BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supporting Information

for

Electrophilic oligodeoxynucleotide synthesis using dM-Dmoc for amino protection

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HPLC profiles, MALDI–TOF MS spectra, UV spectra, and OD_{260} values of ODNs, and NMR spectra of new compounds

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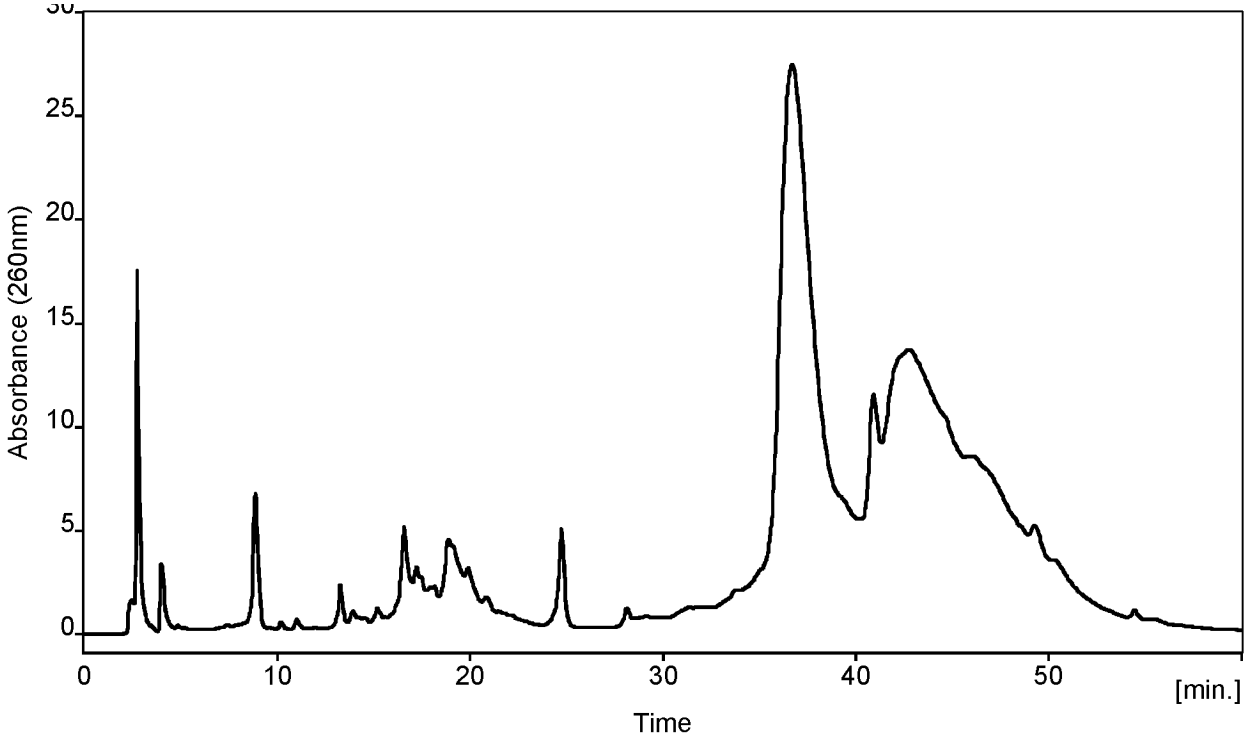
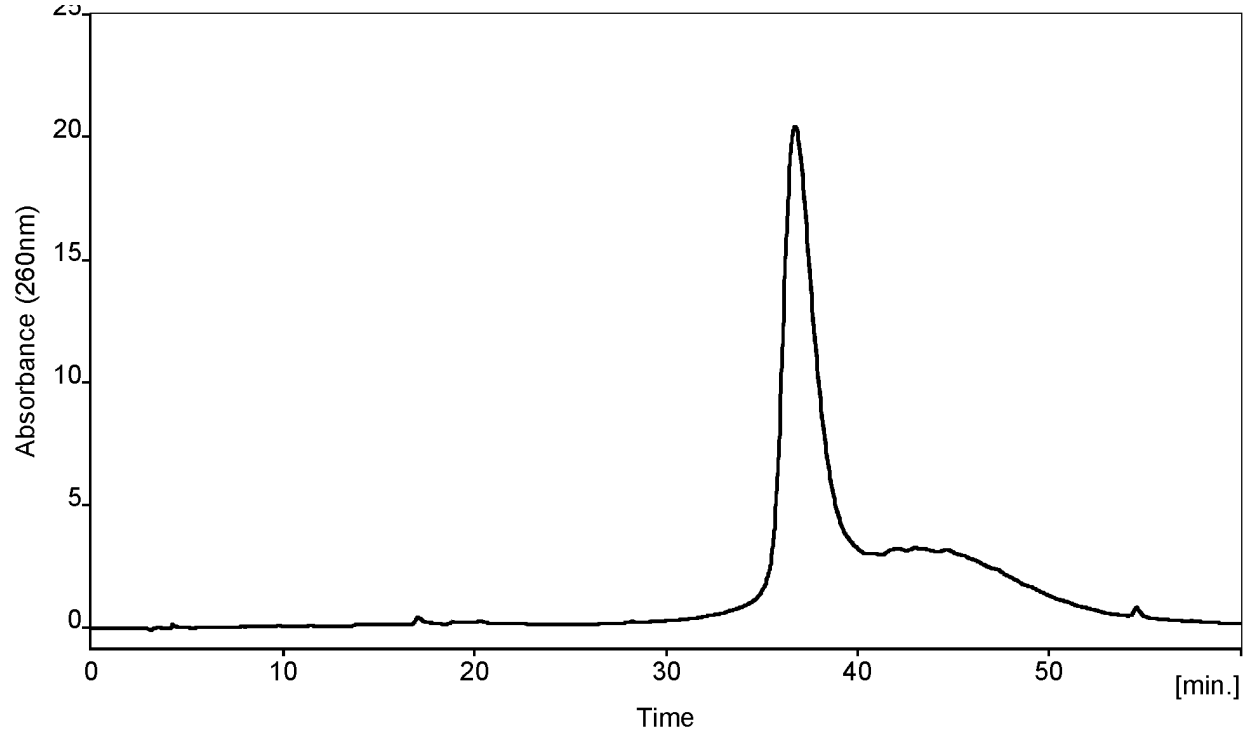
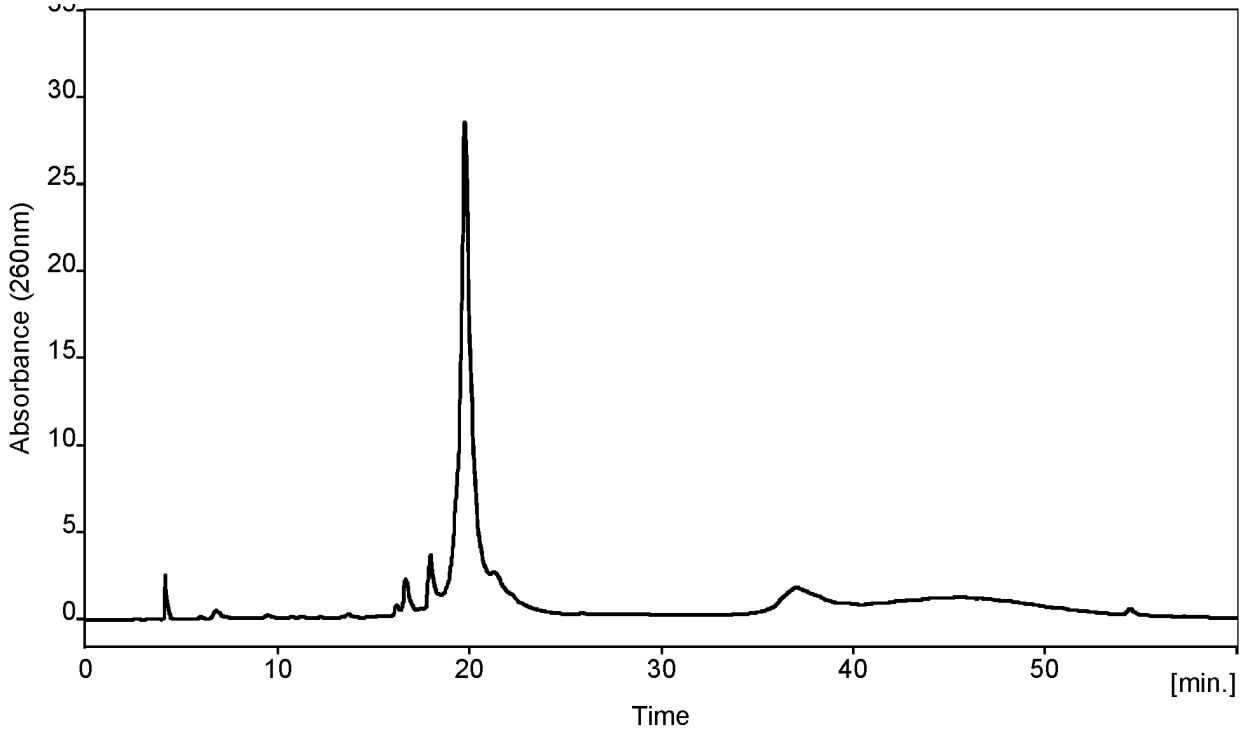


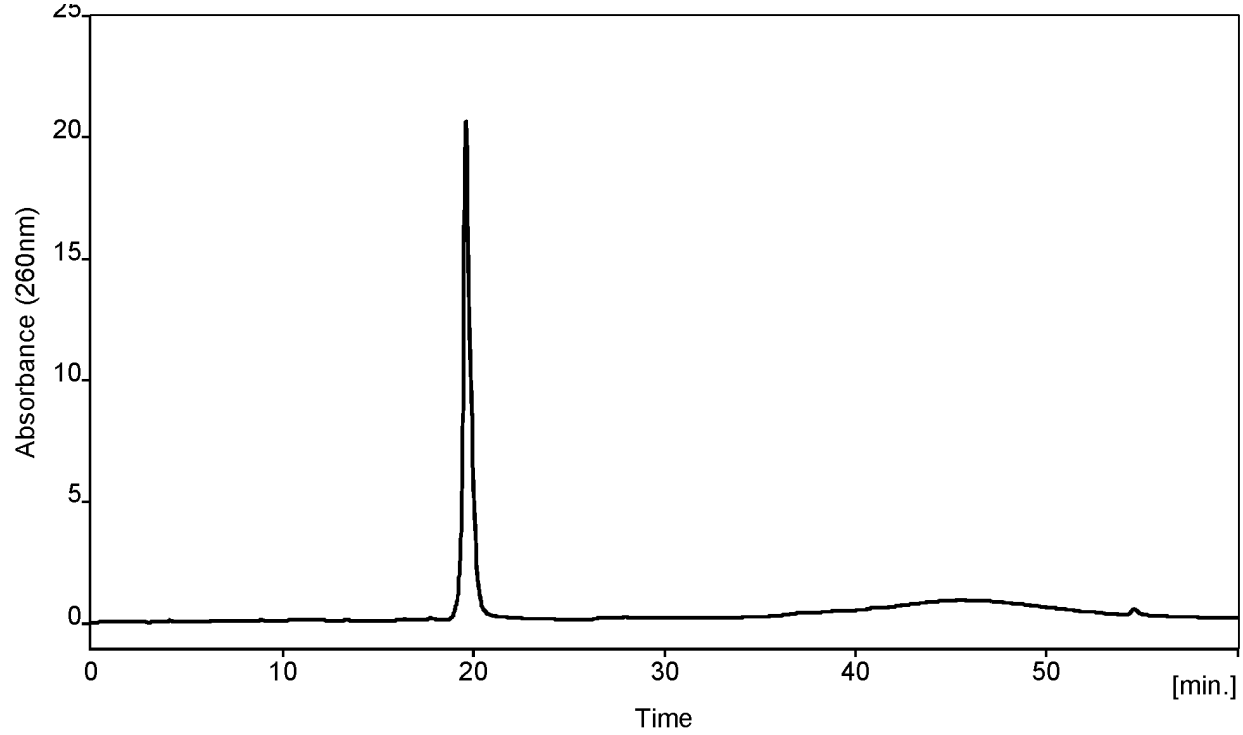
RP HPLC profile of crude trityl-tagged ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**30a-tr**). The peak at 35-39 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.



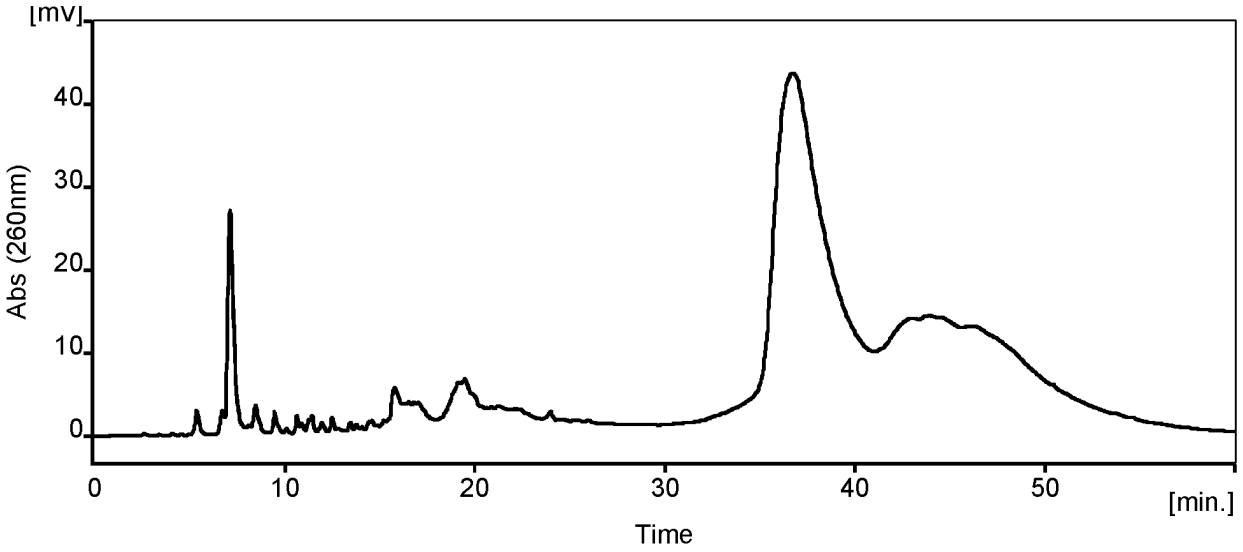
RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**30a-tr**).



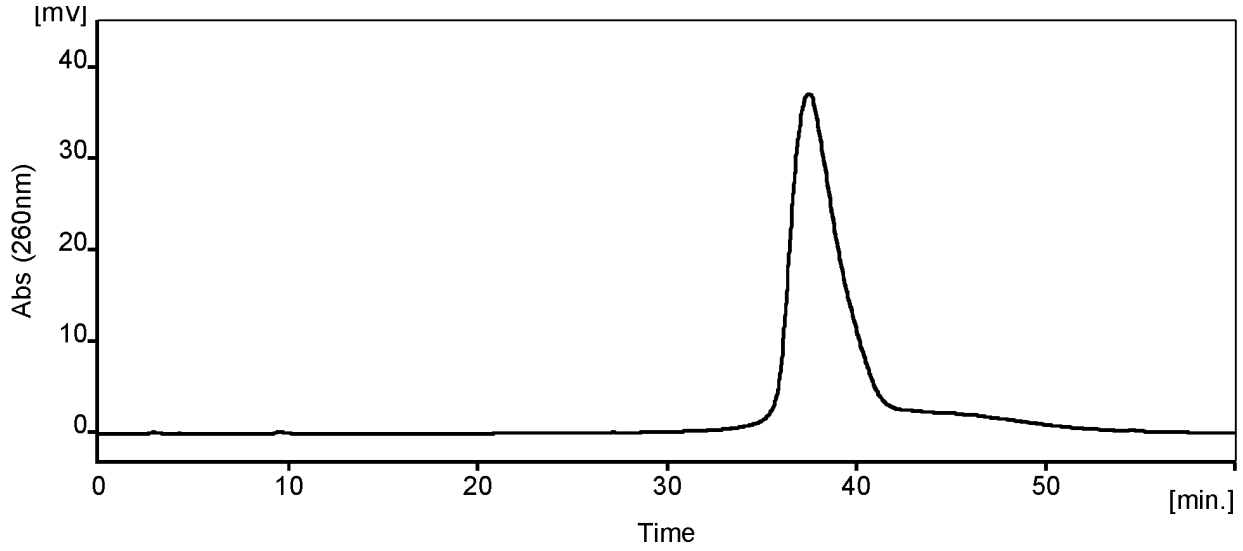
RP HPLC profile of de-tritylated ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**30a**).



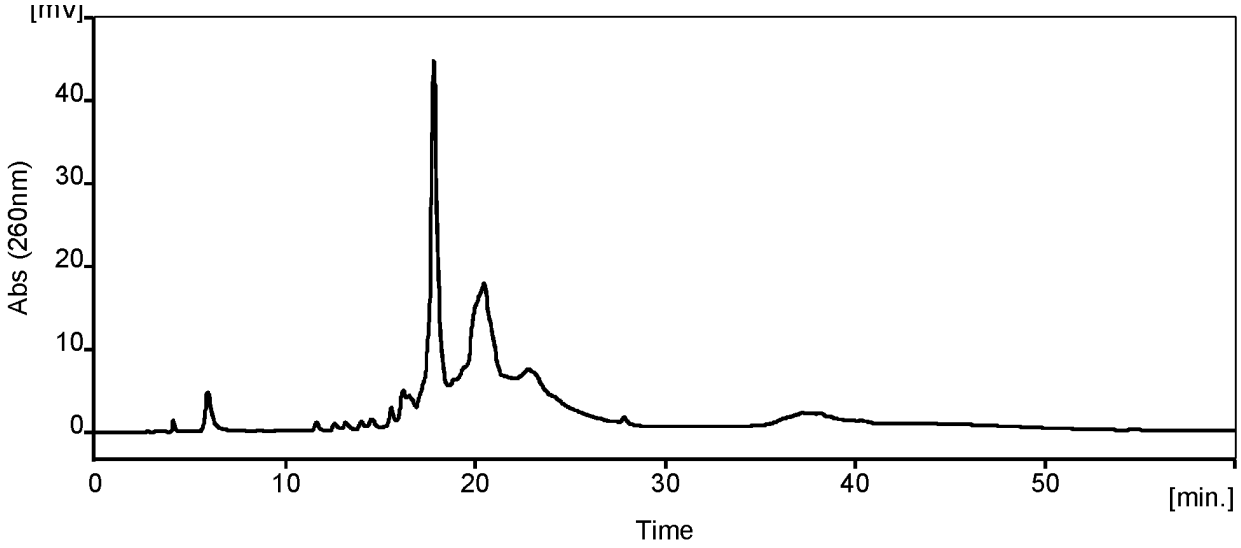
RP HPLC profile of pure de-tritylated ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**30a**).



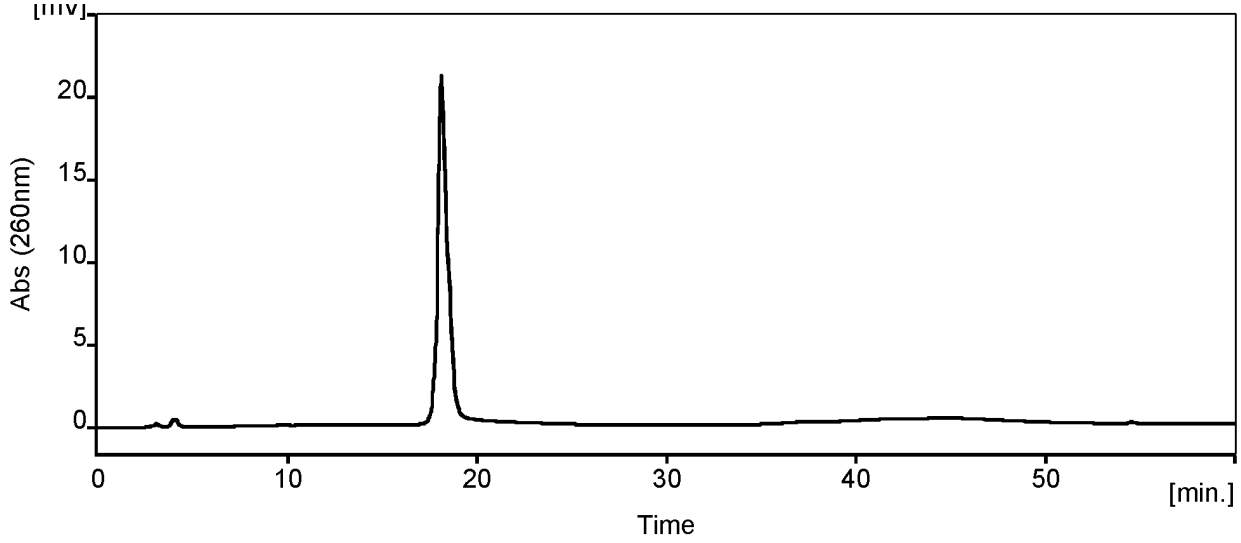
RP HPLC profile of crude trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b-tr**). The peak at 35–39 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.



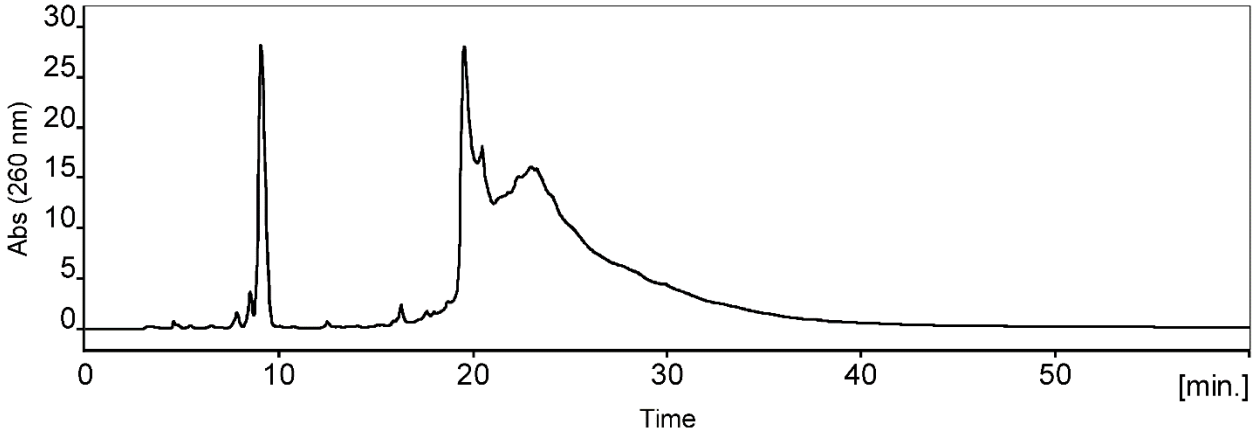
RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b-tr**).



