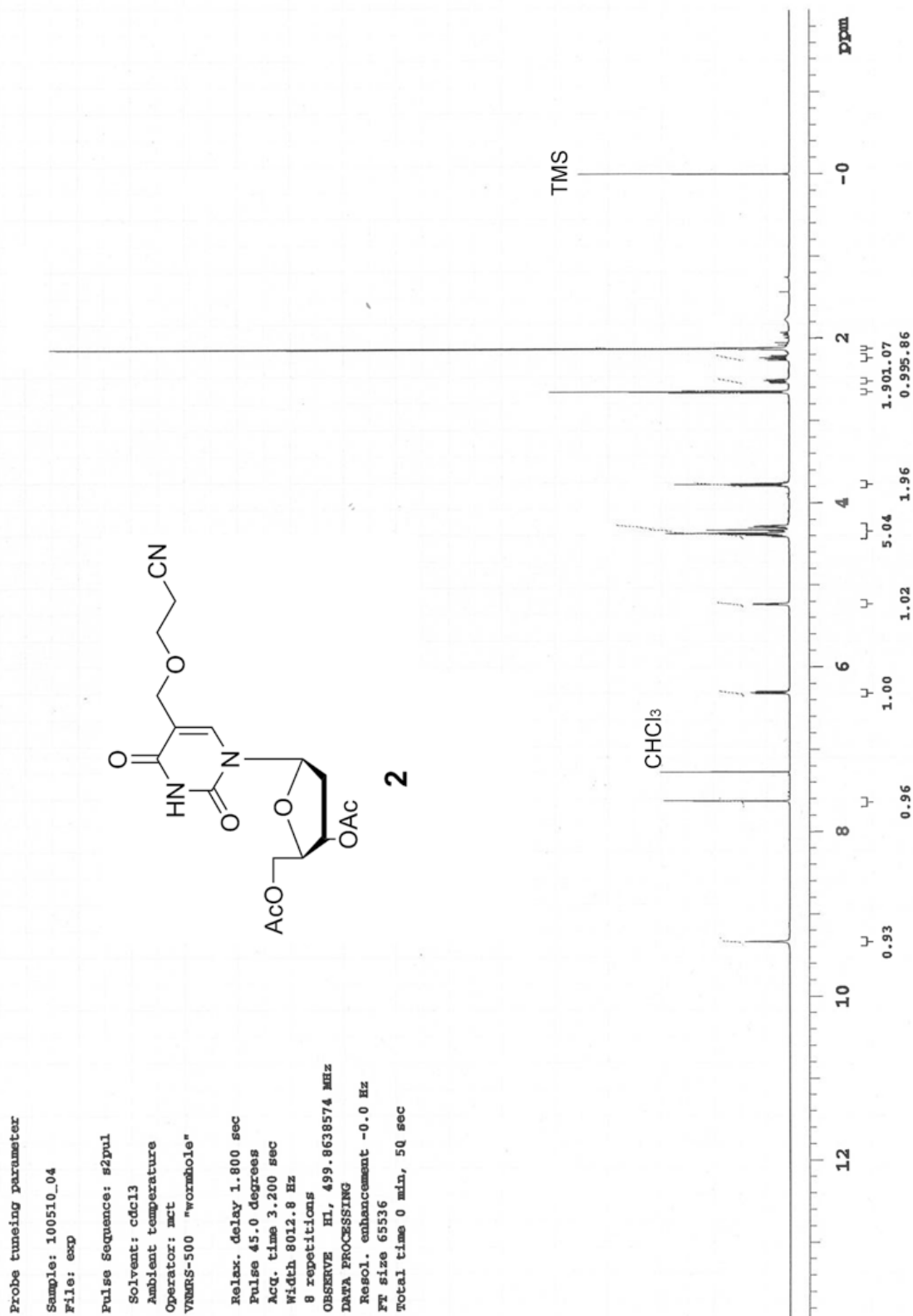


Fig. S3 ^1H NMR of nucleoside 2.

