Supporting Information

A Study on Synthesis and Upscaling of 2'-O-AECM-5-methyl Pyrimidine Phosphoramidites for Oligonucleotide Synthesis

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Contents

*Table S1. Screen for different conditions of MeU 2´alkylation (compound **3**).*

No.	Methyl 2-bromoacetate	Solvent, 5 vol	K_2CO_3	PTC catalyst, 0.2 equiv.	HPLC area%, conver., 40°C, 1h	HPLC area%, conver., 40°C, 4.5 h	HPLC area%, conver., RT, overnight
1.	2 equiv.	MeCN	2 equiv.	TBABr	26%	71%	89%
2.	4 equiv.	MeCN	2 equiv.	TBABr	36%	78%	85%
3.	2 equiv.	MeCN	4 equiv.	TBABr	42%	81%	84%
4.	4 equiv.	MeCN	4 equiv.	TBABr	46%	80%	88%

*Table S2. Screen for different conditions of MeU 2´alkylation (compound **3**).*

No.	Methyl 2-bromoacetate	Solvent, 5 vol	Base, 4 eq	PTC agent, 0.2 equiv.	HPLC area%, conver., RT, over weekend
1.	2 equiv.	Heptane	K_2CO_3	TBABr	96%
2.	2 equiv.	MeCN	K_2CO_3	TBABr	85%
3.	2 equiv.	Heptane	K_3PO_4	TBABr	96%
4.	2 equiv.	MeCN	K_3PO_4	TBABr	85%

*Table S3. Screen for different conditions for N4 acetylation MeC (compound **13**).*

No.	Solvent, 5 vol	A	B	C	D	E	F	G
1.	MeOH	14%	7%*	0%	11%	0%	0%	0%
2.	DCM	55%	82%	84%	92%	92%	90%	80%
3.	Toluene	43%	79%	84%	89%	88%	86%	81%
4.	DMF	67%	86%	83%	86%	82%	80%	83%
5.	MeCN	59%	87%	85%	87%	84%	83%	84%

*degradation

A= HPLC area%, conver., RT, 4.5 h
B= HPLC area%, conver., RT, overnight, +0.5 equiv. Ac_2O
C= HPLC area%, conver., +0.5 equiv. Ac_2O, over weekend
D= HPLC area%, conver., +0.5 equiv. Ac_2O, over weekend, additional 0.5 equiv. Ac_2O, 1h
E= HPLC area%, conver., +0.5 equiv. Ac_2O, over weekend +0.5 equiv. TEA, 1 h
F= HPLC area%, conver., +0.5 equiv. Ac_2O, over weekend, additional 0.5 equiv. Ac_2O, 1h, then 0.5 equiv. TEA
G= HPLC area%, conver., +0.5 equiv. Ac_2O, over weekend, additional 0.5 equiv. Ac_2O, 1h, then 0.5 equiv. Ac_2O

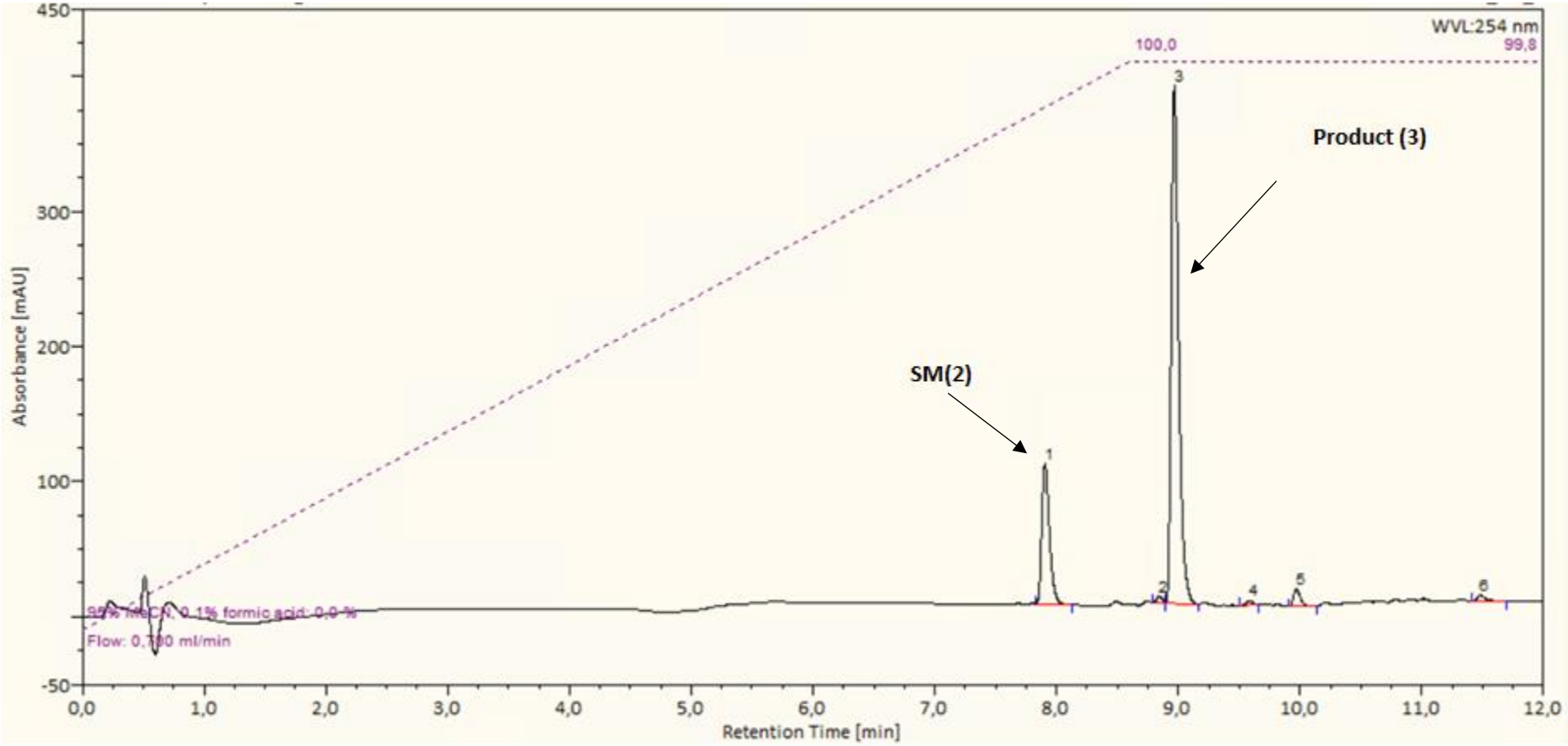

Figure S1.HPLC profile of crude N³ alkylation reaction on MeU in 50 g scale after 5.5 h.

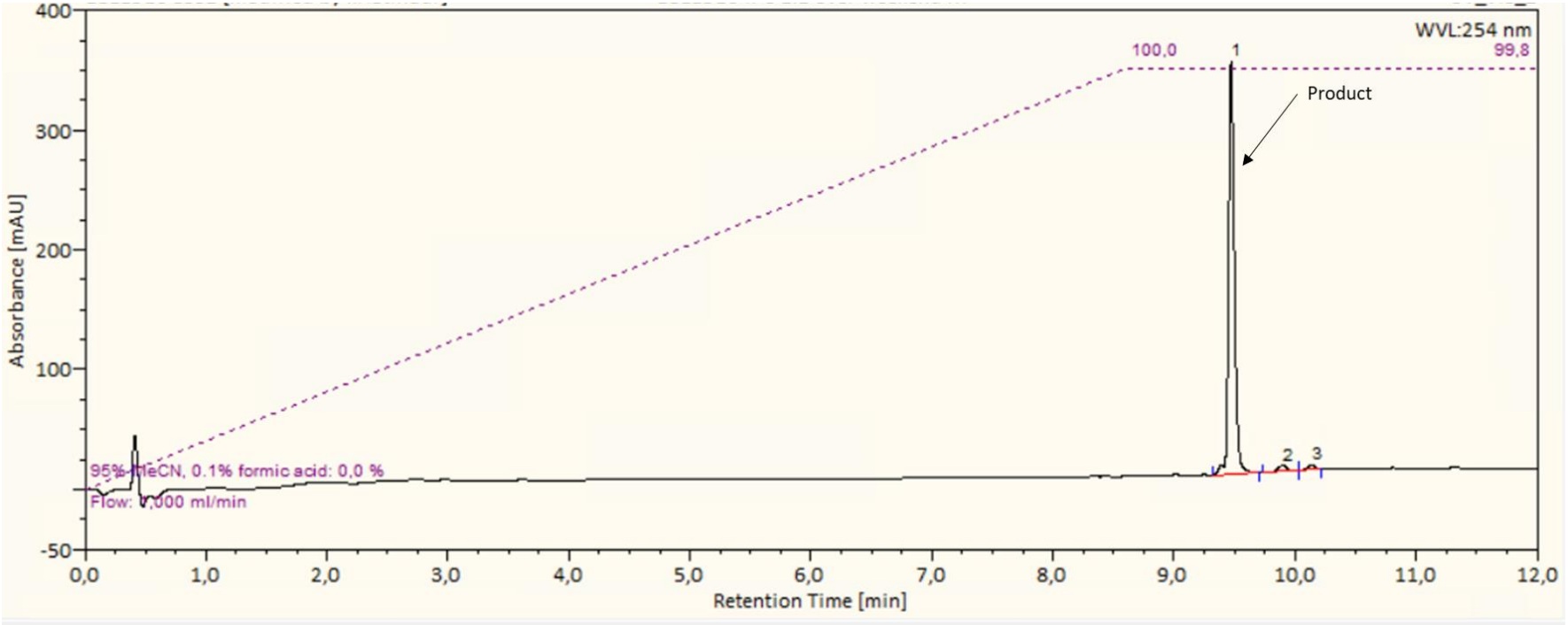

Figure S2. RP-HPLC profile of PTC screen reaction for 2′-OH alkylation of compound **3** *using* K_2CO_3 *as a base and heptane as a solvent on C18 column (Waters XBridge® Oligonucleotide BEH C18 (4.6 x 50 mm)) with a linear gradient from 0-100% of buffer B in buffer A over 12 min at 40 °C. Buffer A: 0.1 M triethylammonium acetate (aq.), buffer B: MeCN+10% (v/v) buffer A.*

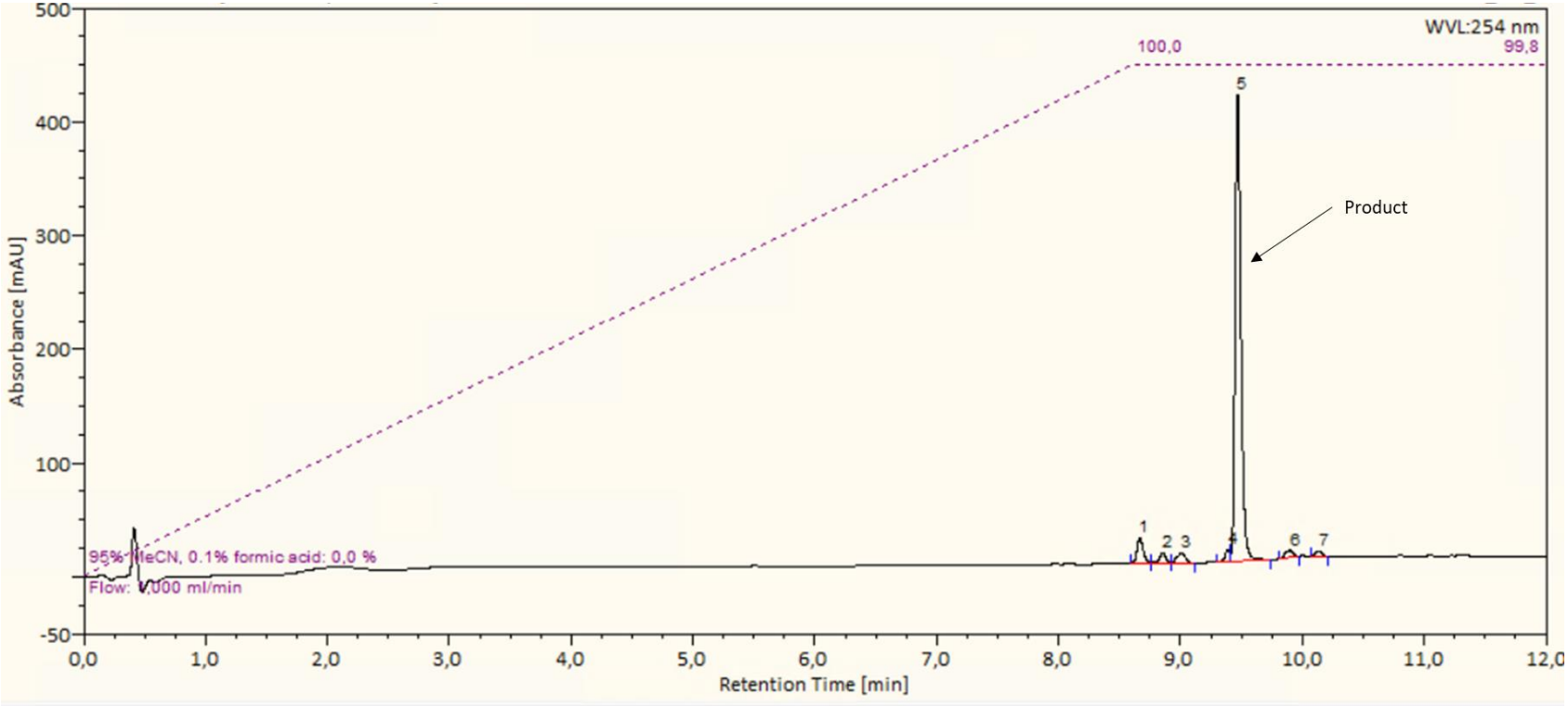

Figure S3. RP-HPLC profile of PTC screen reaction for 2′-OH alkylation of compound **3** *using* K_2CO_3 *as a base and MeCN as a solvent on C18 column (Waters XBridge® Oligonucleotide BEH C18 (4.6 x 50 mm)) with a linear gradient from 0-100% of buffer B in buffer A over 12 min at 40 °C. Buffer A: 0.1 M triethylammonium acetate (aq.), buffer B: MeCN+10% (v/v) buffer A.*

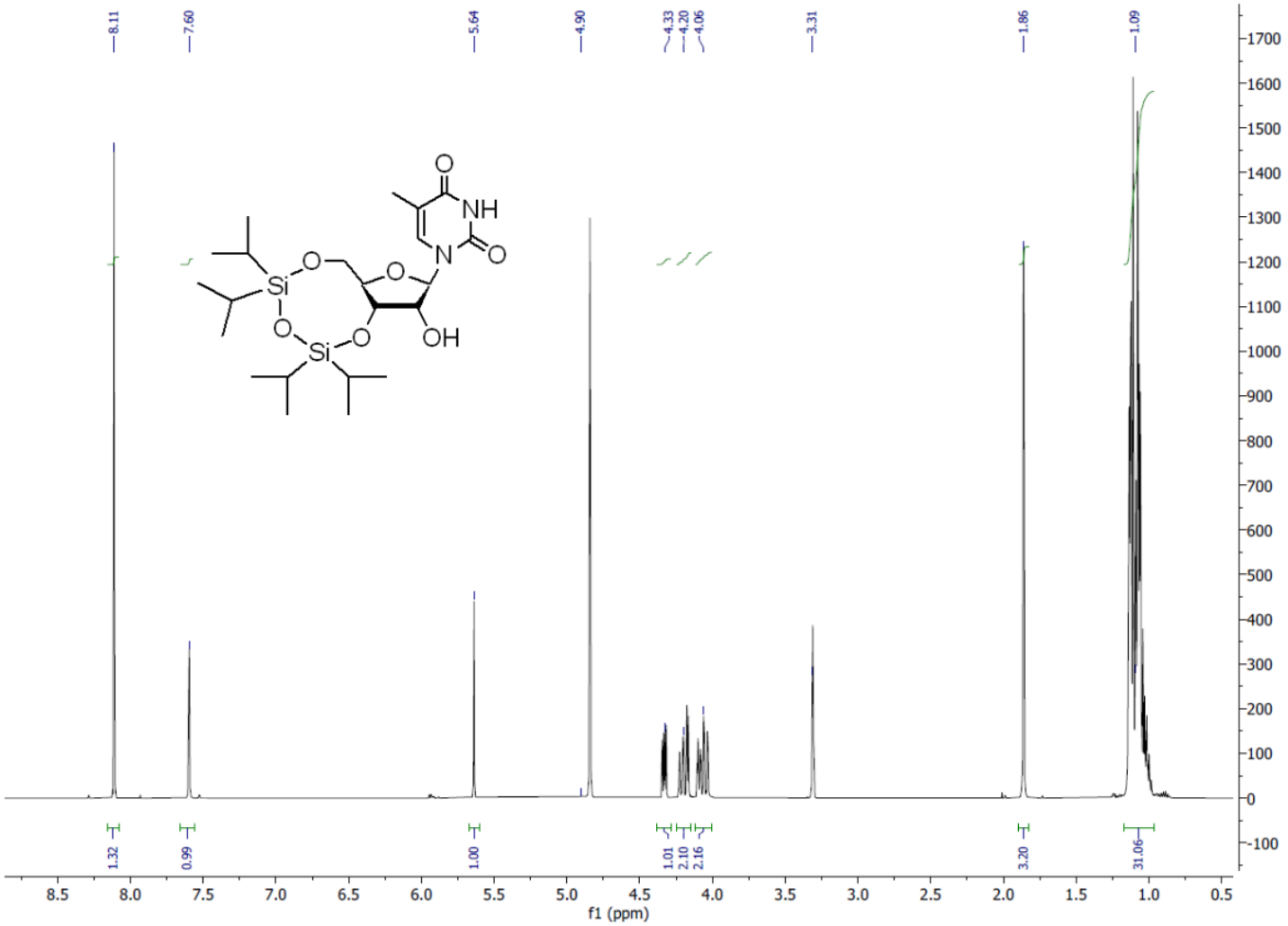

*Figure S4. ^{1}H NMR assay of compound **1** (500 MHz, CD_3OD). 1,2,4,5-Tetrachloro-3-nitrobenzene (δ=8.11) was used as an internal standard.*

