

Phosphorane intermediate vs. leaving group stabilization by intramolecular hydrogen bonding in the cleavage of trinucleoside monophosphates: implications to catalysis by the large ribozymes

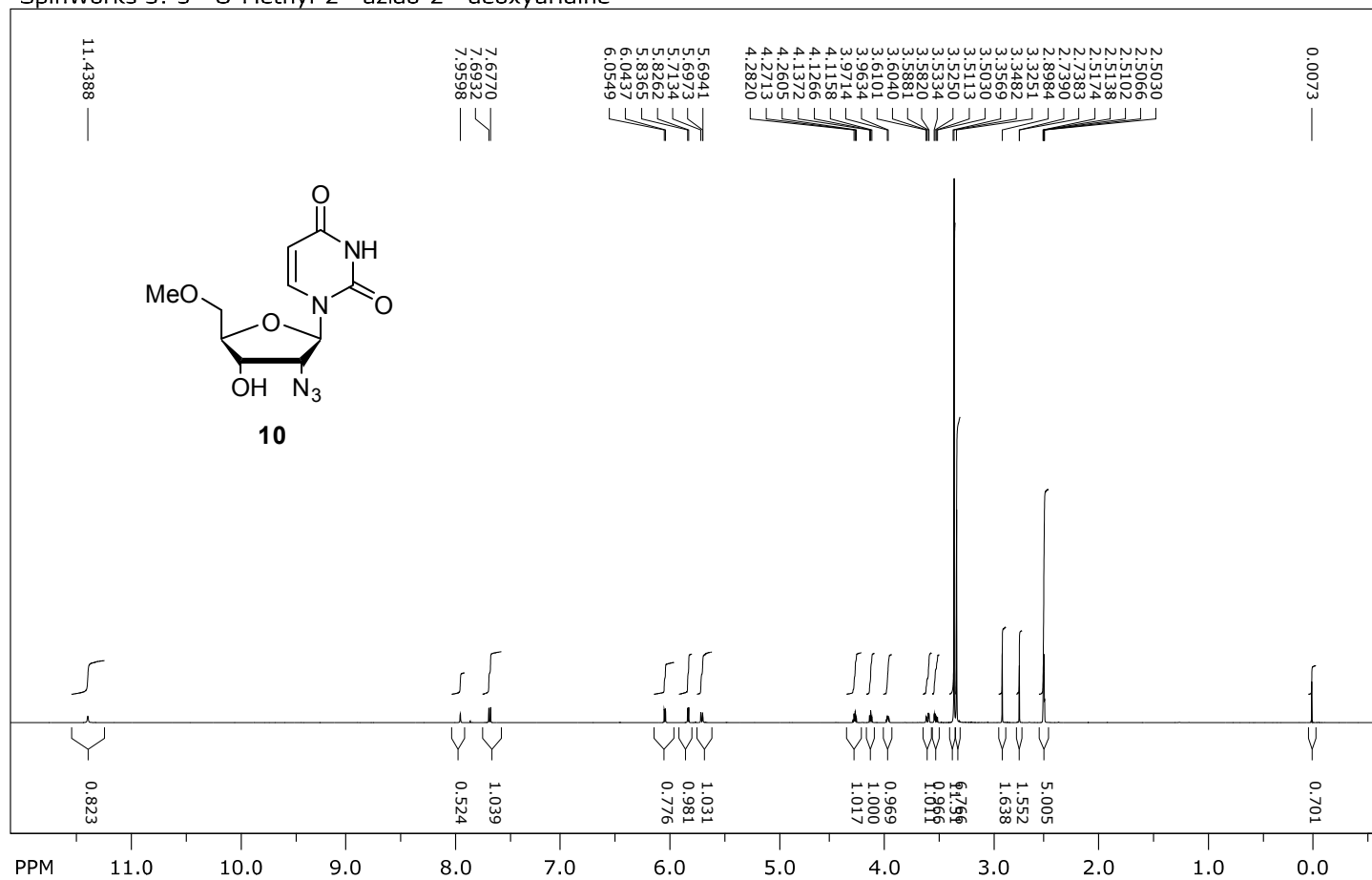
Tuomas Lönnberg and Maarit Laine

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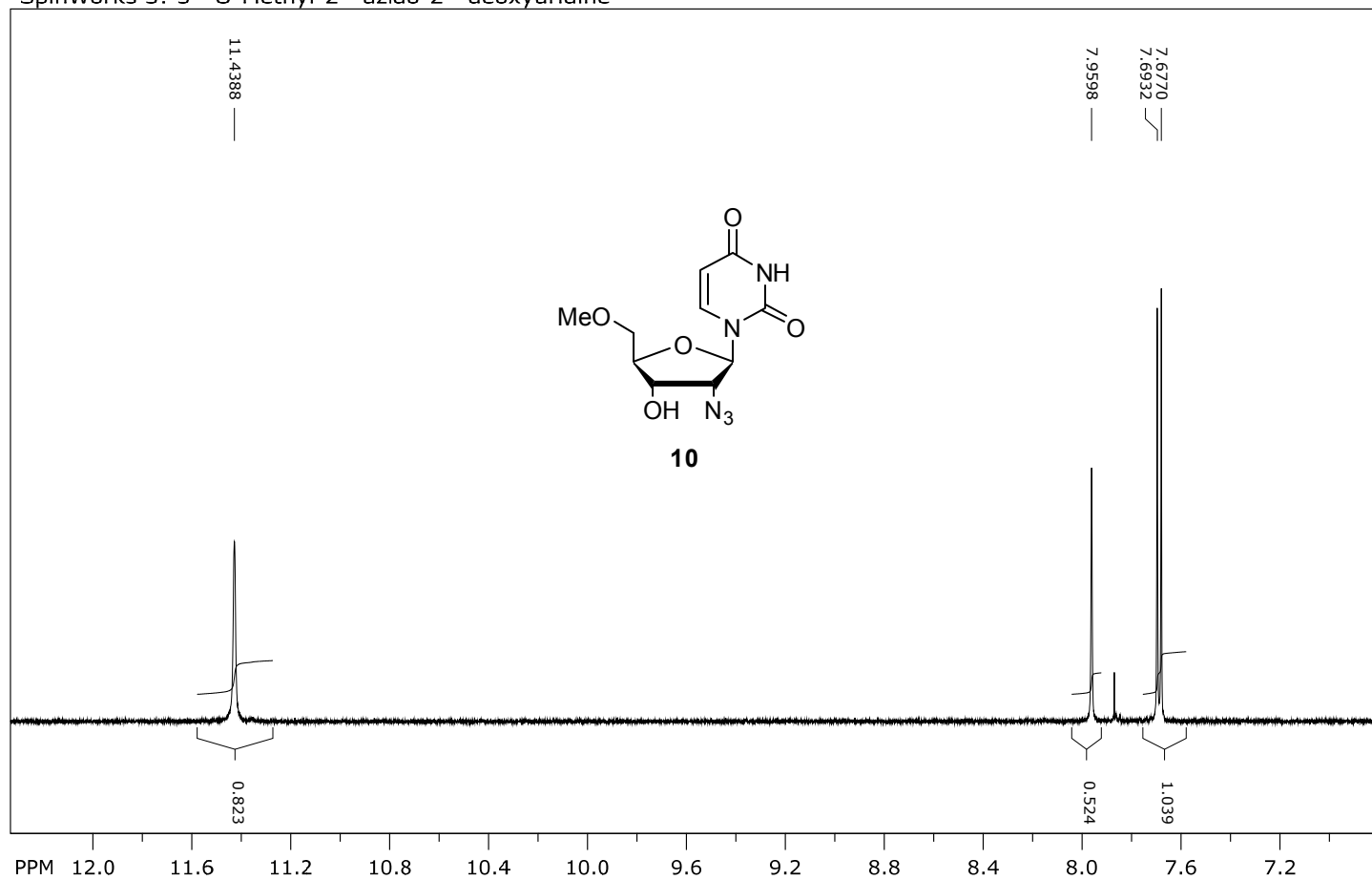
SpinWorks 3: 5'-O-Methyl-2'-azido-2'-deoxyuridine



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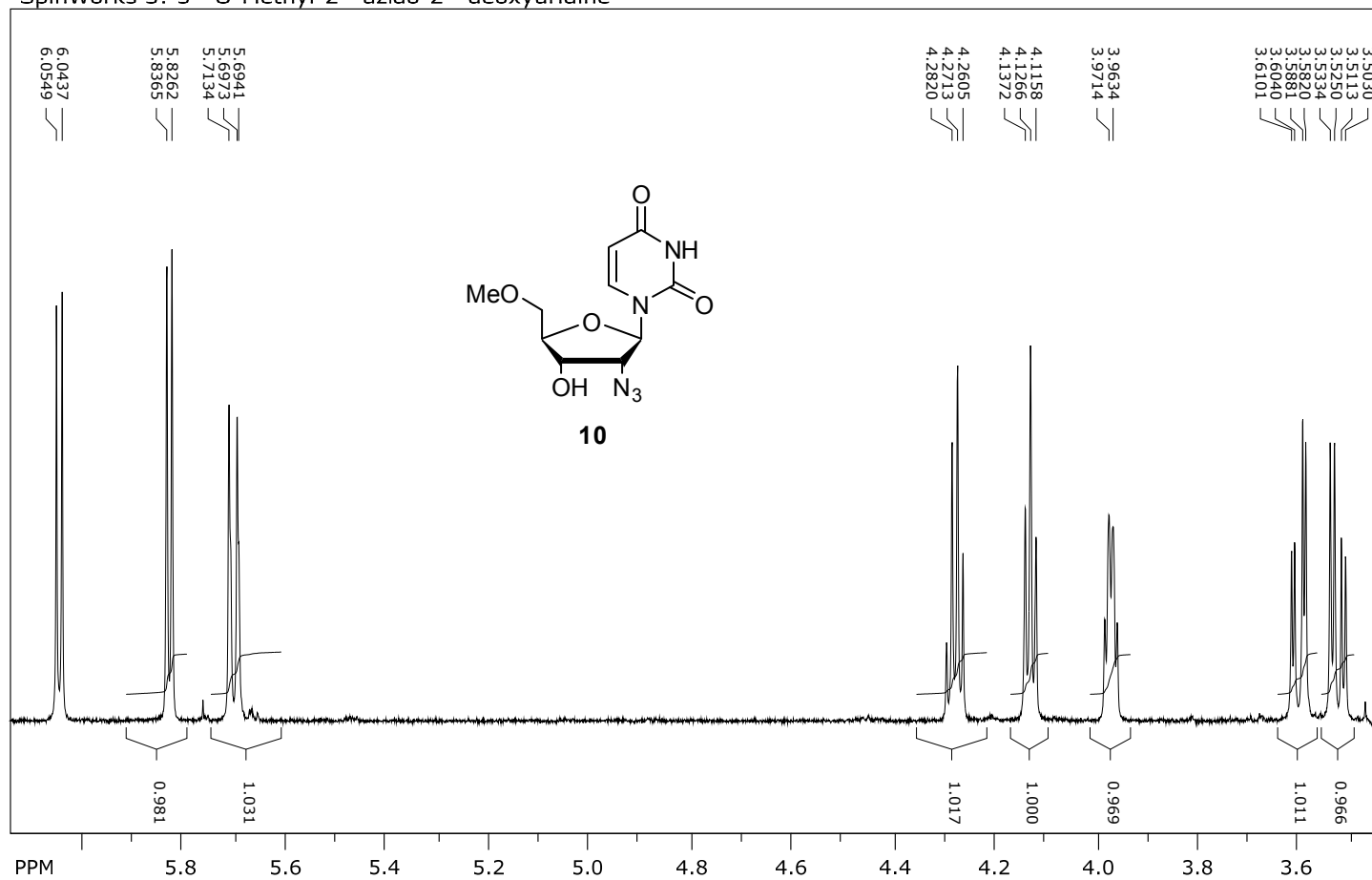
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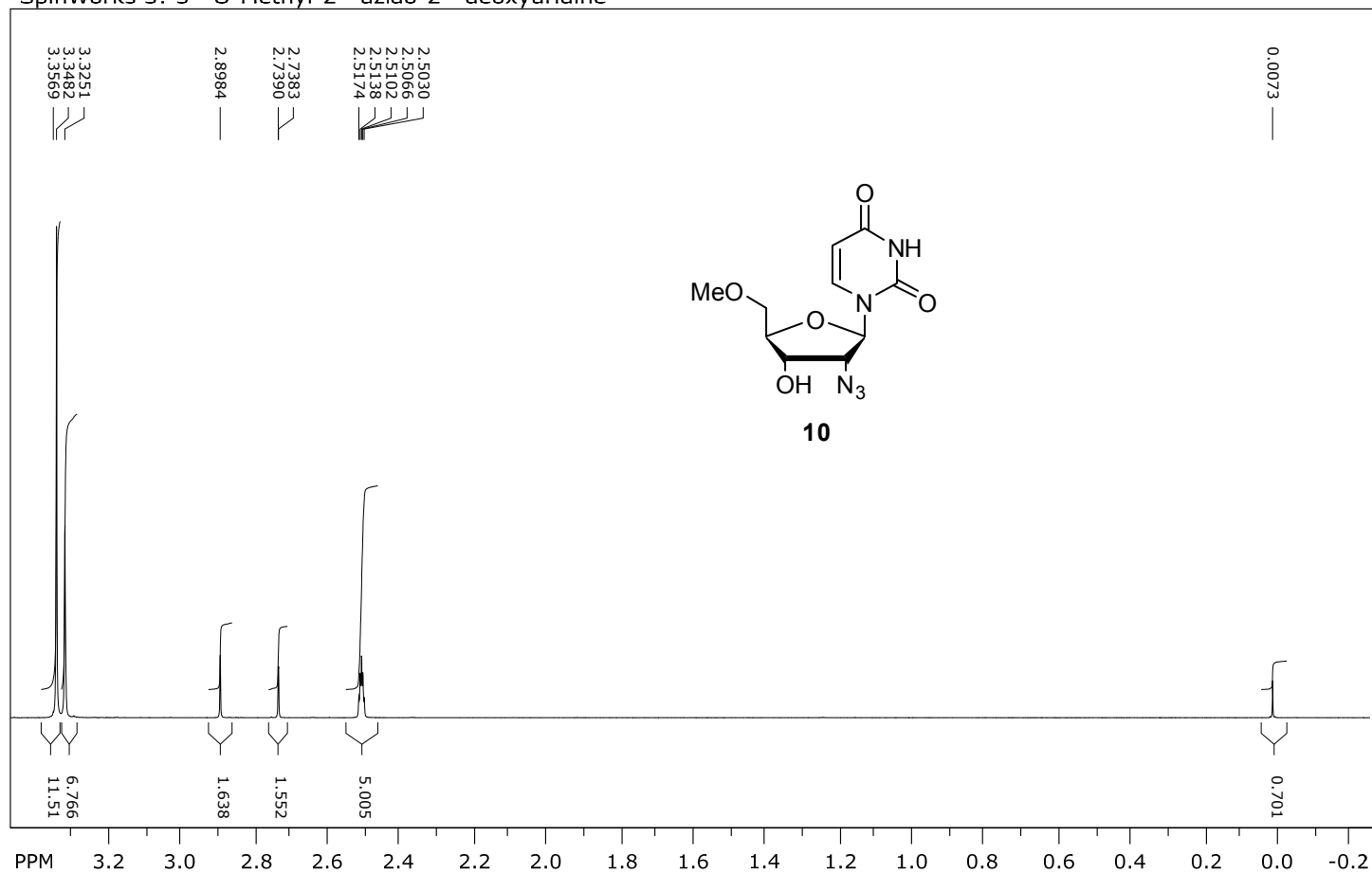
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number of scans: 16

