

**1,3-Dipolar cycloaddition of alkenes to 3'-azido-3'-deoxythymidine
as a route to 3'-deoxythymidin-3'-yl derivatives**

Pavel N. Solyev, Roman A. Novikov, Marina K. Kukhanova and Maxim V. Jasko

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Materials and methods

All reagents and solvents were purchased from Acros (Belgium). AZT was a gift from the “AZT Association” (Moscow, Russia).

^1H and ^{13}C NMR spectra (δ , ppm; J , Hz) were registered on an AMX III-400 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) with the working frequency of 400 MHz for ^1H NMR (Me_4Si as an internal standard for organic solvents), 100.6 MHz for ^{13}C NMR (with carbon-proton interaction decoupling). ^{15}N NMR and 2D NMR spectra were recorded on an AM-300 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) with the working frequency of 300 MHz for ^1H NMR (Me_4Si as an internal standard for organic solvents), 75.5 MHz for ^{13}C NMR (with carbon-proton interaction decoupling), 40.5 MHz for ^{15}N NMR (with nitrogen-proton interaction decoupling) at 27°C. Proton and carbon shifts were additionally determined using HH and CH correlations – COSY, HSQC, HMBC (over ranges of 2 and 3 bonds: $^2J_{\text{H-C-C}}$, $^3J_{\text{H-C-C-C}}$), nitrogen shifts were determined with the help of INEPT (over ranges of 1 and 2 bonds: $^1J_{\text{NH}}$, $^2J_{\text{NH}}$).

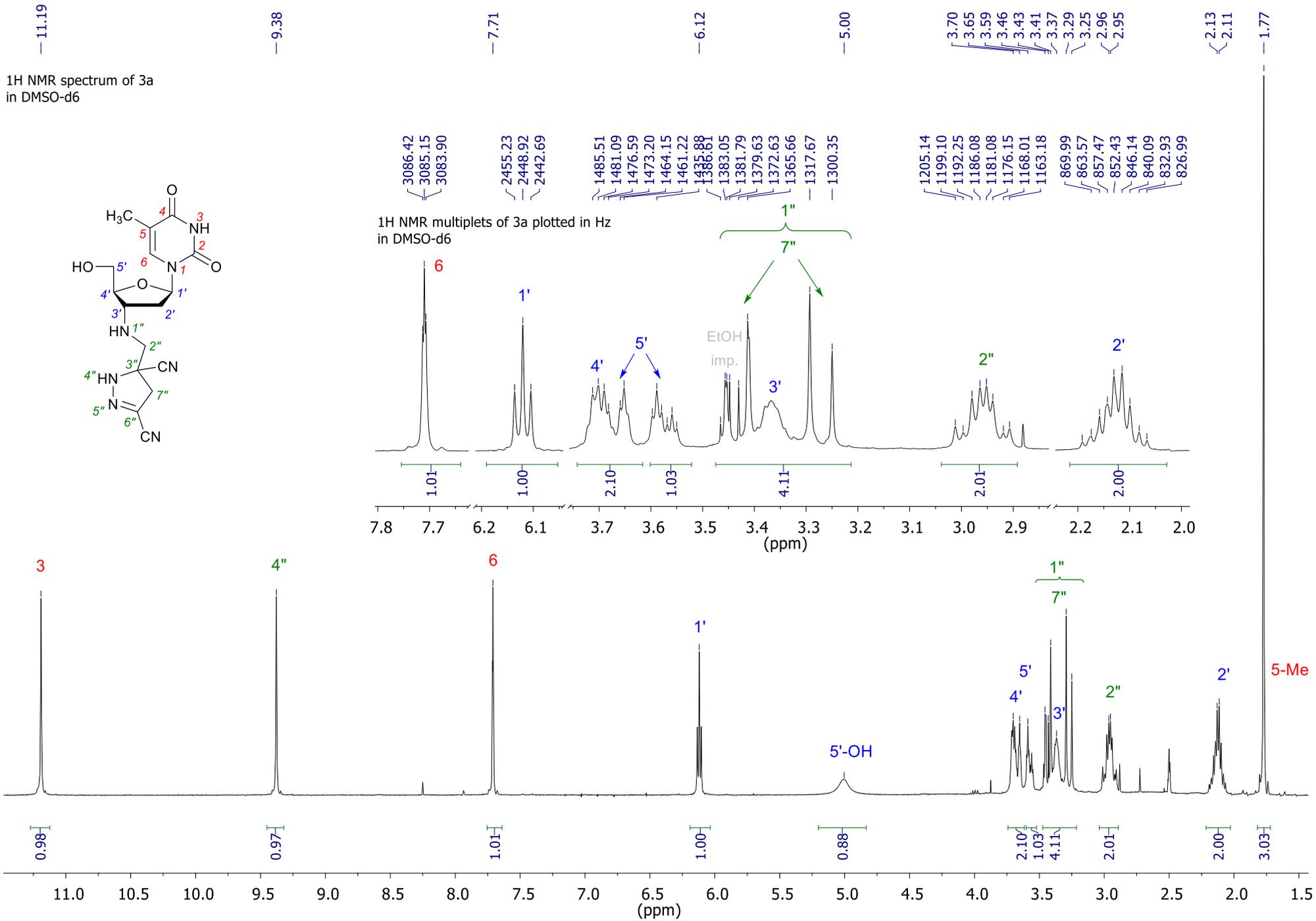
UV spectra were registered on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) in water in the range of 200-300 nm and agreed to those for thymidine derivatives.

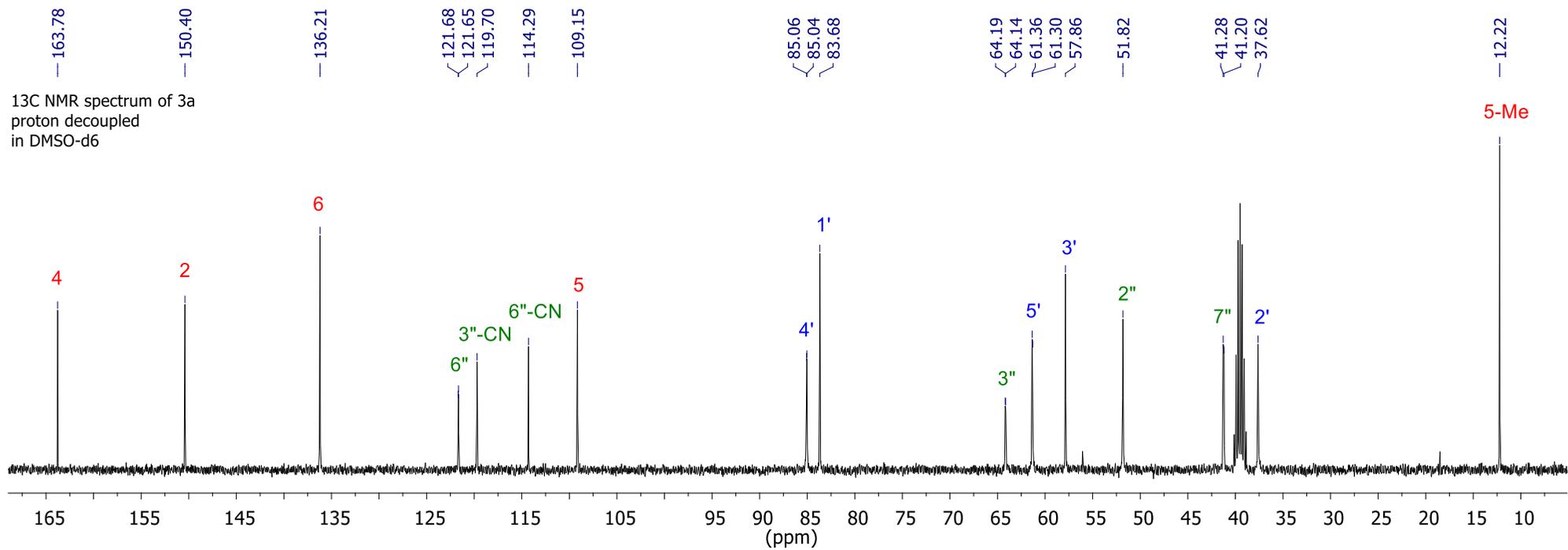
Mass-spectra were registered on a Thermo Finnigan LCQ Advantage spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, USA) using electrospray ionization (ESI). The measurements were acquired in a positive and negative ion mode (interface capillary voltage – 4500 V); mass range from m/z 50 to m/z 3000 Da. A syringe injection was used for solutions in acetonitrile (flow rate 50 $\mu\text{L}/\text{sec}$). Nitrogen was applied as a dry gas; interface temperature was set at 250°C.

High resolution mass spectra (HR MS) were measured on a Bruker maXis instrument (Bruker Daltonik GmbH, Bremen, Germany) using ESI [P.A. Belyakov, V.I. Kadentsev, A.O. Chizhov, N.G. Kolotyrkina, A.S. Shashkov and V.P. Ananikov, *Mendeleev Commun.*, 2010, **20**, 125-131.] for compound **6**. The measurements were acquired in a positive ion mode (interface capillary voltage – 4500 V); mass range from m/z 50 to m/z 3000 Da; external calibration was performed with a Fluka Electrospray Calibrant Solution (Sigma-Aldrich, Buchs, Switzerland). A syringe injection was used for solutions in acetonitrile (flow rate 3 $\mu\text{L}/\text{min}$). Nitrogen was applied as a dry gas; interface temperature was set at 180°C.

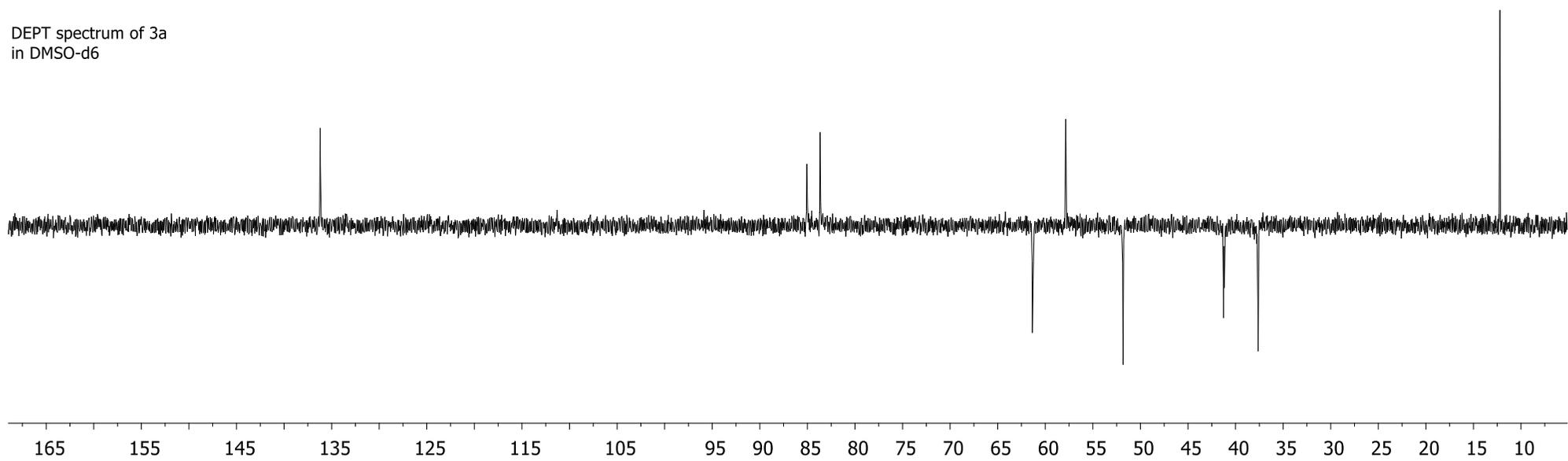
The following materials for HIV-1 RT assays were used: [α - ^{32}P]dATP (5000 Ci/mmol) was from Izotop (Moscow, Russia), activated DNA was purchased from GE Healthcare (Little Chalfont, UK), *E.coli* strain was from Novagen (Madison, WI, USA), 2'-deoxyribonucleoside

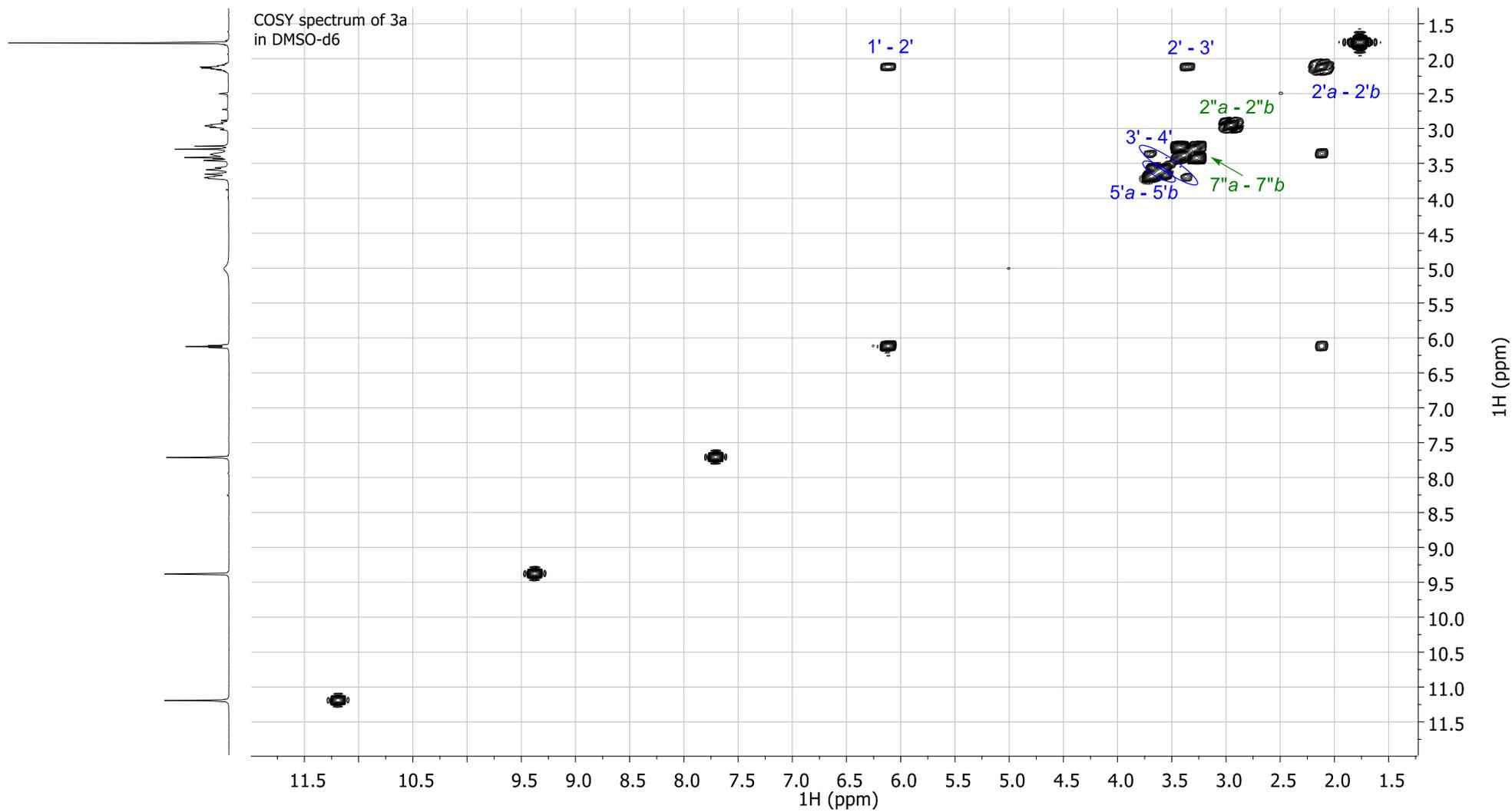
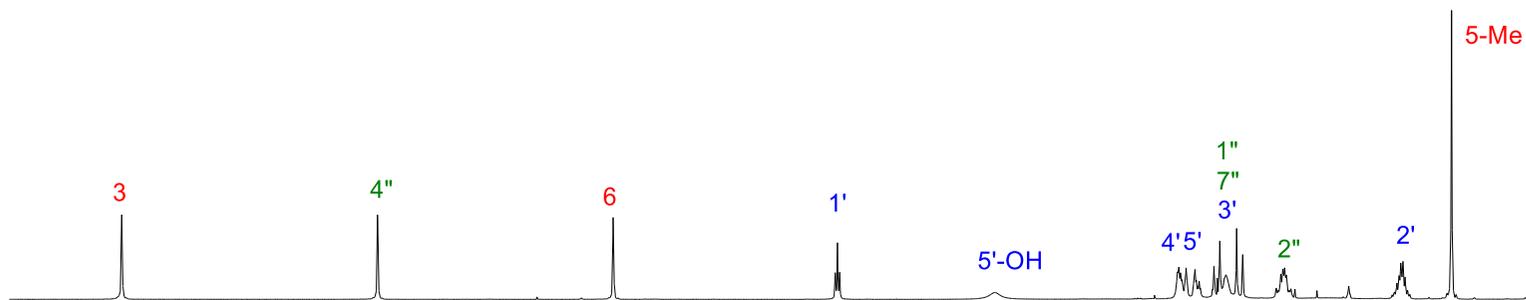
5'-triphosphates were from Promega (Fitchburg, WI, USA), cellulose filters 3MM were from Whatman (GE Healthcare division, Maidstone, UK). All other reagents of highest grade were obtained from Sigma-Aldrich and Fluka. HIV-1 reverse transcriptase was expressed in *E.coli* and isolated as described in [V.O. Rechinskiĭ, S.F. Barbashov, I.L. Degtiarev, S.M. Vorob'ev, D.L. Liakhov, D.A. Kostiuk, A.I. Starov, G.R. Matsevich and S.N. Kochetkov. *Mol. Biol.* (Moscow), 1991, **25**, 1248-1257]. An Intertechnique SL-4000 liquid scintillation counter (Intertechnique, Fairfield, NJ, USA) was used for radioactivity determination.

