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Chemical Synthesis of Sensitive DNA

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CHEMICAL SYNTHESIS OF SENSITIVE DNA

By

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Table of Contents

List of Schemes

List of Figures

List of Appendix Figures

Author Contribution Statement

Initially, all the research ideas of the projects present in chapters 2 and 3 of this dissertation were established under the supervision of Dr. Shiyue Fang. Dissertation writing was done by Komal Chillar and reviewed by Dr. Shiyue Fang.

In chapter 2, Dr. Dhananjani Eriyagama, Yipeng Yin, Dr. Shahien Shahsavari contributed equally to the research experiments under the supervision of Dr. Shiyue Fang. The content has already been published in the *New Journal of Chemistry* **2023**, 47, 8714-8722.

In chapter 3, all the experiments, purification and analysis were performed by Komal Chillar under the supervision of Dr. Shiyue Fang. The content has already been published in *Organic and Biomolecular Chemistry* **2023**, 21, 9005-9010.

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Radhe Radhe

Abstract

Over the past decades, researchers have tried various chemical methods to synthesize modified oligodeoxynucleotides (ODNs, i.e. short segments of DNAs). Traditional ODN synthesis methods require strong basic, and nucleophilic conditions for the deprotection and cleavage of the ODN from the solid support. However, the sensitive ODNs containing labile functionalities are vulnerable to such harsh conditions. Sensitive ODNs have a wide range of applications in research and pharmaceuticals. To synthesize sensitive ODNs, researchers devised different strategies but no practical methods have been developed. To overcome these challenges, we developed alkyl Dim alkyl Dmoc technology. This innovative technology uses weakly basic and non-nucleophilic conditions to synthesize the sensitive ODNs. To demonstrate the feasibility of alkyl Dim alkyl Dmoc technology different ODNs were synthesized, purified using RP-HPLC, and analyzed using MALDI-TOF MS and capillary electrophoresis. Using the method, we successfully synthesized various sensitive ODNs that cannot be synthesized or are highly challenging to synthesize using any known methods. The longest ODN synthesized using methyl Dmoc technology was 23-mer. To further extend the utility and capability of alkyl Dim alkyl Dmoc technology, we aimed to synthesize longer sensitive ODNs. To achieve this goal, PEGylated Dmoc (pDmoc) technology was developed. The introduction of the PEG Dmoc group increased the ODN solubility which facilitated the synthesis of longer ODNs. The longest ODN synthesized was a 49-mer. Moreover, a sensitive 4acC epigenetically modified ODN was synthesized. The success of synthesizing this naturally occurring sensitive ODN will make many projects in the area of epigenetics feasible.

1 Introduction to oligodeoxynucleotides

1.1 DNA

Deoxyribonucleic acid (DNA) is a molecule found in the nucleus of cells in living organisms. DNA serves as a hereditary material responsible for carrying genetic information that determines the growth and function of an organism. Structurally, DNA consists of two polynucleotide chains wound around each other in a twisted ladder 3-D form known as a double helix. Each polynucleotide DNA strand consists of a sugar-phosphate backbone and a nitrogenous base. There are four nitrogenous bases namely adenine (A), cytosine (C), guanine (G), and thymine (T) attached to the sugar molecule which is a 2'-deoxyribose. According to the Watson and Crick base pairing, the two polynucleotide strands are connected via a hydrogen-bond between the nitrogenous bases. Adenine forms a hydrogen bond with thymine while guanine forms a hydrogen bond with cytosine (Figure 1.1). This complimentary base pairing ensures the stability of the DNA. DNA can consist of millions of bases attached in different sequences to determine the genetic information in living organisms. Every organism has a specific sequence of bases attached in DNA which distinguishes one organism from another organism. [1]

Oligodeoxynucleotides (ODNs) are short, single-stranded DNA generally consisting of 8 to 200 nucleotides (nt)/mer. Generally, ODNs can be chemically synthesized using protected phosphoramidites derived from various modified nucleosides or non-nucleoside compounds. The longest ODN synthesized using chemical synthesis is 400 nt.[2] Synthetic ODNs have a wide range of applications in chemical biology and pharmaceuticals. Fomivirsen/Vitravene was the first antisense oligonucleotide (ASO) drug approved by the Food and Drug Administration (FDA) in 1998. Currently, thirteen ASOs have been approved by FDA out of which eight have also been authorized by the European Medicine Agency (EMA) for clinical use. A few of the approved antisense drugs (ASOs) are Givosiran, Inotersen, Patisiran, and many more are still under investigation.[3]

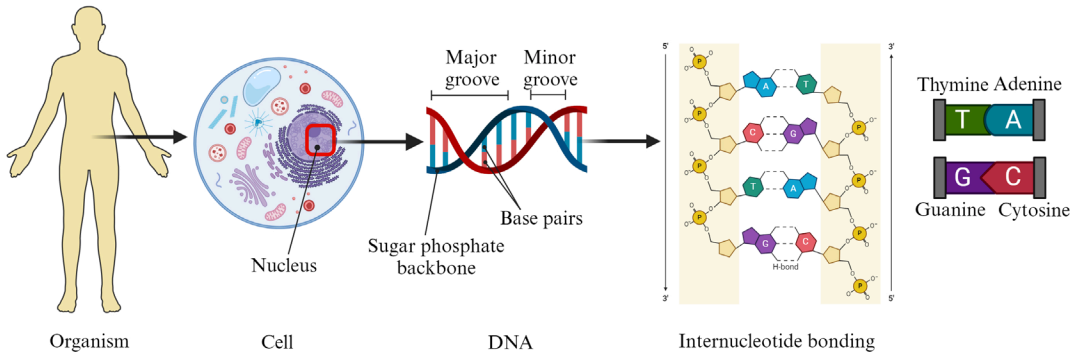


Figure 1.1: Introduction to DNA (Figure is drawn using Biorender.com)

1.2 Sensitive ODN

Over the last few decades, more than one hundred epigenetic modifications while many non-canonical nucleotides have been found in DNA. These modifications serve as an additional layer of genetic information regulation which influences the biological pathways and diseases in the organisms. To understand the biological pathways and disease treatments, modified ODN applications have substantially spread over a wide range of research like antisense drug development,[4, 5] bioconjugation,[6] DNA-protein interaction,[7] nanotechnology,[8] CRISPR genome editing,[9] DNA damage and repair,[10] DNA methylation and demethylation,[11] DNA data storage,[12] synthetic biology,[13] and others. These applications require modified ODNs. Many of these modified ODNs consist of functional groups that cannot survive the known ODN synthesis, deprotection, and cleavage conditions. These sensitive functionalities are unstable under acidic, basic, and strong nucleophilic conditions. Such sensitive functional groups containing modified ODNs are called Sensitive ODNs. The sensitive functional groups include esters, alkyl halides, aldehyde, allyl halides, epoxides, α-halo amides, vinyl ethers, benzyl halides, carbonates, thioesters, tosylates, aziridines, maleimide, chloropurine, N^4-acetyldeoxycytosine (4acC), and many more. One of the most notable sensitive groups found in natural DNA is N^4-acetyldeoxycytosine (4acC). A method for the synthesis of ODNs containing this sensitive group will open a wide range of research opportunities in biology and medicine.

1.3 Chemical synthesis of DNA

DNA is synthesized chemically using solid-phase phosphoramidite chemistry. The synthetic DNA synthesis is done on an automated DNA/RNA synthesizer (like Mermade-6, ABI). Generally, synthesis steps include a series of protection and deprotection steps of the 5'-hydroxyl, exocyclic amino, and phosphate groups of the nucleoside phosphoramidite monomers based on the requirements. The synthesis of DNA occurs on a solid surface like controlled pore glass which is covalently bonded to a suitable linker like succinyl linker. Each DNA synthesis cycle involves four different steps. These four steps are deblocking/detritylation, coupling, capping, and oxidation. During the synthesis, a nucleoside phosphoramidite monomer is sequentially added to the derivatized solid support based on the sequence and the desired length of the DNA. Upon completion of DNA synthesis, the solid-support bound DNA is cleaved and deprotected to give crude DNA. (Figure 1.2)

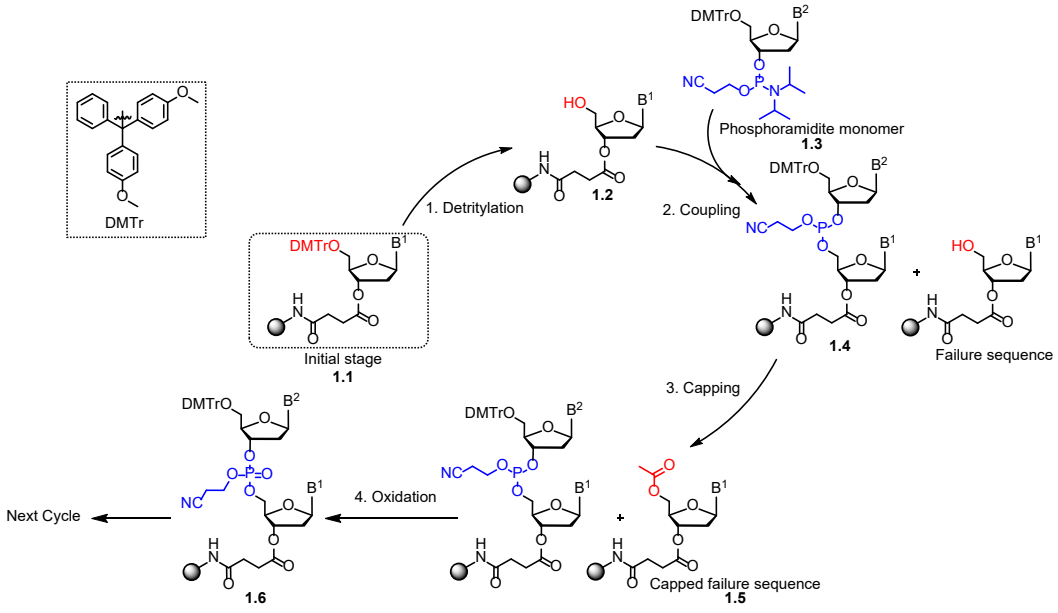


Figure 1.2: Solid-phase DNA synthesis

1.3.1 Deblocking/Detritylation

The acid labile 4,4'-Dimethoxytrityl (DMTr) group present on the 5'-terminal hydroxyl group of the 2'-deoxyribose (**1.1**) is removed using 3% Dichloroacetic acid (DCA) in Dichloromethane (DCM). This step is important for the formation of a free 5'-terminal hydroxyl group (**1.2**) for the next coupling step.

1.3.2 Coupling

A 0.1 M solution of a nucleoside phosphoramidite monomer (1.3) dissolved in acetonitrile (ACN) is activated using a 0.25 M solution of 1*H*-tetrazole in acetonitrile (ACN). In this step, the derivatized solid support with a free 5'-terminal hydroxyl group of the 2'-deoxyribose (**1.2**) displaces the activated diisopropyl amino group (a good leaving group) of the nucleoside phosphoramidite monomer (**1.3**). This forms a phosphite triester linkage between the nucleosides (**1.4**). This step is crucial to determine the yield and purity of the DNA synthesis. However, not all free 5'-terminal hydroxyl group of the 2'-deoxyribose bound to the derivatized solid support (**1.2**) can be coupled to the incoming nucleoside phosphoramidite monomer, a few of the free 5'-terminal hydroxyl group remain unreacted which needs to be capped.

1.3.3 Capping

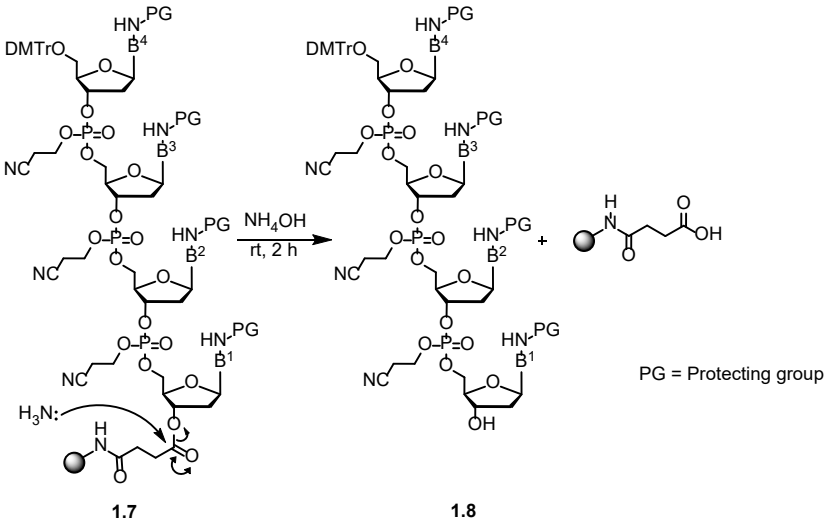
The uncoupled solid support-bound ODN with a free 5'-terminal hydroxyl group (**1.2**) is known as the failure sequence. This failure sequence is capped to avoid further chain elongation. Capping of the failure sequence is done using the acetic anhydride catalyzed by *N*-methyl imidazole to give a capped failure sequence (**1.5**).

1.3.4 Oxidation

The solid support-bound ODN (**1.4**) contains a very labile and unstable phosphite triester linkage between the nucleosides. This phosphite triester linkage is then oxidized to a more stable phosphate triester linkage. This oxidation is achieved by using 0.02 M I_2 in THF/Pyridine/Water to form a phosphate triester solid support-bound ODN (**1.6**). The resulting phosphate group serves as the backbone of the ODN.

1.3.5 Cleavage

Upon reaching the desired sequence and length of the ODN, the ODN is cleaved from the derivatized solid support. Generally, the 3'-terminal hydroxyl group of ODN is attached to a non-nucleosidic or nucleosidic succinate linker which is covalently bonded to the solid support. The ODN on the derivatized solid support (**1.7**) is treated with the 28% aqueous ammonium hydroxide solution at room temperature. This cleavage gives the protected ODN (**1.8**) separated from the solid support.



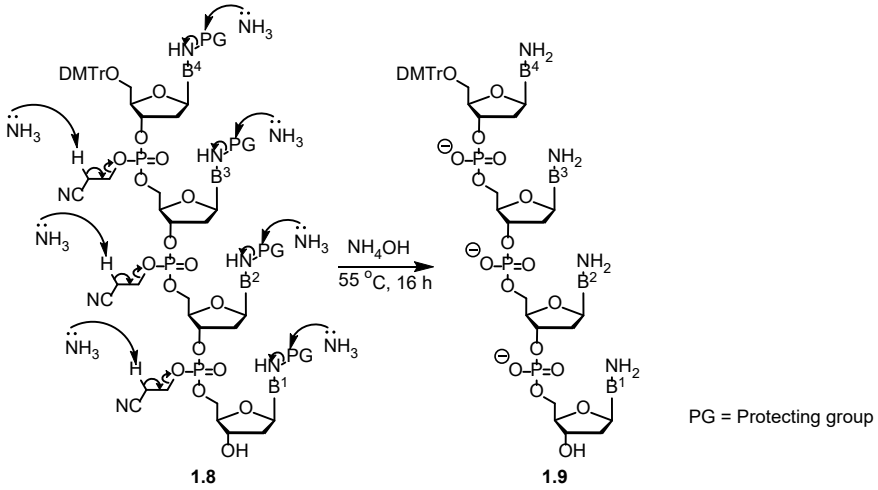
Scheme 1.1: Cleavage of ODN from solid support

1.3.6 Deprotection

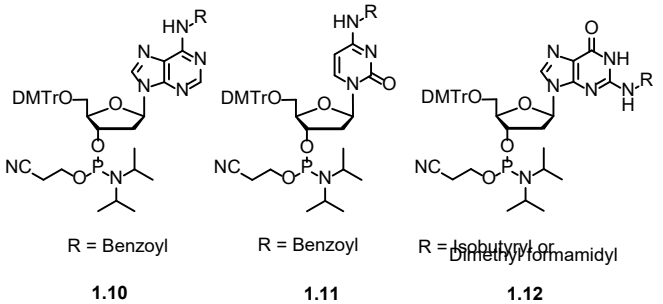
Following the cleavage of the ODN from solid support, the *exo*-amino and phosphate protecting groups are deprotected. The *exo*-amino group present on the base is nucleophilic. These *exo*-amino groups are pre-protected with acyl-protecting groups for the ODN synthesis. After the ODN synthesis, the protected *exo*-amino groups are deprotected to give crude ODN. Typically, dA and dC nucleosides have benzoyl as *exo*-amino protecting group while dG has isobutyrl or dimethylformamidyl as *exo*-amino protecting group. However, dT does not contain a nucleophilic *exo*-amino group and remains free from the protecting group. Additionally, the phosphite triester of the nucleosides is protected by the base-labile 2-cyanoethyl group (Scheme 1.3). Deprotection of these

protecting groups is accomplished by treating the protected ODN (1.8) with 28% aqueous ammonium hydroxide solution at 55 ºC for 16 hours to give crude ODN (**1.9**) (Scheme 1.2).

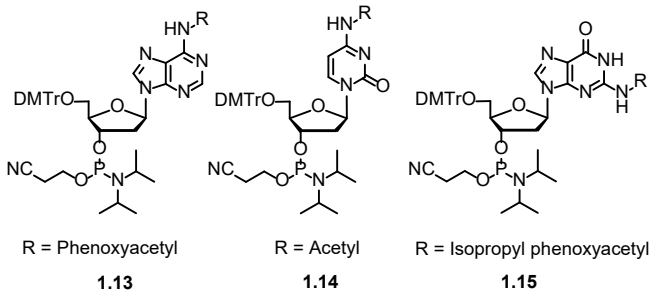
Ultramild deprotection conditions are developed which require more labile *exo*-amino protecting groups. These *exo*-amino protecting groups include phenoxyacetyl, acetyl, and isopropyl phenoxyacetyl for nucleosides dA, dC, and dG respectively (Scheme 1.4). Ultramild deprotection is accomplished by treating protected ODN with 28% aqueous ammonium hydroxide solution at room temperature for 2 hours or 0.05M potassium carbonate solution in methanol at room temperature for 4 hours to give crude ODN.



Scheme 1.2: Deprotection of protecting groups to give crude ODN



Scheme 1.3: Phosphoramidites used for standard deprotection conditions of ODN



Scheme 1.4: Phosphoramidites used for ultramild deprotection conditions of ODN

1.4 Current methods to synthesize sensitive ODN and their limitations

Our objective is to synthesize sensitive DNA that consists of the functional groups unstable under strong acid, base, and nucleophilic conditions. Over the decade, researchers tried various methods and strategies to synthesize these sensitive groups like incorporating protecting groups, linkers, post-synthesis modifications, as well as cleavage and deprotection conditions.[14] Despite all these strategies, all the known methods have limitations. Typically, the standard cleavage and deprotection condition involves the use of concentrated ammonium hydroxide solution which is highly basic and nucleophilic. The labile acyl groups like phenoxyacetyl as an *exo*-amino protecting group and as a linker require mild basic conditions but still nucleophilic conditions.[15] The use of palladium-labile allyl protecting groups poses a challenge too.[16, 17] Palladium is expensive and difficult to remove which is not ideal for the ODN. The *o*-nitrobenzyl functionalized linker is photocleavable but photoirradiation can damage the ODN.[16] The (*p*-nitrophenyl)ethyl (Npe) and (*p*-nitrophenyl)ethyloxycarbonyl (Npeoc) protecting groups require a strong base DBU in aprotic solvent for long duration along with the nucleophilic scavenger.[18] These conditions are also not ideal for the sensitive ODNs. Certain methods require post-synthesis modifications which are case-specific and involve complicated procedures.[5] Additionally, few methods are limited to short ODN synthesis with low yields.[18] However, synthesizing sensitive ODNs without nucleobase protection can be considered but developing a linker that requires mild cleavage conditions is difficult.[19] Also, achieving

selectivity of *O*-phosphitylation over *N*-phosphitylation is challenging. Furthermore, Sekine, etc. reported the synthesis of 4acC containing ODNs, however, the sequence was limited to dT nucleotides.[20] Meier, etc. reported the synthesis of ac4C containing RNAs, using unprotected G phosphoramidite which does not allow the capping of failure sequences.[21] Additionally, Meier, etc. prepared ac4C containing RNAs through in vitro transcription to facilitate ac4C antibody generation, however, the method lacks both efficiency and specificity.[22]

1.5 Non-nucleophilic Dmoc technology to synthesize sensitive ODN

Synthesizing sensitive ODNs is important for numerous applications as mentioned earlier. To overcome the limitations of known methods to synthesize sensitive ODNs, we developed Dmoc technology. In the subsequent sections, different stages of Dmoc technology are explained.

1.5.1 Dmoc Technology

Dmoc technology was developed to incorporate sensitive functionalities in ODNs. Mild deprotection and cleavage conditions were used to retain the sensitive functionality. Dmoc technology uses 1,3-dithiane-2-yl-methoxycarbonyl (Dmoc) as an *exo*-amino protecting group and dT Dmoc linker (**1.16**) attached to the controlled-pore glass (CPG) (Figure 1.3). Dmoc protecting groups are stable under the ODN synthesis conditions. After the desired ODN synthesis on the dT Dmoc linker, the 2-cyanoethyl group on the phosphate triester was removed by adding DBU in acetonitrile at room temperature for 15 minutes. This step involves the β-elimination of the 2-cyanoethyl group to give a hydrophilic phosphate backbone of the ODN. The hydrophilic phosphate backbone promoted the aqueous oxidation of sulfur atoms of the Dmoc groups to sulfoxides or sulfones using 0.4 M sodium periodate solution. These sulfoxides or sulfones drastically lowered the pK_a of α-hydrogen. Under nearly non-nucleophilic conditions, β-elimination of the oxidized Dmoc protecting groups deprotected and cleaved the ODN from the solid support to give crude ODN (Scheme 1.5). Using this technology, we were able to incorporate electrophilic

functionalities into the ODN. However, the limitation of Dmoc technology was the use of DBU and aniline. Aniline served as both a base as well as a scavenger to avoid the formation of the 1,4-Michael adduct (**1.35**) (Scheme 1.6).[23]

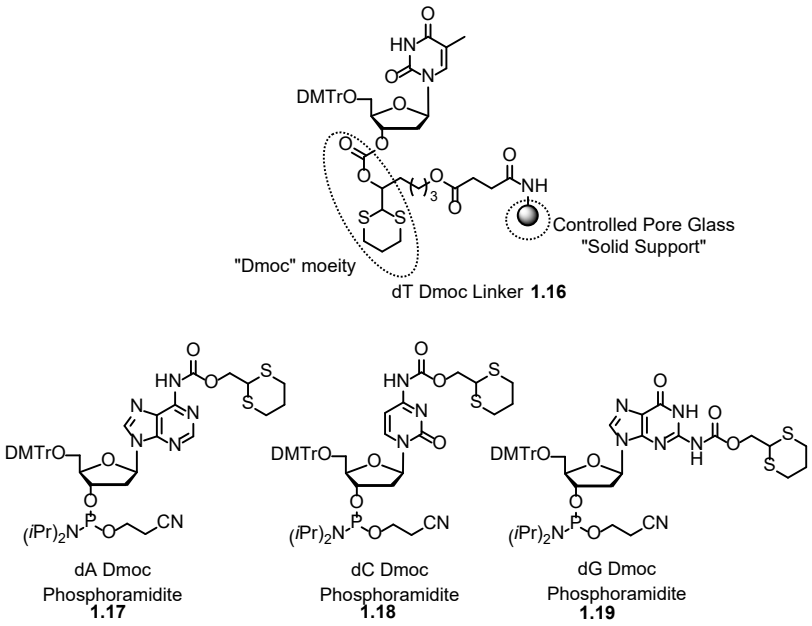
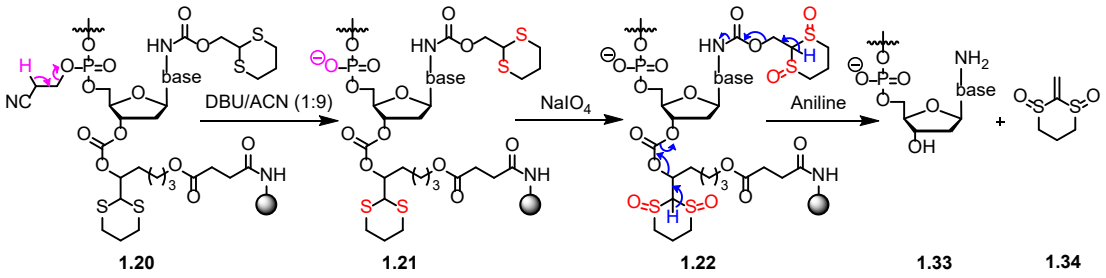
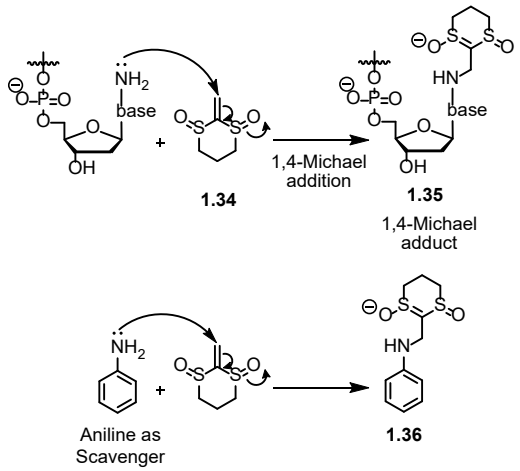


Figure 1.3: Dmoc linker and Dmoc phosphoramidites



Scheme 1.5: Deprotection and cleavage conditions for Dmoc technology



Scheme 1.6: 1,4-Michael addition of ODN with Dmoc β-elimination side product

1.5.2 dM-Dmoc Technology

The limitation of Dmoc technology was the use of aniline, which served both as a base and a scavenger. Aniline is a nucleophile so the synthesis of nucleophile-labile sensitive ODNs was difficult. To overcome this limitation, dM-Dmoc technology was developed. dM-Dmoc technology utilized dimethyl-Dmoc as an *exo*-amino protecting group (Figure 1.4). Using the sterically hindered protecting group prevented the 1,4-Michael reaction (Scheme 1.8). β-Elimination of the oxidized dM-Dmoc protecting groups was achieved using a mild base K_2CO_3. However, a problem observed during the synthesis was the premature cleavage of the dM-Dmoc protecting group. This premature cleavage of the dM-Dmoc group resulted in the formation of branched ODNs. Additionally, DBU which is a strong base was used for the β-elimination of 2-cyanoethyl phosphate protecting group (Scheme 1.7).[24]

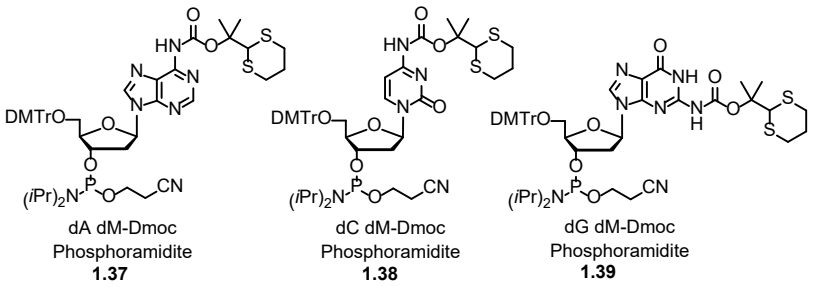
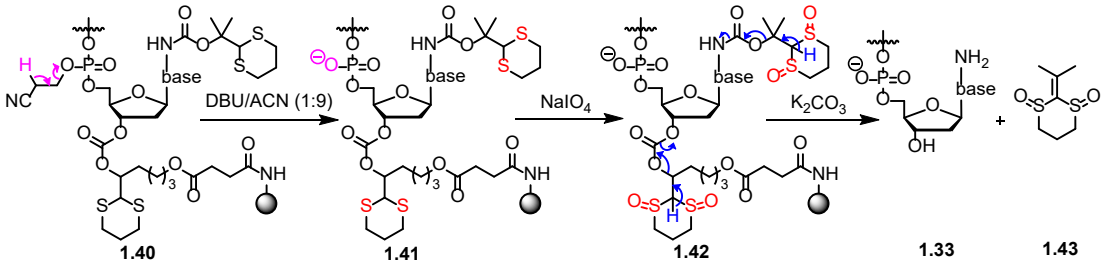
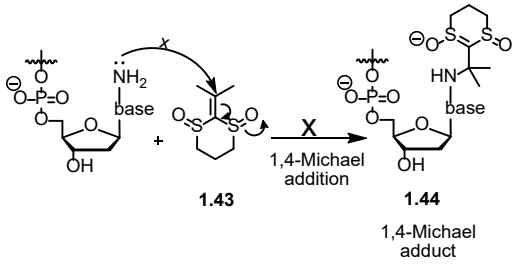


Figure 1.4: dM-Dmoc phosphoramidites



Scheme 1.7: Deprotection and cleavage conditions for dM-Dmoc technology



Scheme 1.8: 1,4-Michael addition of ODN with dM-Dmoc β-elimination side product

1.5.3 Dim-Dmoc Technology

The limitations of dM-Dmoc technology were the use of DBU and the premature cleavage of dM-Dmoc protecting groups. To overcome these limitations, Dim-Dmoc technology was developed. Dim-Dmoc technology used Dmoc as *exo*-amino protecting group while 2-cyanoethyl was replaced by 1,3-dithian-2-yl-methyl (Dim) as a phosphate protecting group (Figure 1.5). Using Dim-Dmoc technology, reduced the post-synthesis steps. Sulfur atoms in the Dim and Dmoc groups were oxidized to sulfoxides or sulfones. β-Elimination

of oxidized Dim and Dmoc protecting groups was achieved using aniline (Scheme 1.9). However, the major drawback of Dim-Dmoc technology was the formation of 1,4-Michael adduct (**1.35**) (Scheme 1.6).[25]

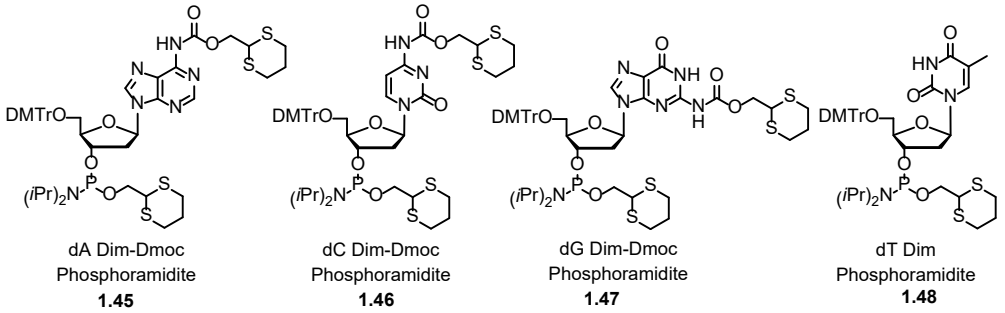
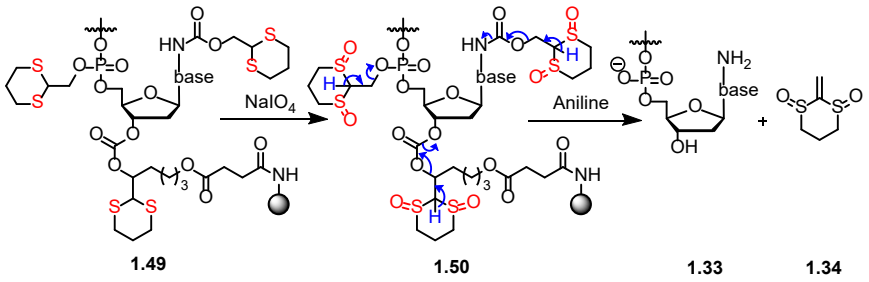


Figure 1.5: Dim-Dmoc phosphoramidites



Scheme 1.9: Deprotection and cleavage conditions for Dim-Dmoc technology

To overcome the limitations of all the Dmoc and modified Dmoc technology, alkyl Dim alkyl Dmoc technology is developed. This innovative approach is very useful to eliminate issues such as the formation of 1,4-Michael adducts, branched ODN, and the need for a strong base. Furthermore, the approach enabled us to synthesize longer ODNs. We successfully synthesized 49-mer. The details are mentioned in the respective chapters.

2 Oligonucleotide synthesis under mild deprotection conditions

Work in this chapter has been published in *New Journal of Chemistry*

Chillar, K. ‡; Eriyagama, D. ‡; Yin, Y. ‡; Shahsavari, S. ‡; Halami, B.; Apostle, A.; Fang, S. "Oligonucleotide synthesis under mild deprotection conditions", *New Journal of Chemistry* **2023**, 47, 8714-8722. (‡Equal Contributions). DOI: 10.1039/D2NJ03845E

The material in this chapter was reprinted with the permission of *New Journal of Chemistry*.

2.1 Abstract

Over a hundred non-canonical nucleosides have been found in DNA and RNA. Many of them are sensitive toward nucleophiles. Because known oligonucleotide synthesis technologies require nucleophilic conditions for deprotection, currently there is no suitable technology for their synthesis. The recently disclosed method regarding the use of 1,3-dithian-2-yl-methyl (Dim) for phosphate protection and 1,3-dithian-2-yl-methoxycarbonyl (Dmoc) for amino protection can solve the problem. With Dim-Dmoc protection, oligodeoxynucleotide (ODN) deprotection can be achieved with $NaIO_4$ followed by aniline. Some sensitive groups have been determined to be stable under these conditions. Besides serving as a base, aniline also serves as a nucleophilic scavenger, which prevents deprotection side products from reacting with ODN. For this reason, excess aniline is needed. Here, we report the use of alkyl Dim (aDim) and alkyl Dmoc (aDmoc) for ODN synthesis. With aDim-aDmoc protection, deprotection is achieved with $NaIO_4$ followed by K_2CO_3. No nucleophilic scavenger such as aniline is needed. Over 10 ODNs including one containing the highly sensitive N^4-acetylcytidine were synthesized. Work on extending the method for sensitive RNA synthesis is in progress.

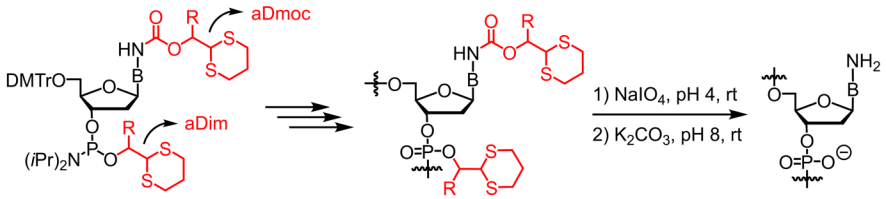


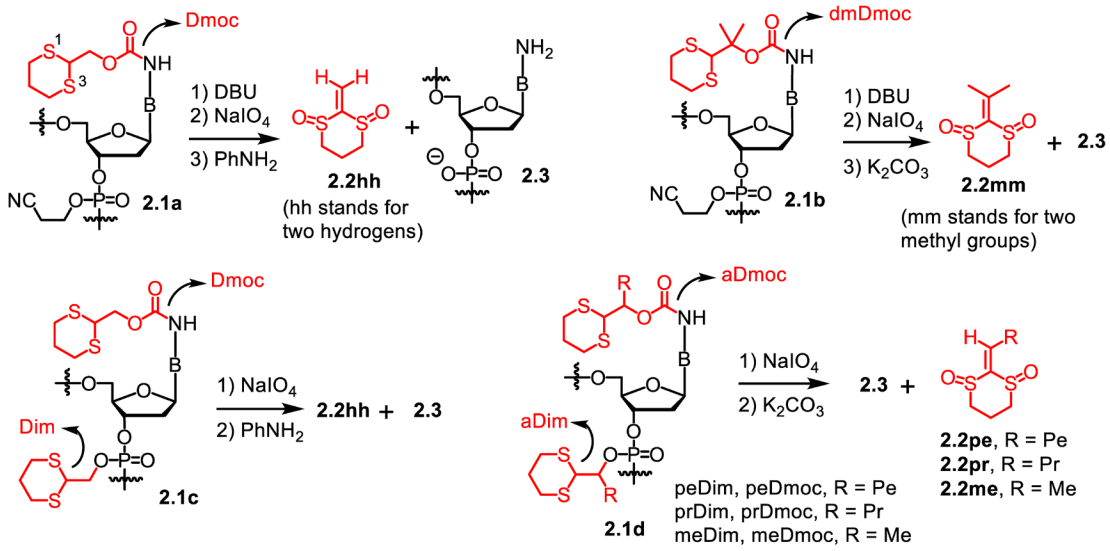
Figure 2.1: Mild deprotection and cleavage conditions for alkyl Dim alkyl Dmoc technology

2.2 Introduction

Many non-canonical nucleotides have been found in DNA and RNA. They serve as an additional layer of regulation of the flow of genetic information in biological systems. Malfunction of this layer of regulation has been found relating to many human diseases.[26, 27] In addition, modified nucleotides can also be produced by reactions of DNA with DNA

modification drugs and toxic compounds from the environment. These DNA adducts have been suggested to be the cause of many cancers.[28] To study the functional mechanisms of the modified nucleotides including those that cause human diseases, chemical synthesis of oligodeoxyribonucleotide (ODN) and oligoribonucleotide (ORN) containing such modified nucleotides is needed. However, known oligonucleotide (ON) synthesis methods use protecting groups and linkers that require harsh basic and nucleophilic conditions for cleavage and deprotection. Many of the modified nucleotides are sensitive to such conditions, and thus ONs containing them cannot be synthesized using known synthesis methods.

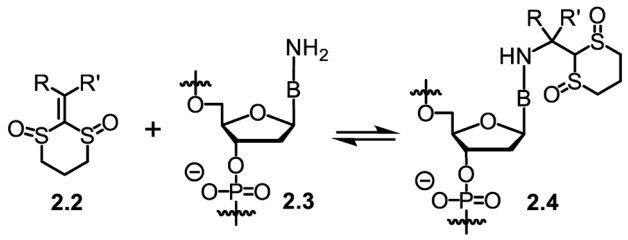
Some existing methods could be considered for the synthesis of ONs containing sensitive nucleotides. However, all of them have limitations. For example, using the phenoxyacetyl-based groups for amino protection, ON deprotection can be achieved under milder conditions than using typical acyl groups. However, conditions such as dilute sodium methoxide in methanol and concentrated ammonium hydroxide at room temperature are still strongly basic and nucleophilic.[29] Using cleavable linkers based on nitrobenzyl groups, ON cleavage can be achieved under non-nucleophilic and non-basic photolytic conditions. However, it is documented that UV light can damage ON.[16] Using allyl and benzyl-based groups for amino protection, ON deprotection can be achieved using palladium. However, excess palladium has to be used, and palladium is toxic, difficult to remove, and expensive.[30] The (*p*-nitrophenyl)ethyl (Npe) and (*p*-nitrophenyl)ethyloxycarbonyl (Npeoc) groups were studied for ON synthesis, but deprotection of these groups requires the strong base DBU with a nucleophilic scavenger.[31] Several other methods in the literature can also be considered for sensitive ON synthesis, which are summarized elsewhere.[32] Again, all of them have limitations.



Scheme 2.1: Summary of deprotection conditions and deprotection side products for ODNs synthesized using Dmoc, dmDmoc, Dim-Dmoc and aDim-aDmoc as protecting groups.

Most recently, Meier's research group at NCI used the cyanoethyloxycarbonyl (Ceoc) group and a photo-cleavable linker for the synthesis of ORNs containing the sensitive ac^{4}C function.[21] Deprotection and cleavage were achieved under non-nucleophilic conditions in three steps consisting of base treatment, fluoride treatment, and photo irradiation. The work constitutes a significant progress in the field. However, many challenges remain. The longest ORN reported was a 15-mer along with several 10-mer ORNs. The guanosine nucleotide was incorporated with a phosphoramidite without amino protection, which requires careful control of coupling conditions to avoid *N*-phosphitylation. The use of an unprotected phosphoramidite also prevents capping failure sequences during solid phase synthesis, which could put a limit on the length of ORN that can be synthesized and may require the ORN product to be purified with gel electrophoresis instead of the more scalable HPLC. In addition, the deprotection needs to use DBU, which prevents the method for the synthesis of ORN containing some sensitive nucleosides such as mchm^{5}U. Therefore, developing new methods for sensitive ON synthesis remains a significant and challenging task.

In 2016, we published our first paper on sensitive ODN synthesis.[33] We used the Dmoc group for amino protection and a Dmoc linker to anchor ODN to solid support. ODNs synthesized can be represented by **2.1a** (Scheme 2.1). Deprotection and cleavage were achieved in three steps by removing the 2-cyanoethyl phosphate protecting group with DBU, oxidation of the Dmoc functions with sodium periodate and aniline induced β-elimination. Besides serving as the base for β-elimination, excess aniline was used as a nucleophilic scavenger for the deprotection side product **2.2hh**, which would otherwise react with the deprotected ODN via Michael addition (Scheme 2.2). ODNs containing sensitive groups such as alkyl ester, aryl ester, thioester, alkyl halide, α-halo amide, and chloropurine were successfully synthesized using the method.



Scheme 2.2: Michael addition side reaction between side product **2.2** and deprotected ODN.

To avoid the use of excess aniline, dmDmoc group was tested for amino protection.[34] The ODN synthesized can be represented with **2.1b**. Deprotection and cleavage were achieved under similar conditions except that the excess aniline could be replaced with dilute potassium carbonate at pH 8. The deprotection side product **2.2mm** was found unreactive toward deprotected ODNs due to steric hindrance. Unfortunately, the dmDmoc protection was not completely stable under the acidic detritylation conditions, and as a result, significant amounts of branched ODNs were formed although the desired ODN could be purified using RP HPLC.[34] Later, we tested the use of Dim for phosphate protection and Dmoc for amino protection.[35, 36] The ODNs synthesized can be represented by **2.1c** (Scheme 2.1). Deprotection and cleavage were achieved in two steps by oxidation with sodium periodate followed by excess aniline.

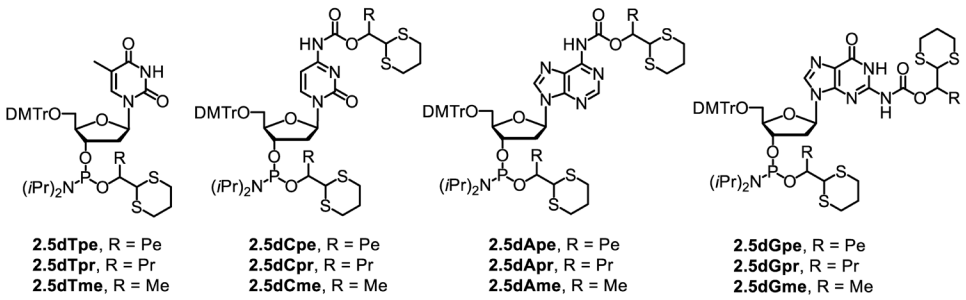
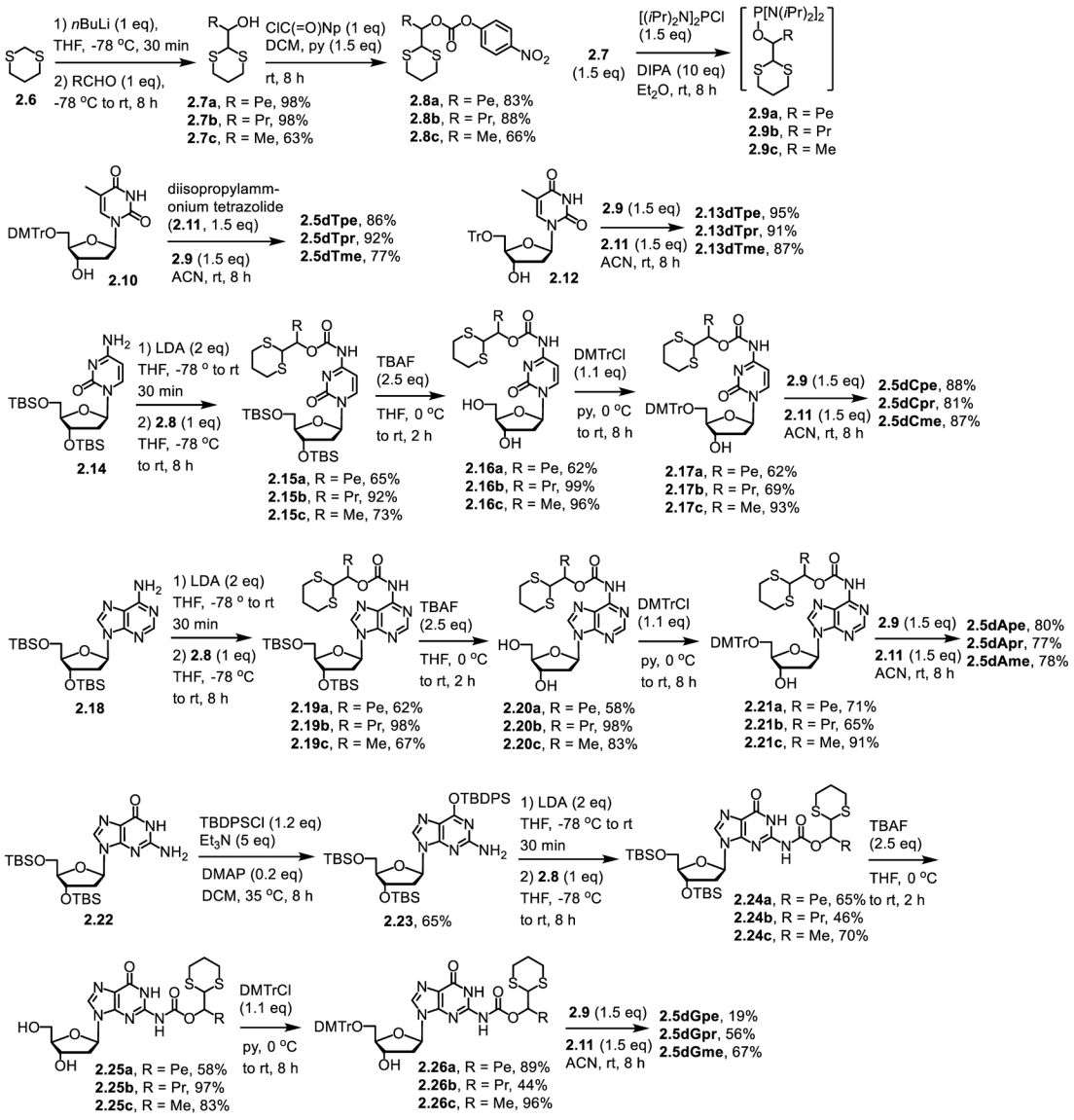


Figure 2.2: aDim-aDmoc phosphoramidite monomers.
The monomers are also called **2.5dNpe**, **2.5dNpr**, **2.5dNme**, wherein N is an abbreviation for nucleosides T, C, A, and G.

In this paper, we report the study of alkyl Dim (aDim) and alkyl Dmoc (aDmoc) as protecting groups for sensitive ODN synthesis. The ODNs synthesized can be represented by **2.1d**. We found that deprotection can be achieved in two steps by oxidation with sodium periodate followed by β-elimination with potassium carbonate (Scheme 2.1). The deprotection side products **2.2pe**, **2.2pr,** and **2.2me**, although less hindered than **2.2mm**, were found unreactive toward deprotected ODN, which indicates that the reaction in Scheme 2.2 favors the left side. As expected, the aDim and aDmoc groups, unlike the dmDmoc group that can form a tertiary carbocation under acidic conditions, were completely stable under the detritylation conditions. Over 10 ODNs including one containing the sensitive N^4-acetyldeoxycytidine group were synthesized and readily purified with RP HPLC. The ODNs were characterized with HPLC and MALDI MS and in selected cases capillary electrophoresis.



Scheme 2.3: Synthesis of aDim-aDmoc phosphoramidite monomers.

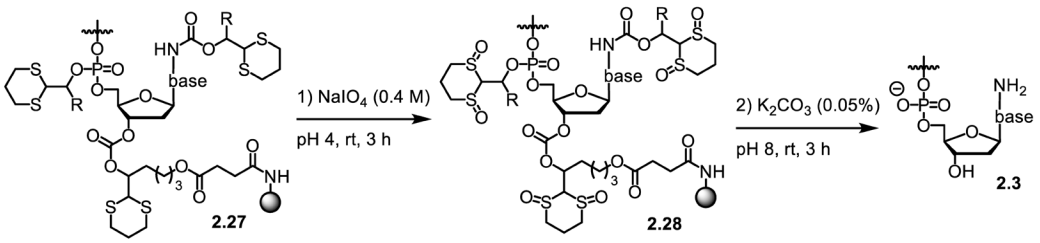
2.3 Results and discussion

2.3.1 peDim-peDmoc phosphoramidites for ODN synthesis

We hypothesized that using the phosphoramidites **2.5dNpe** (N = nucleoside T, C, A or G; Figure 2.2) for ODN synthesis, the ODN deprotection side product **2.2pe**, due to its steric hindrance from the pentyl group, might not react with the deprotected ODN to form adduct **2.4** (Scheme 2.2) in the absence of any nucleophilic scavenger. We further hypothesized

that even if the steric hindrance were not enough to suppress the side reaction, because ODN deprotection is performed in water, the hydrophobicity of the pentyl group could make **2.2pe** barely soluble in water, which could shift the Michael addition side reaction toward free ODN (Scheme 2.2). For these reasons and considering the low chance of instability of the peDim and peDmoc protections under the acidic detritylation conditions during ODN synthesis, we decided to investigate the effectiveness of using peDim and peDmoc as protecting groups for ODN synthesis.

The synthesis of the phosphoramidite monomers **2.5dNpe**, which includes **2.5dTpe**, **2.5dCpe**, **2.5dApe,** and **2.5dGpe**, is shown in Scheme 2.3. The required reagents **2.8a** and **2.9a** were readily synthesized from **2.6** using similar procedures we reported previously.[35] For **2.9a**, due to its sensitivity to oxygen and moisture, it was synthesized at the time of use and used for the next reactions without purification and characterization.



Scheme 2.4: Deprotection and cleavage of ODNs synthesized using aDim-aDmoc protected phosphoramidites.

Compound **2.5dTpe** was synthesized under standard phosphitylation conditions we reported previously for the synthesis of Dim-Dmoc phosphoramidites from **2.10** in 86% yield (Scheme 2.3).[35] We also synthesized **2.13dTpe**, which carries a 5'-Tr group instead of a 5'-DMTr group. The compound was needed for the incorporation of the last nucleoside in ODN synthesis to assist RP HPLC purification of the ODN product. Our earlier studies indicated that the DMTr group could not survive the slightly acidic sodium periodate oxidation conditions for ODN deprotection.[34] For the synthesis of **2.5dCpe** and **2.5dApe**, the introduction of peDmoc to the amino group and the tritylation of 5'-OH group to give **2.17a** and **2.21a** were carried out smoothly under conditions similar to our previously

reported conditions used for the synthesis of dmDmoc phosphoramidites.[34] For the synthesis of **2.5dGpe**, the introduction of peDmoc and DMTr to give **2.26a** was also similar to that for the synthesis of dmDmoc-dG amidites.[34] However, we improved the synthesis by the use of the bulkier TBDPS group instead of the TBS group for the protection of the lactam in the nucleobase. When TBS was used, the product was too labile toward hydrolysis to be isolated. With TBDPS, product **2.23** could be isolated in good yield, which made the subsequent reaction for the installation of the peDmoc group more reproducible. With compounds **2.17a**, **2.21a,** and **2.26a**, the synthesis of the corresponding **2.5dNpe** phosphoramidites were carried out smoothly using our previously reported procedure (Scheme 2.3).[35]

ODN synthesis using the **2.5dNpe** phosphoramidites was carried out under standard conditions with a few modifications.[35] Capping failure sequences was conducted using 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite with 4,5-dicyanoimidazole as the activator. In the last synthetic cycle, phosphoramidite **2.13dTpe** was used so that the full-length sequence was tagged with a trityl group instead of a DMTr group.[34] The resulting ODN can be represented by **2.27** (Scheme 2.4). Deprotection and cleavage were achieved in two steps. In the first step, the sulfide groups in the peDim and peDmoc protecting groups and the Dmoc linker were oxidized with a sodium periodate solution (0.4 M), which has a pH of 4 in the absence of any added acid, at room temperature to give **2.28**. In the second step, β-elimination was induced with potassium carbonate (0.05%), which has a pH of 8, at room temperature to give fully deprotected ODN (**2.3**). The total deprotection and cleavage time was about 6 hours, which could be shortened by raising the reaction temperature. However, we do not suggest doing so because ODNs containing sensitive groups could decompose. The ODN was then purified with Tr-on RP HPLC, and the pure ODN was analyzed with MALDI MS.

2.29a (11-mer), 5'-TTC TCT CTC TT-3'
2.29b (11-mer), 5'-TTA TAT ATA TT-3'
2.29c (11-mer), 5'-TTA TGT ATA TT-3'
2.29d (10-mer), 5'-TCT ATC TCT T-3'
2.29e (19-mer), 5'-TTT AGT CAT CTT CTT TTC T-3'
2.29f (19-mer), 5'-TCG TAC CAT CTT TAA ACA T-3'
2.29g (20-mer), 5'-TTT TTC CAT CCT AGA AAG CT-3'
2.29h (23-mer), 5'-TCA CAT TAT ACC ATT CTC CTA AT-3'
2.29i (19-mer), 5'-TAG TAC TTT ATC CAA CCT T-3'
2.29j (22 Mer), 5'-TCA TAG TA**(ac)C** TTT ATC CAA CCT T-3'
2.29k (22 Mer), 5'-TCA TAG TA**C** TTT ATC CAA CCT T-3'

Figure 2.3: ODN sequences.

ODNs **2.29a–c**, **2.29d–f**, **2.29g–h**, and **2.29i–k** were synthesized using phosphoramidites **2.5dNpe**, **2.5dNpr**, **2.5dNme**, **2.30a–d**, respectively. All were deprotected and cleaved under non-nucleophilic and nearly neutral conditions except for **2.29k**, which was deprotected and cleaved using traditional conditions involving concentrated ammonium hydroxide at elevated temperature.

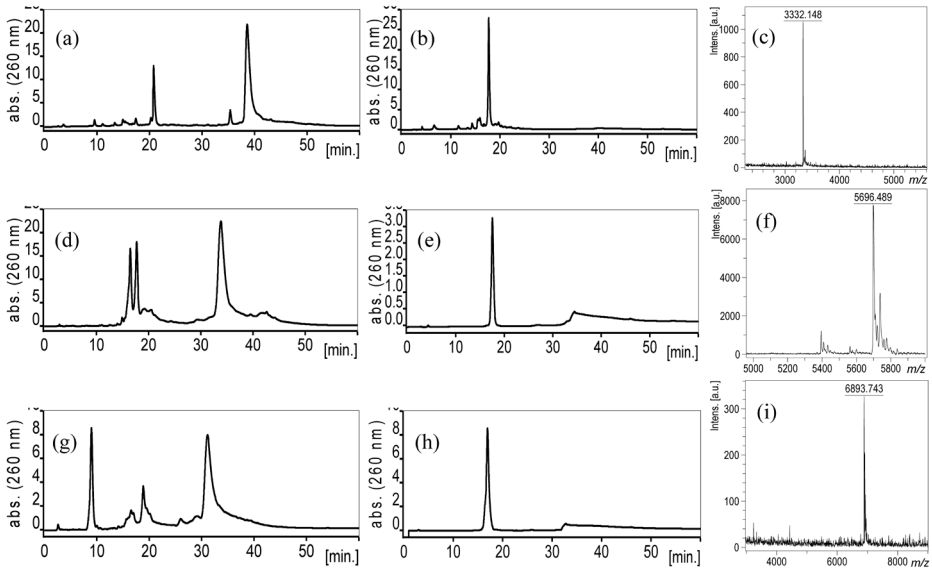


Figure 2.4: RP HPLC profiles and MALDI MS of ODNs synthesized using the aDim–aDmoc technology.

(a) HPLC of crude ODN **2.29c**. (b) HPLC of crude ODN **2.29c** from detritylation of RP HPLC purified trityl-on ODN. (c) MS of pure ODN **2.29c**, calcd for $[M-H]^-$ 3333.6 found 3332.1. (d) HPLC of crude ODN **2.29e**. (e) HPLC of pure ODN **2.29e**. (f) MS of

pure ODN **2.29e**, calcd for $[M-H]^-$ 5696.9 found 5696.5. (g) HPLC of crude ODN **2.29h**. (h) HPLC of pure ODN **2.29h**. (i) MS of pure ODN **2.29h**, calcd for $[M-H]^-$ 6888.2 found 6893.7.

Using the procedure, three 11-mer ODNs (**2.29a-c**, Figure 2.3) were synthesized. Their crude and pure HPLC profiles and images of MALDI MS are given in Appendix 5.1. The RP HPLC profile of crude **2.29c** is also shown in Figure 2.4a, which shows that the synthesis was efficient as only one major peak corresponding to Tr-tagged full length sequence appeared (retention time ~40 minutes). The HPLC profile of crude **2.29c** from detritylation of RP HPLC purified tritylated ODN is shown in Figure 2.4b. The MALDI MS of the purified detritylated **2.29c** is shown in Figure 2.4c. The mass of the molecular ion matches the calculated value.

Encouraged by the results, we proceeded to use the method to synthesize longer ODNs such as 20-mers. However, RP HPLC analysis found that complex mixtures were formed. Careful manipulations such as paying extra attention on drying phosphoramidite monomers and using longer coupling times did not improve the synthesis. After many trials, we concluded that the hydrophobic pentyl groups in the **2.5dNpe** phosphoramidites may have an adverse effect on ODN synthesis. When the ODN on the solid support reached a certain length, the pentyl groups might reduce the solubility of the protected ODN on the solid support, and as a result, the reactions such as coupling, and oxidation were inefficient.

2.3.2 prDim-prDmoc phosphoramidites for ODN synthesis

With the reasoning that the hydrophobicity of the pentyl group in **2.5dNpe** may have an adverse effect on ODN synthesis, we next synthesized the **2.5dNpr** phosphoramidites (Figure 2.2 and Scheme 2.3). We hypothesized that the propyl group, which is less hydrophobic than the pentyl group, in the aDim and aDmoc groups could have a less adverse effect on ODN synthesis, while it is still sufficiently hydrophobic as well as hindered to prevent the Michael addition side reaction between **2.2** and **2.3** (Scheme 2.2) during ODN deprotection. The phosphoramidites were synthesized using the same procedure for the synthesis of **2.5dNpe** (Scheme 2.3). Good to excellent yields were

obtained. Indeed, using these monomers, under the conditions described above for ODN synthesis using **2.5dNpe**, we were able to synthesize longer ODNs (**2.29d-f**), which included two 19-mers. It is noted that potassium carbonate was used to induce β-elimination during deprotection and cleavage. There was no need of nucleophilic scavenger such as aniline to suppress the Michael addition side reaction (Scheme 2.2). The crude and pure HPLC profiles and MALDI MS image of **2.29e** are shown in Figure 2.4d-f. Additional analytical data including those for **2.29d-f** are provided in Appendix 5.1. As can be seen, the syntheses had good yields and the full-length sequences were easy to purify. The results indicate that the hydrophobicity of the pentyl group in **2.5dNpe** may indeed have an adverse effect on ODN synthesis, and the propyl group in **2.5dNpr** has less such adverse effect.

2.3.3 meDim-meDmoc phosphoramidites for ODN synthesis

Encouraged with results using phosphoramidites **2.5dNpr**, we were interested in knowing if phosphoramidites **2.5dNme** would be suitable for ODN synthesis. In this case, the hydrophobicity of methyl group would have much less effect on the equilibrium of the Michael addition side reaction involving **2.2**, **2.3,** and **2.4** (Scheme 2.2), and there is a possibility that the ODN adduct **2.4** could be formed in significant quantity rendering the need of a scavenger such as aniline. If that were the case, aDim-aDmoc would not offer any advantage over Dim-Dmoc we reported earlier.[35] However, if the steric hindrance from the methyl group alone or together with the limited hydrophobicity provided by the methyl group can be sufficient to suppress the formation of **2.4**, phosphoramidites **2.5dNme** would have significant advantages over other aDim-aDmoc phosphoramidites. They are more atom-economic, and their lower hydrophobicity may have no or little effect on the efficiency of ODN synthesis. With these considerations, we decided to synthesize **2.5dNme** phosphoramidites.

As shown in Scheme 2.3, the procedure for the synthesis of **2.5dNme** was the same as that for the synthesis of **2.5dNpe** and **2.5dNpr**. The yields for the transformations were good to excellent. Using these less hydrophobic phosphoramidites, we successfully synthesized ODNs **2.29g-h**, among which **2.29h** contains 23 nucleotides. The conditions for ODN

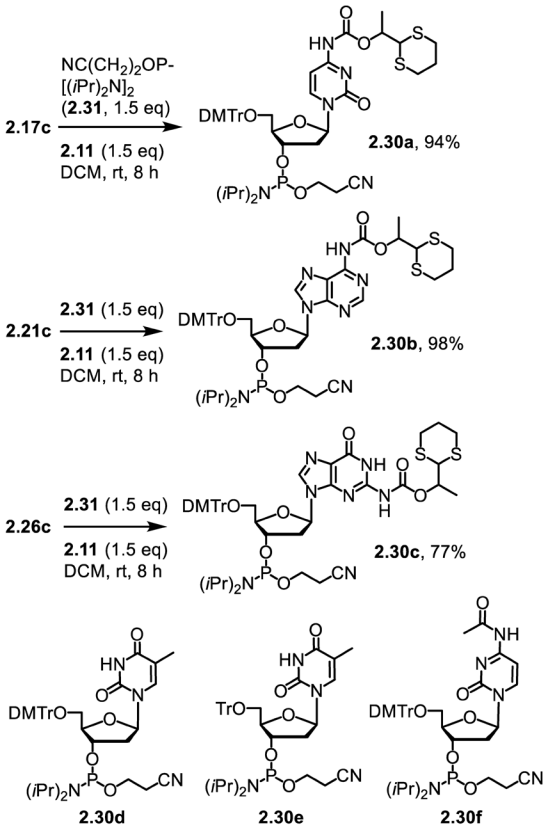
synthesis, and deprotection and cleavage as well as HPLC purification were the same as described for the synthesis of ODNs **2.29a-f** (Scheme 2.4). No scavengers such as aniline was needed to suppress the Michael addition side reaction (Scheme 2.2). The HPLC profiles of crude and pure ODN **2.29h** are shown in Figure 2.4g-h. The image of its MALDI MS is also given (Figure 2.4i). More HPLC and MALDI MS data for the ODNs are provided in Appendix 5.1. As can be seen, the ODNs synthesized are easy to purify as the trityl tagged full-length sequence is well separated from impurities in the RP HPLC profile. The results confirm that the steric hindrance of the methyl group along with the limited hydrophobicity from the methyl group in **2.5dNme** is sufficient to suppress the Michael addition side reaction between **2.2** and **2.3** (Scheme 2.2). Therefore, due to the advantages of methyl group over propyl and pentyl groups discussed earlier, we conclude that **2.5dNme** phosphoramidites are the best choice for sensitive ODN synthesis using the aDim and aDmoc protecting groups.

2.3.4 CE-meDmoc phosphoramidites for ODN synthesis

Although phosphoramidites **2.5dNme** would be more ideal for sensitive ODN synthesis than the CE-meDmoc phosphoramidites **2.30a-c** (Scheme 2.5), their synthesis requires the preparation of the oxygen and moisture sensitive phosphitylation agent **2.9c**, which may be challenging for inexperienced individuals. Because CE-meDmoc phosphoramidites are expected to meet most of the needs of sensitive ODN synthesis and their synthesis is much easier due to the commercial availability of the phosphitylation agent **2.11**, we decided to synthesize **2.30a-c** and demonstrate their use for sensitive ODN synthesis.

With the availability of **2.17c**, **2.21c**, and **2.26c**, phosphoramidites **2.30a-c** were synthesized under standard conditions using the commercially available phosphitylation agent **2.31** (Scheme 2.5). The ODNs **2.29i** was synthesized using **2.30a-d** under the same conditions described for **2.29a-h** except that the last nucleotide at the 5'-end was incorporated with **2.30e**. The coupling yields ranged from 86% to 100%. A trityl assay log is included in the Appendix 5.1, which is typical for the successful ODN syntheses in this article. The ODNs synthesized can be represented with **2.32** (Scheme 2.6). Deprotection and cleavage were achieved in three steps. First, the 2-cyanoethyl group was removed with

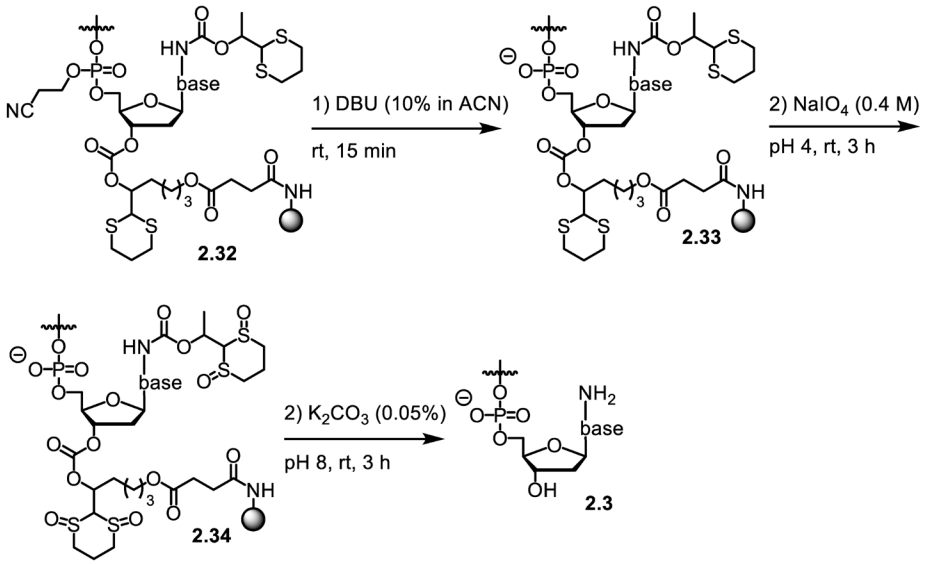
DBU at room temperature; second, the sulfides in meDmoc and linker were oxidized with sodium periodate; and third, the oxidized meDmoc and linker were cleaved with potassium carbonate. The HPLC profiles, capillary electrophoresis profile, and MALDI MS of ODN **2.29i** are provided in Appendix 5.1. As can be seen, the ODNs are easy to purify as the Tr-tagged full length sequences are well separated from impurities in the RP HPLC profiles, and ODNs with good purity can be obtained.



Scheme 2.5: Synthesis of CE-meDmoc phosphoramidites.

We were interested in testing if the meDmoc groups and the Dmoc linker in ODNs **2.32** could be cleaved by a non-nucleophilic base such as DBU without oxidizing the sulfides. Several 20-mer ODNs including one with three dG and 17 dT were synthesized and subjected to cleavage and deprotection with DBU in different solvents including THF, ACN, DMF, DMSO, and NMP at temperatures as high as 55 ºC for up to 16 hours. No significant amount of ODN could be detected with RP HPLC. Using the stronger non-

nucleophilic phosphazene base P2-Et[37] under similar conditions, no ODN was detected either. Therefore, we were confident to conclude that the Dmoc linker and protecting group have to be oxidized before they can be cleaved with non-nucleophilic bases such as potassium carbonate and DBU.



Scheme 2.6: Deprotection and cleavage of ODNs synthesized using CE-meDmoc protected phosphoramidites.

2.3.5 Sensitive ODN synthesis

In our previous studies, we have already demonstrated that a number of functional groups sensitive to cleavage and deprotection conditions used in known ODN synthesis methods such as concentrated ammonium hydroxide at elevated temperature and potassium carbonate in anhydrous methanol (i.e., dilute potassium methoxide) at room temperature can survive the conditions used for removing Dim and Dmoc groups and cleaving Dmoc linker. The sensitive groups tested included alkyl ester, aryl ester, thioester, alkyl halide, α-halo amide, and chloropurine.[32-36, 38] It should be reasonable to believe that these groups should survive the conditions used here for the deprotection of aDim and aDmoc groups because the conditions are the same. In the current study, we decided to test if N^4-acetyldeoxycytidine could survive the deprotection and cleavage conditions. N^4-

Acetylcytidine (ac^{4}C) has been found in many RNAs including mRNA, tRNA, and rRNA. It has important biological functions and is related to many human diseases.[39] If N^4-acetyldeoxycytidine could indeed survive the conditions involving sodium periodate and potassium carbonate, it would be easy to predict that the aDim-aDmoc ODN synthesis method could be extended to the synthesis of RNAs that contain the ac^{4}C nucleotide.

Accordingly, the 22-mer ODN (**2.29j**) was synthesized under the same conditions used for **2.29i**. The phosphoramidites **2.30a**-**d** were used. The N^4-acetyldeoxycytidine was incorporated with the commercially available **2.30f**, which is one of the most widely used phosphoramidites in standard ODN synthesis. Deprotection and cleavage were also conducted under the same conditions used for **2.29i**. No special attention was needed to prevent the loss of the highly sensitive acetyl group on the deoxycytidine nucleotide.[21] The ODN was purified with RP HPLC. The profiles of crude and pure ODN are in Appendix 5.1. As expected, the ODN was easy to purify, and a highly pure product can be readily obtained. MALDI MS analysis indicated that the acetyl group was not lost during the deprotection and cleavage process, and only molecular peaks including those with one to three charges were observed (Figure 3.5). To confirm the result, ODN **2.29k**, which has an identical sequence with **2.29j** but without the acetyl group, was obtained. A mixture of **2.29j** and **2.29k** was prepared and subjected to MALDI MS analysis. Molecular ions corresponding to both **2.29j** and **2.29k** were observed, and the difference of the two peaks matched well with the mass of an acetyl group (Figure 2.5). As expected, resolving **2.29j** and **2.29k** with other means such as RP HPLC and capillary electrophoresis was not easy. Under the conditions we used, analysis of the mixture gave a single peak in all trials.

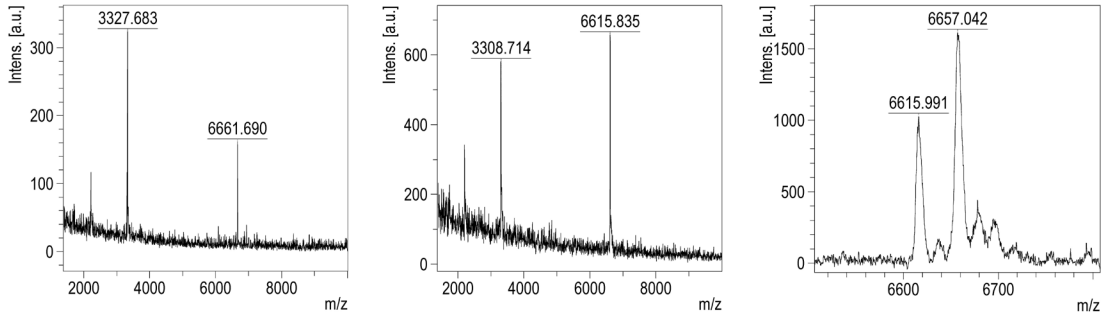


Figure 2.5: MALDI MS of the 22-mer ODN **2.29j** that contains the sensitive N^4-acetyldeoxycytidine nucleoside.

The MS of **2.29k**, which has the same sequence as **2.29j** but lacks the acetyl group, is also included. Left: MS of **2.29j**, calcd for $[M+H]^+$ 6659.1 found 6661.7. Middle: MS of **2.29k**, calcd for $[M-H]^-$ 6615.1 found 6615.8. Right: Mixture of **2.29j** and **2.29k**, calcd for **2.29j** $[M-H]^-$ 6657.1 and **2.29k** $[M-H]^-$ 6615.1, found **2.29j** 6657.0 and **2.29k** 6616.0. The mass difference of 41.0 between **2.29j** and **2.29k** matches the mass 42.0 of CH_3CO-H.

2.4 Conclusions

In summary, aDim–aDmoc monomers (**2.5dNpe**, **2.5dNpr**, and **2.5dNme**) were synthesized and studied for sensitive ODN synthesis. ODN deprotection was achieved under non-nucleophilic and nearly neutral conditions, which are required for sensitive ODN synthesis. Among them, the meDim-meDmoc monomers (*i.e.,* **2.5dNme** monomers) are most ideal for sensitive ODN synthesis. In addition to being most atom economic, they are less hydrophobic than **2.5dNpe** and **2.5dNpr** monomers, and thus have less or no adverse effect on ODN synthesis. We also studied the CE-meDmoc phosphoramidites (**2.30a–c**). These phosphoramidites are easier to synthesize than **2.5dNme** phosphoramidites and can serve most purposes in sensitive ODN synthesis. Finally, we demonstrated that the highly sensitive N^4-acetyldeoxycytidine can survive the deprotection conditions used by the aDim–aDmoc methods. An ODN containing N^4-acetyldeoxycytidine was successfully synthesized and characterized with HPLC, capillary electrophoresis, and MALDI MS. Improving the aDim–aDmoc technology to synthesize

longer and more dG-rich sequences as well as extending the technology for the synthesis of sensitive RNAs are in progress.

2.5 Experimental section

2.5.1 ODN synthesis

ODNs **2.29a–k** were synthesized on dT-Dmoc-CPG (**2.26** μmol g−1 loading, 20 mg, 0.52 μmol) using a MerMade 6 automated synthesizer. Detritylation: DCA (3% in DCM), 90 s × 2. Coupling: phosphoramidite (**2.5dNpe**, **2.5dNpr**, **2.5dNme**, **2.30a–d**, or **2.30f** 0.1 M in ACN), 4,5-dicyanoimidazole (0.25 M in ACN), 60 s × 3. Capping: 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphoramidite (0.1 M, ACN), 4,5-dicyanoimidazole (0.25 M, ACN), 60 s × 3. Oxidation: I_2 (0.02 M, THF/pyridine/H2O, 70/20/10, v/v/v), 40 s × 2. The last nucleotide at the 5′-end was incorporated using **2.13dNpe**, **2.13dNpr**, **2.13dNme**, or **2.30e** under the same conditions as other synthetic cycles. The 5′-trityl group was kept.

2.5.2 ODN deprotection and cleavage

The CPG (0.52 μmol synthesis) was divided into five equal portions, and one portion was subjected to the following deprotection and cleavage conditions. DBU treatment: For ODNs **2.29i–j**, which were synthesized using **2.30a–f** and the ODNs can be represented with **2.32** (Scheme 2.6), to the CPG in a 1.5 mL centrifuge tube was added DBU/ACN (1/9, v/v, 1 mL). The mixture was shaken gently at rt for 15 min. The supernatant was removed with a pipette. The CPG was washed with ACN (1 mL × 5). This removed the 2-cyanoethyl protecting groups and converted **2.32** to **2.33**. For ODNs **2.29a–h**, which were synthesized using **2.5dNpe**, **2.5dNpr**, **2.5dNme**, **2.13dNpe**, **2.13dNpr**, or **2.13dNme** and the ODNs can be represented with **2.27** (Scheme 2.4), the DBU treatment is not needed. $NaIO_4$ oxidation: To the CPG (1/5 of the 0.52 μmol synthesis) represented by **2.27** or **2.33** in a 1.5 mL centrifuge tube, the solution of $NaIO_4$ in water (0.4 M, 1 mL), which has a pH of 4 without adding any acid, was added. The mixture was shaken gently at rt for 3 h. The supernatant was removed with a pipette, and the CPG was washed with water (1 mL × 5). In some instances, the oxidation step was repeated but this did not make much difference.

This step converted **2.27** and **2.33** to **2.28** and **2.34**, respectively. K_2CO_3 treatment: To the CPG represented by **2.28** or **2.34** in a 1.5 mL centrifuge tube, K_2CO_3 (0.05%, pH 8, 1 mL) was added. The mixture was shaken gently at rt for 3 h. The supernatant was transferred into another 1.5 mL centrifuge tube. The CPG was washed with water (200 μL × 5). The supernatant and the washes were combined and concentrated to ~50 μL in a 1.5 mL centrifuge tube. *n*BuOH (450 μL) was added. The mixture was vortexed and then centrifuged (14.5k rpm, 15 min). The supernatant was carefully removed with a pipette without disturbing the ODN precipitate. This converted **2.28** and **2.34** to deprotected ODN **2.3** (Schemes 2.4 and 2.6). ODN **2.29k** was simply cleaved and deprotected by treating the CPG, which can be represented by **2.32**, with concentrated NH_4OH at 55 °C for 16 h.

2.5.3 ODN purification and analysis

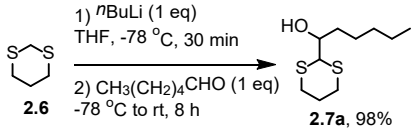
The deprotected ODN (1/5 of the 0.52 μmol synthesis) was dissolved in water (100 μL). A portion of the solution (35 μL in the cases of **2.29a–f** and **2.29i–j**, 20 μL in the cases of **2.29g–h**) was injected into RP HPLC, which generated the profile of crude trityl-tagged ODN. Fractions of the peak corresponding to the full-length trityl-tagged ODN (retention time ~35–40 min) were collected and concentrated to ~100 μL. The solution was injected into HPLC, which generated the profile of pure trityl-tagged ODN. Fractions of the peak were collected and concentrated to dryness. To the residue in a 1.5 mL centrifuge tube was added AcOH (80%, 1 mL). The tube was shaken gently at rt for 3 h. Volatiles were evaporated in a vacuum centrifugal evaporator. The residue was dissolved in water (100 μL) and injected into HPLC, which generated the profile of crude detritylated ODN. Fractions of the peak corresponding to the full-length detritylated ODN (retention time ~19 min) were collected and concentrated to dryness. To the residue in a 1.5 mL centrifuge tube was added water (100 μL). The solution was injected into HPLC, which generated the profile of pure detritylated ODN. Fractions of the ODN were collected and concentrated to dryness. The pure ODN was analyzed with MALDI MS and in selected cases capillary electrophoresis (CE). ODN **2.29k** was purified with trityl-on RP HPLC and analyzed with MALDI MS and CE as well. OD_{260} of all ODNs were determined using a reported

method.[40] HPLC profiles, MS images, CE profiles, and OD_{260} values of all ODNs are in Figure 2.4 and 2.5 or in Appendix 5.1.

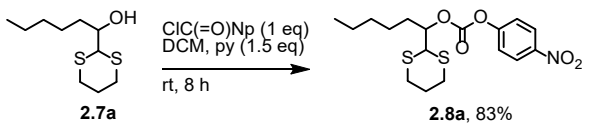
2.5.4 Compounds

General. All the reactions were performed in oven-dried glassware under nitrogen atmosphere using standard Schlenk techniques. All reagents and solvents from commercial sources were used as received with the following exceptions. DCM, pyridine, ACN, diethyl ether and diisopropylamine were distilled over CaH_2 under nitrogen. THF was distilled over CaH_2 and then Na/benzophenone under nitrogen. Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 0.25 μm thickness, were used for thin-layer chromatography (TLC). Selecto Scientific silica gel, particle size 32-63μm, was used for flash column chromatography. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on a Varian UNITYINOVA spectrometer at 400, 100 and 162 MHz, respectively. Chemical shifts (δ) were reported in reference to solvent peaks, residue $CHCl_3$ at 7.24 ppm for ^{1}H, $CDCl_3$ at 77.00 ppm for ^{13}C, and H_3PO_4 at 0.00 ppm for ^{31}P. ODN syntheses were performed on a MerMade 6 solid phase synthesizer. RP HPLC was performed on a JASCO LC-2000Plus System: pump, PU-2089Plus Quaternary Gradient; detector, UV-2075Plus. Column: C-18 reversed phase, analytical, 5 μm diameter, 100 Å, 250 × 4.60 mm. Solvent A: 0.1 M triethylammonium acetate, 5% ACN. Solvent B: 90% ACN. All profiles were generated by detecting absorbance at 260 nm using the linear gradient solvent system: solvent B (0%-45%) in solvent A over 60 min followed by solvent B (45%-100%) in solvent A over 20 min at a flow rate of 1.0 mL/min. Additionally, ODN **2.29j**, which contains N^4-acetylcytidine, was also analyzed under denatured RP HPLC conditions.[41] Capillary electrophoresis (CE) was carried out on an Agilent 7100 CE system with UV-Visible diode-array detector (190–600 nm). Capillary: PVA coated 25 cm × 100 μm (40 cm was cut to 25 cm). Buffer solution: 200 mM Bis-Tris and 200 mM boric acid in CE water, pH 7.2. Sieving solution: 27% or 30% (w/v) PEG 35,000 in buffer solution. Flush regimen: High pressure flush from outlet −8 bar for 5 min. Injection: 0.02-0.07 μM 19- to 22-mer ODNs in buffer solution, −10 KV for 10 sec. Run: −25 KV, 30 °C for 40 min. Detection: alignment interface for standard capillary with 75 μm ID, detected at 260 ± 8 nm. HRMS

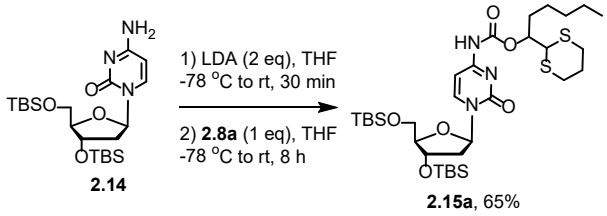
was obtained on a Thermo HR-Orbitrap Elite Mass Spectrometer. LRMS was obtained on a Thermo Finnigan LCQ Advantage Ion Trap Mass Spectrometer. Both MS were calibrated using the Pierce™ ESI Positive/Negative Ion Calibration Solutions and the semi-automatic procedure provided by the instrument software. The HRMS was further checked at *m/z* 138.06619, 195.08765, and 524.26496 (for positive mode) and *m/z* 112.98559, 265.14790, and 514.28440 (for negative mode). MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System. The negative mode of the system was calibrated using unmodified standard ODNs synthesized in house using standard methods as external standards. More details regarding MALDI MS conditions can be found in reference.[42]



*1-(1,3-Dithian-2-yl)hexan-1-ol (**2.7a**):* To a solution of 1,3-dithiane (**2.6**, 7.5 g, 62.5 mmol, 1.0 equiv.) in dry THF (100 mL) was slowly added *n*BuLi (2.5 M in hexanes, 25 mL, 62.5 mmol, 1.0 equiv.) at -78 °C under nitrogen. The mixture was stirred at the same temperature for 30 min. *n*-Hexanal (7.68 mL, 62.5 mmol, 1.0 equiv.) was added. The mixture was stirred for 8 h while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (75 mL) and extracted with EtOAc (50 mL × 3). The extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **2.7a** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 5:1): 13.47 g, 98%; colorless oil; TLC R_f = 0.5 (SiO_2, hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J = 7.0 Hz, 3H), 1.26-1.31 (m, 4H), 1.46-1.55 (m, 2H), 1.73-1.81 (m, 1H), 1.87-1.91 (m, 1H), 2.01-2.09 (m, 1H), 2.41 (t, J = 2.6 Hz, 1H), 2.68-2.78 (m, 2H), 2.86-2.93 (m, 2H), 3.79-3.85 (m, 1H), 3.88 (d, J = 6.4 Hz, 1H), 5.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.8, 25.7, 25.9, 28.2, 28.7, 31.9, 34.3, 52.7, 72.4; HRMS (ESI) *m/z* calcd for $C_{10}H_{20}OS_2Na$ $[M + Na]^+$ 243.0853, found 243.0840.

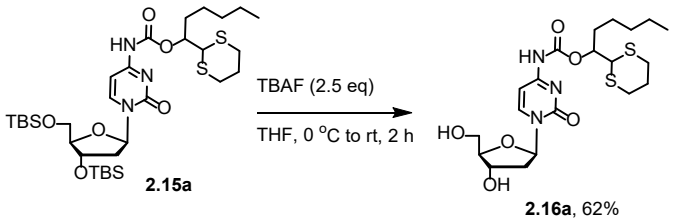


*1-(1,3-Dithian-2-yl)hexyl (4-nitrophenyl) carbonate (**2.8a**):* To a solution of **2.7a** (7.0 g, 36.4 mmol, 1.0 equiv.) in dry DCM (100 mL) under nitrogen was added distilled pyridine (4.39 mL, 54.6 mmol, 1.5 equiv.). After cooling to 0 °C, 4-nitrophenol chloroformate (7.34 g, 36.4 mmol, 1.0 equiv.) was added. The mixture was stirred overnight while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (75 mL) and extracted with DCM (50 mL × 3). The extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **2.8a** was precipitated from DCM by hexanes: 10.18 g, 83%; yellow foam; TLC R_f = 0.6 (SiO_2, hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, J = 6.8 Hz, 3H), 1.27-1.29 (m, 4H), 1.33-1.44 (m, 2H), 1.72-1.81 (m, 1H), 1.91-2.04 (m, 3H), 2.67-2.76 (m, 2H), 2.86-2.96 (m, 2H), 4.01 (d, J = 7.0 Hz, 1H), 5.05-5.09 (m, 1H), 7.36 (d, J = 9.3 Hz, 2H), 8.22 (d, J = 9.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.6, 25.1, 25.6, 28.3, 28.6, 31.6, 31.9, 48.7, 80.1, 122.0, 125.4, 145.5, 152.5, 155.8; HRMS (ESI) *m/z* calcd for $C_{17}H_{23}NO_5S_2Na$ $[M + Na]^+$ 408.0915, found 408.0900.

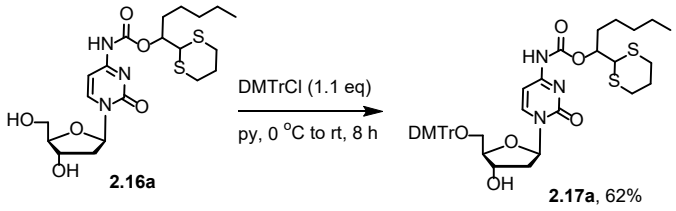


*Compound **2.15a**:* To a solution of diisopropylamine (4.51 mL, 32.02 mmol, 2.0 equiv.) in THF (200 mL) at −78 °C was added *n*BuLi (2.5 M in hexanes, 12.8 mL, 32.02 mmol, 2.0 equiv.). After stirring for 30 min, the freshly prepared LDA solution was added via a cannula to a solution of **2.14** (7.28 g, 16.01 mmol, 1.0 equiv.) in THF (50 mL) at −78 °C. The mixture was stirred at the same temperature for 30 min, and compound **2.8a** (16.01 mmol, 1.0 equiv.) was then added under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt gradually. The contents were poured into a separatory funnel and partitioned between EtOAc (50 mL) and sat. NaCl (50 mL). The aqueous layer was extracted with EtOAc (40 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Product **2.15a** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:1): 7.26 g, 65%; white foam; TLC R_f = 0.55 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.08 (d, J = 4.0

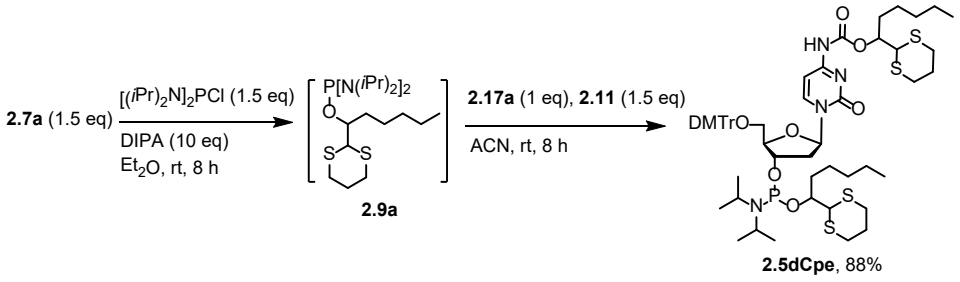
Hz , 6H), 0.84 (s, 10H), 0.89 (s, 8H), 1.15-1.35 (m, 6H), 1.66-1.75 (m, 1H), 1.81-1.93 (m, 2H), 1.99-2.13 (m, 2H), 2.45-2.52 (m, 1H), 2.65-2.77 (m, 2H), 2.83-2.90 (m, 2H), 3.74 (dd, J = 9.4, 2.6 Hz, 1H), 3.90-3.93 (m, 2H), 4.06 (dd, J = 4.6, 1.6 Hz, 1H), 4.33-4.37 (m, 1H), 5.06-5.10 (m, 1H), 6.21 (t, J = 5.5 Hz, 1H), 7.18 (d, J = 7.2 Hz, 1H), 8.36 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.3, -5.2, -4.7, -4.4, 14.2, 18.1, 18.6, 22.6, 25.2, 25.8, 25.9, 26.1, 28.97, 29.00, 29.18, 29.22, 31.6, 31.8, 42.5, 49.72, 49.77, 61.94, 61.98, 70.15, 70.24, 87.03, 87.10, 87.0, 87.99, 88.03, 95.2, 116.1, 126.2, 140.7, 144.7, 152.2, 155.6, 162.4, 164.0; HRMS (ESI) *m/z* calcd for $C_{32}H_{59}N_3O_6S_2Si_2H$ $[M + H]^+$ 702.3462, found 702.3426.



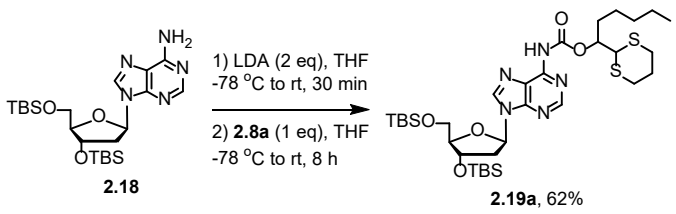
*Compound **2.16a**:* To a solution of **2.15a** (1.95 g, 2.77 mmol, 1.0 equiv.) in THF (50 mL) at 0 °C was added TBAF (1 M in THF, 6.94 mL, 6.94 mmol, 2.5 equiv.). The mixture was stirred for 2 h while warming to rt. THF was evaporated under reduced pressure and the residue was loaded onto a column for flash column chromatography (SiO_2, EtOAc/MeOH 10:1). Compound **2.16a**: 815 mg, 62%; yellow foam; TLC R_f = 0.4 (SiO_2, EtOAc/MeOH 10:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.85-0.88 (m, 3H), 1.22-1.37 (m, 6H), 1.62-1.75 (m, 1H), 1.85-1.91 (m, 2H), 1.97-2.04 (m, 1H), 2.12-2.20 (m, 1H), 2.44-2.50 (m, 1H), 2.71-2.95 (m, 4H), 3.71-3.84 (m, 2H), 3.97-4.00 (m, 1H), 4.13 (dd, J = 6.4, 1.4 Hz, 1H), 4.34-4.38 (m, 1H), 5.09-5.13 (m, 1H), 6.19 (t, J = 6.2 Hz, 1H), 7.26 (d, J = 7.5 Hz, 1H), 8.42 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 13.2, 22.3, 24.9, 25.8, 28.24, 28.51, 28.53, 31.4, 31.7, 41.3, 49.4, 61.3, 70.5, 76.0, 87.4, 88.2, 95.6, 144.7, 153.3, 156.4, 163.6, 164.3; HRMS (ESI) *m/z* calcd for $C_{20}H_{31}N_3O_6S_2Na$ $[M + Na]^+$ 496.1552, found 496.1566.



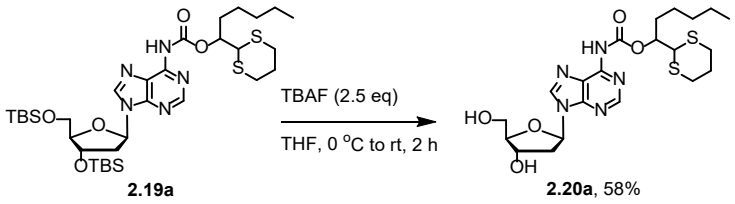
*Compound **2.17a***: To a solution of **2.16a** (534 mg, 1.13 mmol, 1.0 equiv.) in pyridine (10 mL) at 0 °C was added DMTrCl (402.02 mg, 1.18 mmol, 1.1 equiv.) under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt. The volume of the mixture was reduced to about 2 mL under vacuum from an oil pump (the remaining pyridine can help to retain the DMTr group in the product). The residue was poured into a separatory funnel and partitioned between 5% Na_2CO_3 (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. Product **2.17a** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 549.6 mg, 62%; white foam; TLC R_f = 0.6 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.80-0.89 (m, 3H), 1.06-1.24 (m, 6H), 1.68-1.73 (m, 1H), 1.85-1.92 (m, 2H), 2.13-2.21 (m, 1H), 2.60-2.76 (m, 2H), 2.84-2.91 (m, 2H), 3.35 (dd, J = 10.6, 3.6 Hz, 1H), 3.45 (dd, J = 10.4, 2.3 Hz, 1H), 3.76 (s, 6H), 4.02-4.11 (m, 2H), 4.15-4.16 (m, 1H), 4.47-4.48 (m, 1H), 5.05-5.11 (m, 1H), 6.25 (t, J = 5.8 Hz, 1H), 6.81 (d, J = 8.3 Hz, 4H), 7.00 (d, J = 7.5 Hz, 1H), 7.17-7.27 (m, 7H), 7.37 (d, J = 7.5 Hz, 2H), 8.20 (d, J = 5.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.4, 22.6, 25.2, 25.8, 28.8, 29.0, 29.1, 29.2, 31.6, 31.8, 31.9, 42.3, 49.6, 49.8, 55.5, 60.6, 63.0, 71.1, 86.7, 87.1, 87.5, 95.2, 113.5, 127.2, 128.2, 128.4, 130.2, 135.6, 135.7, 144.5, 152.2, 158.8, 162.4; HRMS (ESI) *m/z* calcd for $C_{41}H_{49}N_3O_8S_2H$ $[M + H]^+$ 776.3039, found 776.3063.



*Compound **2.5dCpe***: To a solution of **2.7a** (851.4 mg, 3.87 mmol, 1.5 equiv.) and freshly distilled diisopropyl amine (DIPA, 3.63 mL, 25.8 mmol, 10 equiv.) in dry diethyl ether (30 mL) was added bis(diisopropylamino)chlorophosphine (1.03 g, 3.87 mmol, 1.5 equiv.) at rt under nitrogen. After stirring overnight, a cloudy solution containing the soluble intermediate **2.9a** and insoluble diisopropylamine hydrochloride side product was formed. The intermediate **2.9a** in the supernatant was transferred into a solution of **2.17a** (2.00 g, 2.58 mmol, 1 equiv.) and diisopropylammonium tetrazolide (**2.11**, 661.7 mg, 3.87 mmol, 1.5 equiv.) in dry ACN (60 mL) via a cannula with its inflow end wrapped with a copper wire-secured cotton to avoid transferring of insoluble salts. After stirring overnight, the mixture was concentrated to dryness. The residue was dissolved in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N and loaded onto a column (SiO_2). Eluting with the same solvent mixture gave **2.5dCpe**: mixture of diastereomers; 2.55 g, 2.27 mmol, 88%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76-0.81 (m, 6H), 0.98-1.22 (m, 22H), 1.55-1.60 (m, 1H), 1.63-1.70 (m, 1H), 1.78-1.88 (m, 2H), 1.94-2.00 (m, 2H), 2.18-2.29 (m, 1H), 2.67-2.75 (m, 6H), 2.80-2.86 (m, 2H), 3.39-3.52 (m, 3H), 3.72 (s, 6H), 3.83-3.86 (m, 1H), 4.00 (t, J = 7.2 Hz, 1H), 4.15-4.18 (m, 1H), 4.49-4.59 (m, 1H), 5.01-5.06 (m, 1H), 6.15-6.22 (m, 1H), 6.75-6.79 (m, 4H), 6.83-6.85 (m, 1H), 7.13-7.25 (m, 7H), 7.33 (t, J = 8.2 Hz, 2H), 7.54 (brs, 1H), 8.18-8.23 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 14.4, 22.7, 22.8, 22.90, 22.92, 23.94, 23.96, 24.90, 24.94, 25.01, 25.07, 25.3, 25.91, 26.6, 29.04, 29.17, 29.30, 30.6, 30.9, 31.7, 31.9, 33.4, 43.4, 43.6, 44.7, 44.8, 49.7, 49.8, 53.8, 55.5, 62.5, 76.51, 76.54, 76.64, 76.7, 87.0, 87.2, 87.3, 94.7, 113.4, 127.1, 128.1, 128.3, 130.17, 130.19, 135.47, 135.53, 144.2, 144.30, 144.5, 152.0, 154.9, 158.7, 162.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.8, 149.0, 149.3; HRMS (ESI) *m/z* calcd for $C_{57}H_{81}N_4O_9PS_4H$ $[M + H]^+$ 1125.4702, found 1125.4691.



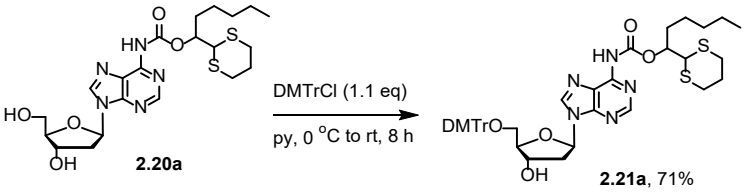
*Compound **2.19a***: Synthesized using the procedure for **2.15a**. Diisopropylamine (4.65 mL, 33.05 mmol, 2.0 equiv.), THF (200 mL), *n*BuLi (2.5 M in hexanes, 13.2 mL, 33.05 mmol, 2.0 equiv.), **2.18** (7.91 g, 16.52 mmol, 1.0 equiv.), THF (50 mL) and **2.8a** (16.52 mmol, 1.0 equiv.) were used. Product **2.19a** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:1): 7.56 g, 62%; light yellow foam; TLC R_f = 0.45 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 (d, J = 3.4 Hz, 12H), 0.72-0.82 (m, 3H), 0.87 (s, 18H), 1.22-1.24 (m, 3H), 1.33-1.36 (m, 1H), 1.70-1.73 (m, 1H), 1.89-1.91 (m, 1H), 1.98-2.02 (m, 1H), 2.41-2.46 (m, 1H), 2.56-2.62 (m, 1H), 2.66-2.75 (m, 1H), 2.82-2.95 (m, 2H), 3.76 (dd, J = 11.1, 2.7 Hz, 1H), 3.87 (dd, J = 11.3, 3.6 Hz, 1H), 4.10 (d, J = 6.0 Hz, 1H), 4.57-4.59 (m, 1H), 5.17-5.21 (m, 1H), 6.47 (t, J = 6.1 Hz, 1H), 6.94 (d, J = 9.0 Hz, 1H), 8.37 (s, 1H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -5.0, -4.5, -4.3, 14.3, 18.3, 18.7, 22.7, 25.4, 26.0, 26.2, 29.00, 29.04, 29.4, 31.7, 31.9, 41.8, 41.9, 50.0, 62.9, 71.95, 71.98, 76.24, 76.28, 85.0, 88.3, 122.2, 141.0, 141.52, 141.55, 149.4, 150.73, 150.79, 152.7, 162.9; HRMS (ESI) *m/z* calcd for $C_{33}H_{59}N_5O_5S_2Si_2H$ $[M + H]^+$ 726.3574, found 726.3597.



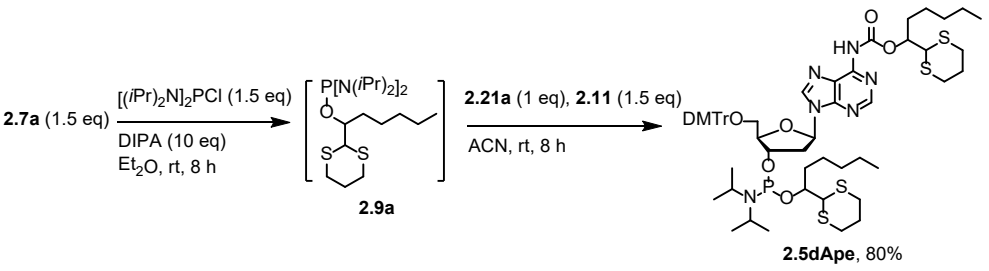
2.19a **2.20a**, 58%

*Compound **2.20c***: Synthesized using the procedure for **2.16a**. Compound **2.19a** (554 mg, 0.75 mmol, 1.0 equiv.), THF (50 mL), and TBAF (1 M in THF, 1.87 mL, 1.87 mmol, 2.5 equiv.) were used. Product **2.20a** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 10:1): 222.4 mg, 58%; light yellow foam; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 10:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.86 (t, J = 6.9 Hz, 3H), 1.22-1.33 (m, 4H), 1.35-1.41 (m, 2H), 1.69-1.76 (m, 1H), 1.81-1.93 (m, 2H), 1.96-2.04 (m, 1H), 2.42-2.47 (m, 1H), 2.72-2.88 (m, 4H), 3.73 (dd, J = 8.8, 3.8 Hz, 1H), 3.82 (dd, J = 8.4, 3.3 Hz, 1H), 4.04 (dd, J = 6.4, 3.3 Hz, 1H), 4.20 (dd, J = 6.2, 1.9 Hz, 1H), 4.56-4.59 (m, 1H), 5.15-5.20 (m, 1H), 6.49 (t, J = 6.8 Hz, 1H), 8.52 (d, J = 1.7 Hz, 1H), 8.55 (d, J = 1.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 13.3, 22.5, 25.1, 26.0, 28.5, 28.8, 31.5, 31.8, 40.4, 49.7,

62.3, 71.6, 75.9, 85.6, 88.6, 122.6, 142.8, 149.8, 150.8, 151.6, 151.8, 164.2; HRMS (ESI) *m/z* calcd for $C_{21}H_{31}N_5O_5S_2H$ $[M + H]^+$ 498.1845, found 498.1836.

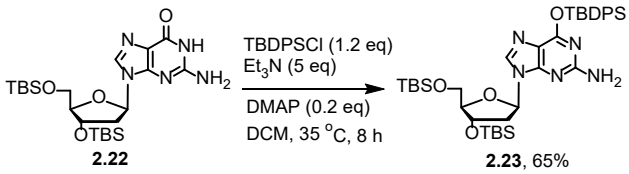


*Compound **2.21a***: Synthesized using the procedure for **2.17a**. Compound **2.20a** (220 mg, 0.43 mmol, 1.0 equiv.), pyridine (10 mL) and DMTrCl (160.5 mg, 0.47 mmol, 1.1 equiv.) were used. Product **2.21a** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 240.5 mg, 71%; white foam; TLC R_f = 0.55 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (t, J = 5.4 Hz, 3H), 1.08-1.26 (m, 4H), 1.31-1.41 (m, 2H), 1.70-1.79 (m, 1H), 1.86-1.92 (m, 2H), 2.53-2.59 (m, 1H), 2.65-2.77 (m, 2H), 2.79-2.88 (m, 2H), 2.90-2.97 (m, 1H), 3.38 (d, J = 4.5 Hz, 2H), 3.73 (s, 6H), 4.14-4.19 (m, 2H), 4.69-4.70 (m, 1H), 5.18-5.23 (m, 1H), 6.45 (t, J = 5.4 Hz, 1H), 6.75 (d, J = 8.7 Hz, 4H), 7.15-7.25 (m, 7H), 7.34 (d, J = 7.1 Hz, 2H), 8.09 (d, J = 0.9 Hz, 1H), 8.66 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 14.5, 22.8, 25.4, 26.1, 29.1, 29.5, 31.8, 31.9, 40.56, 40.60, 50.2, 55.5, 64.0, 72.67, 72.70, 76.1, 84.9, 86.5, 86.8, 113.3, 122.4, 127.1, 128.0, 128.2, 130.1, 135.70, 135.72, 141.4, 144.6, 149.5, 150.6, 150.8, 152.9, 158.7; HRMS (ESI) *m/z* calcd for $C_{42}H_{49}N_5O_7S_2Na$ $[M + Na]^+$ 822.2971, found 822.2999.



*Compound **2.5dApe***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7a** (853.6 mg, 3.88 mmol, 1.5 equiv.), diisopropylamine (3.64 mL, 25.9 mmol, 10 equiv.), diethyl ether (30mL), bis(diisopropylamino)chlorophosphine (1.03 g, 3.88 mmol, 1.5 equiv.),

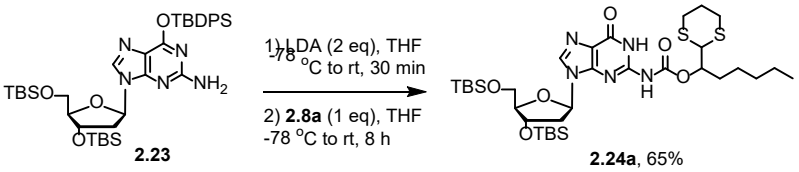
2.21a (2.00 g, 2.59 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 663.5 mg, 3.88 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.5dApe** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 1.04 g, 80%; white foam; TLC R_f = 0.4 and 0.5 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.77-0.79 (m, 6H), 0.94-1.27 (m, 25H), 1.27-1.37 (m, 3H), 1.61-1.74 (m, 3H), 1.81-1.86 (m, 2H), 1.95-2.02 (m, 2H), 2.54-2.92 (m, 10H), 3.29-3.36 (m, 2H), 3.49-3.60 (m, 2H), 3.70 (s, 6H), 3.78-3.90 (m, 1H), 4.12 (t, J = 7.0 Hz, 1H), 4.16-4.19 (m, 0.5H), 4.23-4.26 (m, 1H), 4.29-4.34 (m, 0.5H), 4.66-4.76 (m, 1H), 5.15-5.18 (m, 1H), 6.41 (t, J = 6.4 Hz, 1H), 6.69-6.72 (m, 4H), 7.09-7.22 (m, 7H), 7.32 (d, J = 7.3 Hz, 2H), 8.12-8.14 (m, 1H), 8.64-8.65 (m, 1H), 8.69 (brs, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.3, 14.4, 22.7, 22.9, 24.9, 25.0, 25.04, 25.3, 25.4, 25.5, 26.1, 26.6, 29.1, 29.47, 29.51, 30.64, 30.74, 30.85, 30.97, 31.7, 31.85, 31.9, 33.4, 43.5, 43.6, 46.5, 50.1, 50.14, 53.7, 53.75, 53.83, 53.87, 55.4, 63.77, 63.85, 76.0, 76.6, 76.7, 85.1, 85.2, 86.6, 113.2, 122.5, 127.0, 127.9, 128.2, 130.13, 130.16, 135.7, 135.8, 141.6, 144.57, 144.64, 149.5, 150.6, 150.97, 151.0, 152.8, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.2, 148.5; HRMS (ESI) *m/z* calcd for $C_{58}H_{81}N_6O_8PS_4H$ $[M + H]^+$ 1149.4815, found 1149.4797.



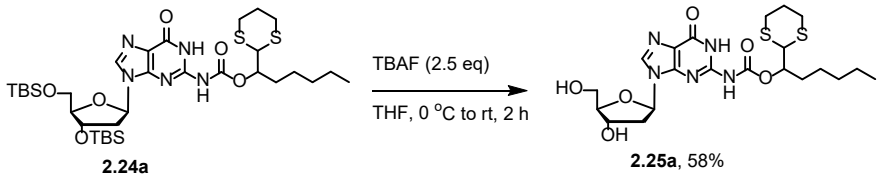
2.22 **2.23**, 65%

*Compound **2.23***: To a solution of TBDPSCl (3.14 mL, 12.12 mmol, 1.2 equiv.), Et_3N (4.82 mL, 50.50 mmol, 5.0 equiv.), 4-dimethylaminopyridine (246.78 mg, 2.02 mmol, 0.2 equiv.) in DCM (150 mL) at 35 °C was added **2.22** (5.0 g, 10.10 mmol, 1.0 equiv.) under positive nitrogen pressure. The mixture was stirred at the same temperature overnight. The crude product was partitioned between DCM (150 mL) and NaH_2PO_4/Na_2HPO_4 buffer (pH 7, 50 mL). The organic phase was washed with the same buffer two times, dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. Product **2.23** was purified with

flash column chromatography (SiO_2, hexanes/ EtOAc 4:1): 4.57 g, 65%; white foam; TLC R_f = 0.5 (SiO_2, hexanes/ EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (d, J = 3.8 Hz, 6H), 0.06 (d, J = 2.9 Hz, 6H), 0.87 (d, J = 2.6 Hz, 18H), 1.15 (s, 9H), 2.23-2.29 (m, 1H), 2.51-2.58 (m, 1H), 3.73 (t, J = 4.3 Hz, 1.5H), 3.91-3.93 (m, 1H), 4.00-4.05 (m, 0.5H), 4.58 (m, 2H), 6.20 (t, J = 6.6 Hz, 1H), 7.22-7.32 (m, 6H), 7.72 (dd, J = 9.0, 2.6 Hz, 4H), 7.83 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -4.9, -4.4, -4.3, 18.3, 18.7, 19.9, 26.1, 26.3, 27.4, 40.7, 63.1, 72.3, 83.8, 87.8, 117.4, 127.57, 127.59, 129.72, 129.75, 133.0, 135.6, 137.9, 154.5, 159.1, 159.5; HRMS (ESI) *m/z* calcd for $C_{38}H_{59}N_5O_4Si_3H$ $[M + H]^+$ 734.3953, found 734.3955.

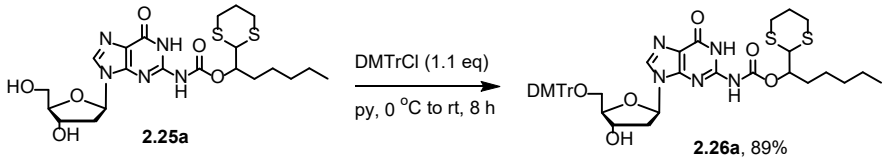


*Compound **2.24a***: Synthesized using the procedure for **2.15a**. Diisopropylamine (0.95 mL, 0.71 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 0.271 mL, 0.68 mmol, 2.0 equiv.), **2.23** (0.249 g, 0.34 mmol, 1.0 equiv.), THF (mL) and **2.8a** (0.13g, 0.34 mmol, 1.0 equiv.) were used. The product was partitioned between EtOAc (50 mL) and NH_4Cl (50 mL). The aqueous layer was extracted with EtOAc (40 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **2.24a** was purified with flash column chromatography (SiO_2, DCM/MeOH, 19:1): 0.16 g, 65%; white foam; TLC R_f = 0.3 (SiO_2, DCM/MeOH 19:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 (d, J = 5.2 Hz, 8H), 0.83-0.93 (m, 21H), 1.24-1.37 (m, 6H), 1.87-1.96 (m, 2H), 2.00-2.06 (m, 1H), 2.31-2.42 (m, 2H), 2.67-2.77 (m, 2H), 2.87-2.97 (m, 2H), 3.74 (d, J = 3.5 Hz, 1H), 3.94-3.97 (m, 2H), 4.53-4.54 (m, 1H), 5.15-5.21 (m, 1H), 6.19-6.24 (m, 1H), 7.77-7.78 (m, 0.5H), 7.92 (d, J = 4.0 Hz, 0.5H), 11.19 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -5.0, -4.4, -4.3, 14.3, 18.3, 18.7, 22.72, 22.74, 25.24, 25.31, 25.8, 26.0, 26.3, 28.44, 28.55, 28.75, 28.83, 31.6, 32.0, 41.7, 41.9, 49.00, 49.13, 63.05, 63.09, 72.17, 72.21, 84.07, 84.08, 88.16, 88.19, 94.6, 121.29, 121.34, 136.80, 136.86, 146.5, 148.1, 153.2, 155.6; HRMS (ESI) *m/z* calcd for $C_{33}H_{59}N_5O_6S_2Si_2H$ $[M + H]^+$ 742.3524, found 742.3554.



2.24a **2.25a**, 58%

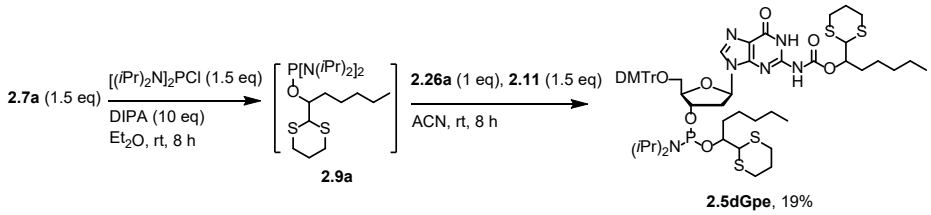
*Compound **2.25a***: Synthesized using the procedure for **2.16a**. Compound **2.24a** (0.48 g, 0.65 mmol, 1.0 equiv.), THF (20 mL) and TBAF (1 M in THF, 1.63 mL, 1.63 mmol, 2.5 equiv.) were used. Product **2.25a** was purified with flash column chromatography (SiO_2, DCM/MeOH 9:1): 222.4 mg, 58%; white foam; TLC R_f = 0.3 (SiO_2, DCM/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.78 (m, 3H), 1.15-1.23 (m, 2H), 1.78-1.87 (m, 1.5H), 1.90-1.97 (m, 1H), 2.48-2.53 (m, 1.5H), 2.57-2.66 (m, 2H), 2.77-2.89 (m, 2H), 3.72-3.82 (m, 1H) 3.90-3.96 (m, 1H), 4.79-4.84 (m, 1H), 5.03-5.09 (m, 1H) , 6.17-6.19 (m, 1H), 7.85 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.7, 24.5, 25.2, 25.7, 28.4, 28.7, 31.6, 32.0, 40.4, 40.5, 48.7, 62.0, 70.36, 70.41, 84.94, 84.96, 87.8, 121.4, 138.0, 138.1, 146.83, 146.88, 148.0, 153.8, 155.8; HRMS (ESI) *m/z* calcd for $C_{21}H_{31}N_5O_6S_2H$ $[M + H]^+$ 514.1794, found 514.1809.



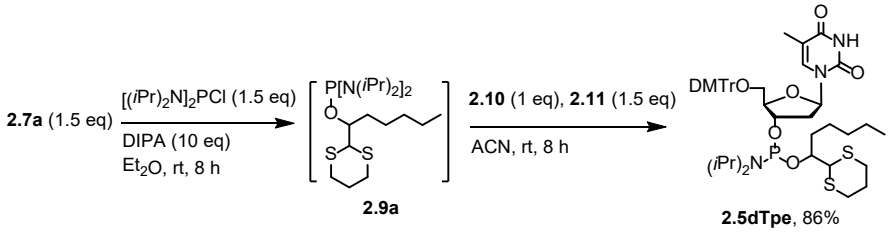
2.25a **2.26a**, 89%

*Compound **2.26a***: Synthesized using the procedure for **2.17a**. Compound **2.25a** (0.419 g, 0.81 mmol, 1.0 equiv.), pyridine (20 mL) and DMTrCl (0.30 g, 0.89 mmol, 1.1 equiv.) were used. Product **2.26a** was purified with flash column chromatography (SiO_2, DCM/MeOH 19:1 with 5% Et_3N): 588 mg, 89%; white foam; TLC R_f = 0.5 (SiO_2, DCM/MeOH 19:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79-0.85 (m, 3H), 1.22-1.26 (m, 4H), 1.62-1.70 (m, 1H), 1.86-2.00 (m, 4H), 2.53-2.71 (m, 4H), 2.85-2.93 (m, 2H), 3.21-3.33 (m, 2H), 3.69 (s, 6H), 3.94 (d, J = 7.2 Hz, 1H), 4.19-4.22 (m, 1H), 4.68-4.74 (m, 1H), 5.13-5.21 (m, 1H), 6.27-6.31 (m, 1H), 6.70-6.72 (m, 4H), 7.08-7.26 (m, 7H), 7.30-7.36 (m, 2H), 7.78 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.1, 14.31, 14.34, 15.1, 22.7, 25.38, 25.40, 25.8, 28.17, 28.23, 28.47, 28.54, 31.7, 32.05, 32.11, 34.8, 40.83, 40.96, 46.2,

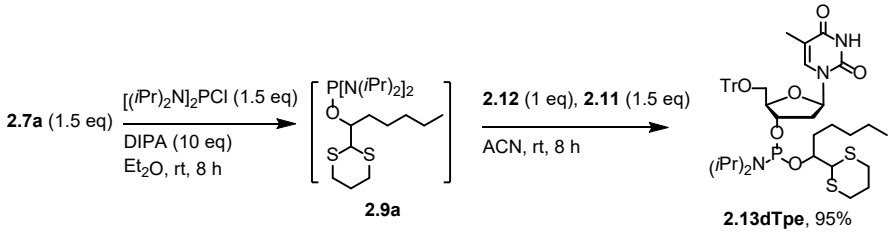
48.62, 48.76, 55.5, 64.5, 72.26, 72.34, 84.59, 84.75, 86.52, 86.56, 86.67, 86.71, 113.3, 120.96, 121.05, 127.0, 127.9, 128.3, 130.1, 135.9, 137.5, 137.6, 144.66, 144.68, 146.96, 146.99, 148.53, 148.56, 153.95, 154.02, 155.85, 155.88, 158.5; HRMS (ESI) *m/z* calcd for $C_{42}H_{49}N_5O_8S_2H$ $[M + H]^+$ 816.3101, found 816.3132.



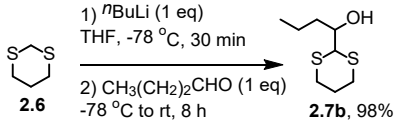
*Compound **2.5dGpe***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7a** (0.128 g, 0.581 mmol, 1.5 equiv.), diisopropyl amine (0.547 mL, 3.88 mmol, 10 equiv.), diethyl ether (10 mL), bis(diisopropylamino)chlorophosphine (0.155 g, 0.581 mmol, 1.5 equiv.), **2.26a** (0.815 g, 0.388 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.099 g, 0.581 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.5dGpe** was purified by dissolving the sample in the solvent mixture of DCM/acetone 19:1 with 5% Et_3N, loading onto a flash chromatography column (SiO_2) and eluting with the same solvent mixture: mixture of diastereomers; 0.224 g, 19%; white foam; TLC R_f = 0.5 and 0.55 (SiO_2, DCM/acetone 19:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78-0.83 (m, 6H), 1.01 (t, J = 7.2 Hz, 9H), 1.10 (dd, J = 6.6, 1.4 Hz, 3H), 1.14-1.23 (m, 16H), 1.61-1.71 (m, 2H), 1.85-1.93 (m, 2H), 1.96-2.04 (m, 2H), 2.72-2.80 (m, 4H), 2.84- 2.94 (m, 2H), 3.22-3.27 (m, 2H), 3.50-3.58 (m, 2H), 3.71 (s, 6H), 3.86-3.92 (m, 1H), 4.17-4.26 (m, 2H), 4.55-4.66 (m, 1H), 5.08-5.15 (m, 1H), 6.13-6.17 (m, 1H), 6.71-6.74 (m, 4H), 7.11-7.26 (m, 7H), 7.33-7.37 (m, 2H), 7.69-7.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.30, 14.33, 14.41, 22.70, 22.73, 22.86, 22.93, 23.93, 24.9, 25.2, 25.3, 25.4, 25.7, 26.6, 28.2, 28.4, 28.7, 30.7, 30.79, 31.0, 31.6, 31.9, 32.0, 33.37, 33.42, 43.4, 43.6, 44.71, 44.77, 45.9, 48.9, 53.7, 53.9, 55.5, 63.91, 63.94, 76.7, 76.8, 84.6, 84.9, 86.5, 86.6, 113.3, 127.0, 128.0, 128.2, 128.3, 130.05, 130.10, 130.16, 135.70, 135.8, 144.56, 144.63, 146.41, 146.49, 148.4, 153.2, 155.7, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.6, 148.5; HRMS (ESI) *m/z* calcd for $C_{58}H_{81}N_6O_9PS_4H$ $[M + H]^+$ 1165.4764, found 1165.4756.



*Compound **2.5dTpe***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7a** (1.21 g, 5.50 mmol, 1.5 equiv.), diisopropyl amine (5.17 mL, 36.7 mmol, 10 equiv.), diethyl ether (30 mL), bis(diisopropylamino)chlorophosphine (1.47 g, 5.50 mmol, 1.5 equiv.), **2.10** (2.00 g, 3.67 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.941 g, 5.50 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.5dTpe** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.82 g, 86%; white foam; TLC R_f = 0.4 and 0.5 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.78-0.83 (m, 3H), 0.99 (d, J = 6.7 Hz, 3H), 1.11-1.19 (m, 12H), 1.22-1.25 (m, 3H), 1.34 (d, J = 4.1 Hz, 4H), 1.58-1.62 (m, 1H), 1.68-1.81 (m, 2H), 1.99-2.05 (m, 1H), 2.22-2.33 (m, 1H), 2.42-2.53 (m, 1H), 2.74-2.81 (m, 4H), 3.25-3.33 (m, 1H), 3.40-3.45 (m, 1H), 3.48-3.58 (m, 2H), 3.74 (s, 6H), 3.84-3.91 (m, 1H), 4.17 (d, J = 4.2 Hz, 0.5H), 4.23 (d, J = 4.2 Hz, 0.5H), 4.58-4.67 (m, 1H), 6.35-6.40 (m, 1H), 6.76-6.80 (m, 4H), 7.16-7.26 (m, 7H), 7.34-7.37 (m, 2H), 7.57 (s, 0.5H), 7.61 (s, 0.5H), 8.98 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 14.4, 22.8, 24.88, 24.95, 25.3, 25.4, 26.6, 30.7, 30.9, 31.83, 31.89, 33.38, 33.49, 43.43, 43.55, 43.60, 53.76, 53.80, 55.5, 63.61, 63.69, 73.49, 73.61, 73.78, 84.9, 85.1, 86.07, 87.1, 111.2, 113.4, 127.2, 128.1, 128.30, 128.31, 130.3, 135.39, 135.48, 135.9, 144.38, 144.46, 150.4, 158.74, 158.76, 164.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1, 148.6; HRMS (ESI) *m/z* calcd for $C_{47}H_{64}N_3O_8PS_2Na$ $[M + Na]^+$ 916.3770, found 916.3755.

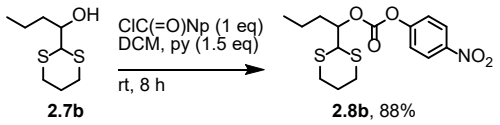


*Compound **2.13dTpe***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7a** (1.36 g, 6.19 mmol, 1.5 equiv.), diisopropyl amine (5.82 mL, 41.3 mmol, 10 equiv.), diethyl ether (30 mL), bis(diisopropylamino)chlorophosphine (414.73 mg, 6.19 mmol, 1.5 equiv.), **2.12** (2.00 g, 4.13 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 1.06 g, 6.19 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.13dTpe** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 2:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 3.27 g, 95%; white foam; TLC R_f = 0.5 and 0.6 (SiO_2, hexanes/EtOAc 2:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.80-0.85 (m, 3H), 1.01 (d, J = 7.2 Hz, 3H), 1.14-1.27 (m, 18H), 1.38 (dd, J = 3.9, 1.1 Hz, 3H), 1.61-1.64 (m, 1H), 1.78-1.87 (m, 1H), 2.03-2.08 (m, 1H), 2.24-2.35 (m, 1H), 2.52 (m, 1H), 2.75-2.85 (m, 4H), 3.31 (dd, J = 10.4, 2.8 Hz, 0.5H), 3.36 (dd, J = 10.5, 2.8 Hz, 0.5H), 3.41-3.47 (m, 1H), 3.48-3.61 (m, 2.5H), 3.76-3.94 (m, 1H), 4.10-4.12 (m, 0.5H), 4.18 (d, J = 4.3 Hz, 0.5H), 4.25 (d, J = 4.0 Hz, 0.5H), 4.61-4.70 (m, 1H), 6.33-6.41 (m, 1H), 7.21-7.31 (m, 9H), 7.37-7.41 (m, 6H), 7.55 (d, J = 1.2 Hz, 0.5H), 7.60 (d, J = 1.2 Hz, 0.5H), 8.64 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.03, 12.04, 14.4, 22.8, 22.9, 23.2, 23.5, 23.97, 23.99, 24.81, 24.89, 24.96, 25.02, 25.3, 25.5, 26.61, 26.64, 30.7, 30.9, 31.85, 31.90, 33.4, 33.5, 40.30, 40.37, 40.57, 40.61, 43.46, 43.51, 43.58, 43.63, 44.70, 44.79, 46.4, 53.78, 53.81, 63.87, 63.94, 73.46, 73.62, 73.73, 76.5, 76.6, 76.8, 84.9, 85.1, 85.57, 85.62, 86.01, 86.05, 87.7, 111.2, 127.5, 128.1, 128.84, 128.86, 135.8, 143.41, 143.47, 150.3, 163.82, 163.85; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.2, 148.6; HRMS (ESI) *m/z* calcd for $C_{45}H_{60}N_3O_6PS_2H$ $[M + H]^+$ 834.3739, found 834.3729.



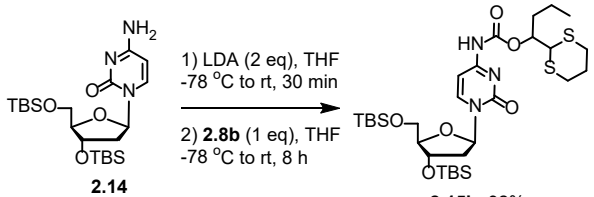
*1-(1,3-Dithian-2-yl)butan-1-ol (**2.7b**)*: Synthesized using the procedure for **2.7a**. 1,3-Dithiane (**2.6**, 7.5 g, 62.5 mmol, 1.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 25 mL, 62.5 mmol, 1.0 equiv.) and *n*-butyraldehyde (5.59 mL, 62.5 mmol, 1.0 equiv.) were used. Product **2.7b** was purified with flash column chromatography (SiO_2, hexanes/EtOAc

5:1): 7.01 g, 98%; colorless oil; TLC R_f = 0.45 (SiO_2, hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (td, J = 7.2, 1.4 Hz, 3H), 1.32-1.41 (m, 1H), 1.46-1.54 (m, 2H), 1.71-1.78 (m, 1H), 1.87-1.96 (m, 1H), 2.00-2.09 (m, 1H), 2.43 (t, J = 3.5 Hz, 1H), 2.68-2.77 (m, 2H), 2.86-2.93 (m, 2H), 3.80-3.85 (m, 1H), 3.88 (d, J = 6.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 19.3, 26.1, 28.5, 28.9, 36.5, 52.9, 72.3; HRMS (ESI) *m/z* calcd for $C_8H_{16}OS_2H$ $[M + H]^+$ 193.0721, found 193.0712.