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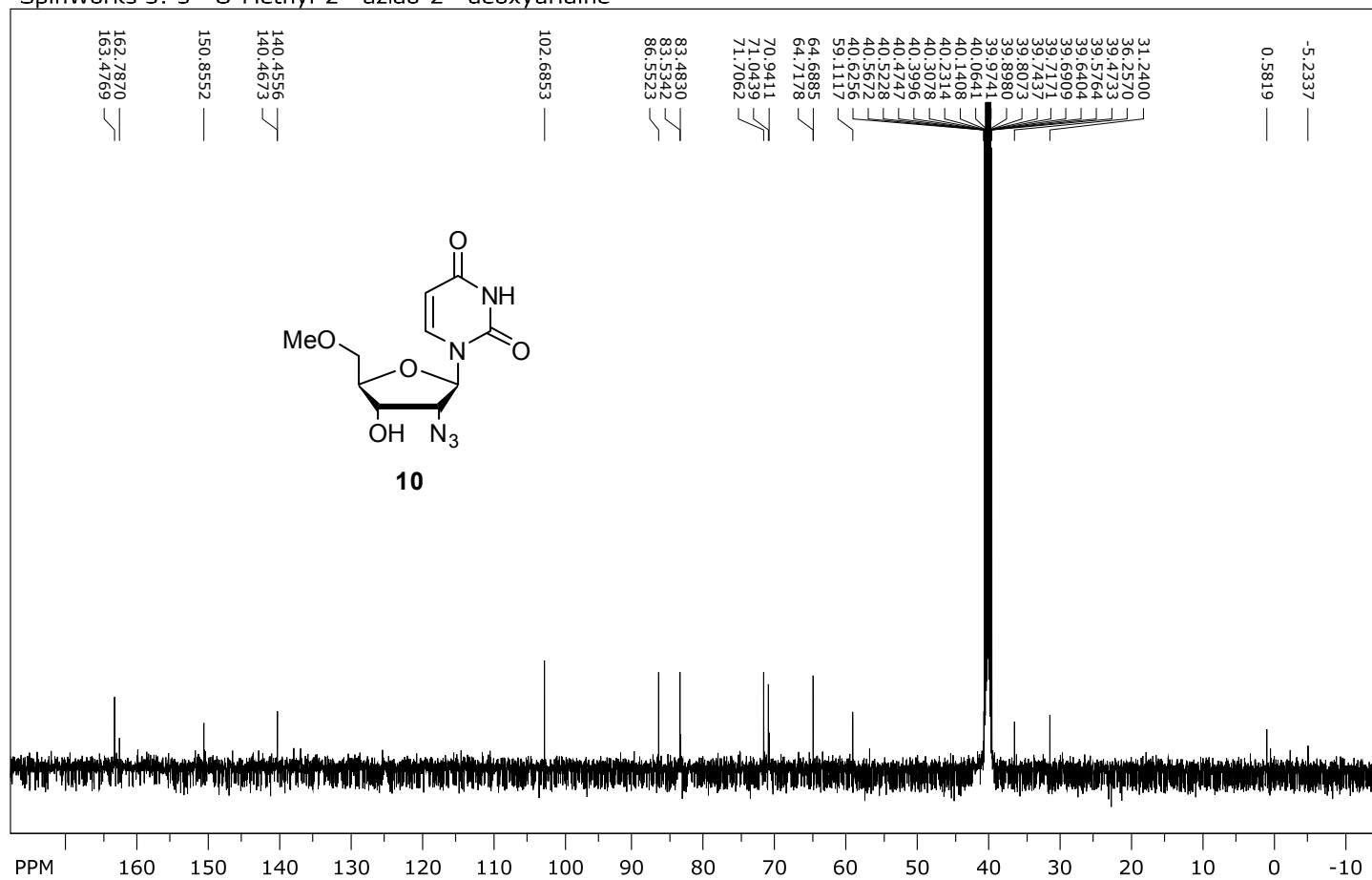
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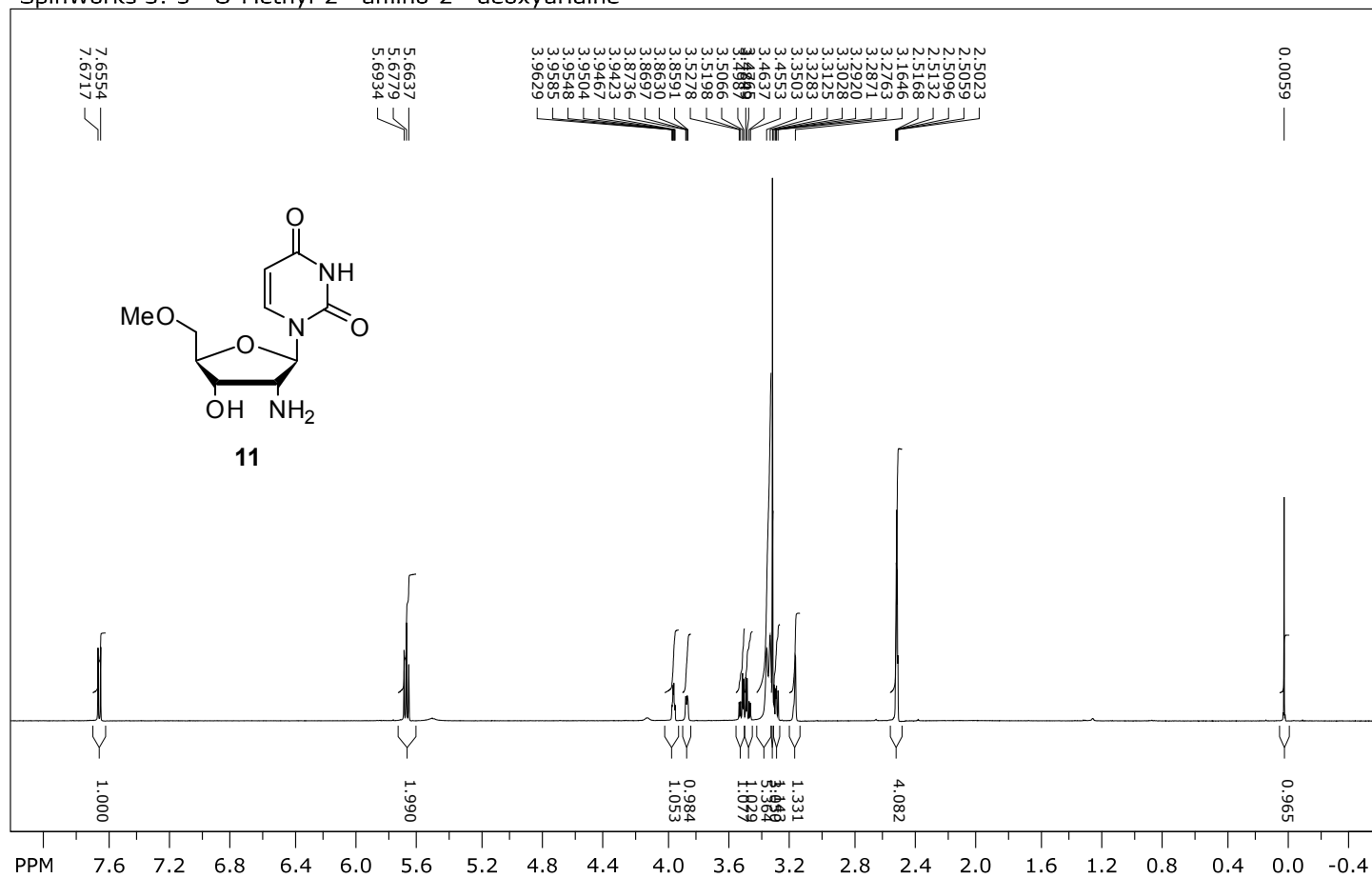
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time domain size: 65536 points
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number of scans: 1115

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 973.091 ppm/cm: 7.73704

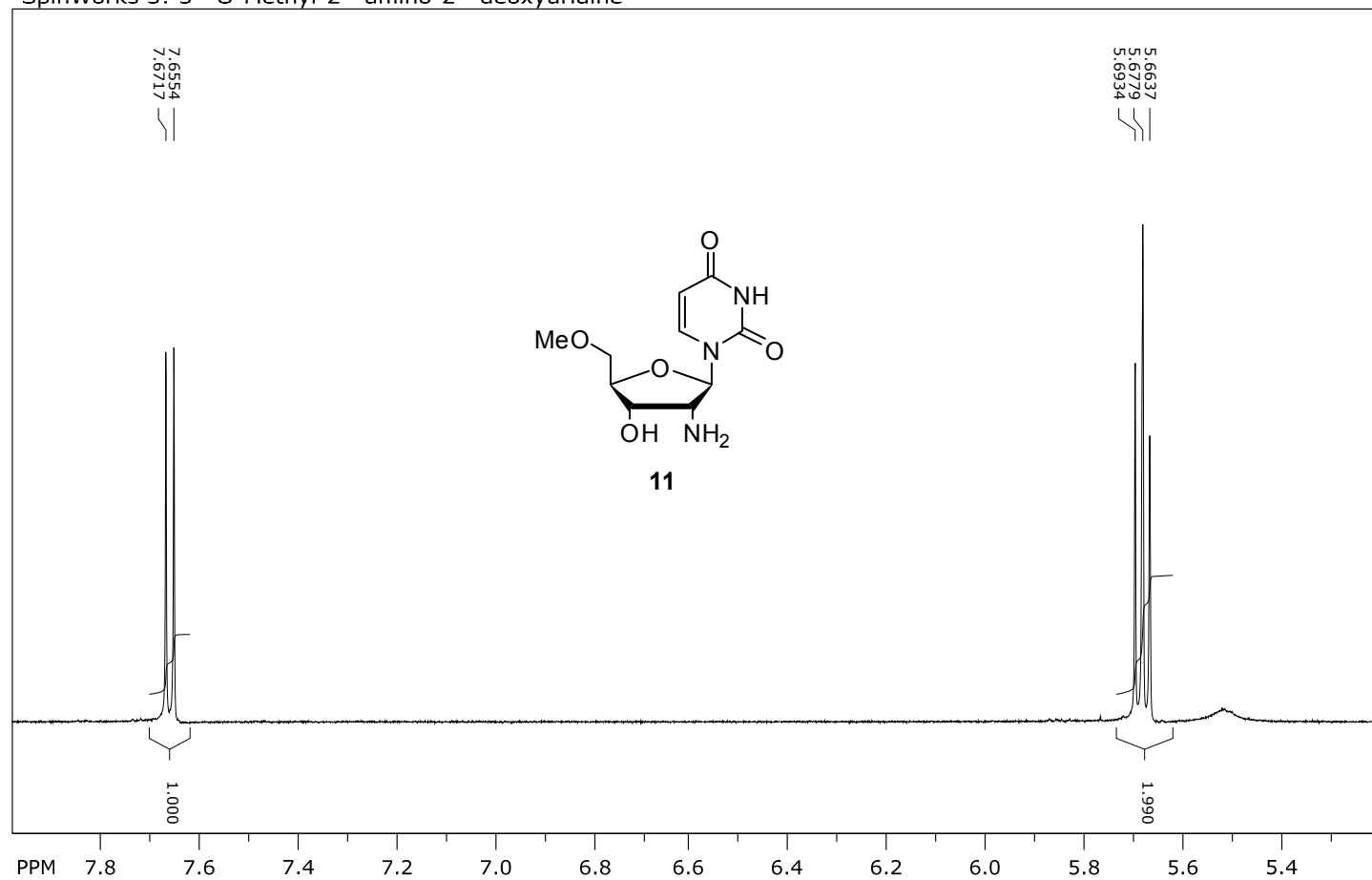
SpinWorks 3: 5'-O-Methyl-2'-amino-2'-deoxyuridine



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 transmitter freq.: 500.133089 MHz
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 number of scans: 16

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 Hz/cm: 176.562 ppm/cm: 0.35303

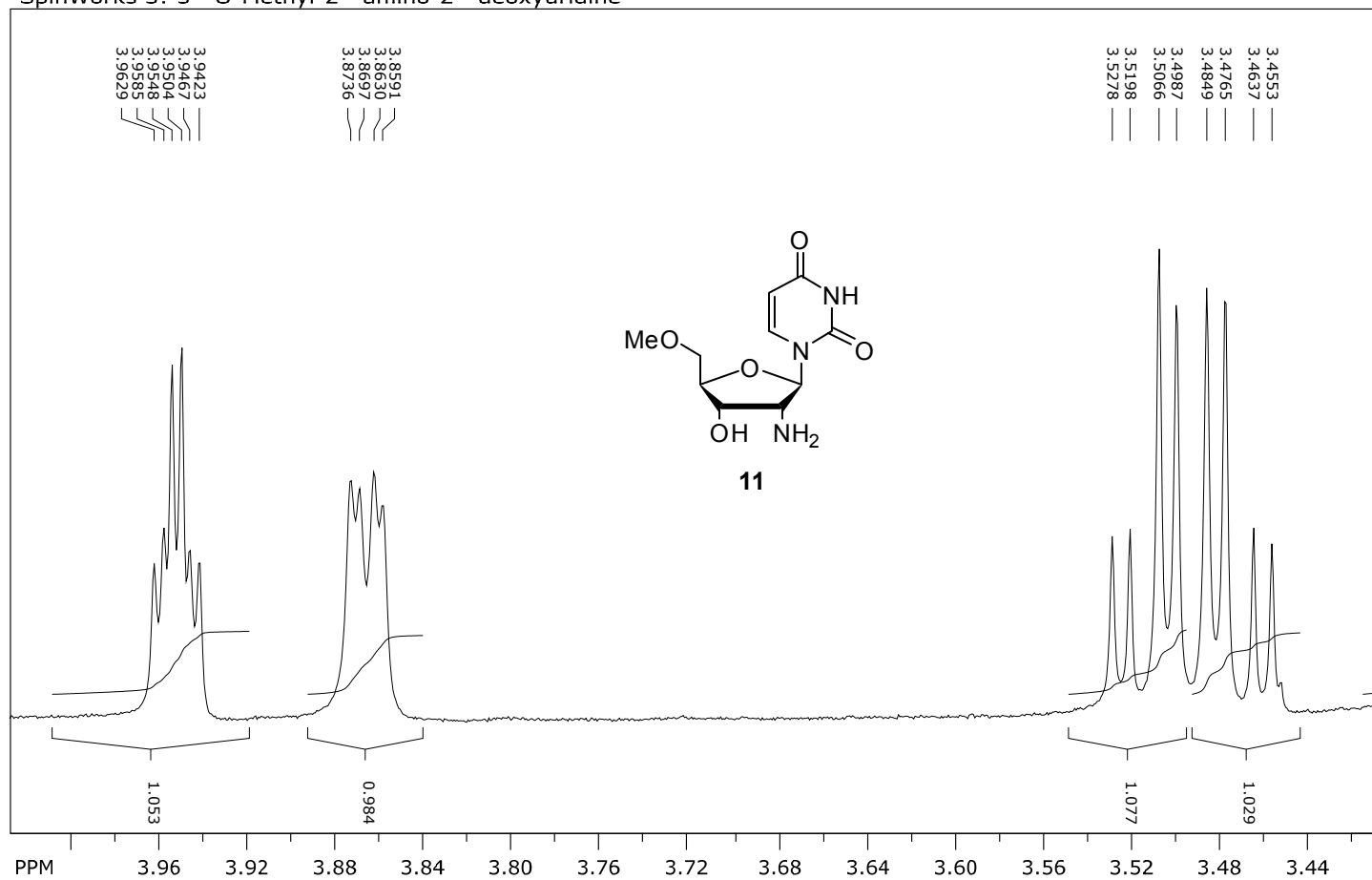
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time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

