

Supplementary Material

Synthesis of N3-Substituted Carboranyl Thymidine Bioconjugates and their Evaluation as Substrates of Recombinant Human Thymidine Kinase 1

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1. HPLC methods.

Preparative HPLC was performed with a Gemini 5 μ C18 column (21.20 mm x 250 mm, 5 μ m particle size) supplied by Phenomenex Inc. CA, USA on a Hitachi HPLC system (L-2130) with a Windows based data acquisition and a Hitachi Diode array detector (L-2455). Purification was accomplished using a water (0.1% trifluoroacetic acid)/methanol (0.1% trifluoroacetic acid) gradient at 7 mL/min flow rate (method: 100:0 to 50:50 over 70 min followed by 50:50 to 0:100 over 40 min).

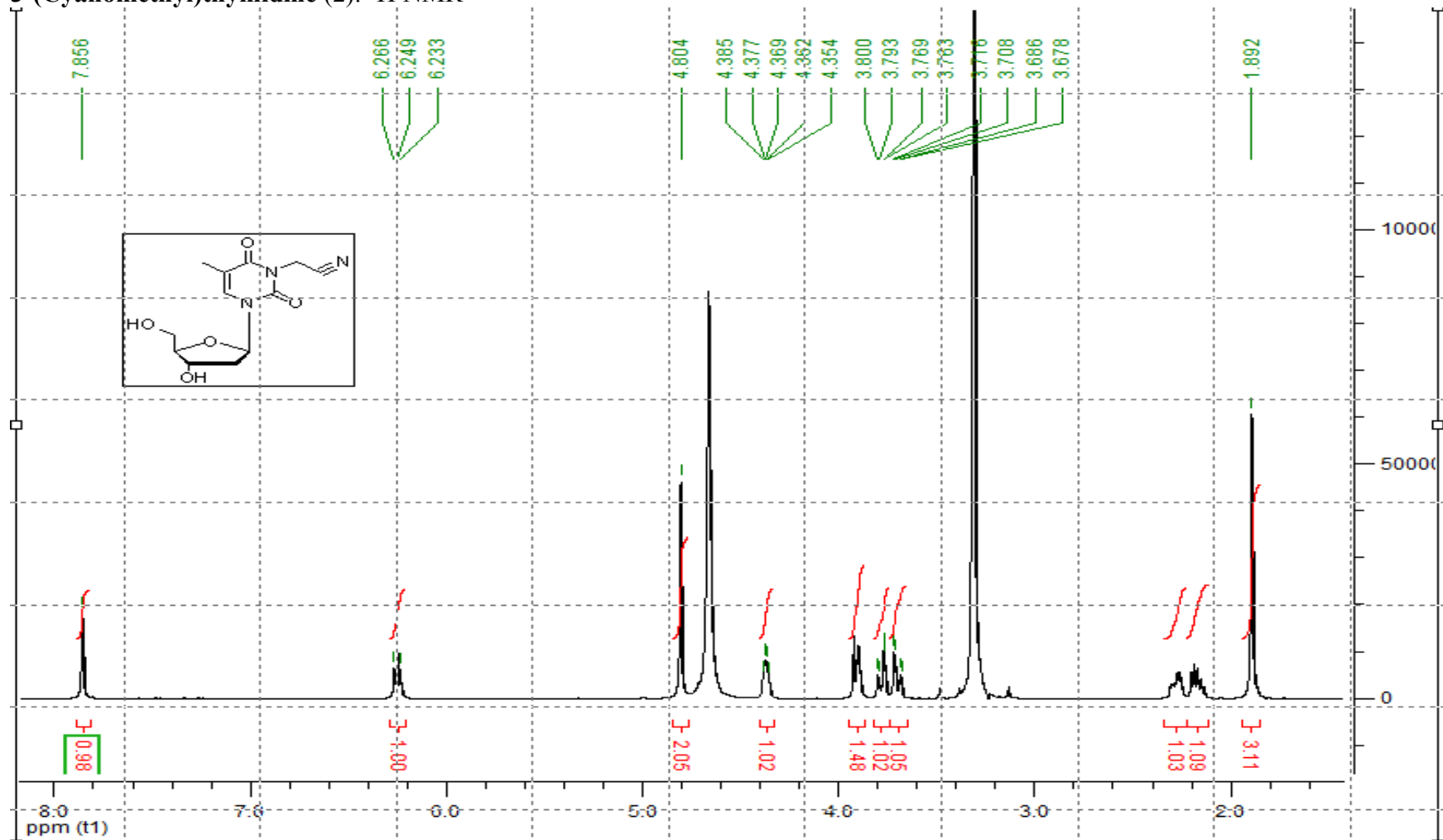
Table S1. HPLC method used for the purification of the compounds.

| Time (minutes) | Water Concentration A (%) | Acetonitrile Concentration B (%) | Flow rate (mL/min) |
|---------------------------|--|---|-------------------------------|
| 0.00 | 100.0 | 0.0 | 1.0 |
| 1.0 | 100.0 | 0.0 | 7.0 |
| 70.0 | 0.0 | 50.0 | 7.0 |
| 110.0 | 0.0 | 100.0 | 7.0 |
| 119.0 | 100.0 | 0.0 | 7.0 |
| 120.0 | 100.0 | 0.0 | 1.0 |

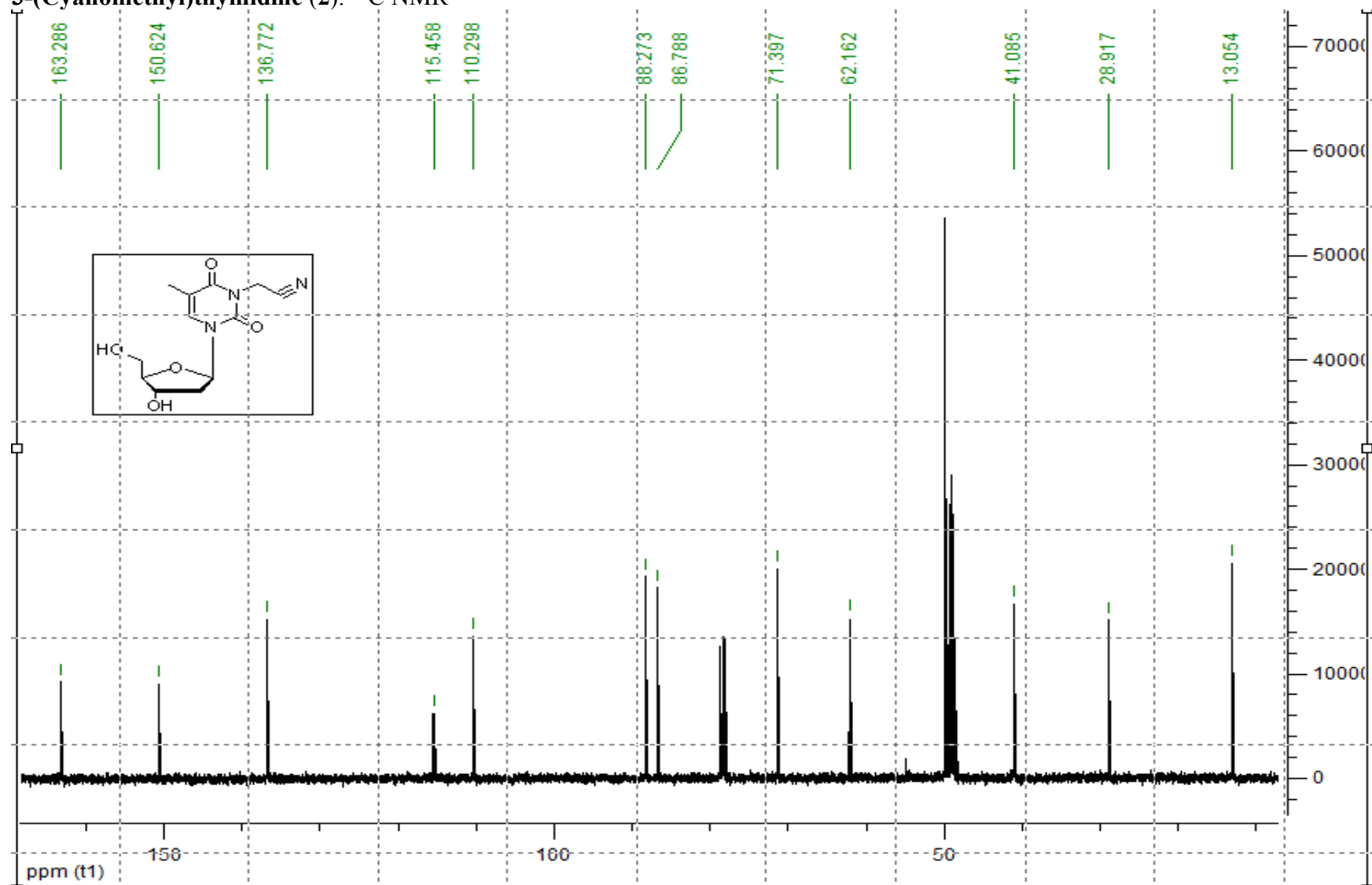
Analytical HPLC was carried out with a Gemini 5 μ C18 110A Column (250 x 4.6 mm) supplied by Phenomenex Inc. CA, USA using the HPLC system above. Two different gradients [water (0.1% trifluoroacetic acid)/acetonitrile (0.1% trifluoroacetic acid) and water (0.1% trifluoroacetic acid)/methanol (0.1% trifluoroacetic acid)] were used at 1 mL/min flow rate (method: 100:0 to 75:25 over 20 min followed by 75:25 to 100:0 over 10 min).

2. High resolution mass-, ^1H -NMR-, and ^{13}C NMR spectra and analytical HPLC traces in two different solvent systems

3-(Cyanomethyl)thymidine (2). ^1H NMR



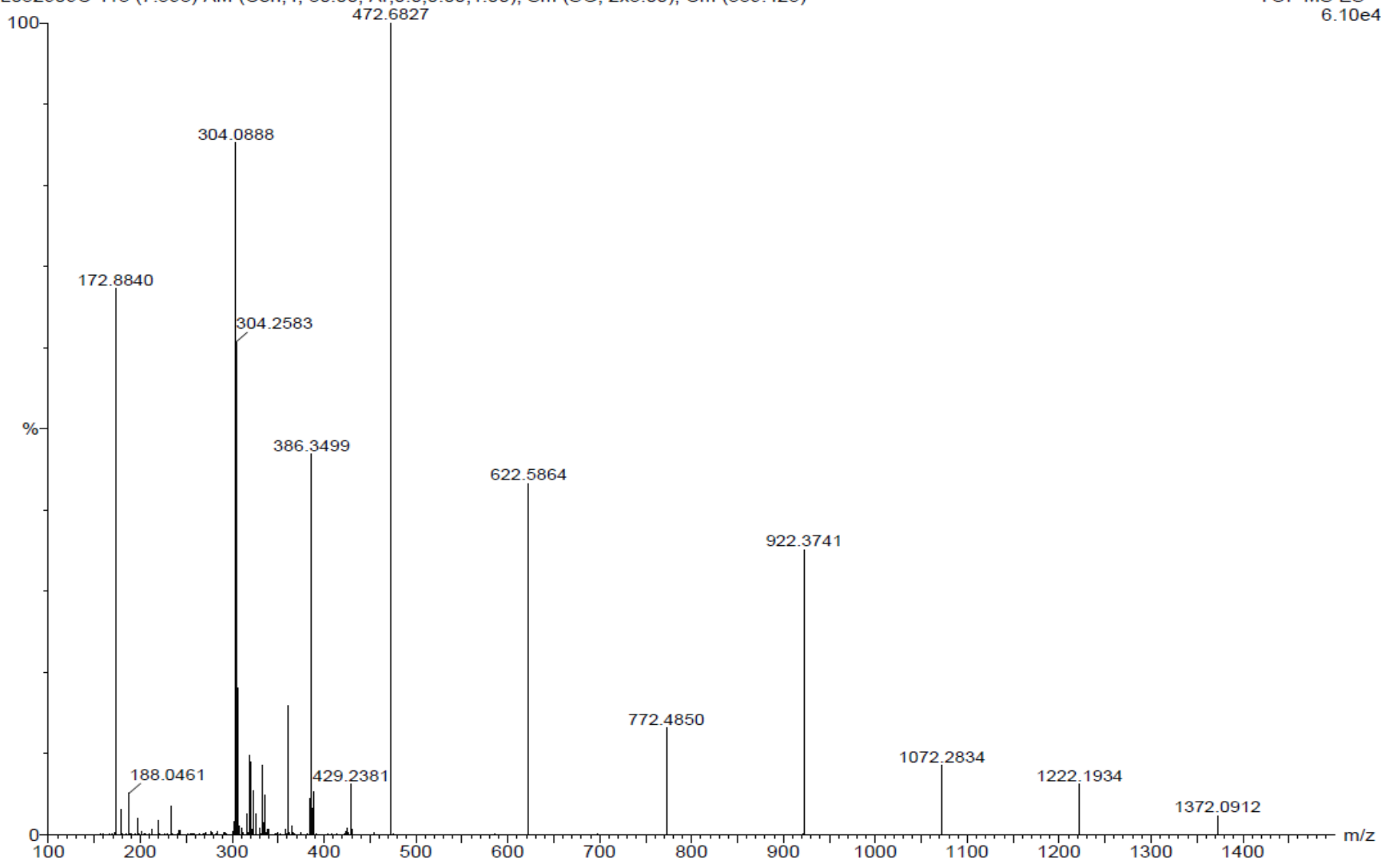
3-(Cyanomethyl)thymidine (2). ^{13}C NMR



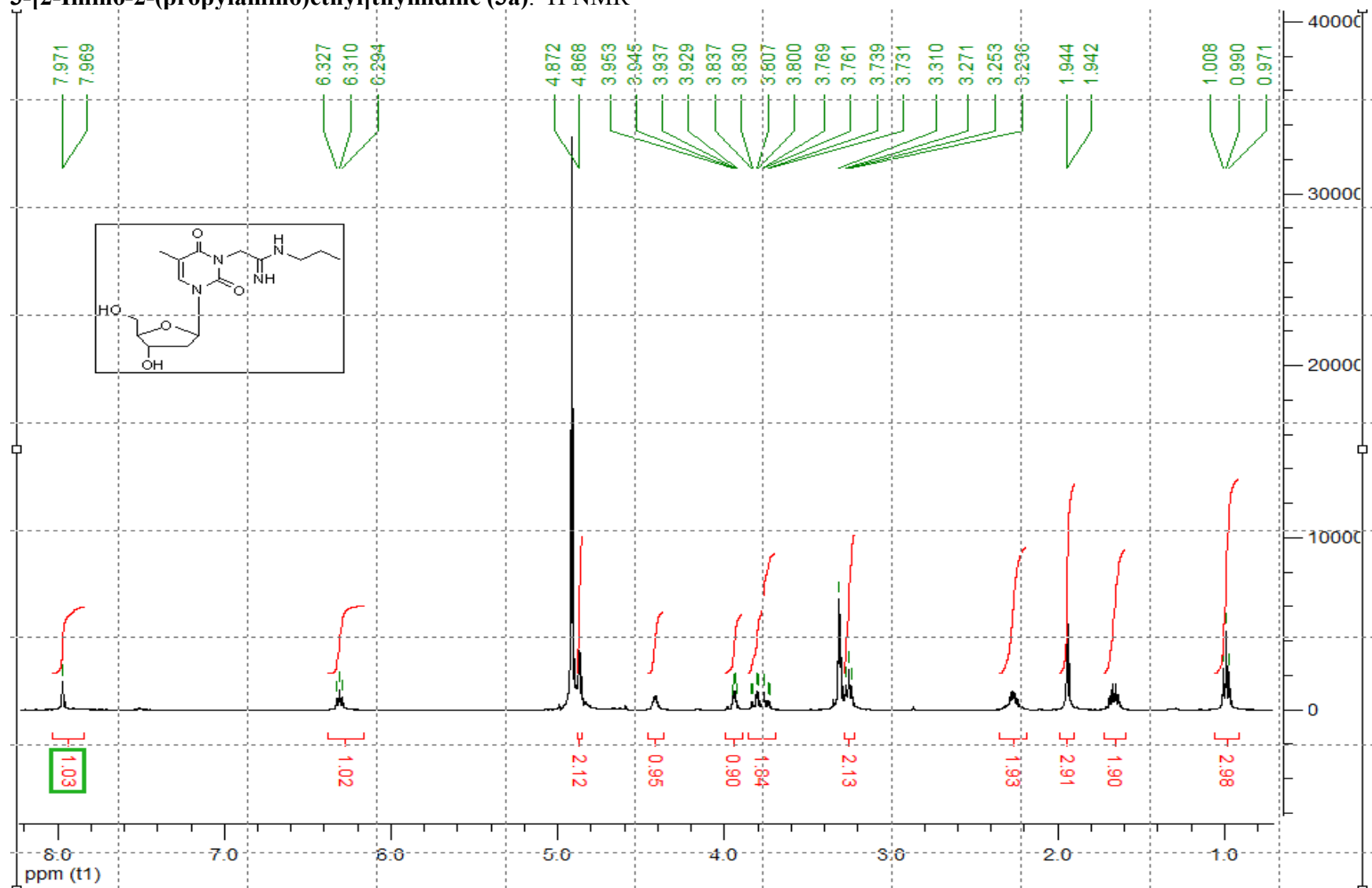
3-(Cyanomethyl)thymidine (2). HR-ESI-MS found: 304.0888.

L052909C 413 (7.593) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (359:425)

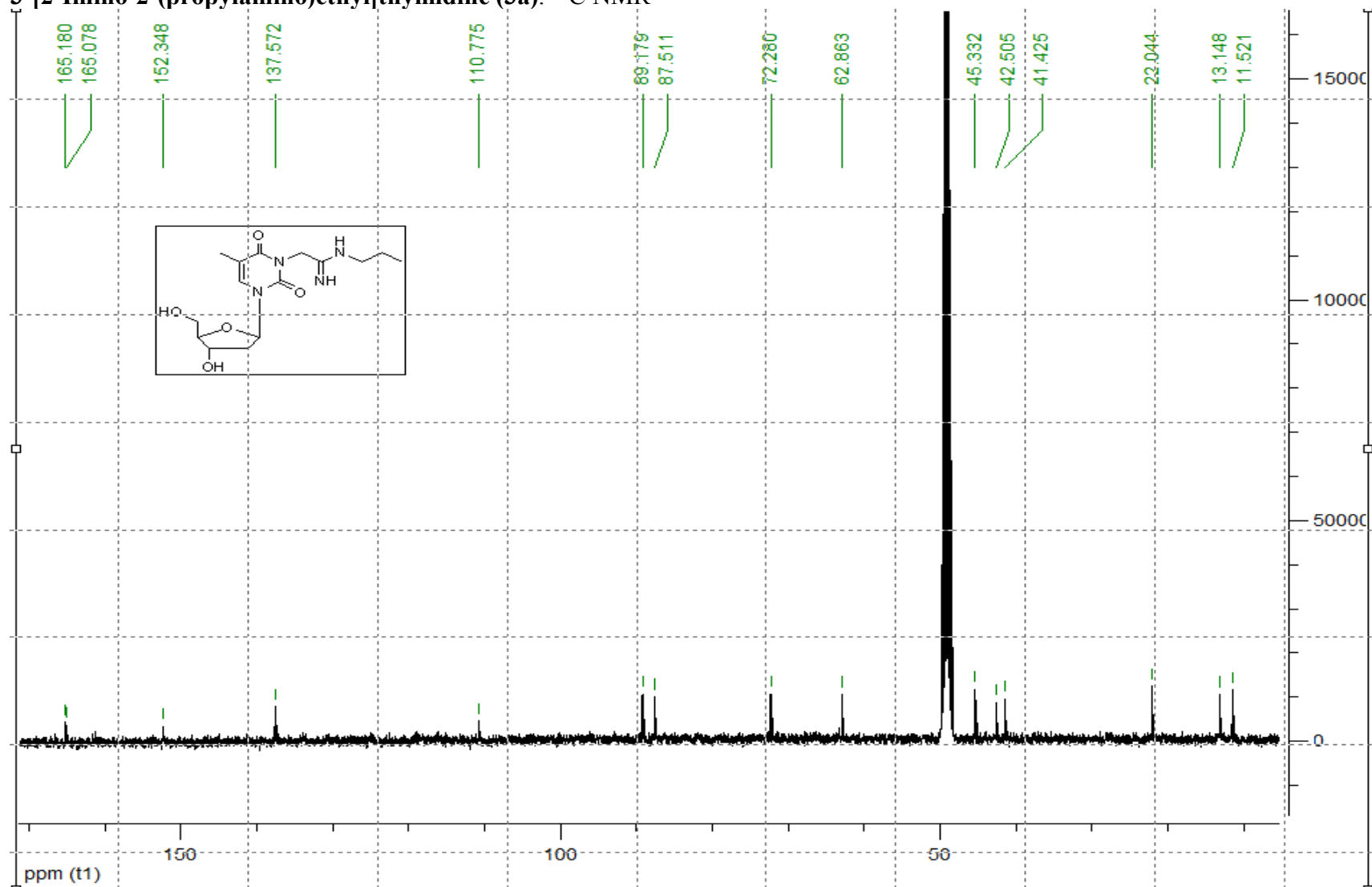
TOF MS ES+
6.10e4



3-[2-Imino-2-(propylamino)ethyl]thymidine (3a). ¹H NMR



3-[2-Imino-2-(propylamino)ethyl]thymidine (3a). ¹³C NMR

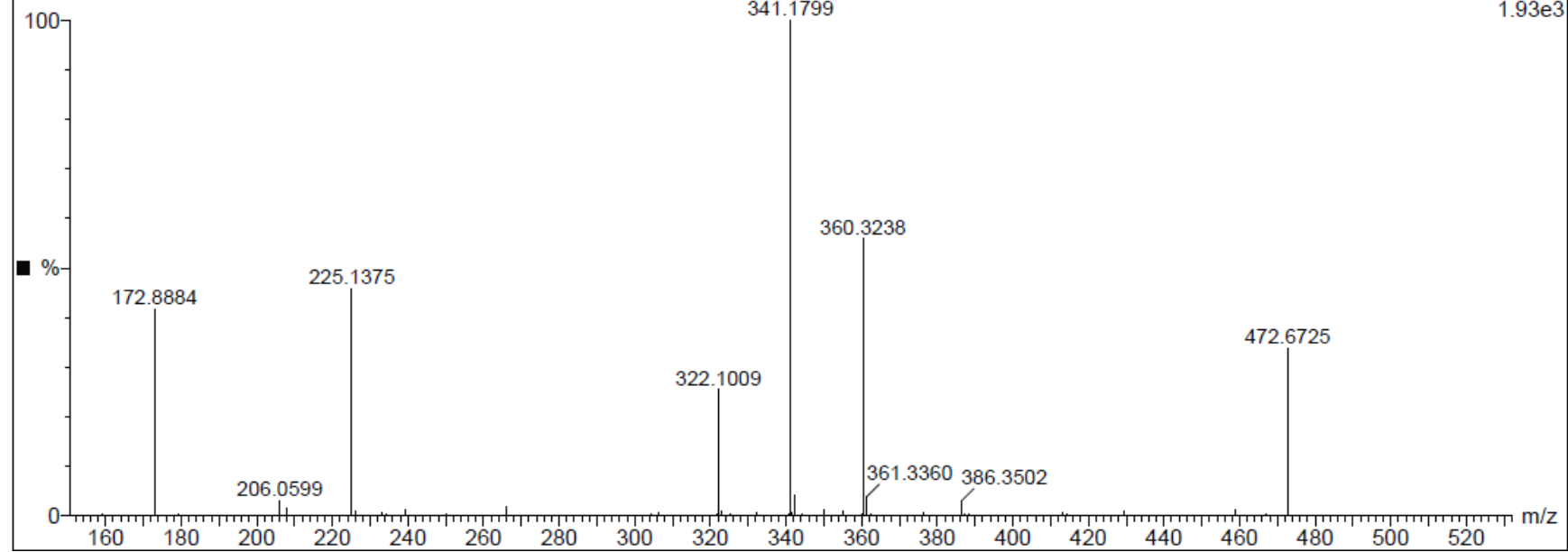


3-[2-Imino-2-(propylamino)ethyl]thymidine (3a). HR-ESI-MS found: 341.1799.

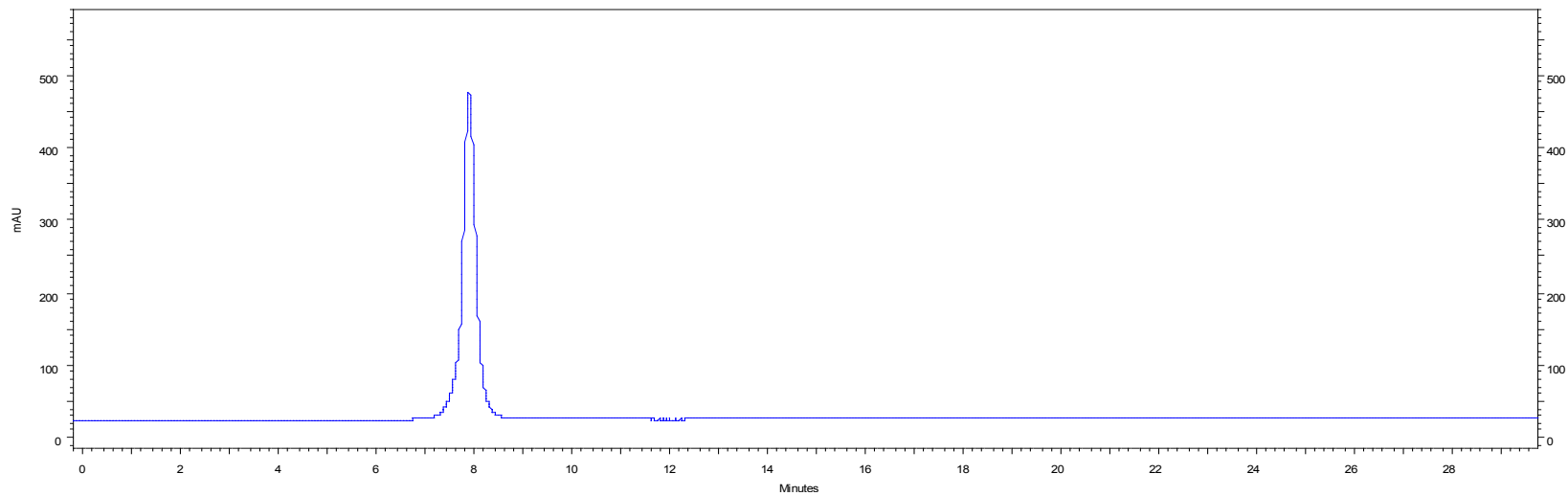
L070209F 107 (1.962) ÅM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (107)

TOF MS ES+

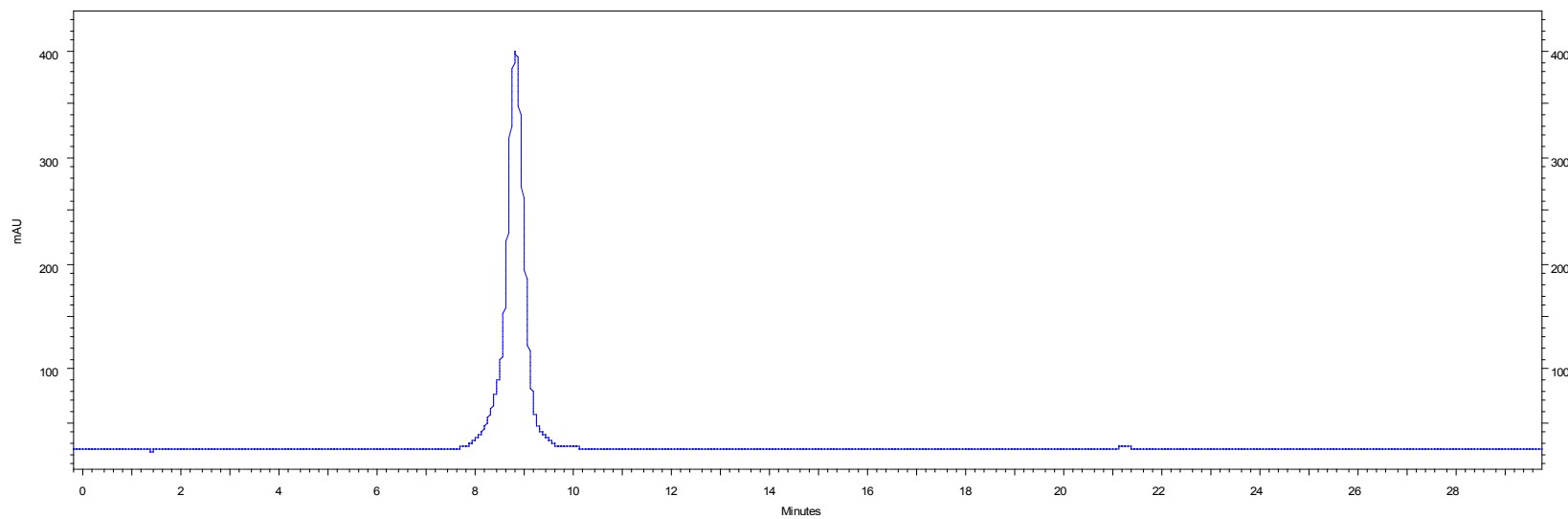
1.93e3



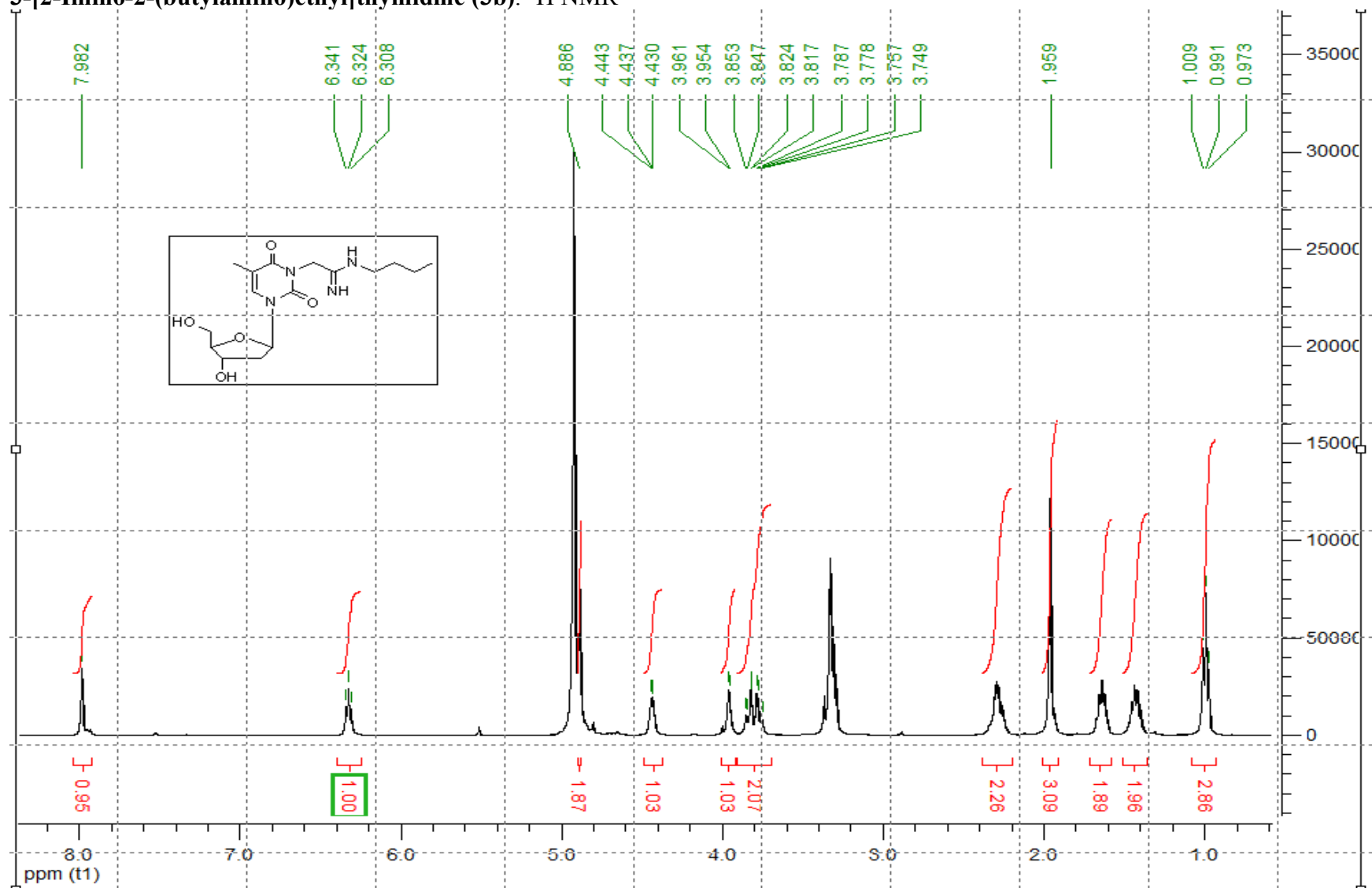
3-[2-Imino-2-(propylamino)ethyl]thymidine (3a). Analytical traces in Water:Acetonitrile gradient



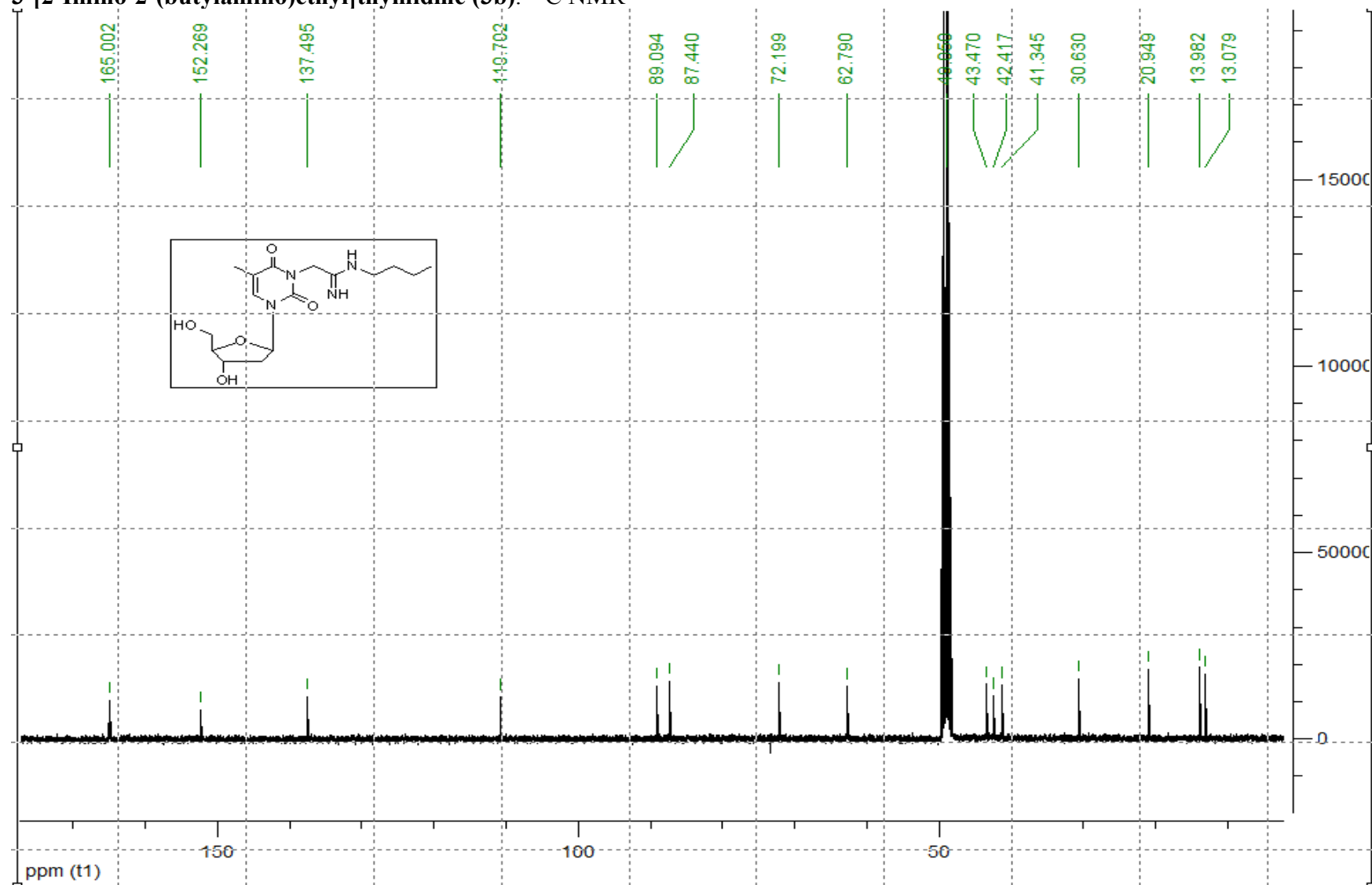
Analytical traces in Water:Methanol gradient



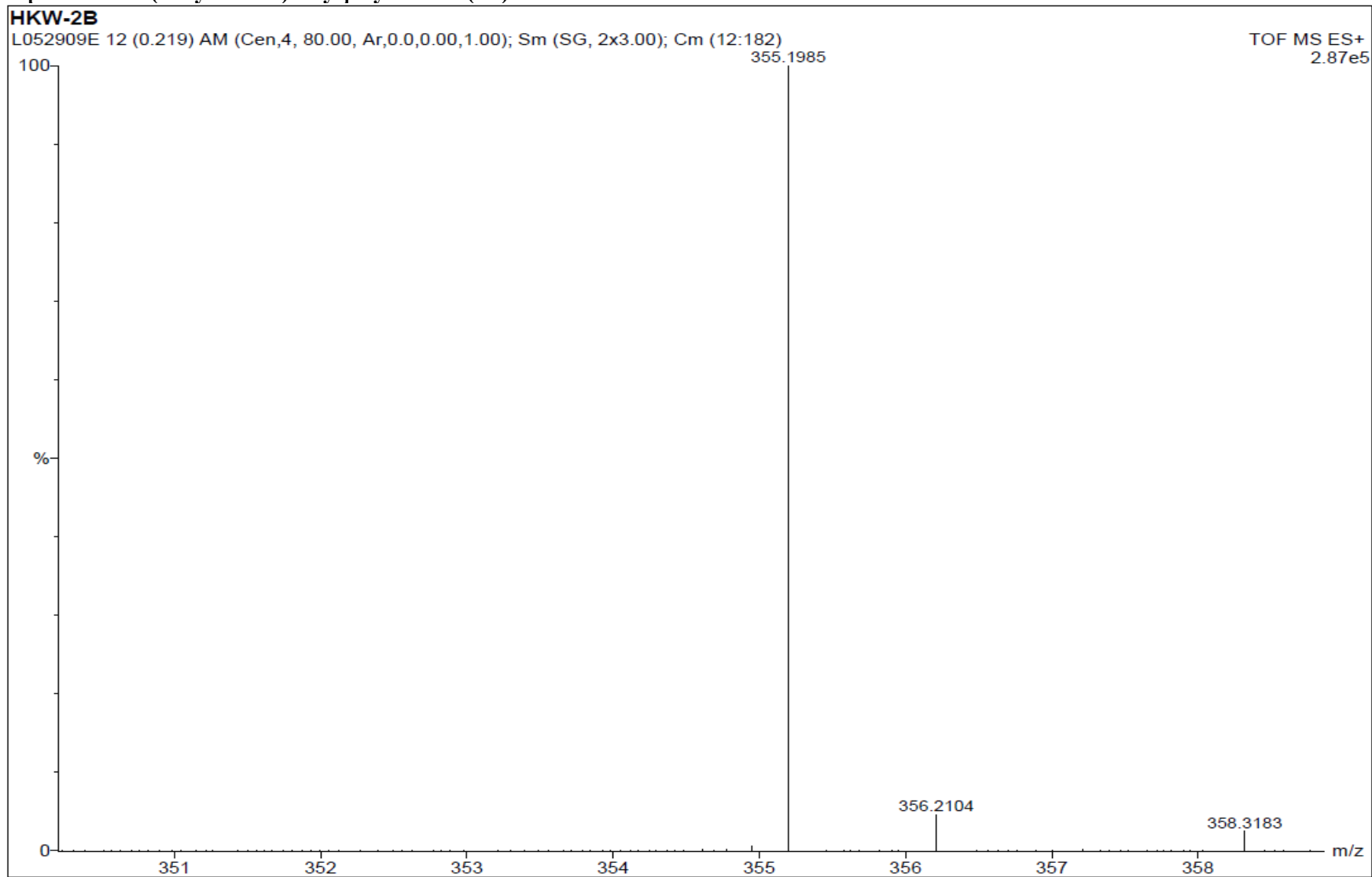
3-[2-Imino-2-(butylamino)ethyl]thymidine (3b). ¹H NMR



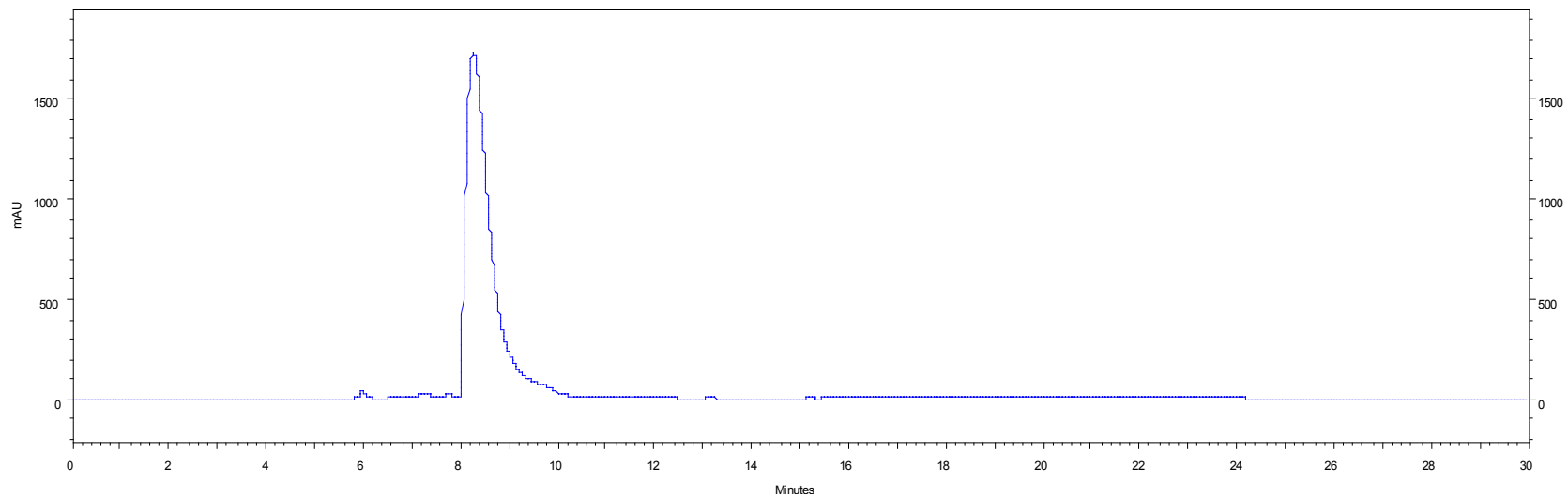
3-[2-Imino-2-(butylamino)ethyl]thymidine (3b). ¹³C NMR



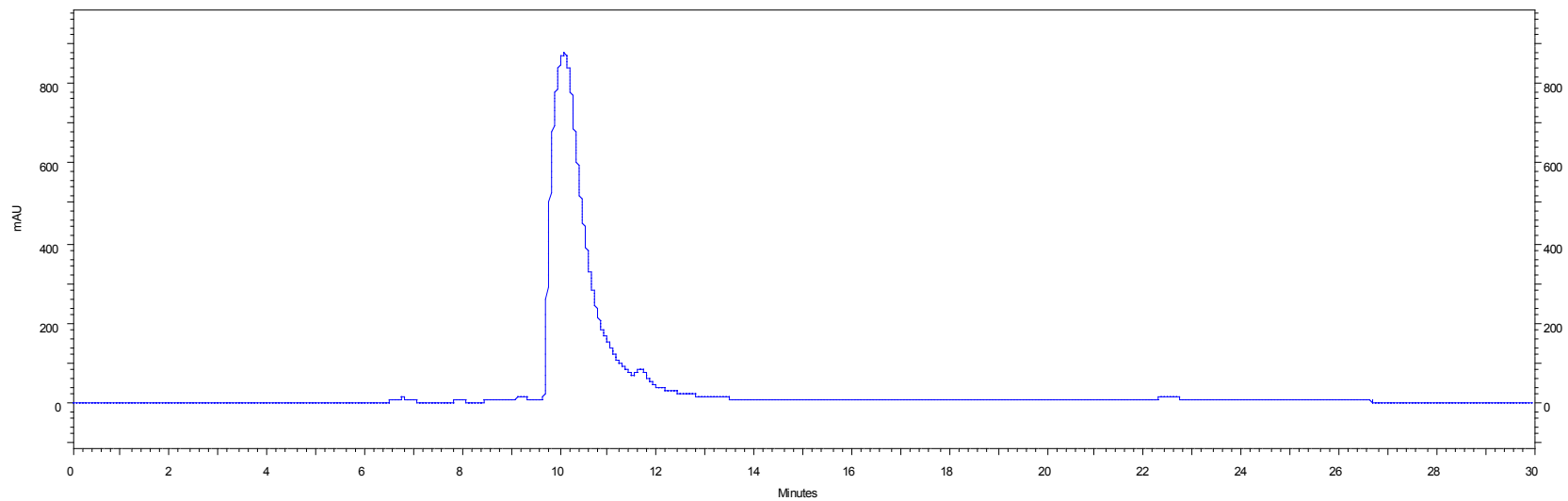
3-[2-Imino-2-(butylamino)ethyl]thymidine (3b). HR-ESI-MS found: 355.1985.



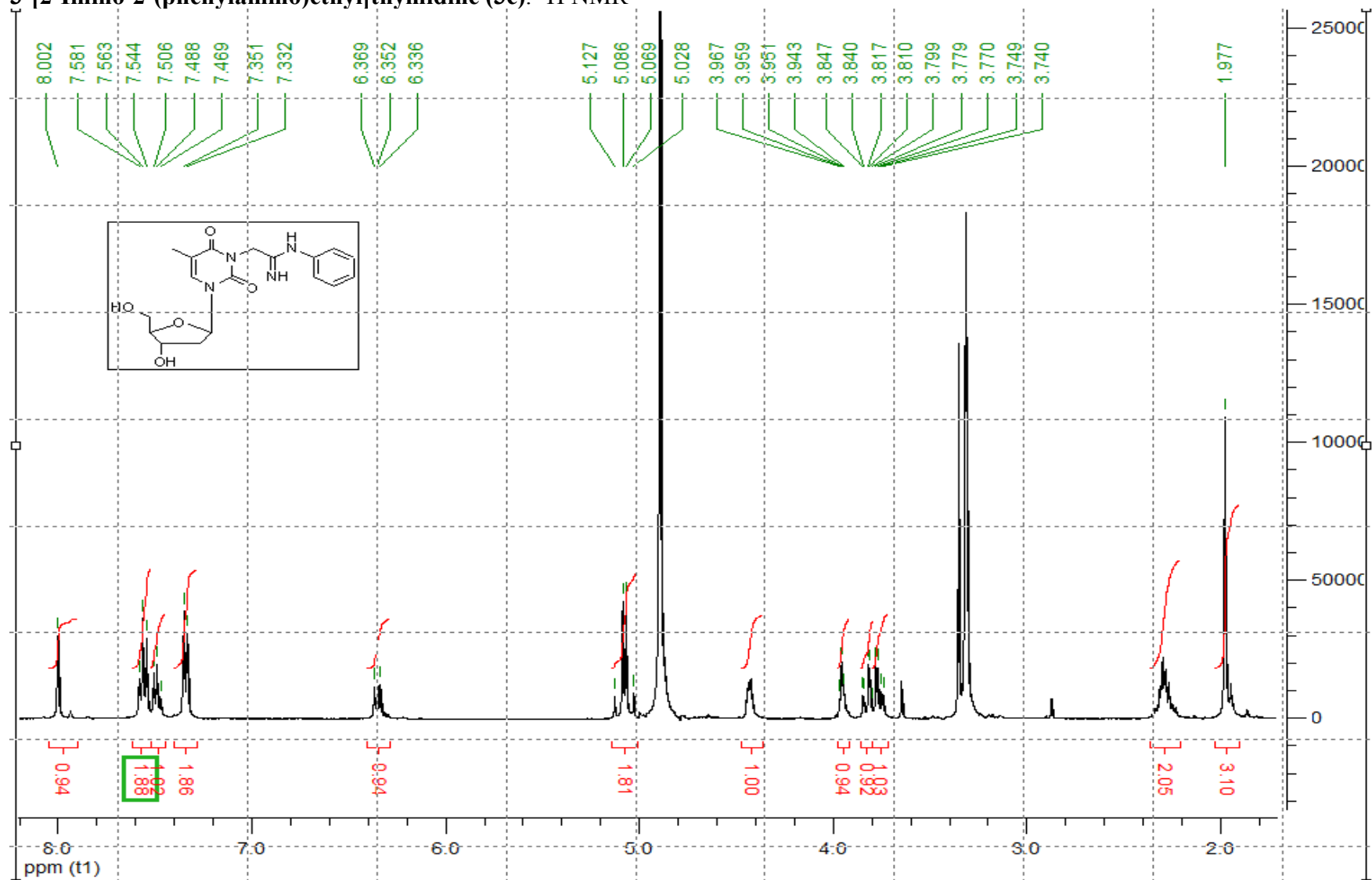
3-[2-Imino-2-(butylamino)ethyl]thymidine (3b). Analytical traces in Water:Acetonitrile gradient



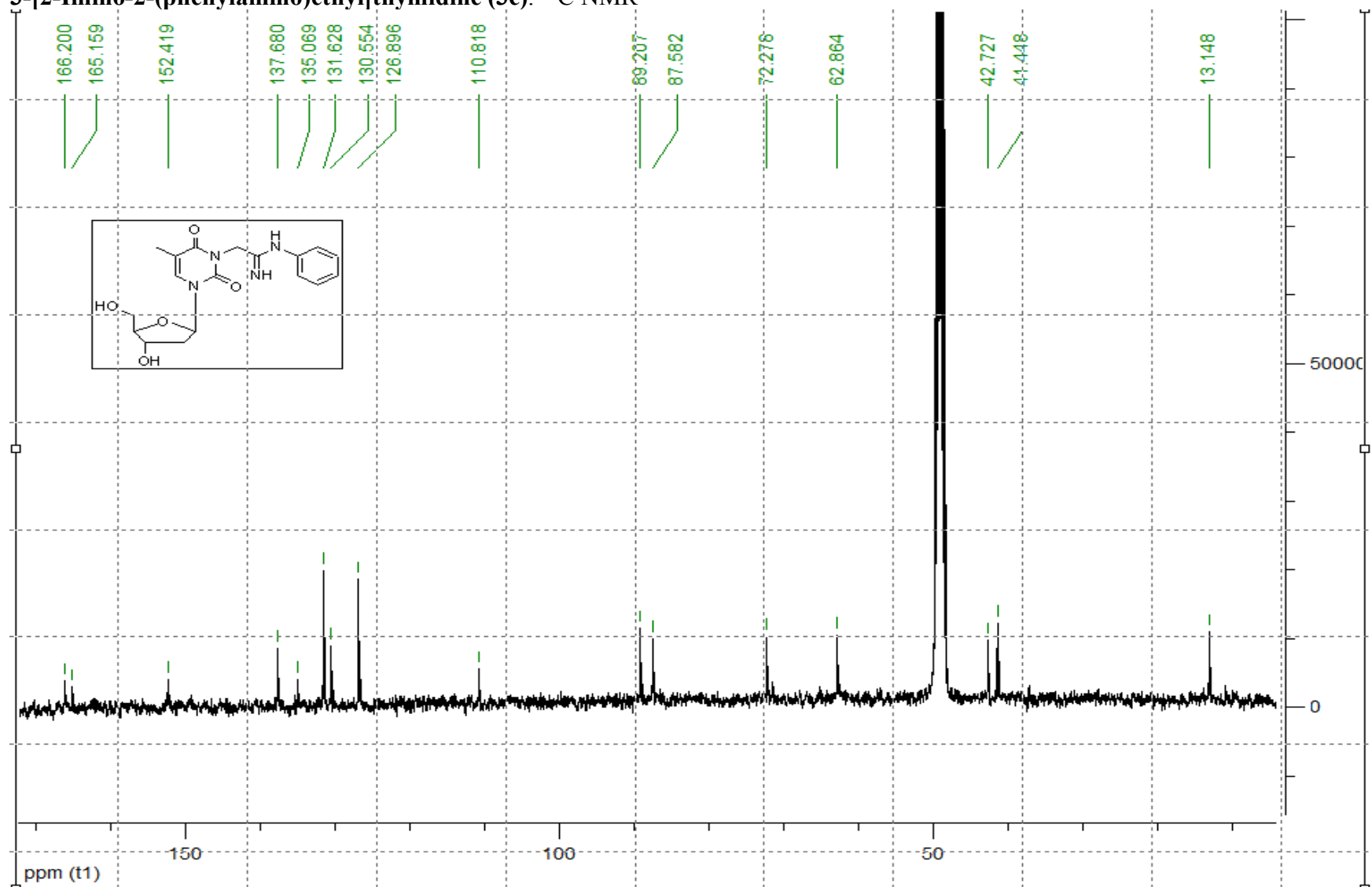
Analytical traces in Water:Methanol gradient



3-[2-Imino-2-(phenylamino)ethyl]thymidine (3c). ¹H NMR



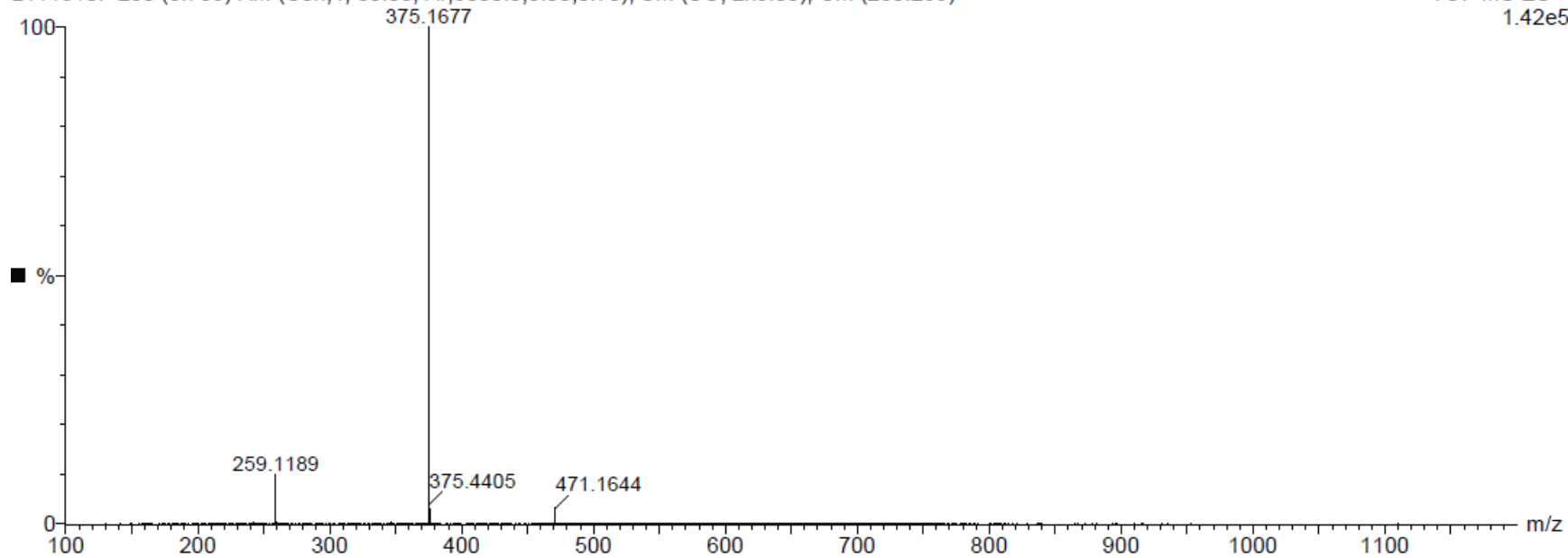
3-[2-Imino-2-(phenylamino)ethyl]thymidine (3c). ¹³C NMR



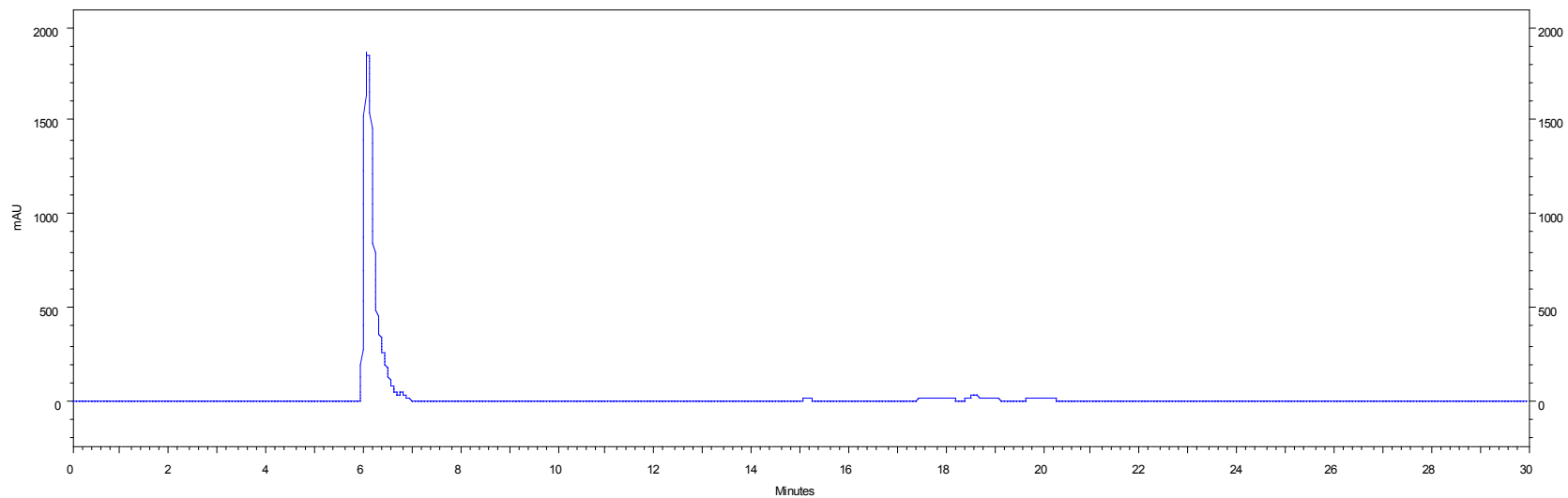
3-[2-Imino-2-(phenylamino)ethyl]thymidine (3c). HR-ESI-MS found: 375.1677.

