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Synthesis of Oligodeoxynucleotides Containing Sensitive Electrophiles

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SYNTHESIS OF OLIGODEOXYNUCLEOTIDES CONTAINING SENSITIVE ELECTROPHILES

By

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Preface

The initial ideas of all research projects in this dissertation were directed under the supervision of Dr. Shiyue Fang. All of the writing of this dissertation (Chapter 1 – 4) was carried out by Mr. Shahien Shahsavari and revised by Dr. Shiyue Fang.

In chapter 2, Dr. Xi Lin and Dr. Jinsen Chen equally contributed to the experiments. The content was published in *The American Chemical Society Organic Letters* **2016**, *18*, 3870–3873.

In chapter 3, all the experiments, purification, and data analysis were conducted by Mr. Shahien Shahsavari. The content has been submitted to *The American Chemical Society Journal of Organic Chemistry*.

In chapter 4, all the experiments, purification, and data analysis were conducted by Mr. Shahien Shahsavari. The content has been accepted in *Beilstein Journal of Organic Chemistry.*

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I would like to express special thanks to my family whose unconditional love and support pushed me through this journey. My parents (Ray and Sarah) and my brother (Ashkan) have been a true blessing for which I thank God Almighty daily. I leave this last section for my dearest friend Vagarshak Begoyan whose love and support has been unimaginable. Thank you all for making this possible.

Abstract

Oligodeoxynucleotides (ODNs) containing electrophilic groups are useful in many studies including antisense drug development and DNA/protein interaction. Due to the use of strong nucleophiles for cleavage and deprotection, traditional ODN synthesis methods are not suitable for their preparation. To solve this problem, a new ODN synthesis technology using the 1,3-dithiane-2-yl-methoxycarbonyl (Dmoc) function as protecting groups and linker has been developed. Furthermore, Dmoc-derivatives were developed to demonstrate the feasibility of the technology. The Dmoc and Dmoc derivative functions are stable under all ODN synthesis conditions using the phosphoramidite chemistry. Upon oxidation of the sulfides in them, because of the drastically increased acidity of H-2, the groups and linker are readily cleaved under nearly non-nucleophilic conditions. Many sensitive electrophiles were able to be incorporated on DNA strands successfully using these technologies. These include but are not limited to sensitive thioester, ethyl ester, alkyl chloride, and α-chloroacetamide moieties.

Chapter 1 Introduction

1.1 Oligodeoxynucleotides (ODNs)

Oligodeoxynucleotides (ODNs) are single-stranded deoxyribonucleic acids, also known as DNA, which consist of four nucleotides – deoxythymidine (dT), deoxycytidine (dC), deoxyadenosine (dA), and deoxyguanosine (dG). All four nucleotides contain their respective nucleobase, a 2'-deoxribose sugar moiety, and are linked together in a 3' to 5' fashion via a phosphate diester backbone. DNA is found in every known living organism, including many viruses, which provides pathways for genetic coding, cellular signaling, and reproduction.

1.2 Applications of ODNs

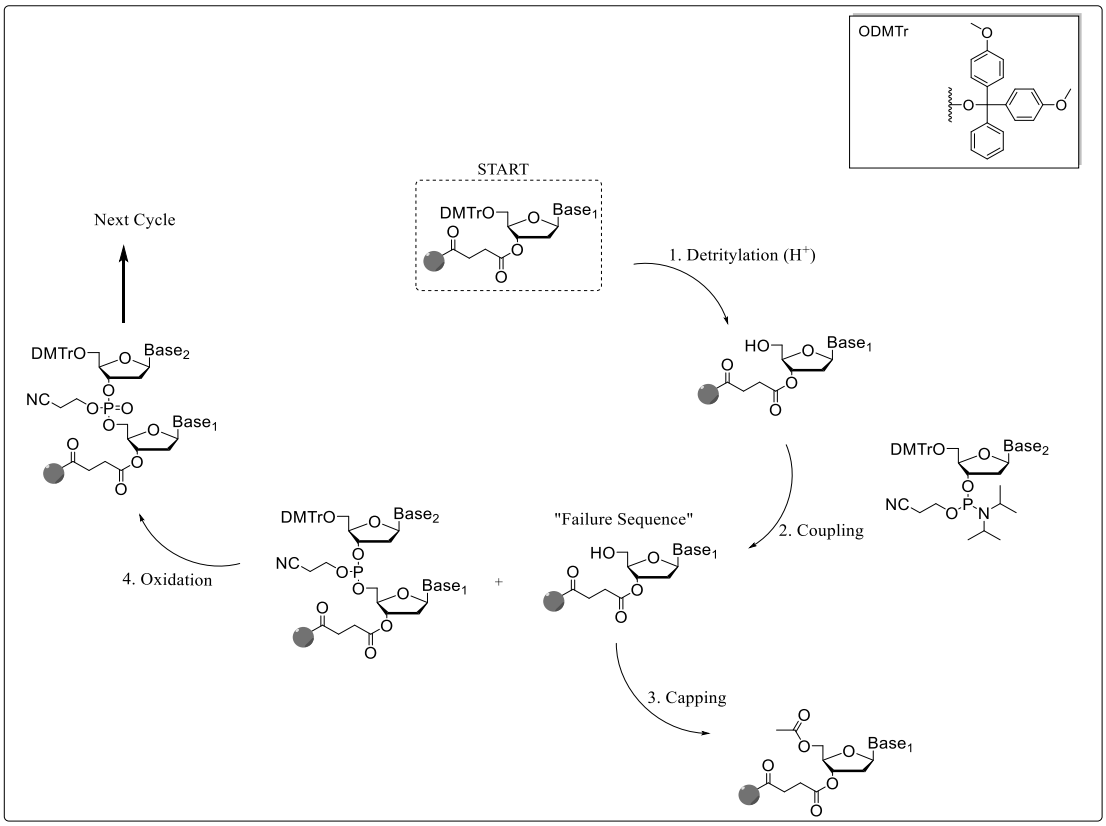
Synthetic ODNs hold a variety of applications in the pharmaceutical world such as antisense drug development, [1] synthetic biology, [2] CRISPR genome editing, [3] as well as DNA data storage. [4] Currently, several FDA approved oligonucleotide based antisense drugs are on the market such as fomivirsen, mipomersen, etelplirsen, and nisinersen.

1.3 Solid Phase ODN Synthesis

Generally, a universal solid support consisting of a controlled pore glass (CPG) covalently bonded to a succinyl linker is used in conjunction with phosphoramidite monomers to synthesize the desired ODN lengths (typically ranging from 15 - 100 nucleotides). The synthetic DNA cycle consists of four different routine steps. Initially, the acid-labile 4,4-dimethoxytrityl protecting group (DMTr) on the 5' alcohol end is removed by the treatment with 3% dichloroacetic acid in dichloromethane. This process is crucial

to unmask the primary alcohol functional group which enables the first coupling step to occur. The 5'-OH group is then reacted with a phosphoramidite monomer along with activator 1*H*-tetrazole. The activator is needed to catalyze and displace the diisisopropyl amino group of the newly added phosphoramidite monomer which creates an enhanced leaving group. A phosphite triester is generated once the 5'-OH group successfully reacts and displaces the activated leaving group. However, since the reaction yield is not quantitative the unreacted free 5'-OH groups on the resin-bound nucleotide, called failure sequences, need to be masked so they do not interfere with the rest of the synthetic cycle. This is generally achieved by capping them with an excess of acetic anhydride catalyzed by *N*-methyl imidazole. The fourth and final step of the cycle consists of the oxidation of the phosphite triester to its more stable pentavalent state. This is accomplished by the addition of iodine in the presence of water and pyridine. The resultant phosphotriester serves as the backbone of the DNA strand. After the desired ODN length is obtained it needs to be cleaved off the solid support. The exo-amino groups of the nucleotides are usually pre-protected with acetyl or benzoyl (for dA and dC) and isobutyryl (for dG) groups. The succinyl linker on the resin as well as these exo-amino protecting groups can easily be removed with concentrated ammonium hydroxide solution at 55 °C for 12 hours. Additionally, Ultramild conditions have also been developed. In these cases, the exo-amino groups of the phosphoramidites are protected with acetyl (for dC), phenoxyacetyl (for dA), and isopropyl phenoxyacetyl (for dG). These more labile groups can be removed with concentrated ammonium hydroxide solution at room temperature in 2 hours or with 0.05M potassium carbonate in methanol at room temperature for 4 hours. However, these

deprotection and cleavage conditions are not suitable for ODNs containing nucleophile-labile moieties.



Scheme 1.1. Solid Phase ODN Synthesis

1.4 Limitations of Solid Phase ODN Synthesis

For most purposes, ODNs are synthesized solely using automated solid-phase methods. While there have been many advances in the protocols, the widely used method still has drawbacks. For instance, the detritylation step uses the abovementioned solution of dichloroacetic acid in dichloromethane. These acidic conditions make it impossible to incorporate acid-sensitive substrates on ODNs. Furthermore, the use of basic and nucleophilic conditions for cleavage and deprotection purposes prevent the practical

incorporation of electrophiles such as activated esters, aziridines, epoxides, α-halo substituted carbonyl functional groups, as well as alkyl halides. Therefore, there is a need for a new technology to overcome these challenges.

1.5 The Need for Modified ODNs

ODNs containing electrophilic functionalities are predicted to have wide applications in fields such as covalent cross-linking studies with messenger ribonucleic acid (mRNA) [5] and protein interactions. [6] Moreover, electrophiles anchored on DNA can enable the synthesis of model compounds which can aid in DNA damage findings such as methylation and demethylation studies. [7] Over the past several years, topics regarding the uptake, metabolism, as well as cellular trafficking of DNA has been noticeably studied. Particularly, there has been emerging interest in the hybridization of antisense oligonucleotides to mRNAs. The incorporation of electrophilic moieties on ODNs can successfully enable the formation of a covalent bond between the modified ODN and the target specific mRNA sequence. However, such DNA-based therapeutics are limited to the integration of the host genome's plasmids. Additionally, there is currently no known commercially viable cross-linking agents available. [8] Thus, there is a strong necessity for nucleophile-labile ODNs.

1.6 Current Methods to Modify ODNs and Their Drawbacks

Currently, there are a small number of approaches mentioned in the literature. The first type attempts the functionalization of electrophilic moieties on ODN after cleavage and deprotection. [8] However, since functionalities are limited in organic chemistry this needs

to be developed on a case-by-case basis and is therefore restrictive. The idea to use protecting groups and linkers that can be cleaved under nearly neutral conditions have been mentioned in the literature. In such cases, palladium-removable allyl groups along with photo-labile linkers have been used. [9] However, transition metals such as palladium are expensive and difficult to remove. Furthermore, it is known that UV irradiation has the potential to damage DNA. There have also been attempts to use enzymatic reactions to access electrophilic ODNs. [10] This strategy has narrow applicable viability and is associated with high costs.

1.7 Our Non-Nucleophilic Technology to Incorporate Electrophiles on ODN

To overcome the aforementioned problems, we have developed a non-nucleophilic technology to install electrophiles on DNA strands. The following paragraphs briefly depict the concepts behind the original technology and its improved derivatives.

1.7.1 Dmoc Technology

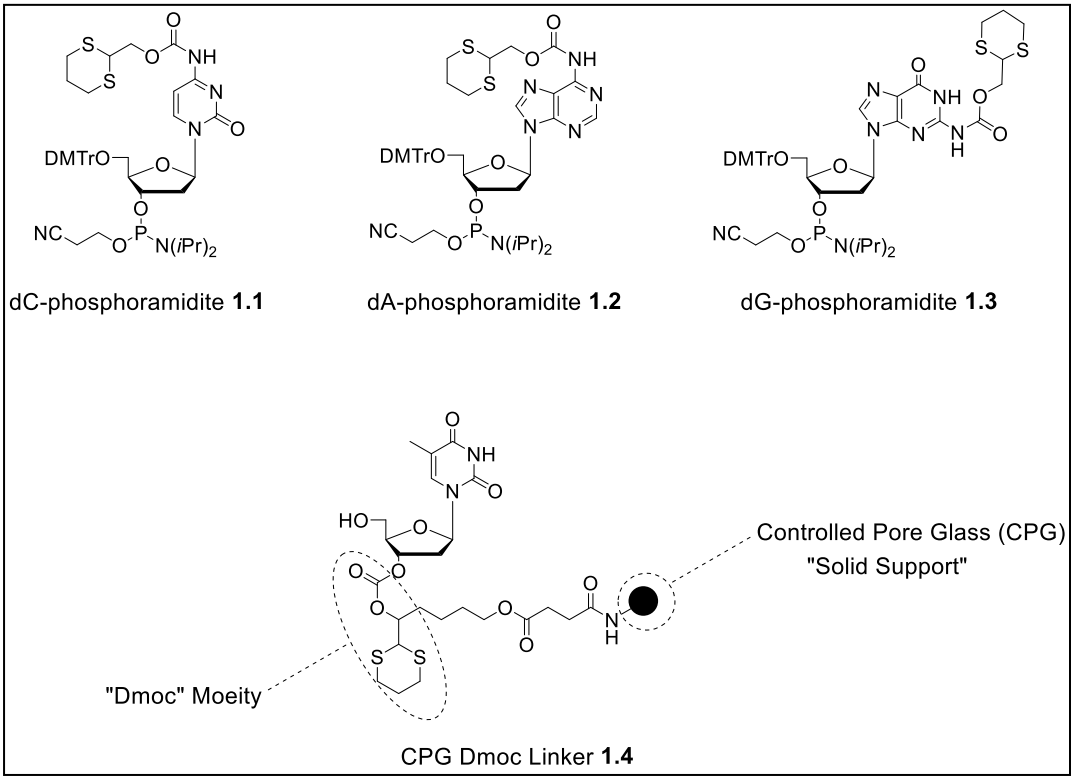


Figure 1.1. Dmoc Phosphoramidites and Dmoc Linker

We have successfully developed a new technology that allows us to incorporate sensitive functionalities on ODNs. As shown in Figure 1.1, the technology is based on the design of 1,3-dithiane-2-yl-methoxycarbonyl (Dmoc) protecting groups (**1.1**-**1.3**) and linkers (**1.4**) used for ODN synthesis. The Dmoc based groups are stable under all standard ODN synthesis conditions. Once the desired ODN length is obtained, the cyanoethyl groups on the phosphate backbone are removed with DBU in acetonitrile at room temperature in 15 minutes. After inducing the beta elimination, the hydrophilic ODN backbone is revealed which can then be subjected to aqueous oxidative conditions to oxidize the sulfur moieties into their respective sulfoxide and sulfone states. Once

oxidation is completed, the Dmoc protecting groups and linker are cleaved under mild nearly non-nucleophilic conditions due to the drastically increased acidity of H-2 in the 1,3-dithiane moieties. Thus, this technology enabled us to incorporate electrophiles on ODNs. Specifics are mentioned in the respective chapter section.

1.7.2 Dmoc Dim Technology

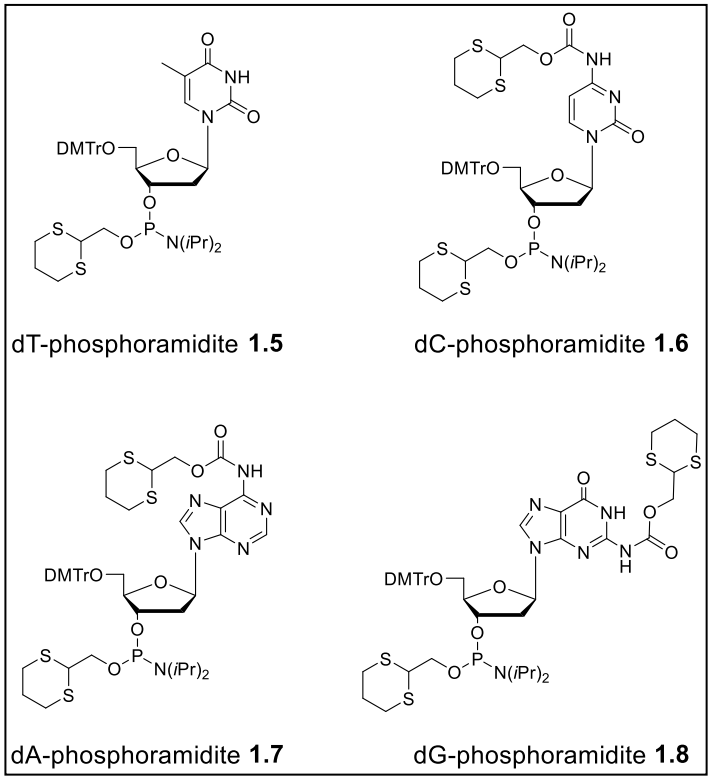
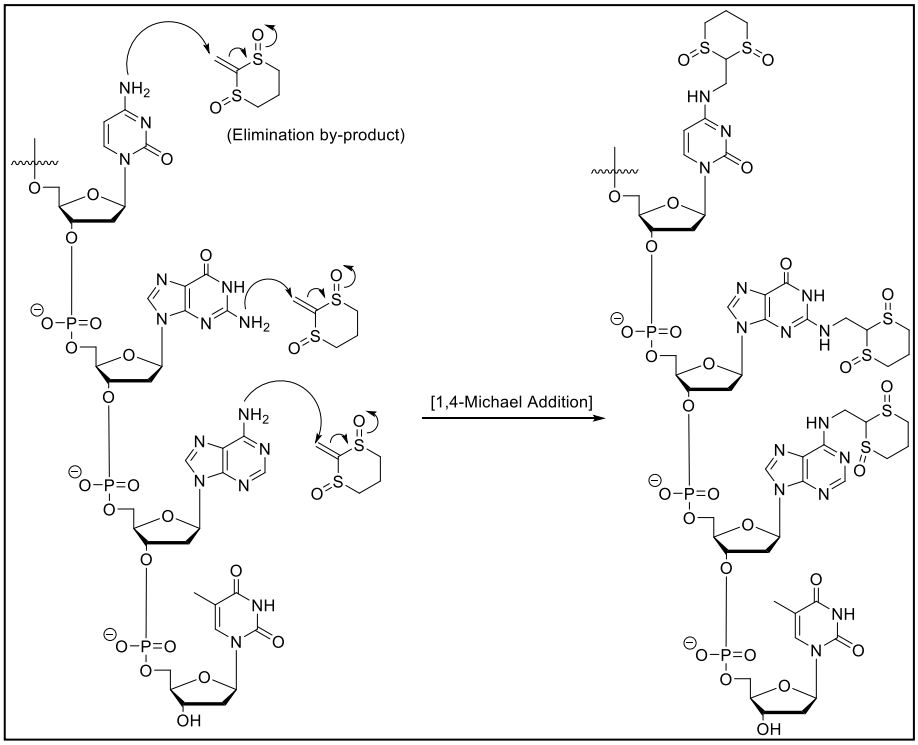


Figure 1.2. Dmoc Dim Phosphoramidites

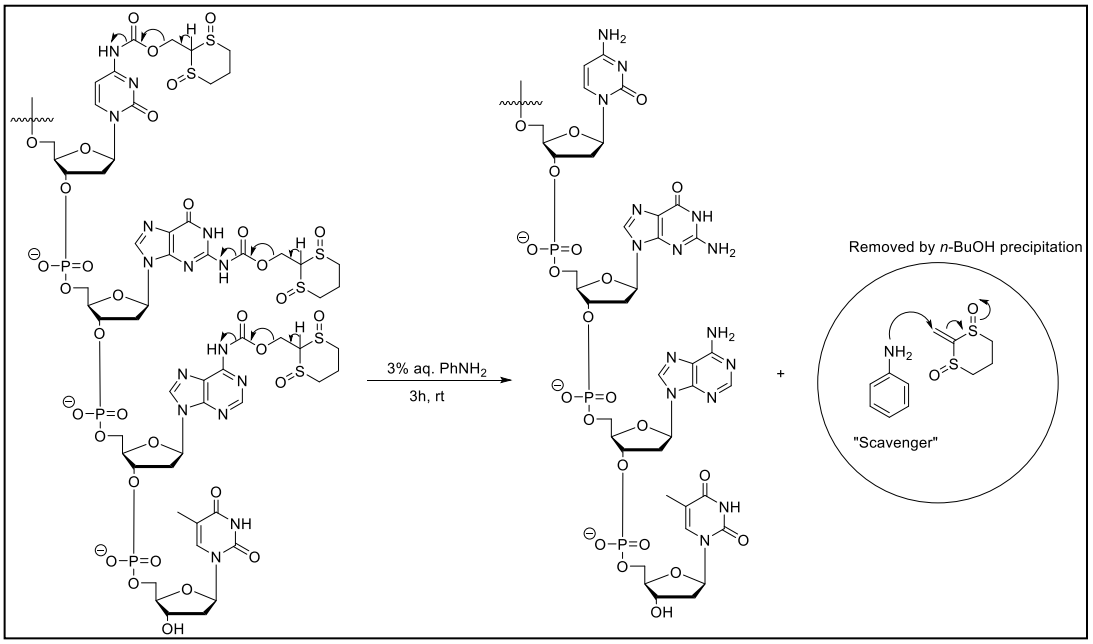
Since DBU is used to remove the cyanoethyl groups, some base-labile ODNs may not be compatible with our technology. Therefore, the Dmoc based protecting groups and linker were then further studied to eliminate the need for DBU in acetonitrile and, thus, improved the technology. As shown in Figure 1.2, we were able to replace the

cyanoethyl group with a 1,3-dithian-2-yl-methyl (Dim) moiety to mask the P(III) of the phosphoramidites (**1.5**-**1.8**). Therefore, this eliminated the need for DBU and reduced the post ODN work up to ultimately two steps – Oxidation of the 1,3-dithiane moieties followed by elimination. Utilizing the CPG Dmoc linker **1.4** along with the Dim modifications enabled us to incorporate additional electrophiles on ODNs. While satisfactory results were obtained using the Dmoc and Dim technology, we still experienced one major drawback – The induced oxidized elimination by-product was undergoing a 1,4-Michael Addition reaction with the free exo-amino groups on the DNA strands, as shown in Scheme 1.2.



Scheme 1.2. Michael Addition of ODN with Dim Elimination By-Product

Therefore, we needed to use aniline as both the base and a scavenger, as shown in Scheme 1.3. Specifics are mentioned in the respective chapter section.



Scheme 1.3. Using Aniline as a Scavenger during ODN Work-Up

1.7.3 dM-Dmoc Technology

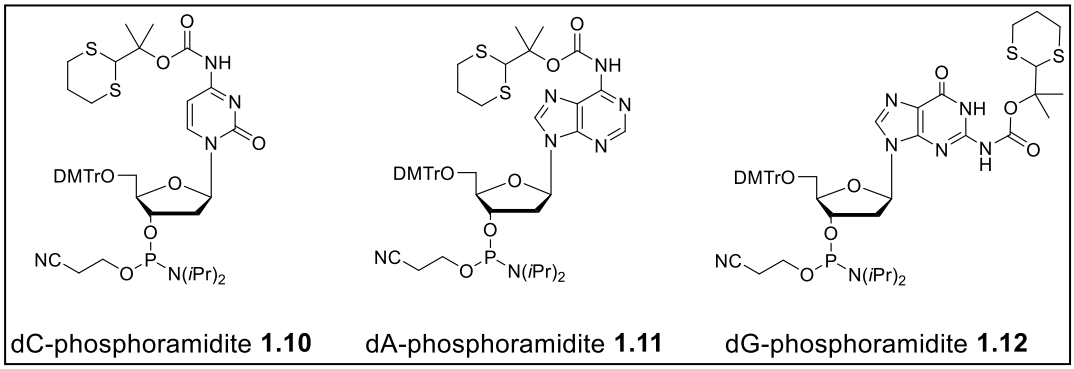
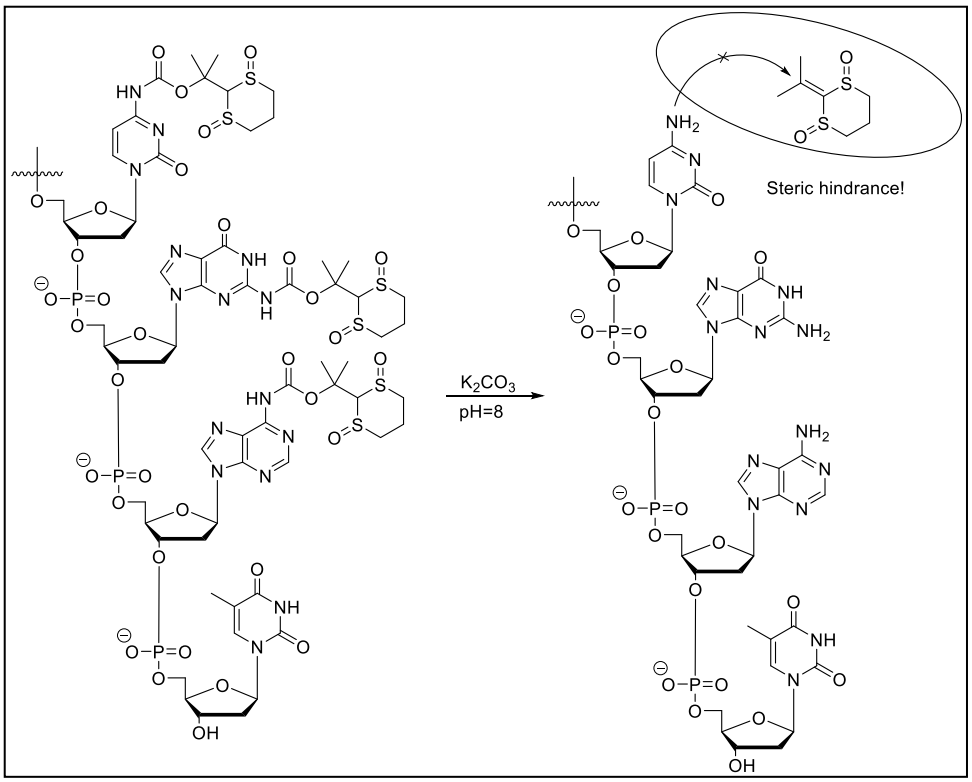


Figure 1.3. dM-Dmoc Phosphoramidite

Since aniline is a potential nucleophile, some ODNs containing nucleophile-labile functionalities may not be compatible with the abovementioned technology. Thus, we diverted our attention to modifying the exo-amino protecting groups to a more robust version. This modified technology uses a dimethyl Dmoc (dM-Dmoc) protecting group for

the phosphoramidites **1.10**-**1.12** (Figure 1.3). Indeed, experimental results showed that the dimethyl dim elimination by-product is too sterically hindered for the troublesome 1,4-Michael Addition reaction to occur (Scheme 1.7). Thus, the usage of scavengers (such as aniline) were avoided and beta elimination was achieved by aqueous potassium carbonate solution (pH=8).



Scheme 1.4. Post ODN Synthesis Work-Up Using dM-Dmoc

However, some drawbacks were experienced via this technology as well – Particularly, premature cleavage of the dM-Dmoc protecting group during the ODN synthesis due to repetitive treatment of dichloroacetic acid in dichloromethane. This resulted in minor branched side sequences which we were able to resolve at the end. Specifics are mentioned in the respective chapter section.

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Chapter 2 Synthesis of Oligonucleotides Containing Electrophilic Groups

Work in this chapter has been published in American Chemical Society Organic Letters

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Abstract

Oligodeoxynucleotides (ODNs) containing electrophilic groups are useful in many studies including antisense drug development and DNA/protein interaction. Due to the use of strong nucleophiles for cleavage and deprotection, traditional ODN synthesis methods are not suitable for their preparation. To solve this problem, a new ODN synthesis technology using the 1,3-dithiane-2-yl-methoxycarbonyl (Dmoc) function as protecting groups and linker has been developed. The Dmoc function is stable under all ODN synthesis conditions using the phosphoramidite chemistry. Upon oxidation of the sulfides in them, because of the drastically increased acidity of H-2, the groups and linker are readily cleaved under nearly non-nucleophilic conditions. Five ODNs including one with a thioester group and another with an α-chloroamide function were successfully synthesized using the strategy. It is predicted that the technique could be adaptable for the synthesis of ODNs containing other electrophiles.

2.1 Introduction

In traditional solid-phase oligodeoxynucleotide (ODN) synthesis, the amino groups on the nucleobases are protected with acyl groups that have to be removed with strongly nucleophilic reagents such as ammonium hydroxide. The widely used succinyl ester linkage is also cleaved under these conditions. For this reason, the methods are not suitable for the synthesis of ODN analogues that contain electrophilic functionalities. However, such analogues have found or are predicted to have wide applications in areas such as covalent cross-linking with messenger ribonucleic acid (mRNA) for antisense drug development,[1] analysis of nucleic acid and protein interactions by detecting cross-linked fragments using mass spectrometry,[2] and the synthesis of model compounds of sensitive nucleic acid intermediates in cells for deoxyribonucleic acid (DNA) damage and DNA methylation and demethylation studies.[3] Current strategies for electrophilic ODN synthesis include two types. One type uses protecting groups and linkers cleavable under less basic or nearly neutral conditions. However, functions in organic chemistry suitable for the need are limited. Those used in the literature include the more base-labile phenoxyacetyl based groups and linker,[4] the palladium-removable allyl groups,[3b,5] and the photolabile o-nitrobenzyl linker.[3b,6] ODN synthesis methods using these functionalities for protecting and linking still have serious drawbacks. For example, the phenoxyacetyl groups and linkers are usually cleaved with dilute K_2CO_3 in methanol or aqueous ammonium hydroxide.[4] These conditions are still strongly nucleophilic. Palladium is expensive and difficult to remove. UV irradiation can damage ODN. The second type uses traditional methods to make an ODN precursor, which is stable under nucleophilic conditions. After cleavage and deprotection, the electrophilic functionality is attached to or uncovered from

the precursor.[1c–e, g–j] These methods are inconvenient, have to be developed case by case, and are not always feasible. Besides the above two strategies, enzymatic reactions have also been used to access electrophilic ODNs.[1a] Drawbacks include narrow applications and high cost. Efforts were also made to search for conditions for ODN synthesis without nucleobase protection.[7] An ideal linker cleavable under non-nucleophilic conditions remains to be developed, and the challenge of achieving complete O-phosphitylation over N-phosphitylation in the coupling step may be nontrivial. In this paper, we report our studies on use of the 1,3-dithian-2-ylmethoxycarbonyl (Dmoc) based protecting group and linker for ODN synthesis. Previously, this and the similar 2-(phenylthio)ethyl group had been studied for peptide synthesis and thymidine protection, but they have not been tested for ODN synthesis.[8] We predicted that the Dmoc function would be stable under all ODN synthesis conditions. However, upon oxidation of the sulfides to sulfoxides or sulfones after synthesis, due to the drastically increased acidity of H-2, they could be cleaved under non-nucleophilic conditions. Using that technology, we successfully synthesized five ODNs including one containing a thioester and another containing an α-chloroacetyl function. The cleavage and deprotection were achieved in three steps under non-nucleophilic conditions. These electrophilic groups would not survive the nucleophilic conditions such as NH_4OH and K_2CO_3/MeOH, while incorporating them into ODNs is desirable due to their potential applications such as antisense drug development, sequence-specific DNA alkylation, and DNA−protein interaction studies.[1g,5a,9] We expect that the new method would be suitable for the synthesis of other electrophilic ODNs as well and, therefore, have a high impact in several research areas.

2.2 Results and Discussion

To carry out an electrophilic ODN synthesis using the Dmoc function for protecting and linking, a Dmoc linker such as that in dT-Dmoc-CPG (controlled pore glass) **2.1** and the phosphoramidite monomers Dmoc-dC-amidite **2.2**, Dmoc-dA-amidite **2.3**, and Dmoc-dG-amidite **2.4** are required (Figure 2.1).

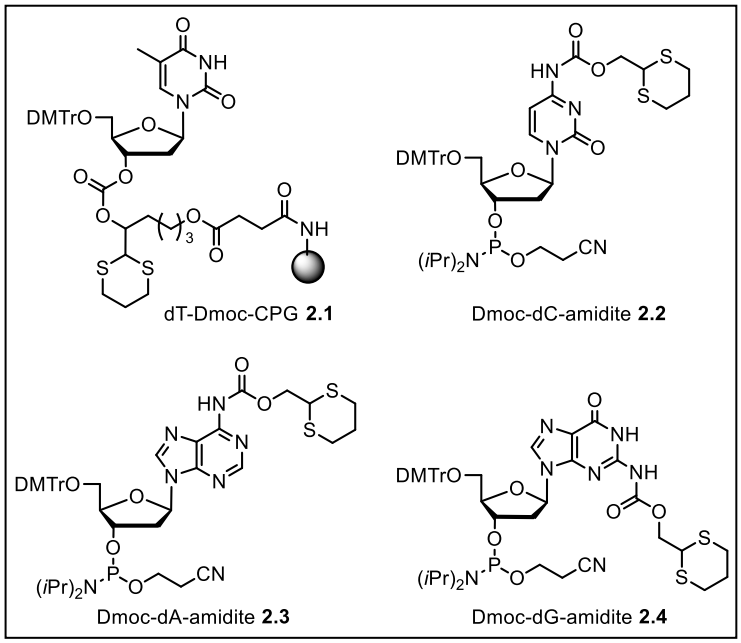
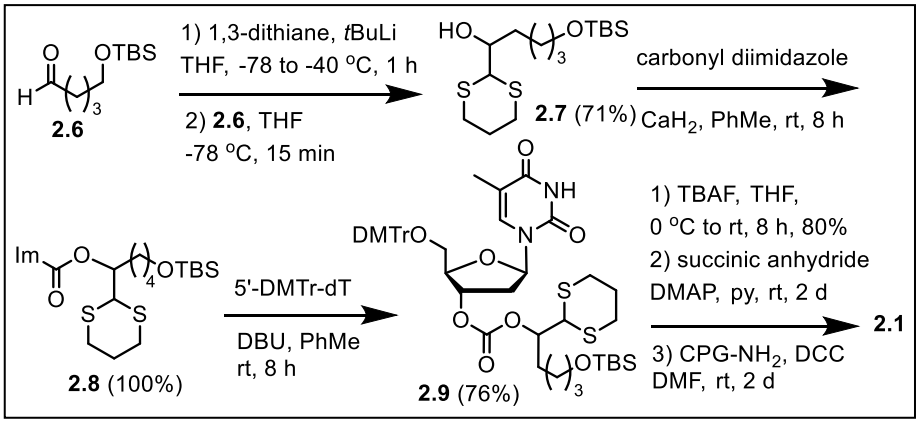


Figure 2.1. CPG with dT-Dmoc linker and Dmoc amidites

The corresponding dT monomer is not required because thymidine does not have an amino group, and a commercial dT amidite (**2.5**) can be used. The preparation of CPG **2.1** is shown in Scheme 2.1. The anion generated by treating 1,3-dithiane with *t*BuLi was reacted with aldehyde **2.6** to give **2.7**. Compound **2.7** was converted to **2.8** by reacting it with 1,1′-carbonyldiimidazole in the presence of calcium hydride. Reaction of **2.9** with 5′-DMTr-dT using DBU as the base gave **2.10**. Removal of the TBS group in **2.10** with TBAF afforded **2.11** (not shown in Scheme 2.1). Attaching **2.11** to CPG to give **2.1** was achieved by

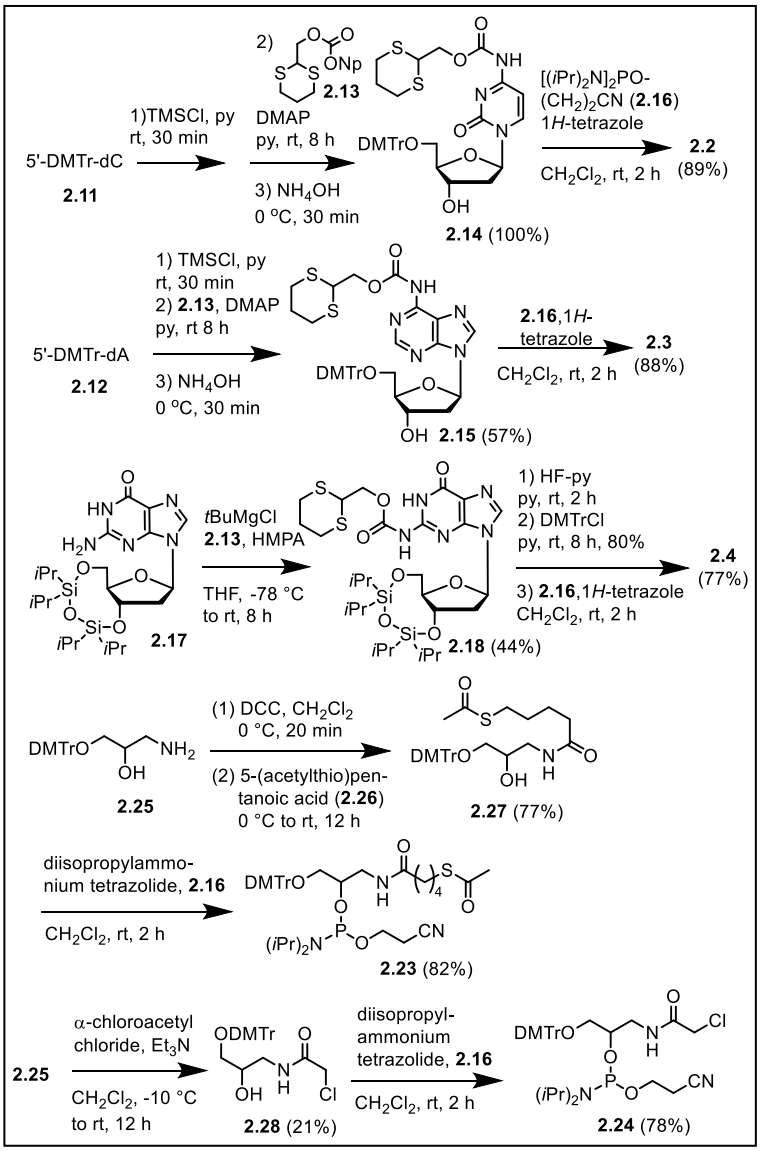
reacting **2.11** with succinic anhydride followed by incubation with amino CPG in the presence of DCC. Because we installed the 1,3-dithiane moiety at the side of the linkage instead of in the linkage, the construction of **2.1** is quite simple.



Scheme 2.1. Synthesis of dT-Dmoc-CPG

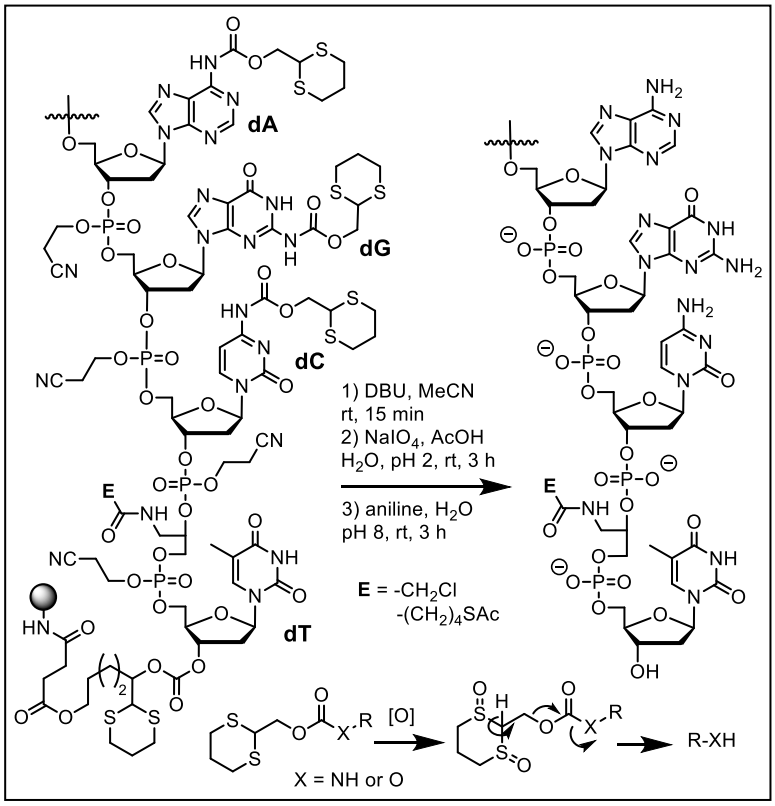
The synthesis of Dmoc amidite **2.2**–**2.4** is shown in Scheme 2.2. The amino groups of 5′-DMTr-dC **2.12** and dA **2.13** were conveniently protected using (1,3-dithian-2-yl)methyl 4-nitrophenylcarbonate (**2.14**)[8c] by first protecting the 3′-hydroxyl group with TMSCl temporarily followed by stirring the reactants at room temperature with DMAP as the catalyst. The products **2.15** and **2.16** were obtained in 100% and 57% yields, respectively. Phosphitylation of **2.15** and **2.16** using 2-cyano-N,N,N′,N′-tetraisopropylphosphorodiamidite (**2.17**) gave the amidite monomers Dmoc-dC-amidite **2.2** and Dmoc-dA-amidite **2.3**, respectively, in excellent yields. However, when the same acylation method was used to synthesize Dmoc-dG, no desired product could be isolated probably due to the lower basicity of the amino group of dG.[10] We tried several other conditions and finally settled with the following procedure. The O-protected dG **2.18**[11] was treated with excess *t*BuMgCl and **2.14** to give **2.19** in 44% yield. The silyl protecting group

was then removed by HF−pyridine,[12] and without purification the product was reacted with DMTr-Cl to give **2.20** (not shown in Scheme 2.2) in 80% yield after flash chromatography. Compound **2.20** was phosphitylated using **2.17** to give Dmoc-dG-amidite **2.4** in 77% yield. With dT-Dmoc-CPG **2.1** and amidite monomers **2.2**−**2.4** in hand, before synthesizing electrophilic ODNs, we tested the technology by synthesizing three unmodified ODNs.



Scheme 2.2. Synthesis of Amidite Monomers

They were 20-mers 5′ HO-TCA TTG CTG CTT ATA CCT CT-OH 3′ (**2.20**), 5′ HO-TCA TTG CTG CTT AGA CCG CT-OH 3′ (**2.21**), and 5′ HO-TTA GTA GGA CCT ACA CCT GT-OH 3′ (**2.22**). The conditions were the same as those in traditional ODN synthesis using the phosphoramidite chemistry. Concentration of the amidites was 0.1M. At the end of synthesis, the DMTr group was removed. According to the trityl assay, the coupling yields were not negatively affected by the Dmoc linker and protecting groups. For deprotection and cleavage, the 2-cyanoethyl groups were first removed by treating with DBU briefly (Scheme 2.3). The sulfides in Dmoc were then oxidized with $NaIO_4$ at pH 2 in 3 h.[13] The acidity of H-2 in Dmoc is now drastically increased. However, due to the acidic conditions, β-elimination did not occur at this stage as indicated by HPLC analysis of the supernatant. After removal of the supernatant, residue $NaIO_4$ was washed away with water at pH 2. Final cleavage and deprotection of ODN were then induced with an aniline solution at pH 8 (Scheme 2.3).



Scheme 2.3. ODN Deprotection and Cleavage

The crude ODNs **2.20**–**2.22** were purified with RP HPLC. The profiles of crude and purified **2.20** are shown in Figure 2.2. Those of **2.21**–**2.22** are shown in the Supporting Information. All of the ODNs were analyzed with MALDI-TOF MS, which gave correct molecular masses (Supporting Information). We next decided to incorporate the nucleophile-sensitive thioester and α-chloroacetyl functions into ODNs. The thioester function was used as a phosphate masking group in ODN prodrugs. For the application, the thioester had to be kept intact during ODN synthesis, cleavage, and deprotection.[5a,9] ODNs containing an α-chloroacetyl function could find applications in sequence-specific alkylation and cleavage of DNA and other areas.[1g–j] We chose to incorporate the electrophilic groups into the middle of the sequences because it is more challenging than

attaching it to the 5′-end. The required amidites **2.23** and **2.24**, which contained the thioester and α-chloroacetyl, respectively, were prepared according to Scheme 2.2. Compound **2.25**[14] was coupled with 5-(acetylthio)pentanoic acid (**2.26**)[15] to give **2.27**, which was phosphitylated to give amidite **2.23**. Amidite **2.24** was also prepared from **2.25**. Acylation of **2.25** with α-chloroacetyl chloride gave **2.28**, which was phosphitylated to give **2.22**. Using the Dmoc-CPG **2.1** and amidites **2.2**−**2.4**, we successfully incorporated **2.23** and **2.24** into ODNs 5′ HO-TCA TTG CTG CTT A-X-A CCT CT−OH3′ (**2.30**) and 5′ HO-TCA TTG CTG CTT A-Y-A CCT CT−OH 3′ (**2.31**), where X and Y are the thioester and α-chloroacetyl units introduced with **2.23** and **2.24**, respectively. The sequences were derived from **2.20** by replacing a T with X or Y. The conditions for ODN synthesis and cleavage and deprotection were the same as described above.

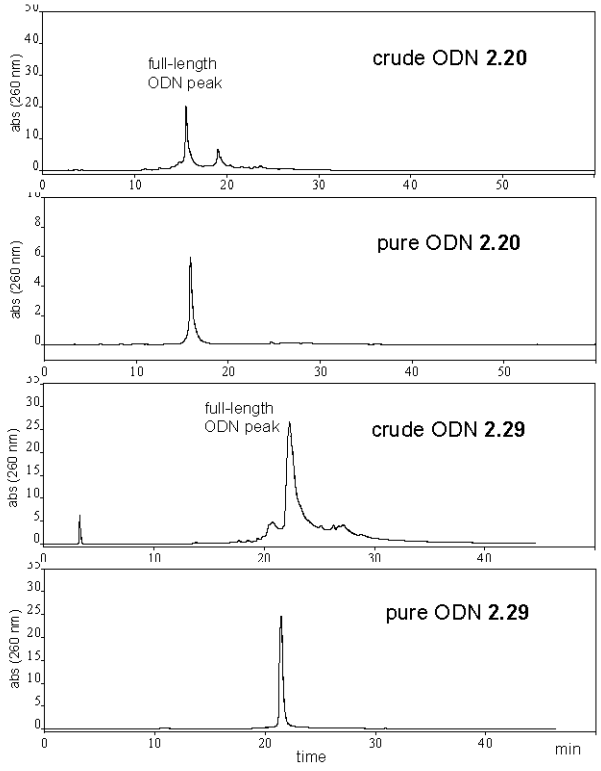


Figure 2.2. RP HPLC profiles of ODN **2.20** and **2.29**

No modification of the procedure was needed except that a simpler precipitation method instead of size-exclusion chromatography was used to separate ODN from small molecules after ODN cleavage and deprotection. The ODNs were purified with RP HPLC. MALDI-TOF MS analyses gave correct molecular masses (Supporting Information). The RP HPLC profiles of crude and purified **2.29** are in Figure 2.2. Those of **2.29** are shown in the Supporting Information. According to trityl assays, the coupling yields using the Dmoc amidites were excellent. To have a direct comparison of these amidites with commercial ones, we synthesized ODN **2.20** two times under identical conditions except that, in one time, **2.1**−**2.4** and commercial dT amidite were used and, in another, **2.1** and commercial dA, dC, dG and dT amidites were used (Supporting Information). In both syntheses, the

CPG **2.1** were used and were from the same batch with identical amounts. A portion of both ODNs on the same weight of CPG were cleaved and deprotected with concentrated NH_4OH under identical conditions (the Dmoc group and linker can also be cleaved with NH_4OH). RP HPLC analysis gave similar peak areas of full-length ODN. The OD_{260} values of purified ODNs were also very close. These experiments further confirmed that the Dmoc protecting groups did not have a negative effect on ODN synthesis efficiency. Cleavage and deprotection of the ODN synthesized with Dmoc amidites were also carried out under the oxidative conditions using the same amount of CPG as the above two experiments. However, the yield of ODN obtained was lower. Both HPLC peak area and OD_{260} values were about one-fourth of those for the experiments involving NH_4OH cleavage and deprotection. The lower yield may be caused by the loss of ODN during removing aniline with Amicon centrifugal filter units. The three-step procedure and the order of the steps for cleavage and deprotection used in new technology are important. The removal of the 2-cyanoethyl groups increases the hydrophilicity of ODNs, which is beneficial for oxidation in water in the next step. Performing oxidation under acidic conditions retains ODN on CPG, which allows easy removal of $NaIO_4$. The acidic conditions in the oxidation step did not cause any noticeable depurination because treating unmodified ODNs from the technology with concentrated NH_4OH did not give peaks of shorter ODNs in HPLC profiles. The excess aniline introduced in the last step is easy to remove due to its small size and high solubility in organic solvents. We achieved this by passing it through a size-exclusion column. We also tested a precipitation method involving adding *n*BuOH to aqueous ODN solutions. ODN was precipitated, and aniline remained in the supernatant, which was removed with a pipette.[16] Finally, ultrafiltration using an Amicon centrifugal

filter unit also proved effective. The Dmoc-based linker and protecting groups are well suited for electrophilic ODN synthesis. Before oxidation, H-2 in the function is not acidic in normal terms of organic chemistry (pKa~ 31), which enables Dmoc to be stable during ODN synthesis. After oxidation, the pKa of H-2 is lowered to ~12. Compared to the Fmoc protecting group, in which case the pKa of H-9 is ~22 and the group can be removed by the weak base piperidine (pKa of conjugate acid ~11), the Dmoc linker and protecting group were predicted to be readily cleavable under nearly neutral and non-nucleophilic conditions. Our results have shown that this is indeed the case. Compared to the ODN synthesis methods that used the allyl and o-nitrobenzyl functionalities, the Dmoc method does not require any expensive and difficult-to-remove transition metal and DNA-damageable UV light for deprotection and cleavage. Instead, the readily available and easily removable $NaIO_4$ and aniline can accomplish the task. We have successfully shown that the technology is suitable for the synthesis of ODNs containing thioester and α-chloroacetyl amide, which we confirmed to be incompatible with the widely known mild deprotection conditions using K_2CO_3 in MeOH (Supporting Information). Besides these two electrophilic groups, other groups such as aldehydes,[13] esters, activated esters, aziridines,[1g] epoxides, alkyl halides, vinyl purines,[17] methides,[18] and maleimides could be incorporated into ODNs as well.

2.3 Conclusion

In conclusion, we have developed a new ODN synthesis method using the Dmoc function as the linker and protecting group. Using the method, deprotection and cleavage are carried out in three steps under non-nucleophilic conditions, and therefore, it is useful

for the synthesis of electrophilic ODNs. Five sequences were successfully synthesized using the strategy. One of them contained a nucleophile-sensitive thioester, and another contained a sensitive α-chloroacetyl. The coupling yields wereexcellent. The products were purified with RP HPLC. MALDI-TOF MS analysis indicated that the ODNs had the correctstructure. We expect that the new technology will find applications in various research fields that need electrophilic ODNs.

2.4 Experimental Section

All reactions were performed in oven-dried glassware under a nitrogen atmosphere using standard Schlenk techniques. Reagents and solvents available from commercial sources were used as received unless otherwise noted. CH_2Cl_2, pyridine, and toluene were distilled over CaH_2. THF was distilled over Na/benzophenone. Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 0.25 μm thickness. Flash column chromatography was performed using Selecto Scientific silica gel, particle size 32-63 μm. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on Varian UNITY INOVA spectrometer at 400, 100 and 162 MHz, respectively; chemical shifts (δ) were reported in reference to solvent peaks (residue $CHCl_3$ at δ 7.24 ppm for ^{1}H and $CDCl_3$ at δ 77.00 ppm for ^{13}C, and H_3PO_4 at δ 0.00 ppm for ^{31}P). ODNs were synthesized on ABI 394 (**2.20**-**2.22**) and MerMade 6 (**2.29**-**2.30**) solid phase synthesizers. RP HPLC was performed on a JASCO LC-2000Plus System: pump, PU-2089Plus Quaternary Gradient; detector UV-2075Plus. A C-18 reverse phase analytical column (5 μm diameter, 100 Å, 250 × 3.20 mm) was used. Solvent A: 0.1 M triethylammonium acetate, 5% acetonitrile. Solvent B: 90% acetonitrile. All profiles were generated by

detection of absorbance of ODN 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 0.5 mL/min. Lcaa-CPG (pore size 497 Å) was a gift from Prime Synthesis, Inc. D-SaltTM dextran desalting column (5K MWCO, 10 mL) was purchased from Pierce Biotechnology, Inc. Polyacrylamide desalting column (5K MWCO, 10 mL) was from Thermo Scientific. Amicon® centrifugal filter units (3K NMWL) were purchased from Sigma-Aldrich.

Compound 2.7

To a solution of 1,3-dithiane (3.25 g, 27.1 mmol) in dry THF (50 mL) was added *t*BuLi (1.7 M in pentane, 16.0 mL, 27.1 mmol) dropwise at -78 °C. The mixture was stirred under nitrogen while warming to -40 °C gradually. After stirring at this temperature for 1 h, it was cooled to -78 °C**,** and a solution of **2.6** (4.88 g, 22.6 mmol) in dry THF (30 mL) was added slowly via cannula. Stirring was continued at -78 °C for 15 min, and the reaction was then quenched with sat. NH_4Cl (50 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Flash column chromatography (SiO_2, 9:1 hexanes/EtOAc) gave **2.8** as a colorless oil (5.40 g, 71%): Rf = 0.2 (9:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 6H, H-1), 0.86 (s, 9H, H-2), 1.38-1.48 (m, 1H, H-3), 1.49-1.59 (m, 4H, H-3), 1.77-1.84 (m, 1H, H-3), 1.89-1.98 (m, 1H, H-4), 2.01-2.10 (m, 1H, H-4), 2.43 (d, J = 3.6 Hz, 1H, H-5), 2.68-2.78 (m, 2H, H-6), 2.86-2.94 (m, 2H, H-6), 3.59 (t, J = 10.4 Hz, 2H, H-7), 3.80-3.85 (m, 1H, H-8), 3.88 (d, J = 6 Hz, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.3, 18.3, 22.1, 25.7,

26.0, 28.0, 28.5, 32.6, 33.8, 52.5, 63.0, 72.2; HRMS (ESI) *m/z* calcd for $C_{15}H_{32}O_2S_2SiH$ $[M+H]^+$ 337.1691, found 337.1695.

Compound 2.8

Carbonyldiimidazole (2.61 g, 16.1 mmol), **2.7** (2.16 g, 6.4 mmol), CaH_2 (90% grade, 0.75 g, 16.1 mmol) and dry toluene (100 mL) were combined and stirred at rt for 8 h. The mixture was filtered, and the filtrate was concentrated. Flash column chromatography (SiO_2, 3:1 hexanes/EtOAc) gave **2.8** as a thick oil (2.83 g, 100%): Rf = 0.2 (3:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (s, 6H, H-1), 0.82 (s, 9H, H-2), 1.39-1.56 (m, 4H, H-3), 1.80-2.08 (m, 4H, H-3 and H-4), 2.67-2.78 (m, 2H, H-5), 2.84-2.95 (m, 2H, H-5), 3.57 (t, *J* = 4 Hz, 2H, H-6), 4.10 (d, *J* = 4 Hz, 1H, H-7), 5.28-5.33 (m, 1H, H-8), 7.04 (s, 1H, H-9), 7.40 (s, 1H, H-10), 8.12 (s, 1H, H-11); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.4, 18.2 21.7, 25.4, 25.8, 28.4, 28.6, 31.4, 32.2, 48.9, 62.4, 78.4, 117.2, 130.6, 137.2, 148.3; HRMS (ESI) *m/z* calcd for $C_{19}H_{34}N_2O_3S_2SiH$ $[M+H]^+$ 431.1858, found 431.1858.

Compound 2.9:

5′-DMTr-thymidine (3.72 g, 6.8 mmol), **2.8** (1.96 g, 4.6 mmol) and DBU (0.21 g, 0.20 mL, 1.4 mmol) and toluene (50 mL) were combined and stirred at rt. After 8 h, the mixture was concentrated and purified with flash column chromatography (SiO_2, 3:1 hexanes/EtOAc with 0.5% Et3N). Compound **2.9** was obtained as a white foam (3.14 g, 76%): m.p. 81.2-82.6 °C; Rf = 0.45 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 6H, H-1), 0.84 (s, 9H, H-2), 1.31 (s, 3H, H-3), 1.36-2.00 (m, 8H, H-4), 2.34-2.74 (m, 4H, H- 4), 2.80-2.89 (m, 2H, H-4), 3.41-3.47 (m, 2H, H-5), 3.57 (t, *J* = 8 Hz, 2H, H-

6), 3.75 (s, 6H, H-7), 3.94 (d, *J* = 8 Hz, 1H, H-8), 4.20 (s, 1H, H-9), 4.91-4.96 (m, 1H, H-10), 5.35 (d, *J* = 8 Hz, 1H, H- 10), 6.43 (dd, *J* = 4 Hz, 8 Hz, 1H, H-11), 6.79 (d, *J* = 8 Hz, 4H, H-12), 7.18-7.31 (m, 8H, H-13), 7.31-7.33 (m, 1H, H-14), 7.54 (s, 1H, H-15), 8.08 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.3, 11.6, 18.3, 21.7, 25.4, 26.0, 28.4, 28.6, 31.6, 32.3, 37.9, 48.9, 55.3, 62.7, 63.7, 78.8, 78.9, 84.0, 84.3, 87.2, 111.5, 113.3, 127.2, 128.0, 128.1, 130.1, 135.2, 135.3, 135.4, 144.2, 150.0, 154.3, 158.8, 163.3; HRMS (ESI) *m/z* calcd for $C_{47}H_{62}N_2O_{10}S_2SiNa$ $[M+Na]^+$ 929.3513, found 929.3497.

Compound 2.10:

To a solution of **2.9** (1.47 g, 1.6 mmol) in THF (40 mL) at 0 °C was added TBAF (1.95 mL, 1.0 M in THF, 1.9 mmol) dropwise. The mixture was stirred for 8 h while warming to rt. The contents were poured into a separation funnel and partitioned between EtOAc (40 mL) and H_2O (40 mL). The aqueous layer was extracted with EtOAc (30 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) gave **2.10** as a white foam (0.96 g, 75%): m.p. 90.6- 92.3 °C; Rf = 0.3 (1:3 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 3H, H-1), 1.41- 1.63 (m, 4H, H-2), 1.69-1.81 (m, 2H, H-2), 1.89-2.08 (m, 2H, H-2), 2.37-2.44 (m, 1H, H-2), 2.56- 2.75 (m, 3H, H-2), 2.84-2.93 (m, 2H, H-2), 3.42-3.51 (m, 2H, H-3), 3.61-3.65 (m, 2H, H-4), 3.77 (s, 6H, H-5), 3.98 (d, *J* = 8 Hz, 1H, H-6), 4.24 (s, 1H, H- 7), 4.97-5.01 (m, 1H, H-8), 5.34 (d, *J* = 4 Hz, 1H, H-8), 6.42 (t, *J* = 4 Hz, 8 Hz, H-9), 6.82 (d, *J* = 8 Hz, 4H, H-10), 7.20-7.34 (m, 8H, H-11), 7.34-7.37 (m, 1H, H-12), 7.58 (s, 1H, H-13), 8.78 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 14.2, 21.0, 21.6, 25.4, 28.4, 28.6, 31.6, 32.1, 38.0, 48.9, 55.2, 60.4, 62.4, 63.7, 78.7, 79.1, 83.7, 84.4, 87.2, 111.6, 113.3, 127.2, 128.0, 128.1, 130.1, 130.1, 135.1, 135.2, 135.3, 144.2, 150.3, 154.2, 158.8, 158.8, 163.5; HRMS (ESI) *m/z* calcd for C41H48N2NaO10S2 $[M+Na]^+$ 815.2648, found 815.2636.

dT-Dmoc-CPG 2.1:

A mixture of **2.10** (0.10 g, 0.13 mmol), succinic anhydride (0.05 g, 0.50 mmol), and DMAP (0.03 g, 0.25 mmol) in anhydrous pyridine (3 mL) was stirred at rt. After 2 days, the contents were partitioned between EtOAc (5 mL) and H_2O (5 mL). The organic layer was washed with sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was dissolved in dry DMF (3 mL) and mixed with amino-lcaa-CPG (0.251 g, 0.027 mmol, 107 μmol/g, 497 Å, Prime Synthesis, Inc.) and DCC (0.027 mL, 1.0 M in CH_2Cl_2, 0.027 mmol). After standing at rt for 2 days, the supernatant was removed, and the CPG was washed with pyridine (3 mL × 5). To the CPG was added a capping solution (0.1 M DMAP in pyridine/Ac_2O, 9:1, v/v; 5 mL), and the mixture was allowed to stand at rt for 2 days. The supernatant was removed, and the CPG was washed with pyridine (3 mL × 5), MeOH (3 mL × 3), DMF (3 mL × 3) and acetone (3 mL × 5), and dried under vacuum.

Compound 2.14:

A solution of 5′-DMTr-dC (**11**, 2.60 g, 4.9 mmol) and TMSCl (1.60 g, 1.87 mL, 14.7 mmol) in pyridine (50 mL) was stirred at rt for 30 min. Compound **13** (3.10 g, 9.8 mmol) and DMAP (0.30 g, 2.5 mmol) in pyridine (15 mL) were added via cannula, and the mixture was stirred for 8 h. After cooling to 0 °C, H_2O (10 mL) was added, and the mixture was stirred for 5 min. Concentrated NH_4OH (15 mL) was then added, and the mixture was stirred at 0 °C for an additional 30 min. The content was poured into a separation funnel containing 5% $NaHCO_3$ (30 mL), and extracted with CH_2Cl_2 (30 mL × 2). The extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column

chromatography (SiO_2, 1:2:5:2:2:1 hexanes/EtOAc/ Et_2O/MeCN/MeOH/Et3N) gave **2.15** as a white foam (4.02 g, 100%): m.p. 121.1- 123.4 °C; Rf = 0.32 (1:2:5:2:2:1 hexanes/EtOAc/Et_2O/MeCN/MeOH /Et3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93-2.09 (m, 2H, H-1), 2.21-2.27 (m, 1H, H-1), 2.41 (br s, 1H, OH), 2.62-2.96 (m, 5H, H-1), 3.37-3.41 (m, 2H, H-2), 3.79 (s, 6H, H-3), 4.09 (t, *J* = 6 Hz, 1H, H-4), 4.46-4.48 (m, 4H, H- 5), 6.23 (t, *J* = 6 Hz, 1H, H-6), 6.83 (d, *J* = 8 Hz, 4H, H-7), 6.96 (d, *J* = 8 Hz, 1H, H-8), 7.20-7.39 (m, 9H, H-9), 8.23 (d, *J* = 8 Hz, 1H, H-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 27.3, 41.9, 42.8, 55.2, 62.6, 65.6, 70.7, 86.3, 86.9, 113.2, 127.0, 128.0, 128.1, 130.0, 135.3, 135.4, 135.4, 144.2, 158.6, 162.1; HRMS (ESI) *m/z* calcd for $C_{36}H_{39}N_3O_8S_2H$ $[M+H]^+$ 706.2251, found 706.2249.

Dmoc-dC-amidite 2.2:

A round-bottom flask containing **2.15** (0.69 g, 1.0 mmol) and a magnetic stirring bar was evacuated and then refilled with nitrogen. The evacuation and nitrogen-filling cycle was repeated for two more times. Dry CH_2Cl_2 (10 mL), 2- cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (**2.17**, 0.33 g, 0.34 mL, 1.09 mmol), and a solution of 1*H*-tetrazole in CH_3CN (0.45 M, 2.41 mL, 1.09 mmol) were added via syringes sequentially. After stirring at rt for 2 h, the mixture was concentrated to dryness by a nitrogen flow over its surface. The residue was purified with flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) giving **2.2** as a white foam (800 mg, 89%): Rf = 0.32 (1:3 hexanes/EtOAc); ^{1}H NMR (400 MHz $CDCl_3$) δ 1.14 (d, *J* = 6 Hz, 12H, H- 1), 1.90-2.07 (m, 1H, H-2), 2.24-2.30 (m, 1H, H-2), 2.41 (t, *J* = 8 Hz, 2H, H-3), 2.64-2.71 (m, 4H, H-2), 2.88-2.93 (m, 2H, H-2), 3.35-3.57 (m, 6H, H-4), 3.77 (s, 6H, H-5), 4.17-4.18 (m, 1H, H-

6), 4.45-4.64 (m, 4H, H-7), 6.22 (t, *J* = 6 Hz, 1H, H-8), 6.81-6.88 (m, 5H, H-9, H-10), 7.22-7.38 (m, 9H, H-11), 8.27 (d, *J* = 8 Hz, 1H, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1, 24.6, 27.2, 29.9, 40.8, 42.7, 43.3, 55.2, 58.1, 58.3, 61.9, 65.6, 71.6, 85.6, 86.8, 94.5, 113.2, 117.3, 127.0, 127.9, 128.2, 129.6, 130.0, 130.1, 135.2, 135.3, 144.0, 144.3, 158.6, 162.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 150.4; HRMS (ESI) *m/z* calcd for $C_{45}H_{56}N_5O_9PS_2H$ [M+H]+ 906.3336, found 906.3342.

Compound 2.15:

The procedure for **2.14** was used: White foam; yield 57%; m.p. 108.2-111.4 °C; Rf = 0.47 (1:2:5:2:2:1 hexanes/EtOAc/Et_2O/MeCN /MeOH/Et3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89- 2.04 (m, 2H, H-1), 2.50-2.97 (m, 6H, H-1), 3.37 (d, *J* = 4 Hz, 2H, H-2), 3.72 (s, 6H, H-3), 4.09-4.16 (m, 1H, H-4), 4.16-4.19 (m, 1H, H-5), 4.52 (d, *J* = 4 Hz, 2H, H-5), 4.68-4.71 (m, 1H, H-5), 6.46 (t, *J* = 6 Hz, 1H, H-6), 6.73-6.75 (d, *J* = 8 Hz, 4H, H-7), 7.12- 7.35 (m, 9H, H-8), 8.13 (s, 1H, H-9), 8.66 (s, 1H, H-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 27.3, 40.2, 43.0, 55.2, 65.3, 72.2, 84.6, 86.2, 86.5, 113.1, 122.3, 126.9, 135.5, 141.4, 149.2, 158.4; HRMS (ESI) *m/z* calcd for $C_{37}H_{39}N_5O_7S_2H$ $[M+H]^+$ 730.2364, found 730.2366.

Dmoc-dA-amidite 2.3:

The procedure for **2.1** was used: White foam; yield 88%; Rf = 0.4 (1:1:1 hexanes/EtOAc/Et3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (d, *J* = 6 Hz, 12H, H-1), 1.94- 2.07 (m, 1H, H-2), 2.41 (t, *J* = 8 Hz, 2H, H-3), 2.52-2.99 (m, 7H, H-2), 3.32-3.41 (m, 2H, H-4), 3.55-3.72 (m, 4H, H-5), 3.76 (s, 6H, H-6), 4.13 (t, *J* = 8 Hz, 1H, H-7), 4.28-4.31 (m, 1H, H-8), 4.56 (d, *J* = 8

Hz, 2H, H-9), 4.74-4.79 (m, 1H, H-8), 6.45 (t, J = 6 Hz, 1H, H-10), 6.77 (d, J = 8 Hz, 4H, H- 11), 7.16-7.37 (m, 9H, H-12), 8.16 (s, 1H, H-13), 8.68 (s, 1H, H-14); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 24.7, 24.8, 25.7, 39.7, 43.2, 43.4, 55.4, 63.5, 65.6, 73.6, 73.8, 85.0, 86.2, 86.3, 86.7, 113.3, 117.6, 122.7, 127.1, 128.0, 128.4, 130.3, 135.8, 135.8, 141.7, 144.7, 149.5, 150.8, 151.1, 152.9, 158.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 150.0; HRMS (ESI) *m/z* calcd for $C_{46}H_{56}N_7O_8PS_2H$ $[M+H]^+$ 930.3448, found 930.3441.

Compound 2.19:

To a solution of compound **2.17** (2.56 g, 5.04 mmol) in HMPA (5 mL) and THF (50 mL) was added *tert*-butylmagnesium chloride (1 M in THF, 15.1 mL, 15.1 mmol) dropwise at -78 °C. After addition, the mixture was allowed to warm to rt slowly, stirred at rt for 30 min, and then cooled to -78 °C again. A solution of **2.13** (3.97 g, 12.6 mmol) in THF (25 mL) was added dropwise. After stirring at rt for 8 h, the reaction was quenched with MeOH (8 mL). Volatiles were evaporated under reduced pressure. The residue was dissolved in EtOAc and washed sequentially with 0.15 M EDTA, saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (SiO_2, 19:1 $CHCl_3$/MeOH) gave **2.18** (1.53 g, 44 %) as a white solid. To the solution of **2.18** (0.57 g, 0.83 mmol) in dry pyridine (10 mL) was added HF-pyridine (70%, 357 mg, 12.5 mmol) at 0 °C. After stirring at rt for 2 h, excess fluoride was quenched with $MeOSiMe_3$ (1.30 g, 12.5 mmol) by stirring at rt for 8 h. Volatiles were evaporated under reduced pressure. The residue was co-evaporated with dry pyridine (5 mL × 3), and then dissolved in dry pyridine (20 mL). To the solution, dimethoxytrityl chloride (0.28 g, 0.83 mmol) was added. After stirring at rt for 8 h, the mixture was partitioned between

CH_2Cl_2 and 5% $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (SiO2, 9:1 EtOAc/MeOH) gave **2.19** (1.06 g, 80%) as a white foam: m.p. 159 – 161 °C; Rf = 0.2 (9:1 EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94-2.06 (m, 2H, H-1), 2.48- 2.59 (m, 2H, H-1), 2.63-2.96 (m, 4H, H-1), 3.27-3.41 (m, 2H, H-2), 3.68 (s, 6H, H-3), 4.10 (t, *J* = 8 Hz, 1H, H-4), 4.15-4.18 (m, 1H, H-5), 4.57 (d, *J* = 8 Hz, 2H, H-6), 4.87-4.91 (m, 1H, H-5), 6.22 (t, *J* = 6 Hz, 1H, H-7), 6.68 (dd, *J* = 9 Hz, 3 Hz, 4H, H-8), 7.07-7.33 (m, 9H, H-9), 7.68 (s, 1H, H- 10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.8, 14.2, 25.4, 27.0, 40.1, 42.6, 45.8, 55.2, 60.4, 64.3, 65.7, 71.7, 84.3, 86.4, 86.5, 113.0, 120.9, 126.8, 135.7, 144.5, 148.4, 154.4, 155.8, 158.4; HRMS (ESI) *m/z* calcd for $C_{37}H_{39}N_5O_8S_2H$ $[M+H]^+$ 746.2313, found 746.2311.

Dmoc-dG-amidite 2.4:

The procedure for **2.1** was used: White foam; yield 77%; Rf = 0.5 (29:1 EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12-1.15 (m, 12H, H-1), 1.97-2.03 (m, 2H), 2.32-2.95 (m, 8H, H-2, H-3), 3.33 (d, *J* = 4 Hz, 2H, H-4), 3.51-3.63 (m, 4H, H-5), 3.73 (s, 6H, H-6), 3.96 (t, *J* = 10 Hz, 1H, H-7), 4.24-4.47 (m, 1H, H-8), 4.50 (d, *J* = 8 Hz, 2H, H-9), 4.68- 4.74 (m, 1H, H-8), 6.19 (t, *J* = 6 Hz, 1H, H-10), 6.73-6.76 (m, 4H, H-11), 7.13-7.38 (m, 9H, H- 12), 7.74 (s, 1H, H-13); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.2, 20.3, 24.5, 24.5, 24.6, 26.8, 39.4, 42.1, 43.2, 43.3, 55.2, 57.8, 58.0, 63.7, 65.6, 73.7, 73.8, 84.6, 86.0, 86.3, 113.1, 117.3, 121.7, 126.9, 127.8, 128.0, 130.0, 135.7, 137.5, 144.5, 146.2, 148.0, 153.0, 158.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.4; HRMS (ESI) *m/z* calcd for $C_{46}H_{56}N_7O_9PS_2H$ $[M+H]^+$ 946.3397, found 946.3405.

Compound 2.27:

To a solution of 5- (acetylthio)pentanoic acid (**2.26**, 100 mg, 0.57 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added DCC (0.63 mL, 1.0 M in CH_2Cl_2, 0.63 mmol) dropwise under argon. After stirring at 0 °C for 20 min, compound **2.25** (223 mg, 0.57 mmol) in dry CH_2Cl_2 (10 mL) was added via cannula and the mixture was stirred for 12 h while warm to rt gradually. The content was poured into a separatory funnel containing 5% $NaHCO_3$ (20 mL), and extracted with CH_2Cl_2 (20 mL × 2). The extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (SiO_2, 1:1:0.05 hexanes/EtOAc/Et3N) gave **27** as a white sticky foam (240 mg, 77%): Rf = 0.35 (1:2 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50-1.69 (m, 4H, H-1), 2.09 (t, J = 7.12 Hz, 2H, H-2), 2.29 (s, 3H, H-3), 2.82 (t, J = 7.12 Hz, 2H, H-4), 3.09-3.22 (m, 3H, H- 5), 3.49-3.55 (m, 1H, H-5), 3.76 (s, 6H, H-6), 3.84-3.88 (m, 1H, H-7), 5.85 (br t, J = 7.12 Hz, NH), 6.81 (d, J = 6.92 Hz, 4H, H-8), 7.19-7.41 (m, 9H, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 28.8, 28.9, 30.9, 35.9, 43.2, 55.4, 64.9, 70.5, 86.4, 113.4, 127.1, 128.1, 128.3, 130.2, 135.9, 144.8, 158.7, 174.0; HRMS (ESI) *m/z* calcd for $C_{31}H_{37}NO_6SNa$ $[M+Na]^+$ 574.2239, found 574.2244.

Compound 2.23:

To the solution of **2.27** (100 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) was added diisopropylammonium tetrazolide (54 mg, 0.32 mmol) and **2.16** (97 mg, 0.32 mmol), and the reaction mixture was stirred under nitrogen at rt for 2 h. The mixture was loaded onto a column (SiO_2) and eluted with the solvent mixture EtOAc/hexanes/Et3N (20:20:1). Compound **2.23** was obtained as a pale yellow oil (130 mg, 82%): two diastereoisomers, Rf = 0.50 (20:20:1 EtOAc/hexanes/Et3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01-1.31 (m,

12H, H-1), 1.45-4.68 (m, 4H, H-2), 2.04 (t, *J* = 6.7 Hz, 1H, H-3), 2.11 (t, *J* = 6.7 Hz, 1H, H-3), 2.29 (s, 3H, H-4), 2.44 (t, *J* = 6.3 Hz, 1H, H-5), 2.62 (t, *J* = 6.2 Hz, 1H, H-5), 2.82 (t, *J* = 7.1 Hz, 1H, H-6), 2.83 (t, *J* = 7.1 Hz, 1H, H- 6), 3.05-3.10 (m, 0.5H, H-7), 3.15-3.24 (m, 1H, H- 7), 3.28-3.36 (m, 0.5H, H-7), 3.41-3.69 (m, 4H, H- 7), 3.69-3.80 (m, 1H, H-8), 3.75 (s, 3H, H-9), 3.76 (s, 3H, H-9), 3.81-3.91 (m, 1H, H-8), 3.95-4.08 (m, 1H, H-10), 5.76 (t, *J* = 5.6 Hz, 0.5H, NH), 6.05 (t, *J* = 5.2 Hz, 0.5H, NH), 6.79 (d, *J* = 7.6 Hz, 2H, H- 11), 6.81 (d, *J* = 5.6 Hz, 2H, H-11), 6.16-7.29 (m, 7H, H-12), 7.41-7.43 (m, 2H, H-12); ^{31}P NMR (162 MHz, CDCl3) δ 149.9, 150.3; HRMS (ESI) *m/z* calcd for C40H54N3O7PSNa [M+Na]+ 774.3318, found 774.3316.

Compound 2.28:

Triethylamine (0.267 mL, 1.92 mmol) was added to a solution of compound **2.25** (630 mg, 1.6 mmol) in dry CH_2Cl_2 (15 mL) and cooled to -10 °C under argon. α-chloroacetyl chloride was added dropwise over 5 min. The mixture was stirred for 12 h while warming to rt slowly. After concentration under reduced pressure, the residue was purified with flash column chromatography (SiO_2, 1:1:0.05 hexanes/EtOAc/Et3N). Compound **2.28** was obtained as a white foam (160 mg, 21%): Rf = 0.30 (1:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (br s, 1H, OH), 3.15-3.23 (m, 2H, H-1), 3.28- 3.34 (m, 1H, H-1), 3.56-3.62 (m, 1H, H-1), 3.80 (s, 6H, H-2), 3.88-3.94 (m, 1H, H-3), 4.00 (s, 2H, H-4), 6.85 (d, *J* = 8.9 Hz, 4H, H-5), 6.91 (t, *J* = 5.4 Hz, 1H, NH), 7.20-7.33 (m, 7H, H-6), 7.43 (dd, *J* = 11.2, 1.4 Hz, 2H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.7, 43.1, 55.4, 65.0, 70.0, 86.6, 113.4, 127.2, 128.1, 129.4, 135.9, 144.7, 158.8, 167.0; HRMS (ESI) *m/z* calcd for $C_{26}H_{28}ClNO_5Na$ $[M+Na]^+$ 492.1554, found 492.1558.

Compound 2.24:

The procedure for **2.23** was used: white foam; yield 78%; two diastereoisomers, Rf = 0.4 and 0.5 (2:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11-1.24 (m, 12H, H-1), 2.41 (t, J = 6.5 Hz, 1H, H-2), 2.63 (t, J = 6.3 Hz, 1H, H-2), 3.05-3.09 (m, 0.5H, H-3), 3.19-3.28 (m, 1H, H-3), 3.33-3.45 (m, 0.5H, H-3), 3.50-3.68 (m, 4H, H-3), 3.50-3.78 (m, 1H, H-5), 3.76 (s, 3H, H-4), 3.77 (s, 3H, H-4), 3.84-3.94 (m, 1H, H-5), 3.96-4.15 (m, 3H, H-6, H-7), 6.70-6.83 (m, 4H, H-8), 7.17-7.48 (m, 9H, H-9); ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.90, 149.94; HRMS (ESI) *m/z* calcd for $C_{35}H_{45}ClN_3O_6PH$ [M+H]+ 670.2813, found 670.2809.

ODN synthesis, deprotection, cleavage and analysis:

The ODNs were synthesized on ABI 394 (**2.20**-**2.22**, 60 mg CPG used) and MerMade 6 (**2.29**-**2.30**, 24 mg CPG used) synthesizers using dT-Dmoc-CPG **2.1**, Dmoc amidites **2.2**-**2.4**, amidites **2.23**-**2.24** and commercial dT amidite. The conditions suggested by synthesizer manufactures for 1 μmol synthesis were used in all cases. The average stepwise coupling yields for **2.20**-**2.22** were 98.6, 98.7, and 98.6%, respectively. Those for **2.29**-**2.30** were not available as the MerMade synthesizer does not have the reading. After synthesis, the CPG was divided into ten (**2.20**-**2.22**) or four (**2.29**-**2.30**) equal portions. One portion was suspended in a solution of DBU in CH_3CN (1:9 DBU/CH_3CN, v/v, 500 μL), and was gently shaken at rt for 15 min. The supernatant was removed with a pipette, and the CPG was washed with CH_3CN (200 μL × 3). To the CPG, an acidic $NaIO_4$ solution (0.1 M in 970 μL H_2O and 30 μL AcOH, pH 2) was added. After shaking in dark at rt for 3 h, the supernatant was transferred into a centrifugal tube. The CPG was washed with

dilute acetic acid (3:97 AcOH/H_2O, v/v, pH 2, 200 μL × 3). HPLC analysis of the supernatant and washes indicated that the ODN was not cleaved from CPG at this time. To the CPG was added a solution of aniline (3:97 aniline/H_2O, v/v, 1 mL, pH 8), and the suspension was shaken at rt for 3 h. The supernatant was transferred into a centrifugal tube. The CPG was washed with water (200 μL × 3). For ODNs **2.20**-**2.22**, the combined solution was concentrated to ~500 μL (but not to dryness, in some cases, no concentration was carried out), loaded onto a dextran or polyacrylamide desalting column (5K MWCO, 10 mL), and eluted with H_2O. Fractions containing ODN were combined and concentrated. The ODN was dissolved in 50 μL H_2O, and 20 μL was injected into RP HPLC to generate the crude ODN trace. The major ODN peak was collected, concentrated, dissolved in 20 μL water, and injected into HPLC to generate the pure ODN trace. In several trials, we used Amicon® centrifugal filter units to remove small molecules. The results were similar. For ODNs **2.29**-**2.30**, the supernatant and water washes were combined and concentrated to ~50 μL (but not to dryness). To the solution, 500 μL *n*BuOH was added. The mixture was vortexed and centrifuged for 10 min. The supernatant was removed without disturbing with a pipette. The residue was dissolved in 50 μL H_2O, and 20 μL was injected into RP HPLC to generate the crude ODN trace. The major ODN peak was collected, concentrated to dryness, dissolved in 20 μL H_2O, and injected into HPLC to generate the pure ODN trace. All pure ODNs were analyzed with MALDI-TOF MS, and correct molecular masses were observed.

ODN deprotection and cleavage protocol

Standard procedure should be used for ODN synthesis. No modification of conditions is required. The deprotection and cleavage of 0.1 μmol crude ODN is used for the description of the

protocol.

1. Place crude ODN on CPG in a 1.5 mL centrifugal tube. Add 500 μL DBU solution in CH_3CN (1:9 DBU/CH_3CN). Shake gently at rt for 15 min.
2. Spin the tube gently and briefly to bring down CPG (and also the liquids).
3. Remove the supernatant with a pipette.
4. Wash the CPG with CH_3CN (200 μL × 3).
5. Add acidic $NaIO_4$ solution (0.1 M in 970 μL H_2O and 30 μL AcOH, pH 2).
6. Wrap the tube with an aluminum foil, and gently shake at rt for 3 h.
7. Transfer the supernatant to another centrifuge tube. Wash the CPG with dilute acetic acid (3:97 AcOH/H_2O, v/v, 200 μL × 3. The ODN is still on CPG at this time, but keep the supernatant and washes in case that the ODN falls off. If it falls off, size-exclusion chromatography and Amicon® ultra filtration are options to separate ODN from $NaIO_4$ and other small molecules).
8. Add aniline solution (3:97 aniline/H_2O, 1 mL, pH 8) to the CPG, and shake at rt for 3 h.
9. Transfer the supernatant to another centrifuge tube. Wash the CPG with water (200 μL × 3). Combine the supernatant and washes.
10. Concentrate (but do not completely dry) the supernatant and washes to ~50 μL and add 500 μL *n*BuOH. Vortex for 30 sec and centrifuge for 10 min.
11. Carefully remove the supernatant with a pipette. The residue is crude ODN, which can be purified with RP HPLC.

12. Alternatively, from step 9, load the combined supernatant and washes (concentration is not needed unless the volume exceeds 1.5 mL) onto a dextran desalting column (5K MWCO, 10 mL), and elute with water. Combine the fractions containing ODN. Evaporate volatiles. The residue is crude ODN, which can be purified with RP HPLC.

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Chapter 3 Sensitive ODN Synthesis Using Dim for Phosphate Protection

Work in this chapter has been submitted to American Chemical Society Journal of Organic Chemistry

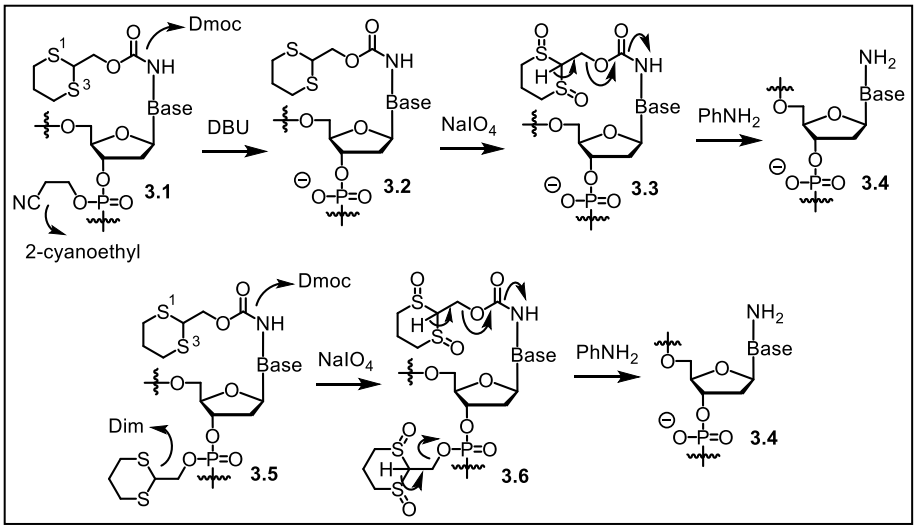
Shahsavari, S.; Eriyagama D.; Halami B.; Fang, S. " Sensitive Oligodeoxynucleotide Synthesis Using Dim for Phosphate Protection", *Journal of Organic Chemistry*, submitted.

