

SN- and NS-puckered sugar conformers are precursors of the (6-4) photoproduct in thymine dinucleotide

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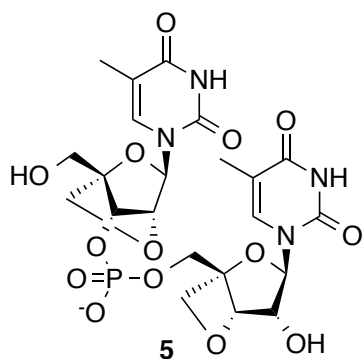
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1. Synthesis of dinucleotide **5**

Materials and Methods. Solvents and chemicals used for the reactions were purchased from commercial suppliers and used without further purification unless otherwise stated. Anhydrous DMF was obtained from Acros Organics. Pyridine was dried by distillation from KOH and kept over KOH. Acetonitrile was dried by distillation from calcium hydride and anhydrous dichloromethane was obtained using Innovative Technology Pure Solv Solvent Purification Systems. Reactions were heated when necessary using an oil bath on a hot plate equipped with a temperature probe. Chromatography was performed on silica gel 60, particle size 35-70 μm , unless otherwise stated. ^1H NMR and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer. Observed chemical shift (δ) values are given in ppm and coupling constants (J) in Hz. ^1H and ^{13}C NMR chemical shifts were calibrated using residual solvent signals at the following values: CDCl_3 δ_{H} 7.26 ppm and δ_{C} 77.16, CD_3OD δ_{H} 3.31 and δ_{C} 49.00. High Resolution Mass Spectra (HRMS) were recorded on a Q-ToF Micromass spectrometer using electrospray ionization (ESI). HPLC purification of dinucleotide **5** was performed on a Sunfire C18 (5 μm , 10 x 250 mm) column using a 67 min, 4 mL/min gradient of 0-20% CH_3CN in 0.05 M aqueous ammonium acetate. The detection was set at 260 nm.

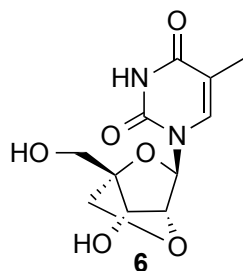
T_{LNP}T_{LS} 5.



Phosphoramidite **10** and alcohol **12** were dried overnight at room temperature in a desiccator over P_2O_5 prior to use. Under argon atmosphere, **10** (140 mg, 0.18 mmol) and **12** (68 mg, 0.22 mmol) were dissolved in anhydrous CH_3CN (2.7 mL). 5-(Ethylthio)-1*H*-tetrazole (78 mg, 0.6 mmol) was added to the solution. The mixture was stirred for 30 min at room temperature. A 0.2 M iodine solution (80 mg in 900 μL THF/ H_2O /2,6-lutidine (2/1/1)) was then added. After 50 min stirring at room

temperature, a saturated aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3$ was added until discoloration. The solution was diluted with CH_2Cl_2 (8 mL) and washed with water (7 mL). The organic layer was collected then dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was dissolved in conc. NH_4OH (4 mL) and stirred at room temperature overnight. The solution was concentrated and the residue dissolved in 80% aqueous acetic acid (4.6 mL). The resulting solution was stirred at room temperature for 4 h and concentrated. Water (10 mL) and CH_2Cl_2 (10 mL) were added, and the aqueous phase separated, extracted with CH_2Cl_2 (2 x 10 mL), concentrated under high vacuum and purified by HPLC. Fractions of interest were pooled, then lyophilized three times to afford **5** in 8% yield, four steps (9 mg, 0.015 mmol). Characterization of **5** has been reported ref 1.

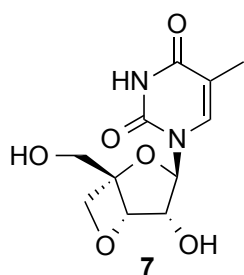
2'-O-4'-C-methylene-5-methyluridine (6).



To a solution of **17** (150 mg, 0.27 mmol) in a mixture of water/ CH_3CN (1.8/3.5 mL) at room temperature were added 4 portions of ceric ammonium nitrate (4 x 151 mg, 4 x 0.27 mmol). After each addition, the mixture was stirred at room temperature for 1.5 h, 7.5 h, overnight, and 8 h, respectively. Then, the mixture was concentrated under reduced pressure, the residue dissolved in ethyl acetate (19 mL) and washed with water (2 x 9 mL). The organic layer was dried over anhydrous MgSO_4 and concentrated in vacuo. The residue was dissolved in anhydrous *N,N*-dimethylformamide (3.2 mL) and dry sodium benzoate (112 mg, 0.78 mmol), was added to the solution. The mixture was stirred at 95°C for 3 h and then dissolved in ethyl acetate (19 mL) and washed with water (2 x 10 mL). The organic layer was dried over anhydrous MgSO_4 and concentrated. The residue was dissolved in conc. NH_4OH (3.5 mL). The mixture was stirred at room temperature for 4 h and diluted with CH_2Cl_2 (3.5 mL) then extracted with water (4 mL). The aqueous phase was concentrated under high pressure. The

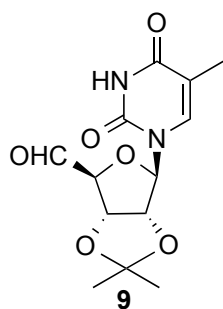
residue was purified by silica gel chromatography using a gradient of MeOH in CH₂Cl₂ (2-10%) to give **6** in 33% yield (24 mg, 0.09 mmol). ¹H NMR (CD₃OD, 300 MHz): δ 7.74 (1H, br q, *J* = 1.2 Hz), 5.54 (1H, s), 4.27 (1H, s), 4.07 (1H, s), 3.95 (1H, d, *J* = 7.9 Hz), 3.91 (2H, s), 3.75 (1H, d, *J* = 7.9 Hz), 1.89 (3H, d, *J* = 1.2 Hz).² ¹³C{¹H} NMR (CD₃OD, 75 MHz): δ 166.5, 151.8, 136.8, 110.7, 90.4, 88.3, 80.8, 72.4, 70.3, 57.6, 12.6.² HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₁₁H₁₄N₂O₆Na 293.0750, found 293.0755.

3'-O-4'-C-methylene-5-methyluridine (**7**).



Compound **19** (172 mg, 0.48 mmol) was dissolved in conc. NH₄OH (6 mL). The mixture was stirred at room temperature for 4.5 h then concentrated. The residue was dissolved in CH₂Cl₂ (6 mL) and the solution was extracted with water (2 x 7 mL). The aqueous phase was concentrated under high pressure. The residue was purified by silica gel chromatography using a gradient of MeOH in CH₂Cl₂ (2-10%) to give **7** in 89% yield (115 mg, 0.43 mmol). ¹H NMR (CD₃OD, 300 MHz): δ 7.50 (1H, br q, *J* = 1.2Hz), 6.40 (1H, d, *J* = 7.6 Hz), 5.02 (1H, d, *J* = 4.6 Hz), 4.80 (1H, d, *J* = 7.9 Hz), 4.48 (1H, d, *J* = 7.9 Hz), 4.10 (1H, dd, *J* = 4.6, 7.6 Hz), 3.80 (2H, m), 1.86 (3H, d, *J* = 1.2Hz).³ ¹³C{¹H} NMR (CD₃OD, 75 MHz): δ 166.2, 152.9, 138.0, 112.4, 88.8, 86.9, 86.7, 79.2, 75.3, 62.6, 12.3.³ HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₁₁H₁₄N₂O₆Na 293.0750, found 293.0744.

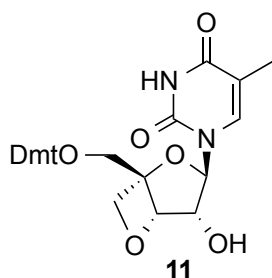
5'-deoxy-2',3'-O-isopropylidene-5'-oxo-5-methyluridine (**9**).



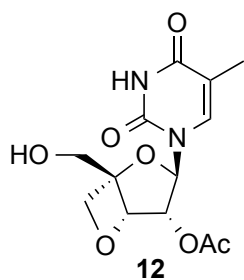
To a solution of 2',3'-isopropylideneribothymidine (**8**)⁴ (2.00 g, 6.71 mmol) in CH₃CN (56 mL) was added 2-iodoxybenzoic acid (IBX, 4.51 g, 16.10 mmol). The solution was stirred at 80°C for 2.5 h then cooled at 0°C for 15 min and filtered through celite. The solid residue was washed

with cold ethyl acetate. Filtrates were combined and after concentration in vacuo, the resulting foam was purified by silica gel chromatography using a gradient of MeOH in CH₂Cl₂. Fractions eluted with 2 to 6% of MeOH were pooled then evaporated to afford **9** as a white foam in 94% yield (1.86 g, 6.28 mmol). ¹H NMR (CDCl₃, 300 MHz): δ 9.42 (1H, s), 8.92 (1H, s), 7.08 (1H, d, *J* = 1.5 Hz), 5.46 (1H, s), 5.21 (1H, dd, *J* = 1.5, 6.2 Hz), 5.10 (1H, br d, *J* = 6.4 Hz), 4.55 (1H, br d, *J* = 1.5 Hz), 1.94 (3H, d, *J* = 1.1 Hz), 1.53 (3H, s), 1.36 (3H, s). ¹³C{¹H} NMR (CDCl₃, 75 MHz): δ 199.5, 164.1, 150.7, 140.2, 113.6, 111.6, 100.1, 94.1, 85.0, 83.9, 26.5, 24.8, 12.4. HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₁₃H₁₆N₂O₆Na 319.0906, found 319.0921.

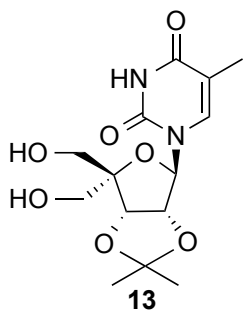
3'-O-4'-C-methylene-5'-O-dimethoxytrityl-5-methyluridine (11).



To a solution of **7** (70 mg, 0.26 mmol) in anhydrous pyridine (5 mL) was added dry dimethoxytrityl chloride (176 mg, 0.52 mmol). The solution was stirred at room temperature for 24 h then diluted with ethyl acetate (7 mL) and washed with water (2 x 7 mL). The organic layer was collected then dried over anhydrous MgSO₄ and concentrated. The residue was purified by silica gel chromatography using a gradient of ethyl acetate in petroleum ether (30 to 80%) to give **11** in 54% yield (80 mg, 0.14 mmol). ¹H NMR (CDCl₃, 300 MHz): δ 7.31 (10H, m), 6.85 (4H, d, *J* = 8.7 Hz), 6.44 (1H, d, *J* = 6.7 Hz), 5.12 (1H, d, *J* = 4.8 Hz), 4.72 (1H, d, *J* = 8.1 Hz), 4.52 (1H, d, *J* = 8.1 Hz), 4.21 (1H, br dd, *J* = 4.8, 6.7 Hz), 3.79 (3H, s), 3.80 (3H, s), 3.76 (1H, d, *J* = 2.6 Hz), 3.47 (2H, br s), 1.54 (3H, br s). ¹³C{¹H} NMR (CDCl₃, 75 MHz): δ 163.3, 158.9, 150.7, 144.3, 135.5, 135.2, 130.1, 128.2, 128.1, 127.4, 113.5, 112.3, 89.3, 86.7, 85.5, 85.2, 79.2, 76.1, 63.1, 55.4, 12.0. HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₃₂H₃₂N₂O₈Na 595.2056, found 595.2064.

3'-O-4'-C-methylene-2'-O-acetyl-5-methyluridine (12).

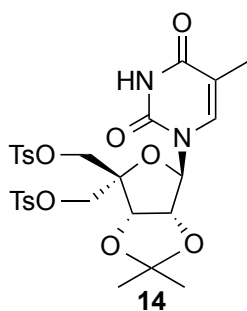
Compound **11** (80 mg, 0.14 mmol) was dissolved in anhydrous pyridine (200 μ L). Acetic anhydride (66 μ L, 0.07 mmol) was added to the solution. The mixture was stirred at room temperature for 2 h then diluted with CH_2Cl_2 (2 mL) and washed with brine (2 x 1 mL). The organic layer was collected then dried over anhydrous MgSO_4 and concentrated. The residue was dissolved in anhydrous CH_2Cl_2 (800 μ L) then trifluoroacetic acid (43 μ L, 0.57 mmol) was added. The mixture was stirred at room temperature for 1.5 h then MeOH was dropped into until discoloration. The solution was concentrated and the residue purified by silica gel chromatography using a gradient of ethyl acetate in petroleum ether (10 to 80%) to afford quantitatively **12** (44 mg, 0.14 mmol). ^1H NMR (CD_3OD , 300 MHz): δ 7.60 (1H, br q, $J = 1.3$ Hz), 6.56 (1H, d, $J = 7.2$ Hz), 5.29 (1H, d, $J = 4.6$ Hz), 5.08 (1H, dd, $J = 4.6, 7.2$ Hz), 4.85 (1H, d, $J = 7.9$ Hz), 4.54 (1H, d, $J = 7.9$ Hz), 3.83 (2H, m), 2.08 (3H, s), 1.90 (3H, d, $J = 1.2$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (CD_3OD , 75 MHz): δ 171.7, 166.1, 152.6, 137.9, 112.6, 87.7, 85.3, 79.4, 76.7, 62.4, 20.3, 12.4. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_7\text{Na}$ 335.0855, found 335.0850.

2',3'-O-isopropylidene-4'-hydroxymethyl-5-methyluridine (13).

To a solution of **9** (629 mg, 2.12 mmol) in dioxane (25 mL) was added formaldehyde 37% (500 μ L, 6.69 mmol). A 2N NaOH solution (2.12 mL, 4.24 mmol) was added dropwise in 2 portions 30 min apart. The mixture was stirred at room temperature overnight, then neutralized by acetic acid and concentrated under reduced pressure. The residue was purified by silica gel chromatography. Elution with 0 to 6% gradient of MeOH in CH_2Cl_2

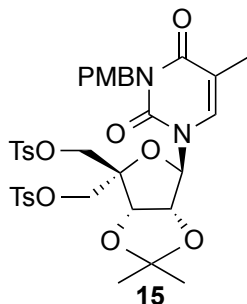
afforded **13**, after evaporation, as white foam in 42% yield (293 mg, 0.89 mmol). ^1H NMR (CDCl_3 , 300 MHz): δ 8.94 (1H, s), 7.16 (1H, s), 5.50 (1H, d, $J = 3.8$ Hz), 5.24 (1H, dd, $J = 3.8, 6.7$ Hz), 5.08 (1H, d, $J = 6.7$ Hz), 3.84 (2H, m), 3.75 (2H, m), 1.91 (3H, s), 1.61 (3H, s), 1.37 (3H, s). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 75 MHz): δ 164.2, 150.8, 139.3, 114.9, 111.4, 95.6, 89.2, 83.7, 81.9, 64.8, 63.3, 26.6, 24.8, 12.4. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{14}\text{H}_{21}\text{N}_2\text{O}_7$ 329.1349, found 329.1344.

2',3'-O-isopropylidene-4'-(*p*-toluenesulfonyloxymethyl)-5'-O-*p*-toluenesulfonyl-5-methyl uridine (14**).**



To a solution of **13** (293 mg, 0.89 mmol) in anhydrous CH_2Cl_2 (9.3 mL) were added 4-dimethylaminopyridine (372 mg, 3.05 mmol) and tosyl chloride (428 mg, 2.24 mmol). The mixture was stirred at room temperature overnight then successively washed with an aqueous saturated solution of NaHCO_3 (8 mL), with water (8 mL) and with brine (8 mL). The organic layer was dried over anhydrous MgSO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography using a gradient of ethyl acetate in petroleum ether (30 to 60%) to afford **14** as white foam in 80% yield (455 mg, 0.71 mmol). ^1H NMR (CDCl_3 , 300 MHz): δ 8.25 (1H, s), 7.76 (2H, d, $J = 8.3$ Hz), 7.75 (2H, d, $J = 8.3$ Hz), 7.37 (2H, d, $J = 8.0$ Hz), 7.35 (2H, d, $J = 8.0$ Hz), 7.10 (1H, s), 5.54 (1H, d, $J = 3.2$ Hz), 5.0 (1H, dd, $J = 3.2, 6.4$ Hz), 4.79 (1H, d, $J = 6.4$ Hz), 4.16 (2H, m), 4.09 (2H, m), 2.47 (3H, s), 2.46 (3H, s), 1.93 (3H, s), 1.43 (3H, s), 1.26 (3H, s). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 75 MHz): δ 163.7, 150.1, 145.7, 145.3, 137.7, 132.3, 132.0, 130.3, 130.0, 128.2, 128.1, 115.1, 111.8, 93.2, 85.3, 84.2, 82.0, 69.2, 68.0, 26.1, 24.7, 21.9, 12.4. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{28}\text{H}_{32}\text{N}_2\text{O}_{11}\text{S}_2\text{Na}$ 659.1345, found 659.1351.

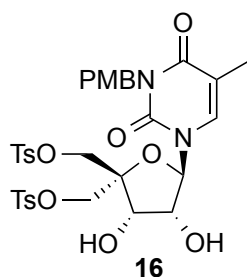
2',3'-*O*-isopropylidene-4'-(*p*-toluenesulfonyloxymethyl)-5'-*O*-*p*-toluenesulfonyl-3-*N*-(*p*-methoxybenzyl)-5-methyluridine (15**).**



To the solution of **14** (100 mg, 0.16 mmol) in anhydrous *N,N*-dimethylformamide (1.6 mL), at 0°C, were added K₂CO₃ (64 mg, 0.47 mmol) and *p*-methoxybenzyl chloride (45 μL, 0.33 mmol) dropwise. The mixture was stirred at room temperature for 2 h then concentrated under high vacuum. The residue was dissolved in ethyl acetate (8 mL)

and the organic phase washed with water (4 mL) and brine (4 mL). The organic layer was dried over anhydrous MgSO₄ and concentrated. The residue was purified by silica gel chromatography using a gradient of ethyl acetate in petroleum ether (10 to 50%) to afford **15** as pink foam in 87% yield (106 mg, 0.14 mmol). ¹H NMR (CDCl₃, 300 MHz): δ 7.73 (4H, d, *J* = 8.2 Hz), 7.42 (2H, d, *J* = 8.6 Hz), 7.34 (2H, d, *J* = 8.2 Hz), 7.32 (2H, d, *J* = 8.2 Hz), 7.01 (1H, br d, *J* = 1.5 Hz), 6.83 (2H, d, *J* = 8.6 Hz), 5.48 (1H, d, *J* = 2.8 Hz), 4.99 (1H, dd, *J* = 2.8, 6.4 Hz), 4.98 (2H, m), 4.83 (1H, d, *J* = 6.4 Hz), 4.13 (4H, m), 3.78 (3H, s), 2.44 (6H, s), 1.91 (3H, s), 1.43 (3H, s), 1.26 (3H, s). ¹³C{¹H} NMR (CDCl₃, 75 MHz): δ 163.2, 159.2, 150.8, 145.7, 145.3, 135.8, 132.3, 132.0, 131.0, 130.2, 130.0, 128.7, 128.2, 128.1, 114.9, 113.8, 111.0, 94.3, 85.4, 84.5, 82.1, 68.8, 68.3, 55.4, 44.0, 26.1, 24.6, 21.9, 21.8, 13.2. HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₃₆H₄₀N₂O₁₂S₂Na 779.1920, found 779.1924.

4'-(*p*-toluenesulfonyloxymethyl)-5'-*O*-*p*-toluenesulfonyl-3-*N*-(*p*-methoxybenzyl)-5-methyluridine (16**).**

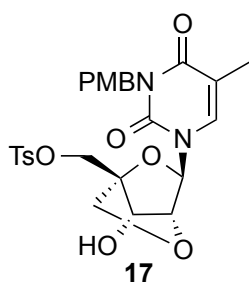


Compound **15** (138 mg, 0.18 mmol) was dissolved in trifluoroacetic acid and water (9/1, 587/65 μL). The solution was stirred at room temperature for 3.5 h then concentrated. The residue was purified by

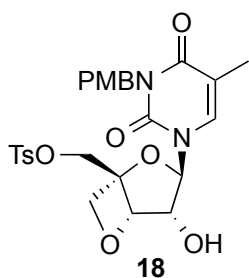
silica gel chromatography using a gradient of ethyl acetate in petroleum ether (20 to 70%) to afford, after concentration of the adequate fractions, **16** as pink foam in 91% yield (118 mg, 0.16 mmol). ^1H NMR (CDCl_3 , 300 MHz): δ 7.74 (2H, d, $J = 8.5$ Hz), 7.72 (2H, d, $J = 8.5$ Hz), 7.39 (2H, d, $J = 8.6$ Hz), 7.32 (5H, m), 6.81 (2H, d, $J = 8.6$ Hz), 5.68 (1H, d, $J = 5.0$ Hz), 5.00 (2H, m), 4.29 (2H, m), 4.20 (2H, m), 4.14 (2H, m), 3.77 (3H, s), 2.46 (3H, s), 2.41 (3H, s), 1.93 (3H, s). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 75 MHz): δ 163.2, 159.3, 152.1, 146.0, 145.6, 133.4, 132.0, 131.9, 130.8, 130.4, 130.1, 128.8, 128.2, 128.0, 113.9, 111.2, 90.7, 85.2, 75.1, 72.1, 69.8, 68.3, 55.4, 44.1, 21.9, 21.8, 13.3. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{33}\text{H}_{36}\text{N}_2\text{O}_{12}\text{NaS}_2$ 739.1607, found 739.1606.

2'-O-4'-C-methylene-5'-O-*p*-toluenesulfonyl-3-N-(*p*-methoxybenzyl)-5-methyluridine (17) and 3'-O-4'-C-methylene-5'-O-*p*-toluenesulfonyl-3-N-(*p*-methoxybenzyl)-5-methyluridine (18).

To a solution of **16** (200 mg, 0.28 mmol) in anhydrous *N,N*-dimethylformamide (5 mL) at 0°C were added 4 portions of sodium hydride 60% in oil (8 mg, 0.20 mmol; 4 mg, 0.10 mmol; 4 mg, 0.10 mmol and 2 mg, 0.05 mmol). After each addition, the mixture was stirred at room temperature for 2 h, 3.5 h, 5.5 h and overnight, respectively. Then, the solution was neutralized with acetic acid, then concentrated. The residue was dissolved in ethyl acetate (15 mL). The organic layer was washed with water (2 x 10 mL), dried over anhydrous MgSO_4 and concentrated. The residue was purified by silica gel chromatography using a gradient ethyl acetate in petroleum ether (5 to 40%) to give **17** in 18% yield (27 mg, 0.05 mmol) and **18** in 72% yield (109 mg, 0.20 mmol).

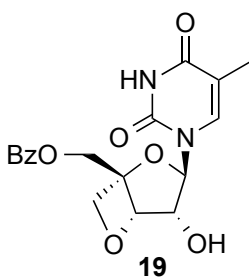


Compound **17**: ^1H NMR (CDCl_3 , 300 MHz): δ 7.81 (2H, d, $J = 8.3$ Hz), 7.45 (2H, d, $J = 8.7$ Hz), 7.39 (1H, s), 7.38 (2H, d, $J = 8.3$ Hz), 6.82 (2H, d, $J = 8.7$ Hz), 5.63 (1H, s), 5.02 (2H, m), 4.43 (1H, s), 4.41 (2H, m), 4.16 (1H, br s), 3.96 (1H, d, $J = 8.1$ Hz), 3.79 (1H, d, $J = 8.1$ Hz), 3.77 (3H, s), 2.83 (1H, br s), 2.46 (3H, s), 1.89 (3H, s). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 75 MHz): δ 163.4, 159.3, 150.5, 146.0, 132.4, 132.3, 131.1, 130.4, 129.1, 128.0, 113.8, 110.3, 87.5, 86.5, 79.2, 70.8, 70.3, 64.0, 55.4, 43.8, 21.9, 13.4. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_9\text{SNa}$ 567.1413, found 567.1412.



Compound **18**: ^1H NMR (CDCl_3 , 300 MHz): δ 7.75 (2H, d, $J = 8.3$ Hz), 7.44 (2H, d, $J = 8.6$ Hz), 7.33 (2H, d, $J = 8.3$ Hz), 7.07 (1H, br d, $J = 1.4$ Hz), 6.83 (2H, d, $J = 8.6$ Hz), 6.12 (1H, d, $J = 5.3$ Hz), 5.15 (1H, d, $J = 5.3$ Hz), 5.02 (2H, m), 4.78 (1H, d, $J = 8.3$ Hz), 4.50 (1H, d, $J = 8.3$ Hz), 4.33 (2H, s), 4.24 (1H, dt, $J = 5.3, 9.1$ Hz), 3.78 (3H, s), 2.44 (3H, s), 1.94 (3H, br s). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 75 MHz): δ 163.2, 159.2, 151.5, 145.7, 134.5, 132.4, 130.9, 130.2, 128.9, 128.0, 113.8, 111.5, 93.5, 85.0, 84.1, 78.2, 75.0, 68.3, 55.3, 44.2, 21.8, 13.3. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_9\text{SNa}$ 567.1413, found 567.1414.

3'-O-4'-C-methylene-5'-O-benzoyle-5-methyluridine (**19**).



To a solution of **18** (452 mg, 0.83 mmol) in a mixture of water/ CH_3CN (1.4/13.5 mL) was added ceric ammonium nitrate (2.05 g, 3.73 mmol). The solution was stirred at room temperature for 37 h then concentrated. The residue was dissolved in ethyl acetate (89 mL) and washed with water (2 x 45 mL). The organic layer was dried over anhydrous MgSO_4 and concentrated. The

residue was dissolved in anhydrous *N,N*-dimethylformamide (10 mL) and dry sodium benzoate (358 mg, 2.49 mmol) was added to the solution. The mixture was stirred at 95°C for 2.75 h, then diluted with ethyl acetate (59 mL) and washed with water (30 mL). The aqueous phase was extracted with ethyl acetate (2 x 59 mL). The organic layers were combined, dried over anhydrous MgSO₄, and concentrated. The residue was purified by silica gel chromatography. Elution with 0-7% MeOH in CH₂Cl₂ afforded **19** in 58% yield (172 mg, 0.48 mmol). ¹H NMR (CD₃OD, 300 MHz): δ 8.08 (2H, m), 7.66 (1H, m), 7.53 (2H, m), 7.21 (1H, br s), 6.48 (1H, d, *J* = 7.5 Hz), 5.24 (1H, d, *J* = 4.6 Hz), 4.98 (1H, d, *J* = 8.1 Hz), 4.67 (2H, m), 4.62 (1H, d, *J* = 8.1 Hz), 4.21 (1H, dd, *J* = 4.6, 7.5 Hz), 1.59 (3H, br s). ¹³C{¹H} NMR (CD₃OD, 75 MHz): δ 167.3, 166.0, 152.7, 137.1, 134.8, 130.8, 130.7, 129.9, 112.6, 88.8, 86.9, 84.6, 79.2, 75.1, 64.6, 12.1. HRMS (ESI) *m/z*: [M+Na]⁺ calcd. for C₁₈H₁₈N₂O₇Na 397.1012, found 397.1006.