L111510F 250 (8.789) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (250:269)

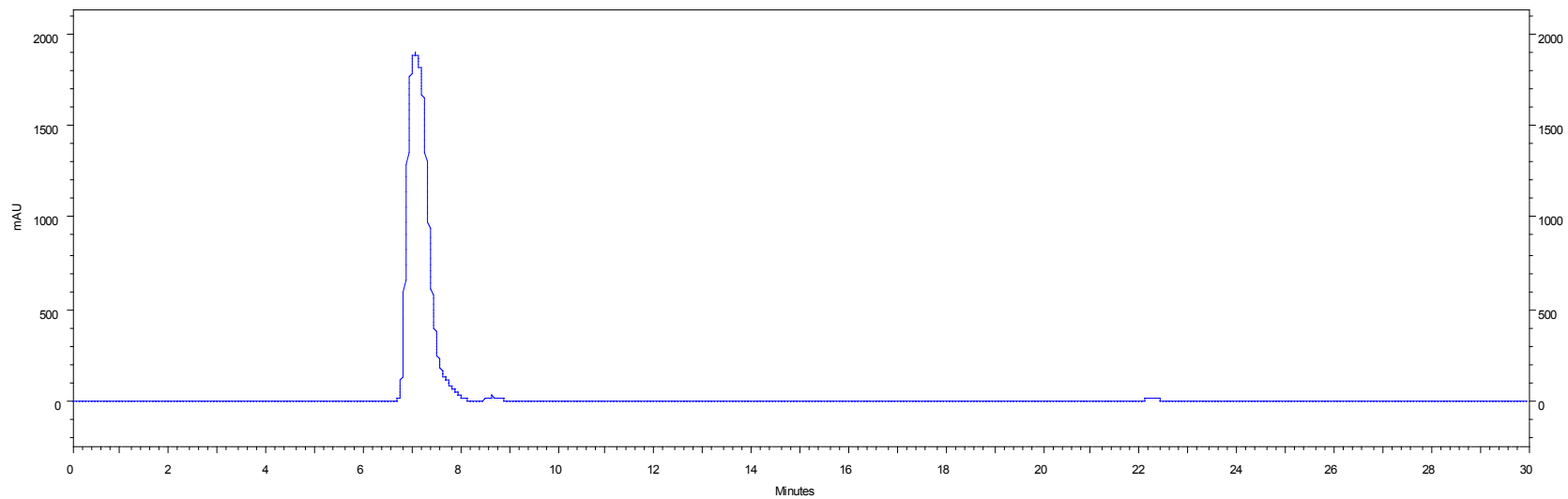
TOF MS ES+
1.42e5



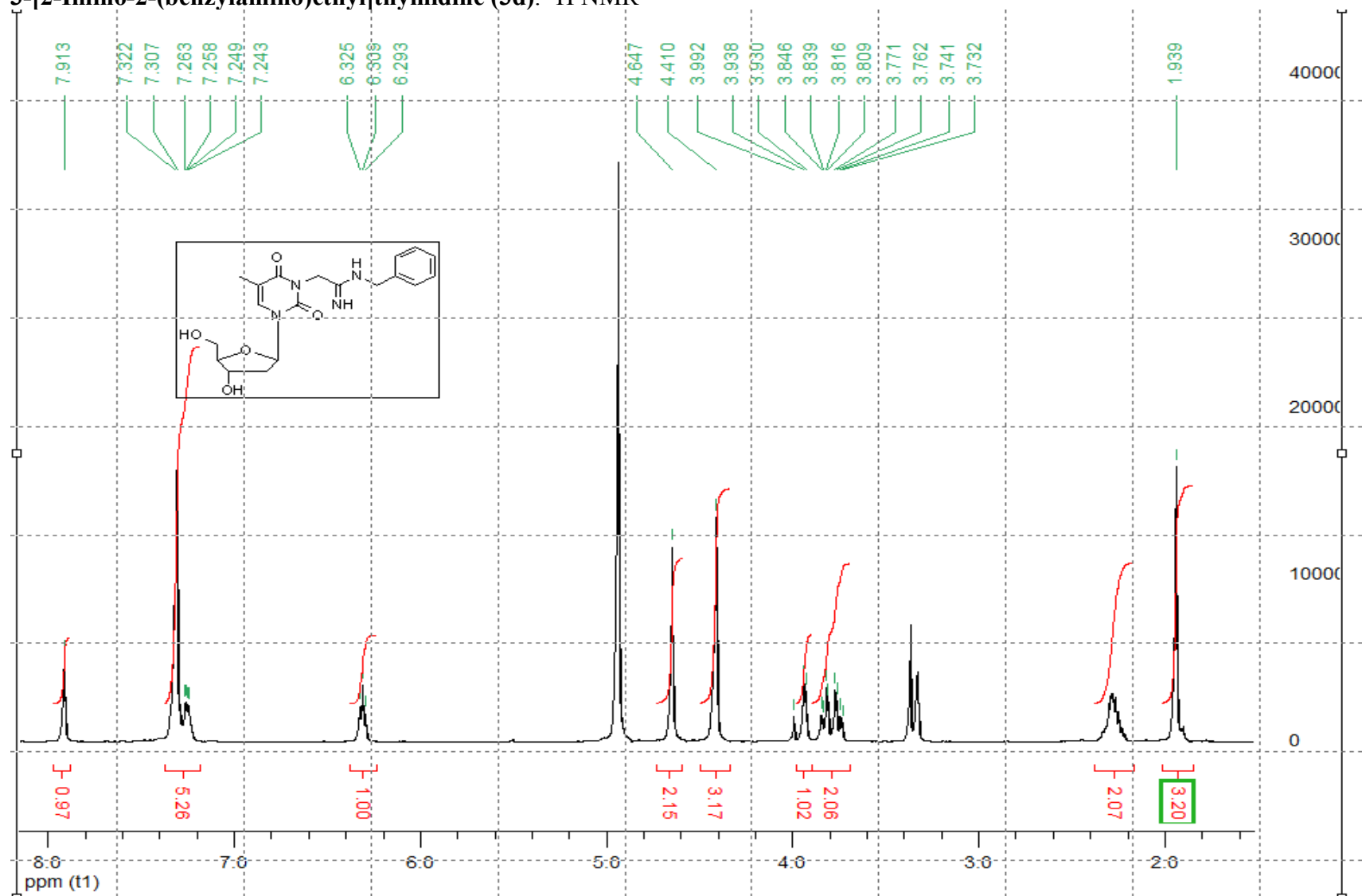
3-[2-Imino-2-(phenylamino)ethyl]thymidine (3c). Analytical traces in Water:Acetonitrile gradient



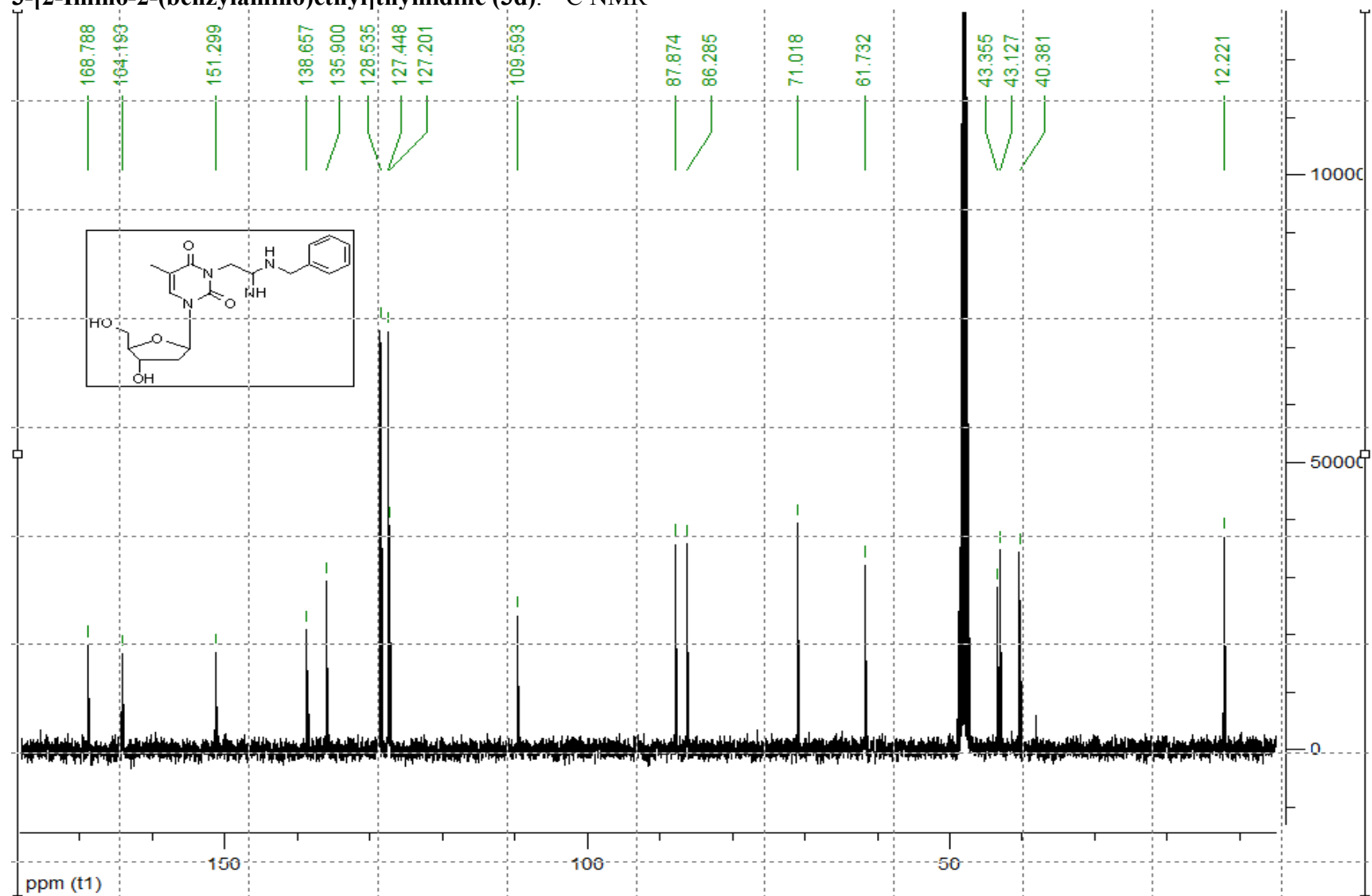
Analytical traces in Water:Methanol gradient



3-[2-Imino-2-(benzylamino)ethyl]thymidine (3d). ¹H NMR



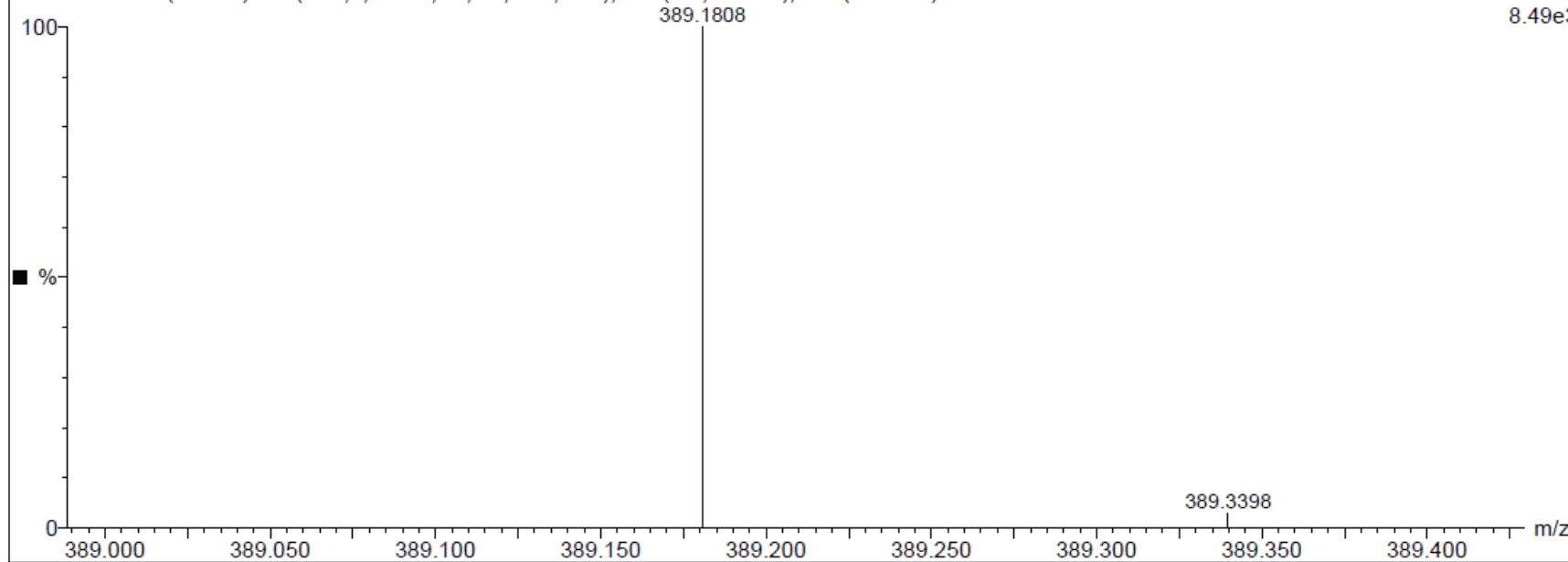
3-[2-Imino-2-(benzylamino)ethyl]thymidine (3d). ¹³C NMR



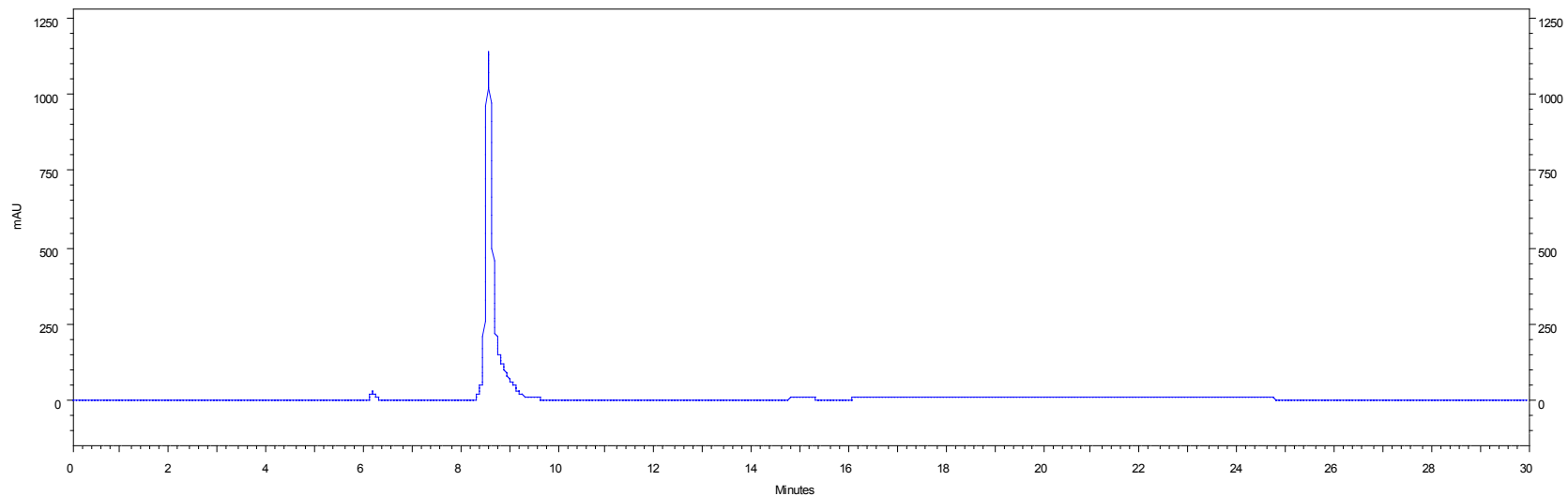
3-[2-Imino-2-(benzylamino)ethyl]thymidine (3d). HR-ESI-MS found: 389.1808

L052509N 350 (11.115) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (295:351)

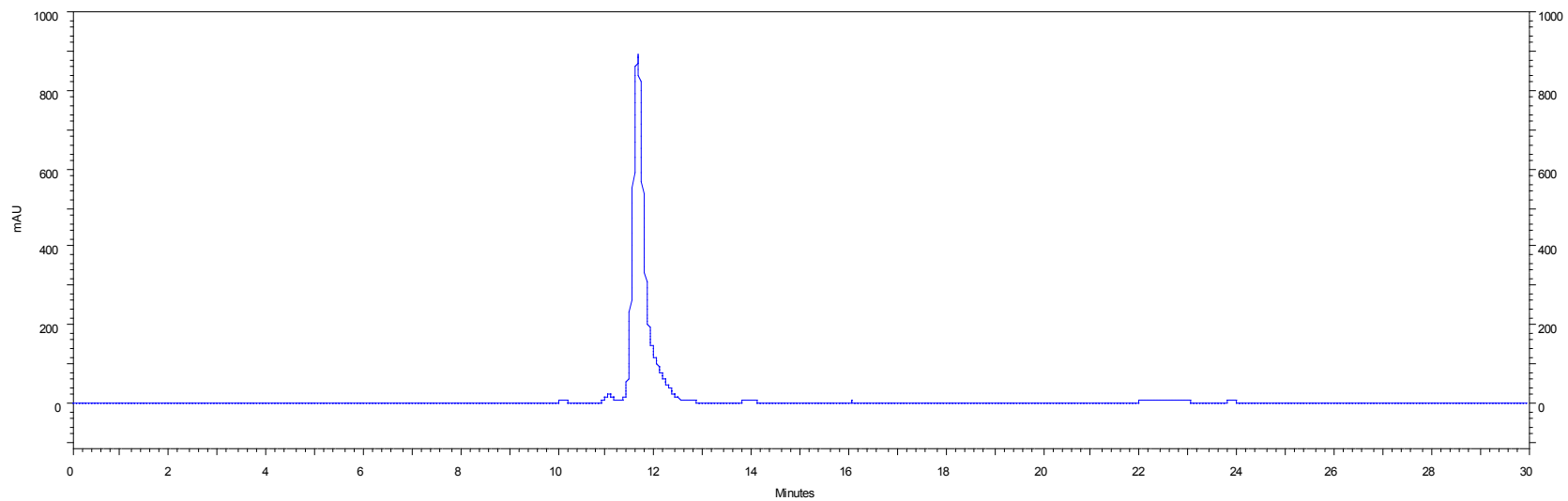
TOF MS ES+
8.49e3



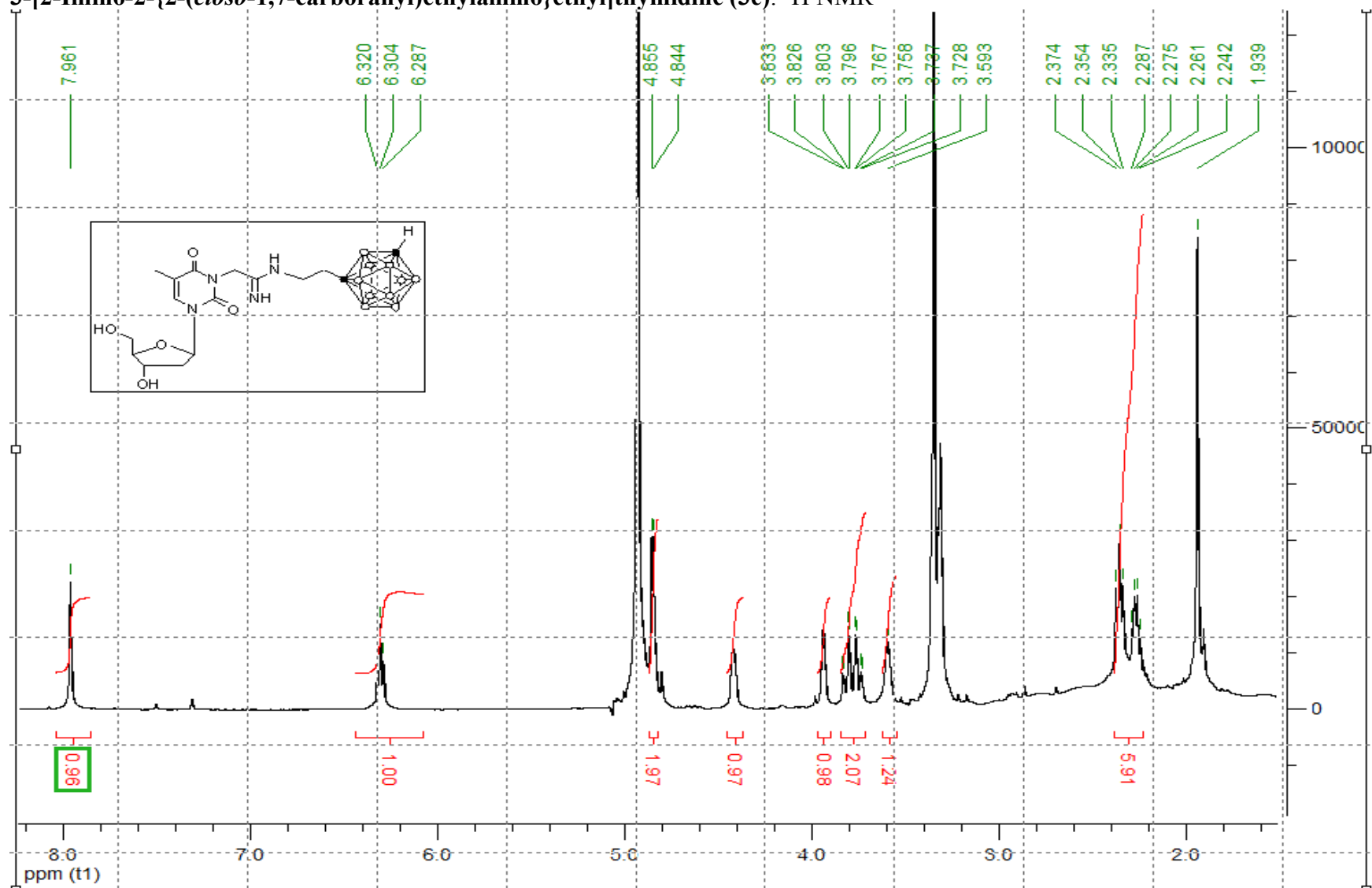
3-[2-Imino-2-(benzylamino)ethyl]thymidine (3d). Analytical traces in Water:Acetonitrile gradient



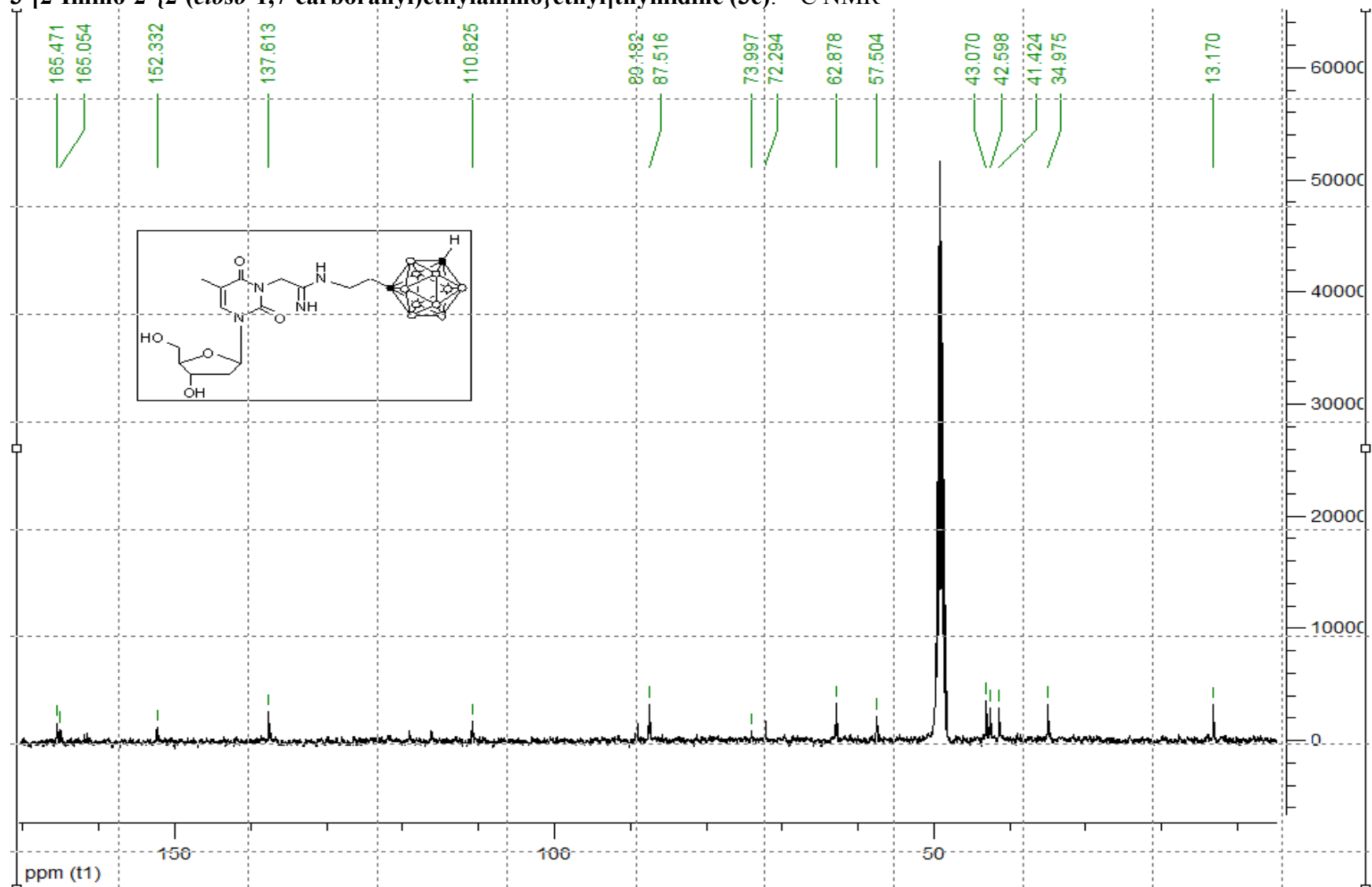
Analytical traces in Water:Methanol gradient



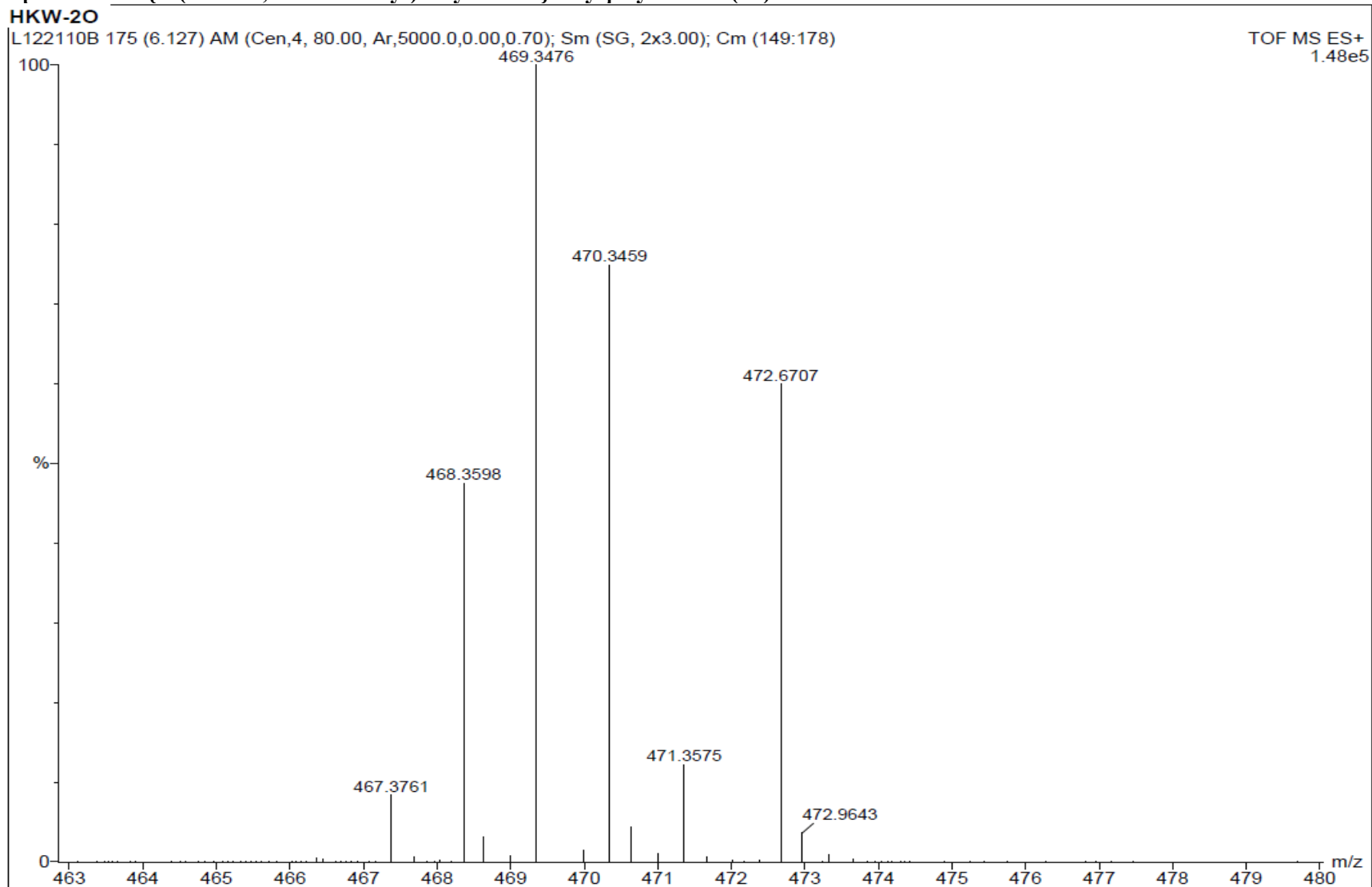
3-[2-Imino-2-{2-(*closo*-1,7-carboranyl)ethylamino}ethyl]thymidine (3e). ¹H NMR



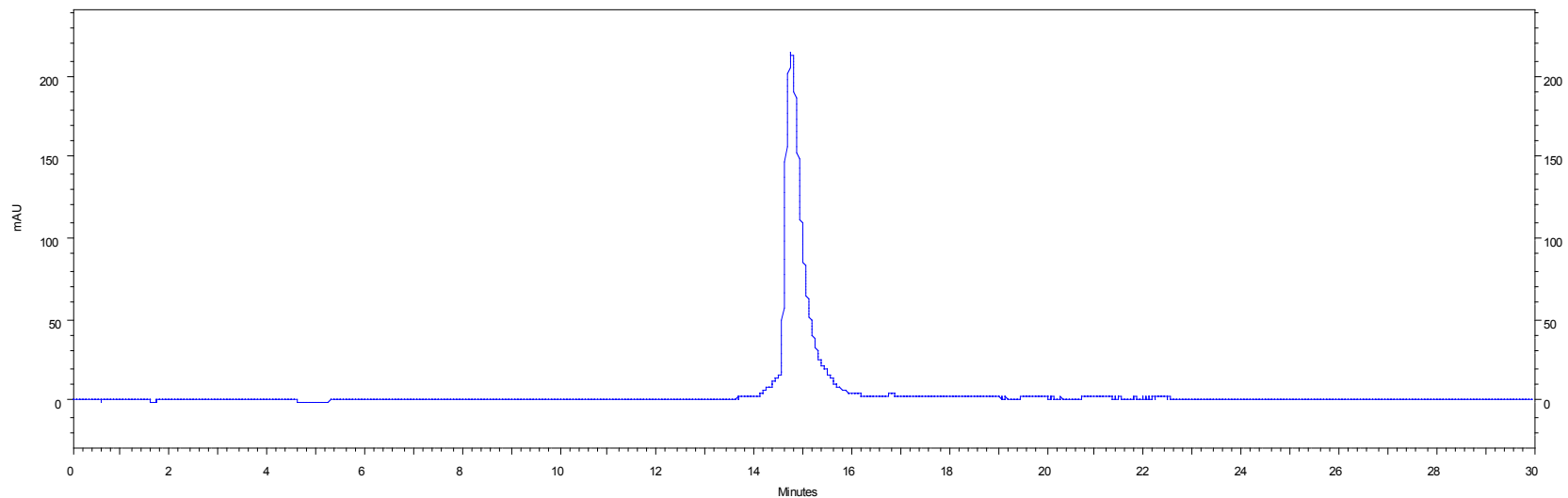
3-[2-Imino-2-{2-(*closo*-1,7-carboranyl)ethylamino}ethyl]thymidine (3e). ¹³C NMR



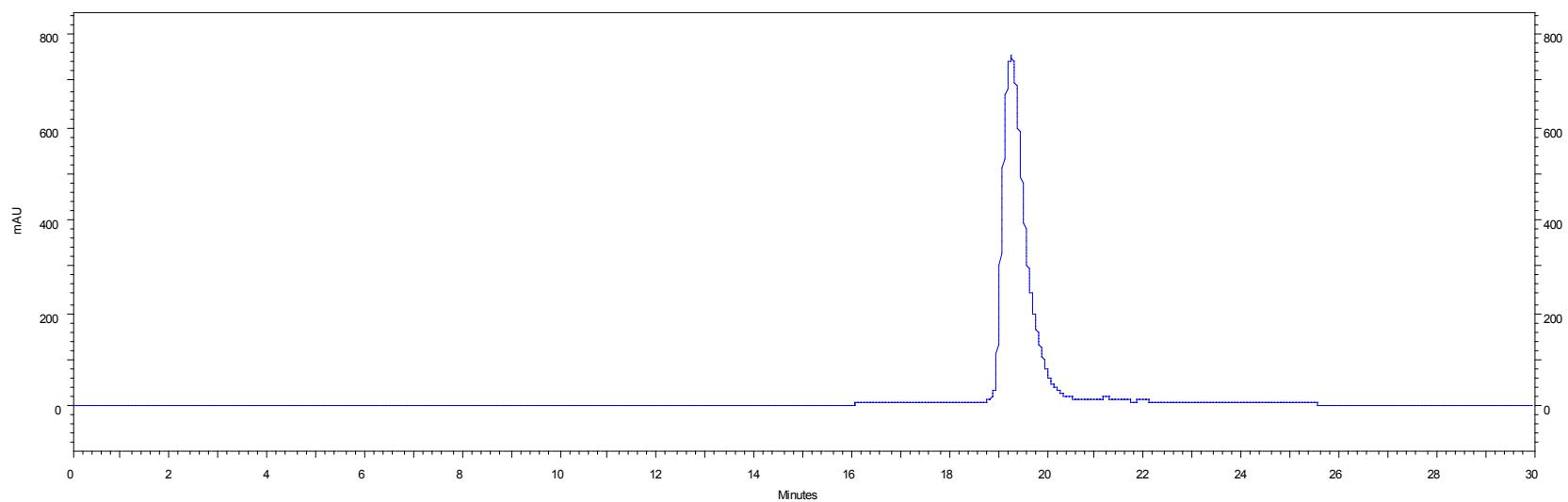
3-[2-Imino-2-{2-(*closo*-1,7-carboranyl)ethylamino}ethyl]thymidine (3e). HR-ESI-MS found: 469.3476.



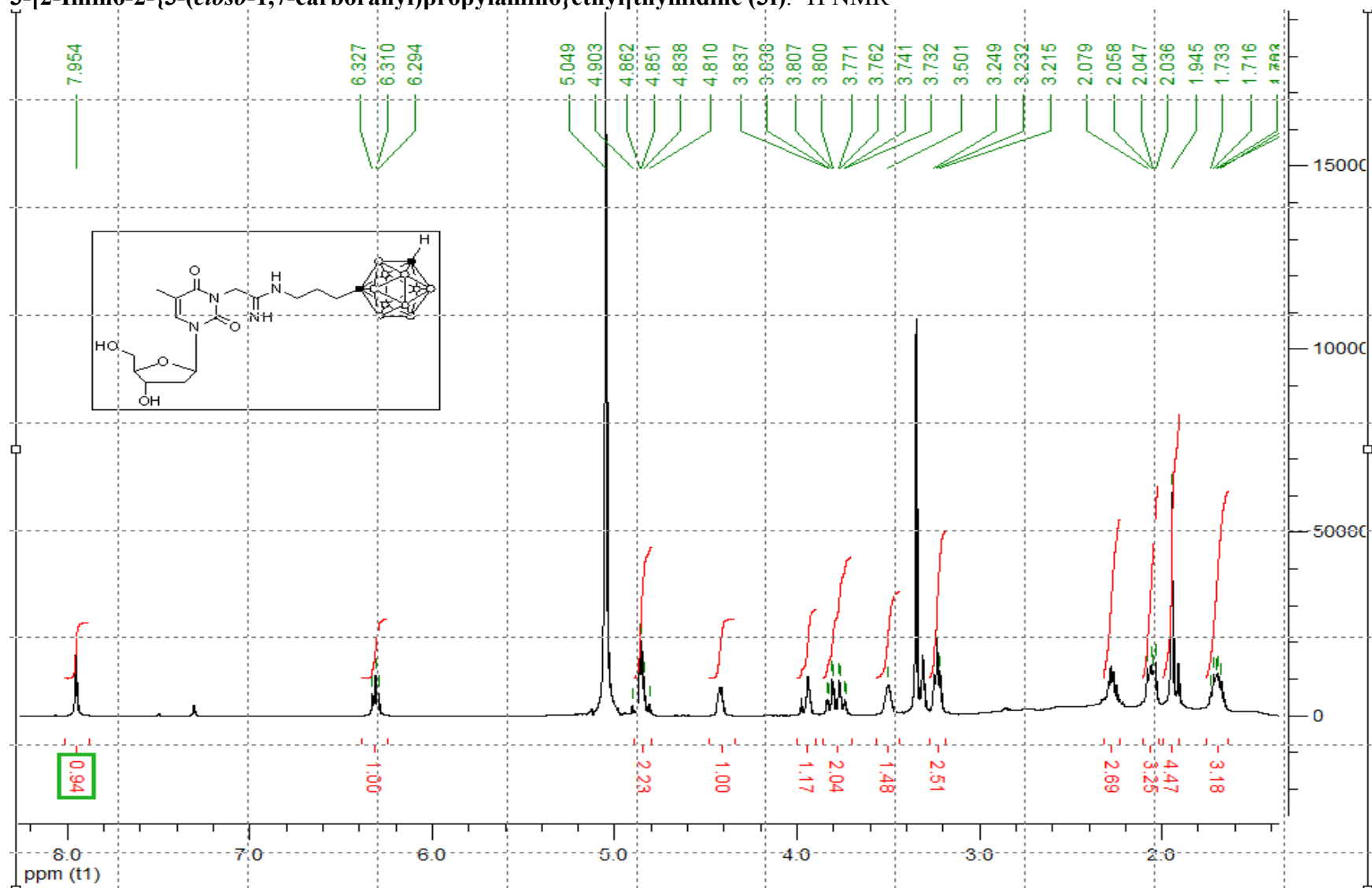
3-[2-Imino-2-{2-(*closo*-1,7-carboranyl)ethylamino}ethyl]thymidine (3e). Analytical traces in Water:Acetonitrile gradient



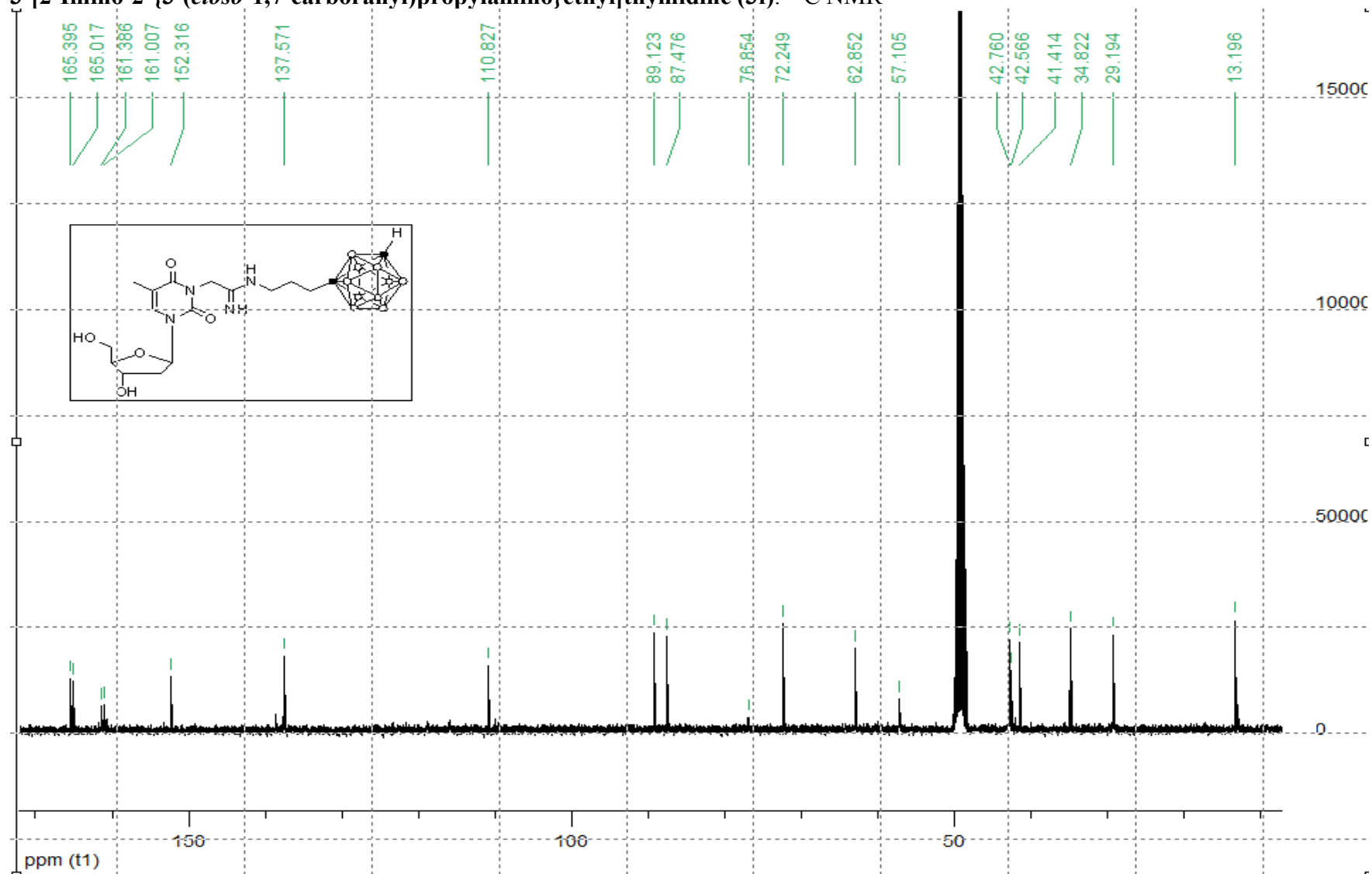
Analytical traces in Water:Methanol gradient



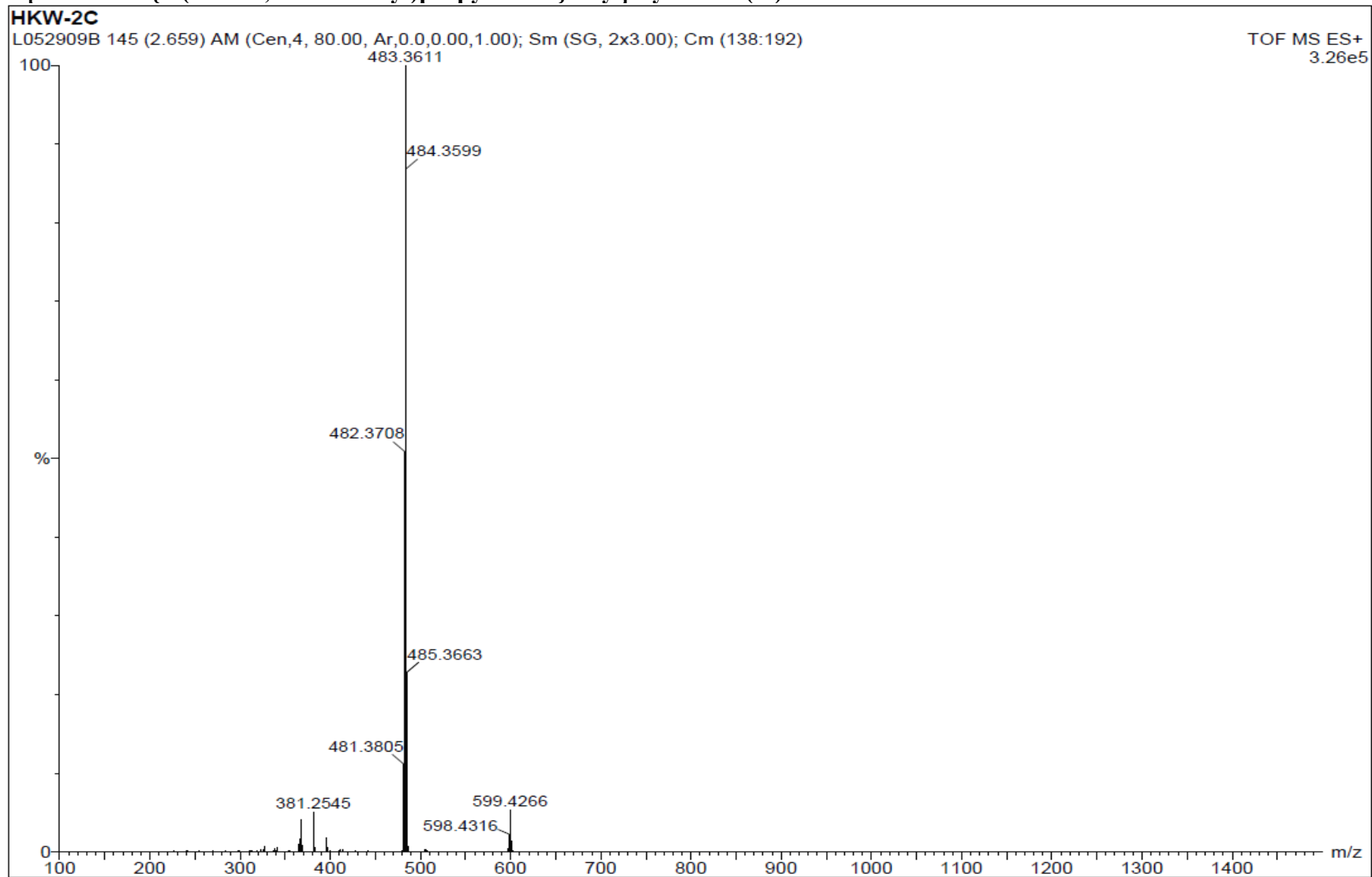
3-[2-Imino-2-{3-(*closo*-1,7-carboranyl)propylamino}ethyl]thymidine (3f). ¹H NMR



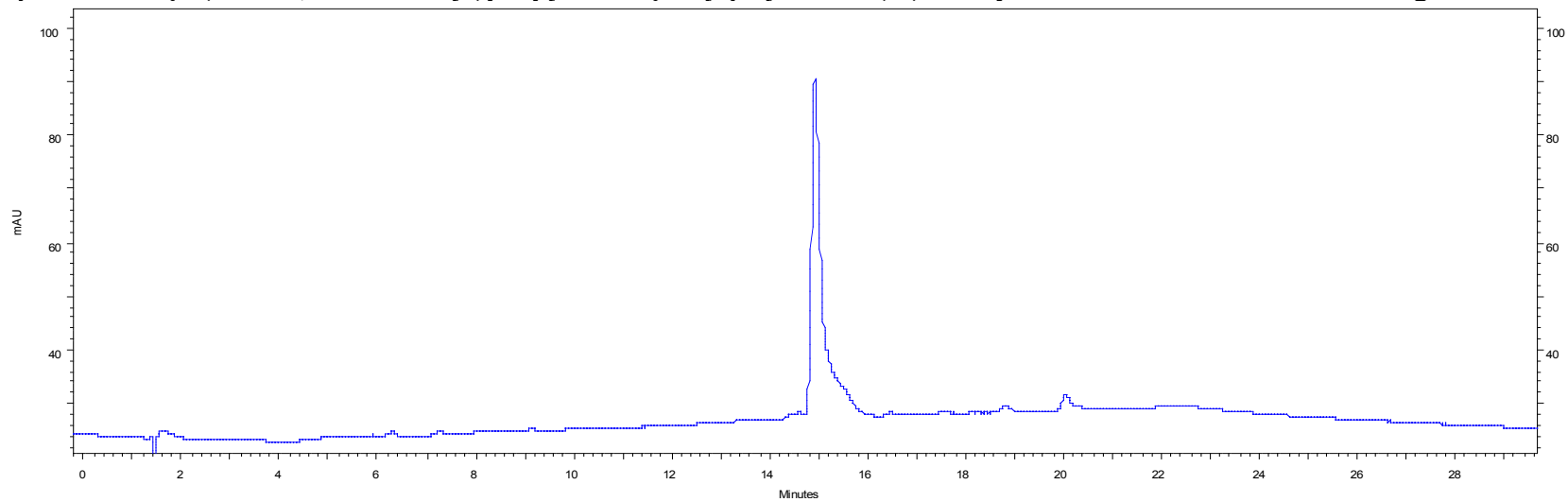
3-[2-Imino-2-{3-(*closo*-1,7-carboranyl)propylamino}ethyl]thymidine (3f). ^{13}C NMR



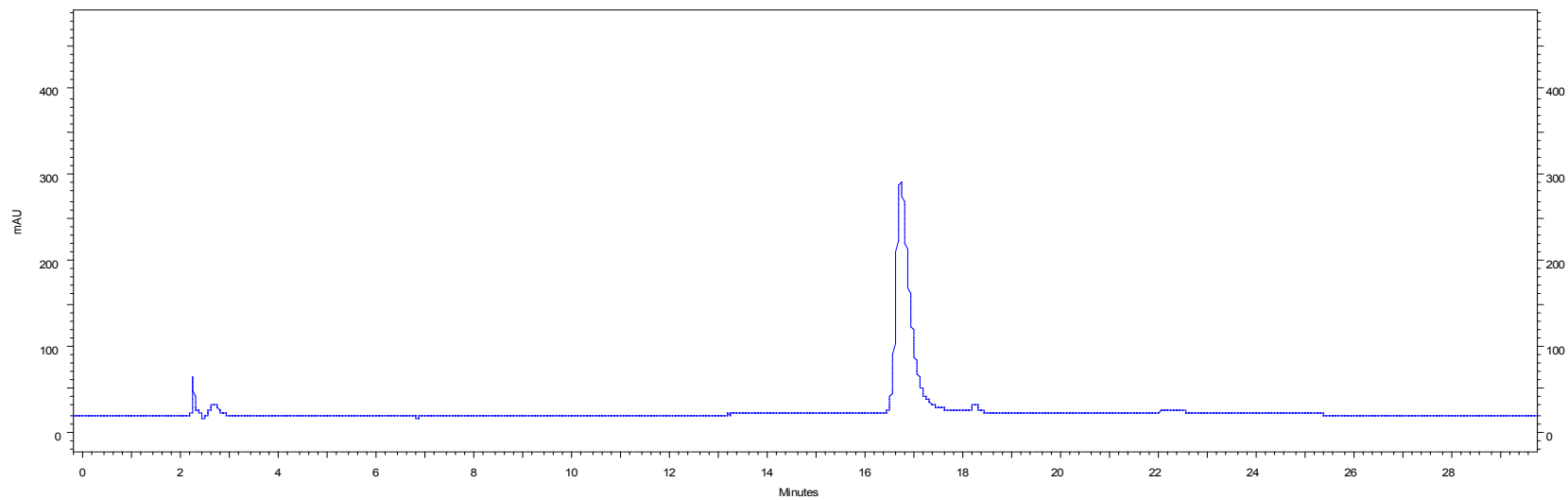
3-[2-Imino-2-{3-(*closo*-1,7-carboranyl)propylamino}ethyl]thymidine (3f). HR-ESI-MS found: 483.3611.