Abstract

In traditional oligodeoxynucleotide (ODN) synthesis, phosphate groups are protected with 2-cyanoethyl group, and amino groups are protected with acyl groups. At the end of ODN synthesis, deprotection is achieved with strong bases and nucleophiles. Therefore, traditional technologies are not suitable for the synthesis of ODNs containing sensitive functionalities. To address the problem, we report the use of Dim and Dmoc groups, which are based on the 1,3-dithian-2-yl-methyl function, for phosphate and amine protection for solid phase ODN synthesis. Using the new Dim-Dmoc protection, deprotection was achieved under mild oxidative conditions without using any strong bases and nucleophiles. As a result, the new technology is suitable for the synthesis of ODNs containing sensitive functions. To demonstrate feasibility, seven 20-mer ODNs including four that contain the sensitive ester and alkyl chloride groups were synthesized, purified with RP HPLC and characterized with MALDI-TOF MS. High purity ODNs were obtained in good yields

3.1 Introduction

Synthetic oligodeoxynucleotides (ODNs) and their analogs have found wide applications in many areas. Examples include antisense drug development,1 DNA-protein interactions,[2] nanotechnology,[3,4] bioconjugation,[5] CRISPR genome editing,[6] DNA damage and repair,[7] DNA methylation and demethylation,[8] DNA data storage,[9] and synthetic biology.[10] It is projected that ODN analogs that contain sensitive functional groups have the potential to greatly expand the scope of the applications and bring about new research directions. Example sensitive ODN analogs include those containing functional groups such as alkyl halides, benzyl halide, allyl halides, α-halo amides, esters, activated esters, carbonates, thioesters, tosylates, sulfonic esters, sultones, phosphates, α,β-unsaturated carbonyl compounds, epoxides, aziridines, maleimides, vinyl arenes, methides, vinyl ethers, acetals, and hemiacetals. These groups are generally stable under typical chemical and biological conditions and can co-exist with functional groups of natural ODNs. However, they cannot survive the harsh acidic and basic conditions used in traditional ODN synthesis and deprotection. Therefore, traditional ODN synthesis technologies cannot be used to synthesize such sensitive ODNs. Some efforts have been made to address the problem, but limited success has been achieved.[11-20] Owing to the high potential of modified ODNs to bring transformative impact to many research areas, it is therefore significant to develop synthetic technologies that can be used to install any sensitive functional groups that are compatible with natural ODNs into any positions of ODNs.



Scheme 3.1 Deprotection of ODNs assembled with 2-cyanoethyl-Dmoc and Dim-Dmoc phosphoramidite monomers.

To achieve this goal, we recently introduced the 1,3-dithian-2-yl-methoxycarbonyl function (Dmoc) as amino protection groups and cleavable linker for solid phase ODN synthesis.[21-23] Mainly due to the concern of low efficiency of inorganic oxidizing agents to penetrate into the relatively hydrophobic fully protected ODNs to oxidize the dithioketals for deprotection and cleavage, we used the 2-cyanoethyl group for phosphate protection. At the end of synthesis, deprotection and cleavage were achieved in three steps. First, the 2-cyanoethyl groups were removed with the non-nucleophilic organic base DBU in acetonitrile (Scheme 3.1). This converted the hydrophobic fully protected ODN **3.1** into the hydrophilic **3.2**. The hydrophilic anionic phosphate groups were believed to be beneficial for the inorganic oxidizing agent in water to penetrate into ODN in the next step. Second, the dithioketals in **3.2** were oxidized with sodium periodate to give **3.3**. This drastically increased the acidity of H-2 in the 1,3-dithane function. Third, after washing away the inorganic materials, β-eliminations were induced with the weak base aniline, and

the ODNs were cleaved from the solid support and fully deprotected to give **3.4**. In this paper, we report our results on the study of the feasibility of using the Dim instead of 2-cyanoethyl function to protect the phosphate group. ODNs synthesized with this protection strategy should appear as **3.5** (Scheme 1). Deprotection and cleavage can then be achieved in two steps by oxidation of the dithioketals to give **3.6** followed by β-elimination. Besides reducing one step during deprotection, another advantage is that the use of the strong base DBU is avoided, which is expected to expand the scope of sensitive functions that can be incorporated into ODNs. Indeed, our results showed that the new protecting strategy was feasible, and the concern of inefficient oxidation of dithioketals in the relatively hydrophobic **3.5** was unnecessary. Using the new Dim-Dmoc technology, ODNs including those that contain sensitive functions can be synthesized in good yields and high purity under finely tuned but reliable conditions.

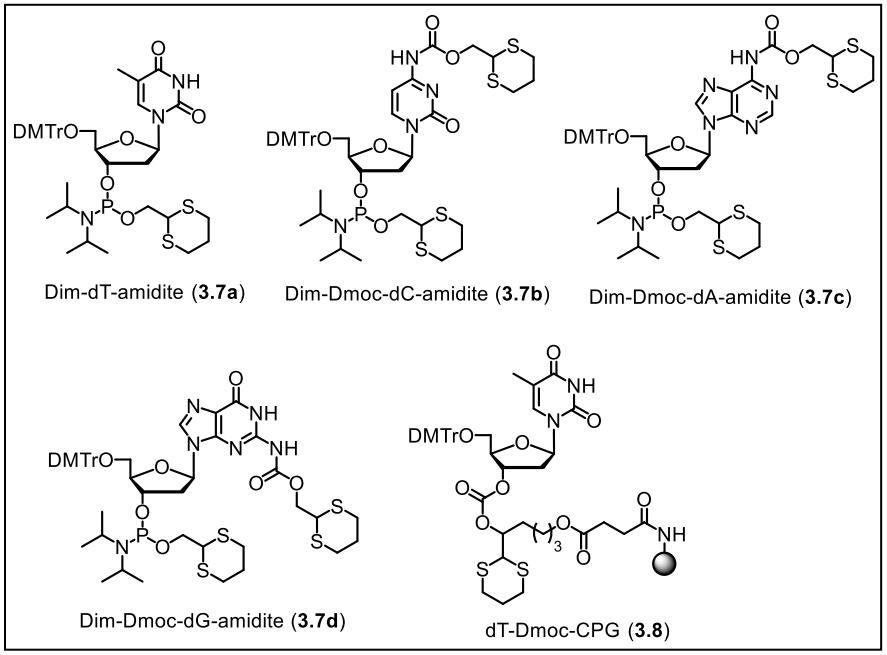
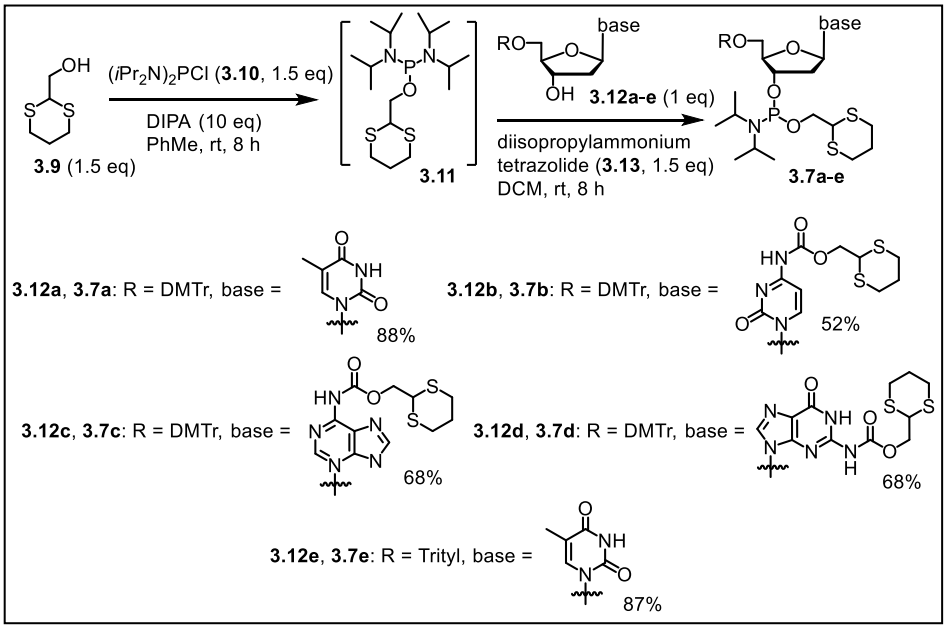


Figure 3.1. Dim-Dmoc phosphoramidite monomers and CPG with Dmoc linker



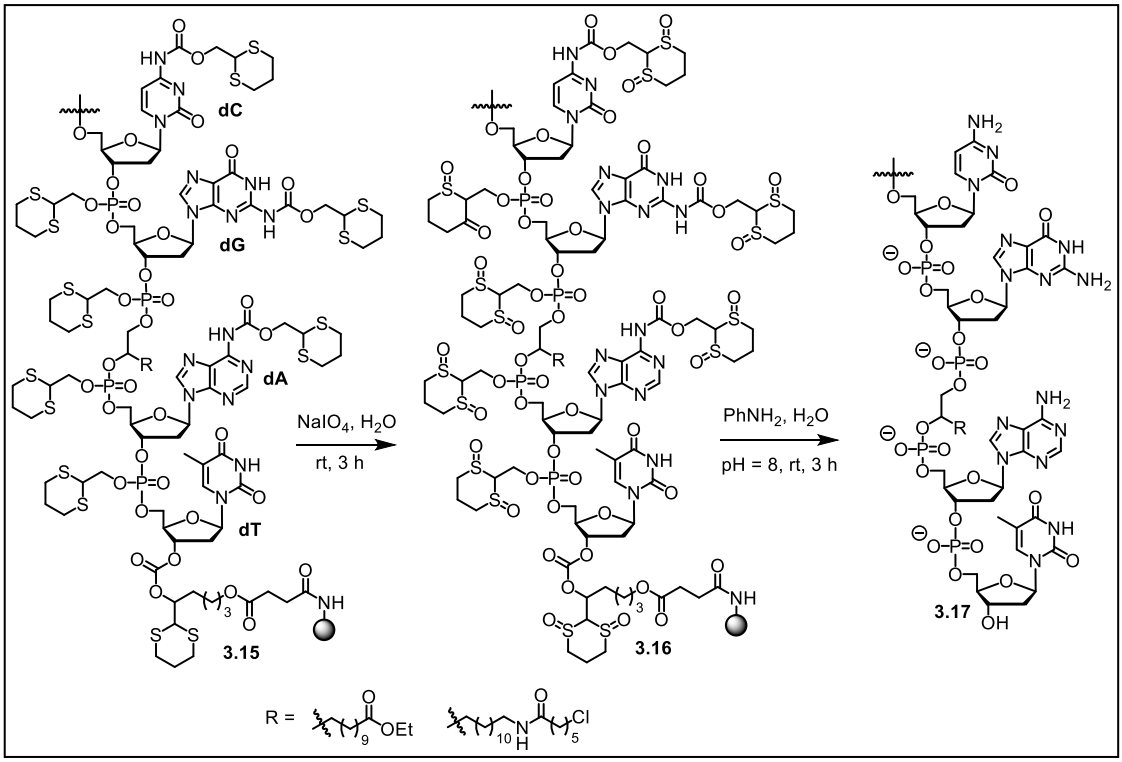
Scheme 3.2 Synthesis of Dim-Dmoc phosphoramidite monomers

3.2 Results and Discussion

To use the Dim-Dmoc technology to synthesize ODNs, the phosphoramidite monomers **3.7a-d** and the solid support with Dmoc linker **3.8** were needed (Figure 3.1). Preparation of **3.8** was reported previously.[21] The synthesis of **3.7a-d** is shown in Scheme 3.2. Compound **3.9** in toluene was reacted with commercially available bis(diisopropylamino)chlorophosphine (**3.10**) in the presence of the amine base diisopropylamine at room temperature overnight under an inert atmosphere.[24, 25] This gave the intermediate **3.11**, which was not isolated. A nucleoside with 5'-OH protected with a 4,4'-dimethoxytrityl (DMTr) or trityl group and amino group protected with the Dmoc group (**3.12**) and the activator diisopropylammonium tetrazolide (**3.13**) were dissolved in DCM. The intermediate **3.11** in the supernatant was transferred via a cannula with its inflow end wrapped with a copper wire-secured filter paper into the solution of **3.12** and activator. The reaction was allowed to proceed at room temperature overnight. The crude product was purified with flash column chromatography without aqueous workup. Good to excellent yields of the Dim-Dmoc phosphoramidites **3.7a-d** were obtained (Scheme 3.2). With the Dim-Dmoc phosphoramidites in hand, we tested the feasibility of using them as building blocks for ODN synthesis under weakly nucleophilic and weakly basic deprotection and cleavage conditions by the synthesis of the unmodified ODN **3.14a** (Figure 3.2). CPG with a Dmoc linker (**3.8**) was used as the solid support.[21] The phosphoramidites **3.7a-d** were used as nucleoside monomers. The syntheses were conducted on a MerMade 6 DNA synthesizer using typical scripts with some modifications. Briefly, detritylation was achieved with 3% DCA in DCM. In coupling, 0.1 M solutions of **3.7a-d** in acetonitrile were used with 5-(ethylthio)-1H-tetrazole as activator.

Capping was accomplished using 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite with the same activator for coupling. Typical conditions involving iodine was used for oxidation. In the last coupling step, the 5'-trityl protected instead of 5'-DMTr protected phosphoramidite **3.7e** was used to incorporate the nucleotide at the 5'-end of the ODN. The synthesis of 3.7e is shown in Scheme **3.2** and was similar as the synthesis of **3.7a-d**. Deprotection and cleavage of ODN was achieved in two steps (Scheme 3.3). First, the dithioketal bonds in the Dim and Dmoc functions in the fully protected ODN **3.15** were oxidized with a solution of sodium periodate in water at room temperature to give **3.16**. Excess oxidizing agents and other materials were simply removed by washing the CPG with water. Second, the CPG was suspended in a solution of aniline in water. This induced β-eliminations of the oxidized Dim and Dmoc functions in **3.16**, which cleaved the ODN from CPG and gave ODN **3.17**. At this stage, **3.17** was fully deprotected except for a trityl tag at its 5'-end, which was desirable for the purpose of assisting RP HPLC purification of the ODN. To remove small organic molecules, the ODN was precipitated from water with butanol. The residue was injected into RP HPLC to generate the profile of crude ODN, in which the tagged full-length ODN was well separated from other materials (Figure 3.3). The trityl-tagged ODN was collected and analyzed with HPLC giving a single peak (profile in supporting information). Removing the tag was achieved with 80% acetic acid, which is the typical condition for detritylation of DMTr-tagged ODNs. The ODN with trityl tag removed was purified with RP HPLC, and the purified fully deprotected ODN was analyzed again with RP HPLC. As shown in Figure 3.3, a single sharp peak was observed. The pure ODN **3.14a** was analyzed with MALDI-TOF MS. Correct molecular peak was found (Figure 3.4). The amount of pure ODN

obtained was estimated by UV, and an OD260 of 2.15 was given for a 0.52 µmol synthesis (supporting information). Besides **3.14a**, two additional unmodified ODNs **3.14b-c** were synthesized and analyzed under similar conditions to further confirm the viability of the new technology. Analysis data are in supporting information.



Scheme 3.3 ODN deprotection and cleavage.

Next, we tested the feasibility of the Dim-Dmoc technology for the synthesis of sensitive ODNs by incorporating the ester and alkyl chloride functions into ODNs. The synthesis of the required Dim phosphoramidite monomers (**3.18a-b**) is shown in Scheme 3.4. The known compound **3.19** was converted to **3.24** in five simple steps. Compounds **3.19** and **3.24** were then converted to their corresponding Dim phosphoramidites **3.18a-b**, respectively using the similar conditions for the synthesis of **3.7a-e**. The phosphoramidites

3.18a-b contain the sensitive ethyl ester and alkyl chloride groups, which are sensitive to traditional ODN cleavage and deprotection conditions involving heating in a concentrated ammonium hydroxide solution. The alkyl chloride is also sensitive to bases via β-elimination. The ODN sequences **3.14d-g** (Figure 3.2) were selected for the studies, of which the long chain chloroalkane-containing **3.14f-g** could provide a means to prepare protein-DNA conjugates via the bioorthogonal reaction between haloalkane dehalogenase and chloroalkanes.[26] The ODNs were synthesized under the same conditions described for the synthesis of unmodified ODNs. Deprotection and cleavage conditions were also the same. RP HPLC profiles of crude and pure ODNs **3.14d** and **3.14f** are in Figure 3.3. Their MALDI-TOF MS spectra are in Figure 3.4. All other analytical data are in supporting information. The data proved that the Dim-Dmoc technology can be used to synthesize the sensitive ODNs that contain the ester and alkyl chloride functionalities in high yields and purity.

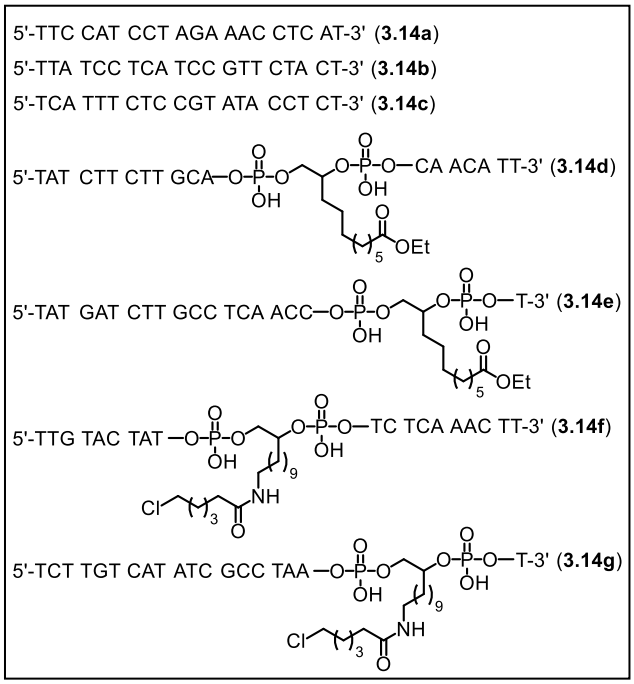


Figure 3.2. ODN sequences

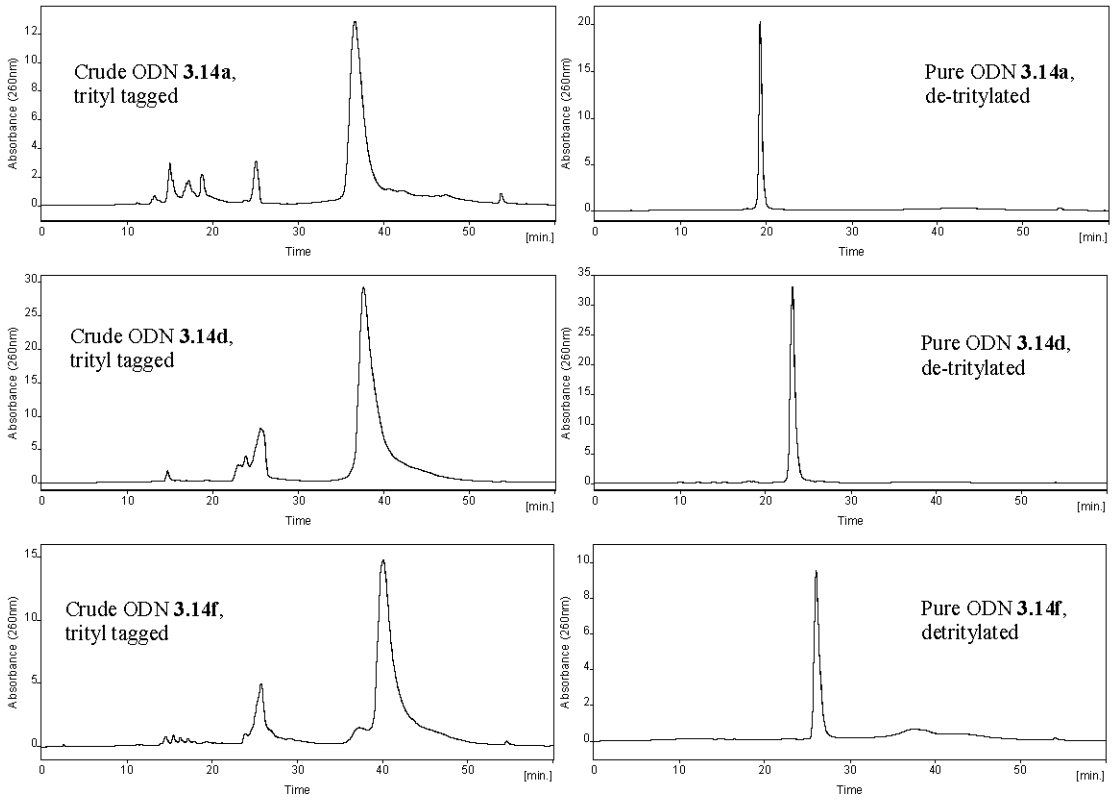


Figure 3.3. HPLC profiles. In the profiles of crude ODNs, the major peak at around 40 minutes is the fully deprotected ODN with a 5'-trityl tag. In the profiles of pure ODNs, the single sharp peak is the fully deprotected ODN without a 5'-trityl tag.

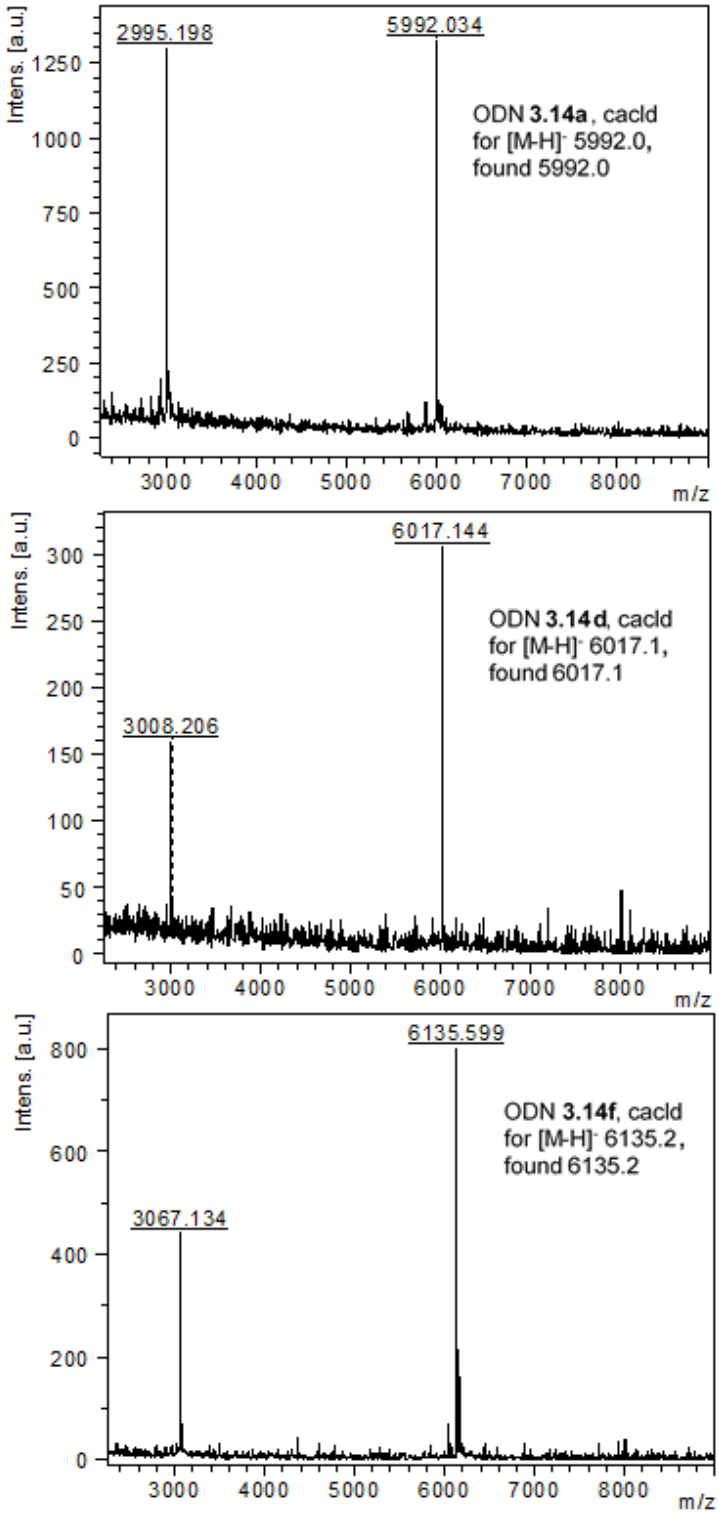
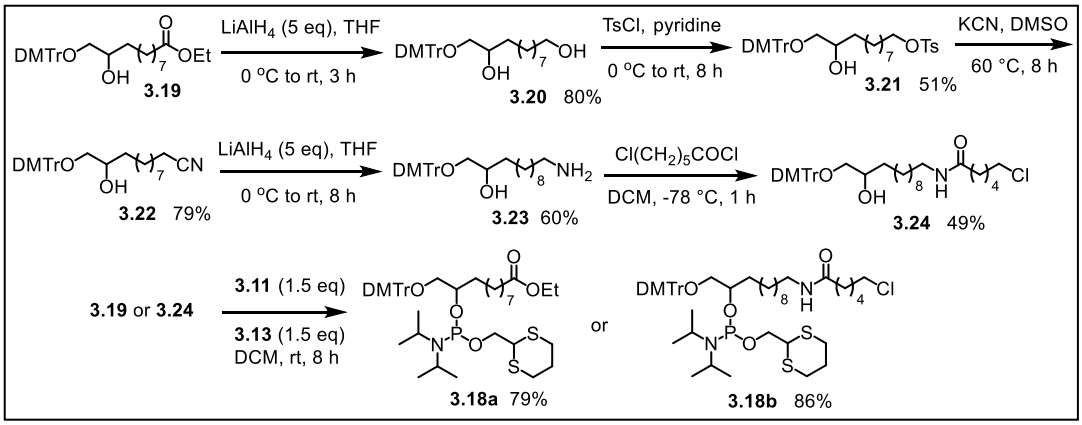


Figure 3.4. MALDI-TOF MS of ODNs **3.14a**, **3.14d** and **3.14f**.



Scheme 3.4. Synthesis of Dim phosphoramidites **3.18a-b** that contain sensitive groups.

The use of the Dim group to protect the phosphate groups in ODN synthesis has two advantages over the method in our previous reports,[21-23] in which the 2-cyanoethyl group was used. First, the number of steps in deprotection and cleavage is reduced from three to two, which significantly simplifies the procedure. Second, the use of the strong organic base DBU to remove the 2-cyanoethyl groups is avoided, which can expand the scope of sensitive groups to be incorporated into ODNs. Earlier, our decision to use the 2-cyanoethyl group instead of Dim for phosphate protection was based on several considerations including the complex nature of chemical ODN synthesis, difficulty to make highly pure Dim phosphoramidites (**3.7a-d**) required for repeated use in a multistep linear synthesis with satisfactory overall yield, and as mentioned earlier the concern of inefficient oxidation of dithioketals during ODN deprotection and cleavage. ODN synthesis is a highly complex process. After careful engineering by many chemists in several decades, the standard procedure is robust. However, slight modification of the procedure can cause significant problems, and those problems are usually very difficult to diagnose and address. For the

synthesis of Dim phosphoramidites **3.7a-d**, it was a concern too. Unlike their 2-cyanoethyl counterparts, which can be synthesized using the commercially available 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphordiamidite as the phosphitylation agent, these compounds have to be synthesized using a new phosphitylation agent such as **3.11**, which is difficult to prepare and purify. For concerns on the inefficiency of oxidation of the dithioketals during ODN deprotection and cleavage, the fully protected ODNs on the CPG are relatively hydrophobic; which excludes the use of oxidizing agents that can only function in water. However, to oxidize multiple dithioketals with complete conversion, the reaction must be highly efficient, and thus the broadness of the scope of oxidizing agents that can be tested is important for the technology to be successful. Therefore, during our initial studies, we chose to use the much simpler and well established 2-cyanoethyl protection chemistry. Indeed, we met many problems during the studies. For example, at the beginning of the project, our RP HPLC profiles were messy. After testing many hypotheses, we finally found that one of the problems was cap exchange, in which the Dmoc groups used for amino protection were replaced by acyl groups during capping involving using reagents such as acetic anhydride under traditional capping conditions. This was counter intuitive because the donation of the lone pair of electrons from the oxygen atom to the carbonyl carbon in the Dmoc function would make the Dmoc protection more stable than acyl protections. Once the problem was diagnosed, it was solved elegantly by using the phosphorylation chemistry instead of the acylation chemistry for capping. The synthesis of **3.7a-d** and making them highly pure for ODN synthesis were indeed difficult too. We screened many conditions and were finally able to identify a procedure involving using toluene as the solvent and diisopropylamine as base to prepare the phosphitylation agent

3.11. With toluene and diisopropylamine, **3.11** was soluble while the side product diisopropylammonium salt was not. This allowed us to obtain **3.11** with sufficient purity for the phosphitylation reaction without aqueous workup and chromatography purification.[24, 25] Of surprise was that the oxidation of dithioketals in the fully protected relatively hydrophobic **3.5** during ODN deprotection and cleavage was achieved with ease using the aqueous solution of sodium periodate. The efficiency was similar to the oxidation of **3.2**, which was highly hydrophilic due to the anionic phosphate groups. Probably, the fully protected ODNs had limited but sufficient solubility in water for the oxidation reaction to occur at the outermost sphere of the ODN coated CPG. Once the dithioketals in the outermost layer were oxidized, the solubility increased, and the reaction gradually penetrated into the inner layer and all dithioketals were oxidized efficiently. However, one observation still puzzles us. We synthesized a simple model oligosulfoxide compound, which contained six sulfoxide groups. We thought that this compound would be highly soluble in water. To our surprise, it was almost insoluble or had very limited solubility in any solvents including water.[27] During the development of the technology, we found that using the trityl group instead of the DMTr group as the 5'-tag to assist RP HPLC purification was needed. When DMTr group was used, the tag was easy to fall off in the sodium periodate oxidation step. The trityl group was able to survive the conditions. Importantly, we found that the trityl group could be removed efficiently with 80% acetic acid under similar conditions used for removing the DMTr group after HPLC purification, which was inconsistent to the report that deprotection of trityl group required two days at room temperature.[28] One concern on developing the Dim-Dmoc technology was the difficulty to identify selective oxidative conditions for the oxidation of phosphite triesters

to phosphate triesters during ODN synthesis and for oxidation of dithioketals during deprotection and cleavage. For the former, we were gratified to find that the standard iodine oxidation conditions were highly selective, and premature oxidation of the dithioketals had never been observed. For the latter, the sodium periodate solution elegantly accomplished an otherwise highly challenging task, which required highly efficient and selective oxidation of the multiple dithioketal groups while not damaging the ODNs via oxidation of the nucleobases and other portions of the molecules.

3.3 Conclusion

In conclusion, a new method for solid phase ODN synthesis has been developed. The method uses Dim for phosphate protection, Dmoc for amino protection, and a Dmoc linker for anchoring the ODN to solid support. With the new protection and linking strategy, ODN deprotection and cleavage can be achieved under oxidative conditions without using any strong bases and nucleophiles. Therefore, the new method is suitable for the synthesis of ODN analogs containing base labile and electrophilic groups, a task that cannot be accomplished or is highly challenging to accomplish using traditional technologies. We expect that the new method will be able to provide a wide range of sensitive ODN analogs to researchers in research areas such as antisense drug development, DNA-protein interaction studies, nanotechnology and bioconjugation.

3.4 Experimental Section

General information:

All reactions were performed in oven-dried glassware under argon using standard Schlenk techniques. Reagents and solvents available from commercial sources were used as received unless otherwise noted. Lcaa-CPG (pore size 497 Å) was purchased from Prime Synthesis. THF, toluene, and CH_2Cl_2 were dried using an Innovative Technology Pure-Solv™ system. Pyridine and diisopropylamine were distilled over CaH_2 under nitrogen. Compounds **3.12a-e** were prepared according to reported procedure.[21, 22, 29] Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 250 μm thickness. Flash column chromatography was performed using SiliCycle silica gel, particle size 40-63 μm. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on a Varian UNITY INOVA spectrometer at 400, 100 and 162 MHz, respectively; chemical shifts (δ) were reported in reference to solvent peaks (residue $CHCl_3$ at δ 7.24 ppm for ^{1}H and $CDCl_3$ at δ 77.00 ppm for ^{13}C) and to H_3PO_4 (δ 0.00 ppm for ^{31}P). HRMS was obtained on a Thermo HR-Orbitrap Elite Mass Spectrometer. LRMS was obtained on a Thermo Finnigan LCQ Advantage Ion Trap Mass Spectrometer. MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System. ODNs were synthesized 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. A C-18 reversed phase analytical column (5 μm diameter, 100 Å, 250 × 3.20 mm) was used. Solvent A: 0.1 M triethylammonium acetate, 5% acetonitrile. Solvent B: 90% acetonitrile. 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.

Compound 3.7a:

To a solution of **3.9** (1.57 g, 10.48 mmol, 1.5 eq.) and freshly distilled diisopropyl amine (9.85 mL, 69.9 mmol, 10 eq.) in dry toluene (25 mL) was added bis(diisopropylamino)chlorophosphine (3.10, 2.80 g, 10.48 mmol, 1.5 eq.) at rt under argon. After stirring overnight, the intermediate **3.11** in the supernatant was transferred into a solution of **3.12a** (3.80 g, 6.99 mmol, 1 eq.) and diisopropylammonium tetrazolide (**3.13**, 1.80 g, 10.48 mmol, 1.5 eq.) in dry DCM (50 mL) via a cannula with its inflow end wrapped with a copper wire-secured filter paper. The reaction mixture was stirred overnight, and then concentrated to dryness. The residue was loaded directly on a column for flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N). Compound **3.7a** was obtained as a white foam (5.04 g, 88%): Mixture of two diastereoisomers; R_f = 0.2 and 0.3 (SiO_2, 1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.16 (m, 12H), 1.38 (s, 3H), 1.77-1.87 (m, 1H), 1.96-2.07 (m, 1H), 2.28-2.42 (m, 1H), 2.45-2.58 (m, 1H), 2.60-2.69 (m, 2H), 2.65-2.84 (m, 4H), 3.29-3.46 (m, 2H), 3.47-3.69 (m, 2H), 3.76 (s, 6H), 3.80-3.89 (m, 1H), 4.04-4.23 (m, 1H), 4.74-4.77 (m, 1H), 6.38 (t, J = 5.76 Hz, 1H), 6.81 (dd, J = 8.81, 3.16 Hz, 4H), 7.20-7.29 (m, 7H), 7.40 (d, J = 7.60 Hz, 2H), 7.60 (s, 0.5H), 7.63 (s, 0.5 H), 8.84 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 24.81, 24.88, 24.95, 25.0, 26.1, 26.2, 28.8 (d, Jcp = 9.15 Hz), 29.0 (d, Jcp = 17.15 Hz), 40.5 (d, Jcp = 5.35 Hz), 40.6 (d, Jcp = 1.84 Hz), 43.4 (d, Jcp = 3.38 Hz), 43.5 (d, Jcp = 3.41 Hz), 47.1 (d, Jcp = 7.04 Hz), 47.8 (d, Jcp = 6.77 Hz), 55.5, 63.3, 63.7, 64.8 (d, Jcp = 18.15 Hz), 65.0 (d, Jcp = 18.90 Hz), 73.6 (d,

Jcp = 15.59 Hz), 74.1 (d, Jcp = 15.19 Hz), 84.8, 85.0, 85.4 (d, Jcp = 6.69 Hz), 86.0 (d, Jcp = 2.83 Hz), 87.0, 87.1, 111.2, 113.4, 127.2, 128.1, 128.4, 130.4, 135.5, 135.6, 135.7, 136.0, 136.1, 144.5, 144.6, 150.4, 158.8, 164.0; 31P NMR (162 MHz, CDCl3) δ 149.4, 149.6 ppm; HRMS (ESI) m/z calcd for $C_{42}H_{55}N_3O_8PS_2$ $[M+H]^+$ 824.3168, found 824.3170.

Compound 3.7b:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N) gave 3.7b as a white foam (1.25 g, 52%): Mixture of two diastereoisomers; R_f = 0.2 and 0.3 (SiO_2, 1:2 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.26 (m, 12H), 1.72-1.84 (m, 2H), 1.90-2.09 (m, 4H), 2.30-2.47 (m, 2H), 2.59-2.74 (m, 6H), 2.85-2.93 (m, 2H), 3.39-3.60 (m, 4H), 3.61-3.89 (m, 1H), 3.77 (s, 6H), 3.91-4.16 (m, 2H), 4.17-4.22 (m, 1H), 4.40-4.49 (m, 1H), 6.18-6.22 (m, 1H), 6.81 (d, J = 7.4 Hz, 4H), 7.18-7.29 (m, 7H), 7.7.39 (d, J = 7.60 Hz, 2H), 8.27-8.29 (m, 0.5H), 8.34-8.35 (m, 0.5H); 13C NMR (100 MHz, CDCl3) δ 23.3 (d, Jcp = 2.21 Hz), 23.4 (d, Jcp = 1.59 Hz), 24.85, 24.89, 24.92, 24.98, 25.1, 25.7, 25.9, 26.1, 26.2, 27.5, 27.6, 28.7 (d, Jcp = 13.00 Hz), 29.1 (d, Jcp = 21.54 Hz), 41.2 (d, Jcp = 5.73 Hz), 41.5, 43.1, 43.4, 43.5, 45.4, 45.5, 47.1 (d, Jcp = 6.88 Hz), 47.7 (d, Jcp = 8.28 Hz), 55.5, 61.9, 62.4, 64.7 (d, Jcp = 19.91 Hz), 64.8 (d, Jcp = 18.5 Hz), 65.8, 65.9, 71.4 (d, Jcp = 9.27 Hz), 71.9 (d, Jcp = 10.13 Hz), 85.2 (d, Jcp = 7.30 Hz), 86.1, 87.0, 94.5, 113.4, 127.2, 128.1, 128.4, 130.2, 130.3, 135.5, 135.6, 135.7, 135.8, 144.3, 144.4, 144.9, 145.0, 151.9, 155.0, 158.7, 161.9, 162.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.2, 149.5; HRMS (ESI) m/z calcd for $C_{47}H_{62}N_4O_9PS_4$ $[M+H]^+$ 985.3137, found 985.3130.

Compound 3.7c:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N) gave **3.7c** as a white foam (1.30 g, 68%): Mixture of two diastereoisomers; R_f = 0.3 and 0.4 (SiO_2, 1:2 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09-1.25 (m, 12H), 1.75-1.84 (m, 2H), 1.95-2.04 (m, 4H), 2.59-2.75 (m, 6H), 2.87-2.98 (m, 4H), 3.31-4.00 (m, 4H), 3.75 (s, 6H), 4.00 (t, J = 6.48 Hz, 0.5H), 4.05-4.18 (m, 1.5H), 4.21-4.27 (m, 0.5H), 4.30-4.39 (m, 0.5H), 4.55 (d, J = 7.1 Hz, 2H), 4.80-4.88 (m, 1H), 6.46 (t, J = 6.5 Hz, 1H), 6.74-6.77 (m, 4H), 7.14-7.30 (m, 7H), 7.36 (d, J = 11.96 Hz, 2H), 8.16 (s, 0.5H), 8.19 (s, 0.5H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.27 (d, Jcp = 2.57 Hz), 23.33 (d, Jcp = 2.00 Hz), 24.86, 24.92, 24.99, 25.8, 26.1, 27.6, 28.8 (d, Jcp = 11.54 Hz), 29.1 (d, Jcp = 14.52 Hz), 40.1 (d, Jcp = 14.77 Hz), 43.3, 43.4, 43.5, 45.45, 45.51, 47.2 (d, Jcp = 7.48 Hz), 47.7 (d, Jcp = 7.55 Hz), 55.5, 63.4, 63.7, 64.7 (d, Jcp = 13.84 Hz), 65.4 (d, Jcp = 18.48 Hz), 65.6, 73.9 (d, Jcp = 13.71 Hz), 74.0 (d, Jcp = 15.26 Hz), 84.8, 85.1, 85.9, 86.4, 86.6, 86.7, 113.3, 122.6, 127.0, 128.0, 128.3, 130.2, 135.78, 135.85, 141.6, 141.7, 144.67, 144.72, 149.2, 150.5, 151.06, 151.12, 152.8, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.4, 149.6; HRMS (ESI) m/z calcd for $C_{48}H_{62}N_6O_8PS_4$ $[M+H]^+$ 1009.3249, found 1009.3255.

Compound 3.7d:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 8:1:1 EtOAc/ACN/Et_3N) gave **3.7d** as a white foam (1.30 g, 68%): Mixture of two diastereoisomers; R_f = 0.2 and 0.3 (SiO2, 8:1:1 EtOAc/ACN/Et_3N). ^{1}H NMR (400 MHz,

$CDCl_3$) δ 1.07-1.16 (m, 12H), 1.77-1.86 (m, 2H), 1.97-2.08 (m, 4H), 2.59-2.94 (m, 10H), 3.25-3.31 (m, 2H), 3.52-3.58 (m, 2H), 3.75 (s, 6H), 3.58-4.21 (m, 2.5H), 4.29-4.32 (m, 0.5H), 4.50 (d, J = 3.48 Hz, 1H), 4.52 (d, J = 3.44 Hz, 1H), 4.72-4.81 (m, 1H), 6.18-6.23 (m, 1H), 6.72-6.78 (m, 4H), 7.16-7.30 (m, 7H), 7.37 (d, J = 7.04 Hz, 1H), 7.39 (d, J = 7.76 Hz, 1H), 7.8 (s, 0.5H), 7.82 (s, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.86, 24.91, 24.94, 24.98, 25.6, 26.1, 27.1, 28.88 (d, Jcp = 11.11 Hz), 29.16 (d, Jcp = 8.47 Hz), 39.9, 42.5, 43.4, 43.5, 47.2 (d, Jcp = 6.85 Hz), 47.6 (d, Jcp =7.36 Hz), 55.5, 63.6, 63.9, 64.8 (d, Jcp = 6.59 Hz), 65.0 (d, Jcp = 6.47 Hz), 66.0, 73.9 (d, Jcp = 11.09 Hz), 74.1 (d, Jcp = 16.48 Hz), 84.3, 84.4, 85.7 (d, Jcp =6.62 Hz), 86.2 (d, Jcp = 2.85 Hz), 86.6, 113.3, 121.6, 127.0, 128.0, 128.3, 128.4, 130.18, 130.24, 135.8, 135.9, 137.4, 137.5, 144.6, 144.7, 146.3, 148.30, 148.32, 153.11, 153.13, 155.7, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.9, 149.6; HRMS (ESI) m/z calcd for $C_{48}H_{62}N_6O_9PS_4$ $[M+H]^+$ 1025.3198, found 1025.3205.

Compound 3.7e:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N) gave **3.7e** as a white foam (233 mg, 87%): Mixture of two diastereoisomers; R_f = 0.2 and 0.3 (SiO_2, 1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04-1.27 (m, 12H), 1.40 (s, 3H), 1.78-1.86 (m, 1H), 1.96-2.05 (m, 1H), 2.29-2.98 (m, 8H), 3.30-3.99 (m, 5H), 4.05-4.25 (m, 1H), 4.74-4.81 (m, 1H), 6.38 (t, J = 7.1 Hz, 1H), 7.18-7.35 (m, 9H), 7.36-7.45 (m, 6H), 7.56 (s, 0.5H), 7.60 (s, 0.5H), 9.11 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 24.84, 24.88, 24.91, 24.95, 25.01, 26.1, 26.2, 28.8 (d, Jcp = 8.40 Hz), 29.0 (d, Jcp = 17.34 Hz), 40.4 (d, Jcp = 5.12 Hz), 40.6, 43.4, 43.5, 47.0 (d, Jcp = 7.16 Hz), 47.5 (d, Jcp = 7.43 Hz), 63.5, 63.9, 64.8 (d, Jcp = 17.88 Hz), 65.0 (d, Jcp =

18.41 Hz), 73.6 (d, Jcp = 15.28 Hz), 73.9 (d, Jcp = 14.43 Hz), 84.8, 85.0, 85.3 (d, Jcp = 6.74 Hz), 85.9, 87.55, 87.61, 111.1, 111.2, 127.5, 128.1, 128.9, 135.9, 136.0, 143.5, 143.6, 150.5, 164.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.4, 149.7; HRMS (ESI) m/z calcd $C_{40}H_{51}N_3O_6PS_2$ $[M+H]^+$ 764.2956, found 764.2960.

Compound 3.20:

To a suspension of lithium aluminum hydride (1.15 g, 30.29 mmol, 5 eq.) in dry THF (25 mL) was added a solution of **3.19** (3.15 g, 6.06 mmol, 1 eq.) in dry THF (50 mL) dropwise via cannula at 0 °C under nitrogen. The reaction mixture was stirred for 3 h, and then quenched by dropwise addition of H_2O (1.15 mL), 15% NaOH (1.15 mL), and H_2O (3.45 mL), sequentially. The white precipitate was removed by filtration over Celite. The filtrate was concentrated to dryness. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N) gave **3.20** as a colorless oil (2.45 g, 80%): R_f = 0.2 (SiO_2, 1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20-1.42 (m, 14H), 1.53 (p, J = 5.80 Hz, 2H), 1.65 (brs, 1H), 2.45 (brs, 1H), 3.02 (dd, J = 9.28, 7.56 Hz, 1H), 3.16 (dd, J = 9.60, 3.56 Hz, 1H), 3.59 (t, J = 6.6 Hz, 2H), 3.73-3.75 (m, 1H), 3.76 (s, 6H), 6.82 (d, J = 8.9 Hz, 4H), 7.20 (tt, J = 7.40, 1.16 Hz, 1H), 7.28 (t, J = 7.24 Hz, 2H), 7.32 (d, J = 8.96 Hz, 2H), 7.43 (d, J = 9.64 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.1, 29.72, 29.78, 29.8, 29.9, 33.0, 33.7, 55.5, 63.2, 67.9, 71.2, 86.2, 113.3, 126.9, 127.9, 128.3, 130.2, 136.2, 145.0, 158.5; HRMS (ESI) m/z calcd for $C_{32}H_{43}O$ $[M+H]^+$ 507.3110, found 507.3122.

Compound 3.21:

To a solution of **3.20** (2.06 g, 4.07 mmol, 1 eq.) in freshly distilled pyridine (50 mL) was added TsCl (0.814 g, 1.05 eq.) at 0 °C under nitrogen. The mixture was stirred at the same temperature for 8 h. The majority of pyridine was evaporated on a rotary evaporator under vacuum generated by an oil pump. The remaining content was poured into a separatory funnel containing 5% $NaHCO_3$ (100 mL) and extracted with EtOAc (50 mL × 3). The extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. Flash column chromatography (SiO_2, 2:1 hexanes/EtOAc with 5% Et_3N) gave **3.21** as a pale-yellow oil (1.37 g, 51%): R_f = 0.4 (SiO_2, 1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12-1.41 (m, 14H), 1.61 (p, J = 6.72 Hz, 2H), 2.42 (s, 3H), 3.01 (t, J = 9.16 Hz, 1H), 3.16 (dd, J = 9.40, 3.28 Hz, 1H), 3.70-3.74 (m, 1H), 3.76 (s, 6H), 4.00 (t, J = 6.5 Hz, 2H), 6.81 (d, J = 8.8 Hz, 4H), 7.20 (t, J = 7.12 Hz, 1H), 7.26 (t, J = 4.80 Hz, 2H), 7.31 (d, J = 8.80 Hz, 6H), 7.43 (d, J = 7.28 Hz, 2H), 7.77 (d, J = 8.32 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 25.6, 25.7, 29.1, 29.2, 29.60, 29.67, 29.8, 33.6, 55.5, 67.8, 70.9, 71.2, 86.2, 113.3, 126.9, 127.9, 128.3, 129.9, 130.2, 133.4, 136.2, 144.7, 145.0, 158.5; HRMS (ESI) m/z calcd for $C_{39}H_{49}O_7S$ $[M+H]^+$ 661.3199, found 661.3204.

Compound 3.22:

To a solution of **3.21** (6.78 g, 10.28 mmol, 1 eq.) in dry DMSO (25 mL) was added KCN (0.802 g, 12.34 mmol, 1.2 eq.) at rt under nitrogen. The reaction mixture was stirred at 60 °C overnight. After cooling to rt, EtOAc (100 mL) was added, and the organic phase was washed with brine (100 ml), dried over anhydrous Na_2SO_4, filtered, and concentrated. Flash column chromatography (SiO_2, 4:1 hexanes/EtOAc with 5% Et_3N) gave **3.22** as a colorless oil (4.20 g, 79%): R_f = 0.2 (SiO_2, 4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz,

$CDCl_3$) δ 1.22-1.26 (m, 10H), 1.37-1.42 (m, 4H), 1.61 (p, d = 7.12 Hz, 2H), 2.27 (t, J = 7.12 Hz, 2H), 2.41 (brs, 1H), 3.02 (dd, J = 9.24, 7.52 Hz, 1H), 3.16 (dd, J = 9.32, 3.32 Hz, 1H), 3.76 (s, 6H), 6.81 (d, J = 8.9 Hz, 4H), 7.20 (t, J = 7.36 Hz, 1H), 7.28 (t, J = 7.76 Hz, 2H), 7.32 (d, J = 8.48 Hz), 7.43 (d, J = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.4, 25.7, 25.8, 28.9, 29.0, 29.5, 29.7, 29.9, 33.7, 55.5, 67.9, 71.2, 86.3, 113.3, 120.0, 126.9, 127.95, 127.98, 128.3, 130.2, 136.3, 145.0, 158.6; HRMS (ESI) m/z calcd for $C_{33}H_{42}NO_4$ $[M+H]^+$ 516.3113, found 516.3120.

Compound 3.23:

To a suspension of lithium aluminum hydride (1.55 g, 40.8 mmol, 5 eq.) in dry THF (50 mL) was added a solution of **3.22** (4.20 g, 8.16 mmol, 1 eq.) in dry THF (50 mL) dropwise via cannula at 0 °C under nitrogen. The mixture was stirred overnight while warming to rt gradually. The reaction was then quenched by dropwise addition of H_2O (1.55 mL), 15% aq. NaOH (1.55 mL), and H_2O (4.65 mL), sequentially. The white precipitate was removed by filtration over Celite and the filtrate was concentrated to dryness. Flash column chromatography (SiO_2, 8:1:1 EtOAc/MeOH/Et_3N) gave 3.23 as a pale-yellow oil (2.50 g, 60%): R_f = 0.2 (SiO2, 8:1:1 EtOAc/MeOH/Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15-1.49 (m, 18H), 2.11 (brs, 2H), 2.67 (t, J = 7.08 Hz, 2H), 2.99 (dd, J = 9.08, 7.68 Hz, 1H), 3.14 (dd, J = 9.28, 3.12 Hz, 1H), 3.70-3.73 (m, 1H), 3.77 (s, 6H), 6.81 (d, J = 8.84 Hz, 4H), 7.19 (t, J = 6.56 Hz, 1H), 7.27 (t, J = 7.16 Hz, 2H), 7.30 (d, J = 8.64 Hz, 4H), 7.41 (d, J = 7.44 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 27.2, 29.7, 29.82 (2C), 29.88, 29.9, 33.7 (2C), 42.4, 55.5, 67.9, 71.2, 86.3, 113.3, 126.9, 128.0, 128.3, 130.2, 136.2, 145.0, 158.6; HRMS (ESI) m/z calcd for $C_{33}H_{46}NO_4$ $[M+H]^+$ 520.3426, found 520.3429.

Compound 3.24:

To a solution of **3.23** (220 mg, 0.423 mmol, 1 eq.) and triethylamine (88 µL, 0.635 mmol, 1.5 eq.) in dry DCM (15 mL) was added 6-chlorohexanoyl chloride (0.051 mL, 0.423 mmol, 1 eq.) at -78 °C under nitrogen. The mixture was stirred for 1 h while warming to rt slowly. Water (15 mL) was added and the organic contents were extracted with DCM (15 mL × 3). The extracts were combined and dried over anhydrous Na_2SO_4, filtered, and concentrated. Flash column chromatography (SiO_2, 2:1 hexanes/EtOAc with 5% Et_3N) gave **3.24** as a pale-yellow oil (0.134 g, 49%): R_f = 0.5 (SiO2, 1:1 hexanes/EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 1.19-1.51 (m, 20H), 1.64 (p, J = 8.84 Hz, 2H), 1.75 (p, J = 6.68 Hz, 2H), 2.14 (t, J = 7.36 Hz, 2H), 2.35 (brs, 1H), 3.00 (dd, J = 9.28, 7.60 Hz, 1H), 3.14 (dd, J = 9.32, 3.32 Hz, 1H), 3.20 (q, J = 7.08 Hz, 2H), 3.50 (t, J = 6.60 Hz, 2H), 3.70-3.74 (m, 1H), 3.76 (s, 6H), 5.49 (brs, 1H), 6.80 (d, J = 8.92 Hz, 4H), 7.19 (tt, J = 7.20, 2.12 Hz, 1H), 7.26 (t, J = 7.76 Hz, 2H), 7.30 (d, J = 8.80 Hz, 4H), 7.41 (d, J = 8.72 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 25.8, 26.8, 27.2, 29.6, 29.77, 29.79 (2C), 29.89, 29.97, 32.6, 33.7, 36.9, 39.8, 45.1, 55.5, 67.9, 71.2, 86.3, 113.3, 126.9, 127.9, 128.3, 130.2, 136.3, 145.0, 158.6, 172.6; HRMS (ESI) m/z calcd for $C_{39}H_{55}ClNO_5$ $[M+H]^+$ 652.3768, found 652.3770.

Compound 3.18a:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 9:1 hexanes/EtOAc with 5% Et_3N) gave **3.18a** as a colorless oil (412 mg, 79%): Mixture of two diastereoisomers; R_f = 0.6 and 0.7 (SiO_2, 3:1 hexanes/EtOAc); ^1H NMR (400 MHz,

$CDCl_3$) δ 1.05 (d, J = 6.76 Hz, 3H), 1.11-1.35 (m, 23H), 1.45-1.79 (m, 3H), 1.79-1.95 (m, 1H), 1.95-2.12 (m, 1H), 2.259 (t, J = 7.68 Hz, 1H), 2.263 (t, J = 7.48 Hz, 1H), 2.57-2.68 (m, 1H), 2.69-2.89 (m, 3H), 2.96 (q, J = 2.96 Hz, 1H), 3.06 (q, J = 5.76 Hz, 1H), 3.22 (q, J = 5.20 Hz, 1H), 3.22 (q, J = 5.04 Hz), 3.47-3.65 (m, 2H), 3.65-3.80 (m, 1H), 3.766 (s, 3H), 3.773 (s, 3H), 3.84-3.92 (m, 1H), 3.92-4.05 (m, 1H), 4.11 (q, J = 7.12 Hz, 2H), 4.10-4.21 (m, 1H), 6.78 (d, J = 11.72 Hz, 2H), 6.81 (d, J = 7.48 Hz, 2H), 7.13-7.21 (m, 1H), 7.24 (t, J = 7.88 Hz, 1H), 7.26 (t, J = 7.20 Hz, 1H), 7.33 (d, J = 8.64 Hz, 2H), 7.35 (dd, J = 8.04, 1.60 Hz, 2H), 7.45 (d, J = 5.08 Hz, 1H), 7.46 (d, J = 5.24 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 24.84, 24.86, 24.91, 24.94, 25.00, 25.04, 25.07, 25.11, 25.17, 25.31, 25.34, 25.39, 26.30, 26.34, 28.6 (d, Jcp = 7.00 Hz), 28.9 (d, Jcp = 8.96 Hz), 29.47, 29.51, 29.59, 29.72, 29.76, 29.91, 30.02, 33.76, 33.9 (d, Jcp = 6.28 Hz), 34.7, 43.2 (d, Jcp = 4.18 Hz), 43.4 (d, Jcp = 4.01 Hz), 46.9 (d, Jcp = 5.52 Hz), 47.3 (d, Jcp = 6.98 Hz), 55.5, 60.4, 64.9 (d, Jcp = 7.38 Hz), 65.1 (d, Jcp = 18.48 Hz), 66.3 (d, Jcp = 1.82 Hz), 66.4 (d, Jcp = 3.34 Hz), 73.7 (d, Jcp = 14.99 Hz), 74.3 (d, Jcp = 18.69 Hz), 85.9, 113.1, 126.7, 127.8, 128.45, 128.53, 130.30, 130.37, 136.6, 136.7, 145.3, 145.4, 158.4, 174.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.0, 149.2; HRMS (ESI) m/z calcd for $C_{45}H_{67}NO_7PS_2$ $[M+H]^+$ 828.4096, found 828.4099.

Compound 3.18b:

The same procedure for **3.7a** was used. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc with 5% Et_3N) gave **3.18b** as a pale-yellow oil (294 mg, 86%): Mixture of two diastereoisomers; R_f = 0.2 and 0.3 (SiO_2, 1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (d, J = 6.80 Hz, 2H), 1.10-1.35 (m, 22H), 1.40-1.51 (m, 4H), 1.56-1.69 (m,

4H), 1.77 (p, J = 7.12 Hz), 1.81-1.94 (m, 1H), 1.95-2.10 (m, 3H), 2.15 (t, J = 7.44 Hz, 2H), 2.56-3.15 (m, 6H), 3.21 (t, J = 6.80 Hz, 1H), 3.22 (t, J = 6.52 Hz, 1H), 3.52 (t, J = 6.64 Hz, 2H), 3.55-4.18 (m, 4H), 3.76 (s, 1H), 3.77 (s, 3H), 4.35-4.57 (m, 2H), 5.47 (brs, 1H), 6.78 (d, J = 8.84 Hz, 2H), 6.80 (d, J = 7.32 Hz, 2H), 7.13-7.21 (m, 1H), 7.21-7.28 (m, 2H), 7.32 (dd, J = 6.52, 2.64 Hz, 2H), 7.34 (dd, J = 8.36, 1.72 Hz, 2H), 7.44 (dd, J = 5.44, 1.61 Hz, 1H), 7.46 (dd, J = 7.20, 1.61 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.84, 24.86, 24.90, 24.94, 25.00, 25.07, 25.11, 25.17, 25.28, 25.38, 25.6, 25.9, 26.1, 26.30, 26.34, 26.7, 26.8, 27.0, 27.2, 27.3, 28.6, 28.7, 28.85, 28.88, 29.6, 29.81, 29.85, 29.88, 29.93, 29.99, 30.03, 32.6, 33.79 (d, Jcp = 2.99 Hz), 33.86 (d, Jcp = 5.24 Hz), 36.9, 39.8, 43.2, 43.4, 45.1, 46.93 (d, Jcp = 7.09 Hz), 47.29 (d, Jcp = 7.27 Hz), 55.5, 64.9 (d, Jcp = 17.44 Hz), 66.3 (d, Jcp = 6.76 Hz), 74.4, 85.9, 113.2, 126.7, 127.8, 128.45, 128.53, 130.3, 136.6, 136.7, 145.3, 145.4, 158.4, 172.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.0, 149.2; HRMS (ESI) m/z calcd for $C_{50}H_{77}ClN_2O_6PS_2$ $[M+H]^+$ 931.4649, found 931.4650

ODN Synthesis, Cleavage and Deprotection, and Analysis:

All ODNs were synthesized on dT-Dmoc-CPG (26 μmol/g loading, 20 mg, 0.52 μmol) using a MerMade 6 Synthesizer. Dim-Dmoc phosphoramidites were used as monomers. The conditions suggested by synthesizer manufacturer for 1 μmol synthesis were used except that coupling was optionally increased from 2 to 3 times and capping was achieved using 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphordiamidite instead of acetic anhydride. Briefly, detritylation, DCA (3%, DCM), 90 sec × 2; coupling, phosphoramidite (**3.7a-e**, **3.18a** or **3.18e**, 0.1 M, MeCN), 5-(ethylthio)-1H-tetrazole (0.25 M, MeCN), 60 sec × 2 (or 3); capping, 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphordiamidite (0.1 M,

MeCN) and 5-(ethylthio)-1H-tetrazole (0.25 M, MeCN), 60 sec × 3; oxidation, I2 (0.02 M, THF/pyridine/H2O, 70/20/10, v/v/v), 40 sec. For incorporating the last nucleoside, **3.7e** instead of **3.7a** was used. At the end of synthesis, the 5'-trityl group was kept on. The CPG was divided into 5 equal portions. One portion was gently shaken in a solution of aqueous $NaIO_4$ (0.4 M, 1 mL) at rt for 3 h. The supernatant was removed with a pipette, and the CPG was rinsed briefly with water (1 mL × 4). To the CPG was added aqueous aniline solution (3%, 1 mL) and the mixture was shaken at rt for 3 h. The supernatant was transferred into a centrifugal tube, which was concentrated to ~100 μL. To the tube was added 1-butanol (900 μL). The tube was vortexed briefly and centrifuged (14.5K rpm, 5 min). The supernatant was removed with a pipette carefully without sucking the ODN precipitate. The ODN was dissolved in H_2O (100 μL) and ~35 μL was injected into RP HPLC to generate the crude ODN. Fractions of the major ODN peak at ~39 min were collected, concentrated to ~100 μL, and injected into HPLC to give the profile of purified trityl-tagged ODN. To the dried trityl-tagged ODN was added 1 mL of 80% AcOH, and the mixture was shaken gently at rt for 3 h. Volatiles were evaporated. The residue was dissolved in ~100 μL water and injected into RP HPLC. The major peak of de-tritylated ODN at ~21 min was collected and concentrated to dryness. The residue was the pure de-tritylated ODN, which was dissolved in 100 μL water and injected into HPLC to generate the profile of pure de-tritylated ODN. The pure ODN was analyzed MALDI-TOF MS. Information about OD260 of the ODNs are provided in the UV spectra section of the Supporting Information.

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Chapter 4 Electrophilic ODN Synthesis Using dM-Dmoc

Work in this chapter has been submitted and accepted by Beilstein Journal of Organic Chemistry

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Abstract

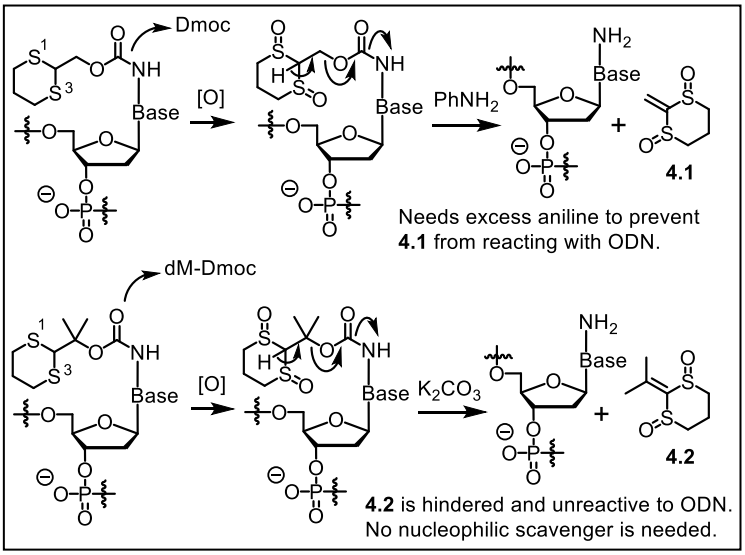
Solid phase synthesis of electrophilic oligodeoxynucleotides (ODNs) was achieved using dimethyl Dmoc (dM-Dmoc) as amino protecting groups. Due to the high steric hindrance of the 2-(propan-2-ylidene)-1,3-dithiane side product from deprotection, the use of excess nucleophilic scavengers such as aniline to prevent Michael addition of the side product to the deprotected ODN during ODN cleavage and deprotection was no longer needed. The improved technology was demonstrated by the synthesis and characterization of five ODNs including three modified ones. The modified ODNs contained the electrophilic groups ethyl ester, α-chloroamide, and thioester. Using the technology, the sensitive groups can be installed at any location within the ODN sequences without using any sequence- or functionality-specific conditions and procedures.

4.1 Introduction

After over 60 years of intensive research, the challenges for chemical oligodeoxynucleotide (ODN) synthesis have been considered largely overcome. [1-4] However, this is only true for unmodified ODNs at limited synthesis scales. For modified ODNs that contain sensitive functionalities including those that are unstable under acidic, basic and strongly nucleophilic conditions, many formidable challenges remain. [2] The reason is that during ODN synthesis using traditional technologies, the 5'-hydroxyl group of nucleoside monomers is protected with the 4,4'-dimethoxytrityl (DMTr) group, which has to be removed with an acid in each synthetic cycle. The *exo*-amino groups of nucleosides dA, dC and dG are protected with acyl groups, the nascent ODN is anchored to a solid support via a base- or nucleophile-cleavable linker, and in the most widely used phosphoramidite technology the phosphate groups are protected with the 2-cyanoethyl group. These protecting groups and the linker have to be cleaved under strongly basic and nucleophilic conditions. As a result, many sensitive groups including acetal, hemiacetal, vinyl ethers, enol ethers, aldehydes, esters, activated esters, thioesters, aziridines, epoxides, alkyl halides, α-halocarbonyls, vinyl purines, methides and maleimides cannot or are difficult to be incorporated into ODNs, or cannot be installed at the desired locations in the ODNs. For example, to synthesize oligos that contain the epigenetically modified 5-formylcytosine, the aldehyde group had to be protected as a cyclic acetal instead of the more labile acyclic acetal. [5,6] The maleimide group was incorporated into ODNs as a Diels-Alder adduct with dimethylfuran. Besides the need of an additional step for deprotection, only examples of 5'-end modification were given probably due to the instability of the adduct under acidic conditions during ODN synthesis. [7]

In recent years, applications of ODNs have extended to emerging areas such as nanotechnology, [8,9] antisense drug development, [10-12] DNA damage and repair, [13,14] DNA methylation and demethylation, [15-18] DNA-protein interactions, [19,20] CRISPR genome editing, [21-23] DNA data storage, [24,25] synthetic biology, 26 bioconjugation, [27] and others. [28-30] These applications frequently require modified ODNs that contain a wide variety of functional groups including those that cannot survive known ODN synthesis, cleavage and deprotection conditions. To meet these demands, some work on developing new technologies suitable for the synthesis of sensitive ODNs has been carried out. [28,31] A common method is to use more labile acyl functions such as the phenoxyacetyl group for amino protection and as linker to enable deprotection and cleavage under milder basic conditions. [32] The palladium-labile allyl groups were also used for amino protection. [33,34] The *o*-nitro benzyl function was used as linker to enable photo cleavage. [34] However, these methods are still not ideal. The phenoxyacetyl group and linker still needs nucleophilic cleavage. Palladium is expensive and difficult to remove from ODN. Photo irradiation can damage ODN. The (*p*-nitrophenyl)ethyl (Npe) and (*p*-nitrophenyl)ethyloxycarbonyl (Npeoc) were also explored for sensitive ODN synthesis under non-nucleophilic conditions. [35-38] The requirement of the strong base - DBU in aprotic solvents over long hours in the presence of a nucleophilic scavenger for their cleavage could limit their application. In addition, in some cases the sequences synthesized by the method were short and the yields of the ODNs were low. [35-38] In the literature, there are also examples using post-synthesis modifications to introduce sensitive groups to ODNs. [12] However, these methods are case-specific, and their procedures are usually complicated. The ODN synthesis method without nucleobase protection could be considered for the incorporation

of sensitive functionalities into ODNs. [39] However, a linker that can be cleaved under mild conditions and is suitable for the purpose has not been identified. More seriously, high selectivity of *O*-phosphitylation over *N*-phosphitylation, which is crucial for practical applications especially for the synthesis of ODNs approaching 20-mer or longer, may not be easy to achieve.



Scheme 4.1. Comparison of Dmoc and dM-Dmoc as nucleobase protecting groups for ODN synthesis.

To develop a universal technology for the synthesis of ODNs that contain a wide variety of sensitive functionalities, we recently reported the use of 1,3-dithian-2-yl-methoxycabonyl (Dmoc) as protecting groups and linkers for ODN synthesis. [40,41] Due to the low acidity of H-2 (pKa ~31) in the Dmoc function, these groups and linkers were expected to be stable under ODN synthesis conditions. However, once the dithioketal in the group is oxidized, the acidity of H-2 (pKa ~12) is drastically increased. [42,43] Considering that the widely used Fmoc protecting group, of which the H-9 has a pKa of ~22, [42] can be readily removed with a weak base such as piperidine, we hypothesized that the oxidized

Dmoc groups and linkers could be cleaved under weakly basic and non-nucleophilic conditions via β-elimination. Indeed, we found that the deprotection and cleavage could be achieved by oxidation with sodium periodate followed by treating with the mild base aniline at room temperature. Due to the mild deprotection and cleavage conditions, we concluded that the technology was suitable for the synthesis of sensitive ODNs that contain electrophilic groups. However, at the current state of art one drawback of the technology is that large excess aniline has to be used as a scavenger to prevent the deprotection side product **4.1** from reacting with the deprotected ODNs via Michael addition. Aniline is a weak nucleophile, but using large excess is not ideal for a technology aimed to be practically and universally useful. In this paper, we report the use of dimethyl Dmoc (dM-Dmoc), which we previously studied for alkyl and aryl amine protections, [44] in place of Dmoc for nucleobase protection for ODN synthesis (Scheme 4.1). Due to the steric hindrance of the side product **4.2** from deprotection, we found that a nucleophilic scavenger was no longer needed during deprotection, and the β-elimination step could be achieved using the non-nucleophilic weak base potassium carbonate.

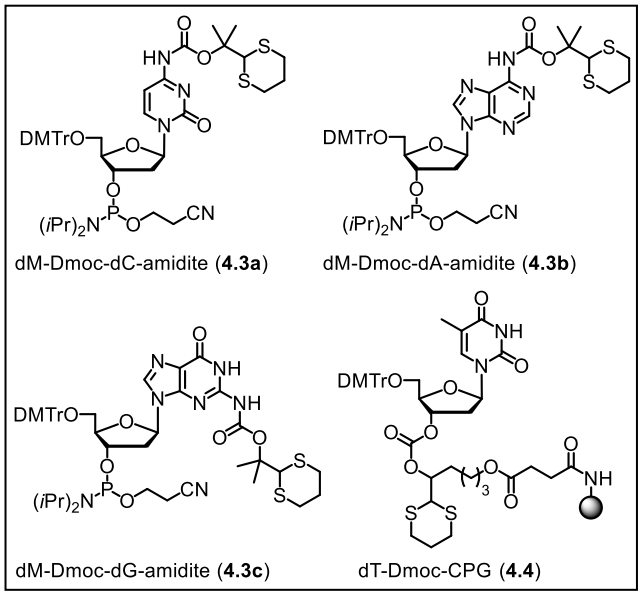
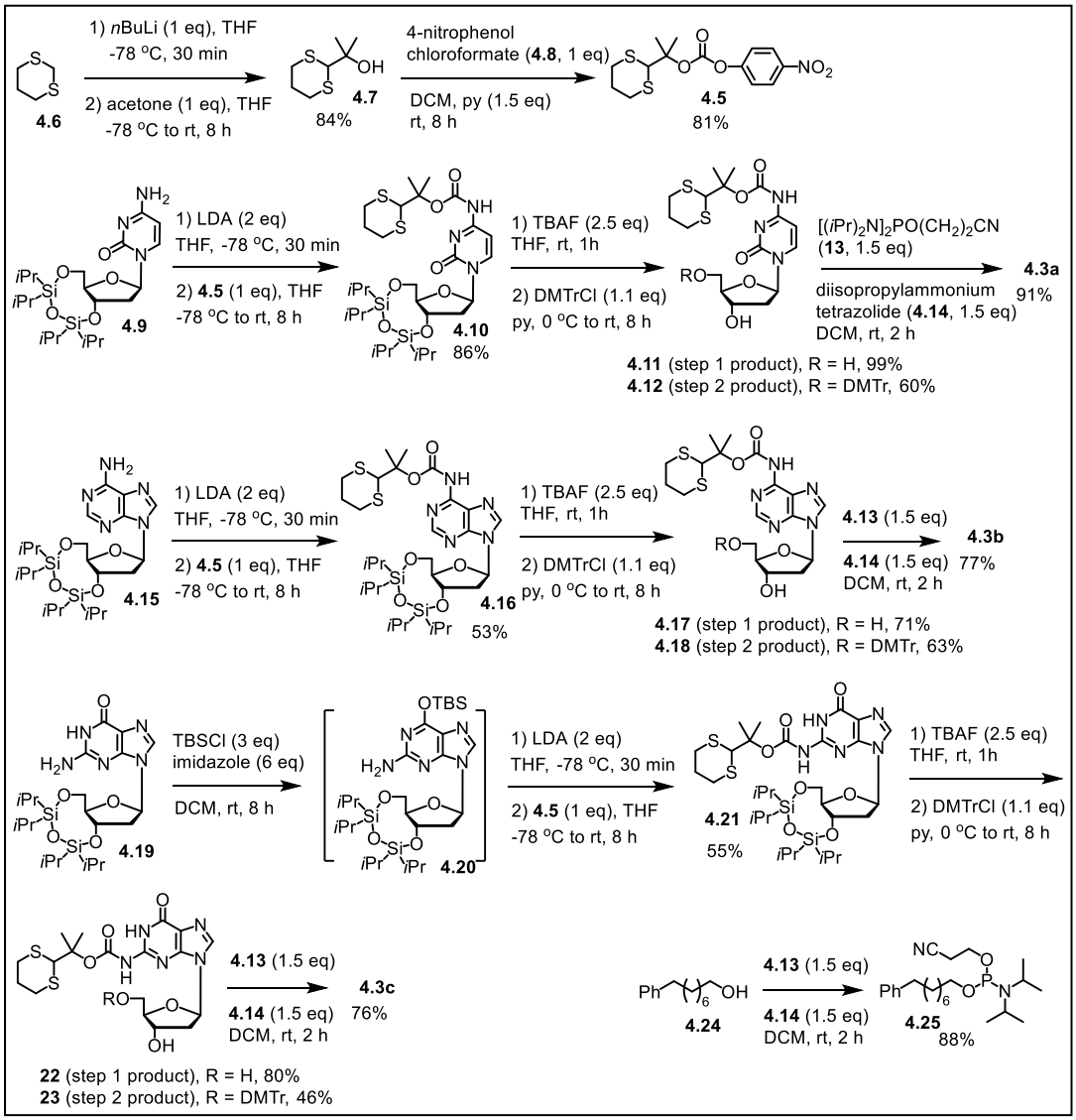


Figure 1: dM-Dmoc phosphoramidite monomers and CPG with Dmoc linker.

4.2 Results and Discussion

To develop the dM-Dmoc electrophilic ODN synthesis technology, the phosphoramidite monomers **4.3a-c** and the linker **4.4** (Figure 4.1) were needed. The construction of linker **4.4** was reported previously. [40] The synthesis of **4.3a-c** is shown in Scheme 4.2. The reagent **4.5** needed for protecting the *exo* amino groups of nucleobases was prepared in two steps from 1,3-dithiane (**4.6**) according to a procedure we reported previously. [44] The dC phosphoramidite monomer **4.3a** was synthesized from compound **4.9**. [45] The formation of the hindered *O*-*tert*-alkyl *N*-arylcarbamate **4.10** was found highly challenging. [44,46,47] We tried many conditions and finally found that acceptable yields could be achieved under the highly reactive conditions involving two equivalents LDA and one equivalent **4.5**. The silyl protecting groups were then removed with TBAF giving compound **4.11** in 99% yield. Tritylation of **4.11** with DMTrCl gave **4.12**, which was phosphitylated with reagents **4.13** and **4.14** to give the target monomer **4.3a** (Scheme 4.2). The dA phosphoramidite monomer **4.3b** was synthesized similarly starting from **4.15**. [48] The amino group of **4.15** was carbamylated with **4.5** in the presence of two equivalents LDA to give **4.16**. The silyl groups were removed, and compound **4.17** was tritylated to give **4.18**, which was phosphitylated to give **4.3b**. The dG phosphoramidite monomer **4.3c** had to be synthesized using slightly different procedure (Scheme 4.2). The amide function in the nucleobase in the silyl protected nucleoside **4.19**. [45] was temporarily protected with TBSCl to give **4.20**. [49] This intermediate was not isolated and the *exo* amino group was carbamylated directly with **4.5** in the presence of two equivalents LDA giving **4.21** in 55% yield. The silyl protecting groups were removed to give **4.22**, which was tritylated to give

4.23 and phosphitylated to give the target monomer **4.3c** (Scheme 4.2). As will be discussed later, we also needed the hydrophobic phosphoramidite **4.25** for developing the dM-Dmoc ODN synthesis technology. The compound was simply prepared from the commercially available **4.24** via phosphitylation using the reagents **4.13**-**14** (Scheme 4.2).



Scheme 4.2. Synthesis of compound **4.5**, [44] nucleoside phosphoramidite monomers **4.3a-c** and phosphoramidite capping agent **4.25**.

To demonstrate the capability of the dM-Dmoc ODN synthesis technology for incorporating electrophilic groups, we also needed phosphoramidite monomers **4.26a-c**, which contained the sensitive functionalities ester, α-chloroacetamide and thioester, respectively (Figure 2). The synthesis of **4.26b-c** has been reported [40]. Scheme 3 shows the synthesis of **4.26a**. The required 1,2-diol **4.28** was simply prepared from the commercially available **4.27** by esterification in ethanol. Cyclization or oligomerization of **4.27** was not an issue for the transformation. The primary alcohol of **4.28** was selectively tritylated with DMTrCl to give **4.29**, which was phosphitylated with **4.13** in the presence of **4.14** to give **4.26a**.

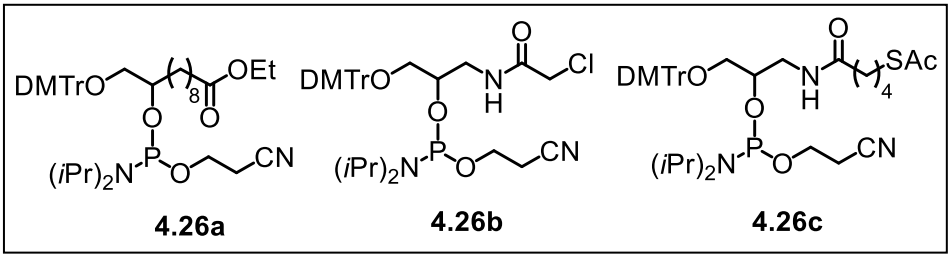
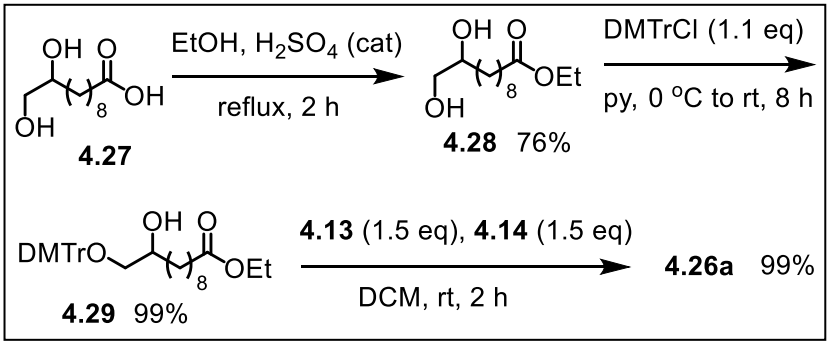


Figure 4.2. Structure of phosphoramidites containing electrophilic groups.



Scheme 4.3. Synthesis of ester-containing phosphoramidite **4.26a**.

With the required phosphoramidite monomers (**4.3a-c**) and linker (**4.4**) in hand, we decided to identify suitable conditions for ODN synthesis, deprotection and cleavage under non-nucleophilic conditions by synthesizing the unmodified ODNs **4.30a-b** (Figure 4.3). The syntheses were conducted at a scale of 0.52 µmol on a MerMade 6 DNA/RNA synthesizer. The dT-Dmoc-CPG (**4.4**) was used as the solid support. Detritylation was carried out under standard conditions suggested by the synthesizer manufacturer for 1 µmol synthesis. The 0.1 M acetonitrile solutions of phosphoramidite monomers **4.3a-c** and the commercially available 5'-DMTr β-cyanoethyl dT phosphoramidite were used for incorporating dA, dC, dG and dT nucleotides, respectively. The coupling conditions were standard except that in some cases, coupling was increased from two to three times. Capping failure sequences was achieved using the phosphoramidite **4.25** with 5-(ethylthio)-1*H*-tetrazole as activator instead of the typically used acetic anhydride. Oxidation was performed under standard conditions. The last nucleotide at the 5'-end of ODN was incorporated with a 5'-trityl nucleoside phosphoramidite instead of a 5'-DMTr counterpart. At the end of the synthesis, the 5'-trityl group was not removed. More details about the synthesis are given in the Experimental Section. For cleavage and deprotection under non-nucleophilic conditions, the ODN on CPG, which should appear as **4.31** (Scheme 4.4) with a 5'-trityl tag, was treated with a DBU solution in acetonitrile at room temperature briefly. This removed the β-cyanoethyl phosphate protecting groups to give **4.32**. HPLC analysis of the DBU solution did not found any ODN that was cleaved prematurely – an observation consistent with the slow rate of cleavage of succinyl-anchored ODNs from solid support under similar conditions. [50] The dithioketal groups in the dM-Dmoc and Dmoc functions of **4.32** were then oxidized with a solution of sodium periodate at room temperature to give **4.33**. The

5'-trityl tag survived the conditions. It should be pointed out that some sulfoxides might be further oxidized to sulfones, which should not affect the overall results of the deprotection and cleavage procedure. Removal of the oxidized dM-Dmoc protection groups and cleavage of the oxidized Dmoc linker were achieved with a solution of the weak non-nucleophilic base potassium carbonate at pH 8 at room temperature giving the fully deprotected 5'-trityl-tagged ODN **4.30** (Scheme 4.4). Purification of the ODN **4.30a** was achieved in two steps – trityl-on RP HPLC followed by trityl-off RP HPLC. For trityl-on HPLC (profile a, Figure 4.4), the desired full-length 5'-trityl-tagged ODN appeared at 36-39 minutes and was well separated from other peaks. This peak was collected, and analyzed with RP HPLC (profile b). The purified 5'-trityl-tagged ODN was detritylated with 80% acetic acid. Even though it was reported that removal of trityl groups from a primary alcohol required two days at room temperature with 80% acetic acid, [51] we found that our detritylation could reach completion or in some cases close to completion in three hours. After the acid was evaporated, the de-tritylated ODN was purified again with RP HPLC (profile c). The major peak at around 20 minutes was collected, the ODN from which showed a single sharp peak when analyzed with RP HPLC (profile d). The purified de-tritylated ODN was further analyzed with polyacrylamide gel electrophoresis (PAGE), a single band was observed (Lane 1, Figure 4.5). The HPLC purified ODN was also analyzed with MALDI-TOF MS, molecular mass corresponding to correct ODN structure was found (Figure 4.6). The unmodified ODN **4.30b** were synthesized, purified and analyzed under the same conditions. Its HPLC profiles and MS are in the Supporting Information, and PAGE image is in Figure 4.5. All the analytical data indicate that the ODNs were pure and had correct identity.

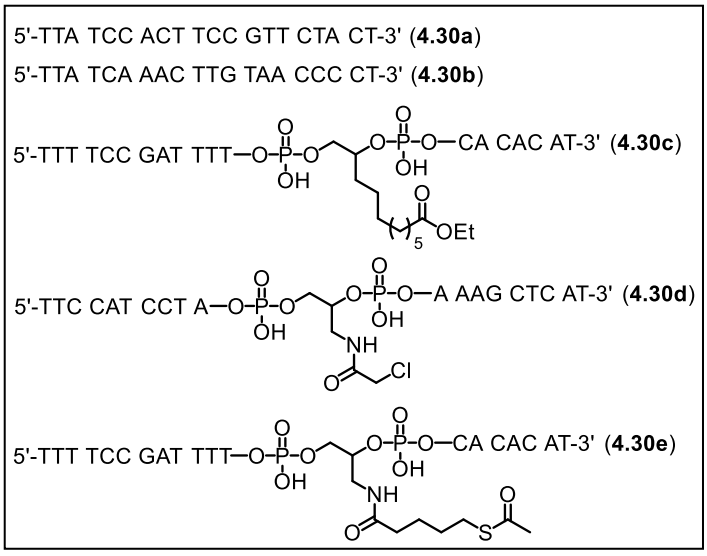
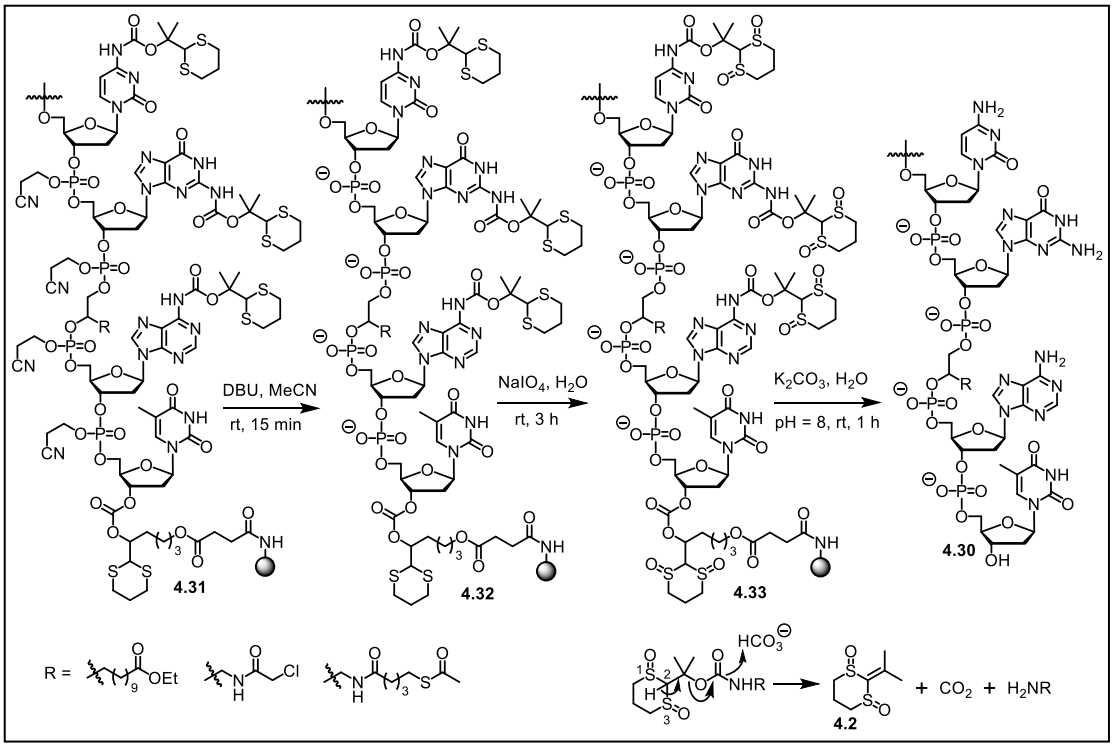


Figure 4.3. ODN sequences **4.30a-e**. Their 5'-tritylated versions are labeled as **4.30a-tr**, **4.30b-tr**, **4.30c-tr**, **4.30d-tr**, and **4.30e-tr**, respectively.