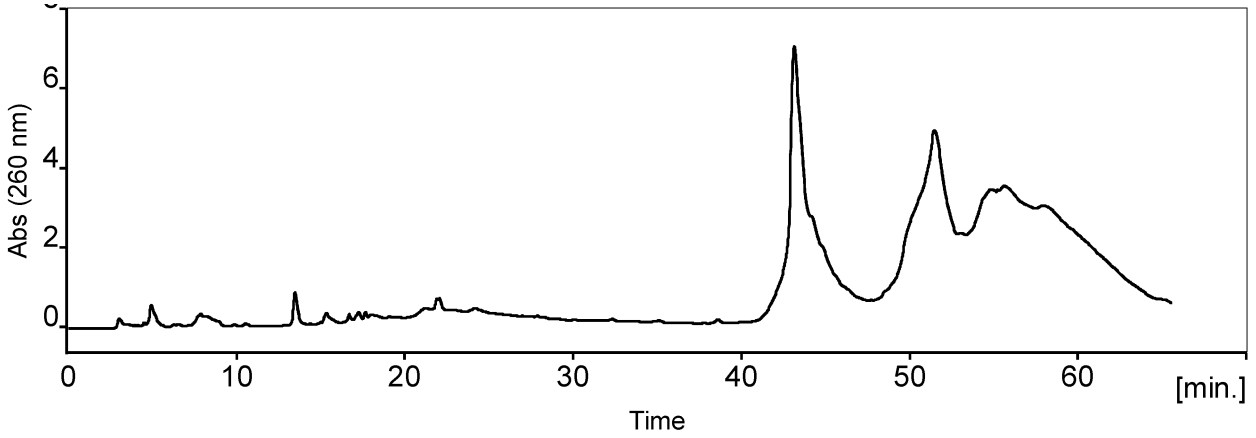
RP HPLC profile of de-tritylated ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b**).



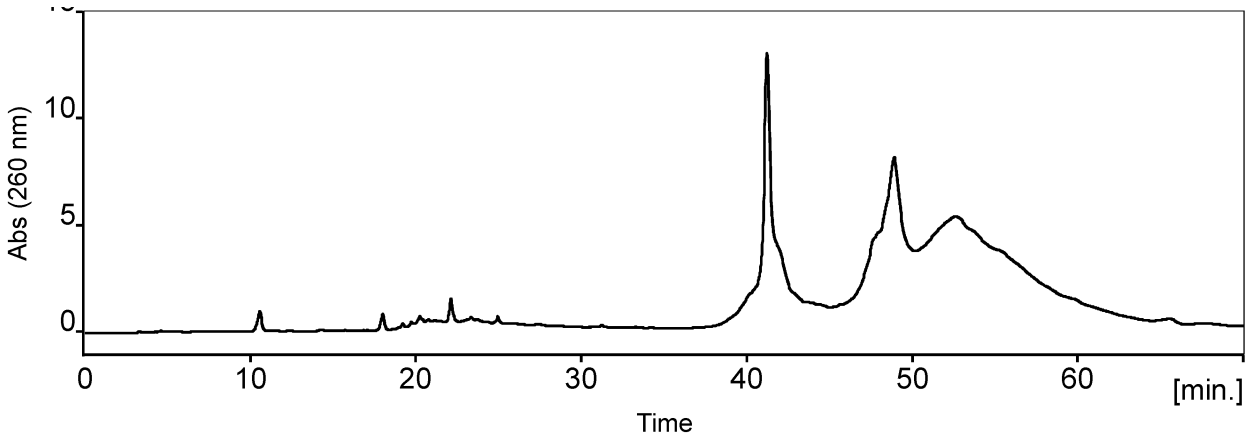
RP HPLC profile of pure de-tritylated ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b**).



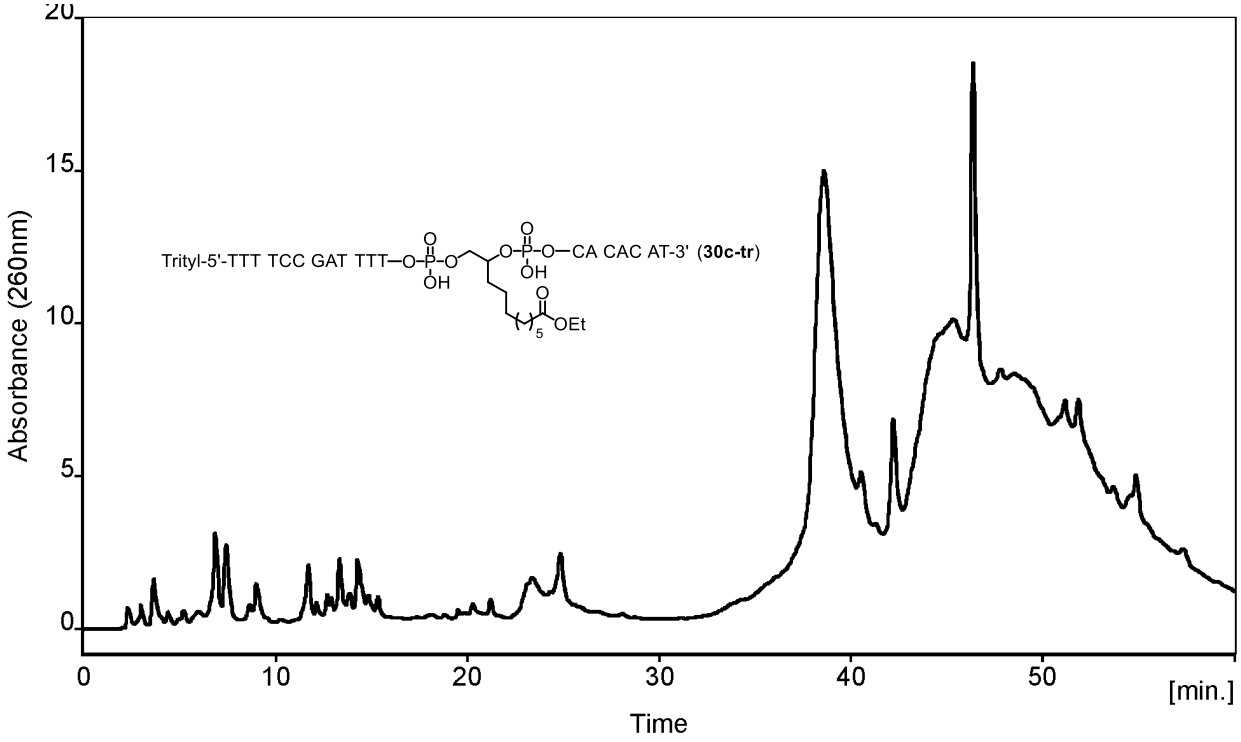
A typical RP HPLC profile of crude ODN (5'-CTA GAT AAC TCA TAG TAC TT-3') synthesized using **3a**–**c** and **4** under standard conditions using acetic anhydride for capping and without 5'-tagging with hydrophobic groups such as trityl and DMTr groups. The peak between 19 and 21 min corresponds to the ODN. The peaks after 21 min correspond to branched sequences. Because the desired ODN and branched sequences were very close, ODN purification was difficult.



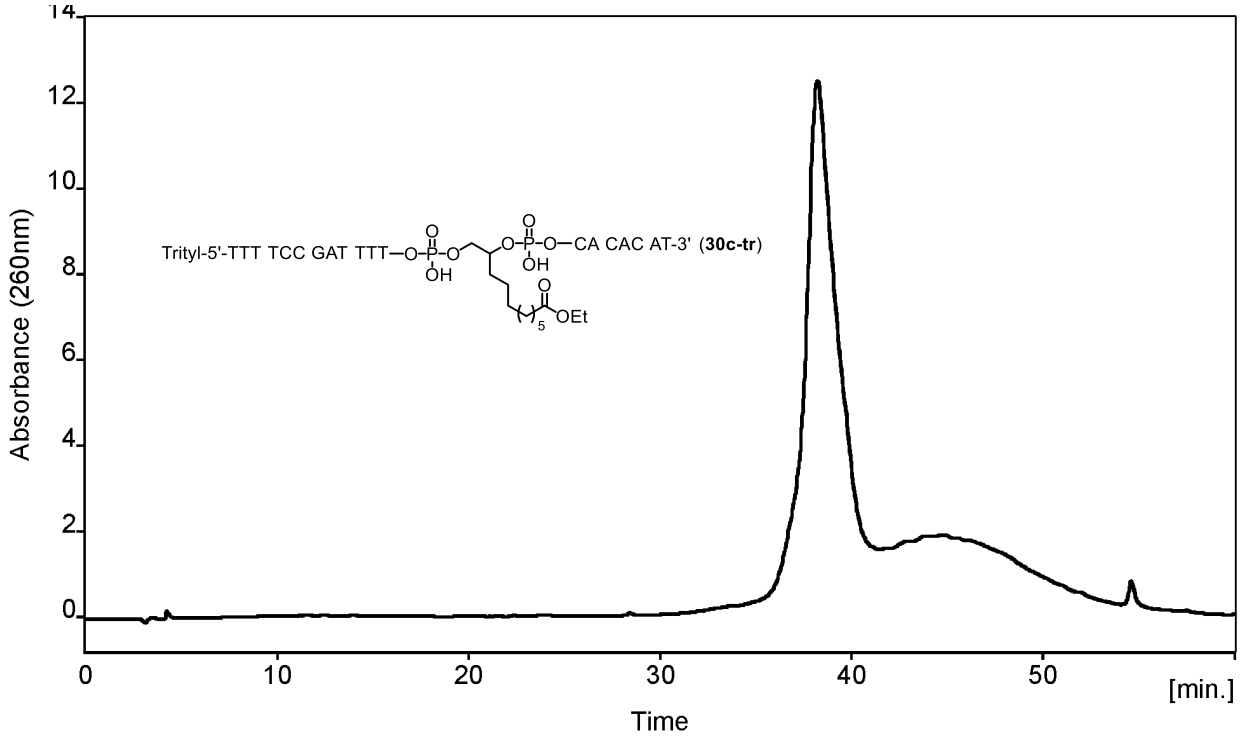
RP HPLC profile of the crude ODN 5'-DMTr-O-TTC CAT CCT AGA AAG CTC AT-3' synthesized using **3a**–**c** and **4** under standard conditions using acetic anhydride for capping. At the end of synthesis, the DMTr group was not removed. Although not always possible, in this case, the DMTr protection survived the cleavage and deprotection conditions involving sodium periodate. The peak in the profile between 43 and 45 min corresponds to the DMTr-tagged ODN. The peaks after 47 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more DMTr groups.



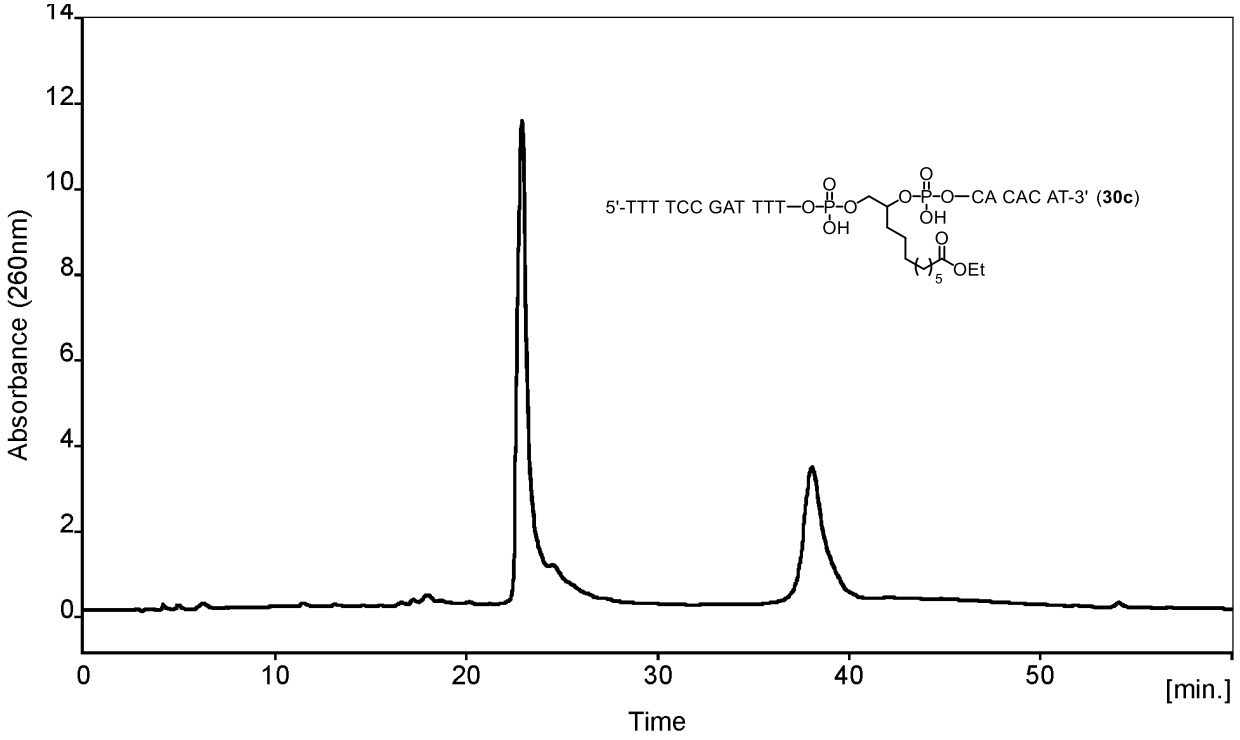
A typical RP HPLC profile of crude ODN (5'-TBDPS-O-CTA GAT AAC TCA TAG TAC TT-3') synthesized using **3a**-**c** and **4** under standard conditions using acetic anhydride for capping and tagged with a TBDPS group at the 5'-end. The TBDPS, which is the *t*-Bu(Ph_2)Si- group, was introduced after solid phase synthesis (5'-DMTr group removed) and before cleavage and deprotection by soaking the CPG in 0.1 M *t*-Bu(Ph_2)SiCl and 0.1 M imidazole in DMF (rt, 12 h). Cleavage and deprotection were then carried out as described in the article. The peak between 41 and 42 min corresponds to the tagged ODN. The peaks after 43 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more TBDPS groups. The approach separated the desired ODN from the branched sequences very well, but at this stage, we cannot identify a mild condition that is compatible with sensitive modifications on ODNs to remove the TBDPS group after the ODN is purified.



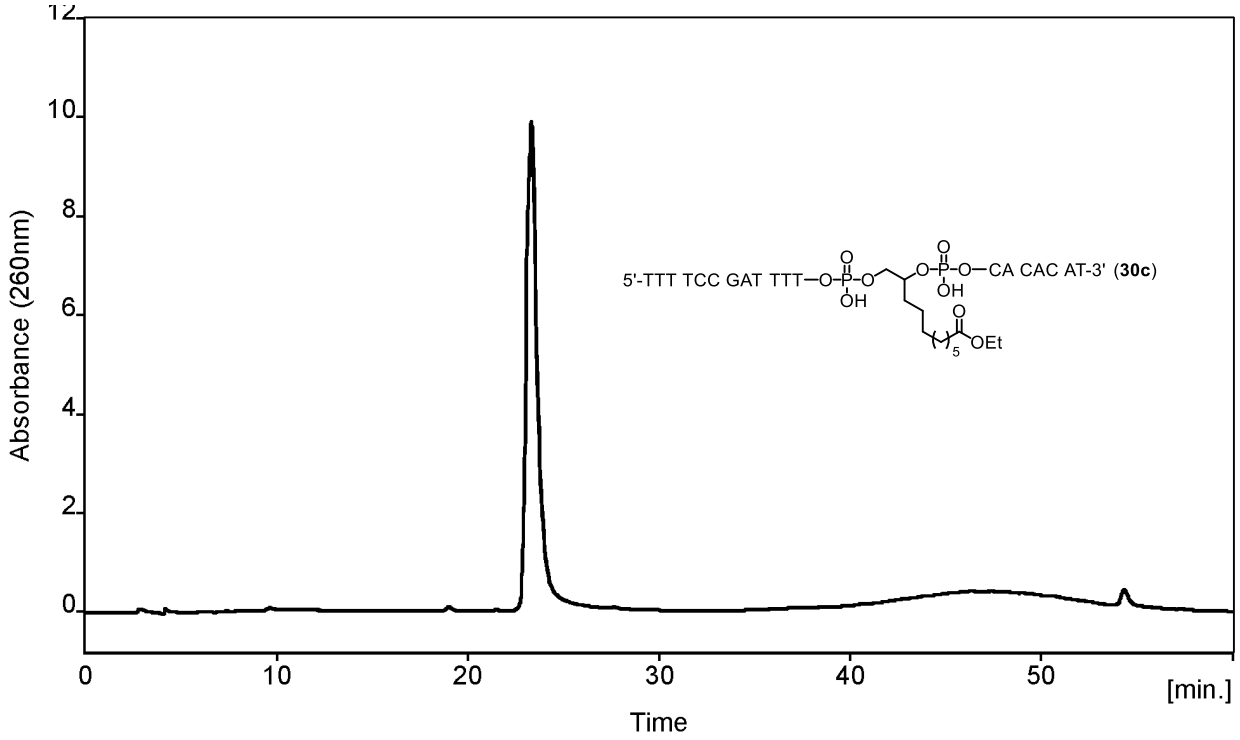
RP HPLC profile of crude trityl-tagged ODN **30c-tr**. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.



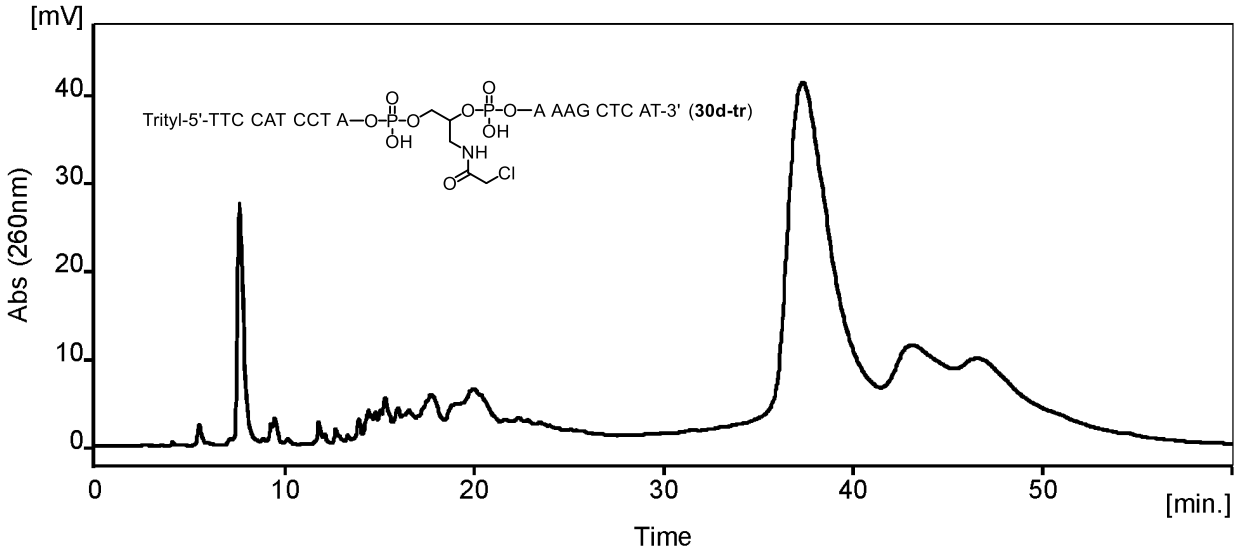
RP HPLC profile of purified trityl-tagged ODN **30c-tr**.



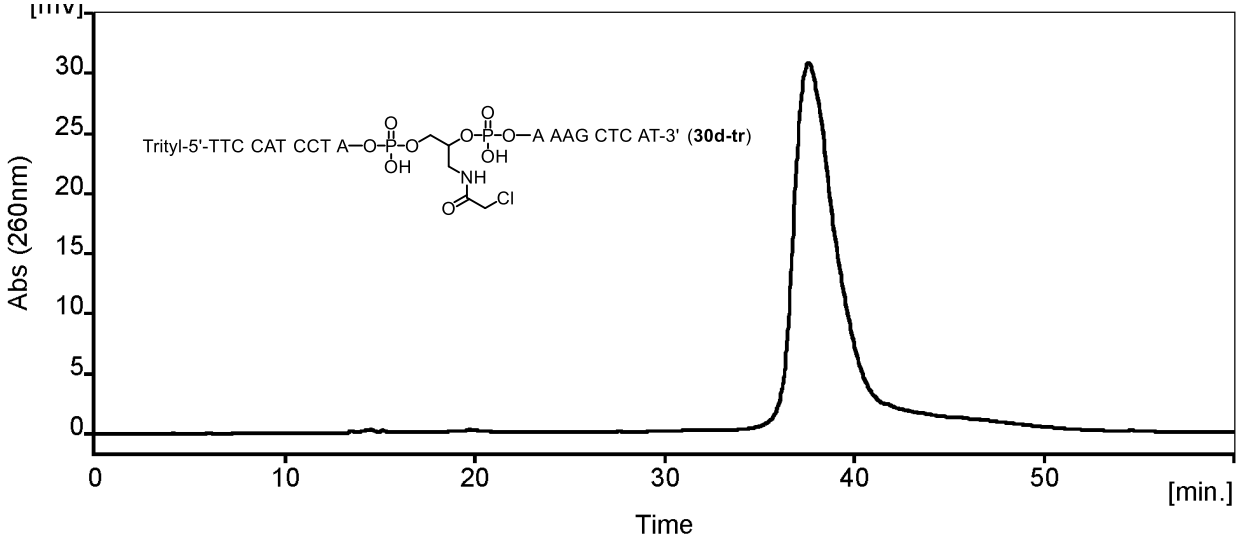
RP HPLC profile of de-tritylated ODN **30c**.



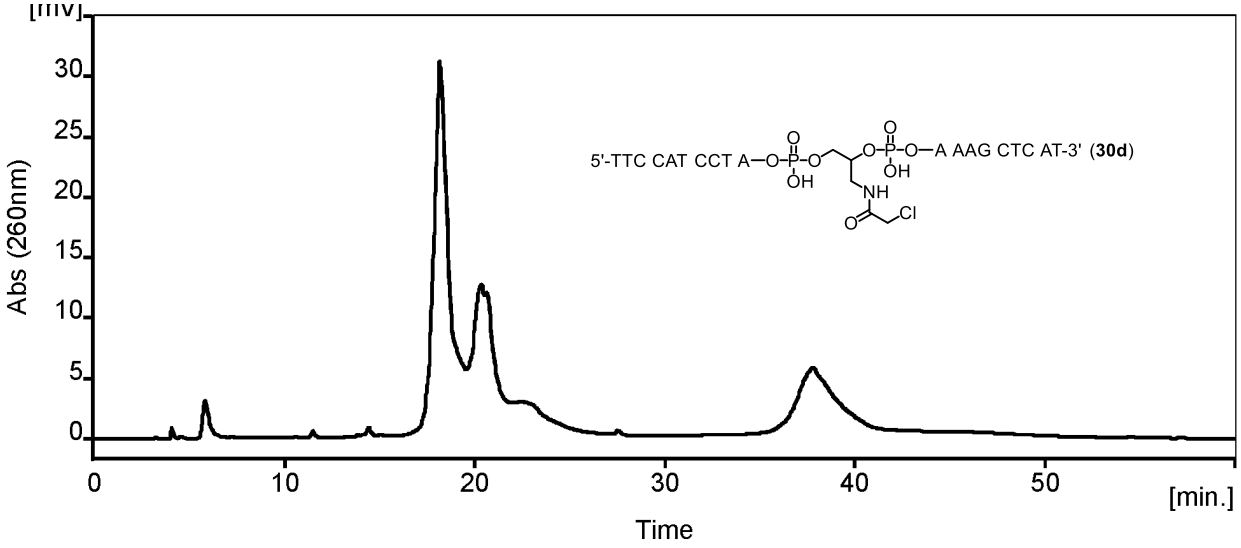
RP HPLC profile of purified de-tritylated ODN **30c**.