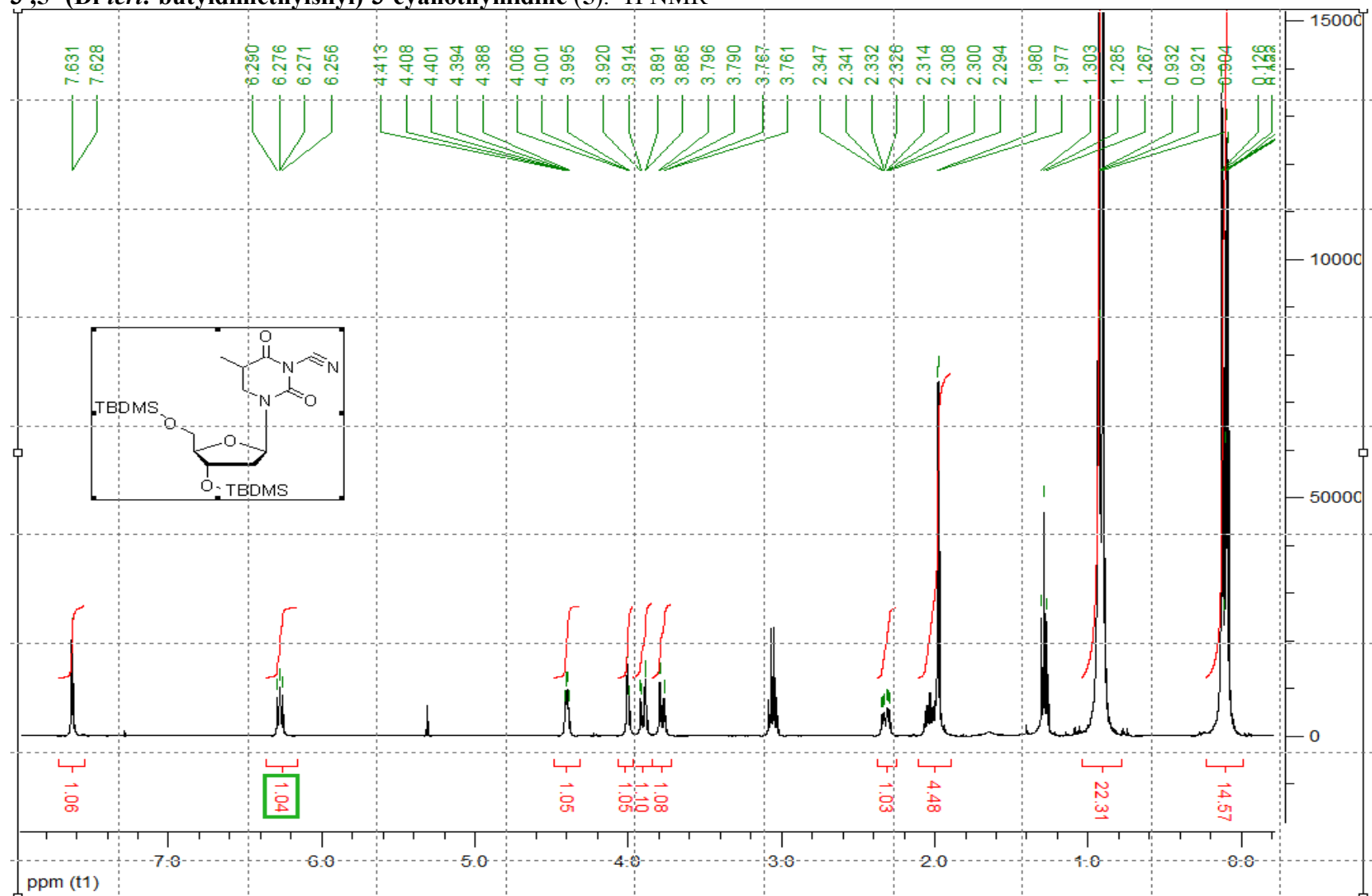
3-[2-Imino-2-{3-(*closo*-1,7-carboranyl)propylamino}ethyl]thymidine (3f). Analytical traces in Water:Acetonitrile gradient



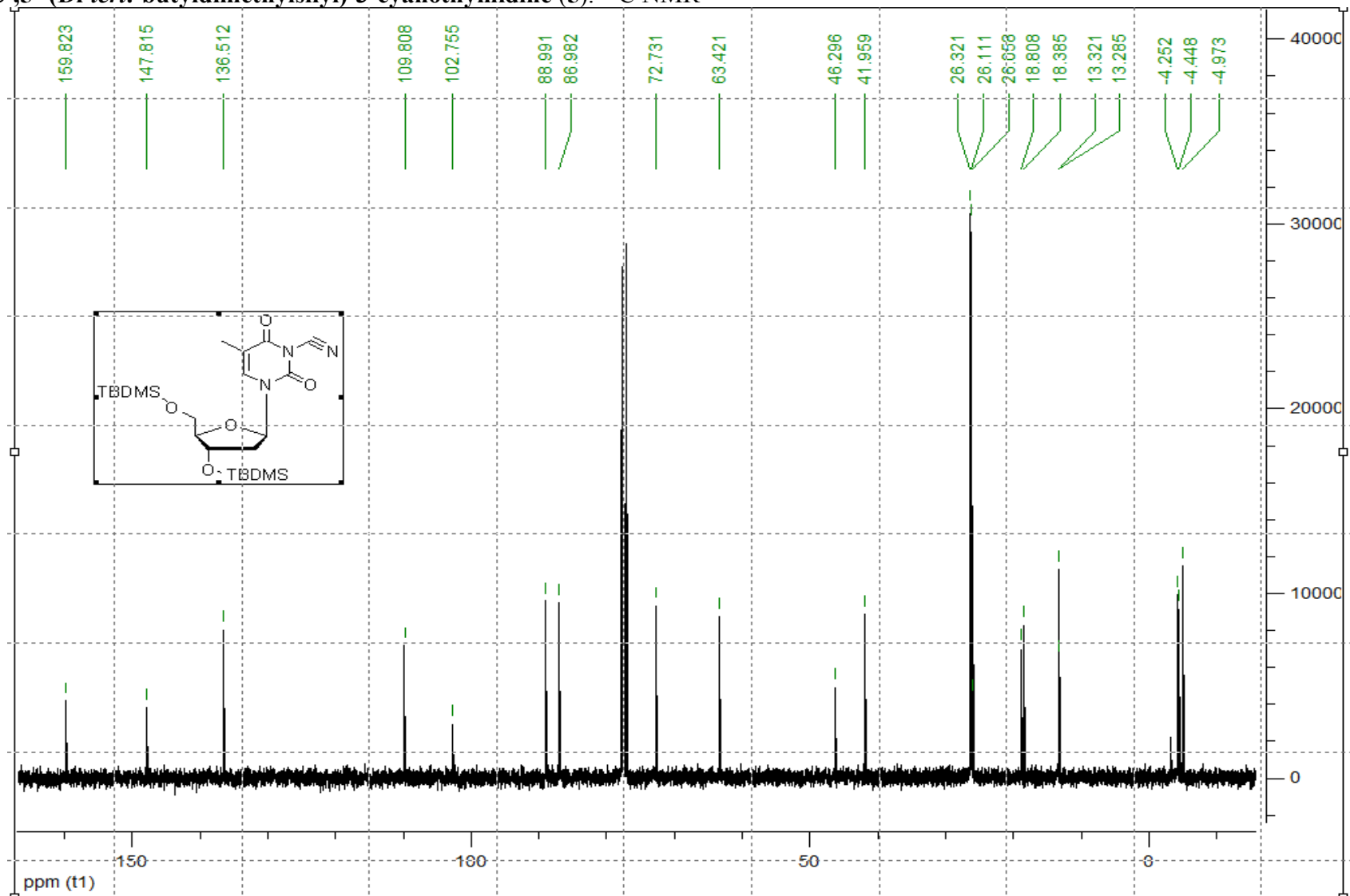
Analytical traces in Water:Methanol gradient



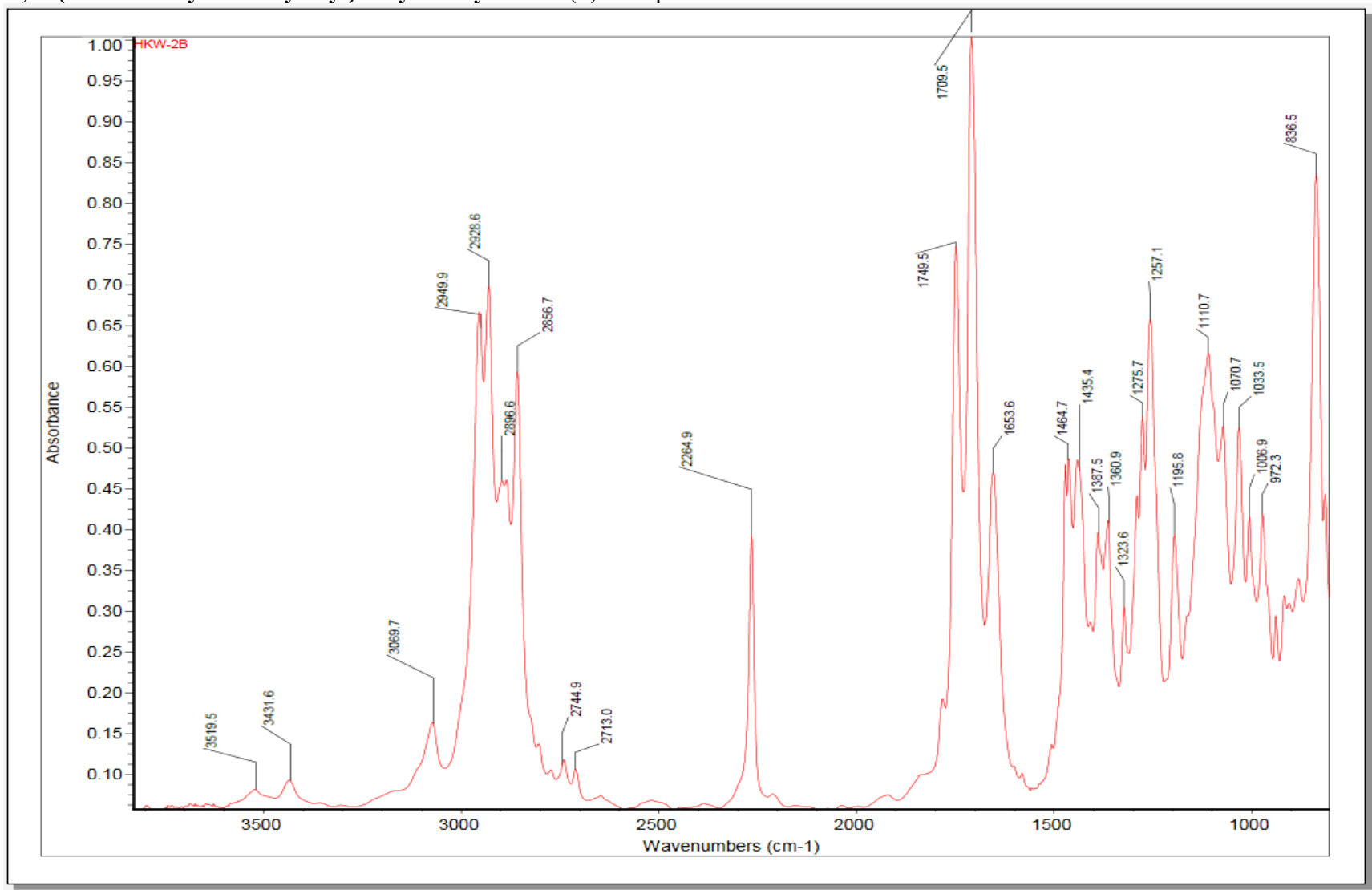
3',5'-(Di-*tert*-butyldimethylsilyl)-3-cyanothymidine (5). ¹H NMR



3',5'-(Di-*tert*-butyldimethylsilyl)-3-cyanothymidine (5). ¹³C NMR



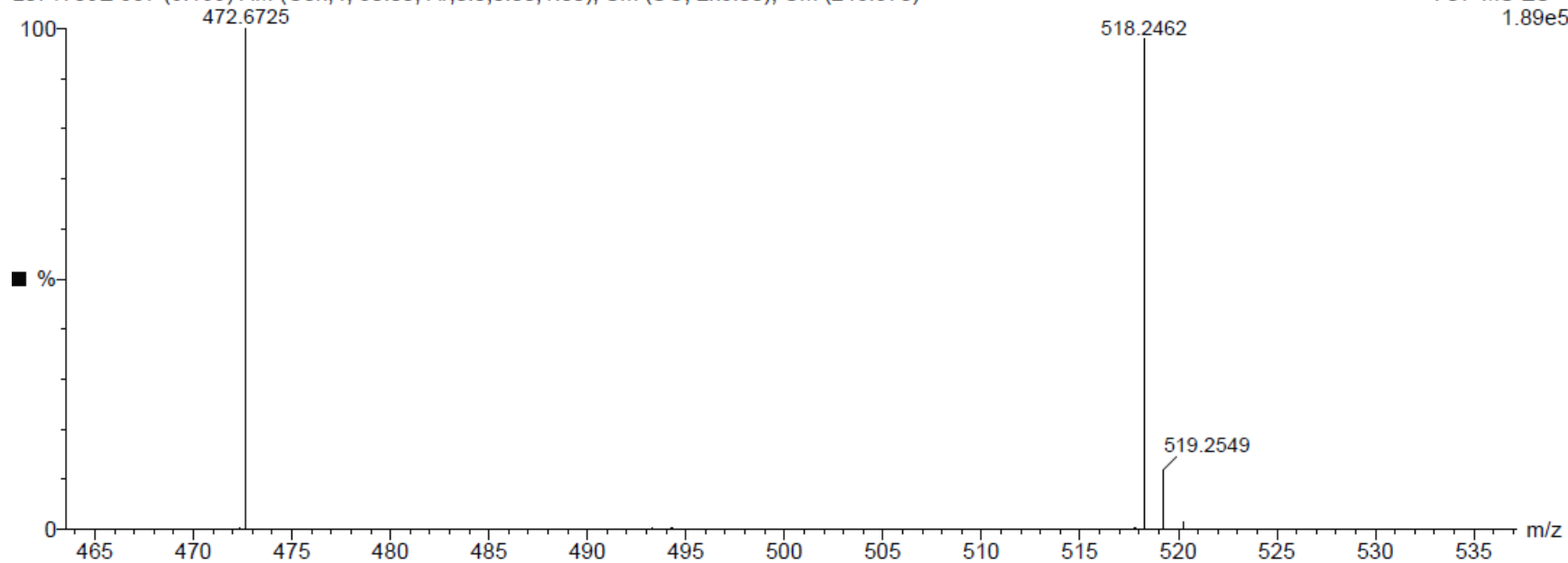
3',5'-(Di-*tert.*-butyldimethylsilyl)-3-cyanothymidine (5). IR-spectra



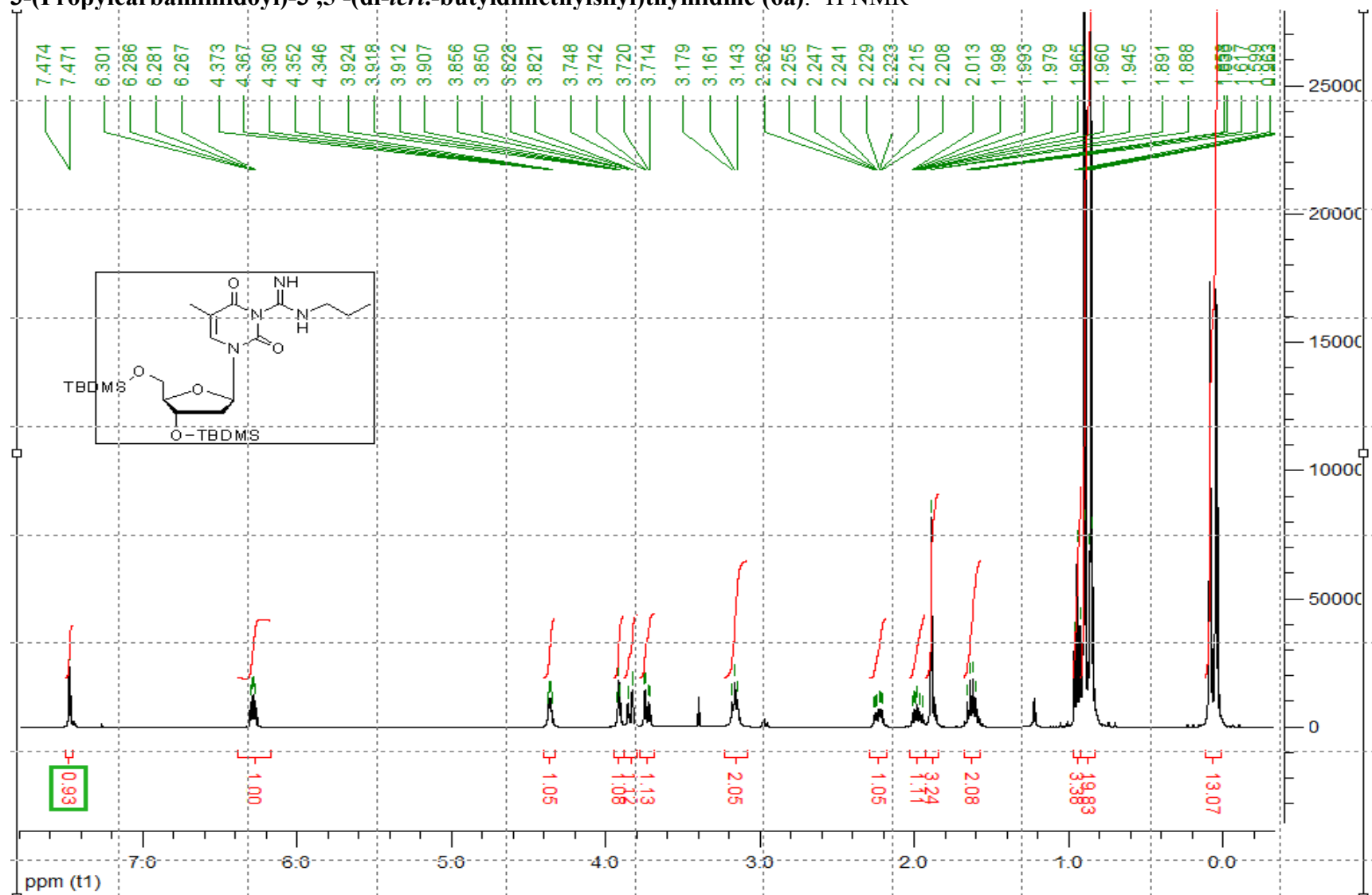
3',5'-(Di-*tert*-butyldimethylsilyl)-3-cyanothymidine. (5). HR-ESI-MS: 518.2462.

L071709E 337 (6.199) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (243:370)

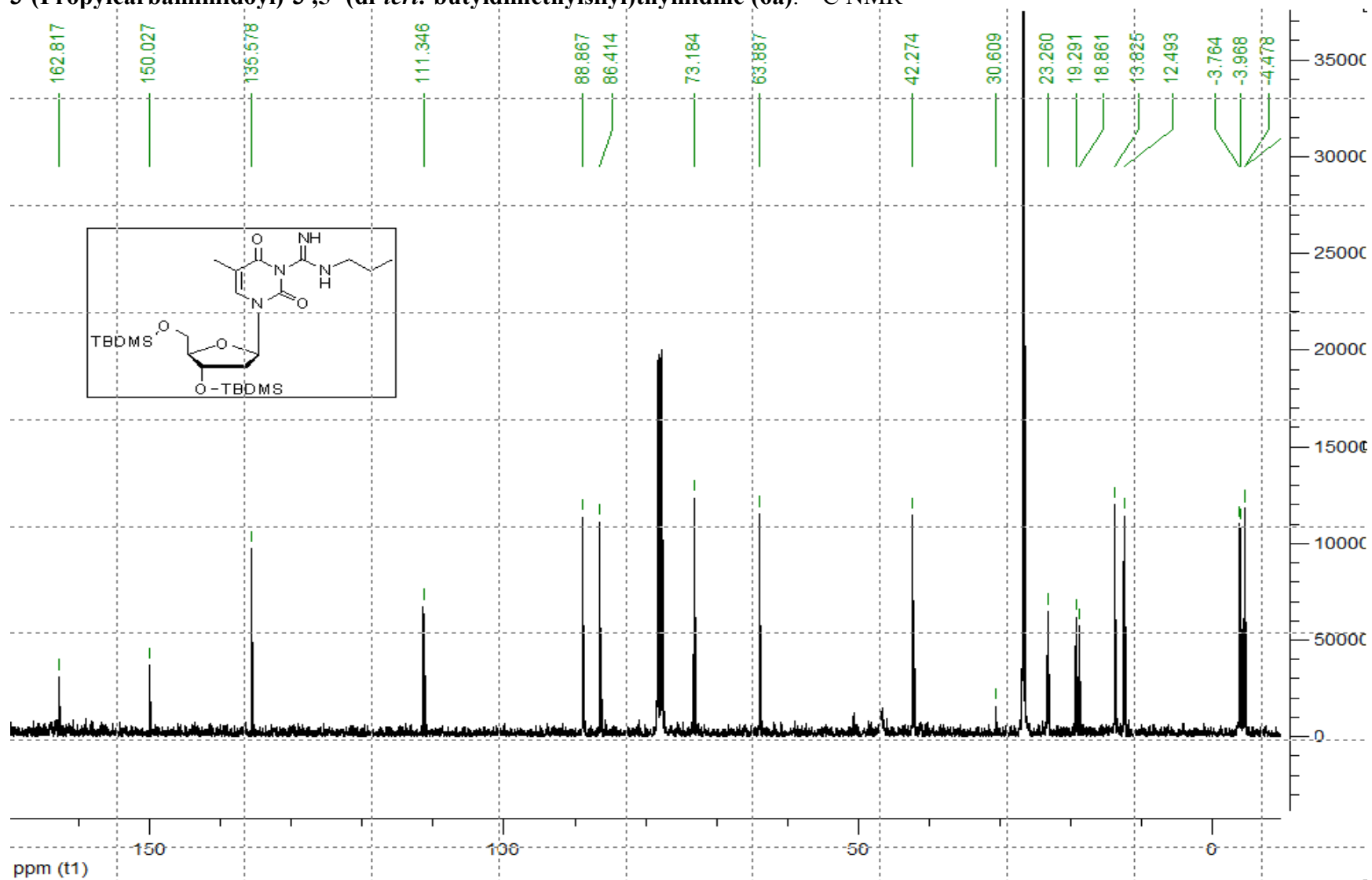
TOF MS ES+
1.89e5



3-(Propylcarbamimidoyl)-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6a). ¹H NMR



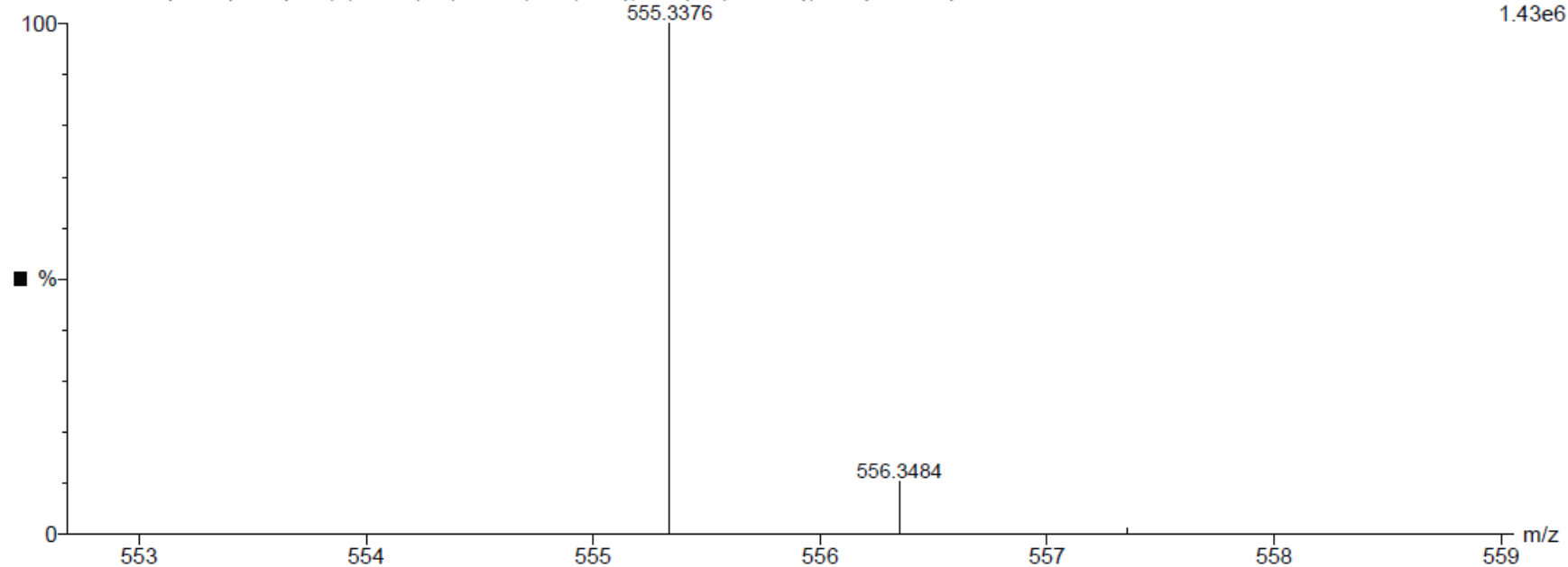
3-(Propylcarbamidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6a). ¹³C NMR



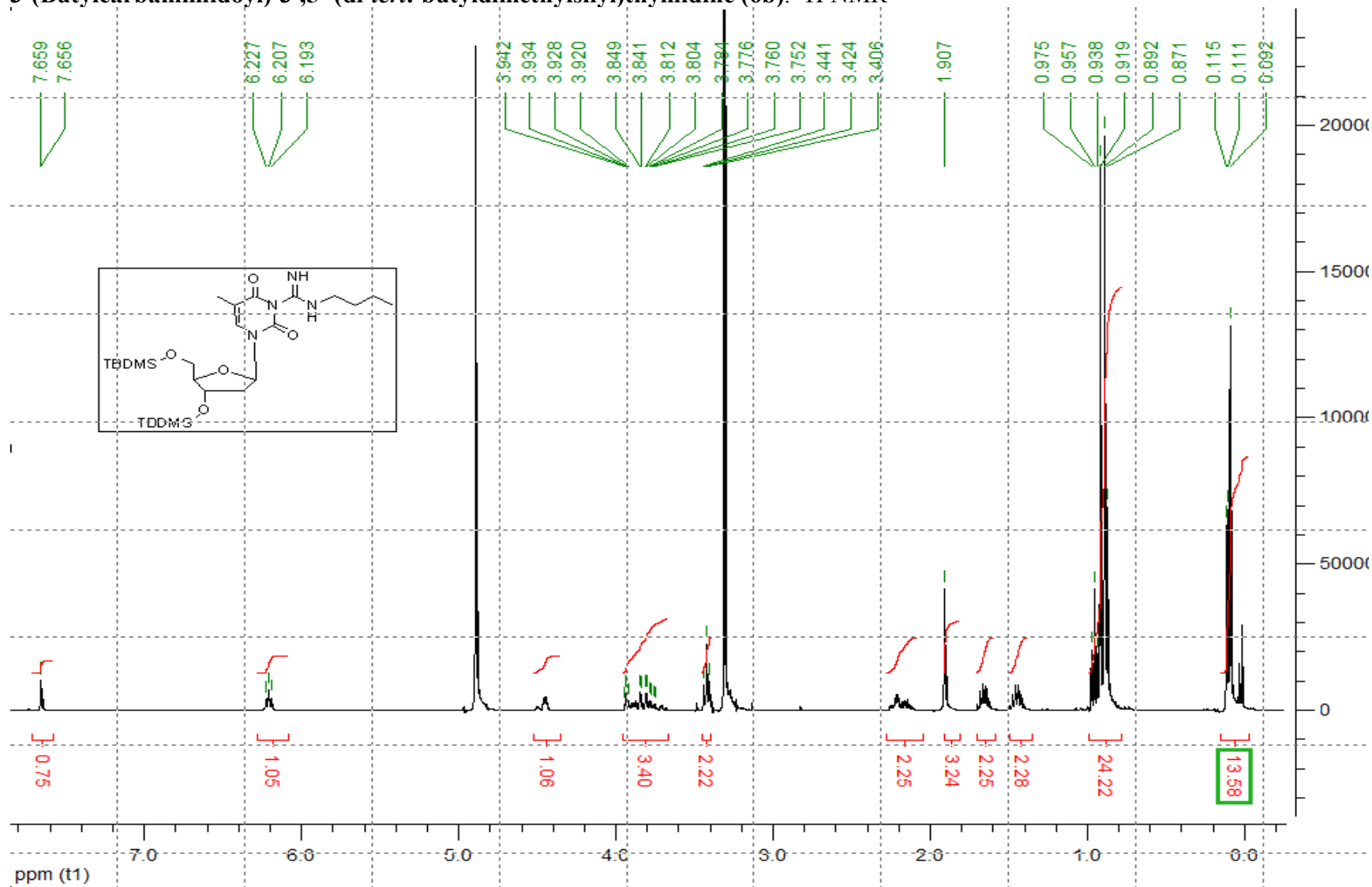
3-(Propylcarbamiidoyl)-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6a). HR-ESI-MS found: 555.3376

L100109C 476 (8.731) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (434:478)

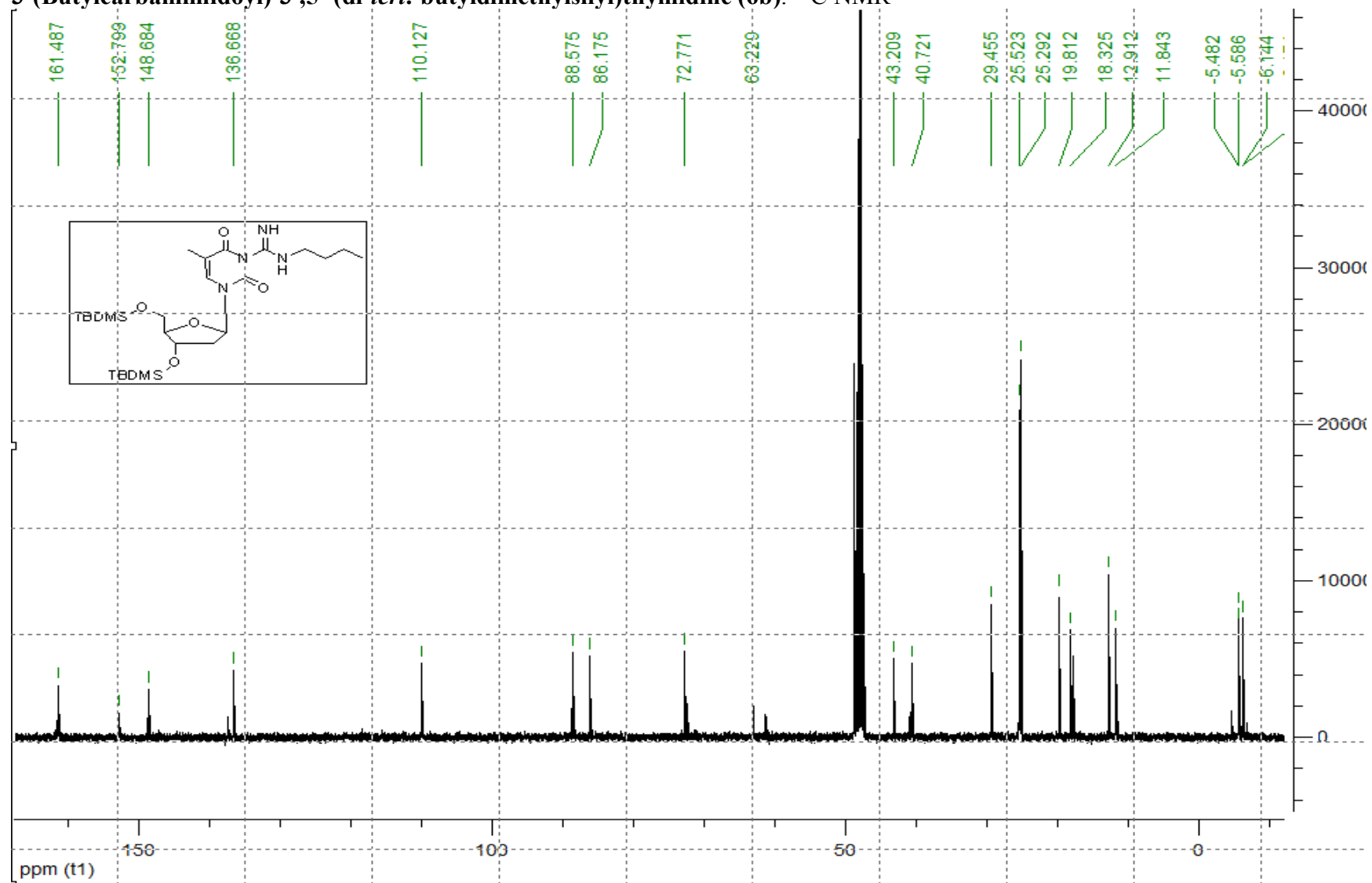
TOF MS ES+
1.43e6



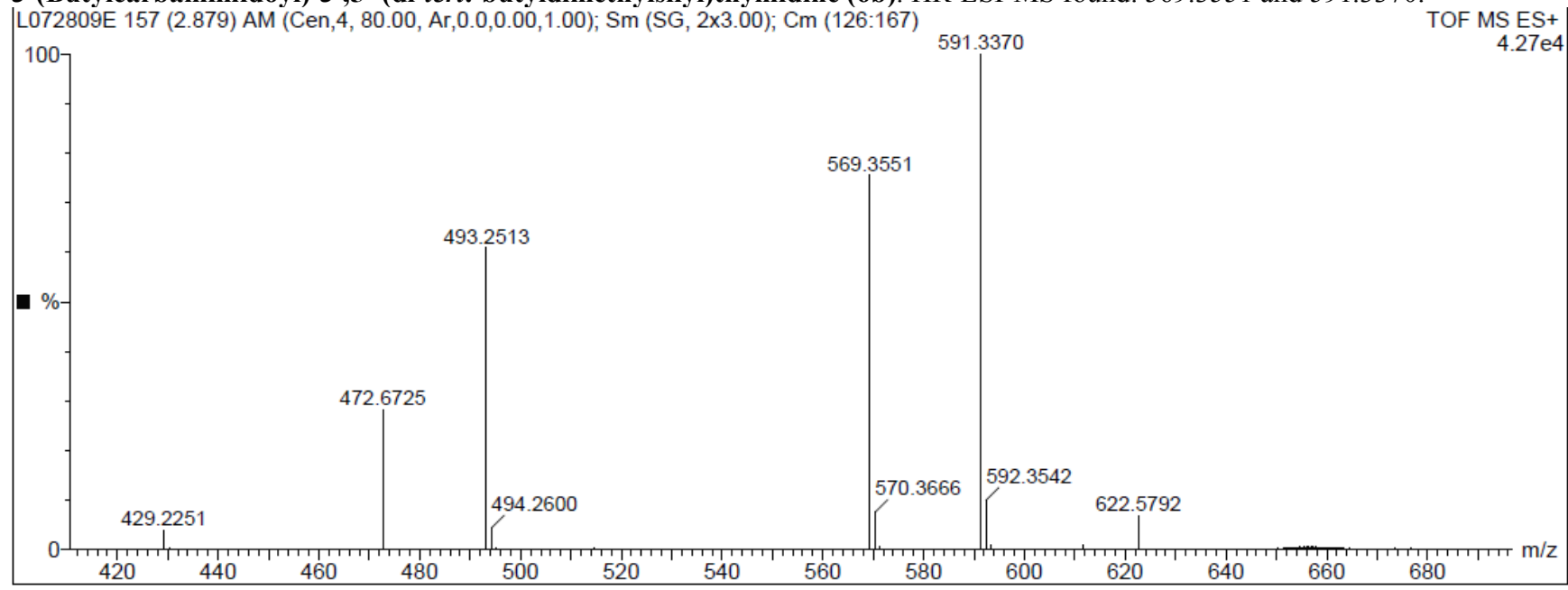
3-(Butylcarbamimidoyl)-3',5'-(di-*tert*.-butyldimethylsilyl)thymidine (6b). ¹H NMR



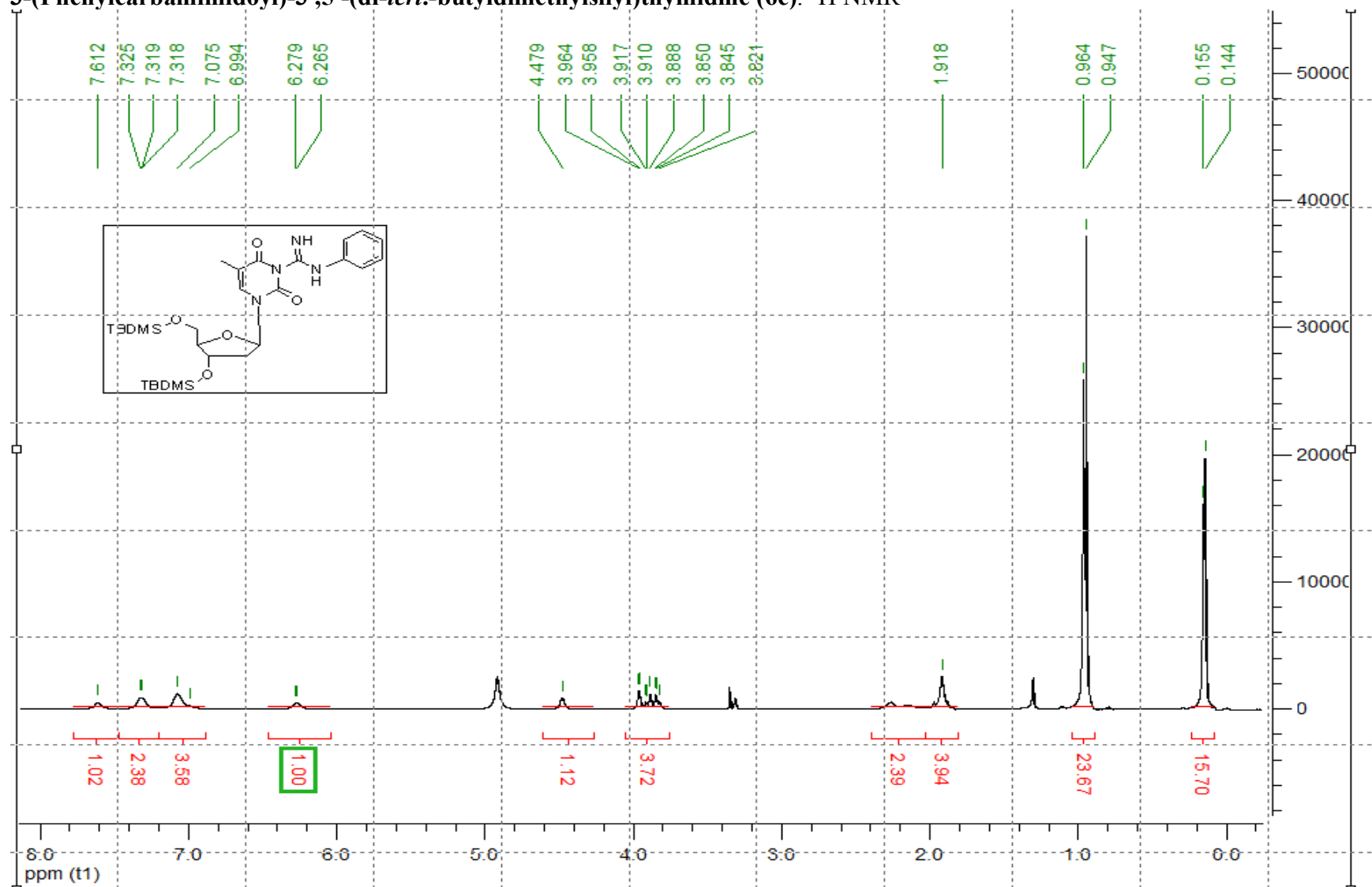
3-(Butylcarbamimidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6b). ^{13}C NMR



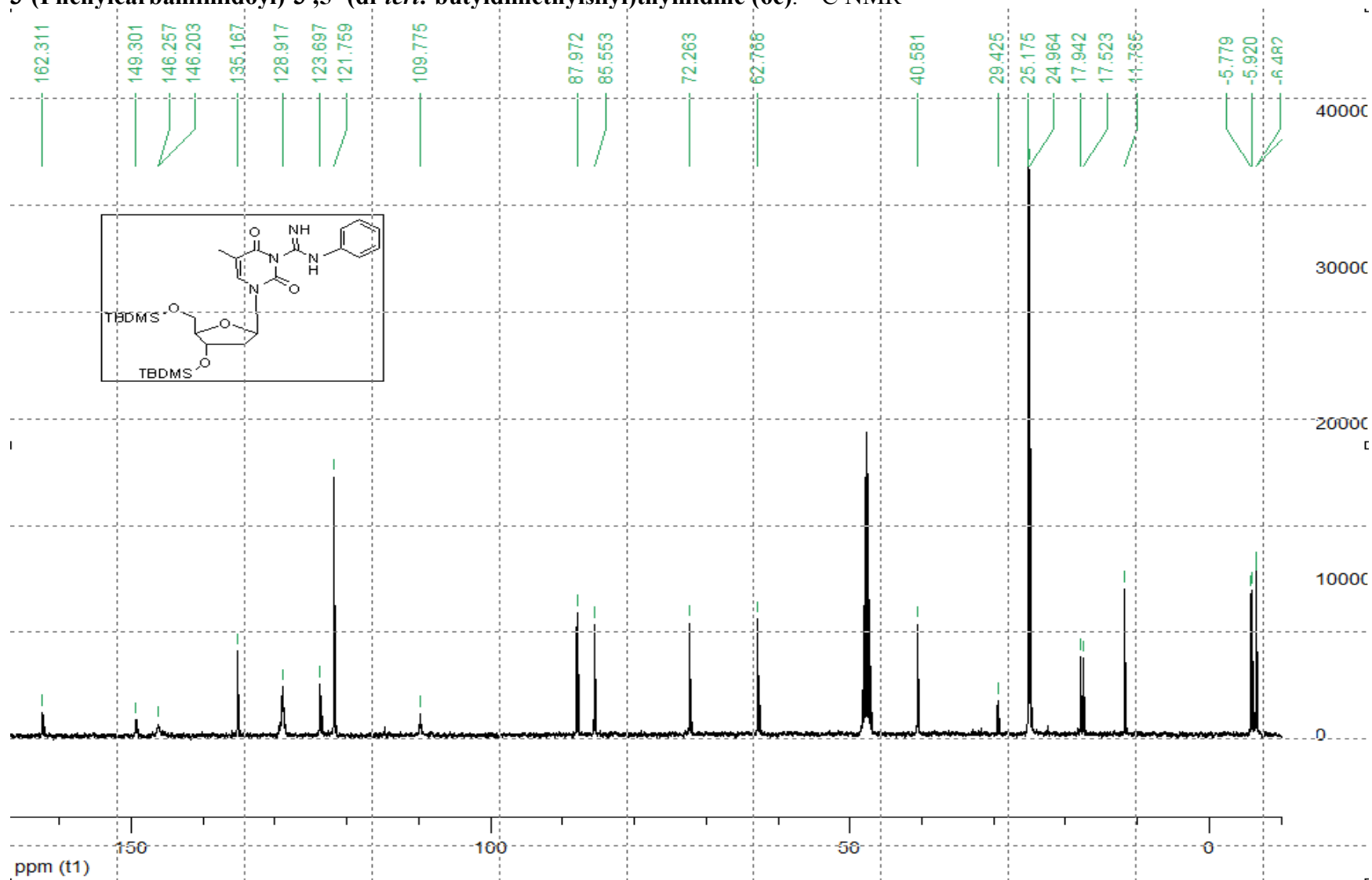
3-(Butylcarbamimidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6b). HR-ESI-MS found: 569.3551 and 591.3370.



3-(Phenylcarbamimidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6c). ¹H NMR



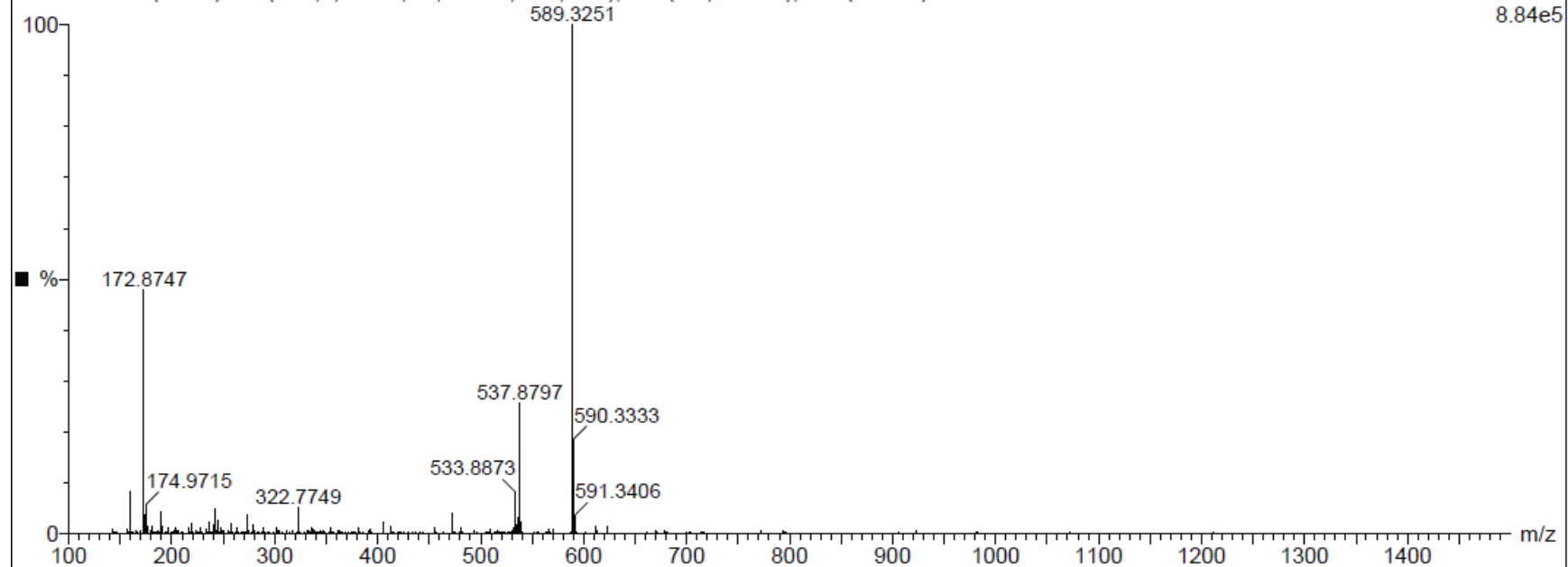
3-(Phenylcarbamidimidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6c). ¹³C NMR



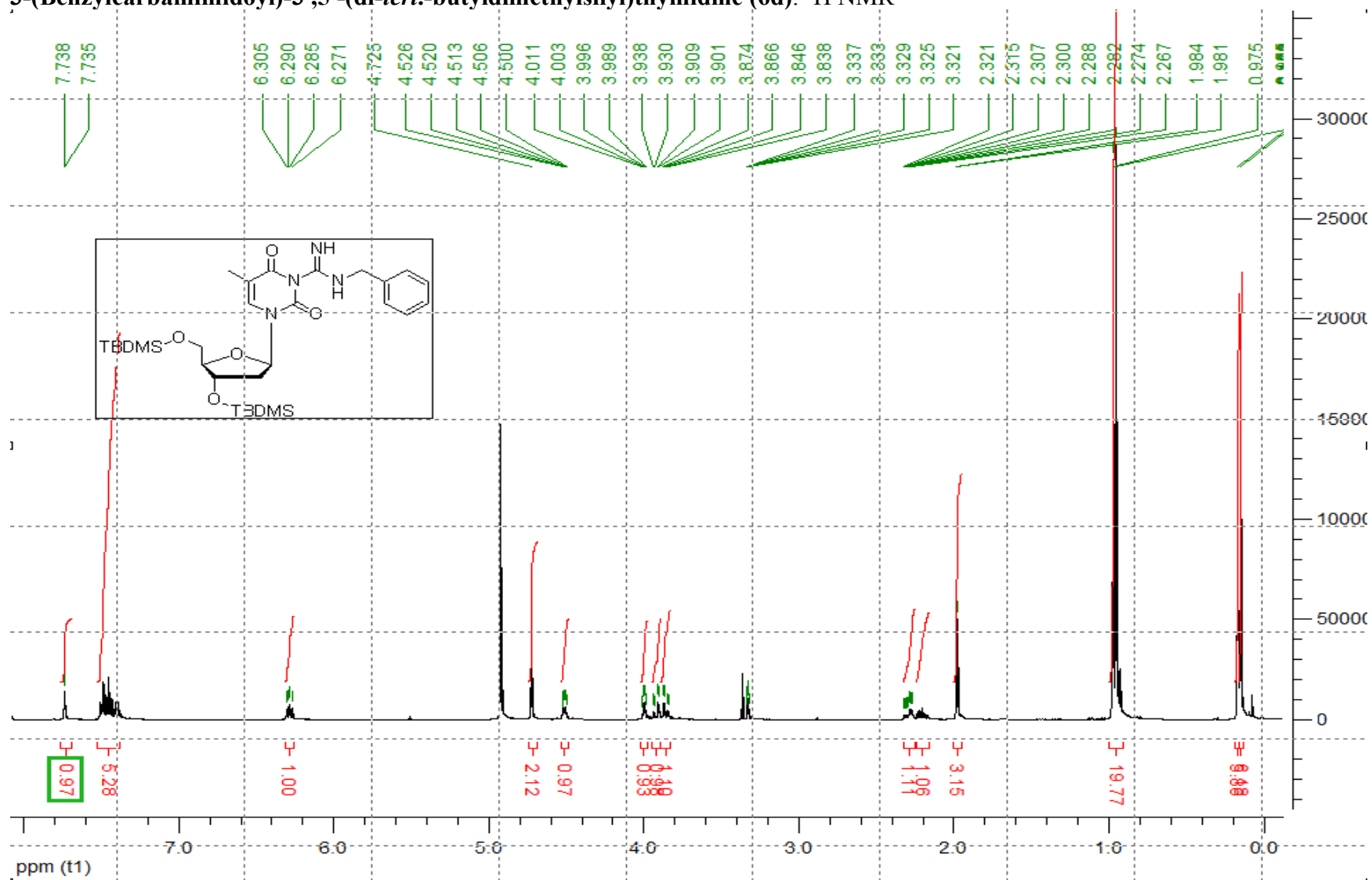
3-(Phenylcarbamidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6c). HR-ESI-MS found: 589.3251.

L090209F 80 (1.485) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (25:182)

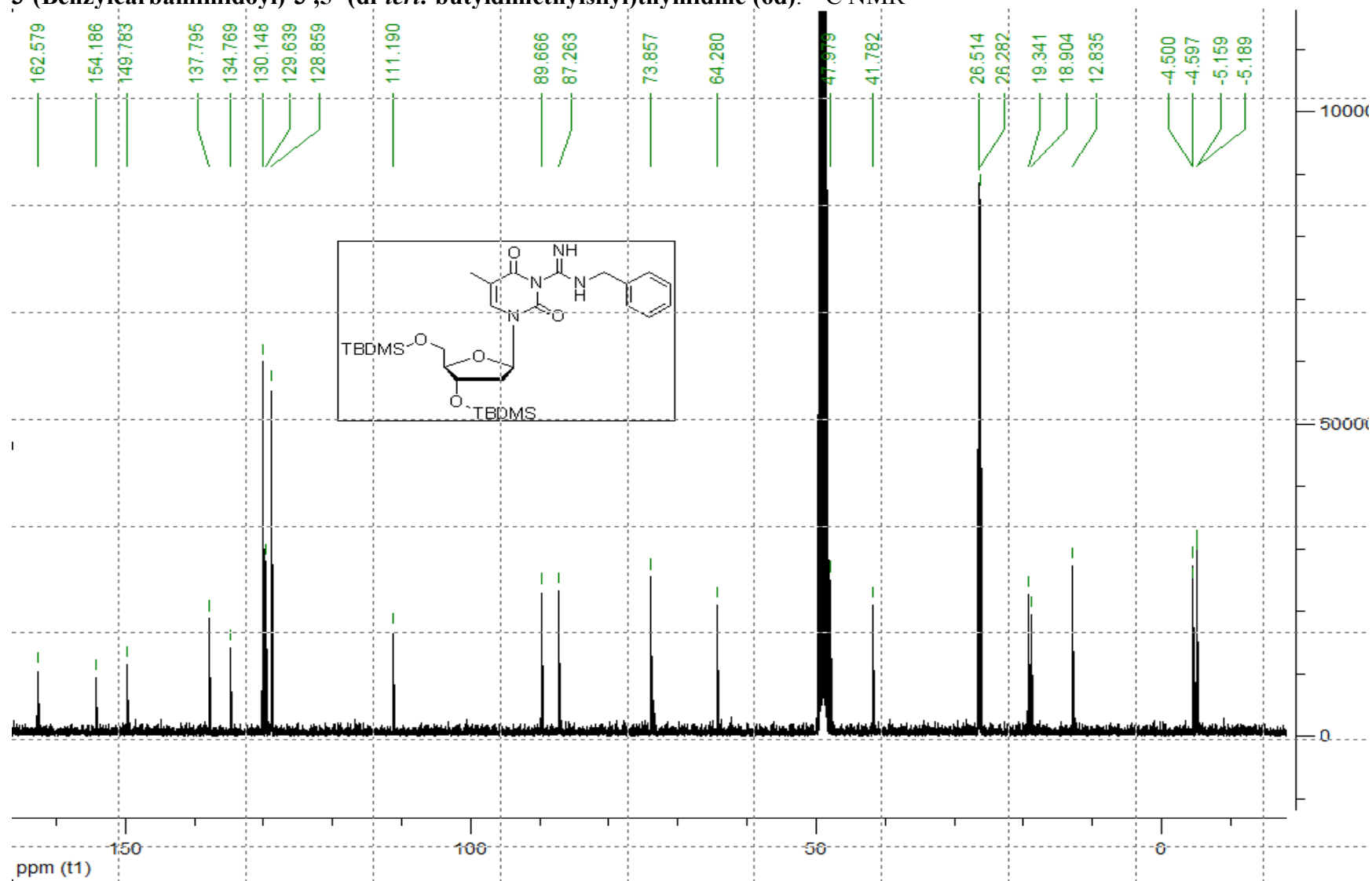
TOF MS ES+
8.84e5



3-(Benzylcarbamidoyl)-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6d). ¹H NMR



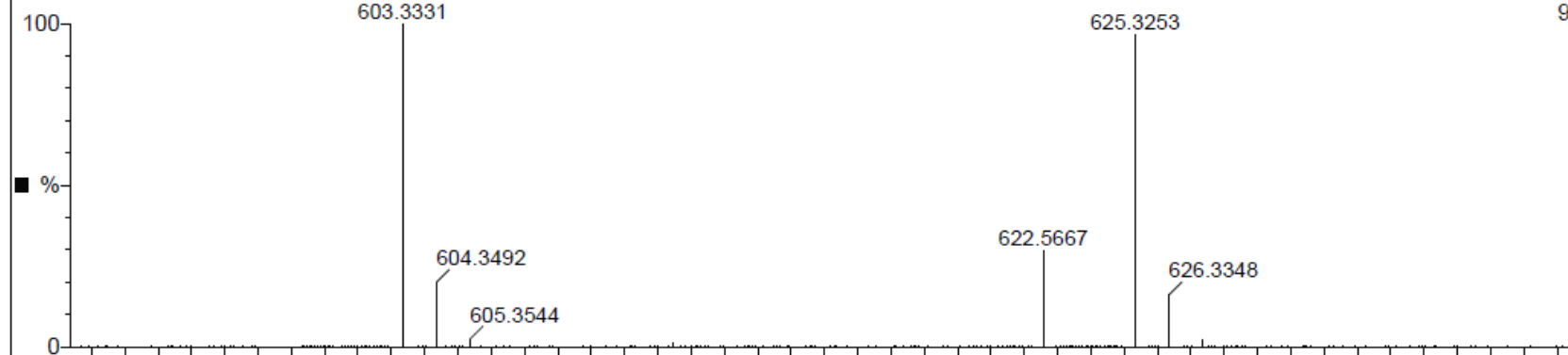
3-(Benzylcarbamimidoyl)-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6d). ¹³C NMR



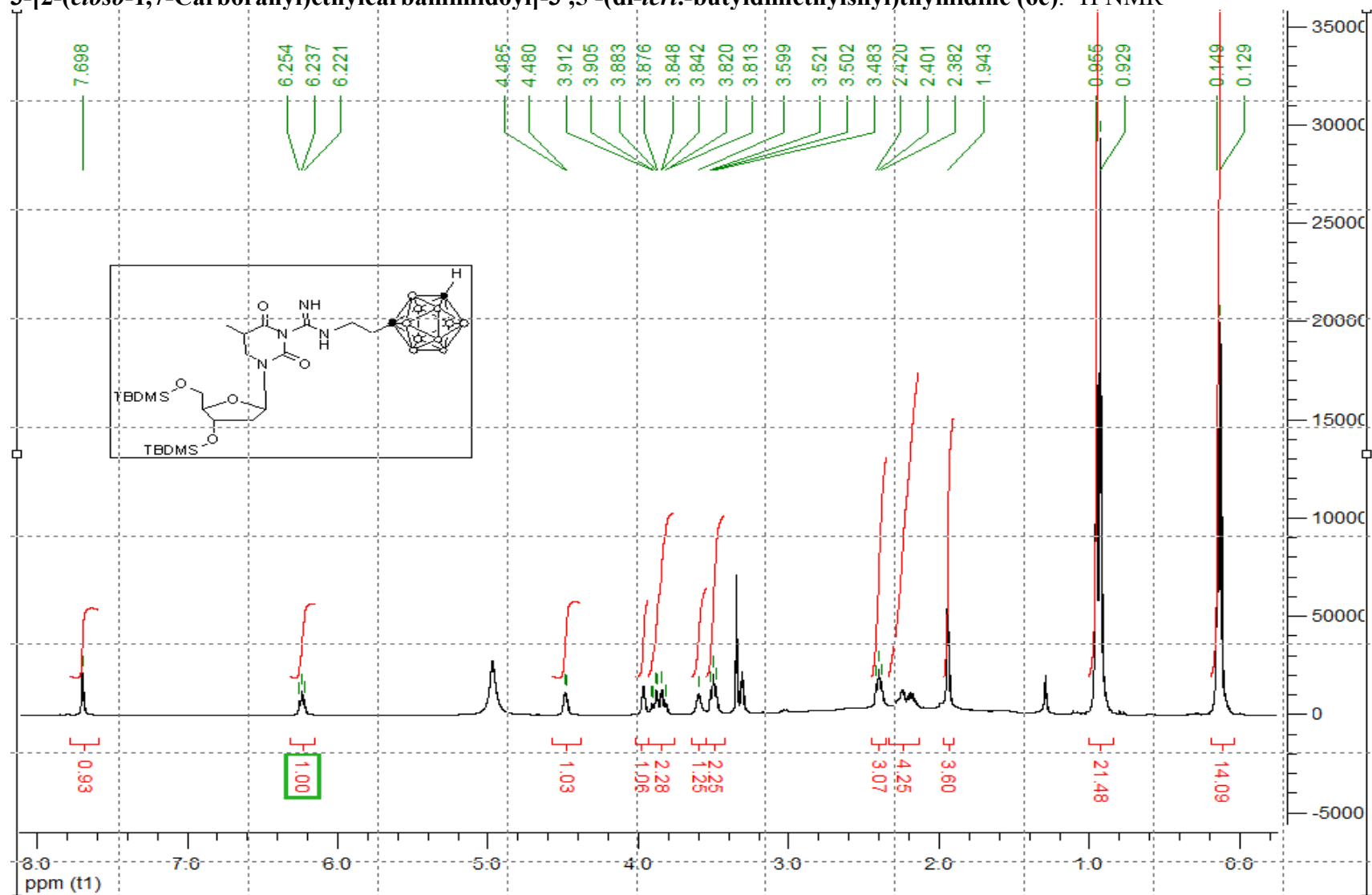
3-(Benzylcarbamiimidoyl)-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6d). HR-ESI-MS found: 603.3331 and 625.3253.

L081008D 166 (3.044) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (102:176)

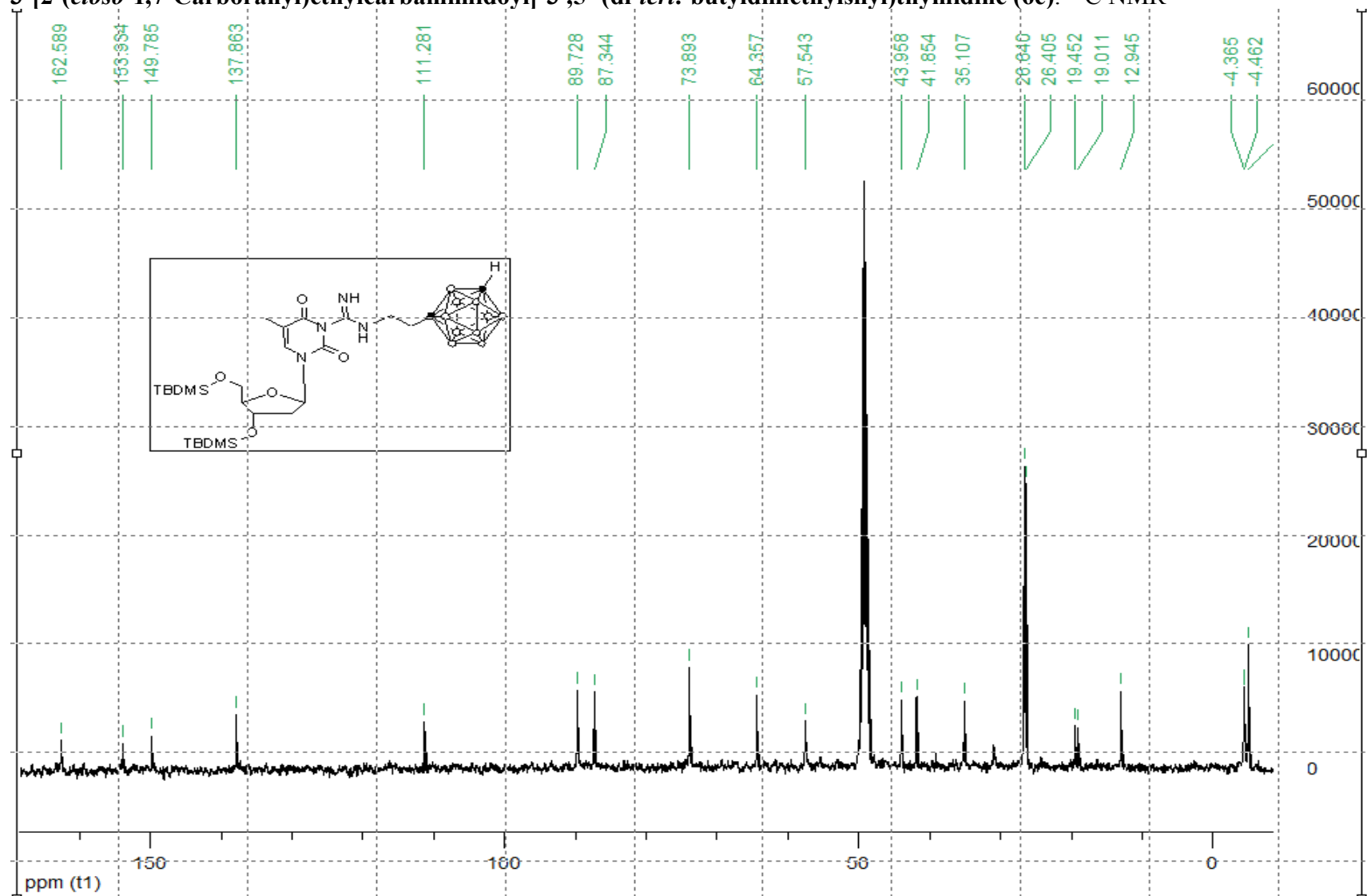
TOF MS ES+
9.98e4



3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6e). ^1H NMR



3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]-3',5'-(di-*tert*.-butyldimethylsilyl)thymidine (6e). ^{13}C NMR

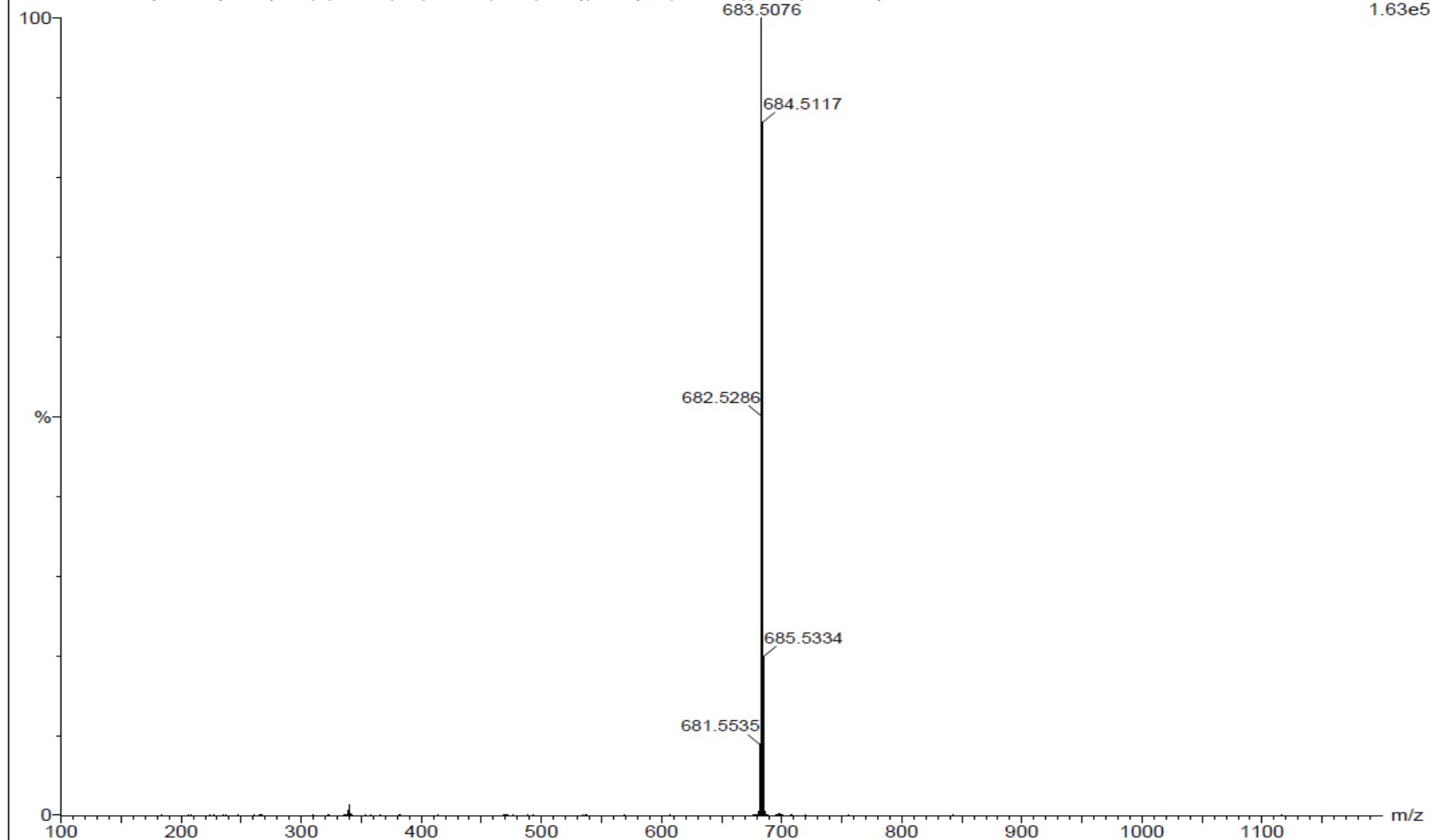


3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6e). HR-ESI-MS found: 683.5076.