2. NMR spectra

Figure S1: ^1H NMR spectrum of 6 (300 MHz, CD_3OD).	S13
Figure S2: ^1H NMR spectrum of 7 (300 MHz, CD_3OD).	S14
Figure S3: ^1H NMR spectrum of 9 (300 MHz, CDCl_3).	S15
Figure S4: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 9 (75 MHz, CDCl_3).	S16
Figure S5: ^1H NMR spectrum of 11 (300 MHz, CDCl_3).	S17
Figure S6: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 11 (75 MHz, CDCl_3).	S18
Figure S7: ^1H NMR spectrum of 12 (300 MHz, CD_3OD).	S19
Figure S8: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 12 (75 MHz, CD_3OD).	S20
Figure S9: ^1H NMR spectrum of 13 (300 MHz, CDCl_3).	S21
Figure S10: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 13 (75 MHz, CDCl_3).	S22
Figure S11: ^1H NMR spectrum of 14 (300 MHz, CDCl_3).	S23
Figure S12: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 14 (75 MHz, CDCl_3).	S24
Figure S13: ^1H NMR spectrum of 15 (300 MHz, CDCl_3).	S25
Figure S14: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 15 (75 MHz, CDCl_3).	S26
Figure S15: ^1H NMR spectrum of 16 (300 MHz, CDCl_3).	S27
Figure S16: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 16 (75 MHz, CDCl_3).	S28
Figure S17: ^1H NMR spectrum of 17 (300 MHz, CDCl_3).	S29
Figure S18: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 17 (75 MHz, CDCl_3).	S30
Figure S19: ^1H NMR spectrum of 18 (300 MHz, CDCl_3).	S31
Figure S20: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 18 (75 MHz, CDCl_3).	S32
Figure S21: ^1H NMR spectrum of 19 (300 MHz, CD_3OD).	S33
Figure S22: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 19 (75 MHz, CD_3OD).	S34

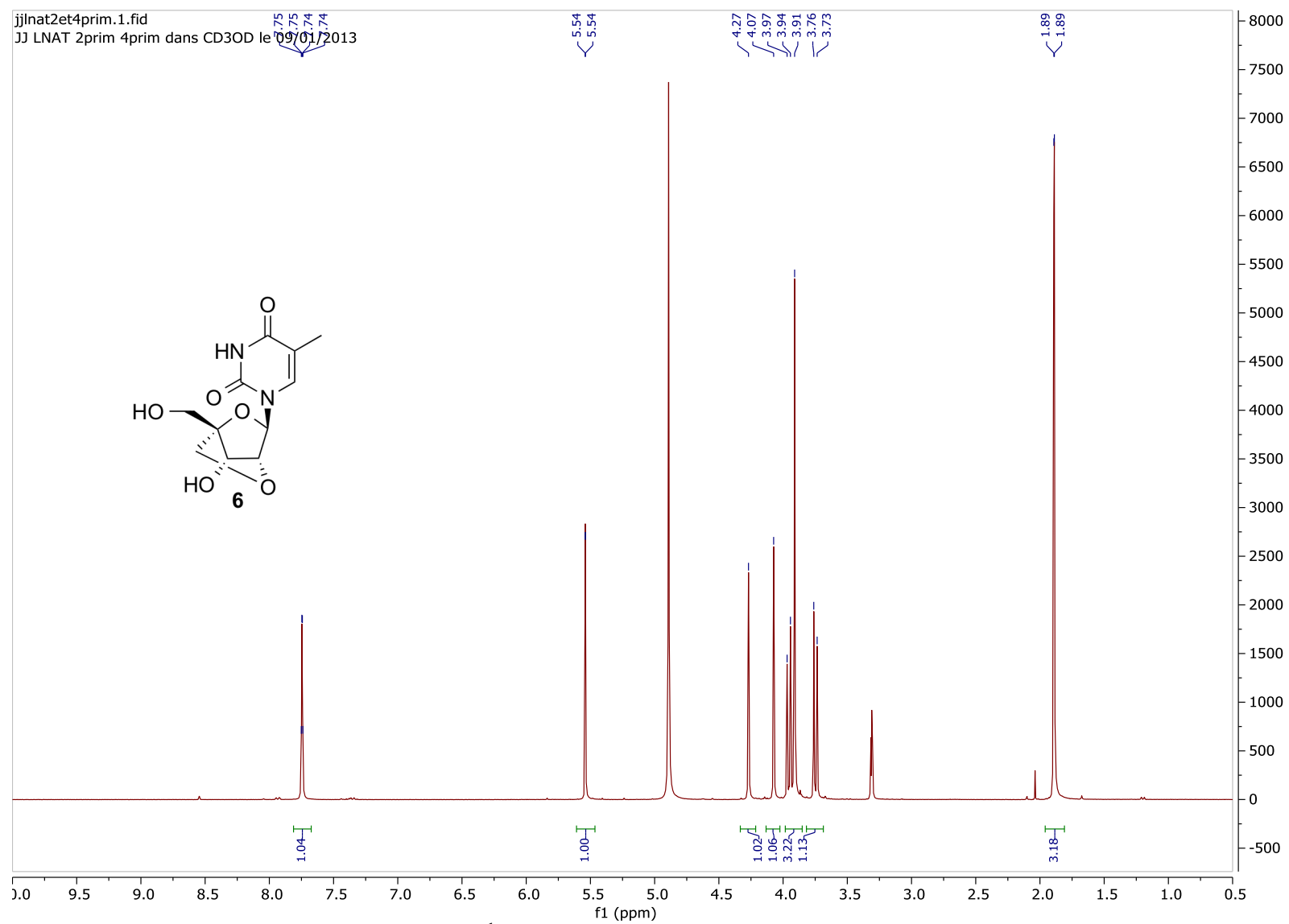


Figure S1: ^1H NMR spectrum of 6 (300 MHz, CD_3OD).

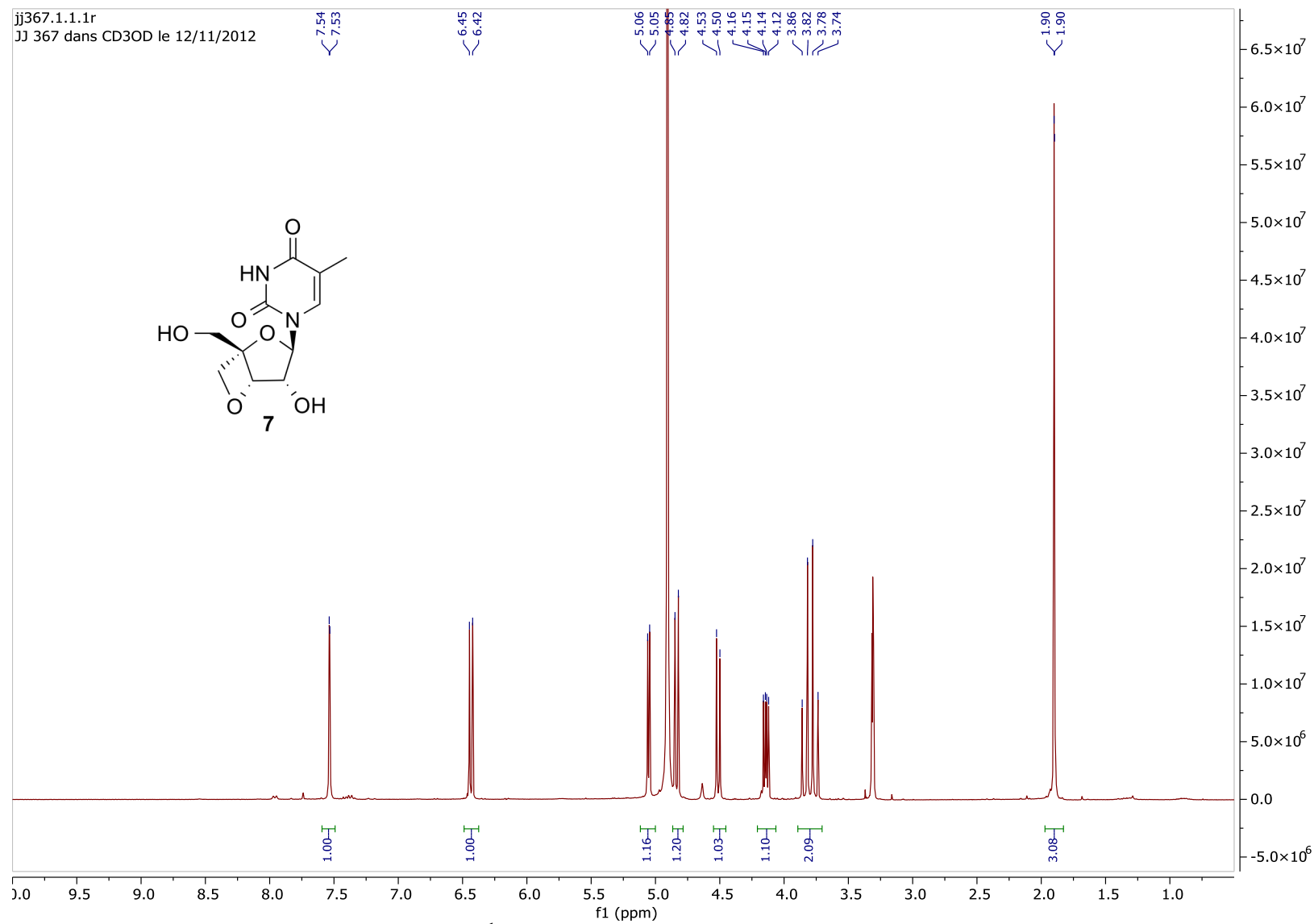


Figure S2: ^1H NMR spectrum of 7 (300 MHz, CD_3OD).

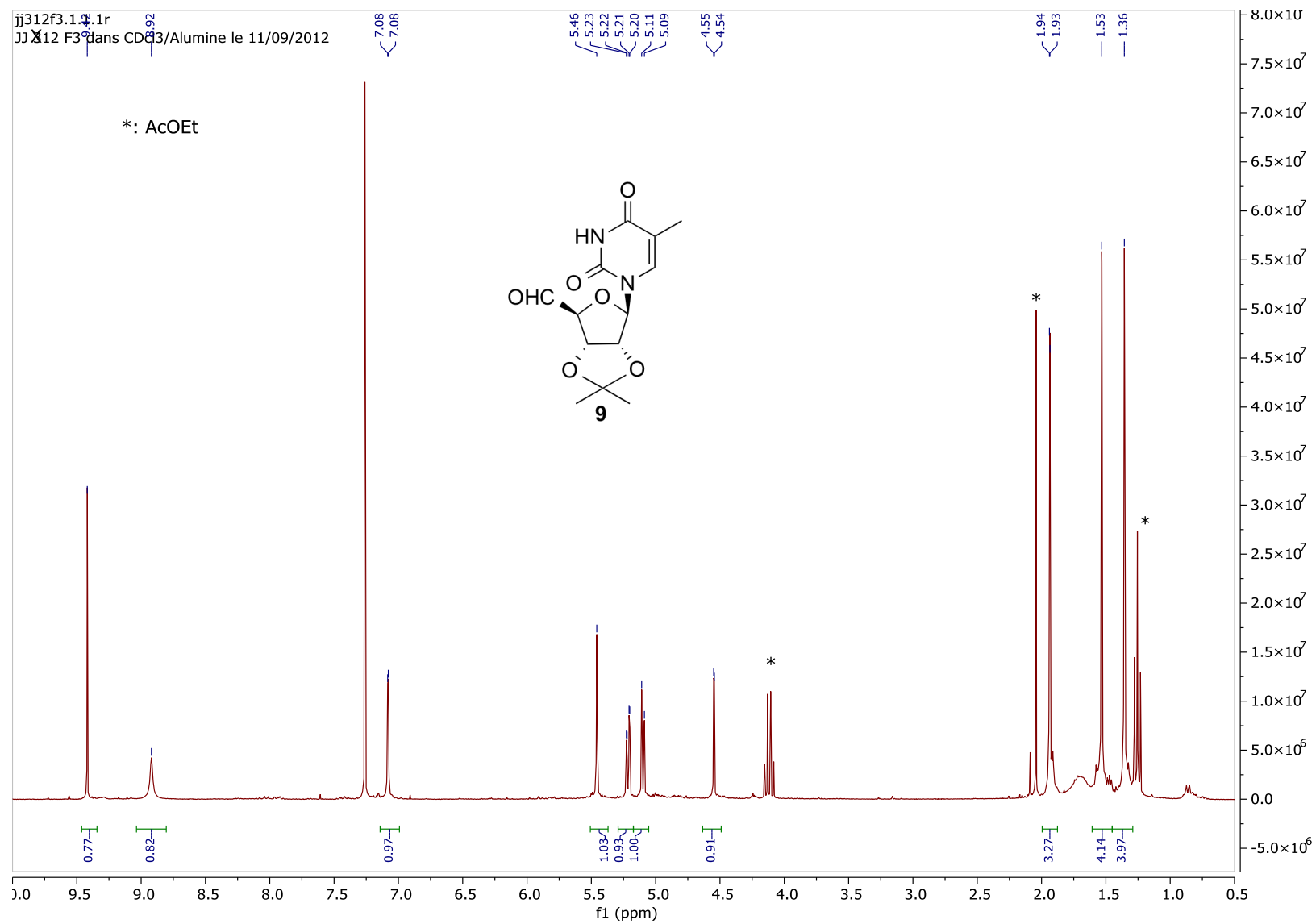


Figure S3: ^1H NMR spectrum of 9 (300 MHz, CDCl_3).

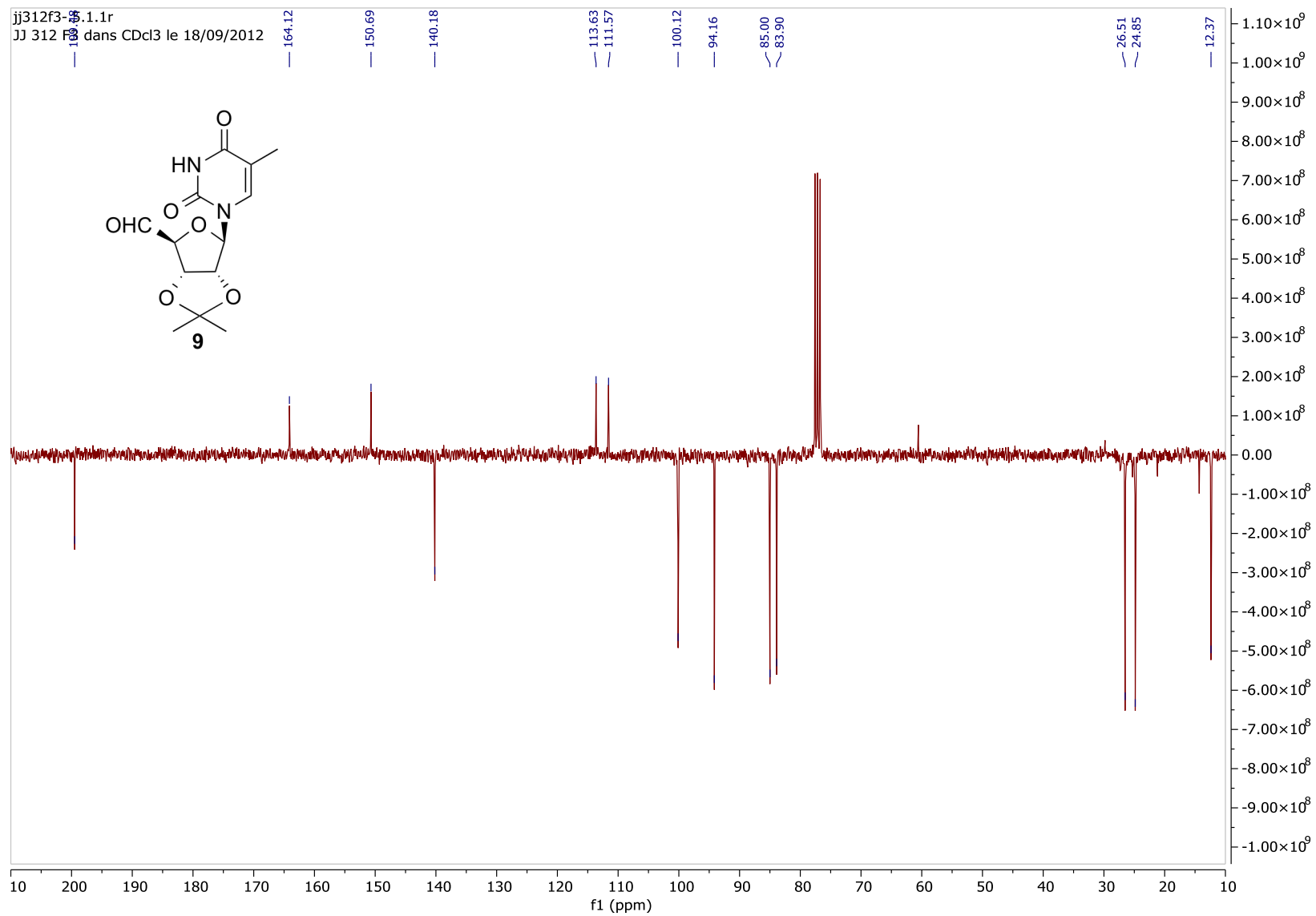


Figure S4: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **9** (75 MHz, CDCl_3).

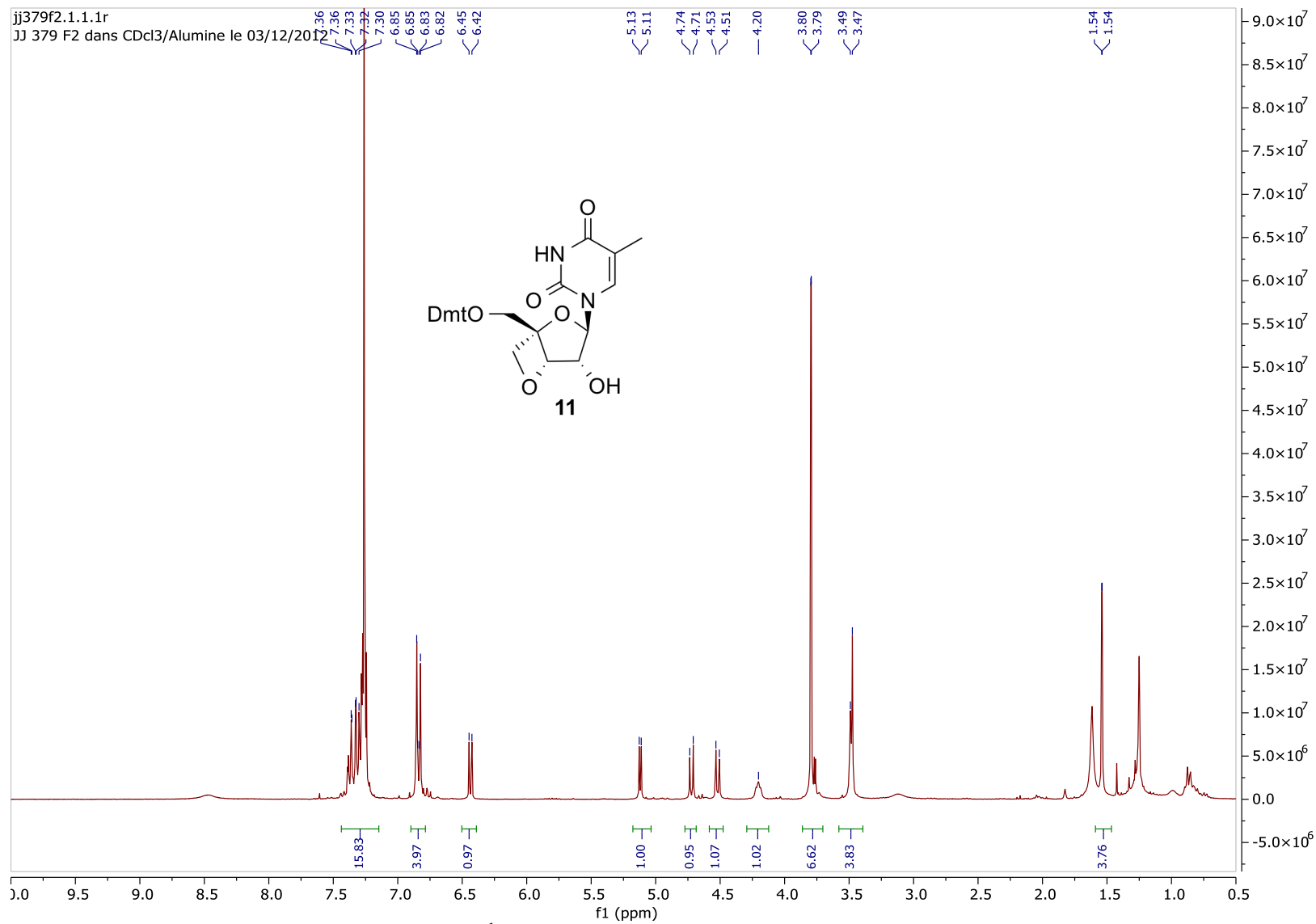


Figure S5: ¹H NMR spectrum of **11** (300 MHz, CDCl₃).

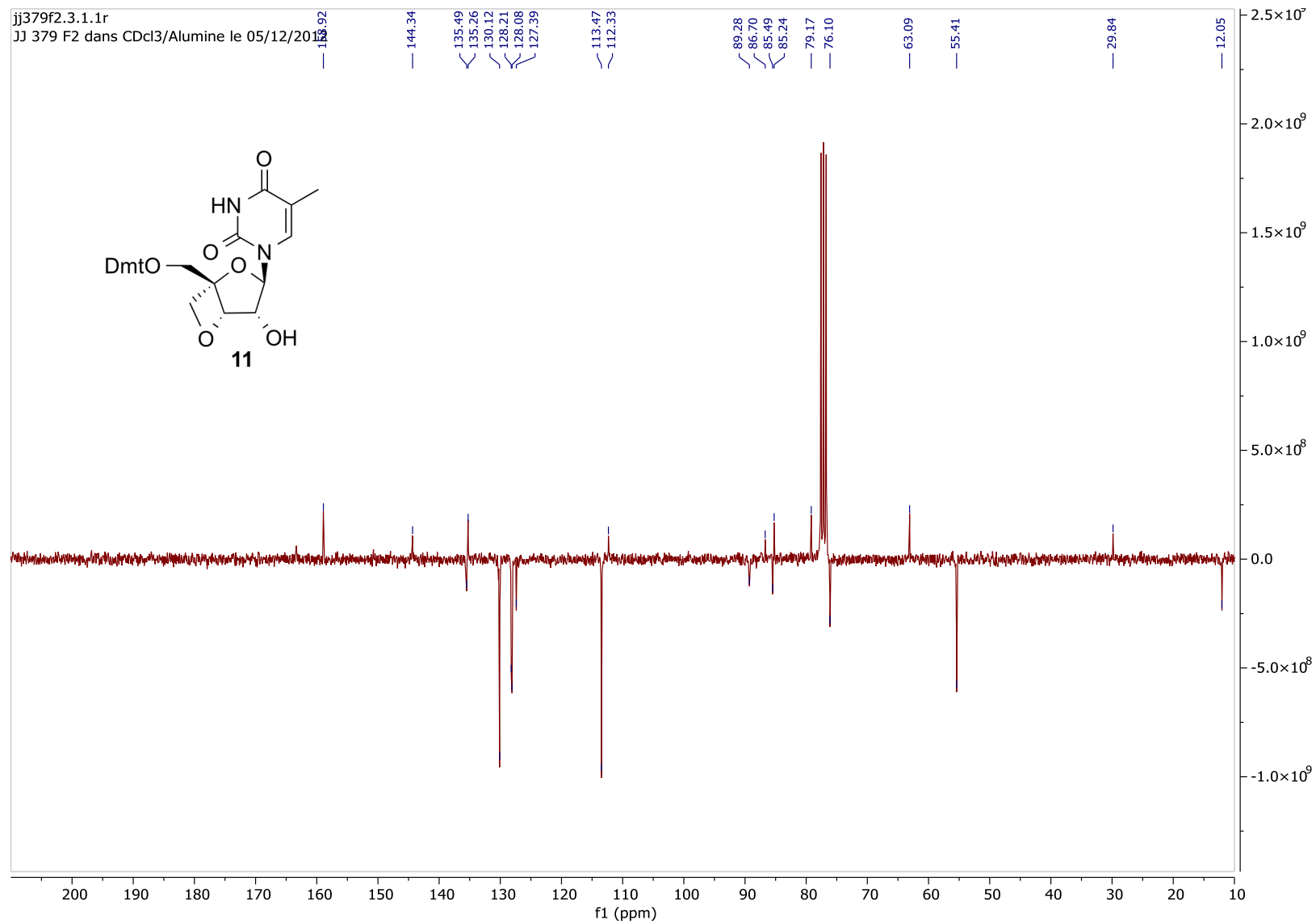


Figure S6: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 11 (75 MHz, CDCl_3).

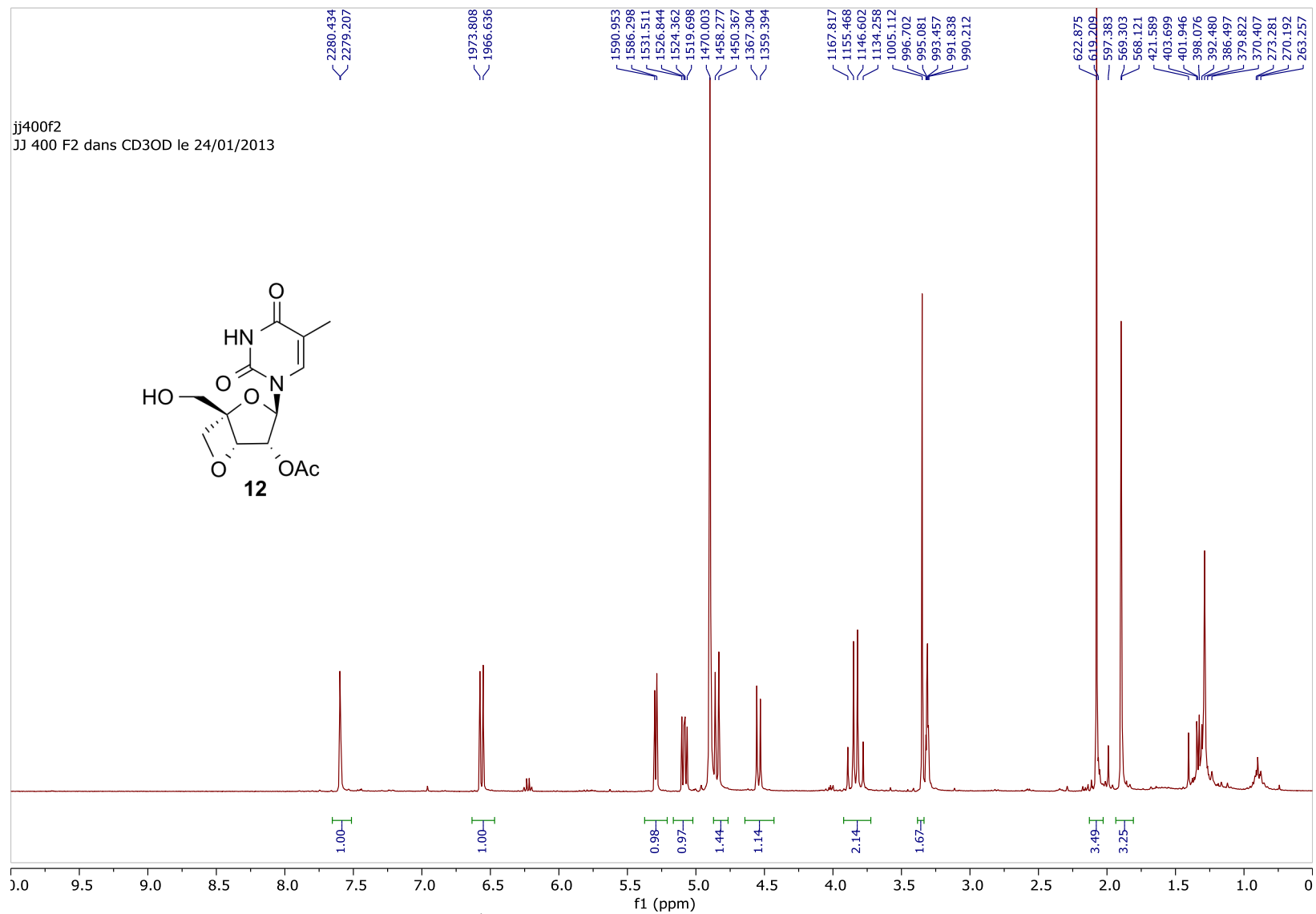


Figure S7: ^1H NMR spectrum of 12 (300 MHz, CD_3OD).