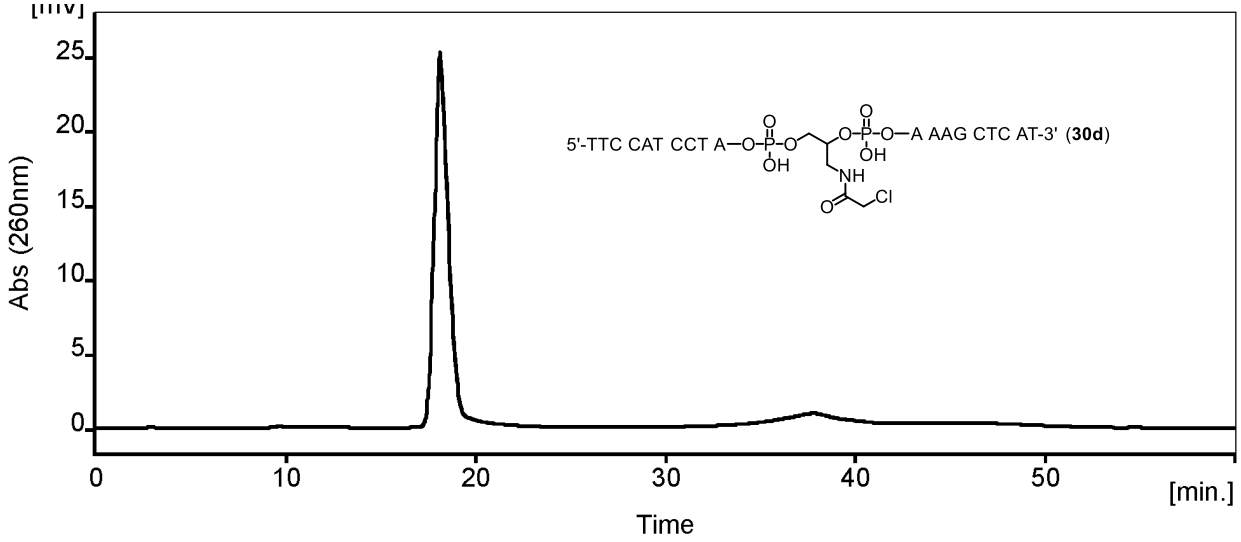
RP HPLC profile of crude trityl-tagged ODN **30d-tr**. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.



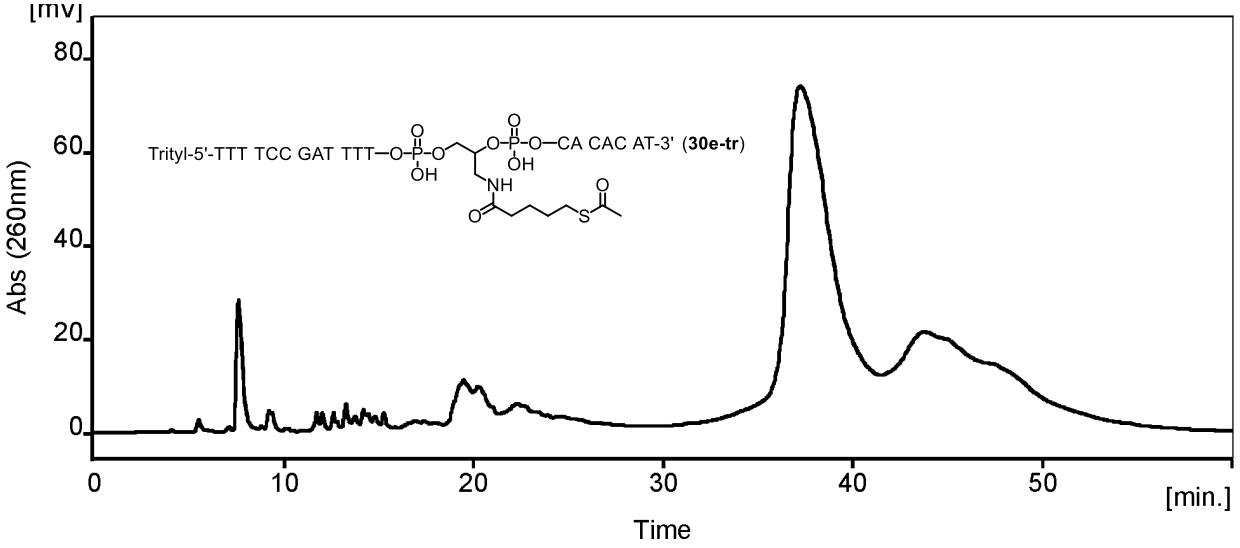
RP HPLC profile of purified trityl-tagged ODN **30d-tr**.



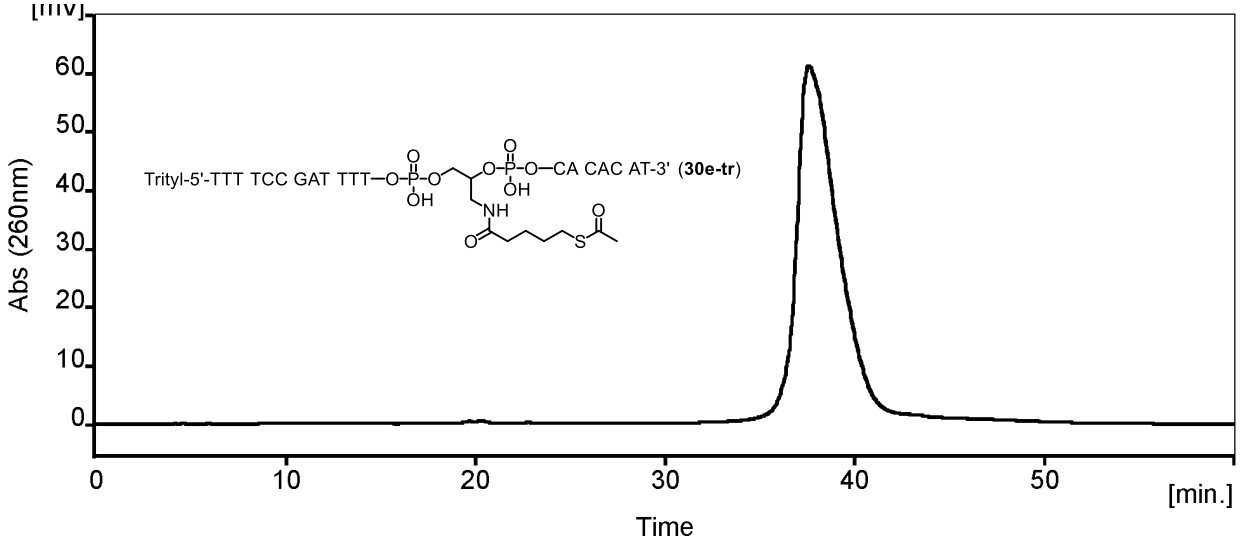
RP HPLC profile of de-tritylated ODN **30d**.



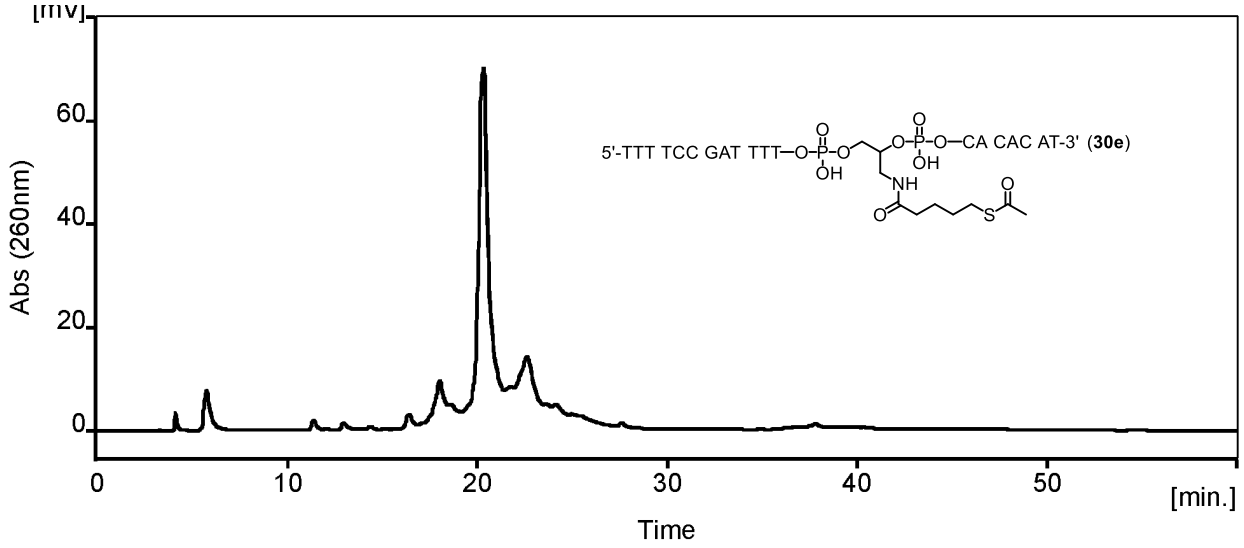
RP HPLC profile of purified de-tritylated ODN **30d**.



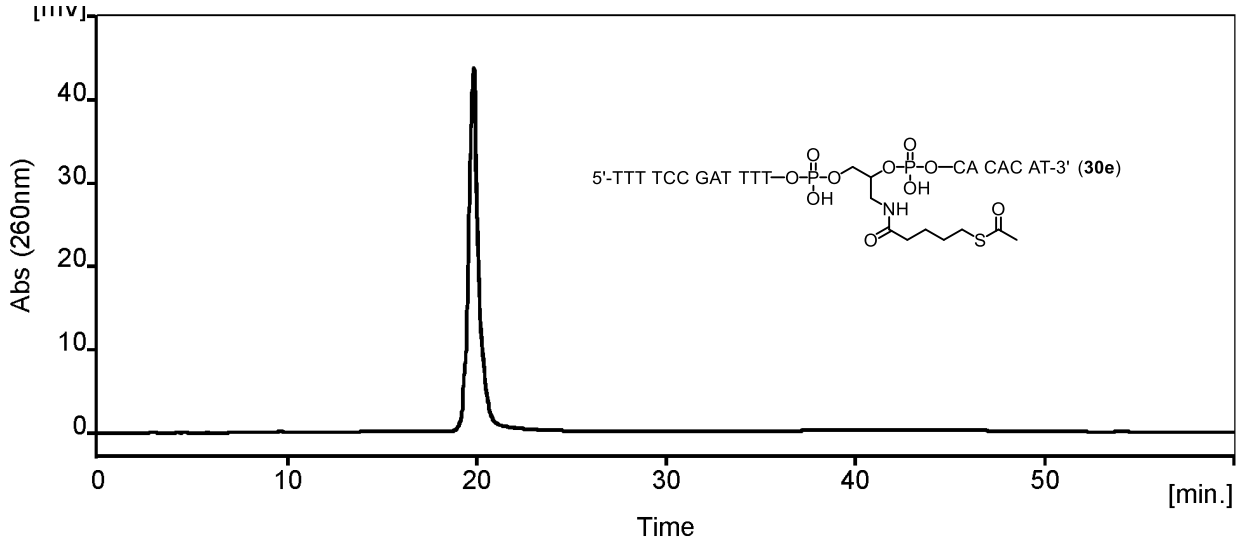
RP HPLC profile of crude trityl-tagged ODN **30e-tr**. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.



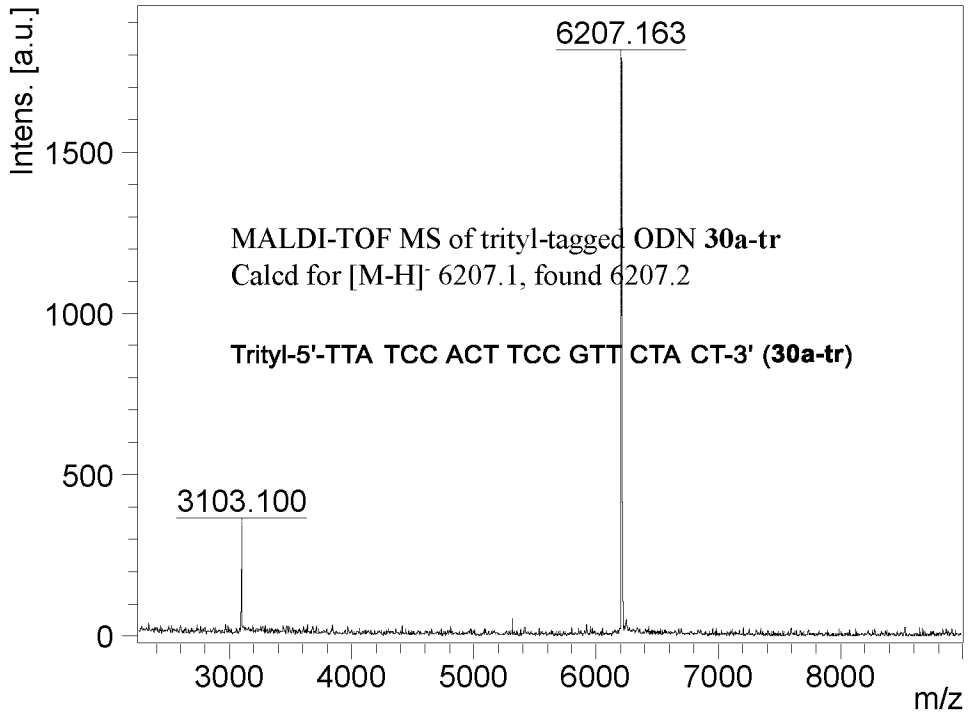
RP HPLC profile of purified trityl-tagged ODN **30e-tr**.

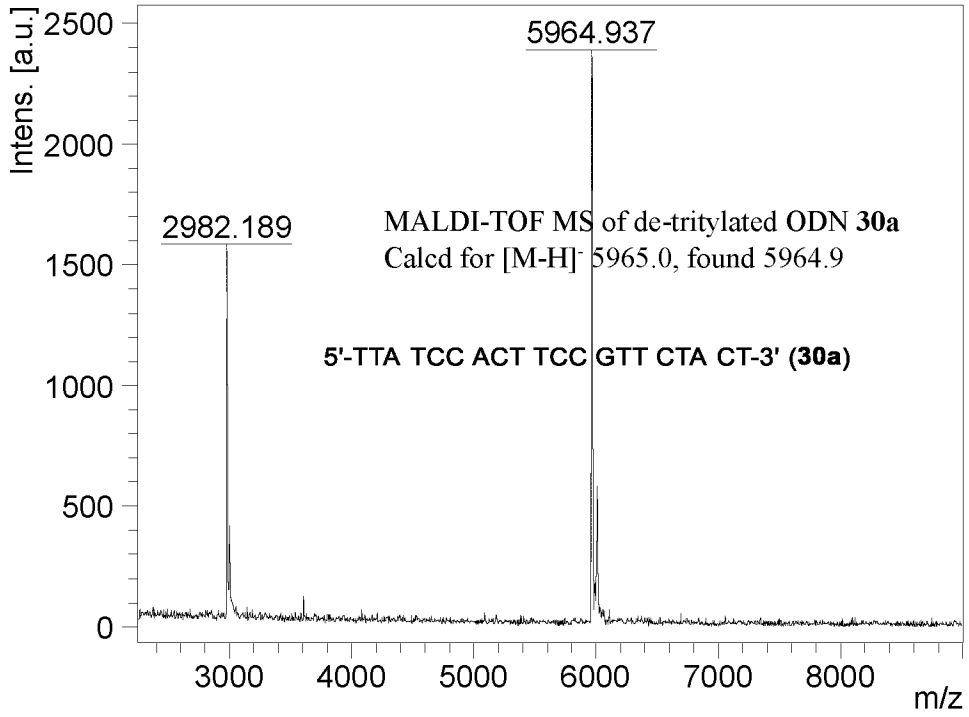


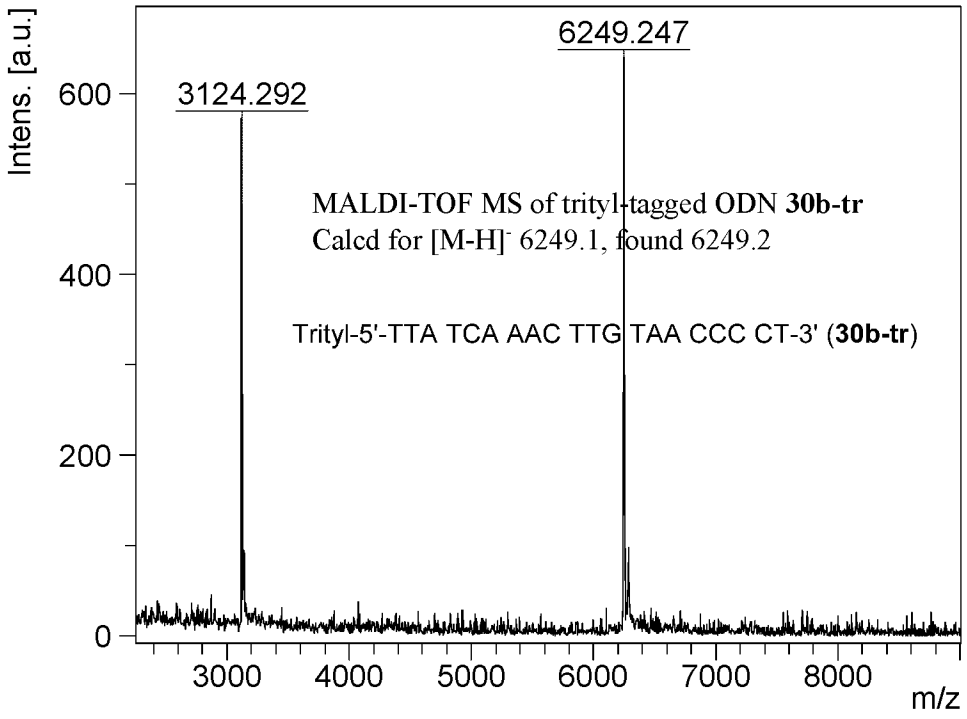
RP HPLC profile of de-tritylated ODN **30e**.

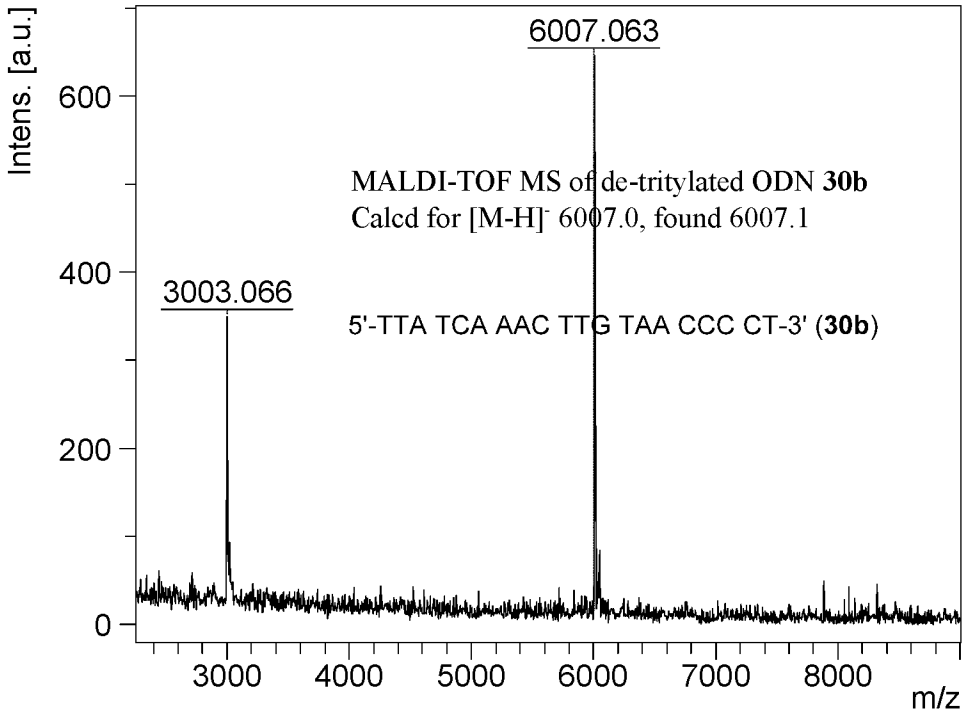


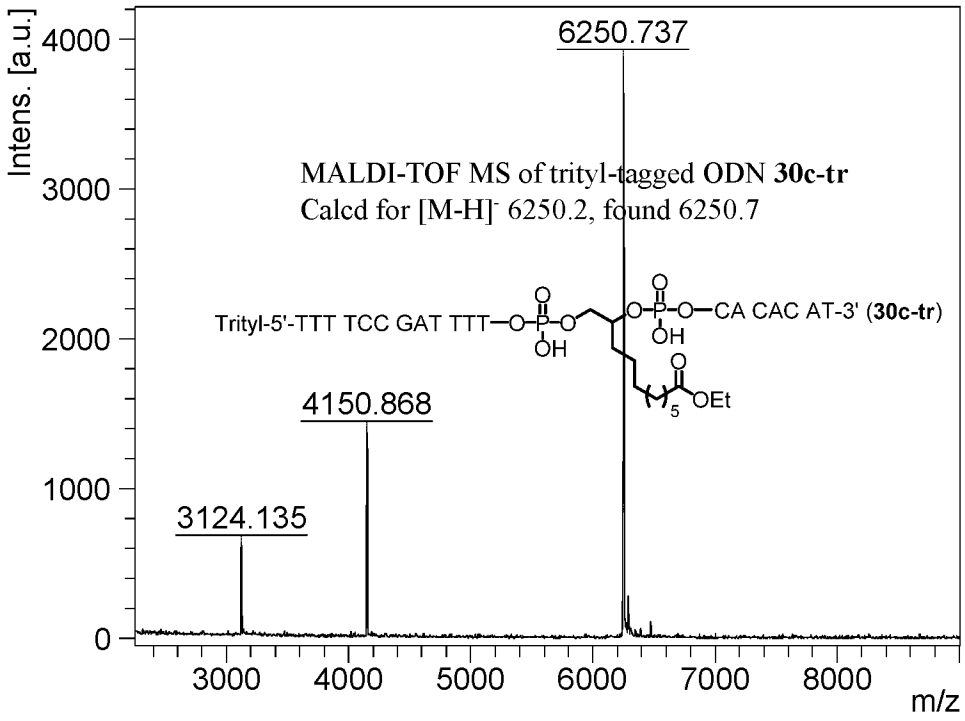
RP HPLC profile of purified de-tritylated ODN **30e**.

