

# CONCORDIA UNIVERSITY

## School of Graduate Studies

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By: **Ernest Palus**

Entitled: **Synthesis of 7-Deaza-deoxyguanosine Analogs as Probes To Investigate DNA Repair**

and submitted in partial fulfillment of the requirements for the degree of

**Master of Science (Chemistry)**

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Synthesis of 7-Deaza-deoxyguanosine Analogs as Probes To  
Investigate DNA Repair

Ernest Palus

A Thesis  
In  
The Department  
Of  
Chemistry and Biochemistry

Presented in Partial Fulfillment of the Requirements  
for the Degree of Master of Science (Chemistry) at  
Concordia University  
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## Abstract

Synthesis of 7-Deaza-deoxyguanosine Analogs as Probes To Investigate DNA Repair

Ernest Palus

Interstrand cross-links can be introduced into DNA as a consequence of chemotherapy. These lesions are considered to be therapeutically relevant and responsible for the efficacy of bifunctional alkylating agents in cancer chemotherapy. There is considerable evidence that indicates interstrand cross-links are recognized by cells and that this recognition can either mediate repair of damage and survival, or accelerate cell death. Development of tumors resistant to these agents is an important factor in the lack of response by some patients to therapy with these agents. Relatively little is known about the mechanism by which interstrand cross-links are recognized and repaired. In order to investigate repair mechanisms, interstrand cross-linked DNA substrates of well defined structure are required. Such substrates are challenging to prepare in quantities sufficient for biochemical and biophysical studies.

A method to synthesize a chemically stable analog of the clinically relevant N7G-alkyl-N7G cross-link was explored. This structural analogue links the C7 atoms of two molecules of 7-iodo-7-deaza-2'-deoxyguanosine with an alkyldiyne linker to form a dimer via the Sonogashira reaction. Several nucleoside dimers were produced using this strategy as proof of principle that it could be employed in the synthesis of interstrand cross-linked DNA. However, in addition to the desired cross-coupling reaction it would appear that a copper catalyzed homocoupling side reaction can also occur.

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I would like to thank Dr. Christopher J. Wilds for providing me the opportunity to work on this interesting and challenging project, for his patient guidance in the course of the work and in the preparation of this thesis.

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*Dedicated to my wife Dana and son Jakub whose patience, support and love have made  
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## List of Abbreviations

<b>A</b>	adenine
<b>Ar</b>	aromatic
<b>br s</b>	broad singlet
<b>C</b>	cytosine
<b>cal</b>	calculated
<b>dCMP</b>	deoxy-cytidine-5'-monophosphate
<b>DCM</b>	dichloromethane
<b>DMAP</b>	N,N-dimethyl aminopyridine
<b>DMF</b>	dimethyl formamide
<b>DMS</b>	dimethylsulfate
<b>DNA</b>	deoxyribose nucleic acid
<b>DTT</b>	dithiothreitol
<b>EtOAc</b>	ethyl acetate
<b>FAPY</b>	formamidopyrimidine
<b>G</b>	guanine
<b>hAGT</b>	human O <sup>6</sup> -alkylguanine-DNA alkyltransferase
<b>iBu</b>	isobutyryl
<b>ICL</b>	interstrand cross-link
<b>m</b>	multiplet
<b>MeOH</b>	methanol
<b>MCPBA</b>	meta-chloroperoxybenzoic acid
<b>MP</b>	melting point
<b>NIS</b>	N-iodosuccinimide
<b>O<sup>6</sup>-MedG</b>	O6-methyldeoxyguanosine
<b>NMR</b>	Nuclear Magnetic Resonance
<b>RT</b>	room temperature
<b>s</b>	singlet
<b>T</b>	thymine
<b>THF</b>	tetrahydrofuran

<b>TMP</b>	2'-deoxythymidine 5'-monophosphate
<b>TMS</b>	trimethylsilyl
<b>TLC</b>	thin layer chromatography
<b>Tol</b>	toluoyl
<b>TTP</b>	2'-deoxythymidine 5'-triphosphate
<b>U</b>	uracil

# CHAPTER 1

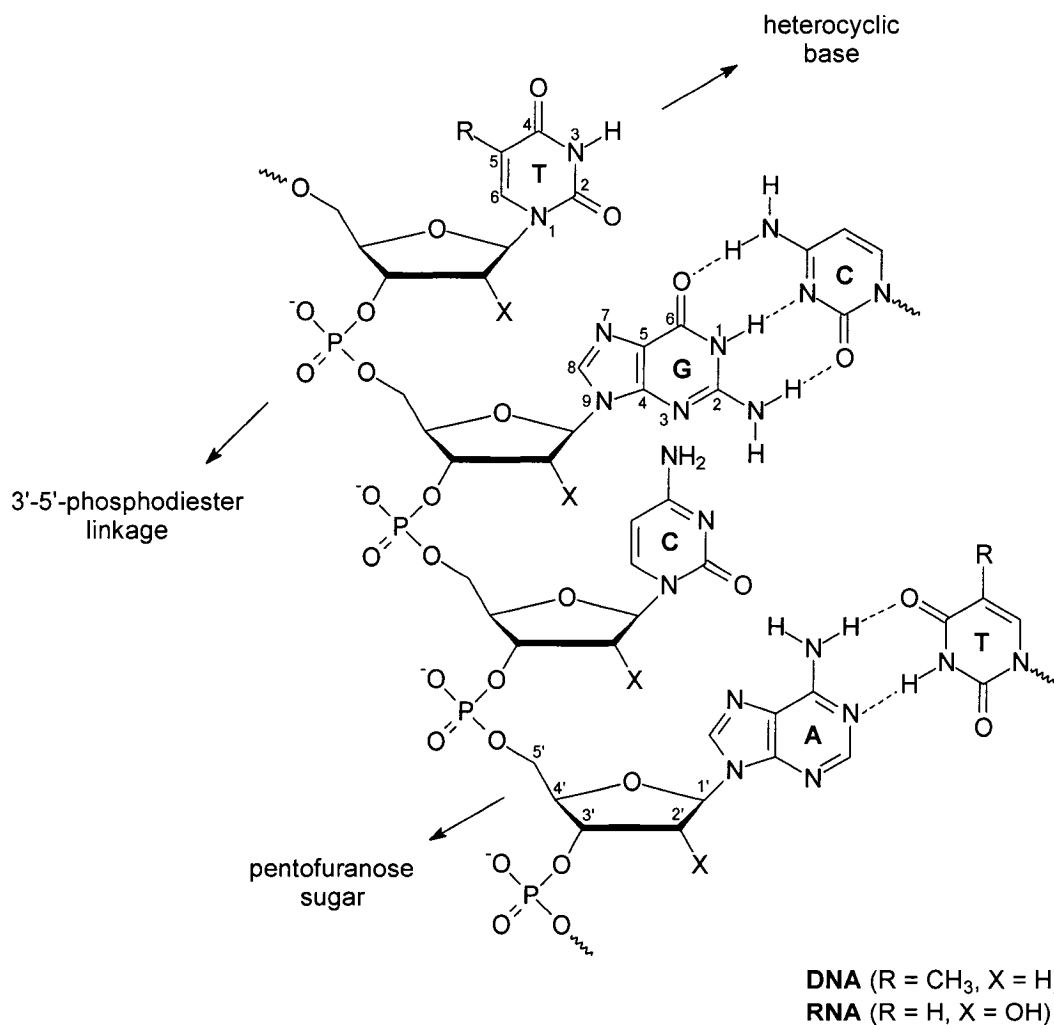
## INTRODUCTION

### 1.1. Importance of Nucleic Acids<sup>1,2,3,4,5</sup>

Nucleic acids are fundamentally important components of all living entities as they contain “all the information” needed by cells to live and propagate. Despite this important role in life’s processes they are by themselves highly unreactive and stable outside the cell. There are two types of nucleic acids: DNA (deoxyribonucleic acids) and RNA (ribonucleic acids), both of which are polymers of alternating pentofuranose sugar and phosphate residues (**Figure 1.1**). Attached to the C1’ of the sugar atom is a purine or pyrimidine base. The configuration of this linkage is  $\beta$ , as the base lies on the same side of the pentofuranose sugar as the C5’ hydroxymethyl group. The DNA chain is said to have directionality - one end of the chain has a free 5’-hydroxyl (OH) group and the other end a free 3’-OH.

DNA usually exists as a double stranded (ds) helix under native conditions. The two strands are held together in an antiparallel fashion by Watson-Crick hydrogen-bonds between composite purine and pyrimidine bases. Three of the bases are common to both DNA and RNA: adenine, cytosine and guanine. The fourth DNA base, thymine, is replaced by uracil in RNA. The genetic information resides in the sequence of these bases and is characteristic for each organism. This complementarity between the bases (G with C and A with T or U) constitutes one of the major stabilizing interactions (hydrogen bonds) and it also contributes to the intrinsic double helical nature of DNA.

The second major interaction is hydrophobic and results in the vertical stacking of pi ( $\pi$ ) electron systems of the coplanar heterocyclic bases along the vertical axis of the double helix. These two base-derived stabilizing interactions, along with steric and hydrophobic interactions, impart to the DNA molecule the stability necessary to preserve life. The four nucleobases ensure fidelity of genetic information transfer while the sugar-phosphate backbone imparts structure, flexibility and hydrophilicity.

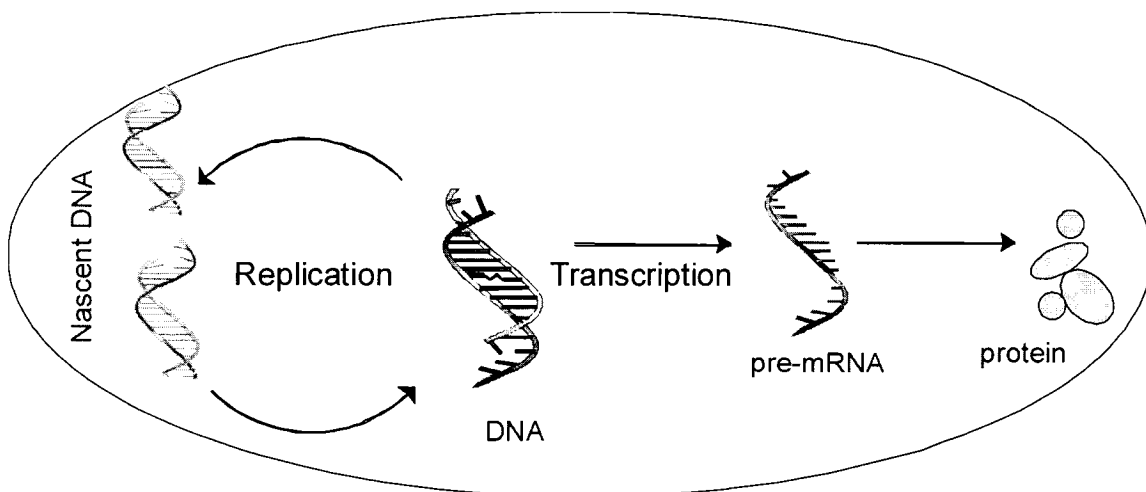


**Figure 1.1:** Schematic representation of the structure of DNA and RNA. The numbering systems for furanose sugar, heterocyclic bases and the Watson-Crick binding of complementary nucleoside bases is also shown.



RNA molecules are usually single stranded (ss) and composed of ribonucleotide units. They are generally shorter than DNA, possessing several different functions and tertiary structures such as messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). RNA acts as an intermediary in gene expression passing on information from DNA to ultimately form protein. Thus DNA, which is chemically more stable than RNA, emerged more ideally suited for the storage of genetic information during the course of evolution.<sup>6</sup>

The path from DNA to protein synthesis begins in a cell's nucleus, when the double-stranded DNA can either be replicated to produce nascent DNA strands (replication) or it can unwind from one point to another to allow for the synthesis of RNA (transcription) (**Figure 1.2**). Finally, the process of translation occurs where RNA is converted to protein. This transfer of biological information from a stored form (typically double helical DNA) to functional polypeptides *via* mRNA intermediates comprises the “central dogma of molecular biology”.<sup>7</sup>



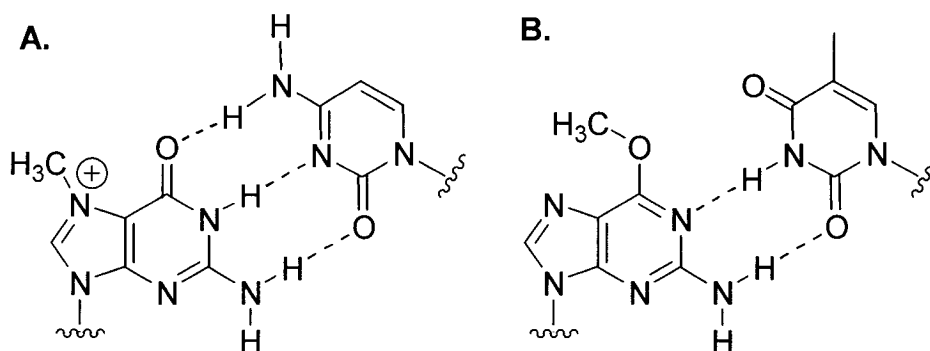
**Figure 1.2:** The flow of biological information in living cells.

## 1.2 DNA Damage and Repair

Despite its stability under physiological conditions, DNA is constantly being assaulted by a large number of agents that can have severe effects if the induced damage persists. Modification of DNA can lead to mutations, which alter the coding sequences of DNA and can lead to cancer in mammals. As a result of this exposure, DNA can be damaged at different levels leading to changes within the structure of DNA such as depurination, oxidation or deamination to name a few.

The ability of alkylating agents to act upon DNA to induce damage has been exploited in various techniques to study DNA. For example, the ability of dimethylsulfate to preferentially alkylate deoxyguanosine at the N7 atom is utilized in the Maxam-Gilbert DNA sequencing technique, where such adducts result in an extremely labile glycosidic bond that may be depurinated followed by cleavage of the DNA strand on either side of the residue under alkaline conditions.<sup>8</sup>

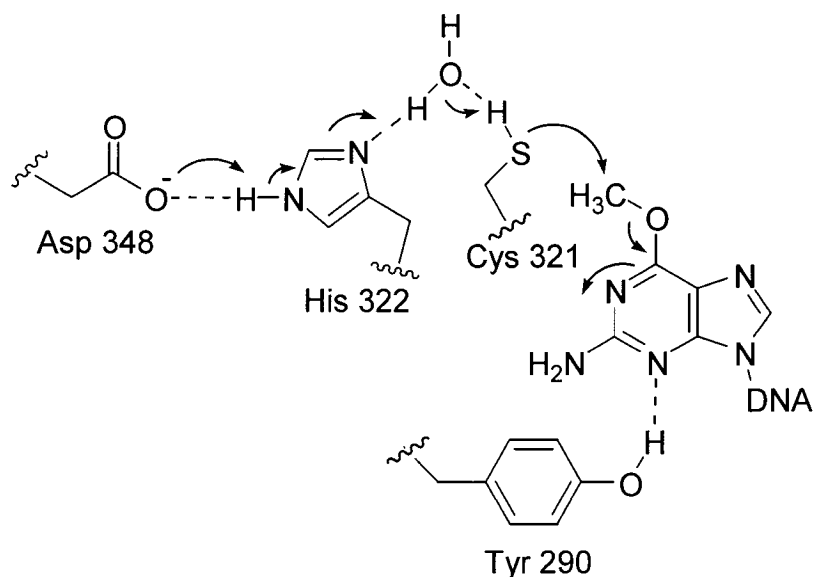
Other alkylating agents such as *N*-methyl-*N*-nitrosourea have been shown to halt tumor growth. Nitrosamines have been shown to alkylate at both the oxygen atoms and endocyclic nitrogen atoms of DNA, with the major site of alkylation (> 85 %) occurring at the non-bridging phosphate oxygens and N<sup>7</sup> position of guanine.<sup>9</sup> NMR studies of DNA duplexes containing a single N<sup>7</sup>-methylguanine base exhibit little change in DNA structure.<sup>10</sup> However, alkylation at the O<sup>6</sup> position of guanine has severe effects by changing the imino nitrogen from a hydrogen bond donor to a hydrogen bond acceptor, preventing the formation of Watson-Crick base pairing arrangements (**Figure 1.3**).<sup>11</sup>



**Figure 1.3:** **A.** N7-methylguanine and **B.** O6-methylguanine residues. Alkylation at the O6 position allows for formation of a stable O6-MedG·T base pair.

It has been shown that bacterial DNA polymerases can incorporate dCMP and TMP opposite O<sup>6</sup>-MedG during *in vitro* primer extension studies, with TTP being the preferential substrate.<sup>12</sup> As a result, this lesion can result in a dG·dC → dA·T transition mutation *in vivo*.

While monoalkylation of a nucleobase may have detrimental effects on an organism, cells are able to reverse this damage relatively easily. For example N<sup>7</sup>-methylguanine can be removed by depurination with the abasic site being eventually repaired.<sup>13</sup> In humans O<sup>6</sup>-alkylated guanines are repaired directly by human O<sup>6</sup>-alkylguanine-DNA alkyltransferase which is capable of removing an alkyl lesion when guanine is base paired with cytosine in a DNA duplex. In the active site of this enzyme a thiolate anion is formed which acts as a nucleophile to transfer the alkyl group from the alkylated guanine base to hAGT (**Figure 1.4**).<sup>14</sup>

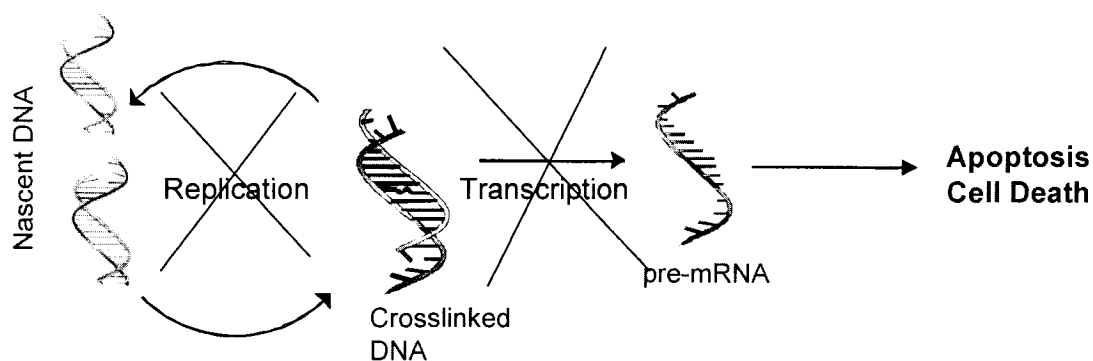


**Figure 1.4:** Reaction site and hydrogen bond network in the active site of hAGT protein (Adapted from Daniels, D. S.; Woo, T. T.; Luu, K. X.; Noll, D. M.; Clarke, N. D.; Pegg, A. E.; Tainer, J. A. *Nat. Struct. Mol. Biol.* 2004, 11, 714.)

### 1.3 Bifunctional Alkylating Agents

Bifunctional alkylating agents represent a much greater threat to the cell, as they give rise to number of different lesions including monoalkylated adducts, intrastrand cross-links where two nucleobases on the same strand are connected by the cross-linking agent and interstrand cross-links (ICL) where the bases on adjacent strands are linked. These adducts represent the most toxic of all alkylation events. Such agents are quite abundant in the environment and within the cell (endogenous). Acrolein and crotonaldehyde are both formed in the cell as a result of lipid peroxidation in addition to being present in tobacco smoke. These molecules possess two electrophilic centres and can thus form crosslinks in DNA.<sup>15,16</sup> There is a high correlation between the ability of bifunctional alkylators to form ICL in DNA and the degree of cytotoxicity. ICL

formation effectively prevents separation of strands and consequently blocks processes of transcription and replication which eventually lead to cell death (**Figure 1.5**). Cells have evolved several ways to neutralize these detrimental effects of damaged DNA and there are various DNA repair pathways that can remove lesions from DNA. The importance of DNA repair is underscored by several human syndromes that are caused by defects in DNA-repair genes. A hallmark feature of these diseases is a dramatically increased predisposition to cancer.

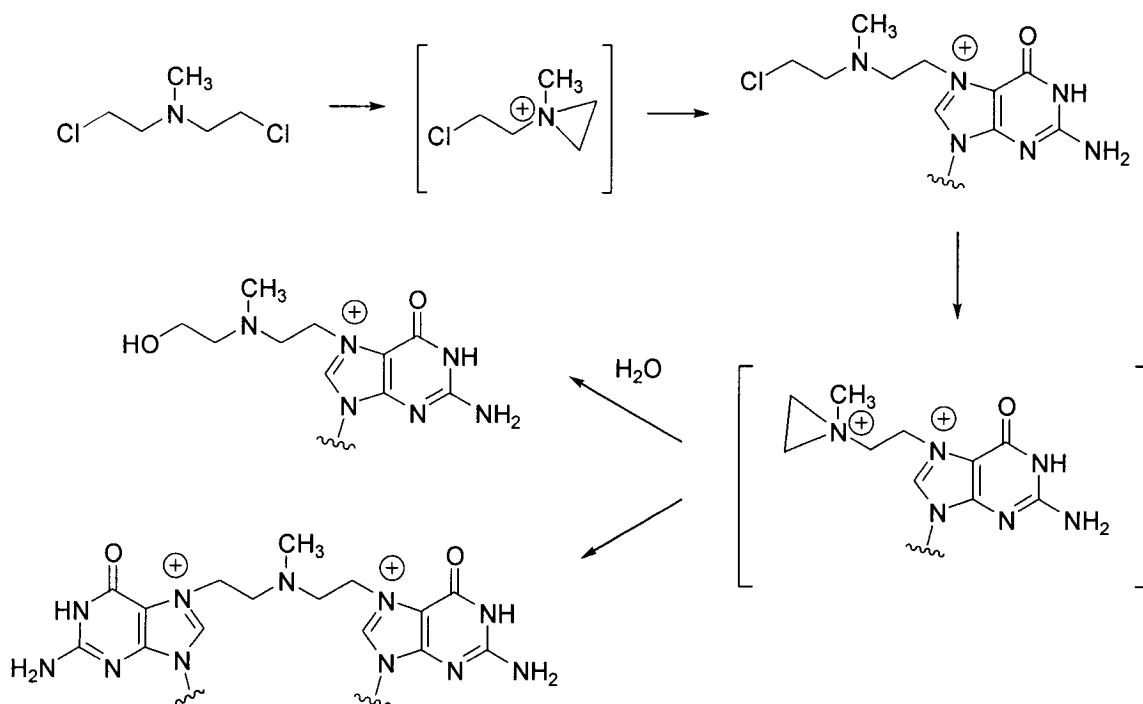


**Figure 1.5:** Proposed mechanism of activation and action of general interstrand crosslinked duplexes.

Formation of ICL lesions within DNA by bifunctional alkylating agents obviously represent serious threats for cell survival. It was estimated that 40 unrepaired ICL lesions per mammalian cell may cause its death.<sup>17</sup> These same toxic properties of such agents mentioned above have been exploited in their extensive clinical use as effective chemotherapeutic agents.<sup>18,19</sup> However the elimination and repair of these very crosslinks are believed to be responsible for development of resistance of the tumor cells to

chemotherapy. Relatively little is known about the mechanisms by which ICL duplexes are recognized and repaired. They are complex DNA lesions that are not easily removed and compared to single strand damage, the repair of ICL is assumed to require more steps since the ICL lesion needs to be excised in order to liberate the covalently linked DNA strands. In addition, no intact template is available for repair synthesis, this again significantly complicates the process of ICL elimination.<sup>20</sup>

Some bifunctional alkylation agents, such as mechlorethamine which falls under the category of the nitrogen mustards, are used in the treatment of advanced Hodgkin's disease. The mechanism of action of mechlorethamine is illustrated in **Figure 1.6**.



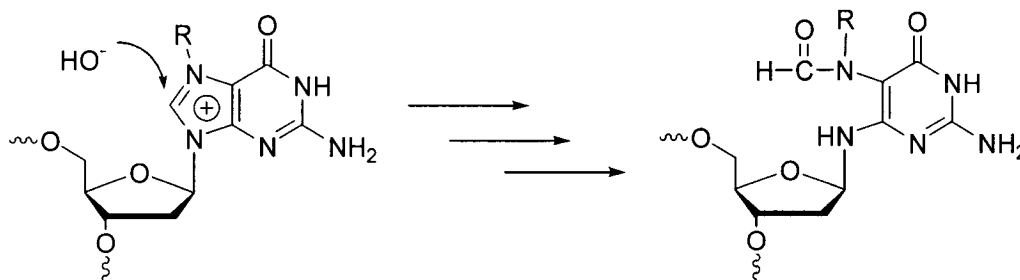
**Figure 1.6:** Mechanism of activation and action of mechlorethamine (Adapted from Noll, D. M.; McGregor Mason, T.; Miller, P. S. *Chem. Rev.* 2006, 106, 277.).

The nitrogen mustards react with guanine bases in DNA via formation of a reactive aziridinium intermediate that alkylates at the N<sup>7</sup>-position to form a reactive monoadduct. At this point a second reactive aziridinium ion may form that can either undergo reaction with water to form a 2-hydroxyethyl monoadduct or react with a second guanine base at the N<sup>7</sup> atom to form an interstrand cross-link.

Loechler's group demonstrated that the preferred target for interstrand crosslinking was at 5'-GXC-3' / 3'-CXG-5' sites (X may be any of the four deoxyribonucleotide bases) by reacting oligonucleotide duplexes with mechlorethamine followed by product isolation via denaturing polyacrylamide gel electrophoresis (PAGE).<sup>21</sup> This finding that the preferential linking was within the 5'-GXC-3' sites was unexpected since the distance between the two N<sup>7</sup> atoms in B form DNA duplex at the 5'-d(GXC) sequence was 8.9 Å which is greater than the actual bridging distance between the reactive sites of the N-methyldiethyleneamine (7.5 Å). In order for the ICL to form in a canonical B-form DNA duplex it was apparent that there had to be some compensating distortion. Computer models confirmed local propeller twisting and buckling at the site of cross-link lesion while PAGE analysis estimated that the deformation caused bending of the helix axis by 12.4-16.8° per lesion.<sup>22</sup>

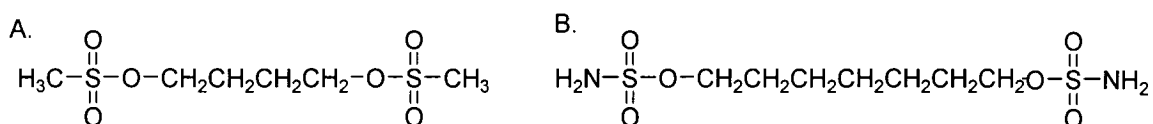
Although mechlorethamine bridges the two 2'-deoxyguanosines through the N-7 positions, this renders the glycosidic bonds labile resulting in depurination, making isolation of the intact ICL difficult. Thus, as a means to increase stability the synthesized ICL duplex was converted to the chemically more stable 7-formamidopyrimidyl (FAPY) adduct (see **Figure 1.7**) by basic hydrolysis and these were then isolated by denaturing

PAGE. Moreover several undesired side products were observed of which the interstrand cross-links only constituted a negligible fraction (typically 1-5%).



**Figure 1.7:** Mechanism of formation of the imidazole ring opened 7-formamidopyrimidyl (FAPY) adduct.

A structurally related class of ICLs are formed by the bis-(methanesulfonyl)alkane esters. Hepsulfam (1-7-heptandiyl disulfamate) which is used clinically in the treatment of chronic myelogenous leukemia was developed as a result of the synthesis of a number of structural analogues of busulfan (**Figure 1.8**).