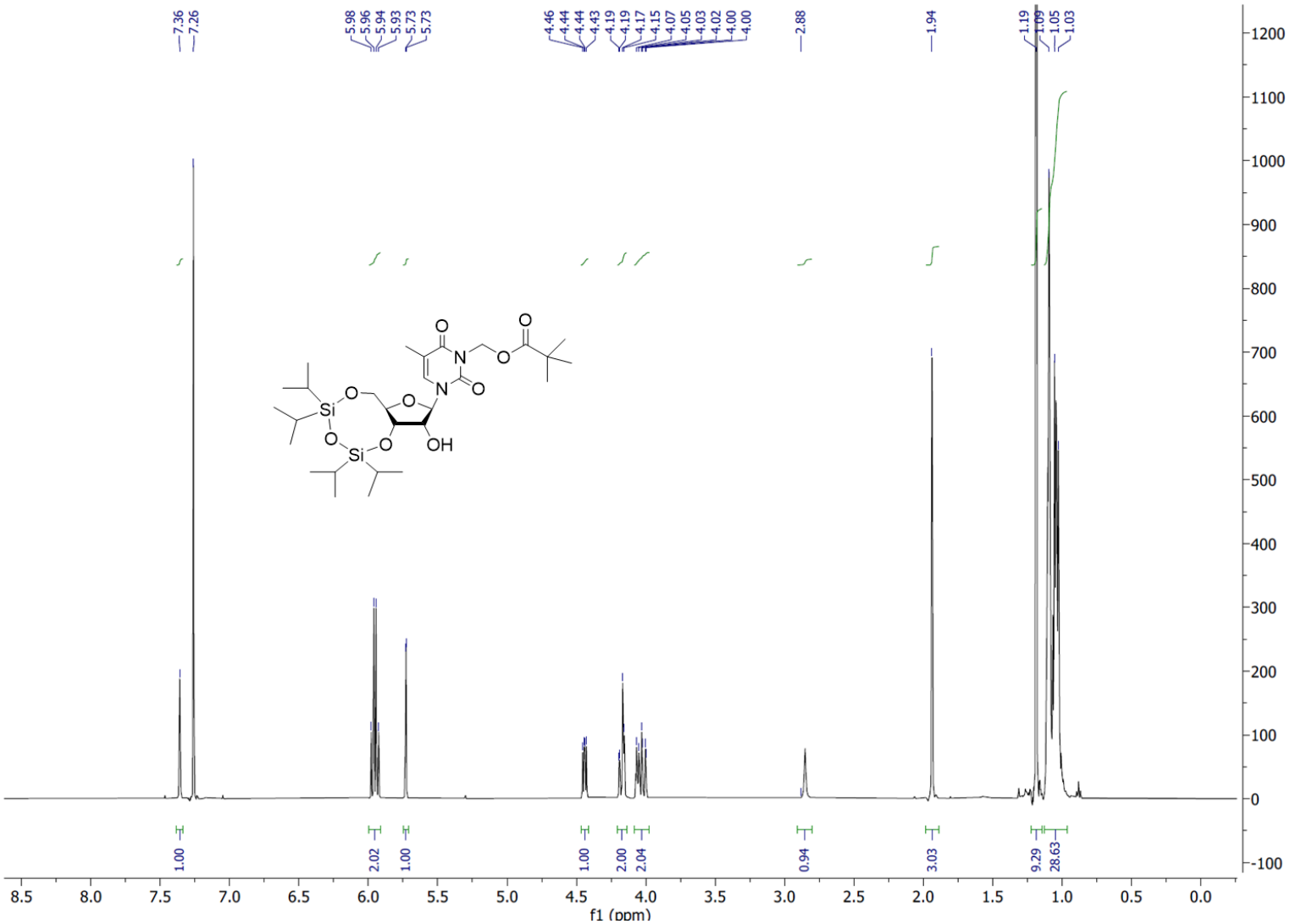

*Figure S5. ^{1}H NMR spectrum of compound **2** (500 MHz, $CDCl_3$).*

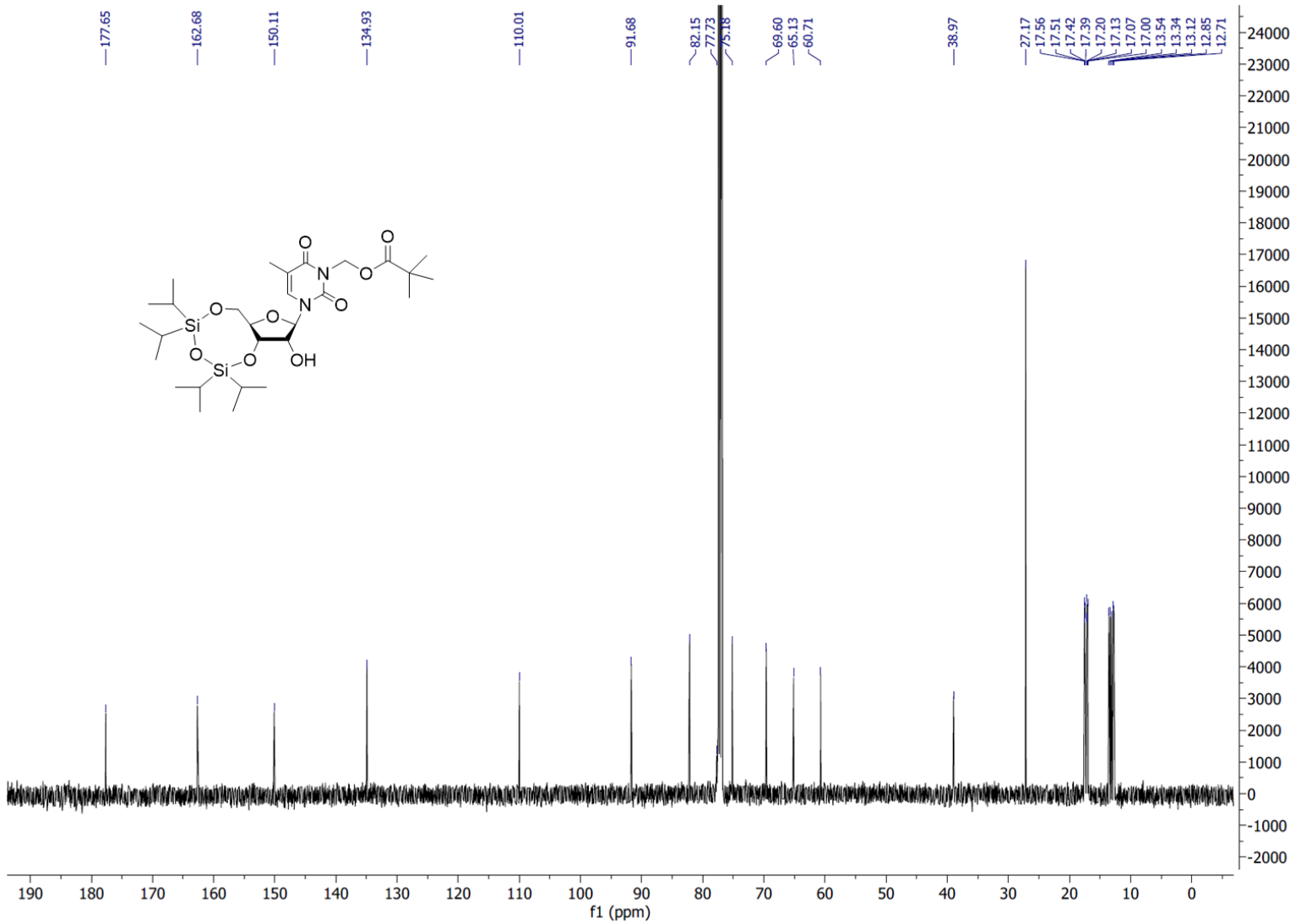

*Figure S6. ^{13}C NMR spectrum of compound **2** (125.76 MHz, $CDCl_3$).*

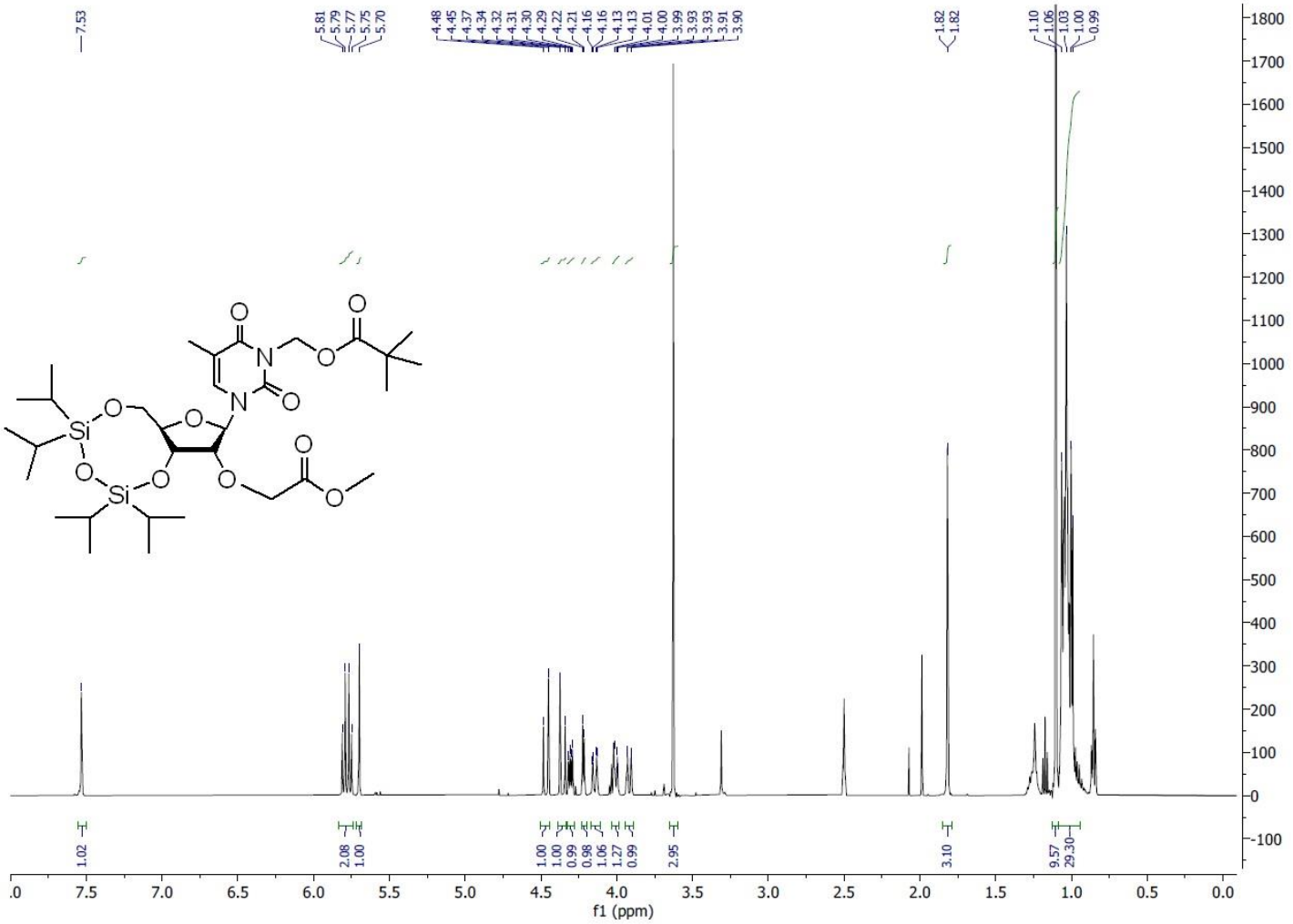

*Figure S7. ^{1}H NMR spectrum of compound **3** (500 MHz, DMSO-d_6).*

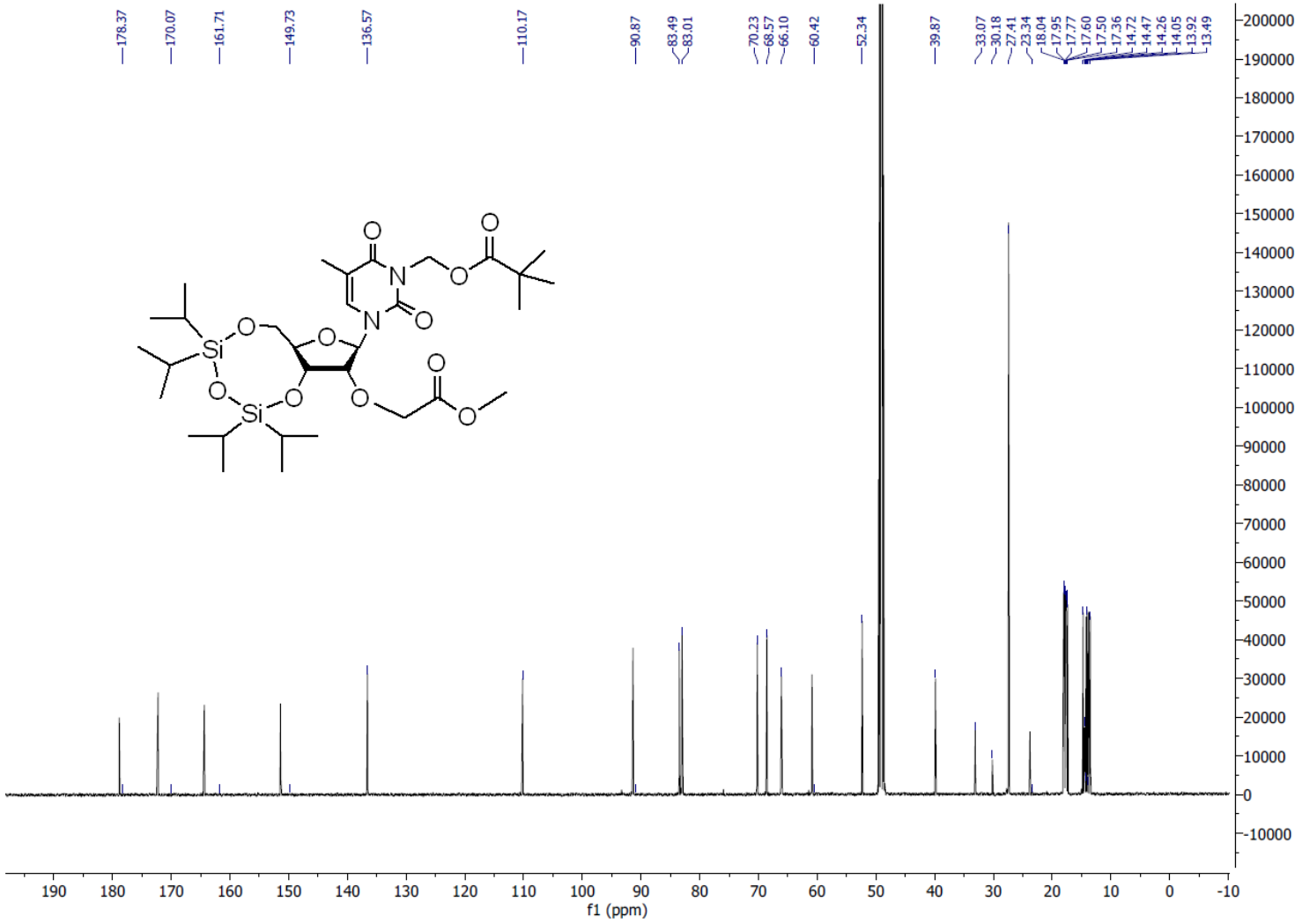

*Figure S8. ^{13}C NMR spectrum of compound **3** (125.76 MHz, CD_3OD).*

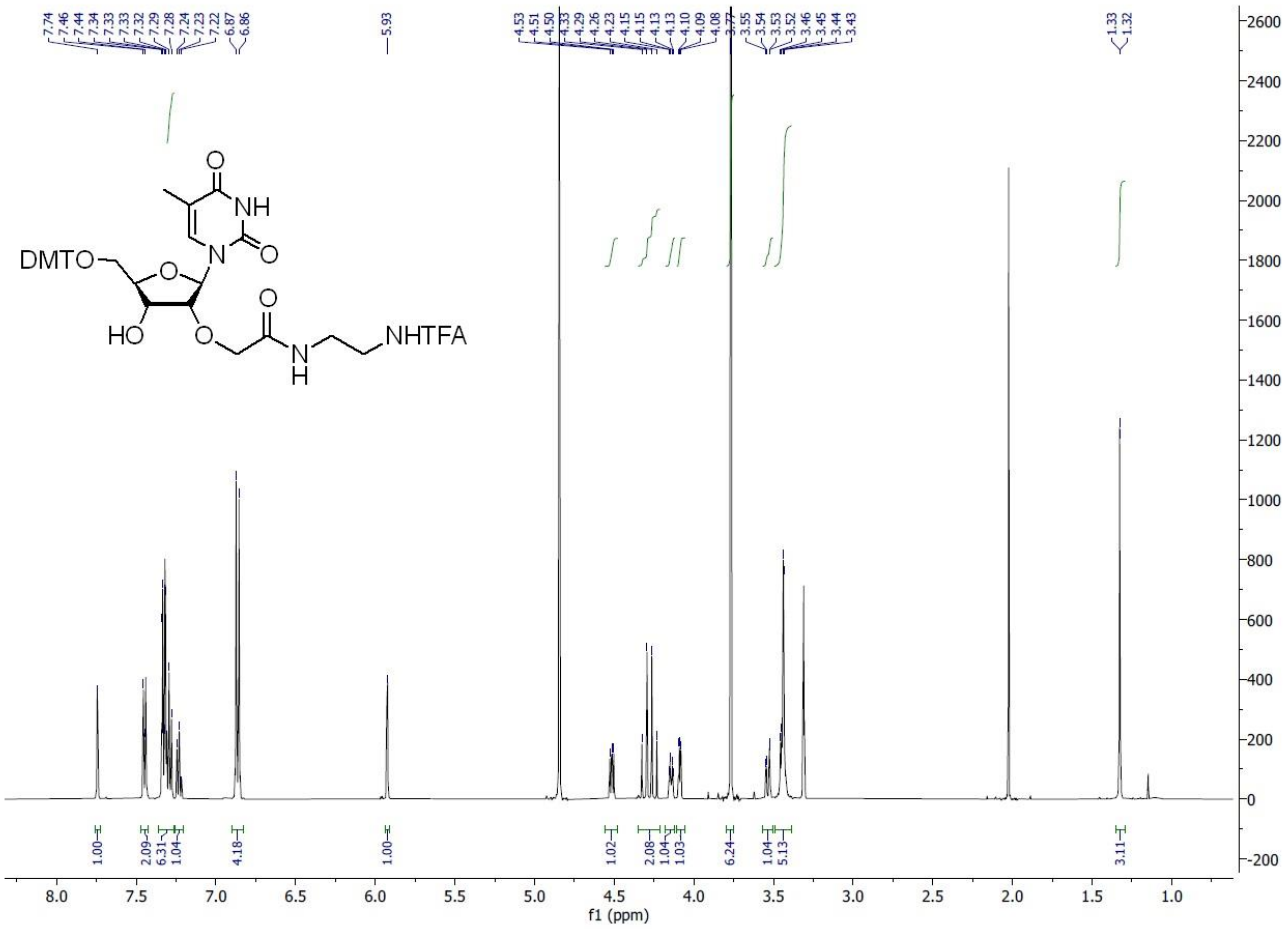

*Figure S9. ^{1}H NMR spectrum of compound **6** (500 MHz, CD_3OD).*

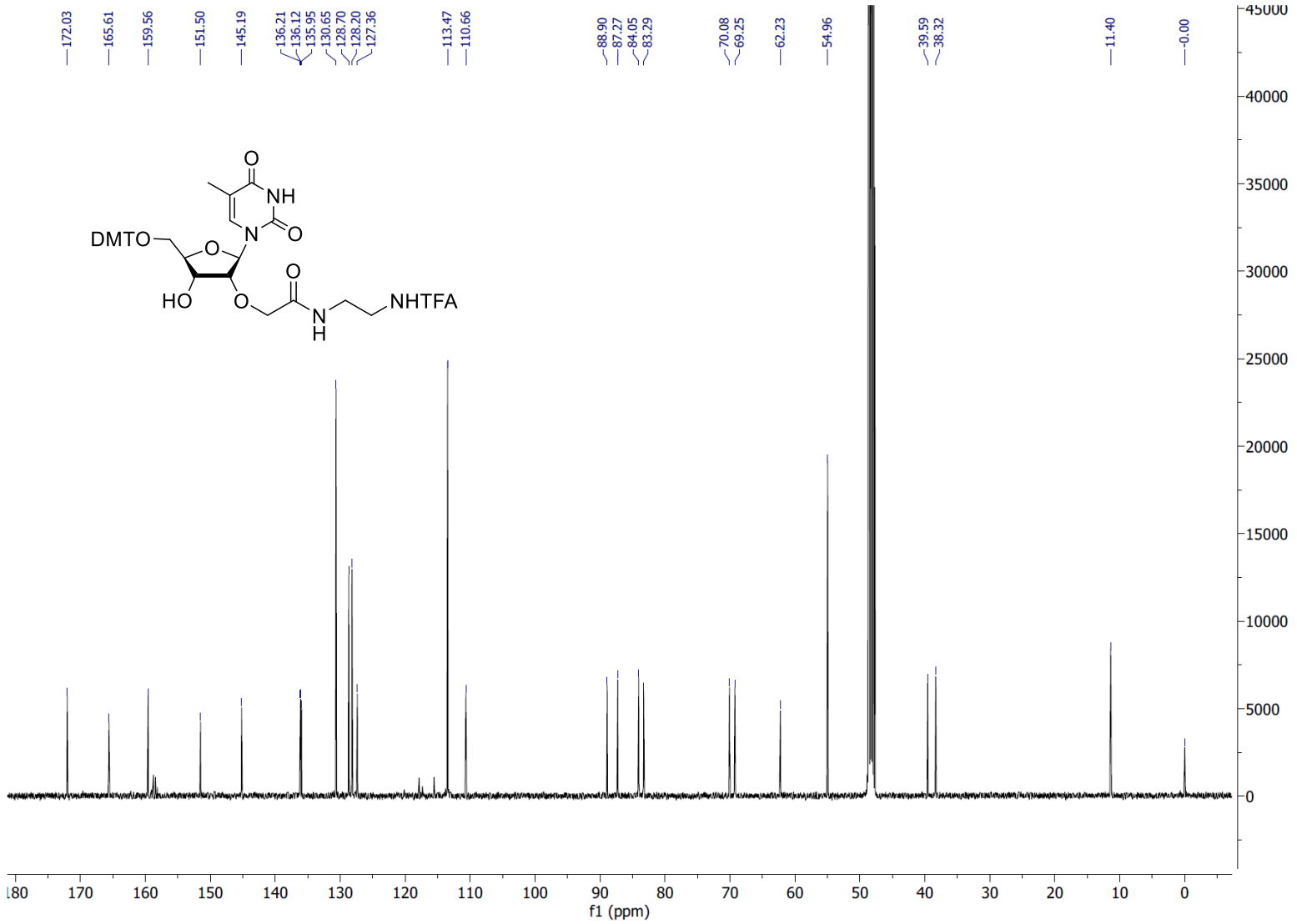

*Figure S10. ^{13}C NMR spectrum of compound **6** (125.76 MHz, CD_3OD).*

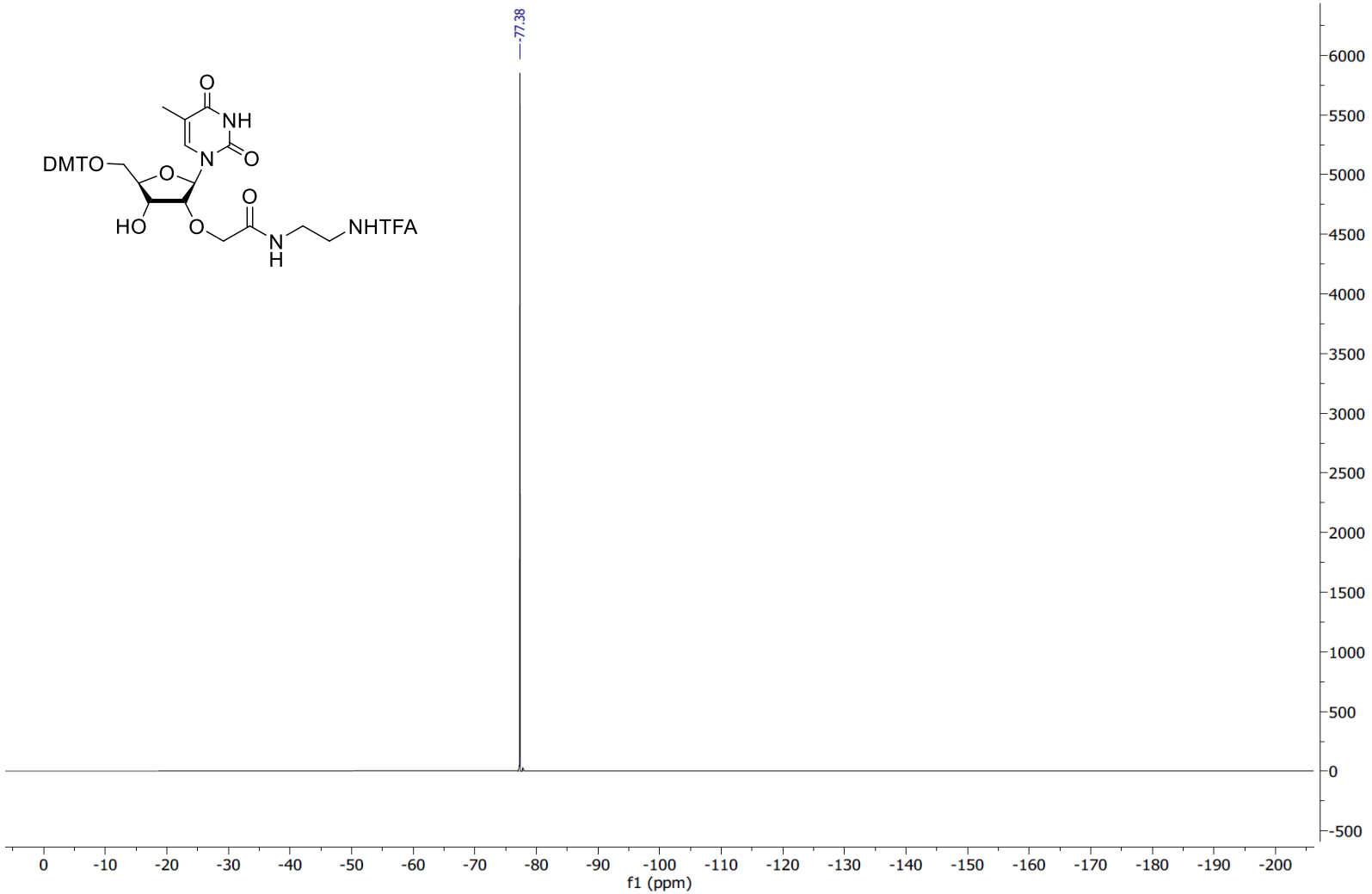

*Figure S11. ^{19}F NMR spectrum of compound **6** (470.56 MHz, CD_3OD).*

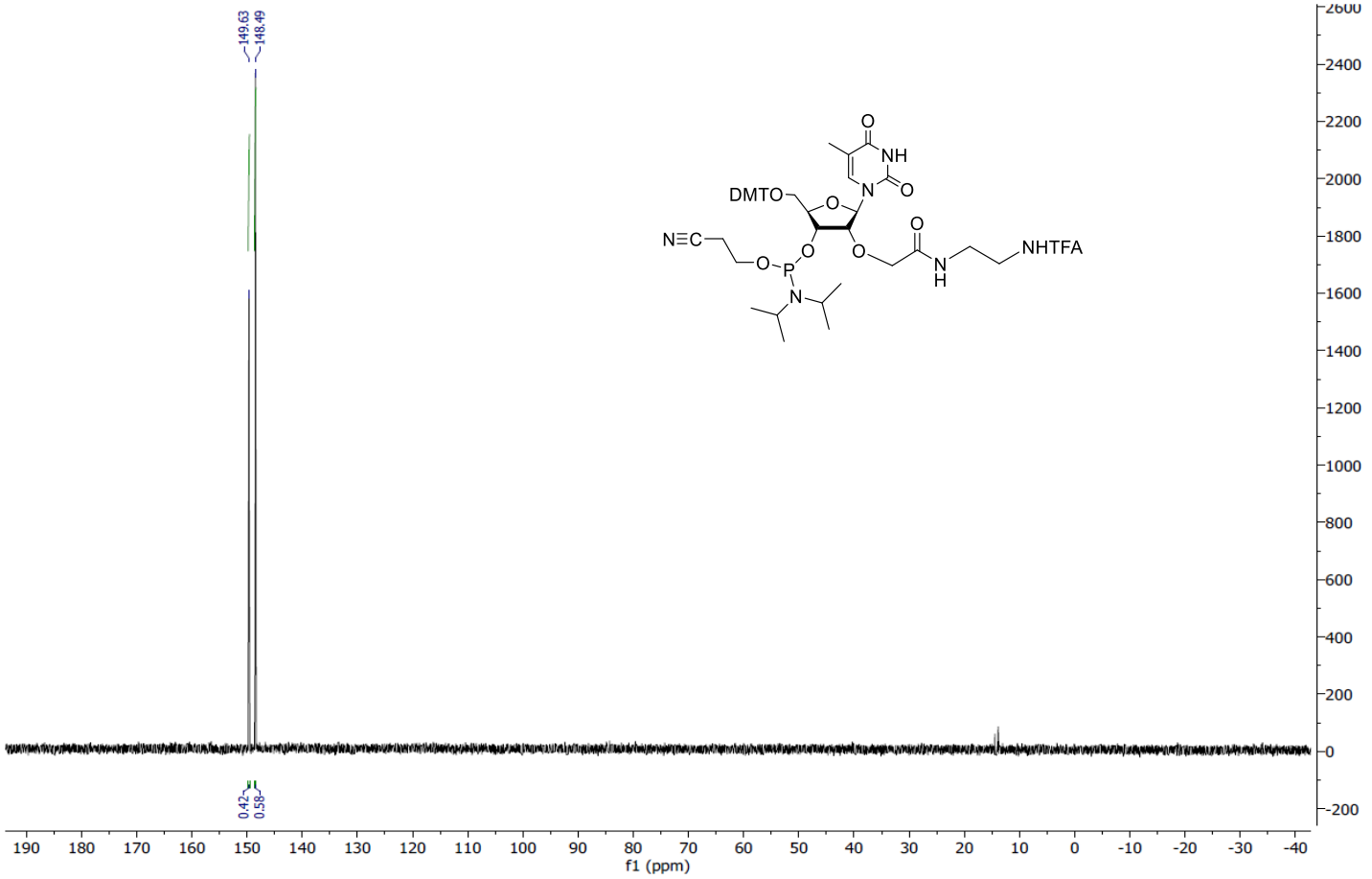

*Figure S12. ^{31}P NMR spectrum of compound **7** (202.47 MHz, CD_3CN).*

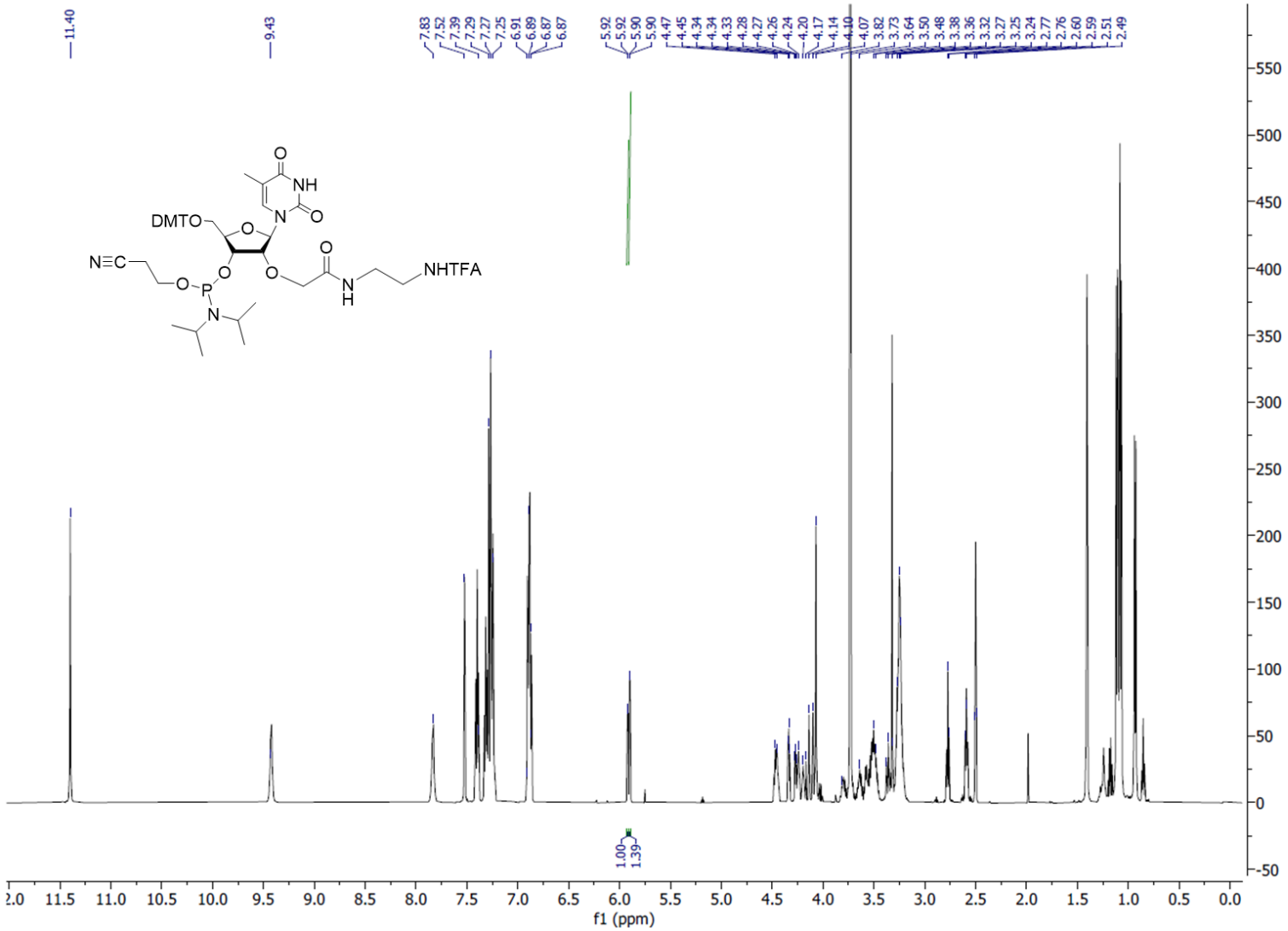