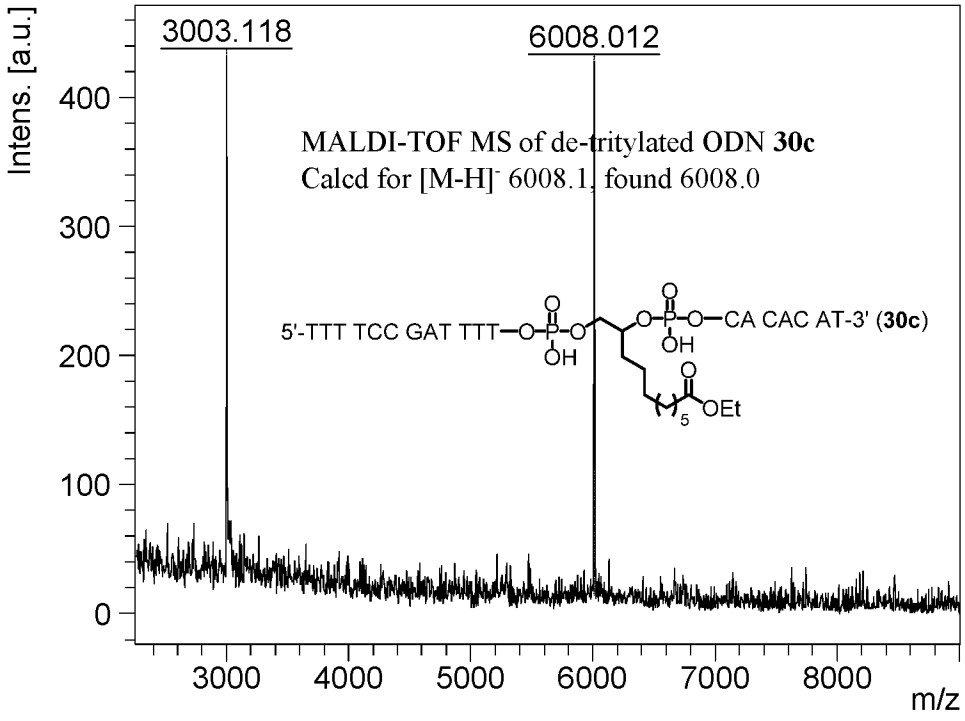

Intens. [a.u.]
6207.163
1500
1000
500
0
MALDI-TOF MS of trityl-tagged ODN 30a-tr
Calcd for [M-H]- 6207.1, found 6207.2
Trityl-5'-TTA TCC ACT TCC GTT CTA CT-3' (30a-tr)
3103.100
3000
4000
5000
6000
7000
8000
m/z

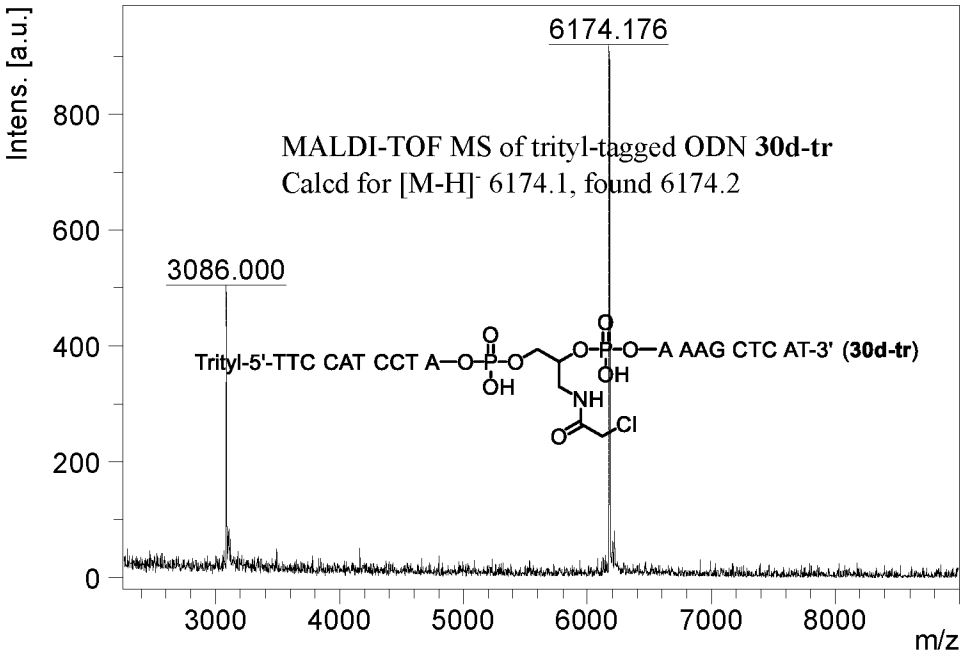

Intens. [a.u.]
2500
2000
1500
1000
500
0
5964.937
2982.189
MALDI-TOF MS of de-tritylated ODN 30a
Calcd for [M-H]- 5965.0, found 5964.9
5'-TTA TCC ACT TCC GTT CTA CT-3' (30a)
3000
4000
5000
6000
7000
8000
m/z

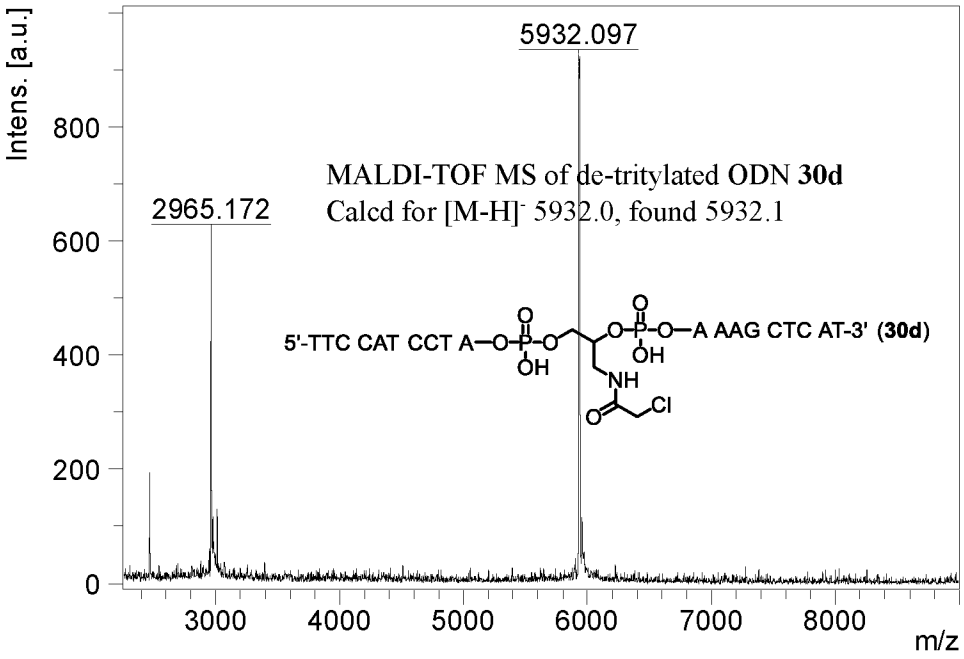

Intens. [a.u.]
600
400
200
0
6249.247
3124.292
MALDI-TOF MS of trityl-tagged ODN **30b-tr**
Calcd for [M-H]⁻ 6249.1, found 6249.2
Trityl-5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b-tr**)
3000
4000
5000
6000
7000
8000
m/z

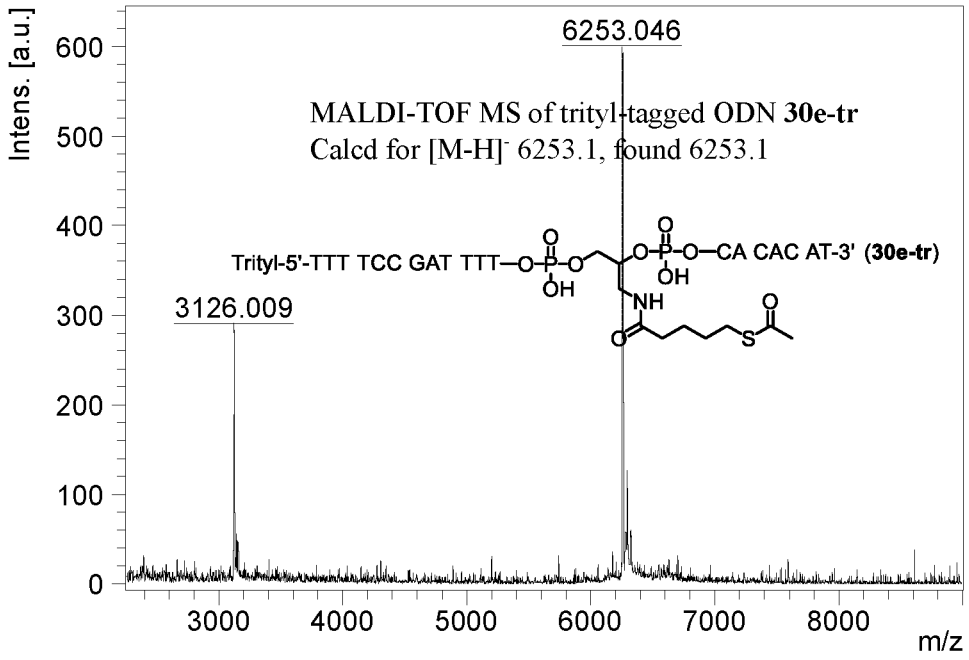

Intens. [a.u.]
600
400
200
0
6007.063
3003.066
MALDI-TOF MS of de-tritylated ODN **30b**
Calcd for [M-H]⁻ 6007.0, found 6007.1
5'-TTA TCA AAC TTG TAA CCC CT-3' (**30b**)
3000
4000
5000
6000
7000
8000
m/z

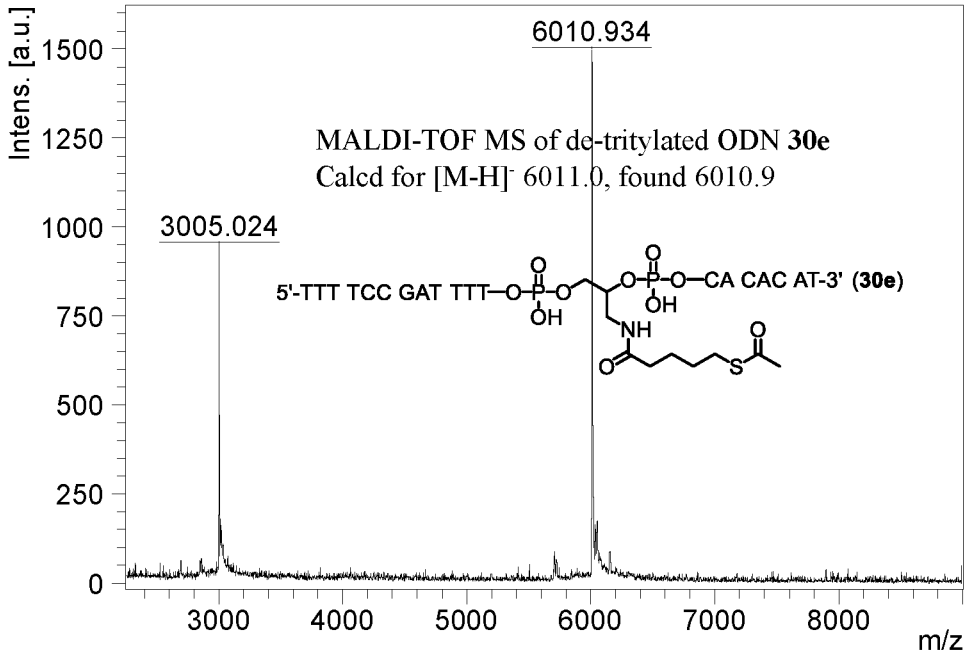

Intens. [a.u.]
4000
3000
2000
1000
0
6250.737
4150.868
3124.135
MALDI-TOF MS of trityl-tagged ODN 30c-tr
Calcd for [M-H]- 6250.2, found 6250.7
Trityl-5'-TTT TCC GAT TTT
CA CAC AT-3' (30c-tr)
OEt
3000
4000
5000
6000
7000
8000
m/z

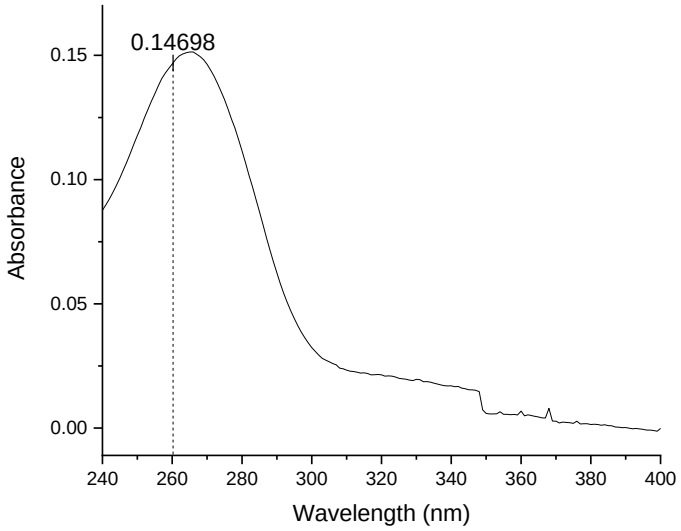

Intens. [a.u.]
400
300
200
100
0
3003.118
6008.012
MALDI-TOF MS of de-tritylated ODN 30c
Calcd for [M-H]- 6008.1, found 6008.0
5'-TTT TCC GAT TTT
CA CAC AT-3' (30c)
OEt
3000
4000
5000
6000
7000
8000
m/z


Intens. [a.u.]
6174.176
MALDI-TOF MS of trityl-tagged ODN 30d-tr
Calcd for [M-H]- 6174.1, found 6174.2
3086.000
Trityl-5'-TTC CAT CCT A
A AAG CTC AT-3' (30d-tr)
800
600
400
200
0
3000
4000
5000
6000
7000
8000
m/z


Intens. [a.u.]
5932.097
MALDI-TOF MS of de-tritylated ODN 30d
Calcd for [M-H]- 5932.0, found 5932.1
2965.172
5'-TTC CAT CCT A
A AAG CTC AT-3' (30d)
800
600
400
200
0
3000
4000
5000
6000
7000
8000
m/z


Intens. [a.u.]
600
500
400
300
200
100
0
6253.046
MALDI-TOF MS of trityl-tagged ODN 30e-tr
Calcd for [M-H]- 6253.1, found 6253.1
Trityl-5'-TTT TCC GAT TTT—O-P-O
OH
O-P-O—CA CAC AT-3' (30e-tr)
OH
NH
3126.009
3000
4000
5000
6000
7000
8000
m/z

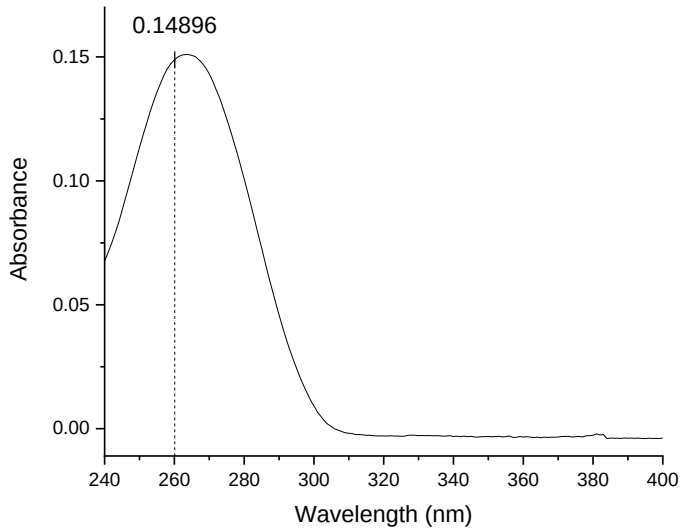

Intens. [a.u.]
1500
1250
1000
750
500
250
0
6010.934
MALDI-TOF MS of de-tritylated ODN 30e
Calcd for [M-H]- 6011.0, found 6010.9
3005.024
5'-TTT TCC GAT TTT—O-P-O
OH
O-P-O—CA CAC AT-3' (30e)
OH
NH
3000
4000
5000
6000
7000
8000
m/z



UV of ODN **30a**.

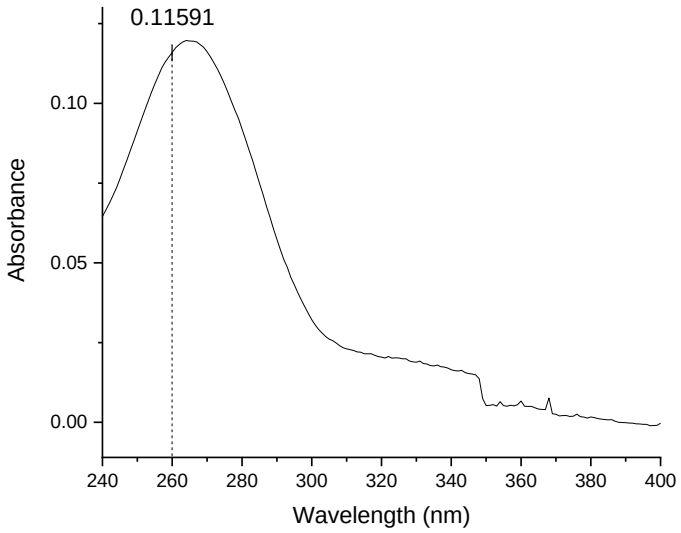
CPG (**4**, loading 26 μmol/g, 20 mg) of 0.52 μmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 μmol synthesis is 2.94 (0.147 × 20), which corresponds to a 3.1% overall yield.

For comparing with standard technology, **30a** was synthesized on the same amount of CPG **4** (loading 26 μmol/g, 20 mg) using commercial phosphoramidites under synthesizer manufacturer recommended conditions. The CPG was divided into 10 portions, one portion was deprotected and cleaved with concentrated NH_4OH (55℃, 12 h). After HPLC purification, the OD_{260} was measured the same way and found to be 8.30 (0.415 × 20), which corresponds to a 8.8% overall yield. As can be seen, even though significant amount branched sequences were observed in the HPLC profile of crude **30a** synthesized with the dM-Dmoc technology, the yield of pure target ODN was not significantly lower than that obtained with standard technology. In addition, it is important to note that the standard technology cannot be used to synthesize electrophilic ODNs such as **30c**–**e**.



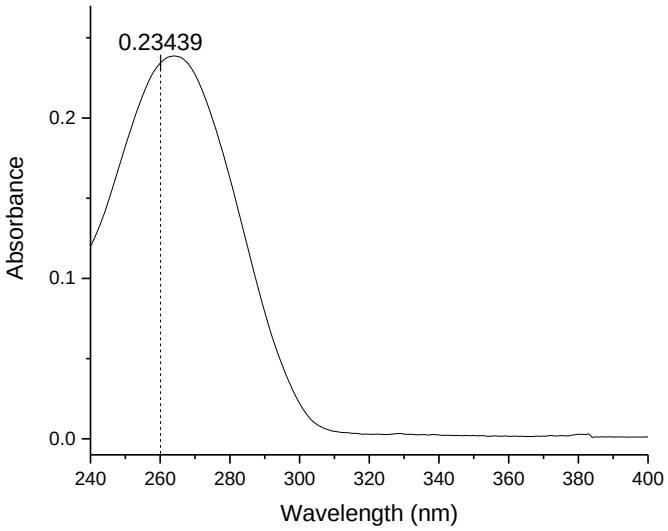
UV of ODN **30b**.

CPG (**4**, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 µmol synthesis is 2.98 (0.149 × 20), which corresponds to a 2.8% overall yield.



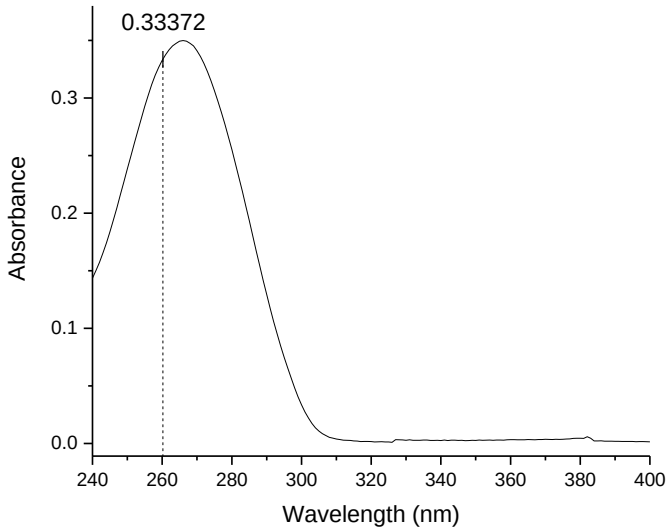
UV of ODN **30c**.

CPG (**4**, loading 26 μmol/g, 20 mg) of 0.52 μmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 μmol synthesis is 2.32 (0.116 × 20), which corresponds to a 2.4% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).



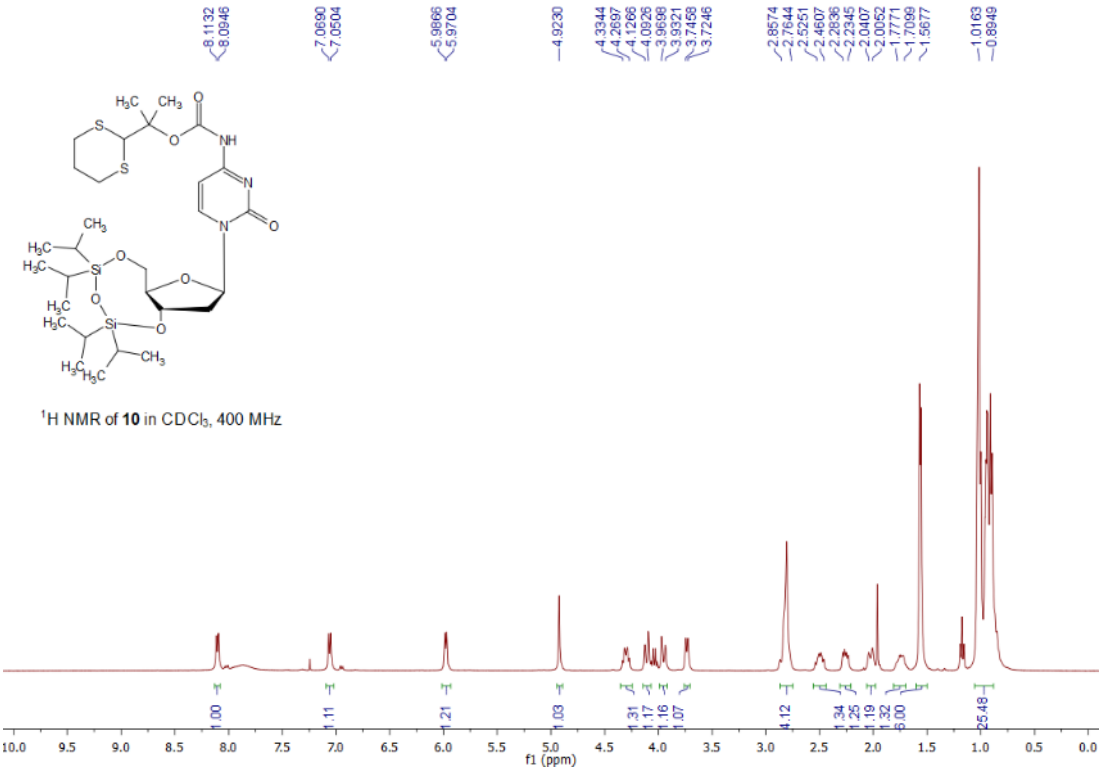
UV of ODN **30d**.

CPG (**4**, loading 26 μmol/g, 20 mg) of 0.52 μmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 μmol synthesis is 4.68 (0.234 × 20), which corresponds to a 4.6% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).