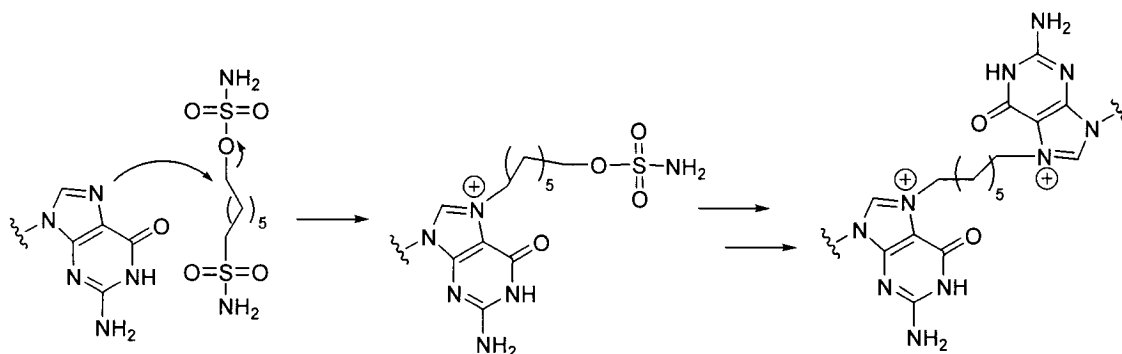
**Figure 1.8:** Structures of the sulfonic acid ester agents busulfan (A) and hepsulfam (B).

Experimental results suggested that hepsulfam also forms ICLs with DNA.<sup>23</sup> Mass spectrometry and UV spectroscopy studies by Colvin and coworkers have shown that hepsulfam is capable of forming various N7 alkylated products with guanosine including 1,7-bis(guanyl)heptane. Experiments with model oligonucleotides revealed that hepsulfam forms interstrand cross-links at 5'-GXC-3' sites, in a sequence orientation



analogous to mechlorethamine.<sup>23</sup> These N-7-alkylated bis adducts are unstable with partial stability imparted by conversion to the formamidopyrimidyl (FAPY) derivative. The 1,7 diaminoheptane linker has a N-N distance of 9.8 Å and the molecular modeling results indicate that introduction of the ICL lesion into DNA duplexes between the N-7 atoms of 5'-GXC-3' sequence causes minimal distortion of canonical B-form of DNA.

Mechanistically hepsulfam alkylates at the N-7 position of deoxyguanosine in DNA (**Figure 1.9**) forming a carbon-nitrogen bond at the N-7 atom with the methylene carbon atom adjacent to the ester oxygen atom of the sulfonyl group. In B-form DNA the N-7 atom of deoxyguanosine protrudes into the major groove and is easily accessible to small electrophilic alkylating agents.



**Figure 1.9:** Mechanism of action and cross-link induced by hepsulfam.

#### 1.4 Synthetic Methods for the Preparation of Interstrand Cross-Linked DNA Duplexes

Our knowledge of the mechanism by which interstrand cross-links are recognized and repaired to date is quite limited. In order to study repair mechanisms, interstrand cross-linked DNA substrates of well-defined structure and sufficient quantities are

required. Unfortunately the limited availability of large quantities of stable ICL DNA duplexes is one of the major challenges of such studies. Currently there are three main strategies used to engineer the cross-linked lesions into DNA duplexes.

#### **1.4.1 Direct Cross-linking Strategy**

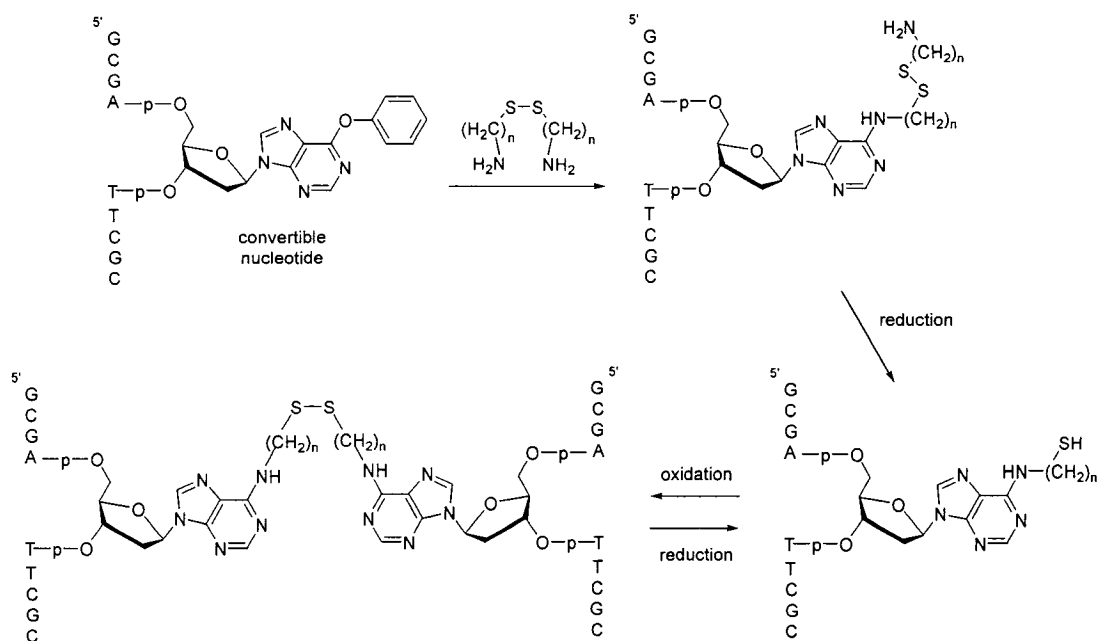
The first and simplest way to introduce cross-linked lesion is by treatment of DNA duplex directly with the cross-linking reagent which typically is a bifunctional alkylator. However, this approach suffers from several important drawbacks as, in addition to the desired ICL DNA, several other side products are formed which now have to be separated *via* PAGE with a low overall yield of the synthesis. This method also lacks target specificity as a result of numerous nucleophilic sites within DNA (exocyclic amine, non-bridging phosphate oxygens, etc.). In addition, these cross-links may be chemically unstable depending on the site of alkylation. For example, alkylation at the N<sup>7</sup> position of guanine produces thermolabile adducts that may depurinate resulting in abasic sites in DNA. Some stability may be imparted to these cross-linked duplexes, for example by alkaline treatment to afford the FAPY derivative. However, this changes the structure of the cross-linked duplex. Examples of cross-linking agents used in the production of ICL DNA duplexes by this strategy include mechlorethamine<sup>21</sup>, hepsulfam<sup>23</sup> and N,N-bis(2-chloroethyl)nitroso-urea.<sup>24</sup>

#### **1.4.2 Hybridization Triggered Cross-Link Formation**

A second strategy for preparation of ICL DNA duplexes is based on site-specific introduction of a modified convertible nucleoside into an oligonucleotide by solid phase synthesis. At the oligonucleotide level, a convertible nucleoside possessing a good leaving group is converted to natural base residue bearing the reactive functional group

either before cleavage of oligonucleotide from the solid support or post cleavage. Then the oligonucleotide is hybridized with a complementary strand to form the DNA duplex. Upon hybridization the reactive functional group is able to form a site-specific cross-link to the complementary base from the opposite strand.

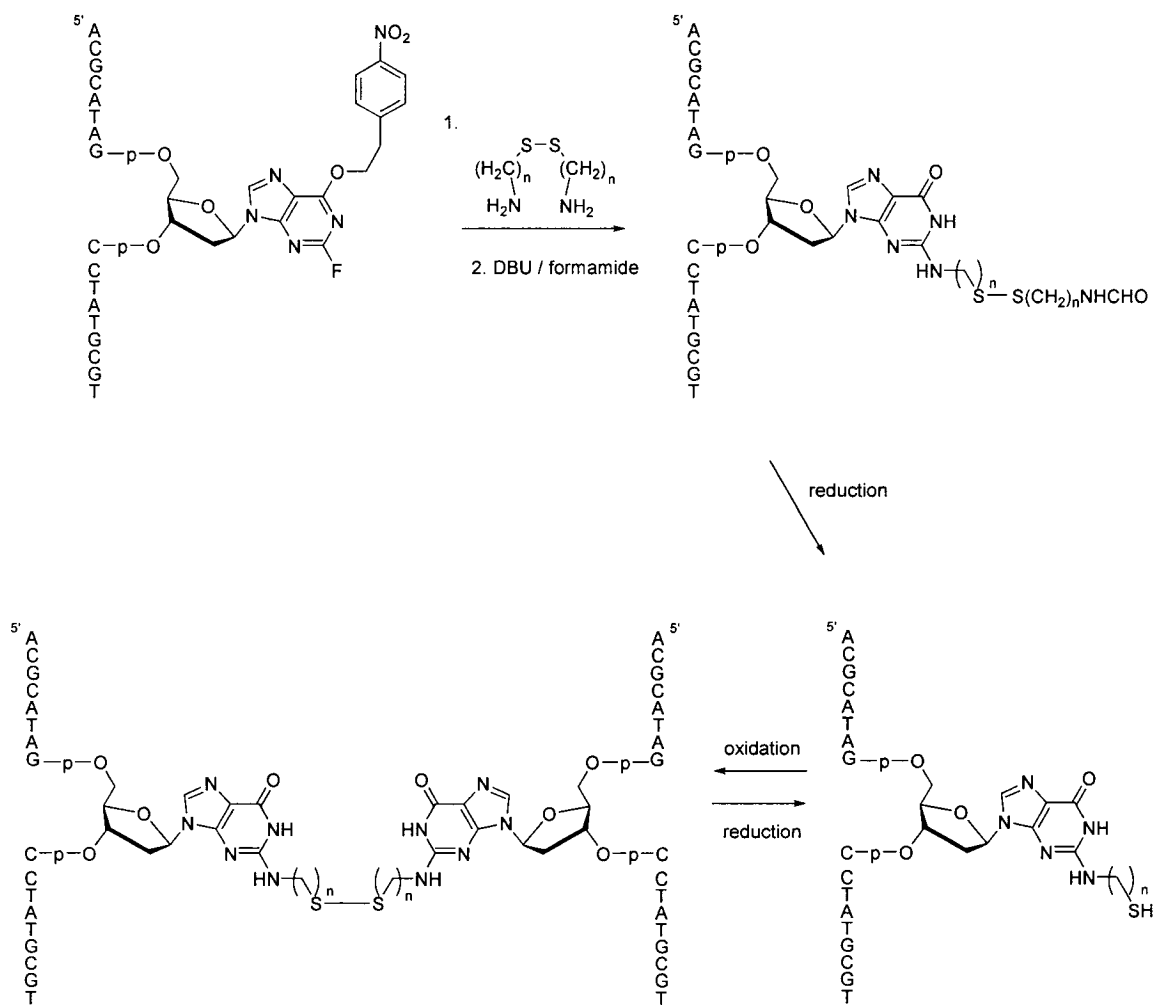
An example of this strategy involves the introduction of O6-phenyl-2'-deoxyinosine into the appropriate position of a self-complementary DNA sequence by solid phase synthesis. After the deprotection and cleavage of the oligonucleotide from the solid support the phenyl group at O6 position of 2'-deoxyinosine residue is displaced by reaction with the disulfide of aminoethanethiol or aminopropanethiol. The product of nucleophilic displacement is an oligodeoxynucleotide that contains a N6-thioalkyl-2'-deoxyadenosine residue. The thioalkyl group protected as a disulfide is reduced with dithiothreitol (DTT) to afford a free thiol. The sequence prepared by this method is self-complementary and thus spontaneously self-pairs to form duplex DNA. In these duplexes the adenines possessing thioalkyl tethers are located on adjacent bases on the opposite strands. Oxidation of the thioalkyl groups triggers ICL DNA formation with an alkyl disulfide linkage (see **Figure 1.10**) between two adenines at 5'-d(AT)-3' sequence.<sup>25</sup> This methodology has been exploited in the synthesis of symmetrical ICL DNA duplexes.



**Figure 1.10:** Strategy for the synthesis of disulfide cross-linked dA residues via hybridization triggered cross-link formation ( $n = 2$  or  $3$ ).

Molecular modeling studies suggest that the introduction of an alkyldisulfide cross-link would cause little distortion in the secondary structure mainly due to its location in major groove of DNA duplex. Moreover  $^1\text{H-NMR}$  data indicates that the cross-link nucleobase residues are still capable of forming Watson-Crick base pairs.

A similar related convertible nucleoside 6-O-[2-(p-nitrophenyl)ethyl]-2-fluoro-2'-deoxyinosine was used to prepare a DNA duplex where two guanine residues were cross-linked by an alkyldisulfide spacer in 5'-d(GC)-3' sequence (**Figure 1.11**).<sup>26</sup>



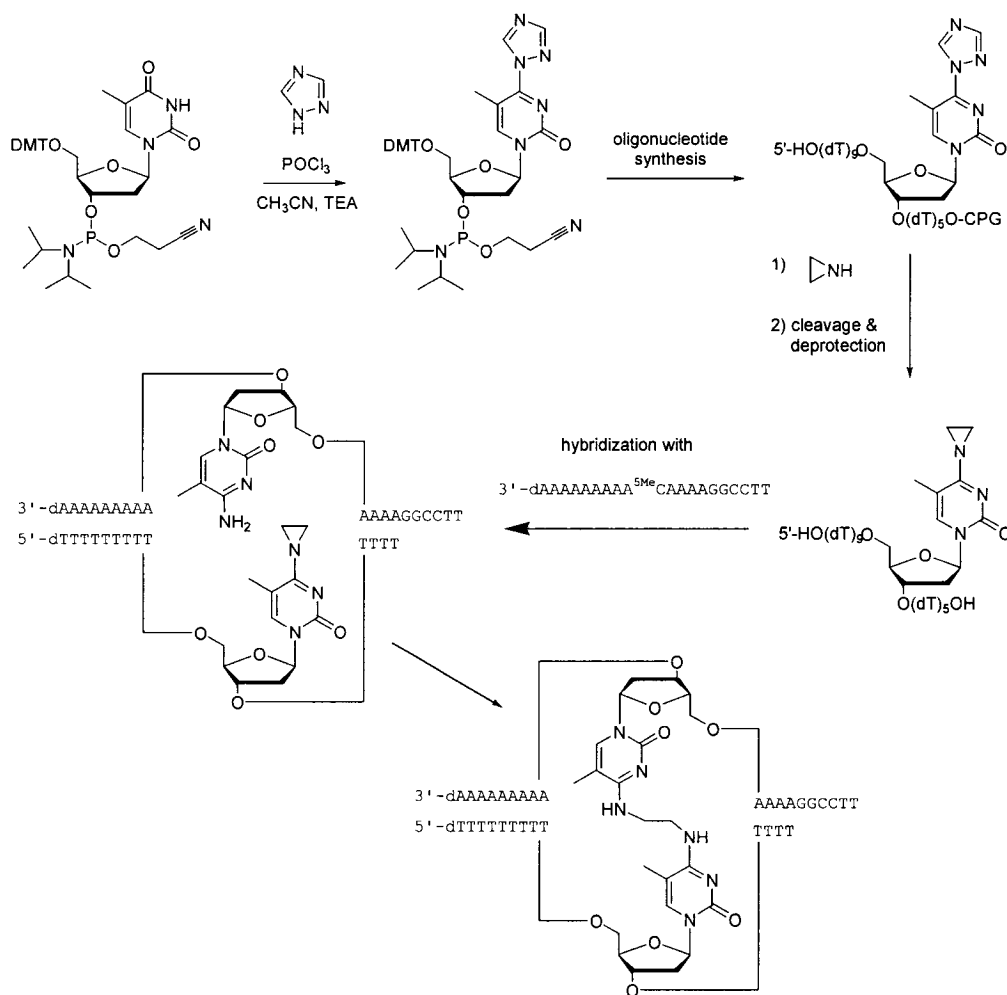
**Figure 1.11:** Strategy for the synthesis of disulfide cross-linked dG residues via hybridization triggered cross-link formation ( $n = 2$  or  $3$ ).

Compared to the previously described cross-link, this synthetic approach requires some additional reaction steps. After the incorporation of convertible nucleoside into an oligonucleotide the fluorine atom is replaced with alkyl disulfide functional group. Then the *para*-nitrophenylethyl (NPE) protecting group was removed by treatment with DBU (1,8-diazabicyclo[5,4,0]undec-7-ene). The remaining two redox reaction steps of this

synthesis are the same as previously described for the preparation of alkyldisulfide cross-linked DNA duplexes containing a 5'-d(AT)-3' sequence.

Another example of the convertible nucleoside strategy involves the introduction of a reactive aziridine group at the 4-position of a 5-methylcytidine base of an oligonucleotide. The reactive aziridine ring system can react with the nucleophilic 4-amino group of mismatched 5-methylcytidine from the opposite strand. As a result of the ring opening reaction a ICL DNA duplex is formed placing an ethylene bridge between two mismatched cytidine residues (**Figure 1.12**).<sup>27</sup> The aziridinyl group was also used to prepare ICL DNA duplexes which possess an ethylene linkage between the 6 position of guanosine and the exocyclic 4-amino group of cytidine base in complementary strand. One advantage of these nucleosides is that aziridine modified deoxyguanosine triphosphate can serve as a substrate of DNA polymerases enabling incorporation in DNA.<sup>28</sup>

Overall the hybridization cross-linking methodology is effective and affords a high yield of the desired cross-link product. It offers greater control over site of the cross-link relative to direct treatment of DNA with bifunctional alkylating agents. However some limitations include a cytosine-cytosine or cytosine-adenosine mismatch at the site of the cross-link for the aziridine ring opening reaction.<sup>28</sup> This problem was partially overcome by using an aziridine-containing guanosine analogue to increase versatility. Another significant disadvantage of this type of ICL DNA structure is the fact that N-N cross-links are not very stable. Also it must be noted that this type of ICL has not been observed when DNA was treated with alkylating agents and therefore is not a clinically relevant ICL for repair studies but serves as a model system.

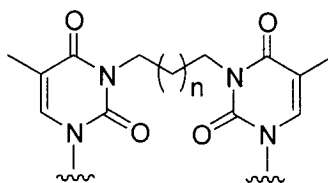


**Figure 1.12:** Hybridization triggered cross-link formation by nucleophilic attack on an aziridinyl modified nucleotide.

### 1.4.3 Nucleoside Dimer Strategy

A more sophisticated approach to synthesize ICL DNA duplexes includes coupling of a protected nucleoside dimer phosphoramidite to the ends of short oligonucleotide strands via standard solid phase synthesis.

An example of a nucleoside dimer used to construct ICL duplexes is shown in **(Figure 1.13)** where the N3 atoms of deoxythymidines are bridged to form a N3T-alkyl-N3T cross-link in the resulting duplex DNA.



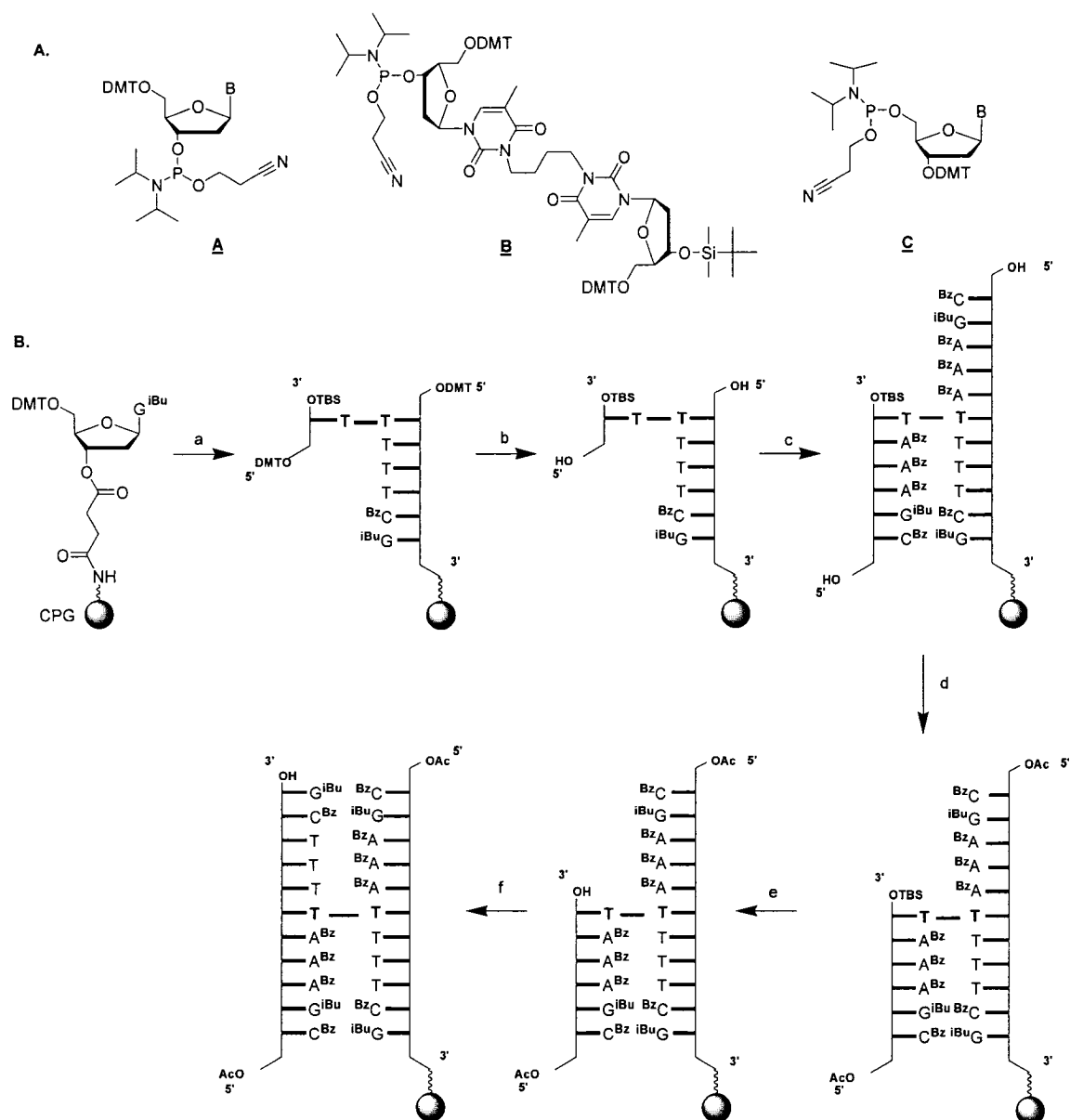
**Figure 1.13:** Structure of the N3T-alkyl-N3T cross-link.

This strategy itself has two approaches that have been used to construct these duplexes. The first involves synthesis of a N3T-alkyl-N3T cross-link phosphoramidite.<sup>29</sup> Assembly of the cross-linked duplex is illustrated in **Figure 1.14**. Oligonucleotide synthesis begins with the first protected nucleoside attached to the solid support (CPG) followed by (a) growth of linear strands with commercially available 3'-O-deoxyphosphoramidites **A** followed by coupling of the cross-link phosphoramidite **B**, (b) acid treatment to liberate the free 5'-OH groups, (c) simultaneous extension of the second and third arms of the duplex by repetitive coupling with 3'-O-deoxyphosphoramidites **A**, (d) capping with acetic anhydride to make the 5'-OH groups of the branched Y-intermediate unreactive (e) removal of the t-butyldimethylsilyl (TBDMS) group at the site of the cross-link by treatment with 1 M tetra-n-butylammonium fluoride (TBAF), in tetrahydrofuran and (f) finally chain extension using 5'-O-deoxyphosphoramidites **C** to afford the fully cross-linked duplex.

These cross-linked oligomers are cleaved from the support by treatment with concentrated ammonium hydroxide: ethanol (3:1) and purified by strong anion exchange HPLC. Although this procedure gives the cross-linked product in only 5-6 % overall

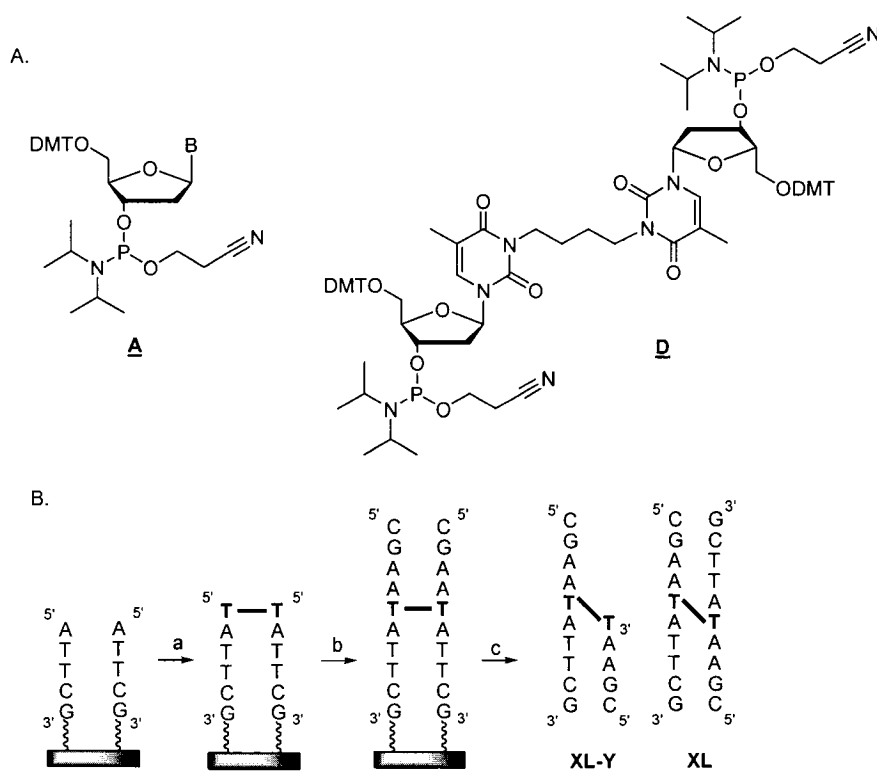


yield, sufficient quantities of cross-linked material were obtained that enabled biological, biophysical and structural studies by high resolution NMR spectroscopy.



**Figure 1.14:** Synthesis of N3T-alkyl-N3T cross-linked duplexes via a monophosphoramidite approach. **A.** Monomers used in cross-linked duplex assembly. **B.** Stepwise solid-phase synthesis of the cross-linked duplex. (Adapted from Wilds, C. J., Noronha, A. M., Robidoux, S. and Miller, P. S. *Journal of the American Chemical Society* 2004, 126, 9257).

Alternatively a newer symmetrical bis-phosphoramidite (**Figure 1.15**) can also be used in the synthesis of this cross-link.<sup>30</sup> The solid-phase synthetic strategy begins with commercially available 3'-O-deoxyphosphoramidites **A**, then the N3T-butyl-N3T bis-phosphoramidite **D** is coupled simultaneously to the 5'-ends of two oligonucleotide chains attached to the solid support. Chain extension around the 5' ends of the cross-linked site with 3'-O-deoxyphosphoramidites completes the synthesis of the cross-linked duplex (**XL**).



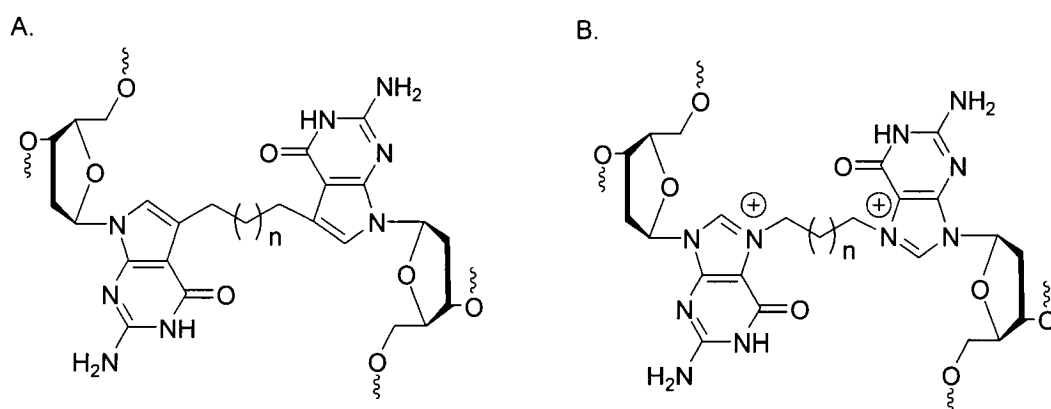
**Figure 1.15:** Synthesis of N3T-alkyl-N3T cross-linked duplexes via a bis-phosphoramidite approach. **A.** Monomers used in cross-assembly. **B.** Stepwise solid-phase synthesis of the cross-linked duplex.<sup>30</sup>

The yields are improved almost two-fold over the monophosphoramidite strategy. However, one inevitable side product is the formation of **XL-Y** type failure sequences

that are formed as a result of only one of the two reactive phosphoramidite moieties successfully coupling to the linear growing oligonucleotide chains attached to the CPG.