2.7b **2.8b**, 88%

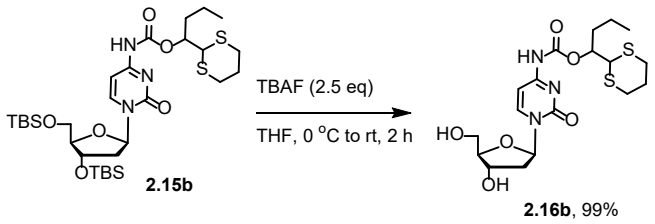
*1-(1,3-Dithian-2-yl)butyl (4-nitrophenyl) carbonate (**2.8b**)*: Synthesized using the procedure for **2.8a**. Compound **2.7b** (7.0 g, 36.4 mmol, 1.0 equiv.), DCM (100 mL), pyridine (4.39 mL, 54.6 mmol, 1.5 equiv.) and 4-nitrophenol chloroformate (7.34 g, 36.4 mmol, 1.0 equiv.) were used. Product **2.8b** was purified by precipitating from DCM by hexanes: 11.40 g, 88%; white foam; TLC R_f = 0.5 (SiO_2, hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (td, J = 7.4, 1.8 Hz, 3H), 1.37-1.55 (m, 2H), 1.75-1.83 (m, 1H), 1.93-2.00 (m, 2H), 2.03-2.10 (m, 1H), 2.70-2.78 (m, 2H), 2.90-3.00 (m, 2H), 4.03 (d, J = 6.9 Hz, 1H), 5.09-5.14 (m, 1H), 7.38 (dd, J = 9.3, 1.4 Hz, 2H), 8.25 (dd, J = 9.1, 1.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 19.0, 25.7, 28.4, 28.7, 34.2, 36.5, 42.9, 48.8, 79.9, 122.0, 125.4, 145.5, 152.5, 155.7; HRMS (ESI) *m/z* calcd for $C_{15}H_{19}NO_5S_2H$ $[M + H]^+$ 358.0783, found 358.0789.



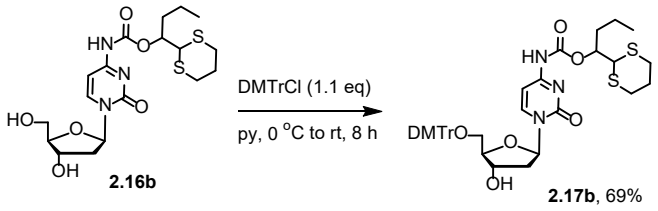
2.14 **2.15b**, 92%

*Compound **2.15b***: Synthesized using the procedure for **2.15a**. Diisopropylamine (4.51 mL, 32.02 mmol, 2.0 equiv.), THF (200 mL), *n*BuLi (2.5 M in hexanes, 12.8 mL, 32.02 mmol, 2.0 equiv.), **2.14** (7.28 g, 16.01 mmol, 1.0 equiv.), THF (50 mL) and **2.8b** (16.01 mmol, 1.0 equiv.) were used. Product **2.15b** was purified with flash column chromatography

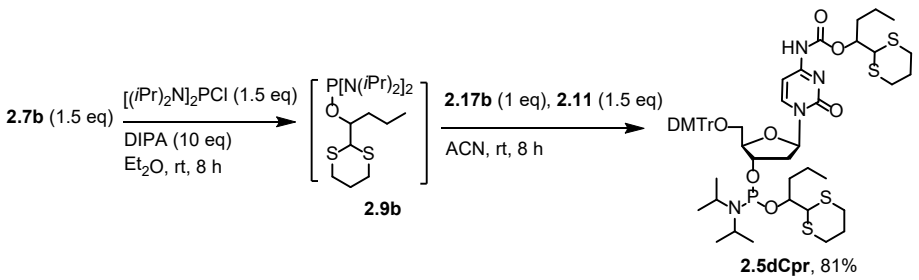
(SiO_2, hexanes/EtOAc 1:1): 9.93 g, 92%; white foam; TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.001 (s, 6H), 0.06 (dd, J = 2.1, 1.6 Hz, 6H), 0.82 (d, J = 1.8 Hz, 9H), 0.87 (d, J = 1.6 Hz, 9H), 1.17-1.21 (m, 3H), 1.26-1.39 (m, 2H), 1.64-1.74 (m, 1H), 1.78-1.89 (m, 2H), 2.03-2.10 (m, 2H), 2.40-2.49 (m, 1H), 2.67-2.75 (m, 2H), 2.82-2.91 (m, 2H), 3.72 (d, J = 11.1 Hz, 1H), 3.89 (d, J = 11.4 Hz, 2H), 4.02-4.08 (m, 3H), 4.30-4.34 (m, 1H), 5.06-5.11 (m, 1H), 6.17 (t, J = 5.4 Hz, 1H), 7.10 (d, J = 7.0 Hz, 1H), 7.73 (brs, 0.5H), 8.29 (d, J = 6.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.3, -4.7, -4.3, 14.0, 14.4, 18.1, 18.6, 18.9, 21.2, 25.9, 26.1, 28.8, 29.1, 34.0, 42.4, 49.6, 60.4, 61.9, 70.1, 70.2, 76.2, 86.7, 87.73, 87.77, 94.6, 144.2, 152.3, 154.8, 162.3, 170.9; HRMS (ESI) *m/z* calcd for $C_{30}H_{55}N_3O_6S_2Si_2H$ $[M + H]^+$ 674.3149, found 674.3143.



*Compound **2.16b***: Synthesized using the procedure for **2.16a**. Compound **2.15b** (13.8 g, 20.5 mmol, 1.0 equiv.), THF (150 mL) and TBAF (1 M in THF, 51.3 mL, 51.25 mmol, 2.5 equiv.) were used. Product **2.16b** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 10:1): 9.0 g, 99%; light yellow foam; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 10:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.88 (t, J = 7.3, 3H), 1.24-1.42 (m, 2H), 1.62-1.71 (m, 1H), 1.80-1.85 (m, 2H), 1.93-2.00 (m, 1H), 2.12-2.19 (m, 1H), 2.43-2.49 (m, 1H), 2.68-2.92 (m, 4H), 3.77 (dd, J = 12.1, 3.3 Hz, 2H), 3.97-4.00 (m, 1H), 4.09 (d, J = 6.4 Hz, 1H), 4.34-4.38 (m, 1H), 5.08-5.11 (m, 1H), 6.18 (t, J = 6.1 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 8.42 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 13.3, 13.6, 18.7, 25.9, 28.4, 28.7, 29.9, 34.0, 41.4, 49.3, 49.5, 60.5, 61.3, 70.4, 70.5, 75.6, 75.8, 87.3, 87.5, 88.1, 88.3, 95.5, 95.7, 144.8, 153.0, 156.3, 163.5, 171.6; HRMS (ESI) *m/z* calcd for $C_{18}H_{27}N_3O_6S_2H$ $[M + H]^+$ 446.1420, found 446.1410.

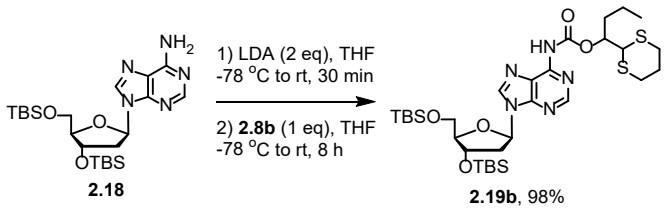


*Compound **2.17b***: Synthesized using the procedure for **2.17a**. Compound **2.16b** (12 g, 26.93 mmol, 1.0 equiv.), pyridine (50 mL) and DMTrCl (10.04 g, 29.62 mmol, 1.1 equiv.) were used. Product **2.17b** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 13.9 g, 69%; white foam; TLC R_f = 0.5 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (td, J = 7.4, 2.1 Hz, 3H), 1.32-1.44 (m, 2H), 1.65-1.76 (m, 4H), 2.18-2.23 (m, 1H), 2.62-2.69 (m, 1H), 2.69-2.78 (m, 2H), 2.84-2.93 (m, 2H), 3.37 (dd, J = 10.7, 3.4 Hz, 1H), 3.47-3.51 (m, 1H), 3.73-3.75 (m, 1H), 3.78 (s, 6H), 4.10 (d, J = 7.1 Hz, 1H), 4.44-4.47 (m, 1H), 5.09-5.12 (m, 1H), 6.22 (t, J = 5.8 Hz, 1H), 6.83 (d, J = 8.8 Hz, 4H), 7.18-7.28 (m, 7H), 7.37 (d, J = 7.6 Hz, 2H), 8.21 (d, J = 7.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.0, 26.0, 28.9, 29.1, 29.3, 31.2, 34.07, 34.13, 42.3, 49.7, 49.9, 55.5, 63.0, 70.99, 71.04, 76.47, 76.49, 86.7, 87.1, 87.4, 95.2, 113.5, 127.2, 128.1, 128.3, 135.57, 135.66, 144.3, 144.4, 152.2, 155.4, 158.7, 162.4; HRMS (ESI) *m/z* calcd for $C_{39}H_{45}N_3O_8S_2H$ $[M + H]^+$ 748.2726, found 748.2722.



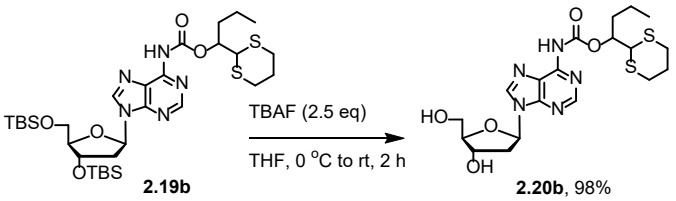
*Compound **2.5dCpr***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7b** (769.36 mg, 4.0 mmol, 1.5 equiv.), diisopropyl amine (3.76 mL, 26.7 mmol, 10 equiv.), diethyl ether (30mL); bis(diisopropylamino)chlorophosphine (1.06 g, 4.0 mmol, 1.5 equiv.), **2.17b** (2.00 g, 2.67 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 684 mg, 4.0 mmol, 1.5 equiv.) and ACN (60mL) were used. Product **2.5dCpr** was purified with flash chromatography by dissolving the sample in the solvent mixture of

hexanes/EtOAc 2:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.29 g, 81%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 2:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69-0.83 (m, 6H), 1.04-1.15 (m, 12H), 1.20-1.36 (m, 4H), 1.56-1.64 (m, 2H), 1.69-1.77 (m, 2H), 1.89-1.97 (m, 2H), 2.14-2.26 (m, 1H), 2.58-2.71 (m, 8H), 2.74-2.84 (m, 2H), 3.28 (td, J = 13.5, 3.2 Hz, 1H), 3.36-3.53 (m, 4H), 3.67 (s, 6H), 3.94 (t, J = 6.6 Hz, 1H), 4.03-4.15 (m, 2H), 4.45-4.57 (m, 1H), 4.98-5.04 (m, 1H), 6.12-6.18 (m, 1H), 6.73 (t, J = 7.8 Hz, 4.5H), 7.09-7.22 (m, 7H), 7.29 (t, J = 6.2 Hz, 2.5H), 8.14 (t, J = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 14.0, 14.19, 14.23, 14.3, 18.5, 18.91, 19.1, 22.9, 23.1, 23.3, 23.9, 24.7, 24.8, 24.9, 25.0, 25.9, 26.2, 26.6, 28.8, 28.9, 29.1, 29.2, 29.5, 30.6, 30.9, 34.0, 34.1, 35.55, 35.59, 35.64, 41.2, 41.6, 43.4, 43.5, 44.65, 44.70, 45.2, 45.3, 46.3, 49.6, 49.7, 51.4, 53.73, 53.76, 53.39, 55.4, 62.5, 71.4, 71.6, 71.9, 76.1, 76.2, 76.23, 76.4, 85.6, 85.7, 86.1, 86.2, 87.0, 87.2, 95.1, 95.2, 113.4, 127.1, 128.0, 128.3, 130.1, 130.2, 135.3, 135.5, 144.1, 144.2, 144.3, 155.5, 158.6, 158.7, 162.2, 162.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1; MS (ESI) *m/z* calcd for $C_{53}H_{73}N_4O_9PS_4Na$ $[M + Na]^+$ 1091.39, found 1091.58.



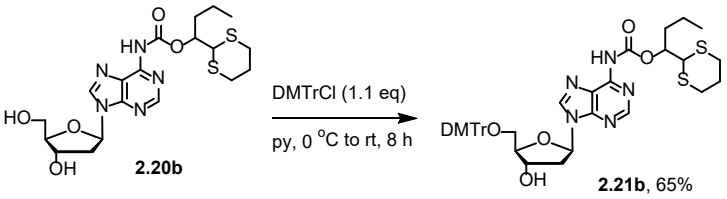
*Compound **2.19b***: Synthesized using the procedure for **2.15a**. Diisopropylamine (4.65 mL, 33.05 mmol, 2.0 equiv.), THF (200 mL), *n*BuLi (2.5 M in hexanes, 13.2 mL, 33.05 mmol, 2.0 equiv.), **2.18** (7.91 g, 16.52 mmol, 1.0 equiv.), THF (50 mL) and **2.8b** (16.52 mmol, 1.0 equiv.) were used. Product **2.19b** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:2): 7.71 g, 98%; light yellow foam; TLC R_f = 0.47 (SiO_2, hexanes/EtOAc 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (d, J = 5.2 Hz, 12H), 0.89 (d, J = 1.4 Hz, 18H), 0.92 (t, J = 7.3 Hz, 3H), 1.35-1.46 (m, 2H), 1.74-1.76 (m, 2H), 1.85-1.93 (m, 2H), 2.41-2.47 (m, 1H), 2.58-2.66 (m, 1H), 2.71-2.79 (m, 2H), 2.86-2.99 (m, 2H), 3.75 (dd, J = 11.4, 3.1 Hz, 1H), 3.86 (dd, J = 10.9, 3.9 Hz, 1H), 4.00-4.01 (m, 1H), 4.15 (d, J = 6.2 Hz, 1H), 4.57-4.61 (m, 1H), 5.21-5.25 (m, 1H), 6.47 (t, J = 6.4 Hz, 1H), 8.29 (d, J = 2.5

Hz, 1H), 8.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.12, -5.05, -4.5, -4.3, 14.06, 14.08, 18.3, 18.7, 19.0, 19.1, 26.0, 26.2, 29.1, 29.5, 40.0, 41.4, 41.5, 50.1, 63.0, 72.1, 75.69, 75.64, 84.9, 88.2, 122.5, 141.6, 149.6, 150.8, 150.9, 152.8; HRMS (ESI) *m/z* calcd for $C_{31}H_{55}N_5O_5S_2Si_2H$ $[M + H]^+$ 698.3261, found 698.3258.



2.19b **2.20b**, 98%

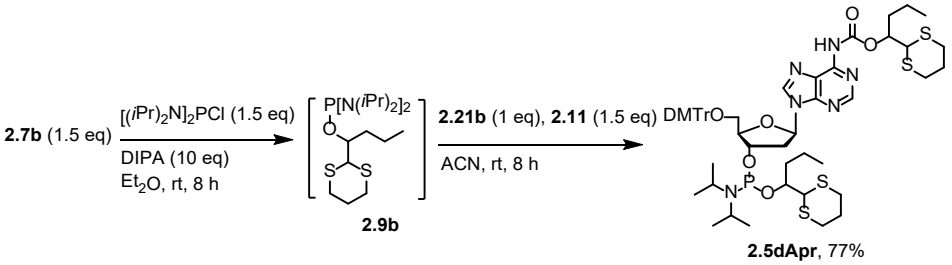
*Compound **2.20b***: Synthesized using the procedure for **2.16a**. Compound **2.19b** (7.71 g, 11.05 mmol, 1.0 equiv.), THF (50 mL) and TBAF (1 M in THF, 27.62 mL, 27.62 mmol, 2.5 equiv.) were used. Product **2.20b** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 10:1): 5.08 g, 98%; pale yellow foam; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88-0.89 (m, 3H), 1.44-1.51 (m, 1H), 1.71-1.79 (m, 1H), 1.84-1.93 (m, 2H), 2.02-2.08 (m, 1H), 2.50-2.56 (m, 1H), 2.70-2.77 (m, 3H), 2.86-2.97 (m, 2H), 3.84-3.90 (m, 2H), 4.06-4.08 (m, 1H), 4.15 (d, J = 6.1 Hz, 1H), 4.71-4.72 (m, 1H), 5.19-5.22 (m, 1H), 6.50 (t, J = 6.1 Hz, 1H), 8.41 (s, 1H), 8.67 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.1, 26.1, 29.1, 29.4, 34.1, 41.0, 50.1, 62.5, 70.8, 71.0, 75.8, 77.5, 85.9, 89.0, 122.9, 142.6, 150.4, 152.3; HRMS (ESI) *m/z* calcd for $C_{19}H_{27}N_5O_5S_2H$ $[M + H]^+$ 470.1532, found 470.1534.



2.20b **2.21b**, 65%

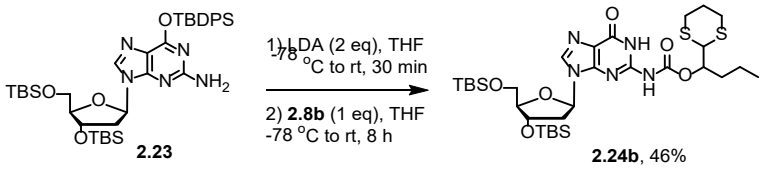
*Compound **2.21b***: Synthesized using the procedure for **2.17a**. Compound **2.20b** (8.71g, 18.65 mmol, 1.0 equiv.), pyridine (40 mL) and DMTrCl (6.95 g, 20.51 mmol, 1.1 equiv.) were used. Product **2.21b** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 9.30 g, 65%; white foam; TLC R_f = 0.62 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (td, J = 7.3, 3.1 Hz,

3H), 1.33-1.46 (m, 2H), 1.71-1.79 (m, 1H), 1.82-1.91 (m, 2H), 2.52-2.58 (m, 1H), 2.68-2.77 (m, 2H), 2.79-2.87 (m, 2H), 2.90-2.99 (m, 2H), 3.38 (d, J = 4.6 Hz, 2H), 3.73 (s, 6H), 4.13-4.18 (m, 2H), 4.67-4.70 (m, 1H), 5.20-5.24 (m, 1H), 6.45 (td, J = 6.4, 1.6 Hz, 1H), 6.75 (d, J = 8.9 Hz, 4H), 7.13-7.25 (m, 7H), 7.34 (dd, J = 8.0, 1.2 Hz, 2H), 8.09 (s, 1H), 8.66 (d, J = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.0, 19.1, 26.0, 29.1, 29.5, 34.0, 40.6, 50.2, 55.4, 64.0, 72.4, 75.8, 85.1, 86.7, 113.3, 122.32, 122.34, 127.0, 127.9, 128.2, 130.1, 135.7, 141.57, 141.61, 144.6, 149.6, 150.8, 152.7, 158.6; HRMS (ESI) *m/z* calcd for $C_{40}H_{45}N_5O_7S_2H$ $[M + H]^+$ 772.2839, found 772.2842.

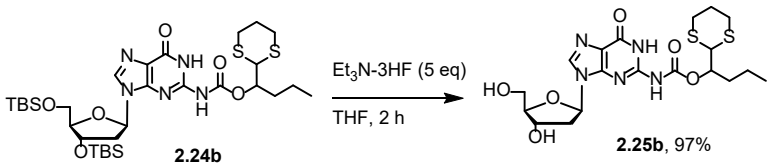


*Compound **2.5dApr***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7b** (750.12 mg, 3.9 mmol, 1.5 equiv.), diisopropyl amine (3.66 mL, 26 mmol, 10 equiv.), diethyl ether (30 mL), bis(diisopropylamino)chlorophosphine (1.04 g, 3.9 mmol, 1.5 equiv.), **2.21b** (2.00 g, 2.6 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 666.9 mg, 3.9 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.5dApr** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.18 g, 77%; white foam; TLC R_f = 0.5 and 0.6 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78-0.87 (m, 6H), 1.09-1.20 (m, 12H), 1.25-1.44 (m, 4H), 1.56-1.83 (m, 6H), 1.90-2.00 (m, 2H), 2.53-2.79 (m, 8H), 2.81-2.90 (m, 2H), 3.42-3.58 (m, 3H), 3.65 (s, 6H), 3.81-3.90 (m, 1H), 4.05-4.10 (m, 1H), 4.19 (dd, J = 16.2, 4.1 Hz, 1.5H), 4.29-4.30 (m, 0.5H), 4.64-4.74 (m, 1H), 5.12-5.17 (m, 1H), 6.39 (t, J = 7.3 Hz, 1H), 6.66-6.68 (m, 4H), 7.07-7.19 (m, 7H), 7.29 (d, J = 6.1 Hz, 2H), 8.15 (d, J = 2.6 Hz, 1H), 8.62 (d, J = 3.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.1, 14.2, 14.4, 18.9, 19.06, 19.11, 22.9, 23.1, 23.4, 23.9, 24.0, 24.9, 26.1, 26.6, 29.1, 29.2, 29.3, 29.5, 29.6, 30.0, 30.6, 30.7, 30.9, 31.0, 33.9, 35.6, 35.7, 43.4, 43.6,

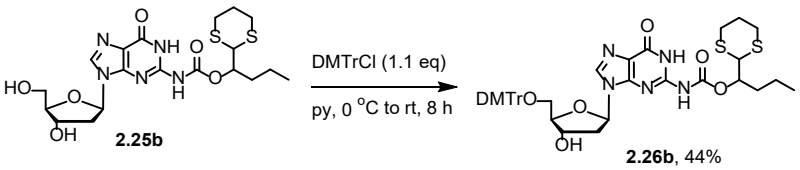
44.67, 44.72, 45.2, 45.3, 46.4, 50.15, 50.23, 53.78, 53.82, 53.9, 55.4, 63.77, 63.80, 75.6, 76.2, 76.3, 76.4, 85.1, 85.2, 86.2, 86.6, 113.2, 122.6, 126.9, 127.9, 128.2, 130.1, 135.6, 135.7, 141.7, 141.8, 144.60, 144.64, 149.7, 150.9, 151.0, 152.8, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.2, 148.5 ppm; MS (ESI) *m/z* calcd for $C_{54}H_{73}N_6O_8PS_4Na$ $[M + Na]^+$ 1115.40, found 1115.58.



*Compound **2.24b***: Synthesized using the procedure for **2.24a**. Diisopropylamine (0.76 mL, 5.44 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 2.17 mL, 5.44 mmol, 2.0 equiv.), **2.23** (2.0 g, 2.72 mmol, 1.0 equiv.), THF (mL) and **2.8b** (972.2 mg, 2.72 mmol, 1.0 equiv.) were used. Product **2.24b** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:3): 2.73 g, 46%; light yellow foam; TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (d, J = 3.9 Hz, 12H), 0.86 (d, J = 2.2 Hz, 18H), 0.89-0.92 (m, 3H), 1.28-1.42 (m, 2H), 1.63-1.72 (m, 1H), 1.85-1.93 (m, 2H), 1.99-2.04 (m, 1H), 2.33-2.40 (m, 2H), 2.65-2.75 (m, 2H), 2.84-2.92 (m, 2H), 3.72-3.74 (m, 2H), 3.91-3.96 (m, 2H), 4.50-4.52 (m, 1H), 5.14-5.21 (m, 1H), 6.23 (dt, J = 9.9, 6.5 Hz, 1H), 7.03 (d, J = 8.0 Hz, 0.5H), 7.97 (d, J = 2.9 Hz, 1H), 8.05 (d, J = 8.0 Hz, 0.5H), 8.19 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.2, -5.1, -4.5, -4.4, 14.0, 18.2, 18.7, 18.8, 18.9, 25.7, 25.9, 26.2, 28.4, 28.6, 28.8, 28.82, 34.1, 41.9, 42.1, 48.9, 49.2, 63.0, 63.1, 72.0, 72.1, 76.8, 84.2, 88.1, 88.2, 116.2, 120.8, 126.1, 137.1, 140.2, 147.0, 148.20, 148.22, 153.6, 155.8, 164.4; HRMS (ESI) *m/z* calcd for $C_{31}H_{55}N_5O_6S_2Si_2Na$ $[M + Na]^+$ 736.3030, found 736.3027.

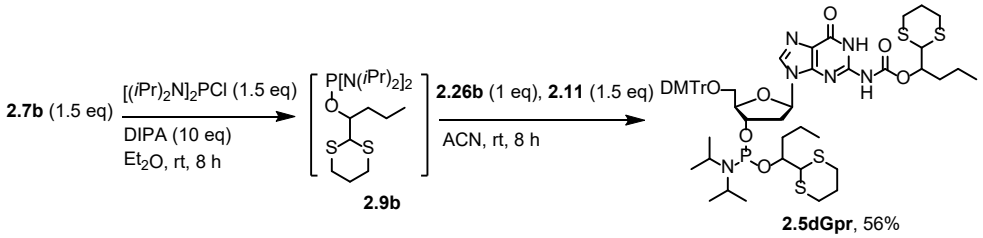


*Compound **2.25b***: To the solution of **2.24b** (2.4 g, 2.52 mmol, 1.0 equiv.) in THF (50 mL) was added triethylamine trihydrogen fluoride (2.05 mL, 12.6 mmol, 5.0 equiv.) at rt. After stirring for 2 h, the reaction was quenched with trimethylmethoxy silane (1.74 mL, 12.6 mmol, 5.0 equiv.). Volatiles were evaporated under reduced pressure. Product **2.25b** was purified with flash chromatography by dissolving the crude product in the solvent mixture of DCM/MeOH 10:1, loading onto a column (SiO_2) and eluting with the same solvent mixture: 1.18 g, 97%; pale yellow foam; TLC R_f = 0.3 (SiO_2, DCM/MeOH 10:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.97 (t, J = 7.4 Hz, 3H), 1.38-1.45 (m, 2H), 1.71-1.79 (m, 1H), 1.89-1.95 (m, 2H), 1.97-2.04 (m, 1H), 2.38-2.43 (m, 1H), 2.62-2.68 (m, 1H), 2.72-2.83 (m, 2H), 2.88-2.97 (m, 2H), 3.69-3.78 (m, 2H), 3.95-3.97 (m, 1H), 4.13 (d, J = 6.6 Hz, 1H), 4.50-4.53 (m, 1H), 5.21-5.26 (m, 1H), 6.33 (t, J = 6.7 Hz, 1H), 8.16-8.18 (m, 0.5H), 8.19 (d, J = 1.0 Hz, 0.5H); ^{13}C NMR (100 MHz, CD_3OD) δ 13.2, 18.6, 25.9, 28.2, 28.5, 29.8, 34.0, 40.5, 49.1, 62.1, 71.3, 76.6, 84.4, 88.2, 119.7, 138.39, 138.40, 148.1, 149.3, 155.1, 156.4; HRMS (ESI) *m/z* calcd for $C_{19}H_{27}N_5O_6S_2H$ $[M + H]^+$ 486.1481, found 486.1474.



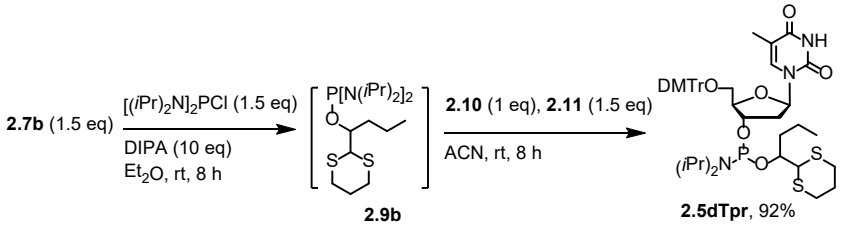
*Compound **2.26b***: Synthesized using the procedure for **2.17a**. Compound **2.25b** (3.09 g, 6.38 mmol, 1.0 equiv.), pyridine (50 mL) and DMTrCl (2.37 g, 7.02 mmol, 1.1 equiv.) were used. Product **2.26b** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 20:1 with 5% Et_3N): 2.20 g, 44%; pale yellow foam; TLC R_f = 0.4 (SiO_2, EtOAc/MeOH 20:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (td, J = 7.3, 2.5 Hz, 3H), 1.30-1.35 (m, 2H), 1.66-1.70 (m, 1H), 1.85-1.92 (m, 2H), 2.01 (s, 1H), 2.44-2.51 (m, 1H), 2.83-2.95 (m, 3H), 3.25-3.34 (m, 2H), 3.71 (s, 6H), 3.99 (dd, J = 6.8, 2.4 Hz, 1H), 4.13 (m, 1H), 4.67-4.71 (m, 1H), 5.16-5.19 (m, 1H), 6.22 (td, J = 6.5, 1.7 Hz, 1H), 6.72 (d, J = 8.8 Hz, 4H), 7.12-7.25 (m, 7H), 7.34 (dd, J = 5.3, 1.7 Hz, 2H), 7.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 19.1, 25.9, 28.4, 28.6, 28.7, 28.8, 34.2, 40.7, 40.8, 49.2, 49.4, 55.5, 64.5, 72.0, 76.1, 84.4, 84.5, 86.48, 86.50, 86.7, 113.2, 120.6, 120.7, 127.0, 127.9,

128.3, 130.1, 130.2, 135.9, 137.1, 137.2, 144.7, 148.8, 149.10, 149.14, 155.35, 155.4, 156.4, 158.52, 158.54; HRMS (ESI) *m/z* calcd for $C_{40}H_{45}N_5O_8S_2H$ $[M + H]^+$ 788.2788, found 788.2800.

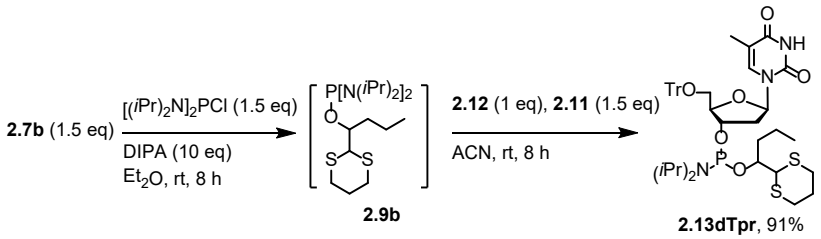


*Compound **2.5dGpr***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7b** (432.7 mg, 2.25 mmol, 1.5 equiv.), diisopropyl amine (2.11 mL, 15.0 mmol, 10.0 equiv.), diethyl ether (20 mL), bis(diisopropylamino)chlorophosphine (600.75 mg, 2.25 mmol, 1.5 equiv.), **2.26b** (1.17 g, 1.5 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 384.75 mg, 2.25 mmol, 1.5 equiv.) and ACN (40 mL) were used. Product **2.5dGpr** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/DCM 1:2 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: 924.5 mg, 56%; white foam; mixture of diastereomers; TLC R_f = 0.2 and 0.3 (SiO_2, hexanes/DCM 1:2 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.76-0.83 (m, 6H), 1.09-1.18 (m, 12H), 1.23-1.33 (m, 4H), 1.50-1.58 (m, 2H), 1.67-1.72 (m, 2H), 1.75-1.82 (m, 2H), 1.91-1.98 (m, 2H), 2.53-2.63 (m, 4H), 2.67-2.78 (m, 8H), 3.16-3.25 (m, 2H), 3.38-3.54 (m, 4H), 3.64 (s, 6H), 3.73-3.76 (m, 1H), 3.79-3.87 (m, 2H), 4.09-4.22 (m, 2H), 4.51-4.62 (m, 1H), 5.01-5.10 (m, 1H), 6.13-6.16 (m, 1H), 6.66-6.68 (m, 4H), 7.06-7.13 (m, 3H), 7.18-7.24 (m, 4H), 7.28-7.34 (m, 2H), 7.71 (d, J = 6.8 Hz, 1H), 7.81 (d, J = 5.8 Hz, 0.5H), 8.04 (d, J = 6.9 Hz, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.2, 14.39, 14.4, 18.5, 18.9, 19.0, 19.1, 19.2, 19.3, 22.9, 23.1, 23.4, 23.5, 23.9, 23.93, 24.8, 24.9, 25.0, 25.7, 26.1, 26.6, 26.7, 28.2, 28.3, 28.7, 29.0, 29.9, 30.6, 30.7, 30.9, 31.0, 34.1, 34.7, 35.2, 35.6, 35.9, 36.5, 43.3, 43.4, 43.5, 43.6, 44.7, 45.2, 45.3, 48.9, 49.0, 51.5, 53.2, 53.8, 53.9, 54.0, 55.4, 63.8, 63.9, 72.4, 76.3, 76.5, 76.6, 84.4, 84.7, 86.4, 86.5, 94.6, 113.2, 121.25, 121.27, 121.5, 125.6, 125.7, 127.0, 127.9, 128.2, 130.1, 130.13, 131.7, 135.7, 135.8, 137.1, 137.4, 137.5, 144.6, 146.9, 147.0, 147.1, 148.5, 153.6, 153.7, 155.9, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$)

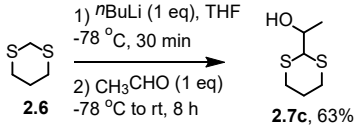
δ 149.2, 148.2 ppm; MS (ESI) *m/z* calcd for $C_{54}H_{73}N_6O_9PS_4Na$ $[M + Na]^+$ 1131.40, found 1131.50.



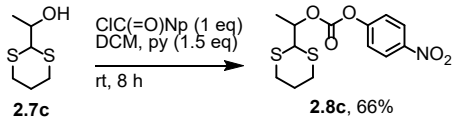
*Compound **2.5dTpr***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7b** (1.06 g, 5.50 mmol, 1.5 equiv.), diisopropyl amine (5.17 mL, 36.7 mmol, 10 equiv.), diethyl ether (30 mL), bis(diisopropylamino)chlorophosphine (1.47 g, 5.50 mmol, 1.5 equiv.), **2.10** (2.00 g, 3.67 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.941 g, 5.50 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.5dTpr** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.92 g, 92%; white foam; TLC R_f = 0.4 and 0.5 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 0.78 (t, J = 7.5 Hz, 1H), 0.85 (t, J = 7.3 Hz, 2H), 1.09-1.22 (m, 12H), 1.33 (d, J = 5.6 Hz, 3H), 1.38-1.46 (m, 1H), 1.50-1.79 (m, 4H), 1.94-2.00 (m, 1H), 2.20-2.31 (m, 1H), 2.39-2.51 (m, 1H), 2.68-2.78 (m, 4H), 3.23-3.32 (m, 1H), 3.38-3.44 (m, 1H), 3.49-3.56 (m, 2H), 3.77 (s, 6H), 3.73-3.81 (m, 1H), 3.85-3.91 (m, 0.5H), 4.05-4.09 (m, 0.5H), 4.14 (d, J = 4.2 Hz, 0.5H), 4.20 (d, J = 3.9 Hz, 0.5H), 4.56-4.65 (m, 1H), 6.33-6.40 (m, 1H), 6.76 (dd, J = 8.0, 4.0 Hz, 4H), 7.19-7.23 (m, 2H), 7.25-7.27 (m, 5H), 7.34 (dd, J = 7.7, 3.0 Hz, 2H), 7.58 (s, 0.5H), 7.62 (s, 0.5H), 9.9 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 14.3, 14.4, 18.9, 19.2, 19.3, 24.9, 25.0, 25.1, 26.1, 26.7, 28.8, 30.7, 30.9, 31.1, 35.7, 35.8, 36.6, 40.3, 43.3, 43.5, 43.6, 53.9, 55.5, 63.7, 63.8, 73.7, 73.9, 85.0, 85.1, 85.7, 86.1, 87.1, 92.2, 111.2, 113.4, 127.3, 128.1, 128.31, 128.35, 130.3, 135.4, 135.5, 135.9, 144.4, 150.2, 158.79, 158.80, 163.6; ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.4, 148.5; MS (ESI) *m/z* calcd for $C_{45}H_{60}N_3O_8PS_2Na$ $[M + Na]^+$ 888.35, found 888.50.



*Compound **2.13dTpr***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7b** (1.19 g, 6.19 mmol, 1.5 equiv.), diisopropyl amine (5.82 mL, 41.3 mmol, 10 equiv.), diethyl ether (30 mL), bis(diisopropylamino)chlorophosphine (414.73 mg, 6.19 mmol, 1.5 equiv.), **2.12** (2.00 g, 4.13 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 1.06 g, 6.19 mmol, 1.5 equiv.) and ACN (60 mL) were used. Product **2.5dTpr** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 2:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 3.02 g, 91%; white foam; TLC R_f = 0.2 and 0.3 (SiO_2, hexanes/EtOAc 2:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (t, J = 7.4 Hz, 1H), 0.90 (dt, J = 10.0, 7.4 Hz, 3H), 1.18-1.26 (m, 12H), 1.37 (dd, J = 7.1, 1.1 Hz, 3H), 1.41-1.49 (m, 1H), 1.58-1.73 (m, 2H), 1.76-1.84 (m, 2H), 2.00-2.10 (m, 1H), 2.24-2.35 (m, 1H), 2.75-2.84 (m, 4H), 2.87-2.96 (m, 1H), 3.32 (td, J = 7.3, 2.9 Hz, 1H), 3.38 (dd, J = 16.8, 2.5 Hz, 1H), 3.44-3.62 (m, 4H), 3.77-3.95 (m, 1H), 4.18 (d, J = 4.3 Hz, 0.5H), 4.24 (d, J = 4.1 Hz, 0.5H), 4.60-4.68 (m, 1H), 6.35-6.40 (m, 1H), 7.20-7.31 (m, 9H), 7.33-7.41 (m, 6H), 7.55 (d, J = 1.2 Hz, 0.5H), 7.59 (d, J = 1.2 Hz, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 14.2, 14.3, 18.5, 18.9, 19.1, 22.9, 23.2, 23.4, 23.9, 24.0, 24.8, 24.9, 25.0, 26.2, 26.6, 29.3, 29.5, 30.7, 30.9, 35.6, 35.8, 36.0, 40.3, 40.6, 43.5, 43.6, 44.8, 45.3, 51.4, 53.8, 53.9, 63.9, 64.0, 73.6, 73.7, 76.2, 76.3, 76.5, 84.9, 85.1, 85.5, 86.0, 87.6, 111.2, 111.3, 127.5, 128.1, 128.81, 128.84, 135.8, 143.4, 143.5, 150.4, 150.5, 164.08, 164.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.3, 148.5; MS (ESI) *m/z* calcd for $C_{43}H_{56}N_3O_6PS_2Na$ $[M + Na]^+$ 828.32, found 828.58.

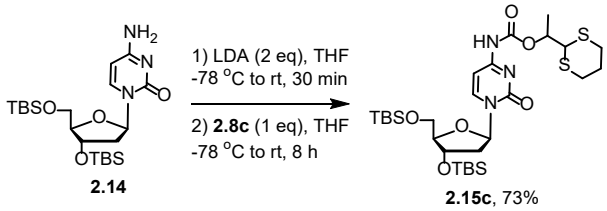


*1-(1,3-Dithian-2-yl)ethan-1-ol (**2.7c**)*: Synthesized using the procedure for **2.7a**. 1,3-Dithiane (**2.6**, 7.51 g, 62.5 mmol, 1.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 25 mL, 62.5 mmol, 1.0 equiv.) and acetaldehyde (2.7 mL, 62.5 mmol, 1.0 equiv.) were used. was added to the reaction mixture. Product **2.7c** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 4:1): 6.29 g, 63%; colorless oil; TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (dd, J = 5.2, 1.0 Hz, 3H), 1.73-1.96 (m, 2H), 2.55-2.63 (m, 2H), 2.73-2.81 (m, 2H), 2.87 (d, J = 3.4 Hz, 1H), 3.70 (d, J = 6.8 Hz, 1H), 3.88-3.92 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 25.9, 28.2, 28.5, 53.7, 68.6; HRMS (ESI) *m/z* calcd for $C_6H_{12}OS_2Na$ $[M + Na]^+$ 187.0227, found 187.0218.



2.7c **2.8c**, 66%

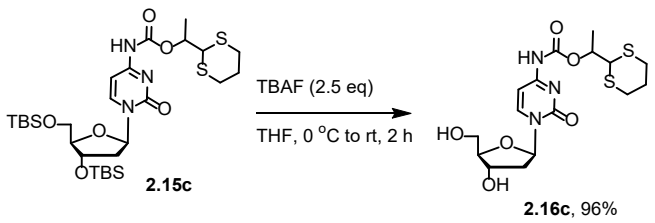
*1-(1,3-Dithian-2-yl)ethyl (4-nitrophenyl) carbonate (**2.8c**)*: Synthesized using the procedure for **2.8a**. Compound **2.7c** (4.51 g, mmol, 1.0 equiv.), DCM (75 mL), pyridine (3.32 mL, 41.25 mmol, 1.5 equiv.) and 4-nitrophenol chloroformate (5.54 g, 27.5 mmol, 1.0 equiv.) were used. Product **2.8c** was purified by precipitating from DCM with hexanes: 4.31 g, 66%; pale white powder; TLC R_f = 0.5 (SiO_2, hexanes/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (dd, J = 5.6, 0.8 Hz, 3H), 1.84-2.04 (m, 2H), 2.65-2.73 (m, 2H), 2.83-2.93 (m, 2H), 3.98 (d, J = 6.8 Hz, 1H), 5.08-5.15 (m, 1H), 7.31 (d, J = 9.4 Hz, 2H), 8.17 (d, J = 9.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3, 25.7, 28.4, 28.8, 49.9, 76.8, 122.0, 125.4, 145.4, 152.0, 155.6; HRMS (ESI) *m/z* calcd for $C_{13}H_{15}NO_5S_2H$ $[M + H]^+$ 330.0470, found 330.0467.



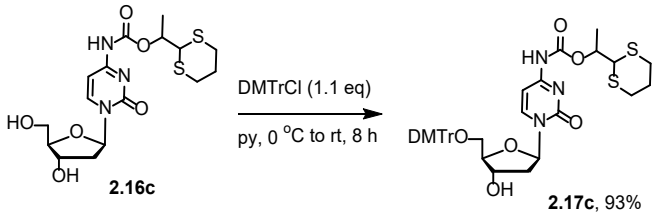
2.14 **2.15c**, 73%

*Compound **2.15c***: Synthesized using the procedure for **2.15a**. Diisopropylamine (1.62 mL, 11.5 mmol, 2.0 equiv.), THF (50 mL), *n*BuLi (2.5 M in hexanes, 4.39 mL, 10.98 mmol,

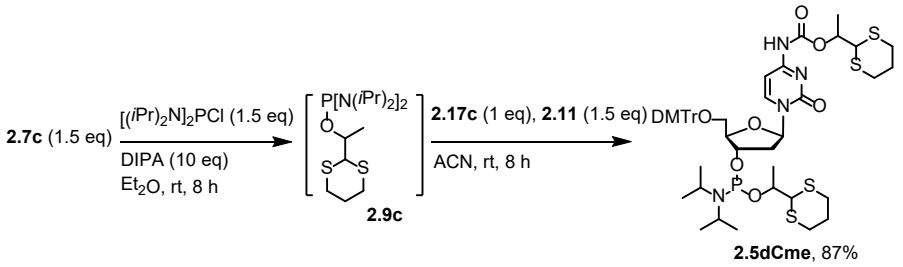
2.0 equiv.), **2.14** (2.5 g, 5.49 mmol, 1.0 equiv.), THF (50 mL) and **2.8c** (1.80 g, 5.49 mmol, 1.0 equiv.) were used. Product **2.15c** were purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:1): 2.60 g, 73%; white foam; TLC R_f = 0.5 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (d, J = 1.5 Hz, 6H), 0.08 (d, J = 4.2 Hz, 6H), 0.84 (s, 9H), 0.89 (s, 9H), 1.44 (d, J = 8.0 Hz, 3H), 1.86-1.95 (m, 1.5H), 2.01-2.13 (m, 2.5H), 2.69-2.78 (m, 2H), 2.84-2.93 (m, 2H), 3.74 (dd, J = 9.5, 2.9 Hz, 1H), 3.90-3.93 (m, 2H), 4.05 (d, J = 6.2 Hz, 1H), 4.32-4.37 (m, 1H), 5.15-5.22 (m, 1H), 6.18-6.21 (m, 1H), 7.12 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.25, -5.30, -4.7, -4.4, 18.1, 18.6, 25.8, 25.9, 26.1, 28.8, 29.2, 42.5, 50.6, 61.9, 62.0, 70.1, 70.2, 73.6, 86.9, 87.0, 87.9, 88.0, 94.6, 94.61, 116.1, 126.2, 144.6, 151.9, 155.2, 162.2; HRMS (ESI) *m/z* calcd for $C_{28}H_{51}N_3O_6S_2Si_2H$ $[M + H]^+$ 646.2836, found 646.2817.



*Compound **2.16c***: Synthesized using the procedure for **2.16a**. Compound **2.15c** (2.4 g, 3.72 mmol, 1.0 equiv.), THF (50 mL) and TBAF (1 M in THF, 9.3 mL, 9.3 mmol, 2.5 equiv.) were used. Product **2.16c** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 19:1): 1.5 g, 96%; white foam; TLC R_f = 0.4 (SiO_2, EtOAc/MeOH 19:1); ^{1}H NMR (400 MHz, CD_3OD) δ 1.43 (dd, J = 6.4, 0.6 Hz, 3H), 1.81-1.91 (m, 1H), 2.01-2.08 (m, 1H), 2.12-2.19 (m, 1H), 2.44-2.50 (m, 1H), 2.74-2.84 (m, 2H), 2.78-2.97 (m, 2H), 3.72 (dd, J = 12.1, 3.8 Hz, 1H), 3.81 (dd, J = 12.1, 3.8 Hz, 1H), 3.96-3.99 (m, 1H), 4.15 (dd, J = 6.3, 2.0 Hz, 1H), 4.35 (dt, J = 6.2, 3.9 Hz, 1H), 5.15-5.22 (m, 1H), 6.19 (t, J = 6.2 Hz, 1H), 7.26 (d, J = 7.6 Hz, 1H), 8.42 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 17.2, 25.8, 28.1, 28.5, 41.3, 50.3, 53.3, 61.3, 70.4, 73.00, 73.02, 87.4, 88.2, 95.5, 144.7, 152.8, 156.5, 163.6; HRMS (ESI) *m/z* calcd for $C_{16}H_{23}N_3O_6S_2H$ $[M + H]^+$ 418.1107, found 418.1107.

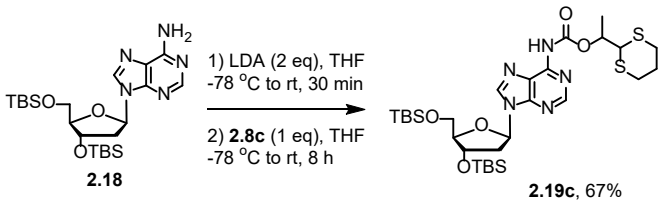


*Compound **2.17c***: Synthesized using the procedure for **2.17a**. Compound **2.16c** (1.5 g, 3.5 mmol, 1.0 equiv.), pyridine (30 mL) and DMTrCl (1.54 g, 3.9 mmol, 1.1 equiv.) were used. Product **2.17c** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 19:1 with 5% Et_3N): 2.3 g, 93%; white foam; TLC R_f = 0.5 (SiO_2, EtOAc/MeOH 19:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (dd, J = 6.4, 1.0 Hz, 3H), 1.84-1.91 (m, 1.5H), 2.03-2.08 (m, 1H), 2.16-2.24 (m, 1.5H), 2.67-2.79 (m, 2H), 2.83-2.91 (m, 2H), 3.37 (dd, J = 10.8, 3.8 Hz, 1H), 3.46 (dd, J = 11.0, 2.6 Hz, 1H), 3.77 (s, 6H), 4.02-4.10 (m, 2H), 4.12-4.15 (m, 1H), 4.46-4.50 (m, 1H), 5.12-5.20 (m, 1H), 6.24 (t, J = 5.8 Hz, 1H), 6.82 (d, J = 8.8, 4H), 6.93-6.98 (m, 1H), 7.00-7.28 (m, 7H), 7.37 (d, J = 8.0 Hz, 2H), 7.76 (brs, 1H), 8.21 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 18.2, 25.8, 28.8, 28.9, 29.1, 29.2, 31.1, 42.2, 50.6, 50.7, 55.4, 55.5, 62.9, 71.0, 73.4, 86.6, 87.1, 87.4, 95.0, 113.5, 127.3, 128.2, 128.4, 130.2, 135.6, 135.7, 144.4, 144.5, 158.8, 162.4; HRMS (ESI) *m/z* calcd for $C_{37}H_{41}N_3O_8S_2H$ $[M + H]^+$ 720.2413, found 720.2403.

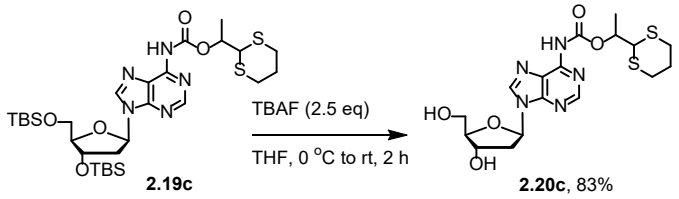


*Compound **2.5dCme***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7c** (0.64 g, 3.9 mmol, 1.5 equiv.), diisopropyl amine (3.6 mL, 26.2 mmol, 10 equiv.), diethyl ether (15 mL), bis(diisopropylamino)chlorophosphine (1.04 g, 3.9 mmol, 1.5 equiv.), **2.17c** (1.89 g, 2.62 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.67 g, 3.9 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.5dCme** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:2

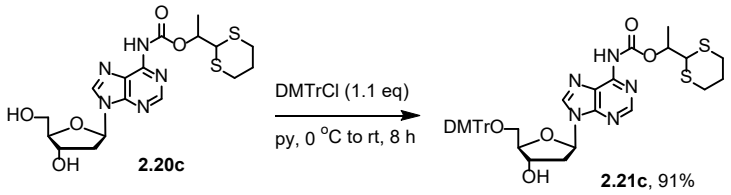
with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.28 g, 87%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 1:2 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99-1.05 (m, 3H), 1.10-1.25 (m, 12H), 1.35-1.37 (m, 1H), 1.43-1.46 (m, 3H), 1.78-1.96 (m, 3H), 2.18-2.36 (m, 2H), 2.70-2.92 (m, 10H), 3.35-3.47 (m, 2H), 3.50-3.65 (m, 3H), 3.78 (s, 6H), 3.81-3.85 (m, 1H), 4.03-4.05 (m, 1H), 4.14-4.19 (m, 1H), 4.55-4.64 (m, 1H), 5.13-5.20 (m, 1H), 6.21-6.25 (m, 1H), 6.80-6.84 (m, 4H), 7.19-7.31 (m, 7H), 7.35-7.39 (m, 2H), 8.23-8.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 14.6, 18.3, 18.4, 20.31, 20.34, 24.85, 24.9, 25.0, 25.9, 26.5, 28.7, 29.3, 29.4, 30.2, 30.3, 30.5, 30.6, 43.4, 43.5, 46.5, 50.7, 50.8, 55.5, 62.5, 62.6, 71.8, 72.5, 73.3, 87.0, 113.4, 127.2, 128.1, 128.3, 128.4, 130.2, 130.3, 135.4, 135.6, 136.7, 139.9, 144.3, 146.0, 158.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.1, 148.1, 148.0; MS (ESI) *m/z* calcd for $C_{49}H_{65}N_4O_9PS_4Na$ $[M + Na]^+$ 1035.33, found 1035.50.



*Compound **2.19c***: Synthesized using the procedure for **2.15a**. Diisopropylamine (1.79 mL, 12.77 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 4.8 mL, 12.0 mmol, 2.0 equiv.), **2.18** (2.9 g, 6.08 mmol, 1.0 equiv.), THF (50 mL) and **2.8c** (2.0 g, 6.08 mmol, 1.0 equiv.) were used. Product **2.19c** was purified with flash column chromatography (SiO_2, hexanes/EtOAc 1:1): 2.72 g, 67%; white foam; TLC R_f = 0.5 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 (d, J = 3.4 Hz, 12H), 1.46 (dd, J = 6.4, 1.4 Hz, 3H), 1.86-1.97 (m, 2H), 2.42-2.61 (m, 2H), 2.67-2.77 (m, 4H), 3.73-3.78 (m, 1H), 3.85-3.89 (m, 1H), 4.05-4.15 (m, 2H), 5.27 (m, 1H), 6.47 (t, J = 6.6 Hz, 1H), 8.37 (d, J = 2.2 Hz, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.0, -4.5, -4.3, 18.2, 18.3, 18.7, 20.6, 25.9, 26.0, 26.2, 27.9, 28.3, 29.0, 29.4, 41.9, 51.0, 53.5, 62.9, 68.6, 72.0, 73.1, 73.14, 85.0, 88.3, 117.5, 122.2, 126.3, 141.6, 149.3, 150.5, 150.8, 152.7, 162.9; HRMS (ESI) *m/z* calcd for $C_{29}H_{51}N_5O_5S_2Si_2H$ $[M + H]^+$ 670.2948, found 670.2937.

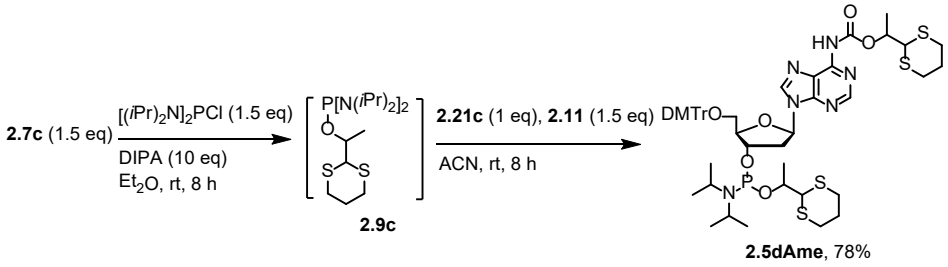


*Compound **2.20c***: Synthesized using the procedure for **2.16a**. Compound **2.19c** (2.6 g, 3.88 mmol, 1.0 equiv.), THF (50 mL) and TBAF (1 M in THF, 8.5 mL, 8.5 mmol, 2.5 equiv.) were used. Product **2.20c** was purified with flash column chromatography (SiO_2, DCM/MeOH 25:1): 1.10 g, 83%; light yellow foam; TLC R_f = 0.5 (SiO_2, DCM/MeOH 25:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (d, J = 6.4 Hz, 3H), 1.85-1.91 (m, 1H), 1.99-2.04 (m, 1H), 2.71-2.91 (m, 4H), 3.57 (m, 1H), 2.92-2.99 (m, 2H), 3.79 (t, J = 12.0 Hz, 1H), 3.93 (d, J =12.0 Hz, 1H), 4.17 (d, J = 5.8 Hz, 1H), 4.23 (m, 1H), 5.24-5.30 (m, 1H), 6.39-6.43 (m, 1H), 8.18 (s, 1H), 8.69 (s, 1H), 9.36 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 18.16, 18.9, 20.5, 25.5, 26.0, 29.2, 29.7, 41.1, 51.3, 52.5, 63.4, 73.0, 73.20, 73.24, 87.6, 89.6, 123.43, 123.44, 142.75, 142.77, 150.3, 150.4, 152.3; HRMS (ESI) *m/z* calcd for $C_{17}H_{23}N_5O_5S_2H$ $[M + H]^+$ 442.1219, found 442.1195.

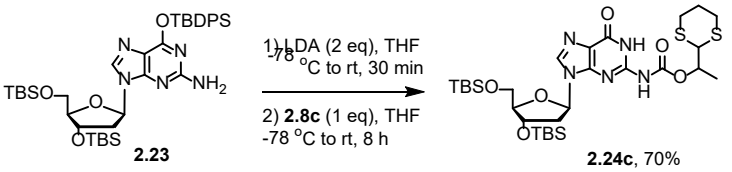


*Compound **2.21c***: Synthesized using the procedure for **2.17a**. Compound **2.20c** (2.2 g, 4.9 mmol, 1.0 equiv.), pyridine (30 mL) and DMTrCl (2.19 g, 5.63 mmol, 1.1 equiv.) were used. Product **2.21c** was purified with flash column chromatography (SiO_2, DCM/MeOH 19:1 with 5% Et_3N): 3.38 g, 91%; white foam; TLC R_f = (SiO_2, DCM/MeOH 19:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 6.4 Hz, 3H), 1.71-1.79 (m, 1H), 1.89-1.96 (m, 2H), 2.53-2.57 (m, 1H), 2.60-2.68 (m, 2H), 2.73-2.81 (m, 2H), 3.33 (d, J = 3.7 Hz, 2H), 3.64 (s, 6H), 4.07-4.11 (m, 1H), 4.19-4.21 (m, 1H), 4.68-4.69 (m, 1H), 5.18-5.25 (m, 1H), 6.44 (t, J = 5.6 Hz, 1H), 6.66 (d, J = 8.8 Hz, 4H), 7.03-7.19 (m, 7H), 7.28 (d, J = 7.0 Hz, 2H), 8.13 (d, J = 2.5 Hz, 1H), 8.62 (d, J = 2.7 Hz, 1H), 9.45 (brs, 1H); ^{13}C NMR

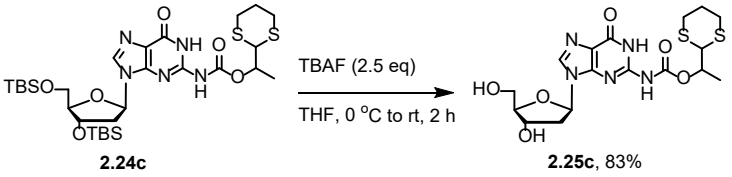
(100 MHz, $CDCl_3$) δ 14.5, 18.2, 26.0, 29.1, 29.5, 40.58, 40.63, 51.2, 55.4, 60.7, 64.0, 72.4, 72.9, 85.05, 85.1, 86.7, 113.3, 122.4, 127.0, 128.0, 128.2, 130.1, 135.7, 141.6, 141.7, 144.6, 149.5, 150.6, 150.9, 152.7, 158.5; HRMS (ESI) *m/z* calcd for $C_{38}H_{41}N_5O_7S_2$ $[M + H]^+$ 744.2526, found 744.2507.



*Compound **2.5dAme***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7c** (0.48 g, 2.97 mmol, 1.5 equiv.), diisopropyl amine (2.8 mL, 19.8 mmol, 10 equiv.), diethyl ether (15 mL), bis(diisopropylamino)chlorophosphine (0.78 g, 2.97 mmol, 1.5 equiv.), **2.21c** (1.47 g, 1.98 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.5 g, 2.97 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.5dAme** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:2 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 0.96 g, 78%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 1:2 with 2% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 1.05-1.16 (m, 12H), 1.32-1.40 (m, 6H), 1.69-1.84 (m, 2H), 1.95-1.99 (m, 2H), 2.63-2.89 (m, 12H), 3.26-3.36 (m, 2H), 3.48-3.58 (m, 1.5H), 3.71 (s, 6H), 3.99-4.04 (m, 1.5H), 4.08-4.11 (m, 2H), 4.65-4.72 (m, 1H), 5.20-5.26 (m, 1H), 6.40 (t, J = 6.7 Hz, 1H), 6.67-6.70 (m, 4H), 7.06-7.21 (m, 7H), 7.30 (d, J = 7.0 Hz, 2H), 8.16-8.18 (m, 1H), 8.64 (t, J = 3.1 Hz, 1H), 9.26 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 18.1, 18.14, 18.2, 20.2, 20.3, 24.7, 24.8, 24.9, 25.0, 26.0, 26.4, 26.6, 29.1, 30.2, 30.3, 30.6, 43.4, 43.5, 51.0, 51.2, 54.6, 54.7, 54.73, 55.3, 55.5, 63.8, 72.3, 72.6, 72.7, 72.9, 85.1, 86.6, 113.1, 113.3, 122.6, 122.7, 126.7, 127.1, 127.8, 128.1, 128.3, 130.0, 130.3, 135.7, 135.8, 144.6, 149.6, 150.5, 151.1, 152.6, 152.8, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$): δ 148.7, 148.6, 148.0; MS (ESI) *m/z* calcd for $C_{50}H_{65}N_6O_8PS_4Na$ $[M + Na]^+$ 1059.34, found 1059.53.

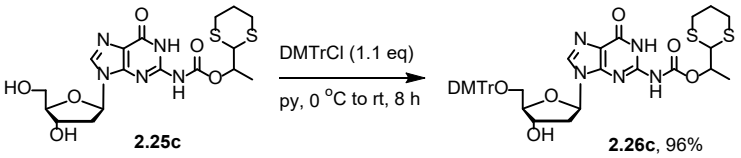


*Compound **2.24c***: Synthesized using the procedure for **2.24a**. Diisopropylamine (2.49 mL, 17.7 mmol, 2.0 equiv.), THF (75 mL), *n*BuLi (2.5 M in hexanes, 6.7 mL, 16.8 mmol, 2.0 equiv.), **2.23** (6.18 g, 8.4 mmol, 1.0 equiv.), THF (50 mL) and **2.8c** (2.77 g, 8.4 mmol, 1.0 equiv.) were used. Product **2.24c** was purified with flash column chromatography (SiO_2, hexanes/EtOAc, 1:1): 3.80 g, 70%; white foam; TLC R_f = 0.4 (SiO_2, hexanes/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.06 (d, J = 6.4 Hz, 12H), 0.87-0.88 (m, 18H), 1.47 (d, J = 6.3 Hz, 3H), 1.90-2.05 (m, 2H), 2.27-2.42 (m, 2H), 2.69-2.77 (m, 2H), 2.86-2.97 (m, 2H), 3.58 (dd, J = 10.5, 3.6 Hz, 0.5H), 3.67 (dd, J = 11.7, 3.8 Hz, 0.5H), 3.94-3.99 (m, 2H), 4.49-4.55 (m, 1H), 5.23-5.32 (m, 1H), 6.17-6.23 (m, 1H), 7.92 (s, 0.5H), 8.16 (s, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -5.0, -4.4, -4.3, 18.3, 18.4, 18.7, 25.7, 26.0, 26.3, 28.6, 29.0, 41.8, 41.9, 50.2, 50.3, 63.1, 72.1, 74.1, 84.1, 88.2, 94.6, 127.8, 131.7, 136.9, 146.6, 148.1, 152.8, 155.7; HRMS (ESI) *m/z* calcd for $C_{29}H_{51}N_5O_6S_2Si_2H$ $[M + H]^+$ 686.2898, found 686.2883.

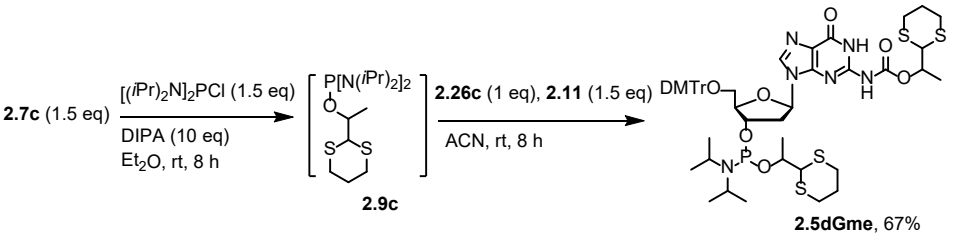


*Compound **2.25c***: Synthesized using the procedure for **2.16a**. Compound **2.24c** (2.6 g, 8.5 mmol, 1.0 equiv.), THF (50 mL) and TBAF (8.5 mL, 8.5 mmol, 5.0 equiv.) were used. Product **2.25c** was purified with flash column chromatography (SiO_2, DCM/MeOH 19:1): 1.1 g, 83%; white foam; TLC R_f = 0.4 (SiO_2, DCM/MeOH 19:1); ^{1}H NMR (400 MHz, CD_3OD) δ 1.44 (d, J = 6.4 Hz, 3H), 1.81-1.89 (m, 1H), 1.96-2.01 (m, 1H), 2.35-2.41 (m, 1H), 2.59-2.67 (m, 1H), 2.70-2.78 (m, 2H), 2.86-2.96 (m, 2H), 3.66-3.75 (m, 2H), 3.91-3.94 (m, 1H), 4.09 (d, J = 6.5 Hz, 1H), 4.48-4.52 (m, 1H), 5.22-5.29 (m, 1H), 6.29 (t, J = 6.6 Hz, 1H), 8.15 (d, J = 1.5 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 17.3, 25.8, 27.9,

28.4, 40.48, 40.49, 49.9, 61.9, 71.2, 73.8, 84.3, 88.0, 119.62, 119.64, 138.2, 138.3, 147.8, 149.2, 154.4, 156.2; HRMS (ESI) *m/z* calcd for $C_{17}H_{23}N_5O_6S_2H$ $[M + H]^+$ 458.1168, found 458.1154.

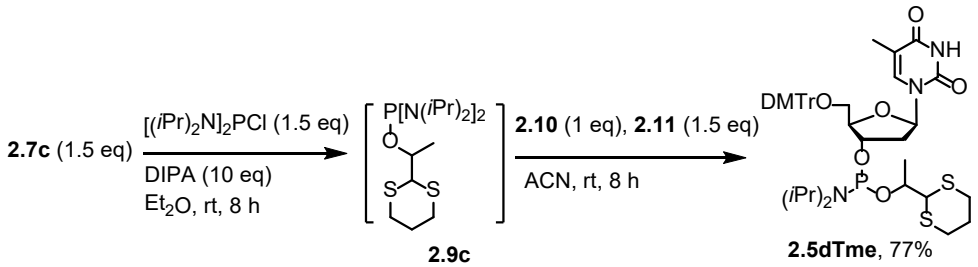


*Compound **2.26c***: Synthesized using the procedure for **2.17a**. Compound **2.25c** (1.19 g, 2.6 mmol, 1.0 equiv.), pyridine (30 mL) and DMTrCl (1.16 g, 2.99 mmol, 1.1 equiv.) were used. Product **2.26c** was purified with flash column chromatography (SiO_2, DCM/MeOH 19:1 with 5% Et_3N): 1.9 g, 96%; white foam; TLC R_f = 0.5 (SiO_2, DCM/MeOH 19:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, J = 5.7 Hz, 3H), 1.62-1.71 (m, 1H), 1.83-1.91 (m, 1H), 2.45-2.61 (m, 2H), 2.66-2.71 (m, 2H), 3.09-3.14 (m, 1H), 3.35-3.41 (m, 1H), 3.55 (s, 6H), 3.95-3.97 (m, 1H), 4.05-4.11 (m, 1H), 4.53-4.56 (m, 1H), 4.98-5.04 (m, 1H), 6.15-6.19 (m, 1H), 6.56 (d, J = 8.4 Hz, 4H), 6.94-7.09 (m, 7H), 7.19 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.1, 18.2, 26.0, 29.1, 29.46, 29.5, 41.2, 41.4, 51.3, 53.0, 55.4, 64.5, 71.8, 72.3, 84.1, 86.3, 86.4, 113.1, 126.8, 127.8, 128.2, 130.1, 135.8, 136.2, 144.7, 150.1, 156.9, 158.4; HRMS (ESI) *m/z* calcd for $C_{38}H_{41}N_5O_8S_2H$ $[M + H]^+$ 760.2475, found 760.2453.



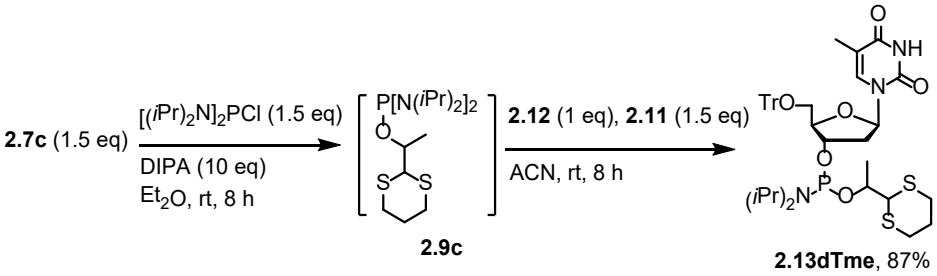
*Compound **2.5dGme***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7c** (0.32 g, 1.97 mmol, 1.5 equiv.), diisopropyl amine (1.85 mL, 13.1 mmol, 10 equiv.), diethyl ether (15 mL), bis(diisopropylamino)chlorophosphine (0.52 g, 1.97 mmol, 1.5 equiv.), **2.26c** (1.0 g, 1.31 mmol, 1.0 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.34 g, 1.97 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.5dGme** was purified with flash

chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:5 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 1.0 g, 67%; white foam; TLC R_f = 0.2 and 0.3 (SiO_2, hexanes/EtOAc 1:5 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 1.11-1.19 (m, 12H), 1.35-1.46 (m, 6H), 1.77-1.94 (m, 2H), 1.99-2.06 (m, 2H), 2.70-2.87 (m, 10H), 3.23-3.30 (m, 2H), 3.55-3.61 (m, 3.5H), 3.74 (s, 6H), 3.97-4.02 (m, 1.5H), 4.18-4.28 (m, 1H), 4.56-4.66 (m, 1H), 5.20-5.24 (m, 1H), 6.19 (t, J = 6.3 Hz, 1H), 6.73-6.77 (m, 4H), 7.21-7.29 (m, 7H), 7.34-7.40 (m, 2H), 7.71 (d, J = 1.4 Hz, 0.5H), 7.74 (d, J = 2.6 Hz, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 18.4, 20.3, 22.9, 24.8, 24.9, 25.1, 26.0, 26.4, 26.5, 28.8, 29.2, 30.2, 30.4, 30.5, 30.6, 43.5, 43.6, 45.8, 50.75, 50.8, 53.1, 55.5, 64.0, 72.5, 84.2, 86.6, 113.1, 113.3, 127.0, 128.0, 128.3, 130.1, 130.2, 131.7, 135.8, 144.3, 144.6, 149.0, 158.5, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.6, 148.5; MS (ESI) *m/z* calcd for $C_{50}H_{65}N_6O_9PS_4Na$ $[M + Na]^+$ 1075.33, found 1075.42.



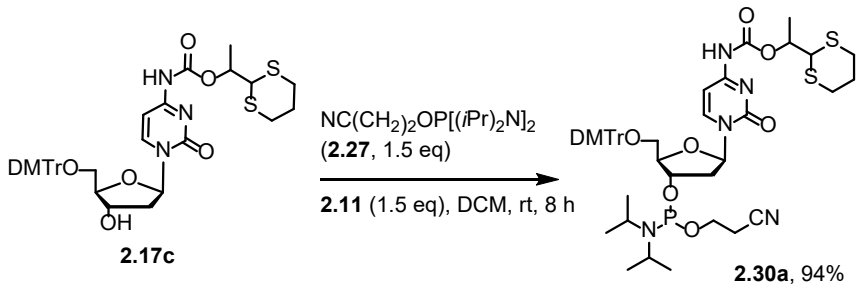
*Compound **2.5dTme***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7c** (0.2 g, 1.2 mmol, 1.5 equiv.), diisopropyl amine (1.7 mL, 8.0 mmol, 10 equiv.), diethyl ether (15 mL), bis(diisopropylamino)chlorophosphine (0.26 g, 1.2 mmol, 1.5 equiv.), **2.10** (0.44 g, 0.80 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.26 g, 1.2 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.5dTme** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 0.78 g, 77%; white foam; TLC R_f = 0.5 and 0.55 (SiO_2, hexanes/EtOAc 1:1 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 1.17-1.26 (m, 12H), 1.48 (dd, J = 4.1, 2.3 Hz, 3H), 1.86-1.93 (m, 2H), 2.66-2.98 (m, 11H), 3.28-3.41 (m, 2H), 3.75 (s, 6H), 4.05-4.10 (m, 1H), 4.12-4.16 (m, 1H), 4.24-4.29 (m, 0.5H), 4.34-4.38 (m,

0.5H), 4.67-4.76 (m, 1H), 5.27-5.33 (m, 1H), 6.45 (t, J = 7.1 Hz, 1H), 6.73-6.77 (m, 4H), 7.14-7.27 (m, 7H), 7.35 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.1 Hz, 1H), 8.41 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 20.3, 23.0, 23.1, 23.4, 24.0, 24.9, 25.0, 25.1, 26.1, 26.5, 29.1, 29.6, 30.4, 30.6, 30.7, 43.5, 43.6, 44.8, 45.4, 51.1, 55.5, 63.7, 72.85, 72.88, 85.0, 85.1, 86.1, 86.7, 94.6, 113.27, 113.29, 127.0, 128.0, 128.3, 130.18, 130.20, 135.7, 135.8, 141.5, 144.6, 149.4, 150.2, 151.0, 152.8, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.6, 148.5; MS (ESI) *m/z* calcd for $C_{43}H_{56}N_3O_8PS_2Na$ $[M + Na]^+$ 860.31, found 860.50.

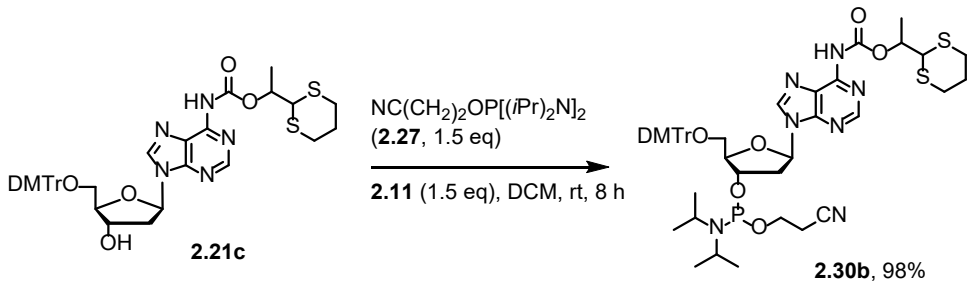


*Compound **2.13dTme***: Synthesized using the procedure for **2.5dCpe**. Compound **2.7c** (0.64 g, 3.9 mmol, 1.5 equiv.), diisopropyl amine (3.6 mL, 26.2 mmol, 10 equiv.), diethyl ether (15 mL), bis(diisopropylamino)chlorophosphine (1.04 g, 3.9 mmol, 1.5 equiv.), **2.12** (1.89 g, 2.62 mmol, 1 equiv.), diisopropylammonium tetrazolide (**2.11**, 0.67 g, 3.9 mmol, 1.5 equiv.) and ACN (10 mL) were used. Product **2.13dTme** was purified with flash chromatography by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:2 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.28 g, 87%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 1:2 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13-1.20 (m, 12H), 1.36-1.40 (m, 3H), 1.78-1.91 (m, 2H), 2.03-2.10 (m, 1.5H), 2.25-2.36 (m, 1H), 2.43-2.55 (m, 1.5H), 2.71-2.91 (m, 5H), 3.31 (dd, J = 10.5, 3.0 Hz, 0.5H), 3.35 (dd, J = 11.6, 2.9 Hz, 0.5H), 3.41-3.61 (m, 4H), 4.02-4.06 (m, 1H), 4.09-4.15 (m, 1H), 4.60-4.67 (m, 1H), 6.38 (dt, J = 7.7, 2.0 Hz, 1H), 7.22-7.31 (m, 9H), 7.38-7.36 (m, 6H), 7.56 (d, J = 1.2 Hz, 0.5H), 7.62 (d, J = 1.2 Hz, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 20.2, 20.3, 20.4, 23.0, 23.1, 23.4, 24.0, 24.9, 25.0, 25.02, 26.1, 26.4, 26.5, 29.1, 30.3, 30.5, 40.5, 40.6, 43.3, 43.5, 43.6, 43.64, 44.8, 45.4, 54.65, 54.71, 63.8, 63.9, 72.3, 72.5, 72.8, 73.5, 73.7, 73.9, 84.9,

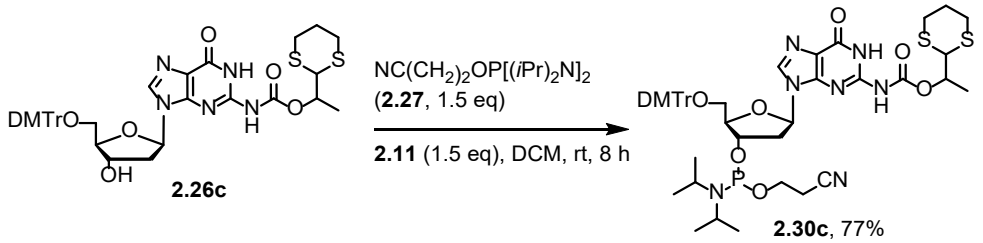
85.1, 85.5, 86.1, 87.7, 111.27, 111.28, 127.6, 128.2, 128.8, 128.9, 135.9, 143.4, 143.5, 150.3, 163.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.7, 148.5; MS (ESI) *m/z* calcd for $C_{41}H_{52}N_3O_6PS_2Na$ $[M + Na]^+$ 800.29, found 800.58.



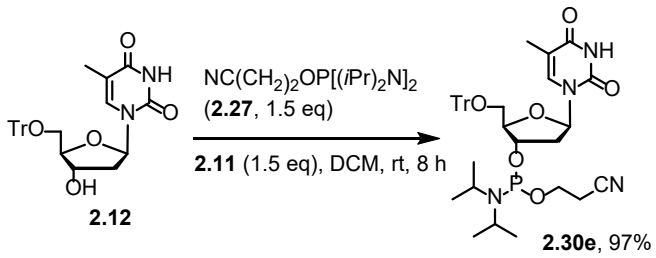
*Compound **2.30a***: To a solution of **2.17c** (1.0 g, 1.39 mmol, 1.0 equiv.) in dry DCM (30mL) was added diisopropylammonium tetrazolide (**2.11**, 356.6 mg, 2.08 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**2.27**, 0.66 mL, 2.08 mmol, 1.5 equiv.) at rt under nitrogen. After stirring overnight, the mixture was concentrated to dryness. Product **2.30a** was purified by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:3 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 1.20 g, 94%; white foam; TLC R_f = 0.4 and 0.45 (SiO_2, hexanes/EtOAc 1:3 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01-1.14 (m, 12H), 1.30 (t, *J* = 5.7 Hz, 3H), 1.66-1.75 (m, 1H), 1.82-1.90 (m, 1H), 2.31 (t, *J* = 6.2 Hz, 0.5H), 2.47 (t, *J* = 6.1 Hz, 1H), 2.55-2.64 (m, 4H), 2.67-2.79 (m, 2H), 3.06-3.14 (m, 0.5H), 3.20-3.28 (m, 1H), 3.31-3.47 (m, 4H), 3.64 (s, 6H), 3.92 (t, *J* = 5.6 Hz, 1H), 4.00-4.10 (m, 2H), 4.44-4.51 (m, 1H), 5.02-5.07 (m, 1H), 6.02 (s, 0.25H), 6.09-6.14 (m, 1H), 6.69-6.74 (m, 4H), 6.78 (brs, 0.5H), 7.06-7.18 (m, 6H), 7.25-7.28 (m, 2H), 7.62 (s, 0.25H), 8.08 (d, *J* = 7.4 Hz, 0.6H), 8.16 (d, *J* = 6.7 Hz, 0.4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7, 14.45, 14.49, 20.28, 20.43, 20.64, 22.5, 23.1, 23.2, 23.2, 23.4, 24.78, 24.84, 24.9, 25.6, 26.3, 28.0, 28.1, 29.1, 41.1, 43.31, 43.35, 43.47, 44.7, 45.42, 46.2, 55.4, 58.4, 58.6, 59.5, 60.5, 62.1, 65.3, 71.53, 71.7, 74.2, 74.39, 85.7, 86.9, 95.3, 113.3, 117.5, 117.7, 117.8, 127.2, 128.1, 128.3, 130.2, 135.25, 135.29, 135.4, 144.07, 144.2, 154.6, 158.6, 162.5, 162.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.7, 150.25, 150.28; HRMS (ESI) *m/z* calcd for $C_{46}H_{58}N_5O_9PS_2H$ $[M + H]^+$ 920.3492, found 920.3477.



*Compound **2.30b***: Synthesized using the procedure for **2.30a**. Compound **2.21c** (1.0 g, 1.34 mmol, 1.0 equiv.), DCM (30 mL), diisopropylammonium tetrazolide (**2.11**, 356.6 mg, 2.08 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**2.27**, 0.66 mL, 2.08 mmol, 1.5 equiv.) were used. Product **2.30b** was purified by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:3 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 1.24 g, 98%; white foam; TLC R_f = 0.3 and 0.4 (SiO_2, hexanes/EtOAc 1:3 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J = 6.8 Hz, 3H), 1.04-1.15 (m, 12H), 1.68-1.75 (m, 1H), 1.87-1.90 (m, 1H), 2.33 (t, J = 6.3 Hz, 1H), 2.46-2.49 (m, 2H), 2.57-2.60 (m, 2H), 2.70-2.80 (m, 2H), 2.84-2.90 (m, 1H), 3.21-3.25 (m, 1H), 3.29-3.42 (m, 2H), 3.34-3.52 (m, 3H), 3.61 (d, J = 2.2 Hz, 6H), 3.68-3.74 (m, 0.5H), 3.93-3.99 (m, 1H), 4.01-4.05 (m, 1.5H), 4.17-4.20 (m, 1H), 4.65-4.70 (m, 1H), 5.14-5.20 (m, 1H), 6.33-6.36 (m, 1H), 6.61-6.65 (m, 4H), 7.02-7.16 (m, 7H), 7.24-7.26 (m, 2H), 7.61 (s, 0.3H), 8.17 (dd, J = 8.4, 2.9 Hz, 1H), 8.57-8.59 (m, 1H), 9.72 (brs, 0.7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 18.0, 18.1, 20.2, 20.3, 20.4, 20.5, 20.6, 20.7, 22.5, 23.11, 23.19, 23.2, 23.7, 24.74, 24.81, 24.88, 25.9, 29.0, 29.5, 29.6, 39.3, 39.4, 43.4, 43.5, 45.4, 45.5, 45.8, 46.3, 51.05, 51.14, 55.4, 58.3, 58.5, 58.6, 58.7, 60.5, 63.5, 63.7, 72.7, 73.6, 73.7, 74.2, 74.4, 85.02, 85.06, 85.88, 85.96, 86.12, 86.15, 86.51, 86.52, 94.61, 113.2, 117.2, 117.6, 117.8, 122.8, 126.9, 127.9, 128.12, 128.15, 130.05, 130.09, 135.7, 142.0, 142.1, 144.6, 149.8, 150.7, 151.08, 151.12, 152.6, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.6, 149.7; HRMS (ESI) *m/z* calcd for $C_{47}H_{58}N_7O_8PS_2H$ $[M + H]^+$ 944.3604, found 944.3589.



*Compound **2.30c***: Synthesized using the procedure for **2.30a**. Compound **2.26c** (1.0 g, 1.32 mmol, 1.0 equiv.), DCM (30 mL), diisopropylammonium tetrazolide (**2.11**, 338.58 mg, 1.98 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**2.27**, 0.63 mL, 1.98 mmol, 1.5 equiv.) were used. Product **2.30c** was purified by dissolving the sample in the solvent mixture of hexanes/acetone 1:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 945 mg, 77%; pale yellow foam; TLC R_f = 0.2 and 0.25 (SiO_2, hexanes/acetone 1:1 with 5% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92-1.09 (m, 12H), 1.26 (d, J = 4.9 Hz, 3H), 1.65-1.81 (m, 2H), 1.95 (s, 1H), 2.19-2.39 (m, 2H), 2.45-2.56 (m, 4H), 2.66-2.75 (m, 2H), 3.05-3.23 (m, 2H), 3.30-3.47 (m, 3H), 3.58 (d, J = 5.4 Hz, 6H), 3.90-4.13 (m, 1H), 4.42-4.60 (m, 1H), 5.06-5.10 (m, 1H), 6.08-6.13 (m, 0.5H), 6.18-6.26 (m, 0.5H), 6.64 (dd, J = 11.2, 8.0 Hz, 4H), 7.01-7.16 (m, 7H), 7.24-7.33 (m, 2.5H), 7.60 (s, 0.15H), 7.67 (s, 0.5H), 8.01 (d, J = 7.6 Hz, 0.35H), 10.28 (brs, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.3, 15.0, 18.2, 20.2, 20.5, 20.6, 23.1, 23.2, 23.4, 24.6, 24.8, 25.7, 28.4, 28.7, 28.8, 29.5, 29.8, 31.1, 34.6, 43.3, 43.4, 45.3, 45.4, 45.5, 50.1, 50.3, 55.4, 58.2, 58.50, 58.55, 63.85, 63.86, 73.40, 73.42, 86.5 , 113.2, 113.3, 117.7, 117.9, 120.5, 126.9, 127.86, 127.94, 128.1, 130.1, 135.65, 135.72, 144.58, 144.6, 144.7, 147.9, 148.8, 153.96, 154.1, 156.4, 158.50, 158.52; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.38, 149.46, 149.50, 149.68, 149.98; HRMS (ESI) *m/z* calcd for $C_{47}H_{58}N_7O_9PS_2H$ $[M + H]^+$ 960.3553, found 960.3528.



*Compound **2.30e***: Synthesized using the procedure for **2.30a**. Compound **2.12** (2.00 g, 4.13 mmol, 1 equiv.), DCM (30 mL), diisopropylammonium tetrazolide (1.06 g, 6.19 mmol, 1.5 equiv.), and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**2.27**, 1.96 mL, 6.19 mmol, 1.5 equiv.) were used. Product **2.30e** was purified by dissolving the sample in the solvent mixture of hexanes/EtOAc 1:2 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.74 g, 97%; white foam; TLC R_f = 0.5 and 0.6 (SiO_2, hexanes/EtOAc 1:2 with 5% Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 1.10-1.16 (m, 12H), 1.41 (d, J = 4.1 Hz, 3H), 2.27-2.35 (m, 2H), 2.49-2.57 (m, 2H), 3.26-3.31 (m, 1H), 3.46-3.61 (m, 4H), 3.66-3.77 (m, 1H), 4.09-4.14 (m, 1H), 4.59-4.66 (m, 1H), 6.35-6.41 (m, 1H), 7.17-7.27 (m, 9H), 7.35-7.38 (m, 6H), 7.52 (s, 0.5H), 7.58 (s, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.17, 2.49, 20.57, 20.8, 24.79, 24.83, 24.86, 24.90, 24.98, 40.3, 43.4, 43.5, 58.3, 58.5, 58.7, 63.5, 63.7, 73.4, 73.6, 73.9, 74.1, 84.80, 84.86, 85.3, 85.7, 87.6, 94.6, 111.5, 111.6, 117.6, 117.8, 127.6, 128.2, 128.3, 135.7, 143.34, 143.37, 150.8, 150.9, 164.37, 164.42; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.5, 149.9; HRMS (ESI) *m/z* calcd for $C_{38}H_{45}N_4O_6PH$ $[M + H]^+$ 685.3155, found 685.3144.

3 PEGylated Dmoc phosphoramidites for sensitive oligodeoxynucleotide synthesis

Work in this chapter has been published in *Organic & Biomolecular Chemistry*

Chillar, K.; Yin, Y.; Apostle, A.; Fang, S. "PEGylated Dmoc phosphoramidites for sensitive oligodeoxynucleotide synthesis", *Organic & Biomolecular Chemistry* **2023**, 21, 9005-9010. DOI: 10.1039/D3OB01495A

3.1 Abstract

Sensitive oligodeoxynucleotides (ODNs) can be synthesized using Dmoc phosphoramidites, but only short ODNs were demonstrated. Here, we report the synthesis of much longer ODNs, which was made possible by the use of PEGylated Dmoc (pDmoc) phosphoramidites. The longer ODNs synthesized include those containing the sensitive 4acC epigenetic modification recently discovered in nature.

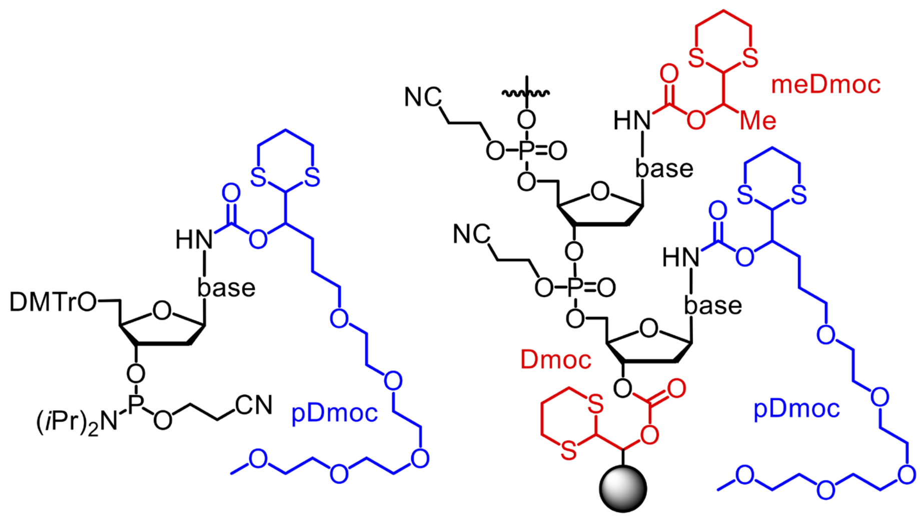


Figure 3.1: pDmoc as *exo*-amino protecting groups for ODN synthesis

3.2 Introduction

Over one hundred epigenetically modified nucleotides have been found in DNA and RNA.[26] These modifications serve as an additional layer of regulation in the biological system. Malfunction of this layer of regulation has been found to be related to many human diseases.[43] Among the modifications, many are sensitive to nucleophiles. For example, N^4-acetylcytosine (ac4C) in RNA[44, 45] and the recently detected N^4-acetyldeoxycytosine (4acC) in DNA[46] cannot survive nucleophilic conditions such as those involving dilute potassium methoxide or ammonia. Standard DNA and RNA synthesis methods use saturated ammonium hydroxide at elevated temperature for deprotection and cleavage, and therefore are unsuitable for the synthesis of oligonucleotides containing 4acC or ac4C.[47]

Some efforts have been made to address the problem,[16, 20-22, 29-31] but a practical solution is lacking. For example, Sekine, etc. were able to synthesize 4acC containing ODNs, but all other nucleotides in the sequences were dT.[20] Meier, etc. reported the synthesis of ac4C containing RNAs, but unprotected G phosphoramidite, which does not allow capping failure sequences, was used.[21] To generate ac4C antibody, Meier, etc. prepared ac4C containing RNAs using *in vitro* transcription, but the method lacks efficiency and specificity.[22]

In 2016, we reported the use of the 1,3-dithian-2-yl-methoxycarbonyl (Dmoc) function as protection groups and linker for oligodeoxynucleotide (ODN) synthesis.[23] Deprotection and cleavage were achieved under non-nucleophilic conditions involving oxidation with sodium periodate at pH 4 followed by β-elimination at pH 8. Subsequently, significant efforts have been devoted to improve the method, and various sensitive groups have been demonstrated to be able to survive the deprotection and cleavage conditions.[25, 38, 48] However, the longest ODN that could be synthesized is a 23-mer.[49] In this paper, we report the use of PEGylated Dmoc (pDmoc) phosphoramidites for sensitive ODN synthesis. Our hypothesis was that the limited length of ODNs that could be synthesized using the Dmoc methods was due to the hydrophobicity of the Dmoc group. When the ODNs on the solid support reached certain length, the cumulative hydrophobic effect of Dmoc groups reduced the solubility of ODN, and therefore, subsequent reactions became less efficient. Because PEGylation can increase solubility of materials, we decided to test if PEGylated Dmoc (pDmoc) phosphoramidites (Figure 3.2) could address the problem. Indeed, using pDmoc phosphoramidites, we were able to synthesize ODNs with length up to 49 nucleotides. In addition, to demonstrate the method for sensitive ODN synthesis, ODN sequences selected from the genomic DNA of *Arabidopsis* containing two 4acC groups have been synthesized.[4] All the ODNs were characterized with HPLC, MALDI MS and capillary electrophoresis.

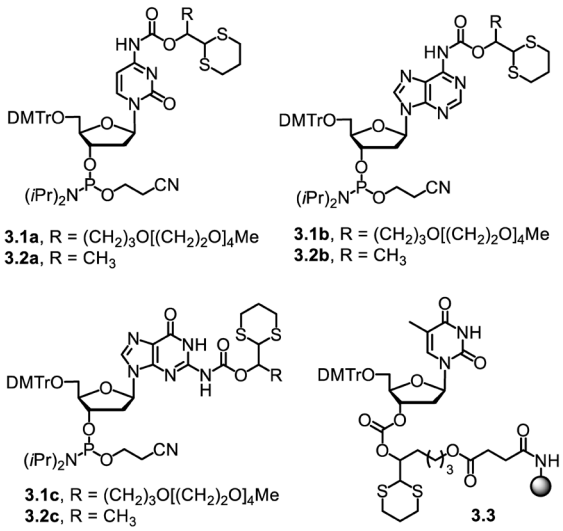
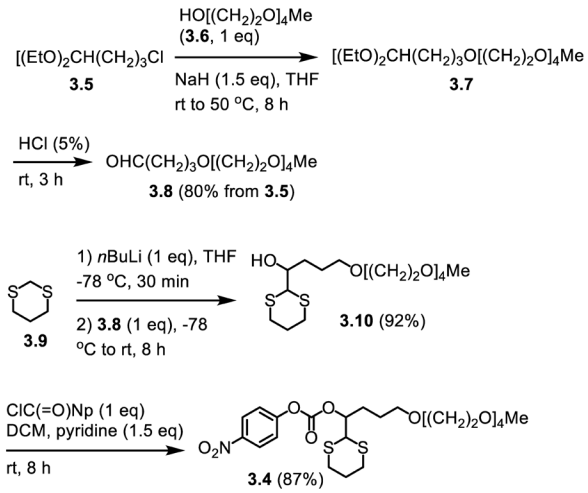


Figure 3.2: pDmoc and meDmoc phosphoramidites and Dmoc linker.

3.3 Results and discussion

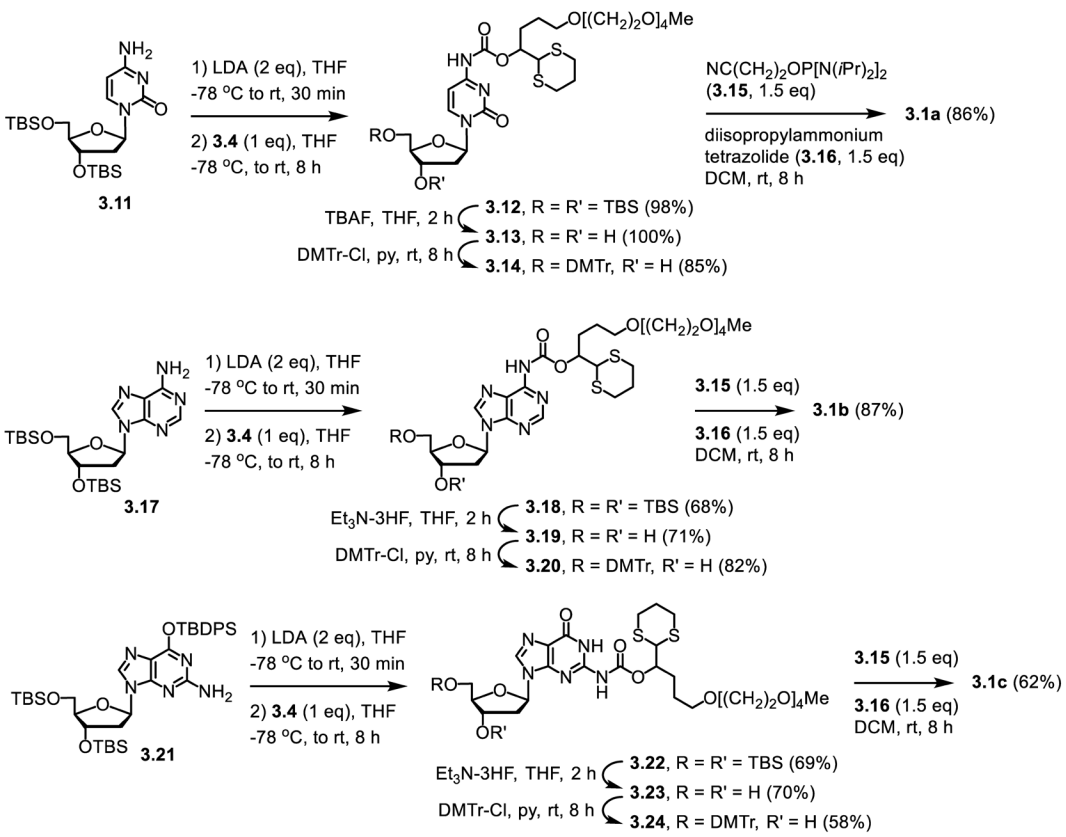
3.3.1 Synthesis of pDmoc phosphoramidites

Our plan was to use pDmoc phosphoramidites **3.1a-c** (Figure 3.2) to increase the solubility of the growing ODN and thus improve the efficiency of reactions for ODN synthesis. The majority of the monomers for the synthesis would still be the non-PEGylated monomers **3.2a-c** as well as the commercial 5'-DMTr-dT-CE phosphoramidite. Monomers **3.1a-c** would only be used occasionally because they are less atom economic. The meDmoc group was chosen over Dmoc because its deprotection does not need a scavenger.[48, 49] All the syntheses were planned to be carried out using linker **3.3**, which allows ODN cleavage under the same non-nucleophilic conditions for Dmoc deprotection. Thus, the plan required monomers **3.1a-c**, **3.2a-c** and linker **3.3** (Figure 3.2). The synthesis of **3.2a-c** and **3.3** has been reported earlier.[23, 49] For the synthesis of **3.1a-c**, the PEGylation agent **3.4** was needed (Scheme 3.1). Commercial **3.5** was reacted with HO(PEG)$_4$OMe (**3.6**) to give **3.7**, which upon treating with an acid gave **3.8**. Reaction of **3.8** with deprotonated **3.9** gave **3.10**. Compound **3.10** was converted to **3.4** by reacting with *p*-nitrophenyl chloroformate. All the reactions were simple, and the yields were high (Experimental section).



Scheme 3.1: Synthesis of reagent (**3.4**) for installing pDmoc.
(a) HO[$(CH_2)_2O]_4$Me (**3.6**, 1 eq), NaH (1.5 eq), THF, rt to 50 °C, 8 h. (b) HCl (5%), rt, 3 h, 85% from **3.5**. (c) nBuLi (1 eq), THF, -78 °C, 30 min, then **3.8** (1 eq), -78 °C to rt, 8 h, 92%. (d) ClC(=O)Np (1 eq), DCM, pyridine (1.5 eq), rt, 8 h, 87%. Np, 4-nitrophenol.

For the synthesis of **3.1a** (Scheme 3.2), cytidine nucleoside derivative **3.11**[49] was treated with 2 equivalents LDA and 1 equivalent **3.4** to give the pDmoc protected dC **3.12**. This condition for the introduction of carbamate groups to arylamines was known.[48, 50] The TBS groups of **3.12** was removed to give **3.13**, the 5'-OH was protected with DMTr-Cl to give **3.14**. Compound **3.14** was phosphitylated to give the target pDomc dC phosphoramidite **3.1a** under standard conditions.[51] The pDmoc dA phosphoramidite **3.1b** was synthesized under similar conditions. For the synthesis of pDmoc dG phosphoramidite **3.1c**, the procedure was similar except that **3.21**, of which the lactam of the nucleobase was protected with a TBDPS group,[49] was used as the starting material. Good to excellent yields were obtained for the steps for the synthesis of **3.1a-b**. For **3.1c**, the yields were slightly lower, but they were still acceptable. We had no difficulty in obtaining sufficient amount of the materials for ODN synthesis.



Scheme 3.2: Synthesis of pDmoc phosphoramidites.

(a) LDA (2 eq), THF, -78 °C to rt, 30 min; then **3.4** (1 eq), THF, -78 °C, to rt, 8 h. (b) $NC(CH_2)_2OP[N(iPr)_2]_2$, (**3.15**, 1.5 eq), diisopropylammonium tetrazolide (**3.16**, 1.5 eq) DCM, rt, 8 h. (c) TBAF, THF, rt, 2 h. (d) DMTr-Cl, pyridine, rt, 8 h. (e) Et_3N-3HF, THF, rt, 2 h.

3.3.2 Longer ODN synthesis using pDmoc phosphoramidites

With monomers **3.1a-c**, **3.2a-c** and linker **3.3**, we started to test the idea of using PEGylation to increase the length of ODN that can be synthesized. The 30-mer **3.25a** (Figure 3.3), which does not contain any sensitive group, was used for the initial study. Without using the PEGylation strategy, the longest ODN that were synthesized was a 23-mer.[49] The current synthesis was carried out under standard conditions with the following modifications. For the coupling step, the majority of the nucleotides dA, dC and dG in the sequence were incorporated with a meDmoc phosphoramidite (**3.2a-c**), but for the

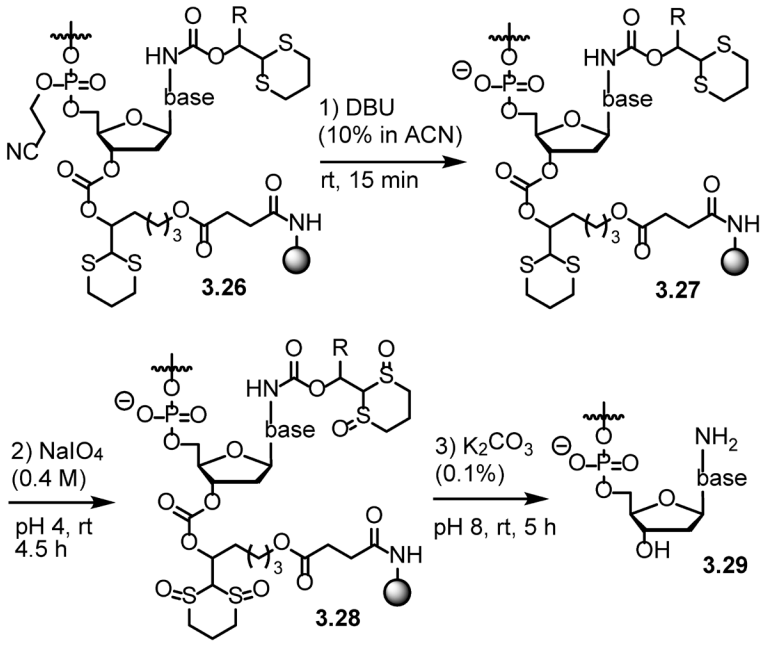
incorporation of roughly every other five nucleotides, the pDmoc phosphoramidite (**3.1a-c**) was used (see Figure 3.2). As stated earlier, the PEG moieties introduced to the ODN was intended to increase ODN solubility. According to trityl assay (see Appendix 6.1), the coupling efficiency was comparable with standard phosphoramidites. Considering the facts that a 30-mer synthesis with 99% coupling efficiency only gives theoretically 74% full-length ODN and besides truncated sequences, the crude ODN contains other impurities such as small molecules and pre-detritylated full-length ODN, the synthesis yields indicated by crude HPLC profiles (see Appendix 6.1) are not inconsistent with results of trityl assay. For capping, instead of acetic anhydride, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite and 4,5-dicyanoimidazole (DCI) were employed. This was intended to prevent cap-exchange. For the last synthetic cycle, a 5'-trityl (Tr) instead of 5'-DMTr phosphoramidite was used for the coupling step. The reason is that the DMTr group is not stable under the sodium periodate oxidation conditions (pH 4) used during ODN deprotection. The Tr group is stable and can assist RP HPLC purification of the ODN product. At the end of the ODN synthesis, the product can be represented as **3.26** (Scheme 3.3), in which the ODN is anchored to the CPG via a Dmoc linker, the phosphate is protected with a 2-cyanoethyl group, and the *exo*-amino groups are protected with Dmoc or pDmoc group.

3.25a 30-mer: TAA CTT TAT CGT ACC ATC TTT AAA CAT ATT
3.25b 30-mer: TGT CCT TAC CTT CAT TCC GTT CAT CTT
3.25c 38-mer: TGG ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TT
3.25d 49-mer: TAC CGA ATG GAT GGA CTT GTA ACT TTA TCG TAC CAT CTT TAA ACA TAT T
3.25e 28-mer: TAC TAG TAC T**C(Ac)**T TCT T**C(Ac)**T TCT TCT TCT T
3.25f 29-mer: TCT TAT CT**C(Ac)** TCT CT**C(Ac)** TTT TTT GGC CTT TT
3.25g 29-mer: TCG AAA CGC **C(Ac)**AT CTC CG**C(Ac)** CGT TAA TCT CT
3.25h 28-mer: TAC TAG TAC T**C**T TCT T**C**T TCT TCT TCT T
3.25i 29-mer: TCT TAT CT**C** TCT CT**C** TTT TTT GGC CTT TT
3.25j 29-mer: TCG AAA CGC **C**AT CTC CG**C** CGT TAA TCT CT

Figure 3.3: ODN sequences.

For **3.25a-g**, nucleotides underlined were incorporated using pDmoc phosphoramidites (**3.1a-c**); those not underlined were incorporated using Dmoc (**3.2a-c**), or standard dT or dC^{Ac} [for C(Ac)] phosphoramidites. For **3.25h-j**, all nucleotides were incorporated using standard phosphoramidites. ODNs **3.25e-g** contain two 4acC each. The sequences of

3.25h-j are identical with **3.25e-g**, respectively, except that 4acC are replaced by dC (bold).



Scheme 3.3: ODN deprotection and cleavage.

ODN deprotection and cleavage were carried out under the same conditions used previously when no pDmoc phosphoramidites were used.[49] Briefly, the CPG (**3.26**, Scheme 3.3) was treated with DBU to remove the 2-cyanoethyl groups giving **3.27**. The Dmoc groups introduced by meDmoc and pDmoc phosphoramidites were oxidized with 0.4 M sodium periodate, which is slightly acidic (pH 4) by itself, to give **3.28**. At this stage, the ODN was still on the solid support, which is important because it allows easy separation of sodium periodate and its reduced product from the ODN by washing with water. Finally, treating **3.28** with dilute potassium carbonate cleaved the ODN from the solid support and gave the fully deprotected ODN **3.29**.

The ODN (**3.25a**) was purified with Tr-on RP HPLC. The trityl group was removed with 80% acetic acid. RP HPLC showed that the ODN was pure (Figure 3.4). The purified ODN

was analyzed with MALDI MS. Correct molecular peak was found (Figure 3.5). The purity of the ODN was further analyzed with capillary electrophoresis (CE, see Appendix 6.1). Because previously without using pDmoc phosphoramidites, we were not able to synthesize ODNs longer than 23-mers, our hypothesis that PEGylation can increase ODN solubility and enable longer ODN synthesis is validated. Encouraged by the results, we made efforts to synthesize different and longer ODNs. Under the same conditions for the synthesis of **3.25a**, the 30-mer **3.25b**, 38-mer **3.25c** and the 49-mer **3.25d** (Figure 3.3) were successfully synthesized. The HPLC profile and MALDI MS of **3.25d** are shown in Figures 3 and 4, respectively. Additional purification and analysis data are in Appendix 6.1.

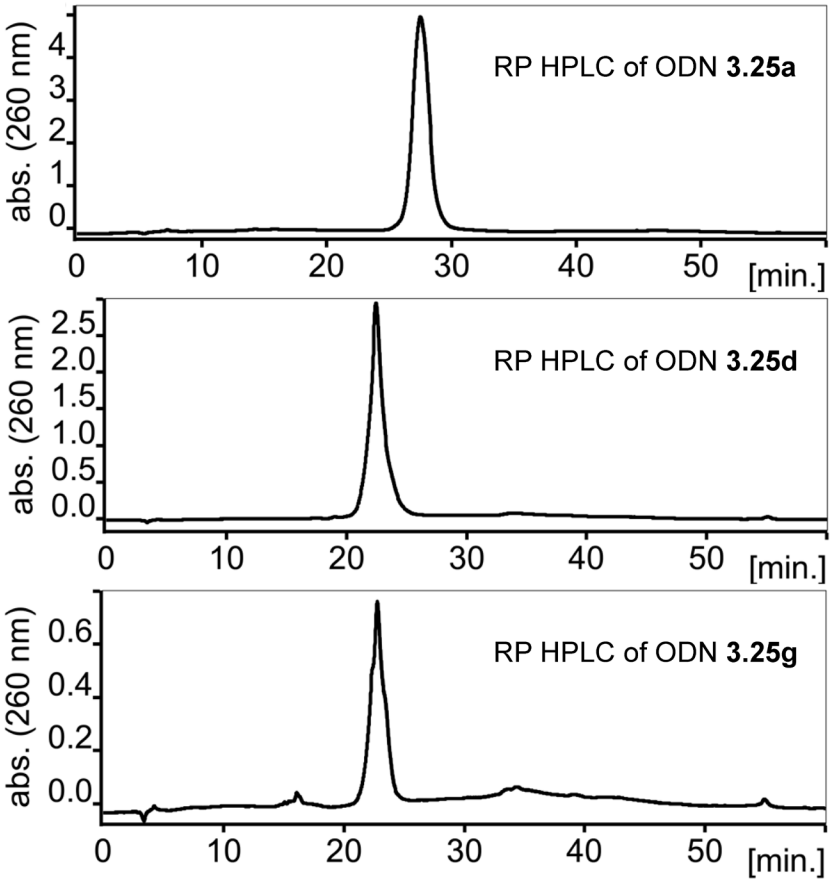


Figure 3.4: RP HPLC of ODNs.

See Appendix 6.1 for detailed conditions for HPLC.

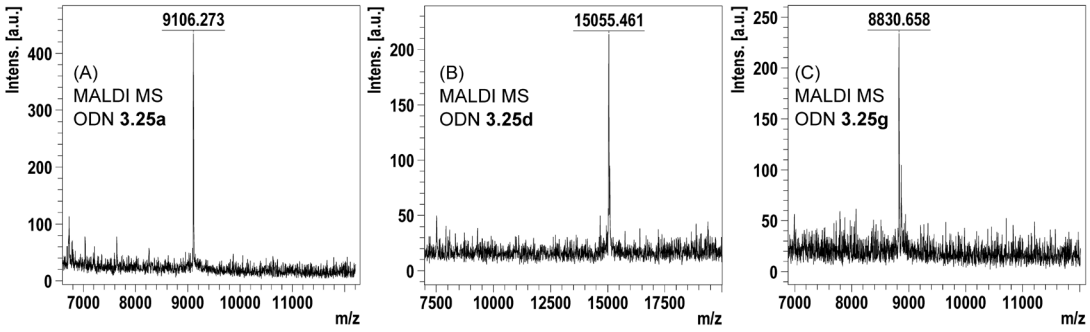


Figure 3.5: MALDI MS of ODNs.

(A) MS of **3.25a**. Calcd for $[M+NH_4]^+$ *m/z* 9105, found 9106. (B) MS of **3.25d**. Calcd for $[M+K]^+$ *m/z* 15054, found 15055. (C) MS of **3.25g**. Calcd for $[M-H]^-$ *m/z* 8831, found 8831.

To see if the method can synthesize even longer ODNs, we briefly tested incorporating all nucleotides with pDmoc (and dT) phosphoramidites. The results were unsatisfactory. Once 20-mer was reached, the synthesis became ineffective as indicated by trityl assay. The large mass introduced by PEGs could be the cause. Evidently, to synthesize longer ODNs, some engineering work for determining the number and location of pDmoc phosphoramidites that should be used in specific sequences is required.

3.3.3 Sensitive ODN synthesis using pDmoc phosphoramidites

Recently Wu's group reported the detection of 4acC in genomic DNAs of *Arabidopsis*, rice, maize, mouse and human, and potential biological roles of the epigenetic modification.[46] Previously, ac4C was known in RNAs, and significant efforts have made to study its biological functions and relations to human diseases.[44, 52] However, until the recent report, there was no information about 4acC in DNAs in nature. Motivated by the report, we synthesized ODNs **3.25e-g** (Figure 3.3). The sequences were from the regions of chromosome 1 of *Arabidopsis*, in which 4acC modifications were detected.[46] They contain the 4acC motifs CDYCDYCDYCDYCDY or YCTCTCTYTCTYYYT (D represents A/G/T; Y represents C/T), and thus chances exist that they may carry 4acC in cells. The motifs are similar to those of many transcription factors suggesting that 4acC may play a role in the regulation of gene expression.[46] To challenge our method, two 4acC

modifications instead of one were placed in each ODN. It is notable that using the Dmoc-pDmoc method, no special manipulations are needed. The HPLC profile and MALDI MS of the 29-mer ODN **3.25g** are shown in Figures 3 and 4, respectively. All data regarding purification and analysis of **3.25e-g** are in Appendix 6.1. The presence of the two acetyl groups of 4acC in the sequences was further confirmed by comparing their MALDI MS with those of ODNs **3.25h-j**, which have identical sequences with **3.25e-g**, respectively, but do not contain acetyl group. As shown in Figure 3.6, the difference of the molecular peaks for **3.25g** and **3.25j** matches closely with the mass of two acetyl groups. It is noted that the peak at *m/z* 8787 may also be from the molecule corresponding to **3.25g** with one acetyl group lost. However, we believe that this is unlikely because this peak is absent in the MS spectrum of **3.25g** without intentionally added **3.25j** (Figure 3.6 and Appendix 6.1). The data regarding **3.25e-f** are provided in Appendix 6.1.

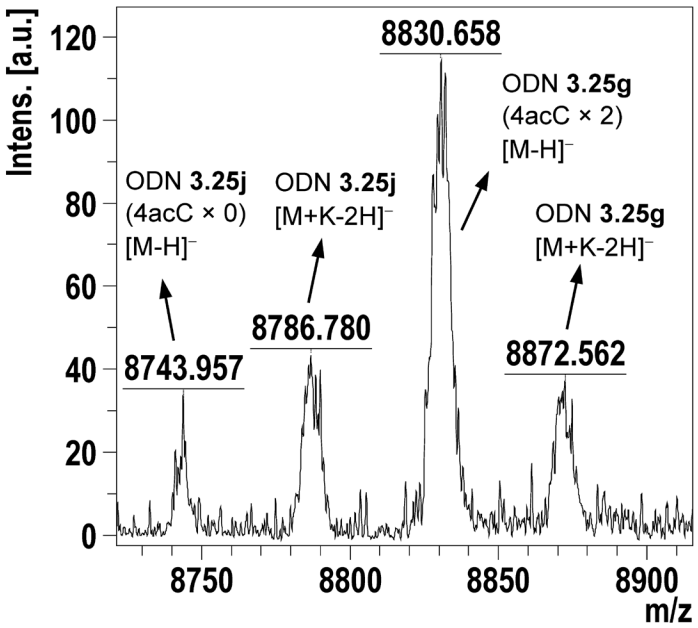


Figure 3.6: MALDI MS of the mixture of ODNs **3.25g** and **3.25j**.
Calcd for **3.25g** $[M-H]^-$ 8830, found 8831; $[M-2H+K]^-$ 8869, found 8873. Calcd for **3.25j** $[M-H]^-$ 8746, found 8744; $[M-2H+K]^-$ 8782, found 8787. The mass difference between the molecular peaks for the two ODNs (87) is close to the mass of two acetyl groups minus two hydrogen (84).

The use of PEGylation to increase the length of ODNs is notable. To our knowledge, for solid phase ODN synthesis, the cause of inability to synthesize longer ODNs has always been attributed to the cumulative effect of non-quantitative stepwise yields. In an unrelated study, we found that even though DMSO is miscible with many solvents, the solubility of oligosulfoxides decreases rapidly as their lengths increase.[53] This prompted us to hypothesize that as ODNs grow, their solubility may decrease, and the reactions may become less efficient. Our success in the present study may inspire the use of PEGylation to improve the efficiency of unmodified DNA and RNA synthesis, which can have a high impact on projects in areas such as synthetic biology,[54] protein engineering,[55] mRNA vaccine development[56] and DNA computer digital data storage[57] in which the availability of long DNA and RNA is crucial for success. In addition, the synthesis of ODNs containing more than one 4acC modification is remarkable. The acetyl group of 4acC and ac4C is notably labile. The present method is expected to be able to provide sensitive ODNs with sufficient length and number of sensitive groups that are practically useful for biological studies. For example, ODNs containing one or more 4acC modifications can help to identify proteins that interact with 4acC. Such proteins include 4acC writers, readers, and erasers. The ODNs can also be used for studying the effects of acetylation of dC nucleotide on cellular stability of DNA, higher order structures of DNA, and efficiency of transcription.[44, 45, 58]

3.4 Conclusions

In summary, using Dmoc as protecting group for ODN synthesis, deprotection can be achieved under non-nucleophilic conditions. As a result, the Dmoc method is useful for the synthesis of epigenetically modified ODNs containing functions sensitive to nucleophiles. However, the hydrophobicity of the Dmoc groups limited the length of ODNs that can be synthesized. Using pDmoc phosphoramidites, due to the increased solubility of the protected ODNs on the solid support, in the present work, synthesis of ODNs with up to 49 nucleotides have been achieved. To demonstrate the application of the method for sensitive ODN synthesis, three ODN sequences selected from the regions of the *Arabidopsis* genome that were found to have the 4acC modification have been successfully

synthesized. All the ODNs were characterized with RP HPLC, MALDI MS and CE. The availability of a method for the synthesis of ODNs with suitable length and multiple sensitive groups is expected to be helpful for projects in the research area of epigenetics.

3.5 Experimental section

3.5.1 ODN synthesis

ODNs were synthesized on an MerMade 6 synthesizer on dT-Dmoc-CPG (**3.3**, 26 μmol/g loading, 20 mg, 0.52 μmol) using the phosphoramidite chemistry. Deblocking: DCA (3%, DCM), 90 sec × 2. Coupling: Phosphoramidite (**3.1a-c**, **3.2a-c**, 5'-DMTr-dT-CE phosphoramidite, 5'-Tr-dT-CE phosphoramidite for last synthesis cycle, 0.1 M, MeCN), 4,5-dicyanoimidazole (DCI, 0.25 M, MeCN), 60 sec × 3. Capping: 2-Cyanoethyl *N*,*N*,*N'*,*N'*-tetraisopropylphosphorodiamidite (0.1 M, MeCN), DCI (0.25 M, MeCN), 60 sec × 3. Oxidation: I_2 (0.02 M, THF/pyridine/H_2O, 70:20:10, v/v/v), 40 sec × 2. At the end of the synthesis, the 5'-Tr group was kept.

3.5.2 ODN deprotection and cleavage

The CPG (0.52 μmol synthesis, **3.26**) was divided into 5 equal portions (~0.104 μmol each). One portion was subject to deprotection and cleavage. Removing 2-cyanoethyl groups: The suspension of CPG (**3.26**, ~0.104 μmol ODN) in the solution of DBU in anhydrous MeCN (1:9, v/v, 1 mL) in a 1.5 mL centrifuge tube was gently shaken at rt for 5 min. The supernatant was removed. The process was repeated two more times. The CPG was washed with anhydrous MeCN (1 mL × 5). Oxidation of Dmoc groups: The suspension of CPG (**3.27**) in the solution of $NaIO_4$ (0.4 M, 1 mL), which has a pH of 4, in a 1.5 mL centrifuge tube was gently shaken at rt for 1.5 h. The supernatant was removed. The process was repeated two more times. The CPG was washed with water (1 mL × 5). Removing oxidized Dmoc groups: The suspension of CPG (**3.28**) in the solution of K_2CO_3 (0.1%, 1 mL), which has a pH of 8, in a 1.5 mL centrifuge tube was gently shaken at rt for 5 h. The supernatant was transferred into a clean 1.5 mL centrifuge tube. The CPG was washed with water (150 μL × 5). The combined supernatant and washes were concentrated to ~50 μL.

To the solution was added *n*BuOH (450 μL). After mixing by vortex, ODN was precipitated via centrifugation (14.5k rpm, ~14k × g, 15 min). The supernatant was removed leaving deprotected ODN (**3.29**) in the tube.

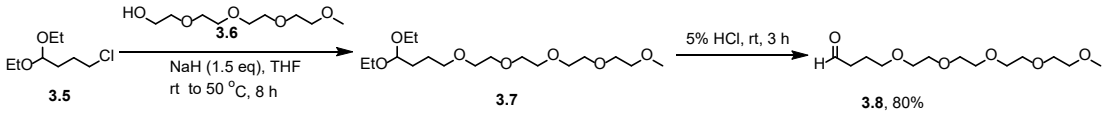
3.5.3 ODN purification and analysis

ODN (**3.29**) was dissolved in H_2O (100 μL). A portion (35 μL) was injected into HPLC to generate the crude ODN profile. Fractions under the major ODN peak at ~35 min were collected, and concentrated to ~100 μL, which was re-injected into HPLC to obtain the profile of pure trityl-tagged ODN. The fractions of the ODN were collected and concentrated to dryness. To the trityl-tagged ODN was added AcOH (80%, 1 mL). The mixture was shaken gently at rt for 3 h. Volatiles were evaporated. The residue was dissolved in water (100 μL) and injected into HPLC to generate the profile of crude de-tritylated ODN. The fractions of the ODN were collected and concentrated to dryness. The fractions of ODN at ~20 min was collected and concentrated to dryness. The residue was dissolved in water (100 μL) and injected into HPLC to generate the profile of pure de-tritylated ODN. The fractions of ODN were collected and concentrated to dryness. ODNs were quantified using a reported method,[59] and analyzed using MALDI-TOF MS and capillary electrophoresis.

3.5.4 Compounds

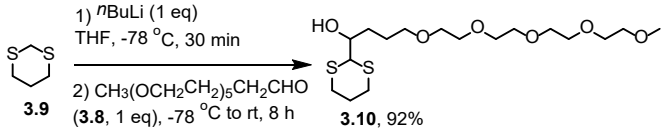
General: All reactions were performed in oven-dried glassware under nitrogen using standard Schlenk techniques. Reagents and solvents from commercial sources were used as received with the following exceptions. DCM, pyridine, ACN, diethyl ether and diisopropylamine were distilled over CaH_2 under nitrogen. THF was distilled over CaH_2 and then Na/benzophenone under nitrogen. Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 0.25 μm thickness, were used for TLC. Selecto Scientific silica gel, particle size 32-63 μm, was used for flash column chromatography. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on a Varian UNITYINOVA spectrometer at 400, 100 and 162 MHz, or Bruker's spectrometer at 500, 126 and 202 MHz, respectively. Chemical shifts (δ) were reported in reference to solvent peaks, residue $CHCl_3$ at 7.24 ppm for ^{1}H, $CDCl_3$ at 77.00

ppm for ^{13}C, and H_3PO_4 at 0.00 ppm for ^{31}P. ODN syntheses were performed on a MerMade 6 solid phase synthesizer. RP HPLC was performed on a JASCO LC-2000Plus System: pump, PU-2089Plus Quaternary Gradient; detector, UV-2075Plus. Column: C-18 reversed phase, analytical, 5 μm diameter, 100 Å, 250 × 4.60 mm. Solvent A: 0.1 M triethylammonium acetate in 5% ACN. Solvent B: 90% ACN. All profiles were generated by detecting absorbance at 260 nm using the linear gradient solvent system: solvent B (0%-45%) in solvent A over 60 min followed by solvent B (45%-100%) in solvent A over 20 min at a flow rate of 1.0 mL/min. Capillary electrophoresis (CE) was carried out on an Agilent 7100 CE system with UV-Visible diode-array detector (190–600 nm). Capillary: PVA coated 25 cm × 100 μm (40 cm was cut to 25 cm). Buffer solution: 200 mM Bis-Tris and 200 mM boric acid in CE water, pH 7.2. Sieving solution: 27% (w/v) PEG 35,000 in buffer solution. Sieving solution injection: −8.0 bar, 5 min. Sample injection: 0.02-0.07 μM ODNs in buffer solution, −10 KV for 10 sec. Run: −25 KV, 30 ºC for 40 min. Detection: alignment interface for standard capillary with 75 μm ID, detected at 260 ± 8 nm. Sieving gel removal (cleaning capillary): −12 bar, 1 min. HRMS were obtained on Thermo HR-Orbitrap Elite or Waters G2-XS QTof Mass Spectrometers. MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System.

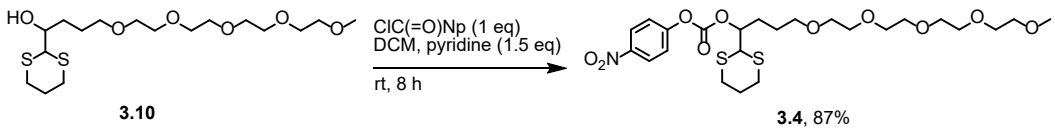


*Compound **3.8***: To NaH (2.16 g, 90.03 mmol, 1.5 equiv.) in dry THF (15 mL) was added the solution of tetraethylene glycol monomethyl ether (**3.6**, 10.0 g, 48.02 mmol, 0.8 equiv.) in dry THF (70 mL) dropwise at rt under nitrogen. The mixture was stirred at rt for 45 min, and then heated to 50 ºC. The solution of **3.5** (10.843 g, 60.02 mmol, 1.0 equiv.) in dry THF (20 mL) was added via a cannula over 5 h under nitrogen. After stirring at 50 ºC for 8 h, water (30 mL) was added slowly. The majority of THF was evaporated under reduced pressure. The mixture was partitioned between water (30 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The crude **3.7** was

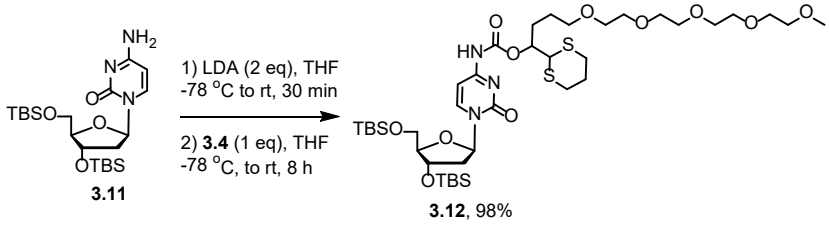
dissolved in suitable volume of 5% HCl. The reaction was allowed to proceed with stirring at rt for 3 h, and then quenched by adding 0.5 M NaOH until pH 7. The product was extracted with DCM (50 mL × 3), and the combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **3.8** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 18:1): 12.95 g, 80%; colorless oil; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 18:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27-1.34 (m, 2H), 1.95 (t, J = 7.0 Hz, 2H), 2.77 (s, 3H), 2.90-2.99 (m, 6H), 3.02-3.05 (m, 12H), 9.18 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 22.3, 22.4, 40.3, 40.4, 40.5, 58.2, 58.4, 69.7, 69.8, 70.0, 70.1, 70.1, 70.2, 70.2, 70.3, 70.5, 71.6, 71.8, 201.3, 201.4; HRMS (ESI) *m/z* calcd for $C_{13}H_{26}O_6Na$ $[M + Na]^+$ 301.1627, found 301.1631.