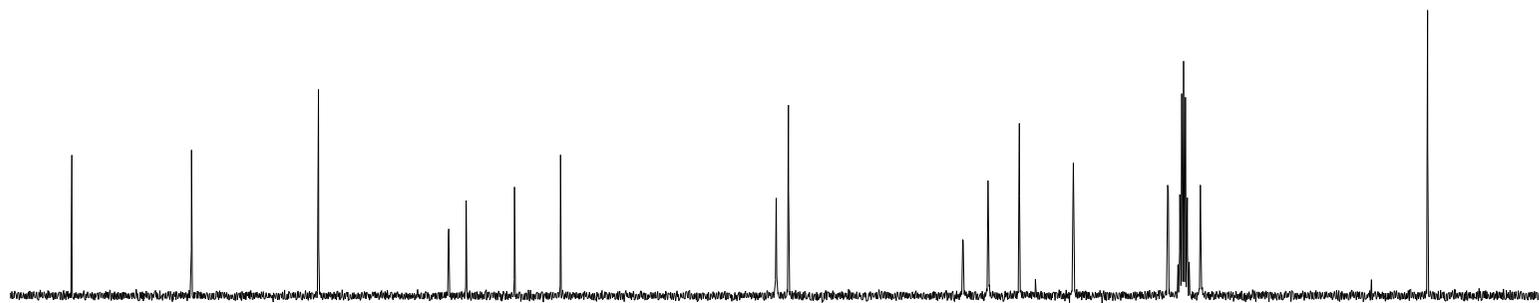


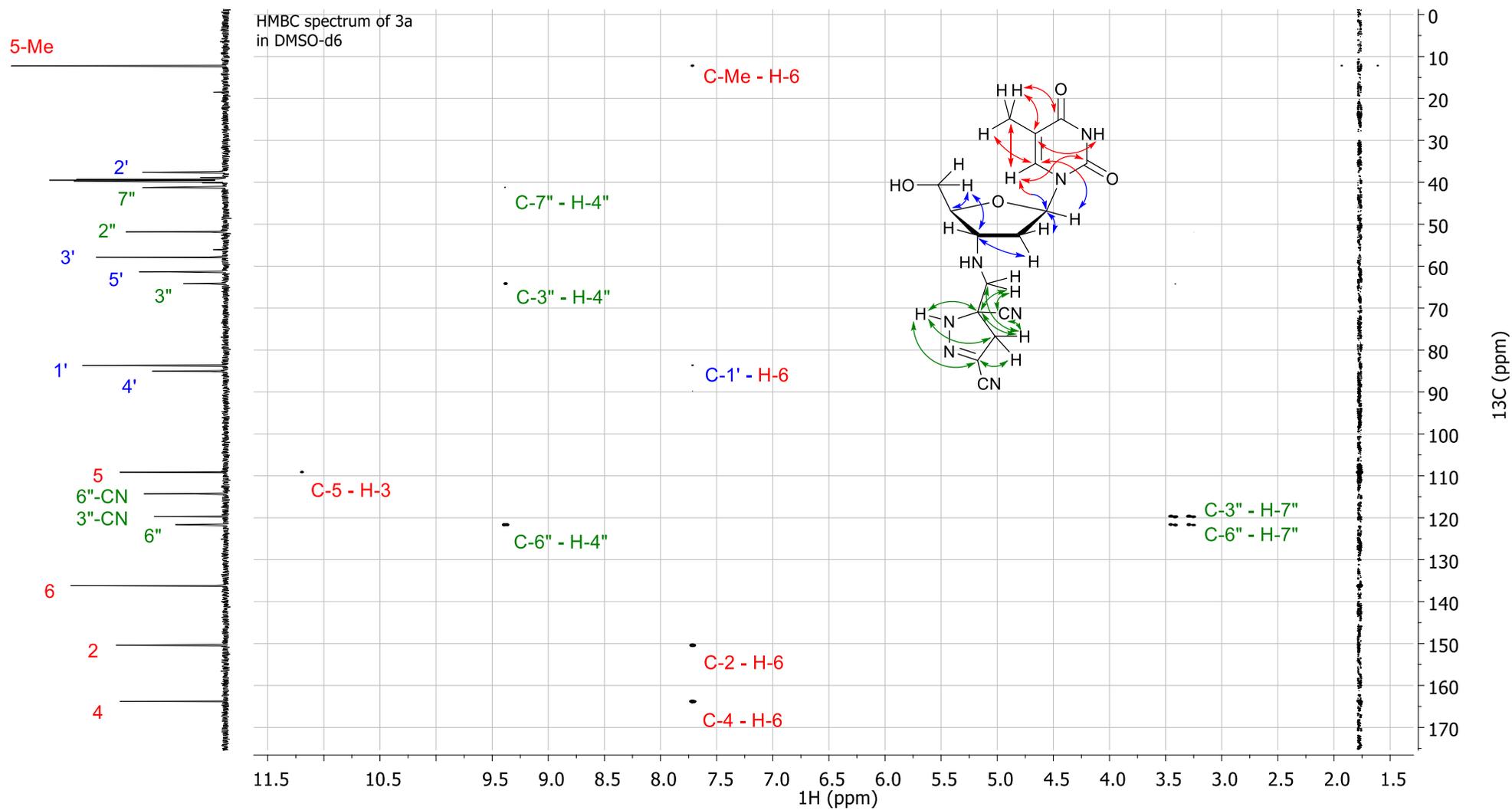
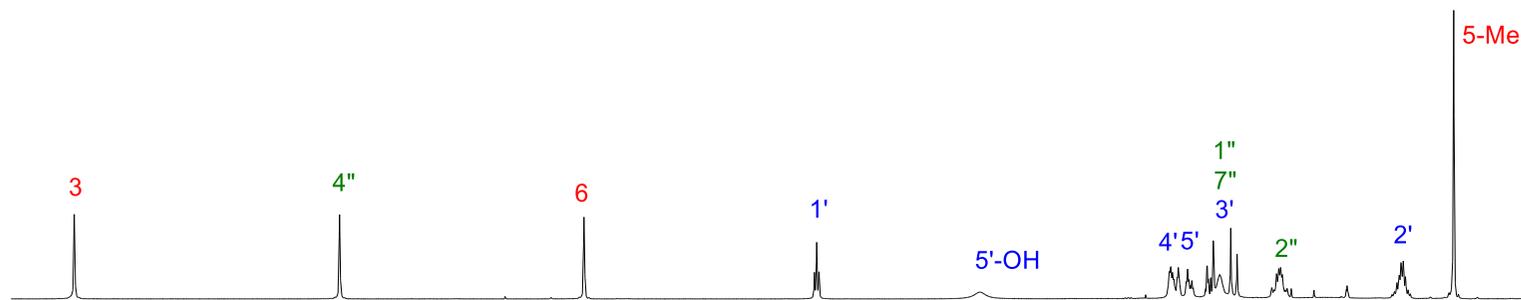


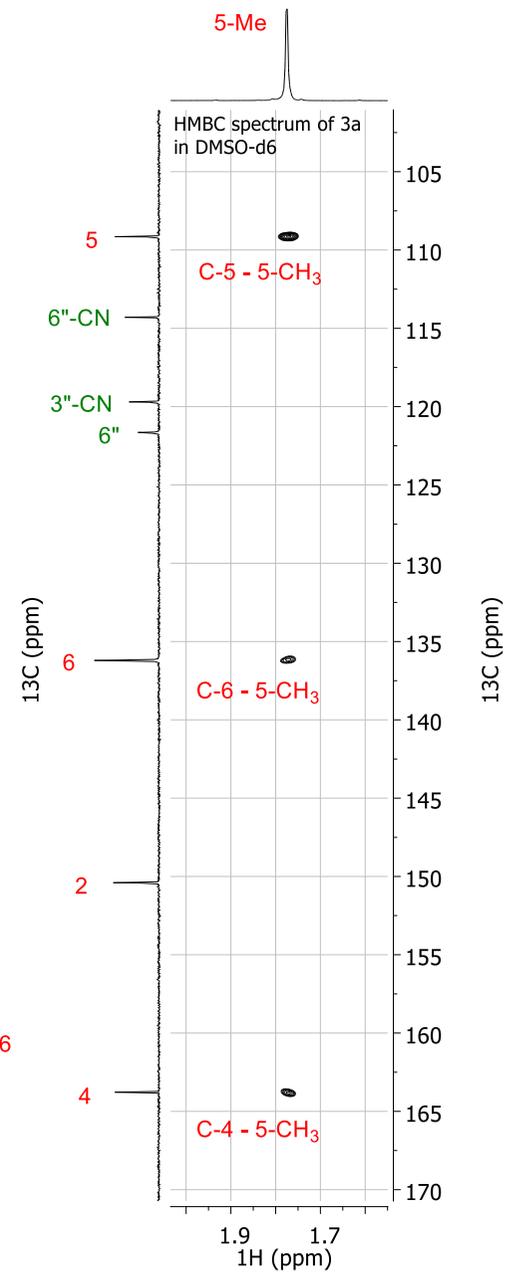
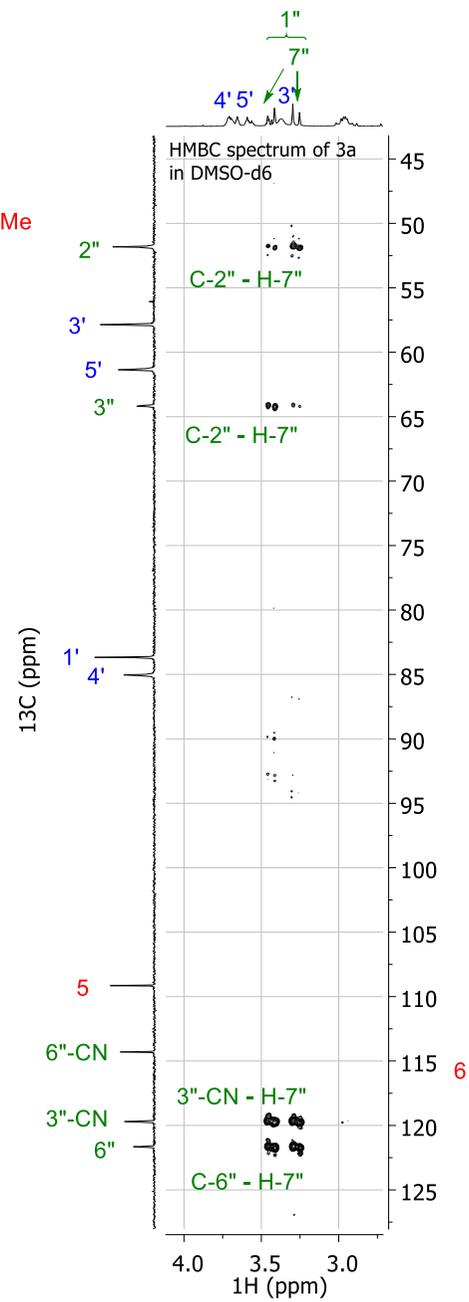
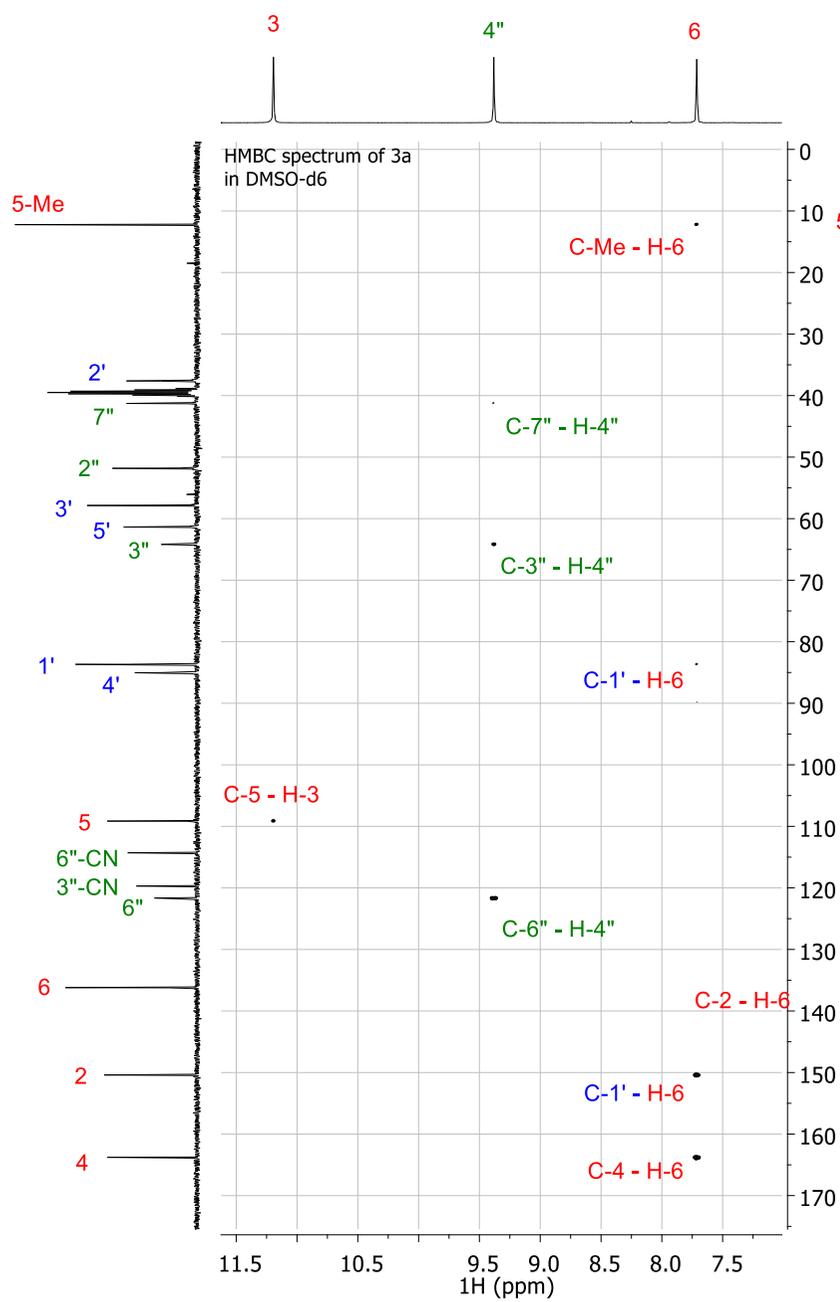
DEPT spectrum of 3a
in DMSO-d6



