

**Novel entry to the synthesis of (*S*)- and (*R*)-5-methoxycarbonylhydroxymethyluridines –
diastereomeric pair of wobble tRNA nucleosides**

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Fig. S1. ^1H NMR (700 MHz, CDCl_3) spectrum of 2',3',5'-tri-*O*-acetyl-*N*³-benzoyluridine-5-malonic acid diethyl ester (**5**).

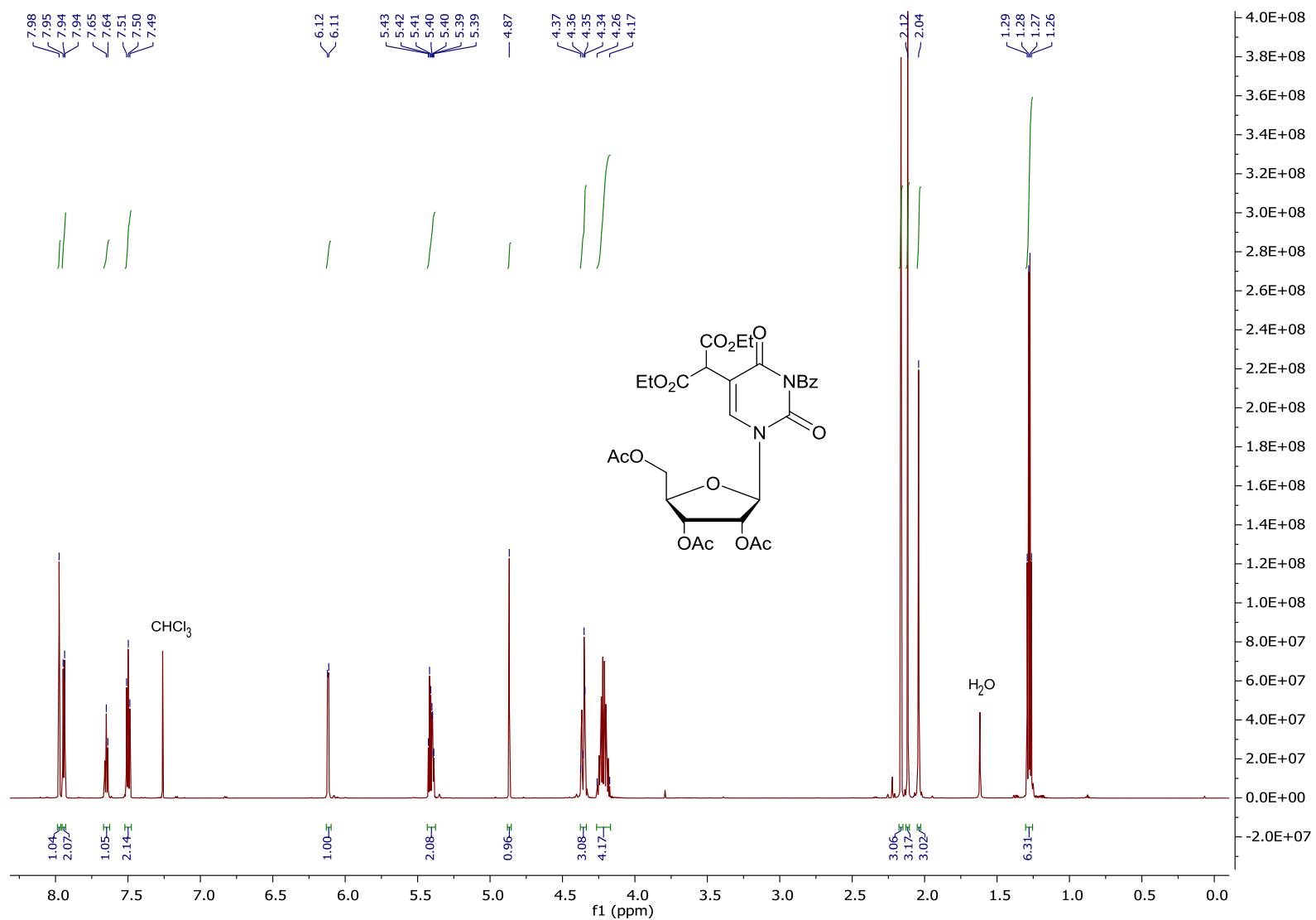


Fig. S2. ^{13}C NMR (176 MHz, CDCl_3) spectrum of 2',3',5'-tri-*O*-acetyl-*N*³-benzoyluridine-5-malonic acid diethyl ester (**5**).

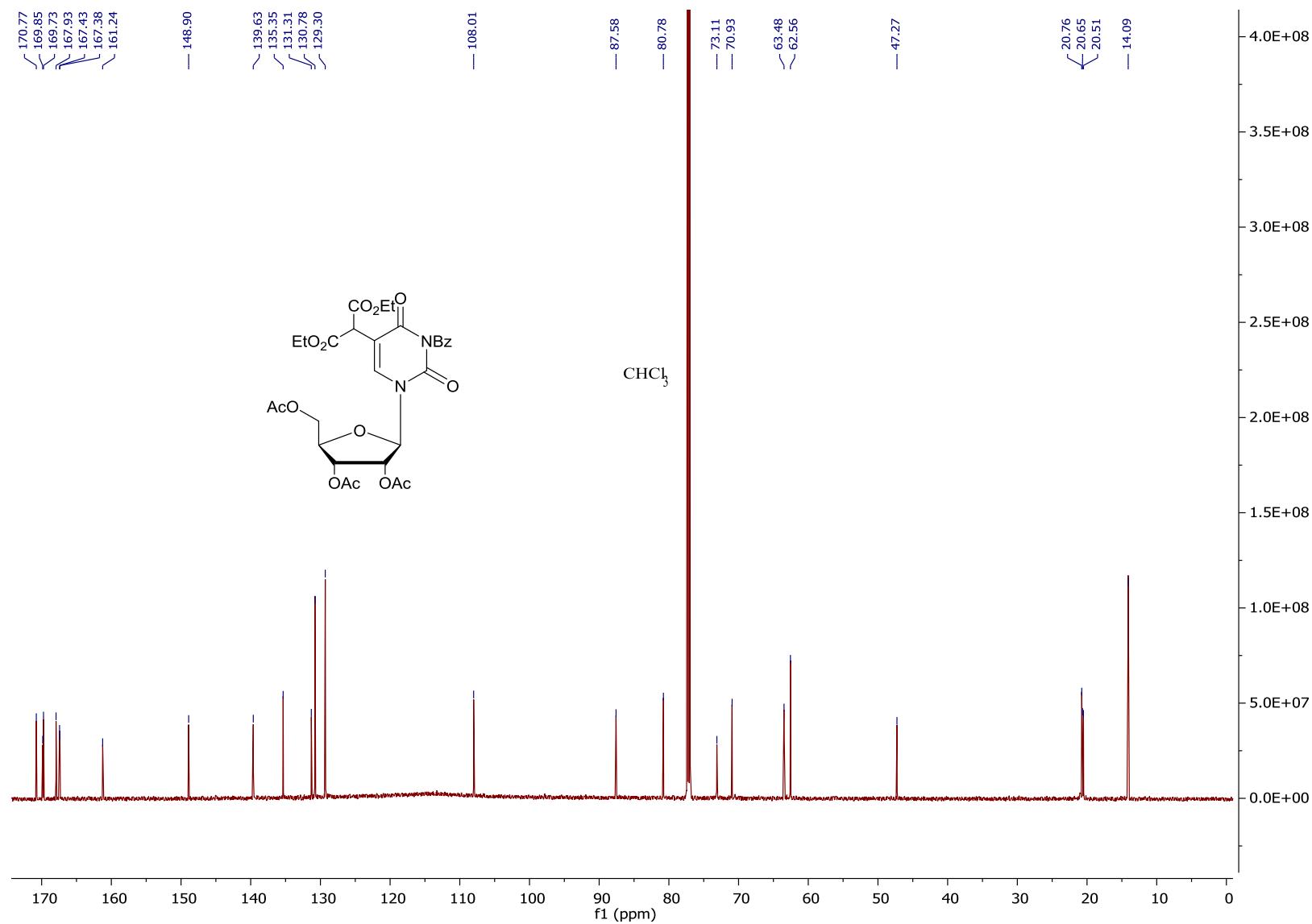


Fig.S3. ^1H NMR (250 MHz, DMSO – d6) spectrum of 2',3',5'-tri-O-acetyl-N³-benzoyluridine-5- α -hydroxymalonic acid diethyl ester (**6**)