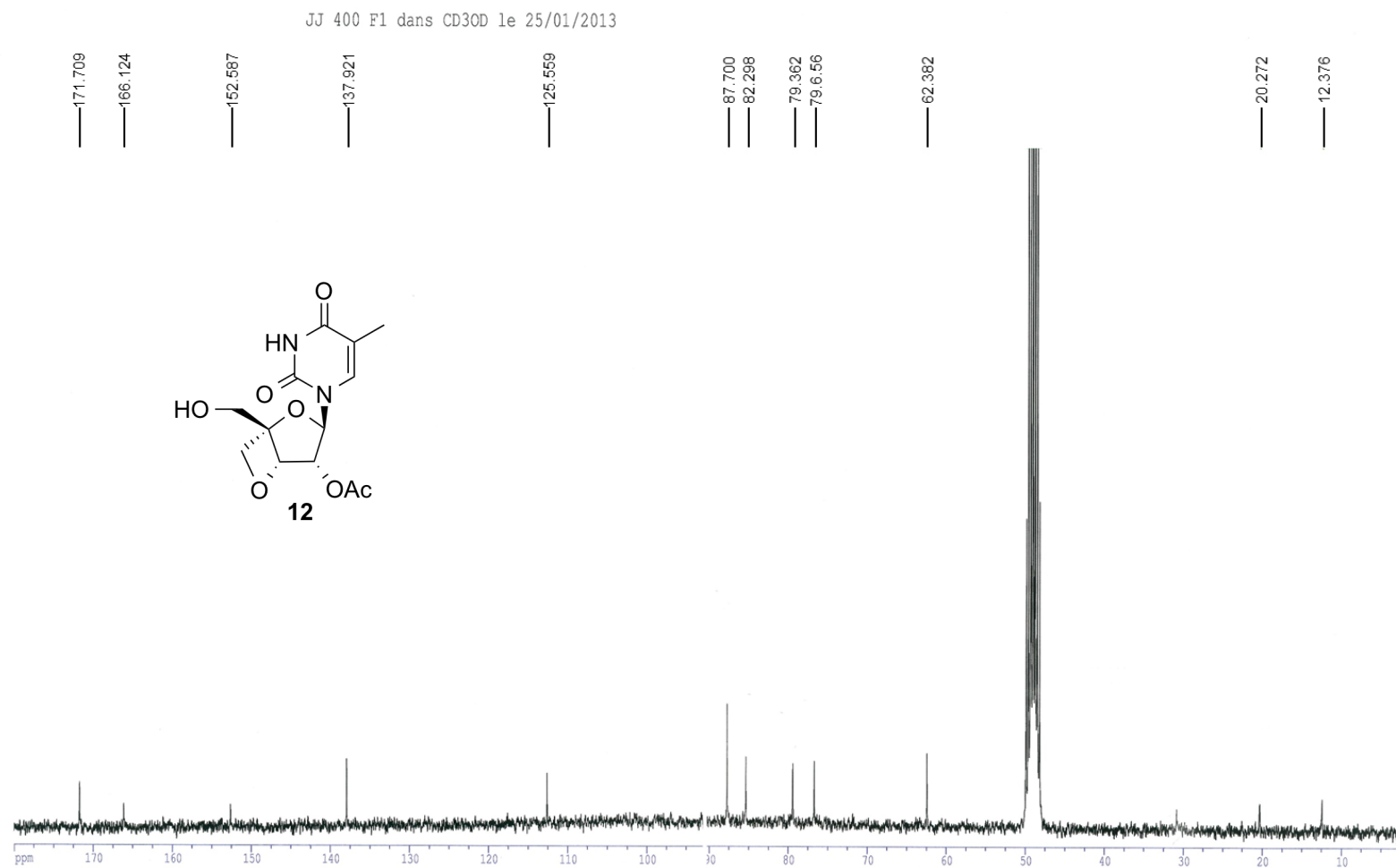


Figure S8: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 12 (75 MHz, CD_3OD).

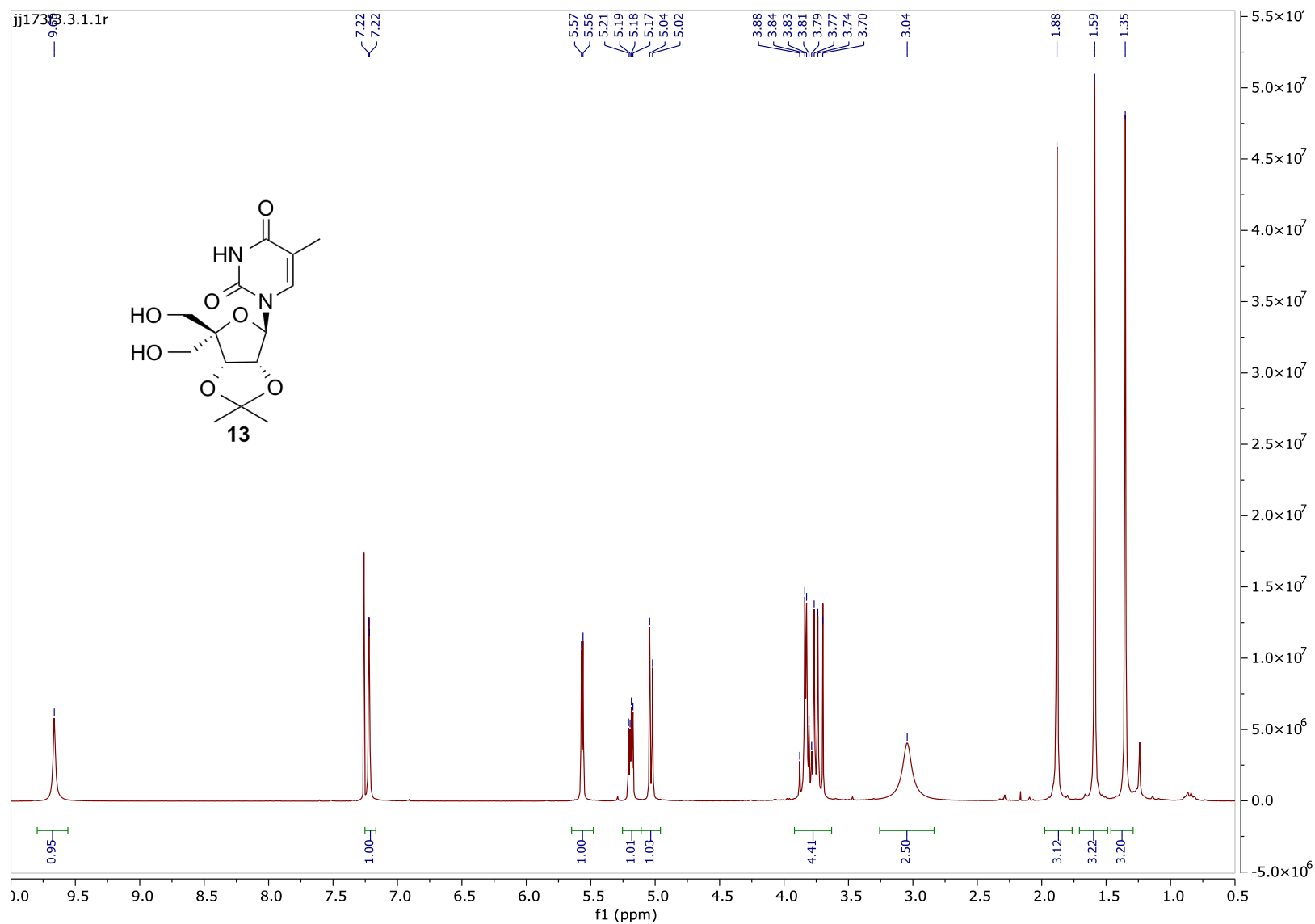


Figure S9: ^1H NMR spectrum of **13** (300 MHz, CDCl_3).

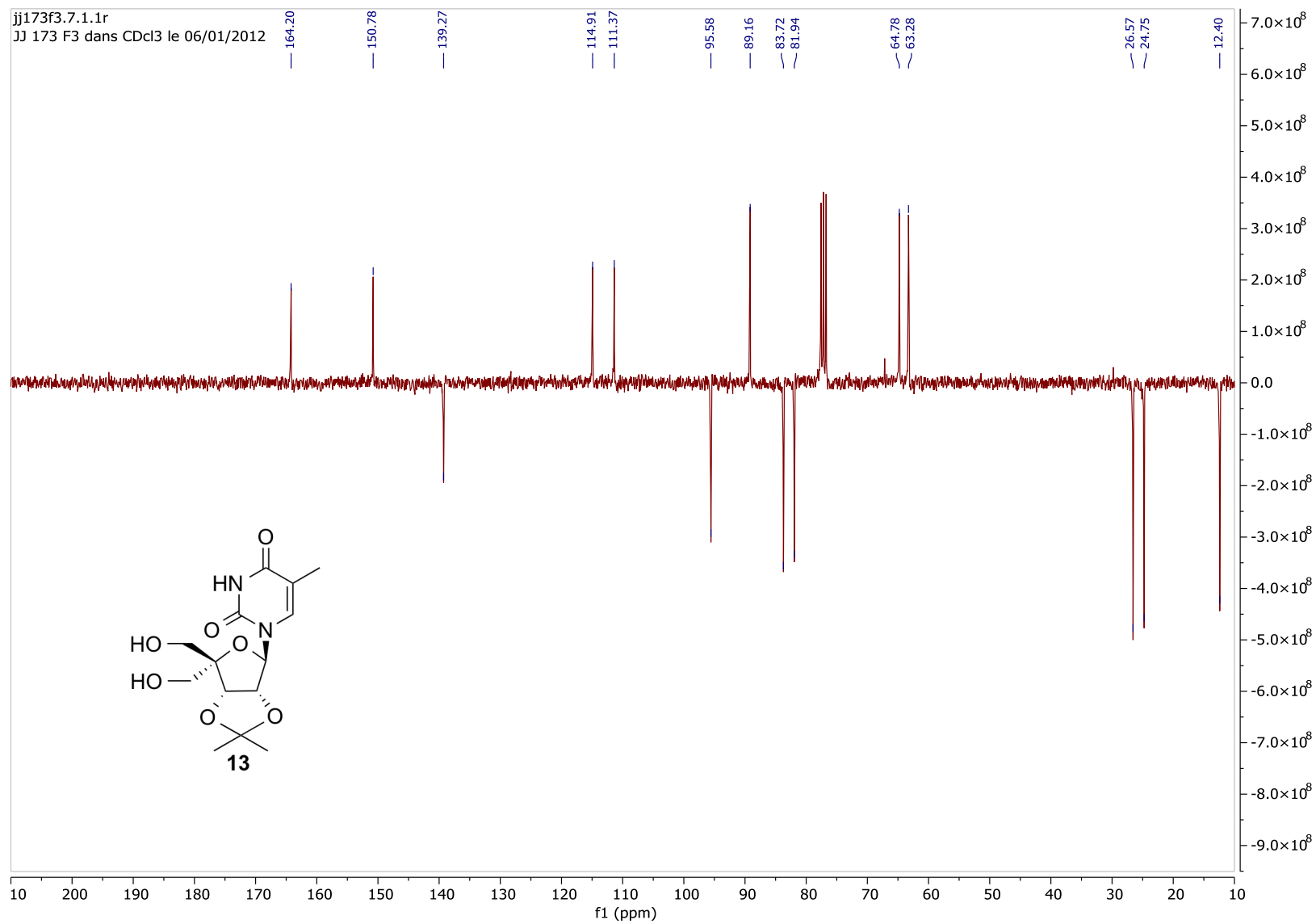


Figure S10: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **13** (75 MHz, CDCl_3).

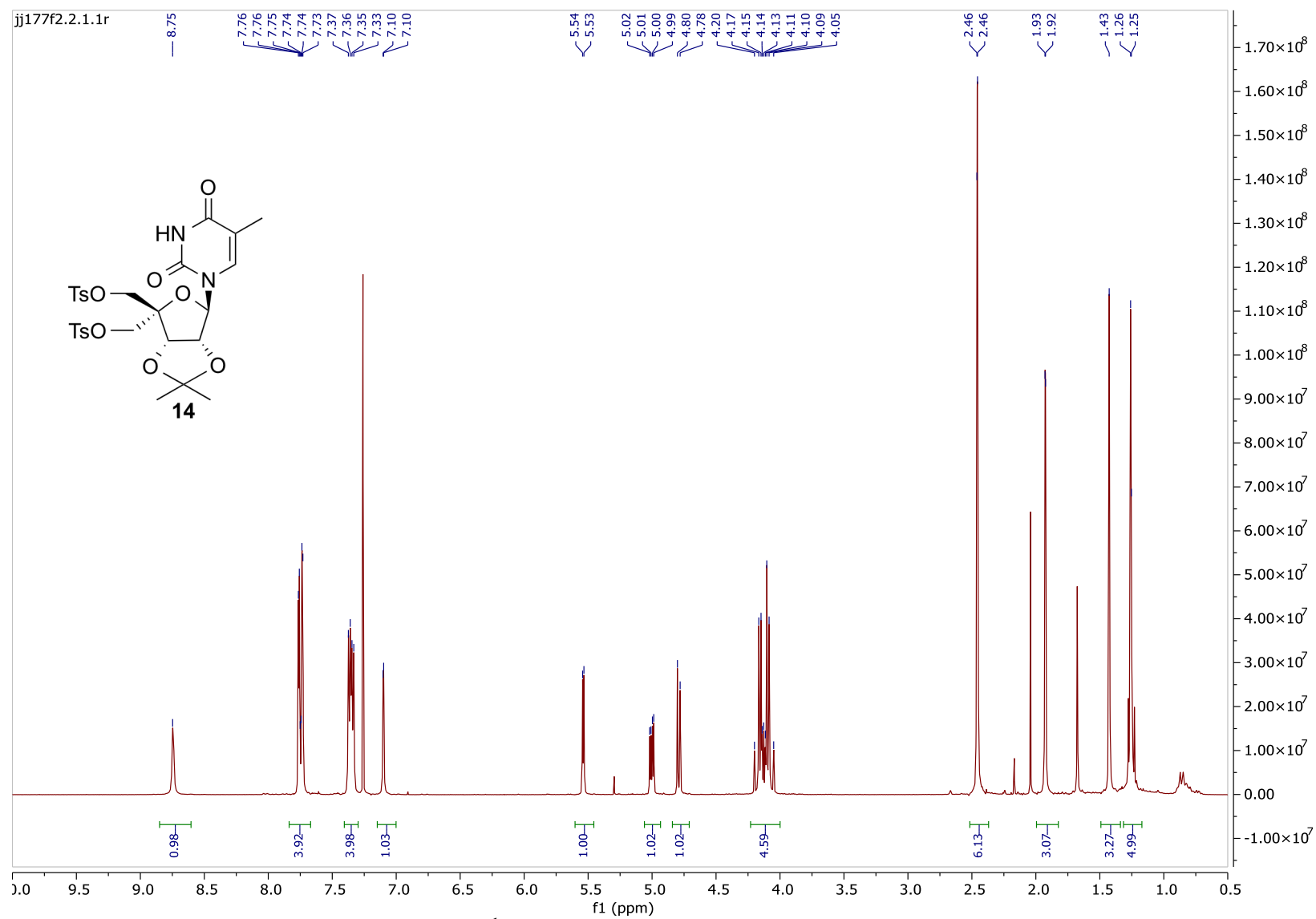


Figure S11: ^1H NMR spectrum of **14** (300 MHz, CDCl_3).

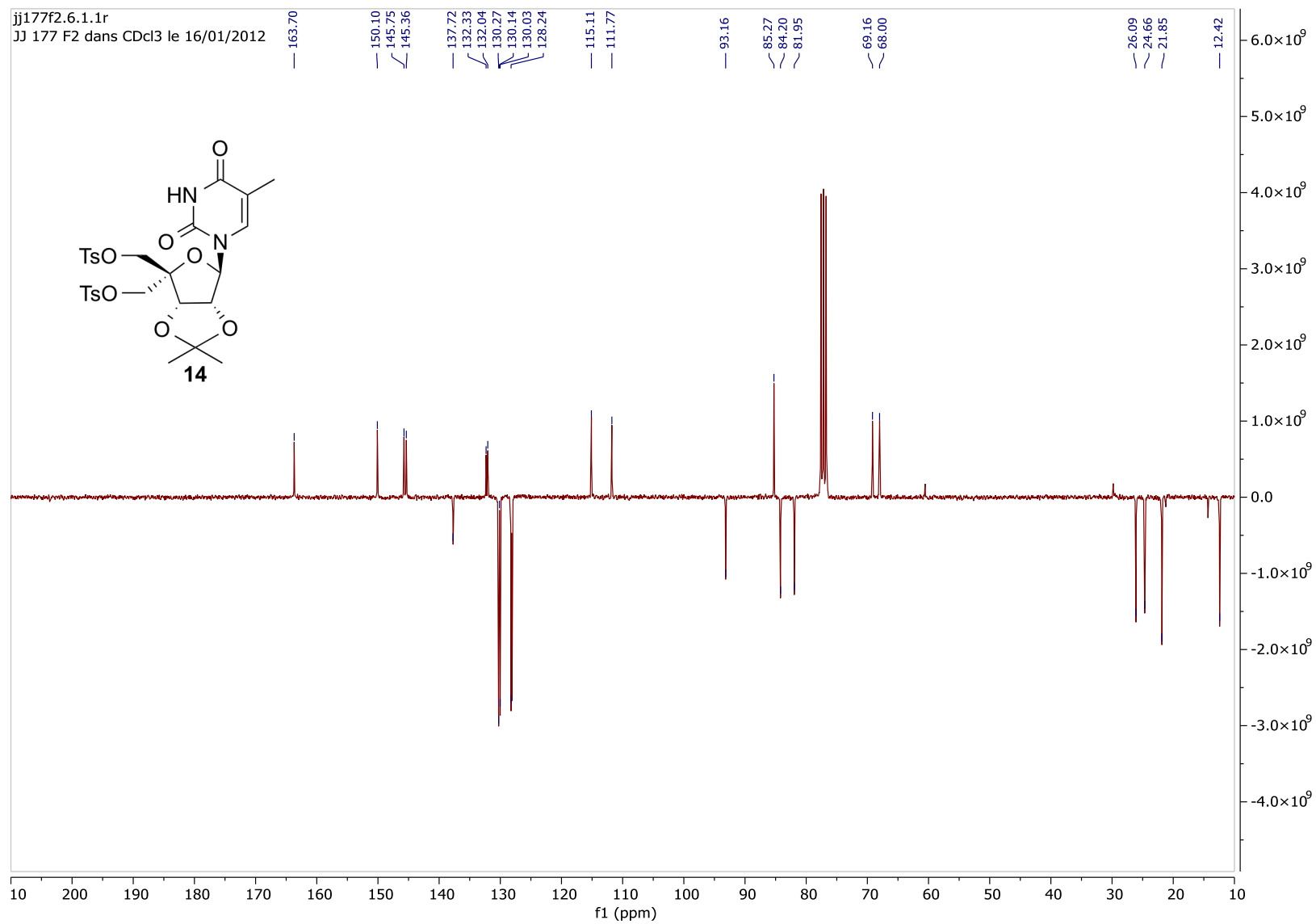


Figure S12: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **14** (75 MHz, CDCl₃).

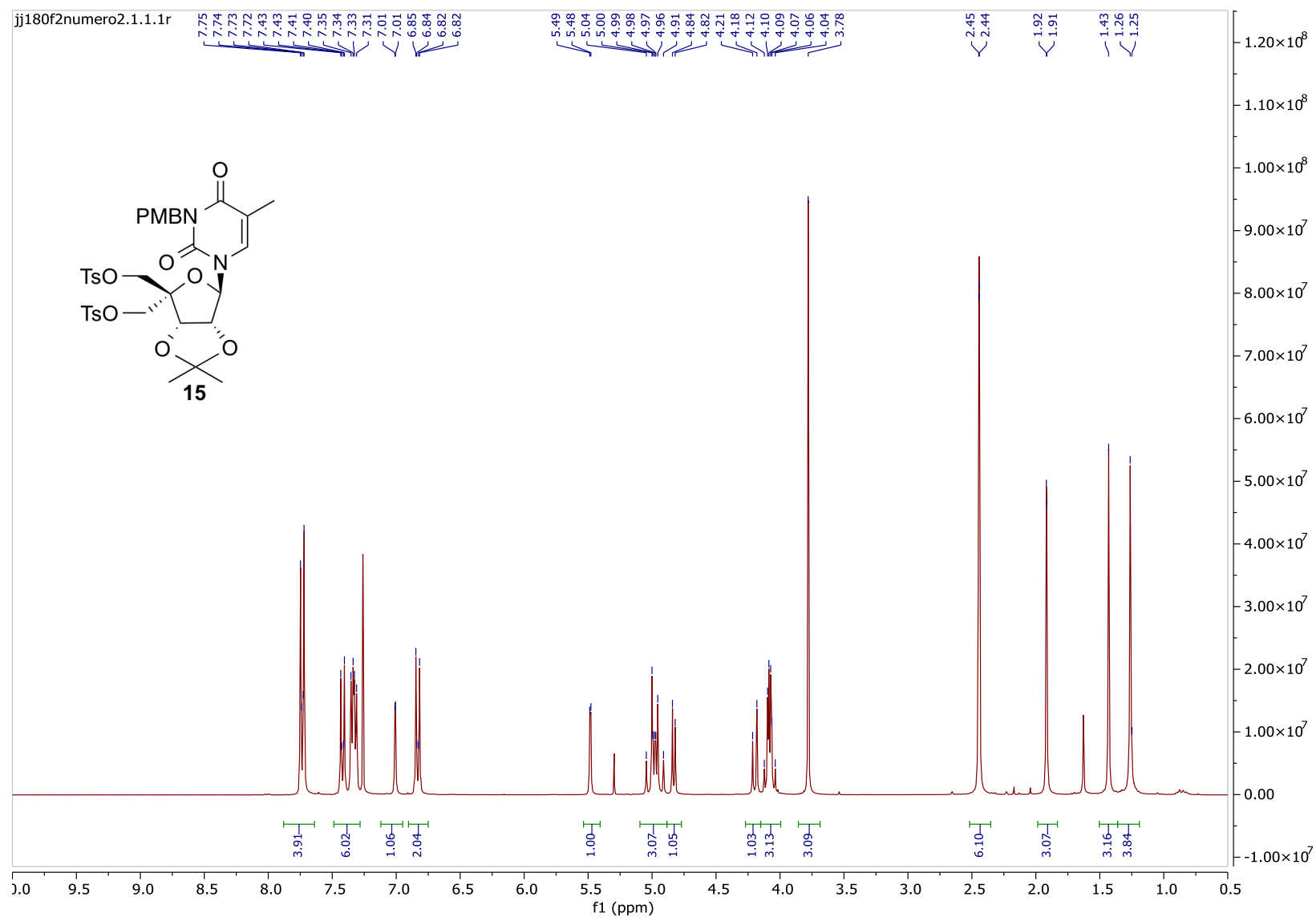


Figure S13: ^1H NMR spectrum of **15** (300 MHz, CDCl_3).

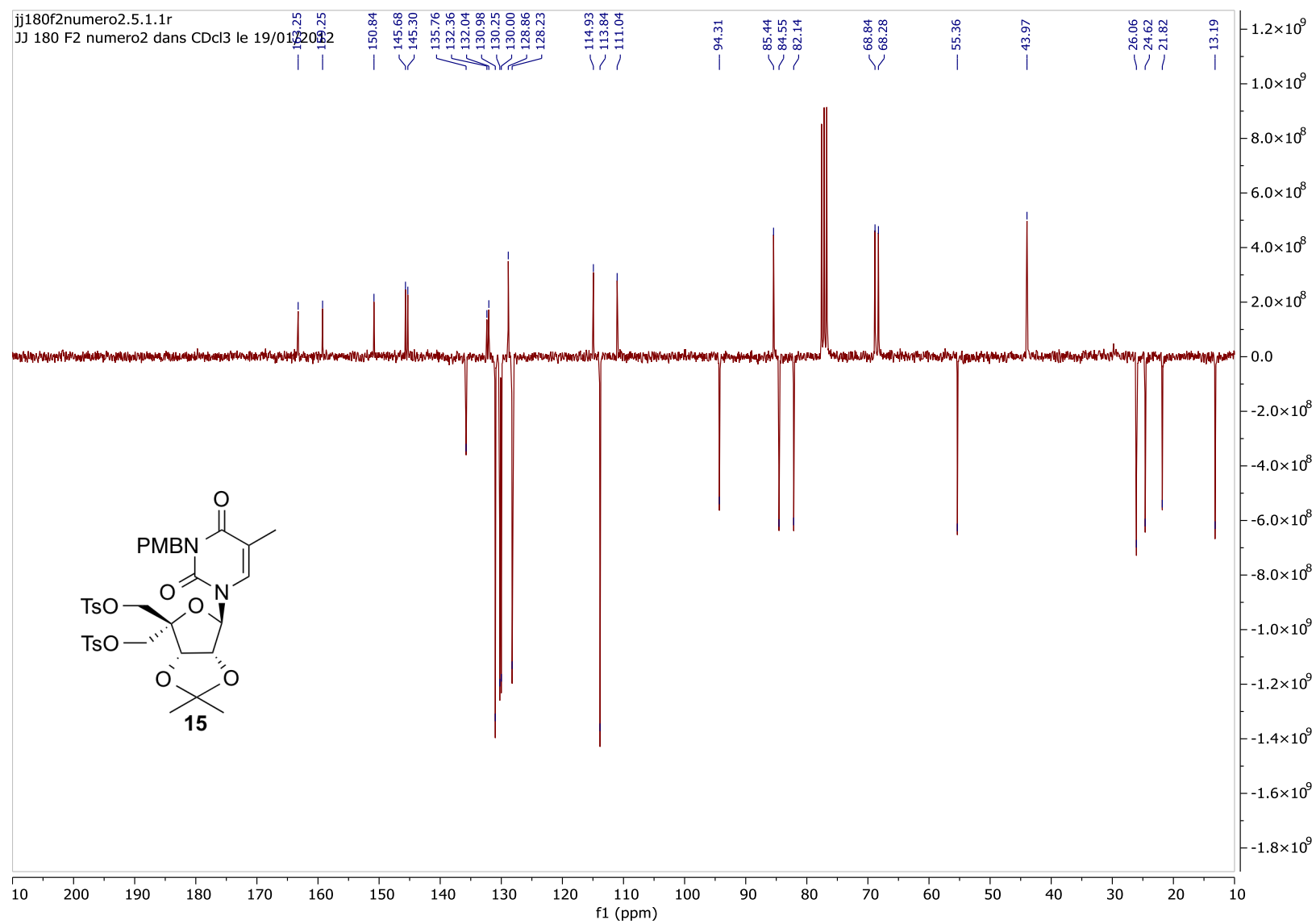


Figure S14: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 15 (75 MHz, CDCl_3).

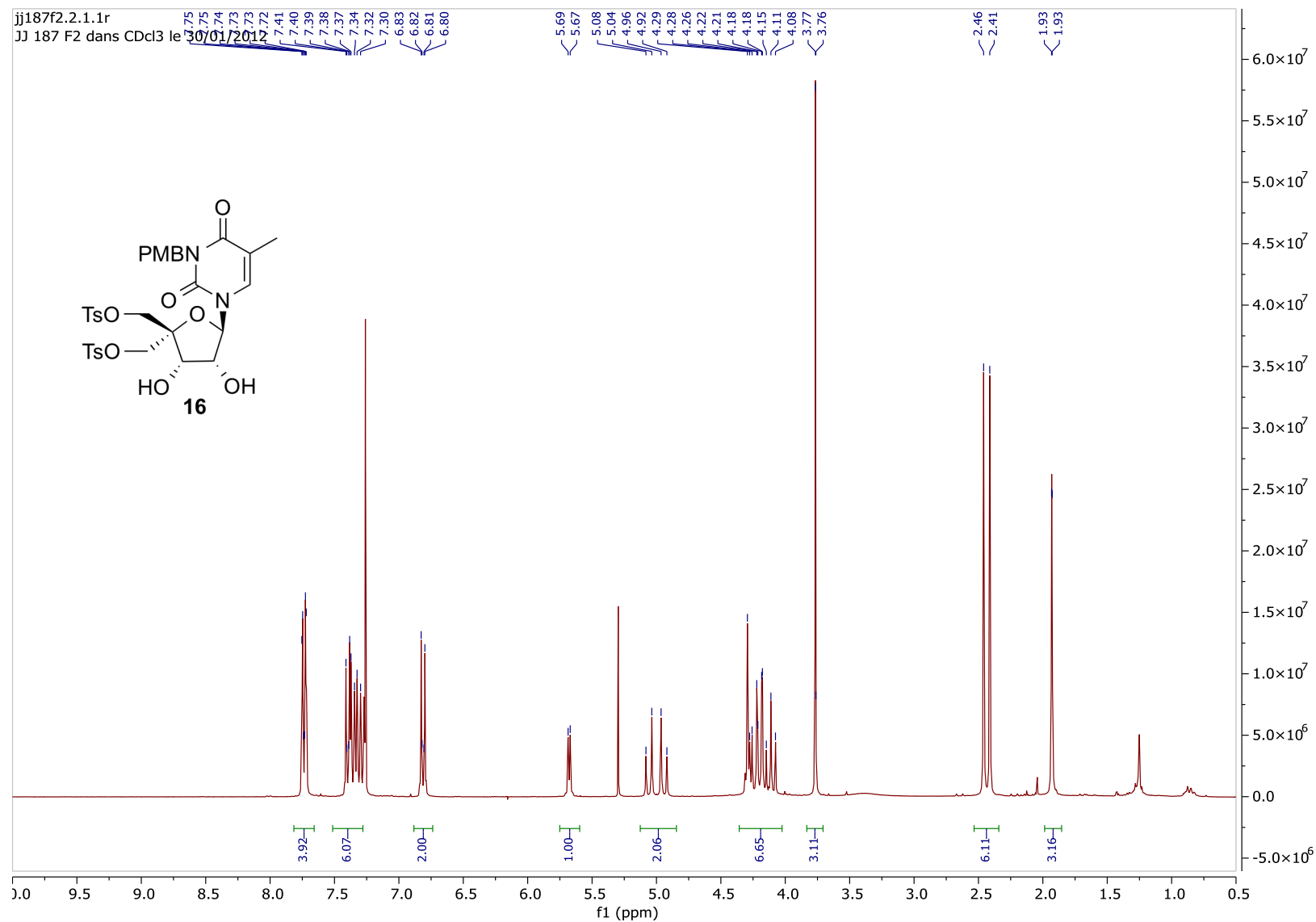


Figure S15: ^1H NMR spectrum of **16** (300 MHz, CDCl_3).