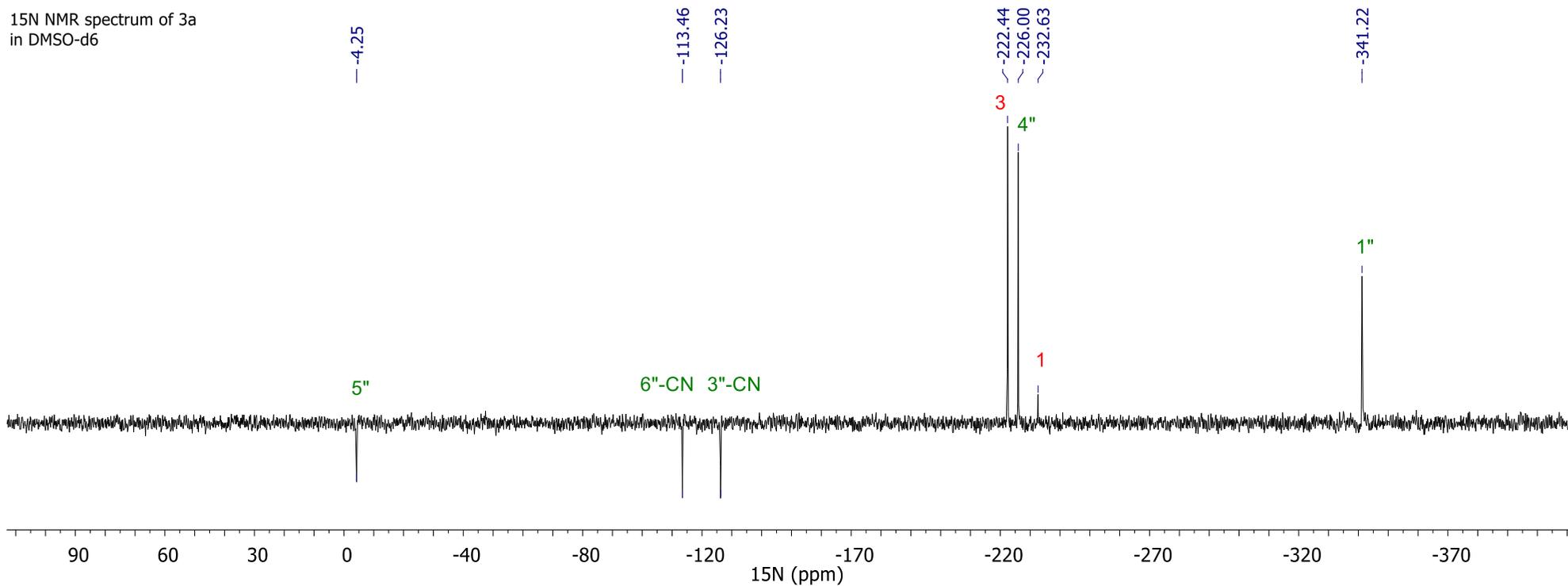




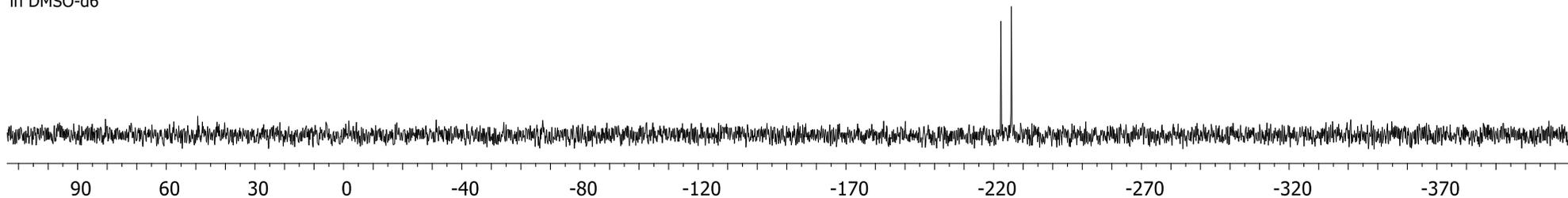




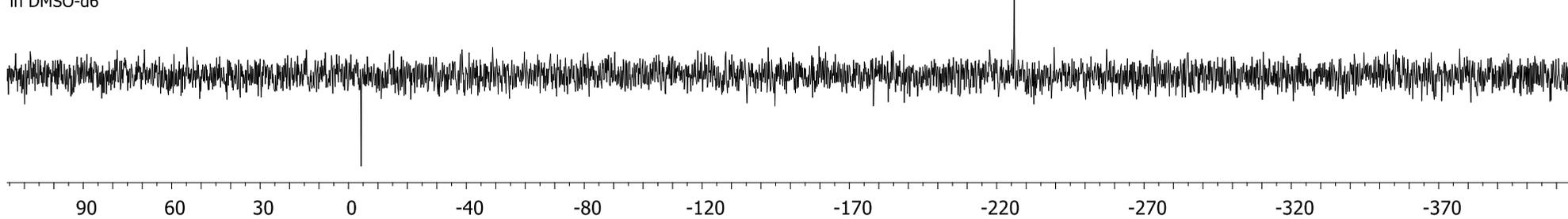
15N NMR spectrum of 3a
in DMSO-d6

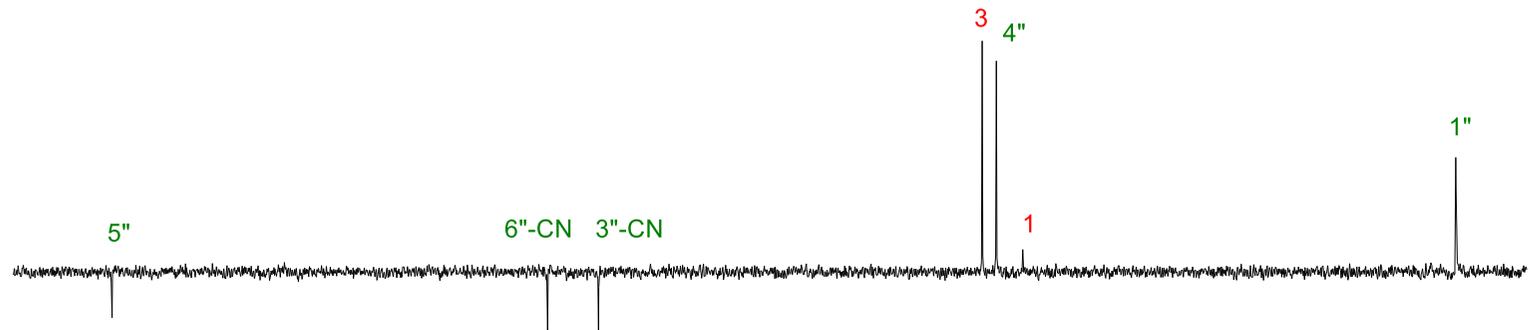


15N-INEPTD (DR) spectrum of 4
(only NH, NH2, NH3)
in DMSO-d6

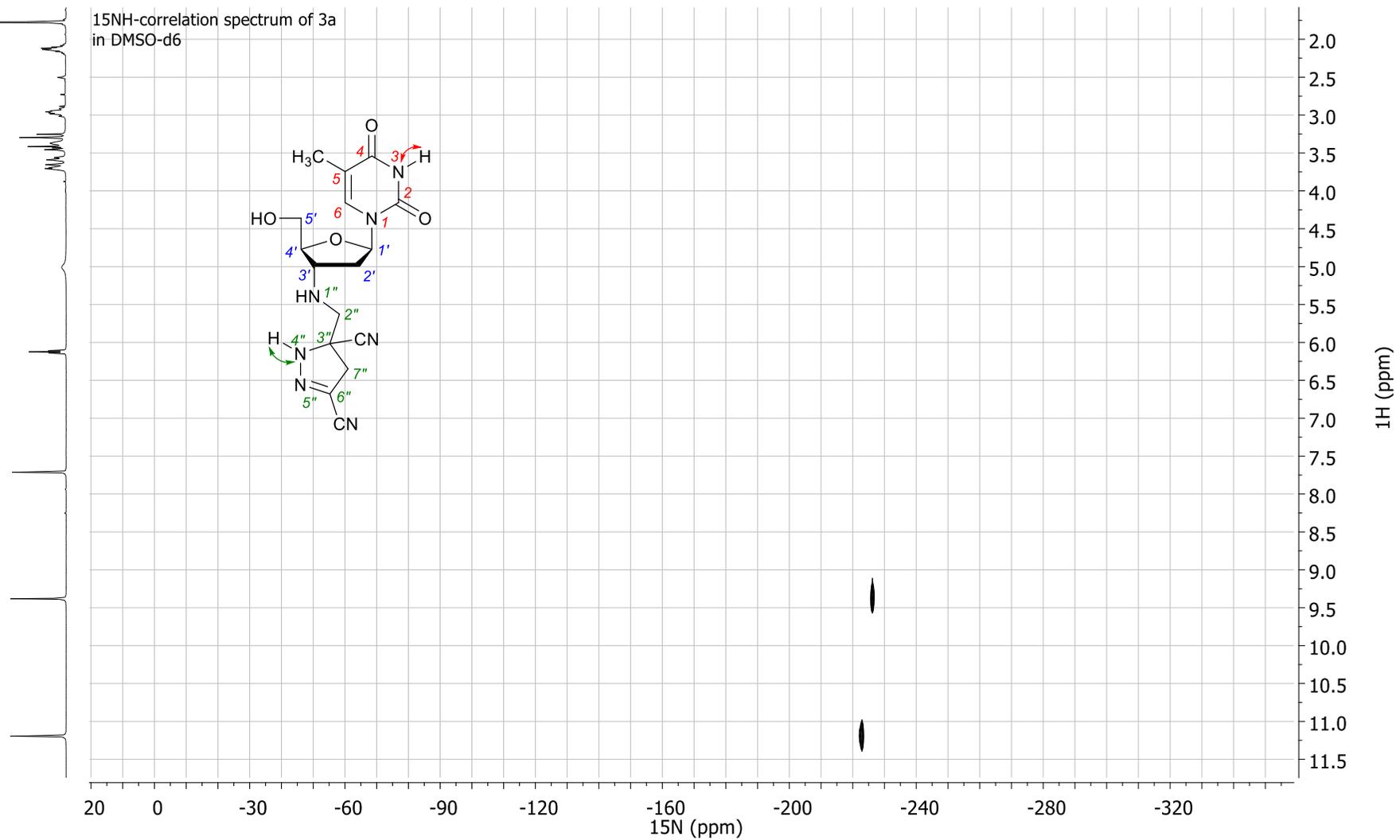


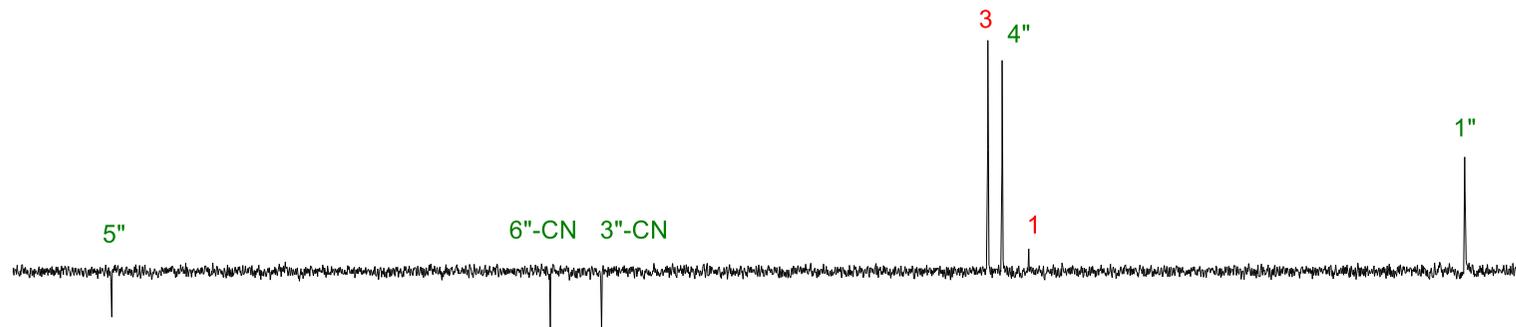
15N-INEPTD (LR) spectrum of 4
(only N with long range 1H-coupling)
in DMSO-d6



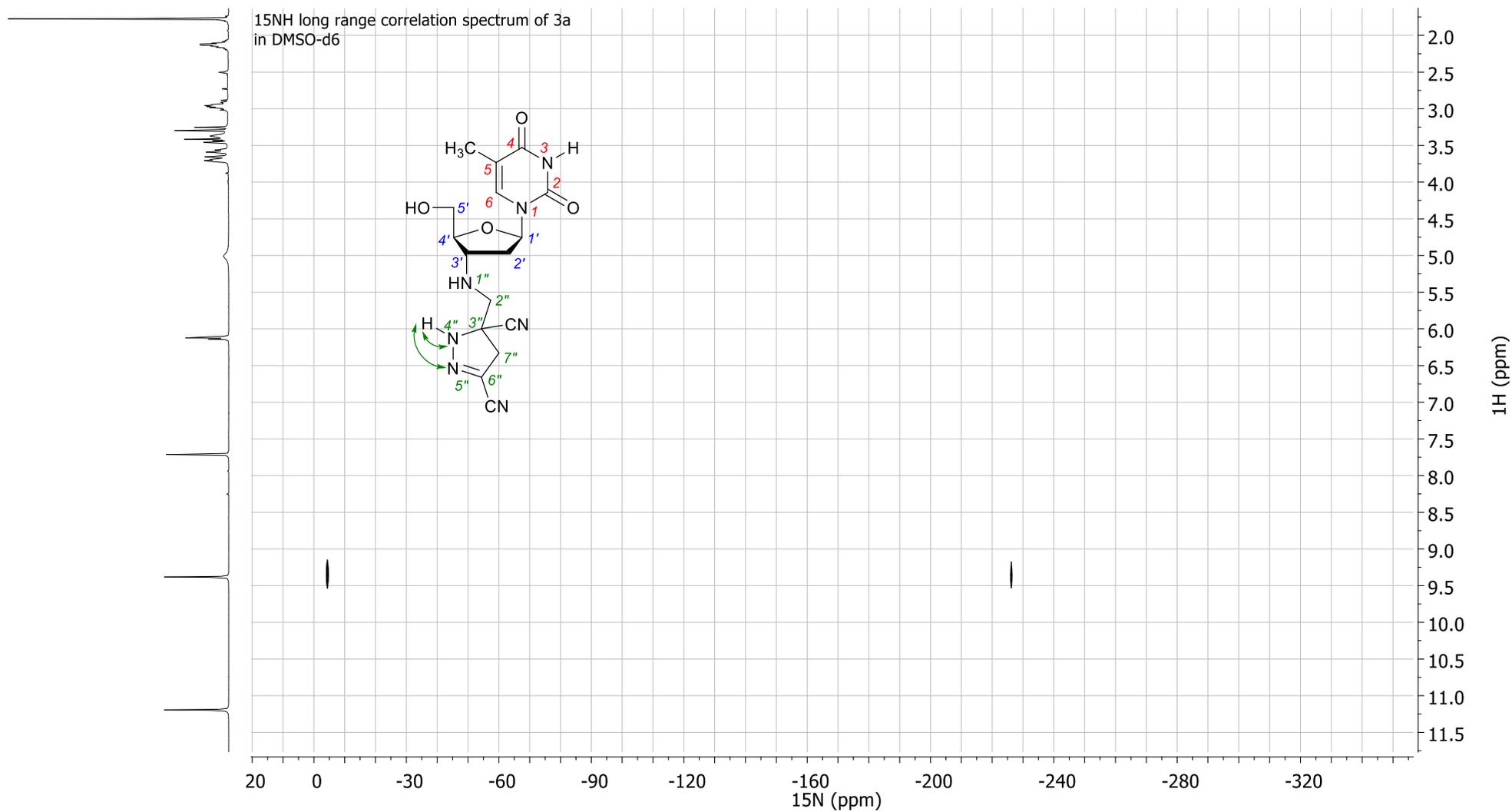


15NH-correlation spectrum of 3a
in DMSO-d6

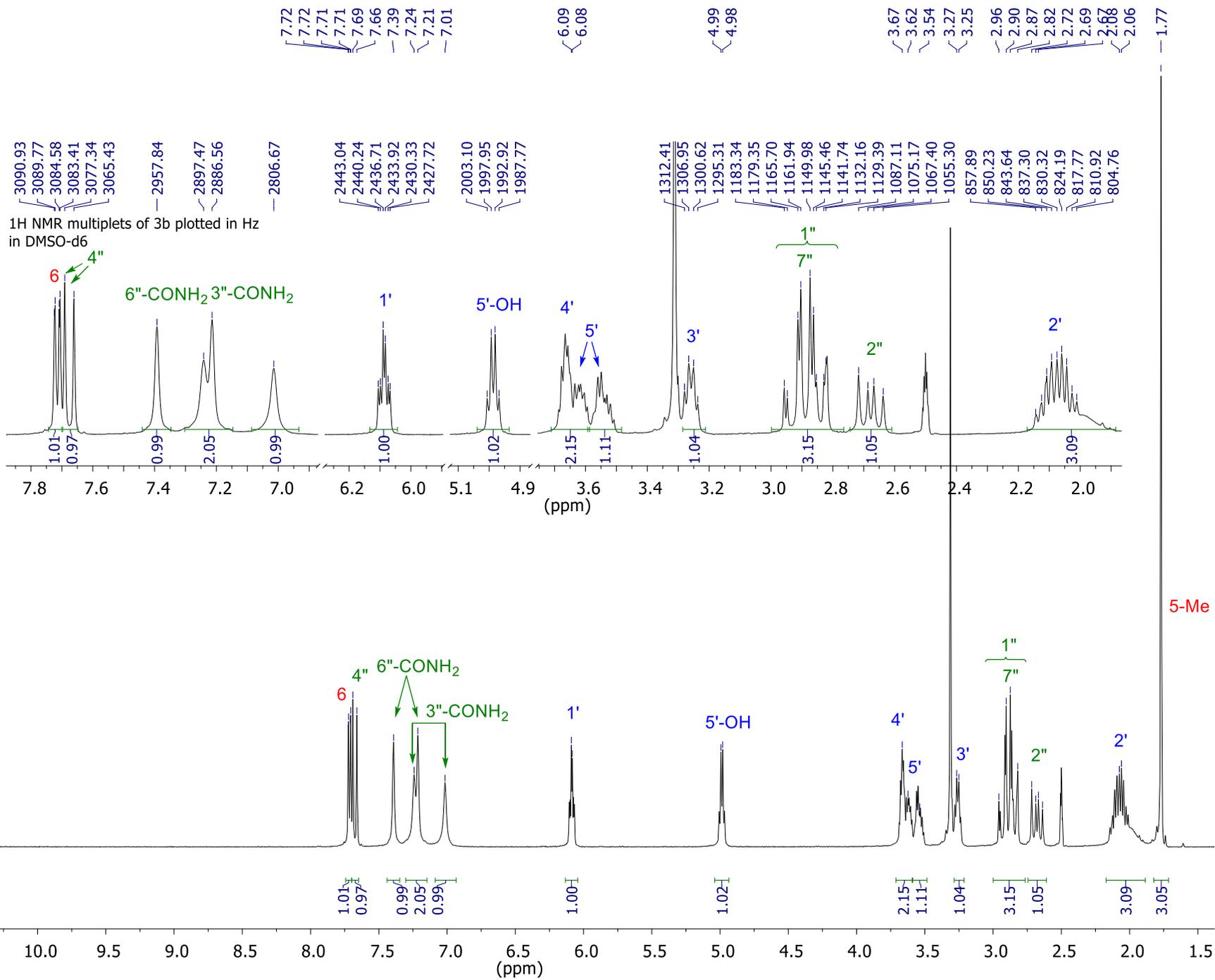
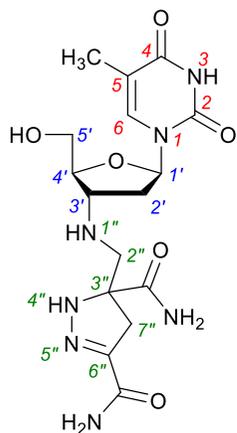