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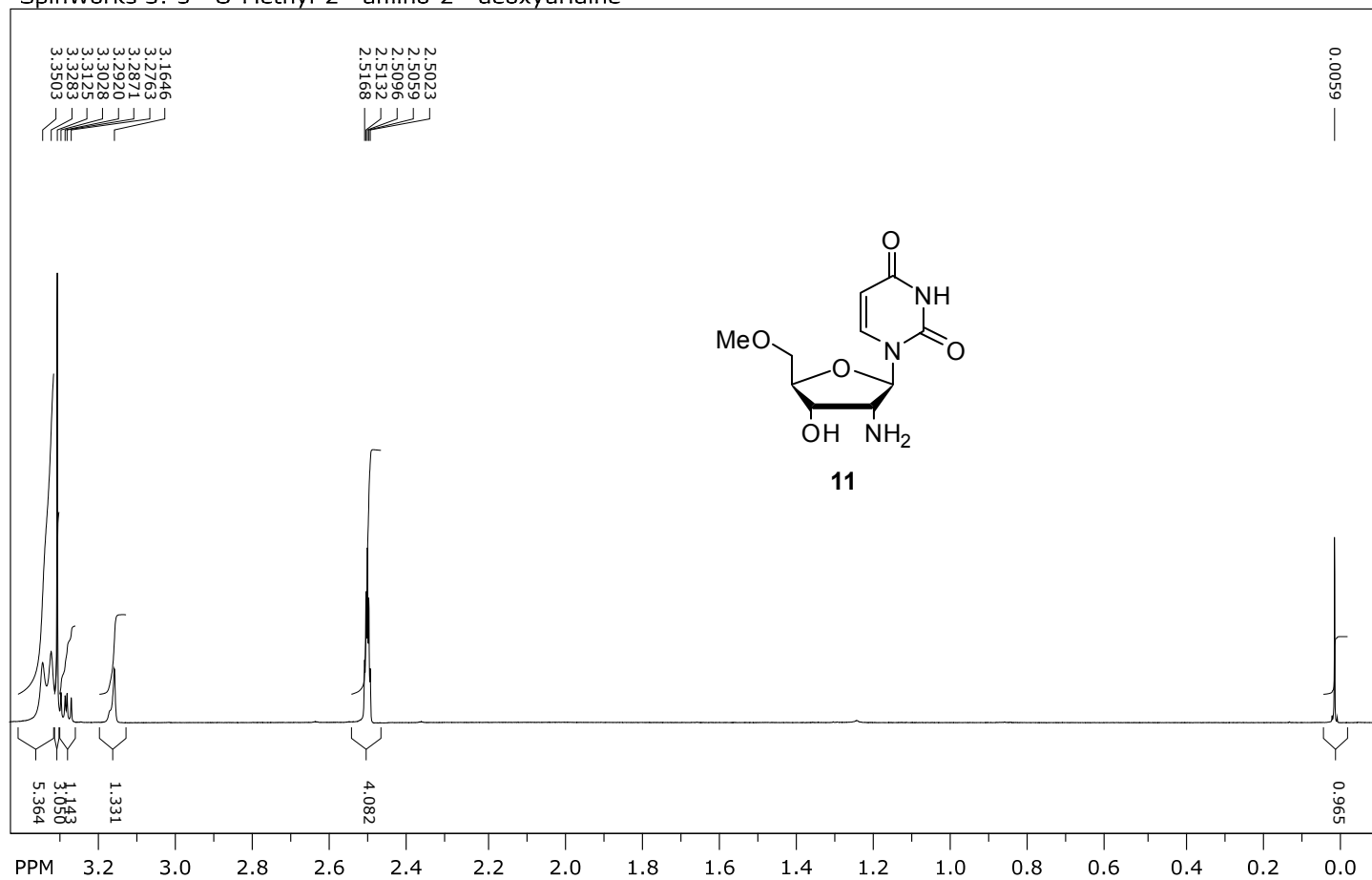
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transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

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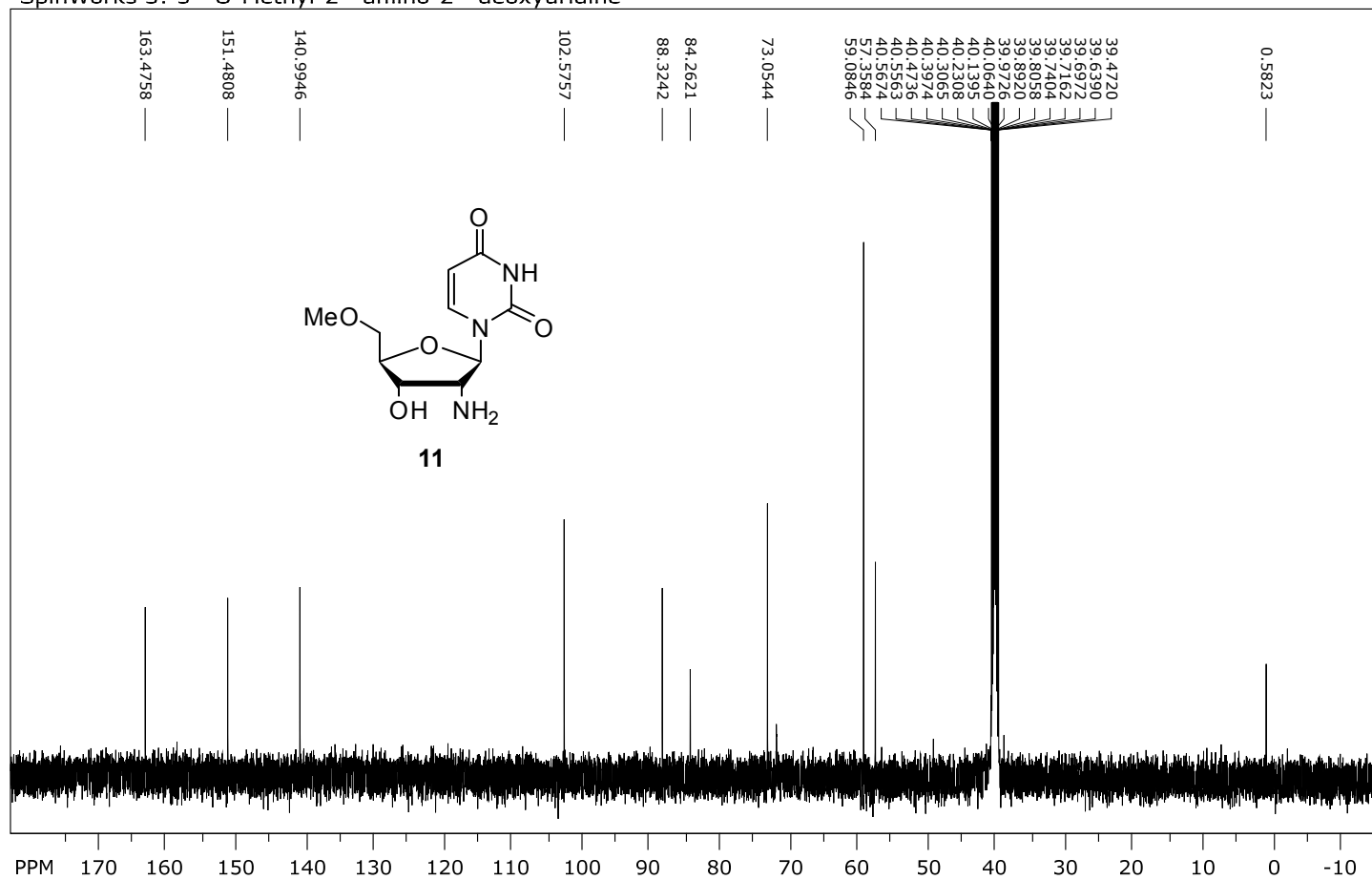
SpinWorks 3: 5'-O-Methyl-2'-amino-2'-deoxyuridine



file: E:\Maaritin spektrit\amino\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 70.863 ppm/cm: 0.14169

SpinWorks 3: 5'-O-Methyl-2'-amino-2'-deoxyuridine



file: E:\Maaritin spektrit\amino1\13\fid expt: <zpgg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1528

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 1000.247 ppm/cm: 7.95296

Fig. S1 HPLC chromatogram of **11** [Hypersil-Keystone Aquasil C18 column (4 × 150 mm, 5 μm); flow rate = 1 mL min⁻¹; 60 mM acetate buffer (pH = 4.3) and a linear gradient of 3→50% MeCN during 60 min, then 50% MeCN for 20 min].

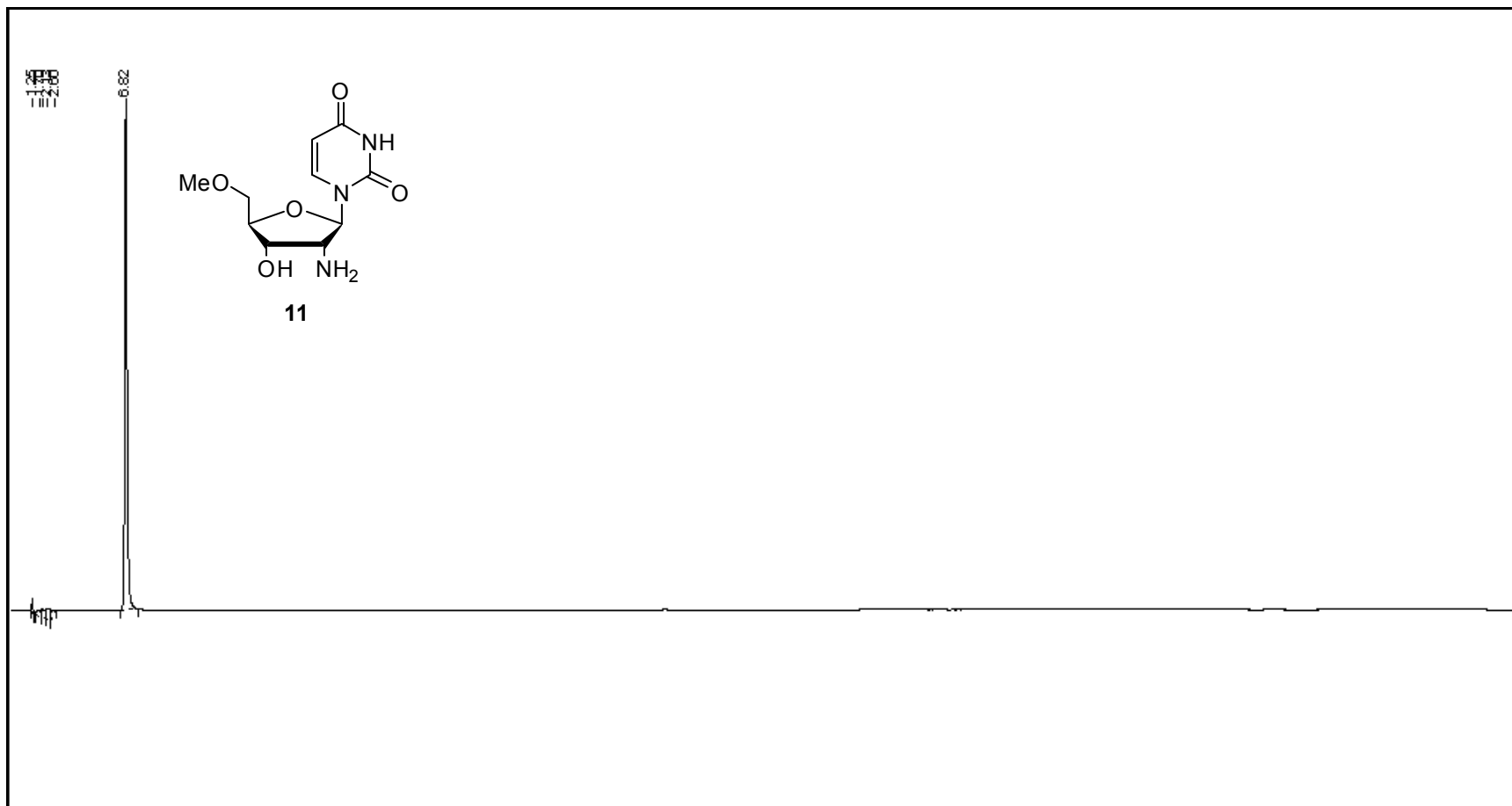
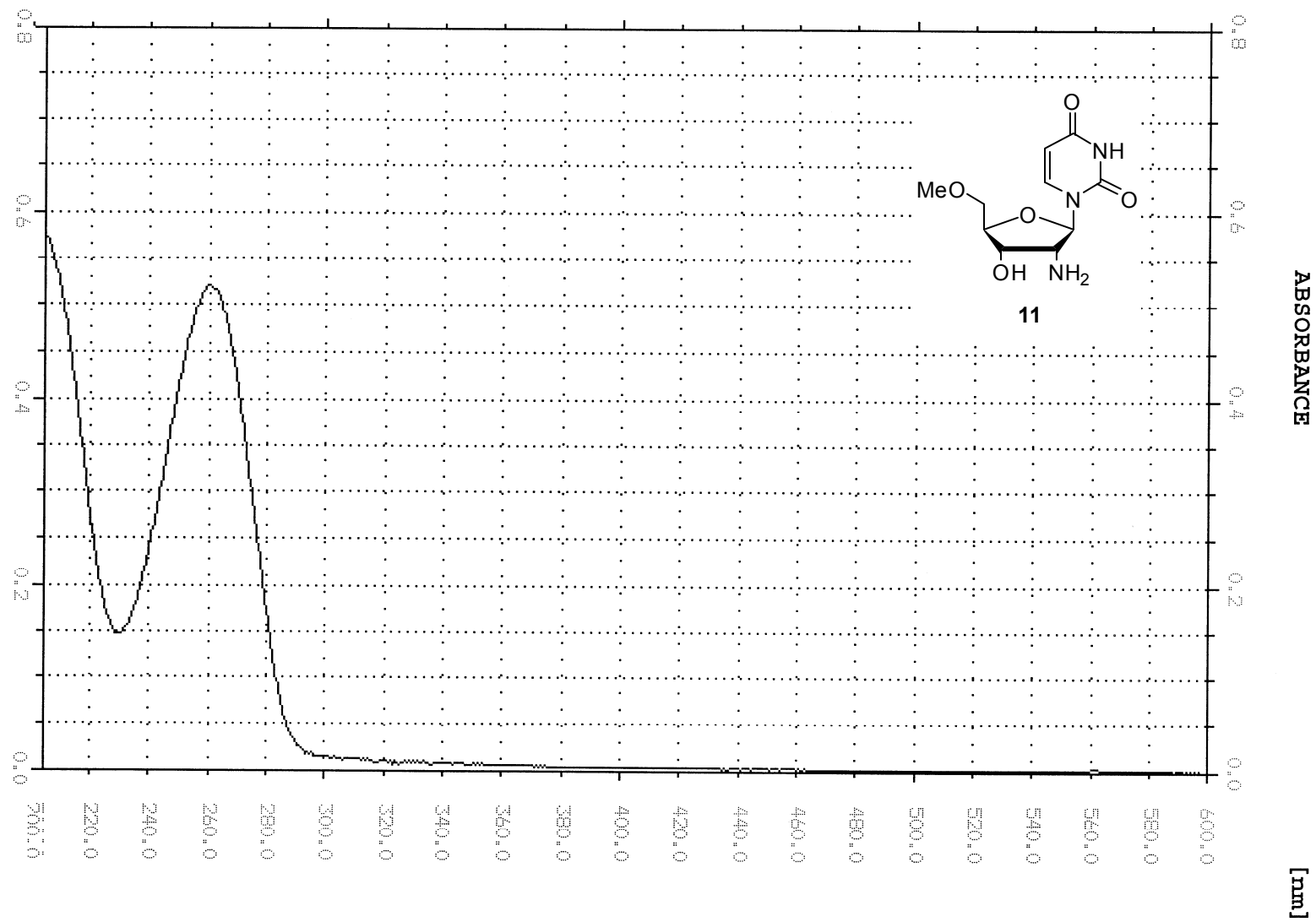
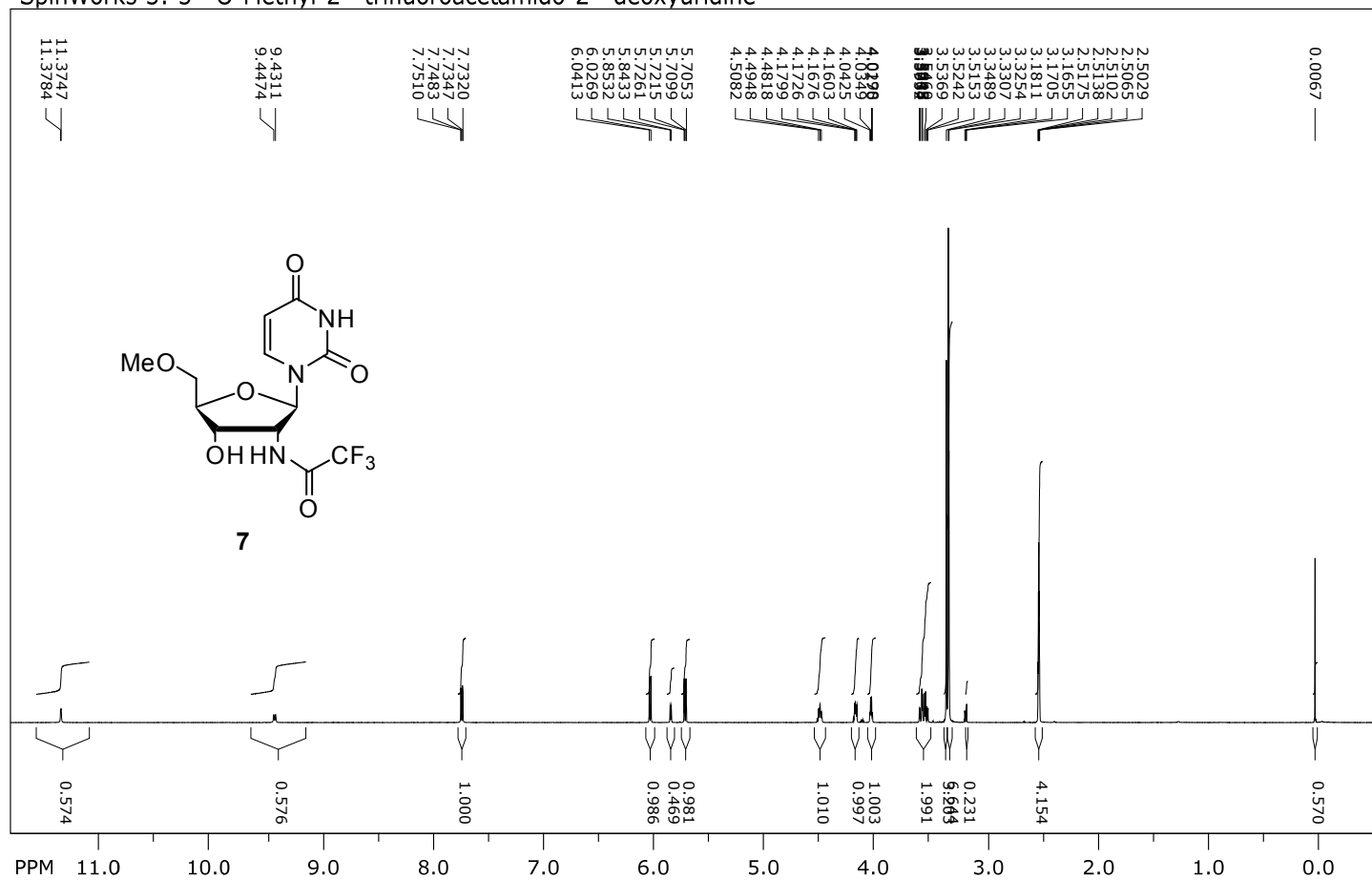