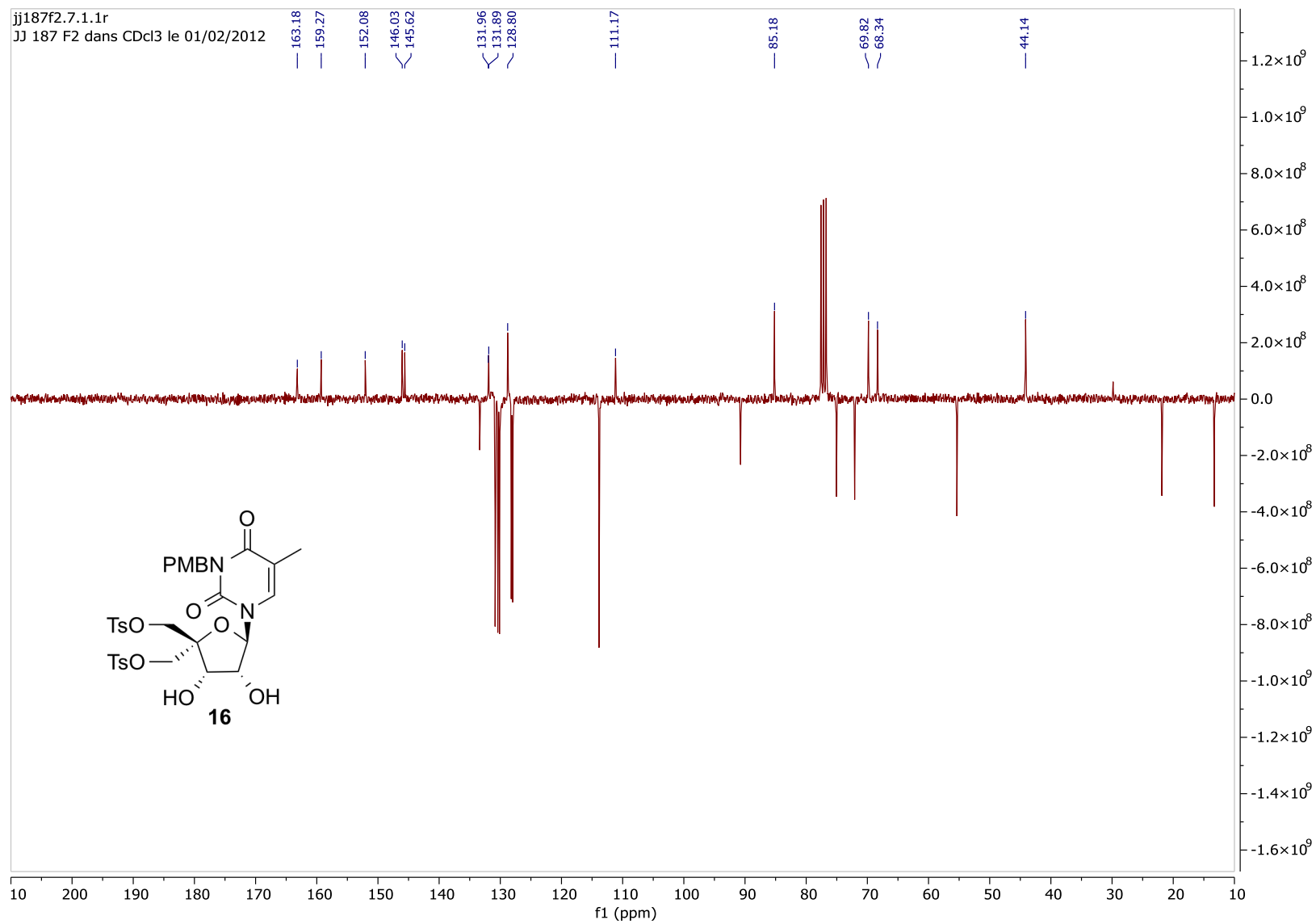


Figure S16: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **16** (75 MHz, CDCl_3).

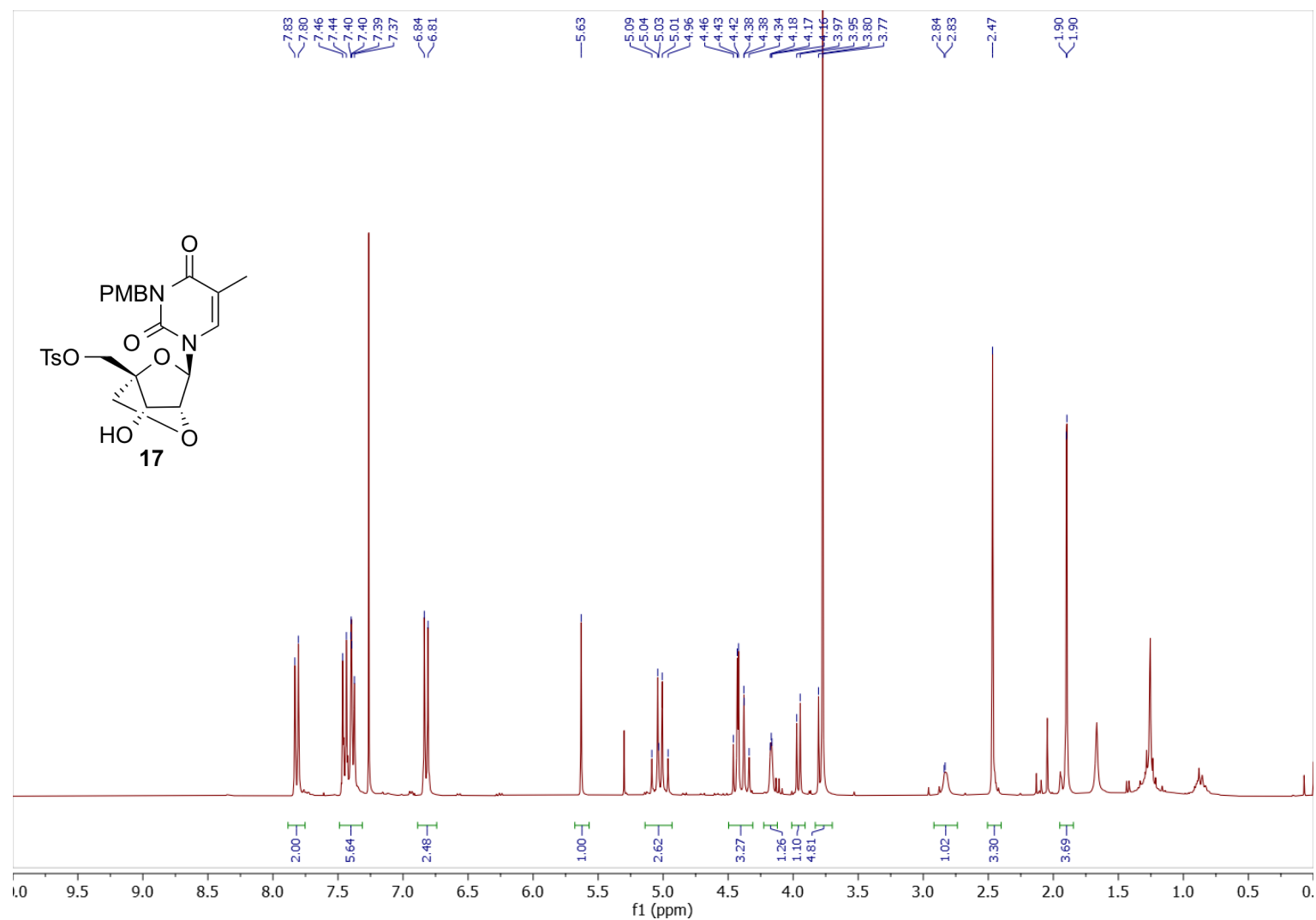


Figure S17: ^1H NMR spectrum of 17 (300 MHz, CDCl_3).

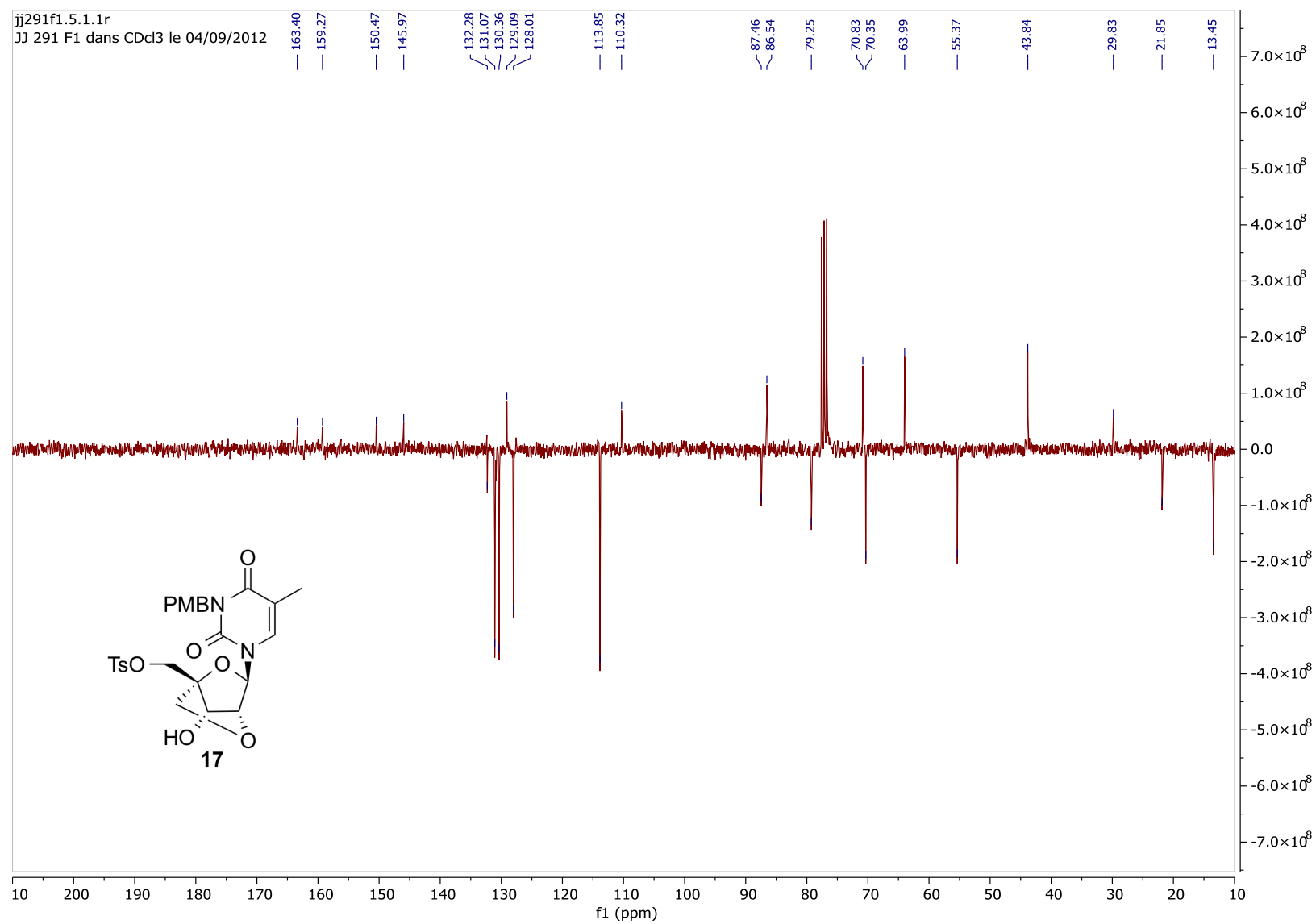


Figure S18: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 17 (75 MHz, CDCl_3).

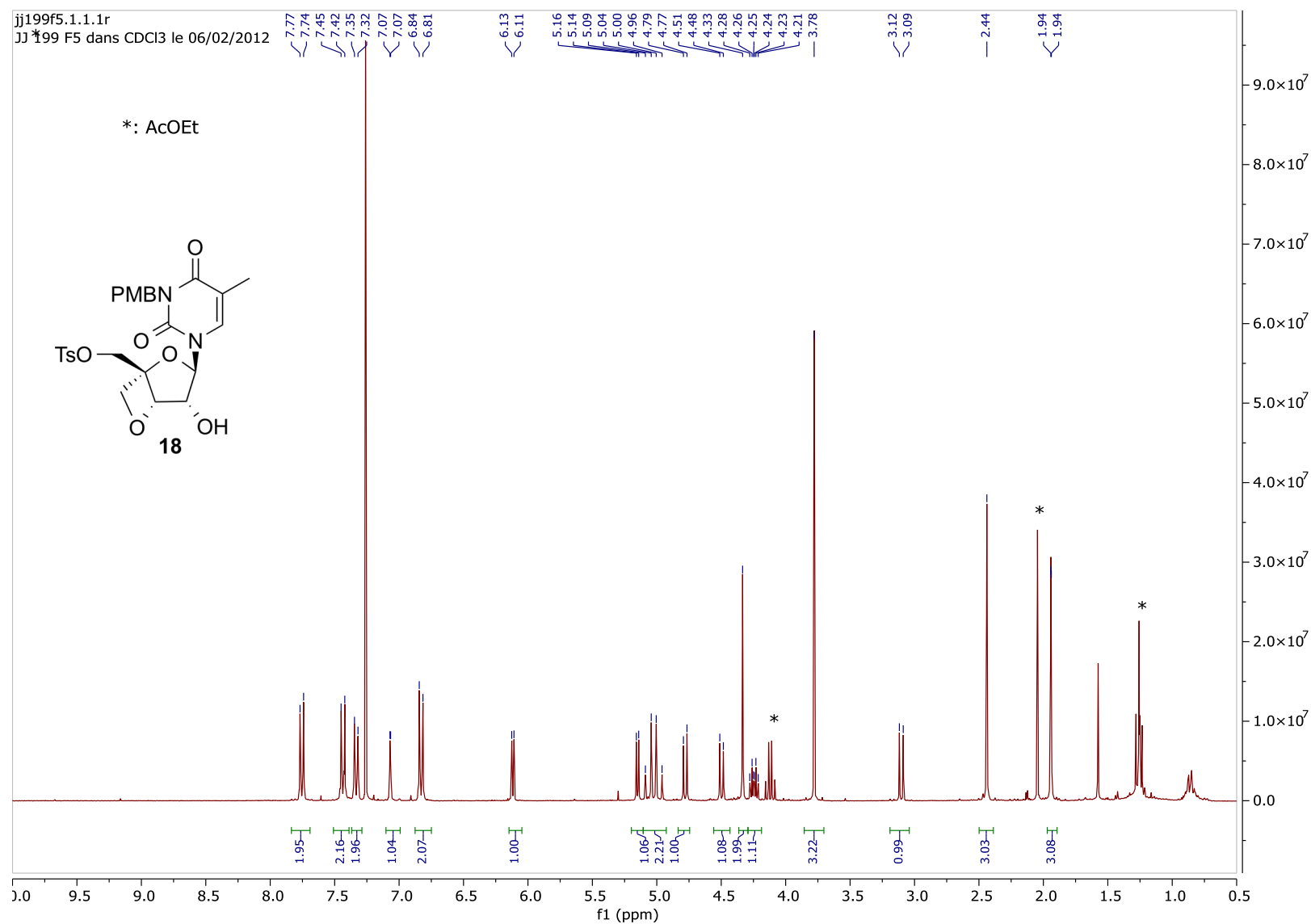


Figure S19: ¹H NMR spectrum of **18** (300 MHz, CDCl₃).

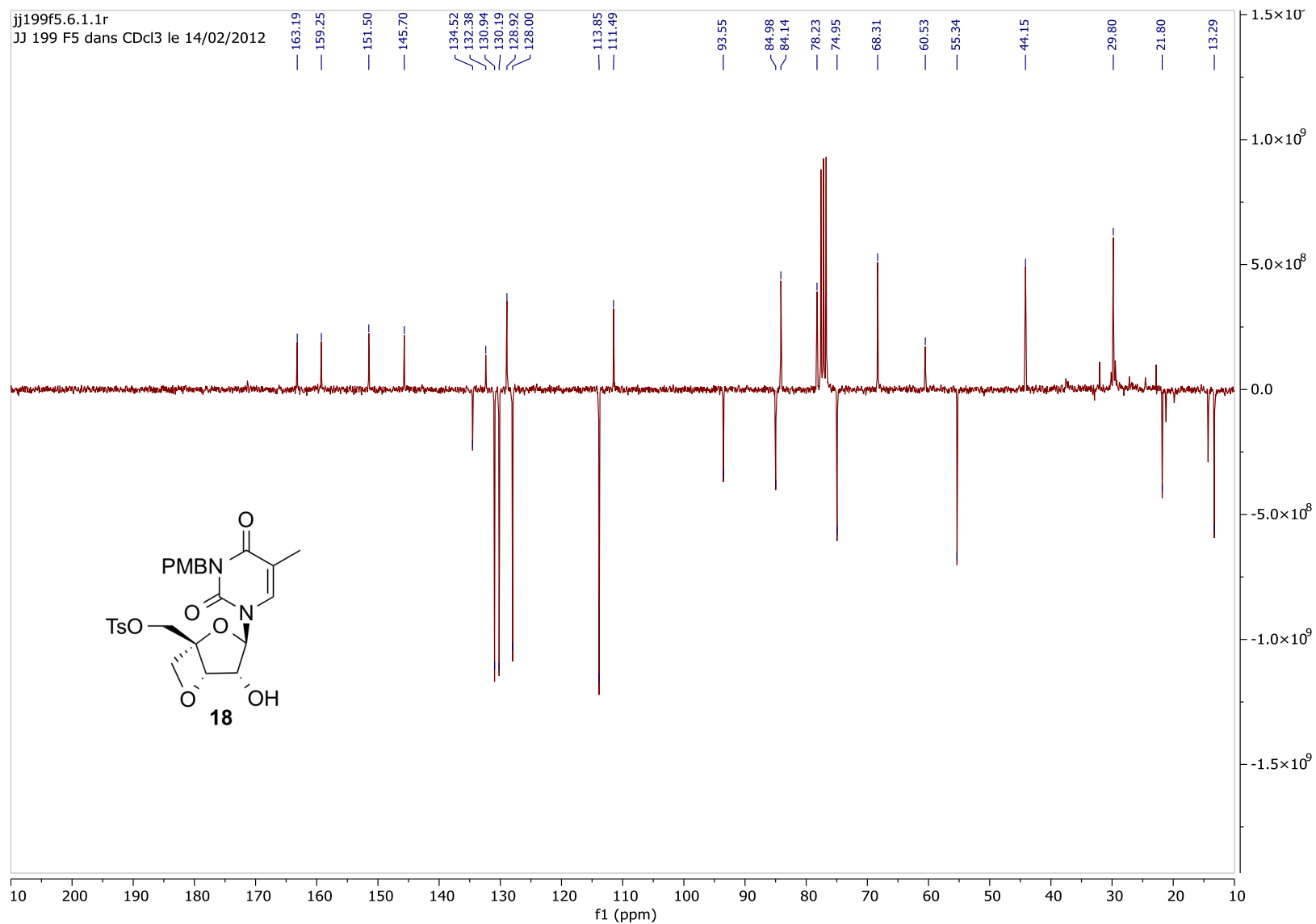


Figure S20: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **18** (75 MHz, CDCl_3).

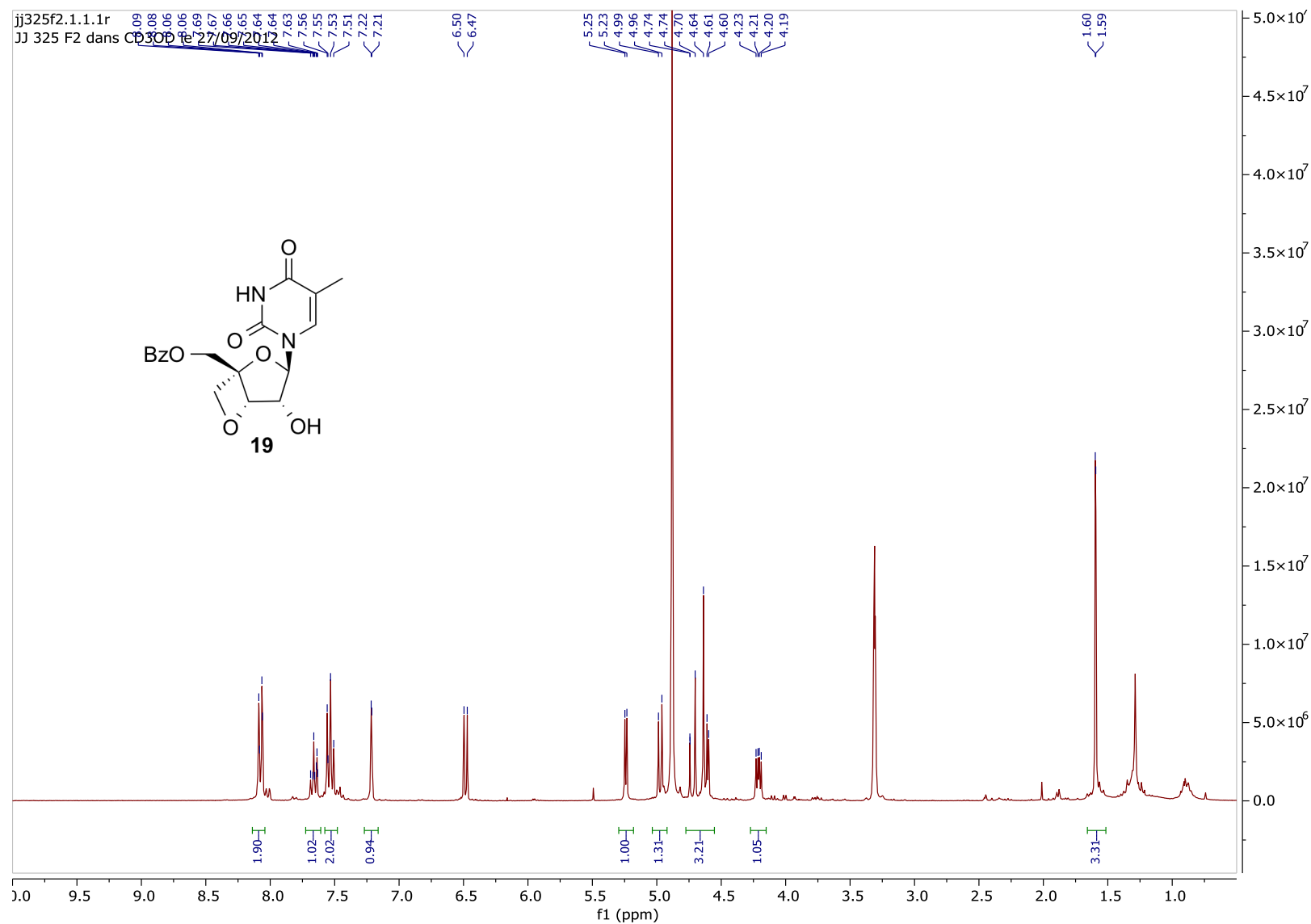


Figure S21: ¹H NMR spectrum of **19** (300 MHz, CD₃OD).

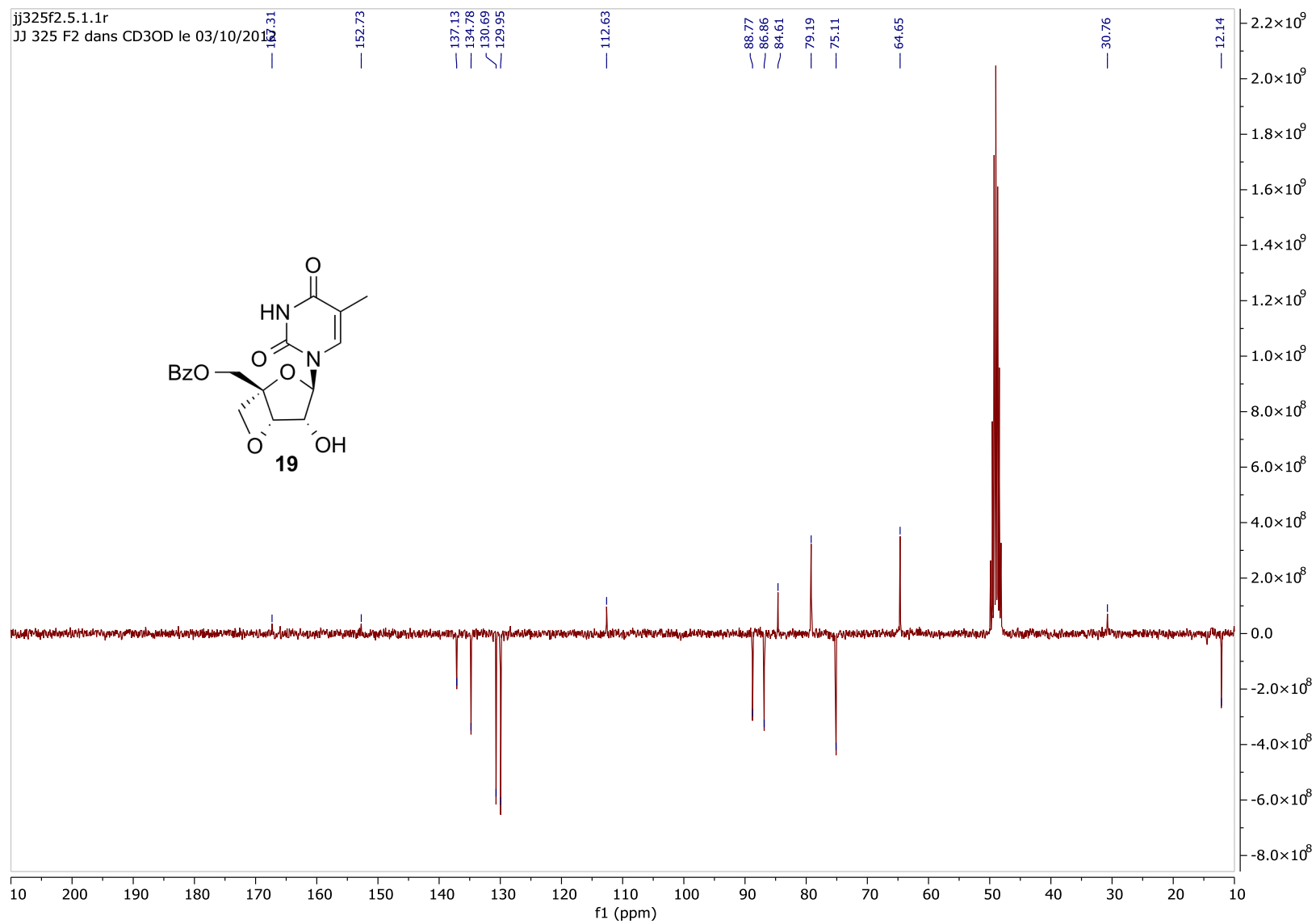


Figure S22: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **19** (75 MHz, CD_3OD).