In the RP HPLC profiles of the crude 5'-trityl-tagged ODNs such as that for **4.30a-tr** (profile a, Figure 4.4), besides failure sequences at around 20 minutes, there were multiple peaks after 40 minutes. We believe that those peaks were from branched ODNs generated from the premature deprotection of dM-Dmoc groups during ODN synthesis. The dM-Dmoc protections, which contained a tertiary butyl carbamate moiety, were not completely stable under the acidic conditions needed for de-tritylation in each synthetic cycle. Once the protection was lost, in the coupling step, incoming phosphoramidites would react with the free amino groups, and branched ODNs would be produced. Fortunately, these branched ODNs had two or more 5'-trityl groups, and therefore were significantly more hydrophobic than the desired ODN. During RP HPLC, they were eluted significantly later than the desired ODN and could be easily removed. We believe that the branching problem was not caused by premature oxidation of the dM-Dmoc groups by iodine in the

oxidation step in ODN synthesis because the problem did not exist when Dmoc was used for ODN synthesis. [41] In addition, we also subjected 1,3-dithiane to the iodine oxidation conditions for over 24 hours, no oxidation could be detected. Before using **4.25** for capping and trityl group for 5'-tagging in ODN synthesis using **4.3a-c** and **4.4**, we tried to synthesize ODNs under standard conditions using acetic anhydride for capping and without tagging the 5'-end of ODNs. RP HPLC analyses showed that the peaks of the desired ODNs and branched sequences were very close and, in some cases, even overlapped, which made HPLC purification of the products difficult. A typical RP HPLC profile of ODNs synthesized in that manner is given in the Supporting Information. We therefore tried to keep the 5'-DMTr group at the end of solid phase synthesis to assist HPLC purification hypothesizing that the desired ODN with one DMTr group would be easy to be separated from any branched sequences that had two or more DMTr groups. This was indeed the case. A RP HPLC profile is given in the Supporting Information. However, the sodium periodate oxidation conditions used for ODN cleavage and deprotection were slightly acidic, and in most cases, we were not able to keep the DMTr groups. This problem made the method unreliable. We also tried to tag the ODN with the hydrophobic *t*-butyldiphenylsilyl (TBDPS) group. In RP HPLC profiles, the desired TBDPS-tagged full-length sequence was also separated very well from the branched sequences (Supporting Information). However, at this time we could not identify a mild condition to remove the tag after purification of the ODN. These experiments directed us to the use of the trityl tag to assist ODN purification as described above. The reason for us to use **4.25** instead of acetic anhydride for capping was based on two considerations. One was that if a branched sequence failed to react at one or more sites during coupling, capping with a hydrophobic

agent would still make the branched sequence more hydrophobic than the desired full-length sequence. Another consideration was that with acetic anhydride for capping, chances existed for replacing the dM-Dmoc groups with acetyl group during capping due to the presence of acids such as pyridinium acetate and large excess of acetic anhydride. Once the capping exchange occurred, the ODN molecule with an acetyl group would not be useful because the acetyl group would not be deprotectable under the mild deprotection conditions. Using **4.25** for capping, such capping exchange would not occur.



Scheme 4.4. ODN deprotection and cleavage under non-nucleophilic conditions.

After identifying suitable conditions for the synthesis of unmodified ODNs under non-nucleophilic conditions using the dM-Dmoc technology, studying the feasibility of the technology for the synthesis of modified ODNs containing ester, α-chloroacetamide and thioester groups was pursued. These groups are sensitive to nucleophiles and cannot

survive the commonly used concentrated ammonium hydroxide deprotection and cleavage conditions. We have demonstrated that the so called UltraMild deprotection and cleavage conditions involving potassium carbonate in anhydrous methanol are incompatible with α-chloroacetamide and thioester. [40] These findings are easily understandable because the species responsible for the cleavage and deprotection under UltraMild conditions is potassium methoxide, which is a strong nucleophile. The ODNs **4.30c**-**e** were chosen as the targets for the current study. The synthesis, deprotection and cleavage conditions were the same as those for the unmodified ODNs. The electrophilic groups were introduced with **4.26a**-**c**, respectively. In all cases, we placed the groups in the middle of the sequences, which is significantly more challenging than placing them at the 5'-end. The fully deprotected crude ODNs **4.30c**-**e** were purified and analyzed as described for **4.30a**. The HPLC profiles of crude and pure **4.30c** are given in Figure 4.4. Its PAGE and MALDI-TOF MS images are in Figures 4.5 and 4.6, respectively. All analytical data for **4.30d-e** are given in the Supporting Information. It is noted that aminolysis and hydrolysis of the sensitive groups in the ODNs, which were found to be a problem previously, [41] were successfully avoided by using the dM-Dmoc protection strategy. For all the five ODNs (**4.30a**-**e**), their OD_{260} after HPLC purification were determined (Supporting Information). They ranged from 2.32 to 6.68 for the 0.52 μmol syntheses. To have a direct comparison with standard ODN synthesis technology, we also synthesized **4.30a** using commercial phosphoramidites and 0.52 μmol **4.4** (Supporting Information). After purification with RP HPLC, the OD of **4.30a** was determined to be 8.30. With these data, we were able to conclude that the dM-Dmoc phosphoramidites had similar coupling efficiency as

commercial phosphoramidites and the overall yields of ODNs from the dM-Dmoc technology were at the same level of those from standard technologies.

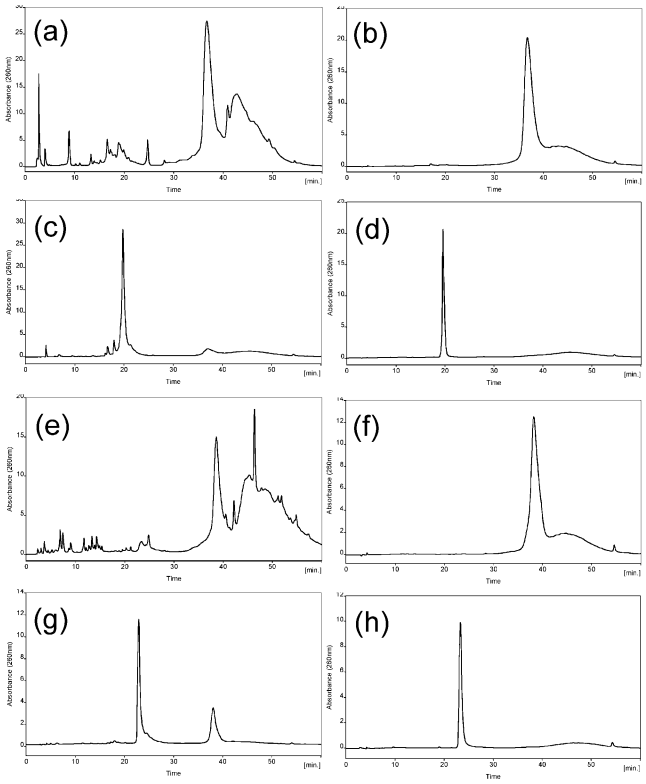


Figure 4.4. RP HPLC profiles of (a) crude **4.30a-tr**, (b) pure **4.30a-tr**, (c) crude **4.30a**, (d) pure **4.30a**, (e) crude **4.30c-tr**, (f) pure **4.30c-tr**, (g) crude **4.30c**, (h) pure **4.30c**. In profiles (a) and (e), the well-separated major peak before 40 minutes is the trityl-tagged full-length ODN. The peaks after 40 minutes are branched sequences with two or more trityl tags.

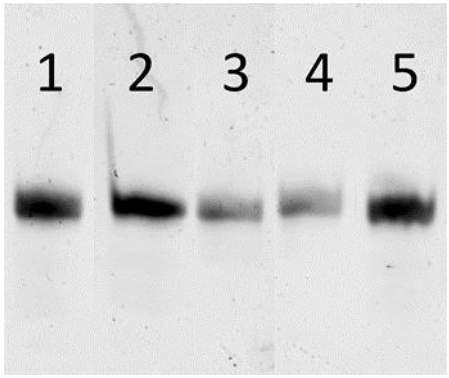


Figure 4.5. PAGE analyses of ODNs **4.30a-e**. Lanes 1-5 are ODNs **4.30a-e**, respectively.

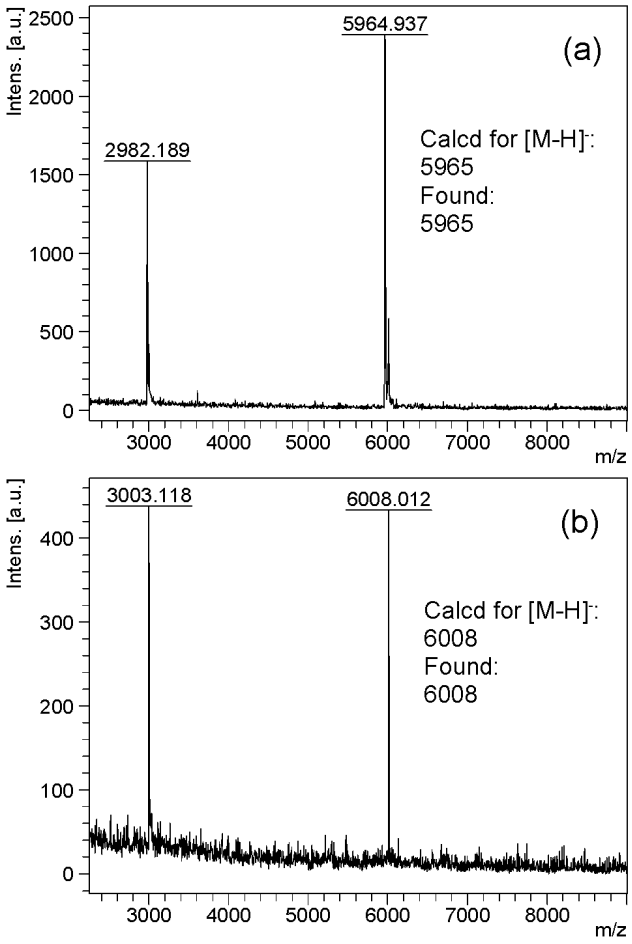


Figure 4.6. MALDI-TOF MS of (a) ODN **4.30a** and (b) **4.30c**.

The successful synthesis and HPLC purification of the above five ODNs demonstrated that dM-Dmoc is a viable choice for amino protection for electrophilic ODN synthesis. Compared with using Dmoc for ODN synthesis, the major advantage of using dM-Dmoc is that deprotection can be achieved without using any nucleophilic scavengers. Using Dmoc, during deprotection after sodium periodate oxidation, large excess aniline is needed to induce β-elimination (see Scheme 4.1) and to prevent the side product **4.1** from reacting with the deprotected ODN via Michael addition. [40] Aniline is a weak base and only mildly nucleophilic. Electrophiles that are compatible with ODNs but reactive toward it are rare. However, using large excess of aniline could be a significant drawback. For example, many electrophiles could be considered unreactive to it, but in the presence of large excess of it, problems might arise. In addition, its boiling point is high, alternative techniques other than simple evaporation has to be used for its removal. In order to accomplish our goal of developing a universally useful technology for electrophilic ODN synthesis, the dM-Dmoc technology is a logical extension of our previous effort. [40] Using dM-Dmoc, the side product of deprotection is **4.2**. We believe that **4.2** could not react with the nucleophiles on ODNs including hydroxyl and amino groups. Even the reaction took place, a hindered four-substituted carbon centre would be formed. Because the Michael addition reaction is reversible, the adducts would easily fall apart to give back un-modified ODNs. Indeed, due to the use of dM-Dmoc, we were able to induce β-elimination with potassium carbonate in the absence of any scavenger under mild conditions. Besides the advantage of avoiding the use of excess aniline as a scavenger, in the new studies, we also found that the acetic acid used in our previous studies for sodium periodate oxidation could be omitted. In that report [40] for oxidation of the dithioketals in Dmoc, an acidic solution of

sodium periodate adjusted to pH 2 with acetic acid was used. Under those conditions, β-elimination did not occur and the ODNs remained on the solid support during oxidation. This greatly facilitated the removal of excess sodium periodate and its reduced salts because they could be easily washed away with water. Otherwise, more expensive means such as size exclusion chromatography had to be used. In our new studies, we tested to perform the oxidation in the absence of acetic acid. We found that the pH of 0.4 M sodium periodate solution was around 4, and this solution did not cause premature β-elimination during oxidation. Therefore, the ODNs remained on the solid support under this significantly less acidic conditions. Because ODNs are inherently unstable under acetic conditions, avoiding the use of acetic acid and performing the cleavage and deprotection at nearly neutral pH could make the technology more useful. In addition, the scope of sensitive functionalities to be introduced to ODNs using the technology could be further extended. The finding of the stability of the Dmoc function in linker **4.4** after oxidation under nearly neutral conditions is also important for considering using the technology for oligoribonucleotides (ORNs) synthesis. One potential problem to use the technology for ORN synthesis is that during oxidation of the Dmoc and dM-Dmoc functions using sodium periodate, if the oxidized Dmoc in the linker were unstable, and the 2' and 3'-OH groups were exposed before sodium periodate were removed, the C-C bond between the 2' and 3' carbons could be cleaved. With the finding of the relatively high stability of the oxidized Dmoc function, we are more confident that the Dmoc associated technologies will be useful for ORN synthesis as well.

4.3 Conclusion

In summary, we have extended the Dmoc-based electrophilic ODN synthesis technology to a new level, at which dM-Dmoc is used for protecting *exo*-amino groups of nucleobases. With this advancement, the previously used large excess aniline for scavenging the Michael acceptor side product during cleavage and deprotection was no longer needed. This makes the technology more convenient to use and could extend its scope on incorporating different sensitive functionalities into ODNs. In addition, we found that the sodium periodate oxidation step for cleavage and deprotection could be performed in the absence of acetic acid at nearly neutral conditions instead of previously used acidic conditions. Because ODNs and many functionalities are sensitive to acid, the significantly less acidic conditions will eliminate concerns of ODN damage and increase the scope of functionalities capable to be incorporated into ODNs. Using the technology, five ODNs including three modified ones containing the sensitive groups – ester, α-chloroamide and thioester – were successfully synthesized. We expect that the technology will become a useful tool for the synthesis of sensitive ODN analogs.

4.4 Experimental Section

General information:

All reactions were performed in oven-dried glassware under argon using standard Schlenk techniques. Reagents and solvents available from commercial sources were used as received unless otherwise noted. Lcaa-CPG (pore size 497 Å) was purchased from Prime Synthesis, Inc. Polyacrylamide desalting column (5K MWCO, 10 mL) was purchased from Thermo Scientific. THF and CH_2Cl_2 was dried using an Innovative Technology Pure-Solv™ system. Pyridine, diisopropylamine and acetone were distilled over CaH_2 under nitrogen. Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 250 μm thickness. Flash column chromatography was performed using SiliCycle silica gel, particle size 40-63 μm. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on a Varian UNITY INOVA spectrometer at 400, 100 and 162 MHz, respectively; chemical shifts (δ) were reported in reference to solvent peaks (residue $CHCl_3$ at δ 7.24 ppm for ^{1}H and $CDCl_3$ at δ 77.00 ppm for ^{13}C) and to H_3PO_4 (δ 0.00 ppm for ^{31}P). HRMS was obtained on a Thermo HR-Orbitrap Elite Mass Spectrometer. LRMS was obtained on a Thermo Finnigan LCQ Advantage Ion Trap Mass Spectrometer. MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System. ODNs were synthesized 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. A C-18 reversed phase analytical column (5 μm diameter, 100 Å, 250 × 3.20 mm) was used. Solvent A: 0.1 M triethylammonium acetate, 5%

acetonitrile. Solvent B: 90% acetonitrile. 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. PAGE of ODNs was run in a gel slide casted with a stock solution prepared using the recipe – 62.5 mL 40% acrylamide and bis-acrylamide (19:1), 52.55 g urea, 6.25 mL 10 × TBE (tris/borate/EDTA) buffer, and suitable amount of DI water for a total 100 mL solution. The gel slide was casted with 7 mL of the stock solution, 70 µL 10% $(NH_4)_2S_2O_4$, and 7 µL TMEDA (tetramethylethylenediamine). Electrophoresis was run in 10 × TBE buffer at 200 V by pre-run (without sample) for 30 min followed by actual run (with sample) for 90 min. The gel was stained with SYBR® Gold, and images were obtained with a BioRad Gel Doc™ XR+ Gel Documentation System.

Compound 4.7: [1]

To a solution of 1,3-dithiane (**4.6**, 5.0 g, 41.6 mmol) in dry THF (100 mL) was slowly added *n*BuLi (2.5 M in pentane, 15.7 mL, 41.6 mmol) under argon at -78 °C. After stirring for 30 min, freshly distilled acetone (3.0 mL, 41.6 mmol) was added dropwise at -78 °C. The reaction was allowed to proceed for 8 h while warming to rt, and then quenched with saturated NH_4Cl (75 mL). The mixture was extracted with EtOAc (50 mL × 2). The extracts were combined and dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified with flash column chromatography (SiO_2, 4:1 hexanes/EtOAc) to afford **4.7** as a white solid (6.24 g, 84%). [1]

Compound 4.5: [1]

To a solution of **4.7** (6.4 g, 36 mmol) and pyridine (2.9 mL, 54 mmol) in DCM (100 mL) was added *p*-nitrophenyl chloroformate (**4.8**, 7.2 g, 36 mmol) at rt under argon. After stirring at rt for 8 h, the contents were poured into a separatory funnel and partitioned between EtOAc (40 mL) and H_2O (80 mL). The aqueous layer was extracted with DCM (50 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (SiO_2, 9:1 hexanes/EtOAc) gave **4.5** as a white solid (10.0 g, 81%). [1]

Compound 4.10:

To a solution of diisopropyl amine (1.2 mL, 8.5 mmol) in THF at -78 °C was added *n*BuLi (2.5 M in pentane, 3.2 mL, 8.1 mmol) and stirred for 30 min. The freshly prepared LDA solution was added via a cannula to a solution of **4.9** (1.9 g, 4.05 mmol) in THF (50 mL) at -78 °C. After stirring for 30 min, compound **4.5** was added as a solid under positive nitrogen pressure at -78 °C. The mixture was stirred for 8 h while warming to rt. The contents were poured into a separatory funnel and partitioned between EtOAc (40 mL) and H_2O (40 mL). The aqueous layer was extracted with EtOAc (30 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) gave **4.10** as a white foam (2.33 g, 86%): R_f = 0.6 (1:2 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89-1.02 (m, 28H), 1.57 (s, 6H), 1.71-1.78 (m, 1H), 2.00-2.04 (m, 1H), 2.23-2.28 (m, 1H), 2.46-2.53 (m, 1H), 2.76-2.86 (m, 4H), 3.73 (d, J = 8.5 Hz, 1H), 3.93-3.97 (m, 1H), 4.09-4.12 (m, 1H), 4.27-4.33 (m, 1H), 4.92 (s, 1H), 5.98 (d, J = 6.5 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 8.10 (d, J = 7.4 Hz, 1H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.5, 13.12, 13.16, 13.6, 16.9, 17.10, 17.12, 17.2, 17.5, 17.64, 17.67, 24.7, 25.9, 31.0, 39.9, 56.8, 60.0, 66.7, 85.2, 85.3, 85.7, 94.5, 143.9, 150.8, 154.9, 162.6; HRMS (ESI): *m/z* calcd for $C_{29}H_{52}N_3O_7S_2Si_2$ $[M + H]^+$ 674.2785, found 674.2783.

Compound 4.11:

To the THF (10 mL) solution of **4.10** (800 mg, 1.19 mmol) at rt was added TBAF (1 M in THF, 3.0 mL, 3.0 mmol). The mixture was stirred for 1 h. THF was evaporated and the residue was loaded directly on a column. Flash column chromatography (SiO_2, 9.5:0.5 EtOAc/MeOH) gave **4.11** as a white foam (0.507 g, 99%): R_f = 0.3 (9.5:0.5 EtOAc/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.60 (s, 6H), 1.70-1.77 (m, 1H), 2.04-2.10 (m, 1H), 2.12-2.18 (m, 1H), 2.43-2.49 (m, 1H), 2.82-2.94 (m, 4H), 3.71 (dd, *J* = 12.1, 3.8 Hz, 1H), 3.81 (dd, *J* = 12.1, 3.2 Hz, 1H), 3.96-3.99 (m, 1H), 4.33-4.37 (m, 1H), 4.81 (s, 2H), 5.07 (s, 1H), 6.19 (t, *J* = 6.2 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 8.40 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 24.0, 26.0, 30.7, 41.3, 56.8, 61.3, 70.4, 84.5, 87.3, 88.2, 95.5, 144.4, 151.6, 156.4, 163.5; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}N_3O_6S_2$ $[M - H]^-$ 430.1107, found 430.1112.

Compound 4.12:

To a solution of **4.11** (513 mg, 1.19 mmol) in pyridine (10 mL) at 0 °C was added DMTrCl (440 mg, 1.31 mmol) 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 (small amount of pyridine was intentionally left to ensure basicity of the residue, which could help to avoid losing DMTr from product). The residue was partitioned

between 5 % Na_2CO_3 (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (15 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. Flash column chromatography (SiO_2, 9.5:0.5:0.5 EtOAc/MeOH/Et_3N) gave **4.12** as a white foam (523 mg, 60%): R_f = 0.5 (9.5:0.5:0.5 EtOAc/MeOH/Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57 (s, 6H), 1.68-1.79 (m, 1H), 1.99-2.03 (m, 1H), 2.14-2.21 (m, 1H), 2.67-2.73 (m, 1H), 2.80-2.83 (m, 4H), 3.31-3.34 (m, 1H), 3.41-3.44 (m, 1H), 3.73 (s, 6H), 4.15 (d, *J* = 3.3 Hz,1H), 4.49 (d, *J* = 4.3 Hz, 1H), 4.93 (s, 1H), 6.2 (t, *J* = 5.2 Hz, 1H), 6.79 (d, *J* = 8.3 Hz, 4H), 6.90 (d, *J* = 7.3 Hz, 1H), 7.16 (t, *J* = 7.0 Hz, 1H), 7.25 (d, *J* = 8.6 Hz, 4H), 7.36 (d, *J* = 7.6 Hz, 2H), 8.03 (bs, 1H), 8.23 (d, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8, 26.0, 31.1, 42.3, 55.4, 56.9, 62.9, 70.6, 85.1, 86.7, 86.9, 87.4, 95.2, 113.5, 127.2, 128.2, 128.4, 130.1, 130.2, 135.7, 135.9, 144.4, 150.9, 155.6, 158.7, 162.6; HRMS (ESI): *m/z* calcd for $C_{38}H_{44}N_3O_8S_2$ $[M + H]^+$ 734.2569, found 734.2565.

Compound 4.3a:

To a solution of **4.12** (500 mg, 0.682 mmol) and diisopropylammonium tetrazolide (**4.14**, 175 mg, 1.02 mmol) in DCM (10 mL) at rt was added 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**13**, 325 µL, 1.02 mmol). After stirring at rt for 2 h, the reaction mixture was concentrated and loaded directly on a column. Flash column chromatography (SiO_2, 4:1:0.25 EtOAc/hexanes/Et_3N) gave **4.3a** as a white foam (580 mg, 91%): Mixture of two diastereoisomers; R_f = 0.3 and 0.4 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (d, *J* = 6.7 Hz, 2H), 1.11-1.20 (m, 12H), 1.23-1.30 (m, 3H), 1.61 (s, 6H), 2.02-2.08 (m, 1H), 1.73-1.83 (m, 1H), 2.02-2.08 (m, 1H), 2.18-2.29 (m, 2H), 2.40 (t, *J* =

6.4 Hz, 1H), 2.57 (t, *J* = 6.4 Hz, 1H), 2.65-2.75 (m, 2H), 2.81-2.89 (m, 4H), 3.29-3.36 (m, 1H), 3.45-3.60 (m, 5H), 3.78 (d, *J* = 3.5 Hz, 6H), 4.14-4.18 (m, 1H), 4.53-4.62 (m, 1H), 4.92 (s, 1H), 6.20-6.26 (m 1H), 6.82 (t, *J* = 7.9 Hz, 5H), 7.26 (t, *J* = 7.7 Hz, 6H), 7.37 (t, *J* = 7.2 Hz, 2H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.3, 24.7, 26.0, 31.0, 41.0, 41.4, 43.4, 55.4, 56.8, 58.5, 62.1, 62.5, 85.2, 85.7, 87.0, 94.8, 113.4, 117.7, 128.1, 128.4, 128.5, 130.21, 130.26, 130.3, 135.6, 144.2, 150.8, 155.0, 158.8, 162.4; ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.7, 150.4; HRMS (ESI): *m/z* calcd for $C_{47}H_{61}N_5O_9PS_2$ $[M + H]^+$ 934.3648, found 934.3652.

Compound 4.16:

The procedure for synthesizing **4.10** was used. After flash column chromatography (SiO_2, 1:2 EtOAc/hexanes) **4.16** was afforded as a white foam in 53% yield: R_f = 0.5 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97-1.05 (m, 28H), 1.61 (d, *J* = 7.5 Hz, 6H), 1.72-1.78 (m, 1H), 2.01-2.07 (m, 1H), 2.60-2.68 (m, 2H), 2.79-2.90 (m, 4H), 3.85-3.88 (m, 1H), 4.01 (t, *J* = 4.0 Hz, 2H), 4.91 (q, *J* = 7.6 Hz, 1H), 5.14 (s, 1H), 6.28-6.30 (m, 1H), 8.19 (s, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 13.0, 13.2, 13.5, 17.0, 17.1, 17.2, 17.3, 17.5, 17.5, 17.6, 17.6, 40.2, 56.9, 61.8, 69.8, 83.6, 84.7, 85.4,122.5, 141.3, 149.4, 149.9, 150.2, 152.9; HRMS (ESI): *m/z* calcd for $C_{30}H_{52}N_5O_6S_2Si_2$ $[M + H]^+$ 698.2897, found 698.2896.

Compound 4.17:

The procedure for synthesizing **4.11** was used. After flash column chromatography (SiO_2, 9:1 EtOAc/MeOH) **4.17** was afforded as a white foam in 71% yield: R_f = 0.3 (9:1

EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (s, 6H), 1.69-1.83 (m, 1H), 2.03-2.09 (m, 1H), 2.34-2.37 (m, 2H), 2.84-2.90 (m, 4H), 2.93-3.08 (m, 1H), 3.81-3.97 (m, 2H), 4.24 (s, 1H), 4.82 (s, 1H), 5.17 (s, 1H), 5.84-5.87 (m, 1H), 6.36-6.40 (m, 1H), 8.02 (s, 1H), 8.42 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 26.1, 31.2, 41.1, 57.1, 63.5, 73.3, 85.0, 87.8, 89.7, 123.4, 142.4, 149.2, 150.1, 150.6, 152.5; HRMS (ESI): *m/z* calcd for $C_{18}H_{26}N_5O_5S_2$ $[M + H]^+$ 456.1375, found 456.1381.

Compound 4.18:

The procedure for synthesizing **4.12** was used. After flash column chromatography (SiO_2, 9:0.5:0.5 EtOAc/MeOH/Et_3N) **4.18** was afforded as a white foam in 63% yield: R_f = 0.4 (9.5:0.5 EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60 (s, 6H), 1.67-1.75 (m, 1H), 1.95-2.04 (m, 1H), 2.49-2.57 (m, 1H), 2.73-2.86 (m, 5H), 3.34 (d, J = 3.8 Hz, 2H), 3.67 (s, 6H), 4.16-4.21 (m, 1H), 4.66-4.70 (m, 1H), 5.10 (s, 1H), 5.21 (s, 1H), 6.44 (t, J = 6.3 Hz, 1H), 6.69 (d, J = 8.7 Hz, 4H), 7.07-7.21 (m, 7H), 7.30 (d, J = 8.0 Hz, 2H), 8.08 (s, 1H), 8.63 (s, 1H), 8.99 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.9, 26.0, 31.1, 40.6, 55.4, 57.0, 63.8, 72.3, 84.6, 84.9, 86.7, 113.3, 122.1, 127.1, 128.0, 128.2, 130.1, 141.3, 144.6, 149.5, 149.9, 150.7, 152.9, 158.6; HRMS (ESI): *m/z* calcd for $C_{39}H_{44}N_5O_7S_2$ $[M + H]^+$ 758.2682, found 758.2685.

Compound 4.3b:

The procedure for synthesizing **4.3a** was used. After flash column chromatography (SiO_2, 2:1:0.15 EtOAc/hexanes/Et_3N) **4.3b** was afforded as a white foam in 77% yield: Mixture of two diastereoisomers; R_f = 0.3 and 0.4 (2:1 EtOAc/hexanes); ^{1}H NMR (400 MHz,

$CDCl_3$): δ 1.15-1.23 (m, 12H), 1.63 (s, 6H), 1.74-1.80 (m, 1H), 2.02-2.07 (m, 1H), 2.42 (t, J = 6.4 Hz, 1H), 2.57 (t, J = 6.4 Hz, 1H), 2.79-2.91 (m, 4H), 3.28-3.33 (m, 1H), 3.38-3.44 (m, 2H), 3.54-3.60 (m, 2H), 3.75 (s, 6H), 3.79-3.87 (m, 1H), 4.08-4.14 (m, 1H), 4.24-4.29 (m, 1H), 4.71-4.77 (m, 1H), 5.14 (s, 1H), 6.40-6.45 (m, 1H), 6.72-6.75 (m, 4H), 7.14-7.25 (m, 7H), 7.33-7.35 (m, 2H), 8.11 (d, J = 9.5 Hz, 1H), 8.38 (bs, 1H), 8.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.1, 20.7, 22.5, 23.4, 24.8, 25.0, 26.2, 31.3, 39.7, 45.3, 45.5, 45.8, 55.4, 57.0, 58.5, 58.7, 63.6, 73.6, 74.4, 84.7, 84.7, 84.9, 86.0, 86.2, 86.7, 113.2, 117.6, 122.4, 127.0, 127.9, 128.2, 130.1, 135.7, 141.3, 144.5, 149.2, 149.7, 150.8, 152.8, 158.6; ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.7, 149.9; HRMS (ESI)**:** *m/z* calcd for $C_{48}H_{60}N_7O_8PS_2H$ $[M + H]^+$ 958.3760, found 958.3769.

Compound 4.21:

The amide functionality in **4.19** was protected with a TBS group by reacting with TBSCl (3 equiv.) in the presence of imidazole (6 equiv.) in DCM at rt for 8 h. [2] The crude intermediate **4.20** was partitioned between DCM and NaH_2PO_4/Na_2HPO_4 buffer (pH 7) and further washed with the buffer two times. The organic phase was dried over Na_2SO_4, filtered and concentrated to dryness. After the crude intermediate was dried under high vacuum over Drierite, **4.20** was converted to **4.21** following the procedure for synthesizing **4.10**. The TBS group probably fell off during partition between EtOAc and saturated NH_4Cl. After flash column chromatography (SiO_2, 1:1 EtOAc/hexanes) **4.21** was afforded as a brown foam in 55% yield: R_f = 0.3 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96-1.08 (m, 28H), 1.63 (s, 6H), 1.77-1.85 (m, 1H), 2.08-2.15 (m, 1H), 2.52-2.55 (m, 2H), 2.85-2.92 (m, 4H), 3.81-3.85 (m, 1H), 3.94-4.04 (m, 2H), 4.71 (q, J = 7.4 Hz, 1H),

4.96 (s, 1H), 6.08 (t, J = 5.2 Hz, 1H), 7.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.8, 13.3, 13.4, 13.7, 17.20, 17.28, 17.3, 17.45, 17.47, 17.5, 17.6, 17.7, 17.8, 24.9, 26.0, 31.4, 40.3, 57.0, 62.0, 70.2, 82.8, 85.4, 86.9, 121.5, 136.7, 146.7, 147.3, 151.8, 155.6; HRMS (ESI)**:** *m/z* calcd for $C_{30}H_{52}N_5O_7S_2Si_2$ $[M + H]^+$ 714.2847, found 714.2842.

Compound 4.22:

The pro-cedure for synthesizing **4.11** was used. After flash column chromatography (SiO_2, 4:1 EtOAc/MeOH) **4.22** was afforded as a brown foam in 80% yield: R_f = 0.2 (9:1 EtOAc/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.79 (s, 6H), 1.70-1.79 (m, 1H), 2.00-2.10 (m, 1H), 2.39-2.44 (m, 1H), 2.60-2.70 (m, 1H), 2.85-2.92 (m, 4H), 3.22 (s, 1H), 3.70-3.76 (m, 2H), 3.92-4.05 (m, 2H), 4.50-4.54 (m, 1H), 5.16 (s, 1H), 8.20 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 23.6, 30.6, 40.4, 56.7, 61.8, 71.1, 84.3, 85.8, 88.0, 119.6, 138.2, 148.0, 153.7, 156.3; HRMS (ESI)**:** *m/z* calcd for $C_{18}H_{26}N_5O_6S_2$ $[M + H]^+$ 472.1324, found 472.1326.

Compound 4.23:

The procedure for synthesizing **4.12** was used. After flash column chromatography (SiO_2, 9:0.5:0.5 EtOAc/MeOH/Et_3N) **4.23** was afforded as a brown foam in 46% yield: R_f = 0.4 (9.5:0.5 EtOAc/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58 (s, 6H), 1.68-1.77 (m, 1H), 1.97-2.03 (m, 1H), 2.50-2.60 (m, 2H), 2.77-2.85 (m, 4H), 3.25-3.29 (m, 2H), 3.67 (s, 6H), 4.14-4.22 (m, 1H), 4.69-4.76 (m, 1H), 4.93 (s, 1H), 6.23 (t, *J* = 6.2 Hz, 1H), 6.69 (d, *J* = 8.7 Hz, 4H), 7.07-7.13 (m, 2H), 7.21 (d, *J* = 8.6 Hz, 4H), 7.31 (d, *J* = 7.3 Hz, 2H), 7.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.7, 25.9, 31.1, 40.6, 55.3, 60.6, 64.4, 72.0, 84.6,

86.5, 86.7, 113.2, 120.8, 127.9, 128.3, 130.1, 135.9, 137.7, 144.7, 147.1, 148.6, 152.7, 155.9, 158.6; HRMS (ESI)**:** *m/z* calcd for $C_{39}H_{44}N_5O_8S_2$ $[M + H]^+$ 774.2631, found 774.2629.

Compound 4.3c:

The procedure for synthesizing **4.3a** was used. After flash column chromatography (SiO_2, 9.5:0.5 EtOAc/Et_3N) **4.3c** was afforded as a brown foam in 76% yield: Mixture of two diastereoisomers; R_f = 0.4 and 0.5 (EtOAc); ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.08-1.25 (m, 12H), 1.59 (d, J = 7.9 Hz, 6H), 1.74-1.85 (m, 1H), 2.06-2.14 (m, 1H), 2.37-2.47 (m, 2H), 2.68-2.74 (m, 1H), 2.83-2.91 (m, 4H), 3.26-3.32 (m, 2H), 3.49-3.60 (m, 2H), 3.74 (s, 6H), 4.08-4.16 (m, 1H), 4.20-4.27 (s, 1H), 4.62-4.71 (m, 1H), 4.97 (s, 1H), 6.11-6.19 (m, 1H), 6.75 (d, J = 8.4 Hz, 4H), 7.15-7.28 (m, 7H), 7.36-7.38 (m, 2H), 7.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.2, 20.6, 23.10, 23.18, 24.7, 24.8, 26.0, 31.2, 39.7, 43.3, 43.5, 45.5, 55.4, 56.8, 58.3, 63.9, 74.7, 84.9, 86.5, 86.6, 113.3, 117.6, 121.6, 127.1, 128.0, 130.1, 135.8, 137.2, 137.5, 144.6, 144.7, 148.3, 152.1, 155.7, 158.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.5, 149.7; HRMS (ESI)**:** *m/z* calcd for $C_{48}H_{61}N_7O_9PS_2$ $[M + H]^+$ 974.3709, found 774.3715.

Compound 4.25:

The procedure for the synthesis of **4.3a** was used. After flash column chromatography (SiO_2, 4:1:0.25 hexanes/EtOAc/Et_3N) **4.25** was afforded as a colorless oil in 88% yield: R_f = 0.3 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14-1.18 (m, 12H), 1.27-1.33 (m, 6H), 1.55-1.61 (m, 4H), 2.56-2.63 (m, 4H), 3.53-3.63 (m, 4H), 3.74-3.86 (m, 4H), 7.14-

7.16 (m, 3H), 7.23-7.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6 (d, J_{C-P} = 6.74 Hz), 24.9, 26.2, 29.6 (d, J_{C-P} = 18.72 Hz), 29.5, 29.7, 31.4, 31.5, 31.7, 36.2, 43.2 (d, J_{C-P} = 12.35 Hz), 58.5 (d, J_{C-P} = 18.95 Hz), 63.9 (d, J_{C-P} = 16.97 Hz), 117.8, 125.7, 128.3, 128.5, 143.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.5; HRMS (ESI): *m/z* calcd for $C_{23}H_{40}N_2O_2P$ $[M+H]^+$ 407.2827, found 407.2812.

Compound 4.28:

A solution of **4.27** (2.0 g, 9.17 mmol) and conc. H_2SO_4 (1 mL) in ethanol (100 mL) was stirred at reflux for 2 h. After cooling to rt, the reaction was quenched with 5% Na_2CO_3 (20 mL) and ethanol was evaporated. The remaining material was partitioned between EtOAc (100 mL) and 5% Na_2CO_3 (50 mL). The organic phase was washed with 5% Na_2CO_3 (50 mL × 2), dried over anhydrous Na_2SO_4, filtered and concentrated. Flash column chromatography (SiO_2, 1:1 hexanes/EtOAc) gave **4.28** as a colorless oil (1.72 g, 76%): R_f = 0.2 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 1.22 (t, J = 7.1 Hz, 3H), 1.30 (s, 10H), 1.42-1.50 (m, 2H), 1.54-1.60 (m, 2H), 2.27 (t, J = 7.4 Hz, 2H), 3.28 (bs, 1H), 3.36-3.46 (m, 2H), 3.53 (bs, 1H), 4.08 (q, J = 7.1 Hz, 2H), 4.80 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 13.3, 24.8, 25.4, 28.9, 29.1, 29.3, 29.5, 33.2, 33.9, 60.1, 66.2, 72.0, 174.3; HRMS (ESI): *m/z* calcd for $C_{13}H_{27}O_4$ $[M + H]^+$ 247.1909, found 247.1907.

Compound 4.29:

The procedure for synthesizing **4.12** was used. After flash column chromatography (SiO_2, 3:2:0.25 hexanes/EtOAc/Et_3N) **4.29** was afforded as a yellow oil in 99% yield: R_f = 0.8 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.28 (m, 10H), 1.35-1.45 (m,

2H), 1.53-1.63 (m, 2H), 2.26 (t, *J* = 7.3 Hz, 2H), 2.47 (bs, 1H), 2.98-3.04 (m, 1H), 3.13-3.17 (m, 1H), 3.74 (s, 6H), 4.10 (q, *J* = 7.1 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 4H), 7.14-7.19 (m, 2H), 7.26 (t, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 4H), 7.43 (d, *J* = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 25.1, 25.6, 29.31, 29.39, 29.5, 29.7, 33.6, 34.5, 55.3, 60.3, 67.8, 71.1, 86.2, 113.3, 126.9, 128.0, 128.3, 130.2, 136.3, 145.1, 158.6, 174.0; HRMS (ESI): *m/z* calcd for $C_{34}H_{44}O_6Na$ $[M + Na]^+$ 571.3035, found 571.3031.

Compound 4.26a:

The procedure for synthesizing **4.3a** was used. After flash column chromatography (SiO_2, 2:1:0.15 hexanes/EtOAc/Et_3N) **4.26a** was afforded as a colorless oil in 99% yield: Mixture of diastereoisomers; R_f = 0.6 and 0.7 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (d, *J* = 6.7 Hz, 4H), 1.14-1.26 (m, 22H), 1.54-1.63 (m, 2H), 2.24-2.29 (m, 2H), 2.35-2.39 (m, 1H), 2.59 (t, *J* = 6.5 Hz, 2H), 2.92-2.99 (m, 1H), 3.09-3.18 (m, 2H), 3.50-3.65 (m, 3H), 3.76 (s, 6H), 3.91-3.99 (m, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 6.80 (t, *J* = 8.7 Hz, 4H), 7.15-7.20 (m, 2H), 7.23-7.27 (m, 2H), 7.32 (d, *J* = 8.9 Hz, 4H), 7.45 (d, *J* = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 20.3, 20.6, 24.6, 24.7, 24.8, 24.9, 25.0, 25.2, 25.3, 29.3, 29.41, 29.44, 29.5, 29.7, 29.8, 33.6, 33.8, 34.5, 43.1, 43.3, 43.4, 55.3, 58.3, 58.6, 60.3, 66.2, 66.5, 73.6, 73.7, 74.4, 74.6, 86.0, 86.1, 117.9, 126.81, 126.87, 127.8, 128.4, 128.5, 130.2, 130.32, 130.36, 136.5, 145.3, 158.6, 174.0 ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.1, 149.7; HRMS (ESI): *m/z* calcd for $C_{43}H_{61}N_2O_7PNa$ $[M + Na]^+$ 771.4114, found 771.4108.

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Appendix A. Supporting Information for Chapter 2

