# New Biocides Based on $N^4$-alkylcytidines: Effects on Microorganisms and Application for the Protection of Cultural Heritage Objects of Painting

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[4] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, 16/10 Miklukho-Maklaya str., Moscow, 117997, Russia; kid1968@yandex.ru (I.D.K.), berzina_maria@mail.ru (M. Ya. B.)

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Table S1. Minimal bacterial growth inhibitory concentration (μg/ml) of $N^4$-alkylcytidine derivatives

| **Bacteria** / **Compounds** | *Bacillus subtilis* ATCC 6633 | *Staphylococcus aureus* FDA 209P | *Staphylococcus aureus* INA 00761 | *Micrococcus luteus* NCTC 8340 | *Leuconostoc mesenteroides* VKPM B-4177 | *Mycobacterium smegmatis* VKPM Ac 1339 | *Mycobacterium smegmatis* $mc^2155$ |
|---|---|---|---|---|---|---|---|
| **1b** | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| **2a** | 64 | 32 | 32 | 32 | 32 | 32 | 32 |
| **2b** | 8 | 8 | 8 | 8 | 8 | 16 | 16 |
| **2c** | 16 | 16 | 8 | 8 | 16 | 8 | 16 |
| **2d** | 32 | 8 | 8 | 16 | 16 | 32 | 32 |
| **2e** | 4 | 4 | 8 | 16 | 16 | 16 | 16 |
| **3a** | >64 | >64 | >64 | >64 | >64 | >64 | >64 |
| **3b** | 8 | 8 | 8 | 32 | 8 | 32 | 32 |
| **3c** | >64 | >64 | >64 | >64 | >64 | >64 | >64 |
| **12a,12b** | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| Antibiotics used in clinics* | AN 4 | AN 2, CIP 4, OX 1 | AN 30, CIP 4, OX 32 | AN 1.7 | VA>400 | AN 30 CIP 4, RFP 8, INZ 0.25 | AN 85 RFP 4, INZ 4 |

*Amikacin (AN), Ciprofloxacin (CIP), Isoniazid (INZ), Rifampicin (RFP), Oxacillin (OX), Vancomycin (VA).

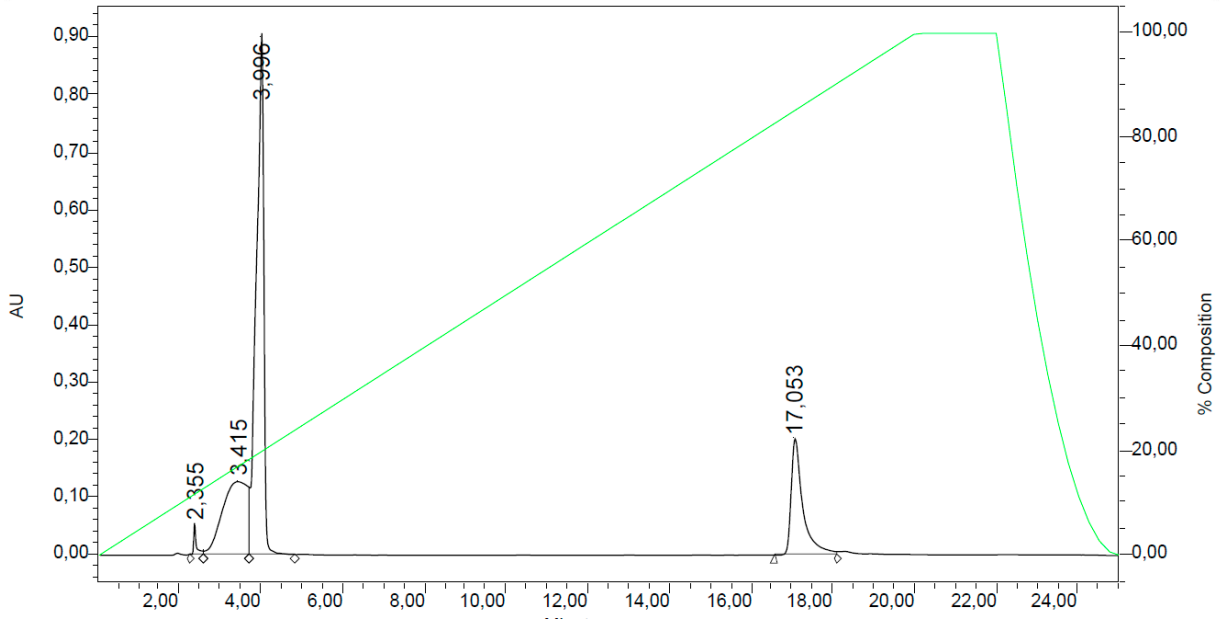
Figure S1. Enzymatic glycosylation of $N^4$-dodecyl-6-methylcytosine (**12b**)

BT
Project Name: TESTLast_1
Reported by User: System

Breeze

SAMPLE INFORMATION

| | | | |
|---|---|---|---|
| Sample Name: | MBf-67 19h + Osk-5 | Acquired By: | System |
| Sample Type: | Unknown | Date Acquired: | 27.06.2023 11:54:02 |
| Vial: | 1 | Acq. Method: | 100B_dual_280 |
| Injection #: | 1 | Date Processed: | 27.06.2023 12:22:49 |
| Injection Volume: | 10,00 ul | Channel Name: | 2487Channel 2 |
| Run Time: | 25,00 Minutes | Sample Set Name: | |
| Column Type: | | | |



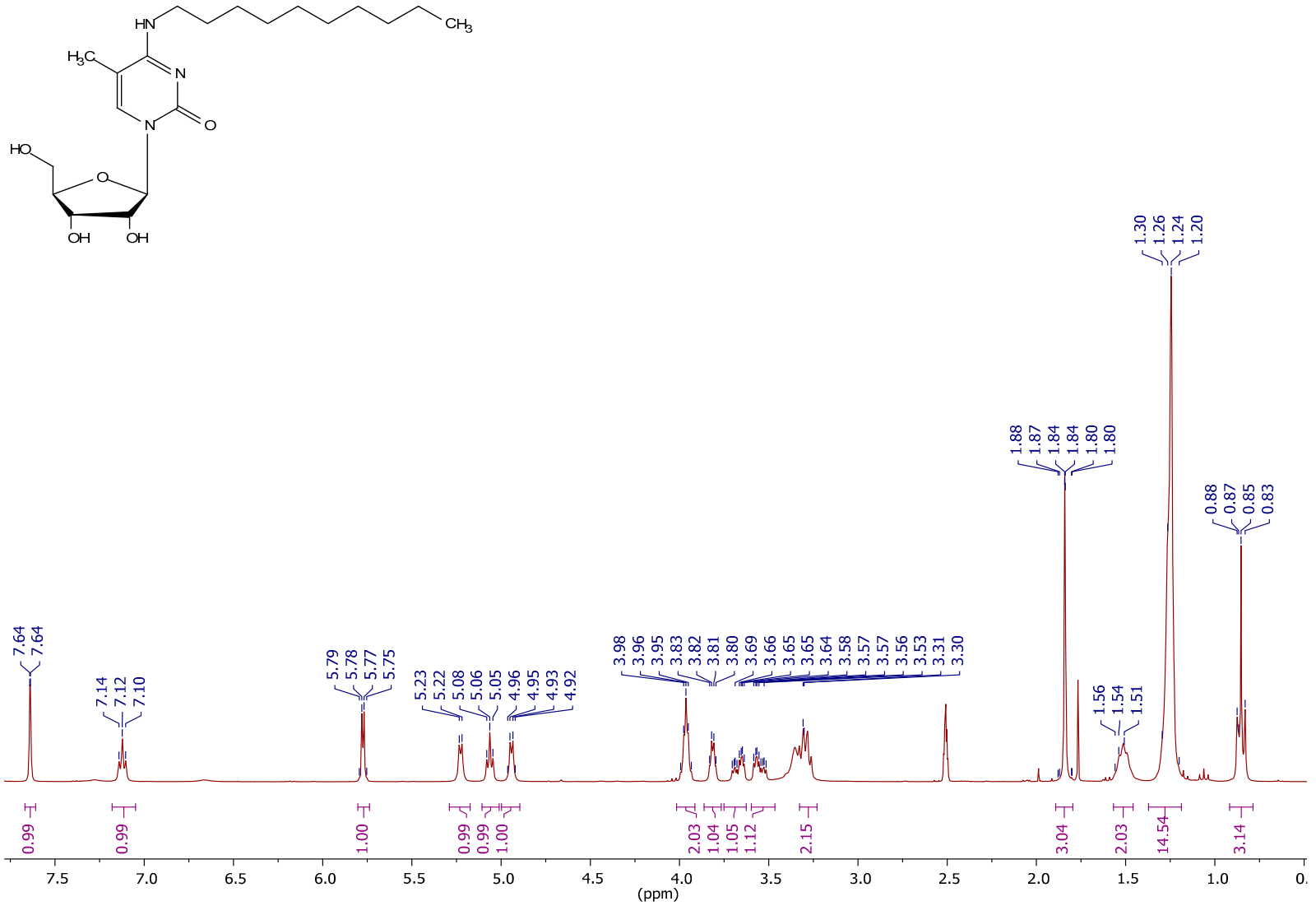

| | RT (min) | Area (µV*sec) | % Area | Height (µV) | % Height |
|---|---|---|---|---|---|
| 1 | 2,355 | 271420 | 1,27 | 51987 | 4,03 |
| 2 | 3,415 | 5621924 | 26,28 | 127774 | 9,90 |
| 3 | 3,996 | 11401358 | 53,29 | 907931 | 70,38 |
| 4 | 17,053 | 4098894 | 19,16 | 202367 | 15,69 |

Figure S1. Chromatogram of reaction mixture with purine nucleoside phosphorylase (PNP). Reaction involving adenosine (peak Rt 3.9 minutes) and $N^4$-dodecyl-6-methylcytosine (**12b**) (Rt 17.0 minutes). Adenine is also present in the reaction mixture (Rt 3.4 minutes), the peak is broadened due to the presence of DMF.