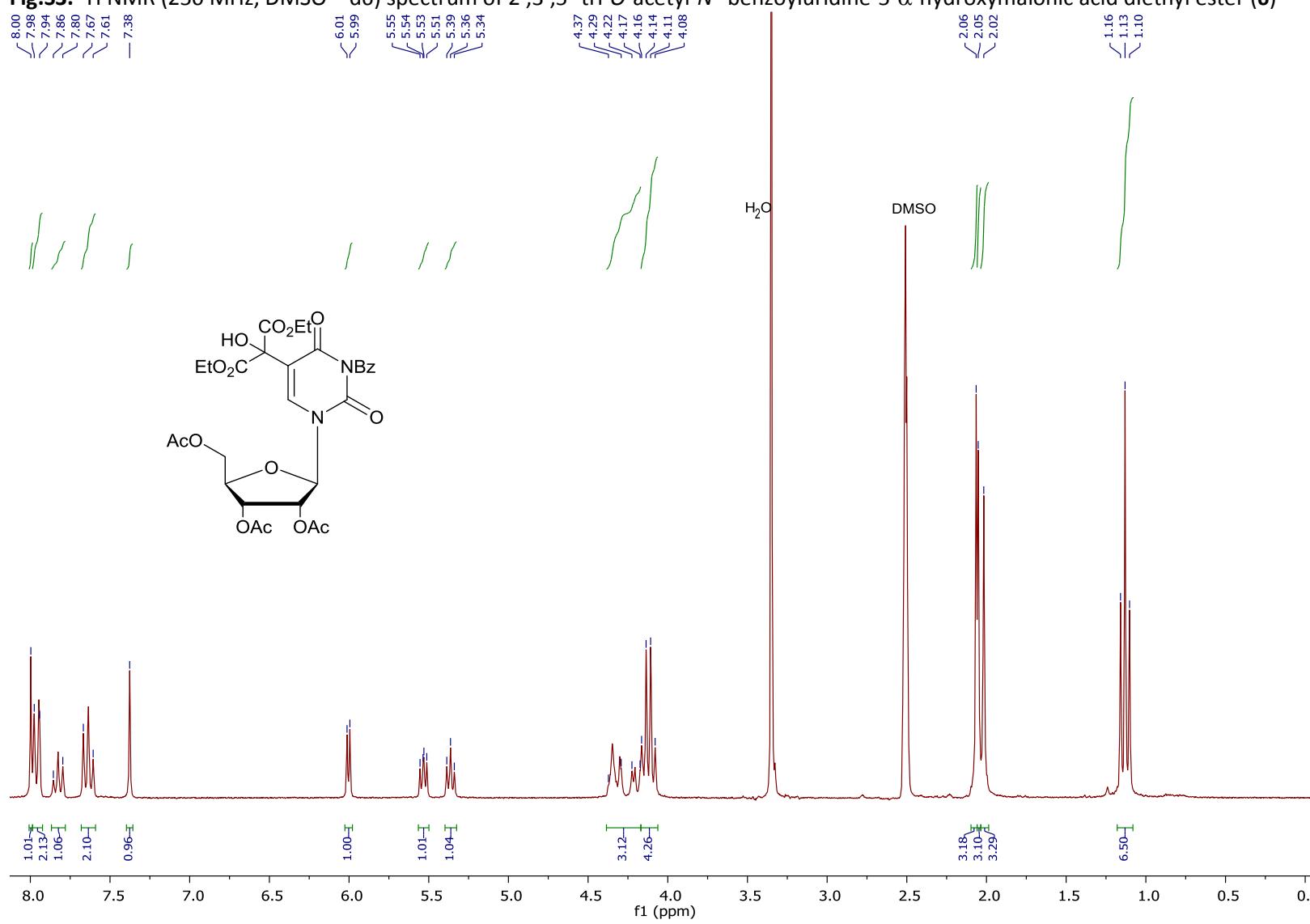


Fig.S4. ^{13}C NMR (176 MHz, DMSO – d6) spectrum of 2',3',5'-tri-O-acetyl- N^3 -benzoyluridine-5- α -hydroxymalonic acid diethyl ester (**6**)

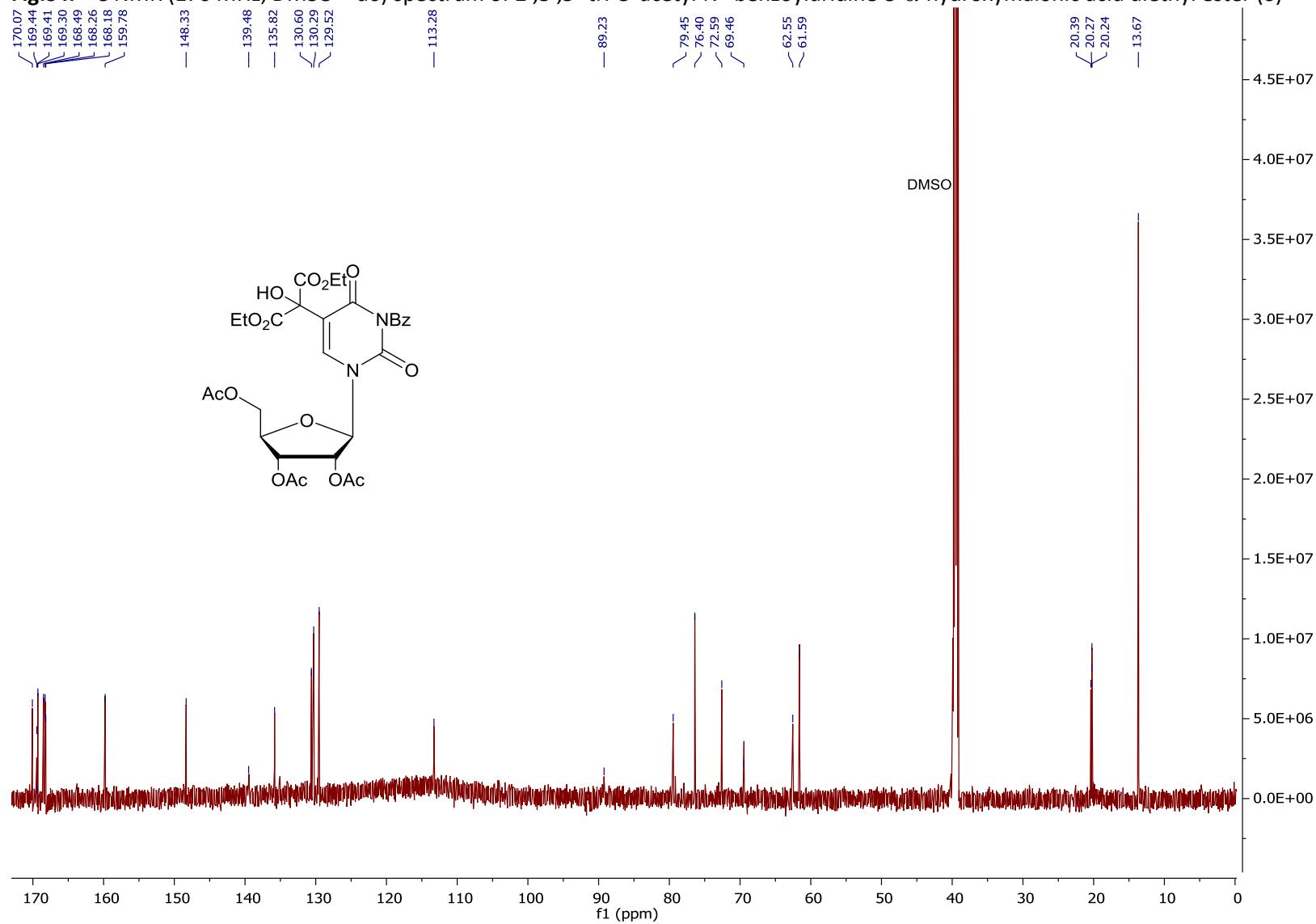


Fig.S5. ^1H NMR (250 MHz, D_2O) spectrum of lactone-containing intermediate **7**