Synthesis of Oligonucleotides Containing Electrophilic Groups

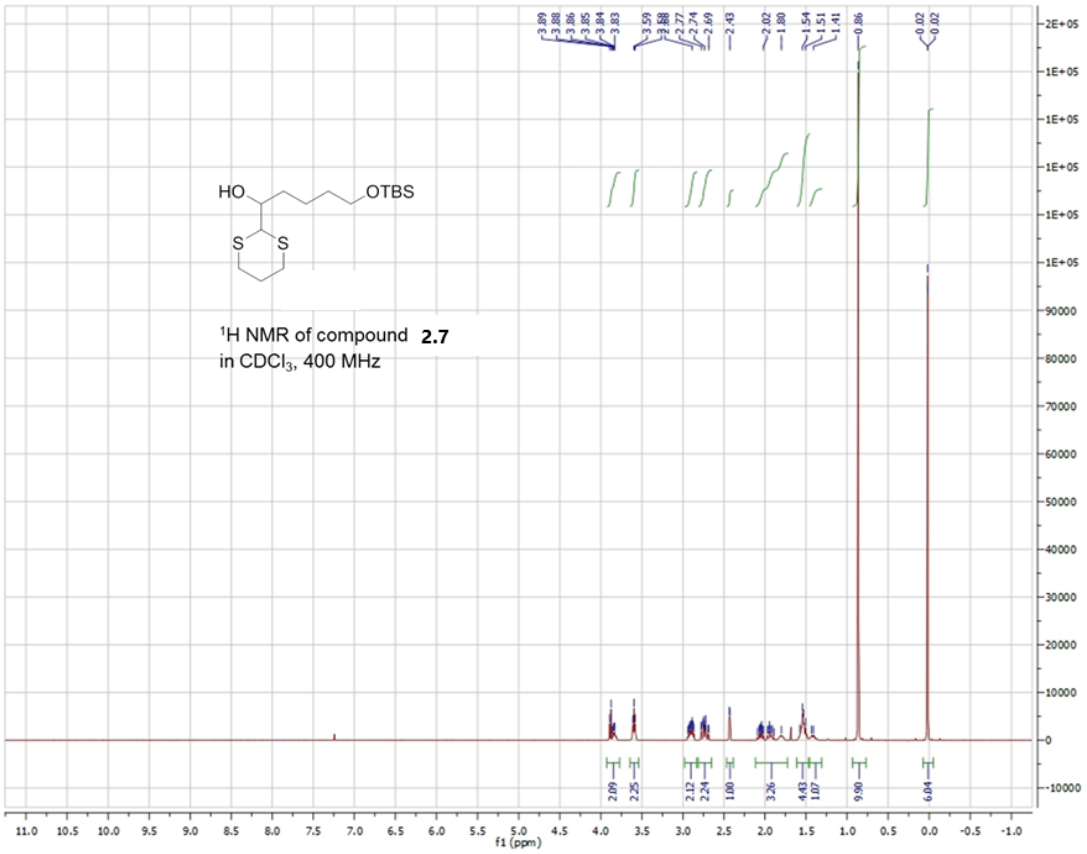


Figure A.1. ^{1}H NMR of Compound **2.7**

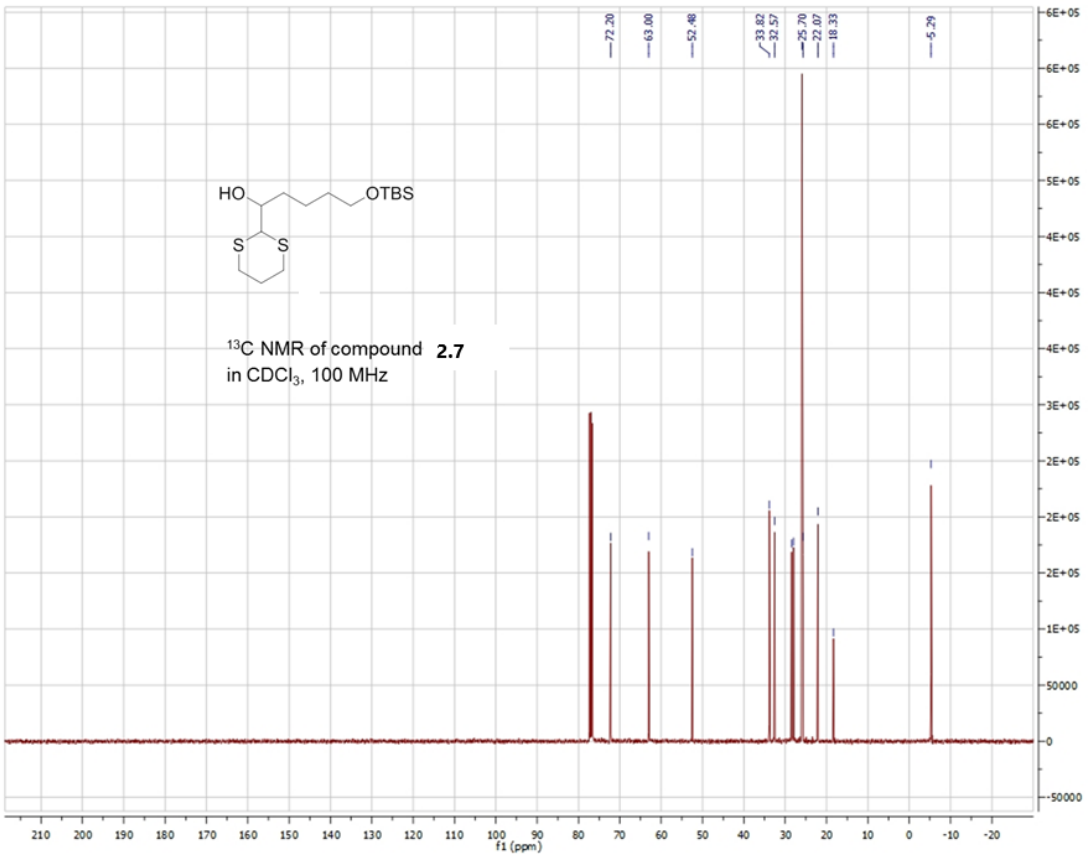


Figure A.2. ^{13}C NMR of Compound **2.7**

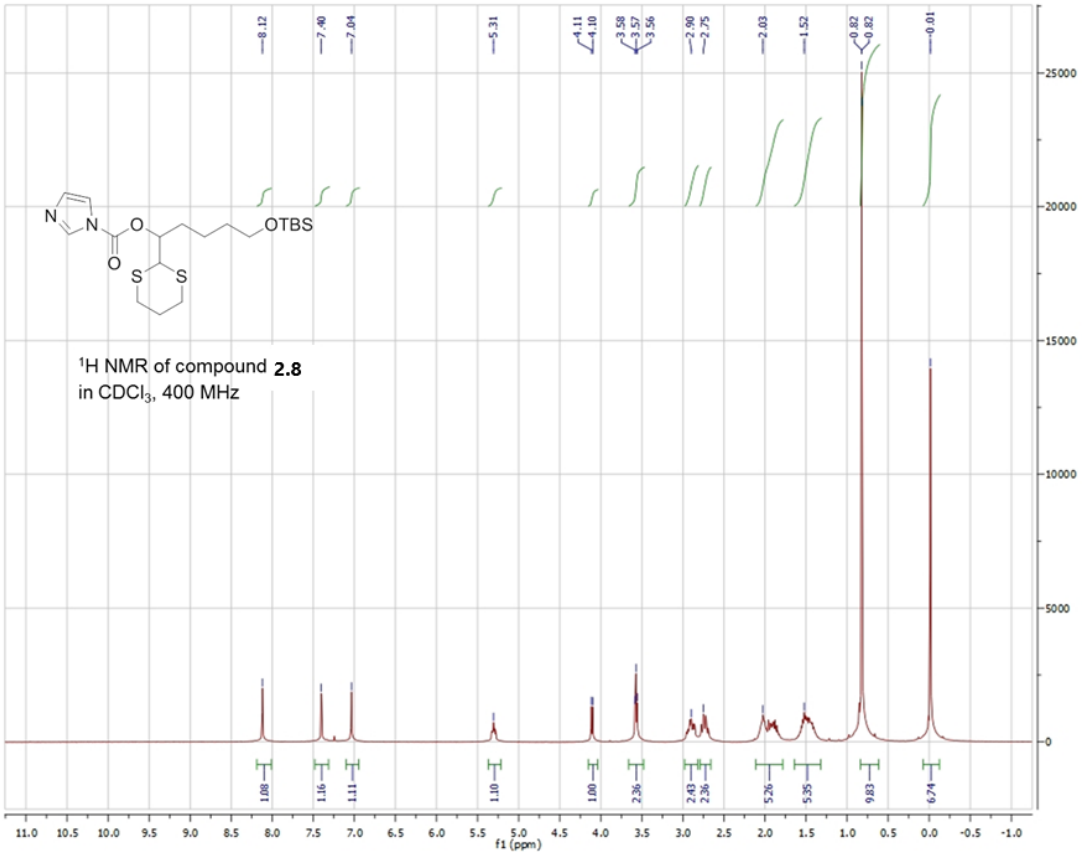


Figure A.3. ^{1}H NMR of Compound **2.8**

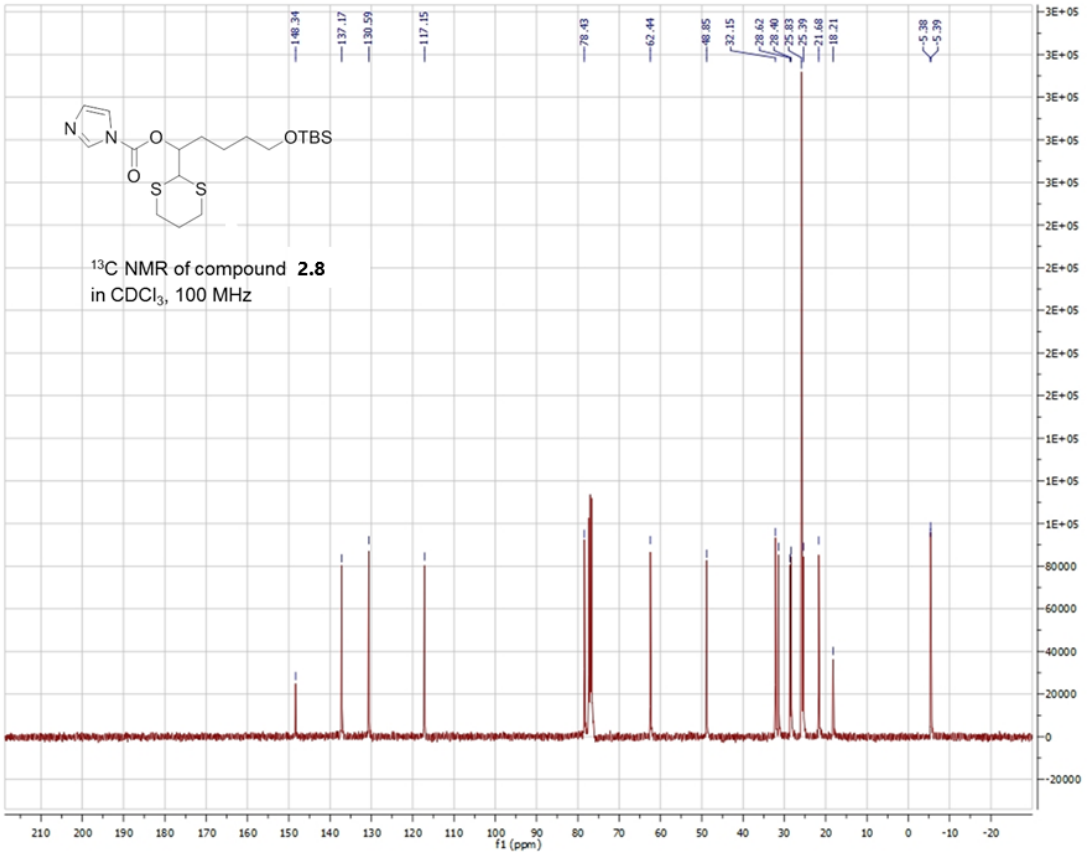


Figure A.4. ^{13}C NMR of Compound **2.8**

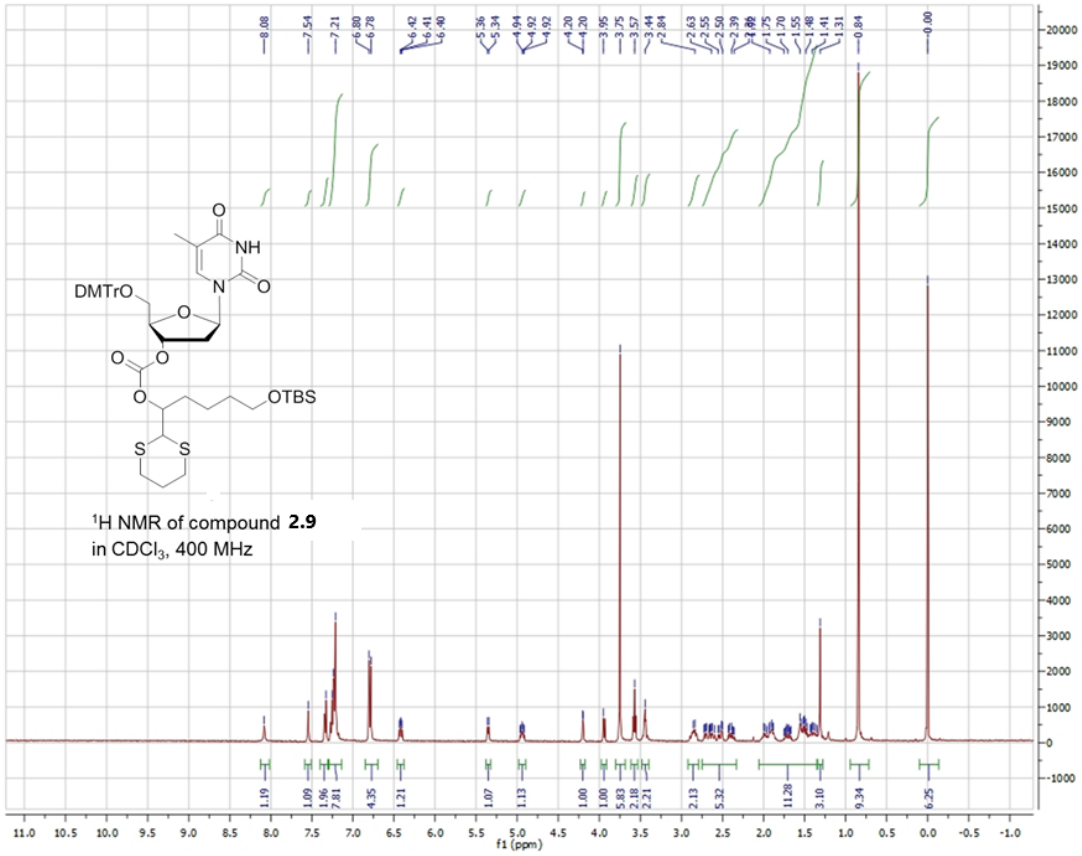


Figure A.5. ^{1}H NMR of Compound **2.9**

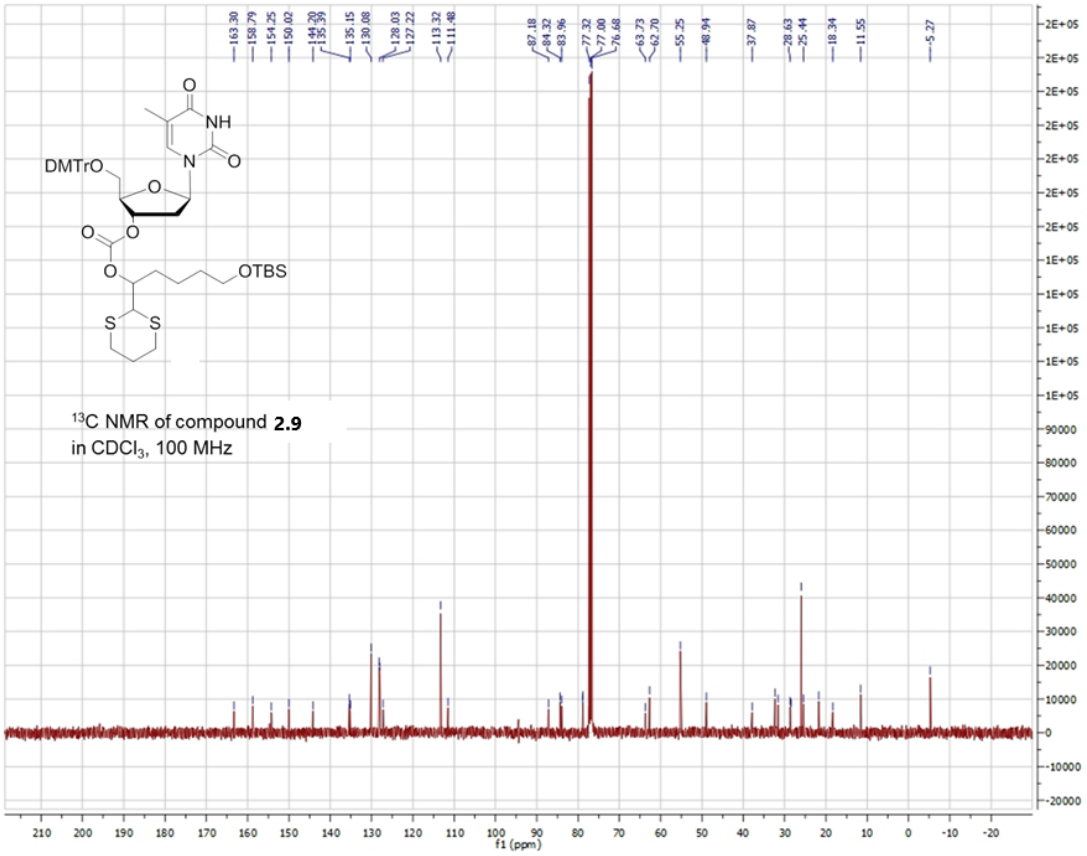


Figure A.6. ^{13}C NMR of Compound **2.9**

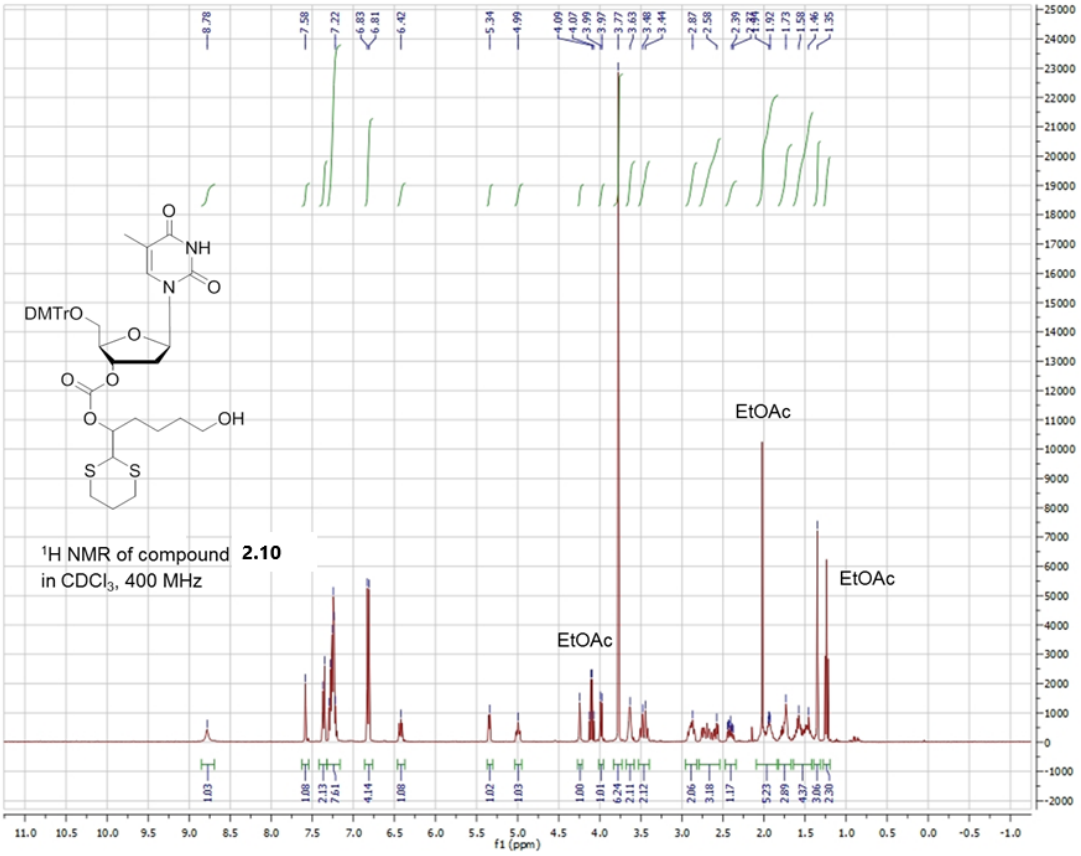


Figure A.7. [1]HNMR of Compound **2.10**

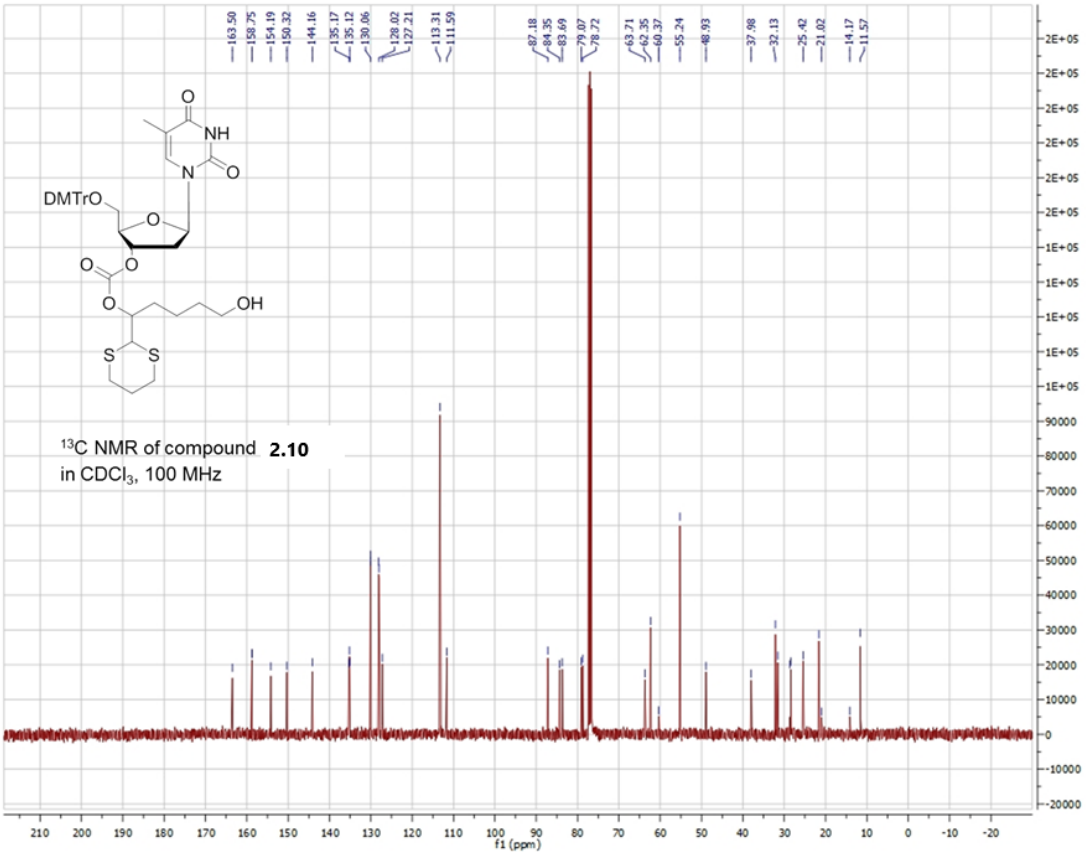


Figure A.8. ^{13}CNMR of Compound **2.10**

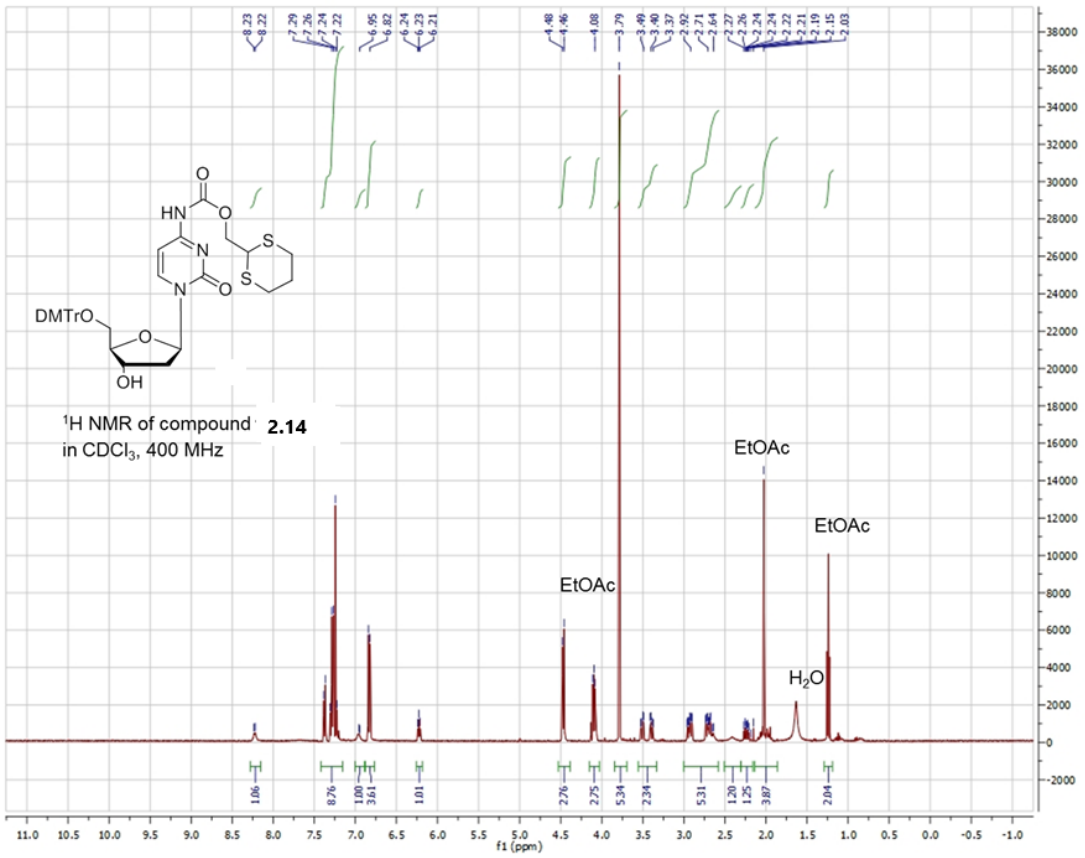


Figure A.9. ^{1}HNMR of Compound **2.14**

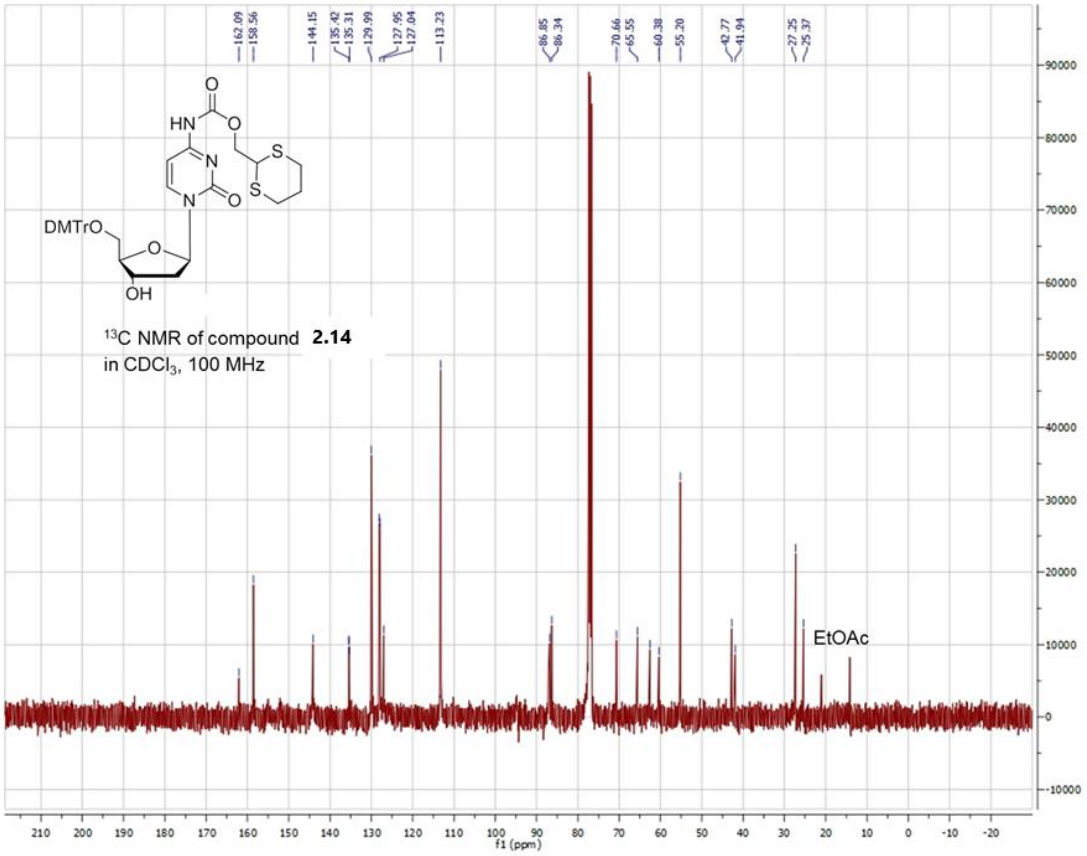


Figure A.10. ^{13}CNMR of Compound **2.14**

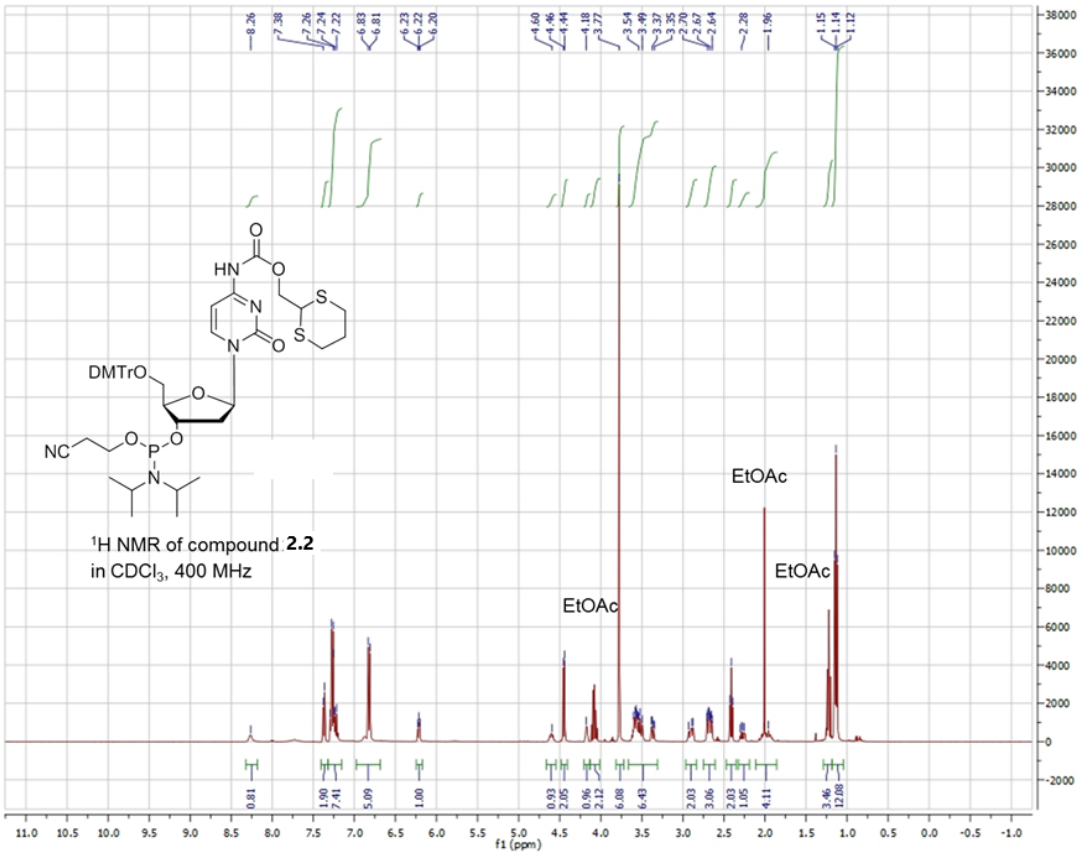


Figure A.11. ^{1}HNMR of Compound **2.2**

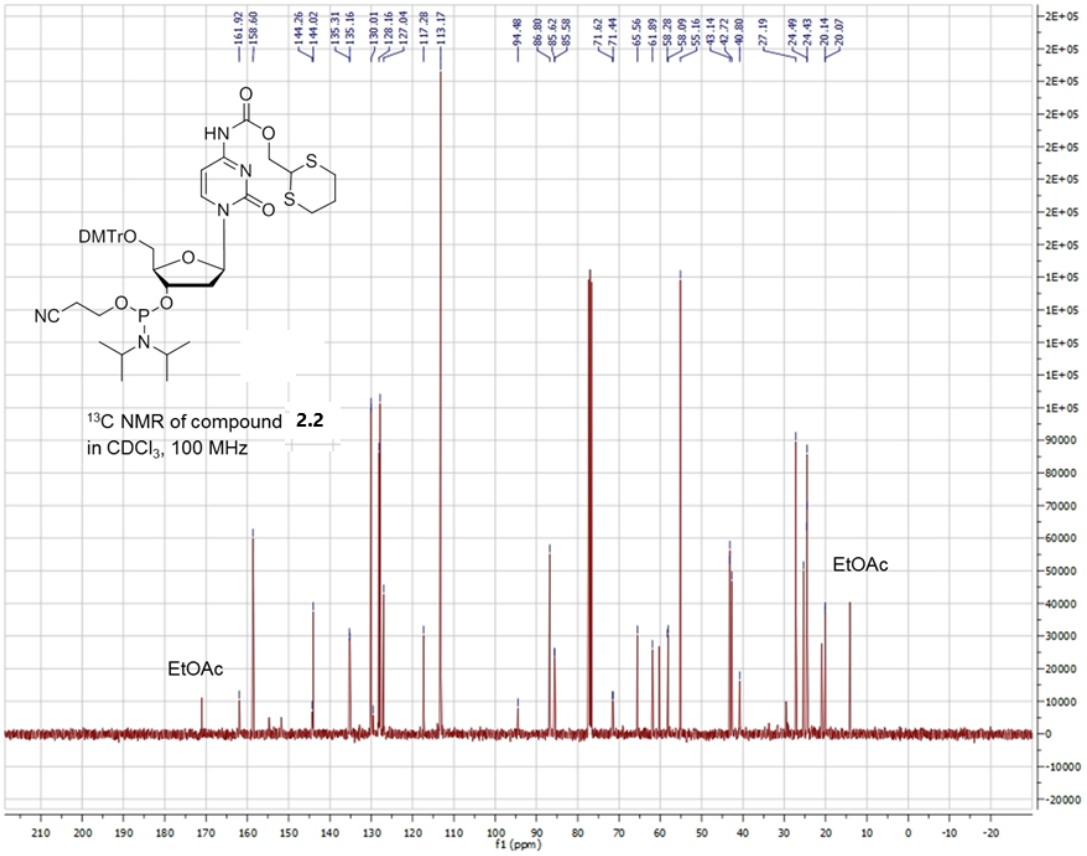


Figure A.12. ^{13}CNMR of Compound **2.2**

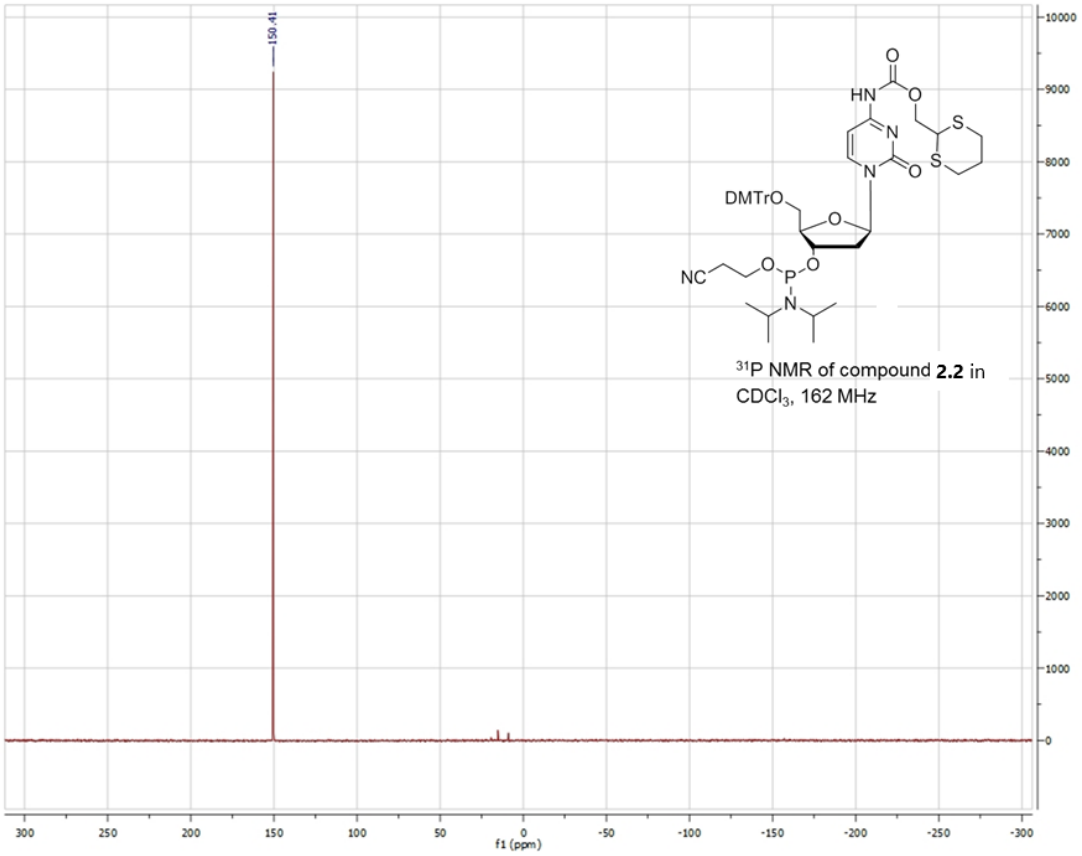


Figure A.13. ^{31}PNMR of Compound **2.2**

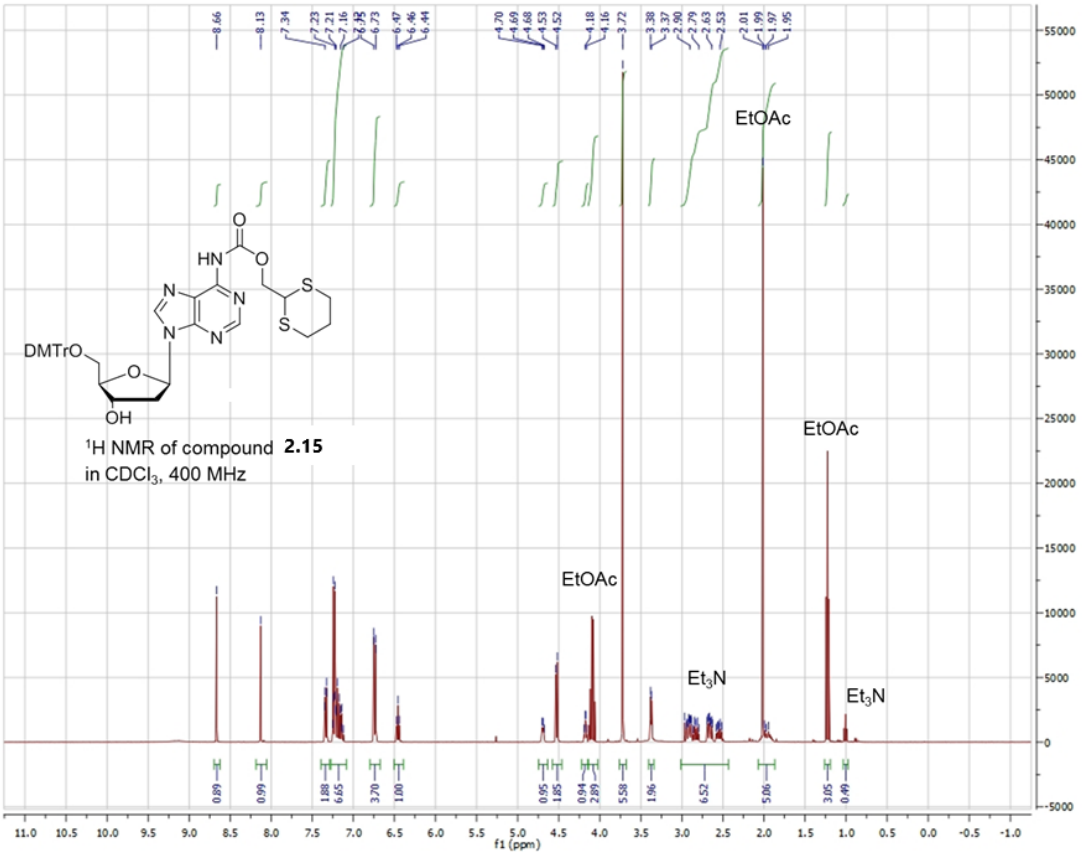


Figure A.14. [1]HNMR of Compound **2.15**

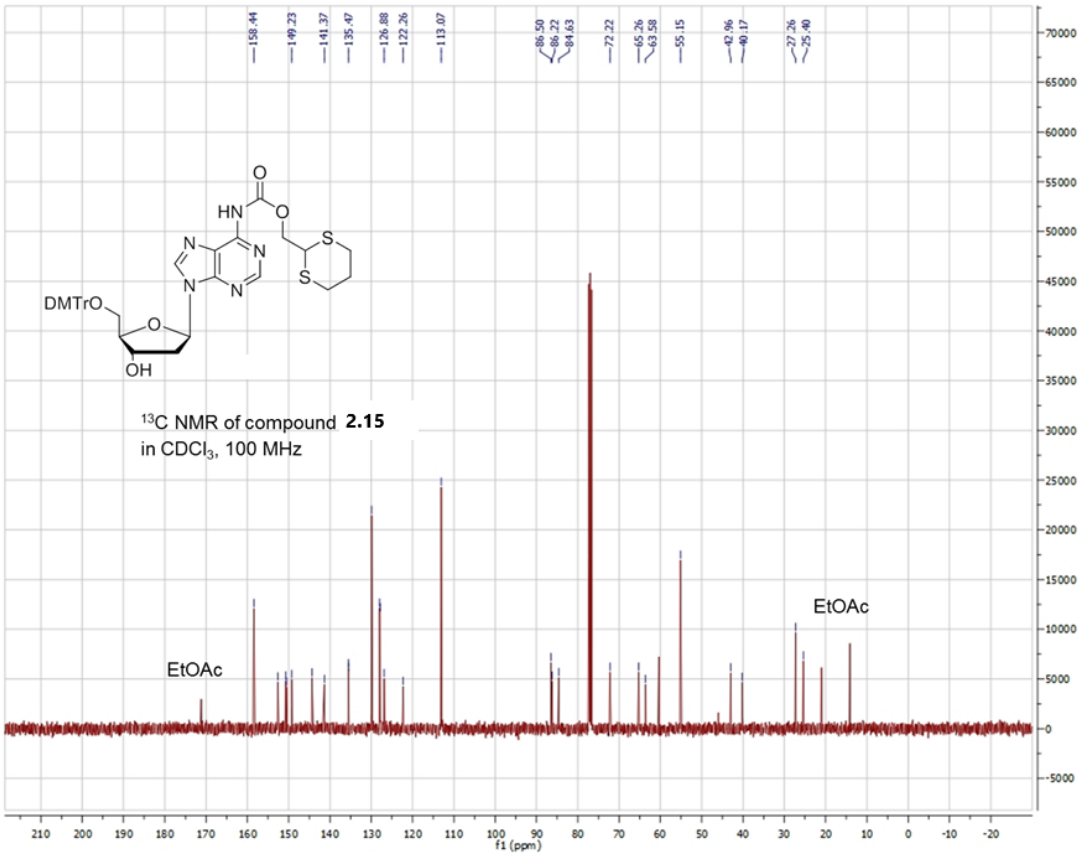


Figure A.15. ^{13}CNMR of Compound **2.15**

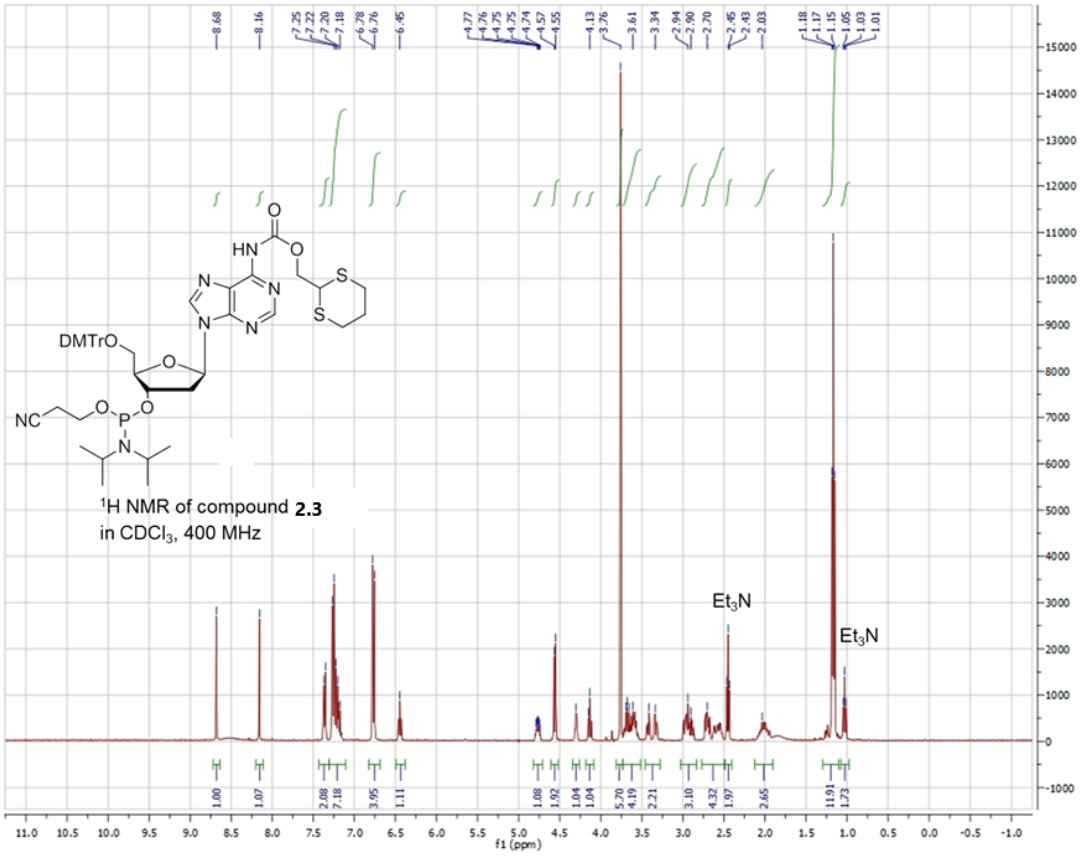


Figure A.16. ^{1}HNMR of Compound **2.3**

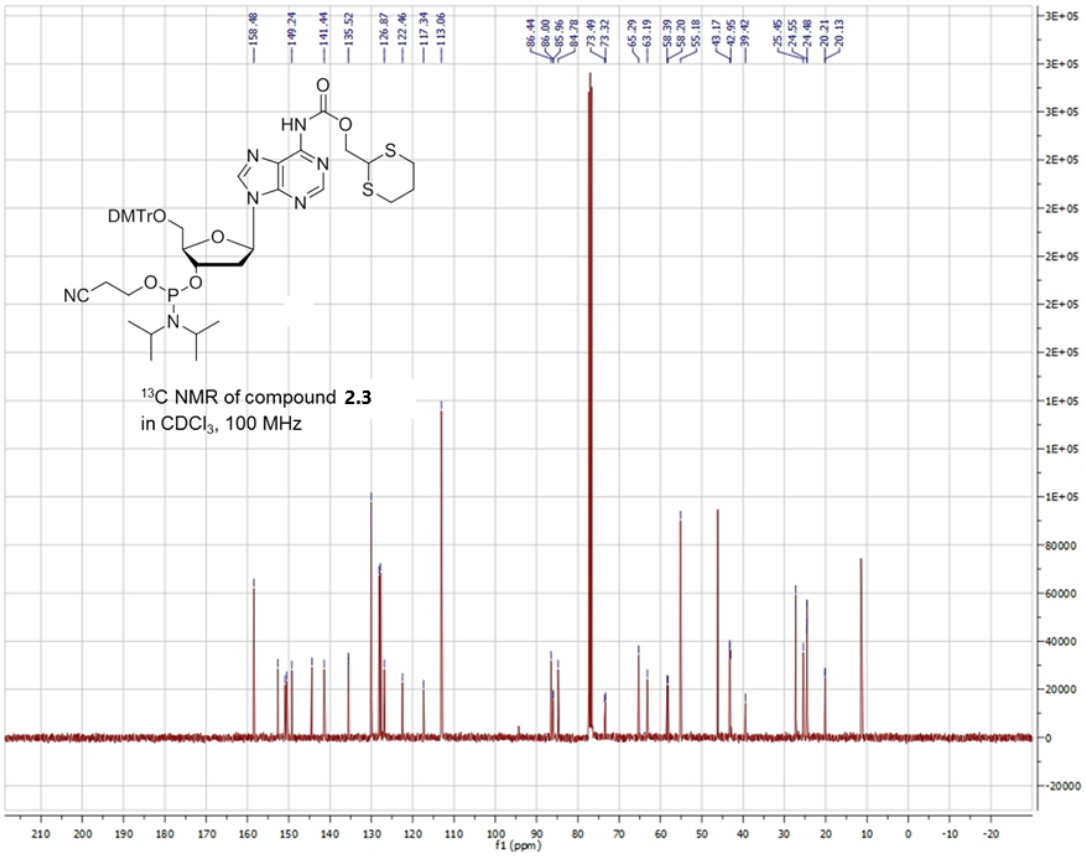


Figure A.17. ^{13}CNMR of Compound **2.3**

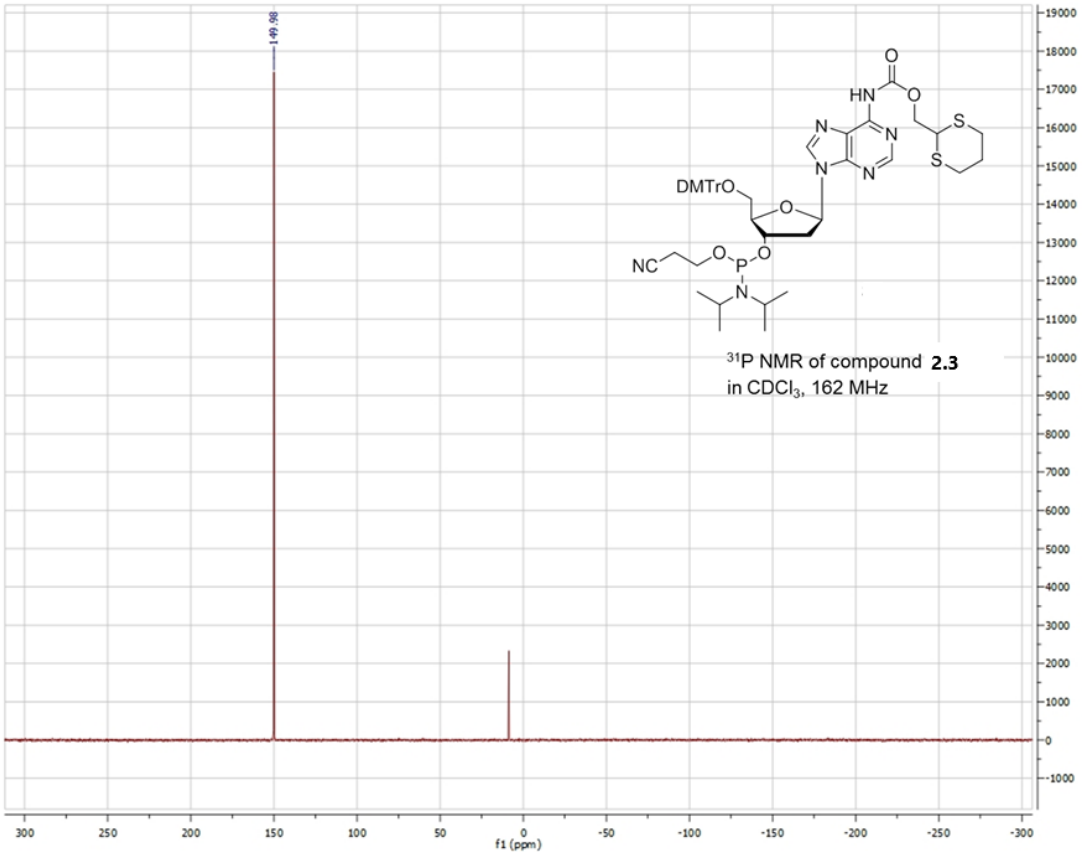


Figure A.18. ^{31}PNMR of Compound **2.3**

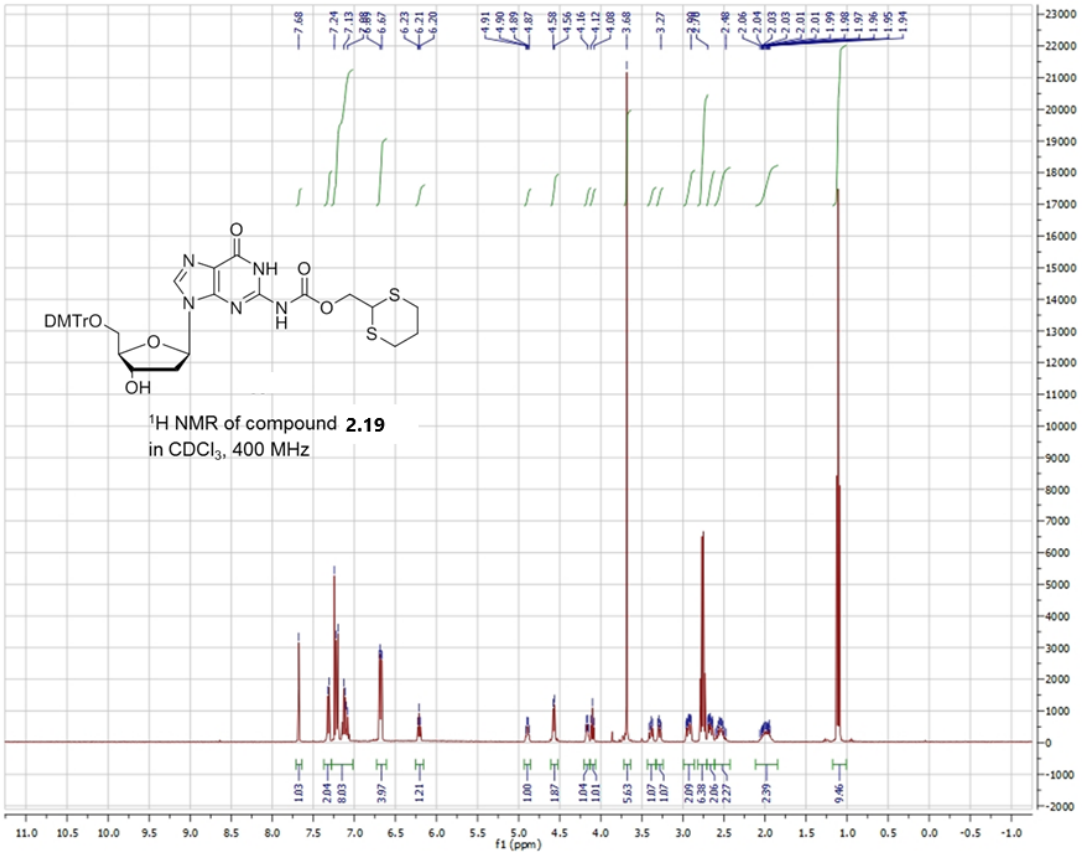


Figure A.19. ^{1}HNMR of Compound **2.19**

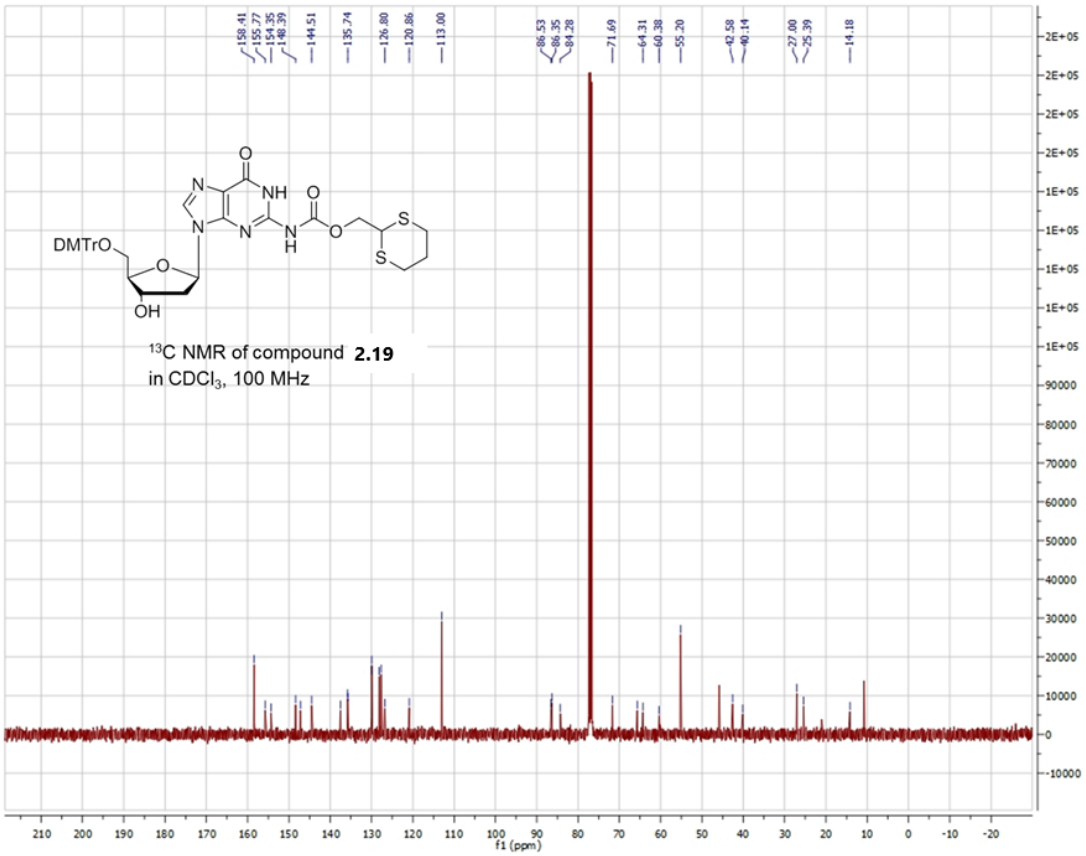


Figure A.20. ^{13}CNMR of Compound **2.19**

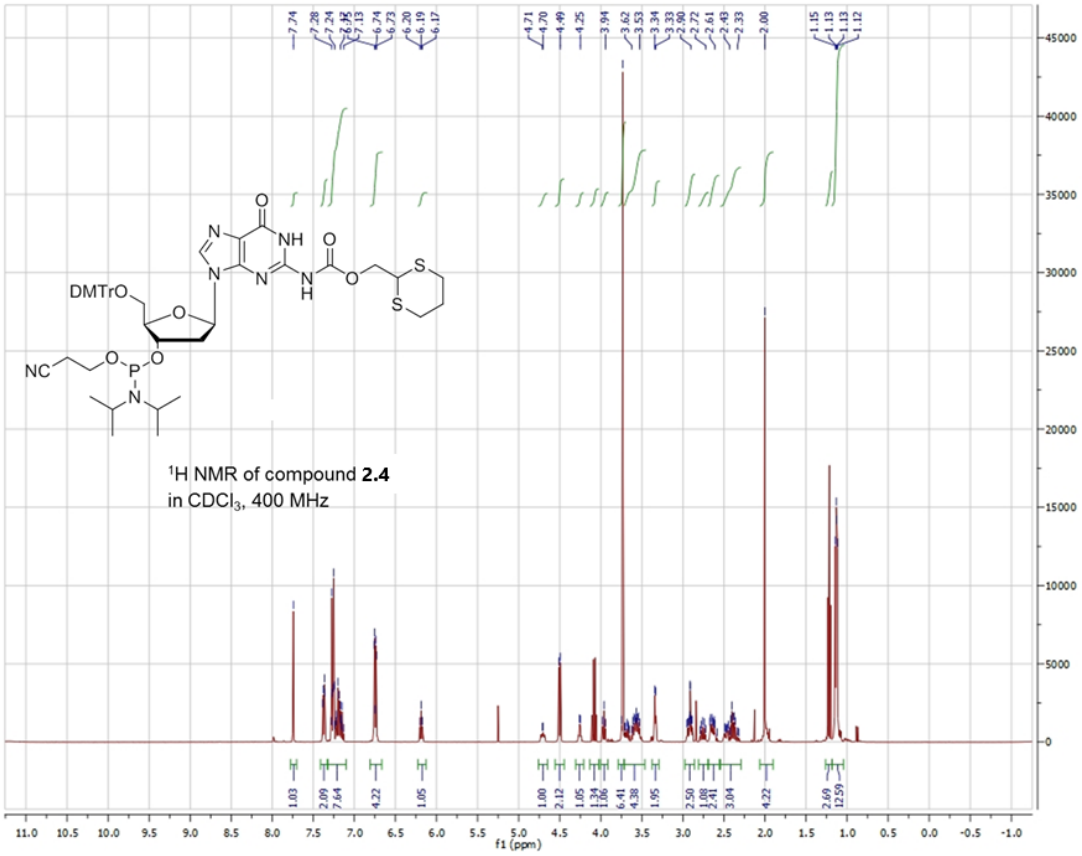


Figure A.21. ^{1}HNMR of Compound **2.4**

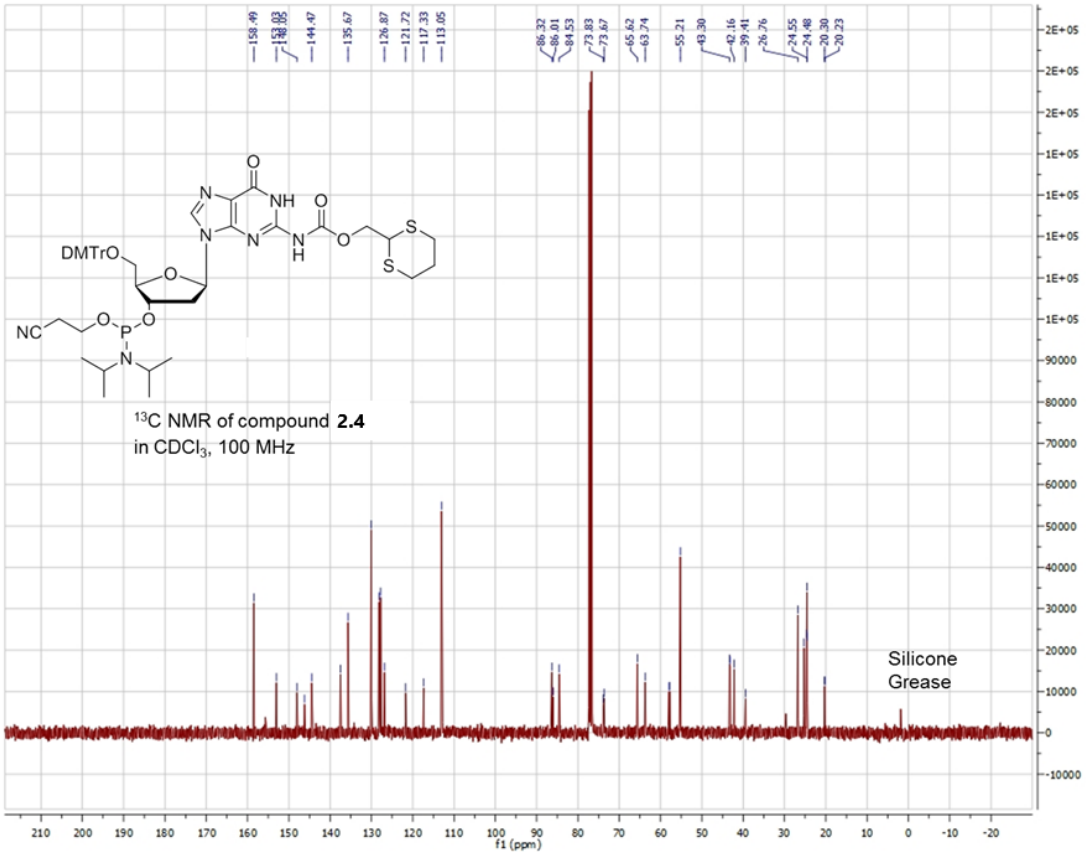


Figure A.22. ^{13}CNMR of Compound **2.4**

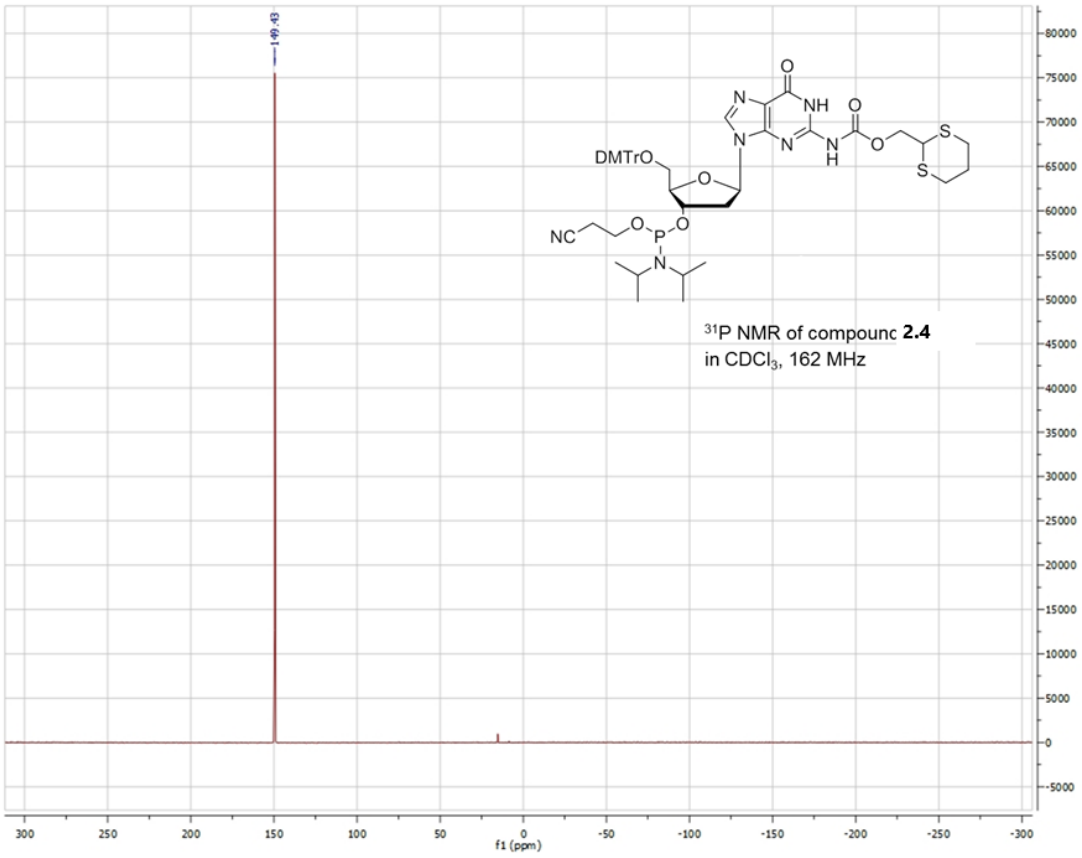


Figure A.23. ^{31}PNMR of Compound **2.4**

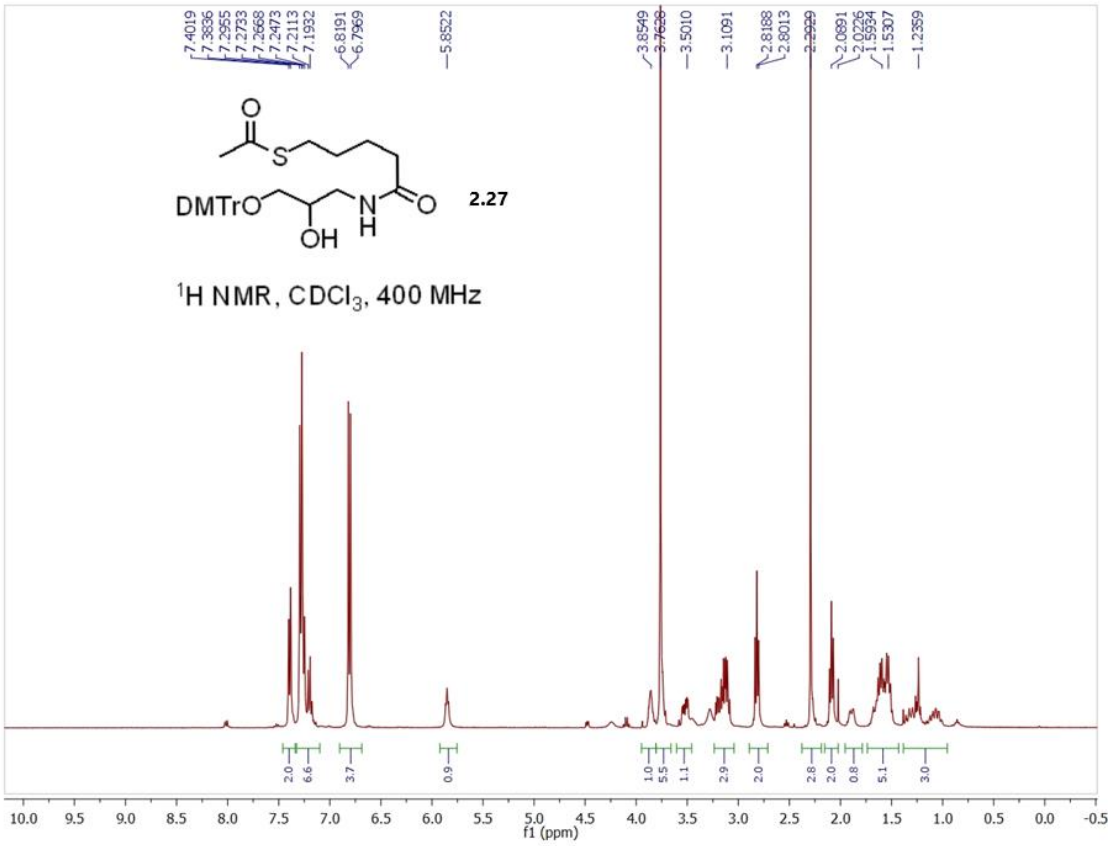


Figure A.24. ^{1}HNMR of Compound **2.27**

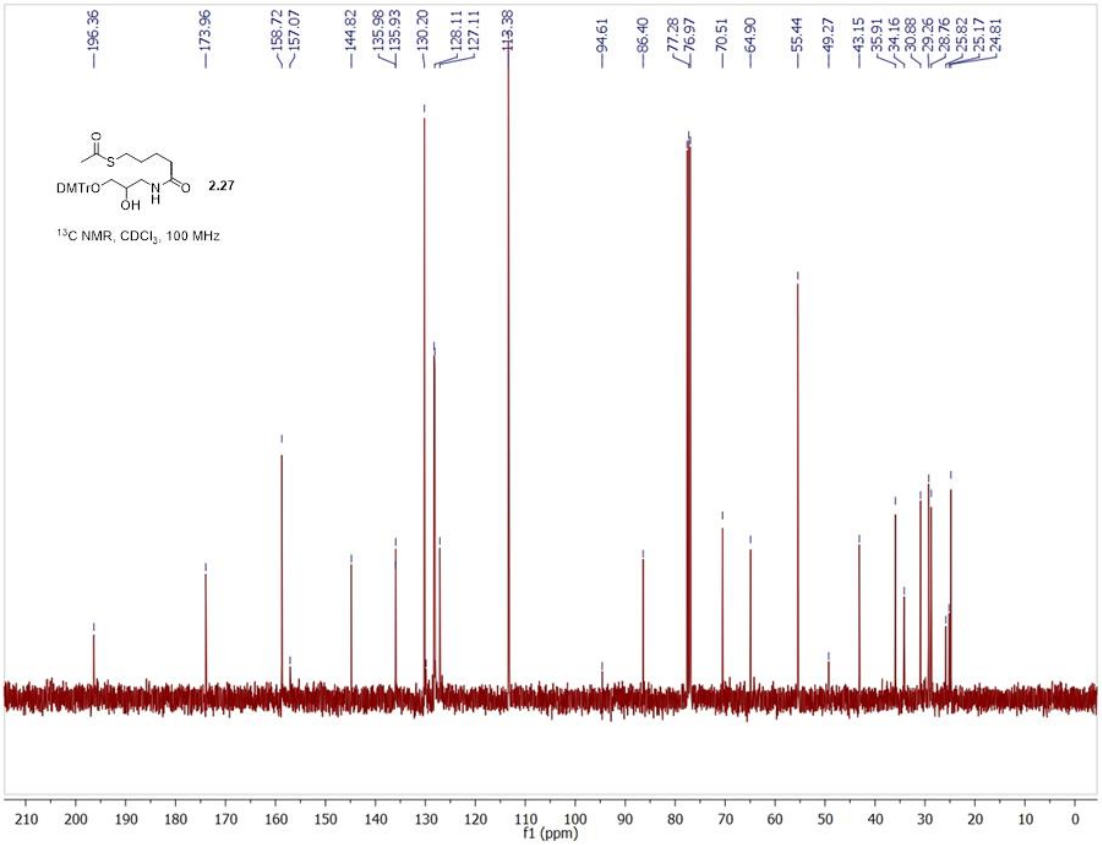


Figure A.25. ^{13}CNMR of Compound **2.27**

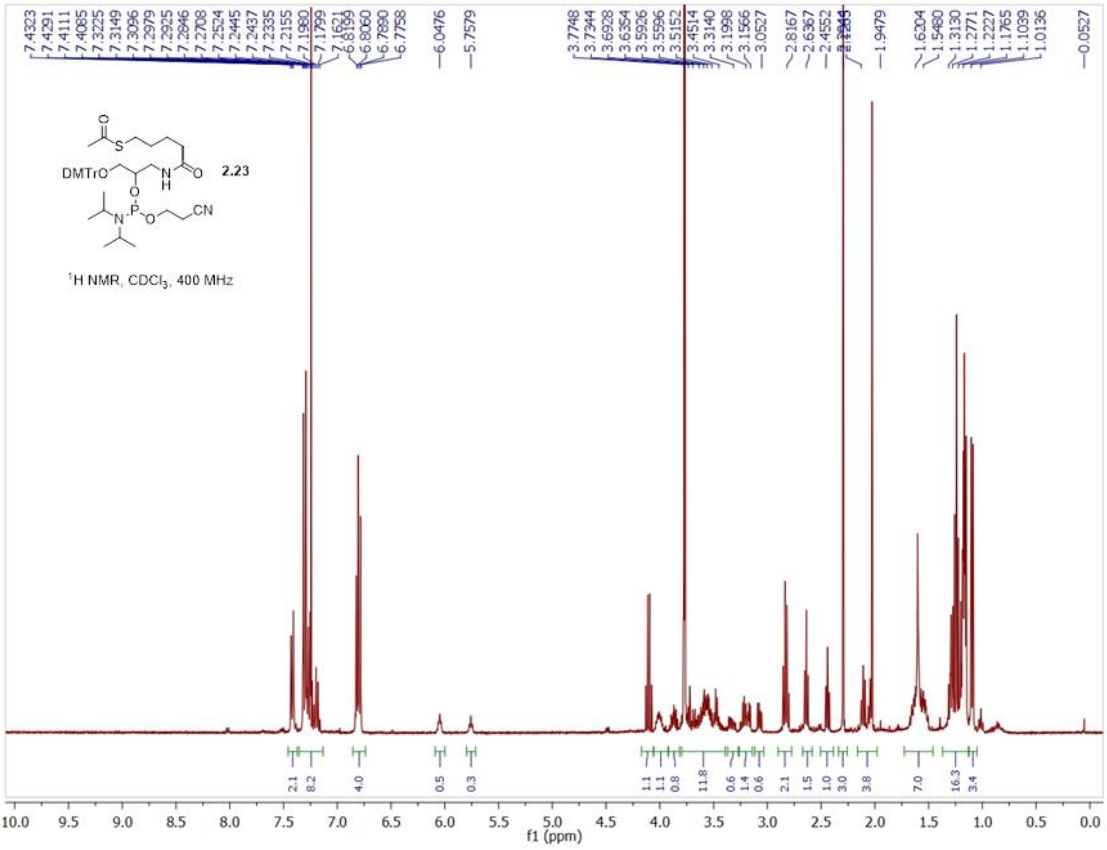


Figure A.26. ^{1}HNMR of Compound **2.23**

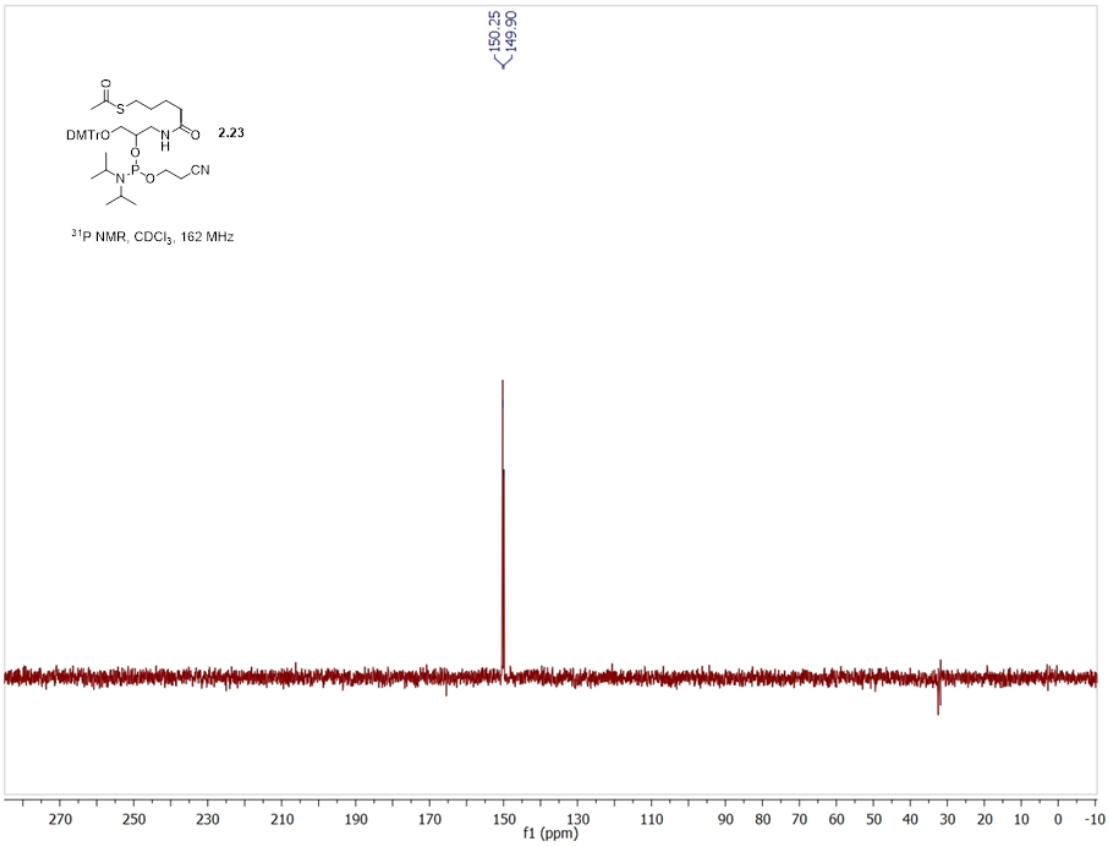


Figure A.27. ^{31}PNMR of Compound **2.23**

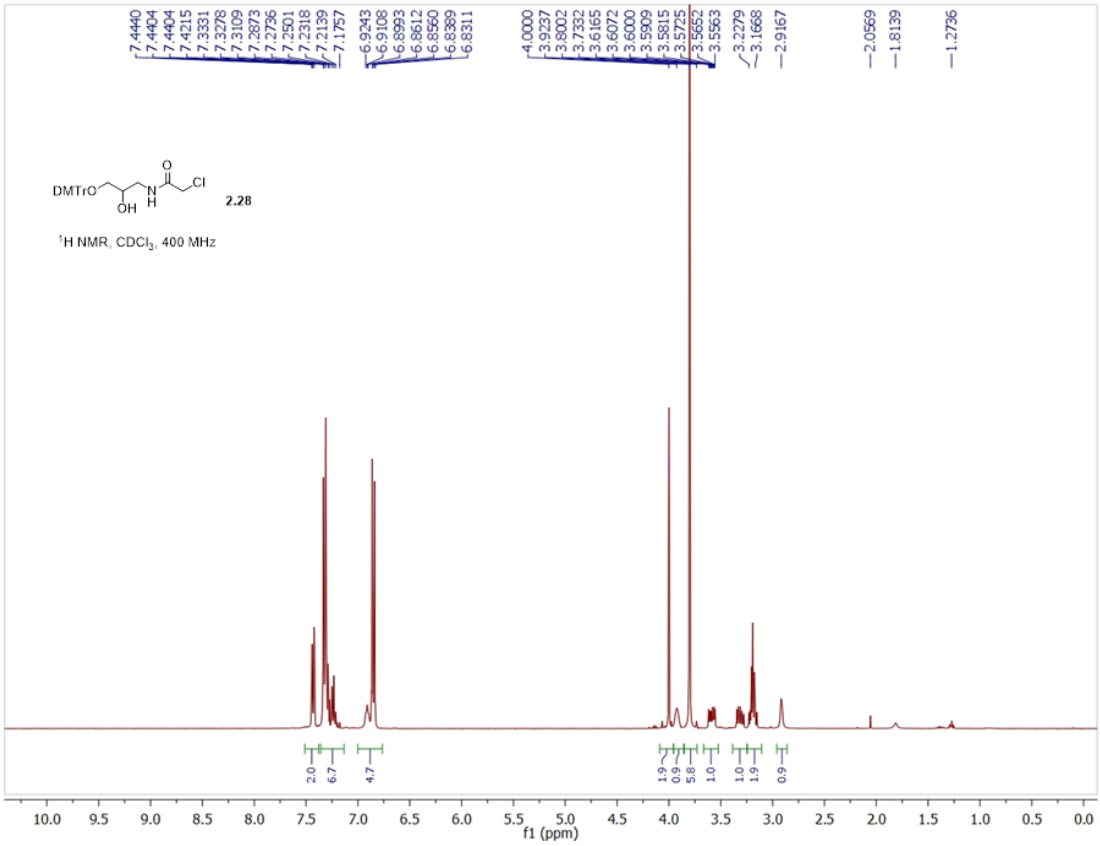


Figure A.28. ^{31}PNMR of Compound **2.28**

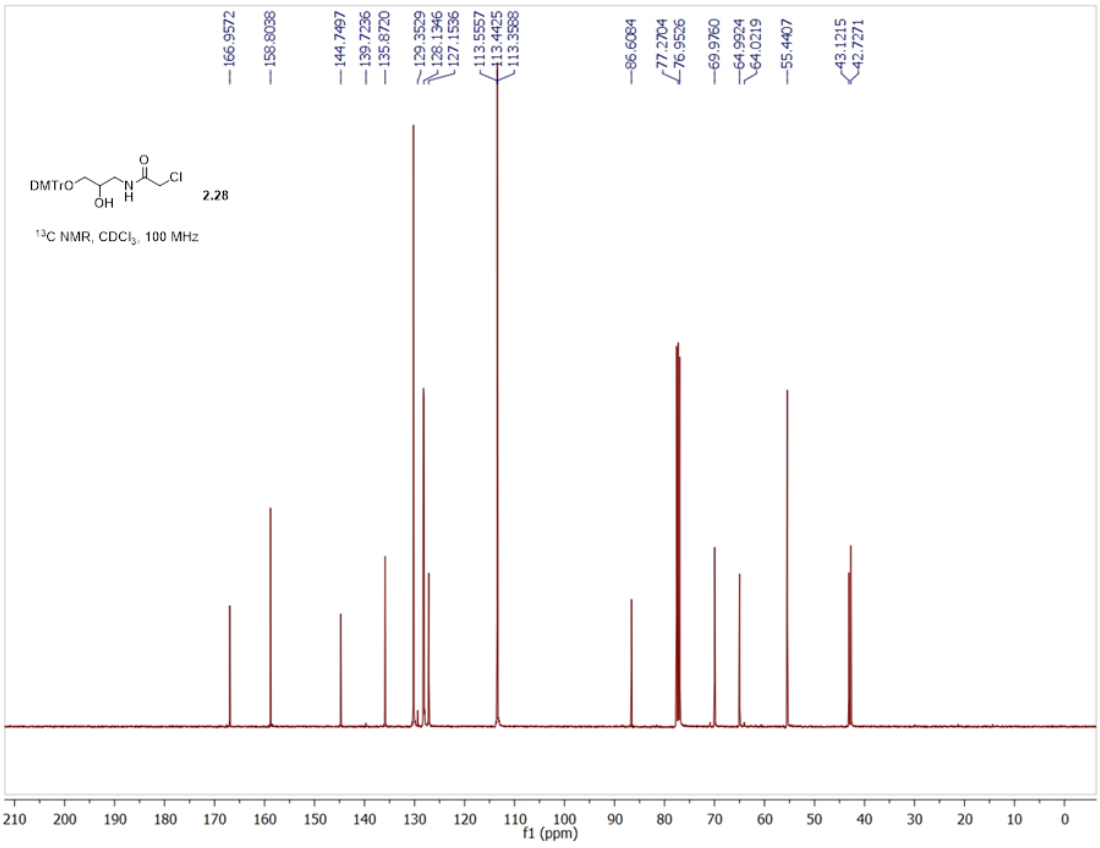


Figure A.29. ^{13}CNMR of Compound **2.28**

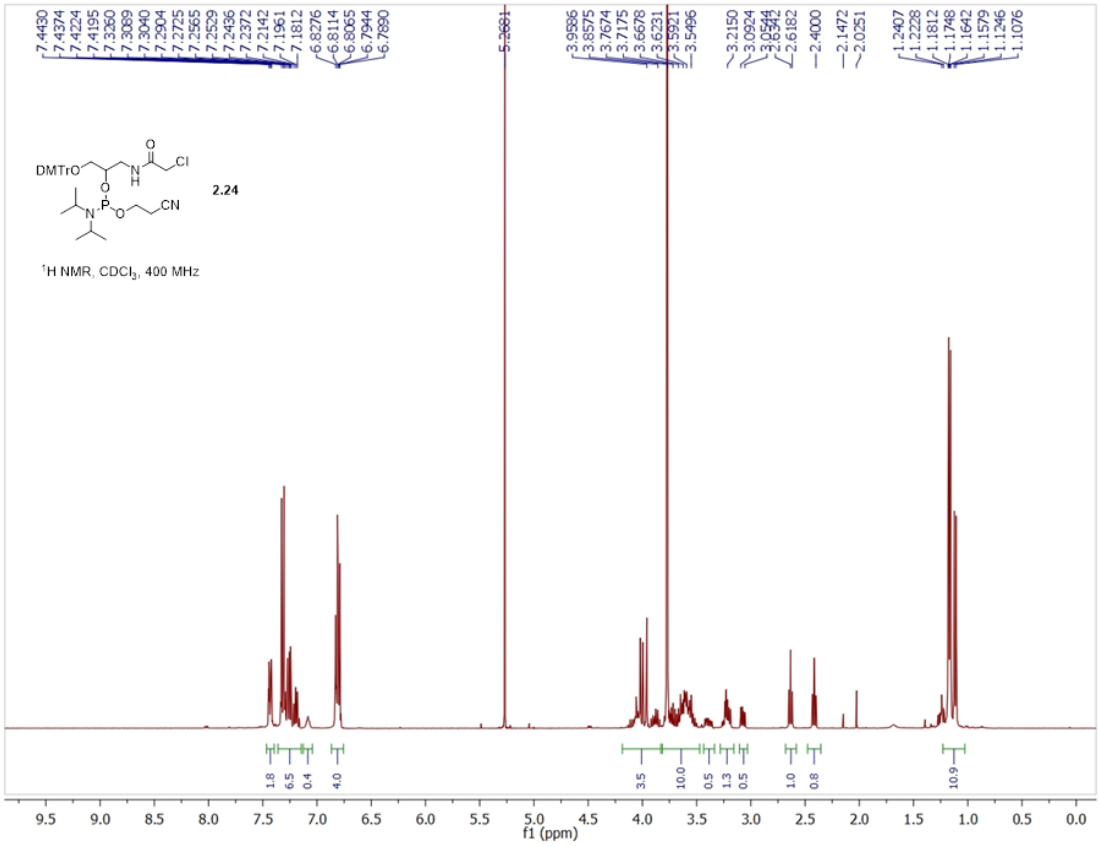


Figure A.30. ^{1}HNMR of Compound **2.24**

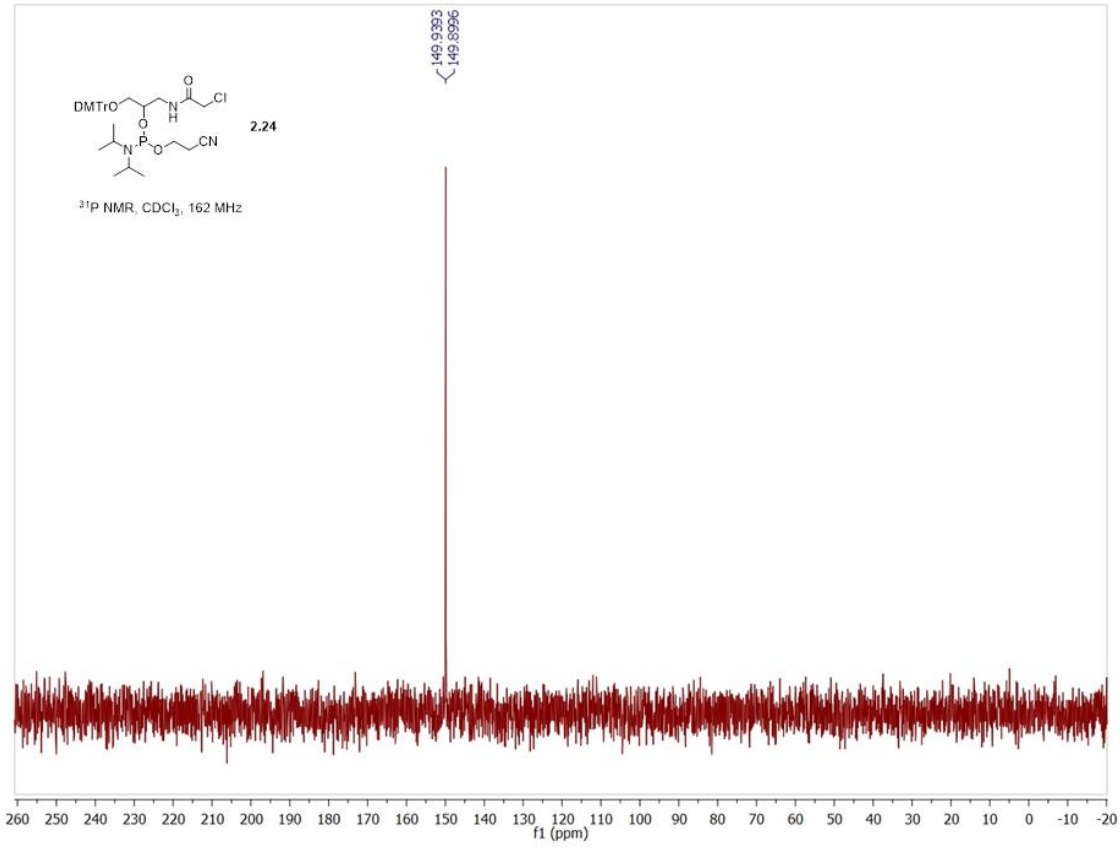


Figure A.31. ^{31}PNMR of Compound **2.24**

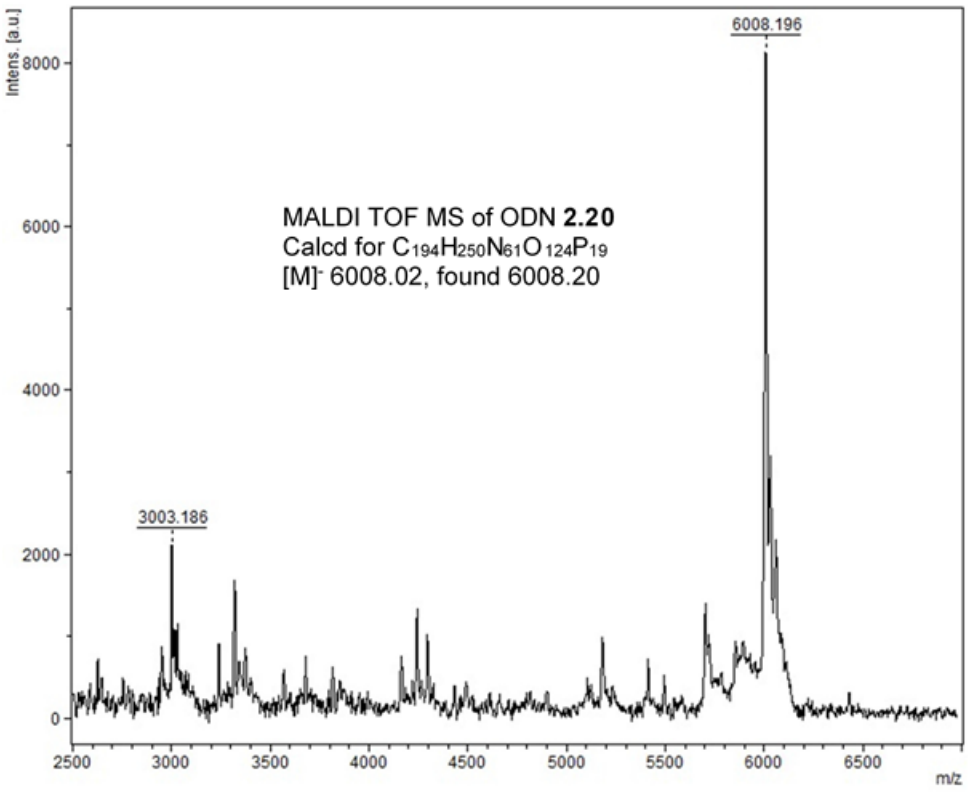


Figure A.32. MALDI-TOF-MS of ODN **2.20**

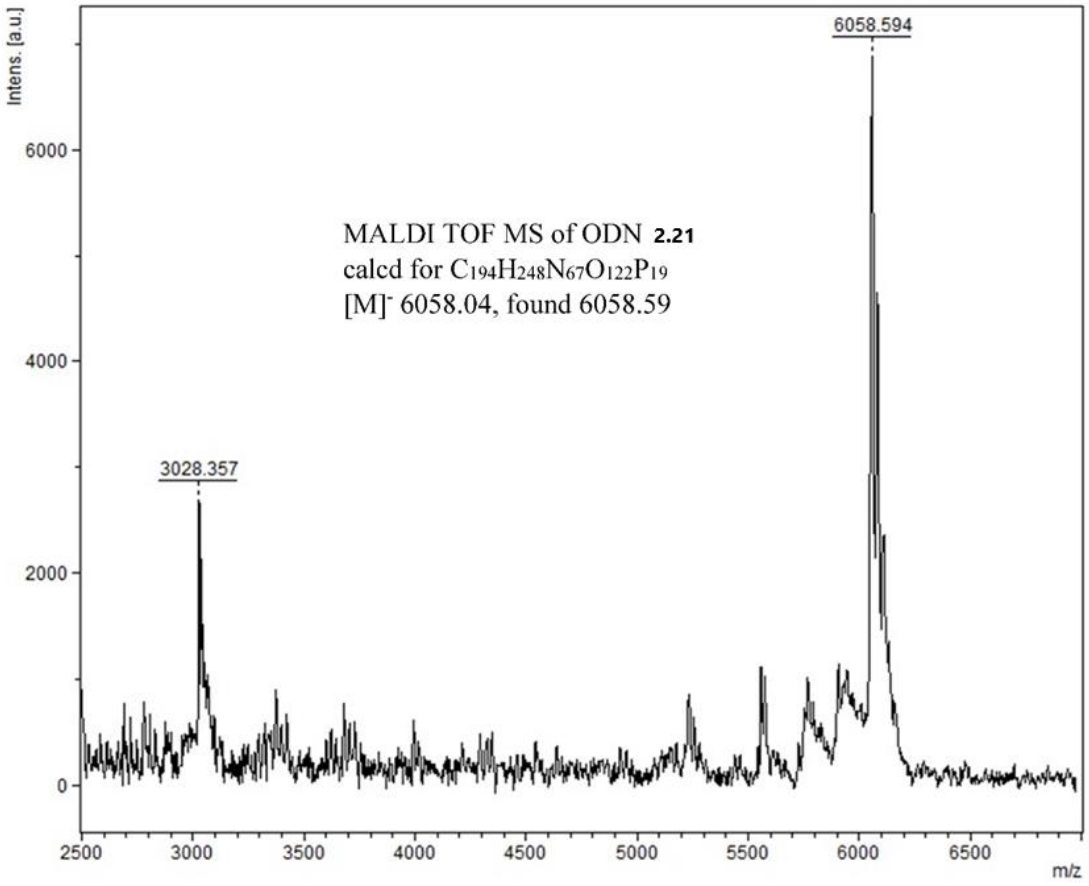


Figure A.33. MALDI-TOF-MS of ODN **2.21**

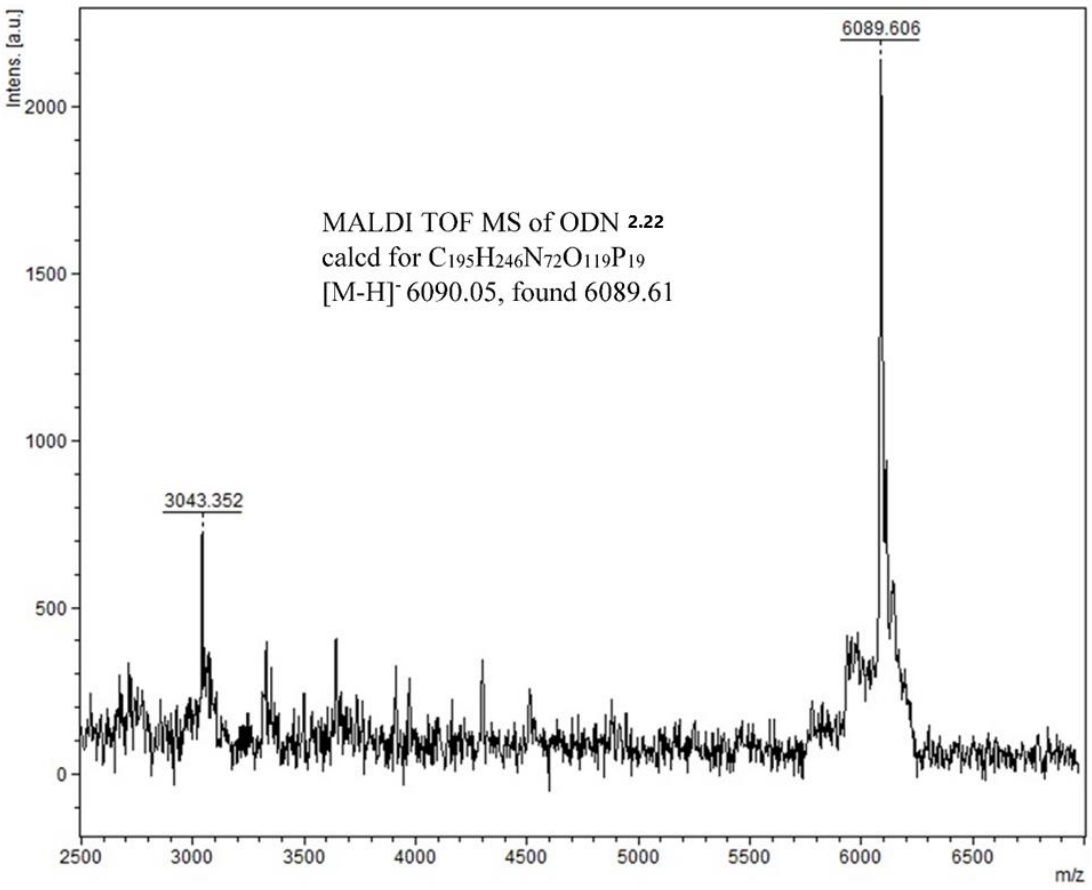


Figure A.34. MALDI-TOF-MS of ODN **2.22**

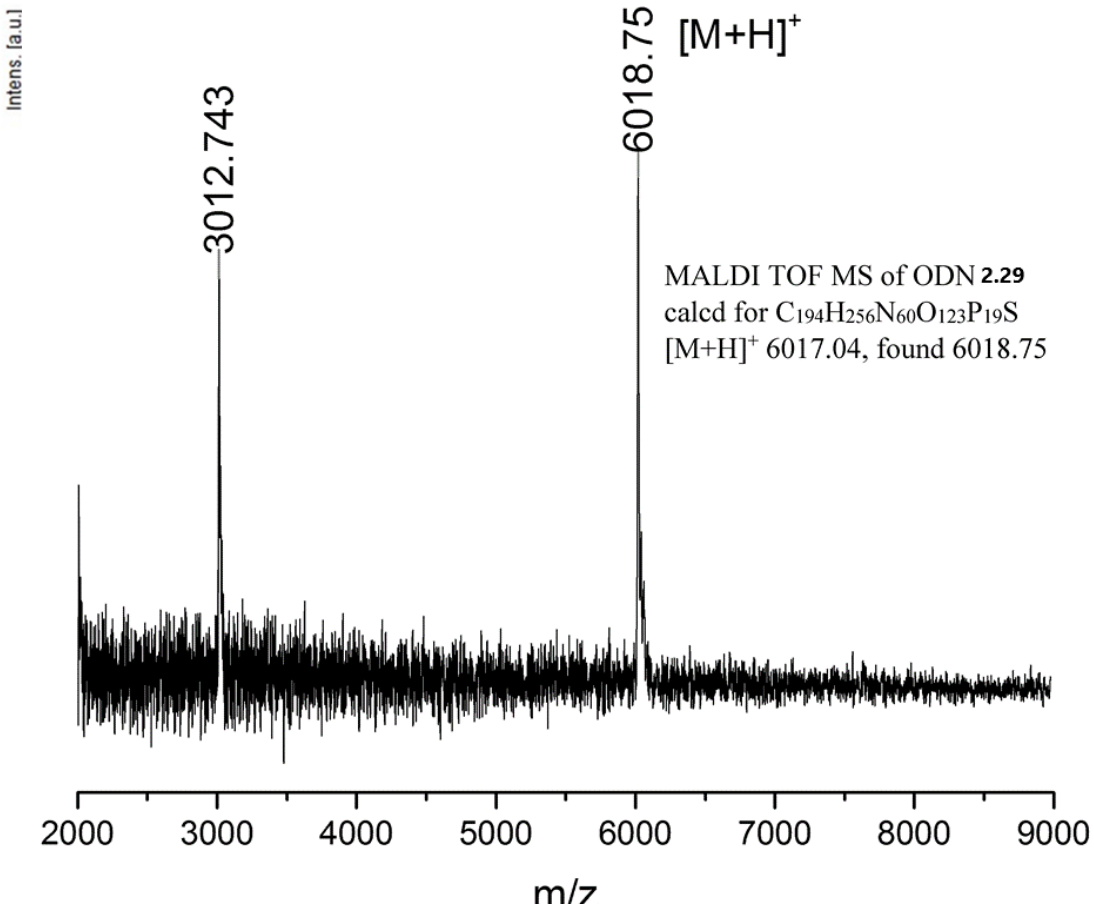


Figure A.35. MALDI-TOF-MS of ODN **2.29**

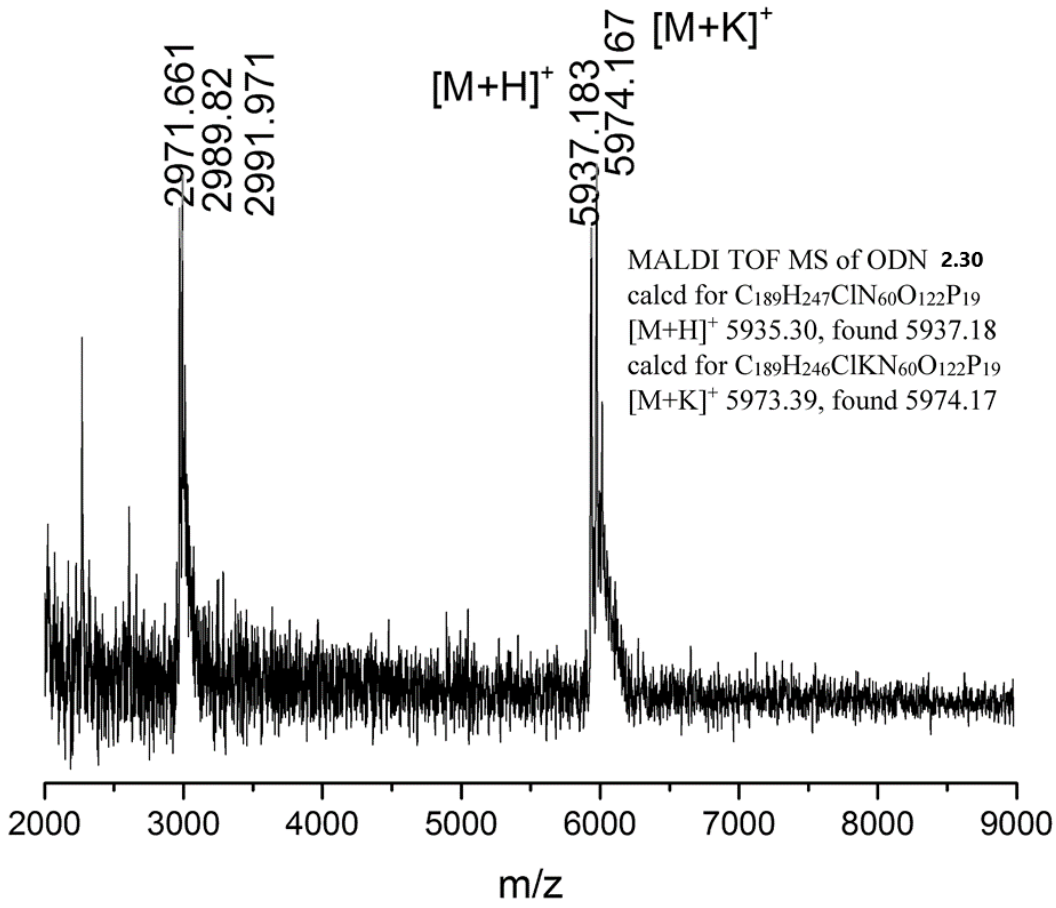


Figure A.36. MALDI-TOF-MS of ODN **2.30**

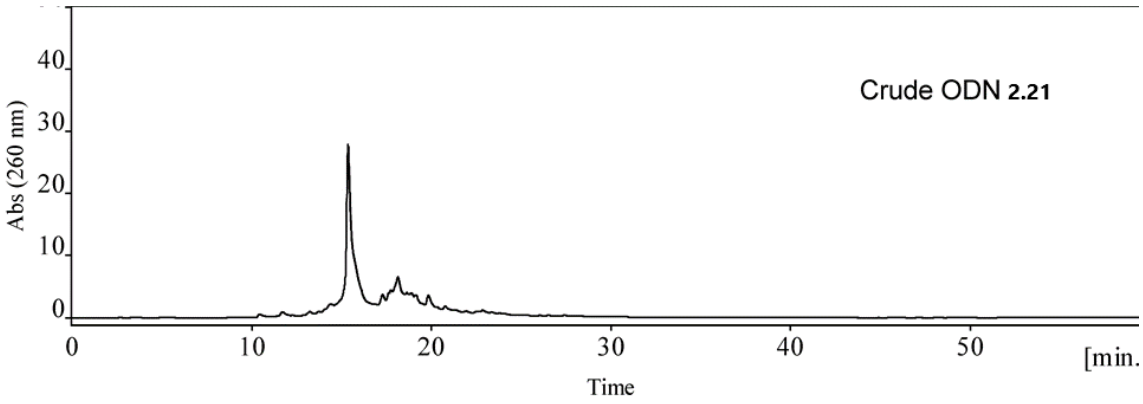


Figure A.37. RP-HPLC profile of crude ODN **2.21**

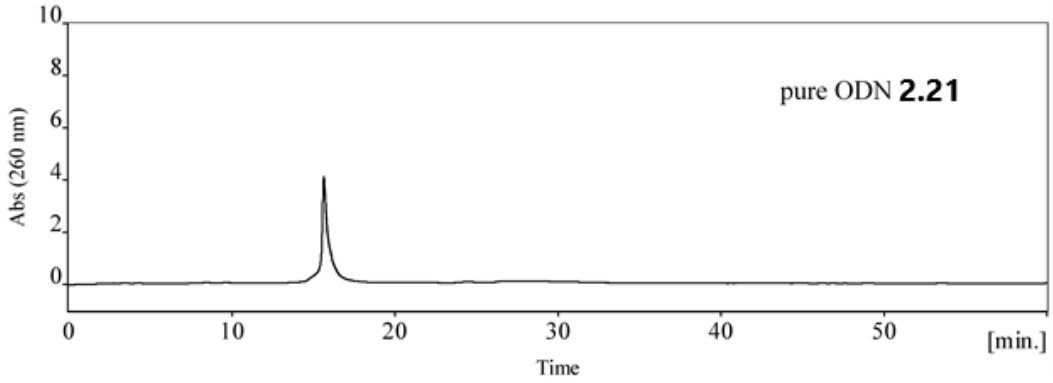


Figure A.38. RP-HPLC profile of pure ODN **2.21**

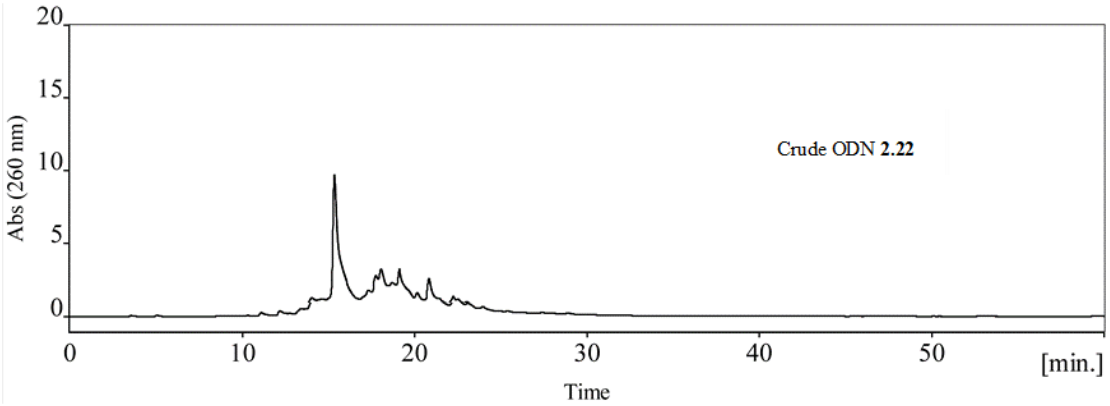


Figure A.39. RP-HPLC profile of crude ODN **2.22**

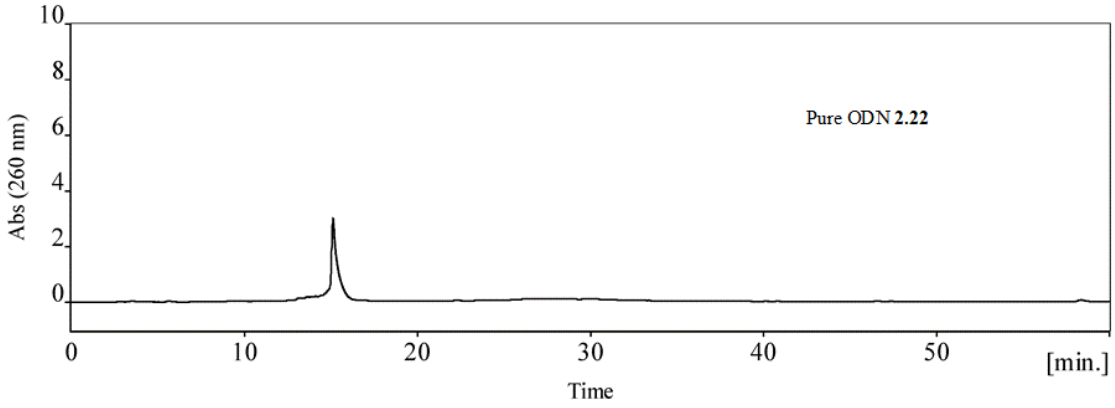


Figure A.40. RP-HPLC profile of pure ODN **2.22**

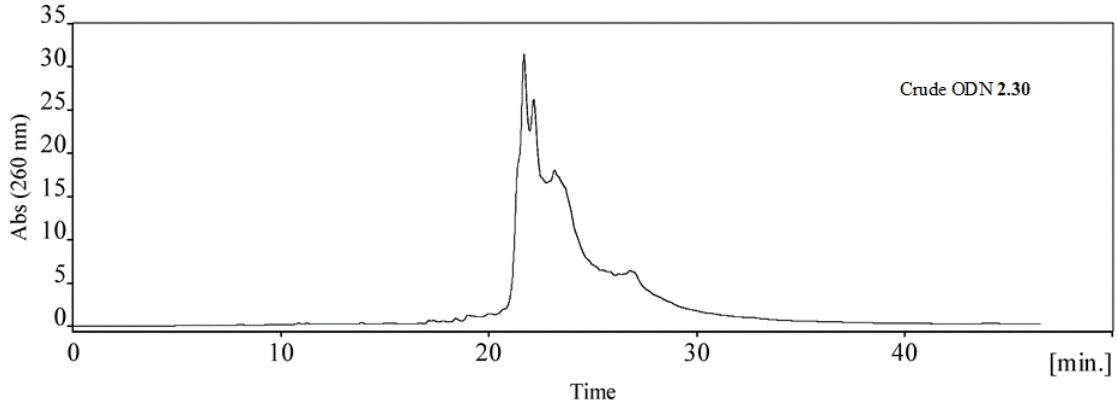


Figure A.41. RP-HPLC profile of pure ODN **2.22**

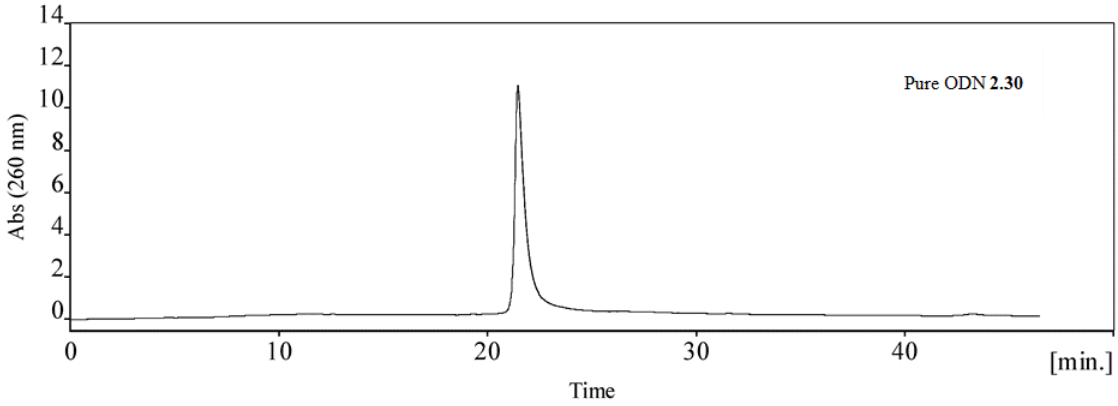
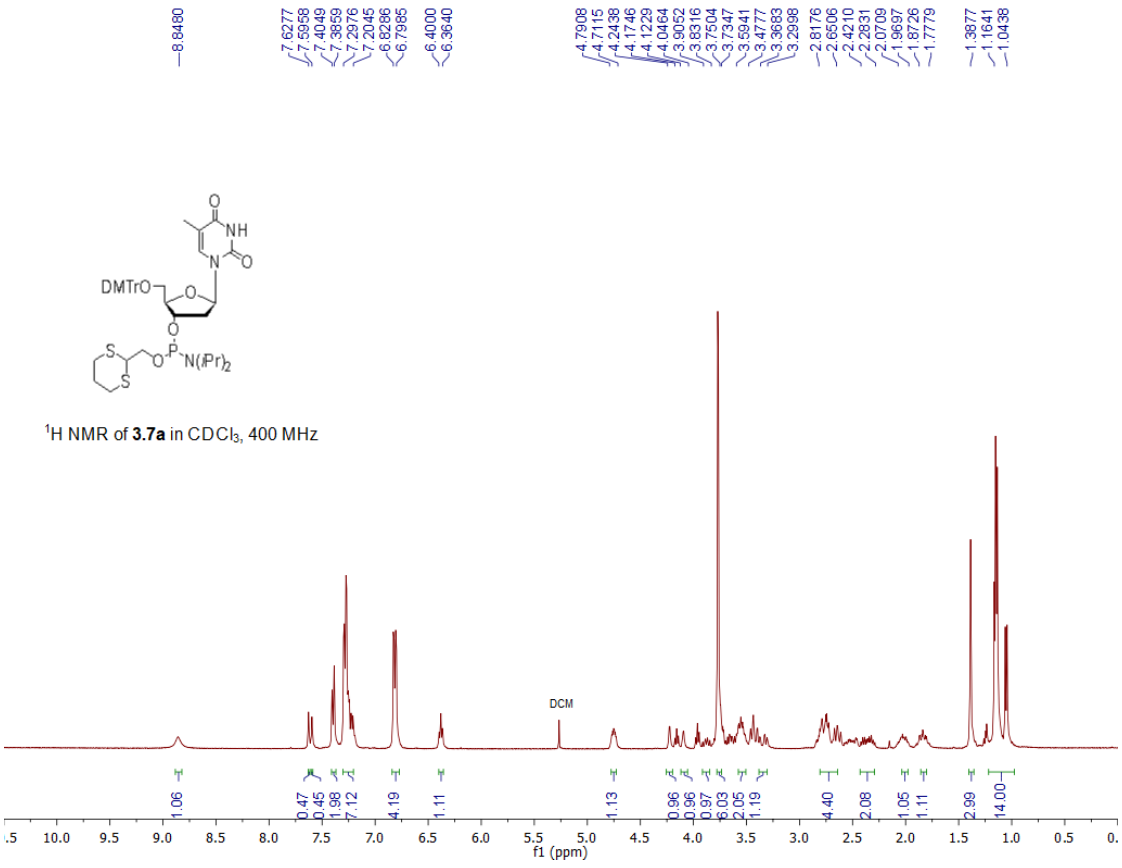