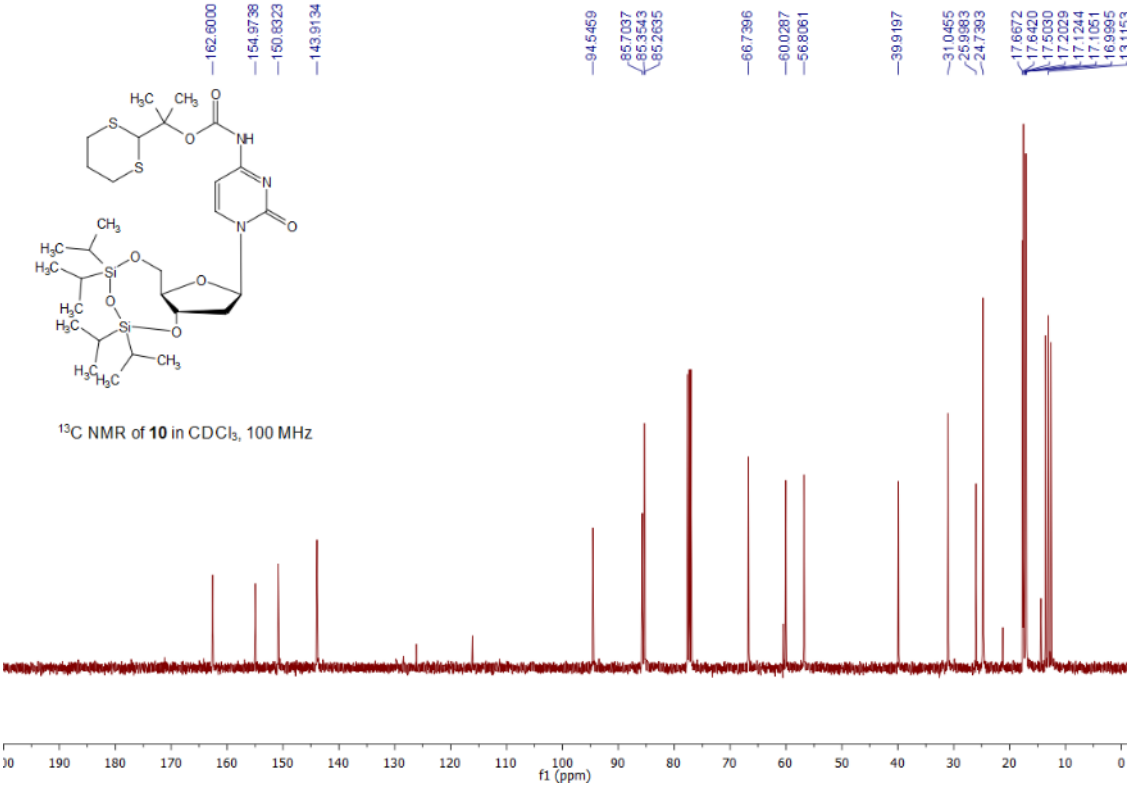


UV of ODN **30e**.

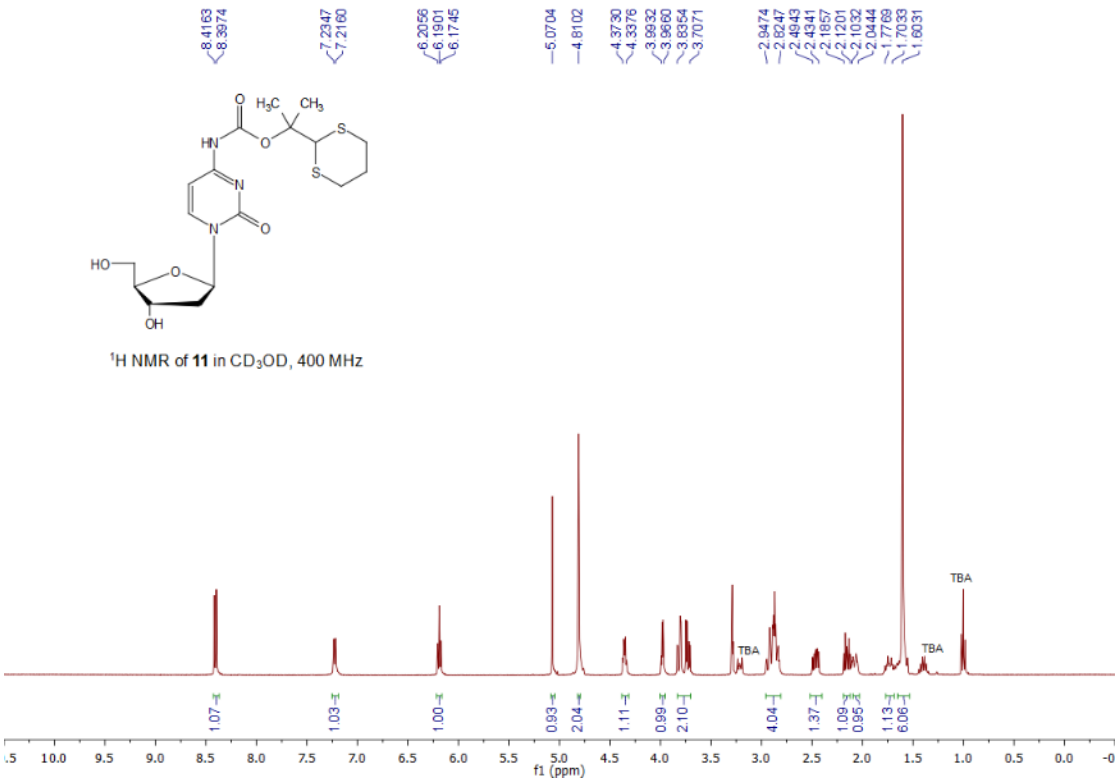
CPG (**4**, loading 26 μmol/g, 20 mg) of 0.52 μmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 μmol synthesis is 6.68 (0.334 × 20), which corresponds to a 7.0% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).



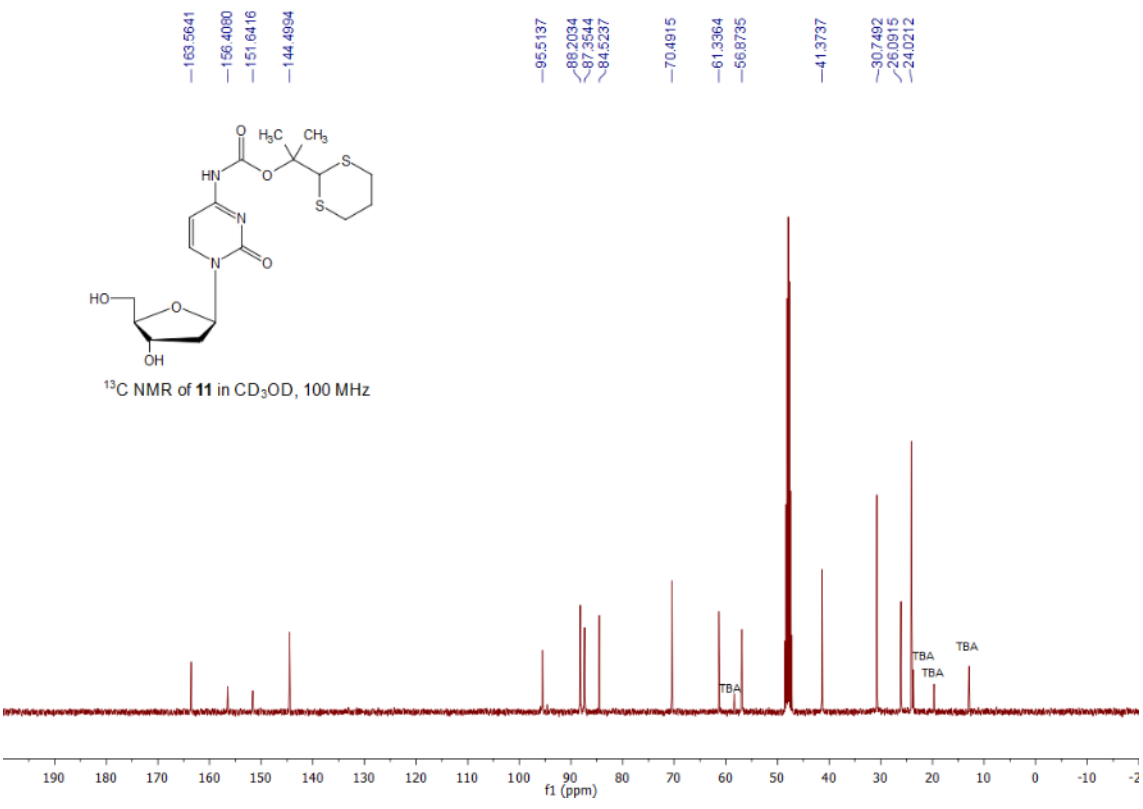
^{1}H NMR of **10** in $CDCl_3$, 400 MHz



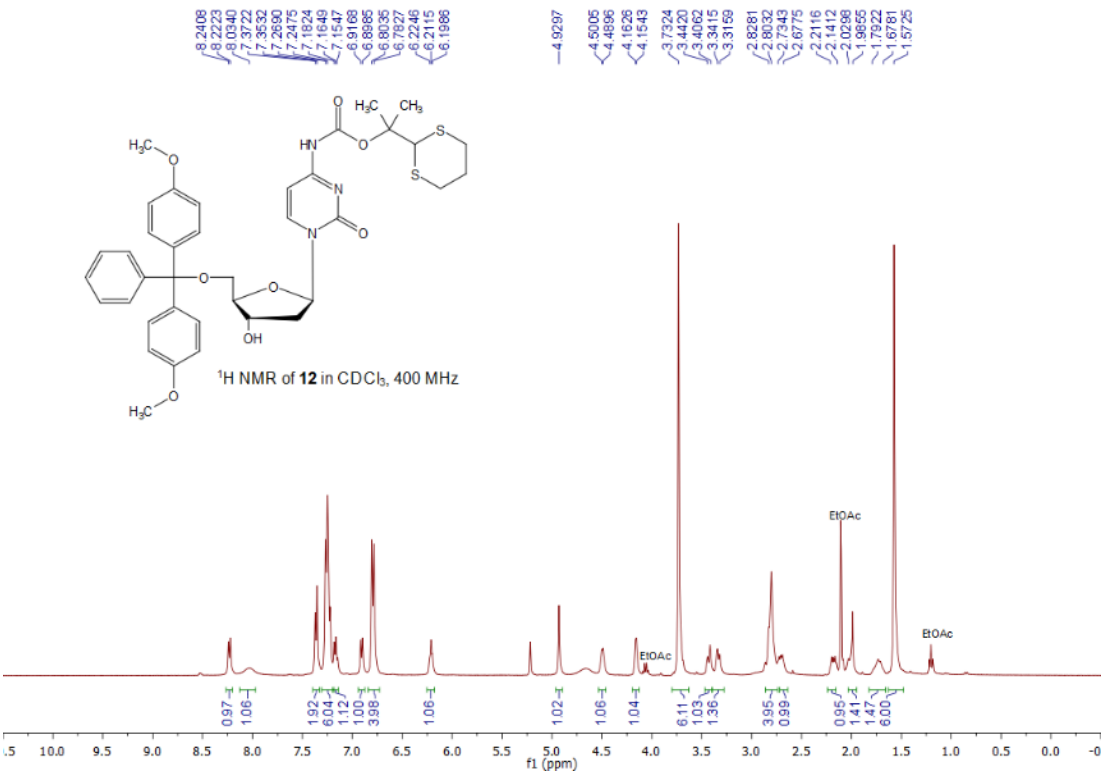
^{13}C NMR of **10** in $CDCl_3$, 100 MHz



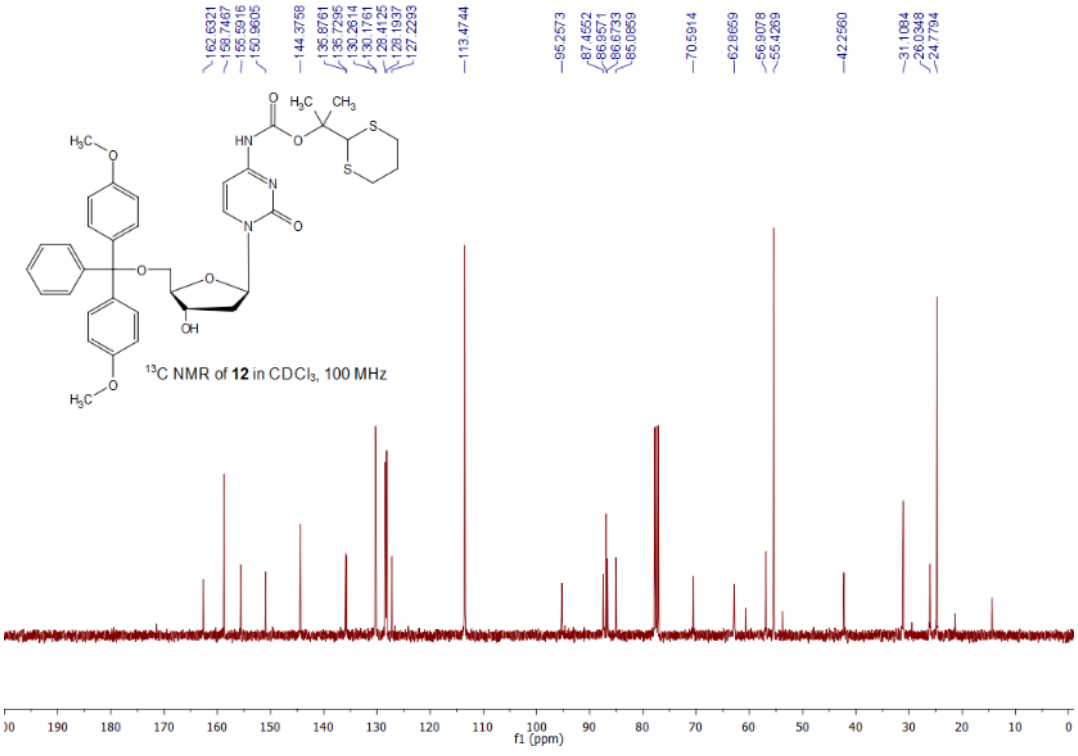
^{1}H NMR of **11** in CD_3OD, 400 MHz



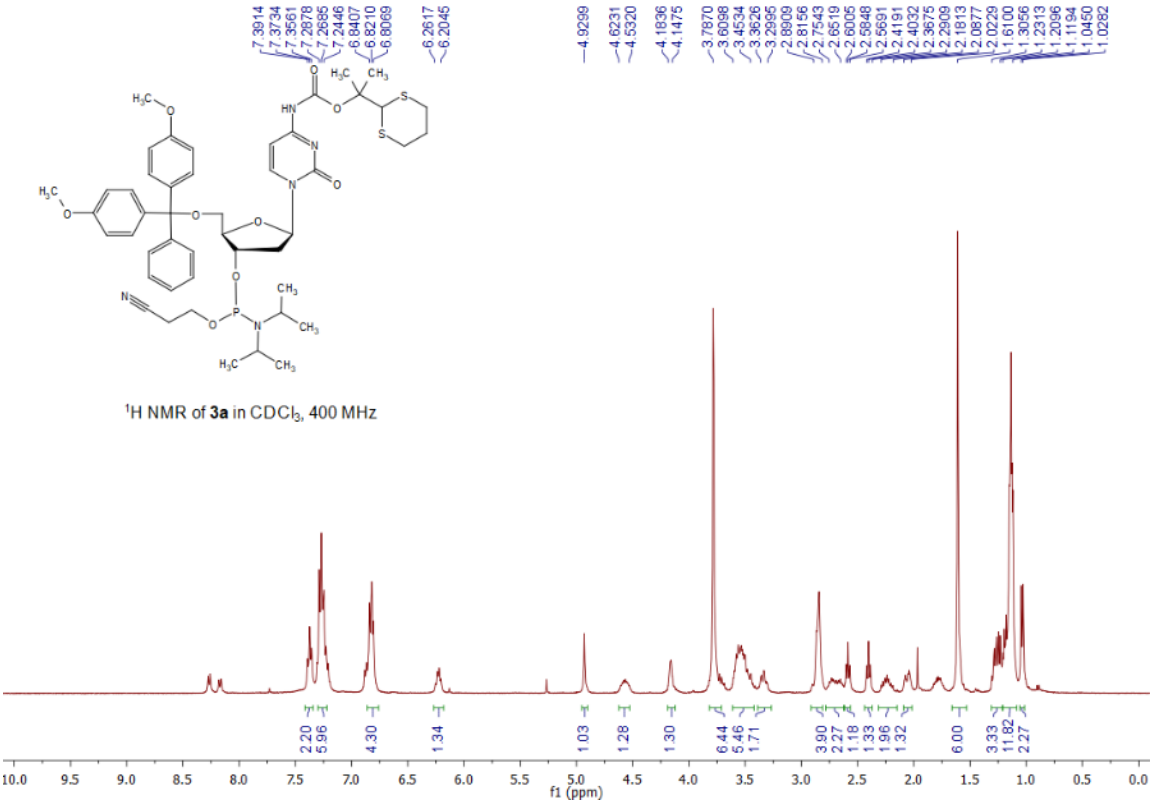
^{13}C NMR of **11** in CD_3OD, 100 MHz



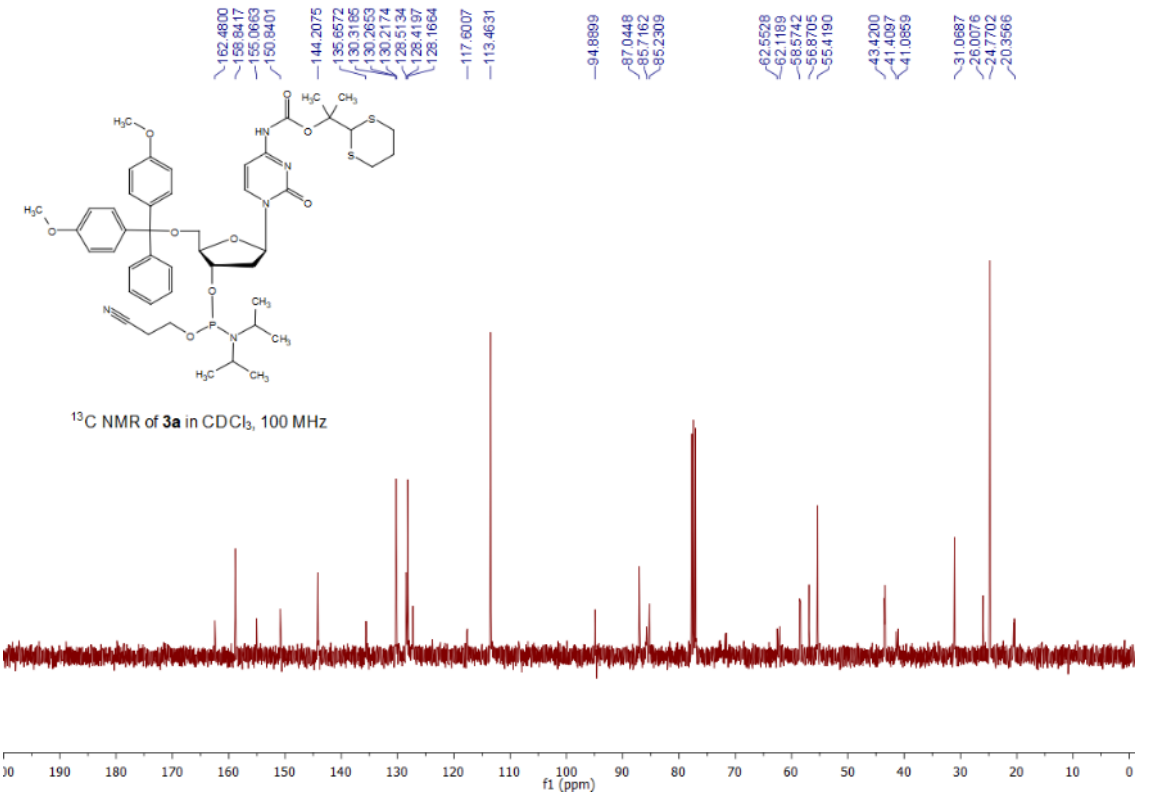
^{1}H NMR of **12** in $CDCl_3$, 400 MHz



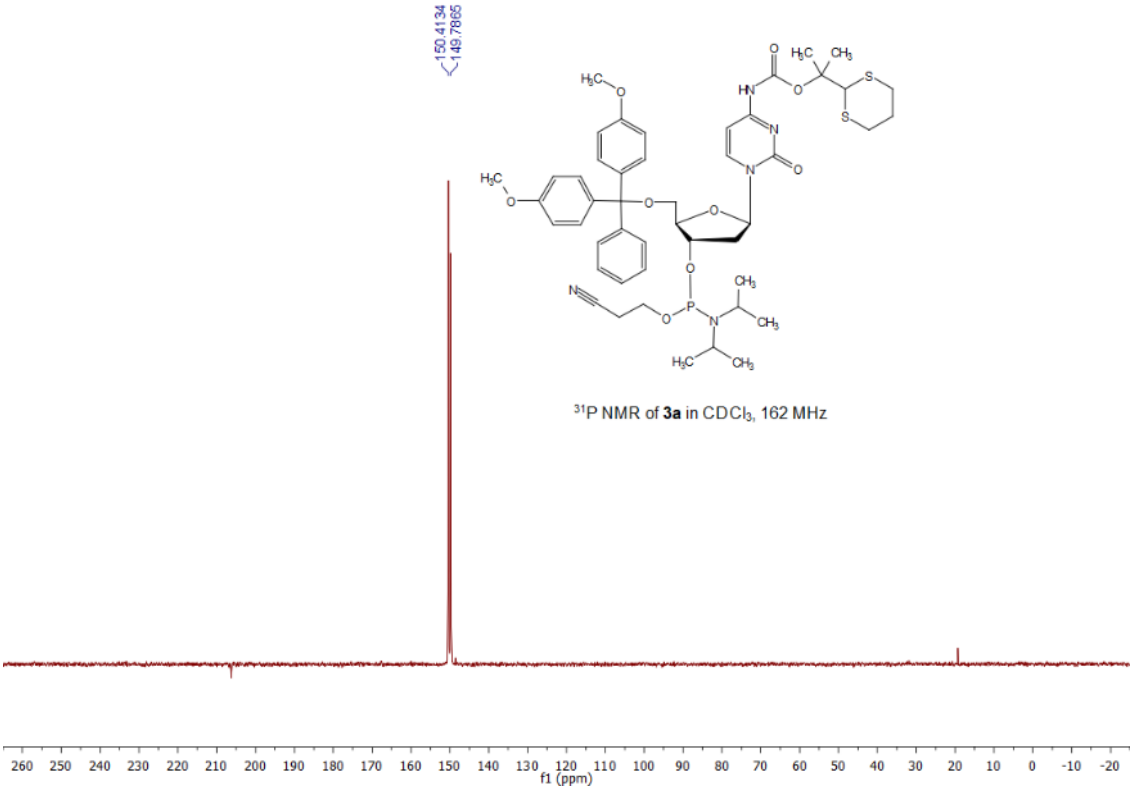
^{13}C NMR of **12** in $CDCl_3$, 100 MHz



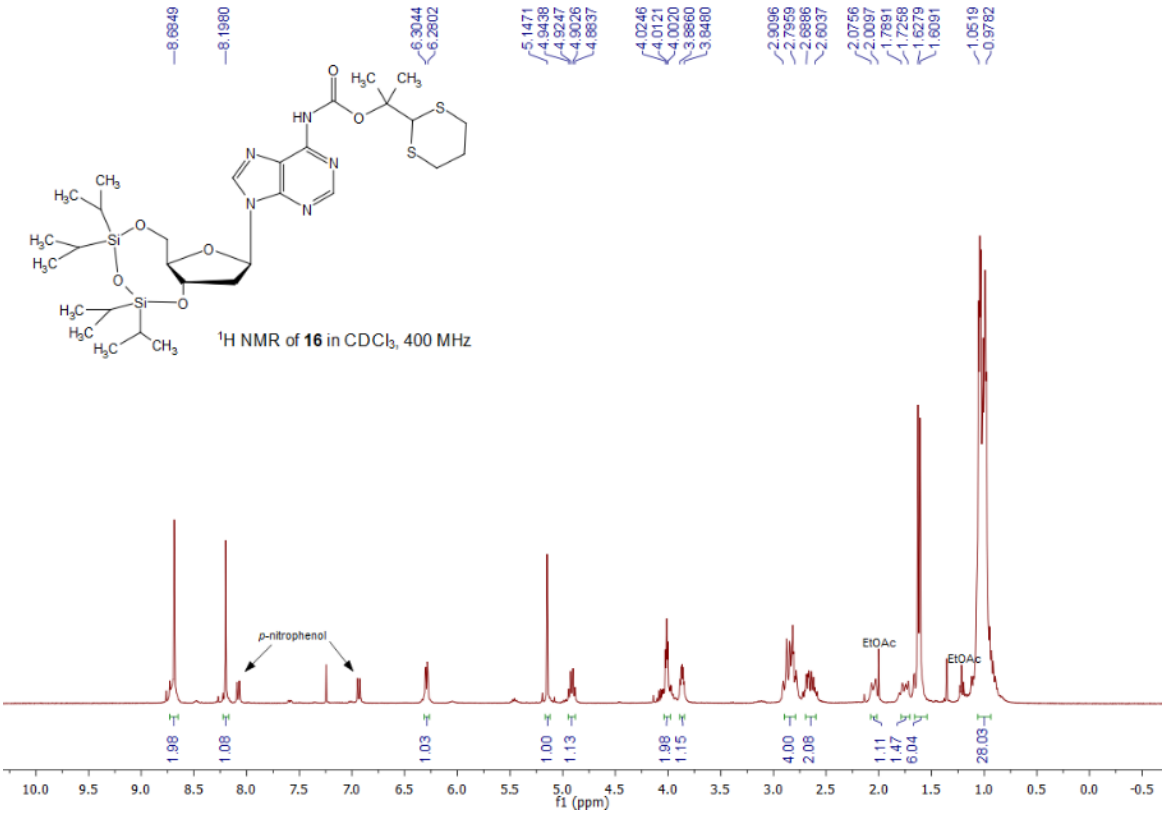
^{1}H NMR of **3a** in $CDCl_3$, 400 MHz