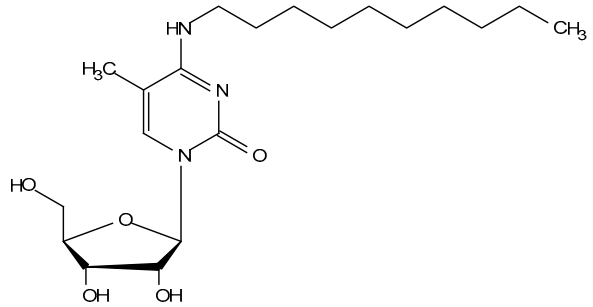
Figure S2. NMR spectra of new compounds

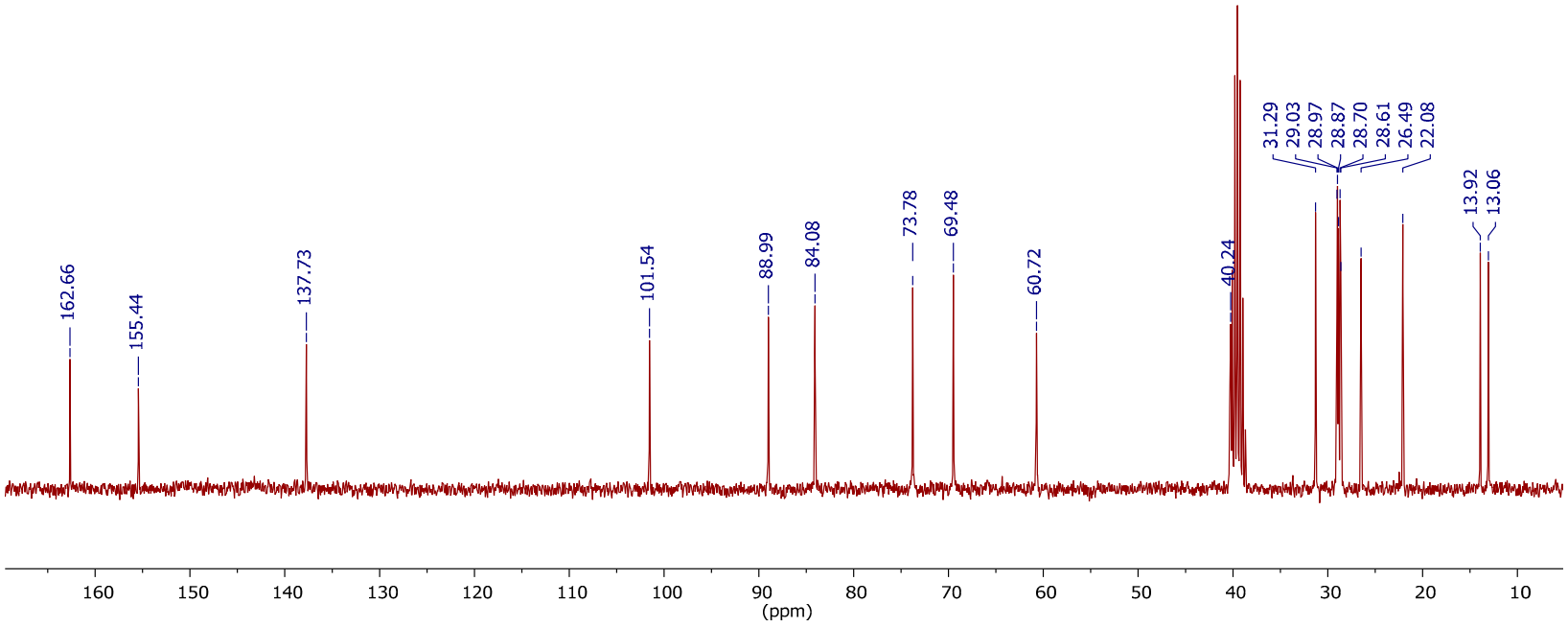
**1) *N*⁴-decyl-5-methylcytidine (2a)**

**$^1$H**



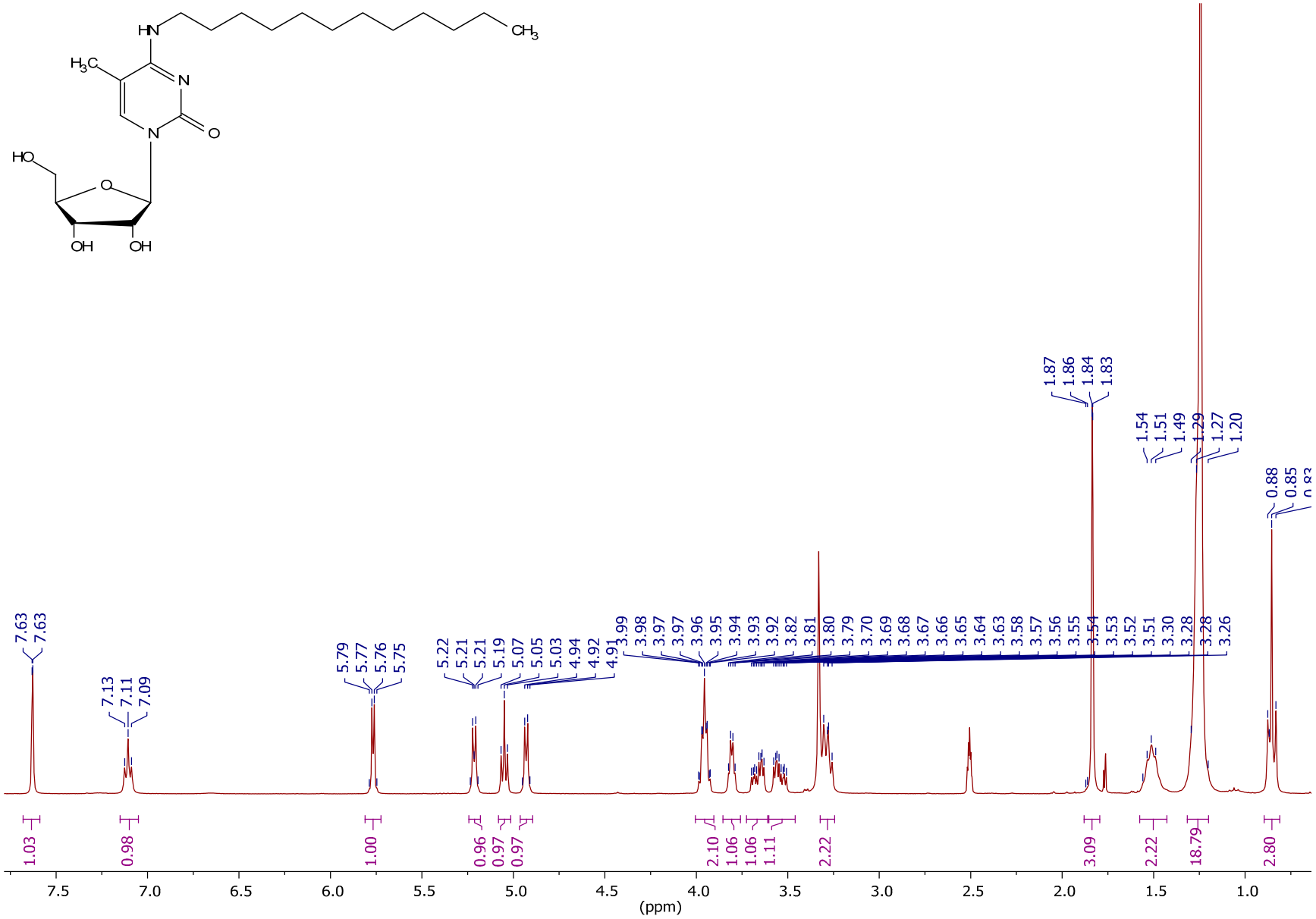
**$^{13}C$**


HN
CH3
H3C
N
N
O
HO
O
OH
OH

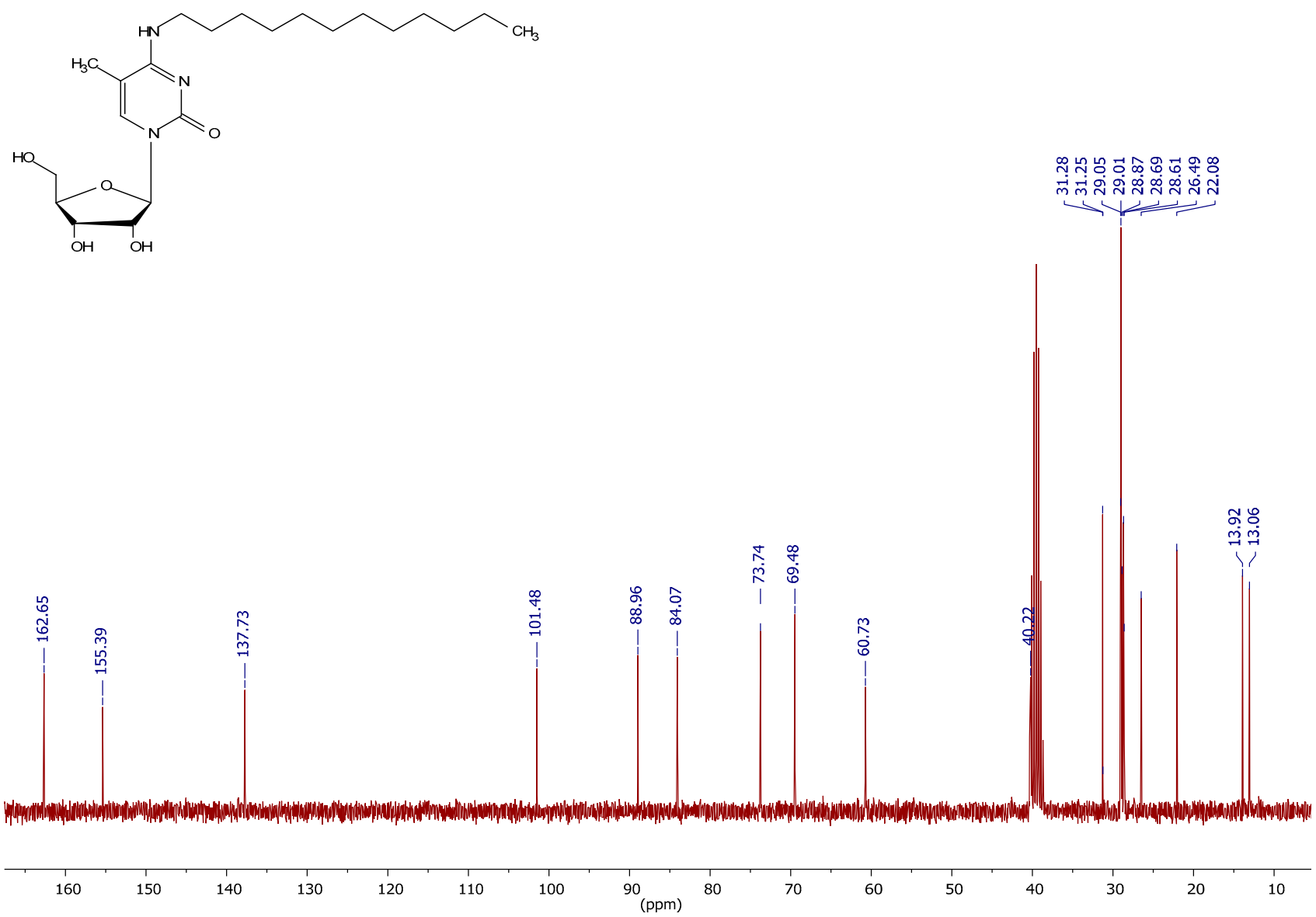

162.66
155.44
137.73
101.54
88.99
84.08
73.78
69.48
60.72
40.24
31.29
29.03
28.97
28.87
28.70
28.61
26.49
22.08
13.92
13.06
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

## 2) $N^4$-dodecyl-5-methylcytidine (2b)

**$^1$H**

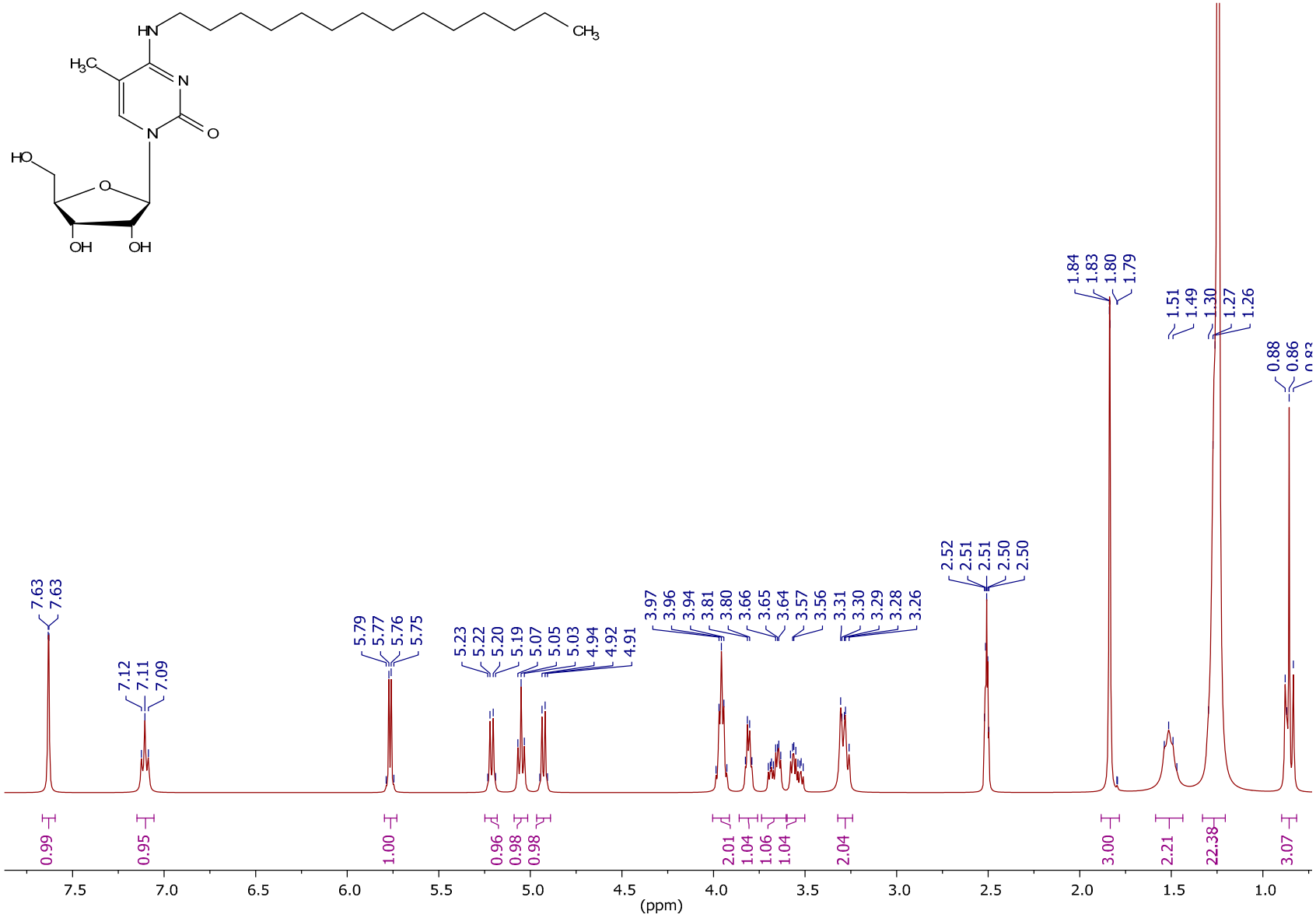


$^{13}C$

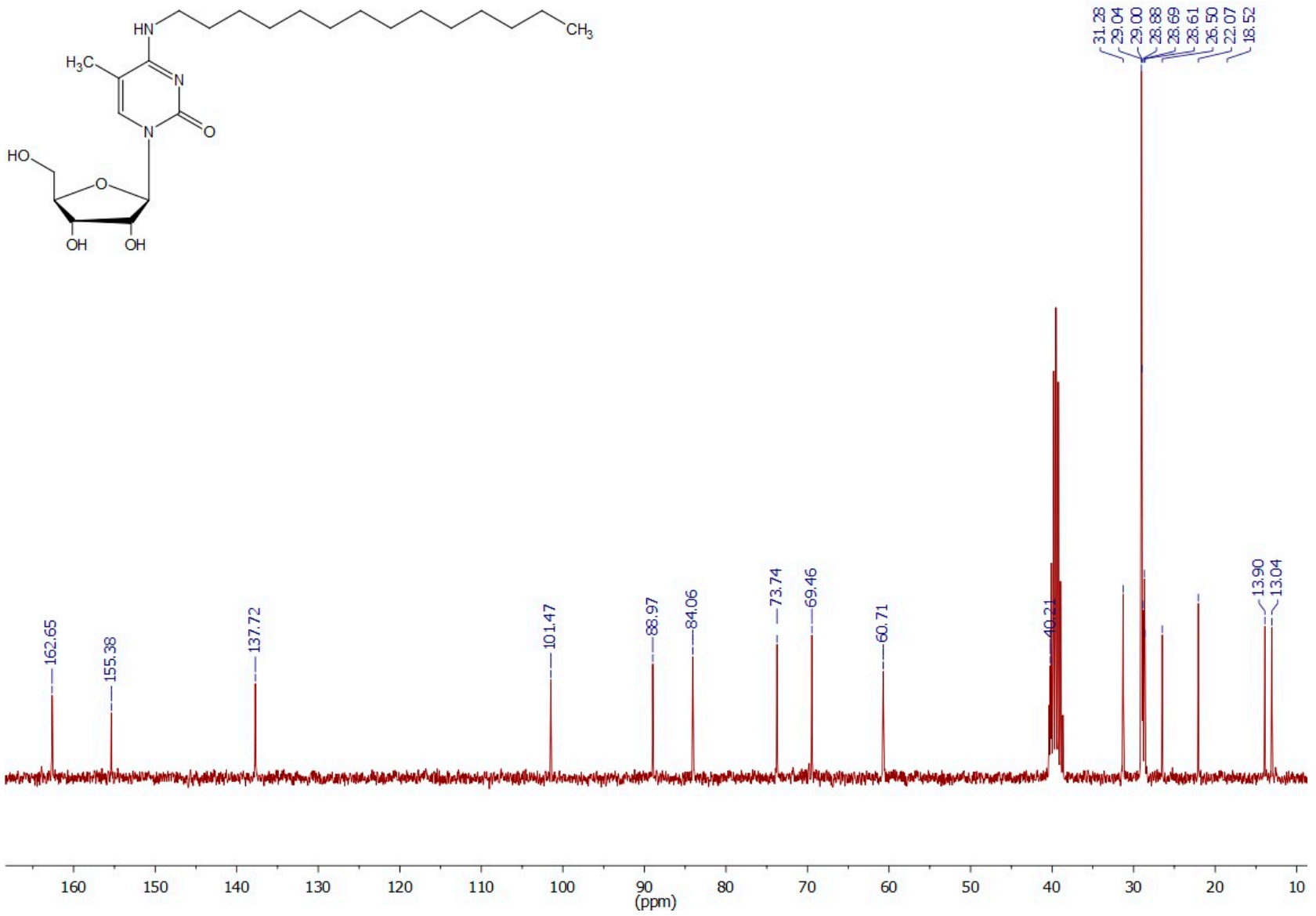

HN
CH3
H3C
N
N
O
HO
O
OH
OH
31.28
31.25
29.05
29.01
28.87
28.69
28.61
26.49
22.08
13.92
13.06
162.65
155.39
137.73
101.48
88.96
84.07
73.74
69.48
60.73
40.22
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

### 3) $N^4$-tetradecyl-5-methylcytidine (2c)

$^1$H

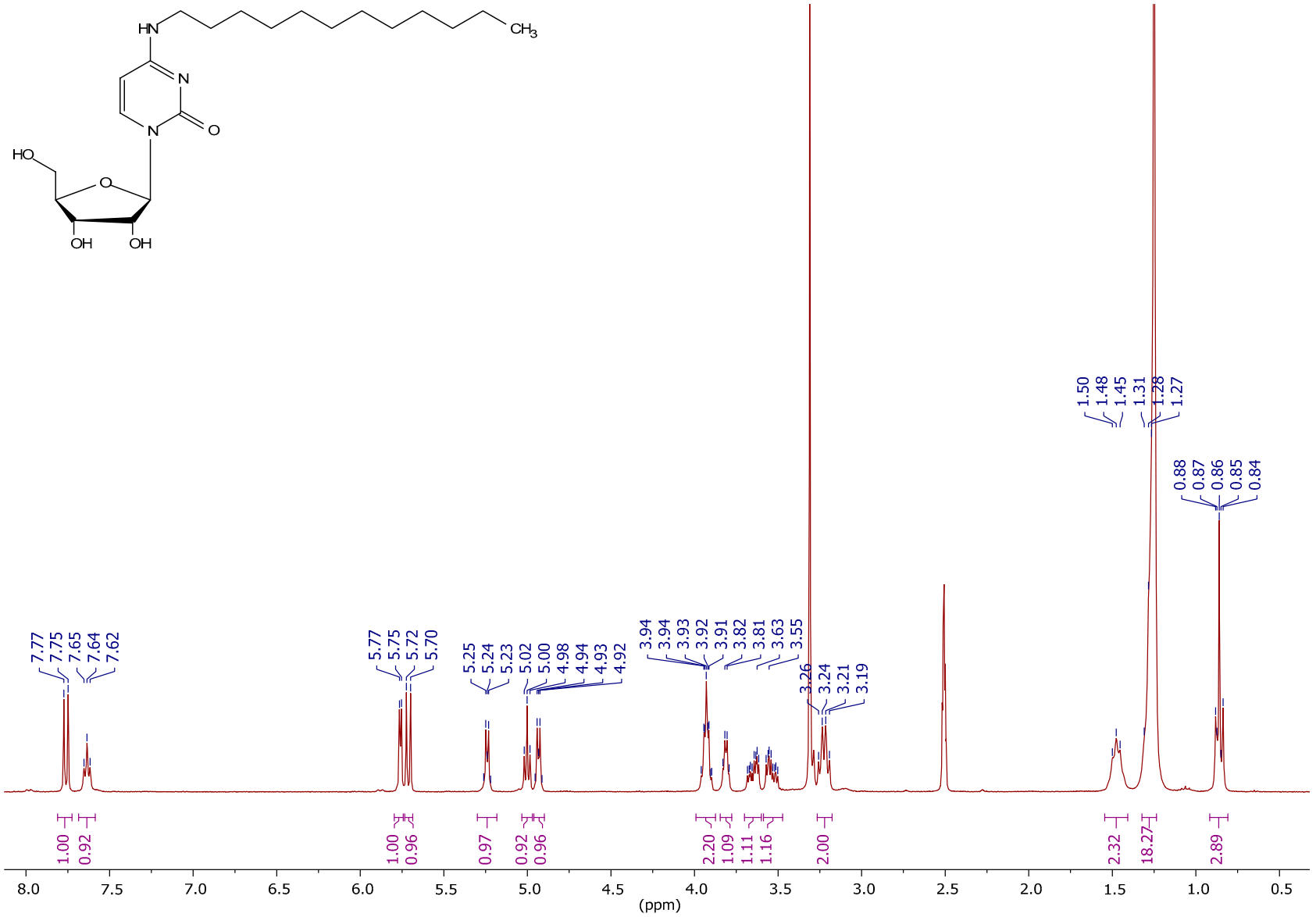


$^{13}C$

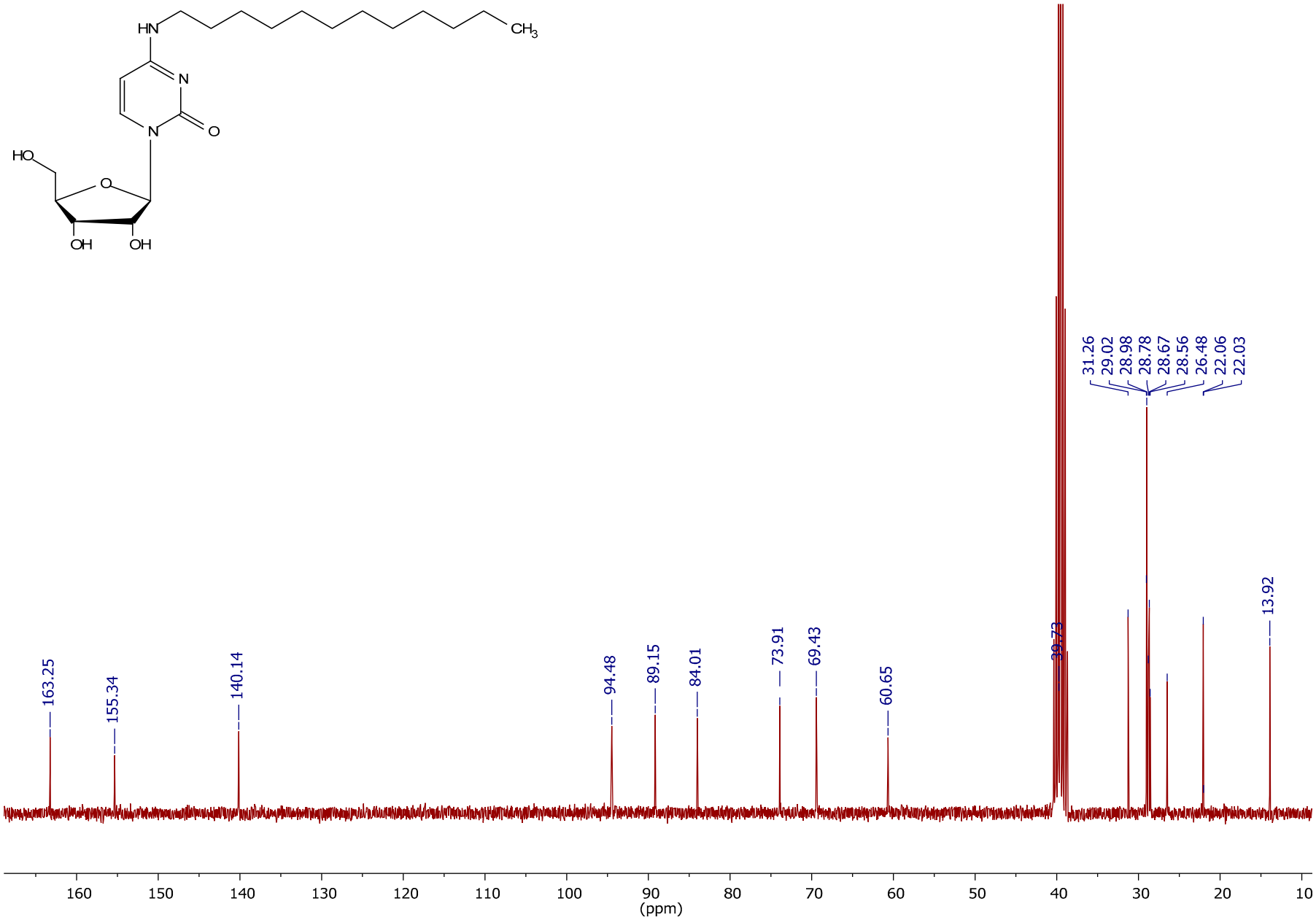

HN
CH3
H3C
N
N
O
HO
O
OH
OH
31.28
29.04
29.00
28.88
28.69
28.61
26.50
22.07
18.52
162.65
155.38
137.72
101.47
88.97
84.06
73.74
69.46
60.71
40.21
13.90
13.04
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