Figure A.42. RP-HPLC profile of crude ODN **2.30**

Appendix B. Supporting Information for Chapter 3

Sensitive ODN Synthesis Using Dim for Phosphate Protection



^{1}H NMR of **3.7a** in $CDCl_3$, 400 MHz

Figure B.1. ^{1}HNMR of Compound **3.7a**

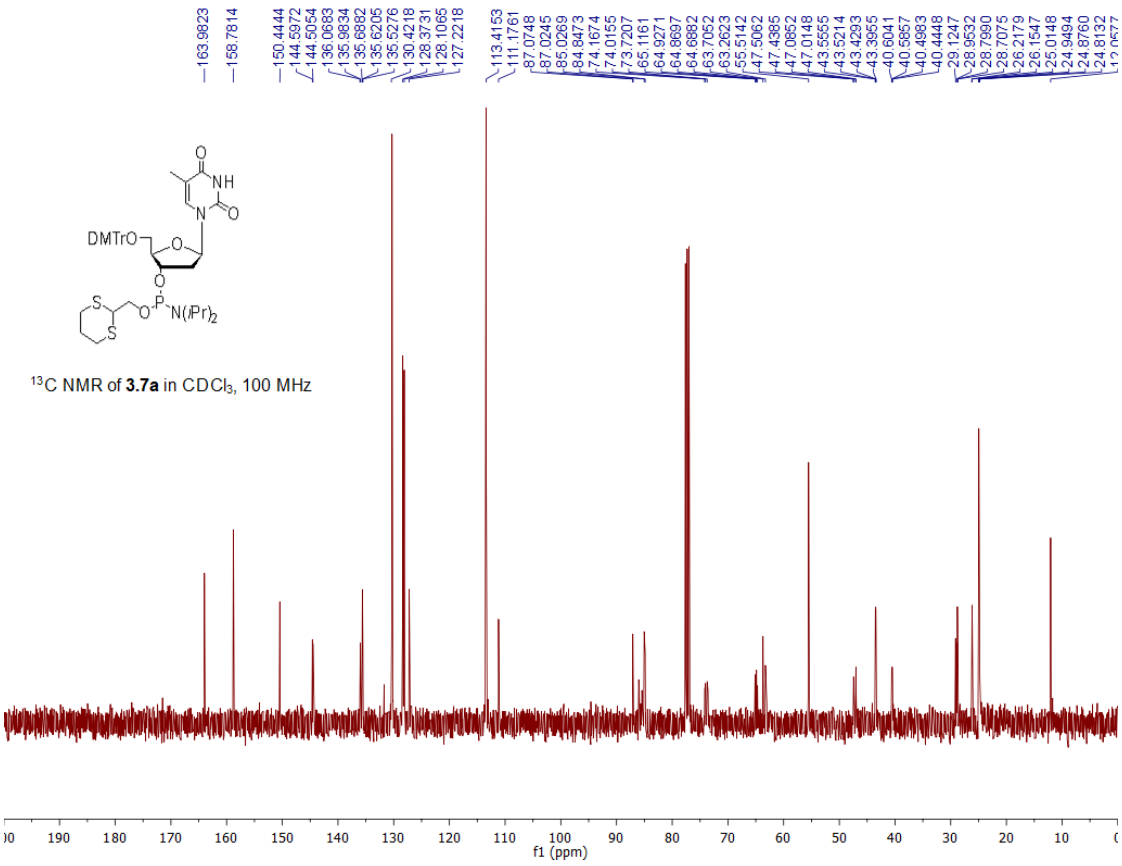


Figure B.2. ^{13}CNMR of Compound **3.7a**

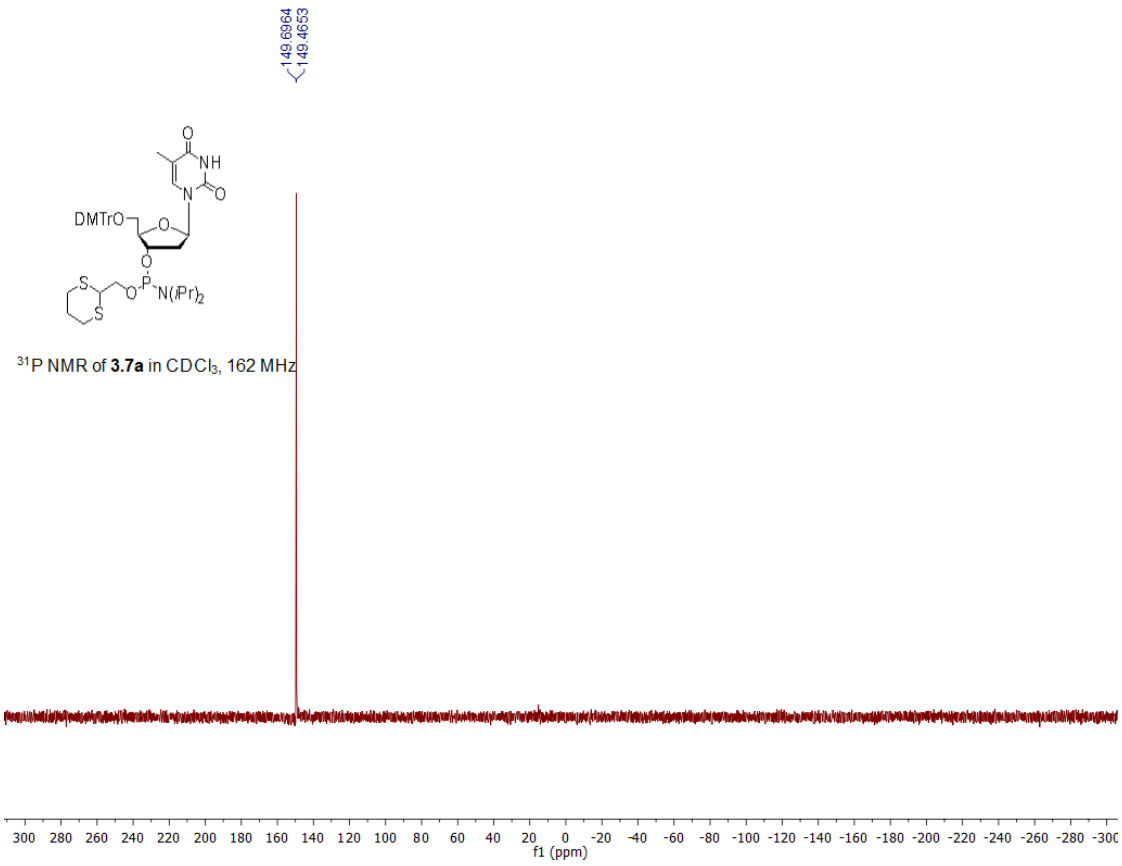


Figure B.3. ^{31}PNMR of Compound **3.7a**

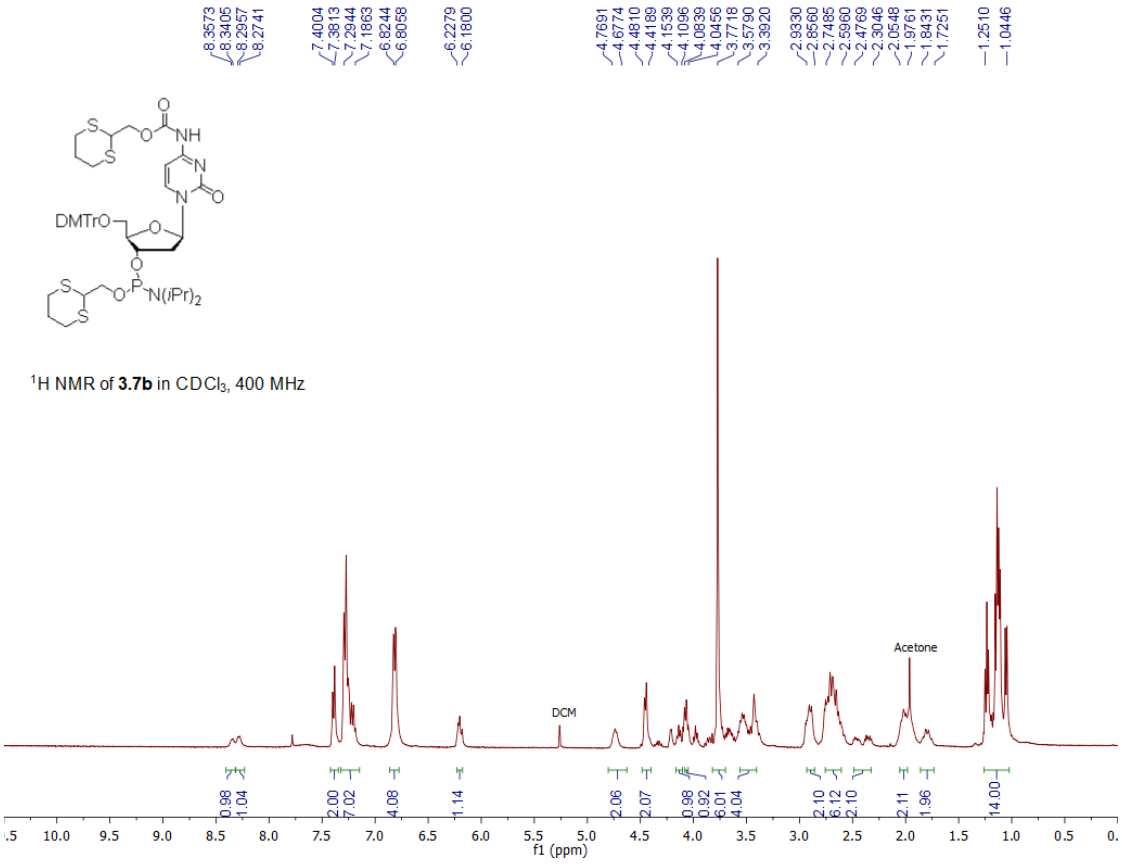


Figure B.4. [1]HNMR of Compound **3.7b**

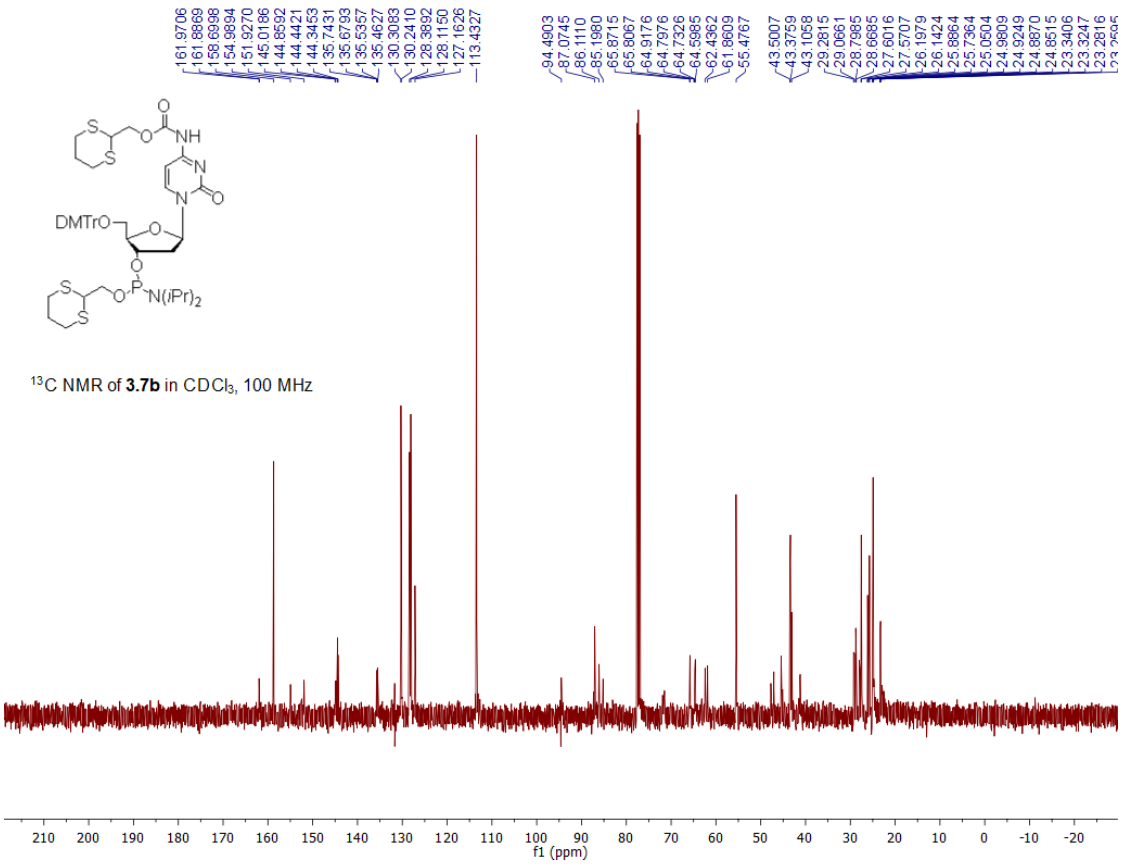


Figure B.5. ^{13}CNMR of Compound **3.7b**

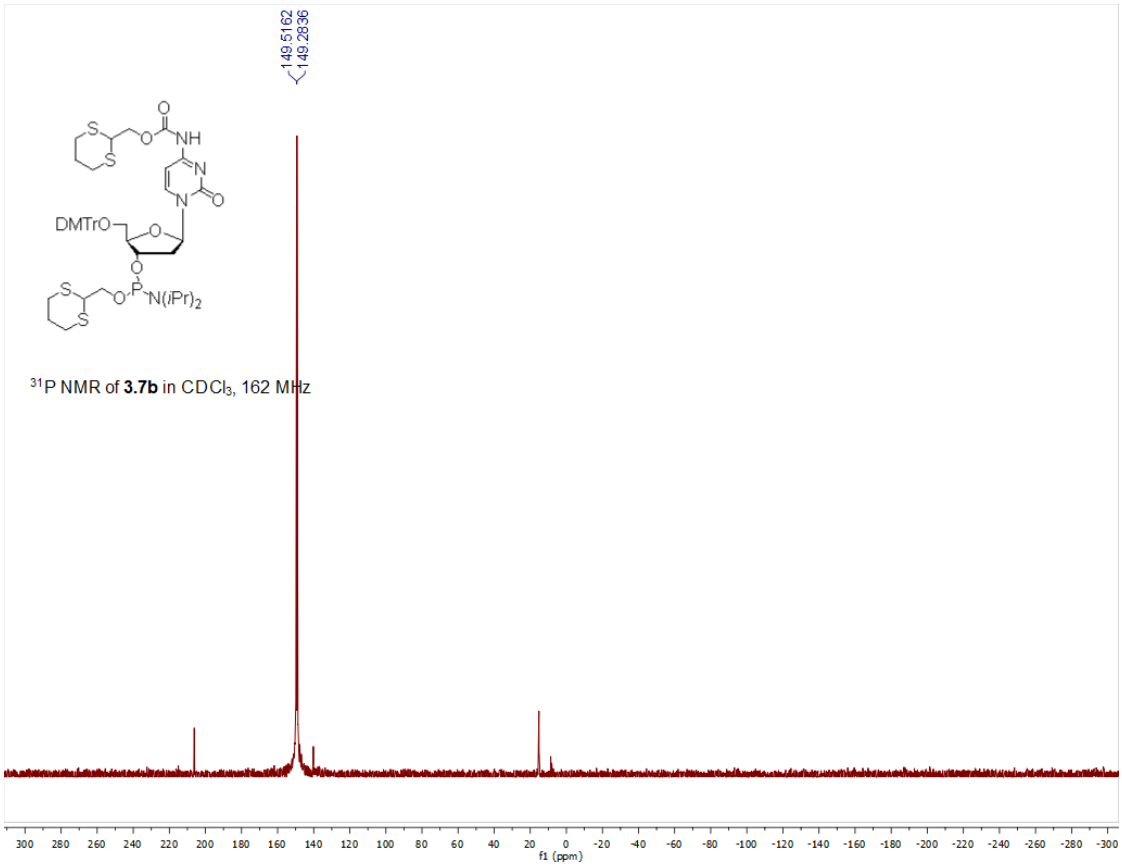


Figure B.6. ^{31}PNMR of Compound **3.7b**

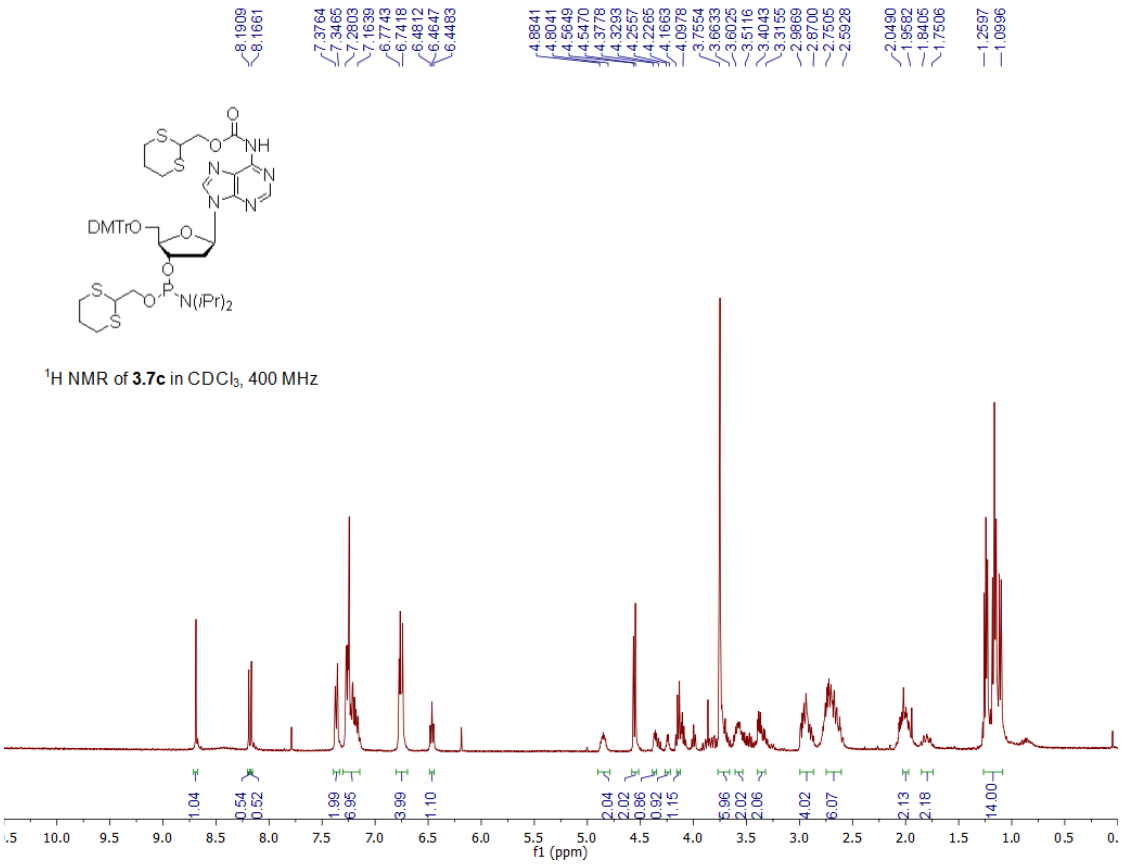


Figure B.7. [1]HNMR of Compound **3.7c**

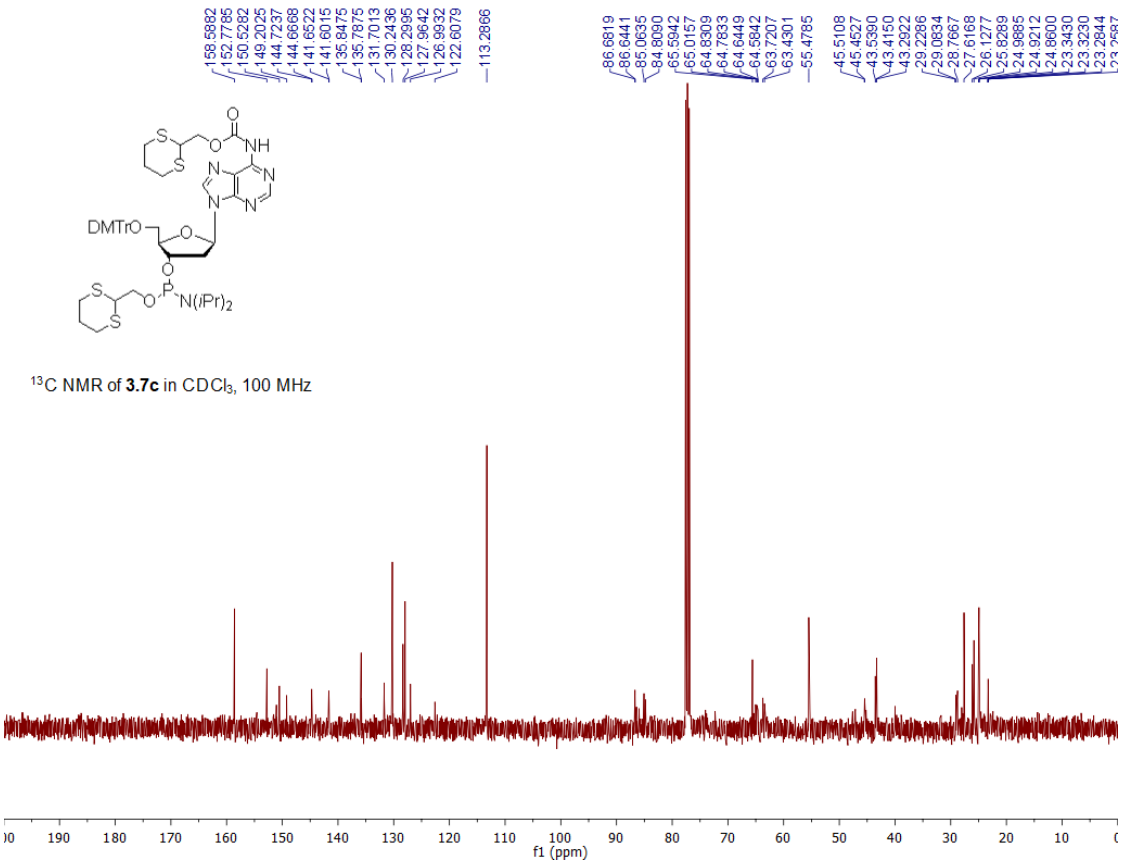


Figure B.8. ^{13}CNMR of Compound **3.7c**

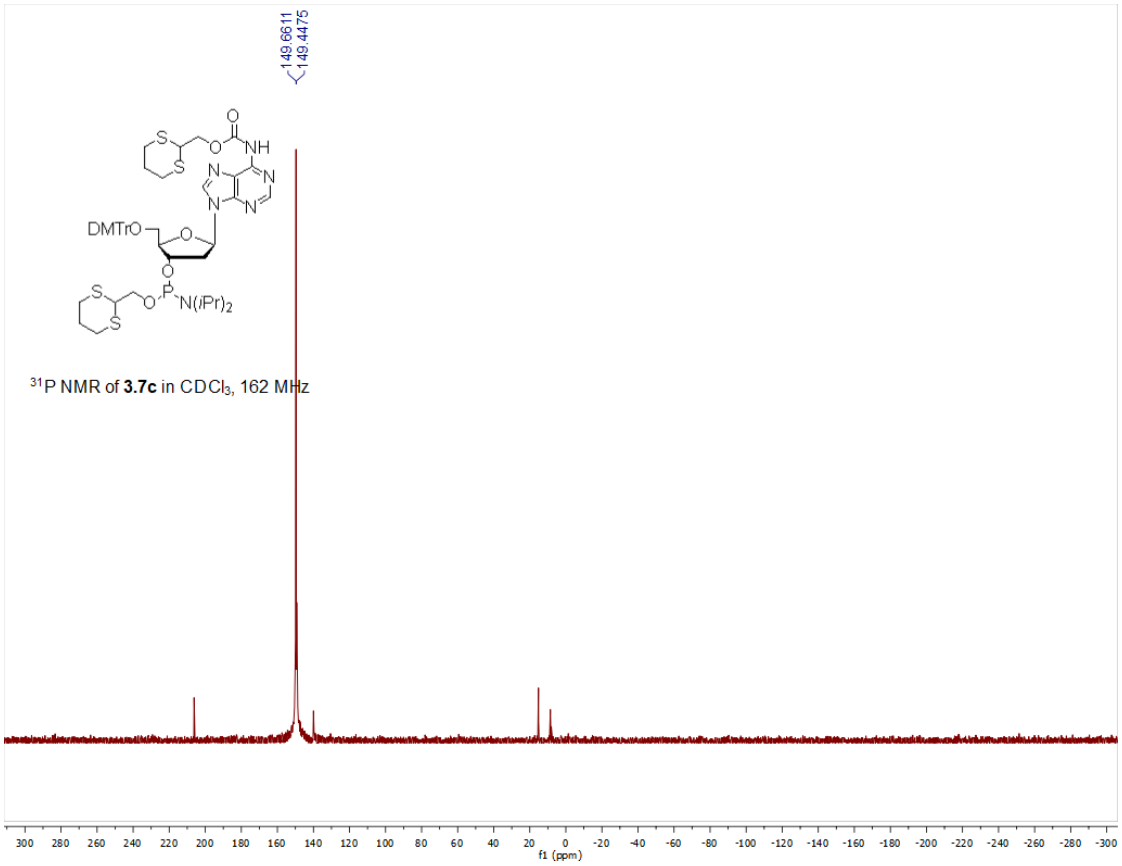


Figure B.9. ^{31}PNMR of Compound **3.7c**

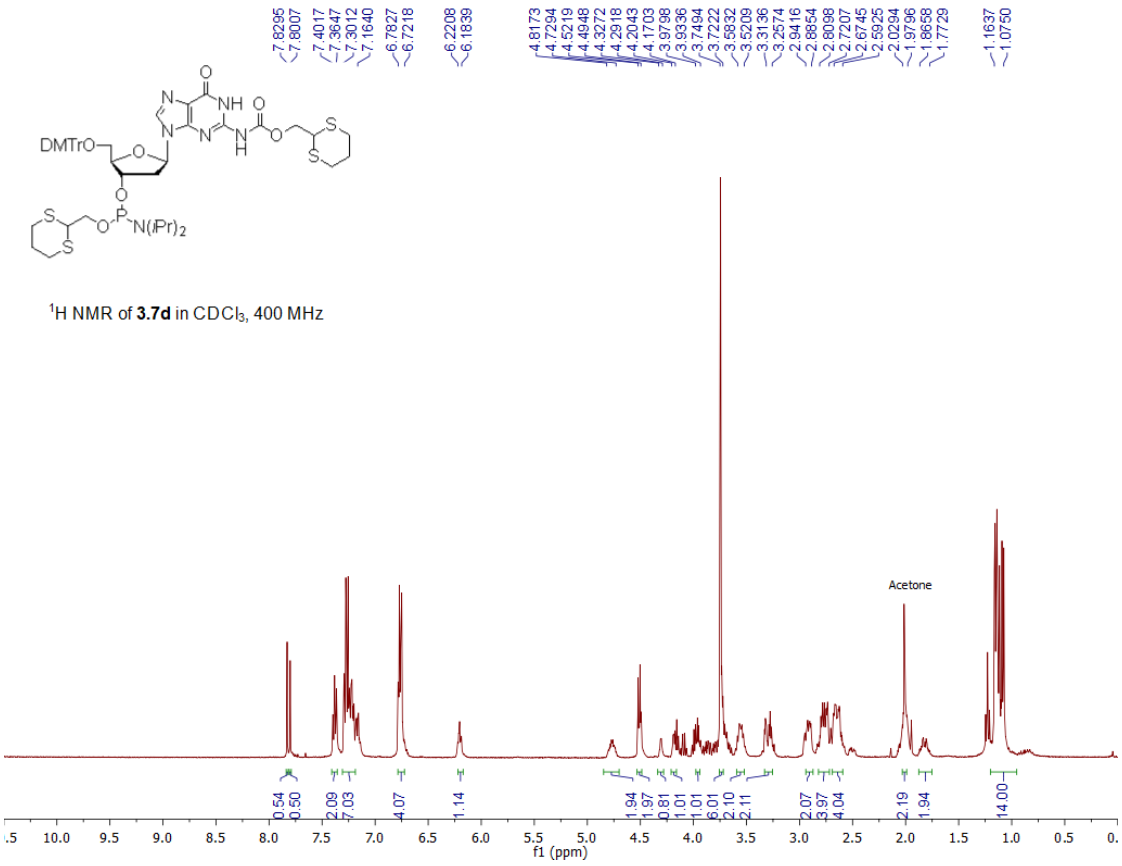


Figure B.10. [1]HNMR of Compound **3.7d**

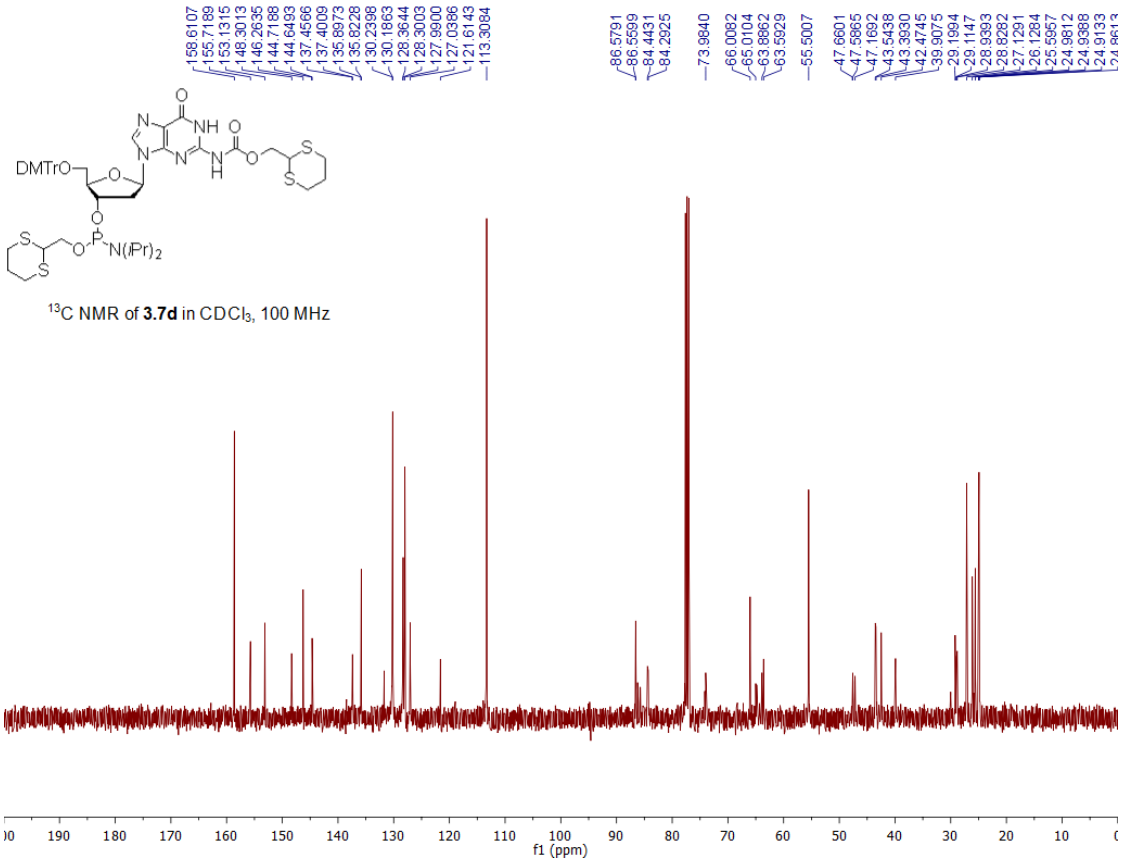


Figure B.11. ^{13}CNMR of Compound **3.7d**

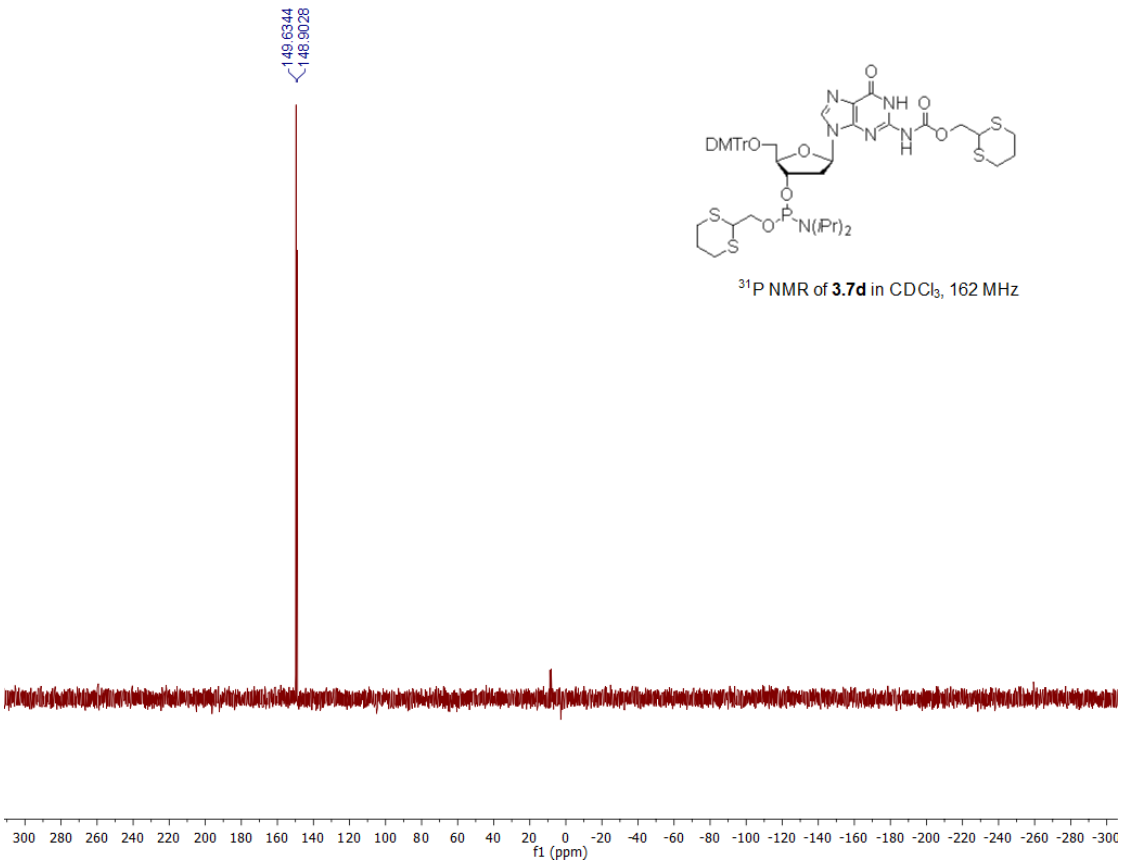


Figure B.12. ^{31}PNMR of Compound **3.7d**

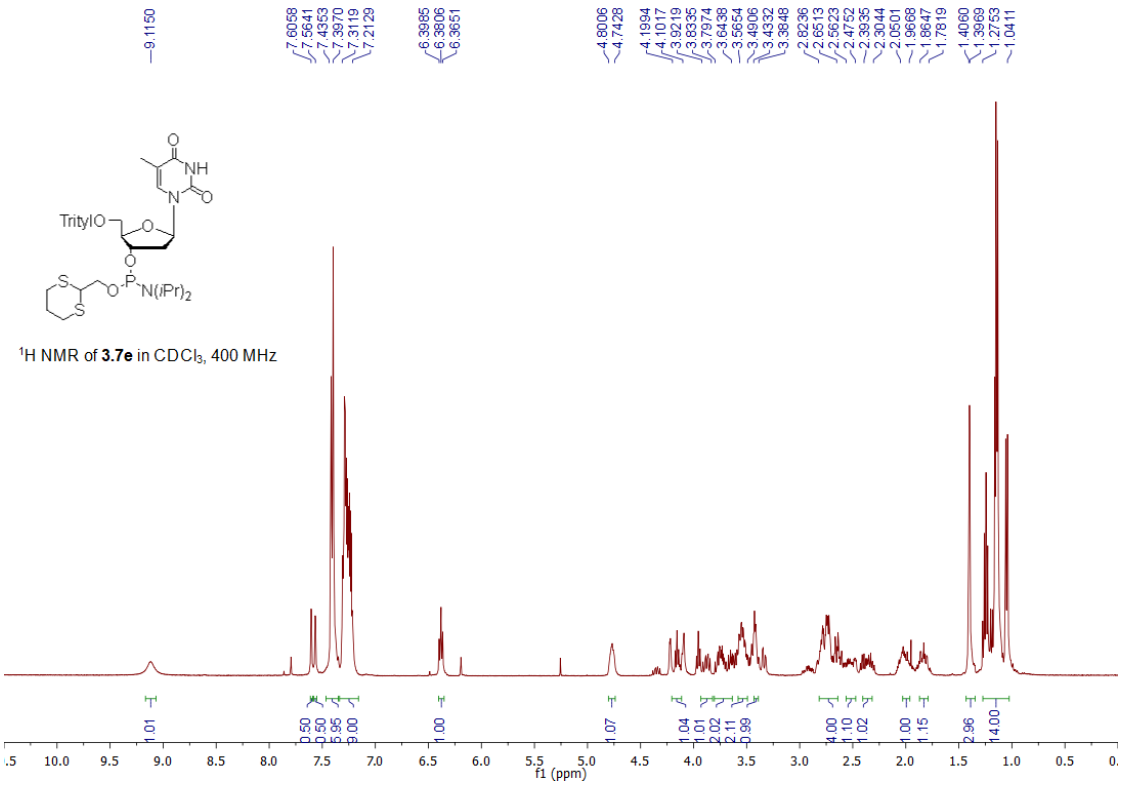


Figure B.13. [1]HNMR of Compound **3.7e**

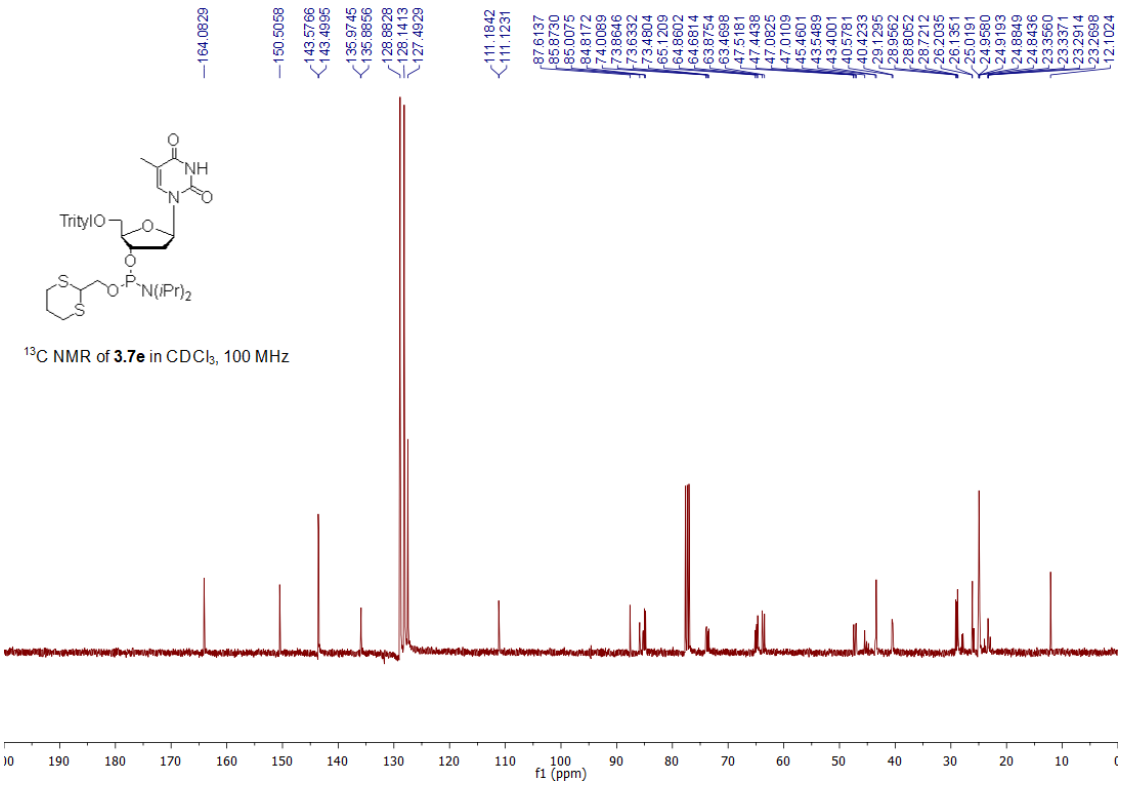


Figure B.14. ^{13}CNMR of Compound **3.7e**

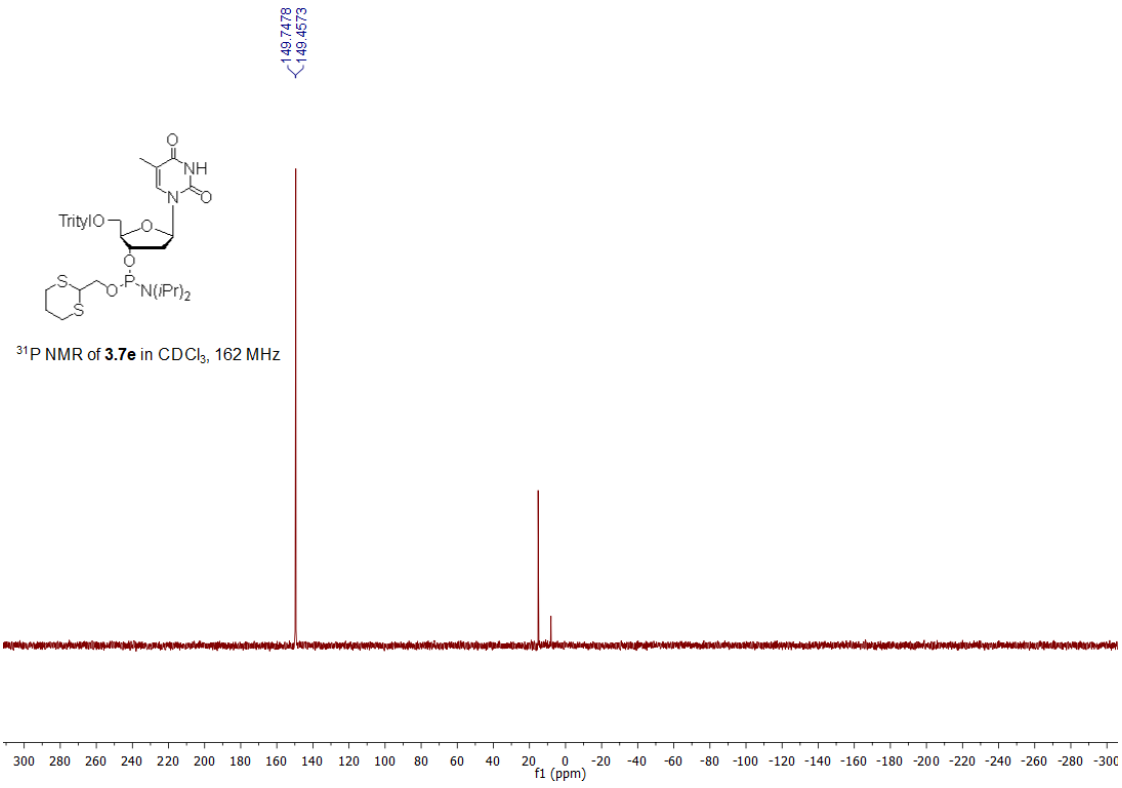


Figure B.15. ^{31}PNMR of Compound **3.7e**

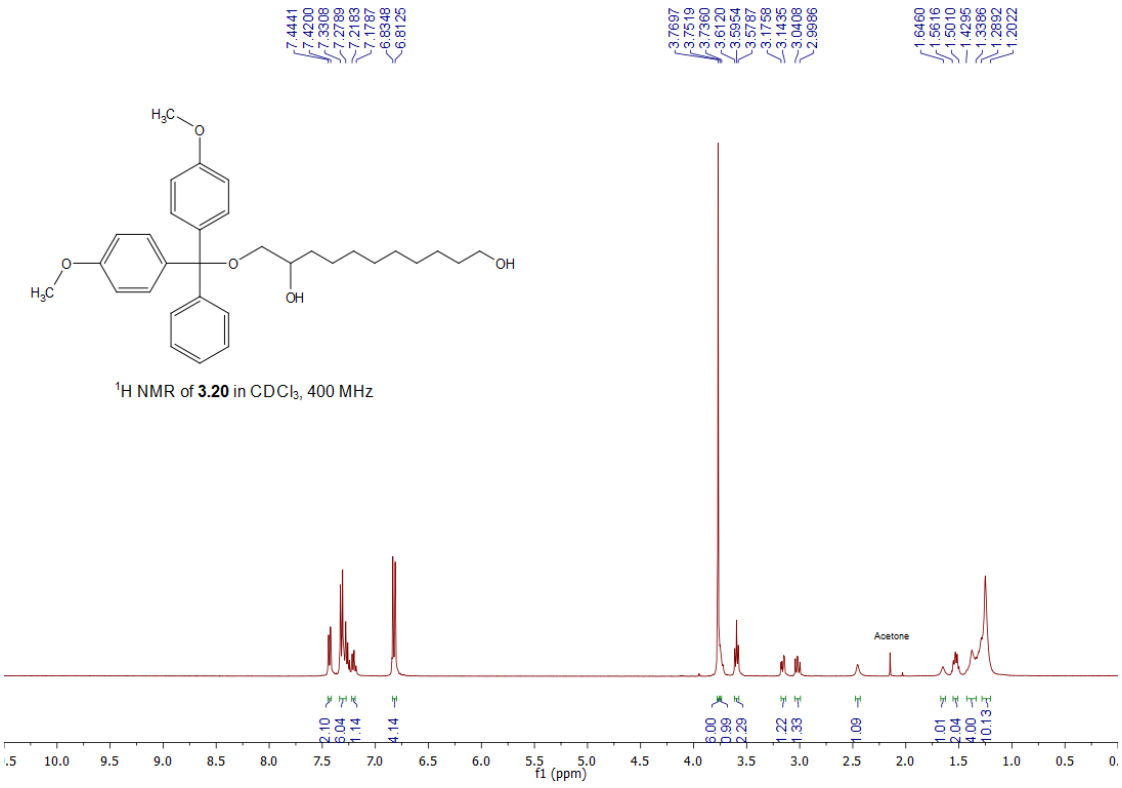


Figure B.16. [1]HNMR of Compound **3.20**

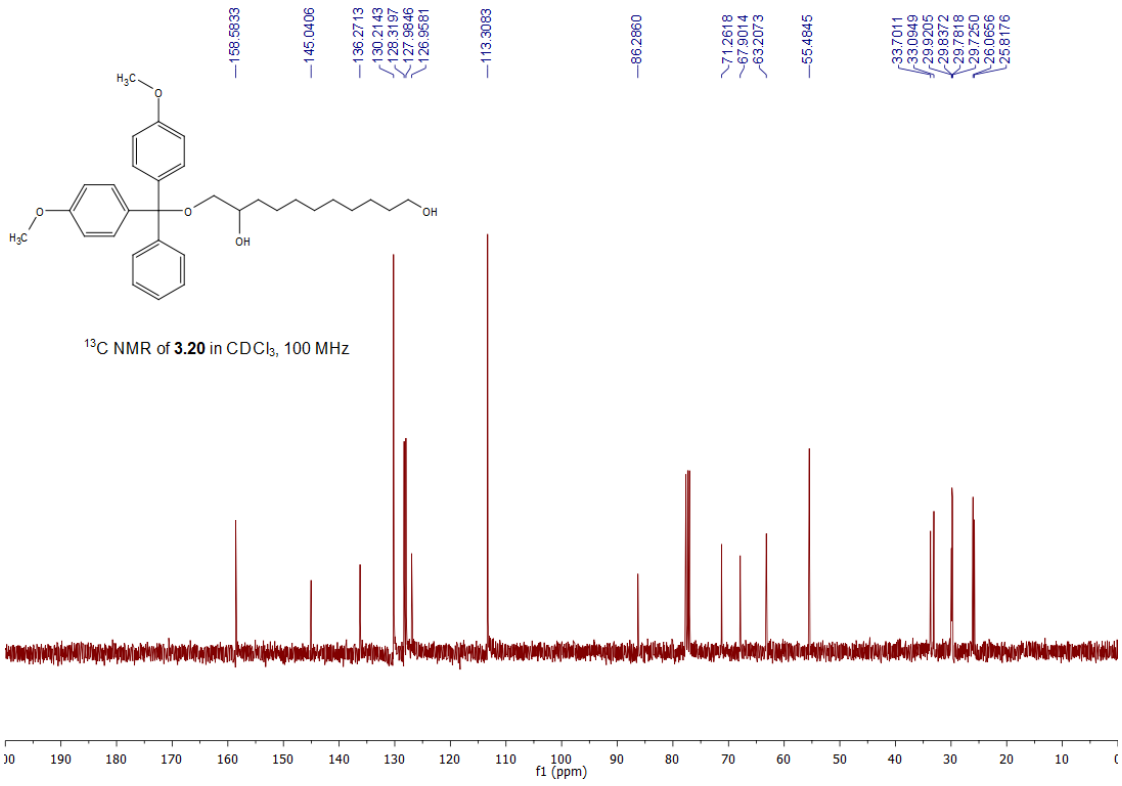


Figure B.17. ^{13}CNMR of Compound **3.20**

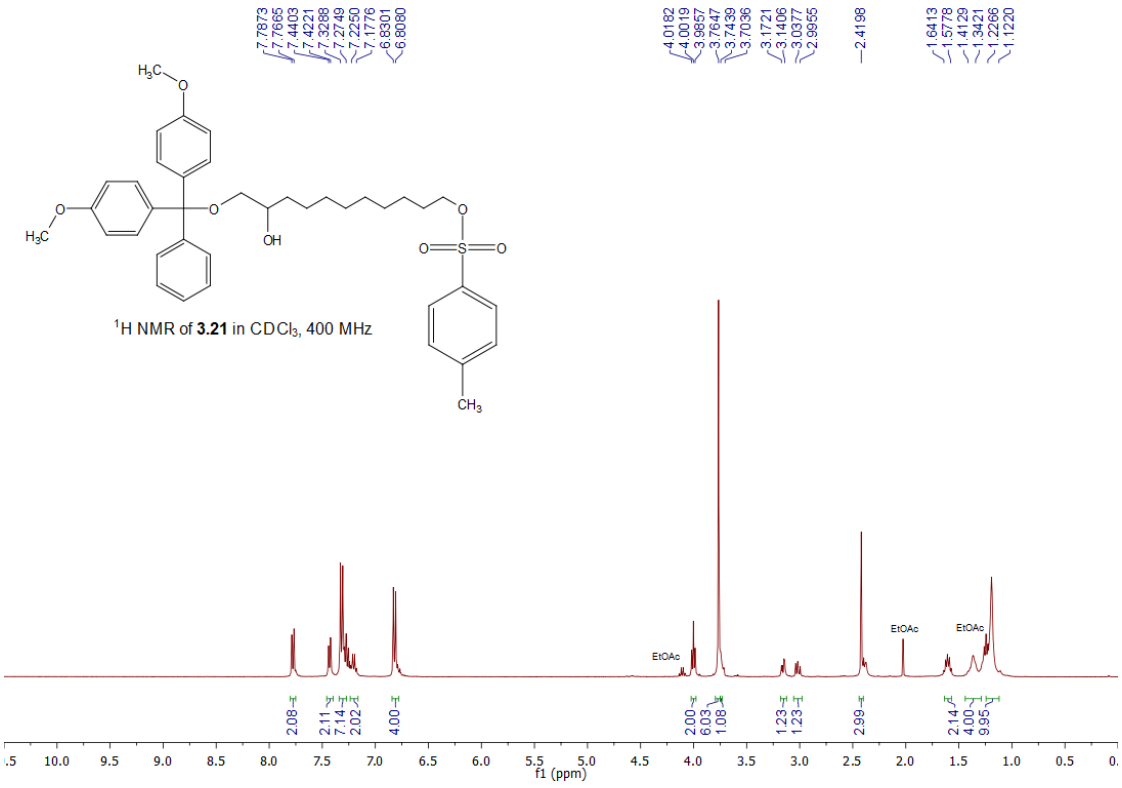


Figure B.18. [1]HNMR of Compound **3.21**

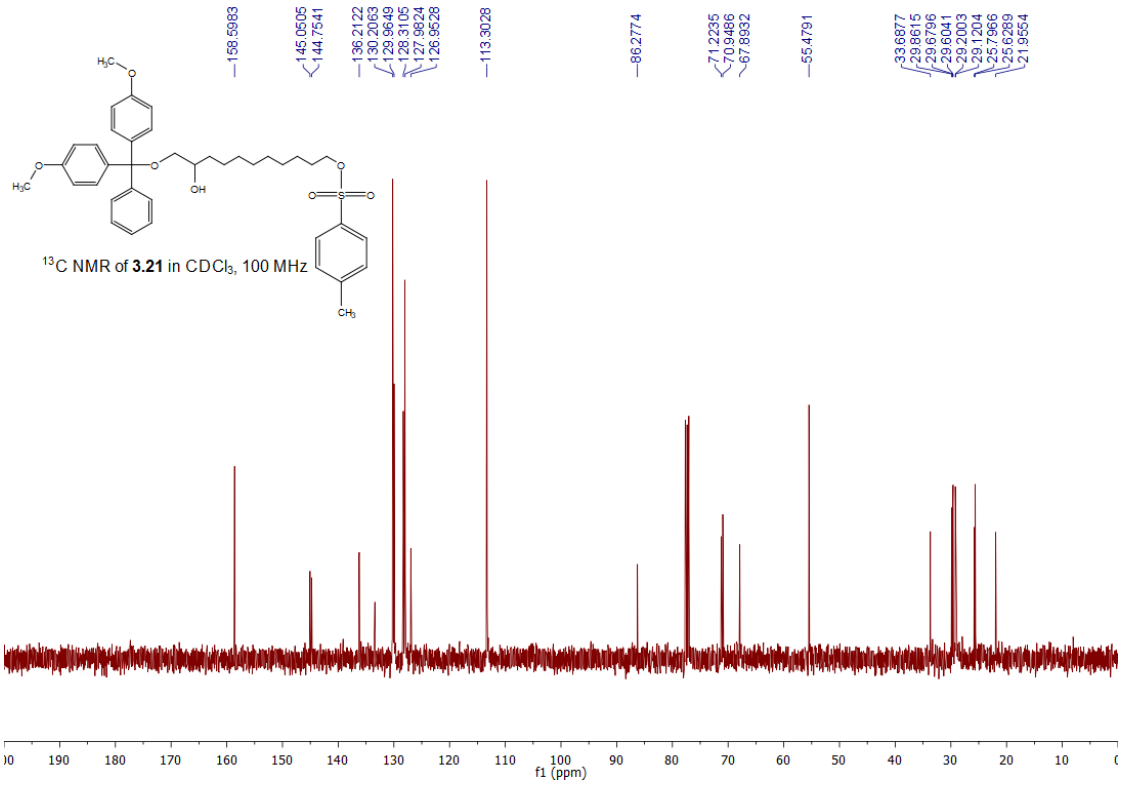
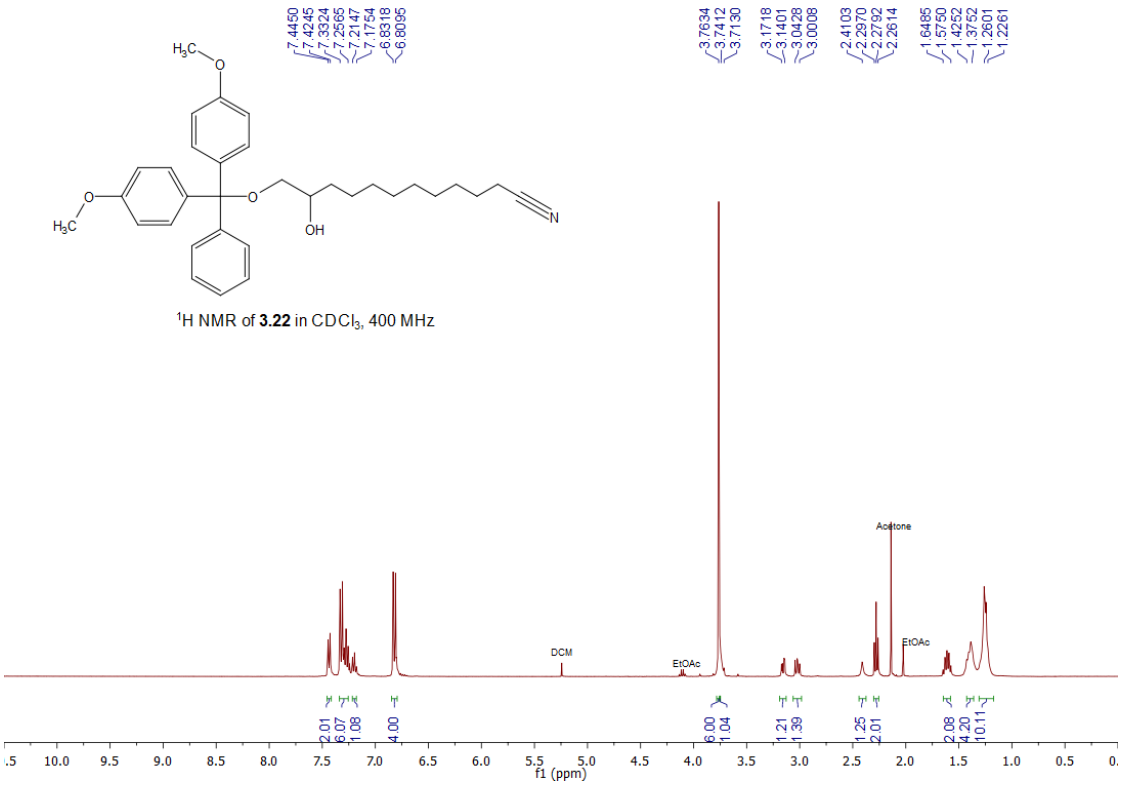


Figure B.19. ^{13}CNMR of Compound **3.21**



^{1}H NMR of **3.22** in $CDCl_3$, 400 MHz

Figure B.20. ^{1}HNMR of Compound **3.22**

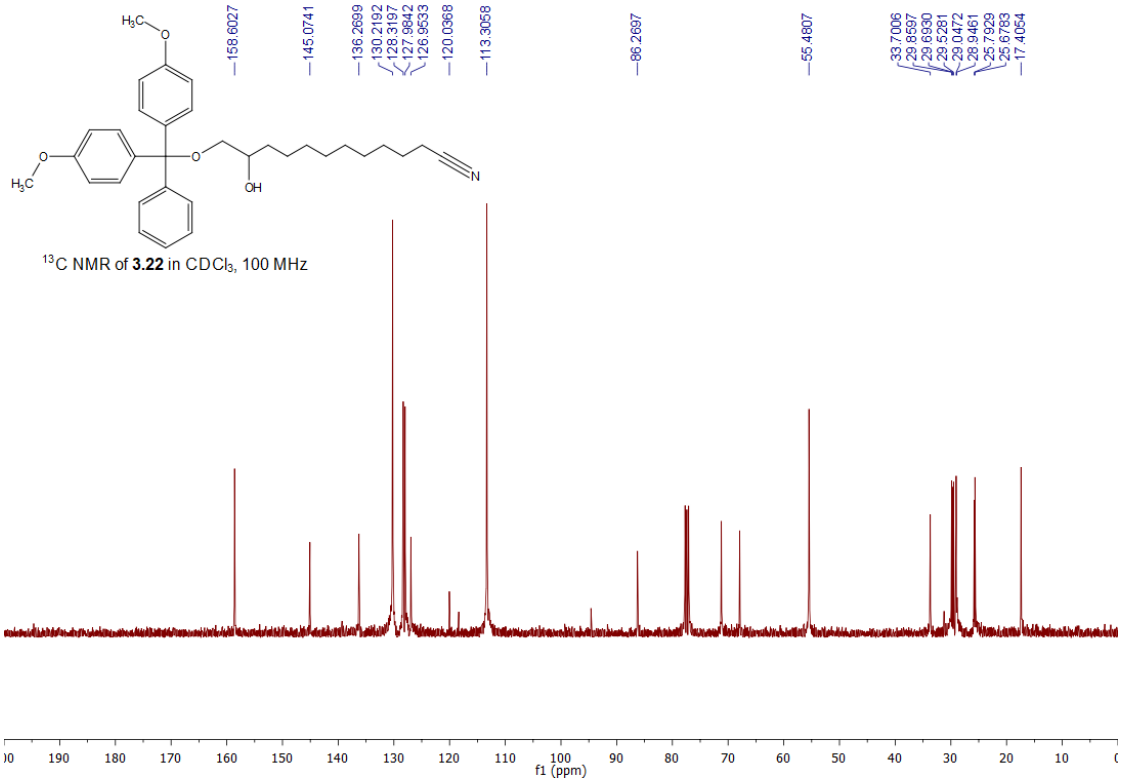


Figure B.21. ^{13}CNMR of Compound **3.22**

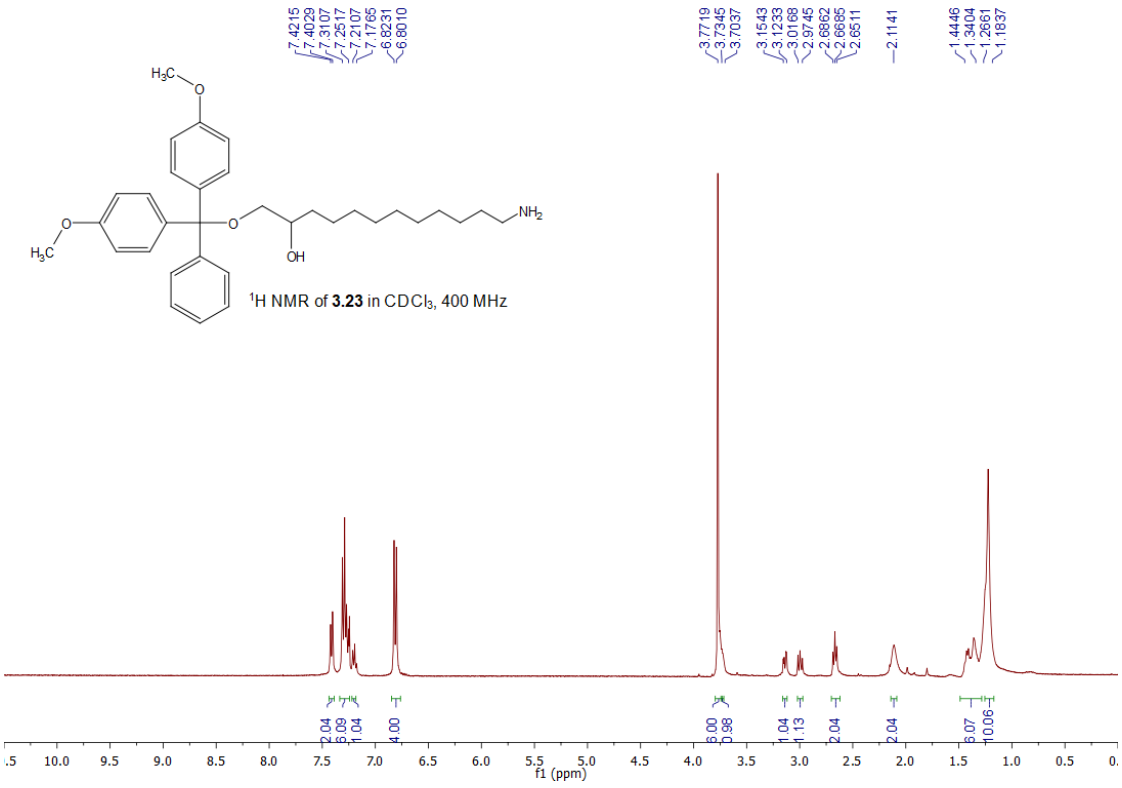


Figure B.22. [1]HNMR of Compound **3.23**

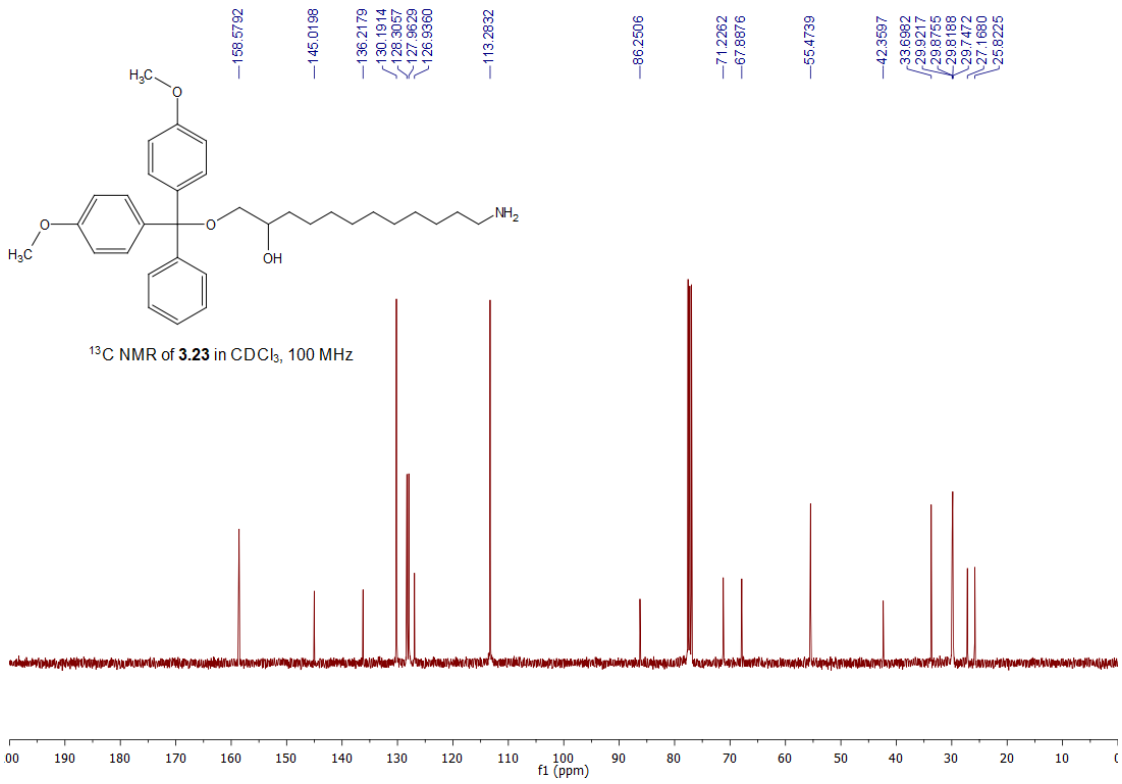


Figure B.23. ^{13}CNMR of Compound **3.23**

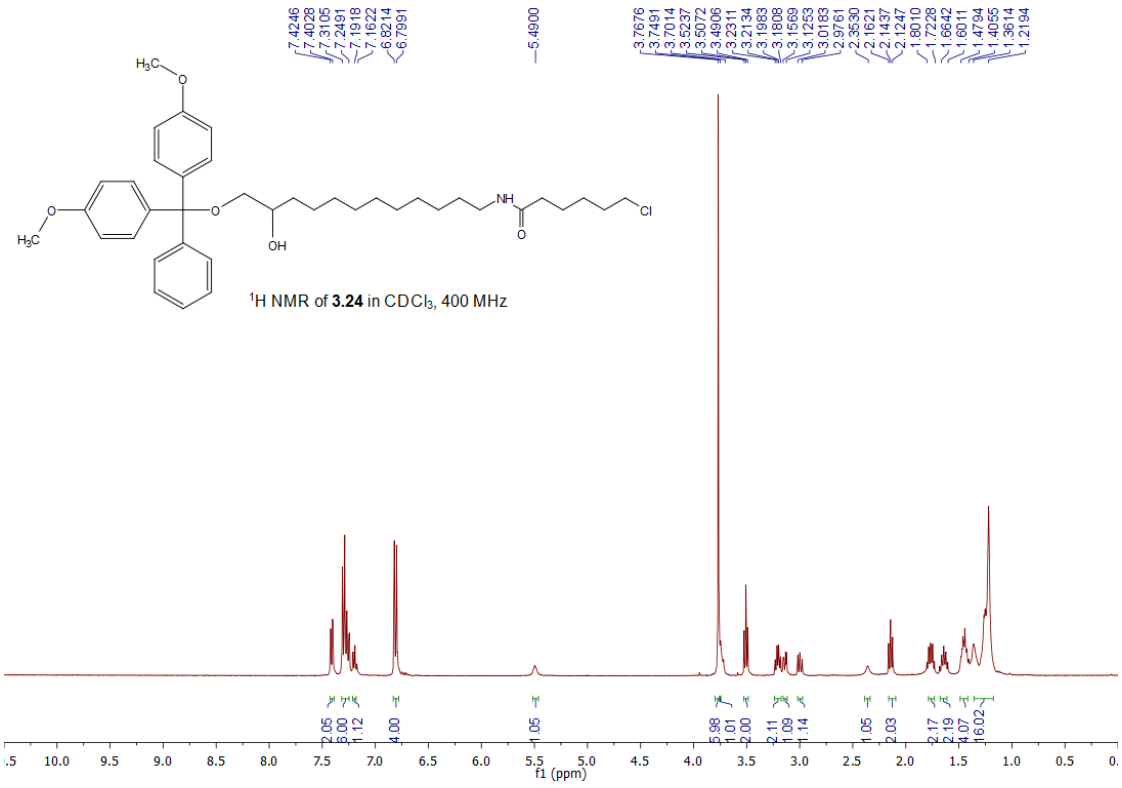


Figure B.24. [1]HNMR of Compound **3.24**

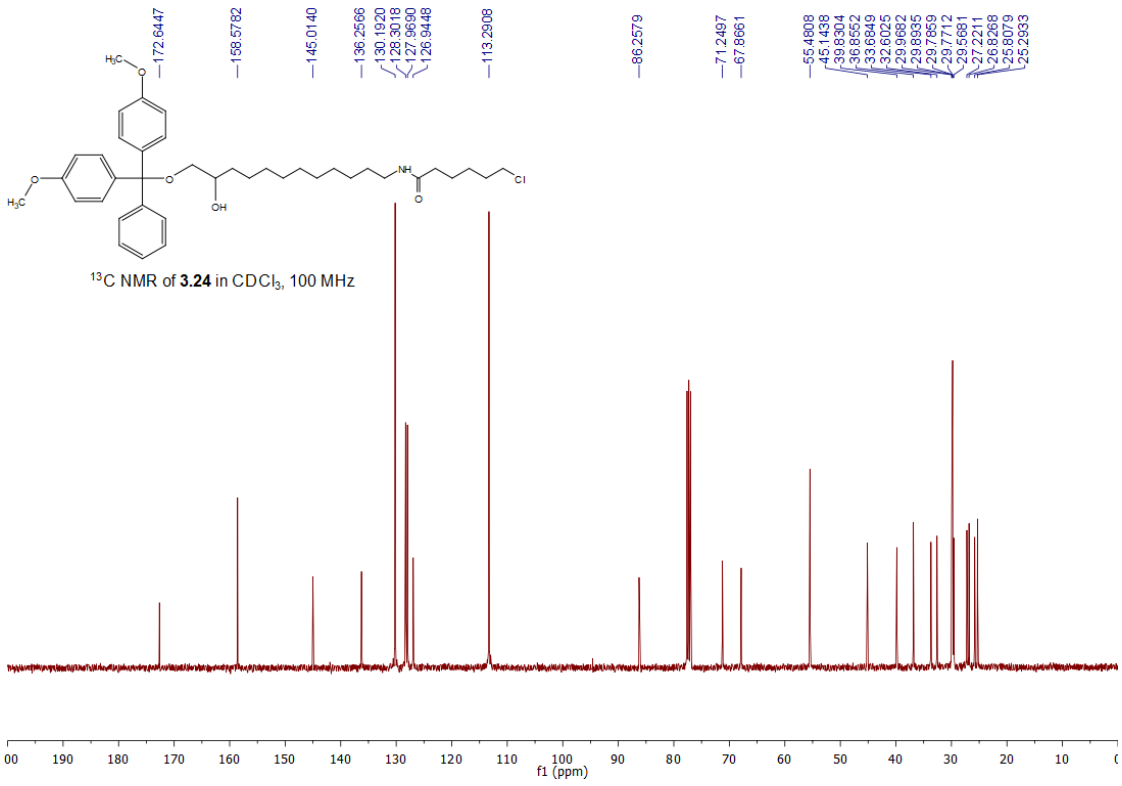
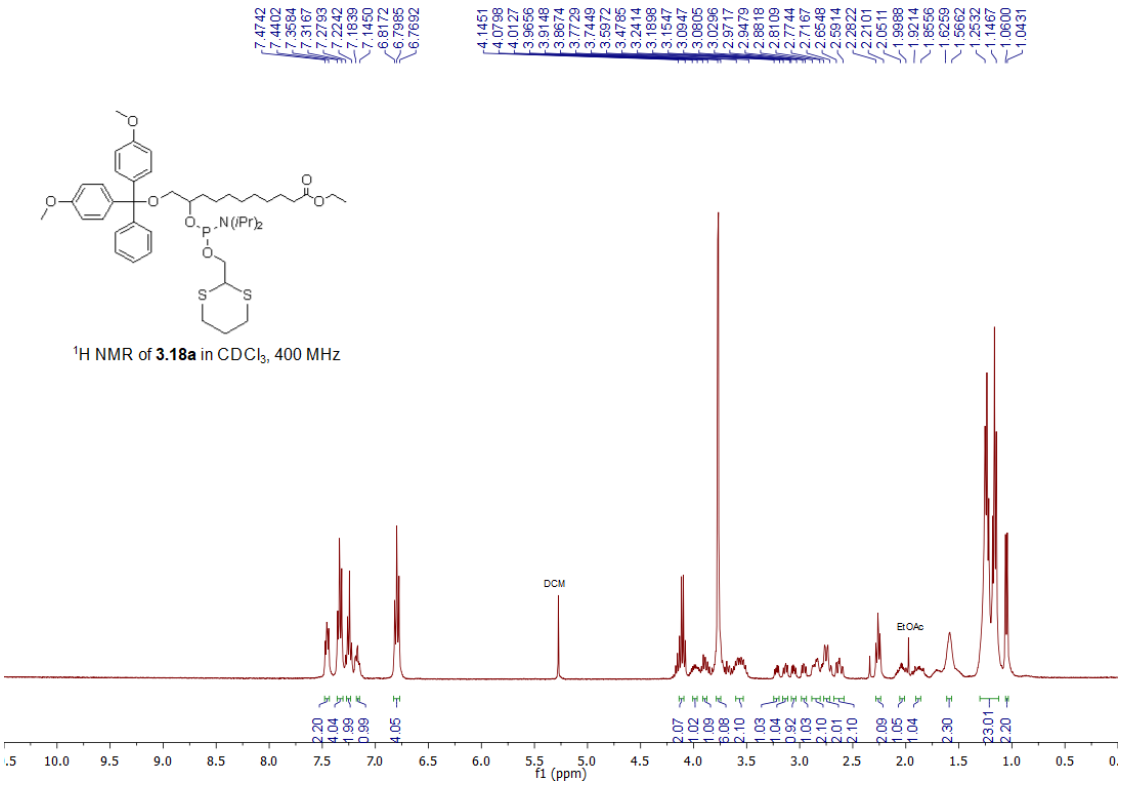