*Figure S13 .[1]H NMR spectrum of compound **7** (500 MHz, DMSO-d_6).*

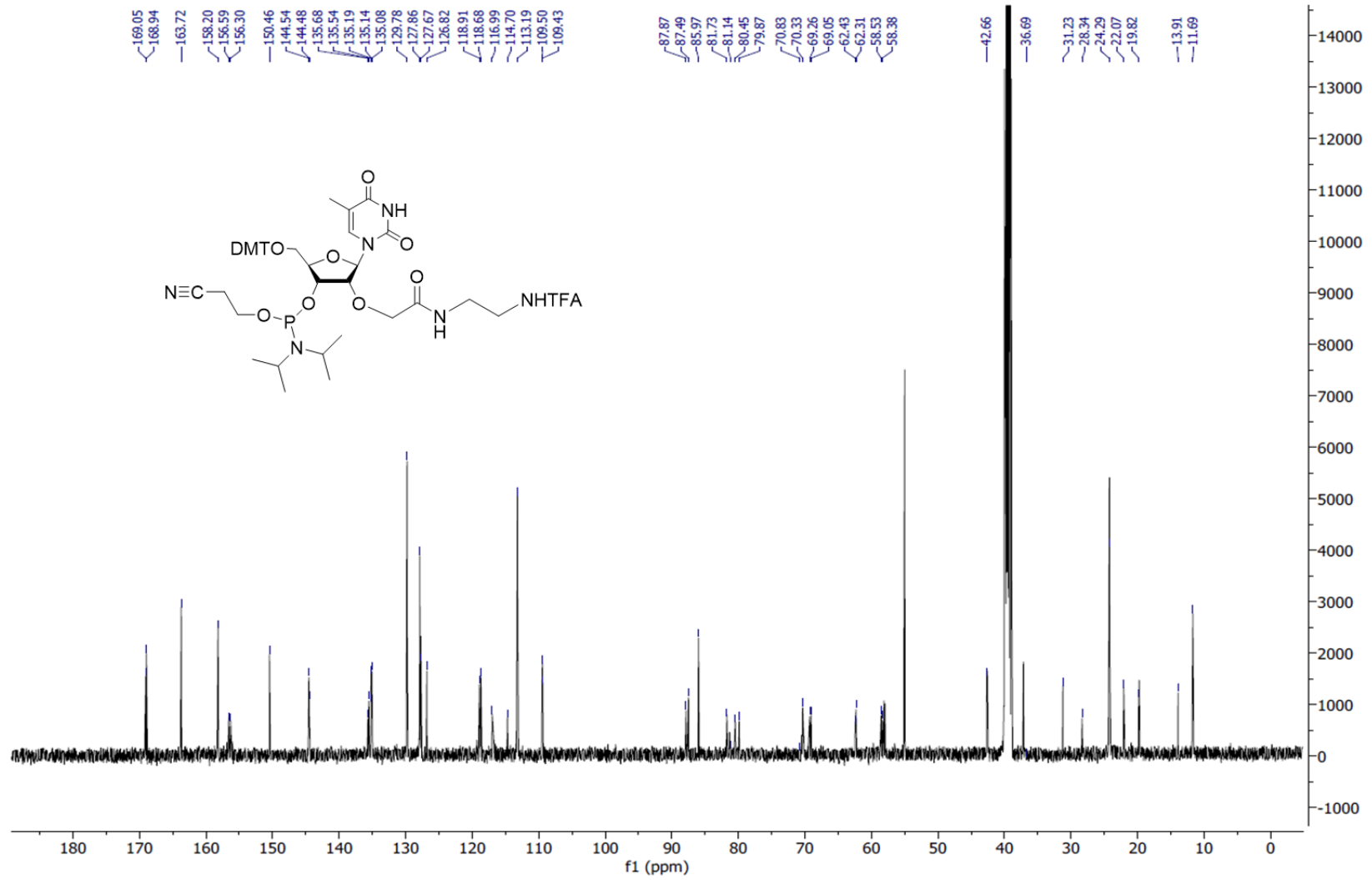

*Figure S14. ^{13}C NMR spectrum of compound **7** (125.76 MHz, DMSO-d_6).*

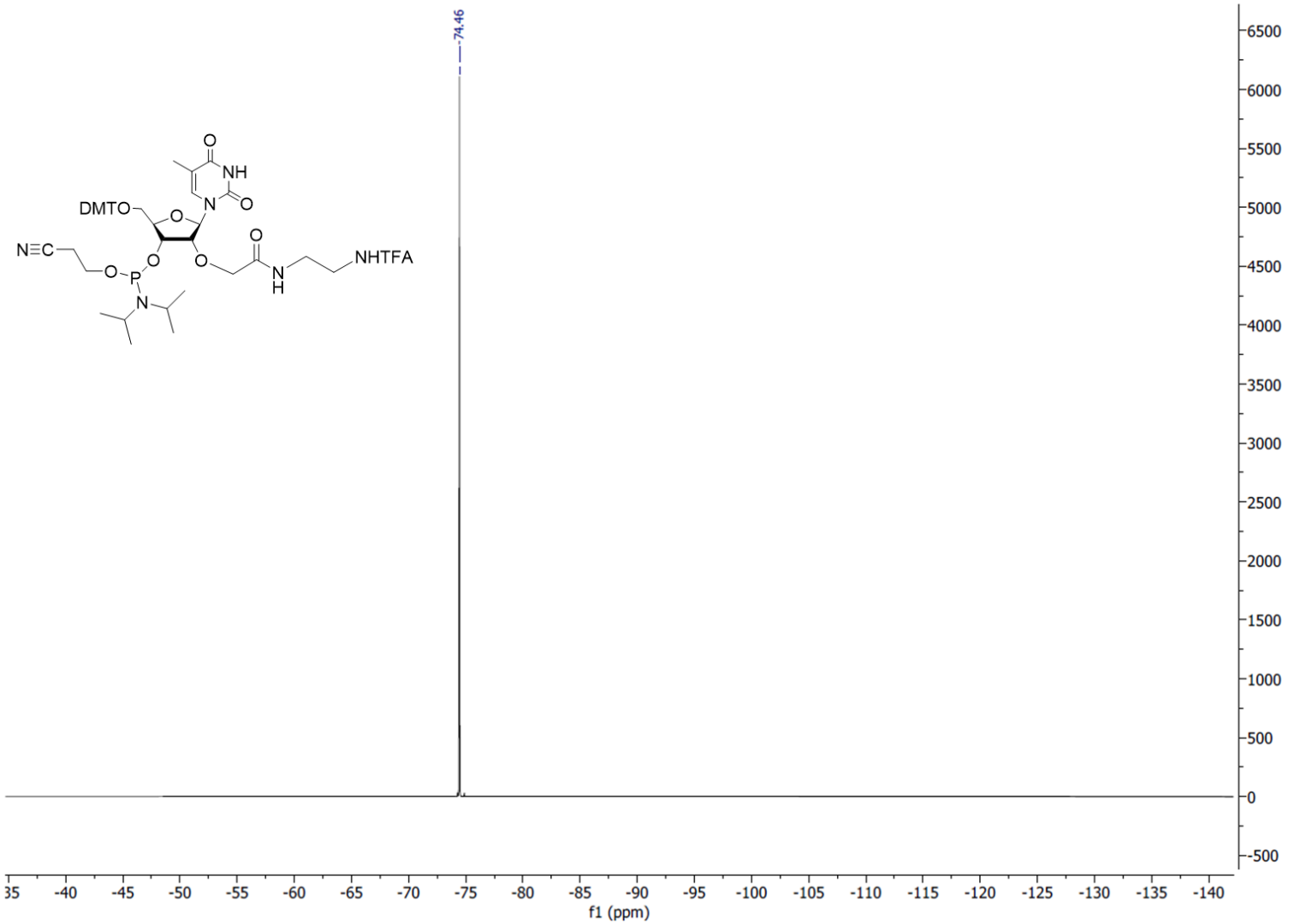

*Figure S15. ^{19}F NMR spectrum of compound **7** (470.56 MHz, DMSO-d_6).*

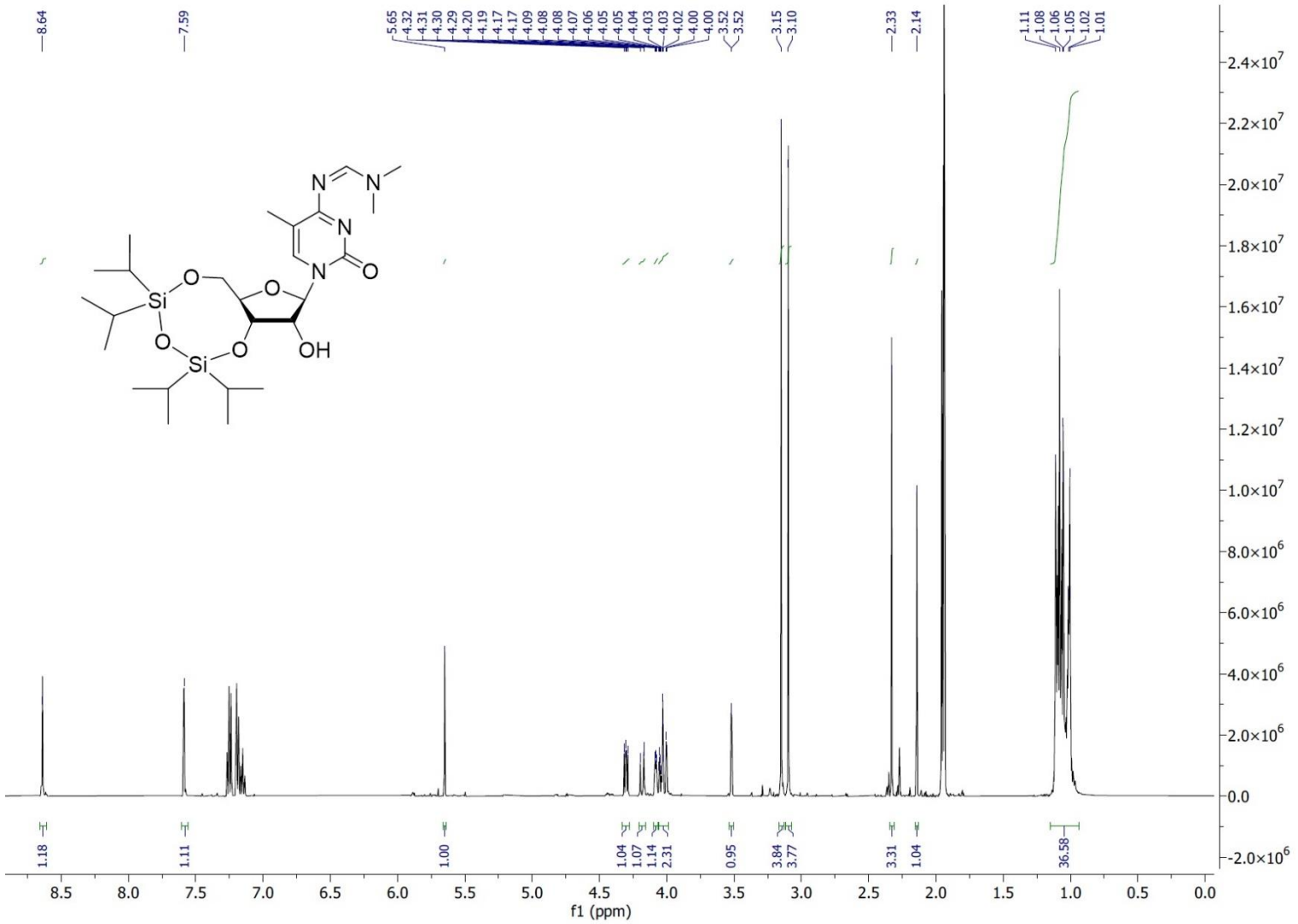

*Figure S16. ^{1}H NMR spectrum of crude compound **9** (500 MHz, CD_3CN).*

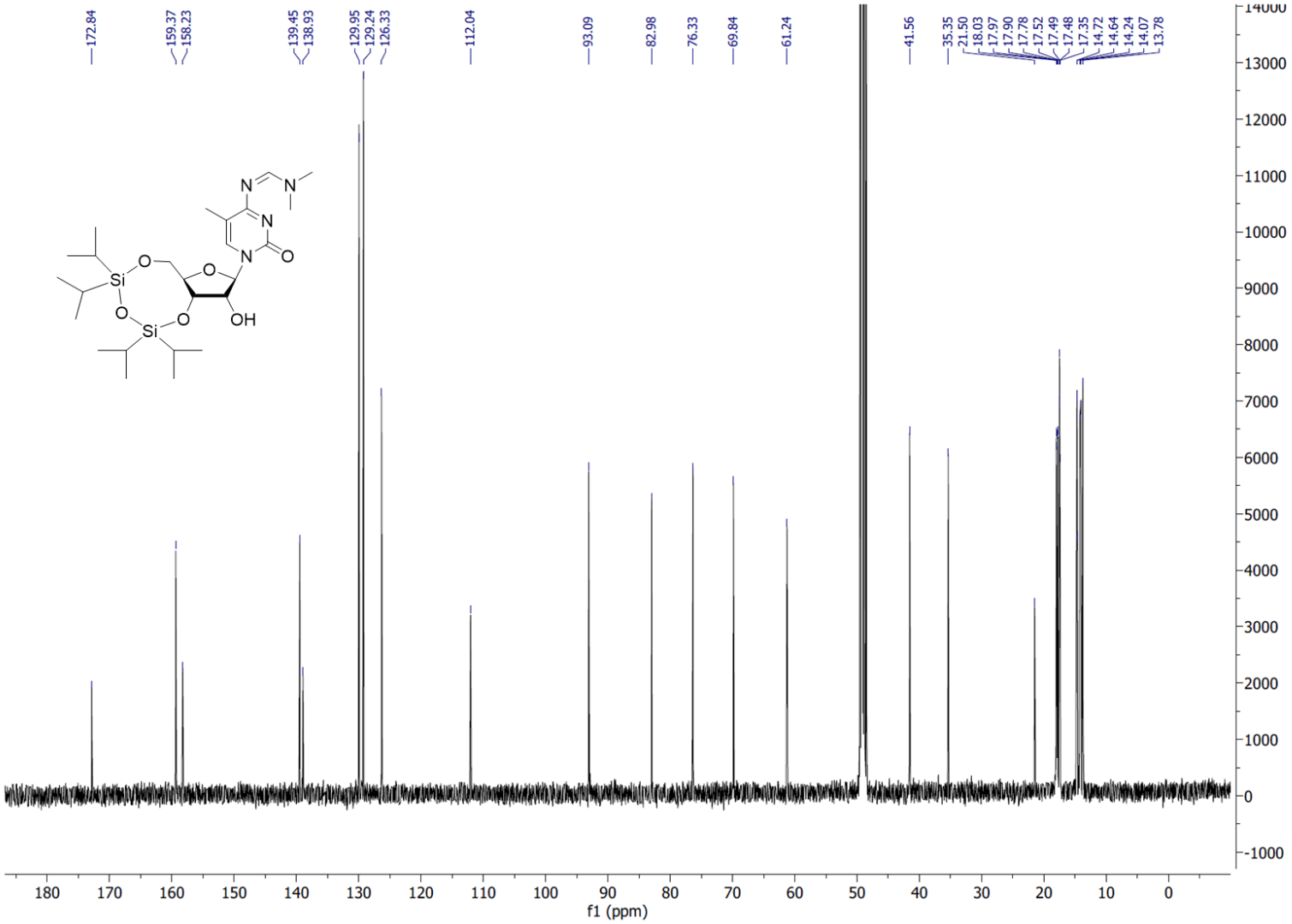

*Figure S17. ^{13}C NMR spectrum of crude compound **9** (125.76 MHz, CD_3OD).*

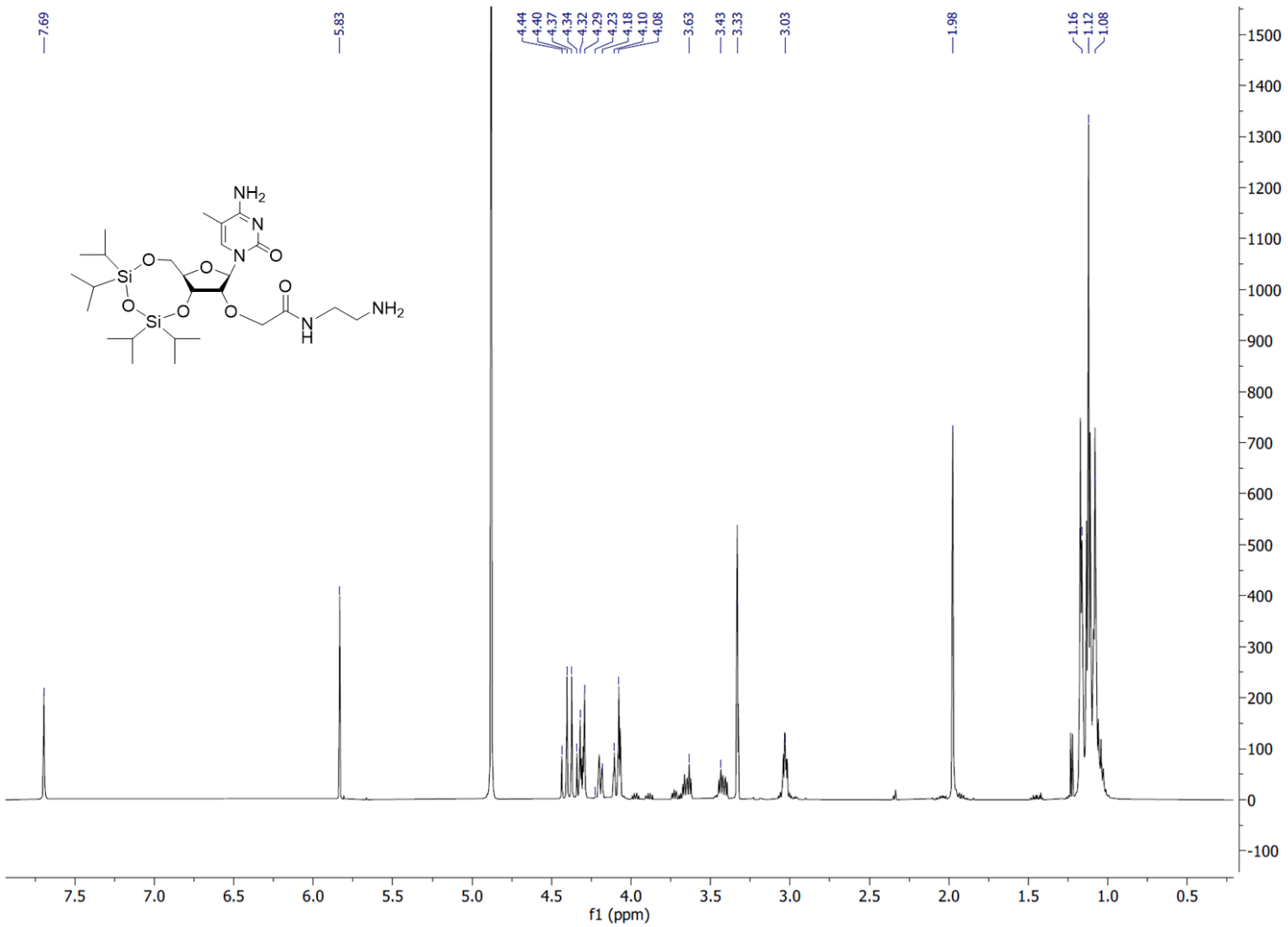

*Figure S18. ^{1}H NMR spectrum of crude compound **11** (500 MHz, CD_3OD).*

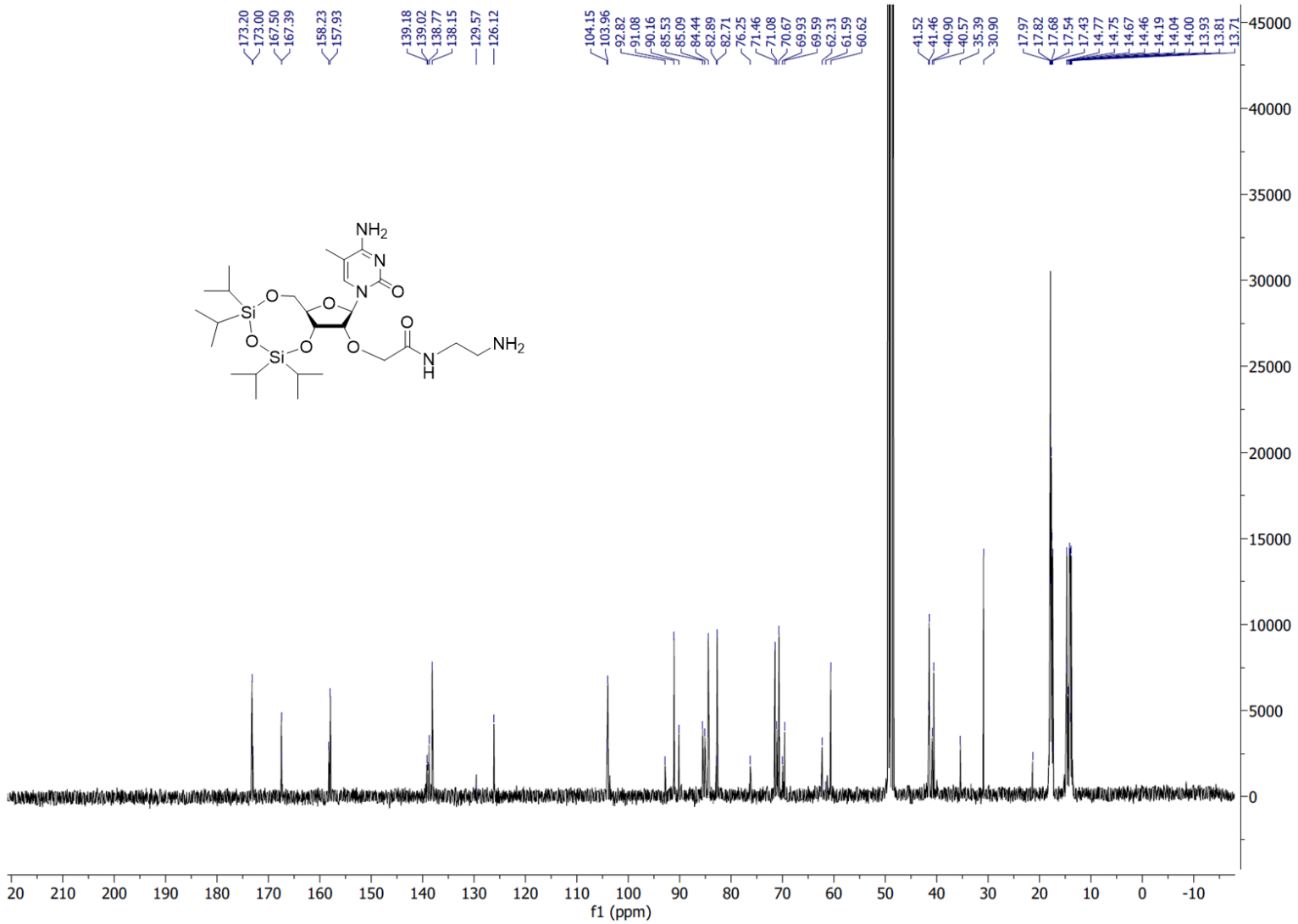

*Figure S19. ^{13}C NMR spectrum of crude compound **11** (125.76 MHz, CD_3OD).*

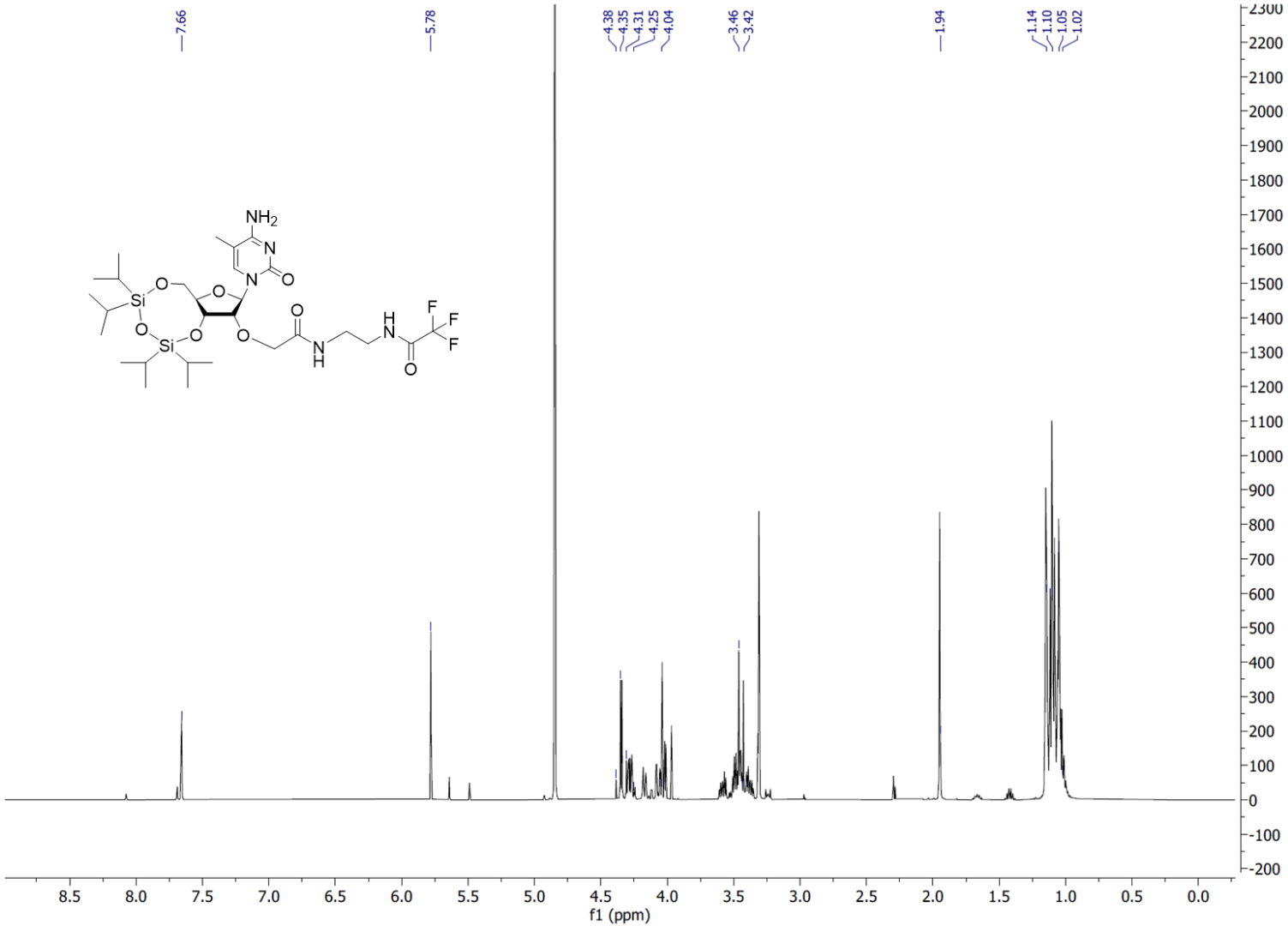

*Figure S20. ^{1}H NMR spectrum of crude compound **12** (500 MHz, CD_3OD).*

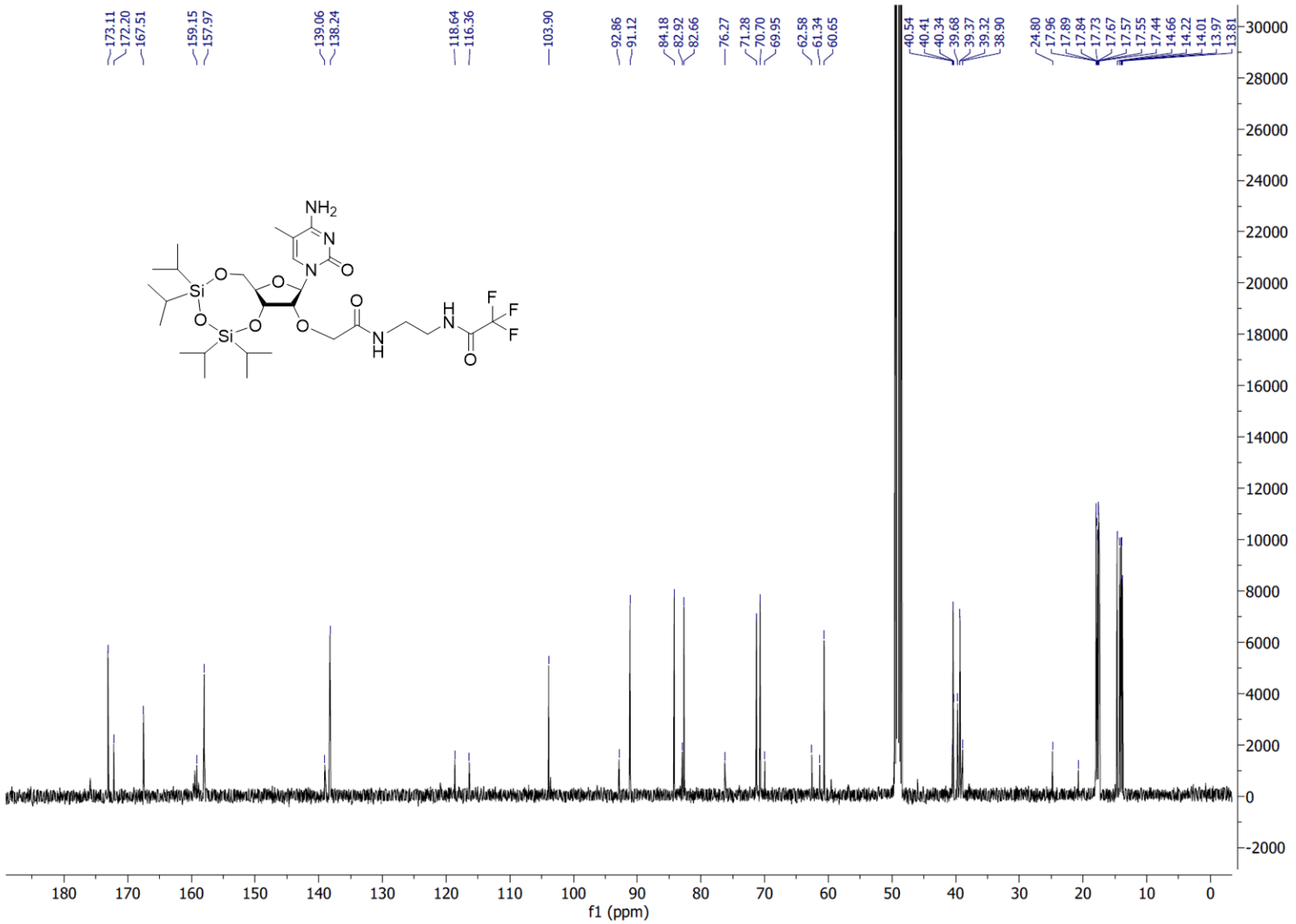