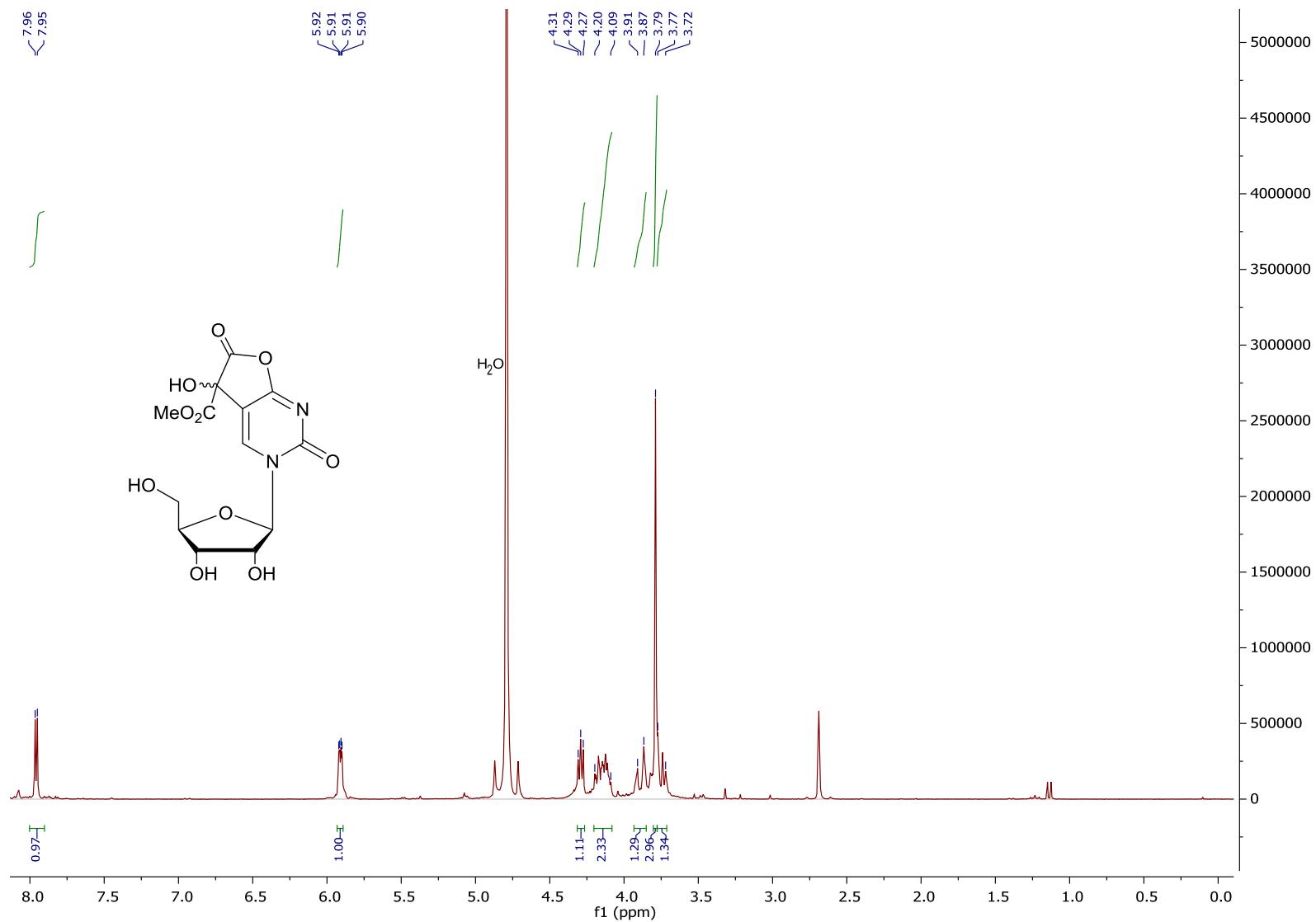


Fig.S6. ^{13}C NMR (176 MHz, D_2O) spectrum of lactone-containing intermediate **7**

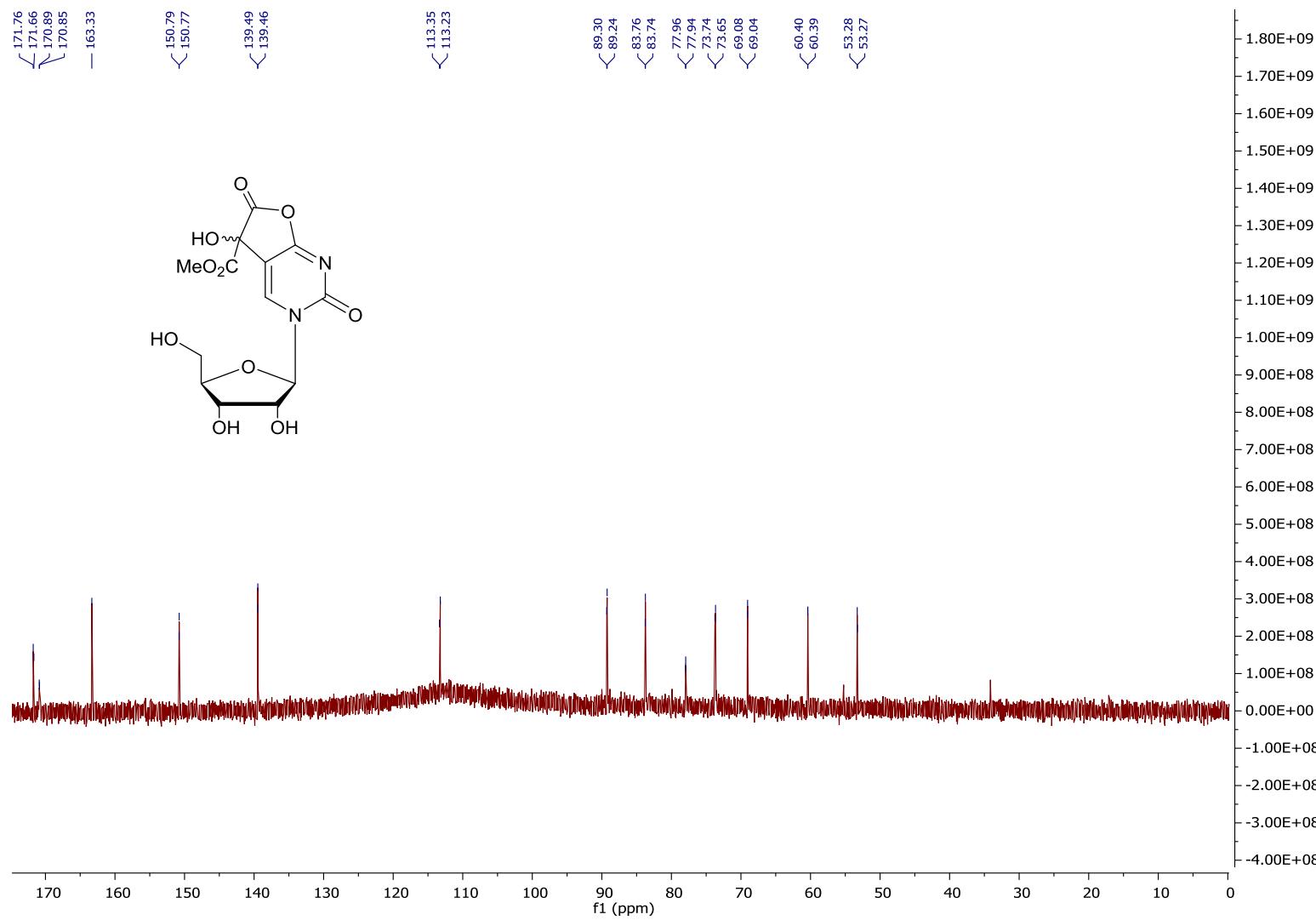


Fig.S7 ^1H NMR (700 MHz, D_2O) spectrum of (*S*)- and (*R*)-5-carboxyhydroxymethyluridine (**8**).

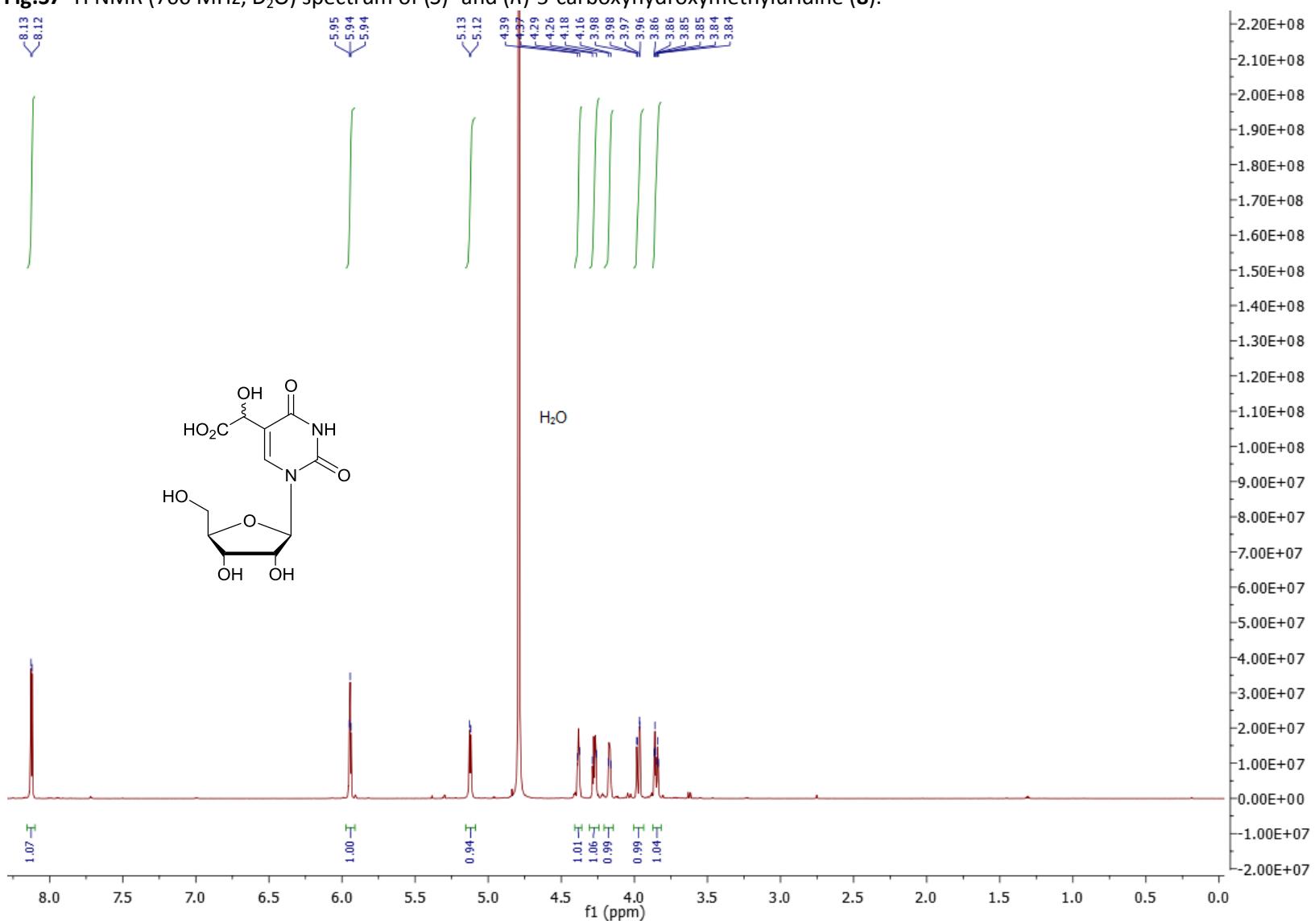


Fig.S8 ^{13}C NMR (176 MHz, D_2O) spectrum of (*S*)- and (*R*)-5-carboxyhydroxymethyluridine (**8**).