Fig. S2 UV spectrum of **11**.



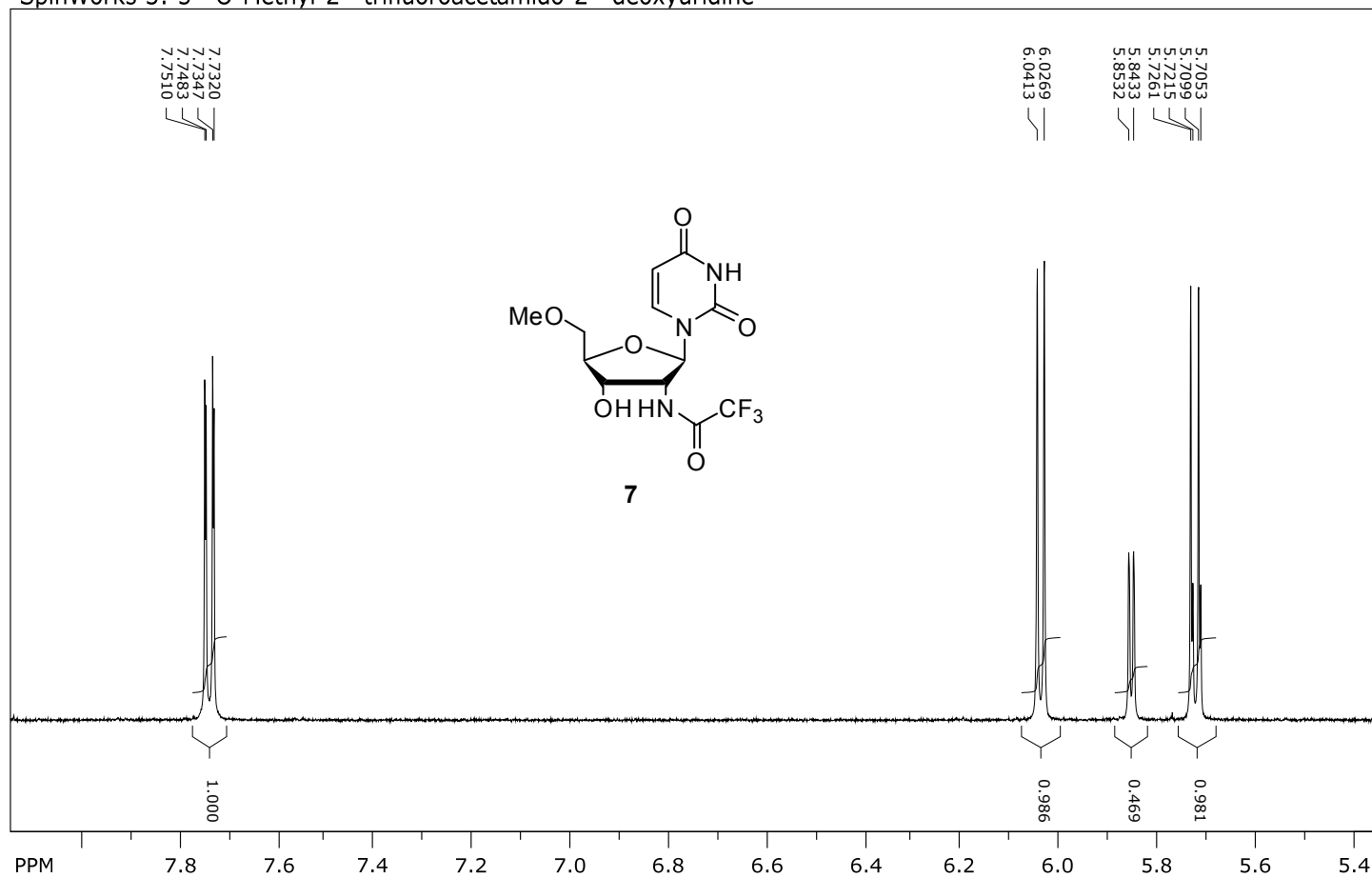
SpinWorks 3: 5'-O-Methyl-2'-trifluoroacetamido-2'-deoxyuridine



file: ...Maartin spektrit(trifluoro1)\1\fid expt: <zg30>
 transmitter freq.: 500.133089 MHz
 time domain size: 65536 points
 width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
 number of scans: 16

freq. of 0 ppm: 500.130000 MHz
 processed size: 32768 complex points
 LB: 0.000 GF: 0.0000
 Hz/cm: 248.183 ppm/cm: 0.49623

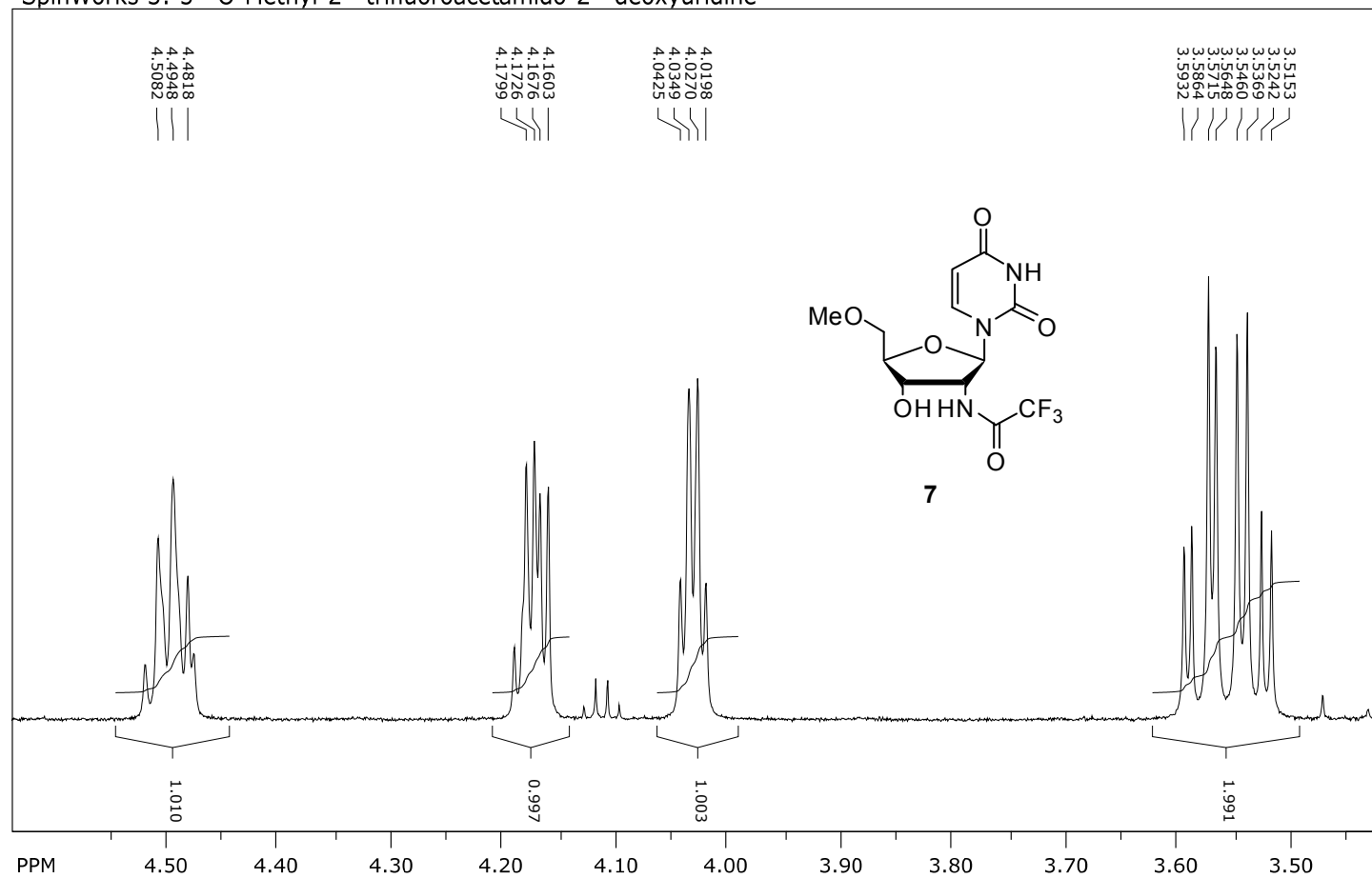
SpinWorks 3: 5'-O-Methyl-2'-trifluoroacetamido-2'-deoxyuridine



file: ...Maartin spektrit(trifluoro1)\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 56.108 ppm/cm: 0.11219

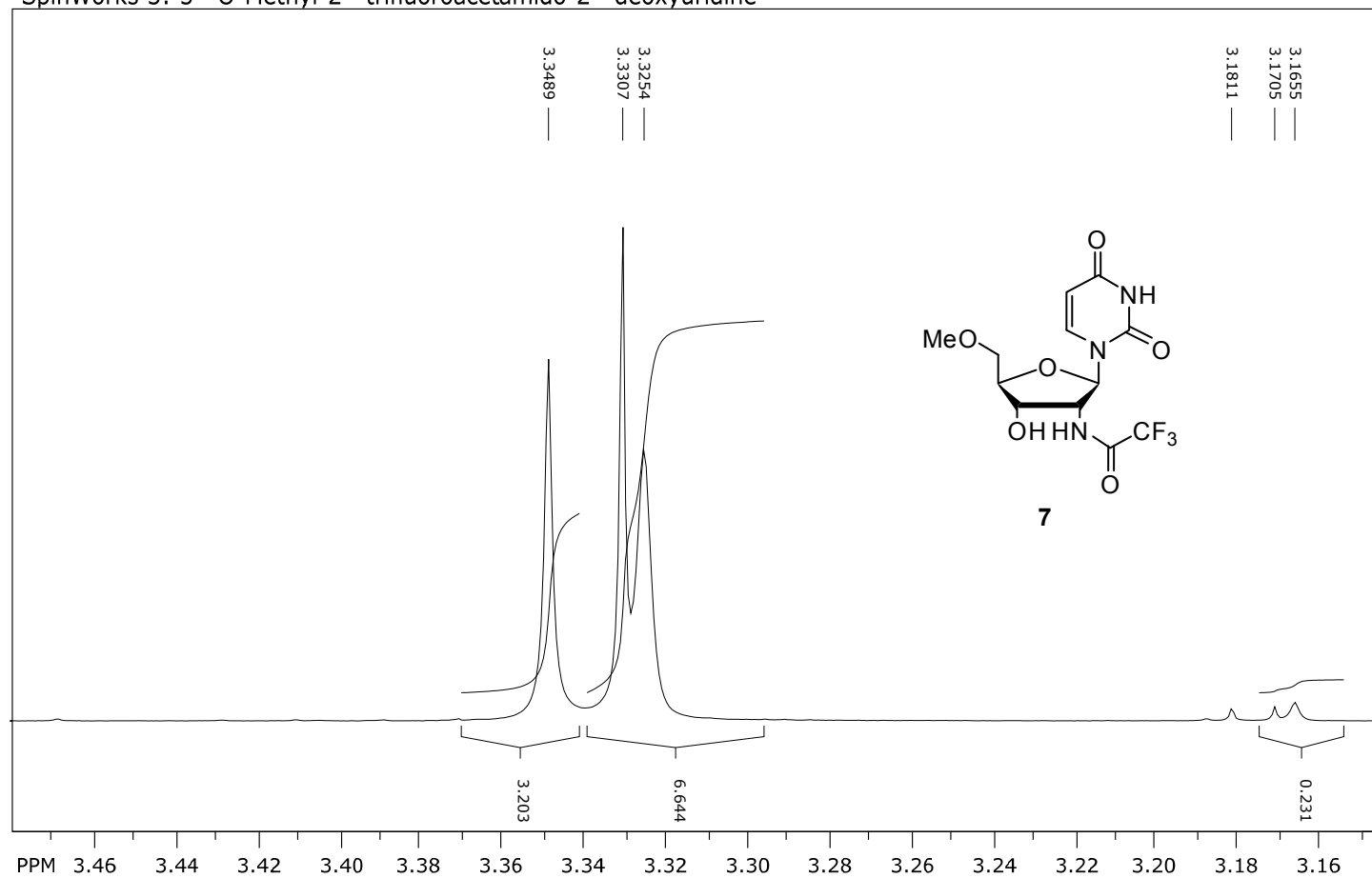
SpinWorks 3: 5'-O-Methyl-2'-trifluoroacetamido-2'-deoxyuridine



file: ...Maaritin spektrit(trifluoro1)\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 24.378 ppm/cm: 0.04874