## 4) $N^4$-dodecylcytidine (2d)

**$^1$H**

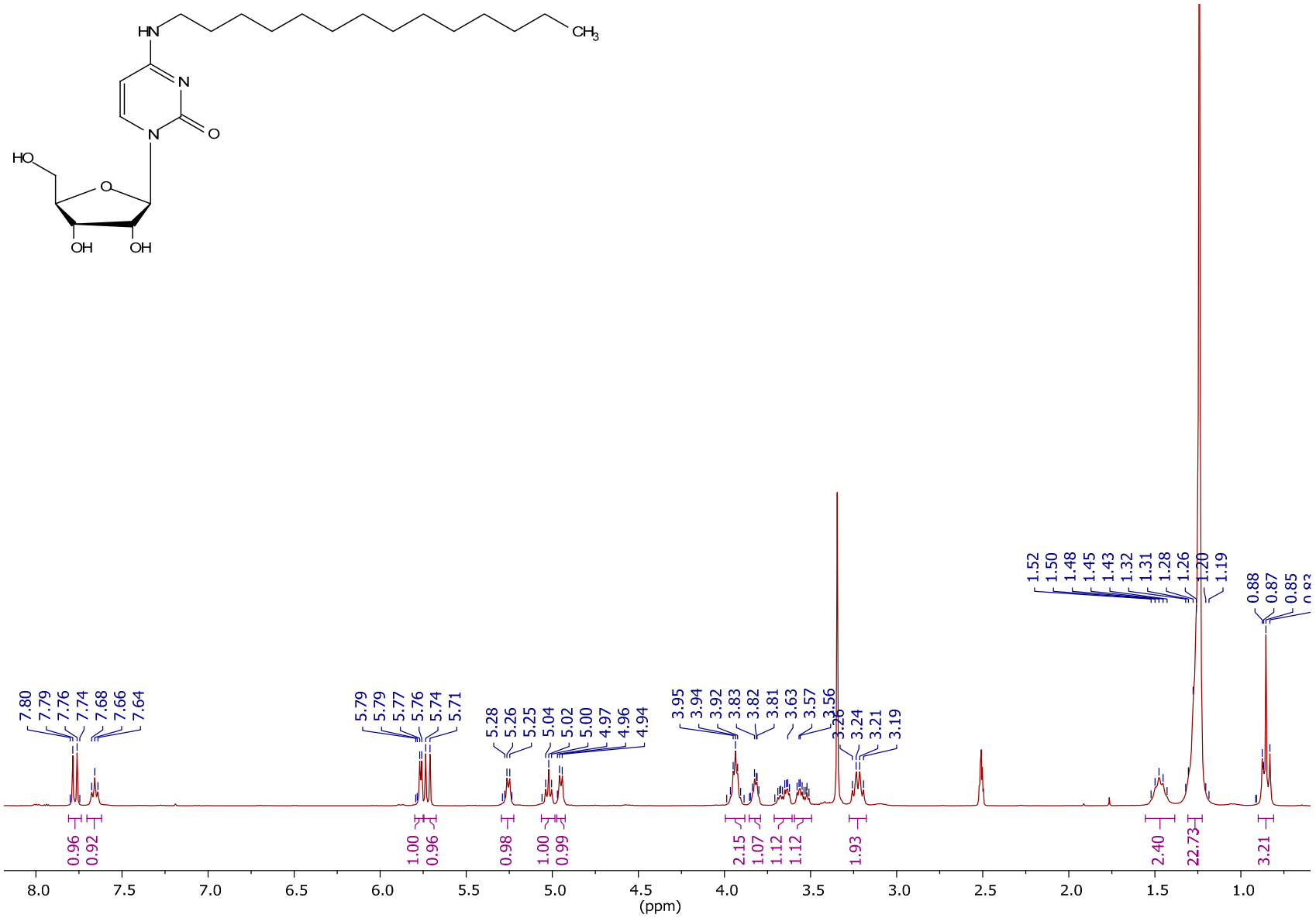


$^{13}C$

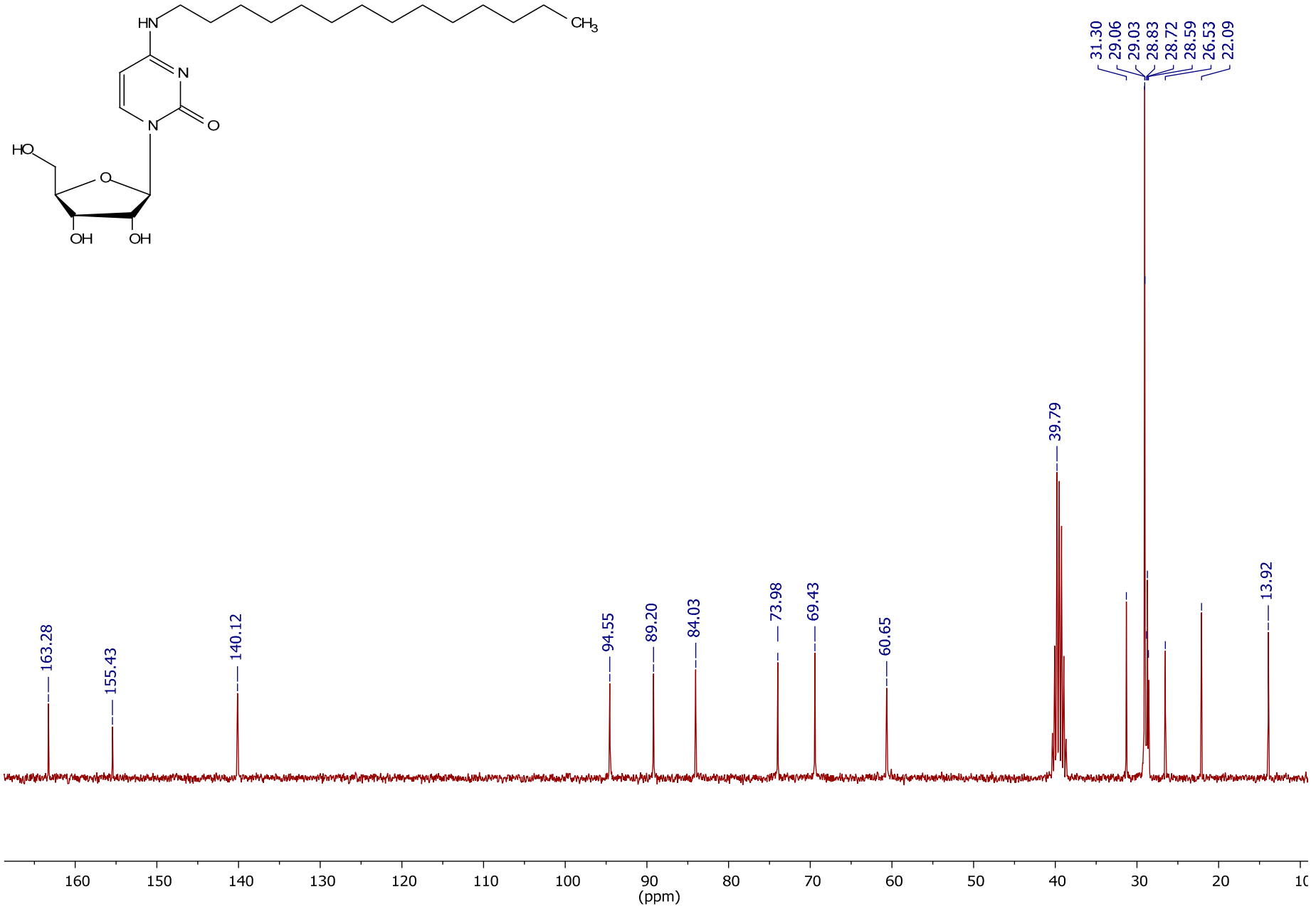

HN
CH3
N
N
O
HO
O
OH
OH
163.25
155.34
140.14
94.48
89.15
84.01
73.91
69.43
60.65
39.73
31.26
29.02
28.98
28.78
28.67
28.56
26.48
22.06
22.03
13.92
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

## 5) $N^4$-tetradecylcytidine (2e)

$^1$H

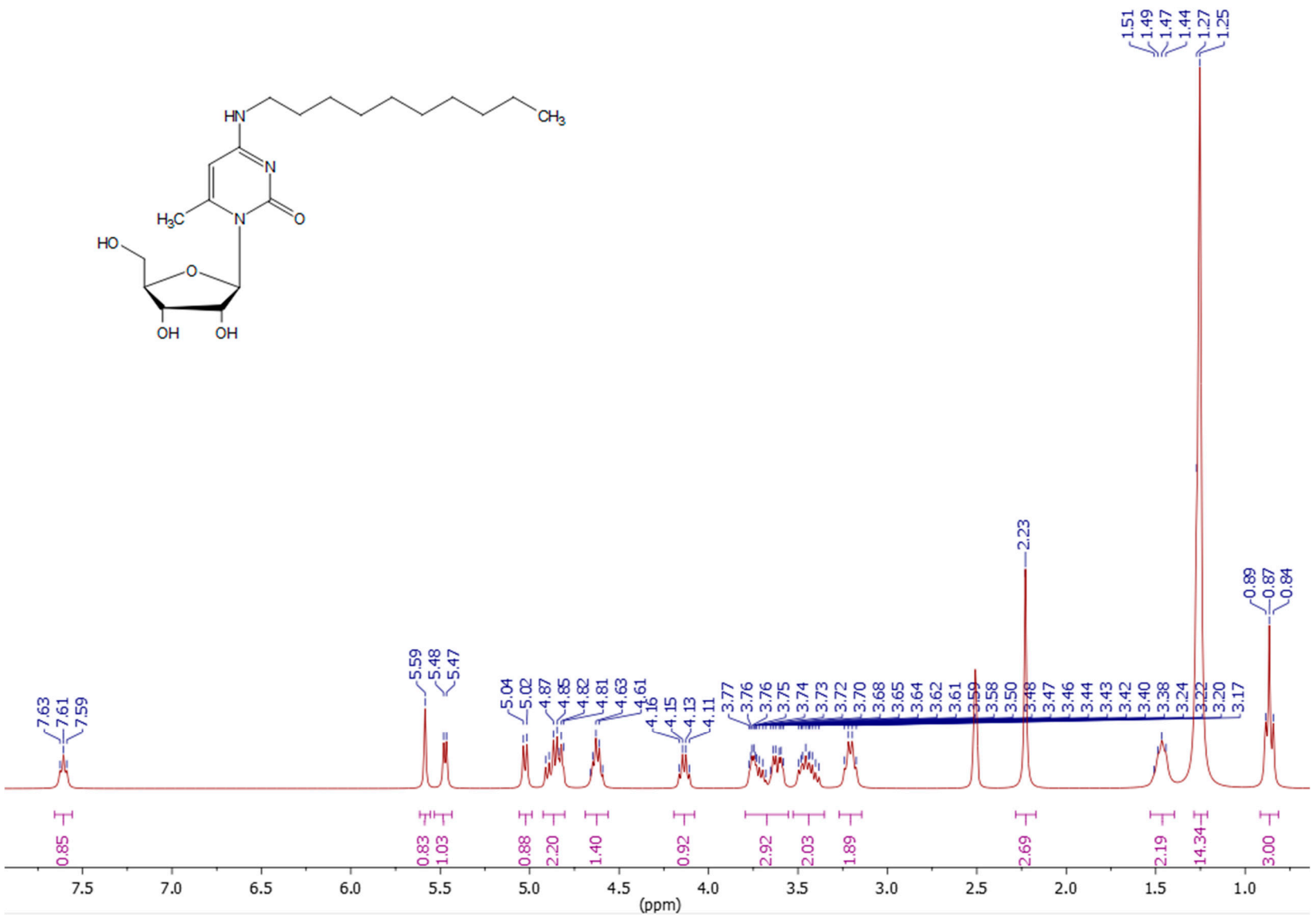


$^{13}C$

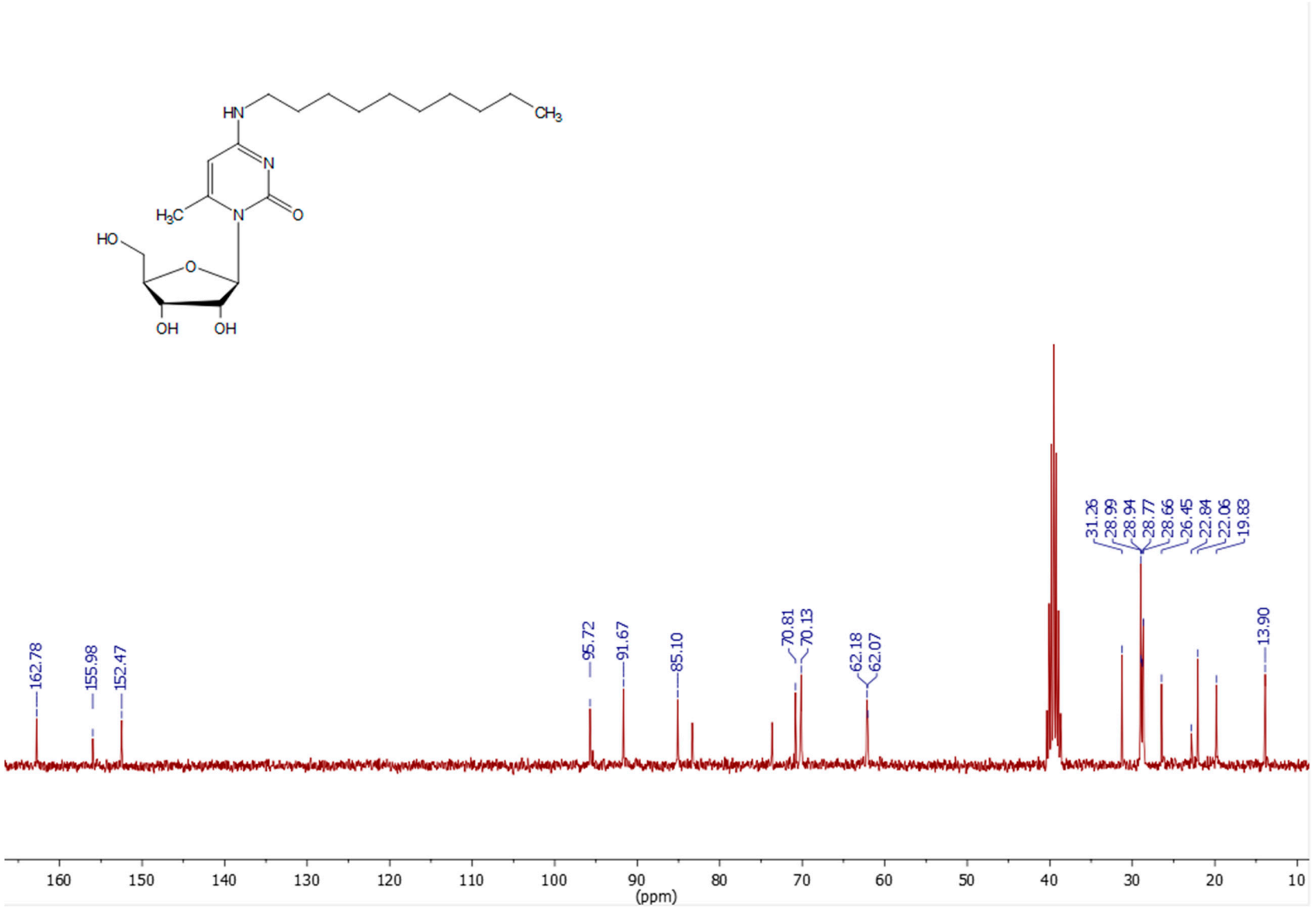

HN
CH3
N
N
O
HO
O
OH
OH
31.30
29.06
29.03
28.83
28.72
28.59
26.53
22.09
39.79
13.92
163.28
155.43
140.12
94.55
89.20
84.03
73.98
69.43
60.65
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