Figure B.25. ^{13}CNMR of Compound **3.24**



^{1}H NMR of **3.18a** in $CDCl_3$, 400 MHz

Figure B.26. ^{1}HNMR of Compound **3.18a**

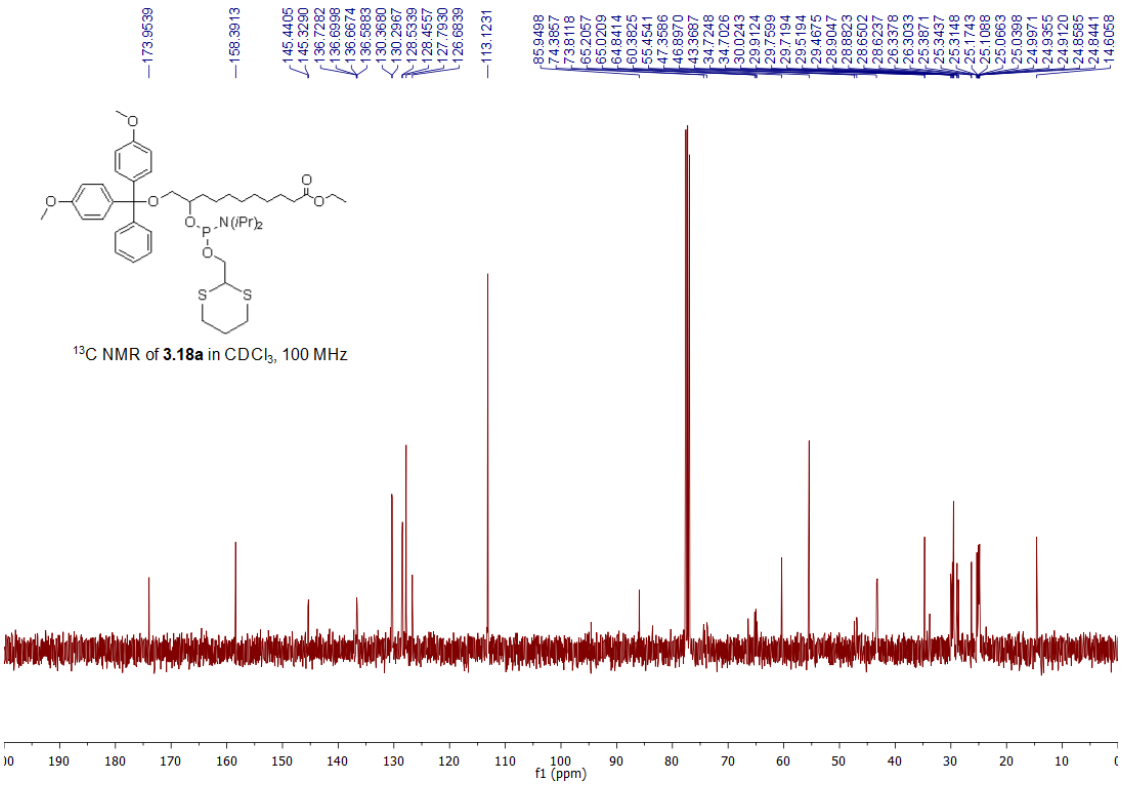


Figure B.27. ^{13}CNMR of Compound **3.18a**

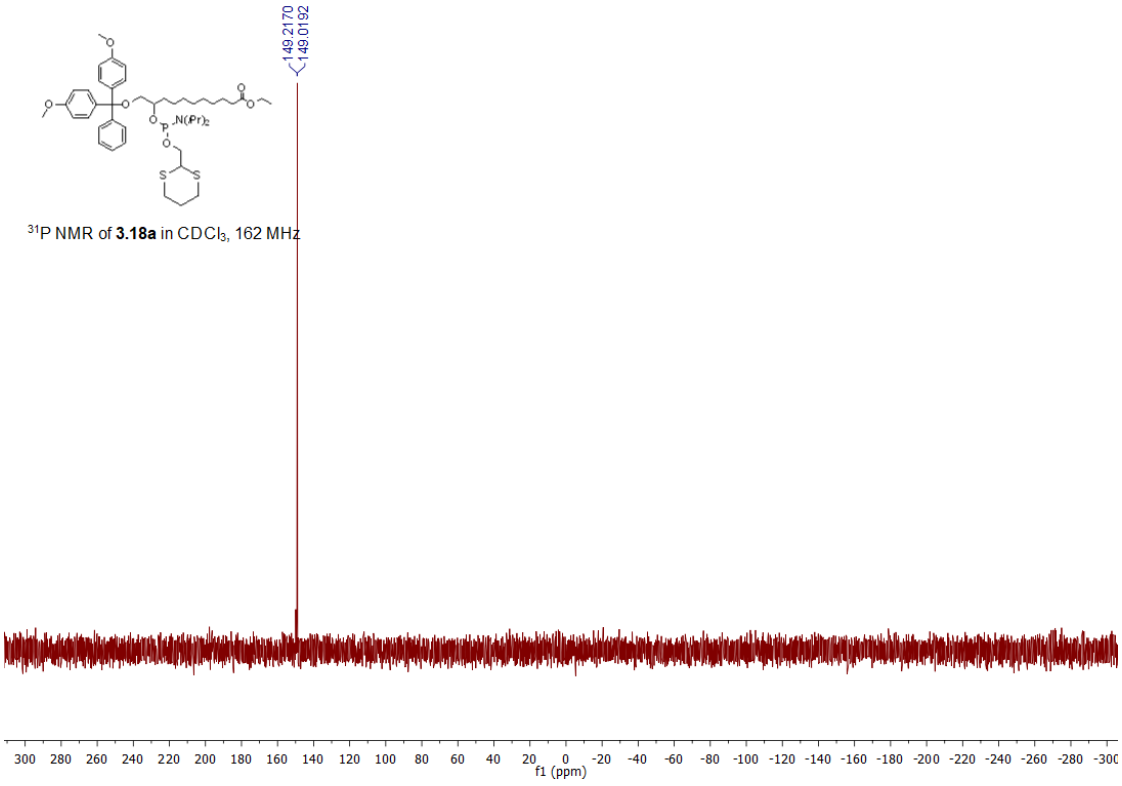


Figure B.28. ^{31}PNMR of Compound **3.18a**

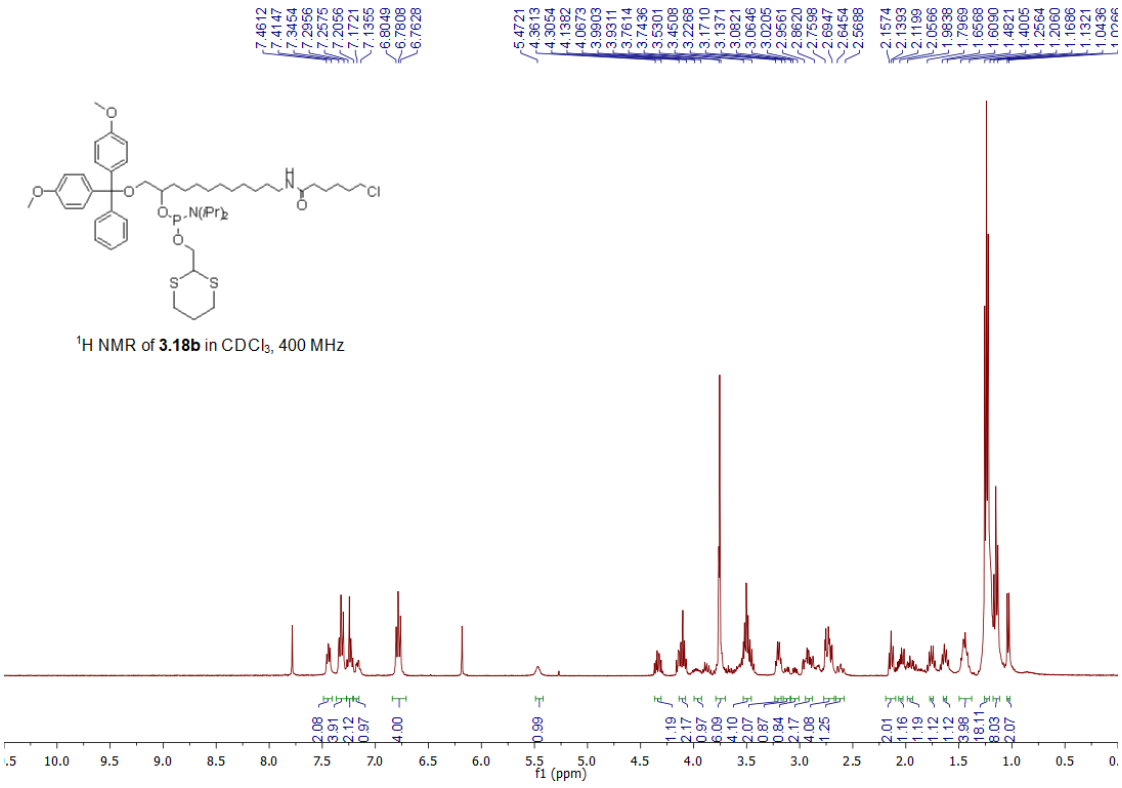


Figure B.29. ^{1}HNMR of Compound **3.18b**

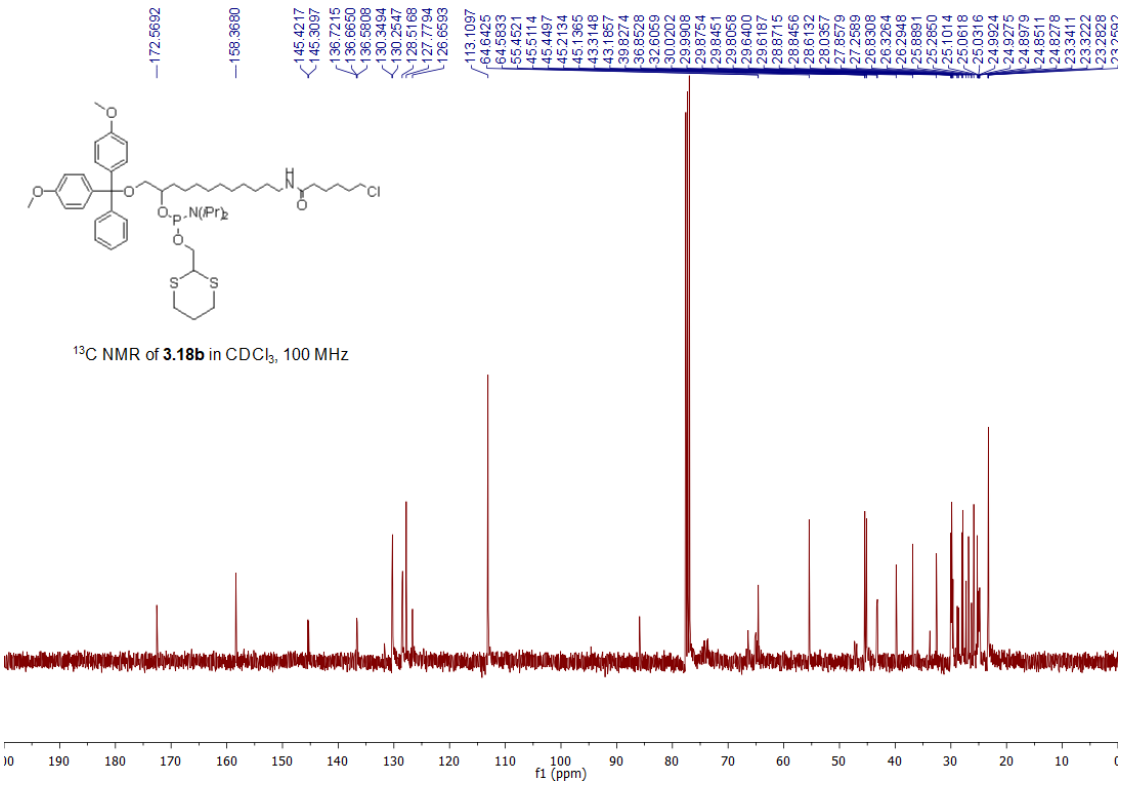


Figure B.30. ^{13}CNMR of Compound **3.18b**

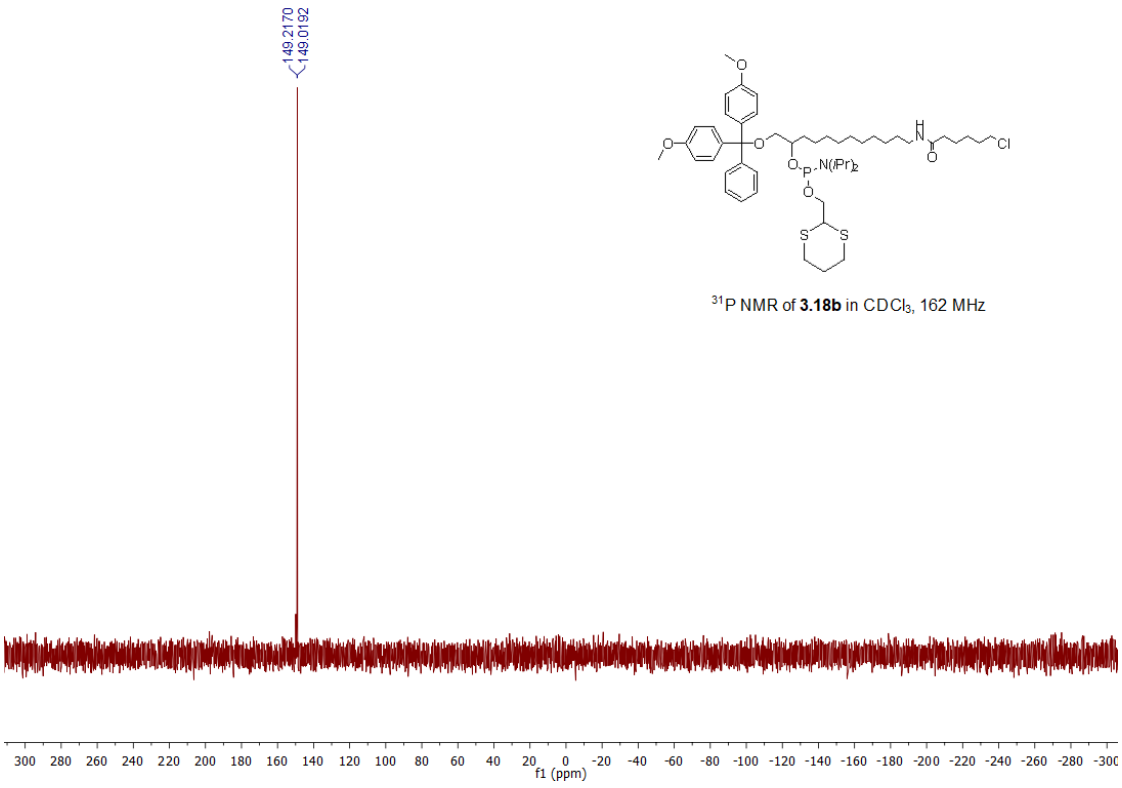


Figure B.31. ^{31}PNMR of Compound **3.18b**

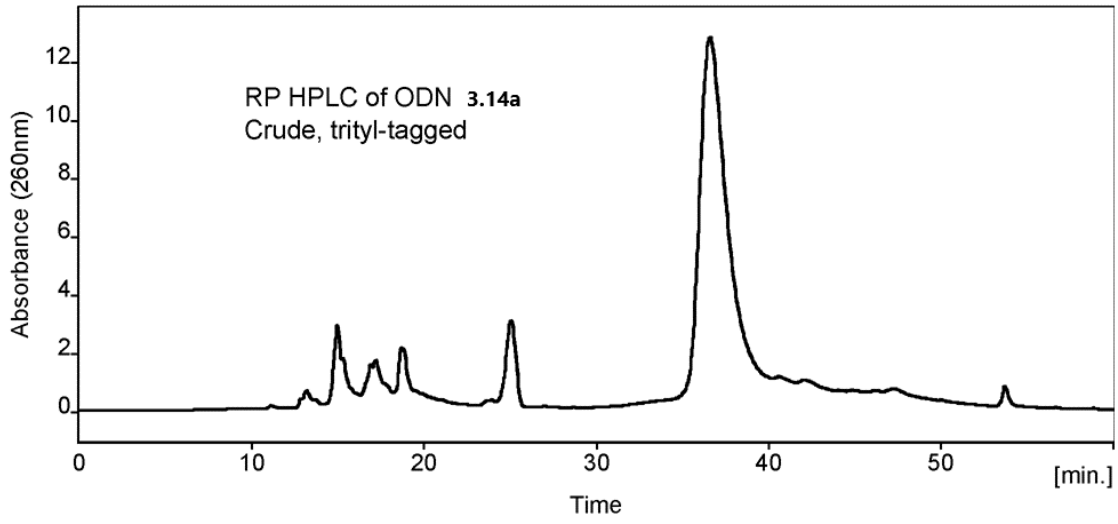


Figure B.32. RP HPLC of compound **3.14a**

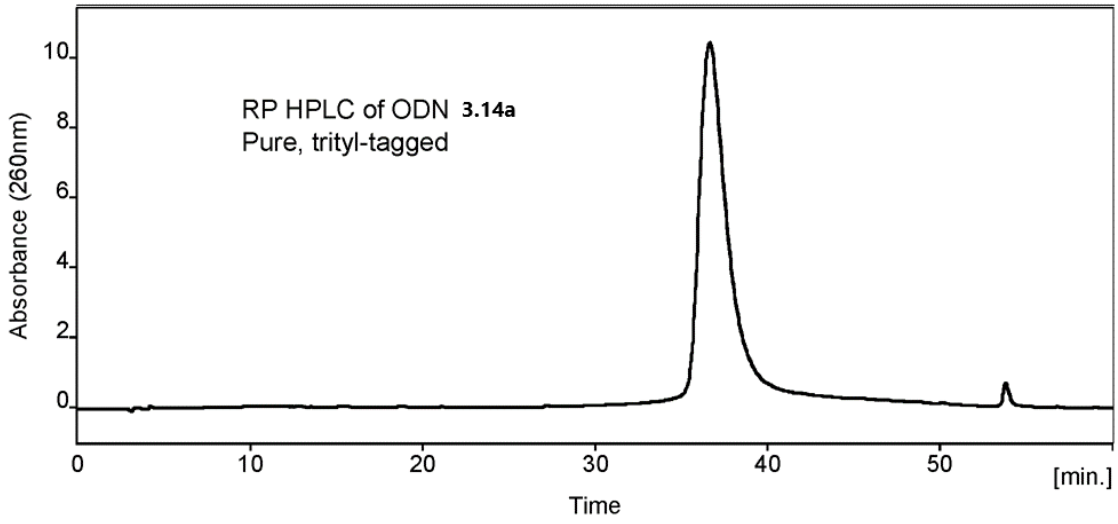


Figure B.33. RP HPLC of compound **3.14a**

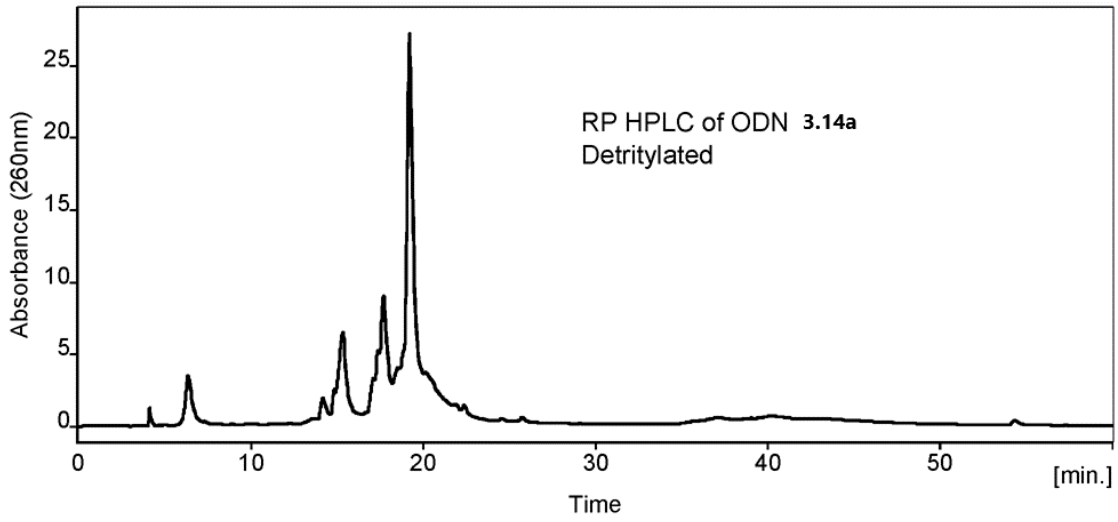


Figure B.34. RP HPLC of compound **3.14a**

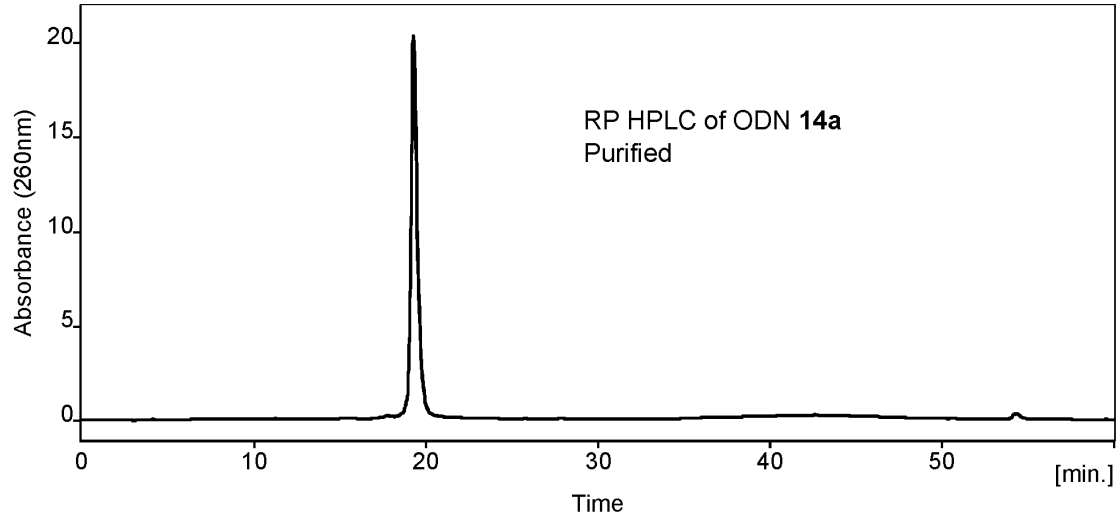


Figure B.35. RP HPLC of compound **3.14a**

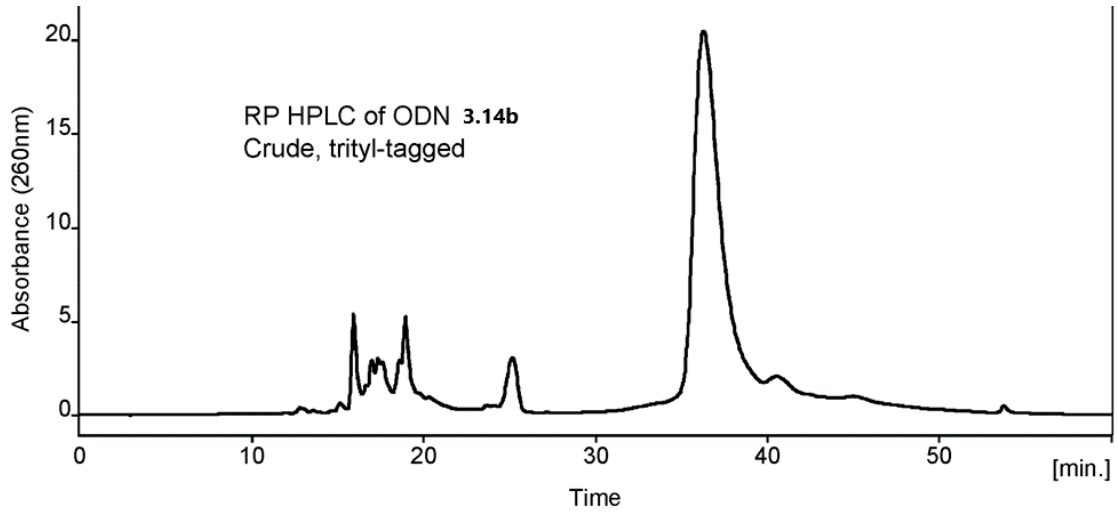


Figure B.36. RP HPLC of compound **3.14b**

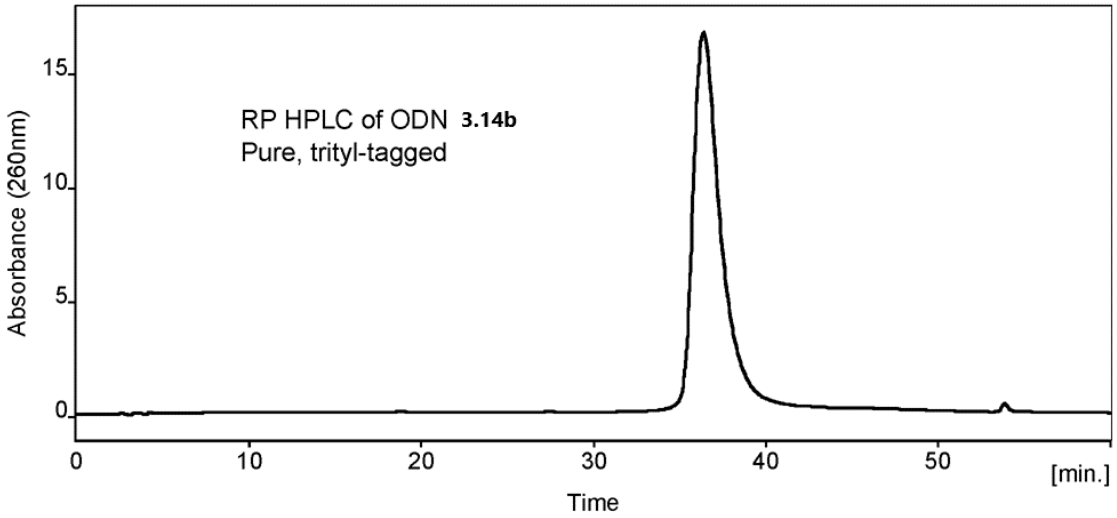


Figure B.37. RP HPLC of compound **3.14b**

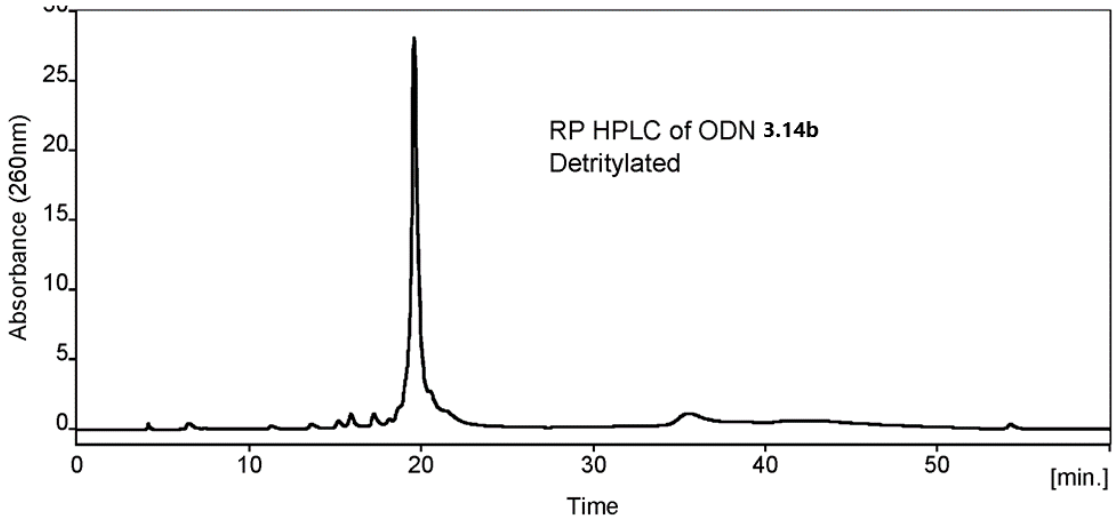


Figure B.38. RP HPLC of compound **3.14b**

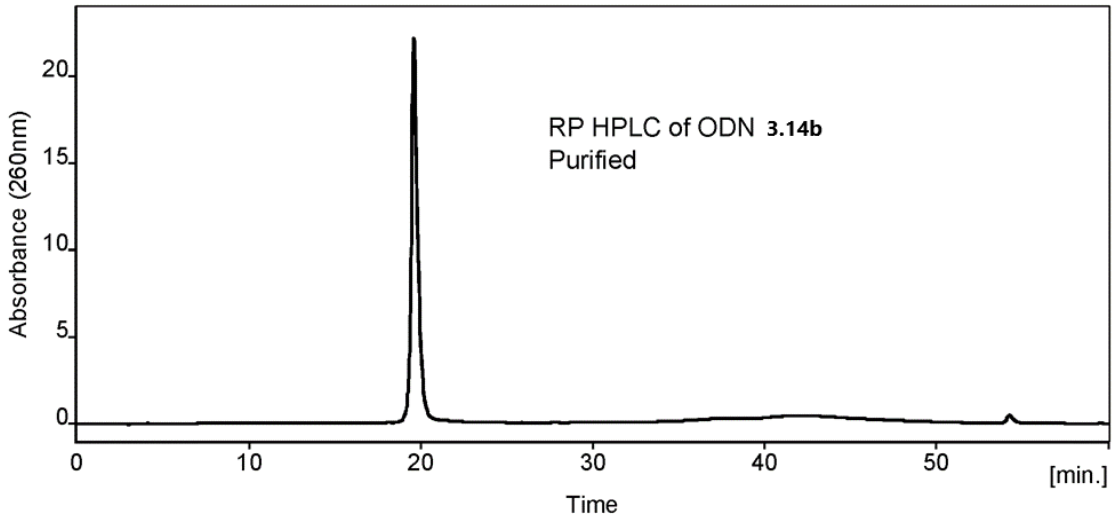


Figure B.39. RP HPLC of compound **3.14b**

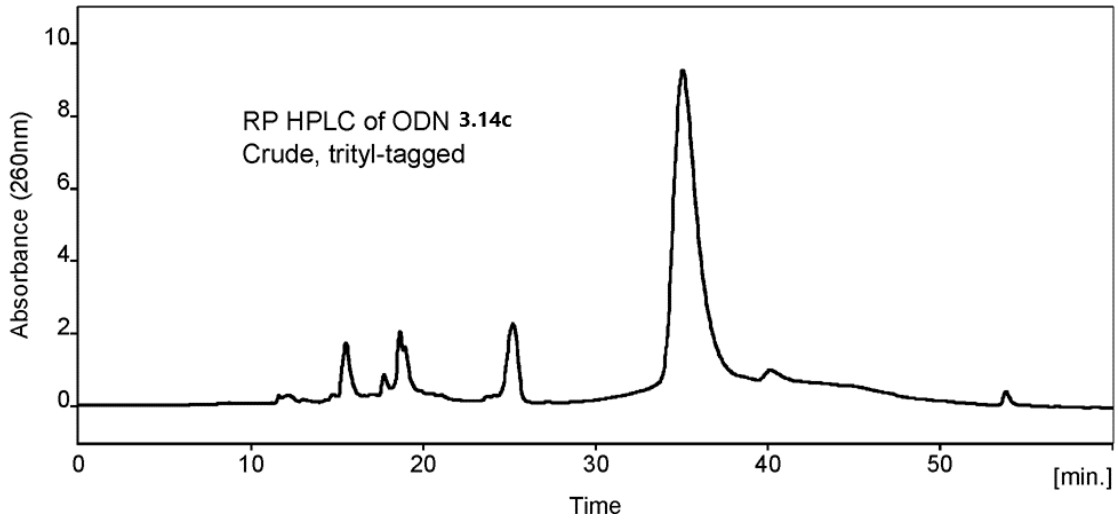


Figure B.40. RP HPLC of compound **3.14c**

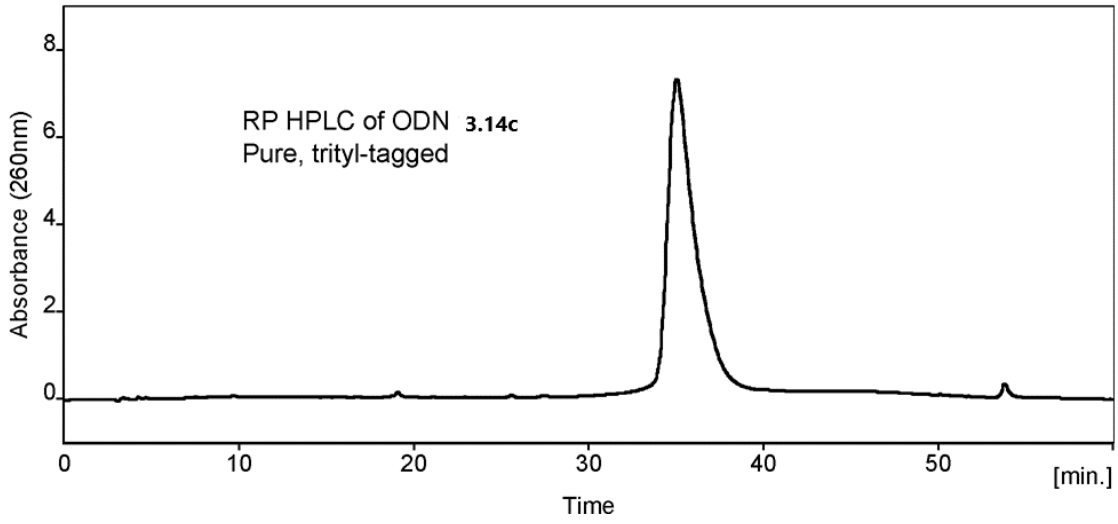


Figure B.41. RP HPLC of compound **3.14c**

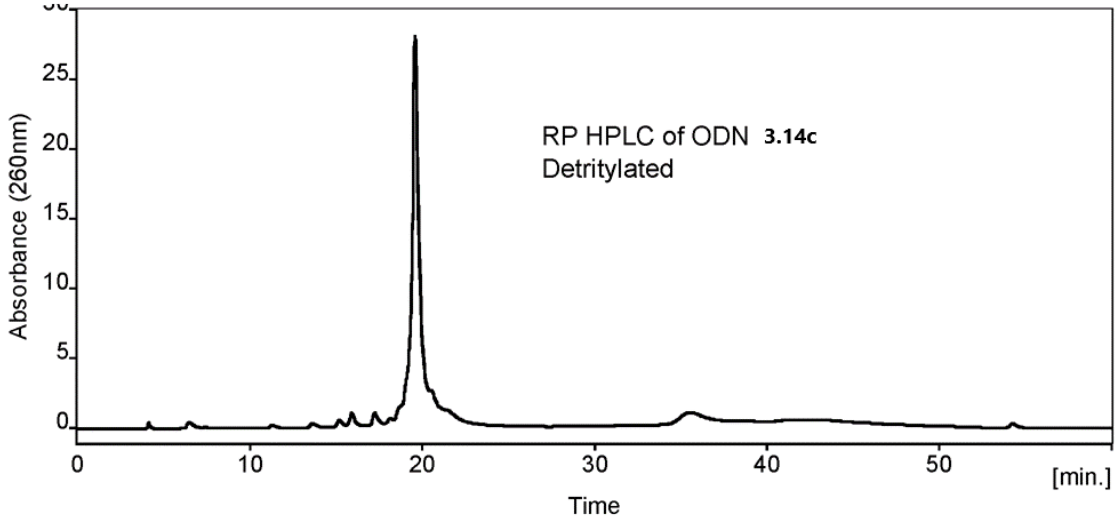


Figure B.42. RP HPLC of compound **3.14c**

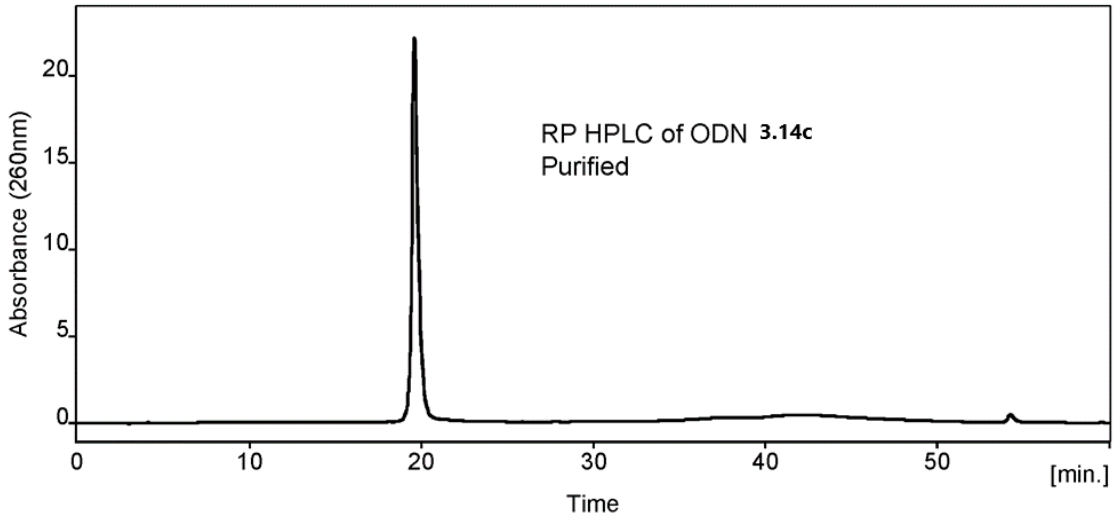


Figure B.43. RP HPLC of compound **3.14c**

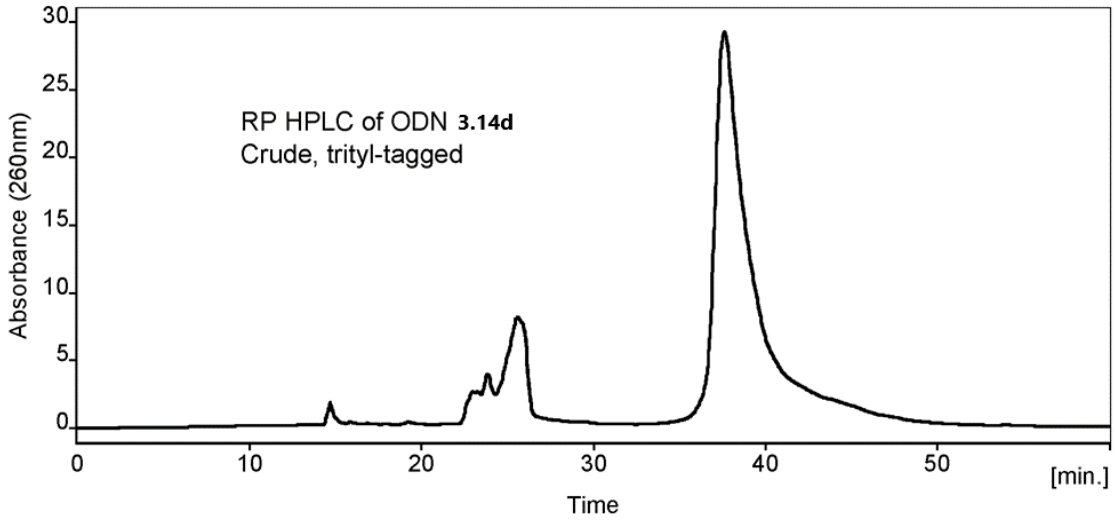


Figure B.44. RP HPLC of compound **3.14d**

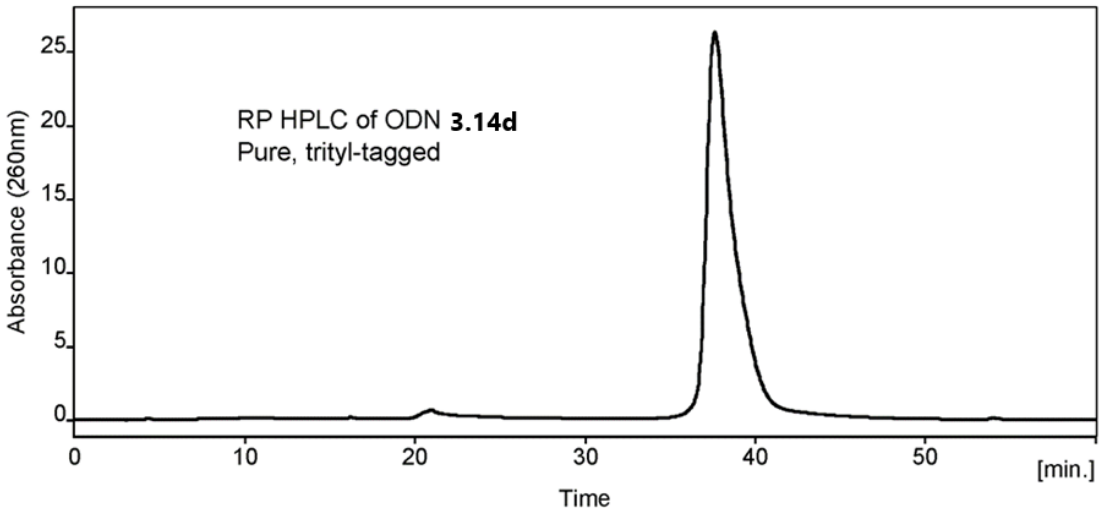


Figure B.45. RP HPLC of compound **3.14d**

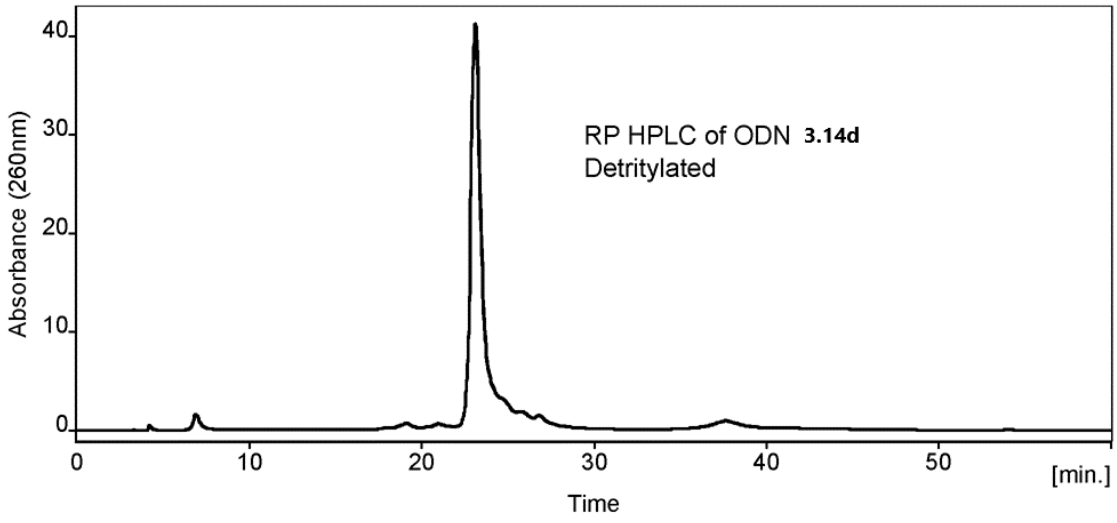


Figure B.46. RP HPLC of compound **3.14d**

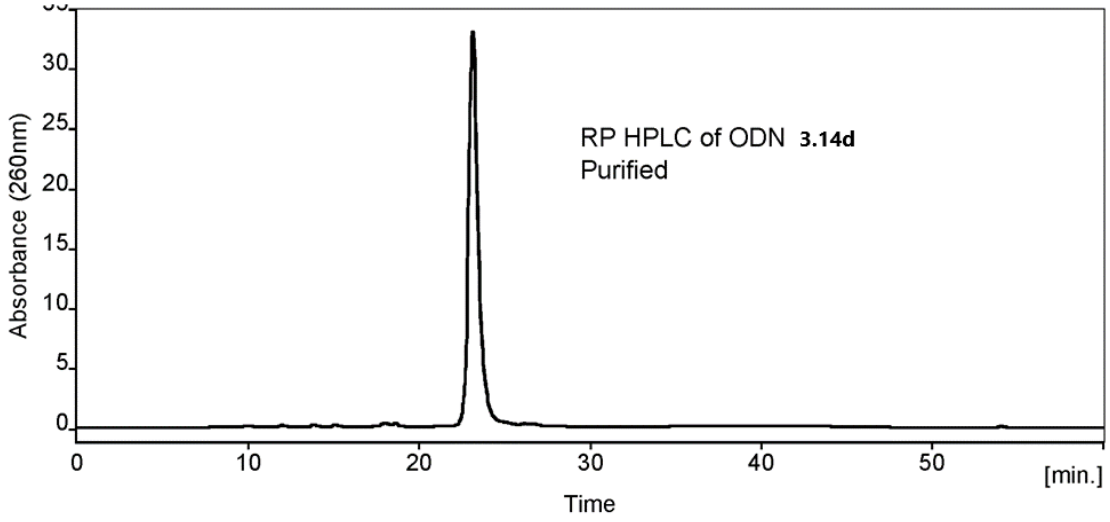


Figure B.47. RP HPLC of compound **3.14d**

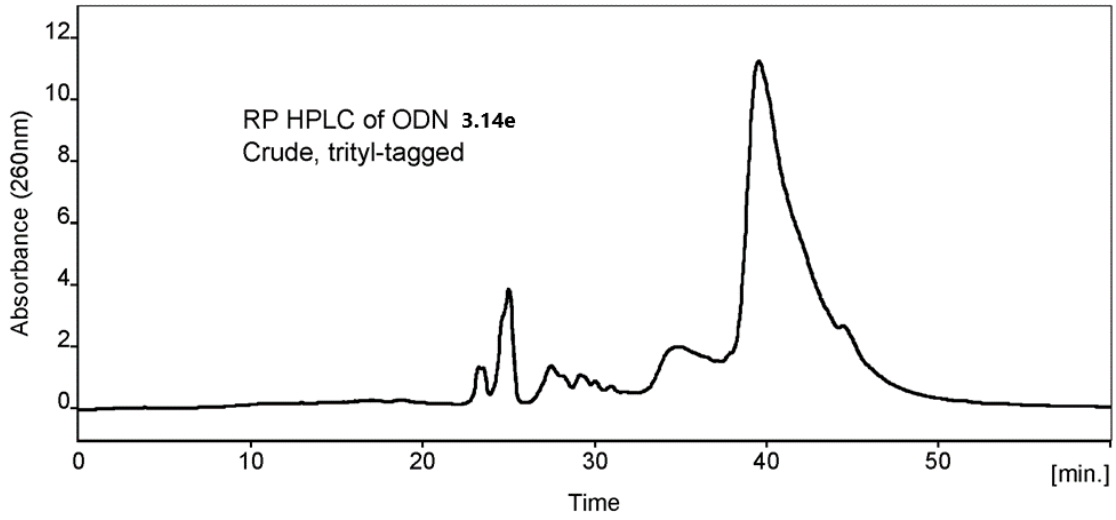


Figure B.48. RP HPLC of compound **3.14e**

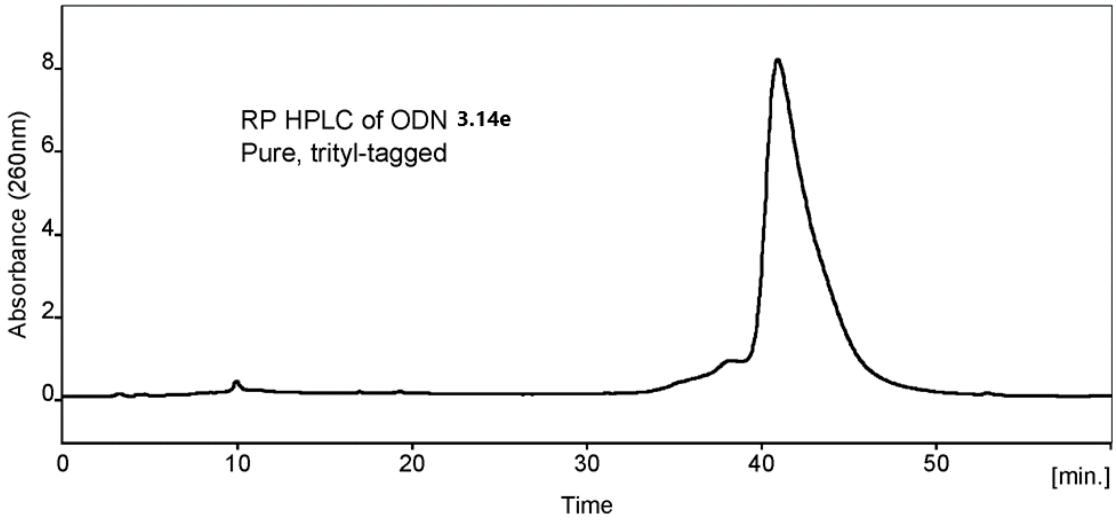


Figure B.49. RP HPLC of compound **3.14e**

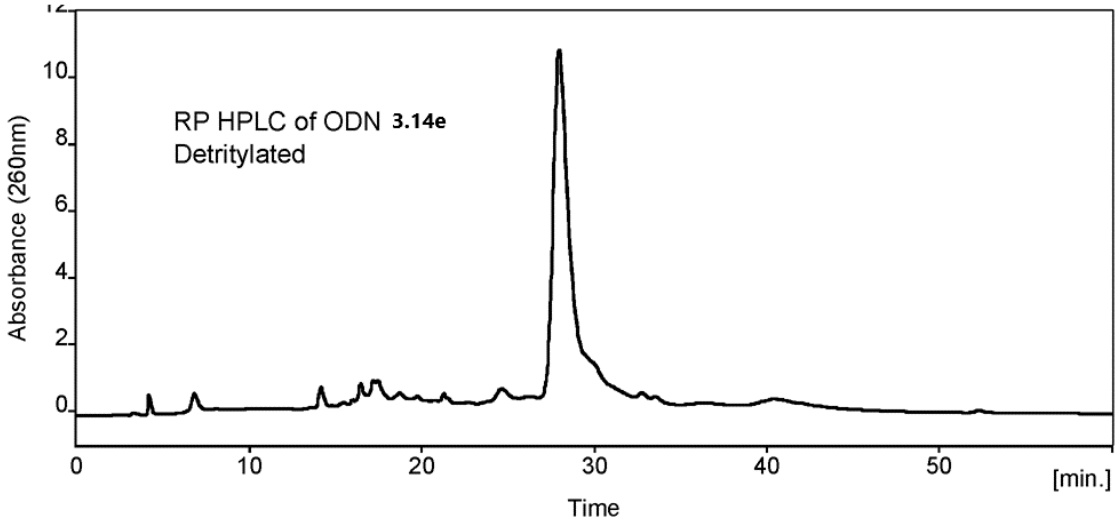


Figure B.50. RP HPLC of compound **3.14e**

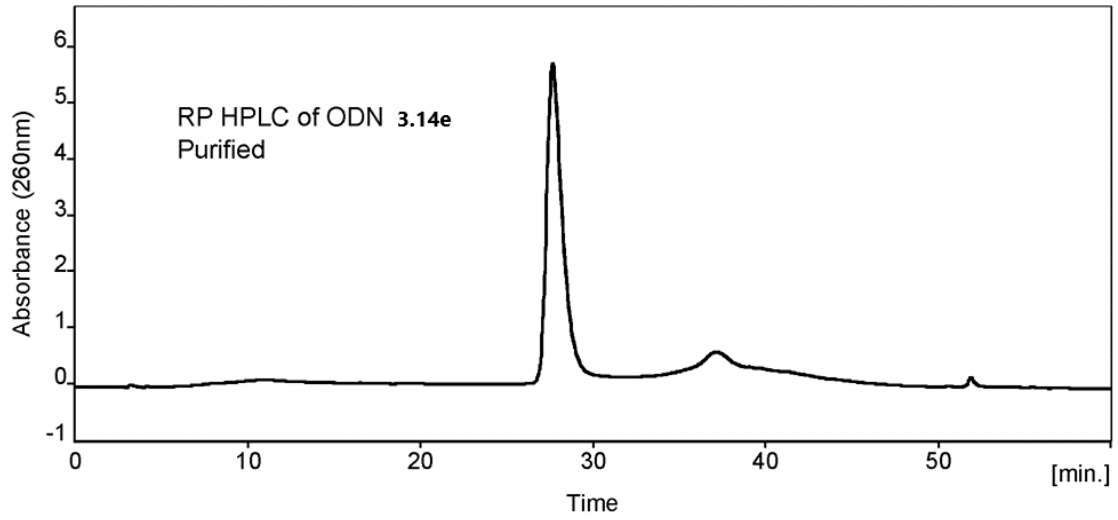


Figure B.51. RP HPLC of compound **3.14e**

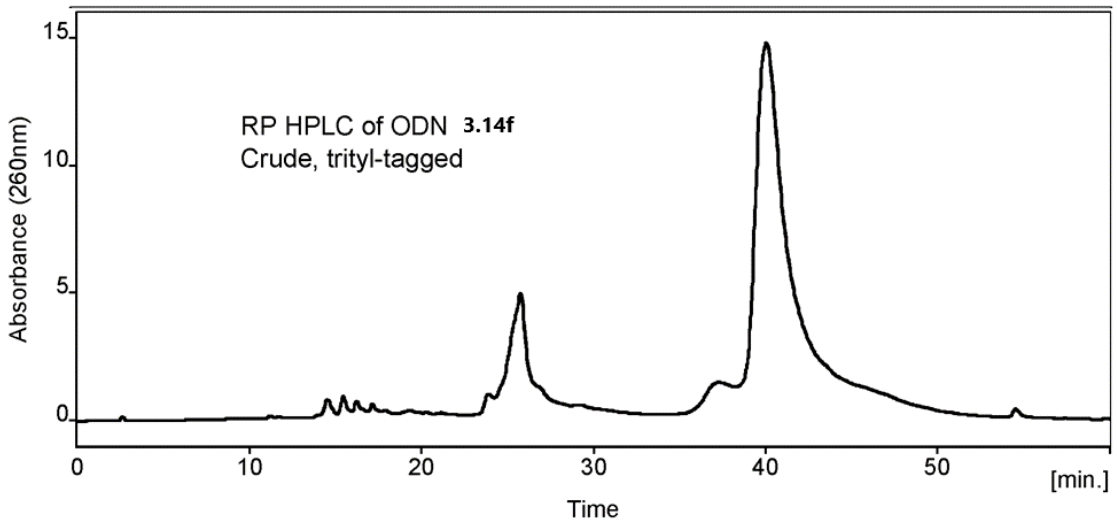


Figure B.52. RP HPLC of compound **3.14f**

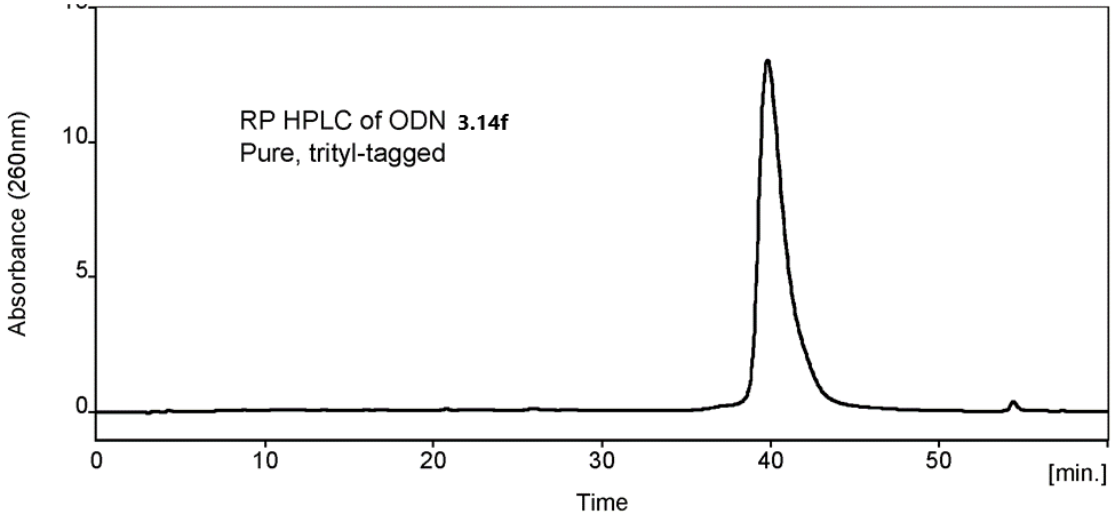


Figure B.53. RP HPLC of compound **3.14e**

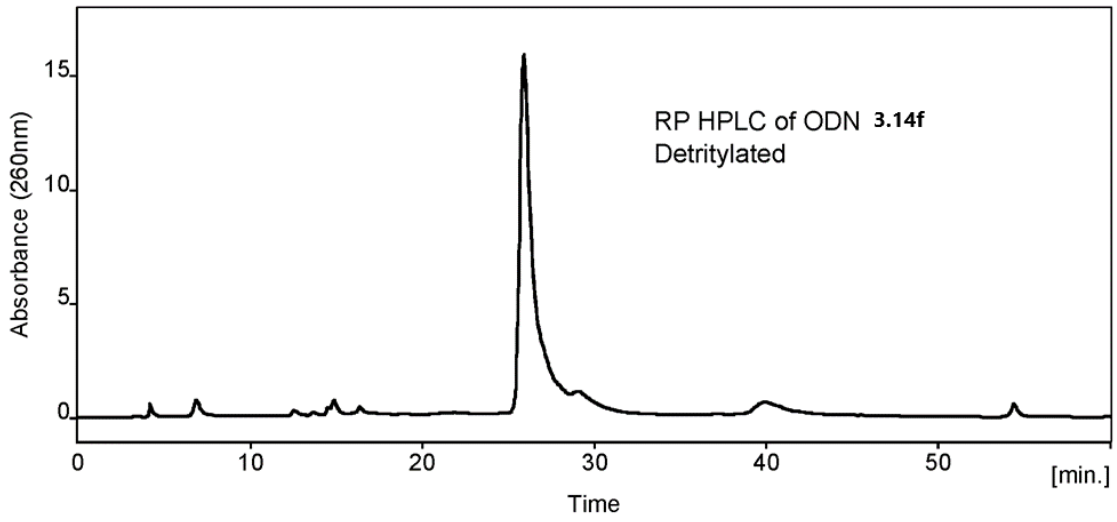


Figure B.54. RP HPLC of compound **3.14f**

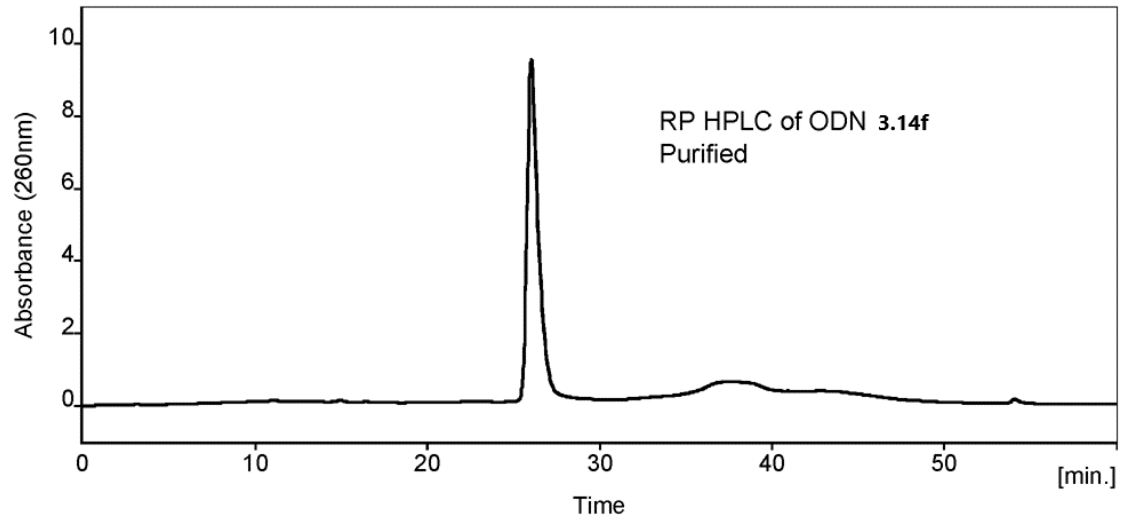


Figure B.55. RP HPLC of compound **3.14f**

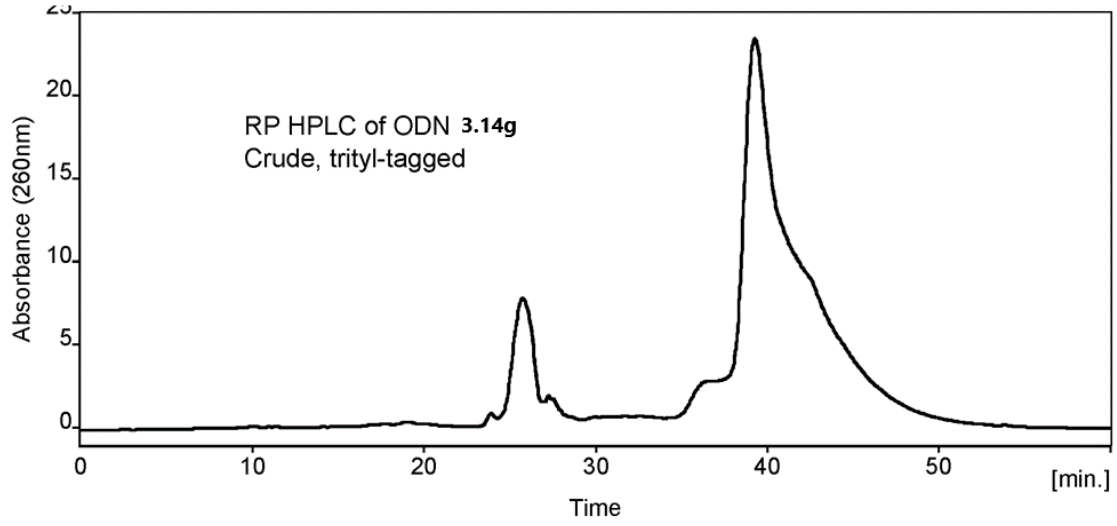


Figure B.56. RP HPLC of compound **3.14g**

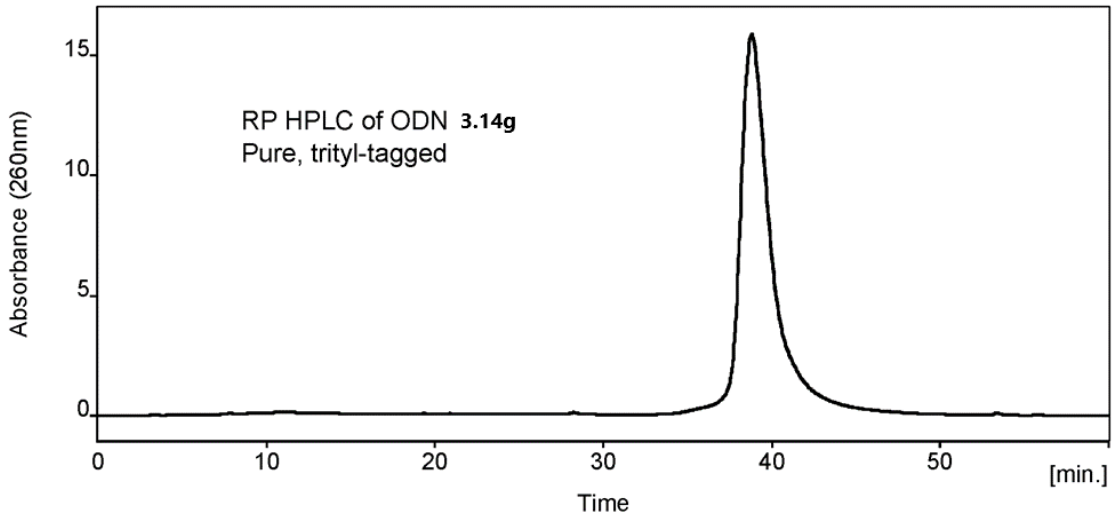


Figure B.57. RP HPLC of compound **3.14g**

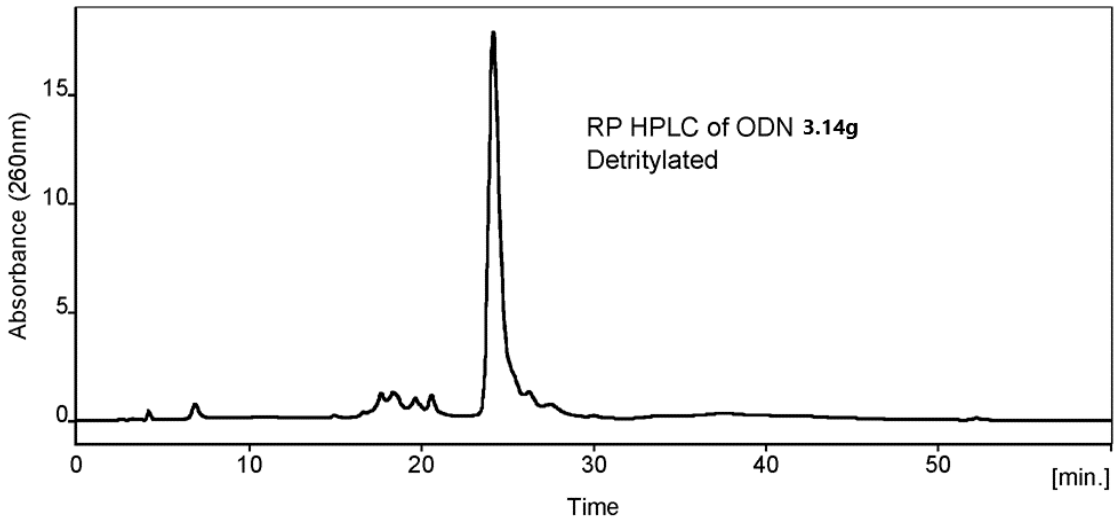


Figure B.58. RP HPLC of compound **3.14g**

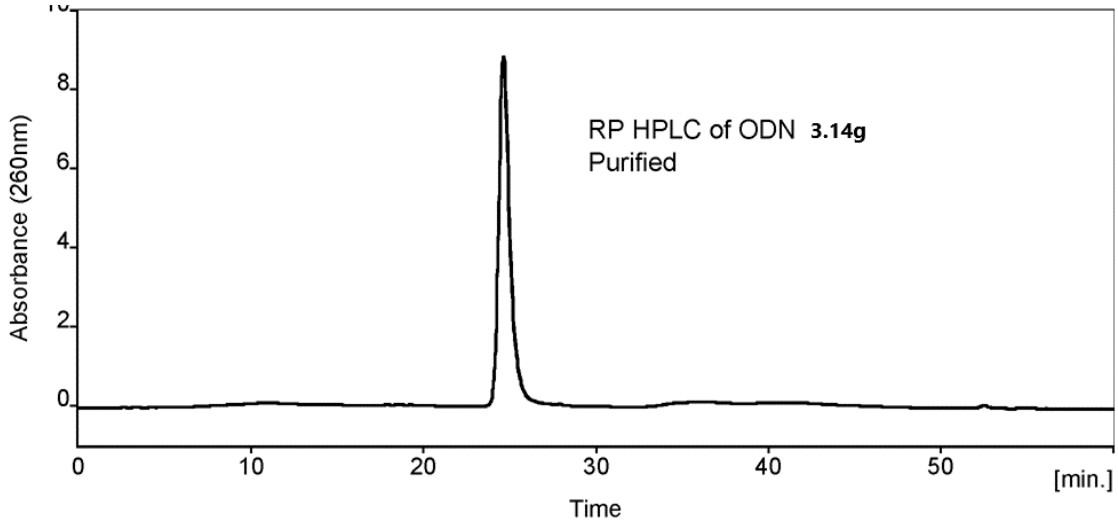


Figure B.59. RP HPLC of compound **3.14g**

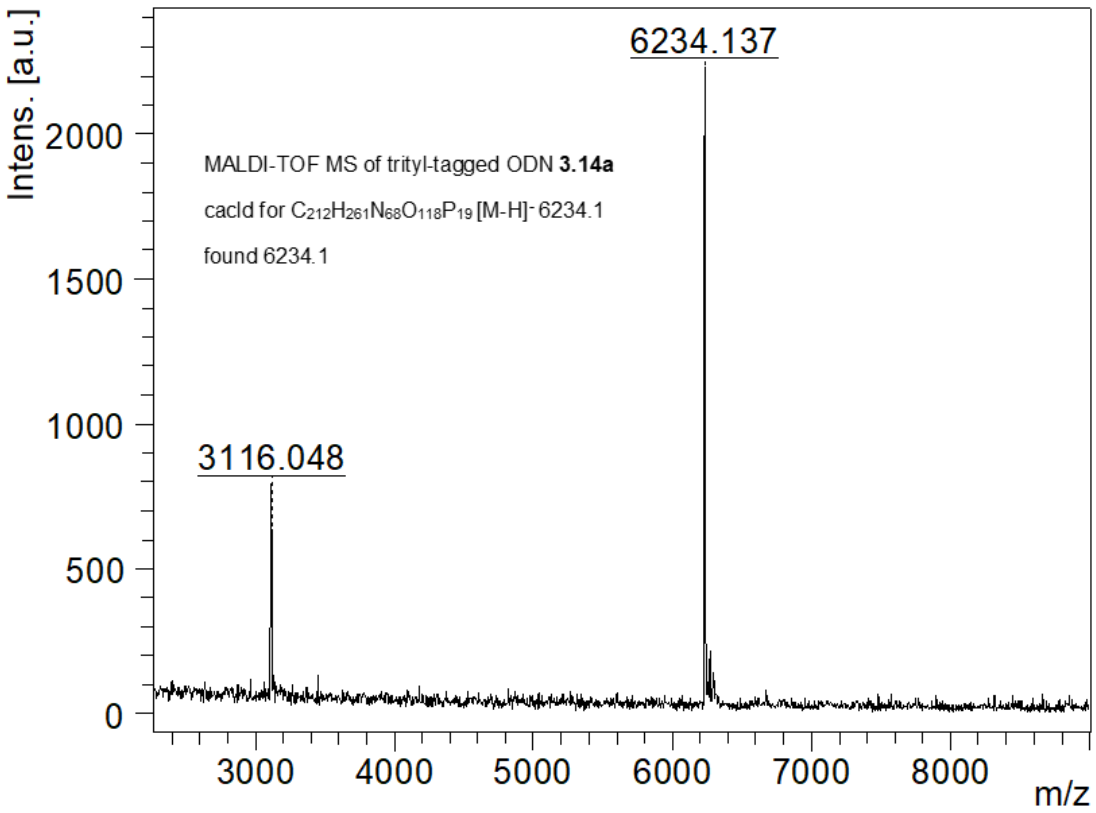


Figure B.60. MALDI-TOF-MS of compound **3.14a**

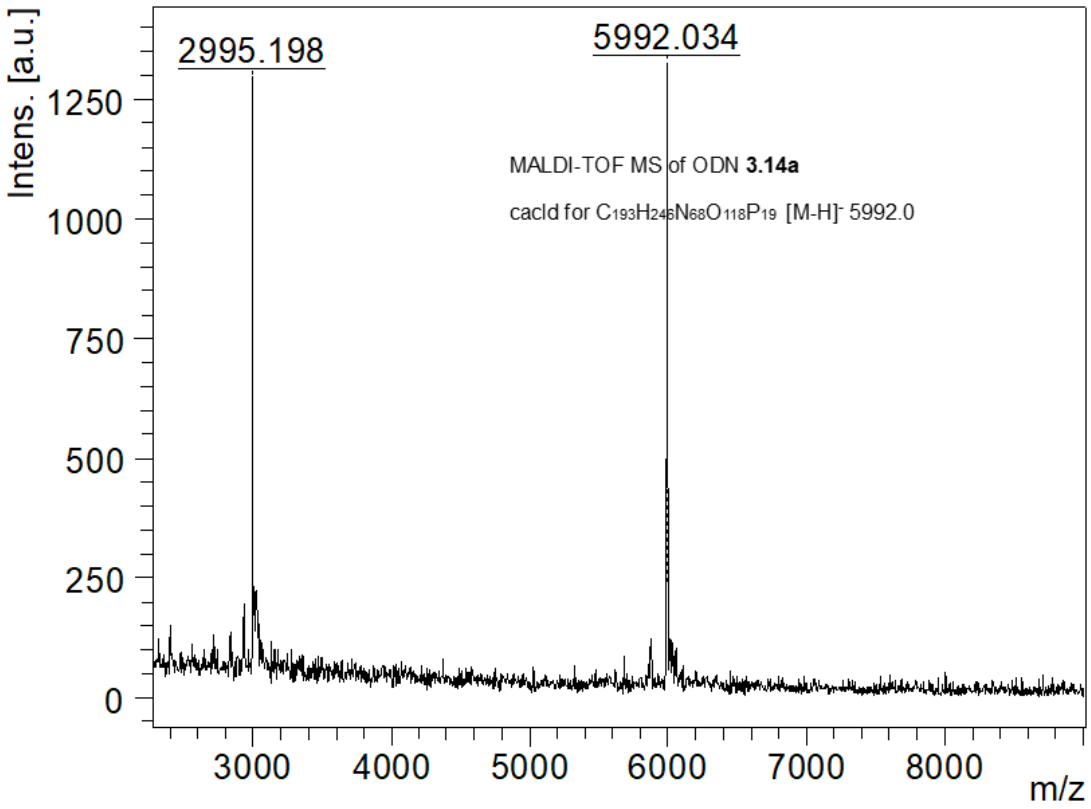


Figure B.61. MALDI-TOF-MS of compound **3.14a**

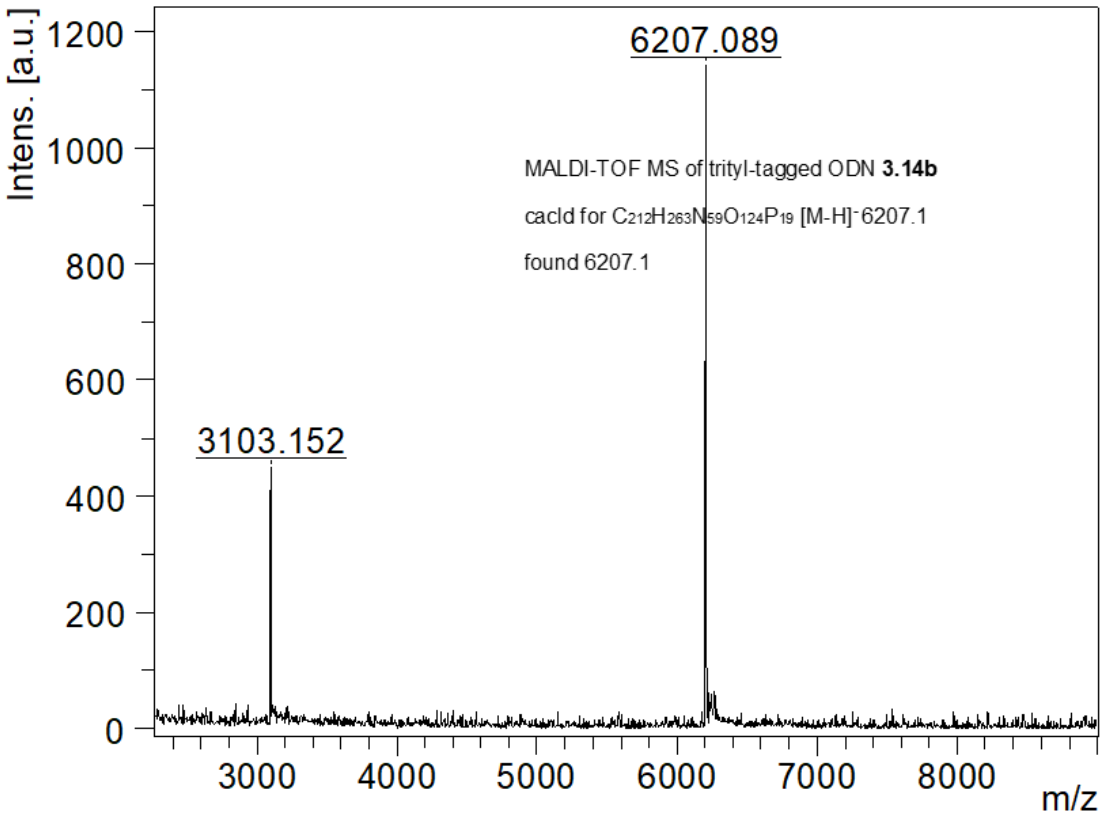


Figure B.62. MALDI-TOF-MS of compound **3.14b**

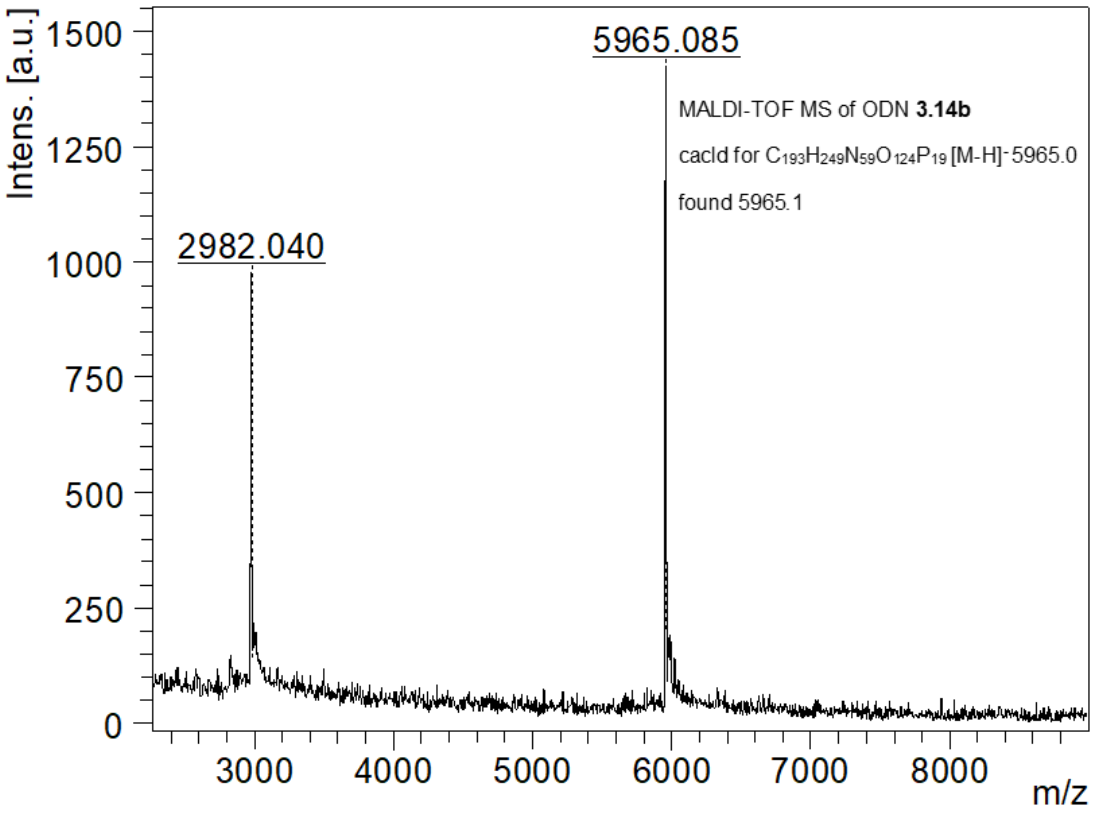


Figure B.63. MALDI-TOF-MS of compound **3.14b**

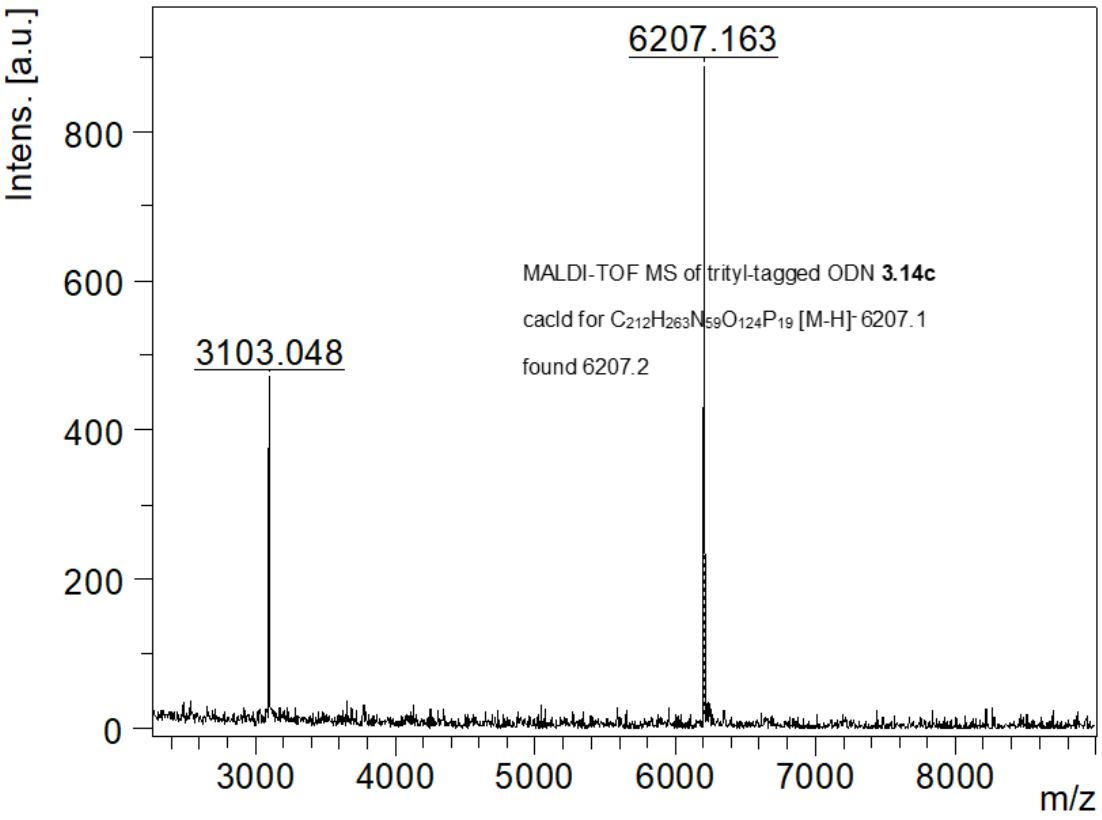


Figure B.64. MALDI-TOF-MS of compound **3.14c**

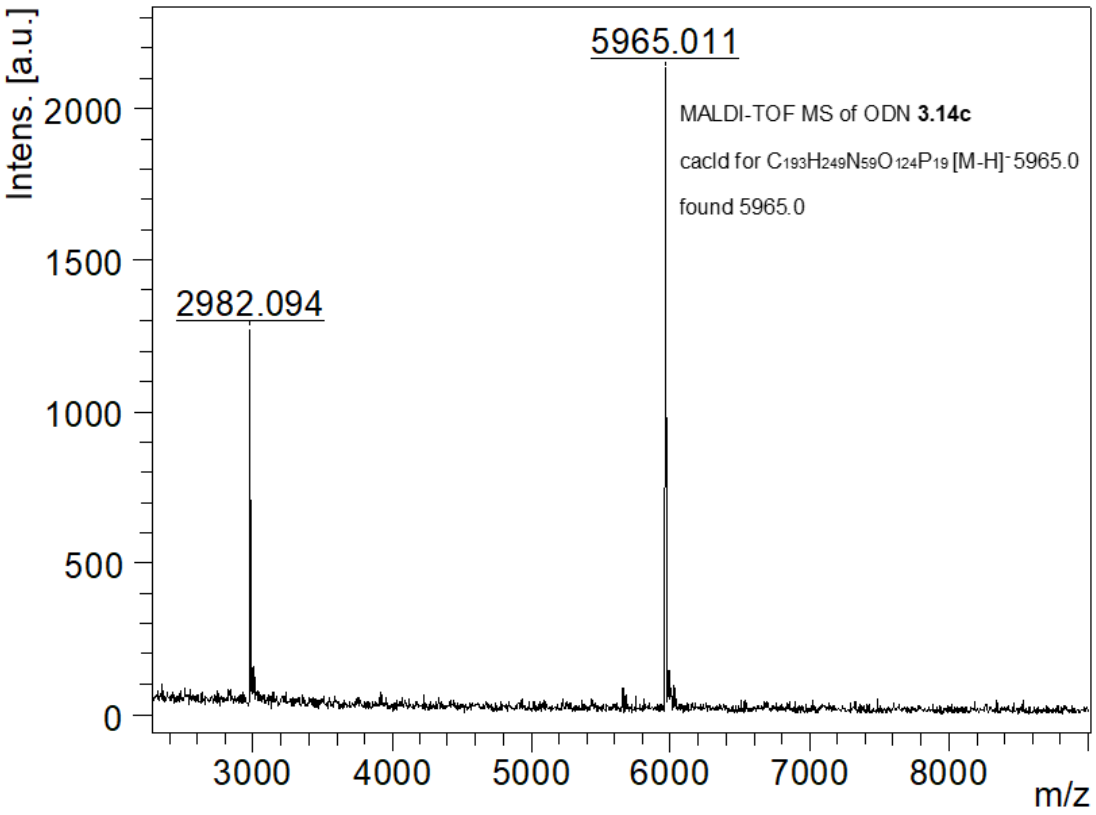


Figure B.65. MALDI-TOF-MS of compound **3.14c**

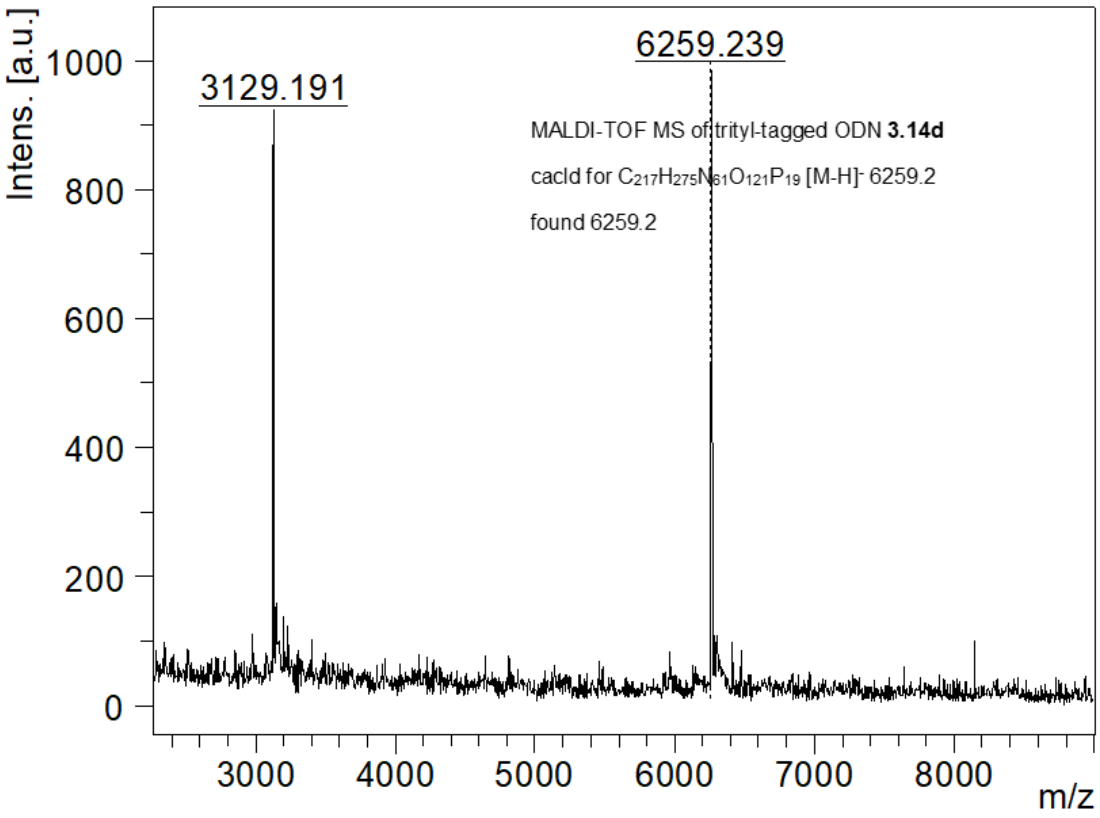


Figure B.66. MALDI-TOF-MS of compound **3.14d**

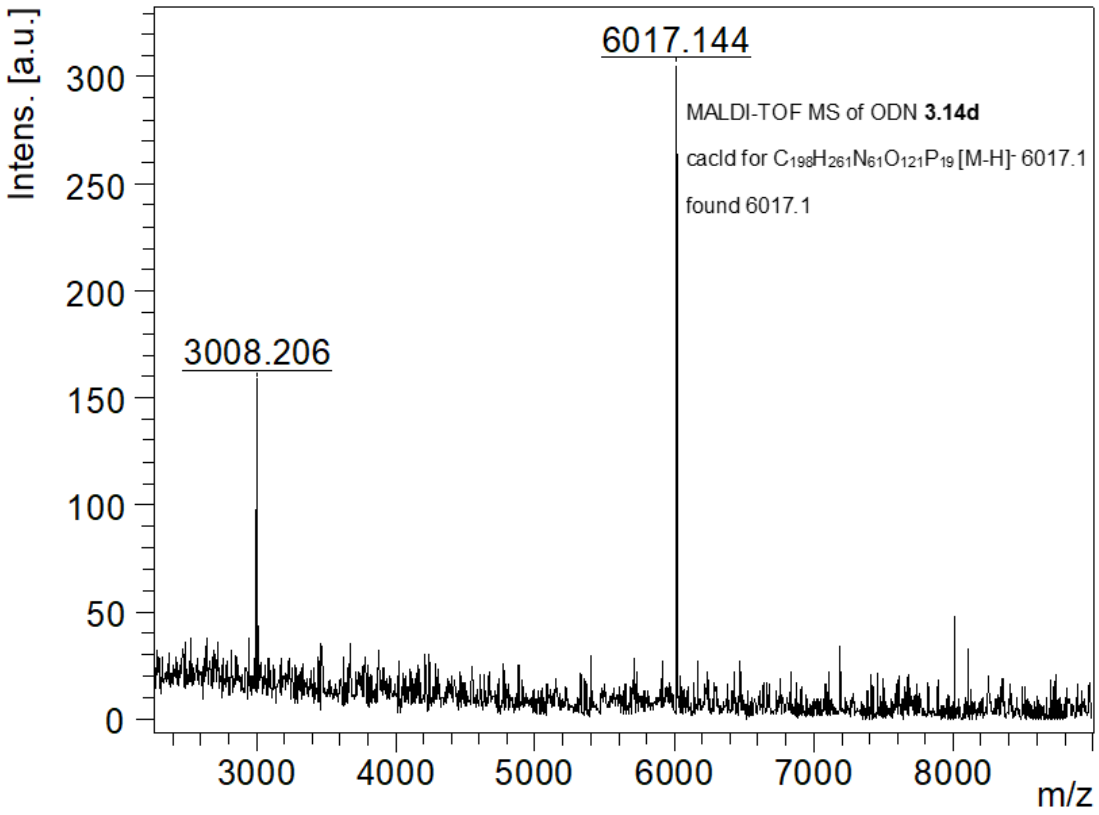


Figure B.67. MALDI-TOF-MS of compound **3.14d**

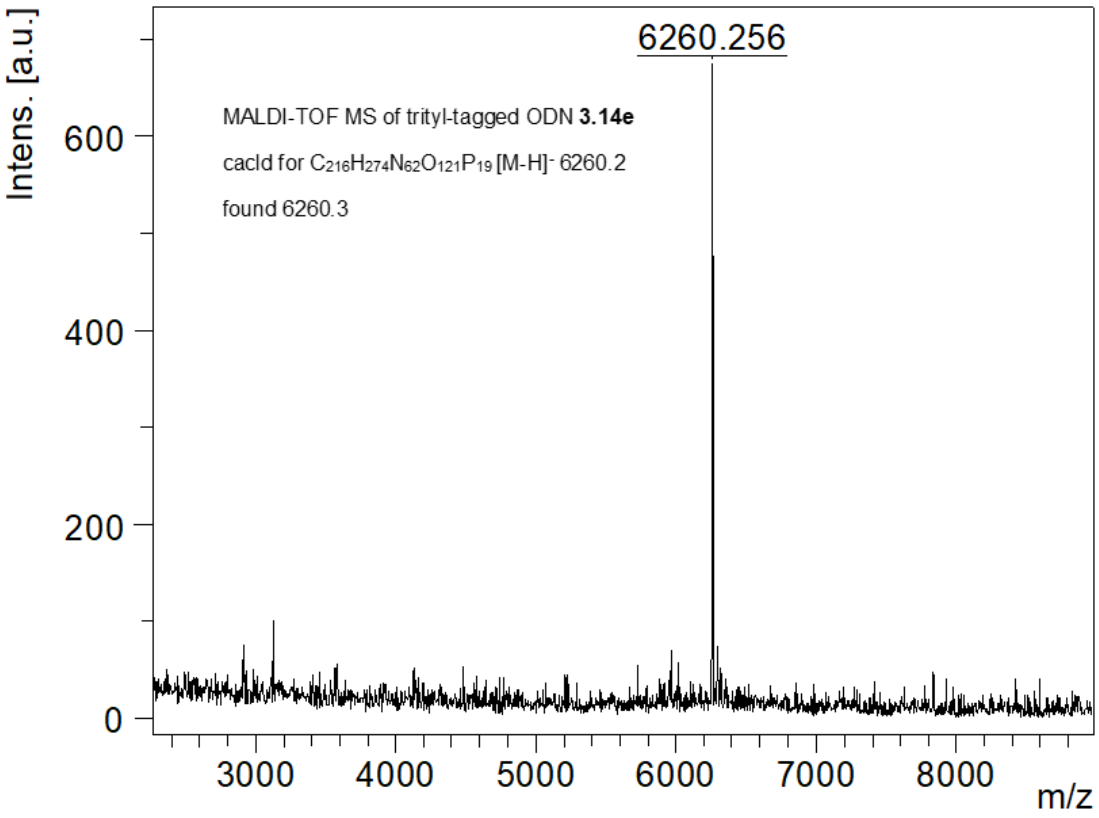


Figure B.68. MALDI-TOF-MS of compound **3.14e**

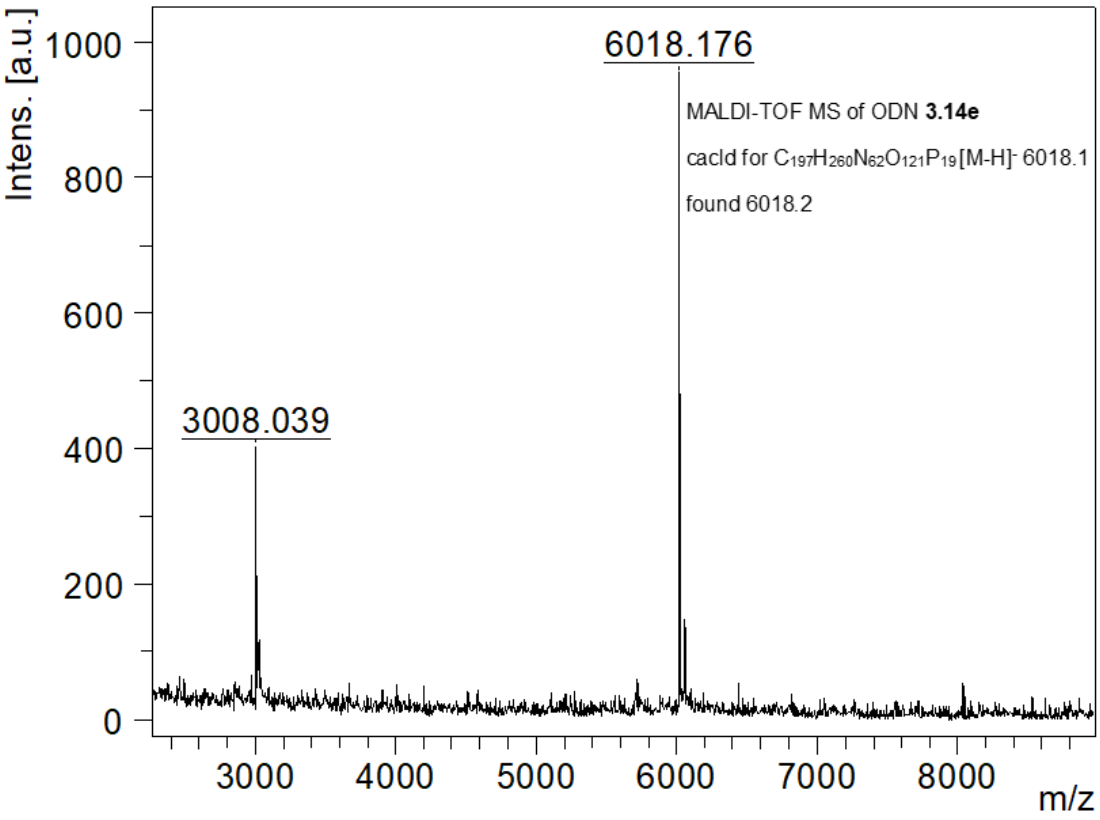


Figure B.69. MALDI-TOF-MS of compound **3.14e**

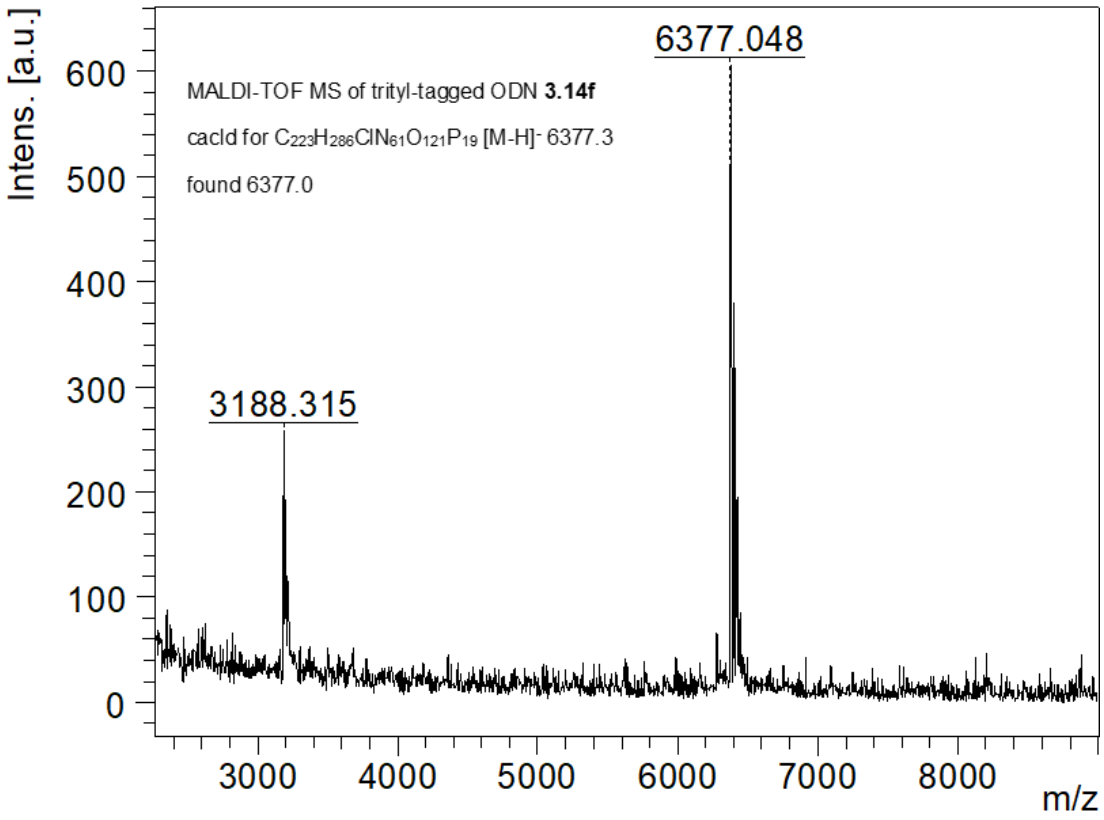


Figure B.70. MALDI-TOF-MS of compound **3.14f**

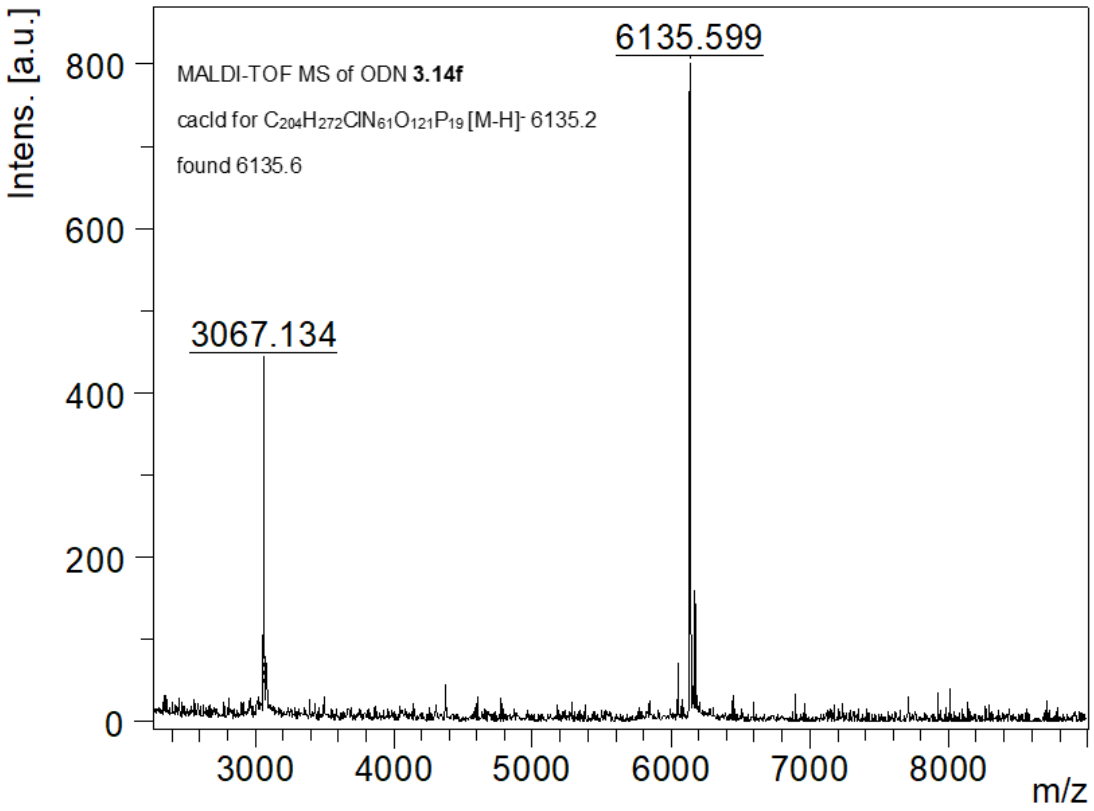


Figure B.71. MALDI-TOF-MS of compound **3.14f**

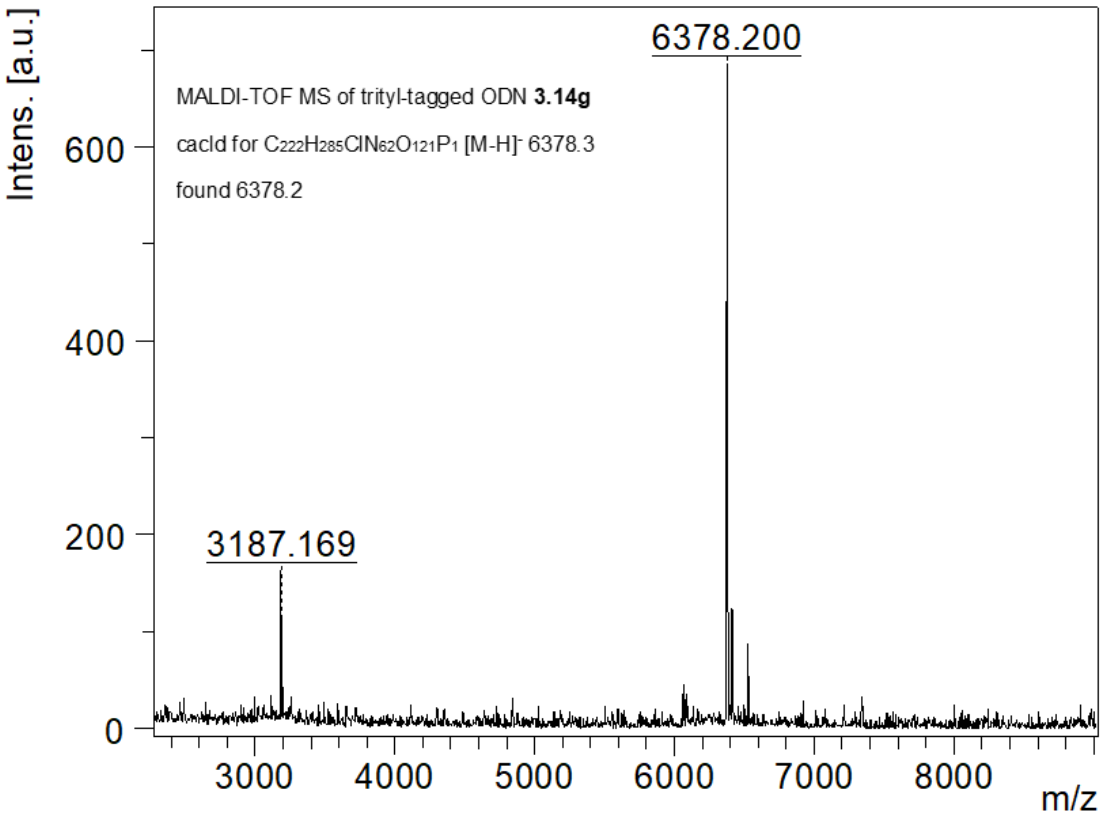


Figure B.72. MALDI-TOF-MS of compound **3.14g**

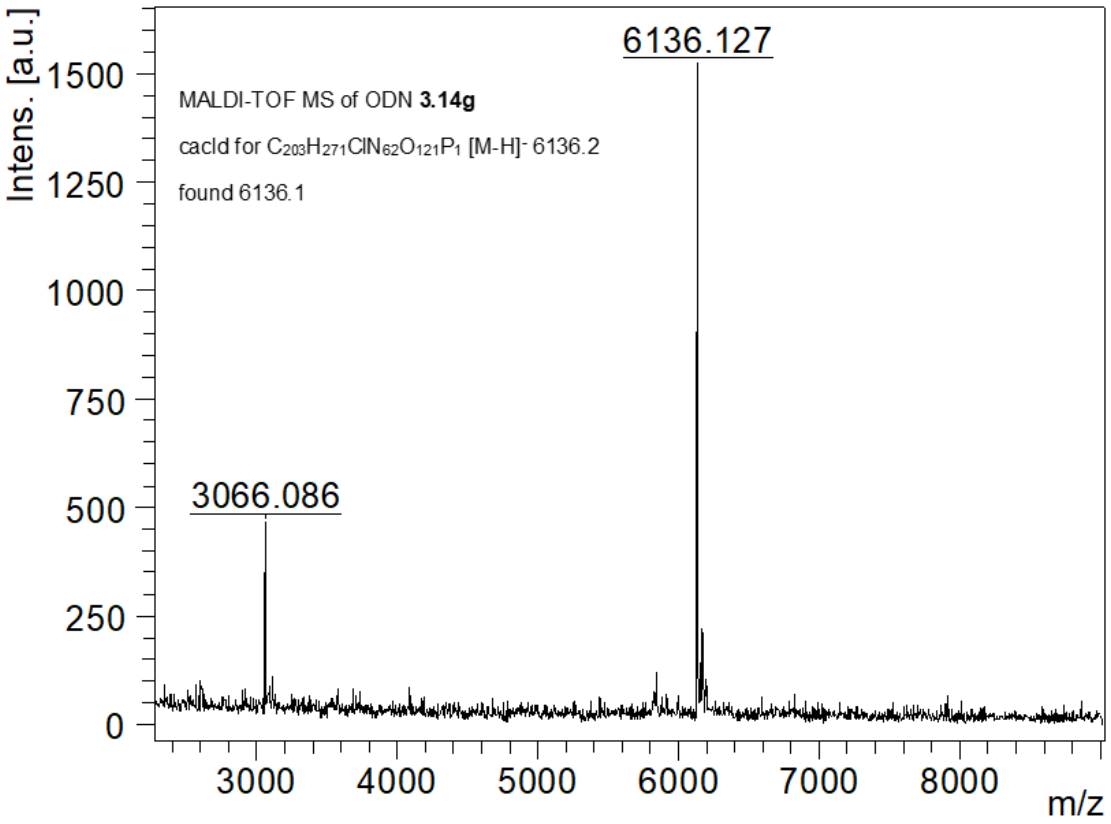
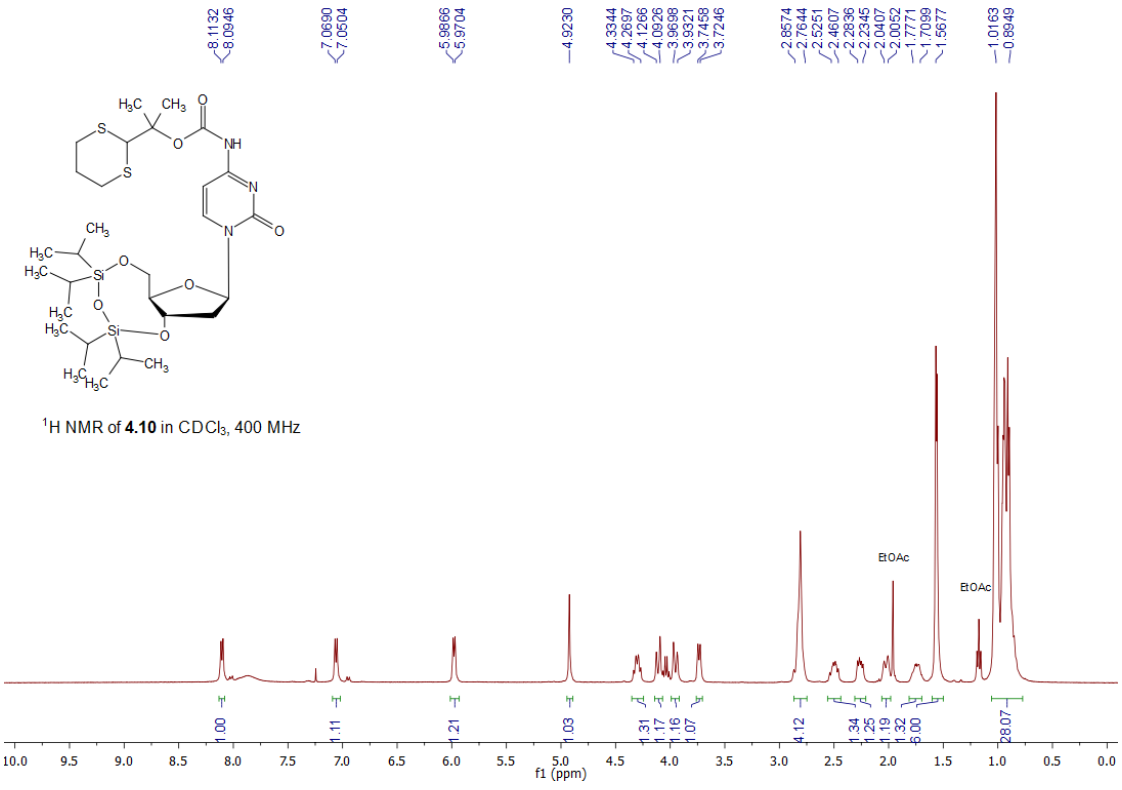