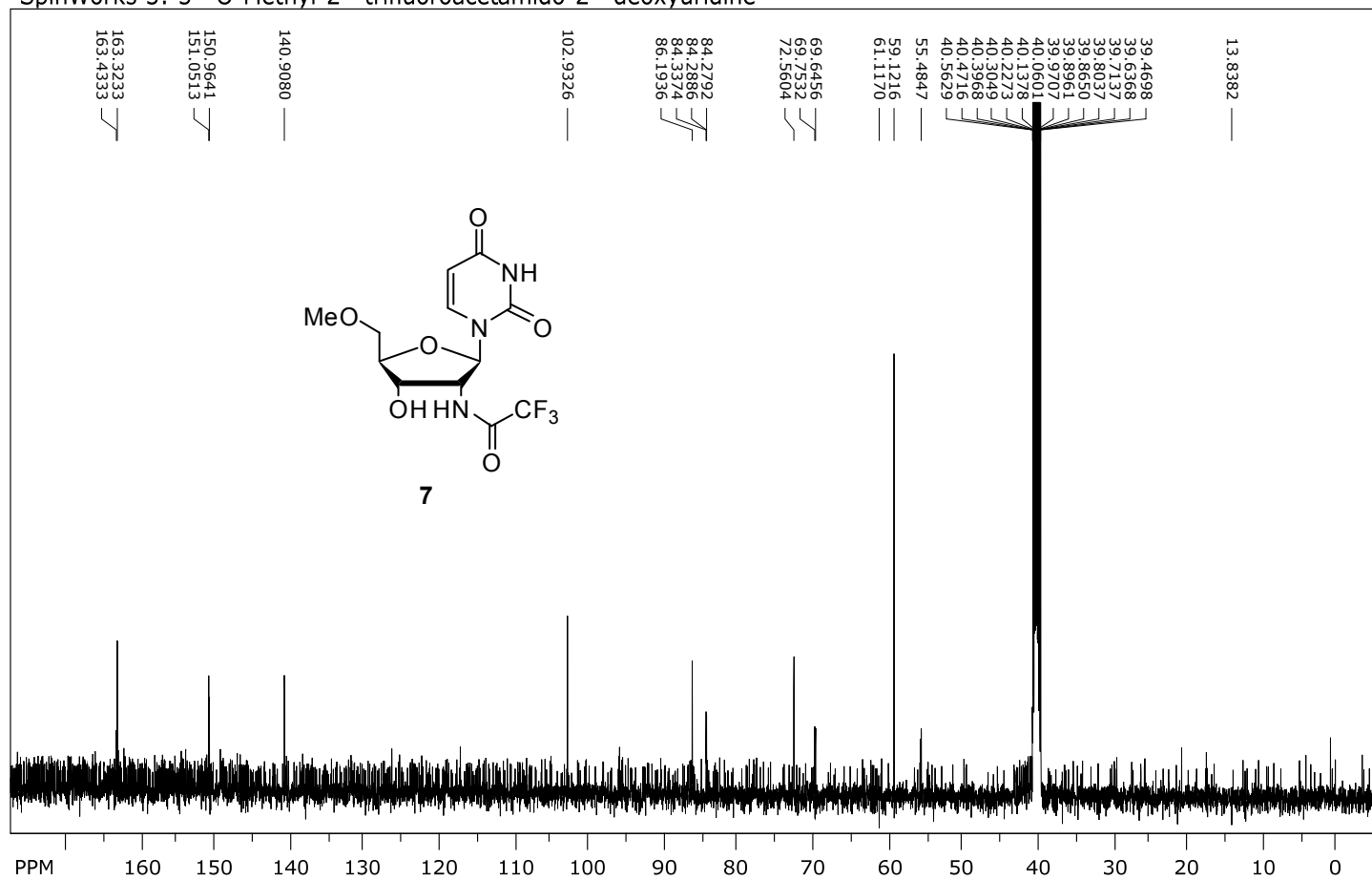
SpinWorks 3: 5'-O-Methyl-2'-trifluoroacetamido-2'-deoxyuridine



file: ...Maartin spektrit(trifluoro1)\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 6.717 ppm/cm: 0.01343

SpinWorks 3: 5'-O-Methyl-2'-trifluoroacetamido-2'-deoxyuridine



file: ...aaritin spektrit\trifluoro1\13\fid exp: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1196

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 923.305 ppm/cm: 7.34119

Fig. S3 HPLC chromatogram of **7** [Hypersil-Keystone Aquasil C18 column (4 × 150 mm, 5 μm); flow rate = 1 mL min⁻¹; 60 mM acetate buffer (pH = 4.3) and a linear gradient of 3→50% MeCN during 60 min, then 50% MeCN for 20 min].

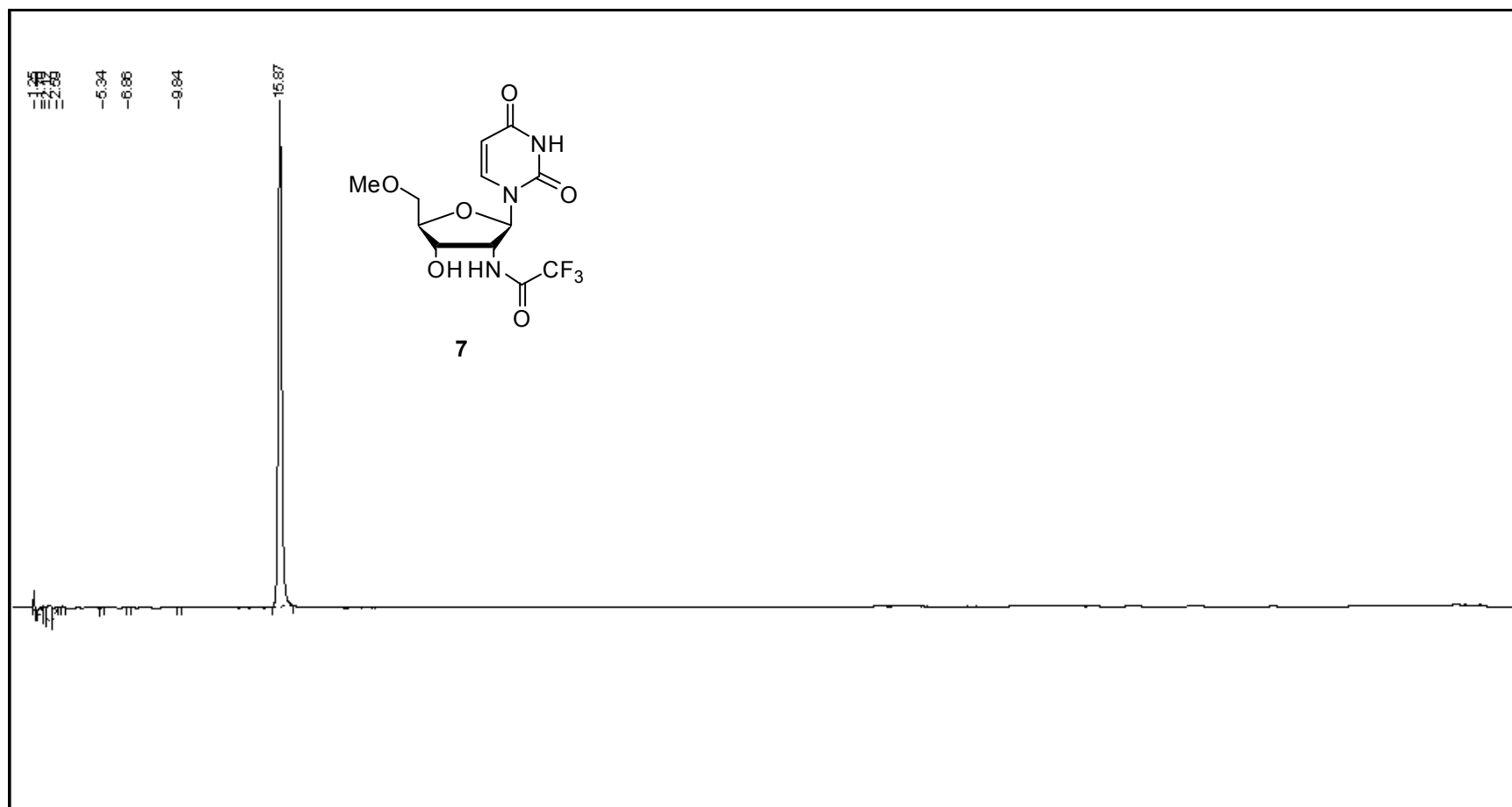
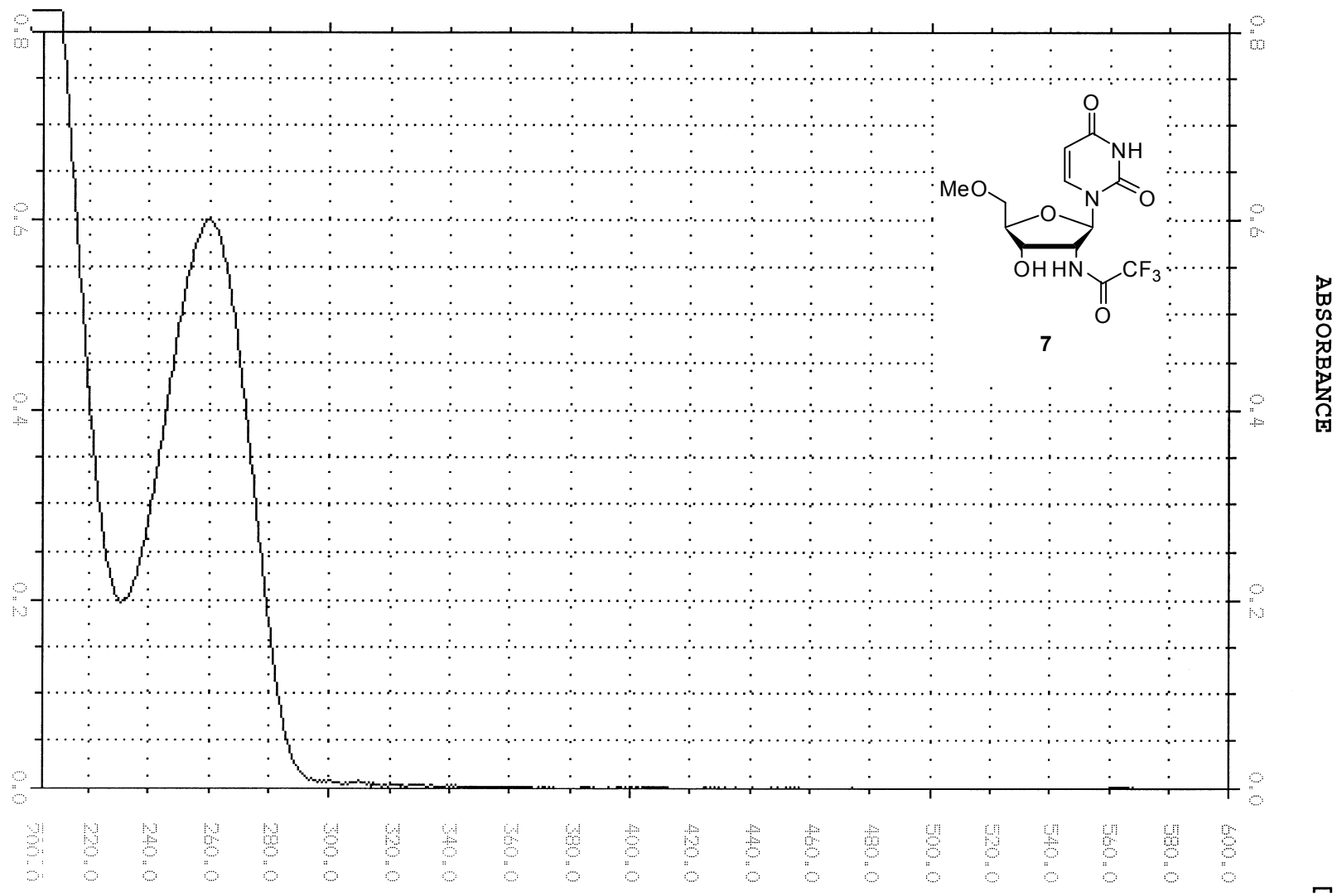
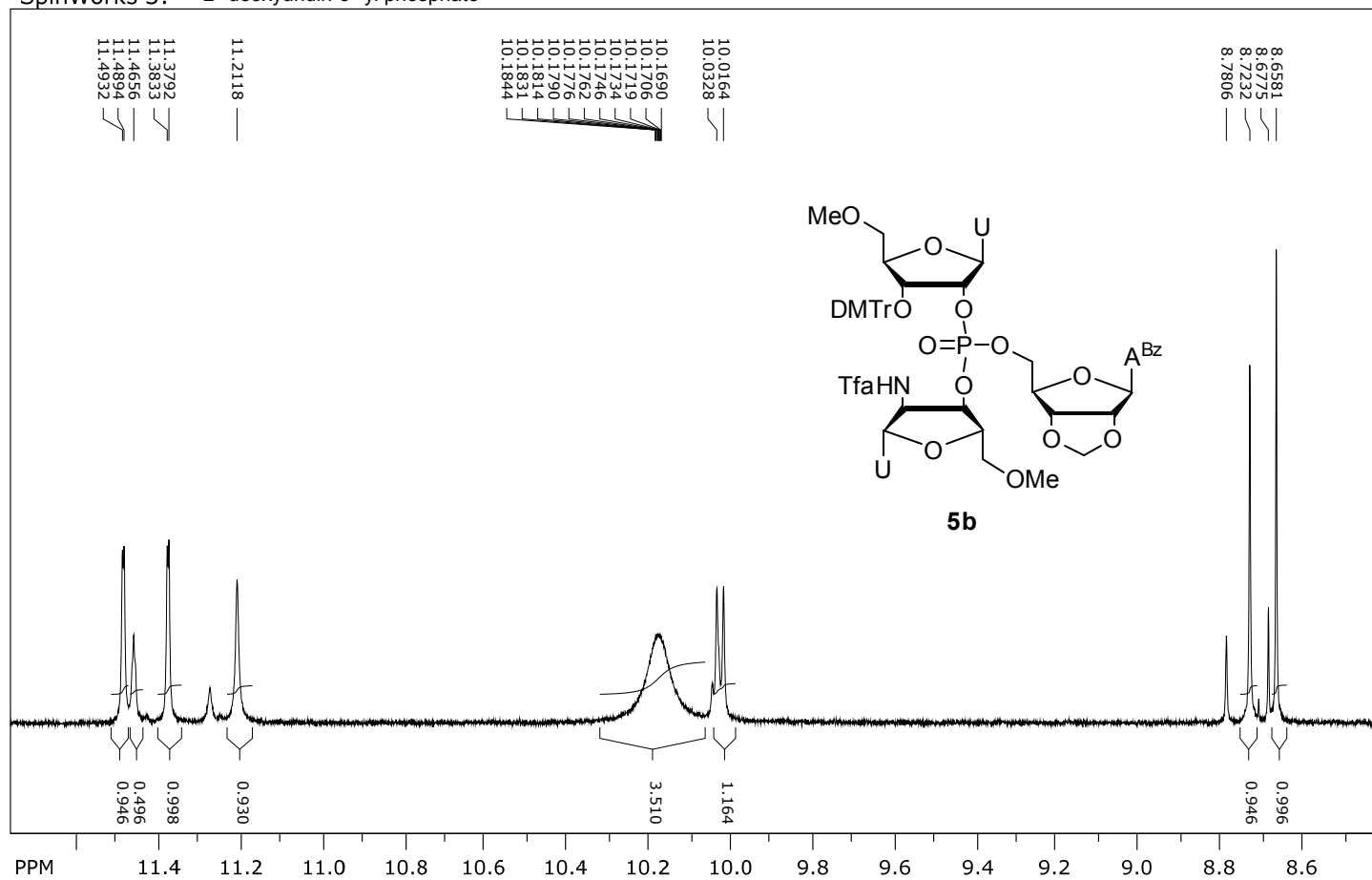


Fig. S4 UV spectrum of 7.