**6) $N^4$-Decyl-6-methylcytidine (3a)**

**$^1$H**

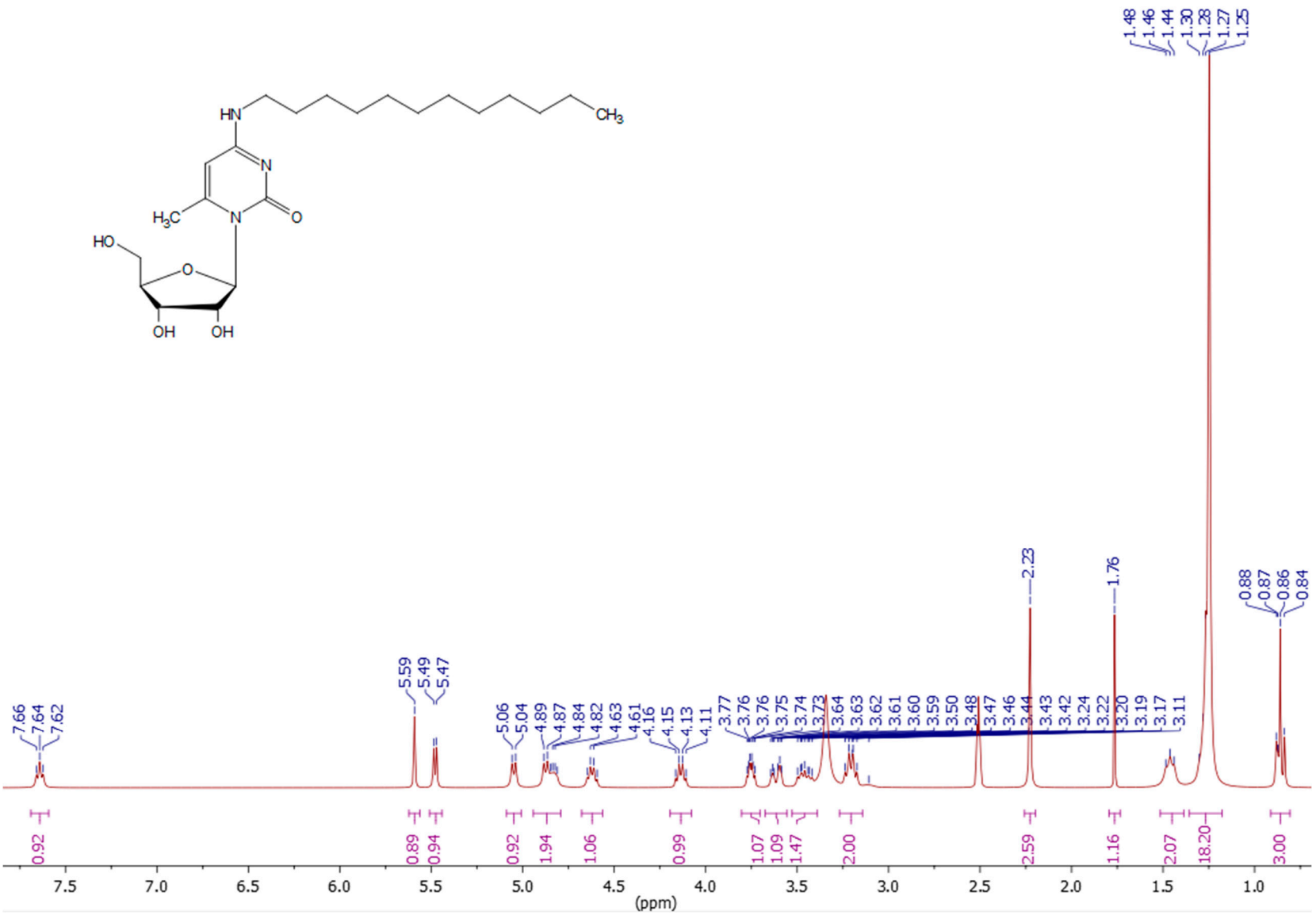


$^{13}C$

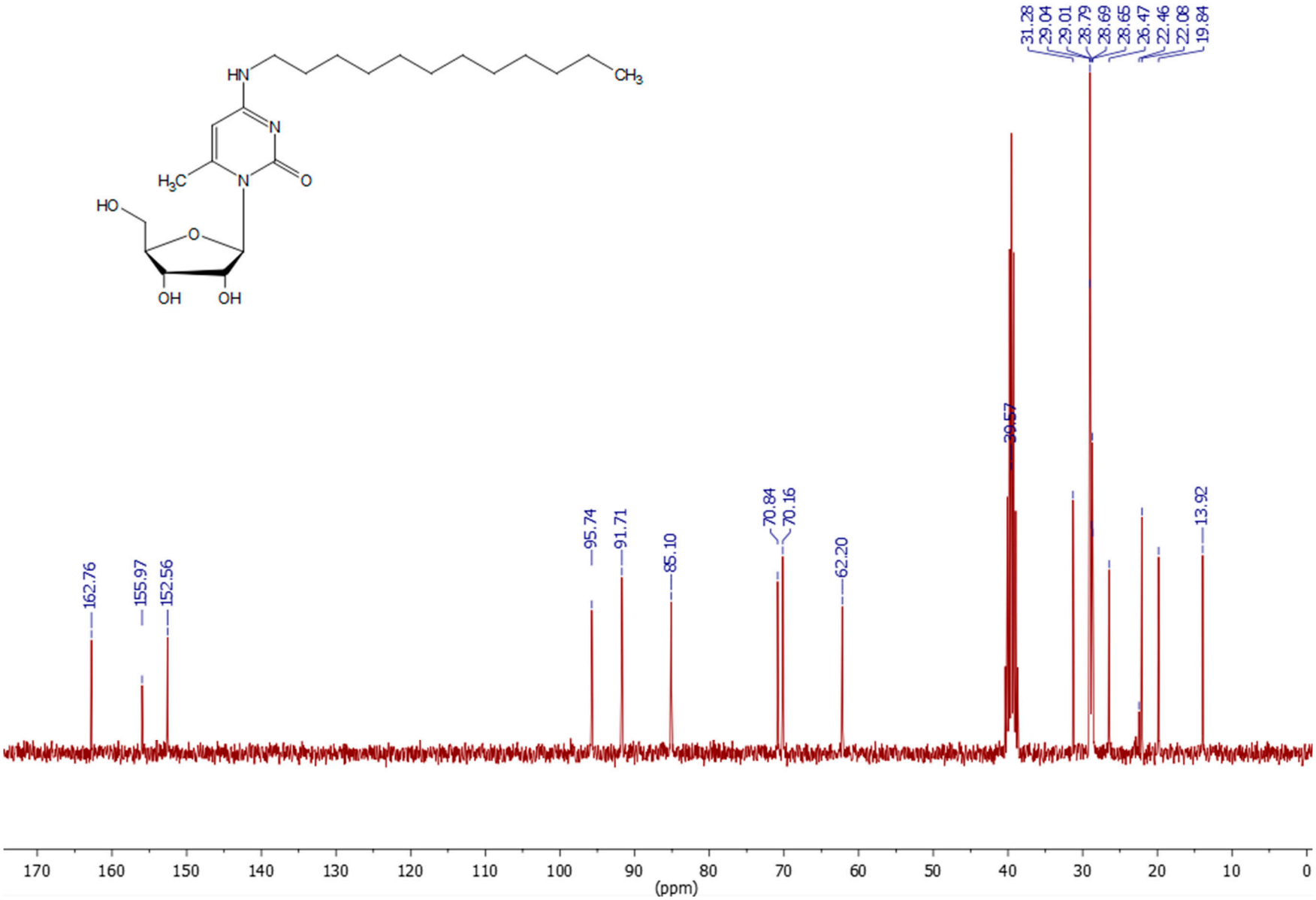

HN
CH3
N
H3C
N
O
HO
O
OH
OH
162.78
155.98
152.47
95.72
91.67
85.10
70.81
70.13
62.18
62.07
31.26
28.99
28.94
28.77
28.66
26.45
22.84
22.06
19.83
13.90
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

**7) $N^4$-Dodecyl-6-methylcytidine (3b)**

**$^1$H**



$^{13}C$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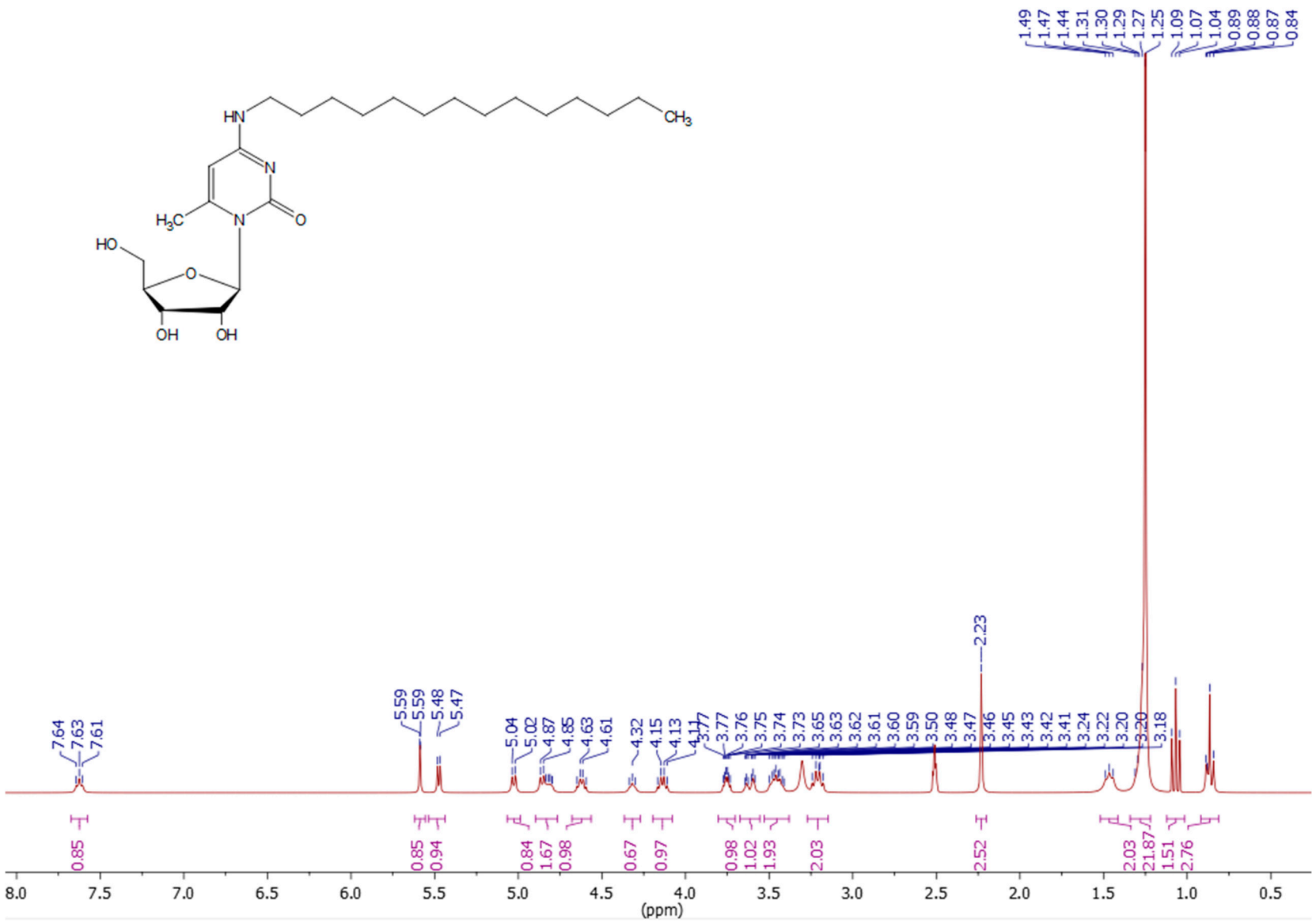
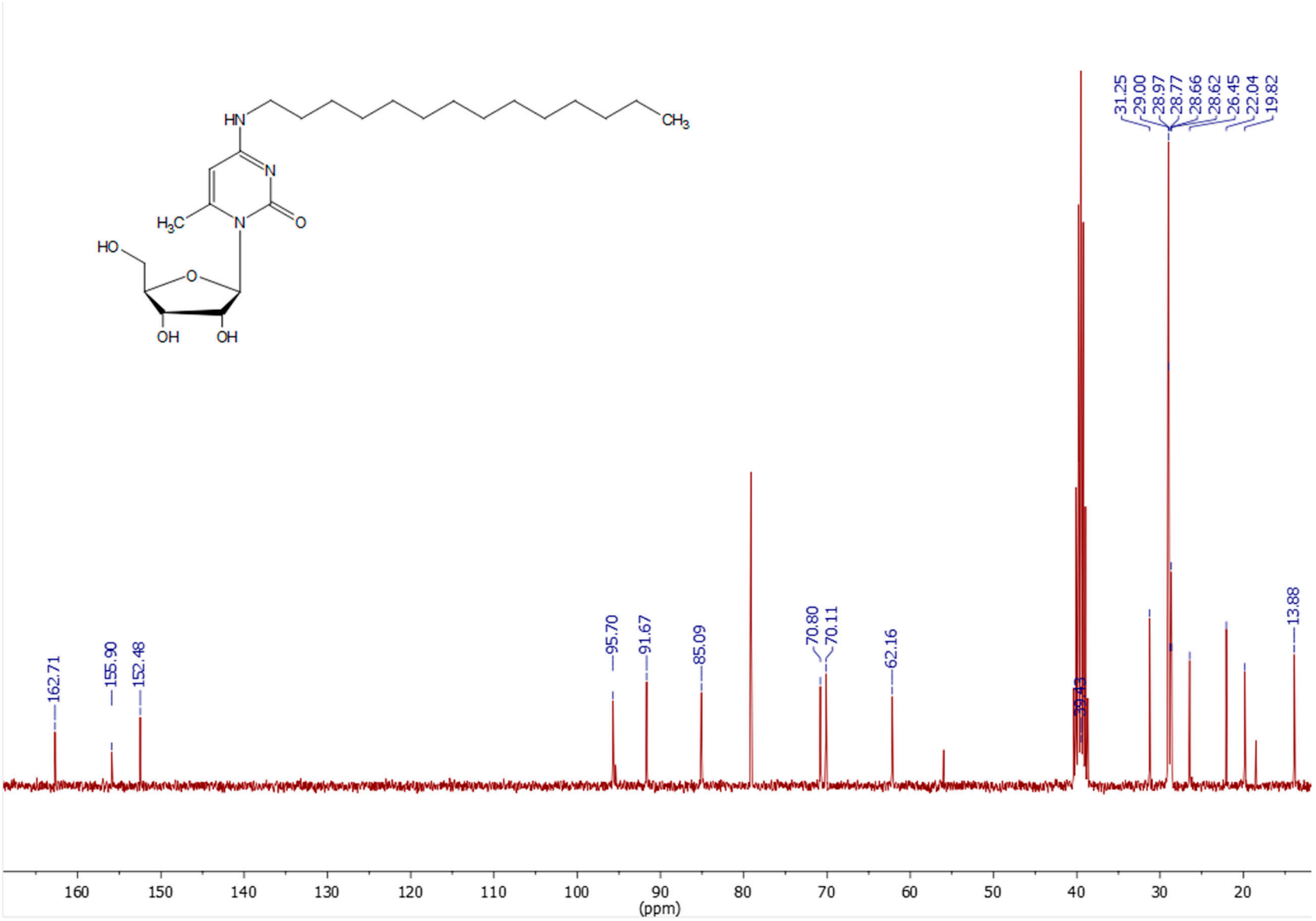
CH3
N
H3C
N
O
HO
O
OH
OH
31.28
29.04
29.01
28.79
28.69
28.65
26.47
22.46
22.08
19.84
30.57
162.76
155.97
152.56
95.74
91.71
85.10
70.84
70.16
62.20
13.92
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

**8) $N^4$-Tetradecyl-6-methylcytidine (3c)**

$^1$H

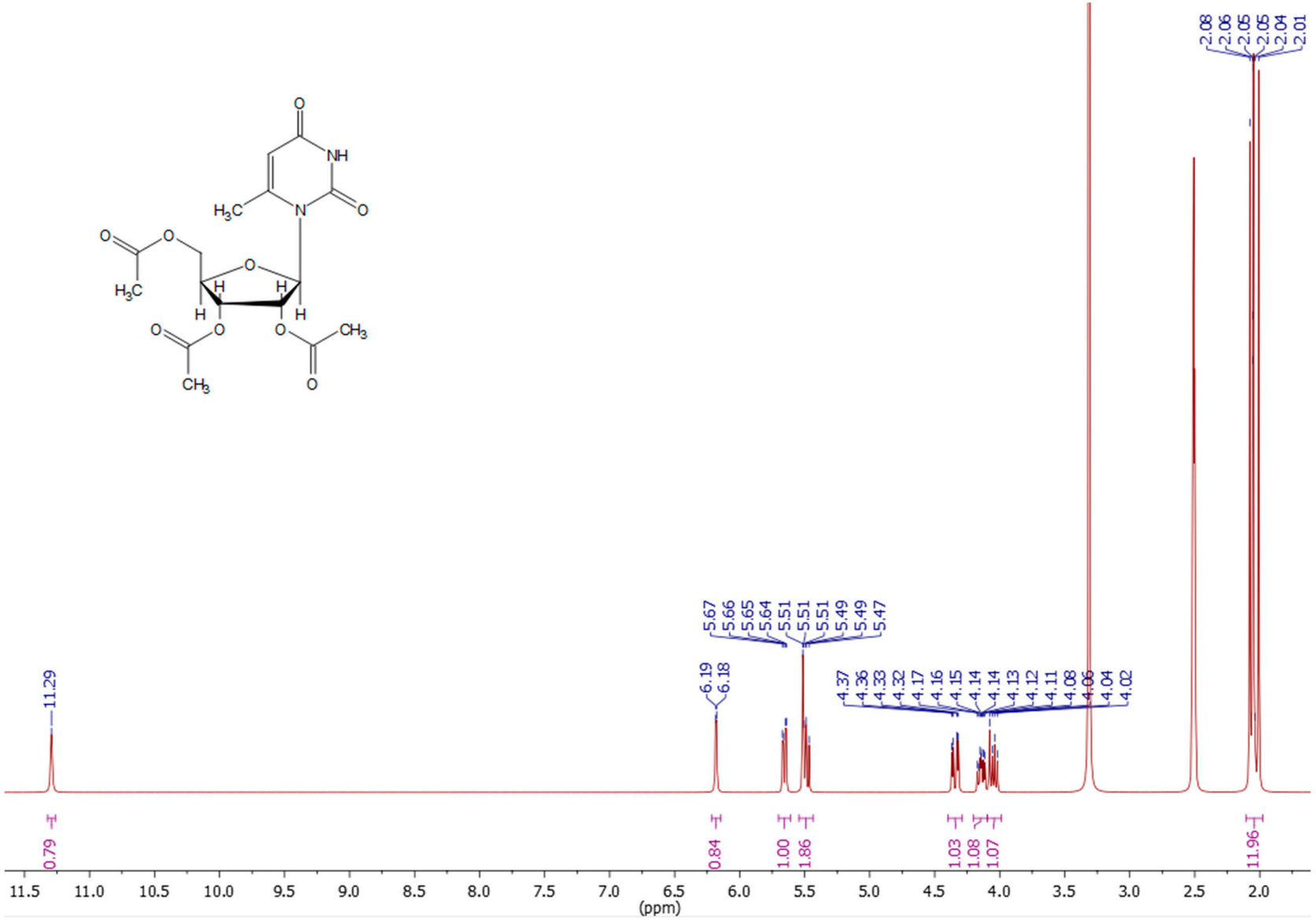


$^{13}C$


HN
CH3
N
H3C
N
O
HO
O
OH
OH
31.25
29.00
28.97
28.77
28.66
28.62
26.45
22.04
19.82
162.71
155.90
152.48
95.70
91.67
85.09
70.80
70.11
62.16
39.43
13.88
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
(ppm)