## 1.5 PROJECT BACKGROUND AND OBJECTIVES

This thesis work focuses on the synthesis of analogues of deoxyguanosine cross-links formed at the N7 position. Instead of using deoxyguanosine, 7-deazadeoxyguanosine which lacks a nitrogen at this position will be used to produce alkyl linked dimers (**Figure 1.16**). The driving force for this particular study was to synthesize a chemically stable interstrand cross-link that would enable biological and structural studies of oligonucleotides containing cross-links at a site analogous to that of the cross-link formed by the agents mechlorethamine (which places 5 atoms between the N7 atoms) or hepsulfam (which places 7). Elaboration of these nucleoside dimers to phosphoramidites for the intent of insertion into DNA duplexes would enable investigation of their physicochemical properties, structures and eventually to correlate structure with the repair of interstrand cross-linked DNA duplexes.



**Figure 1.16:** Structures of the 7-deazadeoxyguanosine-alkyl-7-deazadeoxyguanosine cross-link mimic (**A**) and clinically relevant N7-deoxyguanosine-alkyl-N7-deoxyguanosine cross-link (**B**).

The influence of derivatives of 7-deazapurines bearing different substituents at the 7 position on DNA duplex thermal stability were also studied by Seela and co-workers.<sup>31</sup> It was found that the introduction of small substituents such as chloro, bromo and iodo at the 7-position of 7-deazaadenosine causes stabilization of oligonucleotides duplexes. However the presence of a methyl substituent at position 7 did not exert a significant influence on the base pair stability.<sup>32</sup> These observations implied that substituents at position 7 of deazapurine nucleoside are well accommodated in the major groove of B-form DNA and that their incorporation into DNA did not distort the DNA secondary structure. The 7-iodinated derivatives of 7-deazapurines were then used as a means of introducing reporter groups at the N7 of dG via the Sonogashira cross-coupling reaction, which allows for substitution of side chains into the nucleobase at this sterically favorable position.<sup>33</sup> This strategy was used for preparation of 7-deaza-2',3'-dideoxypurines derivative that carry fluorescent reporter groups at position 7.<sup>34</sup> The triphosphate derivatives of the four chain terminating 2', 3'-dideoxynucleosides bearing different covalently attached fluorescent dyes could then be incorporated into DNA to be further exploited for DNA sequencing techniques. Each base is distinguished by its unique fluorescent emission and these labels circumvent the problematic use of radioisotopes such as <sup>32</sup>P in DNA sequencing. The drawback, however, is the requirement of close to 100% incorporation of modified nucleoside into the transcript DNA. As a result, this process suffers from inefficient incorporation of the fluorescent labelled deazapurines that are not well tolerated by DNA-polymerases.

Base modified nucleoside that are self-fluorescent and that retain the normal Watson-Crick base-pairing properties of natural purine bases have been previously

studied by Seela *et al.*<sup>35</sup> 7-alkynylated 7-deaza-2'-deoxadenosines were prepared via the Sonogashira cross-coupling reaction which all show intensive fluorescence. The fluorescence appears when the triple bond of alkyne side chain is in conjugation with heterocyclic base. Quantum yield and fluorescent decay time significantly increase with increasing polarity of solvent. The compound which exhibits most intensive self-fluorescence properties is 7-hexynyl 7-deaza-2'-deoxyadenosine.<sup>35</sup> In contrast 7-hexenyl-7-deaza-2'-deoxyguanosine does not exhibit significant fluorescence.<sup>32</sup> Other studies involving oligonucleotides that contain this nucleobase with a long hexynyl chain are thermally less stable compare to their unmodified counterparts.<sup>36</sup>

Among the various structural modifications of 7-deazaguanosine that were examined in order to increase base binding affinity the most promising results were achieved with nucleobase bearing the propynyl group. The propynyl side chain was introduced into position 7 of 7-deaza-2'-deoxyguanosine by Sonogashira cross-coupling reaction. The thermal denaturation of oligonucleotides that contains this modified nucleobase, when hybridized to RNA<sup>37</sup> or DNA<sup>38</sup> exhibit an increased stability relative to unmodified oligonucleotides.

All the above mentioned studies involved a key intermediate used in the preparation of 7-deazaguanosine adducts namely 7-iodo-7-deaza-2'-deoxyguanosine. The iodo functional group allows for the formation of a single C-C bond between C7 atom of modified nucleoside and terminal alkyne via palladium (0) catalyzed Sonogashira cross-coupling reaction. Our goal was to adopt this strategy to introduce a dialkynyl side chain at the 7 position of 7-deaza-2'-deoxyguanosine hoping to form a reactive monoadduct-nucleoside. On further subjecting this monoalkynucleoside adduct to a second

Sonogashira cross coupling reaction with a second equivalent of 7-iodo-7-deaza-2'-deoxyguanosine we proposed that a crosslinked dinucleoside dimer would be produced with the relevant protecting groups that make it amenable to solid phase oligonucleotide synthesis. Incorporation of the dimer into oligonucleotides on an automated DNA synthesizer would then produce the desired ICL duplexes which would then be used as probes to carry out various structural and biochemical studies to aid in our understanding of DNA repair and resistance to current chemotherapeutic regimens.

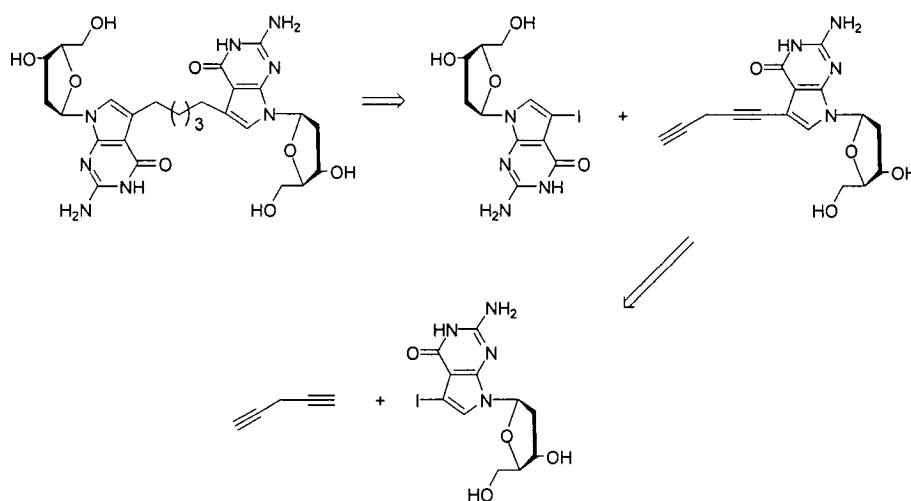
## Chapter 2

### Results and Discussion

#### 2.1 Retrosynthetic Strategy

The objective of this research was to develop a synthetic strategy to prepare a dimer containing two 7-deaza-2'-deoxyguanosine nucleosides connected through the C7 atoms by an alkyl chain (**Scheme 2.1**). This molecule would be incorporated into an oligonucleotide by solid phase synthesis to produce a DNA interstrand cross-link (ICL). When  $n = 3$  the ICL synthesized may be a structural mimic of the lesion formed in DNA by the chemotherapeutic agent mechlorethamine.

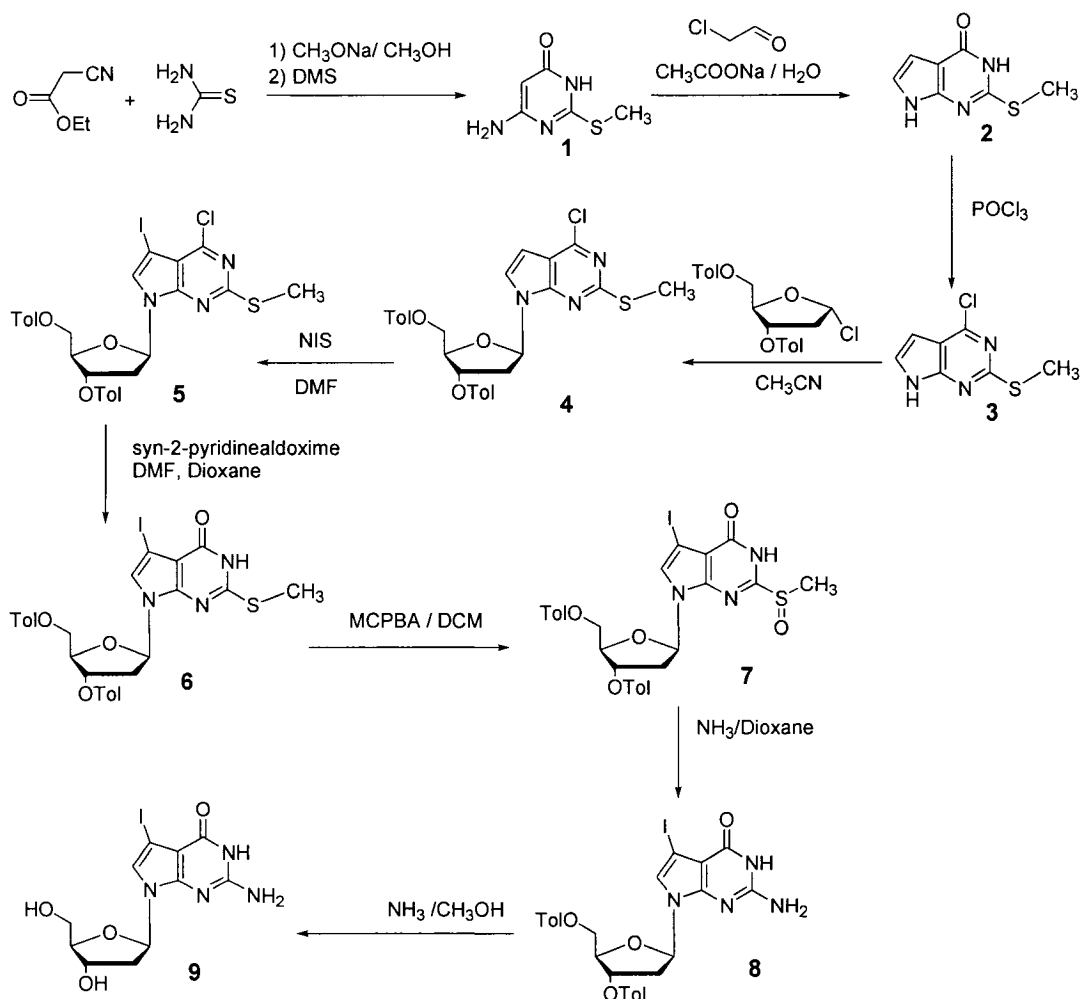
The proposed synthetic approach to form the carbon-carbon bond between the linker and C-7 positions of 7-iodo-7-deaza-deoxy-guanosine would involve the palladium catalyzed Sonogashira cross-coupling reaction between the iodinated nucleoside and an alkyldiyne to form a monoadduct followed eventually by coupling with a second nucleoside to form the dimer according to the retrosynthetic scheme shown below (**Scheme 2.1**).



**Scheme 2.1:** Retrosynthetic strategy to prepare a 7-deaza-2'-deoxyguanosine dimer

## 2.2 Synthesis of 7-iodo-7-deaza-2'-deoxyguanosine

One of the important intermediates in the proposed synthetic strategy (**Scheme 2.1**) is 7-iodo-7-deaza-2'-deoxyguanosine **9**. The synthesis of nucleoside **9** is outlined in **Scheme 2.2** which began with preparation of 2-thiomethyl-4-amino-pyrimidin-6-one **1**. This was formed by the condensation of thiourea with ethyl cyanoacetate in the presence of strong base in absolute ethanol which gave the sodium salt of 2-thio-4-aminopyrimidin-6-one. This intermediate was not isolated, but directly methylated with dimethylsulphate to give compound **1** in good yield (76%).<sup>39</sup>



**Scheme 2.2:** Synthesis of 7-iodo-7-deaza-2'-deoxyguanosine **9**.

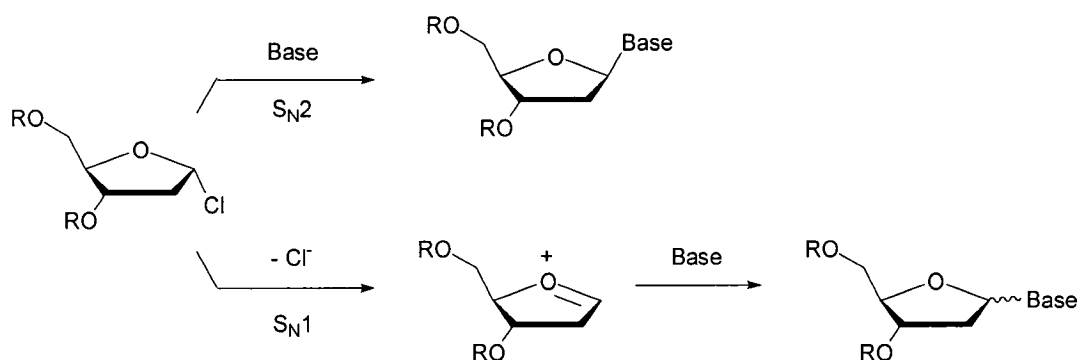


The pyrolo-pyrimidone bicyclic ring system of compound **2** was formed by a condensation reaction of compound **1** with chloroacetaldehyde under aqueous alkaline conditions. The ring closure reaction occurs in a regiospecific manner with compound **2** as the only product isolated in moderate yield (23 %).<sup>40</sup> Attempts to improve the yield of this step included altering reactions conditions such as temperature and carrying out the reaction for an extended amount of time. However, the reaction conditions reported in the literature gave the highest yield.

In order to enhance the reactivity of the 7-deaza-purine heterocycle to the aromatic electrophilic substitution reaction the 6-oxo group has to be masked. One possibility is to methylate at this position, however, it was found that it is very difficult to displace a methoxy group by nucleophiles on 7-deaza-purines.<sup>41</sup> Therefore the 6-oxo group was instead converted to a chloride group which may be readily re-converted to an oxo group. The conversion of compound **2** into its chlorinated derivative was performed by reaction with phosphorous oxychloride in presence of N,N-dimethylaniline. This produced compound **3** with a 48% yield. In our attempts to optimize this step, it was found that the addition of a small portion of acetone in the concentrated crude product improved the efficiency of the extraction step with diethylether and consequently increased, albeit slightly, the overall yield of this step from 43 to 70 %.

In order to synthesize nucleoside **4** the Robins N-glycosylation method was employed. This procedure has been shown to be applicable to 7-deaza-purine nucleosides. Moreover, N-glycosylation readily proceeds in the presence of a methylthio group at the 2-position of the heterocycle.<sup>42</sup> Glycolysation of compound **3** with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl was performed in a one pot

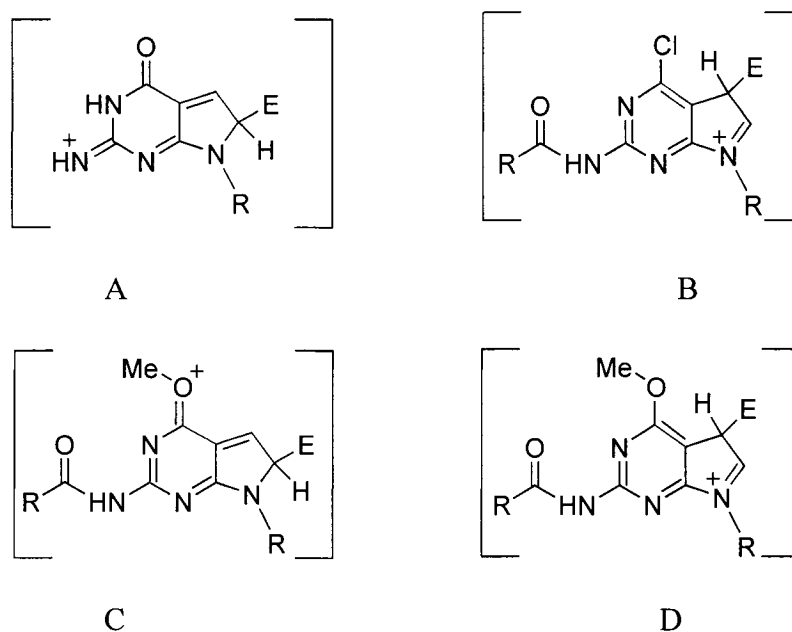
reaction with sodium hydride in anhydrous acetonitrile. The sodium hydride generates the sodium salt of compound **3** in situ which reacts with the chlorinated sugar, presumably via a nucleophilic displacement reaction to produce nucleoside **4** in 65 % yield. This reaction produces the nucleoside with the desired regioselectivity with bonding between C1' of the deoxyribose sugar to the N9 atom of the 7-deaza-purine. In addition, this reaction also proceeds with a high degree of stereoselectivity to produce the  $\beta$  anomer nucleoside at the C1' atom of the deoxyribofuranose. Exclusive formation of the  $\beta$  anomer is believed due to a direct Walden inversion at the C1' carbon by the anionic heterocyclic nitrogen (see **Figure 2.1**).



**Figure 2.1:**  $S_N1$  and  $S_N2$  mechanistic pathways for the attachment of a heterocyclic base to a chlorinated sugar.

Introduction of the iodo substituent at the C7 position of nucleoside **4** was accomplished using N-iodosuccinimide. It was necessary to perform this reaction at this step rather than on a later intermediate as it has been observed that direct iodination of 7-deaza-2' deoxyguanosine leads to mixtures of 7 and 8 iodinated products with the latter as the major product (**Figure 2.2**). This is the result of mesomeric stabilization of the  $\sigma -$

complex formed during electrophilic attack at the C-8 position of 7-deaza-2'deoxyguanosine. Electrophilic attack of the iodide ion is directed onto the undesired C-8 position due to the electron donor ability of the exocyclic amino and oxo functional groups.<sup>43</sup> As a result, the 7-deaza-2'deoxyguanosine is not a favourable intermediate for halogenation. Therefore, iodination of modified nucleoside 4 where the amino group is replaced by a methylthio group and the keto group is masked as a chloride was necessary.



**Figure 2.2:** Mesomeric stabilization of  $\sigma$ -complexes formed during electrophilic attack at 7-deaza-dG (A) and at 2-NH<sub>2</sub> protected 6-Cl-7-deaza-dG (B). Structures (C) and (D) – results of electrophilic attack at 2-NH<sub>2</sub> protected 6-OMe-7-deaza-dG, shows that the 6-OMe group could direct electrophilic attack to the undesired 8 position even though the 2-NH<sub>2</sub> is protected.<sup>44</sup>

Moreover the reaction conditions together with the various substituents on the deazapurine heterocycle play a role in the addition of halogens to the ring. For example, N-bromosuccinimide in anhydrous dichloromethane leads to the formation of three brominated products (7-bromo, 8-bromo and 7,8-dibromo). This problem can be overcome by using DMF, as the solvent for the synthesis of nucleoside **5**.<sup>45</sup> Similarly, this analogous reaction is highly regioselective with exclusive formation of the desired iodo nucleoside **5** in high yield (82 %) in our favour.

Once the iodo substituent was introduced at C7 position of nucleoside **4**, the 6-oxo group could be recovered. Syn-2-pyridinealdoxime and 1,1,3,3-tetramethylguanidine were used for selective nucleophilic displacement of chloride atom to give compound **6** in excellent yield (93.5 %).<sup>46,37</sup>

The displacement of the 2-methylthio group of 7-deazapurines by various nucleophiles is more challenging compared to the corresponding purine.<sup>47</sup> Therefore, transformation of the methylthio group to the amino group was performed by conversion to the more reactive sulfoxide derivative **7**. Nucleoside **6** was oxidized with m-chloroperoxybenzoic acid in DCM. Initially, the temperature of the reaction mixture was kept at 0°C in order to prevent further oxidation of the sulfoxide functional group. Purification of this compound was straightforward by short column chromatography to give compound **7** in excellent yield (97%).

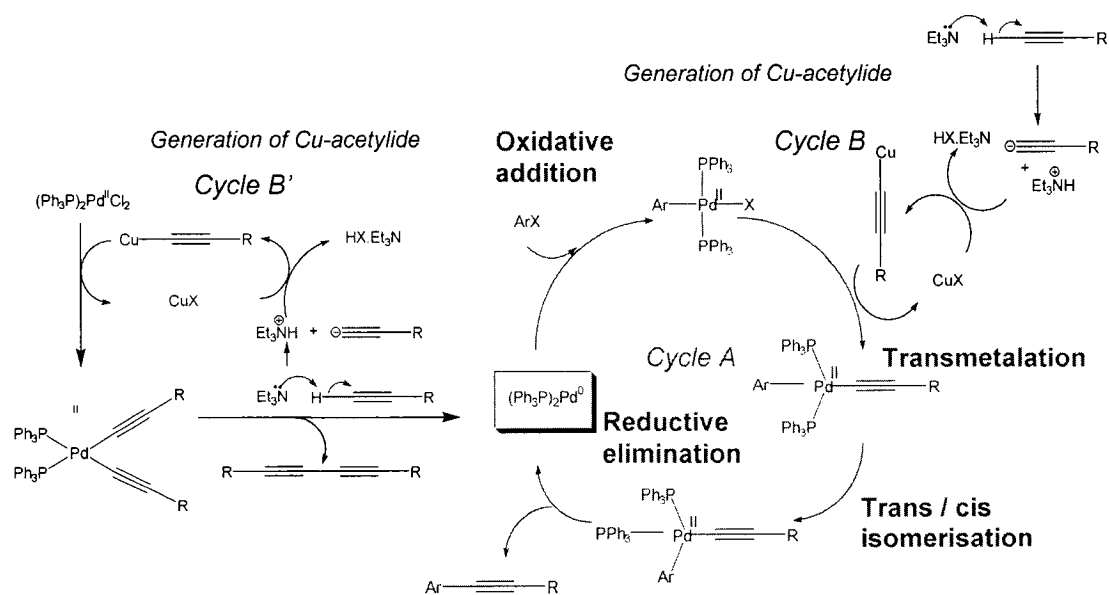
The sulfoxide functional group of nucleoside **7** was replaced with an amino group via reaction with anhydrous ammonia in anhydrous dioxane.<sup>37</sup> However, this transformation requires harsh conditions, and specifically it is carried out in an autoclave at 145°C for 8.5 h. Short column chromatography gave compound **8** in good yield (79%).

The final step in the synthesis of deprotected nucleoside **9** is cleavage of toluoyl protecting groups from nucleoside **8**. The toluoyl protecting groups were removed by treatment with ammonia in methanol in an autoclave at 140°C<sup>37</sup> with the completely deprotected nucleoside obtained after 2 h as confirmed by TLC analysis. Shorter reaction times resulted in incomplete deprotection of the O-toluoyl groups. Initial difficulties were encountered when purifying crude nucleoside **9** by crystallization. However this problem was overcome when the reaction was scaled up to afford unprotected pure nucleoside **9** in 71% yield.

### **2.3 Attempts to Perform the Sonogashira Cross-coupling Reaction with TMS-1,4-pentadiyne**

One of the key steps in the proposed synthetic methodology to produce the alkyl linkage between two 7-iodo-7-deazadeoxyguanosine nucleosides at the C7 position involves the Sonogashira cross-coupling reaction. This reaction enables the formation of a carbon-carbon bond between sp<sup>2</sup> and sp hybridized carbon atoms, a transformation that is otherwise quite difficult to achieve but can be carried out as a simple one pot cross-coupling reaction between an aryl iodide and a terminal alkyne. The reaction is tolerant of a number of functional groups that may be present in the molecules reacting together thereby eliminating the need for various protection-deprotection reactions and generally involves the use of a palladium catalyst in conjunction with CuI (**Figure 2.3**). Literature procedures of the Sonogashira cross-coupling reaction with nucleosides have reported optimal yields of product when the ratio of terminal alkyne to nucleoside is 2 - 2.5

equivalents for pyrimidines<sup>48</sup> and 2 equivalents for 7-deazapurines using 10 mol% of palladium and 20 mol% of CuI catalyst.<sup>33</sup>



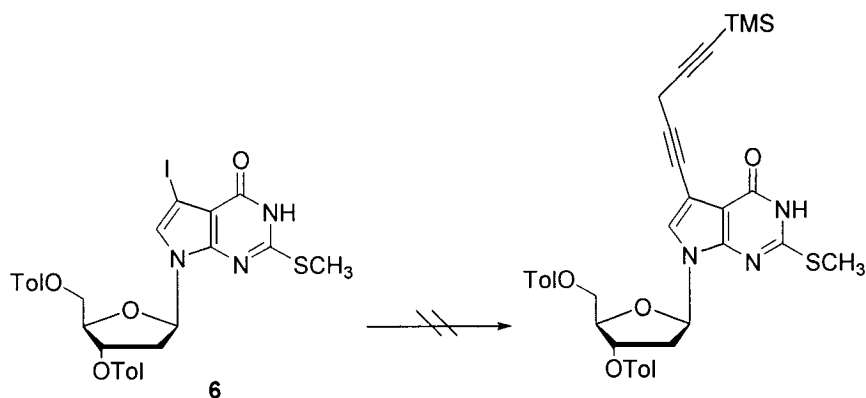
**Figure 2.3:** Proposed mechanism of the Sonogashira reaction.<sup>49</sup>

However a number of reaction parameters including solvent, base, temperature and molar ratio of participating reactants and catalysts had to be optimized for each particular step. In order to conserve valuable nucleoside **9** it was decided to optimize the Sonogashira cross-coupling reaction conditions with the more easily produced nucleoside **6**. The 7-deazapurine ring system of nucleoside **6** slightly differs from nucleoside **9** by the presence of thiomethyl functional group. Moreover the presence of the toluoyl protecting groups should significantly facilitate the isolation of cross-coupled product.

Our original intention was to synthesize the 7-deazapurine nucleoside substituted at the C7 position with an alkyl chain that contains five carbon atoms. The source of the terminal alkyne in our synthetic design is represented by 1,4-pentadiyne. However, the only commercially available source of 1,4-pentadiyne is its TMS derivative - TMS-1,4-

pentadiyne. The successful coupling of TMS protected acetylene to 7-deazaadenosine<sup>35</sup> and pyrimidine<sup>48</sup> nucleosides has been reported, hence the presence of the TMS group should not represent a serious steric obstacle for the Sonogashira cross-coupling reaction. It was reasoned that an additional advantage would be that cross-coupling with TMS protected alkyldiyne would have prevent the second terminal acetylenic group of 1,4-pentadiyne from cross-coupling to form a dimer or react to the give undesired homocoupled product. The acetylenic group would be easily generated by treatment with the desilylating agent TBAF.

The first attempts to couple TMS-1,4-pentadiyne with nucleoside **6** employed reaction conditions similar to Sonogashira reaction conditions previously reported for 7-deazapurines and pyrimidine nucleosides.<sup>33,48</sup> This involved 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI catalyst, 2.5 eq of TMS-1,4-pentadiyne and 2.5 eq of Et<sub>3</sub>N in DMF. The reactions were carried out at RT and at elevated temperature (40°C). However, in both cases after 40 h, only starting nucleoside **6** was recovered from reaction mixture. Increased amounts of palladium catalyst (0.2 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>) had no effect on reaction progress. No reaction was observed when the concentration of the diyne or Et<sub>3</sub>N in DMF was changed. Reaction time was also increased to 4 days with no effect. Finally, high temperature (90°C) where employed but this resulted in complete decomposition of nucleoside **6** with no nucleoside product formed.