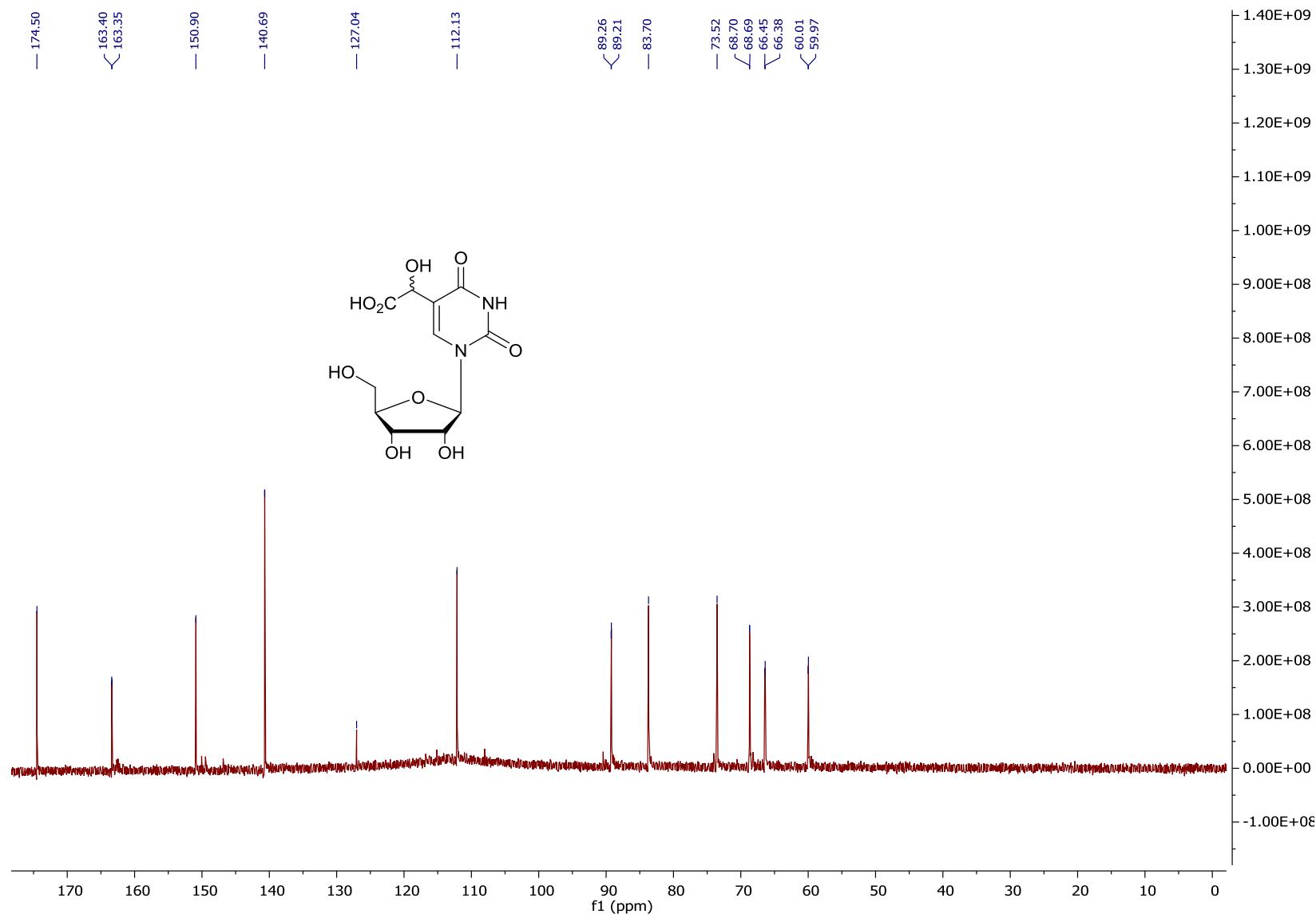


Fig.S9. ^1H NMR (700 MHz, DMSO – d6) spectrum of (*S*)- and (*R*)-5-carboxyhydroxymethyluridine (**8**).

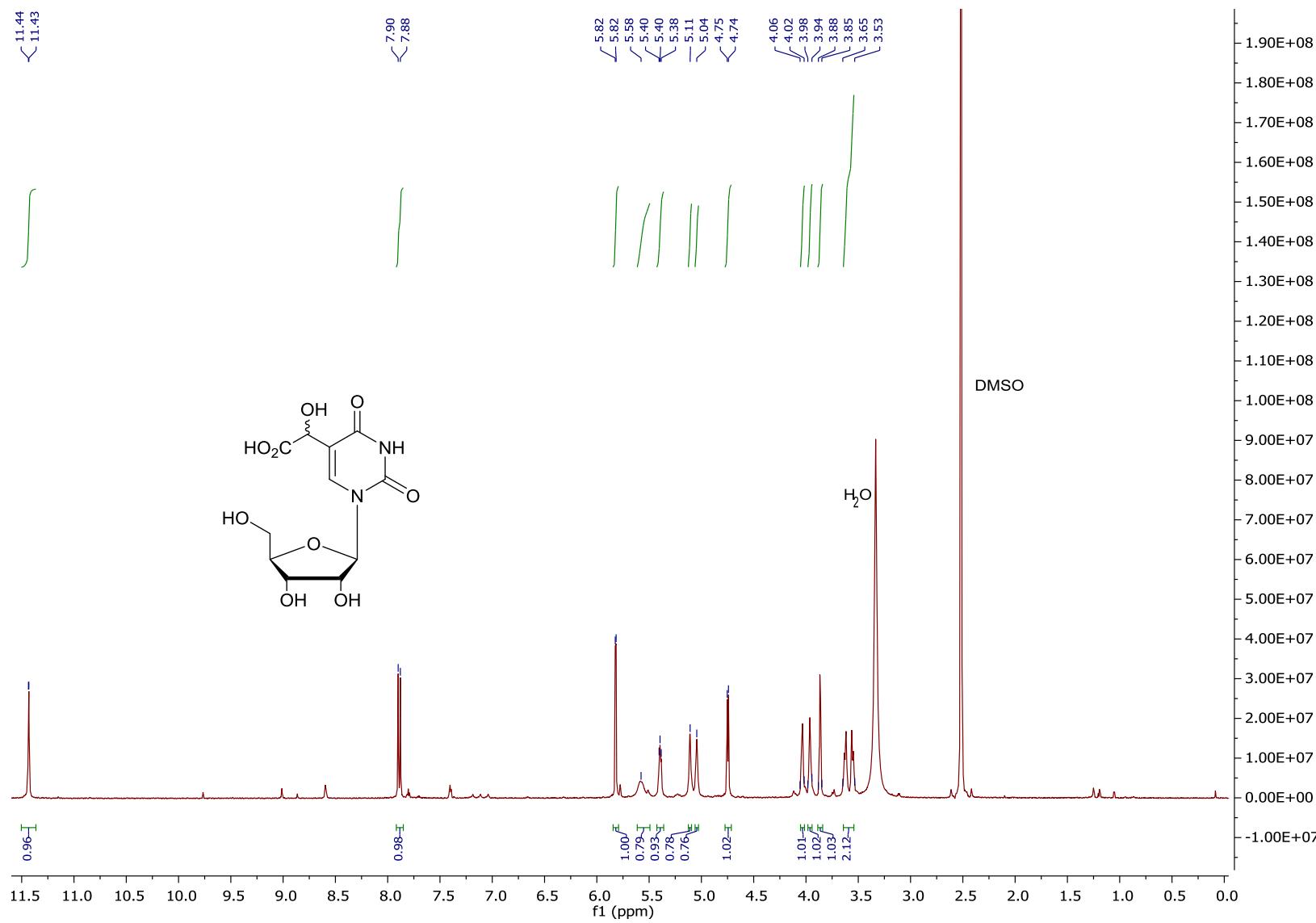


Fig.S10. ^1H NMR (700 MHz, D_2O) spectrum of (*S*)-5-methoxycarbonylhydroxymethylouridine (**1**).