3. NMR conformational analysis

3.1. Variable temperature NMR coupling constants

Attribution of the ^1H NMR signals of **3-5** have been reported.¹ Variable temperature ^1H 1D and ^1H , ^1H 2D J-Res NMR spectra were recorded at 278, 288, 298, 308 and 318 K on a 600 MHz spectrometer in D_2O . Vicinal coupling constants were measured on 1 D spectra (digital resolution 0.2 Hz/pt) and on J-Res spectra when needed. Refinement of $^3J_{\text{HH}}$ couplings was achieved using the PERCH NMR software 2014.⁵

Table S1. $^3J_{\text{HH}}$ Coupling constants (Hz) of the sugar rings of **3-5** at various temperatures (K)

	278	288	298	308	318
T_{LNP}T (3)					
<i>T_{LNP}</i>					
$J_{1'2'}$ ^a	1.4	1.3	1.3	1.3	1.2
$J_{2'3'}$ ^a	1.6	1.6	1.6	1.6	1.6
<i>pT</i>					
$J_{1'2'}$	6.6	6.6	6.6	6.5	6.6
$J_{1'2''}$	5.5	5.5	5.6	5.7	5.9
$J_{2'2''}$	-13.8	-13.8	-13.8	-13.9	-14.0
$J_{2'3'}$	5.6	5.4	5.2	5.2	5.1
$J_{2''3'}$	6.6	6.6	6.6	6.5	6.5
$J_{3'4'}$	6.3	5.9	5.6	5.6	5.4
T_pT_{LN} (4)					
<i>T_p</i>					
$J_{1'2'}$	6.4	6.6	6.7	6.8	6.9
$J_{1'2''}$	6.0	6.1	6.2	6.2	6.2
$J_{2'2''}$	-14.0	-14.0	-14.1	-14.1	-14.1
$J_{2'3'}$	6.3	6.4	6.4	6.5	6.5
$J_{2''3'}$	4.6	4.4	4.2	4.2	4.1
<i>pT_{LN}</i>					
$J_{1'2'}$ ^a	1.1	1.1	1.2	1.2	1.3
$J_{2'3'}$ ^a	1.7	1.6	1.6	1.6	1.4
T_{LNP}T_{LS} (5)					
<i>T_{LNP}</i>					
$J_{1'2'}$ ^a	1.2	1.3	1.2	1.4	1.3
$J_{2'3'}$ ^a	1.7	1.7	1.5	1.4	1.6
<i>pT_{LS}</i>					
$J_{1'2'}$	7.2	7.2	7.2	7.2	7.2
$J_{2'3'}$	4.6	4.6	4.6	4.6	4.7

^aMeasured on J-Res spectra

3. 2. Matlab Pseudorotation GUI analysis of the sugar conformation

General

Analysis of the sugar conformation according to the concept of pseudorotation makes use of vicinal $^3J_{\text{HH}}$ coupling constants to determine the two pseudorotation parameters P and ν_{max} following three steps:

Step 1: Vicinal $^3J_{\text{HH}}$ coupling constants along the bonds of the sugar ring allow to determine the corresponding torsion angles formed between these protons (Φ_j) via a generalized Karplus-type equation that takes into account electronegativities of the ring substituents.

Step 2: Each Φ_j is correlated to its corresponding endocyclic torsion angle (ν_j) through a linear equation $\Phi_j = A_j \nu_j + B_j$ where A_j and B_j are specific to each Φ_j to cope with imperfect tetragonal geometries. These constants can be obtained from DFT-optimized geometries of a family of conformers by plotting Φ_j against its corresponding ν_j .

Step 3: Finally, the five endocyclic torsion angles (ν_0 : C4'-O4'-C1'-C2'; ν_1 : O4'-C1'-C2'-C3'; ν_2 : C1'-C2'-C3'-C4'; ν_3 : C2'-C3'-C4'-O4'; ν_4 : C3'-C4'-O4'-C1') are linked to the two conformational parameters P and ν_{max} . The differences in the cyclic bond lengths can be handled as is the case in the Matlab Pseudorotation GUI program.⁶

Matlab Pseudorotation GUI procedure

Experimental variable temperature $^3J_{\text{HH}}$ of **3-5** were used as inputs in the Matlab Pseudorotation GUI program assuming a two-state sugar conformational equilibrium. Calculations were performed without any constraints unless specifying the starting search parameters for North (N) ($P = 10^\circ$, $\nu_{\text{max}} = 32^\circ$) and South (S) conformers ($P = 140^\circ$, $\nu_{\text{max}} = 32^\circ$). Substituents electronegativities were filled using the electronegativity editor of the software. Parameters A_j and B_j were calculated for models of the sugar residues of **3-5** (Figure S23) by IEFPCM/D3-M062-2X/6-311+G(d,p) potential energy scans (*vide infra*). The pseudorotation parameters that best fitted the experimental data were obtained in the first mode of calculation of the program and the polar plots were obtained using the scan surface mode of the program.

Determination of *Aj* and *Bj* parameters

Ab initio calculations were performed on thymidine 3'- and 5'-phosphate methyl ester models (**TpMe**, **T_{LN}pMe** and **MepT_{LN}**, **MepT_{LS}**, **MepT**, respectively, Figure S23) mimicking the 5'- and 3'-end of dinucleotides **3-5**, respectively.

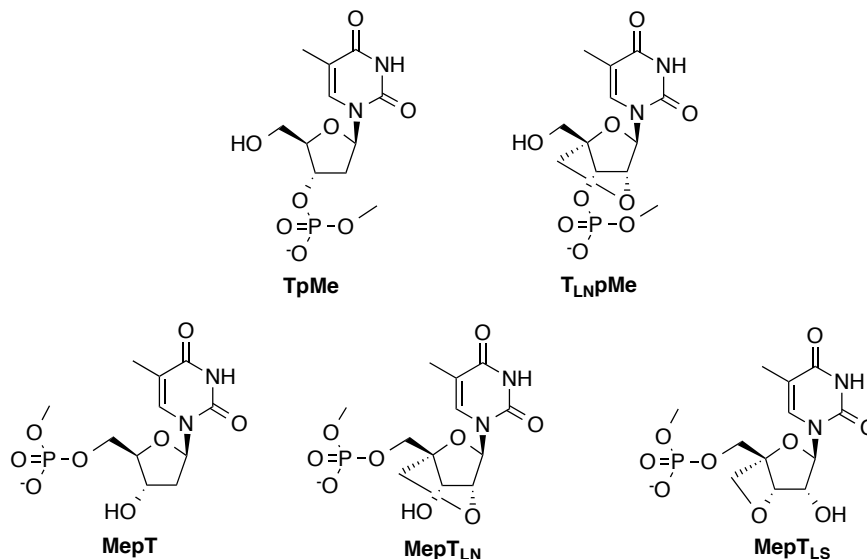


Figure S23. Structure of thymidine 3'- and 5'-phosphate methyl ester models (**TpMe**, **MepT**, **T_{LN}pMe**, **MepT_{LN}**, **MepT_{LS}**).

The N and S conformers were considered for thymidine-containing models (**TpMe**, **MepT**) whereas the N conformer was considered for the N-locked models (**T_{LN}pMe**, **MepT_{LN}**) due to the conformational restriction imposed by the 2'*O*,4'*C* methylene bridge. For the **MepT_{LS}** model, two S conformers were considered. The energy surface defined through the C1'-C2'-C3'-C4' endocyclic torsion angle (ν_2) for the eight conformers was scanned (every 2°) by geometry optimization using the redundant coordinate mode of Gaussian16 defining scan coordinate mode. When two lowest-in-energy conformers of a model are considered, their Boltzmann population (*F*), is given.

All structures were fully optimized with the M06-2X⁷ DFT method with a 6-311+G(d,p) basis set for all atoms. D3 version of Grimme's dispersion with the original D3 damping function⁸ was added for all calculations. The nature of all extrema as minimum or transition state was characterized with analytical calculations of frequencies at 298.15 K and 1 Atm. The solvent effect (water with ϵ of 80.1) was taken in account using the IEFPCM formalism during optimization and frequency calculations. All calculations were done with the Gaussian16 revision B.01 program packages.⁹ Graphical representation of the conformer models was done using CYLview software.¹⁰

Table S2. Geometrical and thermodynamic parameters of the **TpMe** conformers calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory

	<i>TpMe N conformer</i>	<i>TpMe S conformer</i>
<i>P</i>	14.34	166.59
ν_{\max}	38.00	35.43
ν_0	2.91	-19.29
ν_1	-25.60	33.64
ν_2	36.82	-34.46
ν_3	-36.19	24.32
ν_4	21.29	-3.33
ΔH° (kJ/mol)	3.95	0.00
ΔG° (kJ/mol)	1.78	0.00
ΔS° (J/mol.K)	7.26	0.00
<i>F</i>	0.33	0.67

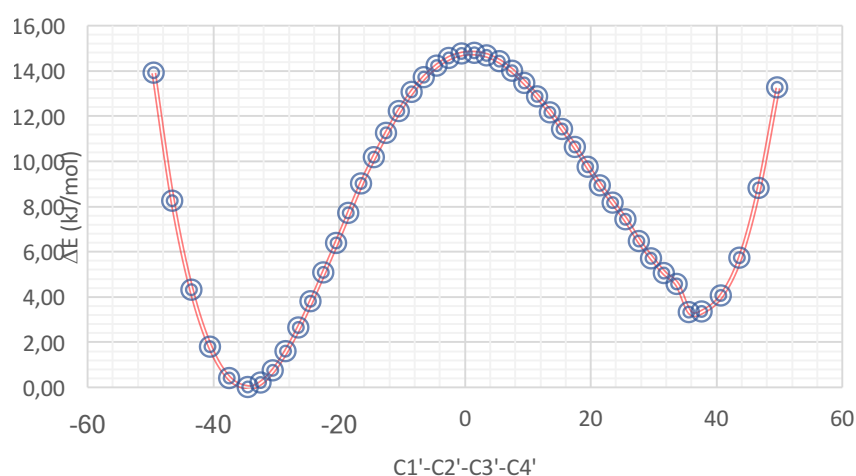


Figure S24. PES along the C1'-C2'-C3'-C4' endocyclic torsion angle (ν_2) of **TpMe** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

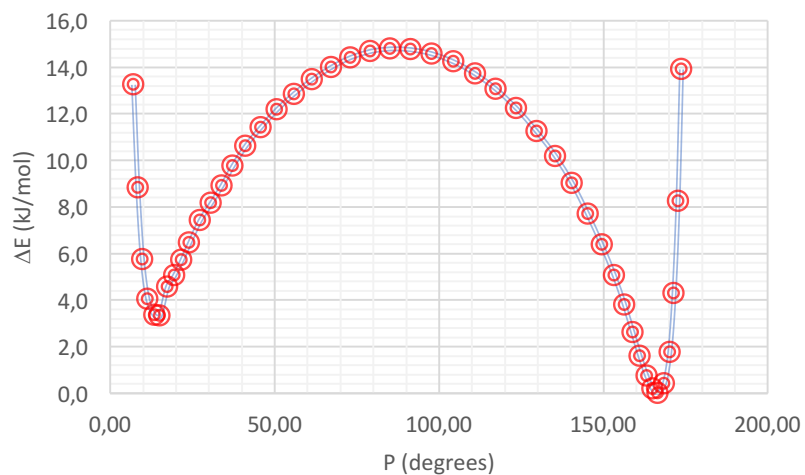


Figure S25. Relation between P and relative energy of **TpMe** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

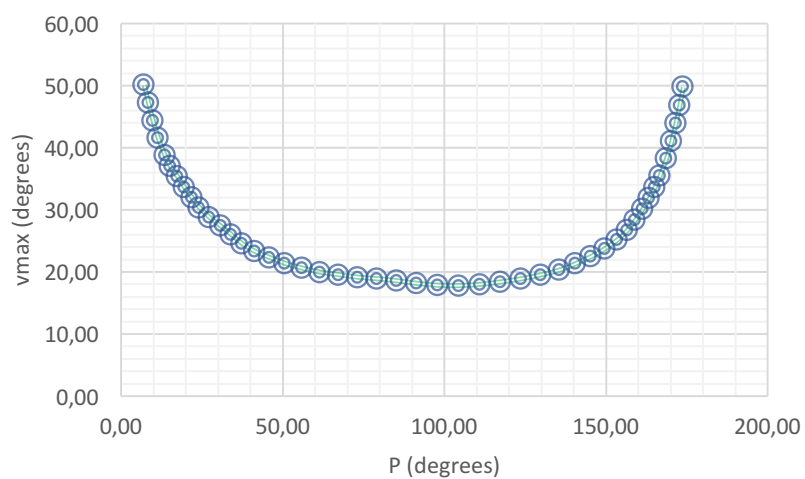


Figure S26. Relation between P and ν_{\max} of **TpMe** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

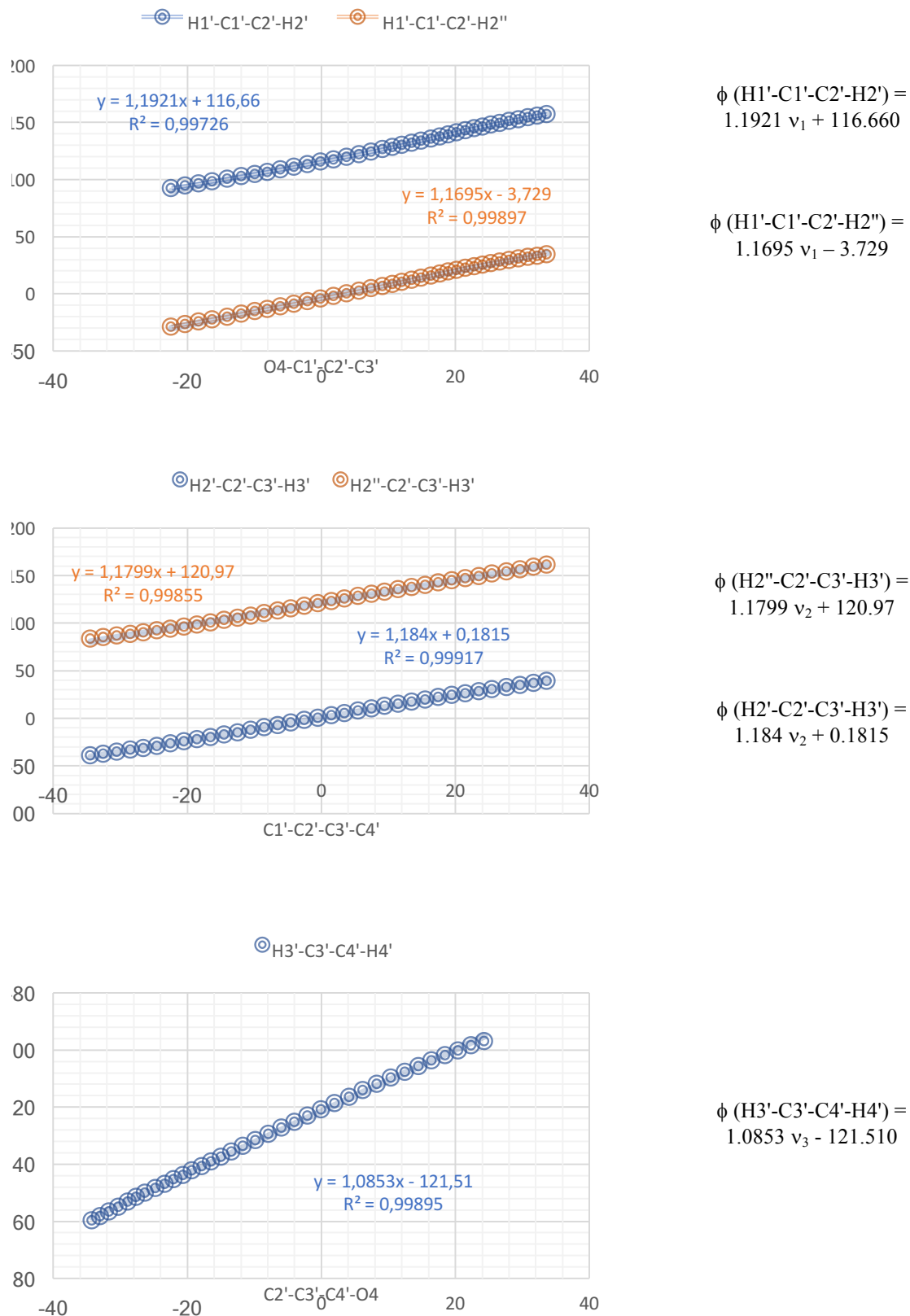


Figure S27. Linear correlations between H-C-C-H exocyclic torsions and respective sugar endocyclic torsions (v_1 - v_3) in degrees, used to calculate A_j and B_j parameters for **TpMe**.

Table S3. Parameters used in Matlab Pseudorotation GUI for the analysis of the Tp sugar moiety of TpT_{LN} (4) and results in terms of puckering and population

<i>Vicinal coupling constant</i>	<i>A</i>	<i>B</i>	<i>Electronegativities</i>			
$J_{1'2'}$	1.192	116.660	0.000	0.620	0.560	1.260
$J_{1'2''}$	1.170	-3.729	0.620	0.000	0.560	1.260
$J_{2'3'}$	1.184	0.182	1.260	0.620	0.560	0.000
$J_{2''3'}$	1.180	120.970	1.260	0.620	0.000	0.560
$J_{3'4'}$	1.085	-121.510	1.270	0.680	0.720	1.260
			<i>N Conformer</i>	<i>S Conformer</i>		
<i>P</i>			3.46	143.14		
v_{max}			36.02	25.68		
v_0			9.82	-21.33		
v_1			-28.31	25.66		
v_2			35.99	-20.19		
v_3			-29.92	7.01		
v_4			12.42	8.85		
<i>Temperature (K)</i>			X_N	X_S		
278			39.1	60.9		
288			37.7	62.3		
298			36.6	63.4		
308			36.0	64.0		
318			35.2	64.8		
<i>RMSD</i>			0.09 Hz			

Table S4. Geometrical and thermodynamic parameters of MepT conformers calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory

	<i>MepT N conformer</i>	<i>MepT S conformer</i>
<i>P</i>	17.68	164.88
<i>v</i> _{max}	35.91	34.98
<i>v</i> ₀	0.57	-19.98
<i>v</i> ₁	-22.59	33.58
<i>v</i> ₂	34.22	-33.77
<i>v</i> ₃	-34.79	23.26
<i>v</i> ₄	21.95	-2.21
ΔH° (kJ/mol)	5.32	0.00
ΔG° (kJ/mol)	2.71	0.00
ΔS° (J/mol.K)	8.75	0.00
<i>F</i>	0.25	0.75

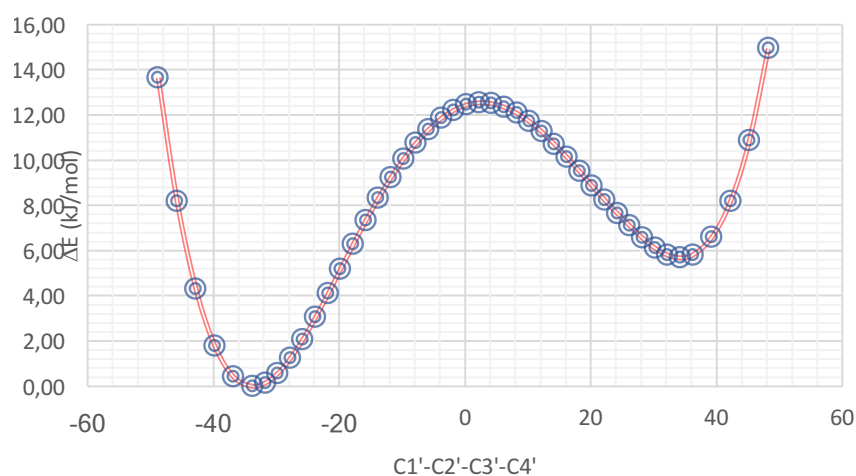


Figure S28. PES along C1'-C2'-C3'-C4' endocyclic torsion angle (*v*₂) of MepT calculated at the IEFPCM/ GD3-M06-2X/6-311+G(d,p) level of theory.

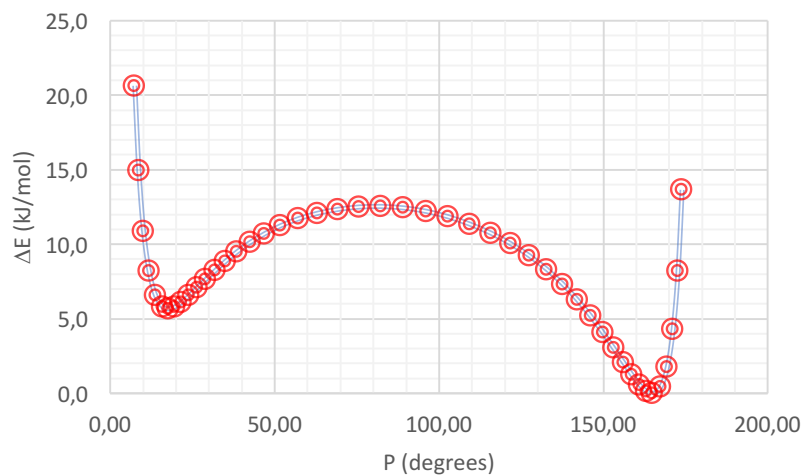


Figure S29. Relation between P and relative energy of **MepT** calculated at the IEFPCM/ GD3-M06-2X/6-311+G(d,p) level of theory.

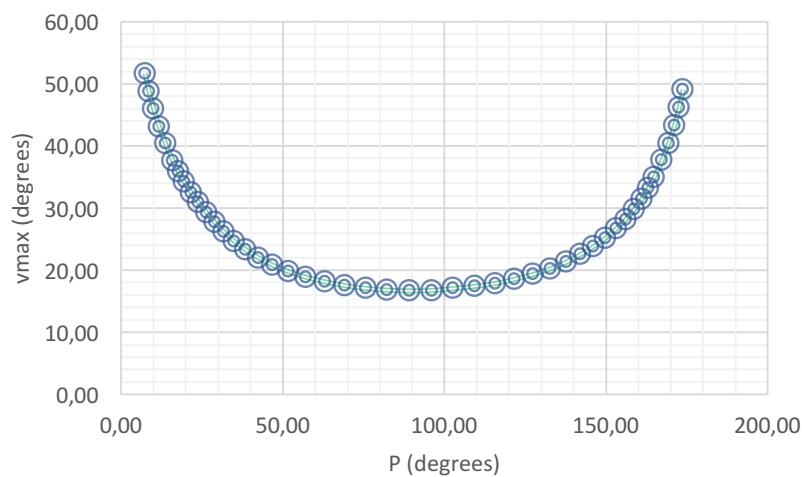


Figure S30. Relation between P and v_{\max} of **MepT** calculated at the IEFPCM/ GD3-M06-2X/6-311+G(d,p) level of theory.

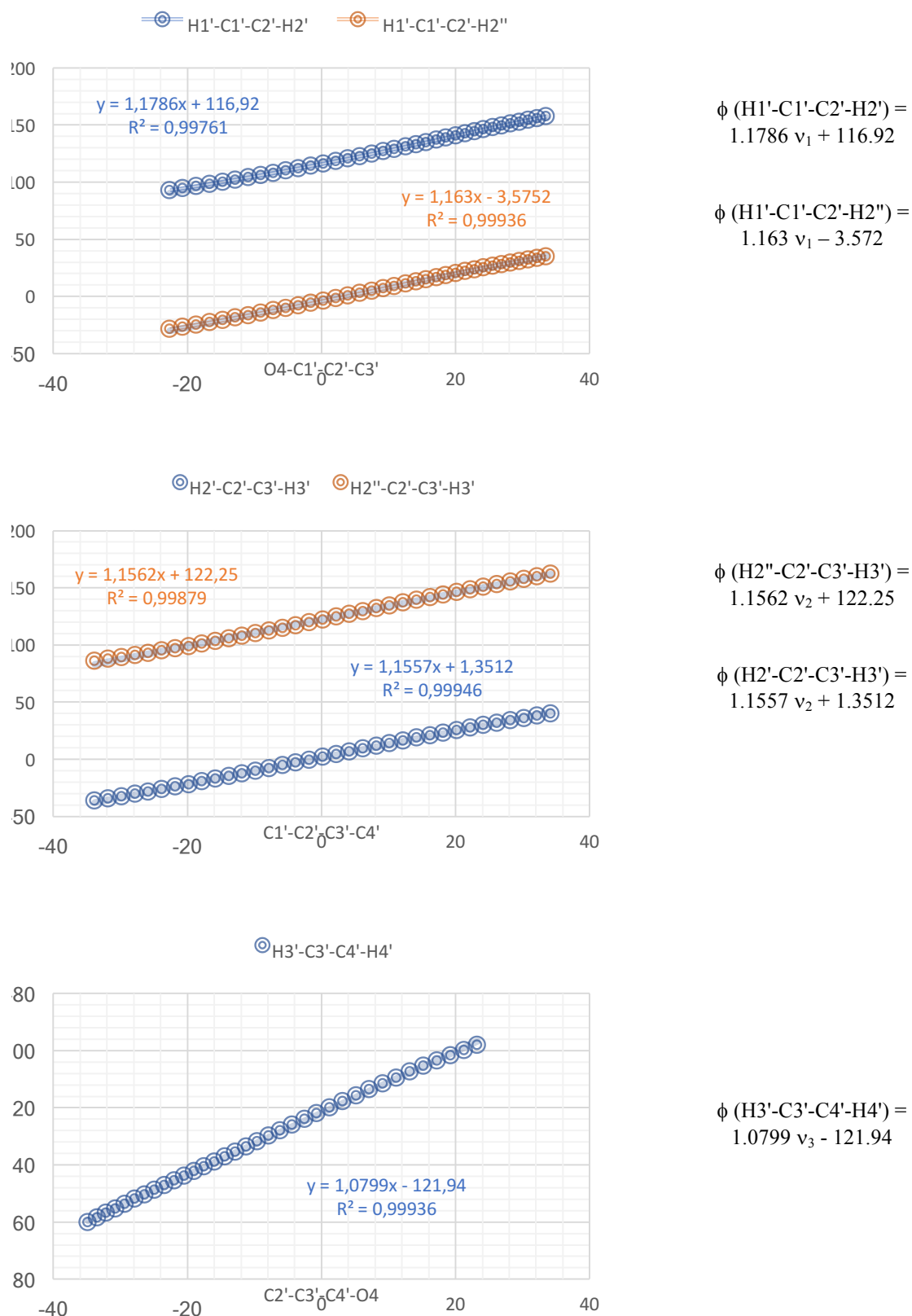


Figure S31. Linear correlations between H-C-C-H exocyclic torsions and respective sugar endocyclic torsions (v_1 - v_3) in degrees, used to calculate A_j and B_j parameters for **MepT**.