**Figure 2.4:** Proposed Sonogashira reaction product between TMS-1,4-pentadiyne and compound **6**.

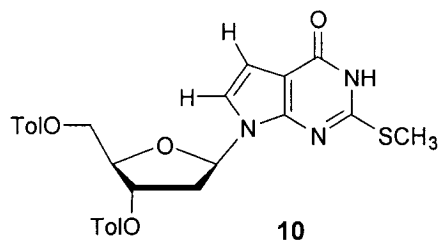
The effect of solvent on the reactivity of nucleoside **6** in the Sonogashira cross-coupling reaction was also investigated. The same cross-coupling reaction conditions (0.1 eq of  $\text{Pd}(\text{PPh}_3)_4$ , 0.2 eq of  $\text{CuI}$  catalyst, 2.5 eq of TMS-1,4-pentadiyne and 2.5 eq of  $\text{Et}_3\text{N}$ ) was carried out in THF. Analysis by TLC did not reveal any new products after 30 h at RT. An additional portion of catalyst (0.1 eq of  $\text{Pd}(\text{PPh}_3)_4$ , 0.2 eq of  $\text{CuI}$ ) was added and the reaction allowed to proceed an additional 40 h at RT, followed by heating to  $40^\circ\text{C}$  for 4 h and then 16 h at RT. This prolonged reaction time did not yield the desired cross-coupled product. Again, TLC indicated the gradual disappearance of starting material with no new UV active compounds formed. The only nucleoside material obtained after column chromatography was recovered starting material nucleoside **6**.

The cross-coupling reaction was also attempted with  $(\text{PPh}_3)_2\text{PdCl}_2$  in THF, but after 4 days only a small portion of starting material nucleoside **6** was isolated after purification.

The reaction was also attempted in piperidine. Nucleoside **6** (1.0 eq), 0.2 eq of  $\text{Pd}(\text{PPh}_3)_4$ , 0.2 eq of  $\text{CuI}$  and 2.6 eq of TMS-1,4-pentadiyne were allowed to react at RT



for 4 days. When the TLC did not indicate any change, the temperature of reaction was gradually increased to 90°C. Two nucleosides were observed by TLC and separated by column chromatography. The major product was recovered nucleoside **6**. Based on <sup>1</sup>H-NMR and ESI-TOF-MS analysis it is believed that minor product of reaction was deiodinated nucleoside **10**. The ESI-TOF-MS analysis has a major peak with a m/z = 534.19 (the calculated MW of deiodinated nucleoside **10** is 533.5). The <sup>1</sup>H-NMR spectrum of this side product reveals that the signal assigned to H6 at 7.03 ppm is doublet ( $J_{H5-H6} = 3.6$  Hz) with a second doublet assigned to H5, which overlaps with the signal for H1' at 6.58 ppm.



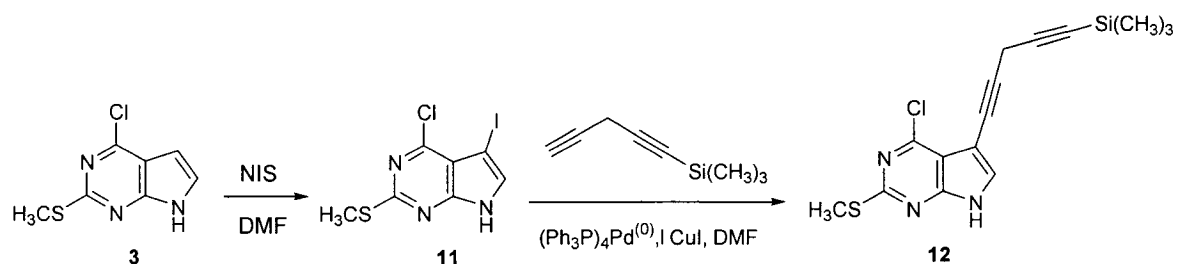
**Figure 2.5:** Biproduct **10** produced by the Sonogashira coupling between **6** and TMS-1,4-pentadiyne.

In order to eliminate the possibility that the TMS group was interfering with the cross-coupling reaction it was decided to remove the TMS protecting group from 1-TMS-1,4-pentadiyne. The attempt to isolate deprotected 1,4-pentadiyne by vacuum distillation failed. Therefore 1,4-pentadiyne was generated in-situ. In order to do this TBAF (3eq) was added drop wise to the TMS-pentadiyne (3 eq) in THF at 0°C. Then, to the THF solution was added 1 eq of nucleoside **6**, 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI and 3.5 eq of

Et<sub>3</sub>N and the reaction mixture allowed to stirred at RT for 5 days. However TLC analysis indicated presence only of starting material.

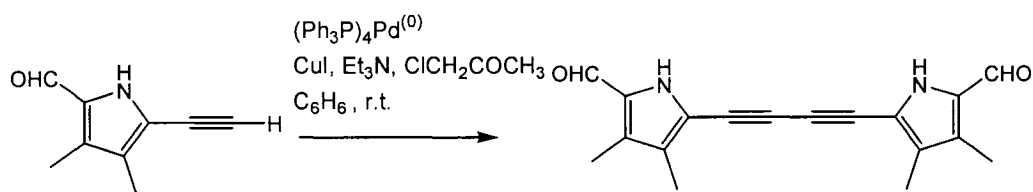
To simplify the reaction and minimize the use of valuable, less easily produced nucleosides, it was decided to attempt the cross-coupling reaction on 7-iodo-7-deazapurine **11** (Scheme 2.3). Compound **11** was prepared by iodination of compound **3** with NIS in DMF.<sup>45</sup> As mentioned above the reaction is highly regioselective due to electronic effects of the functional groups attached to 7-deazapurine ring system. This reaction proceeded in good yield (79 %) to afford compound **11**. This result suggests that deoxyribose sugar does not have any effect on the reactivity of 7-deazapurine for the iodination reaction. It also suggests that the iodo substituent could be introduced at the C-7 position of 7-deazapurine ring system prior to the glycosylation step in order to prepare nucleoside **9**.

The Sonogashira cross-coupling reaction of compound **11** with 2.5 eq of TMS 1, 4-pentadiyne, 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI and 2.5 eq of Et<sub>3</sub>N in DMF was stirred at 40 °C for After 4 days only starting material **11** was recovered. However when the same reaction was repeated with just 1 eq of TMS-1,4-pentadiyne, apart from unreacted compound **10** small portion (3 %) of desired cross-coupled compound **12** was isolated after 19 h treatment.



**Scheme 2.3:** Synthesis of compound **12**.

In our hands, the TMS-1,4-pentadiyne was found not to be reactive enough to enable the formation of C-C bond with **6** and to a very low extent with heterocycle **11** via the palladium catalyzed cross-coupling reaction. We speculate that reason for its inferior reactivity could be due to low stability under basic conditions. Moreover, it was found that palladium–copper iodide catalytic system in presence of oxidative agent may initiate a homocoupling reaction of alkynes (**Figure 2.4**).<sup>50, 51</sup> Performing the Sonogashira cross-coupling reaction under argon atmosphere may not completely eliminate traces of oxygen from reaction mixture. Therefore the homocoupling may be a possible side reaction.

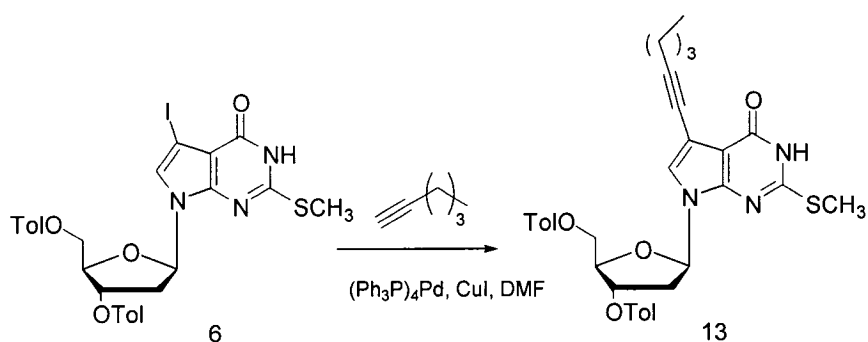


**Figure 2.6:** Example of homocoupling reaction and product.<sup>50</sup>

It has been shown that the presence of electron-withdrawing groups in alkynes, increasing their acidity, promotes more rapid dimerization under alkaline conditions whereas under acidic conditions (pH=3) an inverse relationship was observed and addition of Cu(I) salt became necessary to enhance the reactivity of the alkyne towards the homocoupling reaction.<sup>51</sup> In addition, it has been shown that the homocoupling reaction was promoted for arylalkynes with electron withdrawing groups present on the phenyl ring.<sup>48</sup> However, in spite of the lack of electron-withdrawing groups in 1-TMS-1,4-pentadiyne we believe that the competitive homocoupling reaction or some other process may occur at a rate faster than the desired cross-coupling reaction. The

formation of any homocoupled product could not be detected by TLC. In addition the isolation of the volatile homocoupled product would be difficult as all cross-coupling reactions were carried out on a small scale.

In order to rule out the iodinated nucleoside **6** as the culprit in the unsuccessful cross-coupling reactions with TMS-1,4-pentadiyne, a test reaction with an alkyne was performed. A Sonogashira reaction between nucleoside **6** with 1-hexyne was attempted and found to proceed smoothly to give cross-coupled product **13** in moderate yield (32%) after 20 h treatment at 40°C.



**Scheme 2.4:** Synthesis of compound **13**.

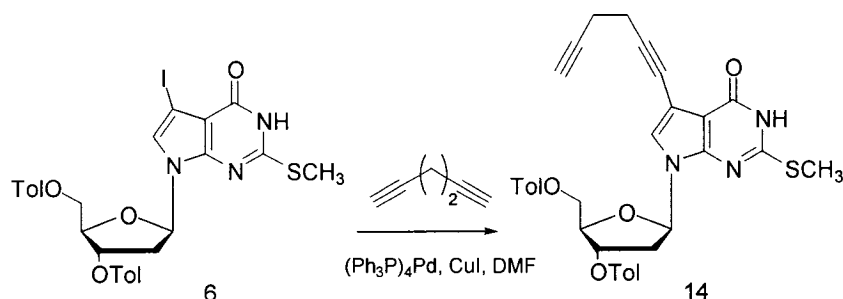
The same reaction carried out at RT did not yield the expected cross-coupled product, only unreacted starting material. Therefore, consequent Sonogashira cross-coupling reactions were performed at elevated temperature (40°C).

#### 2.4 Sonogashira Cross-coupling Reaction with 1,5-hexadiyne

Given the unsuccessful attempts to introduce 1,4-pentadiyne at the C7 position of 7-deazapurine nucleoside it was decided to prepare an analogue that would be the next, most structurally similar candidate to our target molecule. Rather than synthesize the 5

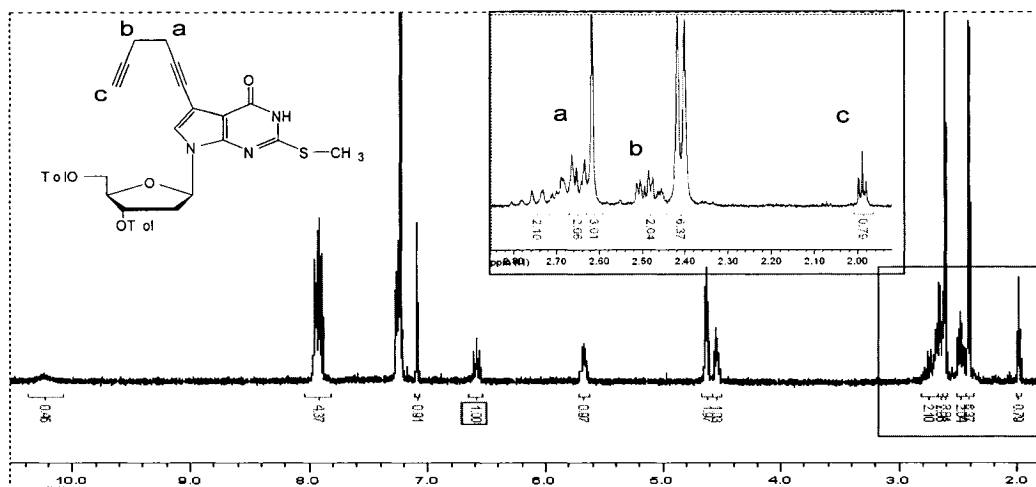
carbon alkyl linker analogue, it was decided to prepare one with a 6 carbon linker, as proof of principle of the synthetic strategy. Therefore, 1,5-hexadiyne, the next closest analogue of the 1,4-pentadiyne, was explored for the synthesis of a C7 adduct of the 7-deazapurine nucleoside via the palladium catalyzed Sonogashira cross-coupling reaction.

First, we verified reactivity of the 1,5-hexadiyne in cross-coupling reaction with nucleoside **6** (**Scheme 2.5**). The Sonogashira reaction was carried out with 2.5 equiv of the diyne, 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.23 eq of CuI and 2.7 eq of Et<sub>3</sub>N in DMF for 16 h at 40°C.



**Scheme 2.5:** Synthesis of compound **14**.

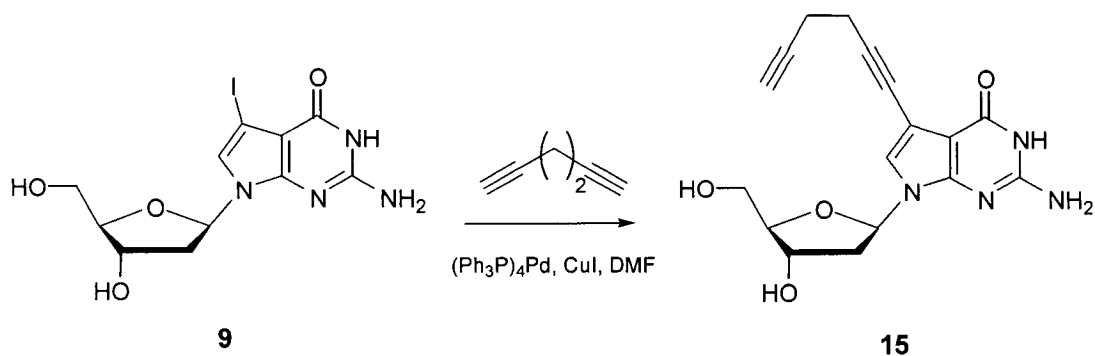
The cross-coupled product **14** was obtained with a 53% yield. These reaction conditions were similar to the one between nucleoside **6** and 1-hexyne illustrating the importance of moderately elevated temperatures in the formation of the desired cross-coupled product. The identity of the cross-coupled product **14** was confirmed by ESI-TOF-MS and <sup>1</sup>H-NMR analysis. In the <sup>1</sup>H-NMR spectrum of compound **14**, apart from two 2 H signals assigned to the methylene groups of hexadiynyl side chain, a 1 H triplet signal assigned to the acetylenic proton was observed at 1.99 ppm (**Figure 2.7**).



**Figure 2.7:**  $^1\text{H}$  NMR spectra of compound **14**.

The signal for one of the methylene groups of the hexadiynyl groups partially overlaps with a signal that belongs to the H2' and H2'' of the deoxyribose moiety. The triplet signal of the acetylenic proton is a characteristic feature of all the  $^1\text{H}$ -NMR spectra of all 1,5 hexadiyne C-7 adducts of 7-deazapurines. The appearance of this signal in  $^1\text{H}$ -NMR spectrum serves as validation that the cross-coupling reaction has occurred.

With the successful coupling of the 1,5-hexadiyne linker to 7-deazapurine via the Sonogashira cross-coupling reaction we attempted to prepare nucleoside **15** (Scheme 2.6), an important intermediate in our strategy of preparation of the cross-linked nucleoside dimer. The reaction conditions recommended for 7-deazapurines were employed. Nucleoside **9** was treated with 2.5 eq of 1,5-hexadiyne, 0.1 eq of  $\text{Pd}(\text{PPh}_3)_4$ , 0.2 eq of  $\text{CuI}$ , and slight excess (2.9 eq) of  $\text{Et}_3\text{N}$  in DMF at  $40^\circ\text{C}$  for 15 h.

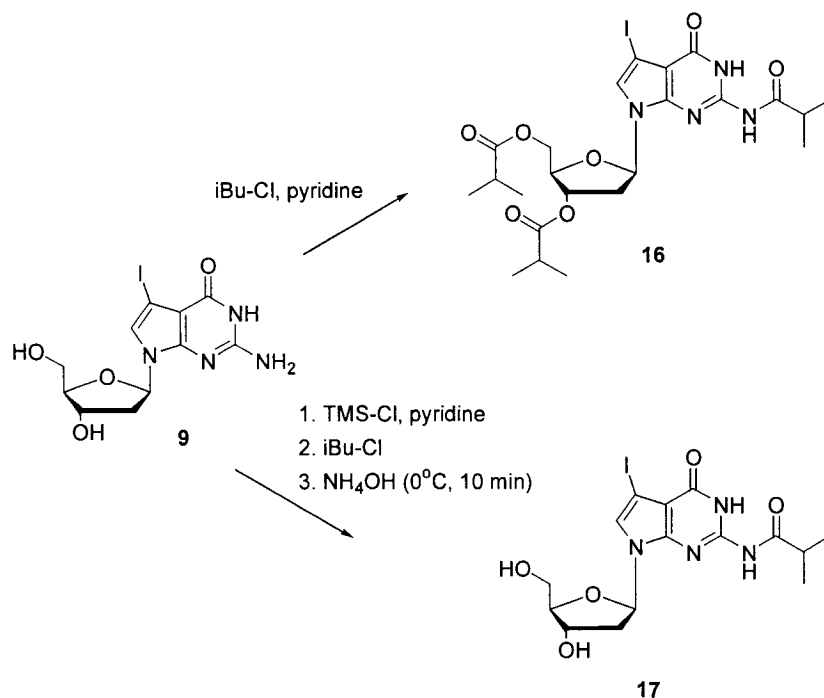


**Scheme 2.6:** Synthesis of compound **15**.

Despite the fact that TLC revealed complete conversion of starting material **9** and formation of new compound with slightly higher  $R_f$  value, the isolation and the purification of nucleoside **15** was not successful. We were unable to separate the crude nucleoside by-products of the Sonogashira cross-coupling reaction. The salts of triethylamine and decomposition products of the palladium catalyst are highly polar species that are not easily removed from polar nucleoside **15** by means of standard normal phase silica gel column chromatography. However, the presence of the 1,5-hexadiyne adduct **15** in the crude product mixture was confirmed by ESI-TOF-MS and  $^1\text{H-NMR}$  analysis. The ESI-TOF-MS spectrum shows the presence of a molecular peak of nucleoside **15** ( $M + \text{H}^+ = 343.37$  and  $M + \text{Na}^+ = 365.37$ ). Moreover in the  $^1\text{H-NMR}$  spectrum a characteristic triplet signal of acetylenic proton of the hexadiynyl side chain was observed at 1.99 ppm.

Due to difficulties encountered during the purification of nucleoside **15** it was theorized that the presence of non-polar protecting groups could significantly facilitate the isolation of cross-coupled product. Therefore we prepared the completely

isobutyrylated nucleoside **16** and nucleoside **17** that is protected only at the exocyclic amino group according to **Scheme 2.7**.



**Scheme 2.7:** Synthesis of isobutyrylated compounds **16** and **17**.

Compound **16** was prepared from nucleoside **9** by reaction with excess of isobutyryl chloride (9 eq) in pyridine. In the best case the yield of the reaction was 96%. Unfortunately the reaction yield of nucleoside **16** significantly fluctuated from case to case. Despite the fact that byproduct of the acylation reaction is the pyridinium hydrochloride salt we were suspicious that prolonged treatment for 20 h under this condition may initiate decomposition of nucleosides present in reaction mixture. However we did not investigate the influence of reaction time on the reaction yield.

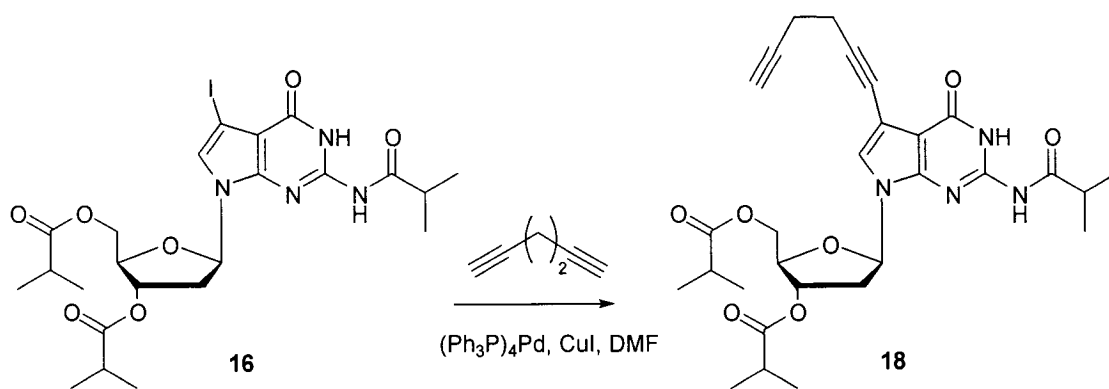
Nucleoside **17** was prepared via transient protection method of Jones.<sup>52</sup> This method is a reliable protecting group methodology that allows for differentiation between



hydroxyl and amino groups of nucleosides. In order to achieve selective protection of exocyclic amino group the hydroxyl groups are protected prior N-acylation. First a TMS protecting group is introduced selectively for the hydroxyl groups. This step is followed by isobutyrylation of exocyclic amino group carried out as a "one pot" reaction. Addition of concentrated aqueous ammonia hydroxide into reaction mixture for a short time (15 min) hydrolyzed the TMS protecting groups to give N-protected nucleoside generally in good yield. The Jones transient approach was successfully applied to prepare deazapurine nucleoside **17** from unprotected nucleoside **9**. After purification by column chromatography the yield of nucleoside **17** was 71 %.

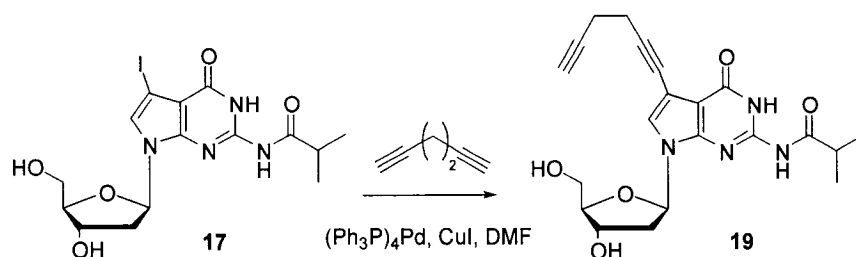
The introduction of these isobutyryl protecting groups renders the nucleoside sufficiently less polar which should facilitate extraction and workup of the Sonogashira cross-coupling reaction.

The fully isobutyrylated nucleoside **16** was cross-coupled with 4.9 eq of 1,5 hexadiyne in presence of 5.4 eq of triethylamine at 40°C (**Scheme 2.8**). It was difficult to monitor the reaction by the TLC due to the fact that retention factor of starting material **16** ( $R_f = 0.61$ ) is almost identical with retention factor of cross-coupled product **18** ( $R_f = 0.63$ , (EtOAc/Hexane/MeOH 4.5:4.5:0.8)). The reaction was allowed to run for 2 days, however TLC analysis of the reaction mixture indicated that the composition of the reaction mixture did not change significantly after the first three hours. We speculate that the longer reaction times may result in decomposition of the reaction products. This could be a reason for obtaining only a moderate yield (25%) of nucleoside **18**. In the future, reduced reaction time should be investigated for the coupling reaction.



**Scheme 2.8:** Synthesis of nucleoside **18**.

The Sonogashira reaction between nucleoside **17** and 2.5 equivalents of 1,5-hexadiyne in the presence of 4.3 equivalents of triethylamine gave the desired cross-coupled nucleoside **19** in 45% yield (**Scheme 2.9**). The reaction mixture was stirred at RT for 3.5 h but TLC revealed that the majority of the product was formed in first 20 minutes. Despite almost identical retention factors of nucleosides **17** and **19**, monitoring of the reaction by TLC indicated the formation of a strong fluorescent spot identified as nucleoside **19**. When the reaction time was reduced to 80 minutes the yield of the reaction was only slightly lower (39 %). This difference may arise from different work up procedures of the crude reaction mixture. We assumed that the evaporation of the DMF at elevated temperature may initiate side reactions and consequently decomposition of the cross-coupled product. In order to eliminate this step we decided to remove DMF by means of column chromatography. In the latter case the reaction mixture was directly purified by the column chromatography. However, in order to obtain sufficiently pure nucleoside product the procedure of column chromatography was repeated twice. This could also account for the slightly lower final yield of nucleoside **19**.



**Scheme 2.9:** Synthesis of nucleoside **19**.

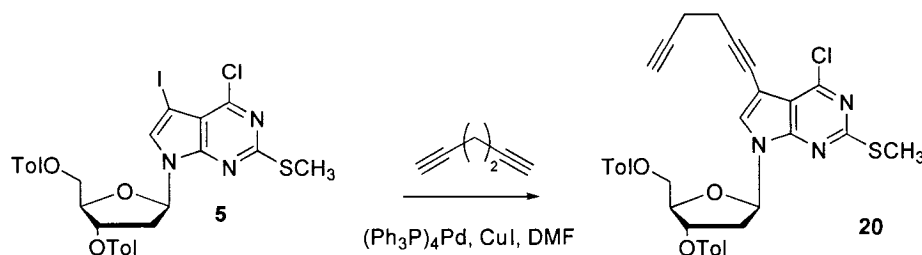
Investigation of the effect of base concentration on the yield of the cross-coupling reaction suggested that small changes in the concentration of triethylamine did not play a profound role in efficiency of cross-coupling reaction. The Sonogashira reaction between nucleoside **17** and 2.5 equivalents of 1,5-hexadiyne in the presence of 2.5 equivalents of triethylamine after 70 min at RT gave the desired cross-coupled nucleoside **19** in various yields (34-45 %).

On the other hand, modified nucleoside **6** that has replaced the exocyclic amino group with a methylmercapto group proved to be more efficient in the cross-coupling reaction with 1,5 hexadiyne with a yield of nucleoside **14** of 53 %. It was observed that the deoxydeazaguanosine nucleosides **9**, **16** and **17** undergo the first step of the cross-coupling reaction, namely oxidative addition, at a rate faster than modified nucleoside **6**. While the Sonogashira reaction mixture of nucleoside **6** was always a transparent orange solution, the reaction mixture for nucleosides **9**, **16** and **17** became dark brown solution immediately after addition of 1,5-hexadiyne, which is known to be sign of rapid consumption of starting iodide.<sup>33</sup>

Our results suggested that complete protection of all polar functional groups is not necessary. Selective protection of exocyclic amino group was sufficient to facilitate isolation of cross-coupled product of Sonogashira reaction with 1,5-hexadiyne. Protecting groups increase solubility by decreasing the polar influence. In addition, it is well

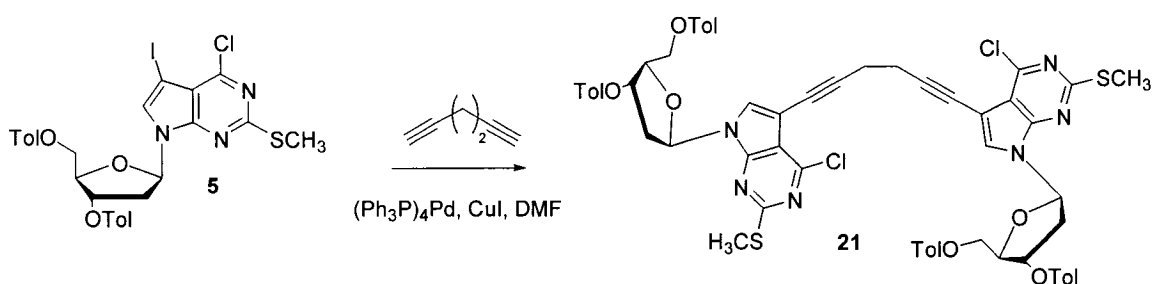
documented that the rate of oxidative addition of palladium (0) catalyst to some aryl-halogen bonds increases as the aromatic ring becomes electron-deficient<sup>33,49</sup> The isobutyryl protecting group eliminates electron donor ability of exocyclic amino group that may in turn facilitate oxidative addition of Pd catalyst to 7-iodo-7-deazaguanosine. Consequently this effect could be also responsible for increased reactivity of nucleoside **17** to Sonogashira cross-coupling reaction compared to its unprotected analogue **9**.