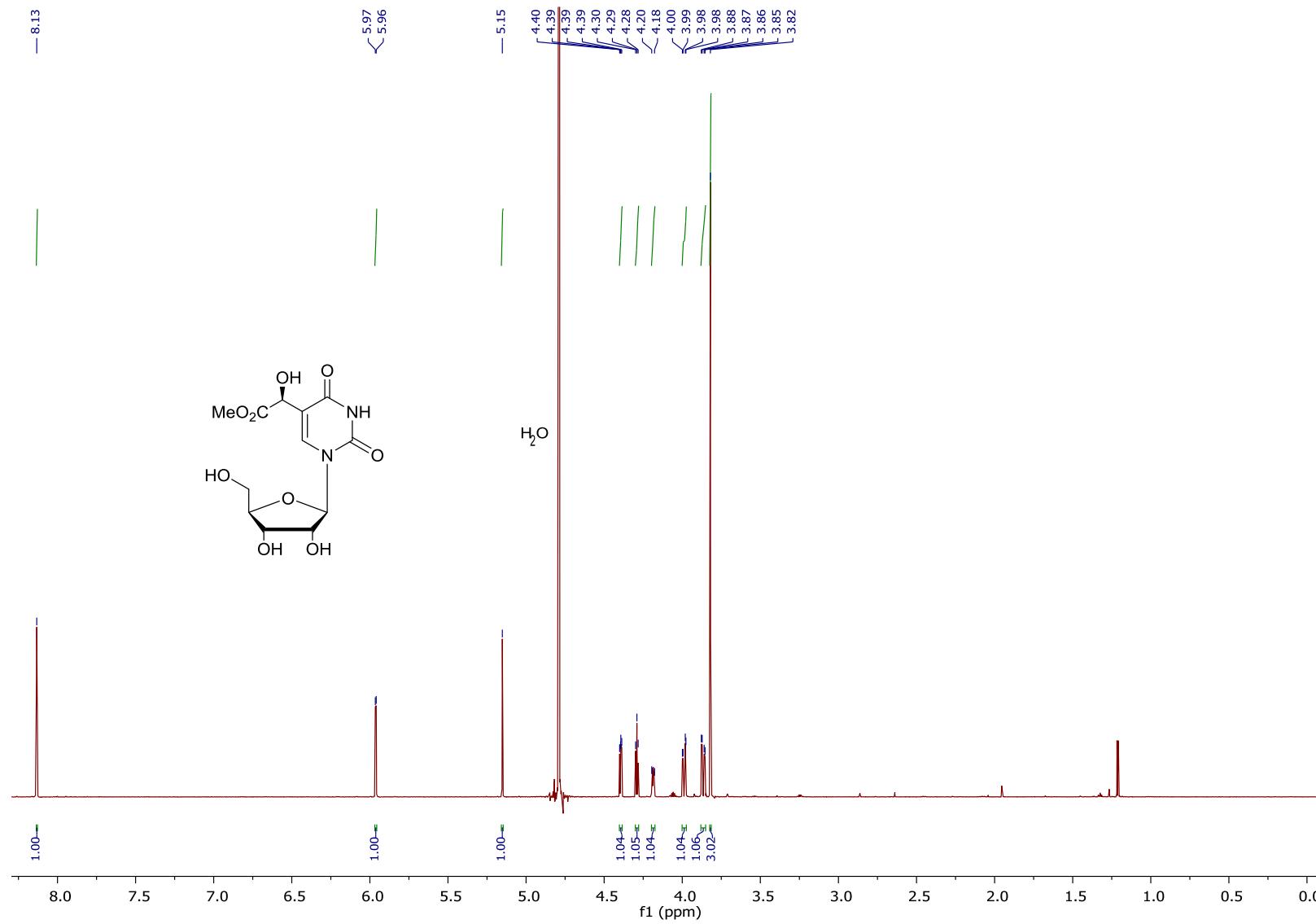


Fig.S11. ^{13}C NMR (176 MHz, D_2O) spectrum of (*S*)-5-methoxycarbonylhydroxymethylouridine (**1**).

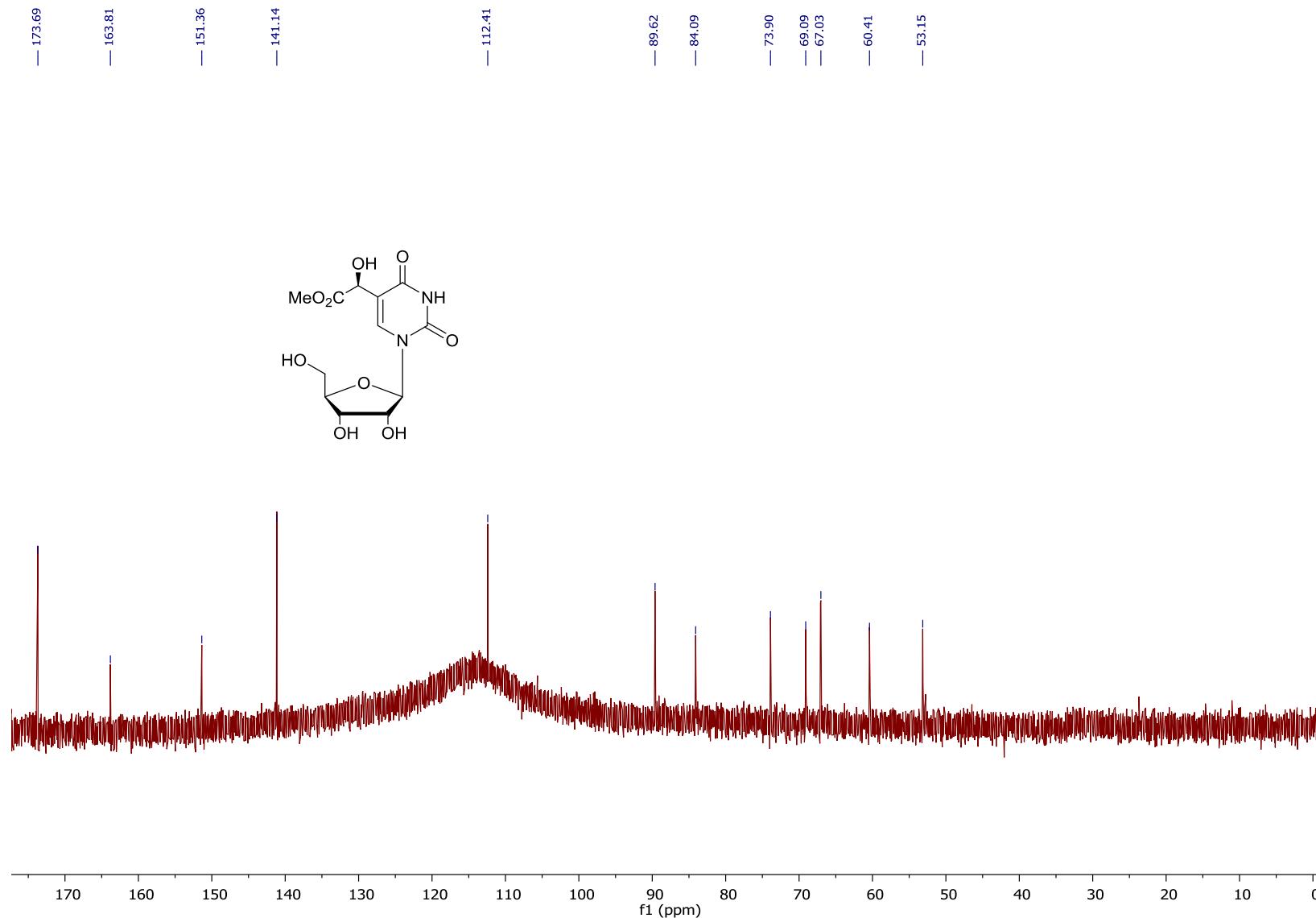


Fig.S12. ^1H NMR (700 MHz, D_2O) spectrum of (*R*)-5-methoxycarbonylhydroxymethylouridine (**2**).