Table S5. Parameters used in Matlab Pseudorotation GUI for the analysis of the pT sugar moiety of T_{LNP}T (3) and results in terms of puckering and population (X, %)

<i>Vicinal coupling constant</i>	<i>A</i>	<i>B</i>	<i>Electronegativities</i>			
$J_{1'2'}$	1.179	116.920	0.000	0.620	0.560	1.260
$J_{1'2''}$	1.163	-3.575	0.620	0.000	0.560	1.260
$J_{2'3'}$	1.166	1.351	1.260	0.620	0.560	0.000
$J_{2''3'}$	1.156	122.250	1.260	0.620	0.000	0.560
$J_{3'4'}$	1.080	-121.940	1.270	0.680	1.000	1.260
			<i>N Conformer</i>	<i>S Conformer</i>		
<i>P</i>			8.03	114.47		
v_{max}			46.49	26.41		
v_0			9.07	-26.29		
v_1			-34.13	22.67		
v_2			46.16	-10.39		
v_3			-40.55	-5.86		
v_4			19.46	19.87		
<i>Temperature (K)</i>			X_N	X_S		
278			42.1	57.9		
288			40.4	59.6		
298			38.6	61.4		
308			37.9	62.1		
318			36.3	63.7		
<i>RMSD</i>			0.11 Hz			

Table S6. Geometrical and thermodynamic parameters of MepT_{LS} calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

	<i>MepT_{LS}-HB</i>	<i>MepT_{LS}-no-HB</i>
<i>P</i>	144.82	153.32
<i>v</i> _{max}	24.30	29.37
<i>v</i> ₀	-20.29	-21.53
<i>v</i> ₁	24.60	29.51
<i>v</i> ₂	-19.86	-26.24
<i>v</i> ₃	9.01	14.81
<i>v</i> ₄	7.20	4.36
ΔH° (kJ/mol)	0.00	6.45
ΔG° (kJ/mol)	0.00	5.41
ΔS° (J/mol.K)	0.00	3.50
<i>F</i>	0.90	0.10

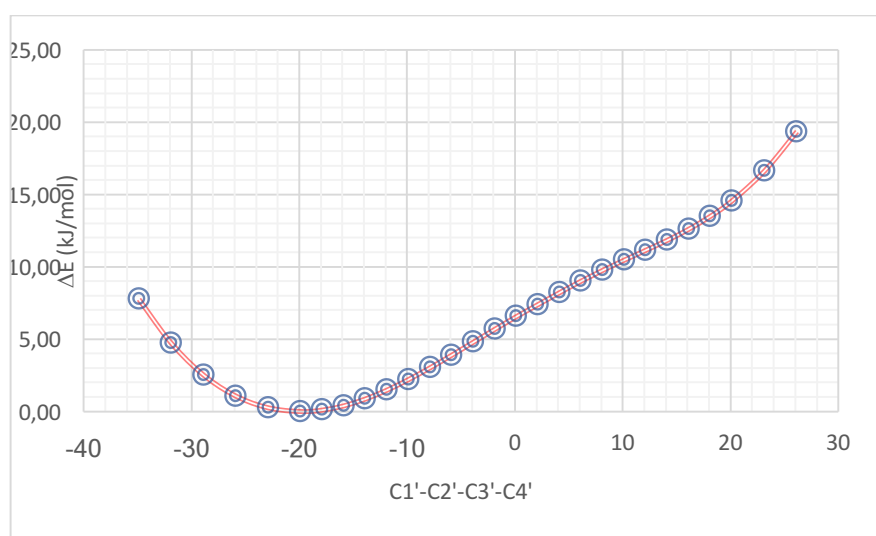


Figure S32. PES along C1'-C2'-C3'-C4' endocyclic torsion angle (*v*₂) of MepT_{LS} calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

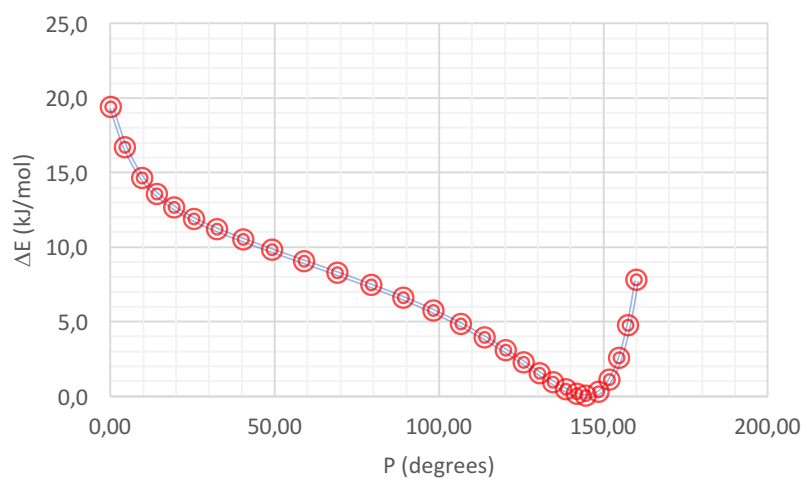


Figure S33. Relation between P and relative energy of **MepT_{LS}** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

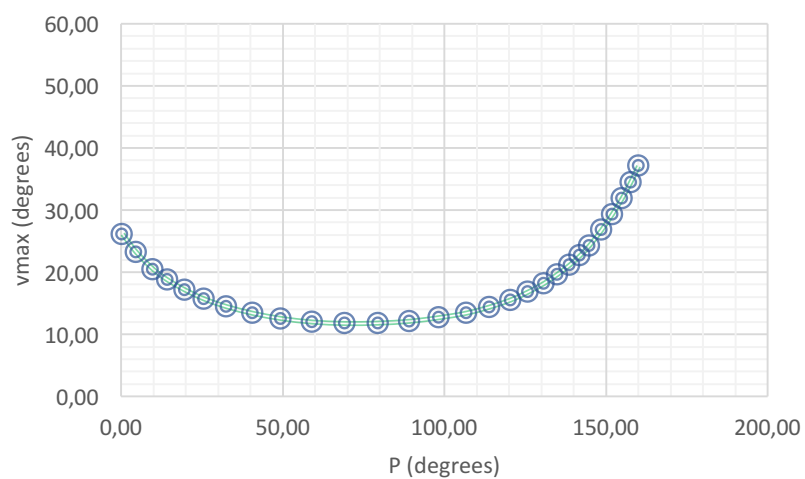
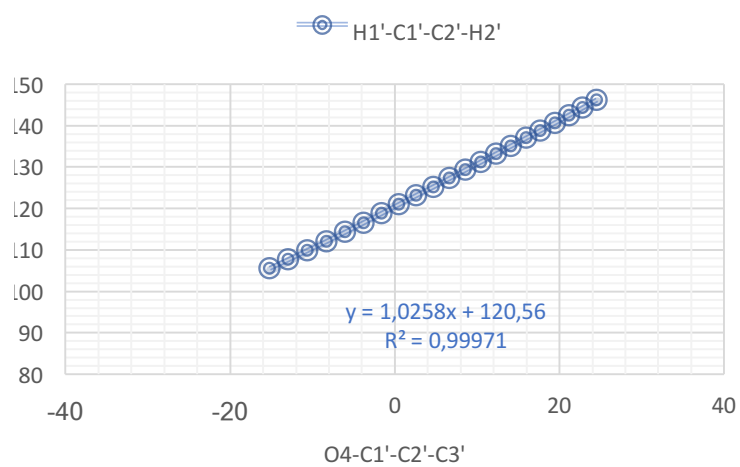
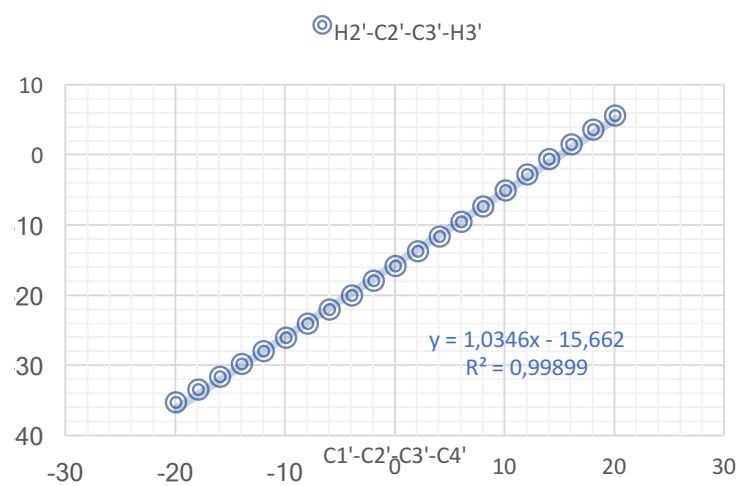


Figure S34. Relation between P and v_{\max} of **MepT_{LS}** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.



$$\phi (H1'-C1'-C2'-H2') = 1.0258 v_1 + 120.56$$



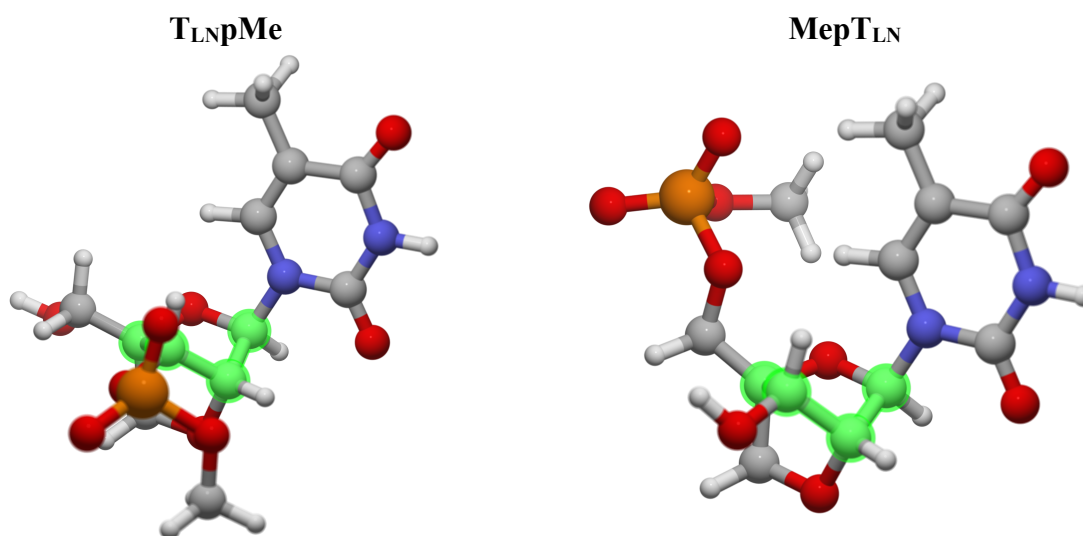
$$\phi (H2'-C2'-C3'-H3') = 1.0346 v_2 - 15.662$$

Figure S35. Linear correlations of H-C-C-H torsions and respective sugar torsions (v_1 and v_2), used to calculate A_j and B_j parameters for **MepT_{LS}**.

Table S7. Parameters used in Matlab Pseudorotation GUI for the pT_{LS} sugar moiety of T_{LNp}T_{LS} (5) and results in terms of puckering and population (X, %)

<i>Vicinal coupling constant</i>	<i>A</i>	<i>B</i>	<i>Electronegativities</i>			
$J_{1'2'}$	1.026	120.560	1.330	0.620	0.560	1.260
$J_{2'3'}$	1.035	-15.662	1.400	0.620	0.560	1.330
			<i>Conformer 1</i>	<i>Conformer 2</i>		
<i>P</i>			131.59	153.65		
v_{\max}			31.94	27.52		
v_0			-29.75	-19.61		
v_1			31.60	27.54		
v_2			-21.38	-24.95		
v_3			2.99	12.83		
v_4			16.54	4.19		
<i>Temperature (K)</i>			X_1	X_2		
278			45.7	54.3		
288			47.4	52.6		
298			49.3	50.7		
308			54.1	45.9		
318			56.0	44.0		
<i>RMSD</i>				0.02 Hz		

Table S8. Geometric and thermodynamic parameters of the N conformer of T_{LN}pMe and MepT_{LN} calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.



<i>P</i>	17.88	13.08
<i>v</i> _{max}	57.53	56.86
<i>v</i> ₀	0.57	5.08
<i>v</i> ₁	-36.91	-40.49
<i>v</i> ₂	54.75	55.39
<i>v</i> ₃	-55.62	-53.50
<i>v</i> ₄	36.21	30.68

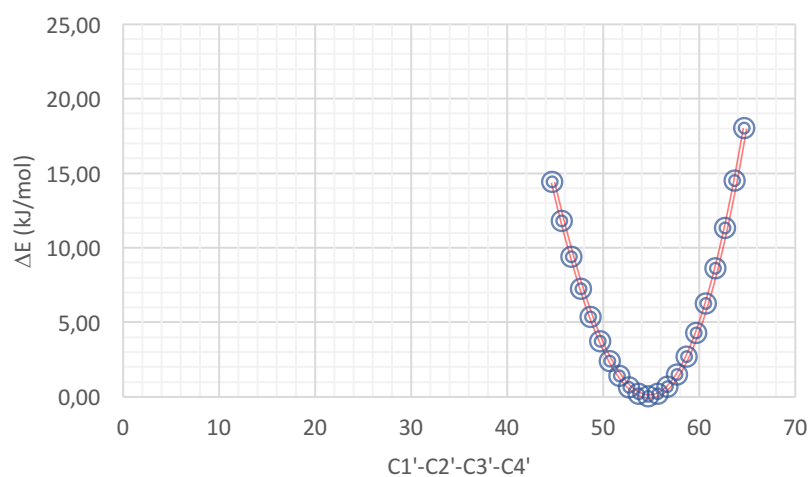


Figure S36. PES along C1'-C2'-C3'-C4' endocyclic torsion angle (*v*₂) of T_{LN}pMe calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

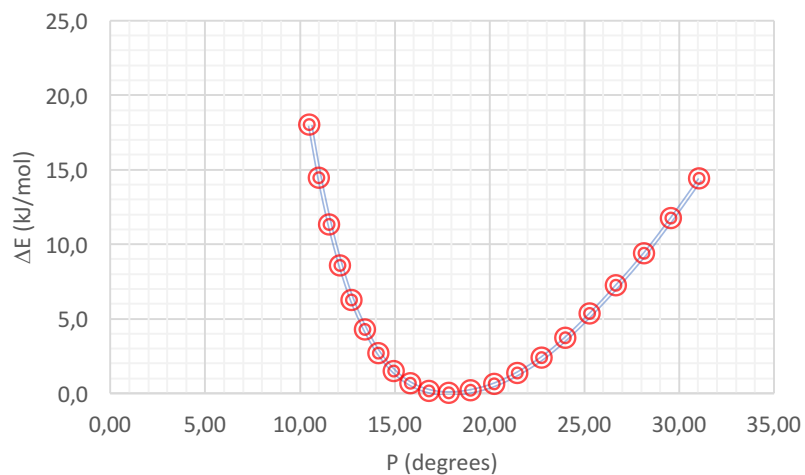


Figure S37. Relation between P and relative energy of $\mathbf{T}_{\text{LNpMe}}$ calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

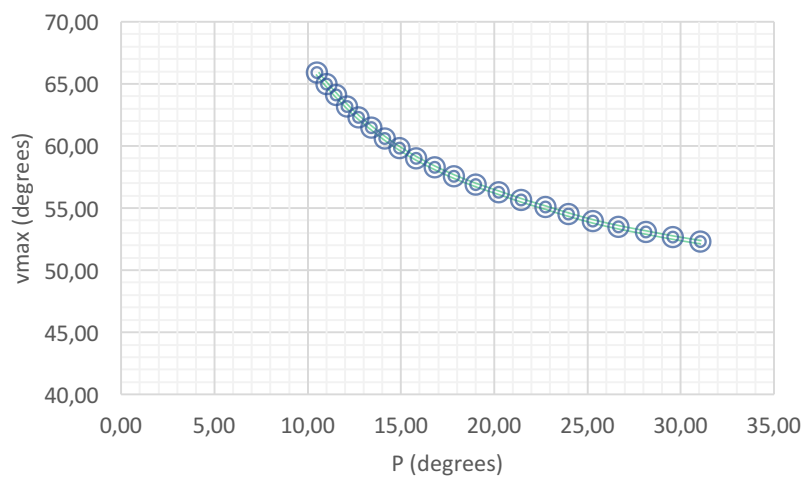


Figure S38. Relation between P and ν_{max} of $\mathbf{T}_{\text{LNpMe}}$ calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

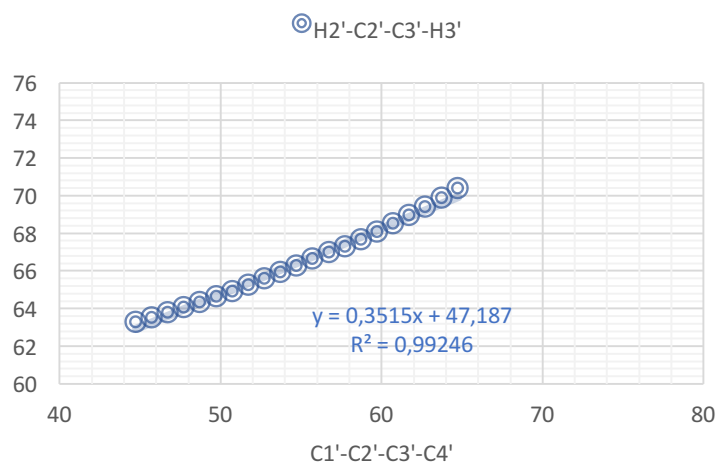
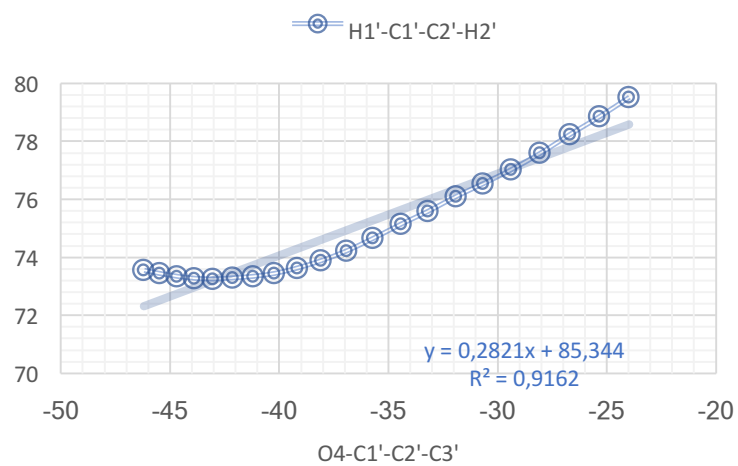


Figure S39. Determination of linear correlations of H-C-C-H torsions and respective sugar torsions (ν_1 and ν_2), used to calculate A_j and B_j parameters for **T_{LN}pMe**.

Table S9. Parameters used in Matlab Pseudorotation GUI for the T_{LNP} sugar moiety of $T_{LNP}T$ (3) and $T_{LNP}T_{LS}$ (5) and results in terms of puckering and population (X, %)^a

<i>Vicinal coupling constant</i>	<i>A</i>	<i>B</i>	<i>Electronegativities</i>			
$J_{1'2'}$	1.192	116.660	1.400	0.620	0.560	1.260
$J_{2'3'}$	1.184	0.182	1.250	0.620	0.560	1.400

	$T_{LNP}T$		$T_{LNP}T_{LS}$	
	<i>Conformer 1</i>	<i>Conformer 2</i>	<i>Conformer 1</i>	<i>Conformer 2</i>
<i>P</i>	16.91	15.85	19.47	18.92
v_{max}	58.35	55.87	59.80	58.63
v_0	1.42	2.41	-1.25	-0.66
v_1	-35.92	-35.22	-34.63	-34.42
v_2	56.71	54.58	57.28	56.34
v_3	-55.83	-53.10	-58.05	-56.75
v_4	33.63	31.33	36.65	35.48
<i>Temperature (K)</i>	X_1	X_2	X_1	X_2
298 ^b	10.20	89.80	8.32	91.68
<i>RMSD</i>	0.04 Hz		0.02 Hz	

^aDue to absence of linear correlation between endocyclic and exocyclic angles, A_j and B_j parameters were taken from TpMe nucleotide.

^bMatlab GUI analysis were performed at 298 K since no significative thermal variation of J -coupling occurred for the T_{LN} sugar residue.

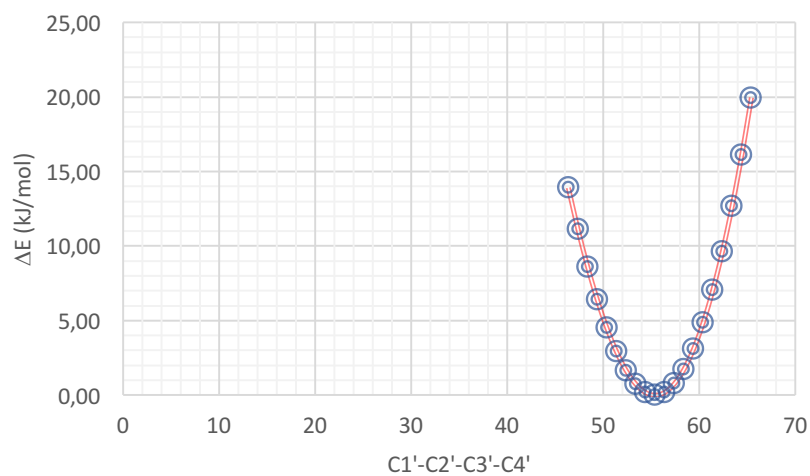


Figure S40. PES along $C1'-C2'-C3'-C4'$ endocyclic torsion angle (ν_2) of **MepT_{LN}** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

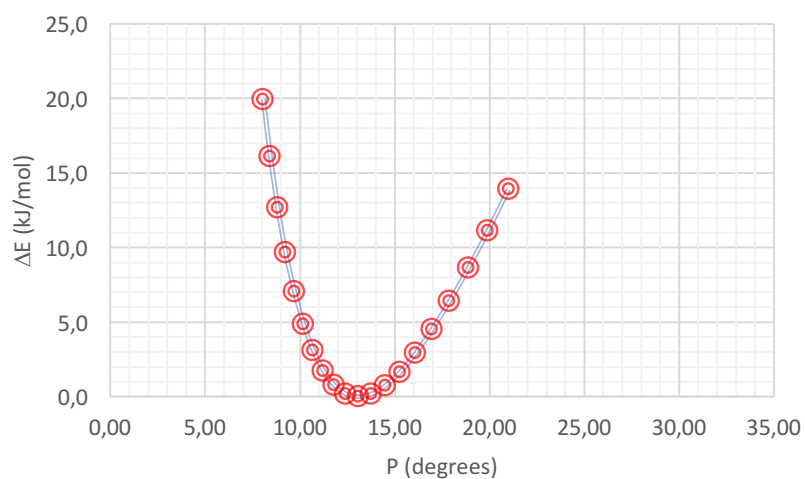


Figure S41. Relation between P and relative energy of **MepT_{LN}** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

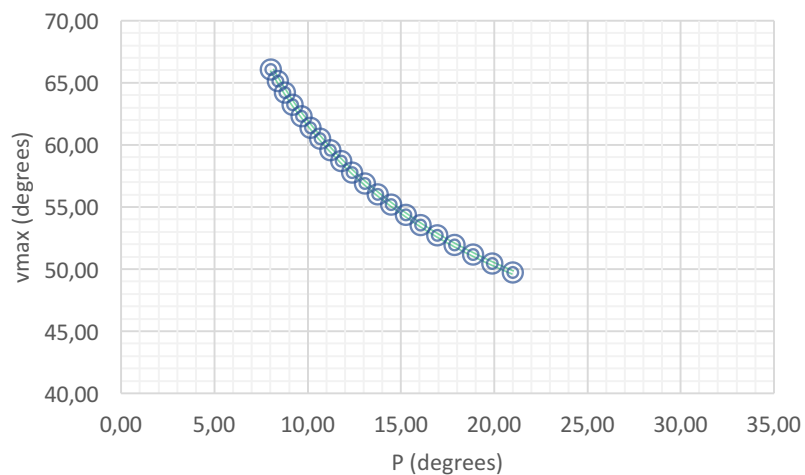


Figure S42. Relation between P and v_{\max} of **MepT_{LN}** calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory.

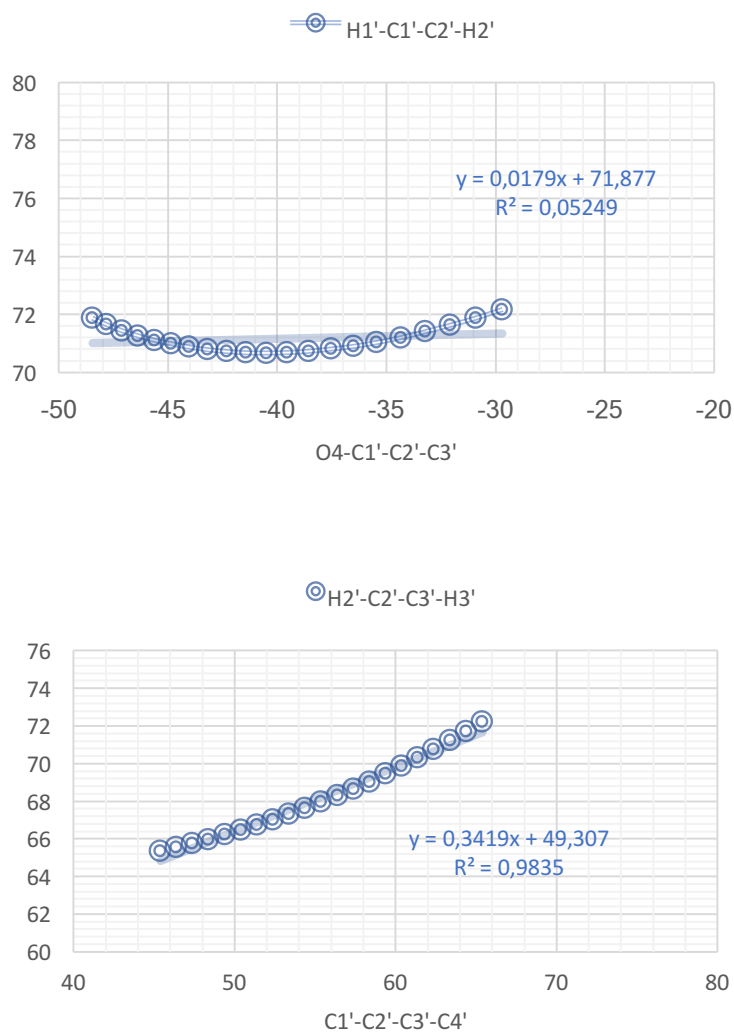


Figure S43. Determination of linear correlations of H-C-C-H torsions and respective sugar torsions (v_1 and v_2), used to determine A_j and B_j parameters for **MepT_{LN}**.

Table S10. Parameters used in Matlab Pseudorotation GUI for the pT_{LN} sugar moiety of TpT_{LN} (4) and results in terms of puckering and population (X, %)^a

<i>Vicinal coupling constant</i>	<i>A</i>	<i>B</i>	<i>Electronegativities</i>			
$J_{1'2'}$	1.179	116.920	1.400	0.620	0.560	1.260
$J_{2'3'}$	1.156	1.351	1.330	0.620	0.560	1.400
			<i>Conformer 1</i>	<i>Conformer 2</i>		
<i>P</i>			18.69	17.96		
ν_{\max}			59.64	58.45		
ν_0			-0.43	0.34		
ν_1			-35.20	-35.12		
ν_2			57.39	56.48		
ν_3			-57.65	-56.27		
ν_4			35.90	34.57		
<i>Temperature (K)</i>			X_1	X_2		
298 ^b			11.29	88.71		
<i>RMSD</i>			0.01 Hz			

^aDue to absence of linear correlation between endocyclic and exocyclic angles A_j and B_j parameters were taken from MepT nucleotide.

^bMatlab GUI analysis were performed at 298 K since nonspecific thermal variation of J -coupling occurred for the T_{LN} sugar residue.