*Compound **3.10**:* To a solution of 1,3-dithiane (**3.9**, 2.23 g, 18.55 mmol, 1.0 equiv.) in dry THF (50 mL) was slowly added *n*BuLi (2.5 M in hexanes, 7.42 mL, 62.5 mmol, 1.0 equiv.) at -78 °C under nitrogen. After stirring for 30 min, compound **3.8** (5.15 g, 18.55 mmol, 1.0 equiv.) dissolved in THF (20 mL) was added to the reaction mixture via a cannula over 15 min at -78 °C under nitrogen. The mixture was stirred for 8 h while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (20 mL). The majority of THF was evaporated under reduced pressure. The mixture was partitioned between sat. NH_4Cl (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **3.10** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 18:1): 6.78 g, 92%; colorless oil; TLC R_f = 0.5 (SiO_2, EtOAc/MeOH 18:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60-1.68 (m, 1H), 1.71-1.85 (m, 2H), 1.88-2.00 (m, 2H), 2.06-2.13 (m, 1H), 2.72-2.2.85 (m, 2H), 2.90-2.95 (m, 1H), 3.39 (s, 3H), 3.51-3.70 (m, 18H), 3.86-3.91 (m, 1H), 3.99 (d, J = 6.2 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.9, 26.1, 28.6, 29.0, 31.6, 53.0, 59.0, 70.1, 70.5, 70.56, 70.61, 71.3, 71.9, 72.5; HRMS (ESI) *m/z* calcd for $C_{17}H_{34}O_6S_2Na$ $[M + Na]^+$ 421.1694, found 421.1684.

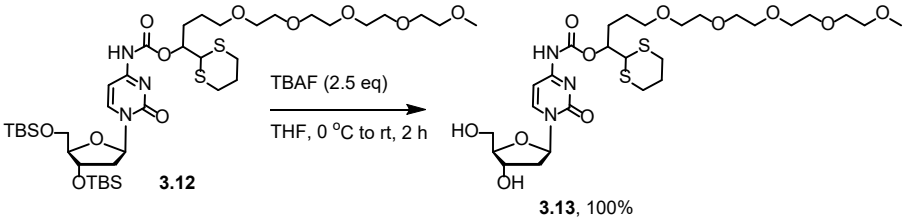


*Compound **3.4**:* To a solution of **3.10** (5.56 g, 14.0 mmol, 1.0 equiv.) in dry DCM (100 mL) under nitrogen was added distilled pyridine (1.7 mL, 21.0 mmol, 1.5 equiv.). After cooling to 0 °C, 4-nitrophenol chloroformate (2.82 g, 14.0 mmol, 1.0 equiv.) was added. The mixture was stirred overnight while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (75 mL) and extracted with DCM (50 mL × 3). The combined extract was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. Product **3.4** was precipitated from DCM with hexanes: 6.85 g, 87%; light yellow oil; TLC R_f = 0.65 (SiO_2, EtOAc/MeOH 5:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35-1.88 (m, 6H), 2.41-2.50 (m, 2H), 2.62-2.72 (m, 2H), 3.03 (s, 3H), 3.19-3.35 (m, 18 H), 3.76 (d, J = 7.0 Hz, 1H), 4.84-4.89 (m, 1H), 7.12 (d, J = 9.2 Hz, 2H), 7.96 (d, J = 9.2 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.4, 27.9, 28.2, 28.7, 48.3, 58.9, 70.2, 70.4, 70.5, 71.8, 79.5, 121.8, 125.2, 145.3, 152.2, 155.5; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}NO_{10}S_2Na$ $[M + Na]^+$ 586.1757, found 586.1756.



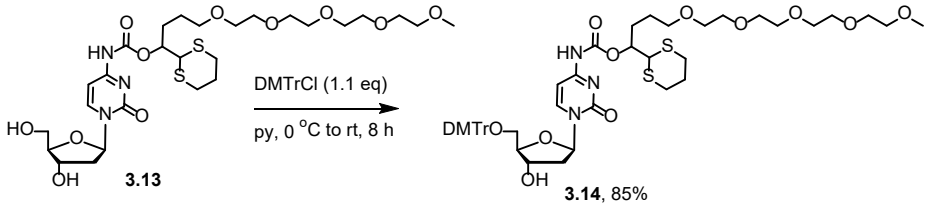
*Compound **3.12**:* To a solution of diisopropylamine (1.27 mL, 9.06 mmol, 2.0 equiv.) in THF (50 mL) at −78 °C was added *n*BuLi (2.5 M in hexanes, 3.62 mL, 9.06 mmol, 2.0 equiv.). After stirring for 30 min, the freshly prepared LDA solution was added via a cannula to the solution of **3.11** (2.061 g, 4.53 mmol, 1.0 equiv.) in THF (50 mL) at −78 °C. The mixture was stirred at the same temperature for 30 min, and compound **3.4** (2.552 g, 16.01 mmol, 1.0 equiv.) was then added under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt gradually. The reaction was quenched with sat. NaCl (20 mL). The majority of THF was evaporated under reduced pressure. The mixture was

partitioned between EtOAc (50 mL) and sat. NaCl (50 mL). The aqueous layer was extracted with EtOAc (40 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Product **3.12** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 18:1): 3.91 g, 98%; yellow oil; TLC R_f = 0.55 (SiO_2, EtOAc/MeOH 18:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.88 (s, 9H), 0.94 (s, 9H), 1.56-2.14 (m, 7H), 2.46-2.55 (m, 1H), 2.71-2.82 (m, 2H), 2.88-2.99 (m, 2H), 3.39 (s, 3H), 3.46-3.71 (m, 18H), 3.78 (d, J = 9.5 Hz, 1H), 3.91-4.01 (m, 2H), 4.10 (t, J = 4.3 Hz, 1H), 4.35-4.45 (m, 1H), 5.12-5.21 (m, 1H), 6.24 (t, J = 5.2 Hz, 1H), 7.16 (d, J = 7.3 Hz, 1H), 8.37 (d, J = 7.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.51, -5.45, -4.9, -4.6, 17.9, 18.3, 25.6, 25.7, 25.9, 28.6, 28.8, 29.0, 31.5, 42.2, 49.4, 53.1, 59.0, 61.7, 69.8, 70.1, 70.2, 70.4, 70.5, 70.6, 71.2, 71.9, 72.4, 76.2, 86.6, 87.7, 94.4, 100.0, 144.3, 152.0, 154.9, 162.1; HRMS (ESI) *m/z* calcd for $C_{39}H_{73}N_3O_{11}S_2Si_2Na$ $[M + Na]^+$ 902.4123, found 902.4107.

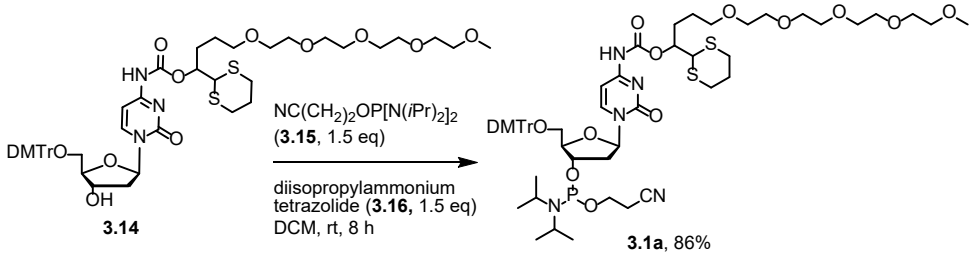


*Compound **3.13**:* To a solution of **3.12** (3.18 g, 3.61 mmol, 1.0 equiv.) in THF (50 mL) at 0 °C was added TBAF (1 M in THF, 9.02 mL, 9.02 mmol, 2.5 equiv.). The mixture was stirred for 2 h while warming to rt. THF was evaporated under reduced pressure and the residue was loaded onto a column for flash column chromatography (SiO_2, EtOAc/MeOH 8:2). Compound **3.13**: 2.454 g, 100%; pale yellow foam; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 8:2); ^{1}H NMR (500 MHz, CD_3OD) δ 1.59-1.75 (m, 2H), 1.76-1.94 (m, 2H), 2.01-2.09 (m, 2H), 2.18-2.2.26 (m, 1H), 2.47-2.2.55 (m, 1H), 2.76-2.88 (m, 2H), 2.89-3.00 (m, 2H), 3.36 (s, 3H), 3.57-3.69 (m, 18H), 3.75-3.89 (m, 2H), 4.02-4.07 (m, 1H), 4.20 (d, J = 6.2 Hz, 1H), 4.43-4.46 (m, 1H), 5.15-5.21 (m, 1H), 6.25 (t, J = 6.2 Hz, 1H), 7.29 (d, J = 7.4 Hz, 1H), 8.50 (d, J = 7.6 Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 25.3, 25.6, 28.0, 28.3, 28.5, 41.2, 49.1, 57.9, 61.1, 69.9, 70.0, 70.16, 70.19, 70.20, 70.29, 71.6, 75.7, 87.2,

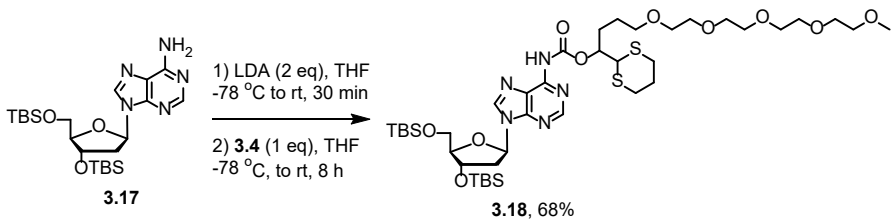
88.1, 95.4, 144.7, 152.9, 156.2, 163.4; HRMS (ESI) *m/z* calcd for $C_{27}H_{45}N_3O_{11}S_2Na$ $[M + Na]^+$ 674.2393, found 674.2376.



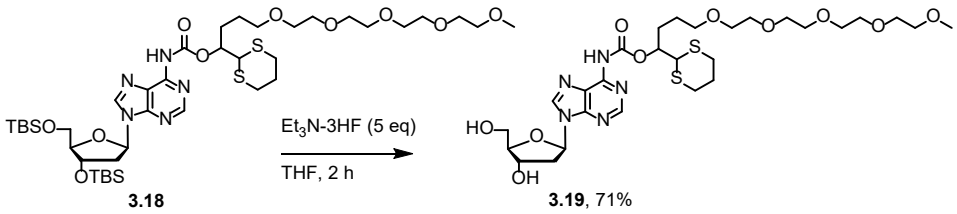
*Compound **3.14***: To a solution of **3.13** (1.6 g, 2.71 mmol, 1.0 equiv.) in pyridine (50 mL) at 0 °C was added DMTrCl (1.01 g, 2.98 mmol, 1.1 equiv.) under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt. The volume of the mixture was reduced to about 2 mL under vacuum from an oil pump (the remaining pyridine can help to retain DMTr on the product). The mixture was partitioned between 5% Na_2CO_3 (20 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. Product **3.14** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 2.20 g, 85%; pale yellow foam; TLC R_f = 0.45 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^1H NMR (500 MHz, $CDCl_3$) δ 1.55-1.91 (m, 5H), 1.92-2.01 (m, 2H), 2.11-2.19 (m, 1H), 2.61-2.69 (m, 2H), 2.70-2.88 (m, 2H), 3.29 (s, 3H), 3.3.29-3.65 (m, 20H), 3.71 (s, 6H), 4.01 (dd, J = 17.9, 6.45 Hz, 1H), 4.13-4.14 (m, 1H), 4.44-4.47 (m, 1H), 5.05-5.11 (m, 1H), 6.19-6.21 (m, 0.8H), 6.28-6.32 (m, 0.2H), 6.77 (d, J = 7.9 Hz, 4H), 6.88-6.89 (m, 1H), 7.15 (t, J = 7.2 Hz, 1H), 7.18-7.27 (m, 6H), 7.34 (d, J = 7.8 Hz, 2H), 8.17-8.21 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.5, 25.6, 28.5, 28.6, 28.7, 42.0, 49.2, 49.4, 55.2, 58.9, 62.7, 70.1, 70.4, 70.8, 71.8, 75.8, 76.0, 86.5, 86.7, 87.2, 95.1, 113.2, 127.0, 127.9, 128.1, 130.0, 135.4, 135.5, 144.2, 152.2, 155.2, 158.5, 162.4; HRMS (ESI) *m/z* calcd for $C_{48}H_{63}N_3O_{13}S_2Na$ $[M + Na]^+$ 976.3700, found 976.3685.



*Compound **3.1a***: To a solution of **3.14** (330 mg, 0.35 mmol, 1.0 equiv.) in dry DCM (10 mL) was added diisopropylammonium tetrazolide (**3.16**, 89.7 mg, 0.525 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**3.15**, 0.17 mL, 0.525 mmol, 1.5 equiv.) at rt under nitrogen. After stirring overnight, the mixture was concentrated to dryness. Product **3.1a** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 9:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 0.340 g, 86%; pale yellow oil; TLC R_f = 0.40 and 0.45 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94-1.19 (m, 12H), 1.47-1.99 (m, 6H), 2.14-2.22 (m, 1H), 2.32 (t, J = 6.1 Hz, 1H), 2.48 (t, J = 6.2 Hz, 1H), 2.41-2.50 (m, 1H), 2.54-2.81 (m, 6H), 3.23 (s, 3H), 3.22-3.75 (m, 22H), 3.66 (s, 3H),3.67 (s, 3H), 3.92-3.94 (m, 1H), 4.09-4.13 (m, 1H), 4.48-4.54 (m, 1H), 5.00-5.06 (m, 1H), 6.11-6.16 (m, 1H), 6.72 (d, J = 7.6 Hz, 2H), 6.74 (d, J = 8.4 Hz), 7.12 (t, J = 7.4 Hz, 1H), 7.12-7.25 (m, 6H), 7.22-7.7.35 (m, 2H), 8.08 (d, J = 7.4 Hz, 0.5H), 8.18 (d, J = 7.1 Hz, 0.5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 19.9, 20.0, 20.07, 20.13, 20.3, 22.8, 22.9, 23.0, 24.4, 24.5, 24.6, 25.5, 28.3, 28.4, 28.5, 28.7, 40.7, 41.1, 43.1, 43.2, 45.2, 45.4, 45.9, 49.1, 49.2, 55.1, 58.2, 58.3, 58.4, 58.82, 58.83, 60.2, 61.9, 62.3, 70.1, 70.3, 70.5, 71.8, 75.7, 75.9, 85.5, 86.7, 95.1, 113.2, 117.3, 117.5, 117.6, 127.0, 127.9, 128.1, 130.0, 135.2, 135.3, 144.0, 158.6, 162.4; ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.47, 148.98, 149.03; HRMS (ESI) *m/z* calcd for $C_{57}H_{80}N_5O_{14}PS_2H$ $[M + H]^+$ 1154.4959, found 1154.4972.



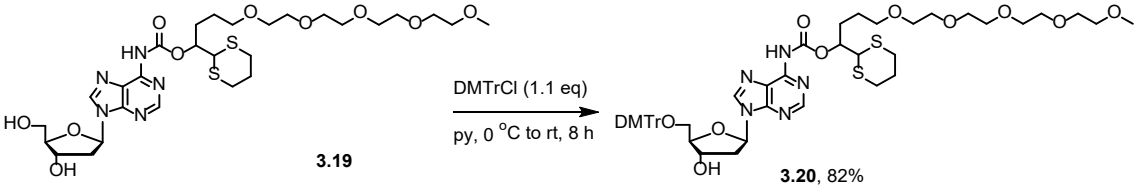
*Compound **3.18***: Synthesized using the procedure for **3.12**. Diisopropylamine (2.25 mL, 16.0 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 6.4 mL, 16.0 mmol, 2.0 equiv.), **3.17** (3.83 g, 8.0 mmol, 1.0 equiv.), THF (20 mL) and **3.4** (4.50 g, 8.0 mmol, 1.0 equiv.) were used. Product **3.18** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 18:1): 4.89 g, 68%; pale yellow oil; TLC R_f = 0.4 (SiO_2, EtOAc/MeOH 18:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.06 (s, 6H), 0.82-0.89 (s, 18H), 1.54-2.10 (m, 6H), 2.37-2.99 (m, 6H), 3.32 (s, 3H), 3.32-3.62 (m, 18H), 3.72 (dd, J = 11.2, 3.10 Hz, 1H), 3.83 (dd, J = 11.1, 3.85 Hz, 1H), 3.94-3.99 (m, 1H), 4.12 (t, J = 5.9 Hz, 1H), 4.56-4.58 (m, 1H), 5.20-5.23 (m, 1H), 6.45 (t, J = 6.7 Hz, 1H), 8.28 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.5, -5.4, -4.8, -4.7, 17.9, 18.4, 25.6, 25.7, 25.86, 25.91, 26.0, 28.5, 28.6, 28.7, 29.0, 30.9, 31.5, 41.2, 49.6, 53.1, 59.0, 62.7, 70.1, 70.4, 70.5, 71.2, 71.9, 72.5, 75.3, 84.6, 88.0, 122.3, 141.3, 149.4, 150.5, 150.7, 152.6; HRMS (ESI) *m/z* calcd for $C_{40}H_{73}N_5O_{10}S_2Si_2H$ $[M + H]^+$ 904.4416, found 904.4413.



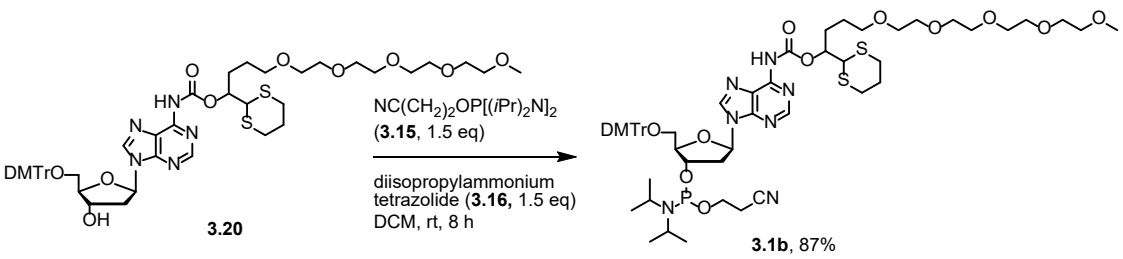
3.18 **3.19**, 71%

*Compound **3.19***: To the solution of **3.18** (3.0 g, 3.32 mmol, 1.0 equiv.) in THF (50 mL) was added triethylamine trihydrogen fluoride (2.7 mL, 16.6 mmol, 5.0 equiv.) at rt. After stirring for 2 h, the reaction was quenched with methoxytrimethylsilane (2.29 mL, 16.6 mmol, 5.0 equiv.).[60] Volatiles were evaporated under reduced pressure. Product **3.19** was purified with flash chromatography by dissolving the crude product in the solvent mixture of EtOAc/MeOH 8:2, loading onto a column (SiO_2) and eluting with the same solvent mixture: 1.590 g, 71%; white foam; TLC R_f = 0.3 (SiO_2, EtOAc/MeOH 8:2); ^{1}H NMR (500 MHz, CD_3OD) δ 1.67-1.80 (m, 2H), 1.84-1.93 (m, 2H), 2.05-2.10 (m, 2H), 2.40-2.51 (m, 1H), 2.78-3.02 (m, 5H), 3.36 (s, 3H), 3.52-3.69 (m, 18H), 3.74-3.78 (m, 1H), 3.84 (t, J = 2.8 Hz, 0.7H), 3.87 (t, J = 2.8 Hz, 0.3H), 4.07-4.09 (m, 1H), 4.27 (d, J = 6.3 Hz, 1H), 4.58-4.62 (m, 1H), 5.26-5.30 (m, 1H), 6.44 (dd, J = 7.9, 6.10 Hz, 0.3H), 6.54 (t, J = 6.8 Hz, 0.7H), 8.19 (s, 0.7H), 8.33 (s, 0.3H), 8.57 (s, 0.3H), 8.60 (s, 0.7H); ^{13}C NMR (126 MHz,

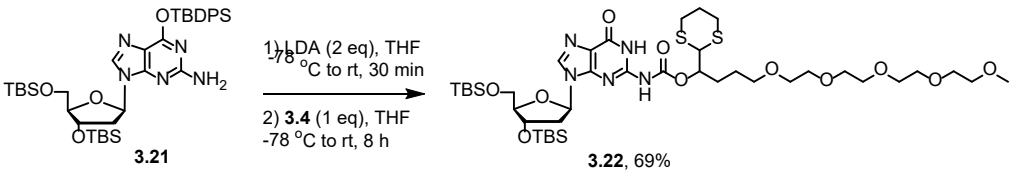
CD_3OD) δ 25.3, 25.7, 28.1, 28.4, 28.5, 40.1, 40.2, 49.3, 57.7, 62.0, 62.3, 69.8, 69.9, 70.1, 71.4, 71.5, 71.7, 75.5, 85.3, 85.8, 88.3, 88.5, 100.0, 119.5, 122.5, 140.2, 142.7, 148.5, 149.7, 150.8, 151.5, 151.7, 152.1, 156.1; HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_5O_{10}S_2H$ [M + H]$^+$ 676.2686, found 676.2688.



*Compound **3.20***: Synthesized using the procedure for **3.14**. Compound **3.19** (1.06 g, 1.60 mmol, 1.0 equiv.), pyridine (20 mL) and DMTrCl (596 mg, 1.76 mg, 1.76 mmol, 1.1 equiv.) were used. Product **3.20** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N): 1.26 g, 82%; pale white foam; TLC R_f = 0.55 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.66-1.76 (m, 2H), 1.79-1.94 (m, 2H), 2.02-2.036 (m, 2H), 2.55- 2.61 (m, 1H), 2.70-2.78 (m, 2H), 2.83-2.91 (m, 2H), 2.94-2.98 (m, 1H), 3.35 (s, 3H), 3.40-3.69 (m, 20H), 3.74 (s, 6H), 4.16 (d, J = 6.1 Hz, 1H), 4.10-4.23 (m, 1H), 4.69-4.71 (m, 1H), 5.20-5.28 (m, 1H), 6.49 (t, J = 6.5 Hz, 1H), 6.76 (d, J = 8.8 Hz, 4H), 7.16 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.8 Hz, 2H), 7.26 (d, J = 8.9 Hz, 4H), 7.36 (d, J = 8.1 Hz, 2H), 8.10 (s, 0.2H), 8.14 (s, 0.8H), 8.66 (s, 0.2H), 8.68 (s, 0.8H), 9.05 (brs, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.6, 25.7, 28.5, 28.7, 29.0, 29.1, 40.3, 49.7, 55.2, 59.0, 63.7, 67.8, 70.2, 70.5, 70.6, 71.9, 72.3, 75.5, 84.7, 86.3, 86.6, 113.2, 122.3, 126.9, 127.9, 128.1, 129.5, 130.0, 135.6, 141.4, 144.5, 149.4, 150.5, 150.7, 152.7, 158.5; HRMS (ESI) *m/z* calcd for $C_{49}H_{63}N_5O_{12}S_2Na$ [M + Na]$^+$ 1000.3812, found 1000.3788.

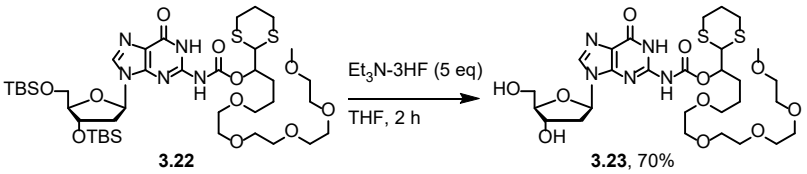


*Compound **3.1b***: Synthesized using the procedure for **3.1a**. Compound **3.20** (2.215 g, 2.27 mmol, 1.0 equiv.), DCM (30 mL), diisopropylammonium tetrazolide (**3.16**, 582.25 mg, 3.40 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**3.15**, 1.08 mL, 3.40 mmol, 1.5 equiv.) were used. Product **3.1b** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 18:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.33 g, 87%; pale yellow oil; TLC R_f = 0.4 and 0.45 (SiO_2, EtOAc/MeOH 18:1 with 5% Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90-1.25 (m, 12H), 1.55-1.81 (m, 4H), 1.94-1.98 (m, 2H), 2.39 (t, J = 6.1 Hz, 1H), 2.50-2.89 (m, 9H), 3.27 (s, 3.5H) 3.10-3.88 (m, 21H), 3.55 (s, 6H), 3.99-4.13 (m, 2H), 4.21-4.24 (m, 1H), 4.66-4.72 (m, 1H), 5.15-5.20 (m, 1H), 6.35-6.46 (m, 1H), 6.60 (m, 4H), 7.08-7.20 (m, 7H), 7.30 (d, J = 7.2 Hz, 2H), 7.50 (s, 0.2H), 8.15 (s, 0.4H), 8.17 (s, 0.4 H), 8.22 (s, 0.2H), 8.61 (s, 0.8H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 19.8,, 20.0, 20.2, 22.7, 22.8, 22.9, 24.4, 25.4, 25.6, 28.3, 28.6, 28.93, 28.92, 38.7, 38.9, 43.0, 43.1, 45.0, 49.6, 49.7, 55.0, 58.1, 58.2, 58.3, 58.7, 63.2, 63.3, 69.9, 70.2, 70.3, 71.7, 75.2, 84.8, 86.2, 112.9, 117.1, 117.5, 117.6, 122.6, 126.6, 127.6, 127.9, 129.8, 135.5, 144.5, 149.6, 150.9, 152.2, 158.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 148.74, 148.78, 148.88, 148.93; HRMS (ESI) *m/z* calcd for $C_{58}H_{80}N_7O_{13}PS_2H$ $[M + H]^+$ 1178.5071, found 1178.5088.



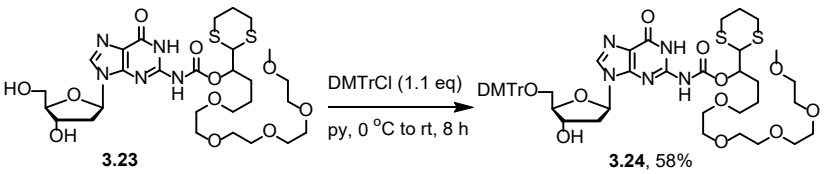
*Compound **3.22***: Synthesized using the procedure for **3.12**. Diisopropylamine (4.70 mL, 33.4 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 13.36 mL, 33.4 mmol, 2.0 equiv.), **3.21**[49] (12.247 g, 16.7 mmol, 1.0 equiv.), THF (50 mL) and **3.4** (9.405 g, 16.7 mmol, 1.0 equiv.) were used. Product **3.22** was purified with flash column chromatography (SiO_2, EtOAc/MeOH, 19:1): 10.62 g, 69%; light brown oil; TLC R_f = 0.5 (SiO_2, EtOAc/MeOH 19:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ -0.06-0.15 (m, 12H), 0.70-1.00 (m, 18H), 1.59-2.11 (m, 6H), 2.31-2.48 (m, 2H), 2.71-2.85 (m, 2H), 2.91-3.05 (m, 2H), 3.39 (s, 3H), 3.44-4.01 (m, 21H), 4.57 (brs, 1H), 5.27 (brs, 1H), 6.24 (t, J = 6.2 Hz, 1H), 8.00

7(s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ -5.6, -5.4, -4.8, -4.7, 17.9, 18.3, 25.3, 25.4, 25.6, 25.7, 25.8, 25.9, 27.7, 28.0, 28.4, 28.6, 28.7, 29.0, 31.3, 41.6, 48.5, 53.1, 58.8, 58.9, 62.7, 70.0, 70.37, 70.39, 70.4, 70.5, 71.1, 71.75, 71.76, 72.4, 78.5, 83.6, 87.7, 120.7, 136.5, 147.1, 148.1, 154.8, 155.6; HRMS (ESI) *m/z* calcd for $C_{40}H_{73}N_5O_{11}S_2Si_2Na$ $[M + Na]^+$ 942.4184, found 942.4151.



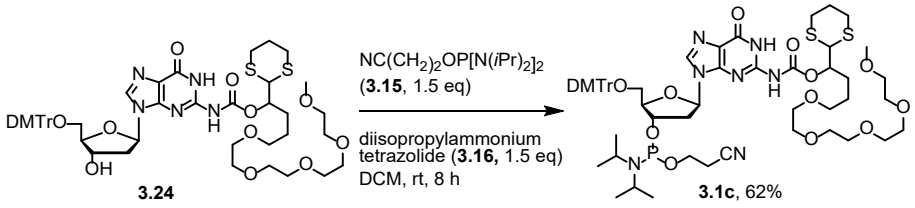
3.22 **3.23**, 70%

*Compound **3.23***: Synthesized using the procedure for **3.19**. Compound **3.22** (7.66 g, 11.08 mmol, 1.0 equiv.), THF (70 mL), triethylamine trihydrogen fluoride (9.03 mL, 55.4 mmol, 5.0 equiv.), and trimethylmethoxy silane (7.66 mL, 55.4 mmol, 5.0 equiv.) were used. Product **3.23** was purified with flash chromatography by dissolving the crude product in the solvent mixture of EtOAc/MeOH 8:2, loading onto a column (SiO_2) and eluting with the same solvent mixture: 4.00 g, 70%; yellow oil; TLC $R_f = 0.3$ (SiO_2, EtOAc/MeOH 8:2); ^{1}H NMR (500 MHz, CD_3OD. Some peaks were buried in the peaks of Et_3NH^+ and H_2O. Attempts to remove these impurities were unsuccessful.) δ 1.83-1.96 (m, 2H), 2.24-2.27 (m, 2H), 2.40-2.45 (m, 1H), 2.69-2.74 (m, 1H), 2.80-2.91 (m, 2H), 2.94-3.06 (m, 4H), 3.51-3.52 (m, 4H), 3.62 (m, 10H), 3.76-3.81 (m, 2H), 3.89-3.95 (m, 1H), 4.01-4.4.11 (m, 1H), 5.27-5.31 (m, 1H), 5.50 (brs, 1H), 6.32 (t, $J = 6.9$ Hz, 1H), 7.10-7.24 (m, 1H), 8.10 (s, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 21.6, 29.2, 29.6, 29.8, 38.2, 40.0, 58.0, 62.4, 63.16, 63.17, 69.9, 70.2, 71.7, 72.5, 84.4, 86.5, 88.2, 88.4, 115.4, 116.5, 136.9, 151.2, 152.4, 153.0, 153.3, 157.8, 157.9; HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_5O_{11}S_2Na$ $[M + Na]^+$ 714.2455, found 714.2456.



3.23 **3.24**, 58%

*Compound **3.24***: Synthesized using the procedure for **3.14**. Compound **3.23** (3.60 g, 5.21 mmol, 1.0 equiv.), pyridine (40 mL) and DMTrCl (1.94 g, 5.73 mmol, 1.1 equiv.) were used. Product **3.24** was purified with flash column chromatography (SiO_2, EtOAc/MeOH 8:2 with 5% Et_3N): 3.00 g, 58%; pale white foam; TLC R_f = 0.45 (SiO_2, EtOAc/MeOH 8:2 with 5% Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55-1.63 (m, 2H), 1.75-1.80 (m, 1H), 1.86-1.92 (m, 1H), 1.97-2.05 (m, 2H), 2.51-2.74 (m, 3H), 2.84-2.99 (m, 2H), 3.07-3.15 (m, 1H), 3.34 (s, 3H), 3.34-3.80 (m, 20H), 3.98-4.22 (m, 3H), 4.70-4.77 (m, 1H), 5.19-5.29 (m, 1H), 6.17-6.32 (m, 1H), 6.23-6.28 (m, 1H), 6.72 (d, J = 8.3 Hz, 4H), 7.05-7.45 (m, 9H), 7.85 (d, J = 7.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.5, 25.6, 27.9, 28.0, 28.2, 28.3, 28.6, 28.7, 40.5, 48.4, 48.6, 55.2, 59.0, 60.4, 64.2, 70.09, 70.12, 70.39, 70.45, 70.48, 70.49, 71.77, 71.84, 76.5, 84.2, 86.27, 86.29, 86.5, 113.1, 120.6, 120.7, 126.8, 127.7, 128.1, 129.96, 130.00, 135.7, 137.6, 144.6, 146.85, 146.87, 148.4, 148.5, 153.89, 153.99, 155.8, 158.40, 158.42; HRMS (ESI) *m/z* calcd for $C_{49}H_{63}N_5O_{13}S_2Na$ $[M + Na]^+$ 1016.3761, found 1016.3763.



*Compound **3.1c***: Synthesized using the procedure for **3.1a**. Compound **3.24** (2.455 g, 2.47 mmol, 1.0 equiv.), DCM (40 mL), diisopropylammonium tetrazolide (**3.16**, 633.55 mg, 3.70 mmol, 1.5 equiv.) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**3.15**, 1.17 mL, 3.70 mmol, 1.5 equiv.) were used. Product **3.1c** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 9:1 with 5% Et_3N, loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 1.81 g, 62%; pale yellow foam; TLC R_f = 0.4 and 0.5 (SiO_2, EtOAc/MeOH 9:1 with 5% Et_3N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16-1.24 (m, 12H), 1.56-2.15 (m, 6H), 2.37-3.03 (m, 8H), 3.25-3.40 (m, 2H), 3.39 (s, 3H), 3.48-3.78 (m, 20H), 3.79 (s, 6H) 3.80-3.88 (m, 1H), 3.90-3.99 (m, 1H), 4.15-4.32 (m, 1.5H), 4.50-4.54 (m, 0.5H), 4.65-4.79 (m, 1H), 5.20-5.28 (m, 1H), 6.22 (t, J = 6.9 Hz, 0.5H), 6.24 (t, J = 6.6 Hz, 0.5H), 6.71-6.82 (m, 4H), 7.15-7.35 (m,

7H), 7.35-7.46 (m, 2H), 7.80 (s, 0.5H), 7.81 (s, 0.5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.12, 20.18, 20.27, 20.31, 20.37, 20.45, 20.50, 24.57, 24.60, 24.62, 24.66, 25.46, 25.47, 27.8, 28.2, 28.73, 28.74, 43.2, 43.27, 43.33, 48.45, 55.21, 55.23, 55.25, 55.27, 58.13, 58.25, 58.40, 59.0, 63.6, 70.2, 70.3, 70.4, 70.5, 70.6, 70.7, 71.9, 76.3, 76.5, 84.0, 84.5, 84.7, 85.9, 86.0, 86.3, 86.4, 86.5, 113.0, 113.1, 113.2, 113.4, 117.4, 117.5, 121.4, 121.57, 121.64, 127.0, 127.83, 127.88, 128.10, 128.15, 128.22, 129.97, 130.01, 130.06, 130.08, 13.60, 135.63, 135.67, 135.69, 135.78, 137.01, 137.02, 137.05, 144.46, 144.48, 144.51, 146.49, 146.50, 148.14, 148.21, 150.1, 151.3, 153.4, 155.7, 158.5; ^{31}P NMR (202 MHz, $CDCl_3$) δ 147.63, 148.22, 148.41, 148.75; HRMS (ESI) *m/z* calcd for $C_{58}H_{80}N_7O_{14}PS_2H$ $[M + H]^+$ 1194.5020, found 1194.5052.

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5 Appendix A. Supporting information for chapter 2

Oligonucleotide synthesis under mild deprotection conditions

5.1 HPLC, MALDI MS and OD_{260}, Capillary electrophoresis, Trityl assay log, Gel electrophoresis

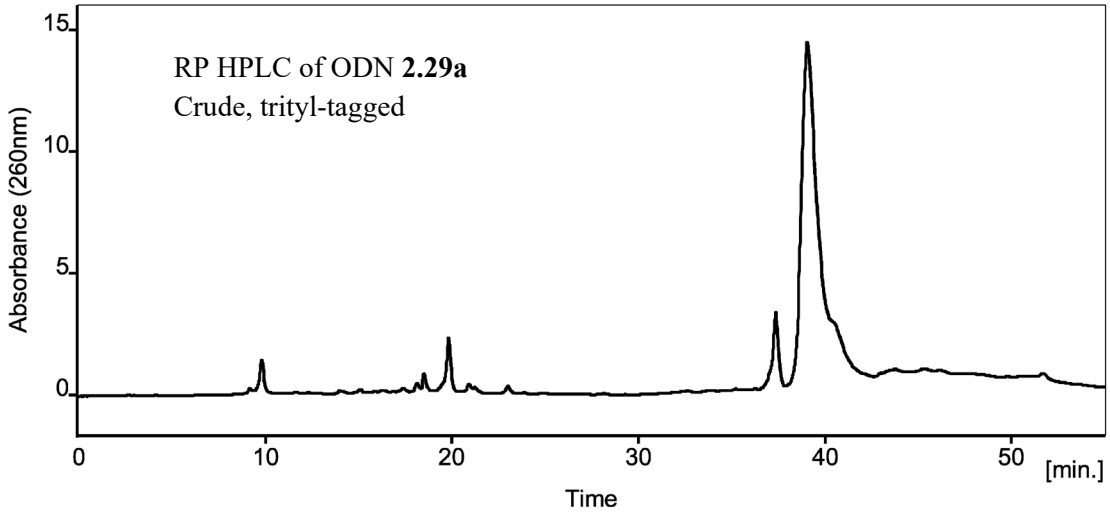


Figure 5.1: RP HPLC of crude trityl-tagged ODN **2.29a**.

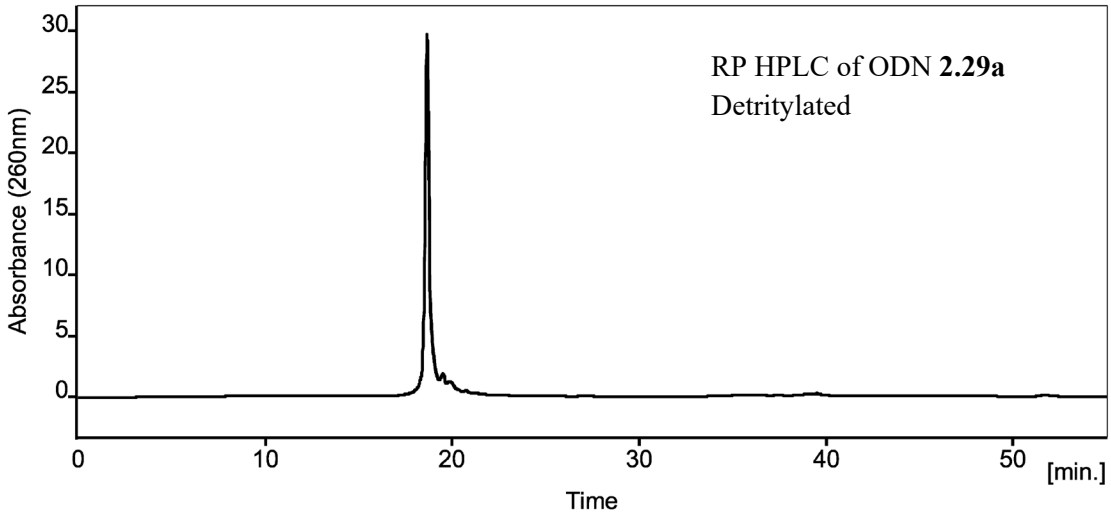


Figure 5.2: RP HPLC of detritylated ODN **2.29a**.

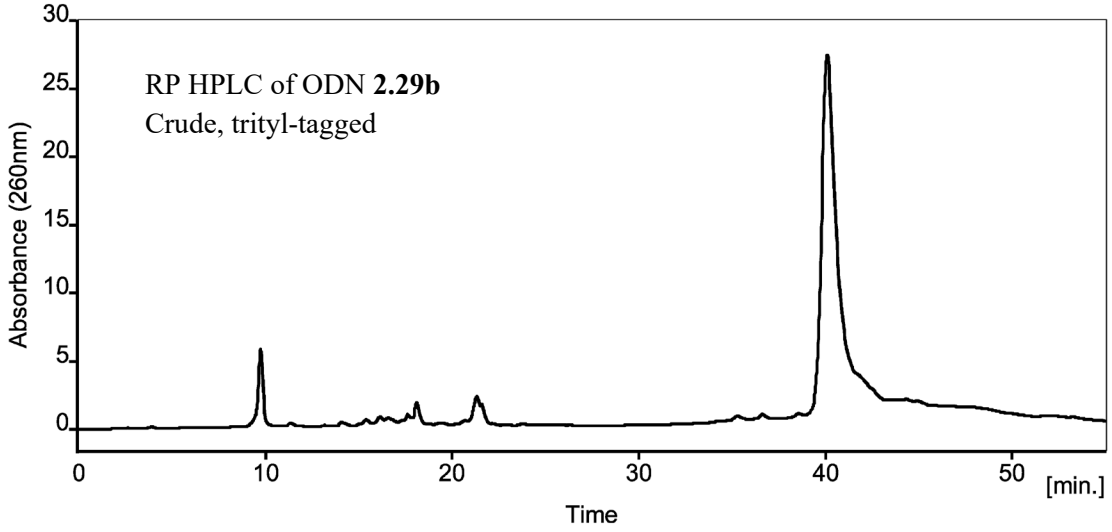


Figure 5.3: RP HPLC of crude trityl-tagged ODN **2.29b**.

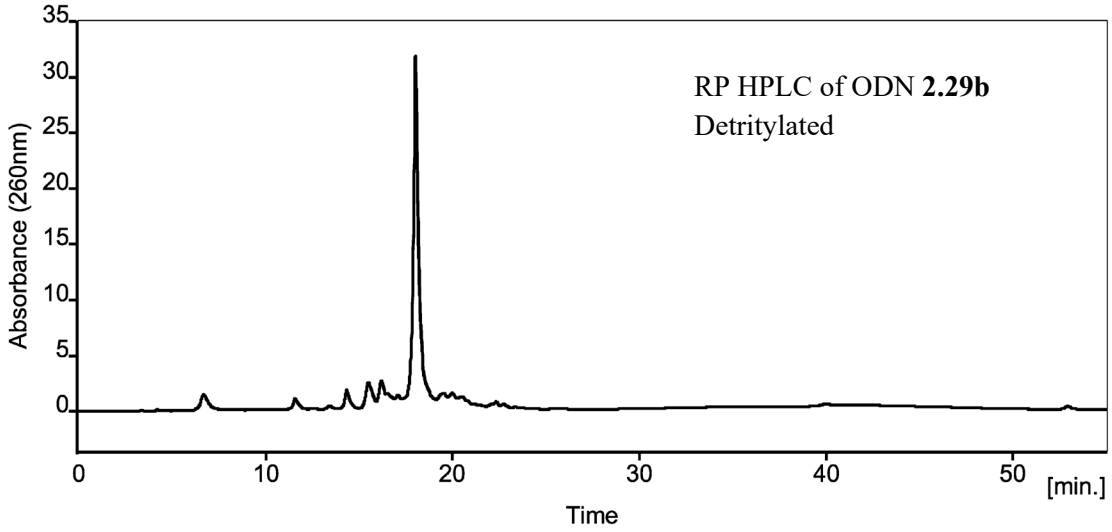


Figure 5.4: RP HPLC of detritylated ODN **2.29b**.

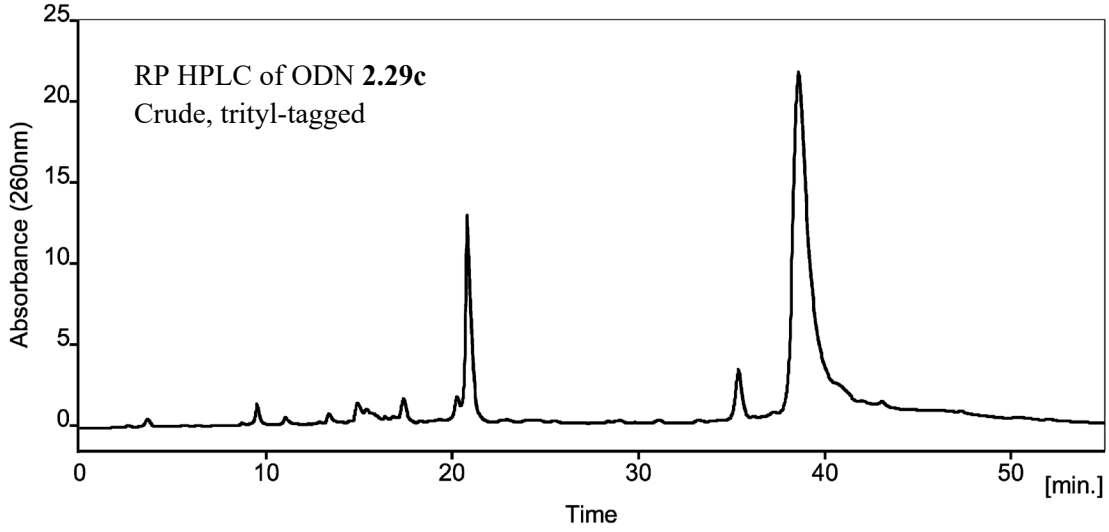


Figure 5.5: RP HPLC of crude trityl-tagged ODN **2.29c**.

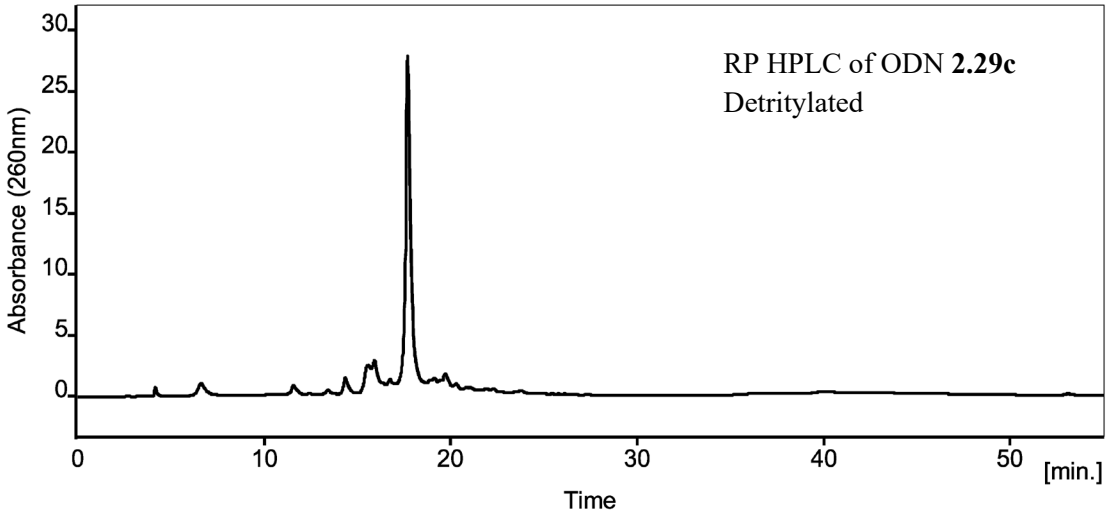


Figure 5.6: RP HPLC of detritylated ODN **2.29c**.

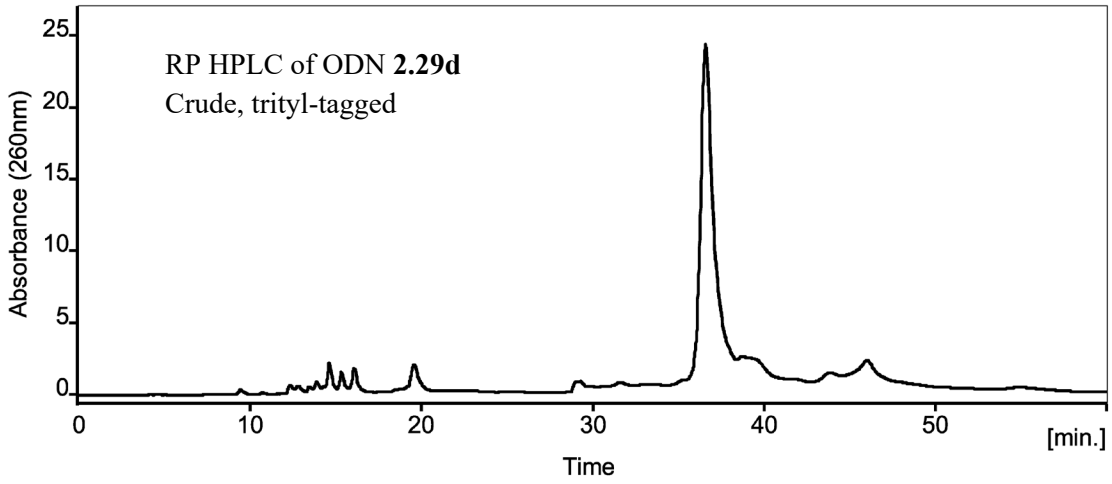


Figure 5.7: RP HPLC of crude trityl-tagged ODN **2.29d**.

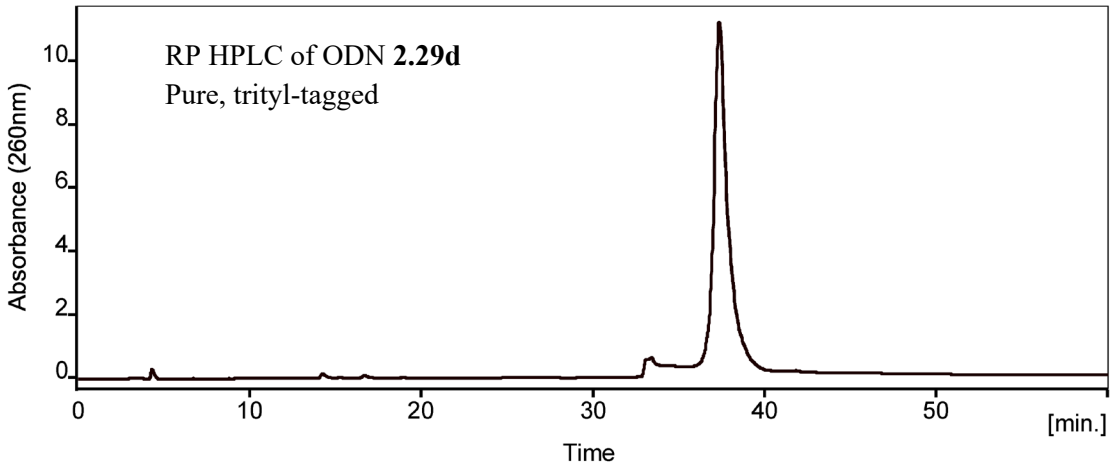


Figure 5.8: RP HPLC of pure trityl-tagged ODN **2.29d**.

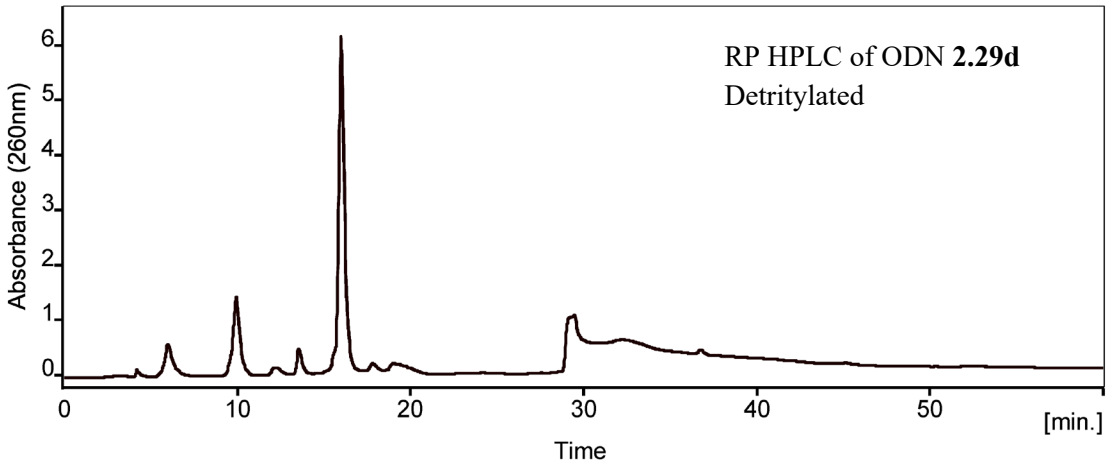


Figure 5.9: RP HPLC of detritylated ODN **2.29d**.

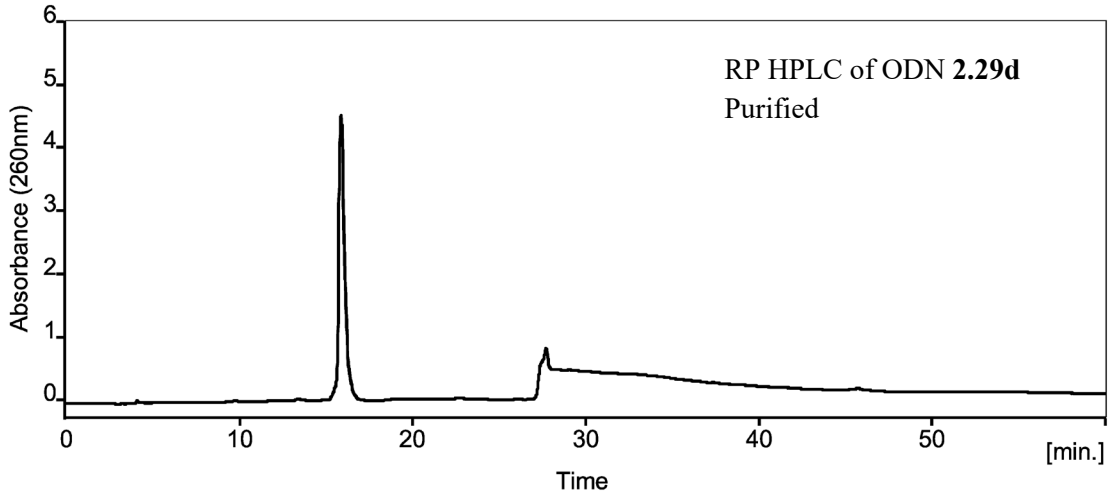


Figure 5.10: RP HPLC of pure ODN **2.29d**.

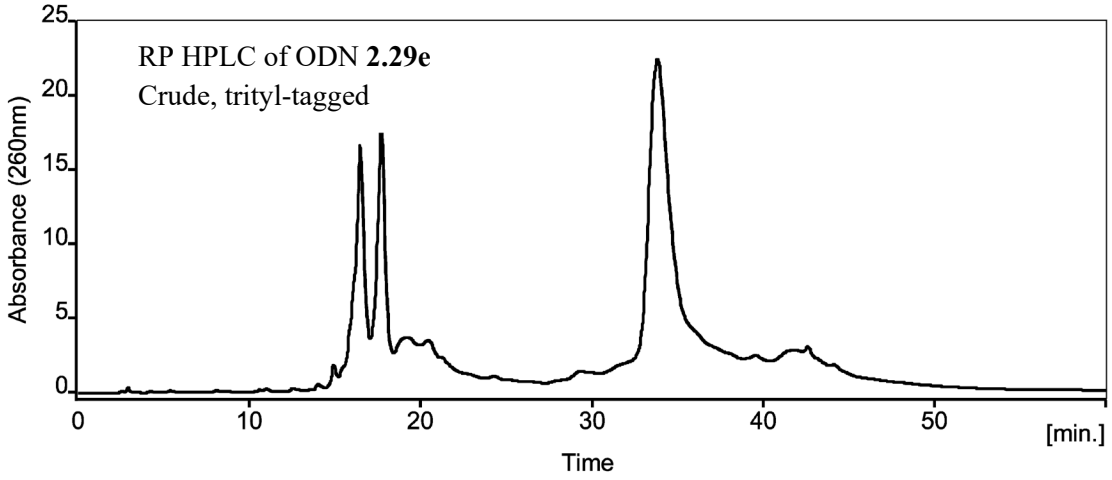


Figure 5.11: RP HPLC of crude trityl-tagged ODN **2.29e**.

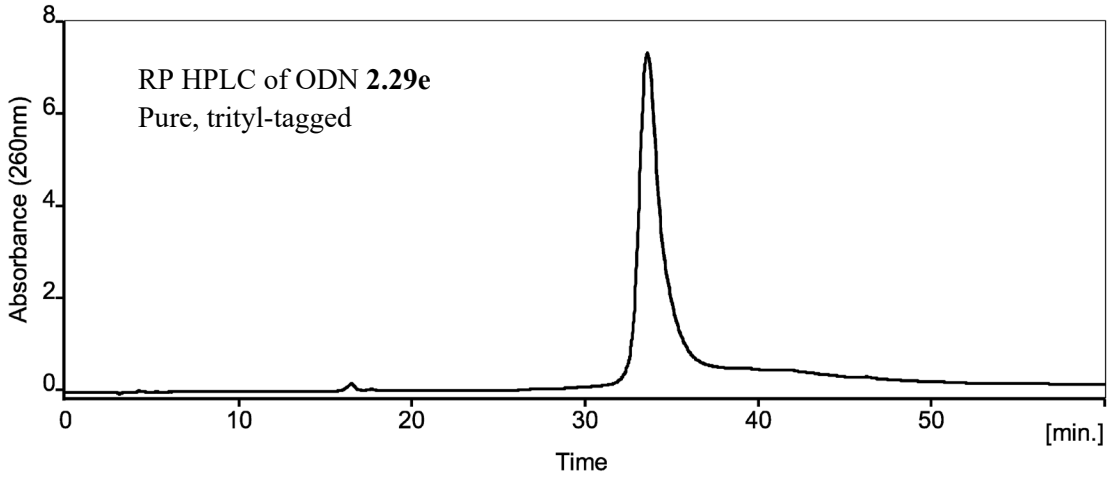


Figure 5.12: RP HPLC of pure trityl-tagged ODN **2.29e**.

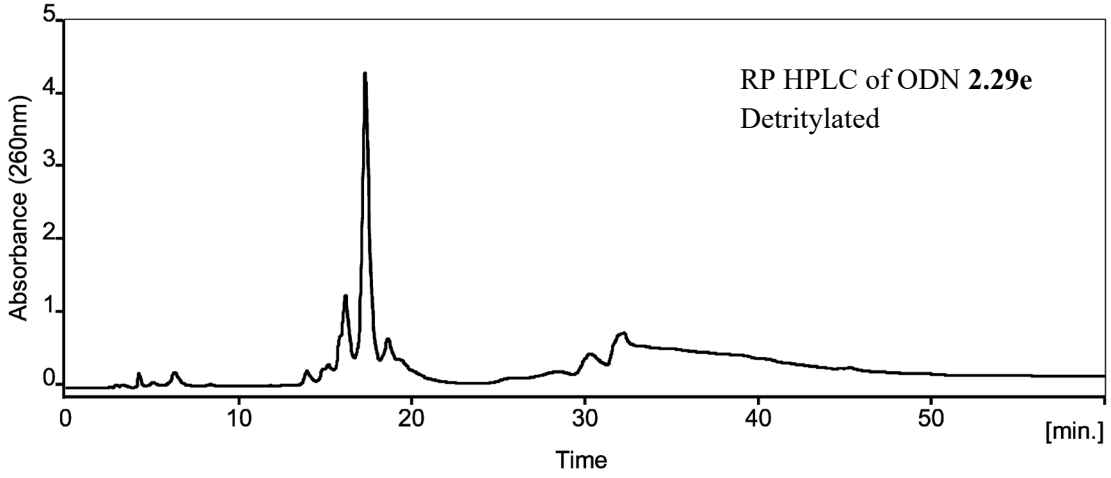


Figure 5.13: RP HPLC of detritylated ODN **2.29e**.

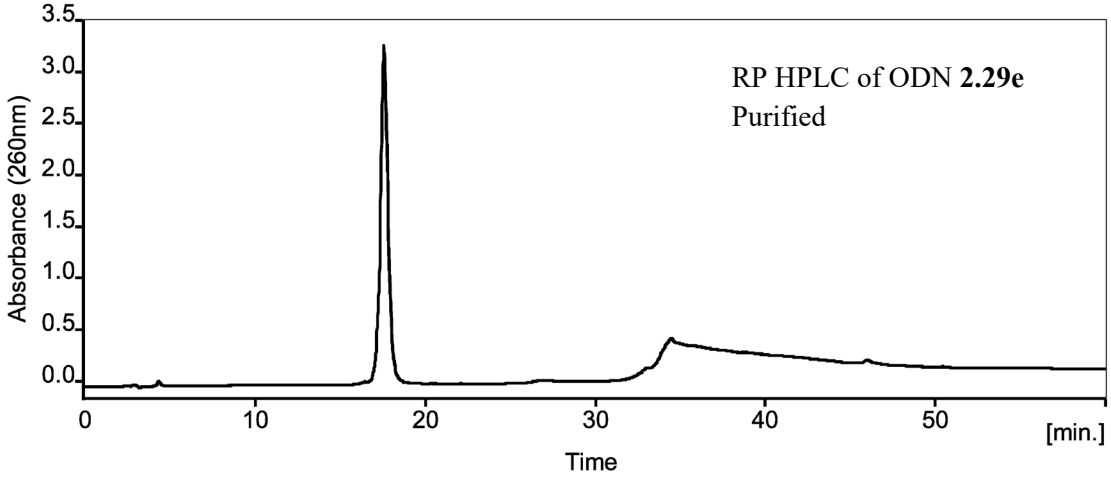


Figure 5.14: RP HPLC of pure ODN **2.29e**.

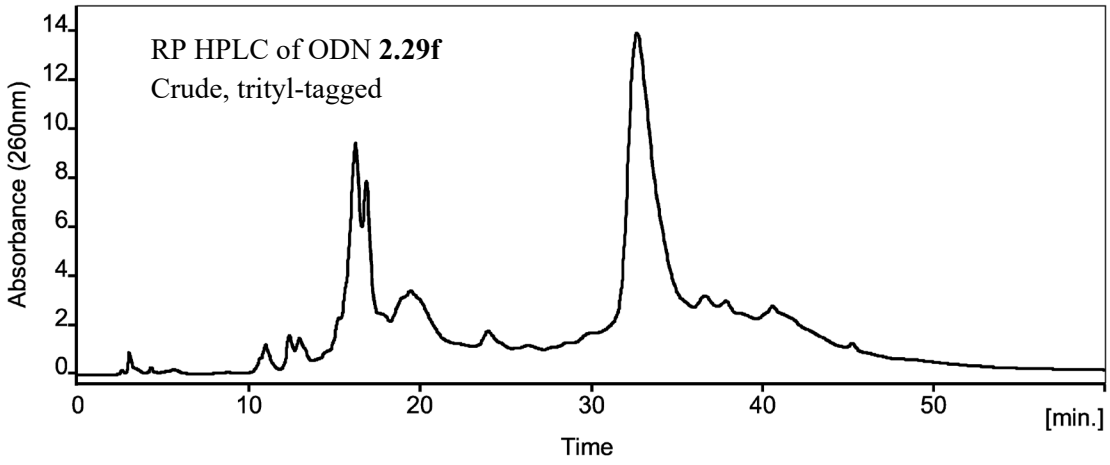


Figure 5.15: RP HPLC of crude trityl-tagged ODN **2.29f**.

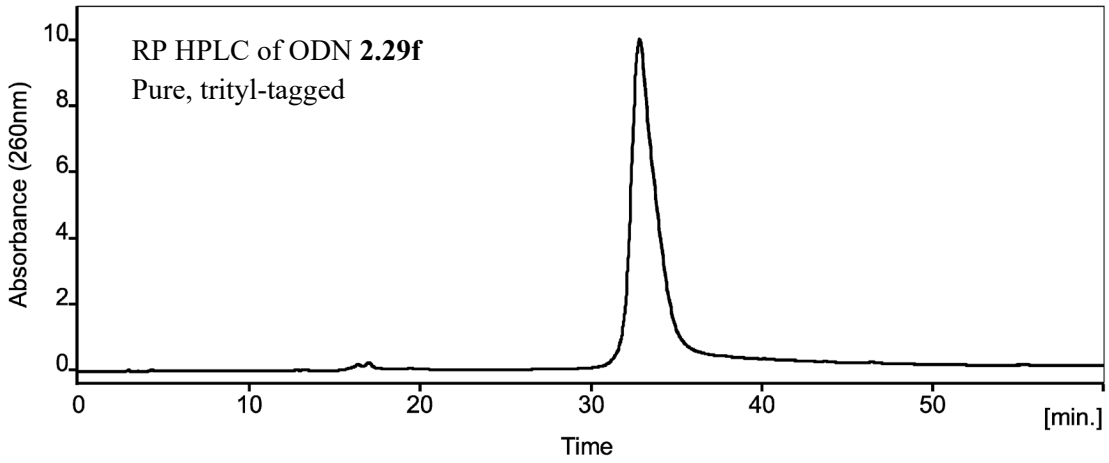


Figure 5.16: RP HPLC of pure trityl-tagged ODN **2.29f**.

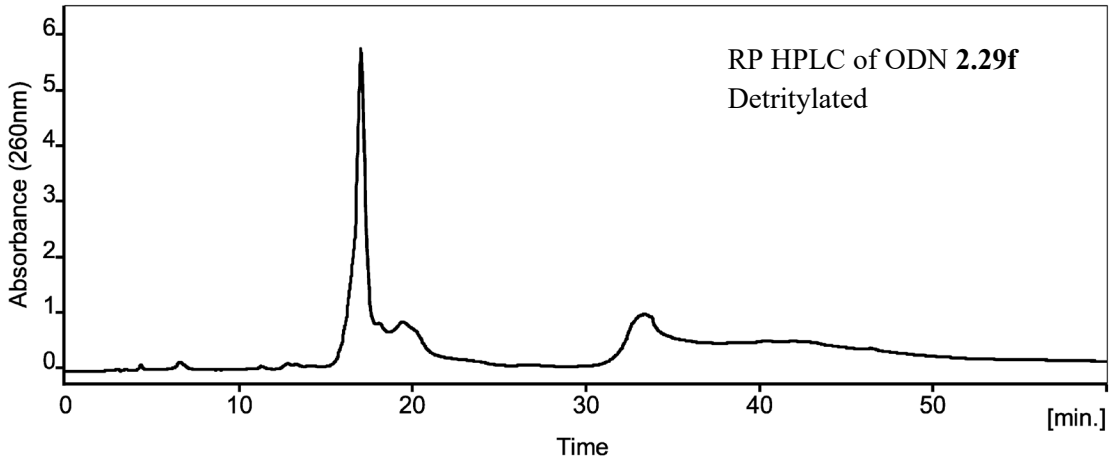


Figure 5.17: RP HPLC of detritylated ODN **2.29f**.

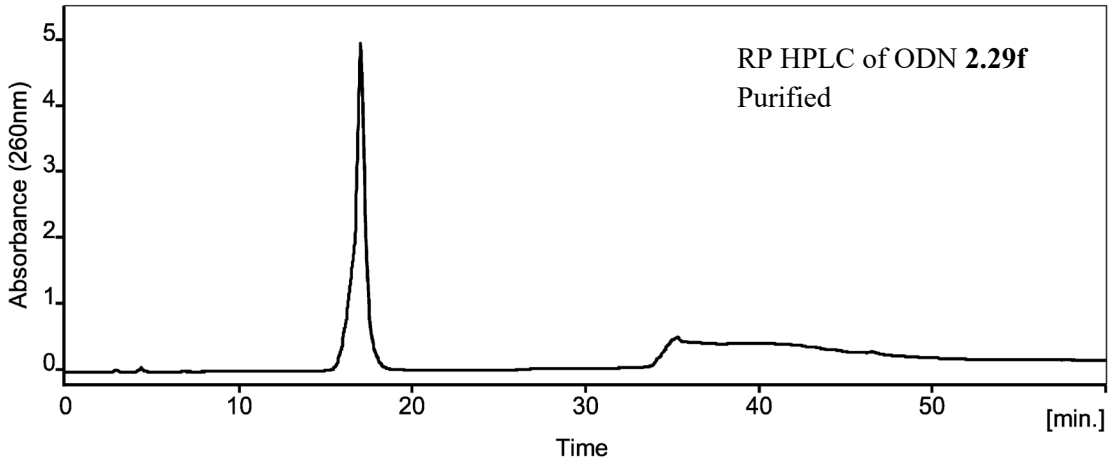


Figure 5.18: RP HPLC of pure ODN **2.29f**.

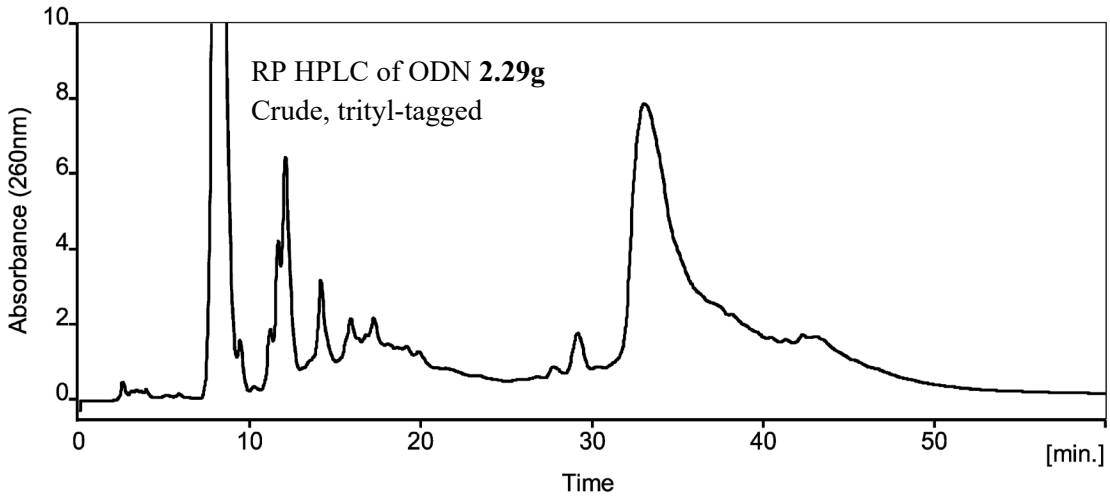


Figure 5.19: RP HPLC of crude trityl-tagged ODN **2.29g**.

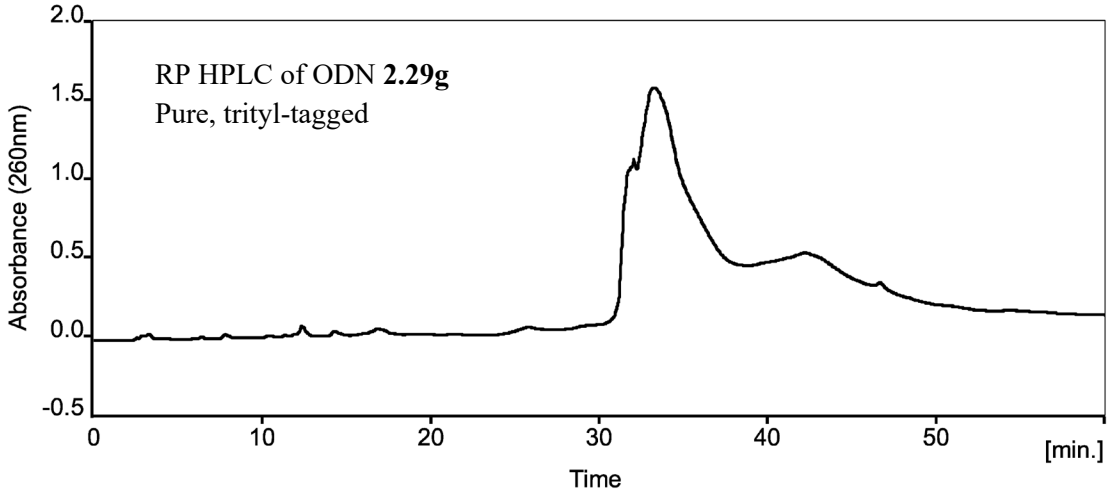


Figure 5.20: RP HPLC of pure trityl-tagged ODN **2.29g**.

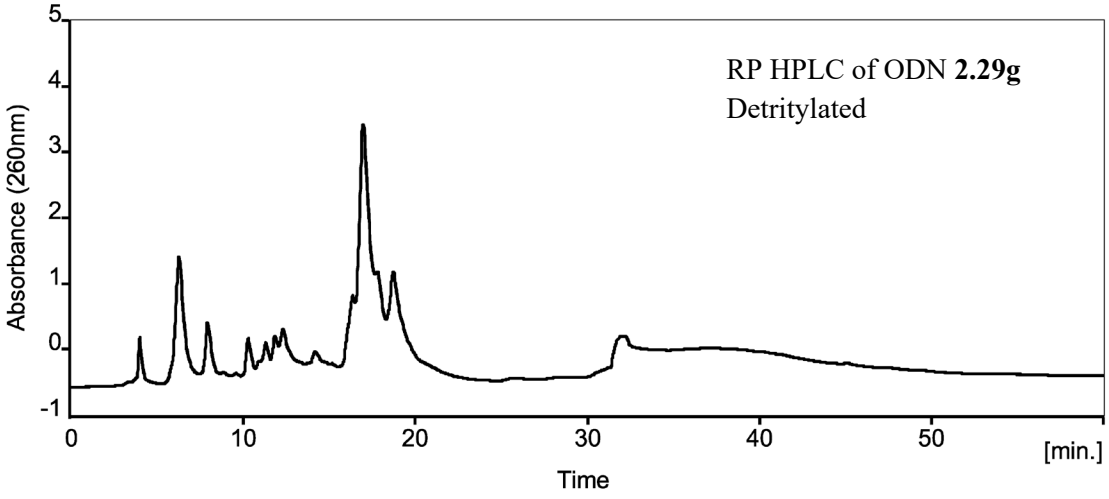


Figure 5.21: RP HPLC of detritylated ODN **2.29g**.

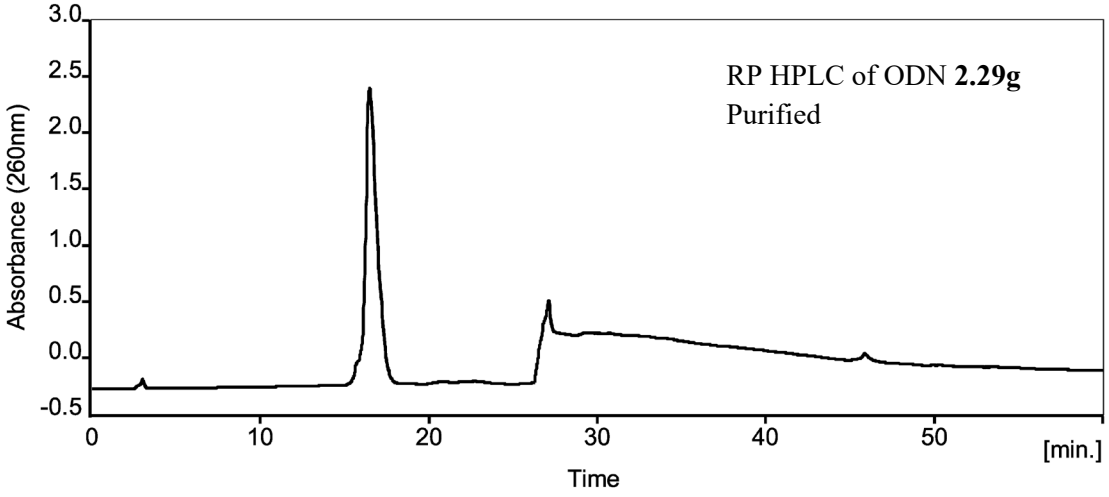


Figure 5.22: RP HPLC of pure ODN **2.29g**.

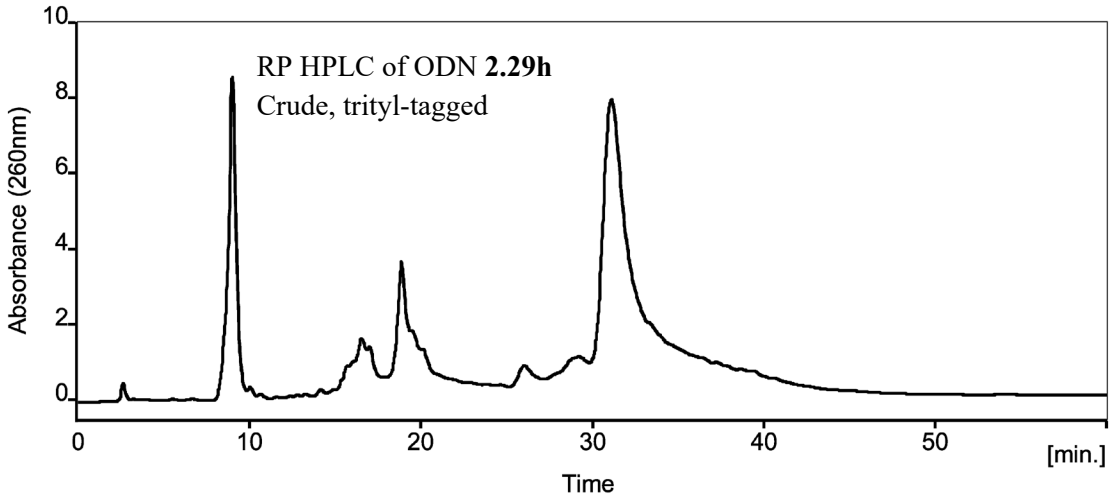


Figure 5.23: RP HPLC of crude trityl-tagged ODN **2.29h**.

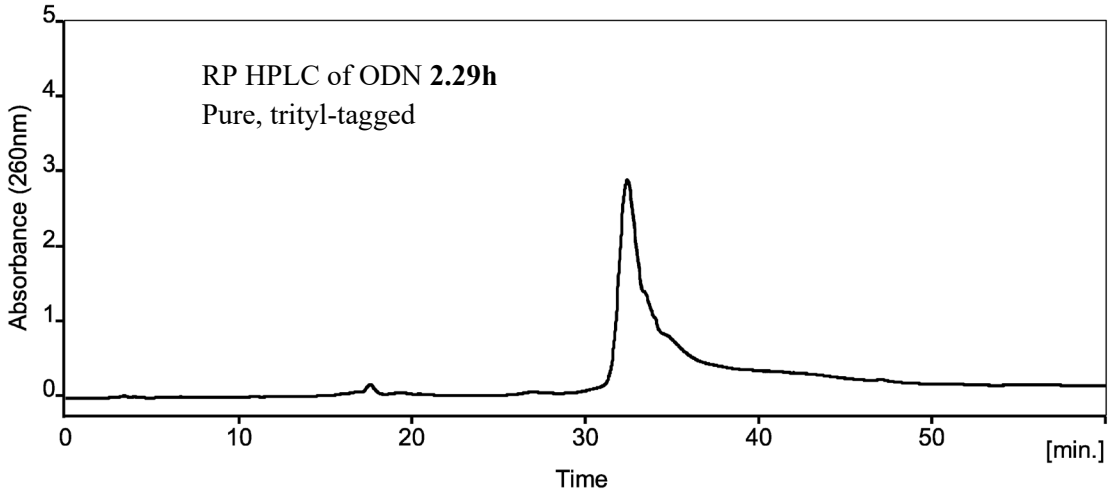


Figure 5.24: RP HPLC of pure trityl-tagged ODN **2.29h**.

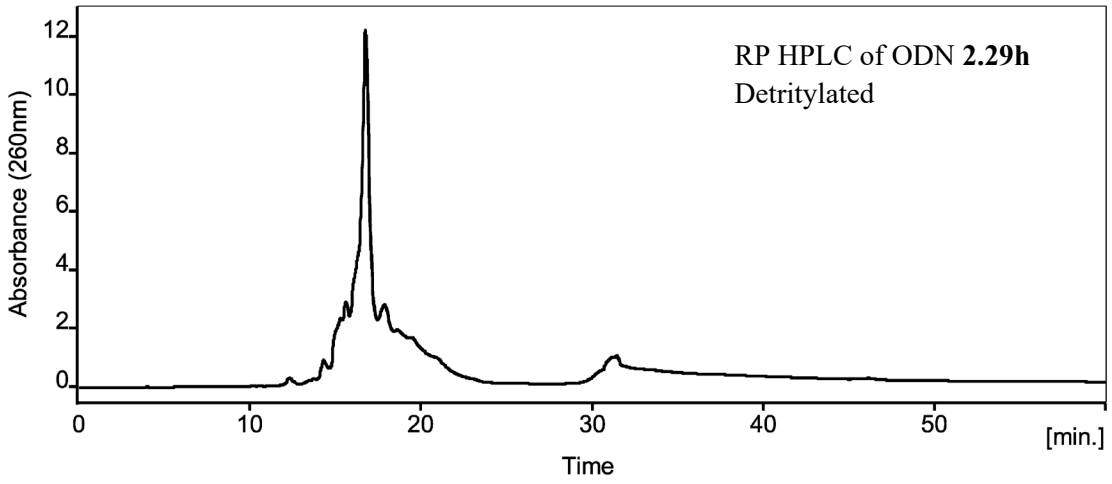


Figure 5.25: RP HPLC of detritylated ODN **2.29h**.

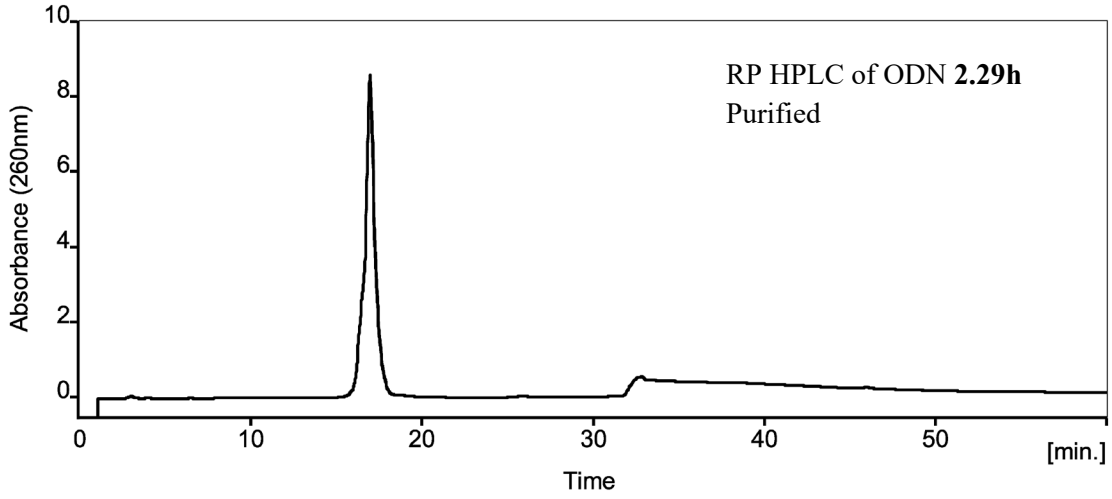


Figure 5.26: RP HPLC of pure ODN **2.29h**.

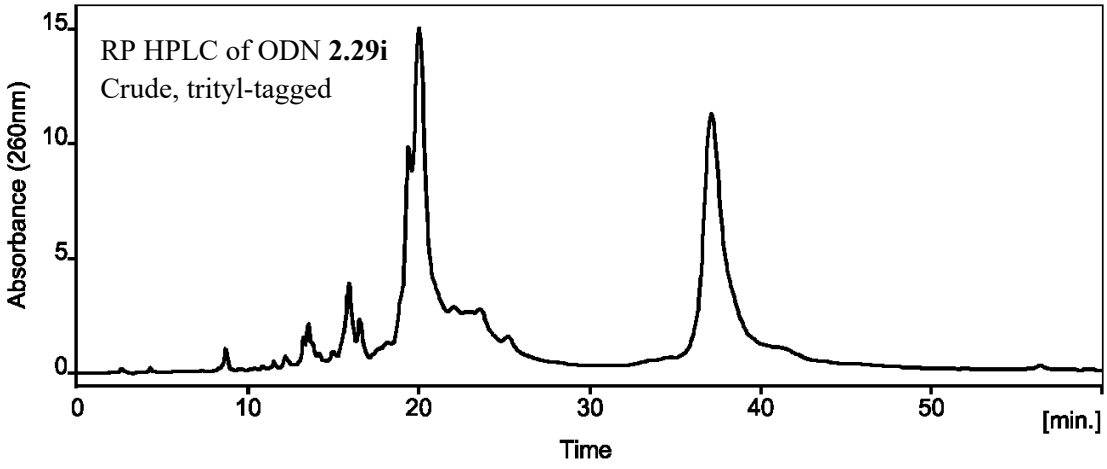


Figure 5.27: RP HPLC of crude trityl-tagged ODN **2.29i**.

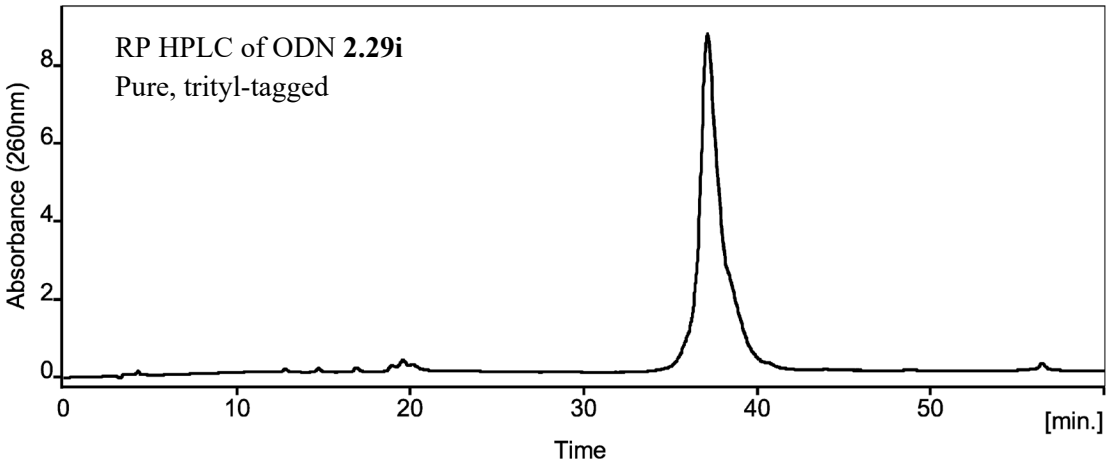


Figure 5.28: RP HPLC of pure trityl-tagged ODN **2.29i**.

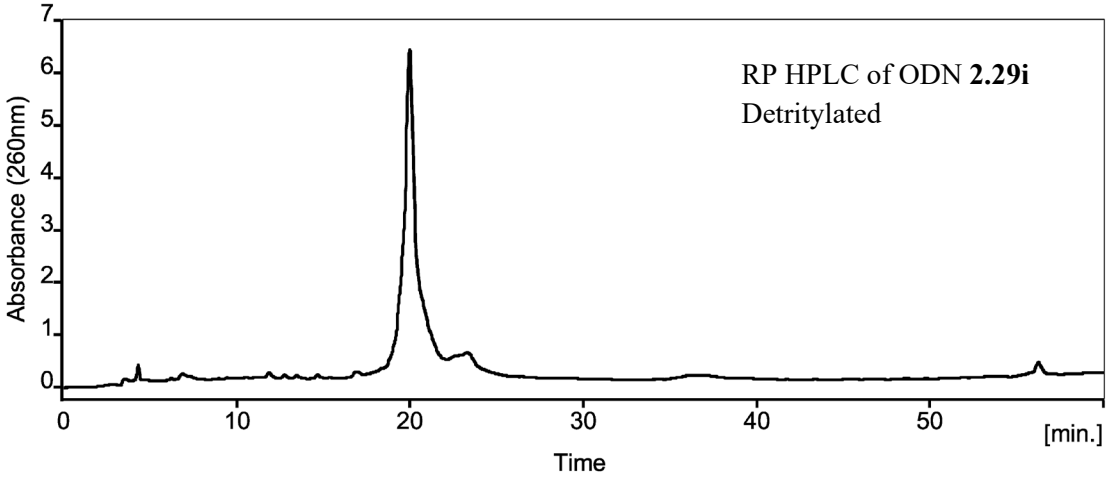


Figure 5.29: RP HPLC of detritylated ODN **2.29i**.

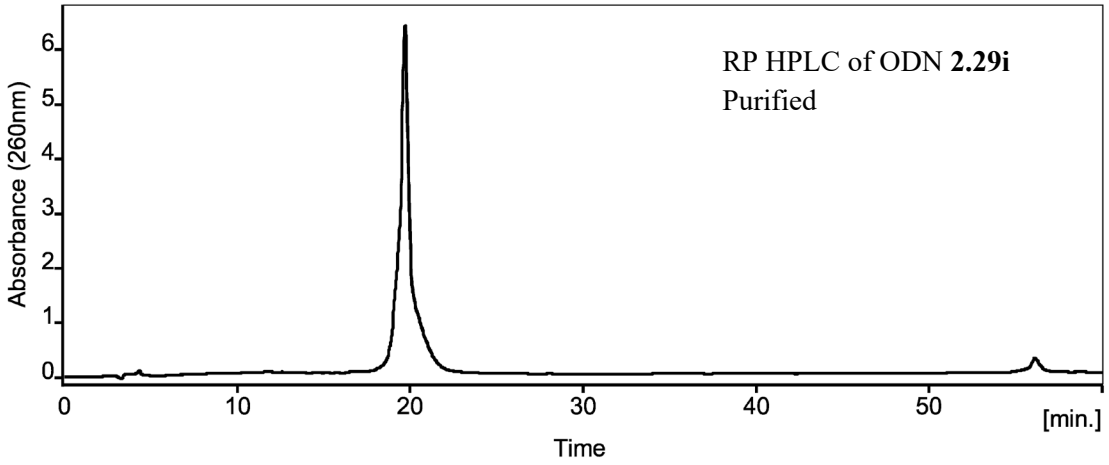


Figure 5.30: RP HPLC of pure ODN **2.29i**.

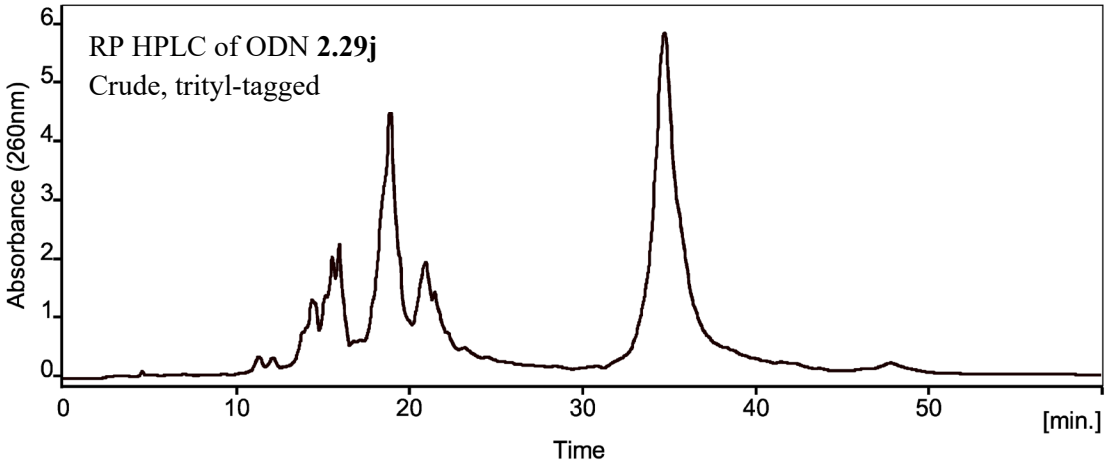


Figure 5.31: RP HPLC of crude trityl-tagged ODN **2.29j**.

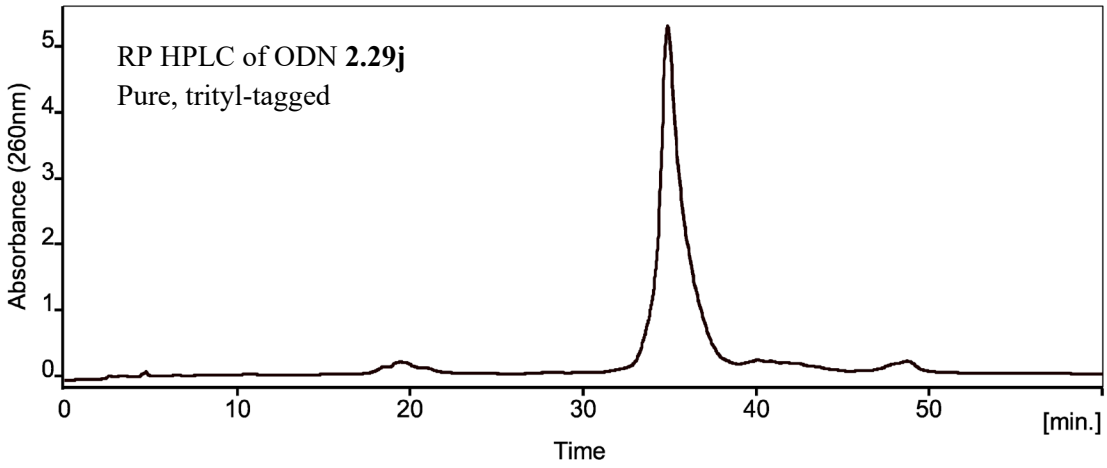


Figure 5.32: RP HPLC of pure trityl-tagged ODN **2.29j**.

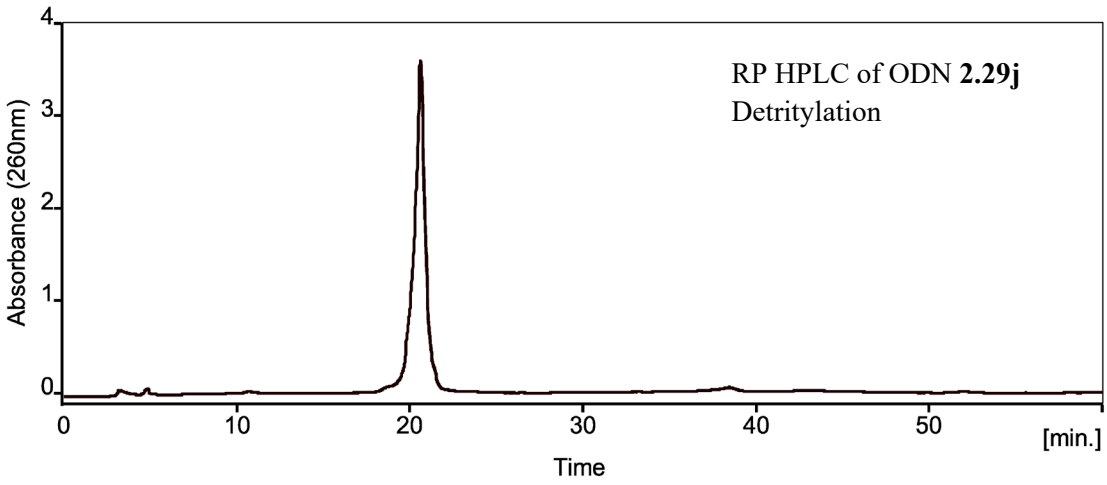


Figure 5.33: RP HPLC of detritylated ODN **2.29j**.

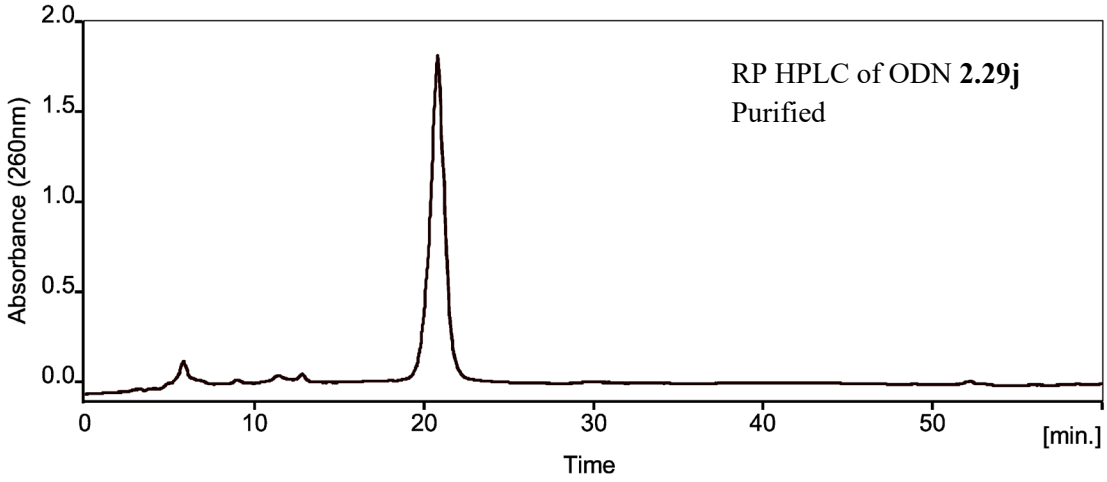


Figure 5.34: RP HPLC of pure ODN **2.29j**.

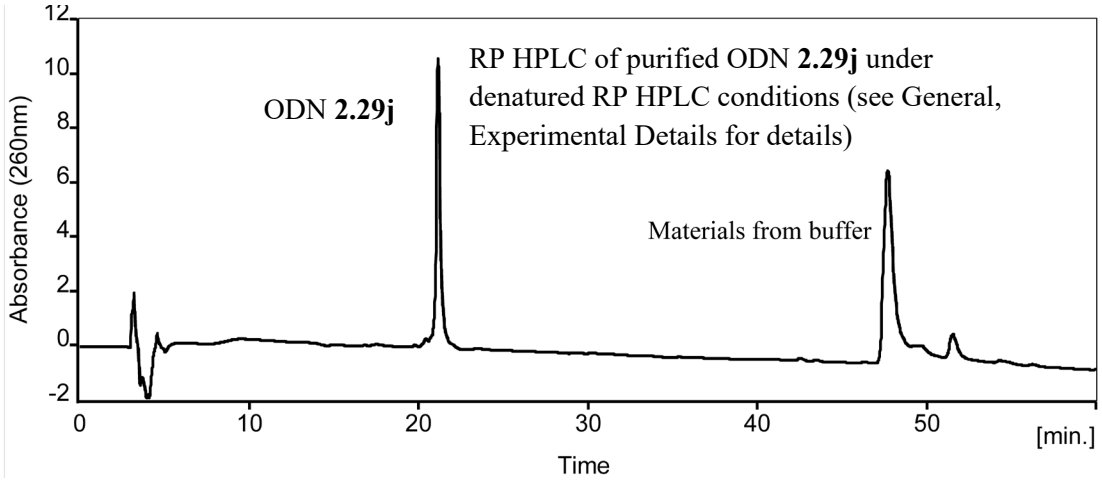


Figure 5.35: RP HPLC of pure ODN **2.29j** under denatured RP HPLC conditions.

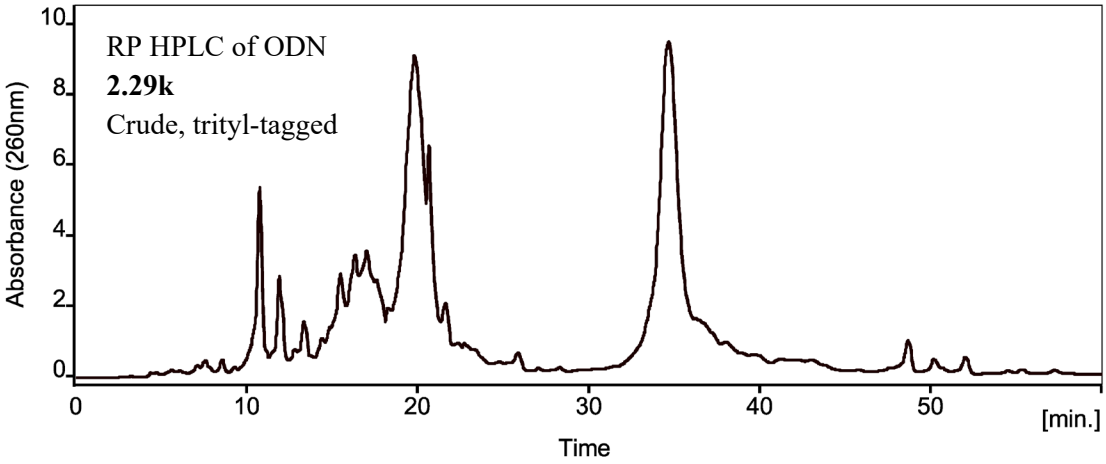


Figure 5.36: RP HPLC of crude trityl-tagged ODN **2.29k**.

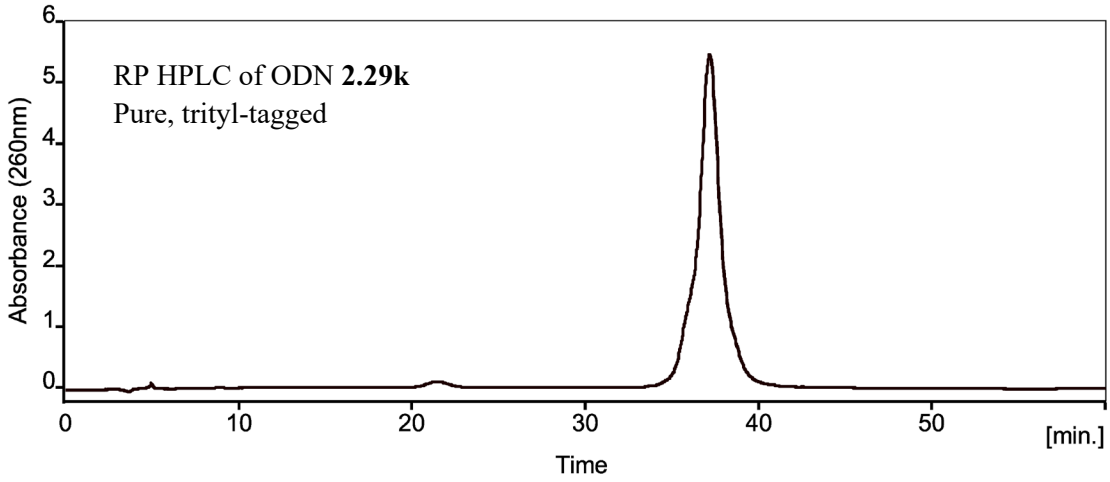


Figure 5.37: RP HPLC of pure trityl-tagged ODN **2.29k**.

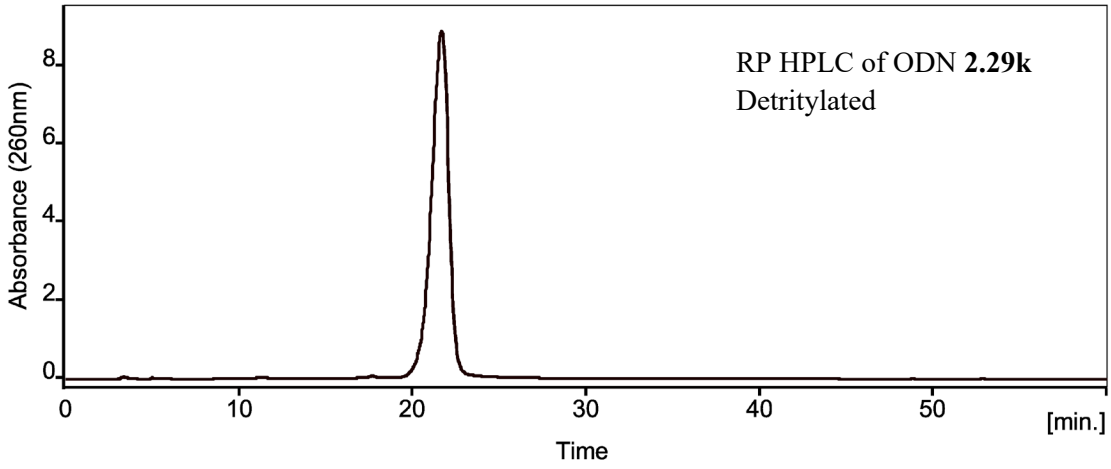


Figure 5.38: RP HPLC of detritylated ODN **2.29k**.

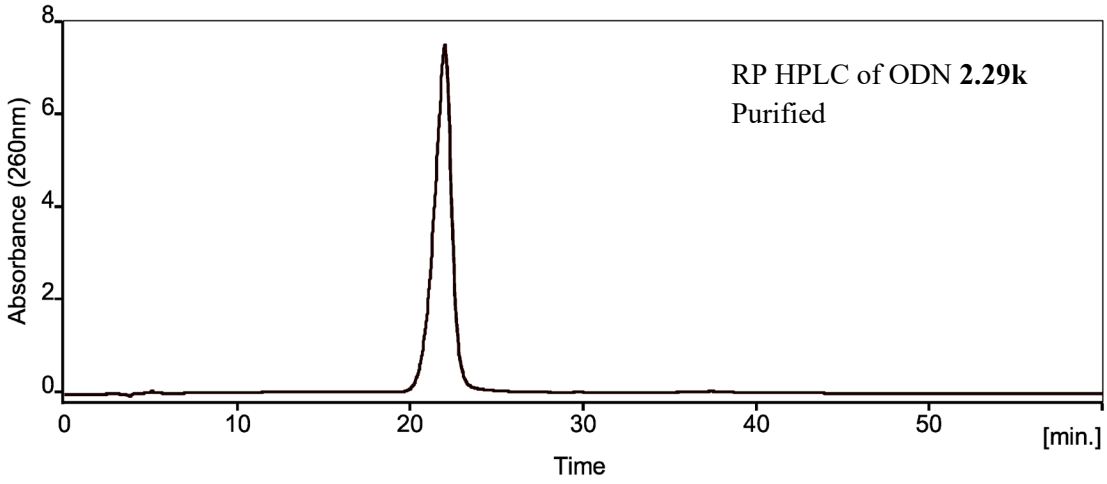


Figure 5.39: RP HPLC of pure ODN **2.29k**.

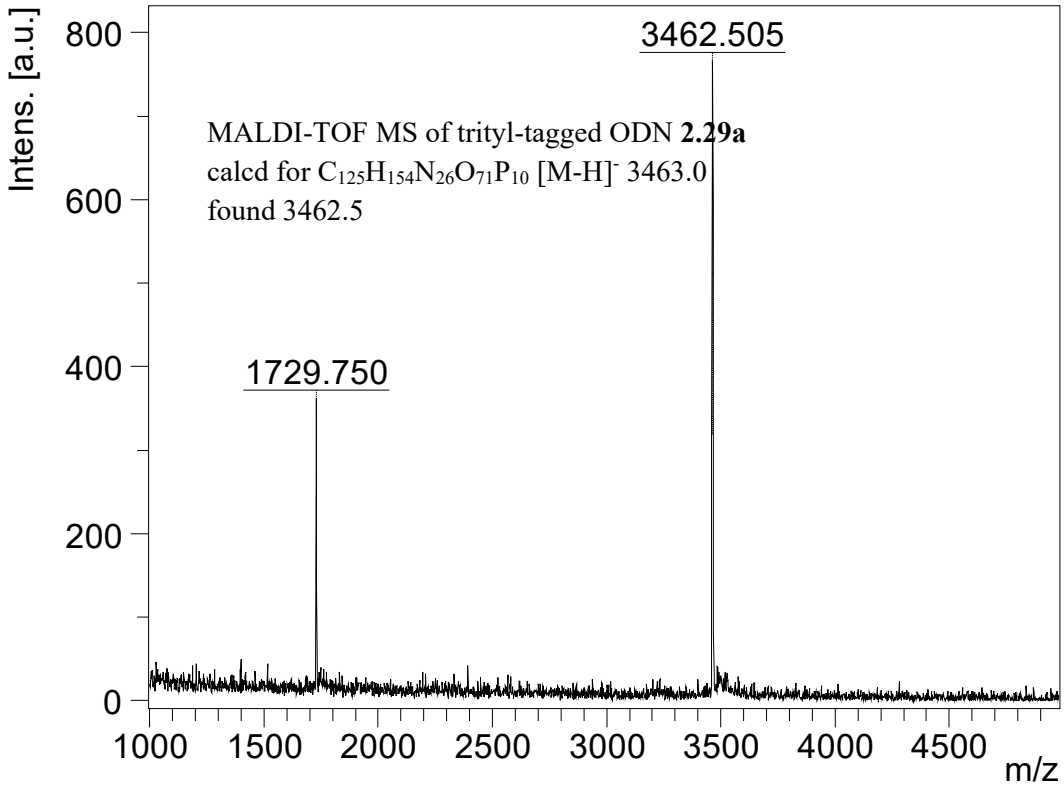


Figure 5.40: MALDI-TOF MS of trityl-tagged ODN **2.29a**.

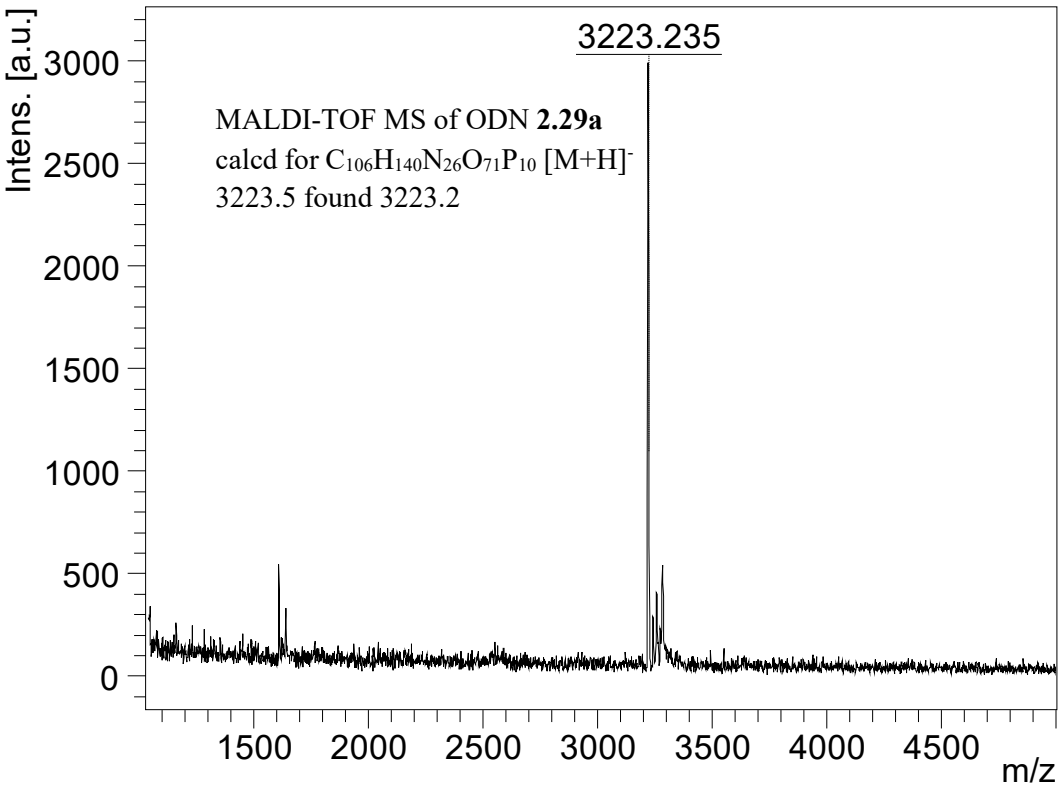


Figure 5.41: MALDI-TOF MS of ODN **2.29a**.

OD_{260} of ODN **2.29a** for the 0.52 μmol synthesis is 2.940.

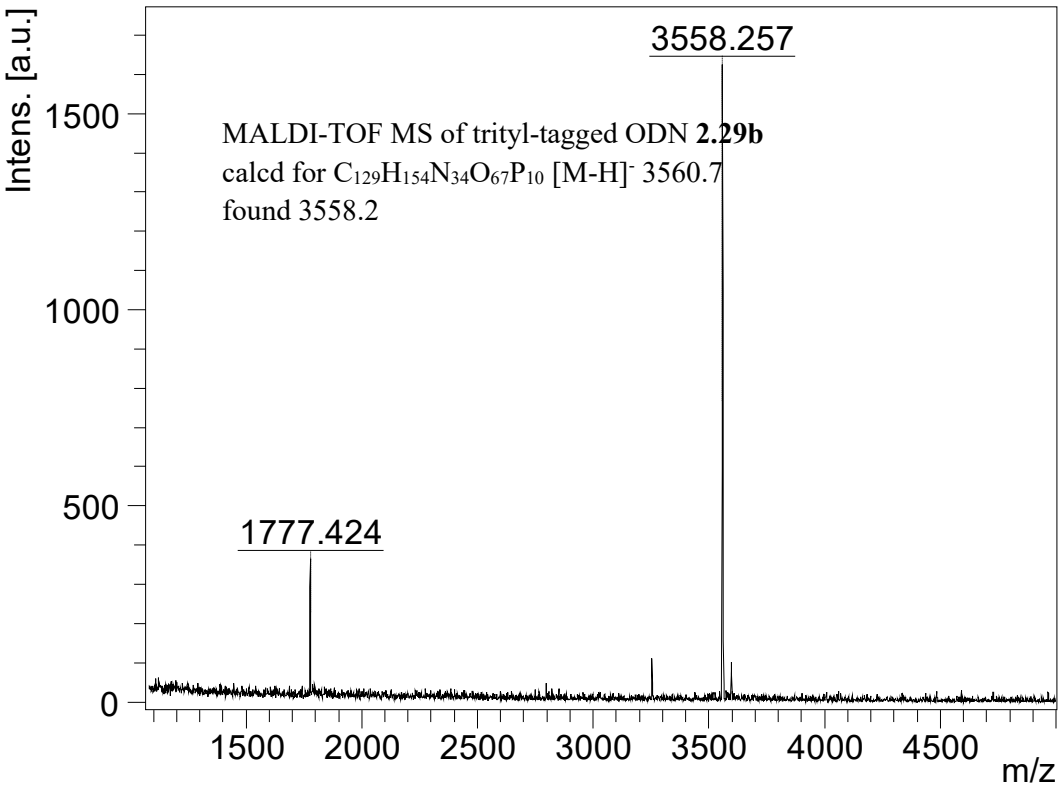


Figure 5.42: MALDI-TOF MS of trityl-tagged ODN **2.29b**.

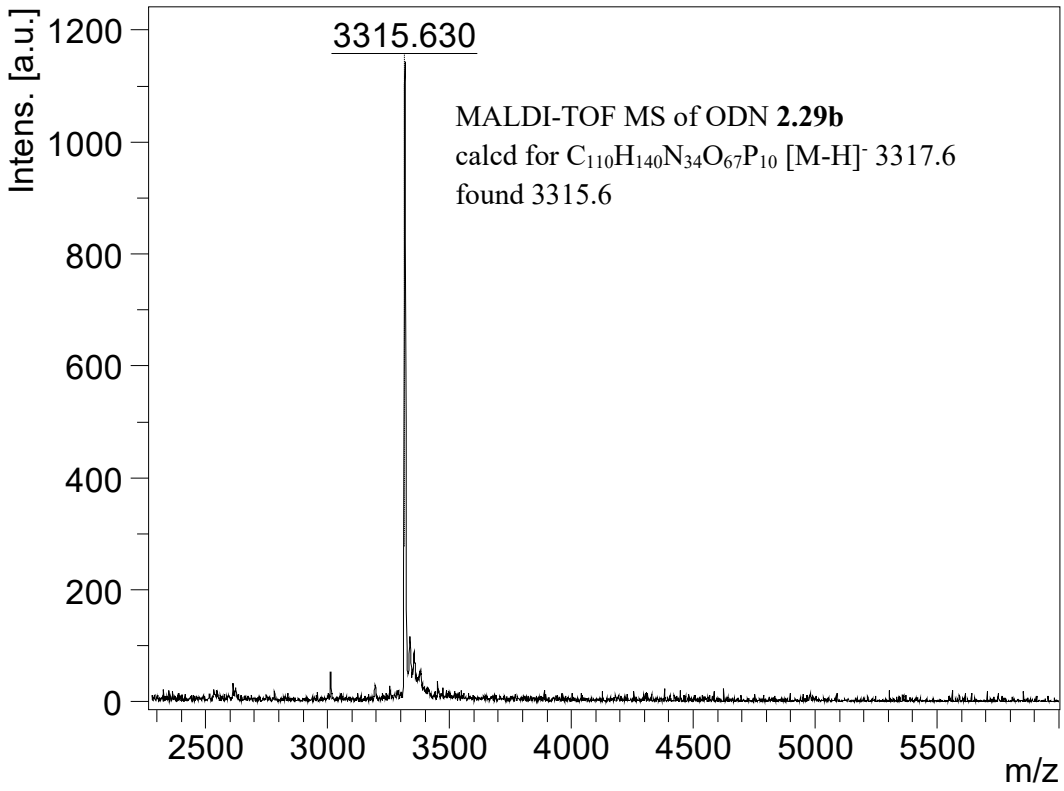


Figure 5.43: MALDI-TOF MS of ODN **2.29b**.

OD_{260} of ODN **2.29b** for the 0.52 μmol synthesis is 3.660.

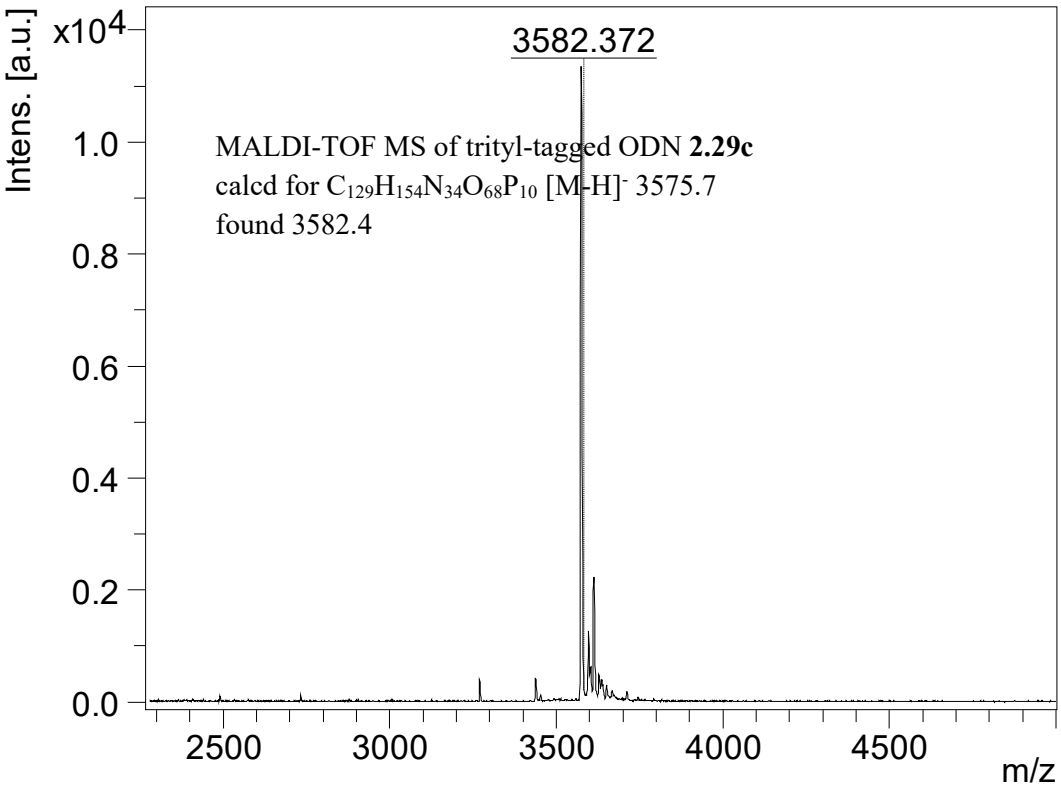


Figure 5.44: MALDI-TOF MS of trityl-tagged ODN **2.29c**.

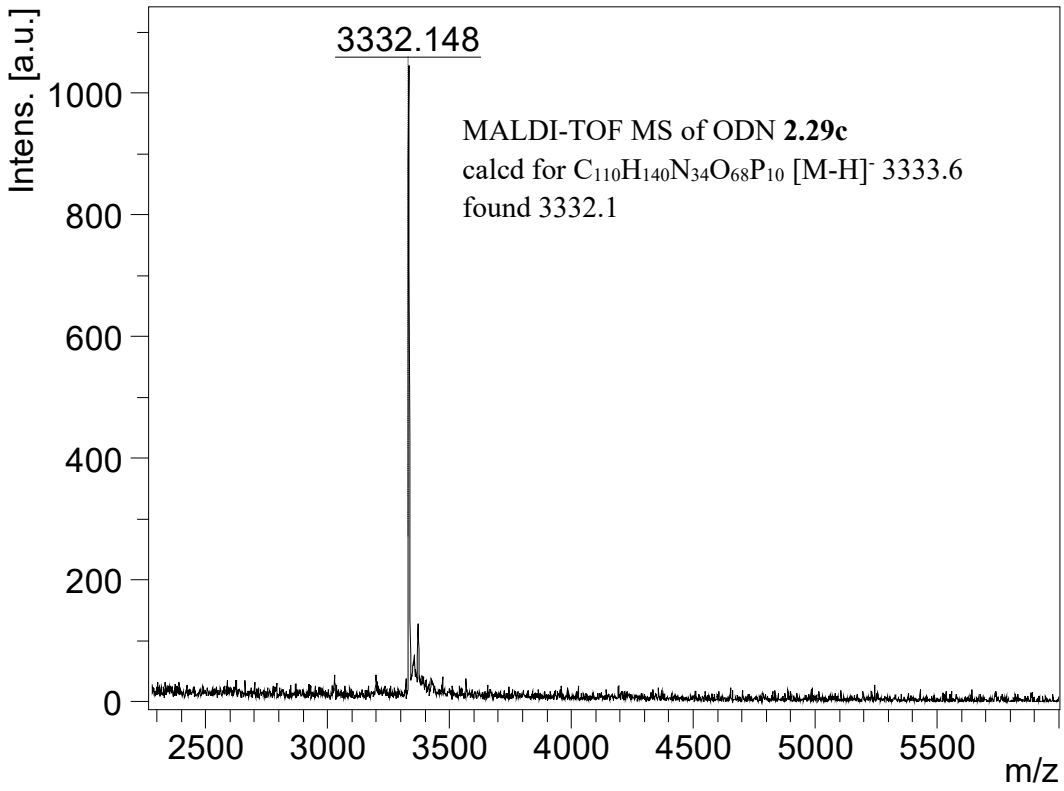


Figure 5.45: MALDI-TOF MS of ODN **2.29c**.

OD_{260} of ODN **2.29c** for the 0.52 μmol synthesis is 3.092.

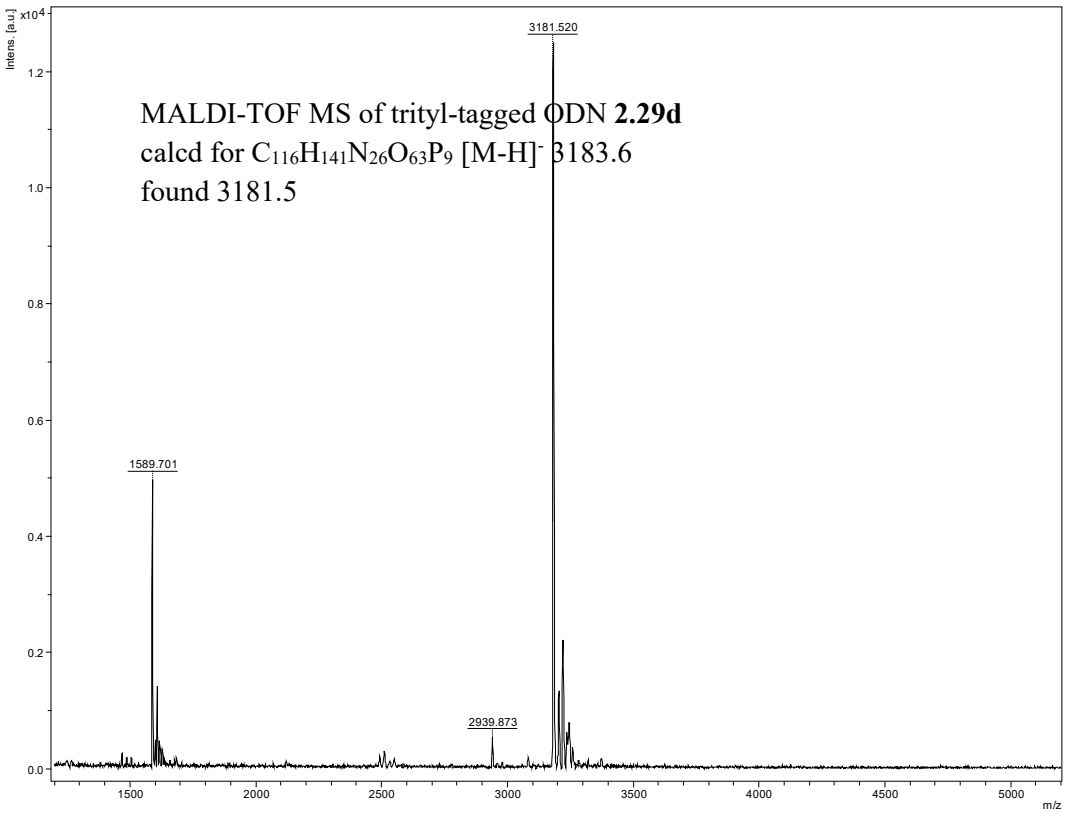


Figure 5.46: MALDI-TOF MS of trityl-tagged ODN **2.29d**.