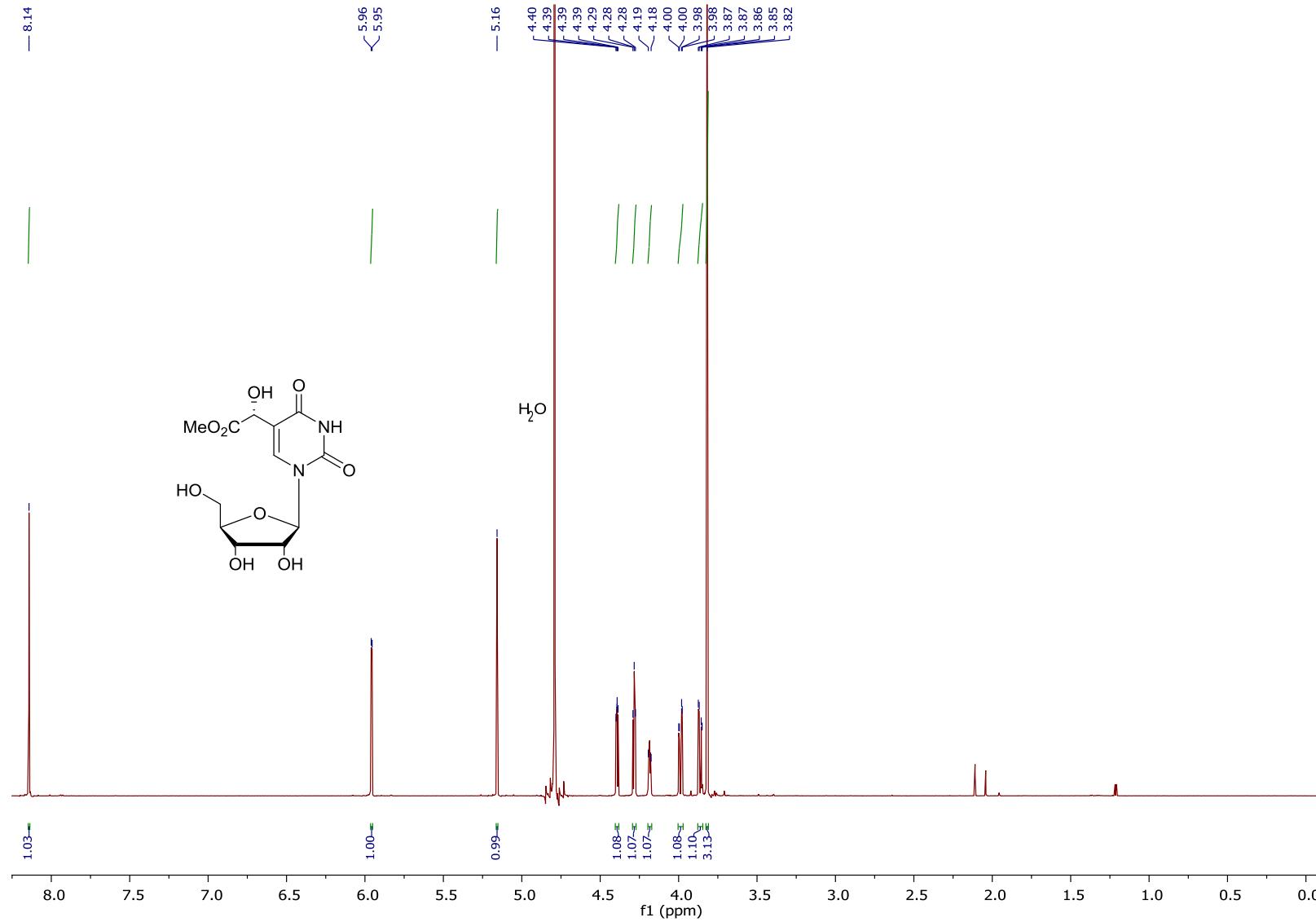


Fig.S13. ^{13}C NMR (176 MHz, D_2O) spectrum of (*R*)-5-methoxycarbonylhydroxymethylouridine (**2**).

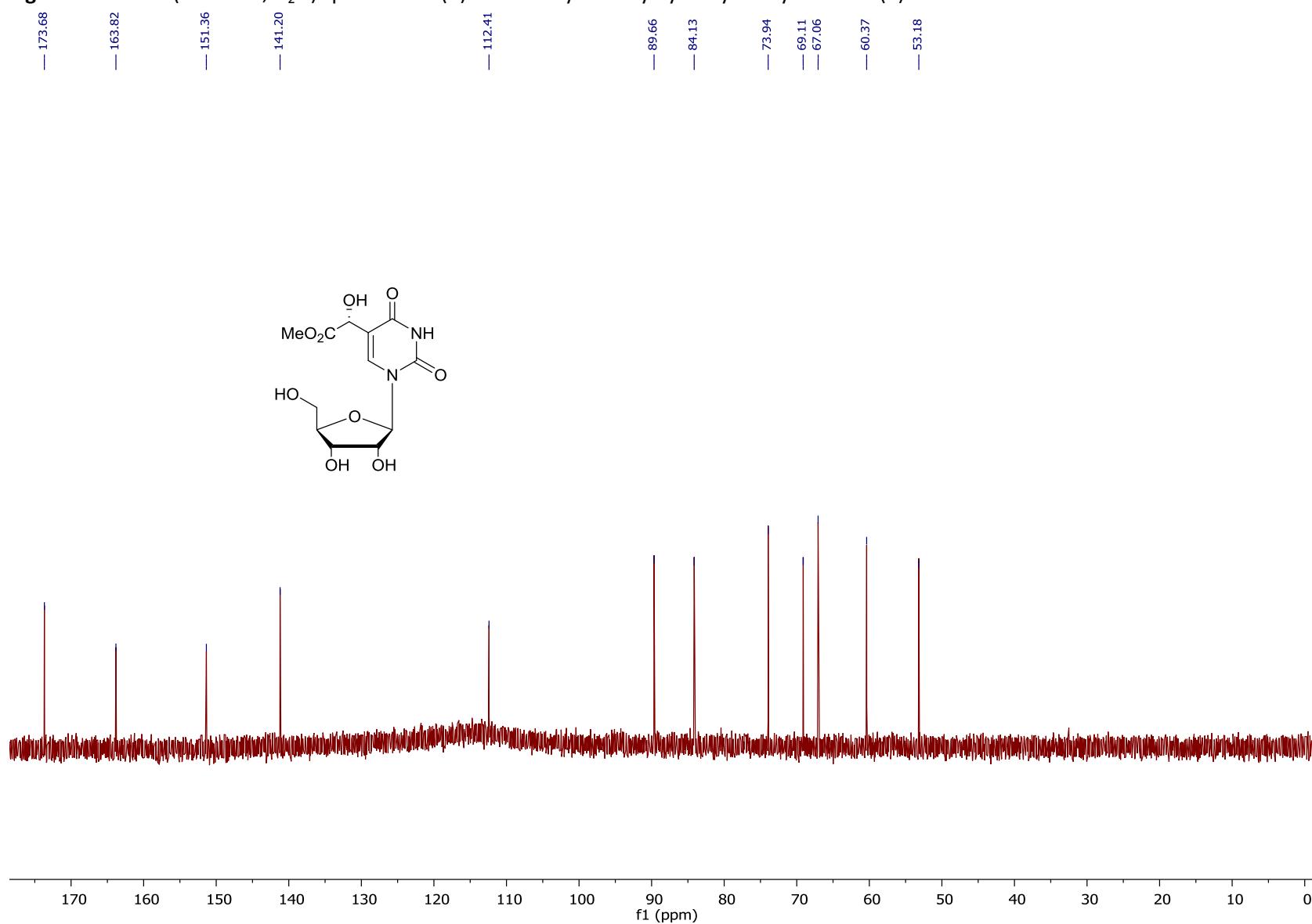


Fig.S14. ^1H NMR (700 MHz, CDCl_3) spectrum of (*R*)- and (*S*)-*O*-(*tert*-butyldimethylsilyl)cyanohydrin of 2',3'-*O*-isopropylidene-5-formyluridine (**10 a**).

NMR (δ [ppm], CDCl_3): ^1H (700 MHz) 0.18 (s, 1.5H), 0.19 (s, 1.5H), 0.25 (s, 1.5H), 0.26 (s, 1.5H), 0.92 (s, 4.5H), 0.93 (s, 4.5H), 1.37 (s, 3H), 1.59 (s, 1.5H), 1.60 (s, 1.5H), 2.58 (bs, 0.4H) 2.63 (bs, 0.4H), 3.82-3.84 (m, 1H), 3.94-3.97 (m, 1H), 4.33-4.34 (m, 0.5H), 4.41-4.41 (m, 0.5H), 4.93-4.96 (dd, 0.5H, $J=2.77$ Hz, $J=6.27$ Hz), 4.96-4.98 (m, 1.4H), 5.49 (d, 0.5H, $J=0.81$ Hz), 5.50 (d, 0.5H, $J=0.88$ Hz), 5.72 (d, 0.5H, $J=2.44$ Hz), 5.76 (d, 0.5H, $J=2.82$ Hz), 7.90 (s, 0.5H), 7.94 (s, 0.5H), 9.23 (bs, 1H).