*Figure S21. ^{13}C NMR spectrum of crude compound **12** (125.76 MHz, CD_3OD).*

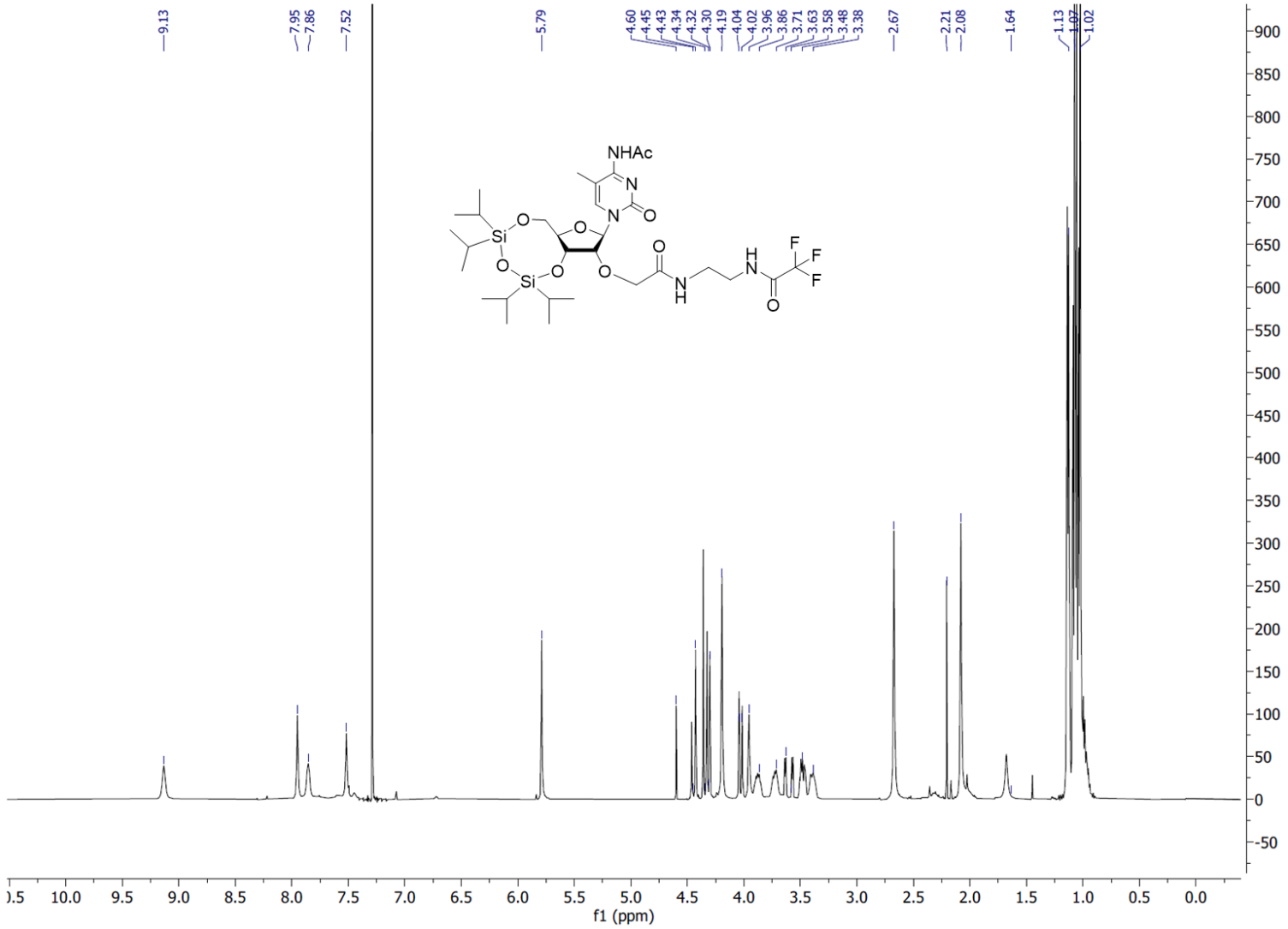

*Figure S22. ^{1}H NMR spectrum of crude compound **13** (500 MHz, $CDCl_3$).*

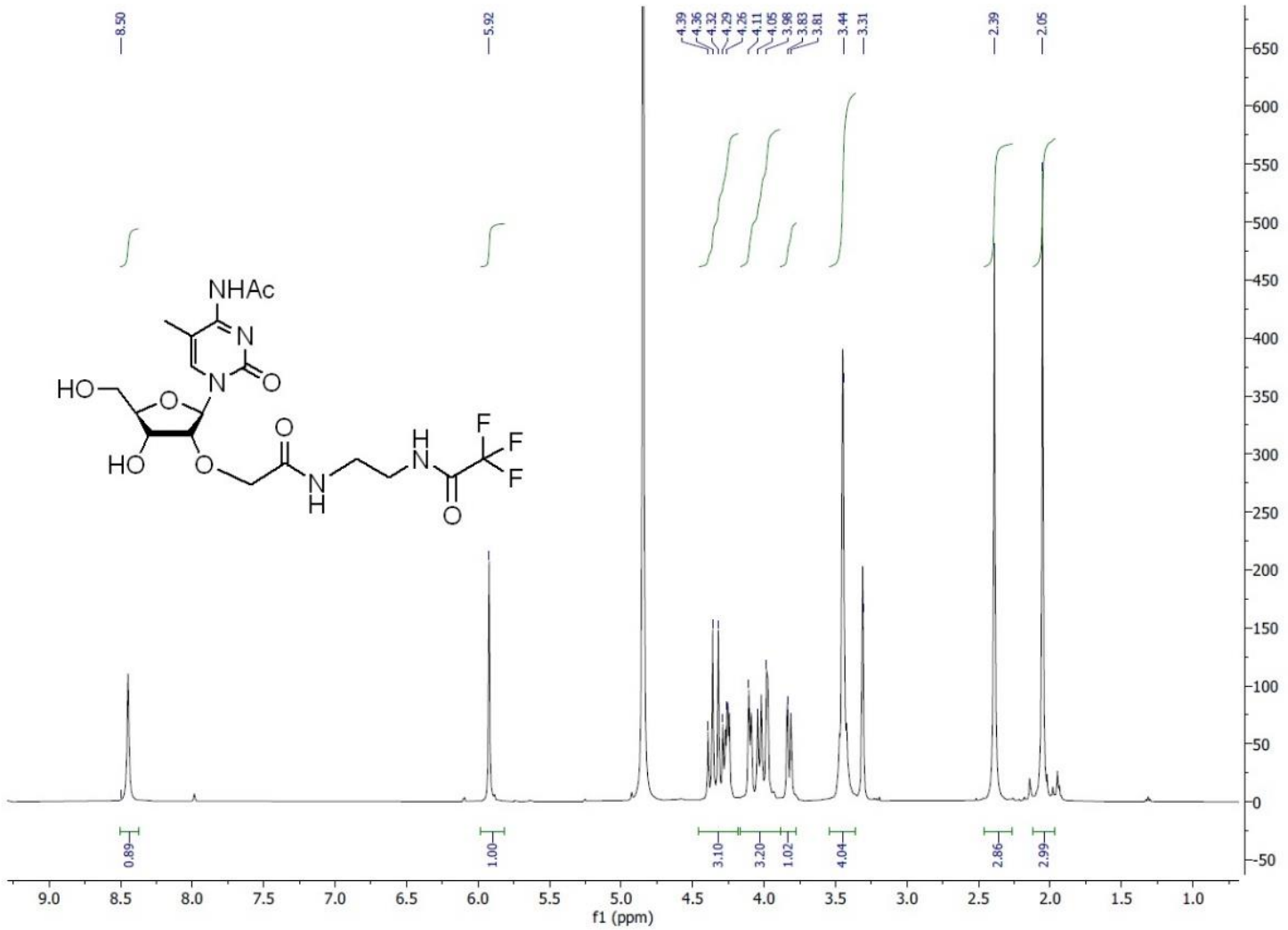

*Figure S23. ^{1}H NMR spectrum of compound **14** (500 MHz, CD_3OD).*

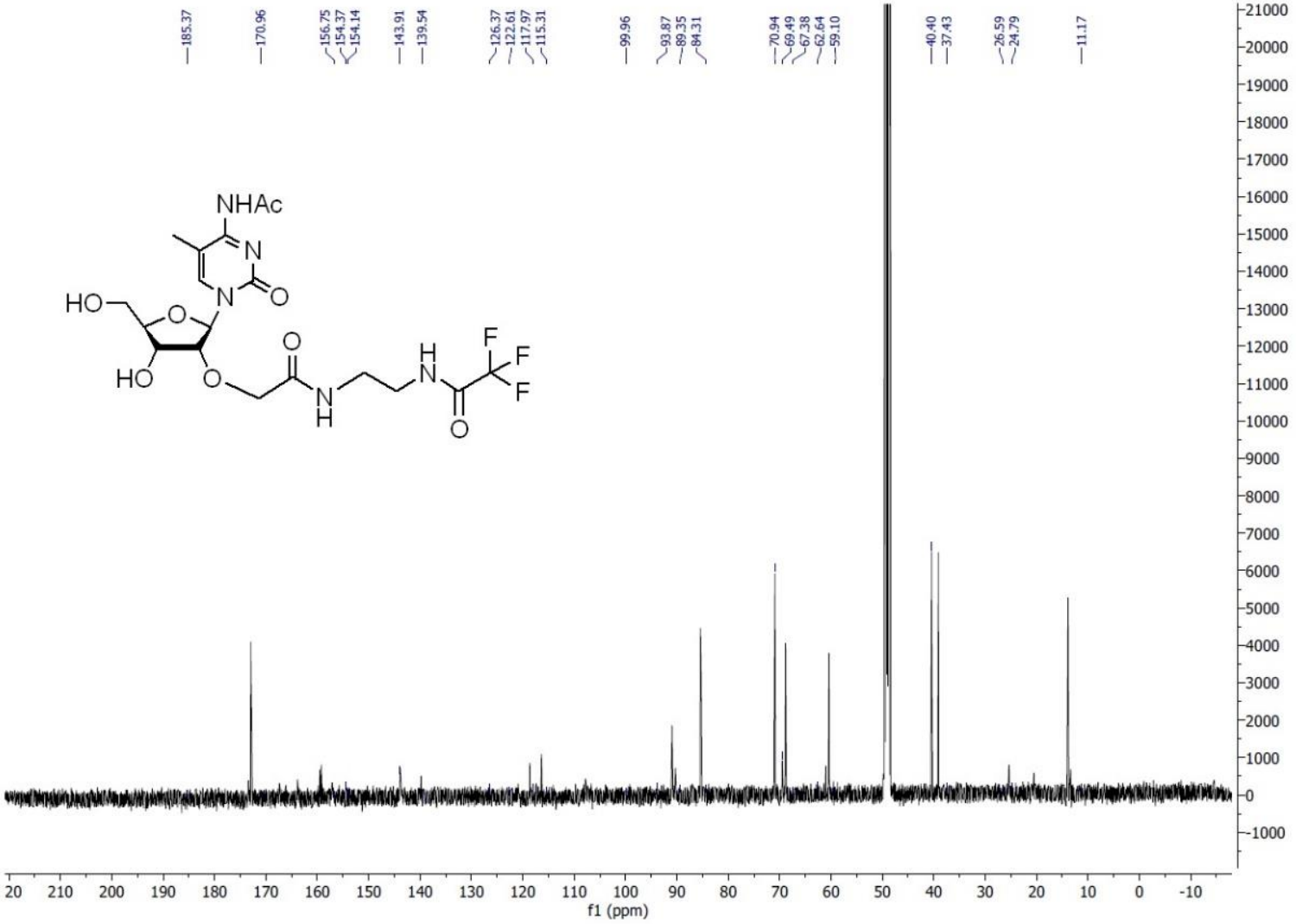

*Figure S24. ^{13}C NMR spectrum of compound **14** (125.76 MHz, CD_3OD).*

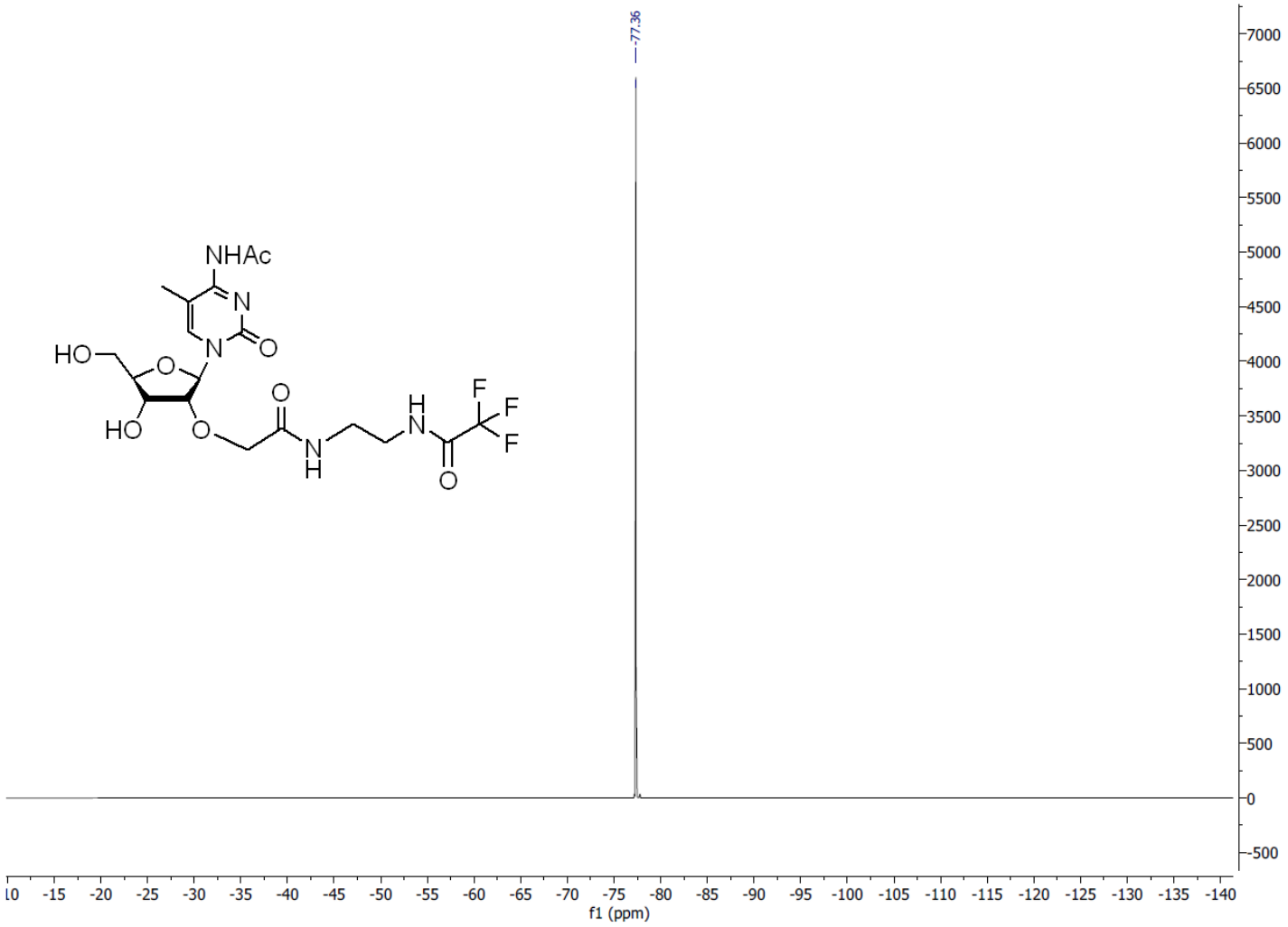

*Figure S25. ^{19}F NMR spectrum of compound **14** (470.56 MHz, CD_3OD).*

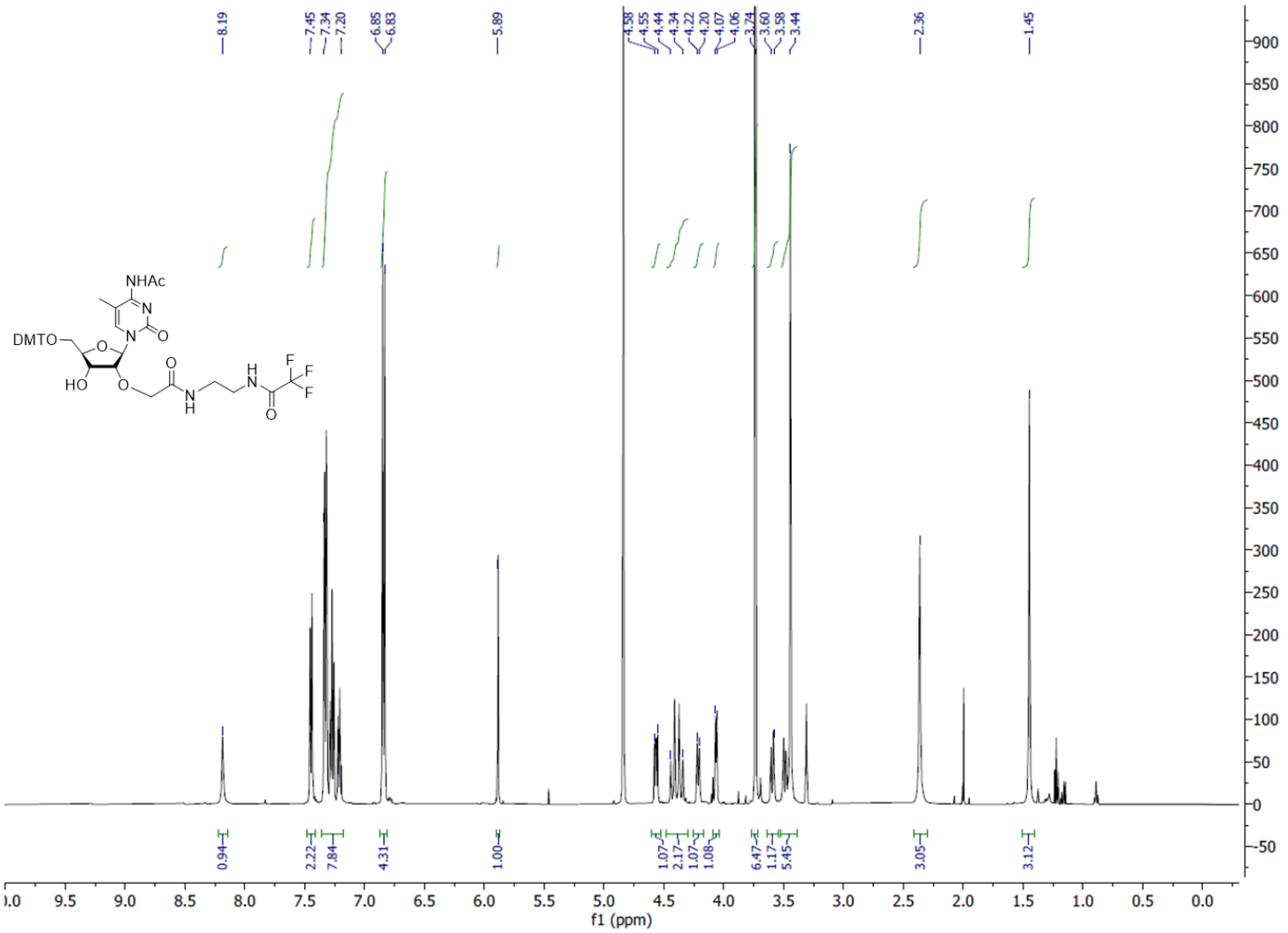

*Figure S26. ^{1}H NMR spectrum of compound **15** (500 MHz, CD_3OD)*

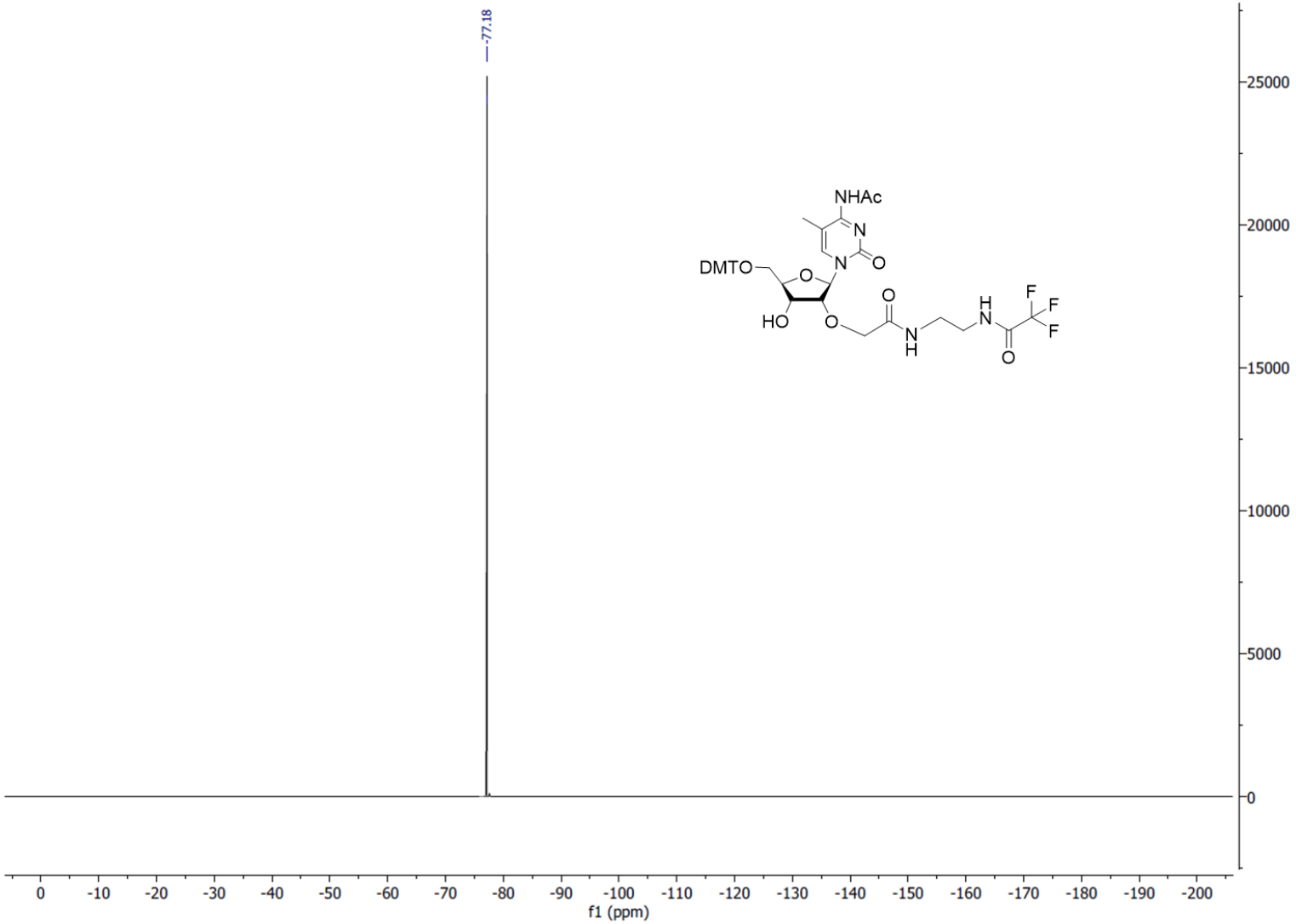

*Figure S27. ^{19}F NMR spectrum of compound **15** (470.56 MHz, CD_3OD).*

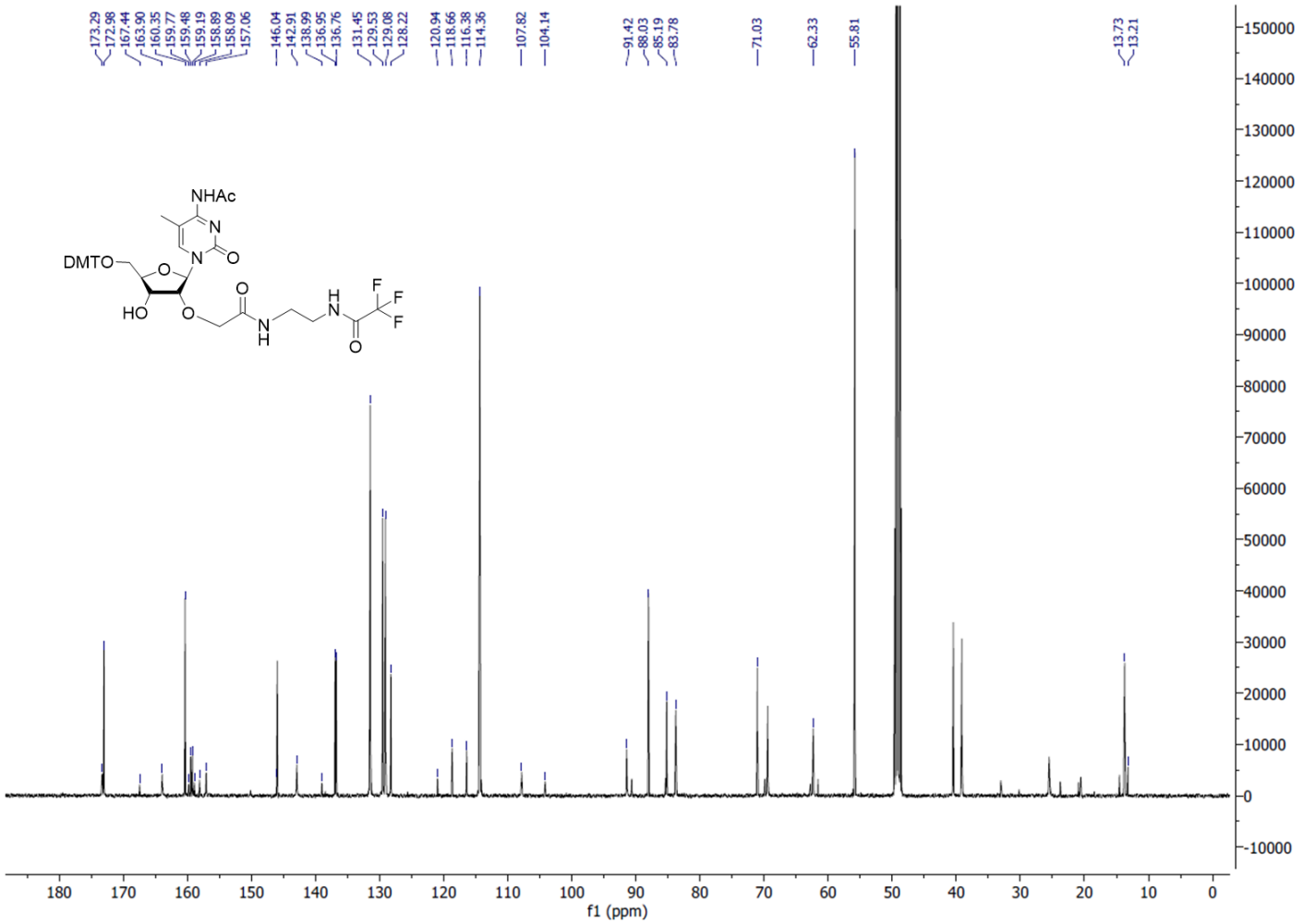

*Figure S28. ^{13}C NMR spectrum of compound **15** (125.76 MHz, CD_3OD).*

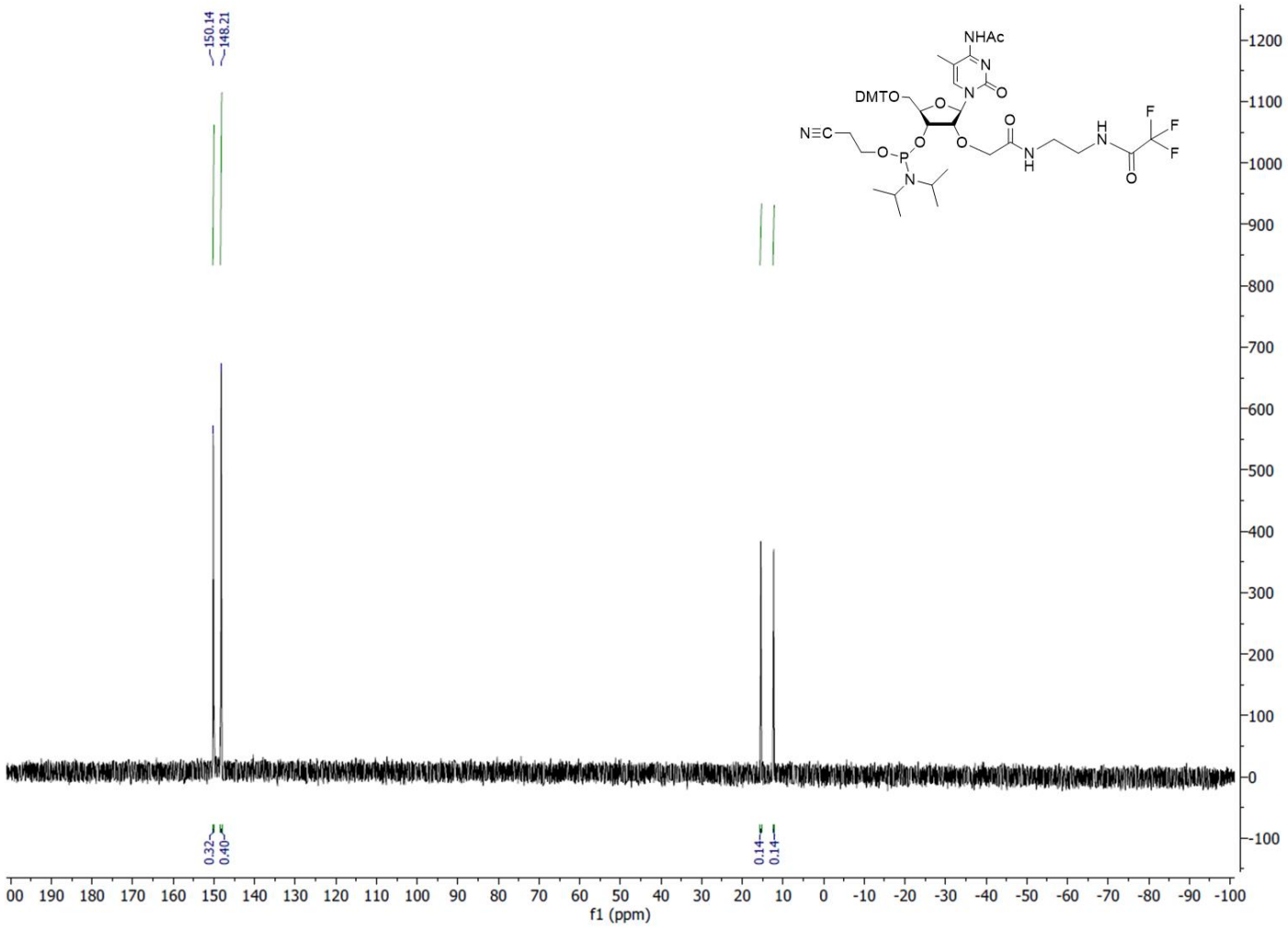