We have shown that the nucleoside **6** can form the C7 adduct with 1,5 hexadiyne with a higher yield of desired product via the Sonogashira reaction compared to the deoxy-7-deazaguanosine nucleosides **9**, **16** and **17**. It is obvious that the presence of the exocyclic amino group can significantly change the reactivity of the 7-deazapurine nucleoside. In order to investigate the effect of the keto group on reactivity of the deazapurine nucleoside the cross-coupling reaction of nucleoside **5** with 1,5-hexadiyne was performed. When nucleoside **5** was reacted with 2.7 eq of 1,5-hexadiyne in presence of 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI and 3.7 eq of Et<sub>3</sub>N in DMF cross-coupled product **20** was isolated in 52% yield after 3 h stirring at RT. The same reaction carried out over 4 h lead to a 61 % yield of nucleoside **20**. In the latter case the DMF evaporation was excluded from the work up and nucleoside **20** was purified directly by column chromatography. This result demonstrates that the method of isolation and work up may also contribute to obtaining the desired product in a higher yield.



**Scheme 2.10:** Synthesis of nucleoside **20**.

Longer reaction times or higher temperature did not improve the efficiency of the cross-coupling reaction. Moreover, prolonged reaction times at elevated temperatures resulted in decomposition of the reactants. For example, when nucleoside **5** was reacted with 2.7 eq of 1,5 hexadiyne in presence of 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI, and 3 eq of Et<sub>3</sub>N in DMF for 40 h at 40°C nucleoside **20** was not isolated. Surprisingly however, a small portion (5%) of nucleoside dimer **21** was obtained (**Scheme 2.11**) as confirmed by MS and <sup>1</sup>H-NMR.



**Scheme 2.11:** Synthesis of dimer **21** as a result of prolonged reaction times and higher temperatures by the Sonogashira reaction.

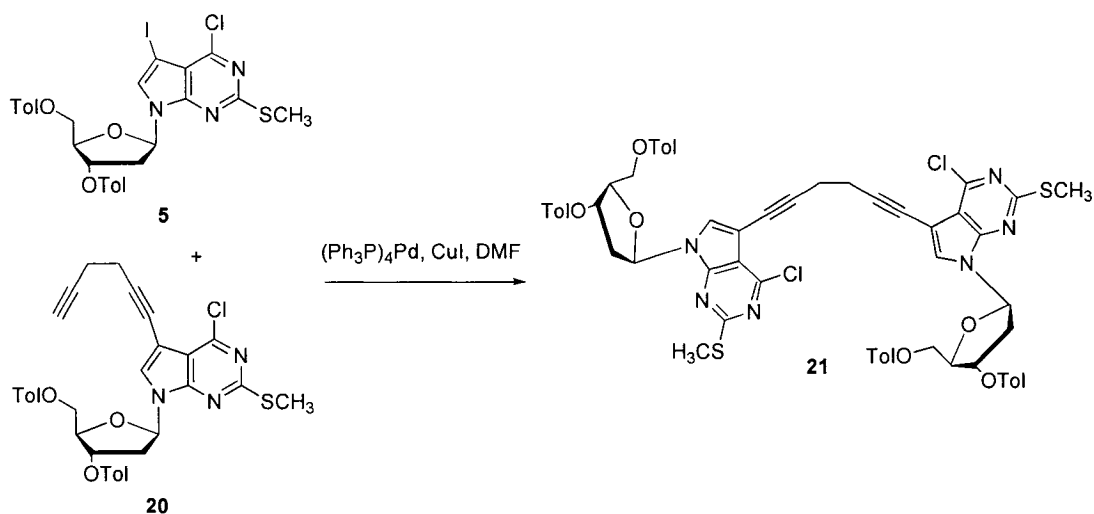
## 2.5 Synthesis of Nucleoside Dimers by the Sonogashira Reaction

Having successfully determined conditions to introduce an alkyldiyne moiety via the Sonogashira reaction at the 7-position of various 7-deazapurines nucleoside, the next major synthetic challenge was to produce a dimer. Due to the difficulties encountered with 1-TMS-1,4-pentadiyne, it was decided to explore reactions between 1,5 hexadiyne and 7-deazapurine nucleosides. Previously reported in the chemical literature were preparation of 7-deazaadenosine nucleosides with the alkyldiynes 1,5-hexadiyne and 1,7-octadiyne.<sup>35</sup> To our knowledge these are the first attempts to synthesize deazaguanosine nucleoside cross-coupled adducts linked through the C7 atoms by reacting a C7-iodo-nucleoside and a nucleoside bearing 1,5-hexadiynyl side chain.

Our experimental results shown that 7-deazaguanosine nucleosides containing an exocyclic amino group gave poor yields of cross-coupled adduct via the Sonogashira reaction. Introduction of isobutyryl protecting group at the exocyclic amino group rendered isolation of cross-coupled adduct more feasible. However only moderate yields of 7-deazaguanosine cross-coupled adducts were achieved.

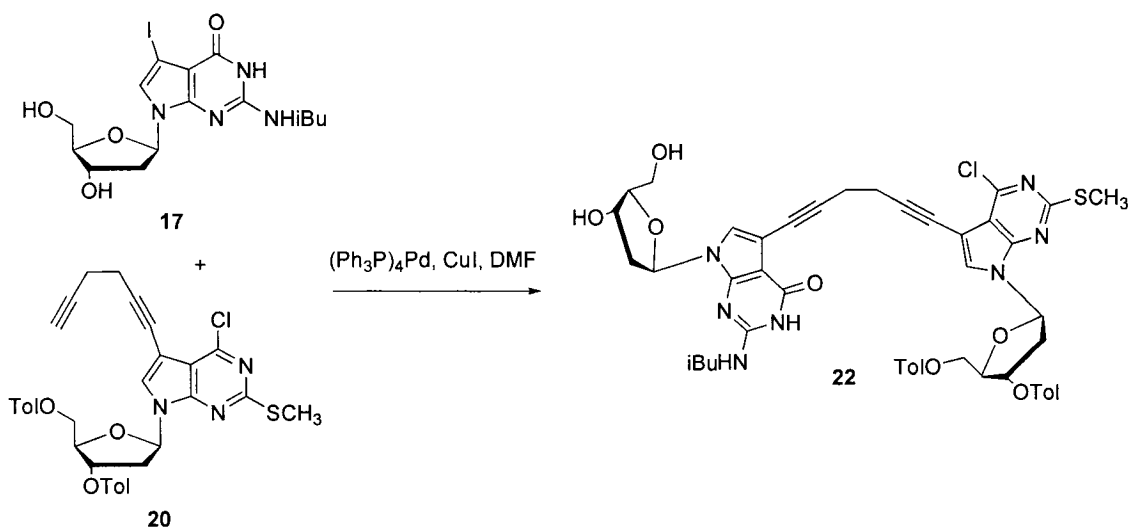
Unlike 7-deazaguanosine, 7-deazapurine nucleosides bearing a methylmercapto group at the C2 position proved to be more reactive. Moreover, a small amount of dinucleoside dimer **21** was obtained in 5% yield when nucleoside **5** was treated with 2.5 eq of 1,5 hexadiyne at 40<sup>0</sup>C for a longer period of time. The same reaction product, dimer **21** could be produced by applying our synthetic strategy, involving the cross-coupling reaction of 7-deazapurine nucleoside **5** and its hexadiynyl derivative nucleoside **20**. We decided to test our synthetic strategy by preparation of nucleoside dimer **21** by treating nucleoside **5** with 2.5 eq of nucleoside **20** in presence of 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI, and 5 eq of Et<sub>3</sub>N in DMF according to **Scheme 2.12**.

TLC analysis indicated complete consumption of iodinated nucleoside **5** after 6 h at RT. The product of reaction was isolated by column chromatography. A single purification step was not sufficient enough to yield a nucleoside dimer of sufficient purity. A second attempt at silica gel column chromatography gave dimer **21** with a 43 % yield. Both <sup>1</sup>H-NMR and MS analysis confirmed identity of nucleoside dimer **21**. The result of this experiment confirmed that our synthetic strategy, the use of two consequent Sonogashira cross-coupling reactions, under favorable circumstances may lead toward nucleoside dimers.



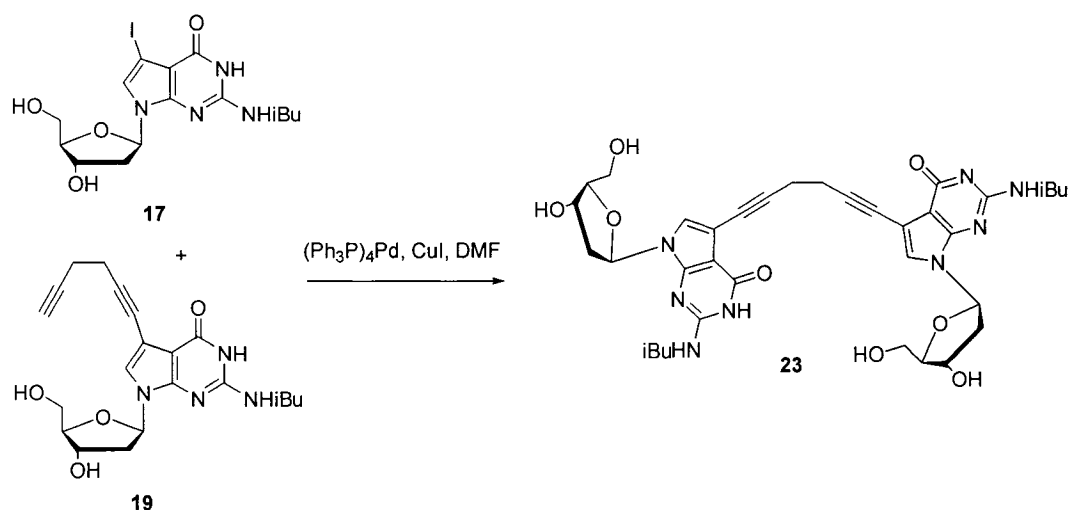
**Scheme 2.12:** Synthesis of nucleoside dimer **21**.

To determine whether the same strategy could be applied to the preparation of the nucleoside dimer from the less reactive 7-deazaguanosine nucleoside N-2 isobutyrylated 7-deazaguanosine **17** was coupled with 2.5 eq of nucleoside **20** according to **Scheme 2.13**. The result of this experiment was encouraging with the nucleoside dimer **22** isolated in good yield (41%) after 7 h treatment at RT.



**Scheme 2.13:** Synthesis of nucleoside dimer **22**.

One of the ultimate goals was the synthesis of 7-deaza-2'-deoxy-guanosine dimer shown below. Due to the challenges encountered with working with unprotected and very polar 7-deazadeoxyguanosine under the Sonogashira reaction conditions it was decided to prepare its analogue 7-deaza-2'-deoxy-guanosine dimer **23** where both exocyclic amino groups are protected by isobutyryl groups by coupling nucleosides **17** and **19** according to **Scheme 2.14**.

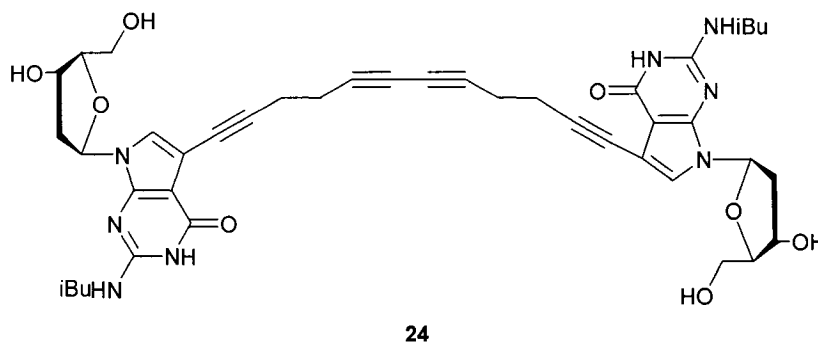


**Scheme 2.14:** Synthesis of nucleoside dimer **23**.

The synthesis of the nucleoside dimer **23** was attempted by a Sonogashira cross-coupling reaction between 1,5-hexadiynyl adduct nucleoside **19** and N-isobutyrylated 7-iodo-7-deaza-2'-deoxyguanosine **17**. In our first attempt we used only 2.1 eq of nucleoside **19** and 7 eq of triethylamine. The TLC analysis revealed complete consumption of nucleoside **17** after 5 h stirring at RT. At the same time the formation of two new compounds with substantial lower retention factors were observed. The retention factor of the major compound measured by normal phase TLC was 0.22 in  $\text{MeOH} / \text{DCM}$  (1:9). In order to increase consumption of the nucleoside **19** another portion 0.25 eq of nucleoside **17** was added into the reaction mixture. The TLC analysis indicated an increase in the formation



of the minor product. The reaction was terminated after 7 h. Our attempt to separate two products of reaction by column chromatography failed. However the  $^1\text{H-NMR}$  analysis of impure product proved that isolated material is mixture of two nucleoside containing products. In the ESI-TOF-MS spectrum two major peaks were found with  $m/z = 769.41$  and  $m/z = 845.46$ . The first peak represents the molecular mass of desired nucleoside dimer **23** ( $M+\text{Na}^+$ ). The molecular mass of the latter peak  $m/z = 845.46$  ( $M+\text{Na}^+$ ) combined with results from  $^1\text{H-NMR}$  analysis suggest that isolated material may also contain product of homocoupling side reaction which we believe may be nucleoside dimer **24** (Figure 2.8).

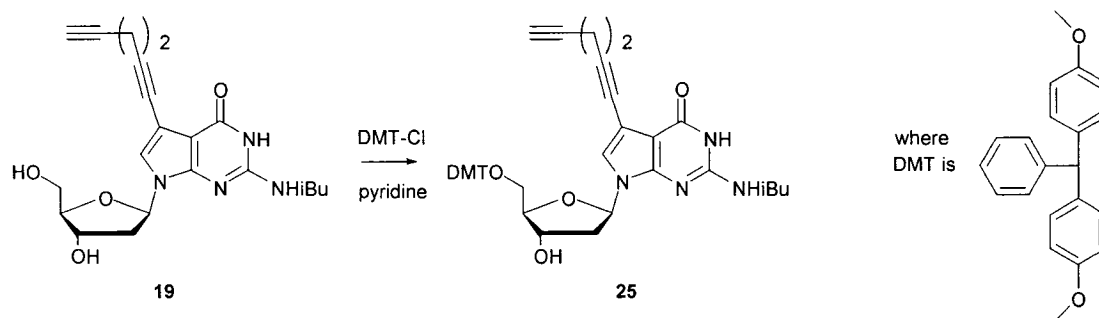


**Figure 2.8:** Proposed structure of the homocoupled side product nucleoside dimer **24**.

From the results, it would appear that the process of homocoupling competes significantly with the desired cross-coupling reaction. Not only does this reaction result in consumption of terminal alkyne but also in consumption of catalyst. As was mentioned earlier it is well known fact that palladium(0)–copper (I) catalytic system in presence of air may initiate homocoupling reactions of a terminal alkyne.<sup>51</sup> However we did not attempt to carry out cross-coupling reaction with different ratios of catalyst. Instead it was speculated that higher amount of triethylamine base (7 eq) may also be involved in the formation of homocoupled dimer.

Therefore in our next attempt to prepare dimer **23** the concentration of triethylamine base was decreased. At the same time, in order to prevent any interruption in the cross-coupling reaction due to premature depletion of terminal alkyne through homocoupling reaction we also decided increase amount of nucleoside **19**. We attempted to couple nucleoside **17** with 2.5 eq of nucleoside **19** in presence of 2.5 eq of triethylamine. In spite of changing the concentration of base and the terminal alkyne the reaction followed the similar reaction pathway. Both new compounds were formed simultaneously. Their similar mobility on TLC and high polarity renders their separation by means of standard silica gel chromatography impossible.

We also explored the use of a nucleoside that contains a dimethoxytrityl protecting group at the 5' position that could facilitate isolation of a dinucleoside dimer and simultaneously significantly facilitate the monitoring of Sonogashira reaction by TLC. Nucleoside **25** was produced by the reaction between nucleoside **19** and dimethoxytrityl chloride (**Scheme 2.15**) in pyridine overnight.



**Scheme 2.15:** Synthesis of tritylated nucleoside **25**.

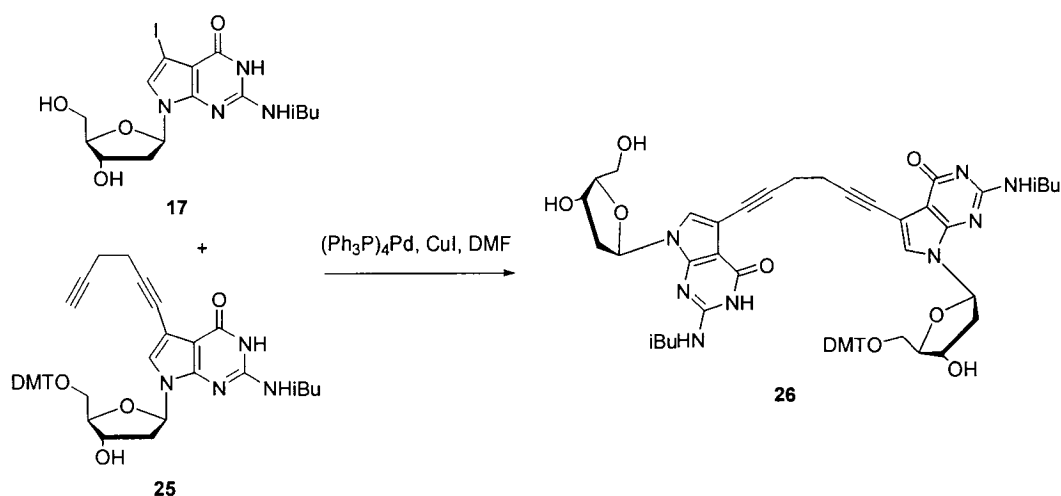
In order to protect the 5'-hydroxyl function of nucleoside **19** we chose the DMT protecting group due to its high lability with acid treatment relative to the MMT. Our final goal was incorporation of target nucleoside dimer into oligonucleotides duplex by

standard solid phase synthesis. Therefore it was assumed that nucleosides bearing easily removable protecting groups compatible with standard solid phase oligonucleotide synthesis would be favoured. From this point of view the DMT protecting group seems to be excellent candidate for protection of 5'-hydroxyl function of 2'-deoxy-7-deazapurines. The treatment of nucleoside **19** with DMT chloride in pyridine afforded tritylated nucleoside **25** in 42% yield.

The different reactivities of primary and secondary hydroxyl group of 2-deoxyribofuranosyl moiety of nucleoside **17** allow for selective dimethoxytrityl protection of the 5' hydroxyl group. Tritylation is usually carried out by treatment of alcohol substrate with DMT chloride in pyridine. The bulky trityl chloride exhibits a high degree of selectivity for the primary hydroxyl group. The trityl ethers are stable to a wide variety of non-acidic conditions but are easily hydrolysed by dilute acid. In addition the acid treatment of nucleosides may also trigger cleavage of glycosidic bonds. This is why more reactive monomethoxytrityl (MMT) and dimethoxytrityl (DMT) groups are used for protection of 5'-hydroxyl functions of 2'-deoxynucleoside rather than trityl. It was found that introduction of each methoxy group into para position of trityl group significantly increase rate of acid hydrolysis.<sup>53</sup> In addition the introduction of electron donor methoxy groups also increase rate of reaction between trityl chlorides and hydroxyl function. Because of these properties the MMT and DMT groups are important protecting groups for primary 5'-hydroxyl functional group of nucleosides.

The proximity of the bulky DMT group relatively close to the reaction centre could generate an additional obstacle for an already spatially congested arrangement for the palladium catalyst which could hinder the cross-coupling reaction. We attempted to

prepare nucleoside dimer **26** from hexadiynyl adduct **25** employing cross-coupling conditions with 1 eq of compound **17**, 1.1 eq of nucleoside **25**, 0.1 eq of Pd(PPh<sub>3</sub>)<sub>4</sub>, 0.2 eq of CuI and 1.5 eq of Et<sub>3</sub>N in DMF at RT according to **Scheme 2.16**. After 6 h treatment TLC analysis showed surprisingly low consumption of the starting nucleosides. Apart from recovered starting nucleosides, a small amount (2% overall yield) of desired nucleoside dimer **26** was isolated by the silica gel column chromatography. However no homocoupled product was obtained, only compound **26** was formed as the single product of the reaction.



**Scheme 2.16:** Synthesis of tritylated dimer **26**.

In our effort to increase the reaction yield of nucleoside dimer **26** we repeated cross-coupling reaction between nucleosides **17** and **25** in presence of 5 eq triethylamine. The TLC analysis revealed that after the first 26 h treatment at RT, the majority of both starting nucleosides were still present. In order to drive the reaction further another portion of palladium (0.1eq) and copper (0.2eq) catalyst was added. After 37 h treatment at RT the reaction mixture did not appear to have progressed any further. Another portion of palladium and copper catalyst were added and the temperature elevated to 40°C for 3

h. The TLC analysis revealed that even elevated temperature and a higher dose of catalyst did not have a significant impact on the reaction. Therefore after 66 h the reaction was terminated and components of reaction mixture isolated by silica gel column chromatography. The desired nucleoside dimer **26** was isolated in 12 % yield. However this compound was highly contaminated with side products of the Sonogashira reaction.

## 2.6 Conclusions and Future Directions

During the course of our studies one of the major challenges involved separating polar nucleoside products of Sonogashira cross-coupling reactions from triethylammonium salts. The formation of both polar byproducts and triethylamine salts significantly complicates the purification process. Another disadvantage of Sonogashira cross-coupling reaction is associated with homogenous character of palladium (0) catalysts. The complete recovery of the catalyst is impossible and consequently the product of cross-coupling reaction contains high levels of palladium contamination. These problems are well known and cited in the literature. Methods to overcome these difficulties may involve use of expensive resins that may neutralize the triethylammonium by-product during work up,<sup>33</sup> the use of semiheterogenous catalytic systems of well dispersed colloidal bimetallic nickel-palladium nanoparticles,<sup>54</sup> or the use of heterogeneous catalyst such as a solid supported Pd/C catalyst.<sup>55</sup> Attempts to completely replace the palladium-copper system by nickel-copper catalysts have also been reported.<sup>56</sup> The idea to immobilize the aryl halide on chloromethyl functionalized polystyrene and subsequently react with acetylenes and catalyst has also been reported.<sup>57</sup>

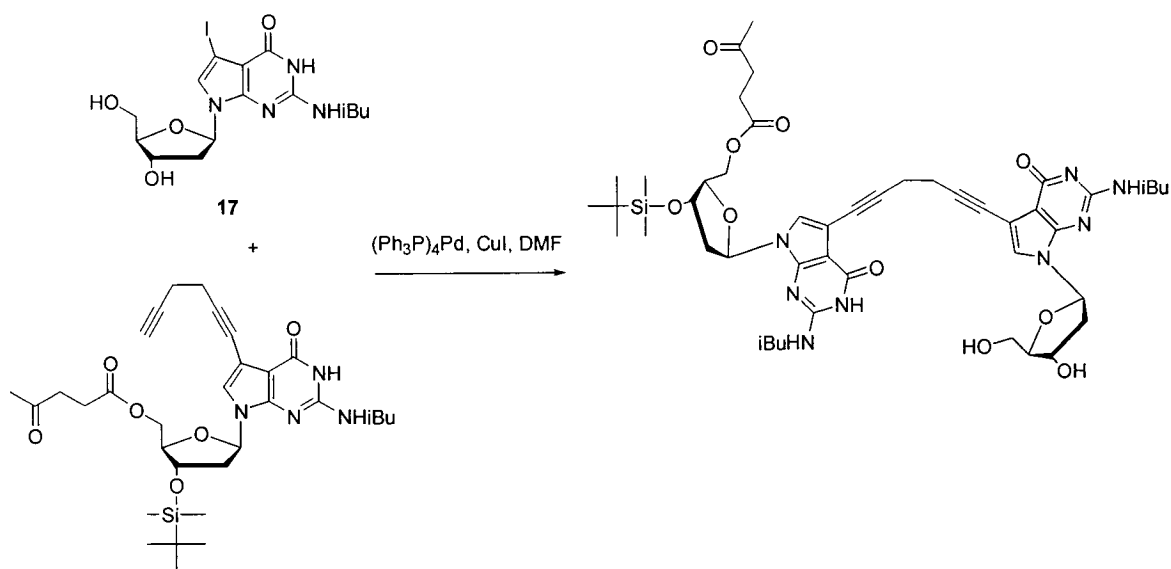
So far only few of these innovations have found practical applications to nucleoside chemistry.

Another interesting idea is to replace the free triethylamine base with inexpensive trialkylamine-bound resin and simultaneously utilize heterogenous Pd/C catalysts.<sup>58</sup> This strategy allows one to remove the alkylammonium salts and Pd catalyst by a simple filtration. The heterogenous Sonogashira protocol that employs a Pd/C catalyst and resin bound tertiary amine as a base was used to prepare cross-coupled adducts of polar unprotected deoxyuridine, deoxyadenosine and deoxyguanosine in good to excellent yields. The idea to anchor nucleoside halides to solid supports was also implemented in order to simplify purification process.<sup>59,60</sup> However in our work we decided to overcome the above mentioned problems by more easily feasible, economical and practical procedures.

The Sonogashira cross-coupling reaction of free unprotected deoxy-7-deazaguanosine with terminal alkynes like 1-propyne, 1-hexyne or 5-trifloracetyl-amino-1-pentyne are well documented and usually give a good yield of cross-coupled adduct.<sup>32,38</sup> However during our work we experienced difficulties with the isolation of the hexadiynyl adducts such as nucleoside **15**. We assumed that the presence of protecting groups may reduce polar character of the nucleoside and consequently facilitate the isolation of cross-coupled product. When the cross-coupling reaction was carried out with nucleoside **16** that has both 3' and 5' hydroxyl groups of sugar moiety and exocyclic amino group of 7-deazapurine protected with the isobutyryl group, a protecting group with a relatively small size compared to the dimethoxytrityl group, a moderate yield of hexadiyne adduct nucleoside **18** was obtained. When larger protecting groups are present

on the nucleoside, such as when attempting to couple tritylated compound **25** with **17**, hardly any product was formed. Given the success as producing dimers **21**, **22** and **23**, it would be advisable to use nucleosides that contain less bulky protecting groups during the homocoupling reaction.

For example, a cross-coupling reaction between compound **17** and a hexadiyne adduct that contains a 5'-O-levulinyl and 3'-O-tert-butyl dimethylsilyl group could give a dimer (**Figure 2.9**).



**Figure 2.9:** Proposed synthesis to produce a nucleoside dimer at the C7 positions of 7-deazaguanosine.

After reduction of the triple bonds in the linker, this dimer could be tritylated selectively at the free 5'-hydroxyl group and elaborated to the phosphoramidite to enable solid phase synthesis of a cross-linked oligonucleotide.

This strategy would require additional deprotection steps to enable cross-link formation, including removal of the levulinyl group by hydrazine, chain extension with

3'-O-deoxyphosphoramidites, removal of the silyl group by TBAF and chain extension with 5'-O-deoxyphosphoramidites.