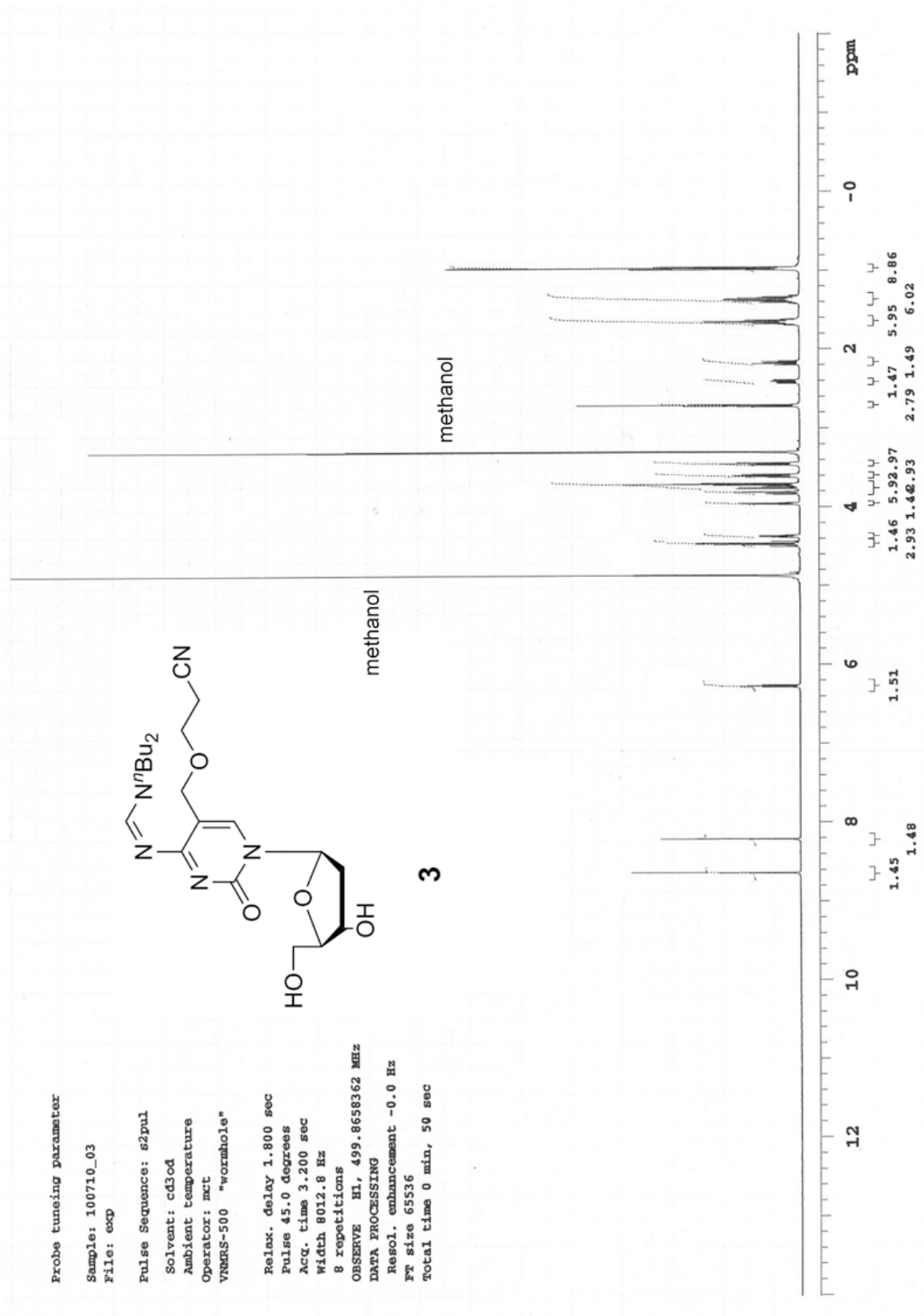


Fig. S4 ¹H NMR of nucleoside 3.