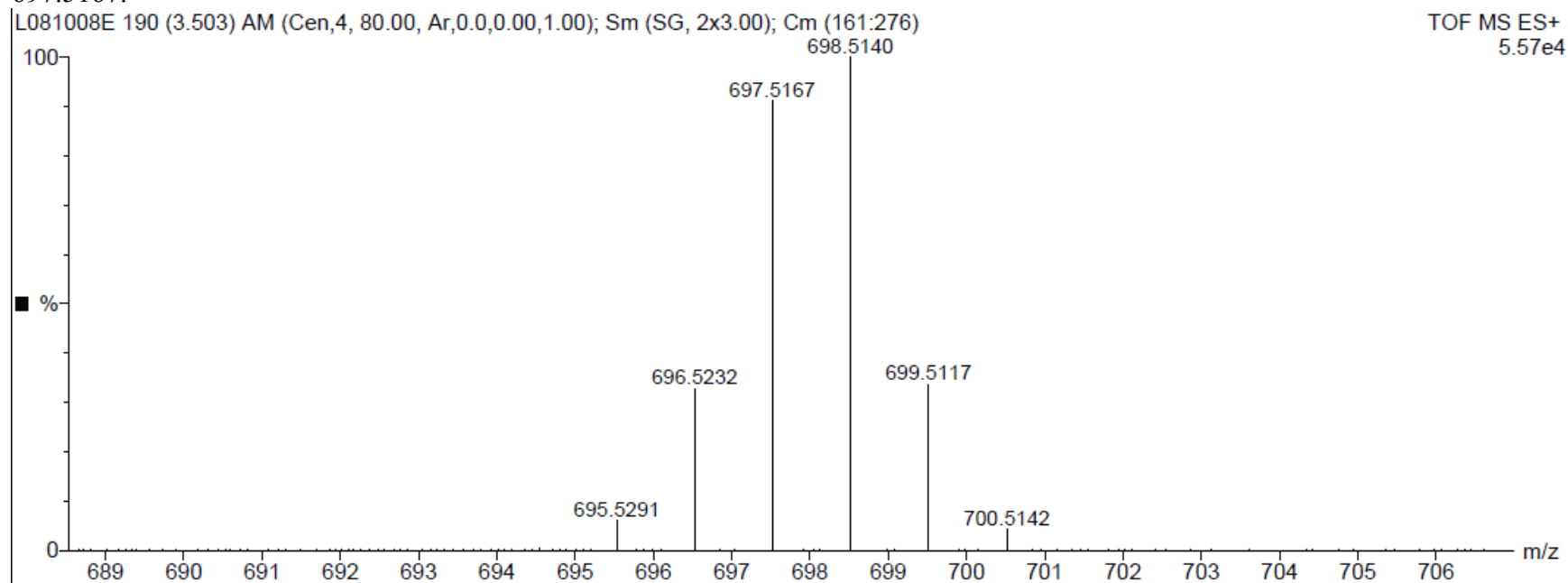
HKW-20

L122110B 482 (16.878) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (482:487)

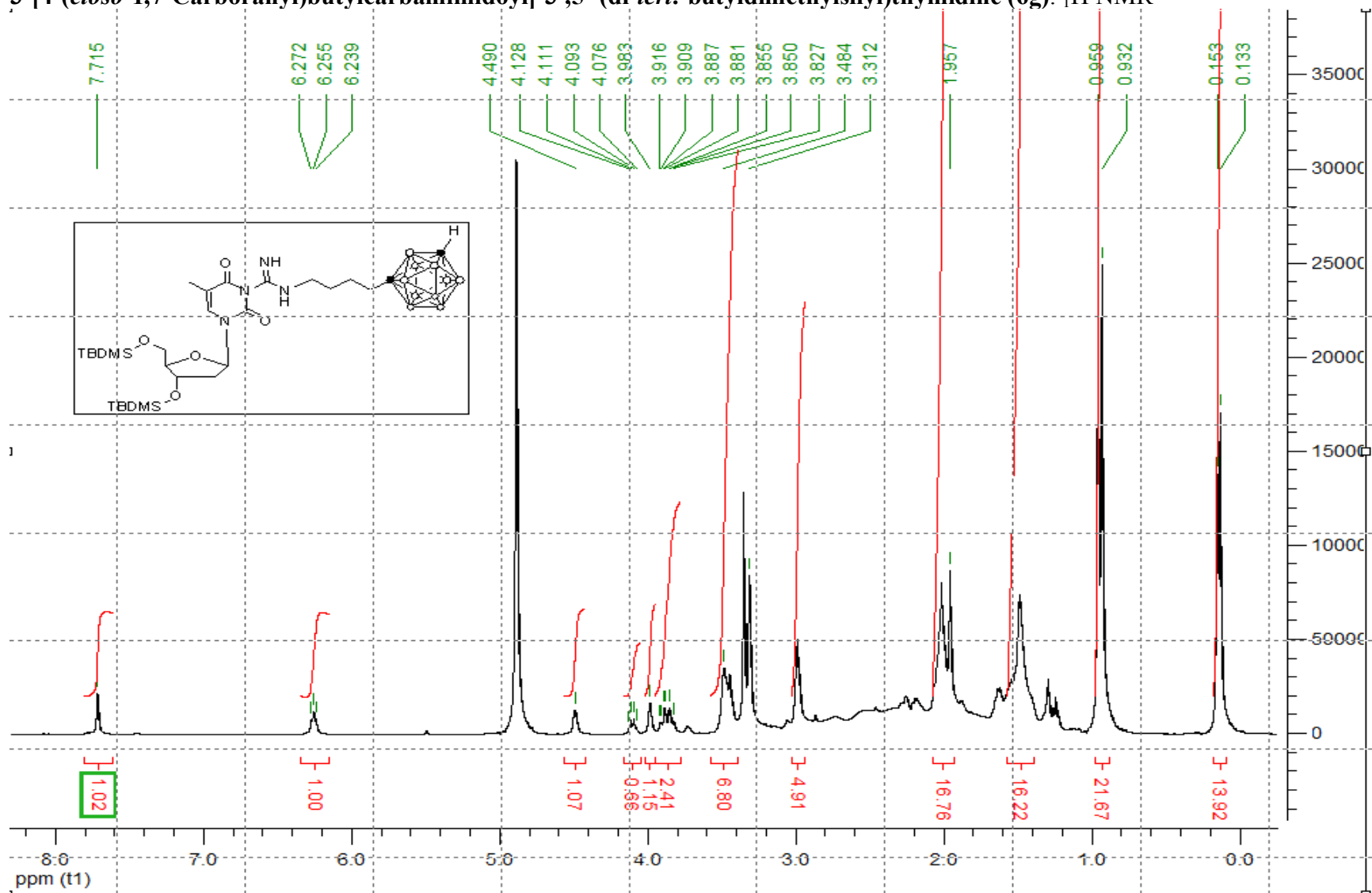
TOF MS ES+
1.63e5



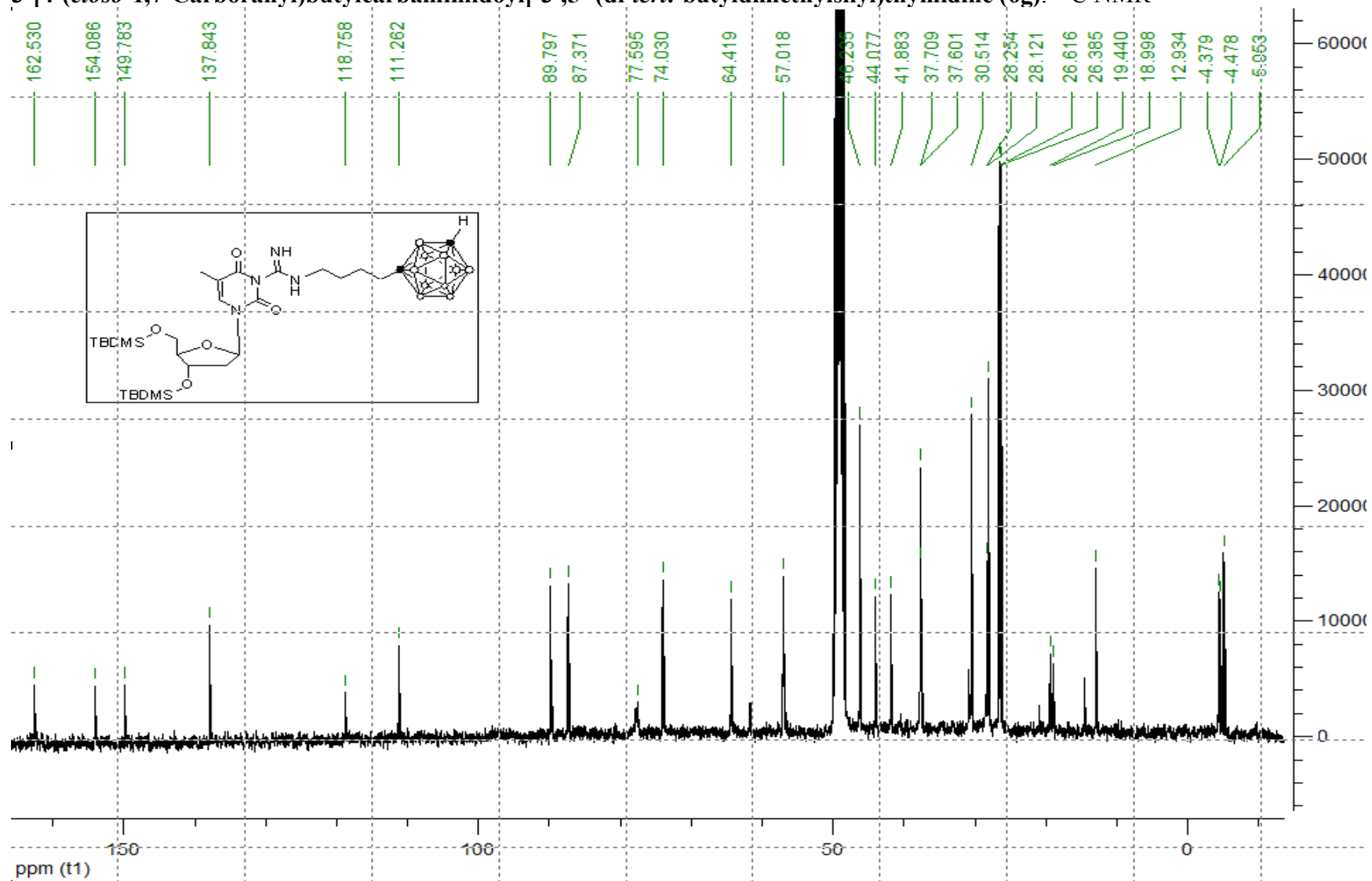
3-[3-(*closo*-1,7-Carboranyl)propylcarbamimidoyl]-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6f). HR-ESI-MS found:
697.5167.



3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6g). ^1H NMR



3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]-3',5'-(di-*tert*-butyldimethylsilyl)thymidine (6g). ^{13}C NMR

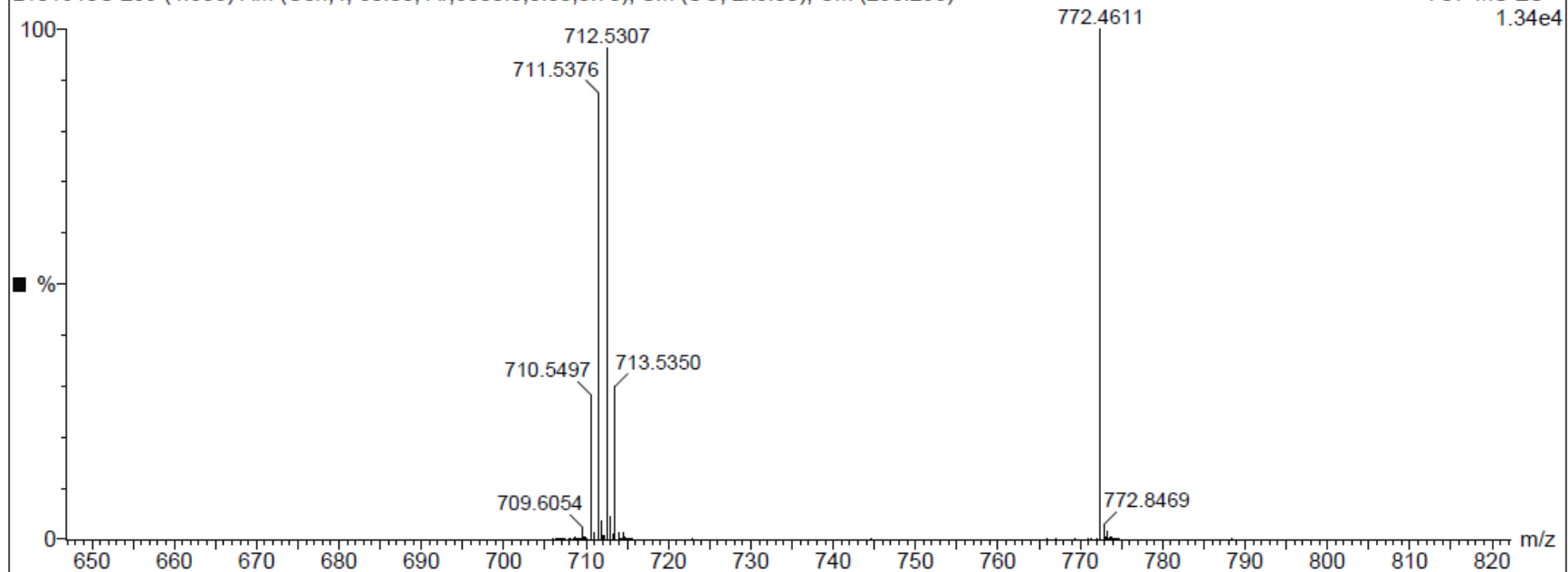


3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]-3',5'-(di-*tert.*-butyldimethylsilyl)thymidine (6g). HR-ESI-MS found: 712.5307.

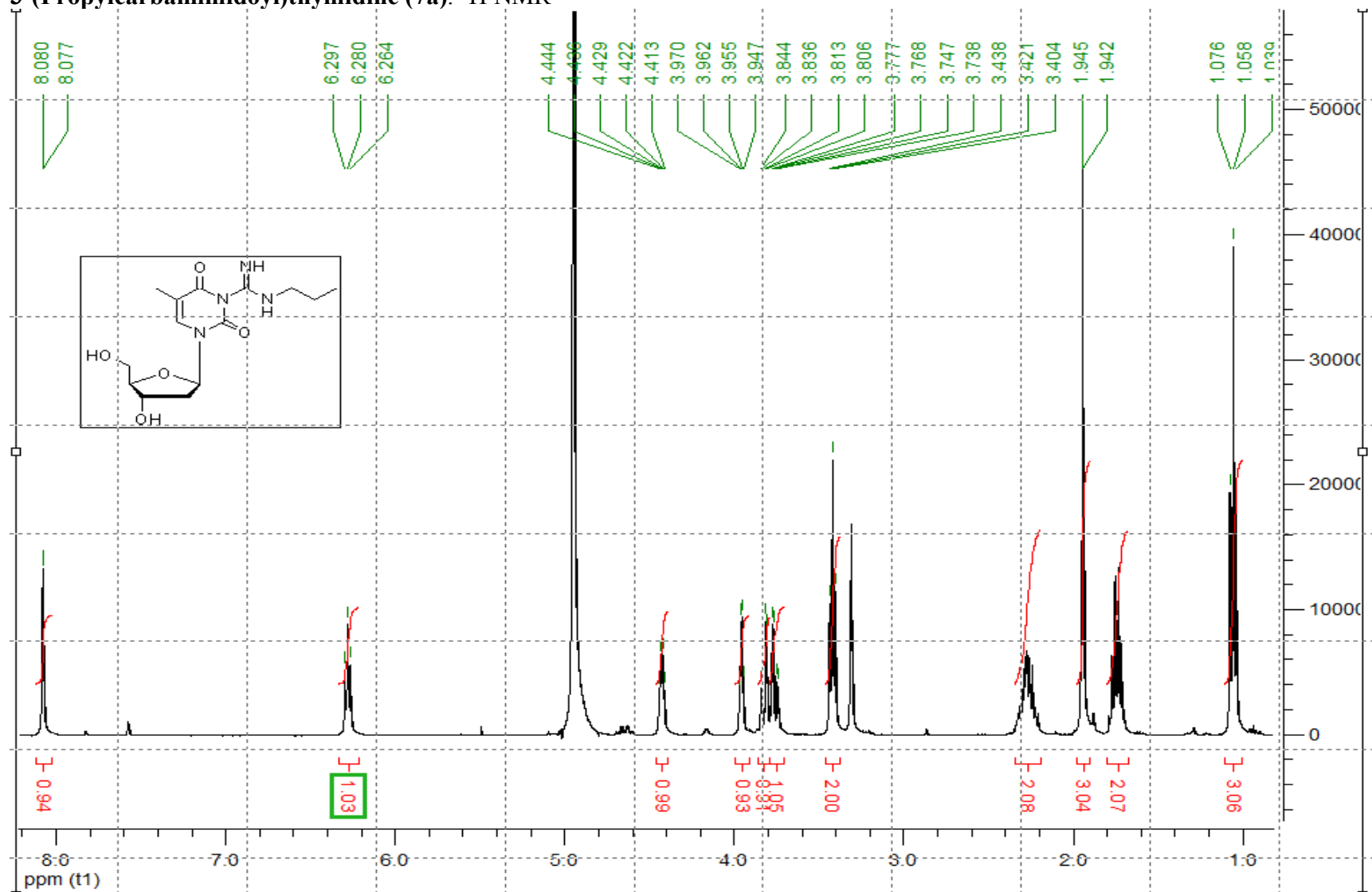
L101910C 239 (4.383) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (238:298)

TOF MS ES+

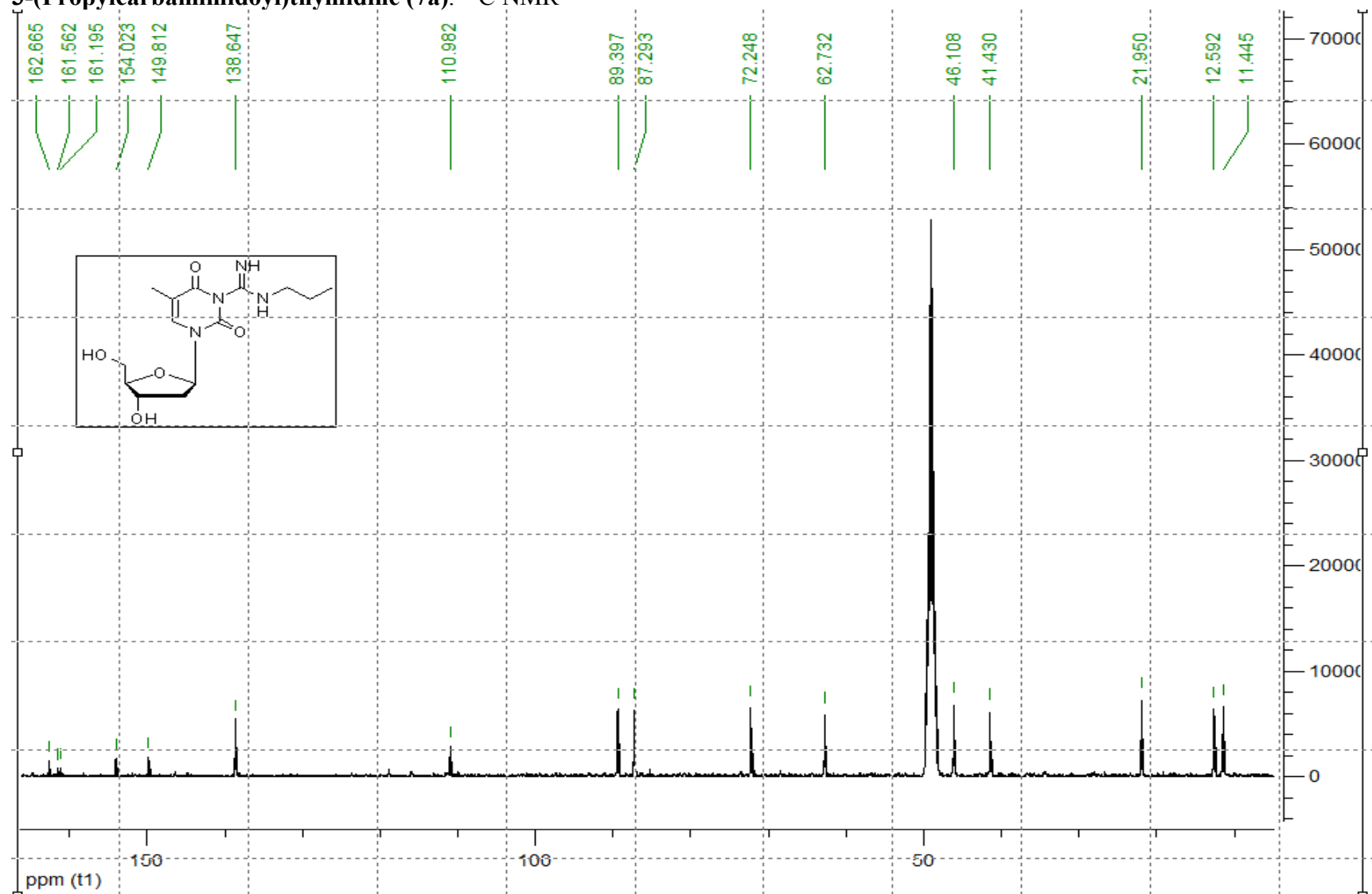
1.34e4



3-(Propylcarbamimidoyl)thymidine (7a). ¹H NMR



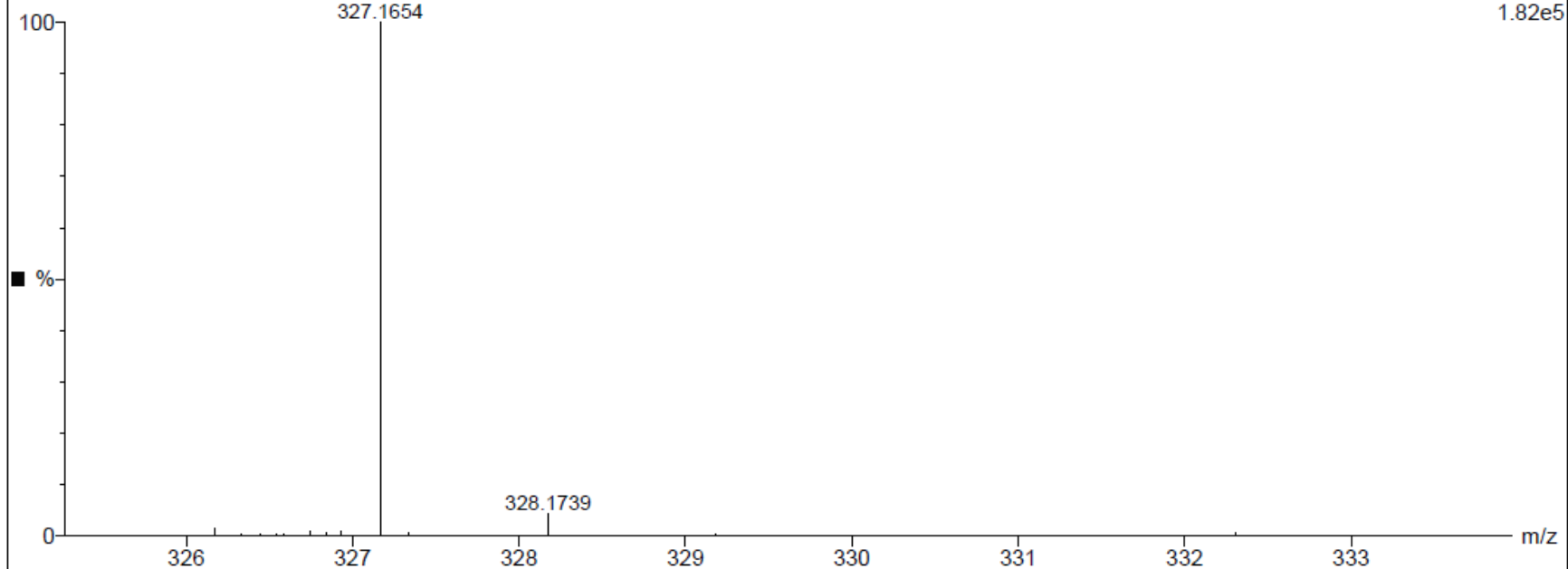
3-(Propylcarbamimidoyl)thymidine (7a). ¹³C NMR



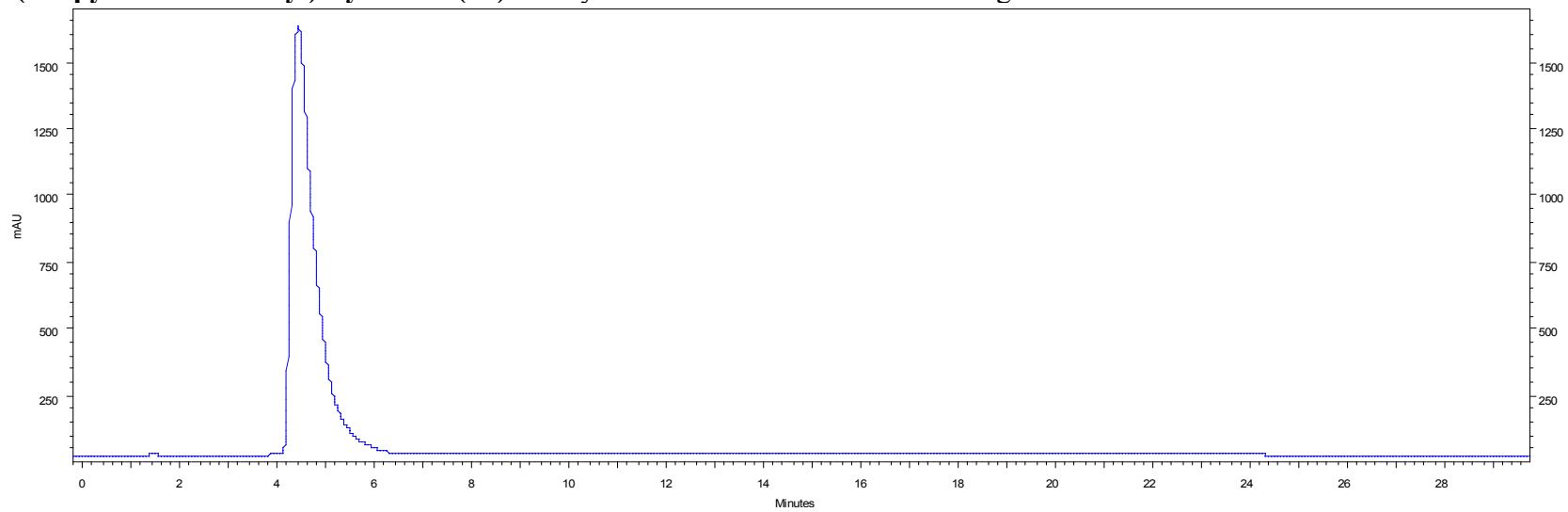
3-(Propylcarbamimidoyl)thymidine (7a). HR-ESI-MS found: 327.1654.

L100109D 565 (10.363) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (560:587)

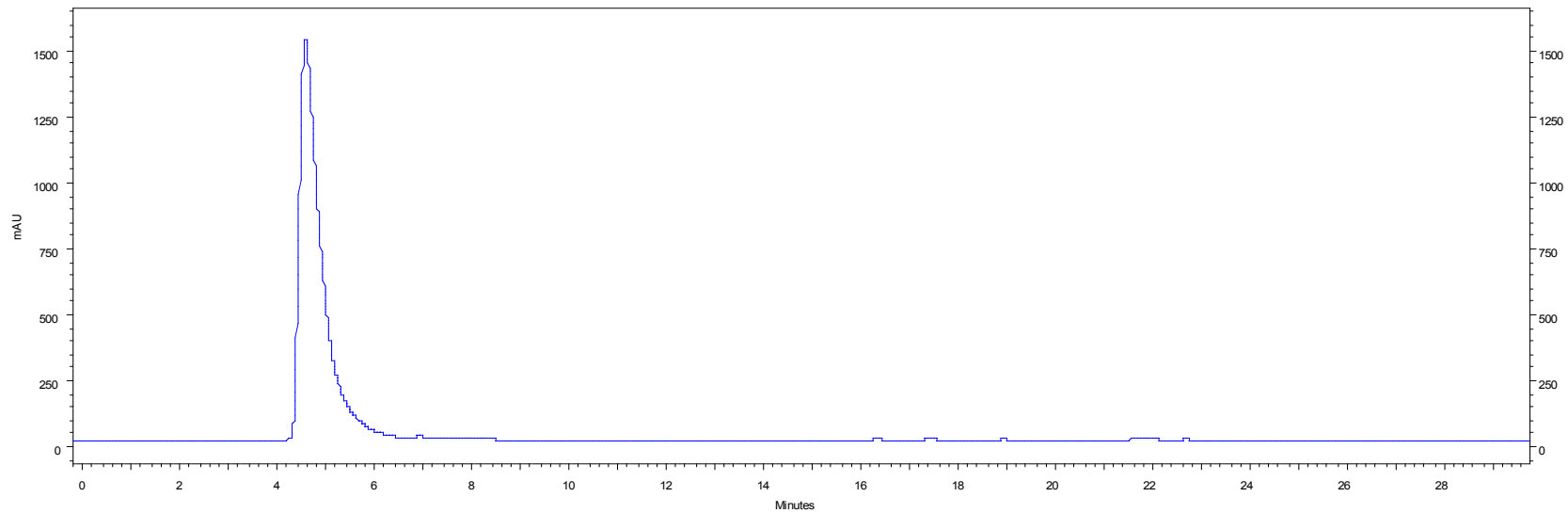
TOF MS ES+
1.82e5



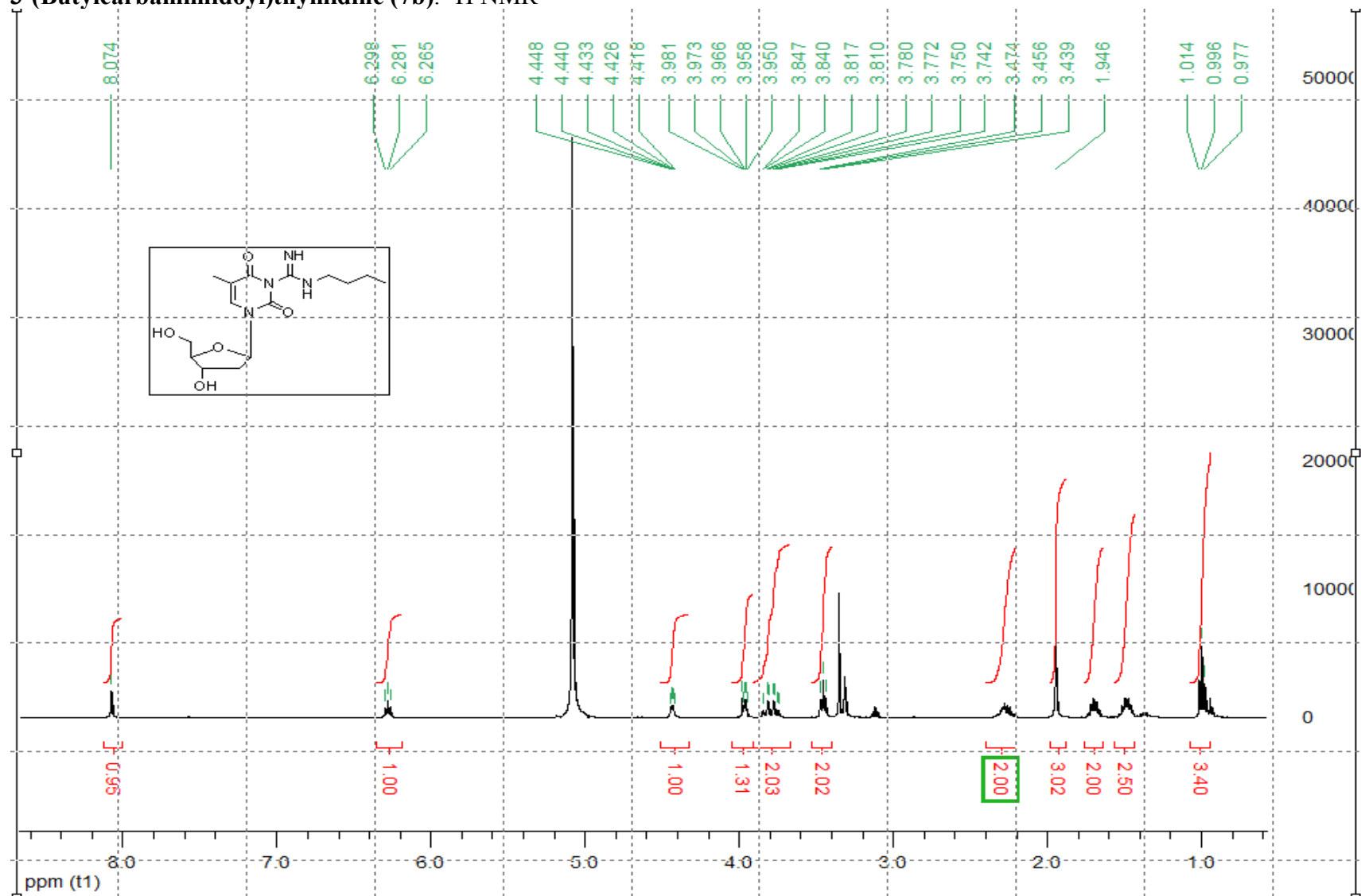
3-(Propylcarbamimidoyl)thymidine (7a). Analytical traces in Water:Acetonitrile gradient



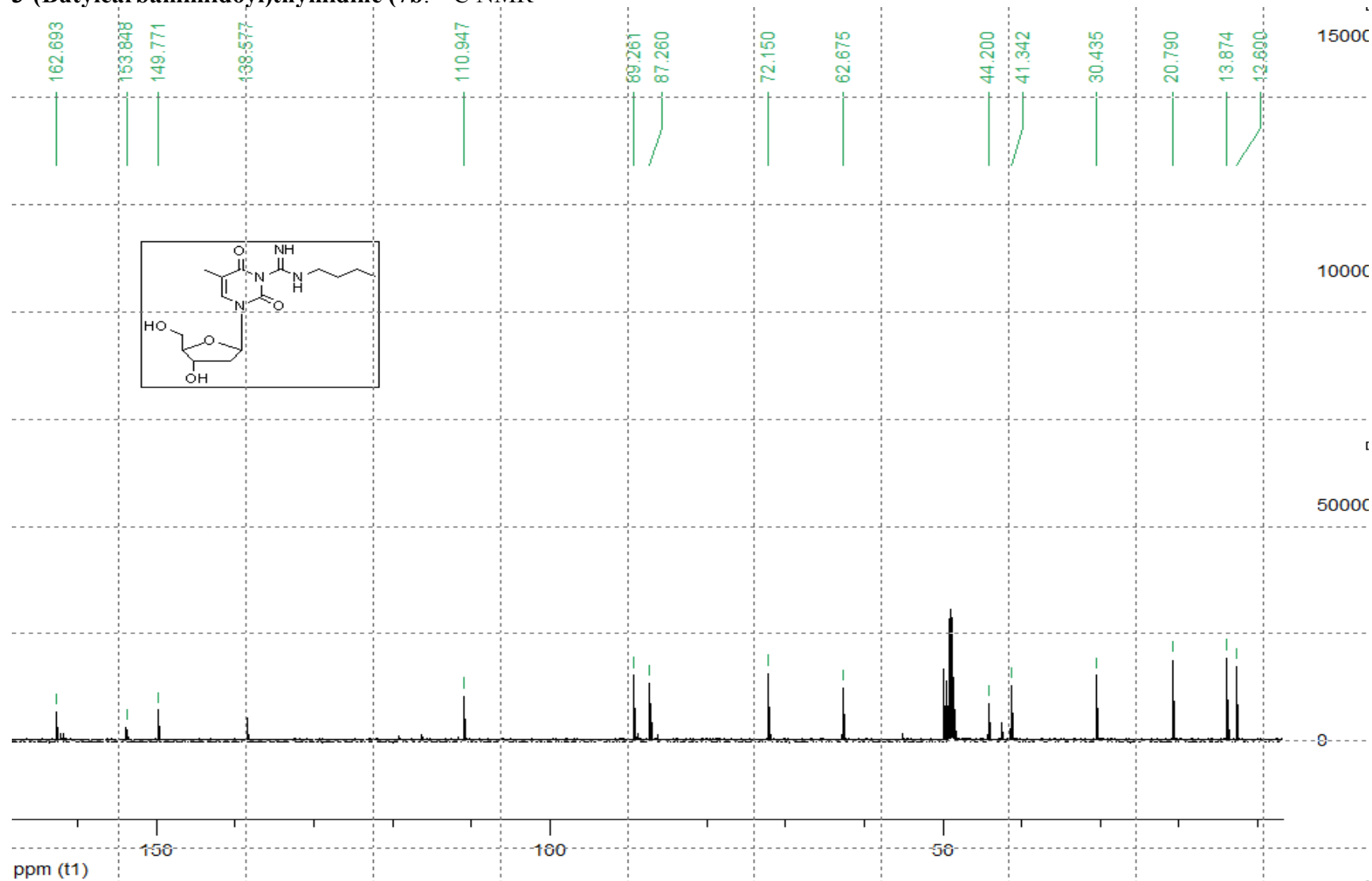
Analytical traces in Water:Methanol gradient



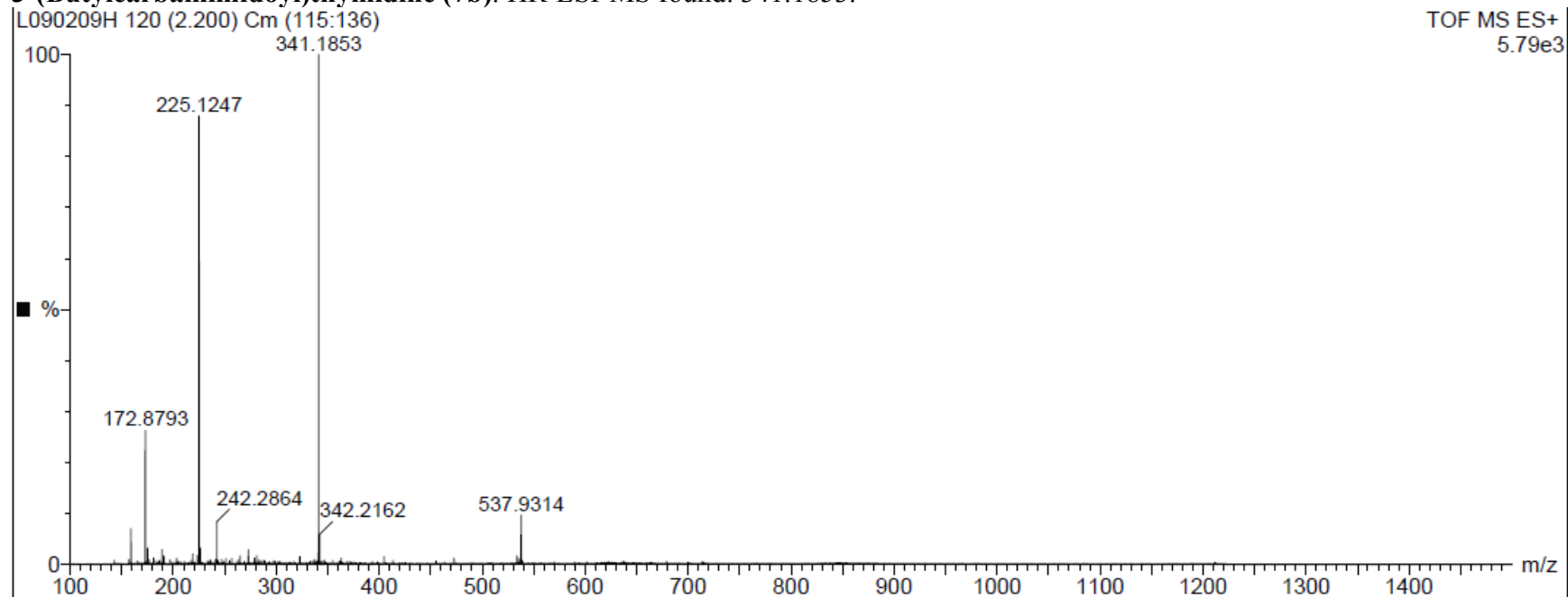
3-(Butylcarbamimidoyl)thymidine (7b). ¹H NMR



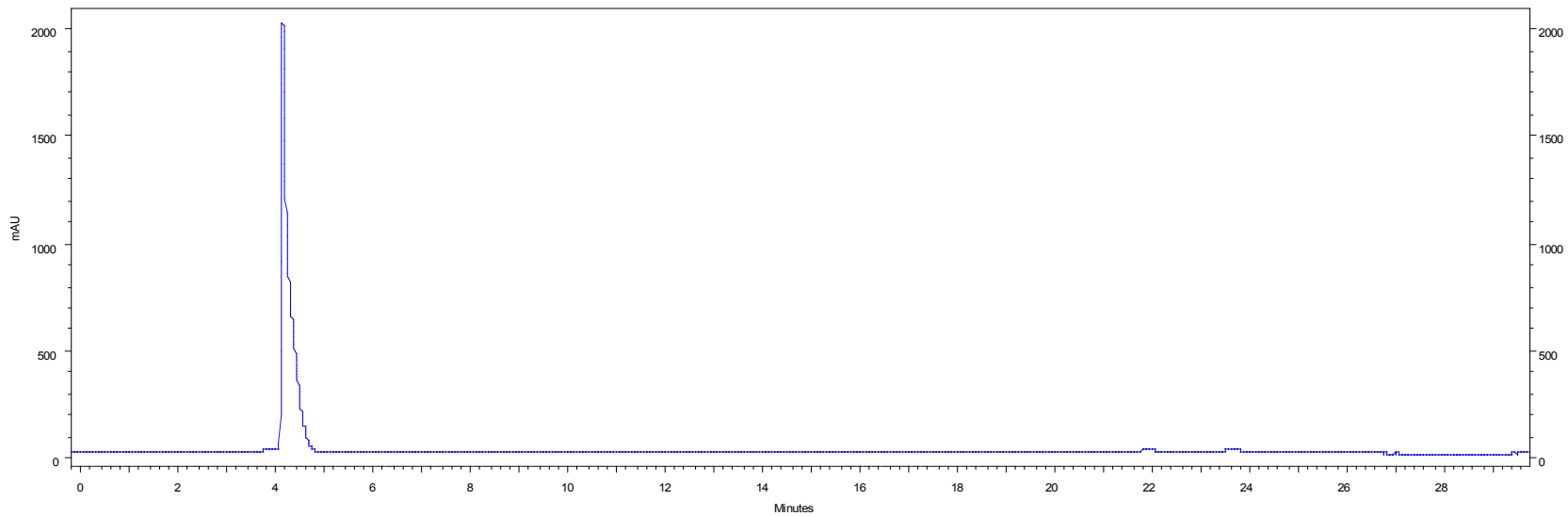
3-(Butylcarbamimidoyl)thymidine (7b). ¹³C NMR



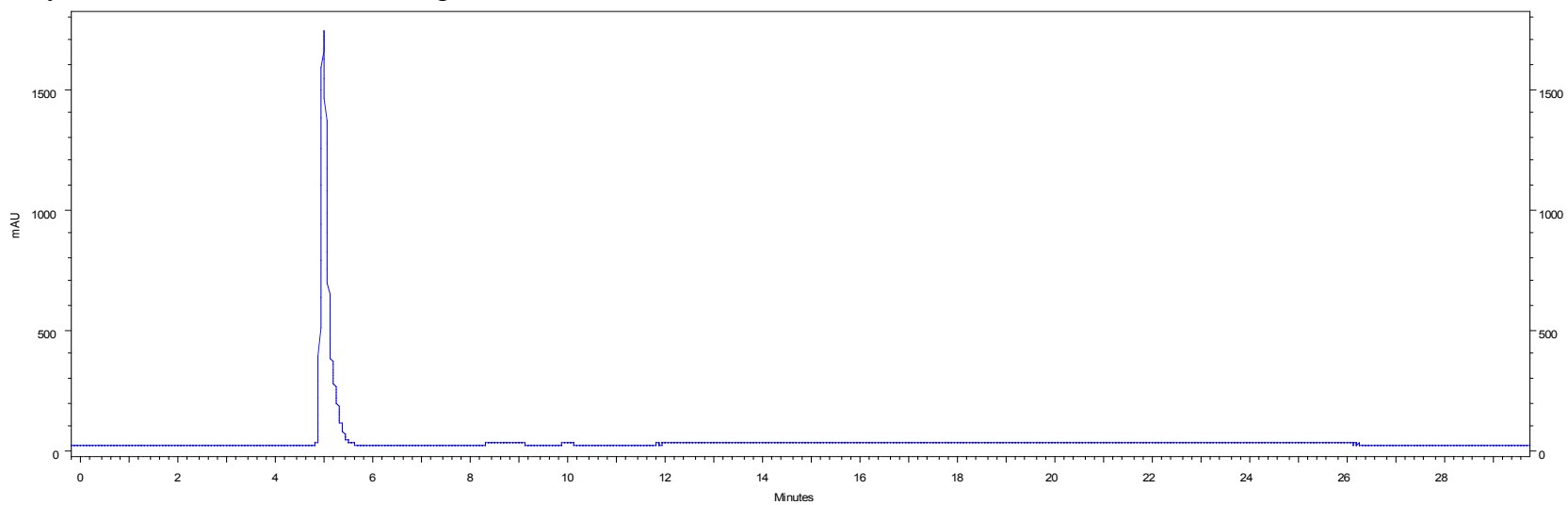
3-(Butylcarbamidoyl)thymidine (7b). HR-ESI-MS found: 341.1853.



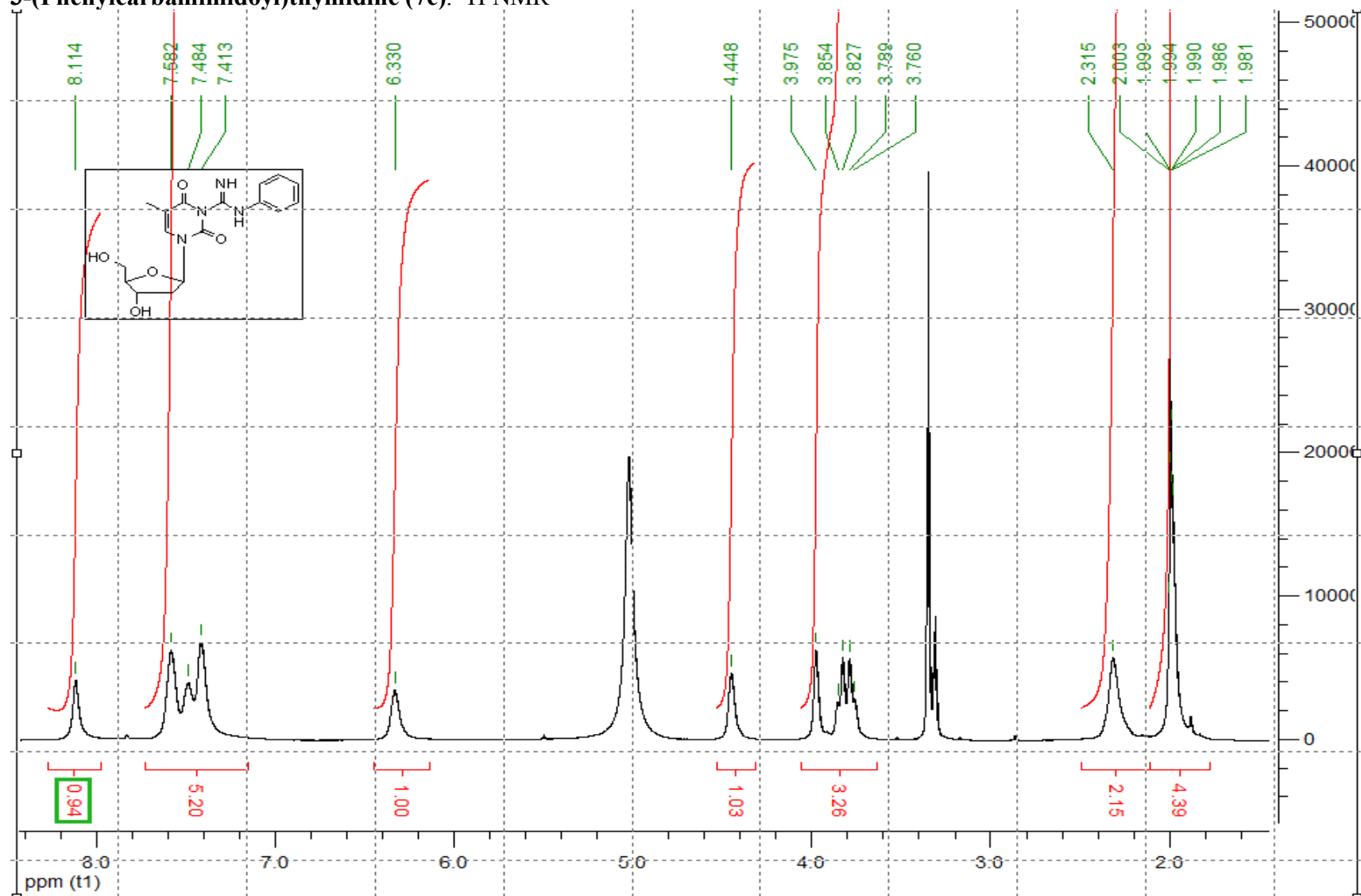
3-(Butylcarbamimidoyl)thymidine (7b). Analytical traces in Water:Acetonitrile gradient



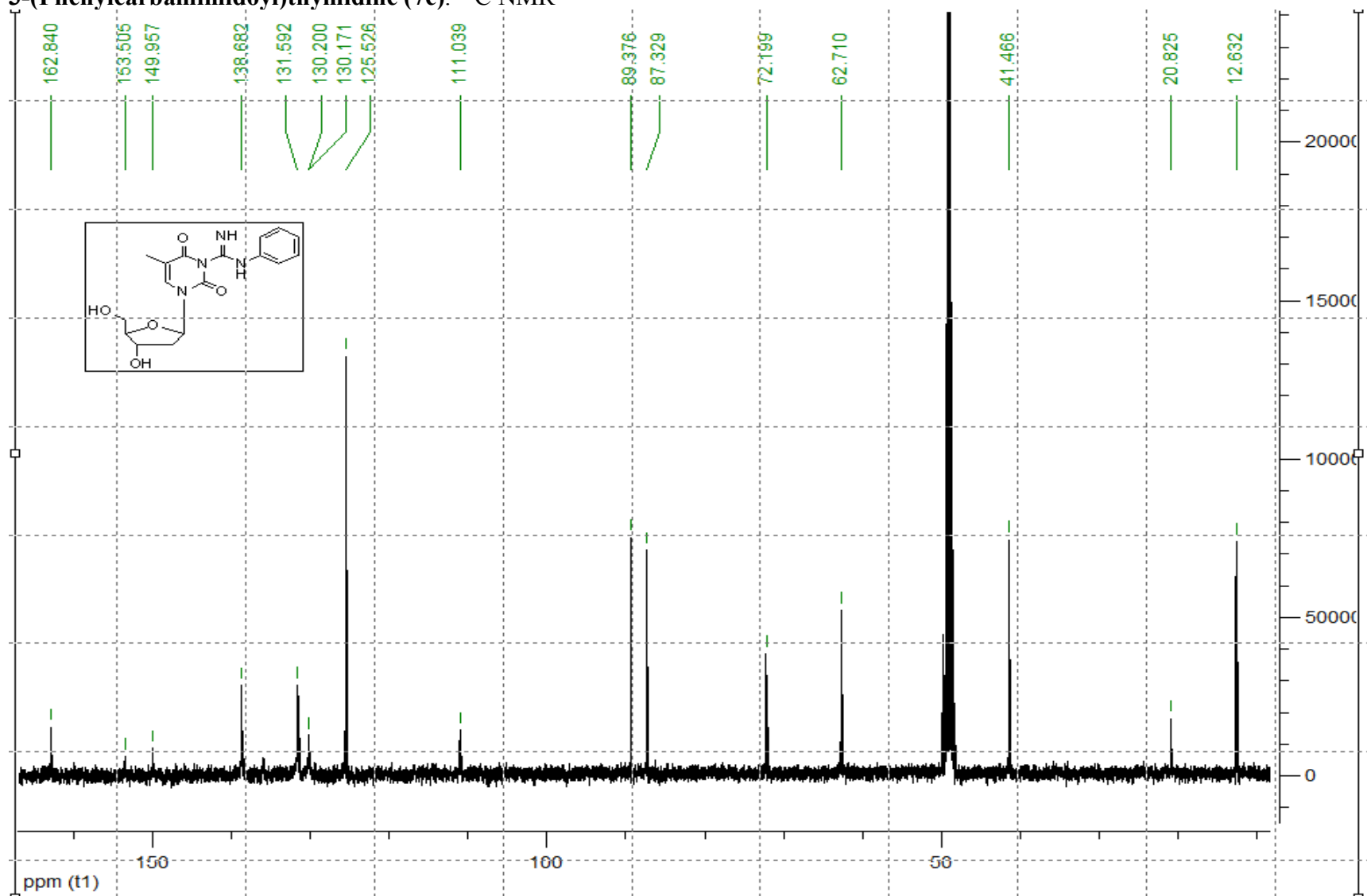
Analytical traces in Water:Methanol gradient



3-(Phenylcarbamimidoyl)thymidine (7c). ¹H NMR



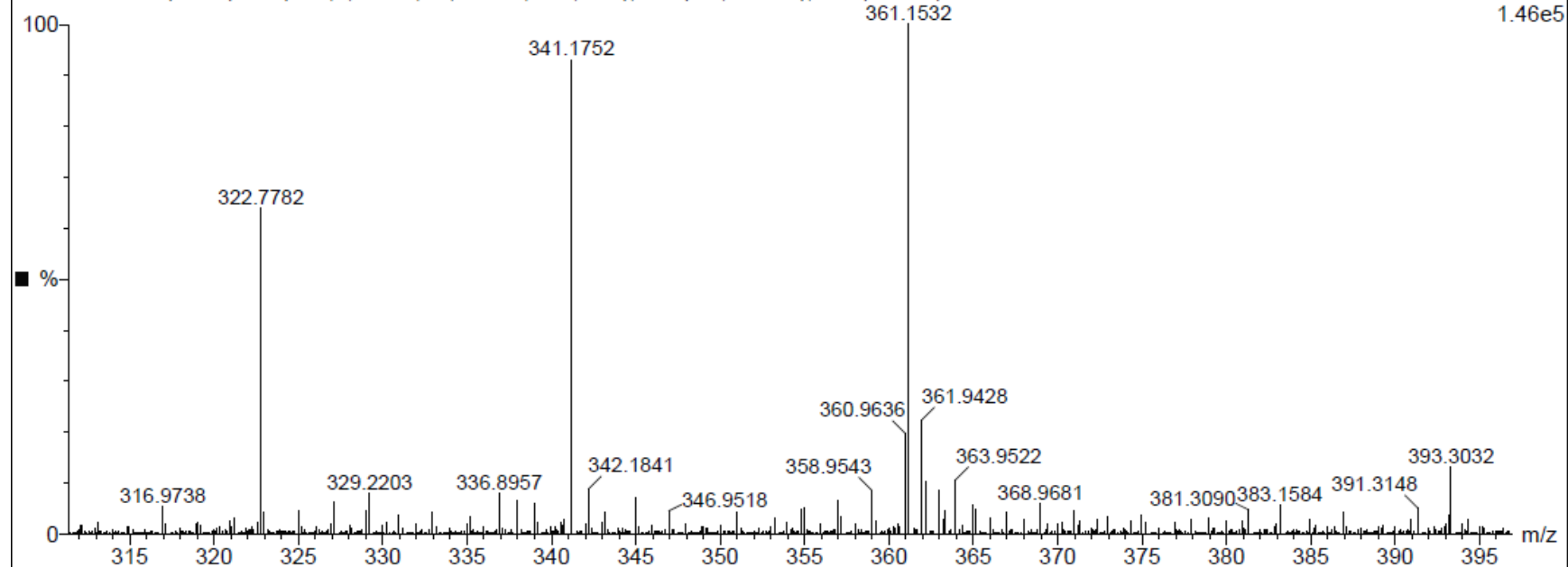
3-(Phenylcarbamimidoyl)thymidine (7c). ¹³C NMR



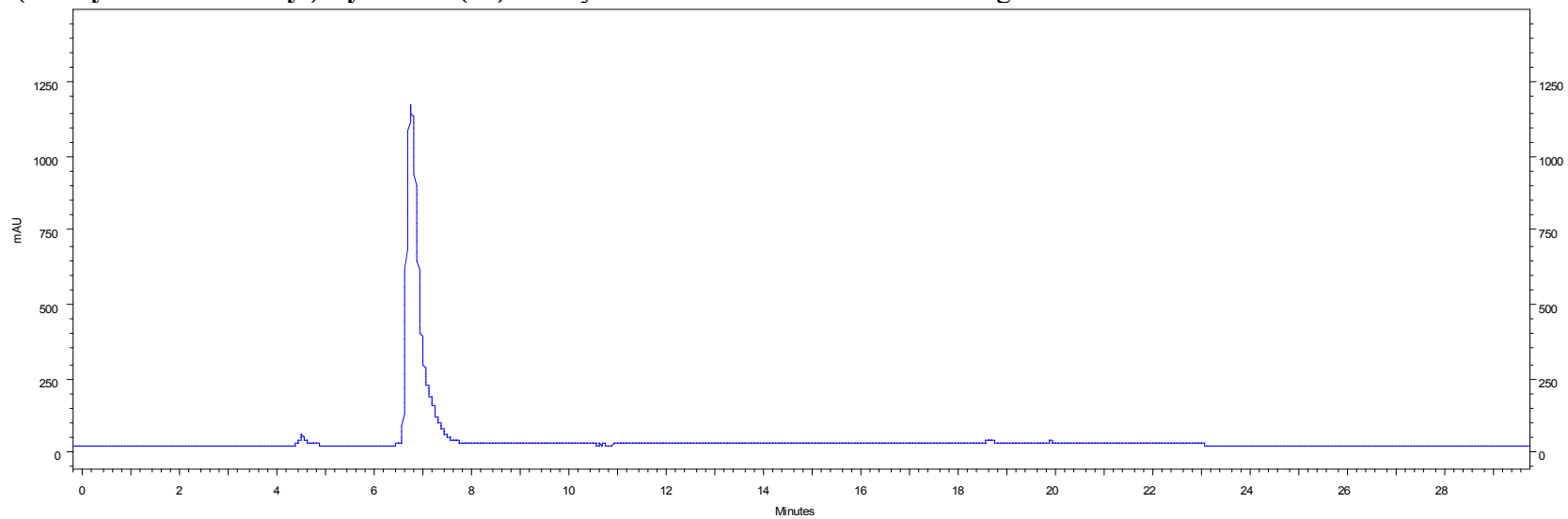
3-(Phenylcarbamimidoyl)thymidine (7c). HR-ESI-MS found: 361.1532

L090209E 231 (4.236) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (15:286)