Figure B.73. MALDI-TOF-MS of compound **3.14g**

Appendix C. Supporting Information for Chapter 4

Electrophilic ODN Synthesis Using dM-Dmoc



^{1}H NMR of **4.10** in $CDCl_3$, 400 MHz

Figure C.1. ^{1}HNMR of Compound **4.10**

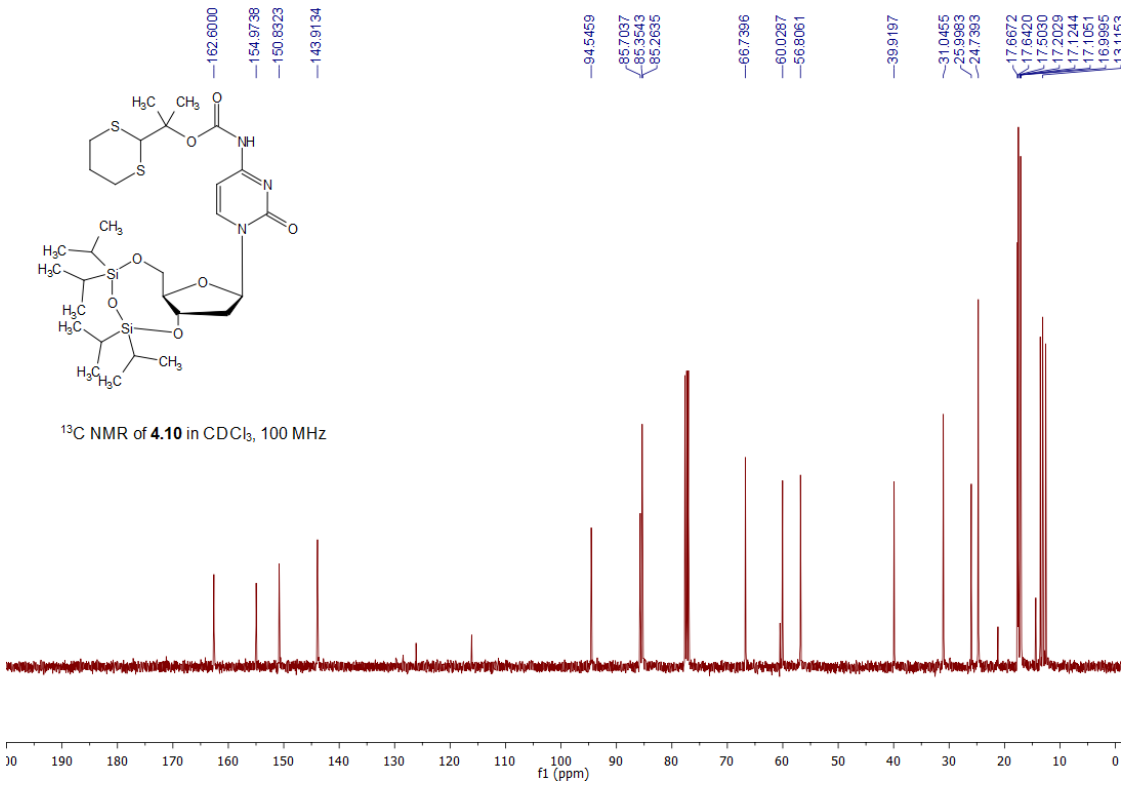
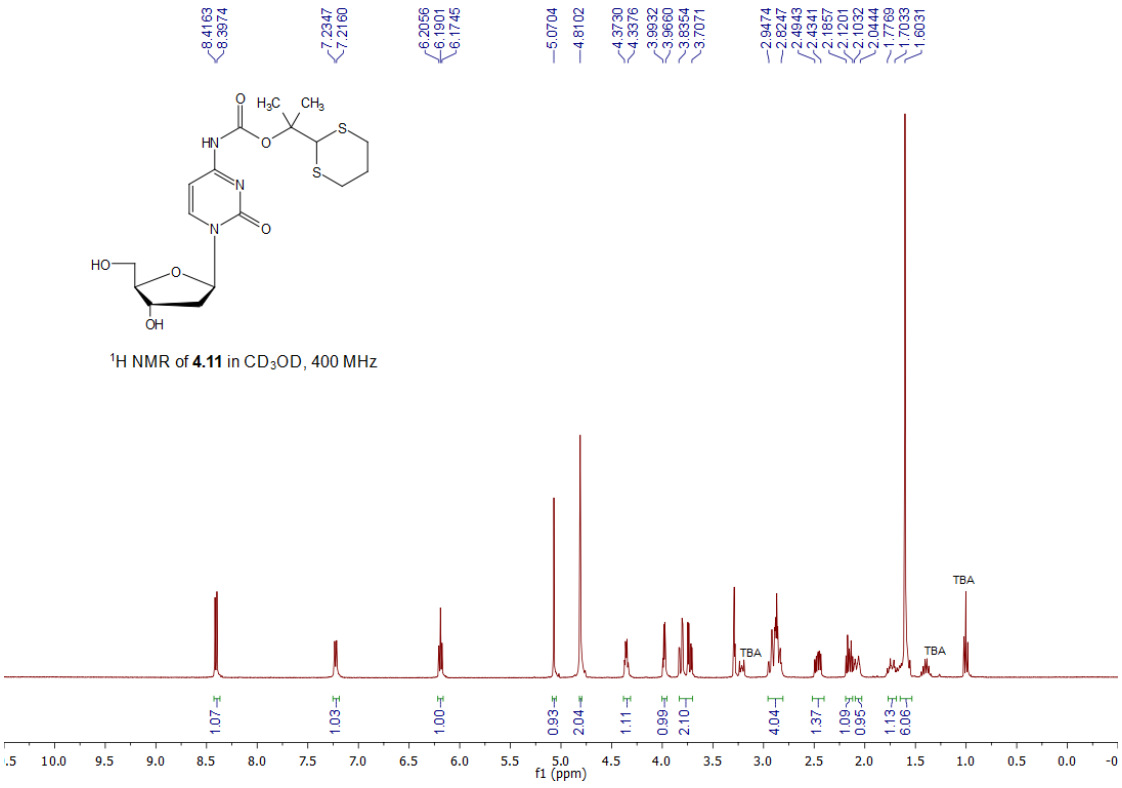
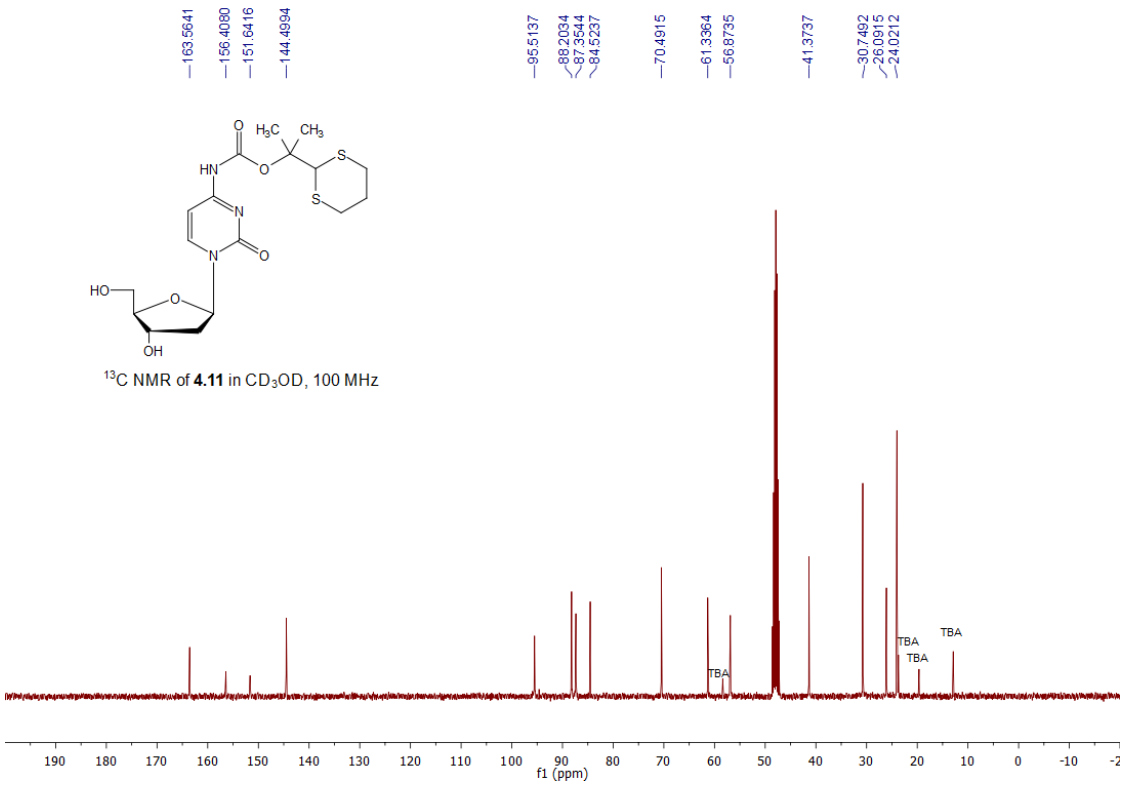


Figure C.2. ^{13}CNMR of Compound **4.10**



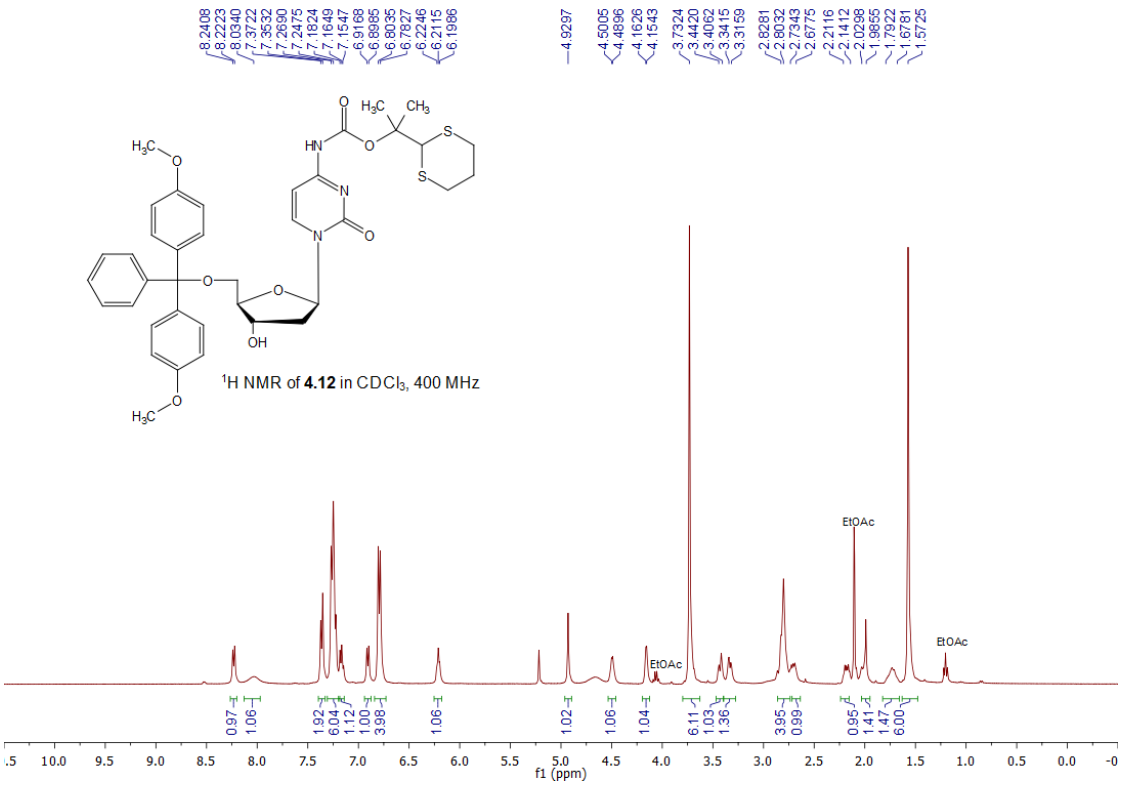
^{1}H NMR of **4.11** in CD_3OD, 400 MHz

Figure C.3. ^{1}HNMR of Compound **4.11**



^{13}C NMR of **4.11** in CD_3OD, 100 MHz

Figure C.4. ^{13}CNMR of Compound **4.11**



^{1}H NMR of **4.12** in $CDCl_3$, 400 MHz

Figure C.5. ^{1}HNMR of Compound **4.12**

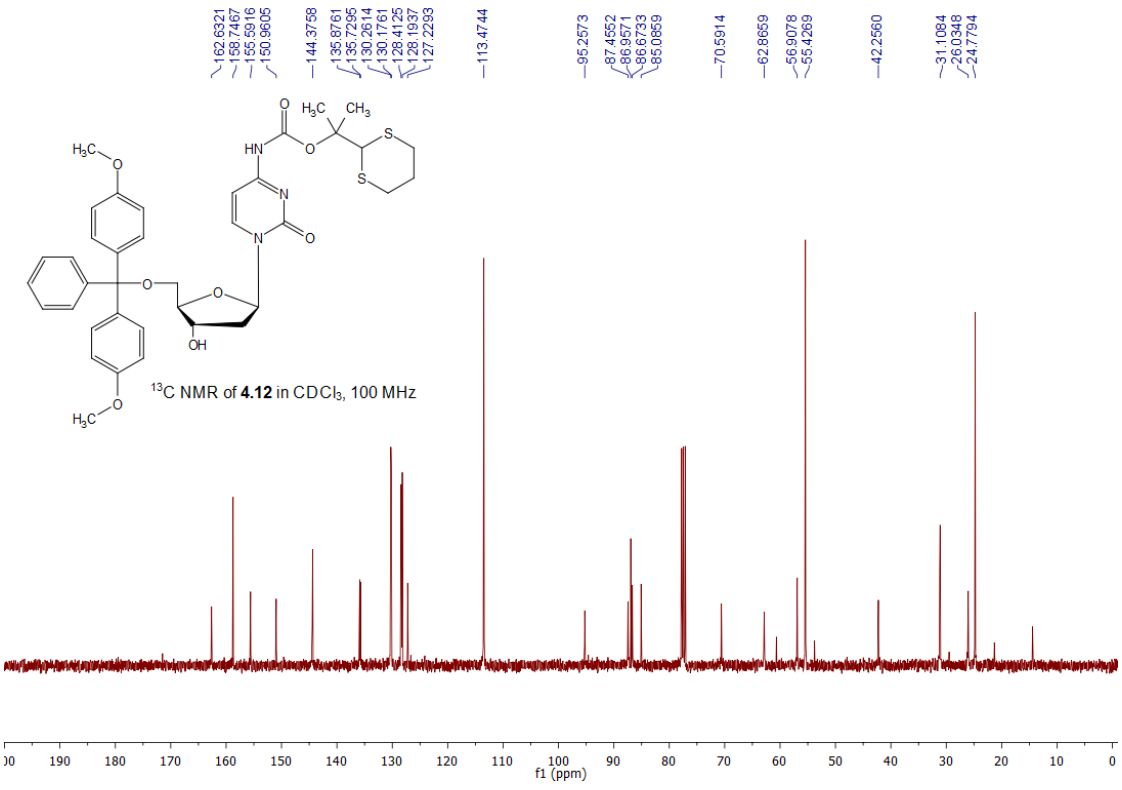


Figure C.6. ^{13}CNMR of Compound **4.12**

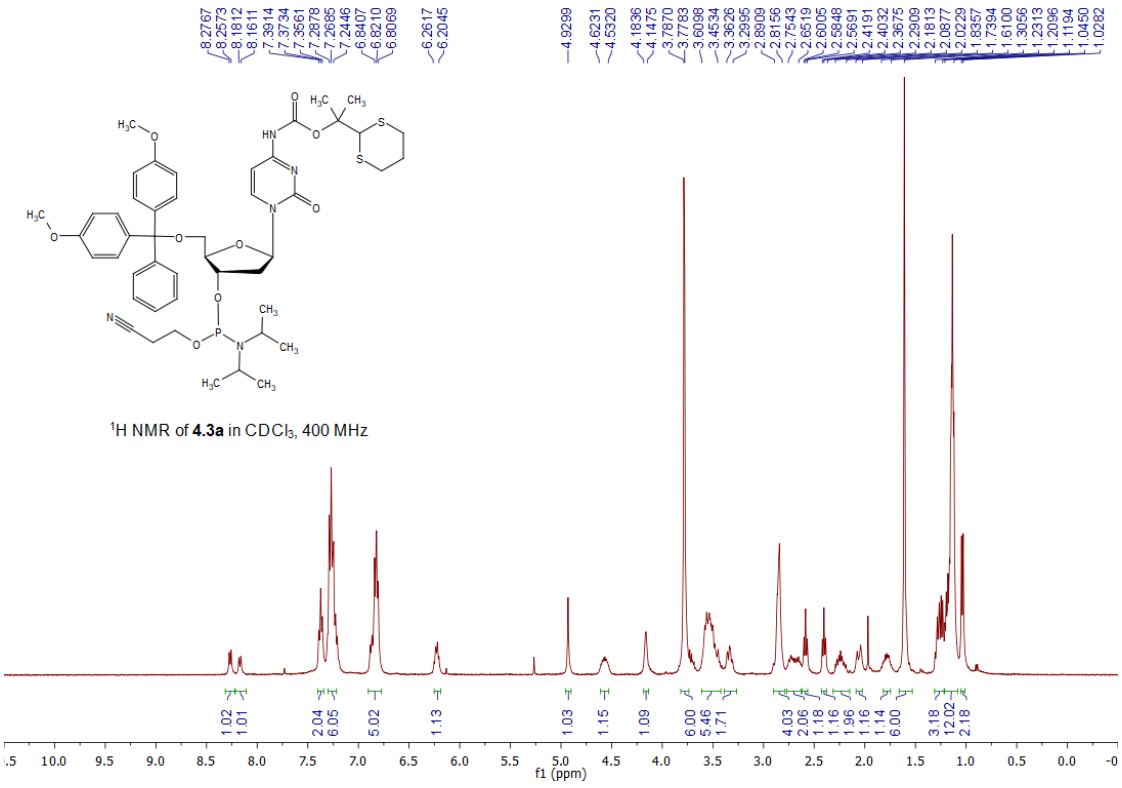


Figure C.7. [1]HNMR of Compound **4.3a**

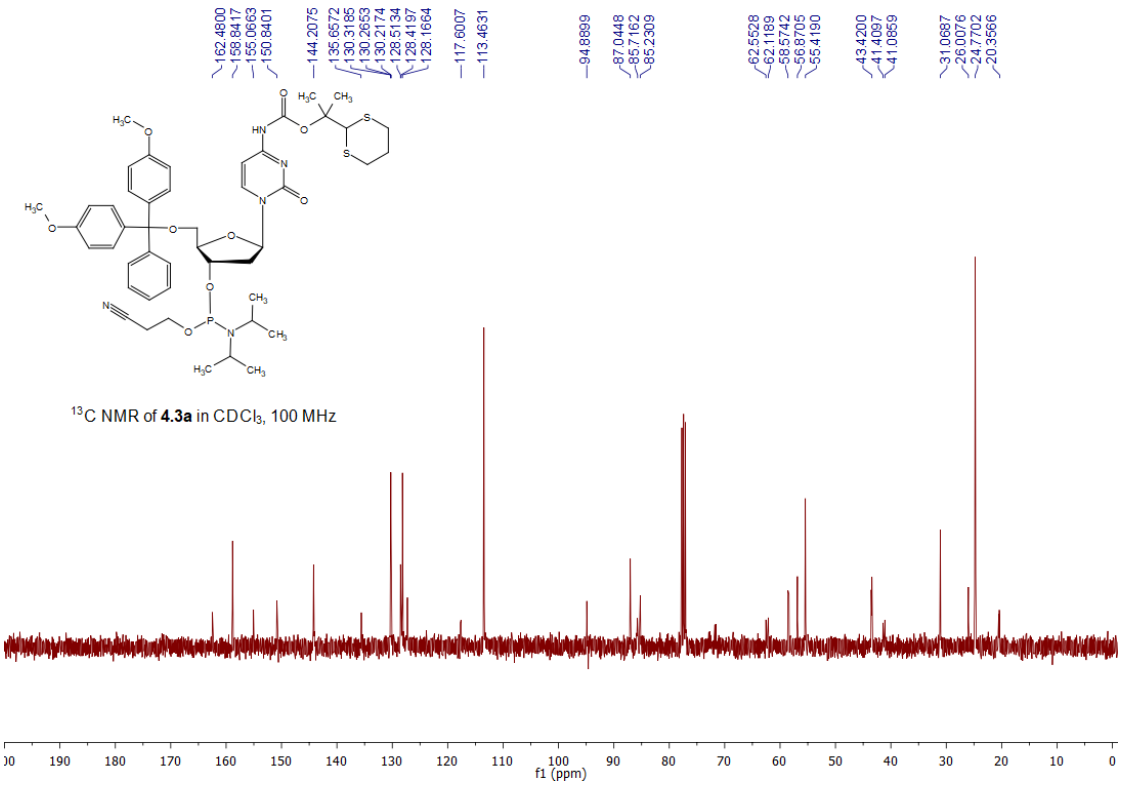


Figure C.8. ^{13}CNMR of Compound **4.3a**

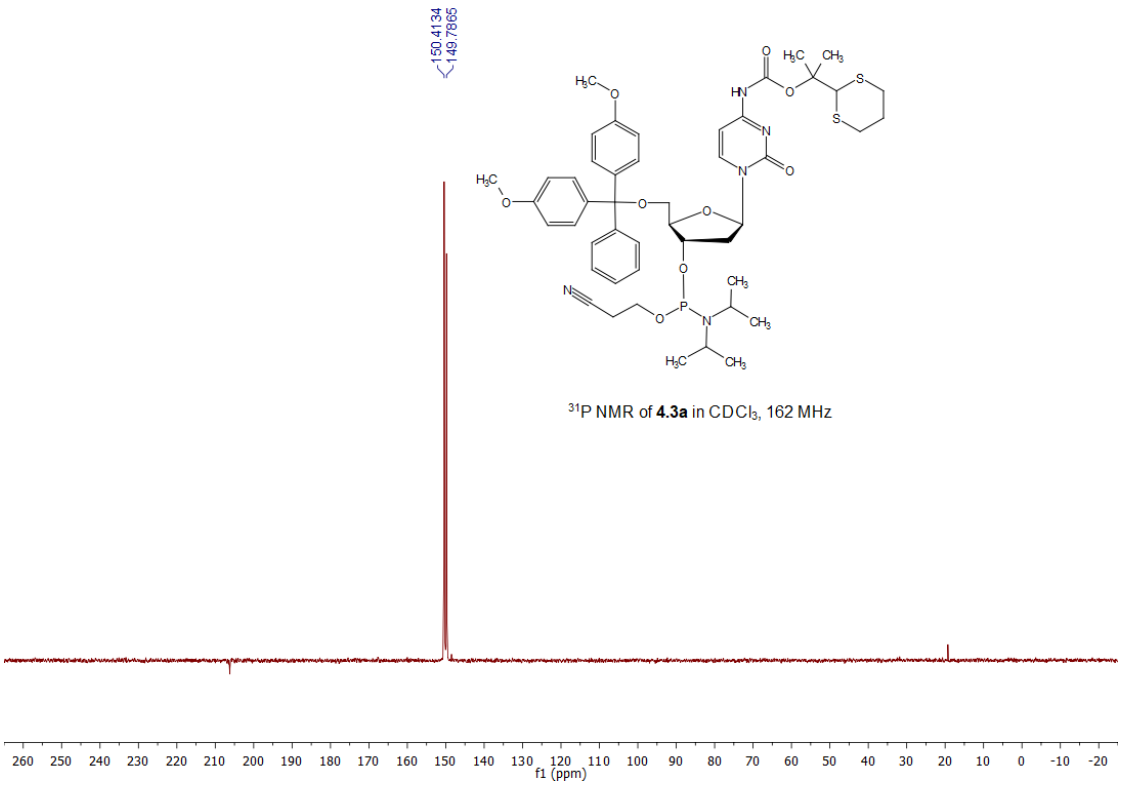


Figure C.9. ^{31}PNMR of Compound **4.3a**

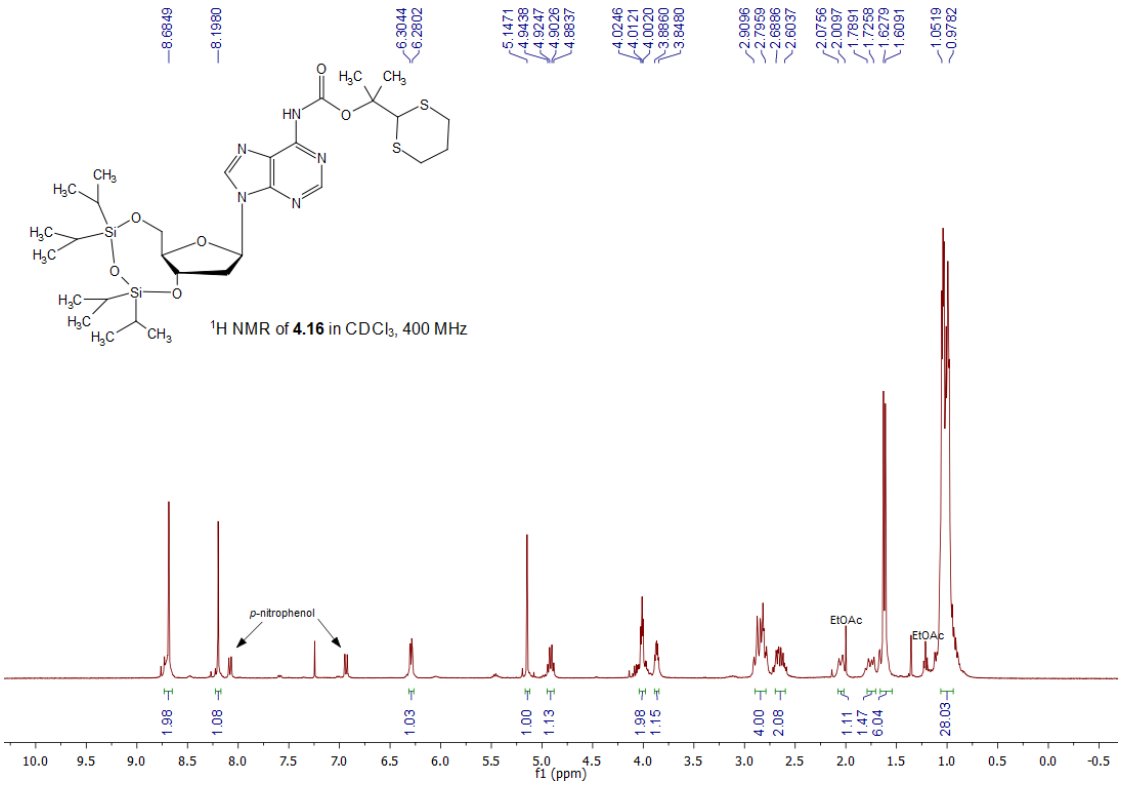


Figure C.10. [1]HNMR of Compound **4.16**

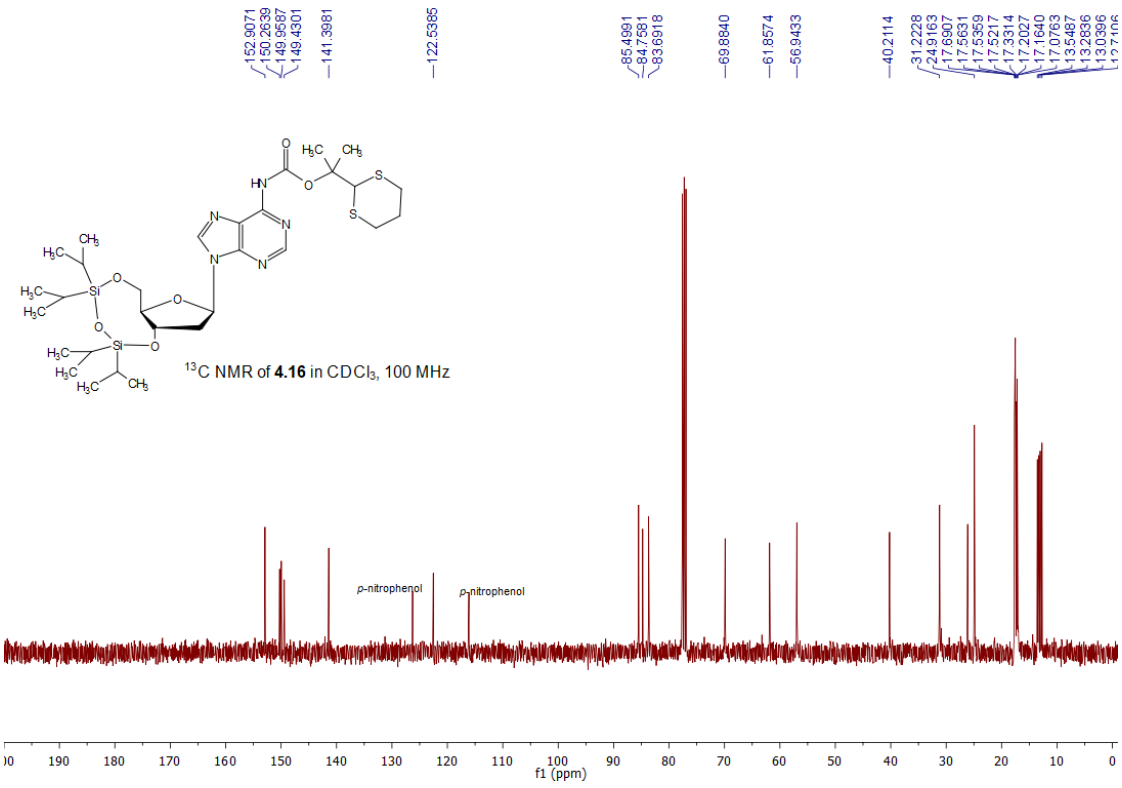


Figure C.11. ^{13}CNMR of Compound **4.16**

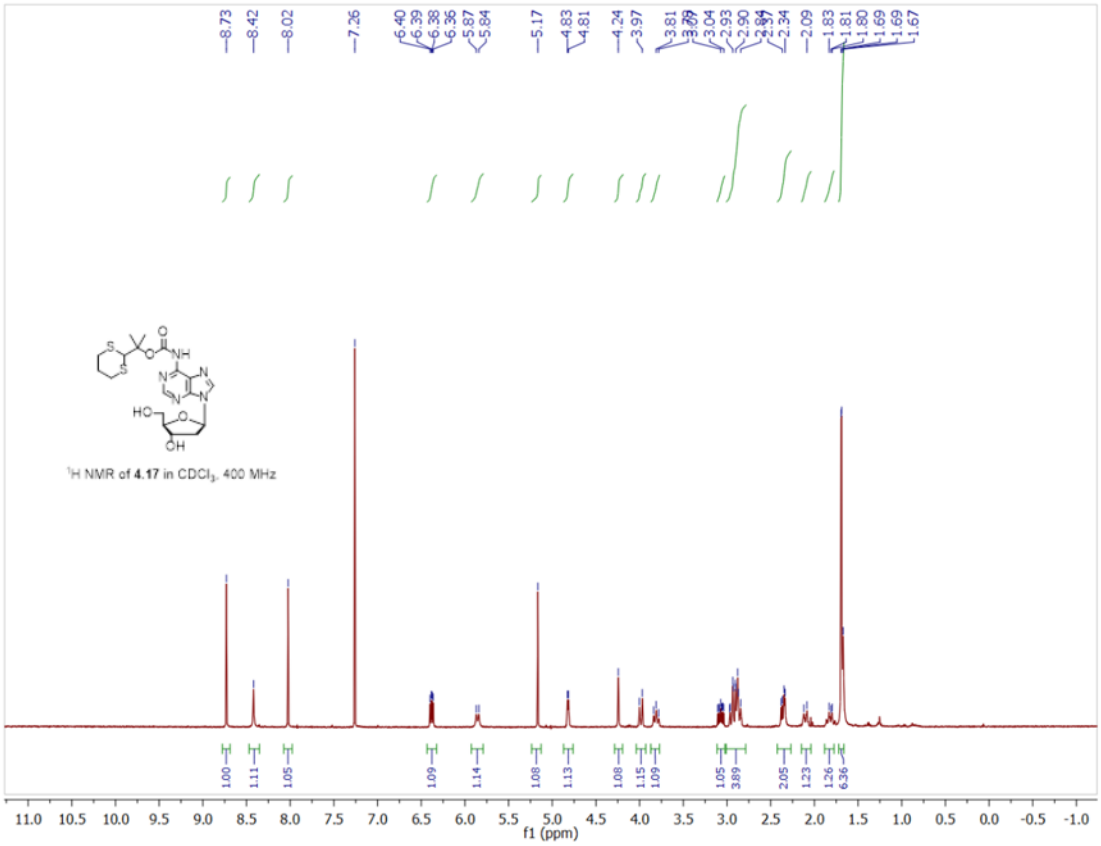


Figure C.12. [1]HNMR of Compound **4.17**

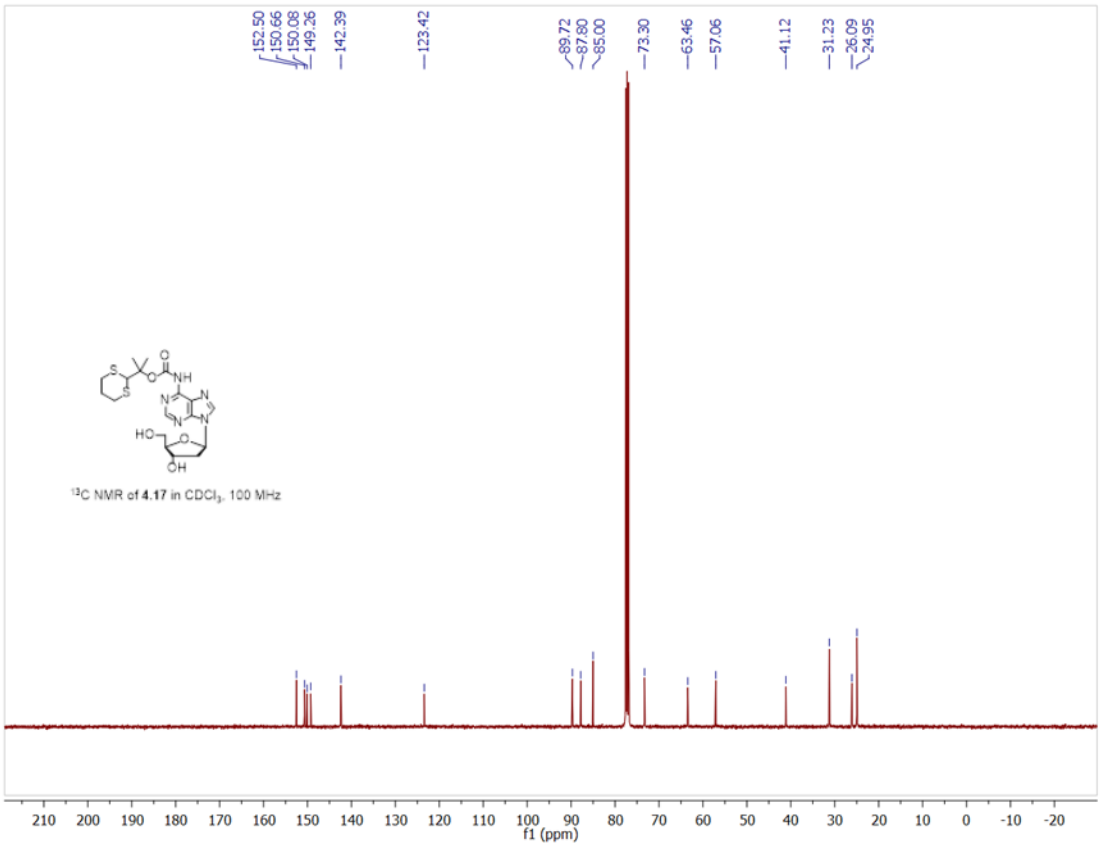
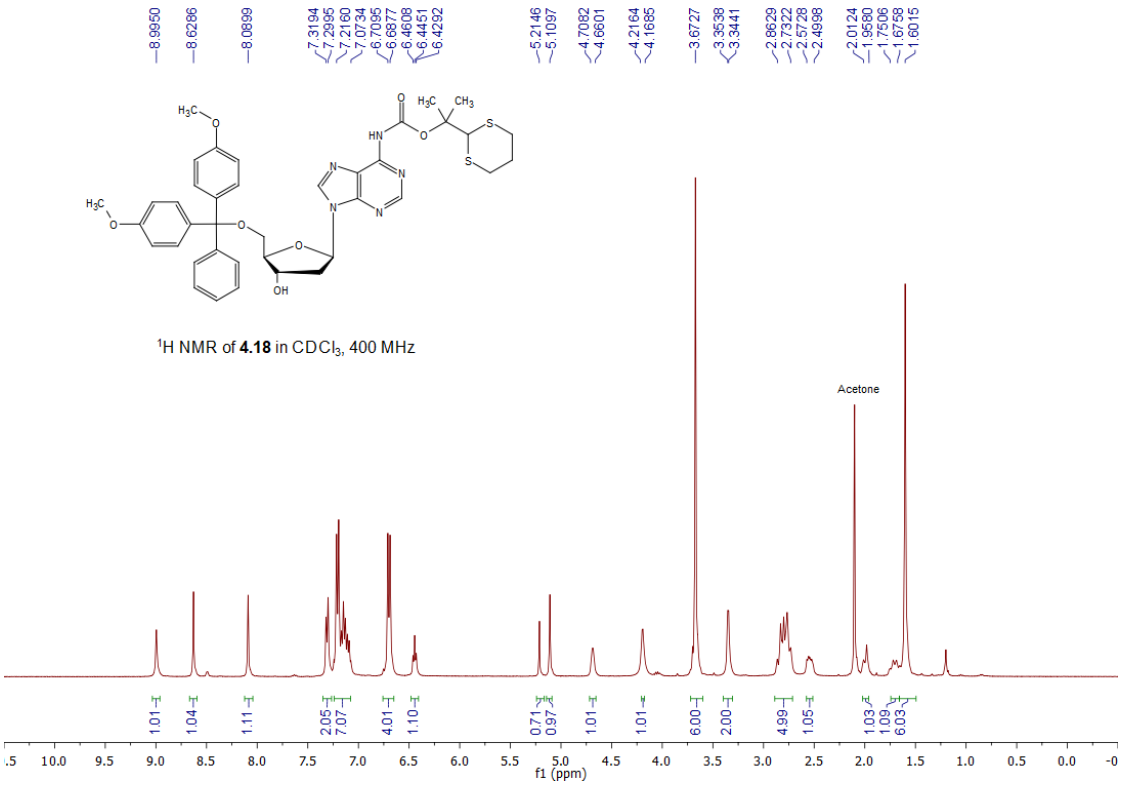


Figure C.13. ^{13}CNMR of Compound **4.17**



^{1}H NMR of **4.18** in $CDCl_3$, 400 MHz

Figure C.14. ^{1}HNMR of Compound **4.18**

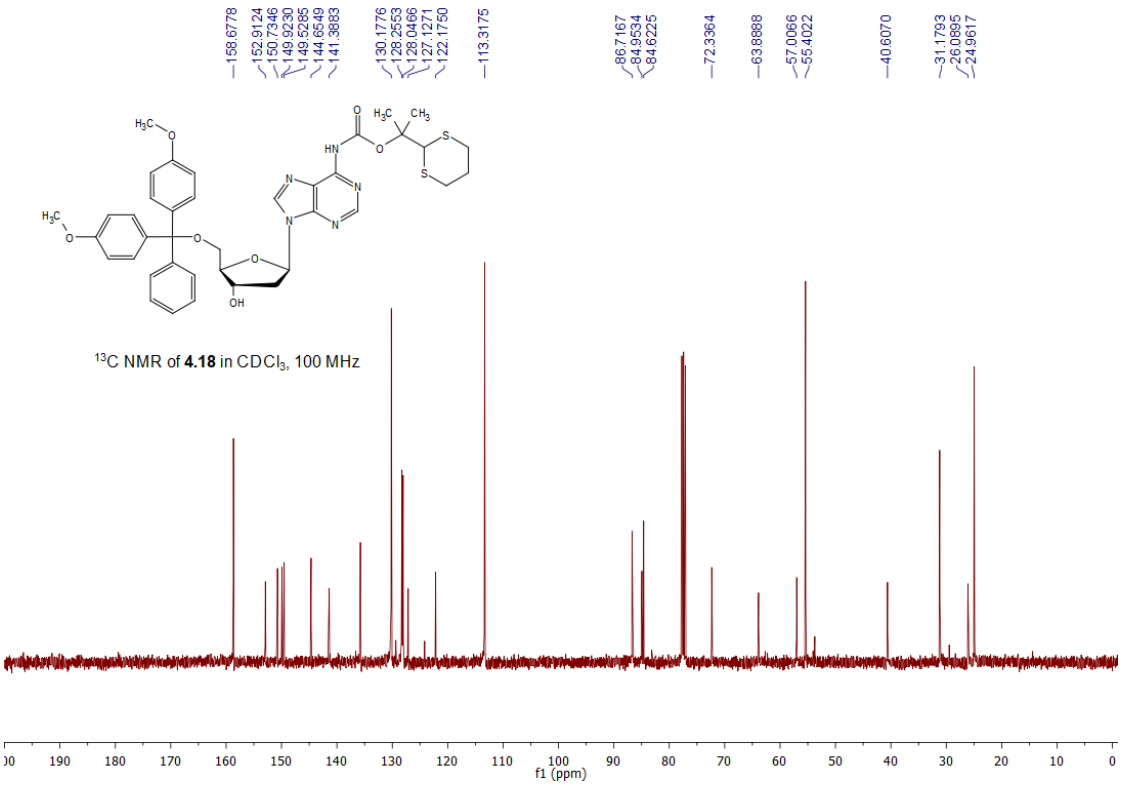


Figure C.15. ^{13}CNMR of Compound **4.18**

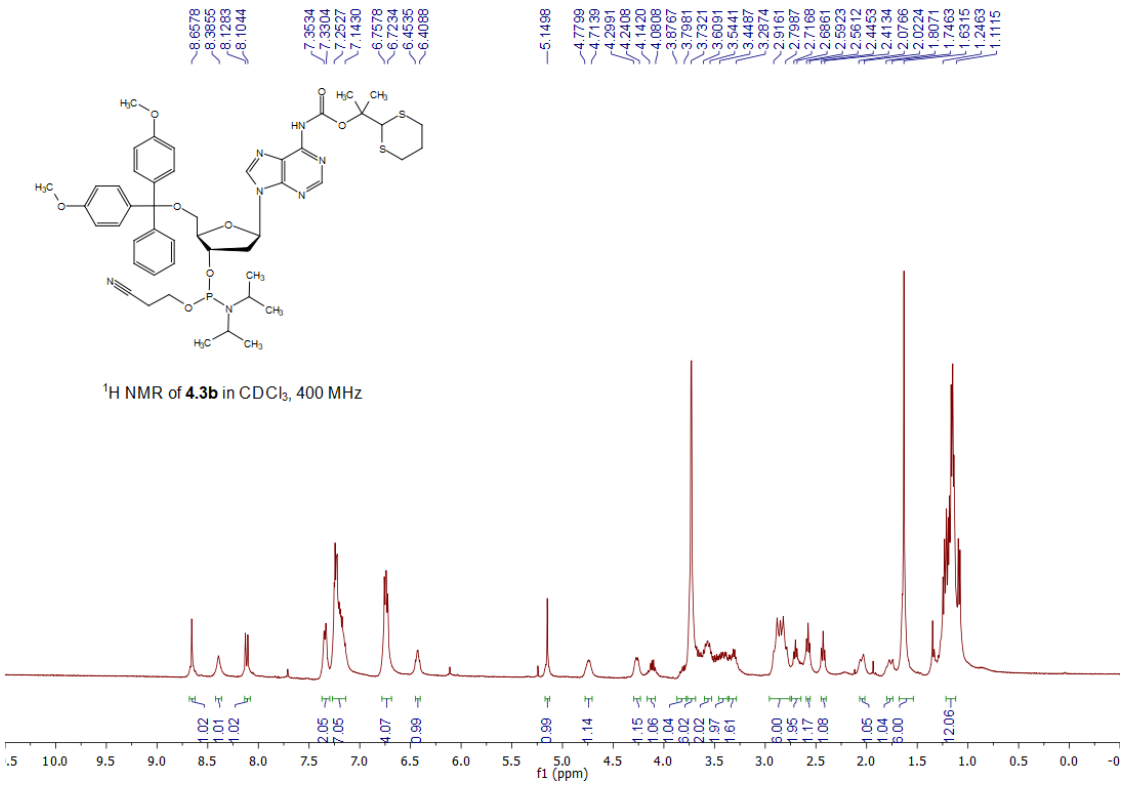


Figure C.16. [1]HNMR of Compound **4.3b**

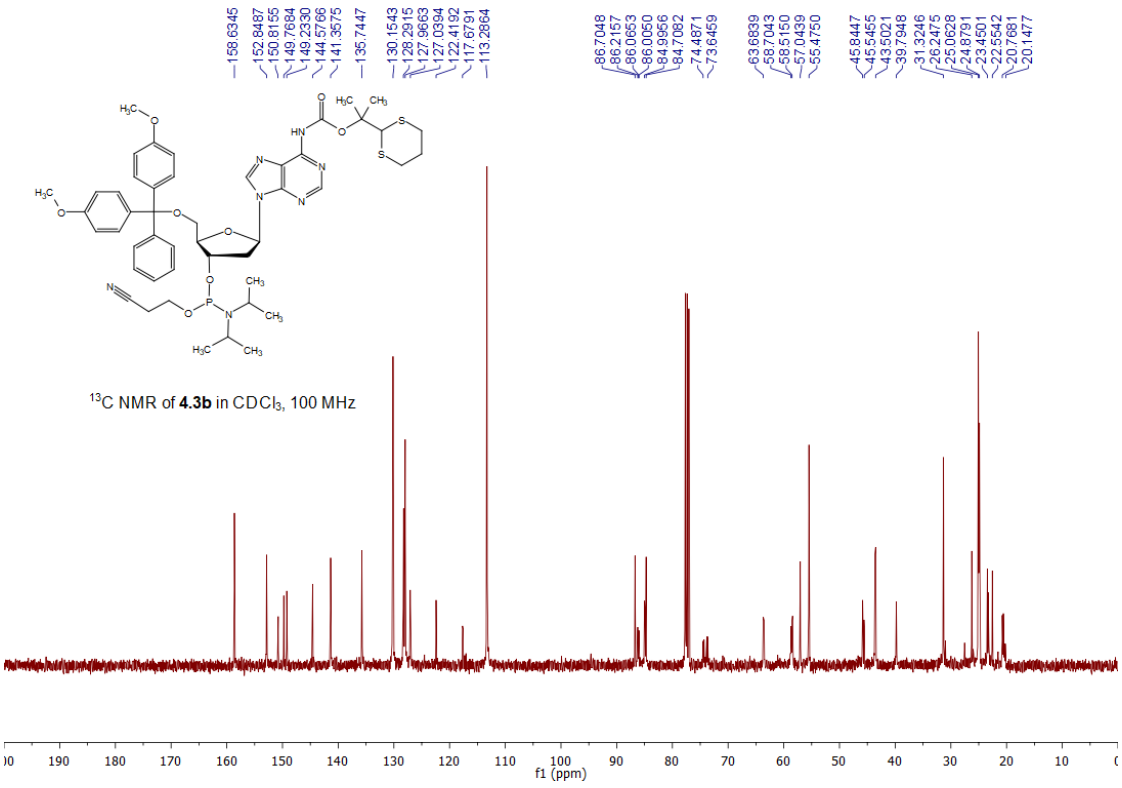


Figure C.17. ^{13}CNMR of Compound **4.18**

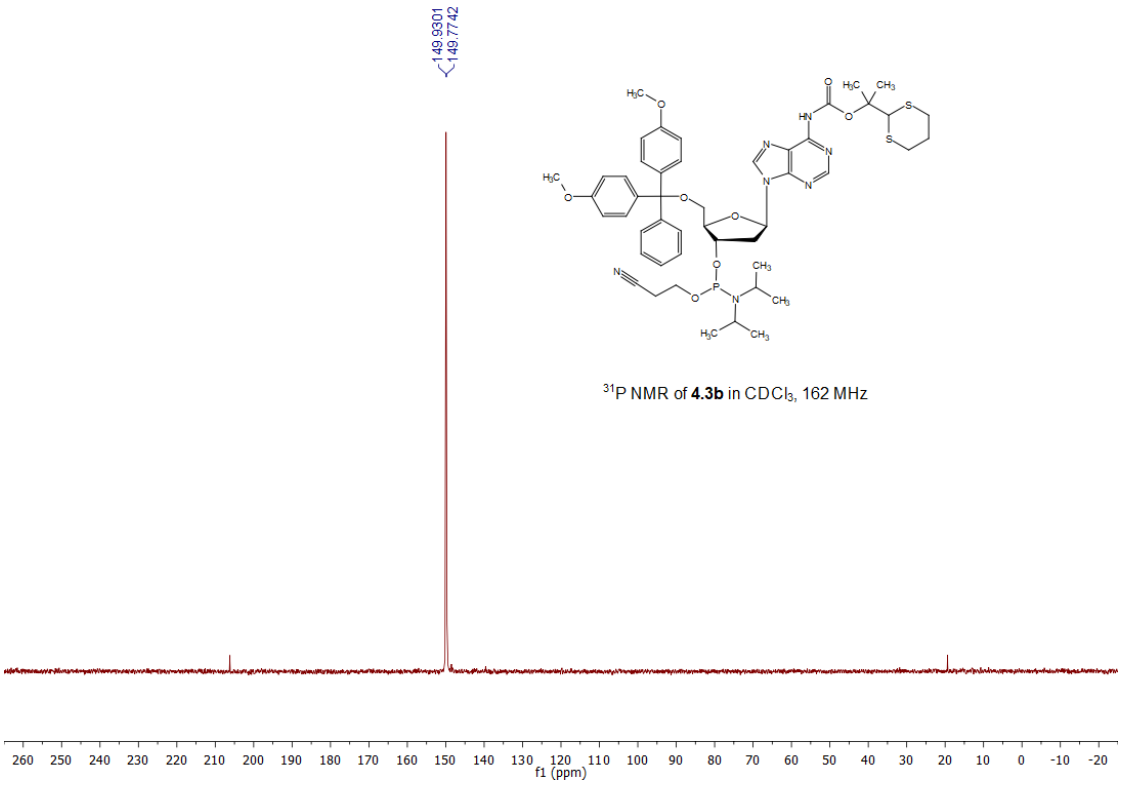


Figure C.18. ^{31}PNMR of Compound **4.3b**

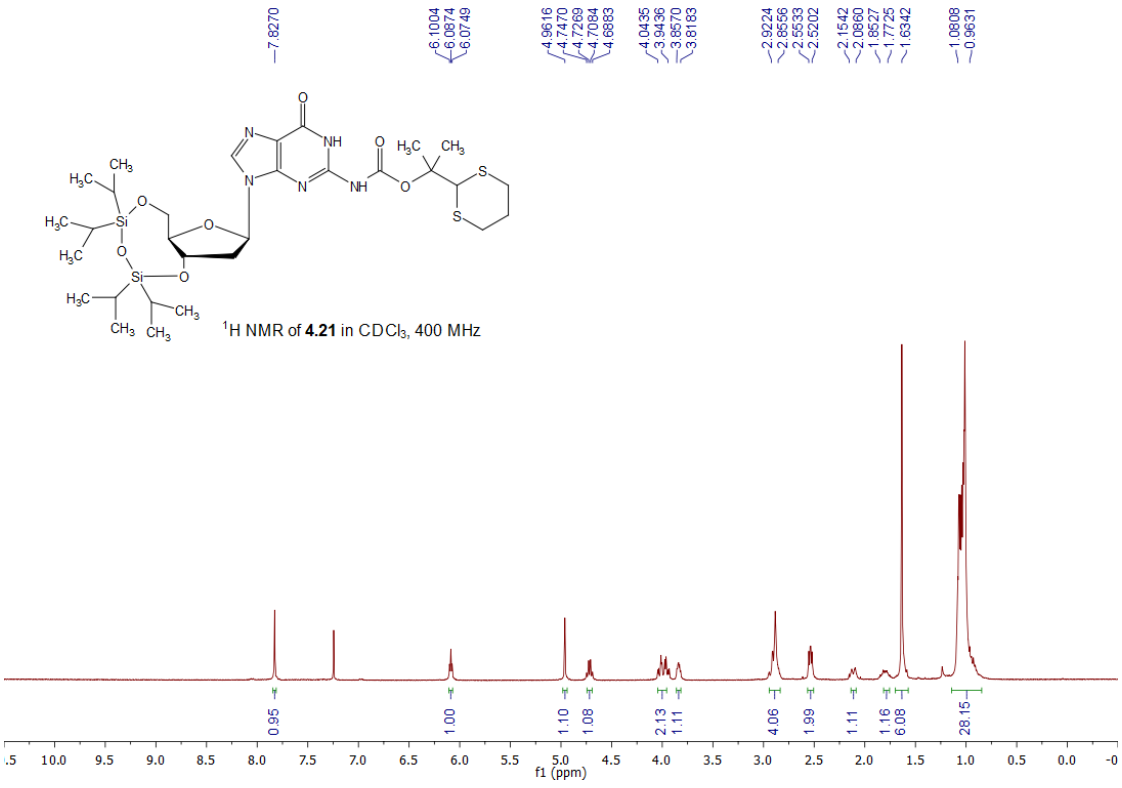


Figure C.19. ^{1}HNMR of Compound **4.21**

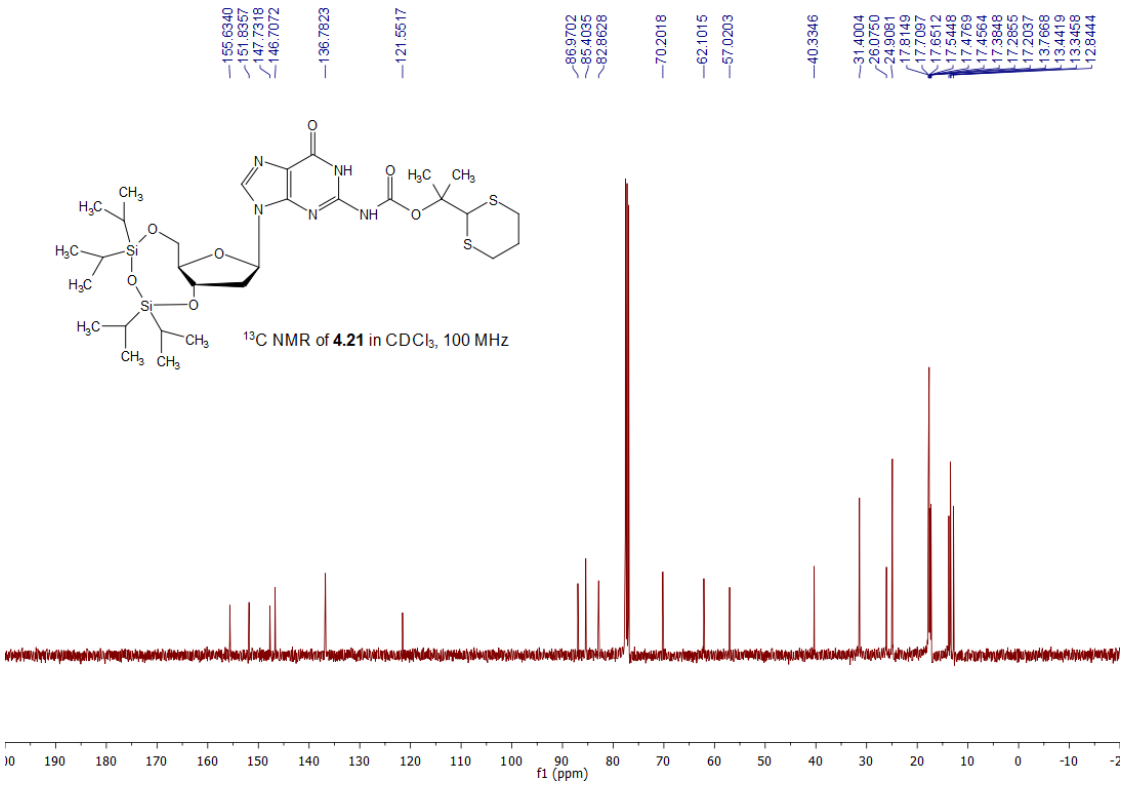


Figure C.20. ^{13}CNMR of Compound **4.21**

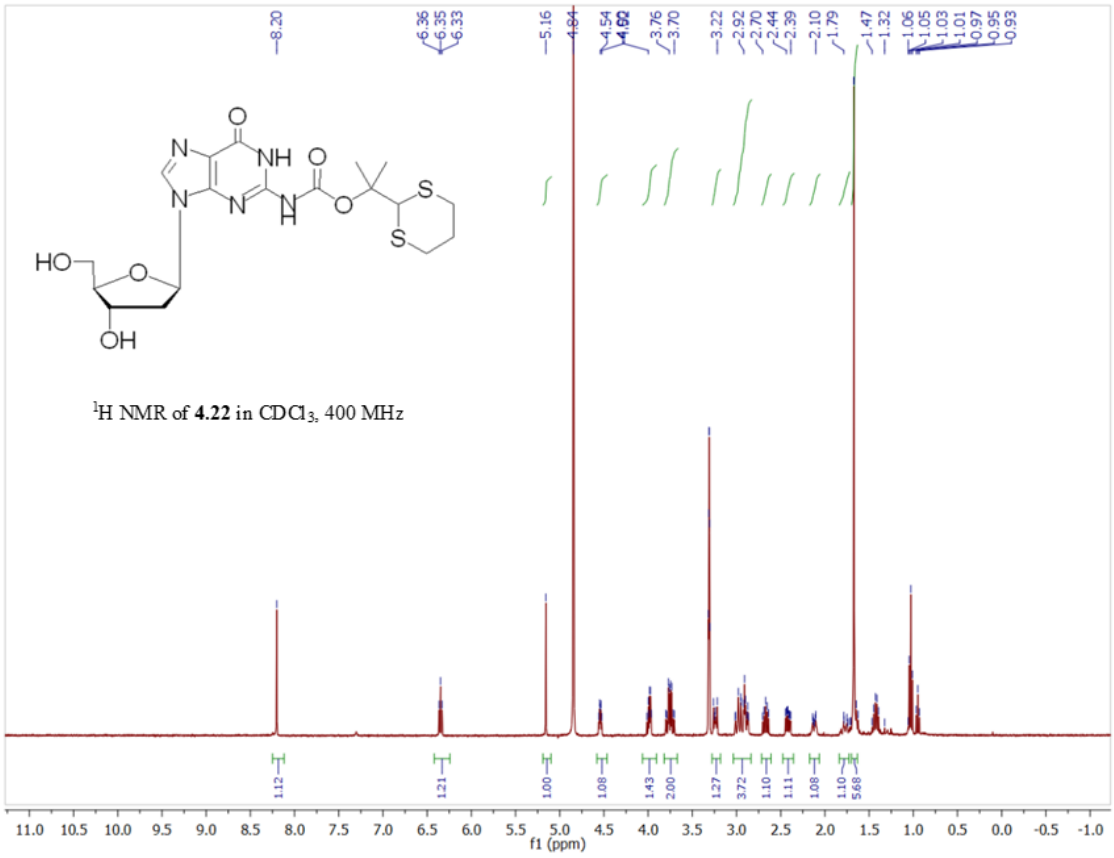


Figure C.21. [1]HNMR of Compound **4.22**

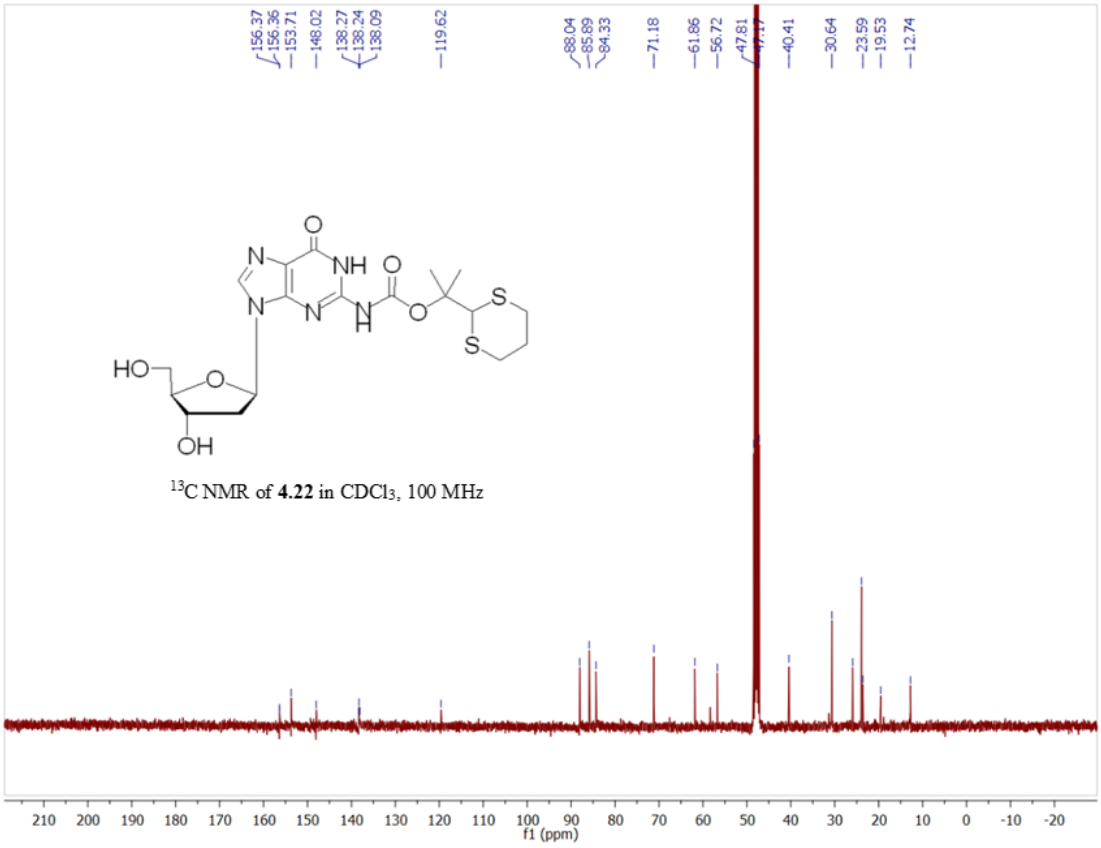


Figure C.22. ^{13}CNMR of Compound **4.22**

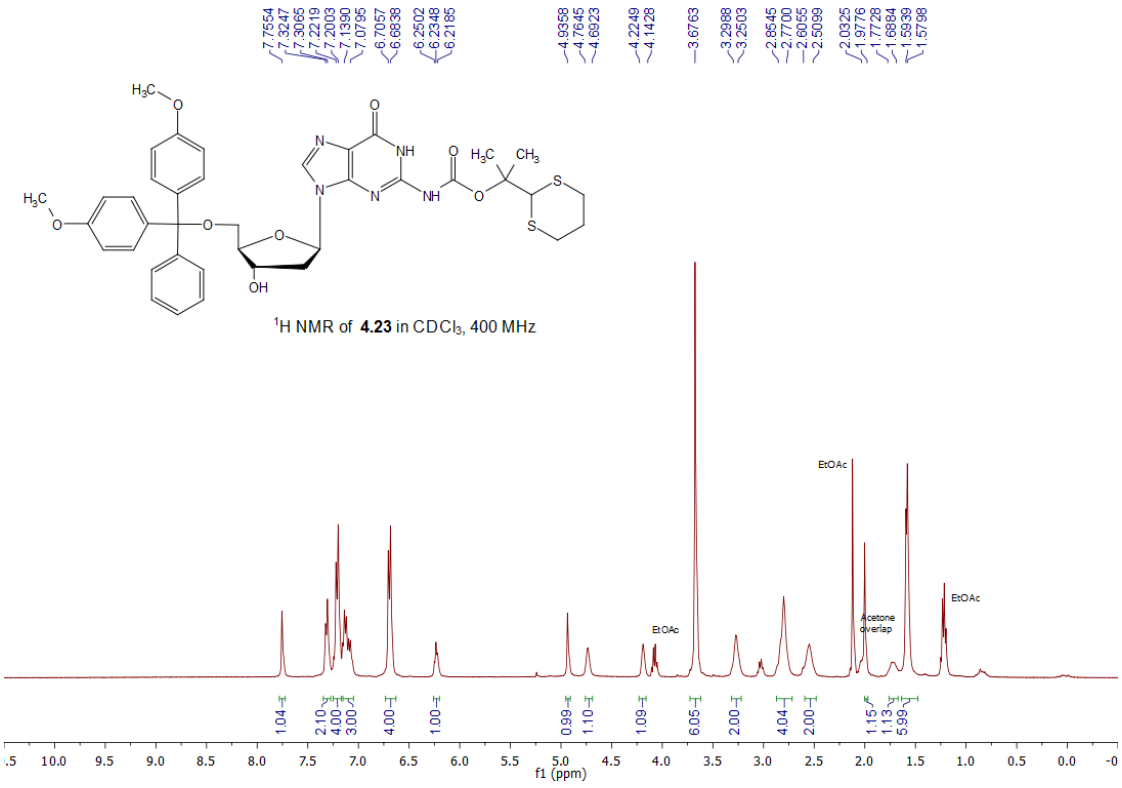
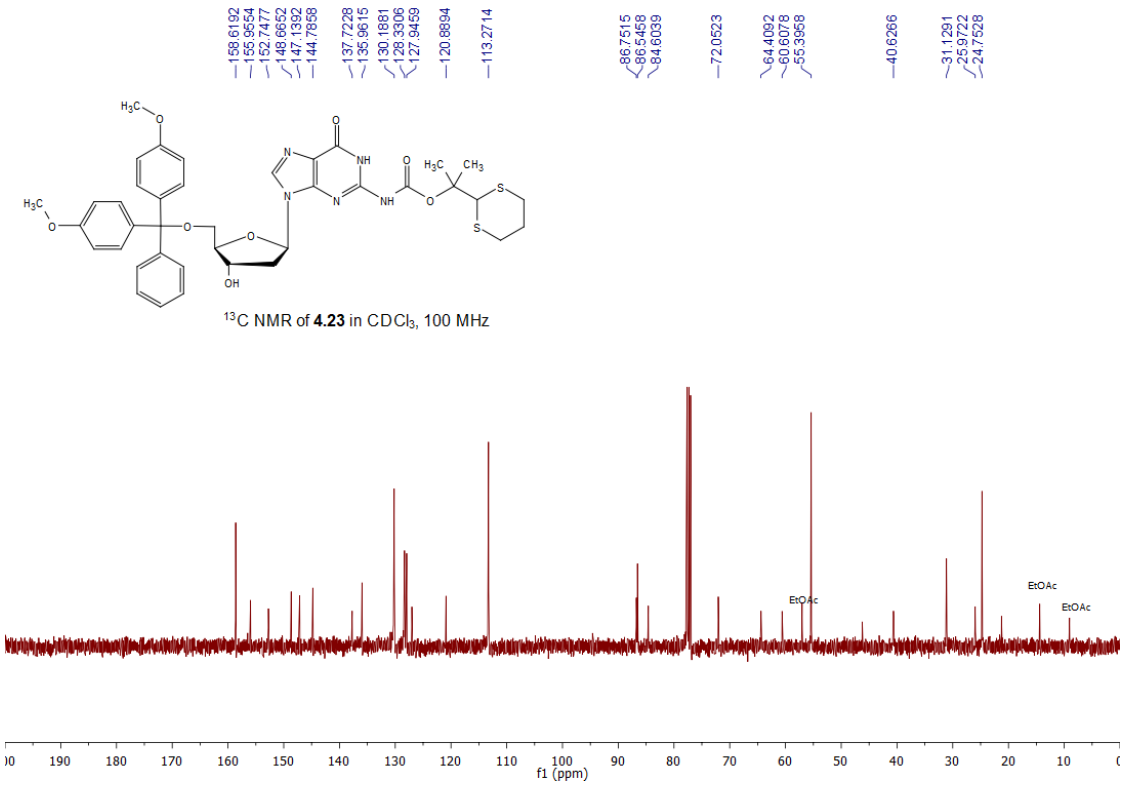