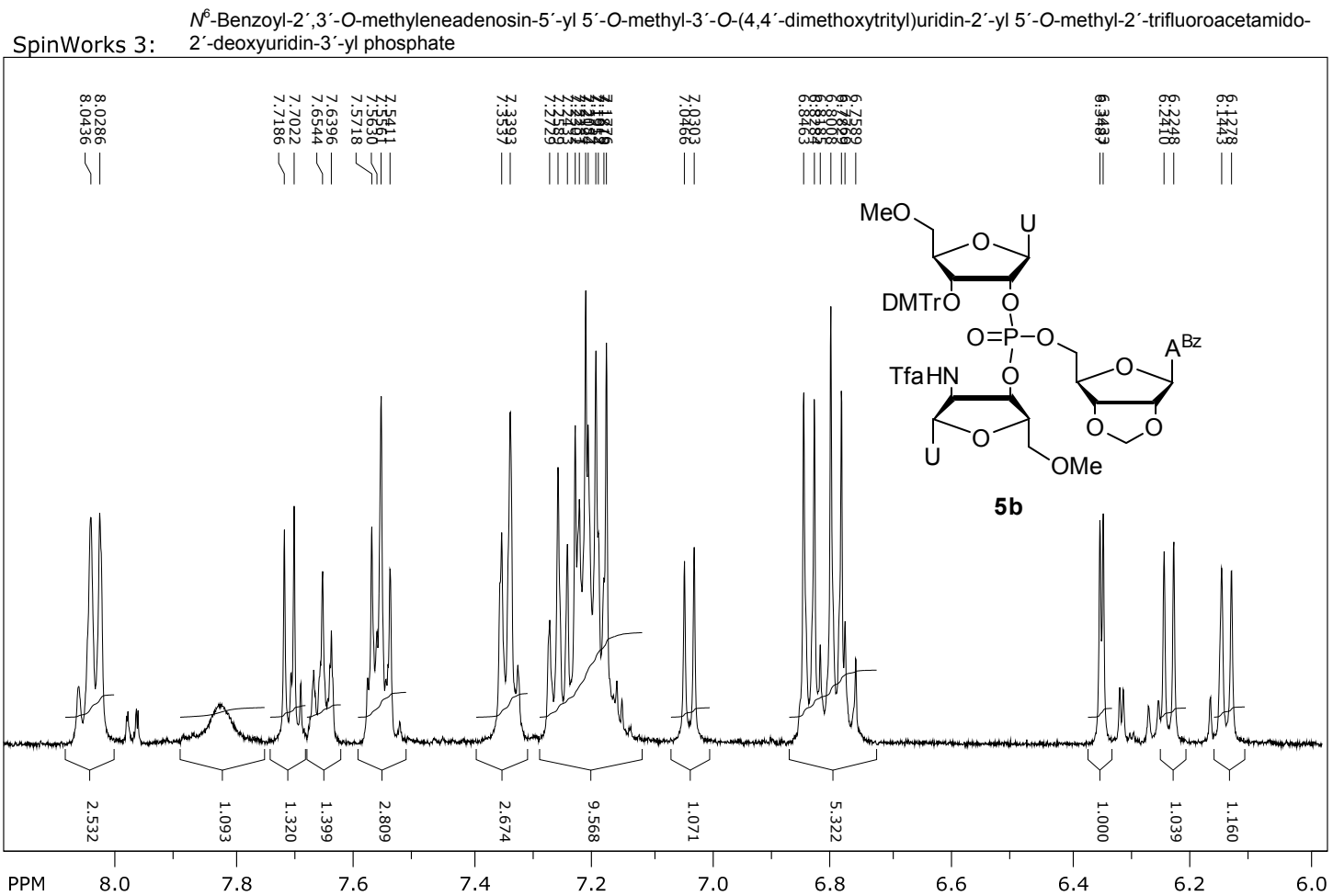


SpinWorks 3: N⁶-Benzoyl-2',3'-O-methyleneadenosin-5'-yl 5'-O-methyl-3'-O-(4,4'-dimethoxytrityl)uridin-2'-yl 5'-O-methyl-2'-trifluoroacetamido-2'-deoxyuridin-3'-yl phosphate



file: ...trit\fosfaatti,1keskimmäinen\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

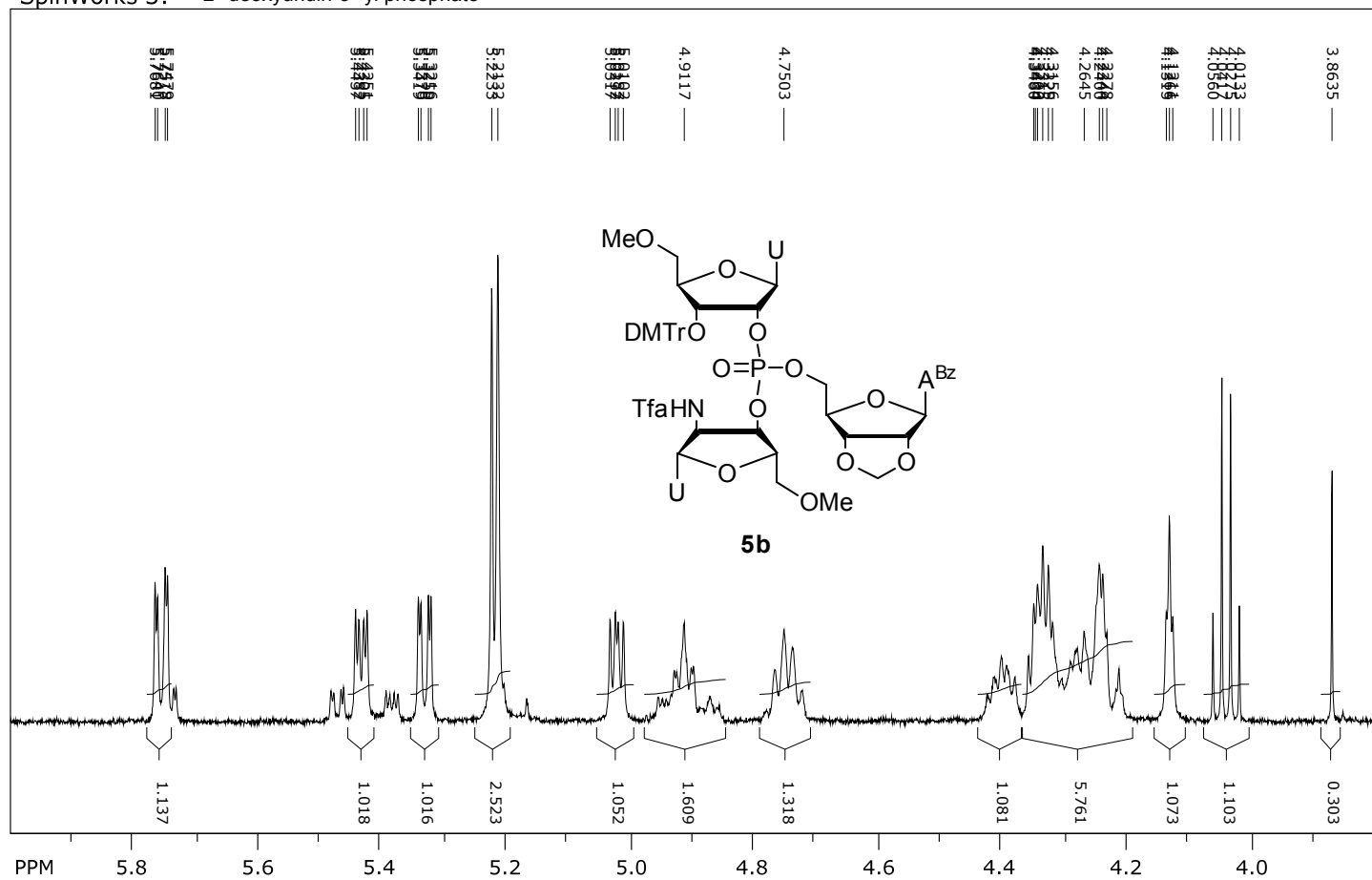
freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 67.262 ppm/cm: 0.13449



file: ...trit(fosfaatti,1keskimmäinen\1)fid expt: <zg30>
 transmitter freq.: 500.133089 MHz
 time domain size: 65536 points
 width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
 number of scans: 16

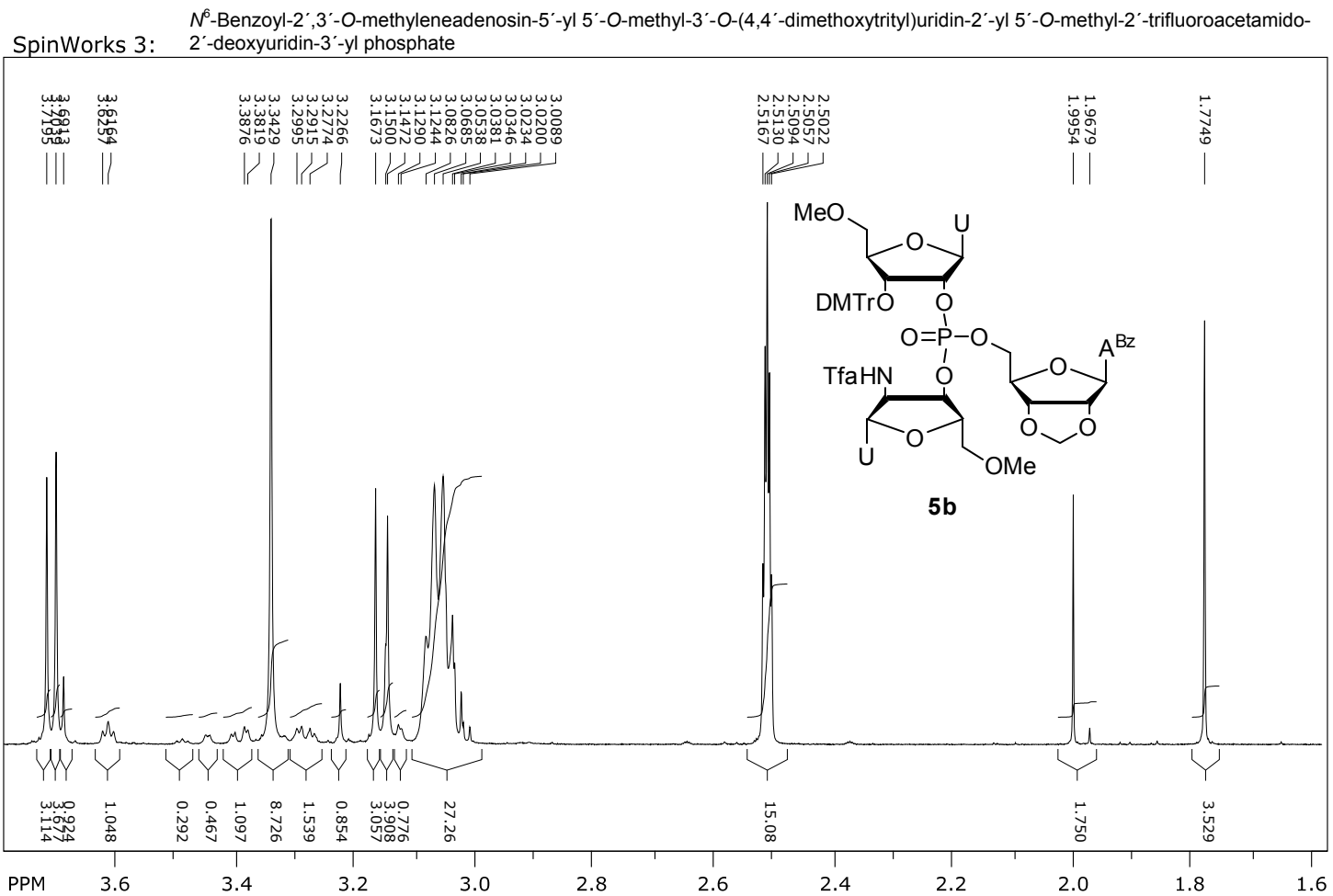
freq. of 0 ppm: 500.130000 MHz
 processed size: 32768 complex points
 LB: 0.000 GF: 0.0000
 Hz/cm: 44.301 ppm/cm: 0.08858

SpinWorks 3: N⁶-Benzoyl-2',3'-O-methyleneadenosin-5'-yl 5'-O-methyl-3'-O-(4,4'-dimethoxytrityl)uridin-2'-yl 5'-O-methyl-2'-trifluoroacetamido-2'-deoxyuridin-3'-yl phosphate



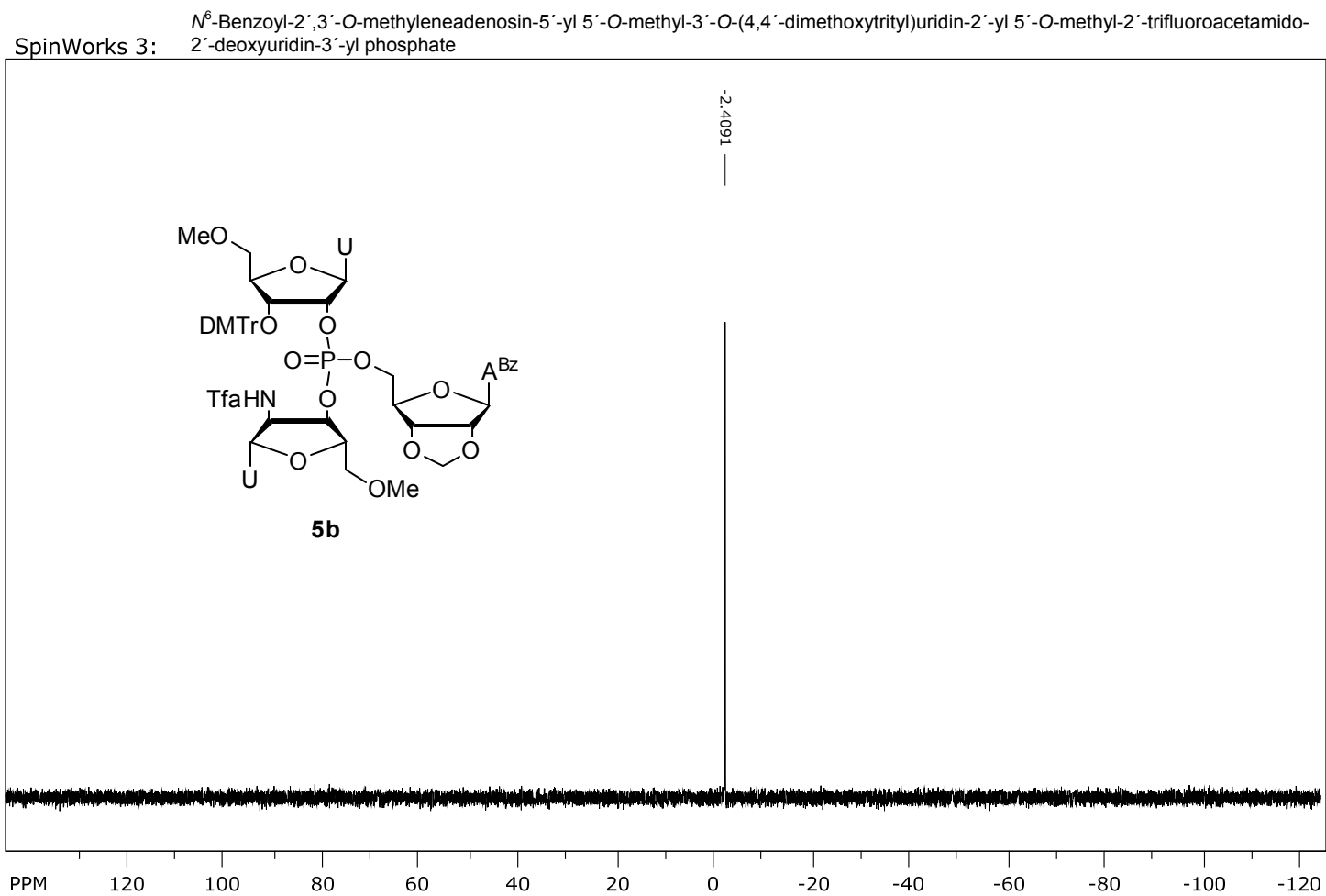
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time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 44.301 ppm/cm: 0.08858