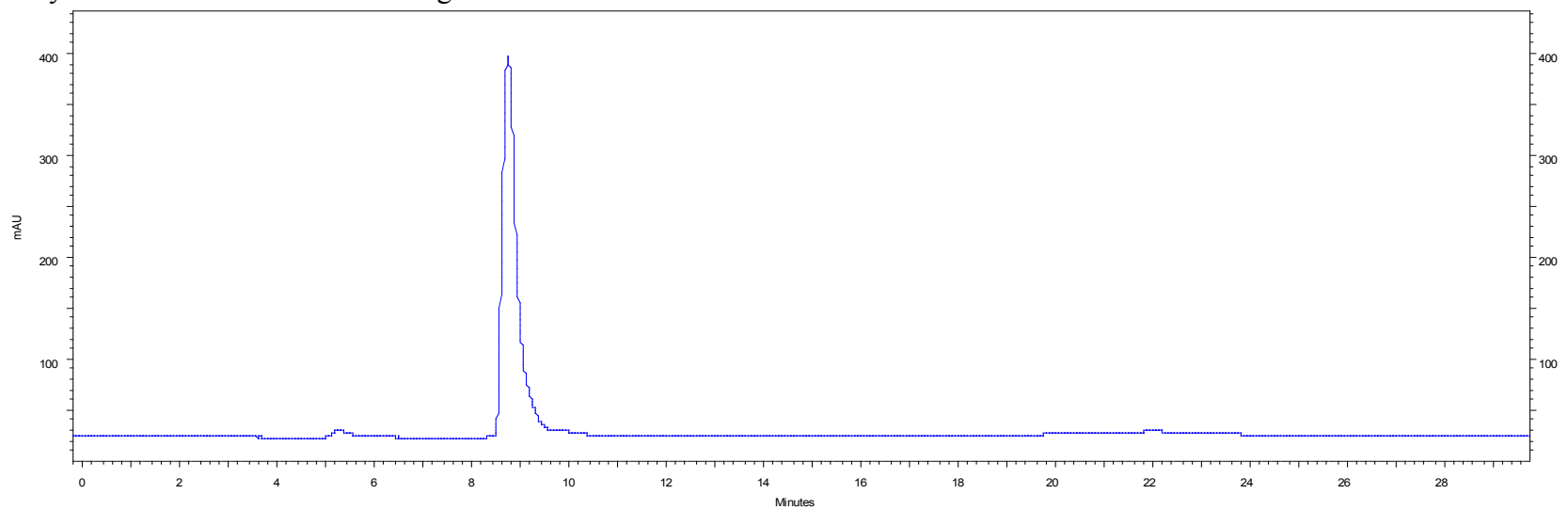
TOF MS ES+
1.46e5



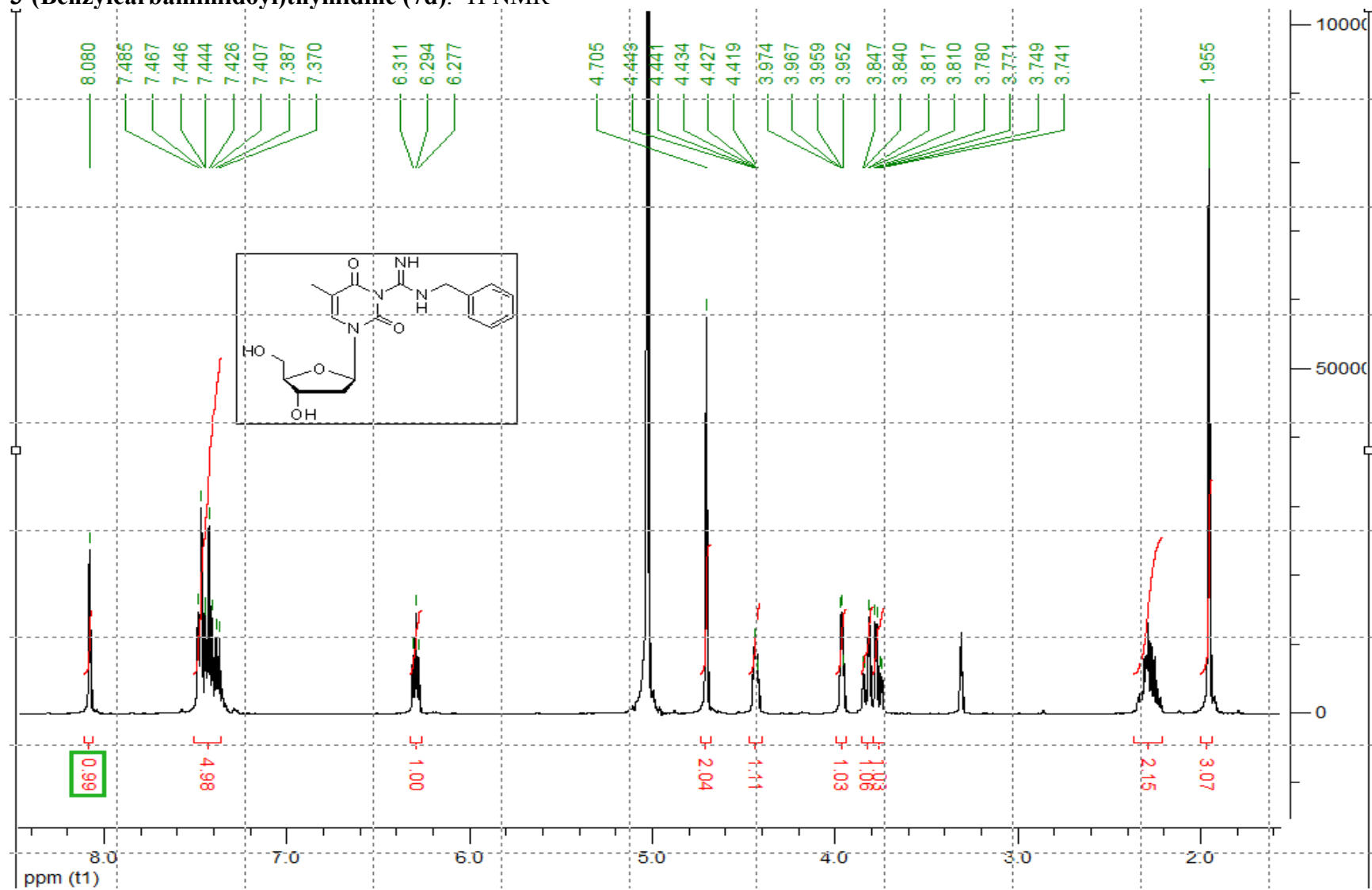
3-(Phenylcarbamidoyl)thymidine (7c). Analytical traces in Water:Acetonitrile gradient



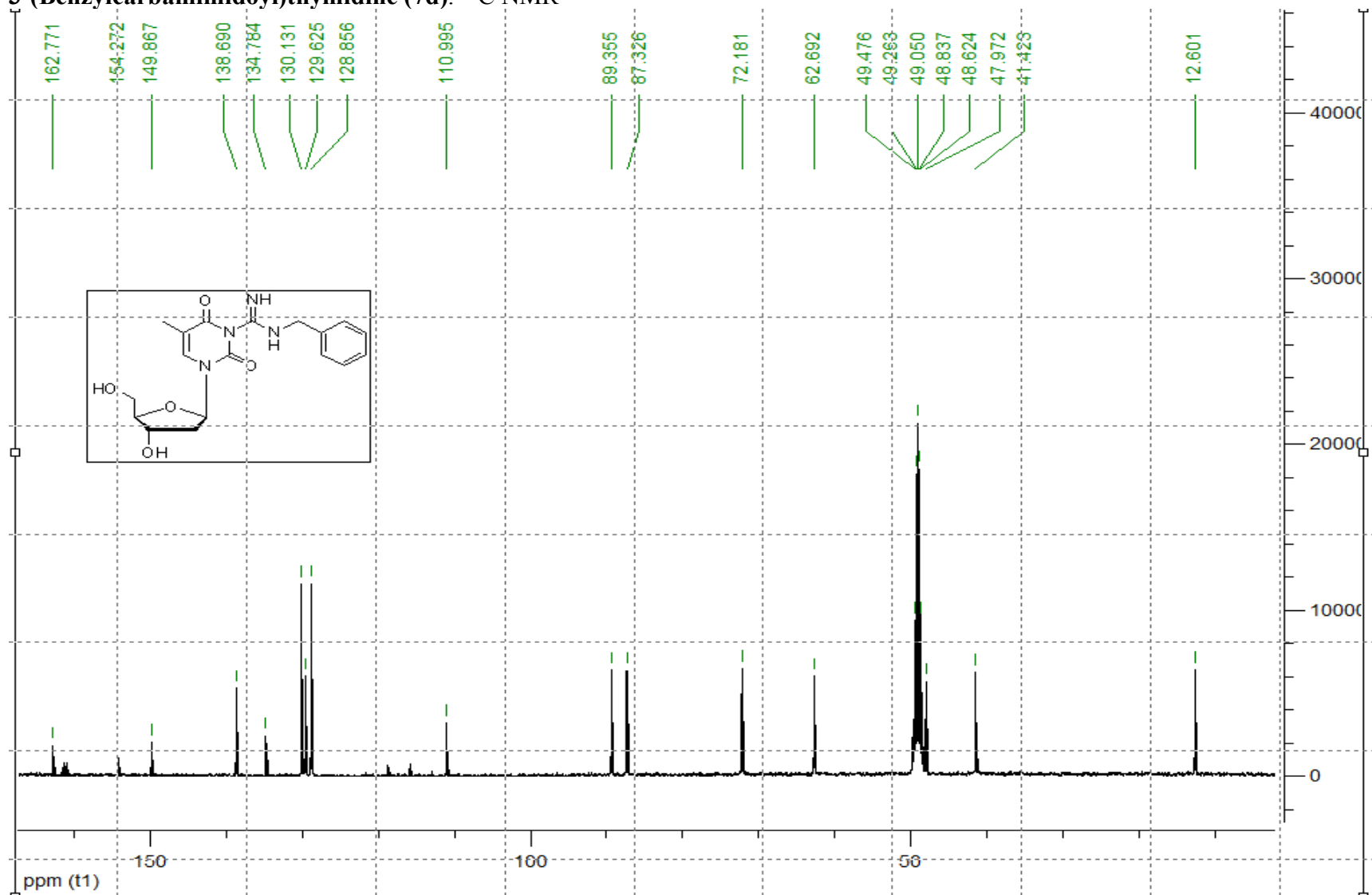
Analytical traces in Water:Methanol gradient



3-(Benzylcarbamimidoyl)thymidine (7d). ¹H NMR



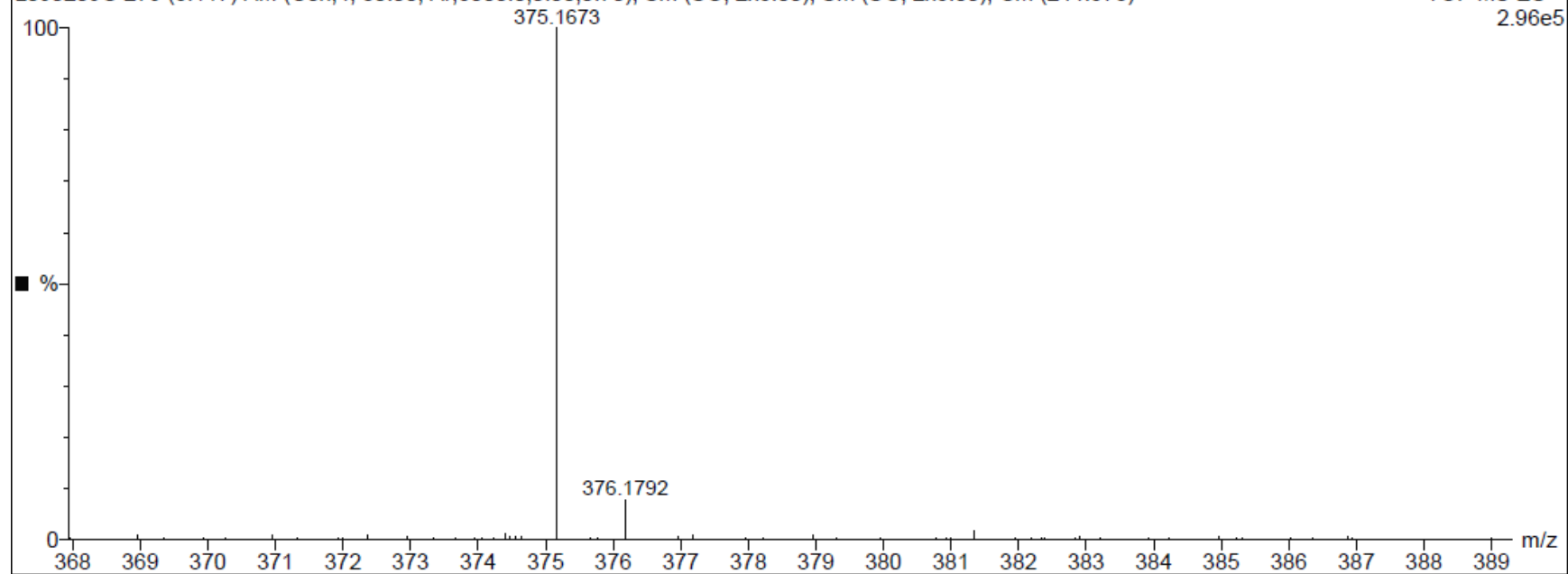
3-(Benzylcarbamidoyl)thymidine (7d). ¹³C NMR



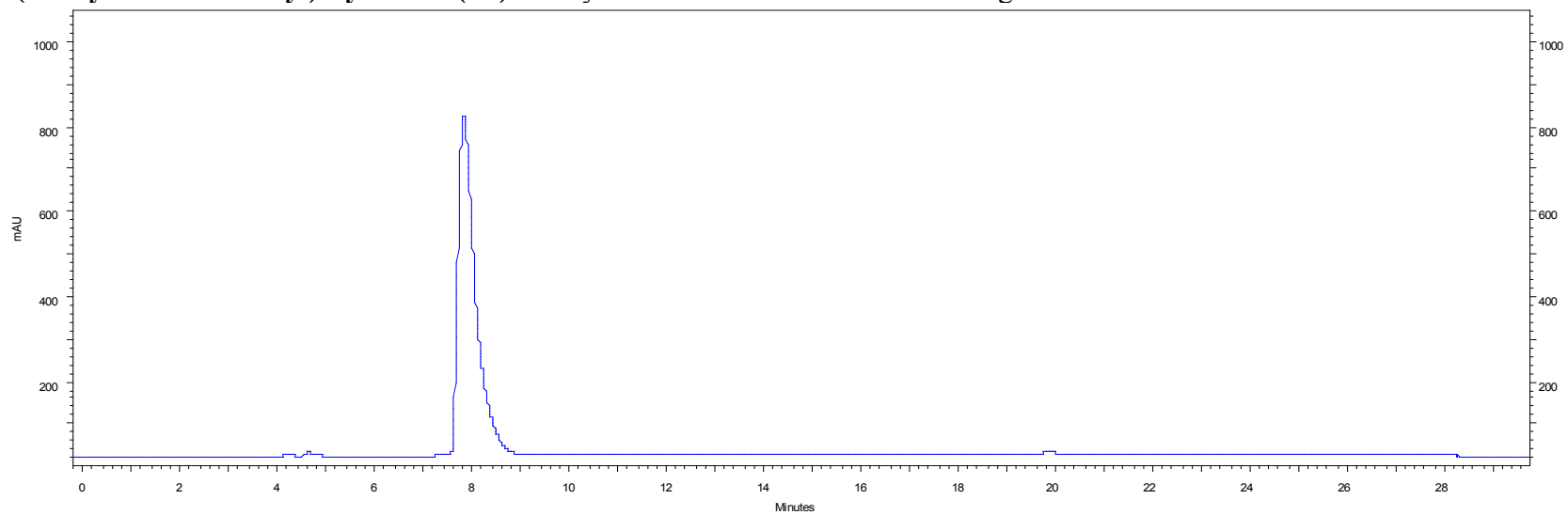
3-(Benzylcarbamimidoyl)thymidine (7d). HR-ESI-MS found: 375.1673

L090209G 279 (5.117) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Sm (SG, 2x3.00); Cm (244:378)

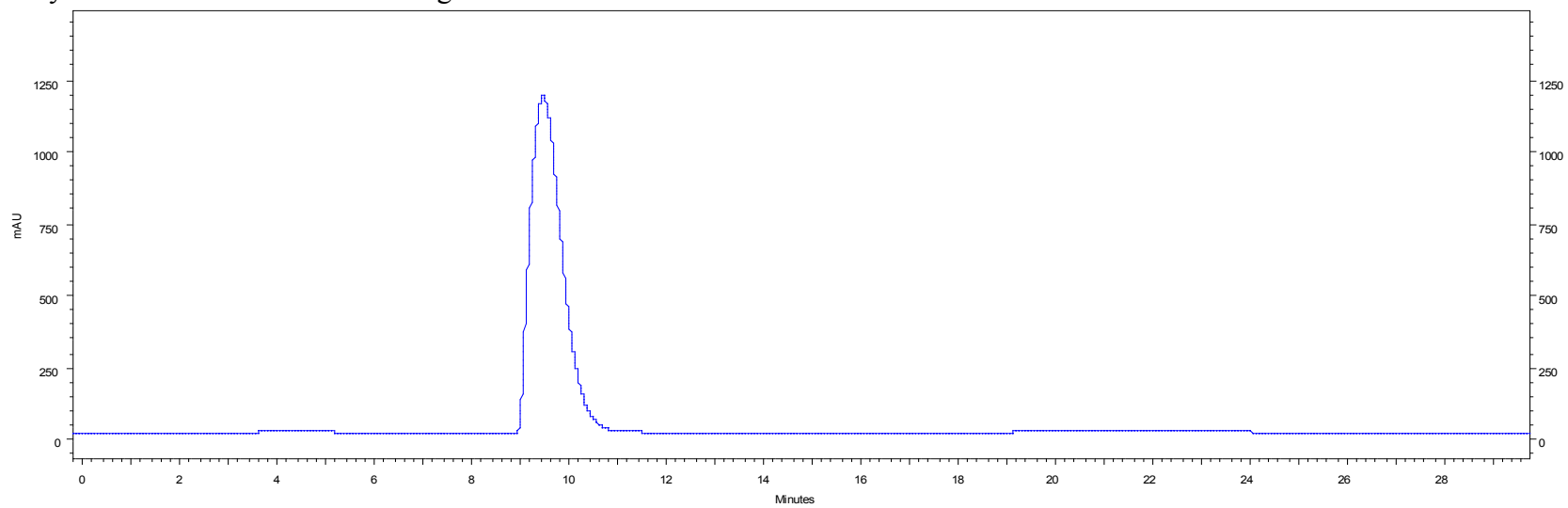
TOF MS ES+
2.96e5



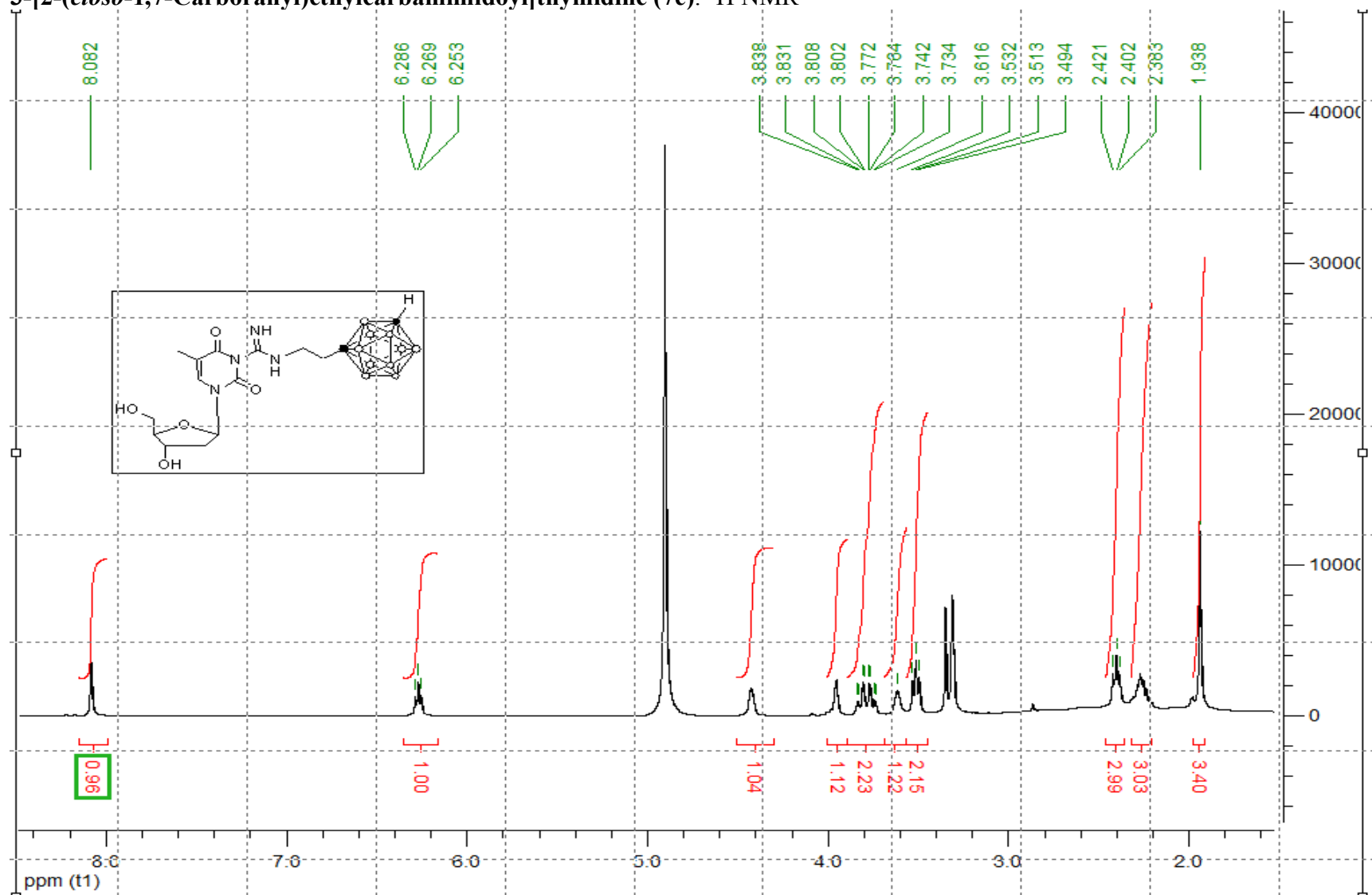
3-(Benzylcarbamimidoyl)thymidine (7d). Analytical traces in Water:Acetonitrile gradient



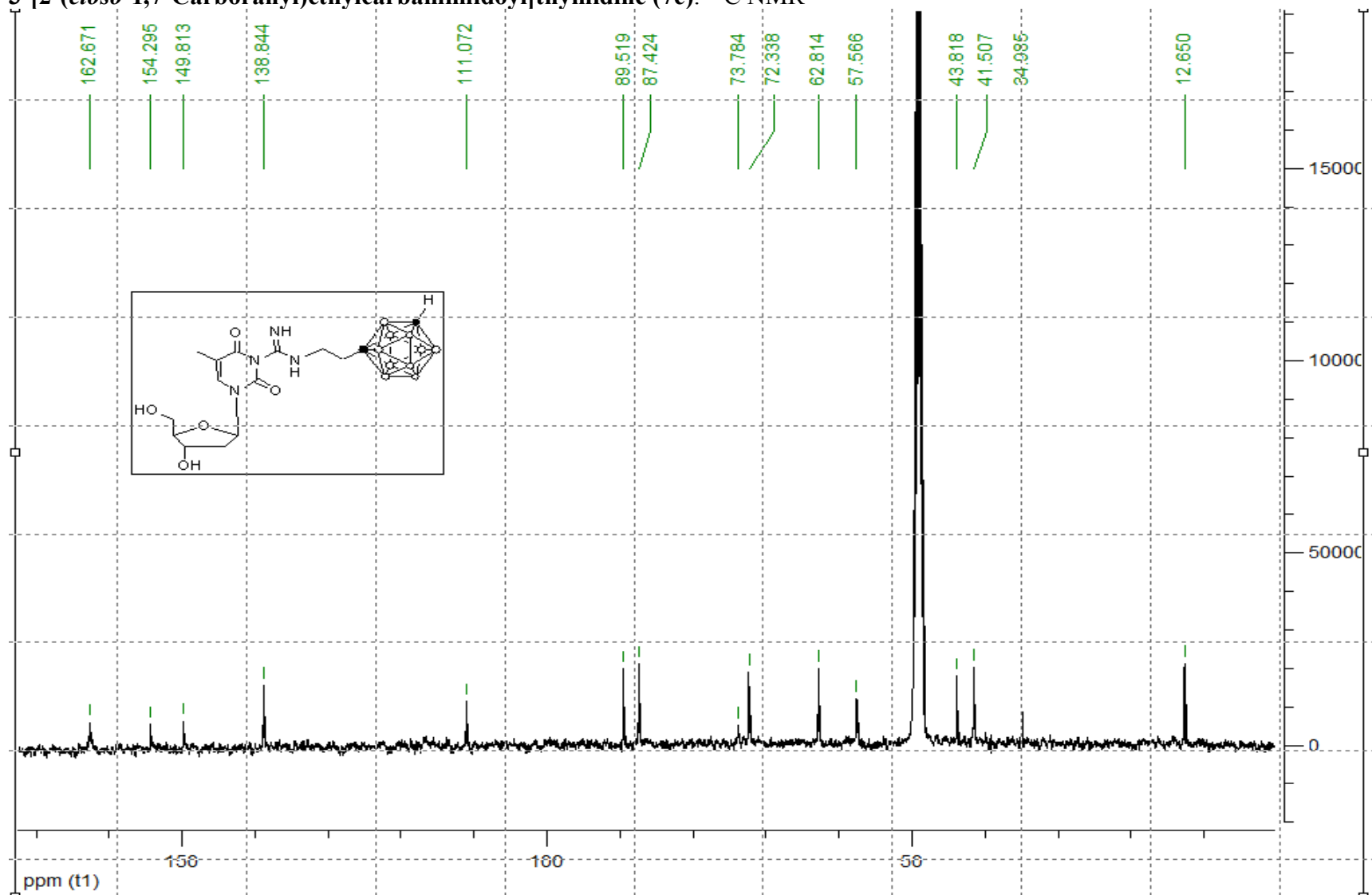
Analytical traces in Water:Methanol gradient



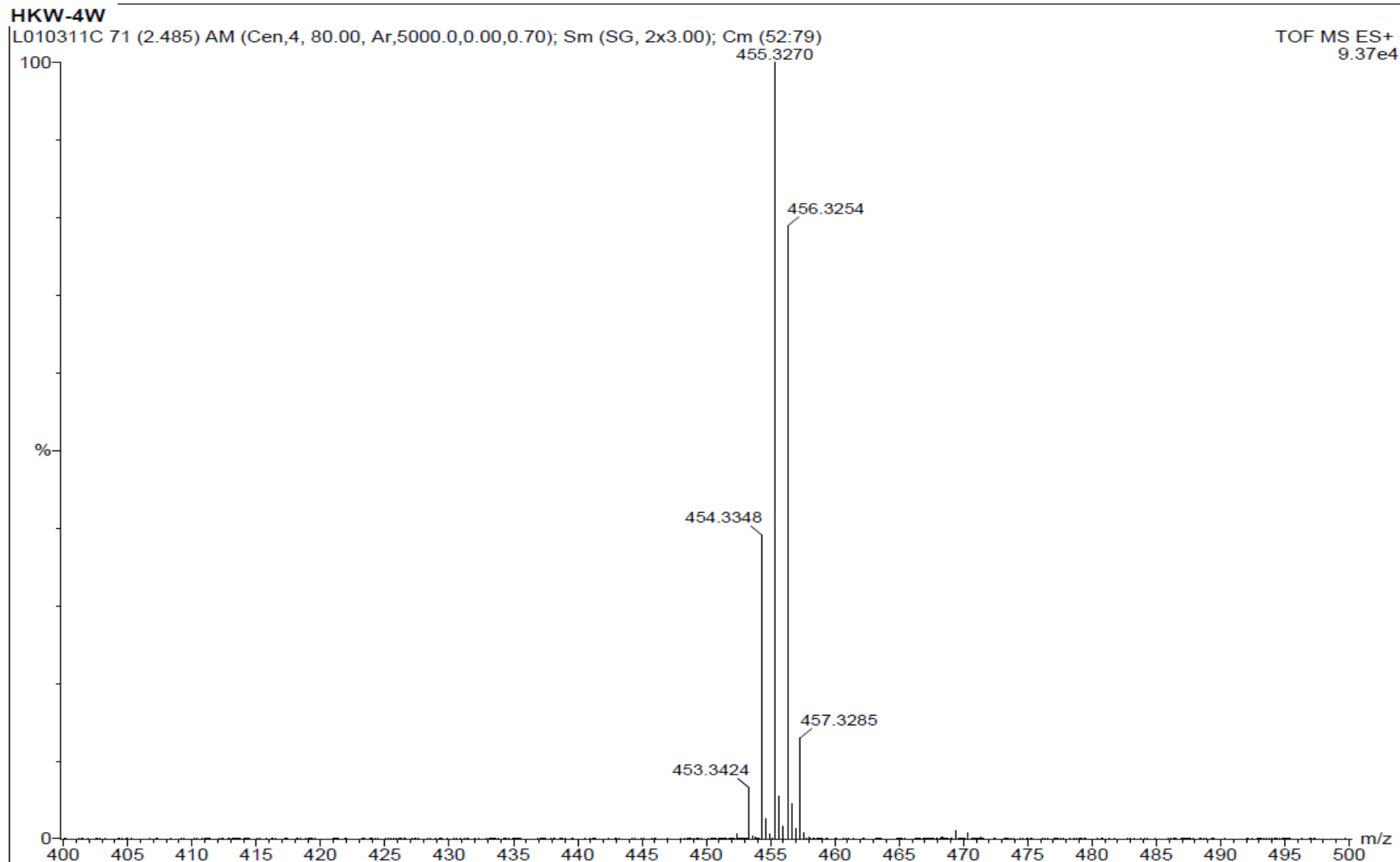
3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]thymidine (7e). ¹H NMR



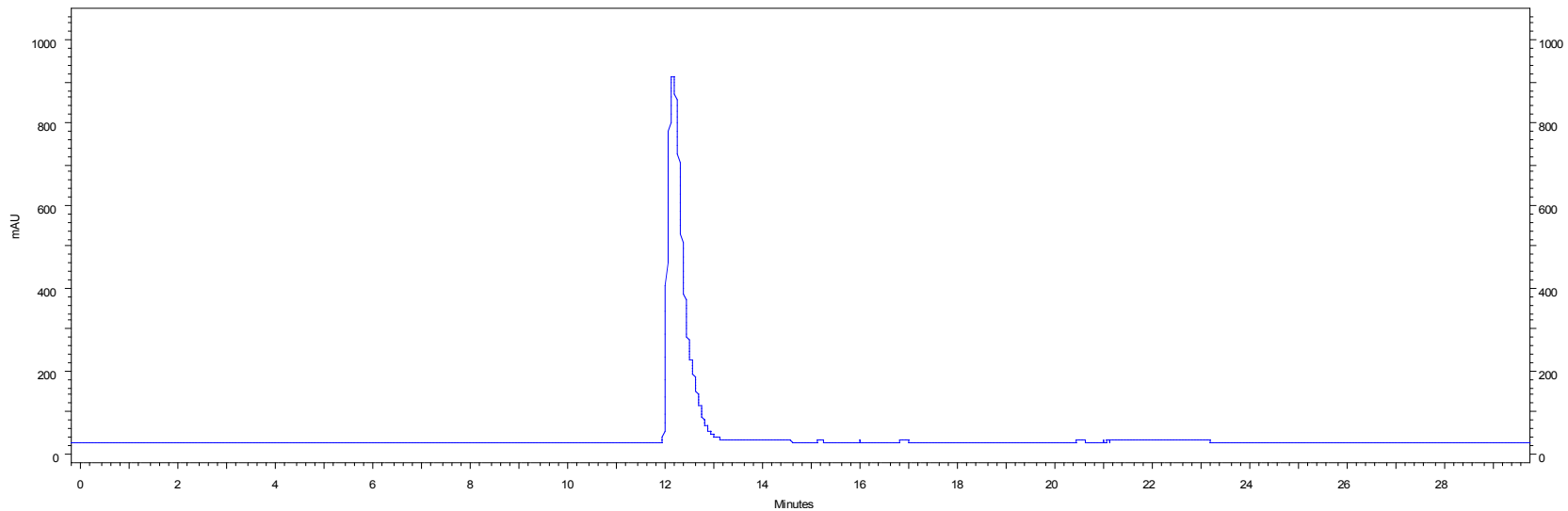
3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]thymidine (7e). ^{13}C NMR



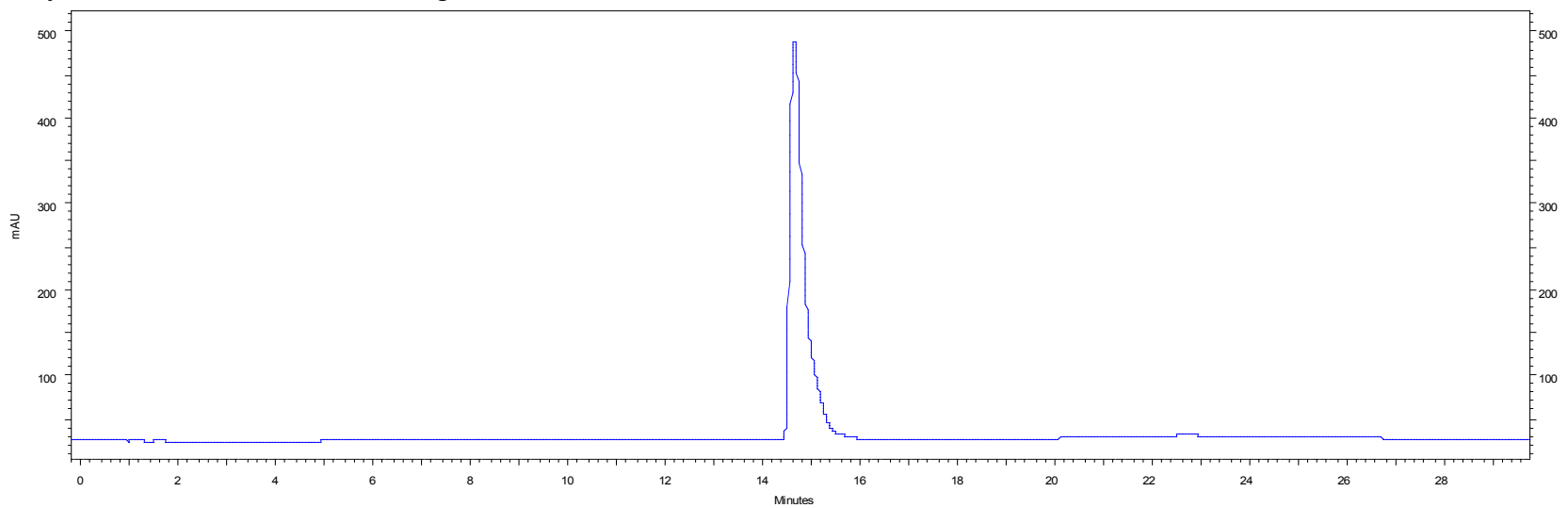
3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]thymidine (7e). HR-ESI-MS found: 455.3270



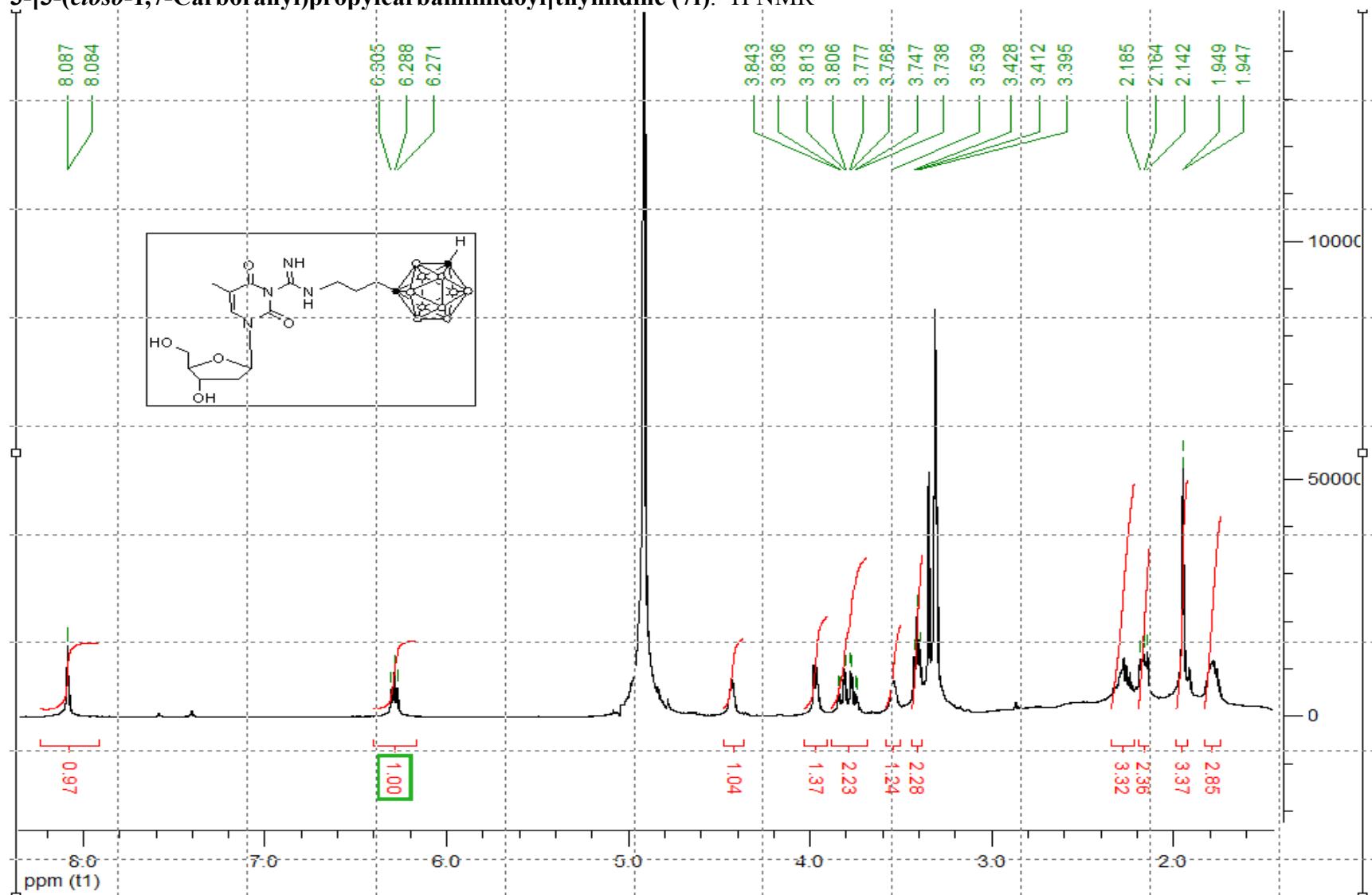
3-[2-(*closo*-1,7-Carboranyl)ethylcarbamimidoyl]thymidine (7e). Analytical traces in Water:Acetonitrile gradient



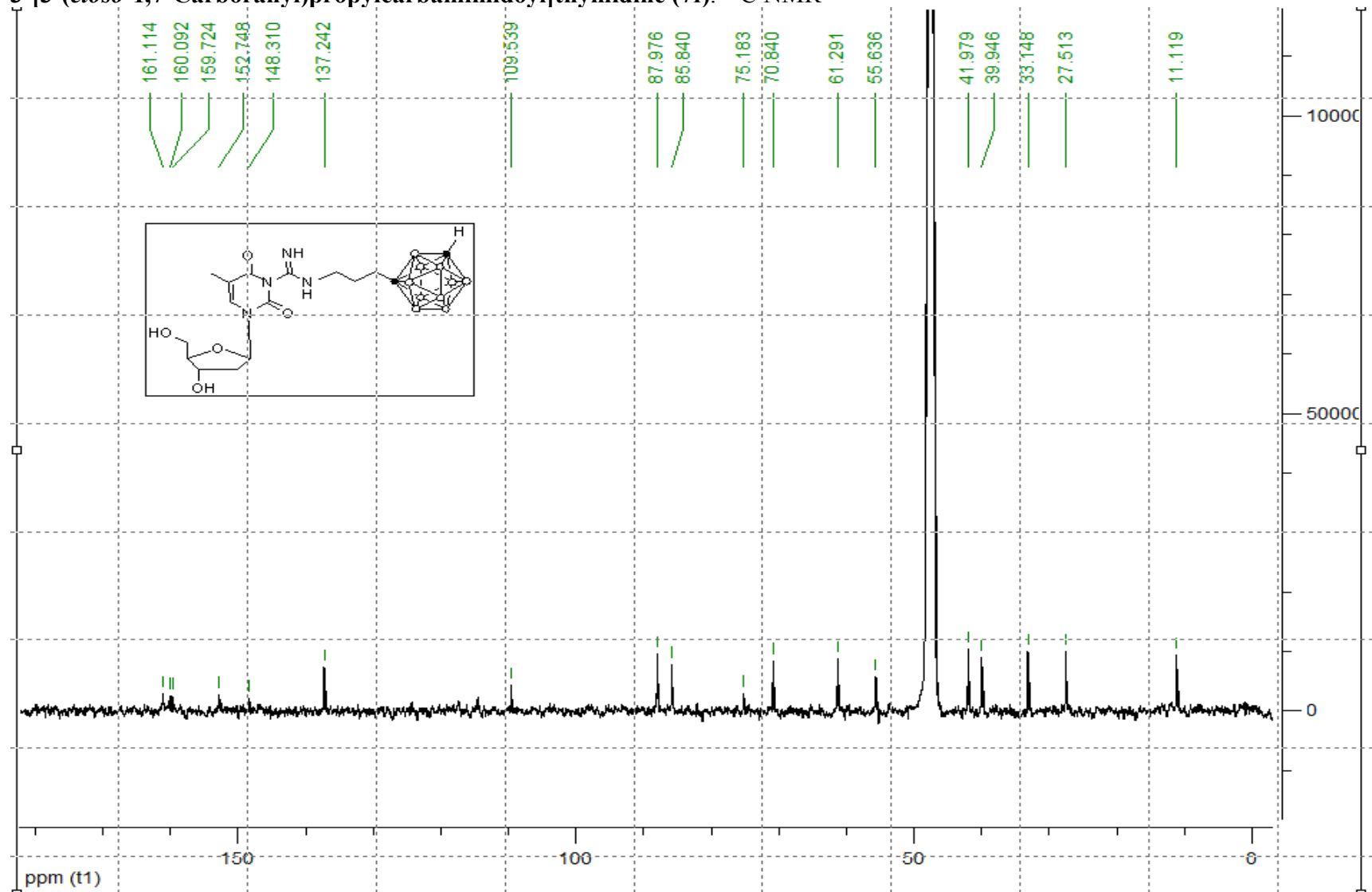
Analytical traces in Water:Methanol gradient



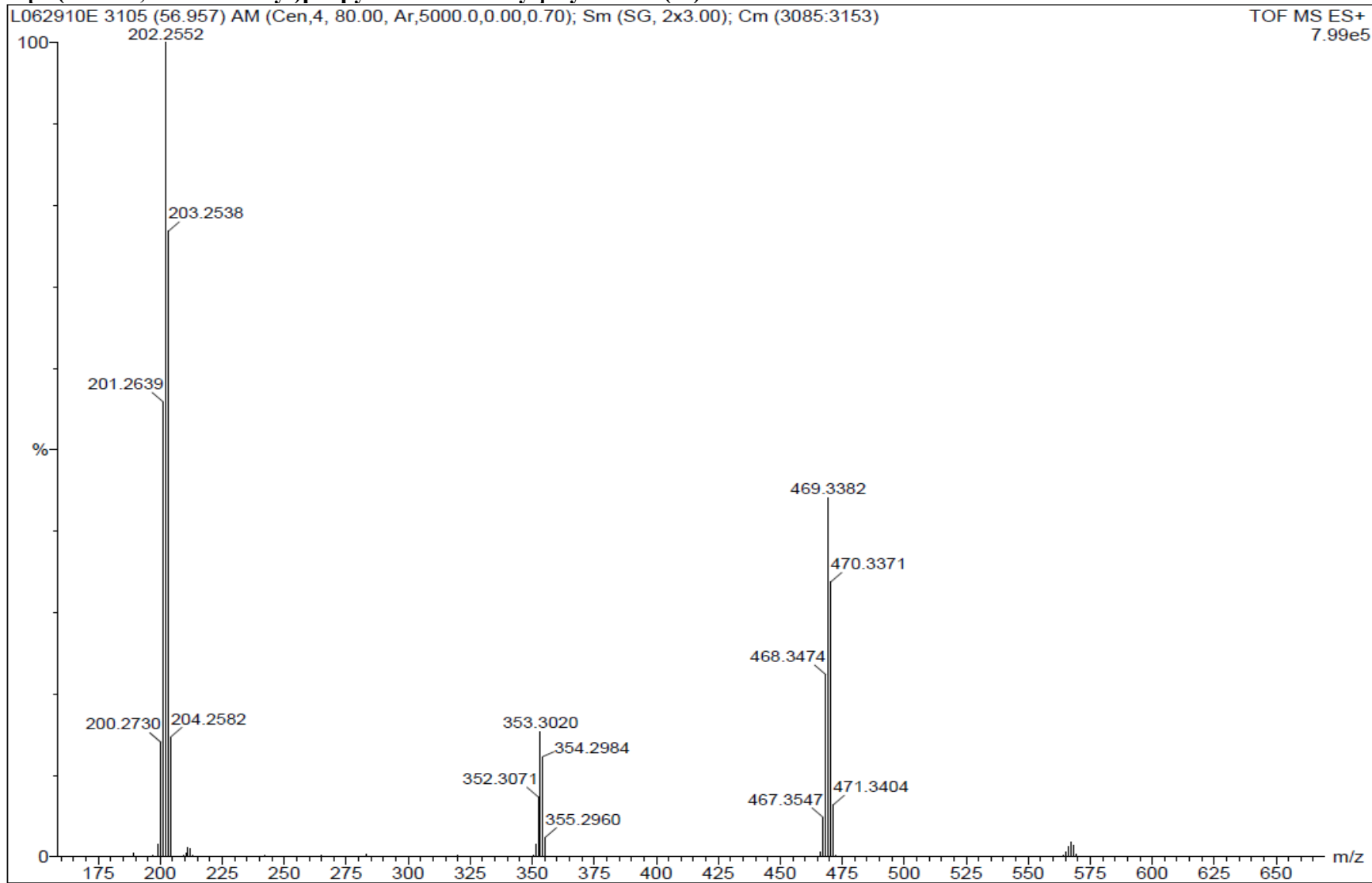
3-[3-(*closo*-1,7-Carboranyl)propylcarbamimidoyl]thymidine (7f). ¹H NMR



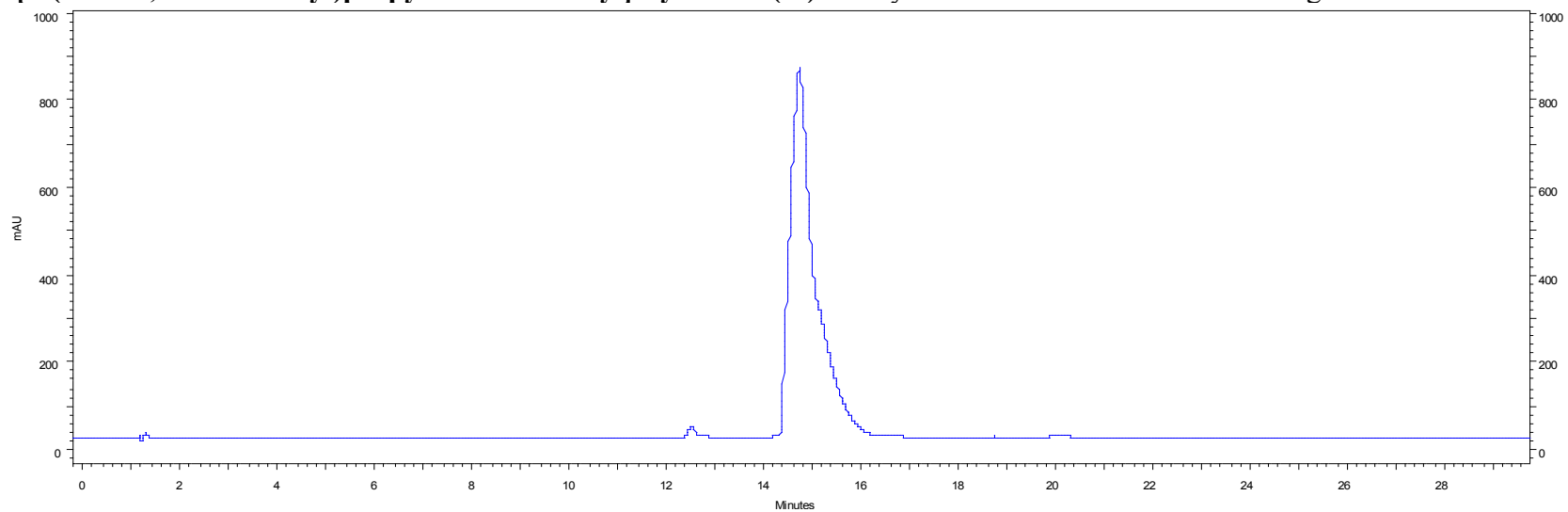
3-[3-(*closo*-1,7-Carboranyl)propylcarbamimidoyl]thymidine (7f). ¹³C NMR



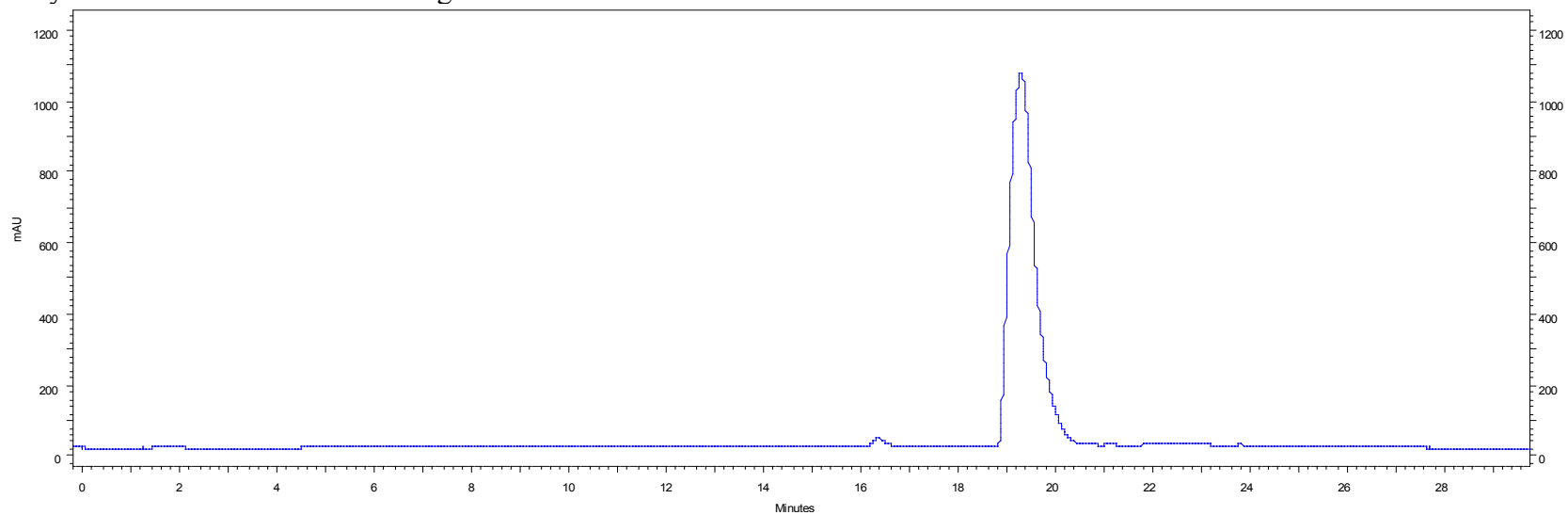
3-[3-(*closo*-1,7-Carboranyl)propylcarbamiidoyl]thymidine (7f). HR-ESI-MS found: 469.3382.



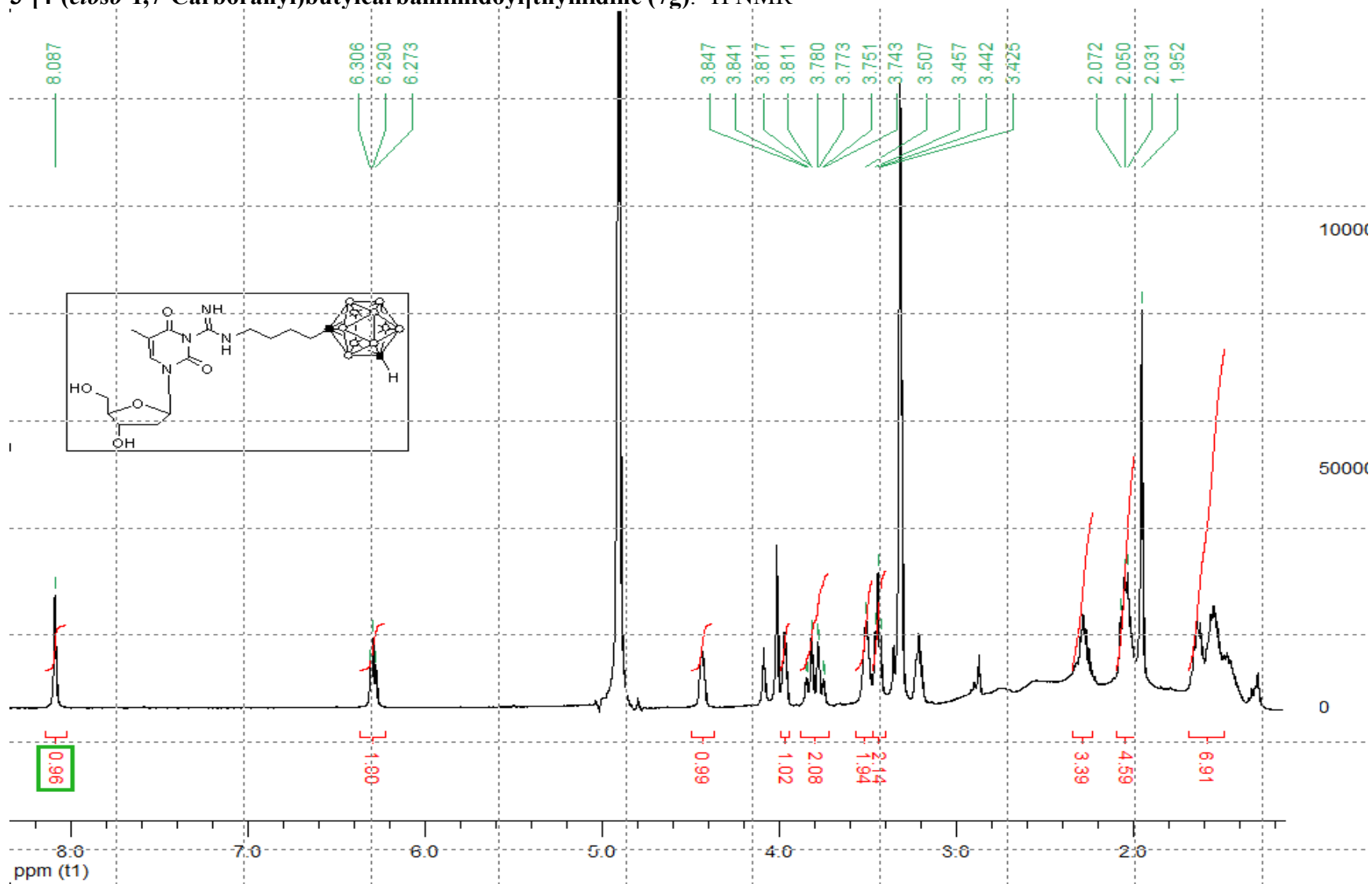
3-[3-(*closo*-1,7-Carboranyl)propylcarbamimidoyl]thymidine (7f). Analytical traces in Water:Acetonitrile gradient



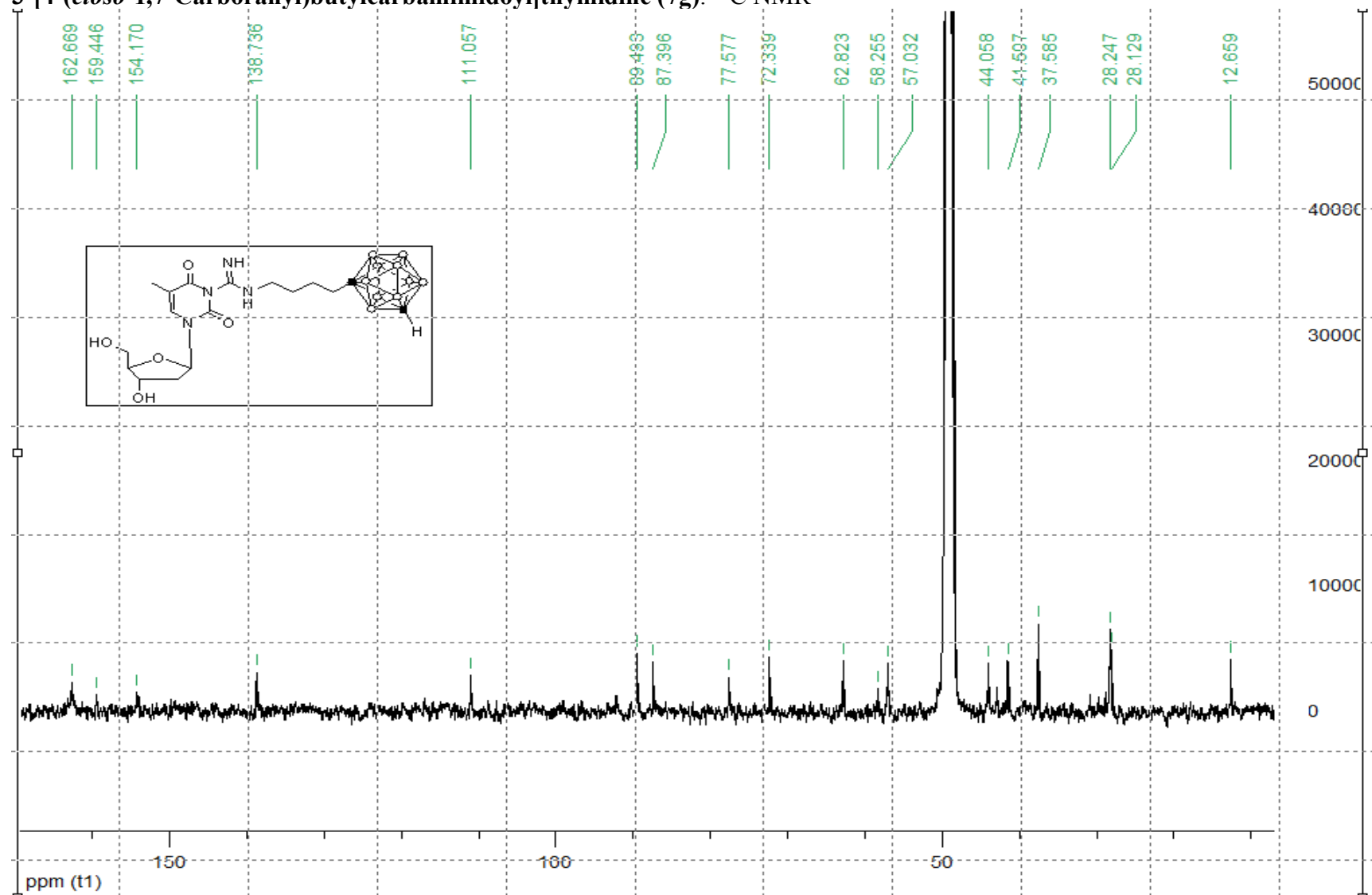
Analytical traces in Water:Methanol gradient



3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]thymidine (7g). ¹H NMR



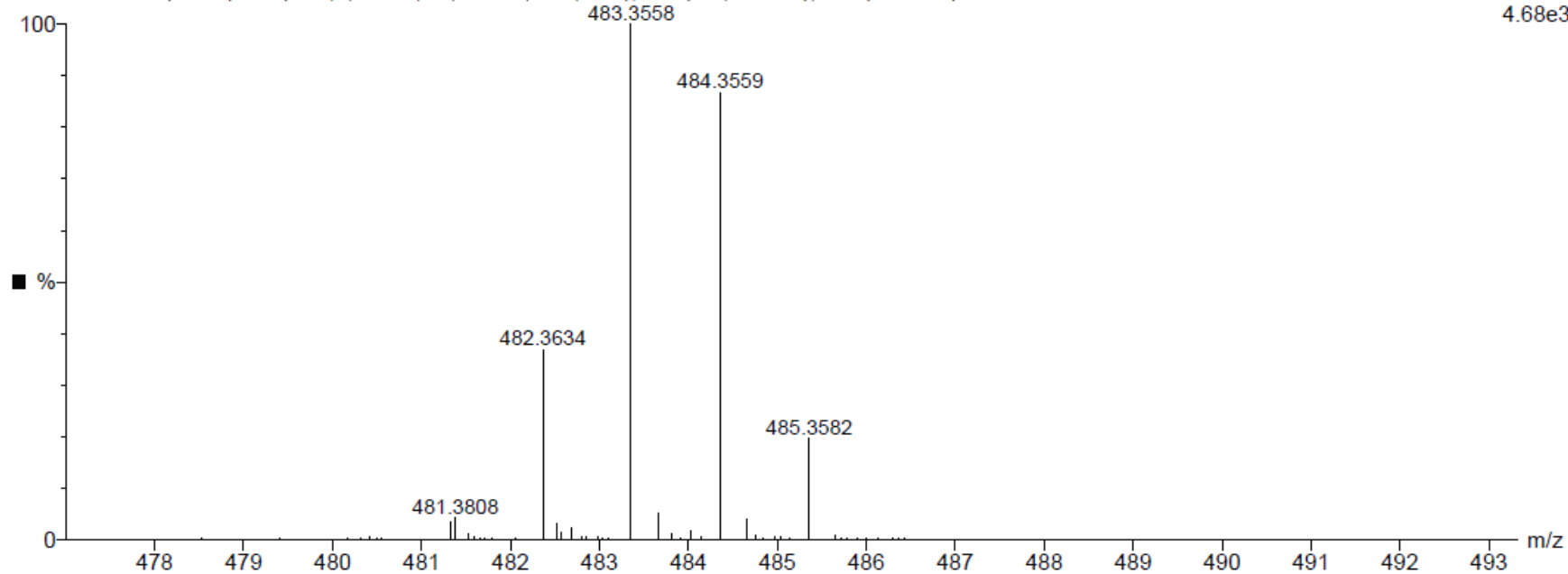
3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]thymidine (7g). ^{13}C NMR



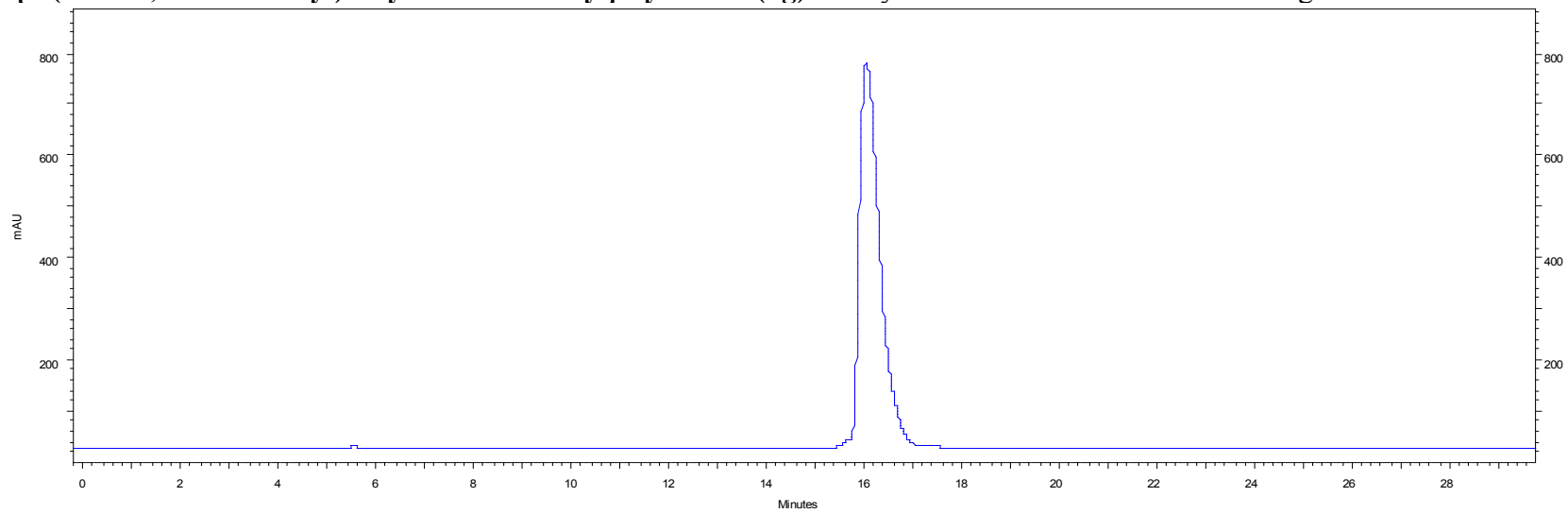
3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]thymidine (7g). HR-ESI-MS found: 483.3558.