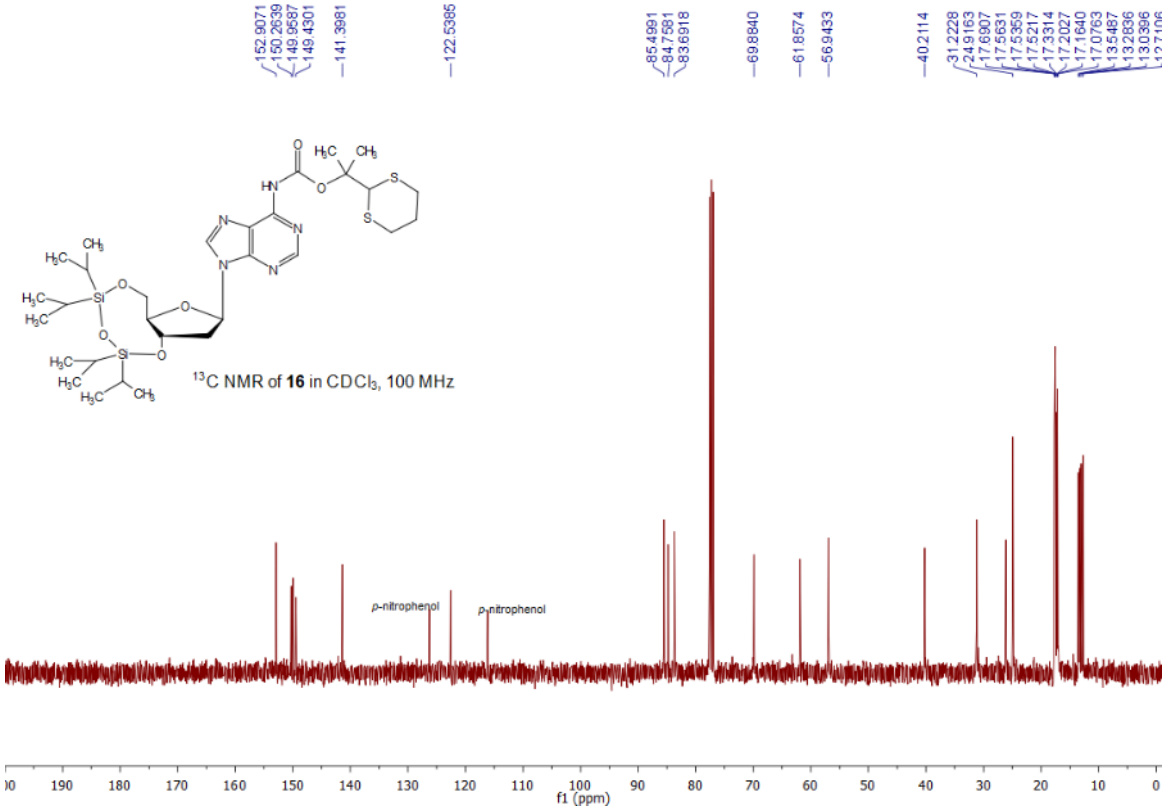
^{13}C NMR of **3a** in $CDCl_3$, 100 MHz



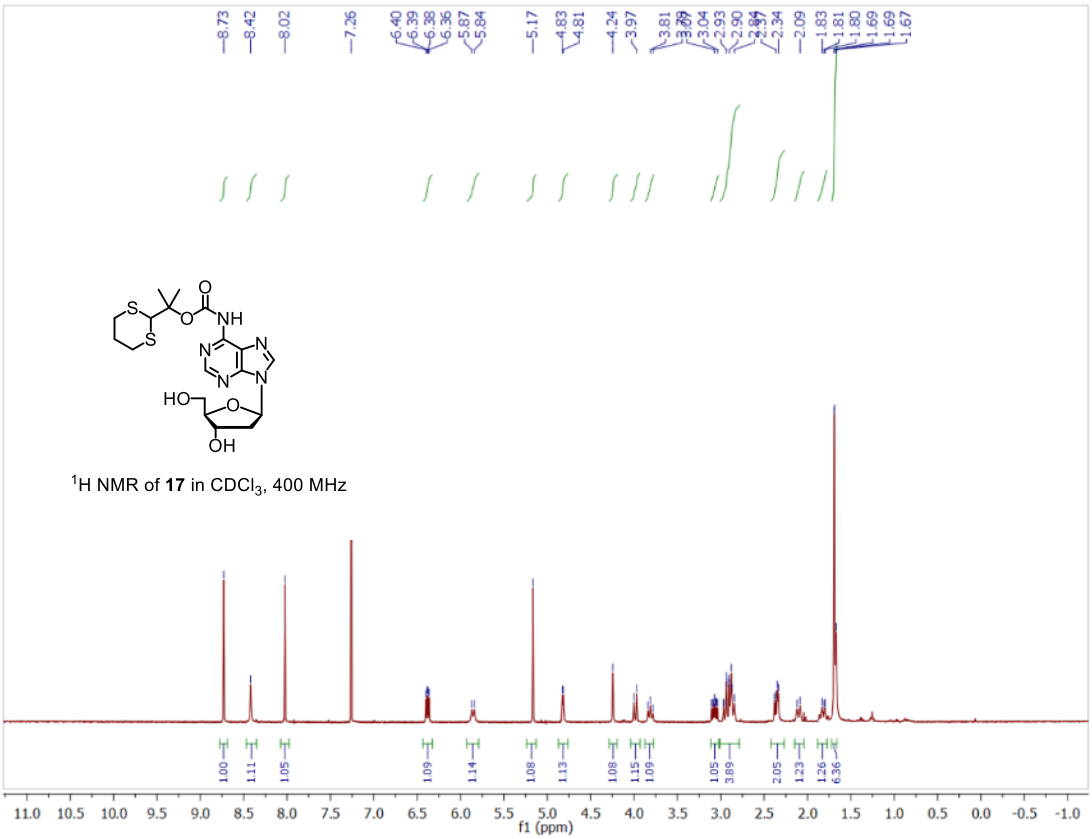
^{31}P NMR of **3a** in $CDCl_3$, 162 MHz



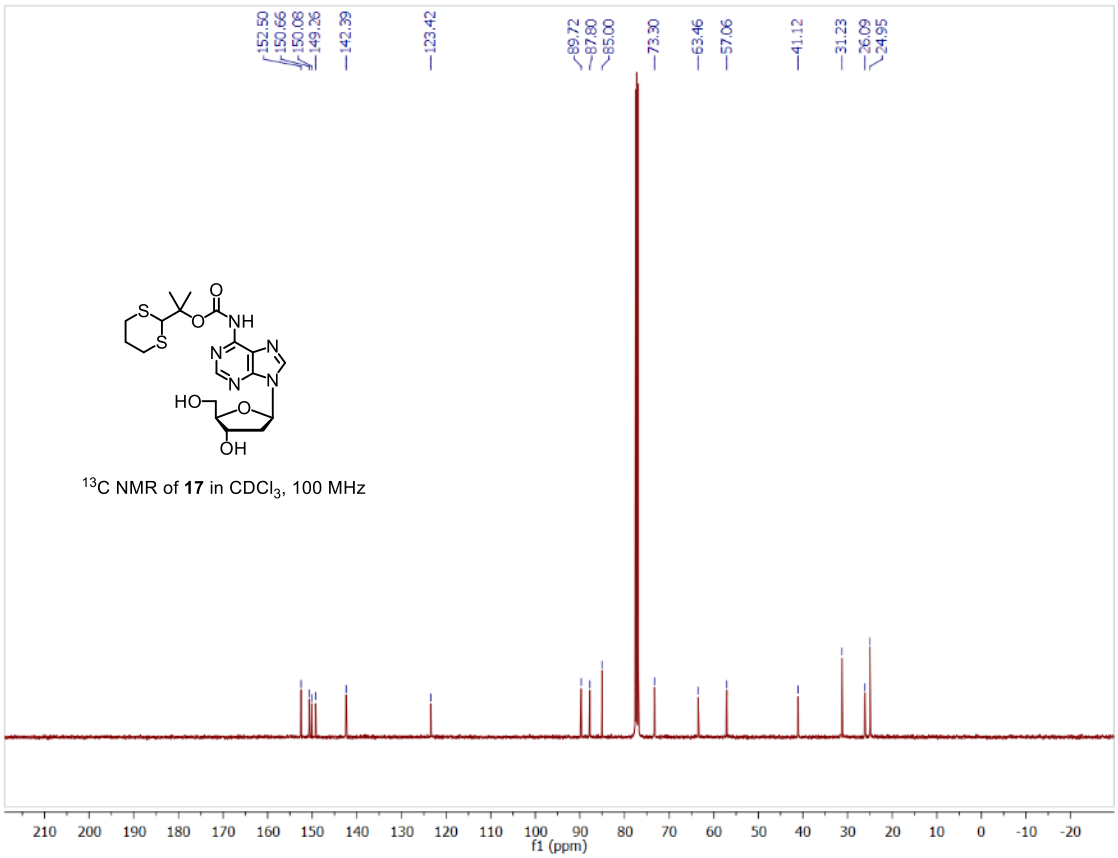
^{1}H NMR of **16** in $CDCl_3$, 400 MHz



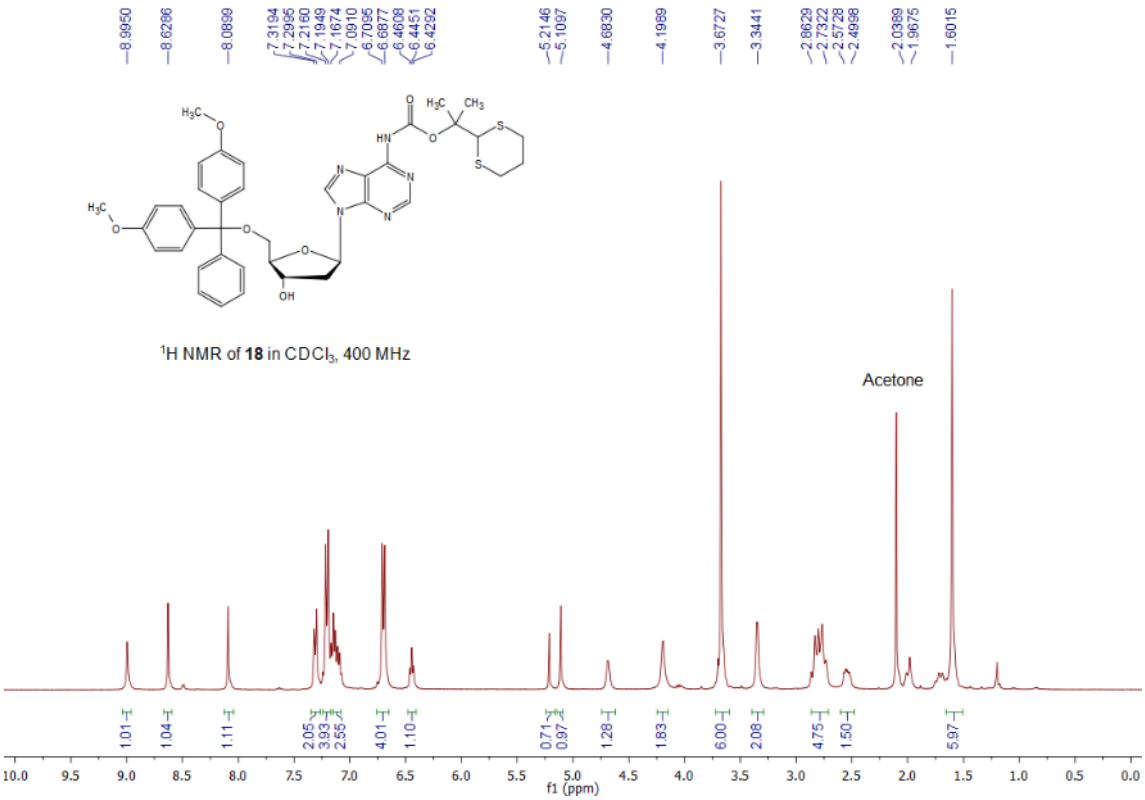
^{13}C NMR of **16** in $CDCl_3$, 100 MHz



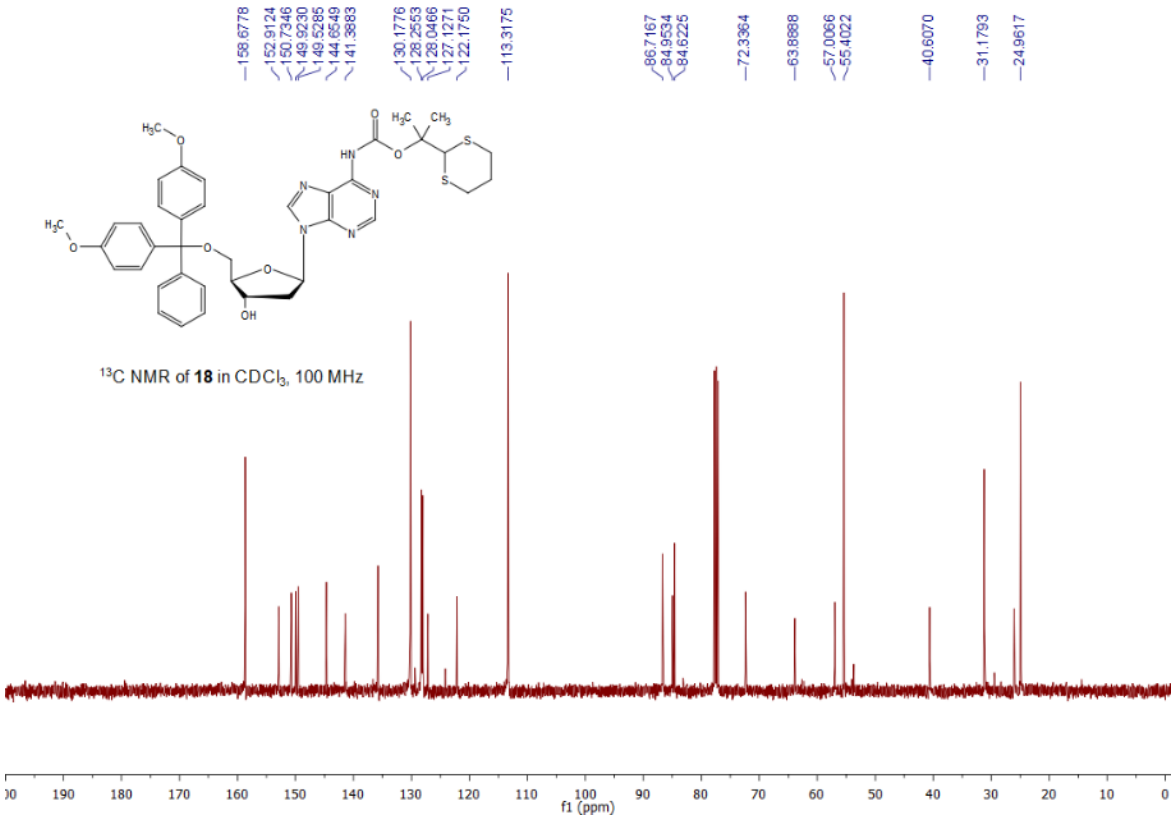
^{1}H NMR of **17** in $CDCl_3$, 400 MHz



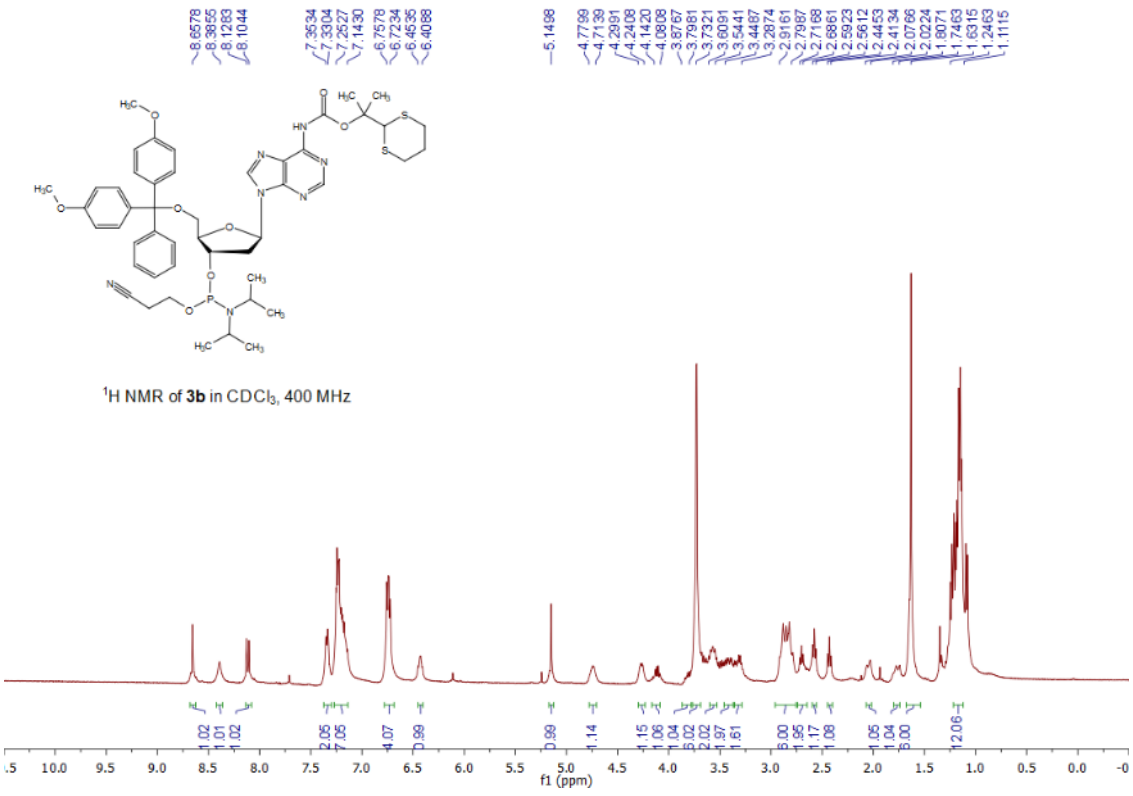
^{13}C NMR of **17** in $CDCl_3$, 100 MHz



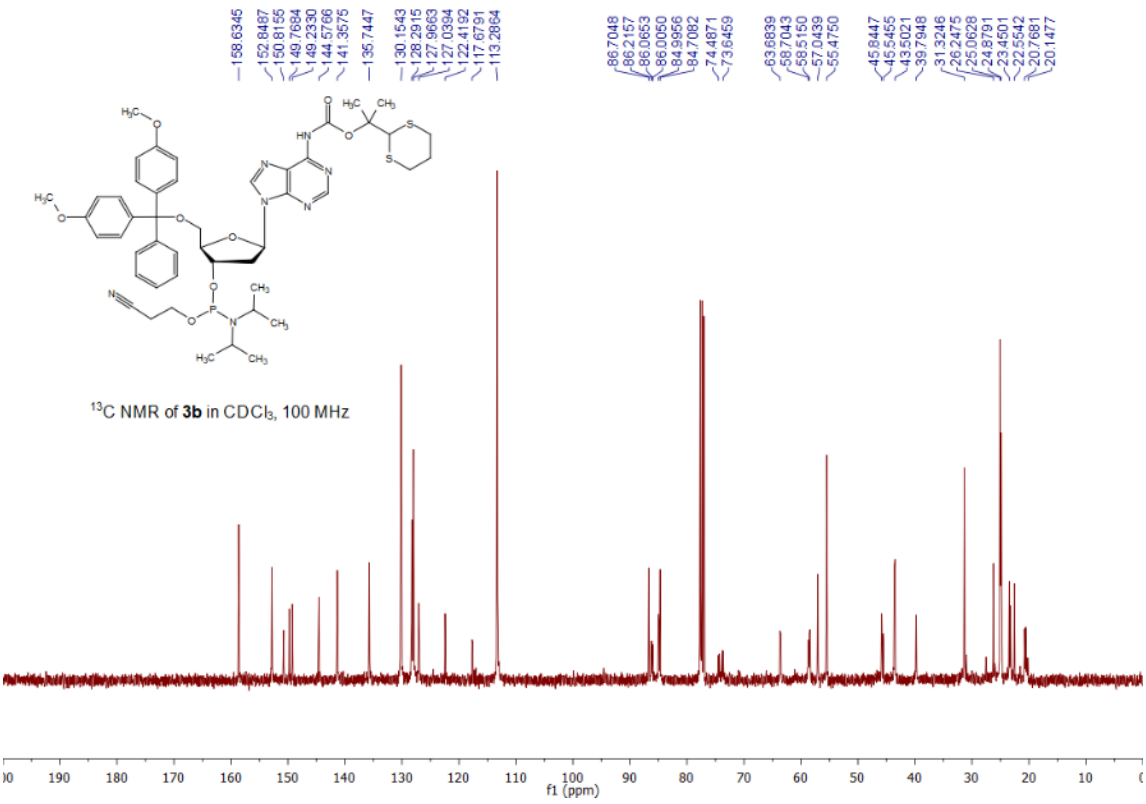
^{1}H NMR of **18** in $CDCl_3$, 400 MHz



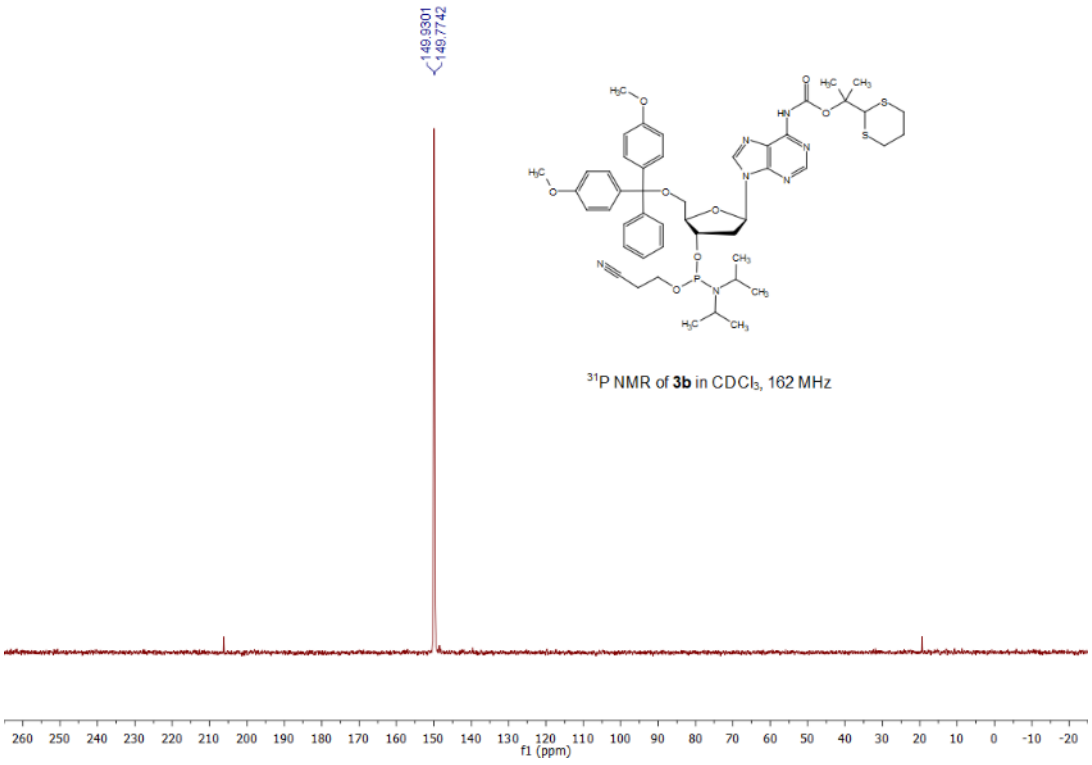
^{13}C NMR of **18** in $CDCl_3$, 100 MHz