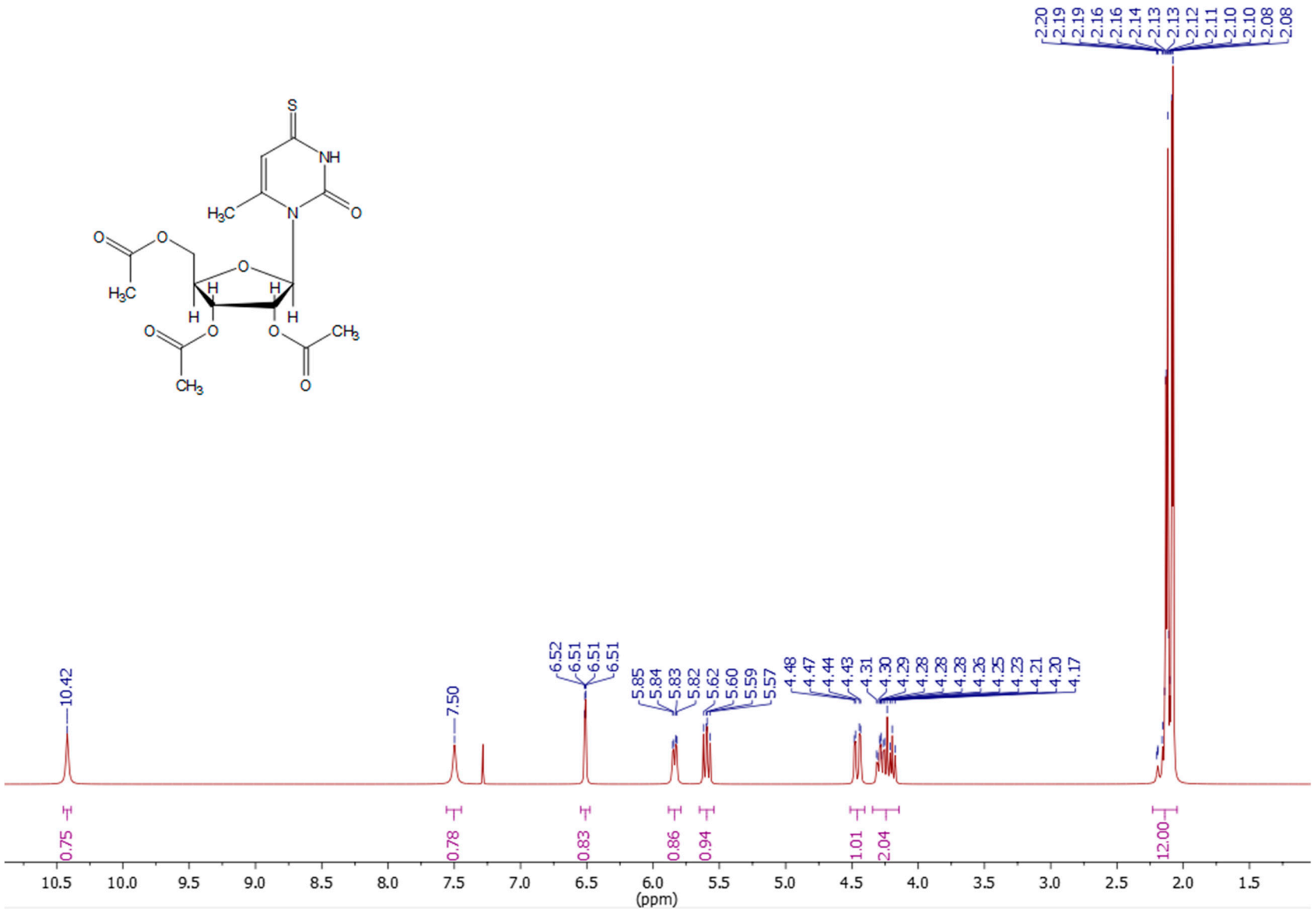
**9) 2′,3′,5′-Tri-*O*-acetyl-6-methyluridine (8)**

$^1$H

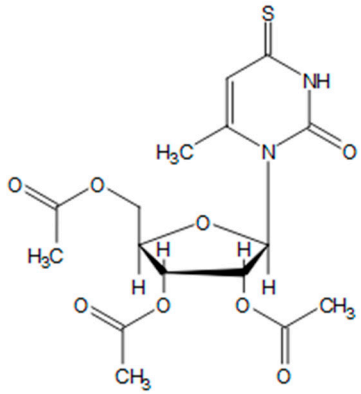


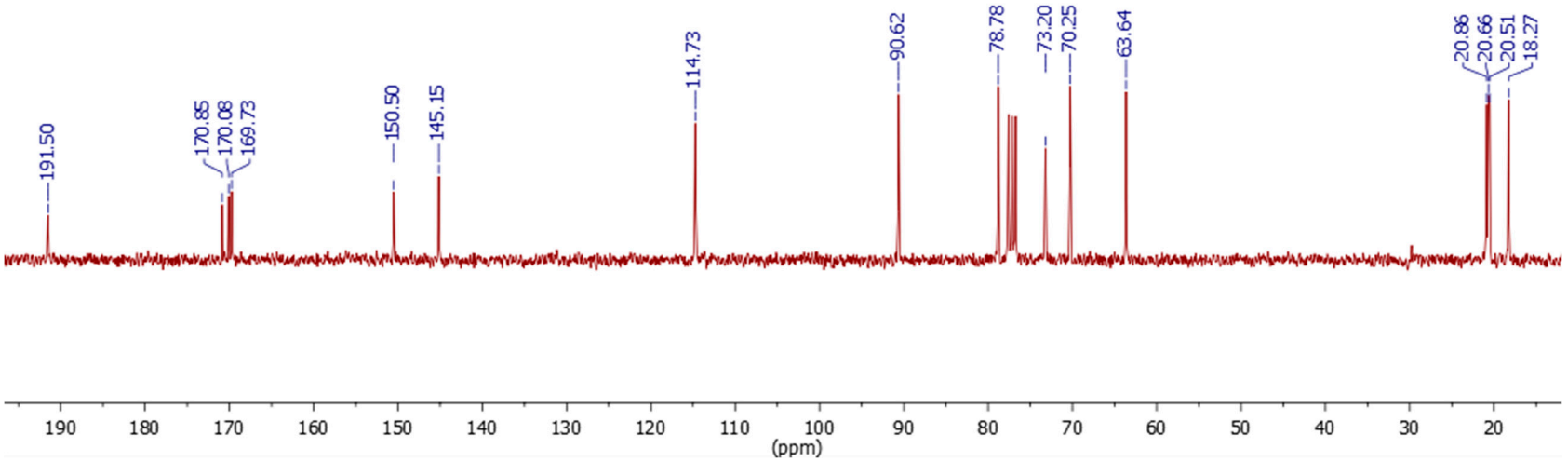
**10) 2′,3′,5′-Tri-*O*-acetyl-6-methyl-4-thiouridine (10)**

$^1$H



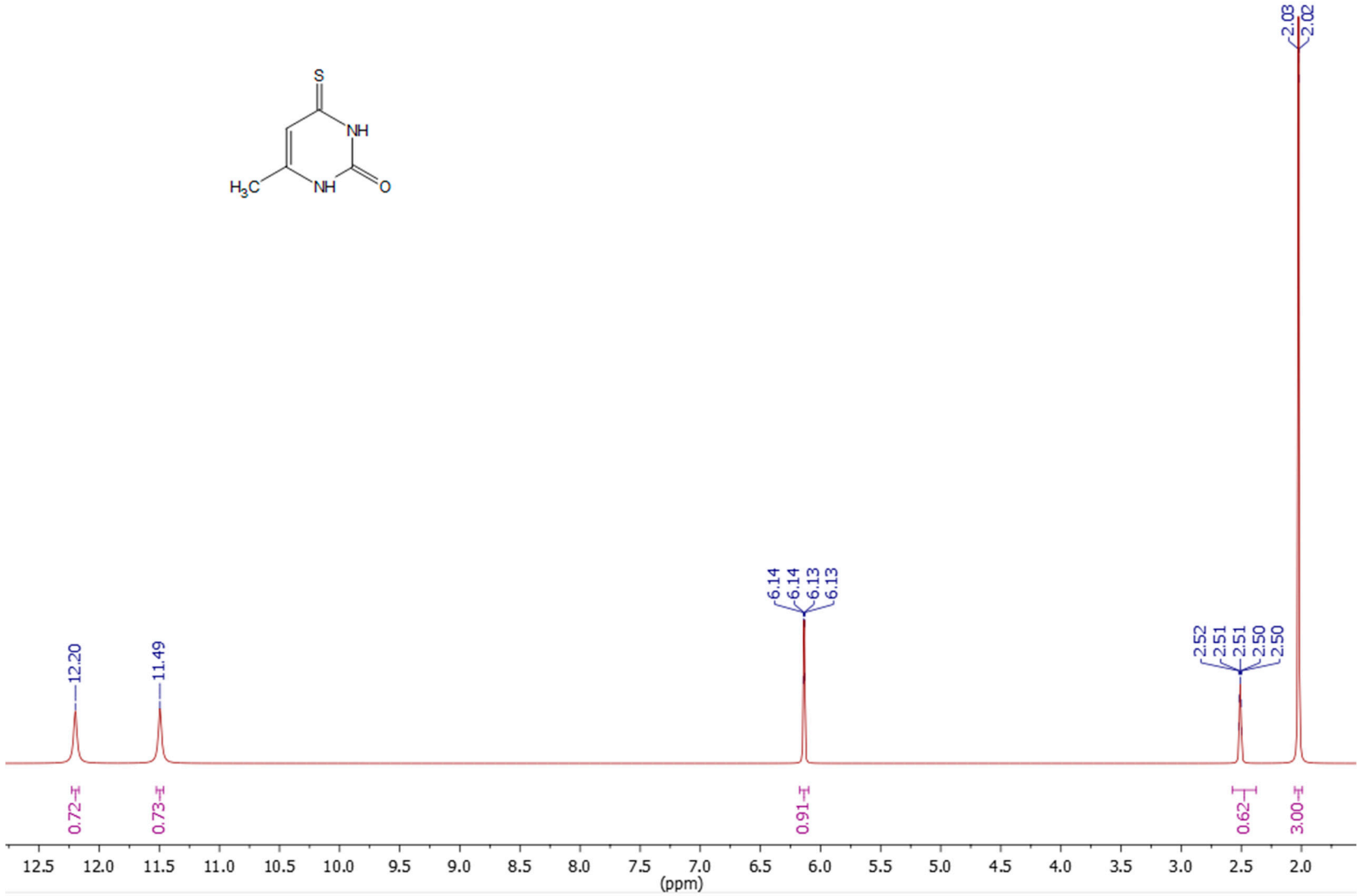
$^{13}C$


S
NH
H3C
N
O
O
O
H3C
O
H
H
H
H
O
O
O
CH3
CH3
O


191.50
170.85
170.08
169.73
150.50
145.15
114.73
90.62
78.78
73.20
70.25
63.64
20.86
20.66
20.51
18.27
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
(ppm)

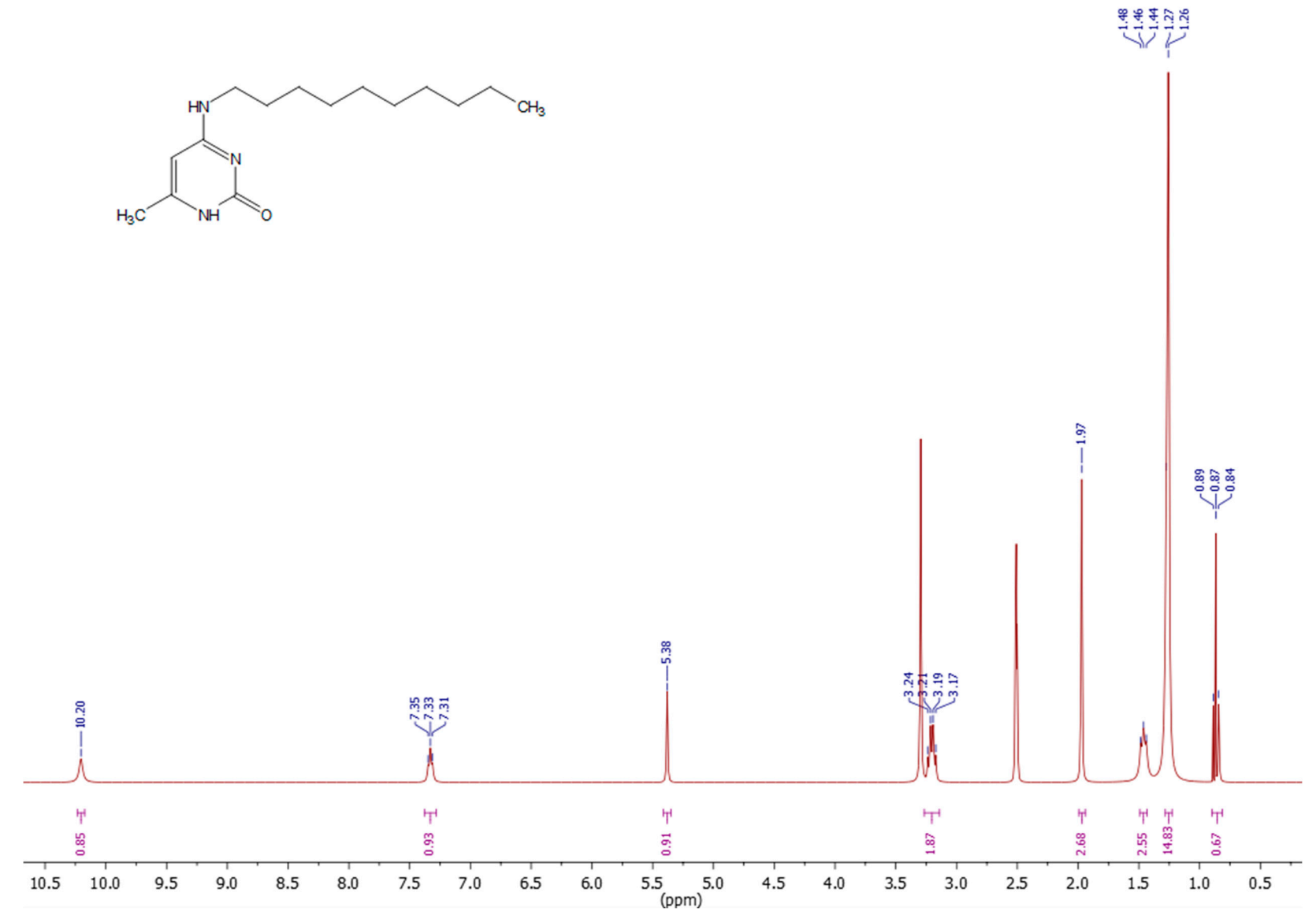
**11) 4-Thio-6-methyluracyl (11)**

**$^1$H**



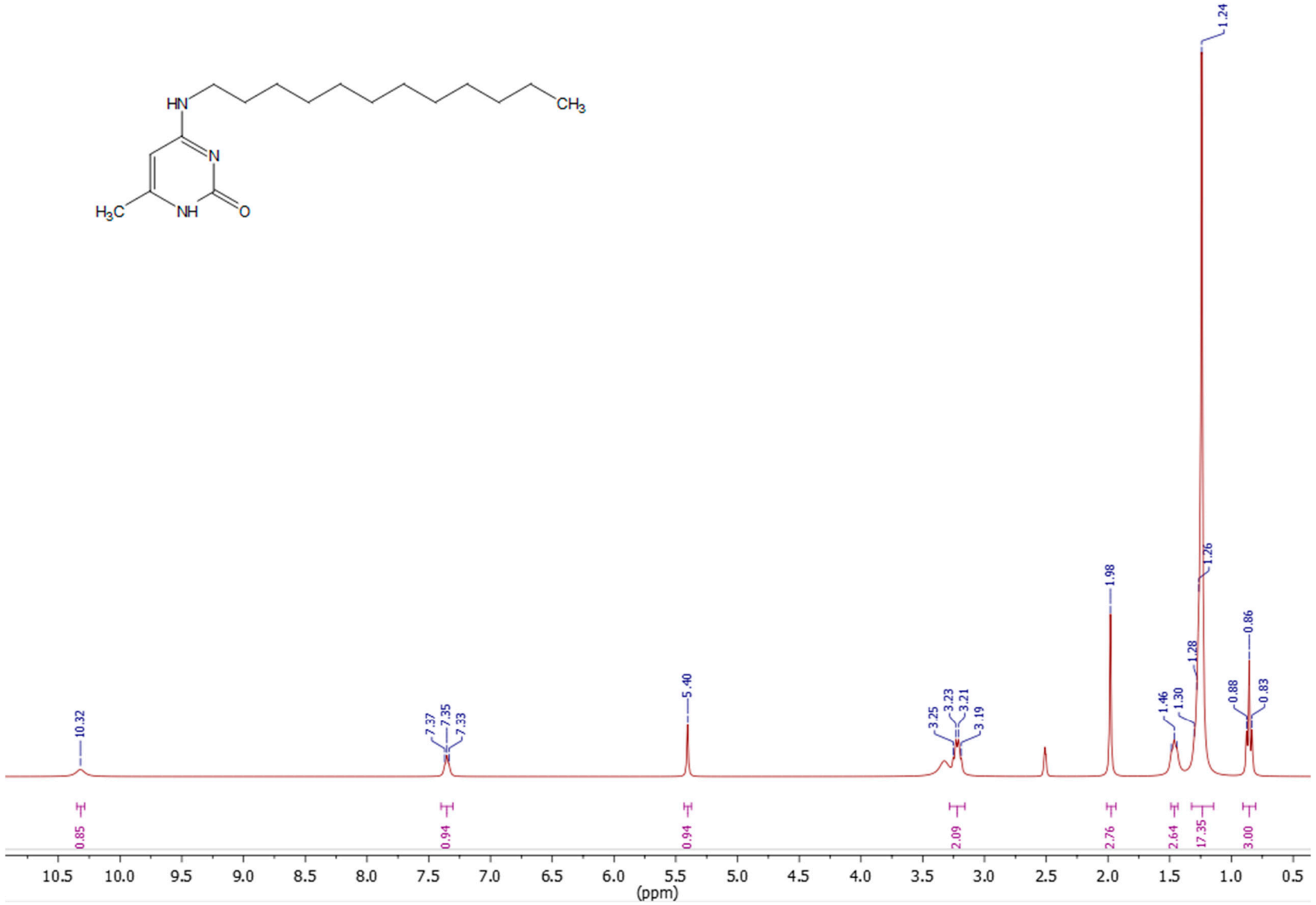
**12) $N^4$-Decyl-6-methylcytosine (12a)**