Figure S29. ^{31}*P NMR spectrum of compound* ***16*** *(202.47 MHz, CD_3CN) before reversed phase chromatography.*

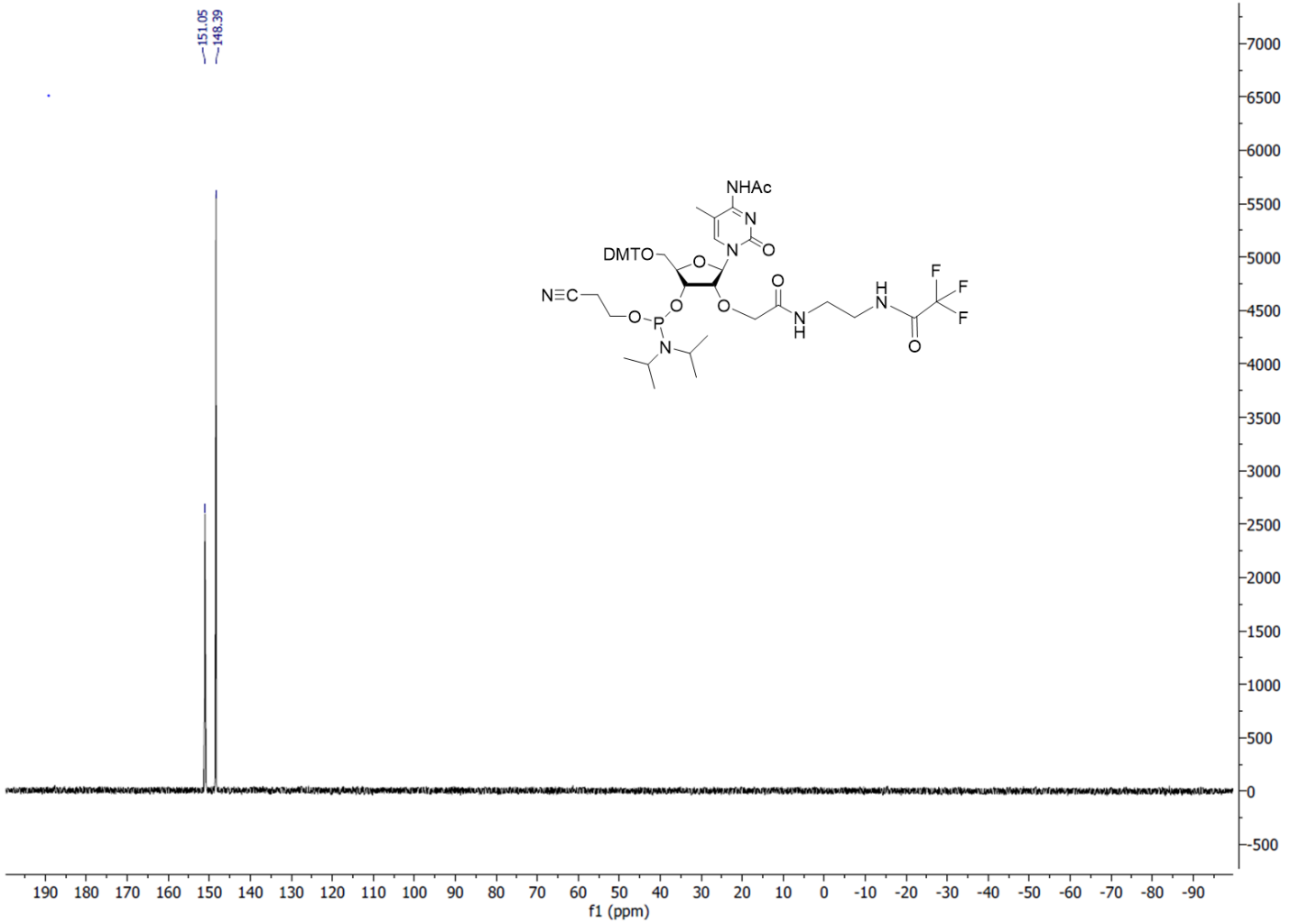

Figure S30. ^{31}*P NMR spectrum of compound* ***16*** *(202.47 MHz,* CD_3CN*) after reversed phase chromatography.*

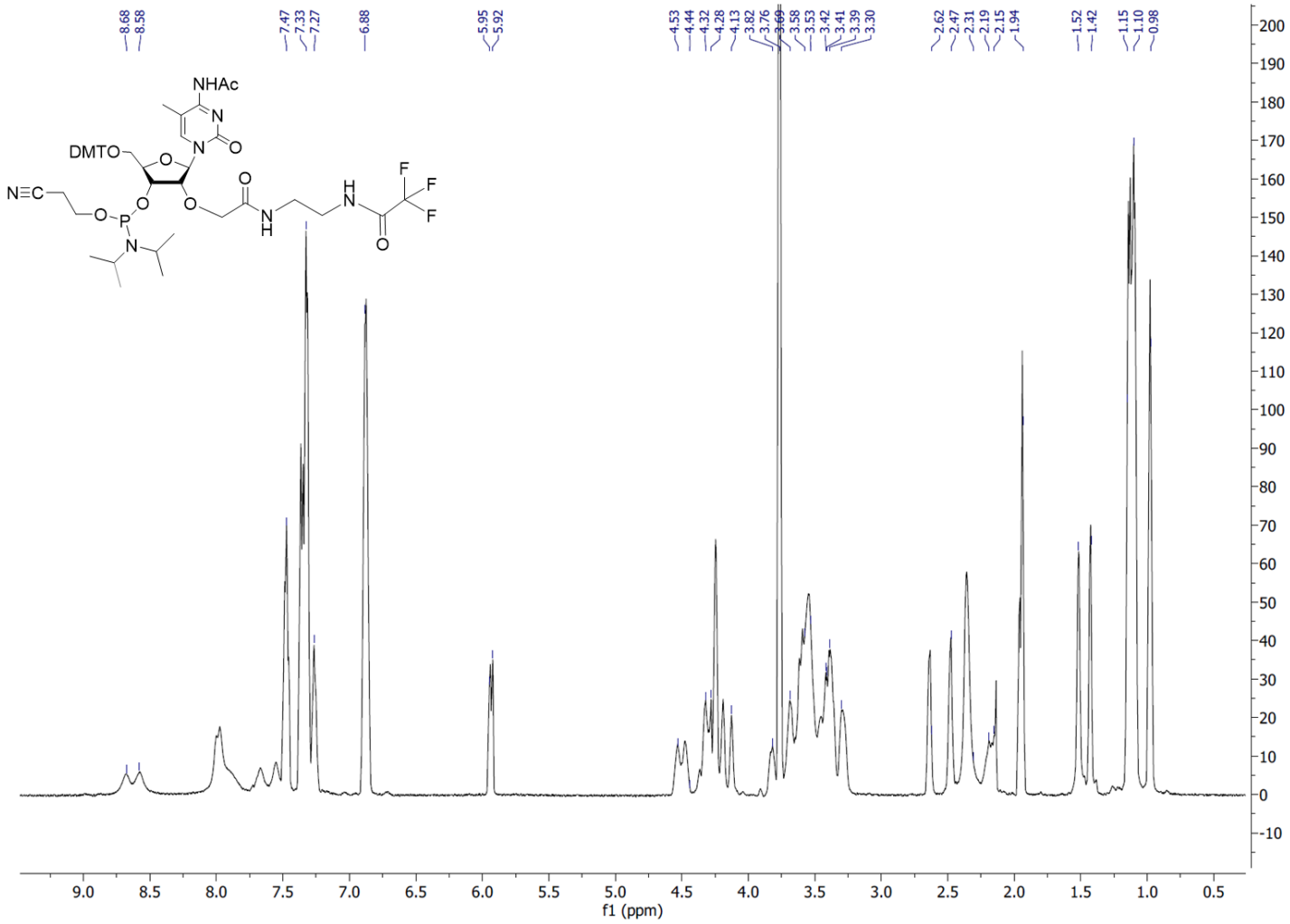

*Figure S31. ^{1}H NMR spectrum of compound **16** (500 MHz, CD_3CN) after reversed phase chromatography.*

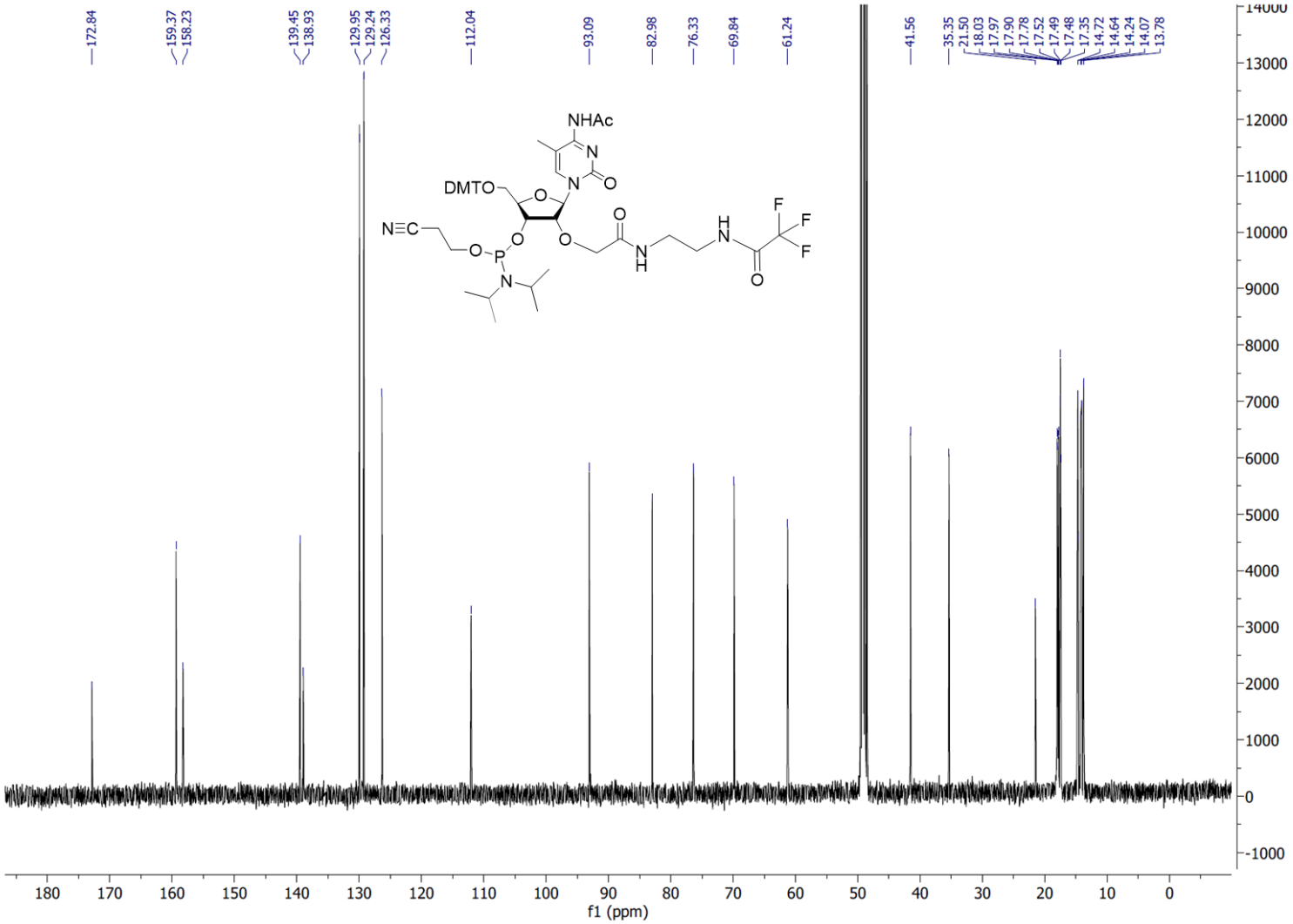

*Figure S32. ^{13}C NMR spectrum of compound **16** (125.76 MHz, CD_3CN) after reversed phase chromatography.*

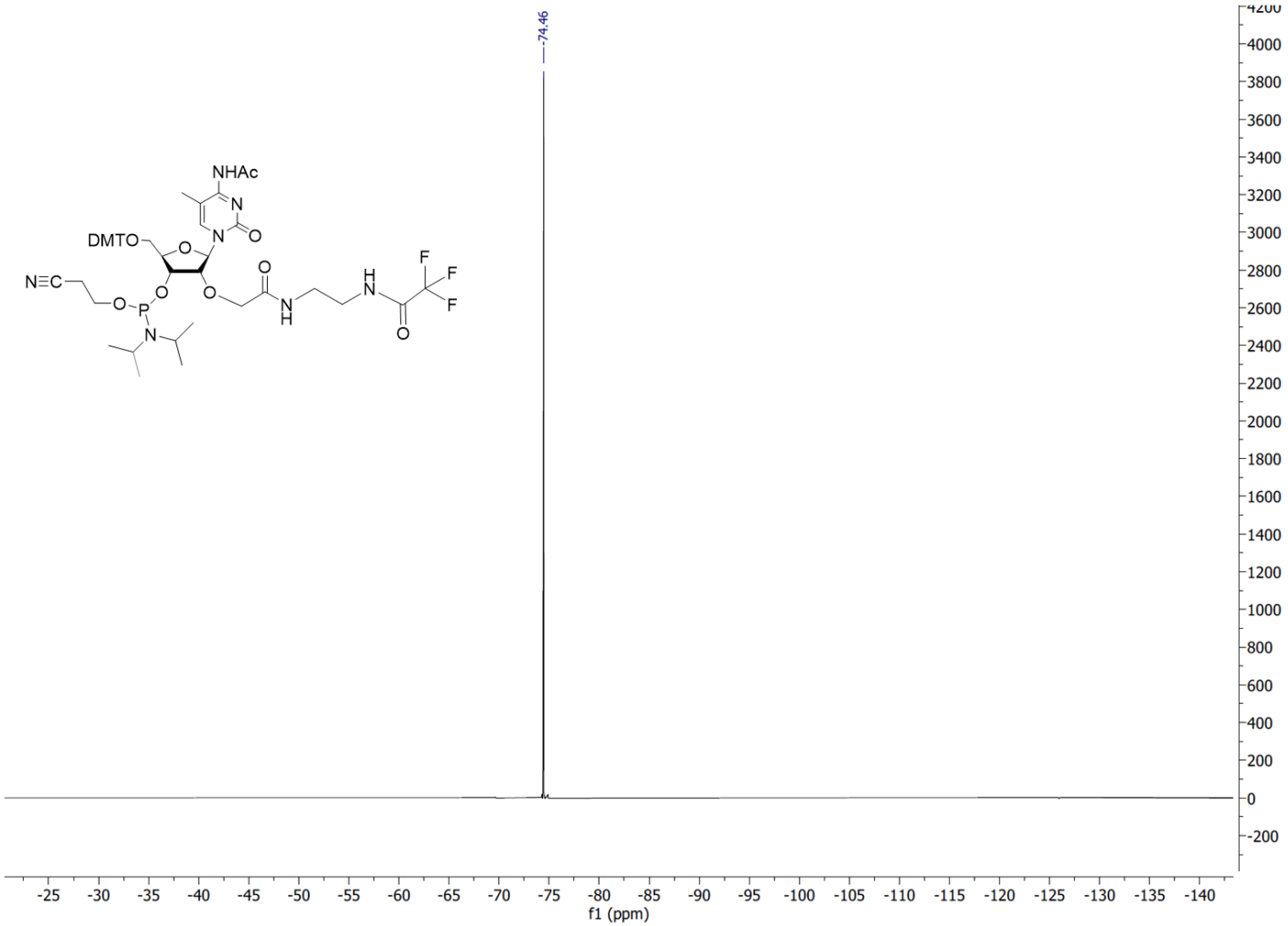

*Figure S33. ^{19}F NMR spectrum of compound **16** (470.56 MHz, DMSO-d_6) after reversed phase chromatography.*

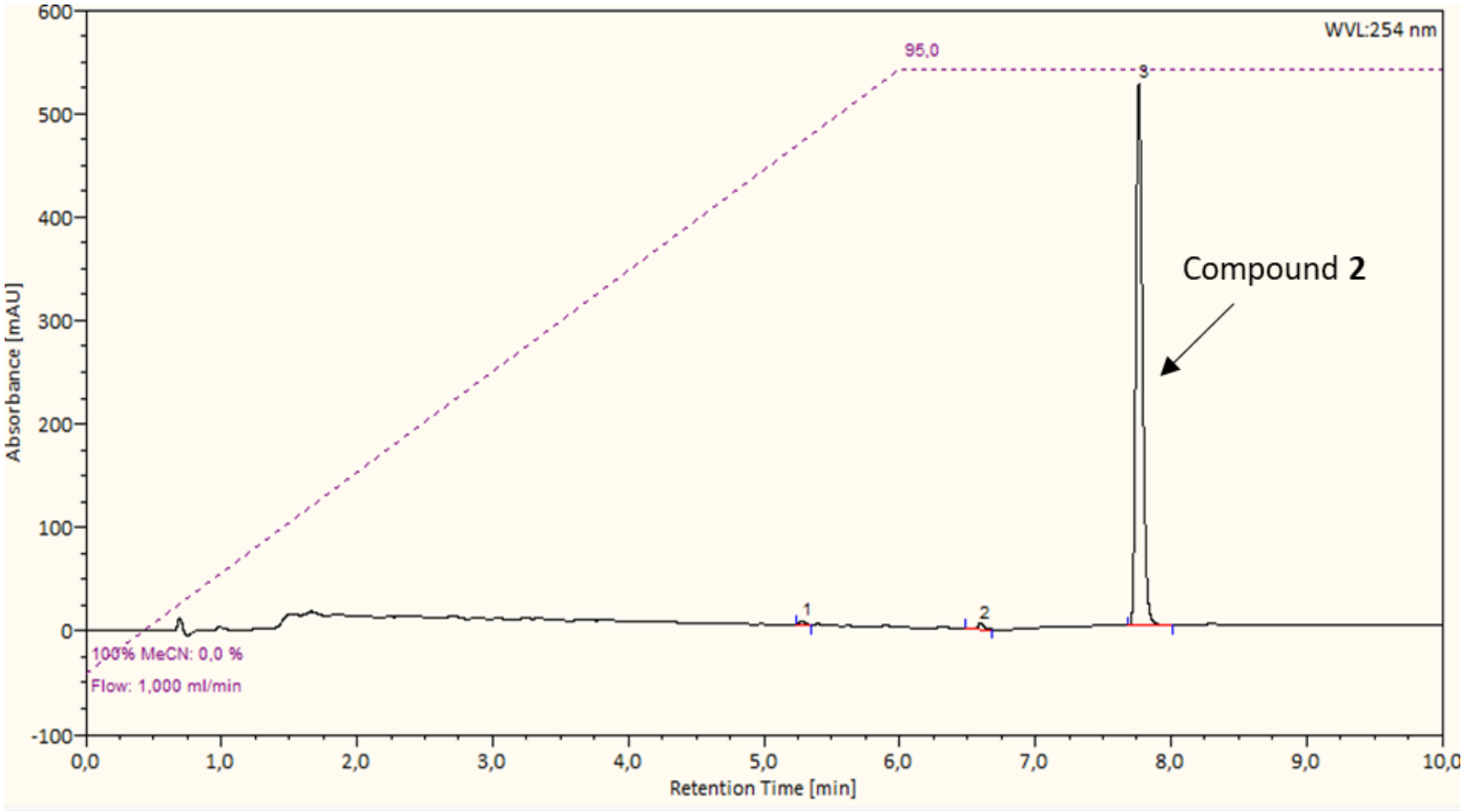

Figure S34 RP-HPLC profile of compound **2** *using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

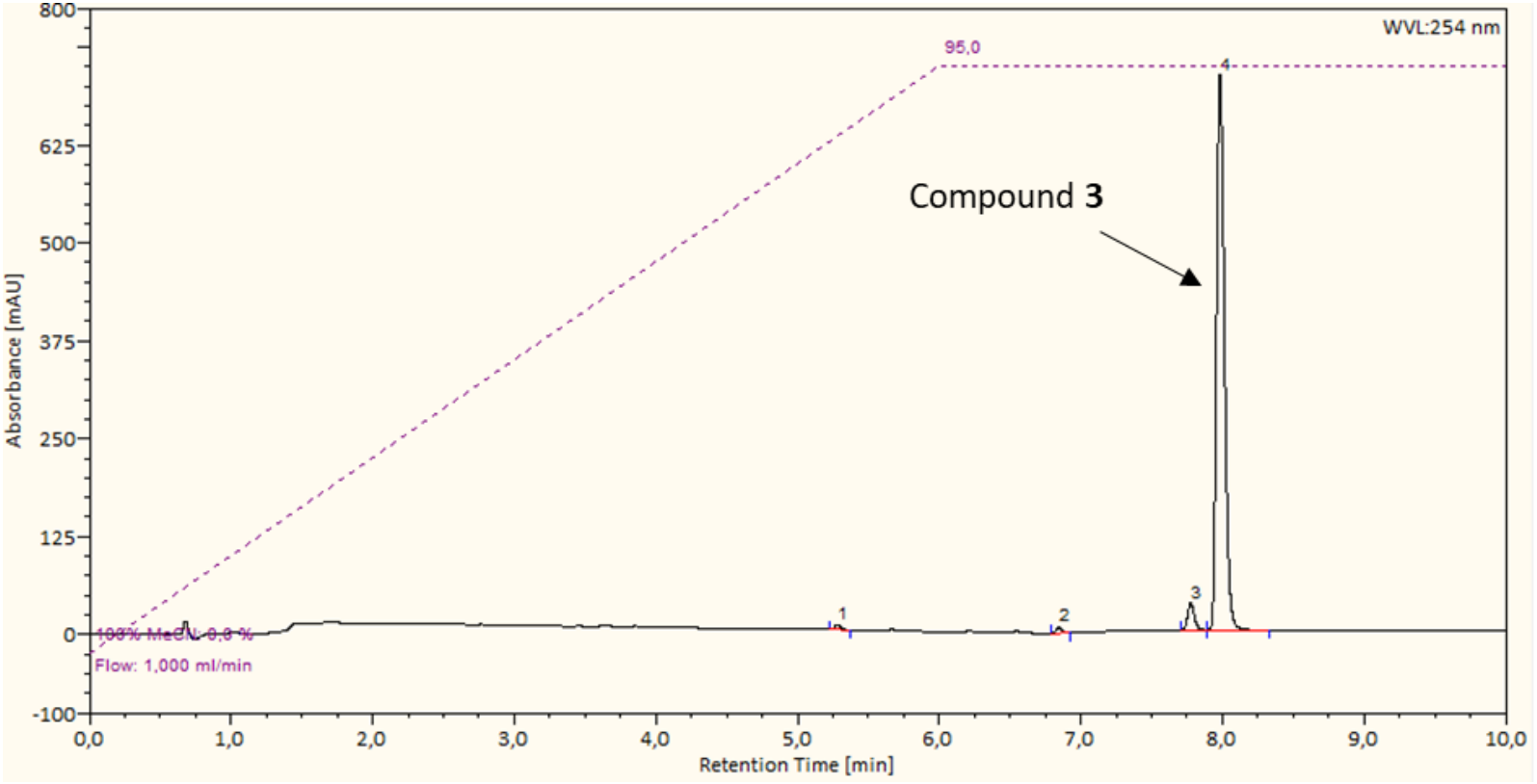

*Figure S35. RP-HPLC profile of compound **3** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