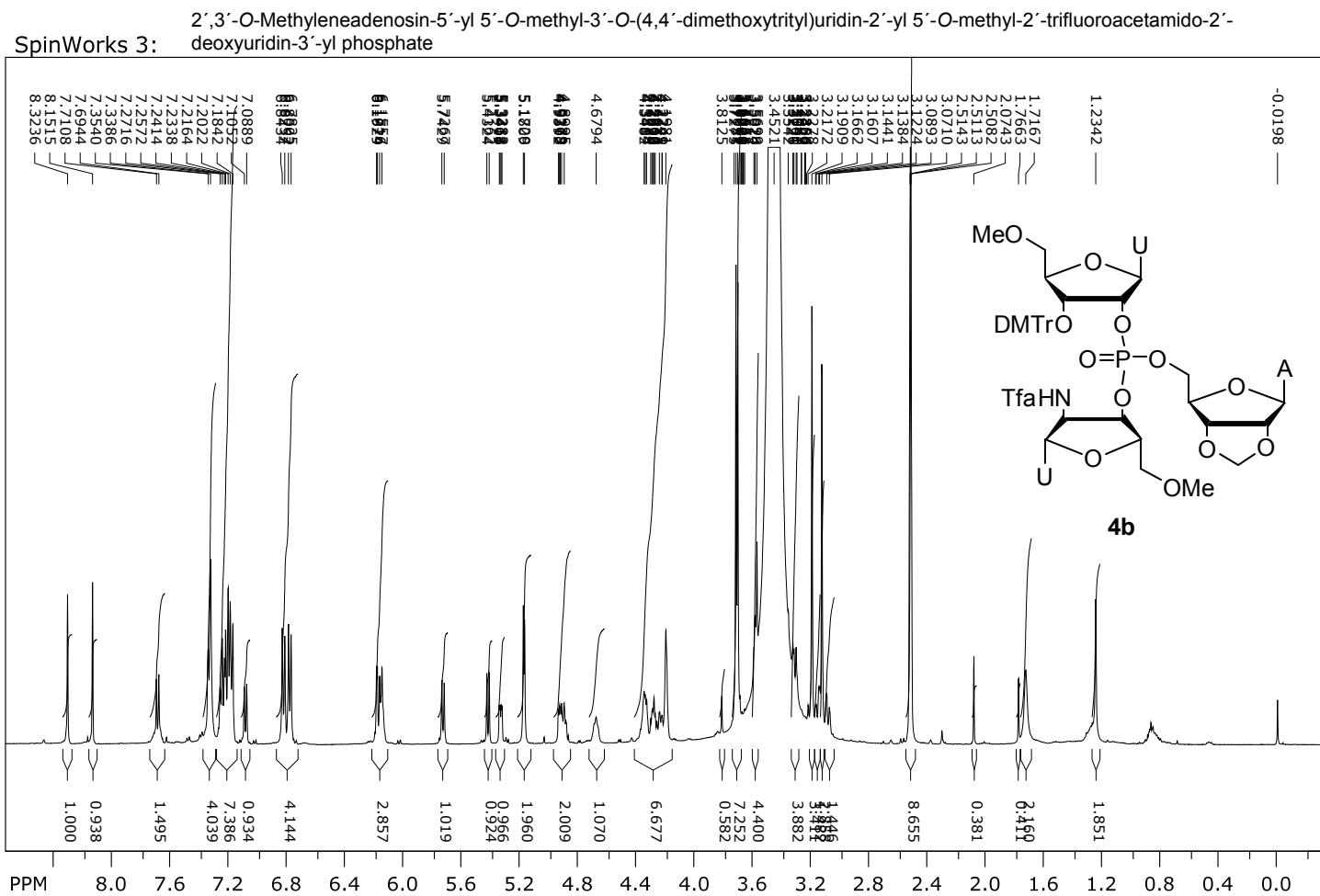
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 time domain size: 65536 points
 width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
 number of scans: 16

freq. of 0 ppm: 500.130000 MHz
 processed size: 32768 complex points
 LB: 0.000 GF: 0.0000
 Hz/cm: 44.301 ppm/cm: 0.08858



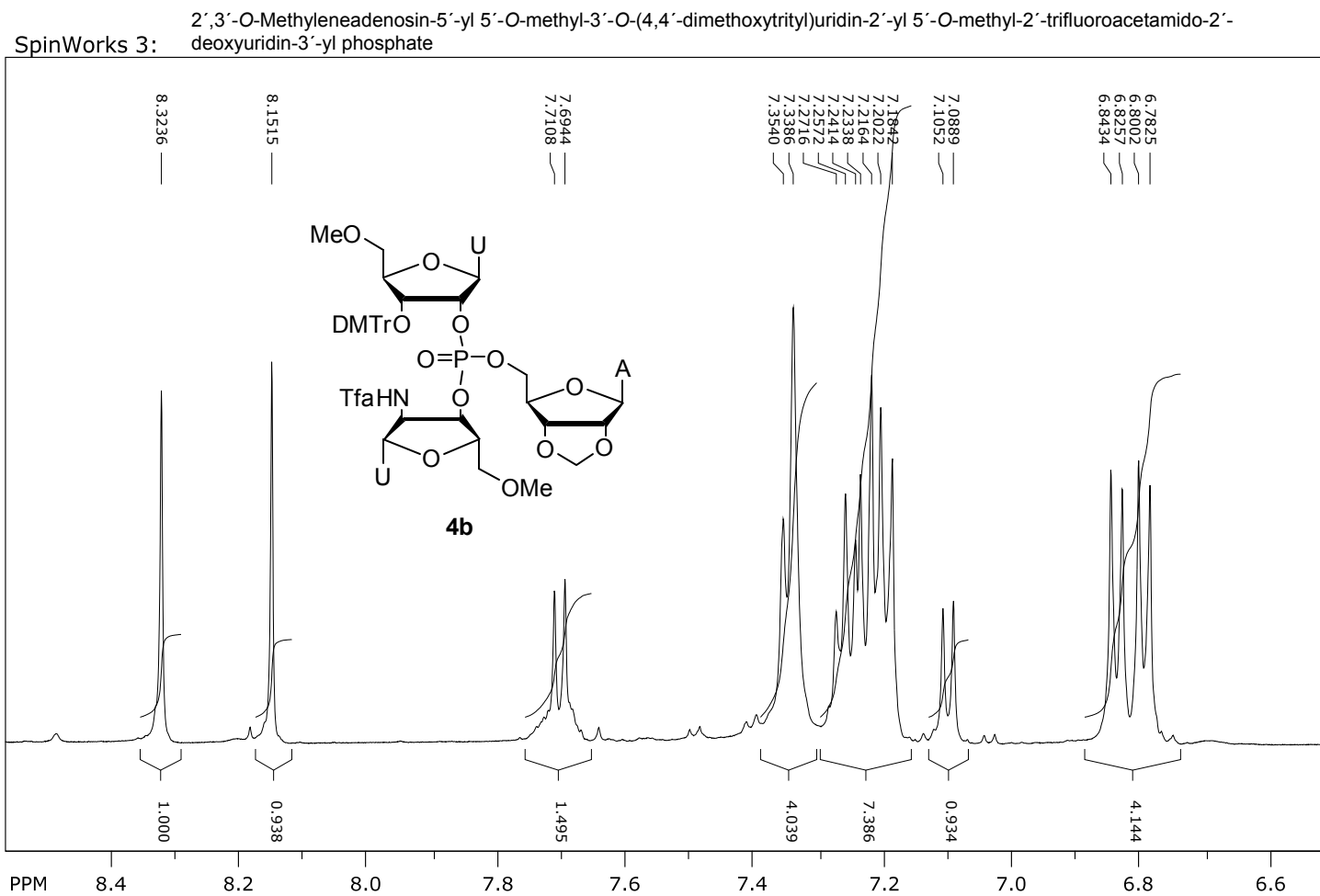
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transmitter freq.: 202.446187 MHz
time domain size: 65536 points
width: 80645.16 Hz = 398.3536 ppm = 1.230548 Hz/pt
number of scans: 154

freq. of 0 ppm: 202.456310 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 2185.381 ppm/cm: 10.79487



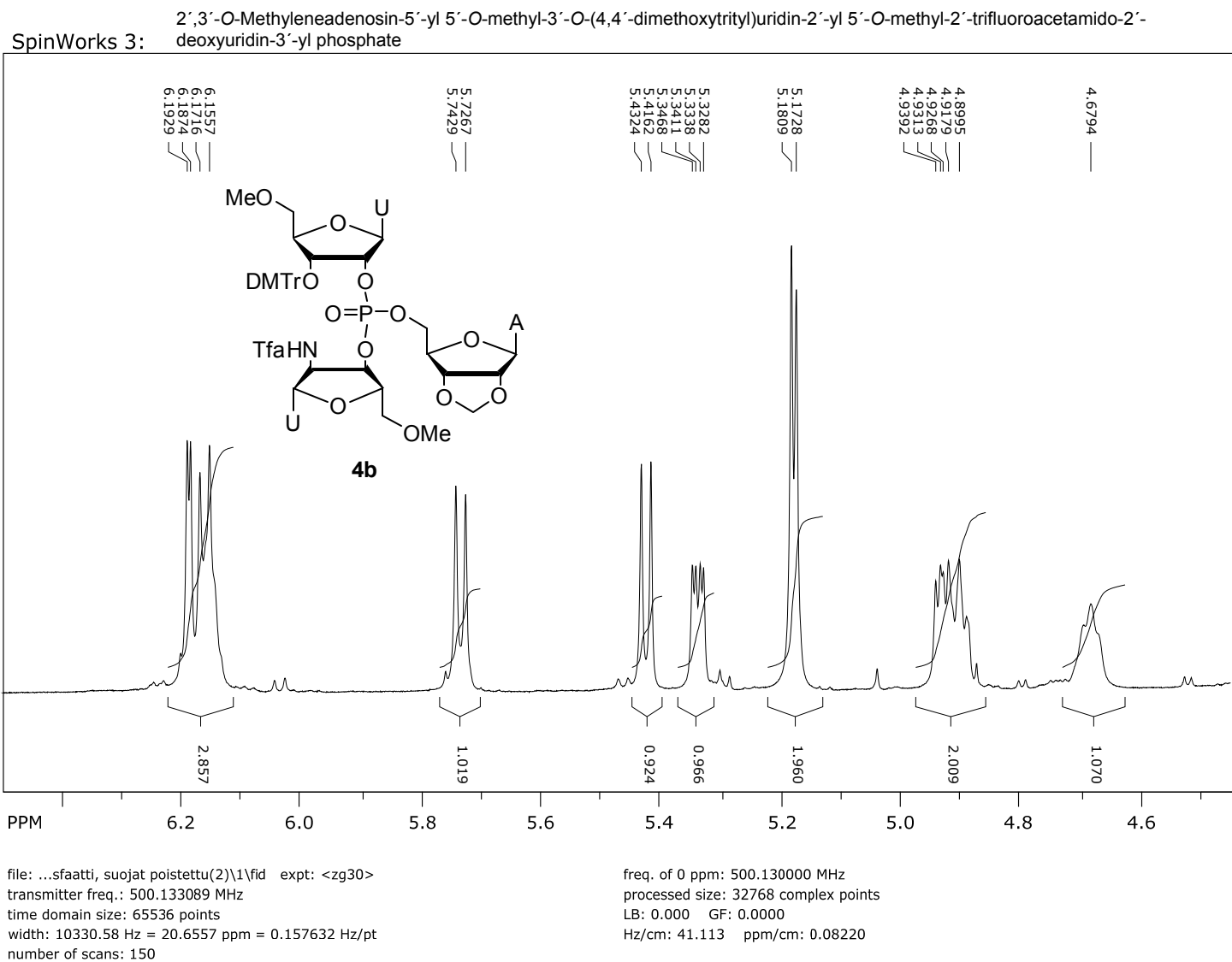
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 time domain size: 65536 points
 width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
 number of scans: 150

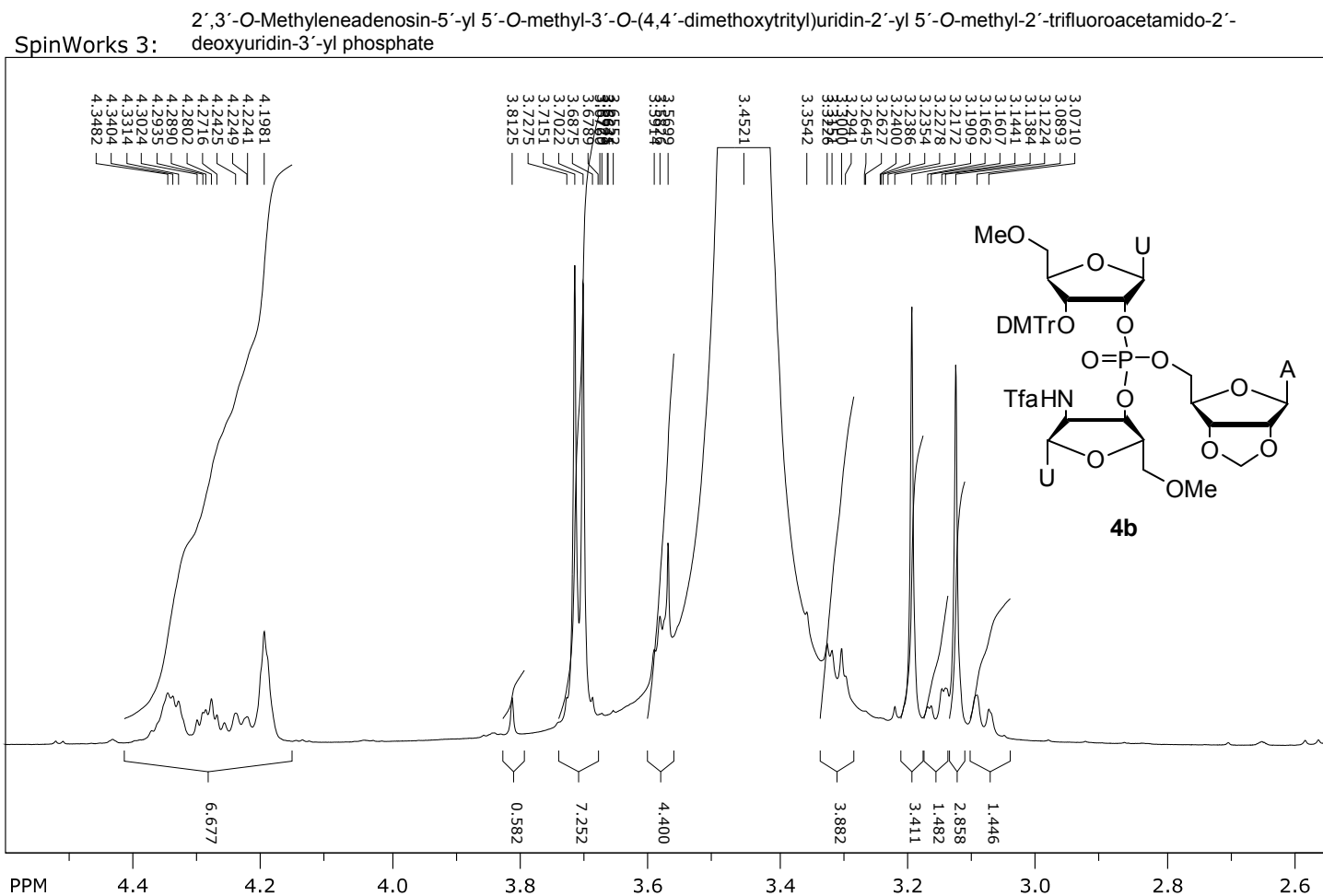
freq. of 0 ppm: 500.130000 MHz
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 LB: 0.000 GF: 0.0000
 Hz/cm: 181.856 ppm/cm: 0.36361



file: ...sfaatti, suojat poistettu(2)\1\fid expt: <zg30>
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time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 150