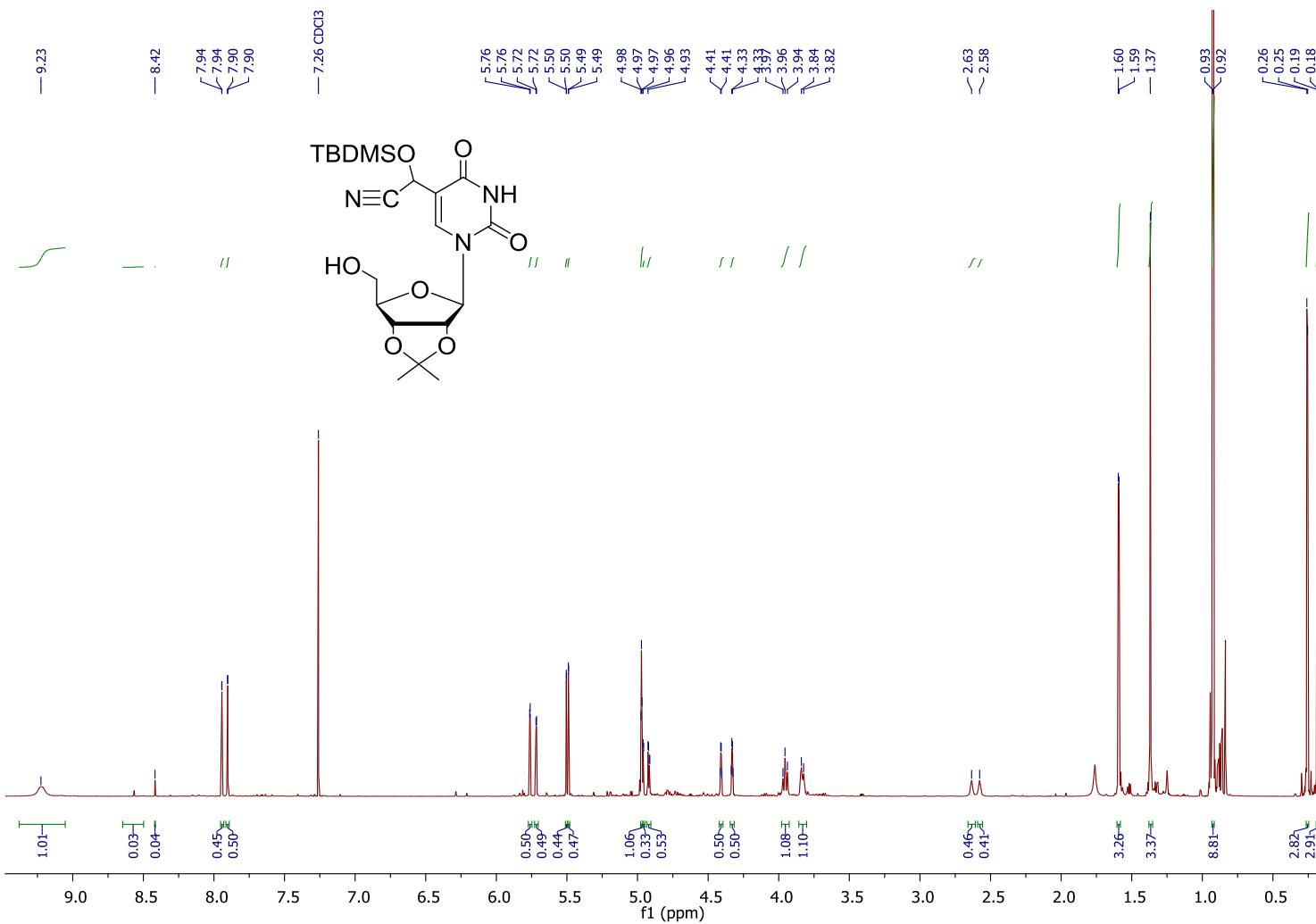


Fig.S15. ^1H NMR (700 MHz, CDCl_3) spectrum of (*S*)- and (*R*)- $2',3'-O$ -izopropylidene- $5'-O$ -tert-butyldimethylsilyl- $5-(O$ -tert-butylidemethylsilyl)cyanohydroxymethyluridine (**10b**).

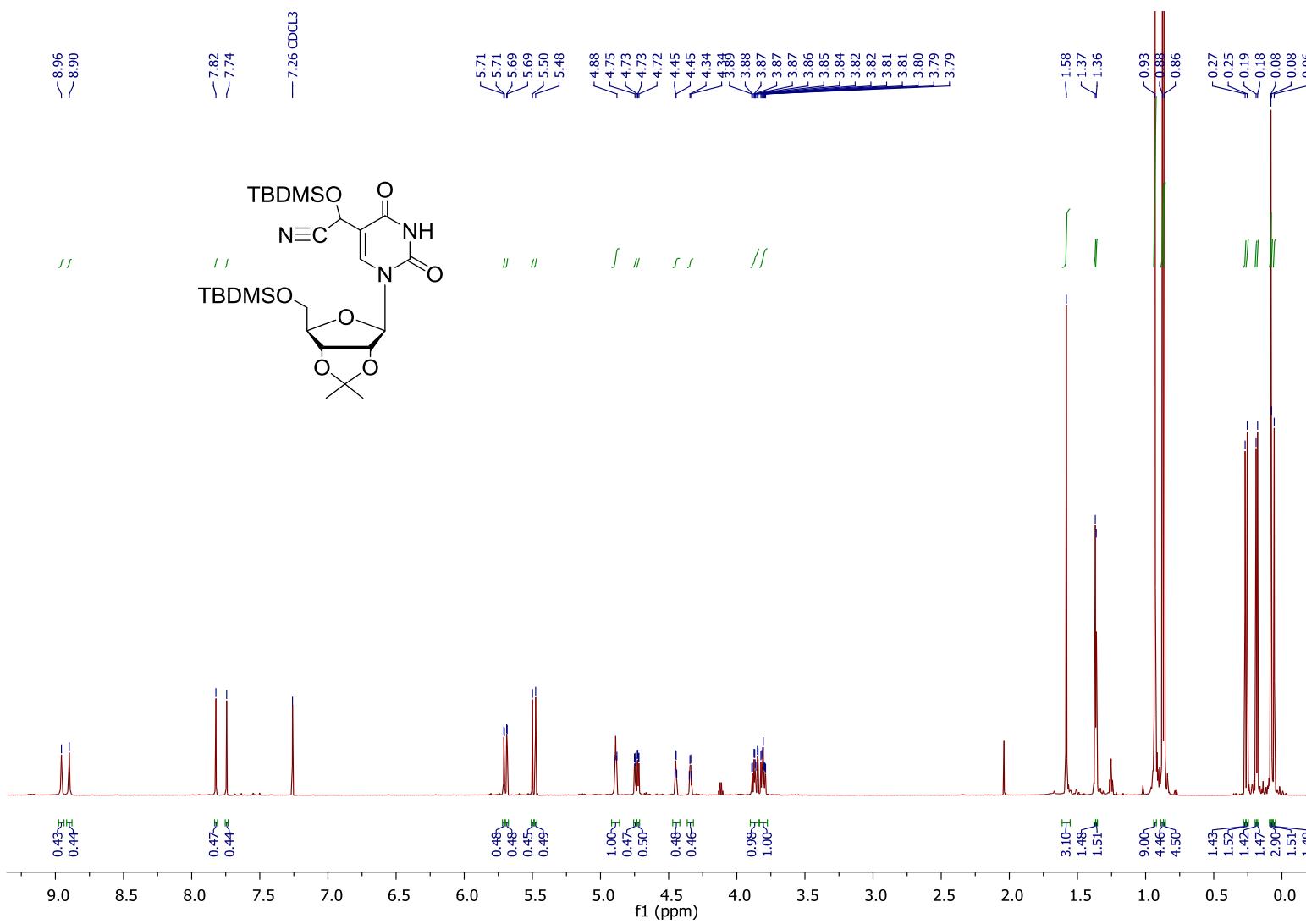
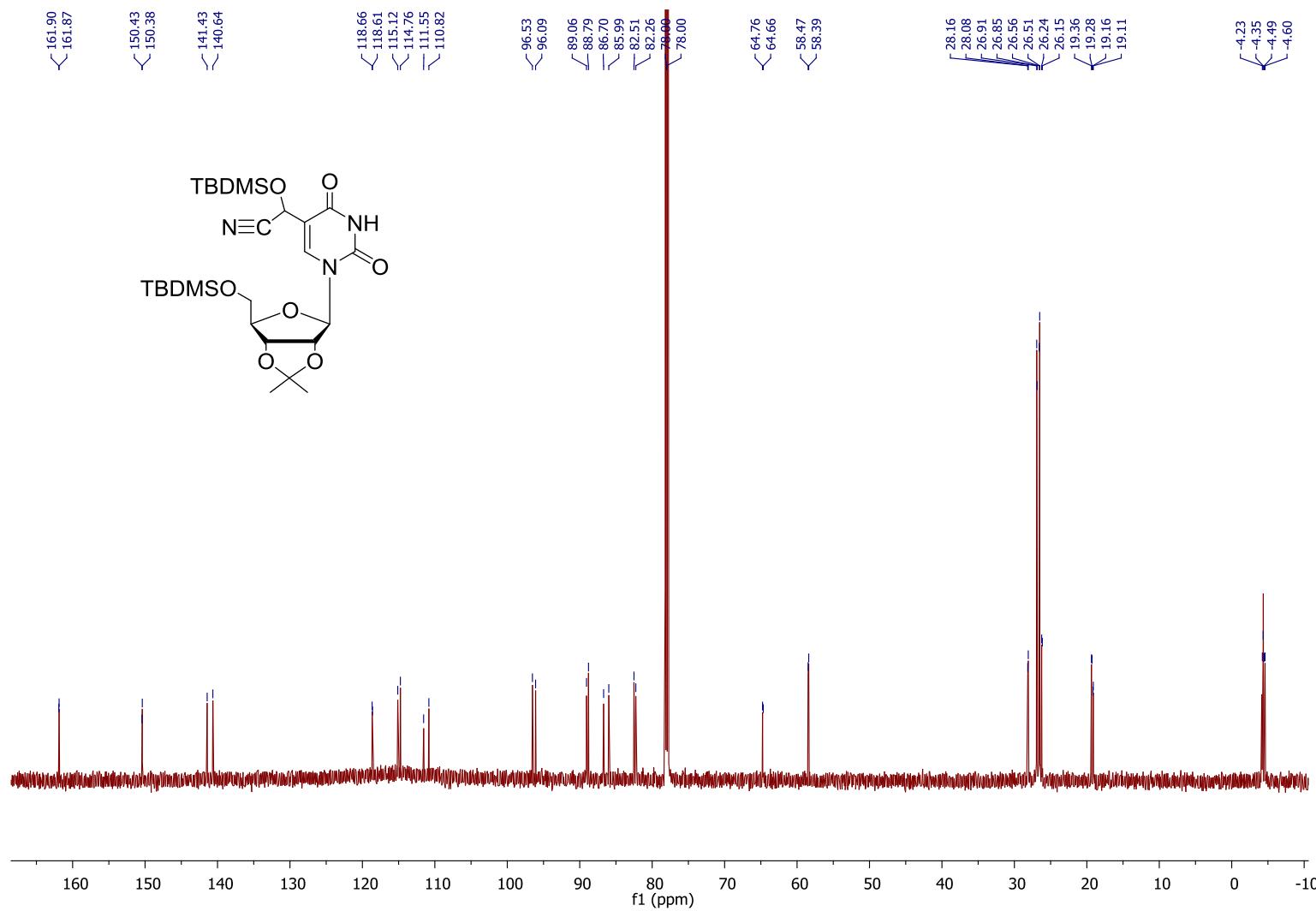
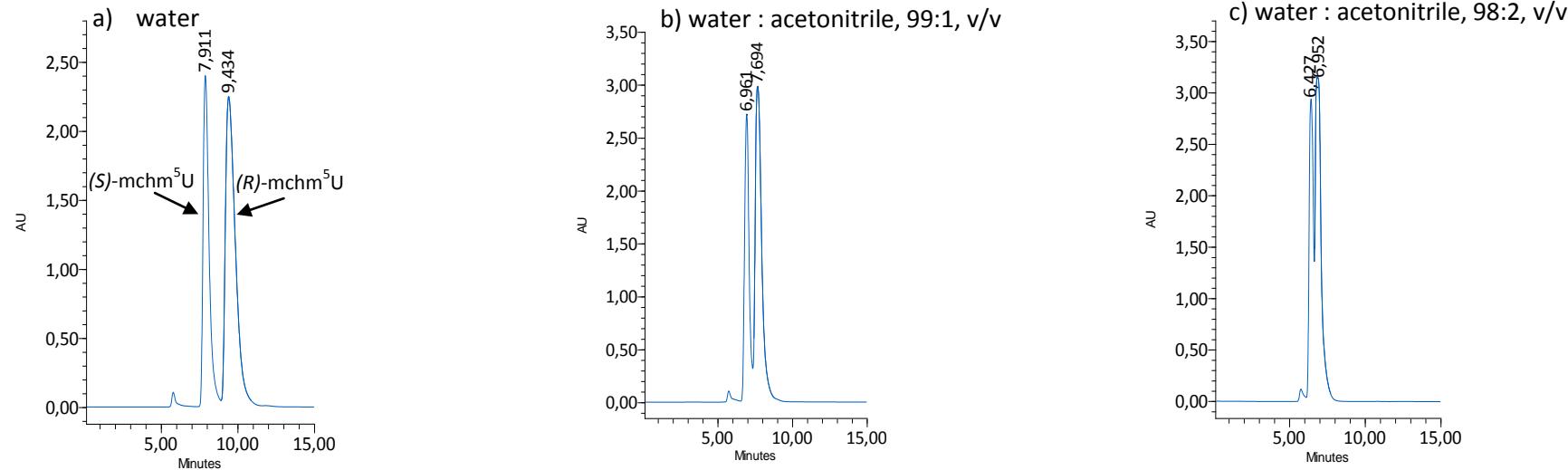