3. 3. Cartesian coordinates of minima calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory

N conformer of TpMe

O	4.995390000	-0.957080000	1.559129000
H	4.810510000	1.935112000	1.413074000
H	3.955368000	-2.628210000	0.069513000
C	4.016985000	-0.729165000	0.865805000
H	3.763201000	1.390222000	2.718543000
C	3.780995000	1.668100000	1.662370000
N	3.496135000	-1.724907000	0.042444000
C	3.302284000	0.535158000	0.806834000
H	3.148341000	2.544646000	1.520986000
C	2.399682000	-1.642259000	-0.787356000
C	2.242571000	0.632637000	-0.022262000
O	1.997103000	-2.591648000	-1.437700000
N	1.800289000	-0.403889000	-0.813424000
H	1.674761000	1.551425000	-0.116381000
O	0.410503000	1.082631000	-1.991894000
C	0.596433000	-0.274714000	-1.677230000
H	0.816425000	-0.842403000	-2.578665000
H	-0.408274000	3.593339000	-1.922261000
H	0.306998000	4.030115000	0.225031000
O	0.186796000	3.101534000	0.007525000
C	-0.736329000	2.996151000	-1.066070000
C	-0.847389000	1.549873000	-1.481993000
C	-0.672006000	-0.745925000	-0.964438000
H	-1.607309000	1.473428000	-2.268868000
H	-1.388974000	-1.121102000	-1.699080000
H	-1.733169000	3.334480000	-0.763326000
H	-3.861852000	-1.436105000	-1.415652000
C	-1.200020000	0.561681000	-0.374554000
C	-4.049589000	-2.096916000	-0.564586000
H	-0.669393000	0.817565000	0.547624000
O	-3.194544000	-1.767842000	0.526963000
O	-2.593590000	0.603034000	-0.151328000
H	-5.097512000	-2.014102000	-0.269986000
P	-3.232885000	-0.243253000	1.110964000
O	-2.277462000	-0.214865000	2.260316000
O	-4.648137000	0.224619000	1.223633000
H	-3.826838000	-3.123727000	-0.849676000
H	-0.474148000	-1.525112000	-0.230748000

SCF Done: E(RM062X) = -1481.65953382

of imaginary frequency = 0

S conformer of TpMe

O	-5.605023000	-1.144104000	-0.461374000
H	-5.516901000	1.734778000	-0.307650000
H	-3.961184000	-2.767982000	0.398181000
C	-4.435715000	-0.872709000	-0.247627000
H	-5.102933000	1.281861000	-1.957095000
C	-4.681120000	1.542824000	-0.984015000
N	-3.569593000	-1.846072000	0.242537000
C	-3.823384000	0.430999000	-0.463329000
H	-4.098524000	2.457857000	-1.089416000
C	-2.227868000	-1.719833000	0.534717000
C	-2.515012000	0.567879000	-0.179023000
O	-1.567841000	-2.653614000	0.951274000
N	-1.724131000	-0.457062000	0.303104000
H	-1.990996000	1.511164000	-0.299964000
O	-0.260484000	0.947798000	1.449140000
C	-0.318172000	-0.181877000	0.596653000
H	0.063556000	-1.063560000	1.109446000
H	-0.251561000	3.553228000	1.549997000
H	-0.685594000	3.957522000	-0.711724000
O	-0.380506000	3.078391000	-0.470006000
C	0.368380000	3.165941000	0.735447000
C	0.856850000	1.782092000	1.107125000
C	0.515329000	0.176139000	-0.630132000
H	1.509937000	1.862552000	1.978784000
H	0.915296000	-0.699892000	-1.137930000
H	1.236113000	3.821921000	0.609500000
H	2.051297000	-2.389082000	0.340766000
C	1.602622000	1.051397000	-0.017586000
C	2.804878000	-2.665310000	-0.403289000
H	2.052192000	1.742383000	-0.732079000
O	3.243392000	-1.518250000	-1.126220000
O	2.616063000	0.255527000	0.594278000
H	3.644531000	-3.147122000	0.100956000
P	3.900269000	-0.276665000	-0.286992000
O	4.268051000	0.757977000	-1.301892000
O	4.876673000	-0.806825000	0.713632000
H	2.363606000	-3.352064000	-1.123633000
H	-0.080113000	0.767907000	-1.327051000

SCF Done: E(RM062X) = -1481.66115293

of imaginary frequency = 0

N conformer of MepT

O	3.949978000	1.416906000	0.866506000
H	1.866658000	2.245705000	1.514657000
C	1.251509000	1.828807000	0.720975000
C	3.267338000	0.485168000	0.476833000
H	4.819205000	-0.798840000	0.221065000
H	-1.278026000	3.190016000	0.904951000
C	1.086754000	2.786136000	-0.462899000
O	-0.041911000	1.550227000	1.203730000
H	1.835943000	2.618931000	-1.235582000
N	3.812480000	-0.729727000	0.124977000
N	1.898158000	0.549374000	0.348018000
C	-1.032312000	2.233377000	0.427184000
C	-0.352690000	2.524560000	-0.908104000
H	-2.630874000	1.168422000	1.370722000
C	3.150420000	-1.847187000	-0.377741000
C	-2.284412000	1.393066000	0.356524000
C	1.167233000	-0.502583000	-0.165408000
H	-0.414358000	1.641691000	-1.551986000
O	3.778385000	-2.856175000	-0.654303000
H	-3.064574000	1.955578000	-0.164957000
H	0.099532000	-0.323039000	-0.241471000
C	1.713315000	-1.680461000	-0.530171000
O	-1.991494000	0.191820000	-0.344717000
O	-2.801630000	-1.449358000	1.402322000
C	0.912246000	-2.823351000	-1.074422000
P	-3.007983000	-1.091100000	-0.179074000
H	1.032017000	-3.707802000	-0.442807000
H	1.260436000	-3.093862000	-2.074566000
H	-0.147132000	-2.563168000	-1.121687000
O	-4.426674000	-0.641379000	-0.319006000
O	-2.418912000	-2.165094000	-1.037881000
C	-1.483386000	-1.749209000	1.859832000
H	-1.002568000	-2.480638000	1.204010000
H	-0.879288000	-0.837920000	1.895094000
H	-1.575914000	-2.164904000	2.861707000
H	1.158778000	3.817431000	-0.109860000
O	-0.996879000	3.626097000	-1.511646000
H	-0.717581000	3.687075000	-2.429813000

SCF Done: E(RM062X) = -1481.66397840

of imaginary frequency = 0

S conformer of MepT

H	-1.801855000	2.882404000	1.390385000
H	-2.506324000	0.541570000	1.662225000
H	-3.461724000	1.353167000	0.399009000
C	-1.475658000	2.132160000	0.665215000
C	-2.464655000	0.986108000	0.662815000
O	-0.186330000	1.638842000	1.052568000
O	3.475222000	2.068229000	0.667557000
H	1.364848000	2.771488000	0.299159000
C	-1.285394000	2.806408000	-0.699203000
O	-2.387493000	-1.929298000	1.283151000
C	0.769665000	1.898976000	0.036021000
O	-2.019930000	0.025041000	-0.284319000
H	-2.154641000	2.660706000	-1.344846000
C	3.022840000	0.992351000	0.323631000
O	-4.141241000	-1.432644000	-0.427353000
H	4.786477000	0.003529000	0.486611000
N	1.703198000	0.779480000	-0.028446000
P	-2.656274000	-1.489983000	-0.268254000
N	3.812096000	-0.134484000	0.243699000
C	-0.030206000	2.134553000	-1.242630000
H	-0.296459000	1.190586000	-1.717955000
C	1.240163000	-0.478010000	-0.376547000
C	3.434260000	-1.423263000	-0.122395000
H	0.174686000	-0.529250000	-0.578465000
O	4.260865000	-2.319166000	-0.146767000
O	-1.779871000	-2.274798000	-1.194375000
C	2.022689000	-1.571358000	-0.447586000
H	1.695900000	-3.649824000	-0.044711000
C	1.516885000	-2.924701000	-0.843225000
H	0.447394000	-2.878718000	-1.055276000
H	2.046603000	-3.284744000	-1.728693000
H	0.510287000	2.759119000	-1.953858000
C	-1.041875000	-1.959349000	1.756425000
H	-1.066629000	-2.354350000	2.770491000
H	-0.425873000	-2.604934000	1.125825000
H	-0.616106000	-0.950523000	1.766604000
O	-1.072189000	4.189733000	-0.444556000
H	-0.904076000	4.633765000	-1.281846000

SCF Done: E(RM062X) = -1481.66614322

of imaginary frequency = 0

N conformer of T_{LN}pMe

H	-0.870335000	1.329280000	2.301636000
O	-2.010618000	-0.869308000	2.614680000
C	-0.629907000	1.025864000	1.283210000
O	-0.269336000	2.141515000	0.493335000
C	-2.429234000	-0.576141000	1.509647000
C	0.621262000	0.140734000	1.231560000
H	0.496214000	-0.879664000	1.580422000
N	-1.828242000	0.374069000	0.711528000
N	-3.538687000	-1.167378000	0.950468000
H	-1.778813000	1.474581000	-1.047012000
C	-2.324669000	0.693792000	-0.534543000
H	-3.990643000	-1.873576000	1.520503000
C	-3.406279000	0.103669000	-1.078248000
C	1.076394000	1.905020000	0.016442000
C	-4.094352000	-0.918824000	-0.302419000
H	-4.977035000	0.799251000	-2.358436000
C	1.057612000	0.388570000	-0.218785000
O	2.336189000	-0.084729000	-0.537106000
H	-3.342679000	1.229272000	-2.897048000
O	-5.081035000	-1.533531000	-0.668508000
C	-3.945085000	0.449308000	-2.432079000
H	0.317797000	0.081656000	-0.962320000
O	1.566096000	4.113450000	-0.675509000
C	1.400573000	2.787599000	-1.150893000
H	0.588958000	2.724585000	-1.883630000
H	-3.944085000	-0.428565000	-3.081777000
H	1.786706000	4.680621000	-1.419663000
O	2.130938000	-2.476117000	0.284586000
P	2.499236000	-1.644488000	-1.069430000
H	2.321227000	2.410488000	-1.608405000
O	3.948152000	-1.791540000	-1.398548000
O	1.410023000	-1.939533000	-2.048541000
O	1.613880000	0.817556000	1.996742000
C	1.991104000	1.988089000	1.236925000
H	3.045880000	1.922187000	0.964888000
H	1.797377000	2.887334000	1.820842000
C	2.993749000	-2.351029000	1.415833000
H	2.566326000	-2.958735000	2.211634000
H	3.995433000	-2.711943000	1.175029000
H	3.046340000	-1.308321000	1.741875000

SCF Done: E(RM062X) = -1594.96324516

of imaginary frequency = 0

N conformer of MepT_{LN}

H	-2.740341000	1.137270000	1.040075000
O	-3.033007000	-1.452077000	1.400417000
H	1.741726000	1.893575000	1.652333000
O	3.923796000	1.288430000	0.680044000
O	-0.120720000	1.193133000	1.086756000
C	-2.280920000	1.205058000	0.048138000
H	4.902531000	-0.899040000	0.057186000
C	-0.953560000	1.902643000	0.142265000
C	3.290129000	0.290129000	0.387696000
C	1.221625000	1.497709000	0.781408000
H	-2.952713000	1.757484000	-0.615842000
O	-4.460070000	-0.864985000	-0.566684000
N	3.888761000	-0.901964000	0.048552000
P	-3.076660000	-1.316948000	-0.226694000
N	1.913371000	0.258460000	0.366714000
O	-2.010215000	-0.088044000	-0.474356000
C	3.266325000	-2.096830000	-0.310923000
O	3.937887000	-3.075719000	-0.588385000
C	1.084735000	2.543650000	-0.333092000
C	1.216064000	-0.882655000	0.017410000
C	-0.065373000	1.933435000	-1.121935000
O	-0.522384000	2.797708000	-2.125766000
C	1.811466000	-2.042576000	-0.320080000
H	0.136777000	-0.779639000	0.027982000
O	-2.436735000	-2.508112000	-0.866613000
H	1.261647000	-4.089110000	-0.002995000
H	0.144928000	0.933654000	-1.502366000
C	1.041143000	-3.272122000	-0.694626000
H	-1.151722000	2.325653000	-2.680089000
H	-0.032018000	-3.069379000	-0.678072000
H	1.321504000	-3.610783000	-1.694921000
C	-0.885161000	3.369309000	0.551337000
H	-1.534908000	4.006214000	-0.050346000
H	-1.086567000	3.501065000	1.614417000
H	2.000499000	2.811422000	-0.851734000
O	0.499048000	3.694849000	0.277657000
C	-1.789342000	-1.775440000	2.022329000
H	-2.005415000	-2.043722000	3.055004000
H	-1.313720000	-2.620581000	1.517402000
H	-1.120808000	-0.910667000	2.001380000

SCF Done: E(RM062X) = -1594.96671474

of imaginary frequency = 0

S conformer of MepT_{LS} -HB

H	-2.651607000	0.382001000	1.507878000
O	-2.438669000	-2.138123000	1.257397000
H	-3.464136000	1.189475000	0.140684000
C	-2.516471000	0.773266000	0.493572000
C	-1.444456000	1.827347000	0.482659000
O	-0.241330000	1.258655000	0.989731000
O	3.563703000	1.702487000	0.976309000
H	1.425441000	2.466886000	0.710343000
O	-2.057673000	-0.245730000	-0.383428000
P	-2.721317000	-1.749982000	-0.303955000
C	0.875851000	1.659312000	0.224417000
C	3.093006000	0.666668000	0.546924000
O	-4.206803000	-1.661635000	-0.442633000
C	-1.087079000	2.528793000	-0.833885000
H	4.808573000	-0.403823000	0.714320000
N	1.783056000	0.524996000	0.132635000
N	3.840291000	-0.484466000	0.425299000
H	-1.786884000	2.445059000	-1.664833000
O	-1.875618000	-2.584029000	-1.214072000
C	0.339828000	2.128197000	-1.149118000
C	1.287498000	-0.682399000	-0.333158000
H	0.229043000	-0.670991000	-0.580682000
C	3.430359000	-1.728823000	-0.048404000
C	2.029699000	-1.798209000	-0.446851000
O	4.221748000	-2.653763000	-0.100272000
H	1.637323000	-3.888448000	-0.211095000
C	1.488023000	-3.099441000	-0.952879000
H	0.421436000	-3.007022000	-1.166361000
H	2.014811000	-3.406402000	-1.859832000
H	0.343324000	1.295451000	-1.855672000
C	-1.706204000	3.244321000	1.007128000
H	-2.761412000	3.505342000	1.106289000
H	-1.146528000	3.522434000	1.899996000
O	-1.140405000	3.822906000	-0.198704000
O	1.111484000	3.158569000	-1.706198000
H	0.911051000	3.966675000	-1.215046000
C	-1.086107000	-2.196274000	1.709243000
H	-0.494098000	-2.855514000	1.069446000
H	-0.642020000	-1.196200000	1.711493000
H	-1.103160000	-2.590146000	2.723932000

SCF Done: E(RM062X) = -1594.95271560

of imaginary frequency = 0

S conformer of MepT_{LS} -no-HB

P	-2.686240000	-1.769970000	-0.330433000
O	-4.169454000	-1.710980000	-0.504160000
O	-1.802163000	-2.580489000	-1.225471000
O	-2.050251000	-0.252935000	-0.384511000
C	-2.531007000	0.746490000	0.503891000
C	-1.481226000	1.822370000	0.509092000
O	-0.260005000	1.269338000	0.998231000
C	-1.134062000	2.566477000	-0.789171000
O	-1.144850000	3.829760000	-0.097099000
C	0.244064000	2.079877000	-1.168060000
C	0.832648000	1.671515000	0.196768000
N	1.766487000	0.557727000	0.117983000
C	3.074521000	0.738285000	0.526735000
O	3.523052000	1.794220000	0.929496000
N	3.847746000	-0.397855000	0.430387000
C	3.464717000	-1.663339000	-0.008488000
O	4.276770000	-2.571064000	-0.039194000
C	2.064540000	-1.775049000	-0.398436000
C	1.552274000	-3.101298000	-0.868907000
C	1.296776000	-0.674241000	-0.310417000
H	4.814502000	-0.287831000	0.714837000
O	-2.432759000	-2.164225000	1.234496000
H	-3.486850000	1.147064000	0.154813000
H	-2.659824000	0.339622000	1.512887000
H	-1.864595000	2.534088000	-1.598123000
H	0.117866000	1.189891000	-1.795058000
H	1.369843000	2.512362000	0.636002000
H	0.486814000	-3.034472000	-1.096939000
H	2.094449000	-3.425400000	-1.760665000
H	1.708202000	-3.864585000	-0.101954000
H	0.237589000	-0.695188000	-0.553309000
C	-1.090301000	-2.199973000	1.717485000
H	-1.124793000	-2.589747000	2.733305000
H	-0.474177000	-2.853100000	1.094475000
H	-0.660600000	-1.193528000	1.725280000
C	-1.753849000	3.221531000	1.068679000
H	-1.226181000	3.470291000	1.989365000
H	-2.812966000	3.479245000	1.139664000
O	0.998676000	3.068182000	-1.819613000
H	1.727995000	2.651397000	-2.289095000

SCF Done: E(RM062X) = -1594.95004387

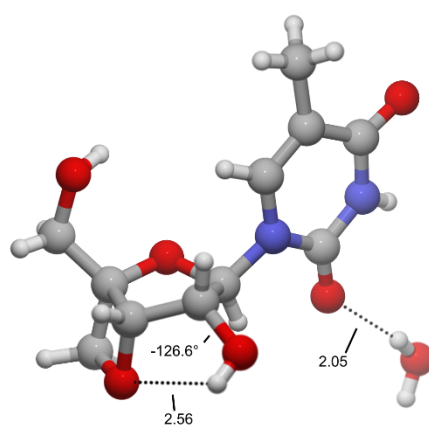
of imaginary frequency = 0

3. 4. Solid and solution state conformation data of the S-locked sugar motif

The geometric parameters of the X-ray structure of **7** and of the conformers of the 3'-sugar residue of $T_{LNP}T_{LS}$ (**5**) are reported Table S11.

Table S11. Conformational parameters of the X-ray structure of **7,^{8,9} of the two Matlab pseudorotation GUI-derived conformers of the pT_{LS} sugar residue of **5**, exocyclic torsion angles H1'-C1'-C2'-H2' and H-2'-C2'-C3'-H3' and the calculated corresponding $J_{1'2'}$ and $J_{2'3'}$ (Hz)**

	<i>X-ray crystal structure of 7</i> ^{3,11}	<i>pT_{LS} residue of 5^a</i>	
		<i>Matlab GUI results</i>	
		Conformer 1	Conformer 2
<i>P</i>	136.18	131.59	153.65
<i>v</i> _{max}	32.51	31.94	27.52
<i>v</i> ₀	-29.63	-29.75	-19.61
<i>v</i> ₁	32.42	31.60	27.54
<i>v</i> ₂	-23.45	-21.38	-24.95
<i>v</i> ₃	7.05	2.99	12.83
<i>v</i> ₄	14.22	16.54	4.19
H1'-C1'-C2'-H2'	155.2	152.98	148.81
H2'-C2'-C3'-H3'	-40.7	-37.78	-41.47
<i>J</i> _{1'2'}	8.01 ^b	7.54 ^c	6.89 ^c
<i>J</i> _{2'3'}	4.45 ^b	4.81 ^c	4.46 ^c



^aExperimental $J_{1'2'}$ and $J_{2'3'}$ (D₂O, 298 K) are 7.21 and 4.63 Hz, respectively.

^bCalculated with the MestReJ software using the Diez-Altona-Donders equation¹²

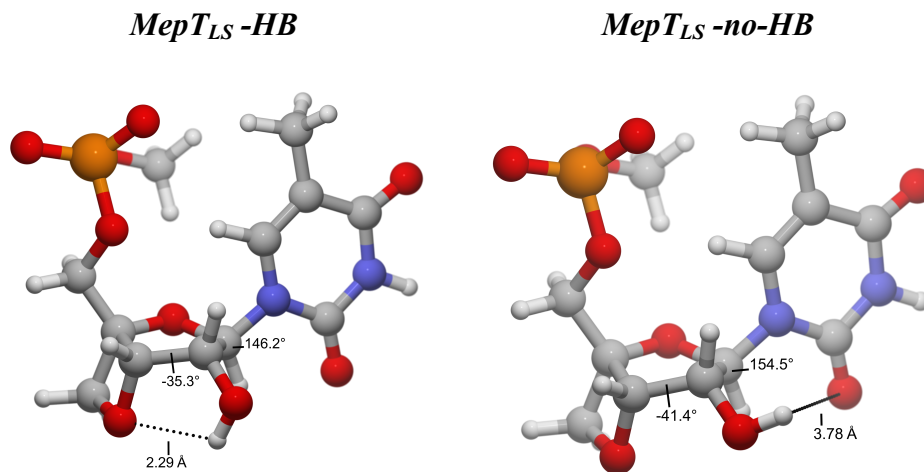
^cDetermined from the MatLab GUI software

3. 5. DFT study using hybrid functional M06-2X of the MepT_{LS} model

During the exploration of the conformational space of the MepT_{LS} model through potential energy scan through the C1'-C2'-C3'-C4' (ν_2) torsion angle, two minima (**MepT_{LS} -HB** and **MepT_{LS} -no-HB**) were located (Table S6). The lowest-in-energy conformer features an intramolecular H-bond between O3' and the proton of the hydroxyl in the 2' position, the other differs by the absence of intramolecular H-bond. Although these models were useful for *Aj* and *Bj* parameters determination, these preliminary DFT results were troublesome since the difference in terms of Gibbs energy between these conformers yielded a Boltzmann population dramatically in favor of **MepT_{LS} -HB** (Table S6). In addition, the calculated coupling constants of these two conformers using MestReJ software weighted by their Boltzmann population failed to fit the experimental ones (Table S12).

As NMR experiments were conducted in deuterated water, we envisioned the hypothesis that MepT_{LS} could exist under the form of a complex with water molecules like in the crystal structure of **7** (CCDC 130640). Using one water molecule forming a H-bond with the proton of the 2' hydroxyl group, we have generated an ensemble of four water-complexed conformers. Exploration of the energy surface was conducted by the scan of the C1'-C2'-O2'-H' torsion angle from -180° to 180°. From these calculations, two minima **MepT_{LS} -HB-H₂O** and **MepT_{LS} -no-HB-H₂O**, close in term of Gibbs energy, were obtained (Table S13). The Boltzmann population and geometric parameters of these conformers gave results close to those obtained by Matlab pseudorotation GUI analysis.

Table S12. Conformational parameters (°) of the most stable conformers of MepT_{LS} at 298 K calculated by DFT, Boltzmann population (F), Gibbs energy, exocyclic torsion angles H1'-C1'-C2'-H2' and H-2'-C2'-C3'-H3' (°), and corresponding calculated $J_{1'2'}$ and $J_{2'3'}$ (Hz)

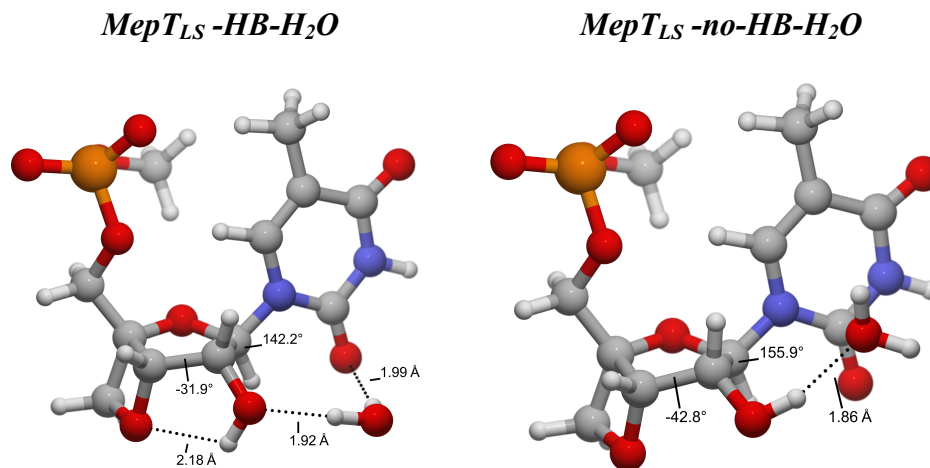


P	144.82	153.32
v_{\max}	24.30	29.37
v_0	-20.29	-21.53
v_1	24.60	29.51
v_2	-19.86	-26.24
v_3	9.01	14.81
v_4	7.20	4.36
F	0.90	0.10
H1'-C1'-C2'- H2'	146.20	154.48
$J_{1'2'}^a$	6.99	8.12
H2'-C2'-C3'- H3'	-35.33	-41.43
$J_{2'3'}^a$	5.15	4.40
$J_{1'2'}w^b$	7.10 ($J_{1'2'}$ pT _{LS} of 5 = 7.21)	
$J_{2'3'}w^b$	5.07 ($J_{2'3'}$ pT _{LS} of 5 = 4.63)	

^aCalculated with the MestReJ software using the Diez-Altona-Donders equation¹²

^b J weighted (w) at 298 K using $J_w = (J_{\text{Conf1}} \cdot X_1) + (J_{\text{Conf2}} \cdot X_2)$ equation with $X_1 + X_2 = 1$

Table S13. Conformational parameters (°) of the two most stable conformers of MepT_{LS} (298 K) hydrogen bonded to a water molecule calculated by DFT, Boltzmann population (*F*), Gibbs energy, exocyclic torsion angles H1'-C1'-C2'-H2' and H-2'-C2'-C3'-H3' (°), corresponding calculated *J*_{1'2'} and *J*_{2'3'} (Hz) and population (*X*, %)



<i>P</i>	141.63	152.94
<i>v</i> _{max}	21.79	29.95
<i>v</i> ₀	-18.91	-22.09
<i>v</i> ₁	22.00	30.18
<i>v</i> ₂	-17.09	-26.67
<i>v</i> ₃	6.93	14.95
<i>v</i> ₄	7.65	4.62
ΔH° (kJ/mol)	-0.46	0
ΔG° (kJ/mol)	0.51	0
ΔS° (J/mol.K)	-3.27	0
<i>F</i>	0.44	0.55
H1'-C1'-C2'- H2'	142.23	155.92
<i>J</i> _{1'2'} ^a	6.39	8.30
H2'-C2'-C3'- H3'	-31.94	-42.83
<i>J</i> _{2'3'} ^a	5.56	4.22
<i>J</i> _{1'2'} <i>w</i> ^b	7.46 (<i>J</i> _{1'2'} pT _{LS} of 5 = 7.21)	
<i>J</i> _{2'3'} <i>w</i> ^b	4.80 (<i>J</i> _{2'3'} pT _{LS} of 5 = 4.63)	

^aCalculated with MestReJ software using the Diez-Altona-Donders equation¹²

^b*J* weighted (*w*) at 298 K using $J_w = (J_{\text{Conf1}} \cdot X_1) + (J_{\text{Conf2}} \cdot X_2)$ equation with $X_1 + X_2 = 1$

Cartesian coordinates of the two minima of MepT_{LS} hydrogen bonded to a water molecule calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory

MepT_{LS}-HB-H₂O

H	-2.629311000	1.177871000	1.480260000
O	-3.360261000	-1.230332000	1.101812000
H	-3.030767000	2.264690000	0.124506000
C	-2.313698000	1.524440000	0.490033000
C	-0.936370000	2.122285000	0.565512000
O	-0.043210000	1.148700000	1.094733000
O	3.651197000	0.147168000	1.249107000
H	1.964088000	1.663993000	0.958095000
O	-2.209264000	0.436637000	-0.416763000
P	-3.391480000	-0.709872000	-0.446095000
C	1.184892000	1.141527000	0.400391000
C	2.844519000	-0.627293000	0.755847000
O	-4.724680000	-0.062734000	-0.637320000
C	-0.290641000	2.682145000	-0.707876000
H	4.020419000	-2.263824000	0.959759000
N	1.611624000	-0.246873000	0.275123000
N	3.111123000	-1.967088000	0.622987000
H	-0.924636000	2.846569000	-1.578137000
O	-2.868975000	-1.766995000	-1.366762000
C	0.945597000	1.839782000	-0.962302000
C	0.726476000	-1.164065000	-0.274271000
H	-0.232064000	-0.745572000	-0.570288000
C	2.295381000	-2.951753000	0.065538000
C	1.001266000	-2.473519000	-0.406821000
O	2.683218000	-4.103822000	0.007700000
H	-0.152878000	-4.270608000	-0.300868000
C	0.047835000	-3.460097000	-1.006364000
H	-0.892609000	-2.969679000	-1.264558000
H	0.481101000	-3.912664000	-1.901738000
H	0.752360000	1.098973000	-1.740116000
C	-0.710465000	3.528071000	1.136533000
H	-1.612231000	4.135695000	1.226406000
H	-0.115484000	3.570032000	2.048633000
O	0.055634000	3.910525000	-0.037281000
O	2.050781000	2.610543000	-1.371137000
H	2.014790000	3.445229000	-0.880788000
C	-2.150663000	-1.800515000	1.600502000
H	-1.810395000	-2.611124000	0.951279000
H	-1.369466000	-1.037155000	1.668920000
H	-2.365417000	-2.192346000	2.593097000
H	3.832218000	1.894469000	-1.298011000
O	4.695768000	1.505572000	-1.098185000
H	4.576808000	1.123100000	-0.219358000