# Chapter 3

## Experimental

### 3.1 Reagents

In general, all solvents used in organic synthesis were anhydrous. Acetonitrile, dichloromethane (DCM), N,N-dimethylaniline, dimethylformamide (DMF), dioxane, methanol, pyridine, and triethylamine were purchased from the Aldrich Chemical Company (Milwaukee, WI, USA). All solvents used in extractions and column chromatography including acetone, acetonitrile, cyclohexane, dichloromethane, diethylether, ethyl acetate, ethanol, hexanes and methanol were of reagent quality and purchased from EMD Chemicals, Inc. (San Diego, CA, USA).

The following reagents were all reagent grade, purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and used as received: Chloroacetaldehyde (50 % in water), copper (I) iodide, meta-chloro peroxybenzoic acid, 2,5-dihydroxybenzoic acid, dimethylaminopyridine, dimethyl sulphate, dithranol, ethyl cyanoacetate, 1,5-hexadiyne (50% in pentane), N-iodo-succinimide, isobutyryl chloride, phosphorus oxychloride, *syn*-2-pyridinealdoxime, sodium hydride, sodium methoxide, tetrakis(triphenylphosphine) palladium(0), 1,1,3,3-tetramethylguanidine, thiourea and TMS-chloride. The compound 1-chloro-2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranose was purchased from Berry & Associates, Inc. (Dexter, MI, USA). Anhydrous ammonia was purchased from Praxair (St. Laurent, QC, Canada). The compound 1-TMS-1,4-pentadiyne was purchased from GFS Chemicals Inc. (Powell, OH, USA). Dimethoxytrityl chloride was purchased from Chemgenes Corporation (Wilmington, MA, USA). Ammonium hydroxide (30 % in

water), ammonium molybdate, celite, ceric sulphate, citric acid, sodium bicarbonate, sodium hydrogen sulfite, sodium sulfate (anhydrous), sulphuric acid and trifluoroacetic acid were purchased from EMD Chemicals, Inc. (SanDiego, CA, USA).

### **3.2 Chromatography**

Column chromatography was performed with silica gel (40-63 micron silica gel 60) purchased from Silicycle (Quebec City, Quebec). Thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F-254 aluminium-back analytical silica gel sheets (0.2 mm thickness) from EMD Chemicals, Inc. (SanDiego, CA, USA). Compounds were visualized on the TLC plate by illumination with a UV light source (Mineralite, emission wavelength *ca.* 254 nm), and further stained by exposure to HCl or trifluoroacetic acid vapours for trityl-containing compounds. Both UV and non UV active compounds could also be visualized with Mohr's solution (2.5 g of ammonium molybdate and 1 g of ceric sulphate in 10% sulphuric acid (w/v)). This was achieved by first dipping the plate in the solution followed by heating to obtain purple coloured spots/regions.

### **3.3 Instrumentation**

#### **3.3.1 NMR Spectroscopy**

All spectra were obtained at ambient temperature, on a Varian XL-300 (Concordia University) or a Varian XL-500 (McGill University) spectrometer, and the chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). For the new compounds synthesized, all <sup>1</sup>H assignments were made using the 2-D NMR experiment homonuclear correlated spectroscopy (COSY). Deuterated solvents were purchased from ACP Chemicals

Inc. (Montreal, Quebec) and include acetone-D<sub>6</sub>, chloroform-CDCl<sub>3</sub>, dichloromethane-D<sub>2</sub>, dimethylsulfoxide-D<sub>6</sub>, methanol-CD<sub>3</sub>OD.

### 3.3.2 FAB-Mass Spectrometry

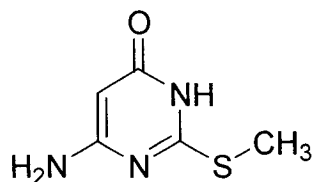
Fast atom bombardment mass spectra were collected by the McGill University Analytical Services using a Kratos MS25RFA mass spectrometer. Nitrobenzyl alcohol (NBA) matrix was used.

### 3.3.3 MALDI TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization time of flight mass spectra were recorded on a Waters TOF instrument with a minimum laser output of 6 mW at a wavelength of 337 nm light, 3 ns pulse width, 1 mm diameter spot). The MALDI instrument was operated in a positive (reflection and linear) mode. The matrix used was either 2,5-dihydroxybenzoic acid or dithranol.

## 3.4 Synthesis

### Synthesis of compound 1



### 2-thiomethyl-4-amino-pyrimidin-6-one

Ethyl cyanoacetate (16.968g, 1eq, 0.15mol) and thiourea (13.083g, 1.14eq, 0.172 mol) were added to a solution of sodium methoxide (9.403g, 1.16eq, 0.174 mol) in

absolute methanol. The mixture was stirred and refluxed for 2 h. After the addition of water (60 ml) the reaction mixture was treated dropwise with dimethyl sulphate (19.638g, 1.04eq, 156mmol). The reaction mixture was stirred and refluxed for an additional 10 minutes and then cooled down in an ice-bath. Crystallized product **1** was collected by filtration and washed with water. An additional amount of product **1** was obtained by concentration of the methanol filtrate to about one-half its volume followed by crystallization and filtration steps. The overall yield of compound **1** was 76 %.

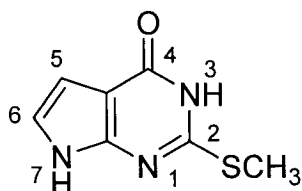
MP: 274-276 °C

R<sub>f</sub> = 0.21 (4.5:4.5:0.8, EtOAc / hexane / methanol)

<sup>1</sup>H NMR (300MHz, CD<sub>3</sub>OD) δ: 5.09 (s, H5, 1H), 2.55 (s, SCH<sub>3</sub>, 3H)

ESI-TOF MS: 158.1 (calc. for [C<sub>5</sub>H<sub>7</sub>N<sub>3</sub>OS + H<sup>+</sup>] 158.0)

### Synthesis of compound **2**



#### 2- thiomethyl-pyrrolo[2,3-d]pyrimidin-4-one

Sodium acetate (19.5g, 0.238 mol) was dissolved in water (150ml) and the solution heated to 80°C. Then compound **1** (10g, 1 eq, 0.064 mol) was added followed by chloroacetaldehyde (50% in water, 10.4 g, 1.04 eq, 0.066mol). The suspension formed was stirred at 80°C for 20 min. After cooling to room temperature the crude product was isolated by filtration, washed with water followed by acetone. The product was recrystallized from water to give compound **2** in 23 % yield.

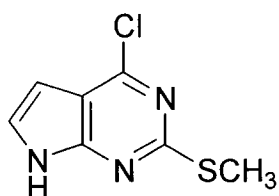
MP: > 300°C

R<sub>f</sub> = 0.44 (4.5:4.5:0.8, EtOAc / hexane / methanol)

<sup>1</sup>H NMR (300MHz, DMSO-d<sub>6</sub>) δ: 12.05 (br s, NH), 11.78 (br s, NH) 6.9(dd, H<sub>6</sub>,1H),6.35 (dd, H<sub>5</sub>, 1H), 3.3 (s, SCH<sub>3</sub>, 3H)

ESI-TOF MS: 181.0 (calc. for [C<sub>7</sub>H<sub>7</sub>N<sub>3</sub> OS ] 181.0)

### Synthesis of compound 3



2- thiomethyl-4-chloro pyrrolo[2,3-d] pyrimidine

Compound **2** (12.847g, 1eq, 0.071 mol), phosphorus oxychloride (150 ml) and freshly distilled N,N-dimethylaniline (20ml) were combined and heated under reflux for 4 h. The excess phosphorus oxychloride was removed in vacuo on a steam bath. The residue (~ 50 ml) was poured with stirring onto crushed ice (~350g). A dark brown tar was formed on the bottom of the beaker with the majority of the product being soluble in the water. The aqueous solution was brought to pH 3-4 with a saturated sodium bicarbonate solution, decanted and extracted with diethyl ether (4 x 400ml). The organic extracts were combined, washed with water, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The diethyl ether was removed by evaporation under reduced pressure. The crude product was recrystallized from cyclohexane–acetone (1:1 v/v) to give the desired compound **3** in 43.4 % yield. The dark brown tar was taken up in acetone (5 ml), diluted with water (150 ml) and extracted with diethyl ether (4x150 ml). The organic extracts were combined,

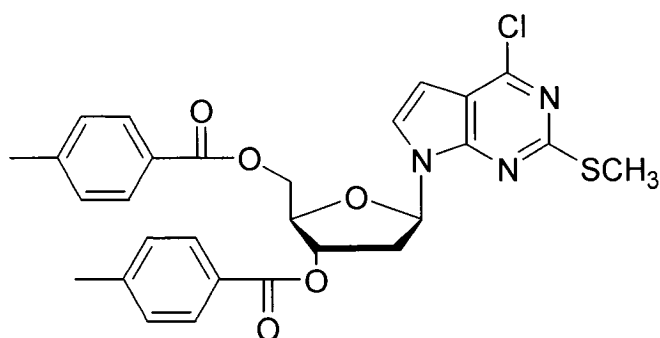
washed with water and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The diethyl ether was removed by evaporation under reduced pressure. The crude product was purified by silica gel column chromatography using 5% acetone in toluene to afford an additional portion of desired compound **3** (4.5 % yield) for a total yield of 6.78 g (47.9 %).

$R_f(\text{SiO}_2) = 0.43$  (1:9, acetone / toluene)

$^1\text{H NMR}$  (300MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$ : 9.35 (br s, NH), 7.25 (dd, H6, 1H), 6.58 (dd, H5, 1H), 2.6 (s,  $\text{SCH}_3$ , 3H)

ESI-TOF MS: 200.1 (calc. for  $[\text{C}_7\text{H}_6\text{ClN}_3\text{S} + \text{H}^+]$  200.0)

#### Synthesis of compound 4



4-Chloro-7-(2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl)-2-(methylthio)pyrrolo [2,3-d]pyrimidine

Compound **3** (0.659g, 1eq, 3.3 mmol) was suspended in anhydrous acetonitrile (25 ml), followed by the addition of sodium hydride (0.091g, 1.1 eq, 3.6 mol). The reaction mixture was stirred at room temperature under a nitrogen atmosphere. After 30 min 1-Chloro-2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl (1.283g, 1eq, 3.3 mmol) was added portion wise. The temperature was increased to  $50^\circ\text{C}$  and the reaction was stirred for an additional 2 h. The mixture was subsequently filtered to remove a small

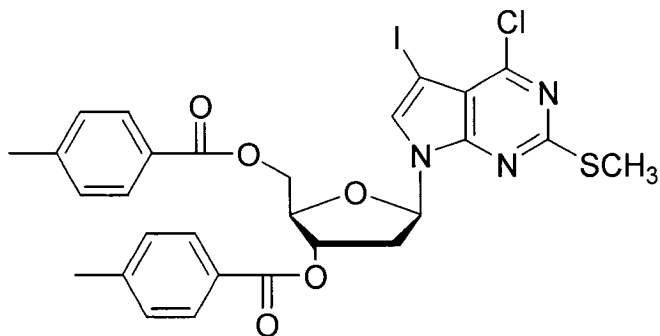
amount of insoluble material and the acetonitrile was removed by distillation under reduced pressure. The oily residue was purified by silica gel column chromatography using 10% acetone in toluene to give desired compound **4** in 65 % yield.

$R_f(\text{SiO}_2)=0.84$  (1:9, acetone / toluene), 0.34 (1:4, EtOAc / hexane)

$^1\text{H NMR}$  (300MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$ : 7.93 (2xd, Tol,  $J_{\text{H-H}}=8.2\text{Hz}$ , 4Ar H ), 7.32-7.25(m, Tol, H6, 4ArH +1H), 6.73 (dd, H1',1H), 6.56 (d, H5,  $J_{\text{H-H}}=3.6\text{Hz}$ ,1H), 5.75 (m, H3',1H), 4.68 (m, H4',1H), 4.58 (m, 5'- $\text{CH}_2$ , 2H), 2.7-3.0 (m, H2', H2'', 2H), 2.63 (s,  $\text{SCH}_3$ , 3H), 2.44 (s,  $\text{CH}_3$ , 3H), 2.42 (s,  $\text{CH}_3$ , 3H)

ESI-TOF MS: 574.1 (calc. for  $[\text{C}_{28}\text{H}_{26}\text{ClN}_3\text{O}_5\text{S} + \text{Na}^+]$  574.1)

### Synthesis of compound **5**:



4-Chloro-7-(2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-erythro-pentofuranosyl)-5-iodo-2-(methylthio) pyrrolo[2,3-d]pyrimidine

Compound **4** (0.50 g, 1 eq, 0.92mmol) was dissolved in anhydrous DMF (27 ml) and NIS (1.48 g, 7.2 eq, 6.59 mmol) was added. The mixture was heated to 95°C for 6 h. On completion of the reaction, as observed by TLC, the mixture was allowed to cool to

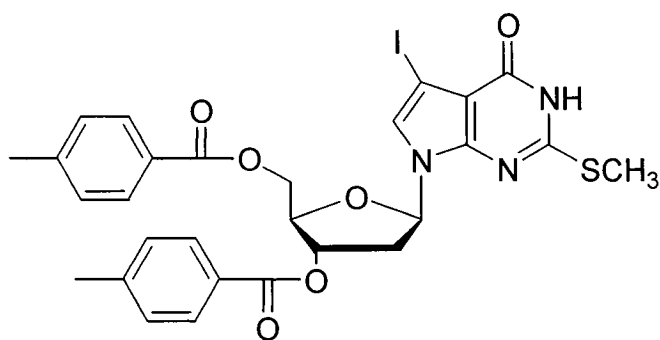
rt. Aqueous sodium bicarbonate (10%, 135  $\mu$ l) was added and the mixture was concentrated to 5 ml. The syrup-like residue was diluted with ethyl acetate (40ml) and washed with water (20ml) followed by 5% aqueous sodium hydrogen sulfite (NaHSO<sub>3</sub>, 2 x 20ml), saturated NaCl (20ml) and then dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The yellow residue was purified by silica gel chromatography using 75% CH<sub>2</sub>Cl<sub>2</sub> in hexane to afford colorless fine crystals of the desired iodinated compound **5** (82 % yield).

R<sub>f</sub> (SiO<sub>2</sub>)= 0.38 (1:4, EtOAc / hexane)

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>)  $\delta$ : 7.95 (2xd, Tol, J<sub>H-H</sub>=8.1Hz 4Ar H ), 7.39 (s, H<sub>6</sub>, 1H), 7.29 (m, Tol, 4Ar H), 6.72 (dd, H<sub>1'</sub>, 1H), 5.73 (m, H<sub>3'</sub>, 1H), 4.57-4.77 (m, H<sub>4'</sub>, 5'-CH<sub>2</sub>, 3H), 2.78(m, H<sub>2'</sub>, H<sub>2''</sub>, 2H), 2.63 (s, SCH<sub>3</sub>, 3H), 2.455 (s, CH<sub>3</sub>, 3H), 2.437 (s, CH<sub>3</sub>, 3H)

ESI-TOF MS: 700.0 (calc. for [C<sub>28</sub>H<sub>25</sub> ClIN<sub>3</sub>O<sub>5</sub> S + Na<sup>+</sup>] 700.0)

#### Synthesis of compound 6:



7-(2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl)-5-iodo-2-(methylthio) -  
pyrrolo[2,3-d]pyrimidin-4-one

To a solution of **5** (0.970 g, 1eq, 1.43 mmol) in DMF (14.4 ml) and dioxane (9.8 ml) was added *syn*-2-pyridinealdoxime (0.888 g, 5.5 eq, 7.80 mmol) and 1,1,3,3-tetramethylguanidine (990 $\mu$ l, 5.5 eq, 7.80 mmol). The mixture was stirred at rt for 24 h.



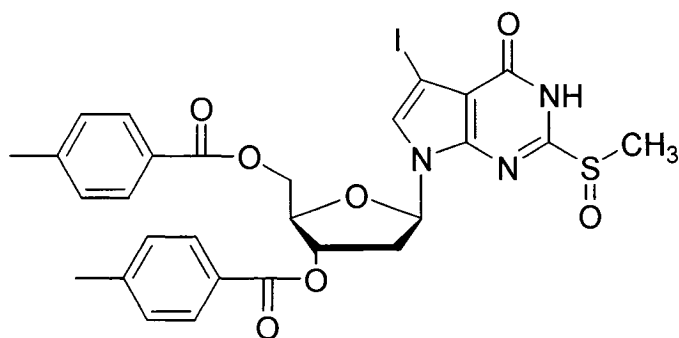
The reaction was concentrated in vacuo and the residue was taken up in DCM (55 ml), washed with 0.1M aqueous citric acid (2 x 35 ml), water (35 ml), saturated aqueous sodium bicarbonate (35 ml), dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was purified by silica gel chromatography using 10 % ethyl acetate in DCM to afford the desired compound **6** (93.5 % yield).

R<sub>f</sub> (SiO<sub>2</sub>) = 0.38 (1:9, EtOAc / DCM), 0.47 (4.5:4.5:0.8, EtOAc / hexane / methanol)

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ: 9.88(br ,NH), 7.95 (2xd, J<sub>H-H</sub>=6.4Hz Tol, 4Ar H ), 7.27(m,Tol, 4Ar H), 7.06(s, H6, 1H) 6.62 (dd, H1', 1H), 5.69 (m, H3', 1H), 4.69-4.67(m, 5'-CH<sub>2</sub>, 2H), 4.58-4.56 (m, H4',1H), 2.81-2.74 (m, H2',H2'', 2H), 2.64 (s, SCH<sub>3</sub>, 3H), 2.45(s, CH<sub>3</sub>, 3H), 2.44 (s, CH<sub>3</sub>, 3H)

ESI-TOF MS: 660.0 (calc. for [C<sub>28</sub>H<sub>26</sub>IN<sub>3</sub>O<sub>6</sub>S + H<sup>+</sup>] 660.1)

### Synthesis of compound 7:



2-Methylsulfoxide-7-(2-deoxy-3,5-di-O-p-toluoyl-β-D-*erythro*-pentofuranosyl)-5-iodopyrrolo[2,3-d]pyrimidin-4-one

Compound **6** (1.32 g, 1 eq, 2.0 mmol) was dissolved in DCM (55 ml) and solution cooled down to 0 °C. Then MCPBA (0.67g, 1.35 eq., 2.71mmol) was added to the stirred solution. After 15 min the ice bath was removed and stirring continued at rt for an

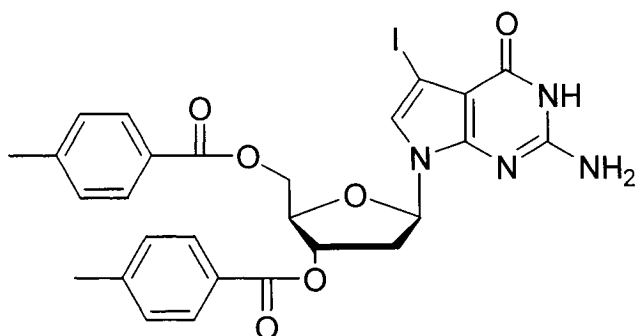
additional 2 hrs. The reaction mixture was diluted with DCM (55ml) and washed with saturated aqueous sodium bicarbonate resulting in the formation of an emulsion. This emulsion was extracted with additional volumes of DCM. All DCM extracts were combined, concentrated to 20 ml and dried over Na<sub>2</sub>SO<sub>4</sub>. After removal of the Na<sub>2</sub>SO<sub>4</sub> by filtration, methanol was added to the filtrate to make a 4% methanol in DCM solution which was directly chromatographed on a short silica gel column to yield sulfoxide 7 (96 % yield).

R<sub>f</sub>(SiO<sub>2</sub>) = 0.24 (4.5:4.5:0.8, EtOAc / hexane / MeOH)

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ: 9.86 (br.s,NH), 7.94 (2xd, J<sub>H-H</sub>=8.1Hz, Tol, 4Ar H ), 7.28 (m,Tol, 4Ar H), 7.20(s, H6,1H), 6.50 (dd, H1',1H), 5.72 (m, H3',1H), 4.71-4.65 (m, 5'-CH<sub>2</sub>, 2H), 4.58 (m, H4', 1H), 2.97, 2.94 (2 x s, SOCH<sub>3</sub>, 3H), 2.80-2.71 (m, H2',H2'', 2H), 2.45 (s, CH<sub>3</sub>, 3H), 2.44 (s, CH<sub>3</sub>, 3H)

ESI-TOF MS: 676.1 (calc. for [C<sub>28</sub>H<sub>26</sub>IN<sub>3</sub>O<sub>7</sub>S + H<sup>+</sup>] 676.1)

### Synthesis of compound 8



2-Amino-7-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)-5-iodo-pyrrolo  
[2,3-d]pyrimidin-4-one

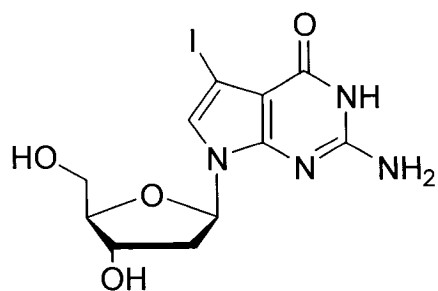
Sulphoxide **7** (1.3g, 1.92 mmol) was suspended in dioxane (25 ml) which was saturated with anhydrous ammonia at 10°C. The cold suspension was transferred to a Parr reactor (a sealed stainless steel vessel with 35 ml volume glass inlet) and anhydrous ammonia was bubbled through the suspension for an additional 10 min at 10°C. The reaction vessel was sealed, the atmosphere inside the reactor replaced by anhydrous ammonia and heated to 145°C for 8.5 h. After this time the reaction mixture was cooled to rt, concentrated and the residue taken up in DCM and extracted with saturated aqueous sodium bicarbonate. The organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. Purification by silica gel column chromatography using 5% methanol in DCM gave product **8** as colorless crystals (79 % yield).

R<sub>f</sub>(SiO<sub>2</sub>) = 0.17 (4.5:4.5:0.8, EtOAc/ hexane /MeOH)

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ: 10.87 (br.s,NH), 7.96 (2xd, J<sub>H-H</sub>=8.1 Hz Tol, 4Ar H ), 7.27 (m,Tol, 4Ar H), 6.87 (s, H<sub>6</sub>, 1H) 6.49(dd, H<sub>1</sub>' , 1H), 6.07 (br s, NH<sub>2</sub>), 5.69 (m, H<sub>3</sub>' , 1H), 4.75-4.61(m, 5'-CH<sub>2</sub>, 2H), 4.55 (m, H<sub>4</sub>' , 1H), 2.78-2.58 (m, H<sub>2</sub>' ,H<sub>2</sub>'', 2H), 2.44 (s, CH<sub>3</sub>, 3H), 2.42 (s, CH<sub>3</sub>, 3H)

ESI-TOF MS: 629.1 (calc. for [C<sub>27</sub>H<sub>25</sub>IN<sub>4</sub>O<sub>6</sub> + H<sup>+</sup>] 629.1)

### Synthesis of compound **9**



2-Amino-7-(2-deoxy- β-D-erythro-pentofuranosyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-4-one

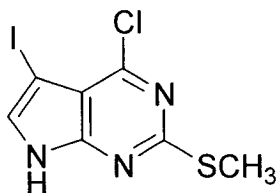
Compound **8** (0.803 g, 1.28 mmol) was suspended in methanol (25 ml) that was saturated with anhydrous ammonia at 0°C. The suspension was transferred to a Parr reactor and anhydrous ammonia was bubbled through the solution at 0°C for an additional 10 min. The reaction vessel was sealed and the atmosphere inside the reactor replaced by anhydrous ammonia. The reaction mixture was heated to 140°C for 2 h, cooled to rt and concentrated. The residue was taken up in water and extracted with diethyl ether. Solid impurities were removed by filtration and the aqueous filtrate concentrated to a solid. The crude product was purified by recrystallization from water (3ml). Precipitated crystals were collected by filtration and washed with cold water and diethyl ether. After drying under vacuum, nucleoside **9** was obtained in a 71 % yield.

R<sub>f</sub>(SiO<sub>2</sub>) = 0.08 (4.5:4.5:0.8, EtOAc / hexane /MeOH), 0.30 (9:1, EtOAc / MeOH)

<sup>1</sup>H NMR (300MHz, DMSO-d<sub>6</sub>) δ: 10.46 (br, s, NH), 7.11(s, H6,1H), 6.32 (b,r s, NH<sub>2</sub>)  
6.26 (dd, H1', 1H), 5.20 (d, J=3.6,3'OH), 4.90 (t, J=5.4Hz, 5'OH), 4.26 (m, H3', 1H),  
3.74 (m, H4', 1H), 3.51-3.46(m, 5'-CH<sub>2</sub>, 2H), 2.35-2.62 , 2.08-2.01(m, H2',H2'', 2H)

ESI-TOF MS: 391.1 (calc. for [C<sub>11</sub>H<sub>13</sub>IN<sub>4</sub>O<sub>4</sub> - H<sup>+</sup>] 391.1)

### Synthesis of compound **11**



4-chloro-5-iodo-2-(methylthio)-pyrrolo[2,3d]pyrimidine

To a solution of compound **3** (0.504g, 1eq, 2.52 mmol) in DMF (10 ml) was added NIS (0.619g, 1.1eq, 2.75mmol). After stirring for 18 h at RT some precipitate,

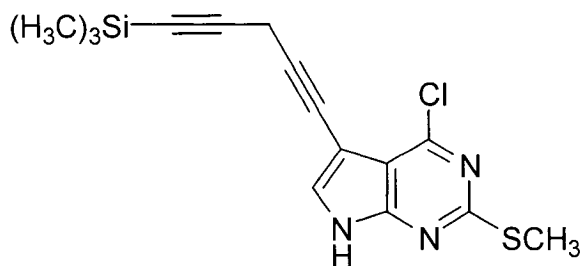
which was crystalline in nature, was formed. These crystals were filtered and the remaining solution was evaporated under vacuum. The solid residue and precipitated crystals were combined and recrystallized from methanol to give compound **11** in 79 % yield.

$R_f(\text{SiO}_2) = 0.45$  (1:3, EtOAc / hexane)

$^1\text{H NMR}$  (300MHz, DMSO- $d_6$ )  $\delta$ : 12.75 (br s, NH), 7.76 (s, H6, 1H), 2.54 (s, SCH<sub>3</sub>, 3H)

ESI-TOF MS: 325.9 (calc. for [C<sub>7</sub>H<sub>5</sub>ClIN<sub>3</sub>S + H<sup>+</sup>] 325.9)

### Synthesis of compound **12**



#### 4-chloro-5-(5-TMS-1,4-pentendiynyl)-2-(methylthio)-pyrrolo[2,3-d]pyrimidine

A mixture of compound **11** (66.2 mg, 1 eq, 0.203 mmol), tetrakis(triphenylphosphine) palladium (0) (23.4 mg, 0.1 eq, 0.020 mmol) and copper iodide (7.8 mg, 0.2 eq, 0.041 mmol) in DMF (2.5 ml) was placed in round bottom flask (5ml) and bubbled with argon for 5 min. Then, 1-TMS-1,4-pentadiyne (35  $\mu\text{l}$ , 27.6mg, 1 eq, 0.203 mmol) and triethylamine (56  $\mu\text{l}$ , 40.7 mg, 2eq, 0.402 mmol) were added. The reaction vessel was sealed with a rubber septum and the atmosphere was replaced with argon. The reaction mixture was kept at 40°C on a sand bath. After 19 h the mixture was transferred to an oil bath and stirred at 40°C for an additional 24 h. The reaction mixture was concentrated, taken up with EtOAc and absorbed onto silica gel (1 g). This material was

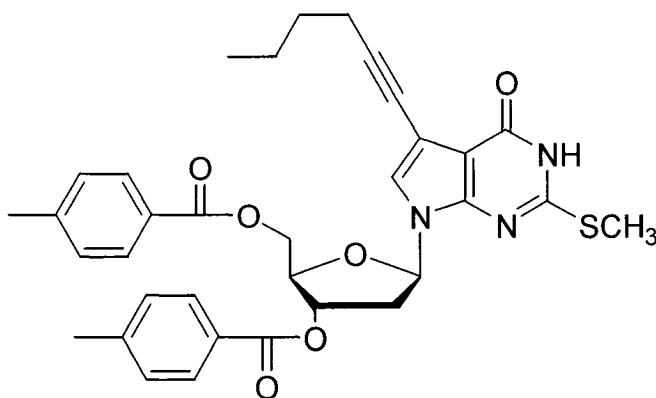
purified by silica gel column chromatography using a gradient of 5-10 % EtOAc in hexane to isolate compound **12** in 3% yield.