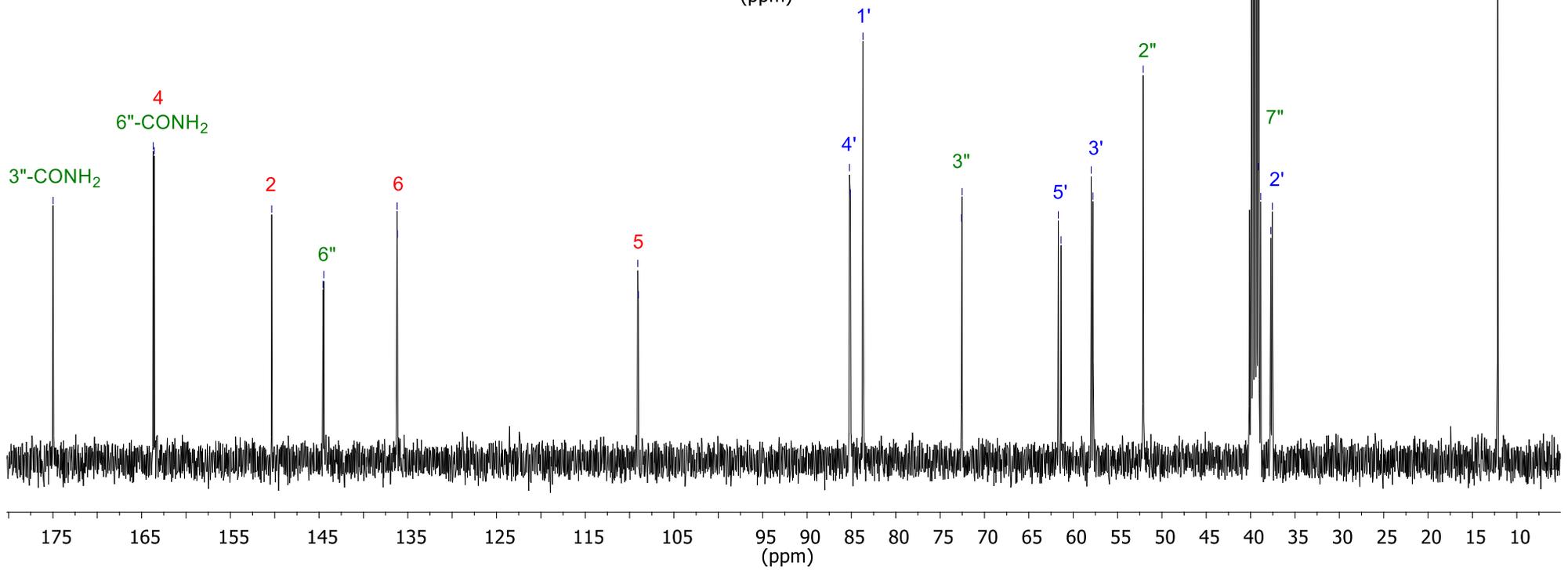
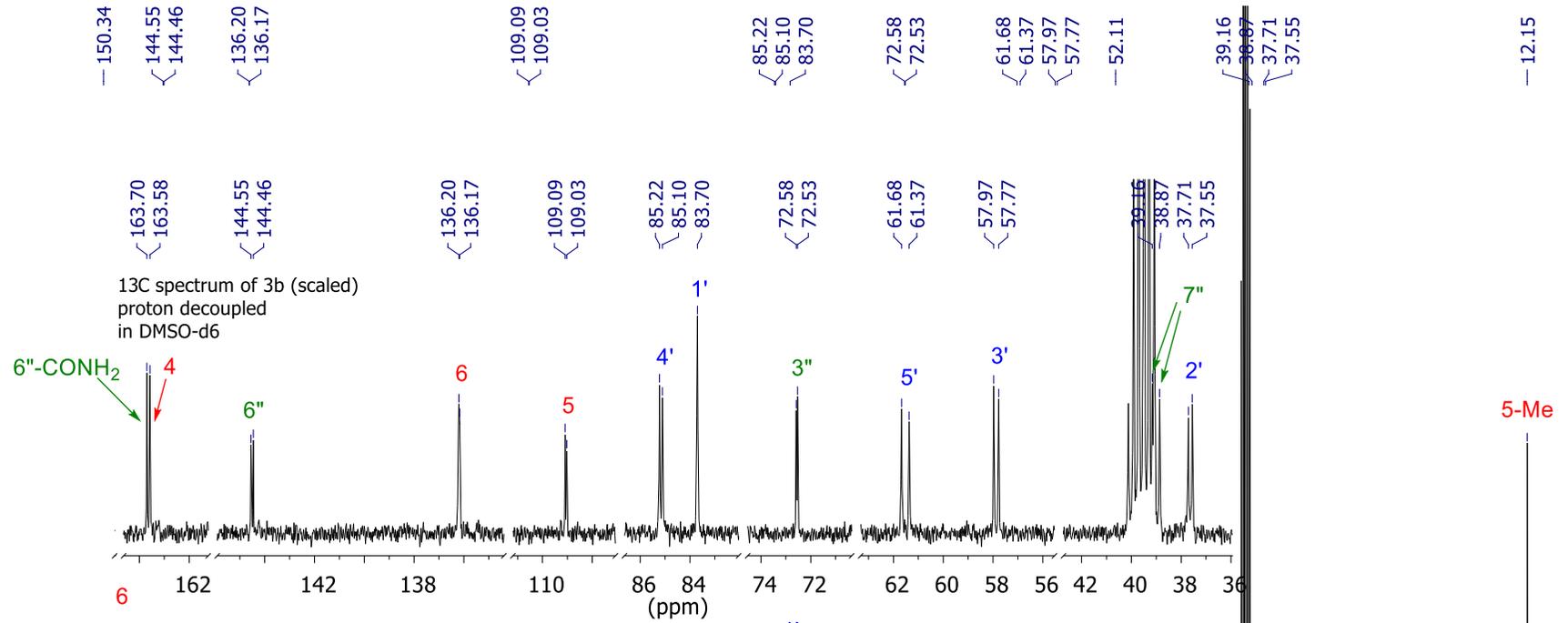
15NH long range correlation spectrum of 3a in DMSO-d6



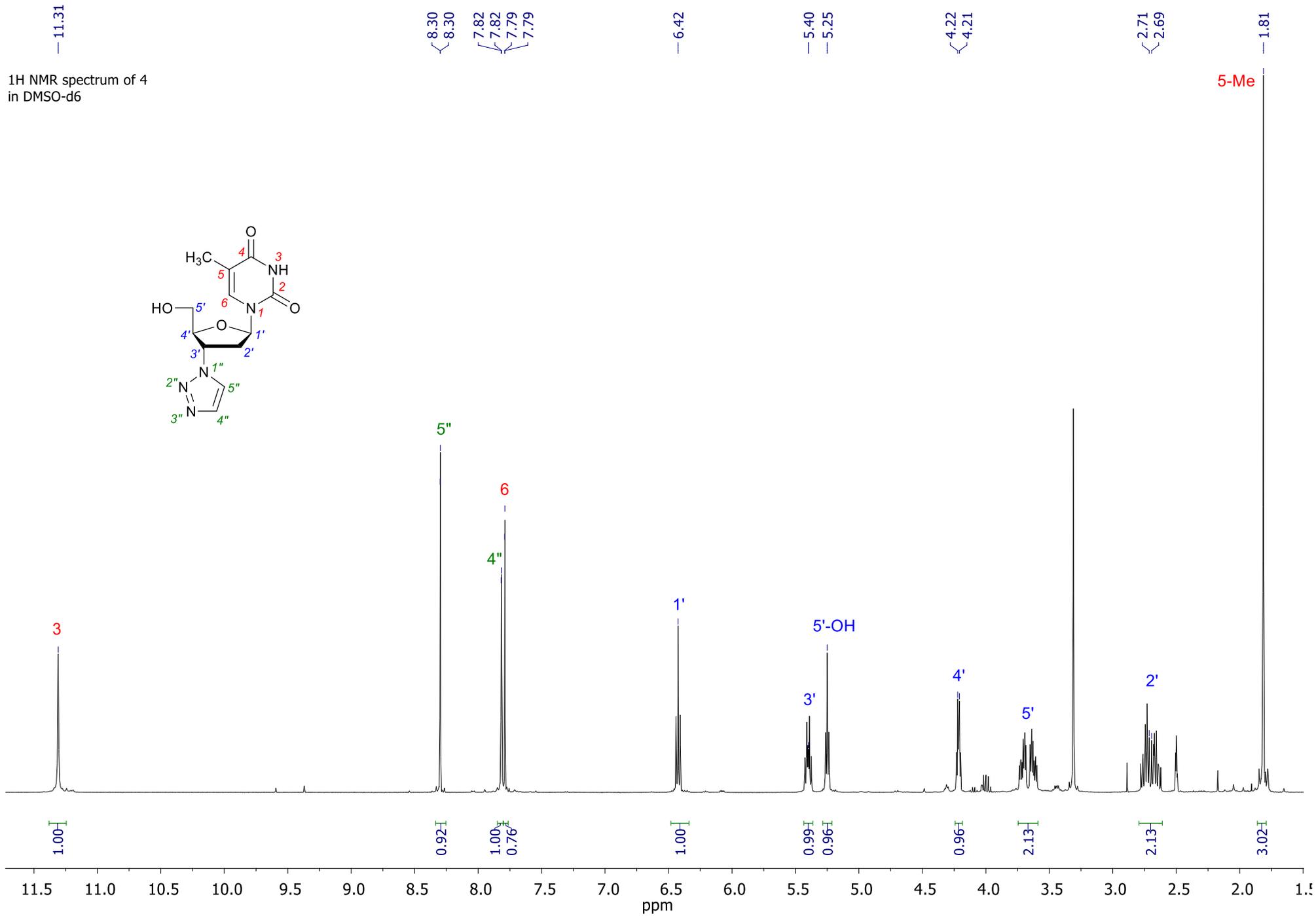
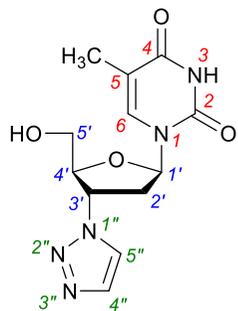
1H NMR spectrum of 3b
in DMSO-d6

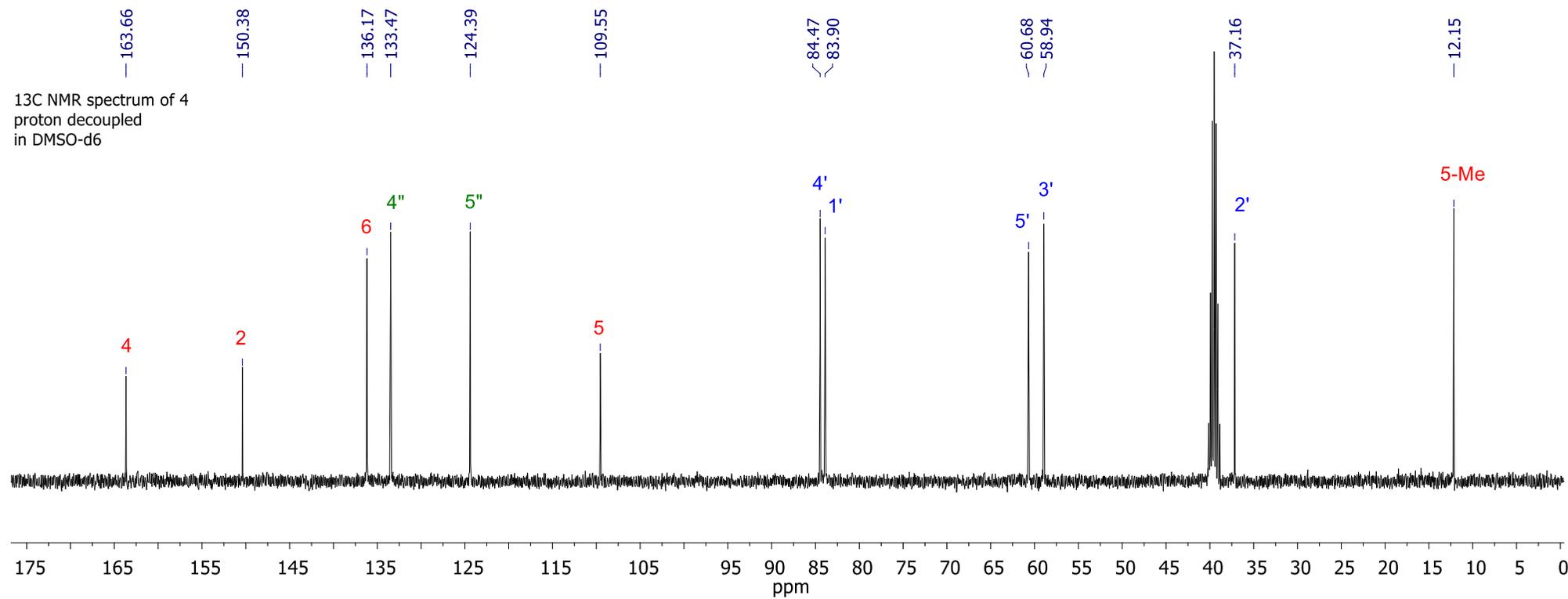
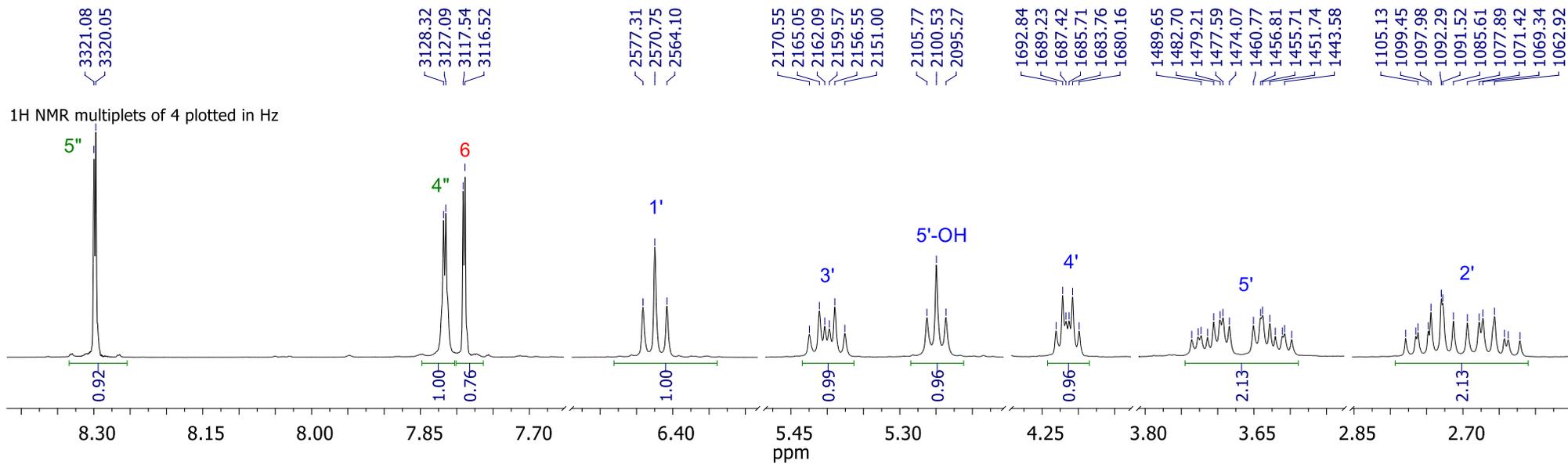