**$^1$H**



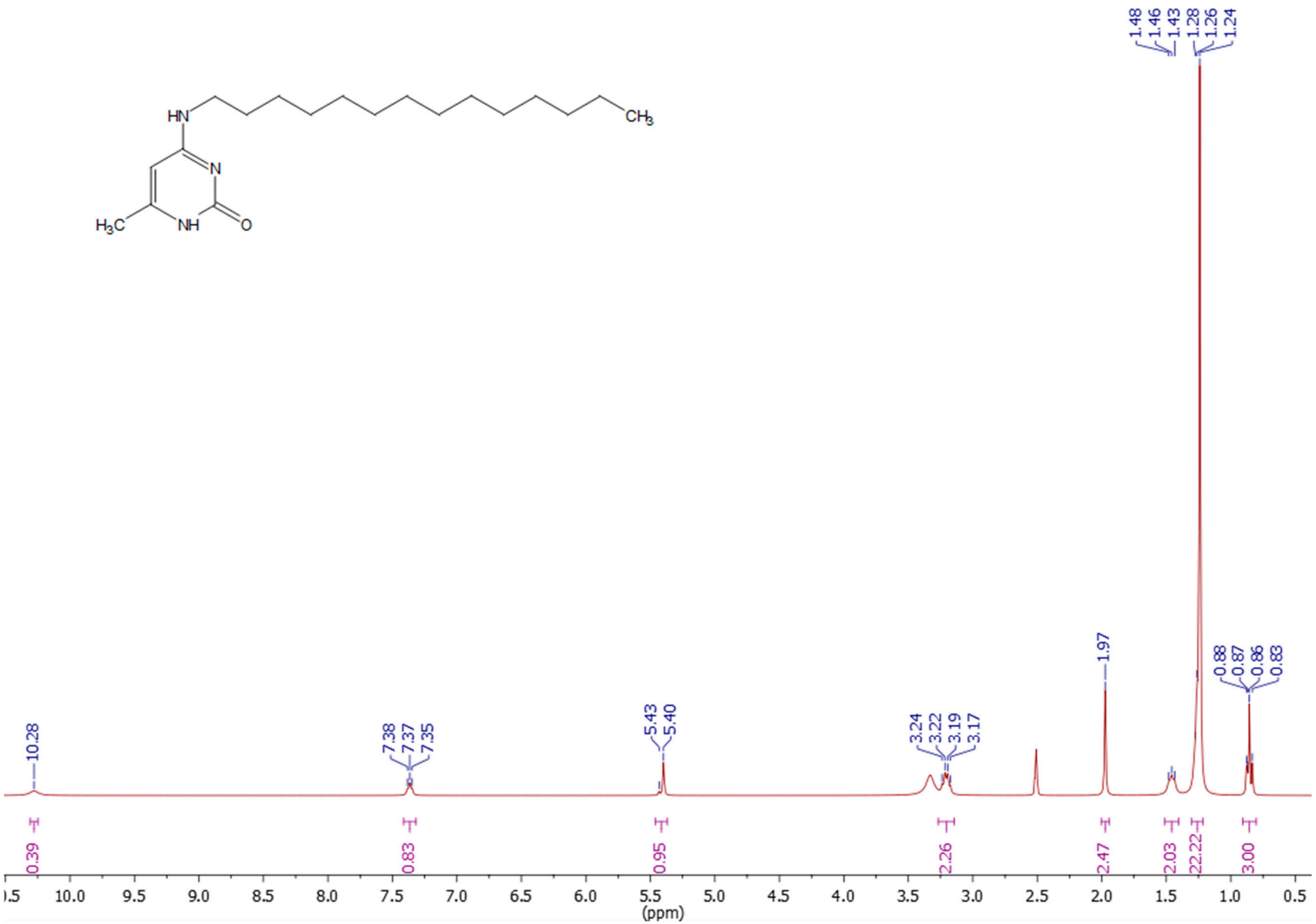
**13) $N^4$-Dodecyl-6-methylcytosine (12b)**

**$^1$H**



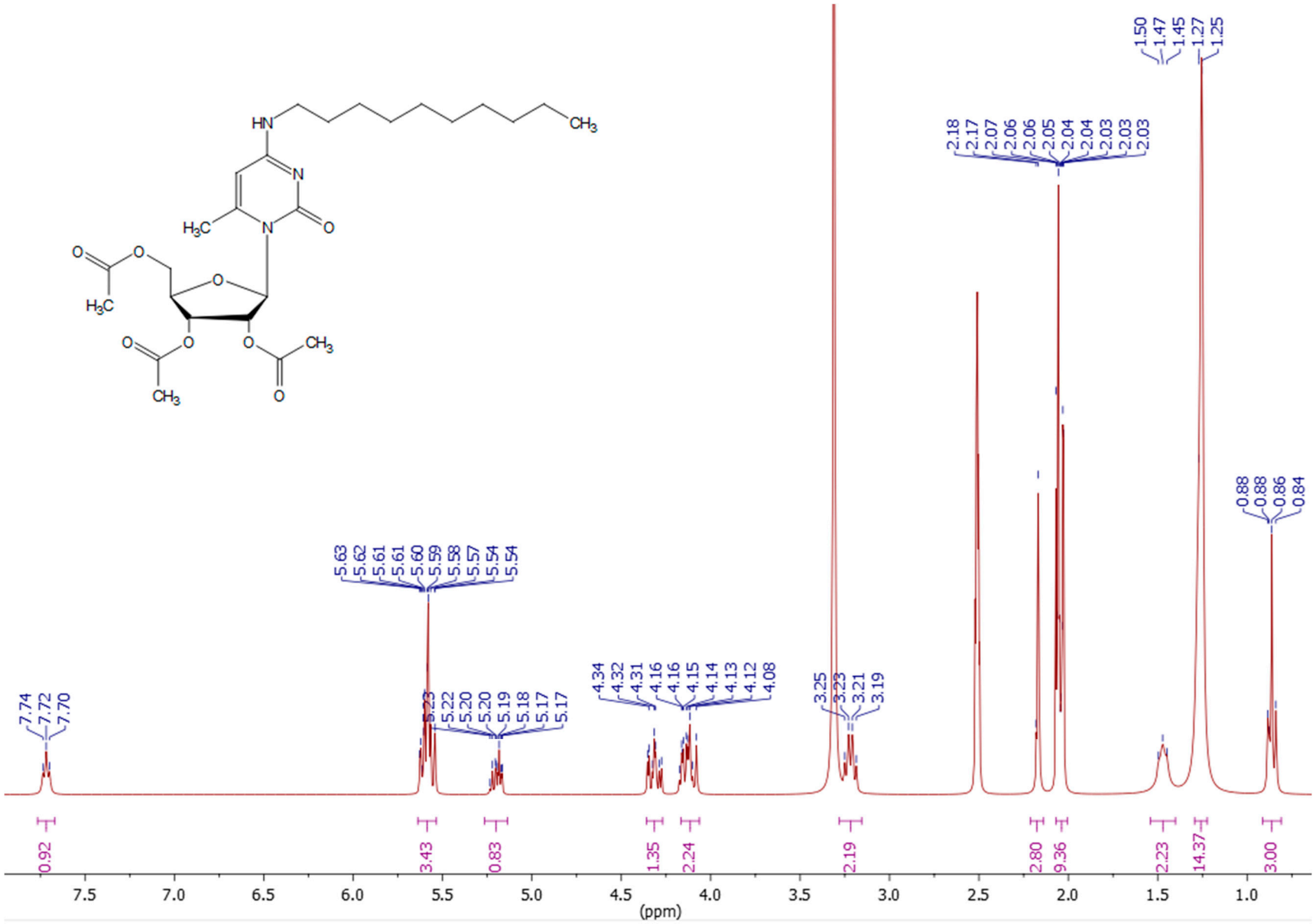
**14) $N^4$-Tetradecyl-6-methylcytosine (12c)**

**$^1$H**



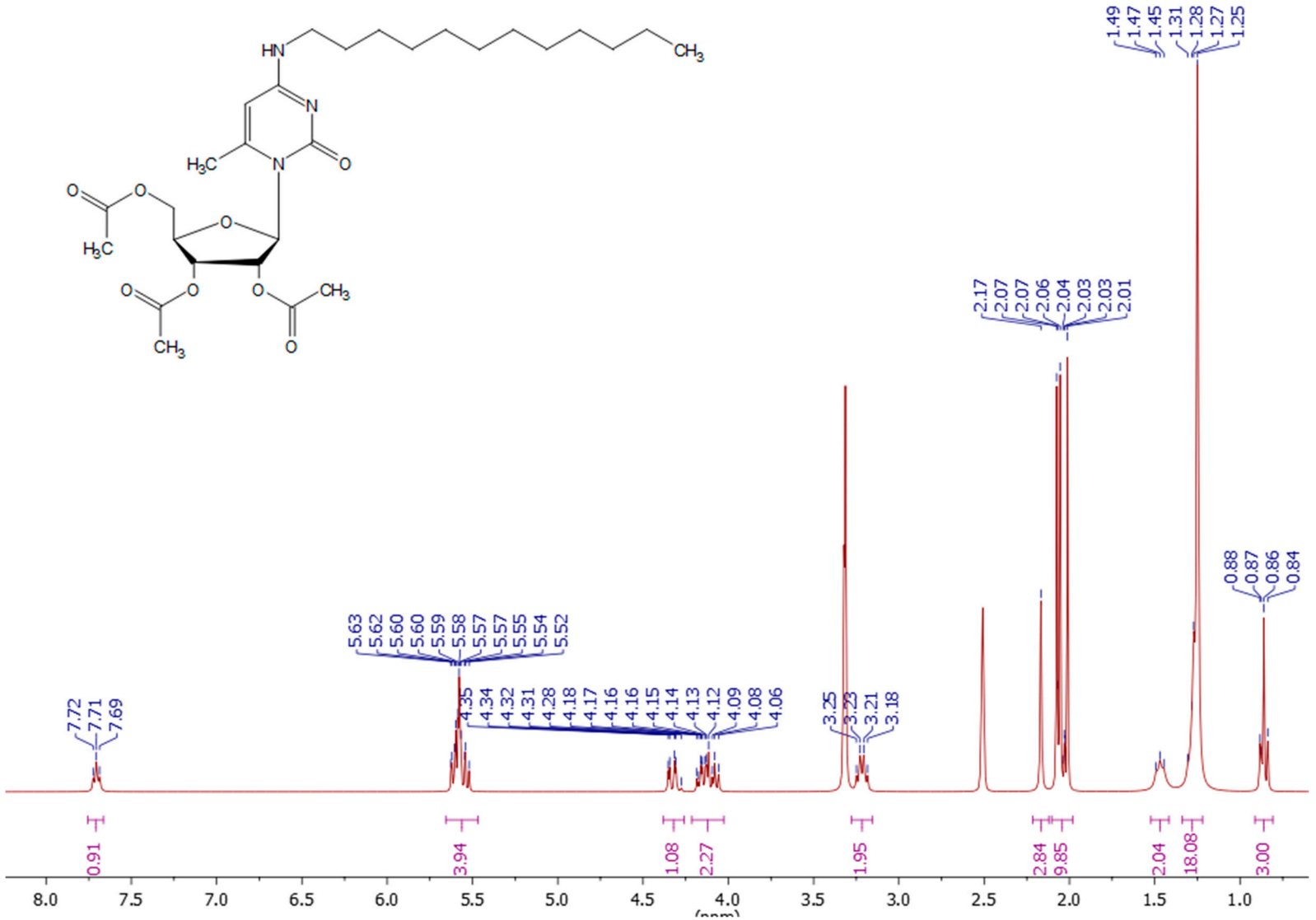
**15) 2′,3′,5′-Tri-*O*-acetyl-*N*$^4$-decyl-6-methylcytidine (13a)**

$^1$H



**16) 2′,3′,5′-Tri-*O*-acetyl-*N*$^4$-dodecyl-6-methylcytidine (13b)**

$^1$H



**17) 2′,3′,5′-Tri-*O*-acetyl-*N*$^4$-tetradecyl-6-methylcytidine (13c)**

$^1$H

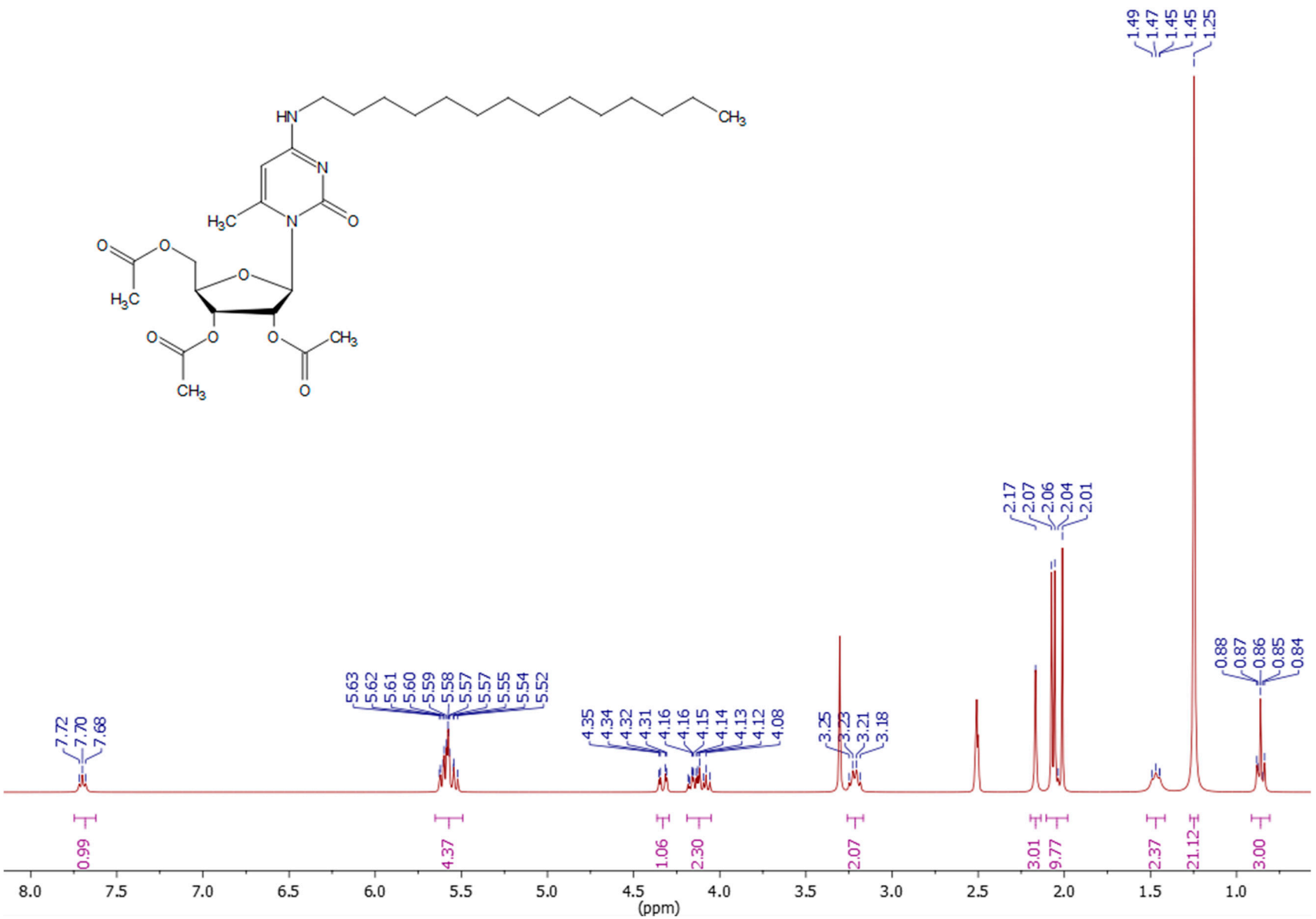
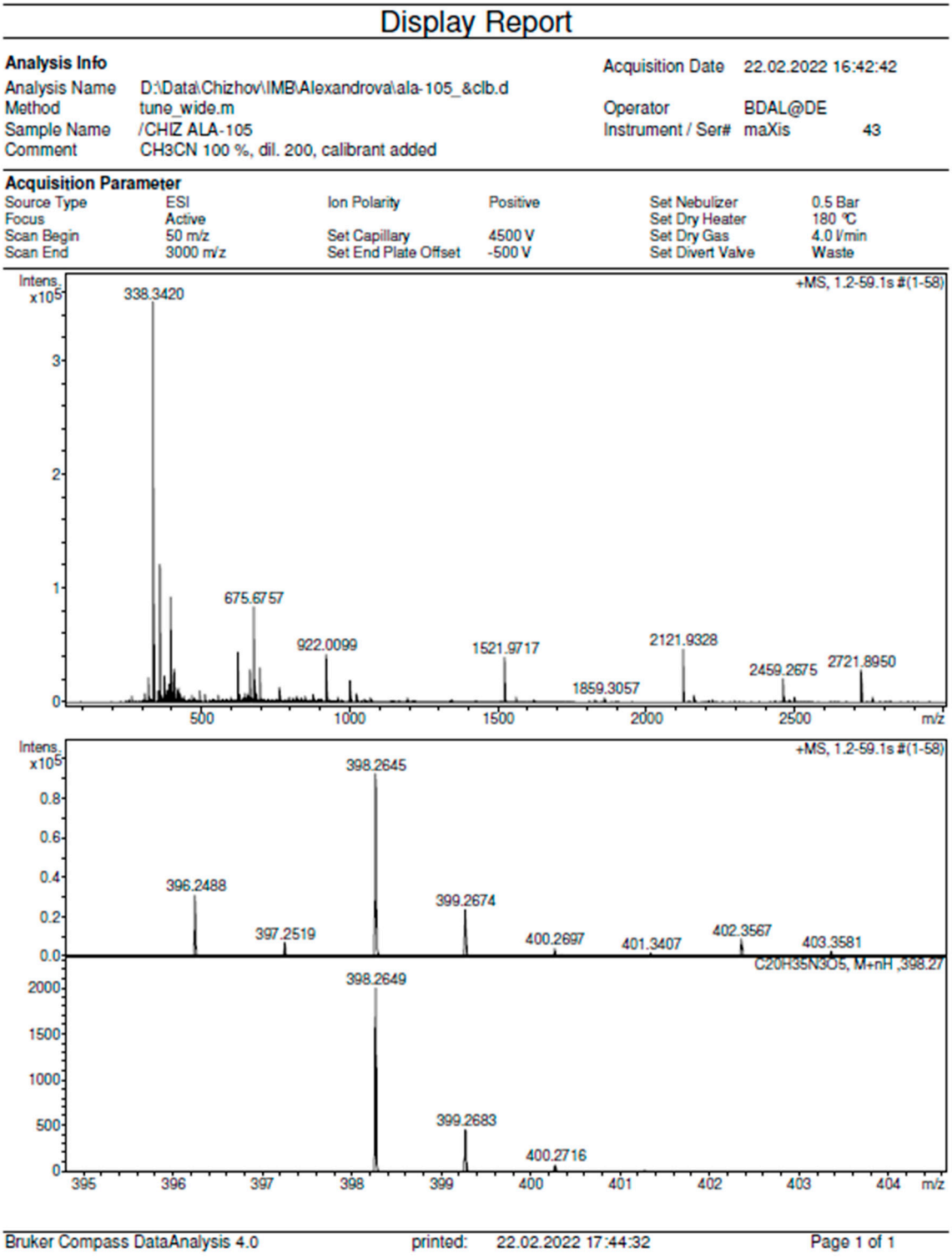