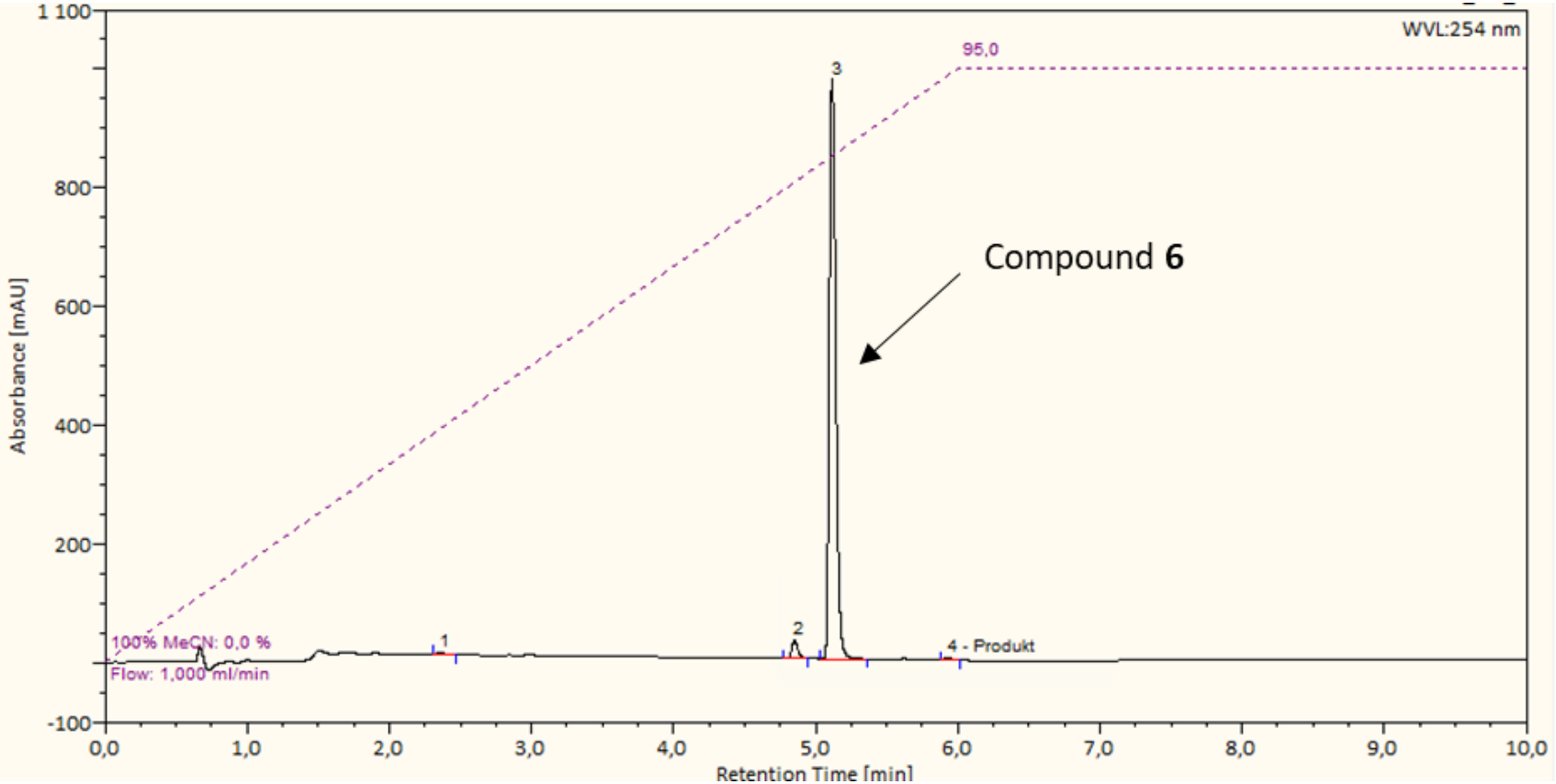

*Figure S36. RP-HPLC profile of compound **6** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

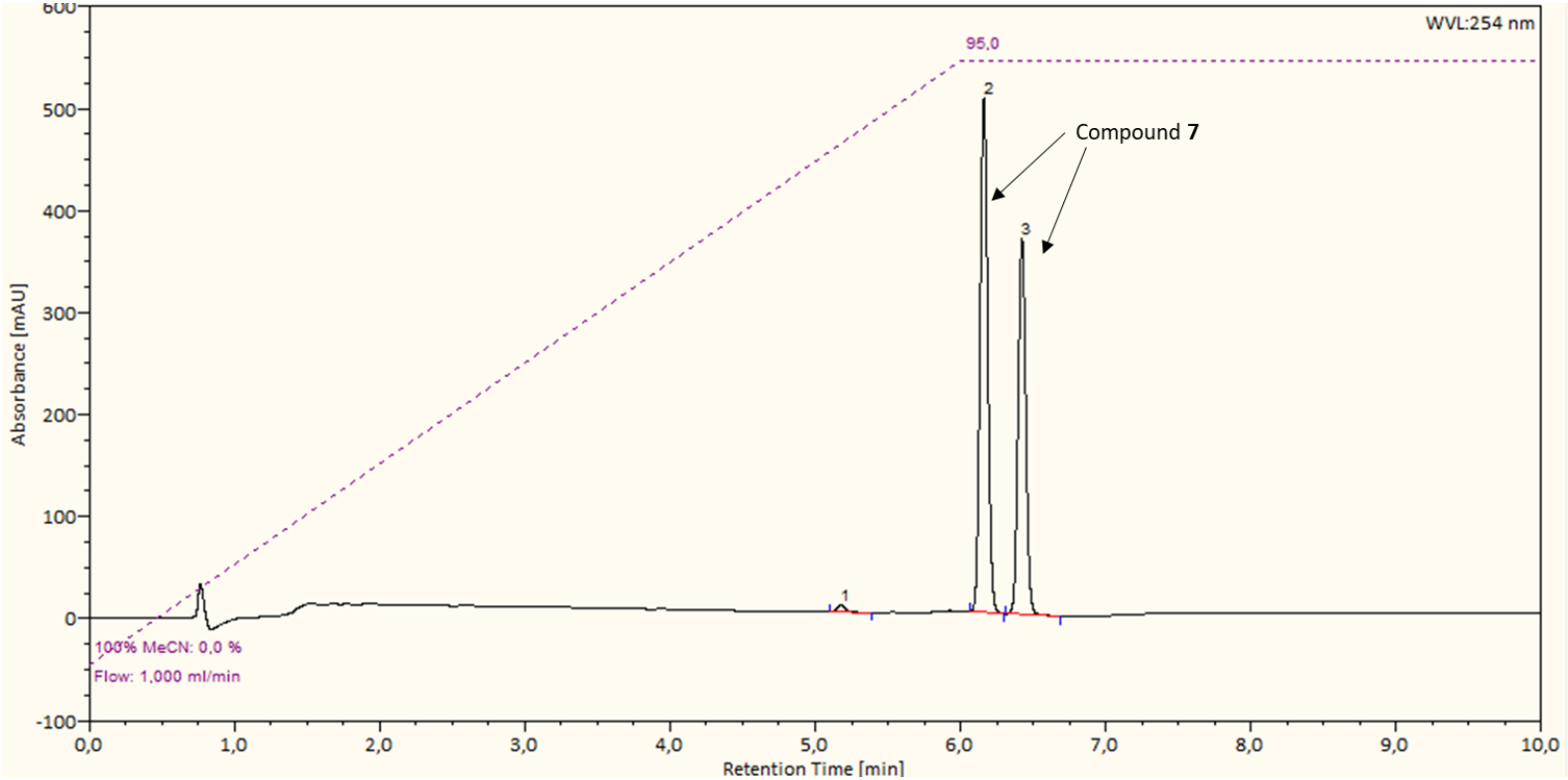

*Figure S37. RP-HPLC profile of compound **7** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

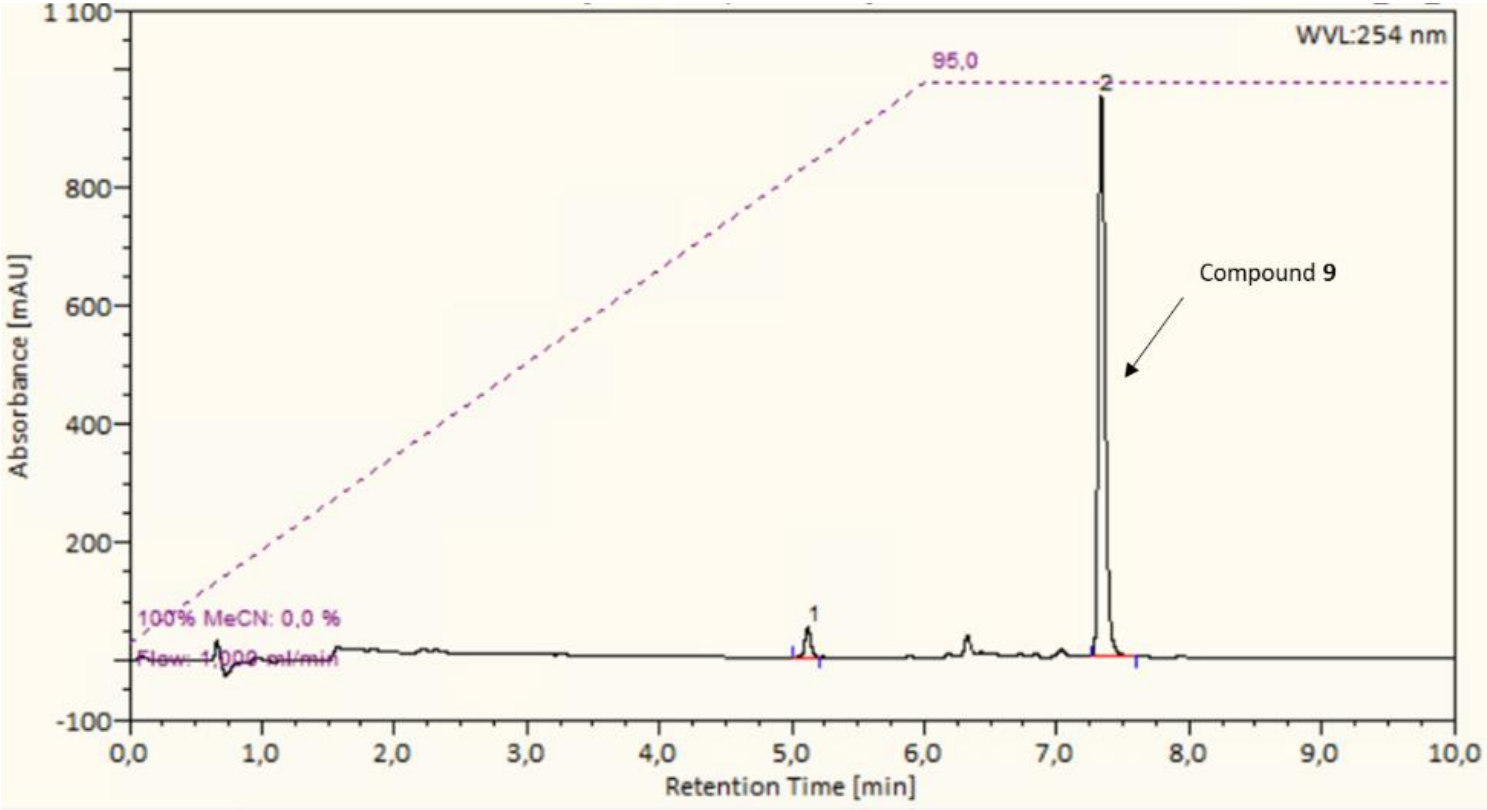

*Figure S38. RP-HPLC profile of compound **9** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

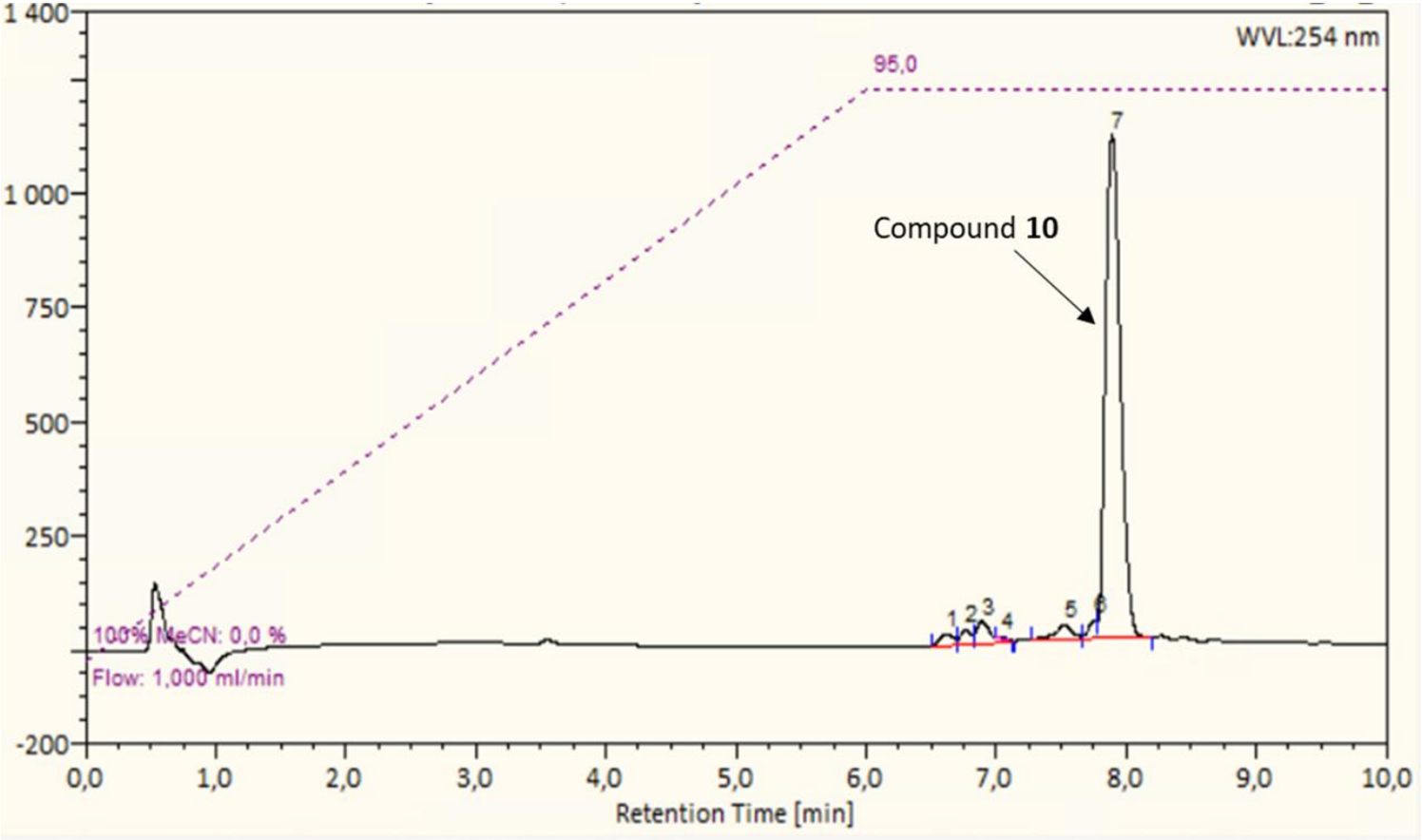

*Figure S39. RP-HPLC profile of compound **10** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

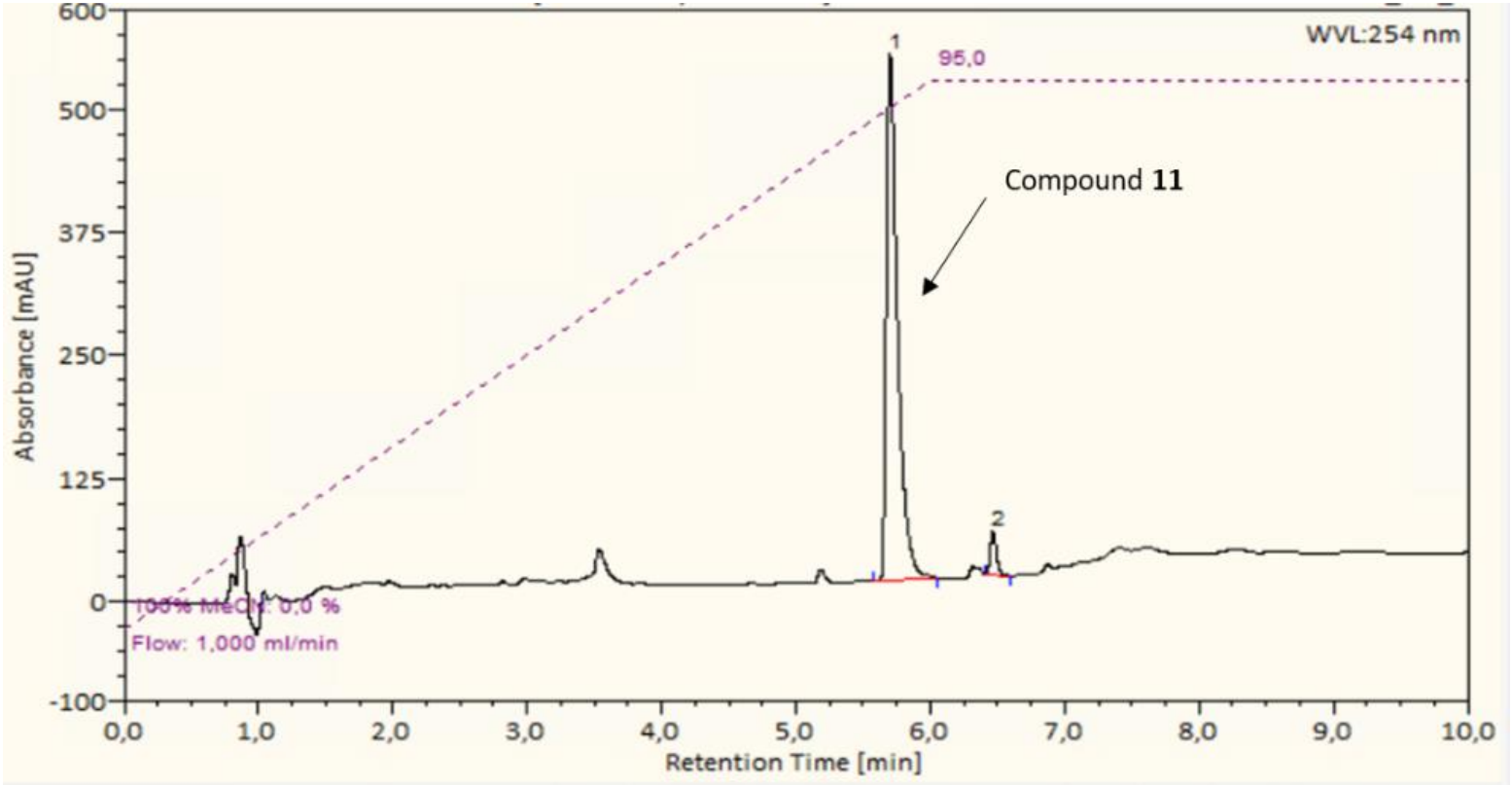

*Figure S40. RP-HPLC profile of compound **11** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

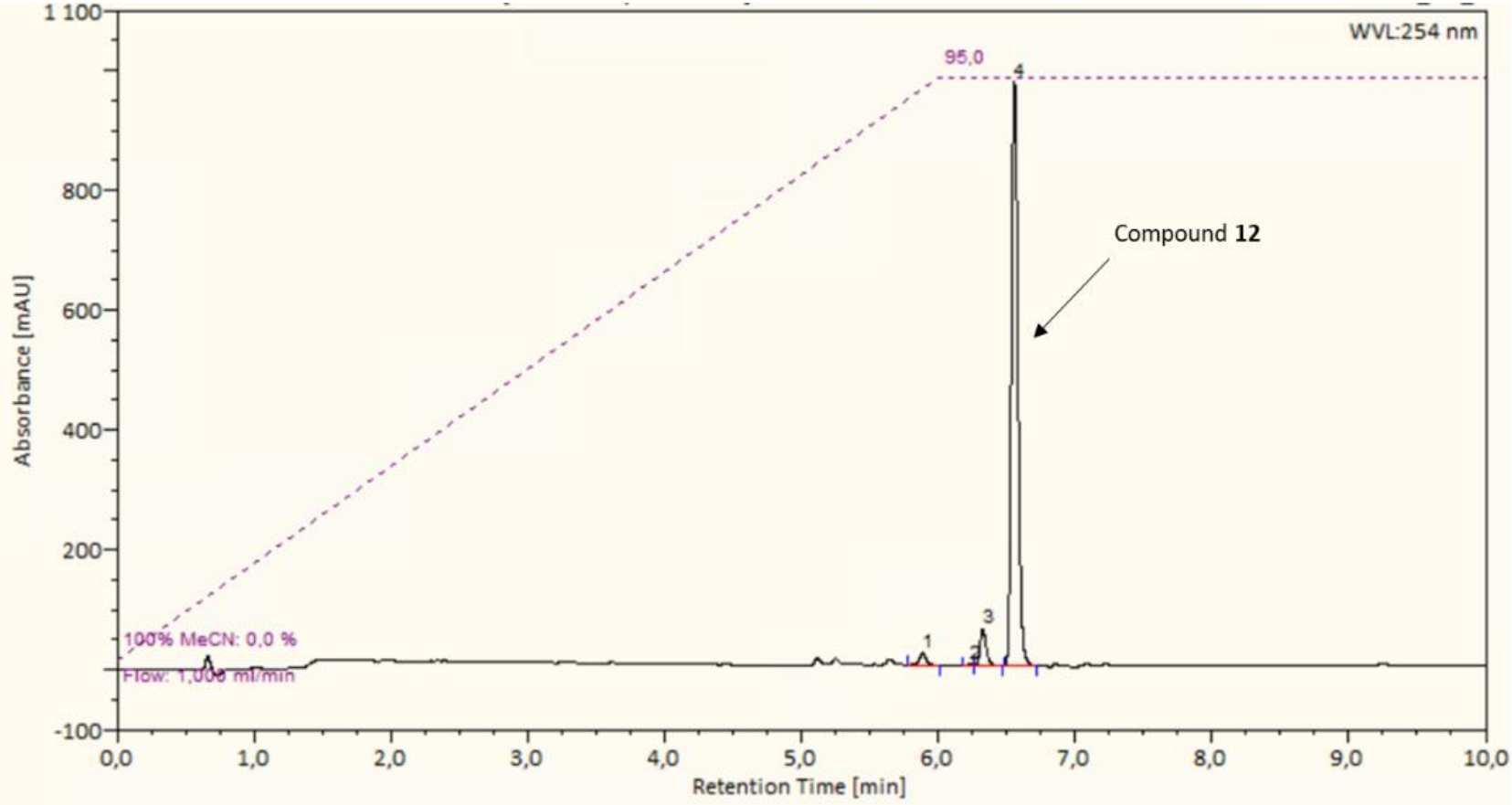

Figure S41. RP-HPLC profile of compound **12** *using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

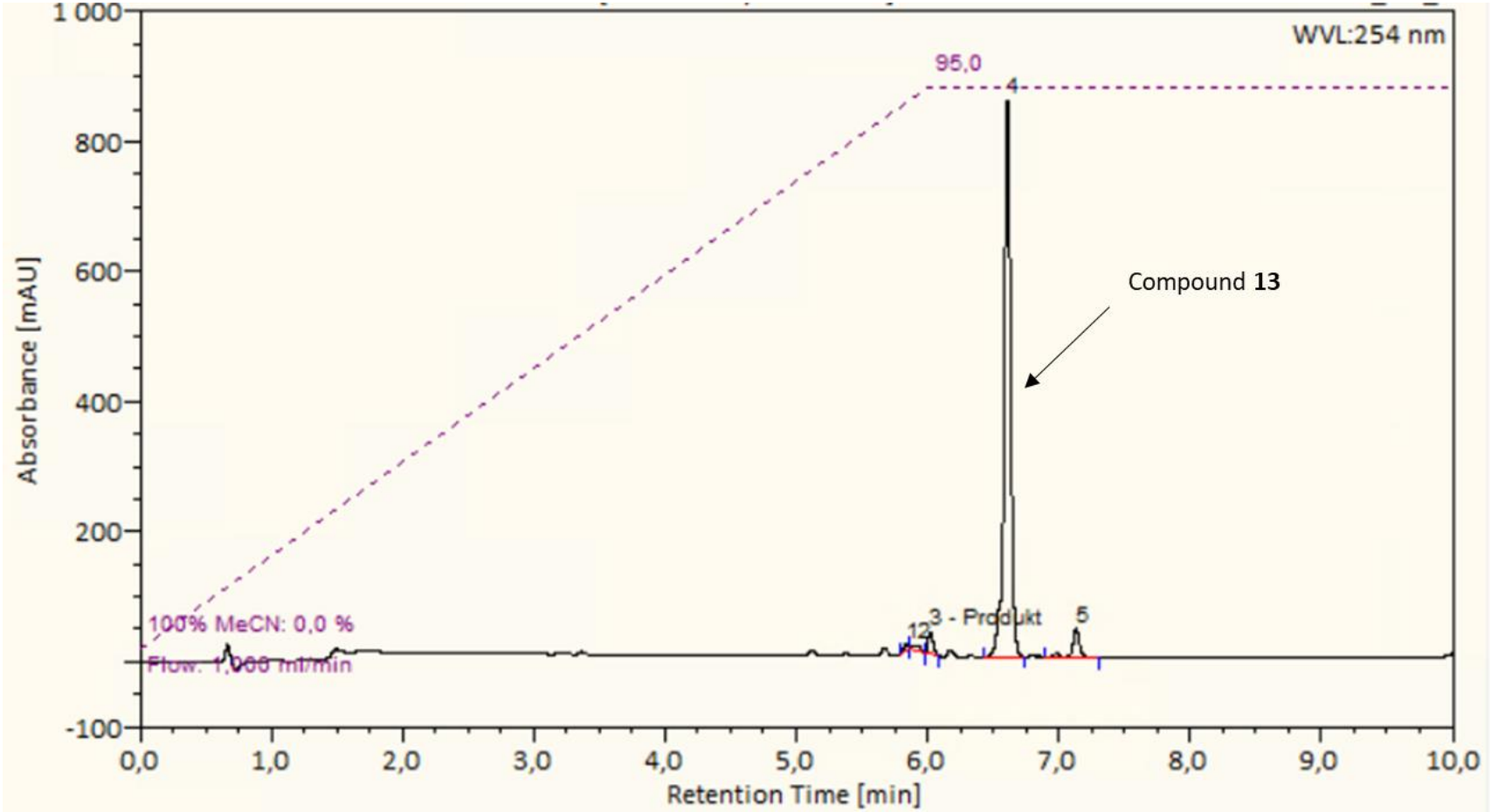

*Figure S42. RP-HPLC profile of compound **13** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