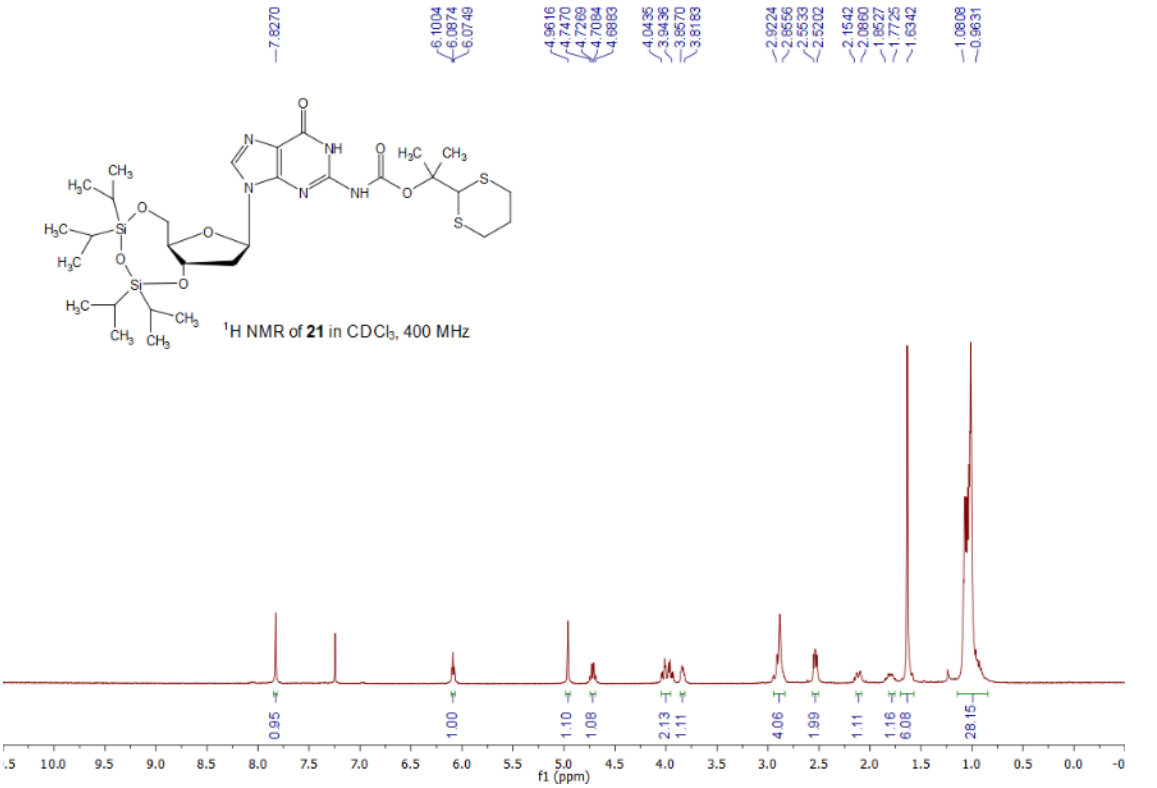
^{1}H NMR of **3b** in $CDCl_3$, 400 MHz



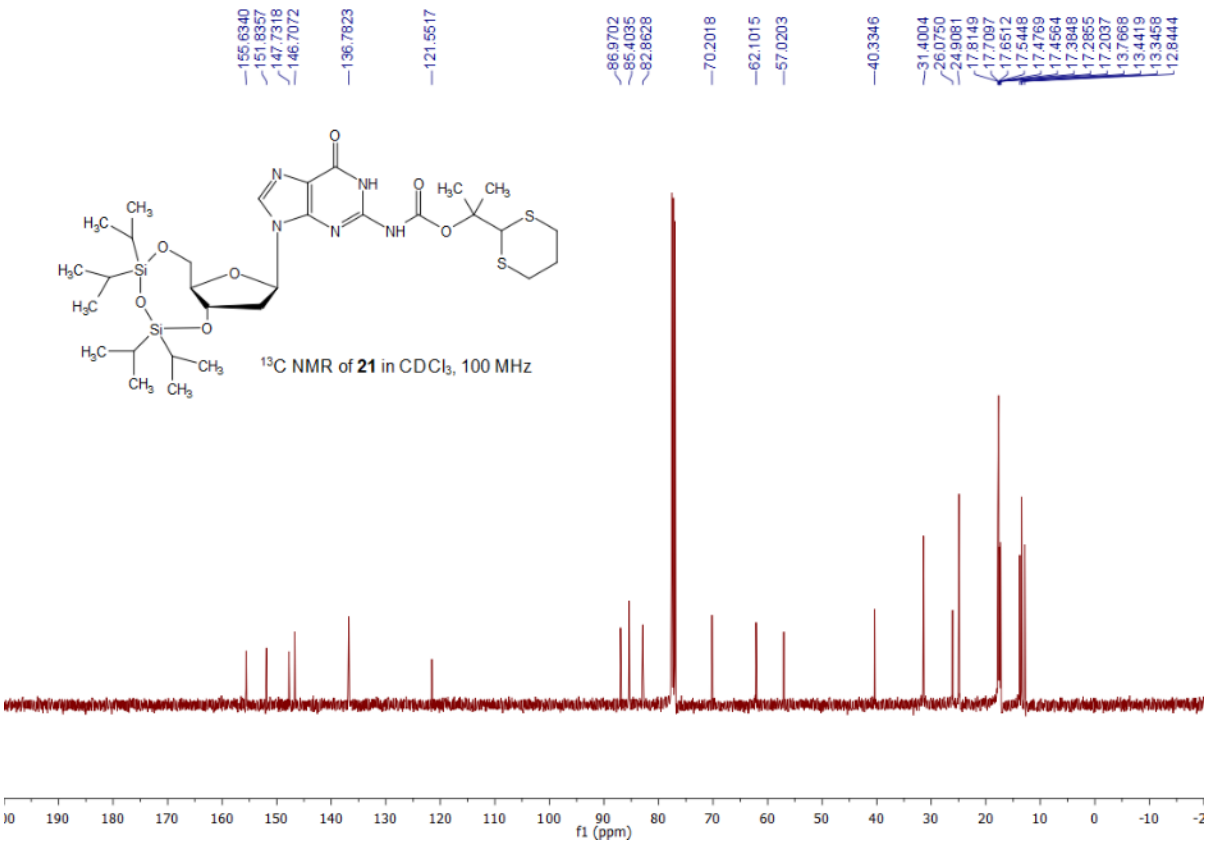
^{13}C NMR of **3b** in $CDCl_3$, 100 MHz



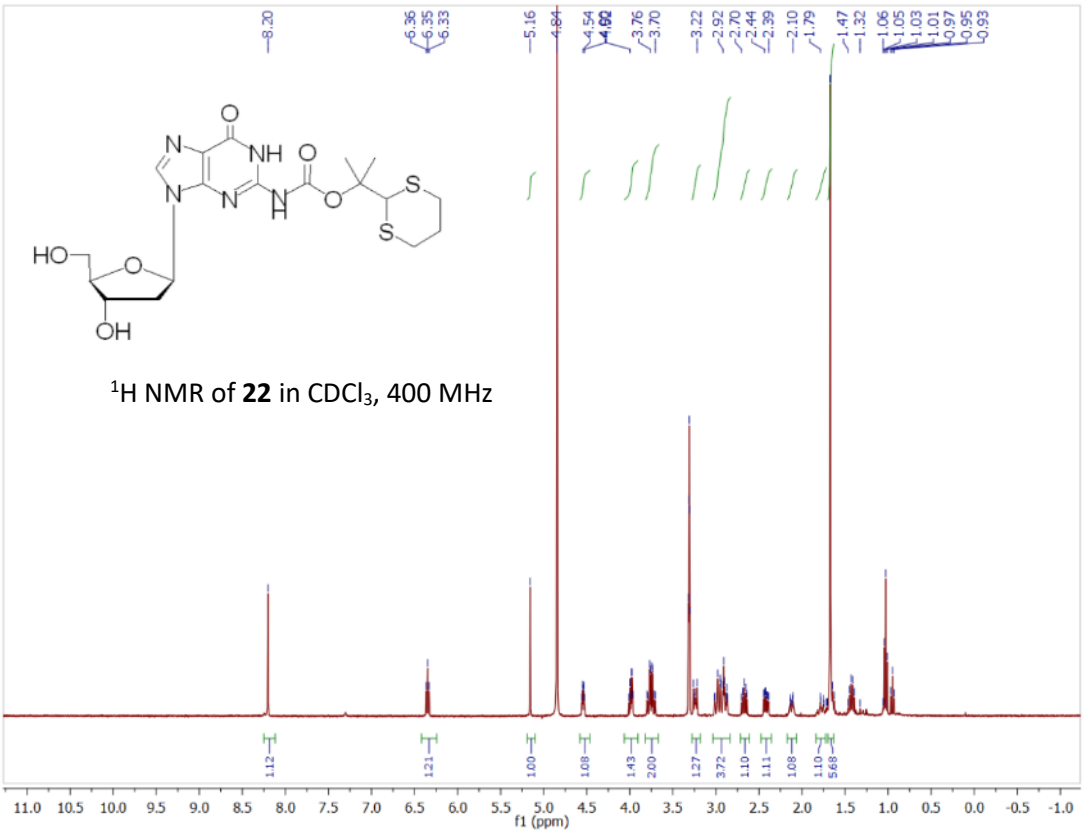
^{31}P NMR of **3b** in $CDCl_3$, 162 MHz



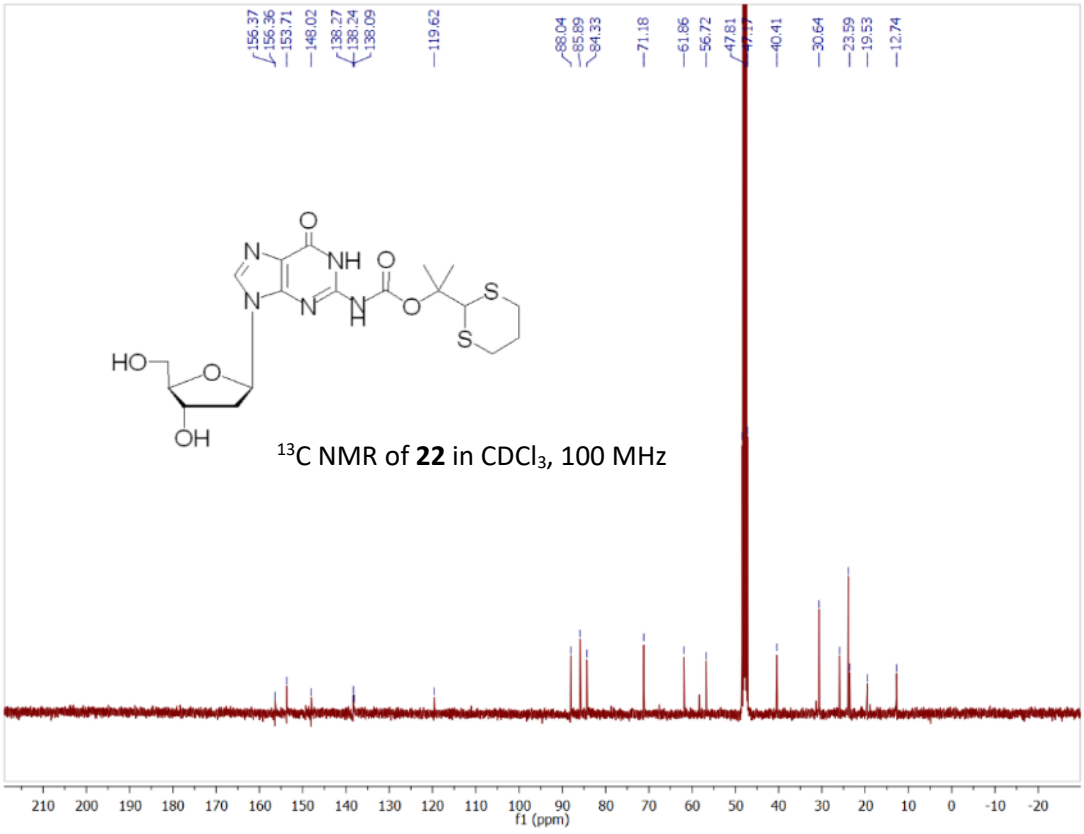
^{1}H NMR of **21** in $CDCl_3$, 400 MHz



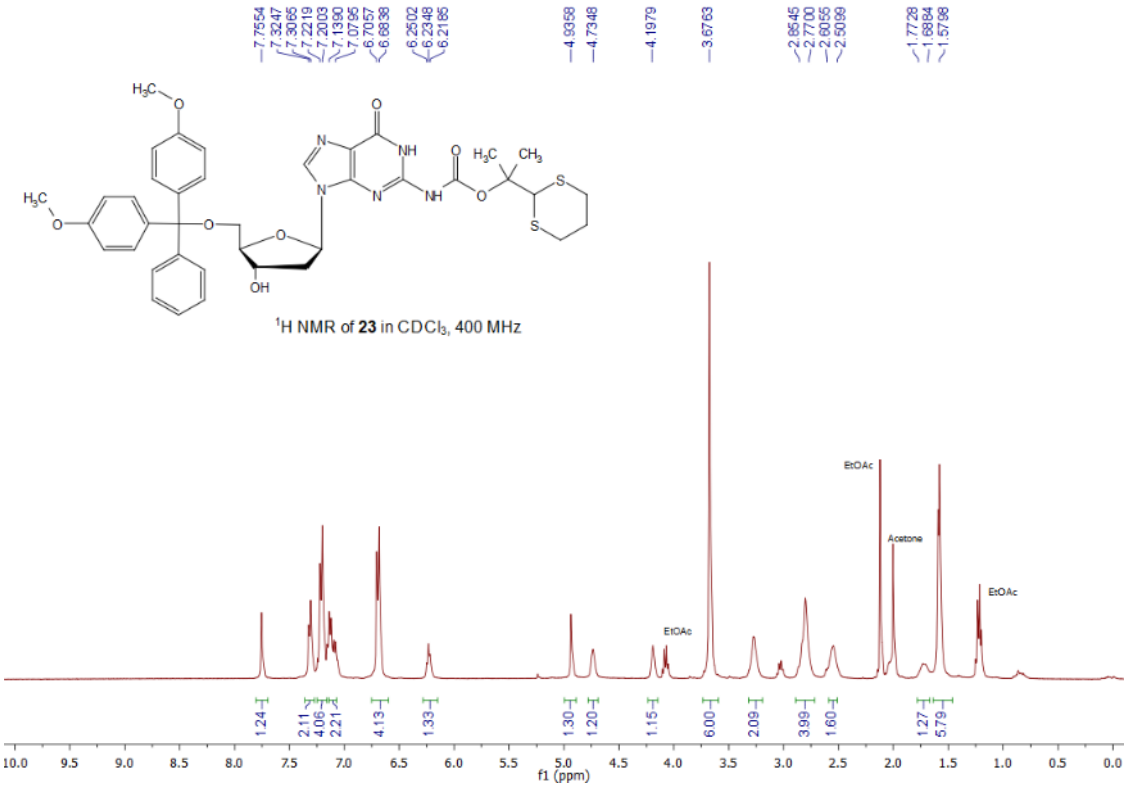
^{13}C NMR of **21** in $CDCl_3$, 100 MHz



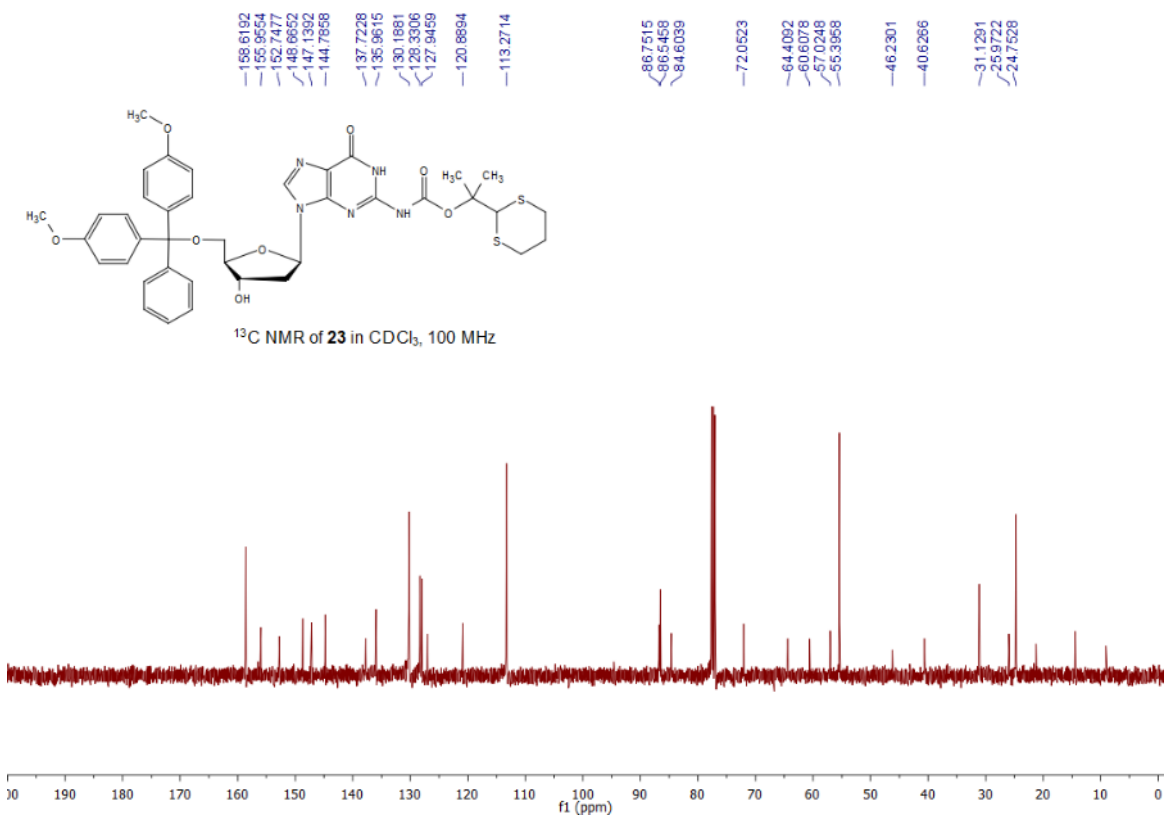
^{1}H NMR of **22** in $CDCl_3$, 400 MHz



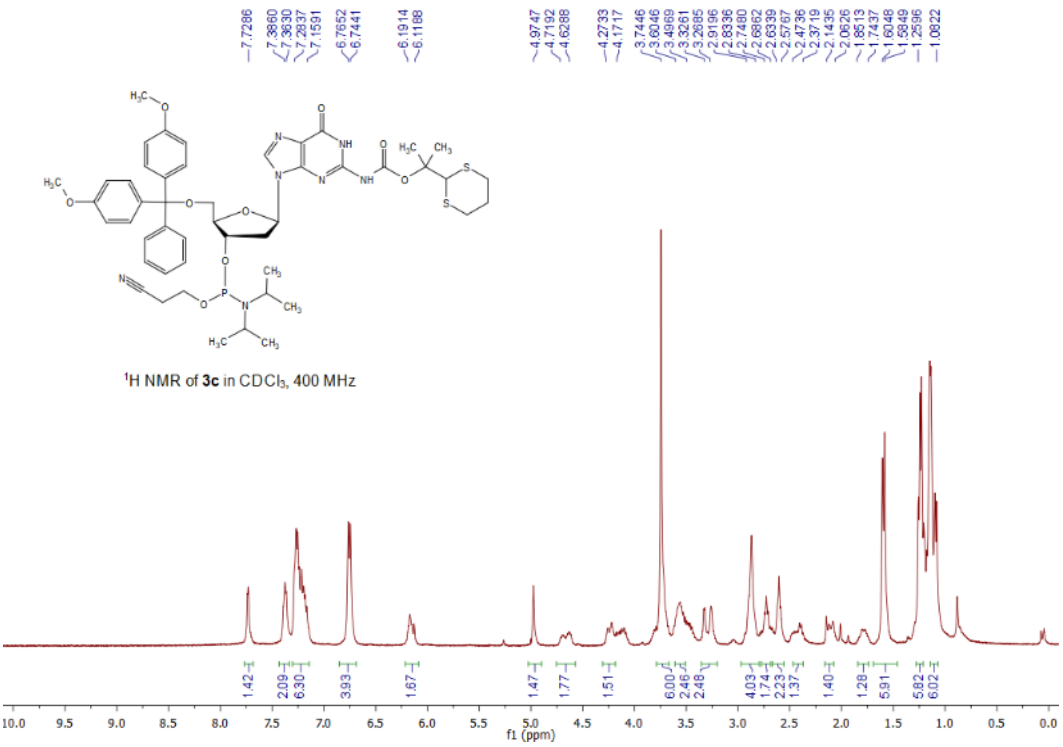
^{13}C NMR of **22** in $CDCl_3$, 100 MHz



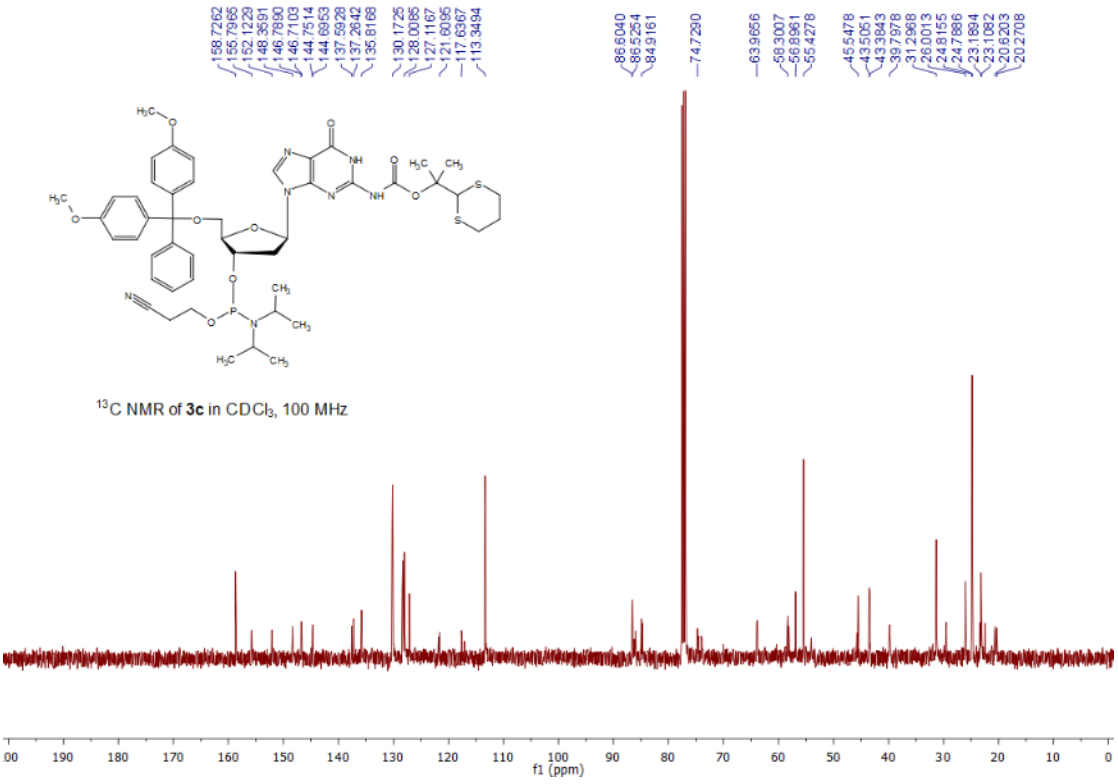
^{1}H NMR of **23** in $CDCl_3$, 400 MHz