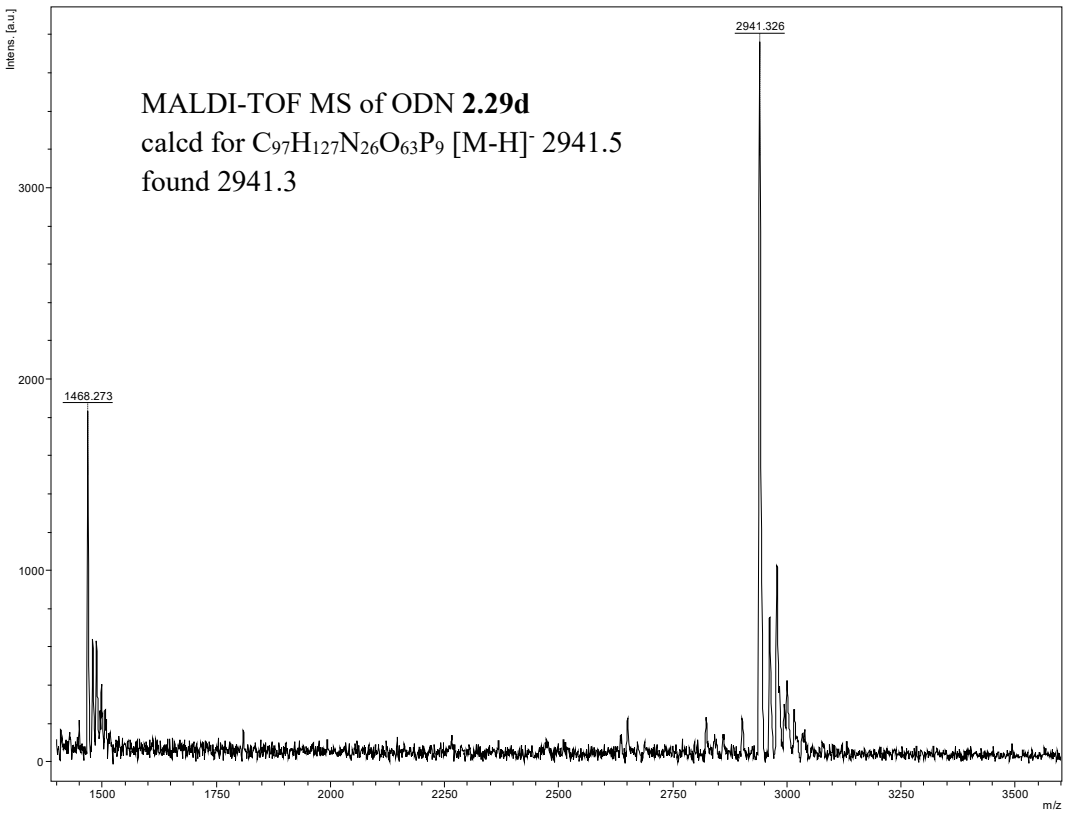


Figure 5.47: MALDI-TOF MS of ODN **2.29d**.

OD_{260} of ODN **2.29d** for the 0.52 μmol synthesis is 0.612.

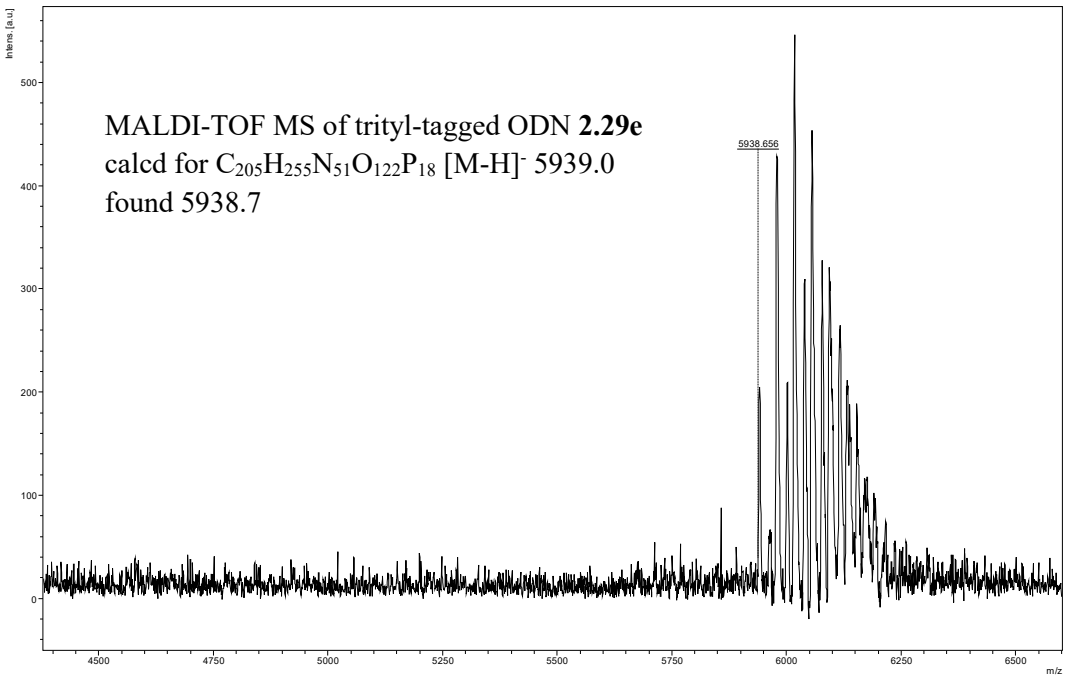


Figure 5.48: MALDI-TOF MS of trityl-tagged ODN **2.29e**.

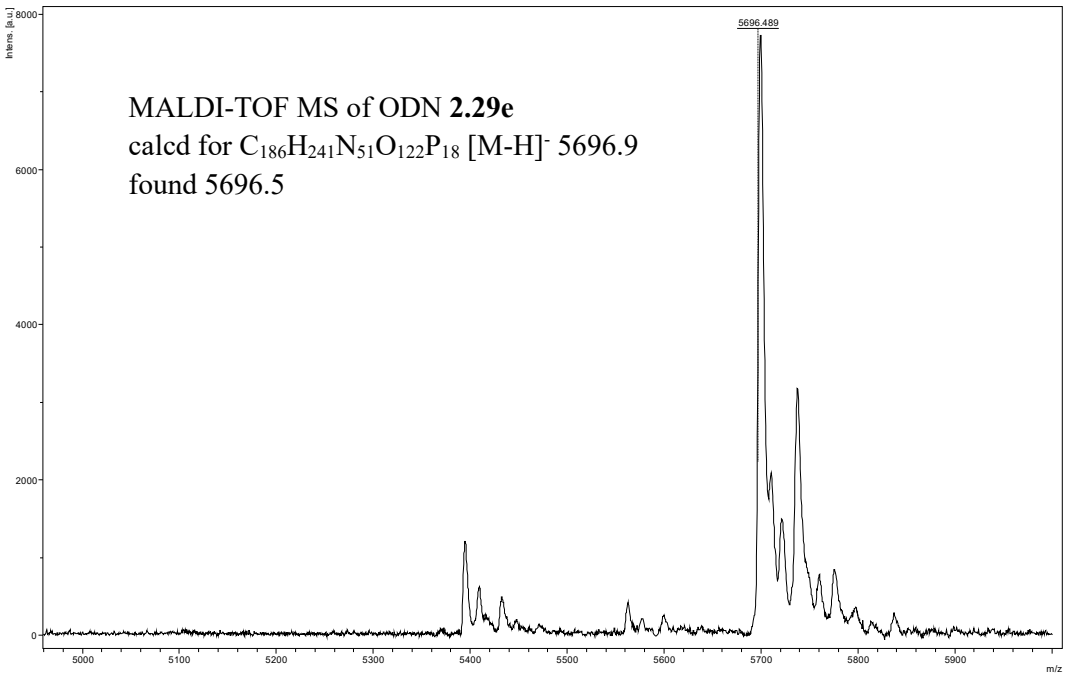


Figure 5.49: MALDI-TOF MS of ODN **2.29e**.

OD_{260} of ODN **2.29e** for the 0.52 μmol synthesis is 0.542.

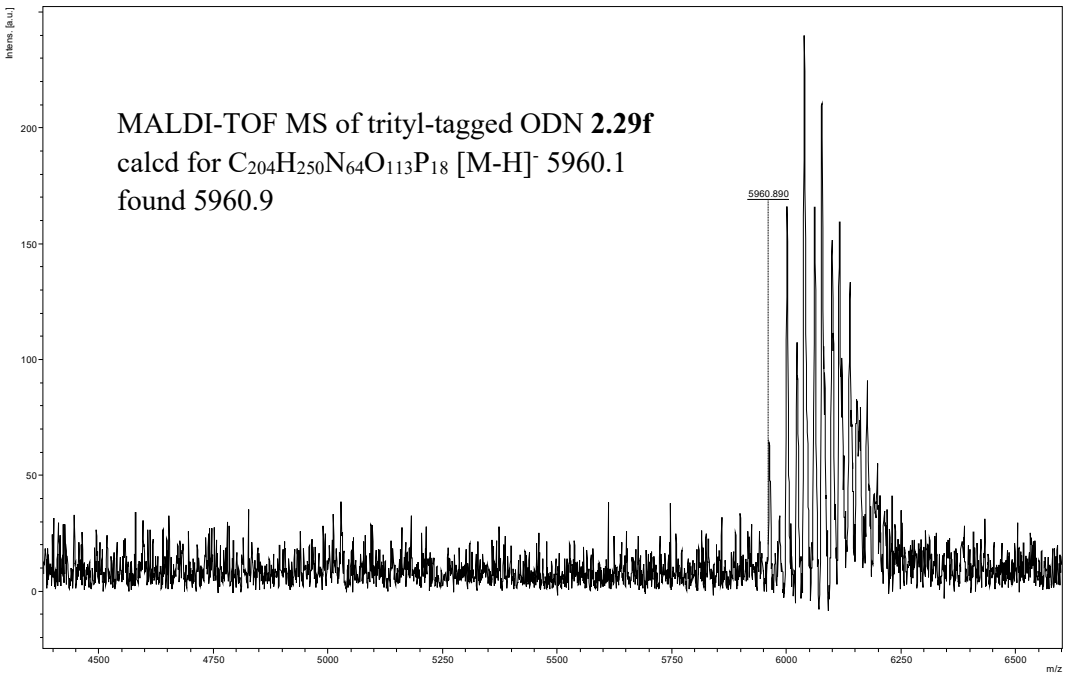


Figure 5.50: MALDI-TOF MS of trityl-tagged ODN **2.29f**.

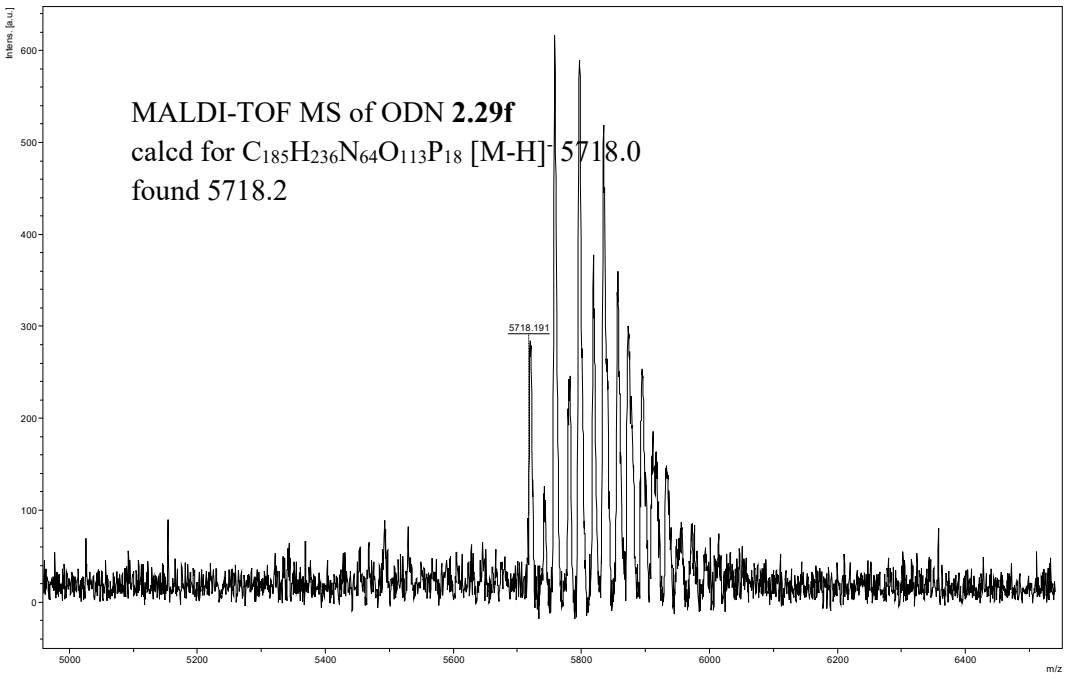


Figure 5.51: MALDI-TOF MS of ODN **2.29f**.

OD_{260} of ODN **2.29f** for the 0.52 μmol synthesis is 1.119.

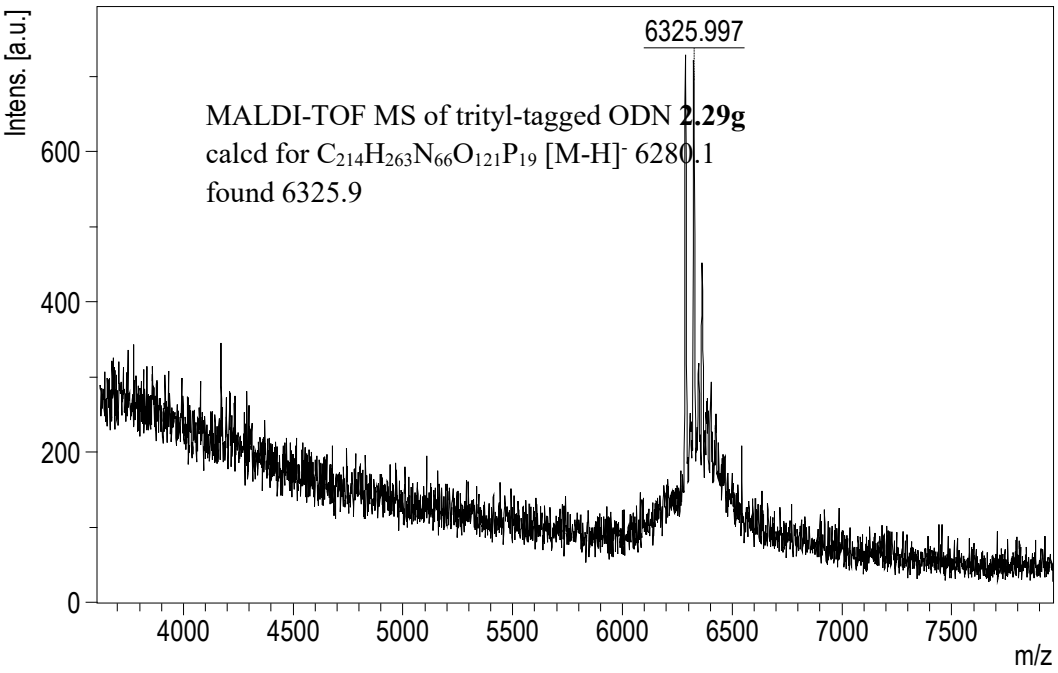


Figure 5.52: MALDI-TOF MS of trityl-tagged ODN **2.29g**.

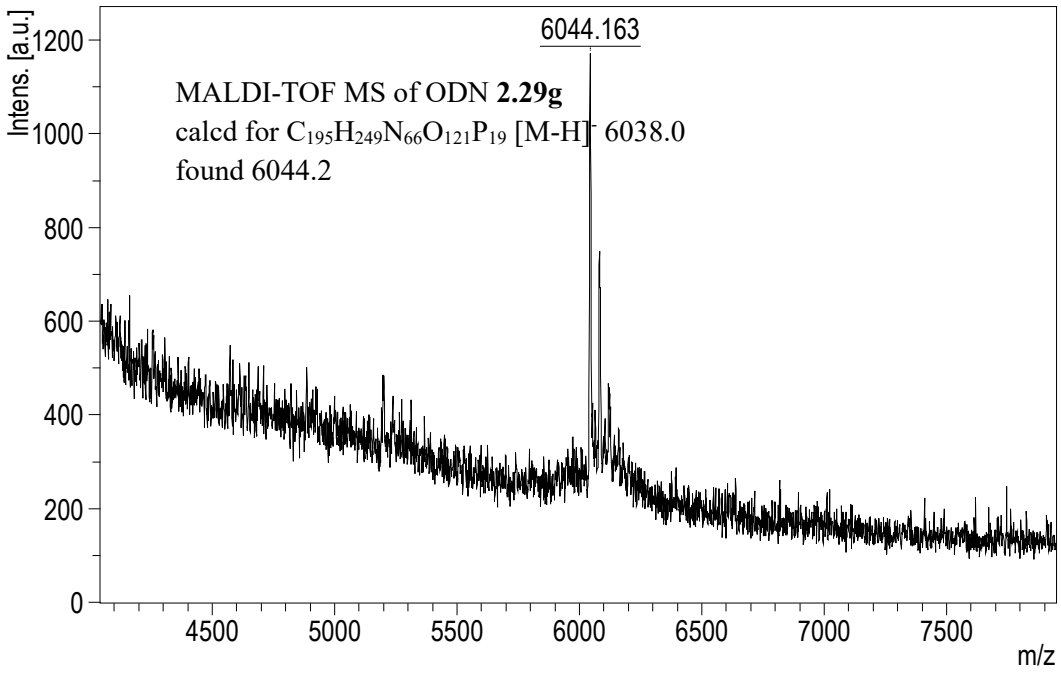


Figure 5.53: MALDI-TOF MS of ODN **2.29g**.

OD_{260} of ODN **2.29g** for the 0.52 μmol synthesis is 1.144.

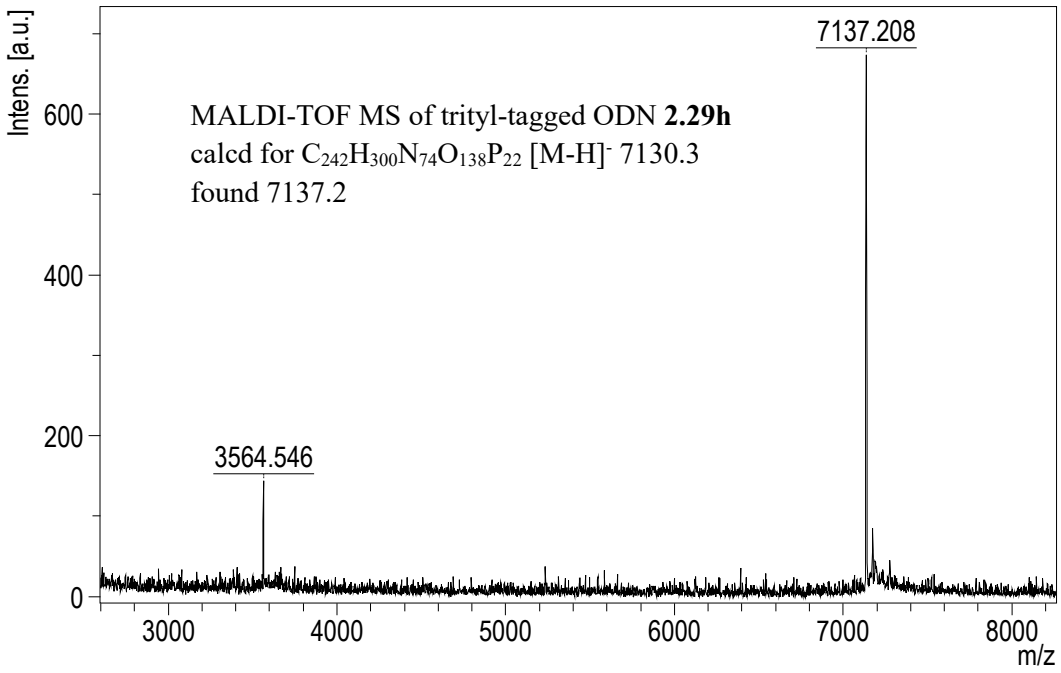


Figure 5.54: MALDI-TOF MS of trityl-tagged ODN **2.29h**.

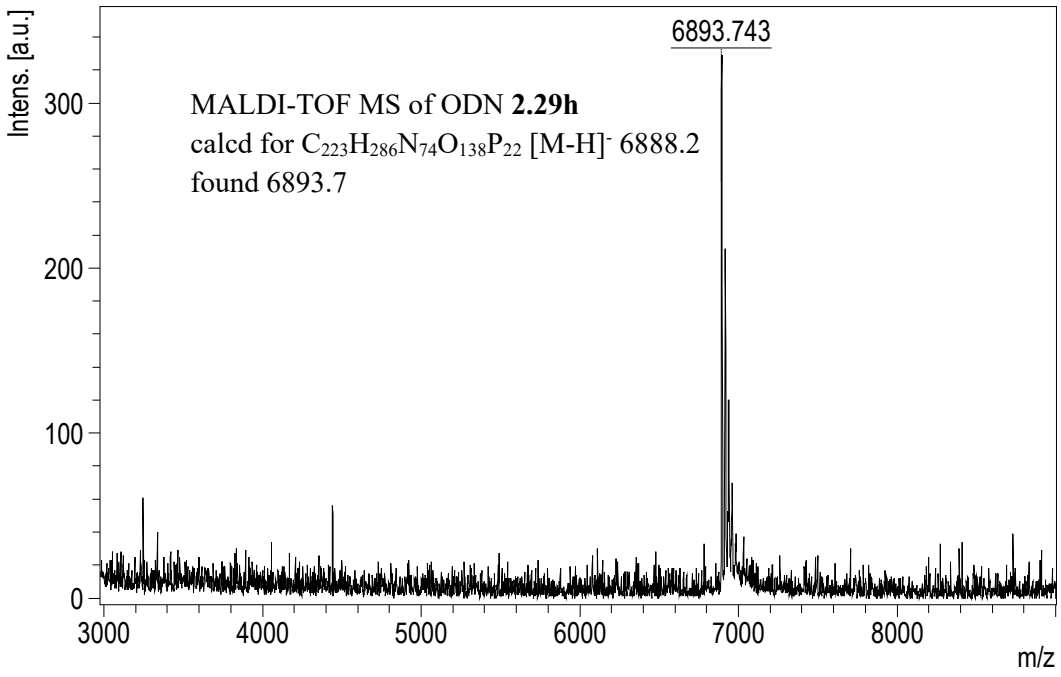


Figure 5.55: MALDI-TOF MS of ODN **2.29h**.

OD_{260} of ODN **2.29h** for the 0.52 μmol synthesis is 3.243.

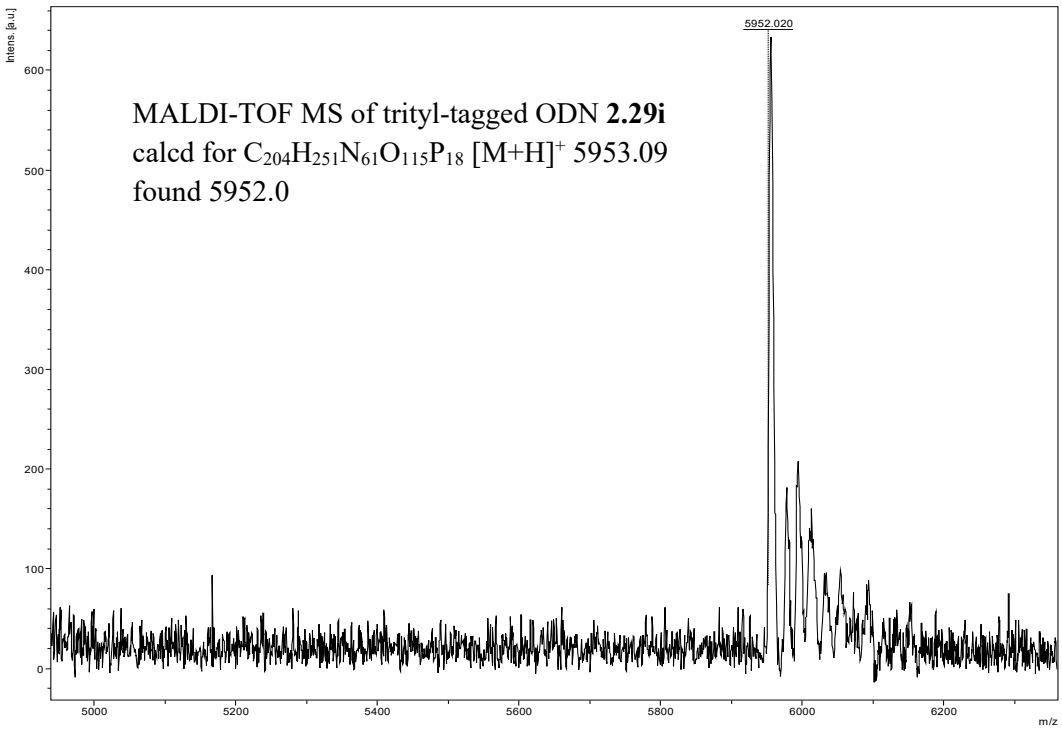


Figure 5.56: MALDI-TOF MS of trityl-tagged ODN **2.29i**.

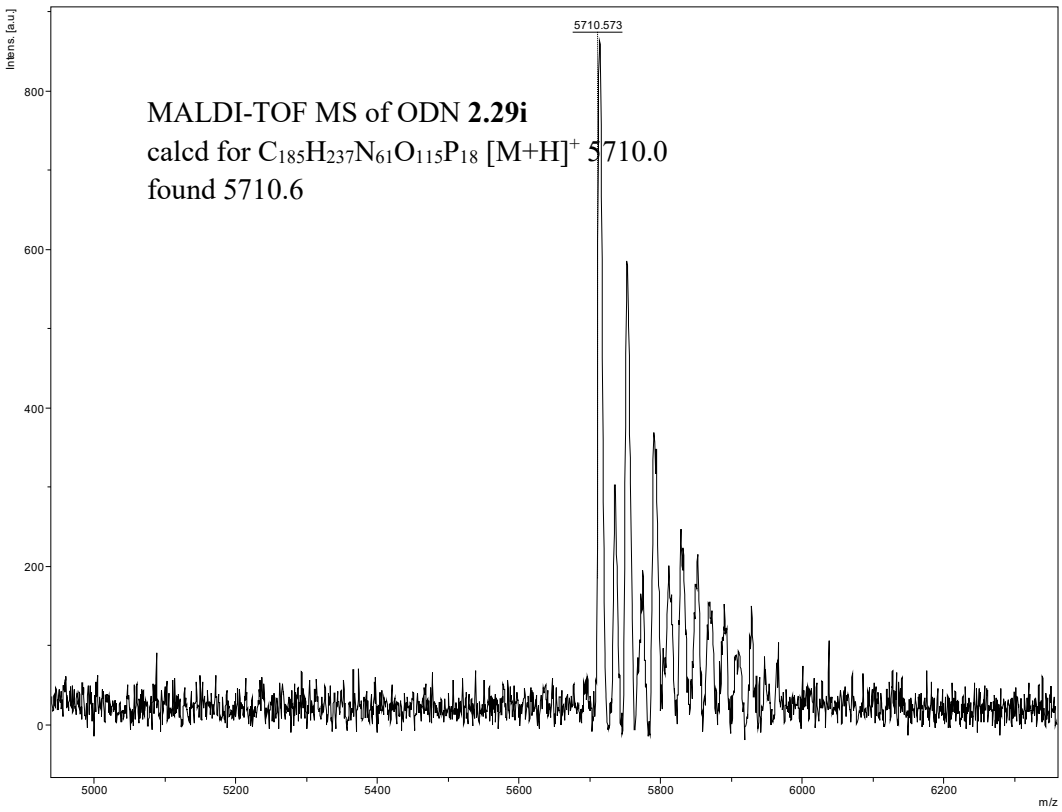


Figure 5.57: MALDI-TOF MS of ODN **2.29i**.

OD_{260} of ODN **2.29i** for the 0.52 μmol synthesis is 1.461.

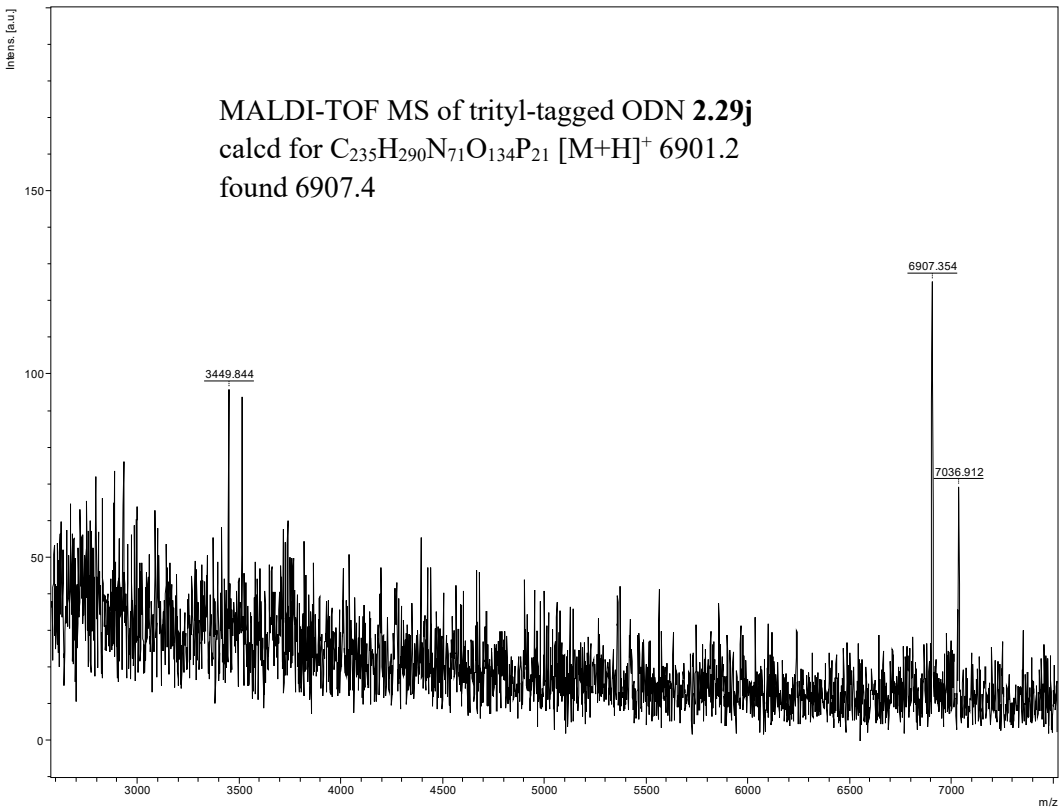


Figure 5.58: MALDI-TOF MS of trityl-tagged ODN **2.29j**.

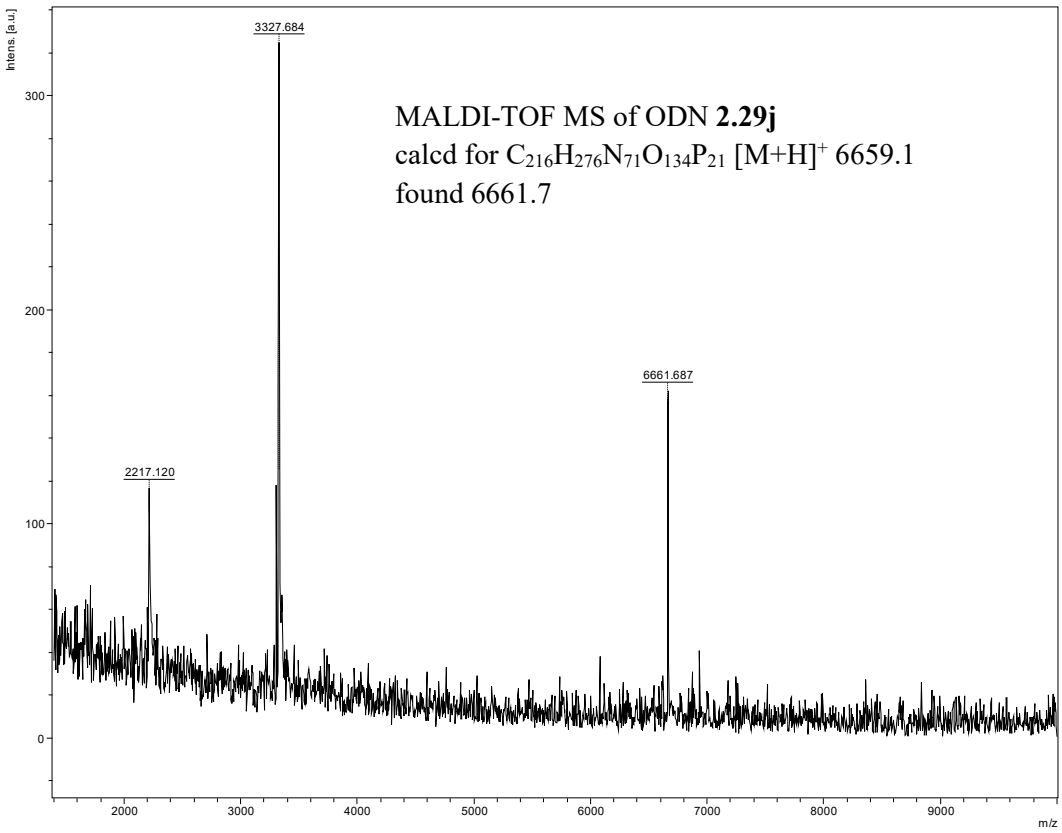


Figure 5.59: MALDI-TOF MS of ODN **2.29j**.

OD_{260} of ODN **2.29j** for the 0.52 μmol synthesis is 0.536.

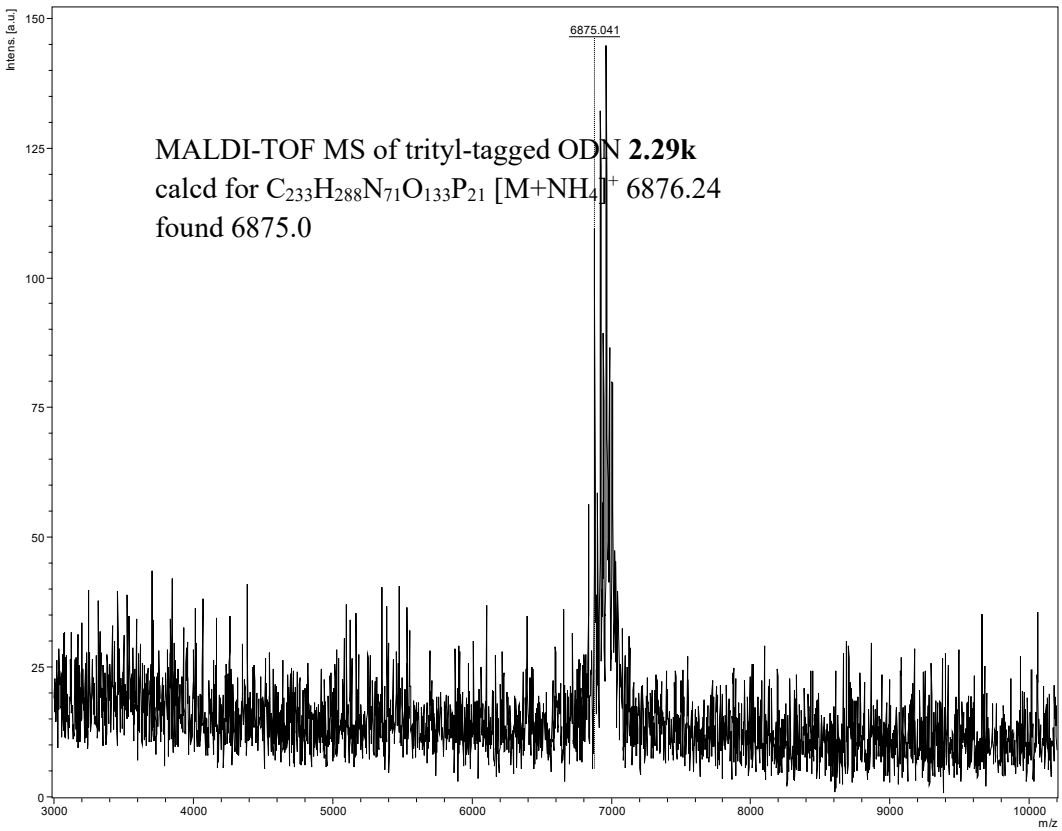


Figure 5.60: MALDI-TOF MS of trityl-tagged ODN **2.29k**.

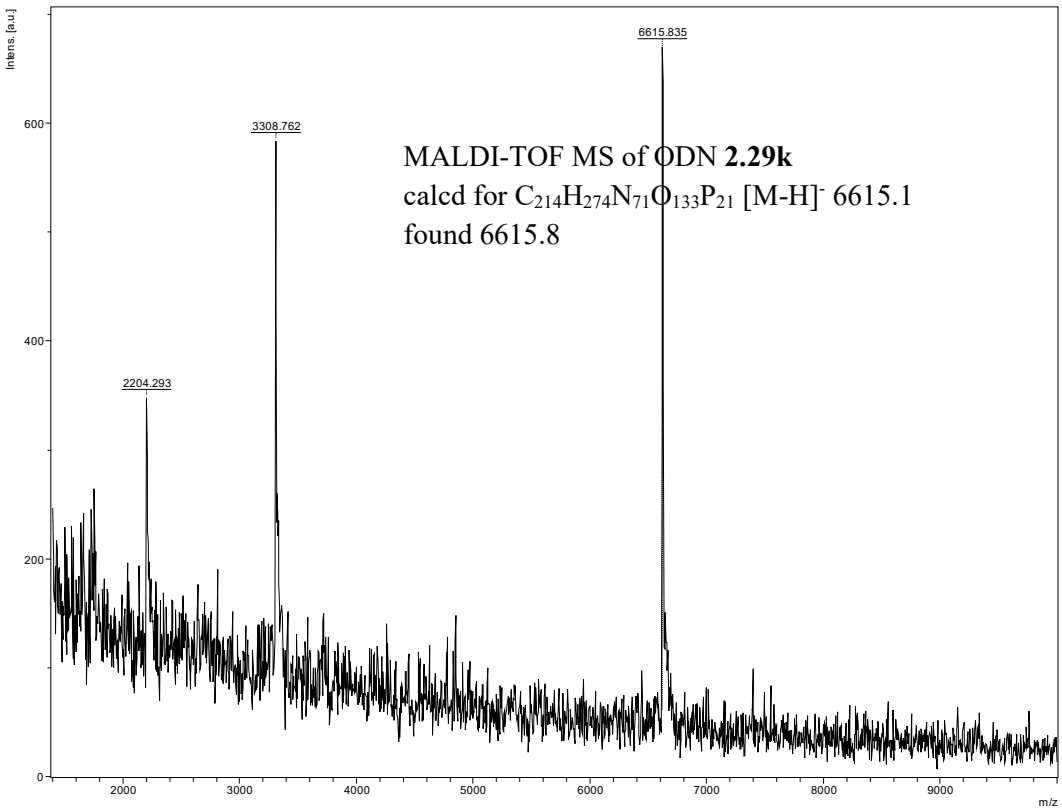


Figure 5.61: MALDI-TOF MS of ODN **2.29k**.

OD_{260} of ODN **2.29k** for the 0.52 μmol synthesis is 2.717.

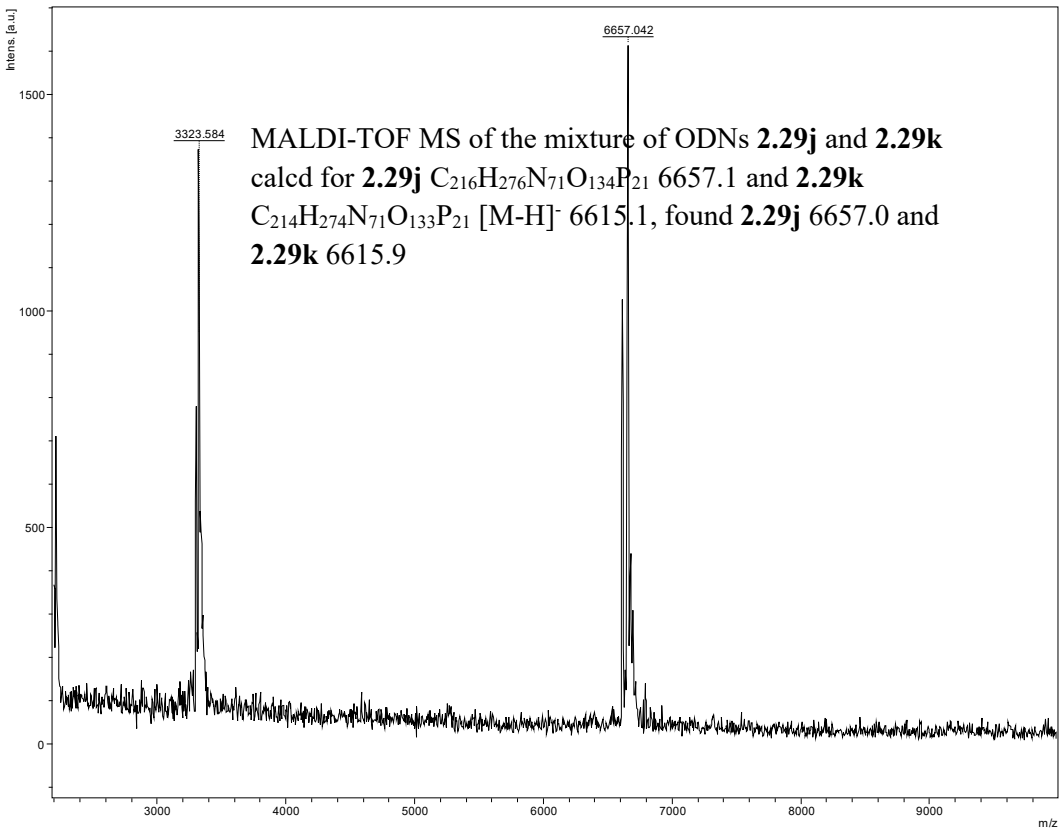


Figure 5.62: MALDI-TOF MS of the mixture of ODNs **2.29j** and **2.29k**.

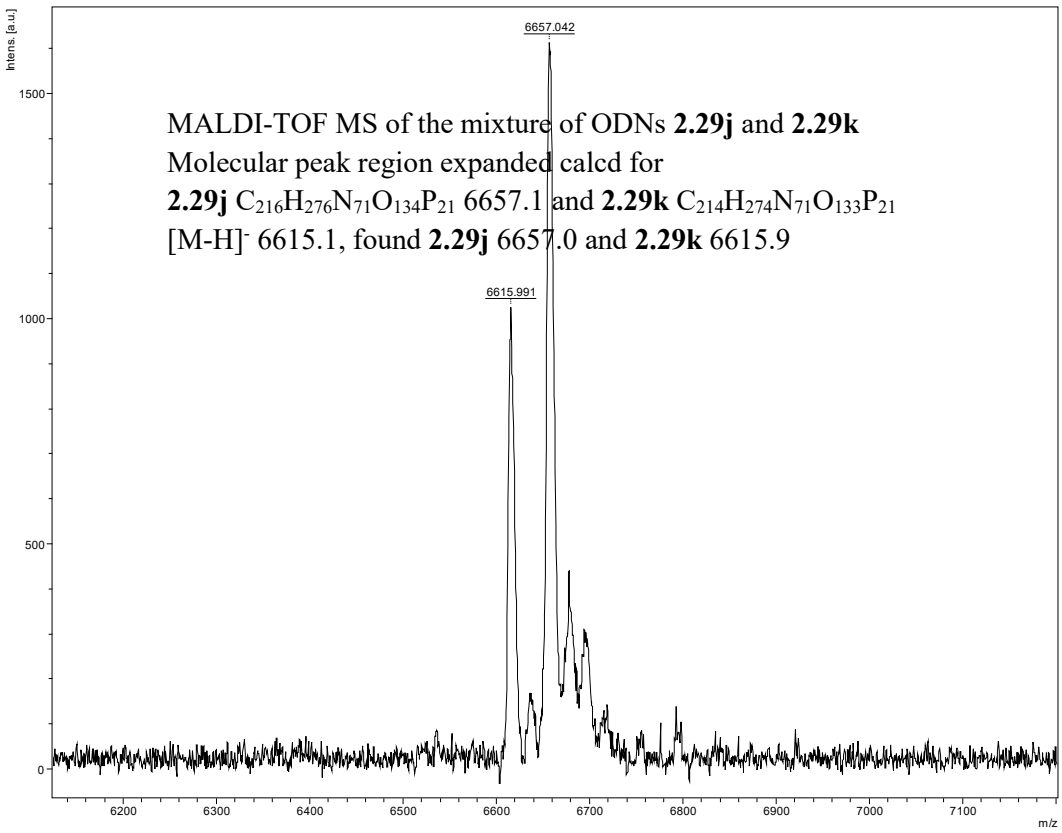


Figure 5.63: Zoomed MALDI-TOF MS of the mixture of ODNs **2.29j** and **2.29k**.

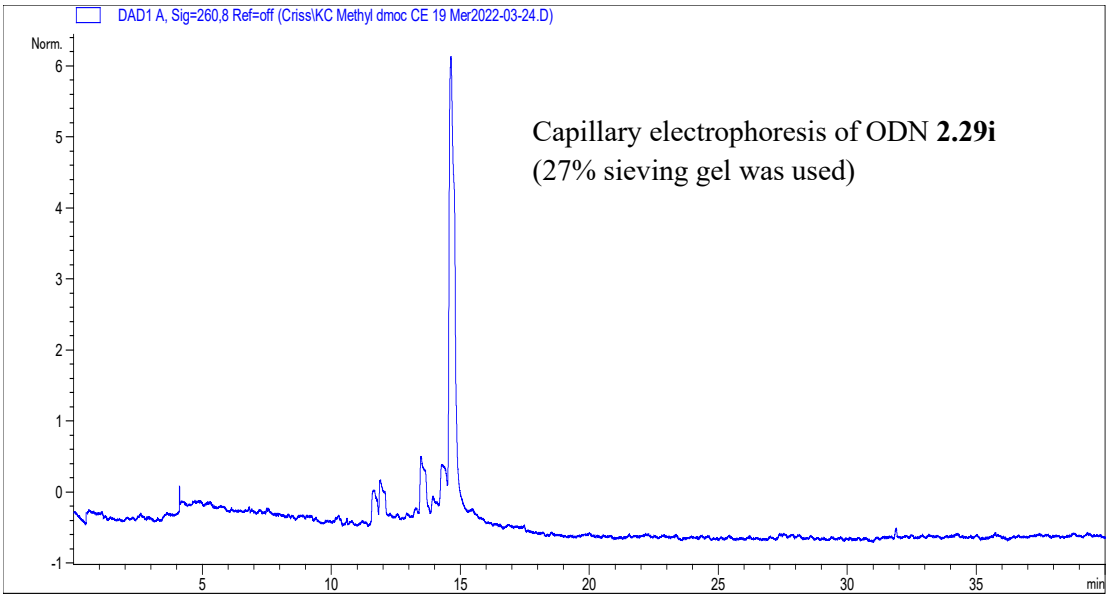


Figure 5.64: Capillary electrophoresis of ODN **2.29i** (using 27% sieving gel).

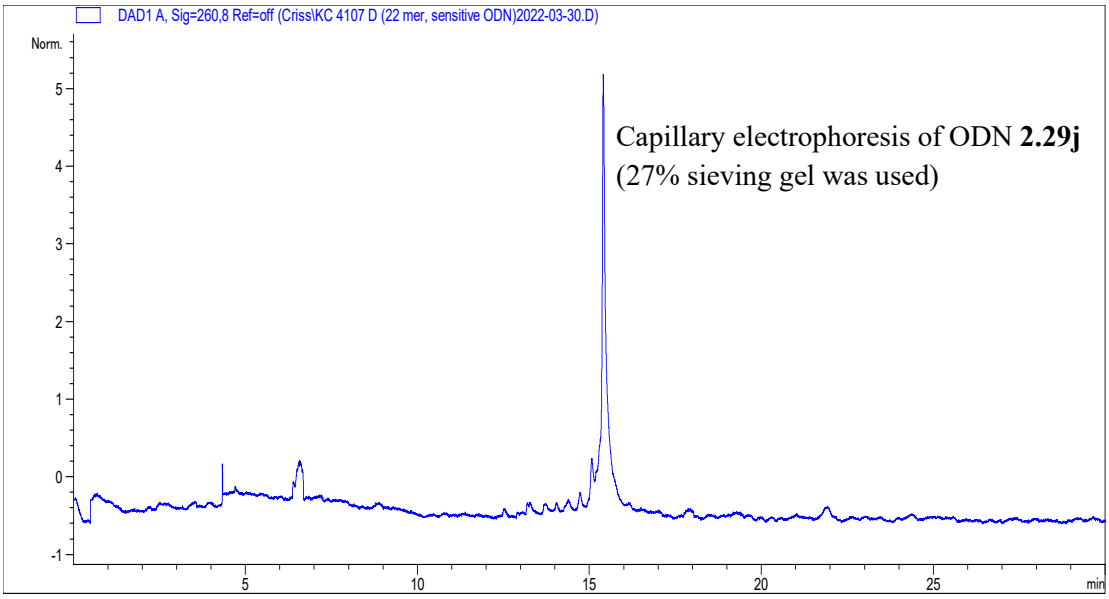


Figure 5.65: Capillary electrophoresis of ODN **2.29j** (using 27% sieving gel).

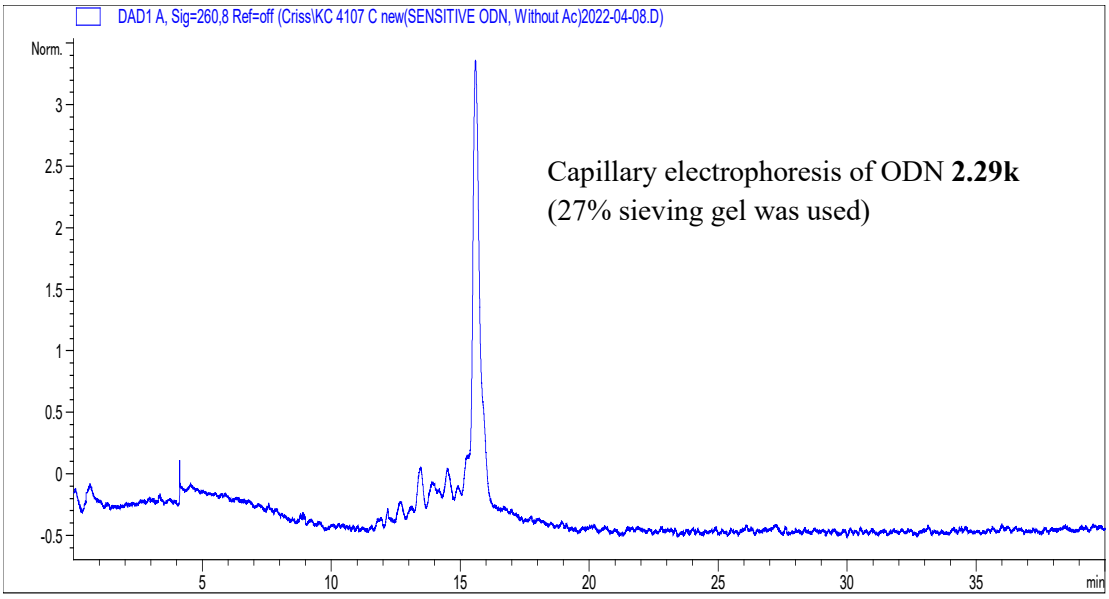


Figure 5.66: Capillary electrophoresis of ODN **2.29k** (using 27% sieving gel).

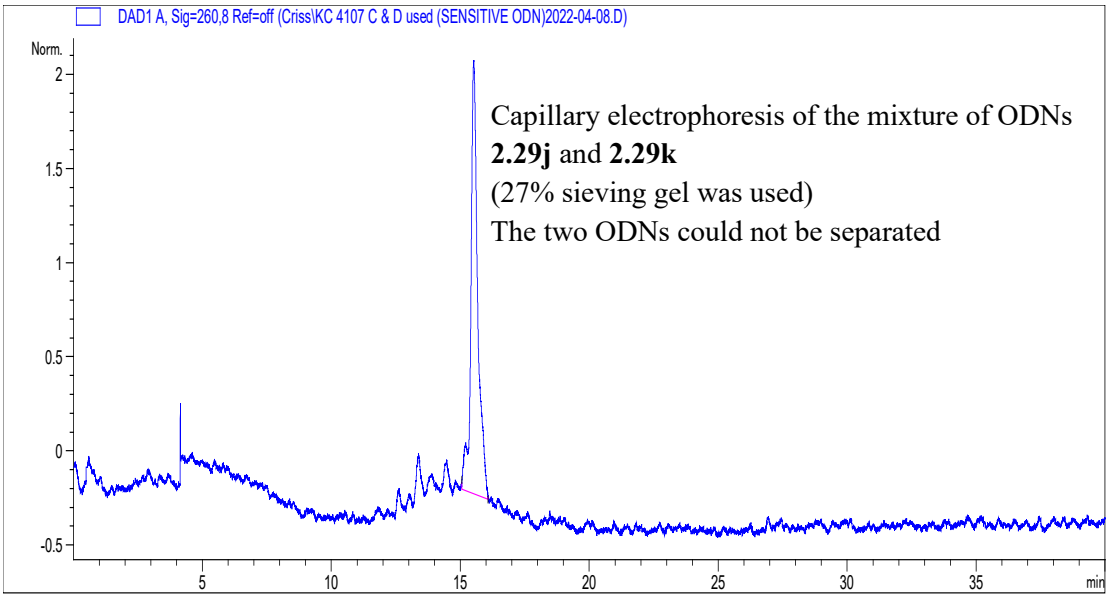


Figure 5.67: Capillary electrophoresis of the mixture of ODNs **2.29j** and **2.29k** (using 27% sieving gel).

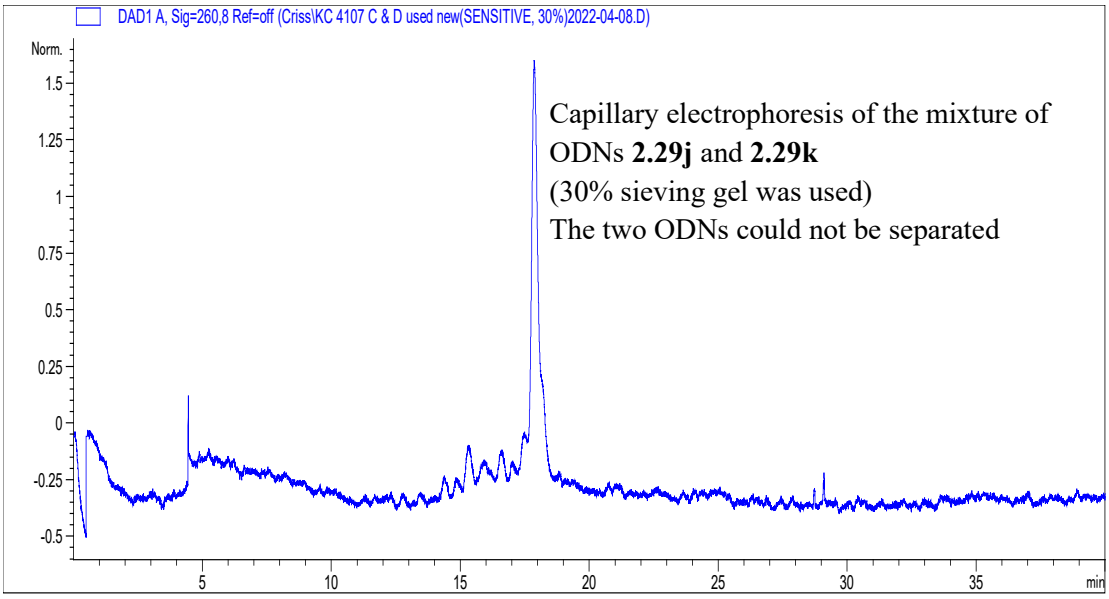


Figure 5.68: Capillary electrophoresis of the mixture of ODNs **2.29j** and **2.29k** (using 30% sieving gel).

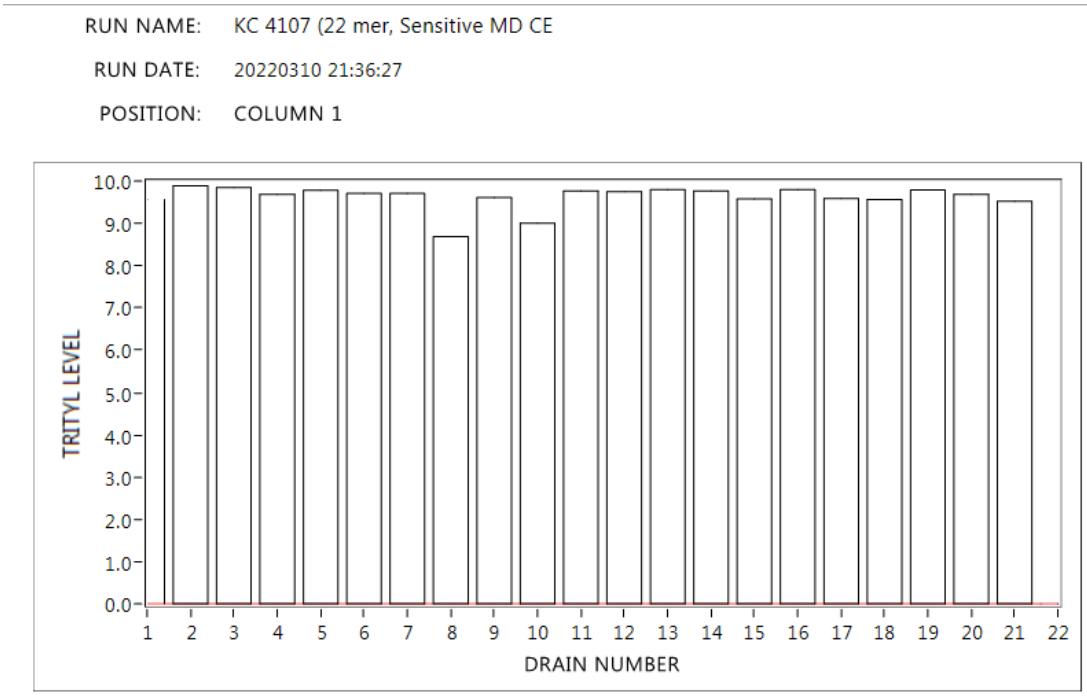


Figure 5.69: An example trityl assay log of ODN synthesis**.**

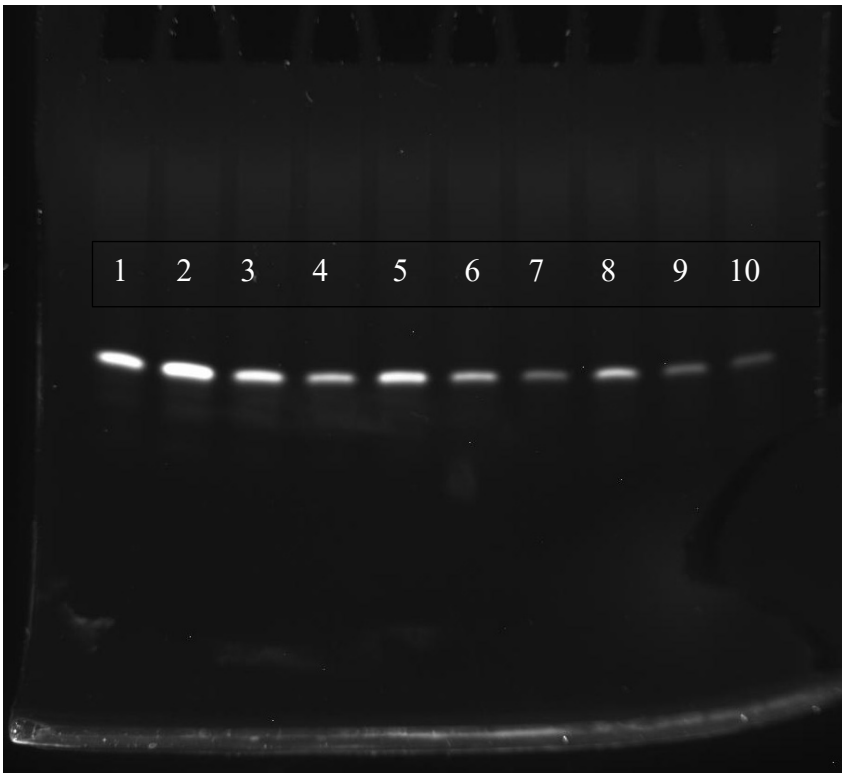


Figure 5.70: Image of denatured gel electrophoresis of the mixture of ODN **2.29j** in comparison with **2.29k**.

The two ODNs have the same sequence but **2.29j** has a 4acC modification while **2.29k** does not. Polyacrylamide gel (15%, 7 M urea), 1X TBE buffer, 190 V, 45 min, stained with Gel Red.

Lane 1: 48 ng **2.29j**. Lane 2: 48 ng **2.29j** and 46.5 ng **2.29k**. Lane 3: 46.5 ng **2.29k**.

Lane 4: 24 ng **2.29j**. Lane 5: 24 ng **2.29j** and 23.3 ng **2.29k**. Lane 6: 23.3 ng **2.29k**.

Lane 7: 12 ng **2.29j**. Lane 8: 12 ng **2.29j** and 11.6 ng **2.29k**. Lane 9: 11.6 ng **2.29k**.

Lane 10: 7.2 ng **2.29j**

Although the two ODNs could not be resolved, the single bands indicate that they do not contain any sequences that are shorter or longer than them. In addition, as presented in other parts of the paper, MALDI MS indicates that **2.29j** contains the 4acC modification

while **2.29k** does not (Figure 3.5). Considering these and other evidence, which include CE and HPLC analyses, presented in the paper, **2.29j** is pure.

5.2 Images of ^{1}H, ^{13}C and ^{31}P NMR spectra of compounds

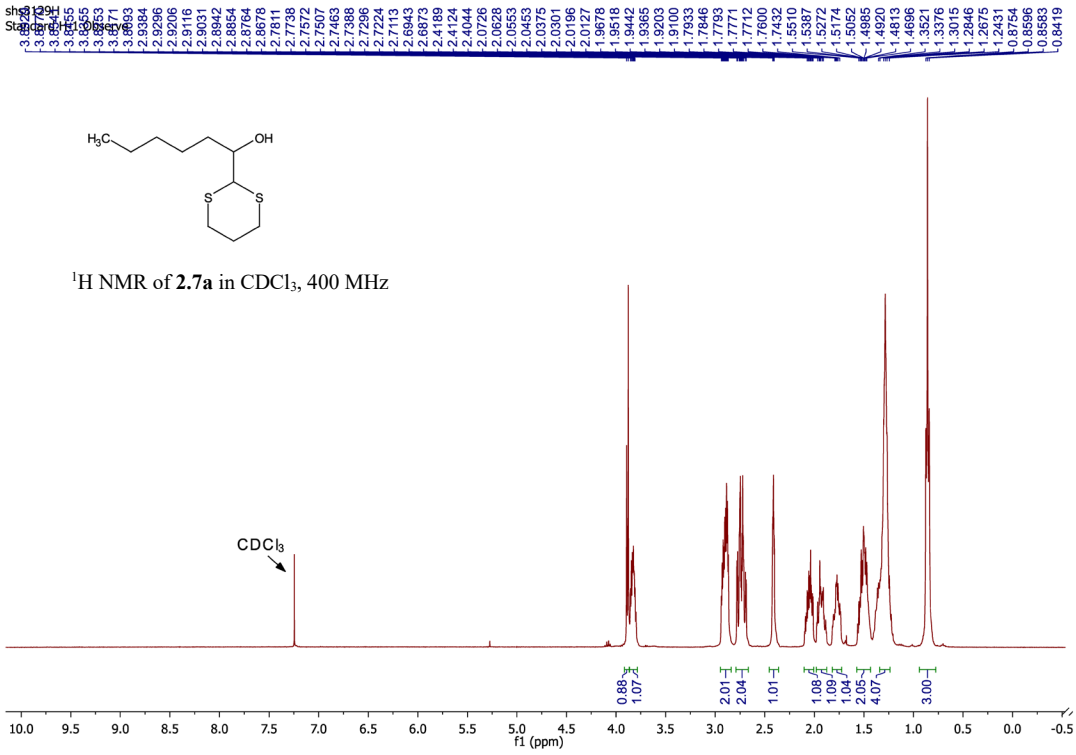


Figure 5.71: ^{1}H NMR spectrum of compound **2.7a**.

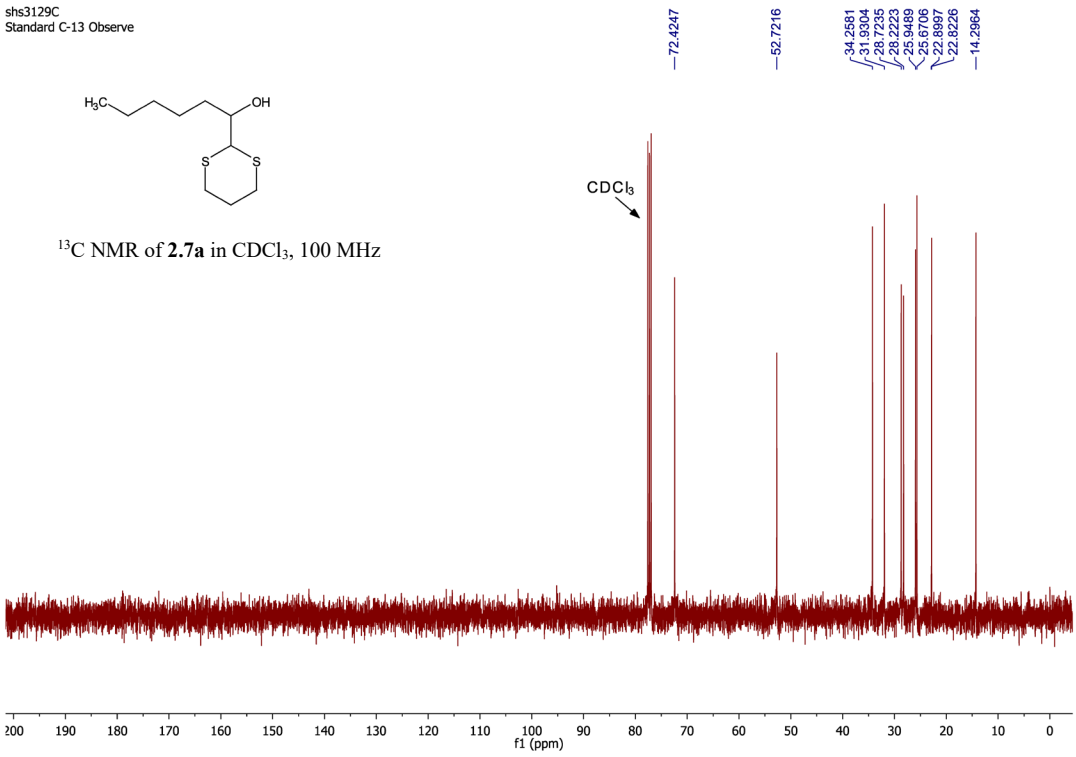
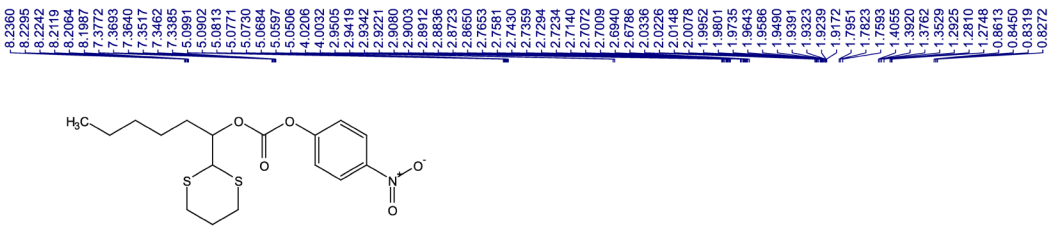


Figure 5.72: ^{13}C NMR spectrum of compound **2.7a**.



^{1}H NMR of **2.8a** in $CDCl_3$, 400 MHz

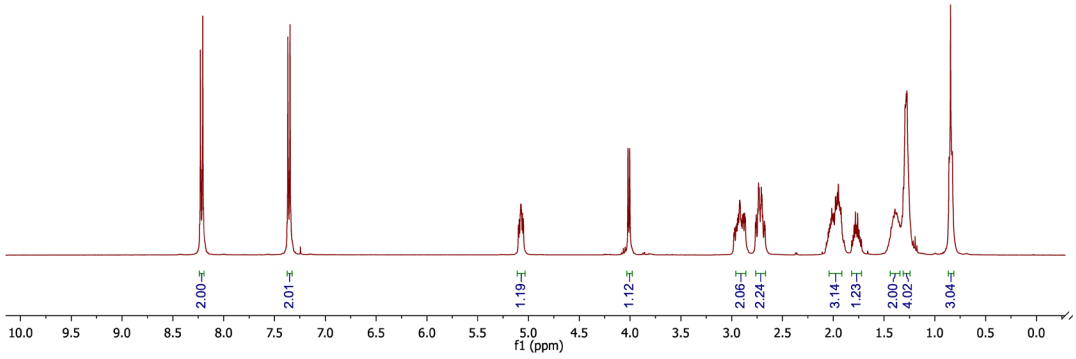


Figure 5.73: ^{1}H NMR spectrum of compound **2.8a**.

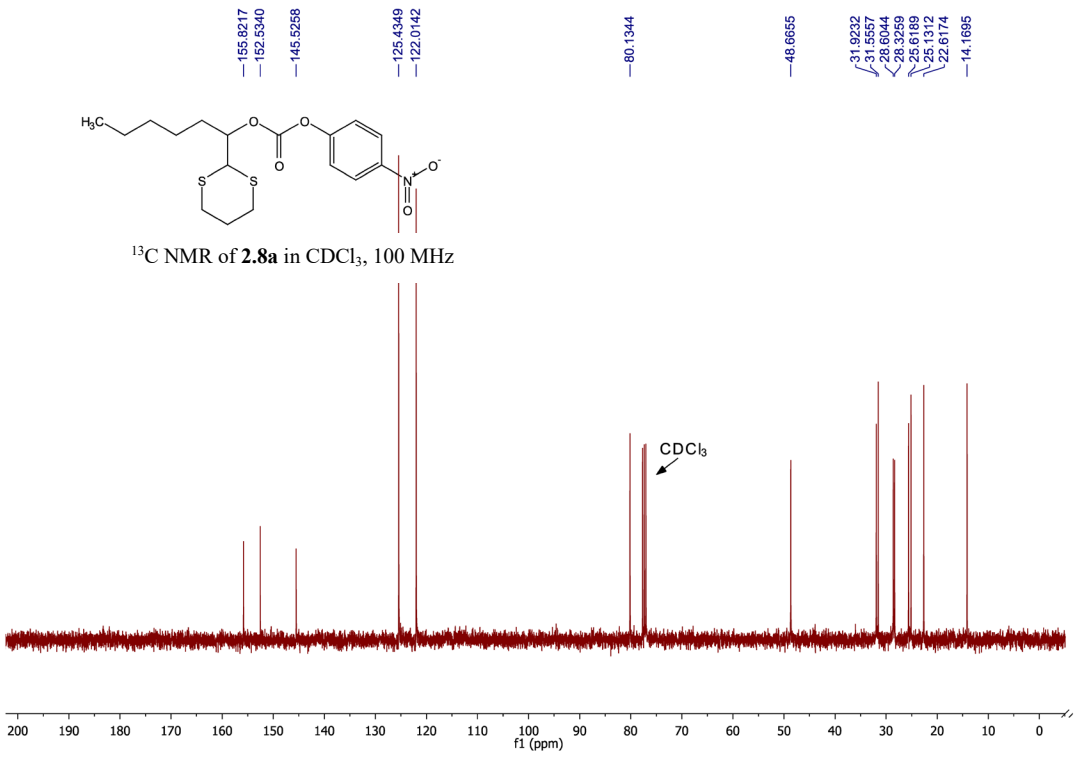
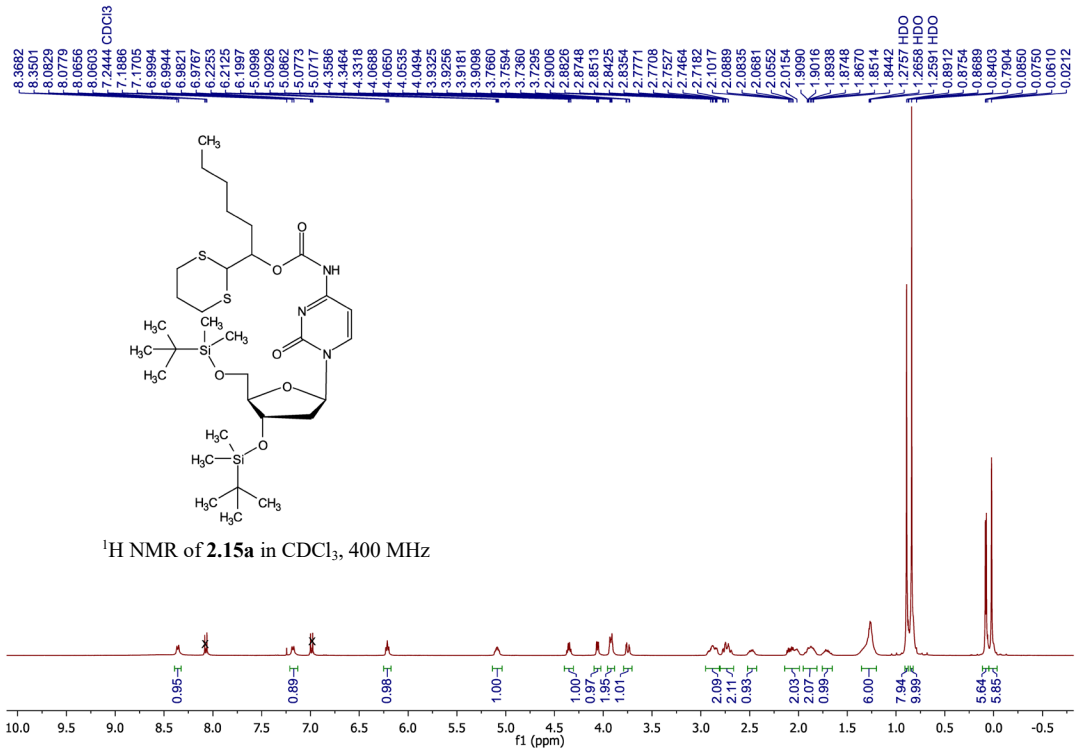


Figure 5.74: ^{13}C NMR spectrum of compound **2.8a**.



^{1}H NMR of **2.15a** in $CDCl_3$, 400 MHz

Figure 5.75: ^{1}H NMR spectrum of compound **2.15a**.

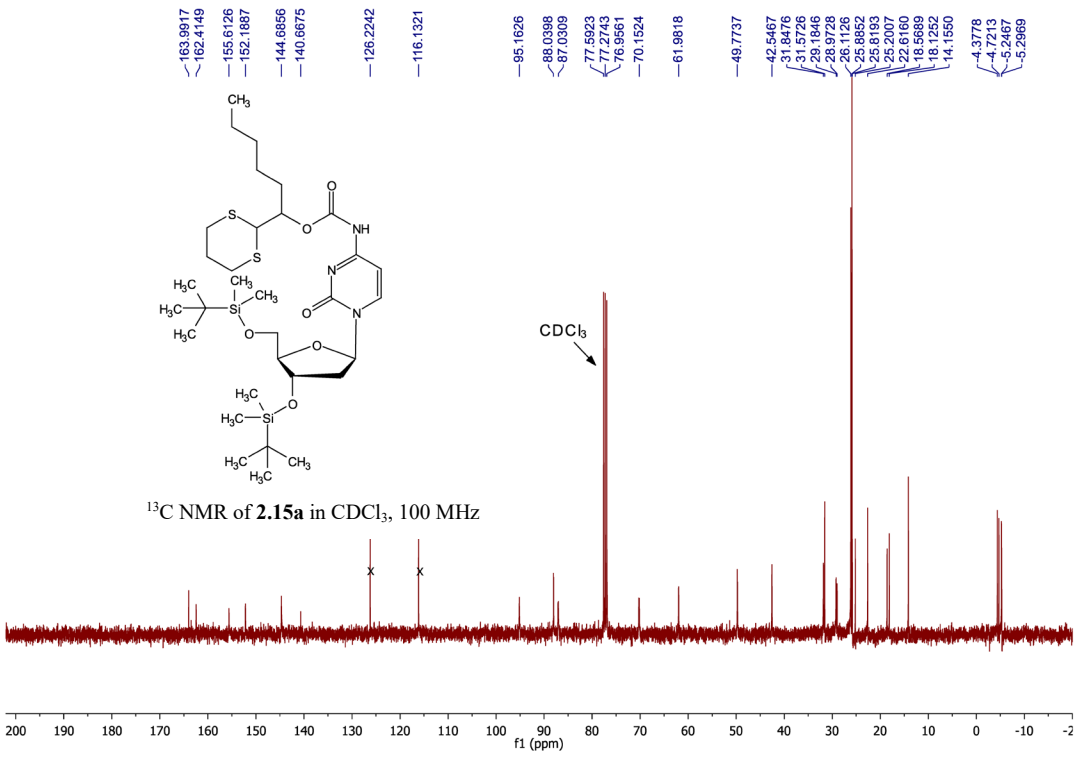


Figure 5.76: ^{13}C NMR spectrum of compound **2.15a**.

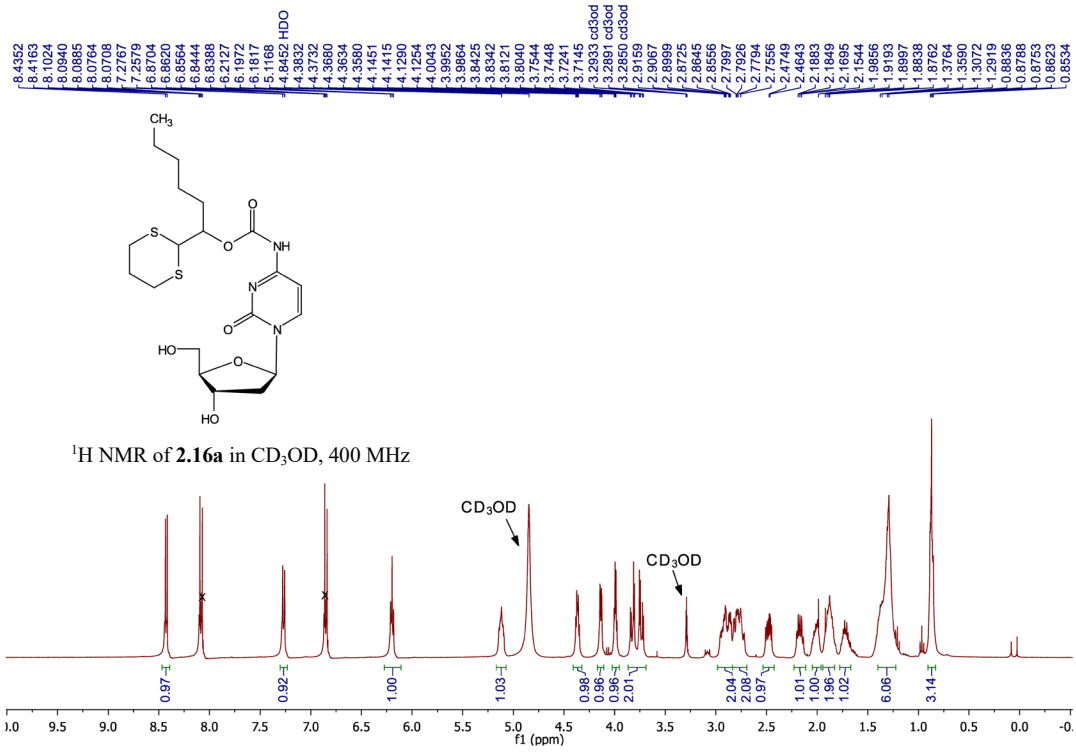
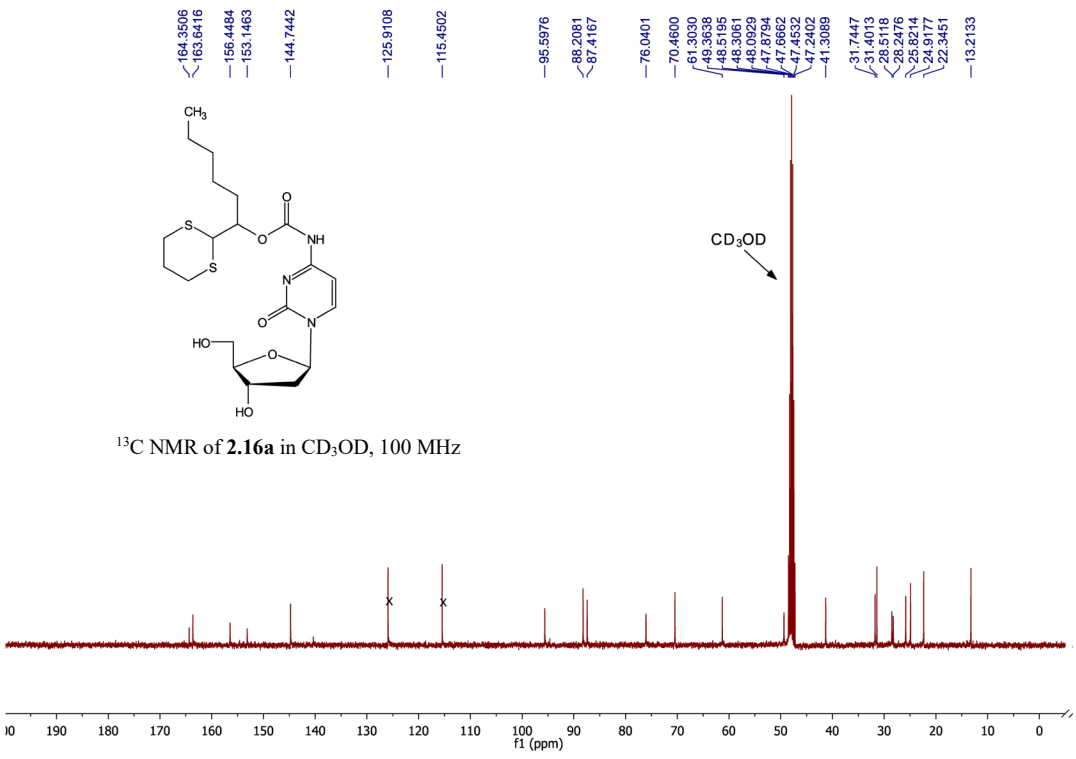
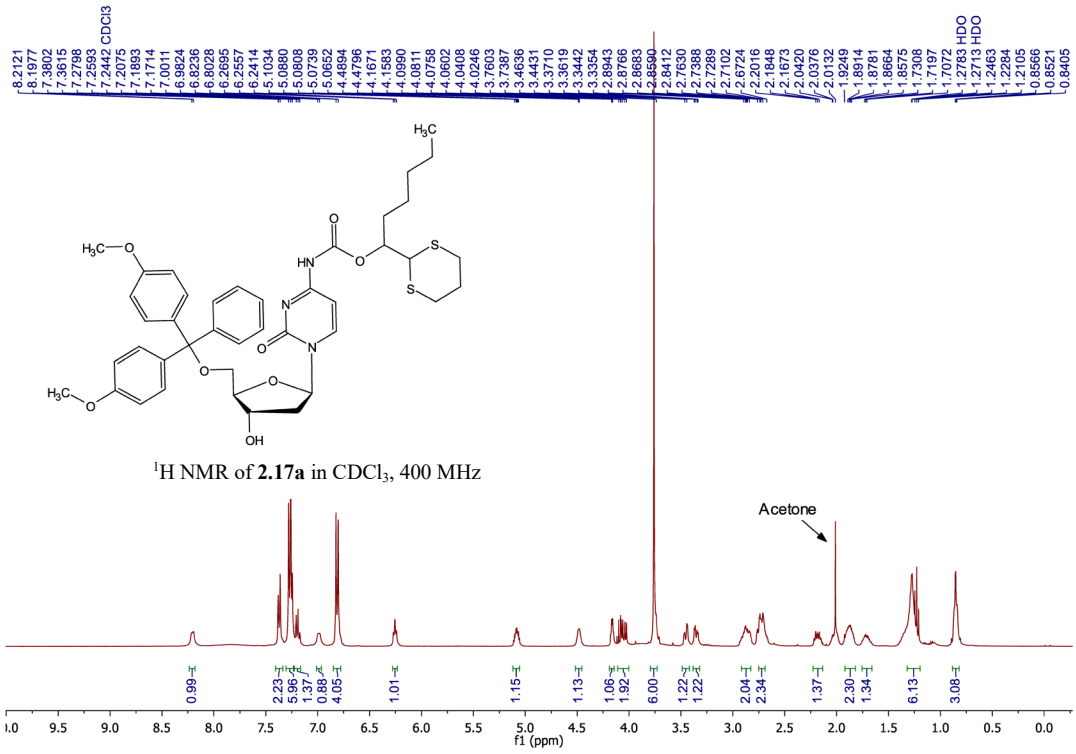


Figure 5.77: [1]H NMR spectrum of compound **2.16a**.



^{13}C NMR of **2.16a** in CD_3OD, 100 MHz

Figure 5.78: ^{13}C NMR spectrum of compound **2.16a**.



^{1}H NMR of **2.17a** in $CDCl_3$, 400 MHz

Figure 5.79: ^{1}H NMR spectrum of compound **2.17a**.

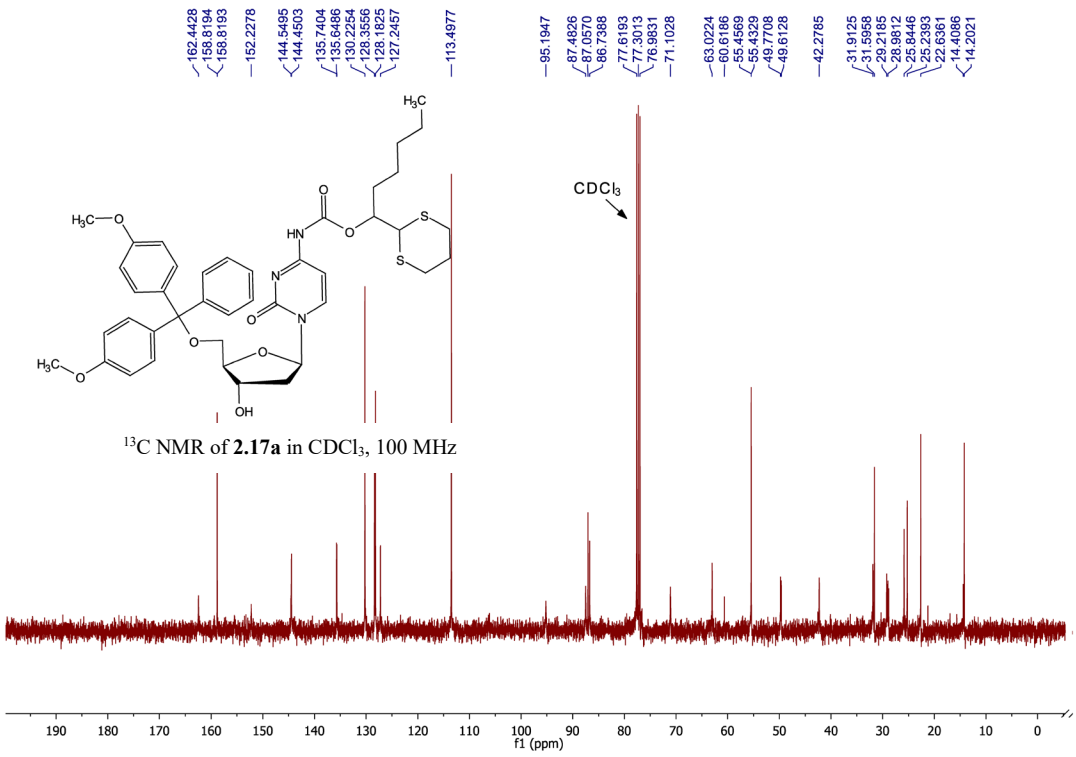
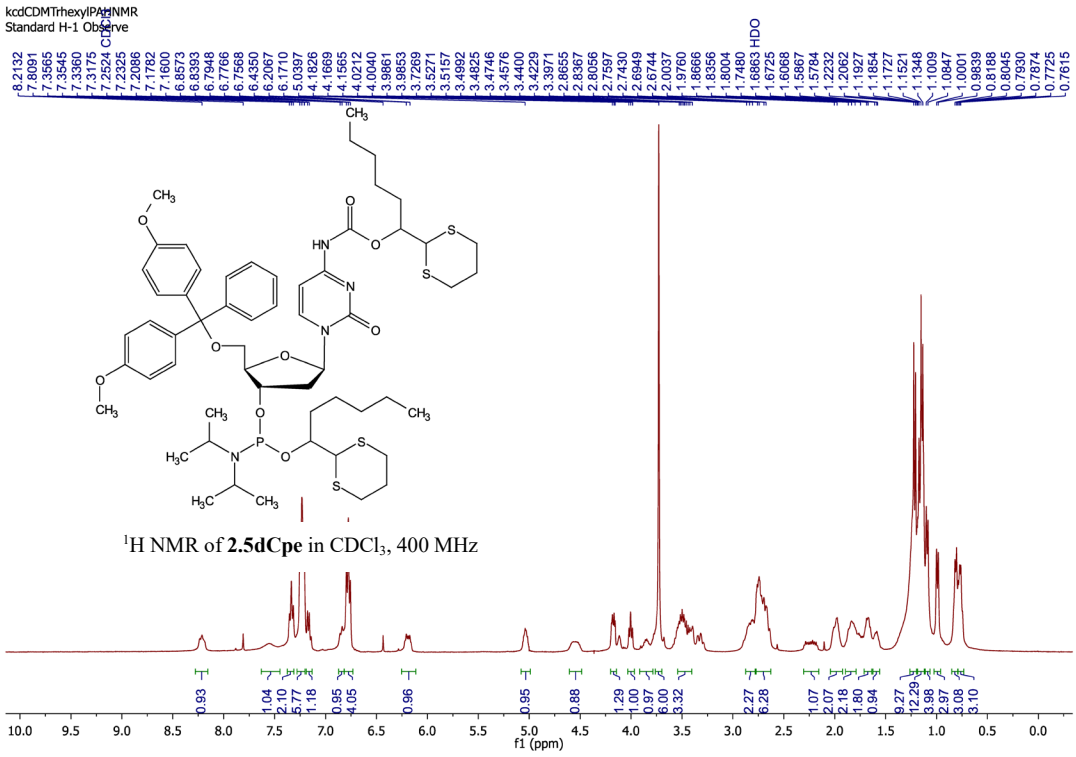
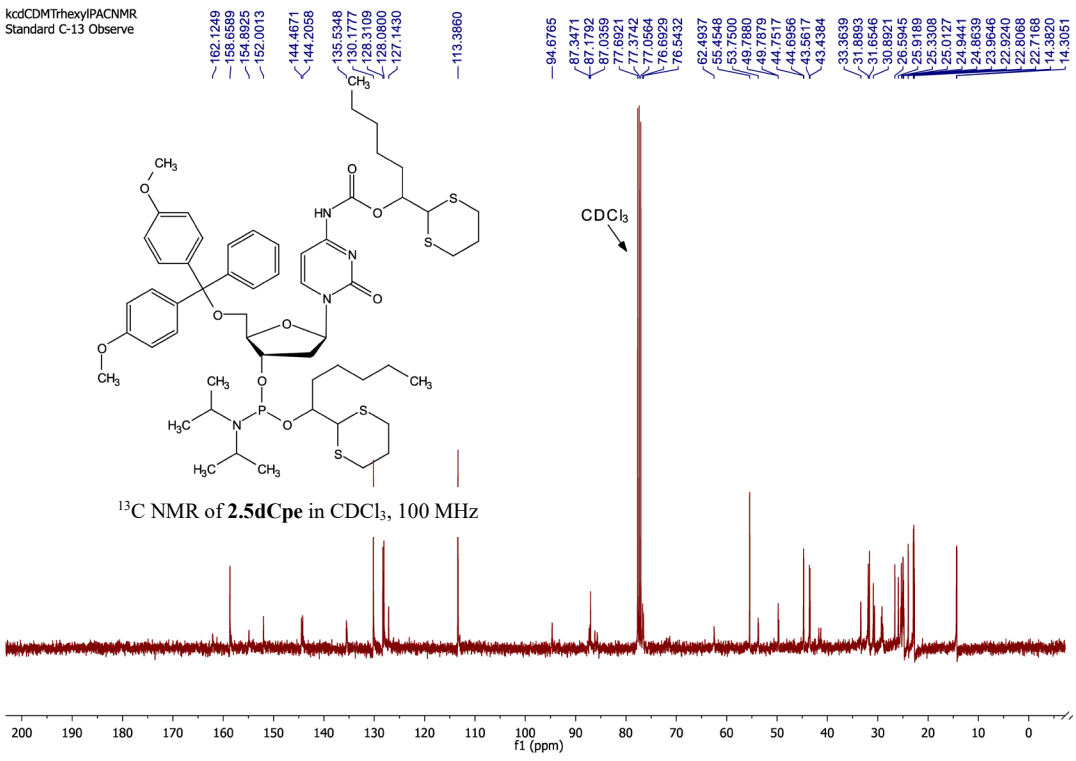


Figure 5.80: ^{13}C NMR spectrum of compound **2.17a**.



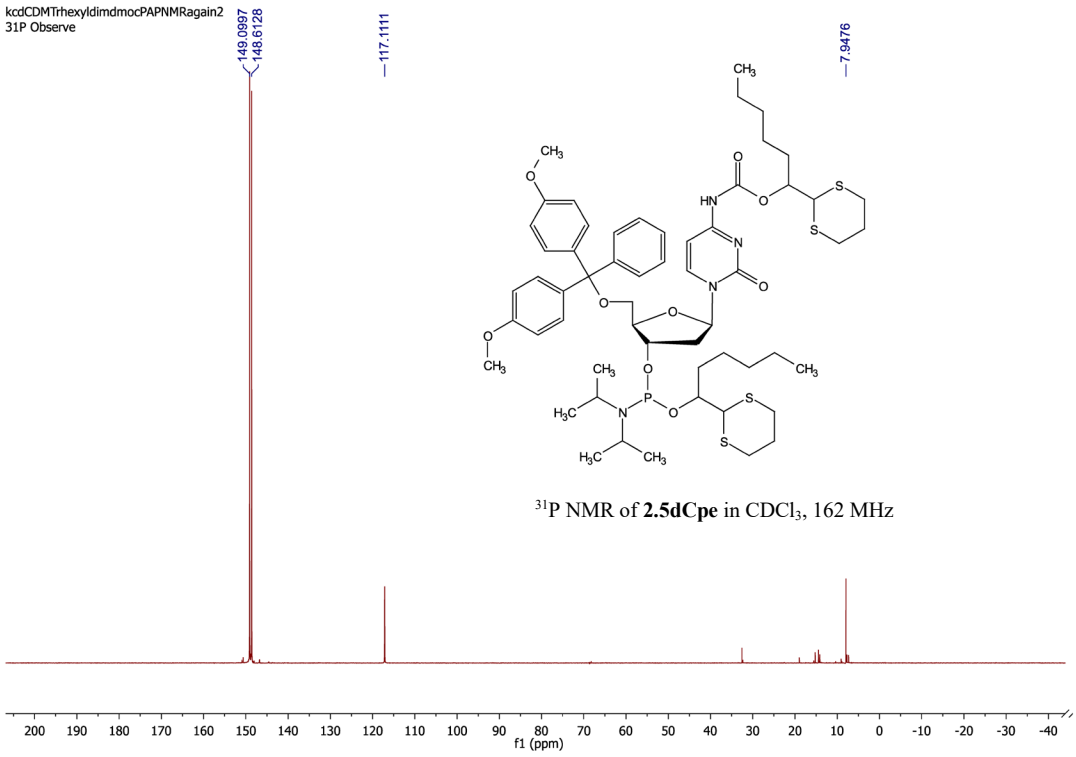
^{1}H NMR of **2.5dCpe** in $CDCl_3$, 400 MHz

Figure 5.81: ^{1}H NMR spectrum of compound **2.5dCpe**.



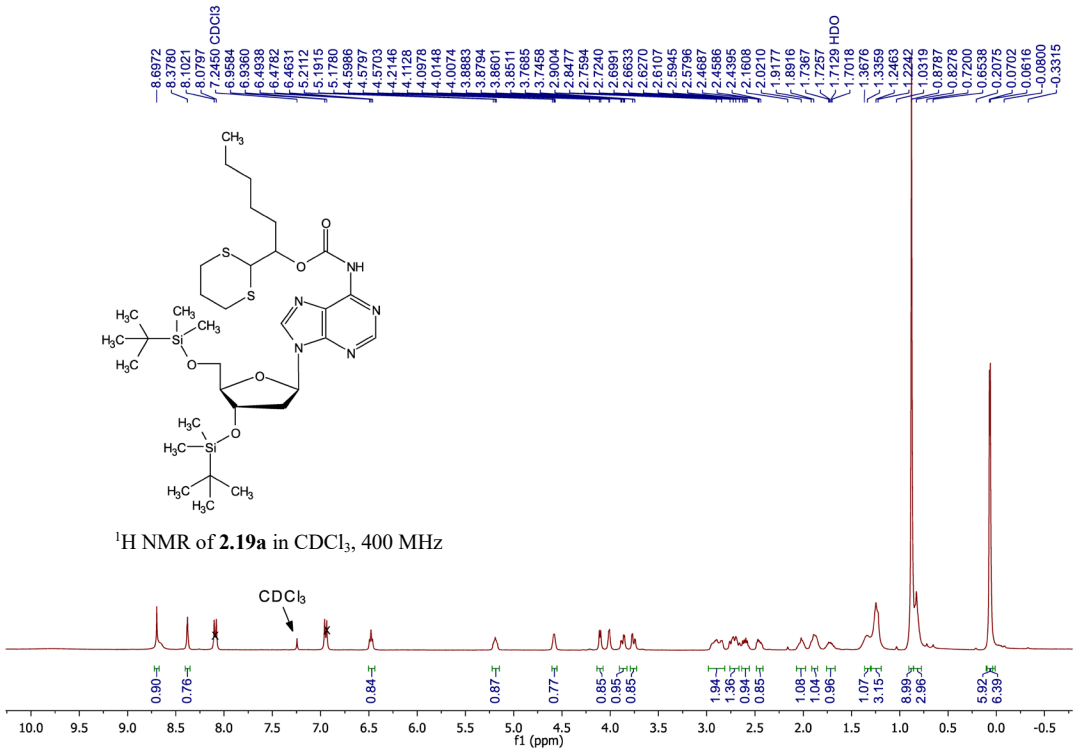
^{13}C NMR of **2.5dCpe** in $CDCl_3$, 100 MHz

Figure 5.82: ^{13}C NMR spectrum of compound **2.5dCpe**.



^{31}P NMR of **2.5dCpe** in $CDCl_3$, 162 MHz

Figure 5.83: ^{31}P NMR spectrum of compound **2.5dCpe**.



^{1}H NMR of **2.19a** in $CDCl_3$, 400 MHz

Figure 5.84: ^{1}H NMR spectrum of compound **2.19a**.

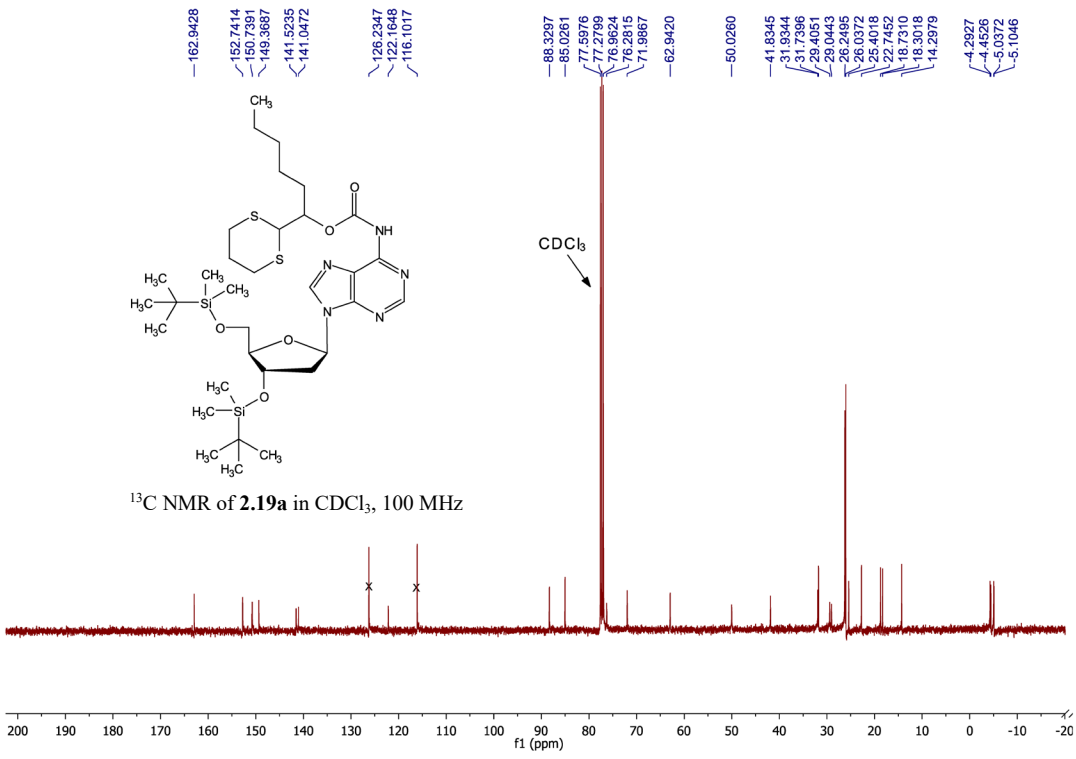
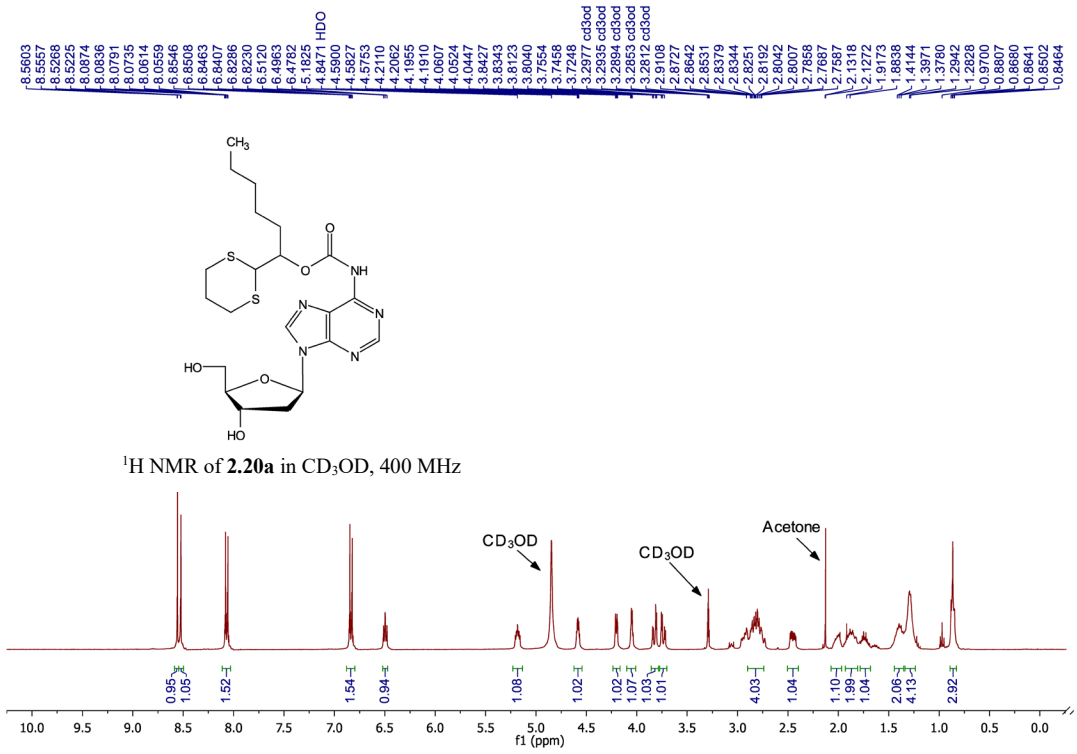
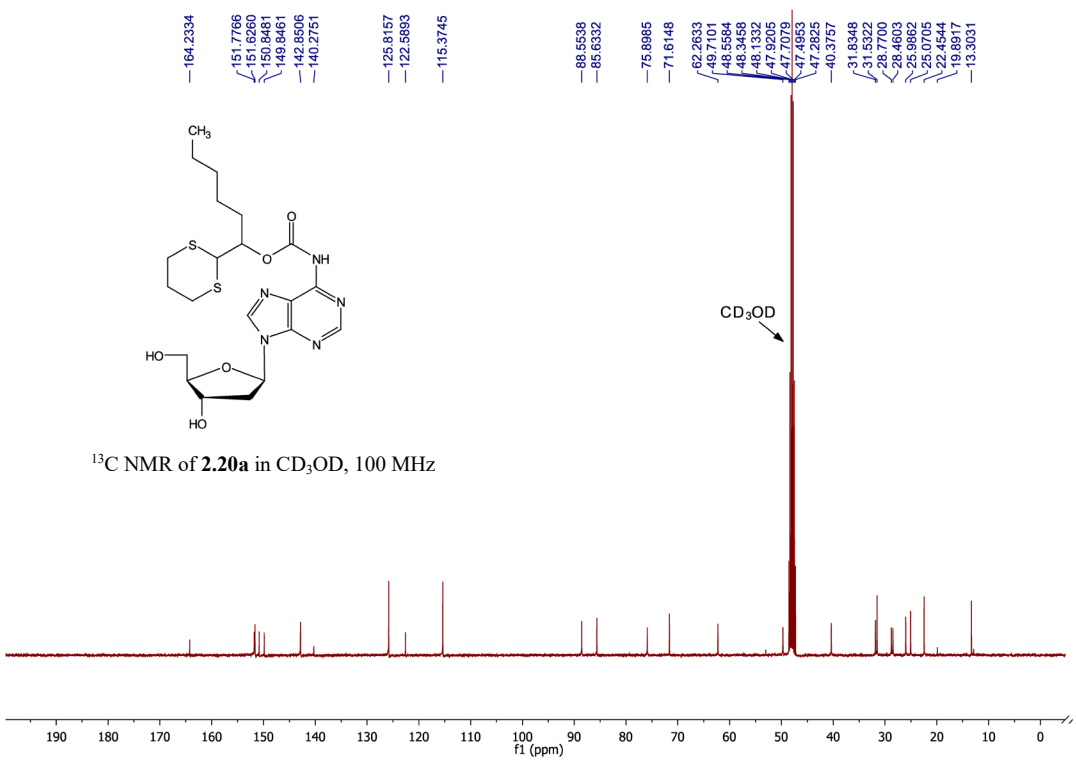


Figure 5.85: ^{13}C NMR spectrum of compound **2.19a**.



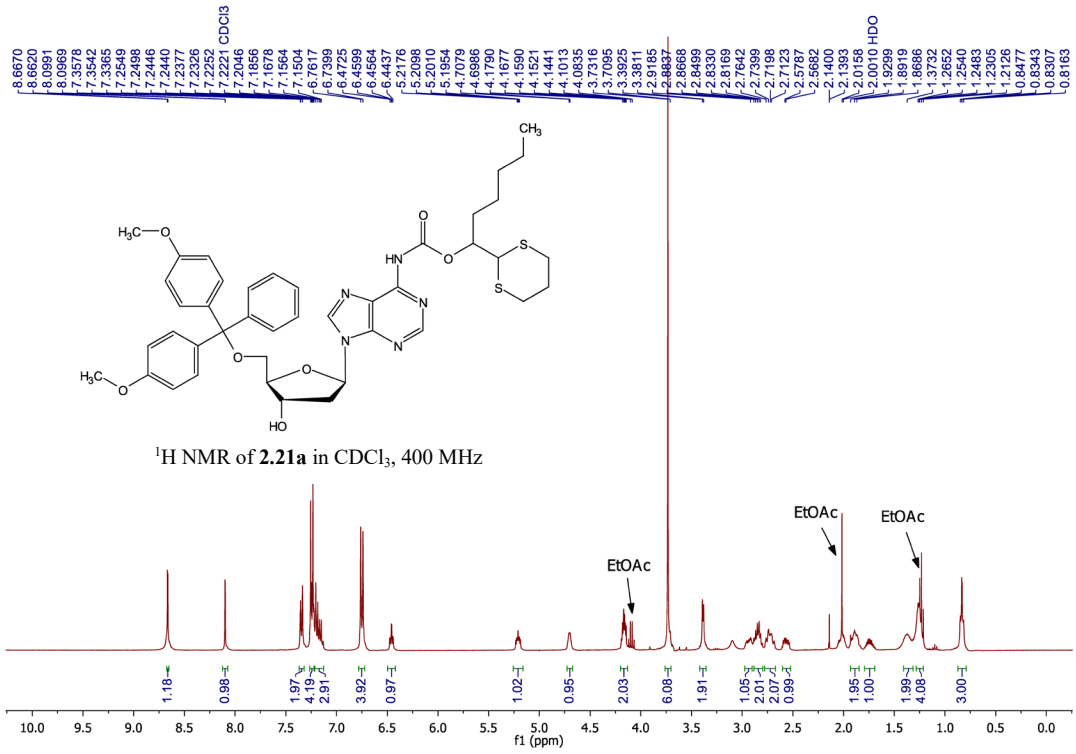
^{1}H NMR of **2.20a** in CD_3OD, 400 MHz

Figure 5.86: ^{1}H NMR spectrum of compound **2.20a**.



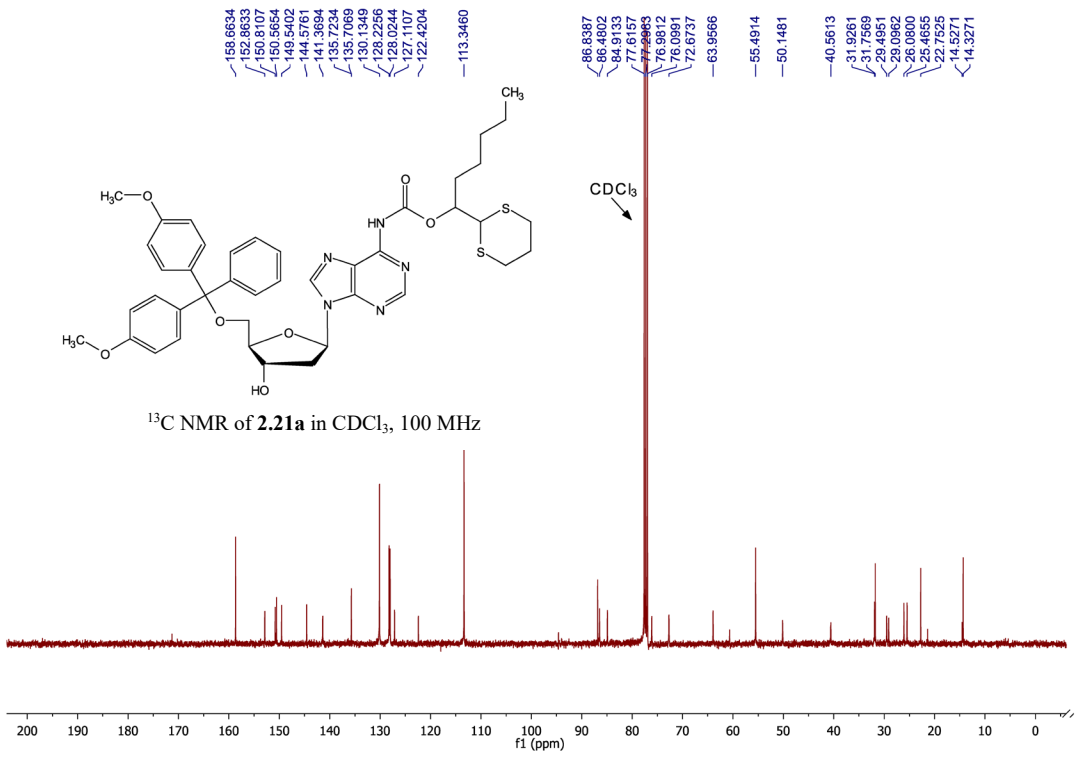
^{13}C NMR of **2.20a** in CD_3OD, 100 MHz

Figure 5.87: ^{13}C NMR spectrum of compound **2.20a**.



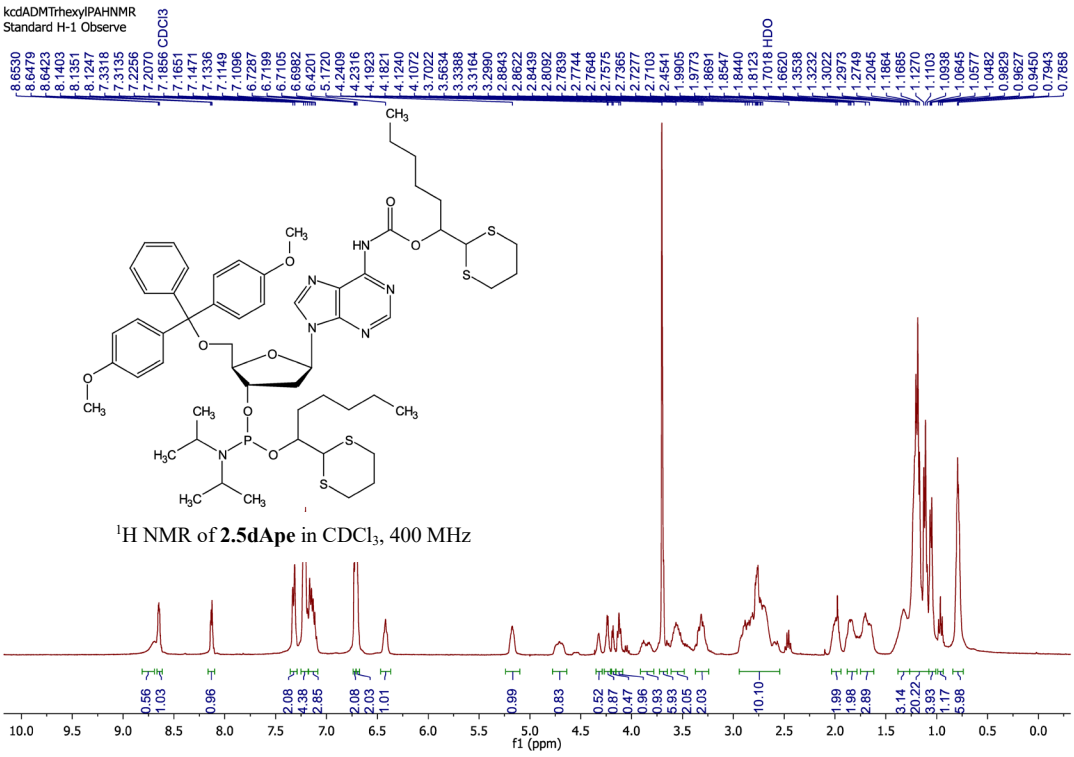
^{1}H NMR of **2.21a** in $CDCl_3$, 400 MHz

Figure 5.88: ^{1}H NMR spectrum of compound **2.21a**.



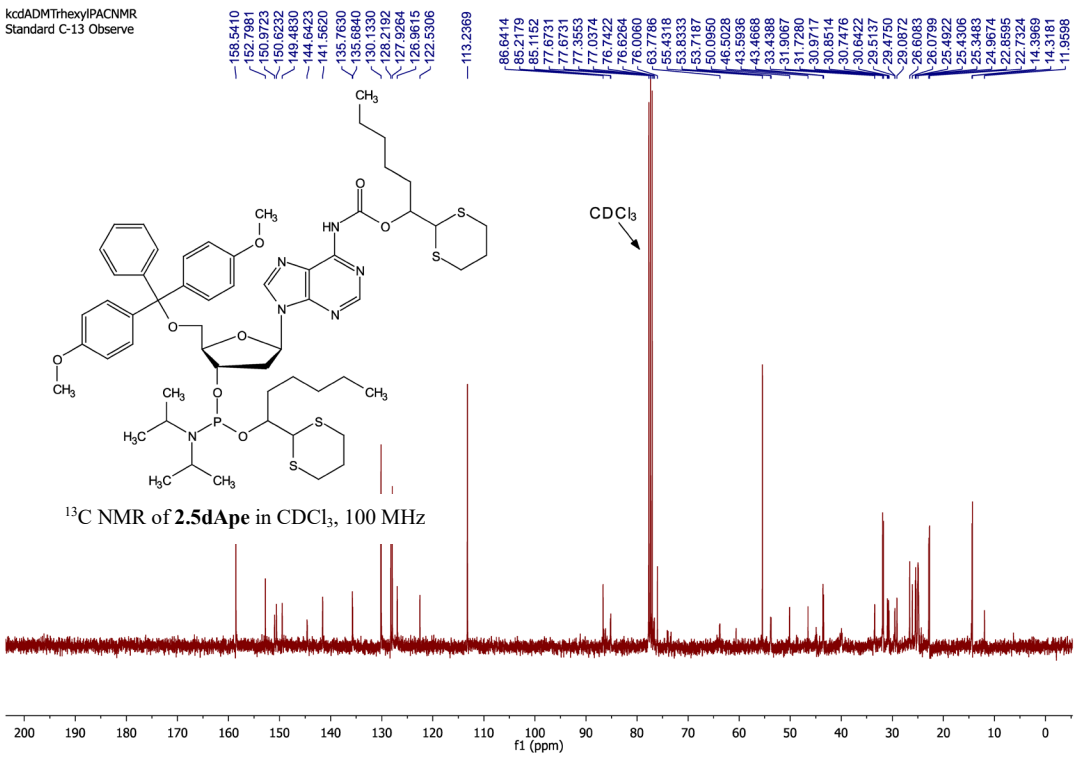
^{13}C NMR of **2.21a** in $CDCl_3$, 100 MHz

Figure 5.89: ^{13}C NMR spectrum of compound **2.21a**.



^{1}H NMR of **2.5dApe** in $CDCl_3$, 400 MHz

Figure 5.90: ^{1}H NMR spectrum of compound **2.5dApe**.



^{13}C NMR of **2.5dApe** in $CDCl_3$, 100 MHz

Figure 5.91: ^{13}C NMR spectrum of compound **2.5dApe**.

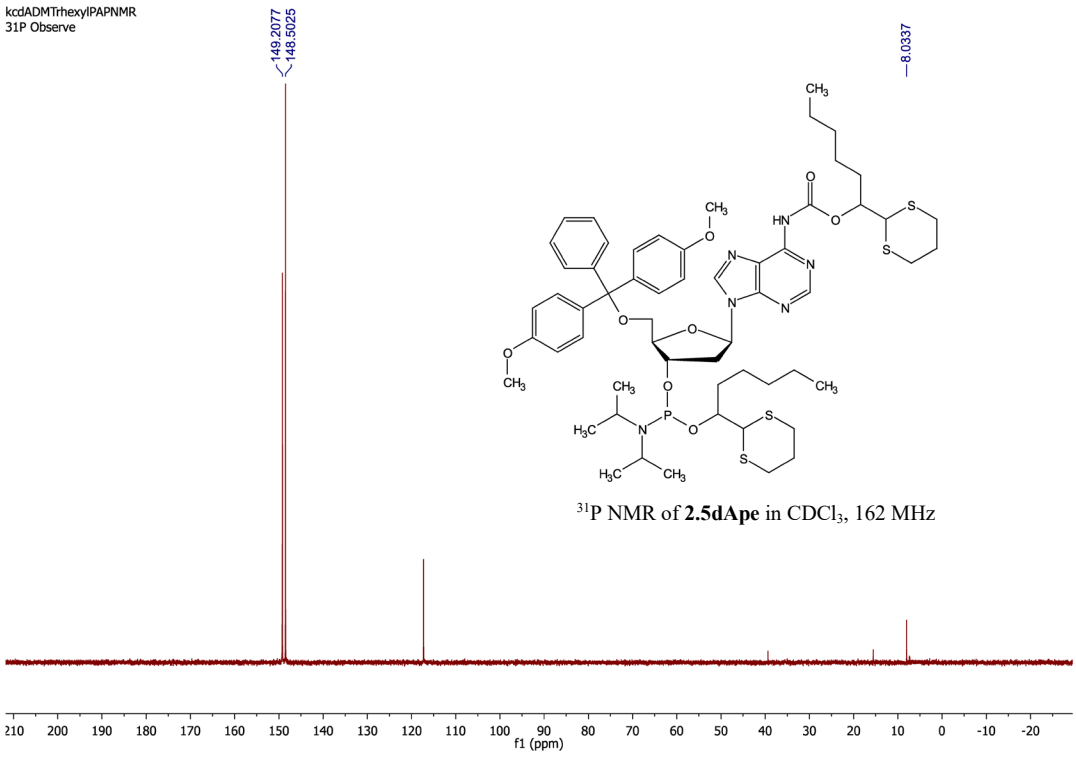
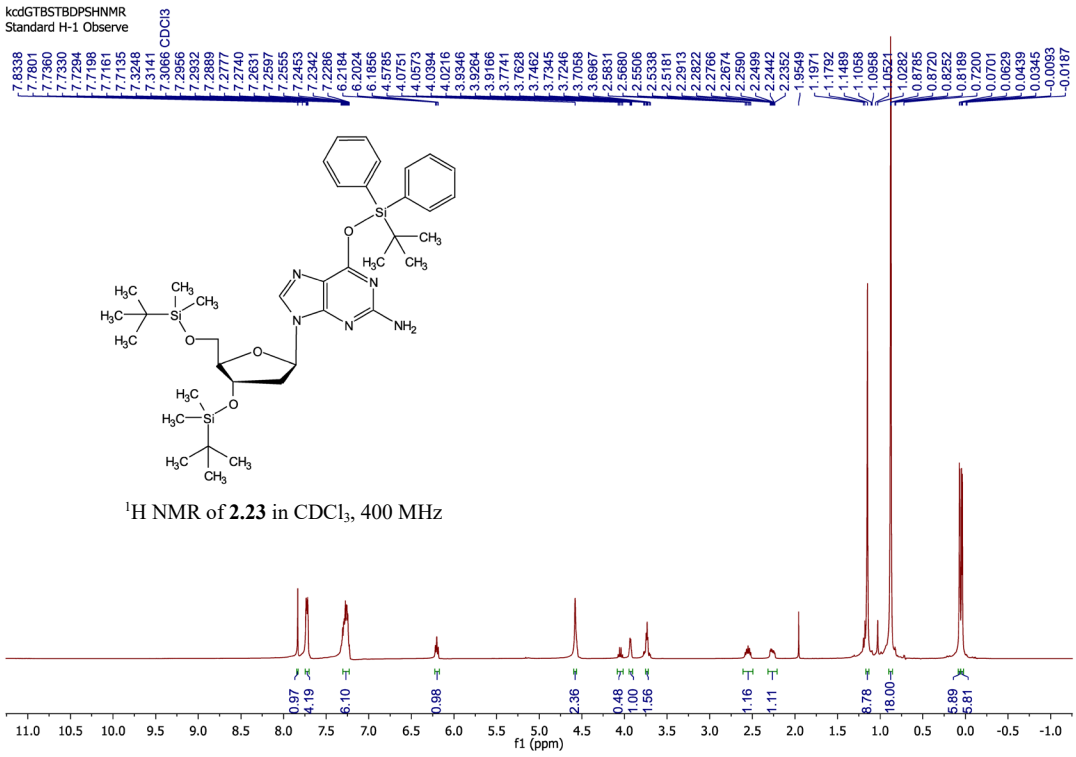
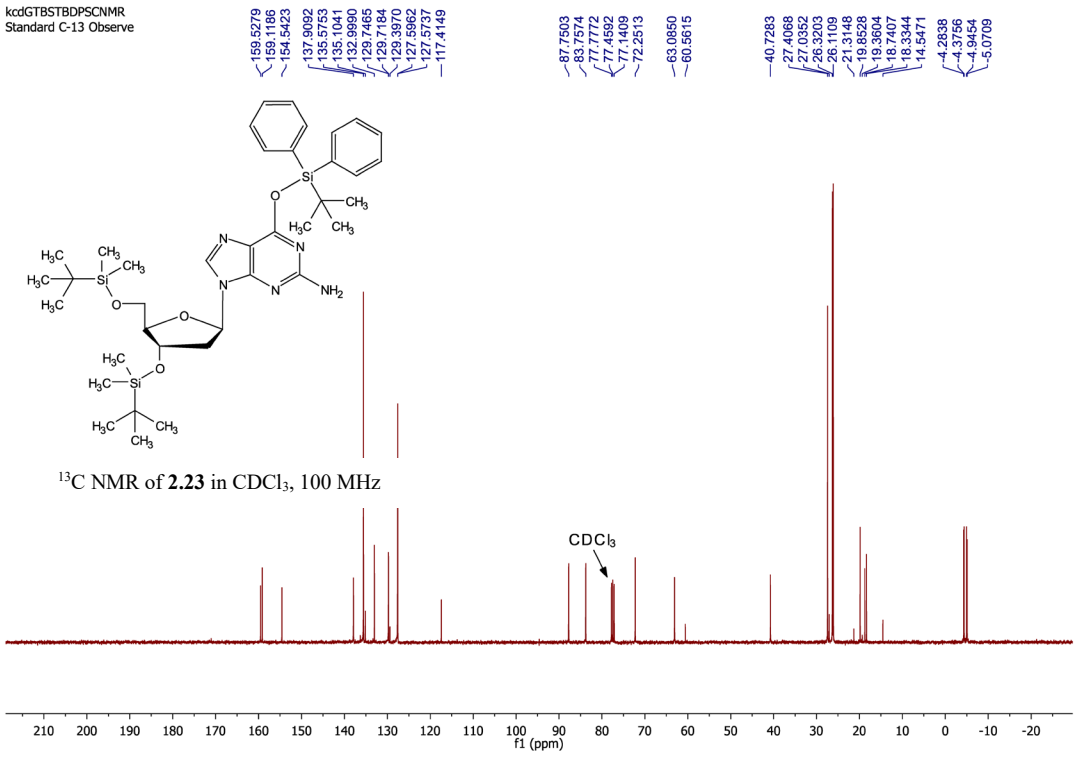


Figure 5.92: ^{31}P NMR spectrum of compound **2.5dApe**.