$R_f(\text{SiO}_2)=0.5$  (1:3, EtOAc / hexane)

$^1\text{H NMR}$  (300MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.8 (br s, NH), 7.2(m, H6, 1H), 3.95(s,  $\text{CH}_2$ , 2H), 2.6 (s,  $\text{SCH}_3$ , 3H), 0.2 (s,  $\text{CH}_3$ , 9H)

ESI-TOF MS: 334 (calc. for  $[\text{C}_{15}\text{H}_{16}\text{ClN}_3\text{SSi} + \text{H}^+]$  334.1)

### Synthesis of compound 13



2-Methylthio-7-(2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl)-5-(1-hexynyl)-pyrrolo[2,3-d] pyrimidin-4-one

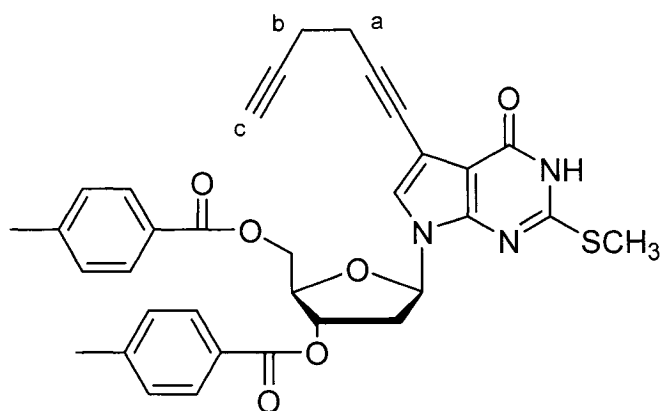
A mixture of **6** (66.9 mg, 1eq, 0.101 mmol), tetrakis(triphenylphosphine)-palladium ( $\text{Ph}_3\text{P}$ ) $_4$ Pd (11.8 mg, 0.1 eq, 0.010mmol), copper iodide (4.0mg, 0.2eq, 0.021 mmol) in DMF (1ml) was bubbled with argon for 5 min in order to eliminate oxygen from the system. Then 97% solution of 1-hexyne (21.2 mg, 29  $\mu\text{l}$ , 0.25 mmol) and triethylamine (25.3 mg, 35  $\mu\text{l}$ , 0.25 mmol) were added. Reaction flask was sealed with a rubber septum, the atmosphere inside was replaced with argon. The mixture was stirred at 40°C for 16 h, after which it was concentrated, taken up with ethyl acetate and absorbed

on the surface of Celite (2g). This material was purified by normal silica gel column chromatography using 10-40 % ethylacetate in hexane to give compound **13** in 32% yield.

$R_f(\text{SiO}_2) = 0.54$  (EtOAc/Hexane/Methanol 4.5:4.5:0.8)

$^1\text{H NMR}$  (300MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$ : 10.15 (br s, NH), 7.95 (2xd,  $J_{\text{H-H}}=8.25$ , Tol, 4Ar H), 7.29(m, Tol, 4Ar H), 7.10 (s, H6, 1H) 6.63 (dd, H1', 1H), 5.69 (m, H3', 1H), 4.62(m, 5'-CH<sub>2</sub>, 2H), 4.56 (m, H4', 1H), 2.82-2.64 (m, H2', H2'', 2H), 2.64 (s, SCH<sub>3</sub>, 3H), 2.43(s, CH<sub>3</sub>, 3H), 2.42 (s, CH<sub>3</sub>, 3H), 1.65-1.4 (m, 3x CH<sub>2</sub>, 6H), 0.93 (t,  $J_{\text{H-H}} = 7.05$  Hz, CH<sub>3</sub>, 3H)  
ESI-TOF MS: 614.23 (calc. for  $[\text{C}_{34}\text{H}_{35}\text{N}_3\text{O}_6\text{S} + \text{H}^+]$  614.2)

#### Synthesis of compound 14



2-Methylthio-7-(2-deoxy-3,5-di-O-p-toluoyl- $\beta$ -D-*erythro*-pentofuranosyl)-5-(1,5-hexadiynyl)-pyrrolo[2,3-d] pyrimidin-4-one

A mixture of **6** (67.1 mg, 1eq, 0.102 mmol), tetrakis(triphenylphosphine)-palladium (0) (11.6 mg, 0.1 eq, 0.010mmol), copper iodide (4.4mg, 0.2eq, 0.023 mmol) in DMF (1ml) was bubbled with argon. Then, 1,5-hexadiyne (a 50% w/w solution in pentane, 0.25 mmol) and triethylamine (27.6mg, 0.273mmol) were added. The reaction

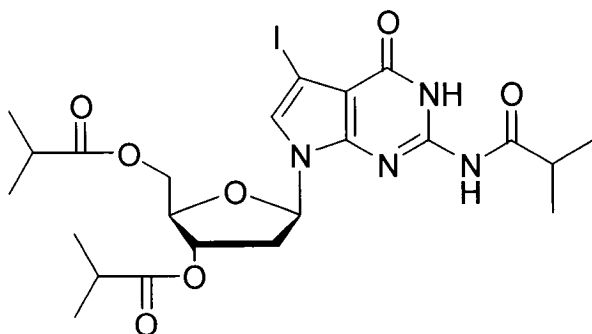
flask was sealed with a rubber septum and the atmosphere inside of the flask replaced with argon. The reaction mixture was stirred at 40°C for 16 h. Then, the reaction mixture was concentrated, taken up with ethyl acetate and absorbed on the surface of Celite (2g). This material was purified by normal silica gel column chromatography using 10-40 % ethyl acetate in hexane to give compound **14** in 53 % yield.

$R_f(\text{SiO}_2) = 0.41$  (4.5:4.5:0.8, EtOAc / hexane / methanol)

$^1\text{H NMR}$  (300MHz,  $\text{CDCl}_3$ )  $\delta$ : 10.25 (br s, NH), 7.92 (2xd,  $J_{\text{H-H}}=6\text{Hz}$ , Tol, 4Ar H), 7.25 (m, Tol, 4Ar H), 7.09(s, H6, 1H) 6.59 (dd, H1', 1H), 5.67 (m, H3', 1H), 4.62(m, 5'-CH<sub>2</sub>, 2H), 4.54 (m, H4', 1H), 2.8- 2.65 (m, H2', H2'', 2H), 2.66 (m, CH<sub>2</sub>-a, , 2H), 2.62 (s, SCH<sub>3</sub>, 3H), 2.49 (m, CH<sub>2</sub>-b, 2H), 2.42 (s, CH<sub>3</sub>, 3H), 2.40 (s, CH<sub>3</sub>, 3H), 1.99 (t,  $J_{\text{H-H}}=2.7$ , CH-c, 1H).

ESI-TOF MS: 610.23 (calc. for  $[\text{C}_{34}\text{H}_{31}\text{N}_3\text{O}_6\text{S} + \text{H}^+]$  610.2)

### Synthesis of compound 16



2-(N-isobutyryl)-amino-7-(2-deoxy-3,5-di-O-isobutyryl- $\beta$ -D-*erythro*-pentofuranosyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-4-one

To a solution of compound **9** (0.269g, 1eq, 0.686mmol) in pyridine (6ml) was added DMAP (4mg, 0.05eq, 0.034 mmol) and isobutyryl chloride (0.658g, 9eq,



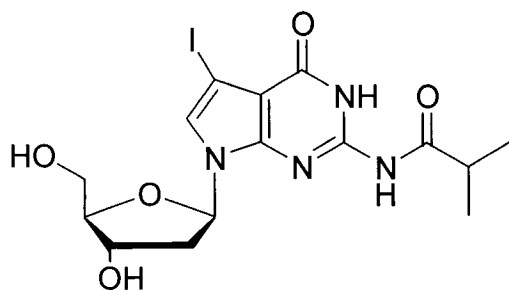
6.171mmol). The reaction mixture was stirred for 5 minutes, allowed to cool to rt and then placed in an oil bath at 35°C. After 20 h, the byproduct pyridinium hydrochloride was filtered and the filtrate was evaporated under reduced pressure. The residue was taken up in ethyl acetate and absorbed onto Celite (1.5g). This material was purified by normal silica gel column chromatography using a gradient of 20-30 % ethylacetate in hexane to give compound **16** in 96% yield.

R<sub>f</sub>(SiO<sub>2</sub>): 0.63 (4.5:4.5:0.8, EtOAc/Hexane/MeOH)

<sup>1</sup>H NMR (300MHz, acetone-d<sub>6</sub>) δ: 11.84 (br.s, NH), 10.33 (br.s, NH), 7.31(s, H6,1H) 6.37 (dd, H1', 1H), 5.39 (m, H3', 1H), 4.32(m, 5'-CH<sub>2</sub>, 2H), 4.25(m, H4', 1H), 2.96-2.46 (m, CH(N-iBu), H2', H2'', CH(O-iBu), 5H), 1.24-1.15 (m, CH<sub>3</sub>, iBu, 18H)

ESI-TOF MS: 603.104, 625.146 (calc. for [C<sub>23</sub>H<sub>31</sub>IN<sub>4</sub>O<sub>7</sub> + H<sup>+</sup>] 603.1, [C<sub>23</sub>H<sub>31</sub>IN<sub>4</sub>O<sub>7</sub> + Na<sup>+</sup>] 625.1)

### Synthesis of compound 17



2-(N-isobutyryl)amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-4-one

Compound **9** (1.027 g, 1 eq, 2.618 mmol) was coevaporated with pyridine (3x) then suspended in pyridine (29ml). Then, TMS-Cl (3.6 ml, 10.9 eq, 28.465 mmol) was

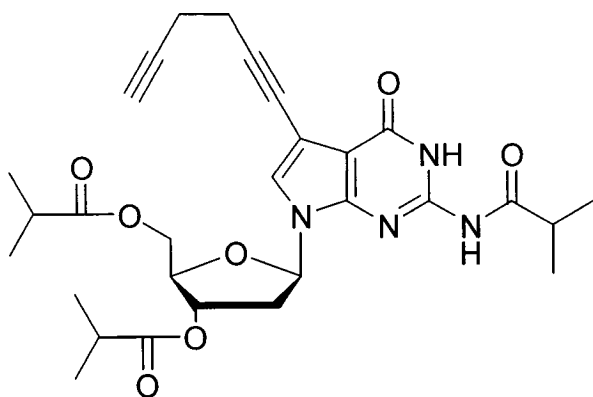
added and the mixture was allowed to stir for 30 min. This was followed by adding and stirring continuously *i*Bu-Cl (1.4 ml, 5 eq, 13.258 mmol). After 2h water (6ml) was added with stirring for 5 min at 0°C. Cold NH<sub>4</sub>OH (30 % in water, 5.72ml) was introduced and allowed to stir for an additional 15 min. The solvent was removed in vacuo, the crude taken up in ethyl acetate, dried over sodium sulphate and adsorbed onto the silica gel. This material was purified by silica gel column chromatography using a gradient of 40-0% hexane in ethyl acetate to give compound **17** in 71 % yield.

R<sub>f</sub>(SiO<sub>2</sub>): 0.53 (EtOAc/MeOH 9:1)

<sup>1</sup>H NMR (300MHz, acetone-d<sub>6</sub>) δ: 11.80 (br s, NH), 10.33(br s, NH), 7.38(s, H<sub>6</sub>, 1H), 6.38(dd, H<sub>1'</sub>, 1H), 4.55(m, H<sub>3'</sub>, 1H), 4.47(br s, 3'OH), 4.14(br s, 5'OH), 3.96(m, H<sub>4'</sub>, 1H), 3.72(m, 5'-CH<sub>2</sub>, 2H), 2.90 (m, CH(*i*Bu), 1H), 2.51-2.421 and 2.28- 2.21 (m, H<sub>2'</sub>, H<sub>2''</sub>), 1.23(d, J=6.9Hz, CH<sub>3</sub>(*i*Bu), 6H)

MALDI TOF MS: 463.0, 485.0 (calc. for [C<sub>15</sub>H<sub>19</sub>IN<sub>4</sub>O<sub>5</sub> + H<sup>+</sup>] 463.0, [C<sub>15</sub>H<sub>19</sub>IN<sub>4</sub>O<sub>5</sub> + Na<sup>+</sup>] 485.0)

## Synthesis of compound 18



2-(N-isobutyryl)-amino-7-(2-deoxy-3,5-di-O-isobutyryl- $\beta$ -D-*erythro*-pentofuranosyl)-5-(1,5-hexadiynyl)-pyrrolo[2,3-d]pyrimidin-4-one

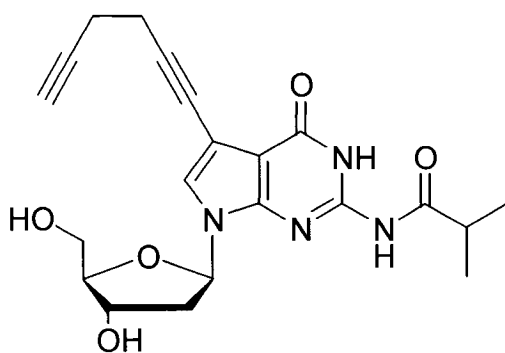
A mixture of **16** (60.2mg, 1eq, 0.1 mmol), tetrakis(triphenylphosphine)-palladium (0) (11.5mg, 0.1 eq, 0.01mmol), copper iodide (3.8mg, 0.2eq, 0.02mmol) in DMF (0.5ml) was bubbled with argon for 5 min with sonication in order to eliminate oxygen from system. The reaction vessel was sealed with a rubber septum and the atmosphere inside the vessel was replaced with argon. Then, a solution of 1,5-hexadiyne in pentane (50%, 76.7mg, 4.9eq, 0.491 mmol) and triethylamine (54.3mg, 5.4eq, 0.537mmol) were injected into the reaction flask. The reaction mixture was stirred at 40 °C for 48 h. The reaction mixture was loaded directly onto a silica gel column and chromatography performed using a gradient of 50-100% DCM in hexane followed by 0-50% ethyl acetate in DCM. All fractions containing compound **18** were combined, concentrated under reduced pressure at 50°C and adsorbed onto silica gel (4g). After a second purification by silica gel column chromatography using 10% ethyl acetate in hexane compound **18** was isolated in 25 % yield.

R<sub>f</sub>(SiO<sub>2</sub>): 0.63 (EtOAc/Hexane/MeOH 4.5:4.5:0.8)

$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.68(br.s,NH), 8.58(br.s,NH), 7.0(s, H6, 1H) 6.17 (dd, H1', 1H), 5.40 (m, H3', 1H), 4.75 (m, H4', 1H), 4.33-4.23(m, 5'- $\text{CH}_2$ , 2H), 2.90 (m, H2', 1H), 2.73-2.3(m, CH( N-iBu ),CH( O-iBu ), H2'',  $\text{CH}_2$ -a, $\text{CH}_2$ -b, 8H ), 2.03 (t,  $J=2.7$  Hz, CH-c, 1H), 1.31-1.18 (m, $\text{CH}_3$ ( iBu), 18H)

ESI-TOF MS: 552.95, 574.92 (calc. for  $[\text{C}_{29}\text{H}_{36}\text{N}_4\text{O}_7 + \text{H}^+]$  553.3,  $[\text{C}_{29}\text{H}_{36}\text{N}_4\text{O}_7 + \text{Na}^+]$  575.3)

### Synthesis of compound 19



2-(N-isobutyryl)amino-7-(2-deoxy- $\beta$ -D-erythro-pentofuranosyl)-5-(1,5-hexadiynyl)-pyrrolo [2,3-d] pyrimidin-4-one

A mixture of **17** (0.327g, 1eq, 0.707 mmol), tetrakis(triphenylphosphine) palladium (0) (82.5 mg 0.1 eq, 0.071mmol), copper iodide (27.7mg, 0.2eq, 0.145mmol) in DMF (1.8ml) was bubbled with argon for 5 min and sonicated in order to eliminate oxygen from system. The reaction vessel was sealed with a rubber septum and the atmosphere in the vessel was replaced with argon. Then, a solution of 1,5-hexadiyne in pentane ( 50 %, 0.295 g, 2.7 eq, 1.891 mmol ) and triethylamine (0.273 g, 3.8 eq, 2.694 mmol ) were injected into the reaction flask. The reaction mixture was stirred at RT for 80mins. The crude mixture was purified by silica gel chromatography using a gradient of

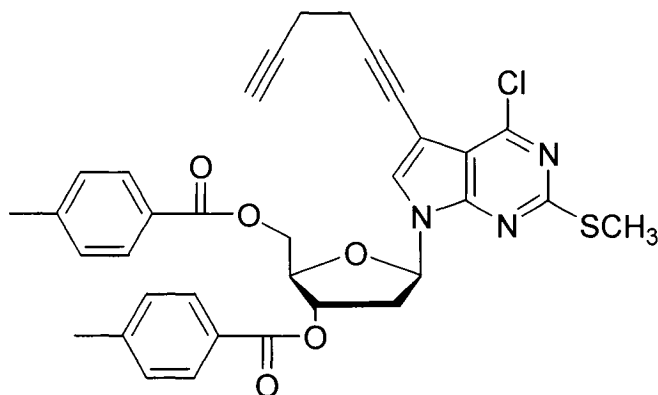
0-2% MeOH in DCM. Fractions containing compound **19** were combined, concentrated under reduced pressure at 50°C and absorbed on to silica gel (2g). Pure compound **19** was isolated in 39% yield after a second column purification using gradient of 0-3% MeOH in DCM.

R<sub>f</sub>(SiO<sub>2</sub>): 0.43 (DCM/MeOH 9:1)

<sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ: 11.67(br s, NH), 8.04(br s, NH), 6.97(s, H6, 1H) 6.12(dd, H1', 1H), 4.69(m, H3', 1H), 4.14 (m, H4', 1H) 3.96-3.81 (m, 5'-CH<sub>2</sub>, 2H), 2.84-2.48(m, H2', CH<sub>2</sub>-a, CH(iBu), CH<sub>2</sub>-b, 6H), 2.29(m, H2'', 1H), 2.04(t, J<sub>H-H</sub>=2.7, CH-c, 1H), 1.29 (2xd, J<sub>H-H</sub> =2.1Hz, CH<sub>3</sub>(iBu))

MALDI TOF MS: 412.51, 434.52 (calc. for [C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub> + H<sup>+</sup>] 413.2, [C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub> + Na<sup>+</sup>] 435.2)

### Synthesis of compound **20**



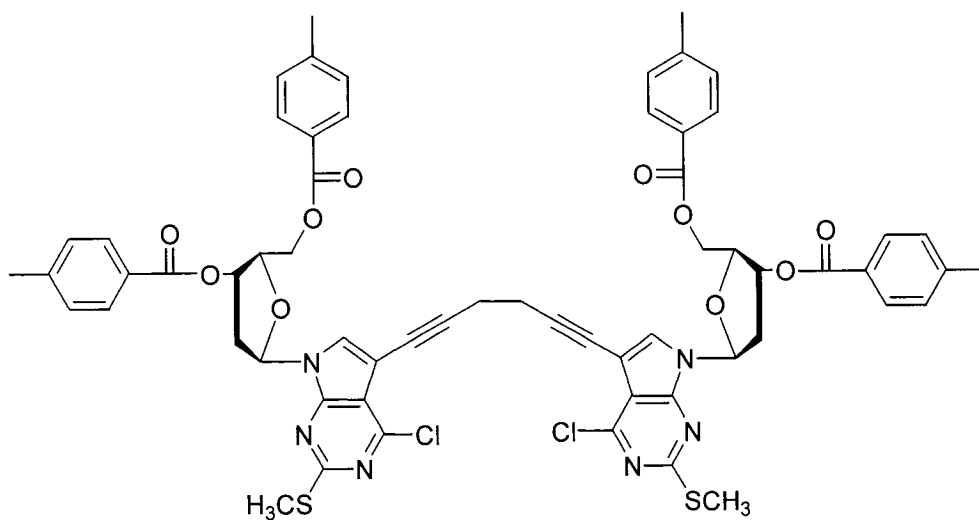
2-Methylthio-4-chloro-7-(2-deoxy-3,5-di-O-p-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(1,5-hexadiynyl)-pyrrolo[2,3-d] pyrimidine

To a mixture of **5** (0.55g, 1eq, 0.811 mmol), tetrakis(triphenyl phosphine) palladium (0) (0.093 g , 0.1 eq, 0.081 mmol), copper iodide (0.031g, 0.2 eq, 0.164 mmol) in DMF ( 11.5 ml ) was bubbled with argon for 5 min while sonicated in order to eliminate oxygen from system. Reaction vessel was sealed with a rubber septum and the atmosphere inside the vessel was replaced with argon. Then 50% pentane solution of 1,5-hexadiyne (0.336g, 2.65eq, 2.153mmol) and triethylamine (0.304g, 3.7eq, 3.01mmol) were injected into the reaction flask. The reaction mixture was stirred at RT for 4 h. The reaction mixture was purified by silica gel chromatography using first 50-100% DCM in hexane then 0-50% ethyl acetate in DCM. All fractions containing compound **20** were combined, concentrated at reduced pressure at 50°C and absorbed into SiO<sub>2</sub> (4g). Pure compound **20** was isolated in 61% yield after an additional silica gel column chromatography using 10% ethyl acetate in hexane.

$R_f(\text{SiO}_2)=0.34$  (EtOAc/Hexene 1:4), <sup>1</sup>H NMR (500MHz, CDCl<sub>3</sub>)  $\delta$ :7.95 (2xd,  $J_{\text{H-H}}=8.1\text{Hz}$ , Tol, 4ArH), 7.39(s, H6, 1H), 7.29 (m,Tol, 4Ar H), 6.70 (dd, H1', 1H), 5.72(m, H3', 1H), 4.67(m, 5'-CH<sub>2</sub>, 2H), 4.59 (m, H4', 1H), 2.77 (m, H2' H2'', 2H), 2.67(t,  $J_{\text{H-H}}=7.4\text{ Hz}$ , CH<sub>2</sub>-a, 2H), 2.63 (s, SCH<sub>3</sub>, 3H), 2.50 (m, CH<sub>2</sub>-b, 2H), 2.45 (s, CH<sub>3</sub>, 3H), 2.43 (s,CH<sub>3</sub>, 3H), 2.04(t,  $J_{\text{H-H}}=2.65\text{ Hz}$ , CH-c, 1H)

MALDI TOF MS : 628.17 (calc. for [C<sub>34</sub>H<sub>30</sub>ClN<sub>3</sub>O<sub>5</sub>S + H<sup>+</sup>] 628.17)

## Synthesis of compound **21**



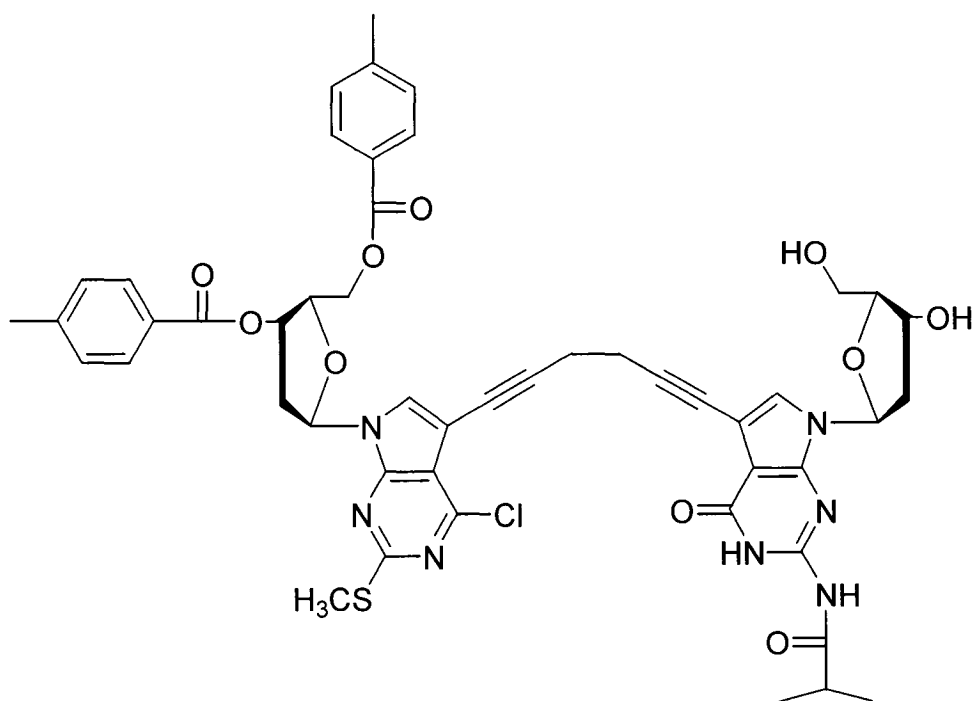
A mixture of **5** (67.8mg, 1eq, 0.1 mmol), **20** (157 mg, 2.5eq, 0.25mmol), tetrakis(triphenylphosphine)palladium (11.6mg, 0.1 eq, 0.01mmol), copper iodide (4mg, 0.21eq, 0.021mmol) in DMF (2.75ml) was bubbled with argon for 5 min with sonication in order to eliminate oxygen from system. The reaction vessel was sealed with a rubber septum and the atmosphere in the vessel was replaced with argon. Then, triethylamine (56 mg, 5.53 eq, 0.55 mmol) was injected into the reaction flask. The reaction mixture was stirred at RT for 6 h. The crude mixture was directly purified by silica gel column chromatography using a gradient of 0-30 % ethyl acetate in hexane. All fractions containing compound **21** were combined, concentrated under reduced pressure at 50°C and adsorbed onto silica gel. Pure compound **21** was isolated in 42.9 % yield after a second column purification using a gradient of 10-20 % ethyl acetate in hexane.

$R_f(\text{SiO}_2) = 0.31$  (EtOAc/Hexane 1:3), 0.2 (EtOAc/Hexane 1:4)

$^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.99-7.89 (2xd,  $J_{\text{H-H}}=8.1\text{Hz}$ , Tol, 8ArH), 7.40(s, H6, 2H), 7.31-7.23(m, Tol, 8Ar H), 6.70 (dd, H1', 2H), 5.72(m, H3', 2H), 4.71-4.58 (m, H4', 5'-CH<sub>2</sub>, 6H), 2.82-2.50 (m, H2', H2'', 4H), 2.74(s, CH<sub>2</sub>-a, CH<sub>2</sub>-b, 4H), 2.62 (s, SCH<sub>3</sub>, 6H), 2.45 (s, CH<sub>3</sub>, 6H), 2.41 (s, CH<sub>3</sub>, 6H)

MALDI TOF MS: 1177.48 (calc. for  $[\text{C}_{62}\text{H}_{54}\text{Cl}_2\text{N}_6\text{O}_{10}\text{S}_2 + \text{H}^+]$  1177.3)

### Synthesis of compound 22



A mixture of **17** (48mg, 1 eq, 0.104 mmol), **20** (155 mg, 2.4 eq, 0.246 mmol) tetrakis(triphenylphosphine)palladium (12.3 mg, 0.1 eq, 0.011 mmol) and copper iodide (3.8 mg, 0.19 eq, 0.02 mmol) in DMF (1ml) was bubbled with argon for 5 min with sonication in order to eliminate oxygen from the system. The reaction vessel was sealed with a rubber septum and the atmosphere in the vessel was replaced with argon. Then,



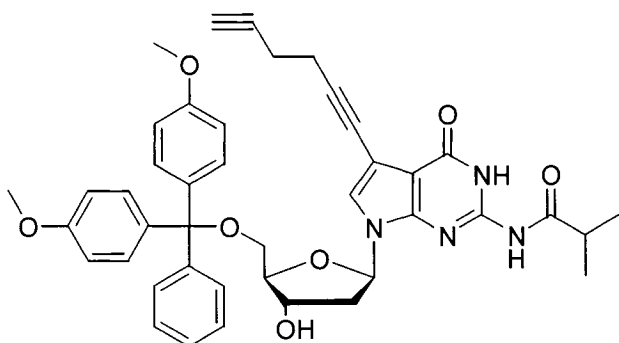
triethylamine (71.3 mg, 6.8 eq, 0.705 mmol) was injected into the reaction flask. The mixture was stirred at RT for 7 h, after which it was directly applied to a silica gel column and purified by chromatography using a gradient of 5-15 % ethyl acetate in hexane. Compound **22** was isolated in 41 % yield.