Figure S3. Mass spectra of new compounds

**1) $N^4$-decyl-5-methylcytidine (2a)**



## 2) $N^4$-dodecyl-5-methylcytidine (2b)

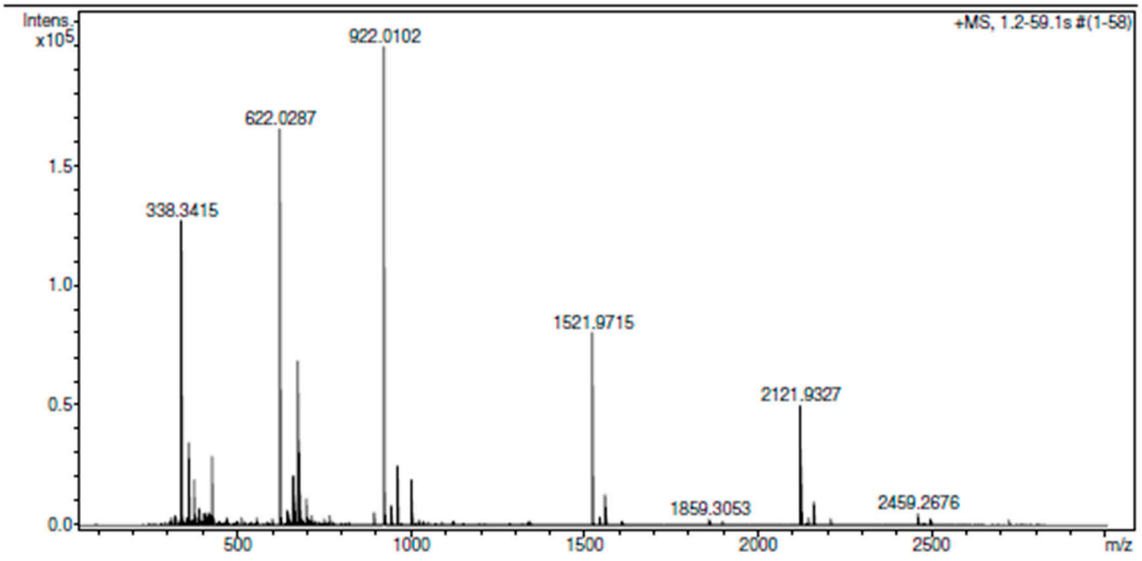
# Display Report

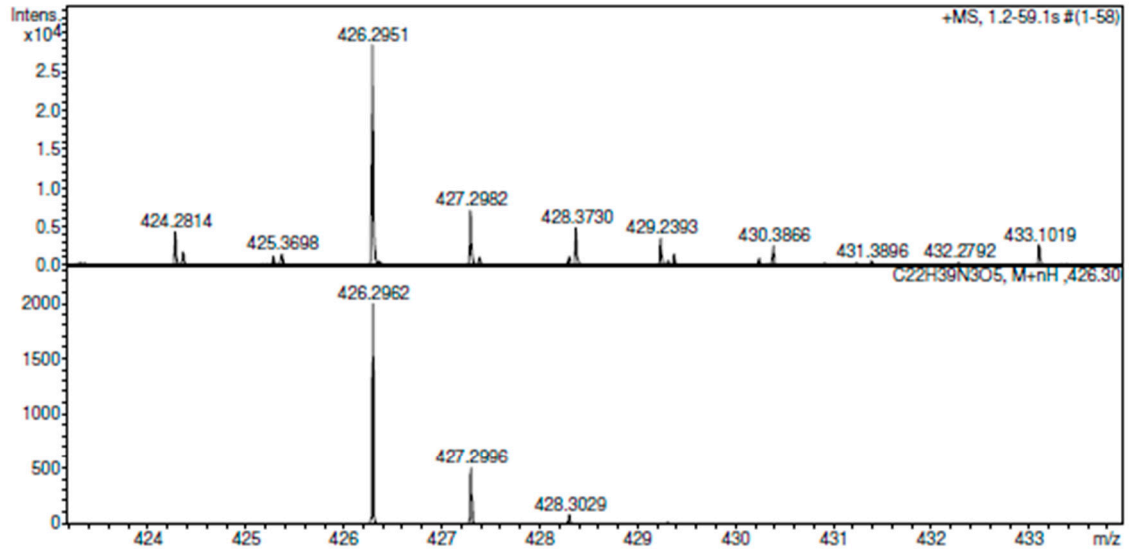
**Analysis Info**

| | | | |
|---|---|---|---|
| Analysis Name | D:\Data\Chizhov\IMB\Alexandrova\ala-106_&clb.d | Acquisition Date | 22.02.2022 16:52:26 |
| Method | tune_wide.m | Operator | BDAL@DE |
| Sample Name | /CHIZ ALA-106 | Instrument / Ser# | maXis 43 |
| Comment | CH3CN : H2O 50/50 %, dil. 400, calibrant added | | |

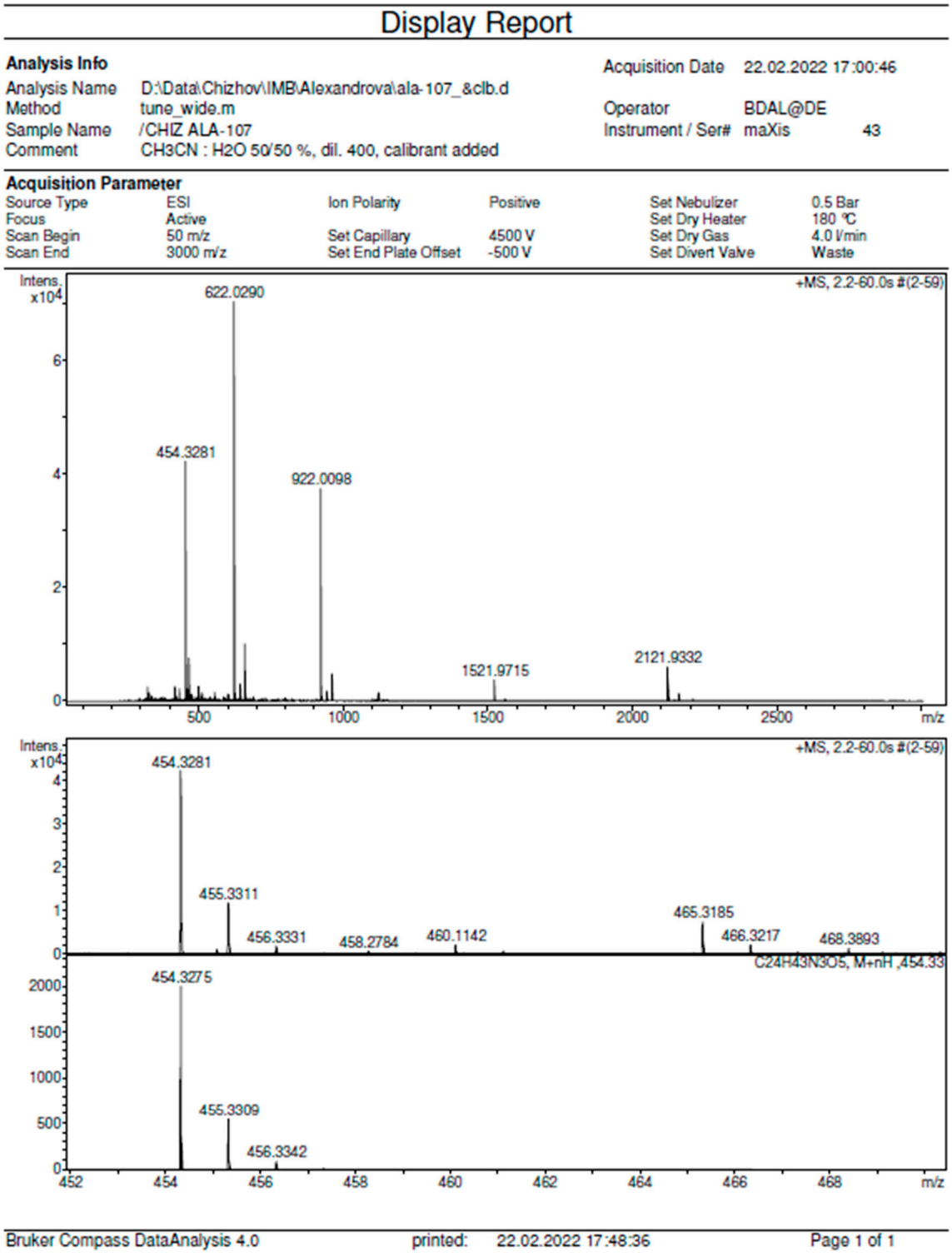
**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.5 Bar |
| Focus | Active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 3000 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |



### 3) $N^4$-tetradecyl-5-methylcytidine (2c)



## 4) $N^4$-dodecylcytidine (2d)

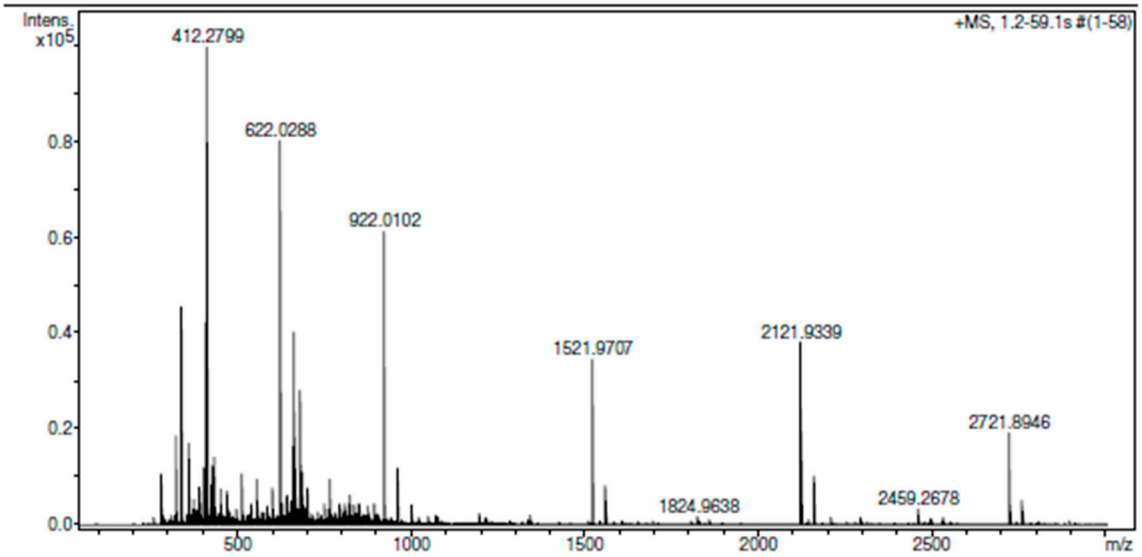
# Display Report

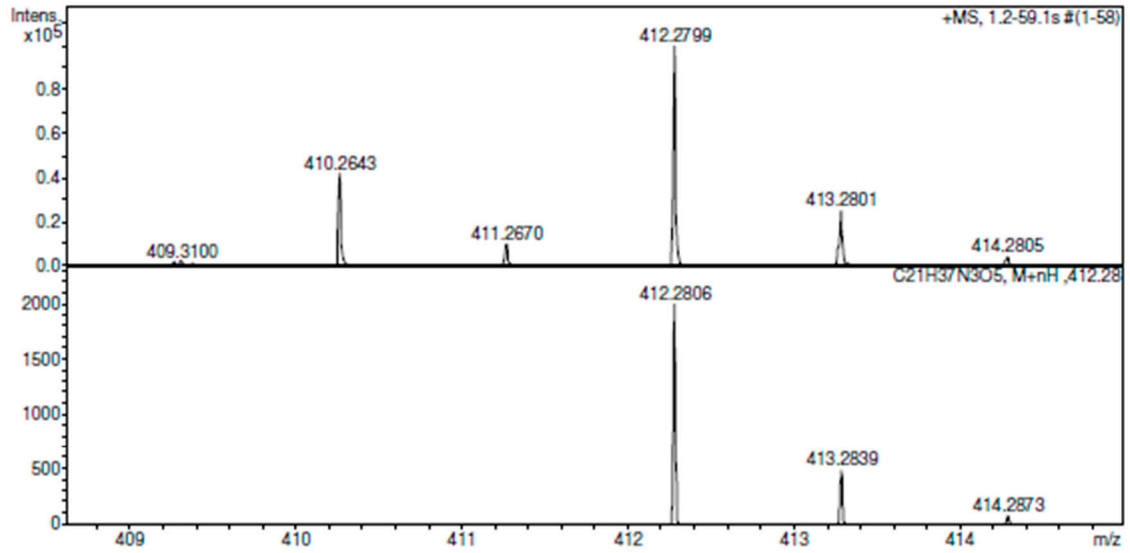
**Analysis Info**

| | | | |
|---|---|---|---|
| Analysis Name | D:\Data\Chizhov\IMB\Alexandrova\ala-103_&clb.d | Acquisition Date | 22.02.2022 15:05:27 |
| Method | tune_wide.m | Operator | BDAL@DE |
| Sample Name | /CHIZ ALA-103 | Instrument / Ser# | maXis 43 |
| Comment | CH3CN 100 %, dil. 200, calibrant added | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.5 Bar |
| Focus | Active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 3000 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |

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## 5) $N^4$-tetradecylcytidine (2e)

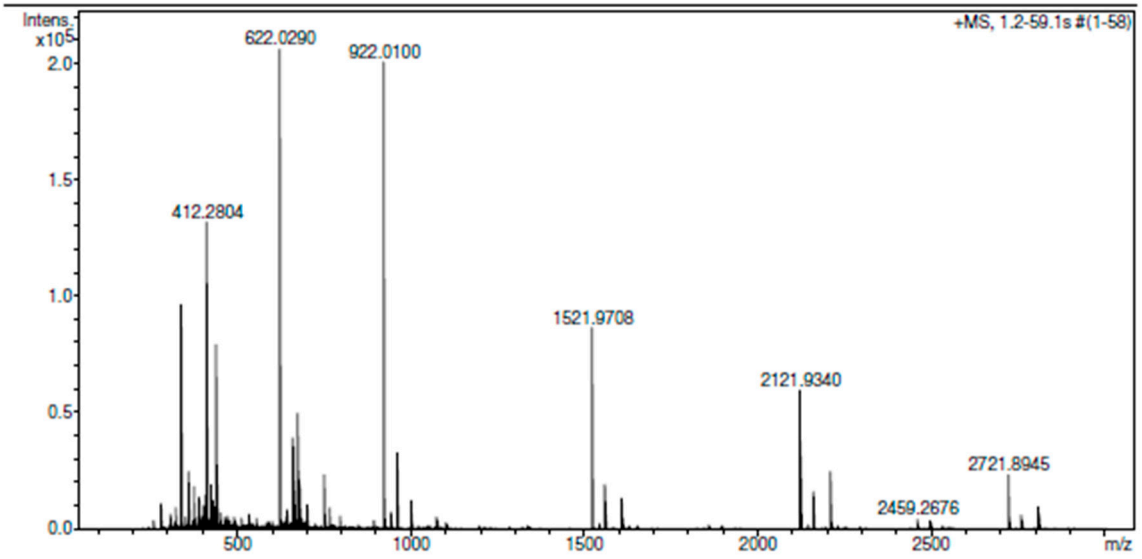
# Display Report

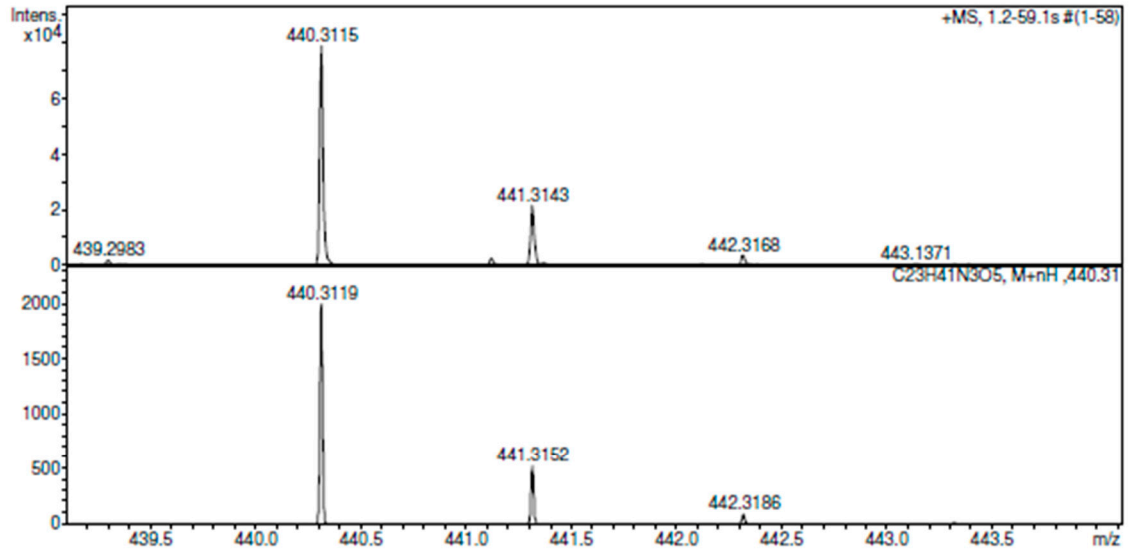
**Analysis Info**

| | | | |
|---|---|---|---|
| Analysis Name | D:\Data\Chizhov\IMB\Alexandrova\ala-104_&clb.d | Acquisition Date | 22.02.2022 15:12:37 |
| Method | tune_wide.m | Operator | BDAL@DE |
| Sample Name | /CHIZ ALA-104 | Instrument / Ser# | maXis 43 |
| Comment | CH3CN 100 %, dil. 2000, calibrant added | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.5 Bar |
| Focus | Active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 3000 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |