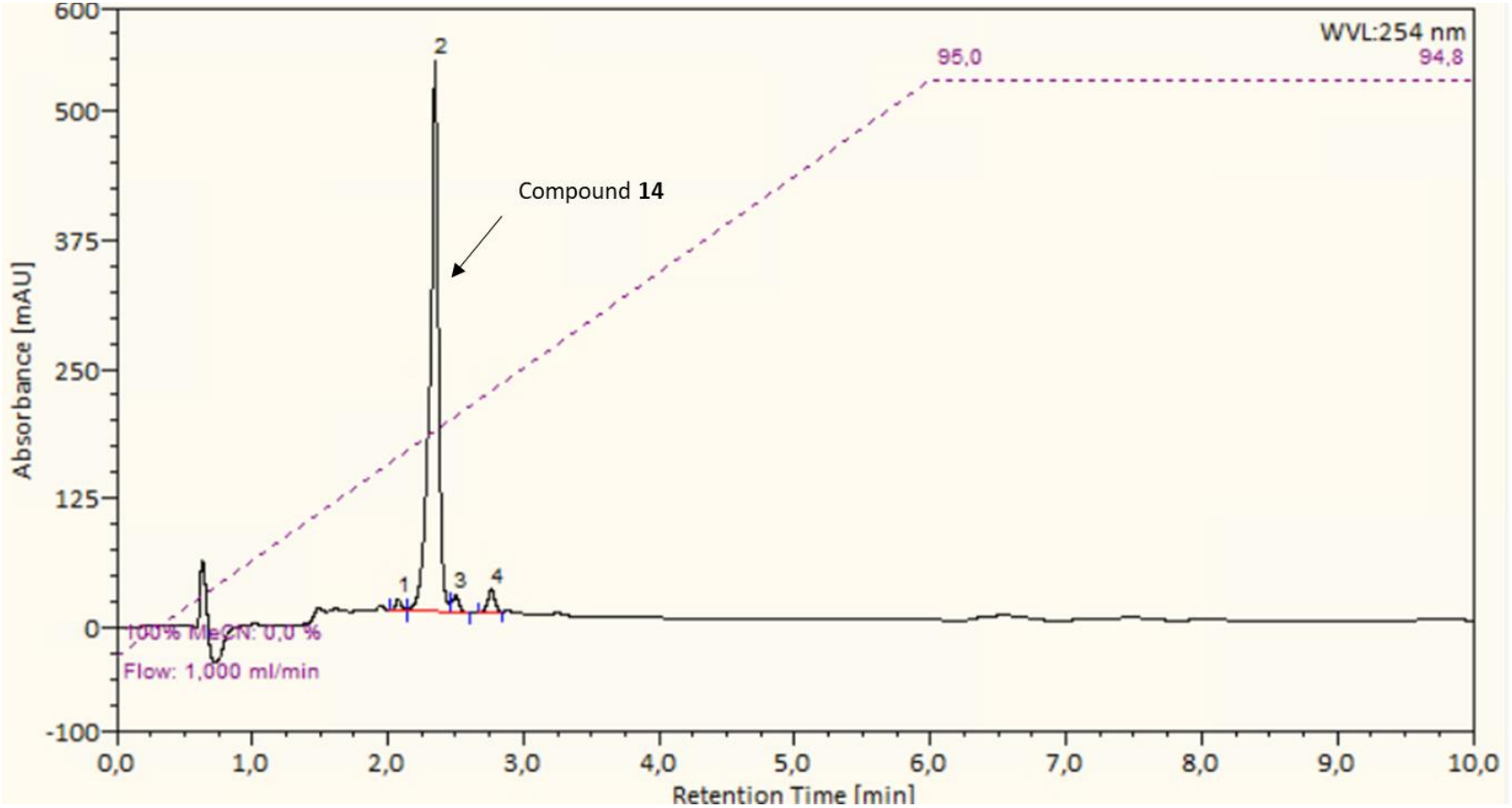

*Figure S43. RP-HPLC profile of compound **14** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

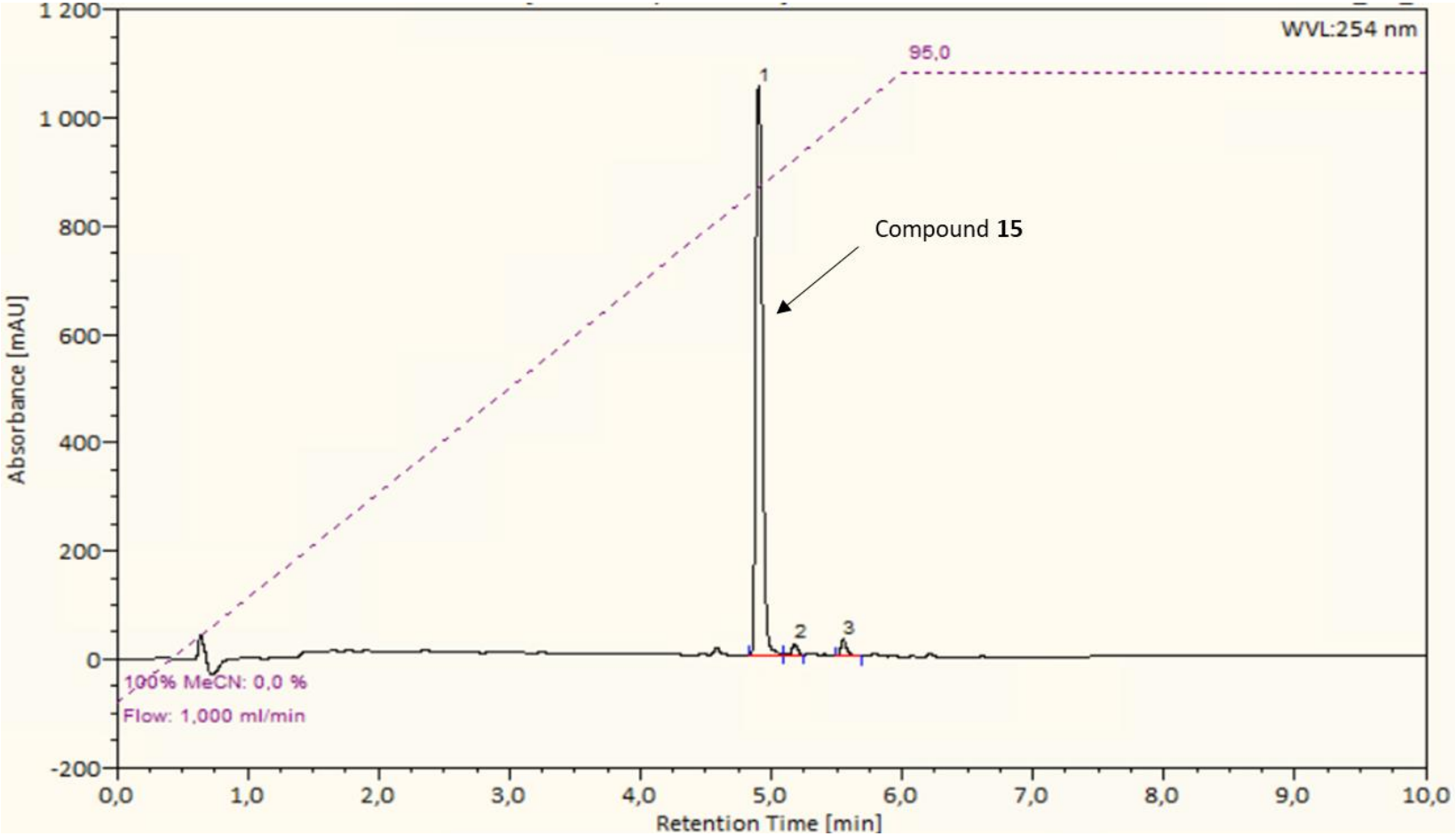

*Figure S44. RP-HPLC profile of compound **15** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN.*

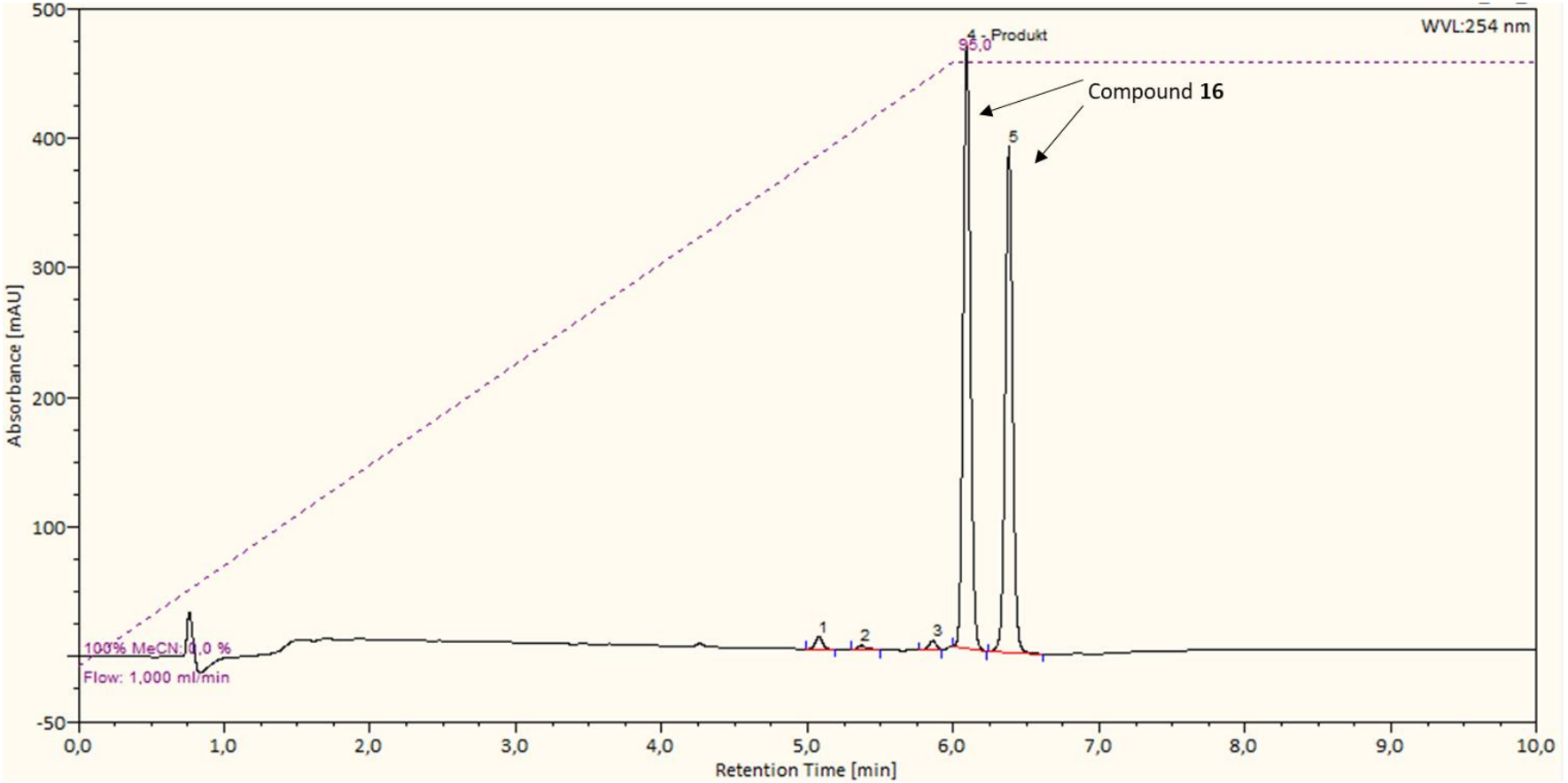

*Figure S45. RP-HPLC profile of compound **16** using RP HPLC on C18 column (Waters XBridge® Oligonucleotide BEH C18) with a linear gradient from 0-100% of buffer B in buffer A over 10 min at 40 °C. Buffer A: 5 mM ammonium acetate, buffer B: MeCN*

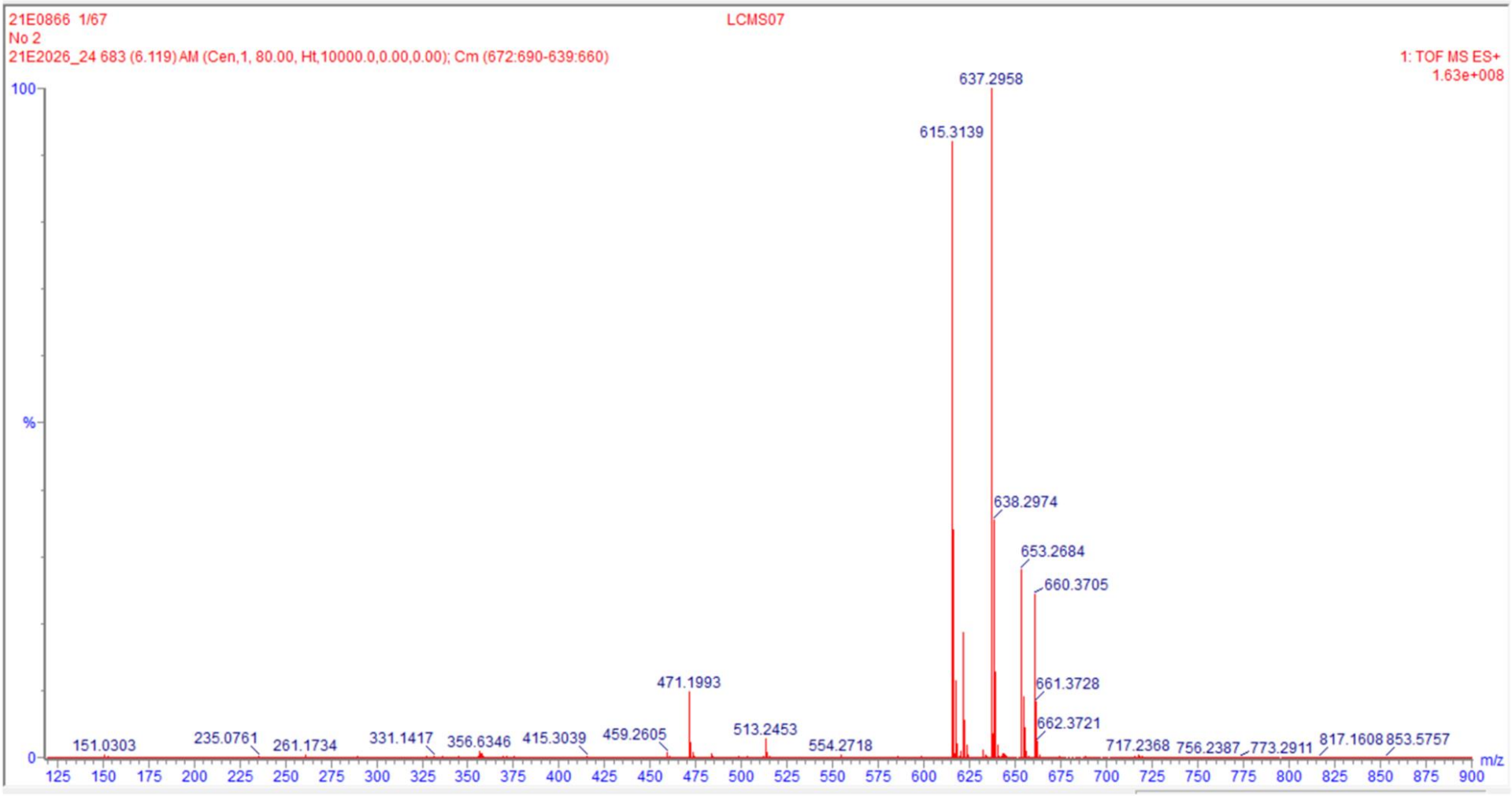

Figure S46. ESI-TOF mass spectrum of compound **2**. *Masses above [MH]+ 615 are adducts.*

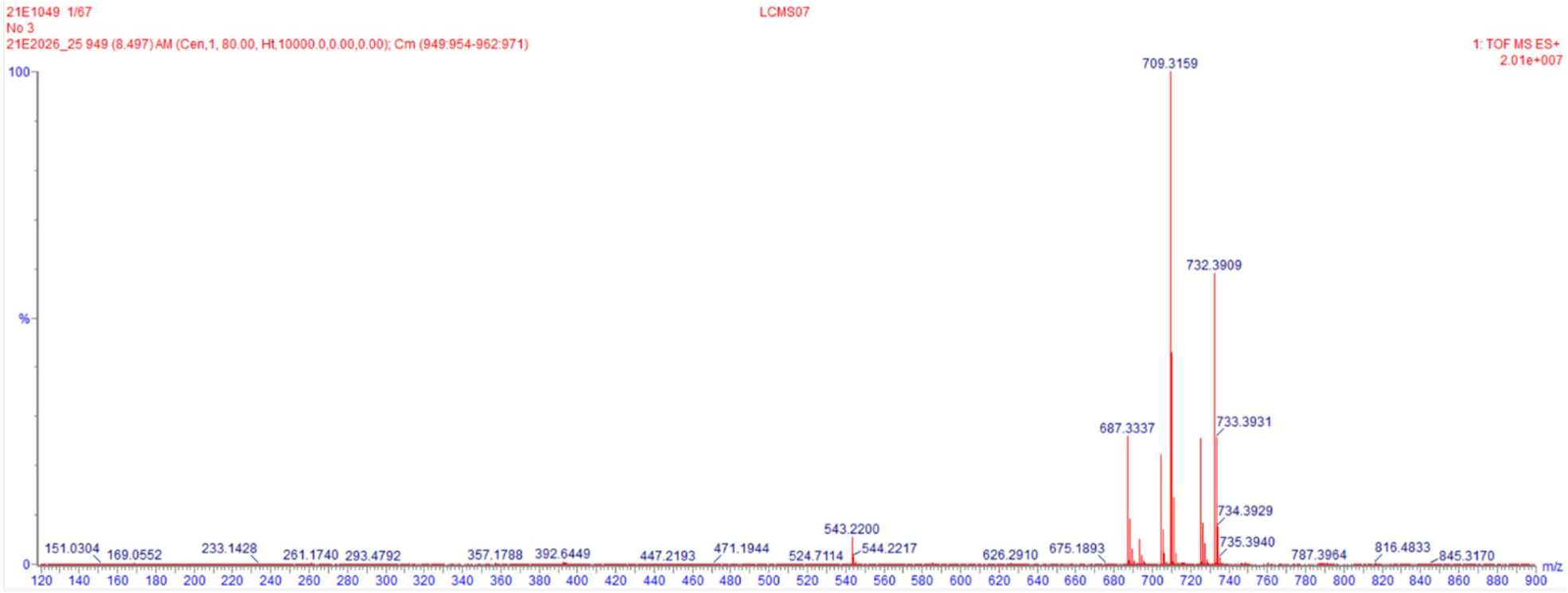

*Figure S47. ESI-TOF mass spectrum of compound **3**. Masses above [MH]+ 687 are adducts.*

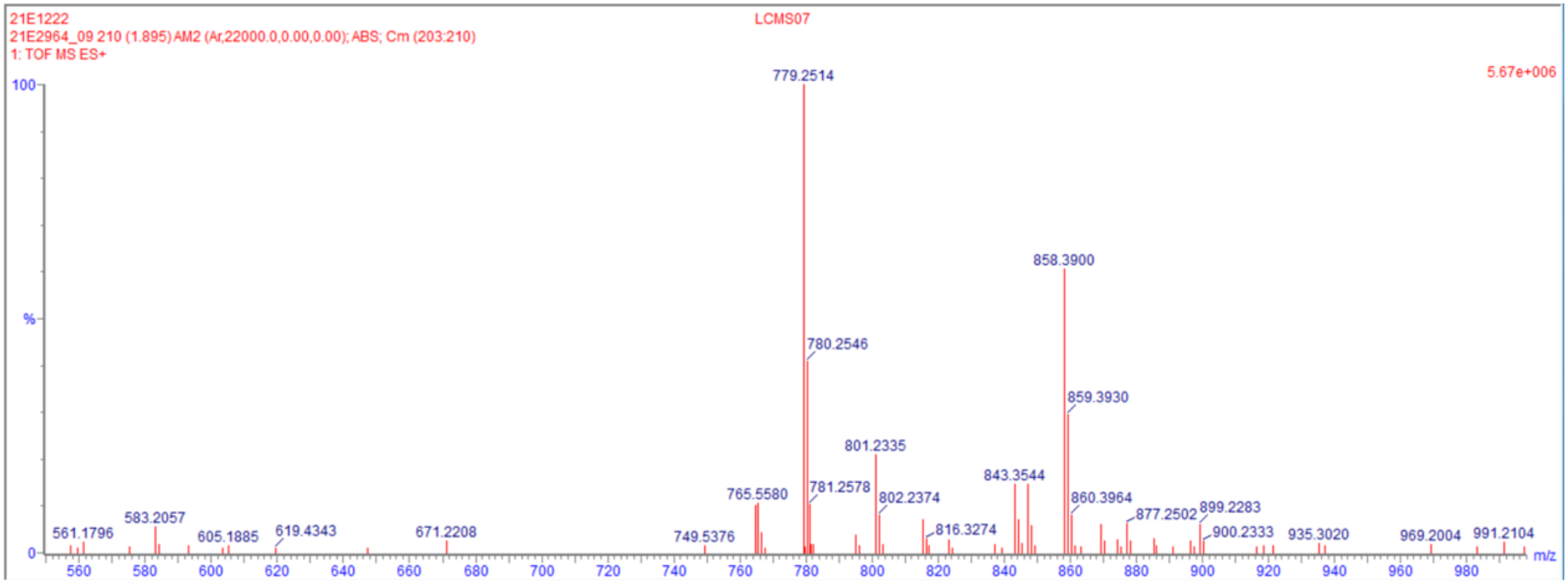

Figure S48. ESI-TOF mass spectrum of compound ***6****. M/z 779 = [MNa]+.*

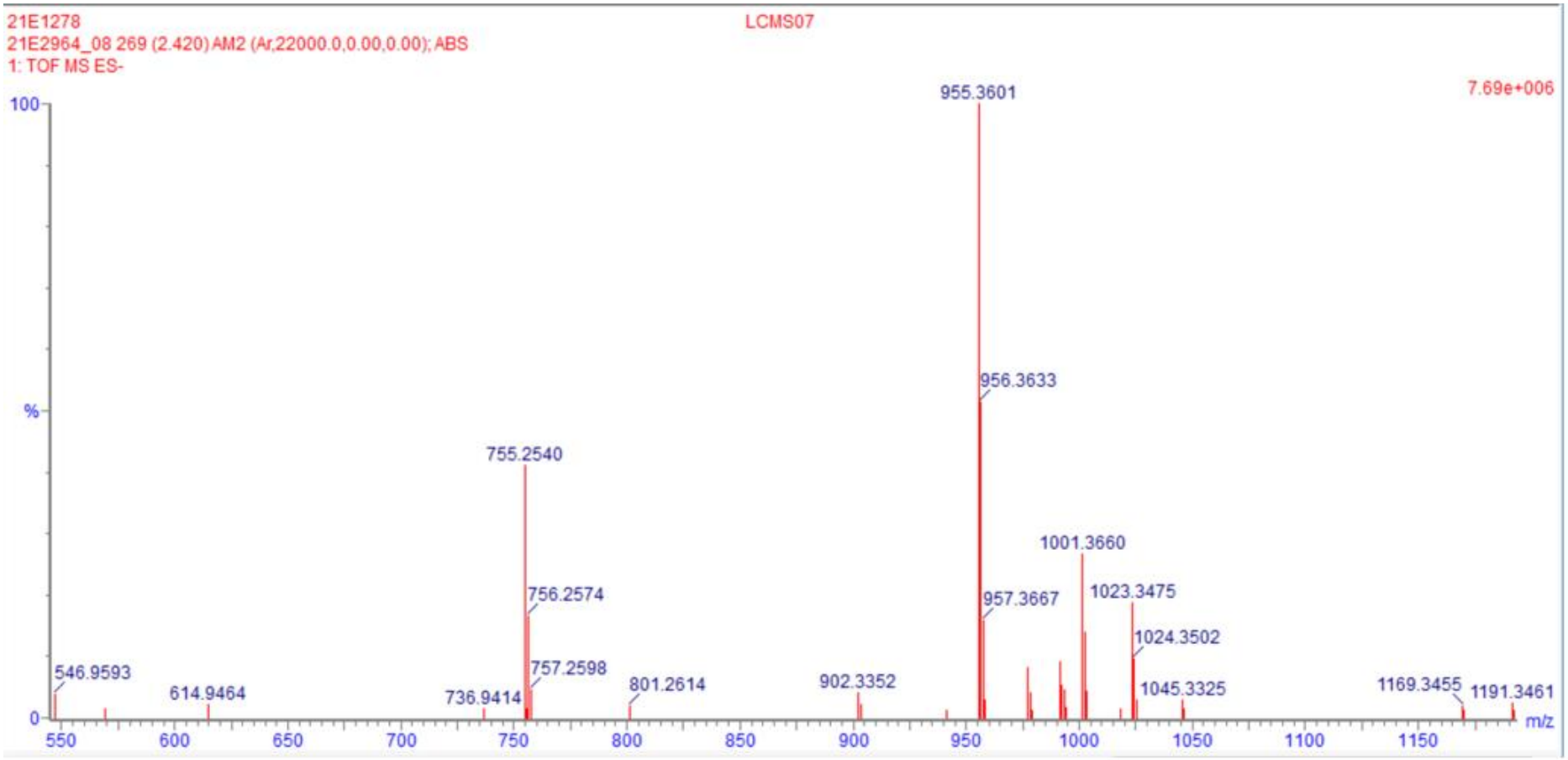

Figure S49. ESI-TOF mass spectrum of compound **7**.