Fig.S16. ^{13}C NMR (176 MHz, CDCl_3) spectrum of (*S*)- and (*R*)-2',3'-*O*-izopropylidene-5'-*O*-*tert*-butyldimethylsilyl-5-(*O*-*tert*-butyldimethylsilyl)cyanohydroxymethyluridine (**10b**).



A) Large scale separation of (*S*)-mchm⁵U and (*R*)-mchm⁵U diastereomers



B) Analytical injections of (*S*)- and (*R*)-mchm⁵U mixture

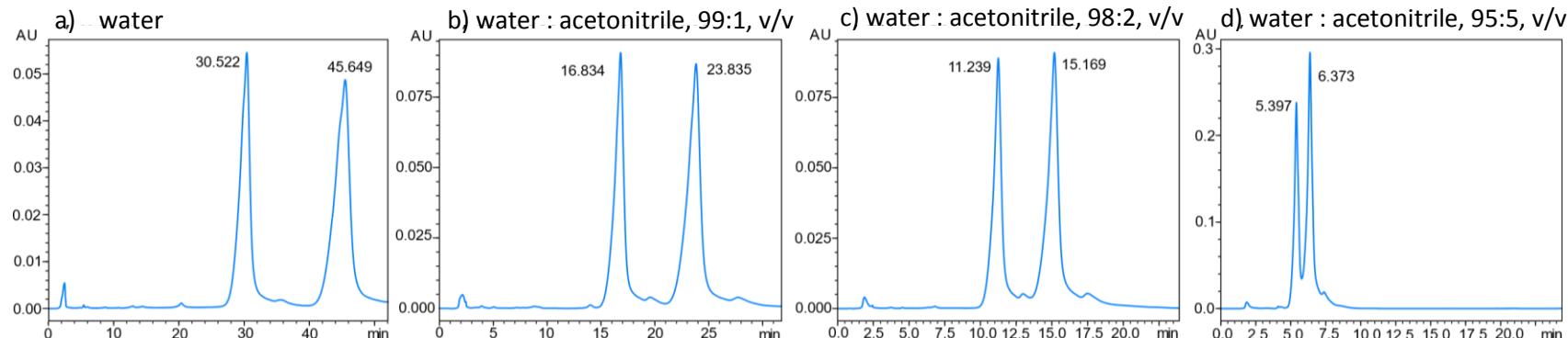


Figure S17. A) Large scale separation of (*S*)-mchm⁵U and (*R*)-mchm⁵U using isocratic RP-HPLC equipped with preparative C18 column (SUPELCO; Ascentis[®]; 25 cm/21.2 mm; 10 μ m; flow 6 ml/min). Three types of eluents were tested: a) water; b) water:acetonitrile, 99:1, v/v; c) water:acetonitrile, 98:2, v/v; **B)** Analytical injections of (*S*) and (*R*) diastereomers mixture on Reprosher 100 C18 column (5 μ m 250x 4,6mm, flow 1 ml/min) to select conditions for separation of (*S*)-mchm⁵U and (*R*)-mchm⁵U on a large scale.

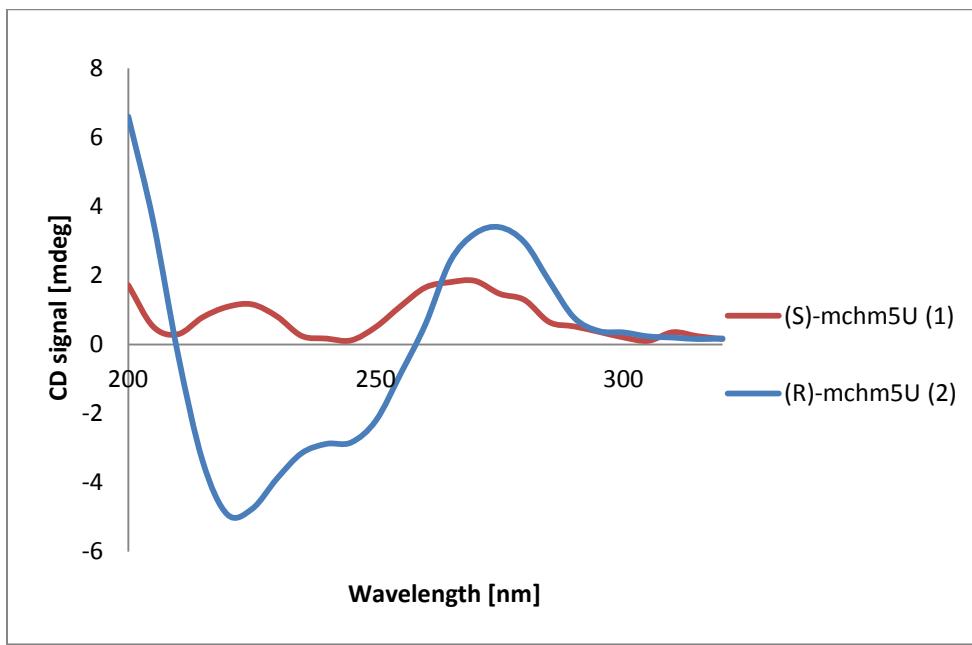


Figure S18. CD spectra of (*S*)-mchm⁵U (**1**, red line) and (*R*)-mchm⁵U (**2**, blue line).