$R_f(\text{SiO}_2) = 0.75$  (EtOAc/MeOH 9:1)

$^1\text{H NMR}$  (300MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.66(br s, NH), 8.06 (br s, NH), 7.94 (2 x d,  $J_{\text{H-H}} = 8.1\text{Hz}$ , Tol, 4ArH), 7.53 (s, H6, 1H), 7.25 (m, 4Ar H, Tol), 7.0 (s, H6, 1H), 6.73 (dd, H1', 1H), 6.14(m, H1', 1H) 5.78(m, H3', 1H), 4.74-4.52 (m, H4', 5'-CH<sub>2</sub>, H3', 4H), 4.12(m, H4', 1H), 3.95-3.75(m, 5'-CH<sub>2</sub>, 2H), 2.95-2.48 (m, H2'-a, H2''-a, H2'-b, CH(iBu), 4H), 2.76(m, CH<sub>2</sub>-a, CH<sub>2</sub>-b, 4H), 2.63 (s, SCH<sub>3</sub>, 3H), 2.45 (s, CH<sub>3</sub>, 3H), 2.41 (s, CH<sub>3</sub>, 3H), 2.28 (m, H2''-b, 1H), 1.26 (d,  $J_{\text{H-H}} = 6.9\text{Hz}$ , CH<sub>3</sub>(iBu), 6H)

MALDI TOF MS: 984.25 (calc. for  $[\text{C}_{49}\text{H}_{48}\text{ClN}_7\text{O}_{10}\text{S} + \text{Na}^+]$  984.3)

### Synthesis of compound **25**



2-(N-isobutyryl)amino-7-(2-deoxy-5-O-(4,4'-dimethoxytrityl)- $\beta$ -D-erythro-pentofuranosyl)-5-(1,5-hexadiynyl)-pyrrolo [2,3-d] pyrimidin-4-one

Compound **19** (110 mg, 1eq, 0.267 mmol) was coevaporated with pyridine (3 x 5 ml) and taken up in pyridine (2ml). To the solution was added DMAP (0.7mg) and DMT-Cl (129 mg, 1.4eq, 0.380 mmol). The reaction mixture was stirred at RT for 24 h. After this time, the biproduct pyridinium hydrochloride was filtered and the filtrate evaporated under reduced pressure. The residue was taken in DCM and the organic layer washed with NaHCO<sub>3</sub> (5%), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by silica gel column chromatography using a gradient of 0-3 % methanol in DCM to give compound **25** in a 58 % yield.

R<sub>f</sub>(SiO<sub>2</sub>): 0.47(EtOAc/Hexane/MeOH 4.5:4.5:1)

<sup>1</sup>H NMR (300MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ: 11.57(br s, NH), 8.08 (br s, NH), 7.04(s, H6, 1H) 7.45-6.82 (m, DMT, 13H), 6.35(m, H1', 1H), 4.53(m, H3', 1H), 4.02(m, H4', 1H), 3.78 (s, CH<sub>3</sub>O, 6H) 3.34-3.21 (m, 5'-CH<sub>2</sub>, 2H), 2.66(m, CH<sub>2</sub>-a, 2H), 2.49-2.23 (m, CH(iBu), H2', H2'', CH<sub>2</sub>-b, 5H), 2.05(t, J<sub>H-H</sub> = 2.7Hz, CH-c, 1H), 1.2(m, CH<sub>3</sub>(iBu), 6H)

MALDI TOF MS: 737.52 (calc. for [C<sub>42</sub>H<sub>42</sub>N<sub>4</sub>O<sub>7</sub> + Na<sup>+</sup>] 737.3)

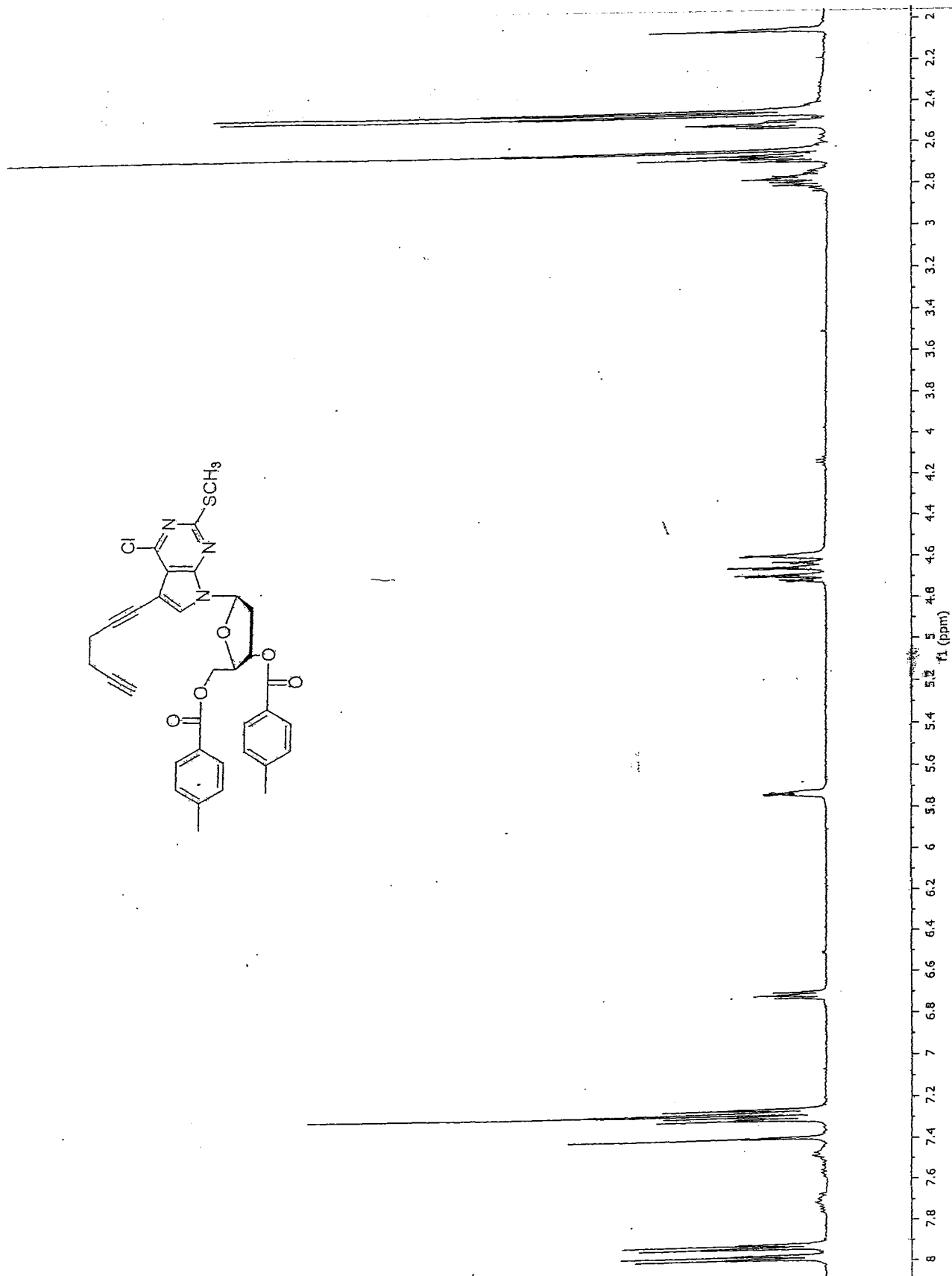
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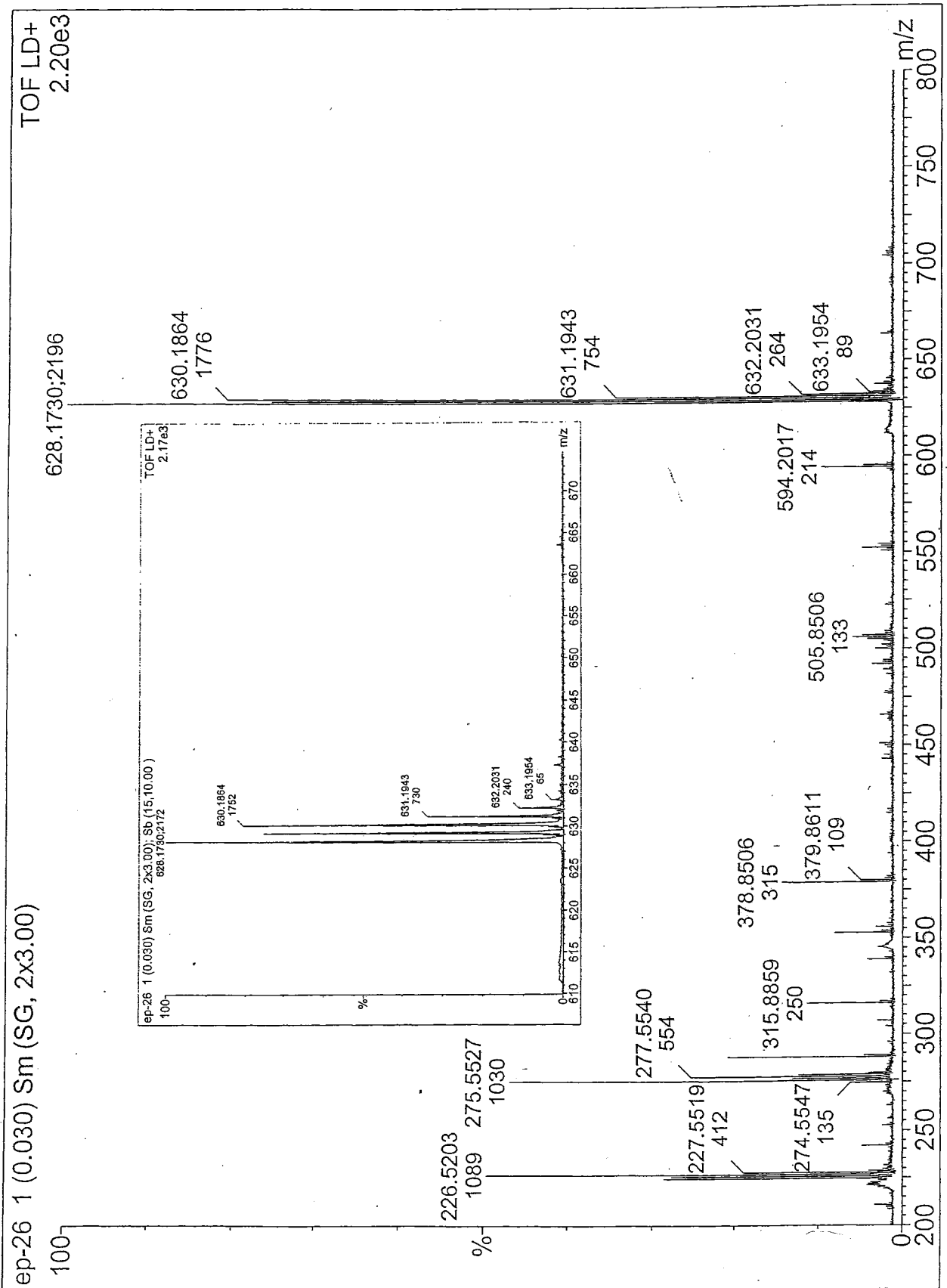
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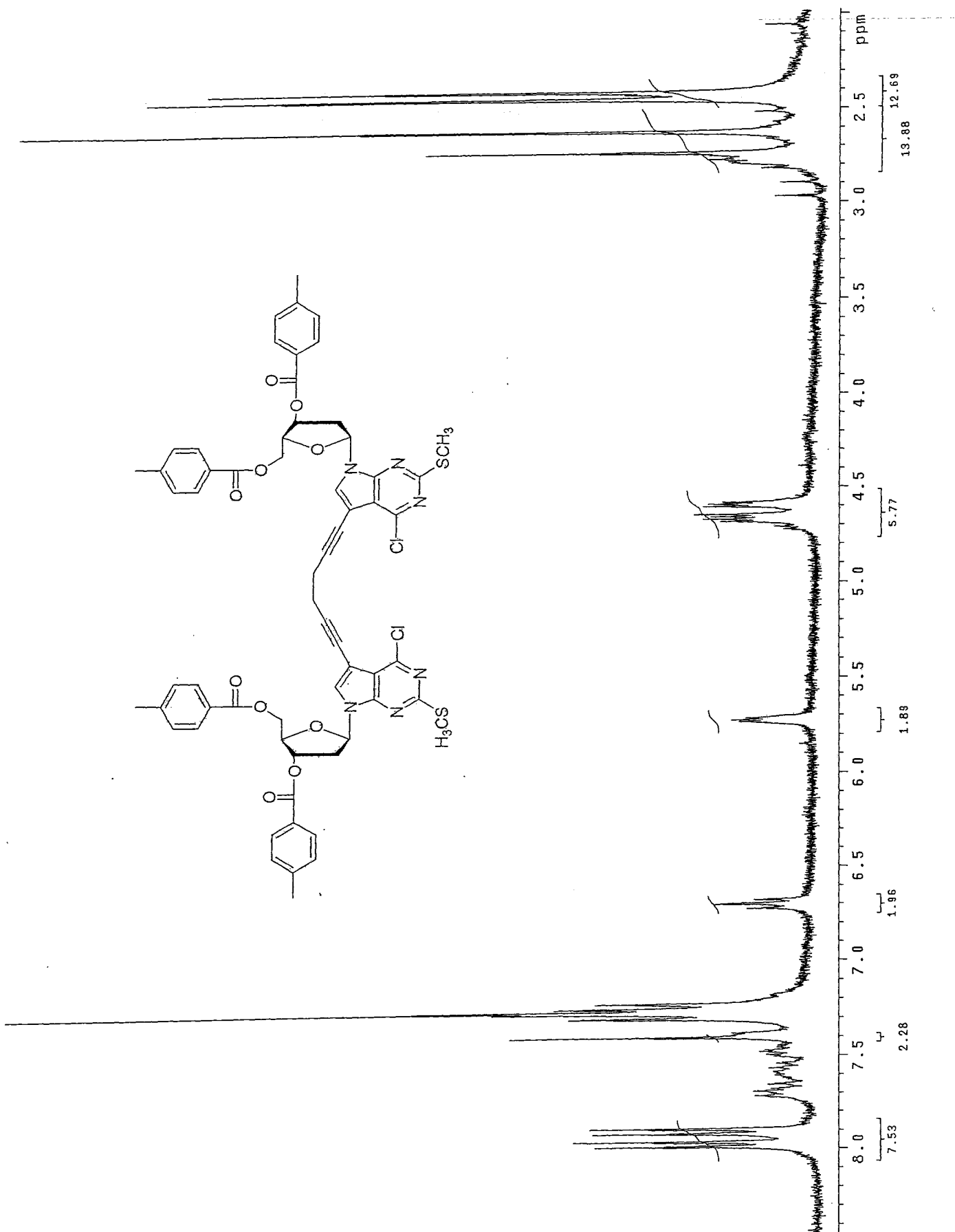


Appendix A:  $^1\text{H}$  NMR Spectrum of Compound 20 (in  $\text{CDCl}_3$ )

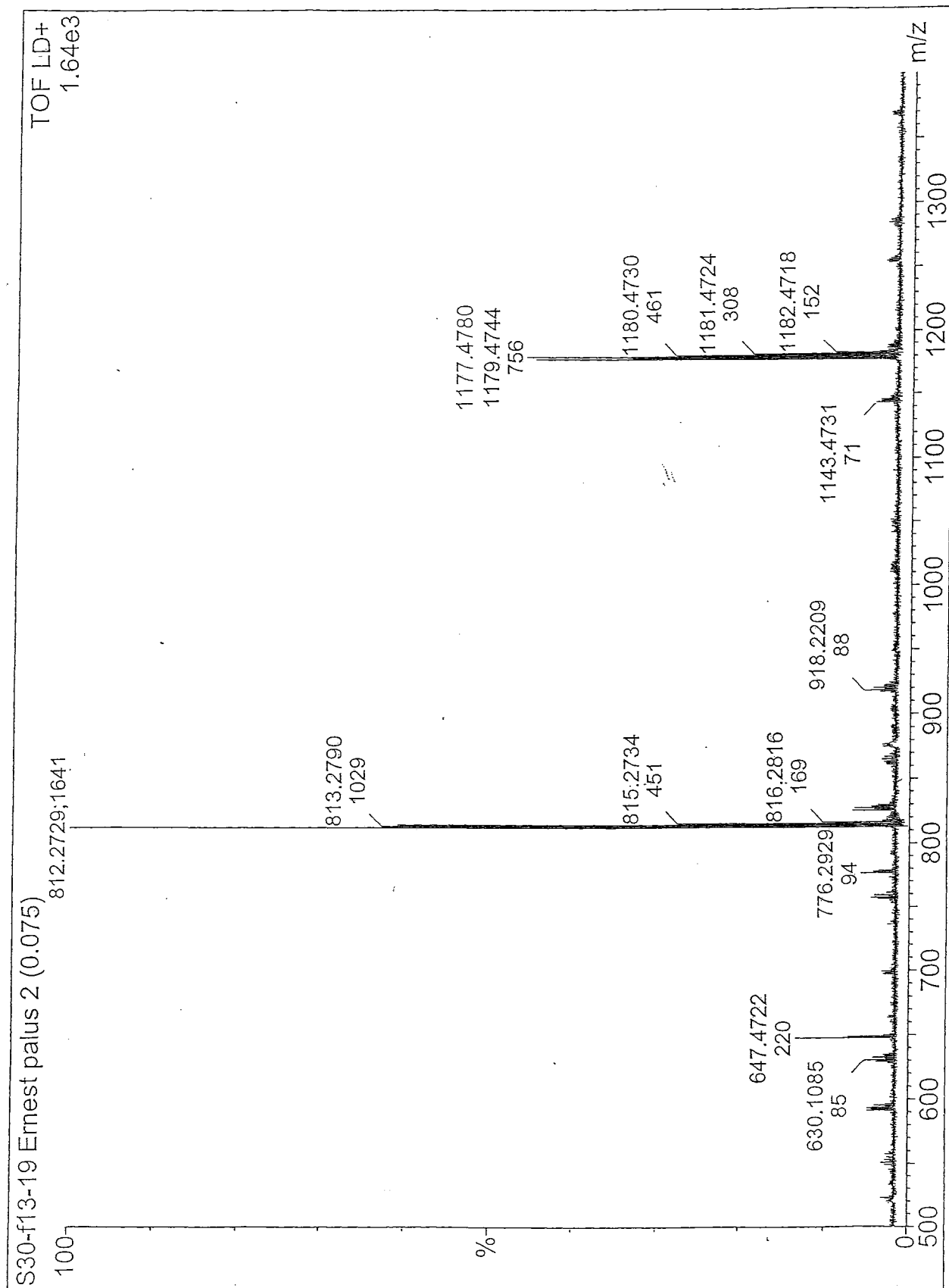


Appendix B: Mass Spectrum of Compound 20

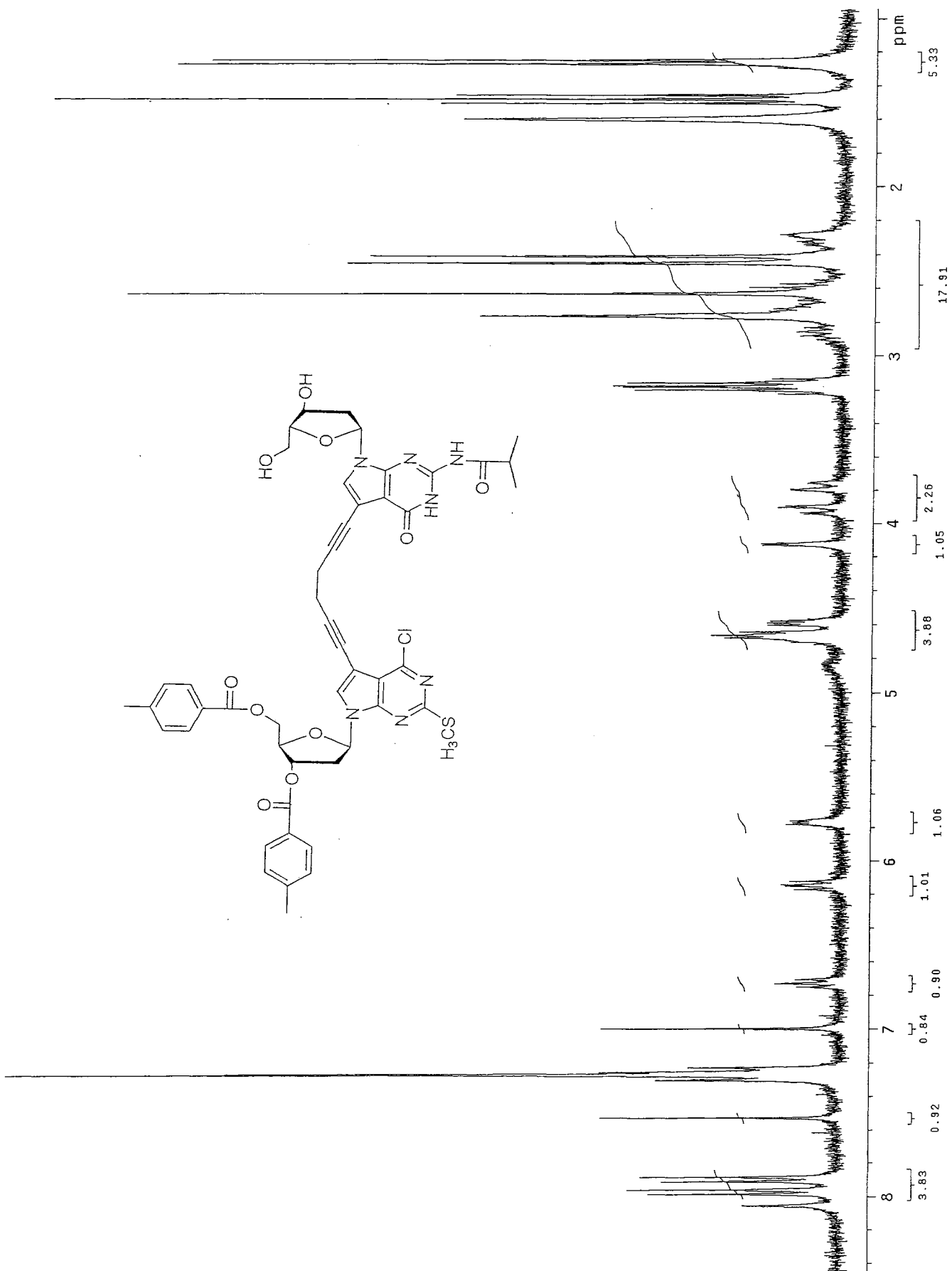




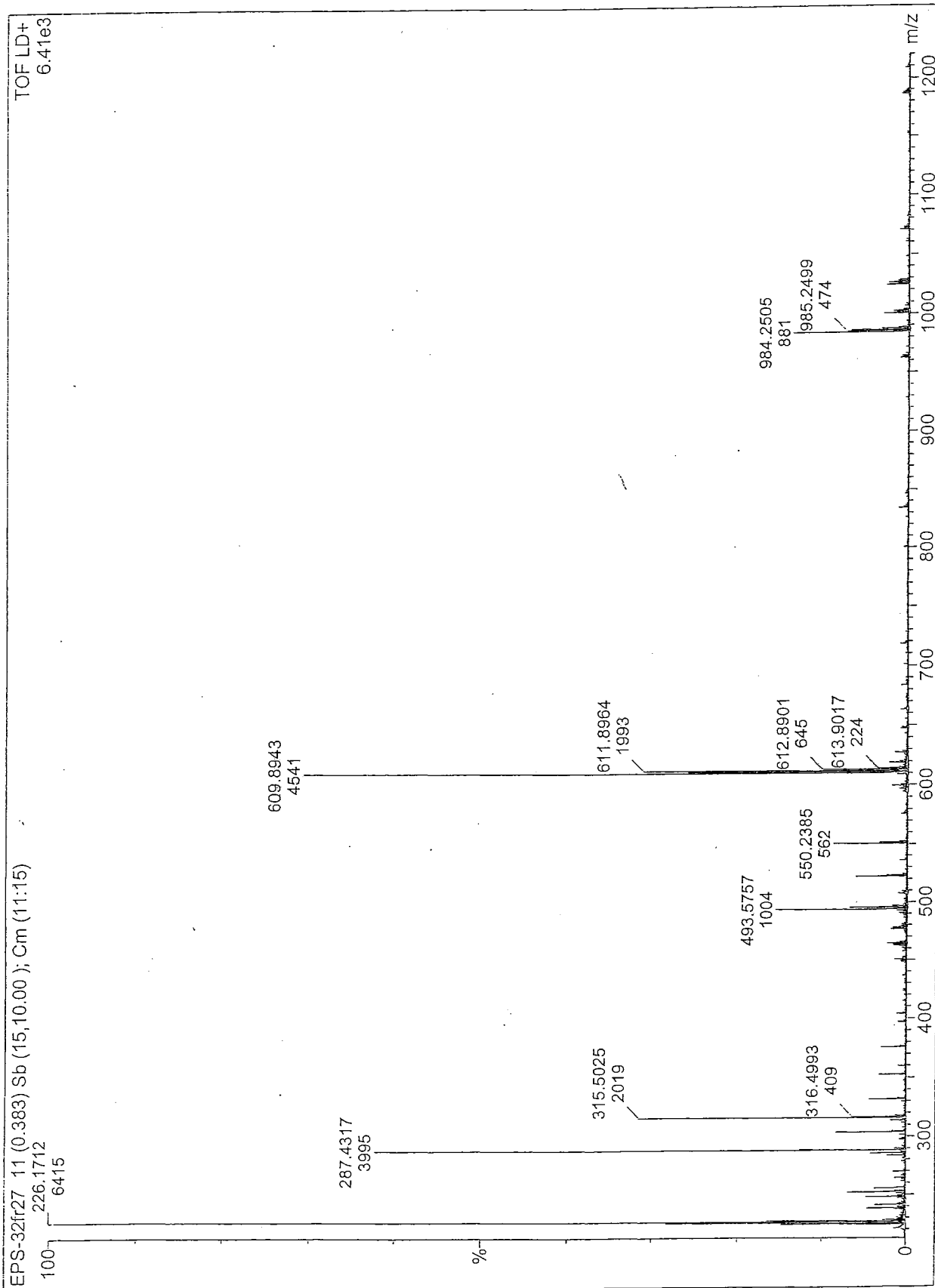
Appendix C:  $^1\text{H}$  NMR Spectrum of Compound 21 (in  $\text{CDCl}_3$ )



Appendix D: Mass Spectrum of Compound 21



Appendix E:  $^1\text{H}$  NMR Spectrum of Compound **22** (in  $\text{CDCl}_3$ )



Appendix F: Mass Spectrum of Compound 22