SCF Done: E(RM062X) = -1671.39298928

of imaginary frequency = none

MepT_{LS} -no-HB-H₂O

H	2.971613000	0.622601000	-1.347953000
O	2.975455000	-1.903857000	-1.098483000
H	3.514362000	1.514849000	0.097617000
C	2.667335000	1.001486000	-0.365934000
C	1.500813000	1.938948000	-0.512966000
O	0.429608000	1.238376000	-1.143881000
O	-3.457273000	1.367954000	-1.309153000
H	-1.368147000	2.279624000	-1.011997000
O	2.209018000	-0.058891000	0.461929000
P	3.018259000	-1.491412000	0.481832000
C	-0.795996000	1.509337000	-0.494689000
C	-2.914894000	0.357268000	-0.902553000
O	4.458829000	-1.263378000	0.808532000
C	0.903309000	2.635717000	0.720387000
H	-4.556702000	-0.818803000	-1.091096000
N	-1.593515000	0.291675000	-0.512553000
N	-3.583468000	-0.843765000	-0.809388000
H	1.526101000	2.694101000	1.613839000
O	2.145885000	-2.405221000	1.284261000
C	-0.438895000	1.978088000	0.929467000
C	-1.045160000	-0.864286000	0.012051000
H	0.000359000	-0.778322000	0.296102000
C	-3.116152000	-2.042950000	-0.281396000
C	-1.723725000	-2.018556000	0.146812000
O	-3.855781000	-3.010523000	-0.211472000
H	-1.162296000	-4.078376000	-0.022632000
C	-1.117399000	-3.267334000	0.709262000
H	-0.075583000	-3.089822000	0.983247000
H	-1.671499000	-3.598767000	1.590931000
H	-0.267552000	1.094786000	1.559303000
C	1.668443000	3.361309000	-1.056015000
H	2.688860000	3.747425000	-1.000413000
H	1.233673000	3.542226000	-2.039164000
O	0.849534000	3.891444000	0.014699000
O	-1.399079000	2.827184000	1.489731000
H	-2.138870000	2.263444000	1.772619000
C	1.703717000	-2.095046000	-1.717483000
H	1.092057000	-2.789362000	-1.135284000
H	1.178996000	-1.139541000	-1.812306000
H	1.887032000	-2.510689000	-2.706750000
O	-3.181065000	0.742831000	2.002978000
H	-4.130788000	0.837480000	2.128983000
H	-2.906047000	0.071707000	2.636280000

SCF Done: E(RM062X) = -1671.39260101

of imaginary frequency = none

Table S14. Thermochemical data of the computed conformers calculated at the IEFPCM/GD3-M06-2X/6-311+G(d,p) level of theory

	<i>TpMe</i>		<i>MepT</i>		<i>T_{LNpMe}</i>	<i>MepT_{LN}</i>	<i>MepT_{LS}</i> <i>-HB</i>	<i>MepT_{LS}</i> <i>-noHB</i>	<i>MepT_{LS}</i> <i>-HB H₂O</i>	<i>MepT_{LS}</i> <i>-noHB H₂O</i>
	<i>North</i>	<i>South</i>	<i>North</i>	<i>South</i>						
Zero-point correction	0.294171	0.294468	0.294980	0.295371	0.305359	0.306095	0.305884	0.305410	0.330847	0.330083
Thermal correction to Energy	0.316003	0.316118	0.316246	0.316386	0.328104	0.328303	0.328191	0.327976	0.356090	0.355877
Thermal correction to Enthalpy	0.316947	0.317062	0.31719	0.317330	0.329048	0.329247	0.329135	0.328920	0.357035	0.356821
Thermal correction to Gibbs Free Energy	0.241279	0.242220	0.244397	0.245530	0.251709	0.254812	0.254251	0.253639	0.274975	0.274390
Sum of electronic and zero-point Energies	-1481.36536	-1481.36669	-1481.36900	-1481.37077	-1594.65789	-1594.66062	-1594.64683	-1594.64463	-1671.06214	-1671.06252
Sum of electronic and thermal Energies	-1481.34353	-1481.34504	-1481.34773	-1481.34976	-1594.63514	-1594.63841	-1594.62452	-1594.62207	-1671.03690	-1671.03672
Sum of electronic and thermal Enthalpies	-1481.34259	-1481.34409	-1481.34679	-1481.34881	-1594.63420	-1594.63747	-1594.62358	-1594.62112	-1671.03596	-1671.03578
Sum of electronic and thermal Free Energies	-1481.41825	-1481.41893	-1481.41958	-1481.42061	-1594.71154	-1594.71190	-1594.69847	-1594.69641	-1671.11802	-1671.11821

4. Photochemical studies

4.1. UV irradiation and HPLC conditions

Studies were conducted as previously described¹³ with an OD of 3 for the 1/1 mixture of TpT **1** and the locked dinucleotide. Each quartz cuvette containing the solution to be irradiated was placed on an ice-cooled aluminium tray. The water used was HPLC-grade (pH 6.2-6.8) and the HPLC buffer was at pH 6.8. An aliquot of the solution was sampled at $t = 0$, 1, 2, 4, 6, 8, 10 and 20 min and analysed by reversed-phase HPLC. Thirty μL of the irradiation mixture were injected on an XBridge C18 (5 μm , 4.6 x 250 mm) column using a 70 min, 1 mL/min gradient of 0-7% CH_3CN in 0.05 M aqueous ammonium acetate for **3** (Run 1) and using a 50 min, 1 mL/min gradient of 0-15% CH_3CN in 0.05 M aqueous ammonium acetate for **4** and **5** (Run 2). The detection was set at 230 nm. A photodiode array detector was used. Peak area was measured at 230 nm (and 320 nm for the irradiated solution of **3**, see below). Retention times (min), Run 1: TpT (**1**): 64.4, CPD_{TpT} : 9.4, $(6-4)_{\text{TpT}}$: 15.3; TLNpT (**3**): 63.0, $\text{CPD}_{\text{TLNpT}}$: 26.6, $(6-4)_{\text{TLNpT}}$: 26.6; Run 2: TpT (**1**): 30.5, CPD_{TpT} : 9.2, $(6-4)_{\text{TpT}}$: 13.0; TpTLN (**4**): 32.9, $\text{CPD}_{\text{TpTLN}}$: 15.4, $(6-4)_{\text{TpTLN}}$: 14.5; TLNpTLS (**5**): 31.1, $\text{CPD}_{\text{TLNpTLS}}$: 13.5, $(6-4)_{\text{TLNpTLS}}$: 17.5.

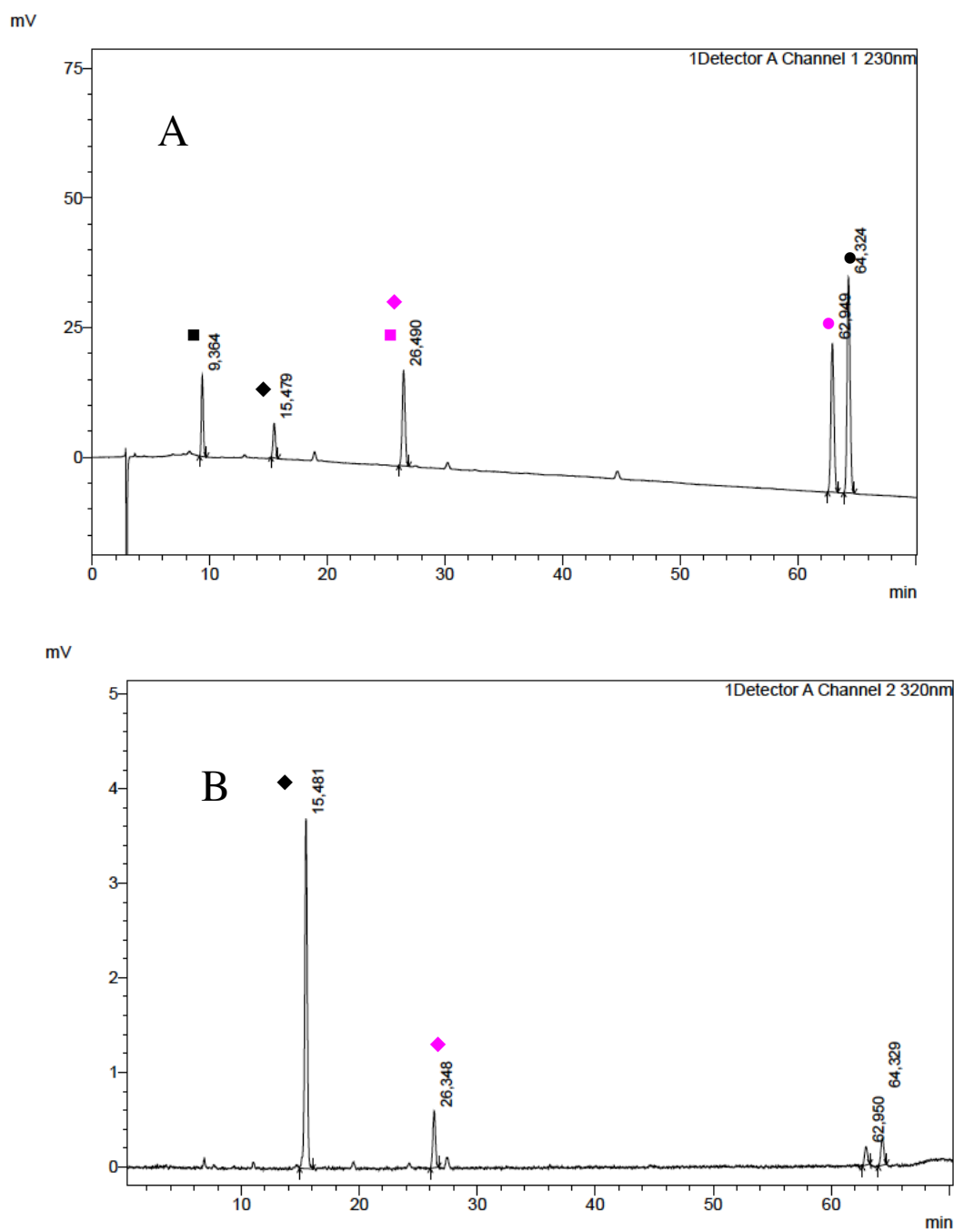


Figure S44. HPLC chromatogram of the 8 min irradiation mixture of **3** and **1** at 230 nm (A) and 320 nm (B): TpT **1** (●), TpT CPD (■), TpT (6-4) (◆), T_{LNP}T **3** (●), T_{LNP}T CPD (■), T_{LNP}T (6-4) (◆).

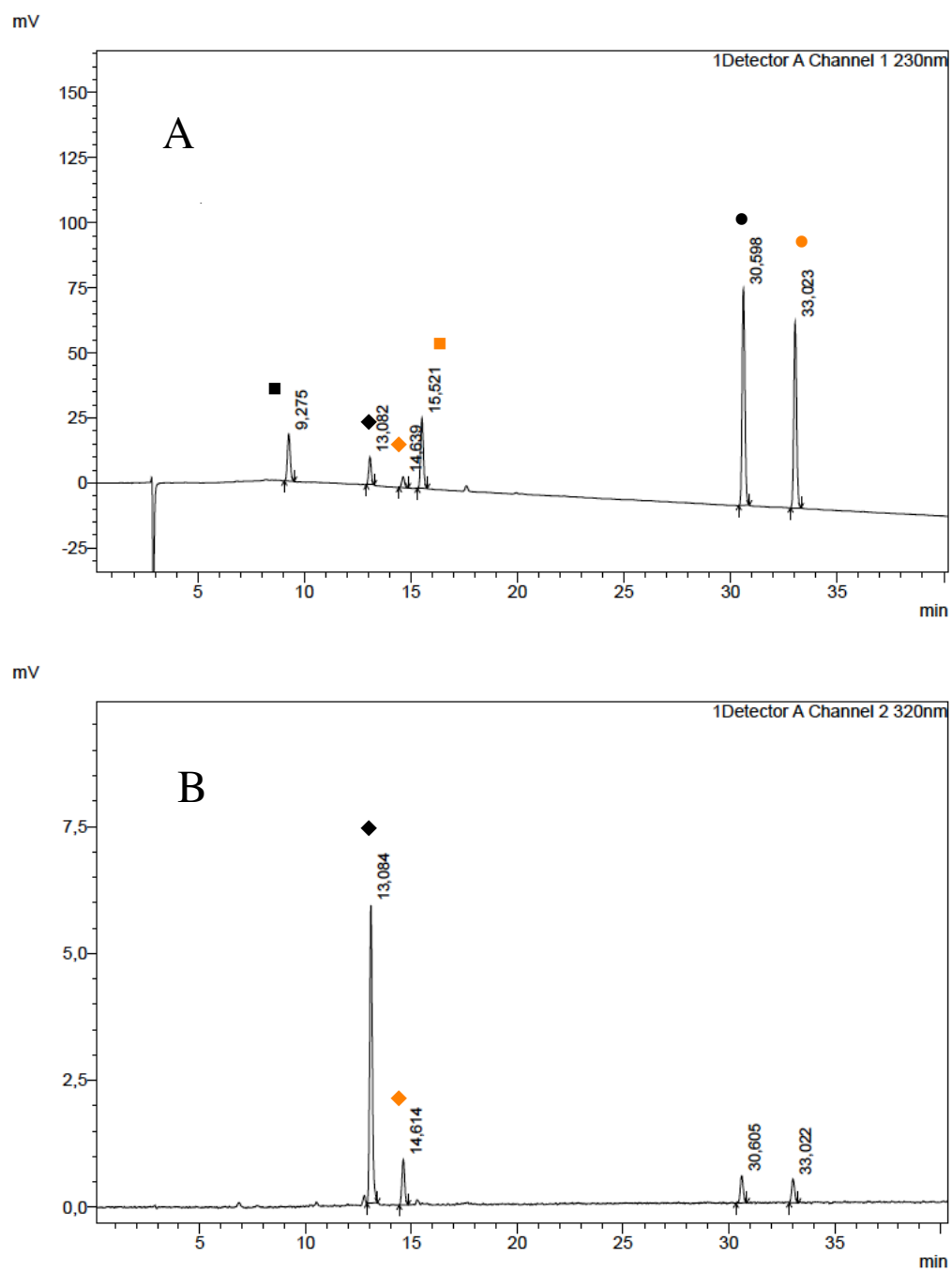


Figure S45. HPLC chromatogram of the 8 min irradiation mixture of 4 and 1 at 230 nm (A) and 320 nm (B): TpT 1 (●), TpT CPD (■), TpT (6-4) (◆), TpT_{LN} 4 (●), TpT_{LN} CPD (■), TpT_{LN} (6-4) (◇).

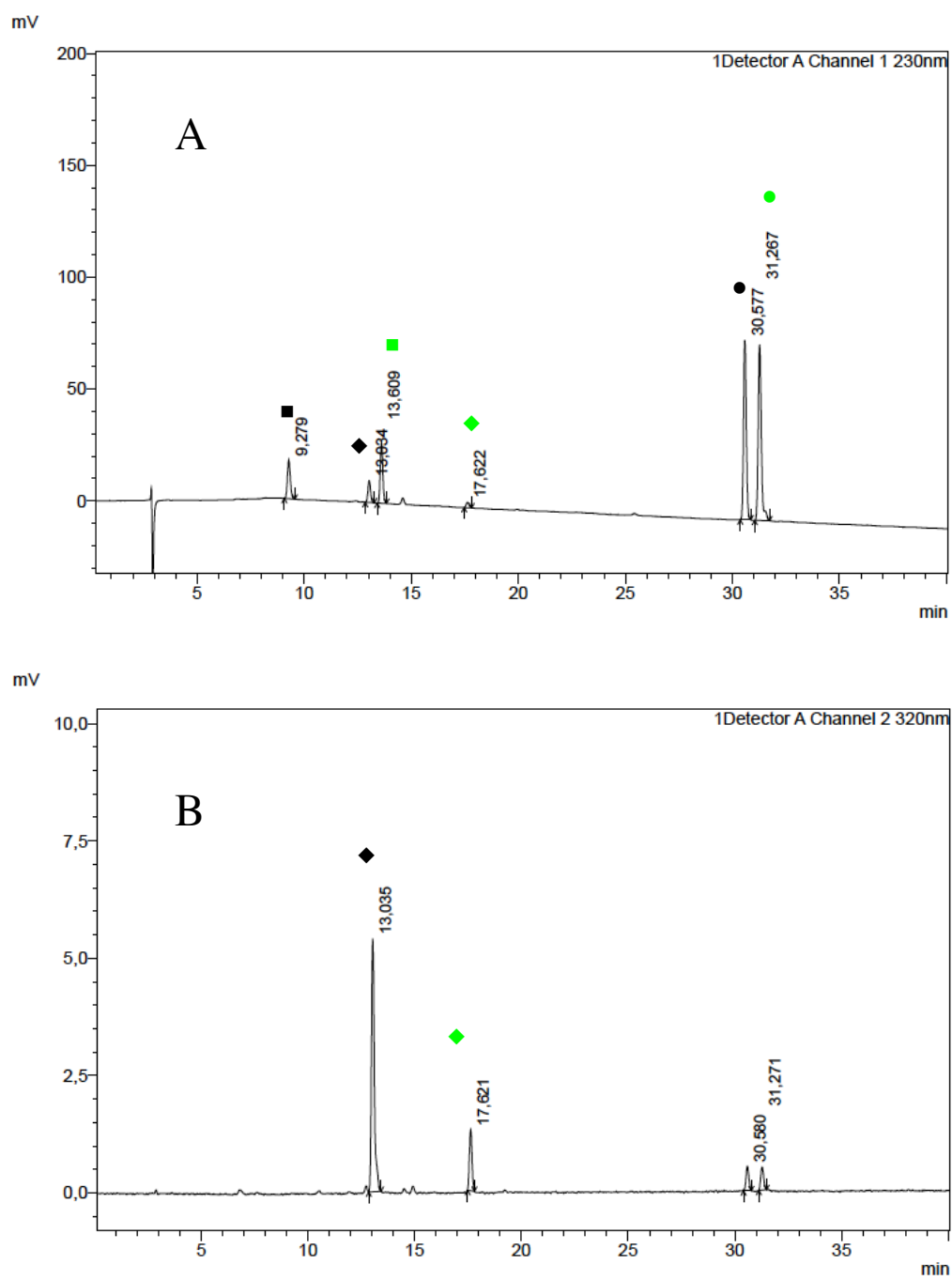


Figure S46. HPLC chromatogram of the 8 min irradiation mixture of **5** and **1** at 230 nm (A) and 320 nm (B): TpT **1** (●), TpT CPD (■), TpT (6-4) (◆), T_{LNP}T_{LS} **5** (●), T_{LNP}T_{LS} CPD (■), T_{LNP}T_{LS} (6-4) (◆).

4. 2. Photoproduct identification by HPLC-ES-MS/MS

Aqueous solutions of dinucleotides **3-5** (800 μ L, OD: 6.3) were degassed with argon for 30 min then irradiated under 254 nm for 20 min. An aliquot of each solution was sampled at $t = 0$ and 20 min. Crude irradiation samples of dinucleotides **3-5** were injected on a C18 Uptisphere (2x150 mm, 3 μ m) column using a 30 min gradient of 0 to 15 % acetonitrile in 2 mM ammonium formate that reached 40% after 20 min at flow rate of 200 μ L/min. Samples were solubilized in 20 mM ammonium formate.

Retention times: TpT (**1**): RT: 24.3 min, CPD_{TpT}: 6.8 min, (6-4)_{TpT}: 12.0 min; T_{LNpT} (**3**): RT: 24.5 min, CPD_{T_{LNpT}}: 15.5 min, (6-4)_{T_{LNpT}}: 15.5 min; TpT_{LN} (**4**): RT: 25.9 min, CPD_{TpT_{LN}}: 13.7 min, (6-4)_{TpT_{LN}}: 12.8 min; T_{LNpT_{LS}} (**5**): 24.9 min, CPD_{T_{LNpT_{LS}}}: 12.2 min, (6-4)_{T_{LNpT_{LS}}}: 15.3 min.

Spectra were recorded on a API 3000 triple quadrupolar spectrometer (SCIEX, Framingham MA). After a first analysis performed in the MS1 mode to determine the molecular weight of each PP, samples were injected a second time to record their fragmentation spectra. For this purpose, the spectrometer was used in the product ion scan mode. The pseudo molecular ion of the PP [M-H]⁻ was isolated in the first quadrupole and fragmented in the second (collision cell). The fragments were identified in the third quadrupole. A collision energy of 30 was used for all samples irrespectively of the stability of the analytes.

Two main fragmentations at 573 \rightarrow 475 ([M-H-98]⁻) and 573 \rightarrow 460 ([M-H-113]⁻), corresponding to specific fragments of CPD and (6-4) PP, respectively, were observed for T_{LNpT} (**3**) and TpT_{LN} (**4**). For T_{LNpT_{LS}} (**5**), the fragmentation at 601 \rightarrow 475 ([M-H-126]⁻) and

601 → 488 (M-H-113) corresponding to specific fragments of the CPD and (6-4) PP, respectively, were observed.

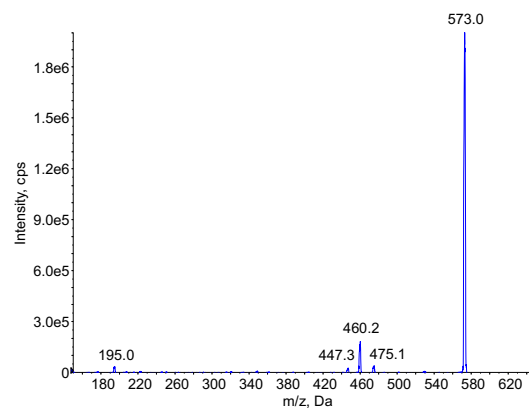


Figure S47. ES/MS-MS spectra (negative mode) of the CPD and the (6-4) PP of $T_{LNP}T$ (**3**). As the two photoproducts coelute, their fragmentation spectrum overlap.

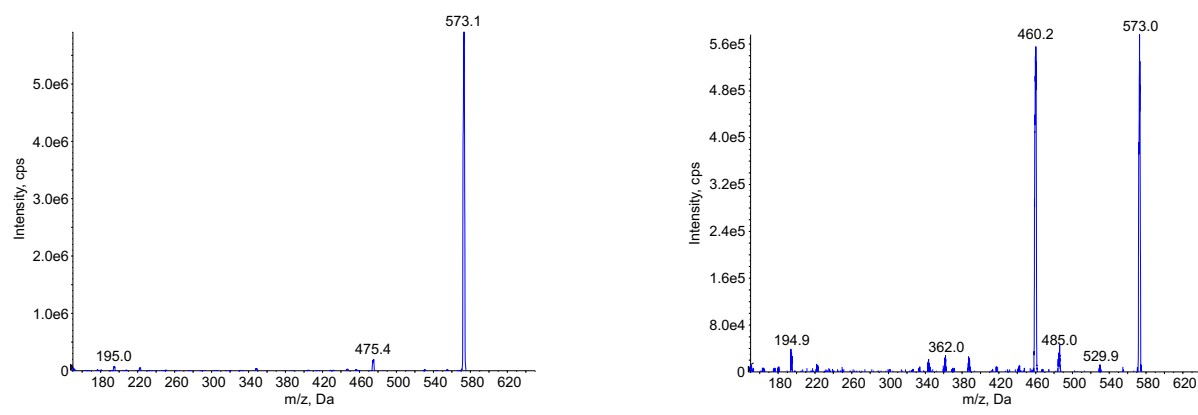


Figure S48. ES/MS-MS spectra (negative mode) of the CPD (left panel) and the (6-4) PP (right panel) of TpT_{LN} (**4**).

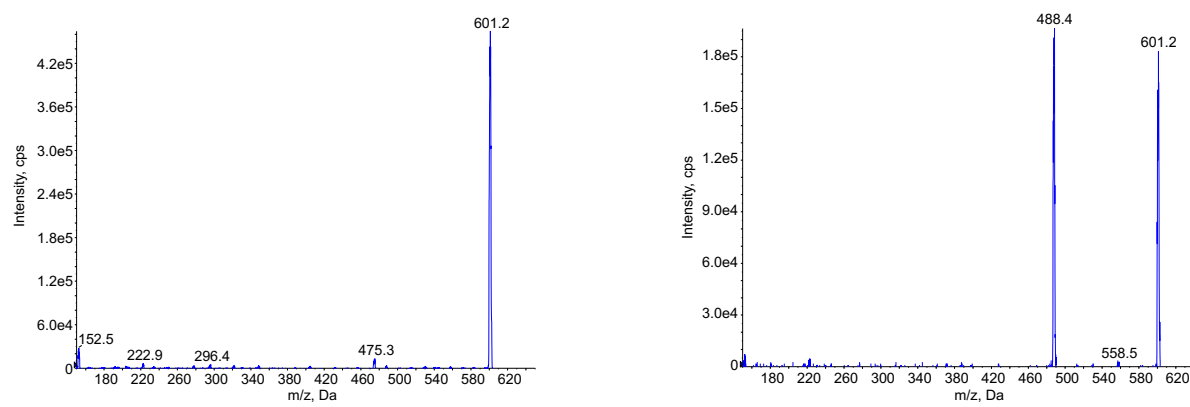
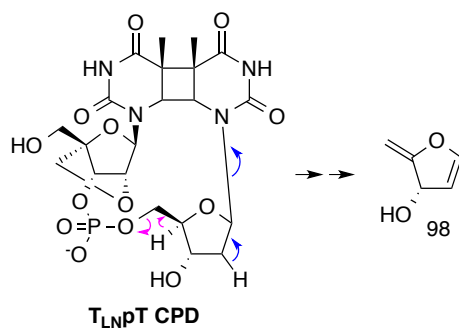
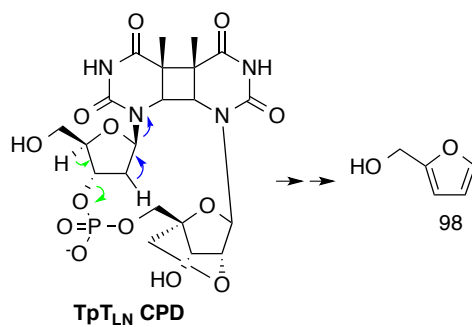


Figure S49. ES/MS-MS spectra (negative mode) of the CPD (left panel) and the (6-4) PP (right panel) of $T_{LNP}T_{LS}$ (**5**).

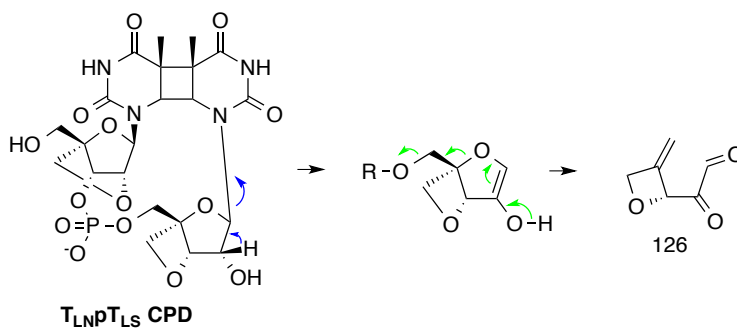
Scheme S1. Proposed fragmentation pathway for the $[M-H-98]^-$ fragment ion for the CPD of $T_{LNP}T$ (3)



Scheme S2. Proposed fragmentation pathway for the $[M-H-98]^-$ fragment ion for the CPD of $T_{p}T_{LN}$ (4)



Scheme S3. Proposed fragmentation pathway for the $[M-H-126]^-$ fragment ion for the CPD of $T_{LNP}T_{LS}$ (5)



4. 3. Kinetic of the 254 nm photoreaction of 3-5 and of PP formation

The kinetic studies were performed as previously described.¹³ Briefly, the HPLC peak of each compound was integrated at 230 nm at sampled irradiation times. The fractional amount of each compound at time t of the reaction was calculated as the peak area of this compound at time t divided by the peak area of the reactant at $t = 0$ and taking into account of their respective molar extinction coefficient at 230 nm.¹³ It was assumed that ϵ at 230 nm of **3-5** were identical as were those of their corresponding PPs within a same class of photoproduct.¹³ The CPD and (6-4) PP originating from T_{LNP}T (**3**) coeluting in different HPLC conditions, the selective 320 nm ($\epsilon_{320} = 4830 \text{ M}^{-1} \cdot \text{cm}^{-1}$)¹⁴ UV absorption of the (6-4) PP was used to calculate the respective peak area of the (6-4) PP and the CPD at 230 nm.

4. 4. Quantum yield determination

Quantum yields were determined relative to those of the TpT photoproducts¹⁴ as previously described.¹³

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