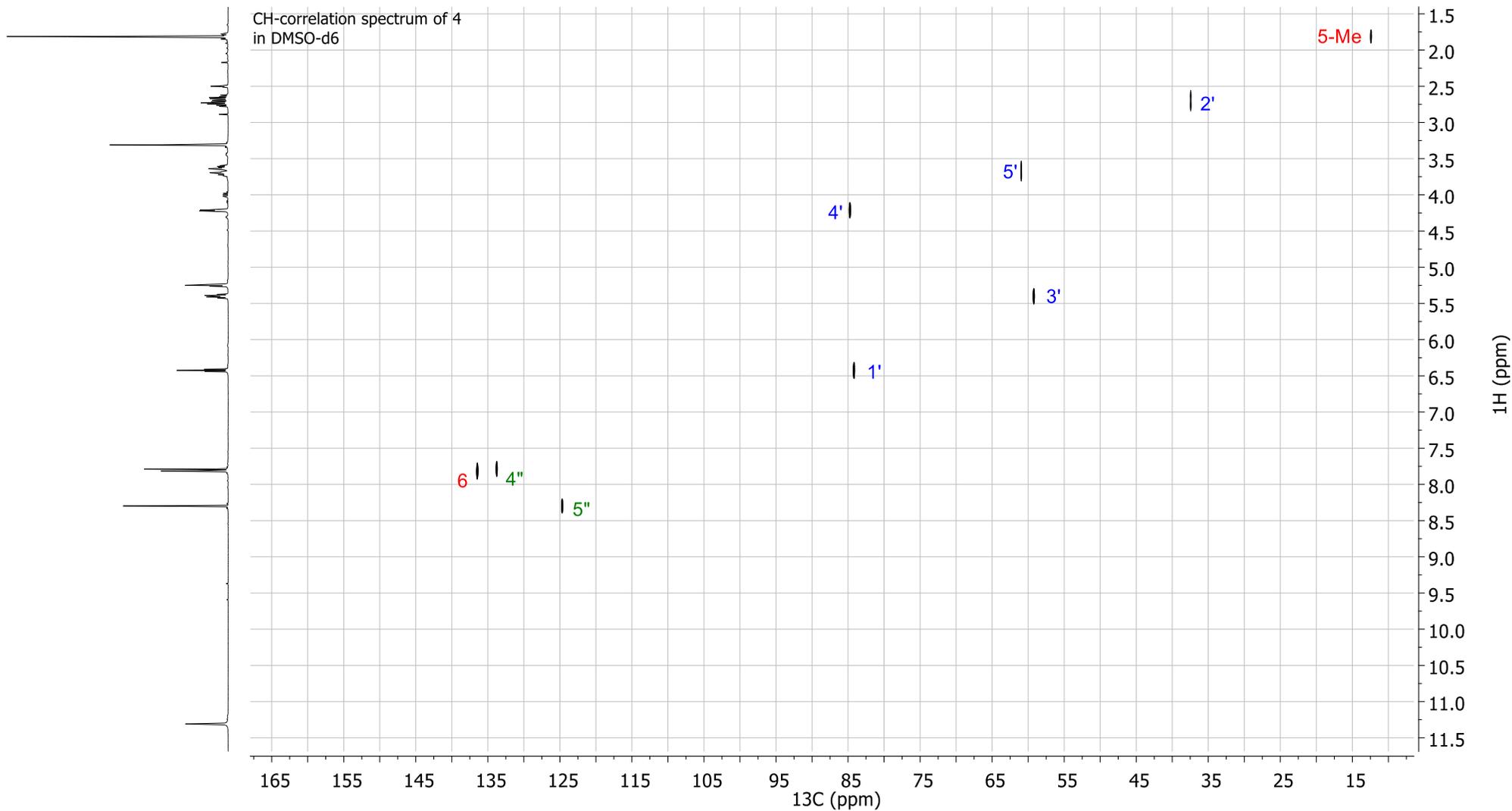
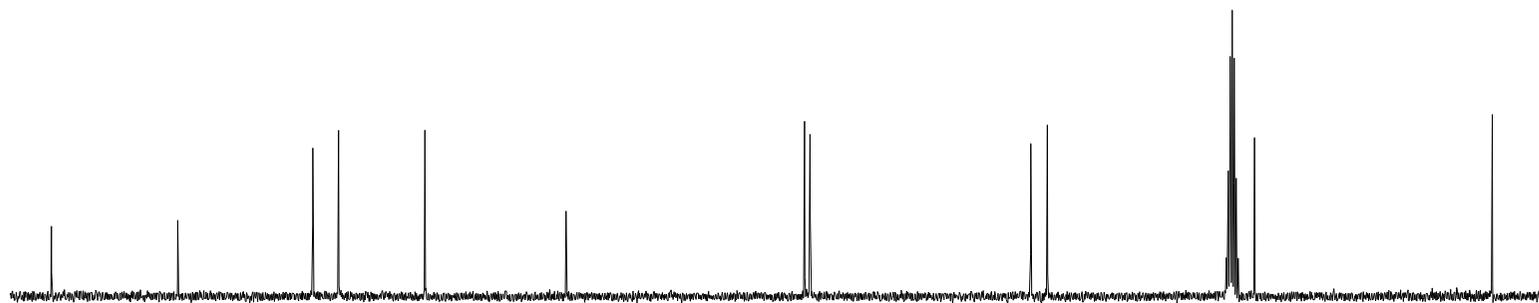
^{13}C spectrum of 3b
proton decoupled
in DMSO-d₆



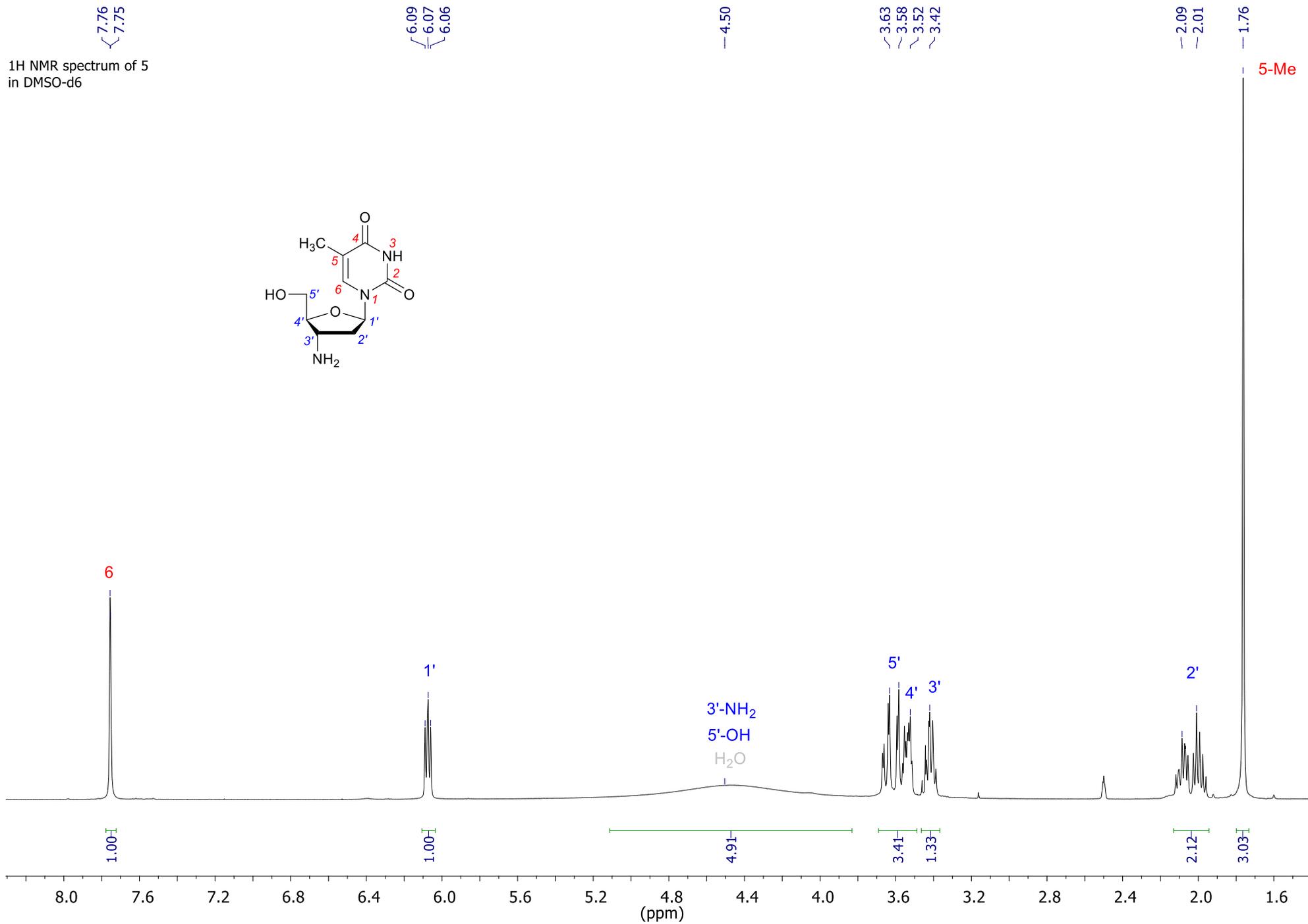
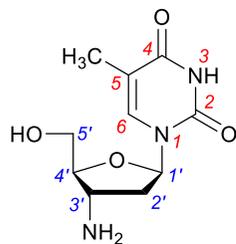
1H NMR spectrum of 4
in DMSO-d6

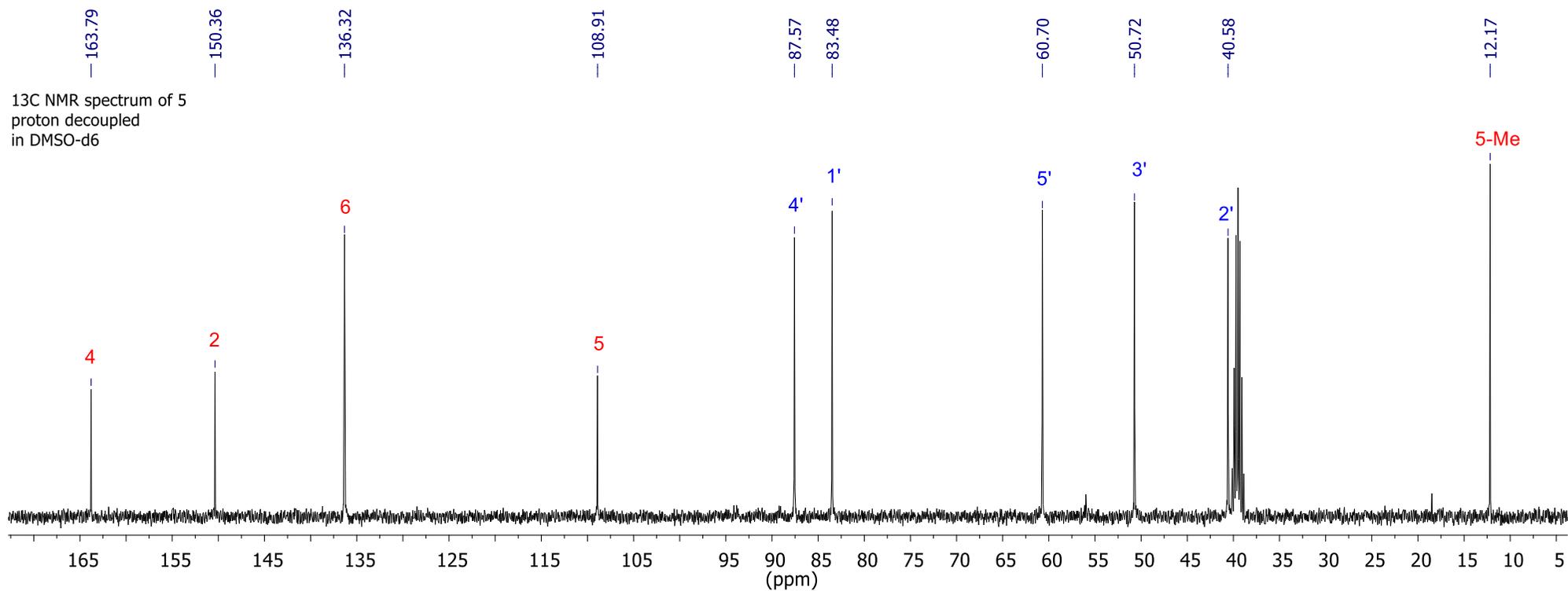
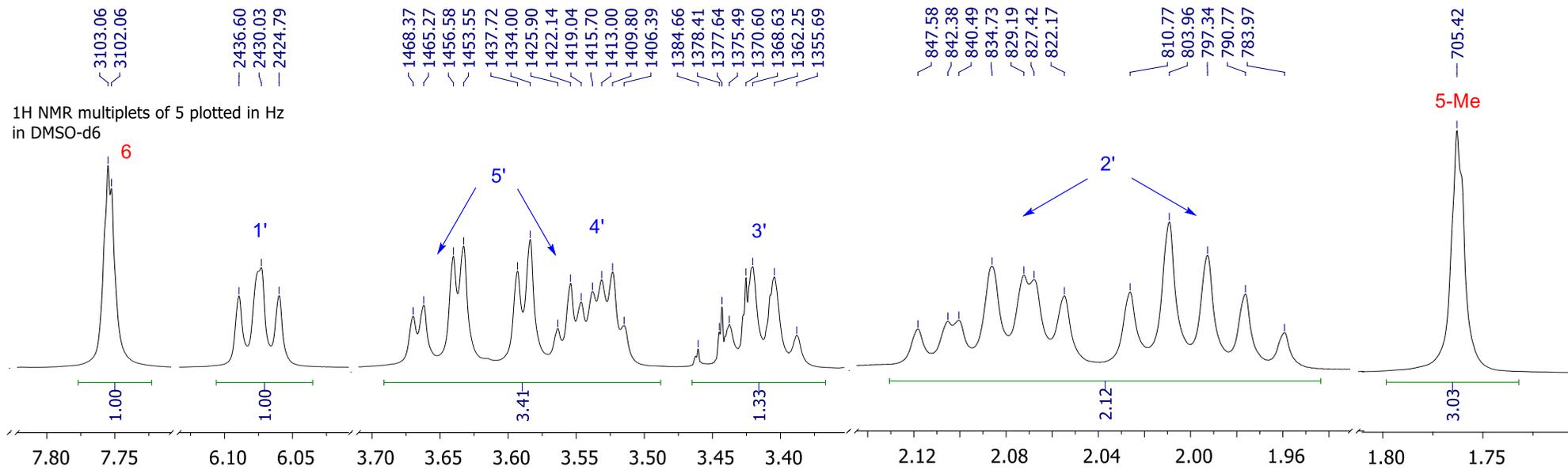