**6) $N^4$-Decyl-6-methylcytidine (3a)**

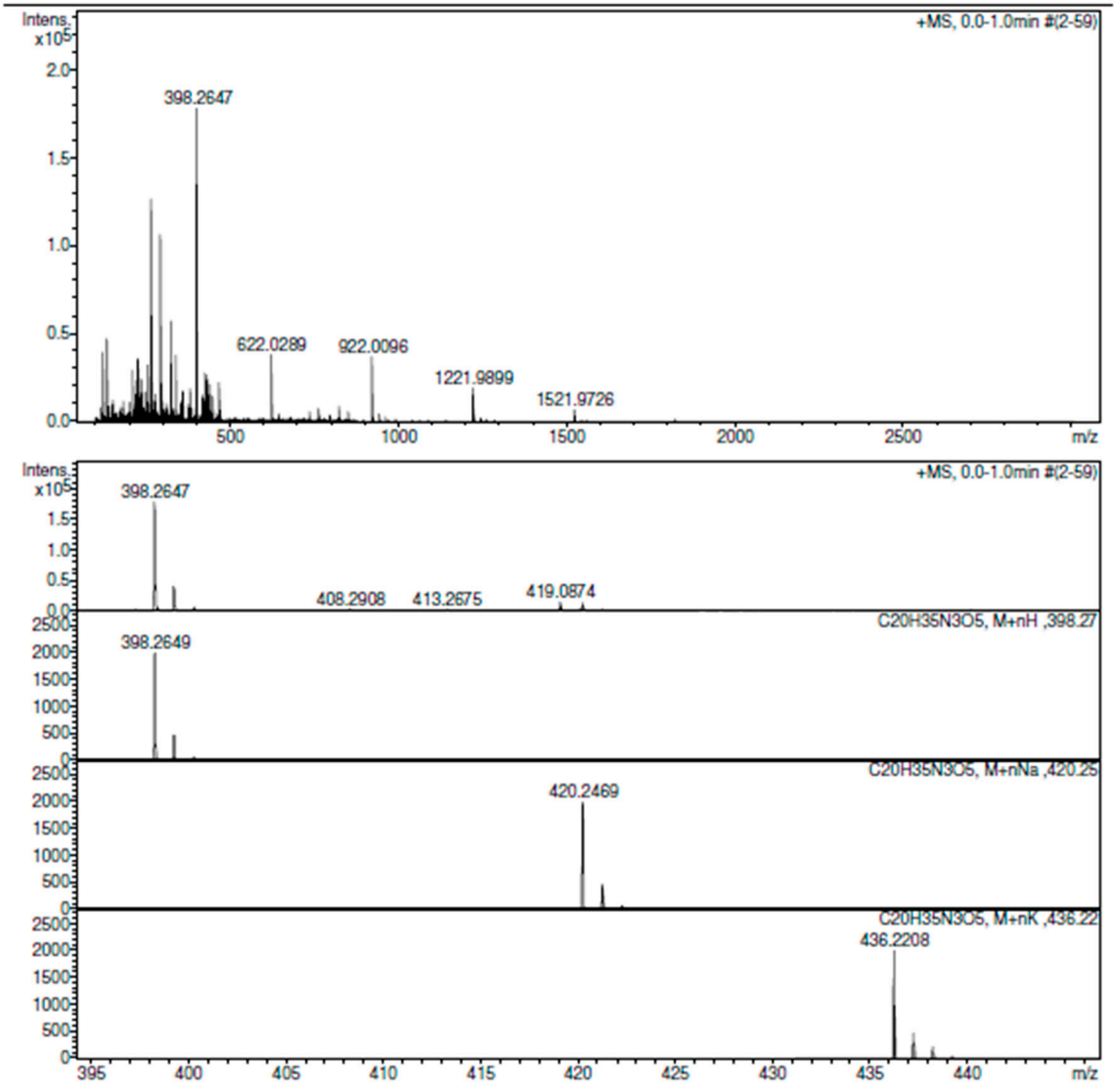
Display Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Analysis Name | D:\Data\Chizhov\IMB\Aleksandrova\Oskol'skii\osk-1_&clblow.d | Acquisition Date | 19.01.2024 17:57:39 |
| Method | tune_low.m | Operator | BDAL@DE |
| Sample Name | /CHIZ OSK-1 | Instrument / Ser# | micrOTOF 10248 |
| Comment | CH3CN:H2O 50/50 %, dil. 20, low conc. calibrant added | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.4 Bar |
| Focus | Not active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 3000 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |

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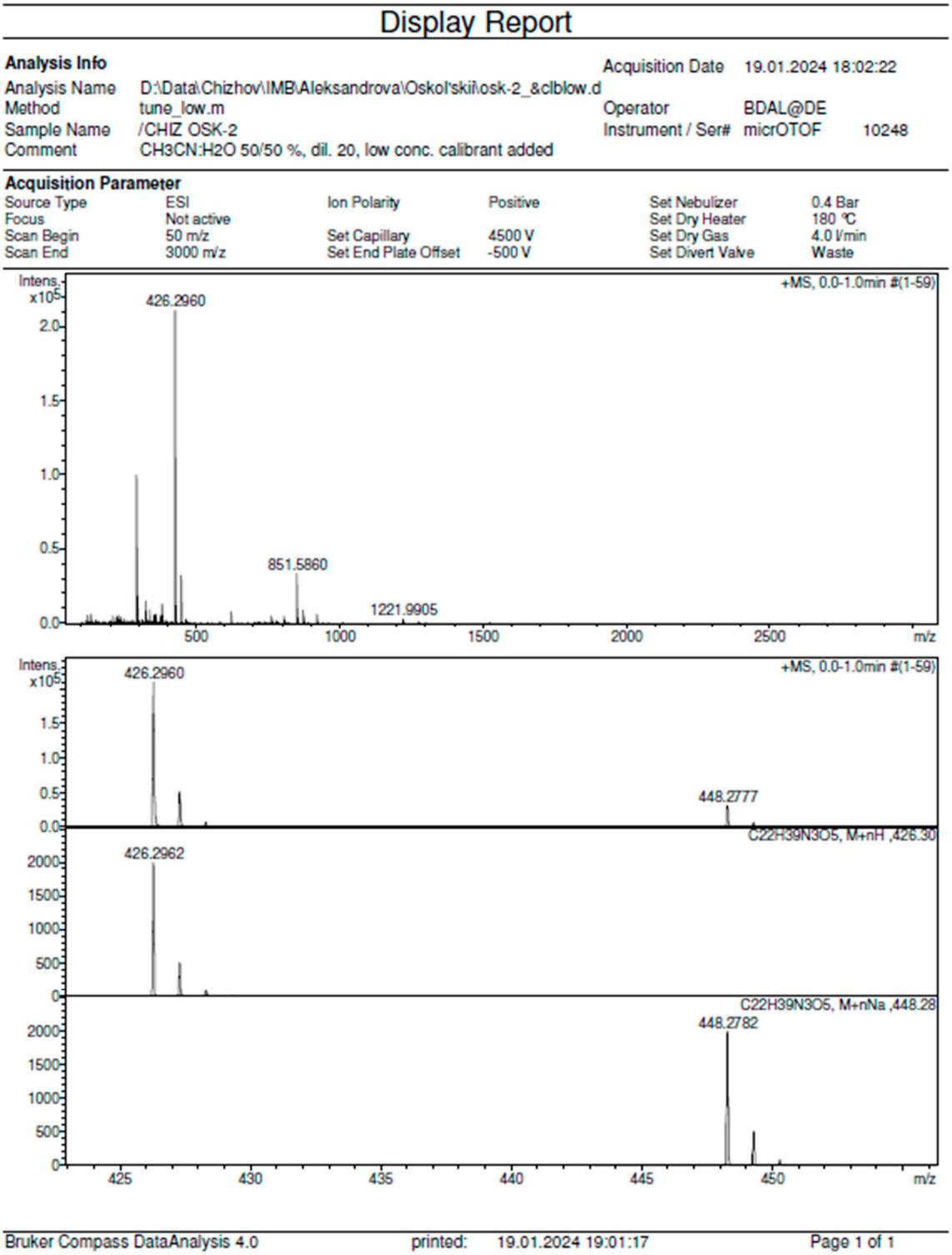
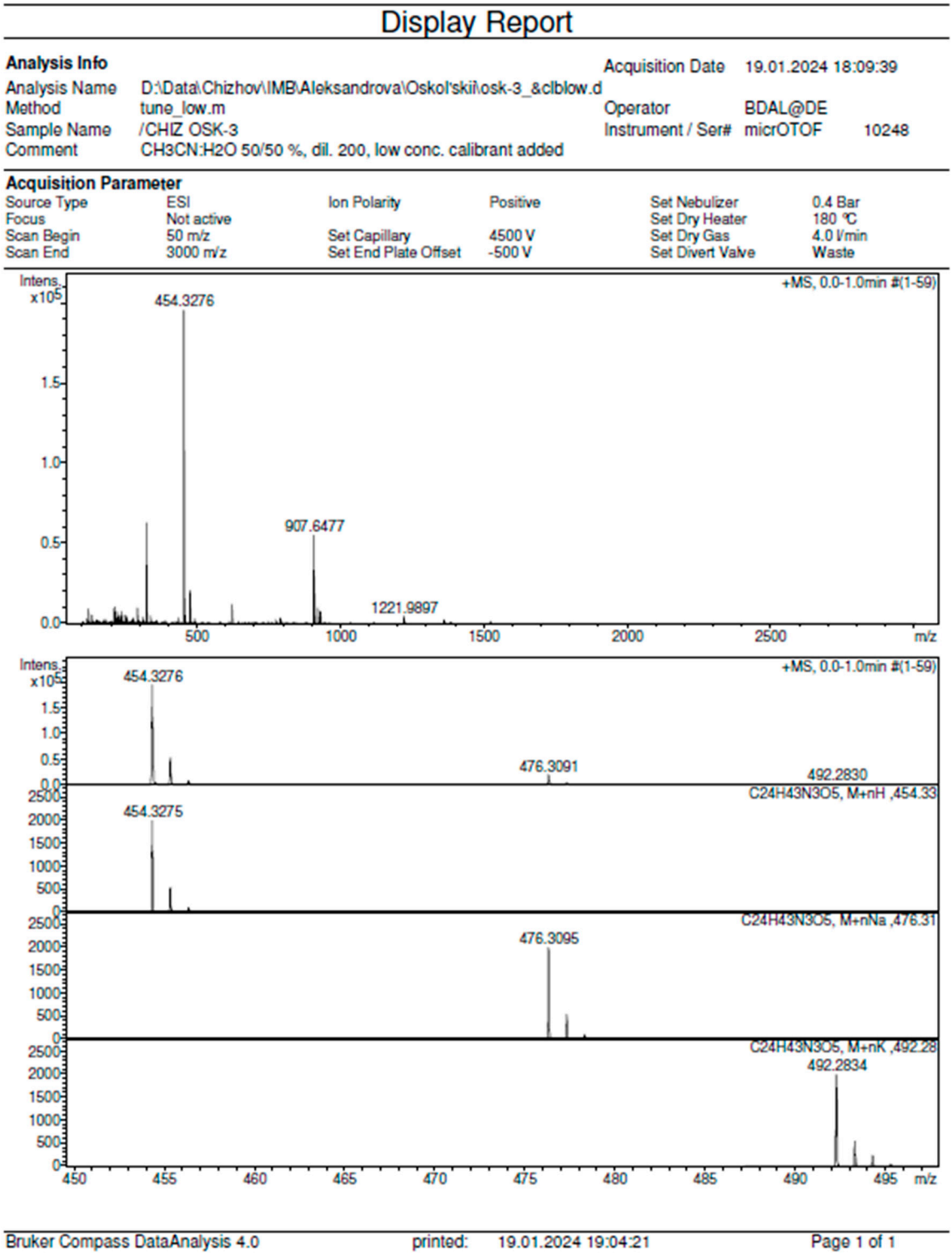
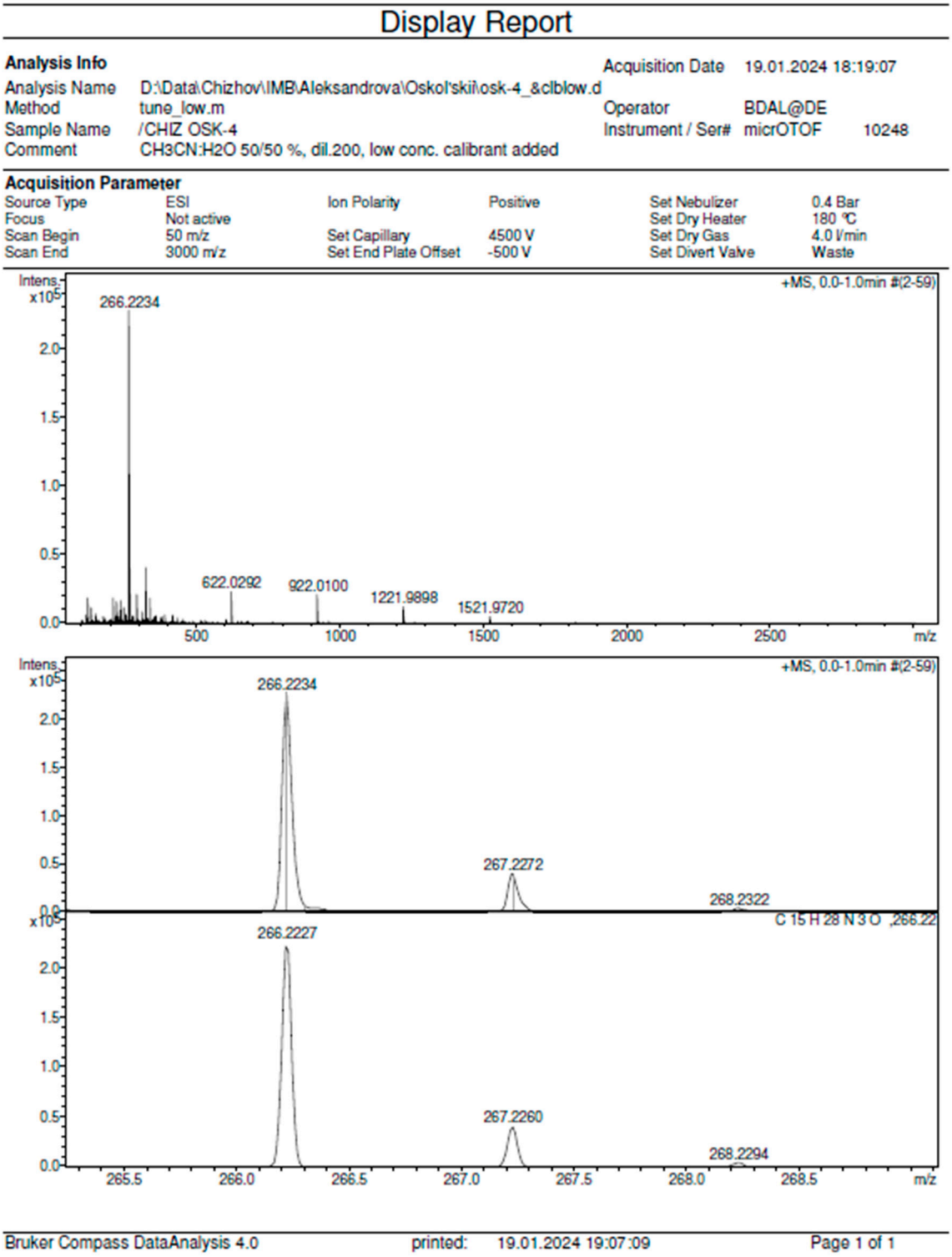
**7)** ***N*$^4$-Dodecyl-6-methylcytidine (3b)**



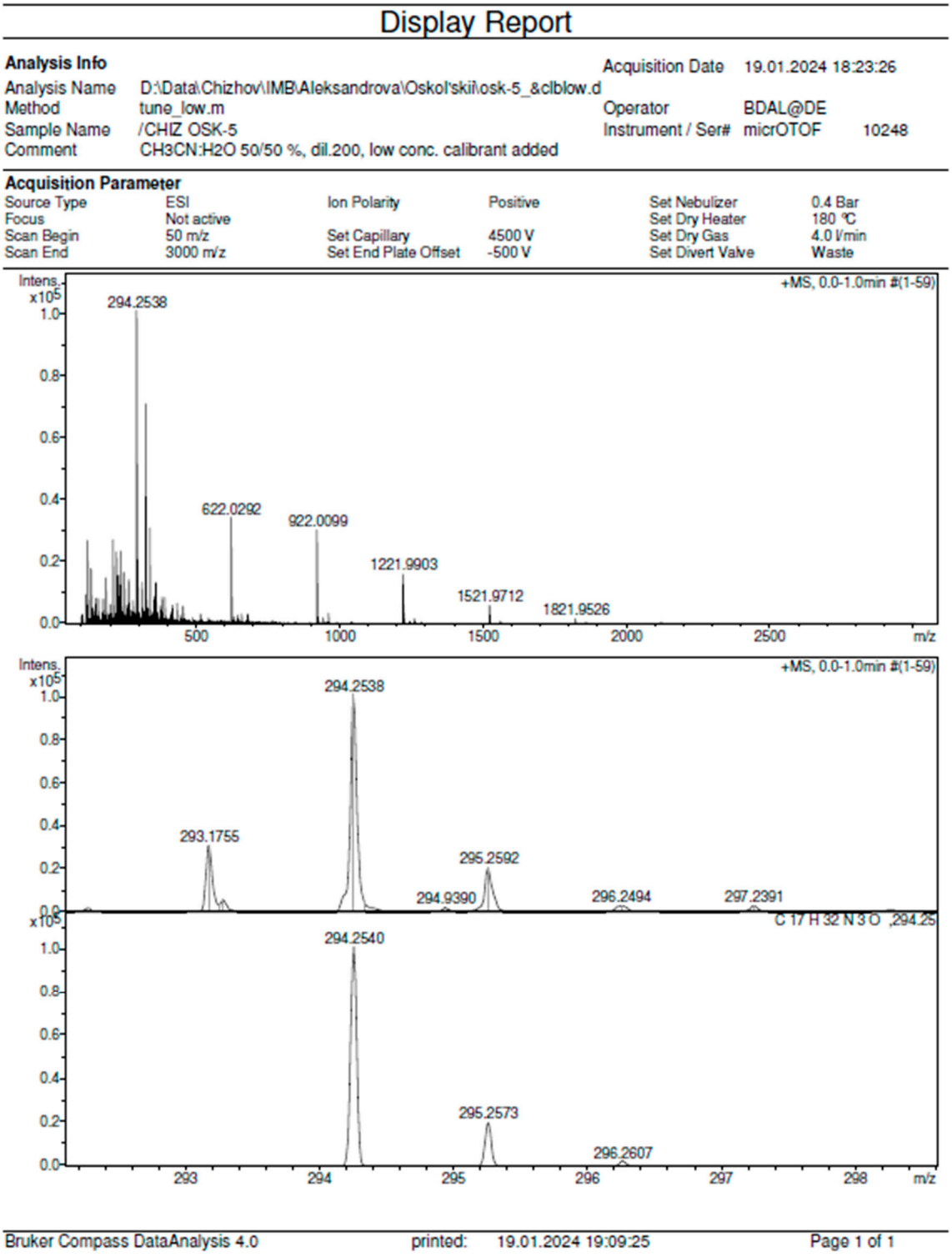
**8) $N^4$-Tetradecyl-6-methylcytidine (3c)**



**9)** ***N*$^4$-Decyl-6-methylcytosine (12a)**



**10) $N^4$-Dodecyl-6-methylcytosine (12b)**



**11) $N^4$-Tetradecyl-6-methylcytosine (12c)**