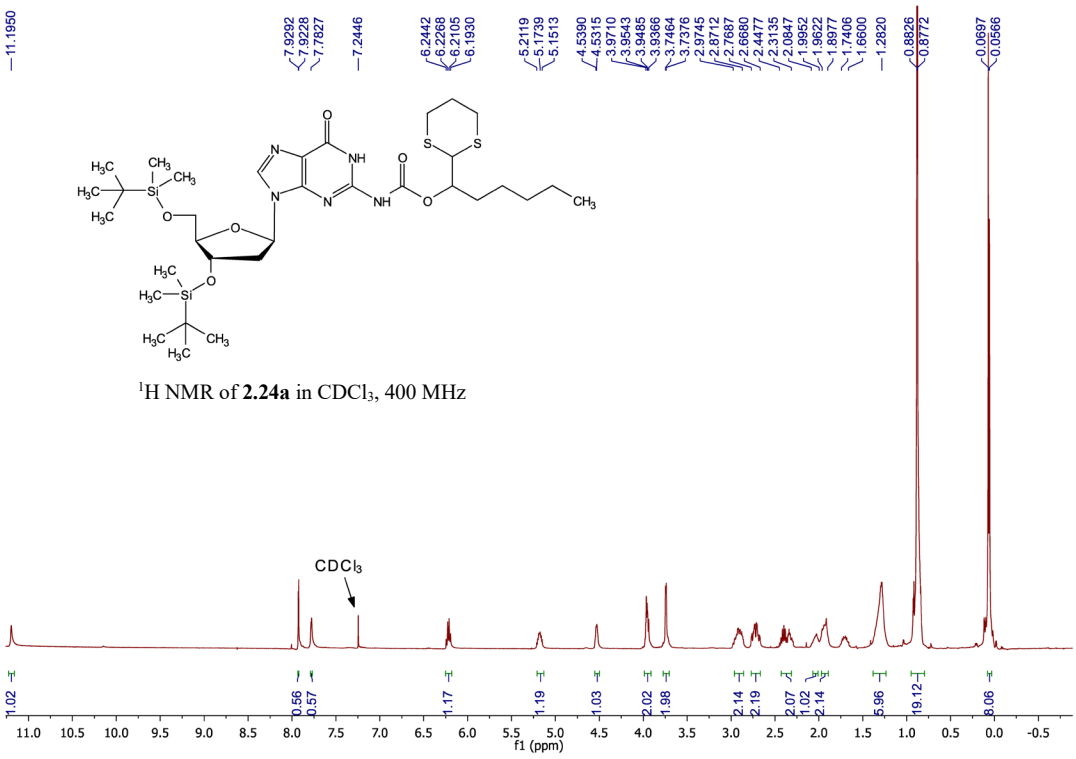
^{1}H NMR of **2.23** in $CDCl_3$, 400 MHz

Figure 5.93: ^{1}H NMR spectrum of compound **2.23**.



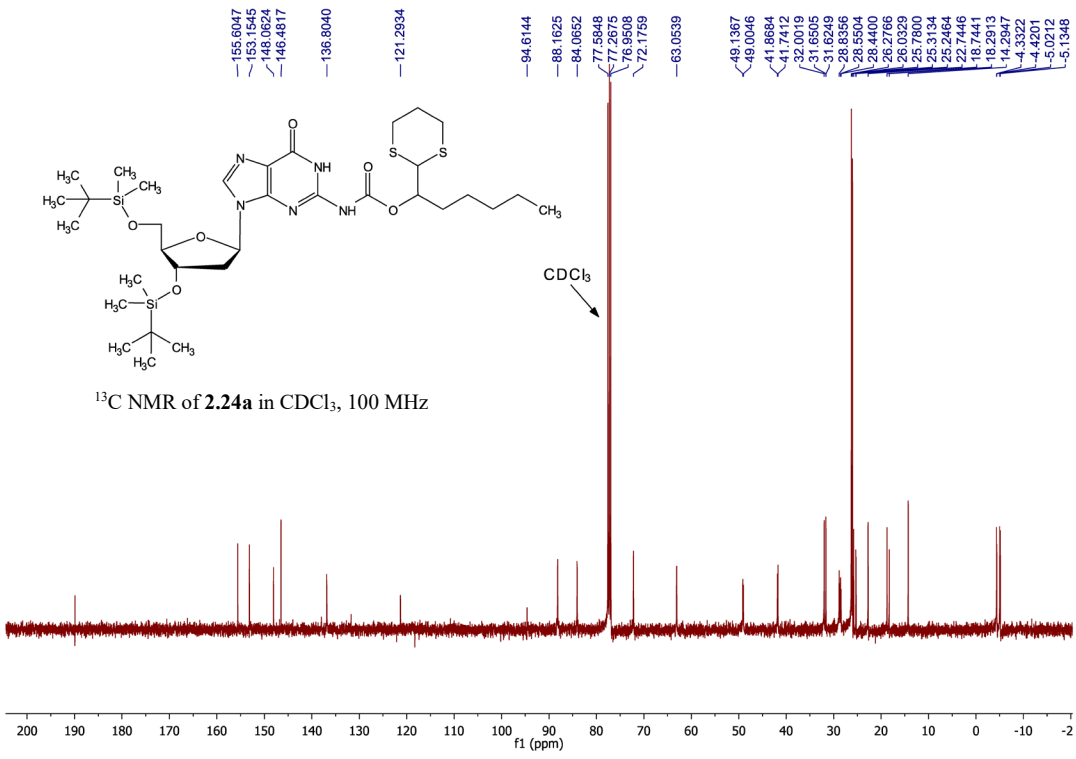
^{13}C NMR of **2.23** in $CDCl_3$, 100 MHz

Figure 5.94: ^{13}C NMR spectrum of compound **2.23**.



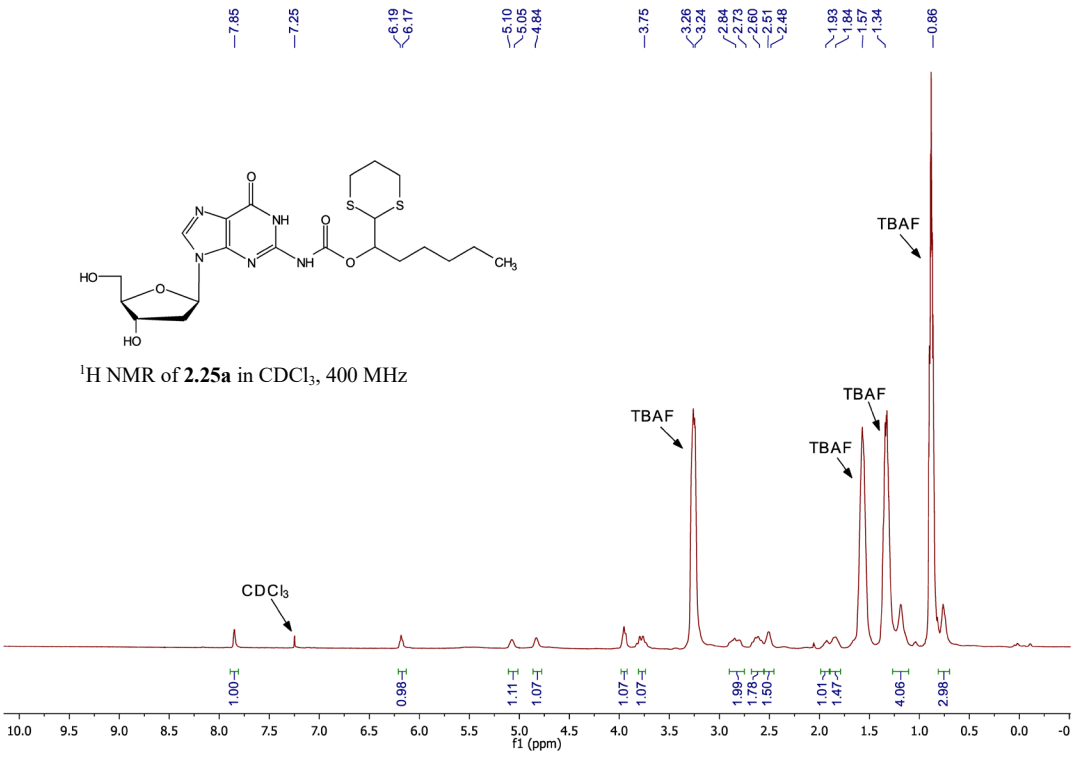
^{1}H NMR of **2.24a** in $CDCl_3$, 400 MHz

Figure 5.95: ^{1}H NMR spectrum of compound **2.24a**.



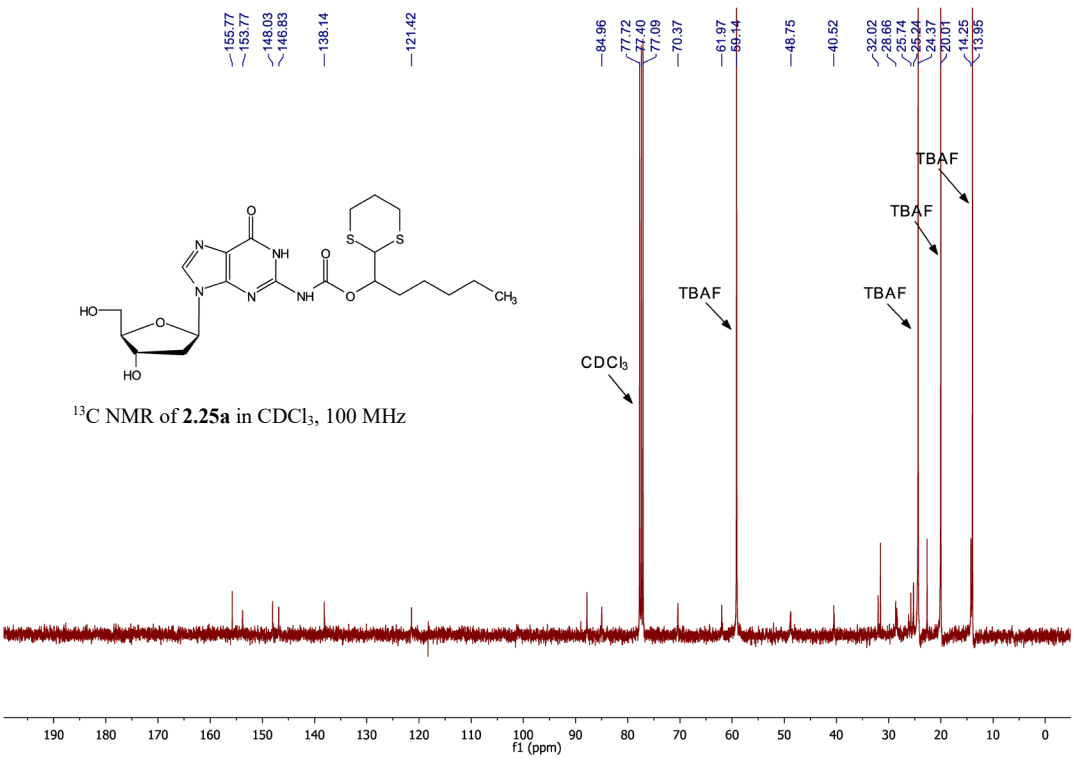
^{13}C NMR of **2.24a** in $CDCl_3$, 100 MHz

Figure 5.96: ^{13}C NMR spectrum of compound **2.24a**.



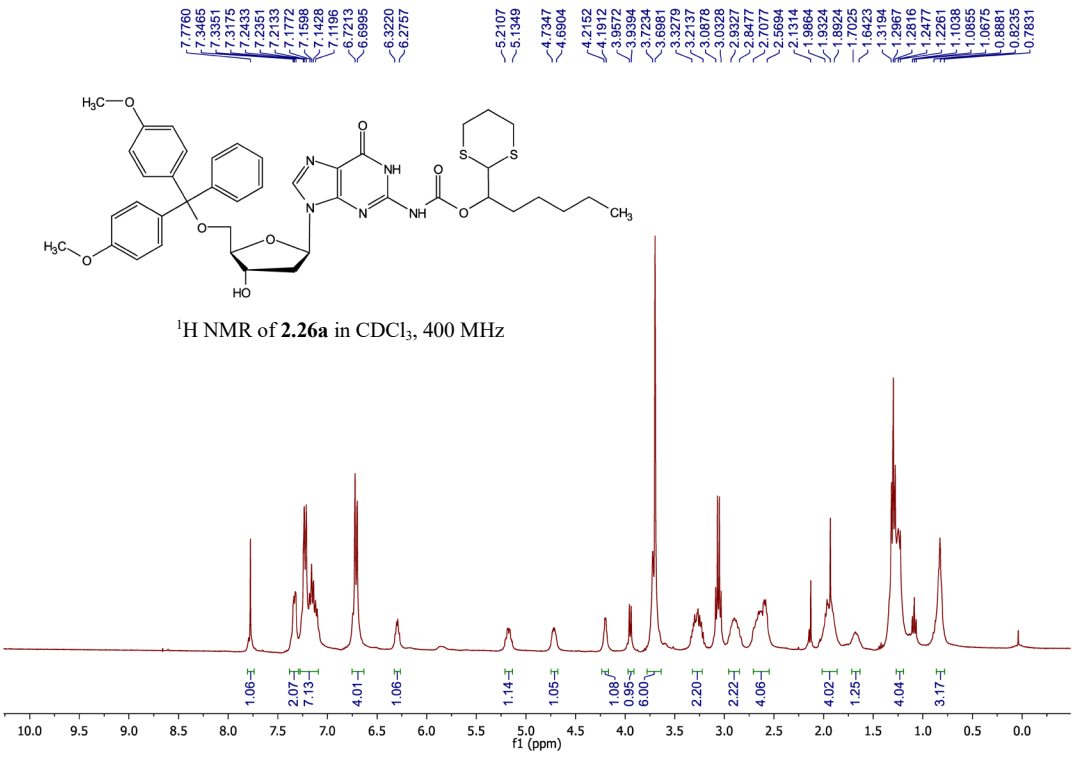
^{1}H NMR of **2.25a** in $CDCl_3$, 400 MHz

Figure 5.97: ^{1}H NMR spectrum of compound **2.25a**.



^{13}C NMR of **2.25a** in $CDCl_3$, 100 MHz

Figure 5.98: ^{13}C NMR spectrum of compound **2.25a**.



^{1}H NMR of **2.26a** in $CDCl_3$, 400 MHz

Figure 5.99: ^{1}H NMR spectrum of compound **2.26a**.

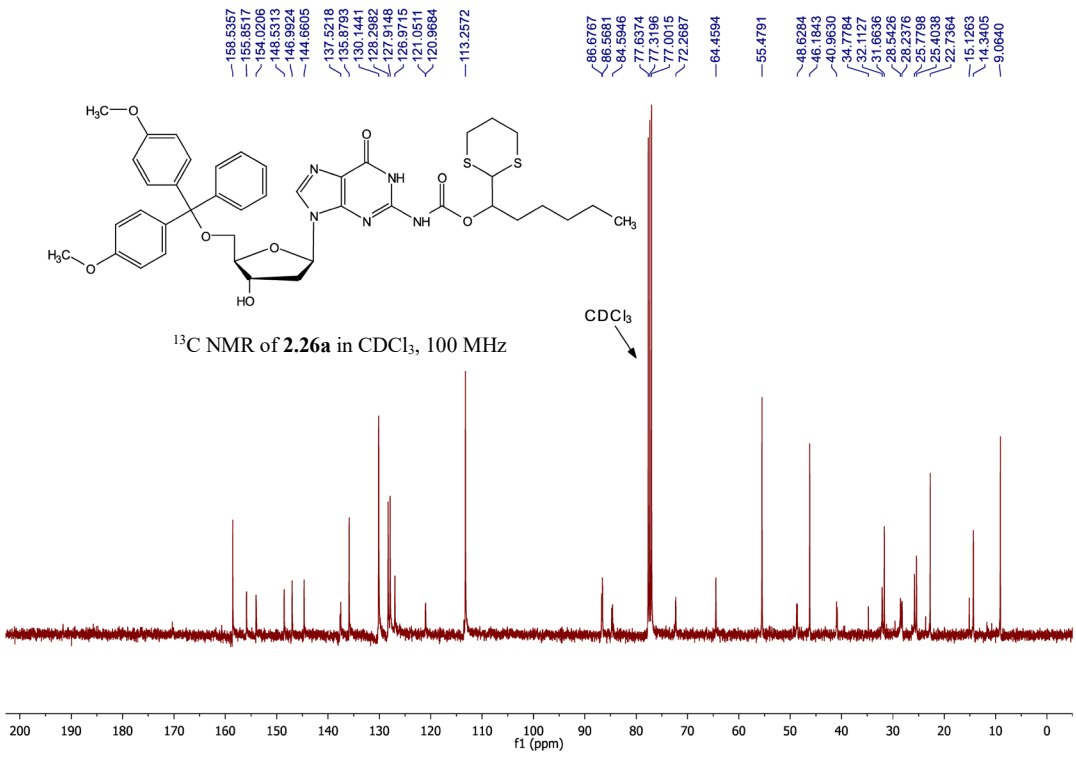
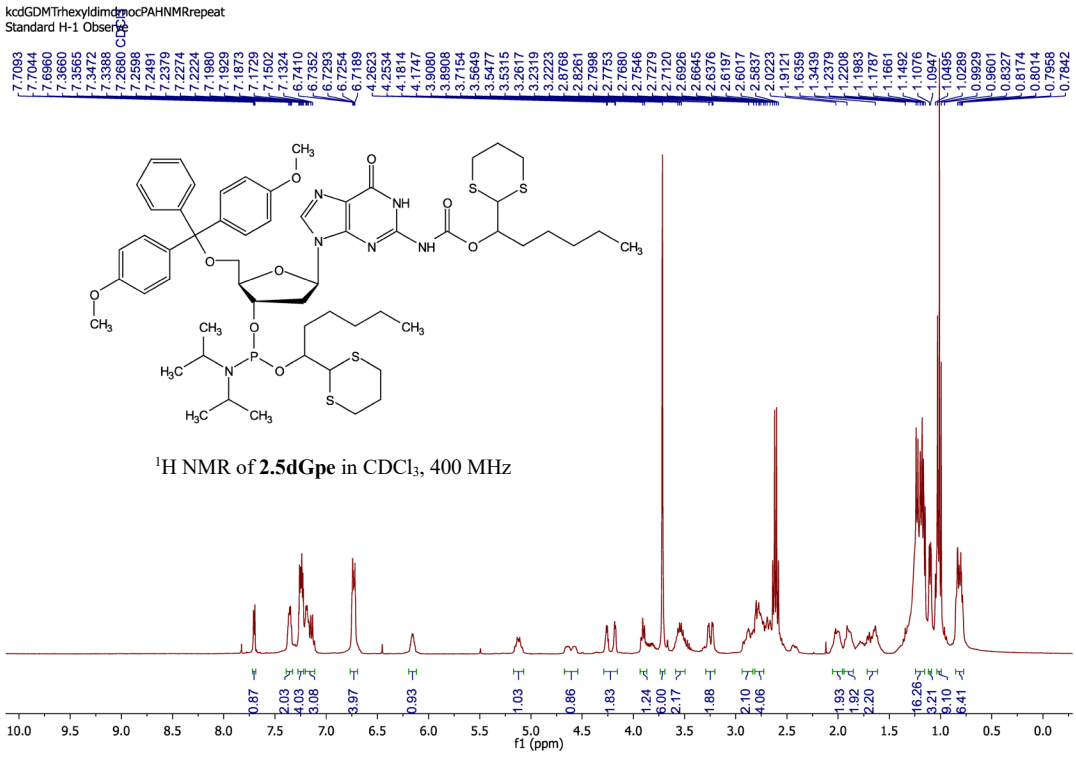
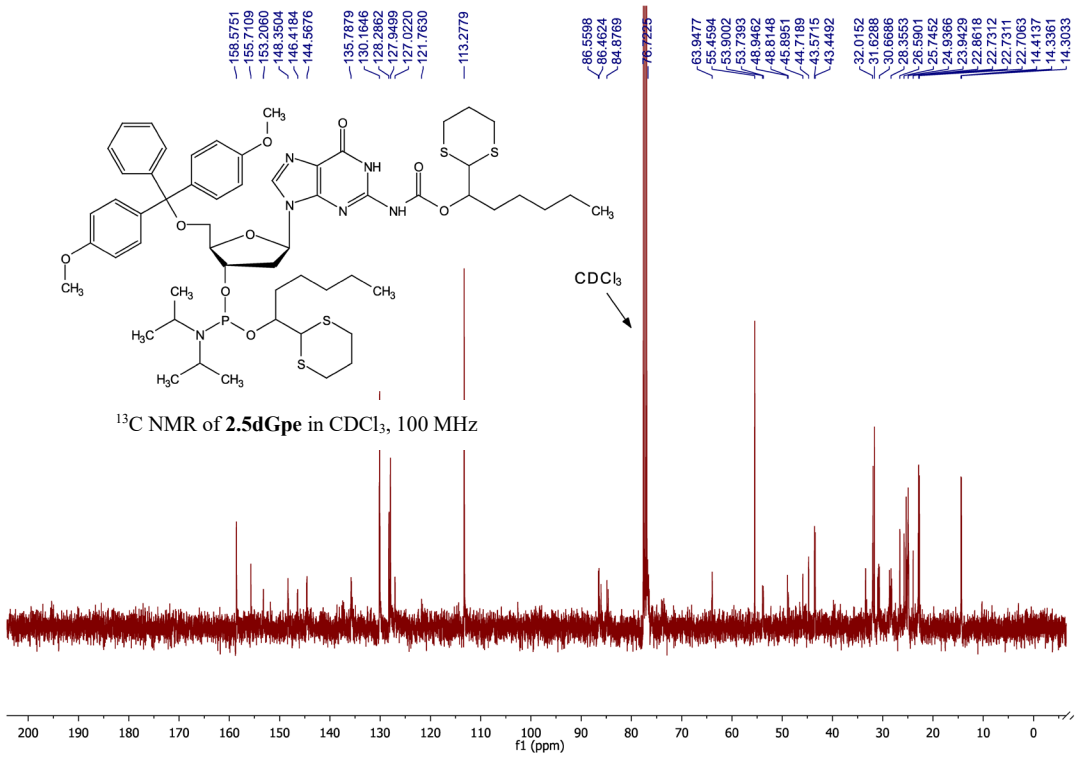


Figure 5.100: ^{13}C NMR spectrum of compound **2.26a**.



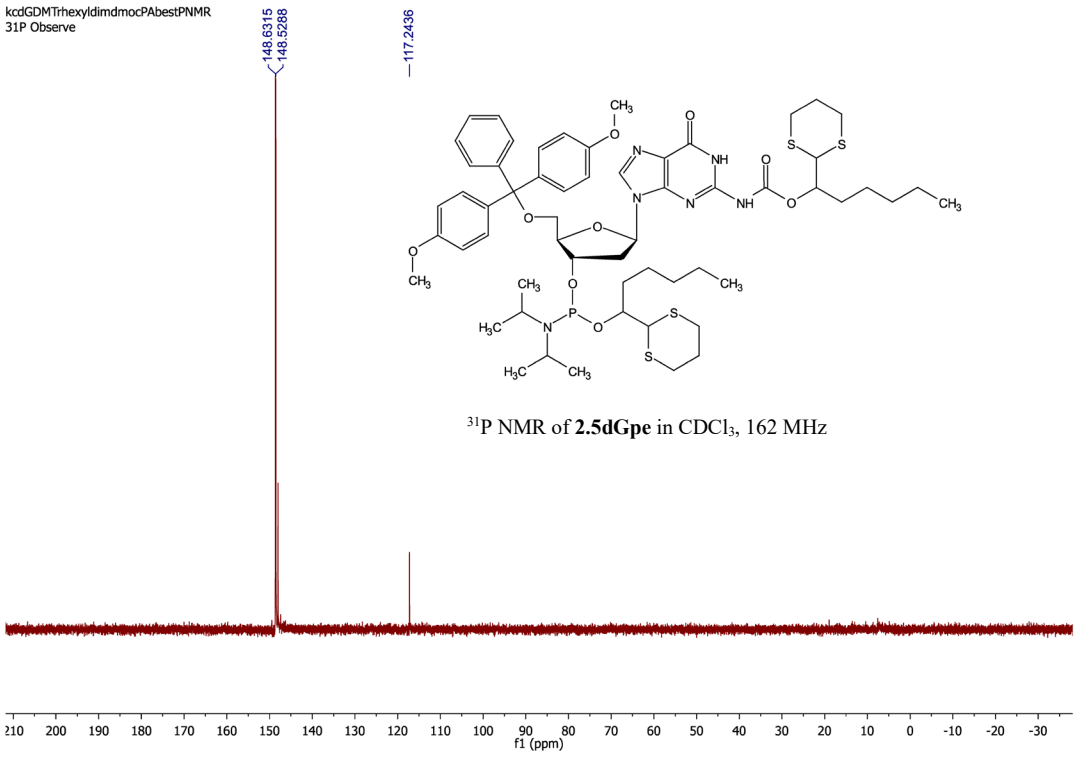
^{1}H NMR of **2.5dGpe** in $CDCl_3$, 400 MHz

Figure 5.101: ^{1}H NMR spectrum of compound **2.5dGpe**.



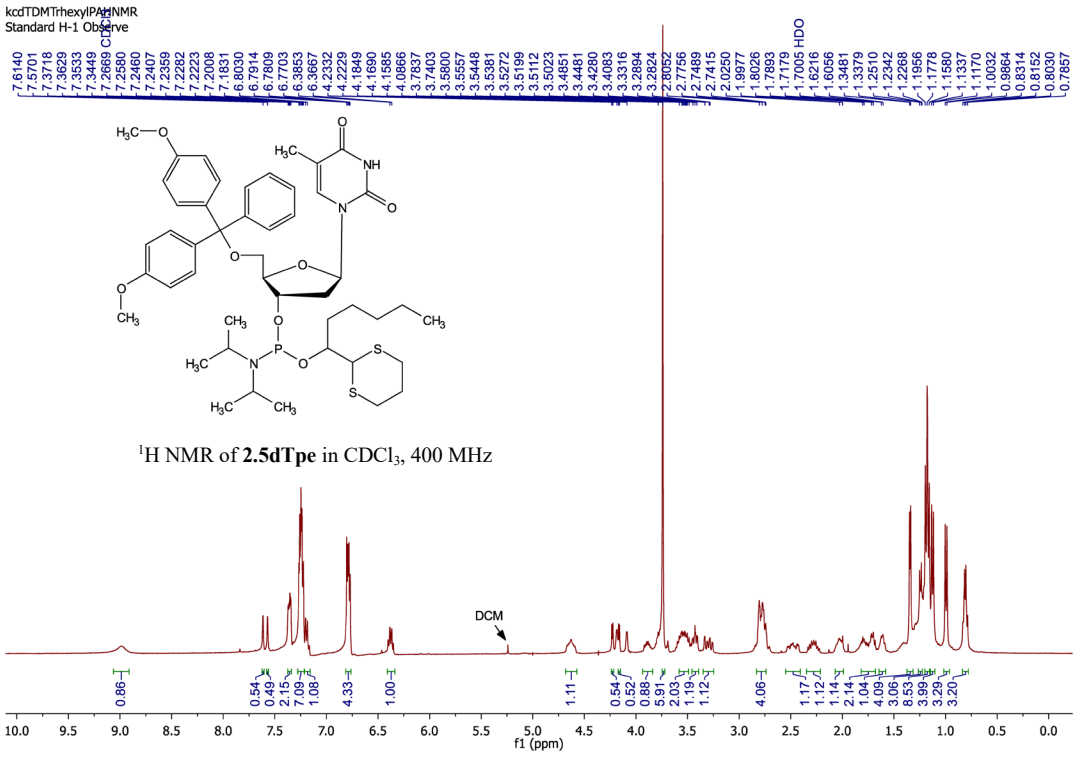
^{13}C NMR of **2.5dGpe** in $CDCl_3$, 100 MHz

Figure 5.102: ^{13}C NMR spectrum of compound **2.5dGpe**.



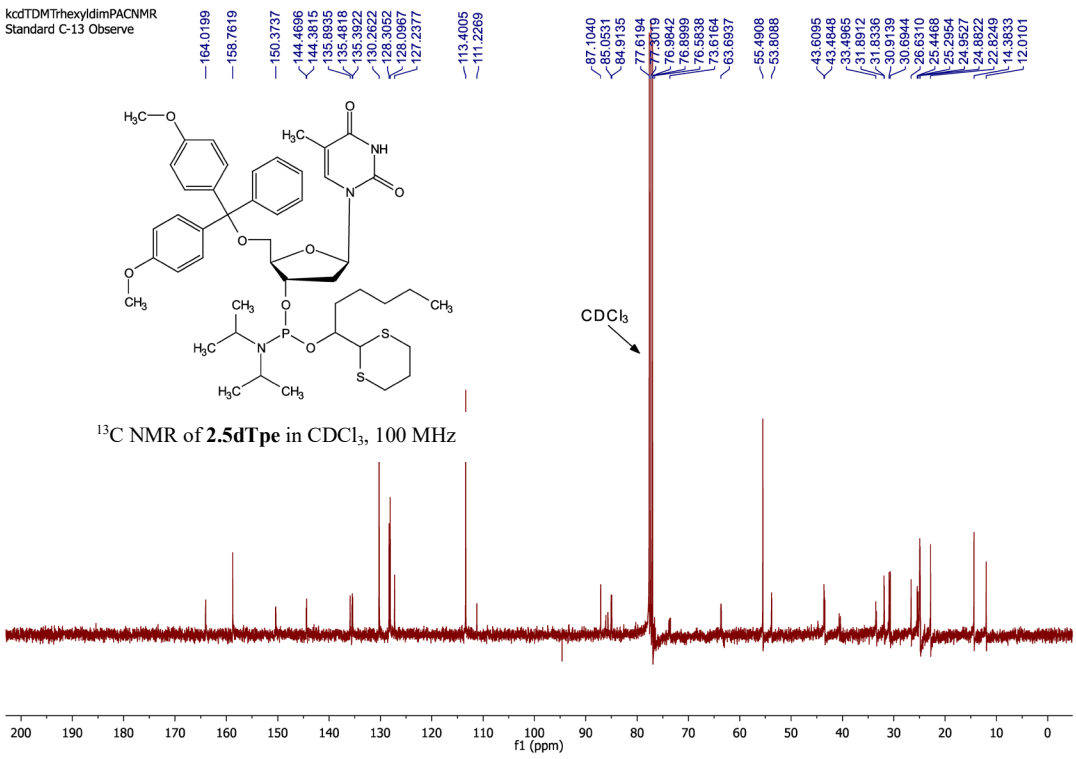
^{31}P NMR of **2.5dGpe** in $CDCl_3$, 162 MHz

Figure 5.103: ^{31}P NMR spectrum of compound **2.5dGpe**.



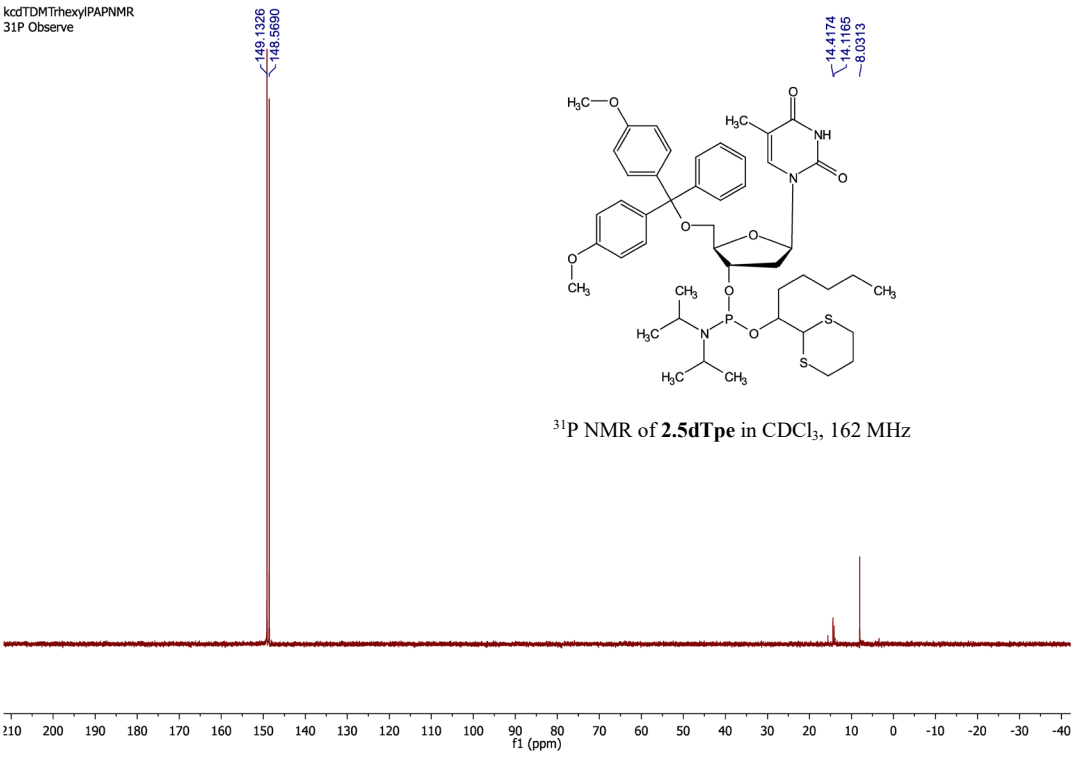
^{1}H NMR of **2.5dTpe** in $CDCl_3$, 400 MHz

Figure 5.104: ^{1}H NMR spectrum of compound **2.5dTpe**.



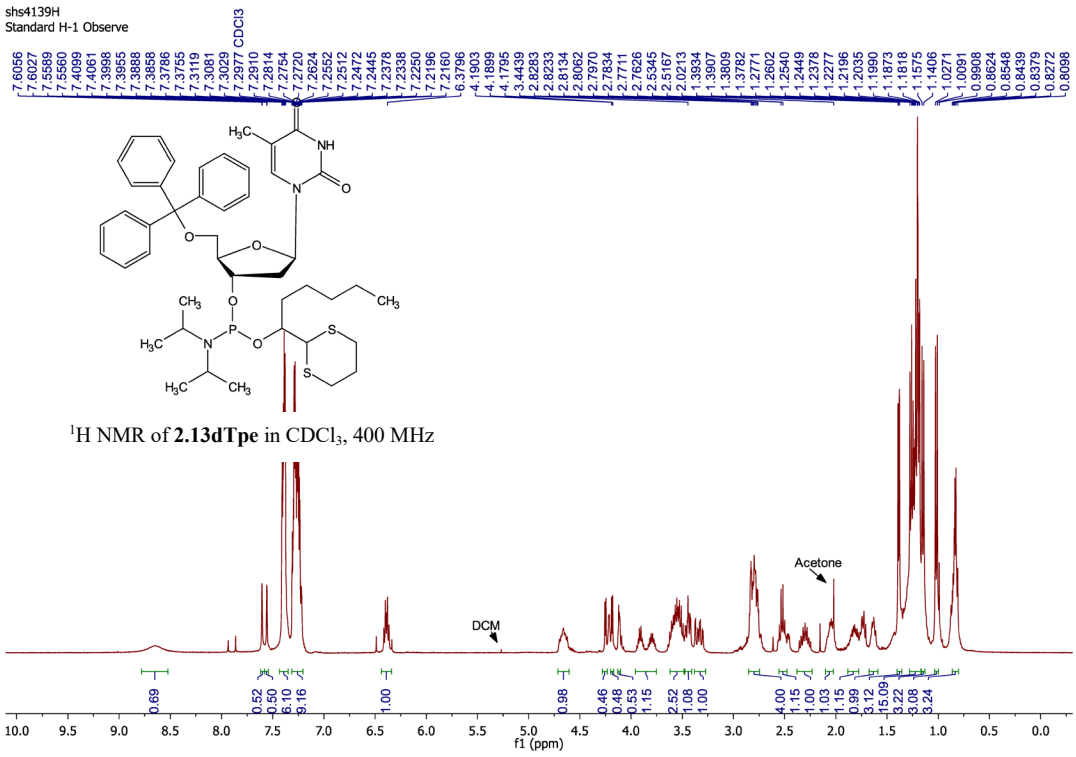
^{13}C NMR of **2.5dTpe** in $CDCl_3$, 100 MHz

Figure 5.105: ^{13}C NMR spectrum of compound **2.5dTpe**.



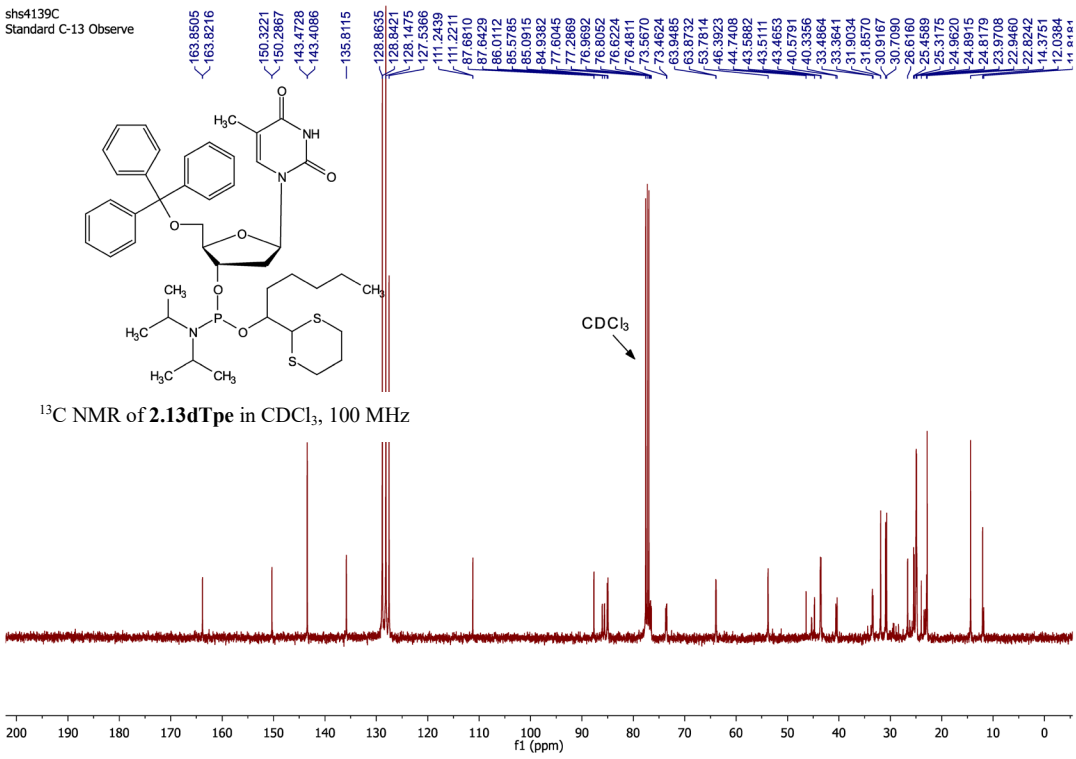
^{31}P NMR of **2.5dTpe** in $CDCl_3$, 162 MHz

Figure 5.106: ^{31}P NMR spectrum of compound **2.5dTpe**.



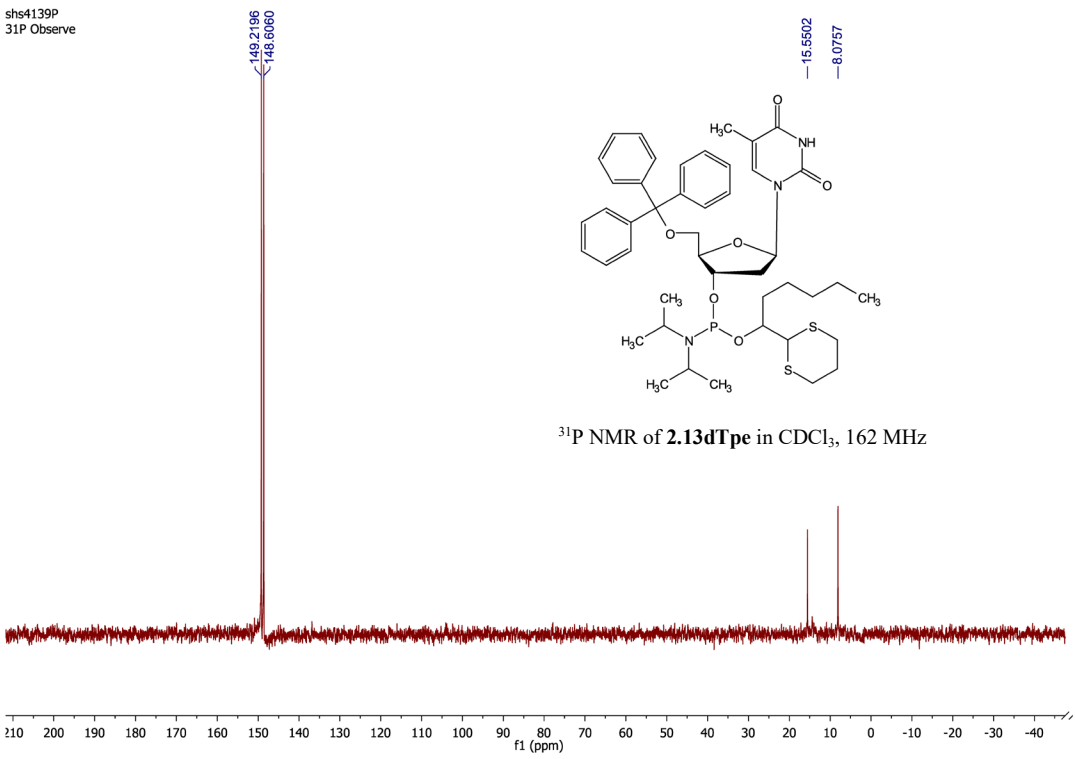
^{1}H NMR of **2.13dTpe** in $CDCl_3$, 400 MHz

Figure 5.107: ^{1}H NMR spectrum of compound **2.13dTpe**.



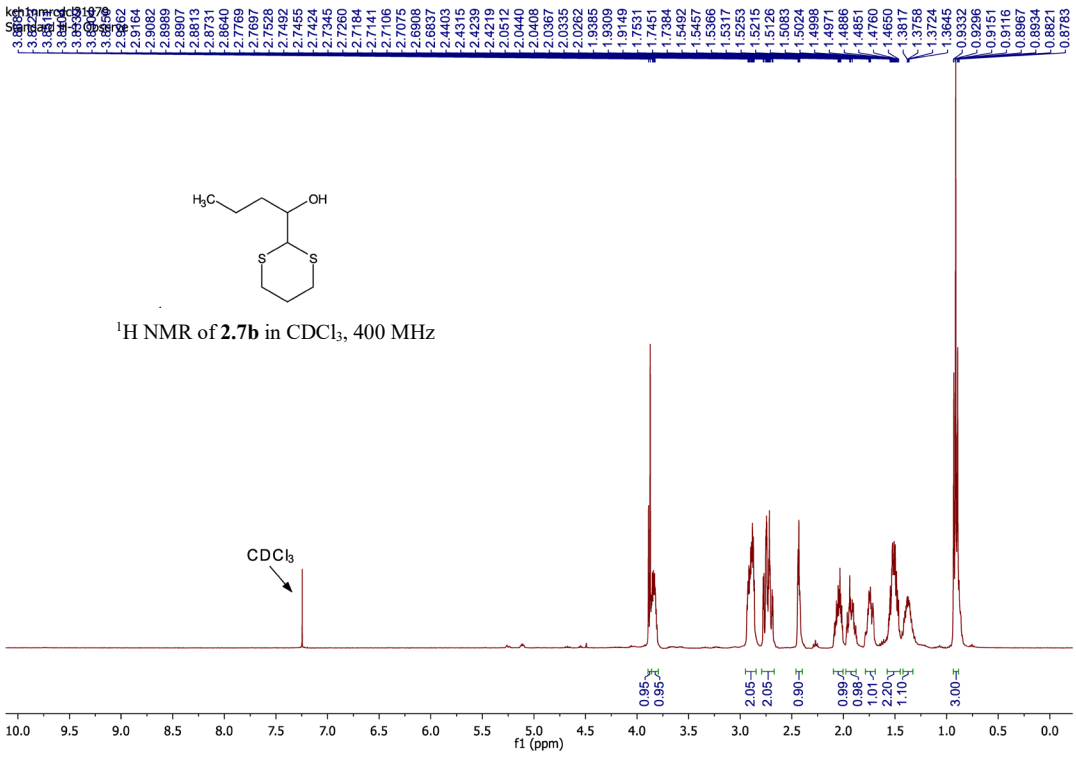
^{13}C NMR of **2.13dTpe** in $CDCl_3$, 100 MHz

Figure 5.108: ^{13}C NMR spectrum of compound **2.13dTpe**.



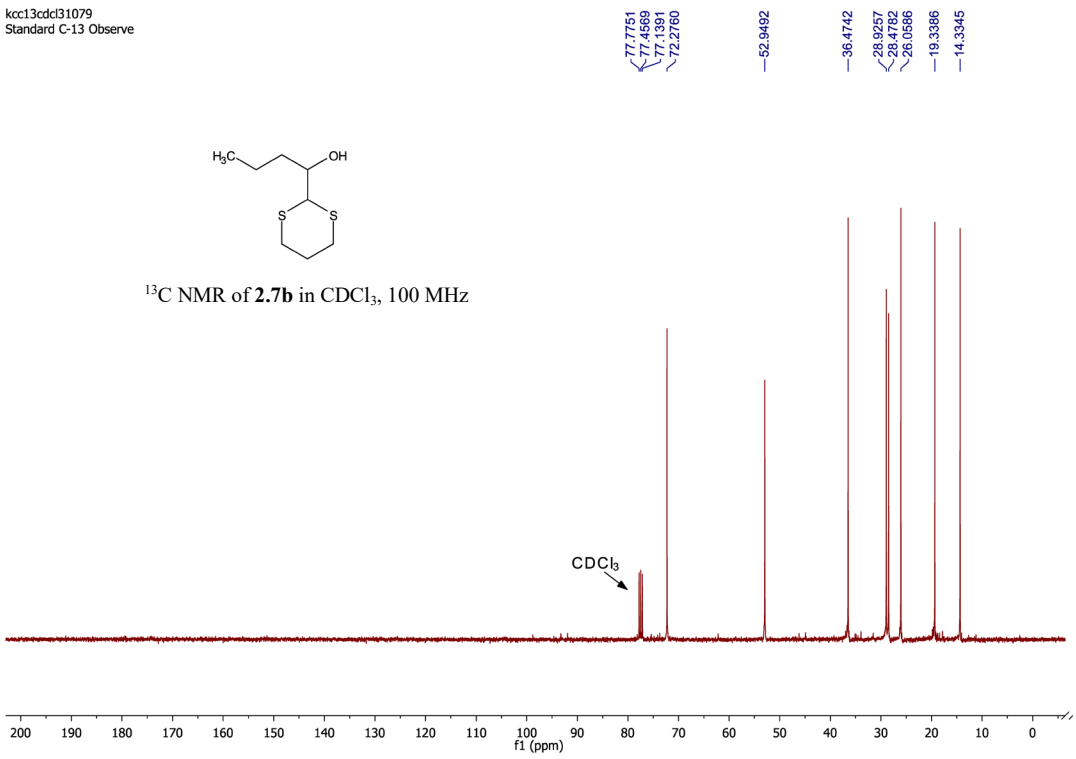
^{31}P NMR of **2.13dTpe** in $CDCl_3$, 162 MHz

Figure 5.109: ^{31}P NMR spectrum of compound **2.13dTpe**.



^{1}H NMR of **2.7b** in $CDCl_3$, 400 MHz

Figure 5.110: ^{1}H NMR spectrum of compound **2.7b**.



^{13}C NMR of **2.7b** in $CDCl_3$, 100 MHz

Figure 5.111: ^{13}C NMR spectrum of compound **2.7b**.

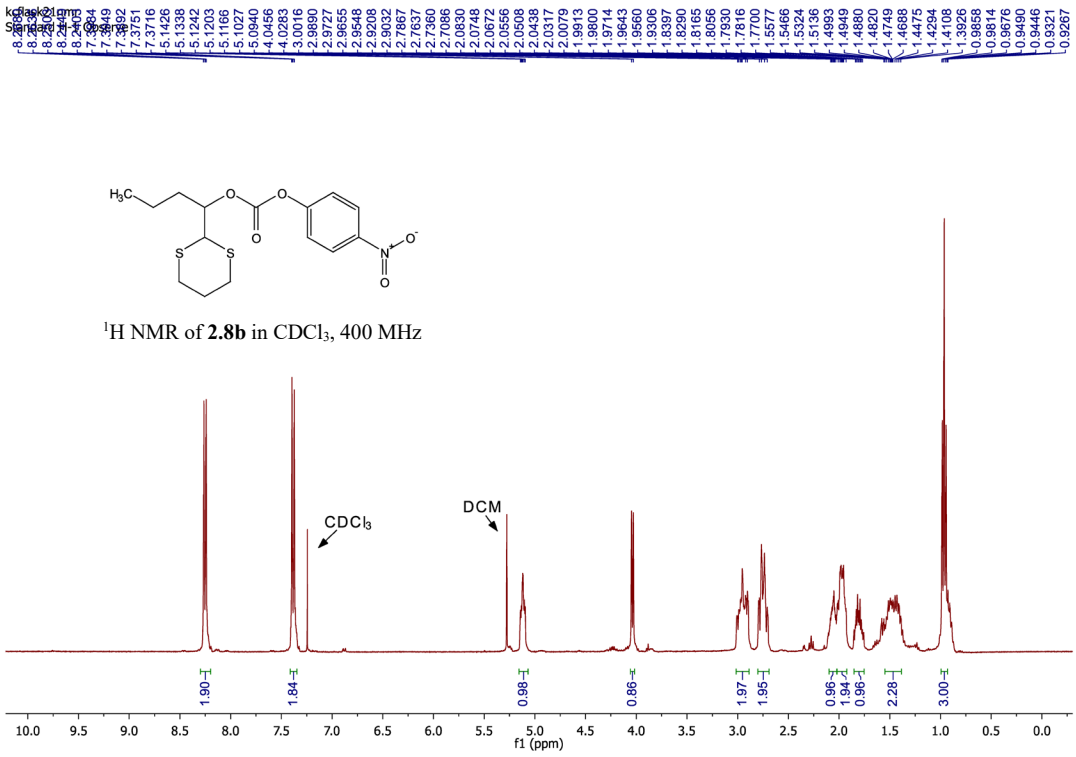
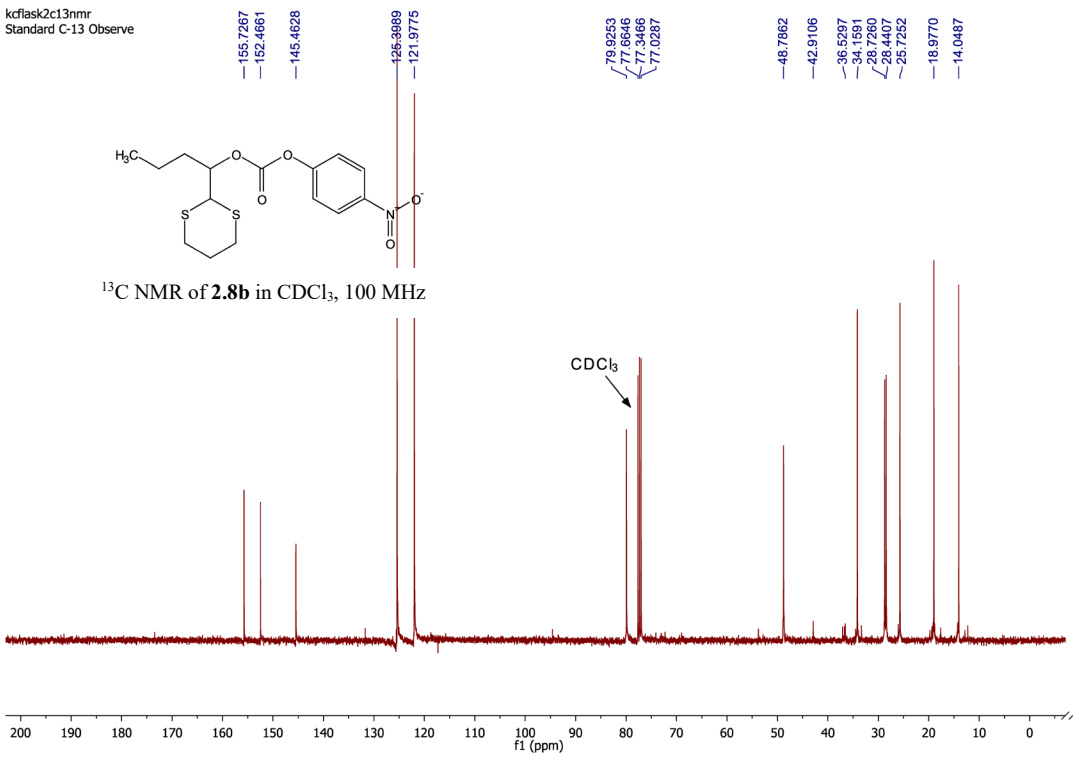
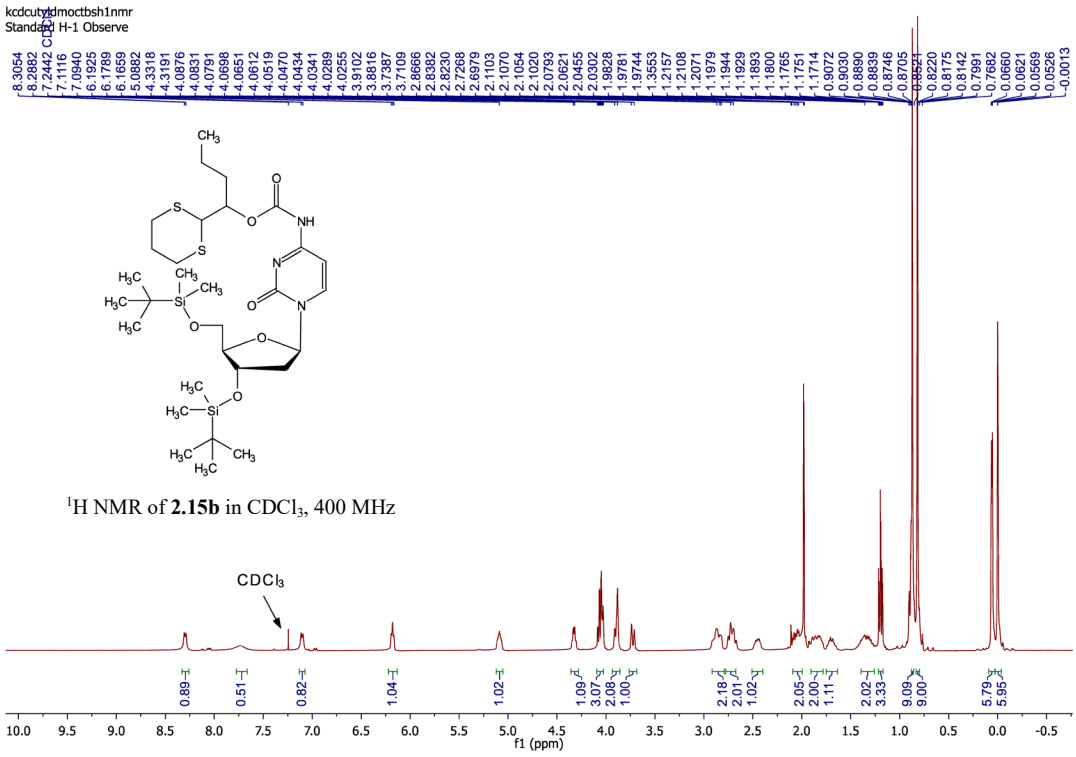


Figure 5.112: ^{1}H NMR spectrum of compound **2.8b**.



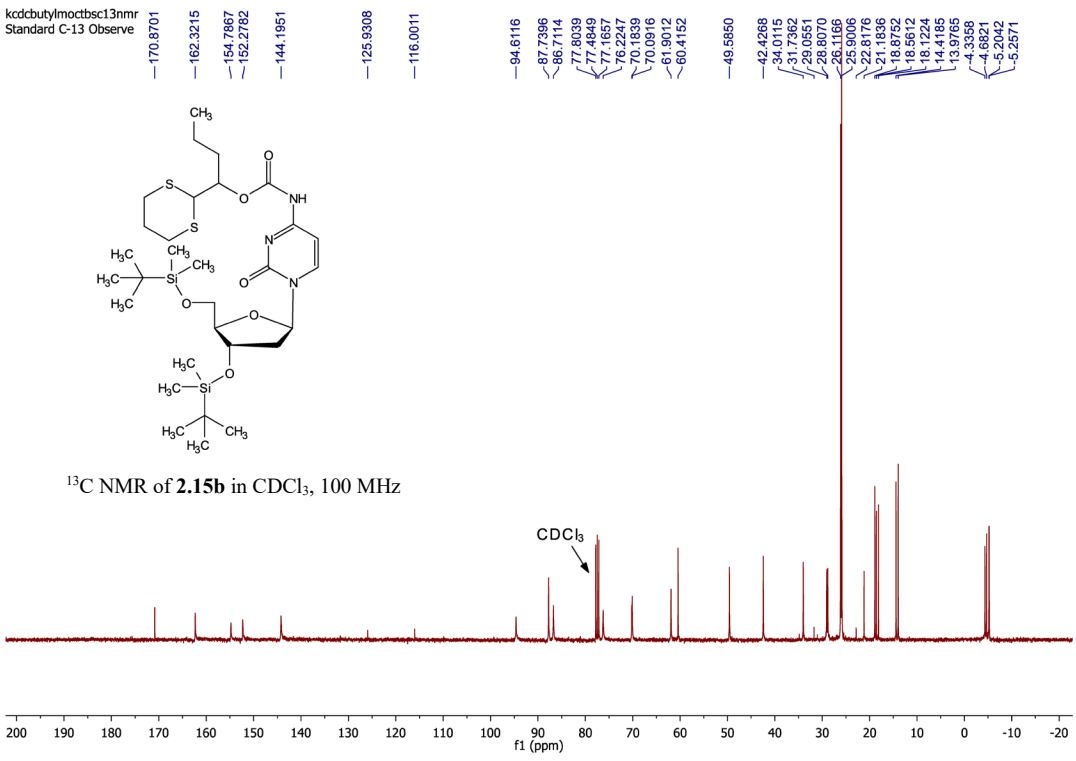
^{13}C NMR of **2.8b** in $CDCl_3$, 100 MHz

Figure 5.1133: ^{13}C NMR spectrum of compound **2.8b**.



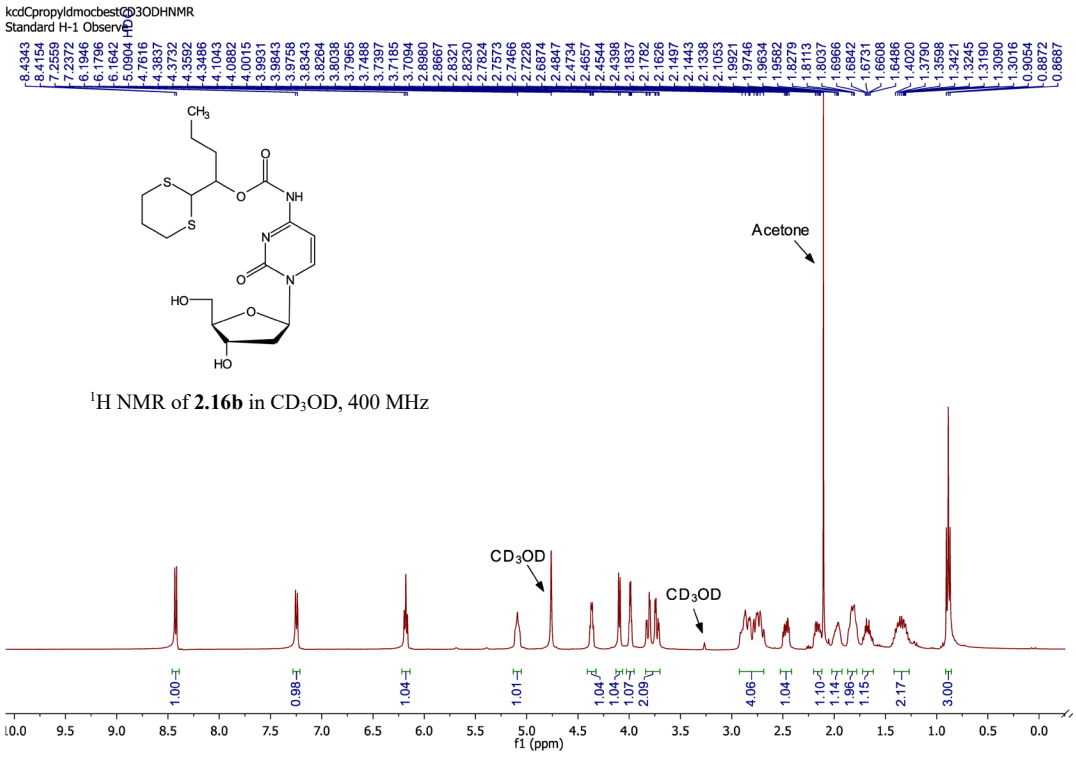
^{1}H NMR of **2.15b** in $CDCl_3$, 400 MHz

Figure 5.114: ^{1}H NMR spectrum of compound **2.15b**.



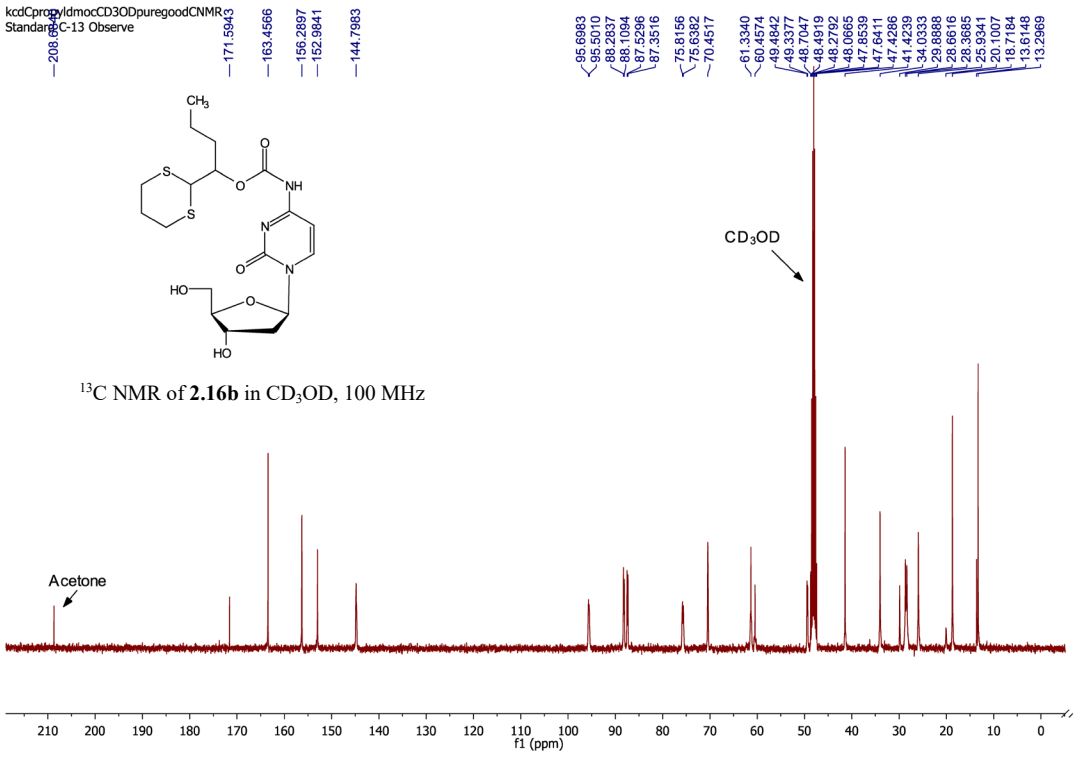
^{13}C NMR of **2.15b** in $CDCl_3$, 100 MHz

Figure 5.115: ^{13}C NMR spectrum of compound **2.15b**.



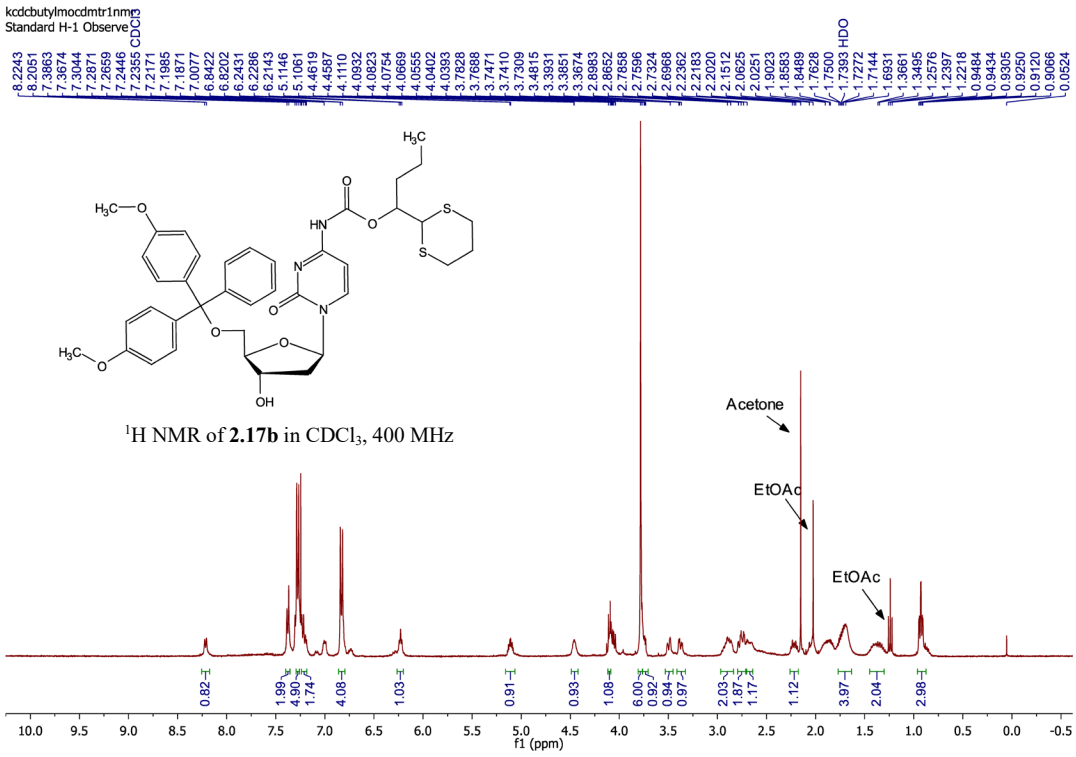
^{1}H NMR of **2.16b** in CD_3OD, 400 MHz

Figure 5.116: ^{1}H NMR spectrum of compound **2.16b**.



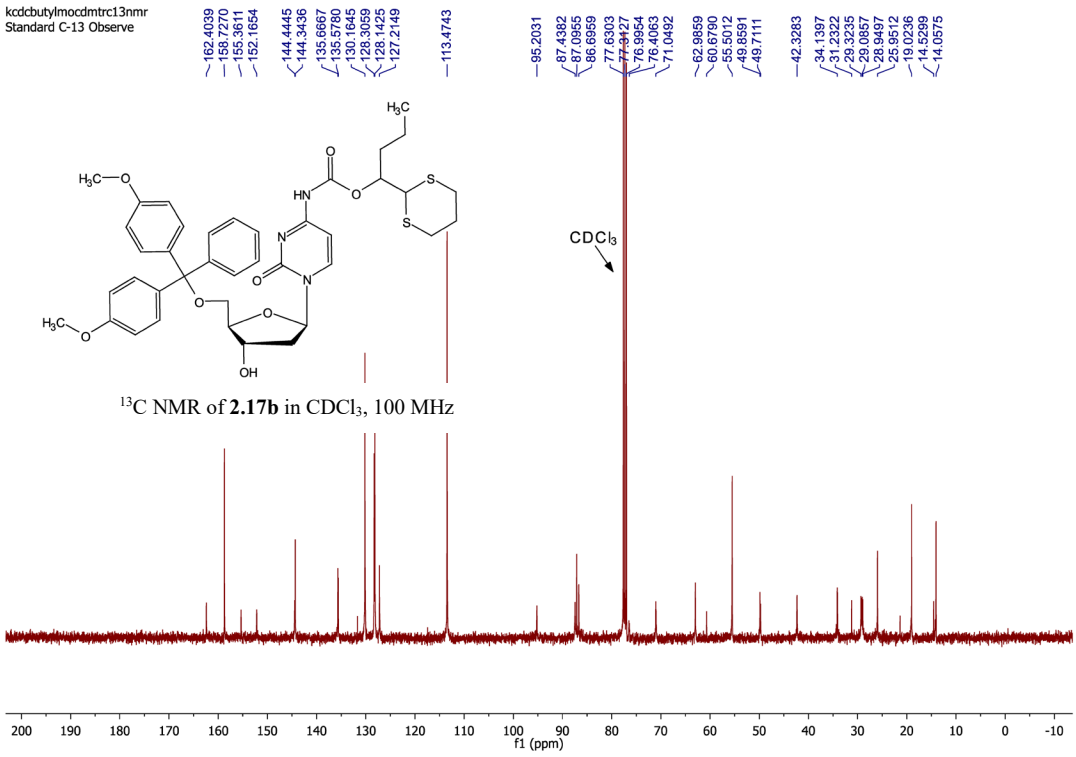
^{13}C NMR of **2.16b** in CD_3OD, 100 MHz

Figure 5.117: ^{13}C NMR spectrum of compound **2.16b**.



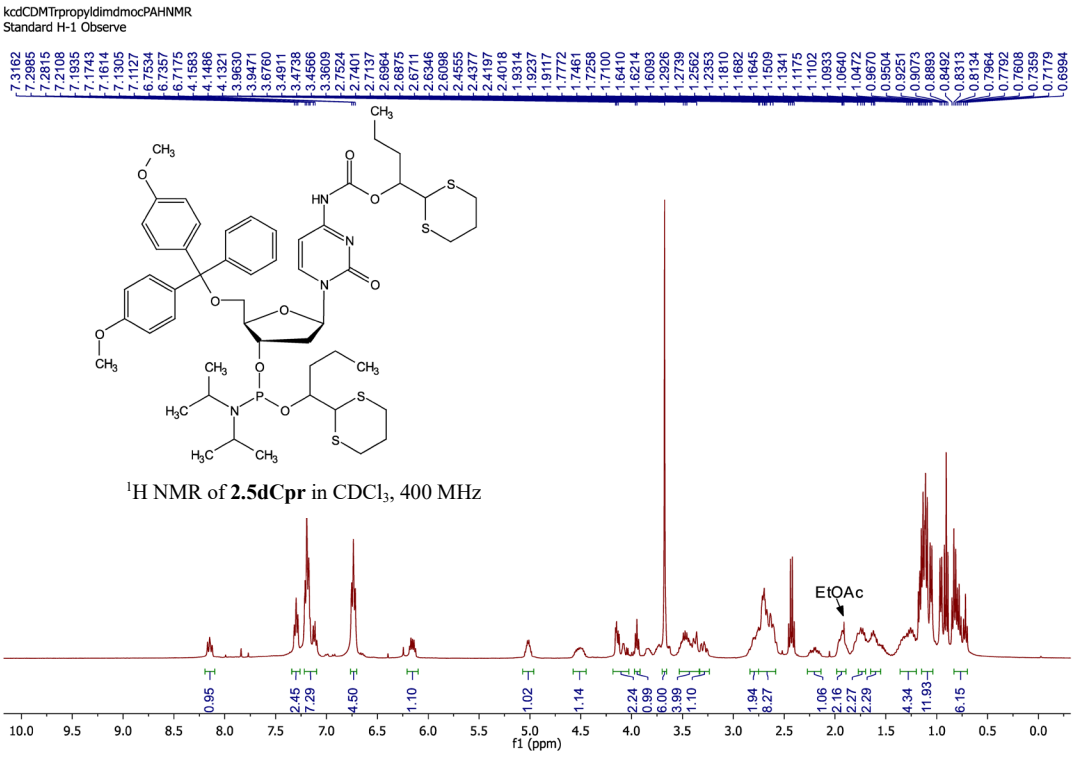
^{1}H NMR of **2.17b** in $CDCl_3$, 400 MHz

Figure 5.118: ^{1}H NMR spectrum of compound **2.17b**.



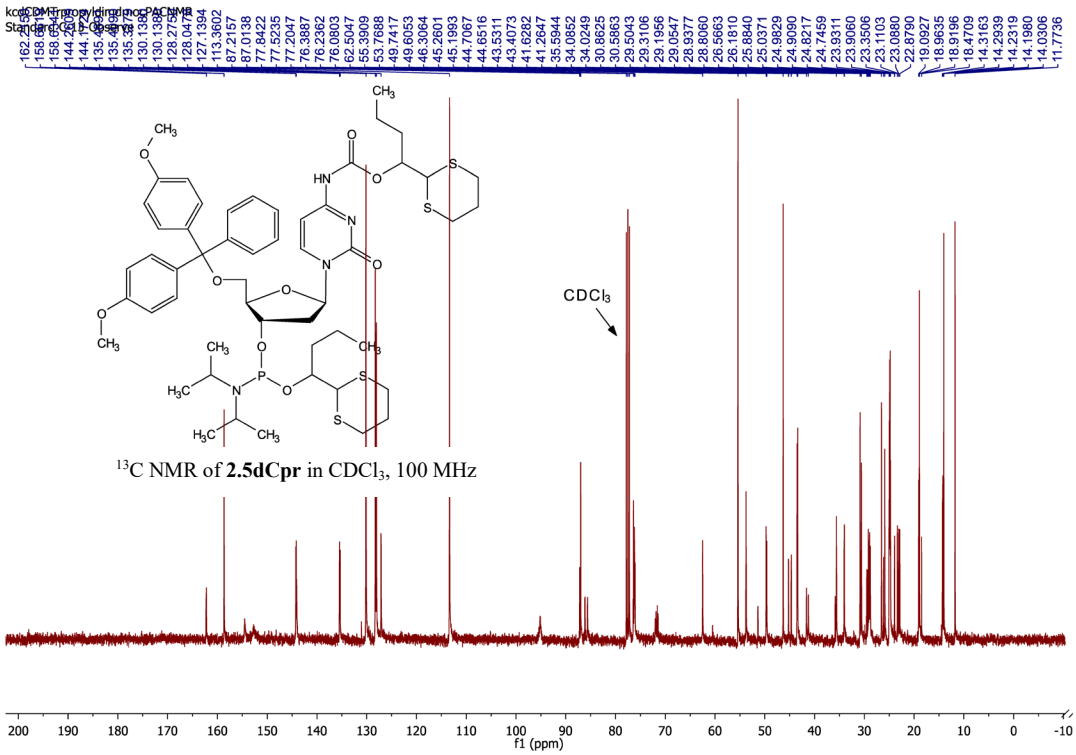
^{13}C NMR of **2.17b** in $CDCl_3$, 100 MHz

Figure 5.119: ^{13}C NMR spectrum of compound **2.17b**.



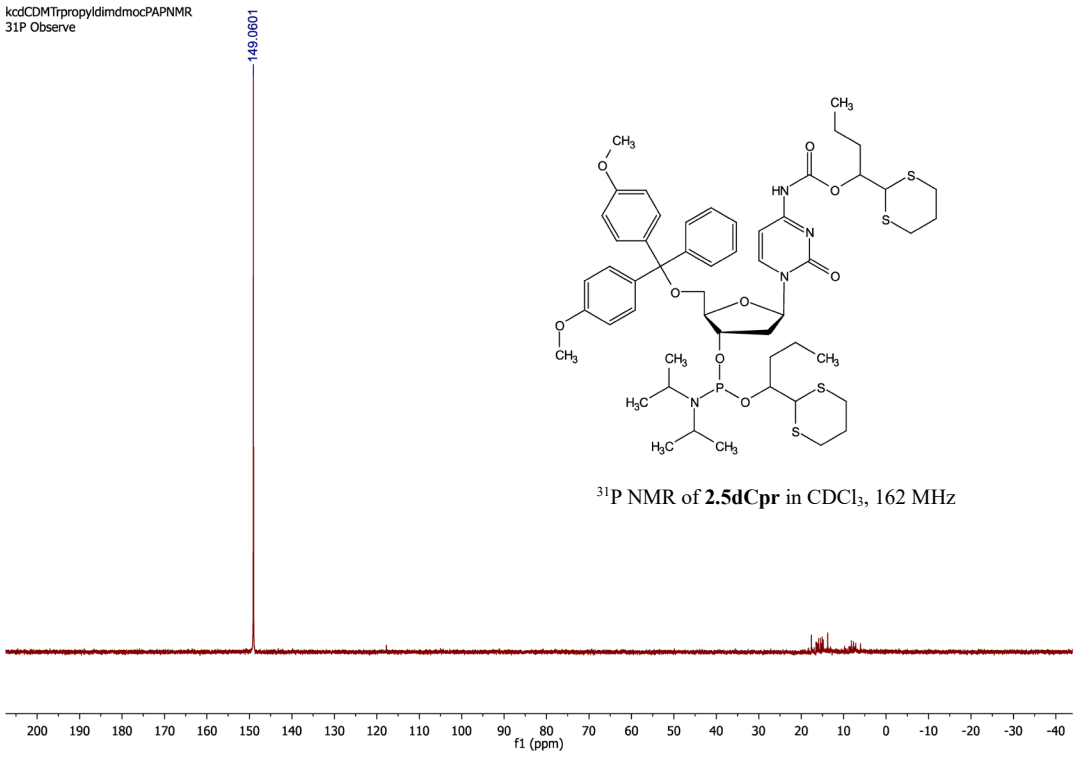
^{1}H NMR of **2.5dCpr** in $CDCl_3$, 400 MHz

Figure 5.120: ^{1}H NMR spectrum of compound **2.5dCpr**.



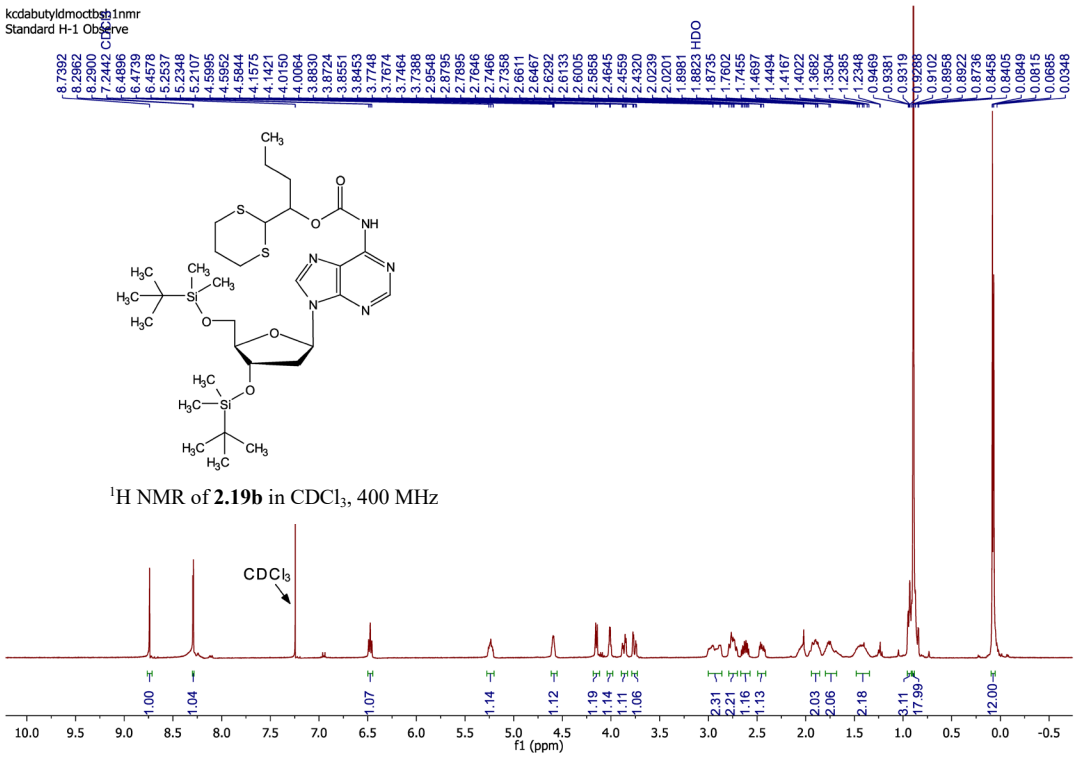
^{13}C NMR of **2.5dCpr** in $CDCl_3$, 100 MHz

Figure 5.121: ^{13}C NMR spectrum of compound **2.5dCpr**.



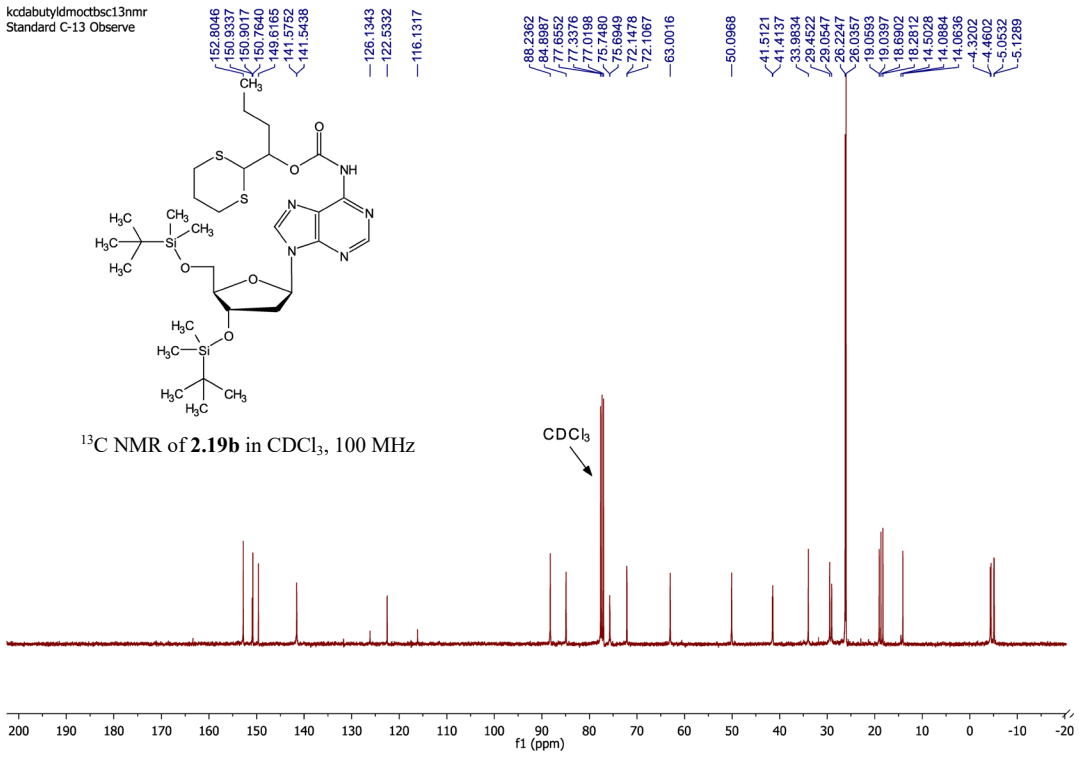
^{31}P NMR of **2.5dCpr** in $CDCl_3$, 162 MHz

Figure 5.122: ^{31}P NMR spectrum of compound **2.5dCpr**.



^{1}H NMR of **2.19b** in $CDCl_3$, 400 MHz

Figure 5.123: ^{1}H NMR spectrum of compound **2.19b**.



^{13}C NMR of **2.19b** in $CDCl_3$, 100 MHz

Figure 5.124: ^{13}C NMR spectrum of compound **2.19b**.

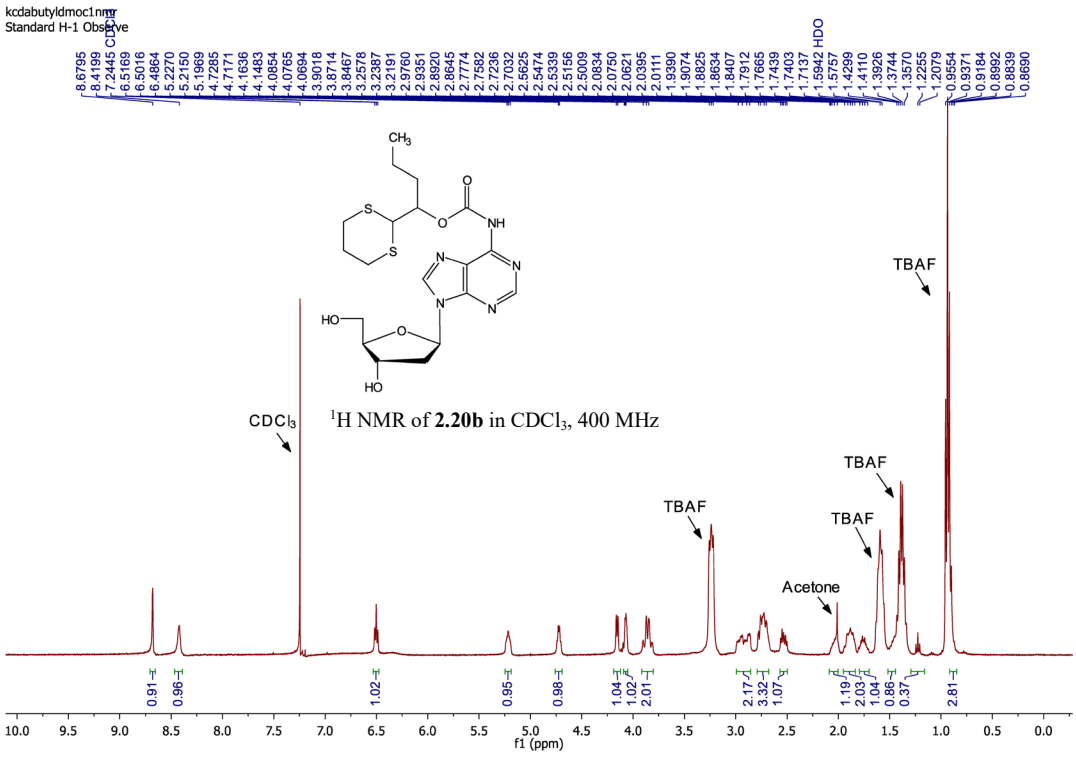
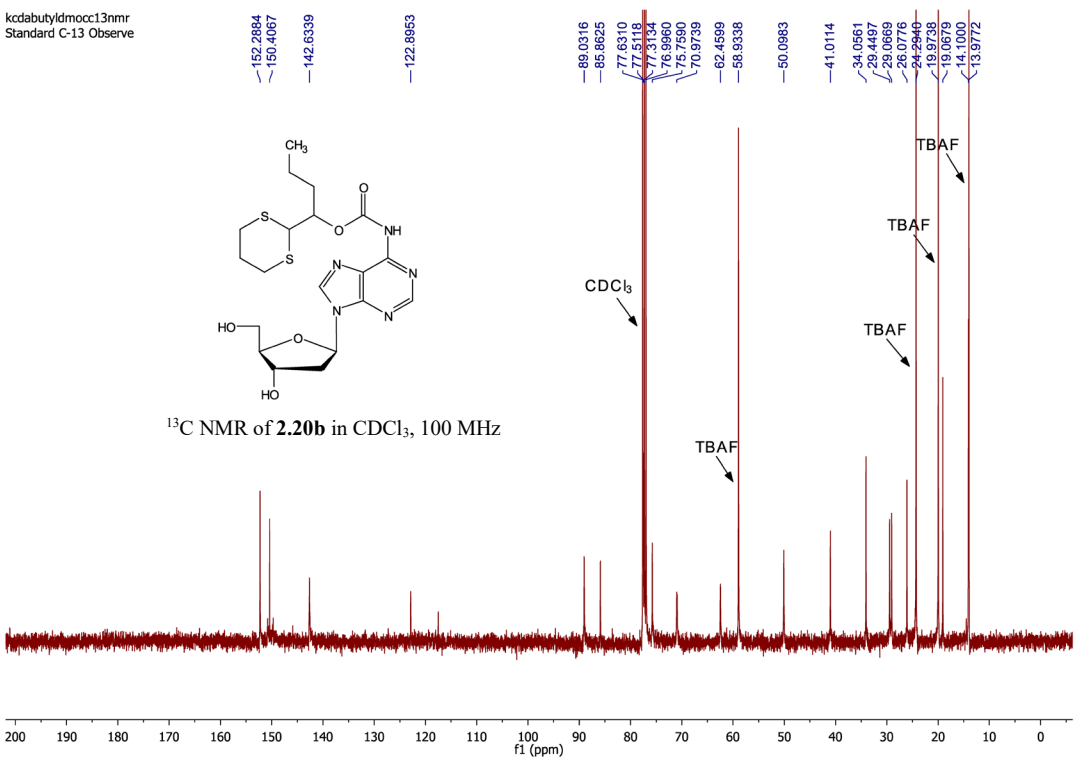
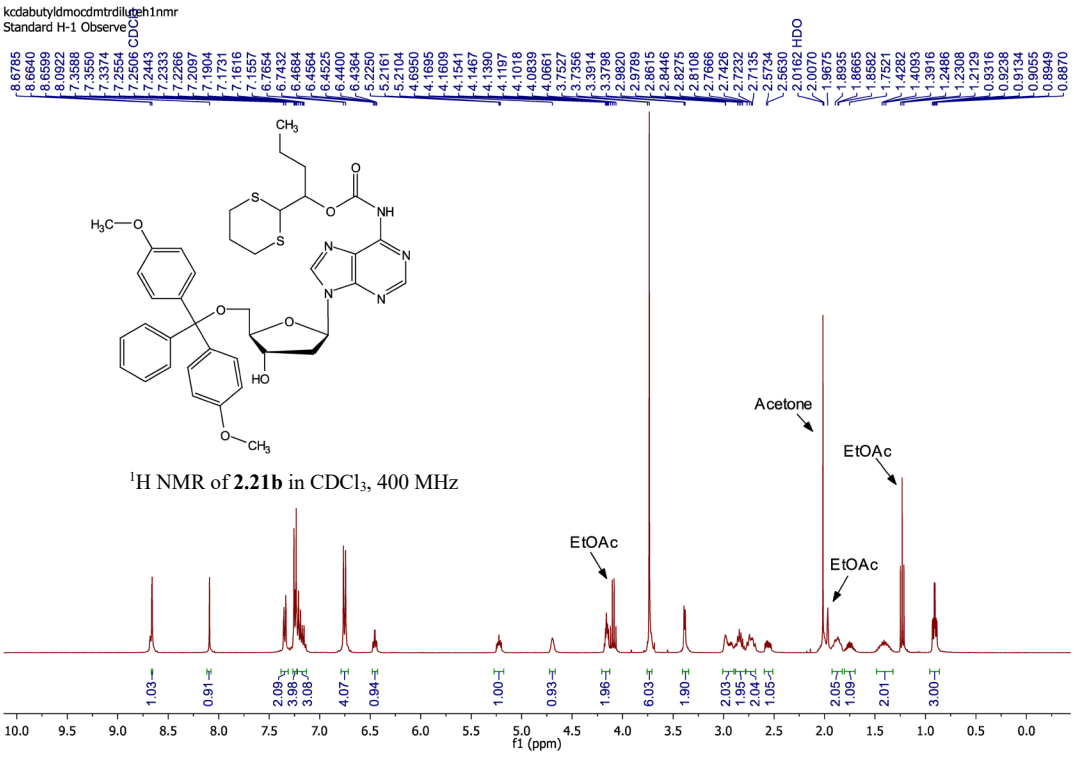


Figure 5.125: ^{1}H NMR spectrum of compound **2.20b**.



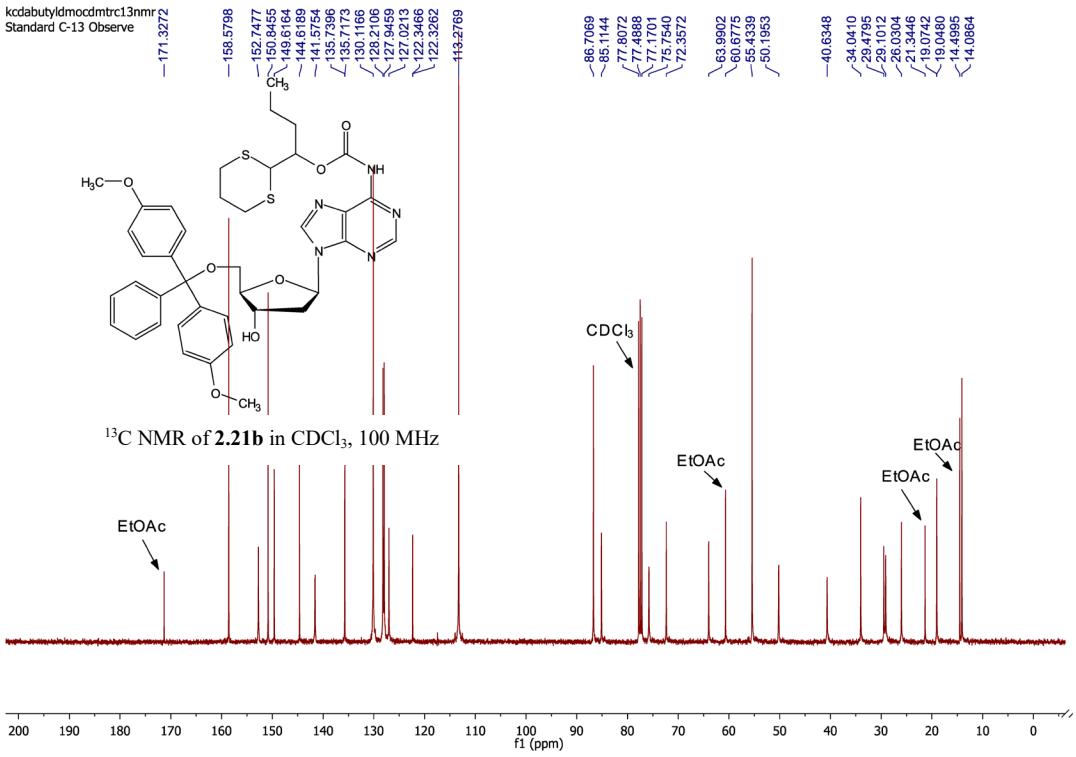
^{13}C NMR of **2.20b** in $CDCl_3$, 100 MHz

Figure 5.126: ^{13}C NMR spectrum of compound **2.20b**.



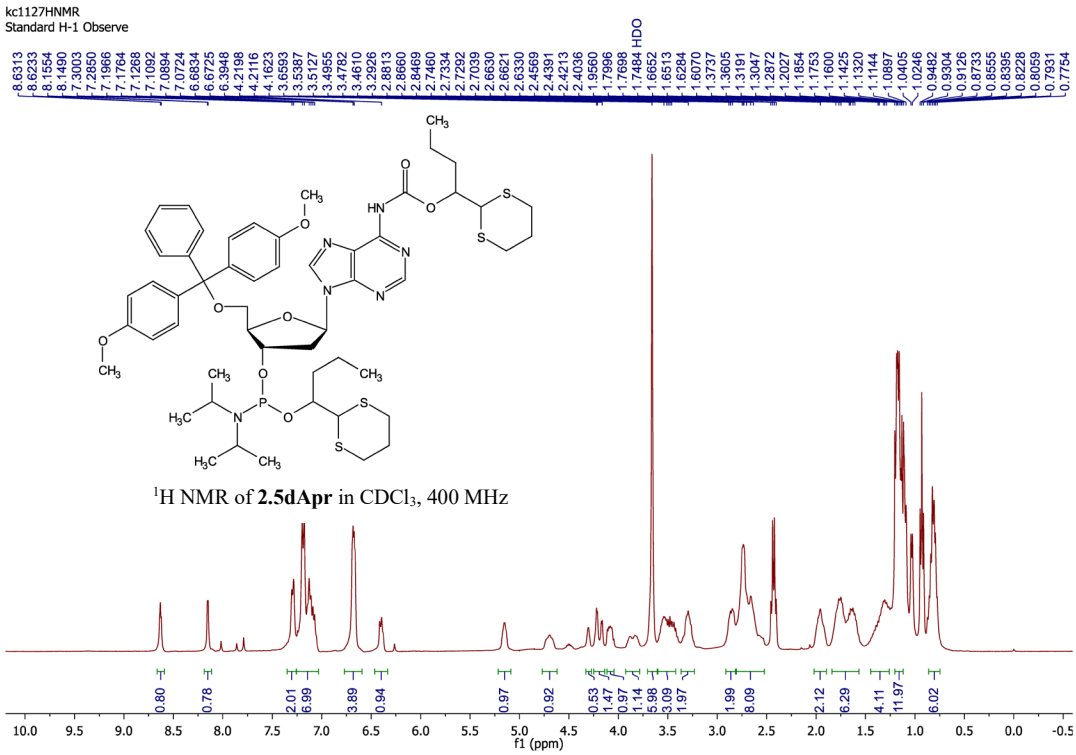
^{1}H NMR of **2.21b** in $CDCl_3$, 400 MHz

Figure 5.127: ^{1}H NMR spectrum of compound **2.21b**.



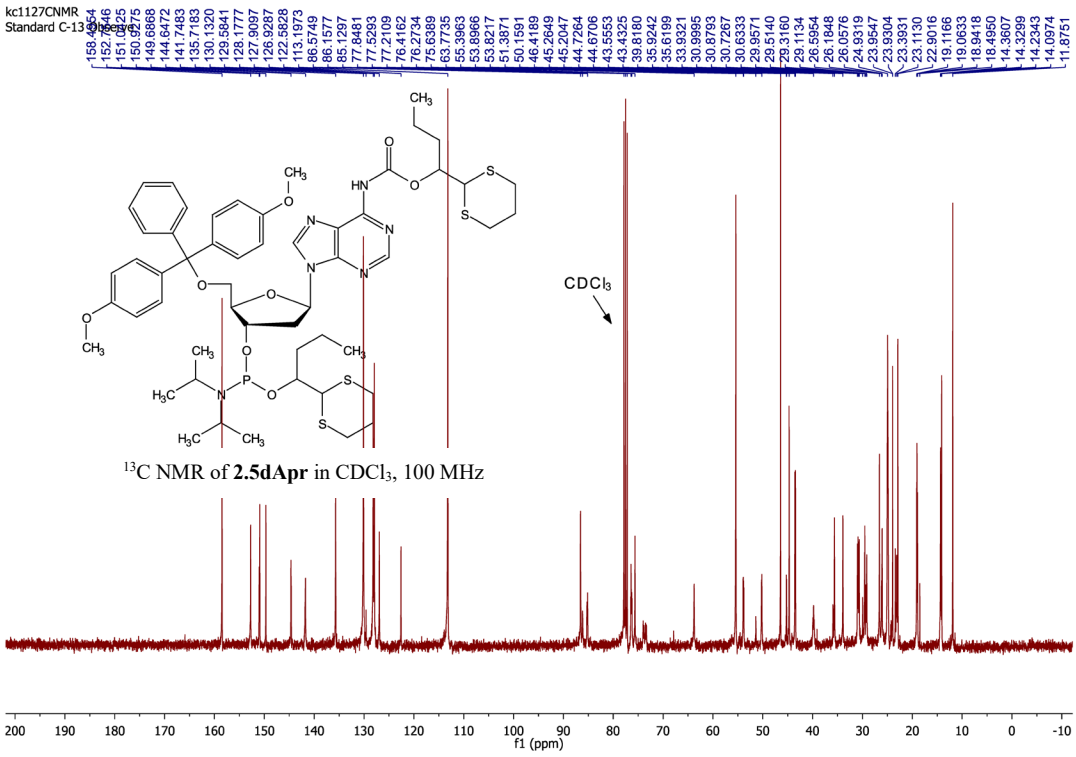
^{13}C NMR of **2.21b** in $CDCl_3$, 100 MHz

Figure 5.128: ^{13}C NMR spectrum of compound **2.21b**.



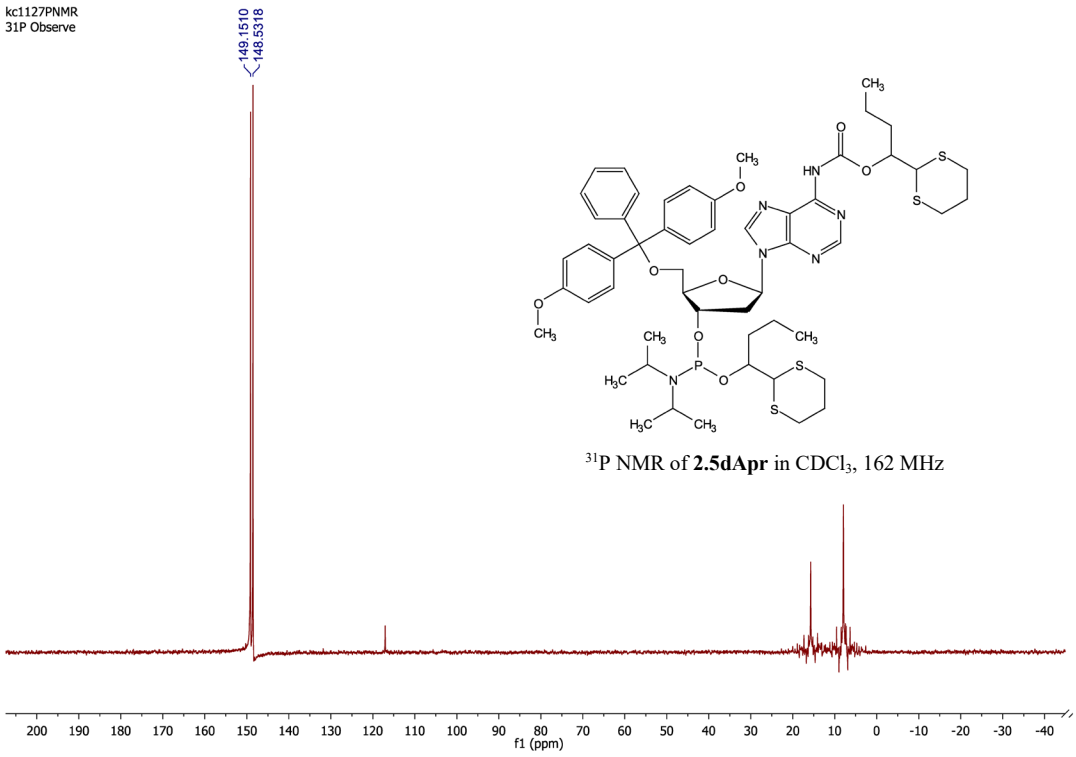
^{1}H NMR of **2.5dApr** in $CDCl_3$, 400 MHz

Figure 5.129: ^{1}H NMR spectrum of compound **2.5dApr**.



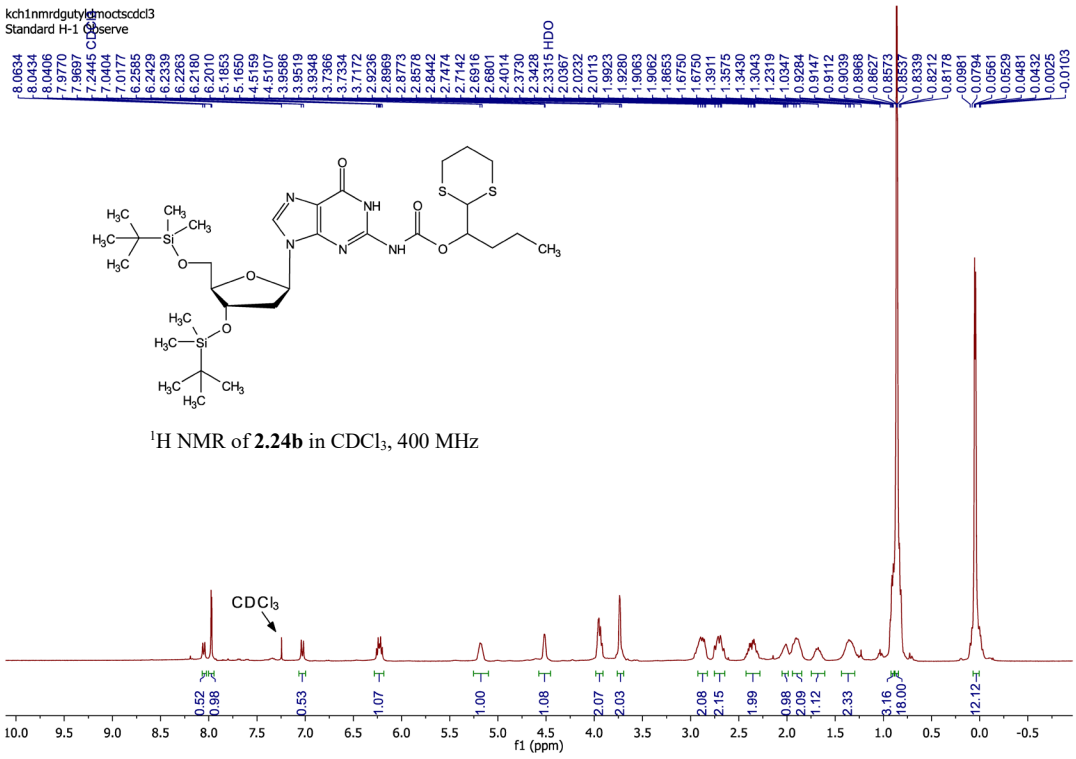
^{13}C NMR of **2.5dApr** in $CDCl_3$, 100 MHz

Figure 5.130: ^{13}C NMR spectrum of compound **2.5dApr**.



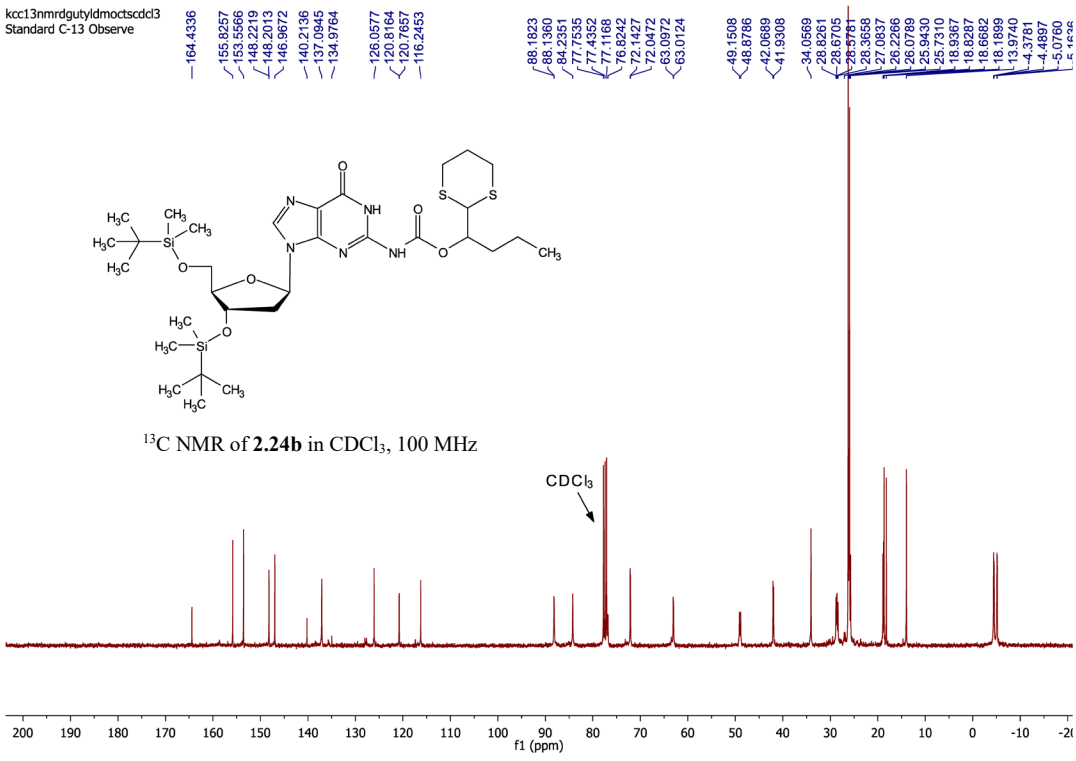
^{31}P NMR of **2.5dApr** in $CDCl_3$, 162 MHz

Figure 5.131: ^{31}P NMR spectrum of compound **2.5dApr**.



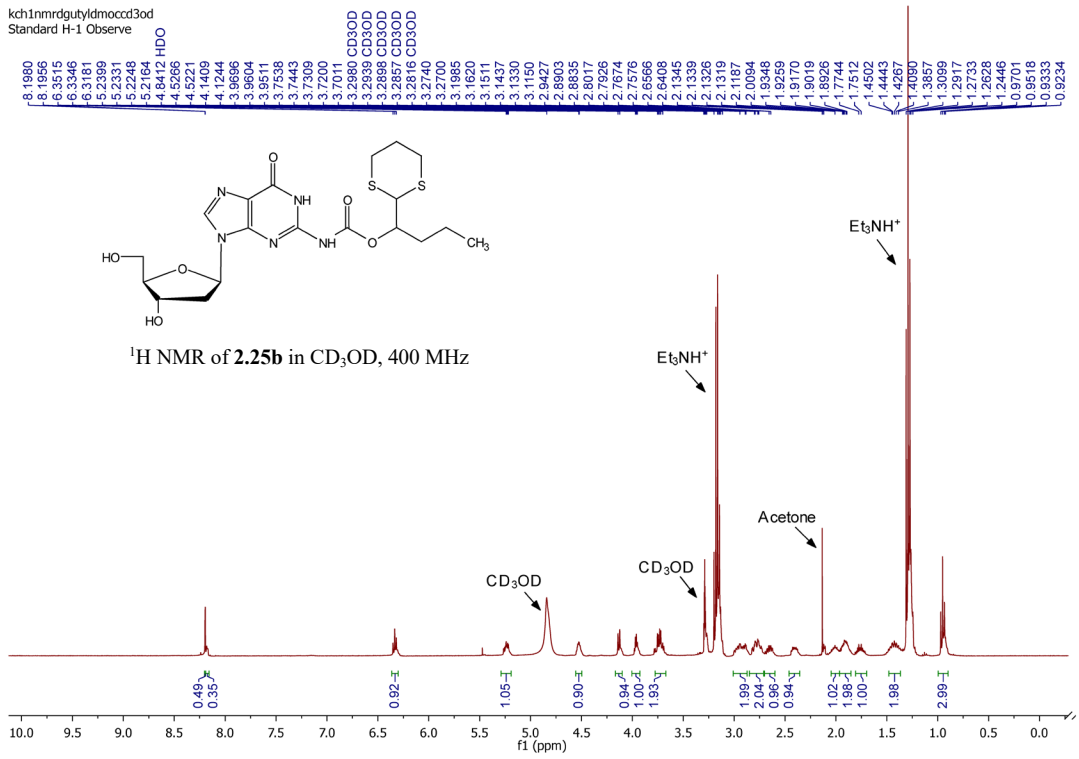
^{1}H NMR of **2.24b** in $CDCl_3$, 400 MHz

Figure 5.132: ^{1}H NMR spectrum of compound **2.24b**.



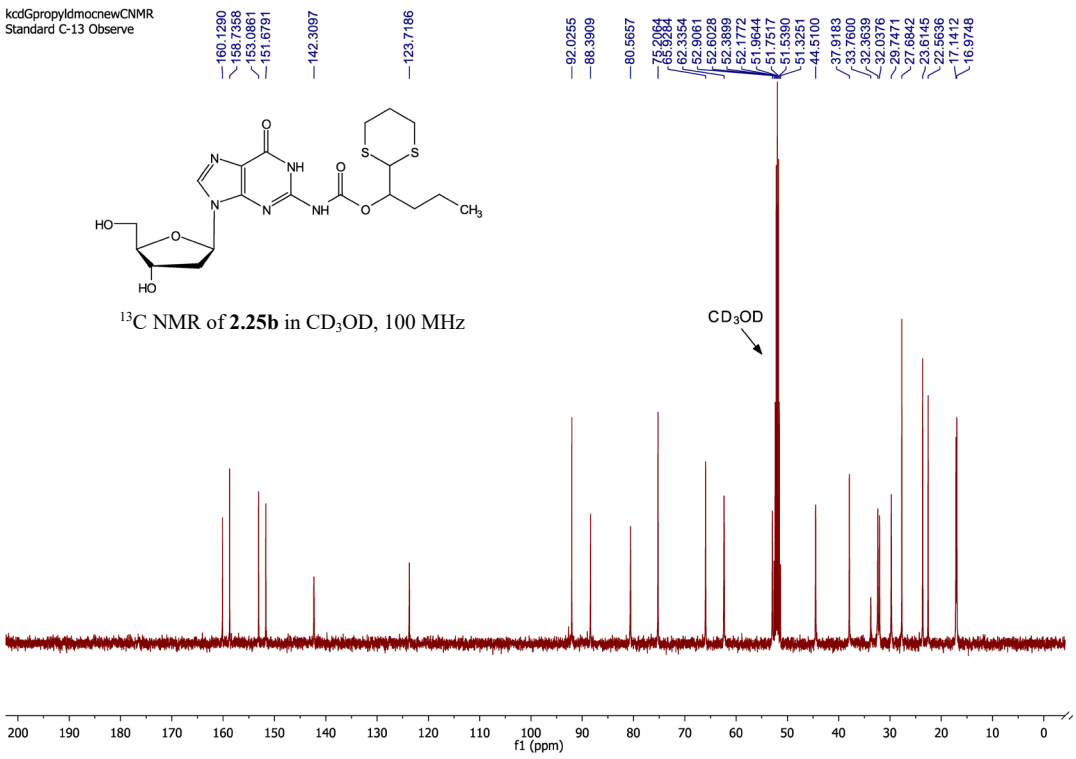
^{13}C NMR of **2.24b** in $CDCl_3$, 100 MHz

Figure 5.133: ^{13}C NMR spectrum of compound **2.24b**.



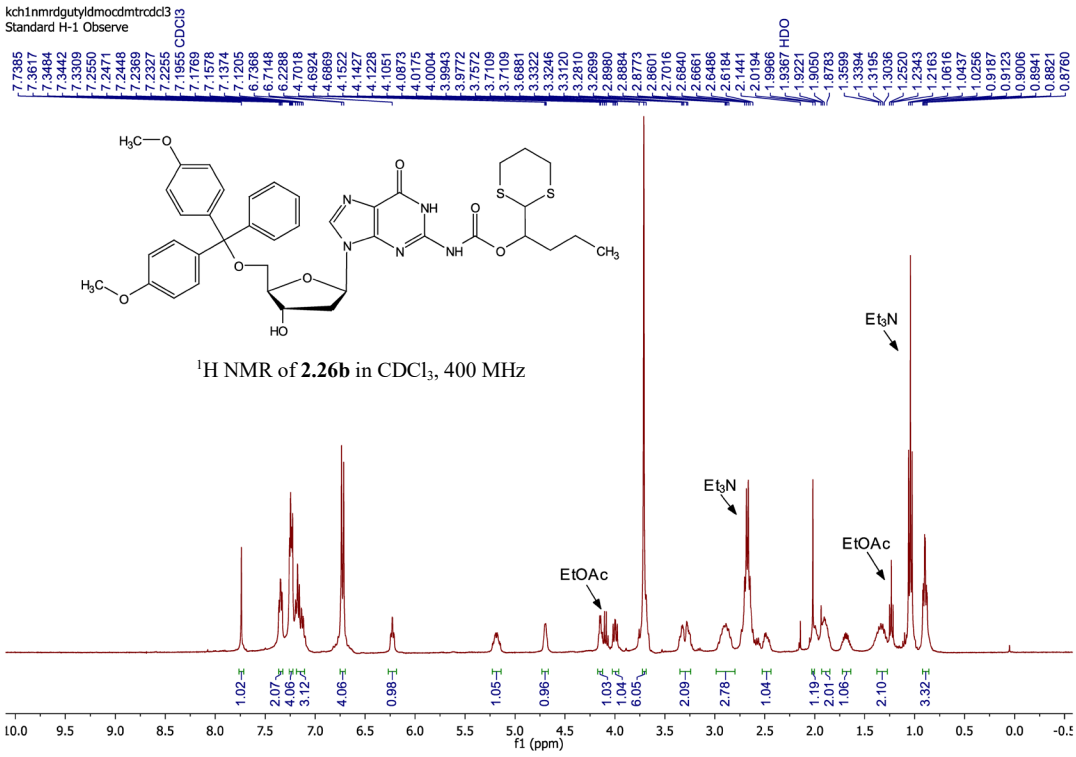
^{1}H NMR of **2.25b** in CD_3OD, 400 MHz

Figure 5.134: ^{1}H NMR spectrum of compound **2.25b**.



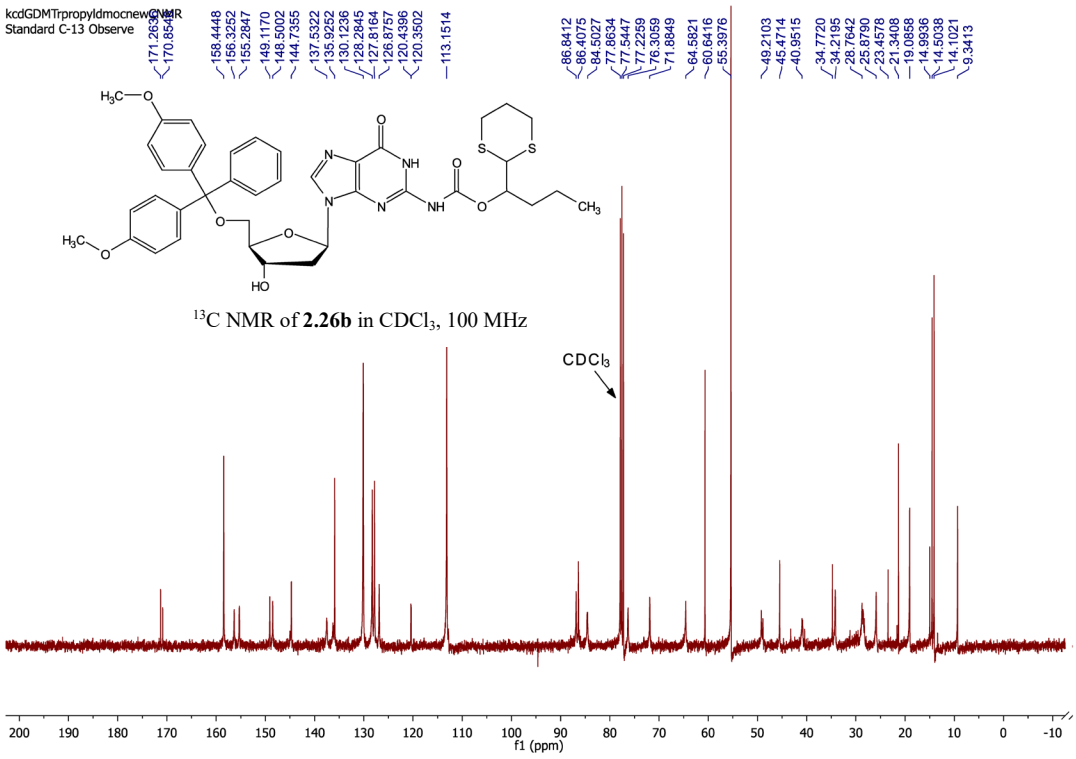
^{13}C NMR of **2.25b** in CD_3OD, 100 MHz

Figure 5.135: ^{13}C NMR spectrum of compound **2.25b**.



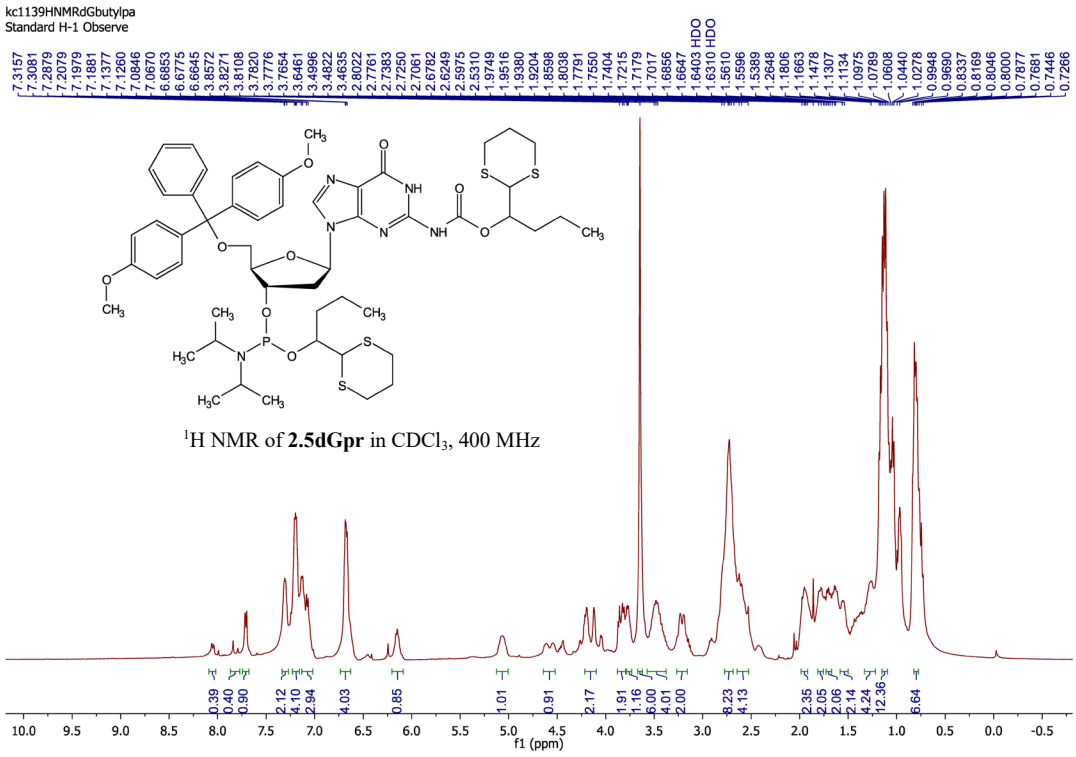
^{1}H NMR of **2.26b** in $CDCl_3$, 400 MHz

Figure 5.136: ^{1}H NMR spectrum of compound **2.26b**.



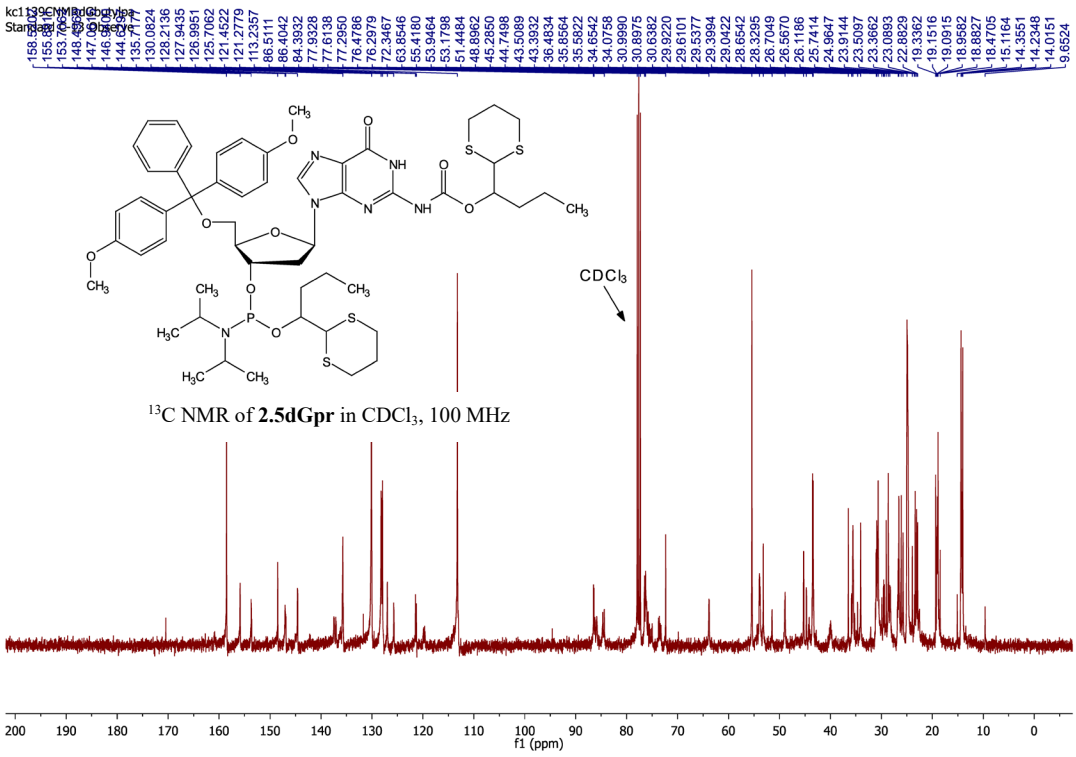
^{13}C NMR of **2.26b** in $CDCl_3$, 100 MHz

Figure 5.137: ^{13}C NMR spectrum of compound **2.26b**.



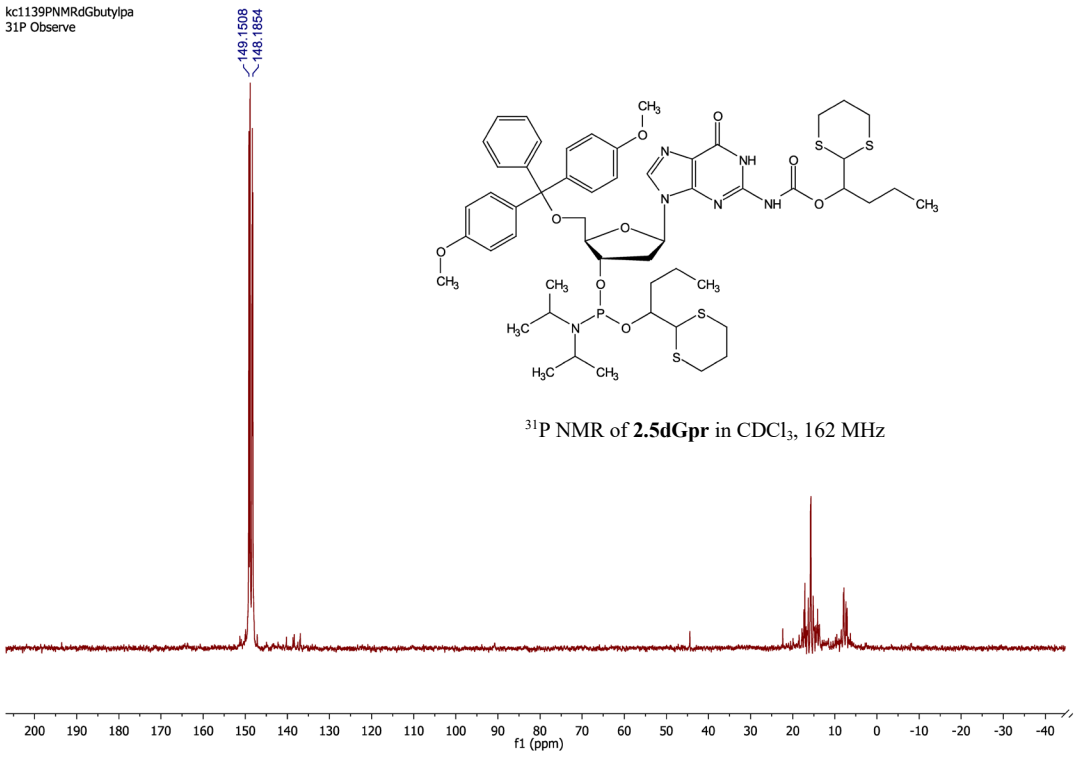
^{1}H NMR of **2.5dGpr** in $CDCl_3$, 400 MHz

Figure 5.138: ^{1}H NMR spectrum of compound **2.5dGpr**.