L102210C 231 (4.236) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (173:236)

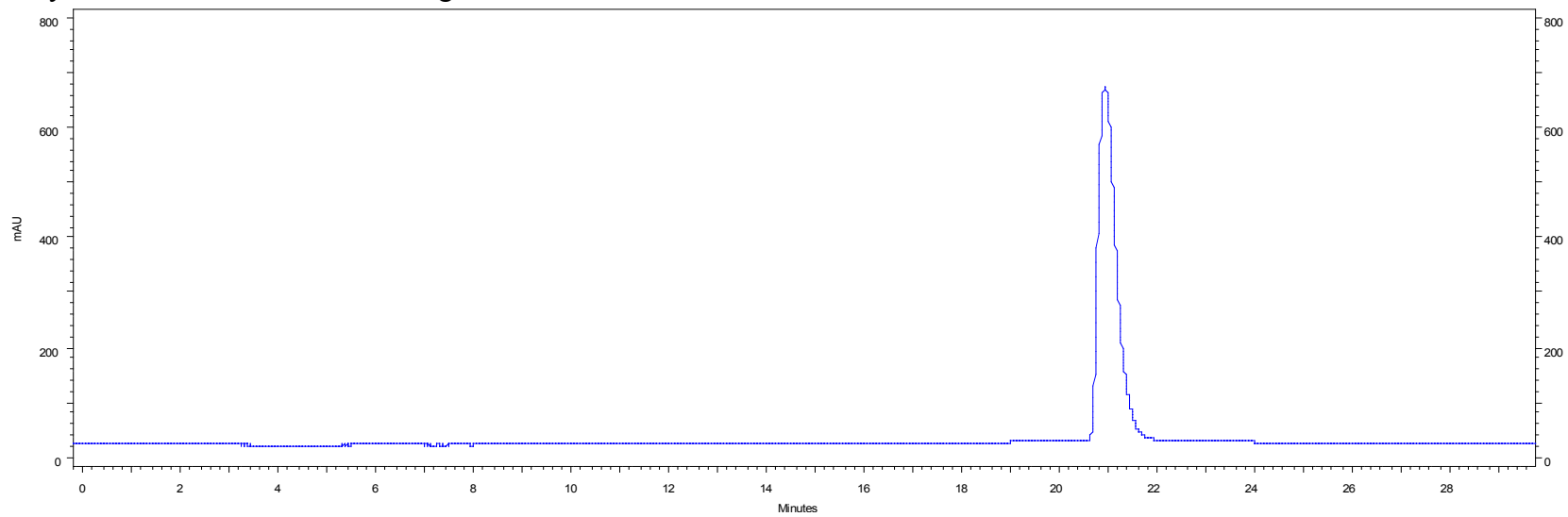
TOF MS ES+
4.68e3



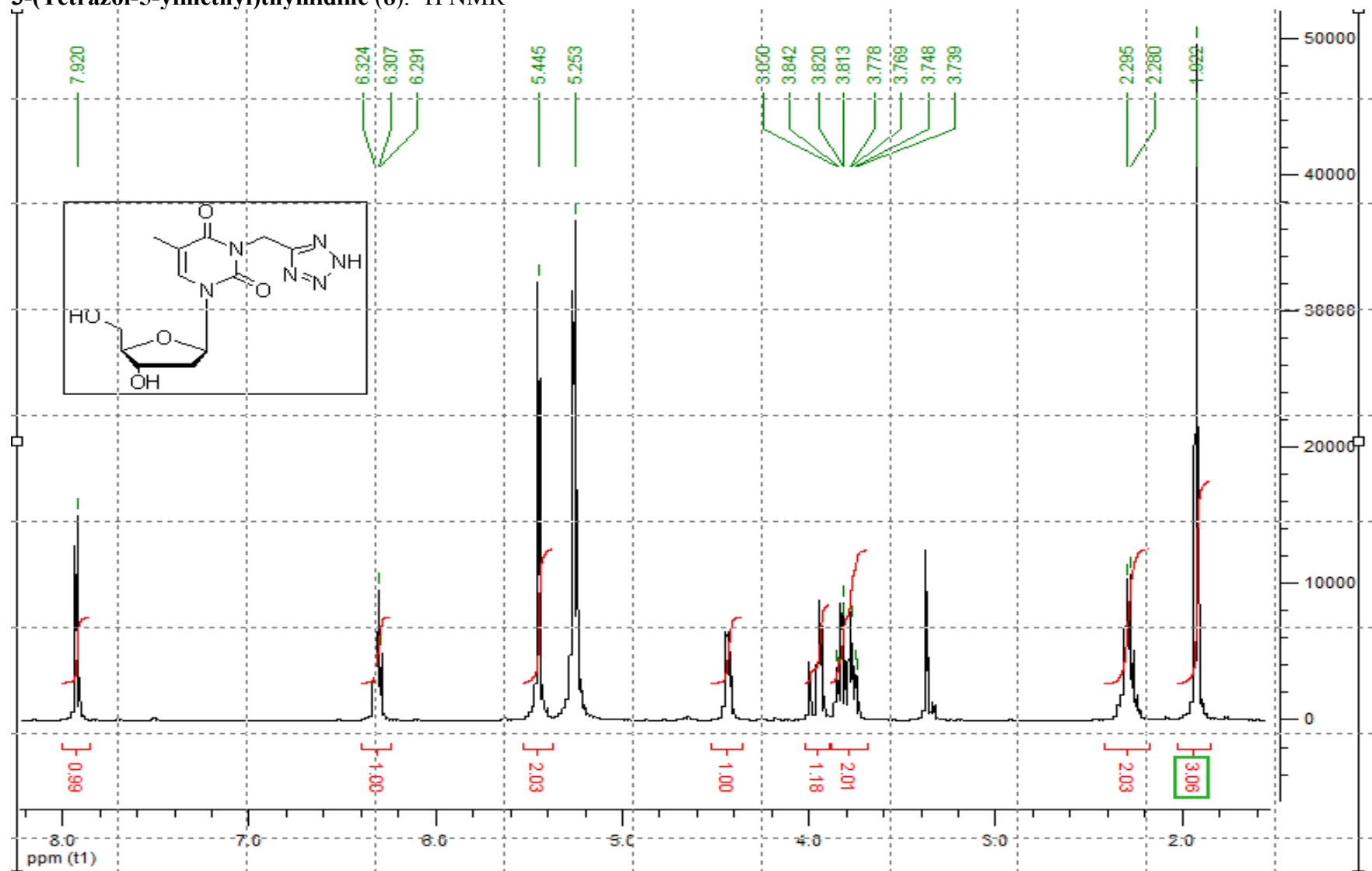
3-[4-(*closo*-1,7-Carboranyl)butylcarbamimidoyl]thymidine (7g). Analytical traces in Water:Acetonitrile gradient



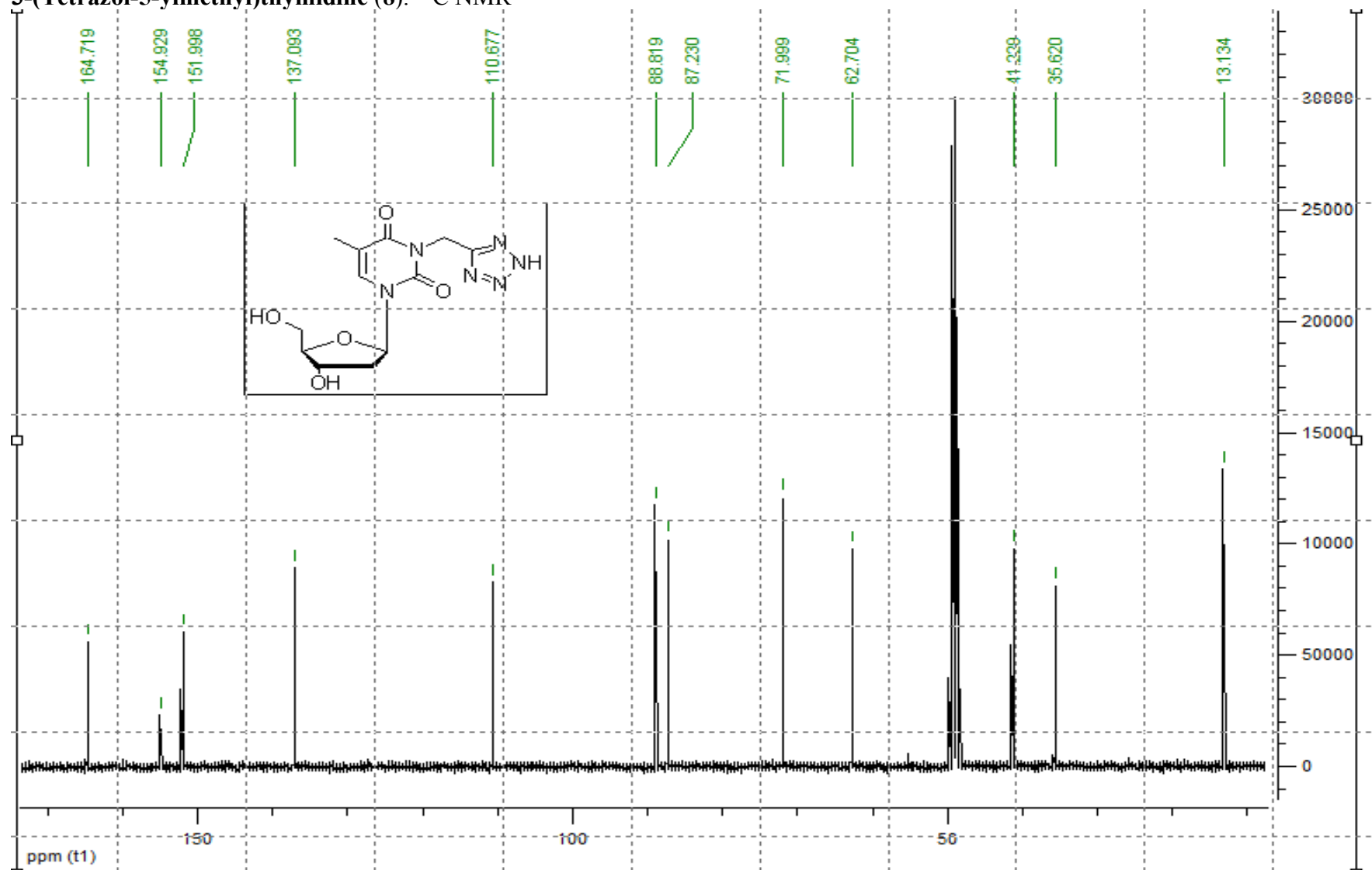
Analytical traces in Water:Methanol gradient



3-(Tetrazol-5-ylmethyl)thymidine (8). ¹H NMR



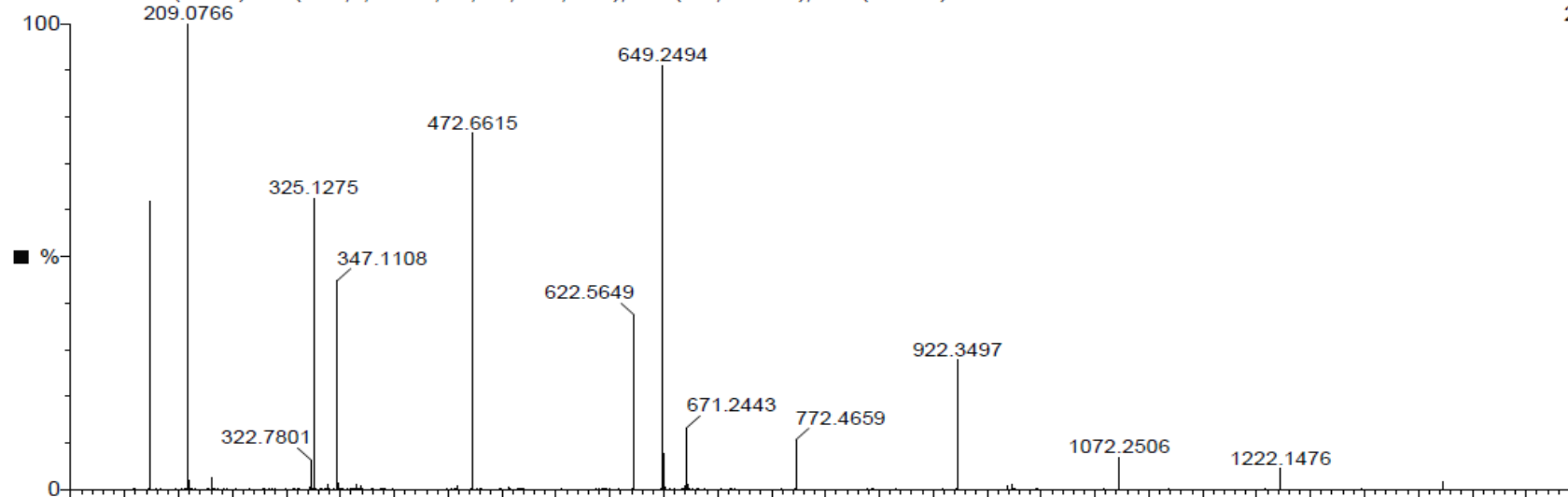
3-(Tetrazol-5-ylmethyl)thymidine (8). ^{13}C NMR



3-(Tetrazol-5-ylmethyl)thymidine (8). HR-ESI-MS found: 325.1275, 347.1108 and 649.2494.

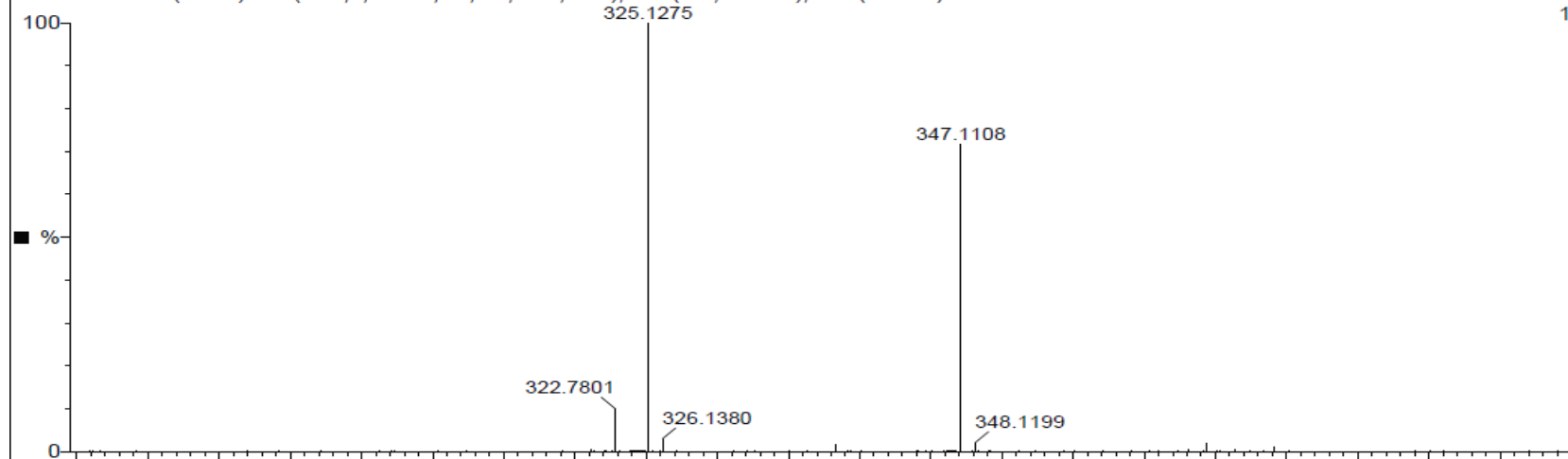
L070209D 178 (3.264) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (21:186)

TOF MS ES+
2.95ef

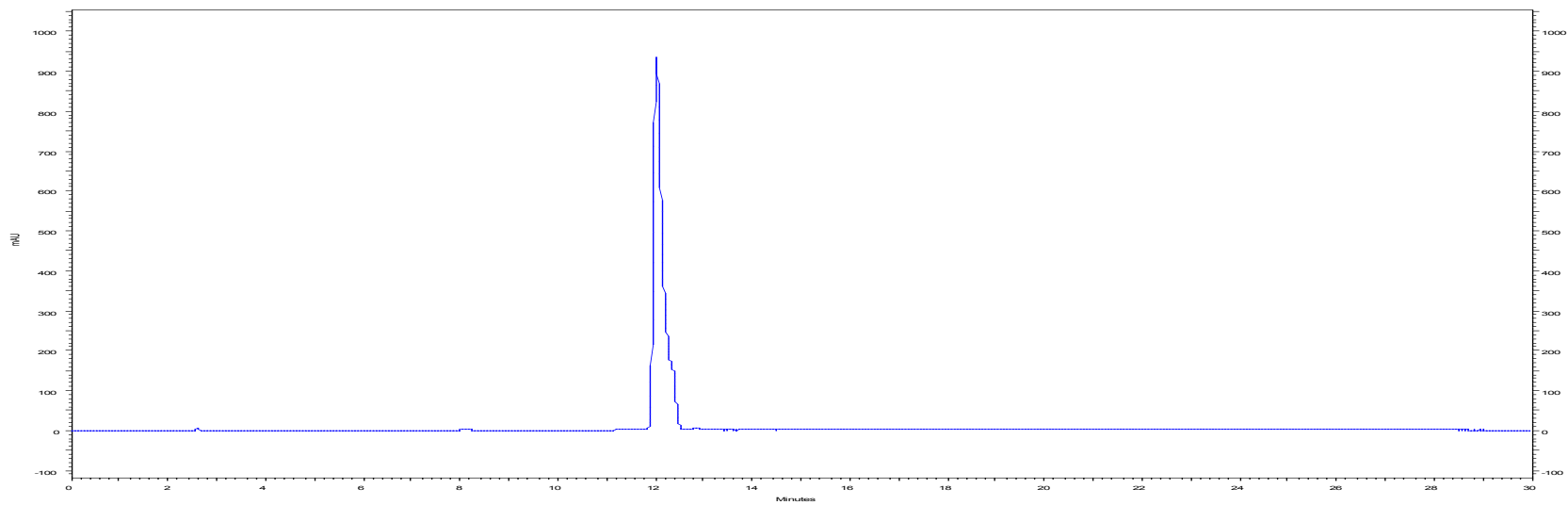


L070209D 178 (3.264) AM (Cen,4, 80.00, Ar,0.0,0.00,1.00); Sm (SG, 2x3.00); Cm (21:186)

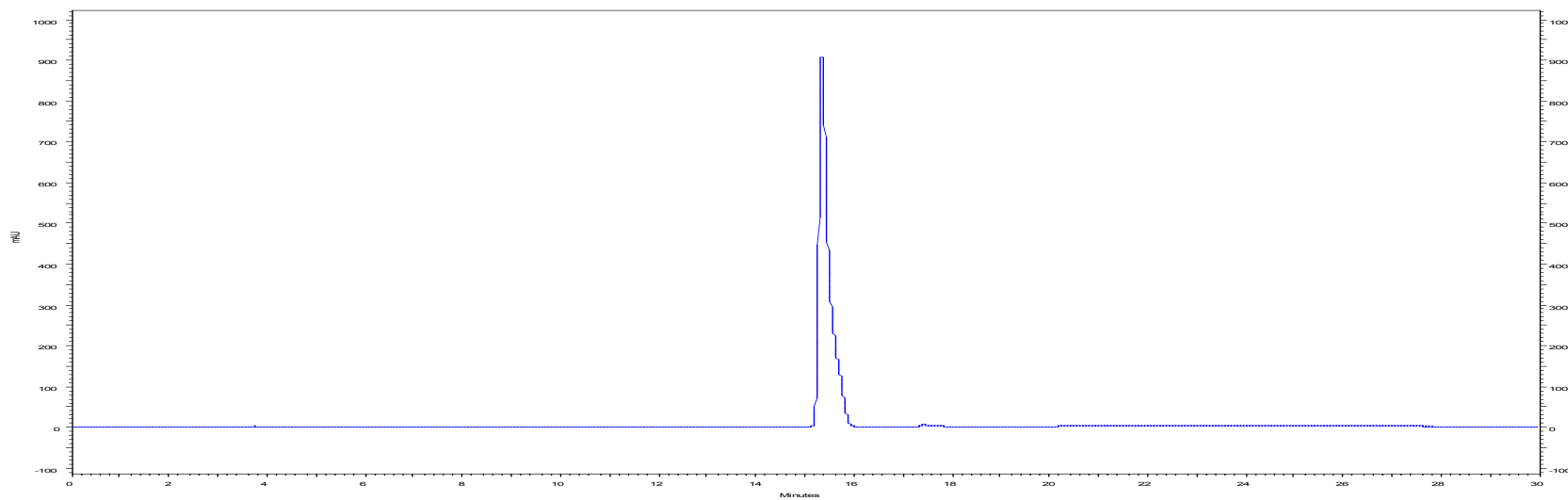
TOF MS ES+
1.84e5



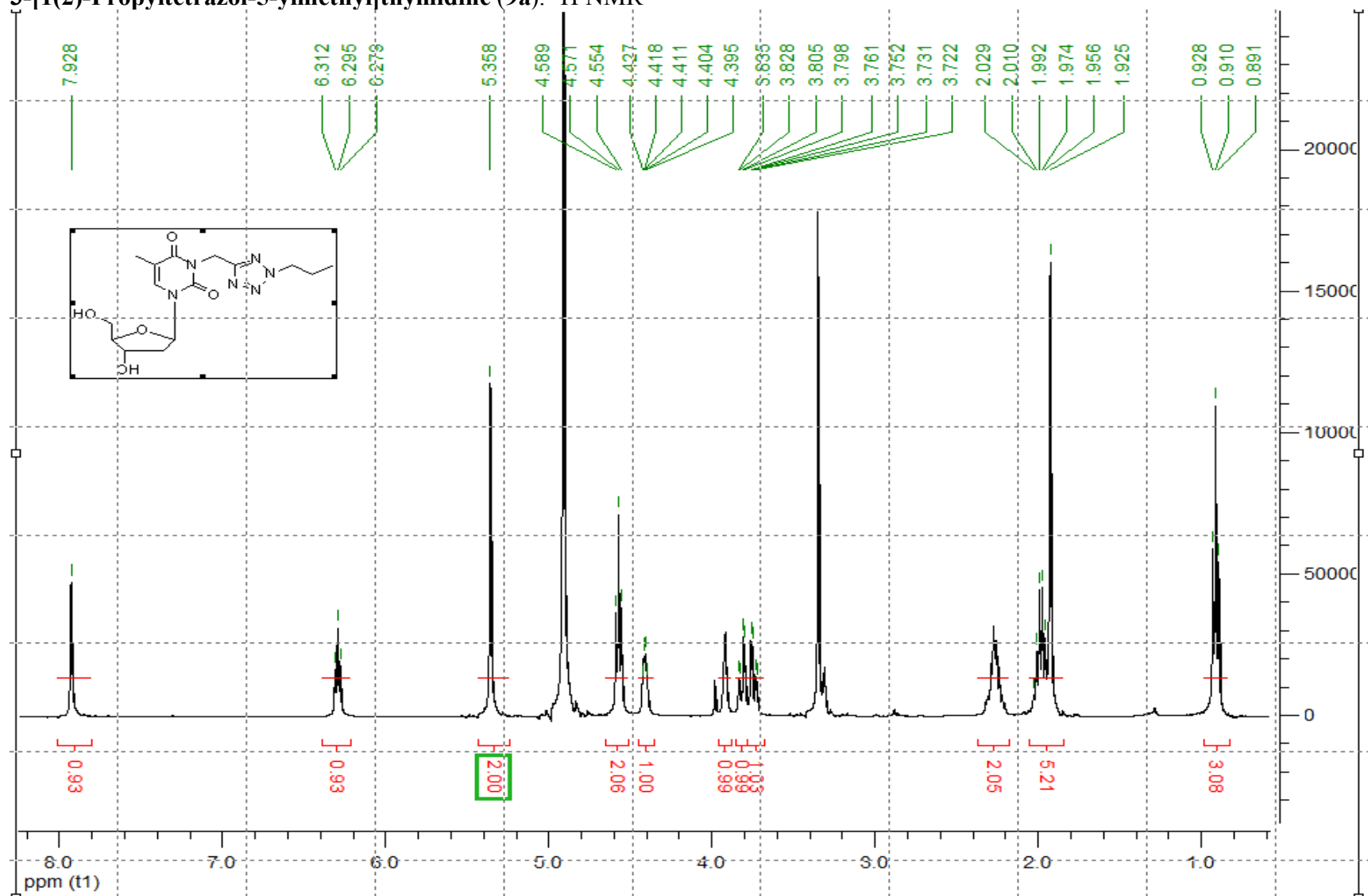
3-(Tetrazol-5-ylmethyl)thymidine (8). Analytical traces in Water:Acetonitrile gradient



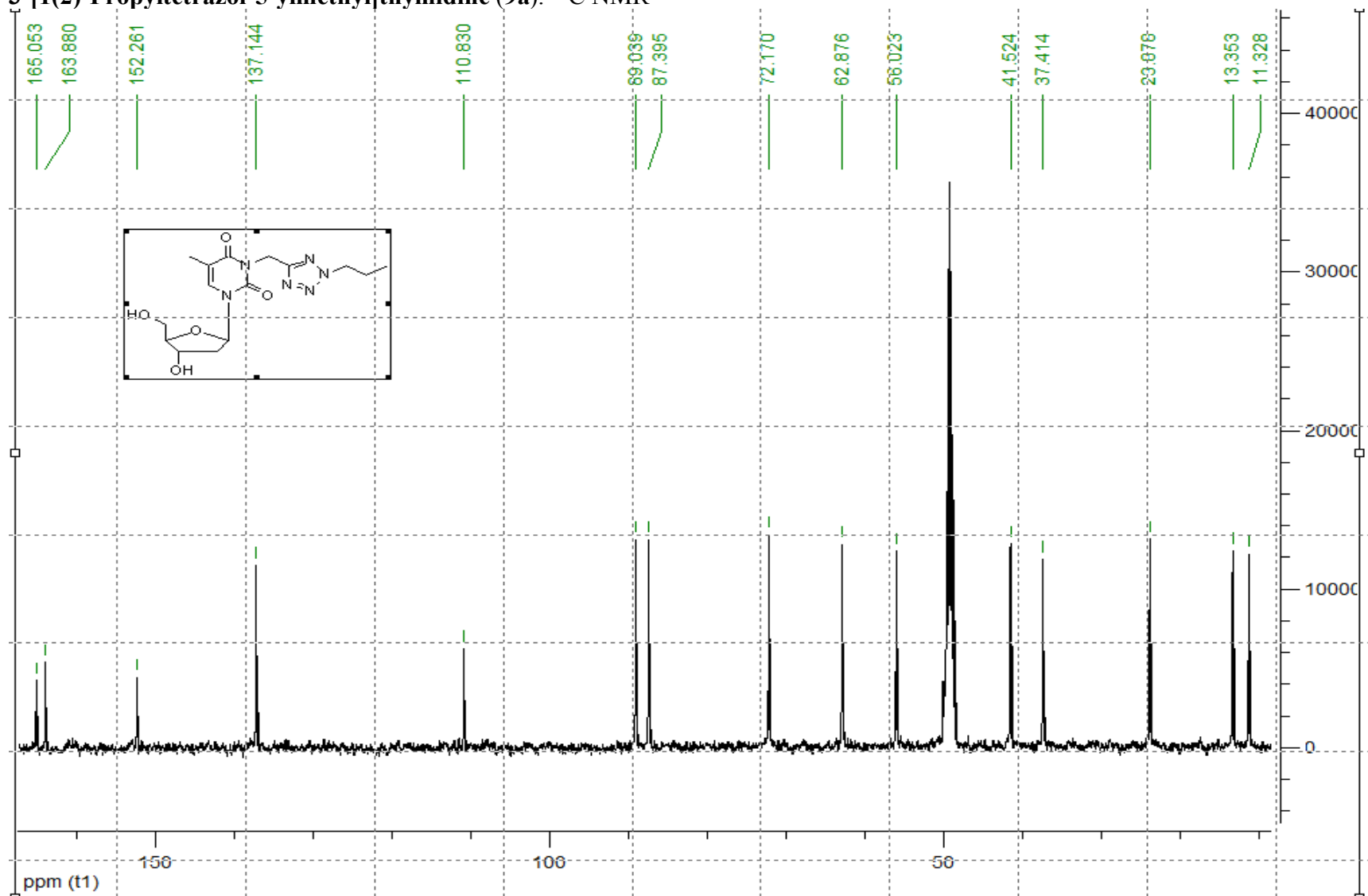
Analytical traces in Water: Methanol gradient



3-[1(2)-Propyltetrazol-5-ylmethyl]thymidine (9a). ¹H NMR



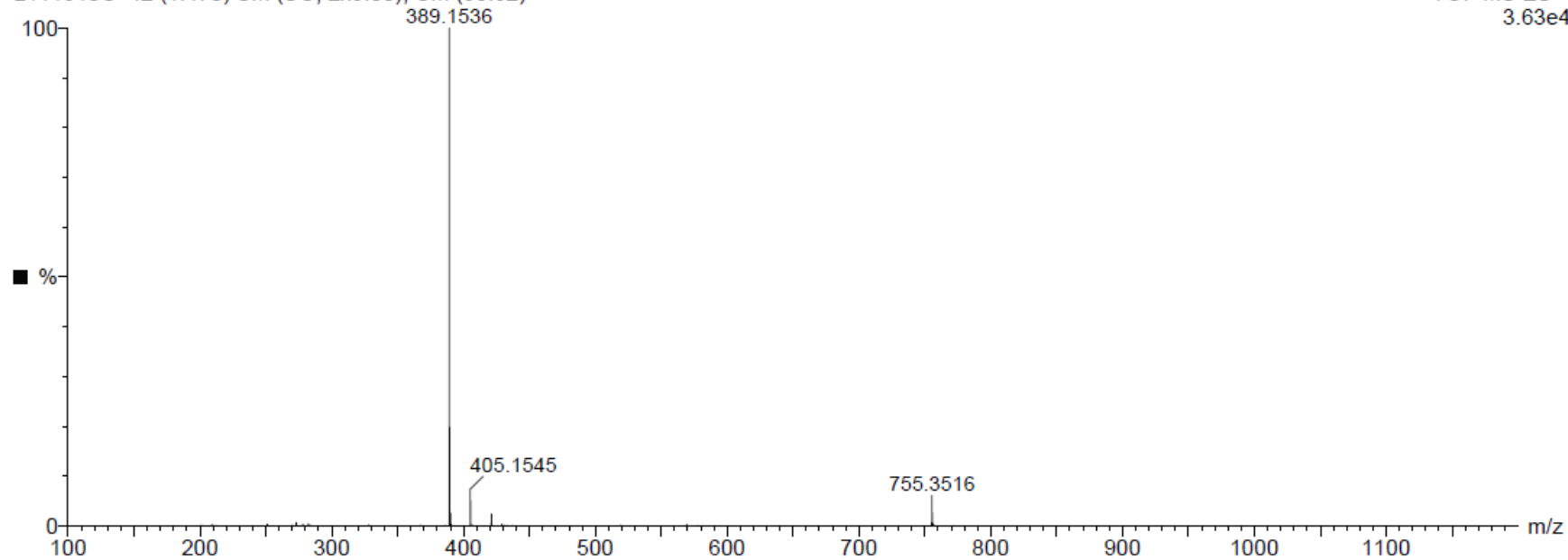
3-[1(2)-Propyltetrazol-5-ylmethyl]thymidine (9a). ¹³C NMR



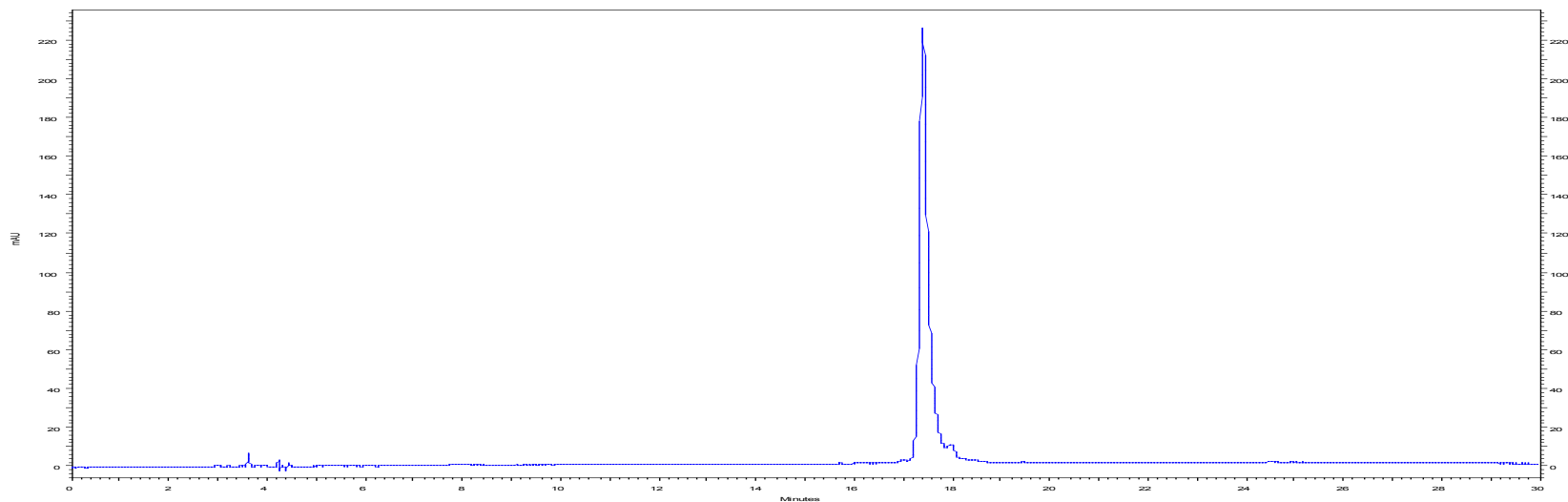
3-[1(2)-Propyltetrazol-5-ylmethyl]thymidine (9a). HR-ESI-MS found: 389.1536, 405.1545 and 755.3516)

L111910C 42 (1.470) Sm (SG, 2x3.00); Cm (35:62)

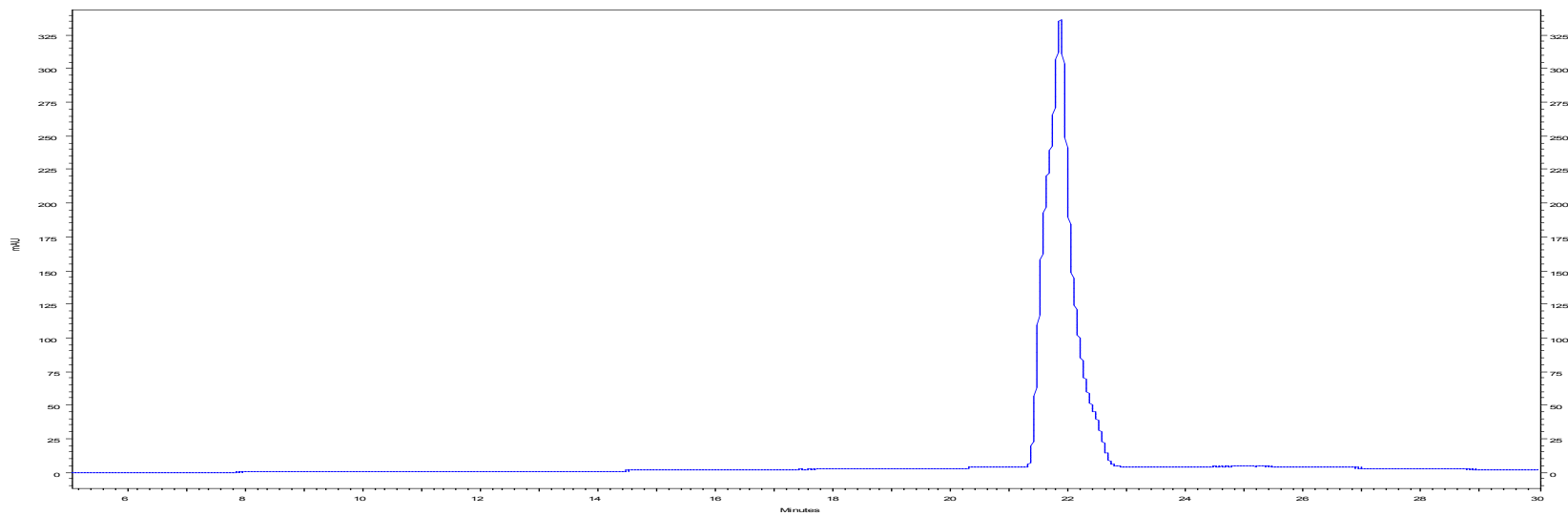
TOF MS ES+
3.63e4



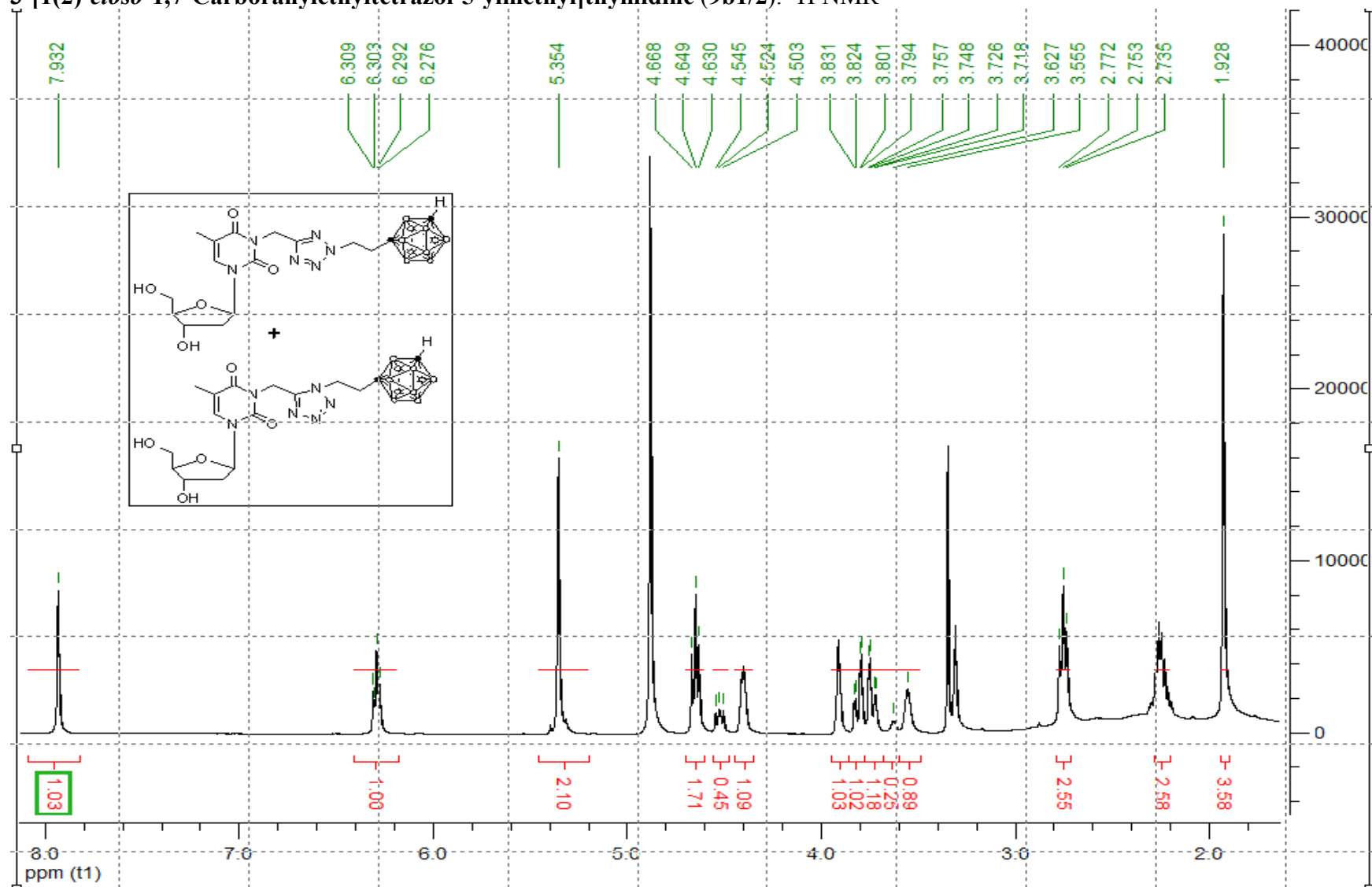
3-[1(2)-Propyltetrazol-5-ylmethyl]thymidine (9a). Analytical traces in Water:Acetonitrile gradient



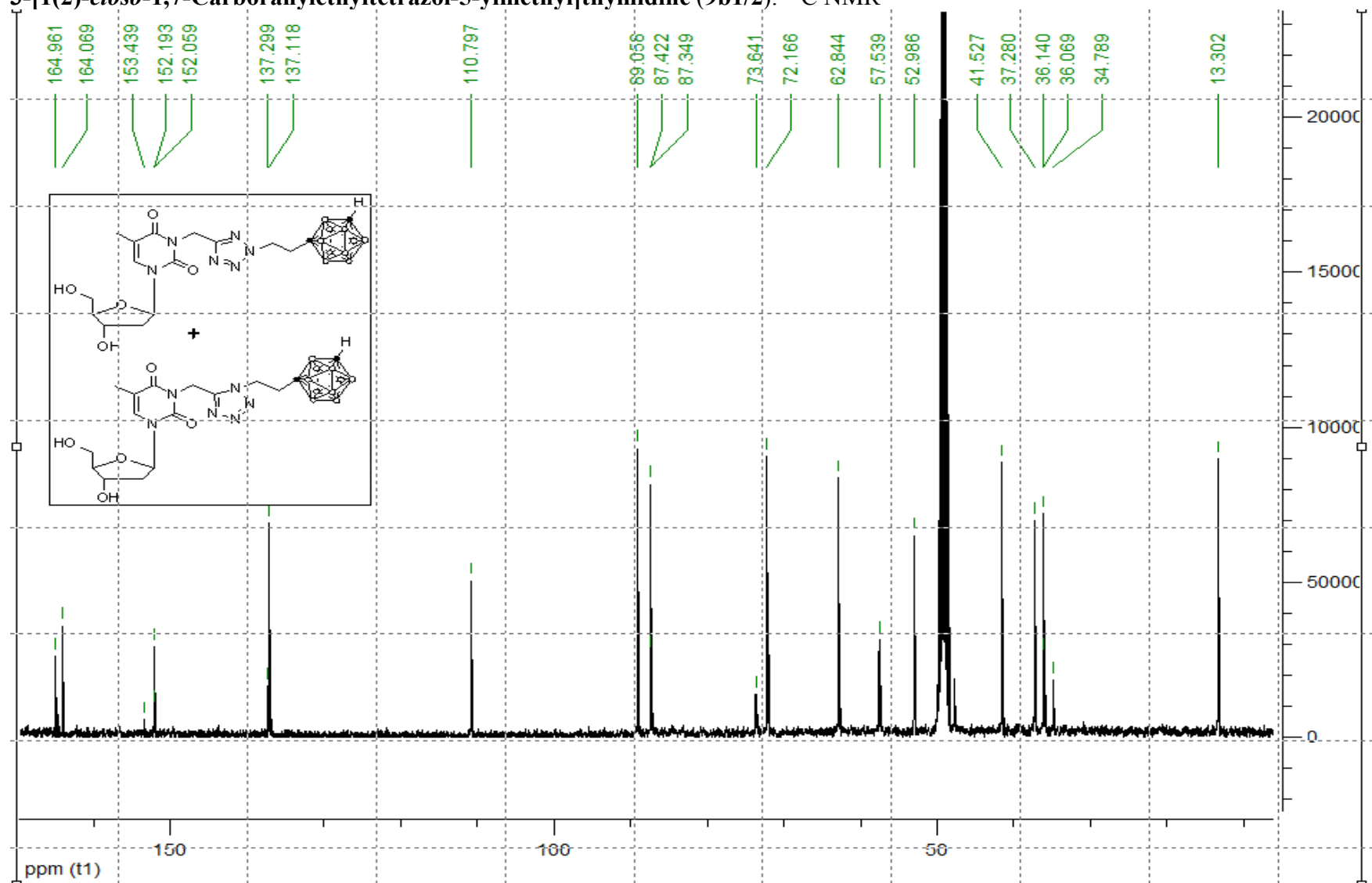
Analytical traces in Water: Methanol gradient



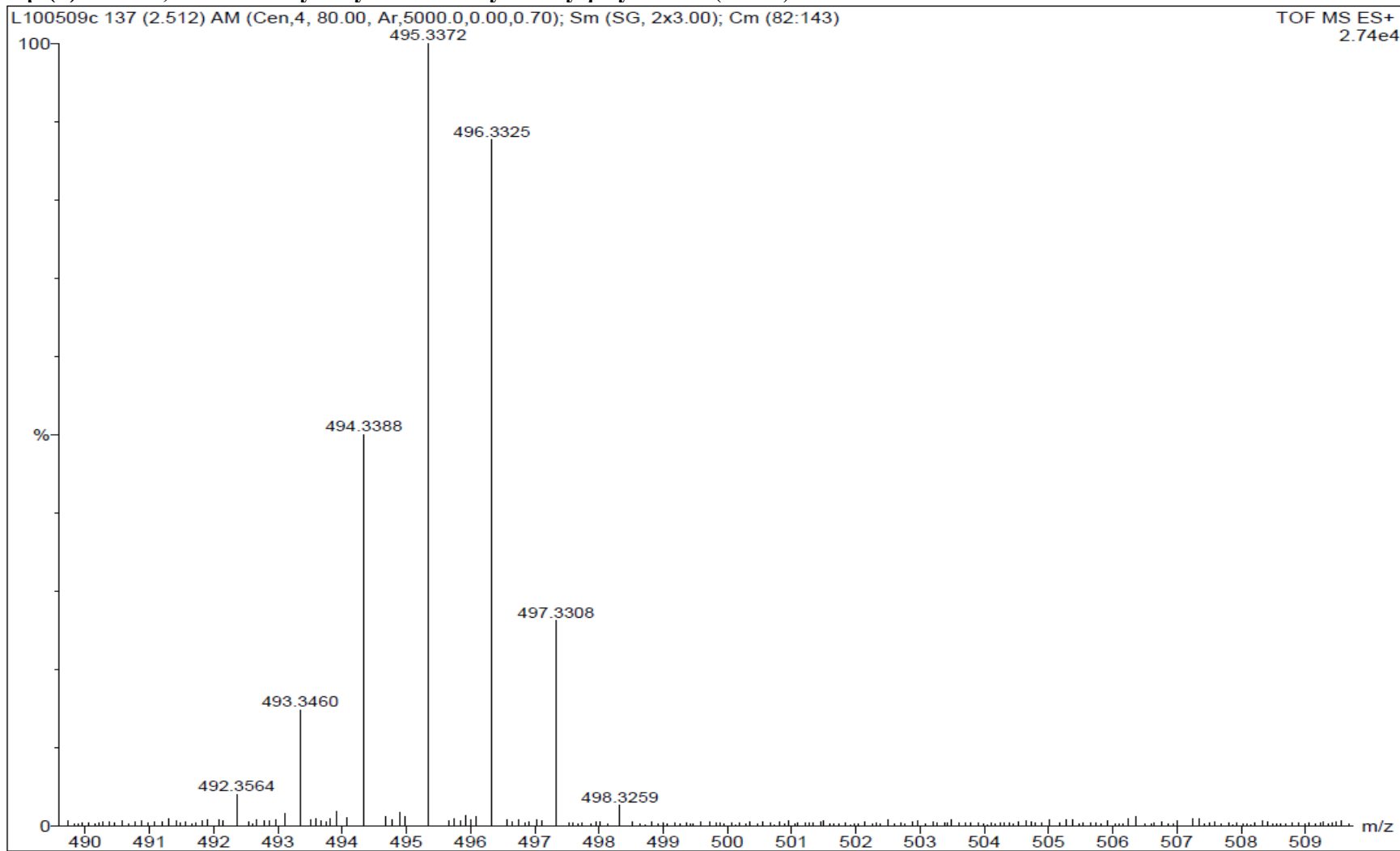
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-ylmethyl]thymidine (9b1/2). ¹H NMR



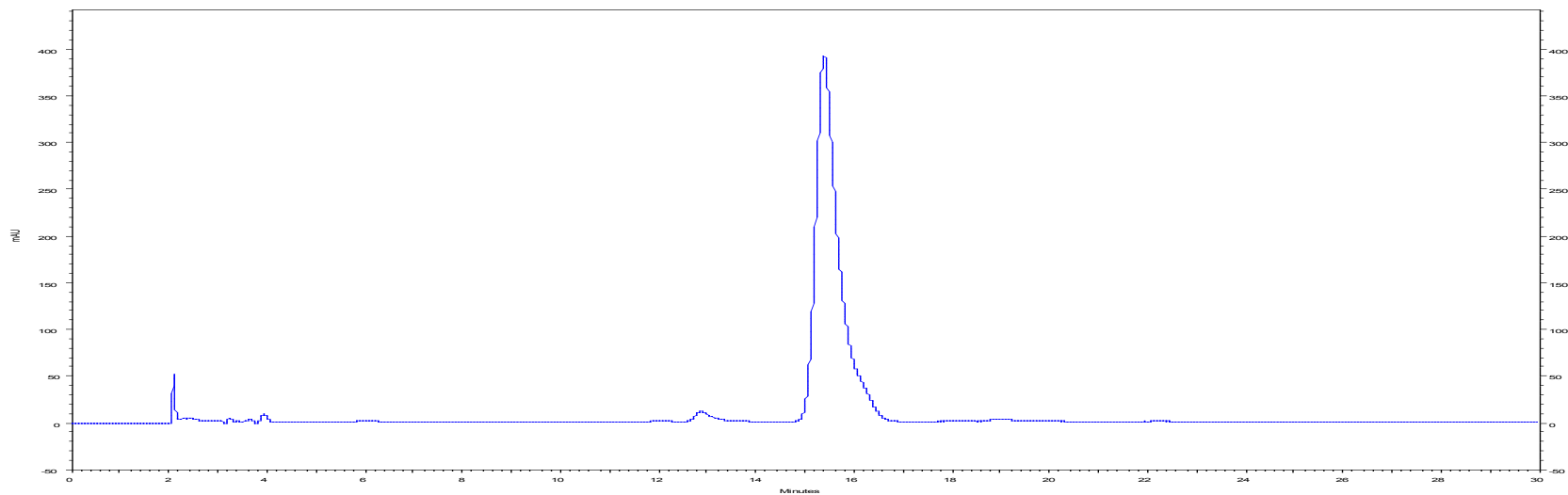
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-ylmethyl]thymidine (9b1/2). ^{13}C NMR



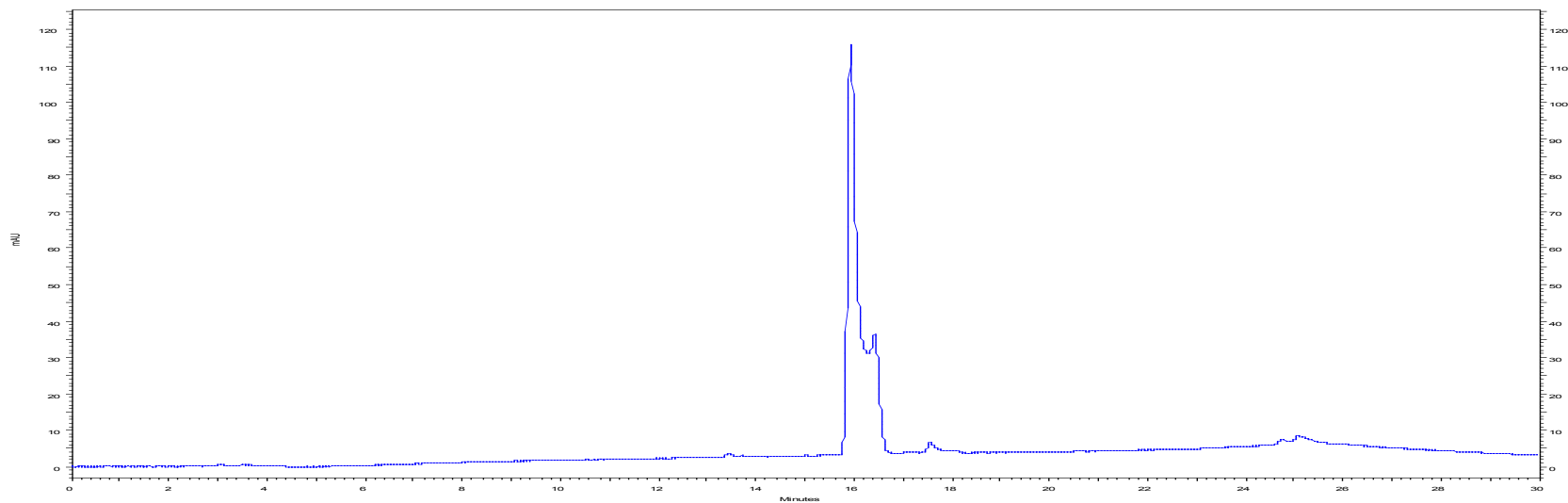
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-ylmethyl]thymidine (9b1/2). HR-ESI-MS found: 495.3372.