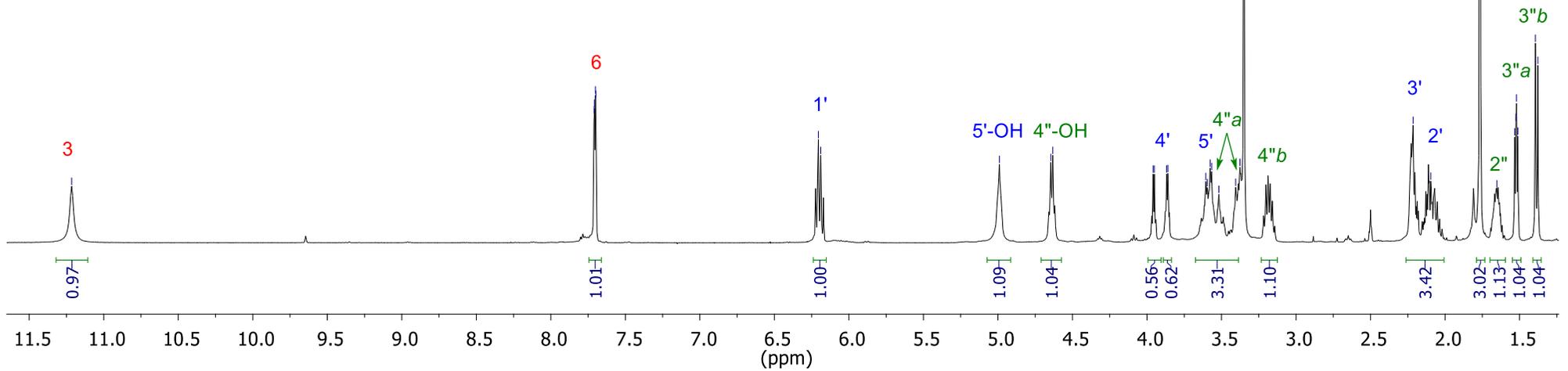
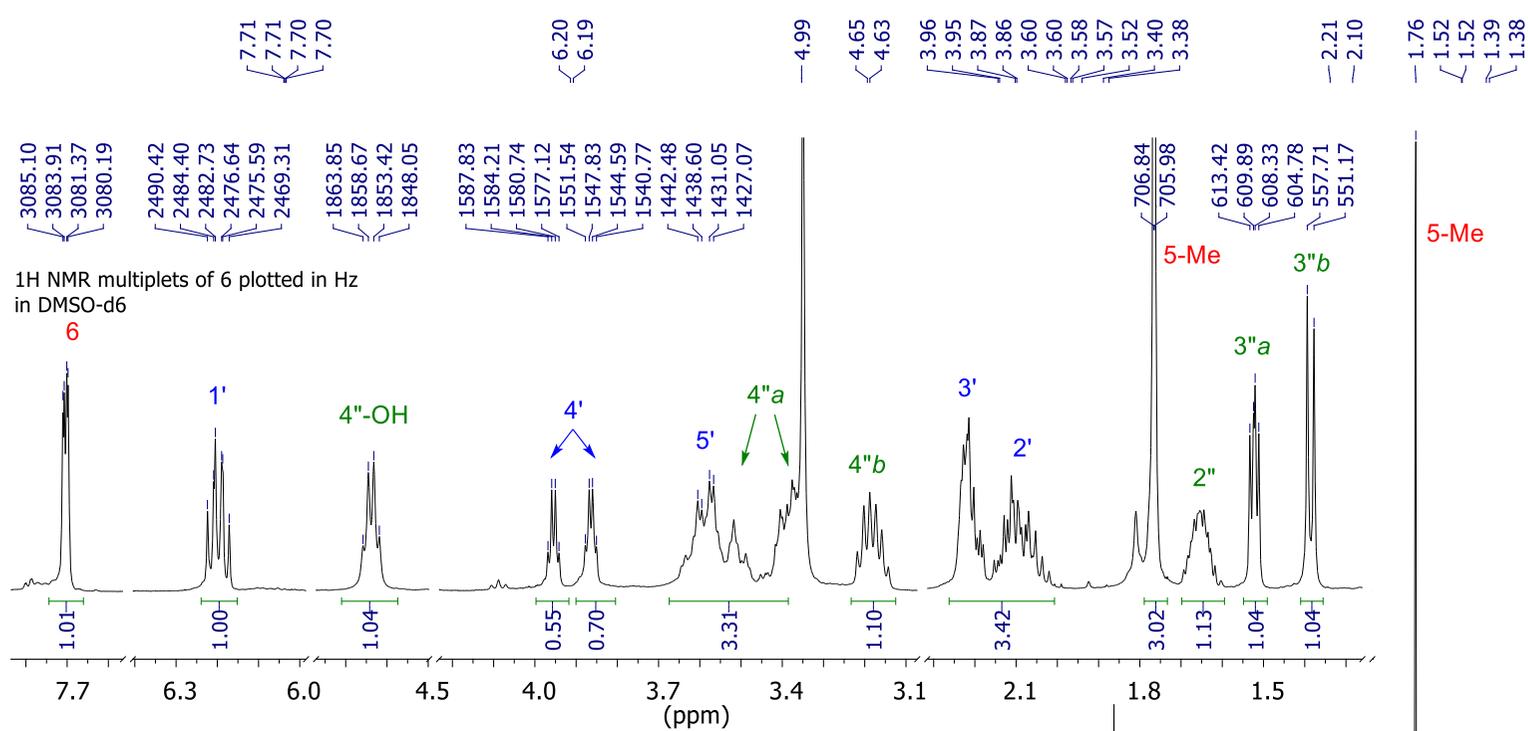
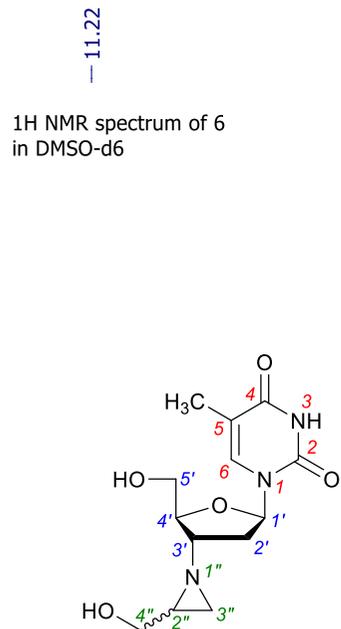




¹H NMR spectrum of 5
in DMSO-d₆







7.71
7.71
7.70
7.70

6.20
6.19
6.19
6.17

3.95
3.95
3.64
3.63
3.61
3.60
3.58
3.57
3.55
3.54
3.40
3.39
3.37
3.36
3.20
3.18
3.17
3.15

-2.22
-2.09

1.77
1.77
1.53
1.52
1.40
1.38

¹H NMR spectrum of **6**
in D₂O
Diastereomeric excess: 53%

3086.96
3085.79
3082.80
3081.57
2482.78
2476.63
2475.61
2469.49
1586.03
1582.42
1578.95
1575.27
1454.98
1450.98
1443.16
1439.22
1431.70
1427.95
1419.84
1416.26
1359.64
1355.16
1348.25
1343.74
1279.03
1272.33
1267.59
1260.89

5-Me

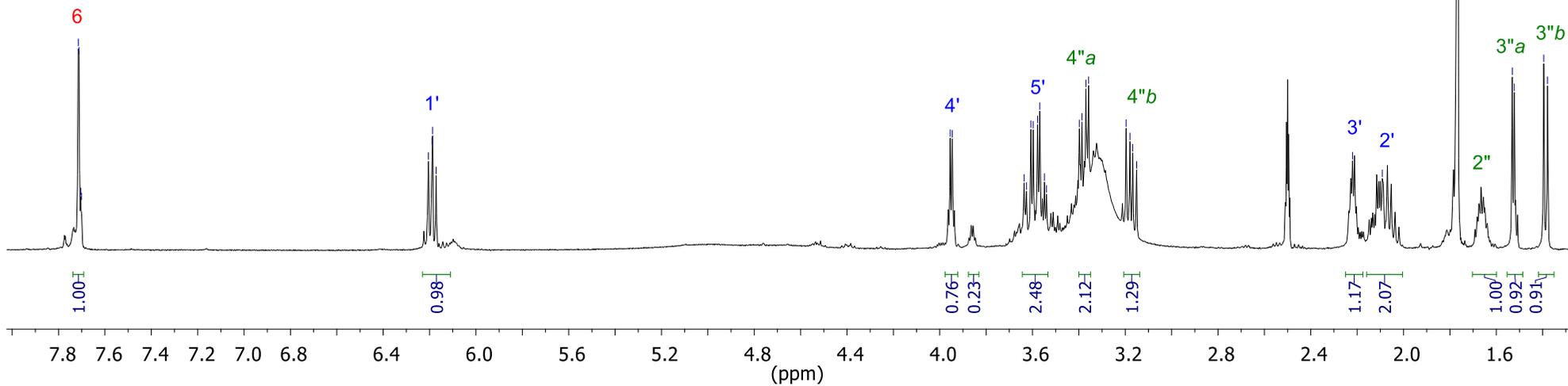
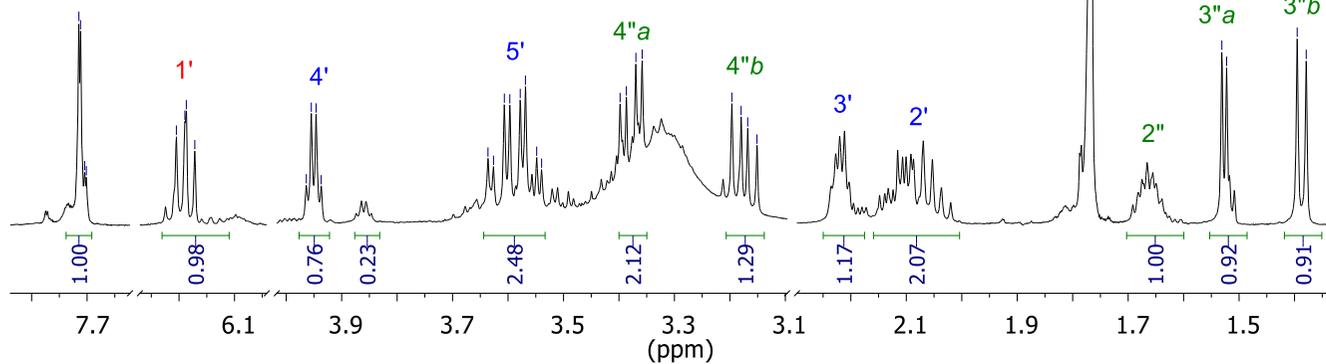
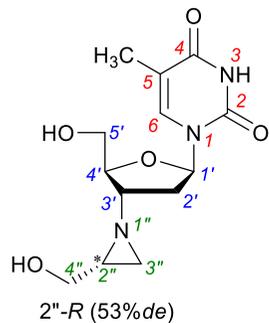
3''a

3''b

5-Me

¹H NMR multiplets of **6** plotted in Hz
in D₂O

6 Diastereomeric excess: 53%



13C spectrum of 6
proton decoupled
in DMSO-d6

163.74

150.49
150.46

136.16
136.10

109.27
109.20

85.43
85.18
84.06
83.92

68.95
68.52
63.17
63.13
61.92
61.65

39.83
37.43
37.23

30.16
30.07

12.22

150.49
150.46

136.16
136.10

109.27
109.20

85.43
85.18

84.06
83.92

63.17
63.13

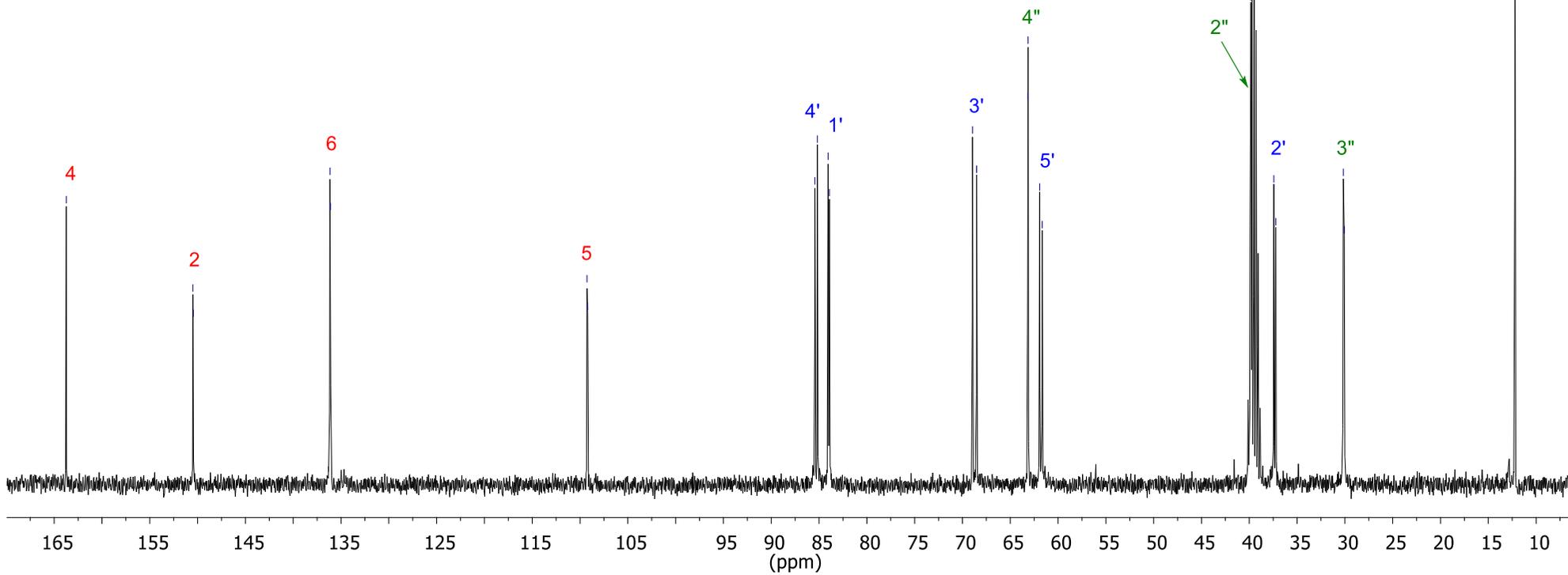
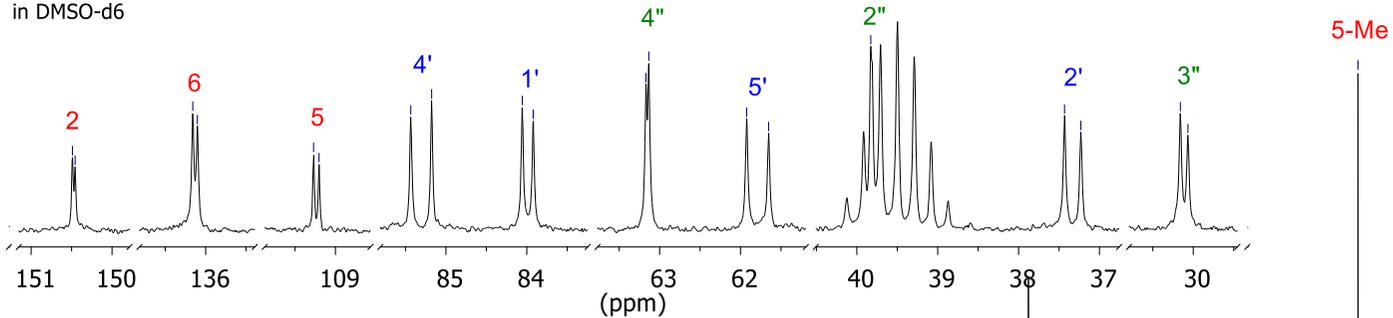
61.92
61.65