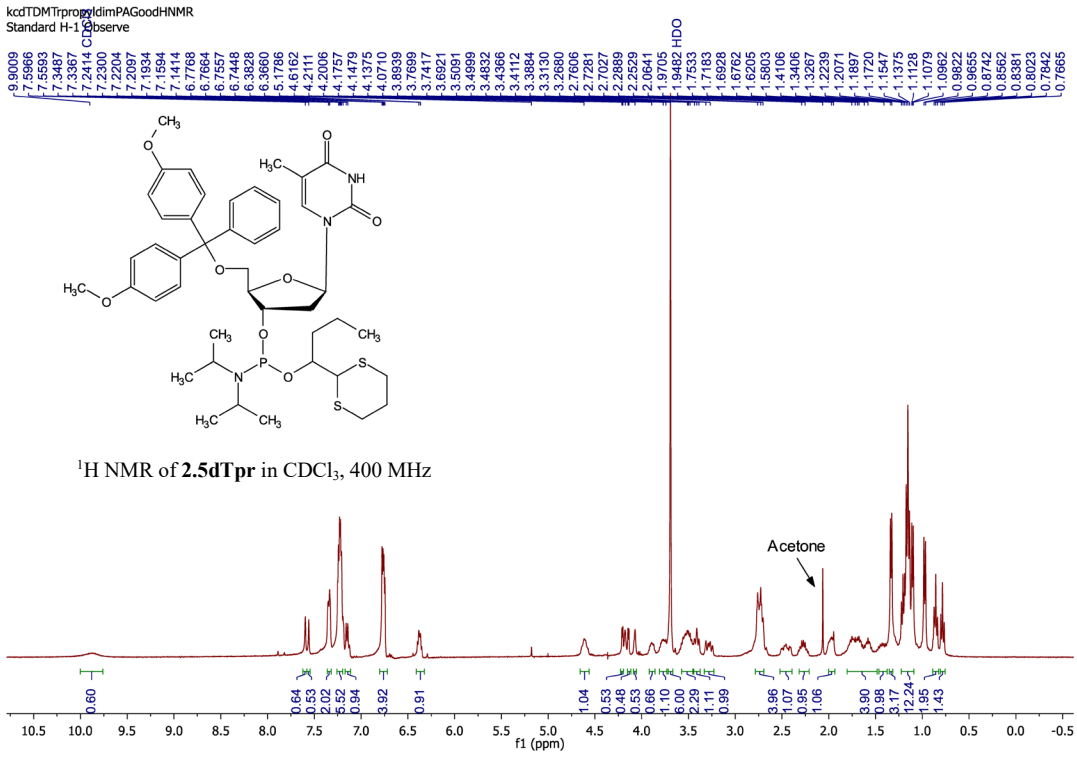
^{13}C NMR of **2.5dGpr** in $CDCl_3$, 100 MHz

Figure 5.139: ^{13}C NMR spectrum of compound **2.5dGpr**.



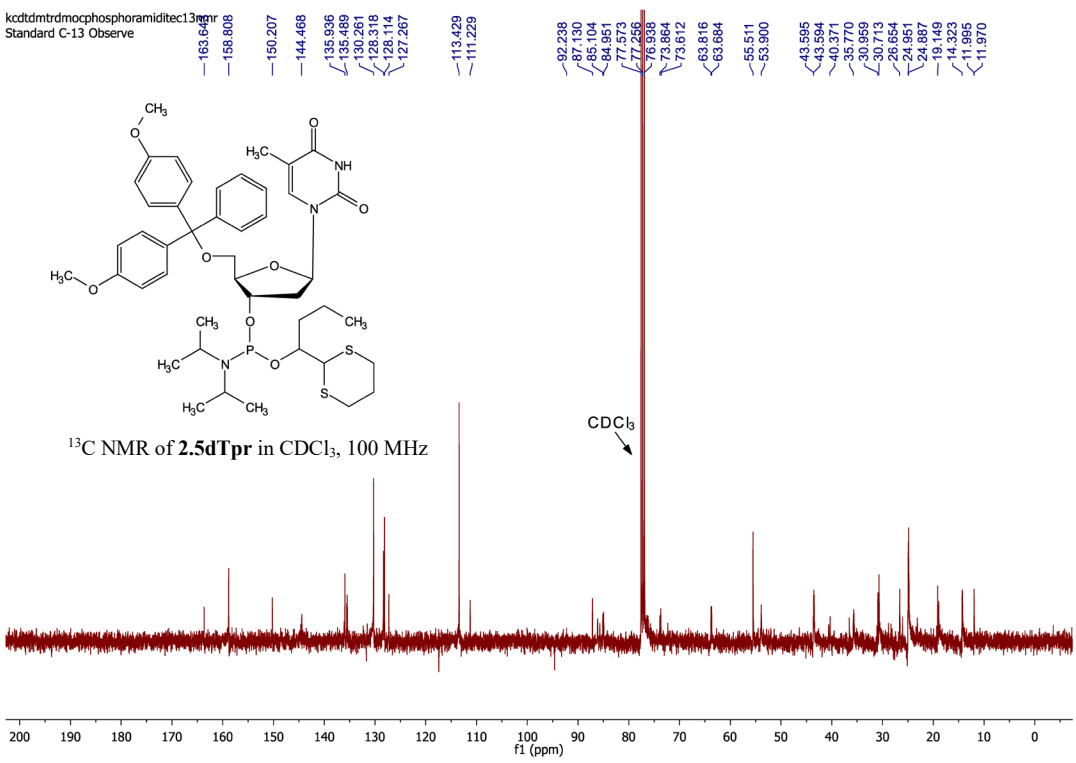
^{31}P NMR of **2.5dGpr** in $CDCl_3$, 162 MHz

Figure 5.140: ^{31}P NMR spectrum of compound **2.5dGpr**.



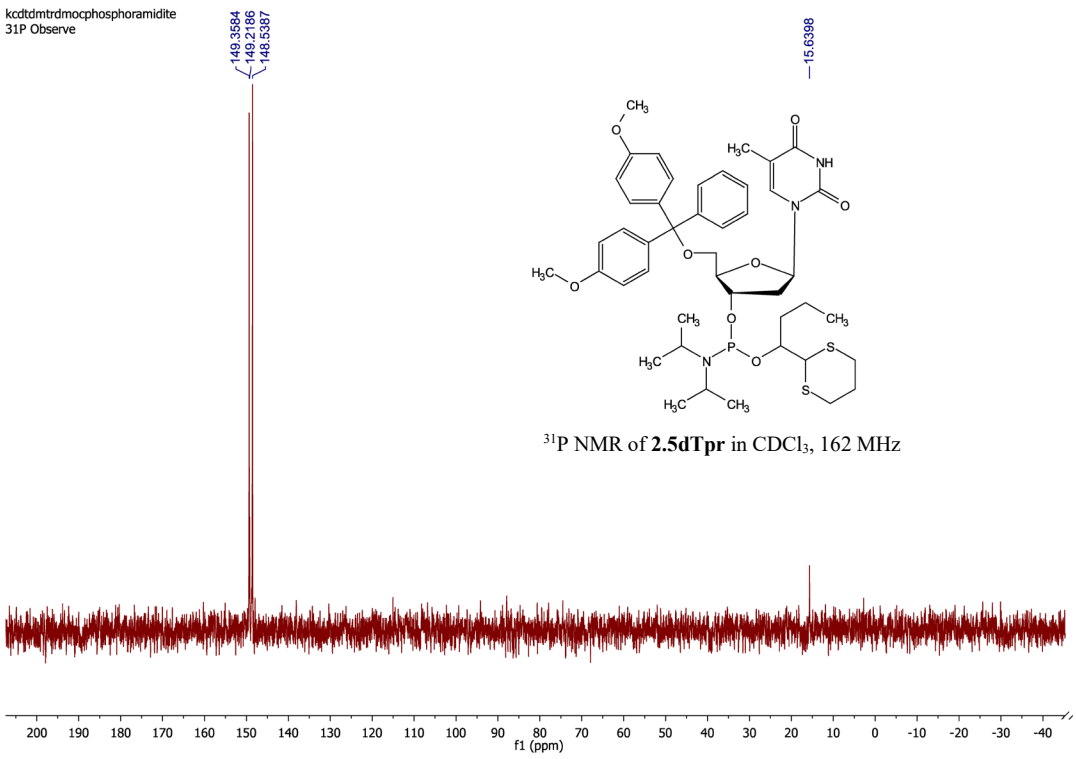
^{1}H NMR of **2.5dTpr** in $CDCl_3$, 400 MHz

Figure 5.141: ^{1}H NMR spectrum of compound **2.5dTpr**.



^{13}C NMR of **2.5dTpr** in $CDCl_3$, 100 MHz

Figure 5.142: ^{13}C NMR spectrum of compound **2.5dTpr**.



^{31}P NMR of **2.5dTpr** in $CDCl_3$, 162 MHz

Figure 5.143: ^{31}P NMR spectrum of compound **2.5dTpr**.

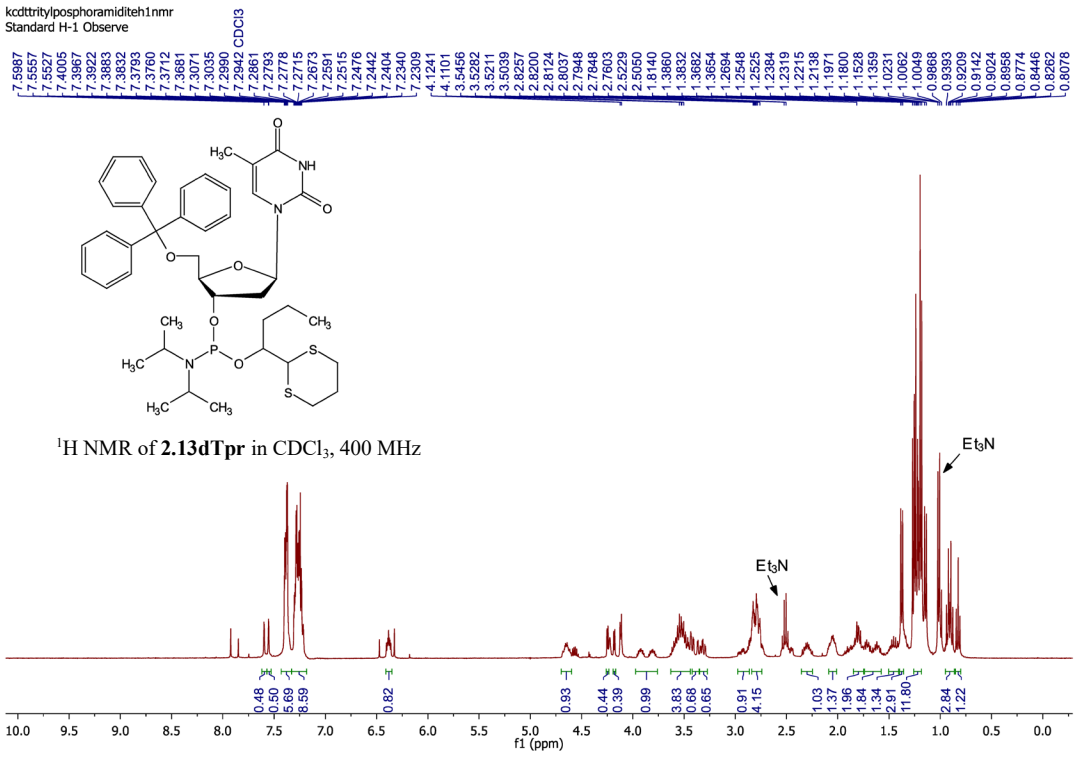
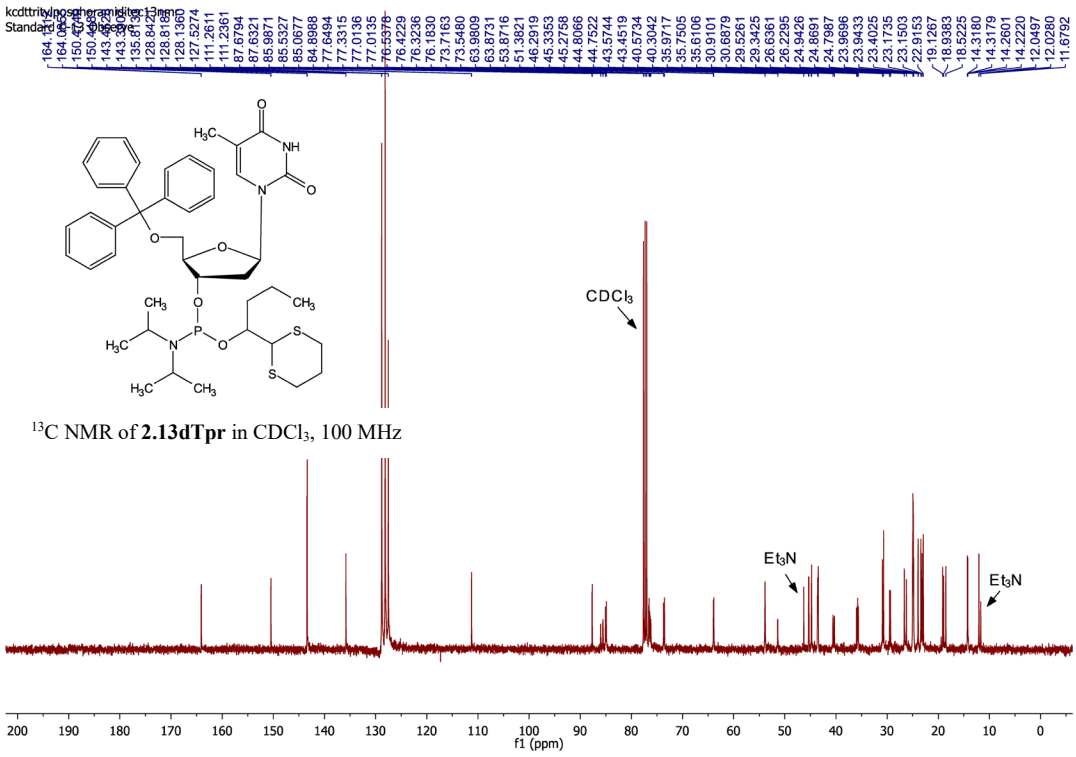
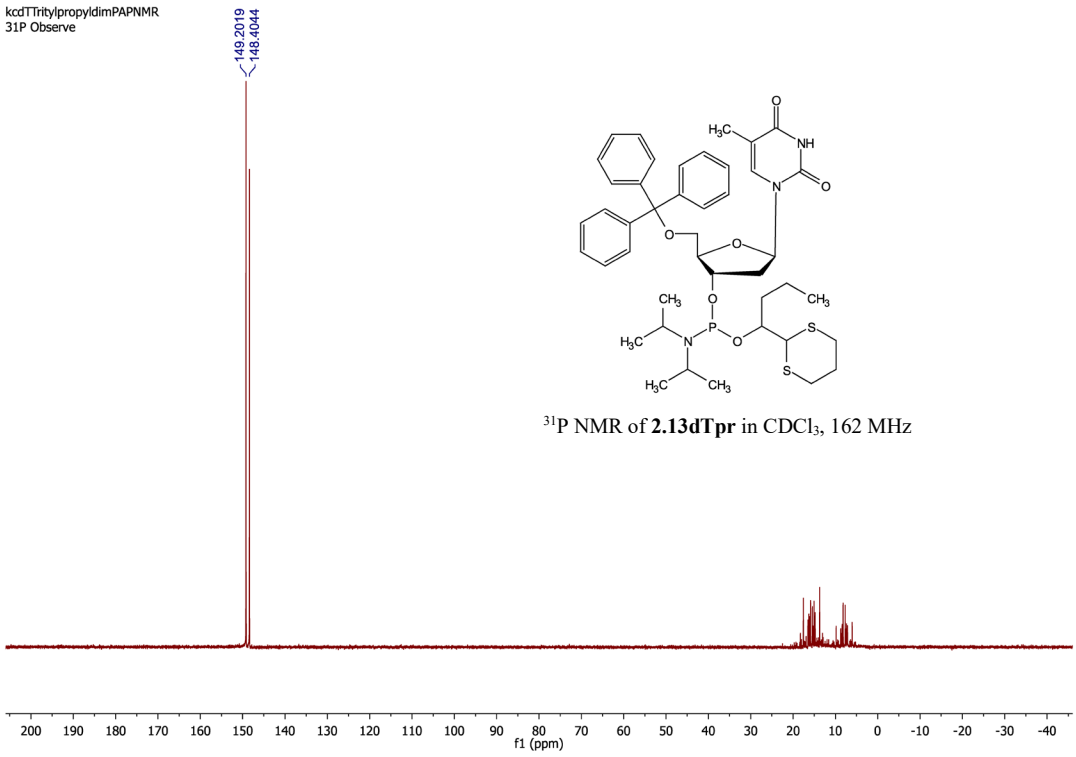


Figure 5.144: [1]H NMR spectrum of compound **2.13dTpr**.



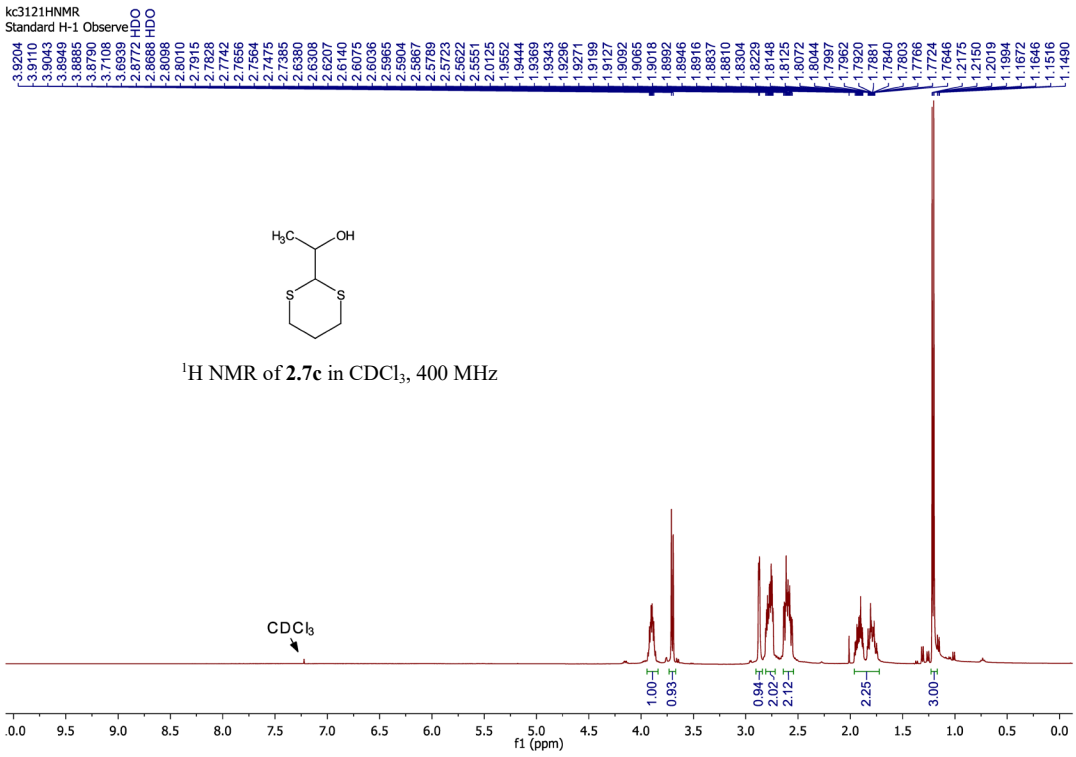
^{13}C NMR of **2.13dTpr** in $CDCl_3$, 100 MHz

Figure 5.145: ^{13}C NMR spectrum of compound **2.13dTpr**.



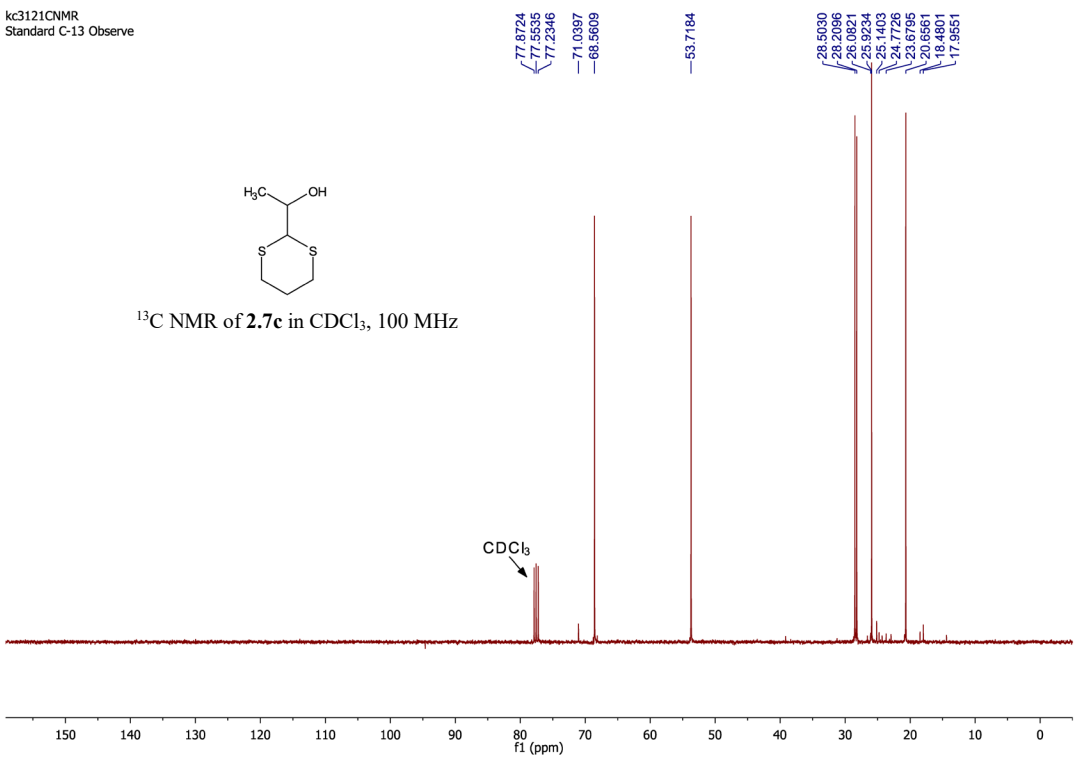
^{31}P NMR of **2.13dTpr** in $CDCl_3$, 162 MHz

Figure 5.146: ^{31}P NMR spectrum of compound **2.13dTpr**.



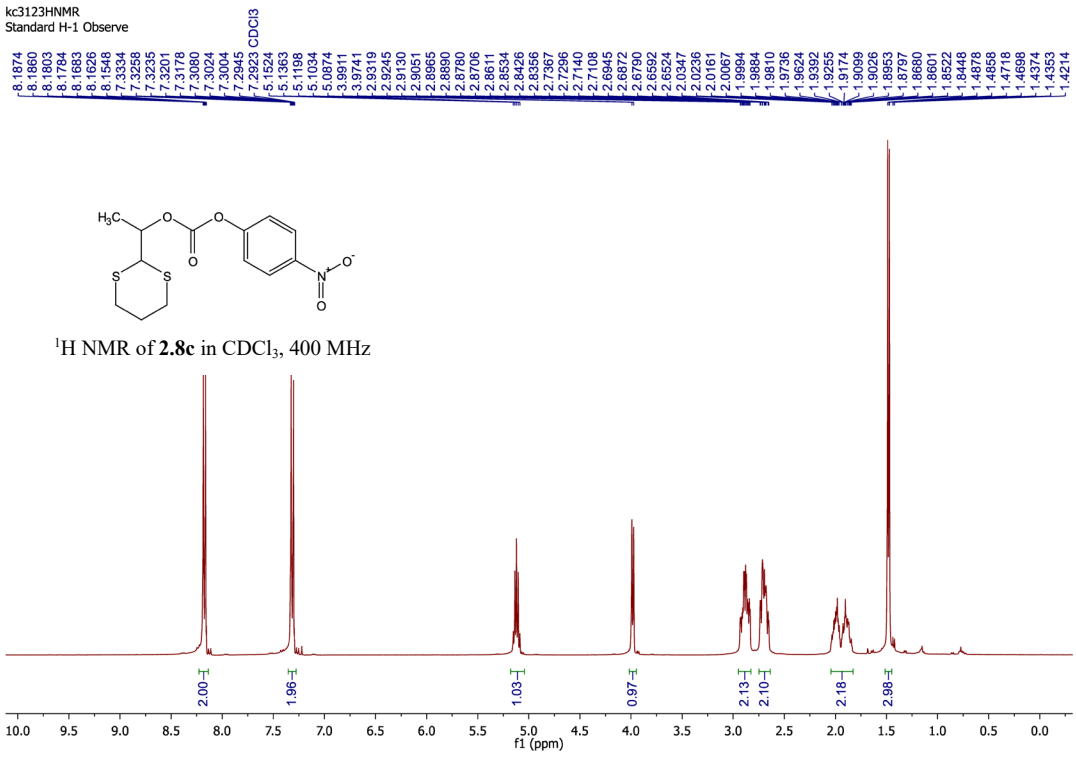
^{1}H NMR of **2.7c** in $CDCl_3$, 400 MHz

Figure 5.147: ^{1}H NMR spectrum of compound **2.7c**.



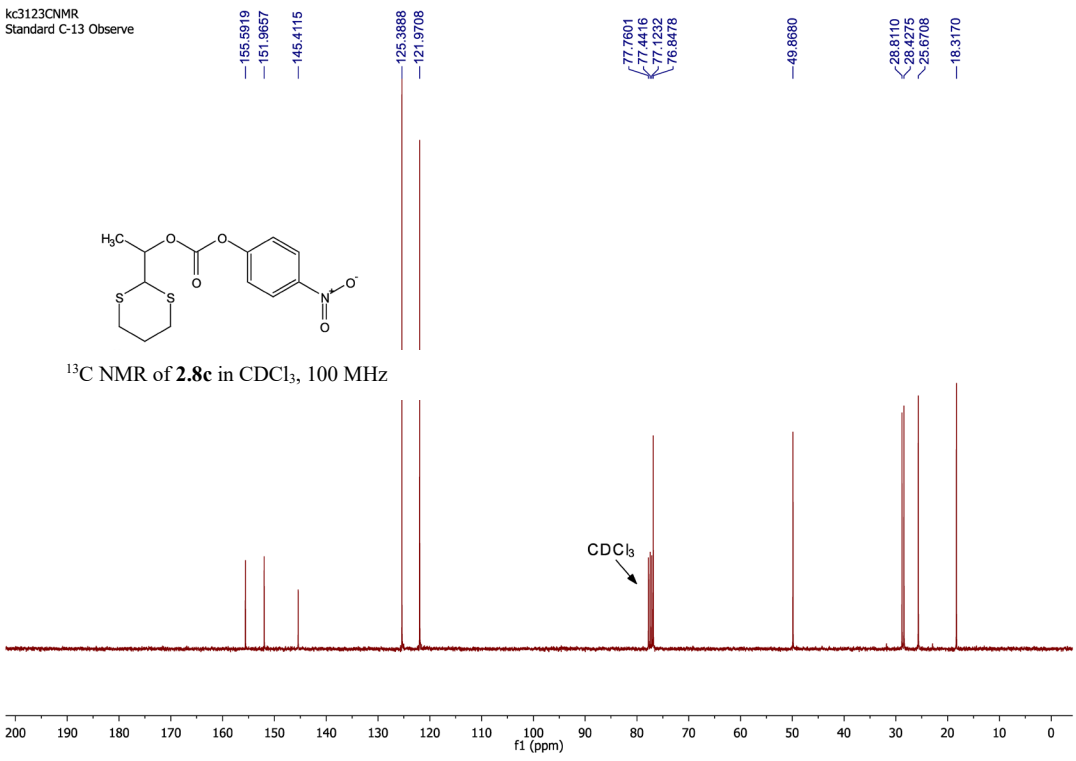
^{13}C NMR of **2.7c** in $CDCl_3$, 100 MHz

Figure 5.148: ^{13}C NMR spectrum of compound **2.7c**.



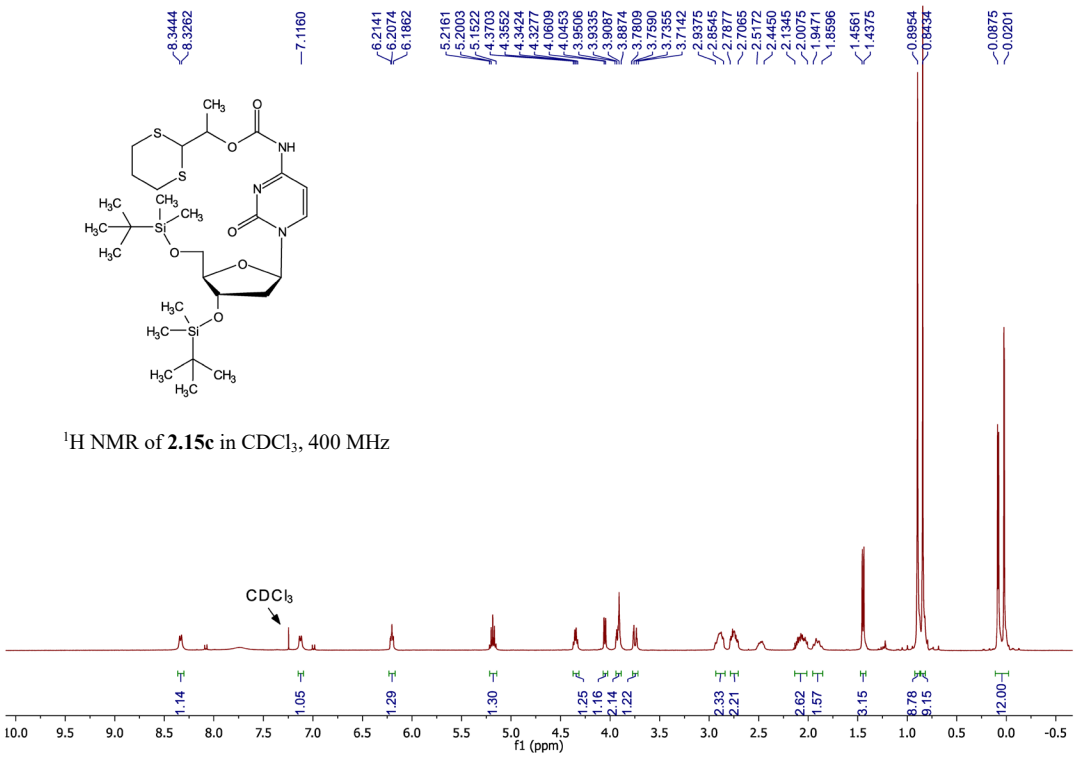
^{1}H NMR of **2.8c** in $CDCl_3$, 400 MHz

Figure 5.149: ^{1}H NMR spectrum of compound **2.8c**.



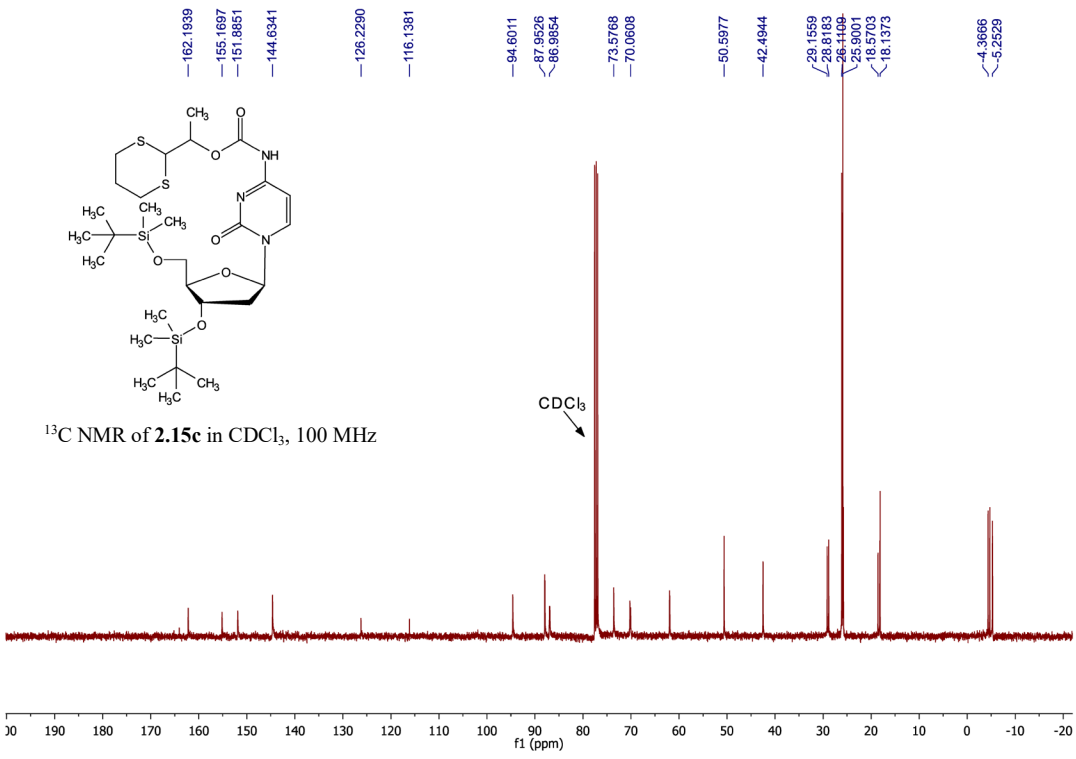
^{13}C NMR of **2.8c** in $CDCl_3$, 100 MHz

Figure 5.150: ^{13}C NMR spectrum of compound **2.8c**.



^{1}H NMR of **2.15c** in $CDCl_3$, 400 MHz

Figure 5.151: ^{1}H NMR spectrum of compound **2.15c**.



^{13}C NMR of **2.15c** in $CDCl_3$, 100 MHz

Figure 5.152: ^{13}C NMR spectrum of compound **2.15c**.

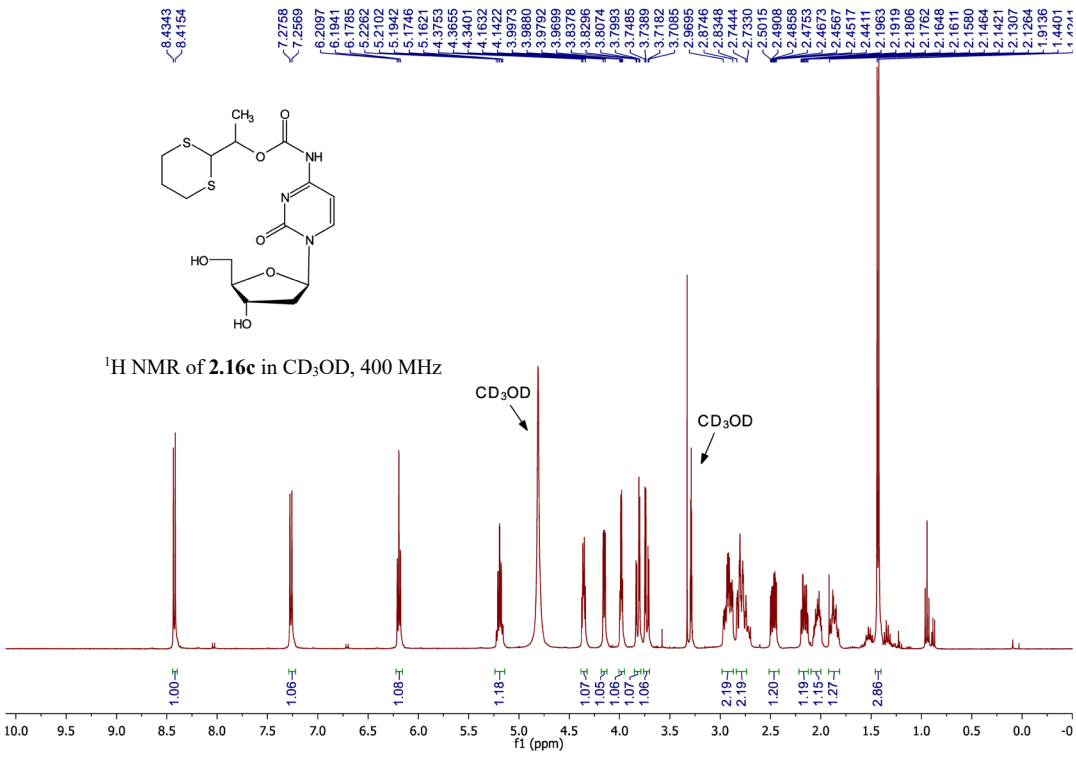
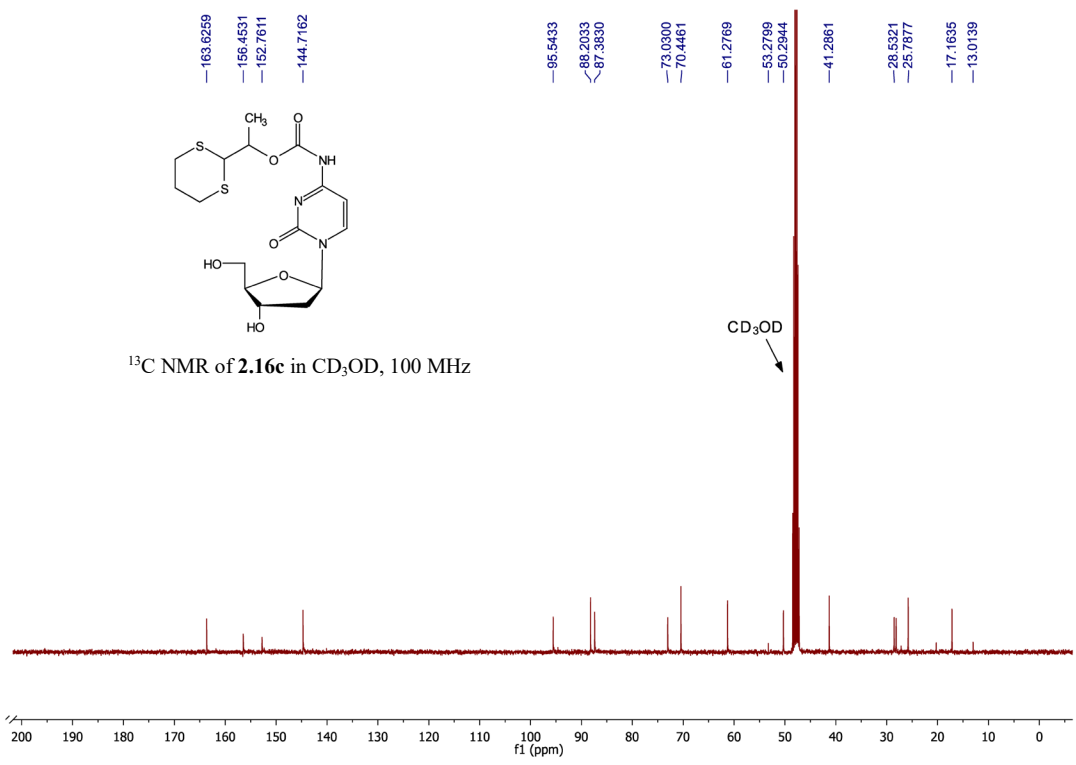
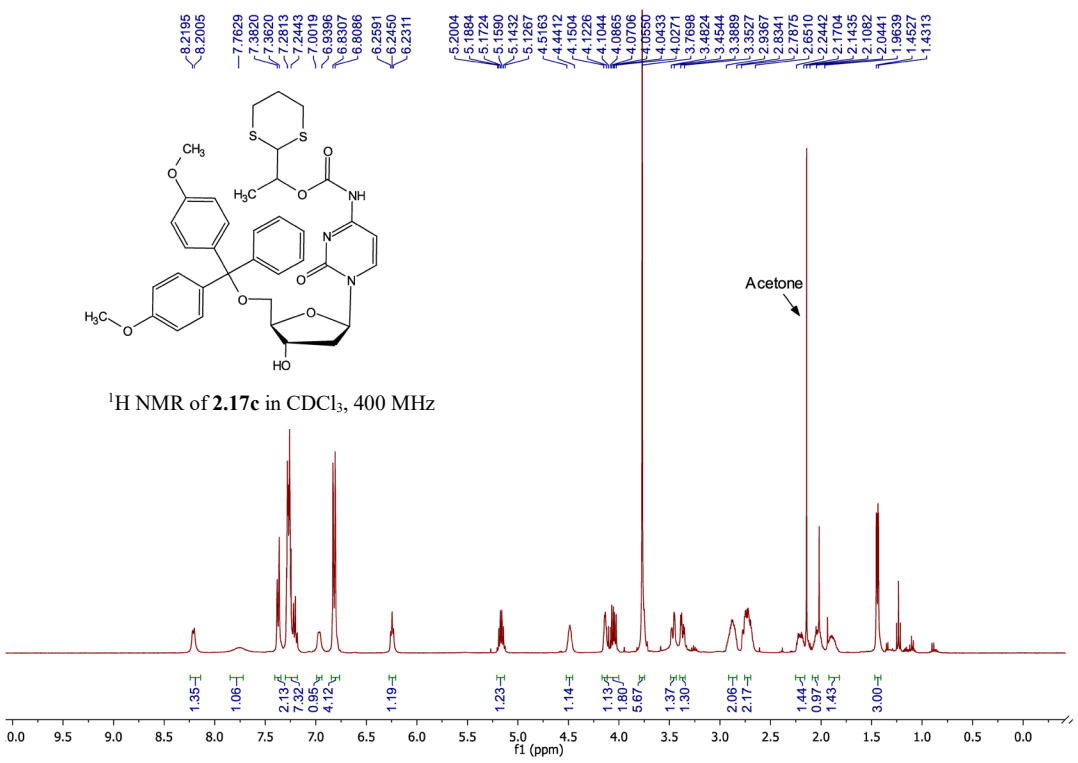


Figure 5.153: ^{1}H NMR spectrum of compound **2.16c**.



^{13}C NMR of **2.16c** in CD_3OD, 100 MHz

Figure 5.154: ^{13}C NMR spectrum of compound **2.16c**.



^{1}H NMR of **2.17c** in $CDCl_3$, 400 MHz

Figure 5.155: ^{1}H NMR spectrum of compound **2.17c**.

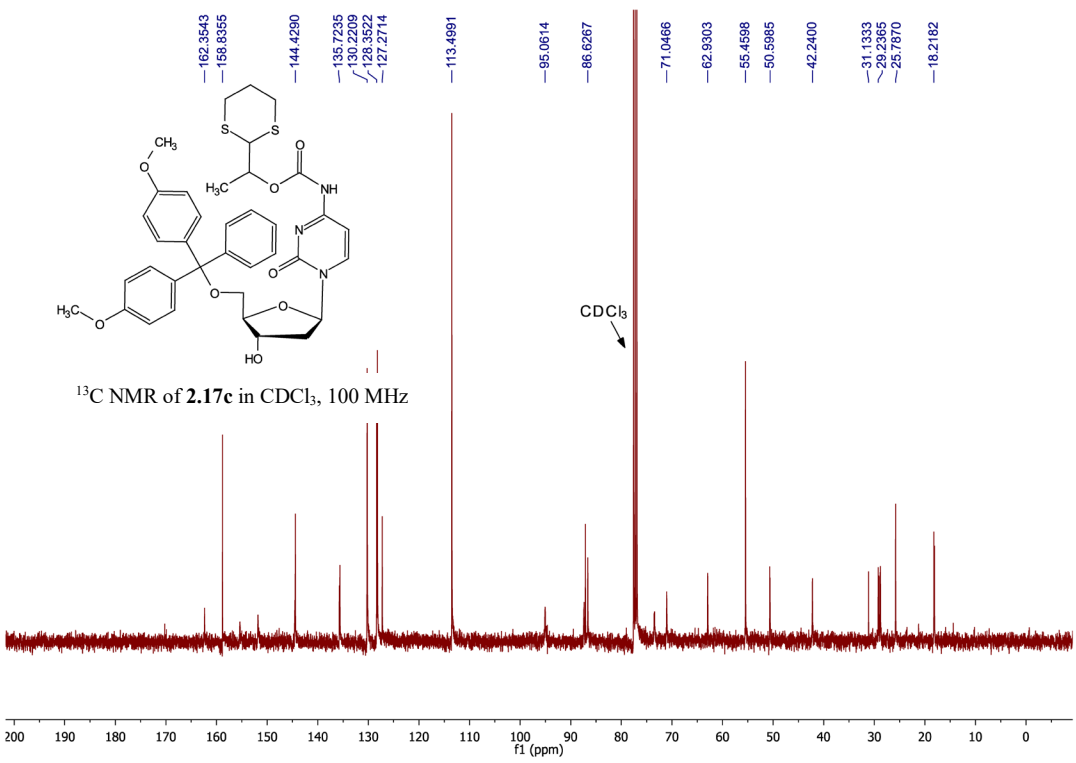
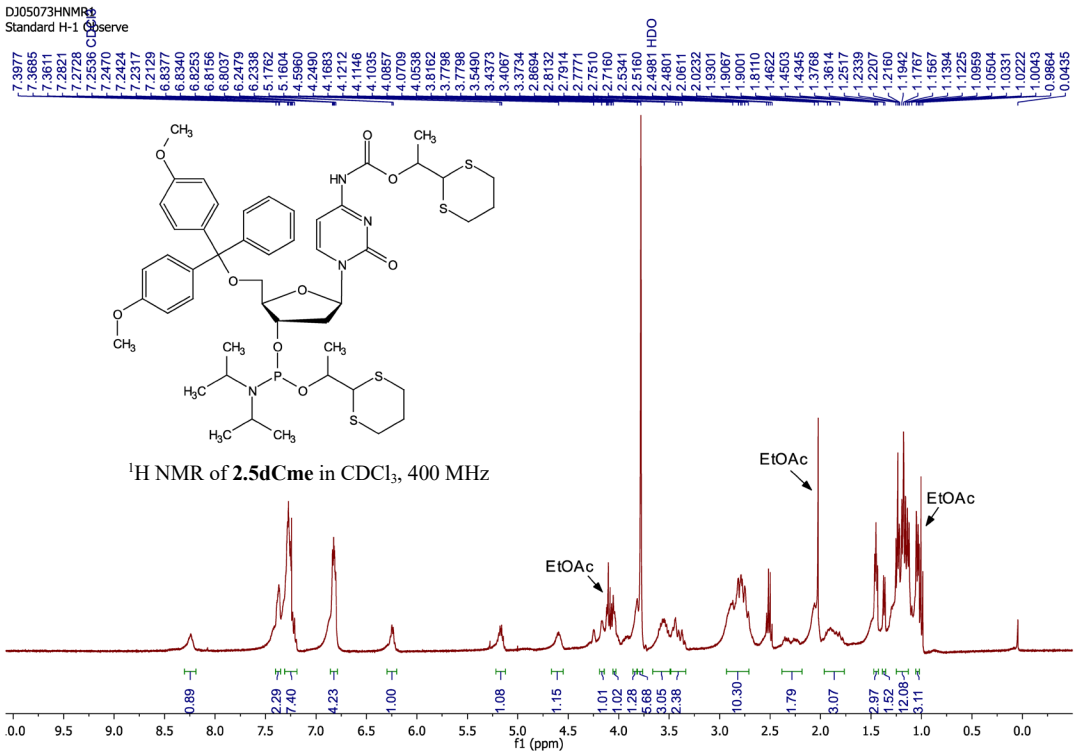
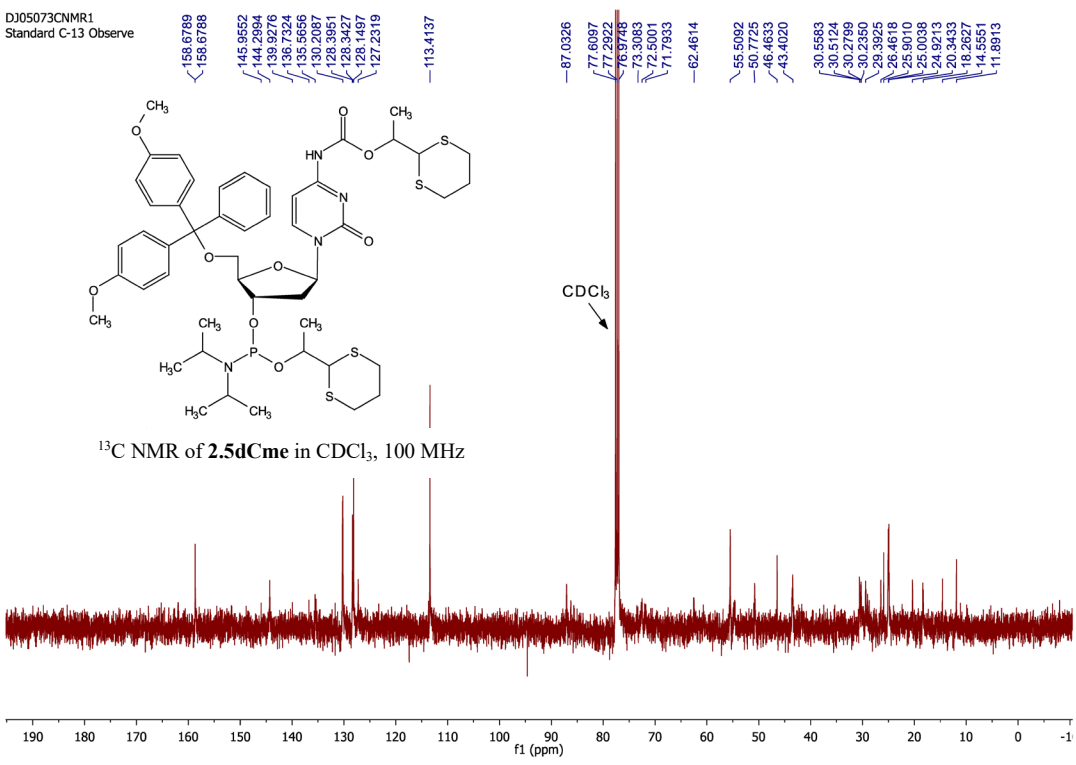


Figure 5.156: ^{13}C NMR spectrum of compound **2.17c**.



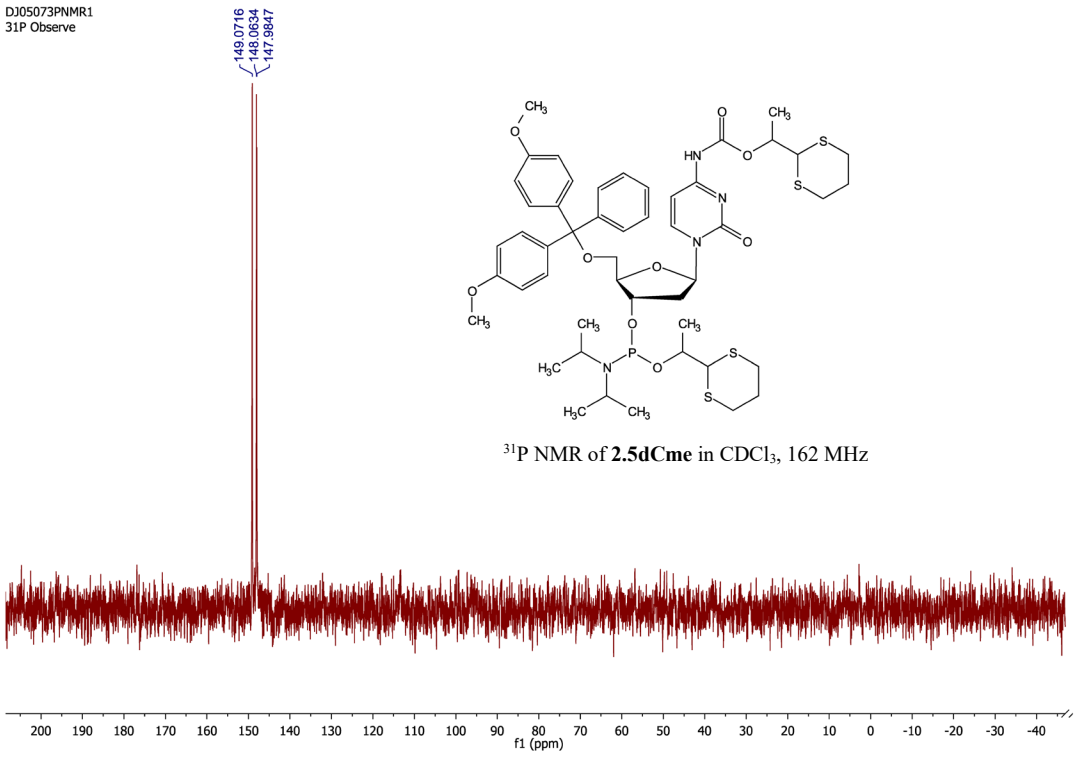
^{1}H NMR of **2.5dCme** in $CDCl_3$, 400 MHz

Figure 5.157: ^{1}H NMR spectrum of compound **2.5dCme**.



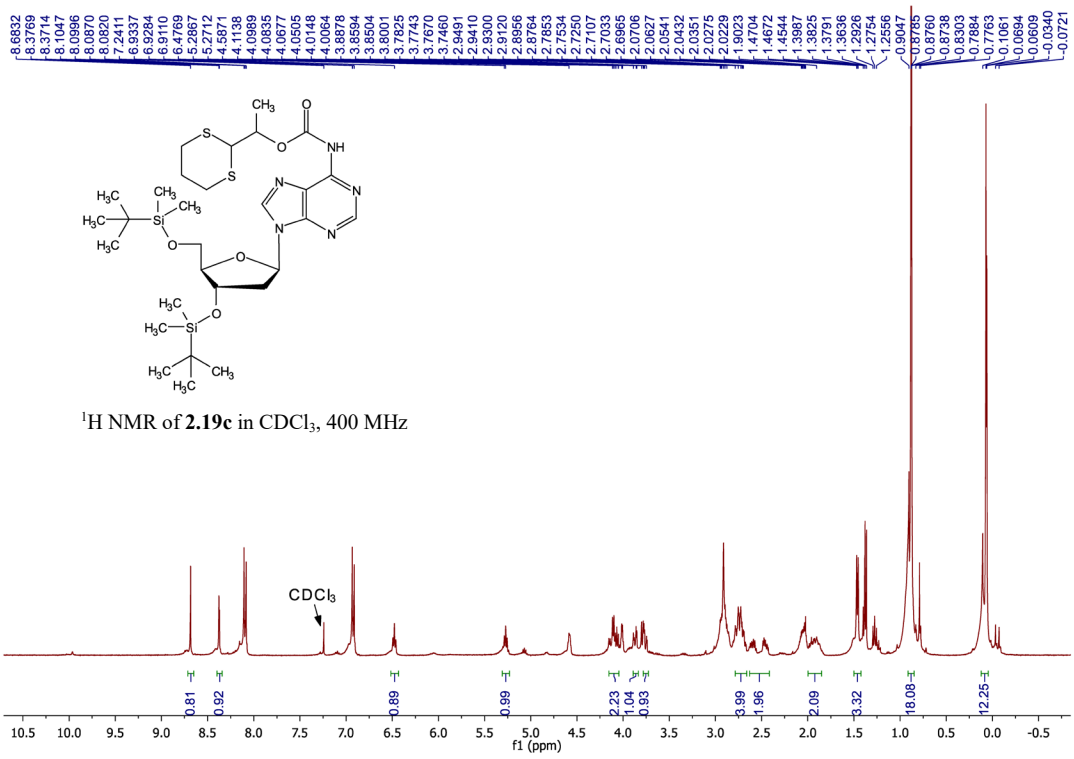
^{13}C NMR of **2.5dCme** in $CDCl_3$, 100 MHz

Figure 5.158: ^{13}C NMR spectrum of compound **2.5dCme**.



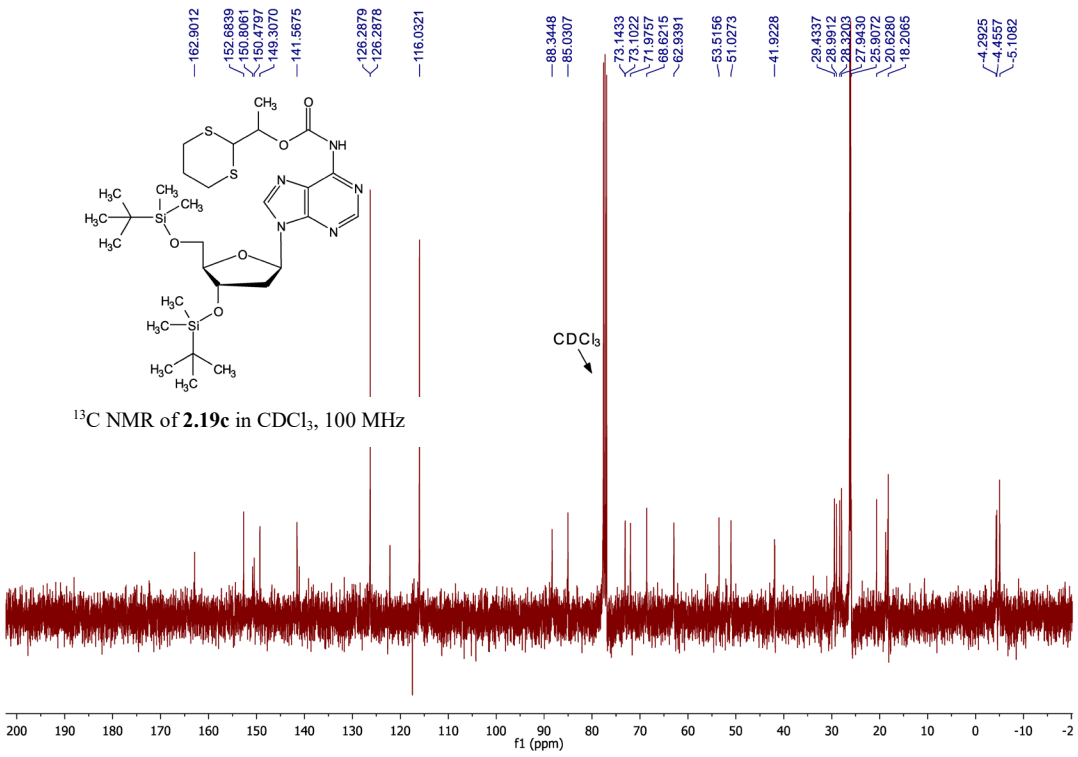
^{31}P NMR of **2.5dCme** in $CDCl_3$, 162 MHz

Figure 5.159: ^{31}P NMR spectrum of compound **2.5dCme**.



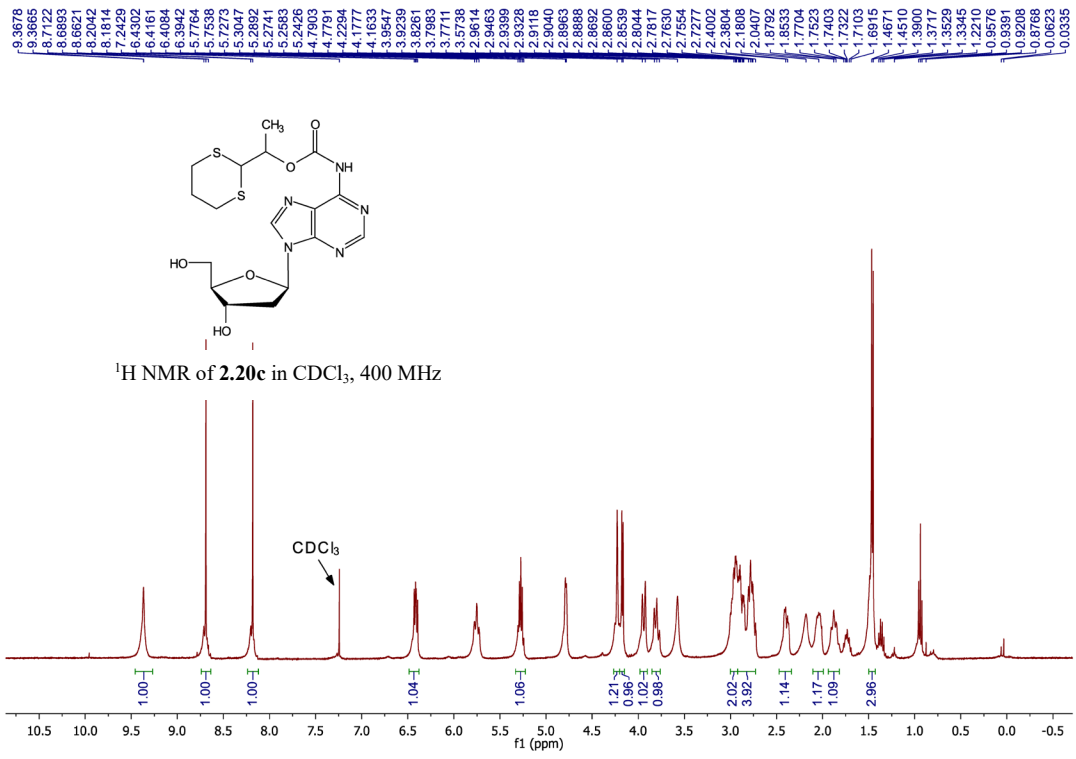
^{1}H NMR of **2.19c** in $CDCl_3$, 400 MHz

Figure 5.160: ^{1}H NMR spectrum of compound **2.19c**.



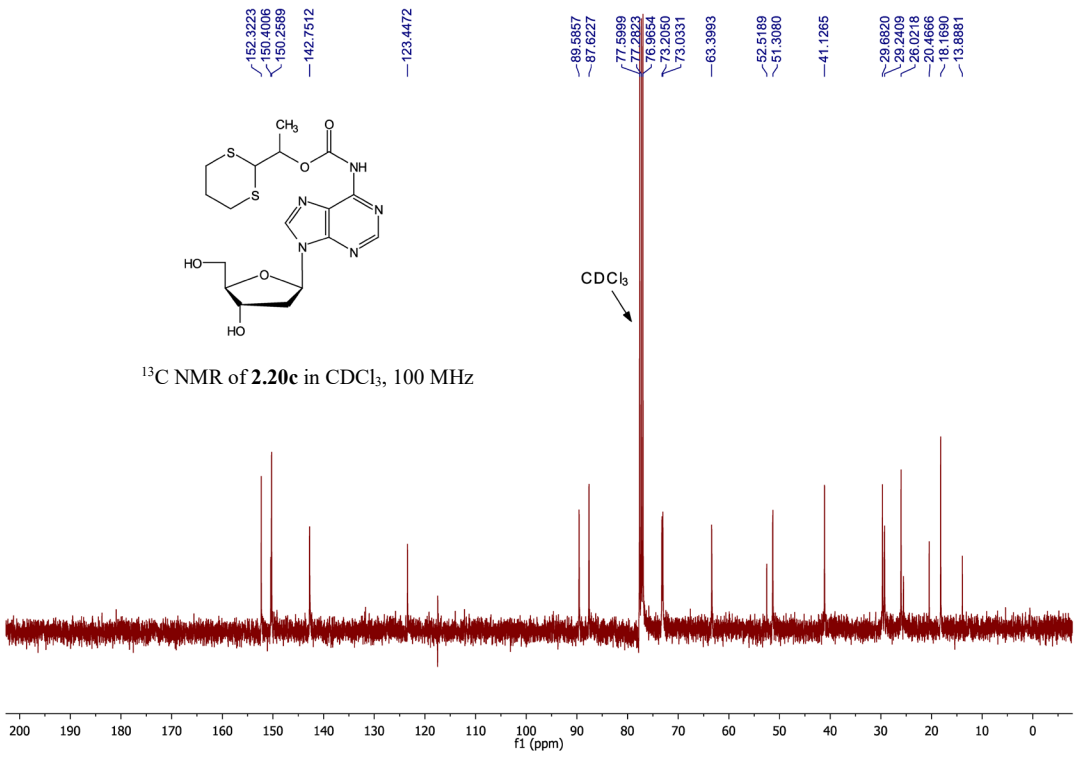
^{13}C NMR of **2.19c** in $CDCl_3$, 100 MHz

Figure 5.161: ^{13}C NMR spectrum of compound **2.19c**.



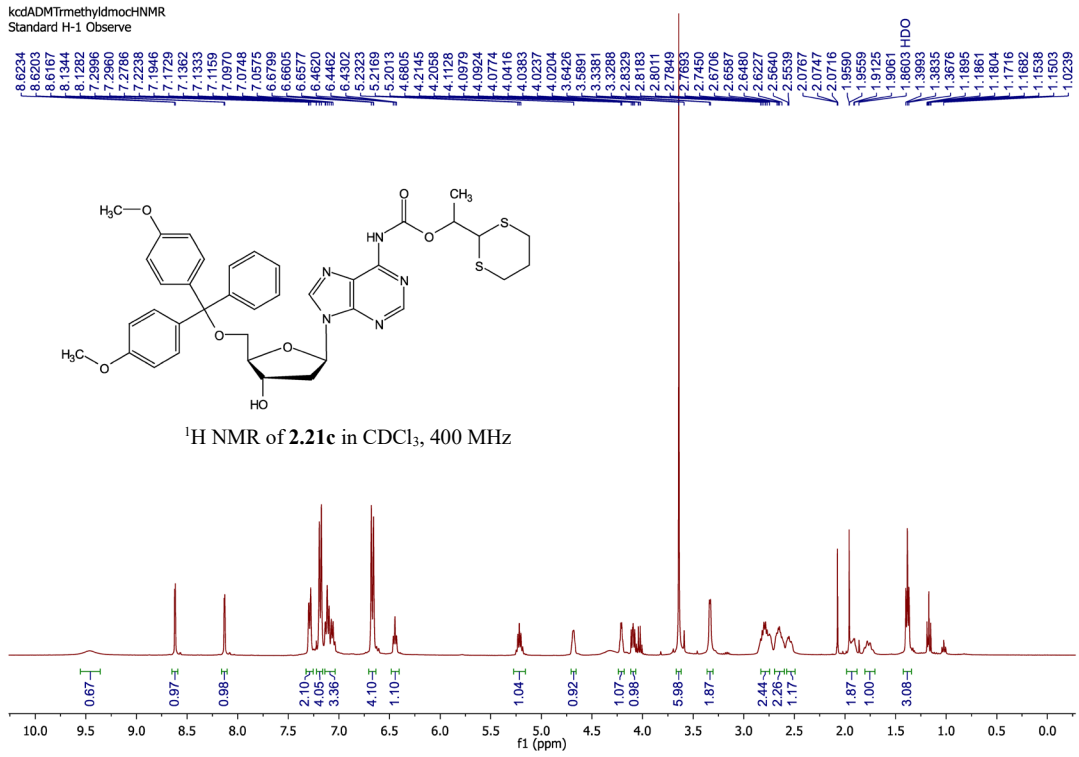
^{1}H NMR of **2.20c** in $CDCl_3$, 400 MHz

Figure 5.162: ^{1}H NMR spectrum of compound **2.20c**.



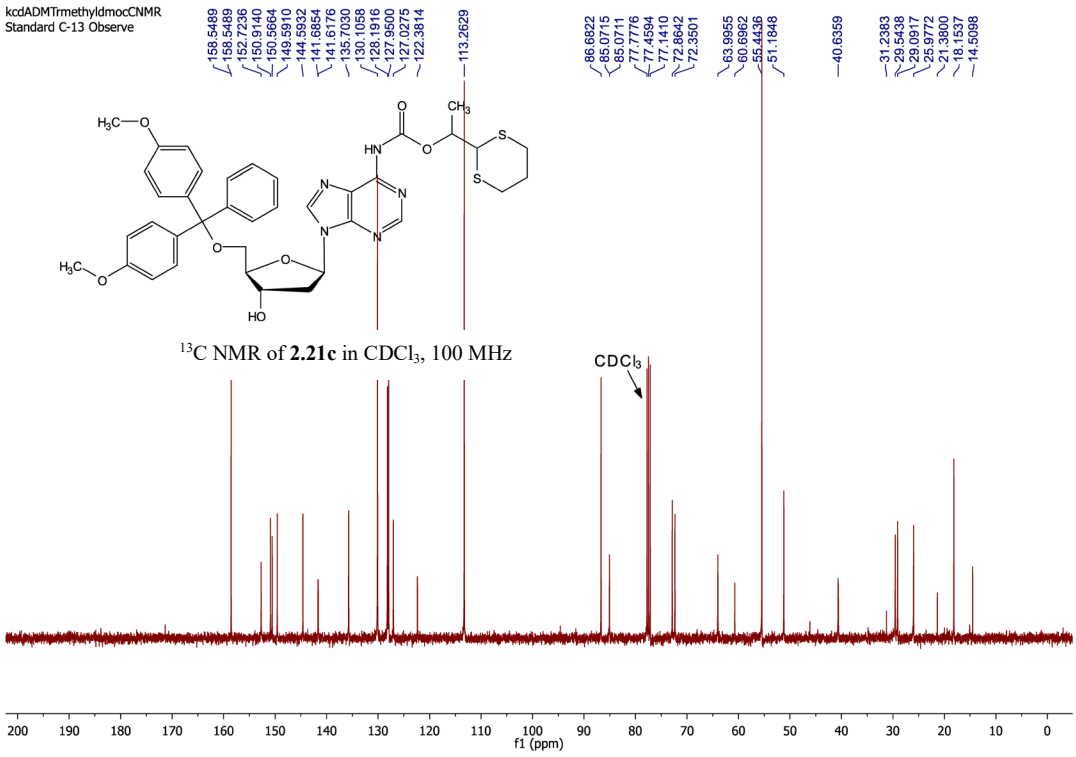
^{13}C NMR of **2.20c** in $CDCl_3$, 100 MHz

Figure 5.163: ^{13}C NMR spectrum of compound **2.20c**.



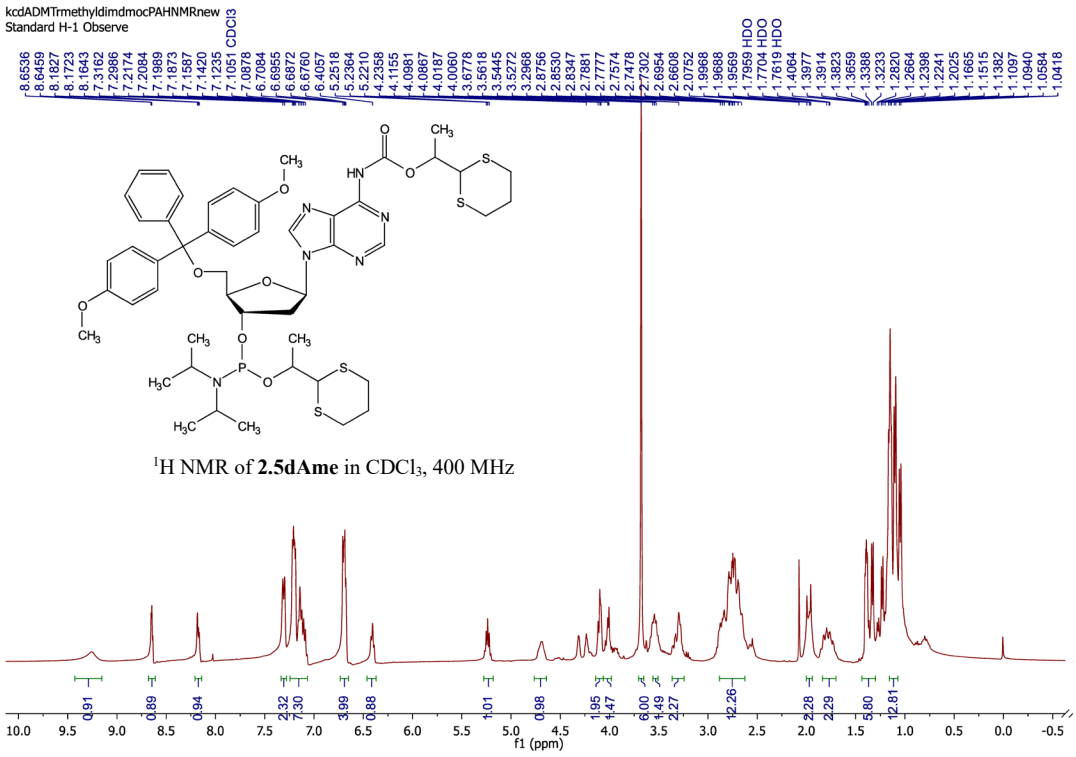
^{1}H NMR of **2.21c** in $CDCl_3$, 400 MHz

Figure 5.164: ^{1}H NMR spectrum of compound **2.21c**.



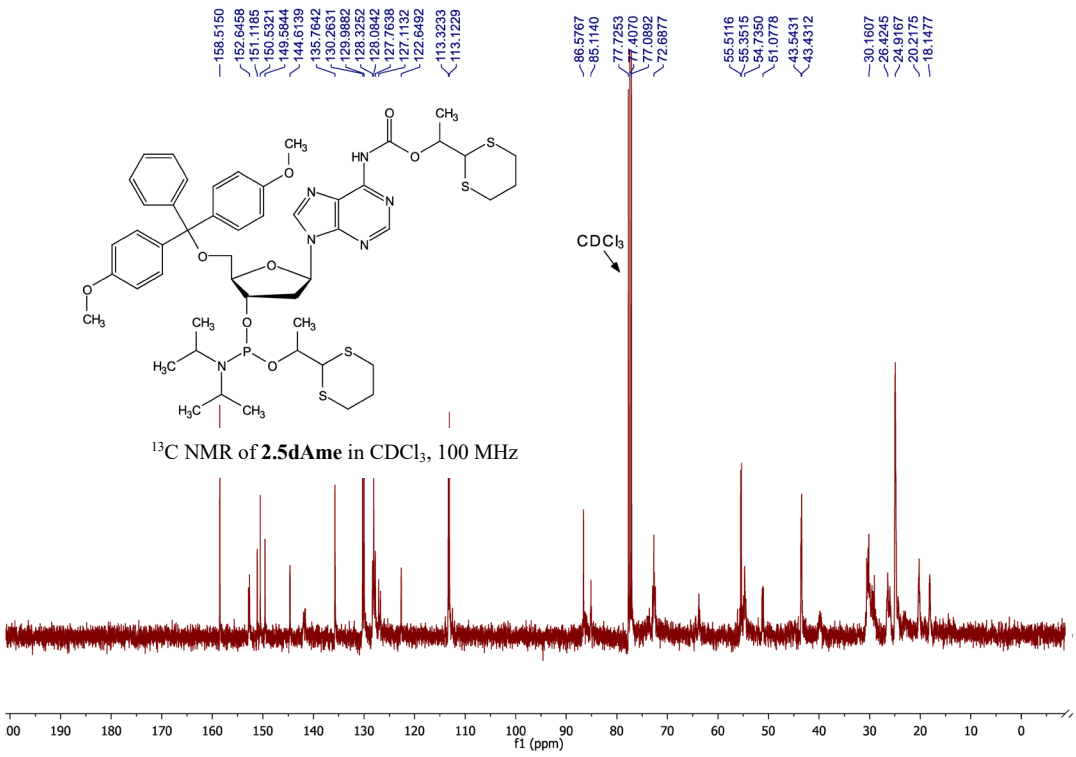
^{13}C NMR of **2.21c** in $CDCl_3$, 100 MHz

Figure 5.165: ^{13}C NMR spectrum of compound **2.21c**.



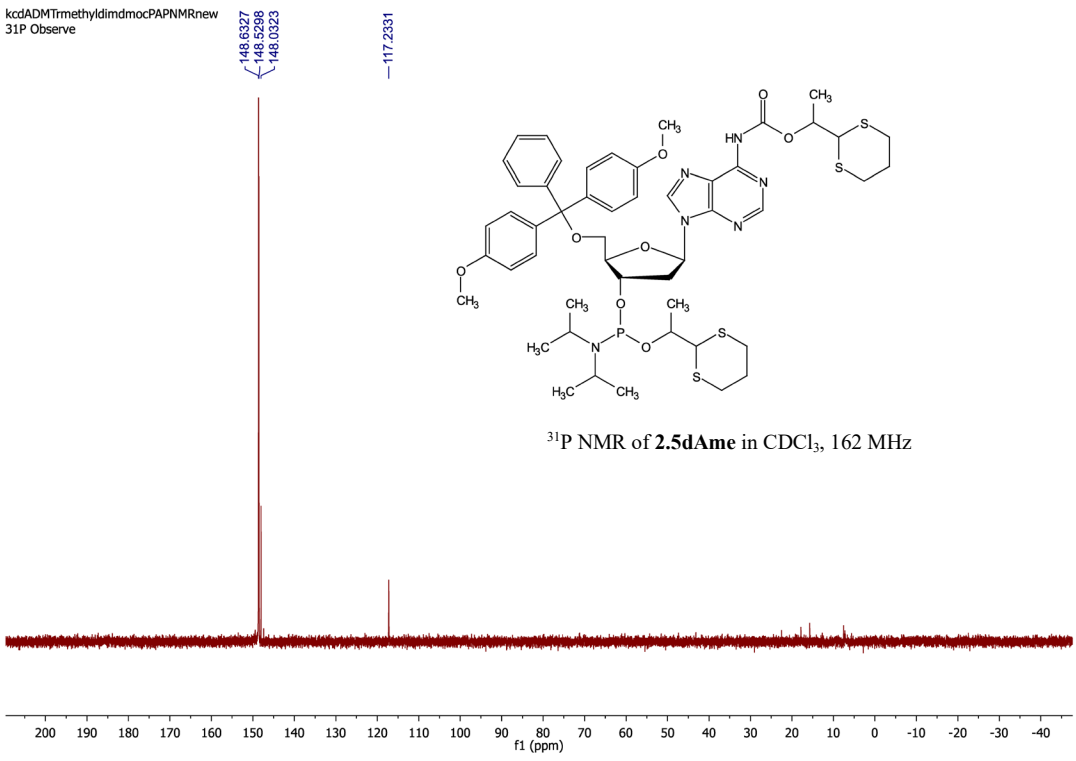
^{1}H NMR of **2.5dAme** in $CDCl_3$, 400 MHz

Figure 5.166: ^{1}H NMR spectrum of compound **2.5dAme**.



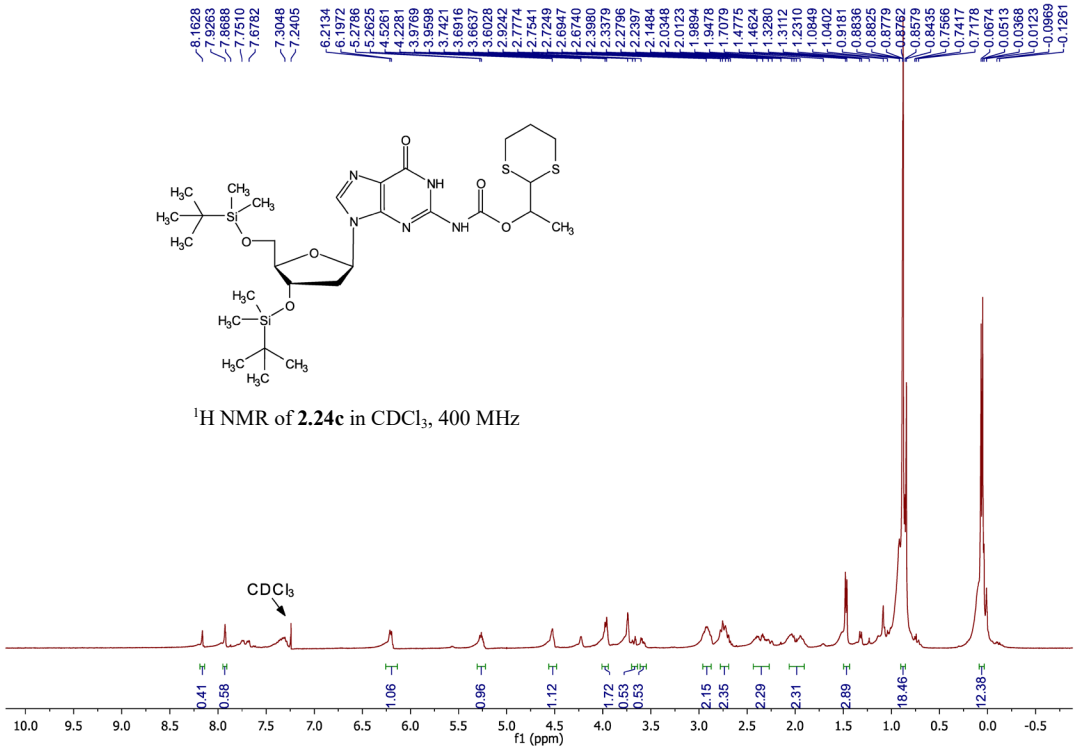
^{13}C NMR of **2.5dAme** in $CDCl_3$, 100 MHz

Figure 5.167: ^{13}C NMR spectrum of compound **2.5dAme**.



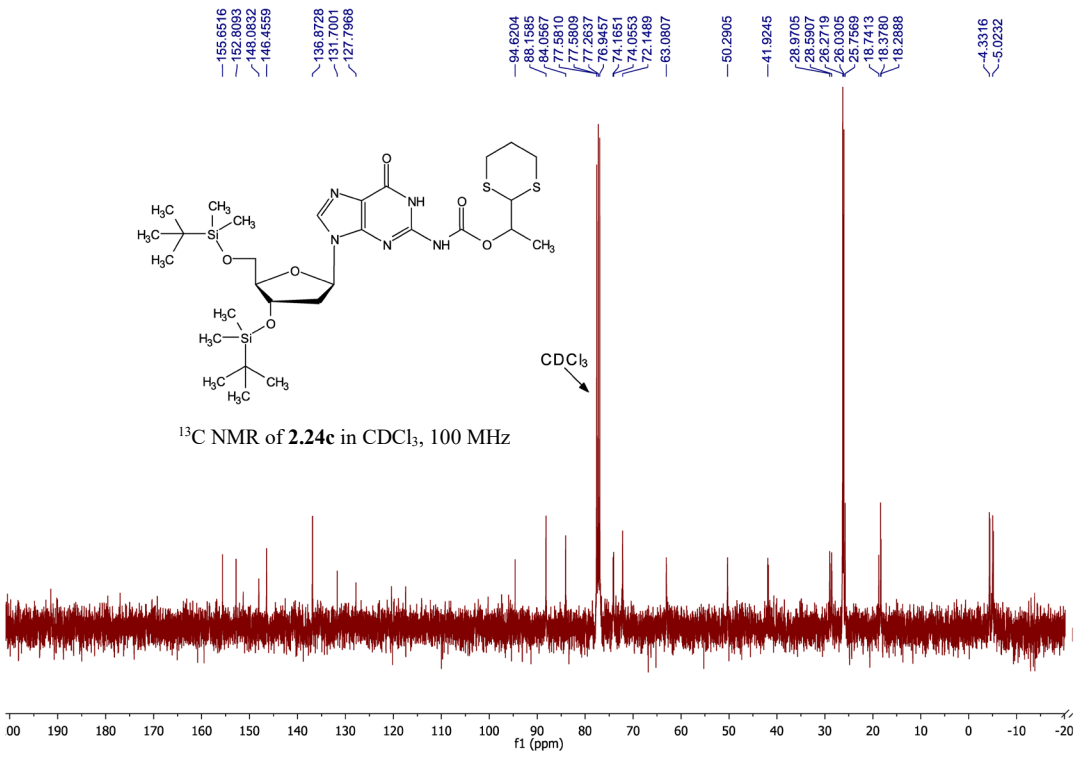
^{31}P NMR of **2.5dAme** in $CDCl_3$, 162 MHz

Figure 5.168: ^{31}P NMR spectrum of compound **2.5dAme**.



^{1}H NMR of **2.24c** in $CDCl_3$, 400 MHz

Figure 5.169: ^{1}H NMR spectrum of compound **2.24c**.



^{13}C NMR of **2.24c** in $CDCl_3$, 100 MHz

Figure 5.170: ^{13}C NMR spectrum of compound **2.24c**.

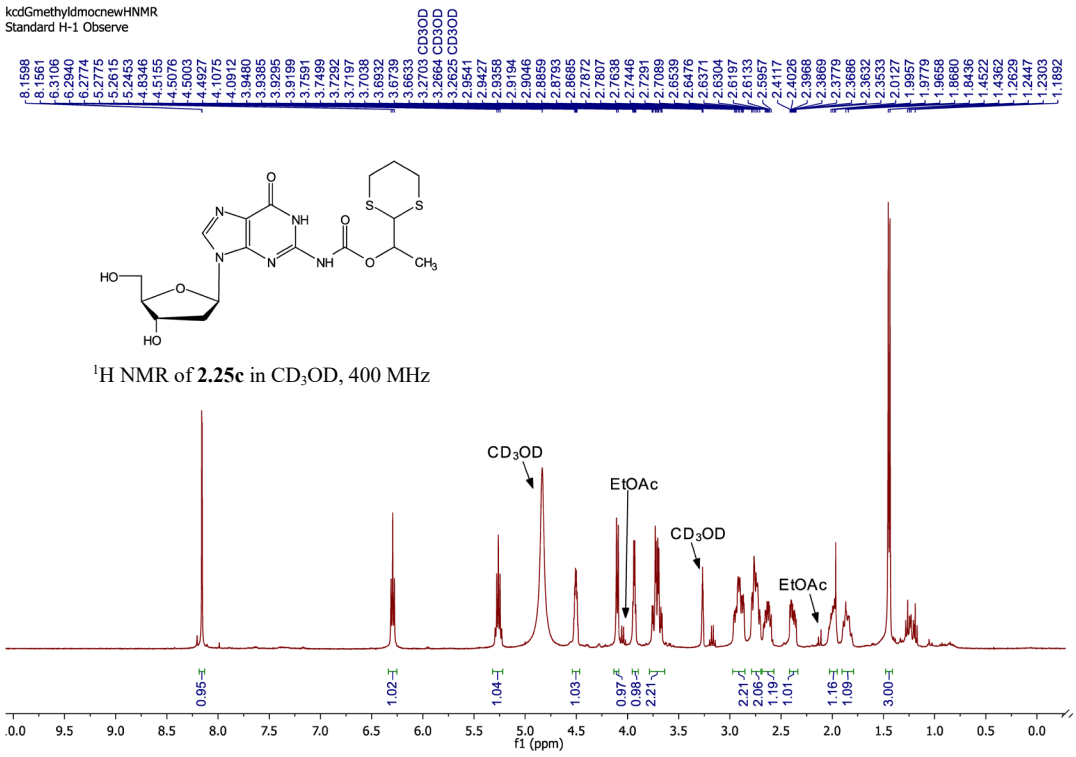
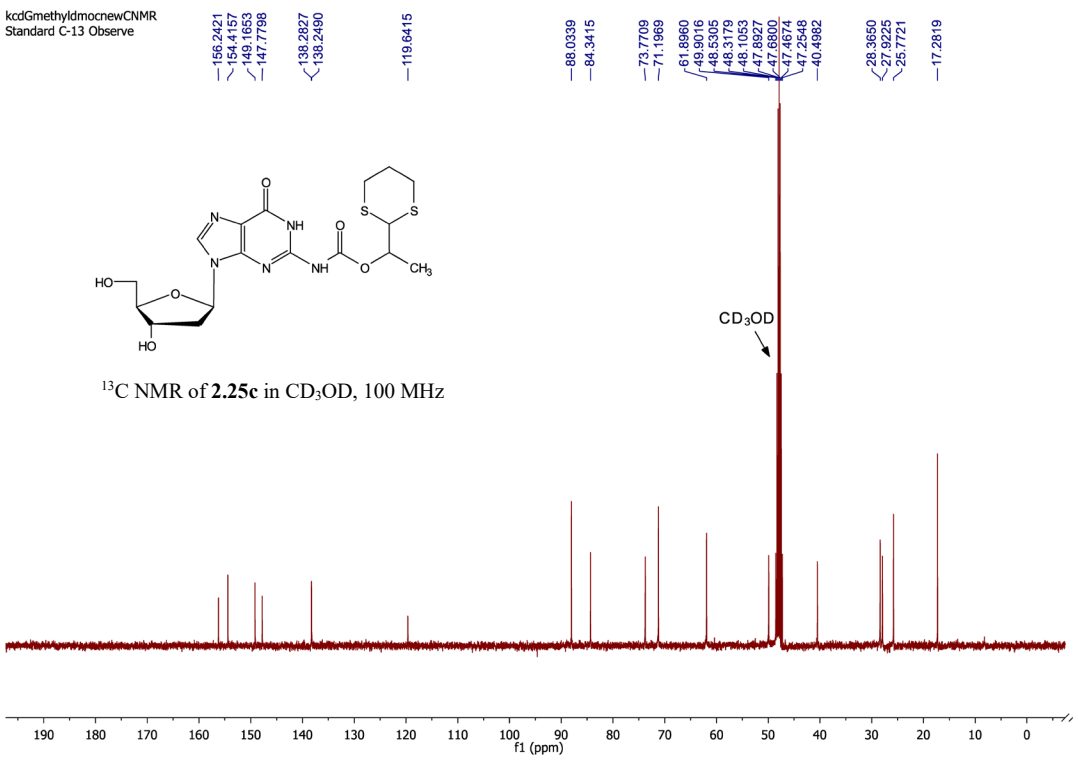
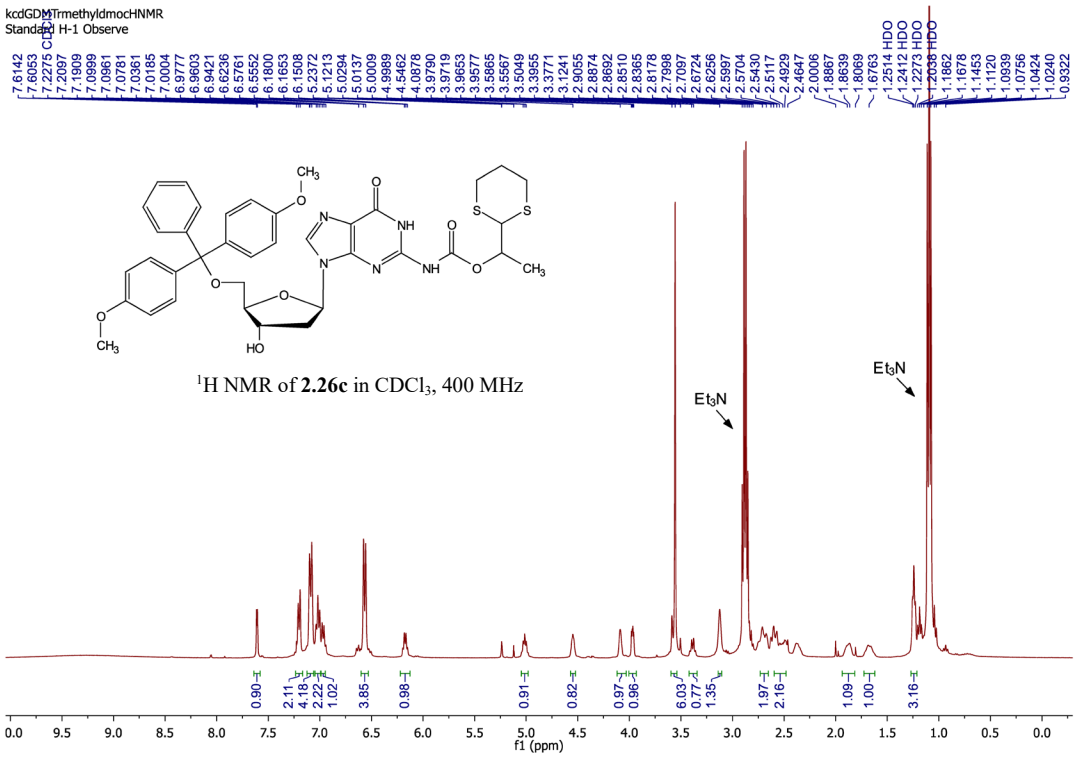


Figure 5.171: ^{1}H NMR spectrum of compound **2.25c**.



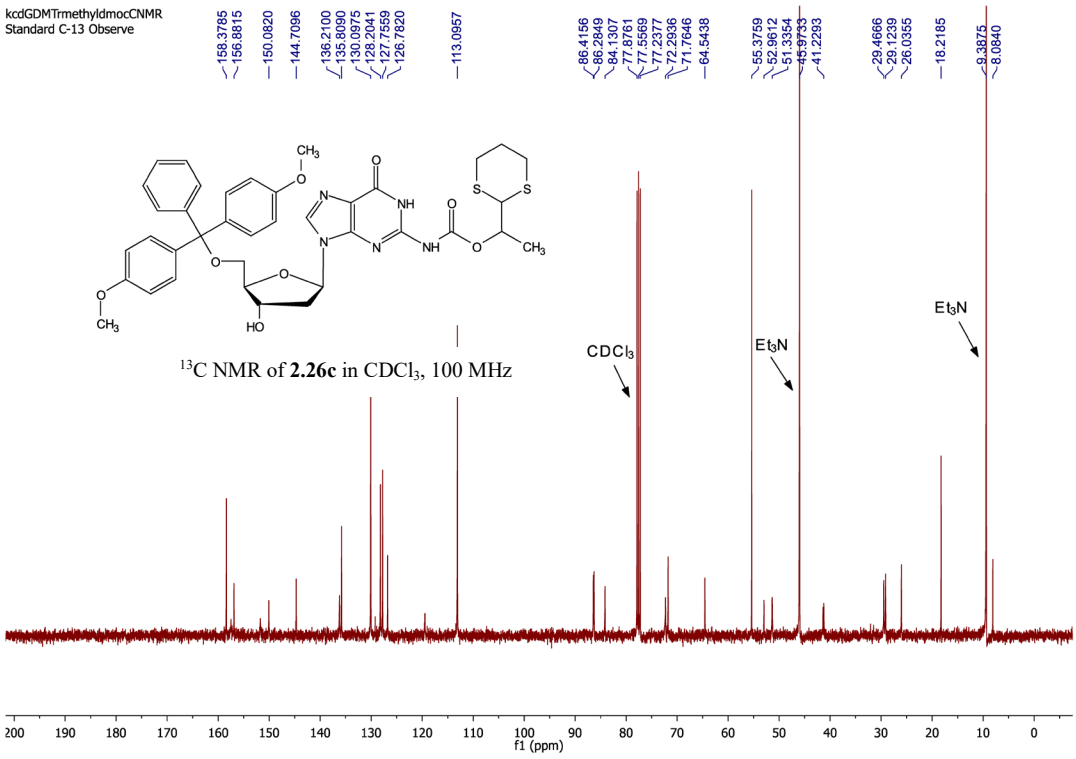
^{13}C NMR of **2.25c** in CD_3OD, 100 MHz

Figure 5.172: ^{13}C NMR spectrum of compound **2.25c**.



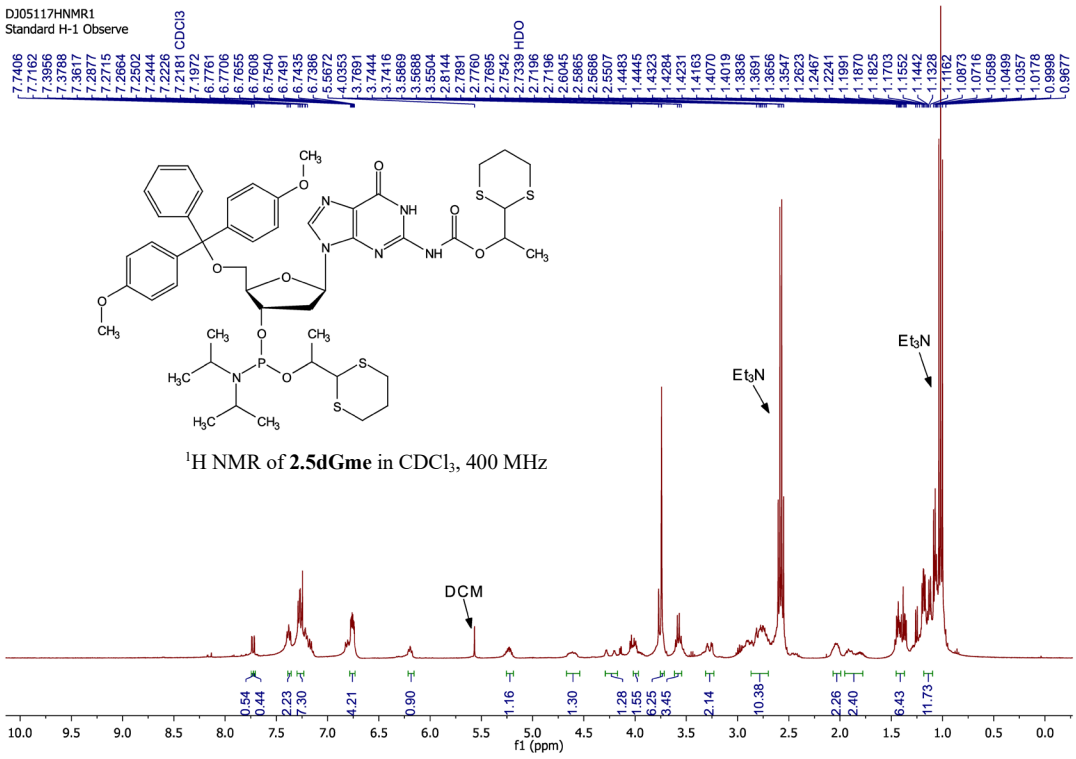
^{1}H NMR of **2.26c** in $CDCl_3$, 400 MHz

Figure 5.173: ^{1}H NMR spectrum of compound **2.26c**.



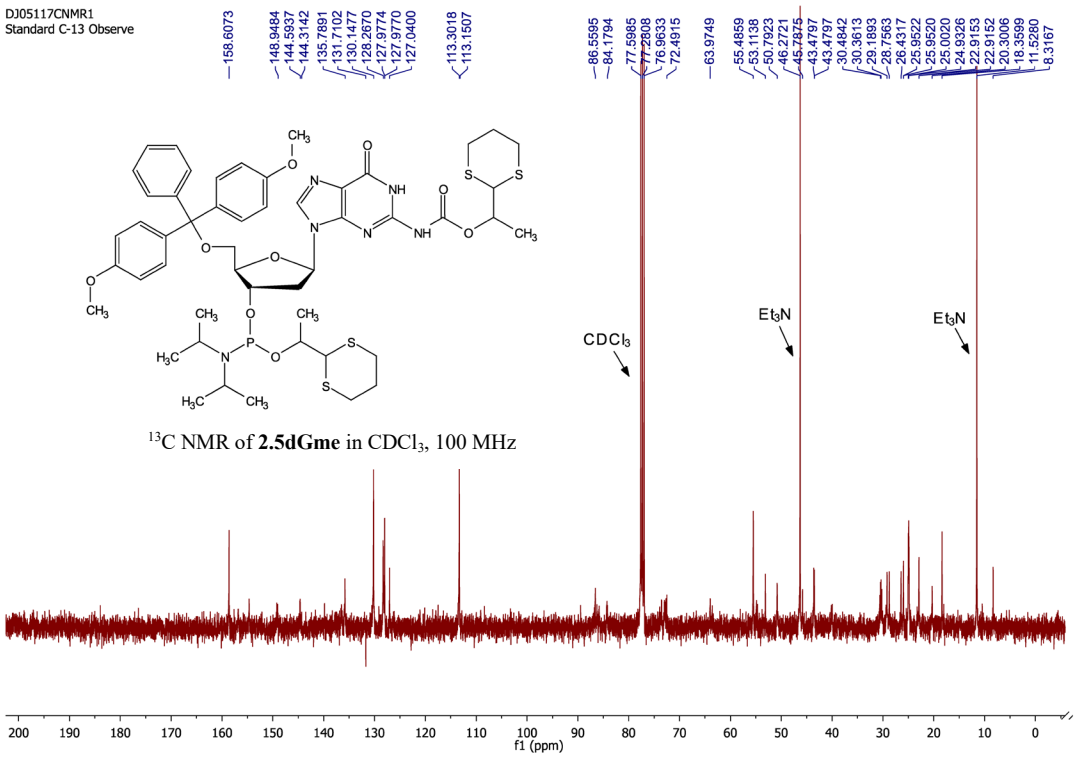
^{13}C NMR of **2.26c** in $CDCl_3$, 100 MHz

Figure 5.174: ^{13}C NMR spectrum of compound **2.26c**.



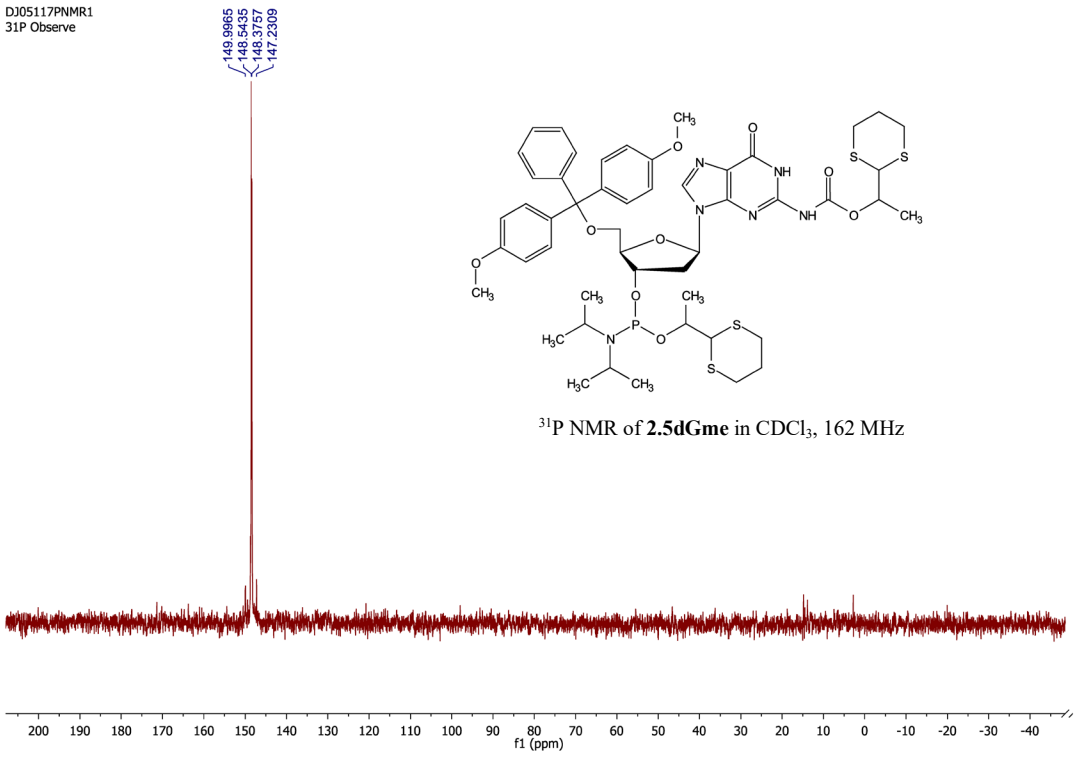
^{1}H NMR of **2.5dGme** in $CDCl_3$, 400 MHz

Figure 5.175: ^{1}H NMR spectrum of compound **2.5dGme**.



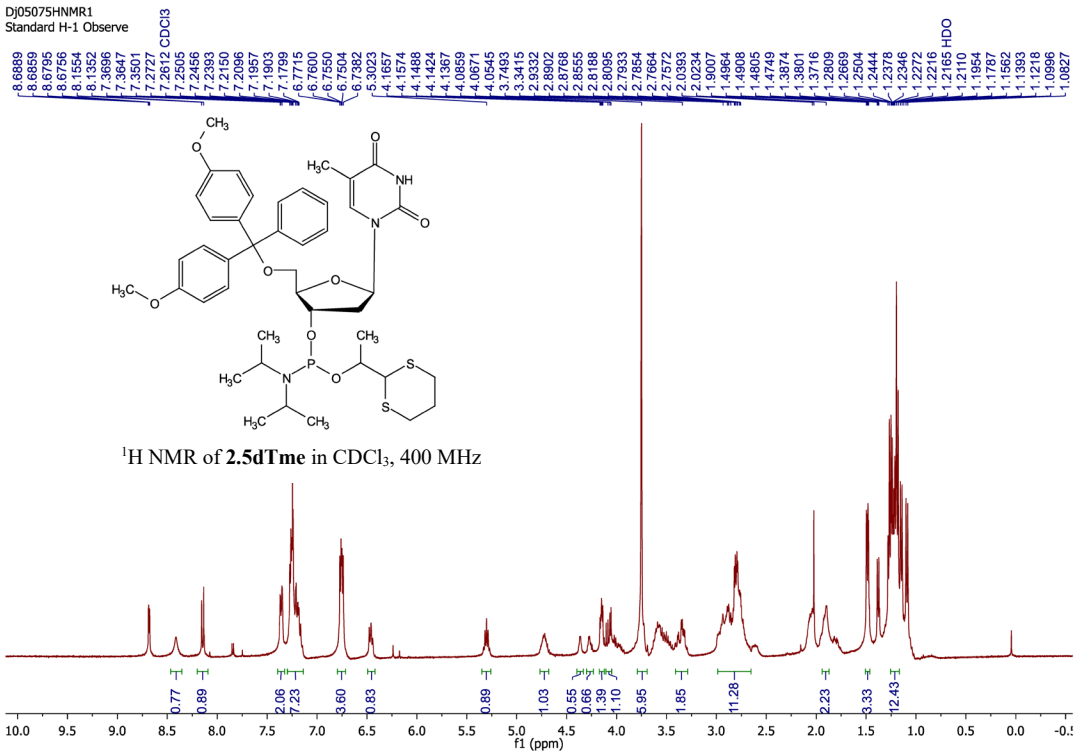
^{13}C NMR of **2.5dGme** in $CDCl_3$, 100 MHz

Figure 5.176: ^{13}C NMR spectrum of compound **2.5dGme**.



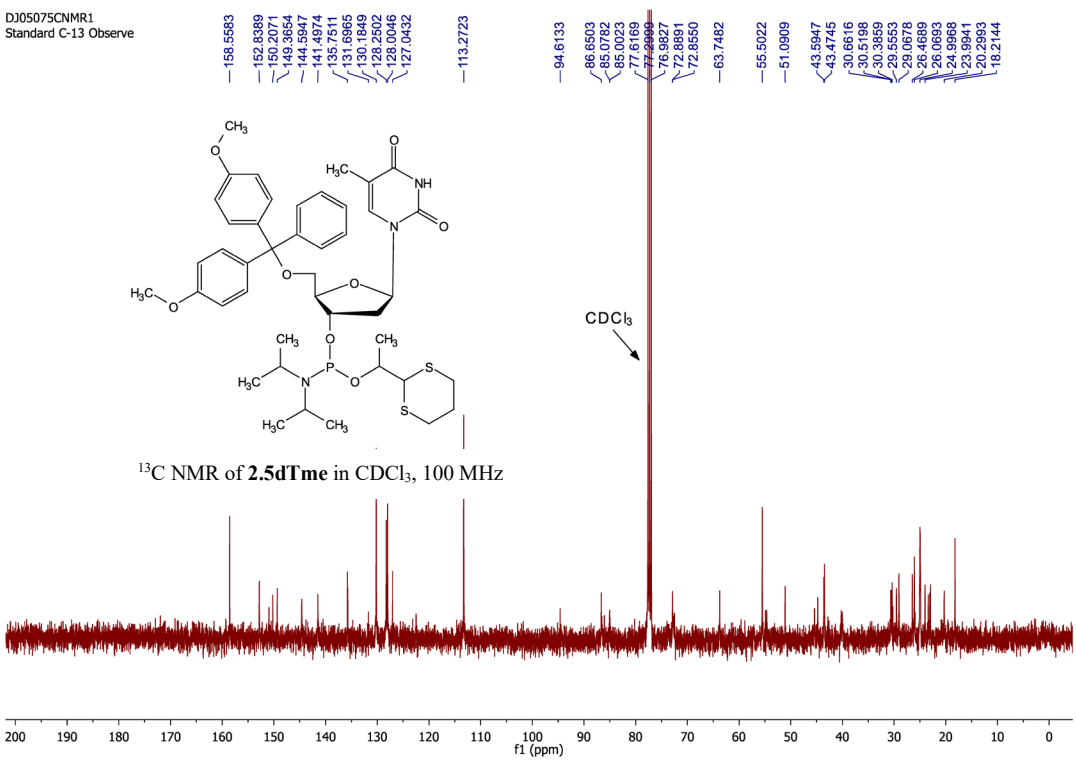
^{31}P NMR of **2.5dGme** in $CDCl_3$, 162 MHz

Figure 5.177: ^{31}P NMR spectrum of compound **2.5dGme**.



^{1}H NMR of **2.5dTme** in $CDCl_3$, 400 MHz

Figure 5.178: ^{1}H NMR spectrum of compound **2.5dTme**.



^{13}C NMR of **2.5dTme** in $CDCl_3$, 100 MHz

Figure 5.179: ^{13}C NMR spectrum of compound **2.5dTme**.

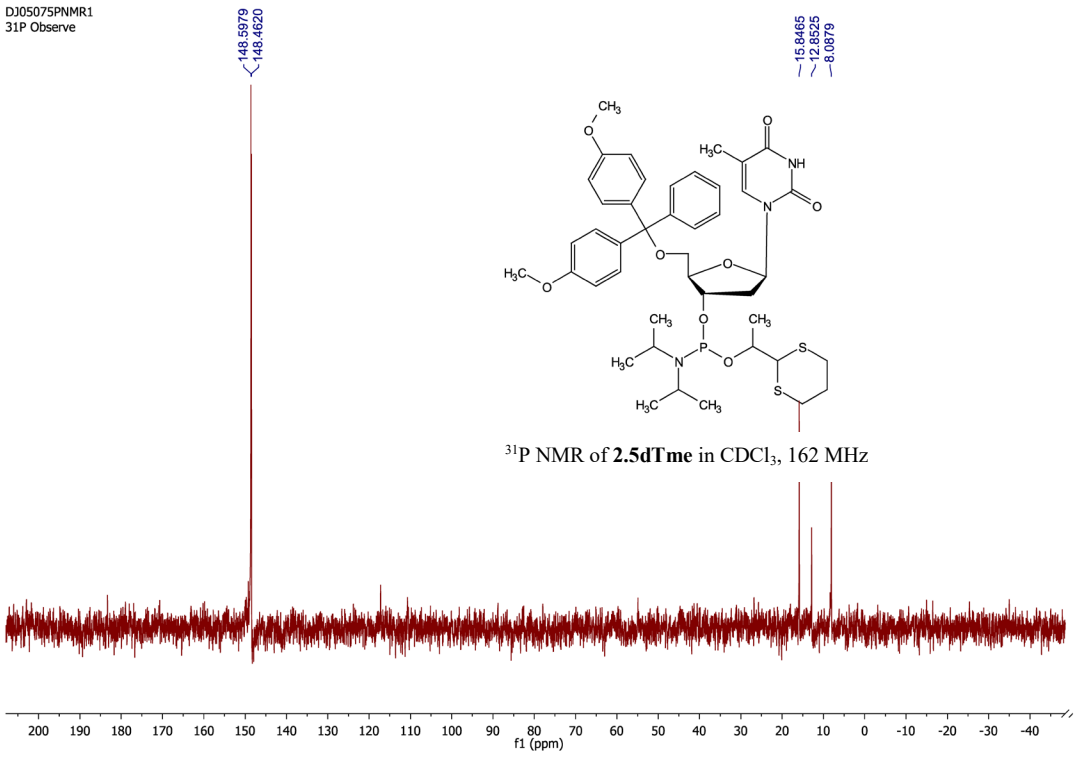
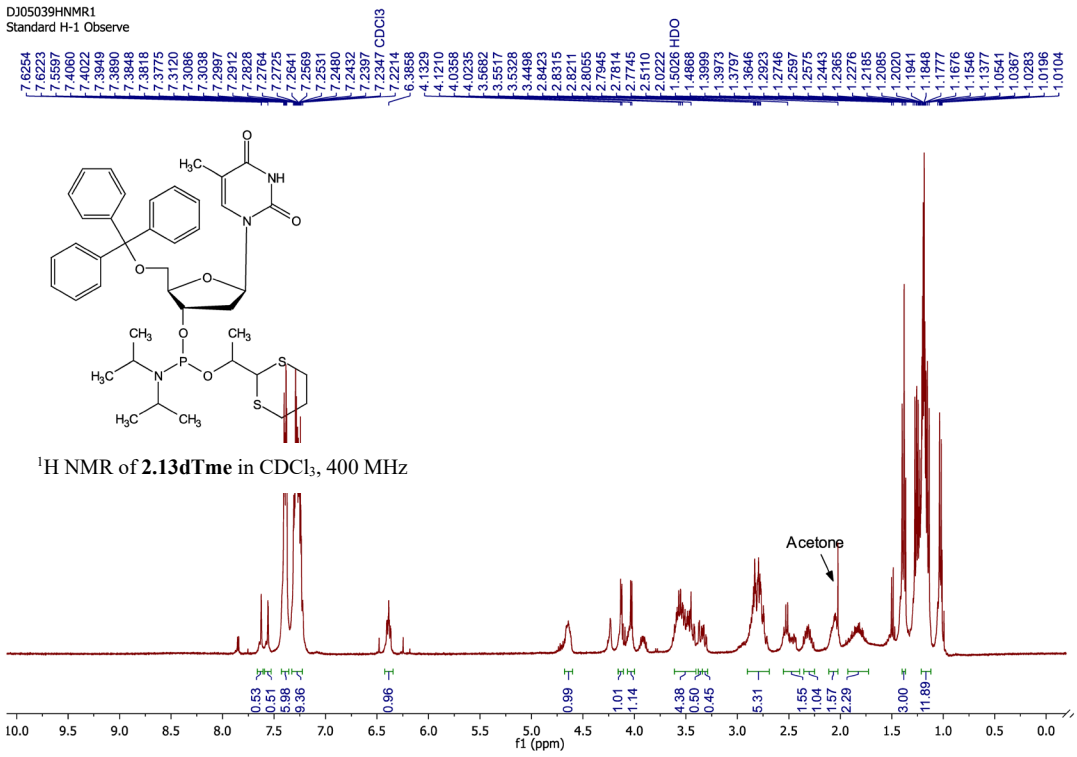
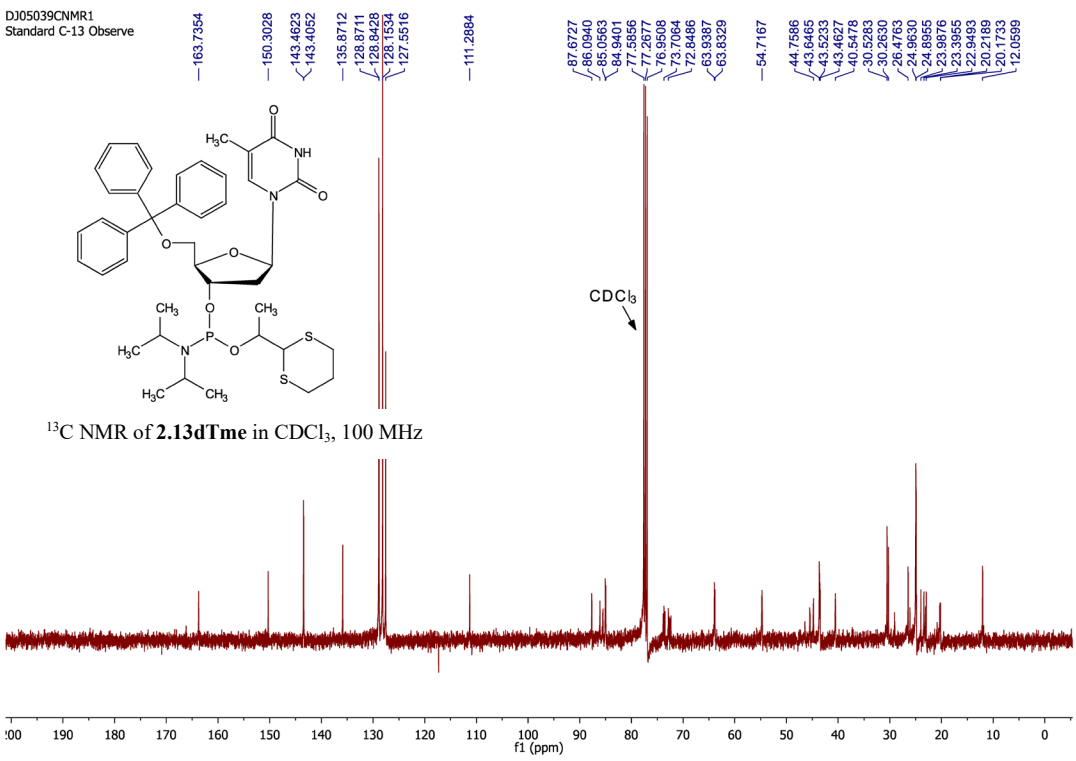


Figure 5.180: ^{31}P NMR spectrum of compound **2.5dTme**.



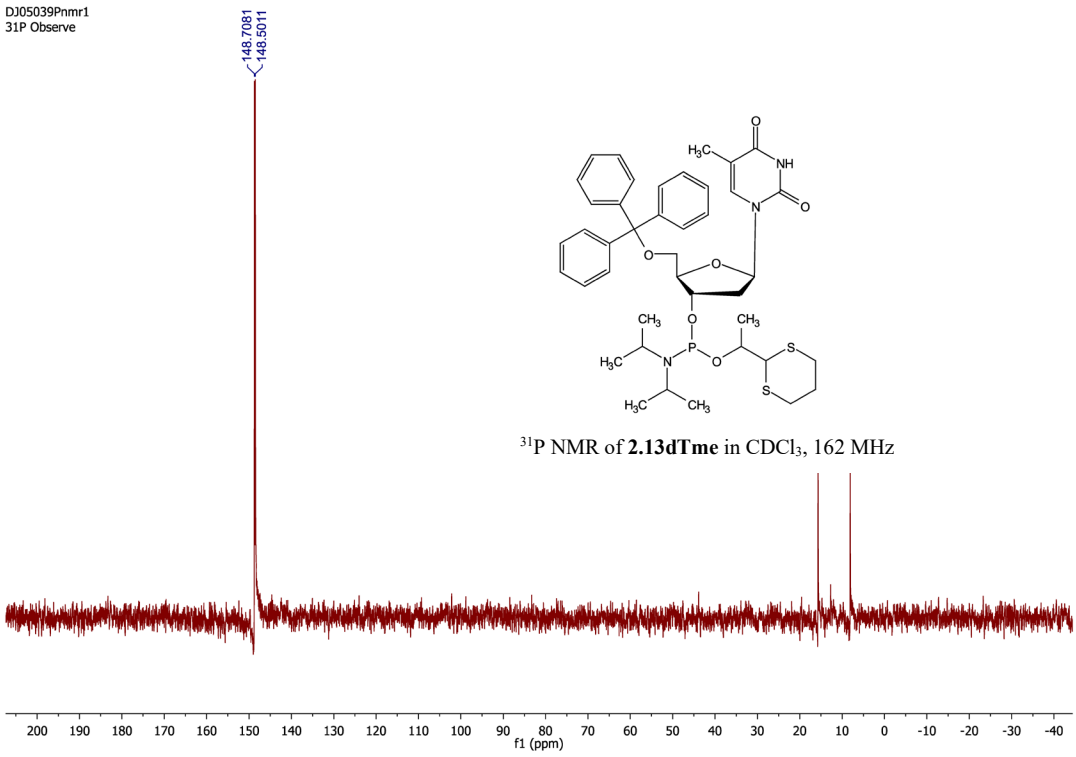
^{1}H NMR of **2.13dTme** in $CDCl_3$, 400 MHz

Figure 5.181: ^{1}H NMR spectrum of compound **2.13dTme**.



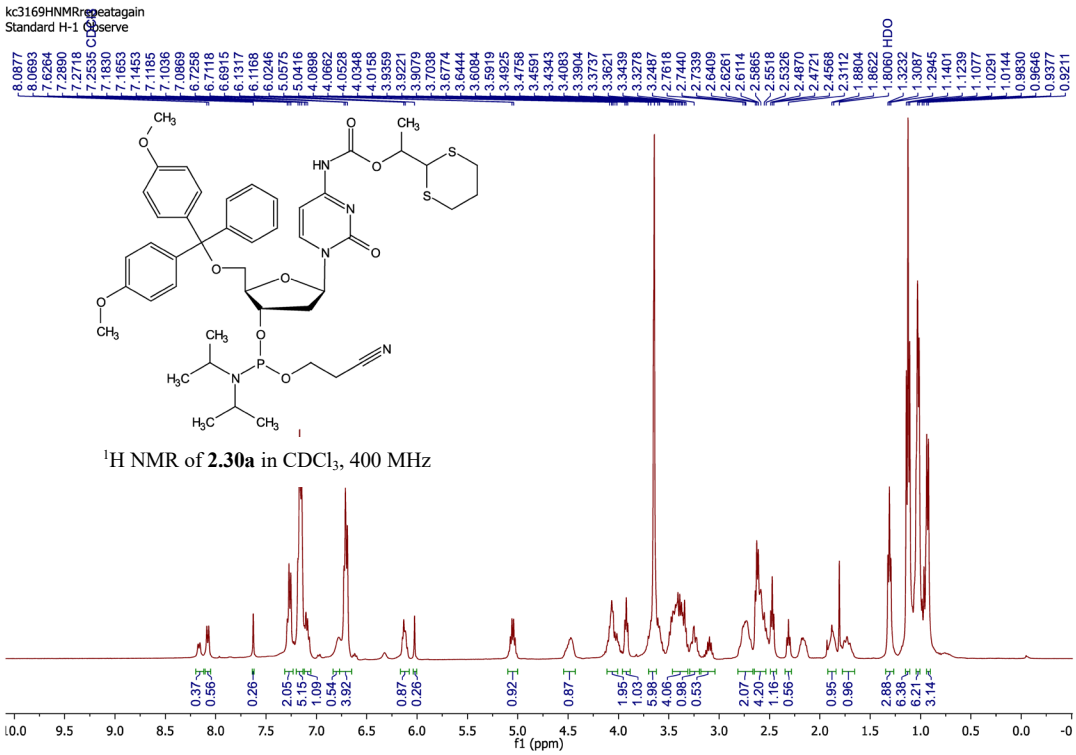
^{13}C NMR of **2.13dTme** in $CDCl_3$, 100 MHz

Figure 5.182: ^{13}C NMR spectrum of compound **2.13dTme**.



^{31}P NMR of **2.13dTme** in $CDCl_3$, 162 MHz

Figure 5.183: ^{31}P NMR spectrum of compound **2.13dTme**.



^{1}H NMR of **2.30a** in $CDCl_3$, 400 MHz

Figure 5.184: ^{1}H NMR spectrum of compound **2.30a**.