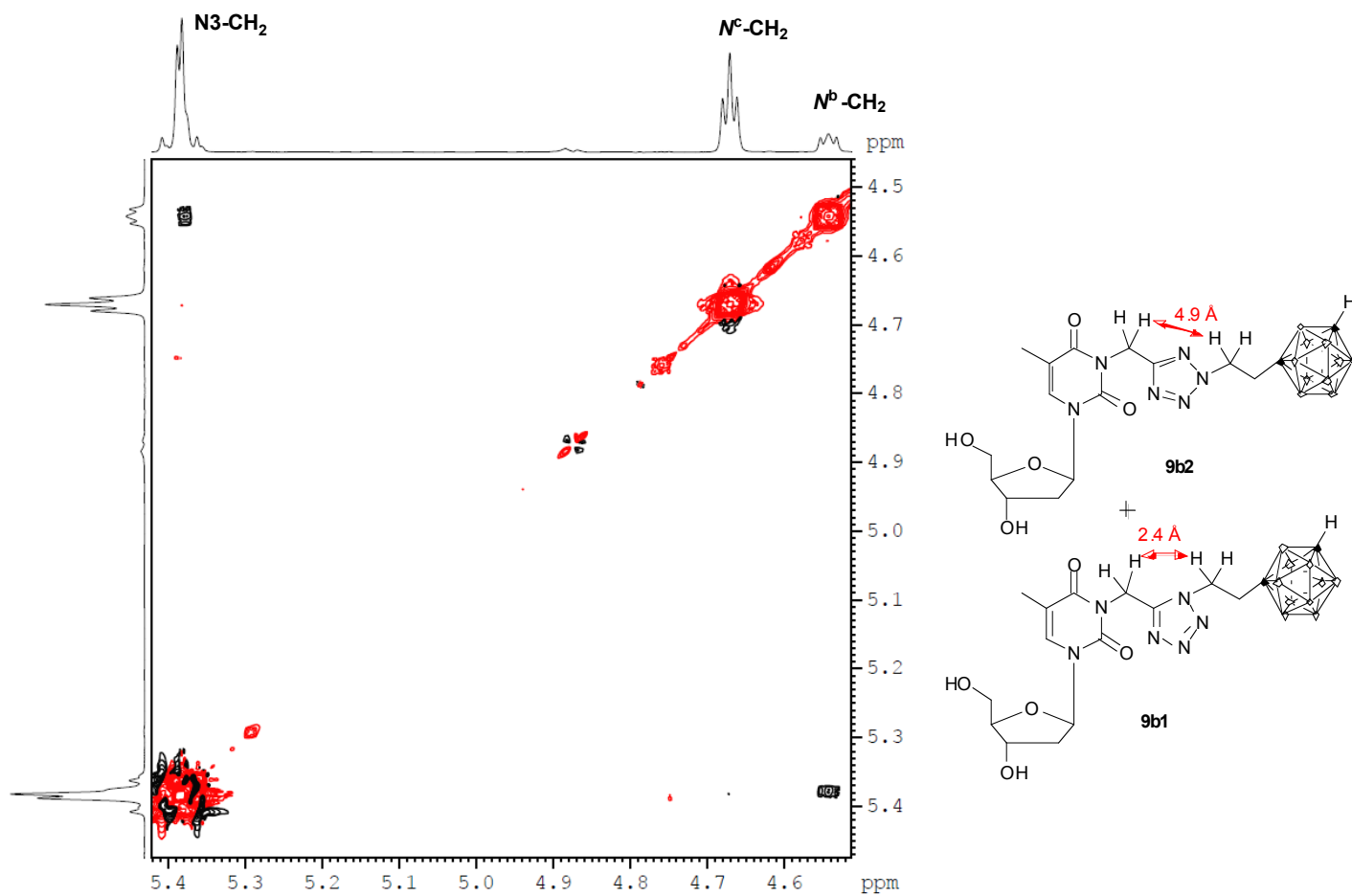
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-ylmethyl]thymidine (9b1/2). Analytical traces in Water:Acetonitrile gradient



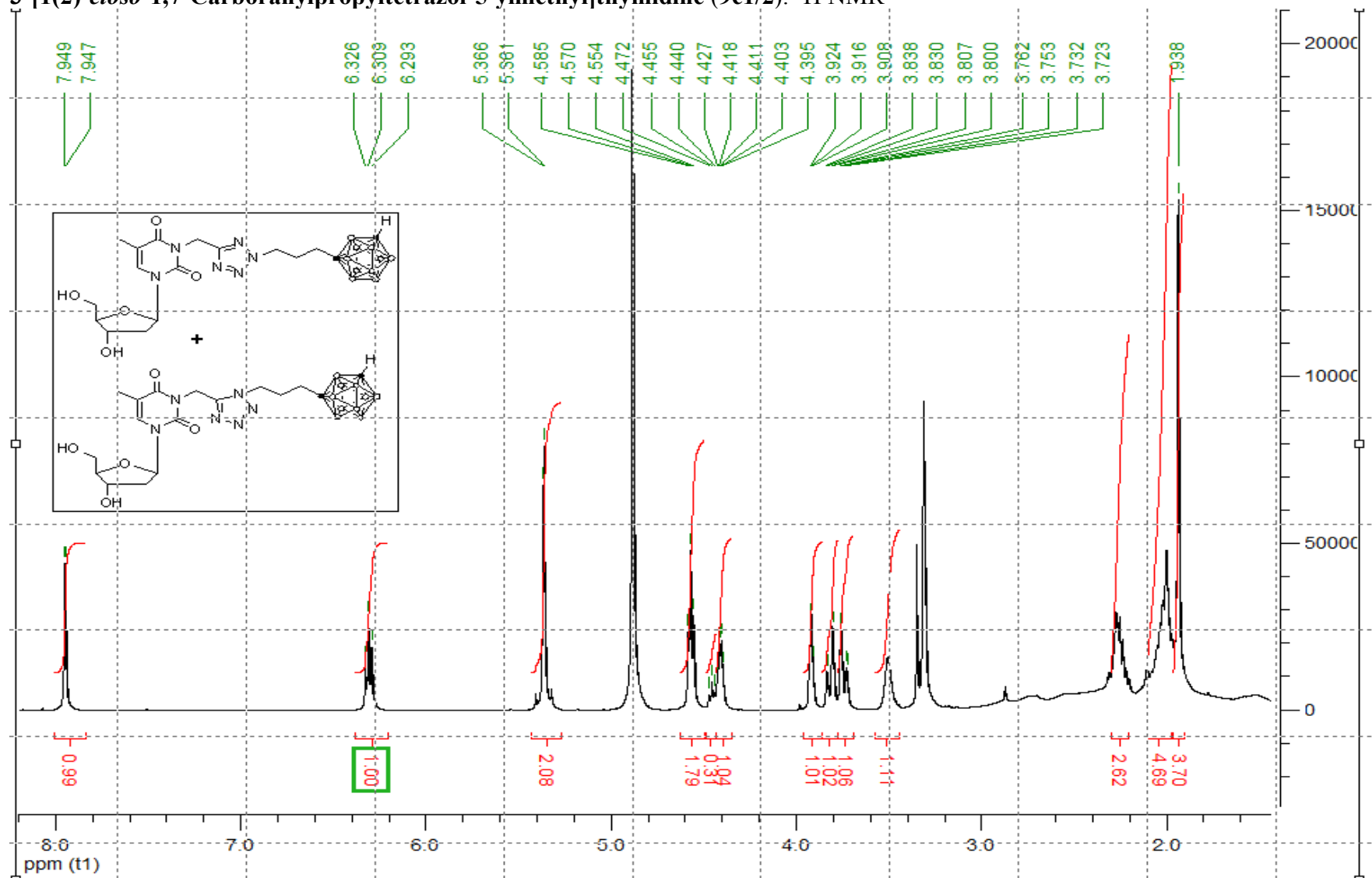
Analytical traces in Water:Methanol gradient



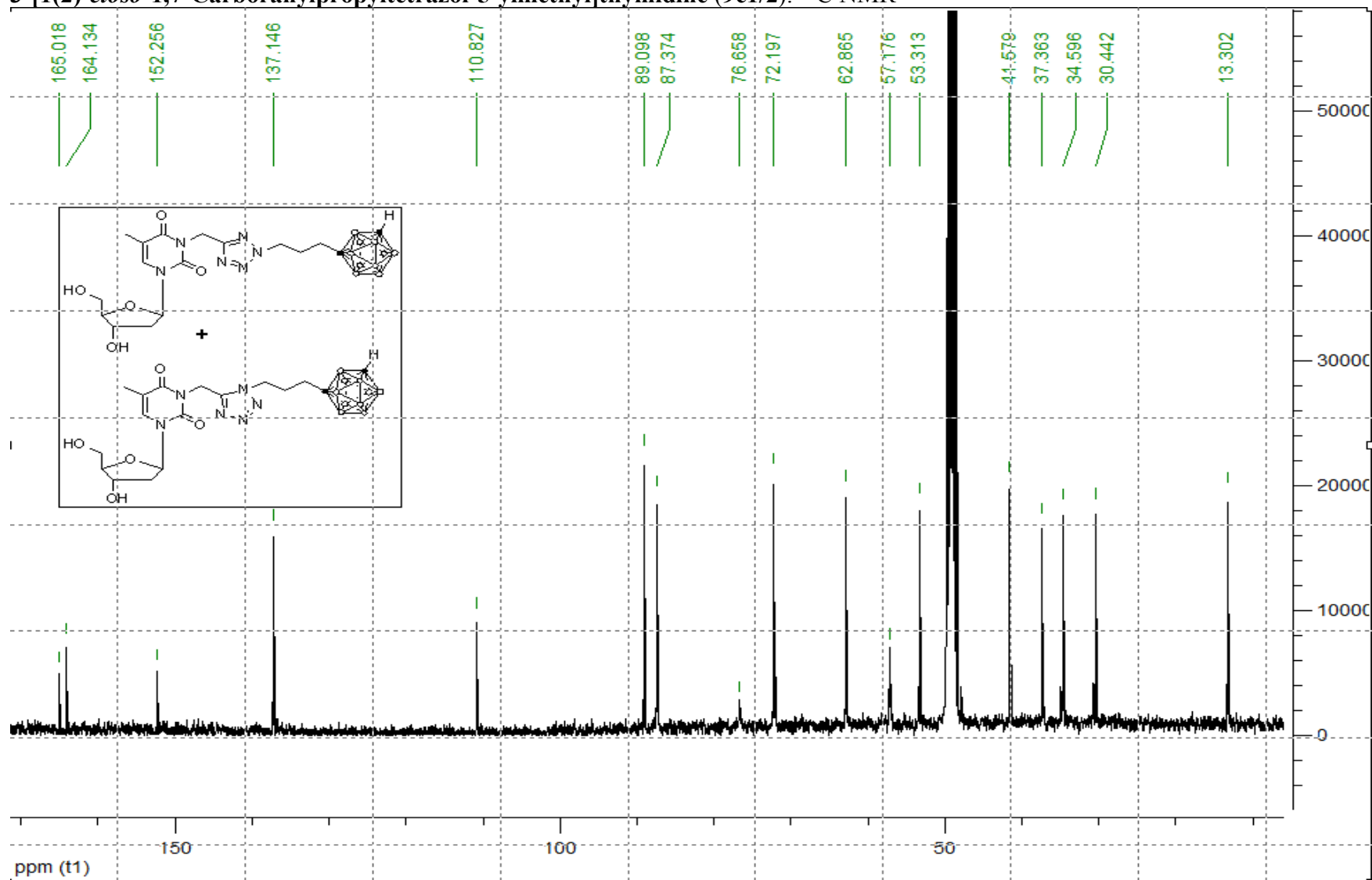
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-ylmethyl]thymidine (9b1/2). ^1H - ^1H NOSEY NMR



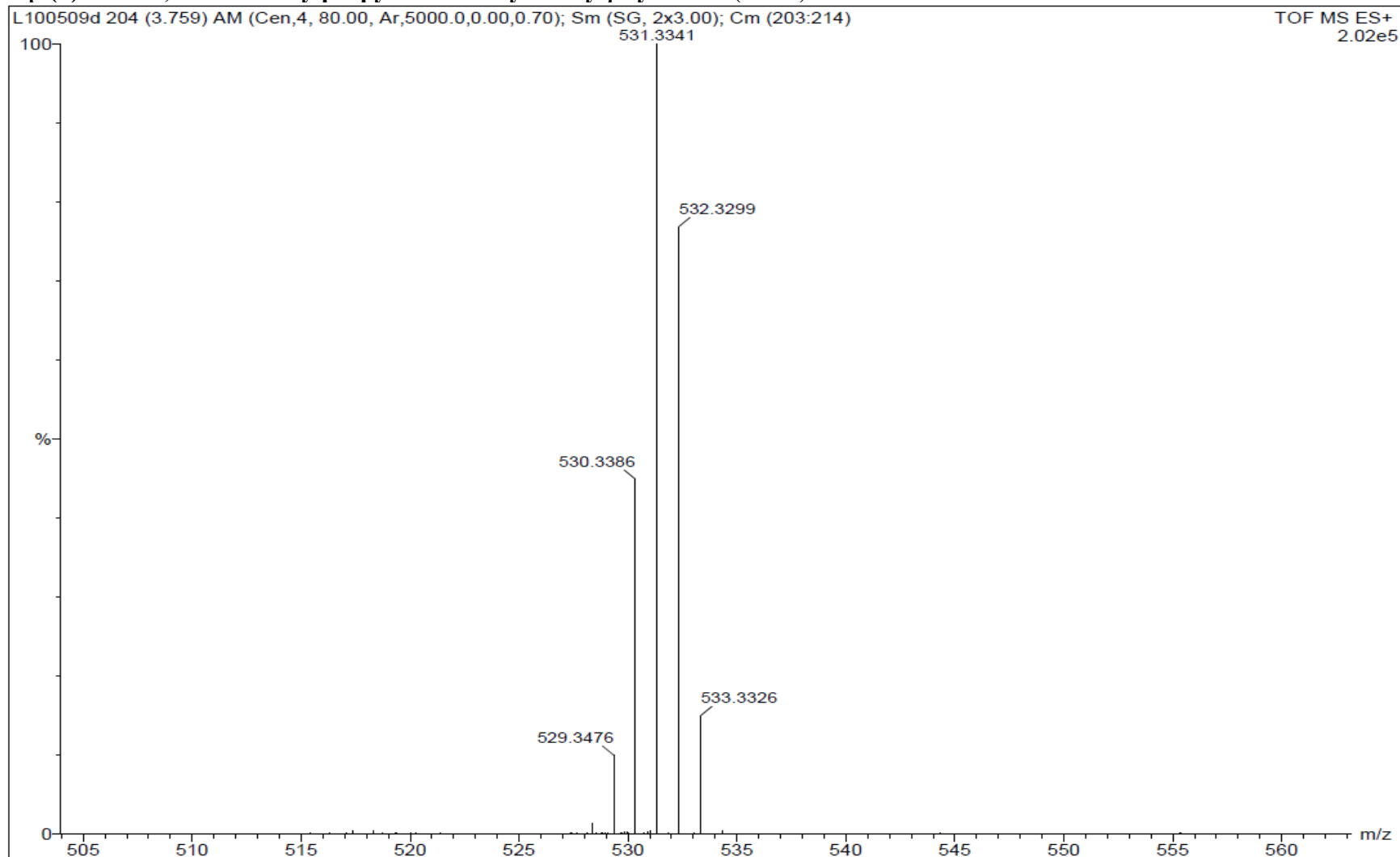
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-ylmethyl]thymidine (9c1/2). ¹H NMR



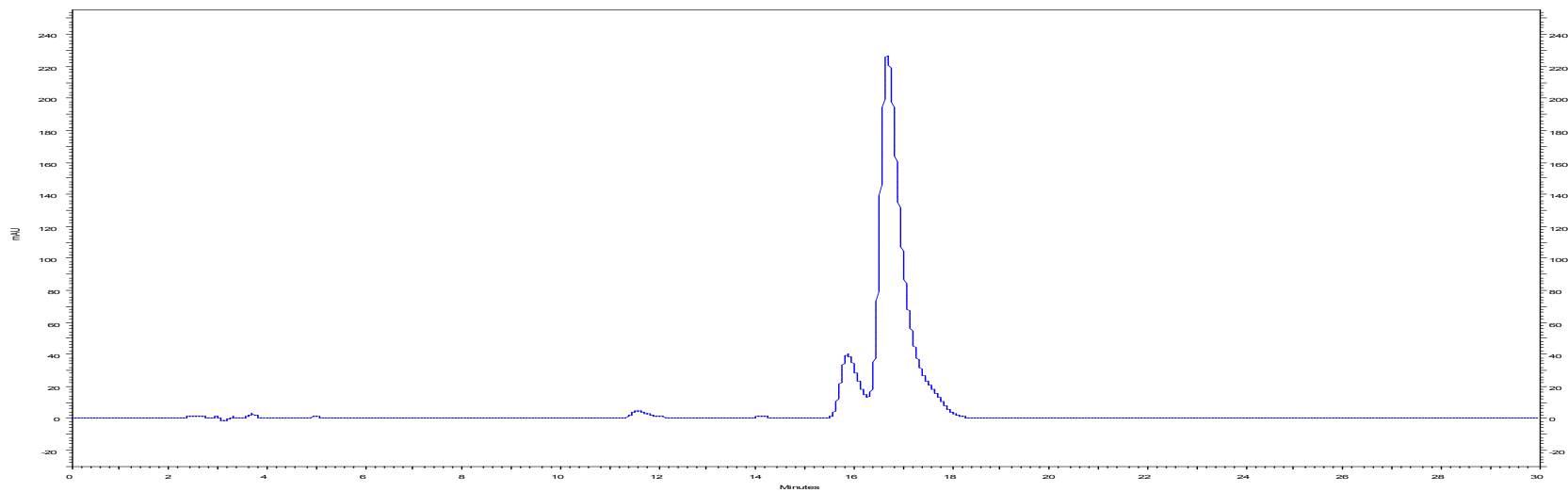
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-ylmethyl]thymidine (9c1/2). ^{13}C NMR



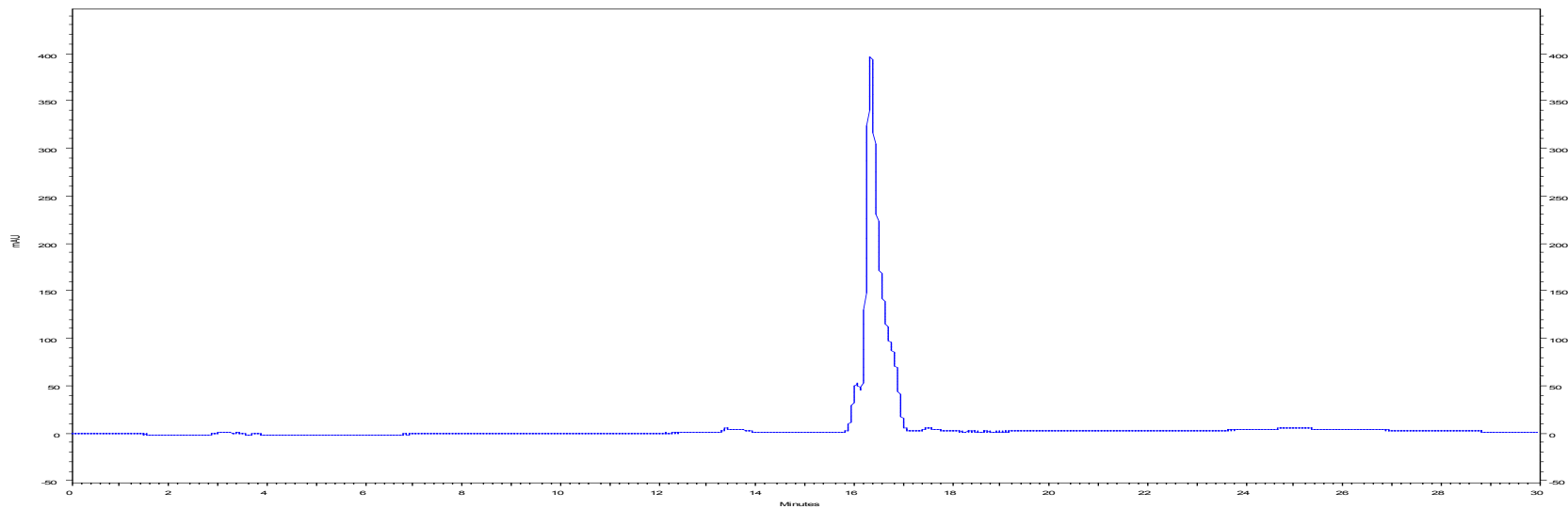
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-ylmethyl]thymidine (9c1/2). HR-ESI-MS found: 531.3341.



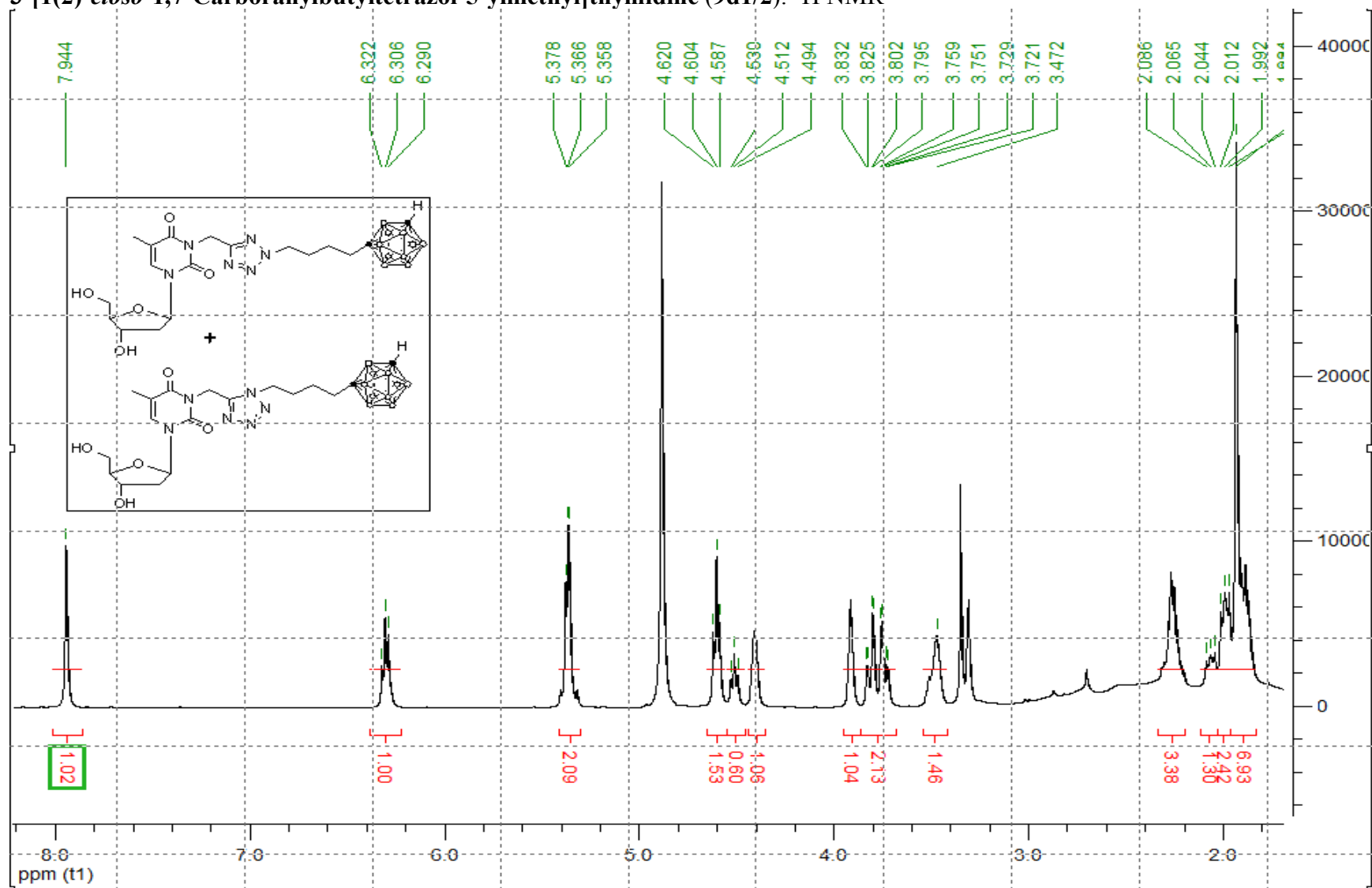
3-[1(2)-*ciso*-1,7-Carboranylpropyltetrazol-5-ylmethyl]thymidine (9c1/2). Analytical traces in Water:Acetonitrile gradient



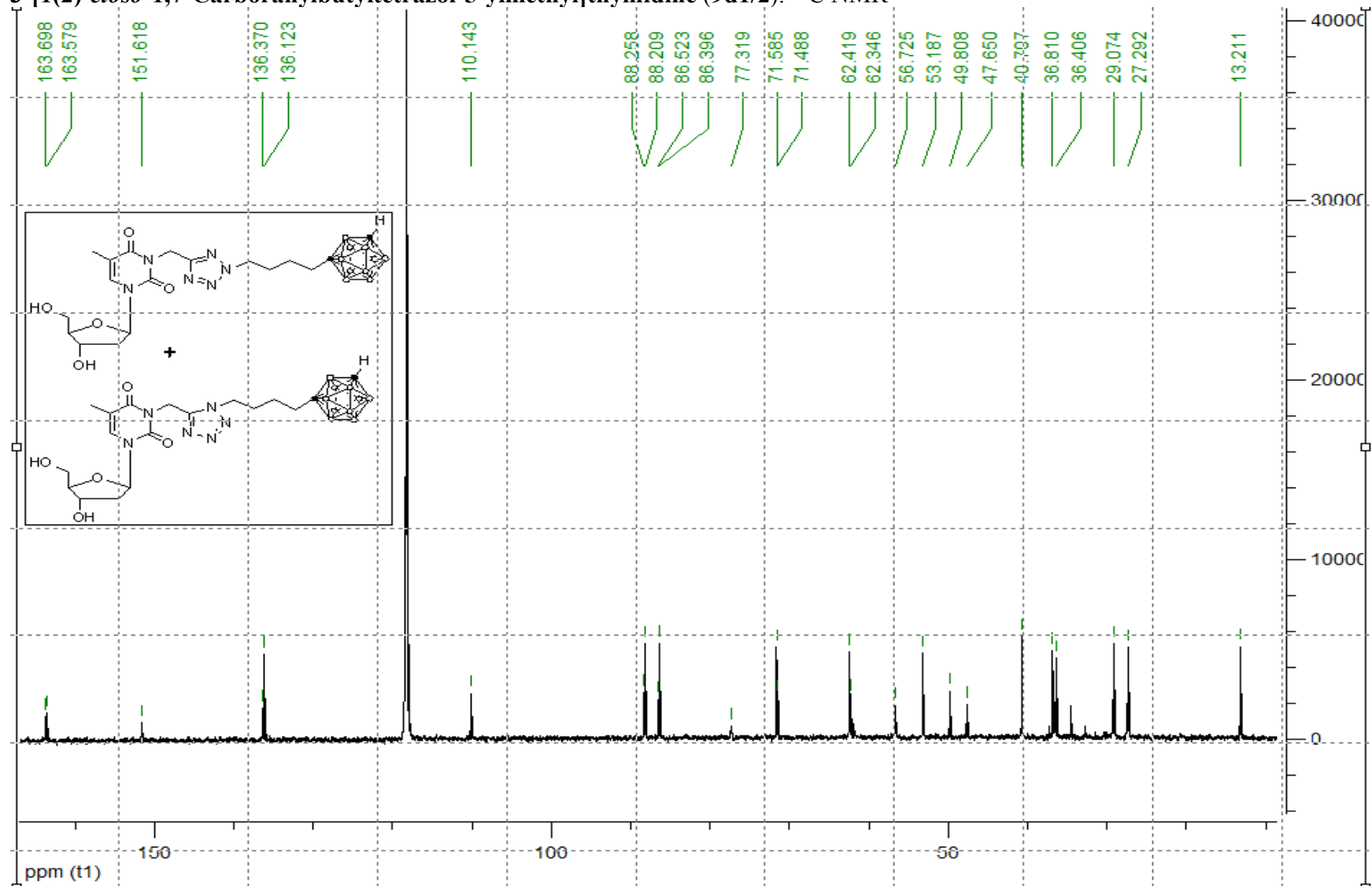
Analytical traces in Water:Methanol gradient



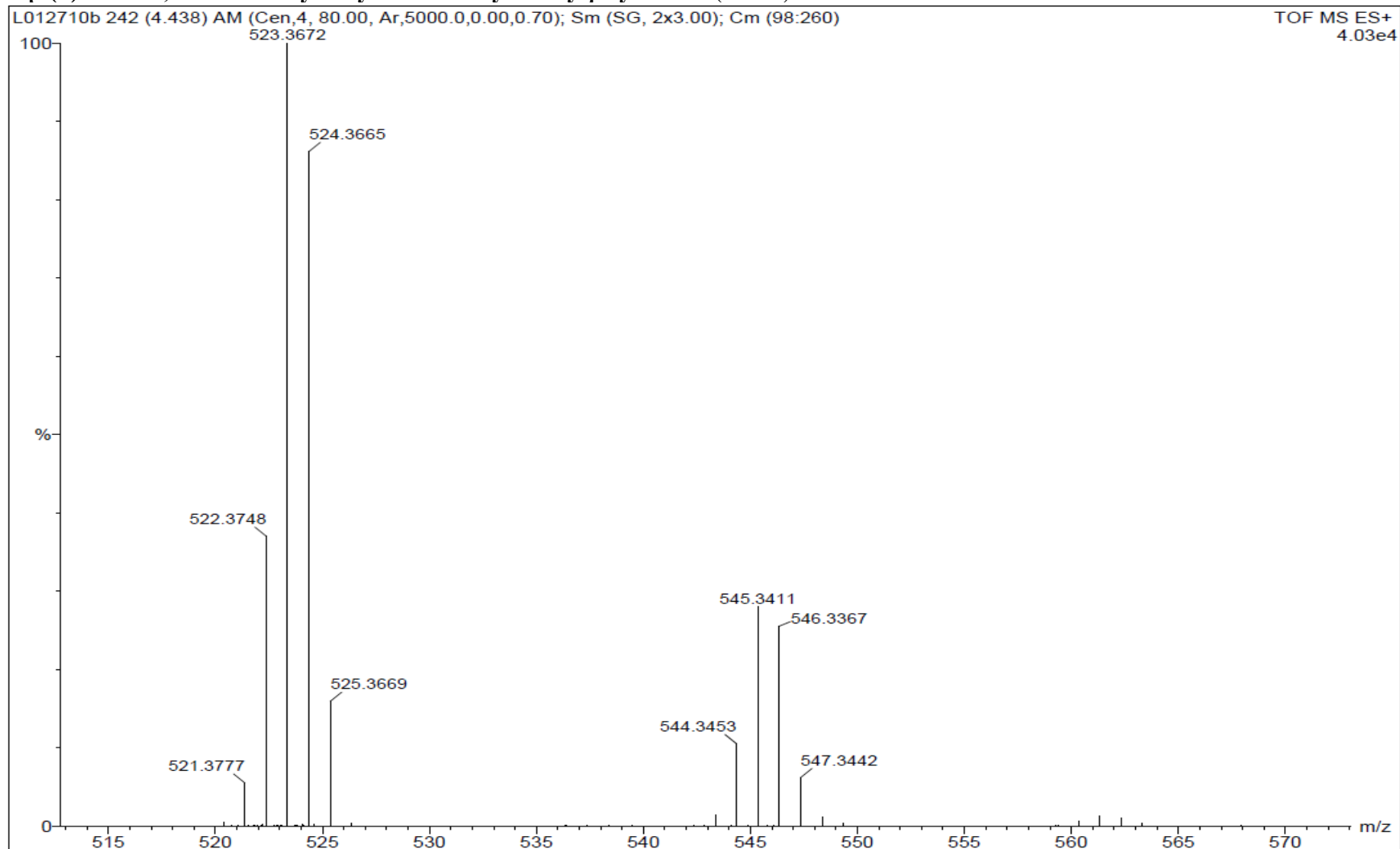
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-ylmethyl]thymidine (9d1/2). ¹H NMR



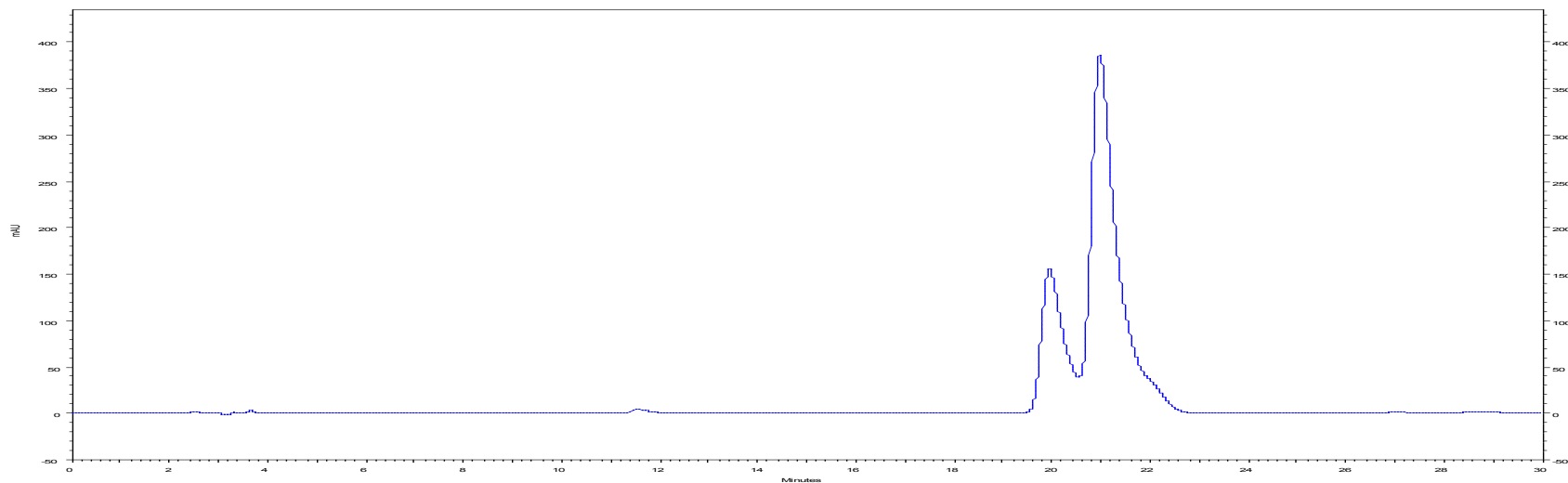
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-ylmethyl]thymidine (9d1/2). ¹³C NMR



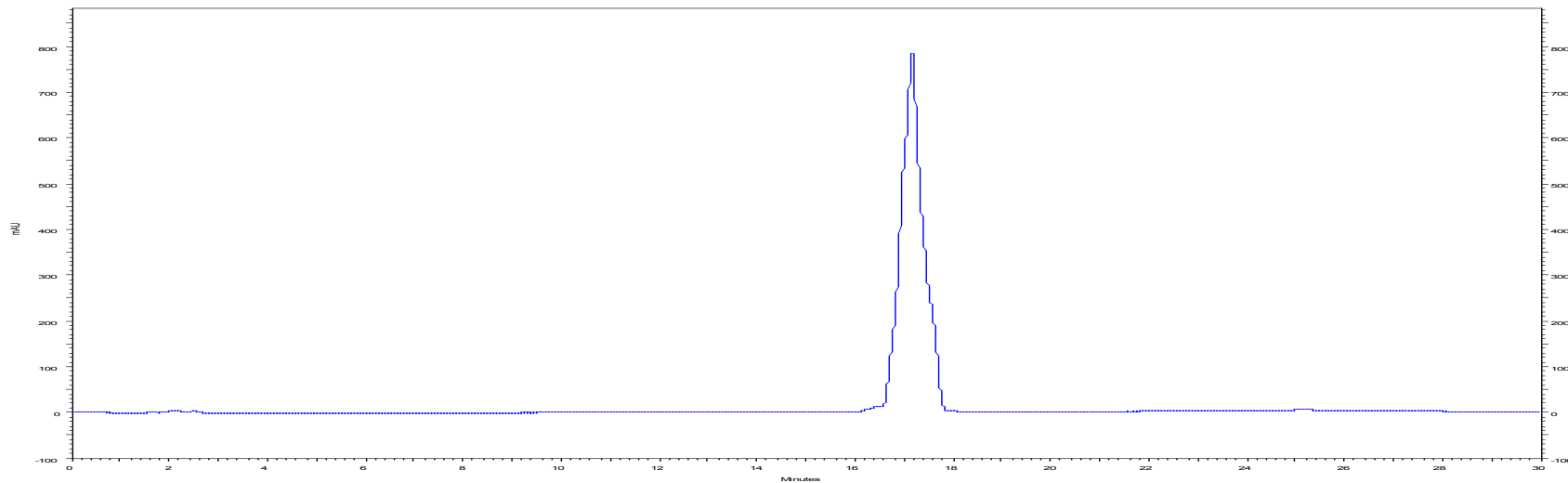
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-ylmethyl]thymidine (9d1/2). HR-ESI-MS found: 523.3672 and 545.3411.



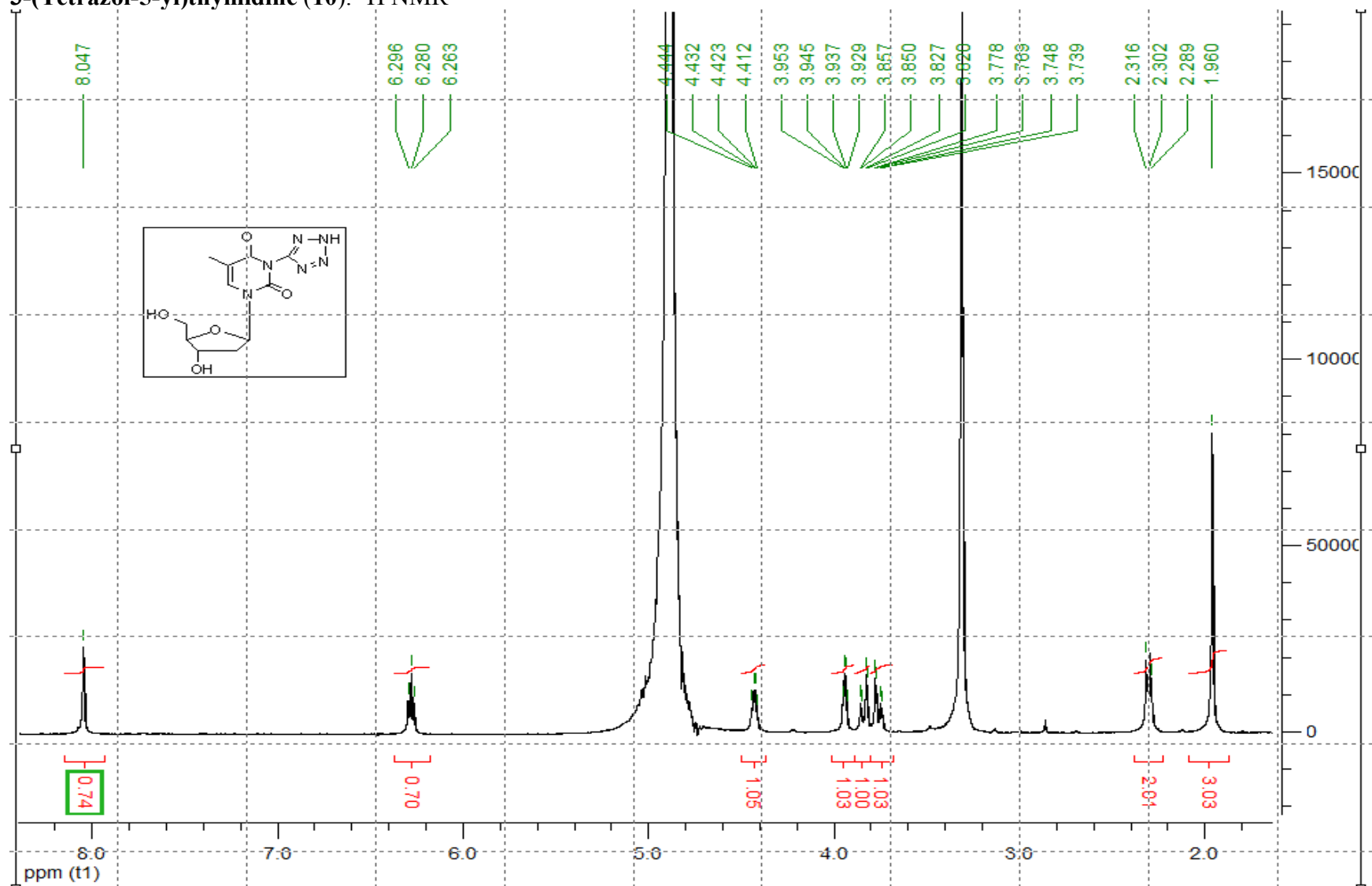
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-ylmethyl]thymidine (9d1/2). Analytical traces in Water:Acetonitrile gradient



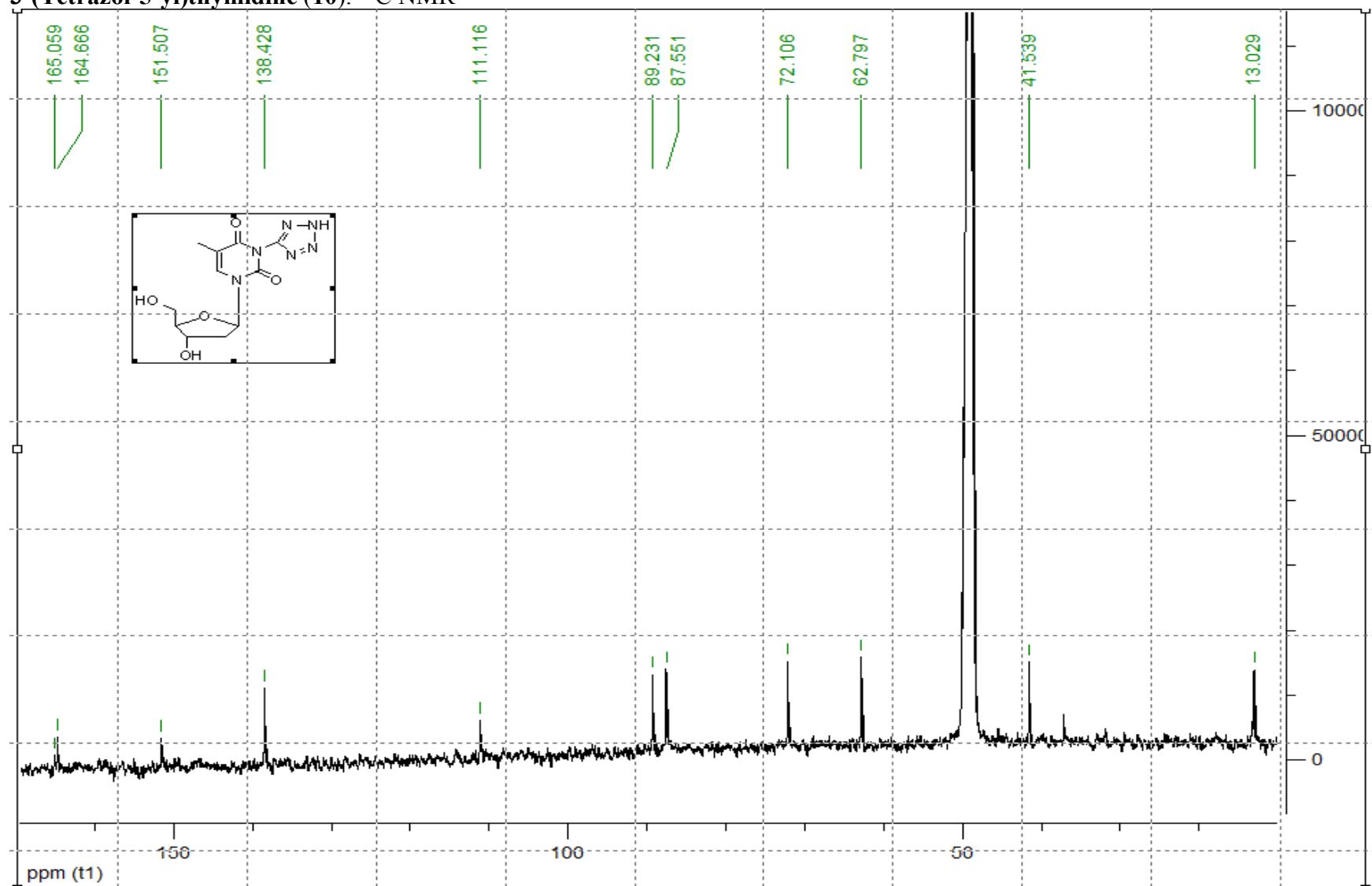
Analytical traces in Water:Methanol gradient



3-(Tetrazol-5-yl)thymidine (10). ¹H NMR



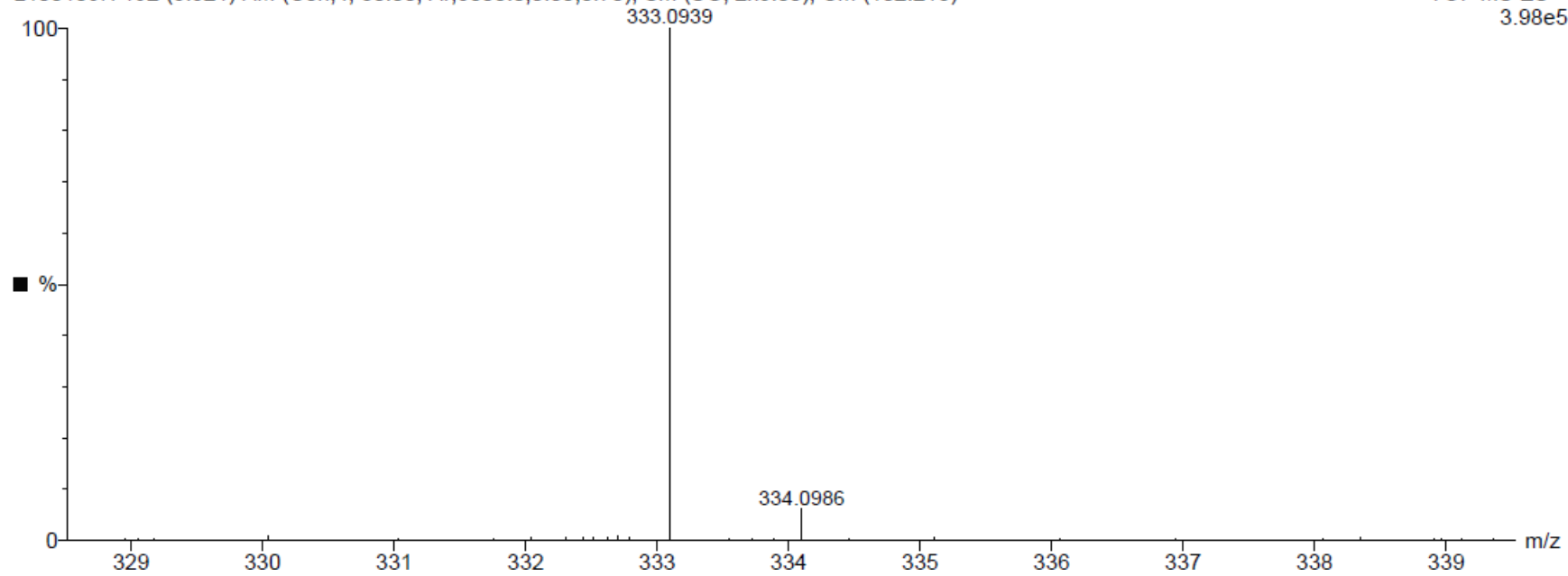
3-(Tetrazol-5-yl)thymidine (10). ¹³C NMR



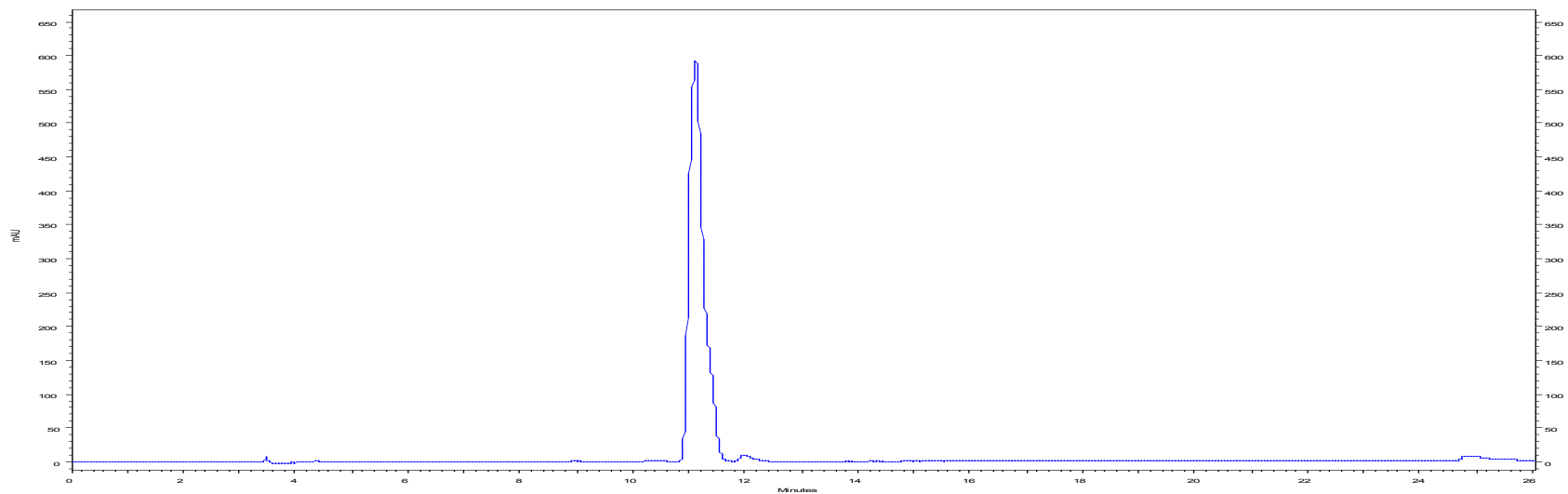
3-(Tetrazol-5-yl)thymidine (10). HR-ESI-MS found: 333.0939.

L100109H 192 (3.521) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (102:218)

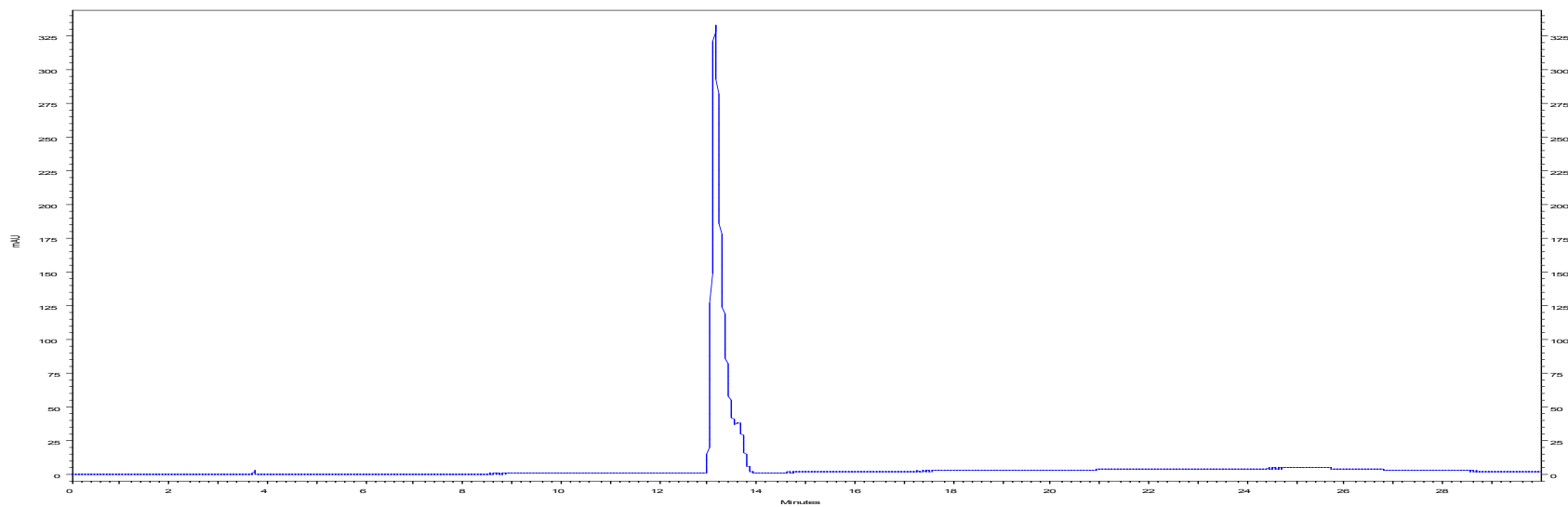
TOF MS ES+
3.98e5



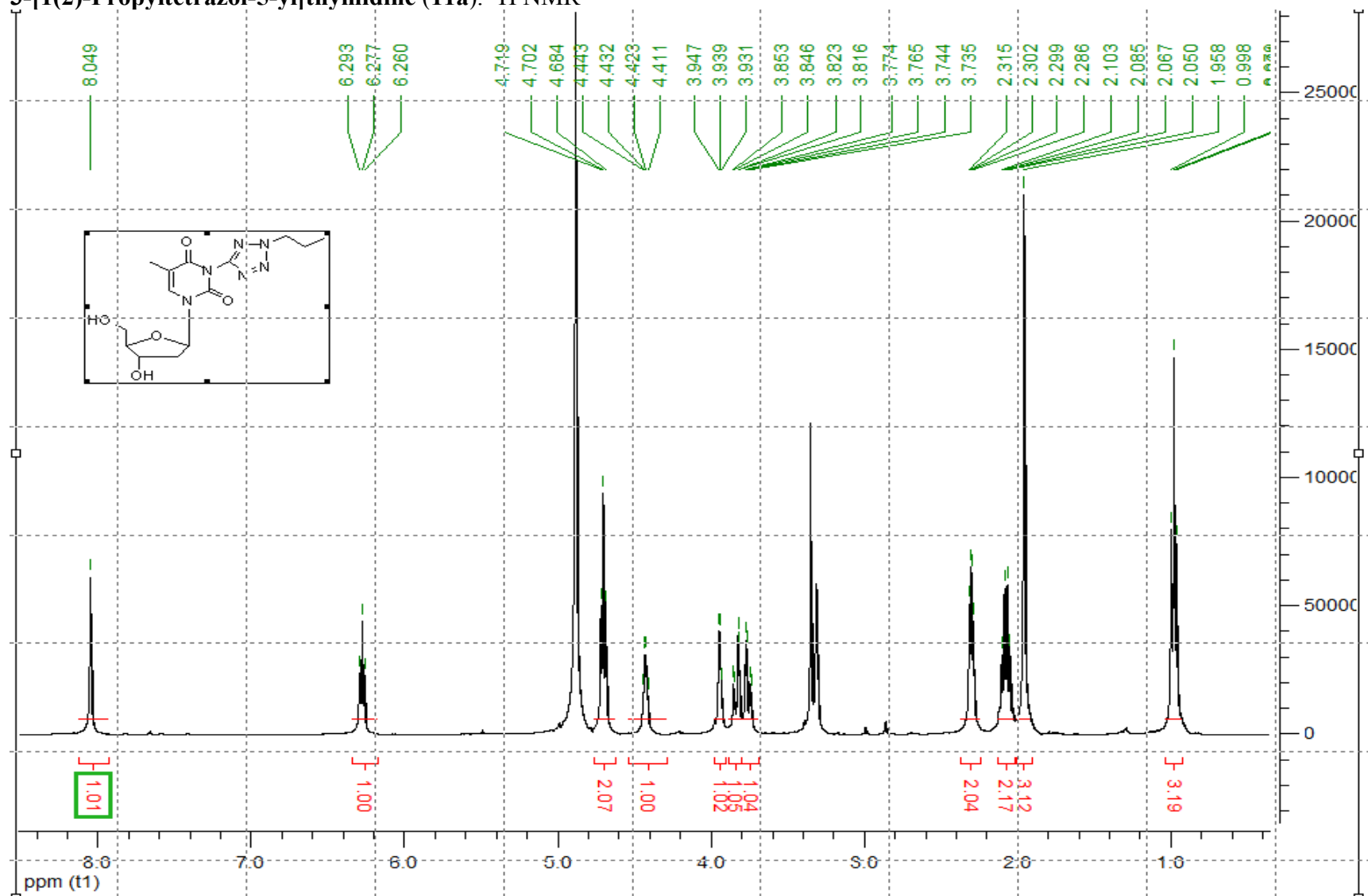
3-(Tetrazol-5-yl)thymidine (10). Analytical traces in Water:Acetonitrile gradient



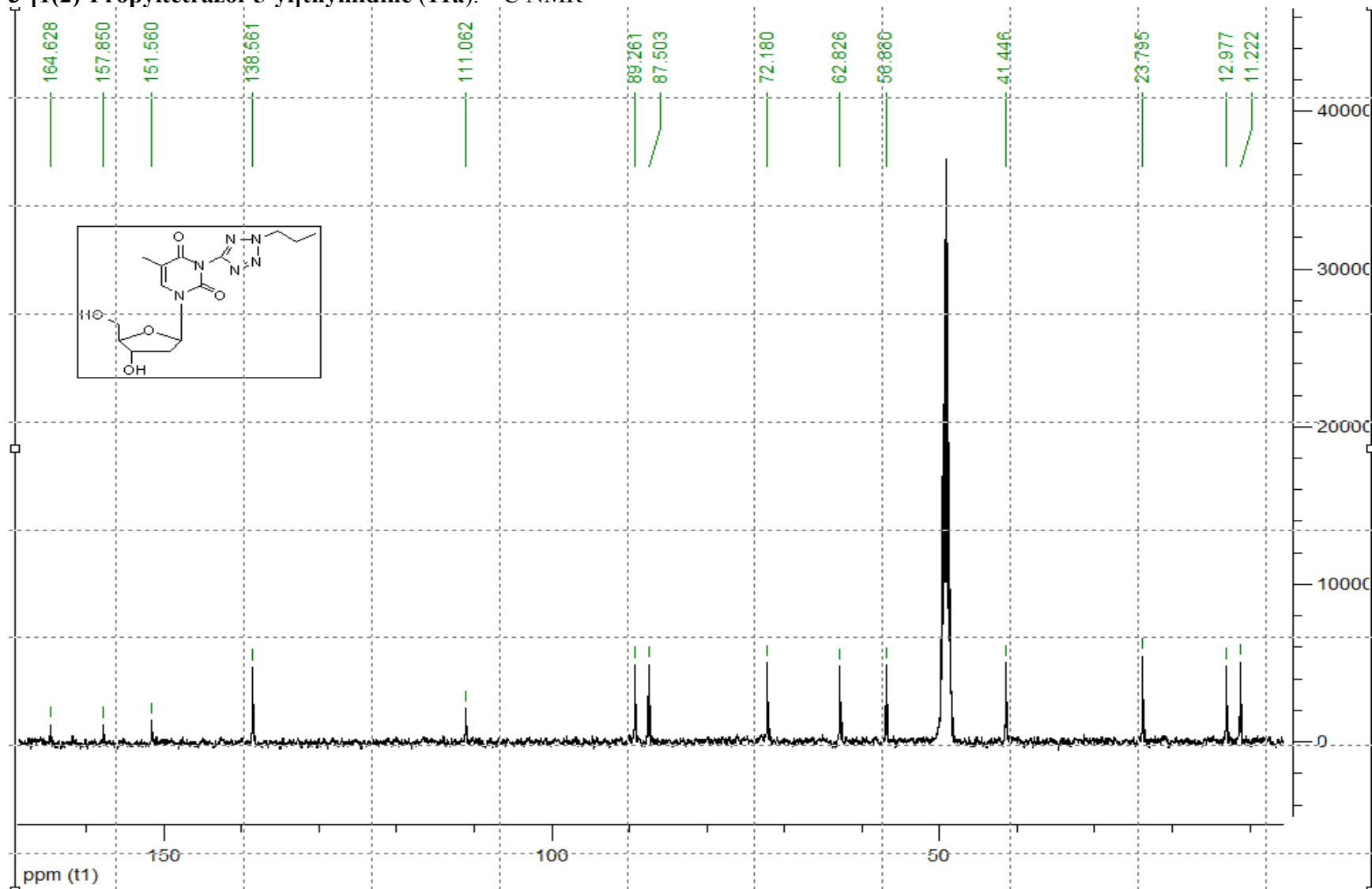
Analytical traces in Water:Methanol gradient



3-[1(2)-Propyltetrazol-5-yl]thymidine (11a). ¹H NMR



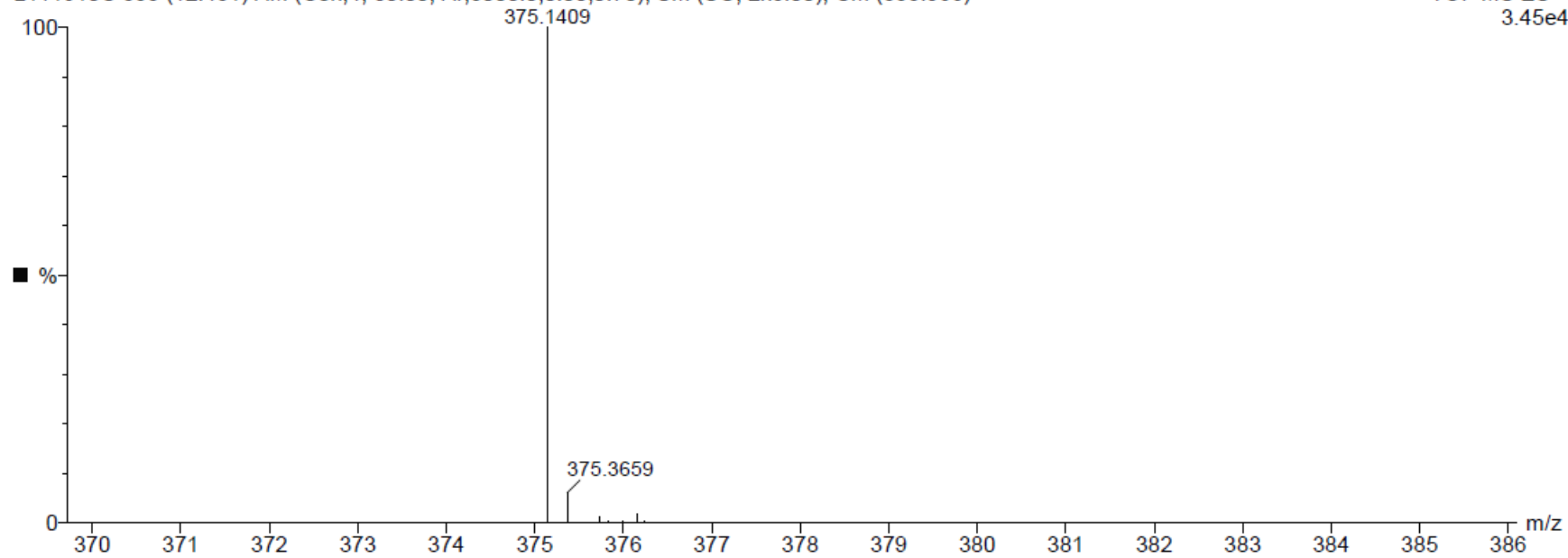
3-[1(2)-Propyltetrazol-5-yl]thymidine (11a). ¹³C NMR



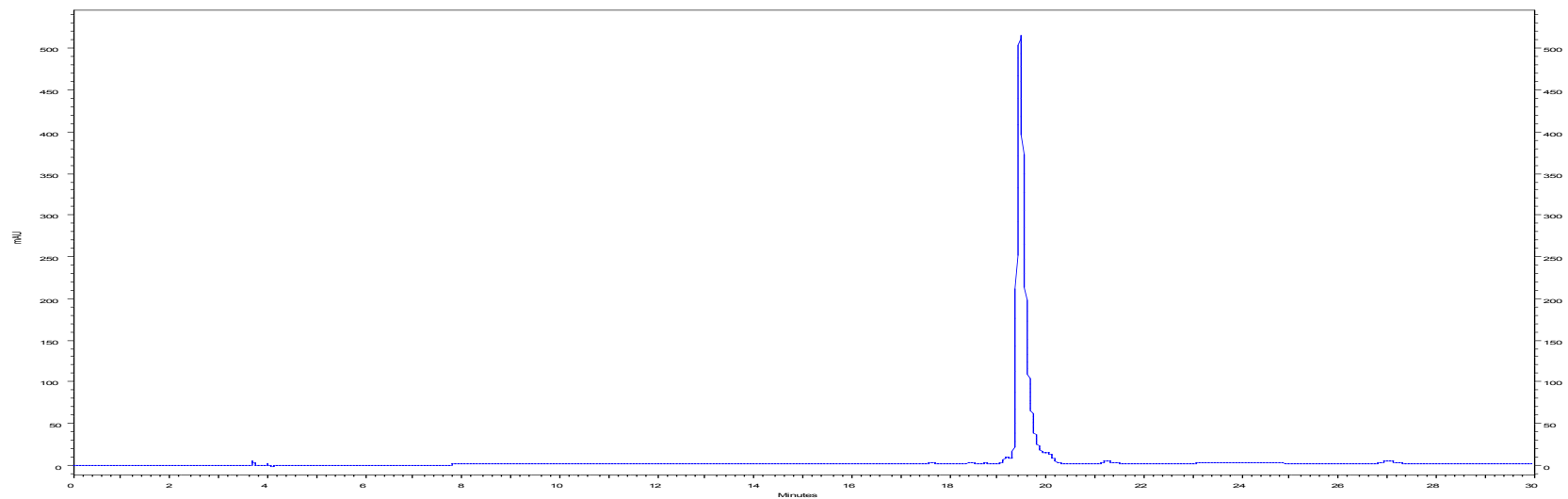
3-[1(2)-Propyltetrazol-5-yl]thymidine (11a). HR-ESI-MS: 375.1409.