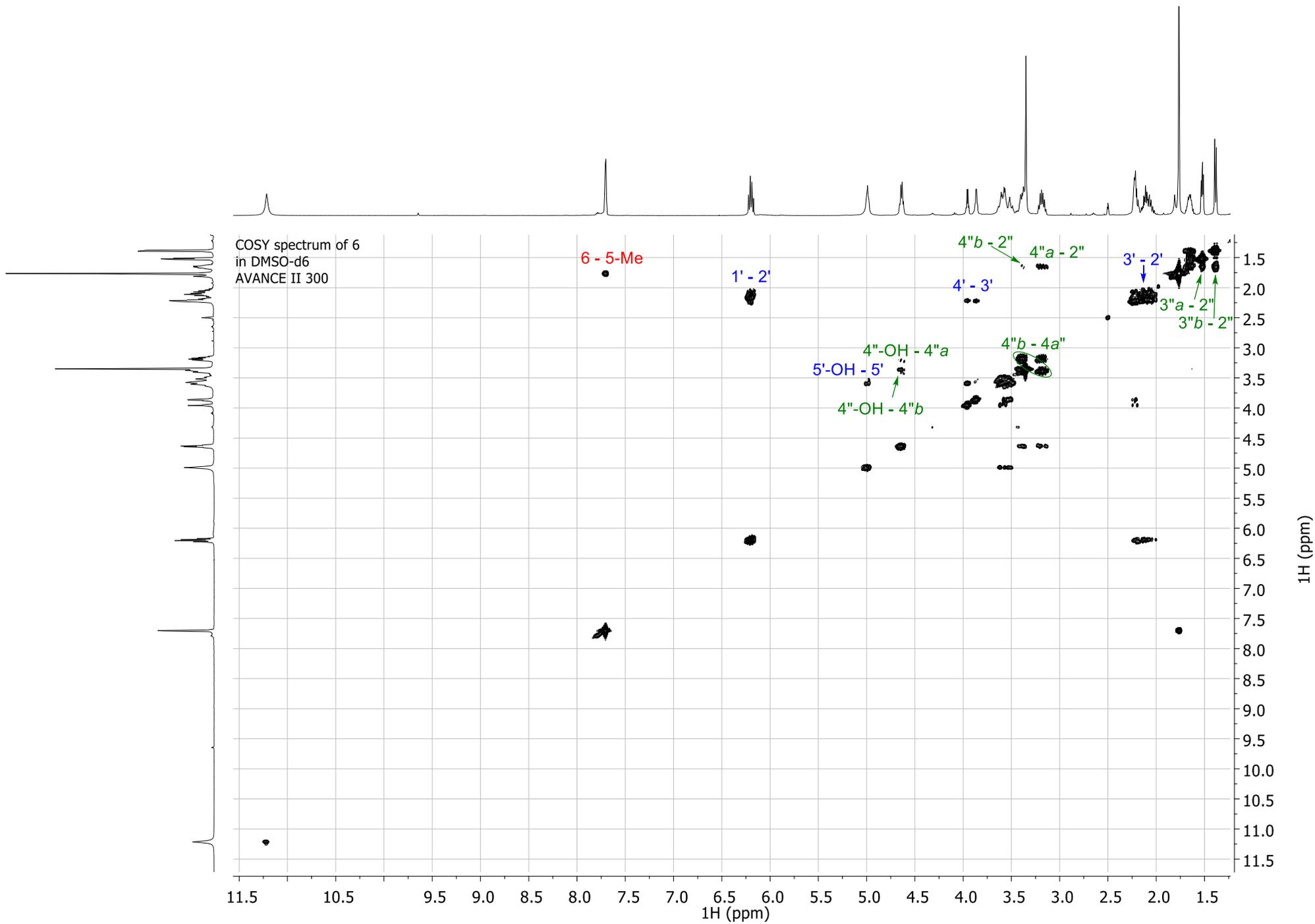
39.83

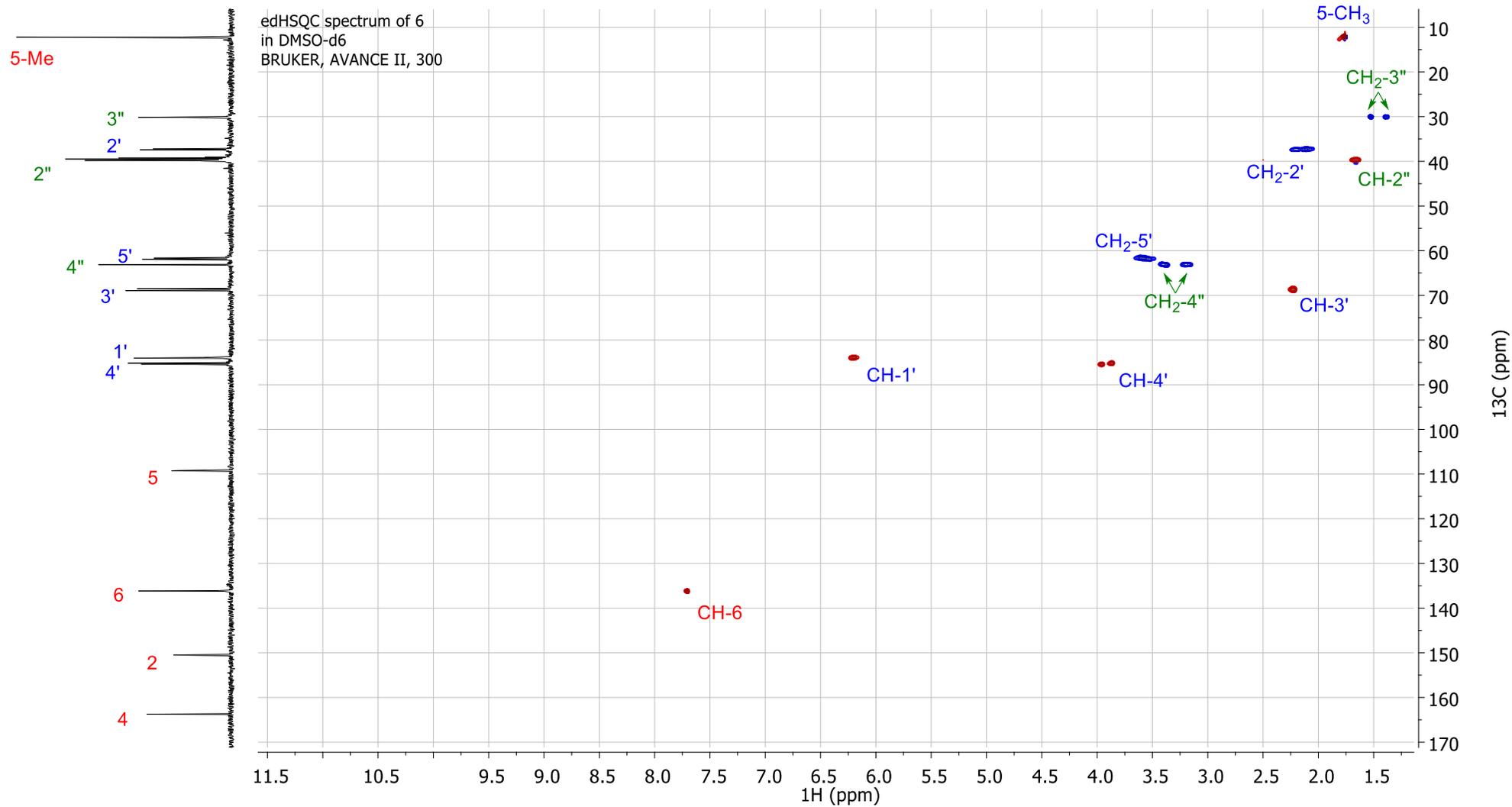
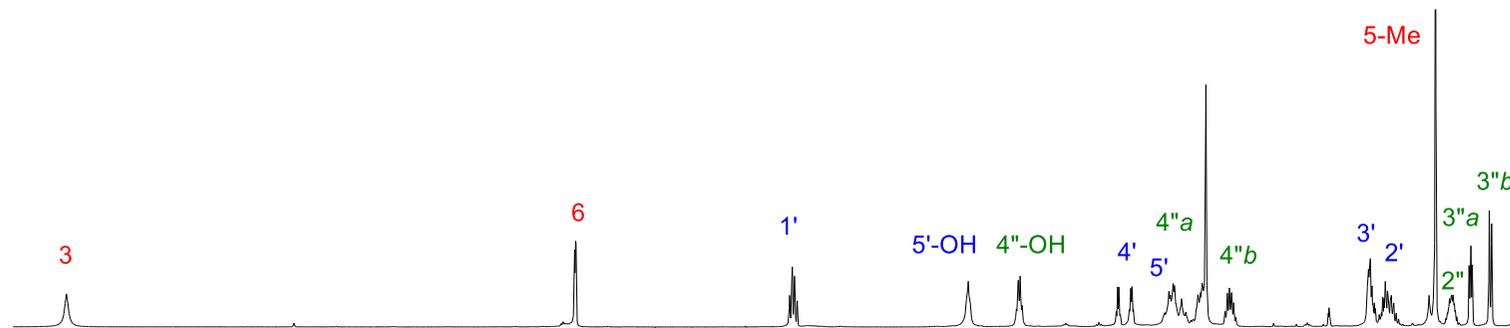
37.43
37.23

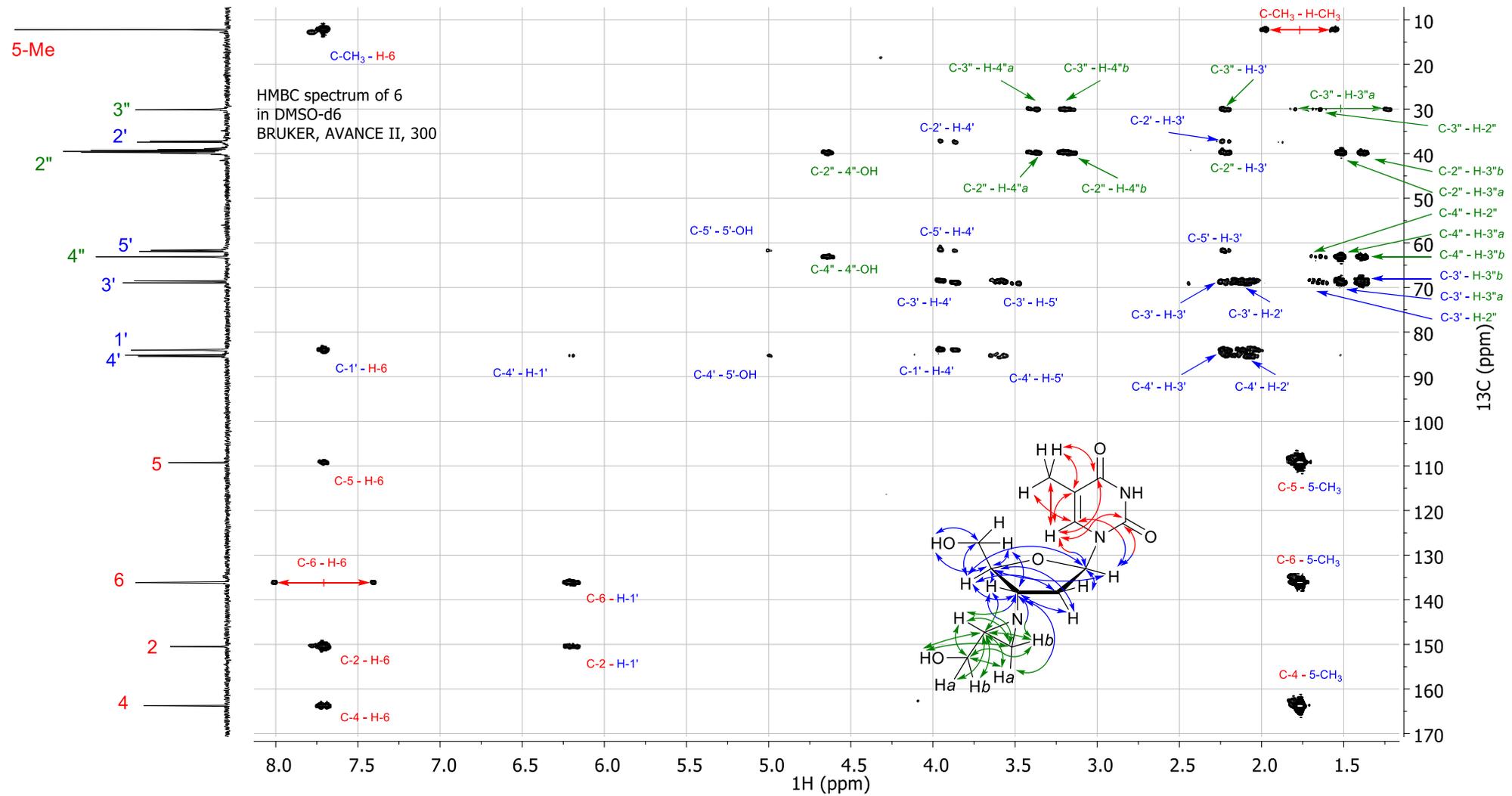
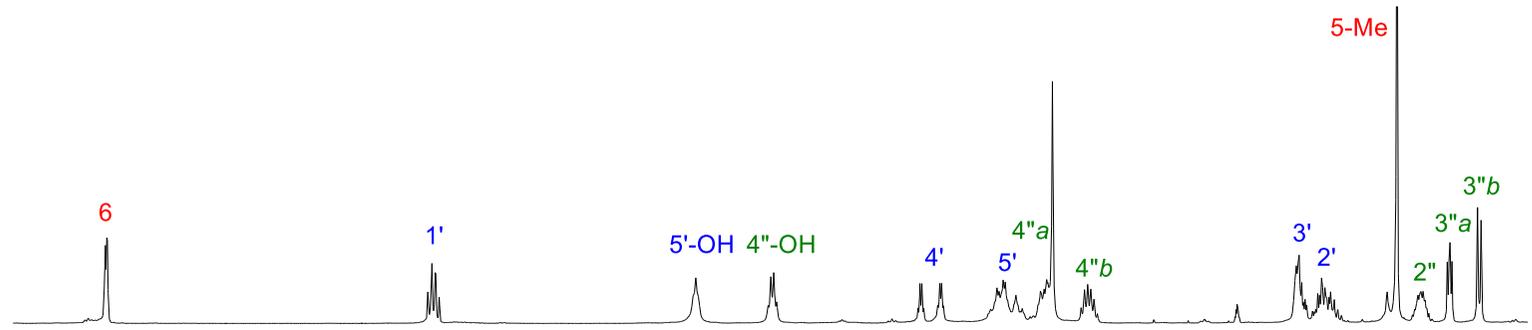
30.16
30.07

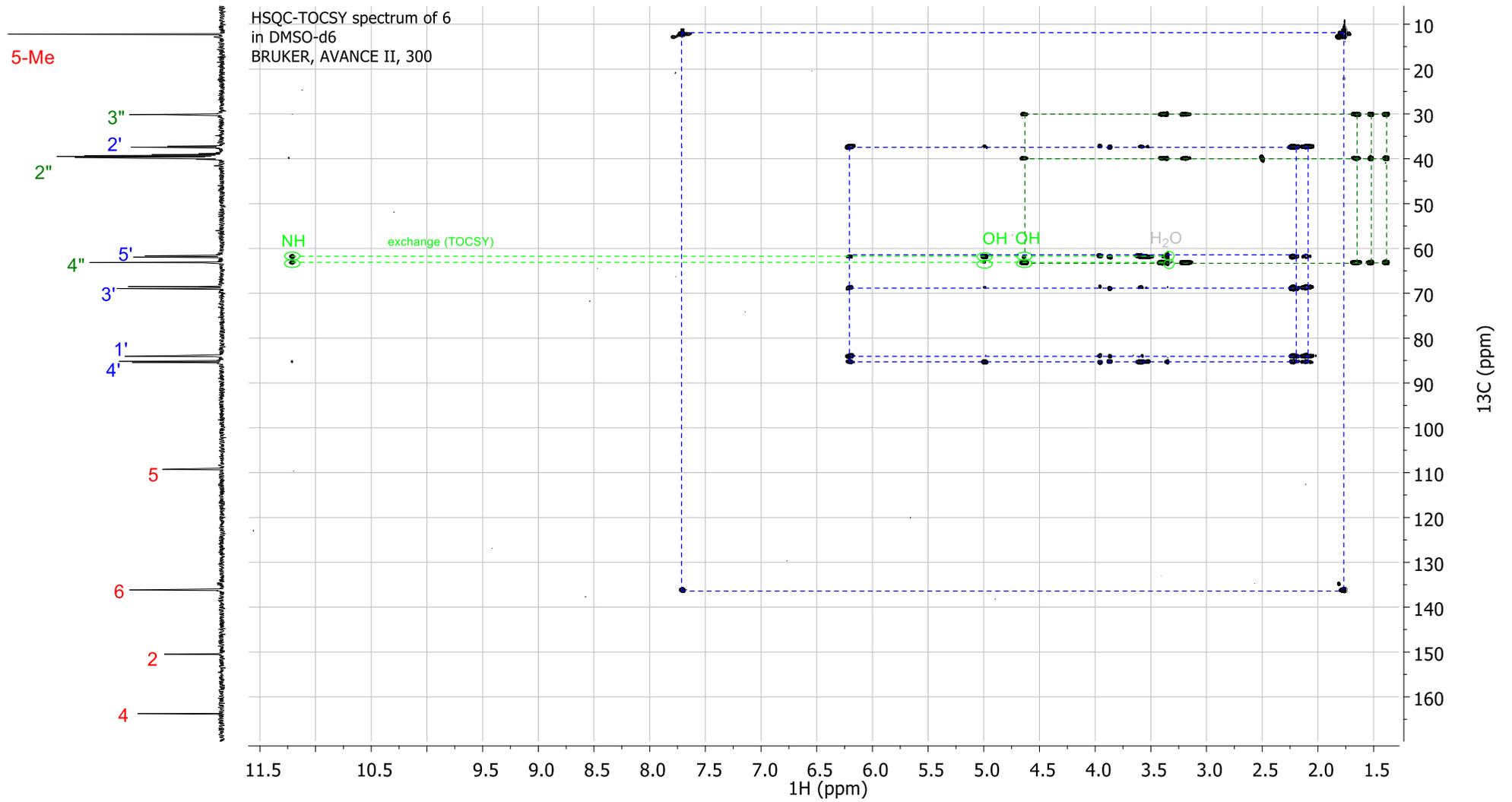
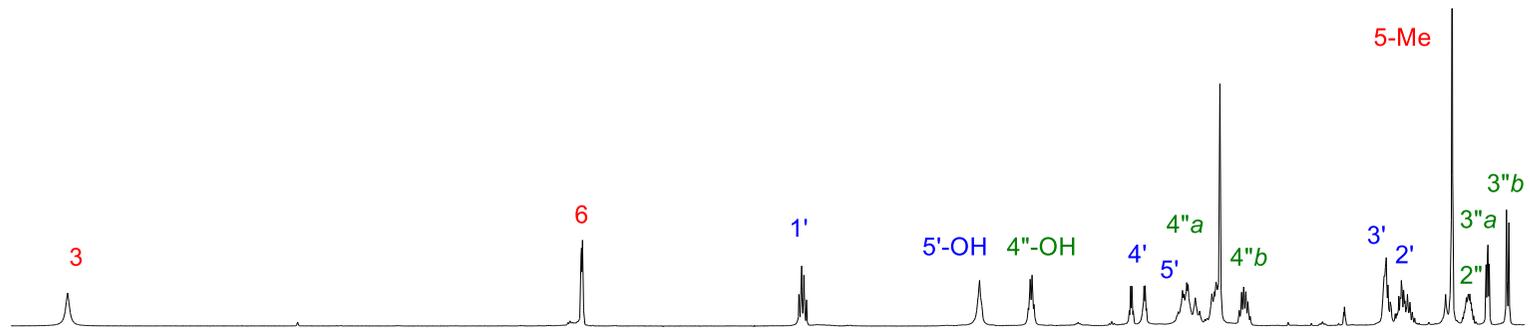
13C spectrum of 6 (scaled)
proton decoupled
in DMSO-d6