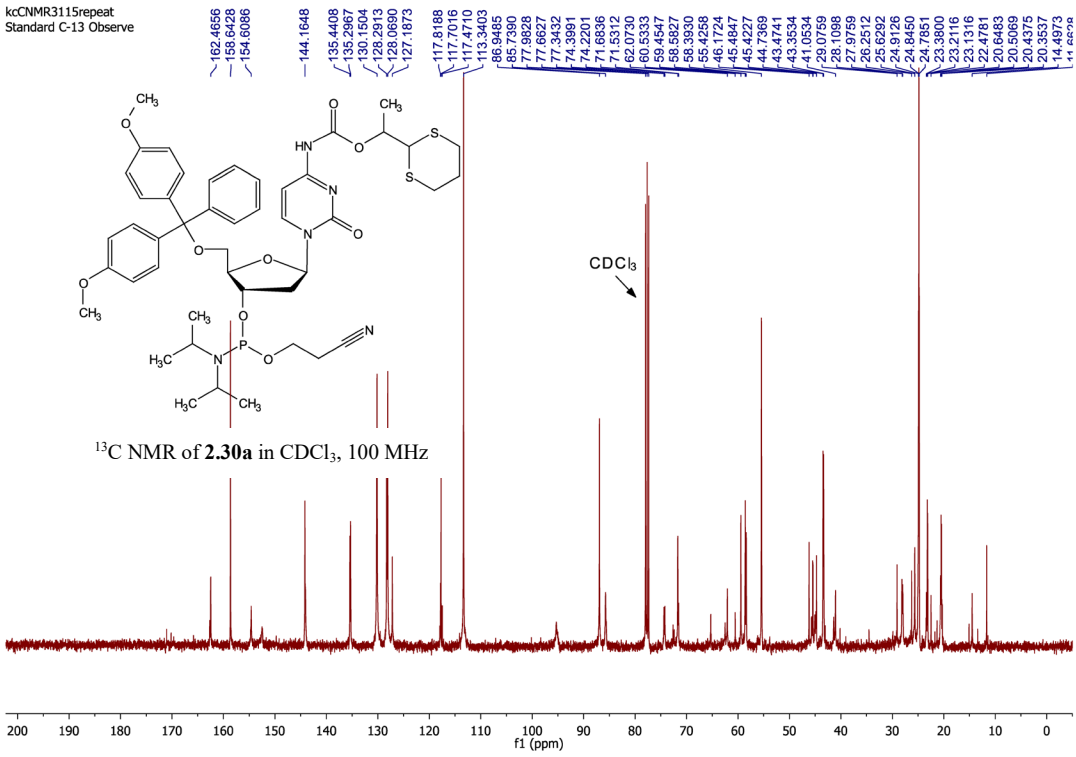
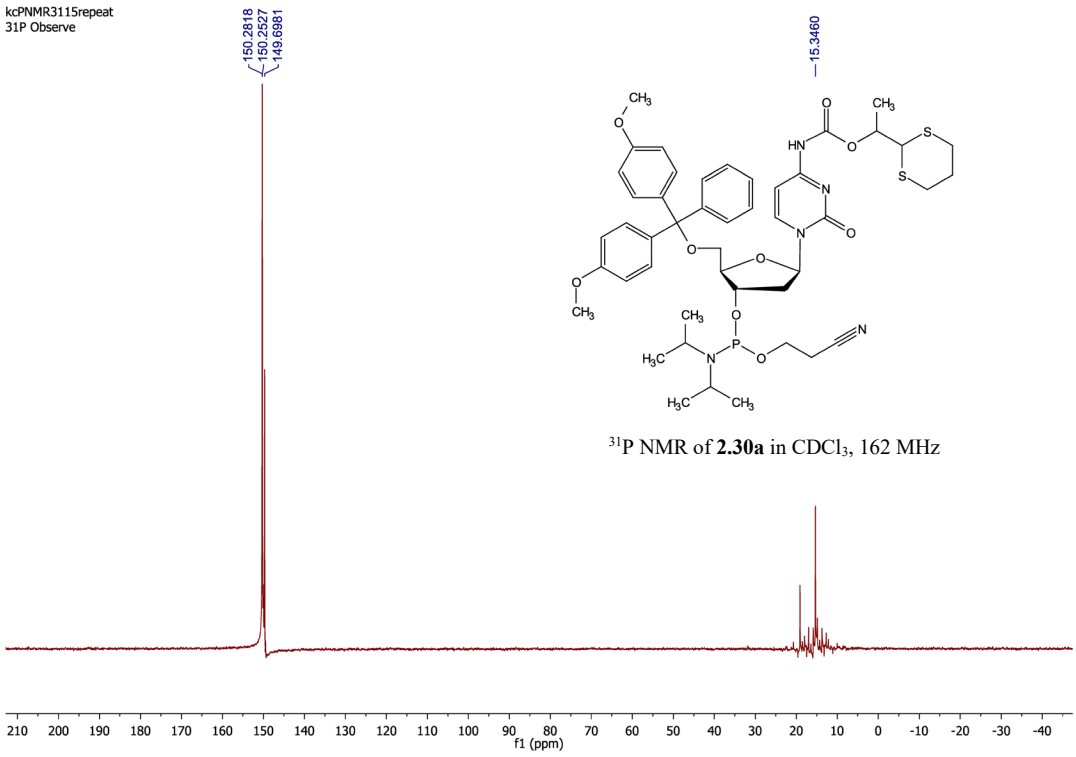
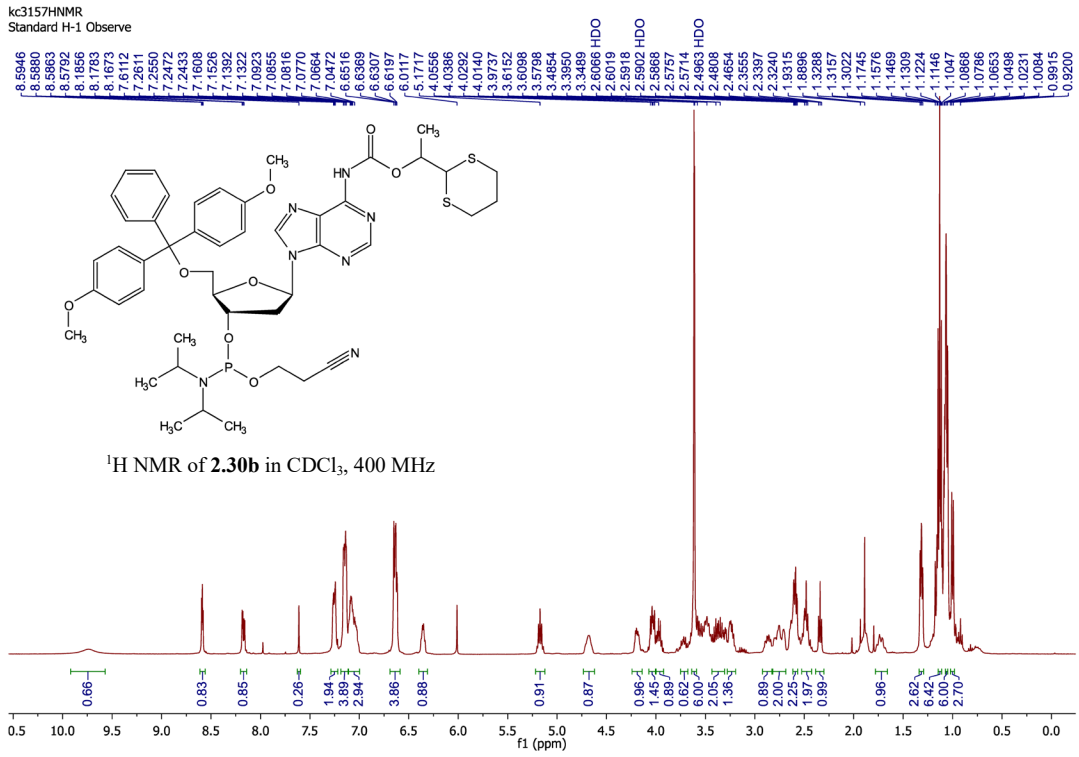


Figure 5.185: ^{13}C NMR spectrum of compound **2.30a**.



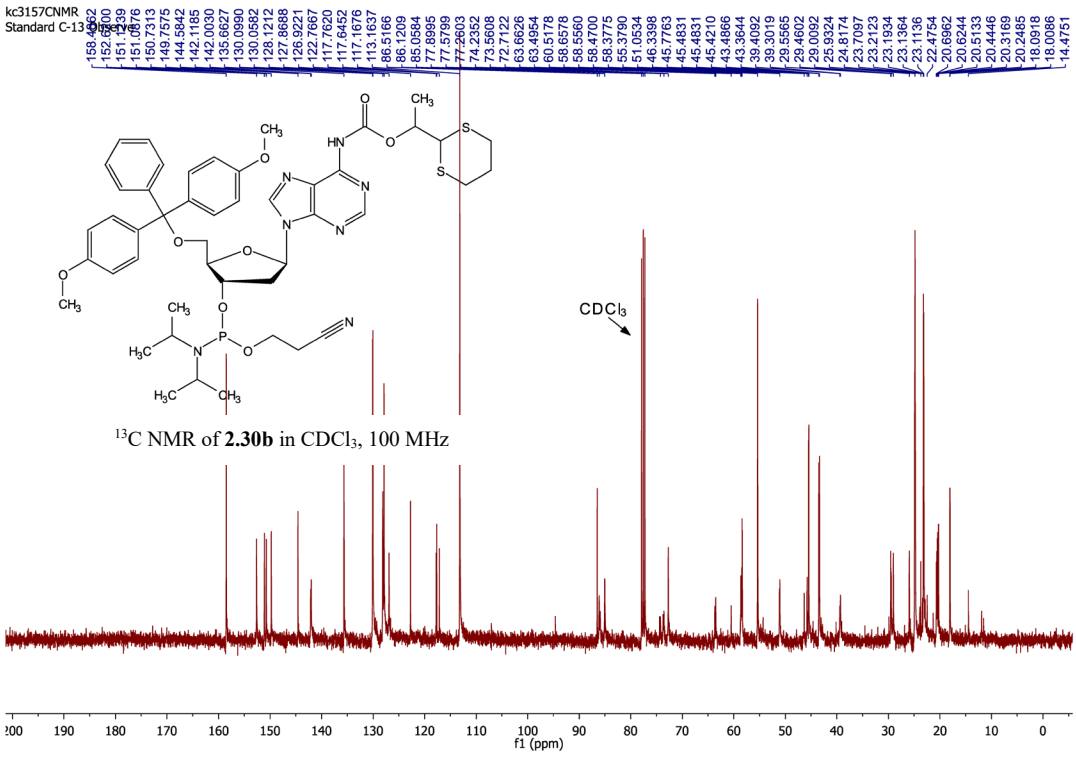
^{31}P NMR of **2.30a** in $CDCl_3$, 162 MHz

Figure 5.186: ^{31}P NMR spectrum of compound **2.30a**.



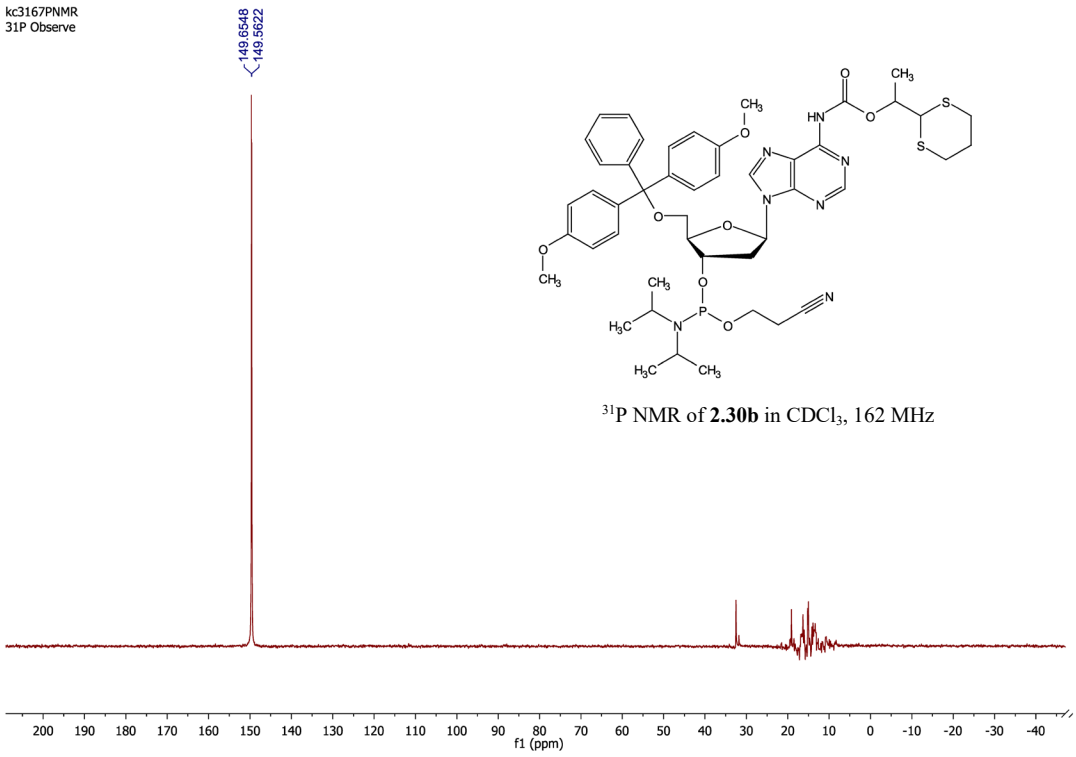
^{1}H NMR of **2.30b** in $CDCl_3$, 400 MHz

Figure 5.187: ^{1}H NMR spectrum of compound **2.30b**.



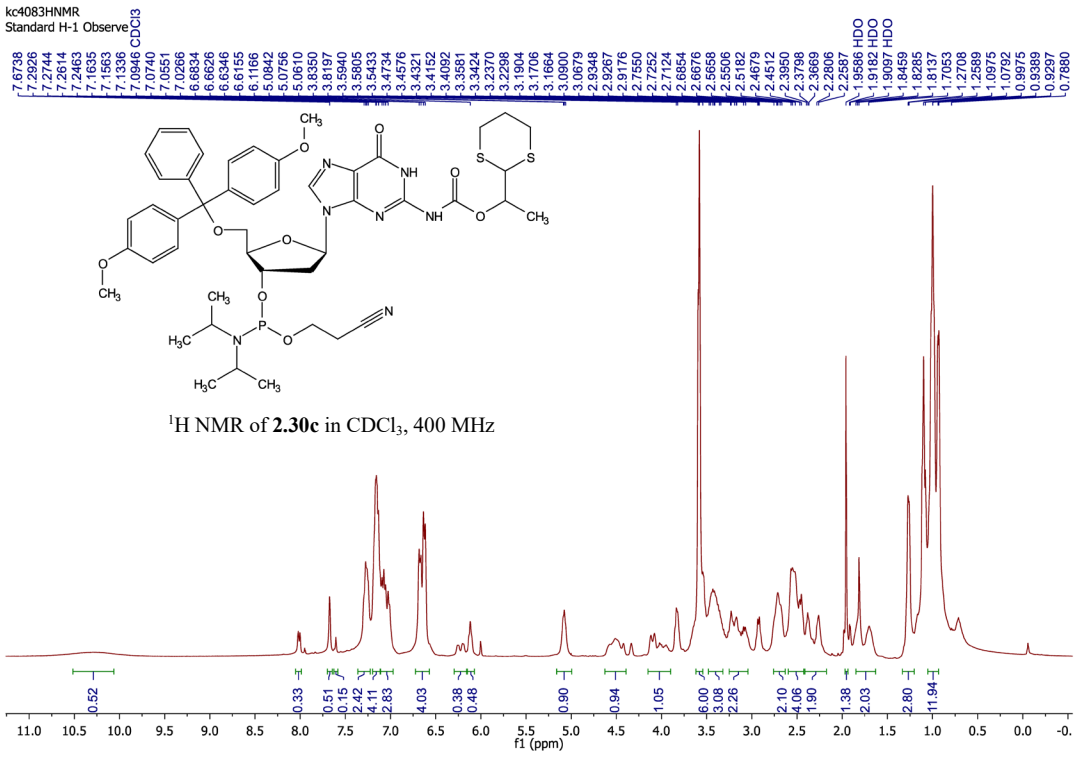
^{13}C NMR of **2.30b** in $CDCl_3$, 100 MHz

Figure 5.188: ^{13}C NMR spectrum of compound **2.30b**.



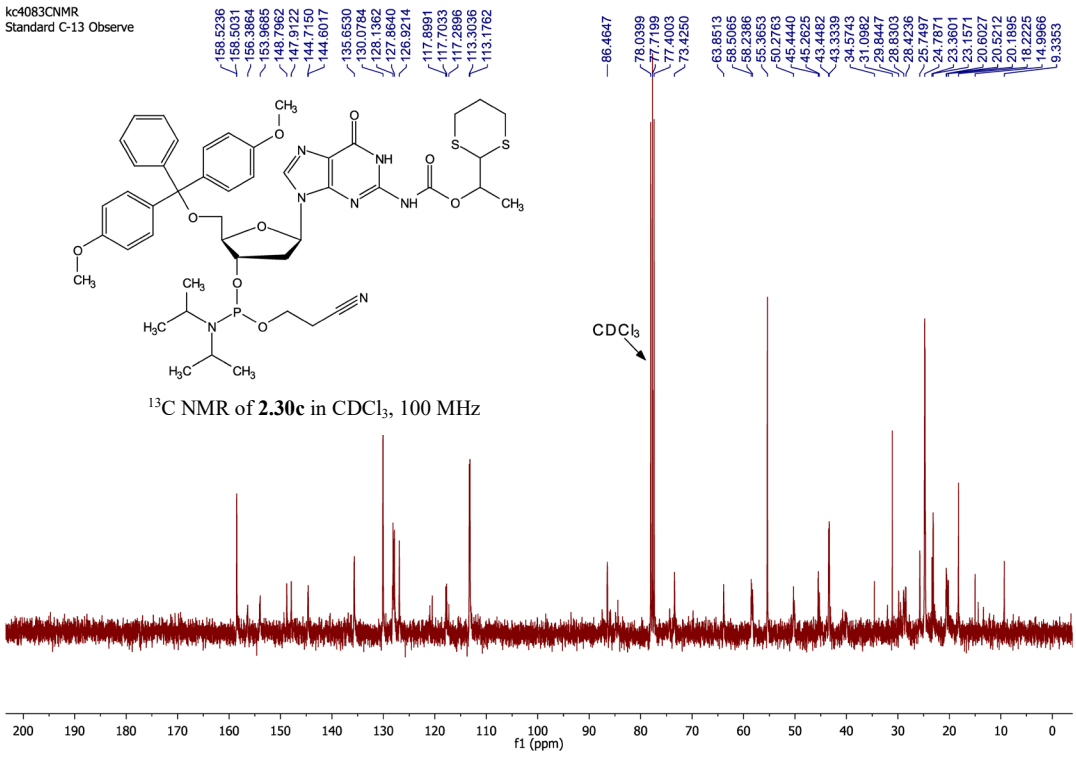
^{31}P NMR of **2.30b** in $CDCl_3$, 162 MHz

Figure 5.189: ^{31}P NMR spectrum of compound **2.30b**.



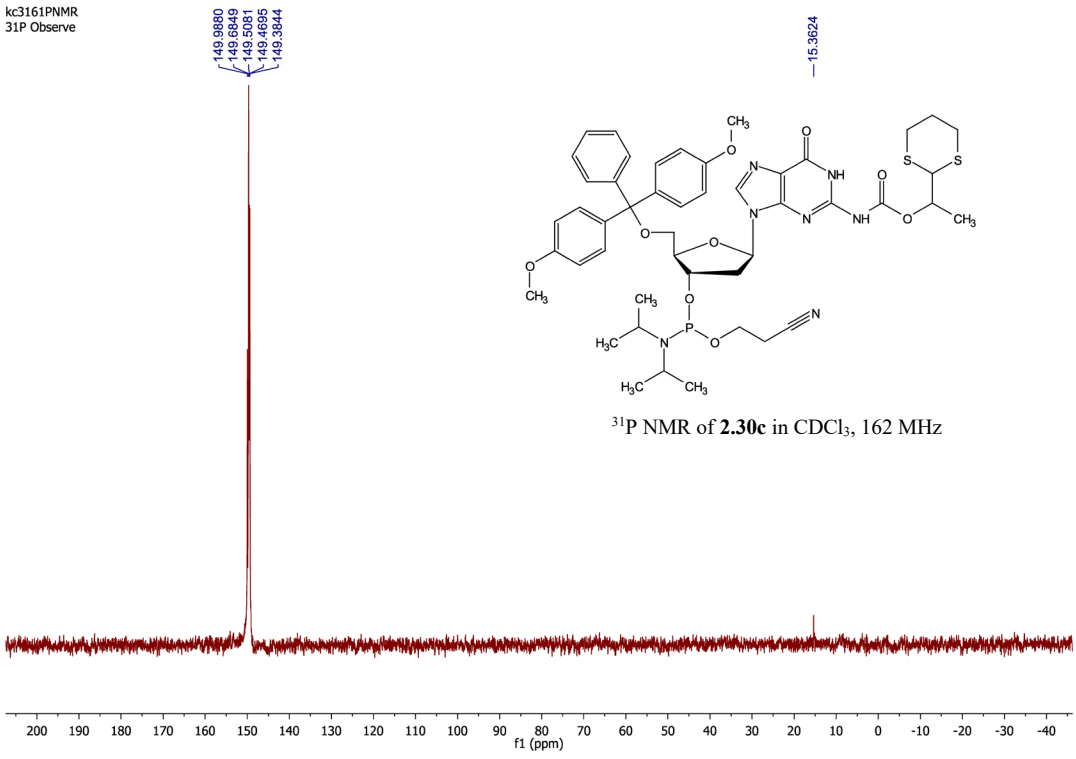
^{1}H NMR of **2.30c** in $CDCl_3$, 400 MHz

Figure 5.190: ^{1}H NMR spectrum of compound **2.30c**.



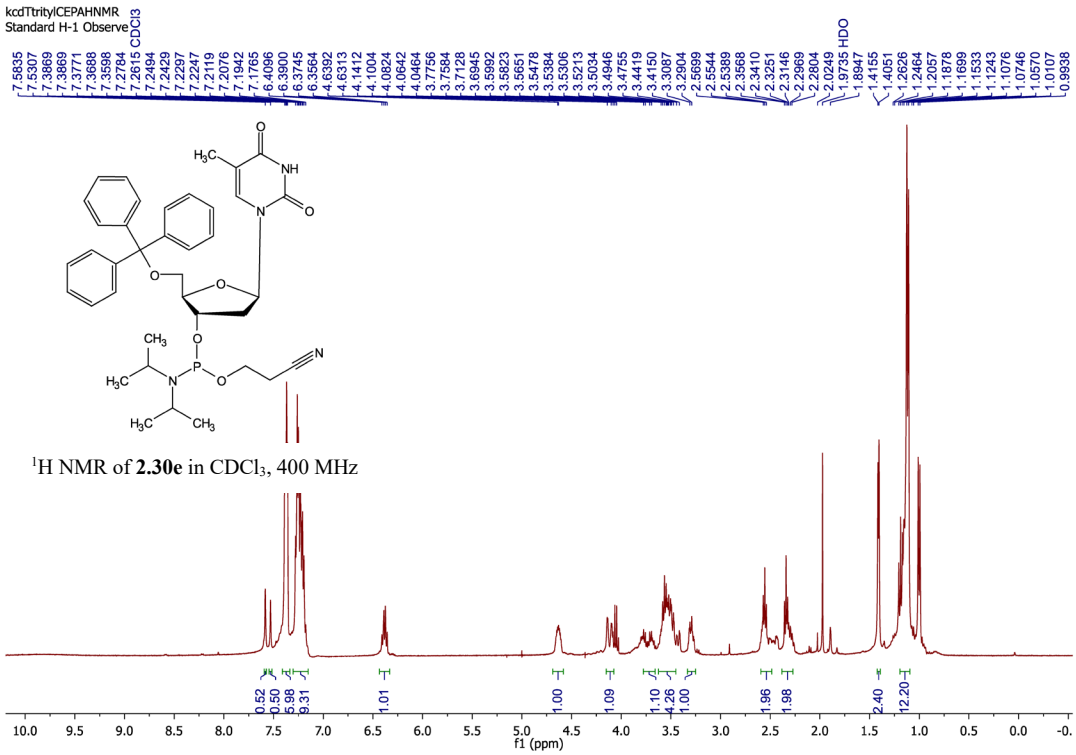
^{13}C NMR of **2.30c** in $CDCl_3$, 100 MHz

Figure 5.191: ^{13}C NMR spectrum of compound **2.30c**.



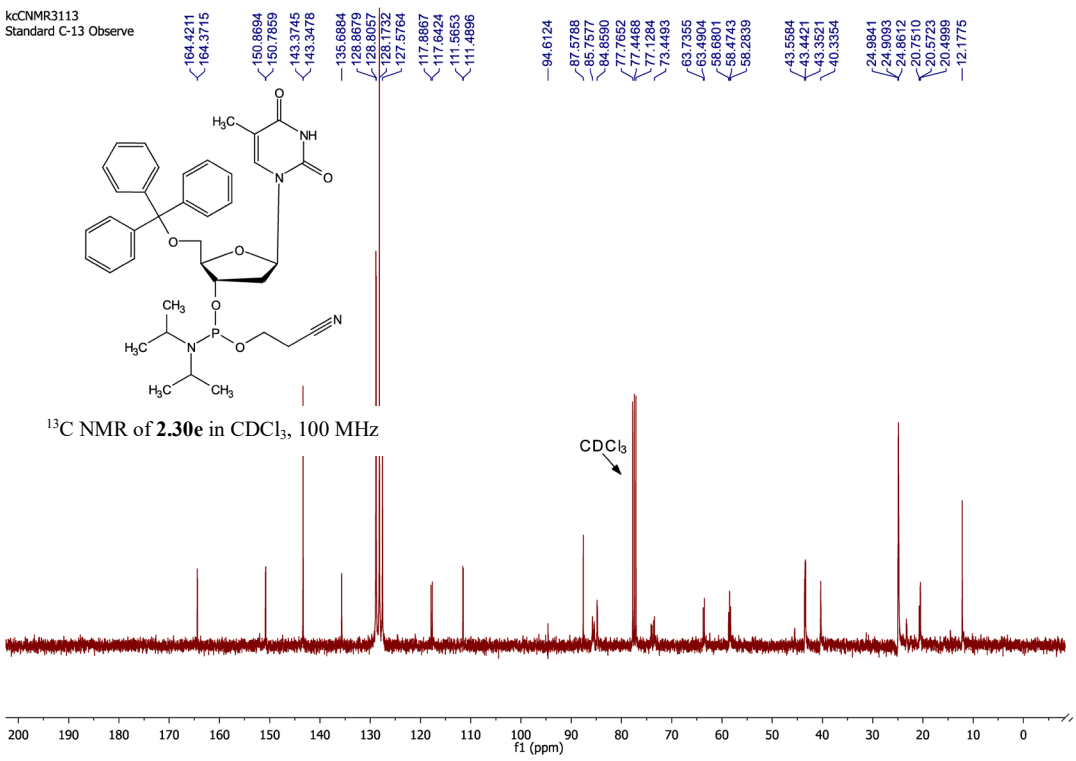
^{31}P NMR of **2.30c** in $CDCl_3$, 162 MHz

Figure 5.192: ^{31}P NMR spectrum of compound **2.30c**.



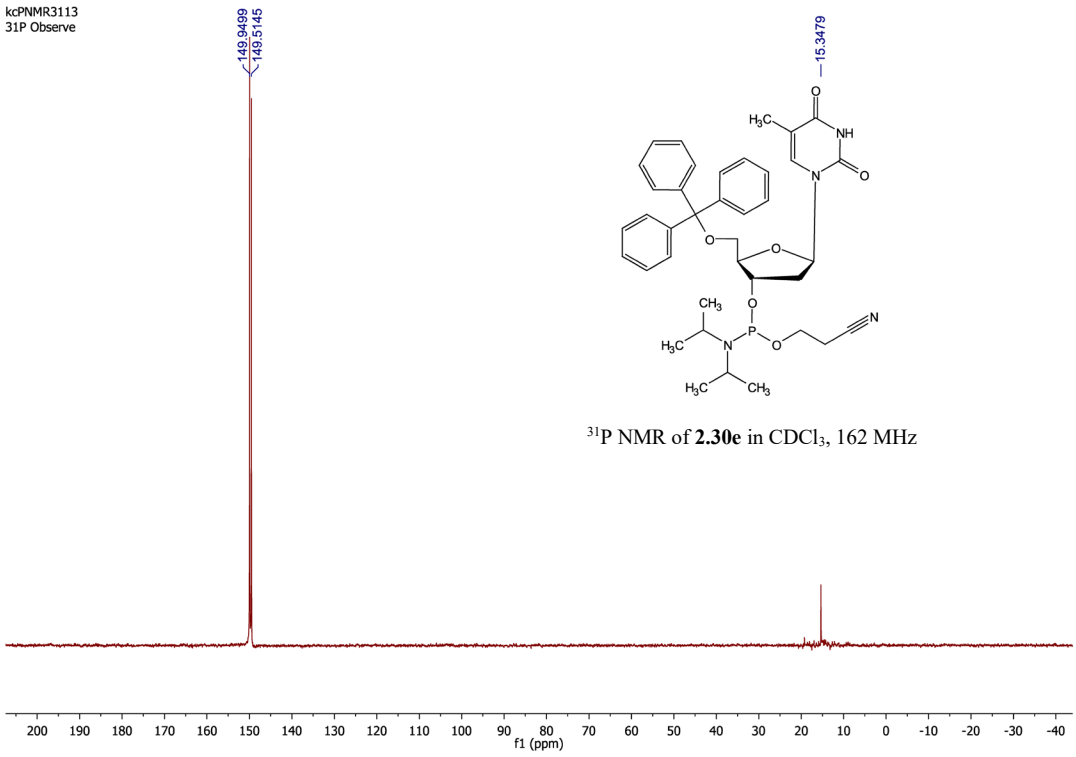
^{1}H NMR of **2.30e** in $CDCl_3$, 400 MHz

Figure 5.193: ^{1}H NMR spectrum of compound **2.30e**.



^{13}C NMR of **2.30e** in $CDCl_3$, 100 MHz

Figure 5.194: ^{13}C NMR spectrum of compound **2.30e**.



^{31}P NMR of **2.30e** in $CDCl_3$, 162 MHz

Figure 5.195: ^{31}P NMR spectrum of compound **2.30e**.

6 Appendix B. Supporting information for chapter 3

PEGylated Dmoc phosphoramidites for sensitive oligodeoxynucleotide synthesis

6.1 HPLC, MALDI MS and OD_{260}, Capillary electrophoresis, Trityl assay log

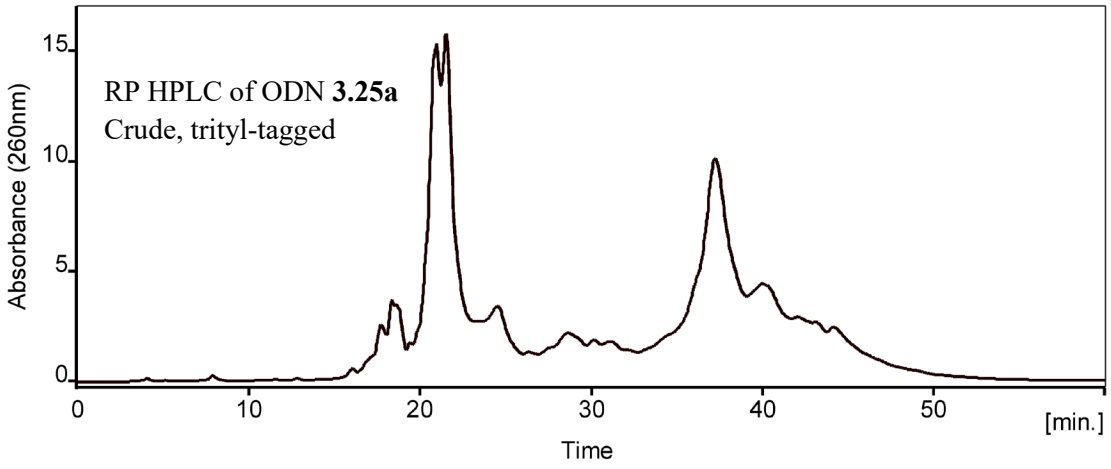


Figure 6.1: RP HPLC of crude trityl-tagged ODN **3.25a**.

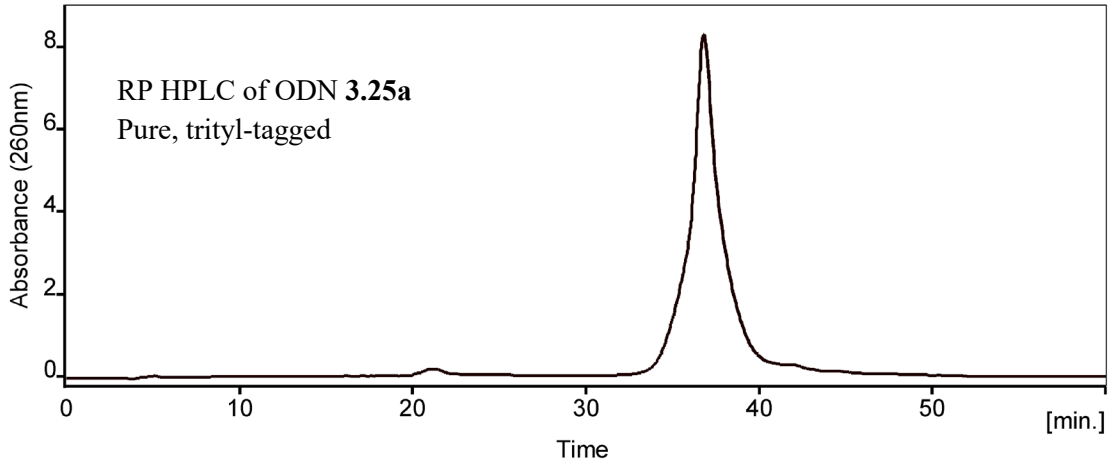


Figure 6.2: RP HPLC of pure trityl-tagged ODN **3.25a**.

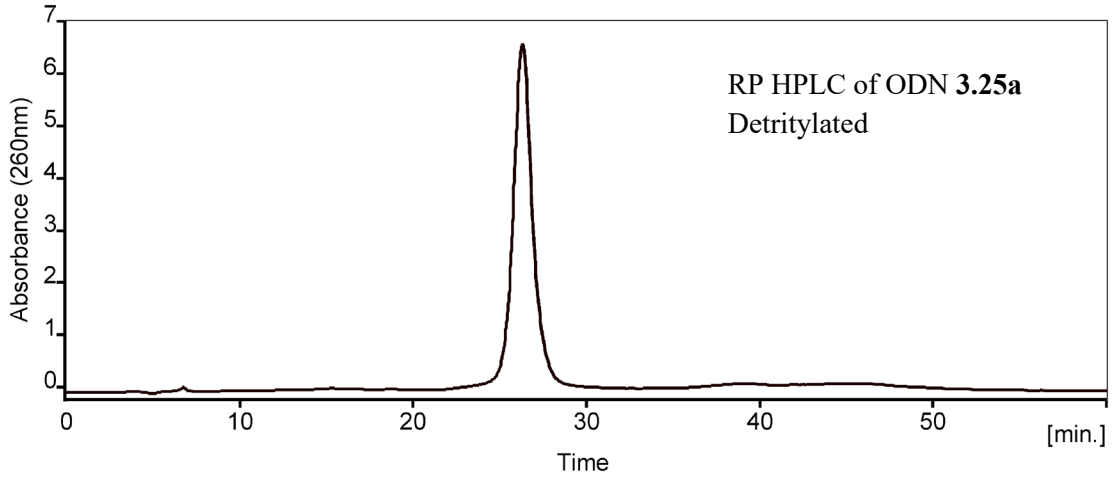


Figure 6.3: RP HPLC of detritylated ODN **3.25a**.

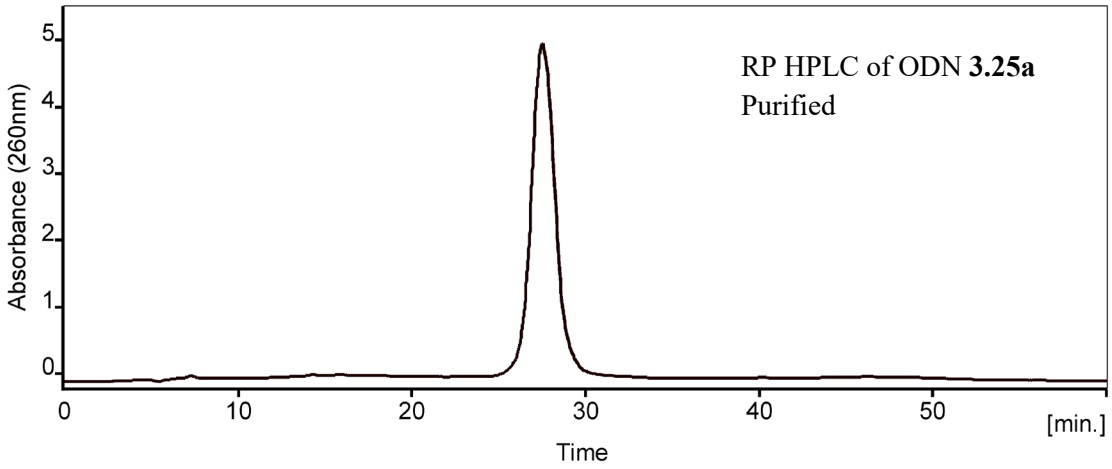


Figure 6.4: RP HPLC of pure ODN **3.25a**.

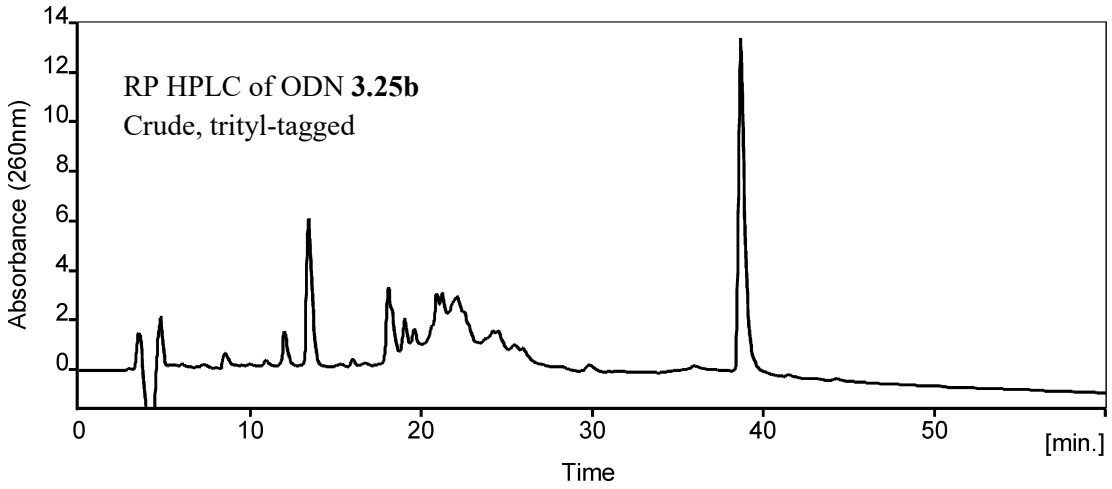


Figure 6.5: RP HPLC of crude trityl-tagged ODN **3.25b**.

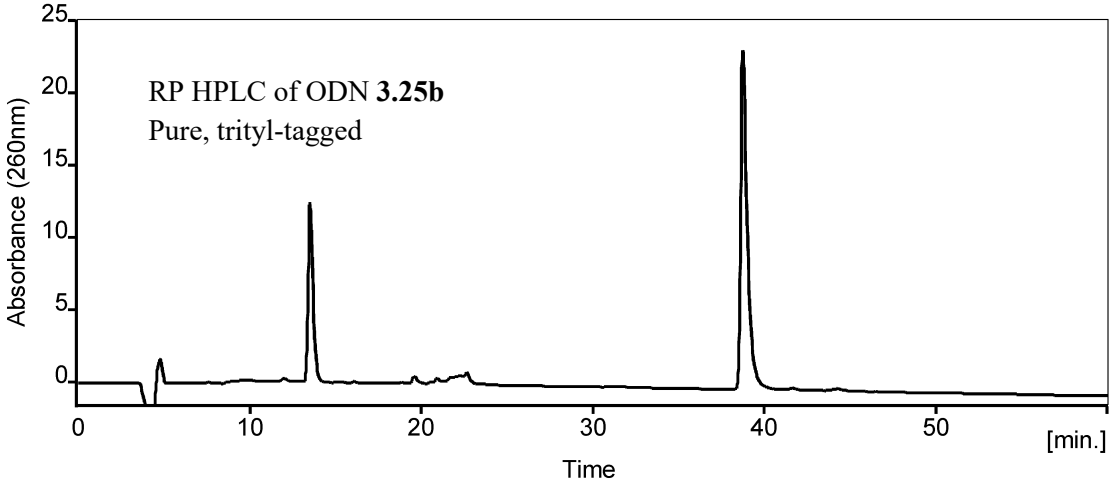


Figure 6.6: RP HPLC of pure trityl-tagged ODN **3.25b**.

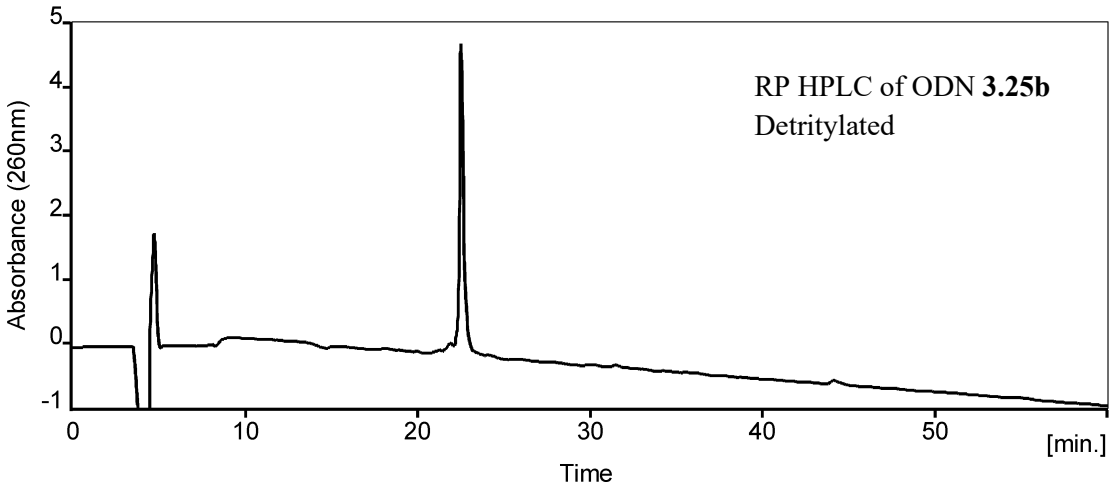


Figure 6.7: RP HPLC of detritylated ODN **3.25b**.

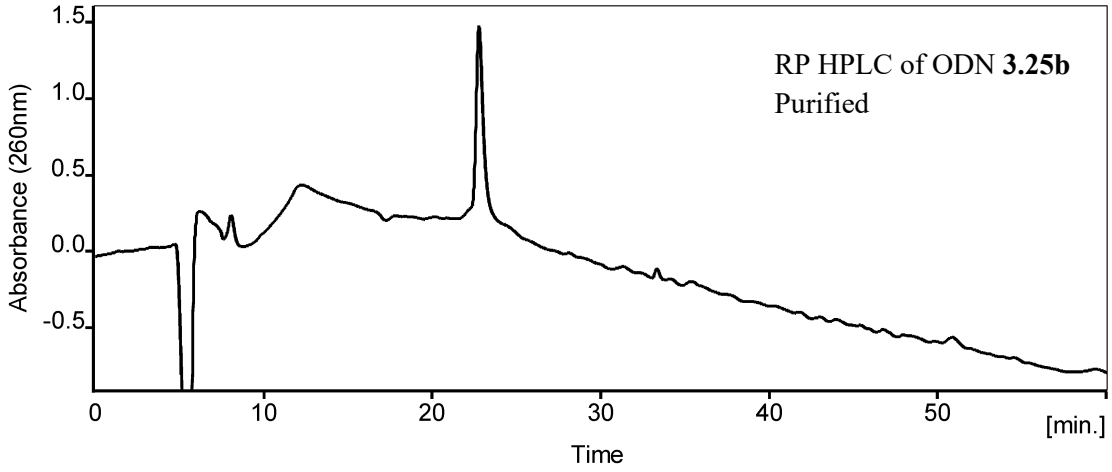


Figure 6.8: RP HPLC of pure ODN **3.25b**.

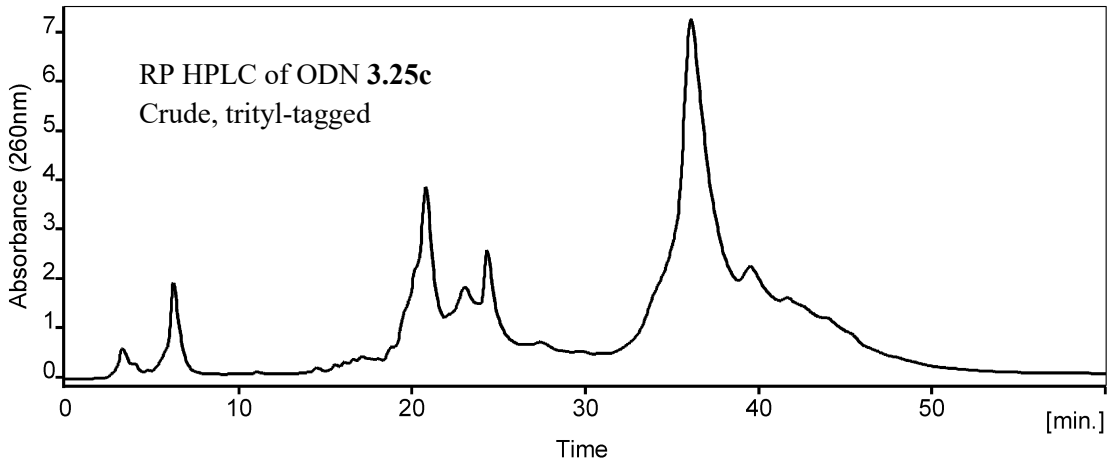


Figure 6.9: RP HPLC of crude trityl-tagged ODN **3.25c**.

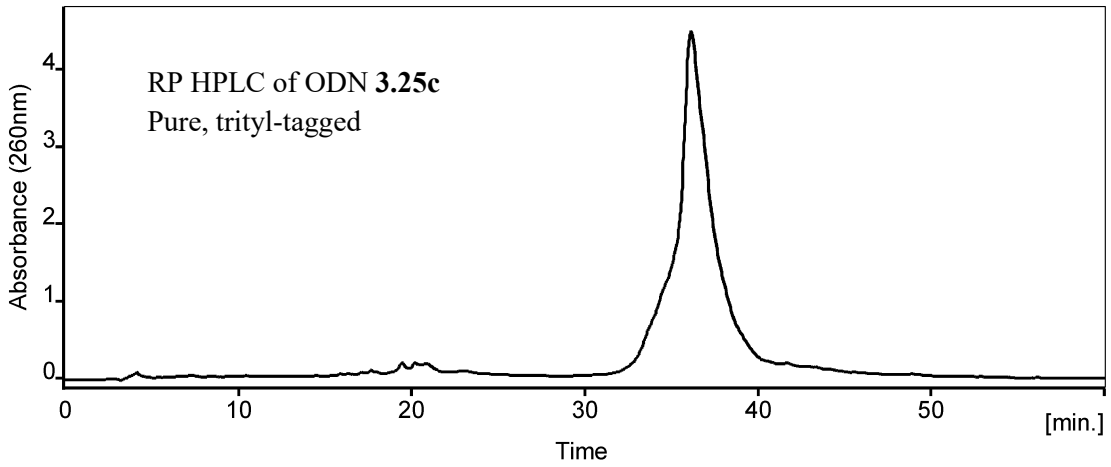


Figure 6.10: RP HPLC of pure trityl-tagged ODN **3.25c**.

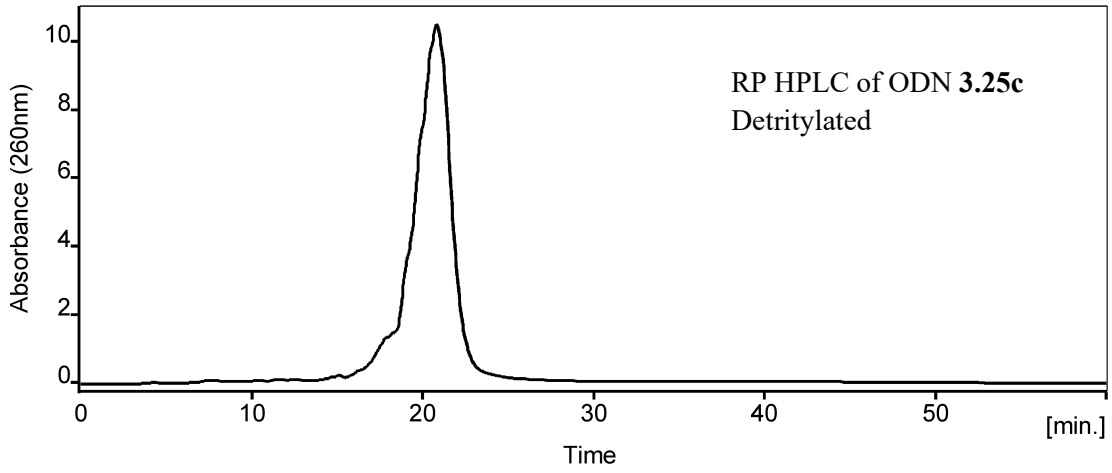


Figure 6.11: RP HPLC of detritylated ODN **3.25c**.

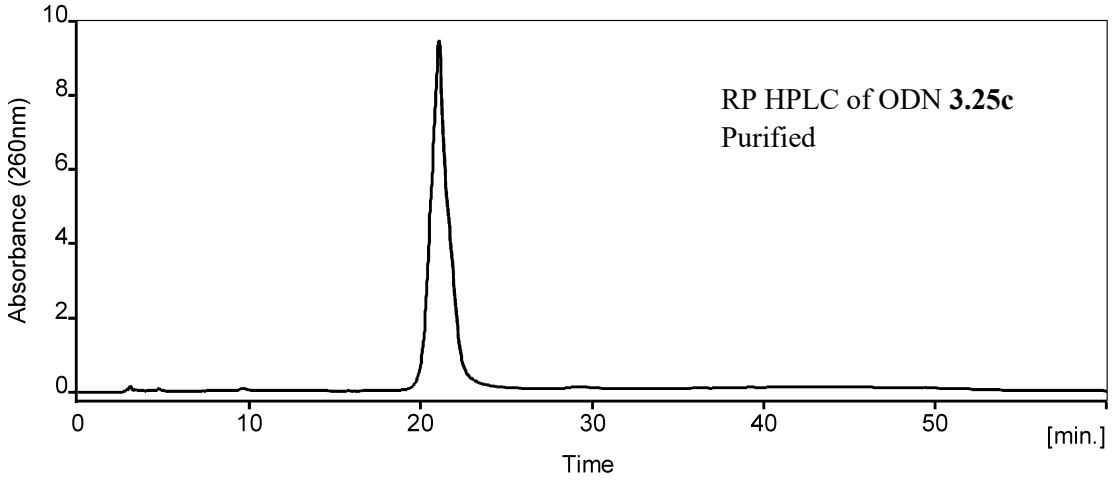


Figure 6.12: RP HPLC of pure ODN **3.25c**.

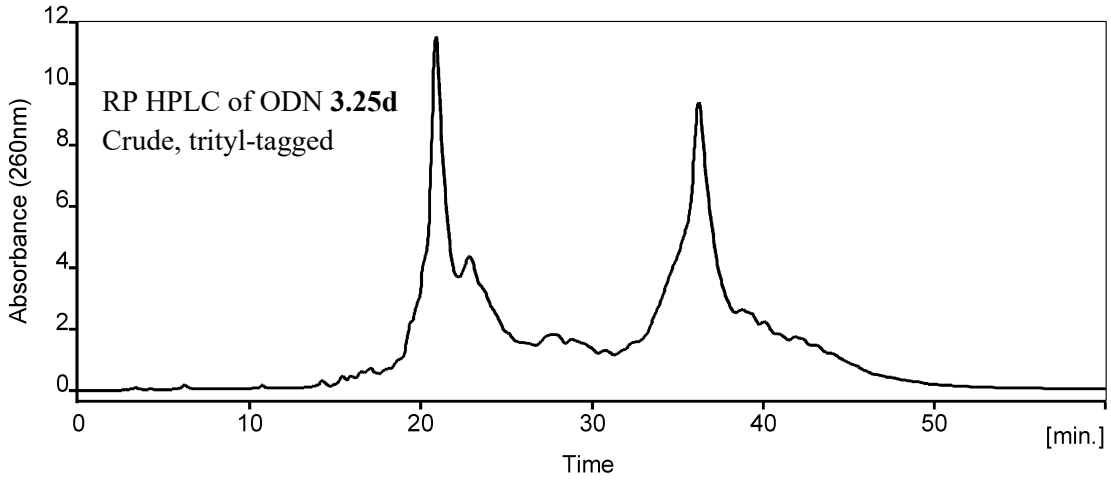


Figure 6.13: RP HPLC of crude trityl-tagged ODN **3.25d**.

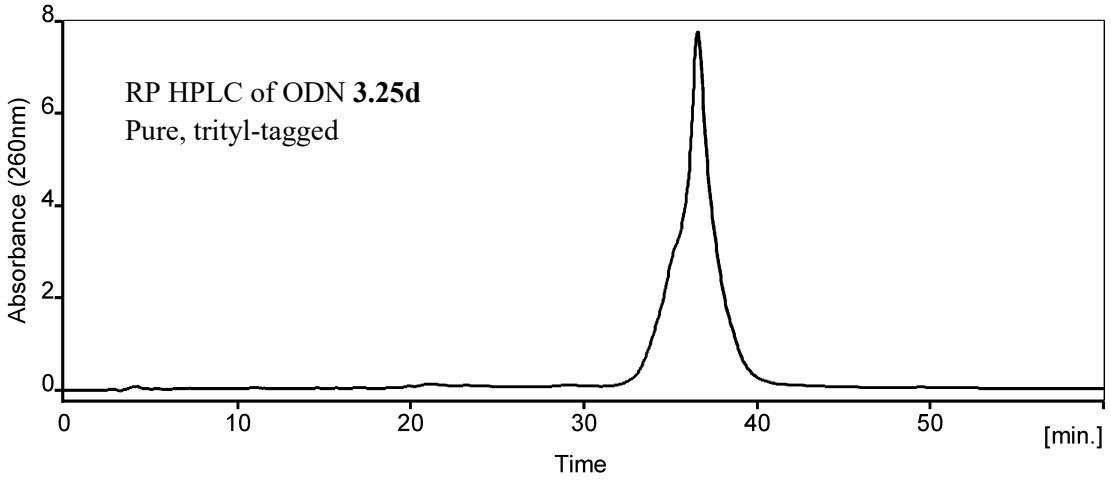


Figure 6.14: RP HPLC of pure trityl-tagged ODN **3.25d**.

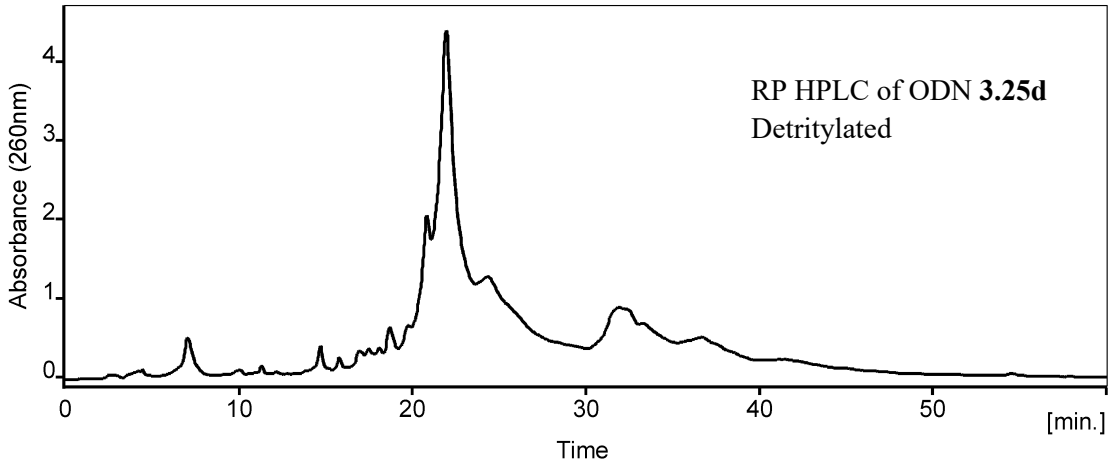


Figure 6.15: RP HPLC of detritylated ODN **3.25d**.

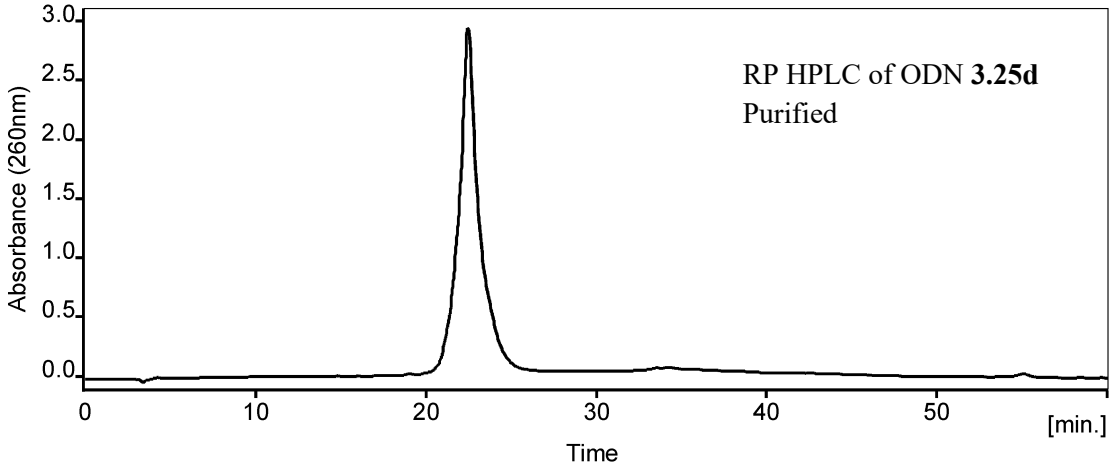


Figure 6.16: RP HPLC of pure ODN **3.25d**.

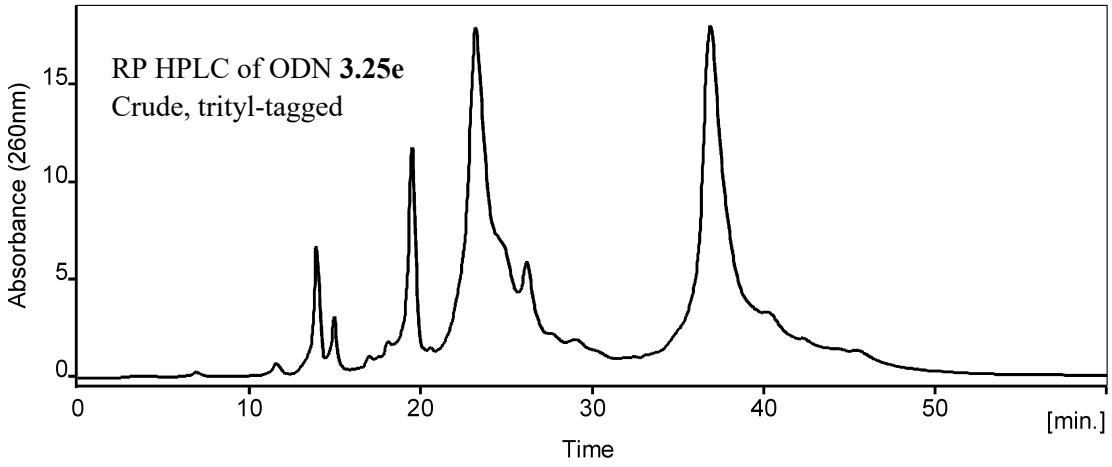


Figure 6.17: RP HPLC of crude trityl-tagged ODN **3.25e**.

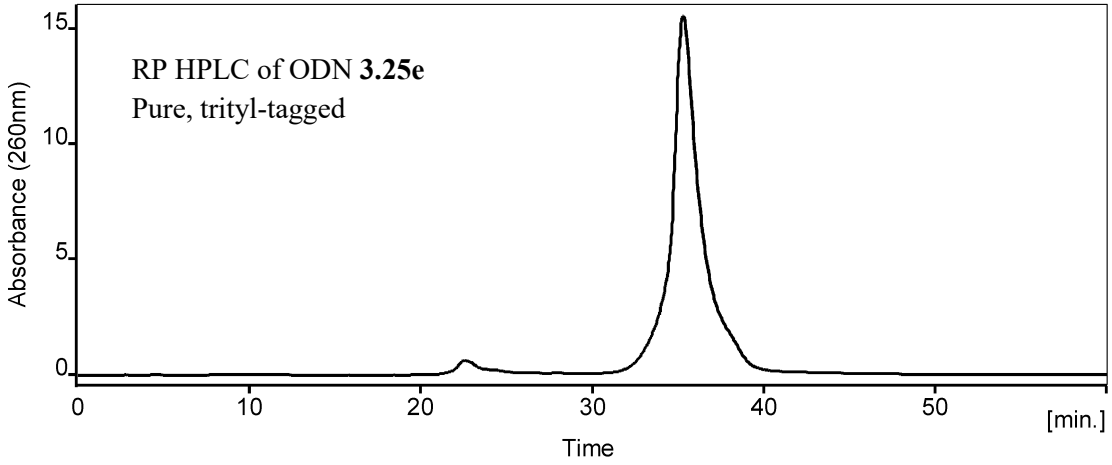


Figure 6.18: RP HPLC of pure trityl-tagged ODN **3.25e**.

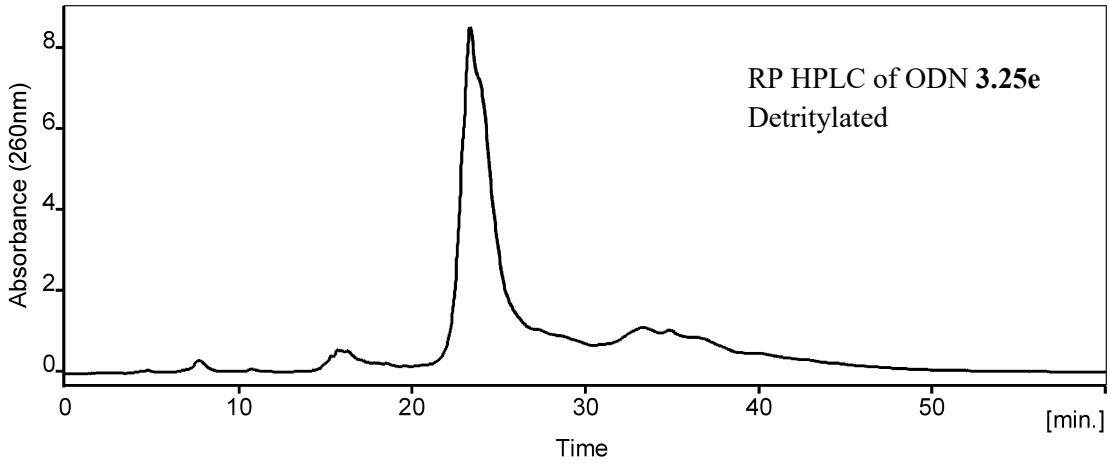


Figure 6.19: RP HPLC of detritylated ODN **3.25e**.

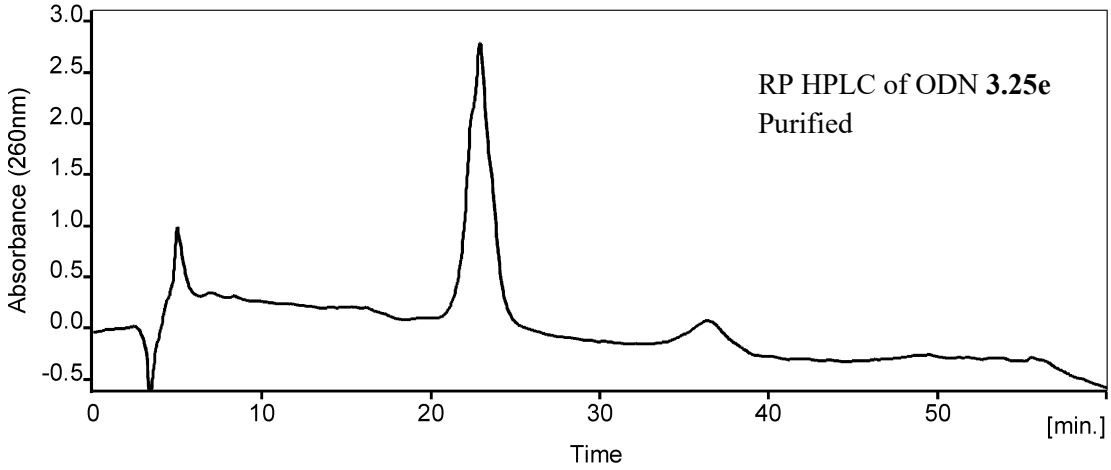


Figure 6.20: RP HPLC of pure ODN **3.25e**.

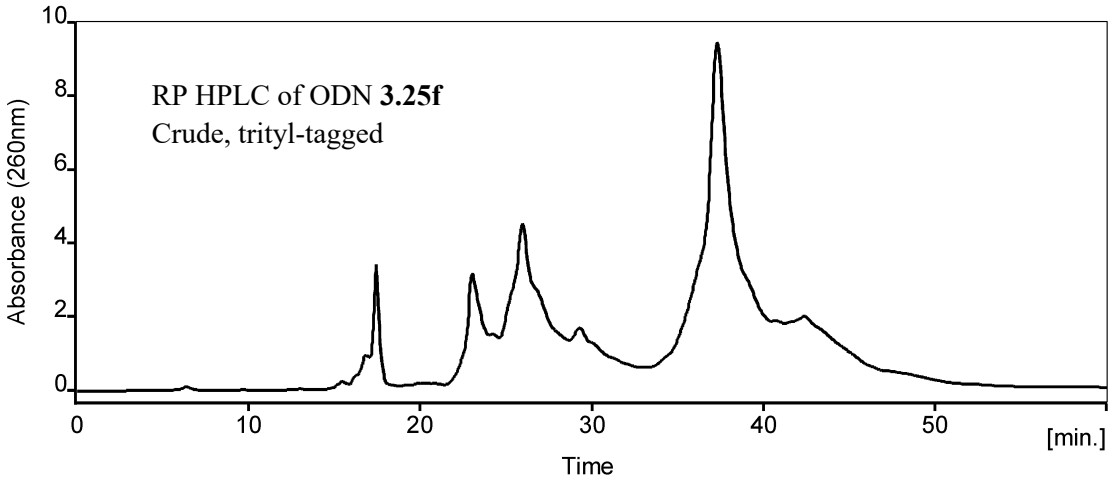


Figure 6.21: RP HPLC of crude trityl-tagged ODN **3.25f**.

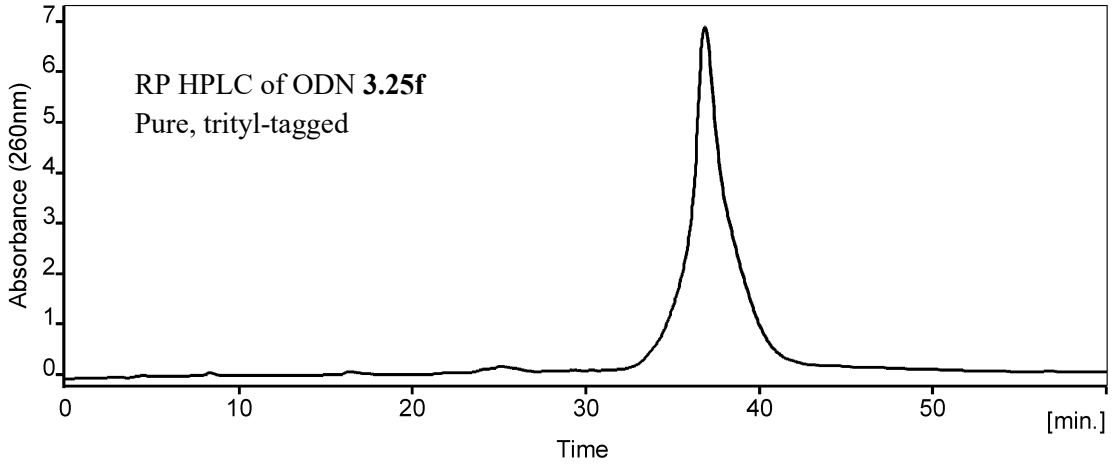


Figure 6.22: RP HPLC of pure trityl-tagged ODN **3.25f**.

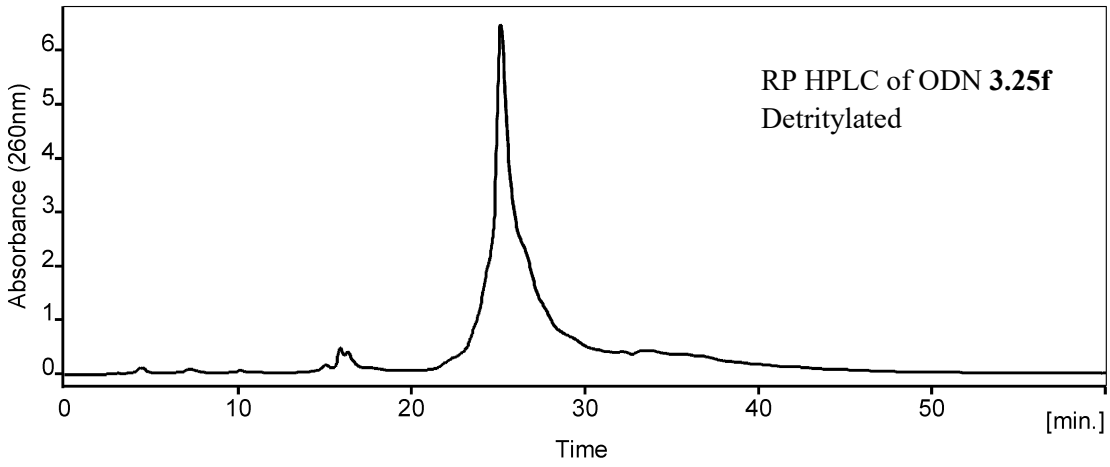


Figure 6.23: RP HPLC of detritylated ODN **3.25f**.

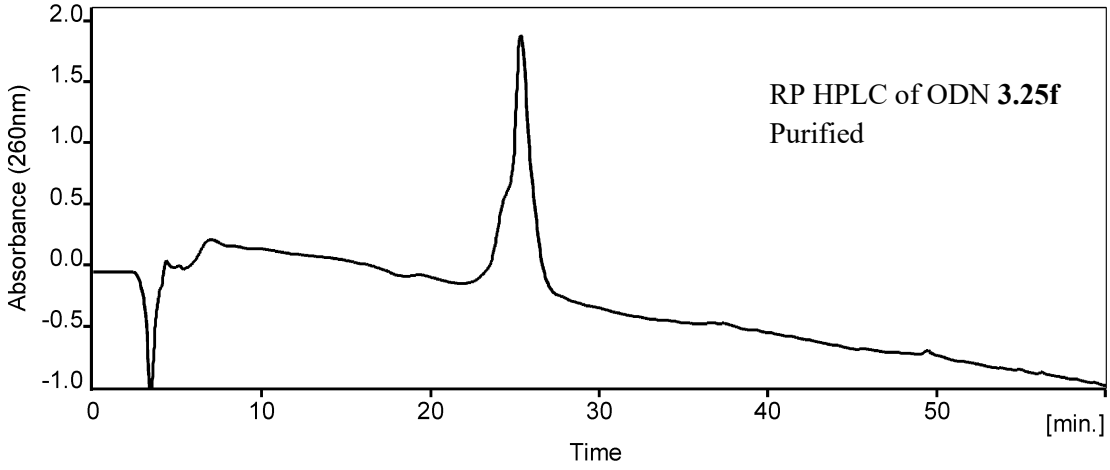


Figure 6.24: RP HPLC of pure ODN **3.25f**.

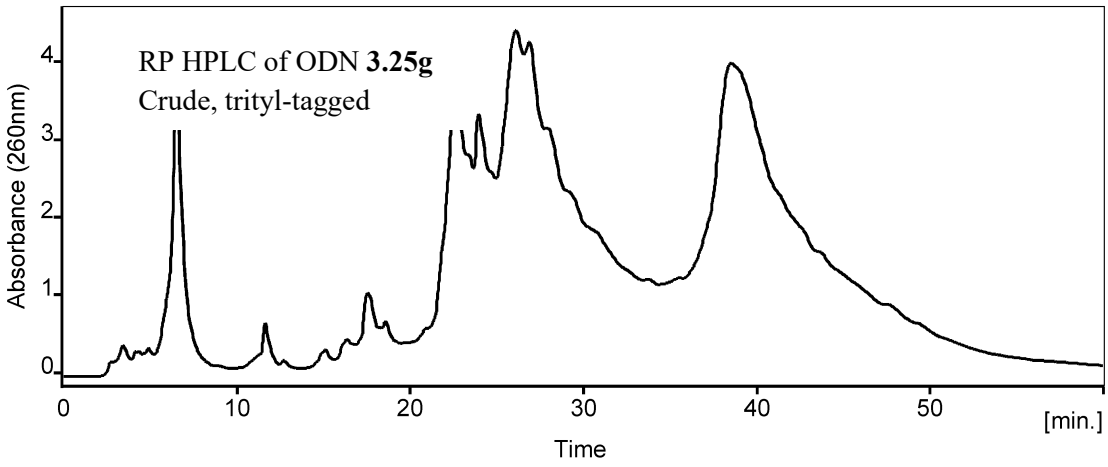


Figure 6.25: RP HPLC of crude trityl-tagged ODN **3.25g**.

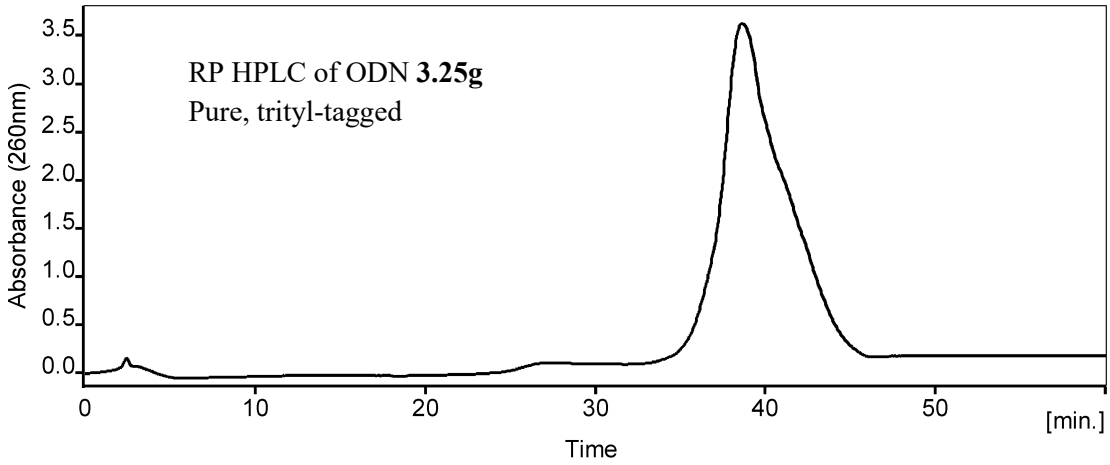


Figure 6.26: RP HPLC of pure trityl-tagged ODN **3.25g**.

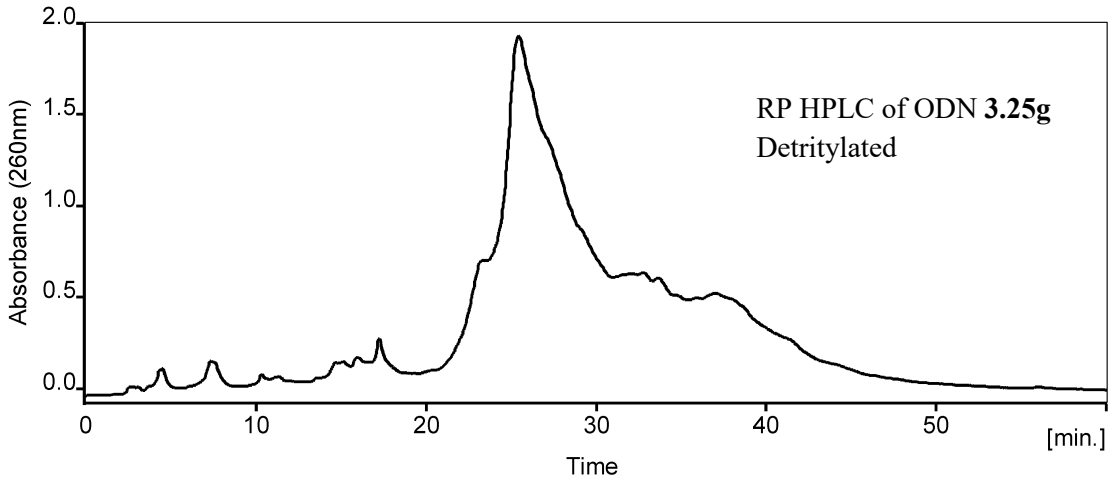


Figure 6.27: RP HPLC of detritylated ODN **3.25g**.

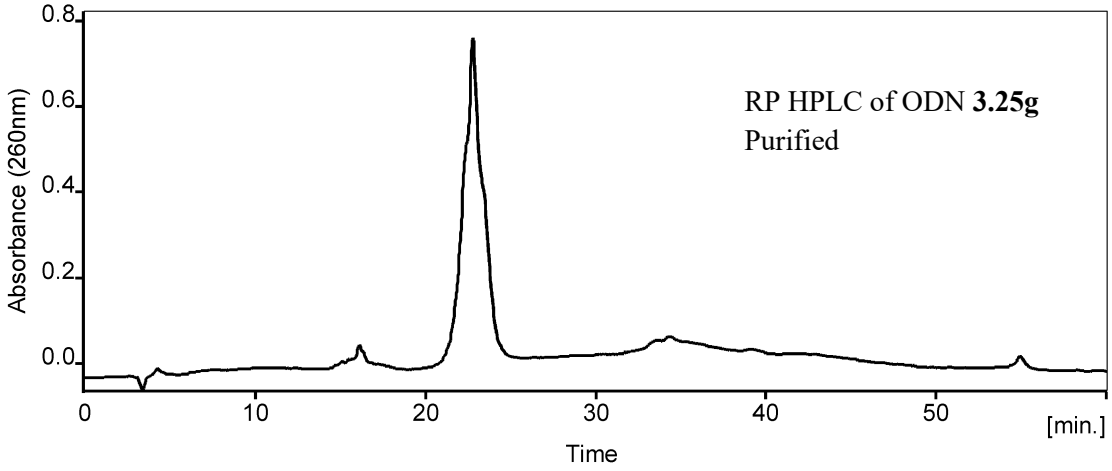


Figure 6.28: RP HPLC of pure ODN **3.25g**.

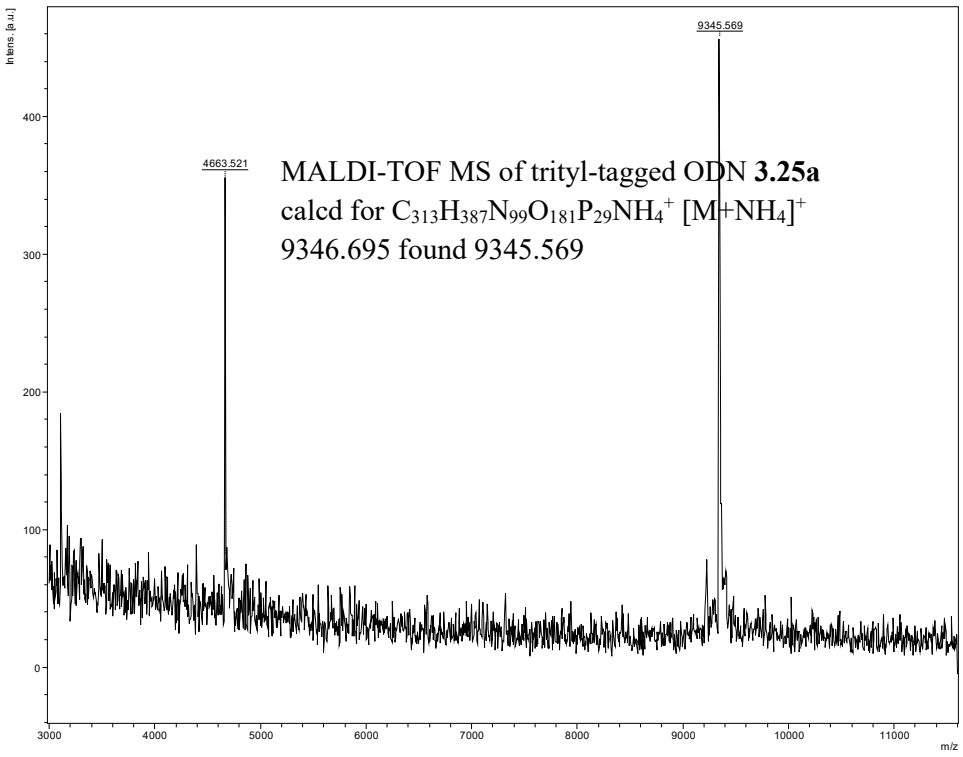


Figure 6.29: MALDI-TOF MS of trityl-tagged ODN **3.25a**.

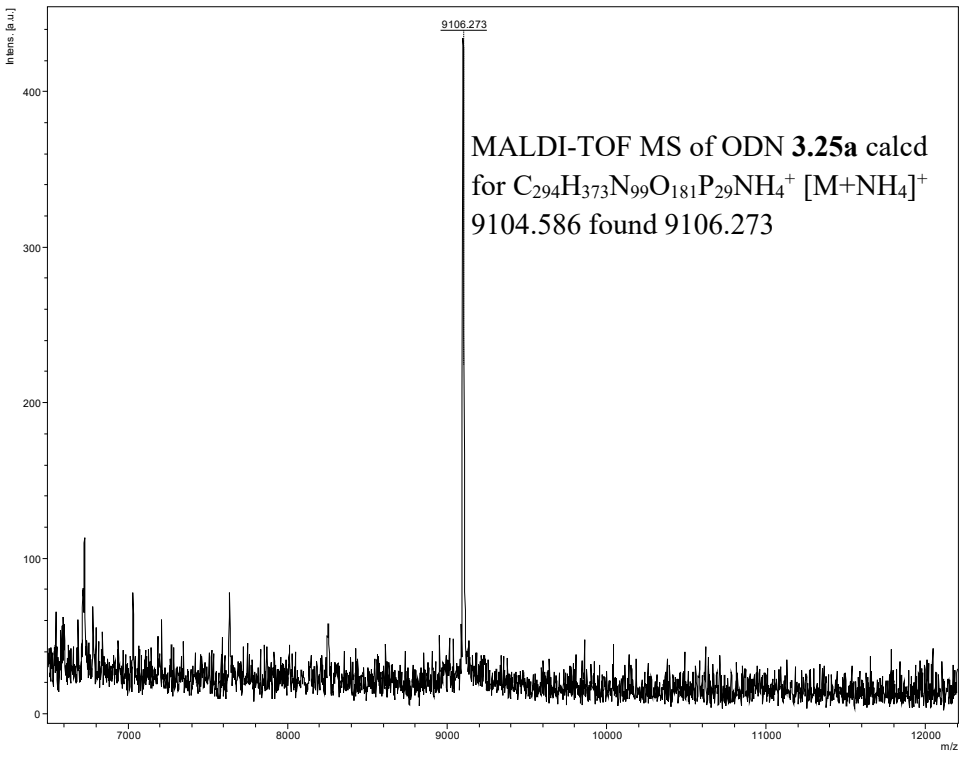


Figure 6.30: MALDI-TOF MS of ODN **3.25a**.

OD_{260} of the ODN **3.25a** (30-mer) obtained from the 0.52 µmol synthesis is 2.17.

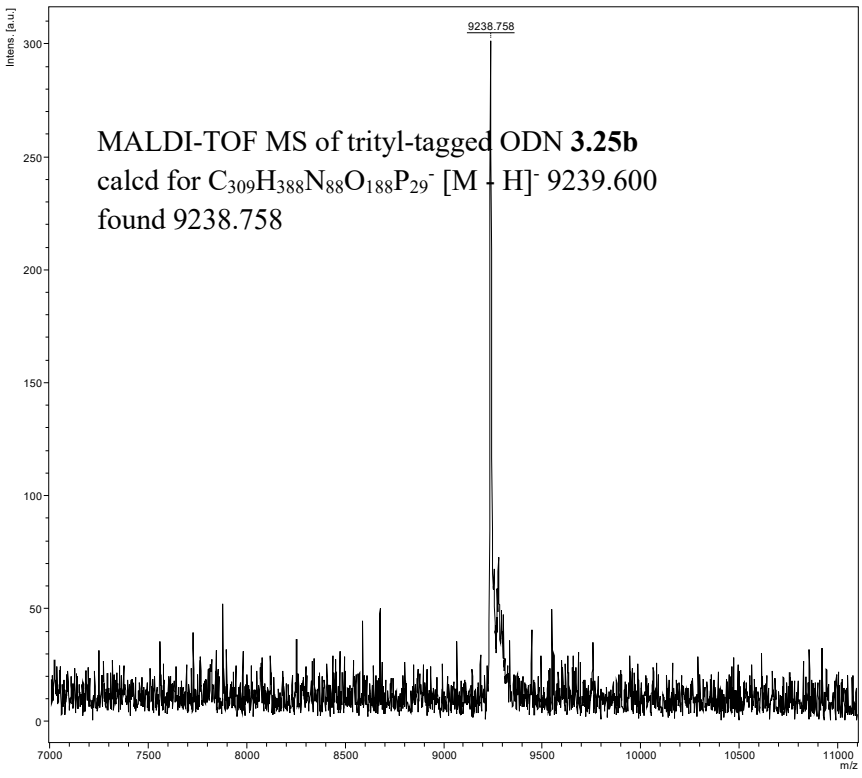


Figure 6.31: MALDI-TOF MS of trityl-tagged ODN **3.25b**.

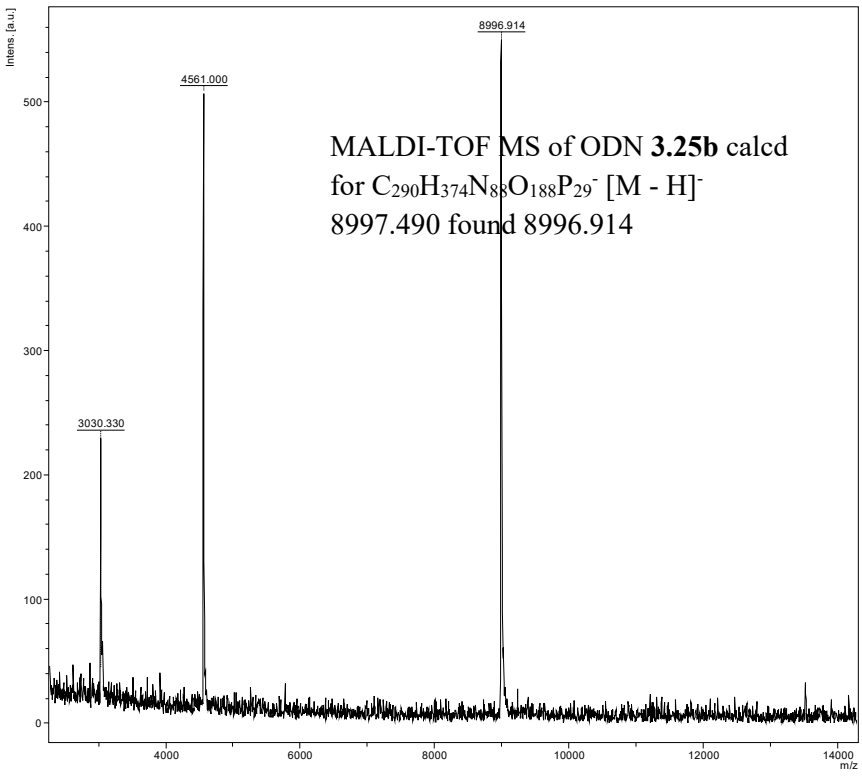


Figure 6.32: MALDI-TOF MS of ODN **3.25b**.

OD_{260} of the ODN **3.25b** (30-mer) obtained from the 0.52 μmol synthesis is 0.196.

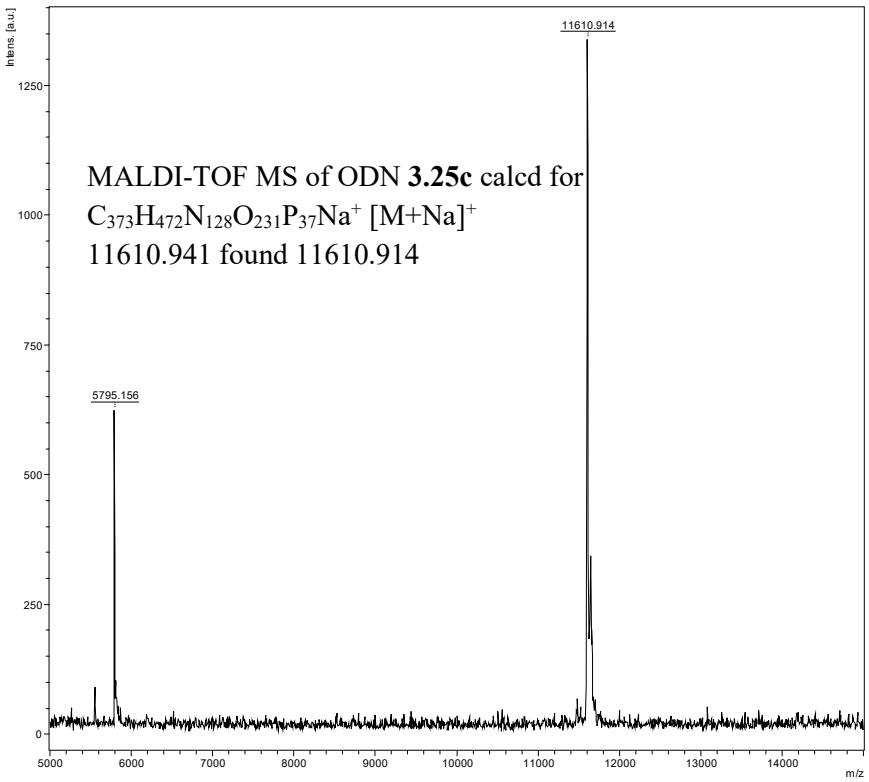


Figure 6.33: MALDI-TOF MS of ODN **3.25c**.

OD_{260} of the ODN **3.25c** (38-mer) obtained from the 0.52 μmol synthesis is 3.15.

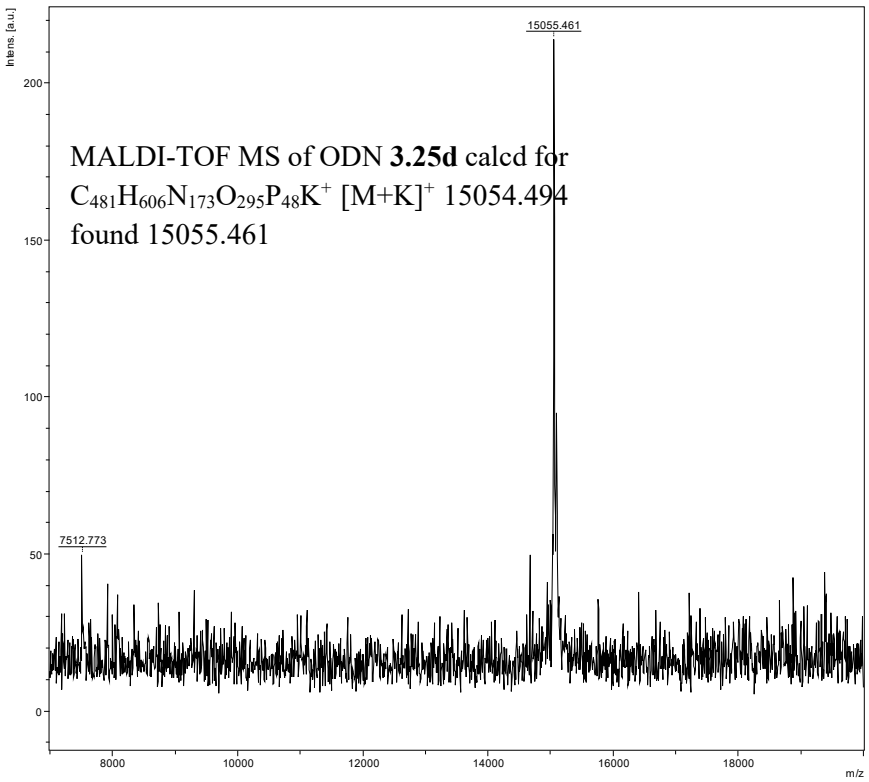


Figure 6.34: MALDI-TOF MS of ODN **3.25d**.

OD_{260} of the ODN **3.25d** (49-mer) obtained from the 0.52 μmol synthesis is 1.10.

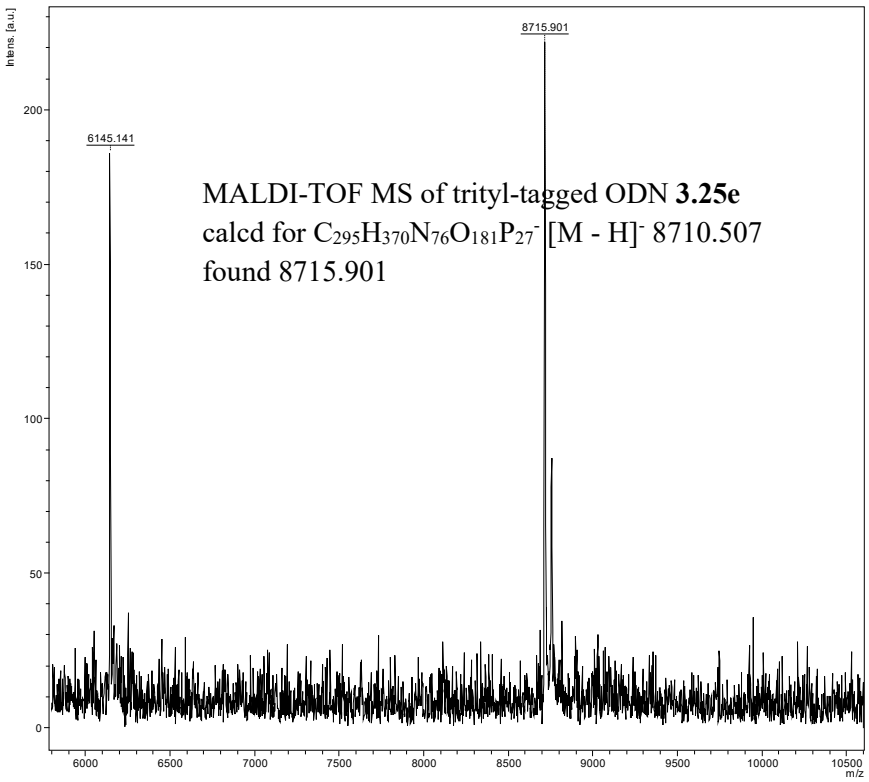


Figure 6.35: MALDI-TOF MS of trityl-tagged ODN **3.25e**.

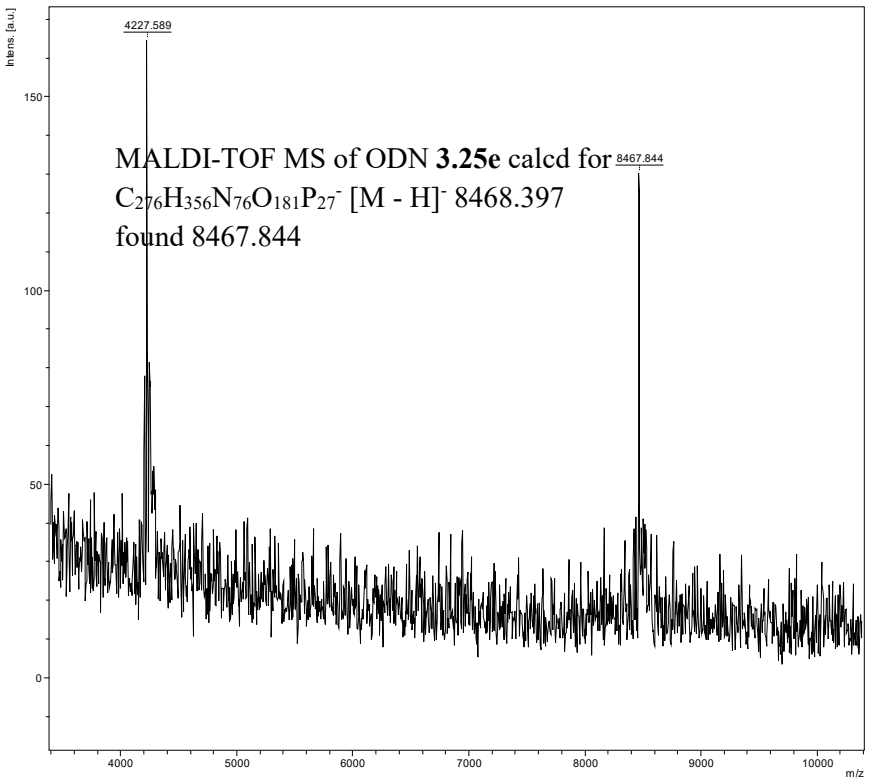


Figure 6.36: MALDI-TOF MS of ODN **3.25e**.

OD_{260} of the ODN **3.25e** (28-mer) obtained from the 0.52 μmol synthesis is 1.23.

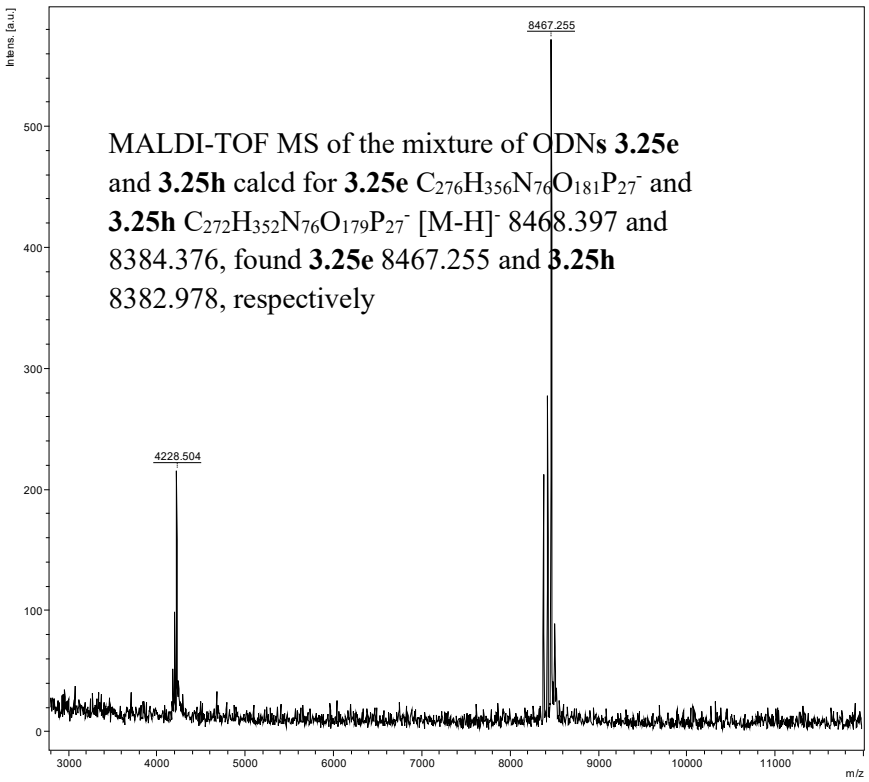


Figure 6.37: MALDI-TOF MS of the mixture of ODNs **3.25e** and **3.25h.**

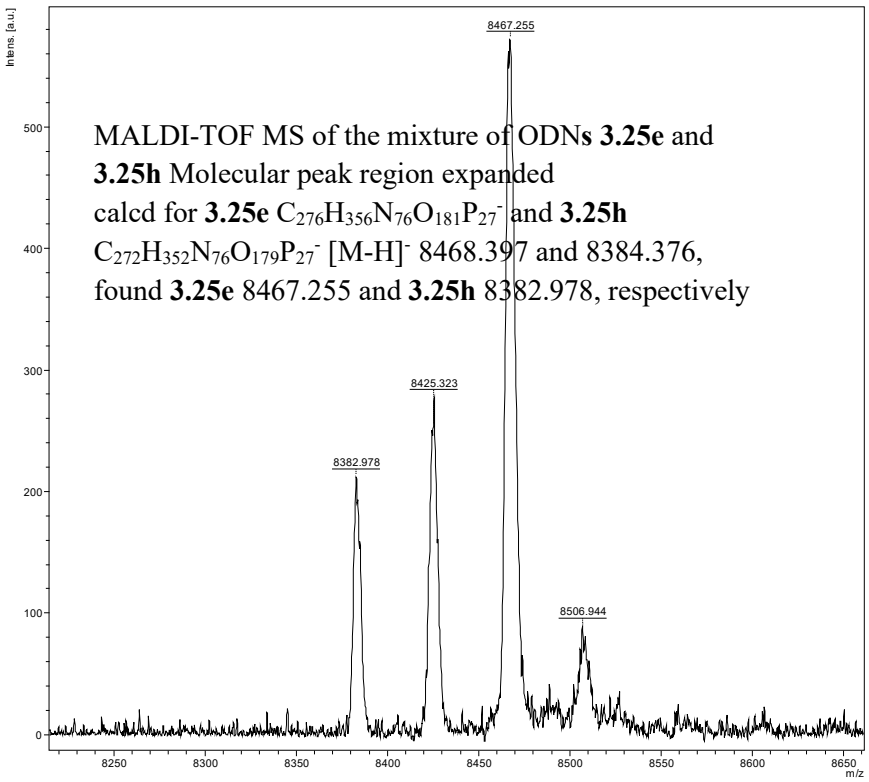


Figure 6.38: Zoomed MALDI-TOF MS of the mixture of ODN**s 3.25e** and **3.25h.**

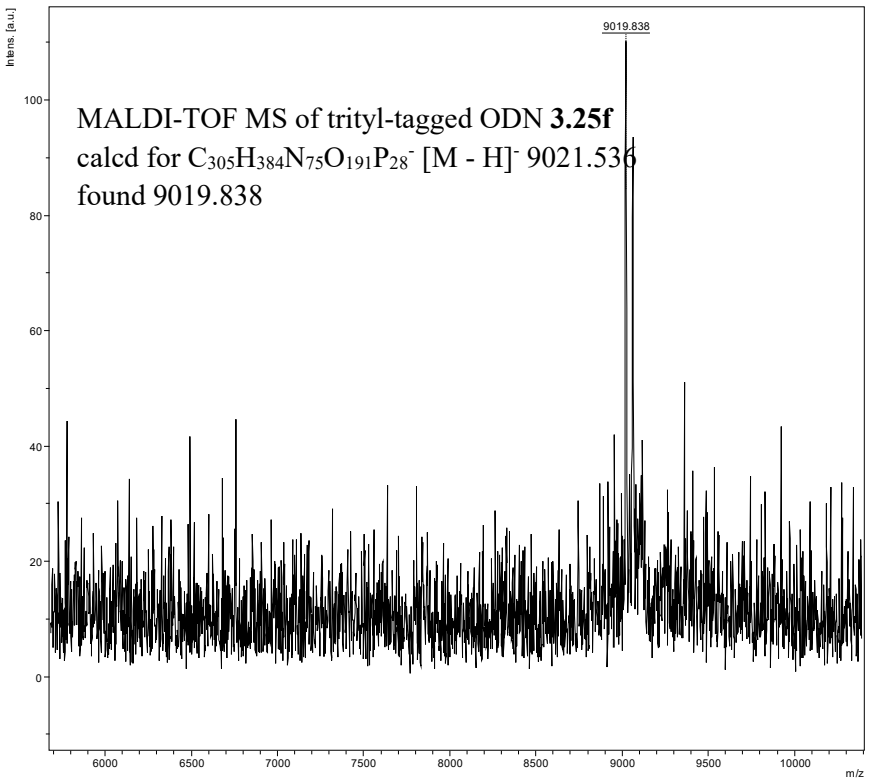


Figure 6.39: MALDI-TOF MS of trityl-tagged ODN **3.25f**.

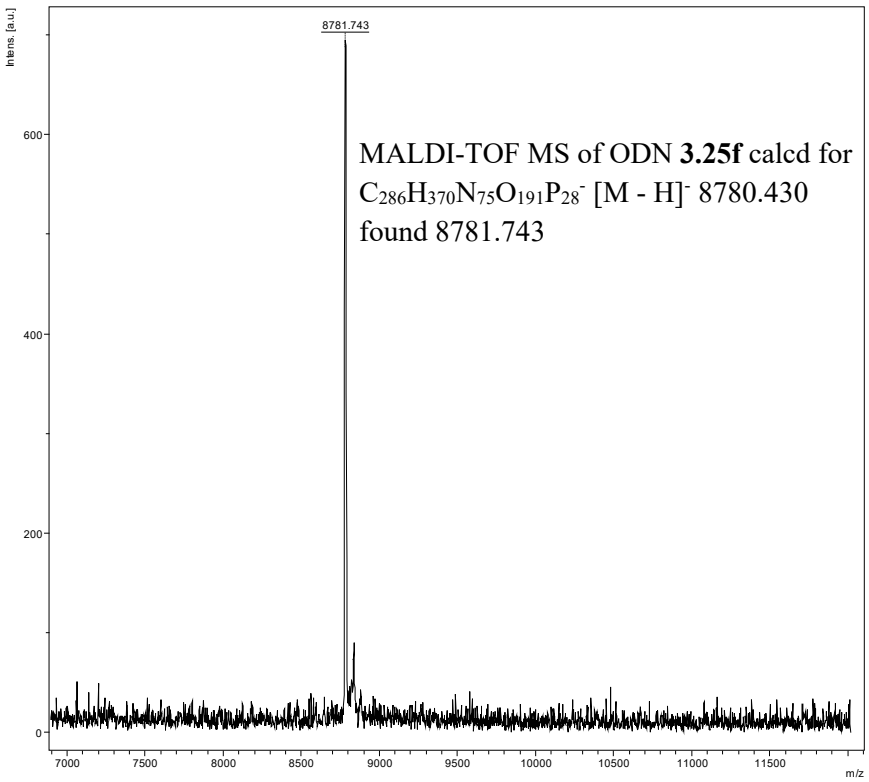


Figure 6.40: MALDI-TOF MS of ODN **3.25f**.

OD_{260} of the ODN **3.25f** (29-mer) obtained from the 0.52 μmol synthesis is 0.91.

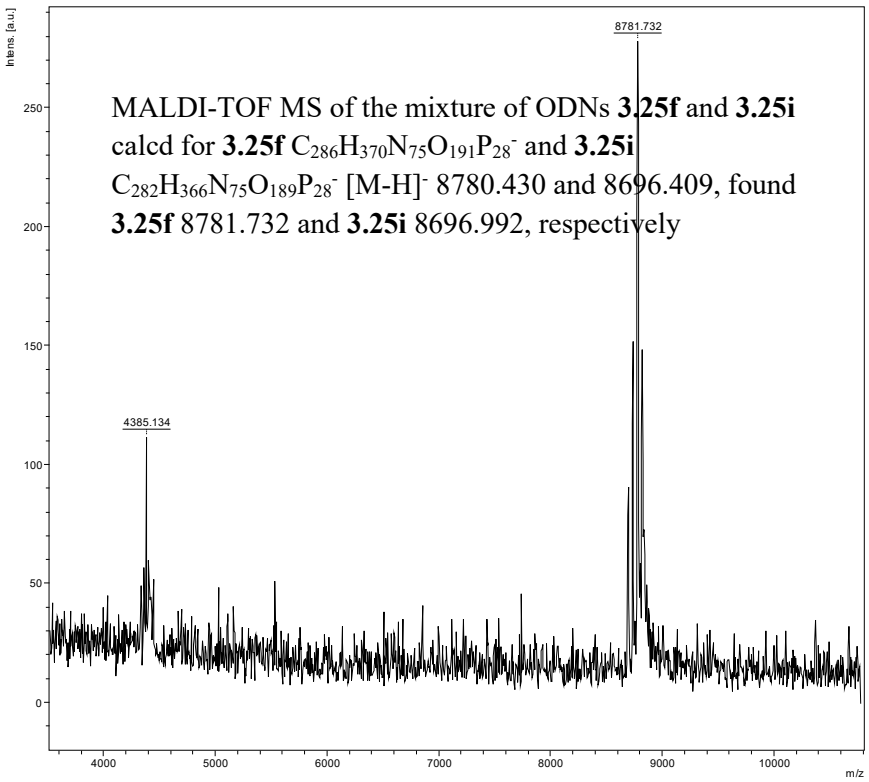


Figure 6.41: MALDI-TOF MS of the mixture of ODNs **3.25f** and **3.25i**.

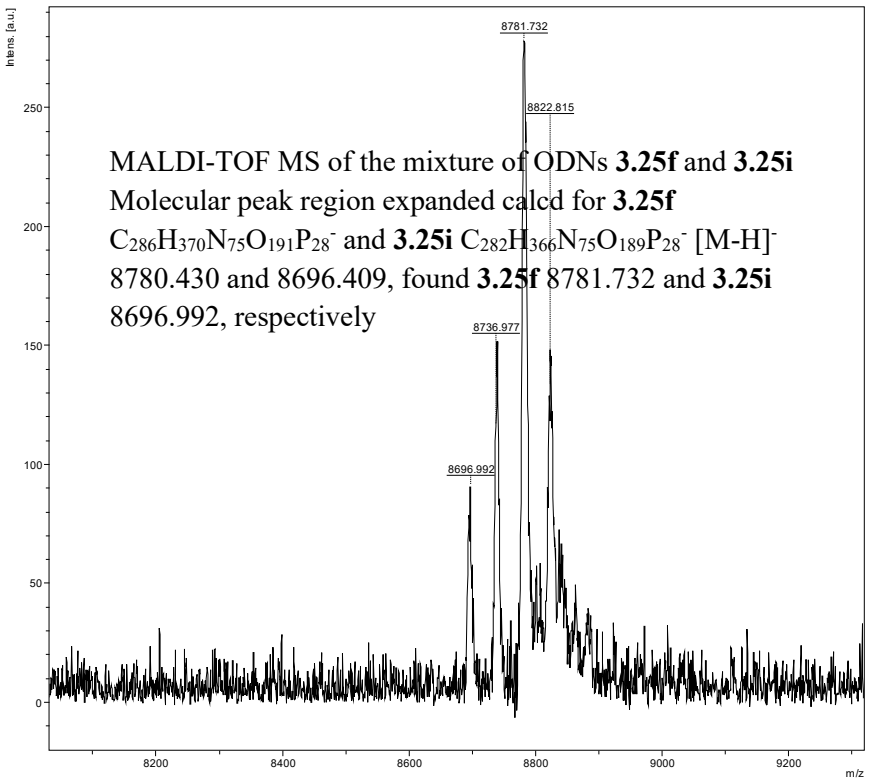


Figure 6.42: Zoomed MALDI-TOF MS of the mixture of ODNs **3.25f** and **3.25i**.

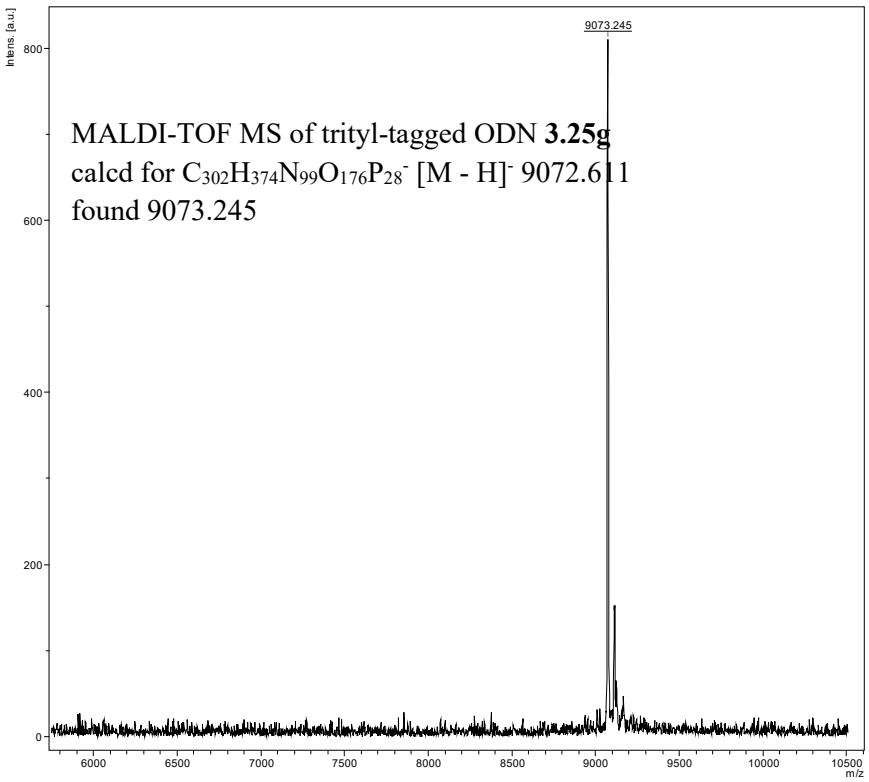


Figure 6.43: MALDI-TOF MS of trityl-tagged ODN **3.25g**.

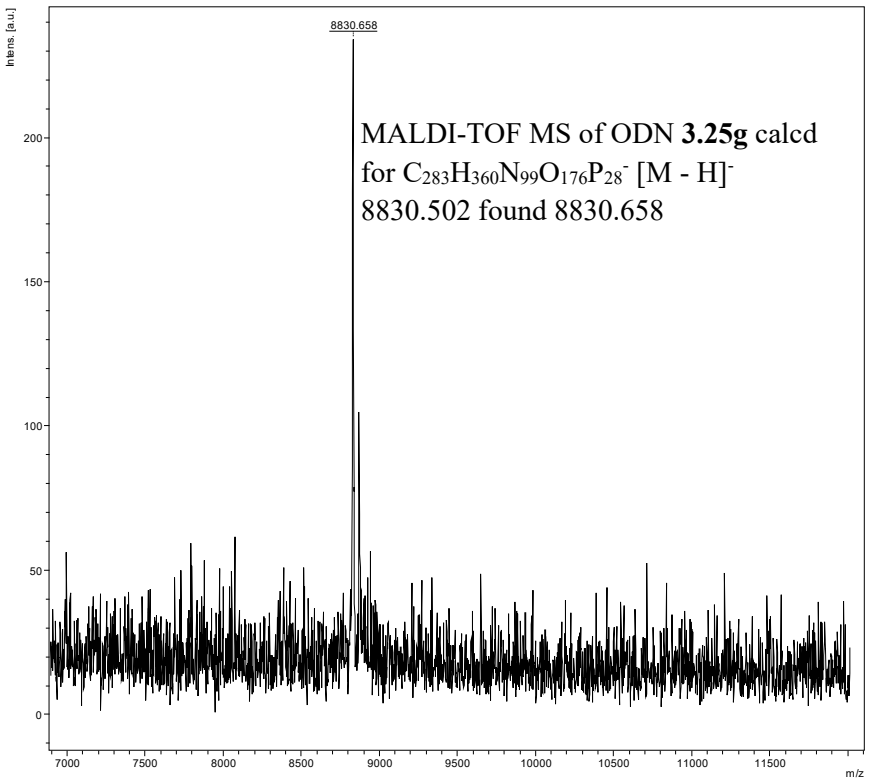


Figure 6.44: MALDI-TOF MS of ODN **3.25g**.

OD_{260} of the ODN **3.25g** (29-mer) obtained from the 0.52 µmol synthesis is 0.24.

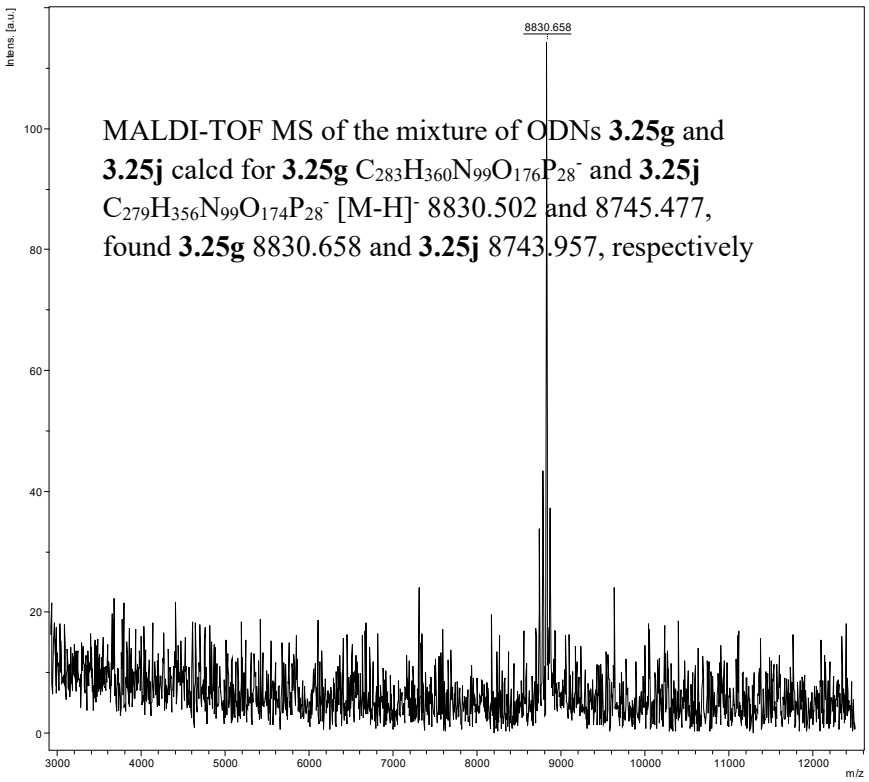


Figure 6.45: MALDI-TOF MS of the mixture of ODNs **3.25g** and **3.25j**.

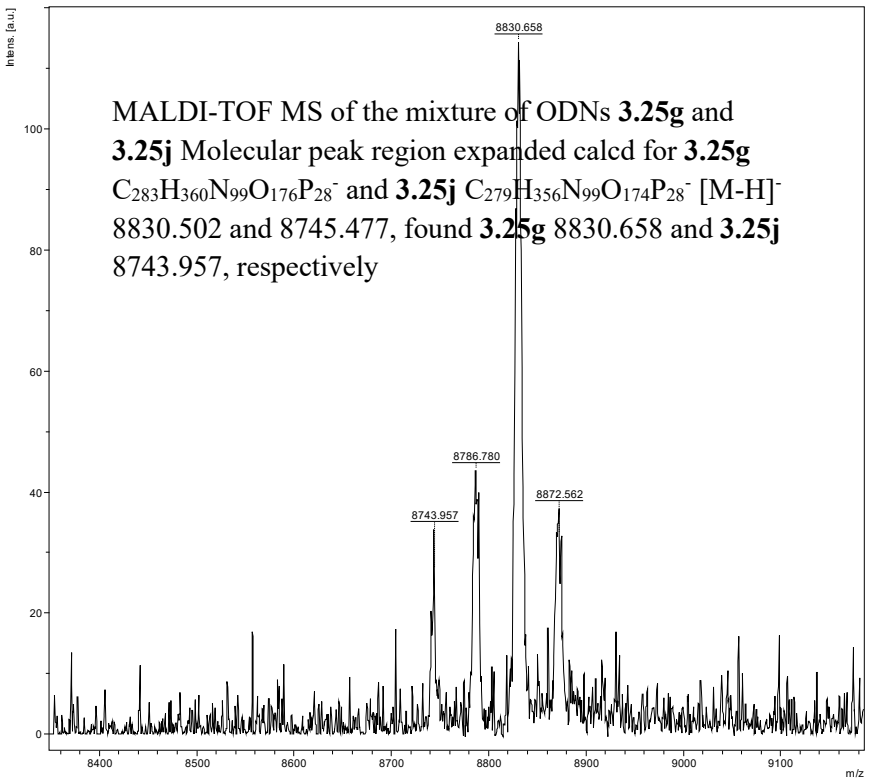


Figure 6.46: Zoomed MALDI-TOF MS of the mixture of ODNs **3.25g** and **3.25j**.

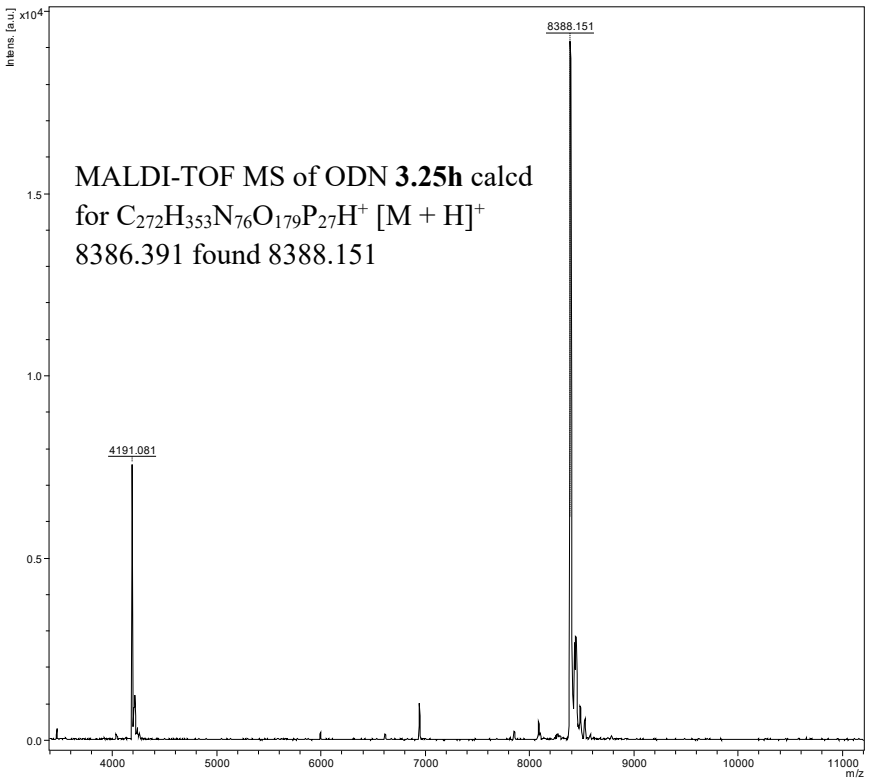


Figure 6.47: MALDI-TOF MS of ODN **3.25h**.

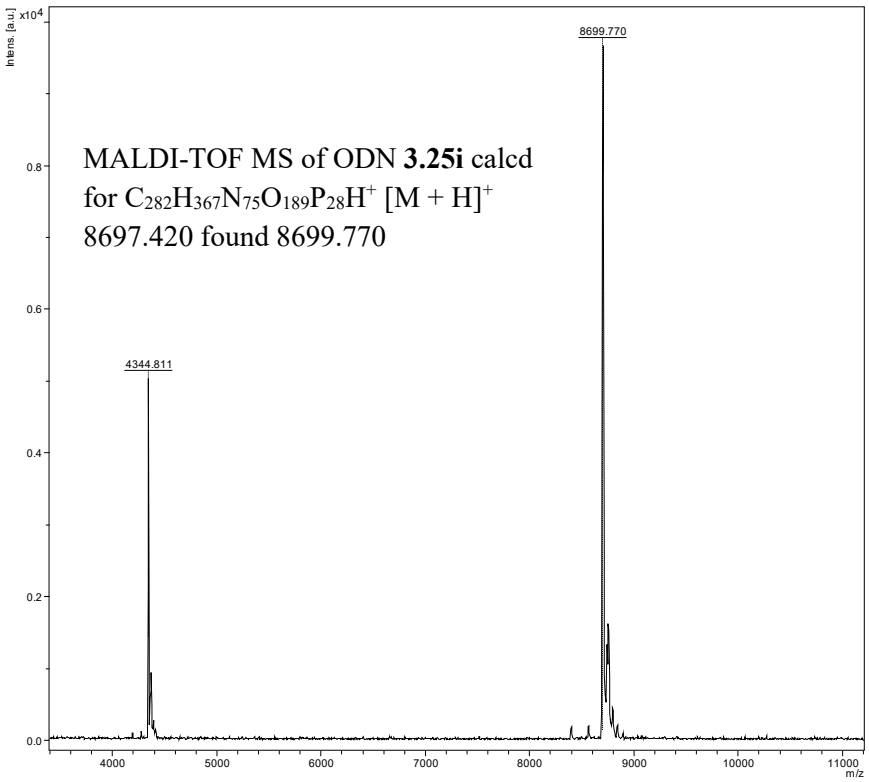


Figure 6.48: MALDI-TOF MS of ODN **3.25i**.

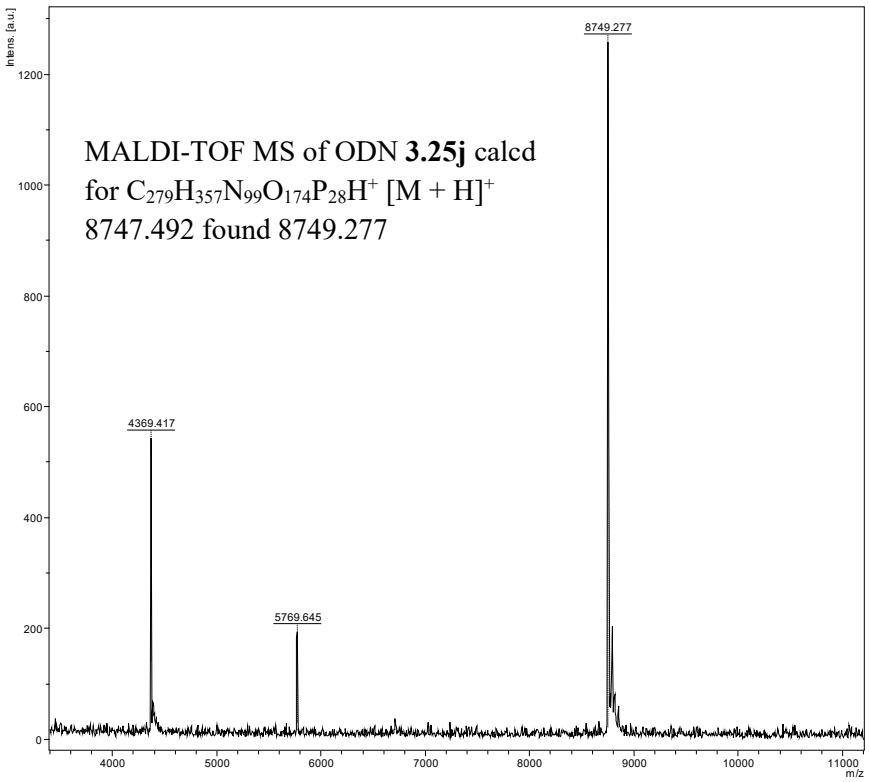


Figure 6.49: MALDI-TOF MS of ODN **3.25j**.