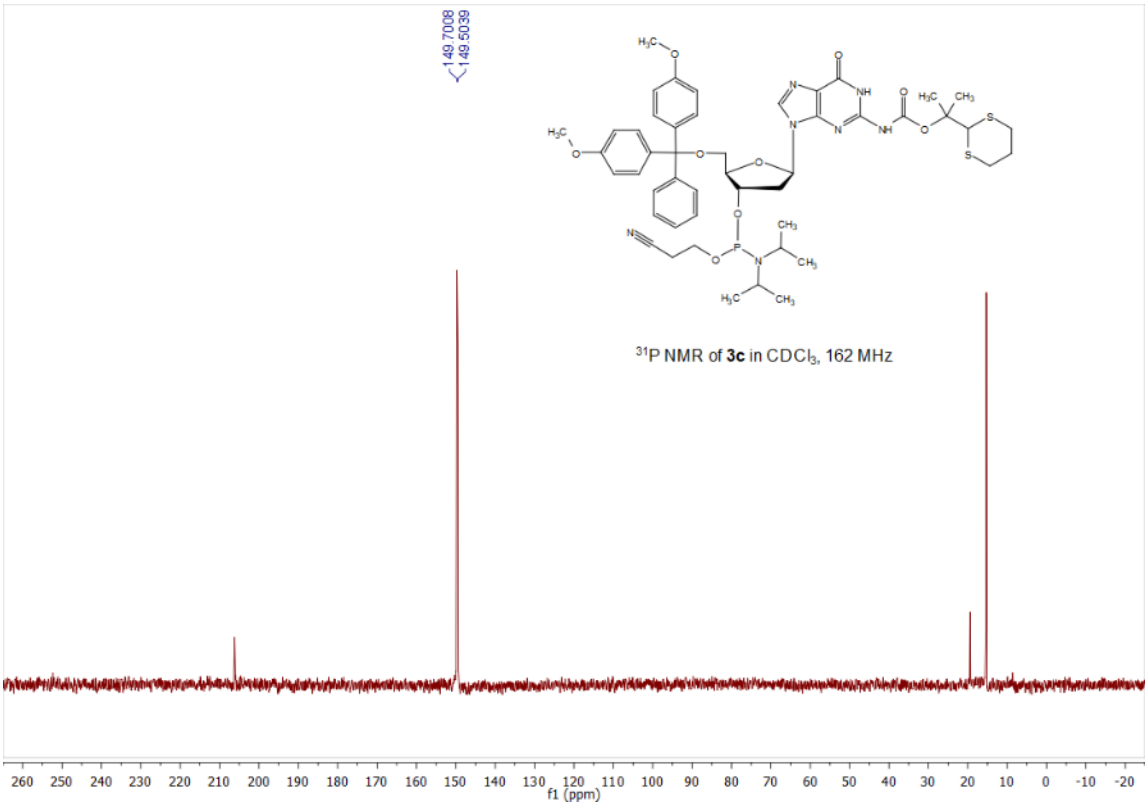
^{13}C NMR of **23** in $CDCl_3$, 100 MHz



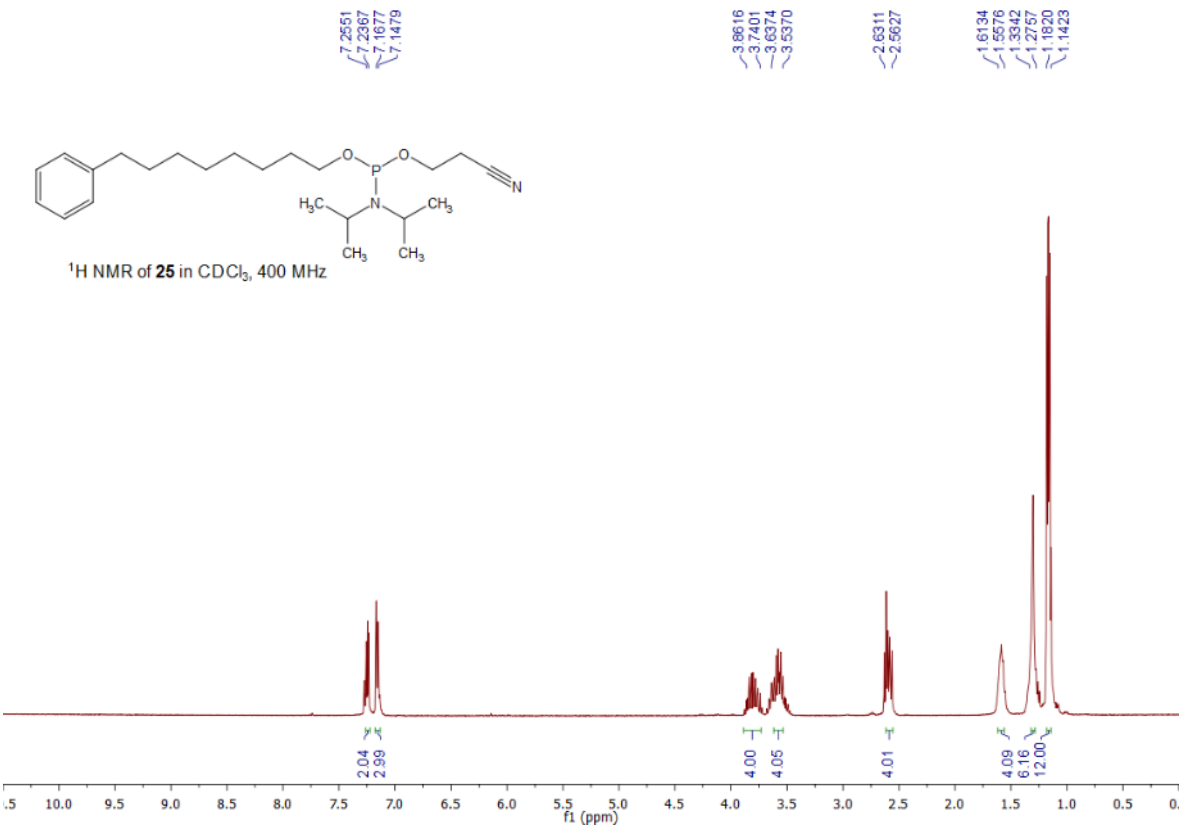
^{1}H NMR of **3c** in $CDCl_3$, 400 MHz



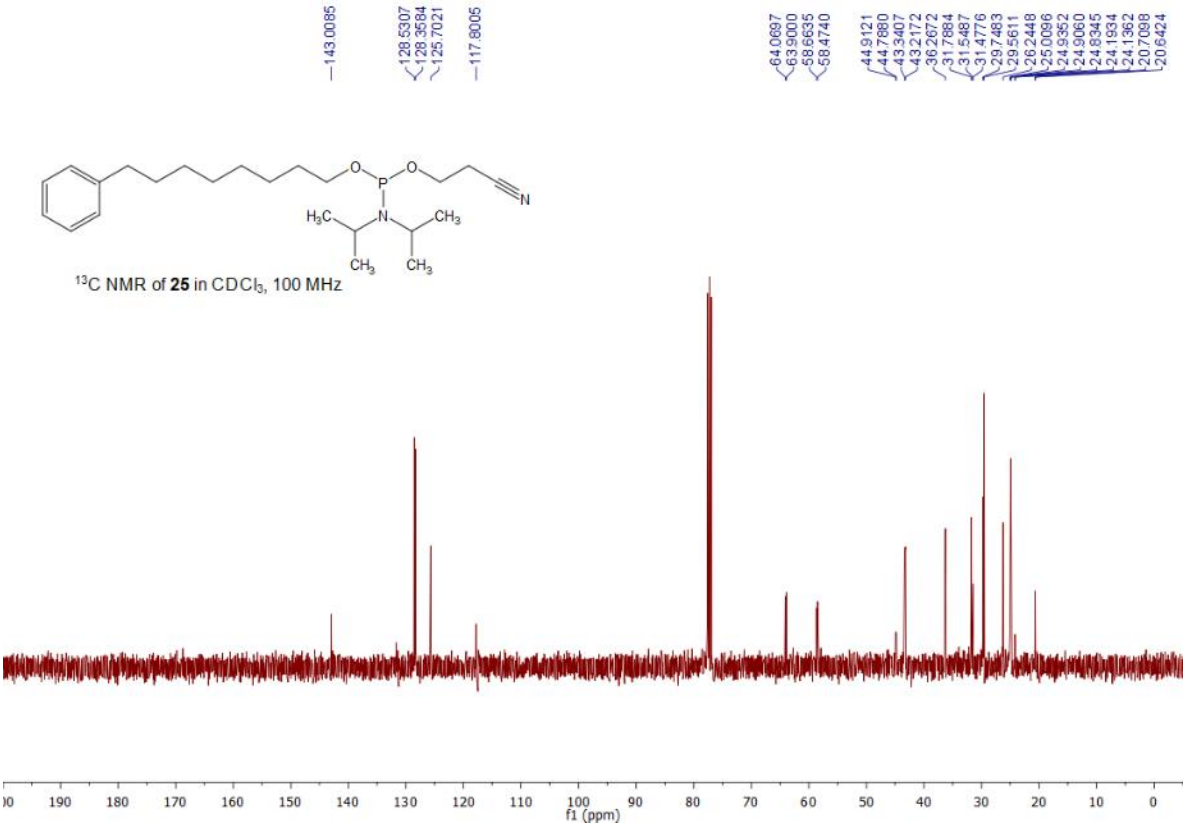
^{13}C NMR of **3c** in $CDCl_3$, 100 MHz



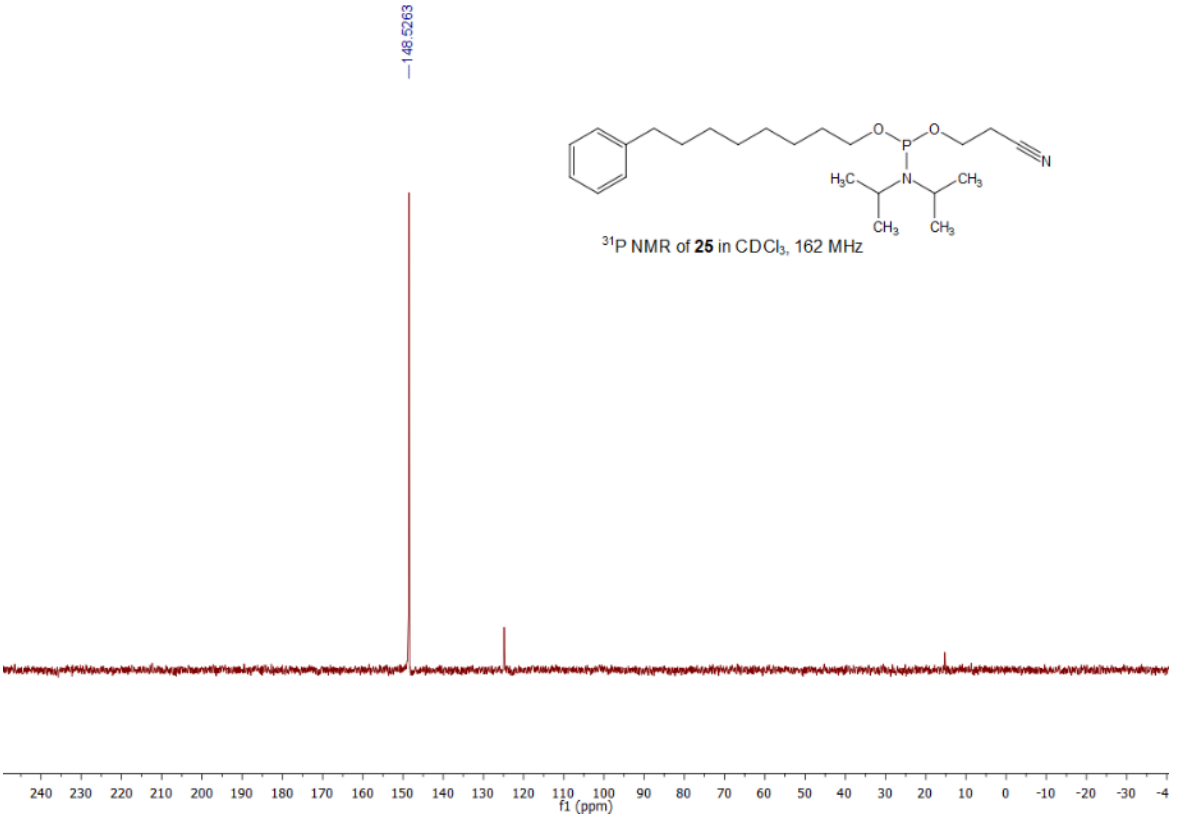
^{31}P NMR of **3c** in $CDCl_3$, 162 MHz



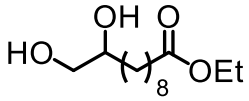
^{1}H NMR of **25** in $CDCl_3$, 400 MHz



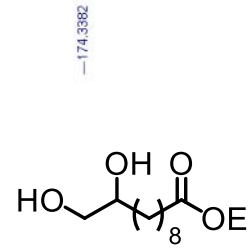
^{13}C NMR of **25** in $CDCl_3$, 100 MHz



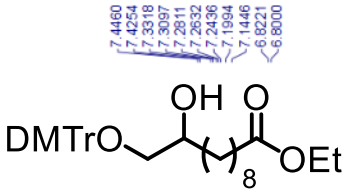
^{31}P NMR of **25** in $CDCl_3$, 162 MHz



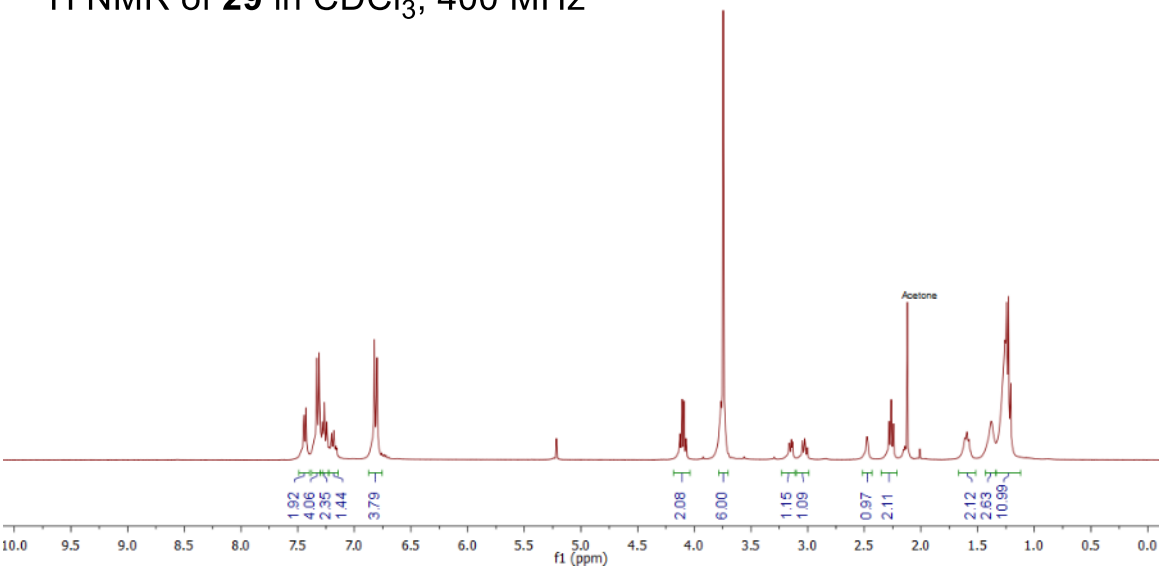
^{1}H NMR of **28** in CD_3OD, 400 MHz

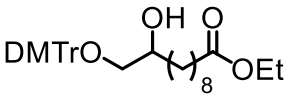


^{13}C NMR of **28** in CD_3OD, 100 MHz

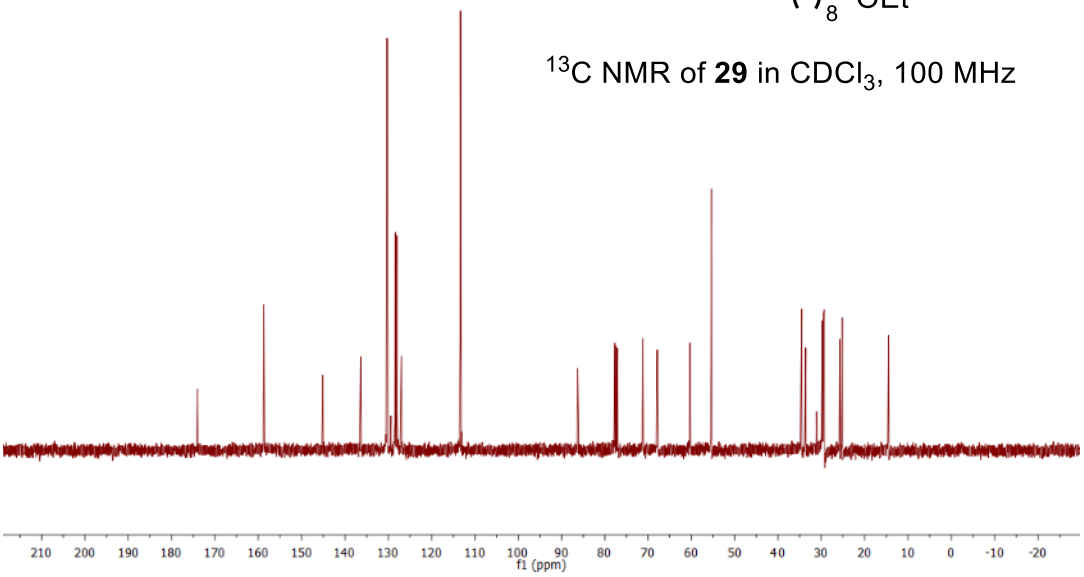


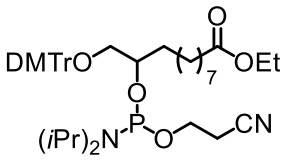
^{1}H NMR of **29** in $CDCl_3$, 400 MHz



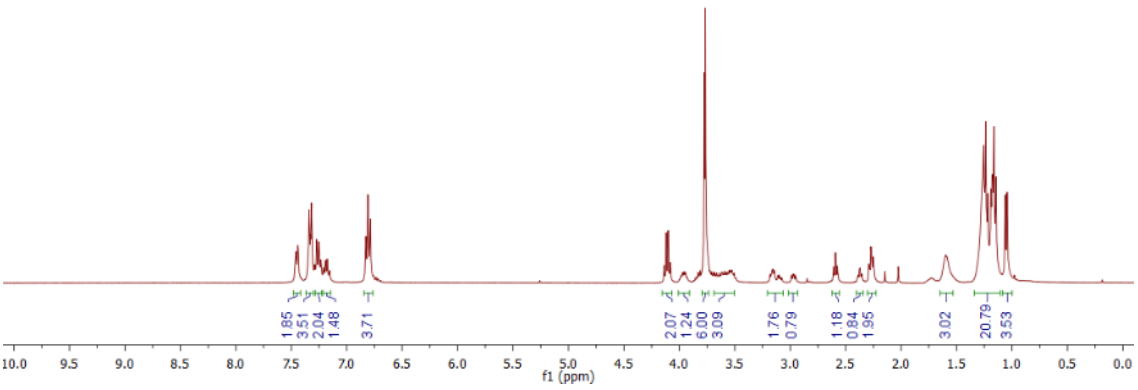


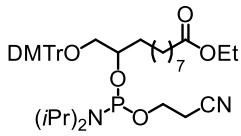
^{13}C NMR of **29** in $CDCl_3$, 100 MHz



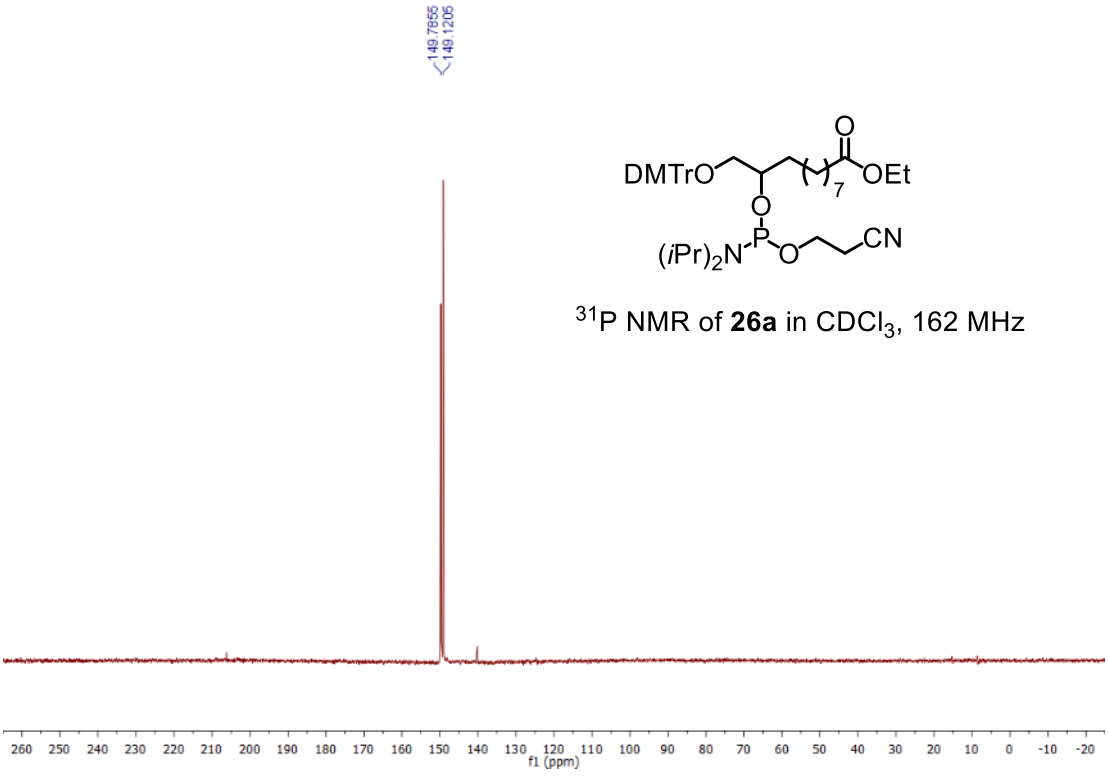


^{1}H NMR of **26a** in $CDCl_3$, 400 MHz





^{13}C NMR of **26a** in $CDCl_3$, 100 MHz



^{31}P NMR of **26a** in $CDCl_3$, 162 MHz