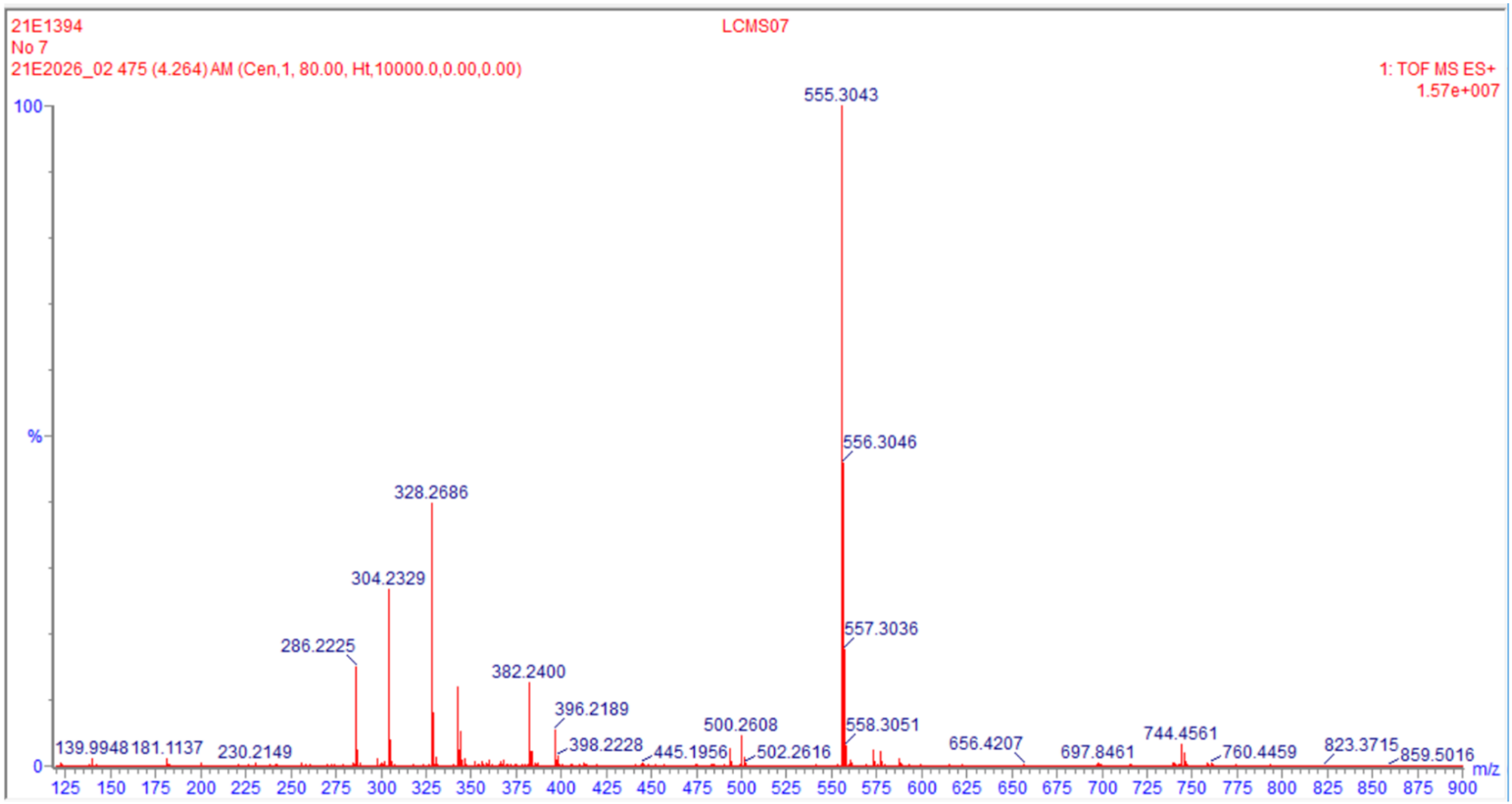

Figure S50. ESI-TOF mass spectrum of compound ***9****.*

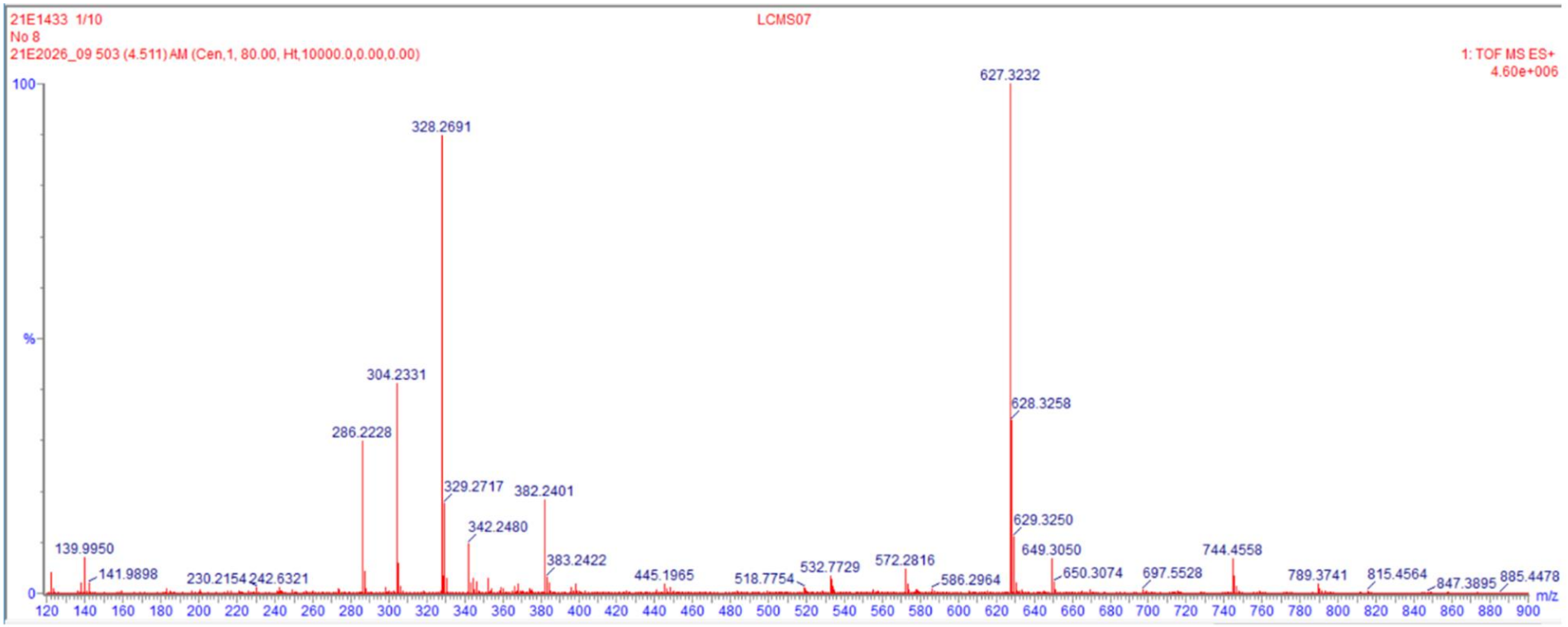

Figure S51. ESI-TOF mass spectrum of compound ***10****.*

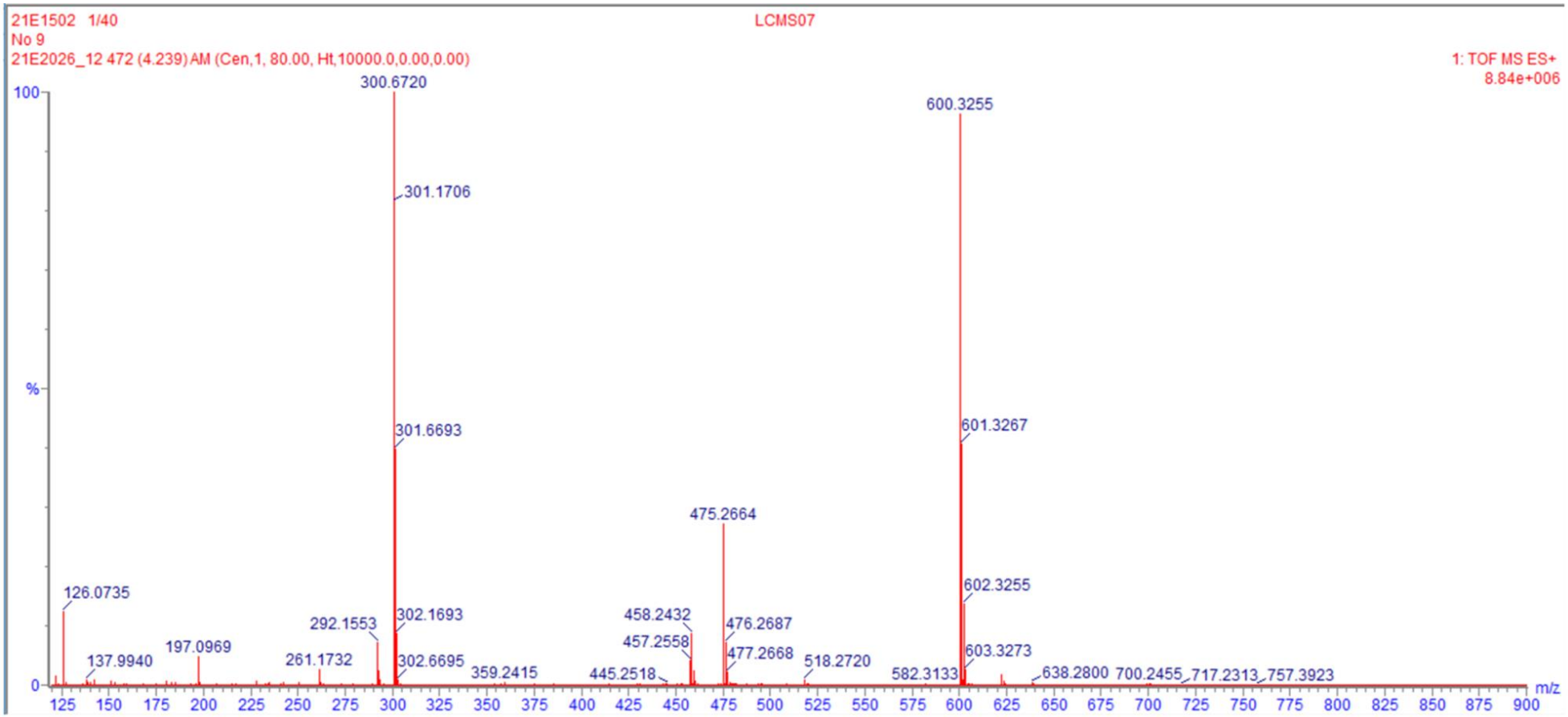

Figure S52. ESI-TOF mass spectrum of compound ***11****. M/z 300 is a fragment of the compound.*

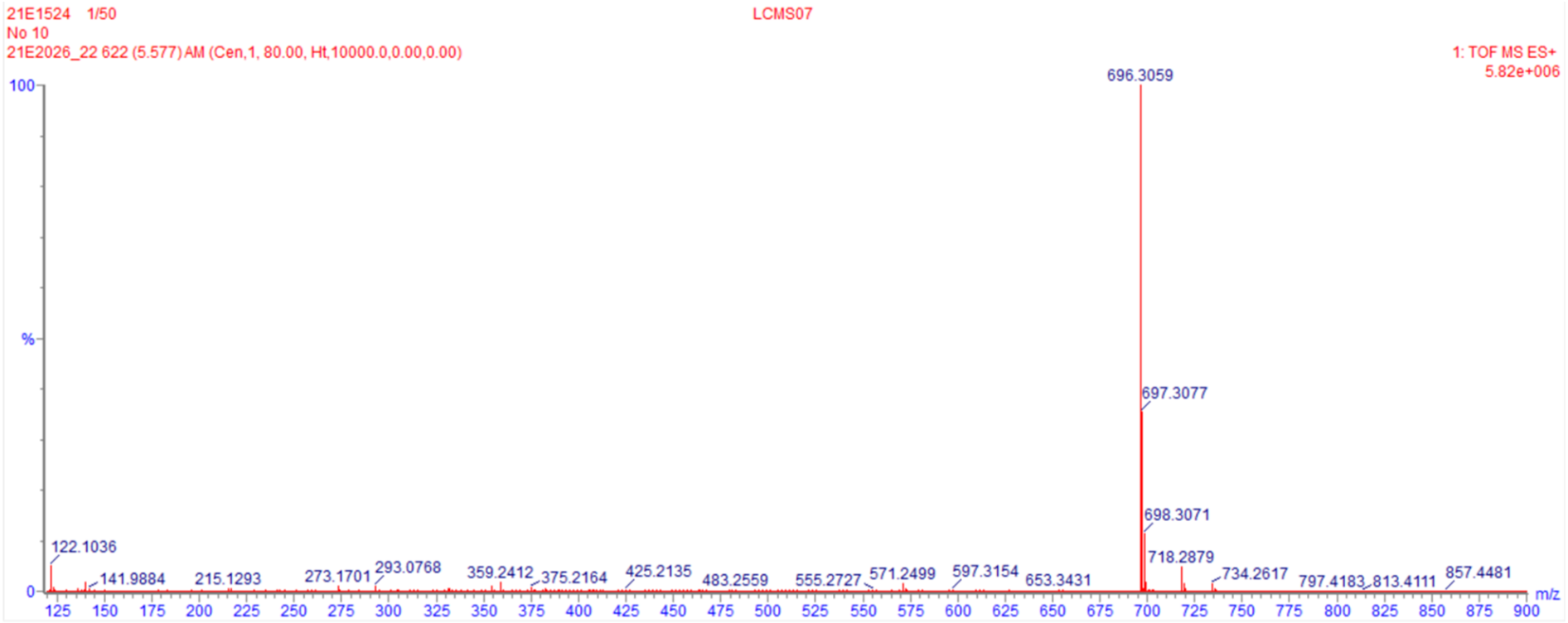

Figure S53. ESI-TOF mass spectrum of compound **12**.

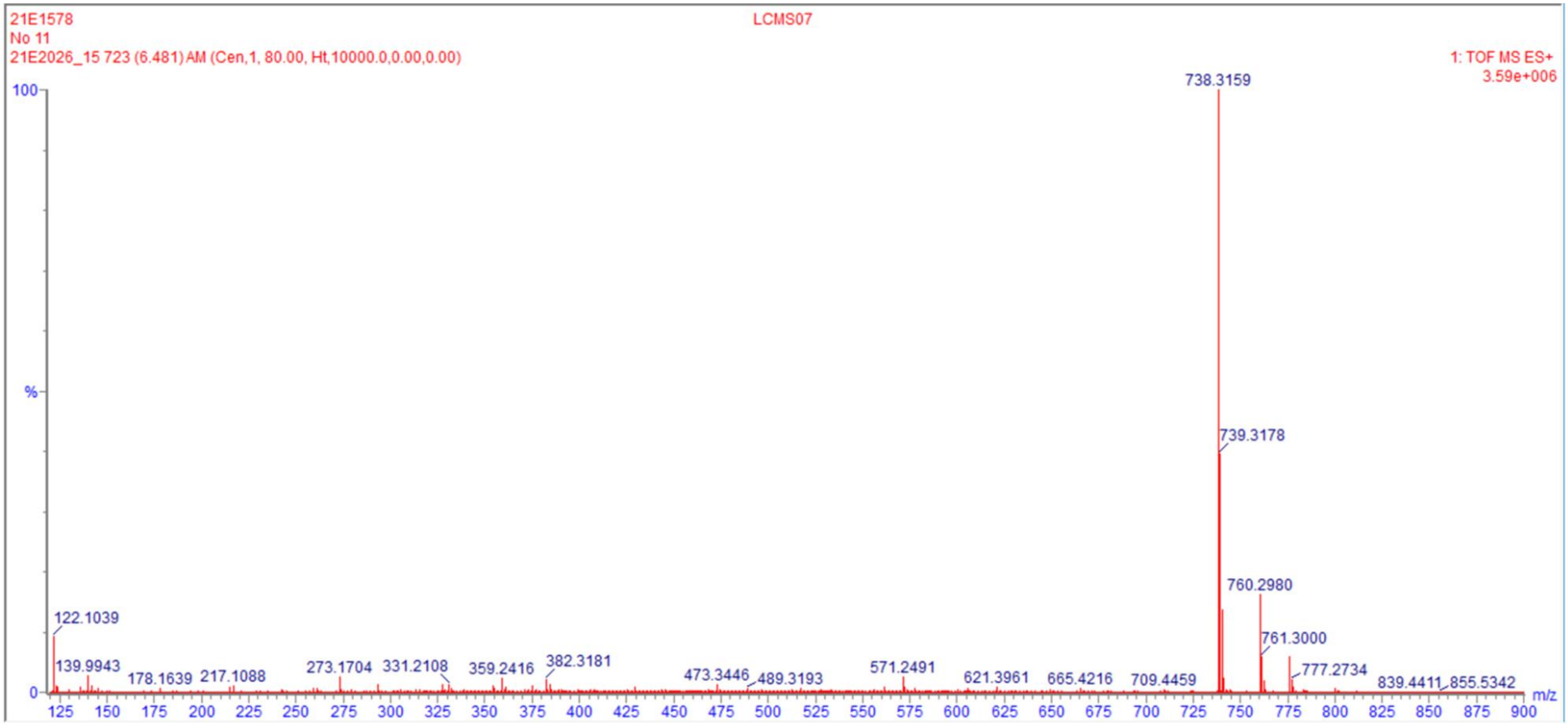

Figure S54. ESI-TOF mass spectrum of compound ***13***.

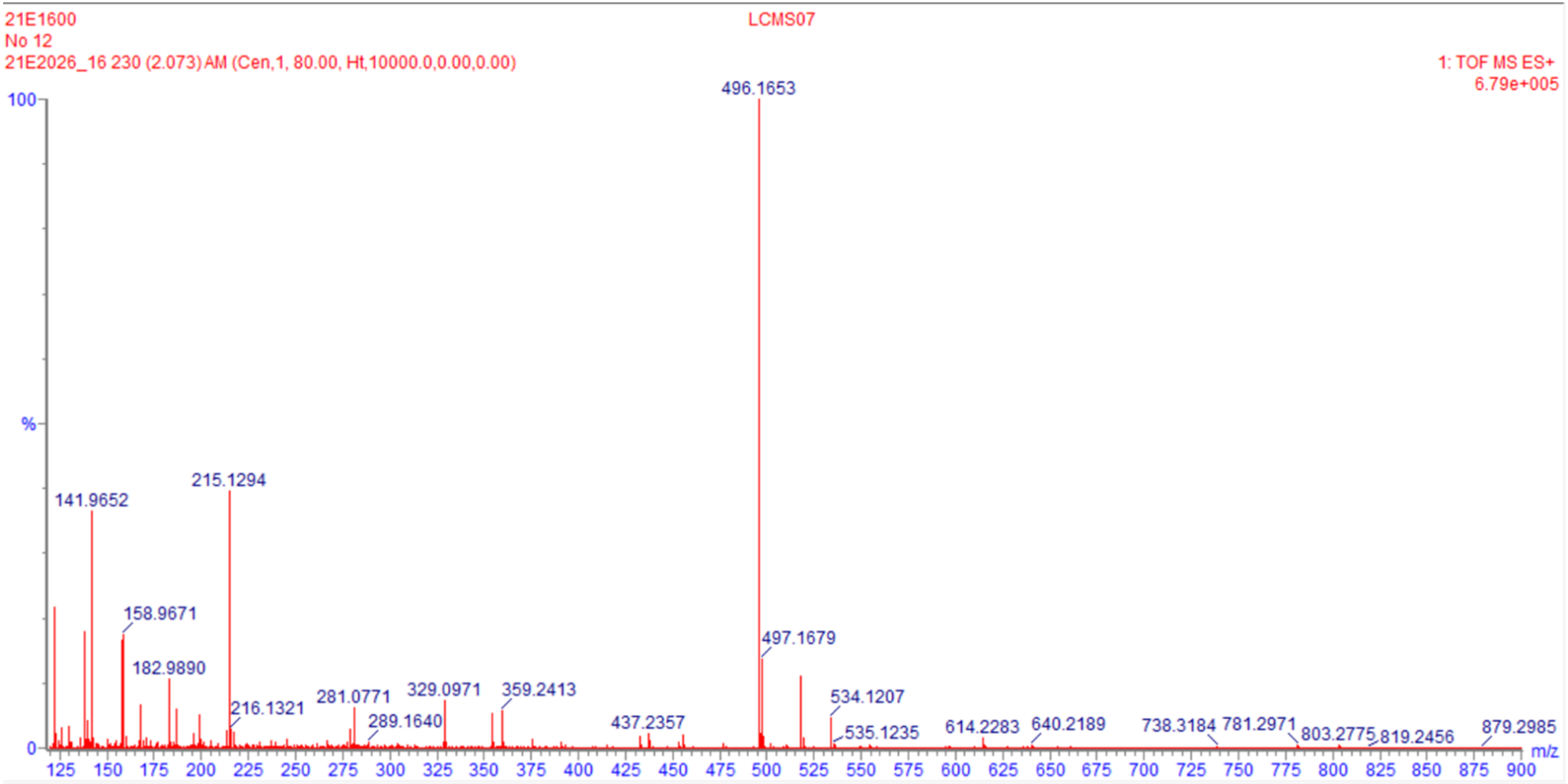

Figure S55. ESI-TOF mass spectrum of compound ***14****.*

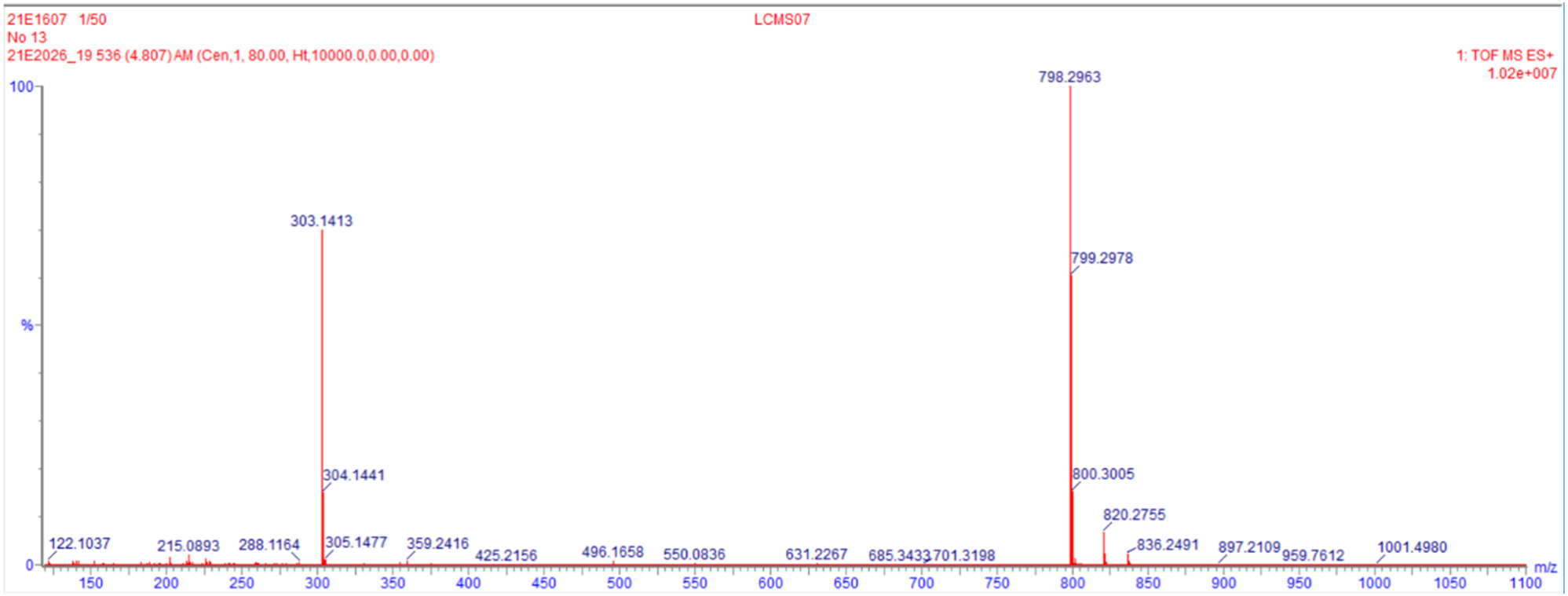

Figure S56. ESI-TOF mass spectrum of compound ***15****. M/z 303 is a fragment of DMT.*

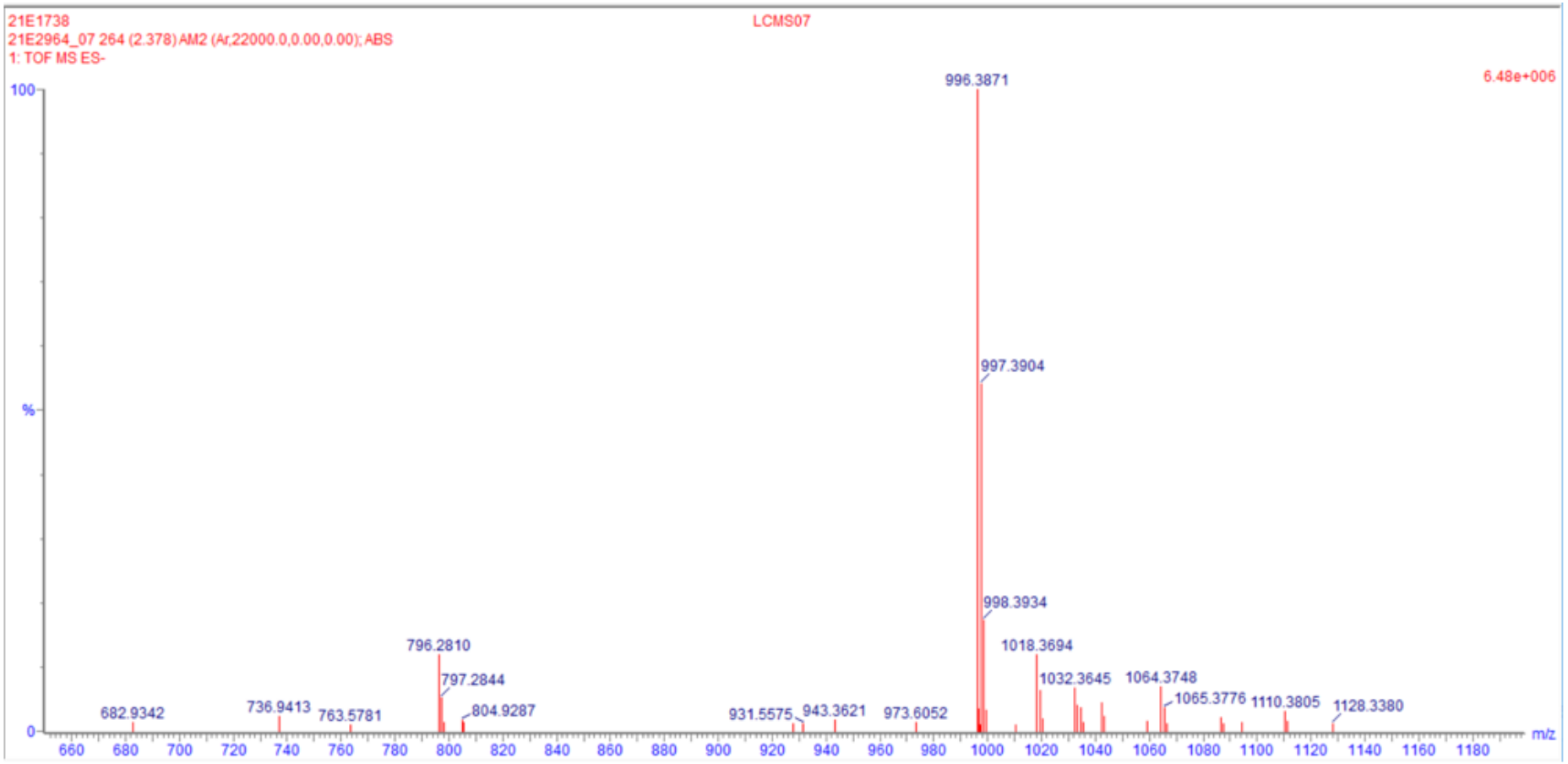

Figure S57. ESI-TOF mass spectrum of compound ***16.***