L111910C 355 (12.431) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (353:366)

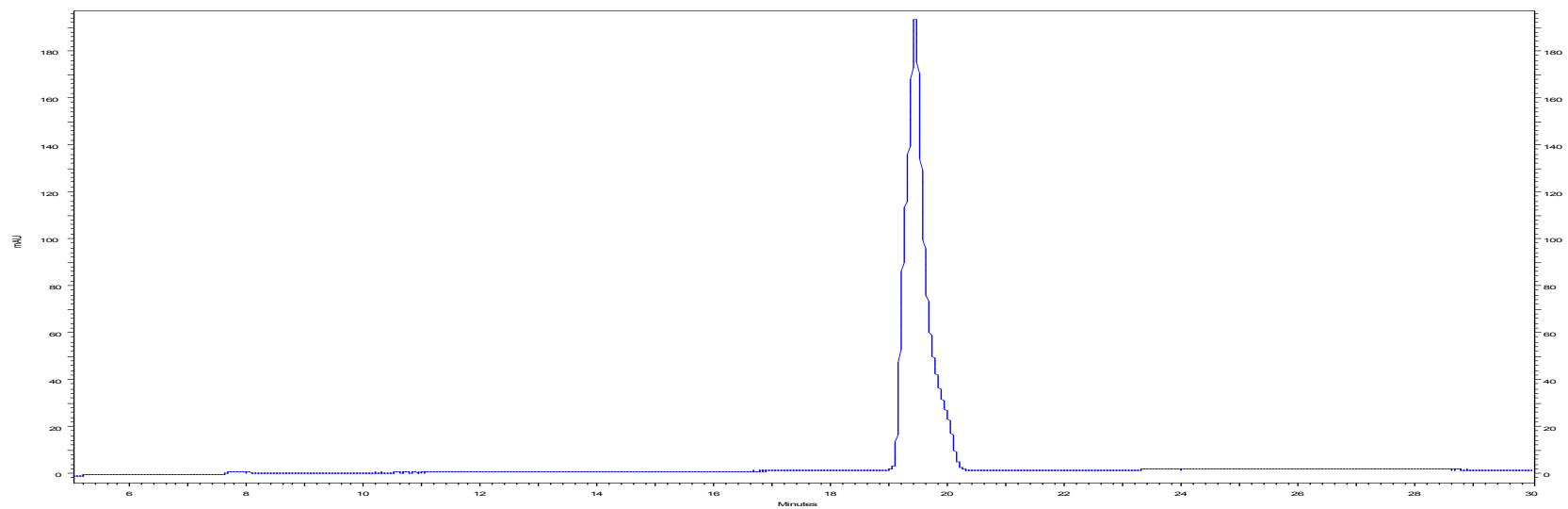
TOF MS ES+
3.45e4



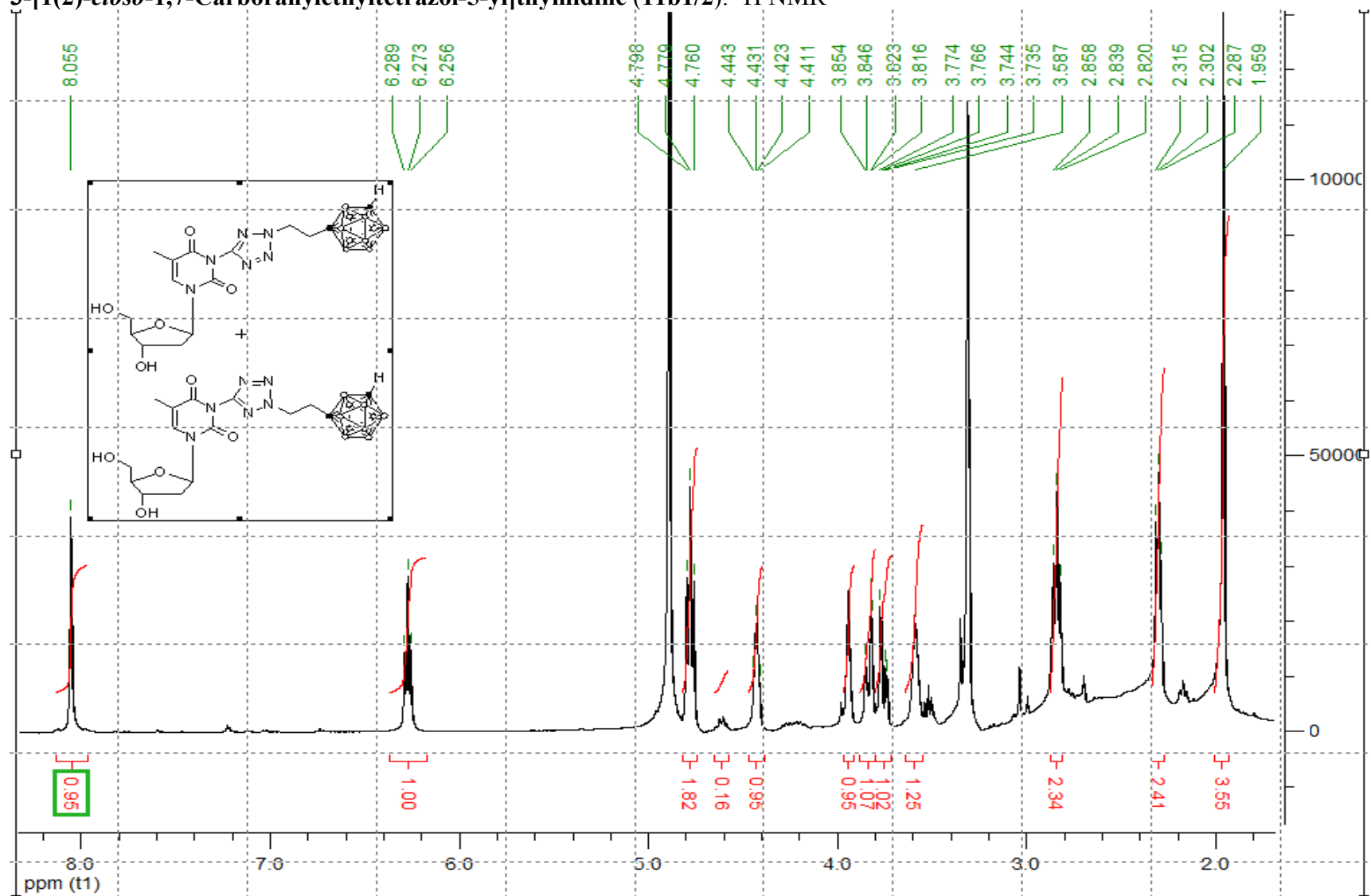
3-[1(2)-Propyltetrazol-5-yl]thymidine (11a). Analytical traces in Water:Acetonitrile gradient



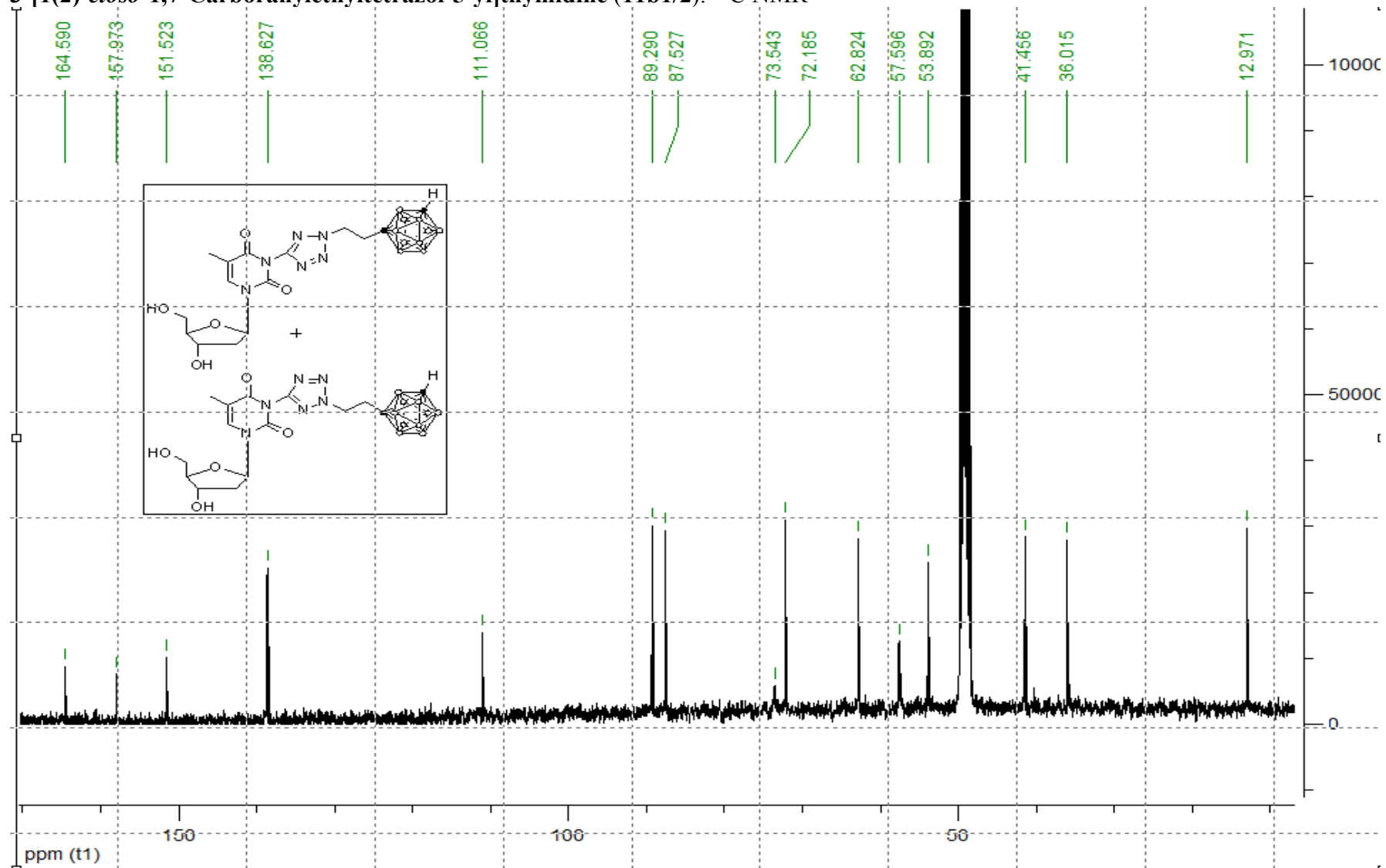
Analytical traces in Water:Methanol gradient



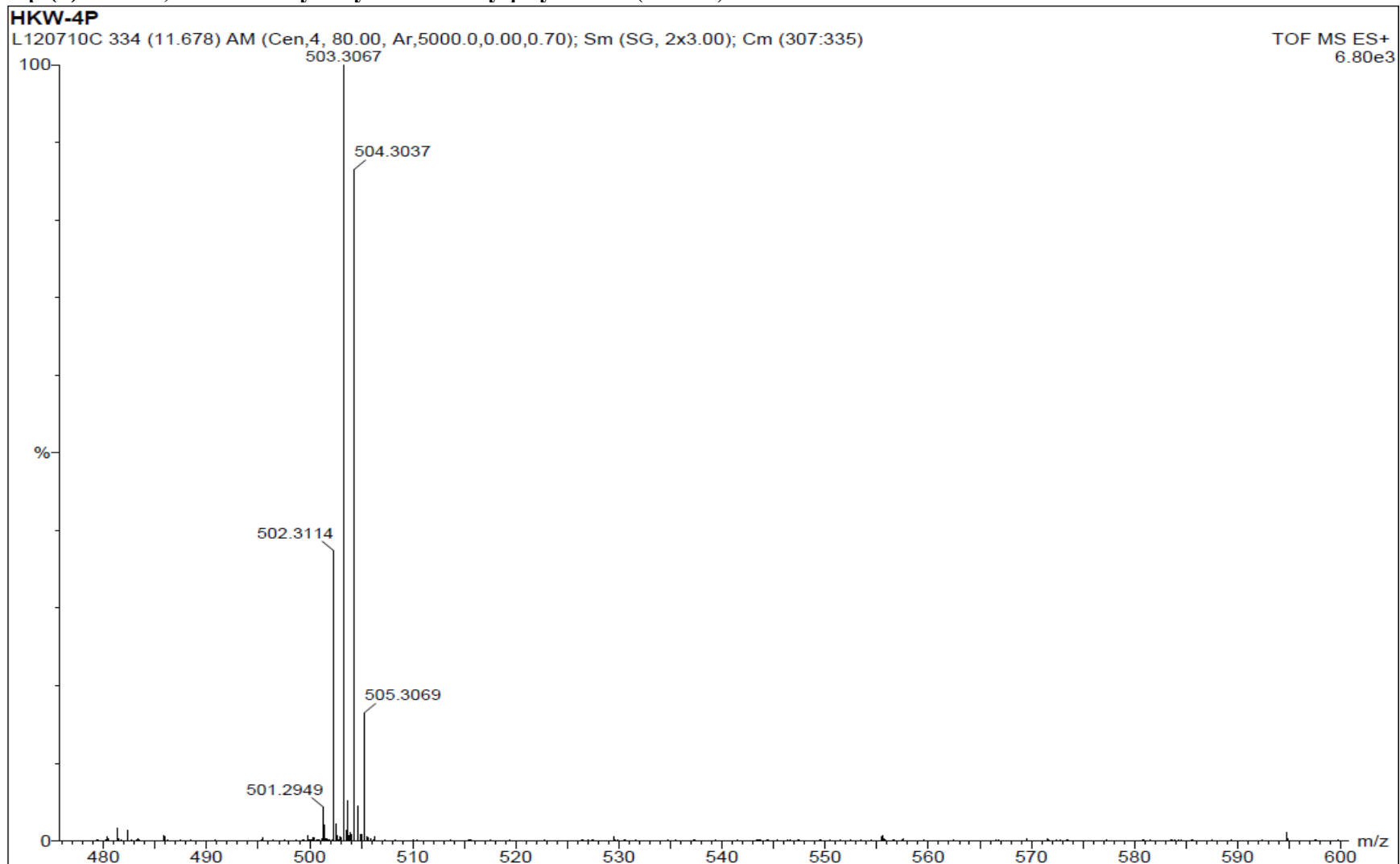
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-yl]thymidine (11b1/2). ¹H NMR



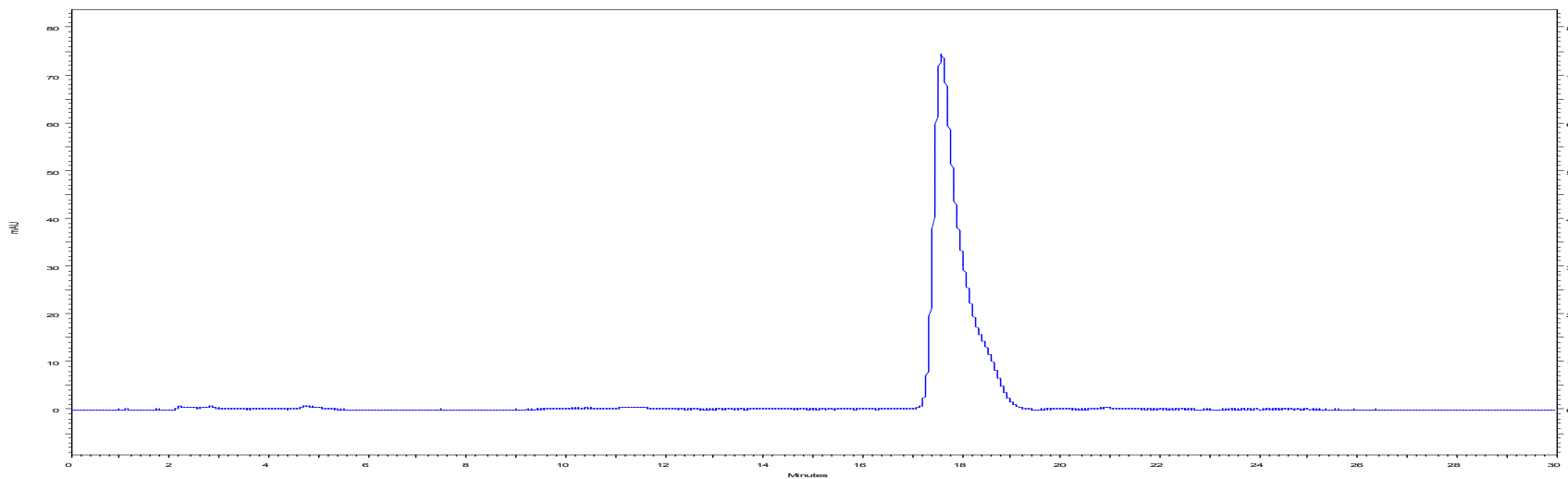
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-yl]thymidine (11b1/2). ¹³C NMR



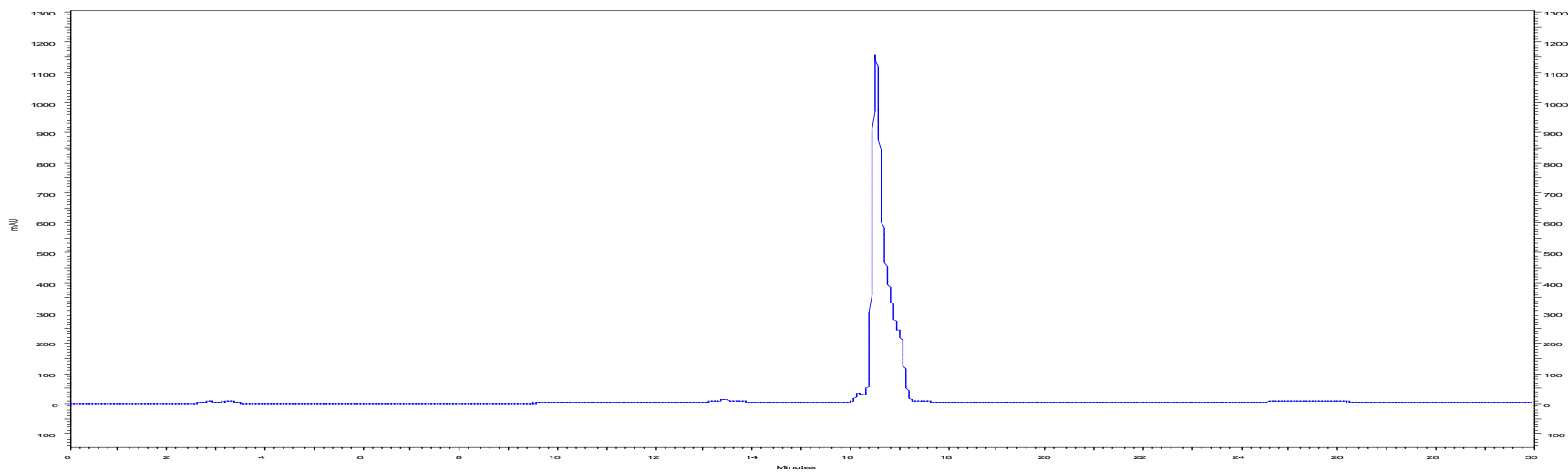
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-yl]thymidine (11b1/2). HR-ESI-MS found: 503.3067.



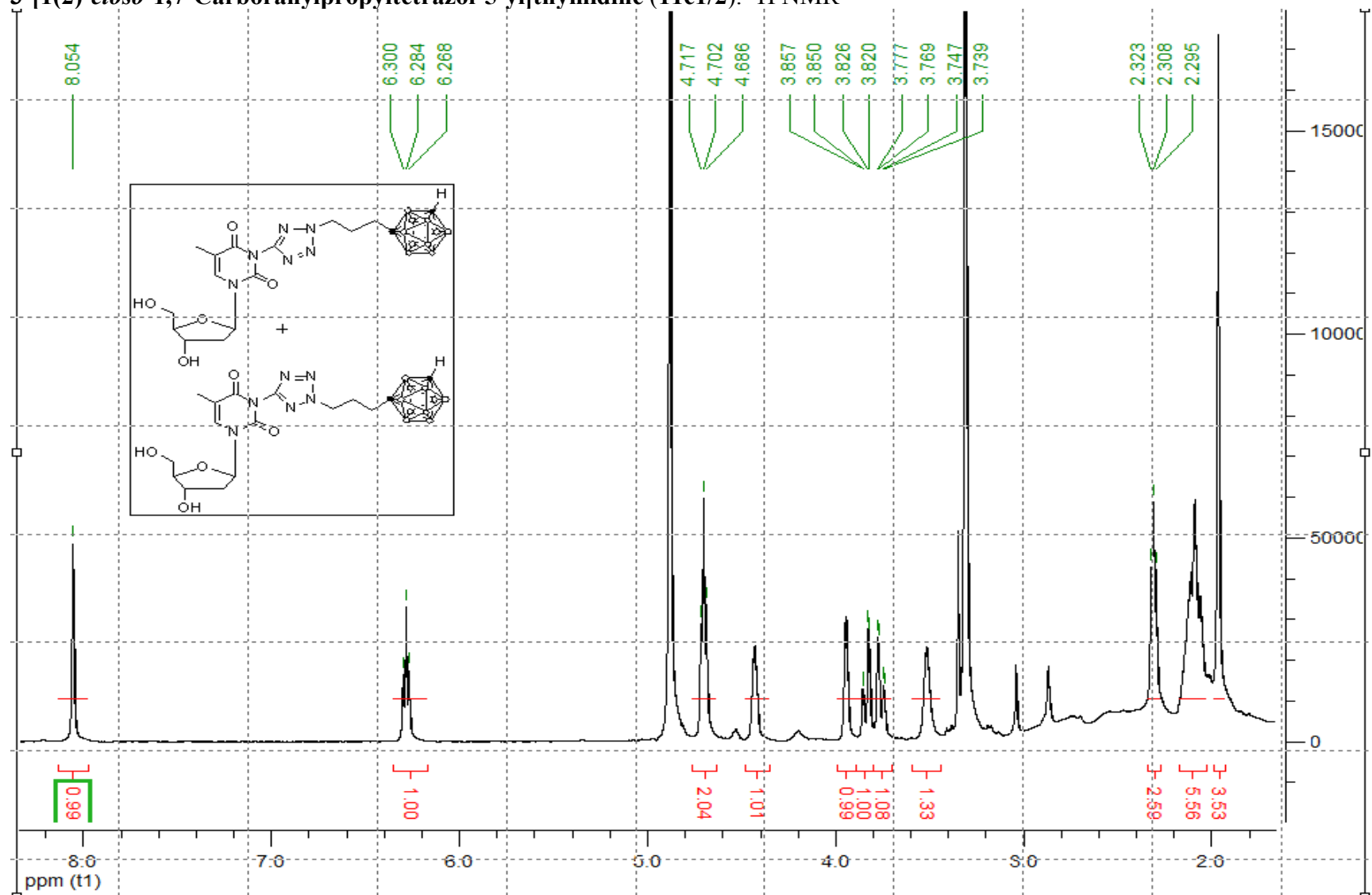
3-[1(2)-*closo*-1,7-Carboranylethyltetrazol-5-yl]thymidine (11b1/2). Analytical traces in Water:Acetonitrile gradient



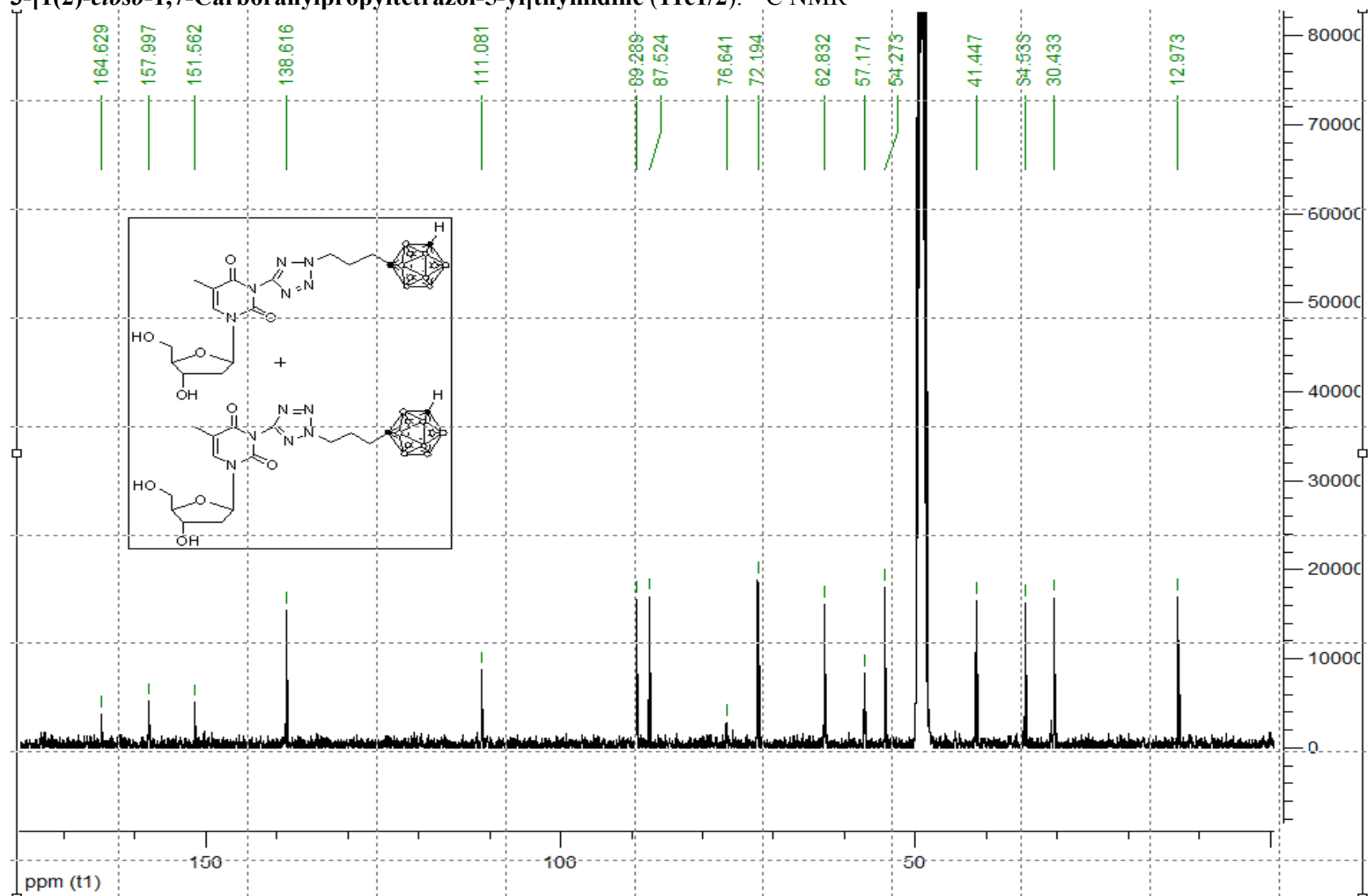
Analytical traces in Water:Methanol gradient



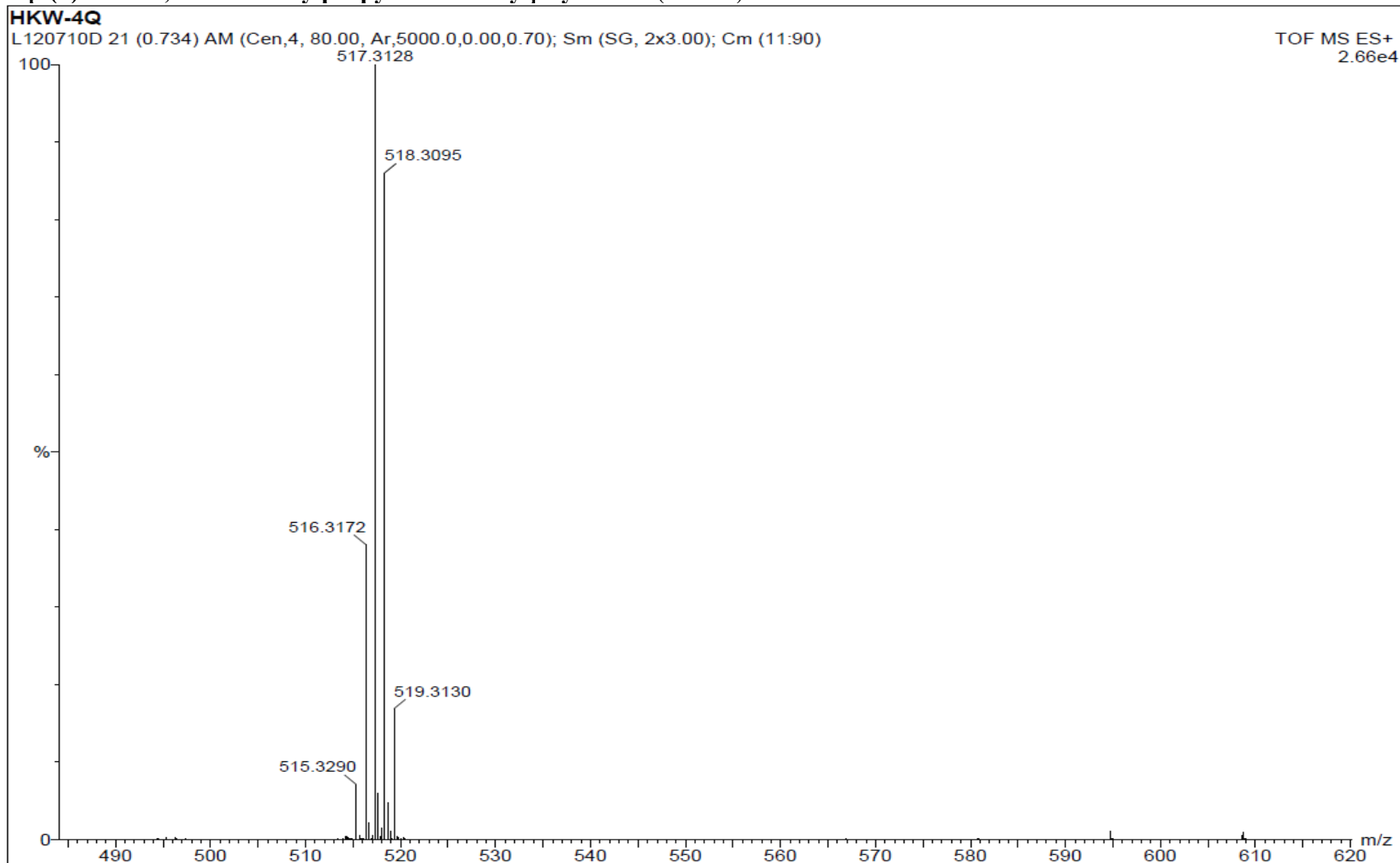
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-yl]thymidine (11c1/2). ¹H NMR



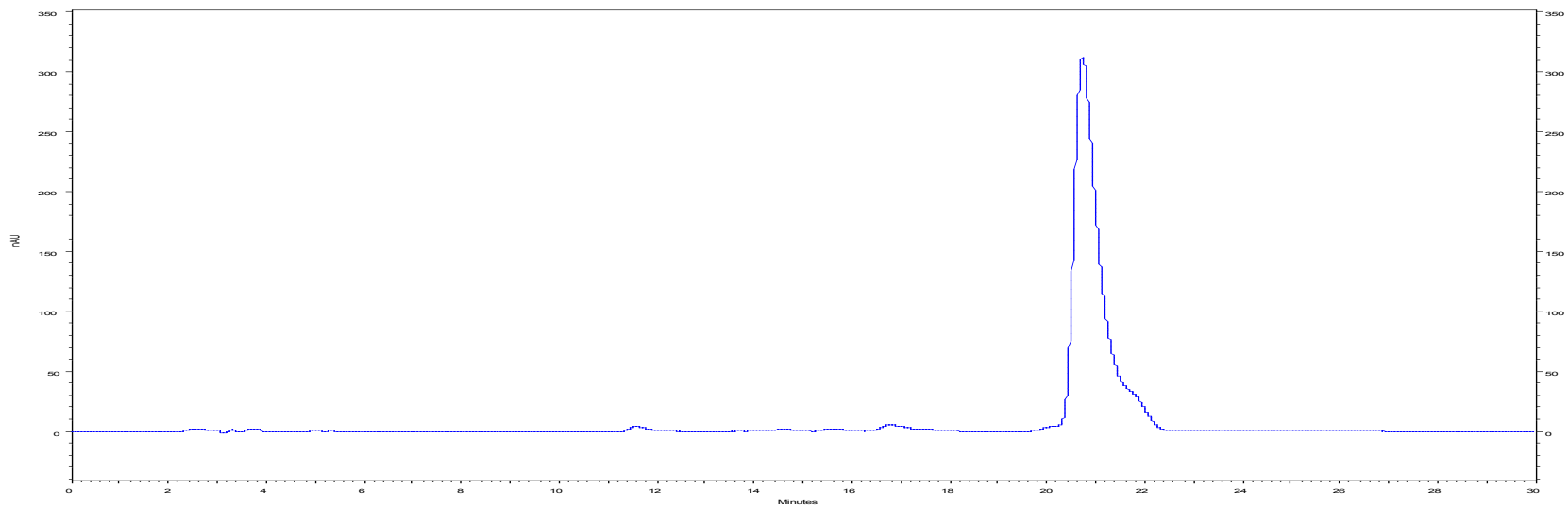
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-yl]thymidine (11c1/2). ¹³C NMR



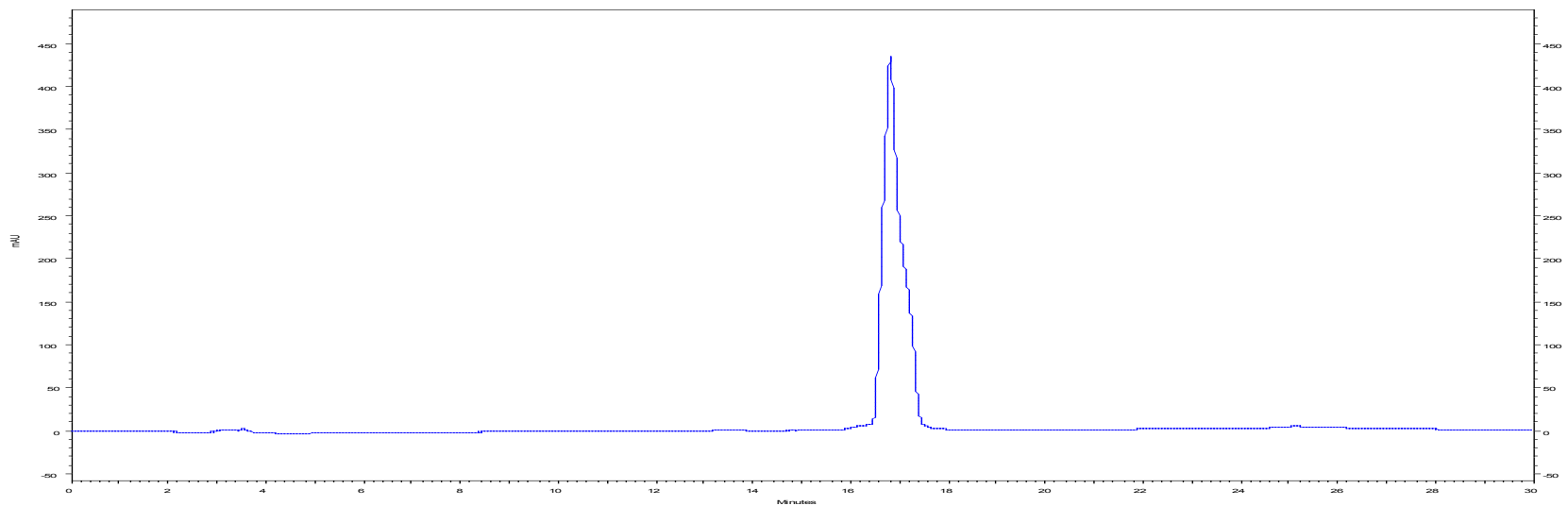
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-yl]thymidine (11c1/2). HR-ESI-MS found: 517.3128.



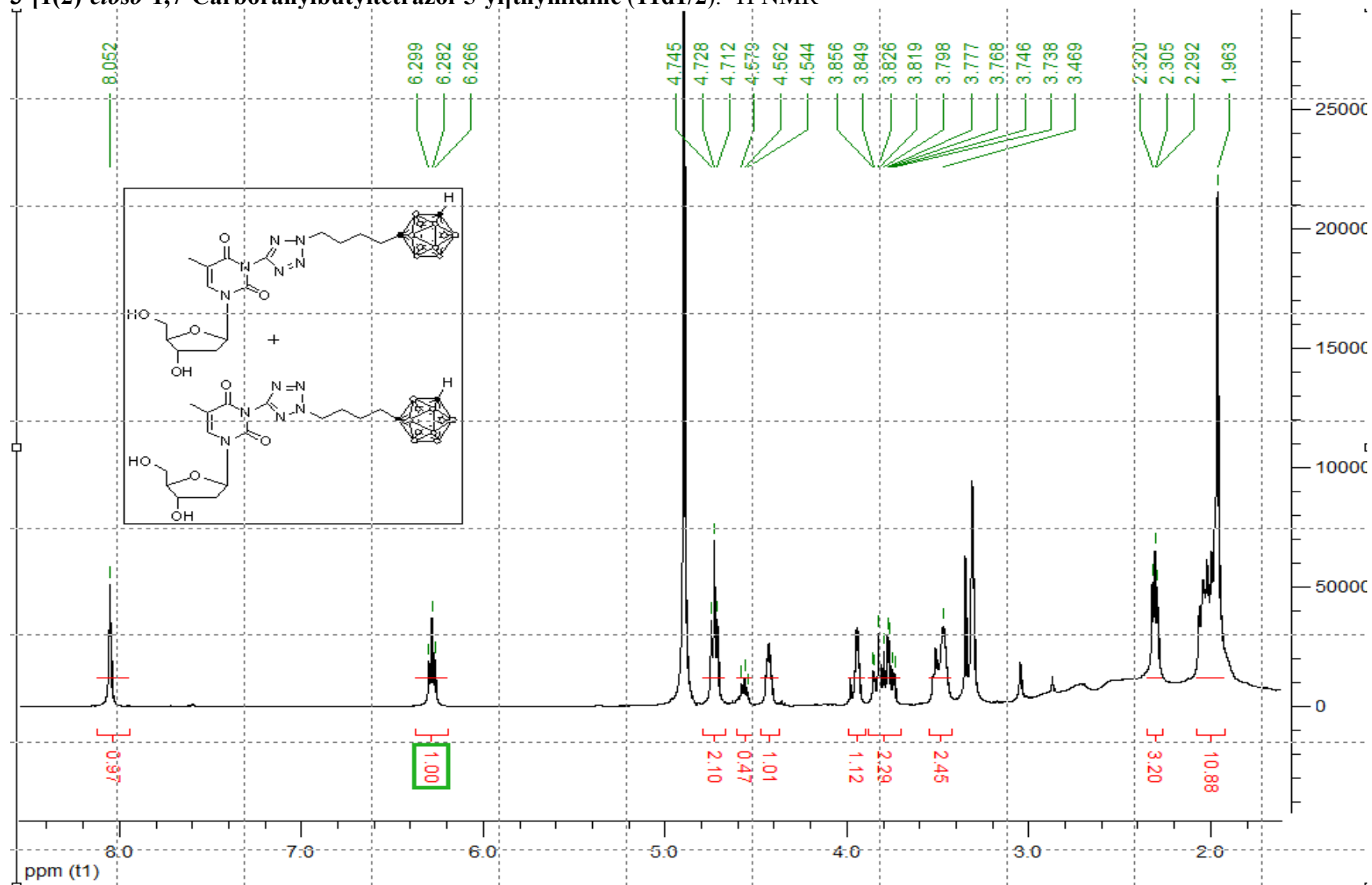
3-[1(2)-*closo*-1,7-Carboranylpropyltetrazol-5-yl]thymidine (11c1/2). Analytical traces in Water:Acetonitrile gradient



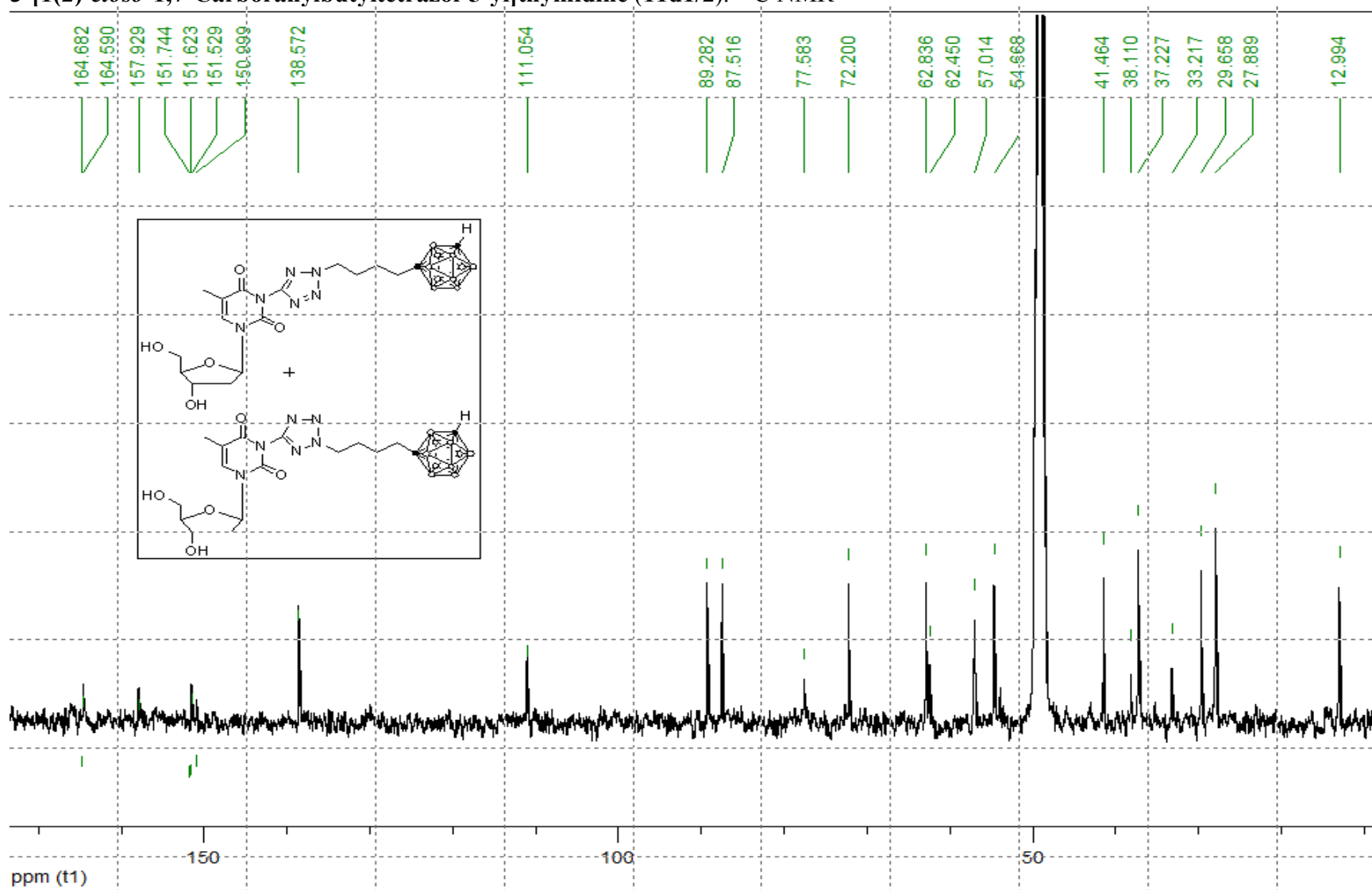
Analytical traces in Water:Methanol gradient



3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-yl]thymidine (11d1/2). ¹H NMR



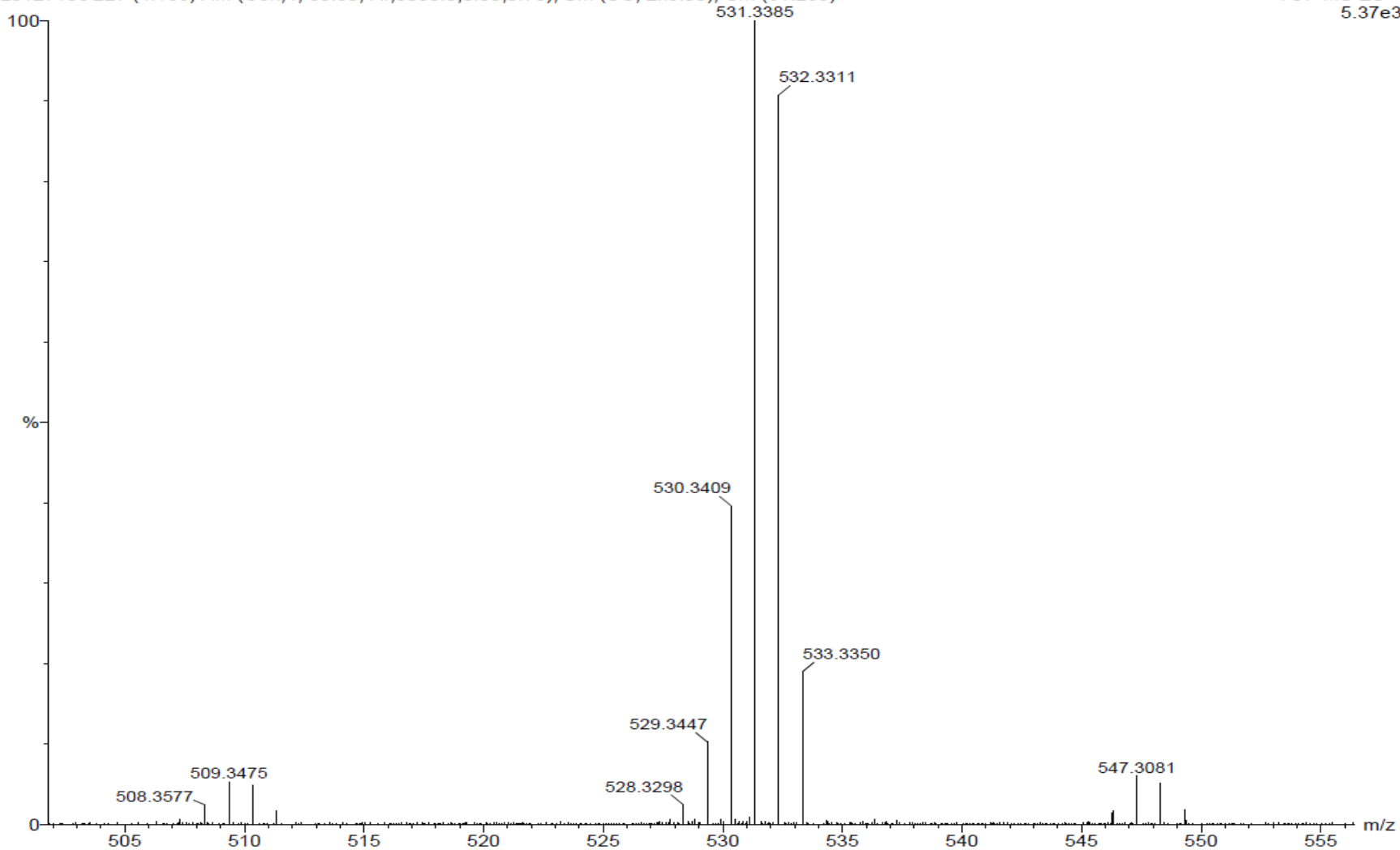
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-yl]thymidine (11d1/2). ¹³C NMR



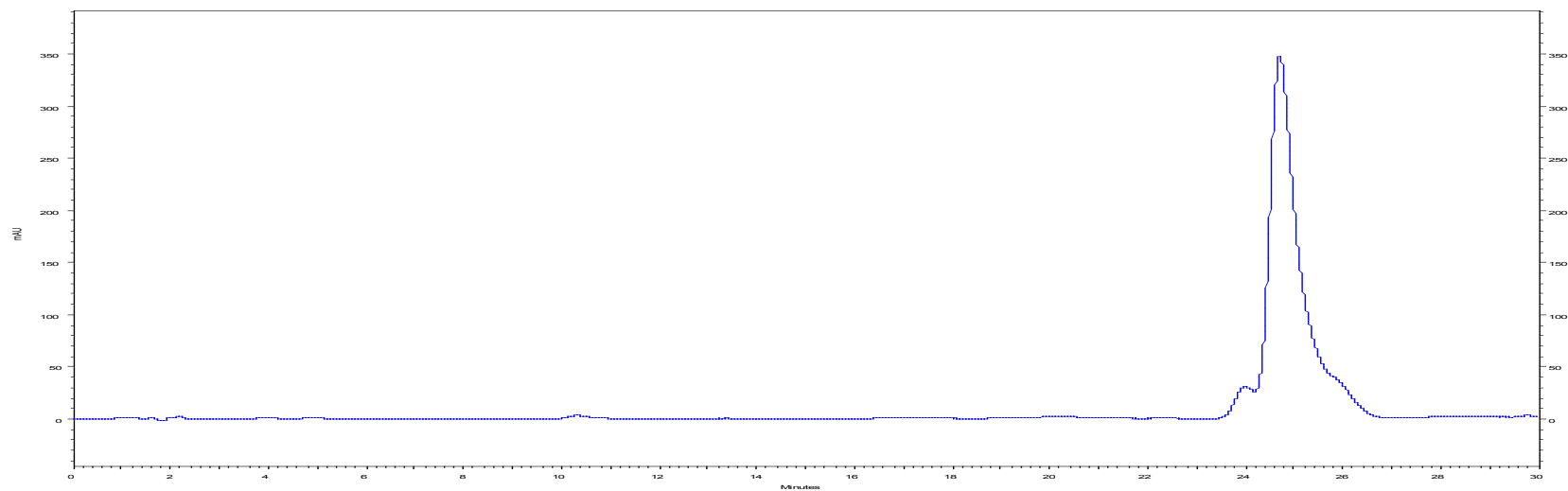
3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-yl]thymidine (11d1/2). HR-ESI-MS found: 509.3475, 531.3385 and 547.3081.

L012710c 227 (4.163) AM (Cen,4, 80.00, Ar,5000.0,0.00,0.70); Sm (SG, 2x3.00); Cm (61:253)

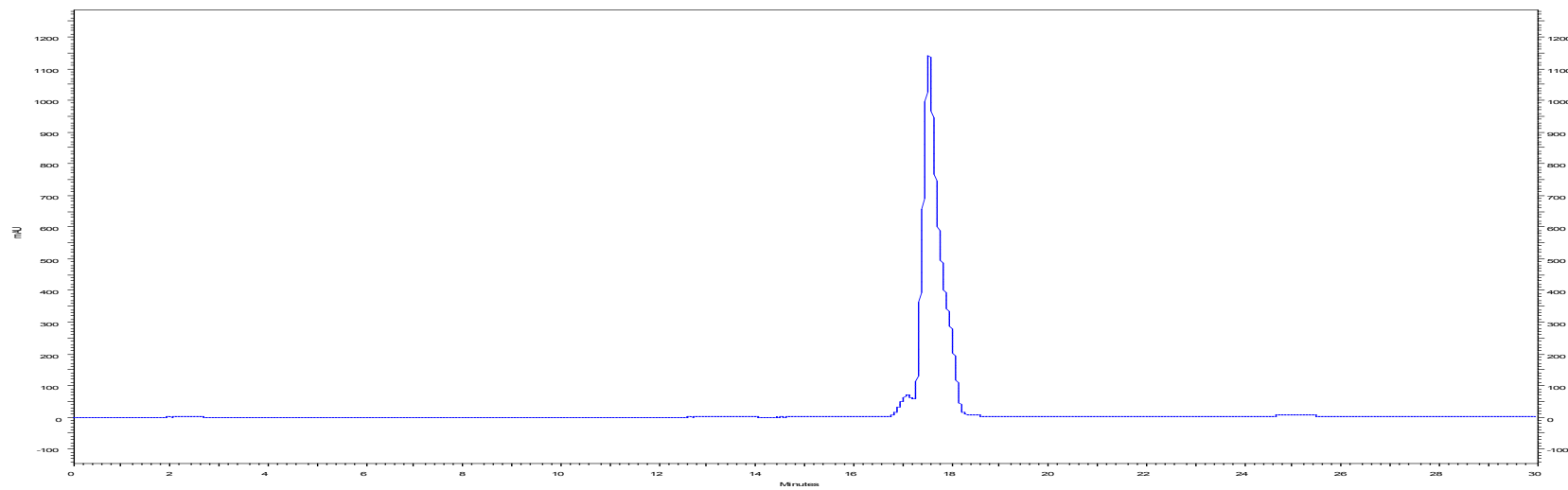
TOF MS ES+
5.37e3



3-[1(2)-*closo*-1,7-Carboranylbutyltetrazol-5-yl]thymidine (11d1/2). Analytical traces in Water:Acetonitrile gradient



Analytical traces in Water:Methanol gradient



3. Purification of hTK1 and determination of its concentration

The hTK1 content in the enzyme preparation was 538.7 µg/mL (Table S2) and was estimated to be ~40% of the purified protein cocktail.

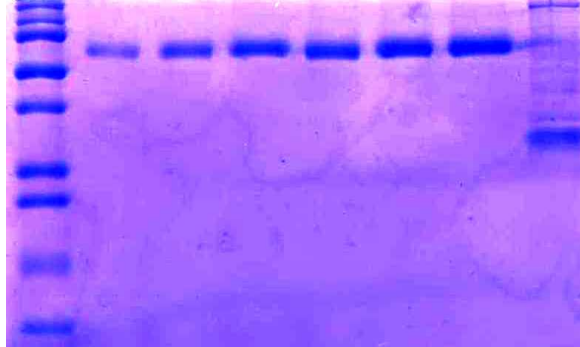


Figure S1. SDS gel with known BSA & hTK1

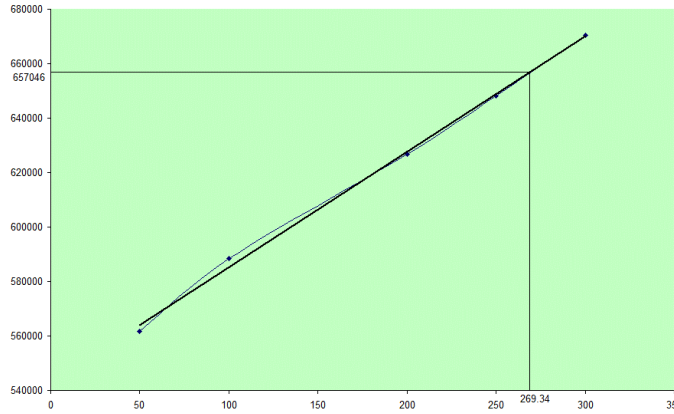


Figure S2. Standard curve for spot intensity of known conc. of BSA & hTK1

Table S2. Protein concentration calculation.

| Protein type | Protein Conc. (µg/mL) | Integrated Density |
|--------------|-----------------------|--------------------|
| BSA | 50 | 561768 |
| BSA | 100 | 588345 |
| BSA | 200 | 626901 |
| BSA | 250 | 648071 |
| BSA | 300 | 670455 |
| hTK1 | Unknown | 657046 |

(50 % solution in glycerol, v/v)

Equation: $Y = 424.65X + 542670$
Conc. of hTK1/mL $X = 269.34 \mu\text{g/mL}$
 (50% in Glycerol, v/v)

Conc. of hTK1 $X = 538.68 \mu\text{g/mL}$

4. LR-ESI-MS for hTK1 enzyme assay of 7f with ^{31}P -ATP

Thymidine monophosphate and 3-[2-(*closo*-1,7-Carboranyl)propylcarbamimidoyl]thymidine monophosphate (7f-MP). LR-ESI-MS found: 323.1 $[\text{M}+\text{H}]^+$ and 549.3 $[\text{M}-\text{PO}_3\text{H}_2 + \text{H}]^+$, respectively.