Figure C.23. [1]HNMR of Compound **4.23**



^{13}C NMR of **4.23** in $CDCl_3$, 100 MHz

Figure C.24. ^{13}CNMR of Compound **4.23**

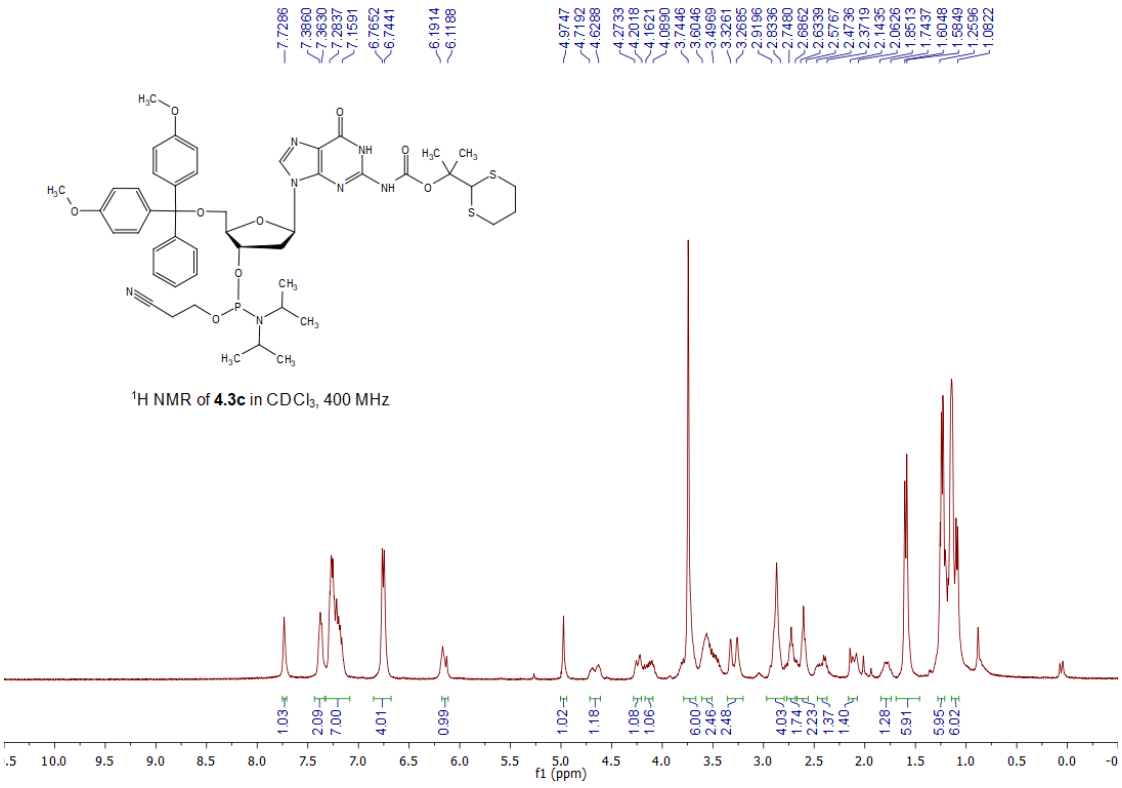


Figure C.25. [1]HNMR of Compound **4.3c**

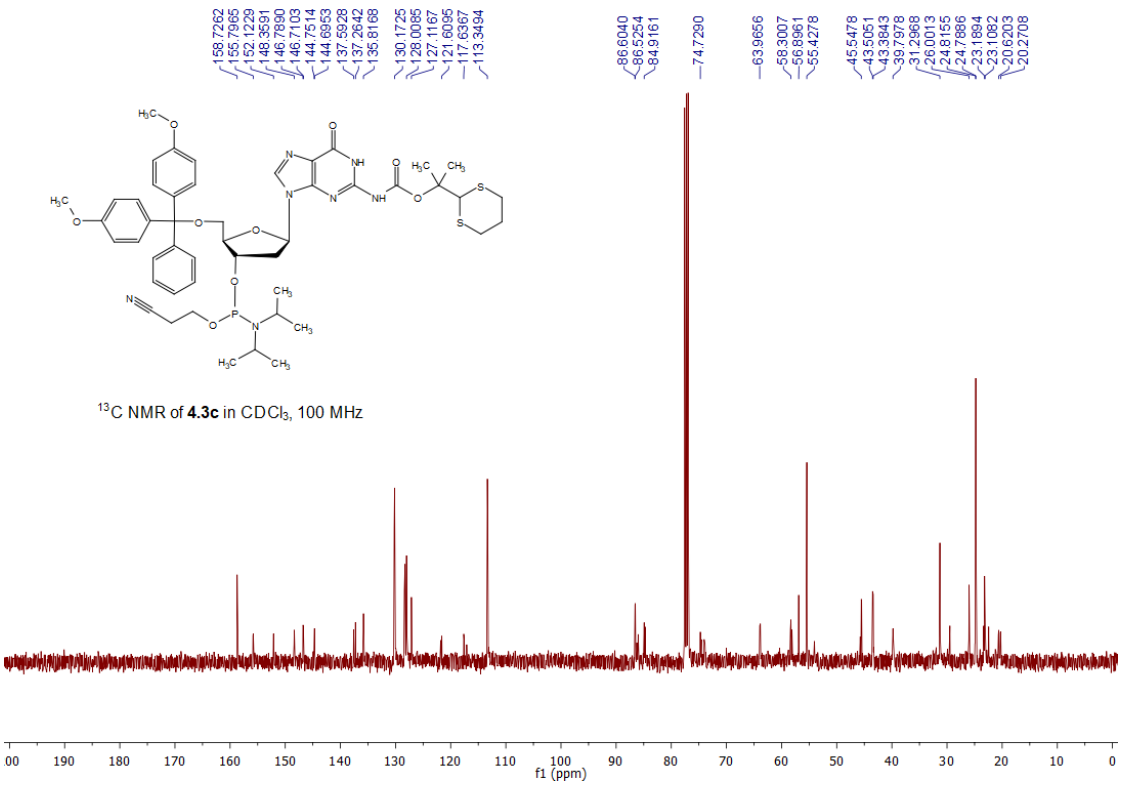


Figure C.26. ^{13}CNMR of Compound **4.3c**

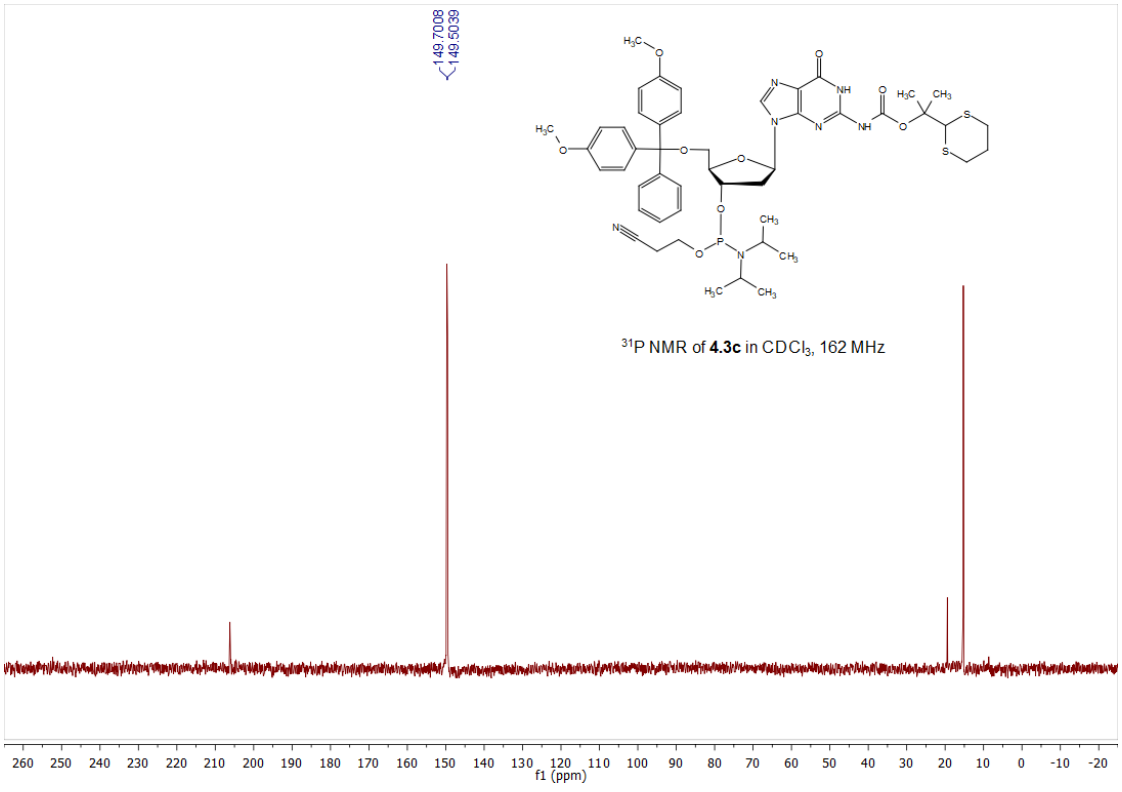


Figure C.27. ^{31}PNMR of Compound **4.3c**

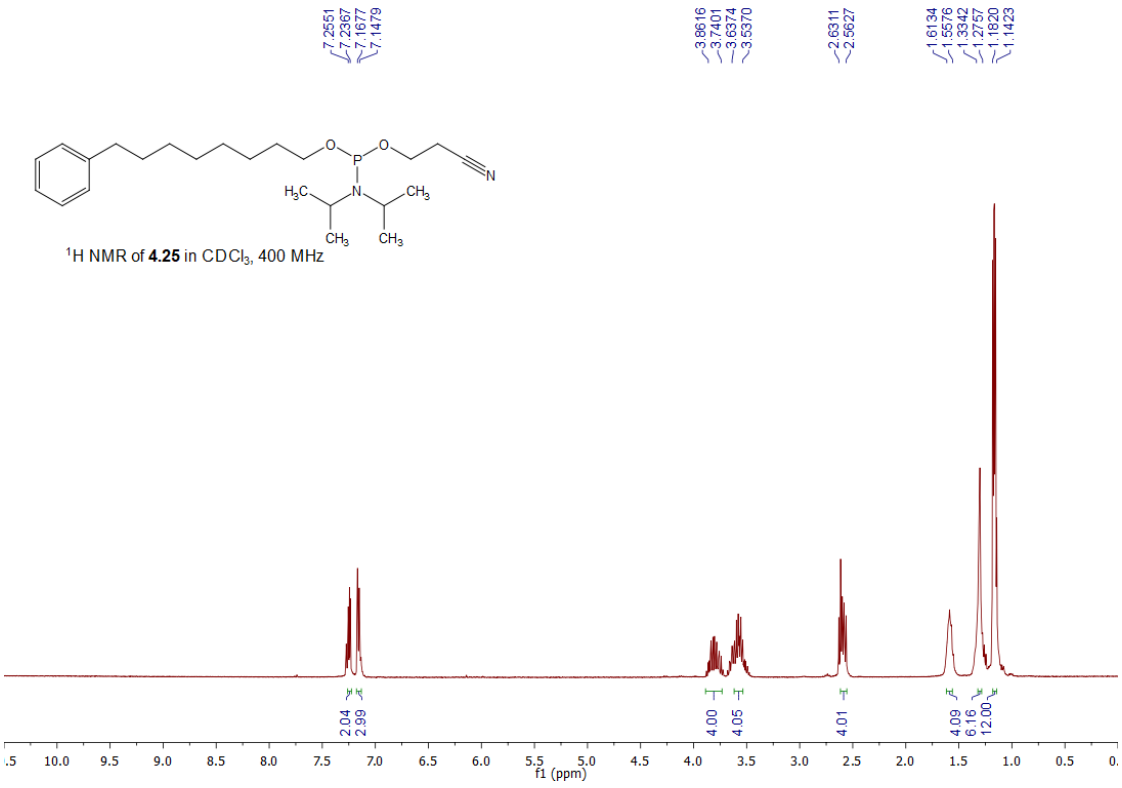


Figure C.28. [1]HNMR of Compound **4.25**

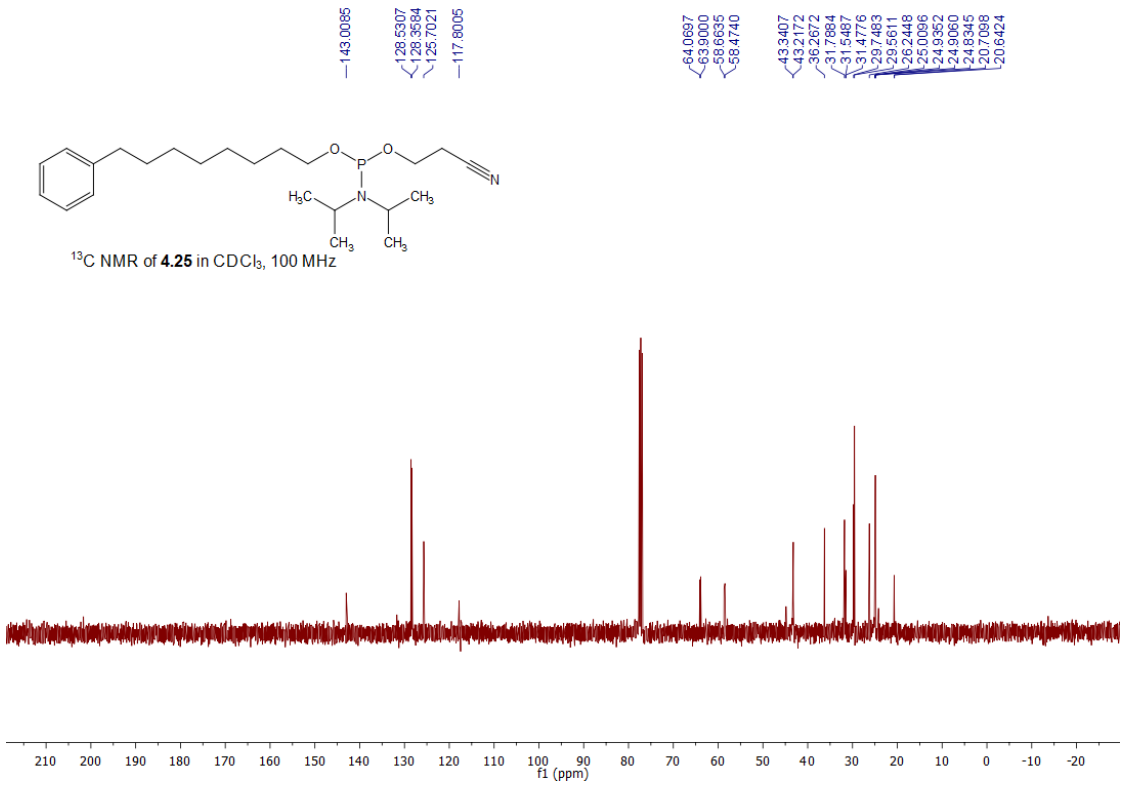


Figure C.29. ^{13}CNMR of Compound **4.25**

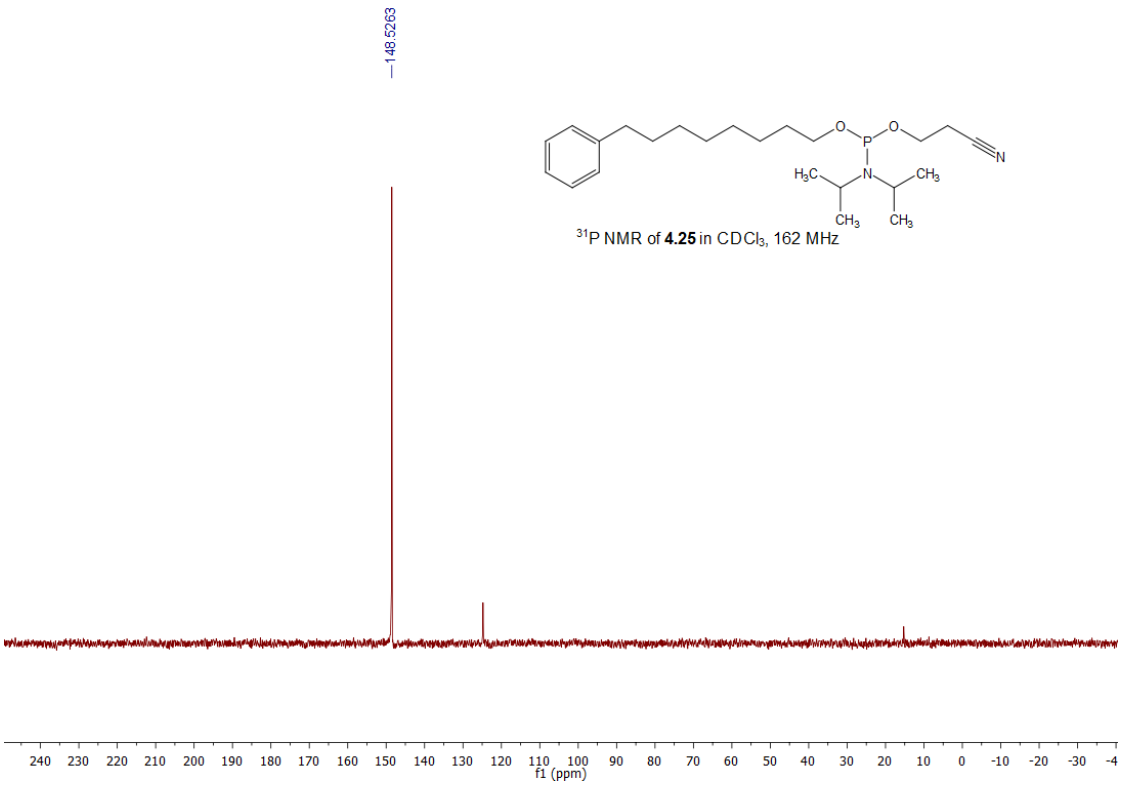


Figure C.30. ^{31}PNMR of Compound **4.25**

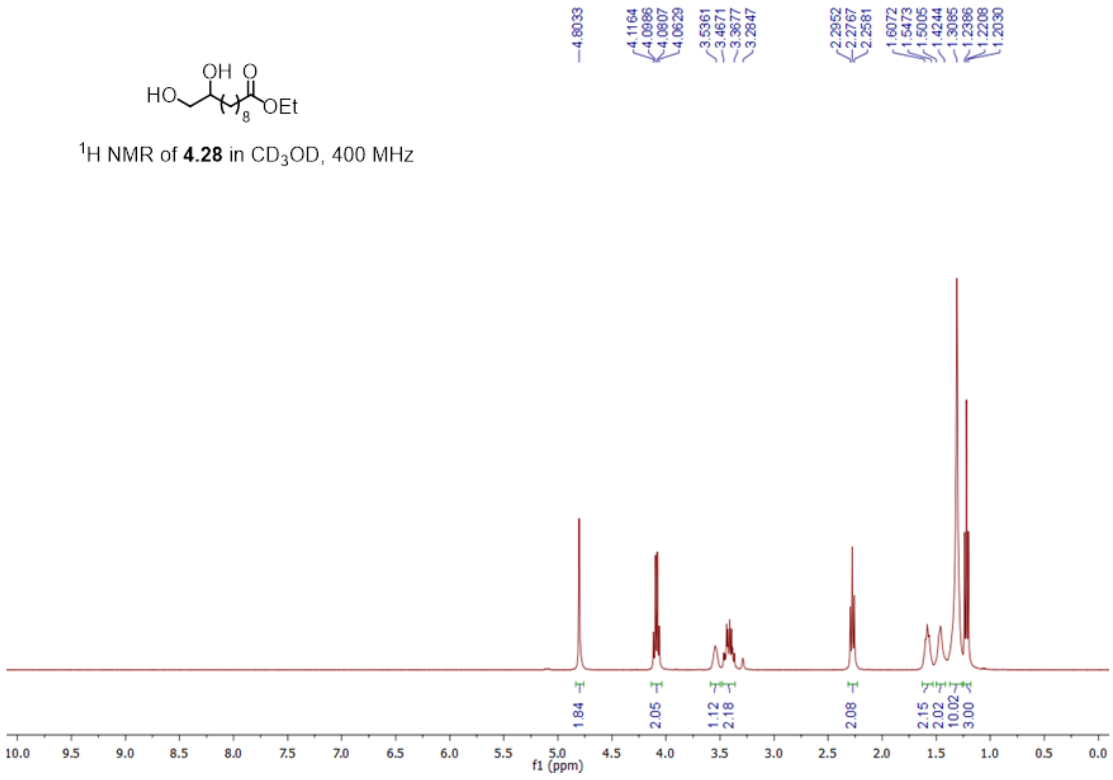


Figure C.31. [1]HNMR of Compound **4.28**

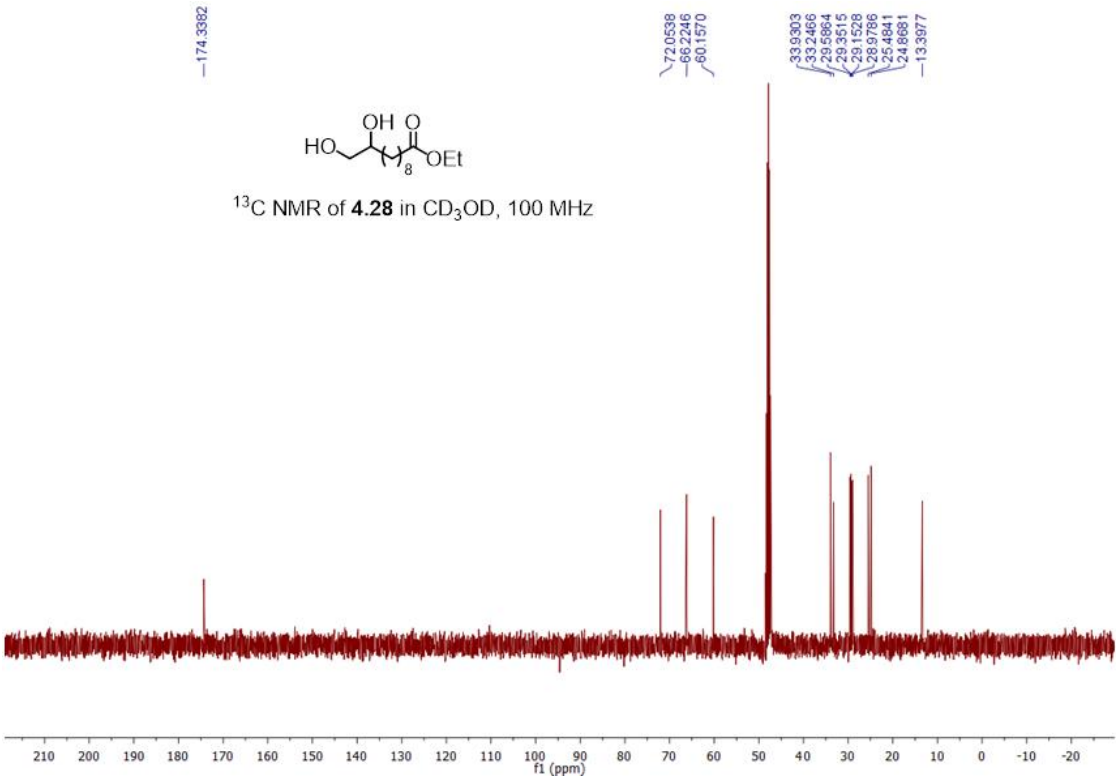


Figure C.32. ^{13}CNMR of Compound **4.28**

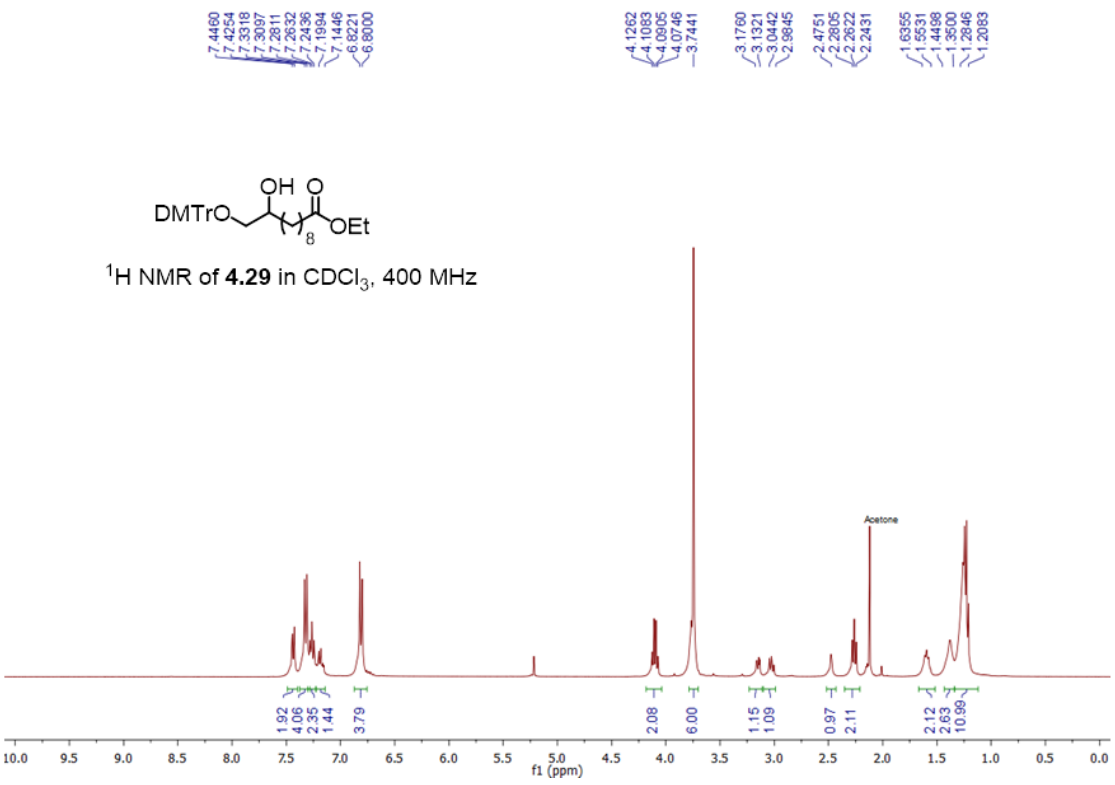


Figure C.33. [1]HNMR of Compound **4.29**

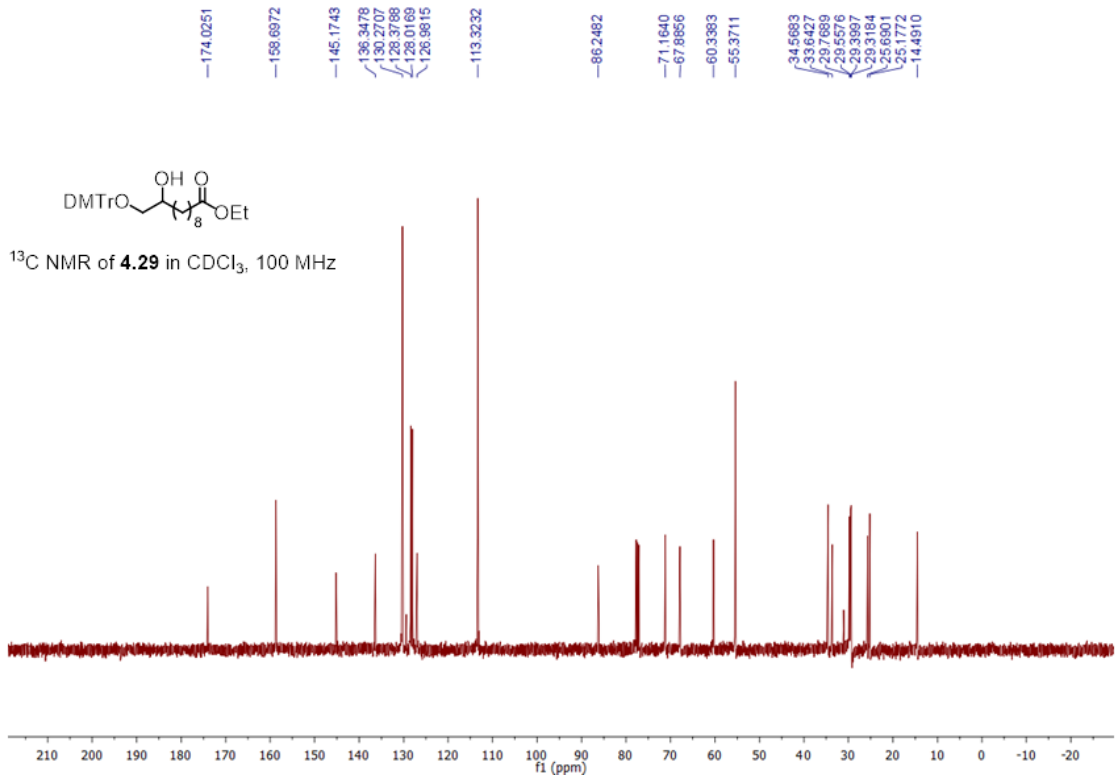


Figure C.34. ^{13}CNMR of Compound **4.29**

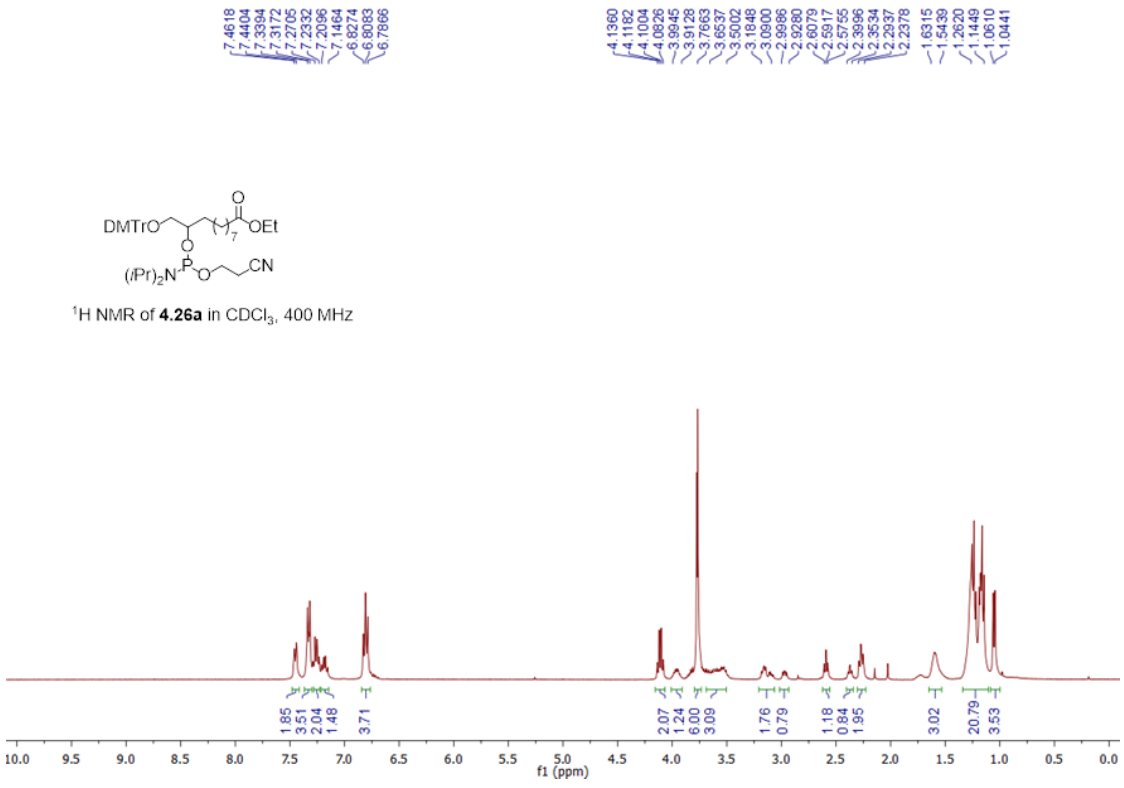


Figure C.35. [1]HNMR of Compound **4.26a**

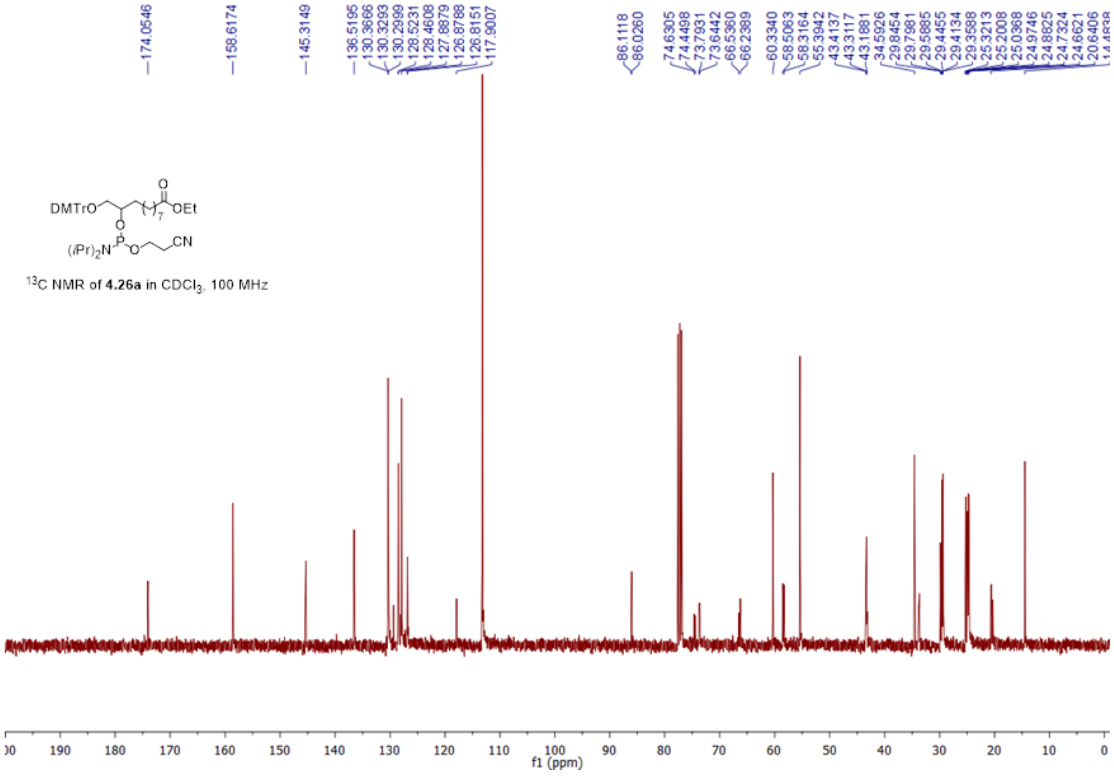


Figure C.36. ^{13}CNMR of Compound **4.26a**

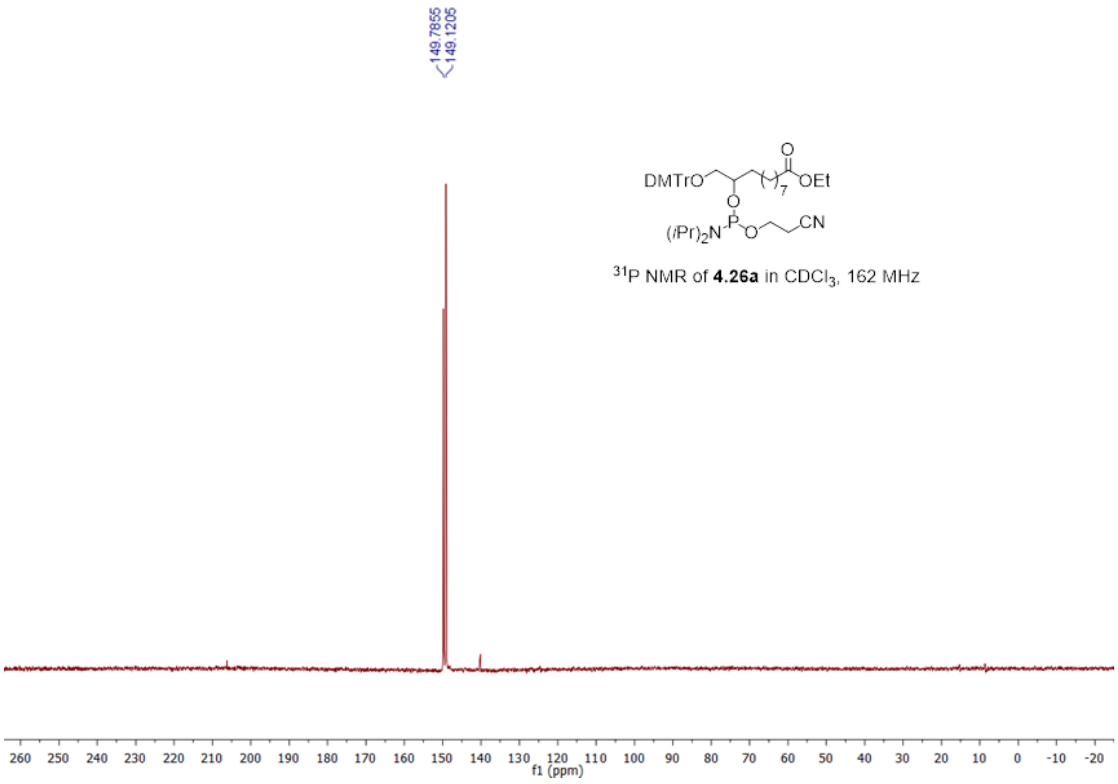


Figure C.37. ^{31}PNMR of Compound **4.26a**

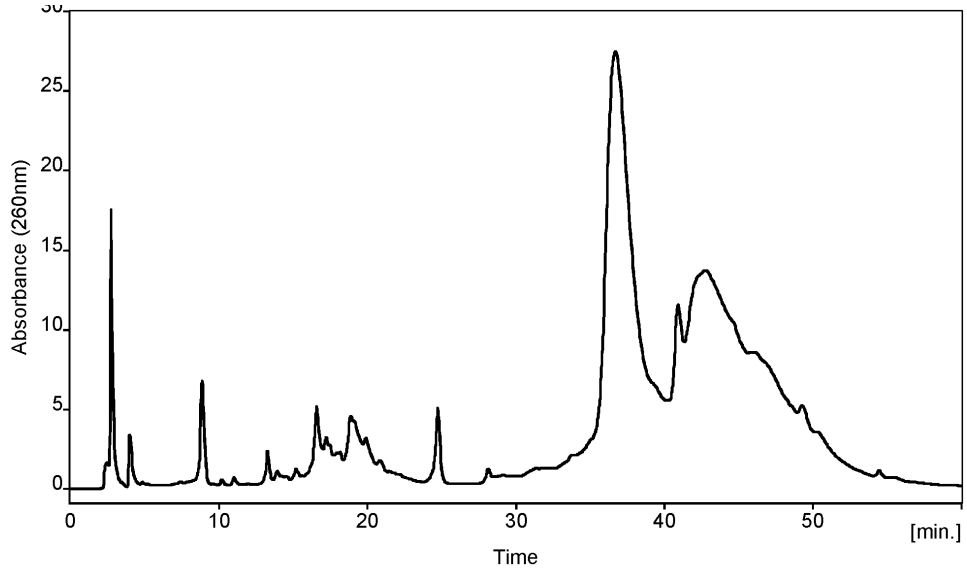


Figure C.38. RP HPLC crude trityl-tagged ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**4.30a-tr**). The peak at 35-39min corresponds to the trityl-tagged ODN. The peaks after 40min correspond to branched sequences.

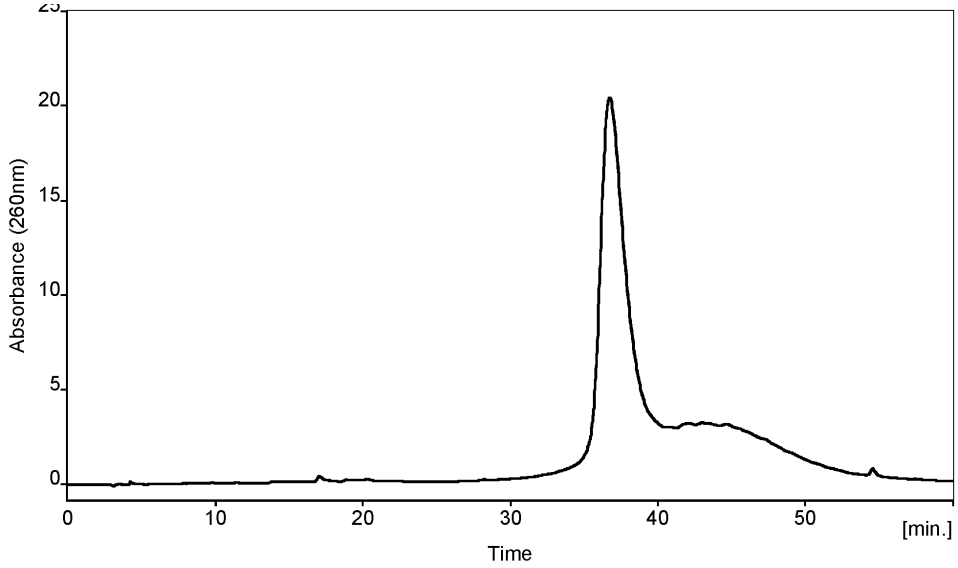


Figure C.39. RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**4.30a-tr**).CTA CT-3' (**4.30a-tr**).

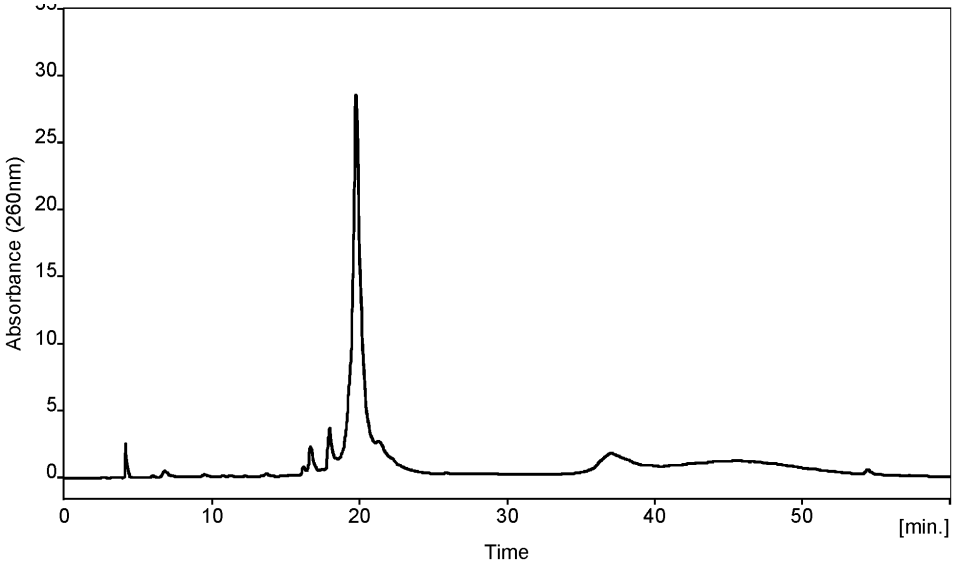


Figure C.40. RP HPLC profile of de-tritylated ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**4.30a**).

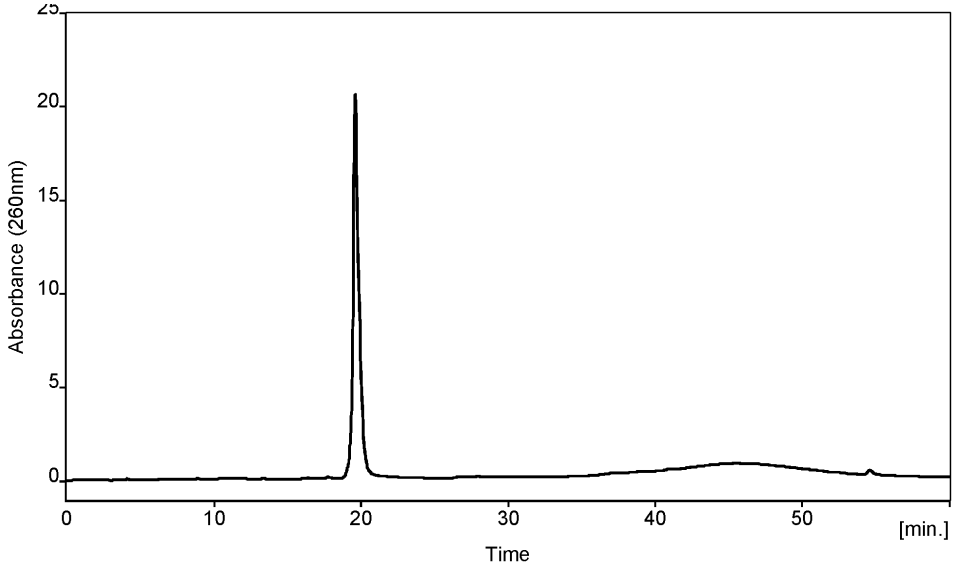


Figure C.41. RP HPLC profile of pure de-tritylated ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (**4.30a**).

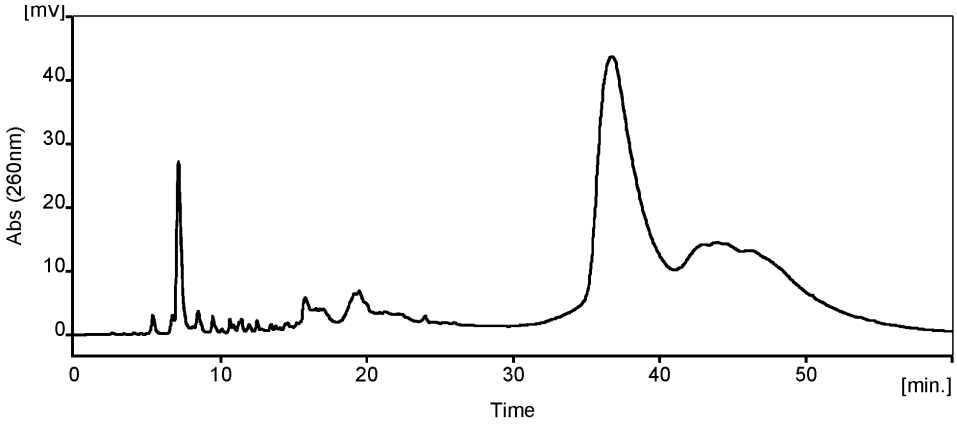


Figure C.42. RP HPLC profile of crude trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**4.30b-tr**). The peak at 35-39 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

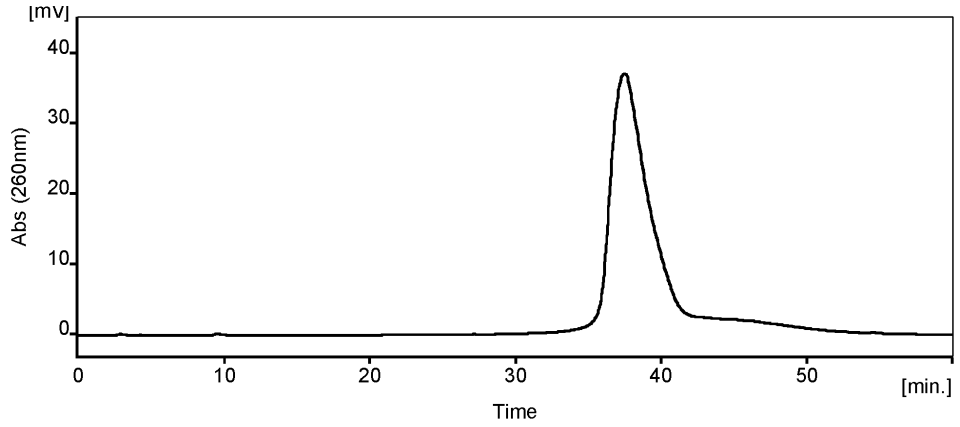


Figure C.43. RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**4.30b-tr**).

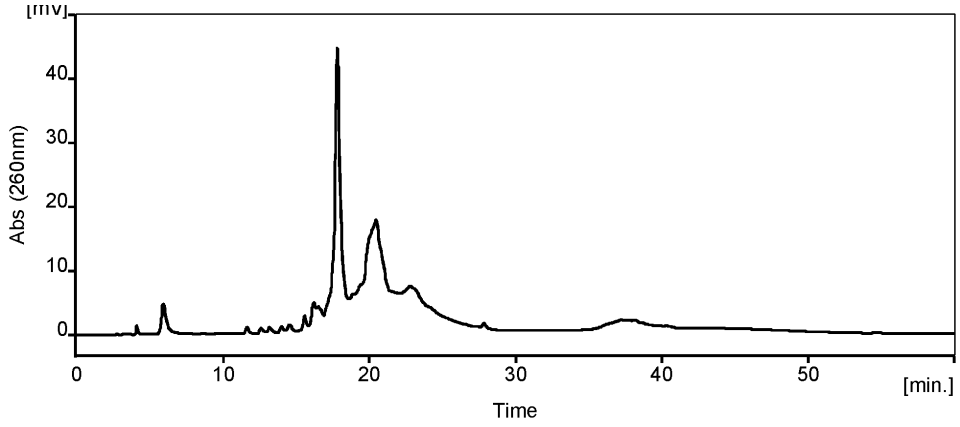


Figure C.44. RP HPLC profile of de-tritylated ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**4.30b**).

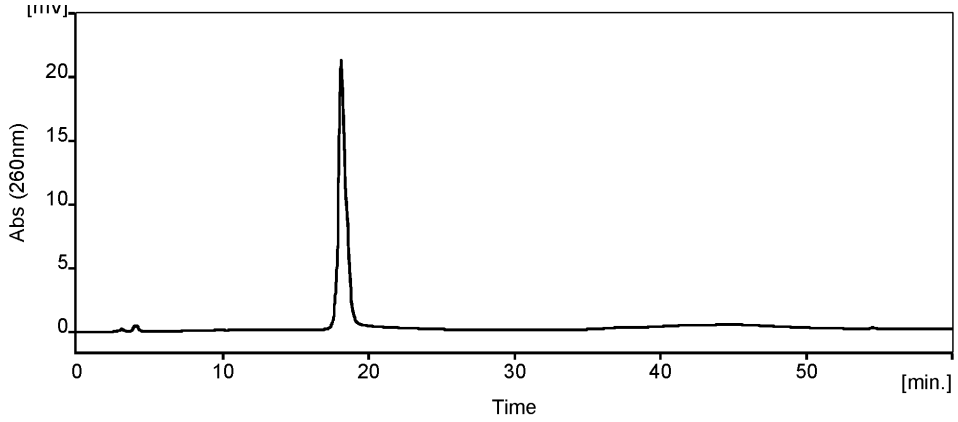


Figure C.45. RP HPLC profile of pure de-tritylated ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (**4.30b**).

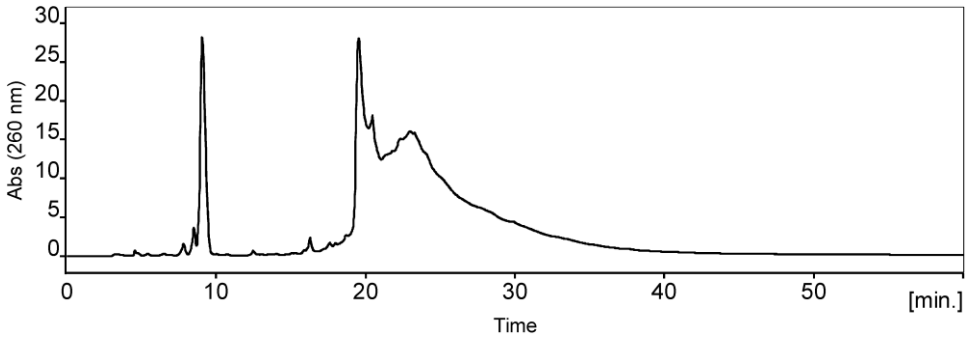


Figure C.46. A typical RP HPLC profile of crude ODN (5'-CTA GAT AAC TCA TAG TAC TT-3') synthesized using **4.3a-c** and **4.4** under standard conditions using acetic anhydride for capping and without 5'-tagging with hydrophobic groups such as trityl and DMTr groups. The peak between 19 and 21 min corresponds to the ODN. The peaks after 21 min correspond to branched sequences. Because the desired ODN and branched sequences were very close, ODN purification was difficult.

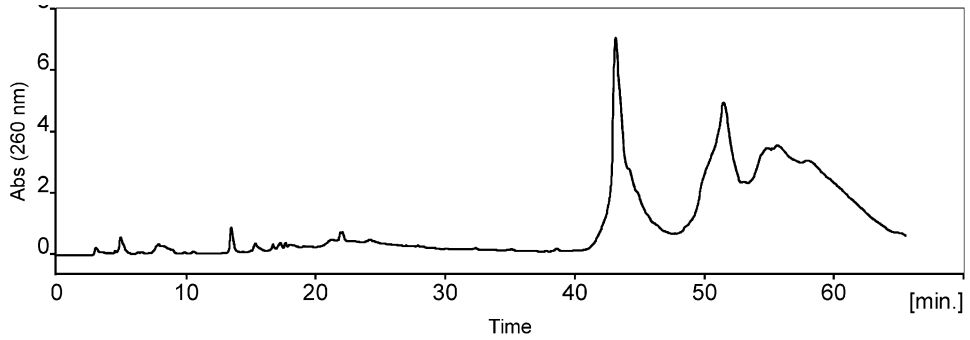


Figure C.47. RP HPLC profile of the crude ODN 5'-DMTr-O-TTC CAT CCT AGA AAG CTC AT-3' synthesized using **4.3a-c** and **4.4** under standard conditions using acetic anhydride for capping. At the end of synthesis, the DMTr group was not removed. Although not always possible, in this case, the DMTr protection survived the cleavage and deprotection conditions involving sodium periodate. The peak in the profile between 43 and 45 min corresponds to the DMTr-tagged ODN. The peaks after 47 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more DMTr groups.

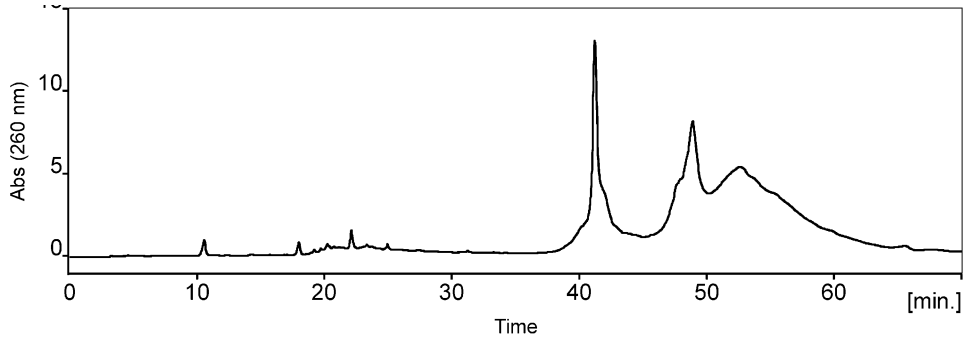


Figure C.48. A typical RP HPLC profile of crude ODN (5'-TBDPS-O-CTA GAT AAC TCA TAG TAC TT-3') synthesized using **4.3a-c** and **4.4** under standard conditions using acetic anhydride for capping and tagged with a TBDPS group at the 5'-end. The TBDPS, which is the *t*Bu(Ph_2)Si- group, was introduced after solid phase synthesis (5'-DMTr group removed) and before cleavage and deprotection by soaking the CPG in 0.1 M *t*Bu(Ph_2)SiCl and 0.1 M imidazole in DMF (rt, 12 h). Cleavage and deprotection were then carried out as described in the article. The peak between 41 and 42 min corresponds to the tagged ODN. The peaks after 43 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more TBDPS groups. The approach separated the desired ODN from the branched sequences very well, but at this stage, we cannot identify a mild condition that is compatible with sensitive modifications on ODNs to remove the TBDPS group after the ODN is purified.

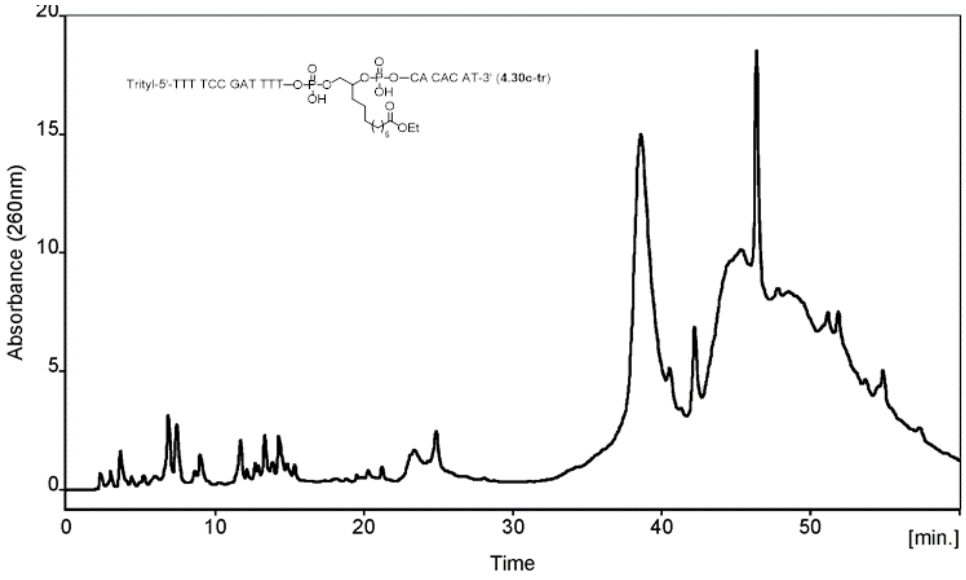


Figure C.49. RP HPLC profile of crude trityl-tagged ODN **4.30c-tr**. The peak at 37-40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

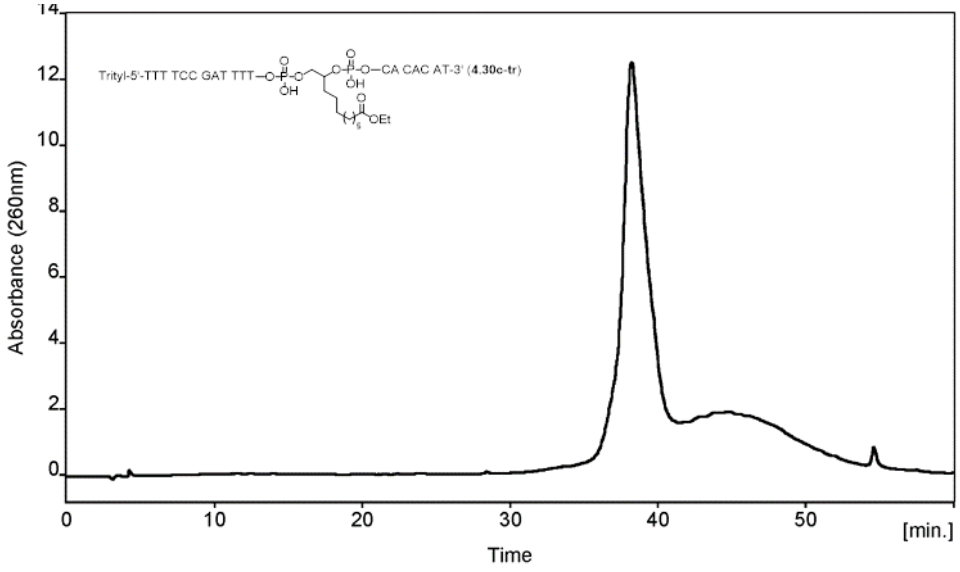


Figure C.50. RP HPLC profile of purified trityl-tagged ODN **4.30c-tr**.

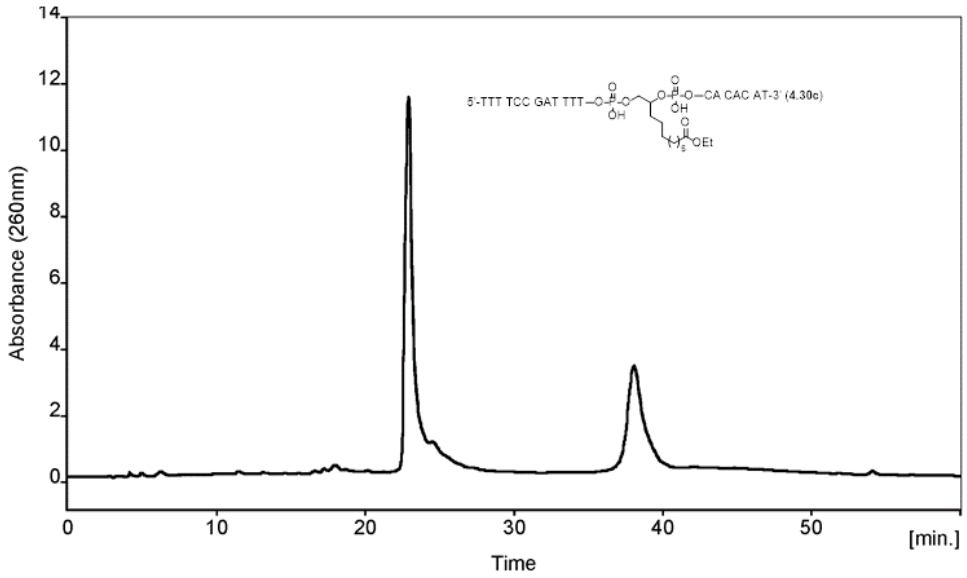


Figure C.51. RP HPLC profile of de-tritylated ODN **4.30c**.

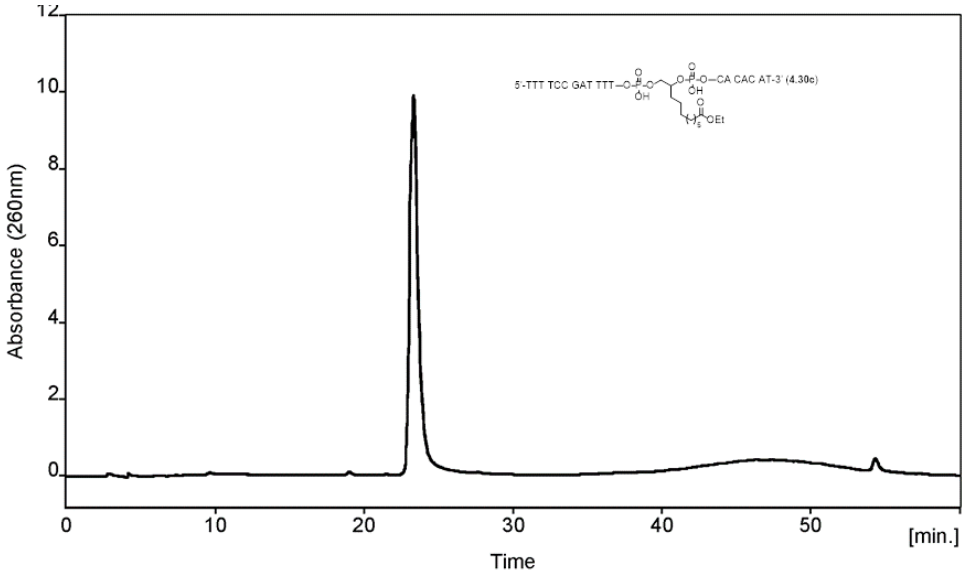


Figure C.52. RP HPLC profile of purified de-tritylated ODN **4.30c**.

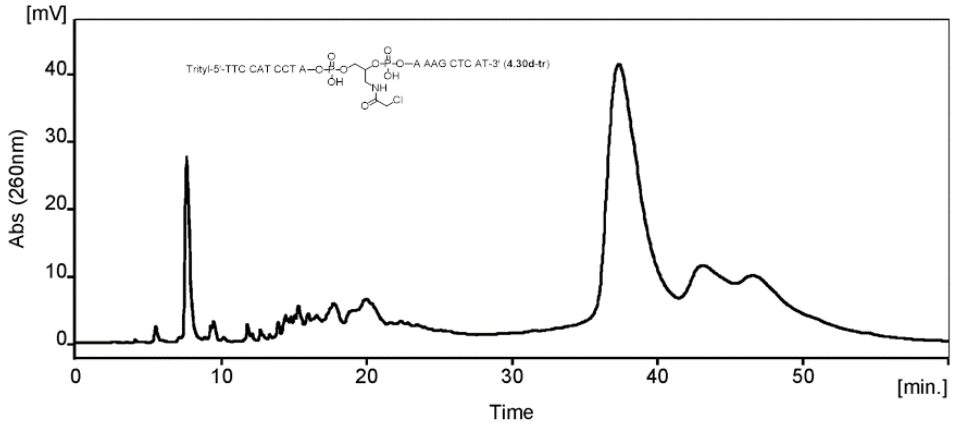


Figure C.53. RP HPLC profile of crude trityl-tagged ODN **4.30d-tr**. The peak at 37-40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

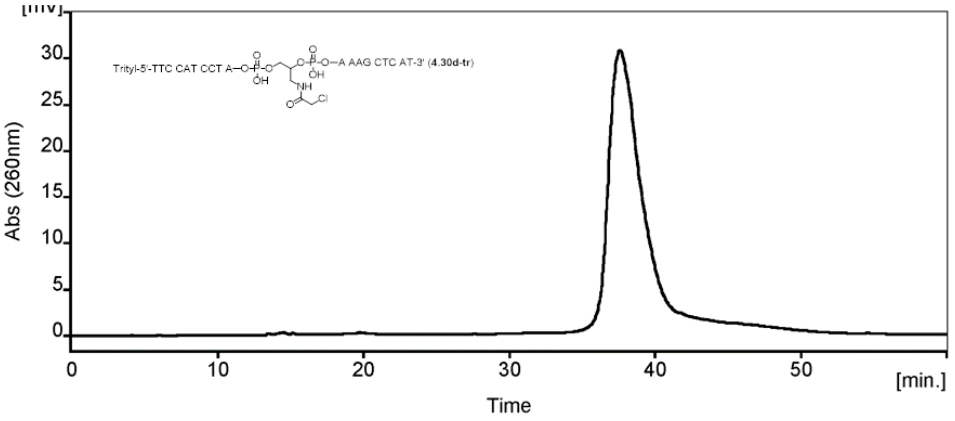


Figure C.54. RP HPLC profile of purified trityl-tagged ODN **4.30d-tr**.

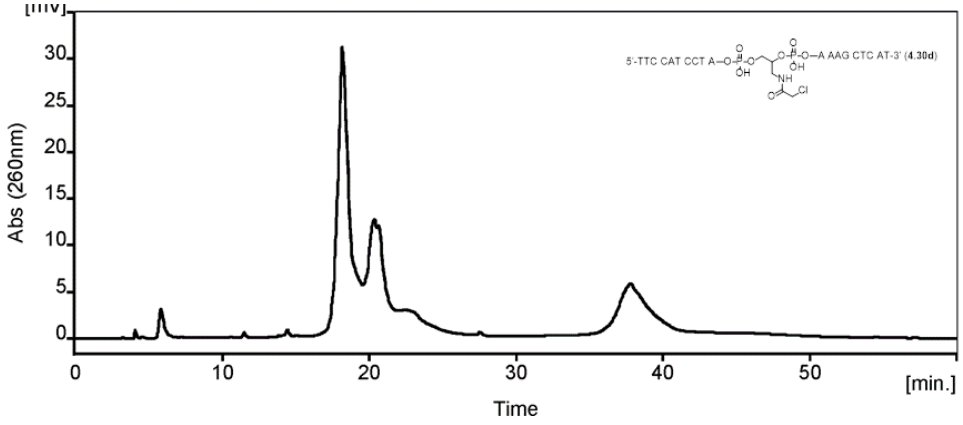


Figure C.55. RP HPLC profile of de-tritylated ODN **4.30d**.

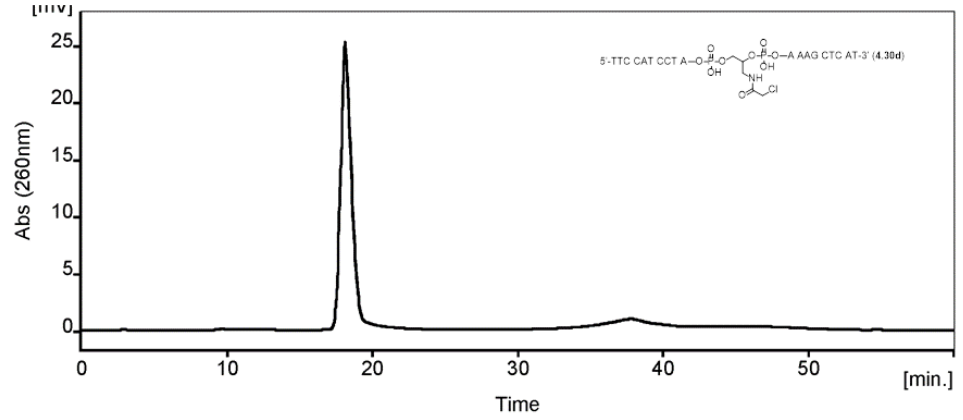


Figure C.56. RP HPLC profile of purified de-tritylated ODN **4.30d**.

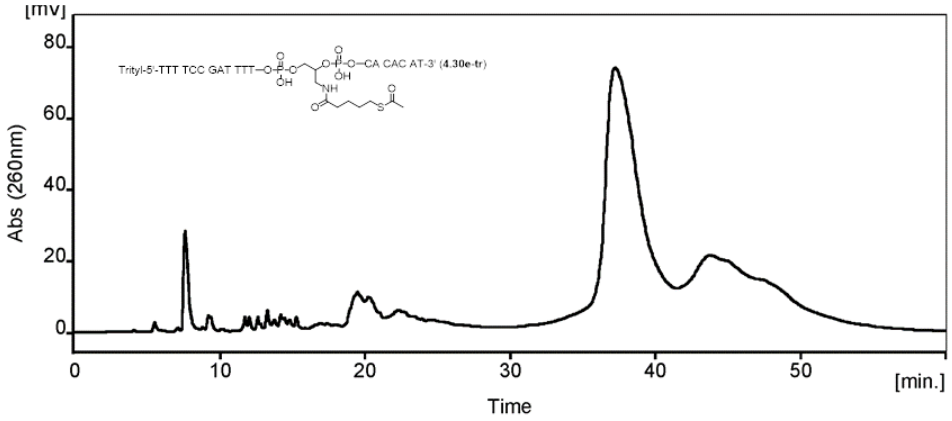


Figure C.57. RP HPLC profile of crude trityl-tagged ODN **4.30e-tr**. The peak at 37-40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

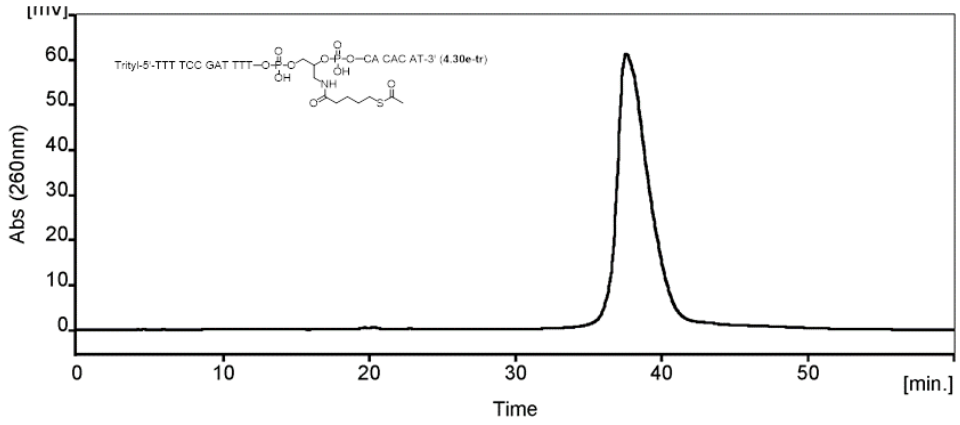


Figure C.58. RP HPLC profile of purified trityl-tagged ODN **4.30e-tr**.

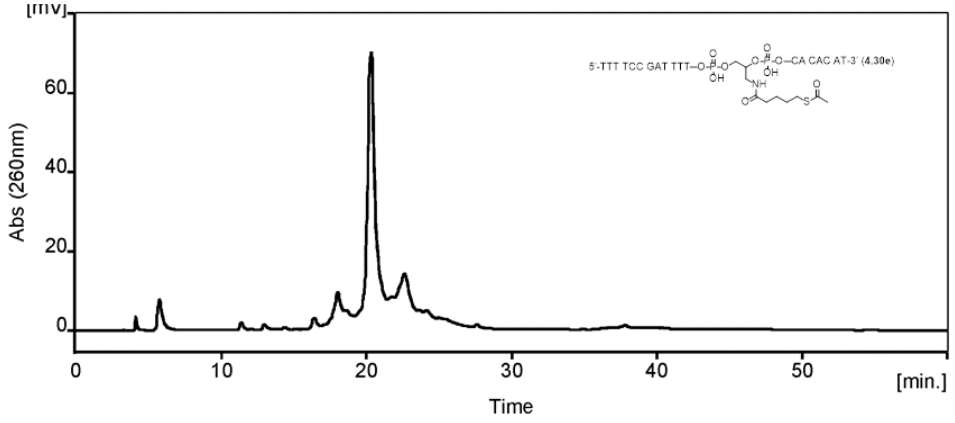


Figure C.58. RP HPLC profile of de-tritylated ODN **4.30e**.

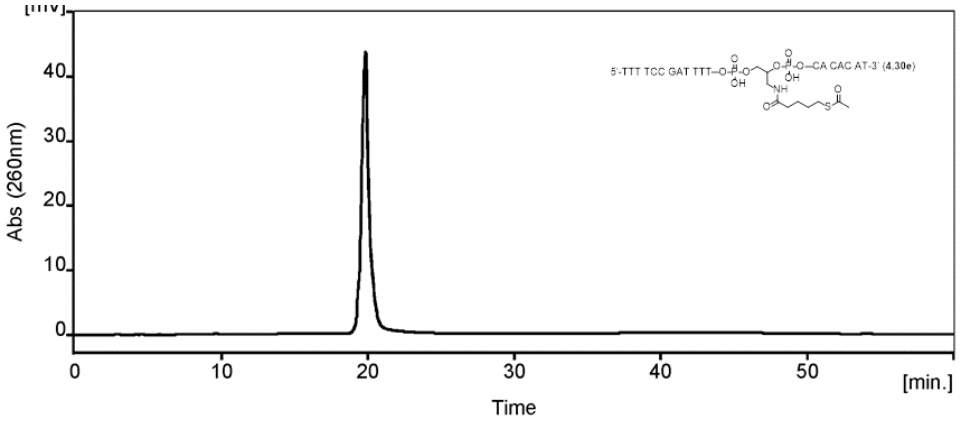


Figure C.59. RP HPLC profile of purified de-tritylated ODN **4.30e**.

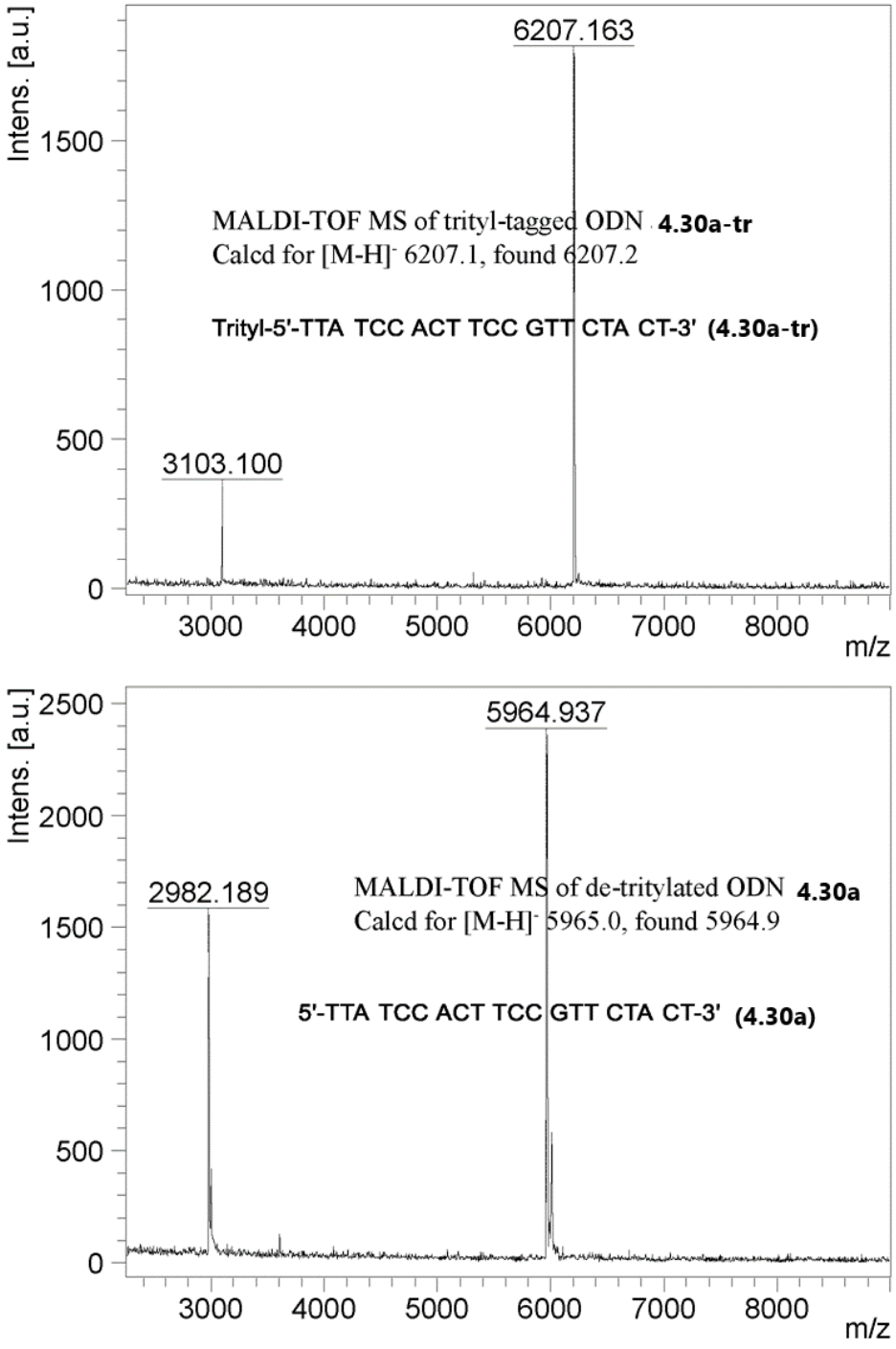


Figure C.59. MALDI-TOF-MS of compound **4.30a-tr** and **4.30a**, respectively

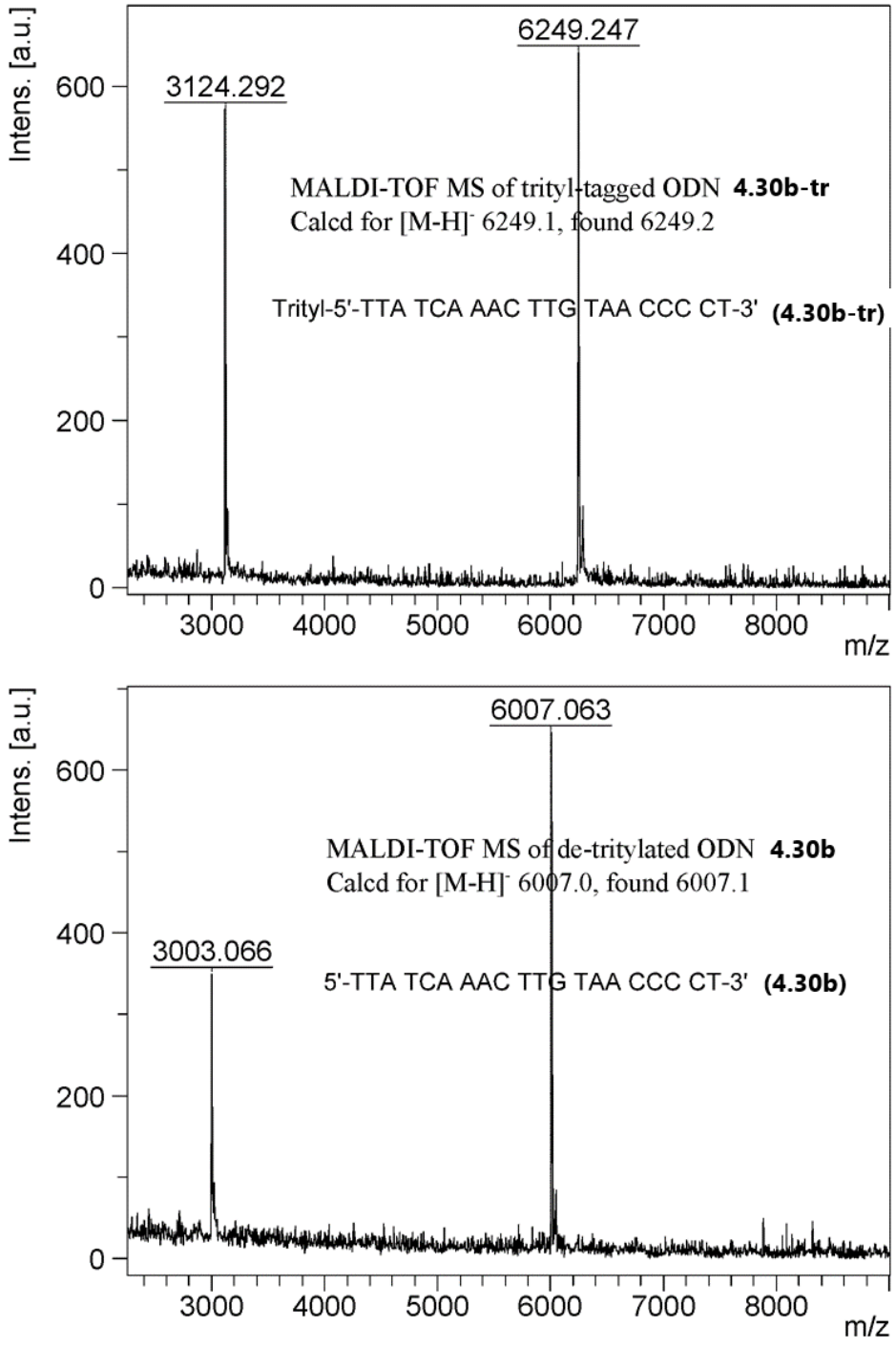


Figure C.60. MALDI-TOF-MS of compound **4.30b-tr** and **4.30b**, respectively

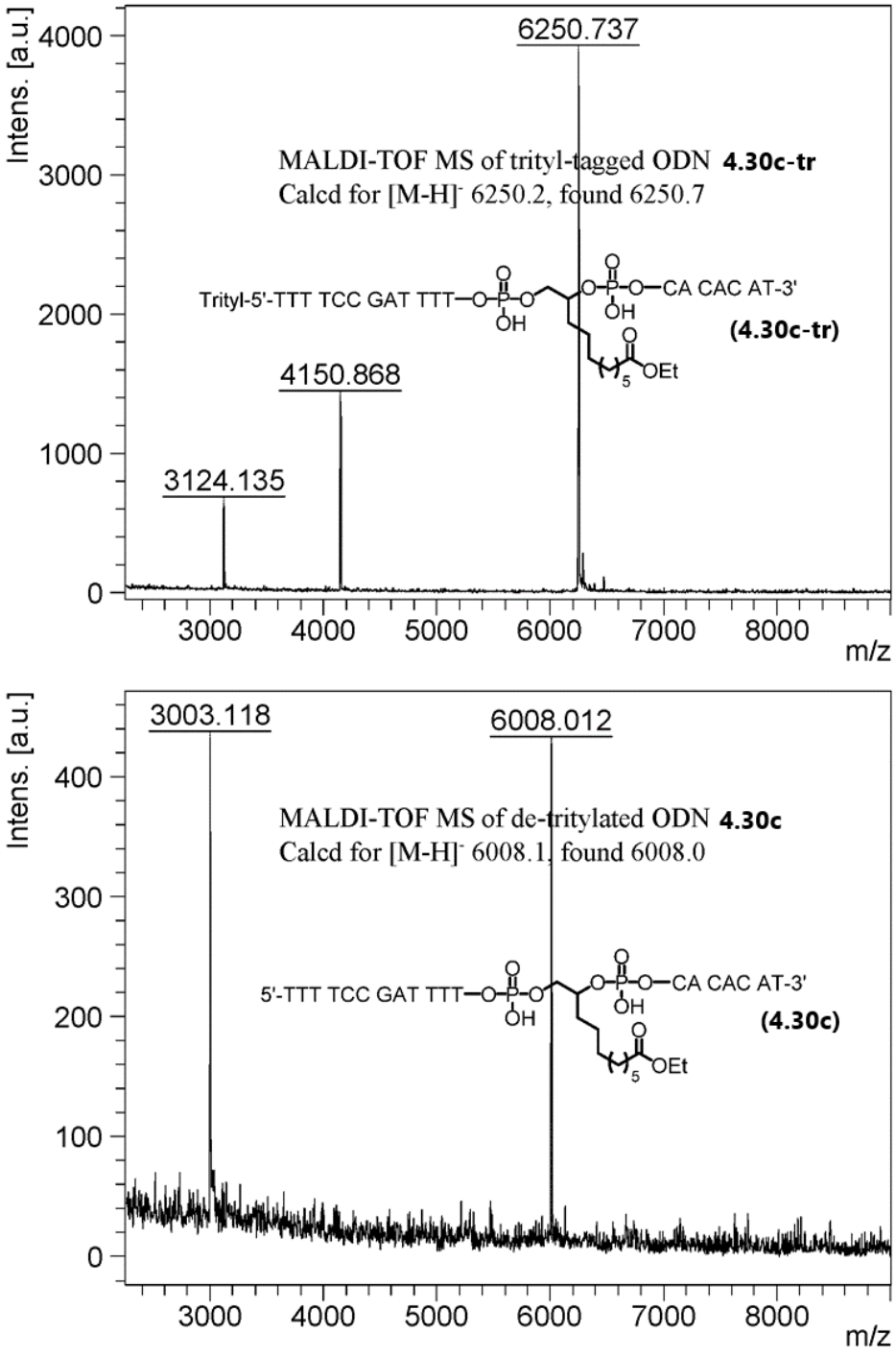


Figure C.61. MALDI-TOF-MS of compound **4.30c-tr** and **4.30c**, respectively

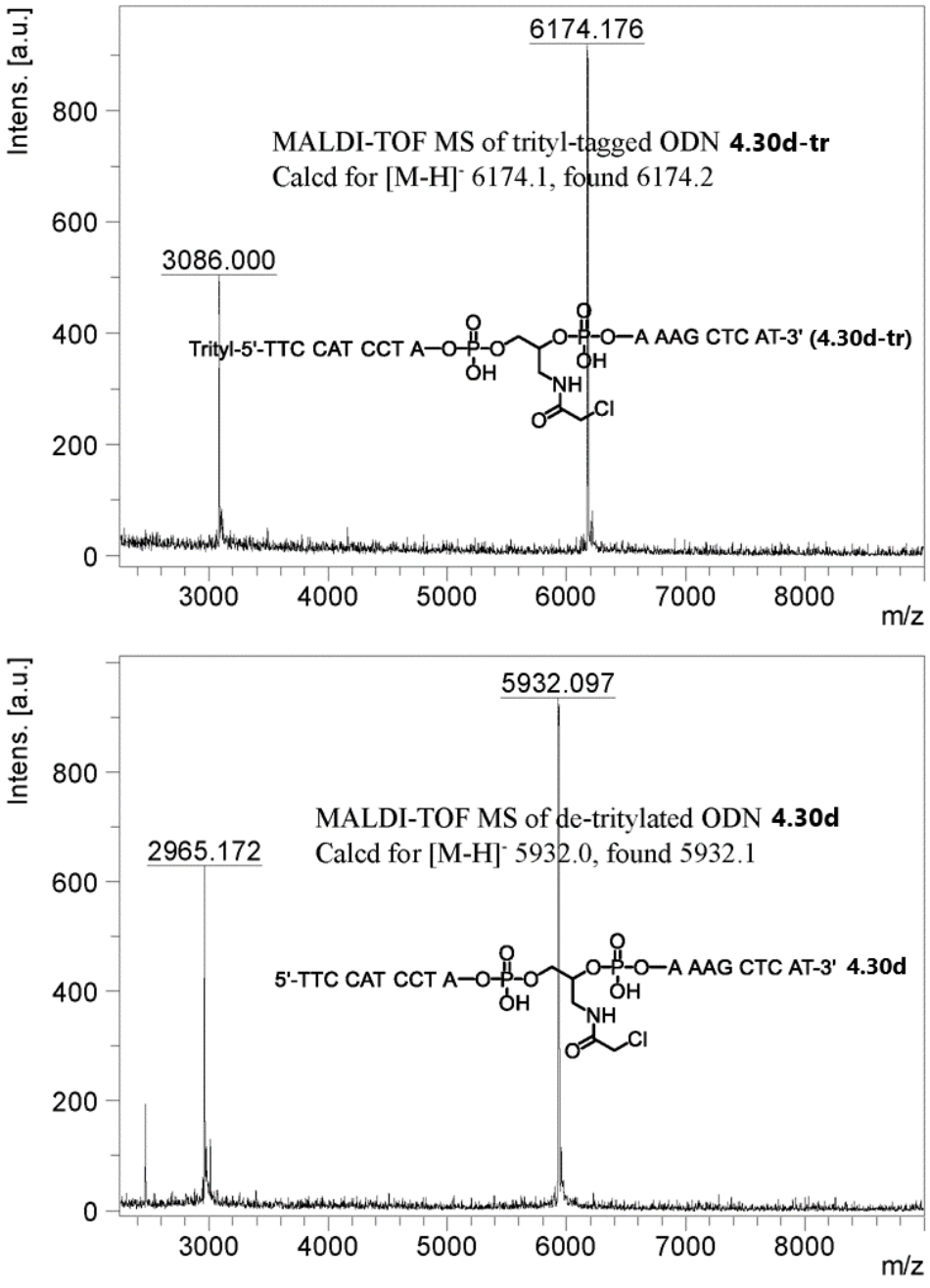


Figure C.62. MALDI-TOF-MS of compound **4.30d-tr** and **4.30d**, respectively

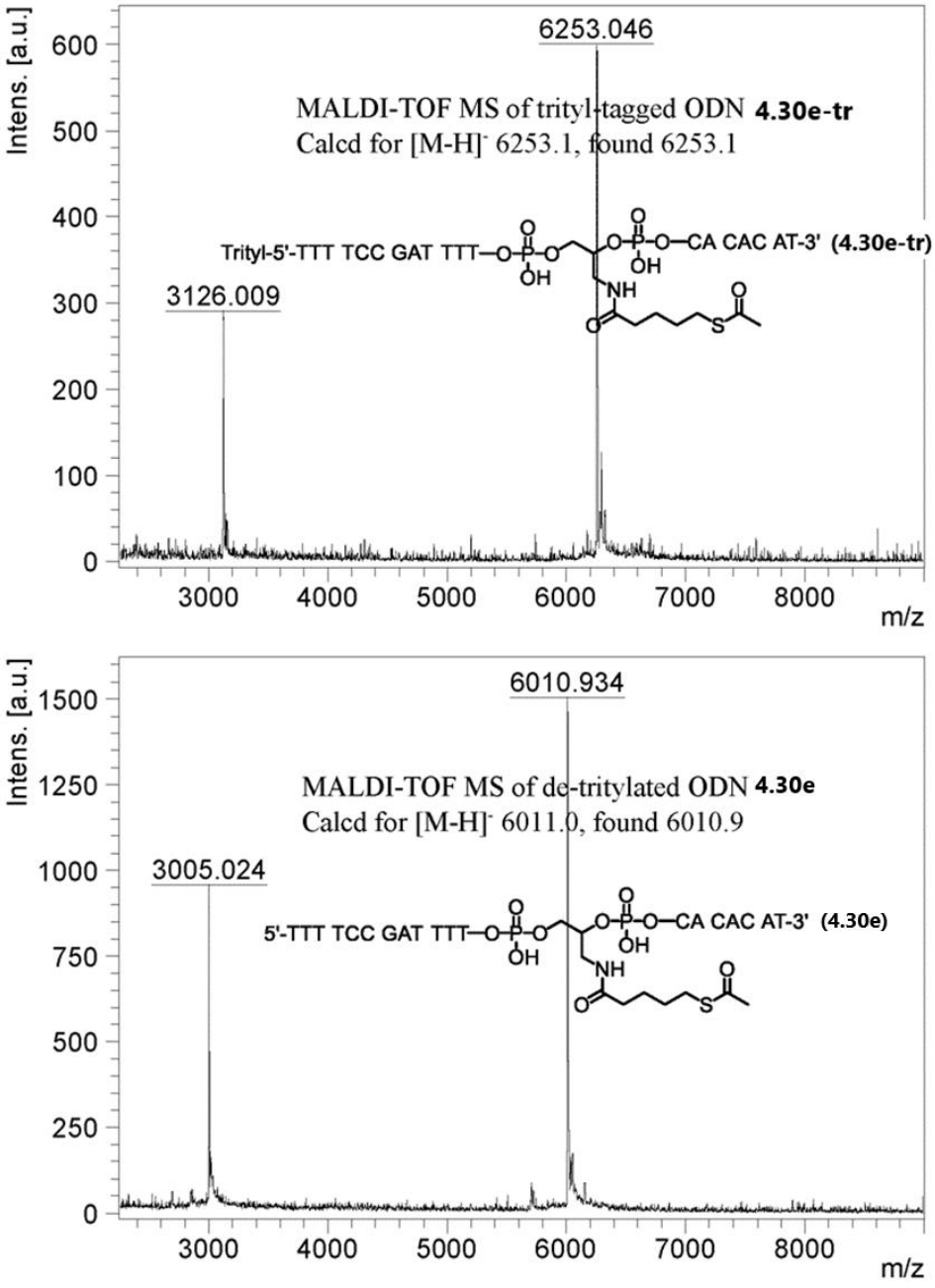


Figure C.63. MALDI-TOF-MS of compound **4.30e-tr** and **4.30e**, respectively