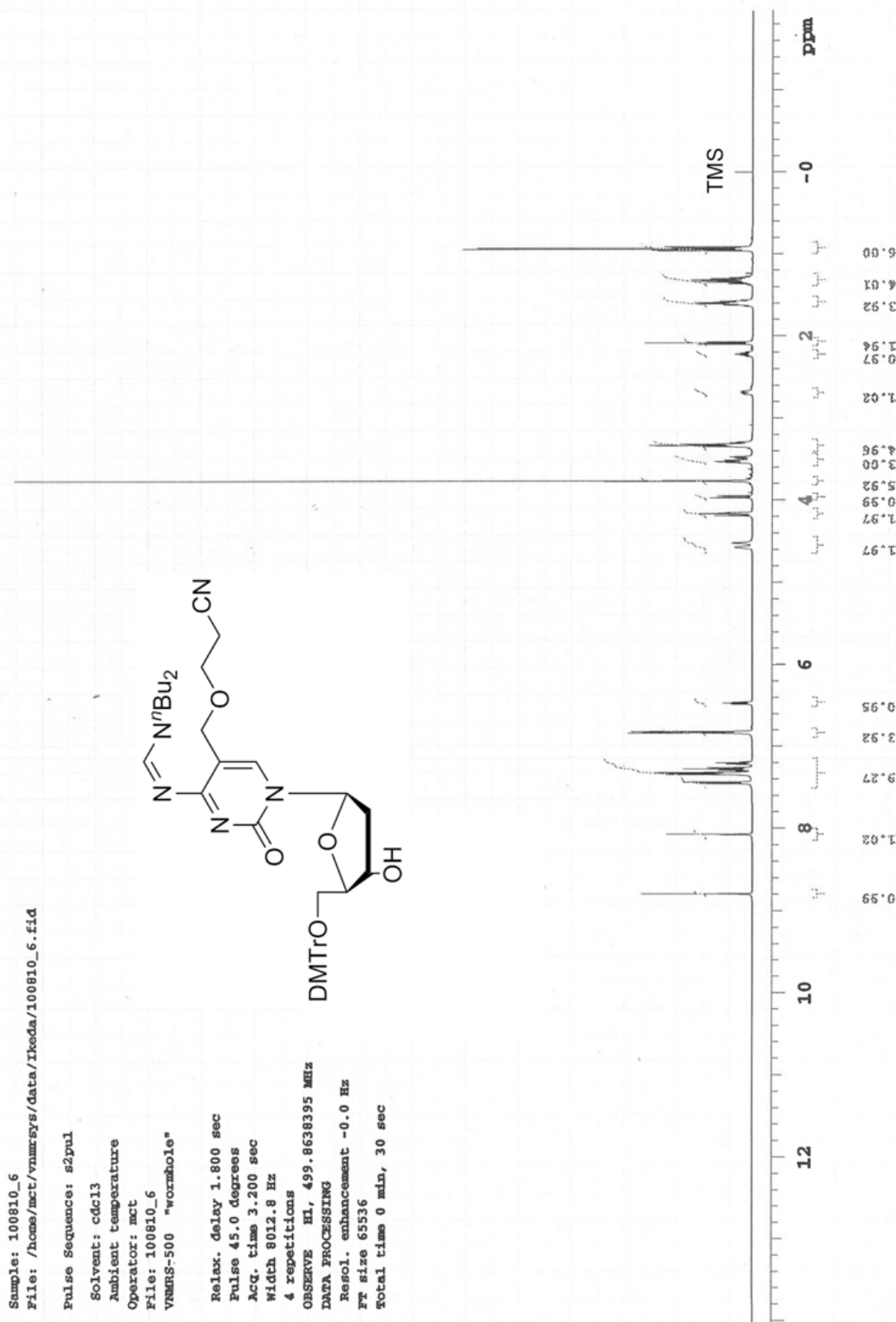


Fig. S5 ^1H NMR of a dimethoxytritylated nucleoside (the precursor of **4**).