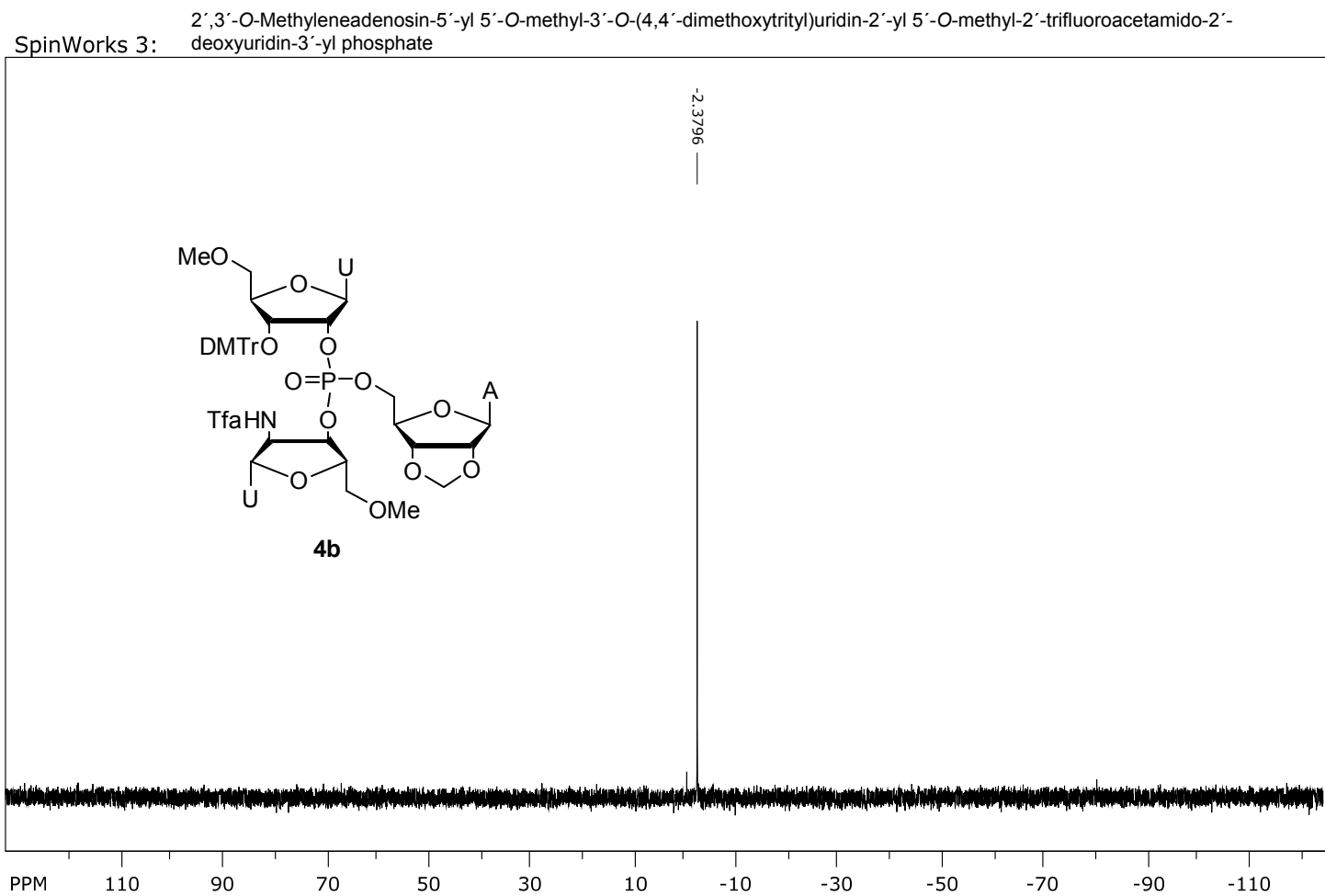
freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 41.113 ppm/cm: 0.08220





file: ...sfaatti, suojat poistettu(2)\1\fid expt: <zg30>
 transmitter freq.: 500.133089 MHz
 time domain size: 65536 points
 width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
 number of scans: 150

freq. of 0 ppm: 500.130000 MHz
 processed size: 32768 complex points
 LB: 0.000 GF: 0.0000
 Hz/cm: 41.113 ppm/cm: 0.08220



file: ...faatti, suojat poistettu(2)\31\fid exp: <zpgg30>
transmitter freq.: 202.446187 MHz
time domain size: 65536 points
width: 80645.16 Hz = 398.3536 ppm = 1.230548 Hz/pt
number of scans: 200

freq. of 0 ppm: 202.456310 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 2088.144 ppm/cm: 10.31457

Fig. S5 HPLC chromatogram of **4b** [Hypersil-Keystone Aquasil C18 column (4 × 150 mm, 5 μm); flow rate = 1 mL min⁻¹; 60 mM acetate buffer (pH = 4.3) and a linear gradient of 3→50% MeCN during 60 min, then 50% MeCN for 20 min].

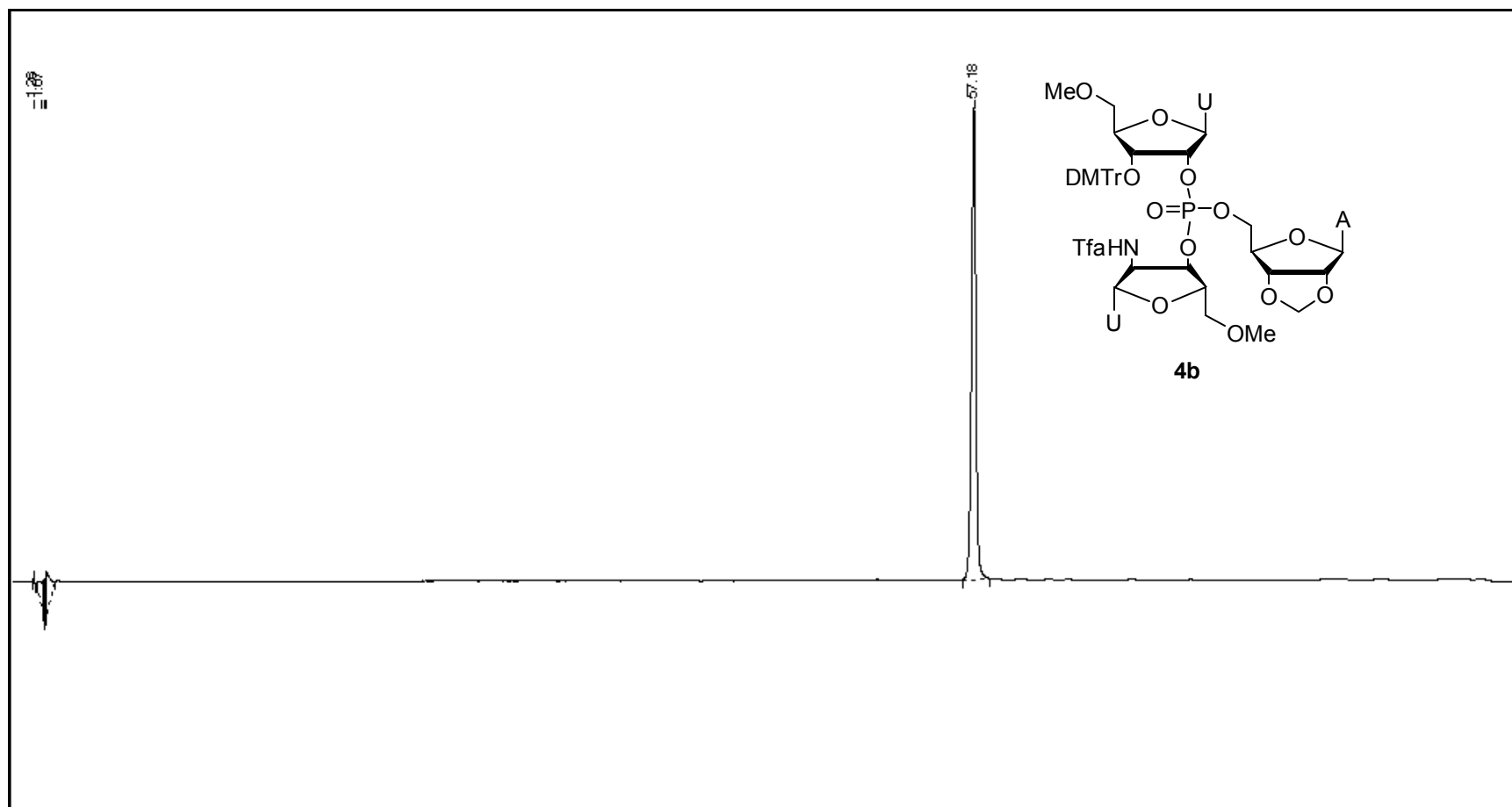


Fig. S6 UV spectrum of 4b.

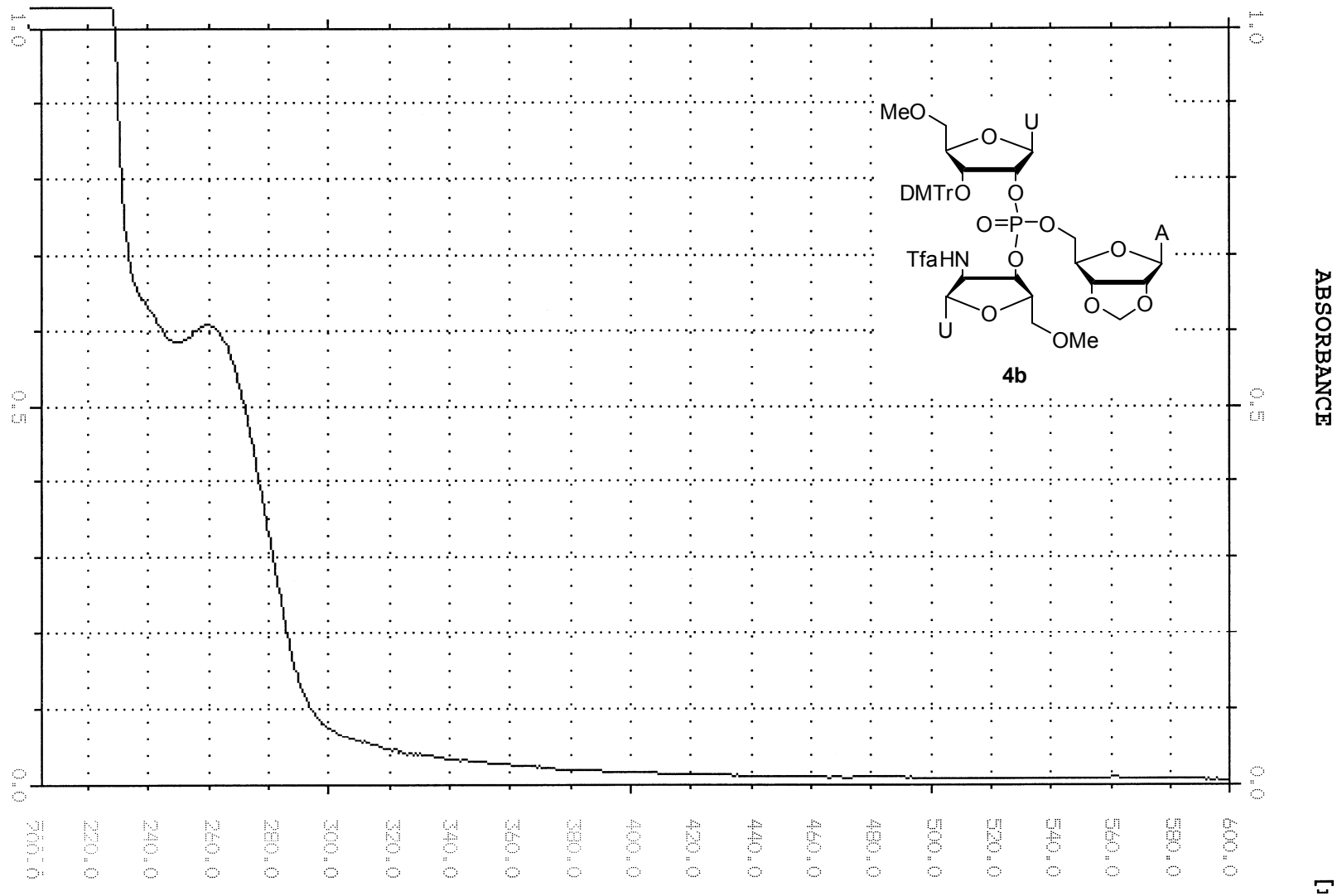


Table S1 Observed pseudo first-order rate constants and product distributions for the hydrolysis of **1a, b**.

pH	[buffer] / mmol L ⁻¹	$k_{\text{obs}} / 10^{-4} \text{ s}^{-1}$	$k_{\text{B}} / (k_{\text{A}} + k_{\text{B}})$
0.02	-	39 ± 2	0.6 ± 0.1
0.39	-	11 ± 3	0.64 ± 0.07
1.00	-	5.6 ± 0.3	0.60 ± 0.06
1.37	-	2.8 ± 0.1	0.62 ± 0.03
2.00	-	3.3 ± 0.5	0.62 ± 0.06
2.81	47.5	4.3 ± 0.4	
	95.0	5.2 ± 0.1	0.60 ± 0.02
	190.0	7.2 ± 0.2	
3.37	47.5	6.2 ± 0.1	
	95.0	8.0 ± 0.2	
	142.5	9.5 ± 0.3	0.61 ± 0.04
	190.0	12.9 ± 0.3	
4.30	47.5	14.4 ± 0.2	
	95.0	22.4 ± 0.6	
	142.5	36 ± 1	0.60 ± 0.06
	190.0	44 ± 3	
4.75	47.5	23.7 ± 0.7	
	95.0	36 ± 1	
	142.5	45 ± 1	0.65 ± 0.02
	190.0	59 ± 2	
5.82	47.5	58 ± 2	0.74 ± 0.08
6.27	47.5	138 ± 7	0.94 ± 0.02
6.73	47.5	270 ± 10	0.98 ± 0.03
11.73	-	-	1.00

Fig. S7 HPLC chromatogram of a reaction solution of the hydrolysis of **1a** and **1b** [Hypersil-Keystone Aquasil C18 column (4 × 150 mm, 5 μm); flow rate = 1 mL min⁻¹; 60 mM formate buffer (pH = 3.0) and a linear gradient of 3→30% MeCN during 40 min].