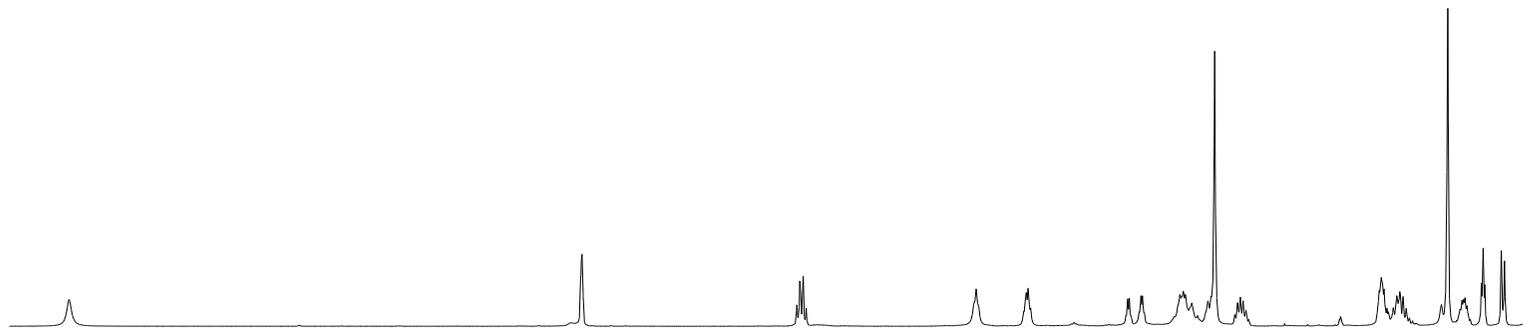




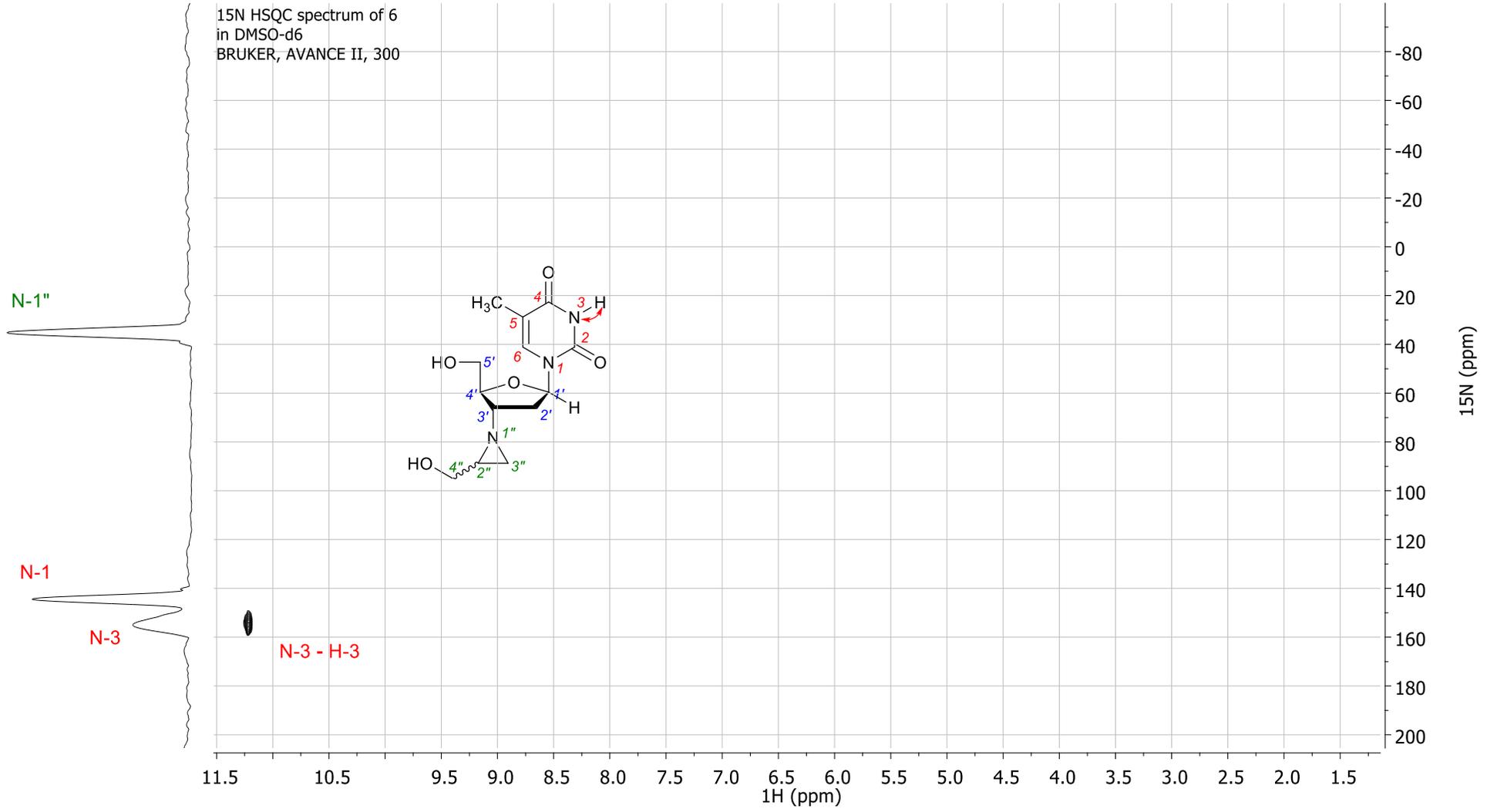


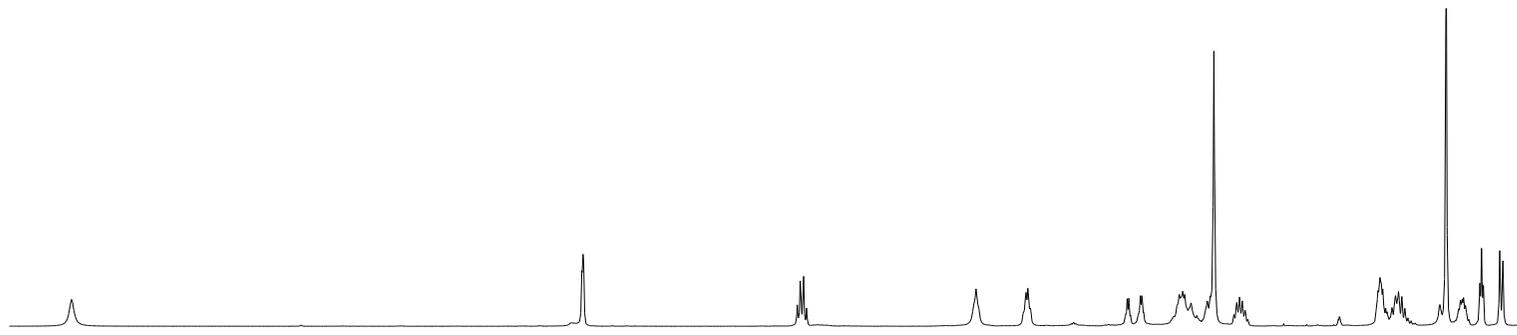




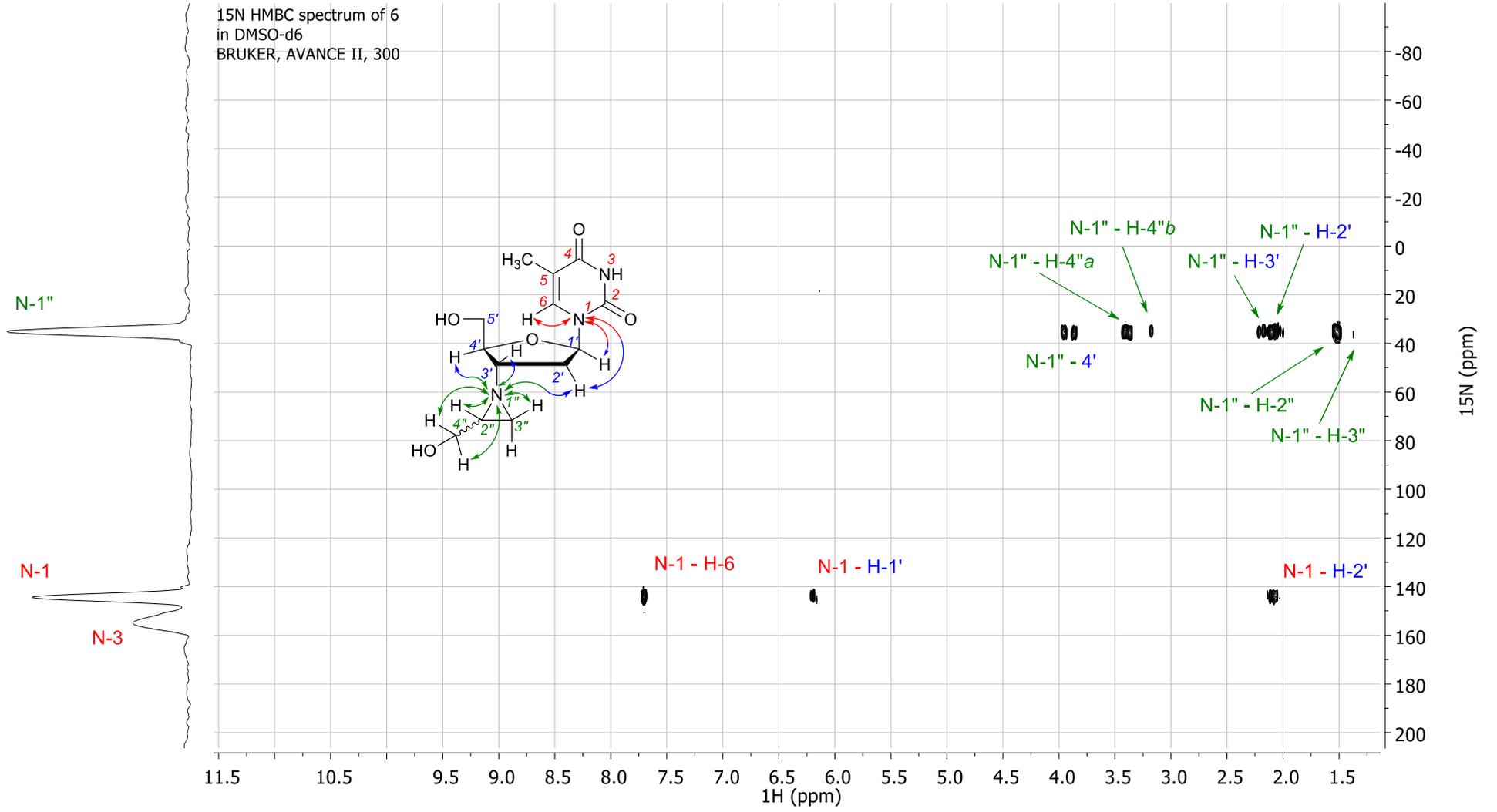


15N HSQC spectrum of 6
in DMSO-d6
BRUKER, AVANCE II, 300





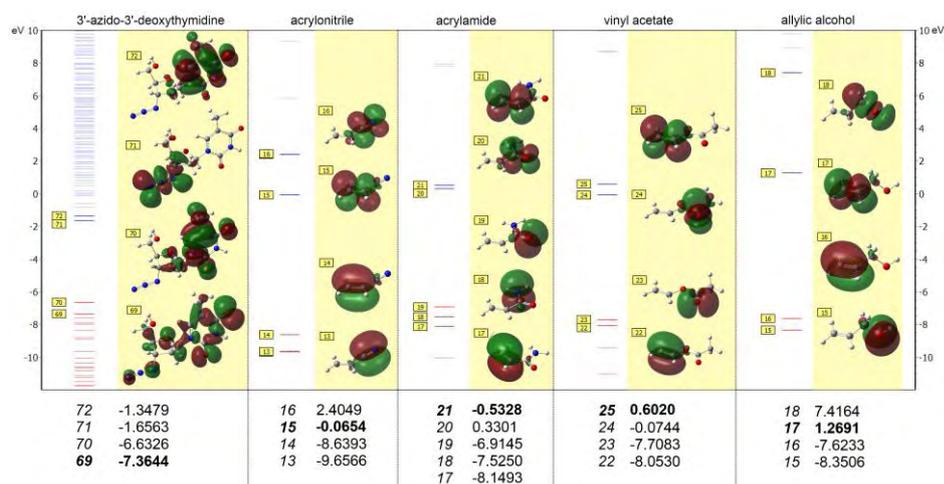
15N HMBC spectrum of 6
in DMSO-d6
BRUKER, AVANCE II, 300



DFT energy calculations

1,3-Dipolar cycloaddition is generally described as a pericyclic reaction that involves the dipole's highest occupied molecular orbital (HOMO) and the dipolarophile's lowest unoccupied molecular orbital (LUMO) interaction. The larger partitioning of charge at the reaction centre the more regioselectively this reaction is directed. The character of a substituent influences the HOMO/LUMO interaction mechanism and specifies the position of the substituent in the product. Initially started on acrylic substrates and aryl azides by Gurvich and Huisgen, later studies were focused on vinyl azides and allyl azides that undergo different domino processes and/or rearrangements leading to rather complex cyclic products [C.-H. Yang and H.-J. Shen, *Tetrahedron*, 1993, **34**, 4051-4054; C.-H. Yang, L.-T. Lee, J.-H. Yang, Y. Wang and G.-H. Lee, *Tetrahedron*, 1994, **50**, 12133-12142].

Starting compounds were optimized in Gaussian[®] 09 program using geometry optimization by DFT (B3LYP) in 6-311++G (d,p) basis. 1,3-Dipolar cycloaddition is of $\pi+2$ type, like Diels-Alder cyclisation. Electron withdrawing substituents neighbouring to alkene double bond are oriented by the *endo*-rule, thus Δ^1 -1,2,3-triazolines are not expected [K. N. Houk, J. Sims, J. R. E. Duke., R. W. Strozier and J. K. George, *J. Am. Chem. Soc.*, 1973, **95**, 7287-7301]. According to the calculated orbital surfaces and energies, the overlapping of azido group LUMO with HOMO of C=C bond of dipolarophile would show lower energy values. But the size of an orbital should also be considered, as well as the electron withdrawing/donating group character of the alkene. Therefore, it appears that AZT HOMO interaction with dipolarophile's LUMO (the first type of interaction according to Huisgen's classification) is more favourable than the reversed symmetry interaction. Alkenes' LUMOs demonstrate the increase of energies from acrylonitrile to allylic alcohol in the mentioned row, and this correlates well with the prolongation of the experimental time required for the completion of the reaction.



RT enzyme assay

The HIV-1 RT assays using activated DNA were performed as follows: the standard reaction mixture (20 μ l) contained 150 μ g/ml of activated DNA, 0.05 μ g of HIV-1 RT, 1.5 μ M dATP, 30 μ M of dCTP, dGTP and dTTP, 1 μ Ci [α -³²P]dATP in a buffer for RT assays (pH 8.1 using 50 mM Tris·HCl, 10 mM MgCl₂ and 200 mM KCl). The test compounds were dissolved in DMSO, and tested in assays with a final 10% DMSO concentration. The same DMSO volumes were added to the control reactions. The reaction mixtures with RT were incubated for 20 min at 37°C, and applied onto Whatman 3MM filters (1 \times 1 cm). After drying on air the filters were washed from non-incorporated into DNA labeled nucleotide with 10% trichloroacetic acid (5 \times 25 ml), once with ethanol, and then air-dried. Radioactivity adsorbed on a filter was determined using Cherenkov counting in a Intertechnique SL-4000 liquid scintillation counter.