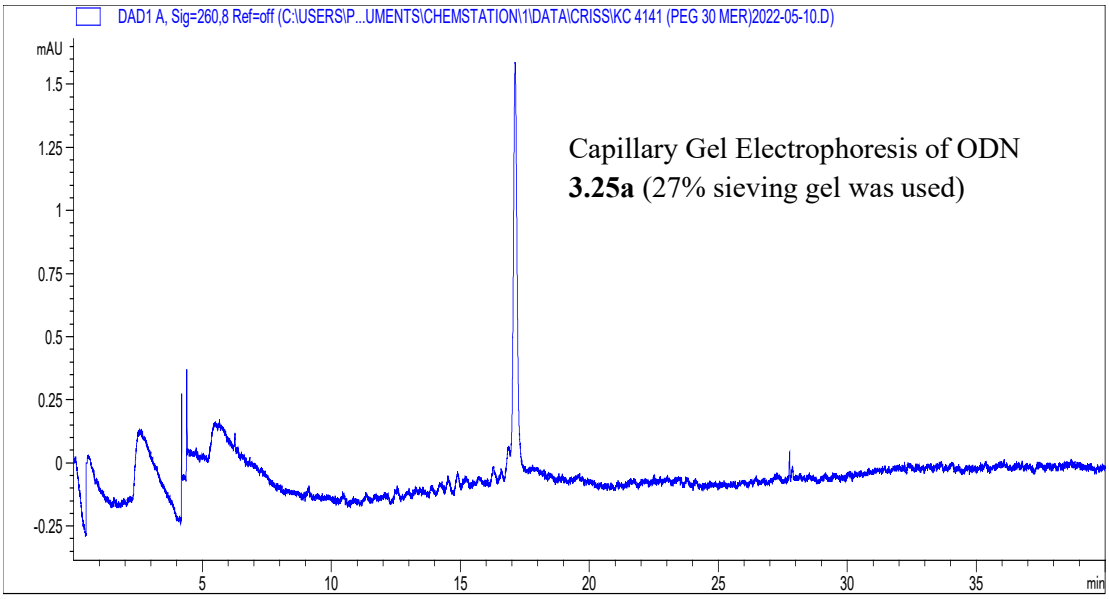


Figure 6.50: Capillary Gel Electrophoresis of ODN **3.25a** (using 27% sieving gel).

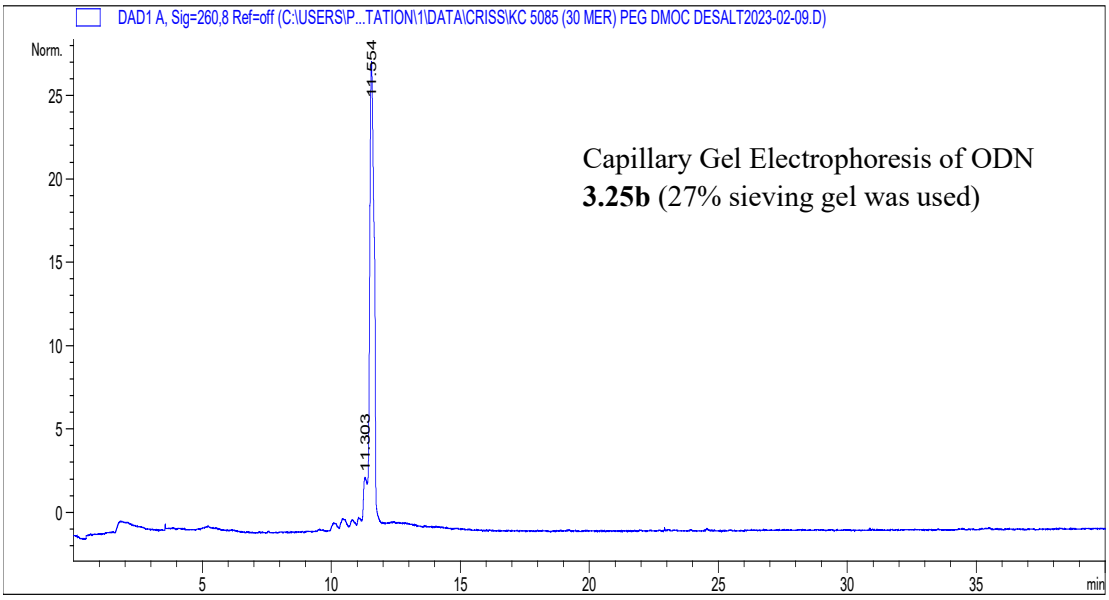


Figure 6.51: Capillary Gel Electrophoresis of ODN **3.25b** (using 27% sieving gel).

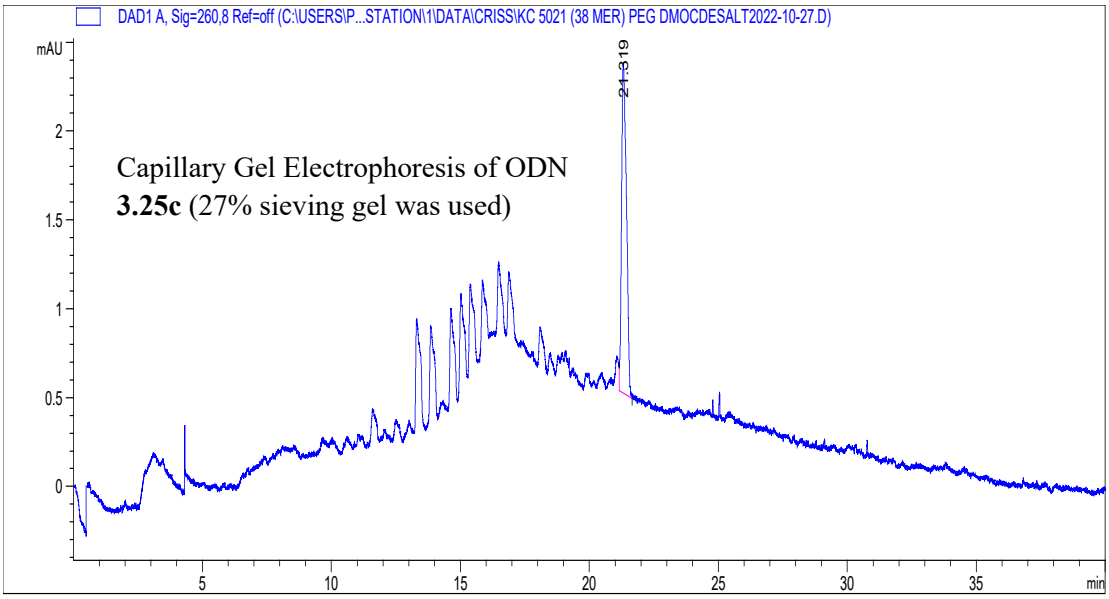


Figure 6.52: Capillary Gel Electrophoresis of ODN **3.25c** (using 27% sieving gel).

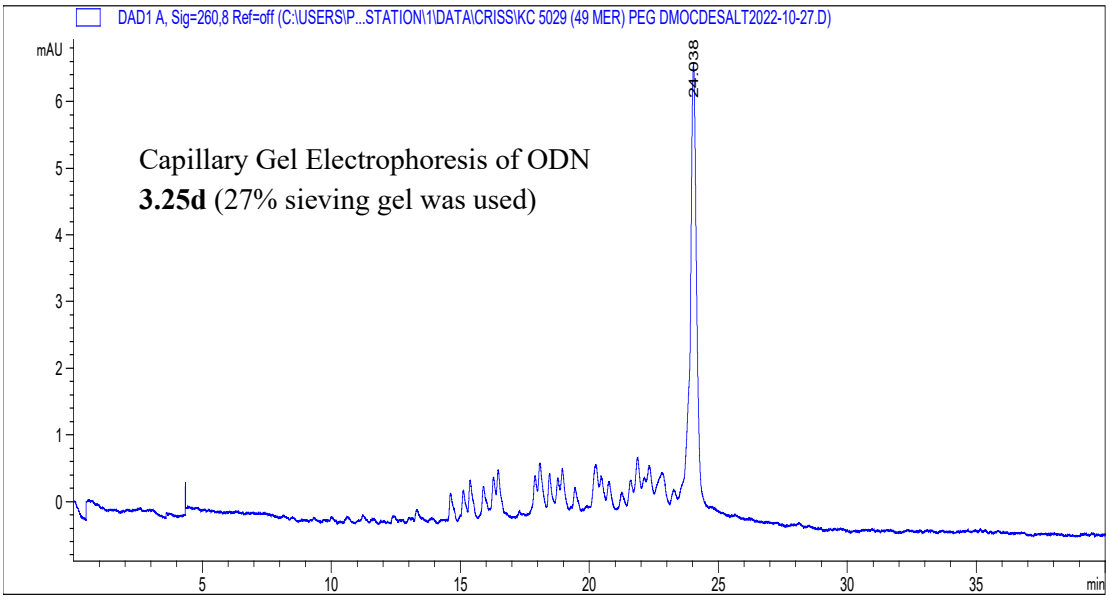


Figure 6.53: Capillary Gel Electrophoresis of ODN **3.25d** (using 27% sieving gel).

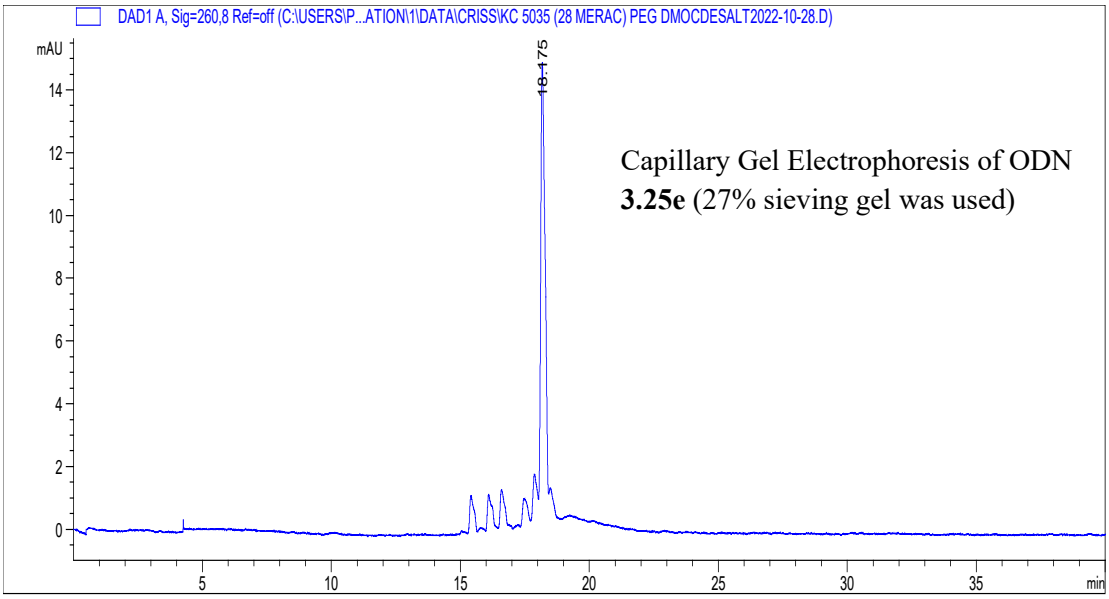


Figure 6.54: Capillary Gel Electrophoresis of ODN **3.25e** (using 27% sieving gel).

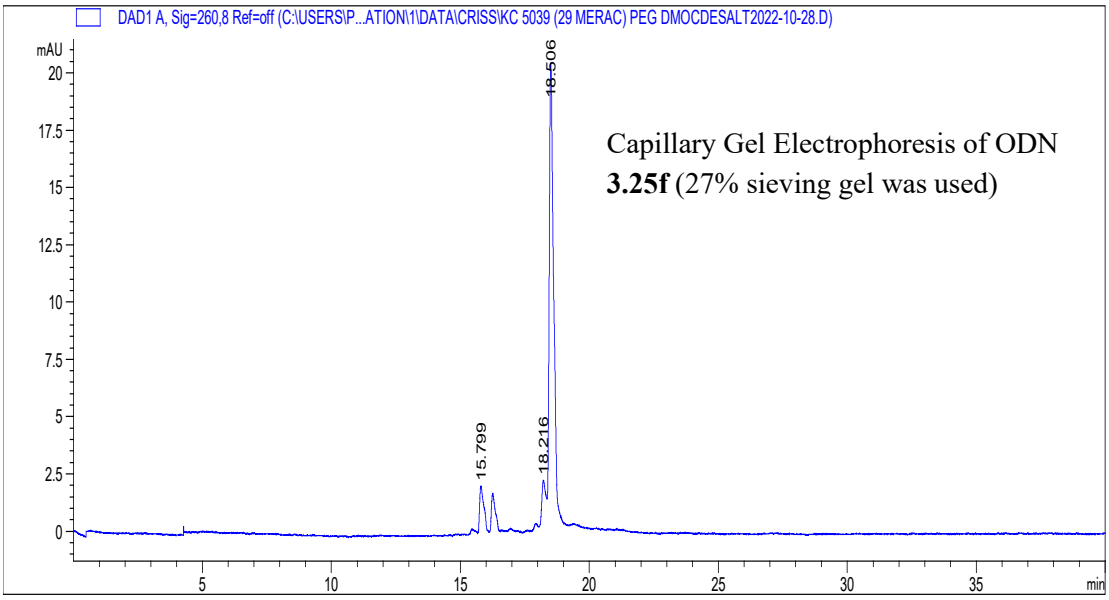


Figure 6.55: Capillary Gel Electrophoresis of ODN **3.25f** (using 27% sieving gel).

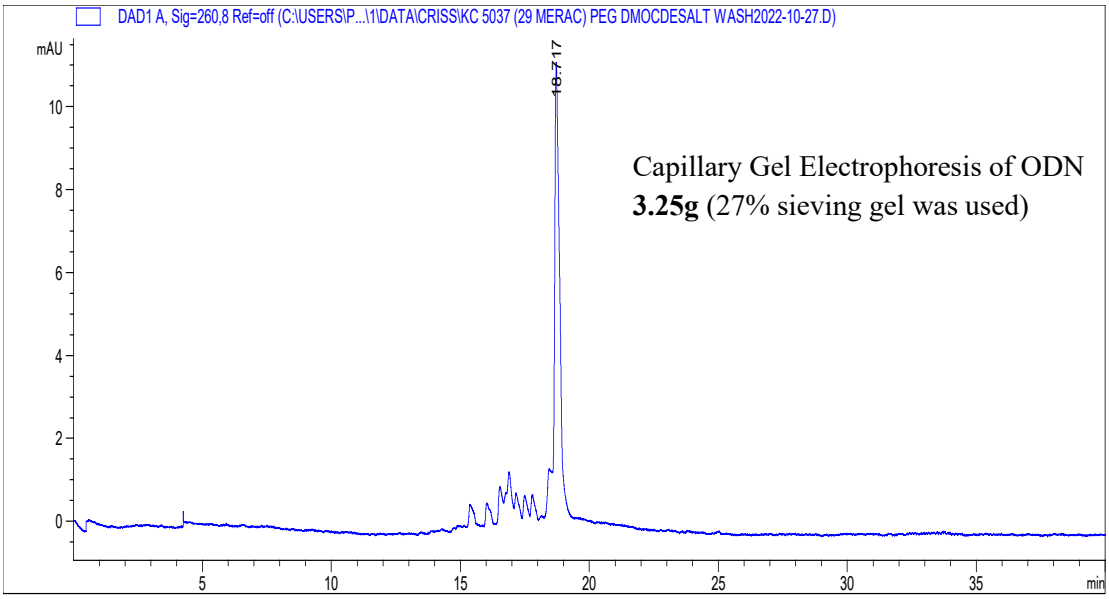


Figure 6.56: Capillary Gel Electrophoresis of ODN **3.25g** (using 27% sieving gel).

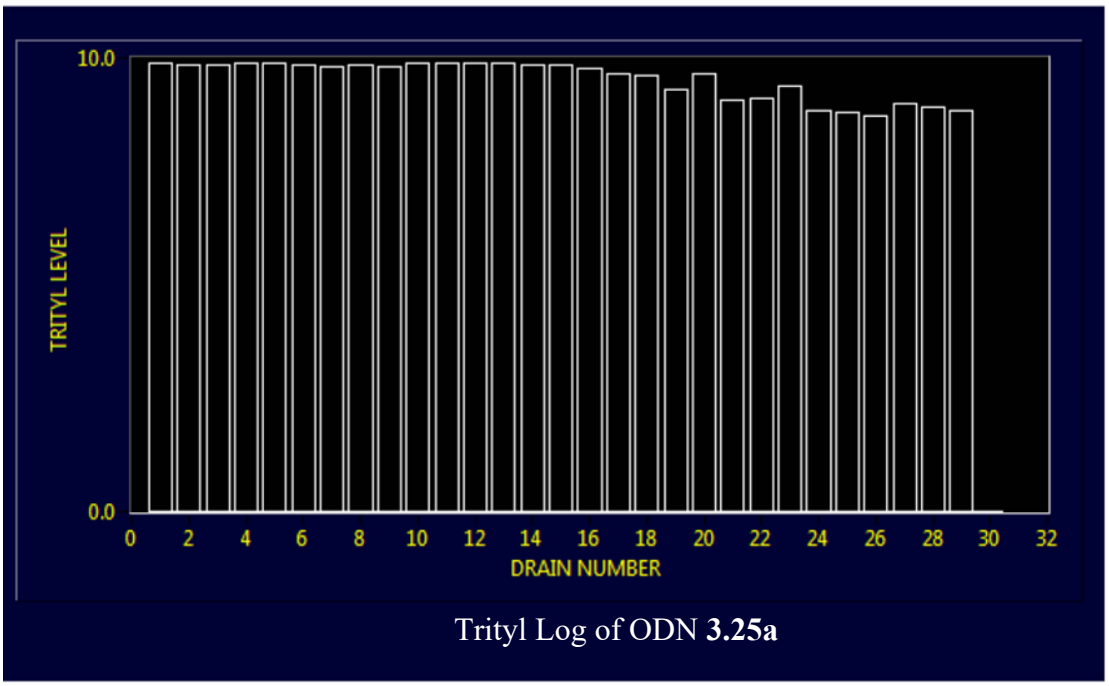


Figure 6.57: Trityl log of ODN **3.25a**.

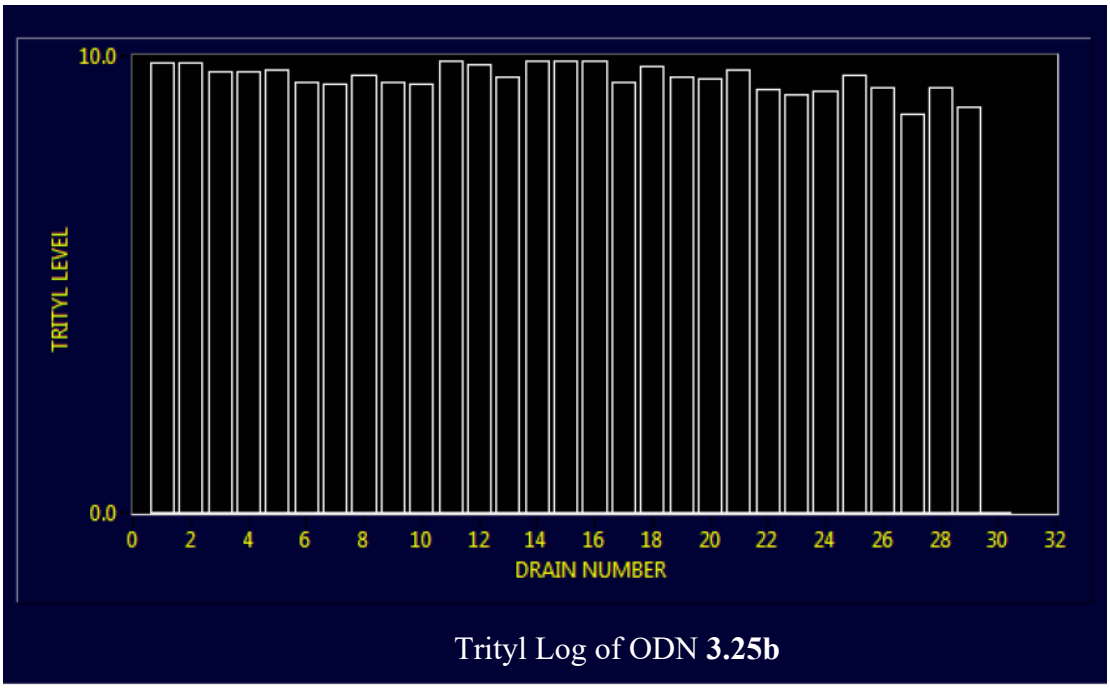


Figure 6.58: Trityl log of ODN **3.25b**.

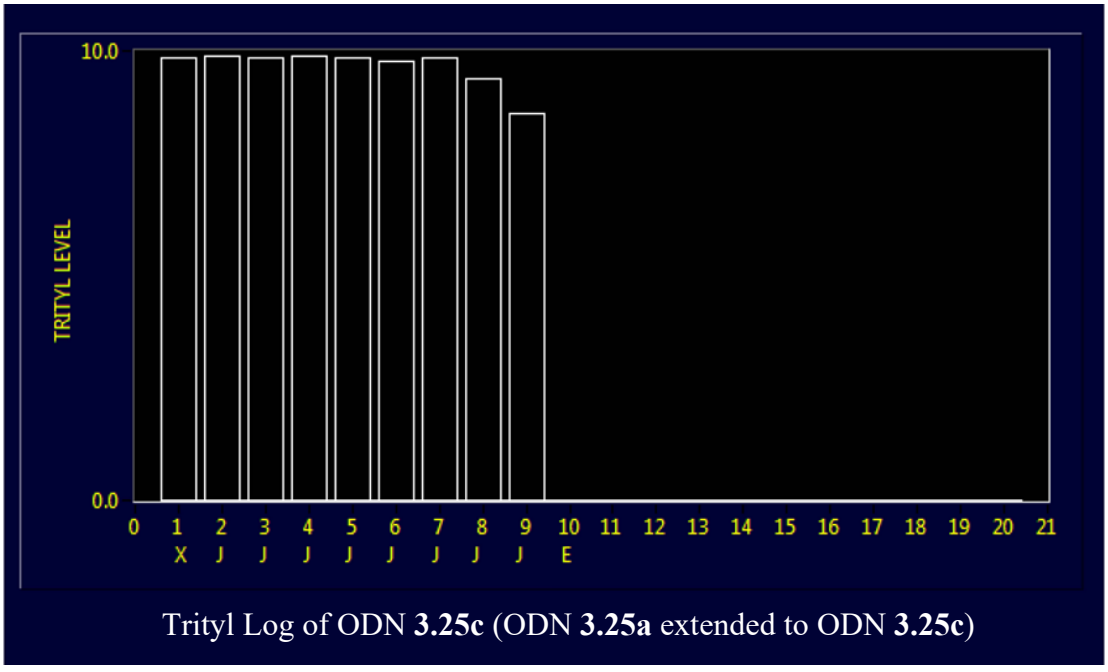


Figure 6.59: Trityl log of ODN **3.25c** (ODN **3.25a** extended to ODN **3.25c**).

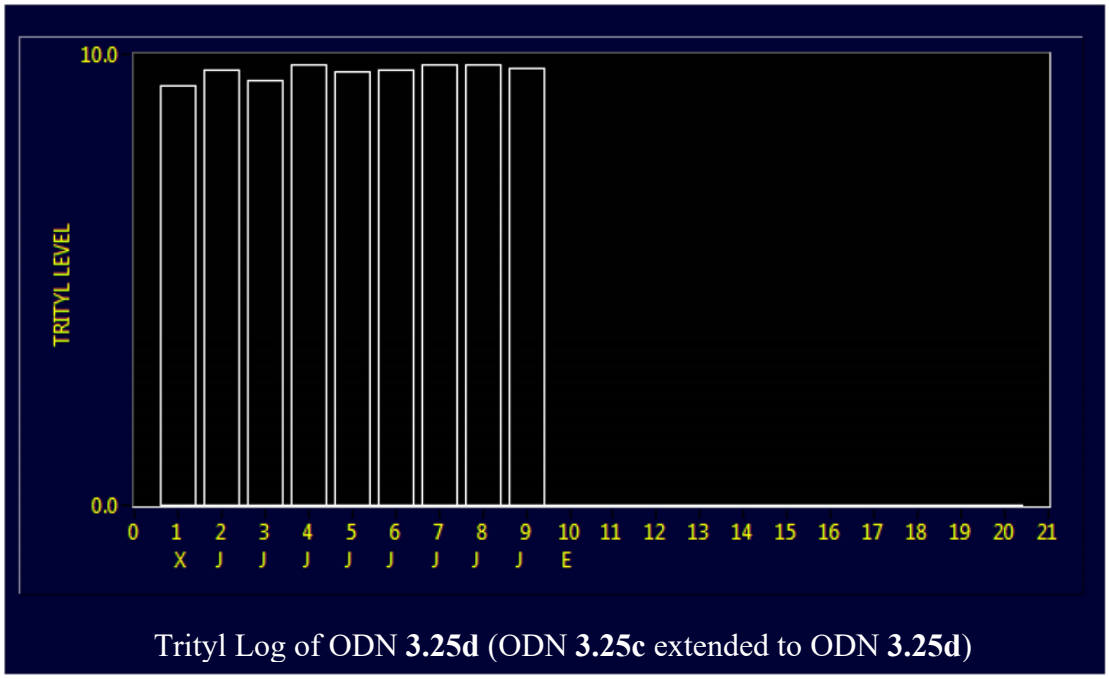


Figure 6.60: Trityl log of ODN **3.25d** (ODN **3.25c** extended to ODN **3.25d**).

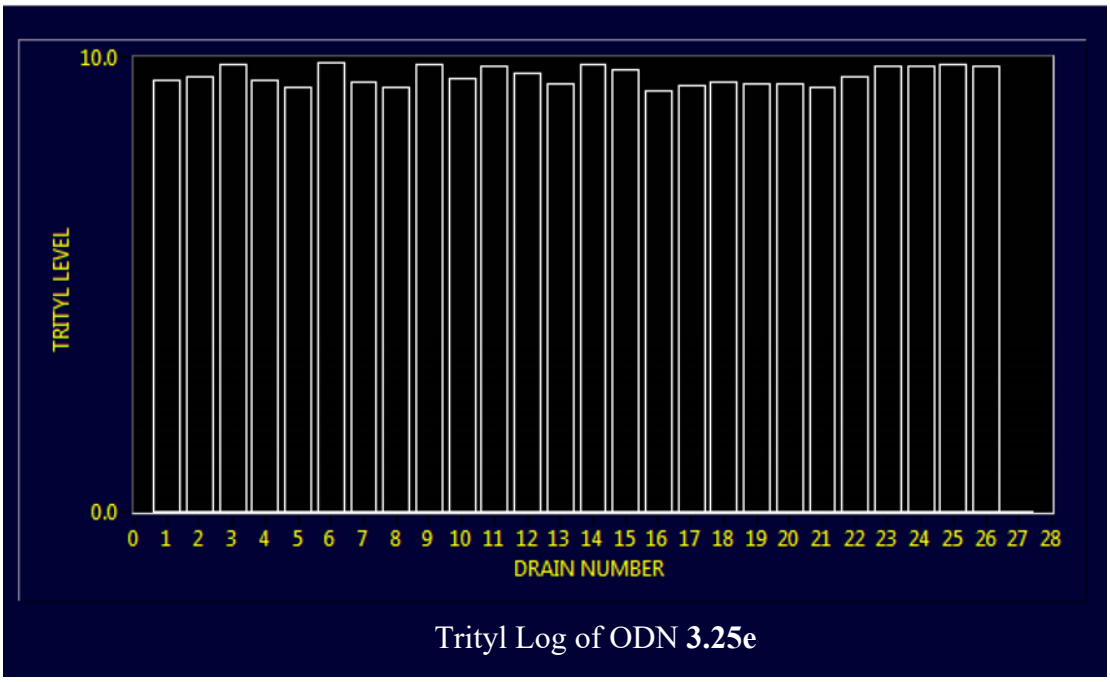


Figure 6.61: Trityl log of ODN **3.25e**.

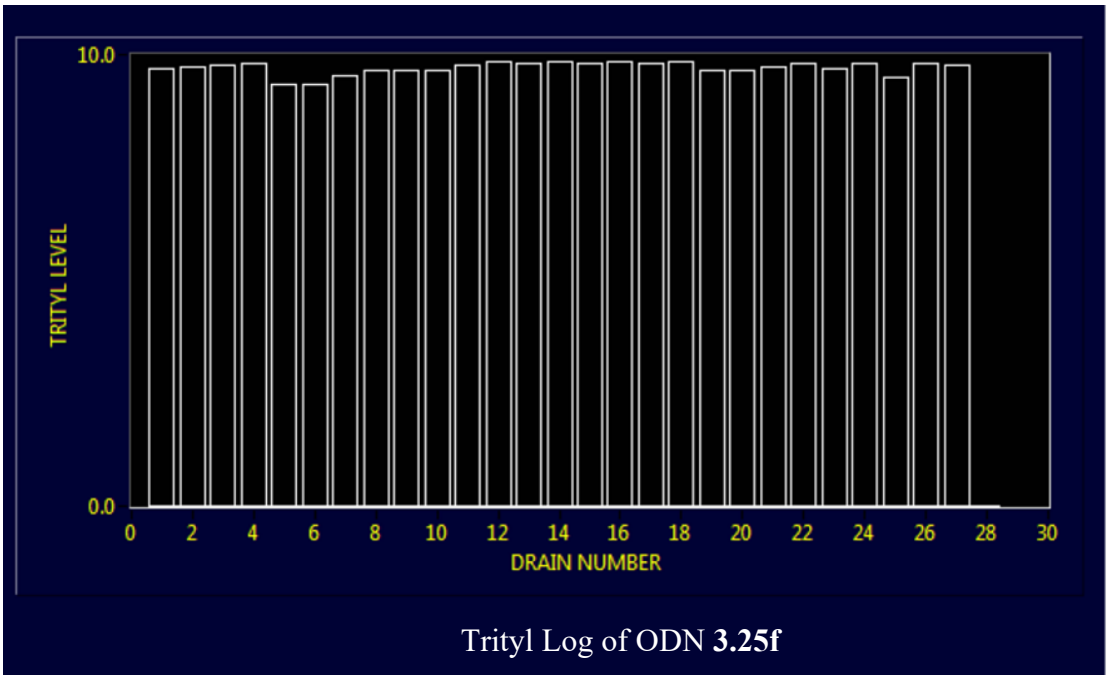


Figure 6.62: Trityl log of ODN **3.25f**.

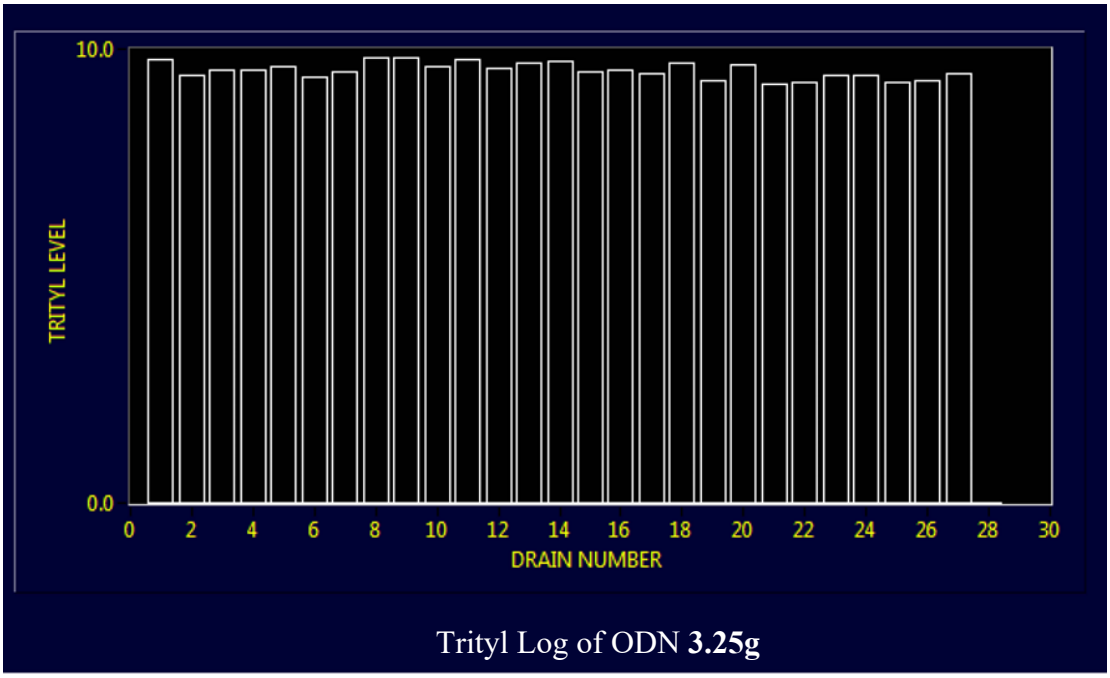
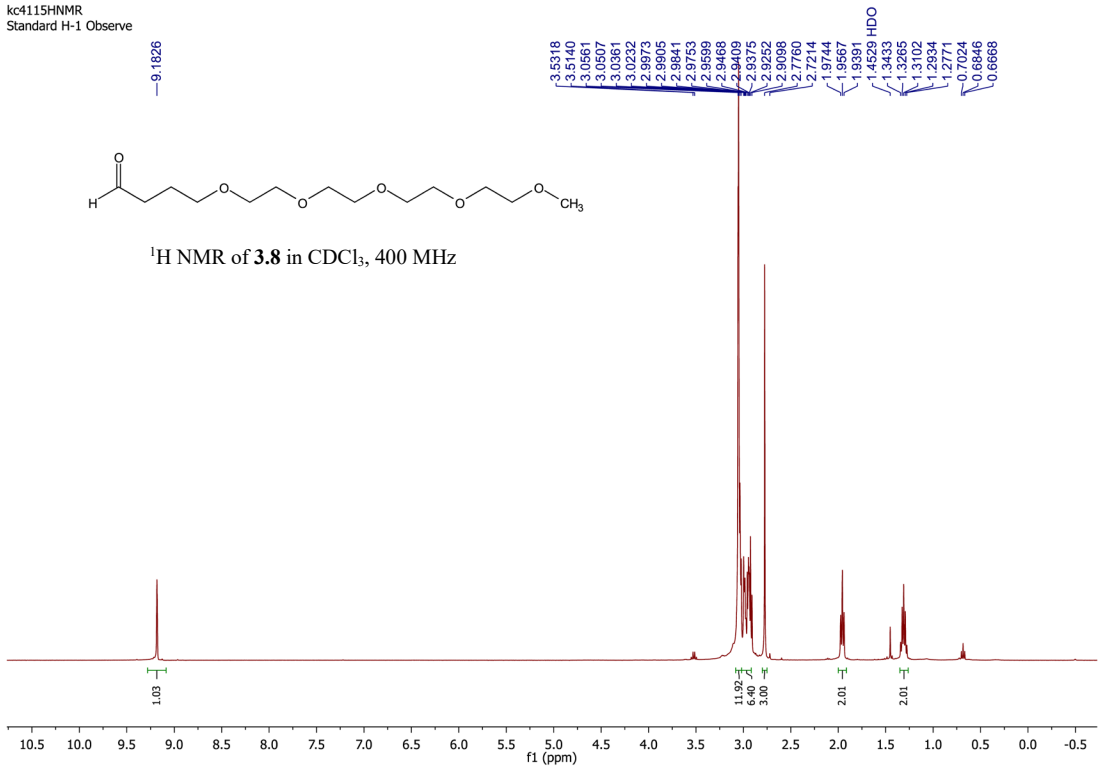


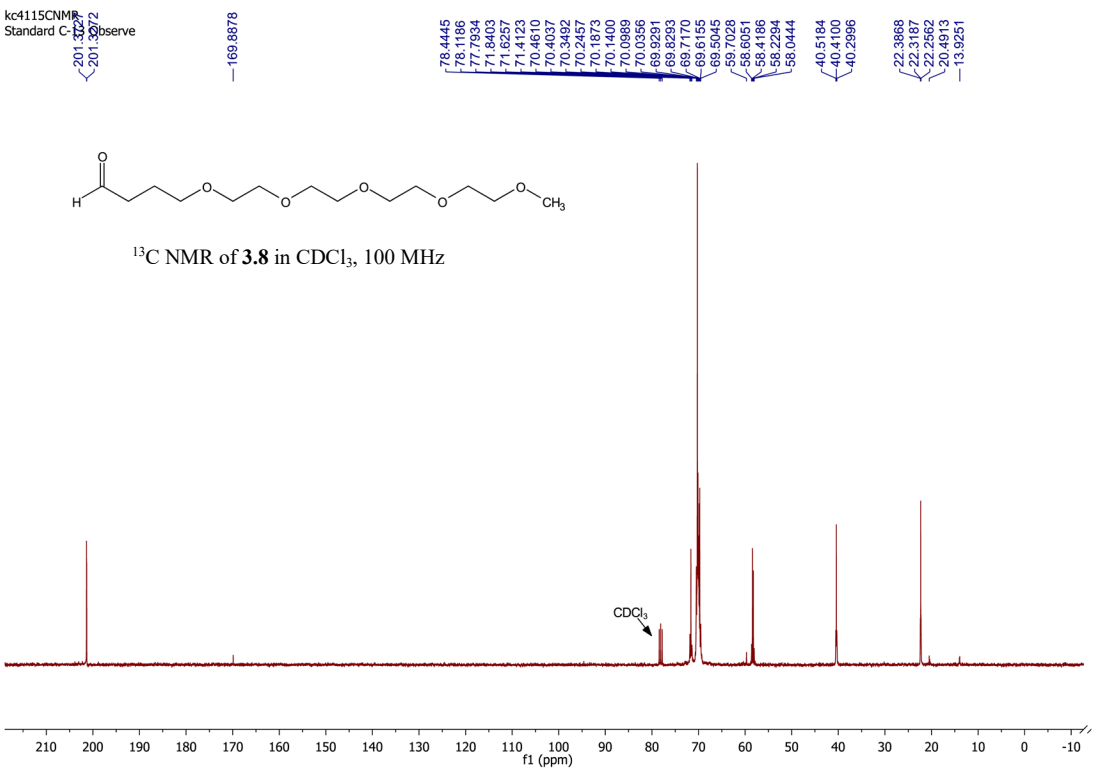
Figure 6.63: Trityl log of ODN **3.25g**.

6.2 Images of ^{1}H, ^{13}C and ^{31}P NMR spectra of compound



^{1}H NMR of **3.8** in $CDCl_3$, 400 MHz

Figure 6.64: ^{1}H NMR spectrum of compound **3.8**.



^{13}C NMR of **3.8** in $CDCl_3$, 100 MHz

Figure 6.65: ^{13}C NMR spectrum of compound **3.8**.

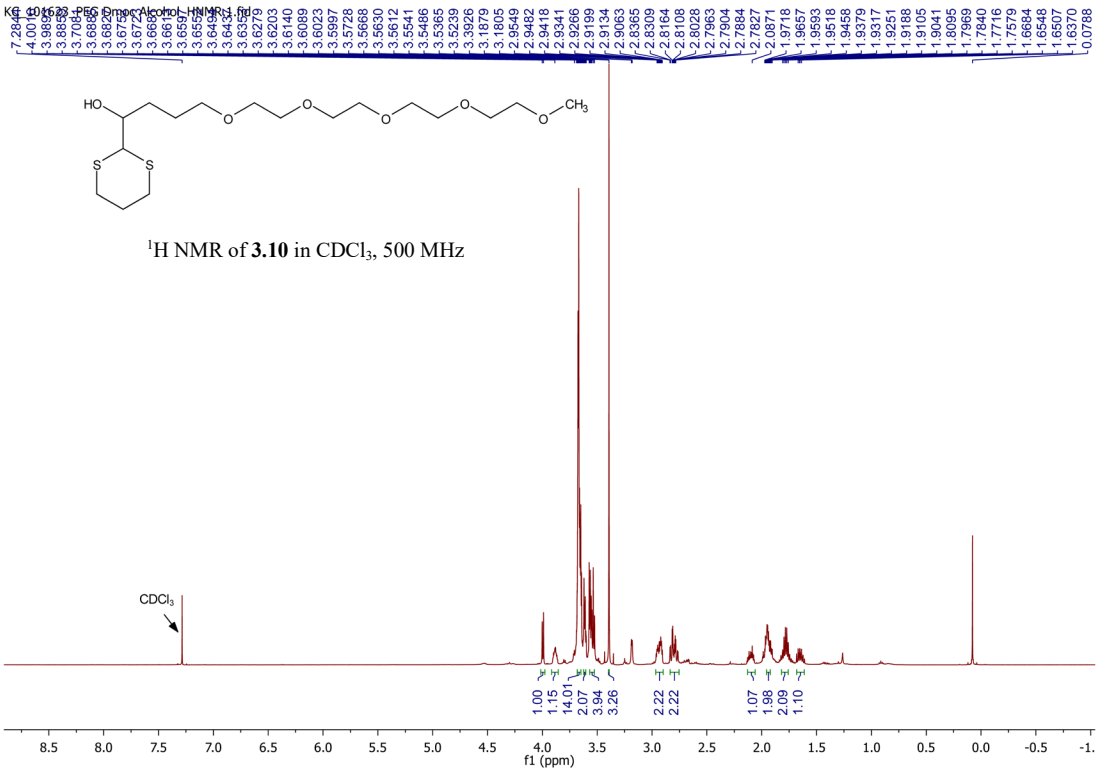


Figure 6.66: ^{1}H NMR spectrum of compound **3.10**.

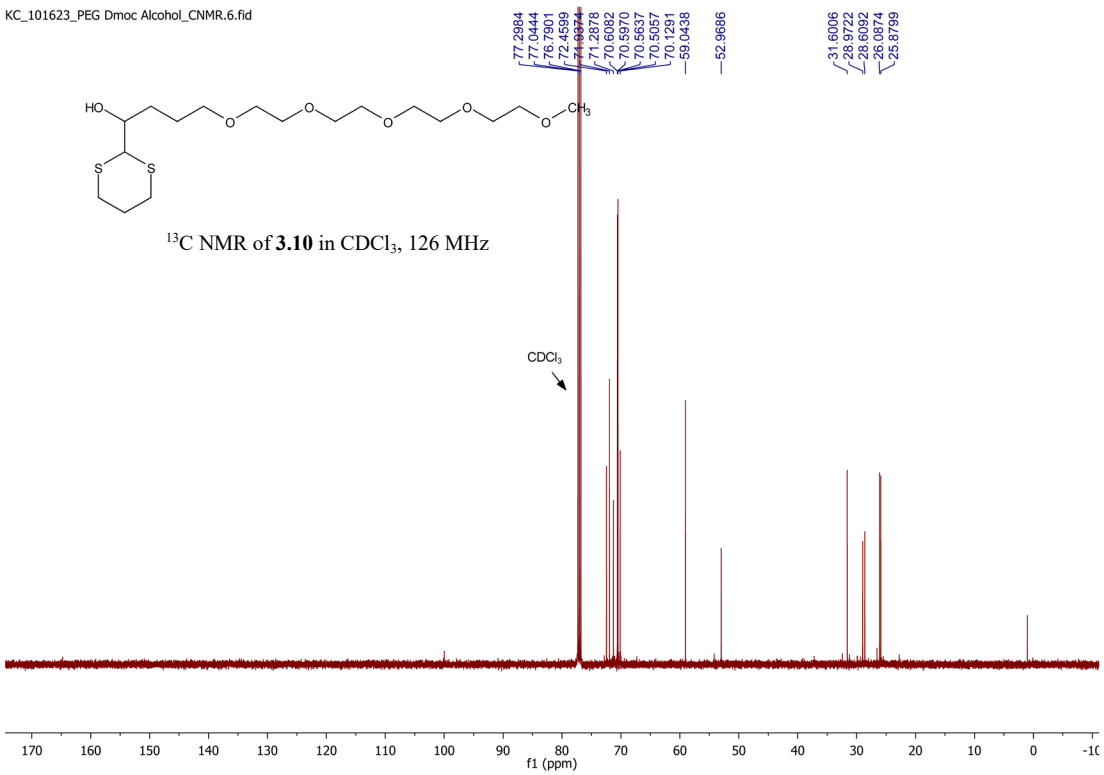
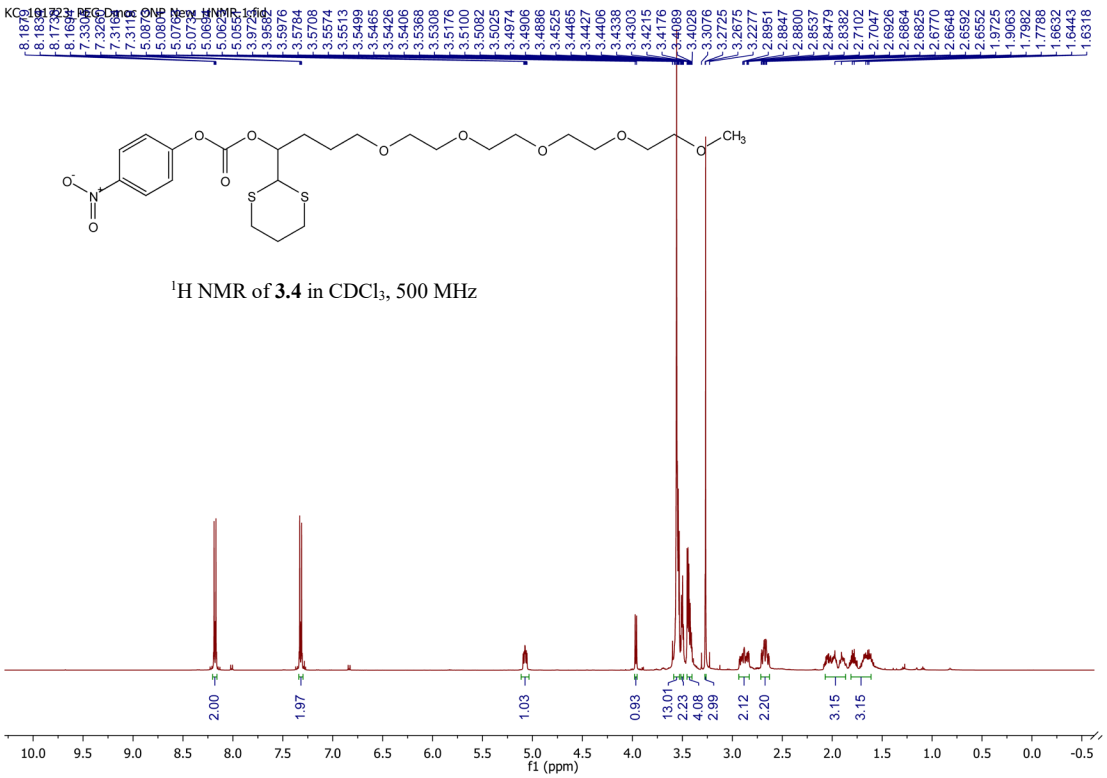
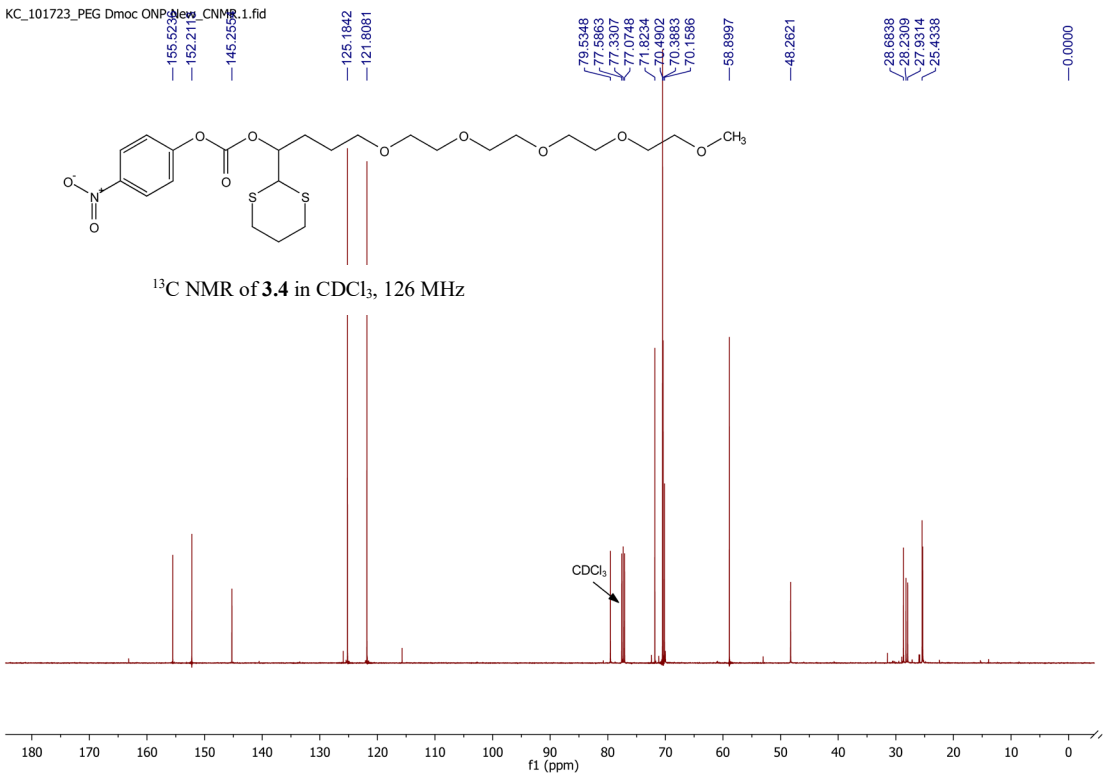


Figure 6.67: ^{13}C NMR spectrum of compound **3.10**.



^{1}H NMR of **3.4** in $CDCl_3$, 500 MHz

Figure 6.68: ^{1}H NMR spectrum of compound **3.4**.



^{13}C NMR of **3.4** in $CDCl_3$, 126 MHz

Figure 6.69: ^{13}C NMR spectrum of compound **3.4**.

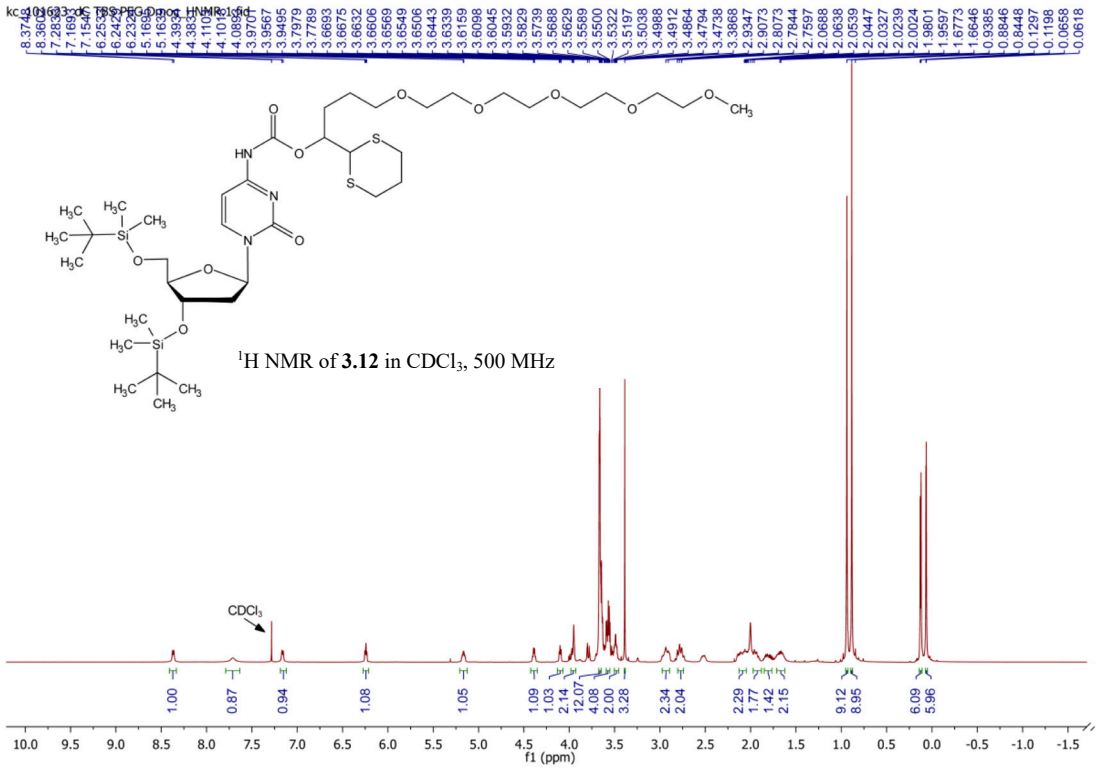


Figure 6.70: ^{1}H NMR spectrum of compound **3.12**.

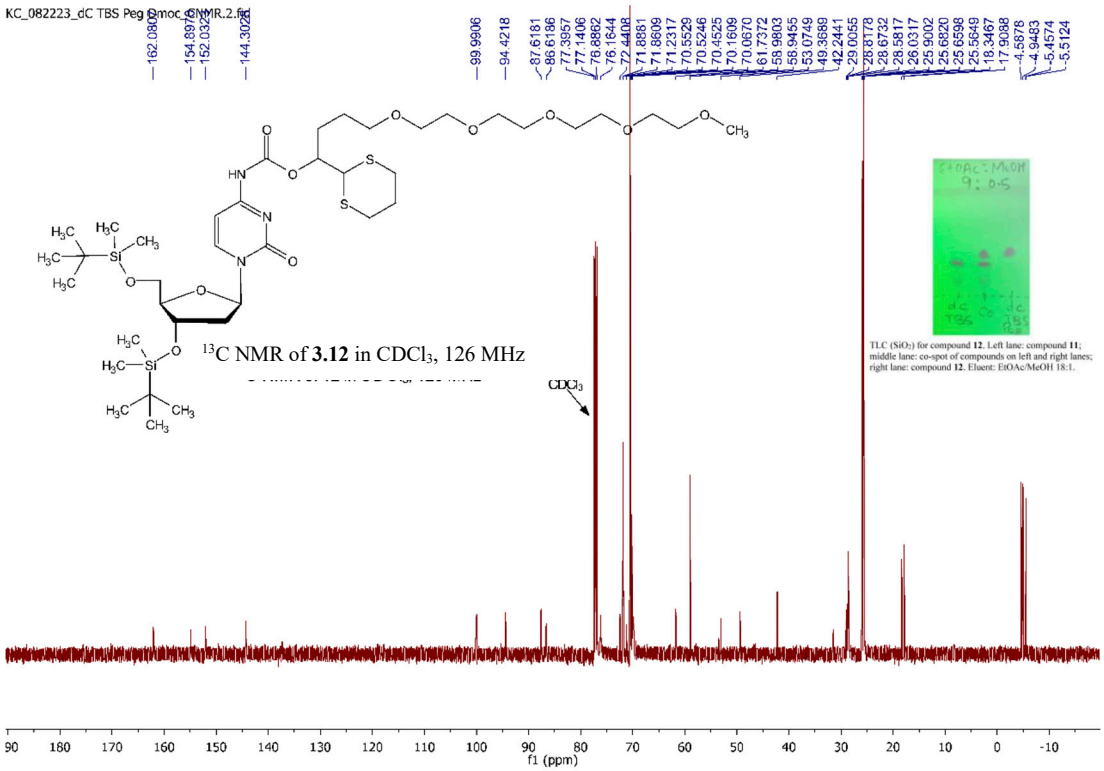
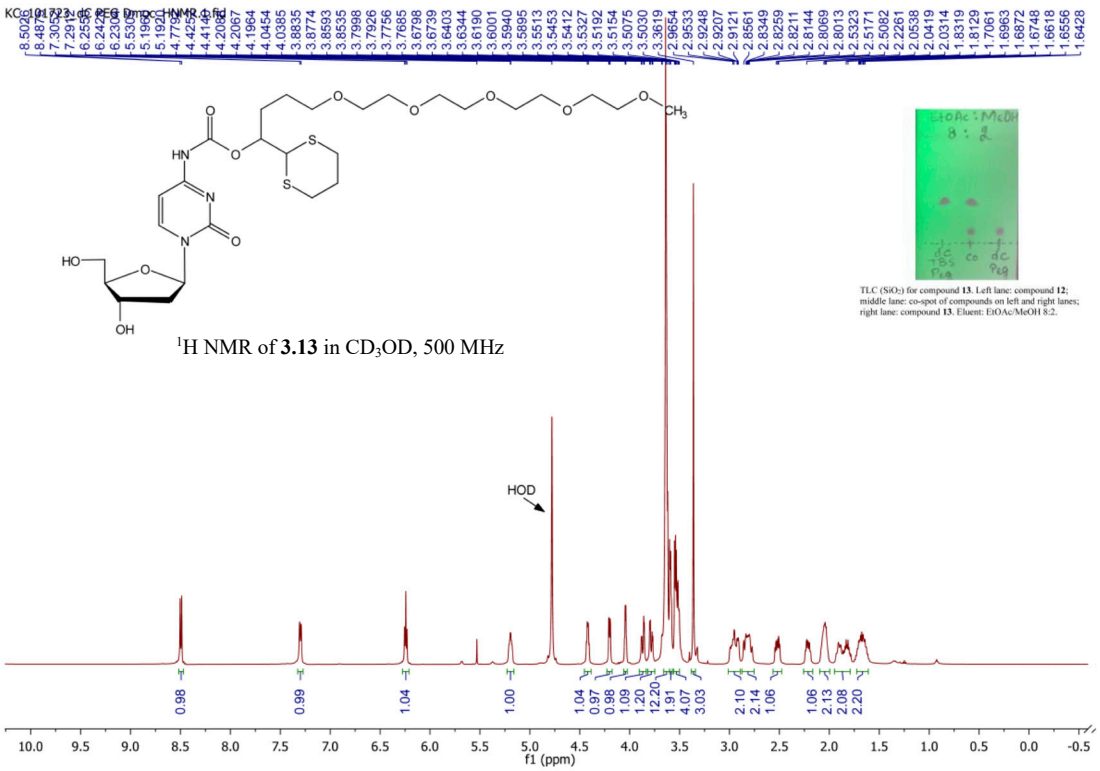


Figure 6.71: ^{13}C NMR spectrum of compound **3.12**.



^{1}H NMR of **3.13** in CD_3OD, 500 MHz

Figure 6.72: ^{1}H NMR spectrum of compound **3.13**.

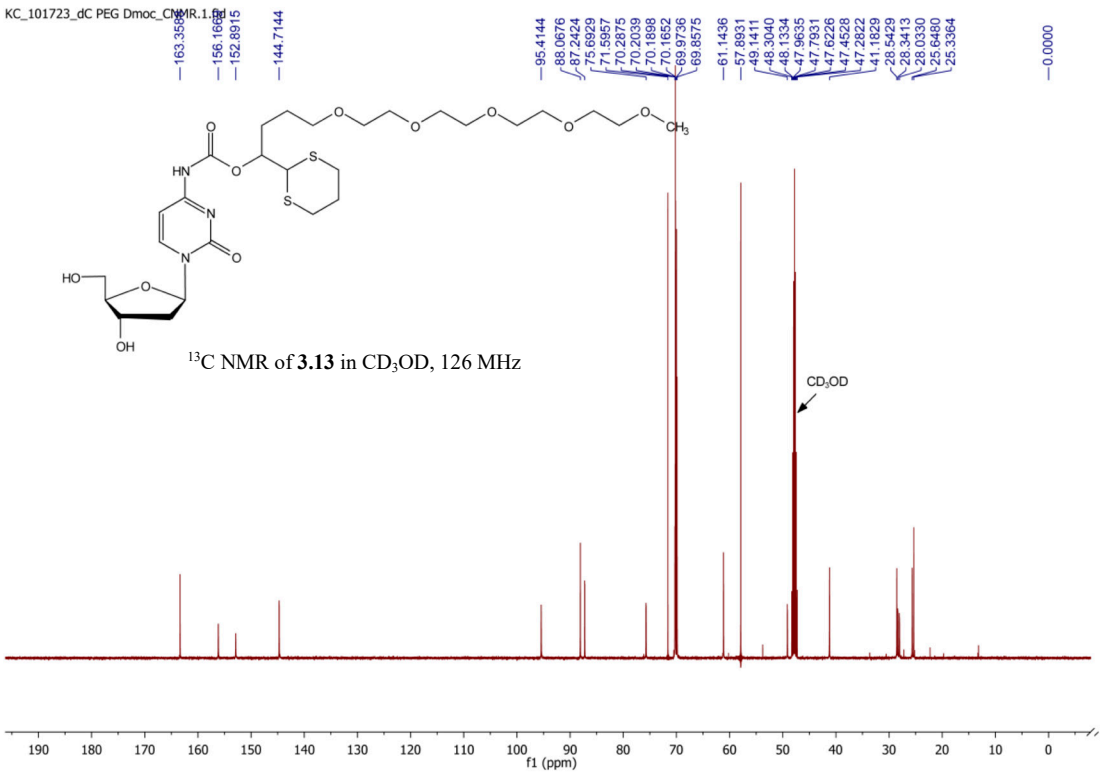


Figure 6.73: ^{13}C NMR spectrum of compound **3.13**.

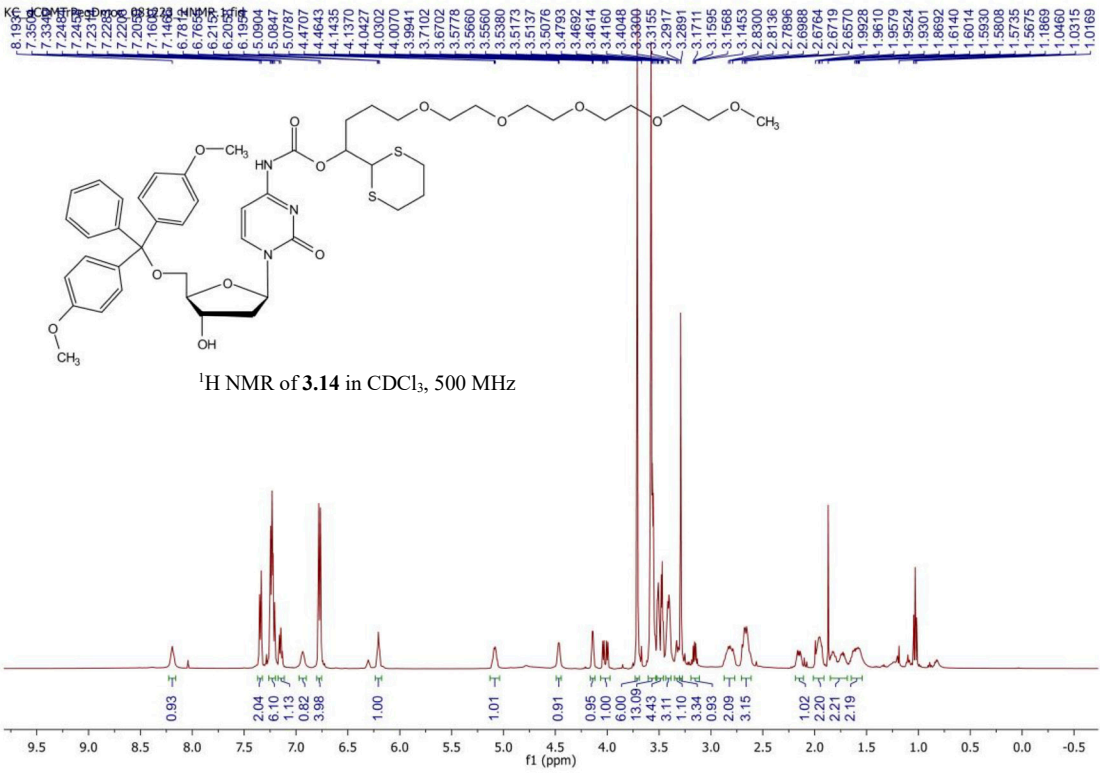
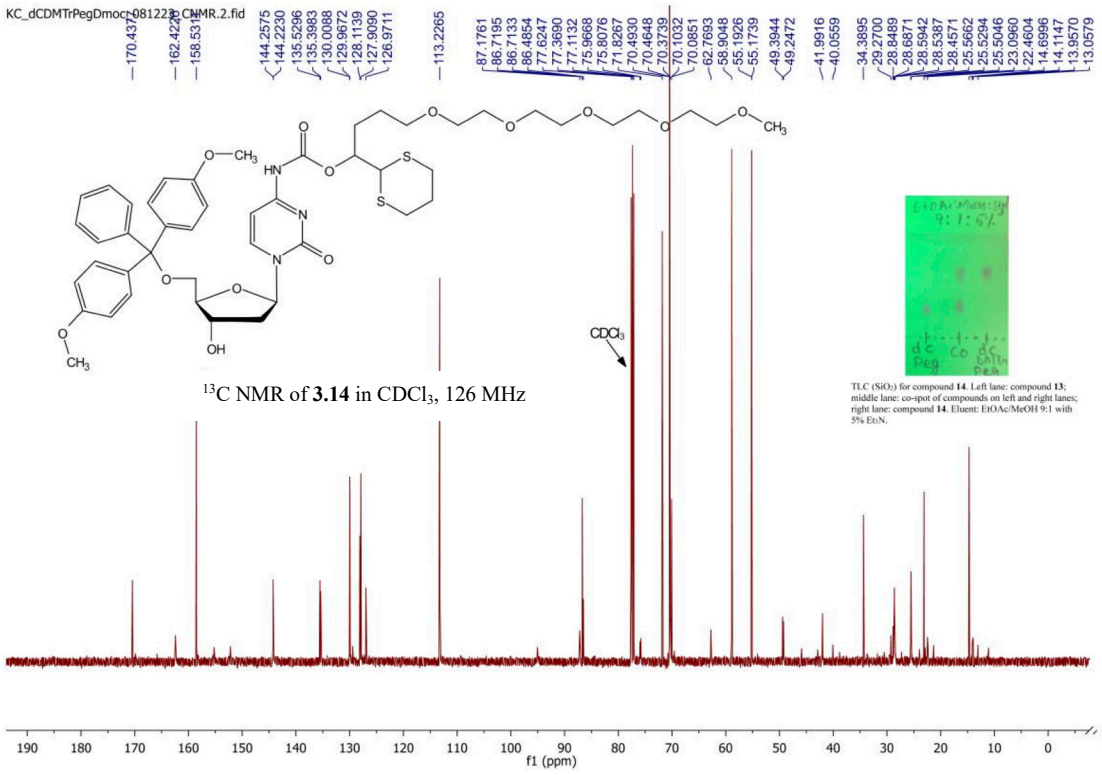


Figure 6.74: ^{1}H NMR spectrum of compound **3.14**.



^{13}C NMR of **3.14** in $CDCl_3$, 126 MHz

TLC (SiO_2) for compound **14**. Left lane: compound **13**; middle lane: co-spot of compounds on left and right lanes; right lane: compound **14**. Eluent: EtOAc/MeOH 9:1 with 5% Et$_3$N.

Figure 6.75: ^{13}C NMR spectrum of compound **3.14**.

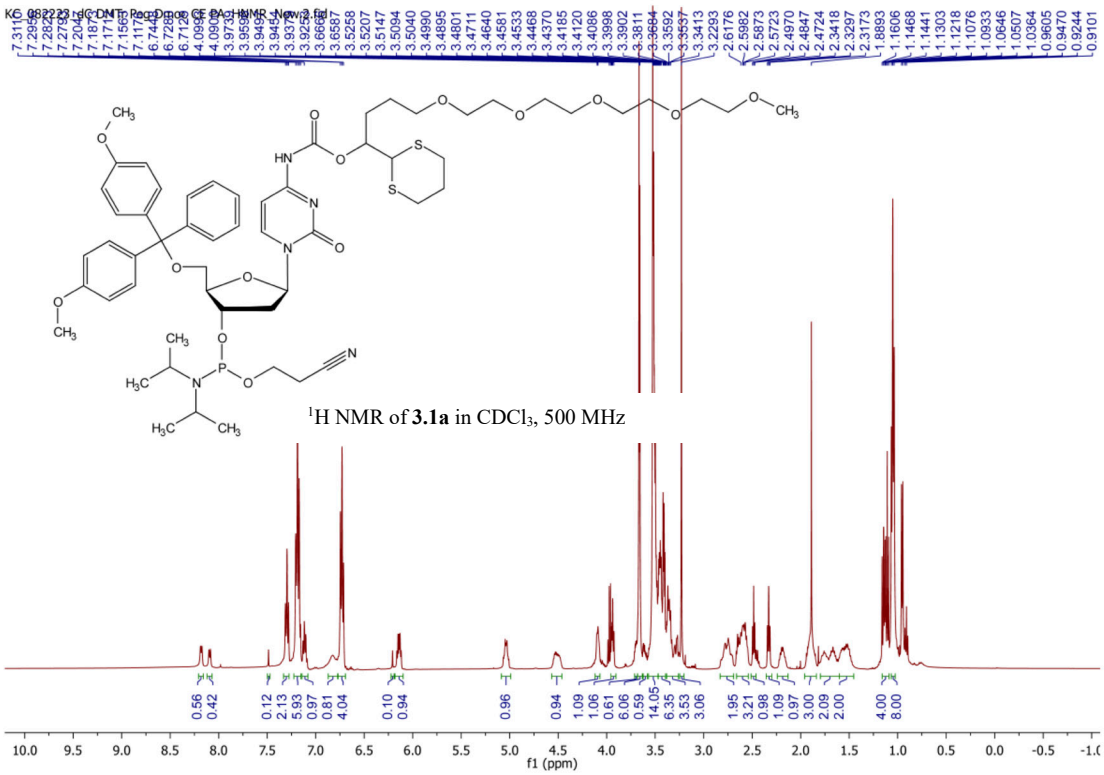
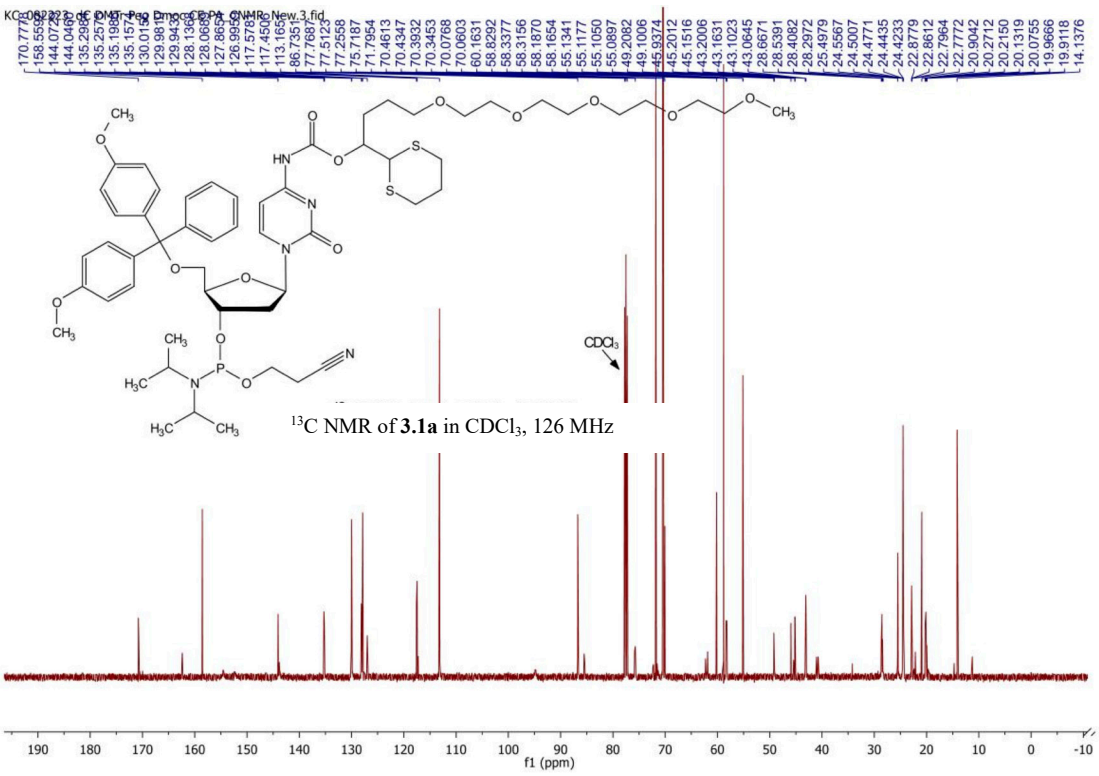
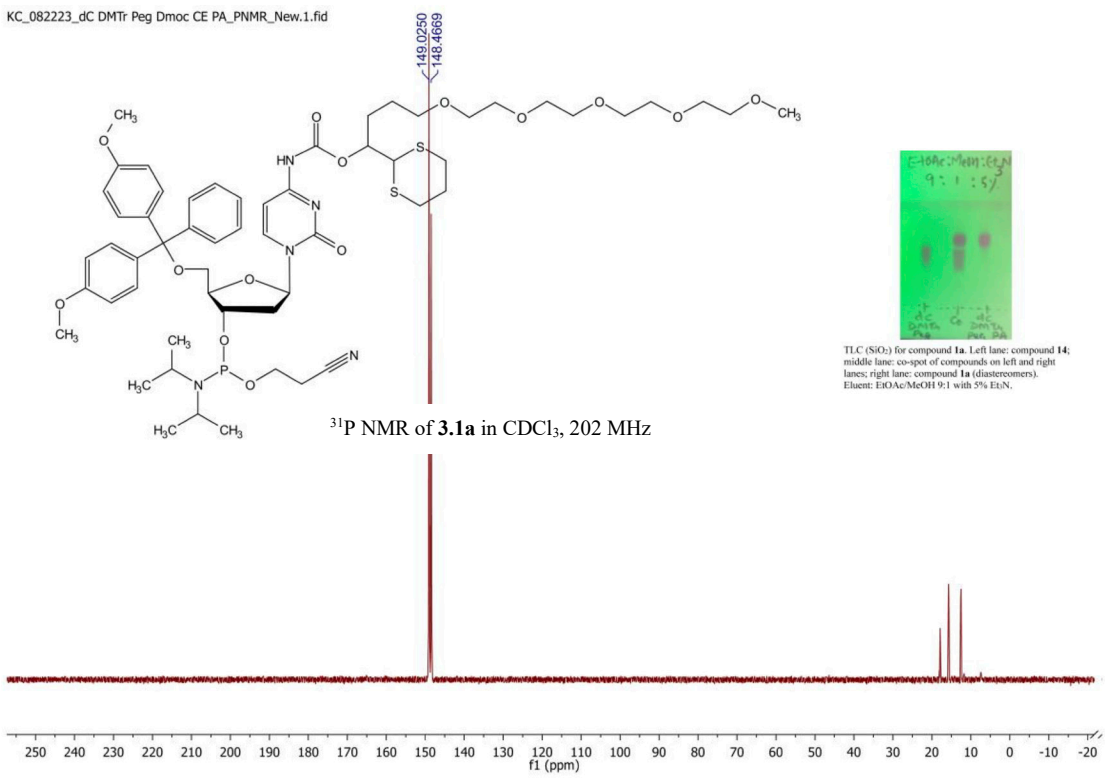


Figure 6.76: ^{1}H NMR spectrum of compound **3.1a**.



^{13}C NMR of **3.1a** in $CDCl_3$, 126 MHz

Figure 6.77: ^{13}C NMR spectrum of compound **3.1a**.



TLC (SiO_2) for compound **1a**. Left lane: compound **14**; middle lane: co-spot of compounds on left and right lanes; right lane: compound **1a** (diastereomers). Eluent: EtOAc/MeOH 9:1 with 5% Et$_3$N.

^{31}P NMR of **3.1a** in $CDCl_3$, 202 MHz

Figure 6.78: ^{31}P NMR spectrum of compound **3.1a**.

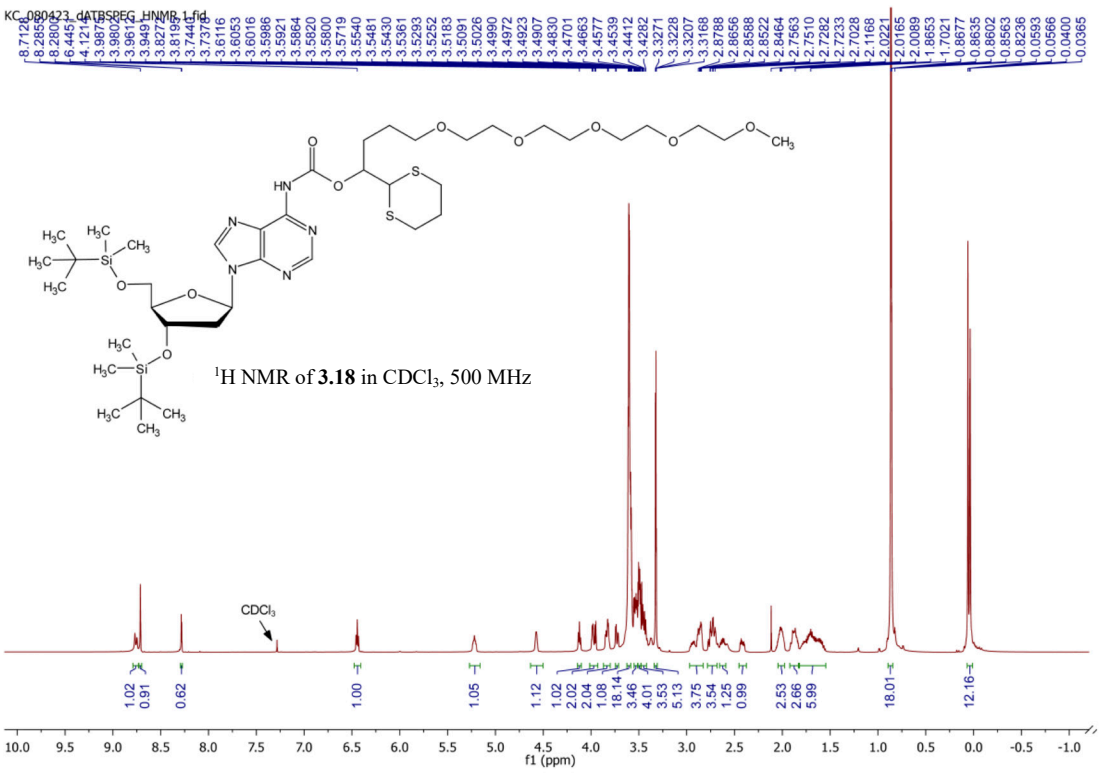


Figure 6.79: ^{1}H NMR spectrum of compound **3.18**.

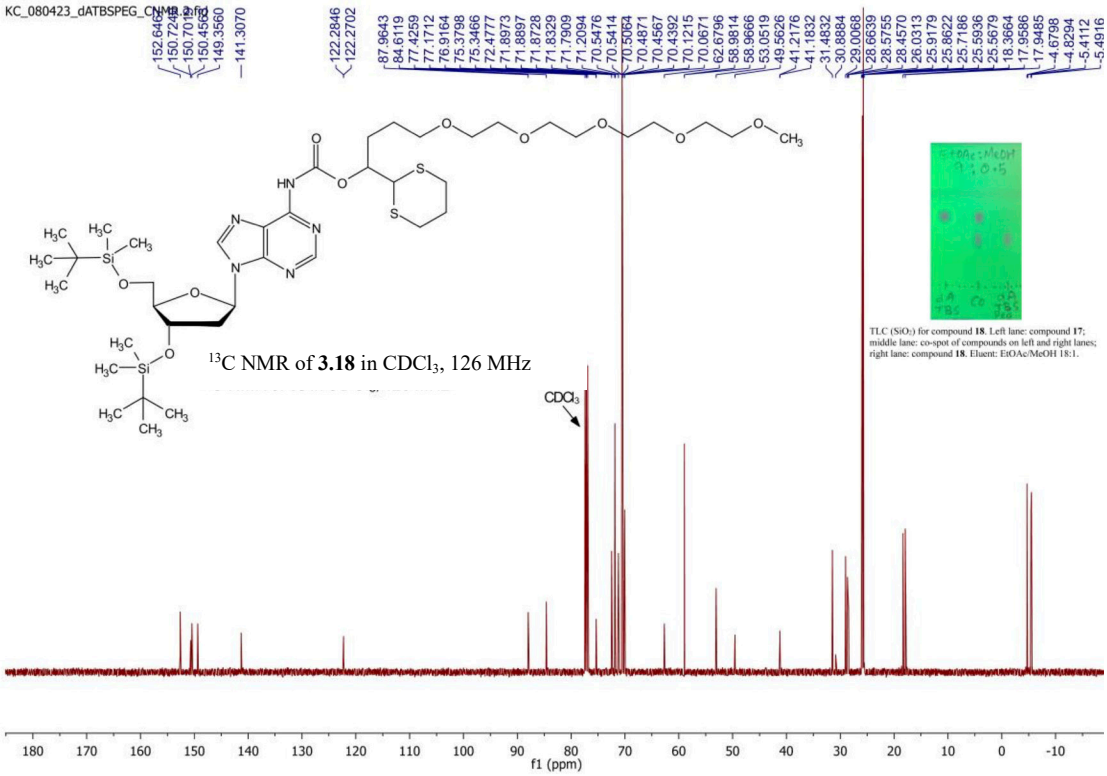


Figure 6.80: ^{13}C NMR spectrum of compound **3.18**.

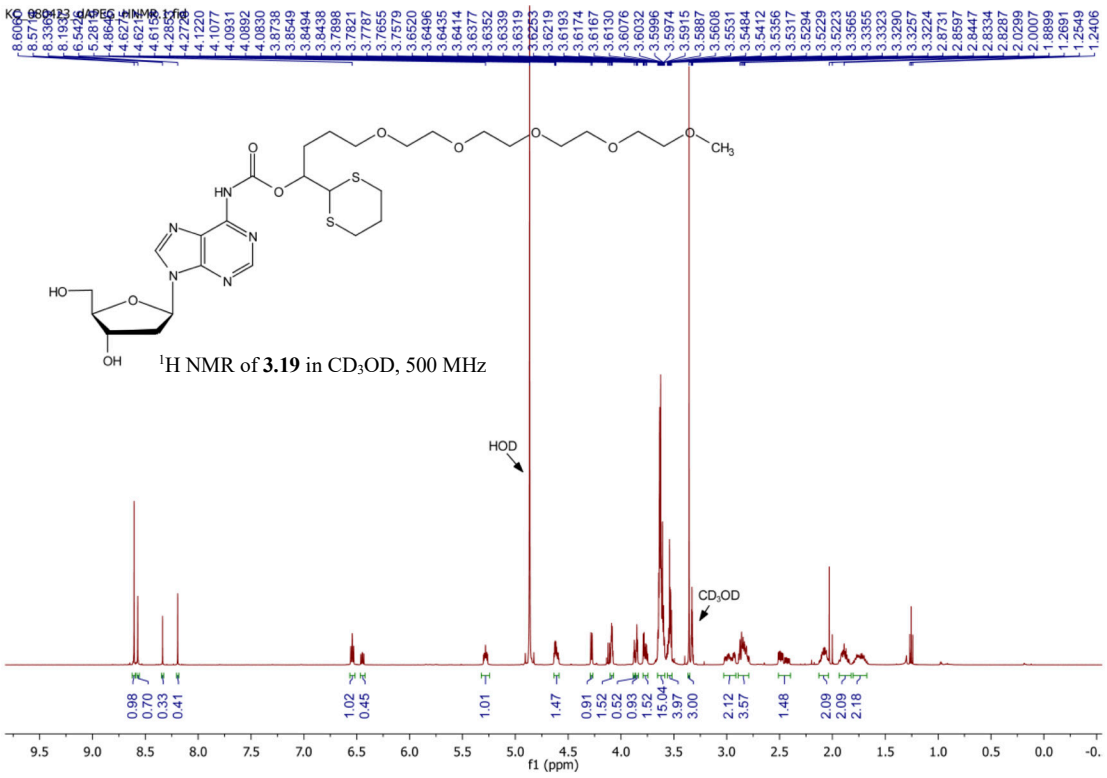


Figure 6.81: ^{1}H NMR spectrum of compound **3.19**.

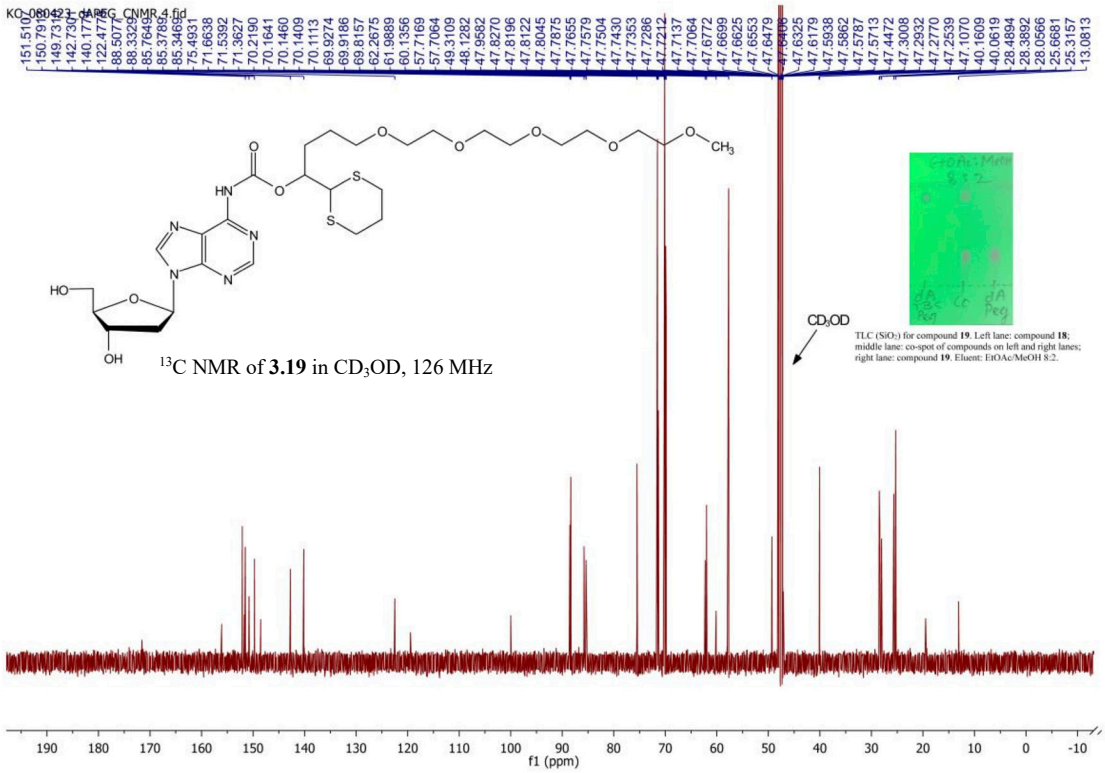
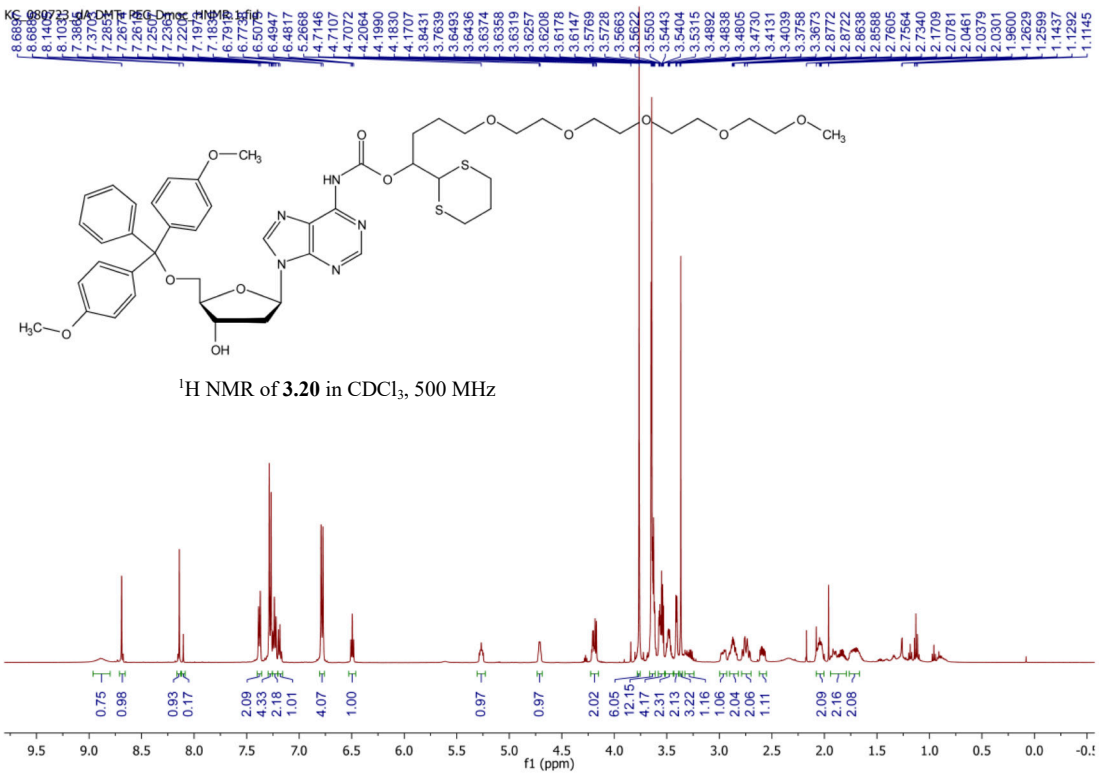


Figure 6.82: ^{13}C NMR spectrum of compound **3.19**.



^{1}H NMR of **3.20** in $CDCl_3$, 500 MHz

Figure 6.83: ^{1}H NMR spectrum of compound **3.20**.

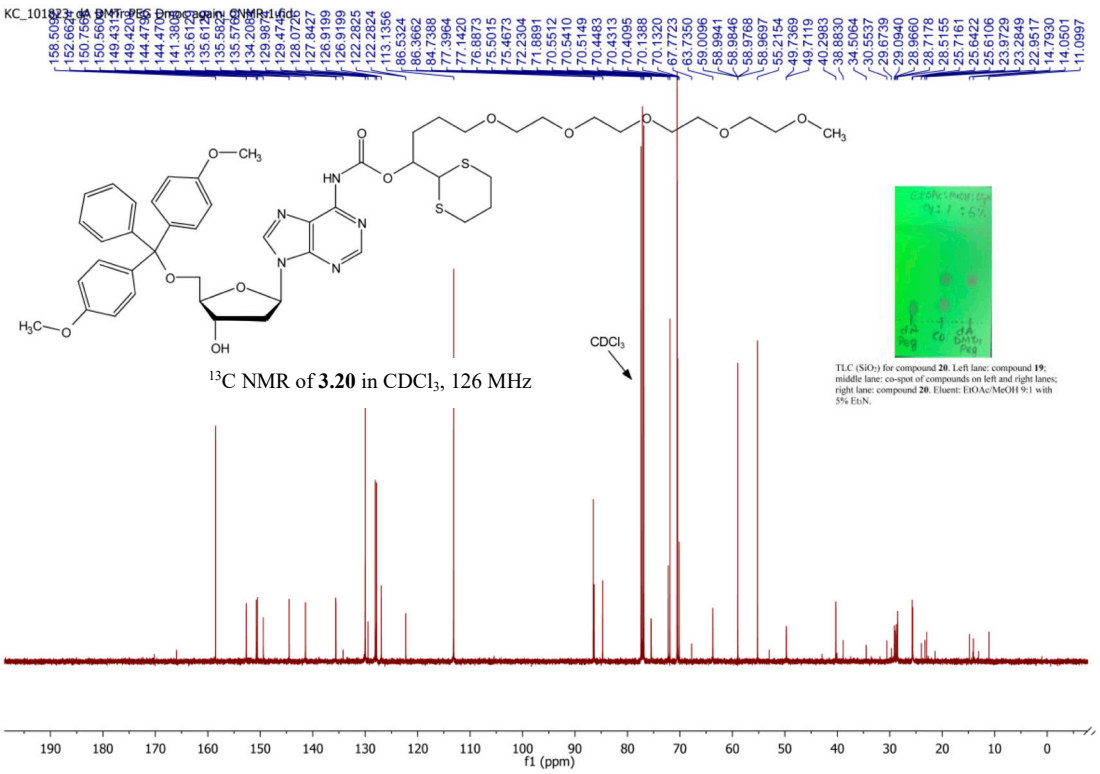


Figure 6.84: ^{13}C NMR spectrum of compound **3.20**.

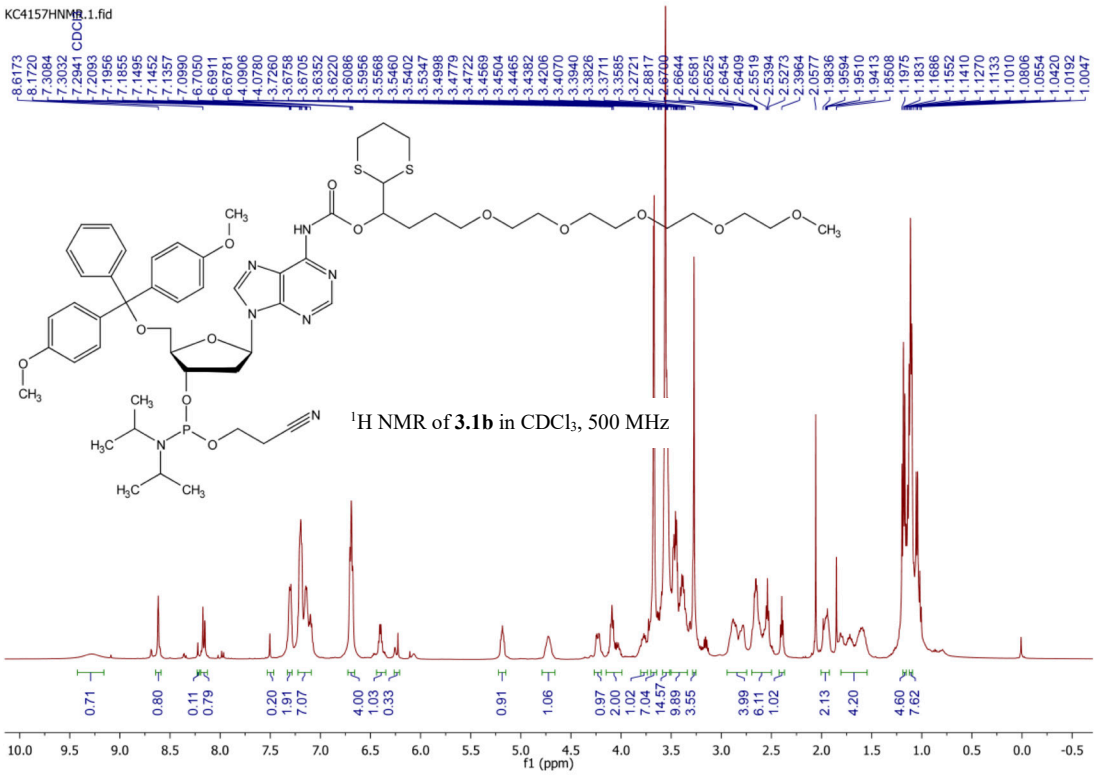


Figure 6.85: ^{1}H NMR spectrum of compound **3.1b**.

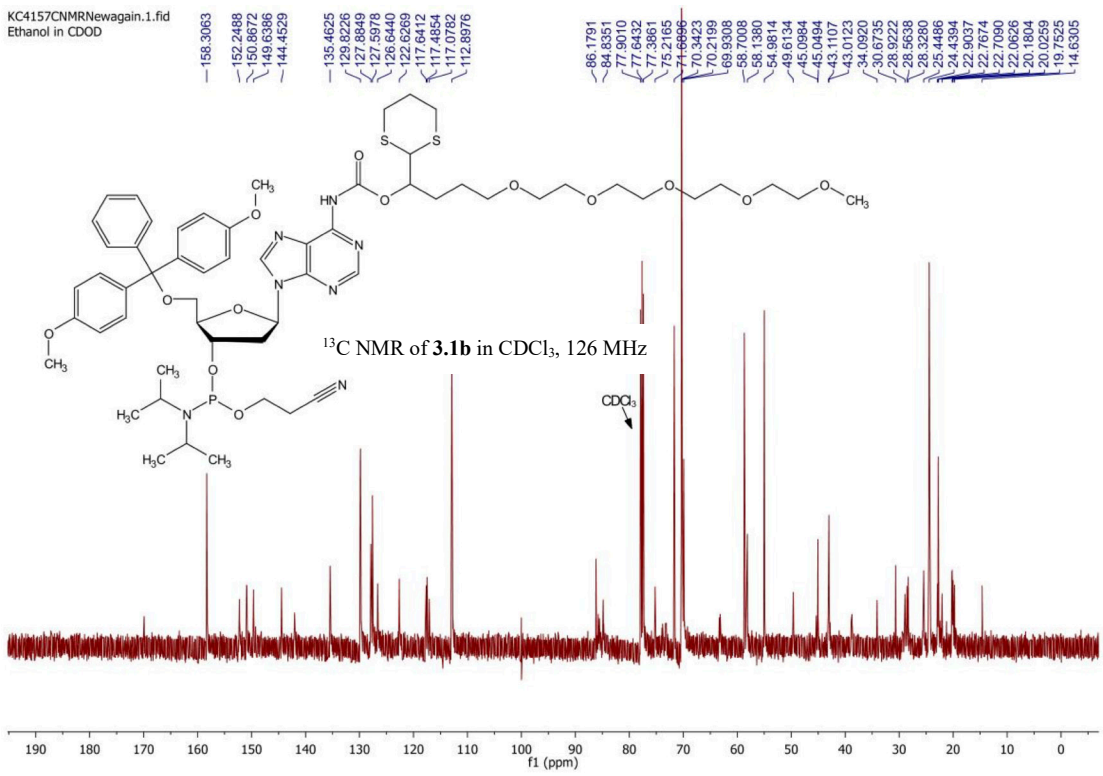


Figure 6.86: ^{13}C NMR spectrum of compound **3.1b**.

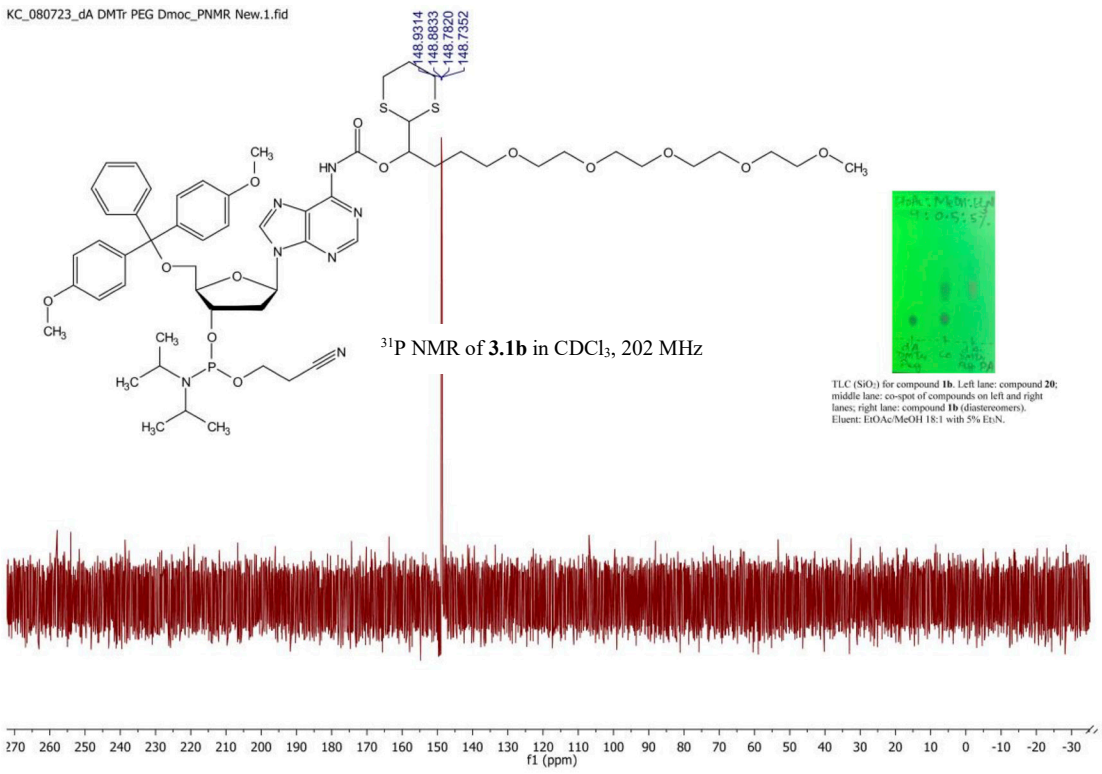
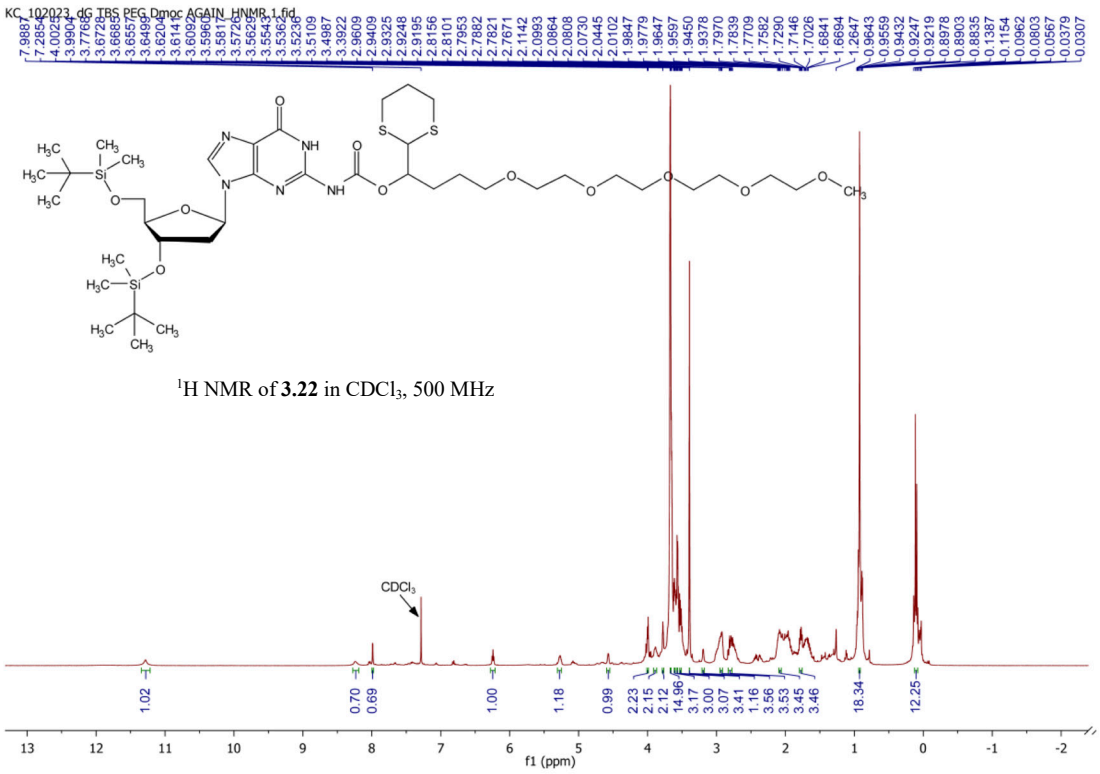
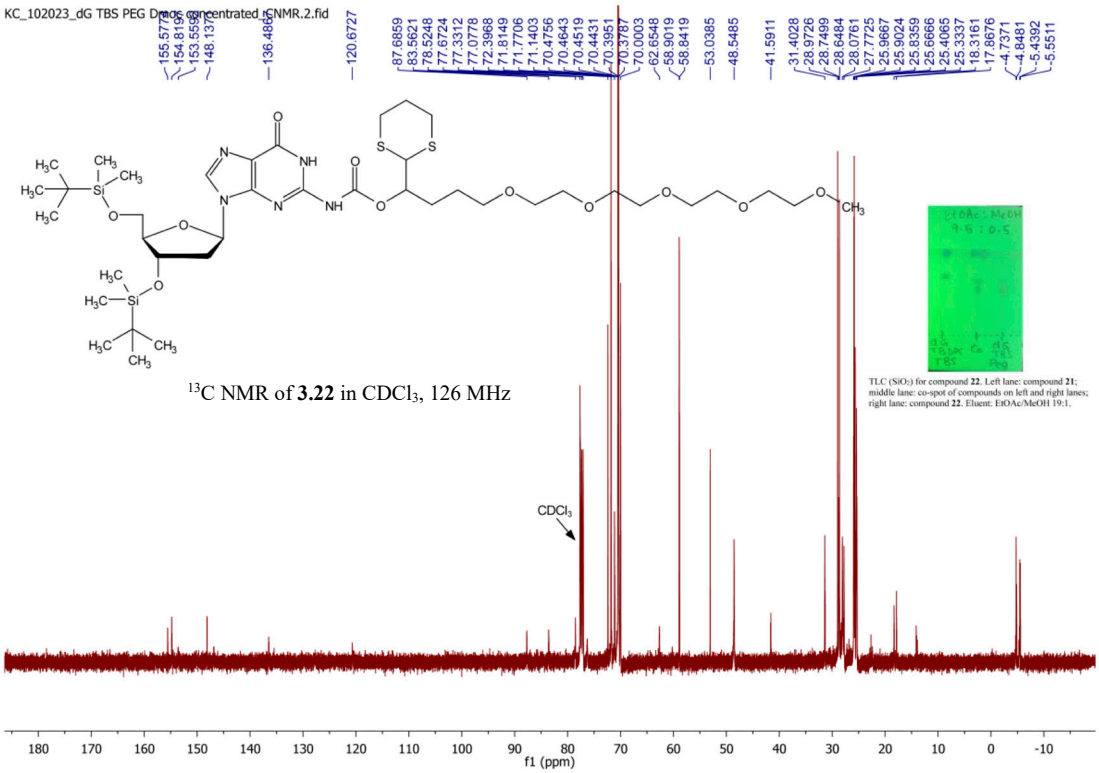


Figure 6.87: ^{31}P NMR spectrum of compound **3.1b**.



^{1}H NMR of **3.22** in $CDCl_3$, 500 MHz

Figure 6.88: ^{1}H NMR spectrum of compound **3.22**.



^{13}C NMR of **3.22** in $CDCl_3$, 126 MHz

TLC (SiO_2) for compound **22**. Left lane: compound **21**; middle lane: co-spot of compounds on left and right lanes; right lane: compound **22**. Eluent: EtOAc/MeOH 19:1.

Figure 6.89: ^{13}C NMR spectrum of compound **3.22**.

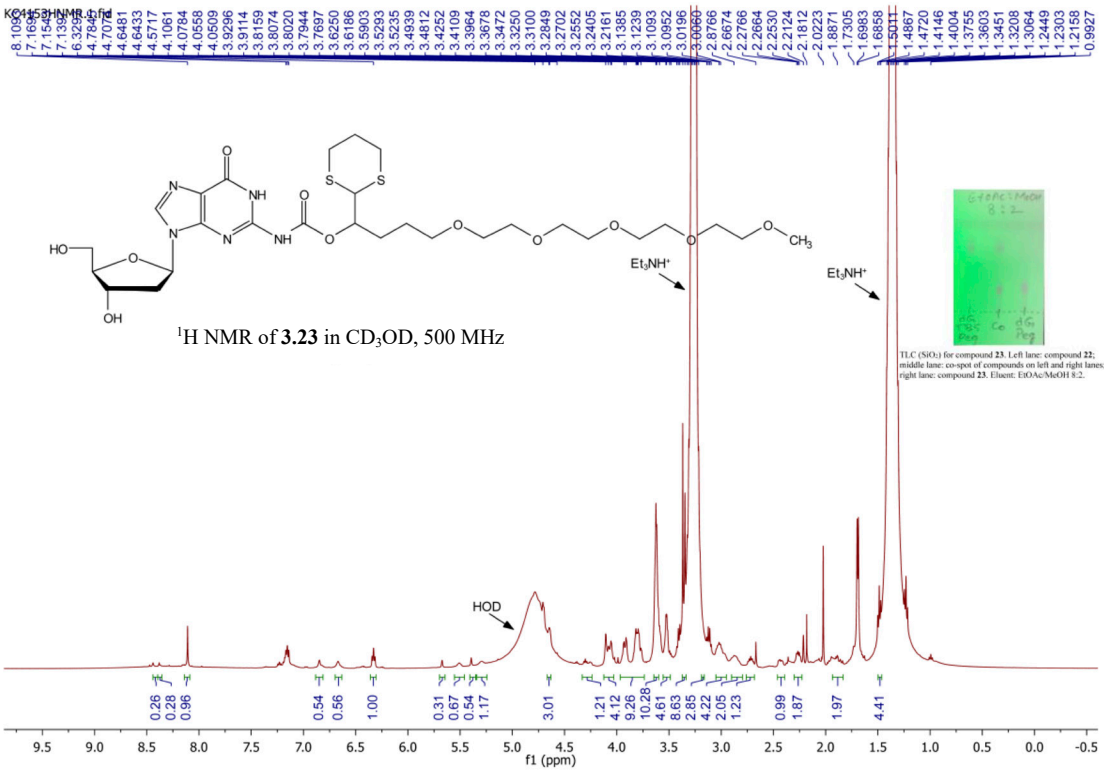
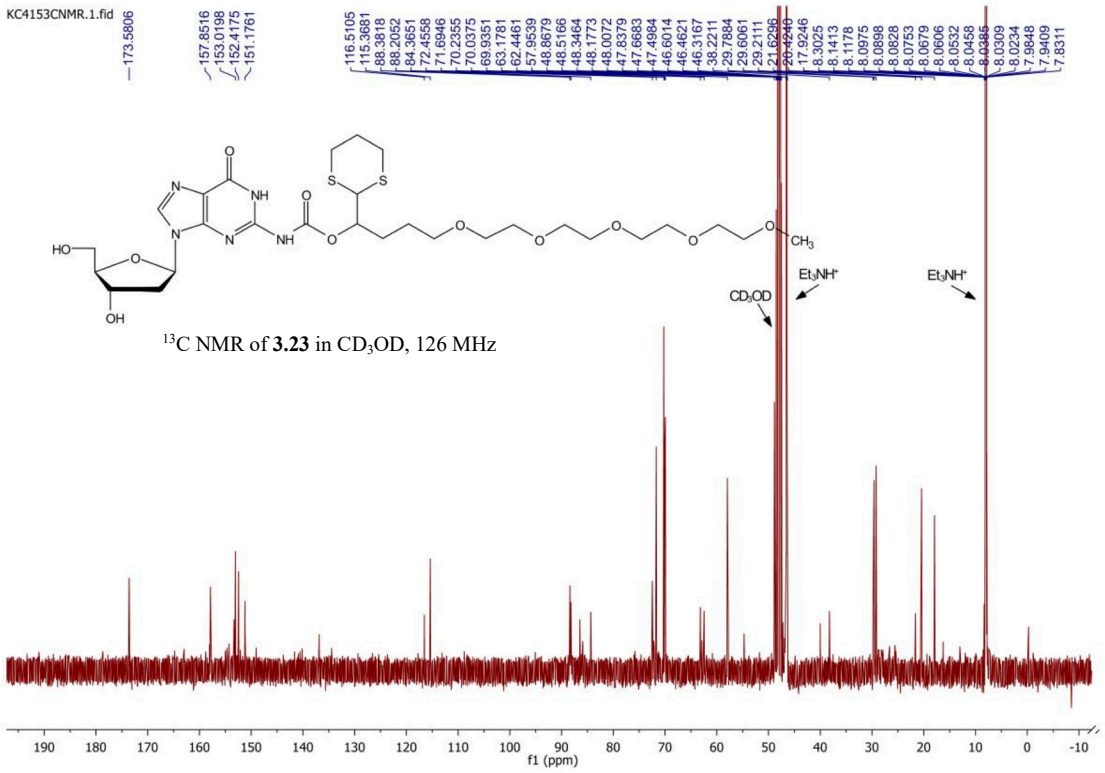
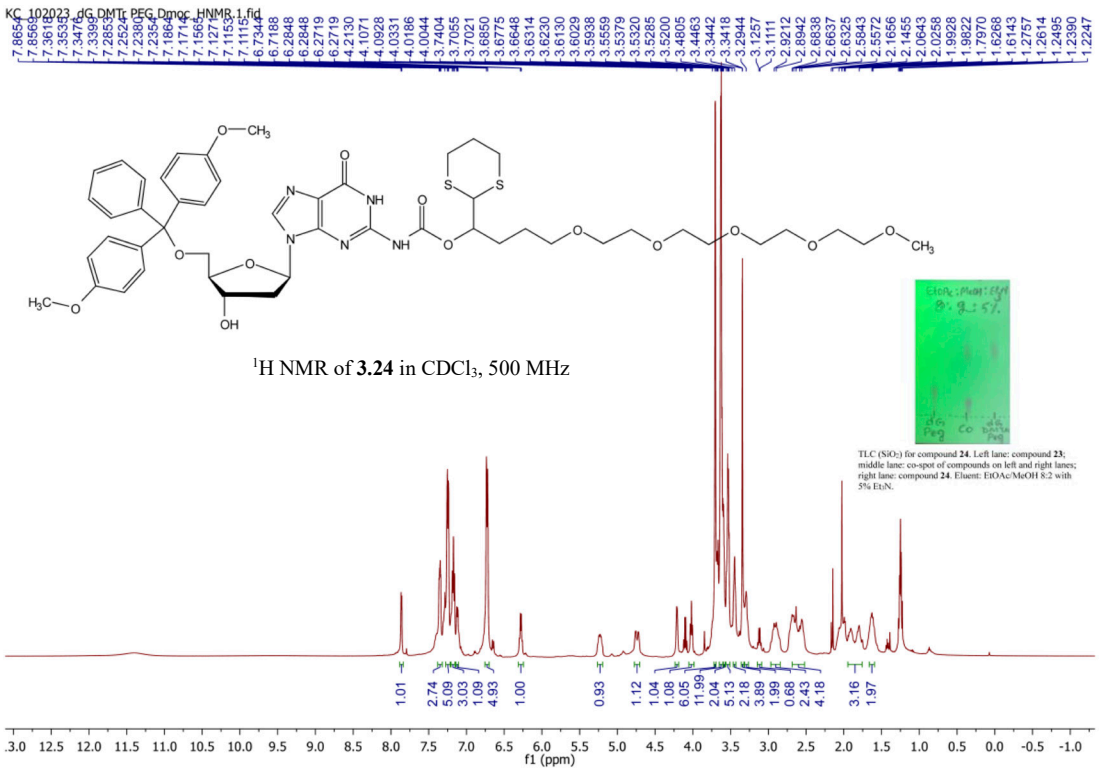


Figure 6.90: ^{1}H NMR spectrum of compound **3.23**.



^{13}C NMR of **3.23** in CD_3OD, 126 MHz

Figure 6.91: ^{13}C NMR spectrum of compound **3.23**.



^{1}H NMR of **3.24** in $CDCl_3$, 500 MHz

TLC (SiO_2) for compound **24**. Left lane: compound **23**; middle lane: co-spot of compounds on left and right lanes; right lane: compound **24**. Eluent: EtOAc/MeOH 8:2 with 5% Et_3N.

Figure 6.92: ^{1}H NMR spectrum of compound **3.24**.

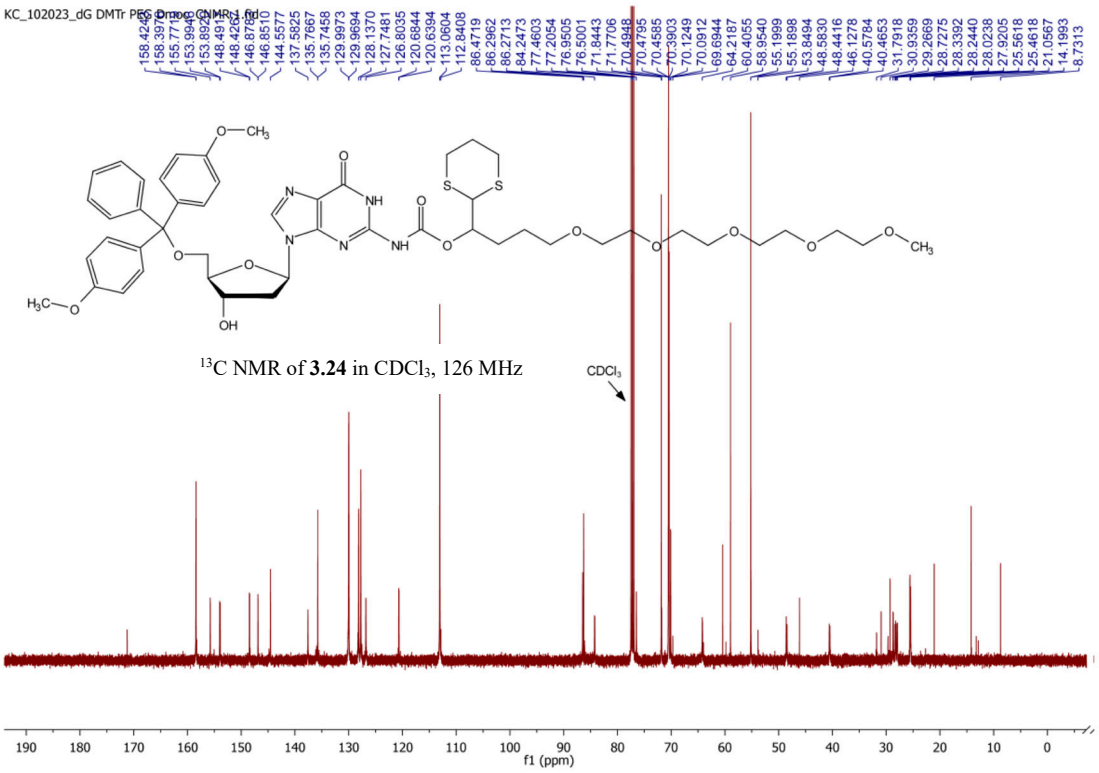


Figure 6.93: ^{13}C NMR spectrum of compound **3.24**.

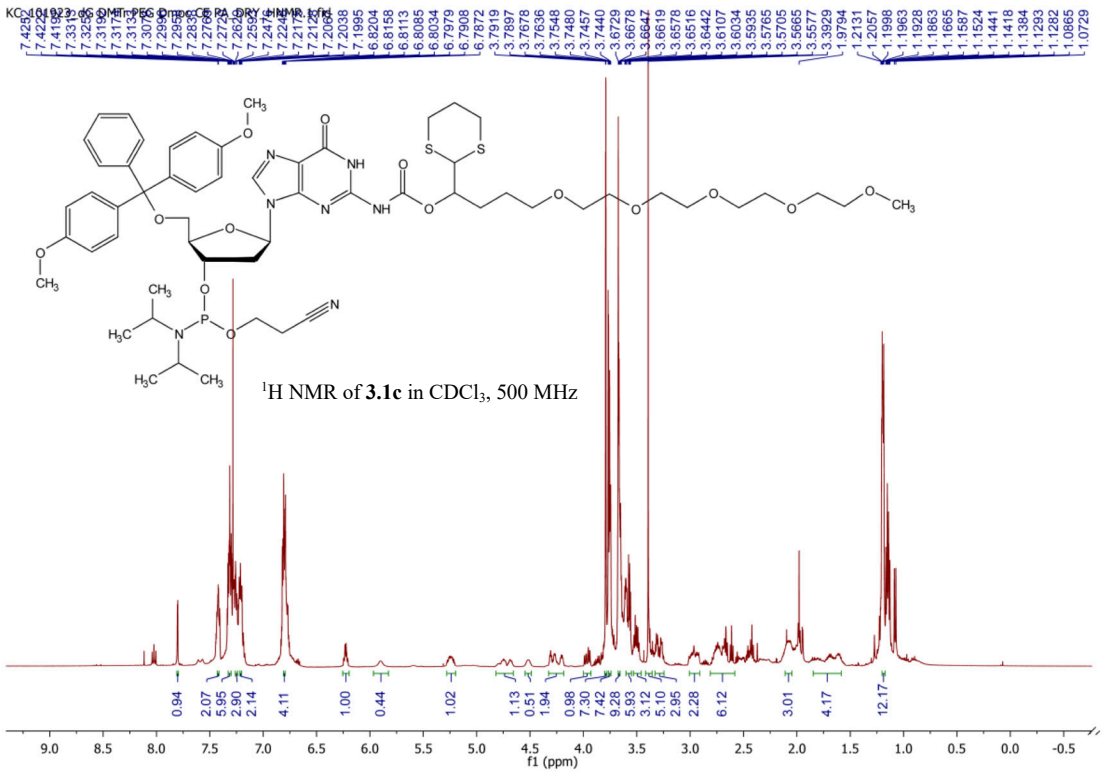


Figure 6.94: ^{1}H NMR spectrum of compound **3.1c**.

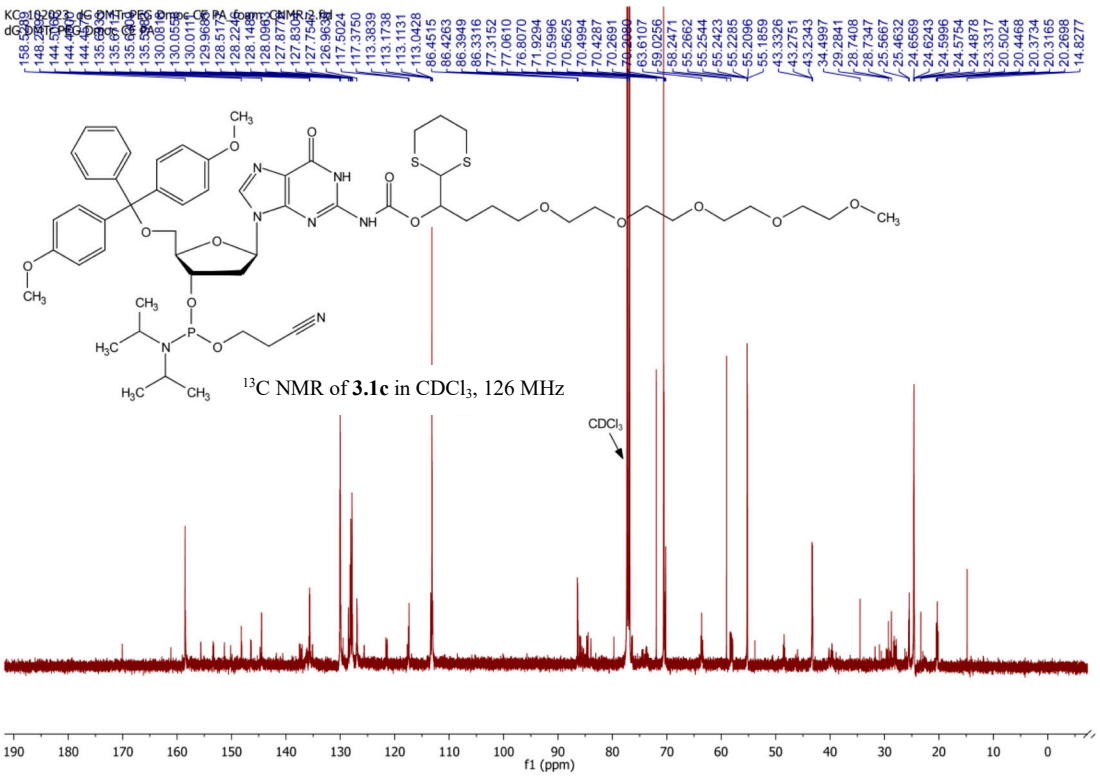
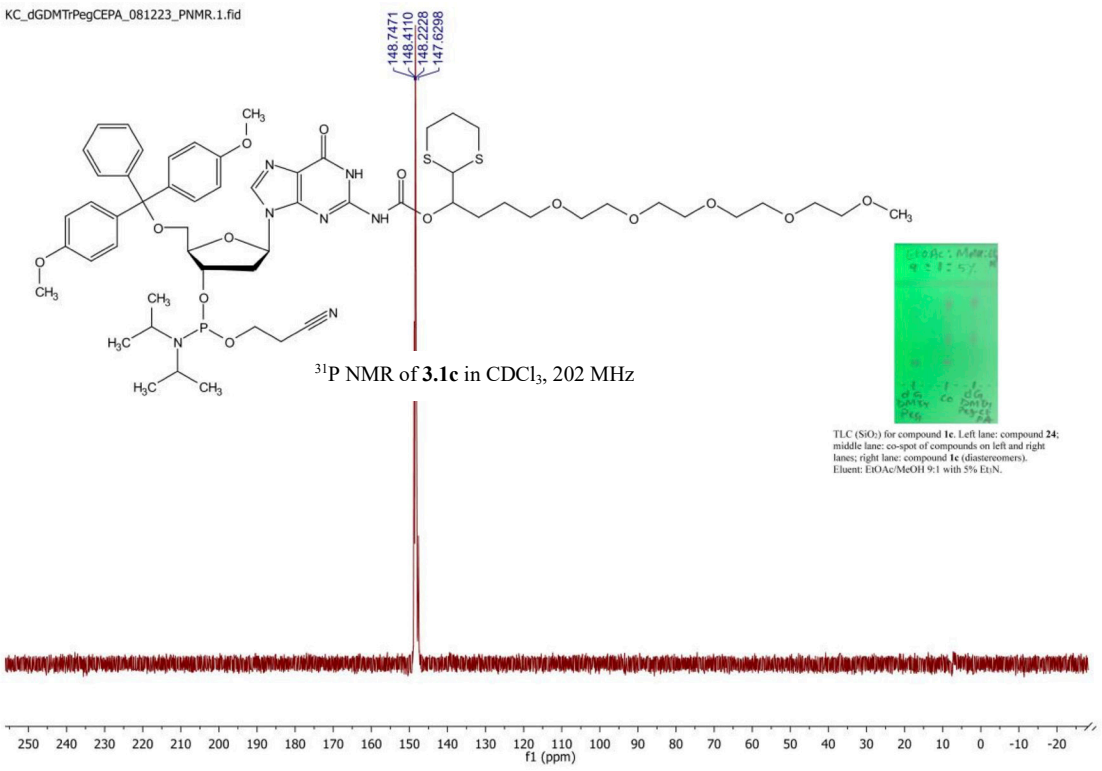


Figure 6.95: ^{13}C NMR spectrum of compound **3.1c**.



^{31}P NMR of **3.1c** in $CDCl_3$, 202 MHz

TLC (SiO_2) for compound **1c**. Left lane: compound **24**; middle lane: co-spot of compounds on left and right lanes; right lane: compound **1c** (diastereomers). Eluent: EtOAc/MeOH 9:1 with 5% Et_3N.

Figure 6.96: ^{31}P NMR spectrum of compound **3.1c**.

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